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ABSTRACT

The compartmentalization of the intracellular milieu into a membrane-bound compartments is the defining feature of the eukaryotic cell. The nucleus is enclosed by the nuclear envelope (NE), which consists of inner and outer nuclear membranes, and serves as a physical barrier between the genome and cytoplasm. The nuclear pore complex (NPC) is the sole gateway mediating the bidirectional trafficking of macromolecules – proteins and RNAs – between the nucleus and cytoplasm. The evolutionarily conserved core scaffold of NPC features a stack of coaxial rings embedded in the membranes of the NE. The core scaffold consists of an inner ring, which is sandwiched between two outer rings, and is anchored to the luminal ring via its pore membrane-spanning transmembrane domains. The core scaffold of NPC serves as a relatively rigid docking platform for the permeability barrier of the NPC, which is responsible for the selective nature of nucleocytoplasmic transport. The functional repertoire of the NPC core scaffold is expanded by peripheral appendages, which contribute to both transport-related and transport-unrelated functions. While the cytoplasmic face of the NPC core scaffold is decorated by flexible cytoplasmic filaments, the nucleoplasmic face is decorated by a prominent fishtrap-like structure known as the nuclear basket. Despite the well-established role of the nuclear basket in coupling transcription to mRNA quality control, the mechanism by which it docks onto the outer rings of the NPC core scaffold has remained elusive. Deciphering the molecular details of this docking mechanism constitutes the major aim of this doctoral thesis. By integrating AlphaFold-based protein–protein interaction screens, membrane-templated biochemical reconstitution, electron microscopy studies of Mlp1 nuclear filaments and live yeast cell fluorescence microscopy, a membrane-anchored tripartite junction between the nuclear basket and NPC core scaffold was uncovered. The basket subunit Nup60 harbours three adjacent short linear motifs (SLiMs), which connect Mlp1—a parallel homodimer composed of coiled-coil segments interrupted by flexible hinges—to the Nup85 subunit of the Y-complex, a key building block of the outer rings. The biochemical dissection of SLiMs revealed an in-built redundancy of the multivalent NPC core–basket junction, which may accommodate for disruptions caused by constrictions/dilations of the NPC core scaffold. The macromolecular assembly consisting of the Y-complex, Nup60 and Mlp1, which represents a minimal core scaffold-nuclear basket junction, was reconstituted on a synthetic membrane, revealing previously unanticipated competition between the Y-complex and Mlp1 for their binding to Nup60. Furthermore, mapped protein–protein interfaces were validated *in vivo*, yielding novel insights into the formation of Mlp1 perinuclear foci upon specific genetic perturbations. In addition, the helical region (HR) of Nup60 was demonstrated to undergo dimerization (or higher order oligomerization) *in vitro*, providing a molecular explanation for the previously proposed nuclear basket-stabilizing effect of the double nuclear

rings. The SLiM-based protein junction at the interface between the core scaffold of NPC and the nuclear basket offers a mechanistic explanation for how NPC structure and function can be reshaped, and how the post-translational modifications could affect the assembly state of the NPC. This work advances our understanding of the compositional and conformational heterogeneity of NPCs and establishes a foundation for future structural-functional studies of the nuclear basket. Moreover, the integrative approach combining high-throughput *in silico* screening of putative protein–protein interactions with *in vitro* characterization and *in vivo* validation of mapped interactions holds great promise for characterizing other complex macromolecular machineries.

ZUSAMMENFASSUNG

Die Kompartimentierung des intrazellulären Milieus in membrangebundenen Kompartimenten ist das entscheidende Merkmal der eukaryotischen Zelle. Der Kern wird von der Kernhülle (NE) umschlossen, die aus der inneren und äußeren Kernmembran besteht und als physische Barriere zwischen Genom und Zytoplasma dient. Der Kernporenkomplex (NPC) ist das einzige Tor, das den bidirektionalen Transport von Makromolekülen wie Proteinen und RNAs zwischen Kern und Zytoplasma vermittelt. Das evolutionär konservierte Kerngerüst des NPC besteht aus einem Stapel koaxialer Ringe, die in die fusionierten Doppelmembranen des NEs eingebettet sind. Das Kerngerüst besteht aus einem inneren Ring der zwischen zwei äußeren Ringen eingebettet ist und über seine porenmembranüberspannenden Transmembrandomänen am luminalen Ring verankert wird. Das Kerngerüst des NPC dient als starre Andockplattform für die Permeabilitätsbarriere des NPC, die für den selektiven Charakter des nukleozytoplasmatischen Transports verantwortlich ist. Das Funktionsrepertoire des NPC-Kerngerüsts wird durch periphere Anhängsel erweitert, die sowohl zu transportbezogenen als auch zu transportunabhängigen Funktionen beitragen. Während die zytoplasmatische Seite des NPC-Kerngerüsts von flexiblen zytoplasmatischen Filamenten geschmückt wird, ist die nukleoplasmatische Seite durch eine auffällige fischfallenartige Struktur – den so genannten nukleären Korb – gekennzeichnet. Trotz der bekannten Rolle des nukleären Korb bei der Kopplung der Transkription an die mRNA-Qualitätskontrolle ist der Mechanismus, mit dem er an die äußeren Ringe des NPC-Kerngerüsts andockt, bislang nicht bekannt. Die Entschlüsselung der molekularen Details dieses Andockmechanismus ist das Hauptziel dieser Doktorarbeit. Durch die Integration von AlphaFold-basierten Protein-Protein-Interaktions-Screens, membrangesteuerter biochemischer Rekonstitution, elektronenmikroskopischen Untersuchungen von Mlp1-Kernfilamenten und Fluoreszenzmikroskopie an lebenden Hefezellen wurde eine membranverankerte dreiteilige Verbindung zwischen dem nukleären Korb und dem NPC-Kerngerüst aufgedeckt. Die Korb-Untereinheit Nup60 besteht aus drei benachbarten kurzen linearen Motiven (SLiMs), die Mlp1, ein paralleles Homodimer aus Coiled-Coil-Segmenten die durch flexible Scharniere unterbrochen sind, mit der Nup85-Untereinheit des Y-Komplexes, einem Schlüsselbaustein der äußeren Ringe, verbinden. Die biochemische Sektion der Nup60-SLiMs ergab eine eingebaute Redundanz der multivalenten NPC-Kern-Korb-Verbindung, die Störungen aufgrund von Verengungen und Dehnungen des NPC-Kerngerüsts ausgleichen kann. Die makromolekulare Baugruppe aus Y-Komplex, Nup60 und Mlp1, die eine minimale Kerngerüst-Korb-Verbindung darstellt, wurde auf einer synthetischen Membran rekonstituiert. Dabei zeigte sich, dass der Y-Komplex und Mlp1 um die Bindung an Nup60 konkurrieren, was zuvor nicht erwartet wurde. Darüber hinaus wurden die kartierten Protein-

Protein-Schnittstellen in vivo validiert, was neue Erkenntnisse über die Bildung von perinukleären Mlp1-Foci bei spezifischen genetischen Störungen lieferte. Darüber hinaus konnte gezeigt werden, dass die helikale Region (HR) von Nup60 in vitro eine Dimerisierung (oder Oligomerisierung höherer Ordnung) durchläuft, was eine molekulare Erklärung für die zuvor vorgeschlagene stabilisierende Wirkung der doppelten Kernringe auf den nukleären Korb liefert. Die SLiM-basierte Proteinverbindung an der Schnittstelle zwischen dem Kerngerüst des NPC und dem nukleären Korb bietet eine mechanistische Erklärung dafür, wie Struktur und Funktion des NPC umgestaltet werden können und wie die posttranslationalen Modifikationen den Montagezustand des NPC beeinflussen könnten. Diese Arbeit verbessert unser Verständnis der kompositorischen und konformationellen Heterogenität von NPCs und schafft eine Grundlage für zukünftige strukturell-funktionelle Studien des nukleären Korbs. Darüber hinaus verspricht der integrative Ansatz, der ein in silico-Hochdurchsatz-Screening mutmaßlicher Protein-Protein-Wechselwirkungen mit einer in vitro-Charakterisierung und in vivo-Validierung der kartierten Wechselwirkungen verbindet, viel für die Charakterisierung anderer komplexer makromolekularer Mechanismen.

TABLE OF CONTENTS

1. INTRODUCTION	7
1.1. Macromolecular trafficking across intracellular membranes.....	7
1.2. Modular structure of NPC.....	8
1.2.1. Molecular architecture of core scaffold of NPC.....	8
1.2.2. Permeability barrier of NPC and nucleocytoplasmic transport.....	12
1.2.3. Expansion of functional repertoire of NPCs by asymmetric appendages	14
1.2.4. Cytoplasmic filaments.....	15
1.2.5. Organization of nuclear basket.....	15
1.2.6. Structure of nuclear basket.....	18
1.3. Compositional heterogeneity of NPCs	20
1.4. Conformational plasticity of NPCs.....	21
1.5. Dynamic profile of NPCs	22
1.6. Biogenesis of NPCs.....	23
2. AIMS	26
3. RESULTS	27
3.1. Results 1	27
3.1.1. Preamble	27
3.1.2. Contribution statement	27
3.1.3. Publication.....	27
3.2. Results 2	28
3.2.1. Preamble	28
3.2.2. Contribution statement	28
3.2.3. Publication.....	28
4. DISCUSSION.....	29
4.1. Comparison of nuclear basket models.....	29
4.2. Functional consequences of interface organized by SLiMs.....	30
4.3. Competition between Mlp1 and Y-complex for Nup60 binding	31
4.4. Function of Nup60 HR.....	32
4.5. Recruitment of nuclear filaments and formation of perinuclear foci	33
4.6. Asymmetric assembly of nuclear baskets	34
4.7. Future applications of approach.....	35
5. REFERENCES	37
6. ABBREVIATIONS.....	51

1. INTRODUCTION

1.1. Macromolecular trafficking across intracellular membranes

The emergence of the endomembrane system and membrane-bound organelles such as mitochondria and chloroplasts during the early evolution of unicellular life led to the subcellular compartmentalization of biochemical reactions and more complex control over cellular processes. The concomitant spatiotemporal separation of transcription and translation gave rise to the first eukaryotic cells (reviewed in *Vosselberg et al., 2024*). The nucleus, a defining feature of eukaryotes, is enclosed by the nuclear envelope (NE), a contiguous double-membrane system consisting of the inner nuclear membrane (INM), facing the genome, and the outer nuclear membrane (ONM), which itself is continuous with the endoplasmic reticulum (ER) (*Franke et al., 1981*). The INM and ONM, collectively known as nuclear membranes, are separated by a ~30 nm-wide luminal space, also known as perinuclear space (PNS) (*Moor&Mühlethaler, 1963*). Consequently, the PNS is connected to the ER lumen via ER–NE junctions (*Bragulat-Teixidor et al., 2024*).

Despite the regulatory advantages conferred by the intracellular membranes, the presence of a lipid bilayer barrier poses a challenge for the chemical communication between different compartments. The macromolecular trafficking across the membranes (herein focussed on protein transport) is facilitated by the compartment-specific transport channels. The first type of transport channels facilitates the translocation of polypeptide chains in their unfolded state. These include Sec61 translocon in the ER membranes and TOM/TIM complexes in the mitochondrial membranes, among others (reviewed in *Rapoport et al., 2017* and *Wiedemann&Pfanner, 2017*, respectively). The second type of transport channels facilitates the translocation of proteins in their native, folded state with the help of shuttling transport receptors. These include PEX13 channels in peroxisomes (*Gao et al., 2022*) and nuclear pore complexes (NPCs) embedded in the NE (reviewed in *Stewart, 2007* and *Wente&Rout, 2010*). Both transport channels harbour semipermeable barriers formed by repeat-containing intrinsically disordered regions (IDRs) of constituent subunits. Cargo proteins, when bound to respective transport receptors, can traverse the semipermeable barrier thanks to a network of weak, multivalent interactions transiently formed between the IDRs and cargo-bound transport receptors.

The nuclear pore complex (NPC) serves as the sole gateway within the NE facilitating bidirectional macromolecular trafficking between the nucleoplasm and cytoplasm. The NPC coats a highly curved pore membrane, which features a saddle-like topology with contours of both positive and negative curvatures. The pore membrane is formed upon fusion of INM and ONM during the biogenesis of the complex (for a more in-depth discussion of membrane

remodelling events during the assembly of NPC, see section 1.6. *Biogenesis of NPCs*). In haploid *Saccharomyces cerevisiae* cells, the total number of NPCs per nucleus varies throughout the cell cycle and increases almost linearly from ~70 NPCs in G1 phase to ~140 NPCs in early M phase (Winey *et al.*, 1997). The estimates derived from serial section electron microscopy (EM) reconstructions of yeast nuclei are in line with recently reported live-cell fluorescence microscopy-based estimate (Zsok *et al.*, 2024). Similarly, the total number of NPCs doubles from ~2000 in G1 to ~4000 at the end of the cell cycle in human (HeLa) cells (Maul *et al.*, 1972; Ribbeck&Görllich, 2001). However, in contrast to yeast, the number of human NPCs does not increase linearly, but instead displays two bursts: first in G1 phase right after mitosis and second shortly before DNA synthesis in S phase (Maul *et al.*, 1972).

1.2. Modular structure of NPC

Both yeast NPCs, with an estimated molecular mass of ~52 MDa, and human NPCs, at ~110 MDa, are composed of approximately 30 different subunits, collectively known as nucleoporins, typically present in multiples of eight (reviewed in Lin&Hoelz, 2019 and Petrovic *et al.*, 2022). The yeast NPC contains roughly 550 individual subunits, whereas human NPC comprises around 1,000 protein building blocks. *In cellulo*, both *S. cerevisiae* and human NPCs have a diameter of ~100 nm (membrane-to-membrane distance), whereas the central channel is ~60 nm in width (Allegretti *et al.*, 2020; Schuller *et al.*, 2021). Despite differences in subunit stoichiometry, the overall framework of the NPC is conserved from yeast to humans. The NPC consists of the symmetric core scaffold, which lines the pore membrane and acts as a structural docking platform for the functional modules; a meshwork of intrinsically disordered phenylalanine-glycine (FG) repeat regions, which forms the central channel permeability barrier of the NPC; and asymmetric appendages, namely, the cytoplasmic filaments and the nuclear basket, decorating the cytoplasmic and nucleoplasmic faces of the core scaffold, respectively (Fig. 1A,B).

1.2.1. Molecular architecture of core scaffold of NPC

The core scaffold of the NPC forms the framework of NE-spanning pore channel and serves as a relatively inert docking site for the functional modules of the NPC, namely, the permeability barrier and asymmetric appendages. The core scaffold of the NPC is the best characterized part of the NPC, partially due to its relative rigidity compared to other modules of the NPC, rotational and bilateral symmetry (covered in detail below), low exchange rates of scaffold nucleoporins (Hakhverdyan *et al.*, 2021) and converging stoichiometric estimates of

constituent nucleoporins independent of the methodology used (*Rout et al., 2000; Alber et al., 2007; Mi et al., 2015; Rajoo et al., 2018; Kim et al., 2018*).

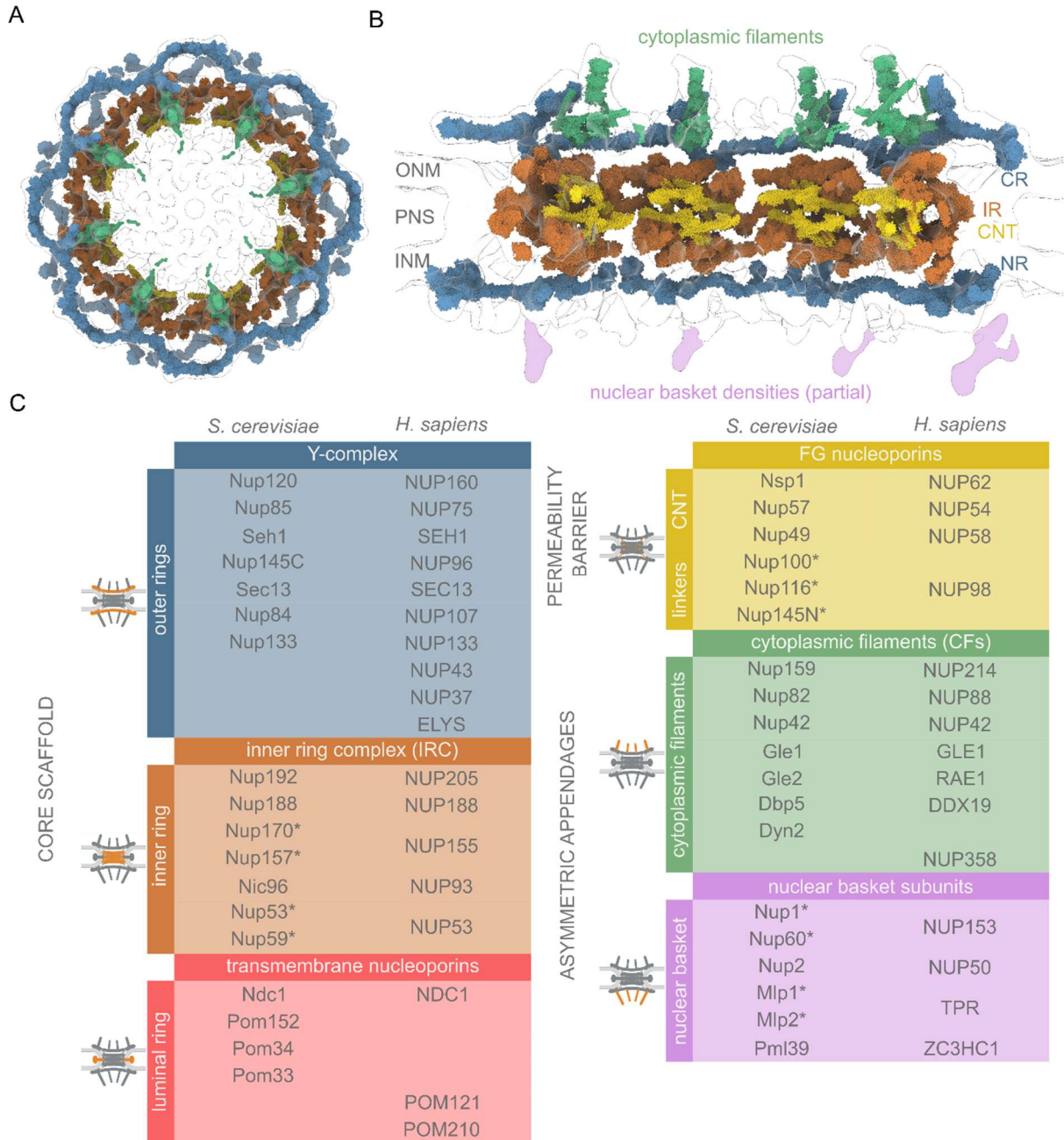


Figure 1. (A-B) Cytoplasmic (A) and cross-sectional (B) view of the *S. cerevisiae* NPC (*Akey et al., 2022*, luminal ring not shown). CNT: channel nucleoporin trimer; CR: cytoplasmic ring; INM: inner nuclear membrane; IR: inner ring; NR: nucleoplasmic ring; ONM: outer nuclear membrane; PNS: perinuclear space. (C) Yeast nucleoporins and their corresponding human orthologs. Asterisk (*) denotes yeast paralogs (e.g., Mlp1 and Mlp2 are yeast paralogs orthologous to human TPR).

Decades of research including biochemical characterization of protein-protein interactions, structural characterization (X-ray crystallography and single-particle cryo-EM) of individual nucleoporins and constituent subcomplexes, quantification of nucleoporin

stoichiometry and cryo-electron tomography (cryo-ET) reconstructions of intact NPCs yielded near-atomic composite structures of core scaffolds for a number of species (*Kim et al., 2018; Allegretti et al., 2020; Akey et al., 2022; Li et al., 2022; Akey et al., 2023; Mosalaganti et al., 2022; Petrovic et al., 2022; Huang et al., 2020; Huang et al., 2022*).

The core scaffold of the NPC displays a high degree of modularity, evident both at a plane of the nucleocytoplasmic transport axis and at a plane perpendicular to it. When viewed from the side, the core scaffold displays bilateral symmetry and appears as a stack of coaxial rings, i.e., an inner ring sandwiched between two outer rings, termed cytoplasmic and nucleoplasmic rings, respectively (Fig. 1B). Additionally, a so-called luminal ring (also referred to as the transmembrane ring) encircles the pore membrane in the perinuclear space of the NE, on the opposite face of the nuclear membrane relative to the inner ring. The inner and luminal rings at the equator of the core scaffold are connected via transmembrane nucleoporins, which span the pore membrane.

The above-described annular modularity of the core scaffold is further complemented by the NPC-defining eightfold rotational symmetry apparent when viewing the structure along the nucleocytoplasmic transport axis (Fig. 1A). The core scaffold is composed of eight protomers, termed spokes, arranged symmetrically around the nucleocytoplasmic transport axis. Each spoke contains subcomplexes constituting inner, luminal and outer rings. The conserved rotational symmetry of the core scaffold greatly facilitated the elucidation of NPC structure and led to the near-atomic models of NPC scaffolds from various species.

An additional level of core scaffold modularity arises from the structural nature of individual rings themselves, which are composed of defined subcomplexes. The pore membrane-proximal inner ring is an assembly of inner ring complexes (IRCs) (*Onischenko et al., 2009; Stuwe et al., 2015*), whereas the outer rings—both cytoplasmic and nucleoplasmic—feature head-to-tail arrangements of the so-called Y-complexes (also known as the Nup84 complex in *S. cerevisiae* and NUP107-160 complex in human) (*Siniosoglou et al., 1996; Siniosoglou et al., 2000; Lutzmann et al., 2002; Kampmann&Blobel, 2009; Fernandez-Martinez et al., 2012; Thierbach et al., 2013; Shi et al., 2014; Stuwe et al., 2015; Kelley et al., 2015*) (Fig. 1C). In *S. cerevisiae*, heteroheptameric Y-complex consists of a stem (Nup145C-Nup84-Nup133) and two short arms (Nup120 and Nup85-Seh1), which converge at a triskelion region (*Siniosoglou et al., 1996; Siniosoglou et al., 2000; Lutzmann et al., 2002; Kampmann&Blobel, 2009*). The head-to-tail oligomerization of Y-complexes is facilitated by the intermolecular interactions between Nup133 and Nup120 subunits belonging to adjacent Y-complexes (*Seo et al., 2009*).

Homozygous deletion of *NUP133* in mouse embryonic stem cells (mESC) results in the structural heterogeneity of NPCs, manifested by increased frequency of sevenfold- and ninefold-symmetric NPCs, which together account for ~17% of all NPCs in mutant mESC, as well as incomplete outer ring architectures (Taniguchi et al., 2025). The observation of NPCs with aberrant symmetries suggests that the outer rings contribute to the establishment of canonical eightfold symmetry, likely templating the interphase NPC assembly during its early steps (Vollmer et al., 2015; Onischenko et al., 2020; Taniguchi et al., 2025).

The subunits of both IRC and Y-complex feature repetitive employment of α -solenoid and/or β -propeller domains, depending on the subunit (Stuwe et al., 2015; Kelley et al., 2015). Both the IRC and Y-complexes are proposed to be anchored to the underlying pore membrane via a combination of amphipathic lipid packing sensor (ALPS) motifs, predicted in the unstructured loops of β -propeller domains (Drin et al., 2007; Kim et al., 2014), and C-terminal amphipathic helices (AHs) of IRC subunit Nup53 and its paralog Nup59 (Eisenhardt et al., 2014). Therefore, the modular nature of the core scaffold is evident across length scales, from individual domains to supramolecular assemblies of subcomplexes.

Although the core scaffold is the most conserved part of the NPC, it displays species-specific structural variations, especially in the stoichiometry of outer rings (for a detailed discussion of the compositional heterogeneity of NPCs, see section 1.3. *Compositional heterogeneity of NPCs*). The most prevalent isoform of *S. cerevisiae* NPC contains 16 copies of the Y-complex per NPC arranged in two octameric outer rings (Kim et al., 2018; Allegretti et al., 2020; Akey et al., 2022; Petrovic et al., 2022). In contrast, human NPC contains 32 copies of the Y-complex arranged in a pair of double outer rings, each composed of 16 copies of the Y-complex (Schuller et al., 2021; Mosalaganti et al., 2022; Petrovic et al., 2022). Each outer ring consists of two staggered octameric Y-complex rings. A similar double-ring configuration is present in *Xenopus laevis* NPCs (Huang et al., 2020; Huang et al., 2022).

The bilateral stoichiometric symmetry of outer rings observed in *S. cerevisiae* (major isoform) and vertebrate cells (8-8 and 16-16, respectively) is broken in other species. The first observation of such structural modification was made in *Chlamydomonas reinhardtii* cells (Mosalaganti et al., 2018). The in-situ cryo-ET structure of *C. reinhardtii* NPC features a single cytoplasmic ring (8 copies of the Y-complex) and a double nucleoplasmic ring (16 copies of the Y-complex) (Mosalaganti et al., 2018). Identical asymmetry in the oligomeric state of outer rings was observed in both *Schizosaccharomyces pombe* and *Toxoplasma gondii* cells (8-16), although the cytoplasmic rings in both species were found to be discontinuous (Zimmerli et al., 2021; Singh et al., 2024). Similarly, the bilateral symmetry of the core scaffold is broken in *Dictyostelium discoideum* cells, but to an even greater extent. The in-situ structure of *D. discoideum* NPC features a unique scaffold architecture with a single cytoplasmic ring (8

copies of the Y-complex) and a triple nucleoplasmic ring (24 copies of the Y-complex) (Hoffmann et al., 2025). The unique triple configuration of *D. discoideum* nucleoplasmic ring has not been observed in any other species to date (Table 1).

Table 1. Copy number of Y-complex per outer ring (CR and NR) across different species

	Sc		Sp	Tg	Cr	Dd	Xl	Hs
CR	8	8	8(?)	8(?)	8	8	16	16
NR	8	16	16	16	16	24	16	16

Abbreviations: *Sc* = *S. cerevisiae*; *Sp* = *S. pombe*; *Tg* = *T. gondii*; *Cr* = *C. reinhardtii*; *Dd* = *D. discoideum*; *Xl* = *X. laevis*; *Hs* = *H. sapiens*. For references, refer to the main text.

Interestingly, all so-far described symmetry breaking scaffold architectures feature a higher copy number of Y-complexes on the nuclear side compared to the cytoplasmic side. The same is also true for the *S. cerevisiae* NPC isoform with a single cytoplasmic ring (8 copies of the Y-complex) and double nuclear ring (16 copies of the Y-complex) (Akey et al., 2022; Akey et al., 2023). Although double nuclear ring in the *S. cerevisiae* NPC has been implicated in the stabilization of the nuclear basket (Singh et al., 2024), whether the stoichiometric mismatches between outer rings across different species indeed favour nuclear basket attachment remains to be addressed in future studies.

1.2.2. Permeability barrier of NPC and nucleocytoplasmic transport

The NPCs display very high translocation capacity with up to 1,000 translocation events per second and a total mass flow of up to ~100 MDa/s, i.e. almost the total mass of a human NPC itself (Ribbeck&Görllich, 2001). The NPCs facilitate the macromolecular trafficking of cargo molecules, which span three orders of magnitude in molecular mass - from ~40 kDa monomeric proteins to ~40 MDa macromolecular assemblies composed of over 1,000 subunits, such as intact HIV-1 nucleocapsids (Bonner, 1975; Zila et al., 2021). Smaller molecules, such as metabolites, hormones and small proteins, diffuse passively in equilibrium between the nucleus and cytoplasm. The transition between passive diffusion and facilitated translocation is fluid, as surface features of cargo proteins can either impede or facilitate their translocation (Frey et al., 2018).

The directional nucleocytoplasmic transport of cargo molecules is dependent on: 1) the presence of a permeability barrier, which keeps the contents of the two compartments unmixed; 2) the RanGTP concentration gradient across the nuclear envelope, which ensures the directionality of the transport (Izaurralde et al., 1997); and 3) shuttling nuclear transport receptors (NTRs), which bind cargo molecules and facilitate their translocation through the

permeability barrier (reviewed in *Stewart, 2007* and *Wente&Rout, 2010*). Some cargo molecules, such as HIV-1 nucleocapsids, have evolved to bypass the NTR requirement by mimicking the engagement of NTR with the permeability barrier (*Fu et al., 2024; Dickson et al., 2024*).

The inner ring of the core scaffold serves as a docking site for the central channel nucleoporins, which feature intrinsically disordered phenylalanine-glycine (FG)-rich repeat regions. A substantial fraction of FG repeats is contributed by channel nucleoporin trimer (CNT), consisting of Nsp1, Nup49, and Nup57 in yeast, and NUP62, NUP58, and NUP54 in metazoans (*Grandi et al., 1995; Stuwe et al., 2015; Chug et al., 2015*) (Fig. 1C). Additional FG repeats are contributed by Nup145N, Nup100, Nup116 in yeast and NUP98 in metazoans, as well as FG-repeat containing subunits of asymmetric appendages, although the asymmetric FG repeats do not perform any essential functions (*Strawn et al., 2004*).

The FG repeats fill the central channel with a diameter of ~60 nm in both *S. cerevisiae* and human NPCs (*Allegretti et al., 2020; Schuller et al., 2021*) and form a multivalent, weak interaction meshwork, which acts as a semi-permeable barrier (*Ribbeck&Görllich, 2001*). The local concentration of FG repeats within the central channel is estimated to be ~50 mM (*Bayliss et al., 1999*). The physicochemical nature of permeability barrier within the NPC remains a hotly debated topic in the field, with proposed models ranging from homogeneous distribution of FG repeats throughout the central channel to the coexistence of regions with varying FG repeat concentrations (reviewed in *Matsuda et al., 2024*). Some insight into the nature of FG repeats has been gathered from *in vitro* experiments. It has been demonstrated that FG nucleoporins undergo liquid-liquid phase separation (LLPS) *in vitro* (*Schmidt&Görllich, 2015; Celetti et al., 2019*). Moreover, bulk condensates of NUP98 and its yeast orthologs recapitulate permeability barrier properties of the intact NPCs (*Frey&Görllich, 2007; Schmidt&Görllich, 2015; Celetti et al., 2019*). However, in-situ FLIM-FRET measurements of fluorescently labelled NUP98 revealed that FG repeats adopt an extended conformation within the intact human NPCs compared to the collapsed solution state *in vitro* (*Yu et al., 2023*). Even though NUP98 condensates recapitulate the permeability barrier properties of the NPC, the molecular dynamics simulations suggest that NUP98 grafted onto the NPC scaffold forms a molecular environment distinct from the bulk condensates (*Yu et al., 2023*). Once formed, the FG phase is mechanically robust. The application of osmotic stress does not affect the permeability barrier of the *D. discoideum* NPCs, suggesting that the FG meshwork remains intact even upon dilation-constriction of the core scaffold (*Hoffmann et al., 2025*).

1.2.3. Expansion of functional repertoire of NPCs by asymmetric appendages

The core scaffold of the NPC is further decorated by asymmetric, compartment-specific appendages on both sides of the core, namely, cytoplasmic filaments on the cytoplasmic face and a nuclear basket on the nucleoplasmic face. Asymmetric appendages harbour docking sites for an NPC accessory proteome, which includes the small GTPase Ran (Yaseen&Blobel, 1999), Ran GTPase-activating protein (RanGAP) (Matunis et al., 1998), 26S proteasomes (Albert et al., 2017), SUMO protease Ulp1 (Zhao et al., 2004), kinases (McCloskey et al., 2018), gene expression and mRNP export machineries (reviewed in Strambio-De-Castillia et al., 2010; Ptak et al., 2014; Bonnet&Palancade, 2014; Bensidoun et al., 2021). Moreover, the nuclear basket has been implicated in peripheral chromatin organization by excluding heterochromatin from the nuclear basket-adjacent region of the nucleoplasm (Krull et al., 2010; Niepel et al., 2013), which has recently been visualized by cryo-ET analysis of mouse NPCs (Singh et al., 2024). In addition to the exclusion effect, chromatin organization can also be remodelled by the nuclear basket-mediated, context-dependent recruitment of either active or repressed genes to the NPCs (Brickner et al., 2004; Cabal et al., 2006). Therefore, asymmetric appendages expand the functional repertoire of NPC core scaffolds by contributing to both transport-related and transport-unrelated functions of the NPC (reviewed in Cho&Hetzer, 2020).

In stark contrast to the core scaffold of the NPC, the peripheral appendages exhibit a high degree of flexibility, which is a major reason why asymmetric appendages remain the last frontier in the structural characterization of the NPC. The conformational heterogeneity of asymmetric appendages became evident during the early structural characterization of the NPC by EM (Goldberg&Allen, 1992, 1993, 1996; Kiseleva et al., 1998; Fahrenkrog et al., 1998). While some of the observed structural variation could be explained by the flux of bulky cargo molecules (Kiseleva et al., 1998), the remaining heterogeneity likely points to the inherent flexibility of the peripheral appendages.

The dissection of cytoplasmic filaments and the nuclear basket was further compounded by the diverging stoichiometric estimates of the nucleoporins constituting the corresponding structures. For example, the stoichiometric estimates of *S. cerevisiae* Nup60, a key subunit organizing the nuclear basket, and its paralog Nup1 range from 4 to 16 copies per NPC depending on the methodology used (Rout et al., 2000; Alber et al., 2007; Mi et al., 2015; Rajoo et al., 2018; Kim et al., 2018). Similarly, the stoichiometric estimates of yeast cytoplasmic filament subunits display a broad range of values (Rout et al., 2000; Alber et al., 2007; Mi et al., 2015; Rajoo et al., 2018; Kim et al., 2018). The estimates of human stoichiometry are equally divergent. For instance, in HeLa cells, 16 copies of TPR were found to be present per NPC based on fluorescence correlation spectroscopy (FCS)-calibrated

confocal microscopy measurements (*Otsuka et al., 2023*). In contrast, an earlier mass spectrometry (MS)-based study of human NPC stoichiometry performed on HeLa cells estimated 32 copies of both TPR and NUP153 per NPC (*Ori et al., 2013*).

Furthermore, several subunits of the asymmetric appendages display some of the highest exchange rates with the free pool of corresponding nucleoporins, further burdening structural characterization (*Hakhverdyan et al., 2021*; for a detailed discussion of structural dynamics, see section 1.5. *Dynamic profile of NPCs*). Despite these obstacles, recent progress has been made in deciphering the organization of both the cytoplasmic filaments and the nuclear basket.

1.2.4. Cytoplasmic filaments

The near-atomic architecture of cytoplasmic filaments has recently been resolved in human NPCs through an integrative approach combining biochemical reconstitution, X-ray crystallography, docking into cryo-ET map, and validation in live cells (*Bley et al., 2022*). Human CFs consist of pentameric bundles of the metazoan-specific NUP358 tethered to the central stalk region of the Y-complex (*Bley et al., 2022*). The CFs extend up to ~60 nm into the cytoplasm and contain a number of Ran-binding and catalytic (E3 ligase and prolyl-isomerase) domains, even though NUP358 is not essential for nuclear import (*Bley et al., 2022*; *Walther et al., 2002*). Additionally, evolutionary-conserved heterohexameric cytoplasmic filament nucleoporin complex (CFNC) is appended to the cytoplasmic ring (*Bley et al., 2022*). The orthologous yeast P-shaped CFNC, also known as Nup82 complex, has also been shown to be tethered to the cytoplasmic ring of *S. cerevisiae* NPC (*Gaik et al., 2015*).

1.2.5. Organization of nuclear basket

The nuclear basket of *S. cerevisiae* NPCs consists of paralogous Nup1 and Nup60 (NUP153 in vertebrates) (*Feuerbach et al., 2002*; *Meszaros et al., 2015*), Nup2 (NUP50 in vertebrates) (*Denning et al., 2001*; *Dilworth et al., 2001*), paralogous Mlp1 and Mlp2 (TPR in vertebrates) (*Strambio-de-Castillia et al., 1999*; *Hase et al., 2001*; *Hase&Cordes., 2003*) and Pml39 (ZC3HC1 in vertebrates) (*Palancade et al., 2005*; *Gunkel&Cordes, 2022*; *Gunkel et al., 2023*) (Fig. 1C). Despite its structural prominence on the nucleoplasmic face of the NPC (*Goldberg&Allen, 1992, 1993, 1996*; *Kiseleva et al., 1998*; *Fahrenkrog et al., 1998*), the nuclear basket is not essential for bulk mRNP export or protein import and export. Mutant yeast cells harbouring single or combinatorial deletions of genes encoding nuclear basket subunits (e.g., $\Delta pml39 \Delta mlp1 \Delta mlp2$) are viable (with the exception of *NUP1* gene, which is

essential in a genetic background-dependent manner) (*Palancade et al., 2005; Niepel et al., 2013; Meszaros et al., 2015*).

Despite its non-essential nature, the nuclear basket has been implicated in numerous cellular processes (reviewed in *Bensidoun et al., 2021*) (Fig. 2). Deletion of either *MLP1* or *PML39* results in the leakage of intron-containing pre-mRNA, although neither splicing nor bulk mRNA export is affected (*Galy et al., 2004; Palancade et al., 2005*), suggesting a role for the nuclear basket in the quality control before the export of mRNPs. In contrast, deletion of *NUP60* leads to both leakage of pre-mRNA and splicing defects (*Galy et al., 2004*). Additionally, the nuclear basket has been implicated in the exclusion of heterochromatin (*Krull et al., 2010; Niepel et al., 2013; Singh et al., 2024*), context-dependent control of gene expression (reviewed in *Strambio-De-Castillia et al., 2010* and *Ptak et al., 2014*), regulation of NPC numbers (*McCloskey et al., 2018*), and recruitment of an NPC accessory proteome, including proteasomes (*Albert et al., 2017*), SUMO protease Ulp1 (*Zhao et al., 2004*), mRNA export factors (reviewed in *Bensidoun et al., 2021*).

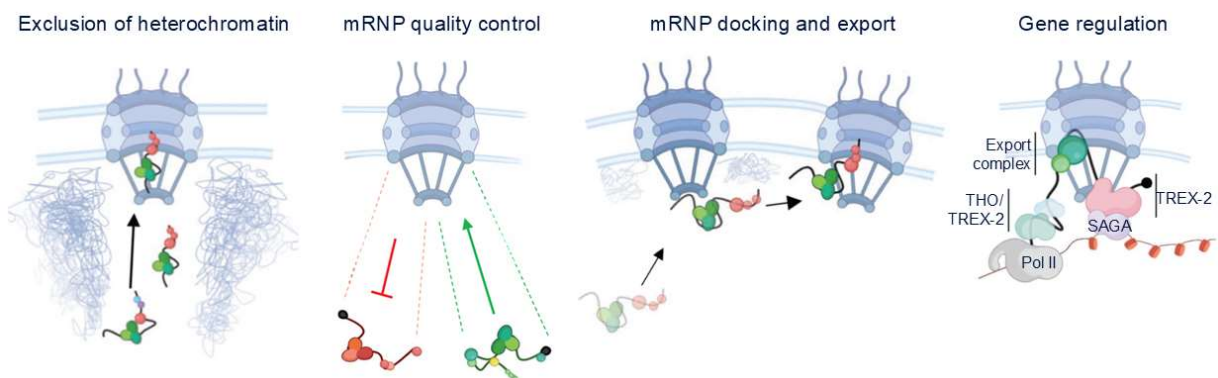


Figure 2. Functional expansion of NPC core scaffolds by the nuclear basket. Figure adapted from *Bensidoun et al., 2021*.

The molecular inventory of the nuclear basket can be subdivided into two subsets: subunits present across all or vast majority of NPCs (Nup60, Nup1, Nup2, hereafter referred to as ‘core nuclear basket subunits’) and subunits present only in the subset of NPCs (Mlp1, Mlp2, Pml39, hereafter referred to as ‘nuclear filament subunits’) (Fig. 1C). During the early characterization of nuclear baskets, nuclear filament subunits were found to be largely excluded from the NE region in proximity to the nucleolus, leading to the proposal of ‘basketless NPCs’ (*Galy et al., 2004; Palancade et al., 2005; Bensidoun et al., 2022*). The classification of nuclear basket subunits into two subsets, initially based on the localization patterns, has since been substantiated by other distinct features. Structurally, core basket subunits – Nup60, Nup1 and Nup2 – contain extensive intrinsically disordered regions and harbour FG repeats (*Meszaros et al., 2015; Cibulka et al., 2022*), while nuclear filament subunits are largely structured (*Hase et al., 2001; Hashimoto et al., 2022*). Moreover, core

basket subunits are constantly exchanging with the free nucleoplasmic pools of corresponding nucleoporins (*Hakhverdyan et al., 2021; Zsok et al., 2024*), whereas basket filament subunits exhibit stable association with the NPC (*Souquet et al., 2018; Hakhverdyan et al., 2021; Zsok et al., 2024*). Finally, even the timing of incorporation during NPC biogenesis differs significantly between the two groups. Core basket subunits are incorporated early during the interphase assembly process, whereas the incorporation of nuclear filaments takes hours and marks the last step of the process (*Onischenko et al., 2020*).

Early studies on nucleoporin localization phenotypes upon individual gene deletions pointed to the central role of Nup60 in the assembly and/or organization of the nuclear basket. Deletion of *NUP60* leads to the mislocalization of Nup2, Mlp1, Mlp2 and Pml39, resulting in either diffuse nucleoplasmic staining (Nup2) or perinuclear foci (Mlp1, Mlp2 and Pml39) (*Denning et al., 2001; Feuerbach et al., 2002; Palancade et al., 2005*). In the case of Nup2, Nup60 plays an essential role in both its recruitment and retention at the NPC (*Dilworth et al., 2001; King et al., 2023*). However, in the case of Mlp1, Nup60 is essential for its recruitment (*Feuerbach et al., 2002; Palancade et al., 2005*), but dispensable for the maintenance of already assembled Mlp1 (*Zsok et al., 2024*). This finding is in line with the observations of meiotic divisions, during which Nup60 becomes nucleoplasmic, whereas Mlp1 is retained at the NPC (*King et al., 2023*). Furthermore, transient conditional depletion of Nup60 does not affect the localization of Mlp1 (*King et al., 2023*). Therefore, in the case of Mlp1, the role of Nup60 is reminiscent of that of an assembly factor. Interestingly, this same dependency between the corresponding human orthologs, i.e. NUP153 and TPR, was reported in the past (*Hase&Cordes, 2003; Aksenova et al., 2020*).

Additional subcellular localization interdependencies were identified between nuclear filament subunits. The deletion of *MLP1* leads to the nucleoplasmic localization of Pml39 and reduced levels of Mlp2 at the NE (*Palancade et al., 2005; Gunkel et al., 2023*), whereas the deletion of *MLP2* has no effect on either Mlp1 or Pml39 (*Gunkel et al., 2023*). The deletion of *PML39* leads to reduced levels of Mlp1 at the NE (*Gunkel et al., 2023; Zsok et al., 2024*). These observations point to a central role of Mlp1 within the nuclear filament subset.

