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The Effect of Mineral Particle Size of Goethite on DNA
Adsorption and Degradation

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Abstract

DNA obtained from environmental samples is an important source of genetic information for a variety of applications. However, when released by organisms into the extracellular environment, it is prone to rapid degradation by several biotic and abiotic decay pathways. DNA adsorption to mineral surfaces has been proposed to protect it against rapid environmental decay and promote its long-term persistence in the environment. The recent discovery of a two-million-year-old DNA from Greenland sediments further validates this hypothesis. As a charged biopolymer (commonly known as a polyelectrolyte), DNA exists in a wide range of polymer lengths, typically ranging from few base pairs (bp) to a few thousand bps in the environment. Varying polymer length of DNA can affect adsorption, thus we hypothesize that it may also play a role in its protection. Additionally, instead of being a homogeneous distribution of particle sizes, mineral crystallites also exist in a wide range of sizes. Despite decades long research in the field of DNA-mineral interactions, the effect of mineral particle size and polymer length on DNA adsorption and ultimately, DNA persistence is not well understood. This master's thesis investigates the effect of DNA polymer length (using 99 and 1000 bp DNA) and mineral particle size using goethite of three different surface areas (30.2, 61.5 and 91.2 m²/g) on adsorption and protection against enzymatic degradation. First goethite-DNA adsorption isotherms were generated using different particle sizes of goethite, then two more isotherm experiments focussing on DNA molecule size and impact of pH-value were performed in a similar manner. Lastly, after complete adsorption, DNA was subjected to two concentrations of DNase I (0.9 and 50 ng/μL), an enzyme that catalyses DNA hydrolytic cleavage, followed by desorption to analyse the fraction that survived. The results were measured using UV-vis spectroscopy, fluorometry and gel electrophoresis and reveal that the larger the specific surface area of the goethite mineral the less DNA mass per μL adsorbs. Furthermore, longer DNA fragments adsorbed better than shorter ones when standardized to same surface area per batch. The influence of solution pH was evident with ≈ 50% increased adsorption at pH 5 and 6 compared to those in the more alkaline range. After 48 hours of incubation an average of 90% of previously adsorbed DNA desorbed, with no significant impact of mineral particle size. The degradation experiments highlighted increased susceptibility

of longer DNA molecules towards degradation than shorter fragments, with stabilization of the polymer occurring at ≈ 100 bp. Additionally, less DNA survived when adsorbed to the smallest mineral particles than polymers adsorbed onto the larger goethite fractions. Based on these results it is concluded that smaller goethite particles' surface sites may be occupied more rapidly than those of e.g. the largest particles used in this study, depending on the way in which DNA fragments adsorb on the surface. Longer DNA fragments degraded more easily than shorter ones, however, further studies on the stabilization of DNA fragments after exposure to DNase I are required.

Kurzfassung

Die aus Umweltproben gewonnene DNA ist eine wichtige Quelle für genetische Informationen für eine Vielzahl von Anwendungen. Wenn sie jedoch von Organismen in die extrazelluläre Umwelt freigesetzt wird, ist sie anfällig für einen schnellen Abbau durch mehrere biotische und abiotische Zerfallswege. DNA-Adsorption an mineralischen Oberflächen wurde als Weg vorgeschlagen, um sie vor schneller Umweltzerstörung zu schützen und ihre langfristige Persistenz in der Umwelt zu fördern. Die jüngste Entdeckung einer zwei Millionen Jahre alten DNA aus grönländischen Sedimenten bestätigt diese Hypothese. Als geladenes Biopolymer (allgemein bekannt als Polyelektrolyt) existiert DNA in einer breiten Palette von Polymerlängen, die typischerweise von wenigen Basenpaaren (bp) bis zu einigen tausend bps in der Umwelt reichen. Die variierende Polymerlänge der DNA kann die Adsorption beeinflussen, daher vermuten wir, dass sie auch eine Rolle bei ihrem Schutz spielen kann. Zusätzlich, anstatt eine homogene Verteilung von Partikelgrößen zu sein, weisen Mineralkristalle ein breites Größenspektrum auf. Trotz jahrzehntelanger Forschung auf dem Gebiet der DNA-Mineral-Wechselwirkungen, ist der Einfluss der Mineralpartikelgröße und Polymerlänge auf die DNA-Adsorption und letztlich auch auf die DNA-Persistenz nicht vollständig geklärt. Diese Masterarbeit untersucht die Wirkung der DNA-Polymerlänge (mit 99 und 1000 bp DNA) und Mineralpartikelgröße unter Verwendung von Goethit mit drei verschiedenen Oberflächengrößen (30.2, 61.5 und 91.2 m²/g) auf das Adsorptionsverhalten und den Schutz gegen enzymatischen Abbau. Zuerst wurden Goethit-DNA-Adsorptionsisothermen unter Verwendung verschiedener Partikelgrößen von Goethit erstellt, dann wurden zwei weitere Isothermen-Experimente durchgeführt, die sich auf die DNA-Molekülgröße und den Einfluss des pH-Wertes konzentrierten. Schließlich wurde die DNA nach vollständiger Adsorption mit zwei Konzentrationen von DNase I (0.9 und 50 ng/μL), einem Enzym, das die hydrolytische DNA-Spaltung katalysiert, behandelt, gefolgt von einer Desorption zur Analyse der überlebenden Fraktion. Die Ergebnisse wurden mit UV-vis-Spektroskopie, Fluorometrie und Gelelektrophorese gemessen und zeigen, dass je größer die spezifische Oberfläche des Goethitminerals ist, desto weniger DNA-Masse pro μL adsorbiert wird. Darüber hinaus adsorbierten längere DNA-Fragmente besser als kürzere, wenn sie auf die gleiche

Oberfläche pro Charge standardisiert wurden. Der Einfluss des pH-Wertes der Lösung war offensichtlich, da die Adsorption bei pH 5 und 6 um 50% gegenüber den Adsorptionswerten im eher alkalischen Bereich erhöht war. Nach 48 Stunden Inkubation wurden durchschnittlich 90% der zuvor adsorbierten DNA desorbiert, ohne dass die Mineralpartikelgröße einen signifikanten Einfluss hatte. Die Abbauversuche zeigten eine erhöhte Anfälligkeit von längeren DNA-Molekülen gegenüber dem Abbau als kürzere Fragmente, wobei sich das Polymer bei ≈ 100 bp stabilisiert. Zusätzlich überlebte weniger DNA, wenn sie an die kleinsten Mineralpartikel adsorbiert wurde gegenüber Polymeren, die an die größeren Goethitfraktionen adsorbiert wurden. Aus diesen Ergebnissen wird geschlossen, dass die Oberflächenstellen von kleineren Goethitpartikeln schneller besetzt werden können als die von z.B. den größten in dieser Studie verwendeten Partikeln, abhängig davon, wie DNA-Fragmente an der Oberfläche adsorbieren. Längere DNA-Fragmente wurden leichter abgebaut als kürzere, jedoch sind weitere Studien zur Stabilisierung von DNA-Fragmenten nach der Exposition mit DNase I erforderlich.

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1 Introduction

1.1 Structure of DNA

Analysis of deoxyribonucleic acid (DNA) obtained from environmental samples provides a unique tool to explore our past and present. The genetic information stored in environmental DNA is used extensively in diverse fields, e.g., palaeoecology (Anderson-Carpenter et al. 2011, Hassan et al. 2022), biodiversity monitoring (DiBattista et al. 2019, Zinger et al. 2019), species distribution (Hobbs et al. 2019, Itakura et al. 2019, Matter et al. 2018), and may even play a role in facilitating horizontal gene transfer (Van Elsas et al. 2003). DNA is a biopolymer with a double-helical structure of two chains of polynucleotides. DNA polymers have three components, deoxyribose sugar, phosphate and nucleobases namely adenine (A), thymine (T), cytosine (C) and guanine (G). The backbone of the DNA polymer is composed of alternating sequence of phosphate groups and deoxyribose sugars, where phosphate is attached to the sugars at the C3' and C5' positions. The nucleobases are attached to the sugar at the C1' position (Figure 1). Thus, a nucleobase, a sugar and a phosphate group combined make a monomeric unit of DNA, termed as a nucleotide. The helical structure of these nucleic acids occurs in different conformations, for example the right-handed A and B form or the left-handed Z-form (Frank-Kamenetskii et al. 1987, Jares-Erijman & Jovin 1996). In aqueous media, DNA predominantly exists as B form. In addition, DNA acquires different three-dimensional shapes, such as the covalent closed circular (CCC) shape of plasmid DNA (Demanèche et al. 2001) and the linear form of chromosomal DNA (Poly et al. 2000). The length of DNA polymers varies from species to species.

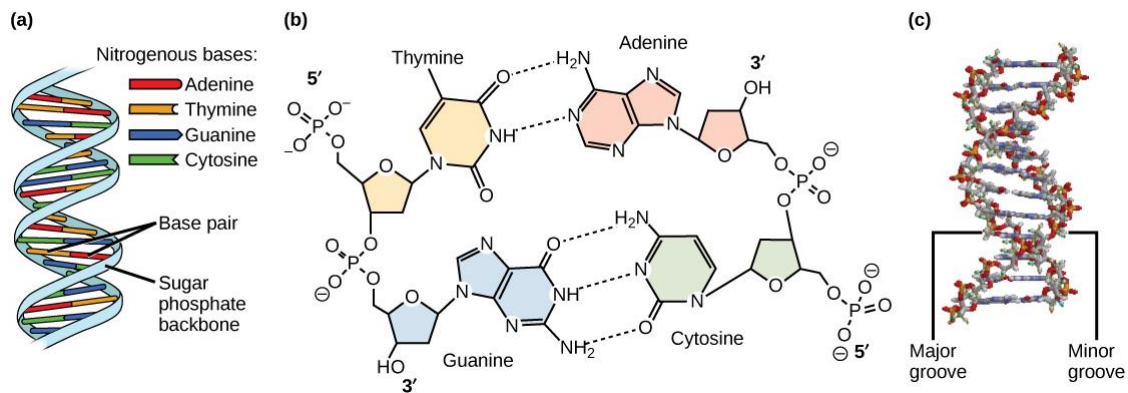


Figure 1. Structure of DNA. (a) Illustration of a double helix and complimentary bases adenine-thymine and cytosine-guanine. (b) Detail of phosphodiester groups connected through hydrogen bonds. (c) Major and minor grooves of sugar-phosphate backbone capable of interacting with DNA-binding proteins.

Source: <https://openintrobiology.pressbooks.tru.ca/chapter/unit4-1-3/>.

After Jerome Walker & Dennis Myts.

1.2 Environmental causes for DNA degradation

The genetic information in DNA is stored in the sequence of nucleotides. The longer the sequence the more the genetic information stored. To use the genetic information stored in DNA at its maximum potential, DNA is desired to be as minimally degraded as possible. However, DNA is a reactive molecule which suffers continuous physical and chemical damages in the environment through a variety of biotic and abiotic pathways. For example, fragmentation by nuclease enzymes that hydrolyse the phosphodiester backbone of the DNA is one of the major pathways of DNA decay in the environment (Lindahl 1993, Pääbo 1989). In plant DNA, mechanical shearing is a result of exposure to molecular stress fields (Levy et al. 1999). Extreme solution conditions such as high temperature and upper- and lower-end pH led to melting and dissociation reactions, entirely destroying the double-helix integrity (Kantidze et al. 2016, Wang et al. 2009). Lack of solution electrolytes also facilitates dissociation of DNA, hence why the composition of the aquatic environment is of great importance (Sidorova & Rau 2001). Reactive oxidative species (ROS) may cause lesions that disrupt the integrity of the double strands, leading to breakage and splitting (Cadet & Wagner 2013). Moreover, oxidative damage such as 5-hydroxy-5-methylhydantoin and 5-hydroxyhydantoin, can inhibit the amplification of DNA by polymerase enzymes (Dabney et al. 2013). One should consider that in the absence of water, there may be less exposure to potentially damaging reactants, however, oxidative damage is more likely to occur in dry

environments (Eglinton & Logan 1991). Since cytosine is susceptible to substitution with guanine, it is the main target for hydrolytic deamination, resulting in coding errors (Dabney et al. 2013). The most common of all DNA-damaging pathways is enzymatic hydrolysis of the DNA backbone induced by proteins like DNase I (Lindahl 1993). Inside the cells of living organisms, these damages are kept in check by a variety of cellular repair mechanisms. However, DNA can be released from the cellular compartments after the cell death or by active release (Pietramellara et al. 2009). Once the DNA is in the extracellular environment, the cellular repair mechanisms cease to exist, and physiochemical damage by a multitude of decay pathways accumulates in the DNA. Many of these damage pathways results in breaking of a long DNA polymer into shorter fragments. Because of this, ancient DNA obtained from the environment usually ranges from 40 to 500 bp in length, which is several orders of magnitude smaller than the DNA polymers present in living organisms (Pääbo 1989), and rapid fragmentation of modern DNA, for example extracellular DNA, has also been observed (Nielsen et al. 2007). Fragmentation of DNA has been commonly reported and is the result of all the deconstruction by microorganisms, intracellular nucleases and enzymatic hydrolysis of the phosphodiester backbone (Levy et al. 1999, Lindahl 1993, Pääbo 1989), with the latter being discussed extensively in this thesis. Once the sequence of nucleotides is disrupted, stored genetic information is lost forever, denying access to valuable information about ancient life and its evolution. Despite the prevalence of numerous damage pathways, a small quantity of ancient DNA is still found to be preserved in sediments and bones over significant timescales spanning human history. For example, DNA obtained from fossil bones have been extensively used to study human evolution and migration by anthropologists (Poinar 1999, Salamon et al. 2005, Slatkin & Racimo 2016, Slon et al. 2017). Findings of DNA in sediments suggest that the interaction of DNA with mineral surfaces might help in long-term persistence of DNA. In fact, the oldest 2-million-years old ancient DNA obtained to date was extracted from Greenland sediments further supports this hypothesis (Kjær et al. 2022)

1.3 DNA adsorption processes

The adsorption of nucleic acids, including DNA, on soil and mineral particles has been widely studied. Sodnikar et al. (2021) conducted dsRNA and DNA adsorption experiments on the iron oxide goethite, showing DNA adsorbed to the mineral surface until saturation of available binding sites was reached. Depending on the mineralogy, the kinetics and the total adsorption capacity varies. For example, Romanowski et al. (1991) found rapid adsorption of DNA to sand particles. After 60 seconds, 85% of the calculated sorption capacity was reached, which increased to 98% after 10 minutes.