The lack of structural information on the organization of the nuclear basket was overcome by the biochemical characterization of relevant protein-protein interactions, leading to an architectural model of nuclear basket organization (*Cibulka et al., 2022*). The nuclear basket is primarily organized by Nup60, which harbours an N-terminal amphipathic helix (AH), through which it is anchored to the nuclear envelope (*Meszaros et al., 2015*), and several conserved short linear motifs (SLiMs), through which it binds other subunits of the nuclear basket (*Cibulka et al., 2020*). The interaction between Nup60 Mlp1-binding motif (MBM) and Mlp1 Nup60-binding domain (N60BD) recruits Mlp1, a coiled-coil subunit of the nuclear basket,

which, together with its paralog Mlp2, forms the nuclear basket filaments ('struts'). Moreover, Nup60 recruits Nup2 via the interaction mediated by the C-terminal Nup2-binding motif (N2BM) (Denning *et al.*, 2001; Dilworth *et al.*, 2001; Cibulka *et al.*, 2020). Due to the nature of nuclear basket organization, the role of Nup60 has been linked to that of a 'suspension cable' tying the megadalton-order structure together.

1.2.6. Structure of nuclear basket

The first in situ cryo-ET map of NPC showing fishtrap-like nuclear basket densities was reported back in 2004 and originated from intact *D. discoideum* nuclei (Beck *et al.*, 2004). The study resolved eight nuclear basket filaments ('struts'), emanating from the nuclear ring and converging ~60 nm away to form another ring structure, termed the distal ring (Beck *et al.*, 2004). However, the low resolution of the map, combined with the lack of high-resolution structures of individual nuclear basket subunits made the interpretation of the densities challenging. Furthermore, it was impossible to rule out the possibility that some of the apparent densities correspond to an NPC accessory proteome, such as NTRs and NTR-cargo complexes, instead of the nuclear basket *per se*. Subsequent subtomogram analysis of *Dd*NPCs with an improved image-processing pipeline increased the resolution of the map, but did not yield further insight into the organization of the nuclear basket (Beck *et al.*, 2007).

In more recent in situ cryo-ET reconstructions of NPCs, only partial, nuclear-ring-adjacent densities of the nuclear basket were resolved (Allegretti *et al.*, 2020; Akey *et al.*, 2022; Hoffmann *et al.*, 2025; Taniguchi *et al.*, 2025). In *S. cerevisiae*, local mask refinement recovered nuclear basket densities centered over the vertex of the Y-complex (Nup145C-Nup120 junction) and extending toward both the stem of the Y-complex (Nup84-Nup133 junction) and its short arm (Nup85-Seh1) (Allegretti *et al.*, 2020). Notably, these nuclear basket densities were resolved in yeast NPC isoform harbouring a single nuclear ring (Allegretti *et al.*, 2020). This positioning of the nuclear basket densities contrasts with a previously reported cryo-ET reconstruction of immunopurified NPCs (also from *S. cerevisiae*), in which a rod-like density, which was interpreted as Mlp1/2, was anchored exclusively to the short arm of the Y-complex (Nup85-Seh1) (Kim *et al.*, 2018). It remains unclear to which extent the observed discrepancy between the positioning and morphology of observed densities can be accounted for by the difference in experimental methodology (in-cell NPCs vs. immunopurified NPCs).

In NPCs of mESC, a protrusion from the nucleoplasmic ring of NPC has been resolved at the NUP107-NUP133 region of the Y-complex and likely corresponds to nuclear basket filaments (Taniguchi *et al.*, 2025). The corresponding density was lost upon homozygous deletion of *NUP133* (Taniguchi *et al.*, 2025), in agreement with earlier report

demonstrating nuclear basket misassembly upon identical genetic perturbation (*Souquet et al., 2018*).

Recent progress has been made in resolving the nuclear basket densities by combining cryo-focussed ion beam (cryo-FIB) milling to produce thin lamellae with in-cell cryo-ET of vitrified cells (*Singh et al., 2024*). Interestingly, *S. cerevisiae* nuclear basket densities were only resolved in the NPC isoform harbouring a double nuclear ring, prompting the authors to suggest that double nuclear ring stabilizes the nuclear basket (*Singh et al., 2024*). Thus, the absence of nuclear basket densities in yeast NPC isoform with single nuclear ring was attributed to the more dynamic nature of the nuclear basket when tethered to the single nuclear ring. Although the proposed explanation is in agreement with the cryo-ET observations, direct biochemical evidence supporting a basket-stabilizing effect of the double nuclear ring is lacking.

In-cellulo nuclear basket densities of ScNPC display a striking similarity to previously resolved nuclear basket densities from *D. discoideum* nuclei, specifically, eight ~10 nm-thick nuclear basket filaments emanating from the nuclear ring at a ~30° angle from the nucleocytoplasmic axis and converging into a ~76 nm-wide distal ring ~54 nm away from the nuclear ring (*Beck et al., 2004; Singh et al., 2024*). In stark contrast, mouse nuclear basket densities run almost parallel to the nucleocytoplasmic axis (~6° angle) and do not converge into a distal ring structure (*Singh et al., 2024*). In both cases, the resolution of cryo-ET reconstructions was too low to unambiguously assign densities without prior structural knowledge.

To this end, combining cryo-ET maps with additional experimental data - such as crosslinking-coupled mass spectrometry (XL-MS) - has enabled the integrative structural modelling of the nuclear basket. In both yeast and mammalian models, coiled-coil subunits of the nuclear basket (Mlp1/TPR) form the struts of the nuclear basket with both N- and C-termini positioned at the distal density, in line with previous observations (*Singh et al., 2024; Niepel et al., 2013*). In the proposed integrative models, nuclear basket anchors (Nup60/NUP153) are associated with the proximal nuclear ring, whereas nuclear basket filaments (Mlp1/TPR) are in contact with Nup84/NUP107 subunits of the distal nuclear ring (*Singh et al., 2024*). The proposed model of interactions provides a mechanistic explanation for the basket-stabilizing effect of double ring, although future studies will have to put the proposed mechanism to experimental test. Moreover, a significant portion of distal ring density remains unaccounted for and may correspond to NPC accessory proteome, such as NTR, NTR-cargo complexes etc.

Despite increasing resolution of nuclear basket densities and elucidation of principles governing nuclear basket organization, the molecular mechanism by which this fishtrap-like structure is anchored onto the core scaffold of the NPC has remained elusive.

1.3. Compositional heterogeneity of NPCs

As noted in preceding sections, the structure of the NPC follows a general blueprint across different species, from yeast to human. The evolutionarily conserved framework of the NPC encompasses the core scaffold, composed of inner and outer rings, onto which the permeability barrier and asymmetric appendages are grafted (Fig. 1A,B). The first level of compositional variation of NPCs arises when comparing even the most conserved part of the NPC, the core scaffold, across different species. The variation in outer ring stoichiometries stands out as the most prominent structural modification of the core scaffold across species (for a detailed discussion of core scaffold variations, see section 1.2.1. *Molecular architecture of core scaffold of NPC*).

The compositional heterogeneity of NPCs arising from inter-species variation in NPC scaffolds is further expanded by intra-species variations. Large-scale proteomic study has revealed that NPC stoichiometry varies across different human tissue cultures and cancer cell lines (*Ori et al., 2013*). The compositional differences between cell type-specific NPCs are most pronounced in the relative abundance of peripheral nucleoporins, such as TPR and NUP50, both of which are also present in significant nucleoplasmic pools (*Ori et al., 2013*).

Variations in NPC structure are observed even within a single cell. Early localization studies of *S. cerevisiae* Mlp1, Mlp2 and Pml39 revealed that these nuclear basket subunits display restricted localization, i.e. they are largely excluded from the NE region in proximity to the nucleolus, in stark contrast to other nucleoporins, which are detectable throughout the NE (*Galy et al., 2004; Zhao et al., 2004; Palancade et al., 2005; Bensidoun et al., 2022; Zsok et al., 2024*). These early observations pointed to the intracellular structural heterogeneity of NPCs, where only a subset of NPCs harbour fully assembled nuclear baskets. This led to the proposal of 'basketless NPCs', although the term 'filamentless NPCs' would better describe this isoform because other nuclear basket subunits, i.e. Nup60, Nup1 and Nup2, are nevertheless present in all NPCs (*Rout et al., 2000; Dilworth et al., 2001; Galy et al., 2004; Niepel et al., 2013; Bensidoun et al., 2022*). Electron microscopy study of yeast mutant carrying double deletion of *MLP1* and *MLP2* revealed the loss of nuclear filaments and nuclear basket-associated exclusion of packed chromatin (*Niepel et al., 2013*). Recent study suggested that 'basketless' NPCs represent the default assembly state, a subset of which is additionally functionalized by appending the nuclear basket (*Bensidoun et al., 2022*).

In yeast (both *S. cerevisiae* and *S. pombe*), the nuclear basket undergoes significant but transient remodelling during meiotic divisions upon which specific subunits or the whole nuclear basket detach from the core scaffold of the NPC (*King et al., 2023*). The meiotic detachment of *S. cerevisiae* Nup60–Nup2 is dependent on Polo kinase Cdc5-mediated phosphorylation of Nup60 (*King et al., 2023*). Resulting NPCs with partially assembled nuclear baskets contribute to the structural heterogeneity of NPCs observed in yeast cells.

The intracellular compositional variation of *S. cerevisiae* NPCs was further corroborated by recent observations of at least two NPC isoforms differing in core scaffold architecture (*Akey et al., 2022; Akey et al., 2023; Singh et al., 2024*). While the majority (~73%) of yeast NPCs harbour a single nuclear ring composed of 8 copies of the Y-complex, a lesser population (~27%) carries a double nuclear ring with 16 copies of the Y-complex (*Akey et al., 2022; Singh et al., 2024*). No significant differences were observed when comparing cytoplasmic and inner ring architectures between the two isoforms. Moreover, the two isoforms displayed no apparent differences in their distribution along the NE (*Singh et al., 2024*), in stark contrast to 'basketless NPCs', which are largely excluded from the NE region adjacent to the nucleolus (*Galy et al., 2004; Zhao et al., 2004; Palancade et al., 2005*). Furthermore, the report that sub-tomogram analysis of *Chaetomium thermophilum* NPCs yielded no meaningful averages points to the possibility of even greater intracellular heterogeneity in other fungi species (*Zimmerli et al., 2021*).

Finally, even the evolutionary conserved eightfold symmetry of the core scaffold of NPC is lost in a small fraction of NPCs as shown by negative stain EM examination of nuclei extracted from *Xenopus laevis* oocytes (*Hinshaw&Milligan, 2003*). Up to 2% of *X. laevis* NPCs exhibit ninefold symmetry and ~0.5% of NPCs display even higher – tenfold - symmetry (*Hinshaw&Milligan, 2003*). However, these NPC subpopulations likely represent atypical or aberrant end products of the assembly process rather than functionally specialized subset of NPCs. Core scaffolds of NPCs with rotational symmetries other than eightfold were also observed in mESC upon homozygous deletion of *NUP133* (*Taniguchi et al., 2025*).

1.4. Conformational plasticity of NPCs

In cellulo, the NPCs undergo reversible global conformational changes involving large-scale movement and bending of constituent subcomplexes in response to physiological cues (*Allegretti et al., 2020; Zimmerli et al., 2021; Schuller et al., 2021; Hoffmann et al., 2025*). Initial indications of NPC plasticity came from a comparison of structural models obtained from purified NPCs with the models of in-situ NPCs (*Allegretti et al., 2020; Schuller et al., 2021*). Both yeast and human in situ NPCs were found to be in the dilated state compared to either

isolated NPCs or purified NE, respectively (*Allegretti et al., 2020; Schuller et al., 2021*). For example, the diameter of human in-situ NPC at IR was measured to be ~57 nm, whereas the diameter of NPC from isolated NE was found at ~43 nm (*Schuller et al., 2021*).

These initial observations suggesting the responsiveness of NPC were later corroborated by obtaining in-situ models of NPCs under different physiological and environmental conditions (*Zimmerli et al., 2021; Hoffmann et al., 2025*). The diameter of *S. pombe* NPC at IR decreases from ~70 nm in exponentially growing cells down to ~50 nm in energy depleted cells when using non-hydrolysable 2-deoxy-glucose in combination with the respiratory chain inhibitor antimycin A (*Zimmerli et al., 2021*). Moreover, NPC constriction of similar degree was reported when *S. pombe* cells were subjected to hyperosmotic stress (*Zimmerli et al., 2021*). Similarly, the *D. discoideum* NPC scaffolds undergo rapid constriction (NPC diameter at IR of ~68 nm) and dilation (NPC diameter at IR of ~78 nm) in response to the osmotic stress, although to a lesser degree compared to *S. pombe* NPC scaffolds (*Hoffmann et al., 2025*). Interestingly, the NPC diameter positively correlates with the NE thickness in both *S. pombe* and *D. discoideum* cells (*Hoffmann et al., 2025; Zimmerli et al., 2021*), in agreement with the proposed model in which the mechanosensitive NPC responds to the changes in the nuclear envelope tension by either constricting or dilating, i.e. acting like an annular spring (*Zimmerli et al., 2021; Taniguchi et al., 2025*).

Moreover, it has been demonstrated that murine NPCs dilate during early neuronal differentiation of mESC into neural progenitor cells, although the change in diameter is of a lesser degree compared to above mentioned examples (*Taniguchi et al., 2025*).

1.5. Dynamic profile of NPCs

The NPC is a dynamic macromolecular assembly with a constant flux of nuclear transport factors and cargo molecules through its permeability barrier (*Kiseleva et al., 1998; Ribbeck&Goerlich, 2001*). However, the dynamic nature of the NPC is also evident in its structure, parts of which undergo constant remodelling.

Despite universally low turnover rates of nucleoporins with essentially negligible degradation as observed in *S. cerevisiae*, *C. elegans* and human cells (*Hakhverdyan et al., 2021; D'Angelo et al., 2009; Daigle et al., 2001*), the nucleoporins display a wide range of exchange rates, i.e. dynamic transition between NPC-bound and free pool of a given nucleoporin. Recent study combining metabolic labelling with quantitative proteomics revealed the dynamic profile of *S. cerevisiae* nucleoporins (*Hakhverdyan et al., 2021*). Among the nucleoporins with the fastest exchange rates (half-life of less than 2.5 h) are core nuclear basket subunits Nup60, Nup1 and Nup2 (*Hakhverdyan et al., 2021*). Generally, connector

nucleoporins harbouring IDR regions and SLiMs as interaction modules with relatively low surface area of contact displayed the highest exchange rates among nucleoporins (*Hakhverdyan et al., 2021*). The core scaffold nucleoporins displayed the lowest exchange rates suggesting their stable association (*Hakhverdyan et al., 2021*). This is in line with previous report showing that scaffold nucleoporins of *X. laevis* NPC does not exchange once inserted into the NE (*D'Angelo et al., 2009*). Recent study employing recombination-induced tag exchange (RITE) in live *S. cerevisiae* cells revealed an almost identical pattern: fast exchange of nuclear basket subunit (Nup60) and stable association of core scaffold nucleoporins (*Zsok et al., 2024*).

Nuclear basket filament subunits Mlp1 and Mlp2 were found to have intermediate exchange rates between ultrafast exchange of aforementioned core nuclear basket subunits and relatively slow exchange of core scaffold nucleoporins (*Hakhverdyan et al., 2021*). However, in contrast to the metabolic labelling approach, RITE-based measurements indicate that Mlp1, Mlp2, and Pml39 are stably associated with the NPCs (*Zsok et al., 2024*), in agreement with observations of their human ortholog TPR (*Souquet et al., 2018*).

1.6. Biogenesis of NPCs

At least two distinct pathways of *de novo* NPC biogenesis have been described, namely, interphase assembly and postmitotic assembly. Cells that undergo open mitosis, such as proliferating human cells, rely on both interphase and postmitotic NPC assembly mechanisms, while *S. cerevisiae* cells, which undergo closed mitosis, rely exclusively on the interphase mechanism. Although the end product of both assembly pathways is the same, namely, functional NPC, the pathways possess distinct order of assembly, distinct membrane remodelling events, different molecular requirements and kinetic features (reviewed in *Otsuka&Ellenberg, 2017*).

In human cells, the interphase NPC assembly is characterized by the *de novo* formation of the pores during nuclear growth. The pores arise from the asymmetric inside-out extrusion of the nuclear envelope (*Otsuka et al., 2016*). The extrusion proceeds via dome-shaped evaginations of the INM and associated mushroom-shaped proteinaceous densities (*Otsuka et al., 2016*). Similar mushroom-shaped evaginations of INM were observed in *S. cerevisiae* cells upon *NUP116* deletion and may represent stalled assembly intermediates (*Allegretti et al., 2020*). The observation of a nuclear ring at the base of INM evaginations in human cells suggests that the eightfold rotational symmetry of the NPC is established early during the biogenesis of the NPC (*Vollmer et al., 2015; Otsuka et al., 2016; Onischenko et al.,*

2020; Taniguchi *et al.*, 2025), likely mediated by the head-to-tail oligomerization of membrane-tethered Y-complexes (Seo *et al.*, 2009).

In contrast, postmitotic NPC assembly is initiated during mitotic exit concomitant with membrane resealing of chromatin and it does not require *de novo* fusion of nuclear membranes (Otsuka *et al.*, 2018). The resealing of chromatin, which is complete within minutes after anaphase onset, is mediated by mitotic ER membranes featuring small membrane discontinuities (Otsuka *et al.*, 2018). These ER fenestrae ('holes') act as precursors to prepores, which subsequently mature into fully assembled NPCs by the radial dilation of NE openings (Otsuka *et al.*, 2018).

In human cells, postmitotic assembly is complete within 15 min after anaphase onset, whereas interphase assembly takes ~100 min to plateau (Otsuka *et al.*, 2023). In haploid *S. cerevisiae* cells, both the number of NPCs and the surface area of the NE increase during the cell cycle progression; however, at different rates with the density of the NPCs peaking in S-phase (Winey *et al.*, 1997). The observed linear increase of NPC number from ~70 in G1-phase to ~140 in early M-phase suggests continuous, cell cycle-independent assembly of NPCs in *S. cerevisiae* cells (Winey *et al.*, 1997).

The temporal order of nucleoporin incorporation is also distinct between the postmitotic and interphase pathways (Otsuka *et al.*, 2023). Interestingly, NUP153, TPR and Y-complex are assembled early in the interphase pathway, followed by inner ring/central channel and cytoplasmic filament subunits (Vollmer *et al.*, 2015; Otsuka *et al.*, 2023; Otsuka *et al.*, 2016). The order between the nuclear filaments and central channel is inverted in postmitotic assembly pathway (Otsuka *et al.*, 2023). The temporal separation between the incorporation of outer ring and inner ring subunits was not observed in *S. cerevisiae* cells (Onischenko *et al.*, 2020), suggesting a distinct temporal order of events between yeast and human interphase NPC assembly pathways. Moreover, yeast nuclear basket subunits Mlp1/2, orthologs of vertebrate TPR, mark the last step of NPC assembly, in stark contrast to the early incorporation of TPR during interphase biogenesis in human cells (Onischenko *et al.*, 2020; Otsuka *et al.*, 2023). Recently, the tethering of nuclear basket filaments in *S. cerevisiae* cells was proposed to be mRNA-dependent, although the molecular mechanism remains to be dissected (Bensidoun *et al.*, 2022). Furthermore, both human NUP107 and SEH1 subunits of the Y-complex were found to assemble simultaneously in both postmitotic and interphase pathways (Otsuka *et al.*, 2023), in contrast to the proposed *S. cerevisiae* NPC assembly model, where Y-complex subunits are incorporated sequentially as two distinct sets of nucleoporins (Onischenko *et al.*, 2020). However, orthogonal evidence for the sequential incorporation of Y-complex subunits is lacking, whereas previous *in vitro* characterization of the Y-complex

pointed to the extremely stable complex with only Nup133 tending to dissociate under specific experimental conditions (*Lutzmann et al., 2002; Stuwe et al., 2015*).

Auxin-induced depletion of NUP96 (yeast Nup145C, subunit of the Y-complex) in human NPCs revealed that inner ring alone is sufficient to maintain the integrity of the NE pore (*Schuller et al., 2021*). However, whether IR-only NPC sub-assemblies arise from depletion of the outer rings from fully assembled NPCs or represent biogenesis intermediates before the assembly of the outer rings remains unknown. Moreover, the permeability barrier of IR-only NPC sub-assemblies has yet to be tested. Similarly, NE-spanning pores are retained by *S. cerevisiae* NPC sub-assemblies containing only IR and NR in the *nup116Δ* strain at non-permissive temperature (*Allegretti et al., 2020*). Whether these sub-assemblies represent intermediates of NPC assembly remain to be elucidated in future studies.

2. AIMS

The overarching aim of this thesis is to shed light how the nuclear basket docks onto the symmetric core scaffold of the nuclear pore complex. To this end, the following objectives served as guideposts toward a mechanistic understanding of this process:

1. A comprehensive biochemical dissection of the molecular interactions at the interface between the nuclear basket and the core scaffold of *S. cerevisiae* NPC;
2. *In vitro* reconstitution of a minimal supramolecular assembly containing subunits of both the nuclear basket and the core scaffold of NPC;
3. Recapitulation of the in-solution findings within a membrane environment;
4. Validation of the findings in live yeast cells.

3. RESULTS

3.1. Results 1

3.1.1. Preamble

The following first-author publication, a primary research article '*Docking a flexible basket onto the core of the nuclear pore complex*' (Stankunas&Köhler, 2024), published in *Nature Cell Biology* on 13th of August 2024, constitutes the core of the thesis by directly addressing the research questions raised in the AIMS section. The publication reports novel empirical findings and conceptual insights into the molecular mechanism, by which the nuclear basket is docked onto the core scaffold of the *S. cerevisiae* NPC. The observations, presented in the study, were derived from a multipronged approach combining state-of-the-art AlphaFold-based interaction screens, electron microscopy, biochemical membrane-templated reconstitution, and live-cell fluorescence microscopy.

3.1.2. Contribution statement

A.K. and E.S. conceived the study. E.S. performed experiments. A.K. and E.S. analysed all data and wrote the paper.

3.1.3. Publication

See next pages.

Docking a flexible basket onto the core of the nuclear pore complex

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The nuclear basket attaches to the nucleoplasmic side of the nuclear pore complex (NPC), coupling transcription to mRNA quality control and export. The basket expands the functional repertoire of a subset of NPCs in *Saccharomyces cerevisiae* by drawing a unique RNA/protein interactome. Yet, how the basket docks onto the NPC core remains unknown. By integrating AlphaFold-based interaction screens, electron microscopy and membrane-templated reconstitution, we uncovered a membrane-anchored tripartite junction between basket and NPC core. The basket subunit Nup60 harbours three adjacent short linear motifs, which connect Mlp1, a parallel homodimer consisting of coiled-coil segments interrupted by flexible hinges, and the Nup85 subunit of the Y-complex. We reconstituted the Y-complex•Nup60•Mlp1 assembly on a synthetic membrane and validated the protein interfaces *in vivo*. Here we explain how a short linear motif-based protein junction can substantially reshape NPC structure and function, advancing our understanding of compositional and conformational NPC heterogeneity.

A basket typically has a framework of interwoven components forming an open, hollow structure. The nuclear pore basket, true to its name, was proposed to share similar characteristics. Composed of proteins known as nucleoporins (Nups), the basket decorates the nucleoplasmic face of the nuclear pore complex (NPC) core scaffold. The basket forms a truncated cone, composed of eight filament-like structures as shown by ultrathin section negative stain and scanning electron microscopy (EM)^{1–3}. The height of the basket varies between species (–60–120 nm)^{4,5}. Whereas the NPC core forms the central conduit for nucleocytoplasmic transport, the nuclear basket adds additional layers of complexity, asymmetry and regulation to the process.

The nuclear basket has been implicated in maintaining free access to the NPC channel by excluding (hetero-) chromatin^{6,7}, docking ribonucleoprotein particles before export^{8,9}, selecting export-competent messenger RNAs and retaining faulty mRNAs¹⁰ and participating in protein quality control¹¹, as well as physically tethering and regulating genes through basket-associated adaptors^{12–16}. The intrinsically disordered phenylalanine–glycine (FG) regions of basket Nups also contribute to the NPC's permeability barrier^{17–19}, and certain basket Nups provide docking sites for transport receptors^{18,20}. Nuclear baskets

assemble only on a subset of NPCs in *Saccharomyces cerevisiae*. The region where the nucleolus meets the inner nuclear membrane (INM) features 'basketless' NPCs^{7,21}.

Basket-containing NPCs recruit a specific RNA and protein interactome, suggesting different transport capabilities compared with basketless NPCs²¹. Thus, the basket is a prominent example of NPC compositional heterogeneity, a poorly understood phenomenon that refers to variations in subunit stoichiometry among different NPCs within a cell or across different cell types^{21–24}. Basket Nups exhibit remarkable dynamics at the NPC. Whereas their protein turnover is relatively slow, the exchange rates of NPC-bound basket Nups with Nups from a free pool are high, suggesting constant swapping at the NPC^{25–28}. Intriguingly, basket assembly depends, directly or indirectly, on RNA polymerase II transcription and subsequent messenger ribonucleoprotein processing²¹, suggesting that gene expression itself can shape NPC structure, which further complicates mechanistic studies. Currently, we do not understand how NPCs become functionalized by the basket because we lack a biochemical and structural understanding of how the basket docks onto the core of the NPC.

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Substantial progress has recently been made in visualizing the structure of the NPC's symmetric core scaffold and the cytoplasmic filaments^{23,24,29–34}. In contrast, the basket has so far resisted a high-resolution structural characterization. An in situ cryo-electron tomography (cryo-ET) study described the basket as a pair of 'bifurcated densities' that associate with each Y-complex in the nuclear outer ring across an extended area²⁴. These densities were interpreted as 'basket anchors'; however, it is currently unclear which proteins account for them and which parts of the basket are missing in the model. Another in situ cryo-ET study found 'rod-like densities' at the connection between the nuclear and inner ring structure and around the Y-complex vertex, which were interpreted as coiled-coil structures³⁴. Nup153 was shown to mediate membrane interactions of the Y-complex during interphase assembly³⁵ and another study proposed a ubiquitin-dependent interaction of Nup60 with the Nup84 subunit of the Y-complex³⁶. Collectively, these studies suggest that basket components are somehow docked onto the outer ring of the NPC core.

In *S. cerevisiae*, the nuclear basket consists of Nup1, Nup2, Nup60, the paralogous Mlp1/2 proteins and Pml39. The metazoan basket comprises only four Nups, namely, Nup153 (a likely orthologue of yeast Nup1/Nup60), Nup50 (Nup2), Tpr (Mlp1/2) and ZC3HC1 (Pml39)^{37–39}. We have previously identified the intrinsically disordered *S. cerevisiae* Nup60 as the basket's flexible 'suspension cable'⁴⁰. Nup60 harbours short linear motifs (SLiMs) as contact sites for the basket subunits Nup2 and the Mlp1 (ref. 40), while interacting with the INM through an N-terminal amphipathic helix (AH)²⁰. How Nup60, as the basket's suspension cable, 'weaves' through the framework to simultaneously connect the INM, Mlp1/2, Nup2 and possibly the NPC core, is unknown. The number of Nup60 molecules per NPC was estimated as 8 (refs. 41,42) or 16 (refs. 43,44). The outer ring of the NPC core, implicated in contacting the basket, is formed by the head-to-tail arrangement of Y-complexes (also known as coat Nup complex or Nup84 complex in yeast and Nup107–160 complex in metazoa), which may be present as a single ring (8 copies) or double ring (16 copies) of Y-complexes depending on the species^{34,41,45–50}. In *S. cerevisiae*, the ~580 kDa Y-complex consists of seven Nups: the two short arms are composed of Nup120 and the Nup85–Seh1 heterodimer, whereas the kinked stem is formed by the Nup145C–Sec13–Nup84–Nup133 heterotetramer^{46,50–52}. The short arms and the stem meet at the triskelion region, which is defined by interactions between Nup120, Nup85 and Nup145C^{46,51}. The Y-complex interacts with the nuclear envelope (NE) membranes via Nup120 and Nup133 (ref. 53). However, the binding strength and purpose of these membrane interactions remain unclear. Of note, the majority of yeast NPCs was reported to contain a single nuclear ring comprising eight Y-complexes, whereas a subset may contain a double nuclear ring with sixteen Y-complexes²⁴. Whether and how this different stoichiometry relates to basket assembly is unknown.

To understand how the functionality of the NPC is tuned, we need to understand the molecular mechanism by which the basket docks onto the NPC core. This is important because it will explain (1) how an NPC becomes fully assembled, (2) how the basket functionalizes the core for transport and gene regulation and (3) how this creates NPC heterogeneity within a cell and across cell types. Understanding the interface between the basket and the NPC core will permit the design

of specific basket separation-of-function mutants that are critical to disentangle the wealth of cellular functions of 'basket-powered' NPCs.

In this Article, we have reconstituted a supramolecular assembly comprising the Y-complex, the Nup60 suspension cable and critical parts of the Mlp1 filaments on a membrane. We have identified the precise docking sites of a tripartite Nup60 region on a conserved groove of Nup85 and a hinge region of Mlp1. In addition, we explain at single residue level how this junction couples Mlp1 filament docking to the Y-complex with INM tethering of the basket in vitro and in vivo. We further demonstrate that Mlp1 forms a parallel homodimer of rigid coiled coils and flexible hinges providing direct evidence for the dual nature of the basket—being both rigid and conformationally flexible. A stoichiometric basket–Y-complex assembly can form independently of external factors, yet, mRNA metabolism may stabilize it.

Results

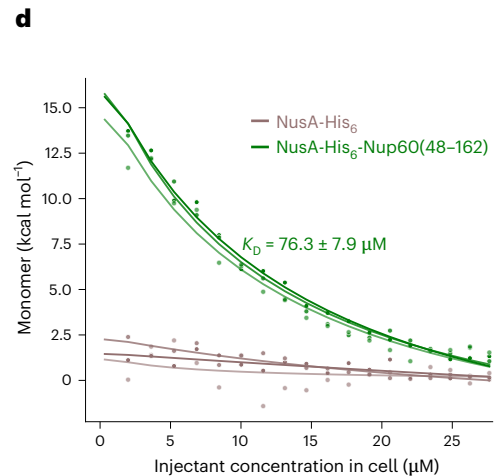
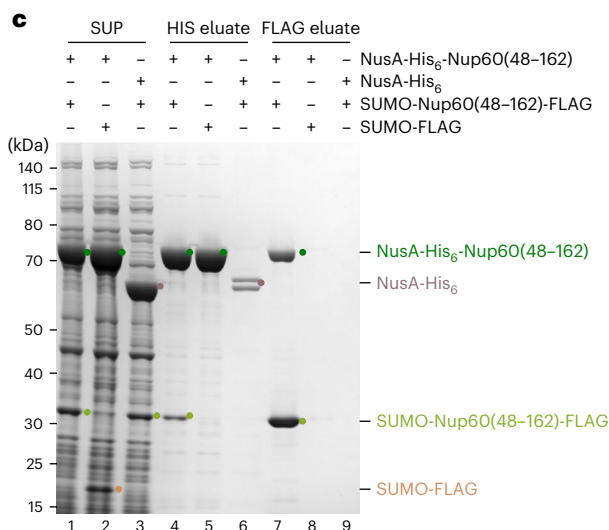
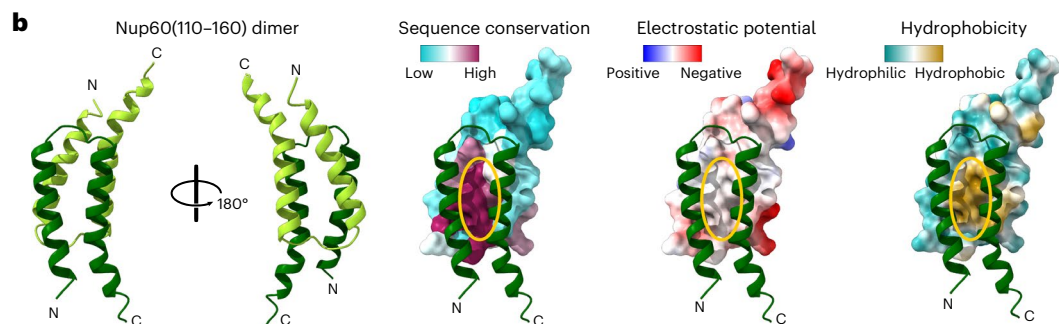
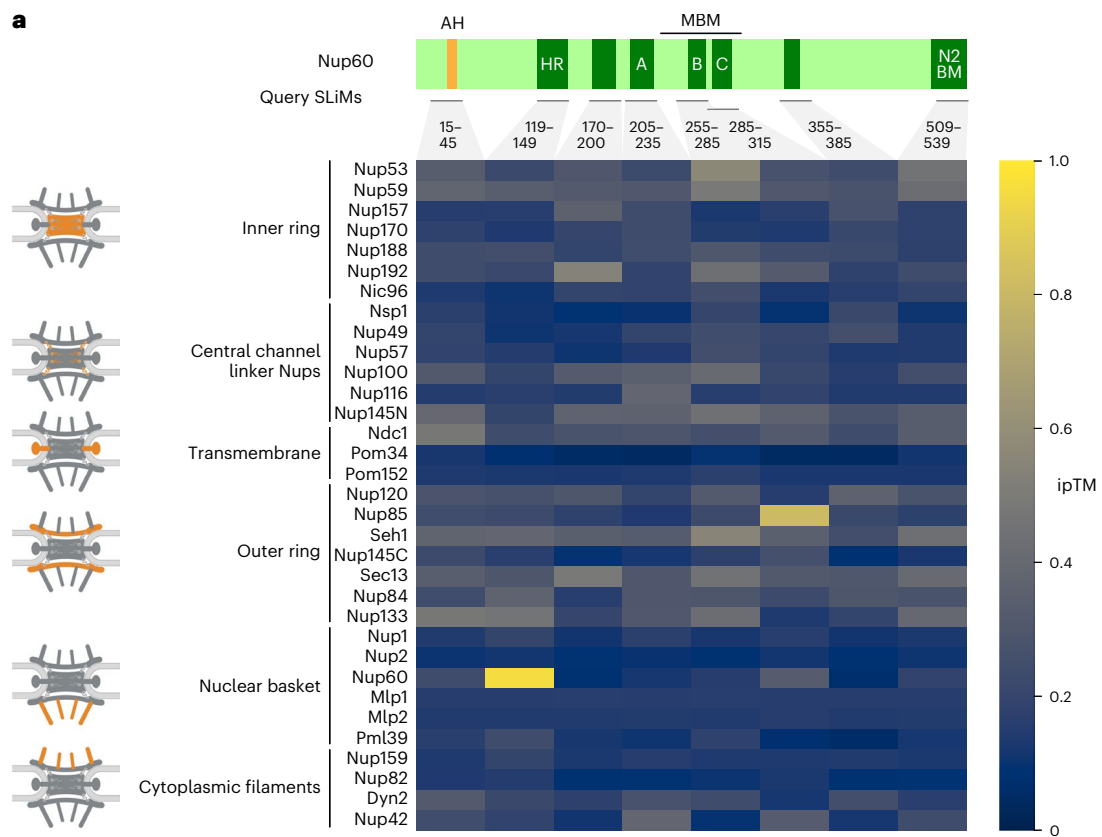
Nup60 'suspension cable' oligomerizes via its helical region

Nup60 was identified as the basket's membrane-anchored suspension cable, containing SLiMs that interact directly with Mlp1 and Nup2 (ref. 40). To determine how the basket docks onto the core of the NPC, we employed an artificial intelligence (AI)-based SLiM screen, extending AlphaFold-Multimer's success in structure prediction towards identifying unknown protein–protein interactions. We segmented Nup60 into pieces of equal length (31 amino acids (aa)) covering all conserved SLiMs and determined the average interface predicted template modelling (ipTM) score for every binary combination with all Nups (Fig. 1a). Out of 264 combinations tested, two pairs stood out by their high ipTM score: the helical region (HR) of Nup60 (HR; aa119–149) with itself, and a SLiM at aa285–315 with Nup85. The HR of Nup60 is predicted to adopt a helix-turn-helix fold with two copies arranged in an antiparallel orientation, held together by a conserved hydrophobic patch that is buried at the dimer interface (Fig. 1b; see Extended Data Fig. 1a,b for model confidence). The solvent-exposed dimer surface is largely hydrophilic (Extended Data Fig. 1c–f). To verify a potential oligomerization of Nup60, we performed a two-step split-affinity purification of differentially tagged Nup60 HR constructs co-expressed in *Escherichia coli*. We fused the Nup60 HR (aa48–162; including an N-terminal linker) with either a His₆ or FLAG tag and appended a NusA (57 kDa) or SUMO (14 kDa) tag to create a size difference. Following a two-step affinity purification on Ni-NTA and anti-FLAG beads, we retrieved both NusA and SUMO-tagged Nup60 species (Fig. 1c), indicating an assembly of at least two Nup60 HR copies.

To characterize Nup60 oligomerization quantitatively, we performed dilution isothermal titration calorimetry (ITC) experiments with the recombinant NusA-His₆-Nup60(48–162) protein. The dilution isotherms displayed a hyperbolic profile, characteristic of a simple dimer dissociation model, whereas NusA-His₆, used as a negative control, did not exhibit such behaviour (Fig. 1d and Extended Data Fig. 1g,h). The dissociation constant (K_D) of the Nup60 HR was $76.3 \pm 7.9 \mu\text{M}$ (mean $\pm$ s.d.). Additionally, we performed size exclusion chromatography coupled with multi-angle light scattering experiments. Consistent with Nup60 dimerization, we observed a concentration-dependent shift of the NusA-His₆-Nup60(48–162) peak towards higher molecular mass (Extended Data Fig. 1i). However, a low

Fig. 1 | The Nup60 suspension cable oligomerizes via its HR. **a**, Heatmap of the average ipTM scores for every possible pairwise combination between *S. cerevisiae* Nup60 SLiMs and full-length Nups as estimated by the AlphaFold-Multimer model. Nup60 query SLiMs are stretches of 31 amino acid residues covering regions evolutionarily conserved across yeast orthologues (yellow and dark green shading in Nup60 cartoon). N2BM, Nup2-binding motif. **b**, The AlphaFold-Multimer model of Nup60(110–160) dimer shown in cartoon representation (left) and a combination of cartoon and surface representations (right) with surface coloured by sequence conservation, Coulombic electrostatic potential and hydrophobicity (from left to right). The oval outline (orange)

depicts a conserved hydrophobic patch buried at the dimer interface. For model confidence and dimer surface representations, see Extended Data Fig. 1. **c**, SDS–PAGE of a two-step split-affinity purification of differentially tagged Nup60(48–162) constructs co-expressed in *E. coli*. The protein eluates were analysed by SDS–PAGE and Coomassie staining. **d**, Dilution ITC profiles (isotherms) of NusA-His₆-Nup60(48–162) and NusA-His₆ samples (170 μM) are shown. By applying a simple dimer dissociation model, the dissociation constant (K_D) of NusA-Nup60(48–162) was estimated to be $76.3 \pm 7.9 \mu\text{M}$ (mean $\pm$ s.d., $n = 3$). Source numerical data and unprocessed gels are available in Source data.



peak resolution prevented us from accurately estimating the molecular mass of the putative dimer peak. We cannot exclude the possibility that Nup60 undergoes higher-order oligomerization, beyond just forming a dimer. Conversely, there was no discernible shift in the NusA-His₆ negative control, thereby ruling out an influence of the tag on Nup60 dimerization (Extended Data Fig. 1j). Dimerization of Nup60, as explained below, is expected to affect the connectivity of the basket, its docking onto the NPC core and may stabilize the head-to-tail arrangement of Y-complexes in the nuclear ring of the NPC.

Nup60 interacts with the Nup85•Seh1 arm of the Y-complex

The second-best hit identified in our AlphaFold-based SLiM screen suggested an interaction between Nup60 and Nup85, a subunit of the Y-complex. Surprisingly, this stretch of 31 amino acids (aa285–315) is embedded in a larger region of Nup60 (aa240–318, termed Nup60 Mlp1-binding motif (MBM)) that was previously shown to recruit the basket subunit Mlp1 (ref. 40). This finding raised the possibility that the Y-complex and the Mlp1 filaments bind to Nup60 in immediate proximity. Mlp1 was not detected in this binary screen, possibly because it interacts with Nup60 only as a coiled-coil homodimer (see further below). Moreover, the expected interaction between Nup60 and Nup2 (ref. 40) was not detected, suggesting that AlphaFold encounters challenges in predicting SLiM-mediated interactions.

To experimentally verify a putative Y-complex–Nup60 interaction, we aimed to reconstitute it *in vitro*. To this end, we engineered a stable *S. cerevisiae* Nup60 construct, which lacks the N-terminal lipid-interacting AH (aa1–47) and the disordered FG-repeat region (aa381–504). This Nup60 construct (aa48–539ΔFG; abbreviated as Nup60*) was purified from *E. coli* via an N-terminal glutathione S-transferase (GST)-tag and immobilized on glutathione (GSH) beads as a bait (Fig. 2a). The Y-complex was affinity purified from an *S. cerevisiae* strain expressing Nup133 with a C-terminal TAP tag (Fig. 2b). When incubated with each other, the Y-complex bound Nup60* (Fig. 2c and Extended Data Fig. 2a), but did not bind to GST used as a negative control. Next, we deleted conserved regions of Nup60, which overlap or lie adjacent to the previously identified direct binding site for Mlp1. Deleting the previously mapped Mlp1 binding site on Nup60 (aa240–309) also reduced the affinity for the Y-complex (Fig. 2c). Deletion of the adjacent Nup60 region (aa200–239) similarly resulted in a partial loss of Y-complex interaction (Fig. 2c). Interestingly, deletion of both Nup60 regions (aa200–309) completely abolished the interaction with the Y-complex (Fig. 2c). This indicates that the region spanning aa200–309 of Nup60 is necessary for direct interactions with the Y-complex. We then asked whether this region of Nup60 is sufficient for binding to the Y-complex. To address this, we generated a stable recombinant GST–Nup60 construct (aa200–318) and performed binding assays with the Y-complex (Fig. 2d,e and Extended Data Fig. 2b). The Y-complex specifically interacted with this minimal Nup60 region, whereas

further N- or C-terminal truncations of the Nup60 construct resulted in a loss of interaction (Fig. 2e). Thus, a region spanning aa200–318 of Nup60 is both necessary and sufficient for a direct interaction with the Y-complex.

To precisely determine the binding site of Nup60(200–318) on the heptameric, ~580-kDa Y-complex, we performed cross-linking coupled to mass spectrometry (XL-MS) experiments using disuccinimidyl suberate (DSS) as a lysine-specific cross-linker. Recombinant Nup60(200–318) cross-linked specifically to the Nup85 and Seh1 subunits of the Y-complex, which constitute one of its short arms (Fig. 2f and Extended Data Fig. 2d). Guided by these results, we tested whether a recombinant Nup85(1–564)•Seh1 heterodimer (termed Nup85*•Seh1), purified via an N-terminal His₆ tag on Seh1 (ref. 54), directly interacts with Nup60* (Fig. 2g and Extended Data Fig. 2c). Indeed, these experiments confirmed that the region between aa200 and aa318 of Nup60 is sufficient to bind to Nup85*•Seh1. Both the regions spanning aa200–239 and aa240–318 play a role in this interaction, with the latter displaying a higher affinity for Nup85*•Seh1. In summary, we have unambiguously identified Nup85•Seh1 as a direct interaction partner of Nup60.

Mlp1 forms a homodimer of coiled coils and flexible hinges

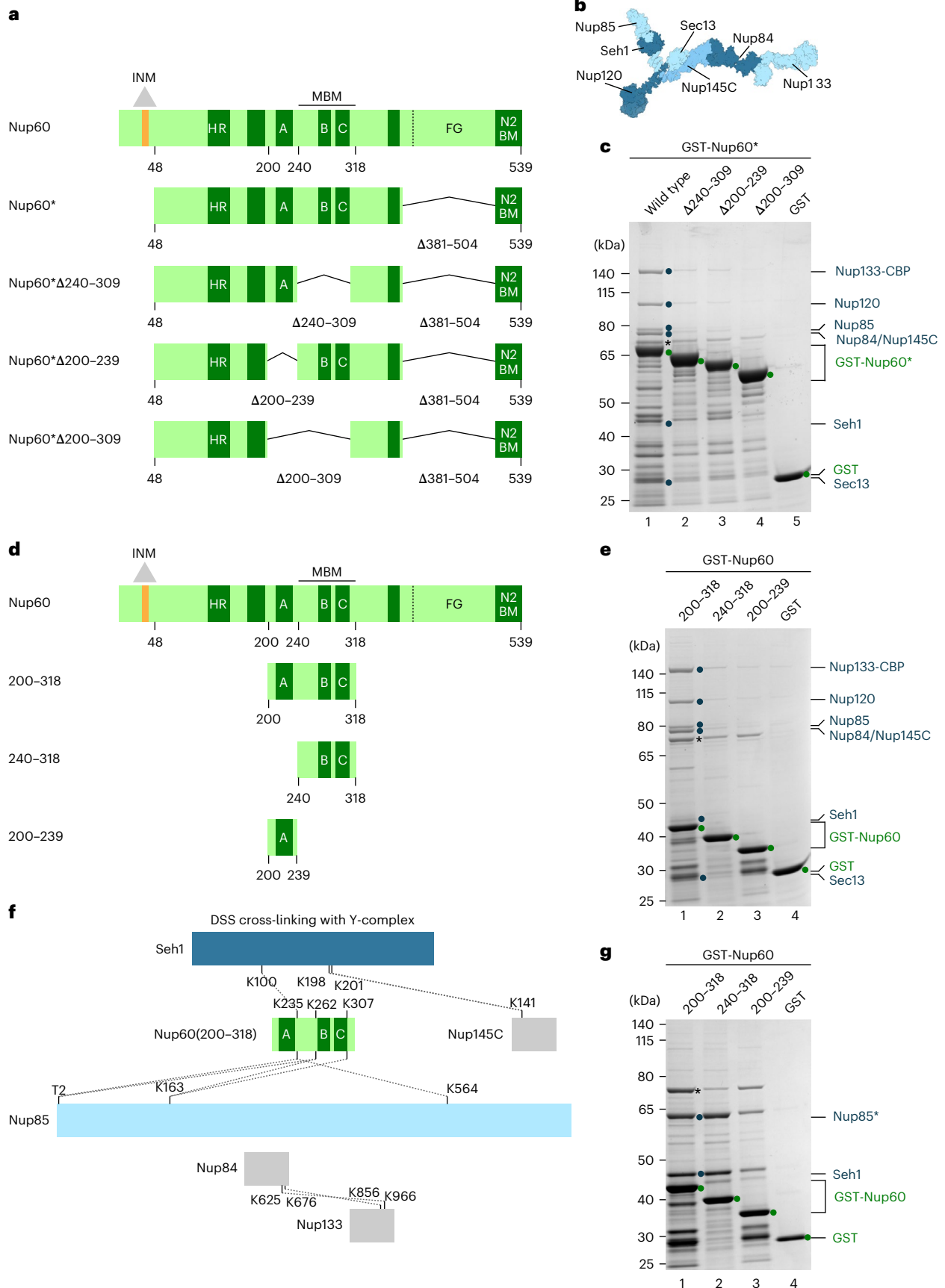
Based on these findings, our objective was to clarify the manner in which the structurally elusive basket filaments are linked to Nup60, and consequently, the Y-complex. Despite decades of effort, the overall topology of the basket filaments remains unknown due to the difficulties of studying these large coiled-coil proteins in isolation or within the NPC context. Earlier EM studies of two separate N-terminal fragments of the human Tpr (aa1–398 and aa774–1370) had reported rectilinear particles⁵⁵. However, the manner in which these fragments are connected remained unclear.

We engineered Mlp1 to carry an N-terminal maltose-binding protein (MBP) tag (40 kDa), large enough to be visualized by EM. We succeeded in purifying a stable *S. cerevisiae* Mlp1(17–1,137) construct, expressed in insect cells. This construct comprises ~60% of the full-length protein and 77% of the structured parts without the disordered C-terminus (Fig. 3a). After MBP-affinity purification on amylose beads and native elution with maltose-containing buffer, the protein exhibited a predicted size of ~174 kDa without major impurities (Fig. 3b). This protein was examined by heavy metal rotary shadowing EM. We observed filamentous particles containing multiple rod-like segments (Fig. 3c; for an overview, see Extended Data Fig. 3a). Notably, we consistently observed two globular knobs at one end of the particles, which we interpret as MBP tags (Fig. 3c), because the knobs were not present in a construct lacking MBP (Extended Data Fig. 3b). This provides direct experimental evidence that Mlp1 forms a parallel homodimer, probably consisting of rigid coiled-coil segments interrupted by flexible hinges. The number of visually distinct segments

Fig. 2 | Nup60 interacts with the Nup85•Seh1 arm of the Y-complex.

a, Top: the motif organization of *S. cerevisiae* Nup60. The motif organization of GST–Nup60*–StrepII constructs used for the *in vitro* binding assays is shown. Motif boundaries and constructs are drawn to scale with relevant amino acid positions indicated. N2BM, Nup2-binding motif. **b**, The cartoon representation of the Y-complex organization is shown. **c**, SDS–PAGE gel of *in vitro* binding assays performed with GSH bead-immobilized recombinant GST–Nup60* proteins (green circles) and the Y-complex (blue circles) affinity purified from *S. cerevisiae* (NUP133–TAP nup60Δ). The protein eluates were analysed by SDS–PAGE, Coomassie staining and immunoblotting against both GST (Nup60) and CBP (Nup133) tags. Bands were identified by MS (black asterisk: *E. coli* chaperone DnaK, which co-purifies with GST–Nup60* fusion proteins). For input, see Extended Data Fig. 2a. **d**, The motif organization of *S. cerevisiae* Nup60 and the GST–Nup60–StrepII constructs used for the *in vitro* binding assays is shown. **e**, SDS–PAGE of *in vitro* binding assays performed with GSH bead-immobilized recombinant GST–Nup60 proteins (green circles) and the Y-complex (blue

circles) affinity purified from *S. cerevisiae* (NUP133–TAP nup60Δ). Protein eluates were analysed by SDS–PAGE, Coomassie staining and immunoblotting against both GST (Nup60) and CBP (Nup133) tags. For input, see Extended Data Fig. 2b. **f**, Inter-protein DSS cross-links identified by MS in the native eluate of GST–Nup60(200–318)•Y-complex. For DSS cross-linking, see Extended Data Fig. 2d. The cross-link between aa141 of Nup145C and aa198 of Seh1 is consistent with a previous report⁴³. Also, aa201 of Nup145C is close to aa198, explaining why it cross-links to Seh1. The cross-link between aa856 of Nup133 and aa676 of Nup84 has also been reported earlier⁴³ and satisfies the DSS distance restraints (<30 Å between Cα atoms) when mapped onto the crystal structure⁶⁶. **g**, SDS–PAGE gel of *in vitro* binding assays performed with GSH bead-immobilized recombinant GST–Nup60 proteins and recombinant Nup85(1–564)•His₆–Seh1 heterodimer. The protein eluates were analysed by SDS–PAGE, Coomassie staining and immunoblotting against both GST (Nup60) and His₆ (Seh1) tags. For input, see Extended Data Fig. 2c. Source numerical data and unprocessed gels are available in Source data.



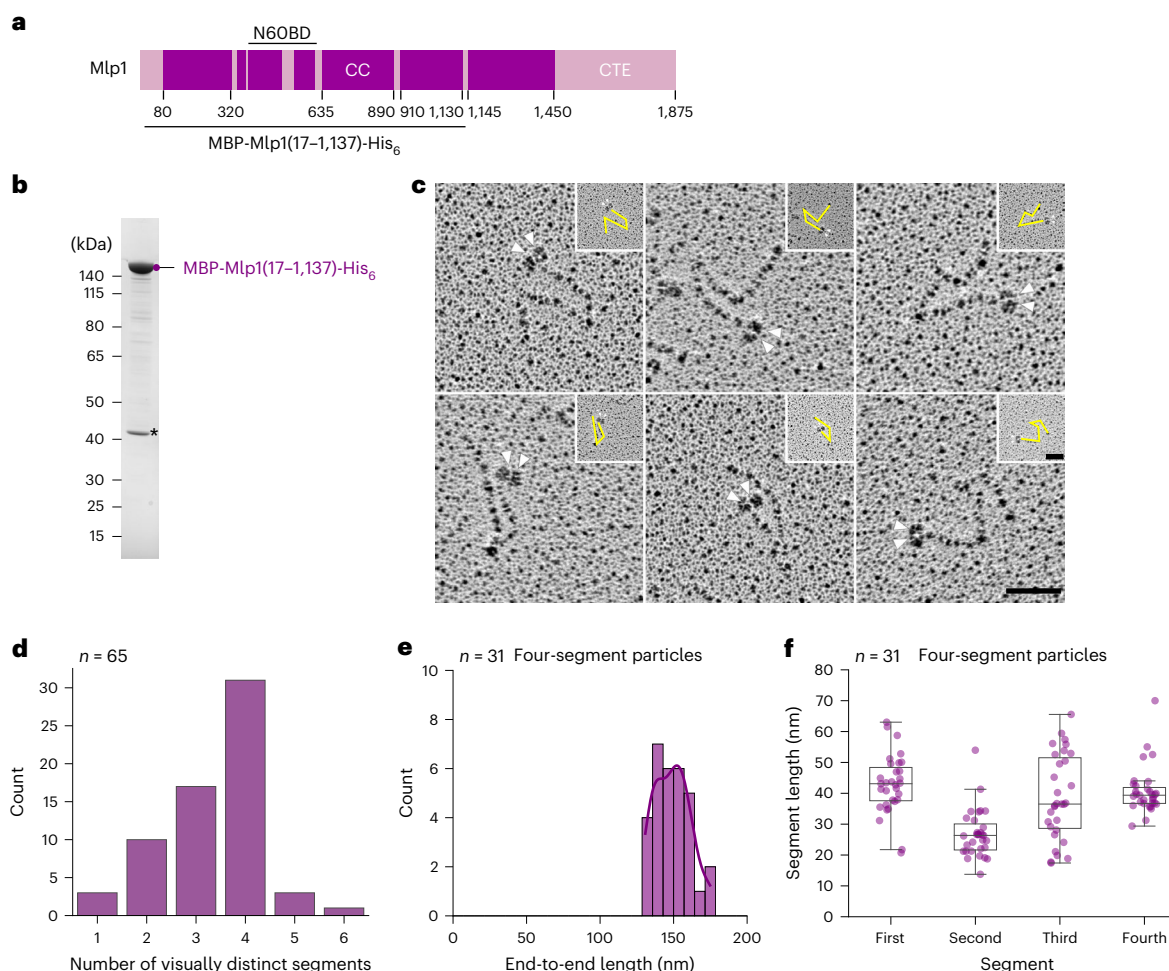


Fig. 3 | Mlp1 forms a homodimer with coiled coils and flexible hinges.

a, Domain organization of *S. cerevisiae* Mlp1 based on previously published reports and AlphaFold-Multimer predictions. Domain boundaries are drawn to scale with relevant amino acid positions indicated. CC, coiled-coil region (dark magenta) and N60BD (bar above the cartoon); CTE, C-terminal extension. For AlphaFold-Multimer predictions of Mlp1, see Extended Data Fig. 4a. **b**, SDS-PAGE gel of recombinant MBP-3C-Mlp1(17-1,137)-His₆ produced in His₆ insect cells. Protein eluate was analysed by SDS-PAGE and Coomassie staining (black asterisk: proteolytically cleaved MBP tag; band was identified by MS). **c**, Representative rotary shadowing EM images of MBP-3C-Mlp1(17-1,137)-His₆ particles (white arrowheads: N-terminal MBP tags; yellow lines in insets, particle traces used for

segment measurements). Scale bar, 40 nm. For a more accurate representation of particle heterogeneity, see a gallery of images in Extended Data Fig. 3a.

d, Histogram of MBP-3C-Mlp1(17-1,137)-His₆ particles based on the number of visually distinct segments ($n = 65$). **e**, Histogram of end-to-end lengths (nm) of four-segment MBP-3C-Mlp1(17-1,137)-His₆ particles ($n = 31$). **f**, Quantification of individual segment lengths (nm) of MBP-3C-Mlp1(17-1,137)-His₆ particles (boxplots, $n = 31$). Segments are counted from the N-terminus (first segment is closest to MBP tags). Data presented in the boxplots as median and interquartile range (IQR) with whiskers extending to points that lie within 1.5 IQRs of the lower and upper quartile. Source numerical data and unprocessed gels are available in Source data.

(defined as a particle segment, which can be traced by a straight line) varied from one up to six; however, most particles contained either four or three segments (Fig. 3d). The end-to-end length (defined as the sum of all segment lengths) of the four-segment particles ranged from 130–170 nm with a median length of ~150 nm (Fig. 3e). We then measured the lengths of individual segments in the largest subpopulation of particles, which contained four distinct segments (Fig. 3f). Counting from the N-terminal knobs of the MBP tag, the first, third and fourth segments displayed similar length distributions with medians between 37 and 43 nm. The second segment was noticeably shorter with a median length of only ~26 nm.

The AlphaFold-Multimer prediction of Mlp1(17-1,137) features three extended coiled coils, each with length of ~34–38 nm (Extended Data Fig. 4a), which closely aligns with the length of the first, third and fourth segments observed through EM. The second segment of Mlp1 is predicted to have a shorter coiled-coil structure and may correspond to the Mlp1 Nup60-binding domain (N60BD) (as explained below). AlphaFold-Multimer predictions also suggest that the overall

architecture is shared between yeast Mlp1 and human Tpr (Extended Data Fig. 4a,b). In sum, we provide experimental evidence that Mlp1(17-1,137) forms a coiled-coil parallel homodimer with flexible hinge regions at distinct positions. Flexible Mlp1 hinges therefore are key features of the basket filaments, contrary to models that portray them as entirely rigid⁵⁶.

Nup60 couples Mlp1 recruitment and docking on the Y-complex

The Nup60 aa200–318 region that we identified as an interaction site with both Mlp1 and the Y-complex consists of three adjacent SLiMs (termed SLiMs A, B and C). The proximity of three SLiMs within a short stretch suggested that Nup60 organizes, and potentially co-regulates, the recruitment of Mlp1 filaments to the Y-complex. To disentangle this complexity, we generated a set of GST-Nup60(200–318) constructs with different SLiM deletions (Fig. 4a), immobilized them on GSH beads and assessed their affinity to either recombinant Nup85*•Seh1 or Mlp1 by in vitro binding assays. Deleting SLiM A reduced the interaction with

Nup85*Seh1 (Fig. 4b, upper panel; compare lane 2 with 4), and correspondingly, SLiM A alone exhibited some affinity for Nup85*Seh1 (compare lane 2 with 3). An additional deletion of SLiM C, retaining SLiM B, completely abolished the interaction with Nup85*Seh1 (compare lane 4 with 5). Conversely, deletion of SLiM B did not affect Nup85*Seh1 binding (compare lane 4 with 6). The linker connecting SLiMs A and B also displayed no detectable affinity for Nup85*Seh1 (compare lane 4 with 8). Importantly, SLiM C was sufficient to robustly interact with Nup85*Seh1 (compare lane 4 with 10).

We then tested the same set of GST-Nup60 constructs for their capacity to bind a recombinant fragment of Mlp1. We used the previously identified N60BD of Mlp1 (aa382–620, Mlp1 N60BD)⁴⁰, which was N-terminally tagged with NusA-His₆ for improved protein stability and expression. The GST-Nup60(200–318) construct exhibited robust affinity for Mlp1 N60BD (Fig. 4b, lane 2 of the lower panel). Deleting SLiM A from Nup60 largely retained the affinity for Mlp1 N60BD (compare lane 2 with 4). Correspondingly, SLiM A alone exhibited only a weak affinity for Mlp1 N60BD (compare lane 2 with 3). An additional deletion of SLiM C had no major effect on Mlp1 N60BD binding (compare lane 4 with 5). Consistently, SLiM C alone exhibited only a minor affinity for Mlp1 N60BD (lane 10). Further dissection of SLiMs B and C showed that deletion of SLiM B had a strong effect on Mlp1 N60BD binding (compare lane 4 with 6). Importantly, when testing the affinities of SLiMs A, B and C for Mlp1 N60BD in isolation, SLiM B displayed the highest affinity for Mlp1 N60BD (compare lanes 3, 9 and 10). This establishes the 18-aa-long Nup60 SLiM B as a critical contact point for the Mlp1 filaments.

Comparing the affinities of the three Nup60 SLiMs for Nup85*Seh1 and Mlp1 N60BD, SLiM B binds exclusively to Mlp1 N60BD, SLiM C binds primarily to Nup85*Seh1 and SLiM A has the capacity to interact with both Nup85*Seh1 and Mlp1 N60BD under the conditions tested (Fig. 4c). These data define the detailed molecular architecture of the junction connecting the NPC basket and the NPC core. The interaction pattern of this tripartite junction suggests that SLiM A may sense the presence of basket filaments and Y-complex simultaneously, acting as a ‘coincidence detector’.

XL-MS confirms Nup60 interactions with Nup85 and Mlp1

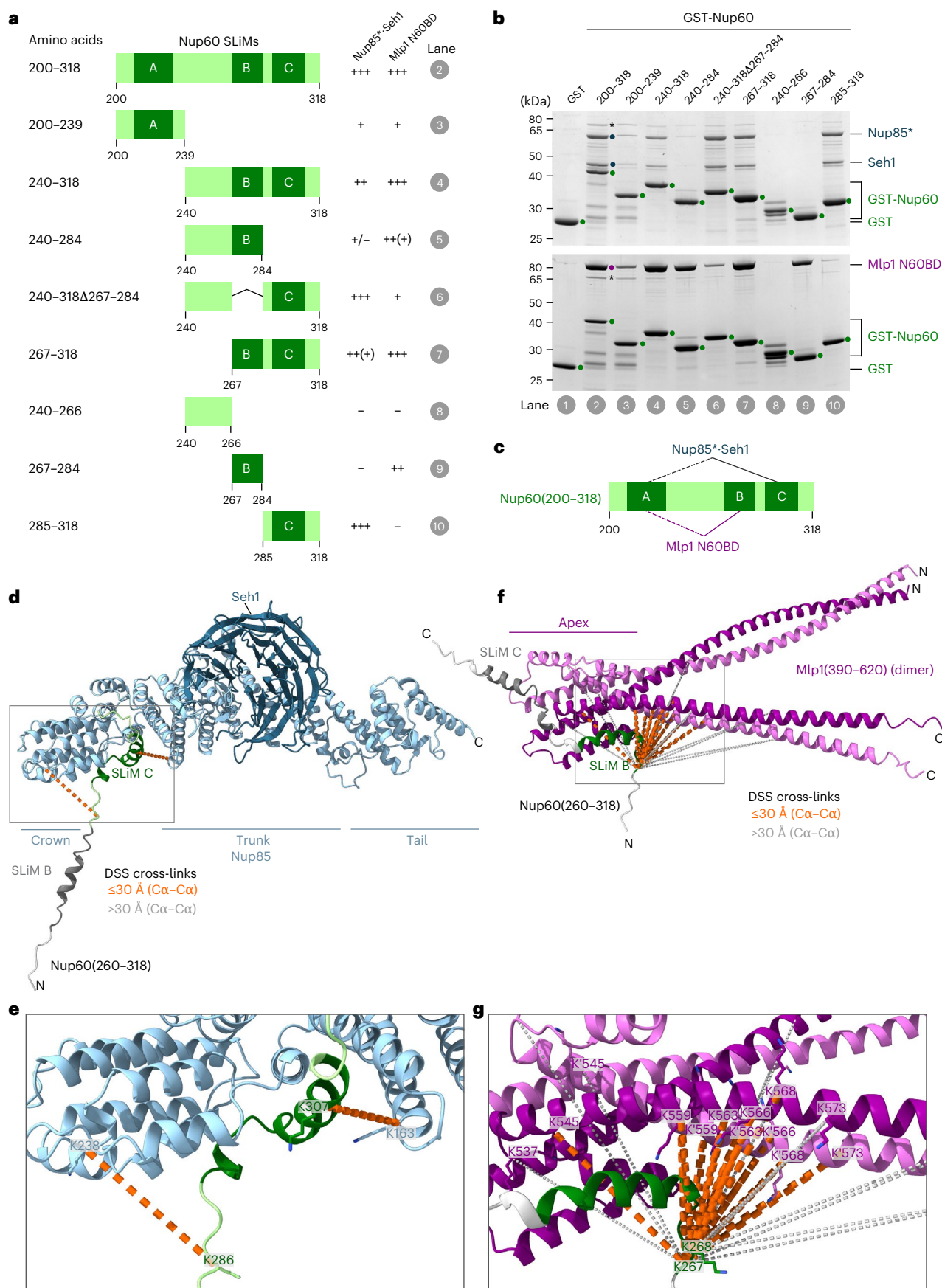
For a refined understanding of the NPC basket-core connection, we modelled Nup60 interactions with either Nup85*Seh1 or Mlp1 by AlphaFold-Multimer. The Nup60 region spanning SLiMs B and C (aa260–318) is predicted to form two α -helices connected by a linker, with each SLiM containing a short α -helix (Fig. 4d). The α -helix of Nup60 SLiM C wedges between the ‘crown’ and ‘trunk’ of the Nup85 α -solenoid fold⁵⁴ (Fig. 4d). In contrast, no contacts were predicted between SLiM B and Nup85 or Seh1, consistent with our in vitro binding assays (Fig. 4b).

AlphaFold-Multimer also did not predict an interaction of Nup60 SLiM A with Nup85*Seh1, possibly because their affinity is too low (Fig. 4b, lane 3). To test the validity of the model, we performed XL-MS experiments with the reconstituted Nup60(285–318)•Nup85*Seh1 complex and mapped the DSS cross-links onto the predicted structure (Fig. 4d,e and Extended Data Figs. 5a,b and 6a–c). Supporting the structural model, the K307 residue of the Nup60 SLiM C α -helix cross-linked specifically to the Nup85 K163 residue, positioned in a groove between the crown and trunk of Nup85. The measured distance between these cross-linked lysine residues satisfies the maximal distance restraints imposed by the DSS cross-linker (<30 Å between C α atoms). A second cross-link was detected between the Nup60 K286 residue and Nup85 K238, providing additional support for the predicted relative orientation of SLiM C with respect to Nup85 (Fig. 4d). Notably, despite the large number of surface-exposed lysine residues in Nup85*Seh1 (29 in Nup85* and 22 in Seh1), only Nup85 K163 and K238 were cross-linked to Nup60 SLiM C, suggesting that the intrinsically disordered Nup60 protein binds to the Y-complex in a defined orientation.

We then modelled Nup60 interactions with Mlp1 following the same approach. The Mlp1 N60BD construct (aa390–620) was predicted to form two dimeric coiled-coil segments, arranged in a V-shape with an acute angle of ~45° (Fig. 4f). The apex of the V-shaped Mlp1 structure, which connects and orients the two coiled-coil segments, is decorated by short α -helices, located on opposite sides of the apex. Nup60(260–318) forms two α -helices connected by a linker similar to the Nup60•Nup85*Seh1 model (Fig. 4d,f). In contrast, SLiM B is predicted to wedge into a groove on the C-terminal coiled-coil segment of Mlp1 N60BD close to the hinge region, whereas SLiM C remains unbound (Fig. 4f). Interestingly, the Mlp2 N60BD is predicted to interact with SLiM B of Nup60 in a similar fashion (Extended Data Fig. 6h). Noting that a segment of human Tpr is predicted to adopt a structure almost identical to Mlp1 N60BD (Extended Data Fig. 4), we asked whether AlphaFold-Multimer predicts a similar interaction mode between human Nup153 and Tpr, which have been shown to interact directly in the past⁵⁷. We ran an unbiased prediction using full-length Nup153 and a Tpr(390–620) homodimer. Interestingly, AlphaFold-Multimer predicted that Nup153(305–320) forms an α -helix that interacts with the Tpr(390–620) homodimer in a fashion identical to the Nup60 SLiM B interaction with the Mlp1 N60BD (for a superposition of the predicted structures, see Extended Data Fig. 6g). The predicted interactions are consistent with previously performed in vitro binding assays demonstrating a direct interaction between recombinant Nup153(228–611) and Tpr(172–651) (ref. 57). In a yeast two-hybrid assay, Tpr(172–651) interacted with Nup153(228–439), but neither interacted with Nup153(1–244) nor Nup153(337–611) (ref. 57), which is in line with our structural modelling. Hence, despite low overall sequence conservation between yeast

Fig. 4 | Nup60 couples Mlp1 filament recruitment and Mlp1 docking onto the Y-complex. a, Motif organization of *S. cerevisiae* GST-Nup60-StrepII constructs used for the in vitro binding assays. **b**, Top: SDS-PAGE gel of in vitro binding assays performed with GSH bead-immobilized recombinant GST-Nup60 proteins (green circles) and Nup85(1–564)•His₆-Seh1 heterodimer (blue circles). Bottom: SDS-PAGE gel of in vitro binding assays performed with GSH bead-immobilized recombinant GST-Nup60 proteins (green circles) and NusA-His₆-Mlp1(382–620) (magenta circle). Protein eluates were analysed by SDS-PAGE and Coomassie staining (black asterisk: *E. coli* chaperone DnaK, which co-purifies with GST-Nup60 fusion proteins). **c**, A cartoon summary of in vitro binding assays with higher affinity interactions depicted in solid lines (Nup60 SLiM B-Mlp1 N60BD and Nup60 SLiM C-Nup85*Seh1) and lower affinity interactions depicted in dashed lines (Nup60 SLiM A-Mlp1 N60BD and Nup60 SLiM A-Nup85*Seh1). **d**, DSS cross-links identified by MS mapped onto AlphaFold-Multimer model of Nup60(260–318)•Nup85*Seh1. The segment of the Nup60 cartoon corresponding to the GST-Nup60(285–318) construct used in the DSS cross-linking experiment is coloured in green, whereas the rest is shown in grey. The identified cross-links are depicted in dashed lines connecting C α atoms

of cross-linked amino acid residues (orange indicates satisfied distance restraint and grey indicates violated distance restraint). For clarity, the unstructured N-terminal extension of Nup85(1–46) and the internal loop of Seh1(248–291) are not shown. For cross-linking, see Extended Data Fig. 5a,b. An AlphaFold3 model of Nup60(260–318)•Nup85*Seh1 recapitulated the positioning of polypeptide chains predicted by AlphaFold-Multimer. **e**, Zoom-in of satisfied cross-links as in **d** with corresponding amino acid residues indicated. **f**, The DSS cross-links identified by MS mapped onto AlphaFold-Multimer model of Nup60(260–318)•Mlp1(390–620) dimer. The segment of Nup60 cartoon corresponding to GST-Nup60(267–284) construct used in the DSS cross-linking experiment is coloured in green, whereas the rest is shown in grey. Identified cross-links are depicted in dashed lines connecting C α atoms of cross-linked amino acid residues (orange indicates satisfied distance restraint and grey indicates violated distance restraint). For cross-linking, see Extended Data Fig. 5a,c. An AlphaFold3 model of Nup60(260–318)•Mlp1(390–620) dimer recapitulated the positioning of polypeptide chains predicted by AlphaFold-Multimer. **g**, Zoom-in of satisfied cross-links as in **f** with corresponding amino acid residues indicated. Source numerical data and unprocessed gels are available in Source data.



Nup60 and human Nup153, as well as between yeast Mlp1 and human Tpr, the predicted interactions are well conserved.

To further test the validity of the model, we performed XL-MS experiments with the reconstituted Nup60(267–284)•Mlp1 N60BD complex and subsequently mapped the identified DSS cross-links onto the structure (Fig. 4f,g and Extended Data Figs. 5a,c and 6d–f). The majority of identified inter-protein cross-links (14 out of 15) mapped onto the C-terminal coiled-coil segment of Mlp1 N60BD (Fig. 4g) even though both coiled-coil segments of the V-shaped Mlp1 model contain a large number of surface-exposed lysine residues (26 in the C-terminal and 12 in the N-terminal segment). The 15-residue stretch (aa559–573) within the Mlp1 N60BD forms a cross-linking hotspot, where Mlp1 K559, K563, K566, K568 and K573 are identified as being cross-linked to both K267 and K268 of the Nup60 SLiM B (Fig. 4g). In each of these instances, at least one chain of the Mlp1 N60BD dimer satisfies the DSS distance restraints. Moreover, the cross-link between K267 of Nup60 and K545 of Mlp1 N60BD is satisfied by one of the Mlp1 chains. The cross-link between K267 of Nup60 and K537 of Mlp1 N60BD, although violated by distance, can be explained by the flexibility of the SLiM B N-terminus. Only two cross-links, involving K267 and K268 of Nup60 and K590 of Mlp1 N60BD, remain unexplained. Thus, the AI-predicted interaction patterns are in excellent agreement with our reconstitution and XL-MS experiments and confirm a division of labour between SLiM B (Mlp1 contact) and SLiM C (Nup85 contact).

Mutational analyses verify the interaction surfaces

To verify the critical interfaces of the Nup60•Nup85•Mlp1 junction, we introduced rationally designed point mutations. The interaction between Nup60 SLiM C and Nup85 is predicted to involve a salt bridge between Nup60 R306 and Nup85 D216 or the adjacent E218 (note that out of the five high-confidence models generated by AlphaFold-Multimer, residue R306 can accommodate two alternative salt bridges) (Fig. 5a). A second salt bridge is formed between Nup60 K309 and Nup85 E177 or E176. Hydrogen bonding between Nup60 N296 and the Nup85 E218–P219 peptide group is expected to further stabilize the interface (Fig. 5a). Notably, the NPY triad (aa296–298) of Nup60 is conserved across yeast species (Extended Data Fig. 7a). Hence, we generated mutations targeting these interactions in Nup60 and Nup85 (alanine substitutions or charge inversions) and tested the ability of recombinant GST-Nup60(285–318), spanning SLiM C, to interact with Nup85•Seh1. The *in vitro* binding assays showed that alanine substitutions of Nup60 N296 and Y298 reduced the affinity for Nup85•Seh1, while mutating P297 to alanine had a minor effect (Fig. 5b and Extended Data Fig. 7b). A double mutant, in which both Nup60 R306 and K309 were mutated to E, also had a disruptive effect (Fig. 5b). Mutating the relevant Nup85 residues also disrupted the Nup60 interaction, although to a lesser extent (Fig. 5b).

For the Nup60 SLiM B interface with Mlp1, AlphaFold predicted a salt bridge between Nup60 D281 and Mlp1 R532, and a network of hydrogen bonds involving Nup60 S270 and N271, and Mlp1 D524, E561 and E564 (Fig. 5c). To assess the relevance of these residues, we examined how mutations impacted the binding strength between recombinant GST-Nup60(267–284), spanning SLiM B, and Mlp1 N60BD. Mutating Nup60 D281 to K reduced the affinity for Mlp1, whereas mutating Nup60 S270 to D abolished binding to Mlp1 (Fig. 5d and Extended Data Fig. 7c). A double mutation, in which both S270 and N271 were mutated to A, had a similarly strong effect (Fig. 5d). Conversely, mutating Mlp1 D524 to A, Mlp1 R532 to E or mutating both E561 and E564 to A strongly reduced the affinity between Mlp1 N60BD and the Nup60(267–284) (Fig. 5d). In summary, our integrative approach, combining *in vitro* binding assays (Fig. 4b), XL-MS (Fig. 4d–g) and point mutagenesis (Fig. 5a–d), provides robust support for the validity of the AlphaFold models.

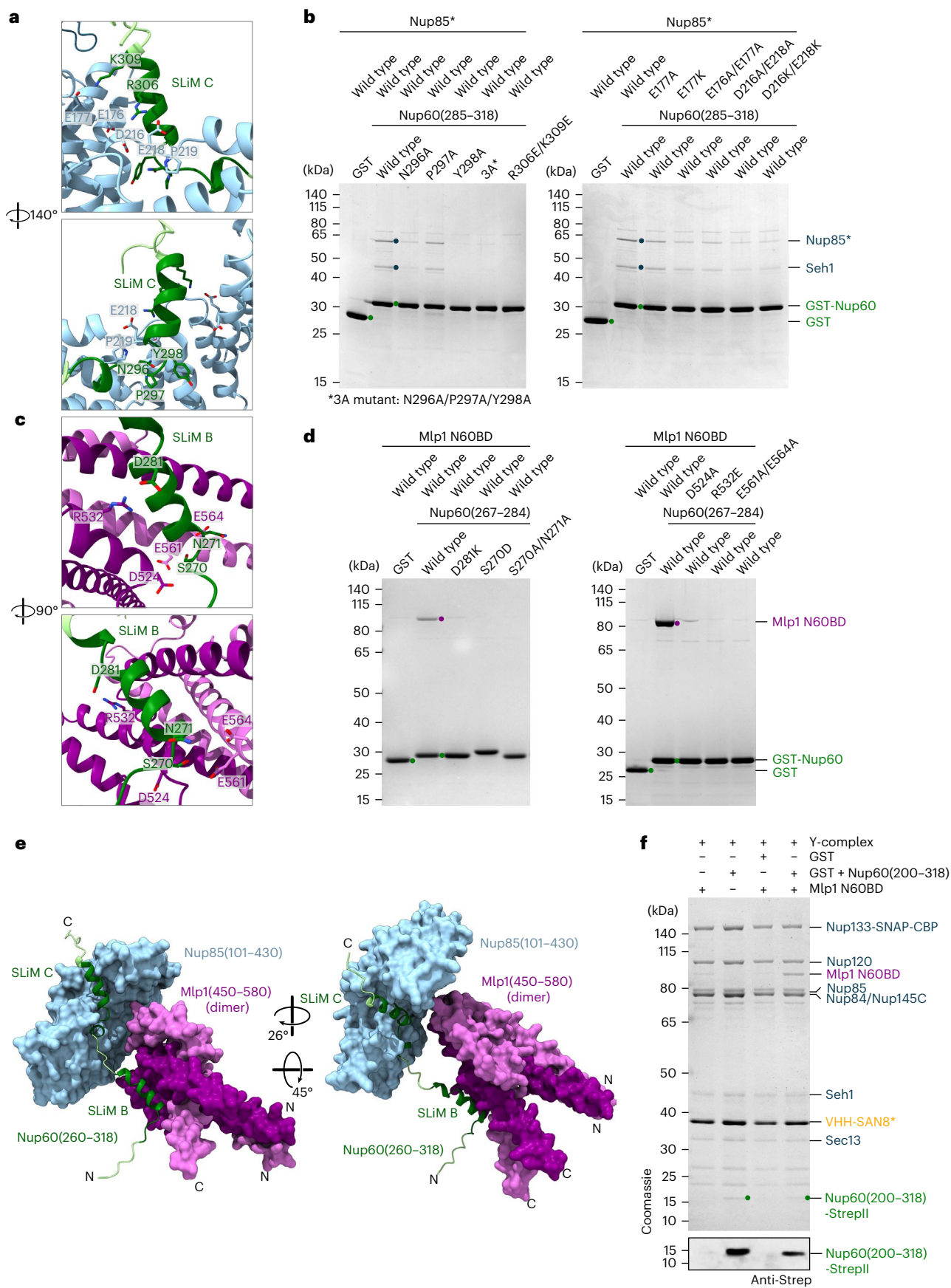
Reconstitution of the basket–outer ring junction *in vitro*

Having identified the Nup60•Mlp1 and Nup60•Nup85 binary interfaces, we set out to obtain a structural model of the entire Nup85•Nup60•Mlp1 junction. Because large multi-protein structures can pose a challenge for AlphaFold, it became necessary to truncate the proteins further. We used the crown-trunk junction (aa101–430) of Nup85 (ref. 54), two copies of Mlp1 covering the hinge region and shortened coiled-coil segments (aa450–580), and Nup60(260–318), spanning SLiMs B and C, as before. The predicted model of the composite ternary complex recapitulates the main features of the binary interactions, yet offers additional insights (Fig. 5e). Nup60 now simultaneously interacts with Mlp1 and Nup85 via SLiM B and SLiM C, respectively. In doing so, it stitches the Y-complex and basket filaments together. The hinge region of Mlp1 lies directly opposite to Nup85, yet, the two proteins exhibit no noticeable contacts, suggesting that the Nup85•Nup60•Mlp1 junction could be intrinsically flexible. These predicted features align with a host of previous observations suggesting that the Mlp1/Tpr filaments can exhibit extensive movements on the NPC core⁵⁸. Of note, the predicted interfaces of the tripartite junction are evolutionarily conserved across yeast species, highlighting their structural relevance: the surface patch of Mlp1, which interacts with Nup60 SLiM B, displays a high degree of conservation across yeast species (Extended Data Fig. 8a–c). Similarly, multiple residues in the groove of Nup85, which accommodates the Nup60 SLiM C, are conserved (Extended Data Fig. 8c).

To reconstitute a supramolecular assembly comprising the Nup60 suspension cable, Y-complex and part of the Mlp1 filaments, we devised a step-wise approach. First, the affinity-purified Y-complex was incubated with recombinant Nup60(200–318) spanning SLiMs A, B and C (generated by TEV protease cleavage of GST-TEV-Nup60(200–318)). The Y-complex was captured on nanobody-coated anti-FLAG beads

Fig. 5 | Mutational analyses verify the interaction surfaces. **a**, Cartoon representation of the interface between Nup60 SLiM C (green) and Nup85 (blue) as predicted by AlphaFold-Multimer model of Nup60(260–318)•Nup85•Seh1. **b**, SDS–PAGE gel of *in vitro* binding assays performed with GSH bead-immobilized recombinant GST-Nup60(285–318) mutants and wild-type Nup85(1–564)•His₆-Seh1 heterodimer (left), and wild-type GST-Nup60(285–318) and Nup85(1–564)•His₆-Seh1 mutants (right). Protein eluates were analysed by SDS–PAGE and Coomassie staining. For inputs, see Extended Data Fig. 7b. **c**, A cartoon representation of the interface between Nup60 SLiM B (green) and Mlp1 dimer (magenta) as predicted by AlphaFold-Multimer model of Nup60(260–318)•Mlp1(390–620) dimer. **d**, SDS–PAGE gel of *in vitro* binding assays performed with GSH bead-immobilized recombinant GST-Nup60(267–284) mutants and wild-type Nup85•His₆-Mlp1(382–620) (left), and wild-type GST-Nup60(267–284) and Nup85•His₆-Mlp1(382–620) mutants (right). The protein eluates were analysed by SDS–PAGE and Coomassie staining. For inputs, see Extended Data Fig. 7c. **e**, The AlphaFold-Multimer model of the Nup85(101–430)•Nup60(260–318)•Mlp1(450–580) dimer complex at the

interface between the NPC core and nuclear basket. For cartoon representation, model confidence and surface conservation, see Extended Data Fig. 8a–c. An AlphaFold3 model of Nup85(101–430)•Nup60(260–318)•dimeric Mlp1(450–580) recapitulated the positioning of polypeptide chains predicted by AlphaFold-Multimer. **f**, SDS–PAGE gel of Y-complex•Nup60(200–318)•Mlp1(382–620) assembly reconstitution. Anti-FLAG beads were coated with VHH-SAN8-SNAP-FLAG (VHH-SAN8*) nanobody specific for the Nup84 subunit of the Y-complex⁵⁹. The Y-complex affinity purified from *S. cerevisiae* (NUP133-SNAP-TAP nup60Δ) was immobilized on VHH-SAN8* beads and co-incubated with recombinant Nup60(200–318)-StrepII and Nup85•His₆-Mlp1(382–620) proteins. To generate Nup60(200–318)-StrepII, purified GST-TEV-Nup60(200–318)-StrepII protein was incubated with TEV protease and the mix was added to the reconstitution mixture. Top: protein eluates were analysed by SDS–PAGE and Coomassie staining. Bottom: the identity of the Nup60(200–318)-StrepII band (green circle) in the Coomassie-stained SDS–PAGE gel was verified by immunoblotting against the C-terminal StrepII tag. It was also verified by MS analysis of the excised band. Unprocessed gels and blot are available in Source data.



using a FLAG-tagged nanobody (VHH-SAN8-SNAP-FLAG), which binds Nup84 (ref. 59). Next, NusA-His₆-Mlp1 N60BD was added, and after washing, the interacting proteins were eluted using a FLAG peptide. As expected, Nup60(200–318) alone bound to the Y-complex (Figs. 2e and 5f). Importantly, Mlp1 N60BD co-purified with the Y-complex at near-stoichiometric amounts in the presence of Nup60(200–318) (Fig. 5f). In contrast, Mlp1 N60BD did not co-purify with the Y-complex when GST was used as a negative control. This shows that docking the Mlp1 basket filaments to the Y-complex critically depends on Nup60, which organizes a tripartite protein junction via adjacent SLiMs.

Nup60 co-recruits Mlp1 and Y-complex to synthetic membrane

Traditionally, Nup protein–protein interactions were studied in solution. However, the behaviour of membrane-anchored Nups may differ substantially⁶⁰. Membrane anchoring can affect local protein concentration, orientation and accessibility towards interacting partners. Thus, membranes can act as platforms for higher-order self-assembly, but may also restrict interactions. Moreover, the lateral diffusion of membrane-tethered Nups could be restricted to specific regions within the membrane, for example, curved membranes⁵³ or laterally segregated lipid domains⁶¹, leading to anisotropic lateral diffusion. We therefore interrogated the assembly of Nup60, Mlp1 and the Y-complex on a chemically defined membrane *in vitro*, using a previously established ‘visual biochemistry’ approach⁴⁰ involving multicolour detection of recombinant proteins on giant unilamellar vesicles (GUVs; 10–20 µm in diameter).