Electrostatic attraction is one of the key mechanisms involved in DNA adsorption. It is dependent on the charge of the mineral surface and the phosphate backbone of DNA (Hou et al. 2014, Romanowski et al. 1991). The surface charge of a mineral may be subject to change, depending on surrounding solution conditions. For example, common clay minerals such as illite, kaolinite and montmorillonite are generally negatively charged, however, their surface charge may change (Yu et al. 2013). This is further backed by Schofield (1950), who at the time already established that depending on clay type edge sites with variable pH occur and may have net positive charge at environmental pH ranges. Tran et al. (2012) showed the influence of solution pH on the clay mineral surface charge as well as ionization state of proteins present. Divalent cations such as Ca^{2+} and Mg^{2+} have been shown to facilitate adsorption of DNA onto negatively charged mineral surfaces owing to charge screening and cation bridging (Romanowski et al. 1991, Hou et al. 2014). The impact of electrostatic forces and ionic strength has been demonstrated in multiple studies. Paget et al. (1992) noted the increase in DNA adsorption on montmorillonite in the presence of Ca^{2+} and Mg^{2+} . This was further confirmed by Saeki et al. (2010) with andosols and Brundin et al. (2013) for adsorption on hydroxyapatite. Cleaves II et al. (2011) adsorbed single-stranded DNA on olivine, pyrite, calcite, hematite and rutile, and concluded that the electrostatic forces and cation bridges are the main mechanisms involved in the DNA binding process. Most studies used clay minerals, as seen in Demanèche et al. (2001), Michalkova et al. (2011) and Hou et al. (2014) who used illite, montmorillonite, kaolinite, rectorite and sericite as sorbents. Michalkova et al. (2011) address another binding mechanism, namely the formation of weak hydrogen bonds in the outer layer of the mineral surface whereas Hou et al. (2014) explained that,

alongside with electrostatic forces and cation bridges, ligand exchange reactions take place at the mineral surface, leading to the formation of inner-sphere complexes. Inner-sphere complexes are covalent bonds between the phosphate ligands of DNA and the surface metal oxygen groups (Stumm 1995). Earlier studies on the adsorption of DNA onto the iron oxide, goethite, have shown that there is a higher affinity towards monodentate than bidentate complex formation (Omoike et al. 2004).

Besides the binding mechanisms involved, many factors influence the quantity of adsorbed DNA, with the most important being mineralogy of the sorbent, the availability of surface sites relative to the concentration of DNA as well as pH and ionic strength of solution. As discussed above, adsorption capacity is highly dependent on the particles selected. Poly et al. (2000) reported an increase in adsorption with increased amount of DNA added. We will confirm this observation in later chapters. It has also been well-established that with increasing pH adsorption decreases, which is attributed to a corresponding increase in negative charge of the mineral surface, which will be further discussed in chapters results and discussion (Antelo et al. 2005, Hou et al. 2014, Romanovski et al. 1991, Saeki et al. 2010, Sodnikar et al. 2021, Yu et al. 2013). In connection with the influence of electrostatic forces on DNA adsorption, the impact of ionic strength of solution has also been investigated. Ionic strength is a measure of ions present in solution. It is widely agreed upon that increased ionic strength positively influences adsorption processes (Cleaves II et al. 2011, Hou et al. 2014, Romanovski et al. 1991, Sodnikar et al. 2021). Lorenz & Wackernagel (1987) suggest the prevalence of weak van der Waals forces in the presence of monovalent cations whereas cation bridging is the dominant binding mechanism in presence of divalent cations. It is therefore evident that adsorption of DNA to minerals is common and may play a role in the preservation of DNA in various environmental compartments.

This variety of binding mechanisms has long been hypothesised to protect DNA from environmental decay. Several studies focussed on DNA adsorption to a variety of mineral surfaces and their effect of preservation against enzymatic degradation. For example, Hou et al. (2014) conducted experiments using salmon sperm DNA on hectorite, montmorillonite and smectite and concluded that the dominant adsorption mechanisms were mineral-specific. This dependence on surface chemistry was further addressed by Valdré et al. (2004) for layered silicates causing varying DNA adsorption patterns.

Brundin et al. (2013) found that DNA binding to hydroxyapatite counteracts decay processes, providing protection from enzymatic hydrolysis by DNase I in human sera. Similar protective effects were observed in studies for illite, kaolinite, montmorillonite, goethite, sand as well as inorganic clay, all reporting at least partial protection from DNase I (Romanowski et al. 1991; Paget et al. 1992; Blum et al. 1997; Demanèche et al. 2001; Cai et al. 2006). Additionally, Demanèche et al. (2001) found that adsorption of DNA to illite, kaolinite and montmorillonite, resulted in a decrease in DNA availability for bacterial degradation. Some studies concluded that protection of DNA is most likely to stem from adsorption of both DNA and DNase I (Cai et al. 2006, Demanèche et al. 2001, Paget et al. 1992), however, little is known about the effects of adsorption order and site competition. In addition, the impact of DNA polymer length on adsorption and protection is not well understood.

1.4 The role iron oxides on DNA preservation

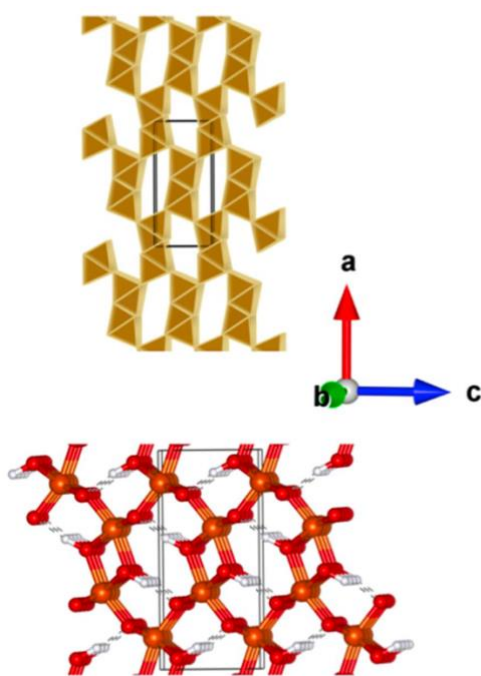


Figure 2. Crystal structure of goethite. Fe atoms are central coordinated surrounded by oxygen with hydrogen bonds in-between. Source: Chen et al. 2017.

Iron oxide minerals dominate the near surface environment due to their higher abundance and they often display a high reactivity owing to their high surface areas. One of these iron oxides, goethite, a crystalline iron (III)-(oxyhydr)-oxide with the chemical formula $\alpha\text{-FeOOH}$, is commonly found in soils and sediments and has been proven to be highly effective in binding DNA fragments to its surface (Schmidt & Martínez 2017). This iron oxide crystallizes according to the orthorhombic crystal class as shown in Figure 2 (Chen et al. 2017). The habitus of goethite resembles needle-shaped crystals with a dominance of the [110] plane and c-axis elongation (Hiemstra & van Riemsdijk 1996).

The tip is located at the [021] plane with alternating rows of singly and doubly coordinated surface groups (Hiemstra & van

Riemsdijk 1996). Hiemstra & van Riemsdijk (1996) also described the surface structure of goethite in three planes (Figure 3). The 0-plane located closest to the mineral surface where iron (Fe) ligands can participate in ligand exchange reactions. Plane 1, also referred to as the Stern-layer is occupied by solution ligands and the last plane, the 2/d diffuse double layer, sits closest to the solution. The charges of the Stern- and diffuse double layer even out the charge of the 0-plane (Sigg & Stumm 1981). Within these planes different binding processes may take place, namely electrostatic force attraction, cation bridging and ligand exchange reactions (Hou et al. 2014). After the latter, DNA binds to the hydroxyl groups of goethite (Sodnikar et al. 2021) and forms covalent P-O-Fe bonds (inner sphere complexes) (Omoike et al. 2004, Omoike & Chorover 2006).

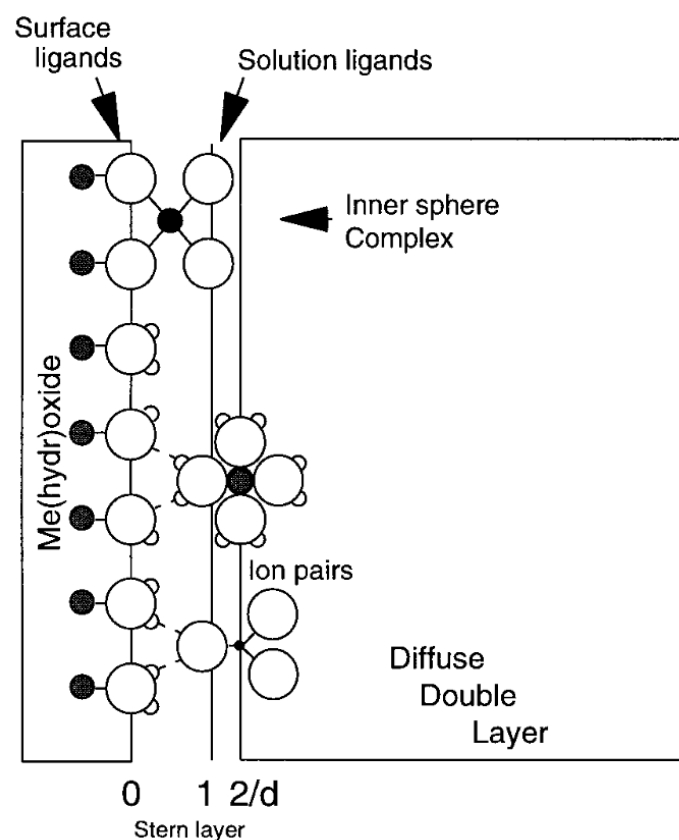


Figure 3. Three-layer surface structure of goethite. Source: Hiemstra & van Riemsdijk 1996.

Due to its small particle size ($< 2 \mu\text{m}$), goethite possesses a high specific surface area (SSA) that offers considerably more surface sites available for adsorption than those common in coarse soil fractions (Romanowski et al. 1991; Paget et al. 1992). For the

same reason, Parker et al. (2019) postulated that an overall finer soil texture should be the cause for increased adsorption of dsRNA. These clay-sized fractions are of great importance to sorption processes in soils and sensitive to environmental parameters with varying particle size. For example, Borggaard et al. (1983) showed how mineral SSA directly correlates to the point of zero charge (PZC) of goethite. Mineral samples with a SSA of 16 m²/g had a PZC of 7.2, whereas for those with a SSA of 82 m²/g it increased to 7.6. Another study observed enhanced aggregation for goethite particles with the largest SSA of 132.6 m²/g (Strauss et al. 1997a) and further noted how reaction time increases with increasing SSA (Strauss et al. 1997b). This gives an overall idea of the impact of SSA when studying the adsorption of DNA, however, it is important to point out that in natural systems homogenic SSA is not achievable and highly depends on the transformation of other iron oxides such as ferrihydrite and hematite (Blesa & Matijević 1989). For better comparability of different SSA, studies under laboratory conditions are an important step towards understanding the impact of particle size on mineral-DNA interactions. On top of this, the type of mineral also plays a major role in DNA adsorption behaviour. Cao et al. (2011) showed in their study that phosphorus has a higher affinity for adsorption to goethite over kaolinite and montmorillonite, respectively. By forming inner-sphere complexes with the nucleic acid phosphate groups, goethite is also capable of strongly binding DNA to its surface (Omoike et al. 2004). Although clay minerals are generally negatively charged, the charge of the iron-bearing goethite mineral surface is dependant on the pH and ionic strength of the surrounding medium (Sodnikar et al. 2021) and the presence of cations (Romanowski et al. 1991, Paget et al. 1992, Poly et al. 2000, Saeki et al. 2010, Cleaves II et al 2011, Michalkova et al. 2011 & Hou et al. 2014).