We employed a set of recombinant Nup60 constructs C-terminally tagged with monomeric green fluorescent protein (mGFP) as well as recombinant Mlp1 N60BD N-terminally tagged with NusA-His₆ and C-terminally tagged with mCherry-FLAG (Fig. 6a,b). The Y-complex was tandem-affinity purified from an *S. cerevisiae* strain expressing Nup133 with a C-terminal SNAP-TAP tag and Nup120 with a C-terminal 6xFLAG tag (Fig. 6b). The Y-complex was fluorescently labelled with Alexa Fluor 647 via the SNAP tag. We chose to fluorescently label Nup133 because it is positioned at one end of the Y-complex, away from Nup85 (Fig. 2b) and tends to dissociate more readily than other subunits⁴⁶. Thus, the labelled Nup133 would serve as a sensitive indicator demonstrating that Nup60 recruits an intact Y-complex through Nup85. We first tested whether recombinant Nup60 (aa27–318ΔHR; abbreviated as Nup60**) can bind to GUVs and recruit both Mlp1 N60BD and the Y-complex. The Nup60** construct contains all relevant SLiMs, but lacks the N-terminus, HR and FG repeats for improved protein stability. Indeed, co-incubation of Nup60**, Mlp1 N60BD and the Y-complex with GUVs resulted in the enrichment of all proteins on the membrane (Fig. 6c). Both Y-complex and Mlp1 fluorescence intensities positively correlated with Nup60 fluorescence intensity (Fig. 6d,e). Membrane recruitment was specifically mediated by the N-terminal AH of Nup60, as the introduction of an I36R mutation, which disrupts the hydrophobic face of the AH, abolished the recruitment of all three proteins (Fig. 6c).

We then tested how deleting the Nup60 SLiMs, which mediate interactions with Mlp1 N60BD and the Y-complex *in vitro*, affects membrane recruitment. Deletion of either SLiM A or SLiM C of Nup60** decreased the co-recruitment of the Y-complex compared with wild-type Nup60** (Fig. 6c), in agreement with the *in vitro* binding assays (Fig. 2c). To gain quantitative insights into the relative recruitment efficiencies of different Nup60 constructs and to account for the slight variation in the levels of membrane-bound Nup60 across different constructs, we fitted linear regression models to these data and extracted the slope values (*m*) as population-wide estimates for the relative recruitment efficiency (Fig. 6d,e). This analysis revealed that deletion of either SLiM A or SLiM C decreased Y-complex recruitment ~4–5-fold compared with wild-type Nup60** (Fig. 6d). Interestingly, the recruitment of Mlp1 N60BD increased upon deletion of either Nup60 SLiM A or SLiM C, suggesting that Y-complex binding attenuates Mlp1

N60BD recruitment under the conditions tested (Fig. 6e). Whether this represents a physiologically relevant weakness of the protein junction remains to be tested. Double deletion of both SLiMs A and C reduced the membrane recruitment efficiency of the Y-complex by a factor of ~50 (Fig. 6d). As predicted, deletion of SLiM B abolished the recruitment of Mlp1 N60BD, but did not affect recruitment of the Y-complex (Fig. 6d,e), consistent with our *in vitro* binding assays (Fig. 4b).

These results confirm that the membrane can act as a platform for the higher-order, SLiM-dependent assembly of the Y-complex–Nup60–basket filament junction. They also suggest that the membrane may attenuate the simultaneous interaction of Mlp1 and the Y-complex with Nup60, potentially creating a built-in instability of the interface between the NPC core and the basket. This may account for the high exchange rate between basket-bound and free nucleoplasmic pools of Mlp1/2 proteins²⁸.

Nup60 SLiMs organize the Mlp1-outer ring junction in cells

Having determined how Nup60 couples Mlp1 filaments with the Y-complex, we sought to verify these findings *in vivo*. To this end, we examined the localization of both Nup60, C-terminally tagged with mCherry, and Mlp1, C-terminally tagged with mGFP. Both Nups were expressed from plasmids under their endogenous promoters in a *nup60Δmlp1Δ* strain and visualized by live cell fluorescence microscopy. Wild-type Nup60 and Mlp1 displayed a punctate NE staining that is characteristic of NPCs (Fig. 7a and Extended Data Fig. 9a). Nup60 is distributed throughout the NE, whereas Mlp1 is absent in the NE region adjacent to the nucleolus⁷²¹. This implies a discrepancy in the quantities of Nup60 and Mlp1 at the NE, with all NPCs containing Nup60, but only a portion containing Mlp1. We determined the NE enrichment factor of both Nups by quantifying the ratio between NE and nucleoplasm (N) fluorescence intensities. Due to some cells losing plasmids, only cells expressing both proteins were taken into account. Notably, upon deletion of SLiM B, Mlp1 mislocalized into the nucleoplasm and formed prominent foci in ~90% of cells, which may reflect an aggregated state of Mlp1 (Fig. 7a,b). By comparison, the localization of Nup60 itself was largely unaffected (Fig. 7a), probably because Nup60 remains tethered to the NE via multiple interactions—to the INM via its AH and to the NPC core via the HR and SLiMs A and C. Collectively, these data confirm the importance of SLiM B for the positioning of the Mlp1 basket filaments *in vivo* and *in vitro*.

Next, we explored under which conditions Nup60 will detach from the NPC. Deleting the HR within the full-length Nup60 enriched Nup60 in the nucleoplasm, but some Nup60 was retained at the NE (Fig. 7a,b), suggesting that Nup60 oligomerization is not strictly required for NPC localization. The positioning of Mlp1 remained mostly unchanged in this mutant. To test for the role of the Nup60–Y-complex connection, we deleted SLiM A and SLiM C, alone or in combination, in a Nup60ΔHR background. While deletion of either SLiM A or SLiM C slightly increased the mislocalization of Nup60 (Fig. 7b), the deletion of both SLiMs strongly mislocalized Nup60 and consequently Mlp1, which formed foci in ~70% of cells (Fig. 7b). Thus, our data indicate a multivalent tethering mechanism, where Nup60 localization is governed by a lipid anchor (AH) and at least two protein–protein interactions with the NPC core (SLiMs A and C), possibly reinforced by oligomerization (HR). In contrast, Mlp1 localization primarily relies on the structurally distinct Nup60 SLiM B, whose efficiency might be tuned by the occupancy of the adjacent SLiMs A and C. Our *in vivo* findings align well with the *in vitro* biochemical reconstitution of the basket–NPC outer ring junction, providing strong support for our model of how the basket is docked to the core of the NPC.

Discussion

A basket-like structure on the nuclear side of the NPC was first observed in the early 1990s^{1–3,62}. However, despite decades of research, key aspects of the basket’s structure remain unclear, positioning the basket

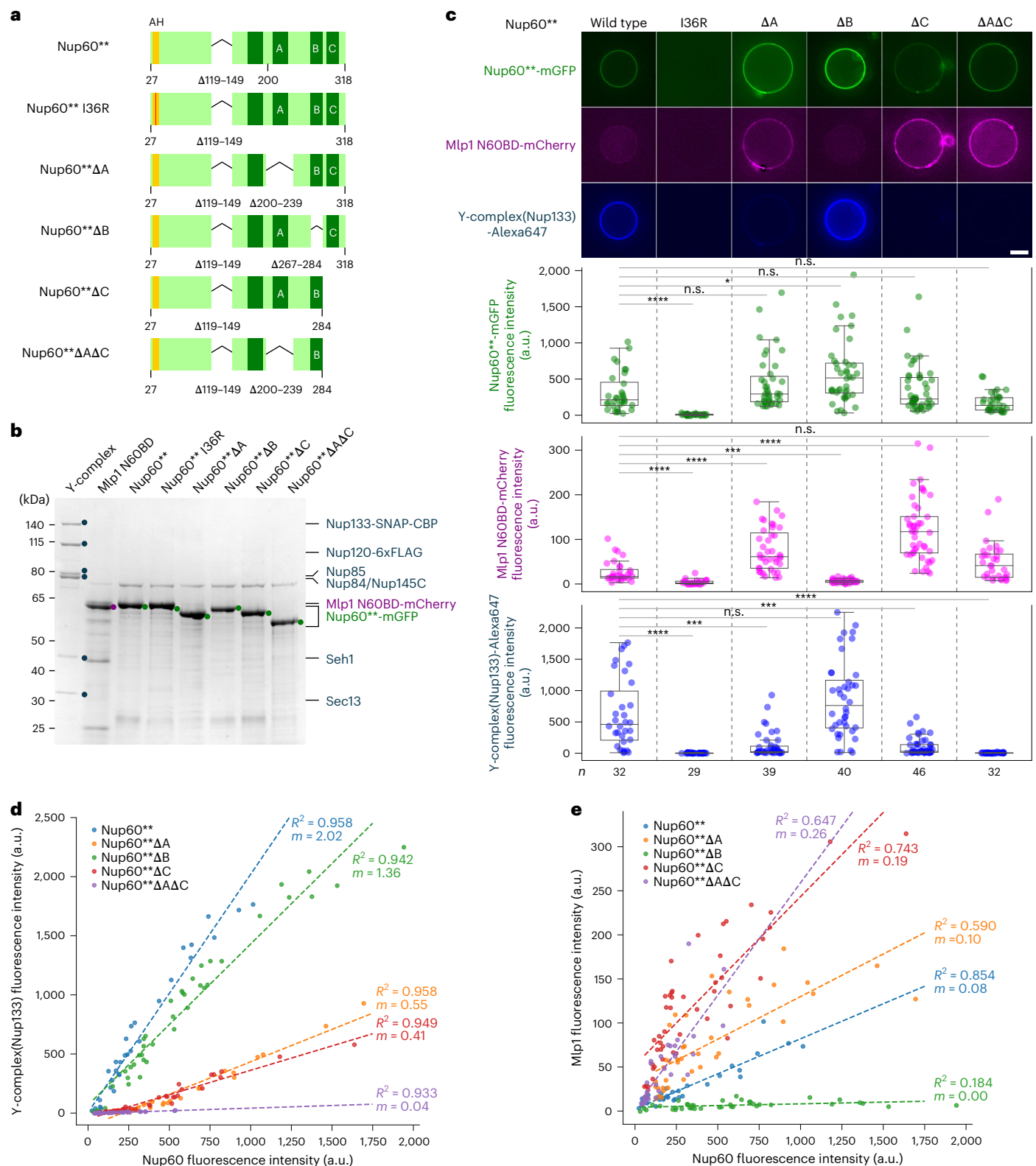


Fig. 6 | Nup60 SLiMs co-recruit basket filaments and Y-complexes to a synthetic membrane. a, The motif organization of *S. cerevisiae* His₆-Nup60**-mGFP-StrepII constructs used for the GUV binding assays. The motif boundaries and constructs are drawn to scale with relevant amino acid positions indicated. **b**, SDS-PAGE gel of input proteins used in GUV binding assays. His₆-Nup60**-mGFP-StrepII and NusA-His₆-Mlp1(382–620)-mCherry constructs were expressed and purified from *E. coli*, whereas the Y-complex was affinity purified via a two-step approach (anti-ProtA (IgG)/anti-FLAG) from *S. cerevisiae* (NUP133-SNAP-TAP NUP120-6xFLAG nup60Δ). **c**, Top: wide-field fluorescence images of GUVs. Bottom: quantification (boxplots) of GUV rim fluorescence intensities (*n*, number of GUVs quantified). Data presented

in the boxplots as median and interquartile range (IQR) with whiskers extending to points that lie within 1.5 IQRs of the lower and upper quartile. *****P* < 0.0001, ****P* < 0.001 determined by two-sided Dunnett's T3 multiple comparisons test ($\alpha = 0.05$). Scale bar, 5 μ m. n.s., not significant. **d**, Linear regression models between Y-complex fluorescence intensity (*y*) and Nup60 fluorescence intensity (*x*) across different Nup60** constructs. *R*², coefficient of determination; *m*, slope of linear regression model. **e**, Linear regression models between Mlp1 fluorescence intensity (*y*) and Nup60 fluorescence intensity (*x*) across different Nup60** constructs. *R*², coefficient of determination; *m*, slope of linear regression model. Source numerical data and unprocessed gels are available in Source data.

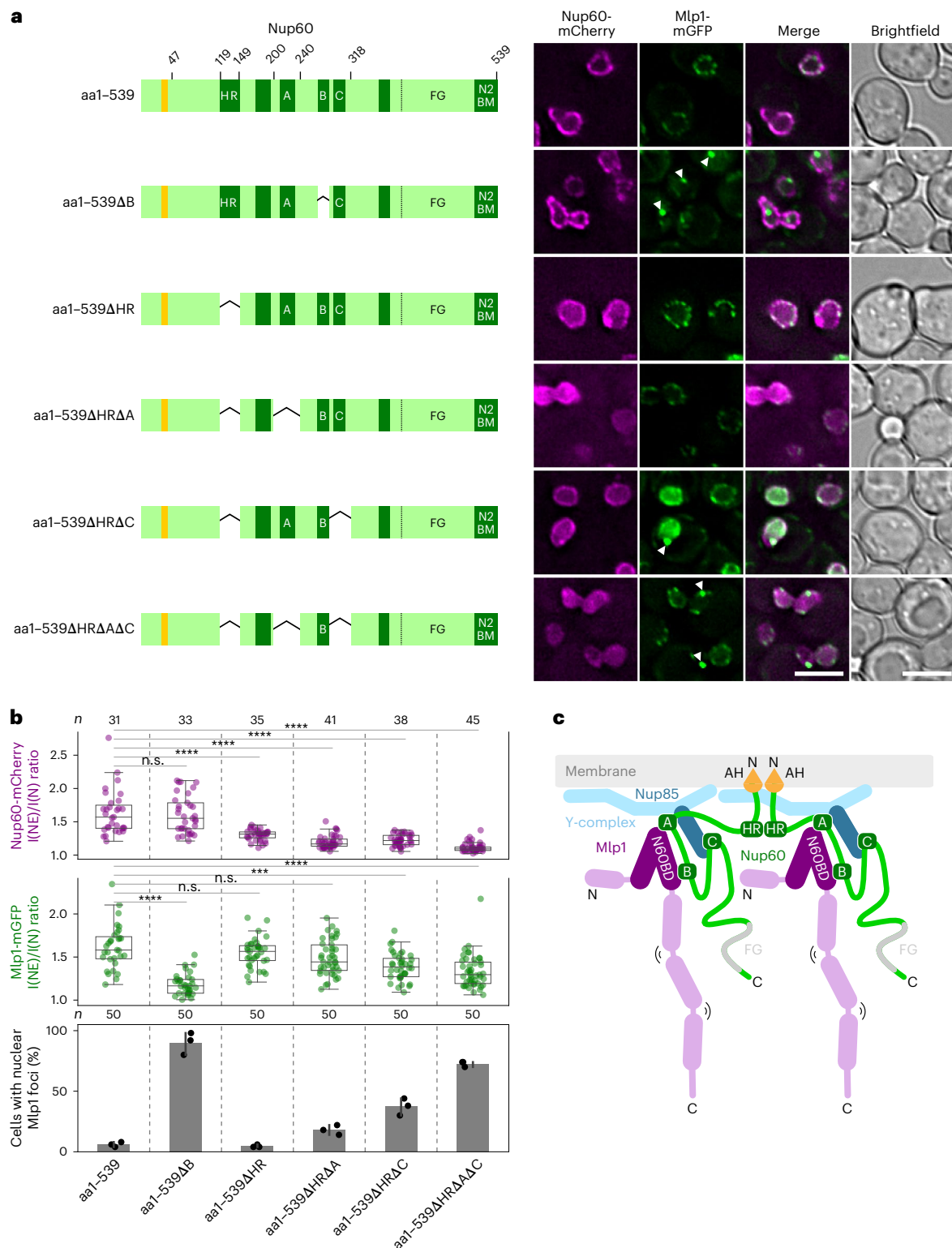


Fig. 7 | Nup60 SLiMs A, B and C organize the basket filament–outer ring junction in cells. a, The motif organization of Nup60-mCherry constructs (left) and representative wide-field fluorescence images of live yeast cells (right). BY4741 *nup60Δ mlp1Δ* strain was transformed with plasmids encoding *NUP60-mCherry* constructs and *MLP1-mGFP* with expression driven by the endogenous promoters. White arrowheads indicate nuclear Mlp1 foci. Scale bars, 5 μ m. **b**, Quantification of NE enrichment (ratio between NE and nuclear fluorescence intensities) of Nup60-mCherry (top, boxplot; *n*, number of cells quantified), Mlp1-mGFP (middle, boxplot) and number of nuclear Mlp1 foci (bottom, bar plot; error bars indicate 95% confidence interval; *n*, number of cells quantified

per replicate with three biological replicates). Data presented in the boxplots as median and interquartile range (IQR) with whiskers extending to points that lie within 1.5 IQRs of the lower and upper quartile. *****P* < 0.0001, ****P* < 0.001 determined by two-sided Dunnett's T3 multiple comparisons test (α = 0.05). n.s., not significant. **c**, Model of NPC core–nuclear basket interface organization. Note that Mlp1 might be organized in a different configuration, for example, the C-terminal coiled-coil segments folding back onto the N-terminal parts. For simplicity, Nup60 is depicted as a dimer but may also form larger oligomers. Source numerical data are available in Source data.

as a final frontier in the structural elucidation of the NPC. Through an integrated approach, including biochemical reconstitution in solution and on synthetic membranes, high-resolution connectivity mapping and AI-guided structure–function interrogation, we have now identified the elusive docking mechanism of basket filaments onto the NPC core. Additionally, we have uncovered a basis for the intrinsic flexibility of basket filaments. These results provide the foundation for understanding at the molecular level how the nuclear side of the NPC is functionalized.

Multivalent network connecting the NPC core and basket

The critical junction between the core scaffold of the NPC and the nuclear basket consists of three adjacent, functionally specialized SLIMs of Nup60, exhibiting affinities to both Nup85 and Mlp1. We consider three potential connection states of Mlp1, each characterized by the Nup60 SLIMs being utilized: Firstly, Mlp1 can independently bind to Nup60 via SLIM B, without involving Nup85. Due to Nup60 interacting with the membrane through its AH, this scenario could lead to an INM-bound Mlp1 filament diffusing on the inner nuclear membrane before interacting with the NPC core, for example, during NPC assembly. In the second state, the Nup60-tethered Mlp1 filament would be anchored to Nup85 through the Nup60 SLIM C. In the third state, Nup60 SLIM A would simultaneously bind Mlp1 and the Y-complex, acting as a coincidence detector. The molecular details of these interactions remain to be identified in future higher-order reconstitutions of the NPC. The cumulative strength of the basket–NPC core interaction would be governed by the avidity among the three identified SLIMs (Fig. 7c).

The nature of these interactions prompts the question of why a seemingly straightforward task—tethering Mlp1 to Nup85—is addressed in such a complex manner. We propose three reasons: Firstly, the remarkably interwoven NPC core–basket junction is conformationally flexible, which explains the ability of basket filaments to undergo axial and radial deformations¹. Secondly, as an architectural principle, the built-in redundancy of the multivalent NPC core–basket junction may accommodate for disruptions caused by constrictions/dilations of the NPC core⁶³. Loss of one SLIM interaction would not entirely break the junction. Thus, the multivalent network may act as a stress-absorber, keeping the basket components in place. Building on our work, future studies can investigate how the SLIM network is utilized and regulated to enable or inhibit basket assembly and to tune basket assembly in response to changes in nuclear RNA metabolism.

Domain architecture and flexibility of basket filaments

The putative nuclear basket densities, as recently revealed by a cryo-ET reconstruction of the *S. cerevisiae* NPC, extend ~20–30 nm into the nucleoplasm when measured from the nuclear outer ring²⁴. However, these densities probably represent only a smaller portion of the entire basket, which is difficult to capture by cryo-ET given its flexibility. Improved biochemistry and EM allowed us to examine 77% of the likely structured parts of Mlp1 (1–1450). Our data indicate that Mlp1 forms a parallel dimer characterized by extended coiled coils interspersed with pliable linkers. We experimentally determined an end-to-end length of 130–170 nm for a construct, which consists of all except one of the extended coiled-coil regions. It is possible that Mlp1 adopts a more compact configuration in cells, where the coiled-coil segments might intertwine, potentially assisted by other Nups or by attachment to the NPC core. This hypothesis requires further investigations.

Importantly, we identify the N60BD of Mlp1 as a key structural element and interaction hub, unresolved in prior studies. This Mlp1 domain may adopt a V-shaped structure, introducing a kink in the Mlp1 filament, thereby orienting the N- and C-terminal coiled-coil segments that emanate from it. Assuming that the Nup85•Nup60•Mlp1 junction forms the NPC core-proximal part of the basket, the apex of the Mlp1 N60BD (Fig. 4f) could function as the structural apex of the entire basket (Fig. 7c). The long C-terminal segment of Mlp1, following the

N60BD, may constitute the primary body of Mlp1 filaments, probably featuring three long coiled-coil segments connected by linkers. Notably, the AlphaFold-predicted V-shaped structure of the Mlp1 N60BD and its hypothetical equivalent in human Tpr exhibit remarkably similar folds (Extended Data Fig. 6g), despite low sequence identity (18%). This indicates an evolutionarily conserved structure–function relationship for this critical element from yeast to human. The overall organization of coiled-coil segments also seems conserved between yeast Mlp1 and human Tpr (Extended Data Fig. 4).

Our data suggest a high degree of intrinsic flexibility facilitated by linkers between the coiled coils. This could explain the observed flexibility of basket filaments, documented back in 1998⁵⁸, when axial compression, extension and radial dilation of the basket was first observed. Our study elucidates the dual nature of the nuclear basket, exhibiting both rigidity in the coiled-coil segments and flexibility in the hinge regions. Drawing parallels with man-made structures such as suspension bridges and space frames, similar engineering principles may apply to Mlp/Tpr coiled-coil proteins, optimizing basket performance and durability under diverse conditions. Linkers between the coiled coils presumably enable the nuclear basket to accommodate cargoes of various sizes⁵⁸. Moreover, the flexible suspension of the filaments on the Y-complex seems adapted to respond to each dilation or constriction of the NPC core, which involves a rearrangement of the Y-complex rings⁶³. On the other hand, the rigid parts may act as spacers that keep basket-associated export adaptors and heterochromatin^{6,21} at a certain distance, preventing the entry point into the NPC's transport channel from getting clogged.

The NPC core–basket filament interactions in a membrane context

Why is it crucial to also anchor the basket–NPC core junction on the pore membrane? It is conceivable that the ALPS motifs of Nup133 and Nup120 lack adequate membrane affinity for the de novo recruitment of the Y-complex to the NE membrane^{35,53}. Notably, we identify a third membrane attachment point for the Y-complex, specifically the tethering of the Nup85•Seh1 arm to the membrane through the Nup60 AH. Consequently, there are at least three distinct membrane attachment sites for the Y-complex. Of note, earlier pulse labelling experiments suggested that the head part of the Y-complex (Nup85•Seh1•Nup120) and Nup60 are incorporated into the maturing NPCs simultaneously⁶⁴. This intuitively points to a lock-and-key mechanism for NPC assembly, where basket filament assembly on a membrane is intricately linked to the assembly and integrity of the NPC core.

In fact, dimerization (or higher-order oligomerization) of Nup60 through its HR is expected to create another layer of membrane-tethered, multivalent interactions. Nup60 dimerization would cross-link two Y-complexes of the nuclear ring (Fig. 7c). So far, the circularization of Y-complexes has been attributed to head-to-tail interactions between the N-terminal extension of Nup133 and Nup120 of a neighbouring Y-complex⁴⁵. Our data suggest that these interactions and Y-complex ring formation could be reinforced by Nup60 connecting the Nup85 arms of neighbouring Y-complexes. As a consequence, both the circularization and membrane attachment of the NPC's nuclear ring is promoted. As reported earlier, besides self-interaction, the Nup60 HR may also bind to as-yet-unidentified components of the NPC core⁴⁰. Multiple lines of evidence suggest that multivalent interactions of membrane proteins can apply compressive stress to membrane surfaces, which can change membrane curvature⁶⁰. How the membrane-tethered NPC core–basket filament junction and other lipid-interacting Nups (for example Nup1) could bend the pore membrane is an interesting avenue for future studies.

Functional implications of basket heterogeneity in cells

Two distinct NPC core scaffold variants have been identified in *S. cerevisiae*: NPCs featuring a single nuclear ring and a recently

discovered isoform with a double nuclear ring^{23,24}. Assuming that all Nup85 binding sites in a double nuclear ring NPC can interact with Nup60•Mlp1, this isoform could accommodate up to 16 copies of Nup60, recruiting up to 16 dimeric Mlp1 filaments (that is, 32 Mlp1 monomers). As Nup60 directly interacts with Nup2 (ref. 40), the Nup2 stoichiometry would also double. Consequently, basket filament stoichiometry in cells could vary from 0 ('basketless' NPC adjacent to the nucleolus) to 8 (single nuclear ring) to 16 (double nuclear ring), resulting in structural heterogeneity on the nucleoplasmic face of the NPC. This may explain discrepancies in basket subunit stoichiometry reported in different studies^{41–44,65}. In principle, a double ring could template a double basket, and vice versa, suggesting a potential reinforcement of the double ring architecture by the basket. This has implications for basket-associated nuclear transport adaptors and enzymes^{13,21}. An intriguing possibility is that doubling the basket filament number, adaptor and enzyme stoichiometry could enhance the export competency of specific NPCs, consequently boosting NPC functionality locally.

In conclusion, our findings shed light on the once enigmatic process of constructing a basket on the nuclear face of the NPC, providing a comprehensive molecular understanding of a missing link of NPC structural biochemistry. Building upon this foundation, future studies can investigate the involvement of the basket in NPC and pore membrane biogenesis, explore the origins and implications of NPC asymmetry, and embark on the question of how NPC heterogeneity encodes cell identity and function.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41556-024-01484-x>.

References

- Goldberg, M. W. & Allen, T. D. The nuclear pore complex and lamina: three-dimensional structures and interactions determined by field emission in-lens scanning electron microscopy. *J. Mol. Biol.* **257**, 848–865 (1996).
- Ris, H. High-resolution field-emission scanning electron microscopy of nuclear pore complex. *Scanning* **19**, 368–375 (1997).
- Ris, H. The nuclear envelope and nuclear pore complex as seen by high-resolution field emission SEM and high voltage TEM. *EMSA Proc.* **53** <https://doi.org/10.1017/s0424820100140178> (Cambridge University Press, 1995).
- Beck, M. et al. Nuclear pore complex structure and dynamics revealed by cryoelectron tomography. *Science* **306**, 1387–1390 (2004).
- Fahrenkrog, B., Hurt, E. C., Aebi, U. & Panté, N. Molecular architecture of the yeast nuclear pore complex: localization of Nsp1p subcomplexes. *J. Cell Biol.* <https://doi.org/10.1083/jcb.143.3.577> (1998).
- Krull, S. et al. Protein Tpr is required for establishing nuclear pore-associated zones of heterochromatin exclusion. *EMBO J.* **29**, 1659–1673 (2010).
- Niepel, M. et al. The nuclear basket proteins Mlp1p and Mlp2p are part of a dynamic interactome including Esc1p and the proteasome. *Mol. Biol. Cell* **24**, 3920–3938 (2013).
- Saroufim, M. A. et al. The nuclear basket mediates perinuclear mRNA scanning in budding yeast. *J. Cell Biol.* **211**, 1131–1140 (2015).
- Green, D. M., Johnson, C. P., Hagan, H. & Corbett, A. H. The C-terminal domain of myosin-like protein 1 (Mlp1p) is a docking site for heterogeneous nuclear ribonucleoproteins that are required for mRNA export. *Proc. Natl Acad. Sci. USA* **100**, 1010–1015 (2003).
- Galy, V. et al. Nuclear retention of unspliced mRNAs in yeast is mediated by perinuclear Mlp1. *Cell* **116**, 63–73 (2004).
- Albert, S. et al. Proteasomes tether to two distinct sites at the nuclear pore complex. *Proc. Natl Acad. Sci. USA* **114**, 13726–13731 (2017).
- Raices, M. & D'Angelo, M. A. Nuclear pore complexes and regulation of gene expression. *Curr. Opin. Cell Biol.* <https://doi.org/10.1016/j.ceb.2016.12.006> (2017).
- Schneider, M. et al. The nuclear pore-associated TREX-2 complex employs mediator to regulate gene expression. *Cell* **162**, 1016–1028 (2015).
- Aksenova, V. et al. Nucleoporin TPR is an integral component of the TREX-2 mRNA export pathway. *Nat. Commun.* **11**, 4577 (2020).
- Buchwalter, A., Kaneshiro, J. M. & Hetzer, M. W. Coaching from the sidelines: the nuclear periphery in genome regulation. *Nat. Rev. Genet.* <https://doi.org/10.1038/s41576-018-0063-5> (2019).
- Sun, J., Shi, Y. & Yildirim, E. The nuclear pore complex in cell type-specific chromatin structure and gene regulation. *Trends Genet.* <https://doi.org/10.1016/j.tig.2019.05.006> (2019).
- Denning, D. P., Patel, S. S., Uversky, V., Fink, A. L. & Rexach, M. Disorder in the nuclear pore complex: the FG repeat regions of nucleoporins are natively unfolded. *Proc. Natl Acad. Sci. USA* **100**, 2450–2455 (2003).
- Pyhtila, B. & Rexach, M. A gradient of affinity for the karyopherin Kap95p along the yeast nuclear pore complex. *J. Biol. Chem.* **278**, 42699–42709 (2003).
- Strawn, L. A., Shen, T., Shulga, N., Goldfarb, D. S. & Wenthe, S. R. Minimal nuclear pore complexes define FG repeat domains essential for transport. *Nat. Cell Biol.* **6**, 197–206 (2004).
- Mészáros, N. et al. Nuclear pore basket proteins are tethered to the nuclear envelope and can regulate membrane curvature. *Dev. Cell* **33**, 285–298 (2015).
- Bensidoun, P., Reiter, T., Montpetit, B., Zenklusen, D. & Oeffinger, M. Nuclear mRNA metabolism drives selective basket assembly on a subset of nuclear pore complexes in budding yeast. *Mol. Cell* **82**, 3856–3871.e6 (2022).
- D'Angelo, M. A., Gomez-Cavazos, J. S., Mei, A., Lackner, D. H. & Hetzer, M. W. A change in nuclear pore complex composition regulates cell differentiation. *Dev. Cell* **22**, 446–458 (2012).
- Akey, C. W. et al. Implications of a multiscale structure of the yeast nuclear pore complex. *Mol. Cell* **83**, 3283–3302.e5 (2023).
- Akey, C. W. et al. Comprehensive structure and functional adaptations of the yeast nuclear pore complex. *Cell* **185**, 361–378.e25 (2022).
- Daigle, N. et al. Nuclear pore complexes form immobile networks and have a very low turnover in live mammalian cells. *J. Cell Biol.* **154**, 71–84 (2001).
- Rabut, G., Doye, V. & Ellenberg, J. Mapping the dynamic organization of the nuclear pore complex inside single living cells. *Nat. Cell Biol.* **6**, 1114–1121 (2004).
- D'Angelo, M. A., Raices, M., Panowski, S. H. & Hetzer, M. W. Age-dependent deterioration of nuclear pore complexes causes a loss of nuclear integrity in postmitotic cells. *Cell* **136**, 284–295 (2009).
- Hakhverdyan, Z. et al. Dissecting the structural dynamics of the nuclear pore complex. *Mol. Cell* **81**, 153–165.e7 (2021).
- Bley, C. J. et al. Architecture of the cytoplasmic face of the nuclear pore. *Science* **376**, eabm9129 (2022).
- Fontana, P. et al. Structure of cytoplasmic ring of nuclear pore complex by integrative cryo-EM and AlphaFold. *Science* **376**, eabm9326 (2022).
- Zhu, X. et al. Structure of the cytoplasmic ring of the *Xenopus laevis* nuclear pore complex. *Science* **376**, eabl8280 (2022).
- Petrovic, S. et al. Architecture of the linker-scaffold in the nuclear pore. *Science* **376**, eabm9798 (2022).

33. Mosalaganti, S. et al. AI-based structure prediction empowers integrative structural analysis of human nuclear pores. *Science* **376**, eabm9506 (2022).
34. Allegretti, M. et al. In-cell architecture of the nuclear pore and snapshots of its turnover. *Nature* **586**, 796–800 (2020).
35. Vollmer, B. et al. Nup153 recruits the Nup107-160 complex to the inner nuclear membrane for interphasic nuclear pore complex assembly. *Dev. Cell* **33**, 717–728 (2015).
36. Niño, C. A. et al. Posttranslational marks control architectural and functional plasticity of the nuclear pore complex basket. *J. Cell Biol.* **212**, 167–180 (2016).
37. Gunkel, P., Iino, H., Krull, S. & Cordes, V. C. An evolutionarily conserved bimodular domain anchors ZC3HC1 and its yeast homologue Pml39p to the nuclear basket. *Mol. Biol. Cell* **34**, ar40 (2023).
38. Palancade, B. B., Zuccolo, M., Loeillet, S., Nicolas, A. & Doye, V. Pml39, a novel protein of the nuclear periphery required for nuclear retention of improper messenger ribonucleoproteins. *Mol. Biol. Cell* **16**, 5258–5268 (2005).
39. Cordes, V. C., Reidenbach, S., Rackwitz, H. R. & Franke, W. W. Identification of protein p270/Tpr as a constitutive component of the nuclear pore complex-attached intranuclear filaments. *J. Cell Biol.* **136**, 515–529 (1997).
40. Cibulka, J., Bisaccia, F., Radisavljević, K., Carrillo, R. M. G. & Köhler, A. Assembly principle of a membrane-anchored nuclear pore basket scaffold. *Sci. Adv.* <https://doi.org/10.1126/sciadv.abl6863> (2022).
41. Alber, F. et al. The molecular architecture of the nuclear pore complex. *Nature* **450**, 695–701 (2007).
42. Mi, L., Goryaynov, A., Lindquist, A., Rexach, M. & Yang, W. Quantifying nucleoporin stoichiometry inside single nuclear pore complexes in vivo. *Sci. Rep.* **5**, 9372 (2015).
43. Kim, S. J. et al. Integrative structure and functional anatomy of a nuclear pore complex. *Nature* **555**, 475–482 (2018).
44. Rajoo, S., Vallotton, P., Onischenko, E. & Weis, K. Stoichiometry and compositional plasticity of the yeast nuclear pore complex revealed by quantitative fluorescence microscopy. *Proc. Natl Acad. Sci. USA* **115**, E3969–E3977 (2018).
45. Seo, H.-S. et al. Structural and functional analysis of Nup120 suggests ring formation of the Nup84 complex. *Proc. Natl Acad. Sci. USA* <https://doi.org/10.1073/pnas.0907453106> (2009).
46. Stuwe, T. et al. Architecture of the nuclear pore complex coat. *Science* <https://doi.org/10.1126/science.aaa4136> (2015).
47. von Appen, A. et al. In situ structural analysis of the human nuclear pore complex. *Nature* **526**, 140–143 (2015).
48. Fernandez-Martinez, J. et al. Structure-function mapping of a heptameric module in the nuclear pore complex. *J. Cell Biol.* **196**, 419–434 (2012).
49. Kelley, K., Knockenhauer, K. E., Kabachinski, G. & Schwartz, T. U. Atomic structure of the Y complex of the nuclear pore. *Nat. Struct. Mol. Biol.* <https://doi.org/10.1038/nsmb.2998> (2015).
50. Lutzmann, M., Kunze, R., Buerer, A., Aebi, U. & Hurt, E. Modular self-assembly of a Y-shaped multiprotein complex from seven nucleoporins. *EMBO J.* <https://doi.org/10.1093/emboj/21.3.387> (2002).
51. Fernandez-Martinez, J. et al. Structural characterization by cross-linking reveals the detailed architecture of a coatmer-related heptameric module from the nuclear pore complex. *Mol. Cell. Proteomics* <https://doi.org/10.1074/mcp.m114.041673> (2014).
52. Kampmann, M. & Blobel, G. Three-dimensional structure and flexibility of a membrane-coating module of the nuclear pore complex. *Nat. Struct. Mol. Biol.* <https://doi.org/10.1038/nsmb.1618> (2009).
53. Drin, G. et al. A general amphipathic α -helical motif for sensing membrane curvature. *Nat. Struct. Mol. Biol.* **14**, 138–146 (2007).
54. Brohawn, S. G., Leksa, N. C., Spear, E. D., Rajashankar, K. R. & Schwartz, T. U. Structural evidence for common ancestry of the nuclear pore complex and vesicle coats. *Science* <https://doi.org/10.1126/science.1165886> (2008).
55. Hase, M. E., Kuznetsov, N. V. & Cordes, V. C. Amino acid substitutions of coiled-coil protein Tpr abrogate anchorage to the nuclear pore complex but not parallel, in-register homodimerization. *Mol. Biol. Cell* <https://doi.org/10.1091/mbc.12.8.2433> (2001).
56. Köhler, A. & Hurt, E. Exporting RNA from the nucleus to the cytoplasm. *Nat. Rev. Mol. Cell Biol.* **8**, 761–773 (2007).
57. Hase, M. E. & Cordes, V. C. Direct interaction with Nup153 mediates binding of Tpr to the periphery of the nuclear pore complex. *Mol. Biol. Cell* **14**, 1923–1940 (2003).
58. Kiseleva, E., Goldberg, M. W., Allen, T. D. & Akey, C. W. Active nuclear pore complexes in *Chironomus*: visualization of transporter configurations related to mRNP export. *J. Cell Sci.* **111**, 223–236 (1998).
59. Nordeen, S. A. et al. A nanobody suite for yeast scaffold nucleoporins provides details of the nuclear pore complex structure. *Nat. Commun.* **11**, 6179 (2020).
60. Stankunas, E. & Köhler, A. The interplay of nuclear pores and lipids. *Curr. Opin. Cell Biol.* <https://doi.org/10.1016/j.ceb.2023.102251> (2023).
61. Romanauska, A. & Köhler, A. Lipid saturation controls nuclear envelope function. *Nat. Cell Biol.* **25**, 1290–1302 (2023).
62. Jarnik, M. & Aebi, U. Toward a more complete 3-D structure of the nuclear pore complex. *J. Struct. Biol.* **107**, 291–308 (1991).
63. Zimmerli, C. E. et al. Nuclear pores dilate and constrict in cellulo. *Science* **374**, eabd9776 (2021).
64. Onischenko, E. et al. Maturation kinetics of a multiprotein complex revealed by metabolic labeling. *Cell* **183**, 1785–1800.e26 (2020).
65. Rout, M. P. et al. The yeast nuclear pore complex. *J. Cell Biol.* <https://doi.org/10.1083/jcb.148.4.635> (2000).
66. Nordeen, S. A., Turman, D. L. & Schwartz, T. U. Yeast Nup84–Nup133 complex structure details flexibility and reveals conservation of the membrane anchoring ALPS motif. *Nat. Commun.* **11**, 6060 (2020).

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Methods

The study complies with all relevant ethical regulations.

The AlphaFold-Multimer screen for protein–protein interactions

The AlphaFold-Multimer deep learning model was used to predict the structures of all possible binary protein complexes between *S. cerevisiae* Nup60 SLIMs and all other Nups (in total, 264 unique pairwise combinations). Nup60 query SLIMs were defined as stretches of 31 consecutive amino acid residues containing at least 10 highly conserved residues across yeast orthologues. The computational results were obtained on the Cloud Infrastructure Platform high-performance computing cluster. For every prediction, the mean ipTM score of models ranked one to five (confidence estimate of protein–protein interface) was calculated and plotted in the heatmap using Python data visualization library seaborn (v0.11.2). AlphaFold3 (ref. 67) models of Nup60(260–318)•Nup85•Seh1, Nup60(260–318)•dimeric Mlp1(390–620) and Nup85(101–430)•Nup60(260–318)•dimeric Mlp1(450–580) recapitulated the positioning of polypeptide chains predicted by AlphaFold-Multimer.

Reagents, plasmids and yeast strains

Reagents, plasmids and yeast strains used in this study are listed in Supplementary Tables 1–3, respectively. All plasmids were verified by either Sanger sequencing (PCR-based molecular cloning approaches) or diagnostic restriction digest (subcloning). Yeast genes were chromosomally tagged by a standard one-step PCR-based technique⁶⁸. Microbiological techniques followed standard procedures.

Recombinant protein expression in *E. coli*

Recombinant proteins were expressed in *E. coli* BL21 CodonPlus (DE3) RIL cells. *E. coli* cultures were grown in lysogeny broth (Miller) medium supplemented with expression vector-compatible antibiotics (140 r.p.m., 37 °C) to optical density at 600 nm of 0.30–0.40, transferred to 130 r.p.m. 23 °C, induced with 0.5 mM IPTG (final concentration) at optical density at 600 nm of 0.70–0.80 for 3 h before harvesting. Pellets were frozen in liquid nitrogen and stored at –20 °C.

Expression of MBP-3C-Mlp1(17–1,137)-His₆ in insect cells

MBP-3C-Mlp1(17–1,137)-His₆ was produced using baculovirus expression vector system. To generate V0 baculovirus stock, EMBAcY bacmid harbouring the open reading frame of MBP-3C-Mlp1(17–1,137)-His₆ was transfected into Sf9 insect cells (Expression Systems). The recombinant fusion protein was expressed in Hi5 insect cells (Expression Systems) at 27 °C. The insect biomass was collected 3 days after proliferation arrest and flash frozen in liquid nitrogen.

Protein purification

Protein purification procedures, including affinity resins used and buffer solution compositions, are summarized in Supplementary Table 4. All steps were performed at 4 °C using pre-chilled buffers and containers. Briefly, frozen pellets of biomass were thawed, resuspended in lysis buffer and lysed using either Avestin Emulsiflex C3 high-pressure homogenizer (for *E. coli*/insect pellets) or Fritsch Pulverisette 6 planetary ball mill (for *S. cerevisiae* pellets). The lysate was cleared by high-speed centrifugation (at 48,000g for 20 min). Cleared lysate was incubated with corresponding beads for 2 h at 4 °C on tube rotator. After incubation, beads were collected by centrifugation and batch washed in 2 ml microcentrifuge tubes using wash buffer (either 3× or 5× 1 ml, for 1 min each with 30 s at 500g centrifugation steps between washes). Beads were then transferred to 1 ml Mobicol columns (MoBiTec) and eluted by adding elution buffer and incubating for 30–90 min at 4 °C. All protein solutions were cleared of aggregates by high-speed centrifugation (10 min at 16,000g) before downstream use. Protein concentration was measured using Bio-Rad protein assay

based on the Bradford dye-binding method using bovine serum albumin (20 mg ml^{–1}, NEB) solutions for calibration.

Nup60 oligomerization assay

NusA-His₆-Nup60(48–162) and SUMO-Nup60(48–162)-FLAG as well as binary combinations with solubility/affinity tags were co-expressed in *E. coli* BL21 CodonPlus (DE3) RIL cells following the procedure described above in ‘Recombinant protein expression in *E. coli*’ section. Pellets of biomass were then subjected to two-step affinity purification as summarized in Supplementary Table 4 and briefly described above in ‘Protein purification’ section.

ITC of Nup60 HR

NusA-His₆-Nup60(48–162) and NusA-His₆ (negative control) were purified as specified in Supplementary Table 4. After 16 h dialysis in HIS-PB (300 mM NaCl, 50 mM Tris pH 7.5, 50 mM imidazole and 1.5 mM MgCl₂) samples were diluted to final concentration of 170 μM and measured in dilution (dissociation) ITC setup on MicroCal PEAQ-ITC calorimeter (Malvern). Baseline correction, thermogram integration and fitting of simple dimer dissociation model was performed using MicroCal PEAQ-ITC Analysis Software.

Size exclusion chromatography coupled with multi-angle light scattering of Nup60 HR

NusA-His₆-Nup60(48–162) and NusA-His₆ (negative control) were purified as specified in Supplementary Table 4. After 16 h dialysis in HIS-PB (300 mM NaCl, 50 mM Tris pH 7.5, 50 mM imidazole, 1.5 mM MgCl₂) samples were diluted to a final concentration in range of 100–200 μM and loaded through a 50 μl injection loop. The samples were analysed at constant flow rate of 0.5 ml min^{–1} on Superdex 200 Increase 10/300 GL column (GE Healthcare) connected to ÅKTA pure 25 chromatography system coupled with miniDAWN MALS detector (Wyatt Technology). The analysis was performed using ASTRA 8.2.0 software package (Wyatt Technology).

In vitro binding assays

In every binding assay setup, purified GST-Nup60 fusion proteins were used as baits (GST alone as negative control). Binding assay reactions contained 25 μl of Glutathione Sepharose 4B (Cytiva) beads (50 μl of 1:1 bead slurry), 0.25–0.75 nmol (depending on the experiment) of either GST or GST-Nup60 fusion proteins and 1–1.3× molar excess of prey proteins (Y-complex, Nup85•Seh1 or Mlp1N60BD) at the final volume of 100–250 μl. Binding reactions with the Y-complex were set using PB-100 buffer solution (100 mM NaCl, 50 mM Tris pH 7.5, 1.5 mM MgCl₂ and 0.5 mM dithiothreitol (DTT)), whereas binding reactions with either Nup85•Seh1 or Mlp1N60BD were set using PB-200 buffer solution (200 mM NaCl, 50 mM Tris pH 7.5, 1.5 mM MgCl₂ and 0.5 mM DTT). After 1 h incubation at 4 °C on tube rotator, beads were batch washed with either PB-100 (Y-complex, 2 × 1 ml for 1 min each) or PB-200 (Nup85•Seh1 or Mlp1N60BD, 5 × 1 ml for 2 min each) buffer solutions. Proteins were eluted by adding 50 μl GSH-EB (100 mM NaCl, 50 mM Tris pH 7.5, 1.5 mM MgCl₂, 0.5 mM DTT and 20 mM reduced GSH) and incubated for 30 min at 4 °C on tube rotator. Native eluates were mixed with 4× sample buffer (SB), boiled for 5 min at 95 °C and analysed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) and Coomassie staining.

Chemical cross-linking of reconstituted protein complexes

Reconstituted complexes were prepared as described above in ‘In vitro binding assays’ section using HEPES-containing elution buffer instead of Tris. Protein complexes were chemically cross-linked with either isotopically coded disuccinimidyl suberate (1:1 molar mix of DSS-H12/D12, Creative Molecules) in case of the Y-complex or isotopically unlabelled DSS (Thermo Scientific) in case of Nup85•Seh1 and Mlp1N60BD for 30 min at 30 °C at 1,000 r.p.m. on a thermomixer. Reaction was

quenched by adding 100 mM ammonium bicarbonate (final concentration) and incubating for 10 min at 30 °C at 1,000 r.p.m. Cross-linked samples were mixed with 4× SB, boiled for 5 min at 95 °C and analysed by SDS–PAGE and Coomassie staining.

Sample preparation for MS analysis

The Coomassie-stained gel band was destained with a mixture of acetonitrile (VWR) and 50 mM ammonium bicarbonate (Sigma). The proteins were reduced using 10 mM dithiothreitol (Roche) and alkylated with 50 mM iodoacetamide (Sigma). Trypsin (Promega; Trypsin Gold, Mass Spectrometry Grade) was used for proteolytic cleavage. Digestion was carried out at 37 °C overnight. Formic acid (Fisher chemical) was used to stop the digestion and peptides were extracted using a mixture of acetonitrile and formic acid. Extracts were speedvaccated until dryness and resuspended in 0.1% trifluoroacetic acid (Thermo Scientific) 2% acetonitrile.

Liquid chromatography MS analysis

Peptides were separated on an ultimate 3000 nano-flow chromatography system (Thermo Fisher), using a pre-column for sample loading (Acclaim PepMap C18, 2 cm × 0.1 mm, 5 µm, Thermo Fisher) and a C18 analytical column (Acclaim PepMap C18, 50 cm × 0.75 mm, 2 µm, Thermo Fisher), applying a segmented linear gradient from 2% to 35% and finally 80% solvent B (80% acetonitrile, 0.1% formic acid; solvent A 0.1% formic acid) at a flow rate of 230 nl min⁻¹ over 120 min.

Eluting peptides were analysed on an Exploris 480 Orbitrap mass spectrometer (Thermo Fisher) coupled with a high-field asymmetric waveform ion mobility spectrometry Pro ion-source (Thermo Fisher) using coated emitter tips (PepSep, MSWil) with the following settings: The mass spectrometer was operated in data-dependent acquisition mode with the compensation voltage set to -40, -55 and -70. The cycle time was set to 1 s. The survey scans were obtained in a mass range of 375–1600 *m/z*, at a resolution of 120,000 at 200 *m/z*, and a normalized AGC target at 100%. The selected ions were isolated with a width of 1.2 *m/z*, fragmented in the HCD cell at 27%, 30% and 33% collision energy, and the spectra recorded for maximum 200 ms at a normalized AGC target of 200% and a resolution of 30,000. Peptides with a charge of +3 to +8 were included for fragmentation, the peptide match feature was set to preferred, the exclude isotope feature was enabled and selected precursors were dynamically excluded from repeated sampling for 30 s.

Data analysis of MS data

Raw data were converted per compensation voltage into raw files in Freestyle and searched using the MaxQuant software package (2.0.3.0)⁶⁹ against the target sequences, the UniProt *E.coli* and yeast reference proteome (www.uniprot.org), as well as a database of most common contaminants. The search was performed with standard identification settings, including full trypsin specificity allowing a maximum of two missed cleavages. Carbamidomethylation of cysteine residues was set as fixed, and oxidation of methionine and acetylation of protein N-termini were set as variable modifications. All other settings were left at default. Results were filtered at a false discovery rate of 1% at protein and peptide spectrum match (PSM) level.

pLink search

To identify cross-linked peptides, the spectra were searched using pLink software (version 2.3.9)⁷⁰ against the sequences of the top ten non-contaminant proteins from the MaxQuant search sorted by intensity-based absolute quantitation values. DSS and DSS-D12 were selected as the cross-linking chemistry. Carbamidomethyl on cysteine was set as fixed, oxidation of methionine and protein N-terminal acetylation as variable modifications. Enzyme specificity was selected according to the protease used for digestion. Search results were filtered for 1% false discovery rate on the PSM level limiting the precursor mass

deviation to 10 ppm. To remove low-quality PSM an additional *e*-value cut-off of <0.001 was applied. Cross-link maps were generated in xiNET⁷¹.

XiSearch search

To identify cross-linked peptides, the spectra were searched using XiSearch software (1.7.6.7)⁷² against the sequences of the top ten non-contaminant proteins from the MaxQuant search sorted by intensity-based absolute quantitation. DSS and DSS-D12 were selected as the cross-linking chemistry. Carbamidomethyl on cysteine was set as fixed, and oxidation of methionine and protein N-terminal acetylation were set as variable modifications. Enzyme specificity was selected according to the protease used for digestion. Search results were filtered in XiFDR with 5% false discovery rate for residue pairs limiting the precursor mass deviation to 15 ppm. Cross-link maps were generated in xiVIEW (<http://xiview.org>).

Reconstitution of the basket–NPC core junction

VHH-SAN8 nanobody (VHH-SAN8-SNAP-FLAG) specific for Nup84 subunit of the Y-complex⁵⁹ was purified as specified in Supplementary Table 4. Nanobody was immobilized on beads by incubating 25 µg of purified VHH-SAN8 nanobody with 250 µl of anti-FLAG beads (Anti-FLAG M2 affinity gel, Cytiva) in a volume of 600 µl for 1 h at 4 °C on a tube rotator. To wash away a free nanobody, slurry was batch washed with PB (100 mM NaCl, 50 mM Tris pH 7.5, 1.5 mM MgCl₂ and 0.5 mM DTT) (3 × 1 ml for 1 min each). After final wash, beads were resuspended in 1,000 µl and aliquoted 100 µl per reaction (each aliquot contained ~25 µl of nanobody-coated beads). Purified Y-complex (Nup133-SNAP-CBP) and equimolar amounts of either GST or Nup60(200–318)-StrepII (that is, a mix of Nup60(200–318)-StrepII and GST, generated by TEV protease cleavage of GST-TEV-Nup60(200–318)-StrepII) were then added to the beads and incubated for 1 h at 4 °C on a tube rotator. Purified Mlp1 N60BD was then added and incubated for 30 min more at 4 °C on a tube rotator. After incubation, beads were batch washed with PB (3 × 1 ml for 1 min each) and eluted with 50 µl of FEB (100 mM NaCl, 50 mM Tris pH 7.5, 1.5 mM MgCl₂ and 0.5 mg ml⁻¹ FLAG peptide). Eluates were mixed with 4 × SB, boiled for 5 min at 95 °C and analysed by SDS–PAGE.

Rotary shadowing EM of Mlp1 samples

Protein samples were first diluted to a concentration of ~0.1 mg ml⁻¹ in maltose elution buffer containing 100 mM NaCl, 50 mM Tris (pH 7.5), 1.5 mM MgCl₂, 0.5 mM DTT and 30 mM maltose. Before freezing, samples were diluted 1:1 in Mabuchi spraying buffer, containing 200 mM ammonium acetate and 60% (v/v) glycerol, with the pH adjusted to 7.4. To obtain a clean surface, mica chips (quality V3; Agar Scientific) were freshly cleaved; the diluted samples were sprayed onto them, and immediately transferred into a BAL-TEC MED020 high vacuum evaporator (BAL-TEC, Liechtenstein), equipped with electron guns. To ensure a uniform coating from all sides, the mica chips were placed on a rotating table. After reaching a vacuum of 2 × 10⁻⁵ mbar, the samples were coated with 0.7 nm platinum (BALTIC) at an angle of 4–5°, followed by 7 nm carbon (Balzers) at 90°. The obtained replicas were floated off from the mica chips, picked up on 400 mesh Cu/Pd grids (Agar Scientific) and inspected in an FEI T20 G2 transmission electron microscope operated at 200 kV. Images were acquired using an FEI Eagle 4k charge-coupled device camera (both TEM and camera: formerly FEI, now Thermo Fisher Scientific). Additionally, grids were inspected in an FEI Morgagni 268D TEM (formerly FEI, now Thermo Fisher Scientific) operated at a high tension of 80 kV. Digital images were acquired using a Megaview III CCD camera (Olympus-SIS).

Lipids

1-Palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC), sodium salt of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine (POPS), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE),

1-2-dioleoyl-*sn*-glycerol (DAG) and brain ammonium salt of L- α -phosphatidylinositol-4,5-bisphosphate (PIP₂) were purchased from Avanti Polar Lipids. The lipid stock solutions were stored in glass containers layered with argon at -80°C . Lipid-containing solutions were handled using glass microliter syringes (Hamilton).

GUV binding assay and image analysis

GUVs were prepared by PVA-assisted method⁷³. A mixture of lipids corresponding to 45 mol% POPE, 30 mol% POPS, 20 mol% POPC, 4 mol% PIP₂ and 1 mol% DAG was used for the ER-like lipid composition. GUVs were pre-incubated with purified proteins (at final concentrations of 100 nM) for 10 min before imaging with a 60×1.42 NA Plan-Apochromat oil-immersion objective using DeltaVision Elite microscope (GE Healthcare) equipped with a cooled CCD camera (CoolSNAP HQ2; Photometrics). All images were taken within an imaging window of 10 min. The imaging system was controlled with softWoRx 6.5.2 software. In-built image restoration by deconvolution (ratio conservative method) was applied for all images collected. Background-corrected fluorescence intensity along the rim of the GUVs was measured manually in FIJI. Linear regression models were fitted using the least squares method.

Live cell fluorescence microscopy of yeast cells

S. cerevisiae $\Delta nup60 \Delta mlp1$ strain was transformed with pRS315_MLP1p_MLP1-yeGFP_NUP1t and pRS316_NUP60p_NUP60(1-539)-mCherry_NUP1t-derived constructs and selected on solid SDC-LEU-URA medium. For live cell imaging, yeast cells were grown in SDC-LEU-URA medium at 30°C to midlog phase and collected by centrifugation. Cells were mounted on agarose pads⁷⁴ and visualized with a 60×1.42 NA Plan-Apochromat oil-immersion objective using DeltaVision Elite microscope (GE Healthcare) equipped with a cooled CCD camera (CoolSNAP HQ2) (Photometrics). The imaging system was controlled with softWoRx 6.5.2 software. In-built image restoration by deconvolution (ratio conservative method) was applied to all images acquired.

Quantification of live cell fluorescence images

To quantify the degree of protein (either Nup60-mCherry or Mlp1-mGFP) enrichment at the NE compared with the N, nuclear rim regions (bands of $1.29\text{ }\mu\text{m}$ in width, corresponding to 12 pixels) were manually traced and straightened in FIJI. Consistent clockwise tracing ensured that after the straightening the nuclear and cytoplasmic sides of the nuclear envelope were consistently oriented across all selections. Only cells expressing both Nup60-mCherry and Mlp1-mGFP were included in the analysis. For each cell, the straightened selection of the nuclear rim was analysed in a row-wise fashion, that is, for each row of pixels the nuclear intensity $I(\text{N})$ and NE intensity $I(\text{NE})$ values were extracted. $I(\text{N})$ was defined as the intensity of a pixel on the nuclear side, which is distalmost from the NE in the given selection (on average, $\sim 600\text{ nm}$ away from the peak fluorescence intensity corresponding to the NE). $I(\text{NE})$ was defined as the maximum intensity value in the row of pixels. The enrichment factor $I(\text{NE})/I(\text{N})$ was defined as the ratio between median $I(\text{NE})$ and $I(\text{N})$ for each cell.

To quantify the nuclear puncta of Mlp1-mGFP, 50 cells from every condition were visually inspected (z-stack) and cells harbouring nuclear foci were counted. Only cells expressing both Nup60-mCherry and Mlp1-mGFP were included in the analysis.

Statistics and reproducibility

No statistical method was used to predetermine sample size. When analysing live cell fluorescence imaging data (Fig. 7a,b), only cells that expressed both the Nup60-mCherry and Mlp1-mGFP plasmids were included (that is, cells expressing only one plasmid due to plasmid loss were excluded). To characterize the effect of Nup60 mutations on the localization of Mlp1, it was necessary for both proteins to be

present in the cells. This exclusion criterion was determined before the experiment. To control for any time-dependent effects during in vitro binding assays, binding experiments were repeated with random sample processing order. Blinding was not implemented during experiments and outcome assessments because the same investigator performed and analysed all the experiments. Experiments were performed the following number of times: twice (Figs. 3c–f, 4b,d–g and 5b,d,f and Extended Data Figs. 1i,j, 2d, 3a,b, 5a–c, 7b,c and 9a), three times (Figs. 1d, 2c,e,g, 3b, 6b–e and 7a,b and Extended Data Fig. 2a–c) and six times (Fig. 1c). The detailed summary of multiple comparison tests (two-sided Dunnett's T3 test, $\alpha = 0.05$) for Figs. 6c and 7b is provided in Source data.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository⁷⁵ under accession number [PXD052015](https://doi.org/10.26434/chemrxiv-2024-01484). All other data supporting the findings of this study are available from the corresponding author on reasonable request. Source data are provided with this paper.

References

- Abramson, J. et al. Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature* **630**, 493–500 (2024).
- Janke, C. et al. A versatile toolbox for PCR-based tagging of yeast genes: new fluorescent proteins, more markers and promoter substitution cassettes. *Yeast* **21**, 947–962 (2004).
- Tyanova, S., Temu, T. & Cox, J. The MaxQuant computational platform for mass spectrometry-based shotgun proteomics. *Nat. Protoc.* **11**, 2301–2319 (2016).
- Chen, Z.-L. et al. A high-speed search engine pLink 2 with systematic evaluation for proteome-scale identification of cross-linked peptides. *Nat. Commun.* **10**, 3404 (2019).
- Combe, C. W., Fischer, L. & Rappsilber, J. xiNET: cross-link network maps with residue resolution. *Mol. Cell. Proteomics* **14**, 1137–1147 (2015).
- Mendes, M. L. et al. An integrated workflow for crosslinking mass spectrometry. *Mol. Syst. Biol.* **15**, e8994 (2019).
- Weinberger, A. et al. Gel-assisted formation of giant unilamellar vesicles. *Biophys. J.* **105**, 154–164 (2013).
- Rines, D. R., Thomann, D., Dorn, J. F., Goodwin, P. & Sorger, P. K. Live cell imaging of yeast. *Cold Spring Harb. Protoc.* **6**, pdb.top065482 (2011).
- Perez-Riverol, Y. et al. The PRIDE database resources in 2022: a hub for mass spectrometry-based proteomics evidences. *Nucleic Acids Res.* **50**, D543–D552 (2022).

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Author contributions

A.K. and E.S. conceived the study. E.S. performed experiments. A.K. and E.S. analysed all data and wrote the paper.

Competing interests

The authors declare no competing interests.

Additional information

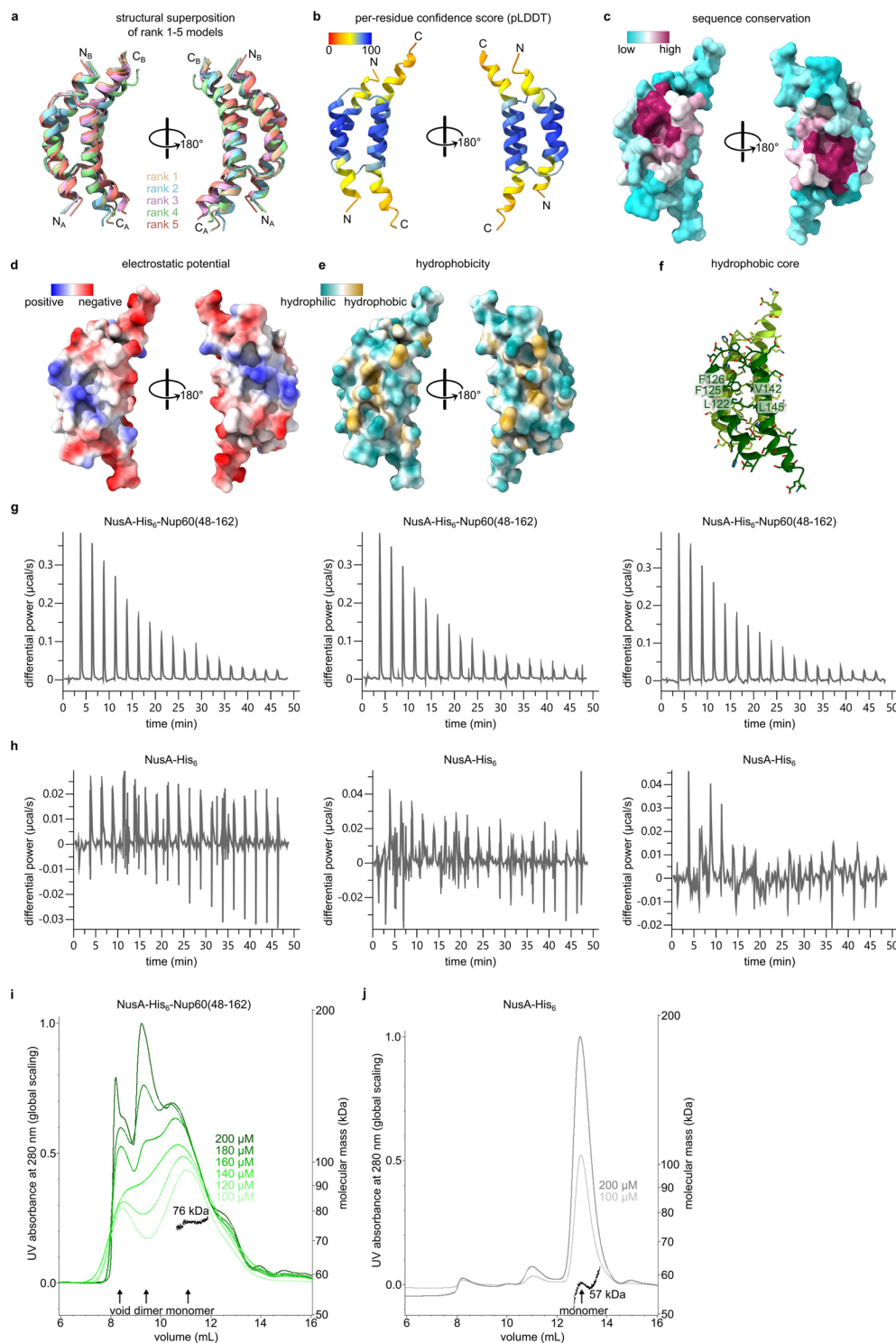
Extended data is available for this paper at <https://doi.org/10.1038/s41556-024-01484-x>.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41556-024-01484-x>.

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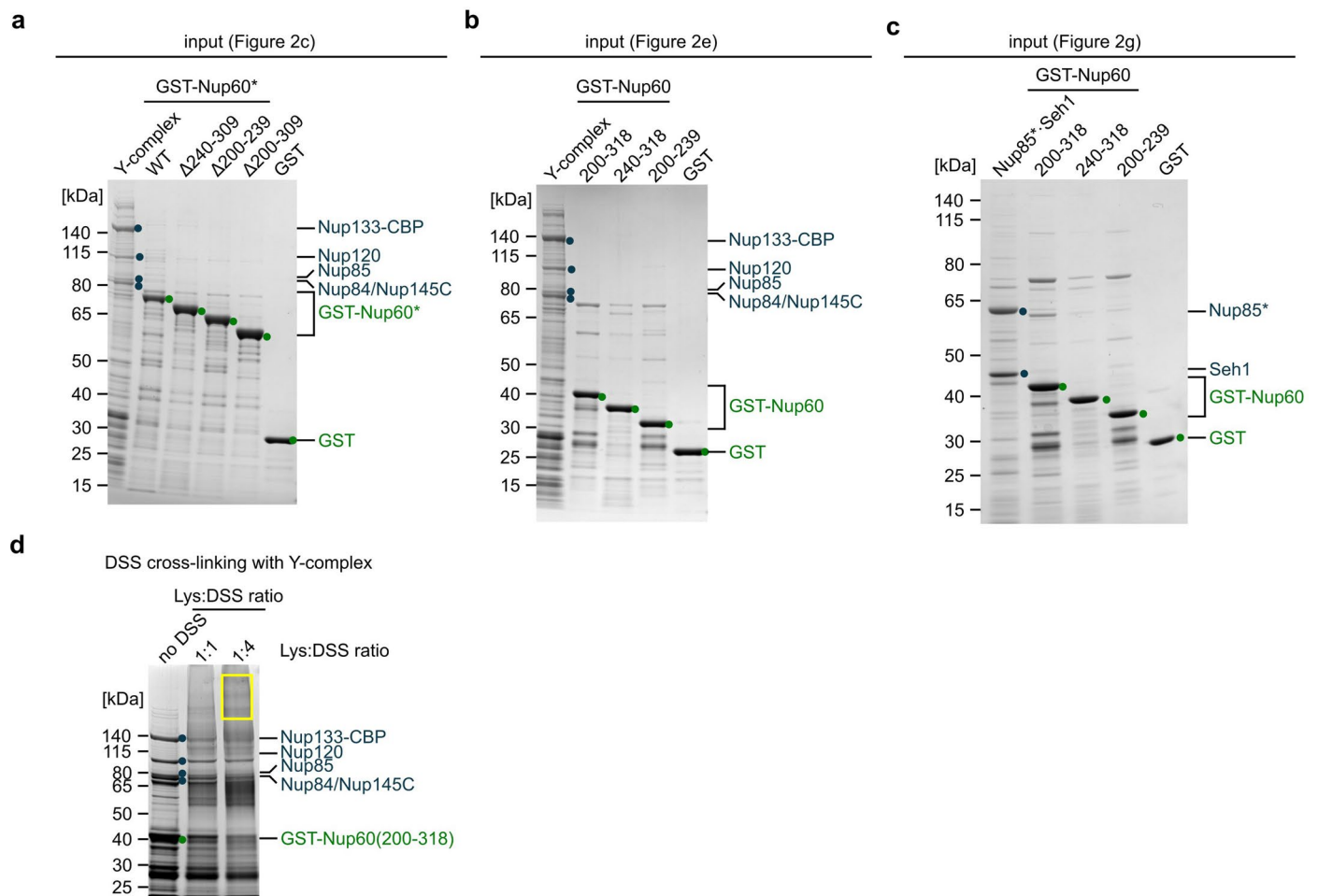
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Extended Data Fig. 1 | AlphaFold-Multimer model of Nup60 HR dimer.

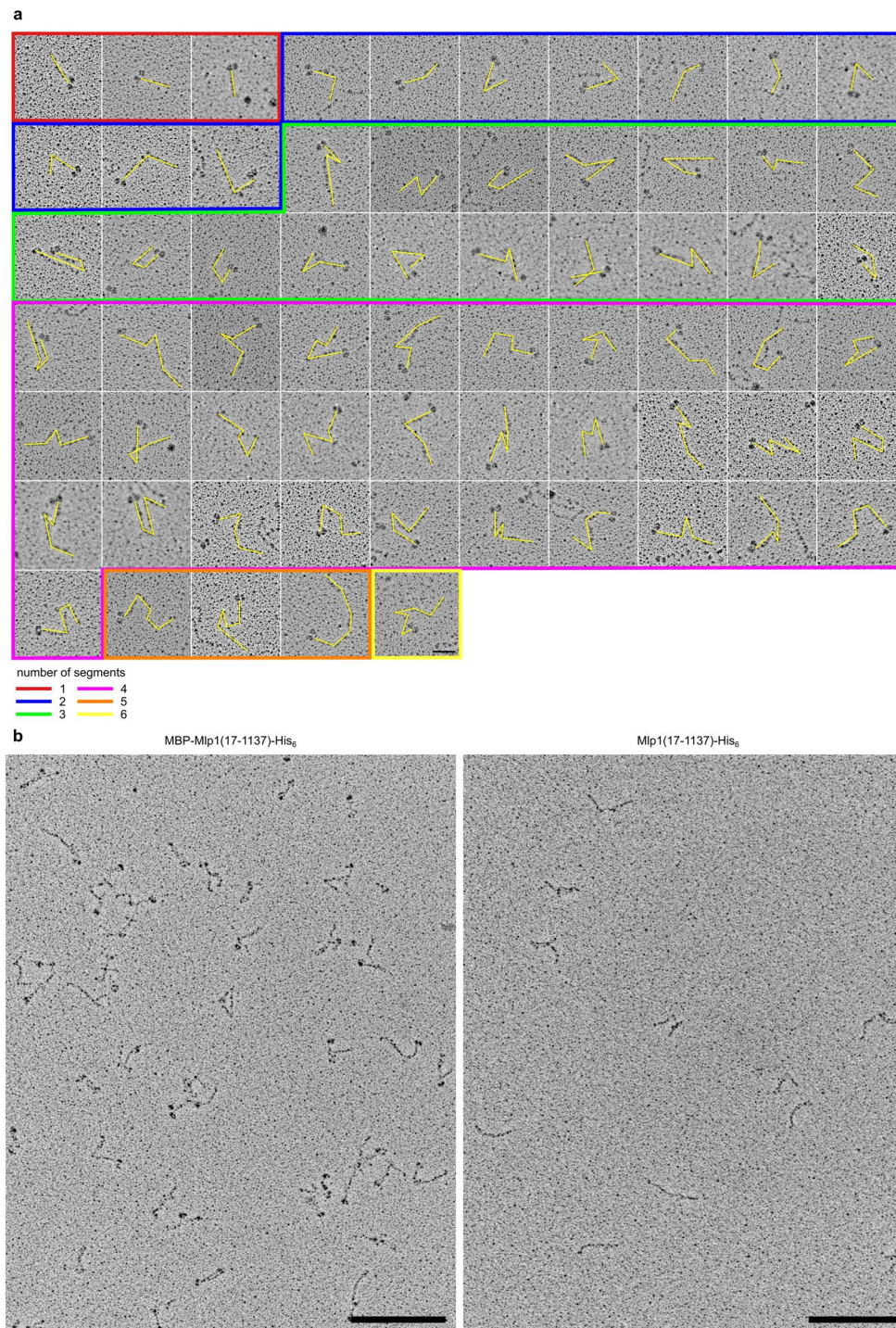
a, Superposition of rank 1-5 AlphaFold-Multimer models of Nup60(110-160) dimer. **b**, AlphaFold-Multimer models of Nup60(110-160) dimer shown in cartoon representation coloured by per-residue confidence score (pLDDT) (blue: high confidence, orange: low confidence). **c**, AlphaFold-Multimer models of Nup60(110-160) dimer shown in surface representation coloured by sequence conservation. **d**, AlphaFold-Multimer models of Nup60(110-160) dimer shown in surface representation coloured by Coulombic electrostatic potential. **e**, AlphaFold-Multimer models of Nup60(110-160) dimer shown in surface representation coloured by hydrophobicity. **f**, AlphaFold-Multimer model of a Nup60(110-160) dimer shown in cartoon (backbone) and ball-and-stick (sidechains) representation. Hydrophobic amino acid residues forming the buried dimeric core are labelled. **g**, Dilution ITC thermograms of

NusA-His₆-Nup60(48-162) sample, corresponding to ITC isotherms provided in Fig. 1d. **h**, Dilution ITC thermograms of NusA-His₆ sample, corresponding to ITC isotherms provided in Fig. 1d. **i**, UV absorbance (280 nm) chromatograms of NusA-His₆-Nup60(48-162) size exclusion chromatography coupled with multi-angle light scattering (SEC-MALS), performed in an injection concentration range of 100-200 μM. Arrows indicate monomer, putative dimer and void volume peaks. The molecular mass of the monomer peak (76 kDa, black trace) was measured at an injection concentration of 100 μM. **j**, UV absorbance (280 nm) chromatograms of NusA-His₆ SEC-MALS runs in an injection concentration range of 100-200 μM. Arrow indicates monomer peak. The molecular mass of the monomer peak (57 kDa, black trace) was measured at an injection concentration of 100 μM. Source numerical data are available in source data.



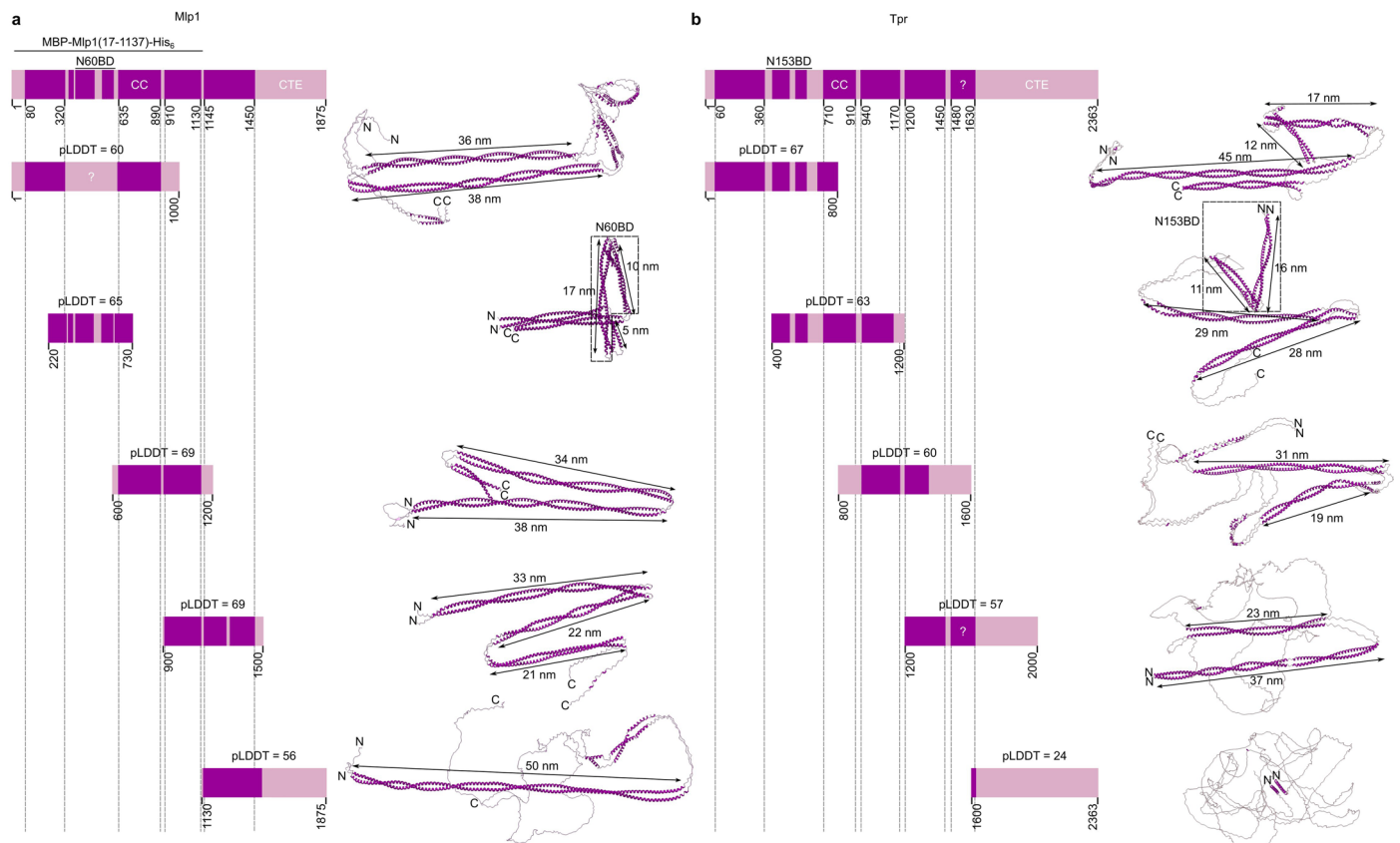
Extended Data Fig. 2 | Input gels for in vitro binding assays. **a**, SDS-PAGE gel showing native eluates of Y-complex and GST-Nup60* constructs used for the in vitro binding assays presented in Fig. 2c. **b**, SDS-PAGE gel showing native eluates of Y-complex and GST-Nup60 constructs used for the in vitro binding assays presented in Fig. 2e. **c**, SDS-PAGE gel showing native eluates of Nup85(1-564)·His₆-Seh1

and GST-Nup60 constructs used for the in vitro binding assays presented in Fig. 2g. **d**, SDS-PAGE gel showing reconstituted complex between Y-complex and GST-Nup60(200-318) (left lane) used as an input for DSS cross-linking (right lanes). Yellow box indicates a band of gel that was submitted for XL-MS analysis. Unprocessed gels are available in source data.



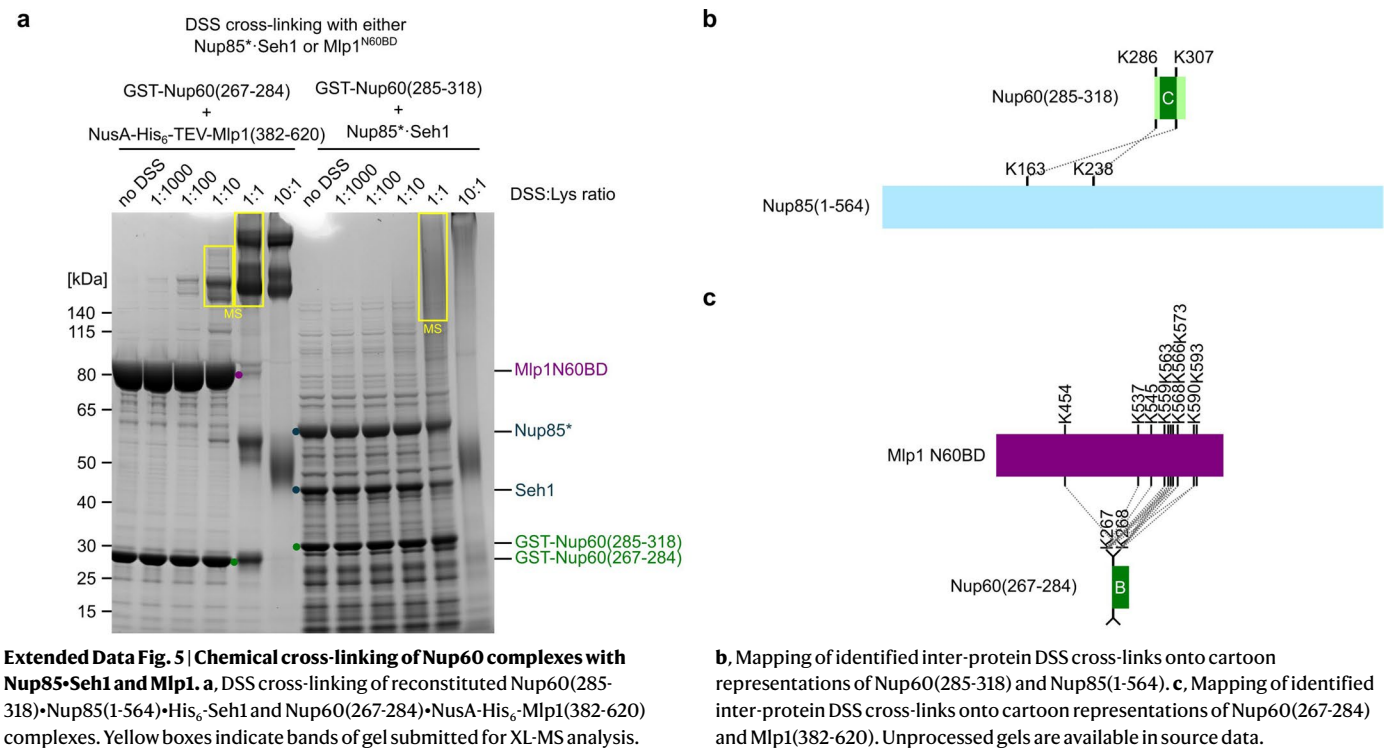
Extended Data Fig. 3 | Gallery of Mlp1 rotary shadowing electron micrographs. a, Selection of MBP-3C-Mlp1(17-1,137)-His₆ particles visible in rotary shadowing electron microscopy images acquired using FEI T20 G2 transmission electron microscope. Particles are manually grouped (boxes of different colours) by the number of visually distinct rectilinear segments

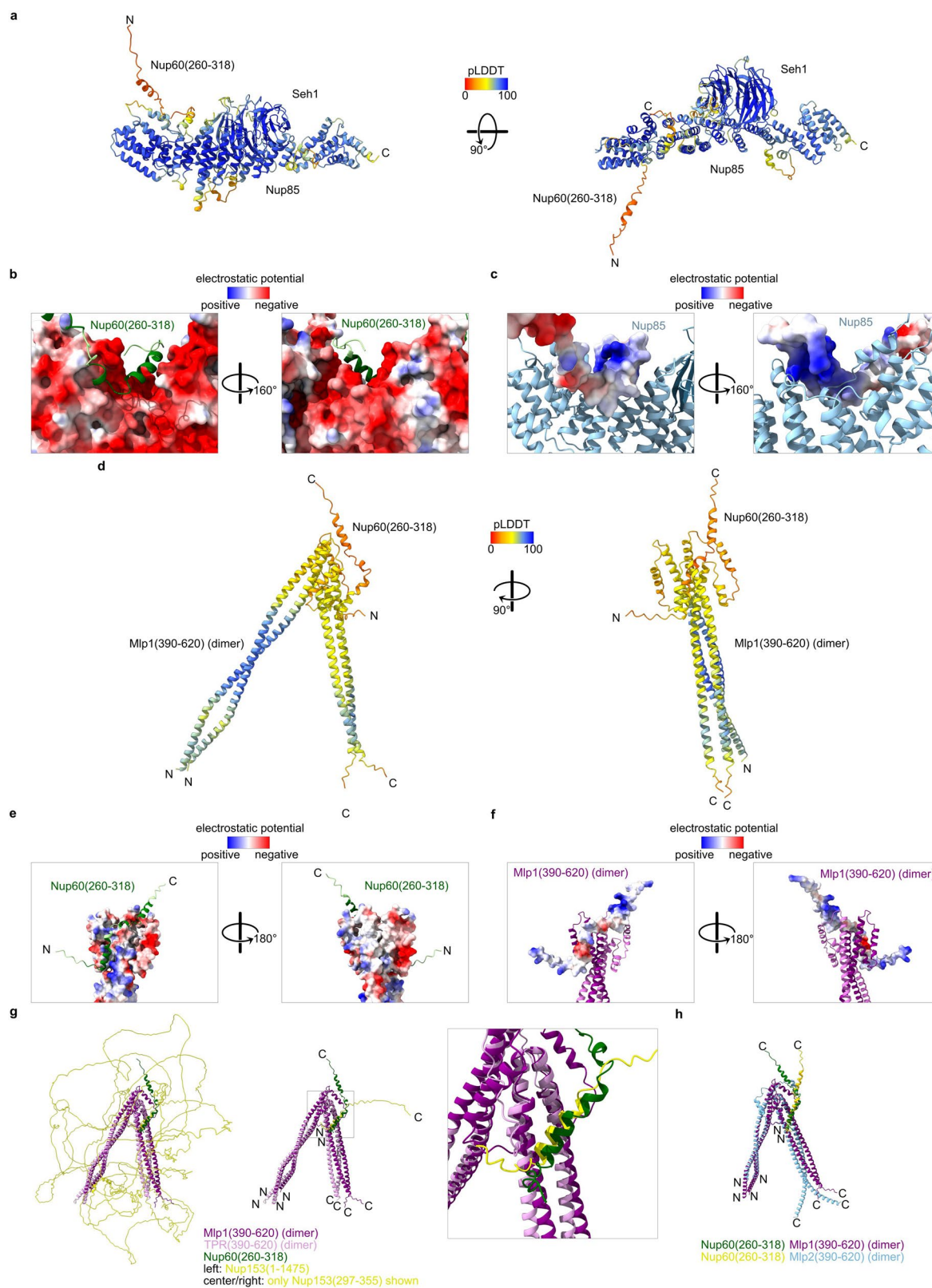
(yellow traces). Scale bar, 40 nm. **b**, Overview images of MBP-3C-Mlp1(17-1,137)-His₆ (uncleaved, left) and Mlp1(17-1,137)-His₆ (cleaved with 3C protease, right) samples. Images were acquired using FEI Morgagni 268D transmission electron microscope. Scale bar, 200 nm.