2 Research objectives

As shown in the previous chapter, research on the adsorption of DNA onto mineral surfaces has been plentiful. However, despite the great knowledge of the adsorption processes of DNA on mineral surfaces, the mechanisms responsible for DNA preservation remain unknown. Given the importance of genetic information encoded in DNA it is crucial to address this matter. Furthermore, many studies focussed on long genomic DNA polymers with lengths of more than 10000 base pairs, for example Blum et al. 1997, Franchi et al. 1999 and Poly et al. 2000. This is problematic as it is unrealistic for such pristine polymers to occur in natural environments, hence why studies with shorter length molecules should be encouraged. Additionally, emphasis should be put on the influence of polymer length on adsorption and degradation processes as research on this parameter is lacking, which is especially urgent when considering that DNA belongs to the group of polyelectrolytes, for which length can correlate with different chemical properties (Frank-Kamenetskii et al. 1987). Lastly, while adsorptive properties on goethite have been well-studied (Omoike et al. 2004, Omoike & Chorover 2006, Sodnikar et al. 2021, Zeng et al. 2004), the effect of varying mineral particle sizes on adsorption and subsequent long-term preservation remains unknown.

This master's thesis aims to address these issues in a set of different experiments:

1. Geochemical factors on DNA adsorption
 - a. Effect of varying mineral particle size
 - b. Effect of DNA polymer length
 - c. Effect of varying solution pH
2. Mineral particle size effect on DNA preservation
3. DNA polymer length effect on its preservation

3 Materials and Methods

3.1 Mineral synthesis

We synthesized goethite of three different crystal sizes using an alkaline precipitation method with differential thermal ageing given by Schwertmann et al. (1985). 50 mL of 1M Fe(NO₃) and 450 mL of 0.7M KOH were mixed in polyethylene flasks under continuous stirring resulting in an immediate precipitation of ferrihydrite. The three batches were separately incubated at 10°C for 35 days, 40°C for 14 days and 70°C for 2.5 days to allow the transformation of ferrihydrite into goethite. Incubation at higher temperatures allows low nucleation and high growth rates for the goethite crystals resulting into larger crystallites, whereas lower temperatures impede the crystal growth but facilitates higher nucleation rate resulting into small goethite crystallites. To remove any excess ions after the incubation period, the resulting goethite suspensions were dialysed against ultrapure water (specific resistance > 18.2 MΩ cm), until the conductivity reached < 2 μS/cm. Minerals were then freeze dried, grounded, and transferred into 50 mL nuclease free falcon tubes and stored at 2°C until use.

3.2 Mineral characterisation

3.2.1 Specific surface area of minerals

The SSAs of the three goethite batches were measured on Quantachrome Nova 2000e using six-point Brunauer-Emmett-Teller (BET) method. The samples were degassed overnight at the room temperature prior to the analysis. The monolayer adsorption of the N₂ was performed at six points between the relative pressure (P/P_o) range of 0.05 to 0.35. The data was then plotted using the following equation, where X is the number of gas molecules adsorbed at the relative pressure (P/P_o). C is a second parameter related to the heat of adsorption.

$$\frac{1}{X[(\frac{P_o}{P}) - 1]} = \frac{1}{X_m C} + \frac{C - 1}{X_m C} \left(\frac{P}{P_o}\right) \quad (1)$$

The SSA was then calculated using the slope and the intercept of linear regression fit to the equation 1, by using equation 2, where CSA is the cross-sectional area of the adsorbate.

$$SSA = \left(\frac{1}{Slope + intercept} \right) CSA \quad (2)$$

The specific surface area of three different goethite minerals were as follows:

Incubation temperature	Incubation time	BET surface area (m ² /g)
70°C	2.5 days	30.2
40°C	14 days	61.5
10°C	35 days	91.2

Table 1. Details of incubation method used for goethite synthesis of varying particle size along with their respective surface areas measured using N₂-BET.

3.2.2 Mineralogy

Mineralogical characterization of goethite was performed by measuring X-ray diffraction (XRD) spectra. The XRD was performed using a Rigaku MiniFlex diffractometer (Rigaku, Miniflex 600) using Cu-K α radiation ($\lambda = 1.5406 \text{ \AA}$). Diffraction patterns were recorded between 10-80°2 θ at 0.05° step size and 0.5°/min scanning speed on a zero-background silica sample holder. Mineral identification was performed using Profex 5.2.1.2. We matched the diffraction peaks of the samples with reference structure files from software and found pure goethite without presence of any other mineralogical phases.

3.3 DNA synthesis and purification

Throughout this study five different lengths of DNA fragments were prepared: 99 bp, 400 bp, 1000 bp, 4000 bp and genomic DNA (gDNA) with a length of ~18000 bp. Commercially available gDNA (extracted from salmon sperm) was purchased from Sigma-Aldrich, USA. gDNA stock solutions were prepared by dissolving in desired pH buffers on a shaker (30 rpm) for 3 hours. All other DNA polymers were synthesized using polymerase chain reaction (PCR). Briefly, we mixed DreamTaq 10x buffer, dNTP mix

10mM, template DNA (from pBR322 plasmid), forward and reverse primers and DreamTaq DNA polymerase in proportions and concentrations indicated in Table 2. All the reagents were kept on ice while mixing to maintain low temperature to avoid polymerase activity. After mixing, the solution was transferred into 50 μ L PCR optical tube strips and loaded into a thermocycler (Thermo-Fischer Scientific, USA). The temperature in thermocycler was varied as a function of time in a controlled manner as given in Table 3 to synthesize the desired PCR products.

Components	99 bp (μ L)	400 bp (μ L)	1000 bp (μ L)	2000 bp (μ L)	4000 bp (μ L)
10x DreamTaq Buffer	5	5	5	5	5
dNTP Mix (10 mM each)	1	1	1	1	1
Forward primer (100 μ M)	5	0.5	0.5	0.5	0.5
Reverse primer (100 μ M)	5	0.5	0.5	0.5	0.5
Template DNA (2 ng/ μ L)	0.5	0.5	0.5	0.5	0.5
DreamTaq DNA Polymerase	0.25	0.25	0.25	0.25	0.25
Water	33.25	42.25	42.25	42.25	42.25

Table 2. Mixing protocol for different polymer lengths for 50 μ L batches with pBR322 plasmid template. Source: Instruction manual by Veer Singh.

Step	No. of cycles	99 bp ($^{\circ}$ C/min)	400 bp ($^{\circ}$ C/min)	1000 bp ($^{\circ}$ C/min)	2000 bp ($^{\circ}$ C/min)	4000 bp ($^{\circ}$ C/min)
Initial denaturation	1	95/1.5	95/1.5	95/1.5	95/1.5	95/1.5
Denaturation	37	95/0.5	95/0.5	95/0.5	95/0.5	95/0.5
Primer annealing		56/0.5	56/0.5	56/0.5	56.5/0.5	56.5/0.5
Extension		72/1	72/1	72/1	72/1	72/3
Final extension	1	72/10	72/10	72/10	72/10	72/10

Table 3. Thermocycler settings dependant on desired polymer lengths. Source: Instruction manual by Veer Singh.

For purification after the PCR synthesis (to remove unreacted dNTPs, salts, primers and polymerase), PCR products with desired DNA polymers were pooled together, mixed with 5x volume of binding buffer (70% 5M Guanidine hydrochloride, 30% isopropanol, pH 5). 750 μ L of PCR-binding buffer mixture was loaded into a silica spin column and centrifuged for DNA binding on silica membrane. The silica bound DNA was then washed

using a washing buffer (80% ethanol, 20% 10mM Tris-hydrochloride, pH 7.5), to remove unreacted dNTPs, salts, primers and polymerase. This resulted into only DNA remaining bound on the silica membrane. The silica bound DNA was then eluted into desired pH buffer in a 1.5 mL protein Lo-Bind tubes. All the centrifugations during PCR cleaning were done for one minute at 17 000 rcf. The resulting DNA solutions were kept at -20°C until use.

After synthesis, DNA polymer lengths in the PCR products were confirmed using an automated gel electrophoresis instrument (Agilent TapeStation 4150) with ready to use D1000 and D5000 screen tapes (equivalent to conventional agarose gels) as per manufacturer's instructions. DNA quantification was performed by measuring absorbance at 260 nm at an extinction coefficient of $0.0256 \text{ (ng/}\mu\text{L)}^{-1}\text{cm}^{-1}$ using UV-visible spectrophotometry on a nanodrop Spectrophotometer (Thermo FisherScientific). To confirm the purity of the DNA solutions obtained from PCR purifications we measured absorbance ratios at 230, 260 and 280 nm wavelengths. Absorbance ratios of A_{260}/A_{280} for all DNA solutions were in range of 1.8–1.9 ensuring no protein contamination. Similarly, absorbance ratios of A_{260}/A_{230} were in range of 2.0–2.2 ensuring no carryover of chaotropic salts in DNA solution during PCR clean-up.

4 Experimental setup

Mineral stock solutions were freshly prepared by suspending minerals in ultrapure water at continuous stirring of 300 rpm for 24 hours for mineral–surface hydration. To achieve better particle dispersion, mineral stocks were ultrasonicated immediately before experiments for three minutes at 35 kHz (Bandelin Sonorex RK 100, Bandelin electronics, Germany). We used acetate for pH 4 and 5, 3-(N-morpholino) propanesulfonic acid (MOPS) for pH 6, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) for pH 7 and 8, and sodium tetraborate for pH 9. Each experiment had 3mM of buffering species and 30mM of NaCl to provide background ionic strength. All the experiments were performed in triplicates run parallelly in dark at 20°C, in 2 mL protein LoBind tubes. All samples were agitated at 30 rpm using an overhead shaker for during competitive adsorption experiments. The samples were centrifuged at 20 000 rcf for 20 minutes followed by supernatant collection and immediate DNA analyses.

4.1 Adsorption isotherms

To understand the effect of varying goethite particle size on DNA adsorption, we performed a series of adsorption isotherms at pH 7 using goethite with SSAs of 30, 60 and 90 m²/g and DNA with polymer length of 1000 bp. We chose the mineral loadings in batch reactors such that each mineral provided 300 cm² surface area per mL of reaction volume (Table 4). The mineral suspensions and DNA solutions were mixed in equal volumes to obtain initial DNA concentrations of 5, 10, 25, 40, 55, 70, 85, and 100 ng/μL in a 60 μL batch volume. The samples were then incubated for 6 hours and analysed for remaining DNA concentration.

<i>Goethite</i>	<i>Specific surface area (m²/g)</i>	<i>Mineral loading (mg/mL) to achieve 300 cm² surface area</i>
Goe-30	30.2	1
Goe-60	61.5	0.49
Goe-90	91.2	0.33

Table 4. Mineral loadings (mg/mL) used to achieve normalized surface area.

4.2 Effect of DNA polymer length on adsorption

To study the effect of varying DNA polymer length on adsorption to goethite, we performed an adsorption experiment at pH 7 using DNA of polymer lengths, 99, 400, 1000, 4000 and ~ 18000 bp (gDNA). Equal volumes of mineral suspensions and DNA solutions were mixed to obtain initial DNA concentrations (20 ng/ μ L of each DNA polymer) and equal surface area (300 cm²/mL) from each mineral. We incubated the mixture for 6 hours and followed by analysis of DNA concentrations to calculate the DNA adsorption as a function of polymer length.

4.3 Effect of pH on DNA adsorption

To understand the effect of varying pH on DNA adsorption capacity on goethite of varying crystal sizes, we performed a pH-dependent adsorption experiment. Here we mixed 1000 bp DNA solutions in pH buffers ranging from pH 4-9 with different goethite to obtain initial DNA and mineral loadings of 20 ng/ μ L and 300 cm²/mL, respectively. We incubated the mixture for 6 hours followed by supernatant collection for DNA analysed and final pH measurements.