Extended Data Fig. 4 | Threading-based AlphaFold-Multimer modelling of *S. cerevisiae* Mlp1 and *H. sapiens* Tpr. **a, AlphaFold-Multimer models of Mlp1 dimers with overlapping amino acid residue boundaries (right) and corresponding domain organization cartoons (left). The boxed area (indicated by dashed line) highlights the V-shaped N60BD of Mlp1, which is structurally**

similar to the V-shaped Nup153BD of Tpr in **b**. The AlphaFold-predicted orientation of the remaining coiled-coil segments in relation to these V-shaped domains varies and is likely devoid of structural significance. **b**, AlphaFold-Multimer models of Tpr dimers with overlapping amino acid residue boundaries (right) and corresponding domain organization cartoons (left).

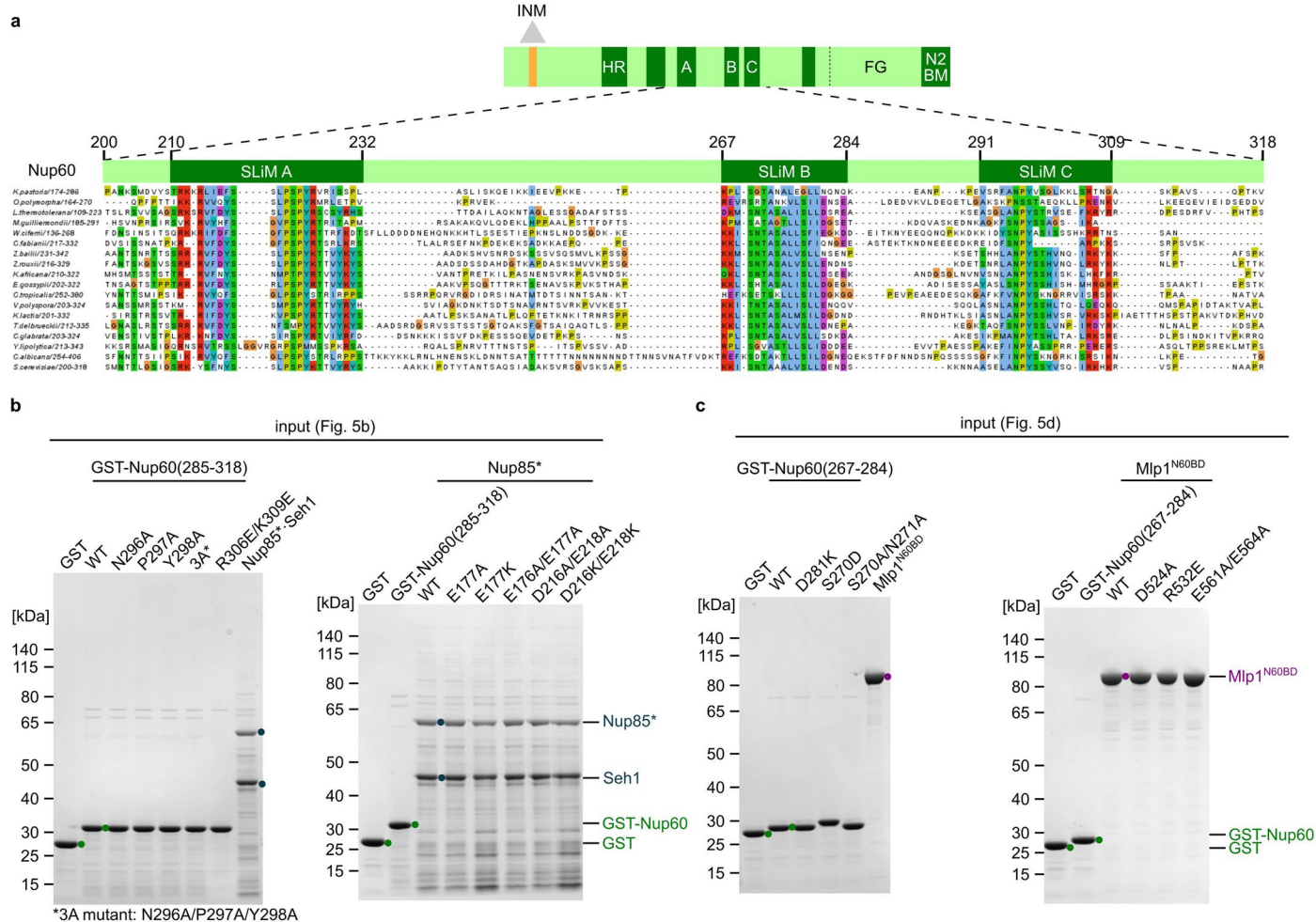




Extended Data Fig. 6 | See next page for caption.

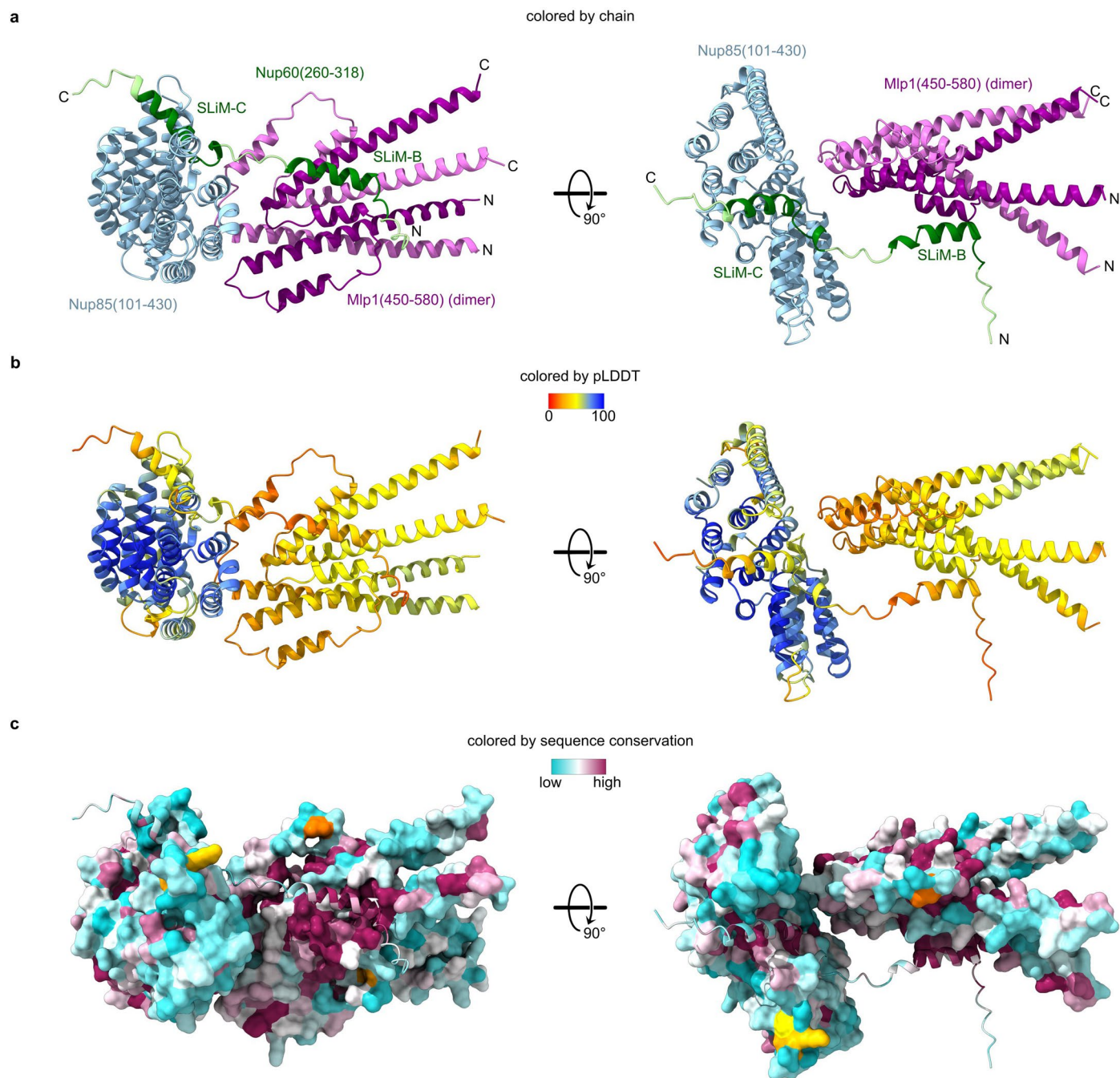
Extended Data Fig. 6 | AlphaFold-Multimer models of Nup60 in complex with Nup85•Seh1 and Mlp1 N60BD. **a**, AlphaFold-Multimer model of Nup60(260-318)•Nup85•Seh1 shown in cartoon representation coloured by per-residue confidence score (pLDDT) (blue: high confidence, orange: low confidence). For clarity, the unstructured N-terminal extension of Nup85 (aa1-46) and internal loop of Seh1 (aa248-291) are not shown. **b**, Predicted interface between Nup60 (cartoon representation, dark green) and Nup85 (surface representation, coloured by Coulombic electrostatic potential). **c**, Predicted interface between Nup60 (surface representation, coloured by Coulombic electrostatic potential) and Nup85 (cartoon representation, light blue). **d**, AlphaFold-Multimer model of Nup60(260-318)•Mlp1(390-620) dimer shown in cartoon representation

coloured by per-residue confidence score (pLDDT) (blue: high confidence, orange: low confidence). **e**, Predicted interface between Nup60 (cartoon representation, dark green) and Mlp1 N60BD dimer (surface representation, coloured by Coulombic electrostatic potential). **f**, Predicted interface between Nup60 (surface representation, coloured by Coulombic electrostatic potential) and Mlp1 N60BD dimer (cartoon representation, magenta). **g**, Superposition of Nup60(260-318)•Mlp1(390-620) dimer and Nup153(1-1475)•Tpr(390-620) dimer AlphaFold-Multimer models. **h**, Superposition of Nup60(260-318)•Mlp1(390-620) dimer and Nup60(260-318)•Mlp2(390-620) dimer AlphaFold-Multimer models suggests a similar interaction mode between Nup60 and Mlp1 and Nup60 and Mlp2.



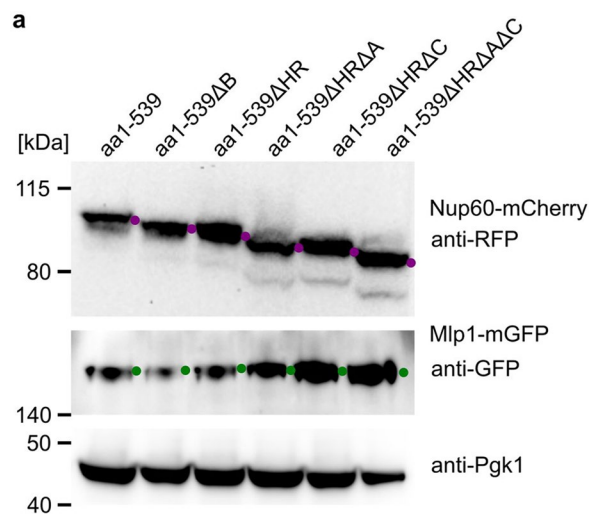
Extended Data Fig. 7 | Input gels for mutational analysis. a, Multiple-sequence alignment (MSA) of Nup60 orthologs across yeast species. Amino acid residue numbering corresponds to *S. cerevisiae* sequence. **b**, SDS-PAGE gel showing native eluates of GST-Nup60(285-318) and Nup85(1-564)•His₆-Seh1 constructs

used for the in vitro binding assays presented in Fig. 5b. **c**, SDS-PAGE gel showing native eluates of GST-Nup60(267-284) and Nup85(1-564)•His₆-Mlp1(382-620) constructs used for the in vitro binding assays presented in Fig. 5d. Unprocessed gels are available in source data.



Extended Data Fig. 8 | AlphaFold-Multimer model of interface between NPC core and nuclear basket. a, AlphaFold-Multimer model of Nup85(101-430)•Nup60(260-318)•Mlp1(450-580) dimer complex shown in cartoon representation and coloured by polypeptide chain. **b,** AlphaFold-Multimer model of Nup85(101-430)•Nup60(260-318)•Mlp1(450-580) dimer complex

shown in cartoon representation and coloured by per-residue confidence score (pLDDT) (blue: high confidence, orange: low confidence). **c,** AlphaFold-Multimer model of Nup85(101-430)•Nup60(260-318)•Mlp1(450-580) dimer complex shown in surface representation and coloured by sequence conservation.



Extended Data Fig. 9 | Immunoblot analysis of Nup60 and Mlp1 expression levels. a, Western blot analysis of Nup60-mCherry and Mlp1-mGFP expression levels from live-cell fluorescence imaging experiment presented in Fig. 7a. Pgk1 was used as loading control. Unprocessed blots are available in source data.

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Sample size

No statistical methods were employed to predetermine the sample size. Instead, sample sizes were selected based on or exceeding the standards commonly accepted in the field.

Data exclusions

When analyzing live cell fluorescence imaging data (Fig. 7a-b), only cells that expressed both the Nup60-mCherry and Mlp1-mGFP plasmids were included (i.e., cells expressing only one plasmid due to plasmid loss were excluded). To characterize the effect of Nup60 mutations on the localization of Mlp1, it was necessary for both proteins to be present in the cells. This exclusion criterion was determined prior to the experiment.

Replication

All findings in this study have been successfully replicated using appropriate negative controls in each replicate. For a detailed overview of the replication process, please refer to the 'Statistics and Reproducibility' section under 'Methods'.

Randomization

In most experiments presented in this study, randomization was not applicable. However, to control for any time-dependent effects during in vitro binding assays, the binding experiments were repeated with a randomized sample processing order.

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Blinding was not implemented during experiments and outcome assessments because the same investigator performed and analyzed all the experiments.

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☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	anti-GFP (mouse, Roche, cat. no. 11814460001, clones 7.1 and 13.1, lot no. 47859600, 1:2000), anti-RFP (mouse, ChromoTek, cat. no. 6g6, clone 6G6, lot. no. 104272-07-02, dilution 1:2000), anti-Pgk1 (mouse, Abcam, cat. no. ab113687, clone 22C5D8, lot. no. GR3286258-2, dilution 1:10000), anti-Strep Tag II (mouse, Qiagen, cat. no. 34850, lot. no. 157030640, dilution 1:2500), AffiniPure IgG (goat anti-mouse, Fcy fragment specific, Jackson ImmunoResearch, cat. no. 115-035-008, lot. no. 142709, dilution 1:5000)
Validation	According to the manufacturer, anti-GFP (Roche) has been tested for Western blot applications (E. coli extract containing recombinant GFP fusion protein). According to the manufacturer, anti-Pgk1 (Abcam) has been tested for Western blot applications (S. cerevisiae cell lysate). According to the manufacturer, anti-RFP (ChromoTek) has been tested for Western blot applications (cell extracts of HEK 293T cells expressing different red fluorescent proteins, including mCherry used in this study). According to the manufacturer, anti-Strep Tag II (Qiagen) has been tested for Western blot applications, the antibody displays negligible cross-reactivity with crude E. coli, mammalian, or insect-cell lysates.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	Both Sf9 and Hi5 insect cells were sourced from Expression Systems (cat. no. 94-001F and 94-002F, respectively).
Authentication	Cell lines were not additionally authenticated.
Mycoplasma contamination	Both cell lines tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Plants

Seed stocks	<i>Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.</i>
Novel plant genotypes	<i>Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.</i>
Authentication	<i>Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.</i>

3.2. Results 2

3.2.1. Preamble

The following first-author publication, a review/opinion piece '*The interplay of nuclear pores and lipids*' (Stankunas&Köhler, 2023), published in *Current Opinion in Cell Biology* (themed issue on Cell Nucleus 2023) on 5th of October 2023, constitutes a second publication prerequisite for the cumulative thesis. The publication examines the current understanding of the mutual interdependence between the proteinaceous scaffold of the NPC and its surrounding lipid environment. It emphasizes the importance of considering membrane component when probing the structure and function of the NPC. The publication provides background knowledge and lays the conceptual foundation for achieving the aims of this thesis, with particular relevance to Aim 3.

3.2.2. Contribution statement

A.K. and E.S. reviewed the literature, prepared the figures and wrote the paper.

3.2.3. Publication

See next pages.



The interplay of nuclear pores and lipids

Edvinas Stankunas^{1,2} and Alwin Köhler¹

Abstract

Nuclear pore complexes (NPCs) mediate the bidirectional transport of cargo across the nuclear envelope (NE). NPCs are also membrane remodeling machines with a capacity to curve and fuse the membranes of the NE. However, little is known about the interplay of NPCs and lipids at a mechanistic level. A full understanding of NPC structure and function needs to encompass how the NPC shapes membranes and is itself shaped by lipids. Here we attempt to connect recent findings in NPC research with the broader field of membrane biochemistry to illustrate how an interplay between NPCs and lipids may facilitate the conformational plasticity of NPCs and the generation of a unique pore membrane topology. We highlight the need to better understand the NPC's lipid environment and outline experimental avenues towards that goal.

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Introduction

Nuclear pore complexes fuse the inner (INM) and outer nuclear membranes (ONM) to create a pore membrane. The membrane-embedded NPCs regulate nucleocytoplasmic transport, as well as chromatin architecture and activity, and their misfunction can cause human diseases. Each NPC is composed of ~30 different proteins (nucleoporins or Nups) present in multiple (8–64) copies, adding up to ~1000 proteins within one NPC in humans [1]. Key structural elements of the NPC are the inner ring (IR), which is located at the crest of the pore

membrane; the cytoplasmic ring (CR) and the nuclear ring (NR), which are both anchored by the inner ring; and peripheral appendages, namely the cytoplasmic filaments and the nuclear basket, which emanate from the cytoplasmic and nuclear ring, respectively. The central channel of the NPC is lined by phenylalanine–glycine (FG) Nups, which establish the NPC permeability barrier [2]. The Y-complex (also termed the Nup84 complex in *S. cerevisiae* and NUP107-160 complex in humans) is the main component of the cytoplasmic and nuclear rings and forms octameric ring structures [3–6]. Other biochemically stable modules have been described [1]. Importantly, short linear motifs (SLiMs) in intrinsically disordered regions of Nups establish critical connections within the NPC scaffold [7–9]. Thus, rigid units are grafted onto flexible “springs”. This mode of NPC connectivity may offer regulatory and structural flexibility during assembly/disassembly or when experiencing mechanical stress (e.g., large cargo or NE deformations during cell migration) [10,11].

Lipid bilayers typically resist both bending and remodeling. Hence, forces have to be applied to curve and fuse the pore membrane during NPC biogenesis. Equally important, the curved pore membrane needs to be stabilized to prevent NE rupture and nuclear leakage. A number of putative membrane-deforming and curvature-stabilizing activities have been mapped to symmetric components of the NPC core (see Table 1). They include the conserved curvature-sensing Amphipathic Lipid Packing Sensor (ALPS) motifs of *Sc*Nup120 and *Sc*Nup133, both of which are subunits of the Y-complex, as well as a putative ALPS-like motif in *Sc*Nup170, and a curvature-inducing amphipathic helix (AH) at the C-terminus of *Sc*Nup53 [12,13]. Several other lipid-interacting motifs were predicted and await experimental validation. How the transmembrane Nups (*Sc*Ndc1, *Sc*Pom152/33/34; *Hs*NDC1, *Hs*POM210/121) influence membrane curvature is still unclear. Within the NE lumen, *Sc*Pom152 forms a massive, membrane-anchored belt around the equator of the pore membrane, whose precise role is still unclear [3,14]. The NPC also contacts the membrane through the nuclear basket, one of its asymmetric appendages. The basket nucleoporins *Sc*Nup1 and *Sc*Nup60, via amphipathic helices, directly bend membranes *in vitro*, reshape the NE *in vivo* and maintain NPC/NE integrity [7,15]. The mechanism appears conserved, as metazoan NUP153 (an ortholog of *Sc*Nup1/Nup60) also binds to the INM

Table 1

Membrane-binding nucleoporins in yeast and human.

S.cerevisiae	H.sapiens	
<i>Peripheral membrane-interacting Nups</i>		<i>lipid-interacting motif</i>
Nup53/59	NUP53	AH
Nup157/170	NUP155	ALPS, WF motif
Nup120	NUP160	ALPS
Nup133	NUP133	ALPS
Nup1	NUP153	AH
Nup60	NUP153	AH
<i>Transmembrane Nups</i>		
Ndc1	NDC1	
Pom33	-	
Pom34	-	
Pom152	-	
-	POM121	
-	POM210	

AH: amphipathic helix, ALPS: amphipathic lipid packing sensor, WF: tryptophan-phenylalanine.

via an N-terminal AH and facilitates interphase NPC biogenesis [16]. Thus, an intricate network of integral membrane proteins (IMPs) and peripheral membrane proteins creates membrane shaping forces within the NPC. Although some functional interactions among lipid-interacting Nups have been established [15,17], we currently lack a systematic understanding of how all Nups (Table 1) work together to shape the pore membrane. To progress, it is essential to gain mechanistic insights into the relationship between NPCs and lipids.

While the diameter of the NPC channel has long been observed to be ~40 nm, recent progress in imaging technology has allowed for snapshots of NPCs captured *in cellulo* [3,4,6]. These studies have revealed striking dilations in the central channel accompanied by conformational changes in the scaffold and underlying pore membrane. Another significant insight from these *in cellulo* snapshots was the observation of an asymmetry in the highly curved pore membrane [4], whose origin is not understood. These new findings highlight the need to understand the interplay of NPCs with their membrane environment. Not only is the influence of the NPC on membranes poorly understood, even less was known about how lipids affect the NPC. Recent work has shown that a finely tuned balance of saturated and unsaturated lipids regulates NE and NPC function, shedding light on the particular lipid needs of the NPC [18]. Decades of research in membrane biochemistry have established that solvating lipids can exert a profound influence on membrane protein structure and function. These aspects of membrane biology are underappreciated in NPC research, yet may hold the key to understanding many features of NPC function, adaptability and evolution.

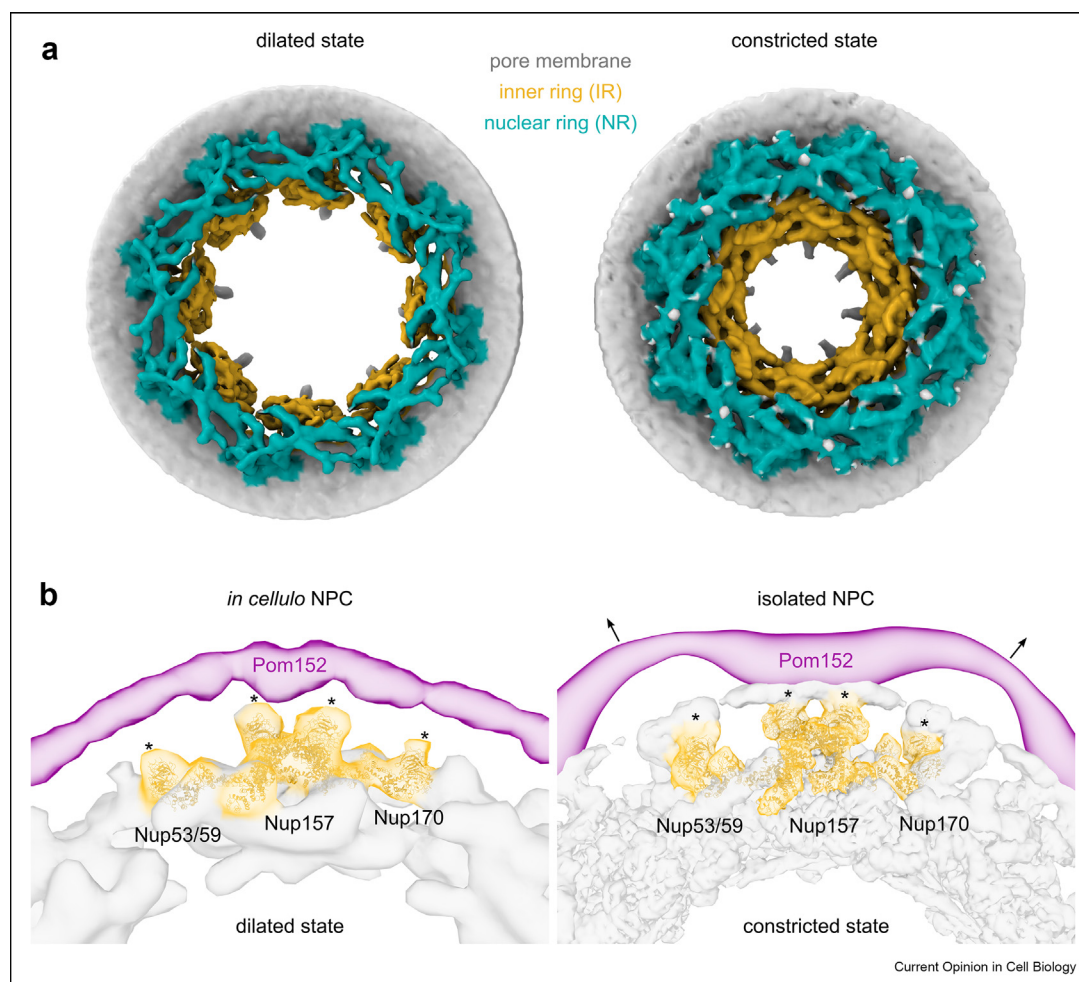
A malleable NPC – force transduction via lipids

To sculpt the NE out of the ER and to populate the NE with the fenestrations occupied by NPCs (~100 in yeast; ~2000 in human cells), membranes must undergo deformation. The amount of work needed to deform the membrane is determined by the membrane's bending rigidity or, conversely, its elasticity [19]. In general, lipids containing long saturated fatty acids tend to thicken, rigidify, and decrease the fluidity of membranes due to the close packing of their hydrophobic tails and stronger interactions between neighboring lipids. Conversely, unsaturated lipids tend to have the opposite effect as their kinked acyl-chains hinder tight packing [20]. In addition, membrane elasticity is influenced by membrane lipid composition and protein content [21]. In principle, the pore membrane connecting the ONM and INM must have elasticity, since it is a saddle-shaped, highly curved membrane segment which exhibits both positive (convex) and negative (concave) curvature. A new study reported that elevated lipid saturation levels decrease the elasticity of the NE, transforming a spherical nucleus into a rigid polyhedron that becomes susceptible to fracturing. Maintaining a balanced lipid saturation was shown to be essential for proper pore membrane curvature and NPC integrity [18]. This study provides evidence that a single double bond in the C9 position of lipid acyl chains, which induces a kink, is linked to NPC function.

Several new studies, which combined cryo-focused-ion-beam milling of whole cells with cryo-electron tomography (ET) and docking of crystal structures have shown that the topological arrangement of subunits and diameter of the NPC transport channel depend on the native NPC environment in cells. *S. pombe* NPCs were shown to adopt dilated and constricted states [6]. When cells were exposed to osmotic shock or depleted of energy, nuclear shrinkage was correlated with NPC constriction, giving rise to the idea that NE membrane tension may play a crucial role in regulating NPC diameter (Figure 1a). Similar conclusions may be drawn from comparisons of cryo-ET structures of purified *S. cerevisiae* NPCs and NPCs *in cellulo* – the native membrane environment keeps NPCs in a dilated state, whereas purified NPCs are constricted [3] (Figure 1b). Also in human cells, the central transport channel *in cellulo* is dilated with a diameter of nearly 60 nm [4]. Whether the constricted state of native and purified NPCs has the same underlying cause (i.e. reduced membrane tension) or reflects other experimental parameters remains to be shown.

Overall, these studies suggest that the NPC scaffold is mechanosensitive. Functionally, the NPC's mechanosensitivity may play a role in cellular processes involving mechanical forces such as cell differentiation, cell

Figure 1



Constricted and dilated state of NPCs. **a**) Isosurface rendering of cryo-EM density maps of the *S.pombe* NPC in dilated (left) and constricted (right) states [6], corresponding to EMDB-11373 and EMDB-11375 entries, respectively, as seen from the nucleoplasm. Densities are colored by their spatial proximity to fitted atomic models with pore membrane colored in grey, inner ring in gold and nuclear ring in dark cyan. **b**) Isosurface rendering of cryo-EM density maps of *S.cerevisiae* in cellulo (left) and isolated (right) NPC [3], corresponding to EMDB-24258 and EMDB-24224 entries, respectively. Views of the pore membrane-proximal densities within the inner ring (gold) as well as the Pom152 luminal ring (magenta) are shown. Models of Nup53, Nup59, Nup157 and Nup170 are shown in cartoon representation (gold) and correspond to PDB 7N9F (*in situ*) and 7N85 (isolated) entries. Membrane contact sites are marked with asterisks (*); the membrane has been omitted for clarity. Note the conformational change of the Pom152 rings between *in situ* and isolated NPCs (arrows).

migration, and changing osmotic conditions. An attractive idea is that membrane changes ultimately change nucleocytoplasmic transport. As an example, NE membrane compression was proposed to trigger nuclear entry of the mechanosensitive transcriptional activator YAP, possibly via changes in NPC transport [22]. How this works is still unclear. At a fundamental level, altered membrane tension must change nucleoporin conformation. Upon NPC constriction, for example, the Pom152 belt in the NE lumen bulges out considerably, whereas NPC dilation straightens the belt (Figure 1b) [3,6]. Such large-scale movements may transduce the membrane state to yet-unidentified response elements, however, the cause-effect relationship between

membrane tension and Pom152 conformation remains to be investigated. A related question is whether NPC mechanosensitivity only occurs on slow time scales (minutes to hours), or whether the NPC harbors mechanosensors with rapid response properties (milliseconds) similar to mechanosensitive ion channels, which rapidly convert mechanical stimuli that act on a membrane into electrical, osmotic, and/or chemical intracellular signals [23,24]. It has been proposed that mechanosensing can be implemented by altered membrane curvature and/or shifted lateral pressure in the membrane, which subsequently induce changes in protein conformation and function [25]. It will be interesting to explore whether specific changes in nuclear

pore membrane curvature are actively sensed by the NPC scaffold to influence NPC function.

Pore membrane curvature – force transduction from proteins

Bending of membranes by proteins is a common mechanism in intracellular membrane dynamics and can be categorized into two types: The first involves the interaction of a protein domain with the lipid bilayer core, such as a hydrophobic insertion or wedging, for example by AH-containing Nups [26]. The second type involves external curvature-generating forces applied to the bilayer surface, while the bilayer itself remains intact, known as scaffolding or crowding mechanisms [27]. Scaffolding occurs when hydrophilic macromolecular complexes with inherent curvature attach to the lipid bilayer, causing it to bend. This mechanism is employed in endomembrane traffic, for example by large protein complexes forming spherical cages such as COPI and COPII [28,29].

To induce membrane curvature via scaffolding, the protein scaffold must be strongly anchored to the membrane and possess a curved shape and greater rigidity than the lipid bilayer. Scaffolding has long been implicated in pore membrane formation, specifically through the Y-complex. The Y-complex may in fact employ both mechanisms, because it also inserts itself into the pore membrane using its ALPS motifs (Figure 2a and b) [3–6,12]. Generally, the extent of curvature generation of any AH depends on its insertion depth into the membrane leaflet: shallow embedding leads to an asymmetric strain and higher curvature (Figure 3b). In contrast, deep insertion leads to a symmetric strain and lower curvature [26]. Curvature generation is expected to be most potent when multiple shallow insertions of AHs occur in close proximity. Perhaps more important than the activity of a single Y-complex is the collective behavior of Y-complexes.

A combination of genetics and cryo-ET in cells has recently allowed to dissect the scaffolding function of the Y-complex in *S. pombe* and humans [4,6]. Notably, deletion of *HsNUP96* (*ScNup145C*), a key component of the Y complex and member of both the nuclear and cytoplasmic rings, produced dilated NPCs containing just the inner ring (IR) *in cellulo* [4]. Strikingly, these NPCs exhibit an increase in positive pore membrane curvature, not a decrease, as one might have predicted (Figure 2c), suggesting that the Y-complex attenuates the generation of positive pore membrane curvature within the NPC. This increased membrane curvature could reflect scaffolding by other parts of the NPC. The remaining pore membrane contacts of the IR are likely mediated by NUP53 (Figure 1b and 2a), which binds membranes directly via its two membrane binding motifs [30]. Additionally, NUP53 interacts with the

transmembrane Nups NDC1 and NUP155 [13], which may contribute yet another membrane affinity. Conceivably, these proteins play a greater role in scaffolding pore membrane curvature than previously thought.

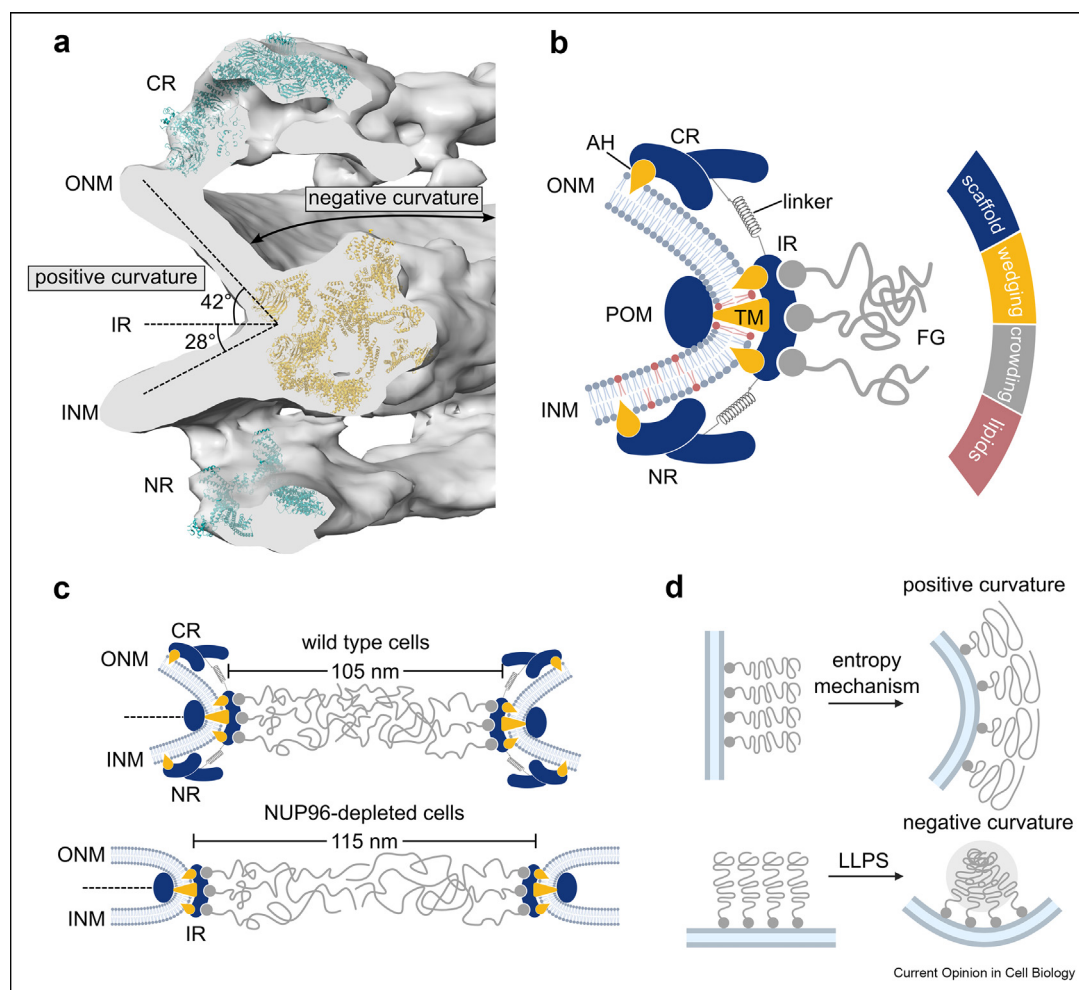
One of the new studies added yet another layer of complexity, as it reported an asymmetric pore membrane in human cells (Figure 2a) [4]. The pore membrane crest beneath the inner ring complex is more curved on the nucleoplasmic than on the cytoplasmic side. This finding raises multiple questions. How come that the curvature is asymmetric, when the majority of putative membrane-deforming and curvature-sensing activities of the NPC localize to symmetric parts of the NPC core? Do NPC-lamina interactions contribute to this asymmetry? How malleable is this membrane structure and is it functionally relevant (e.g. in mechanosensing)? In the NUP96-depleted cells, this asymmetry in the pore membrane was lost. The great unknown is whether, in addition to the loss of the symmetric CR and NR, the basket may have detached. The basket was not resolved, but if it detached, the membrane bending activity of basket NUP153 may explain the pore membrane asymmetry.

Seen in a larger context, NPC rigidity is a critical factor in facilitating efficient curvature generation and must surpass that of the lipid bilayer. Recent work has comprehensively characterized the network of linkers that connect the NPC [5]. These linkers are flexible and may enable the scaffold to undergo major conformational changes. Whereas these characteristics would reduce the NPC's rigidity, constant collisions of the structured parts of the NPC, which are connected by "springs", could nevertheless impose sufficient energy to curve the pore membrane (Figure 2b).

Another important contributor to pore membrane shape could be the FG-Nups at the center of the pore. Their material properties remain a matter of discussion. Since the FG-Nups are anchored to the NPC scaffold [31], their mode of interaction may affect the underlying pore membrane. In this respect, it is interesting that two different protein crowding models make opposite predictions about membrane curvature generation. Entropy-driven interactions are expected to induce positive curvature, whereas attractive multivalent interactions should induce negative curvature (Figure 2d).

Entropy-mediated mechanisms have been implicated as drivers of curvature generation for cilia, filopodia and neuronal axons [32]. In this model, polymer brushes, an assembly of polymers which are tethered by one end to a surface [33], become crowded and must stretch away from the surface to prevent overlapping. The bending force arises from the polymer's inherent drive to

Figure 2



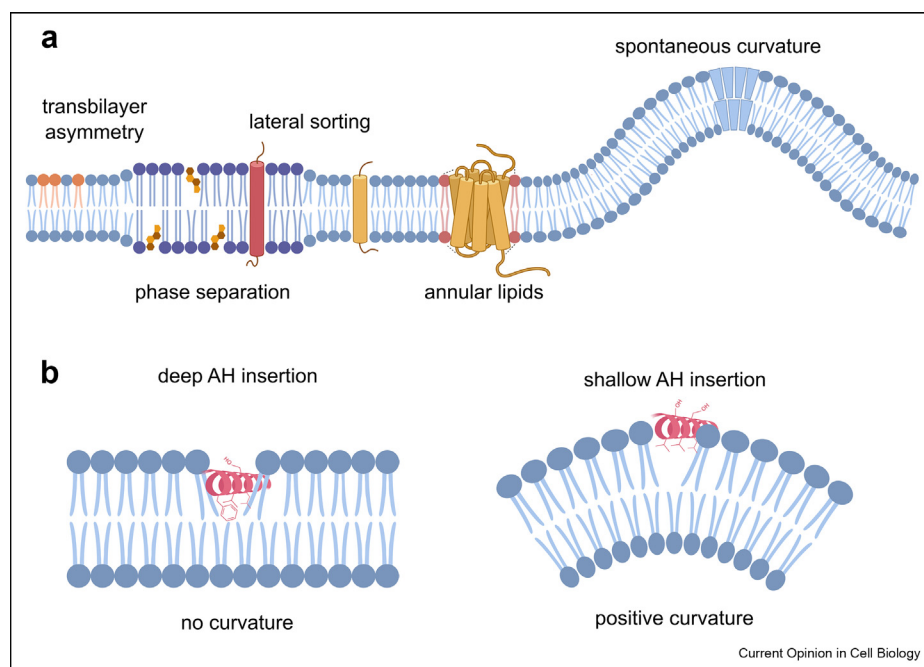
Mechanisms of curvature generation in NPCs. **a)** Isosurface rendering of cryo-EM density map of human NPC [4] shown in a cutaway view, corresponding to EMD-12814 entry. Models of Y-complexes (cyan) and inner ring complex (gold) are shown in cartoon representation and correspond to PDB 7PEQ and 7PER entries, respectively. Note the angular asymmetry within the pore membrane. CR, cytoplasmic ring, ONM, outer nuclear membrane, IR, inner ring, INM, inner nuclear membrane, NR, nuclear ring. **b)** Cartoon representation of NPC organization highlighting the in-built mechanisms of pore membrane shaping. AH, amphipathic helix, TM, transmembrane domain, FG, phenylalanine–glycine repeats. **c)** Cartoon representation of human NPC architecture in wild type cells (top) and NUP96-depleted cells (bottom) as reported in Ref. [4]. Note the dilation of the NPC (115 nm vs 105 nm), loss of pore membrane asymmetry as well as decrease of ONM-INM distance upon NUP96-depletion. **d)** Cartoon representation of membrane curvature induction by protein crowding, namely entropy-driven generation of positive curvature by polymer brushes (top) and multivalent, phase separation-driven generation of negative curvature (bottom). Figure created with BioRender.com.

increase its entropy [34]. Membrane bending provides more space for the polymer chains. The net result is a positive membrane curvature (Figure 2d). FG-Nups have been proposed to form a polymer brush [35] and could thereby aid positive membrane curvature in the plane of the nucleocytoplasmic axis.

In contrast, attractive multivalent interactions, like those that trigger protein phase separation, are expected to bend the membrane into a negative (concave) shape (Figure 2d). Recent experiments have demonstrated that attachment of an intrinsically disordered domain of a model protein to synthetic and

cell-derived membrane vesicles can indeed bend membranes [36]. FG-Nups have been proposed to engage in cohesive, multivalent interactions with each other and to form a condensed phase. This would not readily explain ONM-INM curvature, which is positive (convex), but could contribute to the forces that sculpt the negative curvature of the pore ring. At least from a lipid perspective, the different FG-Nup models are not mutually exclusive. Whether and how entropy-driven and multivalency-driven membrane remodeling could create yet another layer of membrane sculpting activity in the NPC is conceptually interesting and testable.