4.4 Enzymatic hydrolysis of DNA

After establishing an experimental set-up for the desorption of DNA from goethite particle surfaces, we performed enzymatic hydrolysis experiments on DNA adsorbed onto goethite surfaces to understand the adsorptive protection offered by goethite of different crystal sizes for DNA of varying polymer lengths. After all DNA adsorbed onto mineral overnight (proven to be sufficient in single-batch experiments), DNase I dilutions were prepared. Roughly 1 mg of DNase I enzyme (deoxyribonuclease I from bovine pancreas by Sigma-Aldrich) was weighed-in and left to dissolve in 9x DNase I buffer for 5 minutes on shaker. After measuring the concentration on UV-vis the protein solution was diluted down until concentrations of 9 and 450 ng/ μ L were reached. The reagents were kept on ice throughout the experiment to avoid early activation of the enzyme. Immediately after preparation 10 μ L of DNase I dilution were spiked into the DNA-mineral complex still on the shaker and kept at 37°C for one hour. As a final step 10 μ L

of 10x desorption buffer were added to the samples and left at 75°C for 15 minutes before being kept at room temperature for 48 hours. After incubation all samples were centrifuged at 20000 rcf for 20 minutes and the supernatant separated from the mineral. DNA left in solution was diluted 1:20 and analysed with Qubit HS and gel electrophoresis (high sensitivity D1000 and D5000 screen tape). Briefly, we mixed 40 µL of 99 or 1000 bp DNA with 40 µL goethite suspensions with mineral loadings calculated from adsorption isotherms such that nearly all of DNA (> 98.7%) is adsorbed to the mineral surface. We highlight the fact that these mineral loadings did not yield the same surface area from each goethite in the batch reactors. The mixture was incubated overnight and 10 µL of a model non-specific endonuclease were added, DNase I in two concentrations 0.9 and 50 ng/µL, to observe varying degree of hydrolysis. DNase I requires divalent cations for its optimal enzymatic activity; thus, we prepared the enzyme solution in a buffer such that after addition of DNase I, there were 0.5mM Ca^{2+} and 2.5mM Mg^{2+} present in batch reactors at pH 7. Immediately after the addition of enzymes, the batch reactors were placed in shakers at 30 rpm at 37°C. To terminate the enzymatic activity and desorb the degraded and survived DNA after the enzymatic hydrolysis, we added 10 µL of buffer to obtain a final concentration of 3mM tetraborate, 3.3mM EDTA and 20mM phosphate at pH 10. Here, tetraborate acts as a pH buffer for pH 10 to maintain a high pH resulting in enhanced desorption of DNA from goethite. Phosphate acts as a competitive anion which competes for the mineral surface sites and displaces the phosphate of DNA bound to the mineral surface. (for a detailed discussion on desorption protocol optimization, see next section). Finally, EDTA chelates with the divalent cations and terminates the enzymatic activity. To ensure a complete enzymatic inactivation, we also heated the reaction mixture at 75°C for 15 minutes for denaturing of DNase I. We kept the final mixture for the desorption at 20°C for 48 hours and collected the supernatant for analyses of hydrolysed and non-hydrolysed fractions of DNA after the experiment. The samples were then diluted 1:20 using ultrapure water to reduce the concentration of background electrolytes which may interfere with downstream analysis and measured using Qubit high sensitivity and automated gel electrophoresis (HS D1000 and HS D5000). The data obtained from the Qubit measurements was used to determine the percentage of DNA that survived after 48 hours of incubation. Equation 3 shows the calculation dividing the amount of final DNA (DNA_f) measured after incubation by the

initial concentration of DNA (DNA_i) added at the beginning of the experiment. For DNA_f and DNA_i , the averages of the triplicate batches were used. The initial concentration of DNA was 40 ng/ μ L in a volume of 40 μ L, so DNA_i was multiplied by 40. Additionally, 40 μ L of mineral suspension, 10 μ L of DNase I and 10 μ L of desorption buffer were added, resulting in a final volume of 100 μ L as a coefficient for DNA_f .

$$\% \text{ DNA survived} = \left(\frac{(DNA_f * 100)}{(DNA_i * 40)} \right) 100 \quad (3)$$

4.5 Optimization of DNA desorption from mineral surfaces

It is crucial to retrieve the DNA adsorbed onto the mineral surfaces for a variety of analytical purposes. In the scope of present work, we optimised the DNA desorption protocol to analyse DNA after the enzymatic hydrolysis experiments. Here, we first mixed 40 μ L of DNA solution at an initial concentration of 40 ng/ μ L with 40 μ L goethite suspension for 4 hours. For a better comparison two DNA fragment lengths were selected: 99 and 1000 bp. Both PCR products were eluted in pH 7 6mM HEPES buffer and diluted to 40 ng/ μ L. We selected the mineral loadings from adsorption isotherms such that, experiments belonged to vertical region of isotherm with at least 98.7% of DNA adsorption in all cases (Table 5). After 4 hours the concentration of DNA left in solution was measured in control groups on high-sensitivity fluorescence spectroscopy (Qubit HS). The results showed that for 1000 bp up to 1.3% of DNA did not adsorb which had to be considered in later calculations, hence we decided to increase the incubation time to 12 hours to ensure complete adsorption of DNA. All other samples were spiked with 10 μ L of a 9x DNase I buffer solution containing 90 mM Tris*HCl, 22.5mM $MgCl_2$ and 4.5mM $CaCl_2$ (pH 7) and kept in a shaking oven set to 150 rpm at 37°C for one hour. Then 10 μ L of 10x desorption buffer made of 200mM phosphate, 33mM EDTA and 30mM Na-tetraborate with a pH of 10 were mixed into the DNA-mineral complex. In early tests, adjustment to pH 9 has also been sufficient in desorbing DNA, however, better results were achieved with pH 10 desorption buffer. The samples were kept for 48 hours at room temperature ($\approx 20^\circ\text{C}$) on overhead shaker (30 rpm). After centrifugation and separation, the amount of desorbed DNA was determined on Qubit HS.

Goethite surface area (m ² /g)	Mineral loading (mg/mL)
30	3.4
60	2.3
90	1.8

Table 5. Mineral loadings selected for hydrolysis experiments. The amounts represent 2.5x the calculated value required to adsorb all DNA based on results of adsorption isotherms.

5 Results

5.1 Goethite adsorption isotherms

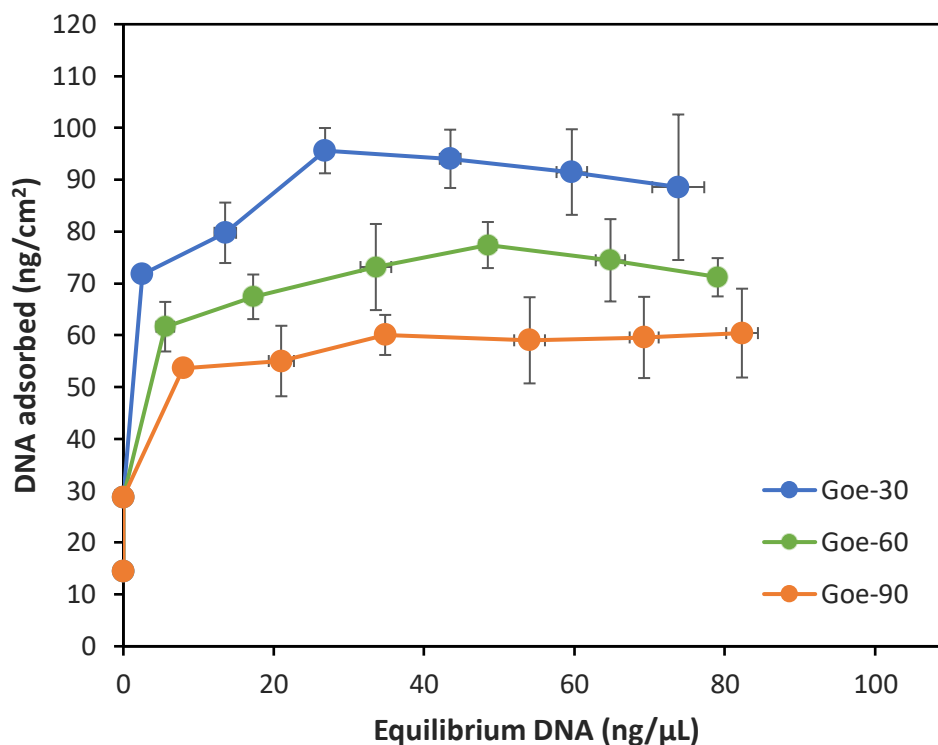


Figure 4. Adsorption isotherms with 1000 bp DNA at various concentrations.

To understand the adsorption affinity of DNA for goethite of varying particle sizes, we performed a series of adsorption isotherms. We mixed varying concentrations of 1000 bp DNA with all three mineral particle sizes, i.e., Goe-30, Goe-60 and Goe-90) and analysed the supernatant for non-adsorbed DNA concentration after 6 hours. The aim was to generate comparable adsorption isotherms as presented in Figure 4. For improved comparability, we chose the mineral loadings such that each goethite sample provide the same surface area in batch experiments and then normalised the adsorbed DNA by surface area. The data show an initial steep increase in adsorbed DNA until eventually a plateau is reached where no significant net change in adsorption can be observed. This trend was consistent within all three specific surface sizes, however, there are differences in the magnitude of adsorption. At lowest initial DNA concentrations of 5 and 10 ng/μL, complete adsorption was achieved, indicating high adsorption affinity. With increasing concentration of DNA adsorption also increased until a plateau was

reached. At that point further increase in DNA concentration did not lead to enhanced adsorption. For Goe-30, a maximum adsorption was achieved at 95.6 ng/cm², whereas maximum adsorption for Goe-60 and Goe-90 was lower, 77.4 and 60.4 ng/cm², respectively. In conclusion, the data shows a distinct systematic trend of increasing adsorption with decreasing mineral SSA.

5.2 DNA length-dependant adsorption

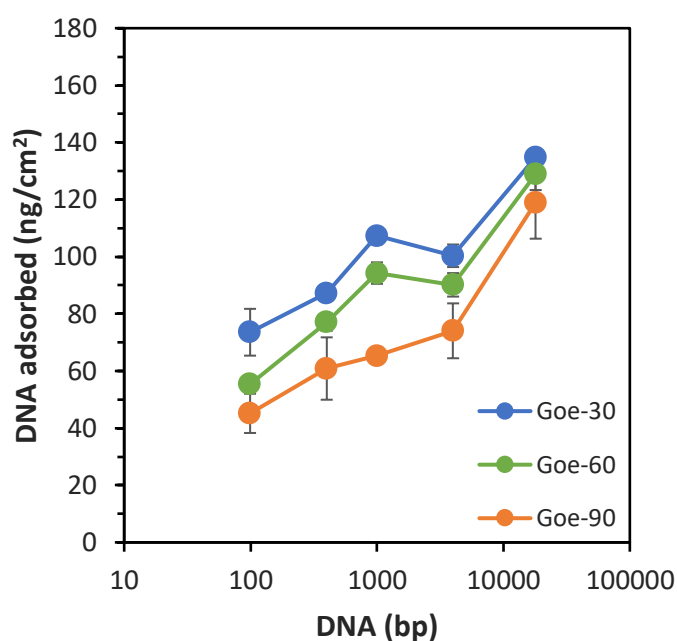


Figure 5. Adsorption isotherms with various DNA fragment lengths.

To evaluate the effect of varying DNA polymer length on DNA adsorption capacity, we incubated 99, 400, 1000, 4000 and gDNA at an initial concentration of 20 ng/μL with goethite of three mineral particle sizes as shown in Figure 5. Two key trends can be observed from this set of data. First, Goe-30 had highest DNA adsorption capacity for any DNA polymer, followed by Goe-60 and Goe-90 as the lowest. Second, DNA adsorption capacity by mass increased with increasing DNA polymer length, regardless of mineral particle size. Across all different mineral SSAs, 99 bp DNA adsorbed the least with 73.6, 55.4 and 45.2 ng/cm², respectively. The largest DNA polymer, gDNA, adsorbed the most with peaking amounts of 134.9, 128.8 and 119 ng/cm². Interestingly, for Goe-30 and 60 the amounts of 1000bp DNA adsorbed are elevated in comparison to the observations at 40 ng/μL in chapter 5.1 by 25.6% average for Goe-30 and 28.5% average

for Goe-60. Higher dilution of the mineral suspension prior to the experiment might be a potential cause for this.

5.3 pH-dependant adsorption isotherms

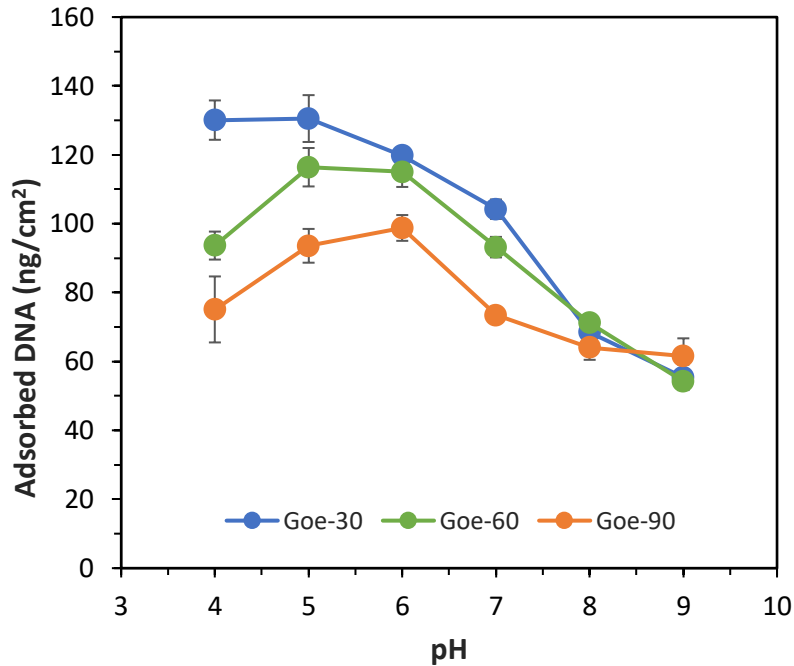


Figure 6. Adsorption isotherms with 1000 bp DNA at various pH-values.

To explore the effect of varying environmental pH on DNA adsorption capacity on goethite of varying particle sizes, we incubated 1000 bp DNA with initial concentration of 20 ng/μL at pH 4, 5, 6, 7, 8 and 9 (Figure 6). As with the previous experiments, the greatest extent of adsorption was observed in Goe-30 samples whereas the lowest amount of DNA adsorbed when exposed to Goe-90. The difference in adsorption capacity across the goethite particle sizes decreases with increasing pH. At pH 8 and 9, the concentrations of adsorbed fragments are highly similar between all three mineral batches, ranging from 64.1 to 71.2 ng/cm² at pH 8 and 54.3 to 61.6 ng/cm² at pH 9. Below the point of zero charge (PZC) of DNA at ≈ 4.5 (Guo et al. 2016, Sherbet et al. 1983), a steep increase in adsorption of +19.5% for both Goe-60 and 90 takes place. The same cannot be observed for the measurement points at pH 4 and 5 for Goe-30 samples, where only a small increase of +0.3% occurs, however, after peaking at pH 5 (pH 6 for Goe-90) the adsorption of DNA decreases steadily, passing the PZC of goethite at around 7.5, until pH 8 where all goethite particles exhibit similar adsorption. With increasing pH

from 6 to 9, adsorbed DNA was observed to reduce by 53.8, 52.8 and 37.6% for Goe-30, 60 and 90, respectively.

5.4 DNA desorption experiments

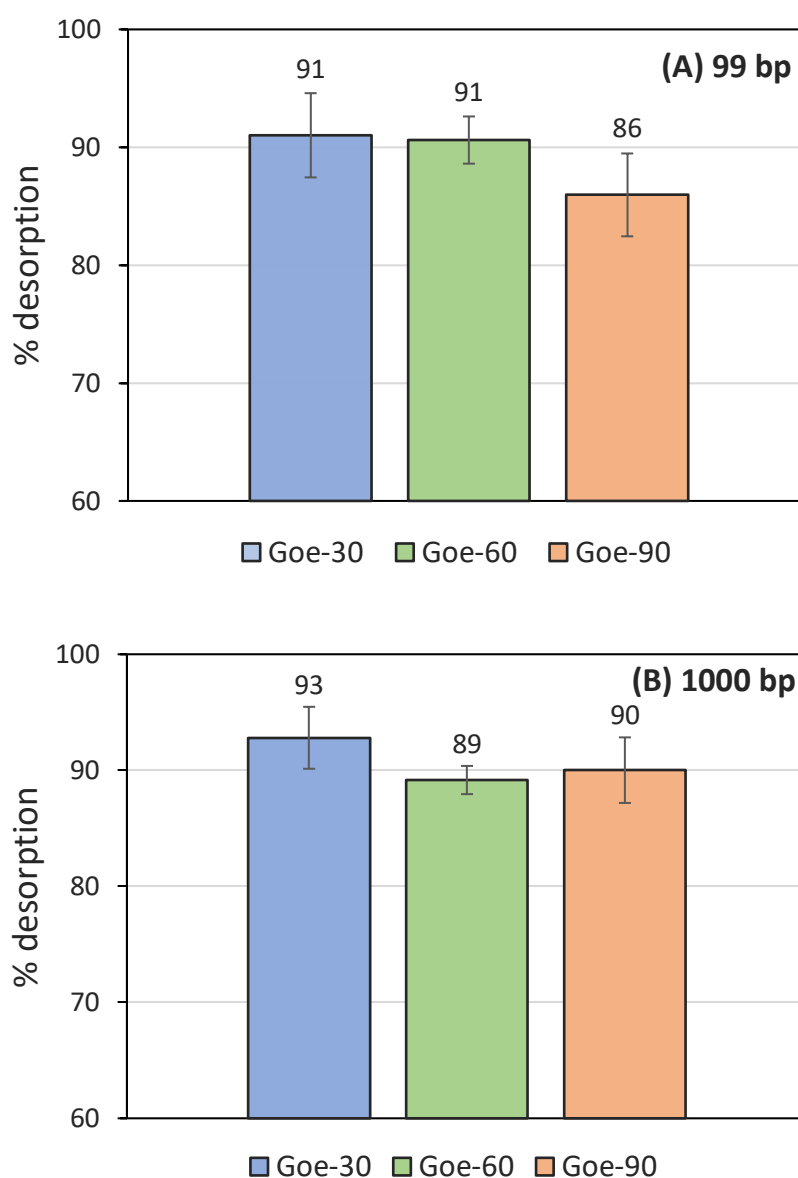


Figure 7. Percentage of desorbed (A) 99 bp and (B) 1000 bp DNA after 48 hours of incubation.

To rule out any biases regarding the recovery rates of DNA, an experimental desorption protocol had to be established prior to the degradation experiments. To extract intact DNA and hydrolysed products resulting from enzymatic activity, multiple desorption tests were performed with varying parameters such as mineral content, adsorption time

and concentration of DNase I. All tests were performed using 99 and 1000 bp DNA in triplicates. The results discussed below refer to the final protocol that – with exception of the adsorption duration (see chapter 4.6) – was later followed in the degradation experiments.

Figure 7 shows the desorbed amount of 99 and 1000 bp DNA after 48 hours of desorption using alkaline phosphate buffer. Desorption was consistently > 90% for both polymer lengths and all particle sizes, with the exception of 99 bp and Goe-90, where only 86% desorption occurred. This indicates that little to no bias in desorption towards DNA polymer length and mineral particle size was present, showing efficient recovery of DNA. Only for the 99 bp Goe-90 batch correlations between SSA of the mineral and desorption of the DNA fragments occurs. The data suggests that for 99 and 1000 bp an average of 11 and 9% of adsorbed DNA is expected to remain bound to mineral surfaces after 48 hours without DNase I present in solution, with average standard deviations ± 3 and 2%, respectively.

5.5 DNA degradation experiment

5.5.1 Percentage of DNA survival after incubation

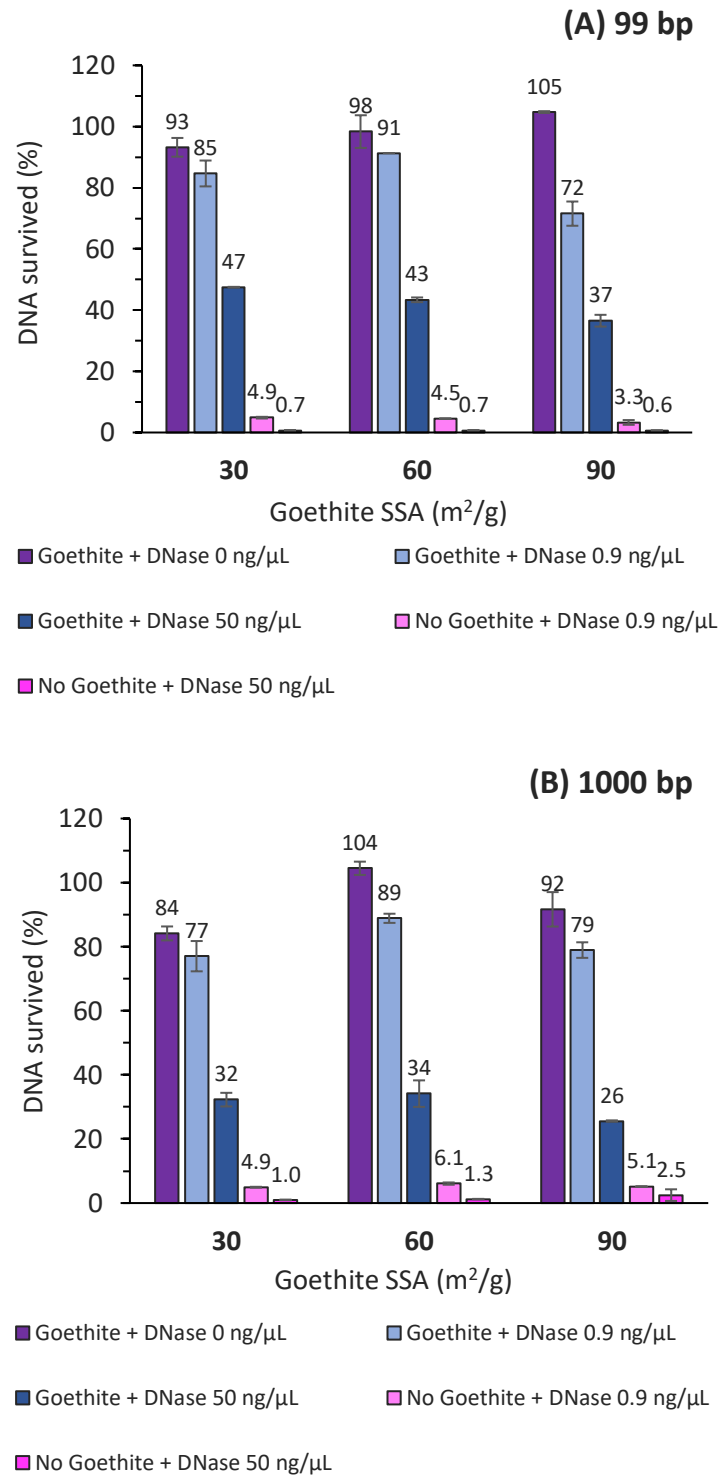


Figure 8. Absolute percentage of (A) 99 bp and (B) 1000 bp DNA that survived after 48 hours of incubation. The blue bars represent the desorption of DNA from goethite when incubated with DNase I. Controls without DNase I are represented by the purple bars, controls containing no goethite by the pink bars.

DNA degradation experiments were performed by adding DNase I in final concentrations of 1 and 50 ng/ μ L, then desorbing the hydrolysed and non-hydrolysed products using the desorption protocol discussed in chapter 4.6. The desorbed DNA was then analysed using fluorometer and automated gel electrophoresis. Figure 8 shows the absolute percentage of DNA that survived after desorption, calculated from the amount of measured DNA concentrations against initial DNA added, as shown in chapter 4.5. In the samples containing 0 ng/ μ L DNase I desorption was consistently > 90%, conforming to the previously developed desorption protocol. Without the presence of mineral almost complete degradation of DNA was observed. For both control sets DNA fragment length did not influence the quantity of degradation.

When DNase I was added, different degrees of degradation occurred. Degradation increased with increasing amounts of DNase I, as represented by the decrease in fluorescent signal in Figure 8. For both 99 and 1000 bp the values are similar for Goe-30 and 60, however, with the exception of 1000 bp DNA treated with 0.9 ng/ μ L DNase I, Goe-90 samples showed decreased survival of DNA. This suggests no significant difference in protection for Goe-30 and 60 and decreased protection of Goe-90. When comparing 0.9 ng/ μ L batches with those that contained 50 ng/ μ L DNase I, the addition of 50 ng/ μ L DNase I resulted in a decrease in survived DNA by 57 and 67% for 99 and 1000 bp, respectively, indicating increased degradation of DNA by DNase I.

While the results were similar for Goe-30 and 60, Goe-90 diverted when mixed with either 99 or 1000 bp DNA. As already addressed, addition of 0.9 ng/ μ L DNase I lead to higher degradation than in samples containing larger mineral particles. When the amount of DNase I was increased to 50 ng/ μ L protection of both DNA polymer lengths was least in samples containing Goe-90. For 99 bp DNA fluorescence decreased from 47% with Goe-30 to 37% with Goe-90 and for 1000 bp DNA from an average of 33% to 26%. Overall the results suggest a positive correlation between degradation and DNase I present as well as increased protection of DNA with decreasing goethite particle size.

5.5.2 Degradation of DNA

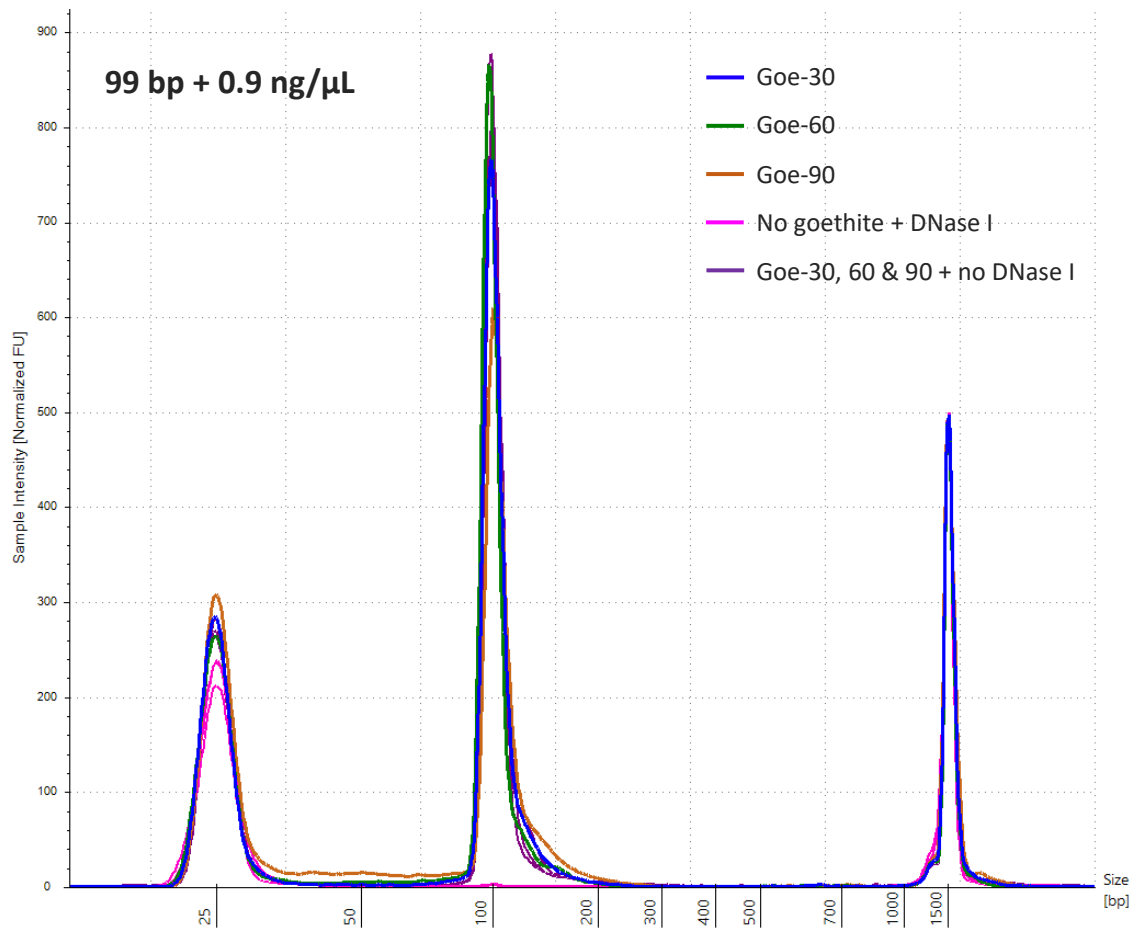


Figure 9. TapeStation data showing degradation of 99 bp DNA from 0.9 ng/μL DNase I. The sample intensity [FU] of detected DNA is plotted against the polymer length [bp].