Figure 3



Physical and compositional variations in membranes. **a)** Selection of lipid-centric mechanisms with potential relevance for NPC structure and function. **b)** Cartoon representation of hydrophobic insertion of an amphipathic helix (AH). Shallow insertion of AH (*right*) induces positive curvature, whereas deep insertion of AH (*left*) produces no membrane curvature. Figure created with [BioRender.com](https://www.biorender.com).

The lipid nano-environment of nuclear pores

Clearly, NPC structure and function is inextricably linked to the surrounding lipids, but whereas the functional lipid environment of IMPs, such as ion channels, has been studied for decades, the emerging concepts have not yet been tested in the context of the NPC. In the purified NPC, a lipid-detergent ring encircles the NPC's inner ring [3]. What these lipids are and whether they differ from the average lipid composition of the NE and ER is a key open question.

In the early 1970s, electron paramagnetic resonance spectroscopy using spin-labelled lipids provided the initial direct proof of lipid-protein interactions [37]. Lipids located in proteins' immediate surroundings behaved differently from those in the bulk membrane, exhibiting reduced dynamics and slower exchange rates. These findings gave rise to the concept of a "lipid annulus" around IMPs, which differs in composition and properties from the bulk membrane [37]. Subsequently, improved techniques for studying membrane proteins resulted in high-resolution structures, some of which showed electron densities consistent with lipid molecules [38,39]. That lipids survived the solubilization, purification, and crystallization processes suggested strong ligand-like, specific binding rather than a cloud of "annular lipids". Indeed, specific lipids were shown to

play crucial roles in regulating protein activity. For instance, the phosphorylated lipid PIP₂ (phosphatidylinositol 4,5-bisphosphate) plays a key role in regulating potassium channels and several recent cryo-EM structures of TRP channels have revealed bound lipids, including PIP₂ and cholesterol [40–43]. As PIP₂ can be rapidly produced, consumed, or sequestered by various cytoplasmic proteins, this enables dynamic regulation of these channels. Similarly, phosphatidic acid [44] and cardiolipin [45], which are rare and highly compartmentalized in cells, have been proposed to regulate protein function. The picture that emerges is that many lipids exhibit non-selective binding to transmembrane proteins, while others impact protein structure [46,47]. Even protein multimerization can be driven by interactions with the lipid membrane [48]: the monomers of a chloride channel cause structural defects in the surrounding membrane, resulting in strain. When a dimer forms, this membrane strain dissipates, making dimerization energetically favorable.

Taken together, membrane protein structure, dynamics, and oligomerization are influenced by their lipid environment. Lipid nano-environments can influence protein conformational equilibria, a topic that could be highly relevant given the NPCs astonishing structural plasticity [49]. In this respect, the reported enrichment of DAG at the INM [50] and a differential regulation of

lipid metabolism across the NE [51] could contribute to an NPC-specific lipid environment. This may be promoted, in part, by specific lipid metabolic enzymes that were detected at NPCs [52]. Indeed, specific lipids can enhance membrane curvature due to their intrinsic conical shape [20], in particular when their distribution in the bilayer is asymmetric as suggested for certain lipids in the ER/NE [53] (Figure 2b and 3a). Moreover, lipids can undergo a segregation into distinct phases, which can contribute to a lateral sorting of IMPs *in vitro* [54] (Figure 3a). Intriguingly, balanced lipid saturation was recently shown to prevent the spontaneous segregation of NE lipids into liquid ordered and liquid disordered phases *in vivo* [18]. NPCs were shown to strongly prefer the disordered phase, while avoiding the ordered territory, suggesting that NPCs require a malleable lipid environment to function. Finally, elegant *in vitro* experiments with tubules, pulled out from giant vesicles, have shown that these highly curved structures have the capacity to secondarily attract lipids that support high curvature formation [54,55]. While all these data are circumstantial, they hint to the possibility of a functionally relevant “lipid fingerprint” of NPCs. If deteriorated, a pathological NPC lipidome may manifest itself as membrane herniations around NPCs, as observed in various mutations of Nups, Nup-associated factors [56,57] and lipid metabolic enzymes [58–61].

Conclusion

In sum, we propose that lipid-nucleoporin interactions may result in the formation of a distinct lipid environment around the NPC, characterized by specific compositional and biophysical properties. This lipid environment can be influenced by external factors, leading to structural and functional alterations of the NPC. Such alterations may result from physical factors, such as changes in membrane elasticity and tension, or biochemical processes, such as the enzymatic conversion of distinct lipid species or metabolic alterations to the lipidome [62]. To test these hypotheses, it is crucial to develop lipidomic techniques that can investigate the immediate NPC lipid environment at high spatial and temporal resolution, integrating *in cellulo* structural biology with membrane biochemistry, genetic perturbations and modeling approaches. To us, answering the following questions is crucial in advancing NPC research.

- What is the qualitative and quantitative contribution of the various NPC membrane interactions to curvature generation and how is this choreographed?
- What is the structure of the NPC’s transmembrane domains and how conformationally flexible are they? Do they act as a wedge?
- Does the NPC have a stable annulus of lipids that moves with it, similar to a swim ring? Is this ‘lipid fingerprint’ functionally important?

- Could metabolic processes drive sufficient lipidomic changes to influence the structure and function of NPCs?
- Do lipids selectively bind to Nups, modulating their function allosterically? Or are lipids merely a solvent for the NPC?
- What other lipid species, besides DAG, could exhibit asymmetric distribution across the NE, and what is their functional relevance for NPC and NE biology?

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

We apologize to those colleagues whose work has not been cited due to space limitations.

Data availability

No data was used for the research described in the article.

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References

Papers of particular interest, published within the period of review, have been highlighted as:

- of outstanding interest

1. Lin DH, Hoelz A: **The structure of the nuclear pore complex (an update)**. *Annu Rev Biochem* 2019, **88**:725–783.
2. Yu M, Heidari M, Mikhaleva S, Tan PS, Mingu S, Ruan H, Reinkemeier CD, Obarska-Kosinska A, Siggel M, Beck M, *et al.*: **Visualizing the disordered nuclear transport machinery in situ**. *Nature* 2023, **617**:162–169.
3. Akey CW, Singh D, Ouch C, Echeverria I, Nudelman I, Varberg JM, Yu Z, Fang F, Shi Y, Wang J, *et al.*: **Comprehensive structure and functional adaptations of the yeast nuclear pore complex**. *Cell* 2022, **185**:361–378.e25.
4. Schuller AP, Wojtynek M, Mankus D, Tatli M, Kronenberg-Tenga R, Regmi SG, Dip PV, Lytton-Jean AKR, Brignole EJ, Dasso M, *et al.*: **The cellular environment shapes the nuclear pore complex architecture**. *Nature* 2021, **598**:667–671.
5. Petrovic S, Samanta D, Perriches T, Bley CJ, Thierbach K, Brown B, Nie S, Mobbs GW, Stevens TA, Liu X, *et al.*: **Architecture of the linker-scaffold in the nuclear pore**. *Science* 1979, **202**:376.
6. Zimmerli CE, Allegretti M, Rantos V, Goetz SK, Obarska-Kosinska A, Zagorij I, Halavatyi A, Hummer G, Mahamid J, Kosinski J, *et al.*: **Nuclear pores dilate and constrict in cellulo**. *Science* 1979, **202**:374.

This study describes the compositional and structural heterogeneity of NPCs in *S.cerevisiae*, comparing structures of purified and *in cellulo* NPCs.

This cryo-ET study describes the angular asymmetry of the pore membrane within human NPCs and probes the role of the Y complex for membrane curvature.

Using an integrative structural biology approach, the authors establish the critical function of linker nucleoporins in tying the scaffold of the NPC together and provide a mechanistic explanation of NPC plasticity.

The authors capture the dilation-constriction cycle of *S.pombe* NPCs *in cellulo* upon osmotic shock and energy-depletion.

7. Cibulka J, Bisaccia F, Radisavljević K, Carrillo RMG, Köhler A: **Assembly principle of a membrane-anchored nuclear pore basket scaffold.** *Sci Adv* 2022;8.
- The authors report multi-step *in vitro* reconstitutions of a membrane-tethered nuclear basket scaffold in a chemically defined setup.
8. Teimer R, Kosinski J, Von Appen A, Beck M, Hurt E: **A short linear motif in scaffold Nup145C connects Y-complex with pre-Assembled outer ring Nup82 complex.** *Nat Commun* 2017, 8.
9. Fischer J, Teimer R, Amlacher S, Kunze R, Hurt E: **Linker Nups connect the nuclear pore complex inner ring with the outer ring and transport channel.** *Nat Struct Mol Biol* 2015, 22: 774–781.
10. Hakhverdyan Z, Molloy KR, Keegan S, Herricks T, Lepore DM, Munson M, Subbotin RI, Fenyő D, Aitchison JD, Fernandez-Martinez J, et al.: **Dissecting the structural dynamics of the nuclear pore complex.** *Mol Cell* 2021, 81:153–165.e7.
11. Onischenko E, Noor E, Fischer JS, Gillet L, Wojtynek M, Vallotton P, Weis K: **Maturation kinetics of a multiprotein complex revealed by metabolic labeling.** *Cell* 2020, 183: 1785–1800.e26.
12. Drin G, Casella JF, Gautier R, Boehmer T, Schwartz TU, Antonny B: **A general amphipathic α -helical motif for sensing membrane curvature.** *Nat Struct Mol Biol* 2007, 14:138–146.
13. Eisenhardt N, Redolfi J, Antonin W: **Interaction of Nup53 with Ndc1 and Nup155 is required for nuclear pore complex assembly.** *J Cell Sci* 2014, 127:908–921.
14. Upla P, Kim SJ, Sampathkumar P, Dutta K, Cahill SM, Chemmama IE, Williams R, Bonanno JB, Rice WJ, Stokes DL, et al.: **Molecular architecture of the major membrane ring component of the nuclear pore complex.** *Structure* 2017, 25: 434–445.
15. Mészáros N, Cibulka J, Mendiburo MJ, Romanauska A, Schneider M, Köhler A: **Nuclear pore basket proteins are tethered to the nuclear envelope and can regulate membrane curvature.** *Dev Cell* 2015, 33:285–298.
16. Vollmer B, Lorenz M, Moreno-Andrés D, Bodenhöfer M, De Magistris P, Astrinidis SA, Schooley A, Flötenmeyer M, Leptihn S, Antonin W: **Nup153 recruits the nup107-160 complex to the inner nuclear membrane for interphasic nuclear pore complex assembly.** *Dev Cell* 2015, 33:717–728.
17. Amm I, Weberuss M, Hellwig A, Schwarz J, Tatarek-Nossol M, Luchtenborg C, Kallas M, Brügger B, Hurt E, Antonin W: **Distinct domains in Ndc1 mediate its interaction with the Nup84 complex and the nuclear membrane.** *J Cell Biol* 2023:222.
18. Romanauska A, Köhler A: **Lipid saturation controls nuclear envelope function.** *Nat Cell Biol* 2023, <https://doi.org/10.1038/s41556-023-01207-8>.
- This study demonstrates how lipid saturation chemistry is critical for NE and NPC structure and function, employing genetic engineering, *in vitro* reconstitution and multimodal imaging.
19. Helfrich W: *Elastic properties of lipid bilayers: theory and possible experiments.* 1973.
20. Ernst R, Ejsing CS, Antonny B: **Homeoviscous adaptation and the regulation of membrane lipids.** *J Mol Biol* 2016, 428: 4776–4791.
21. Fowler PW, Hélie J, Duncan A, Chavent M, Koldsø H, Sansom MSP: **Membrane stiffness is modified by integral membrane proteins.** *Soft Matter* 2016, 12:7792–7803.
22. Elosegui-Artola A, Andreu I, Beedle AEM, Lezamiz A, Uroz M, Kosmalska AJ, Oria R, Kechagia JZ, Rico-Lastres P, Le Roux AL, et al.: **Force triggers YAP nuclear entry by regulating transport across nuclear pores.** *Cell* 2017, 171:1397–1410.e14.
23. Jin P, Jan LY, Jan Y-N: **Mechanosensitive ion channels: structural features relevant to mechanotransduction mechanisms.** *Annu Rev Neurosci* 2020, 43:207–229.
24. Kefauver JM, Ward AB, Patapoutian A: **Discoveries in structure and physiology of mechanically activated ion channels.** *Nature* 2020, 587:567–576.
25. Martinac B, Bavi N, Ridone P, Nikolaev YA, Martinac AD, Nakayama Y, Rohde PR, Bavi O: **Tuning ion channel mechanosensitivity by asymmetry of the transbilayer pressure profile.** *Biophys Rev* 2018, 10:1377–1384.
26. Campelo F, McMahon HT, Kozlov MM: **The hydrophobic insertion mechanism of membrane curvature generation by proteins.** *Biophys J* 2008, 95:2325–2339.
27. Stachowiak JC, Schmid EM, Ryan CJ, Ann HS, Sasaki DY, Sherman MB, Geissler PL, Fletcher DA, Hayden CC: **Membrane bending by protein–protein crowding.** *Nat Cell Biol* 2012, 14: 944–949.
28. Dodonova SO, Diestelkoetter-Bachert P, von Appen A, Hagen WJH, Beck R, Beck M, Wieland F, Briggs JAG: **A structure of the COPI coat and the role of coat proteins in membrane vesicle assembly.** *Science* 2015, 349:195–198.
29. Hutchings J, Stancheva VG, Brown NR, Cheung ACM, Miller EA, Zanetti G: **Structure of the complete, membrane-assembled COPII coat reveals a complex interaction network.** *Nat Commun* 2021, 12:2034.
30. Vollmer B, Schooley A, Sachdev R, Eisenhardt N, Schneider AM, Sieverding C, Madlung J, Gerken U, MacEk B, Antonin W: **Dimerization and direct membrane interaction of Nup53 contribute to nuclear pore complex assembly.** *EMBO J* 2012, 31:4072–4084.
31. Onischenko E, Tang JH, Andersen KR, Knockenhauer KE, Vallotton P, Derrer CP, Kralt A, Mugler CF, Chan LY, Schwartz TU, et al.: **Natively unfolded FG repeats stabilize the structure of the nuclear pore complex.** *Cell* 2017, 171: 904–917.e19.
32. Shurer CR, Kuo JCH, Roberts LDM, Gandhi JG, Colville MJ, Enoki TA, Pan H, Su J, Noble JM, Hollander MJ, et al.: **Physical principles of membrane shape regulation by the glycocalyx.** *Cell* 2019, 177:1757–1770.e21.
33. Zhao B, Brittain WJ: **Polymer brushes: surface-immobilized macromolecules.** *Prog Polym Sci* 2000, 25:677–710.
34. Lipowsky R: **Bending of membranes by anchored polymers.** *Europhys Lett* 1995, 30:197.
35. Lim RYH, Fahrenkrog B, Köser J, Schwarz-Herion K, Deng J, Aebi U: **Nanomechanical basis of selective gating by the nuclear pore complex.** *Science* 2007, 318:640–643.
36. Yuan F, Alimohamadi H, Bakka B, Tremontozzi AN, Day KJ, Fawzi NL, Rangamani P, Stachowiak JC: **Membrane bending by protein phase separation.** *Proc Natl Acad Sci USA* 2021, 118, e2017435118.
- The study describes a novel mechanism of membrane bending by membrane-tethered intrinsically disordered proteins undergoing multivalency-driven liquid-liquid phase separation.
37. Marsh D: **Protein modulation of lipids, and vice-versa, in membranes.** *Biochim Biophys Acta Biomembr* 2008, 1778: 1545–1575.
38. Belrhali H, Nollert P, Royant A, Menzel C, Rosenbusch JP, Landau EM, Pebay-Peyroula E: **Protein, lipid and water organization in bacteriorhodopsin crystals: a molecular view of the purple membrane at 1.9 Å resolution.** *Structure* 1999, 7: 909–917.
39. McAuley KE, Fyfe PK, Ridge JP, Isaacs NW, Cogdell RJ, Jones MR: **Structural details of an interaction between cardiolipin and an integral membrane protein.** *Proc Natl Acad Sci USA* 1999, 96:14706–14711.
40. Long SB, Tao X, Campbell EB, MacKinnon R: **Atomic structure of a voltage-dependent K⁺ channel in a lipid membrane-like environment.** *Nature* 2007, 450:376–382.
41. Hansen SB, Tao X, MacKinnon R: **Structural basis of PIP2 activation of the classical inward rectifier K⁺ channel Kir2.2.** *Nature* 2011, 477:495–498.
42. Gao Y, Cao E, Julius D, Cheng Y: **TRPV1 structures in nanodiscs reveal mechanisms of ligand and lipid action.** *Nature* 2016, 534:347–351.

43. Duncan AL, Song W, Sansom MSP: **Lipid-Dependent regulation of ion channels and G protein-coupled receptors: insights from structures and simulations.** *Annu Rev Pharmacol Toxicol* 2020, **60**:31–50.
44. Hite RK, Butterwick JA, MacKinnon R: **Phosphatidic acid modulation of Kv channel voltage sensor function.** *Elife* 2014, **3**, e04366.
45. Hedger G, Rouse SL, Domański J, Chavent M, Koldsø H, Sansom MSP: **Lipid-loving ANTs: molecular simulations of cardiolipin interactions and the organization of the adenine nucleotide translocase in model mitochondrial membranes.** *Biochemistry* 2016, **55**:6238–6249.
46. Gupta K, Donlan JAC, Hopper JTS, Uzdevinys P, Landreh M, Struwe WB, Drew D, Baldwin AJ, Stansfeld PJ, Robinson CV: **The role of interfacial lipids in stabilizing membrane protein oligomers.** *Nature* 2017, **541**:421–424.
47. Laganowsky A, Reading E, Allison TM, Ulmschneider MB, Degiacomi MT, Baldwin AJ, Robinson CV: **Membrane proteins bind lipids selectively to modulate their structure and function.** *Nature* 2014, **510**:172–175.
48. Chadda R, Bernhardt N, Kelley EG, Teixeira SCM, Griffith K, Gil-Ley A, Öztürk TN, Hughes LE, Forsythe A, Krishnamani V, et al.: **Membrane transporter dimerization driven by differential lipid solvation energetics of dissociated and associated states.** *Elife* 2021, **10**, e63288.
- The study reports that ion channel oligomerization is mediated by the energetics of transmembrane domain solvation by lipids rather than by direct protein binding.
49. Zila V, Margiotta E, Turoňová B, Müller TG, Zimmerli CE, Mattei S, Allegretti M, Börner K, Rada J, Müller B, et al.: **Cone-shaped HIV-1 capsids are transported through intact nuclear pores.** *Cell* 2021, **184**:1032–1046.e18.
- The study provides evidence for the astonishing plasticity of the NPC by directly visualizing intact capsids of HIV-1 translocating through NPCs.
50. Romanauska A, Köhler A: **The inner nuclear membrane is a metabolically active territory that generates nuclear lipid droplets.** *Cell* 2018, **174**:700–715. e18.
51. Romanauska A, Köhler A: **Reprogrammed lipid metabolism protects inner nuclear membrane against unsaturated fat.** *Dev Cell* 2021, **56**:2562–2578.e3.
- This study demonstrates the tightly-controlled metabolic equilibrium between saturated and unsaturated lipids at the nuclear envelope of *S.cerevisiae* cells.
52. Cheng LC, Baboo S, Lindsay C, Brusman L, Martinez-Bartolomé S, Tapia O, Zhang X, Yates JR, Gerace L: **Identification of new transmembrane proteins concentrated at the nuclear envelope using organellar proteomics of mesenchymal cells.** *Nucleus* 2019, **10**:126–143.
53. Tsuji T, Cheng J, Tatematsu T, Ebata A, Kamikawa H, Fujita A, Gyobu S, Segawa K, Arai H, Taguchi T, et al.: **Predominant localization of phosphatidylserine at the cytoplasmic leaflet of the ER, and its TMEM16K-dependent redistribution.** *Proc Natl Acad Sci USA* 2019, **116**:13368–13373.
54. Heinrich M, Tian A, Esposito C, Baumgart T: **Dynamic sorting of lipids and proteins in membrane tubes with a moving phase boundary.** *Proc Natl Acad Sci U S A* 2010, **107**:7208–7213.
55. Sorre B, Callan-Jones A, Manneville J-B, Nassoy P, Joanny J-F, Prost J, Goud B, Bassereau P: **Curvature-driven lipid sorting needs proximity to a demixing point and is aided by proteins.** *Proc Natl Acad Sci USA* 2009, **106**:5622–5626.
56. Kuiper EFE, Gallardo P, Bergsma T, Mari M, Kolbe Muszkopf M, Kuipers J, Giepmans BNG, Steen A, Kampinga HH, Veenhoff LM, et al.: **The chaperone DNAJB6 surveils FG-nucleoporins and is required for interphase nuclear pore complex biogenesis.** *Nat Cell Biol* 2022, **24**:1584–1594.
- The study describes the FG-binding molecular chaperone DNAJB6 in safeguarding against nuclear envelope herniations during NPC biogenesis.
57. Prophet SM, Rampello AJ, Niescier RF, Gentile JE, Mallik S, Koleske AJ, Schlieker C: **Atypical nuclear envelope condensates linked to neurological disorders reveal nucleoporin-directed chaperone activities.** *Nat Cell Biol* 2022, **24**:1630–1641.
- The study describes nuclear envelope blebbing and formation of aberrant FG-Nup condensates within them upon malfunction of the AAA + ATPase TorsinA.
58. Thaller DJ, Patrick Lusk C: **Fantastic nuclear envelope herniations and where to find them.** *Biochem Soc Trans* 2018, **46**:877–889.
59. Kralt A, Wojtynek M, Fischer JS, Agote-Aran A, Mancini R, Dultz E, Noor E, Uliana F, Tatarek-Nossol M, Antonin W, et al.: **An amphipathic helix in Brl1 is required for nuclear pore complex biogenesis in *S. cerevisiae*.** *Elife* 2022, **11**, e78385.
- The study links Brl1, a proposed nuclear envelope fusion factor, with impressive nuclear envelope herniations upon perturbed NPC biogenesis.
60. Vitale J, Khan A, Neuner A, Schiebel E: **A perinuclear α -helix with amphipathic features in Brl1 promotes NPC assembly.** *Mol Biol Cell* 2022, **33**:ar35.
- The study links Brl1, a proposed nuclear envelope fusion factor, with impressive nuclear envelope herniations upon perturbed NPC biogenesis.
61. Thaller DJ, Tong D, Marklew CJ, Ader NR, Mannino PJ, Borah S, King MC, Ciani B, Lusk CP: **Direct binding of ESCRT protein Chm7 to phosphatidic acid-rich membranes at nuclear envelope herniations.** *JCB (J Cell Biol)* 2021:220.
62. Levental KR, Malmberg E, Symons JL, Fan Y-Y, Chapkin RS, Ernst R, Levental I: **Lipidomic and biophysical homeostasis of mammalian membranes counteracts dietary lipid perturbations to maintain cellular fitness.** *Nat Commun* 2020, **11**:1339.

4. DISCUSSION

4.1. Comparison of nuclear basket models

The nuclear basket, a prominent fishtrap-like structure on the nucleoplasmic face of the NPC core, is the final frontier in the structural characterization of the NPC. Although the integration of high-resolution structures of individual nucleoporins or subcomplexes with low-resolution cryo-ET maps of NPCs has yielded near-atomic models of the core scaffold of the NPC (*Kim et al., 2018; Allegretti et al., 2020; Akey et al., 2022; Li et al., 2022; Akey et al., 2023; Mosalaganti et al., 2022; Petrovic et al., 2022; Huang et al., 2020; Huang et al., 2022*), the flexibility of the nuclear basket calls for novel approaches. The study, which dissected the key principles of the nuclear basket organization (*Cibulka et al., 2022*), is now followed by the multi-pronged approach presented in this thesis, which sheds light on the elusive mechanism by which the megadalton structure is docked onto the core scaffold of the NPC (*Stankunas&Köhler, 2024*).

The interface between the core scaffold and the nuclear basket filaments is organized by Nup60 (see section 3.1. *Results 1*), a subunit of the nuclear basket that has been previously ascribed the central role in the organization of the nuclear basket itself (*Cibulka et al., 2022*). The principle of nuclear basket organization, namely, tying the subunits together via interactions mediated by Nup60 SLiMs, extends to the core-basket interface (see section 3.1. *Results 1*). To this end, Nup60 harbours three adjacent SLiMs, which bring together the Y-complex (SLiMs A and C), a key building block of the outer rings, and Mlp1 basket filaments (SLiM B), forming a presumably flexible complex (*Stankunas&Köhler, 2024*). The biochemical mapping of interactions between Nup60 SLiM C and Nup85·Seh1 arm of the Y-complex and spatial proximity between SLiMs B and C position Mlp1 N60BD in vicinity to Nup85·Seh1 arm of the Y-complex (*Stankunas&Köhler, 2024*). This would, in turn, position Mlp1 basket filaments and an NPC accessory proteome over the central channel of the NPC primed for the multitude of functionalities nuclear basket has been implicated in (reviewed in *Bensidoun et al., 2021*).

The positioning of Nup60 density on Nup85·Seh1 arm of the Y-complex in the integrative model of yeast nuclear basket is in full agreement with the model proposed in this thesis (*Singh et al., 2024; Stankunas&Köhler, 2024*). However, the positioning of Mlp1 filaments in relation to the Y-complex diverges: the integrative model features association between Mlp1/TPR and Nup84/NUP107 subunit of Y-complex, whereas the model proposed in the thesis positions Mlp1 close to Nup85·Seh1 arm based on the proximity of Nup60 SLiMs B and C (*Singh et al., 2024; Stankunas&Köhler, 2024*). The proposed positioning of Mlp1 filaments is in better agreement with the partial nuclear basket densities resolved in cryo-ET

reconstruction of immunopurified NPCs from *S. cerevisiae*, where rod-like density, which was interpreted as Mlp1/2, was only anchored to the short arm of the Y-complex (Nup85·Seh1) (Kim *et al.*, 2018). Similarly, recently resolved bifurcated density, which was interpreted as part of the nuclear basket, was in contact with Nup85·Seh1 arm of the Y-complex, among other nucleoporins (Allegretti *et al.*, 2020). It is unknown to what degree the discrepancy reflects differences in experimental methodology (in-cell NPCs vs immunopurified NPCs), image processing or inherent differences between NPC isoforms (double nuclear ring vs single nuclear ring). Future cryo-ET studies will be essential to resolve remaining ambiguities by systematically comparing reconstructions derived from different NPC isoforms and different genetic backgrounds.

Moreover, none of the proposed models so far explicitly account for Nup1, Mlp2 and Pml39 and their role in the nuclear basket structure (for a more in-depth discussion, see section 4.5. *Recruitment of nuclear filaments and formation of perinuclear foci*). Future structural and functional interrogation of the nuclear basket will also have to address recently reported stoichiometry between nuclear basket filament subunits (4:2:1 for Mlp1:Mlp2:Pml39) (Zsok *et al.*, 2024).

4.2. Functional consequences of interface organized by SLiMs

The principle of nuclear basket organization, namely, tying the subunits together via interactions mediated by Nup60 SLiMs, extends to the core scaffold-nuclear basket interface and provides a mechanistic explanation for a plethora of nuclear basket-related observations (see section 3.1. *Results 1*).

First, the low contact surface areas associated with SLiM-mediated interactions at the interface between NPC scaffold and nuclear basket enables Nup60 SLiMs to detach and re-bind, likely contributing to NPC plasticity during transport flux, important for large cargo molecules, such as ribosomal subunits or mRNP (Kiseleva *et al.*, 1998). Moreover, SLiM-mediated attachment of Nup60 to the core scaffold likely aids its fast exchange rate (Hakhverdyan *et al.*, 2022). It is noteworthy that Nup60, Nup1 and Nup2 are among the fastest exchangers of *S. cerevisiae* NPC, in line with the nature of interactions identified in this thesis and previously published reports (Hakhverdyan *et al.*, 2022; Cibulka *et al.*, 2022; Stankunas&Köhler, 2024).

Based purely on the mapped interaction between Nup60 SLiM B and Mlp1 N60BD, the observation that deletion of SLiM B leads to Mlp1-positive perinuclear foci as well as the dynamic nature of Nup60, one would presume that Mlp1, similarly to Nup60, displays high exchange rates. However, the experimental evidence, both from metabolic labelling

experiment and RITE measurements, shows stable association of Mlp1 with NPC core scaffolds (*Hakhverdyan et al., 2021; Zsok et al., 2024*). Moreover, depletion of Nup60 does not lead to the mislocalization of already assembled Mlp1 (*King et al., 2023; Zsok et al., 2024*). The dispensable nature of Nup60 in terms of Mlp1 retention could be explained by additional contact Mlp1 presumably forms upon incorporation into the NPC (although recruitment is totally dependent on Nup60). Previously published XL-MS data on immunopurified NPCs (*Kim et al., 2018*) showed numerous cross-links between Mlp1/2 and core scaffold nucleoporins providing a list of candidate nucleoporins for further characterization in order to dissect the molecular mechanism responsible for the maintenance of Mlp1 upon depletion of Nup60.

Moreover, the low contact surface areas associated with SLiM-mediated interactions enable straightforward regulation of such interactions. The post-translational modifications (PTMs) of amino acid residues constituting the interface between interaction partners could modify the affinity providing an attractive avenue for the regulation of assembly and disassembly of macromolecular machines. The interactions mapped in this thesis open the future avenues to mechanistically dissect previously reported PTMs of Nup60 (*King et al., 2023*) as well as human ortholog NUP153 (*McCloskey et al., 2018*).

4.3. Competition between Mlp1 and Y-complex for Nup60 binding

The in-built redundancy in Nup60 SLiMs follows a broader topic of nucleoporin redundancy, especially in *S. cerevisiae*, which underwent whole-genome duplication during its evolution (*Wolfe&Shields, 1997, Kellis et al., 2004*). Although SLiMs B and C have very defined and distinct binding specificities to Mlp1 N60BD and Nup85, respectively, SLiM A displays dual, although lower affinity to both nucleoporins. The fact that the supramolecular complex between Nup60, Y-complex and Mlp1 could only be reconstituted in the presence of all three SLiMs suggests that overlapping affinities play a critical role in tethering the nuclear basket to the core scaffold of the NPC.

It is important to note that GUV binding assays revealed an unexplored aspect of competition between Mlp1 and Y-complex for the binding of Nup60 (see section 3.1. *Results* 1). Deletions of either Nup60 SLiM A or SLiM C, which significantly reduced the GUV recruitment efficiency of the Y-complex, led to the enhanced recruitment of Mlp1 compared to Nup60 WT (*Stankunas&Köhler, 2024*). One of the possible underlying mechanisms could be steric clashes arising from the co-recruitment of two relatively large and elongated complexes (heteroheptameric Y-complex and dimeric NusA- and mCherry-tagged Mlp1 N60BD). Future cell-based studies are required to validate the *in vivo* relevance of observed competitive behaviour *in vitro*.

4.4. Function of Nup60 HR

Noteworthy is the observation that specific deletions of Nup60 SLiMs (or combination of SLiM deletions) shift the steady-state equilibrium of Nup60 towards INM-unbound nucleoplasmic pool of Nup60 even in the absence of genetic AH perturbations (*Stankunas&Köhler, 2024*). Out of the tested individual deletions, the deletion of HR resulted in the most pronounced shift from INM-bound pool to nucleoplasmic pool (*Stankunas&Köhler, 2024*). The dynamics of Nup60 could be modelled as a system of equilibrium processes, namely:

1. Equilibrium between nucleoplasmic pool of Nup60 and INM-bound pool of Nup60 (membrane binding – unbinding)
2. Equilibrium between nucleoplasmic pool of Nup60 and NPC-bound pool of Nup60 (NPC binding – unbinding)
3. Equilibrium between INM-bound pool of Nup60 and INM-bound, NPC-bound pool of Nup60 (NPC binding – unbinding when bound to INM)

In such a system, any mutation affecting the binding of Nup60 to the core scaffold of the NPC would shift the equilibrium towards the nucleoplasmic pool of Nup60, in agreement with previous observations (*Meszaros et al., 2015; Stankunas&Köhler, 2024*). Moreover, this system is likely modulated by HR-mediated dimerization or higher-order oligomerization of Nup60 (*Stankunas&Köhler, 2024*). Relatively low affinity of HR-HR interactions (K_d of $\sim 80 \mu\text{M}$) suggests that nucleoplasmic Nup60 likely exists in a predominantly monomeric state. However, once 8 or 16 copies of Nup60 are recruited to the nucleoplasmic ring of the NPC, the effective concentration of HRs likely exceeds the K_d and leads to the formation of dimers (oligomers). Although such proposal requires further experimental validation, coupling of Nup60 dimerization with its incorporation into the NPC provides an explanation for how the yeast NPC isoform with double nuclear ring could stabilize the nuclear basket (*Singh et al., 2024*).

Interestingly, an N-terminal cluster of Nup60 phosphosites, previously shown to be upregulated during meiotic divisions, which are accompanied with the transient detachment of Nup60 and Nup2, map to Nup60 HR (*King et al., 2023*). Although the authors argued that phosphorylation of Nup60 HR may disrupt its interaction with the Y-complex based on previously published low-resolution cryo-EM map of yeast NPC (*King et al., 2023; Kim et al., 2018*), the results presented herein raises a possibility of phosphorylation-modulated dimerization or higher-order oligomerization of Nup60, which could in turn regulate its affinity

to the core scaffold of the NPC. Moreover, monoubiquitylation of the HR of Nup60 has been implicated in altering its association with the NPC (*Niño et al., 2016*). It is also interesting to note that fluorescently tagged Nup60 mislocalizes in the yeast cells lacking Nup145C, a subunit of the Y-complex (*Feurbach et al., 2002*). Future studies will have to decipher the molecular mechanism behind these observations and unambiguously identify the core scaffold element that Nup60 HR binds to.

4.5. Recruitment of nuclear filaments and formation of perinuclear foci

It has been previously reported that Nup60 Mlp1-binding motif (aa240-318) is necessary to recruit Mlp1 to the NPCs (*Cibulka et al., 2022*). The work presented in this thesis improves the resolution of mapped interactions and narrows it down to Nup60 SLiM B (aa267-284), a stretch of just 18 amino acid residues, which plays a critical role in recruitment of Mlp1 filaments (*Stankunas&Köhler, 2024*). The fact that deletion of Nup60 SLiM B results in the Mlp1 mislocalization pattern visually indistinguishable from the phenotype of *nup60Δ* strain demonstrates that SLiM B is essential for Mlp1 recruitment to the NPCs. Live-cell imaging of yeast cells expressing combinatorial Nup60 SLiM deletion mutants provided novel insights into the curious mislocalization pattern of Mlp1. The Mlp1-positive perinuclear puncta were also observed when NPC recruitment of Nup60 was perturbed genetically, even though Nup60 retained SLiM B (*Stankunas&Köhler, 2024*). This observation suggests that it is the recruitment of Mlp1 filaments to the NPCs that keep them in an unaggregated state, not a mere interaction with Nup60 SLiM B. Interestingly, Mlp1/2-positive perinuclear foci also appear upon heat shock (*Carmody et al., 2010; Bensidoun et al., 2022*) or upon overexpression of Mlp1 (*Strambio-de-Castillia et al., 1999*).

Recent studies have implicated Pml39 (ZC3HC1) in bridging individual Mlp1 (TPR) molecules together into supramolecular assemblies ('puncta') (*Gunkel et al., 2022; Gunkel et al., 2023*). Deletion of *PML39* leads to the decrease of Mlp1 signal at the NE (*Gunkel et al., 2023; Zsok et al., 2024*). Similarly, targeted auxin-inducible depletion of Pml39 leads to the increase of nucleoplasmic Mlp1 signal, though fraction of Mlp1 still remains at the NE (*Bensidoun et al., 2022*). Most strikingly, whereas deletion of *NUP60* results in the perinuclear foci of Mlp1, combined deletion of *NUP60* and *PML39* leads to the diffuse nucleoplasmic staining of Mlp1 (*Gunkel et al., 2023*), suggesting that Pml39 plays an important role in the formation of perinuclear foci. However, the molecular details of the interactions and structural understanding of resulting complexes remain scarce. Yeast-two-hybrid assay revealed interaction between Pml39 and N-terminal regions of both Mlp1 and Mlp2 (*Palancade et al., 2005*). Interestingly, the measurement of fluorescence intensities of single NPCs in non-

nucleolar region yielded 2:1 stoichiometry between Mlp1 dimers and Pml39 (Zsok *et al.*, 2024), which would be in complete agreement with the model of Pml39-mediated cross-linking of Mlp1 dimers.

The crystal structure of Pml39 has only recently been solved (Hashimoto *et al.*, 2022), whereas the high-resolution structural data on Mlp1 filaments is still missing. The insights gathered from the rotary shadowing EM images reported herein paves the way for future structural characterization of one of the last elements of the NPC.

4.6. Asymmetric assembly of nuclear baskets

The assembly of the nuclear basket must be tightly regulated in order to eventually establish two-level asymmetry: 1) asymmetry across the nuclear envelope, so that nuclear baskets are only assembled onto the nucleoplasmic face of the core scaffold; 2) asymmetry along the INM perimeter, which establishes nuclear basket filament-free area in vicinity to the nucleolus. Whereas the asymmetry across the nuclear envelope is true for all nuclear basket subunits, the asymmetry along the INM perimeter holds true only for the nuclear basket filament subunits (Mlp1, Mlp2 and Pml39).

The two types of asymmetry are likely established independently and at different timepoints. The NPC assembly model based on metabolic labelling experiments puts core nuclear basket subunits (Nup60, Nup1, Nup2) in the intermediate tier (Onischenko *et al.*, 2020), suggesting that asymmetry across the NE is established relatively early during the biogenesis of the NPC. However, the molecular determinants behind the asymmetric docking of its appendages remain elusive, as both cytoplasmic filaments and the nuclear basket are docked onto virtually identical support, i.e. outer rings. Previous attempts to rationalize the asymmetric tethering of cytoplasmic filaments in human NPCs have revealed that mechanisms beyond simple steric hindrance are involved (Bley *et al.*, 2022). The conclusion is drawn from the observation that human nuclear basket component ELYS is found exclusively on the nucleoplasmic face of the NPC, despite the fact that the corresponding site on the cytoplasmic face remains unoccupied (Bley *et al.*, 2022).

Recently reported Ran injection experiments in syncytial blastoderm embryos of *Drosophila melanogaster* and structural characterization of yeast intranuclear NPCs upon *NOT4* deletion suggest that the type of appendage docked onto the core scaffold of the NPC depends on the nucleotide state of small GTPase Ran: RanGTP leads to the formation of the nuclear basket on the nucleoplasmic face, whereas RanGDP promotes the appending of the cytoplasmic filaments (Sachweh *et al.*, 2024). According to the proposed model, Ran exerts its influence on the composition of the NPC via NTR-mediated enrichment of peripheral

nucleoporins in distinct compartments (e.g. nuclear basket components are enriched in nucleus, RanGTP-rich compartment, by the RanGTP-facilitated disassembly of import complexes) (*Sachweh et al., 2024*). Although the effect of Ran was demonstrated convincingly, additional experimental data is required to support the proposed model of core scaffold asymmetrisation.

Metabolic labelling experiments suggest that nuclear basket filaments (Mlp1, Mlp2 and likely Pml39) are the last to be recruited (*Onischenko et al., 2020*) and only to the subset of the core scaffolds embedded in the NE away from the nucleolus (*Galy et al., 2004; Palancade et al., 2005; Bensidoun et al., 2022*). Therefore, a second type of asymmetry is established later during the process of NPC biogenesis, likely in a mechanism dependent on Pol II activity and nuclear mRNA metabolism (*Bensidoun et al., 2022*), a type of RNA that nucleolus is depleted of. However, the depletion of Rpb2, a subunit of Pol II, does not lead to the redistribution of Mlp1-positive NPCs to the nucleolar region (*Zsok et al., 2024*), suggesting that Mlp1-positive NPCs are not retained in non-nucleolar region in an mRNA-dependent manner. The exact mechanism for nucleolus exclusion remains to be unveiled in future studies.

The recruitment of basket filaments is also co-dependent on Nup60 as SLiM B is essential for the tethering of Mlp1 (*Stankunas&Köhler, 2024*). This raises a question how the interaction between Nup60 SLiM B and Mlp1 N60BD is regulated during the assembly of the nuclear basket. It is known that the biogenesis of NPC in *S. cerevisiae* is asynchronous (*Winey et al., 1997*), which suggests that regulation takes place at individual NPCs and does not affect global levels of either Nup60 or Mlp1. One possibility is transcription-coupled regulation, e.g. transcription-coupled post-translational modification of already incorporated Nup60. A recent report suggests that all NPCs are capable of exporting mRNPs via the Mex67-dependent pathway (*Bensidoun et al., 2022*). These observations raise the possibility that regulation of nuclear basket assembly could be coupled to the increased local transcriptional load, and subsequently, increase in export demand. Therefore, appending nuclear basket filaments to the NPCs in vicinity to nuclear region with increased transcription levels could be an evolutionary adaptation to safeguard the quality of mRNP being exported (as nuclear basket, first and foremost, is important for the retention of immature mRNPs). This is a very exciting possibility; however, future studies will have to assess the validity of the proposed mechanism.

4.7. Future applications of approach

The AlphaFold-based screening approach presented in the thesis holds even greater promise with the advent of more sophisticated and accurate deep learning models, such as

AlphaFold3 and its derivatives. The in-silico approach presented in the thesis could be applied to addressing research questions related to the intermolecular interaction networks present in other macromolecular machines, e.g. spindle pole body. Even more exciting future avenues are opened by the possibility to include nucleic acids and small-molecule ligands in the predictions of advanced artificial intelligence (AI) models. The structural predictions and corresponding hypotheses will have to be subjected to rigorous experimental tests in order to verify the proposed models. The experimental dissection of the mechanism underlying the docking of the nuclear basket onto the core scaffold of NPC demonstrated the feasibility of the approach and lays a rich foundation for elucidating nuclear basket structure and function.