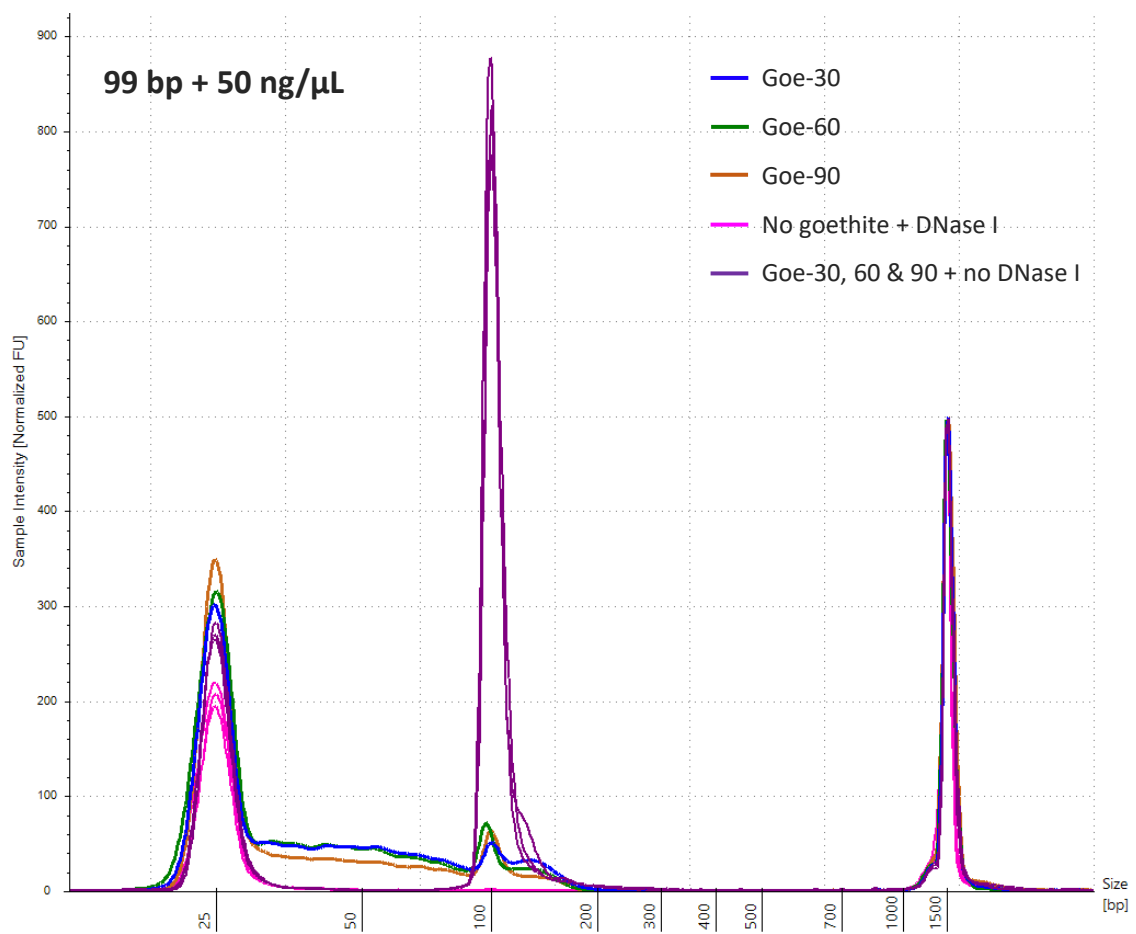


Figure 10. TapeStation data showing degradation of 99 bp DNA from 50 ng/ μ L DNase I. The sample intensity [FU] of detected DNA is plotted against the polymer length [bp].

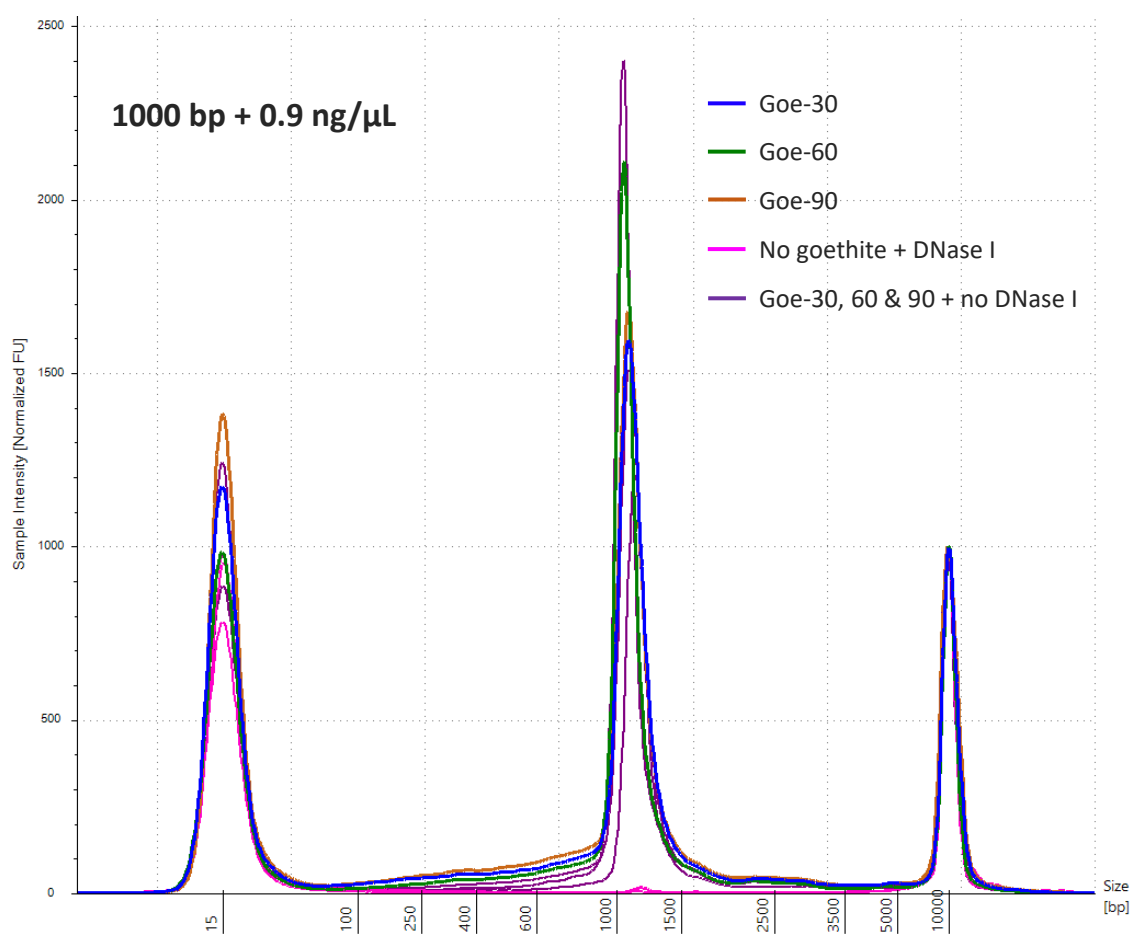


Figure 11. TapeStation data showing degradation of 1000 bp DNA from 0.9 ng/μL DNase I. The sample intensity [FU] of detected DNA is plotted against the polymer length [bp].

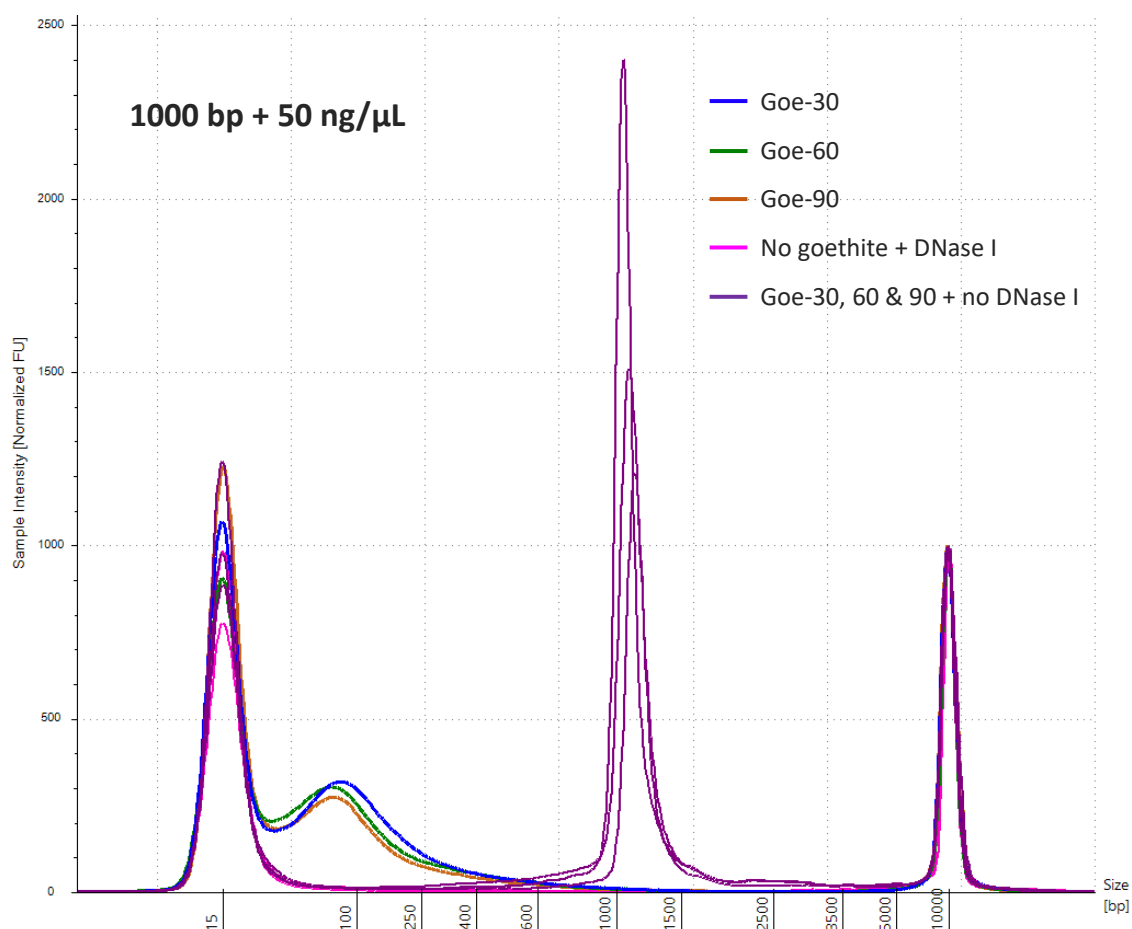


Figure 12. TapeStation data showing degradation of 1000 bp DNA from 50 ng/μL DNase I. The sample intensity [FU] of detected DNA is plotted against the polymer length [bp].

In addition to fluorescent measurements, we also measured sample intensity [FU] on TapeStation, with peaks indicating clusters of similar polymer lengths. The data in Figures 9-12 represent the distribution of DNA fragments sized within the boundaries of the screen tapes (HS D1000 and HS D5000) used. Measurements were performed in triplicates, however, for simplification purposes only singles are depicted as no major variations between triplicates occurred. Negative controls without DNase I and positive controls without mineral followed the trend shown in fluorescence (Figure 8), with negative controls serving as a comparison to samples treated with DNase I.

When subjected to 0.9 ng/μL of Dnase I, in agreement with the slight decrease in fluorescent signal observed, 99 and 1000 bp both showed signs of degradation with broad peaks appearing below the initial DNA size. However, the TapeStation data suggested the degradation was not dependent on particle size, with little difference in the degradation peaks seen. Similar to the results discussed in the previous section

addition of 50 ng/ μ L of DNase I (Figure 10 and 12) gave decreased intensities indicating enhanced degradation, especially of 1000 bp DNA, which was much more affected by the 50 ng/ μ L DNase I treatment than 99 bp DNA polymers. Across all mineral particle sizes 1000 bp DNA formed a new peak at approximately 90 bp. This implies an accumulation in the smaller polymer length range (< 100 bp), indicating better protection of smaller fragments from DNase I-induced degradation. When compared to the results shown in chapter 5.5.1, it appears that the quantity of initial DNA degraded correlates with SSA, however, the polymer length of the degradation products is not affected by SSA, as 1000 bp DNA consistently degraded into fragments < 100 bp across all mineral sizes.

6 Discussion

The focal point of this work is to elucidate the role of mineral particle size and DNA polymer length on adsorption and protection of DNA. Goethite is a common mineral that can be found across various environmentally relevant conditions, hence why it was chosen for this study. DNA is a valuable molecule carrying important information that shapes the history of life itself and has been found to be highly reactive with its surroundings. In the environment, DNA is expected to be highly fragmented (Pääbo 1989). To investigate the impact of polymer size on the fate of DNA, two polymer sizes representing fragmented DNA were chosen, 99 and 1000 bp. The decision to work with pH 7 solution buffer has been made to present a neutral reference pH across the experiments.

The different goethite samples used in this study have SSAs of 30.2, 61.5 and 91.2 m²/g. Although, while these surface areas do not differ vastly from each other, they still represent extreme ends of goethite particle sizes occurring in the environment. Additionally, another important point to notice is the chemical precipitation used to synthesize various goethite in the current study results into formation of polydispersed goethite particle sizes, i.e., having a particle size distribution instead of having all particles of the same size. It is noteworthy that the accuracy of SSA measurements for very fine particles is reduced compared to more coarse materials due to the increased error range in the nanoscale. Determining particle size distribution is also expected to be difficult due to the increasing error with decreasing particle size, however, the error in our BET-measurements was low. Despite these challenges, the results clearly show different amounts of adsorption depending on SSA.

In general, the properties of the mineral surface are the key factor determining adsorption affinity (Saeki et al. 2010). This is supported by the distinct differences all three batches of goethite exhibit when being exposed to varying concentrations of DNA, longer and shorter DNA molecules and varying pH. In adsorption isotherms using 1000 bp, we provided the same mineral surface area of each goethite particle size, which should have resulted in similar (if not same) adsorption due to availability of same number of reactive sites. However, we observed that 1000 bp DNA adsorption capacity increased with decreasing mineral SSA, i.e., with increasing mineral particle size. In

return, smaller mineral particles are attributed with an increased risk to form aggregates, which reduces the effectively available surface area for adsorption. Due to the similarity in SSA for all goethite particle sizes in our experimental system, there will be higher number of smaller mineral particles than bigger ones. The higher number of mineral particles results in higher surface area to volume ratios of the solid, increasing the likelihood of surface-surface interactions via van der Waals, electrostatic interactions and ultimately leading to increasing aggregation. Smaller particles with large SSA tend to form aggregates more easily, reducing the amount of available surface sites. It would be very difficult to quantify aggregation given the complexity of such highly dynamic systems. For example, upon shaking already adsorbed nucleic acid could have collided with an unoccupied surface site of another mineral particle and thus form bonds with both (Van de Ven 1994). Another approach is to look at pore space formation caused by aggregation (Vereecken et al. 1989). These pore spaces would be filled with aqueous solution, trapping DNA (Sposito 2016). Particle size distribution measurements could help provide data-based assumptions on the occurrence of pore spaces. Strauss et al. (1997a) noted enhanced aggregation and decreased particle size in goethite with a large SSA of 132.6 m²/g when investigating the effect of crystallinity on the properties of goethite. In a follow-up paper they also reported longer reaction times with increasing SSA, ranging from 18.2 to 132.6 m²/g as measured with BET analysis (Strauss et al. 1997b). For this thesis, all adsorption experiments were conducted with an incubation time of six hours. Further research could increase the experiment duration to see if the increased timespan induces changes in the observed differences in SSA-related adsorption patterns. For our experiments we tried to minimize the risk of aggregation by ultrasonication of mineral suspensions before use. Dynamic light scattering could be measured to give information on the aggregation state of the goethite-DNA complex but would have exceeded the scope of this study.

Increased aggregation can also be caused by proximity of the PZC of goethite (~ 7.9) to the experimental pH of 7. It is important to note that each crystal face of goethite carry a unique hydroxyl group density (Barrón & Torrent 1996). The differing proportions of crystal faces, influenced by variations in crystal size and shape, result in changes in overall surface hydroxyl group density, which may ultimately lead to small variations in the PZC among goethite samples of different crystal sizes. However, these variations in

PZC are likely to be small and unlikely to be the driving force behind the observed difference in DNA adsorption with varying mineral particle size.

Depending on how DNA fragments adsorb on the mineral surface, surface sites may be more easily occupied on smaller particles. It is not known whether DNA adsorbs parallel to the spherule, at the tips, wrapped around, punctually or partially by forming loops. According to the Coulombic end effect described by Stein et al. (1995), DNA possesses an increased negative charge density at the fragment ends, potentially leading to diverse bonding preference points depending on mineral surface charge. In their research on dsRNA adsorption on goethite, Sodnikar et al. (2021) showed that with increasing initial concentration of nucleic acid available mineral surface sites decrease more rapidly, corresponding with our data. The saturation of goethite surfaces could explain free DNA fragments left in solution throughout all adsorption experiments.

As already addressed in chapter 1, electrostatic forces play an important role in determining adsorption to goethite surfaces. The results presented in chapter 5.3 show decreasing adsorption capacity with increasing pH. Similar adsorption patterns can be found in the literature, namely Sposito (1984) and Antelo et al. (2005), who adsorbed PO_4^{3-} on goethite, or Romanowski et al. (1991) within a pH range from 5 to 9 using plasmid DNA. Strauss et al. (1997a) extended the pH range until 10 with similar outcome for the adsorption of chloride on the same mineral. The reason for this lies in the change of surface charge of the goethite particles. With increasing pH it becomes more negatively charged, limiting the possibilities of the likewise negatively charged DNA molecule to bond, resulting in reduced adsorption. At pH 4 we observed a slight decrease for Goe-60 and 90 samples, highlighting the transition in the bulk DNA charge from negative to positive, below its isoelectric point (pI) at pH ~ 4.5 (Sherbet et al. 1983 & Stotzky 2000). Below this pH, DNA as well as the mineral surface are both positively charged, causing electrostatic repulsion hence reduced adsorption. Once solution pH exceeds the PZC of goethite, its surface possesses opposite charge of DNA, causing decreased repulsion. Sodnikar et al. (2021) determined the PZC of goethite to be at pH 7.85. Other studies determined different PZC for goethite. Borggaard et al. (1983) found that PZC increases with increasing SSA (e.g. 7.2 for SSA of 16 m^2/g , 7.6 for SSA 82 m^2/g), which was later confirmed by Strauss et al. (1997a). Zeltner & Anderson (1988) pointed out how the PZC depends on multiple factors such as amount of background electrolyte,

measuring errors as well as crystallinity and purity of the minerals. After additional purification of goethite from CO₂-contamination, Evans et al. (1979) determined the PZC to be at pH 9.3. Despite the decrease by 50% of the maximum adsorption at pH 8 and 9, both providing a repulsing electrostatic environment, adsorption still occurs. This can be explained when considering that overall surface charge does not equal a complete shift among all surface sites. Some may still be oppositely charged, providing binding sites for DNA. Furthermore, Sodnikar et al. (2021) suggest that at high pH negative electrostatic forces must be overcome by inner-sphere complexation. Although all batches of goethite show a consistent trend of decrease in adsorption with increasing pH, adsorption capacities reach similar values at pH 8 and 9. Such a drop in adsorption until amounts do not differ between SSA batches was also shown between clay minerals by Hou et al. (2014) and goethite by Sodnikar et al. (2021). This could be attributed to aggregation of mineral particles when close to their PZC, eliminating the effect of varying particle sizes. Additionally, Sodnikar et al. (2021) suggest that at high pH inner-sphere complexation dominates over the weakened electrostatic forces, which may act as a limiting factor to the adsorption process.

Another focal point of this study was the effect of DNA polymer length on adsorption. As shown in chapter 5.2, we observed increased adsorption with increasing bp. Throughout all experiments DNA was kept in buffer solutions with a background ionic strength of 60mM NaCl. The amount was chosen to prevent DNA from denaturing in absence of any counterions. As discussed in chapter 1, the DNA helix can undergo conformational shifts depending on the processes taking place at the mineral surface and the composition of the surrounding medium. Given the consistent ionic strength in all systems, no changes in DNA conformation between the different experiments were expected to have occurred (Frank-Kamenetskii et al. 1987). Another parameter closely related to the ionic strength of solution is the persistence length (“stiffness”) of DNA fragments, quantifying its flexibility. Salomo et al. (2006) addressed the correlation between persistence length and ionic strength by investigating the elastic properties of different length DNA with a solution background of 137mM NaCl. Liu et al. (2000) also reported such correlation, though considered 103mM NaCl a low concentration of background electrolyte. Additionally, the lower the salt concentration the greater the persistence length (Liu et al. 2000). In general, persistence length of DNA is considered

to be ~ 50 nm or 150 bp. Thus in our experimental system, 99 bp DNA should be of linear and more rigid conformation whereas 1000 bp DNA is expected to be of more flexible conformation. Cleaves II et al. (2011), who used short ssDNA of 27 to 32 bp length, argued that for dsDNA flexibility must be below that of ssDNA. Since the shortest fragments also adsorbed the least it is likely that the higher persistence length of these molecules leads to increased uptake of available sorption sites as they lay more flat on the mineral surface. On the contrary, Ogram et al. (1994) found favoured adsorption for shorter strands of DNA. These contradicting results may be due to the different properties of the particles investigated in their study, which focussed on various types of soils (clay, loam and sand), none of which contained goethite. The results presented in this thesis correlate better with those presented by Franchi et al. (1999) who mentioned that longer DNA fragments have better chances to adsorb as they are expected to have more available binding sites. Shorter DNA polymers may adsorb flat on the mineral surface thus covering more potential surface sites, whereas the longer ones, being more flexible, may form loops and trails, ultimately attaching to fewer binding sites, giving a higher chance to other DNA polymers to also adsorb.

The results of the degradation experiment (chapter 5.5) confirm the partial protection of DNA from enzyme-induced nucleases compared to free DNA in solution. With increasing concentration of DNase I degradation also increased, which supports the findings of other studies. Cai et al. (2006) attempted degradation of gDNA derived from salmon sperm with varying concentrations of DNase I after prior adsorption to kaolinite and montmorillonite. With 20 ng/ μ L kaolinite samples degraded to an average fragment length of 2000 to 4000 bp and were totally degraded after being spiked with 80 ng/ μ L of enzyme. Samples adsorbed to montmorillonite were more strongly protected; no effect was observed with 40 ng/ μ L DNase I, and with 80 ng/ μ L fragments still were on average < 4000 bp long. The initial higher adsorption affinity to kaolinite did not lead to increased protection against DNase I. Therefore, a different parameter must be responsible for promoting DNA protection. Conformational shifts in DNA helix structure may occur (Demanèche et al. 2001) but were found to be neglectable (Cai et al. 2006). For the degradation of plasmid DNA from illite, Demanèche et al. (2001) treated samples with 5 and 10 ng/ μ L DNase I. These amounts were insufficient in degrading all DNA, similar to the lack of enzymatic nucleases shown in this thesis with 0.9 ng/ μ L DNase. The same

study, however, proposed that DNase may adsorb to mineral surfaces. The deactivation of DNase I upon adsorption has been investigated (Brundin et al. 2013) and widely been addressed as the potential main limiter in enzymatic DNA hydrolysis processes (Sarkar et al. 1989, Demanèche et al. 2001, Cai et al. 2006 and Parker et al. 2019). To ensure complete adsorption mineral was added in excess. It is possible that enough surface sites remained available for DNase I to adsorb onto goethite, however, further investigation on the significance of order in the adsorption process is required to give better understanding of the mechanisms involved in protection of DNA. Overall it can be concluded that DNA adsorption may make it partially inaccessible to degradation by DNase I. Furthermore, DNase I itself may adsorb on mineral surfaces, thus becoming inactive which contributes to the protection of DNA.

For 99 bp DNA, an effect of mineral particle size could be observed between Goe-90 and the larger particle sizes. With 99 bp DNA, the protective properties seem to decrease. Furthermore, great differences in the degradation between 99 and 1000 bp occurred. The enzymatic hydrolysis with 50 ng/μL DNase I affected the integrity of the 1000 bp fragments much more than that of the 99 bp ones. After a long tail of fragmentation of 1000 bp DNA a peak at < 100 bp can be observed. A similar peak location was reported by Veer Singh (unpublished data). It is therefore likely that the stabilization of DNA around a fragment length below 100 bp is a property of DNA and not necessarily the sorbent. The duration of such stabilization effect remains unknown and requires further research for confirmation. As previously explained, shorter DNA fragments are of a stiffer conformation than their longer counterparts. The flat adsorption of the 99 bp samples appears to be more favourable for DNA protection compared to the 1000 bp samples, where DNA may adsorb using less surface binding sites (e.g. forming loops), thus being more susceptible to degradation. The lack of effect of mineral particle size on DNA protection does not follow the trend of adsorption, which indicates that DNA-mineral complexes do not affect the accessibility of DNase I to surface bound DNA.