5. REFERENCES

- Akey, C. W., Echeverria, I., Ouch, C., Nudelman, I., Shi, Y., Wang, J., Chait, B. T., Sali, A., Fernandez-Martinez, J., & Rout, M. P. (2023). Implications of a multiscale structure of the yeast nuclear pore complex. *Molecular Cell*, 83(18), 3283-3302.e5. <https://doi.org/10.1016/j.molcel.2023.08.025>
- Akey, C. W., Singh, D., Ouch, C., Echeverria, I., Nudelman, I., Varberg, J. M., Yu, Z., Fang, F., Shi, Y., Wang, J., Salzberg, D., Song, K., Xu, C., Gumbart, J. C., Suslov, S., Unruh, J., Jaspersen, S. L., Chait, B. T., Sali, A., ... Rout, M. P. (2022). Comprehensive structure and functional adaptations of the yeast nuclear pore complex. *Cell*, 185(2), 361-378.e25. <https://doi.org/10.1016/j.cell.2021.12.015>
- Aksenova, V., Smith, A., Lee, H., Bhat, P., Esnault, C., Chen, S., Iben, J., Kaufhold, R., Yau, K. C., Echeverria, C., Fontoura, B., Arnaoutov, A., & Dasso, M. (2020). Nucleoporin TPR is an integral component of the TREX-2 mRNA export pathway. *Nature Communications*, 11(1). <https://doi.org/10.1038/s41467-020-18266-2>
- Alber, F., Dokudovskaya, S., Veenhoff, L. M., Zhang, W., Kipper, J., Devos, D., Suprpto, A., Karni-Schmidt, O., Williams, R., Chait, B. T., Rout, M. P., & Sali, A. (2007). Determining the architectures of macromolecular assemblies. *Nature*, 450(7170), 683–694. <https://doi.org/10.1038/nature06404>
- Albert, S., Schaffer, M., Beck, F., Mosalaganti, S., Asano, S., Thomas, H. F., Plitzko, J. M., Beck, M., Baumeister, W., & Engel, B. D. (2017). Proteasomes tether to two distinct sites at the nuclear pore complex. *Proceedings of the National Academy of Sciences of the United States of America*, 114(52), 13726–13731. <https://doi.org/10.1073/pnas.1716305114>
- Allegretti, M., Zimmerli, C. E., Rantos, V., Wilfling, F., Ronchi, P., Fung, H. K. H., Lee, C. W., Hagen, W., Turoňová, B., Karius, K., Börmel, M., Zhang, X., Müller, C. W., Schwab, Y., Mahamid, J., Pfander, B., Kosinski, J., & Beck, M. (2020). In-cell architecture of the nuclear pore and snapshots of its turnover. *Nature*, 586(7831), 796–800. <https://doi.org/10.1038/s41586-020-2670-5>
- Bayliss, R., Ribbeck, K., Akin, D., Kent, H. M., Feldherr, C. M., Go, D., & Stewart, M. (1999). Interaction Between NTF2 and xFxFG-containing Nucleoporins is Required to Mediate Nuclear Import of RanGDP. *Journal of Molecular Biology*, 293, 579–593. <http://www.idealibrary.com>
- Beck, M., Förster, F., Ecke, M., Plitzko, J. M., Melchior, F., Gerisch, G., Baumeister, W., & Ohad, M. (2004). Nuclear Pore Complex Structure and Dynamics

Revealed by Cryoelectron Tomography. *Science*, 306, 1387–1390.
<https://www.science.org>

Beck, M., Lůí, V., Förster, F., Baumeister, W., & Medalia, O. (2007). Snapshots of nuclear pore complexes in action captured by cryo-electron tomography. *Nature*, 449(7162), 611–615. <https://doi.org/10.1038/nature06170>

Bensidoun, P., Reiter, T., Montpetit, B., Zenklusen, D., & Oeffinger, M. (2022). Nuclear mRNA metabolism drives selective basket assembly on a subset of nuclear pore complexes in budding yeast. *Molecular Cell*, 82(20), 3856-3871.e6. <https://doi.org/10.1016/j.molcel.2022.09.019>

Bensidoun, P., Zenklusen, D., & Oeffinger, M. (2021). Choosing the right exit: How functional plasticity of the nuclear pore drives selective and efficient mRNA export. In *Wiley Interdisciplinary Reviews: RNA* (Vol. 12, Issue 6). John Wiley and Sons Inc. <https://doi.org/10.1002/wrna.1660>

Bley, C. J., Nie, S., Mobbs, G. W., Petrovic, S., Gres, A. T., Liu, X., Mukherjee, S., Harvey, S., Huber, F. M., Lin, D. H., Brown, B., Tang, A. W., Rundlet, E. J., Correia, A. R., Chen, S., Regmi, S. G., Stevens, T. A., Jette, C. A., Dasso, M., ... Hoelz, A. (2022). Architecture of the cytoplasmic face of the nuclear pore. *Science*, 376(6598). <https://doi.org/10.1126/science.abm9129>

Bonner, W. M. (1975). Protein Migration into Nuclei. I. Frog Oocyte Nuclei In Vivo Accumulate Microinjected Histones, Allow Entry to Small Proteins, and Exclude Large Proteins. *The Journal of Cell Biology*, 64, 421–430. <http://rupress.org/jcb/article-pdf/64/2/421/1629818/421.pdf>

Bonnet, A., & Palancade, B. (2014). Regulation of mrna trafficking by nuclear pore complexes. In *Genes* (Vol. 5, Issue 3, pp. 767–791). MDPI AG. <https://doi.org/10.3390/genes5030767>

Bragulat-Teixidor, H., Ishihara, K., Szűcs, G. M., & Otsuka, S. (2024). The endoplasmic reticulum connects to the nucleus by constricted junctions that mature after mitosis. *EMBO Reports*, 25(7), 3137–3159. <https://doi.org/10.1038/s44319-024-00175-w>

Brickner, J. H., & Walter, P. (2004). Gene recruitment of the activated INO1 locus to the nuclear membrane. *PLoS Biology*, 2(11). <https://doi.org/10.1371/journal.pbio.0020342>

Cabal, G. G., Genovesio, A., Rodriguez-Navarro, S., Zimmer, C., Gadal, O., Lesne, A., Buc, H., Feuerbach-Fournier, F., Olivo-Marin, J. C., Hurt, E. C., & Nehrbass,

U. (2006). SAGA interacting factors confine sub-diffusion of transcribed genes to the nuclear envelope. *Nature*, 441(7094), 770–773. <https://doi.org/10.1038/nature04752>

Carmody, S. R., Tran, E. J., Apponi, L. H., Corbett, A. H., & Wente, S. R. (2010). The Mitogen-Activated Protein Kinase Slt2 Regulates Nuclear Retention of Non-Heat Shock mRNAs during Heat Shock-Induced Stress. *Molecular and Cellular Biology*, 30(21), 5168–5179. <https://doi.org/10.1128/mcb.00735-10>

Celetti, G., Paci, G., Caria, J., VanDelinder, V., Bachand, G., & Lemke, E. A. (2020). The liquid state of FG-nucleoporins mimics permeability barrier properties of nuclear pore complexes. *Journal of Cell Biology*, 219(1). <https://doi.org/10.1083/jcb.201907157>

Cho, U. H., & Hetzer, M. W. (2020). Nuclear Periphery Takes Center Stage: The Role of Nuclear Pore Complexes in Cell Identity and Aging. In *Neuron* (Vol. 106, Issue 6, pp. 899–911). Cell Press. <https://doi.org/10.1016/j.neuron.2020.05.031>

Chug, H., Trakhanov, S., Hülsmann, B. B., Pleiner, T., Görlich, D. (2015). Crystal structure of the metazoan Nup62•Nup58•Nup54 nucleoporin complex. *Science*, 350(6256), 106–110. <https://doi.org/10.1126/science.aac4690>

Cibulka, J., Bisaccia, F., Radisavljević, K., Carrillo, R. M. G., & Köhler, A. (2022). Assembly principle of a membrane-anchored nuclear pore basket scaffold. *Science Advances*, 8. <https://www.science.org>

Daigle, N., Beaudouin, J., Hartnell, L., Imreh, G., Hallberg, E., Lippincott-Schwartz, J., & Ellenberg, J. (2001). Nuclear pore complexes form immobile networks and have a very low turnover in live mammalian cells. *Journal of Cell Biology*, 154(1), 71–84. <https://doi.org/10.1083/jcb.200101089>

D'Angelo, M. A., Raices, M., Panowski, S. H., & Hetzer, M. W. (2009). Age-Dependent Deterioration of Nuclear Pore Complexes Causes a Loss of Nuclear Integrity in Postmitotic Cells. *Cell*, 136(2), 284–295. <https://doi.org/10.1016/j.cell.2008.11.037>

Dickson, C. F., Hertel, S., Tuckwell, A. J., Li, N., Ruan, J., Al-Izzi, S. C., Ariotti, N., Sieracki, E., Gambin, Y., Morris, R. G., Towers, G. J., Böcking, T., & Jacques, D. A. (2024). The HIV capsid mimics karyopherin engagement of FG-nucleoporins. *Nature*, 626(8000), 836–842. <https://doi.org/10.1038/s41586-023-06969-7>

Dilworth, D. J., Suprpto, A., Padovan, J. C., Chait, B. T., Wozniak, R. W., Rout, M. P., & Aitchison, J. D. (2001). Nup2p Dynamically Associates with the Distal Regions of the Yeast Nuclear Pore Complex. In *The Journal of Cell Biology* (Vol. 153, Issue 7). <http://www.jcb.org/cgi/content/full/153/7/1465>

Drin, G., Casella, J. F., Gautier, R., Boehmer, T., Schwartz, T. U., & Antonny, B. (2007). A general amphipathic α -helical motif for sensing membrane curvature. *Nature Structural and Molecular Biology*, 14(2), 138–146. <https://doi.org/10.1038/nsmb1194>

Eisenhardt, N., Redolfi, J., & Antonin, W. (2014). Interaction of Nup53 with Ndc1 and Nup155 is required for nuclear pore complex assembly. *Journal of Cell Science*, 127(4), 908–921. <https://doi.org/10.1242/jcs.141739>

Fahrenkrog, B., Hurt, E. C., Aebi, U., Panté, N., & Müller, M. E. (1998). Molecular Architecture of the Yeast Nuclear Pore Complex: Localization of Nsp1p Subcomplexes. In *The Journal of Cell Biology* (Vol. 143, Issue 3). <http://www.jcb.org>

Fernandez-Martinez, J., Phillips, J., Sekedat, M. D., Diaz-Avalos, R., Velazquez-Muriel, J., Franke, J. D., Williams, R., Stokes, D. L., Chait, B. T., Sali, A., & Rout, M. P. (2012). Structure-function mapping of a heptameric module in the nuclear pore complex. *Journal of Cell Biology*, 196(4), 419–434. <https://doi.org/10.1083/jcb.201109008>

Feuerbach, F., Galy, V., Trelles-Sticken, E., Fromont-Racine, M., Jacquier, A., Gilson, E., Olivo-Marin, J. C., Scherthan, H., & Nehrbass, U. (2002). Nuclear architecture and spatial positioning help establish transcriptional states of telomeres in yeast. *Nature Cell Biology*, 4(3), 214–221. <https://doi.org/10.1038/ncb756>

Franke, W. W., Scheer, U., Krohne, G., & Jarasch, E.-D. (1981). The Nuclear Envelope and the Architecture of the Nuclear Periphery The Nuclear Envelope Is a Membrane Cisterna Interrupted by Pore Complexes. *The Journal of Cell Biology*, 91(3), 39–50. <http://rupress.org/jcb/article-pdf/91/3/39s/1640012/39s.pdf>

Frey, S., & Görlich, D. (2007). A Saturated FG-Repeat Hydrogel Can Reproduce the Permeability Properties of Nuclear Pore Complexes. *Cell*, 130(3), 512–523. <https://doi.org/10.1016/j.cell.2007.06.024>

Frey, S., Rees, R., Schünemann, J., Ng, S. C., Fünfgeld, K., Huyton, T., & Görlich, D. (2018). Surface Properties Determining Passage Rates of Proteins through Nuclear Pores. *Cell*, 174(1), 202–217.e9. <https://doi.org/10.1016/j.cell.2018.05.045>

Fu, L., Weiskopf, E. N., Akkermans, O., Swanson, N. A., Cheng, S., Schwartz, T. U., & Görlich, D. (2024). HIV-1 capsids enter the FG phase of nuclear pores like a transport receptor. *Nature*, 626(8000), 843–851. <https://doi.org/10.1038/s41586-023-06966-w>

Gaik, M., Flemming, D., Von Appen, A., Kastitis, P., Mücke, N., Fischer, J., Stelter, P., Ori, A., Bui, K. H., Baßler, J., Barbar, E., Beck, M., & Hurt, E. (2015). Structural

basis for assembly and function of the Nup82 complex in the nuclear pore scaffold. *Journal of Cell Biology*, 208(3), 283–297. <https://doi.org/10.1083/jcb.201411003>

Galy, V., Gadai, O., Fromont-Racine, M., Romano, A., Jacquier, A., & Nehrbass, U. (2004). Nuclear Retention of Unspliced mRNAs in Yeast Is Mediated by Perinuclear Mlp1. *Cell*, 116, 63–73. <http://www.cell>.

Gao, Y., Skowrya, M. L., Feng, P., & Rapoport, T. A. (2022). Protein import into peroxisomes occurs through a nuclear pore–like phase. *Science*, 378(6625). <https://doi.org/10.1126/science.adf3971>

Goldberg, M. W., & Allen, T. D. (1992). High Resolution Scanning Electron Microscopy of the Nuclear Envelope: Demonstration of a New, Regular, Fibrous Lattice Attached to the Baskets of the Nucleoplasmic Face of the Nuclear Pores. *Journal of Cell Biology*, 119, 1429–1440.

Goldberg, M. W., & Allen, T. D. (1993). The nuclear pore complex: three-dimensional surface structure revealed by field emission, in-lens scanning electron microscopy, with underlying structure uncovered by proteolysis. *Journal of Cell Science*, 106, 261–274.

Goldberg, M. W., & Allen, T. D. (1996). The Nuclear Pore Complex and Lamina: Three-dimensional Structures and Interactions Determined by Field Emission In-lens Scanning Electron Microscopy. In *J. Mol. Biol* (Vol. 257).

Grandi, P., Schlaich, N., Tekotte, H., & Hurt, E. C. (1995). Functional interaction of Nic96p with a core nucleoporin complex consisting of Nsp1p, Nup49p and a novel protein Nup57p. *EMBO Journal*, 14(1), 76–87. <https://doi.org/10.1002/j.1460-2075.1995.tb06977.x>

Gunkel, P., & Cordes, V. C. (2022). ZC3HC1 is a structural element of the nuclear basket effecting interlinkage of TPR polypeptides. *Molecular Biology of the Cell*, 33(9). <https://doi.org/10.1091/mbc.E22-02-0037>

Gunkel, P., Iino, H., Krull, S., & Cordes, V. C. (2023). An evolutionarily conserved bimodular domain anchors ZC3HC1 and its yeast homologue Pml39p to the nuclear basket. *Molecular Biology of the Cell*, 34(5). <https://doi.org/10.1091/mbc.E22-09-0402>

Hakhverdyan, Z., Molloy, K. R., Keegan, S., Herricks, T., Lepore, D. M., Munson, M., Subbotin, R. I., Fenyő, D., Aitchison, J. D., Fernandez-Martinez, J., Chait, B. T., & Rout, M. P. (2021). Dissecting the Structural Dynamics of the Nuclear Pore Complex. *Molecular Cell*, 81(1), 153-165.e7. <https://doi.org/10.1016/j.molcel.2020.11.032>

Hase, M. E., & Cordes, V. C. (1923). Direct Interaction with Nup153 Mediates Binding of Tpr to the Periphery of the Nuclear Pore Complex. *Molecular Biology of the Cell*, 14. <https://doi.org/10.1091/mbc.E02-09>

Hase, M. E., Kuznetsov, N. V., & Cordes, V. C. (2001). Amino Acid Substitutions of Coiled-Coil Protein Tpr Abrogate Anchorage to the Nuclear Pore Complex but Not Parallel, In-Register Homodimerization. In *Molecular Biology of the Cell* (Vol. 12).

Hashimoto, H., Ramirez, D. H., Lautier, O., Pawlak, N., Blobel, G., Palancade, B., & Debler, E. W. (2022). Structure of the pre-mRNA leakage 39-kDa protein reveals a single domain of integrated zf-C3HC and Rsm1 modules. *Scientific Reports*, 12(1). <https://doi.org/10.1038/s41598-022-22183-3>

Hinshaw, J. E., & Milligan, R. A. (2003). Nuclear pore complexes exceeding eightfold rotational symmetry. *Journal of Structural Biology*, 141(3), 259–268. [https://doi.org/10.1016/S1047-8477\(02\)00626-3](https://doi.org/10.1016/S1047-8477(02)00626-3)

Hoffmann, P. C., Kim, H., Obarska-Kosinska, A., Kreysing, J. P., Andino-Frydman, E., Cruz-León, S., Margiotta, E., Cernikova, L., Kosinski, J., Turoňová, B., Hummer, G., & Beck, M. (2025). Nuclear pore permeability and fluid flow are modulated by its dilation state. *Molecular Cell*, 85(3), 537-554.e11. <https://doi.org/10.1016/j.molcel.2024.11.038>

Huang, G., Zhan, X., Zeng, C., Liang, K., Zhu, X., Zhao, Y., Wang, P., Wang, Q., Zhou, Q., Tao, Q., Liu, M., Lei, J., Yan, C., & Shi, Y. (2022). Cryo-EM structure of the inner ring from the *Xenopus laevis* nuclear pore complex. *Cell Research*, 32(5), 451–460. <https://doi.org/10.1038/s41422-022-00633-x>

Huang, G., Zhan, X., Zeng, C., Zhu, X., Liang, K., Zhao, Y., Wang, P., Wang, Q., Zhou, Q., Tao, Q., Liu, M., Lei, J., Yan, C., & Shi, Y. (2022). Cryo-EM structure of the nuclear ring from *Xenopus laevis* nuclear pore complex. *Cell Research*, 32(4), 349–358. <https://doi.org/10.1038/s41422-021-00610-w>

Huang, G., Zhang, Y., Zhu, X., Zeng, C., Wang, Q., Zhou, Q., Tao, Q., Liu, M., Lei, J., Yan, C., & Shi, Y. (2020). Structure of the cytoplasmic ring of the *Xenopus laevis* nuclear pore complex by cryo-electron microscopy single particle analysis. *Cell Research*, 30(6), 520–531. <https://doi.org/10.1038/s41422-020-0319-4>

Izaurralde, E., Kutay, U., Von Kobbe, C., Mattaj, I. W., & Gö Rlich, D. (1997). The asymmetric distribution of the constituents of the Ran system is essential for transport into and out of the nucleus. In *The EMBO Journal* (Vol. 16, Issue 21). <https://www.embopress.org>

Kampmann, M., & Blobel, G. (2009). Three-dimensional structure and flexibility of a membrane-coating module of the nuclear pore complex. *Nature Structural and Molecular Biology*, 16(7), 782–788. <https://doi.org/10.1038/nsmb.1618>

Kelley, K., Knockenhauer, K. E., Kabachinski, G., & Schwartz, T. U. (2015). Atomic structure of the y complex of the nuclear pore. *Nature Structural and Molecular Biology*, 22(5), 425–431. <https://doi.org/10.1038/nsmb.2998>

Kellis, M., Birren, B. W., & Lander, E. S. (2004). Proof and evolutionary analysis of ancient genome duplication in the yeast *Saccharomyces cerevisiae*. *Nature*, 428, 617–624. <http://www.broad.mit.edu/seq/YeastDuplication>

Kim, S. J., Fernandez-Martinez, J., Nudelman, I., Shi, Y., Zhang, W., Raveh, B., Herricks, T., Slaughter, B. D., Hogan, J. A., Upla, P., Chemmama, I. E., Pellarin, R., Echeverria, I., Shivaraju, M., Chaudhury, A. S., Wang, J., Williams, R., Unruh, J. R., Greenberg, C. H., ... Rout, M. P. (2018a). Integrative structure and functional anatomy of a nuclear pore complex. *Nature*, 555(7697), 475–482. <https://doi.org/10.1038/nature26003>

Kim, S. J., Fernandez-Martinez, J., Nudelman, I., Shi, Y., Zhang, W., Raveh, B., Herricks, T., Slaughter, B. D., Hogan, J. A., Upla, P., Chemmama, I. E., Pellarin, R., Echeverria, I., Shivaraju, M., Chaudhury, A. S., Wang, J., Williams, R., Unruh, J. R., Greenberg, C. H., ... Rout, M. P. (2018b). Integrative structure and functional anatomy of a nuclear pore complex. *Nature*, 555(7697), 475–482. <https://doi.org/10.1038/nature26003>

Kim, S. J., Fernandez-Martinez, J., Sampathkumar, P., Martel, A., Matsui, T., Tsuruta, H., Weiss, T. M., Shi, Y., Markina-Inarrairaegui, A., Bonanno, J. B., Sauder, J. M., Burley, S. K., Chait, B. T., Almo, S. C., Rout, M. P., & Sali, A. (2014). Integrative structure-function mapping of the nucleoporin Nup133 suggests a conserved mechanism for membrane anchoring of the nuclear pore complex. *Molecular and Cellular Proteomics*, 13(11), 2911–2926. <https://doi.org/10.1074/mcp.M114.040915>

King, G. A., Wettstein, R., Varberg, J. M., Chetlapalli, K., Walsh, M. E., Gillet, L. C. J., Hernández-Armenta, C., Beltrao, P., Aebersold, R., Jaspersen, S. L., Matos, J., & Ünal, E. (2023). Meiotic nuclear pore complex remodeling provides key insights into nuclear basket organization. *Journal of Cell Biology*, 222(2). <https://doi.org/10.1083/jcb.202204039>

Kiseleva, E., Goldberg, M. W., Allen, T. D., & Akey, C. W. (1998). Active nuclear pore complexes in *Chironomus*: visualization of transporter configurations related to mRNP export. *Journal of Cell Science*, 111, 223–236.

Krull, S., Dörries, J., Boysen, B., Reidenbach, S., Magnius, L., Norder, H., Thyberg, J., & Cordes, V. C. (2010). Protein Tpr is required for establishing nuclear pore-associated zones of heterochromatin exclusion. *EMBO Journal*, 29(10), 1659–1673. <https://doi.org/10.1038/emboj.2010.54>

Li, Z., Chen, S., Zhao, L., Huang, G., Pi, X., Sun, S., Wang, P., & Sui, S. F. (2022). Near-atomic structure of the inner ring of the *Saccharomyces cerevisiae* nuclear pore complex. *Cell Research*, 32(5), 437–450. <https://doi.org/10.1038/s41422-022-00632-y>

Lin, D. H., & Hoelz, A. (2019). The Structure of the Nuclear Pore Complex (An Update). *Annual Review of Biochemistry*, 88, 725–783. <https://doi.org/10.1146/annurev-biochem-062917>

Lutzmann, M., Kunze, R., Buerer, A., Aebi, U., & Hurt, E. (2002). Modular self-assembly of a Y-shaped multiprotein complex from seven nucleoporins. *The EMBO Journal*, 21, 387–397.

Matsuda, A., Mansour, A., & Mofrad, M. R. K. (2024). Deciphering the intrinsically disordered characteristics of the FG-Nups through the lens of polymer physics. In *Nucleus* (Vol. 15, Issue 1). <https://doi.org/10.1080/19491034.2024.2399247>

Matunis, M. J., Wu, J., & Blobel, G. (1998). SUMO-1 Modification and Its Role in Targeting the Ran GTPase-activating Protein, RanGAP1, to the Nuclear Pore Complex. In *The Journal of Cell Biology* (Vol. 140, Issue 3). <http://www.jcb.org>

Maul, G. G., Maul, H. M., Scogna, J. E., Lieberman, M. W., Stein, G. S., Hsu, B. Y.-L., & Borun, T. W. (1972). Time sequence of nuclear pore formation in phytohemagglutinin-stimulated lymphocytes and in HeLa cells during the cell cycle. *Journal of Cell Biology*, 55, 433–447.

McCloskey, A., Ibarra, A., & Hetzer, M. W. (2018). Tpr regulates the total number of nuclear pore complexes per cell nucleus. *Genes and Development*, 32(19–20), 1321–1331. <https://doi.org/10.1101/GAD.315523.118>

Mészáros, N., Cibulka, J., Mendiburo, M. J., Romanauska, A., Schneider, M., & Köhler, A. (2015). Nuclear Pore Basket Proteins Are Tethered to the Nuclear Envelope and Can Regulate Membrane Curvature. *Developmental Cell*, 33(3), 285–298. <https://doi.org/10.1016/j.devcel.2015.02.017>

Mi, L., Goryaynov, A., Lindquist, A., Rexach, M., & Yang, W. (2015). Quantifying Nucleoporin Stoichiometry Inside Single Nuclear Pore Complexes In vivo. *Scientific Reports*, 5. <https://doi.org/10.1038/srep09372>

Moor, H., & Mijhlettaler, K. (1963). Fine structure in frozen-etched yeast cells. *Journal of Cell Biology*, 17, 609–628. <http://rupress.org/jcb/article-pdf/17/3/609/1535007/609.pdf>

Mosalaganti, S., Kosinski, J., Albert, S., Schaffer, M., Strenkert, D., Salomé, P. A., Merchant, S. S., Plitzko, J. M., Baumeister, W., Engel, B. D., & Beck, M. (2018). In situ architecture of the algal nuclear pore complex. *Nature Communications*, 9(1). <https://doi.org/10.1038/s41467-018-04739-y>

Mosalaganti, S., Obarska-Kosinska, A., Siggel, M., Taniguchi, R., Turoňová, B., Zimmerli, C. E., Buczak, K., Schmidt, F. H., Margiotta, E., Mackmull, M. T., Hagen, W. J. H., Hummer, G., Kosinski, J., & Beck, M. (2022). AI-based structure prediction empowers integrative structural analysis of human nuclear pores. *Science*, 376(6598). <https://doi.org/10.1126/science.abm9506>

Niepel, M., Molloy, K. R., Williams, R., Farrar, J. C., Meinema, A. C., Vecchietti, N., Cristea, I. M., Chait, B. T., Rout, M. P., & Strambio-De-Castillia, C. (2013). The nuclear basket proteins Mlp1p and Mlp2p are part of a dynamic interactome including Esc1p and the proteasome. *Molecular Biology of the Cell*, 24(24), 3920–3938. <https://doi.org/10.1091/mbc.E13-07-0412>

Niño, C. A., Guet, D., Gay, A., Brutus, S., Jourquin, F., Mendiratta, S., Salamero, J., Géli, V., & Dargemont, C. (2016). Posttranslational marks control architectural and functional plasticity of the nuclear pore complex basket. *Journal of Cell Biology*, 212(2), 167–180. <https://doi.org/10.1083/jcb.201506130>

Onischenko, E., Noor, E., Fischer, J. S., Gillet, L., Wojtynek, M., Vallotton, P., & Weis, K. (2020). Maturation Kinetics of a Multiprotein Complex Revealed by Metabolic Labeling. *Cell*, 183(7), 1785-1800.e26. <https://doi.org/10.1016/j.cell.2020.11.001>

Onischenko, E., Stanton, L. H., Madrid, A. S., Kieselbach, T., & Weis, K. (2009). Role of the Ndc1 interaction network in yeast nuclear pore complex assembly and maintenance. *Journal of Cell Biology*, 185(3), 475–491. <https://doi.org/10.1083/jcb.200810030>

Ori, A., Banterle, N., Iskar, M., Andrés-Pons, A., Escher, C., Khanh Bui, H., Sparks, L., Solis-Mezarino, V., Rinner, O., Bork, P., Lemke, E. A., & Beck, M. (2013). Cell type-specific nuclear pores: A case in point for context-dependent stoichiometry of

molecular machines. *Molecular Systems Biology*, 9(1).
<https://doi.org/10.1038/msb.2013.4>

Otsuka, S., Buy, K. H., Schorb, M., Hossain, M. J., Politi, A. Z., Koch, B., Eltsov, M., Beck, M., & Ellenberg, J. (2016). Nuclear pore assembly proceeds by an inside-out extrusion of the nuclear envelope. *ELife*, 5. <https://doi.org/10.7554/eLife.19071.001>

Otsuka, S., & Ellenberg, J. (2018). Mechanisms of nuclear pore complex assembly – two different ways of building one molecular machine. In *FEBS Letters* (Vol. 592, Issue 4, pp. 475–488). Wiley Blackwell. <https://doi.org/10.1002/1873-3468.12905>

Otsuka, S., Steyer, A. M., Schorb, M., Hériché, J. K., Hossain, M. J., Sethi, S., Kueblbeck, M., Schwab, Y., Beck, M., & Ellenberg, J. (2018). Postmitotic nuclear pore assembly proceeds by radial dilation of small membrane openings. *Nature Structural and Molecular Biology*, 25(1), 21–28. <https://doi.org/10.1038/s41594-017-0001-9>

Otsuka, S., Tempkin, J. O. B., Zhang, W., Politi, A. Z., Rybina, A., Hossain, M. J., Kueblbeck, M., Callegari, A., Koch, B., Morero, N. R., Sali, A., & Ellenberg, J. (2023). A quantitative map of nuclear pore assembly reveals two distinct mechanisms. *Nature*, 613(7944), 575–581. <https://doi.org/10.1038/s41586-022-05528-w>

Palancade, B. B., Zuccolo, M., Loeillet, S., Nicolas, A., & Doye, V. (2005). Pml39, a Novel Protein of the Nuclear Periphery Required for Nuclear Retention of Improper Messenger Ribonucleoparticles □ *D. Molecular Biology of the Cell*, 16, 5258–5268. <https://doi.org/10.1091/mbc.E05-06>

Petrovic, S., Mobbs, G. W., Bley, C. J., Nie, S., Patke, A., & Hoelz, A. (2022). Structure and Function of the Nuclear Pore Complex. *Cold Spring Harbor Perspectives in Biology*, 14(12). <https://doi.org/10.1101/cshperspect.a041264>

Petrovic, S., Samanta, D., Perriches, T., Bley, C. J., Thierbach, K., Brown, B., Nie, S., Mobbs, G. W., Stevens, T. A., Liu, X., Tomaleri, G. P., Schaus, L., & Hoelz, A. (2022). Architecture of the linker-scaffold in the nuclear pore. *Science*, 376(6598). <https://doi.org/10.1126/science.abm9798>

Ptak, C., Aitchison, J. D., & Wozniak, R. W. (2014). The multifunctional nuclear pore complex: A platform for controlling gene expression. In *Current Opinion in Cell Biology* (Vol. 28, Issue 1, pp. 46–53). Elsevier Ltd. <https://doi.org/10.1016/j.ceb.2014.02.001>

Rajoo, S., Vallotton, P., Onischenko, E., & Weis, K. (2018). Stoichiometry and compositional plasticity of the yeast nuclear pore complex revealed by quantitative

fluorescence microscopy. *Proceedings of the National Academy of Sciences of the United States of America*, 115(17), E3969–E3977. <https://doi.org/10.1073/pnas.1719398115>

Rapoport, T. A., Li, L., & Park, E. (2017). Structural and Mechanistic Insights into Protein Translocation. *Annual Review of Cell and Developmental Biology*, 33, 369–390. <https://doi.org/10.1146/annurev-cellbio-100616>

Ribbeck, K., & Görlich, D. (2001). Kinetic analysis of translocation through nuclear pore complexes. *EMBO Journal*, 20(6), 1320–1330. <https://doi.org/10.1093/emboj/20.6.1320>

Rout, M. P., Aitchison, J. D., Suprpto, A., Hjertaas, K., Zhao, Y., & Chait, B. T. (2000). The Yeast Nuclear Pore Complex: Composition, Architecture, and Transport Mechanism. In *The Journal of Cell Biology* (Vol. 148, Issue 4). <http://www.jcb.org>

Sachweh, J., Börmel, M., Klumpe, S., Becker, A., Taniguchi, R., Kubańska, M. A., Pintschovius, V., Kaindl, E., Plitzko, J. M., Wilfling, F., Beck, M., & Hampoelz, B. (2024). The small GTPase Ran defines Nuclear Pore Complex Asymmetry. *BioRxiv Preprint*. <https://doi.org/10.1101/2024.10.10.617378>

Schmidt, H. B. roder, & Görlich, D. (2015). Nup98 FG domains from diverse species spontaneously phase-separate into particles with nuclear pore-like permselectivity. *ELife*, 4. <https://doi.org/10.7554/eLife.04251>

Schuller, A. P., Wojtynek, M., Mankus, D., Tatli, M., Kronenberg-Tenga, R., Regmi, S. G., Dip, P. V., Lytton-Jean, A. K. R., Brignole, E. J., Dasso, M., Weis, K., Medalia, O., & Schwartz, T. U. (2021). The cellular environment shapes the nuclear pore complex architecture. *Nature*, 598(7882), 667–671. <https://doi.org/10.1038/s41586-021-03985-3>

Seo, H.-S., Ma, Y., Debler, E. W., Wacker, D., Kutik, S., Nter Blobel, G., & Hoelz, A. (2009). Structural and functional analysis of Nup120 suggests ring formation of the Nup84 complex. *Proceedings of the National Academy of Sciences of the United States of America*, 106(34), 14281–14286. www.pnas.org/cgi/doi/10.1073/pnas.0907453106

Shi, Y., Fernandez-Martinez, J., Tjioe, E., Pellarin, R., Kim, S. J., Williams, R., Schneidman-Duhovny, D., Sali, A., Rout, M. P., & Chait, B. T. (2014). Structural characterization by cross-linking reveals the detailed architecture of a coatomer-related heptameric module from the nuclear pore complex. *Molecular and Cellular Proteomics*, 13(11), 2927–2943. <https://doi.org/10.1074/mcp.M114.041673>

Singh, D., Soni, N., Hutchings, J., Echeverria, I., Shaikh, F., Duquette, M., Suslov, S., Li, Z., van Eeuwen, T., Molloy, K., Shi, Y., Wang, J., Guo, Q., Chait, B. T.,

Fernandez-Martinez, J., Rout, M. P., Sali, A., & Villa, E. (2024). The molecular architecture of the nuclear basket. *Cell*. <https://doi.org/10.1016/j.cell.2024.07.020>

Siniosoglou, S., Lutzmann, M., Santos-Rosa, H., Leonard, K., Mueller, S., Aebi, U., & Hurt, E. (2000). Structure and assembly of the Nup84p complex. *Journal of Cell Biology*, 149(1), 41–53. <https://doi.org/10.1083/jcb.149.1.41>

Siniosoglou, S., & Wimmer, C. (1996). A Novel Complex of Nucleoporins, Which Includes Sec13p and a Sec13p Homolog, Is Essential for Normal Nuclear Pores. In *Cell* (Vol. 84).

Souquet, B., Freed, E., Berto, A., Andric, V., Audugé, N., Reina-San-Martin, B., Lacy, E., & Doye, V. (2018). Nup133 Is Required for Proper Nuclear Pore Basket Assembly and Dynamics in Embryonic Stem Cells. *Cell Reports*, 23(8), 2443–2454. <https://doi.org/10.1016/j.celrep.2018.04.070>

Stankunas, E., & Köhler, A. (2023). The interplay of nuclear pores and lipids. In *Current Opinion in Cell Biology* (Vol. 85). Elsevier Ltd. <https://doi.org/10.1016/j.ceb.2023.102251>

Stankunas, E., & Köhler, A. (2024). Docking a flexible basket onto the core of the nuclear pore complex. *Nature Cell Biology*, 26(9), 1504–1519. <https://doi.org/10.1038/s41556-024-01484-x>

Stewart, M. (2007). Molecular mechanism of the nuclear protein import cycle. In *Nature Reviews Molecular Cell Biology* (Vol. 8, Issue 3, pp. 195–208). <https://doi.org/10.1038/nrm2114>

Strambio-De-Castillia, C., Blobel, G., & Rout, M. P. (1999). Proteins Connecting the Nuclear Pore Complex with the Nuclear Interior. In *The Journal of Cell Biology* (Vol. 144, Issue 5). <http://www.jcb.org>

Strambio-De-Castillia, C., Niepel, M., & Rout, M. P. (2010). The nuclear pore complex: Bridging nuclear transport and gene regulation. In *Nature Reviews Molecular Cell Biology* (Vol. 11, Issue 7, pp. 490–501). <https://doi.org/10.1038/nrm2928>

Strawn, L. A., Shen, T., Shulga, N., Goldfarb, D. S., & Wente, S. R. (2004). Minimal nuclear pore complexes define FG repeat domains essential for transport. *Nature Cell Biology*, 6(3), 197–206. <https://doi.org/10.1038/ncb10097>

Stuwe, T., Bley, C. J., Thierbach, K., Petrovic, S., Schilbach, S., Daniel, †, Mayo, J., Perriches, T., Rundlet, E. J., Jeon, Y. E., Collins, L. N., Huber, F. M., Lin, D. H., Paduch, M., Koide, A., Lu, V., Fischer, J., Hurt, E., Koide, S., ... Hoelz, A. (2015). Architecture of

the fungal nuclear pore inner ring complex. *Science*, 350(6256), 56–64.
<https://www.science.org>

Stuwe, T., Correia, A. R., Lin, D. H., Paduch, M., Lu, V. T., Kossiakoff, A. A., & Hoelz, A. (2015). Architecture of the nuclear pore complex coat. *Science*, 347(6226), 1148–1152.

Taniguchi, R., Orniacki, C., Kreysing, J. P., Zila, V., Zimmerli, C. E., Böhm, S., Turoňová, B., Kräusslich, H.-G., Doye, V., & Beck, M. (2025). Nuclear pores safeguard the integrity of the nuclear envelope. *Nature Cell Biology*. <https://doi.org/10.1038/s41556-025-01648-3>

Thierbach, K., Von Appen, A., Thoms, M., Beck, M., Flemming, D., & Hurt, E. (2013). Protein interfaces of the conserved Nup84 complex from chaetomium thermophilum shown by crosslinking mass spectrometry and electron microscopy. *Structure*, 21(9), 1672–1682. <https://doi.org/10.1016/j.str.2013.07.004>

Vollmer, B., Lorenz, M., Moreno-Andrés, D., Bodenhöfer, M., De Magistris, P., Astrinidis, S. A., Schooley, A., Flötenmeyer, M., Leptihn, S., & Antonin, W. (2015). Nup153 Recruits the Nup107-160 Complex to the Inner Nuclear Membrane for Interphasic Nuclear Pore Complex Assembly. *Developmental Cell*, 33(6), 717–728. <https://doi.org/10.1016/j.devcel.2015.04.027>

Vosseberg, J., van Hooff, J. J. E., Köstlbacher, S., Panagiotou, K., Tamarit, D., & Ettema, T. J. G. (2024). The emerging view on the origin and early evolution of eukaryotic cells. In *Nature* (Vol. 633, Issue 8029, pp. 295–305). Nature Research. <https://doi.org/10.1038/s41586-024-07677-6>

Walther, T. C., Pickersgill, H. S., Cordes, V. C., Goldberg, M. W., Allen, T. D., Mattaj, I. W., & Fornerod, M. (2002). The cytoplasmic filaments of the nuclear pore complex are dispensable for selective nuclear protein import. *Journal of Cell Biology*, 158(1), 63–77. <https://doi.org/10.1083/jcb.200202088>

Wente, S. R., & Rout, M. P. (2010). The nuclear pore complex and nuclear transport. In *Cold Spring Harbor perspectives in biology* (Vol. 2, Issue 10). <https://doi.org/10.1101/cshperspect.a000562>

Wiedemann, N., & Pfanner, N. (2017). Mitochondrial Machineries for Protein Import and Assembly. *Annual Review of Biochemistry*, 86, 685–714. <https://doi.org/10.1146/annurev>

Winey, M., Yarar, D., Giddings, T. H., & Mastronarde, D. N. (1997). Nuclear Pore Complex Number and Distribution throughout the *Saccharomyces cerevisiae* Cell Cycle

by Three-Dimensional Reconstruction from Electron Micrographs of Nuclear Envelopes. In *Molecular Biology of the Cell* (Vol. 8).

Wolfe, K. H., & Shields, D. C. (1997). Molecular evidence for an ancient duplication of the entire yeast genome. *Nature*, 387, 708–713.

Yaseen, N. R., & Günter Blobel, G. (1999). Two distinct classes of Ran-binding sites on the nucleoporin Nup-358 (RanBP1-homologous domainsGDP-bound RanGTP-bound RanRanBP1zinc fingers). In *Cell Biology* (Vol. 96). www.pnas.org.

Yu, M., Heidari, M., Mikhaleva, S., Tan, P. S., Mingu, S., Ruan, H., Reinkemeier, C. D., Obarska-Kosinska, A., Siggel, M., Beck, M., Hummer, G., & Lemke, E. A. (2023). Visualizing the disordered nuclear transport machinery in situ. *Nature*, 617(7959), 162–169. <https://doi.org/10.1038/s41586-023-05990-0>

Zhao, X., Wu, C. Y., & Blobel, G. (2004). Mlp-dependent anchorage and stabilization of a desumoylating enzyme is required to prevent clonal lethality. *Journal of Cell Biology*, 167(4), 605–611. <https://doi.org/10.1083/jcb.200405168>

Zila, V., Margiotta, E., Turoňová, B., Müller, T. G., Zimmerli, C. E., Mattei, S., Allegretti, M., Börner, K., Rada, J., Müller, B., Lusic, M., Kräusslich, H. G., & Beck, M. (2021). Cone-shaped HIV-1 capsids are transported through intact nuclear pores. *Cell*, 184(4), 1032-1046.e18. <https://doi.org/10.1016/j.cell.2021.01.025>

Zimmerli, C. E., Allegretti, M., Rantos, V., Goetz, S. K., Obarska-Kosinska, A., Zagoriy, I., Halavatyi, A., Hummer, G., Mahamid, J., Kosinski, J., & Beck, M. (2021). Nuclear pores dilate and constrict in cellulo. *Science*, 374(6573). <https://doi.org/10.1126/science.abd9776>

Zsok, J., Simon, F., Bayrak, G., Isaki, L., Kerff, N., Kicheva, Y., Wolstenholme, A., Weiss, L. E., & Dultz, E. (2024). Nuclear basket proteins regulate the distribution and mobility of nuclear pore complexes in budding yeast. *Molecular Biology of the Cell*, 35(11). <https://doi.org/10.1091/mbc.E24-08-0371>

6. ABBREVIATIONS

AH	amphipathic helix
AI	artificial intelligence
ALPS	amphipathic lipid packing sensor
CF	cytoplasmic filament
CFNC	cytoplasmic filament nucleoporin complex
CNT	channel nucleoporin trimer
EM	electron microscopy
ER	endoplasmic reticulum
ET	electron tomography
FCS	fluorescence correlation spectroscopy
FG	phenylalanine-glycine (repeat)
FIB	focussed ion beam
FLIM-FRET	fluorescence lifetime imaging - Förster resonance energy transfer
GTP	guanosine-5'-triphosphate
HR	helical region
IDR	intrinsically disordered region
INM	inner nuclear membrane
IRC	inner ring complex
LLPS	liquid-liquid phase separation
MBM	Mlp1-binding motif
mESC	mouse embryonic stem cells
mRNA	messenger ribonucleic acid
mRNP	messenger ribonucleoprotein
N2BM	Nup2-binding motif
N60BD	Nup60-binding domain

NE	nuclear envelope
NPC	nuclear pore complex
NTR	nuclear transport receptor
ONM	outer nuclear membrane
PNS	perinuclear space
PTM	post-translational modification
RanGAP	Ran GTPase-activating protein
RITE	recombination induced tag exchange
SLiM	short linear interacting motif
TIM	translocase of the inner membrane
TOM	translocase of the outer membrane
XL-MS	crosslinking-coupled mass spectrometry