7 Conclusion

This study showed the impact of various parameters on DNA adsorption and its preservation. Our results show that with decreasing SSA adsorption of DNA increased. Thus, larger goethite particles are more efficient in protecting DNA. We furthermore highlighted the effect of pH as a determining factor in DNA and mineral surface charge. In the pH-range where the charges of the mineral surface and DNA are expected to be opposite, the highest adsorption occurred, confirming our expectations. With increasing pH, the SSA of goethite becomes less significant due to higher aggregation around the PZC of the mineral. Longer DNA polymers adsorbed in larger quantities as their uptake of surface binding sites may be less than that of shorter fragments, however, they are more susceptible to damage. With increasing amount of DNase I damage to DNA polymers also increased, with 1000 bp DNA degrading until ~ 100 bp length, where we observed stabilization in fragment distribution. It is therefore suggested that shorter fragments are more persistent in environmental systems.

8 Bibliography

- Anderson-Carpenter, L. L., J. S. McLachlan, S. T. Jackson, M. Kuch, C. Y. Lumibao, and H. N. Poinar. 2011. 'Ancient DNA from Lake Sediments: Bridging the Gap between Paleoecology and Genetics'. *BMC Evolutionary Biology* 11: 1-15. <http://www.biomedcentral.com/1471-2148/11/30>.
- Antelo, J., M. Avena, S. Fiol, R. López, and F. Arce. 2005. 'Effects of pH and Ionic Strength on the Adsorption of Phosphate and Arsenate at the Goethite–Water Interface'. *Journal of Colloid and Interface Science* 285 (2): 476–86. <https://doi.org/10.1016/j.jcis.2004.12.032>.
- Barrón, V., and J. Torrent. 1996. 'Surface Hydroxyl Configuration of Various Crystal Faces of Hematite and Goethite'. *Journal of Colloid and Interface Science* 177 (2): 407–10. <https://doi.org/10.1006/jcis.1996.0051>.
- Blesa, M. A., and E. Matijević. 1989. 'Phase Transformations of Iron Oxides, Oxohydroxides, and Hydrous Oxides in Aqueous Media'. *Advances in Colloid and Interface Science* 29 (3–4): 173–221. [https://doi.org/10.1016/0001-8686\(89\)80009-0](https://doi.org/10.1016/0001-8686(89)80009-0).
- Blum, S. A. E., M. G. Lorenz, and W. Wackernagel. 1997. 'Mechanism of Retarded DNA Degradation and Prokaryotic Origin of DNases in Nonsterile Soils'. *Systematic and Applied Microbiology* 20 (4): 513–21. [https://doi.org/10.1016/S0723-2020\(97\)80021-5](https://doi.org/10.1016/S0723-2020(97)80021-5).
- Borggaard, O. K. 1983. 'Effect of Surface Area and Mineralogy of Iron Oxides on Their Surface Charge and Anion-Adsorption Properties'. *Clays and Clay Minerals* 31 (3): 230–32. <https://doi.org/10.1346/CCMN.1983.0310309>.
- Brundin, M., D. Figdor, G. Sundqvist, and U. Sjögren. 2013. 'DNA Binding to Hydroxyapatite: A Potential Mechanism for Preservation of Microbial DNA'. *Journal of Endodontics* 39 (2): 211–16. <https://doi.org/10.1016/j.joen.2012.09.013>.
- Cadet, J., and J. R. Wagner. 2013. 'DNA Base Damage by Reactive Oxygen Species, Oxidizing Agents, and UV Radiation'. *Cold Spring Harbor Perspectives in Biology* 5 (2): 1–18. <https://doi.org/10.1101/cshperspect.a012559>.
- Cai, P., Q.-Y. Huang, and X.-W. Zhang. 2006. 'Interactions of DNA with Clay Minerals and Soil Colloidal Particles and Protection against Degradation by DNase'. *Environmental Science & Technology* 40 (9): 2971–76. <https://doi.org/10.1021/es0522985>.
- Cao, Y., X. Wei, P. Cai, Q. Huang, X. Rong, and W. Liang. 2011. 'Preferential Adsorption of Extracellular Polymeric Substances from Bacteria on Clay Minerals and Iron Oxide'. *Colloids and Surfaces B: Biointerfaces* 83 (1): 122–27. <https://doi.org/10.1016/j.colsurfb.2010.11.018>.
- Chen, Y., E. J. Bylaska, and J. H. Weare. 2017. 'Weakly Bound Water Structure, Bond Valence Saturation and Water Dynamics at the Goethite [100] Surface/Aqueous Interface: Ab Initio Dynamical Simulations'. *Geochemical Transactions* 18 (1): 3. <https://doi.org/10.1186/s12932-017-0040-5>.
- Cleaves II, H. J., E. Crapster-Pregont, C. M. Jonsson, C. L. Jonsson, D. A. Sverjensky, and R. A. Hazen. 2011. 'The Adsorption of Short Single-Stranded DNA Oligomers to Mineral Surfaces'. *Chemosphere* 83 (11): 1560–67. <https://doi.org/10.1016/j.chemosphere.2011.01.023>.

- Dabney, J., M. Meyer, and S. Pääbo. 2013. 'Ancient DNA Damage'. Cold Spring Harbor Perspectives in Biology 5 (7): a012567–a012567. <https://doi.org/10.1101/cshperspect.a012567>.
- Demanèche, S., L. Jocteur-Monrozier, H. Quiquampoix, and P. Simonet. 2001. 'Evaluation of Biological and Physical Protection against Nuclease Degradation of Clay-Bound Plasmid DNA'. Applied and Environmental Microbiology 67 (1): 293–99. <https://doi.org/10.1128/AEM.67.1.293-299.2001>.
- DiBattista, J. D., J. D. Reimer, M. Stat, G. D. Masucci, P. Biondi, M. De Brauwier, and M.I Bunce. 2019. 'Digging for DNA at Depth: Rapid Universal Metabarcoding Surveys (RUMS) as a Tool to Detect Coral Reef Biodiversity Across a Depth Gradient'. PeerJ 7: 1-21. <https://doi.org/10.7717/peerj.6379>.
- Eglinton, G., and G. A. Logan. 1991. 'Molecular Preservation'. Philosophical Transactions of the Royal Society London B 333: 315-28. <https://doi.org/10.1098/rstb.1991.0081>.
- Evans, T. D., J. R. Leal, and P. W. Arnold. 1979. 'The Interfacial Electrochemistry of Goethite (α -FeOOH) Especially the Effect of CO₂ Contamination'. Journal of Electroanalytical Chemistry 105: 161-167. [https://doi.org/10.1016/S0022-0728\(79\)80347-2](https://doi.org/10.1016/S0022-0728(79)80347-2).
- Franchi, M., E. Bramanti, L. Morassi Bonzi, L. Orioli, C. Vettori, and E. Gallori. 1999. 'Clay-Nucleic Acid Complexes: Characteristics and Implications for the Preservation of Genetic Material in Primeval Habitats'. Origins of Life and Evolution of the Biosphere 29: 297-315. <https://doi.org/10.1023/A:1006557832574>.
- Frank-Kamenetskii, M. D., V. V. Anshelevich, and A. V. Lukashin. 1987. 'Polyelectrolyte Model of DNA'. Soviet Physics Uspekhi 30 (4): 317-303. <https://doi.org/10.1070/PU1987v030n04ABEH002833>.
- Guo, Z., Y. Wang, A. Yang, and G. Yang. 2016. 'The Effect of pH on Charge Inversion and Condensation of DNA'. Soft Matter 12 (31): 6669–74. <https://doi.org/10.1039/C6SM01344A>.
- Hassan, S., Z. Khurshid, Sabreena, B. Singh Bali, B. Ah Ganai, R. Z. Sayyed, P. Pocza, et al. 2022. 'A Critical Assessment of the Congruency between Environmental DNA and Paleoecology for the Biodiversity Monitoring and Paleoenvironmental Reconstruction'. International Journal of Environmental Research and Public Health 19 (15): 1-25. <https://doi.org/10.3390/ijerph19159445>.
- Hiemstra, T., and W. H. Van Riemsdijk. 1996. 'A Surface Structural Approach to Ion Adsorption: The Charge Distribution (CD) Model'. Journal of Colloid and Interface Science 179 (2): 488–508. <https://doi.org/10.1006/jcis.1996.0242>.
- Hobbs, J., J. M. Round, M. J. Allison, and C. C. Helbing. 2019. 'Expansion of the Known Distribution of the Coastal Tailed Frog, *Ascaphus Truei*, in British Columbia, Canada, using Robust eDNA Detection Methods'. PLOS ONE 14 (3): 1-16. <https://doi.org/10.1371/journal.pone.0213849>.
- Hou, Y., P. Wu, and N. Zhu. 2014. 'The Protective Effect of Clay Minerals against Damage to Adsorbed DNA Induced by Cadmium and Mercury'. Chemosphere 95 (January):206–12. <https://doi.org/10.1016/j.chemosphere.2013.08.069>.
- Itakura, H., R. Wakiya, S. Yamamoto, K. Kaifu, T. Sato, and T. Minamoto. 2019. 'Environmental DNA Analysis Reveals the Spatial Distribution, Abundance, and Biomass of Japanese Eels at the River-Basin Scale'. Aquatic Conservation: Marine and Freshwater Systems 29 (3): 361–73. <https://doi.org/10.1002/aqc.3058>.

- Jares-Erijman, E. A., and T. M. Jovin. 1996. 'Determination of DNA Helical Handedness by Fluorescence Resonance Energy Transfer'. *Journal of Molecular Biology* 257 (3): 597–617. <https://doi.org/10.1006/jmbi.1996.0188>.
- Kantidze, O. L., A. K. Velichko, A. V. Luzhin, and S. V. Razin. 2016. 'Heat Stress-Induced DNA Damage'. *Acta Naturae* 8 (2): 75–78. <https://doi.org/10.32607/20758251-2016-8-2-75-78>.
- Kjær, K. H., M. Winther Pedersen, B. De Sanctis, B. De Cahsan, T. S. Korneliussen, C. S. Michelsen, K. K. Sand, et al. 2022. 'A 2-Million-Year-Old Ecosystem in Greenland Uncovered by Environmental DNA'. *Nature* 612: 283–91. <https://doi.org/10.1038/s41586-022-05453-y>.
- Levy, M. S., I. J. Collins, S. S. Yim, J. M. Ward, N. Titchener-Hooker, P. Ayazi Shamlou, and P. Dunnill. 1999. 'Effect of Shear on Plasmid DNA in Solution'. *Bioprocess Engineering* 20 (1): 7–13. <https://doi.org/10.1007/s004490050552>.
- Lindahl, T. 1993. 'Instability and Decay of the Primary Structure of DNA'. *Nature* 362 (6422): 709–15. <https://doi.org/10.1038/362709a0>.
- Liu, H., J. Gapinski, L. Skibinska, A. Patkowski, and R. Pecora. 2000. 'Effect of Electrostatic Interactions on the Dynamics of Semiflexible Monodisperse DNA Fragments'. *The Journal of Chemical Physics* 113 (14): 6001–10. <https://doi.org/10.1063/1.1290477>.
- Lorenz, M. G., and W. Wackernagel. 1987. 'Adsorption of DNA to Sand and Variable Degradation Rates of Adsorbed DNA'. *Applied and Environmental Microbiology* 53 (12): 2948–52. <https://doi.org/10.1128/aem.53.12.2948-2952.1987>.
- Matter, A. N., J. A. Falke, J. Andrés Lopez, and J. W. Savereide. 2018. 'A Rapid-Assessment Method to Estimate the Distribution of Juvenile Chinook Salmon in Tributary Habitats using eDNA and Occupancy Estimation'. *North American Journal of Fisheries Management* 38: 223–36. <https://doi.org/10.1002/nafm.10014>.
- Michalkova, A., T. L. Robinson, and J. Leszczynski. 2011. 'Adsorption of Thymine and Uracil on 1 : 1 Clay Mineral Surfaces: Comprehensive Ab Initio Study on Influence of Sodium Cation and Water'. *Physical Chemistry Chemical Physics* 13 (17): 7862. <https://doi.org/10.1039/c1cp00008j>.
- Nielsen, K. M., P. J. Johnsen, D. Bensasson, and D. Daffonchio. 2007. 'Release and Persistence of Extracellular DNA in the Environment'. *Environmental Biosafety Research* 6 (1–2): 37–53. <https://doi.org/10.1051/ebr:2007031>.
- Ogram, A. V., M. L. Mathot, J. B. Harsh, J. Boyle, and C. A. Pettigrew. 1994. 'Effects of DNA Polymer Length on Its Adsorption to Soils'. *Applied and Environmental Microbiology* 60 (2): 393–96. <https://doi.org/10.1128/aem.60.2.393-396.1994>.
- Omoike, A., J. Chorover, K. D. Kwon, and J. D. Kubicki. 2004. 'Adhesion of Bacterial Exopolymers to α -FeOOH: Inner-Sphere Complexation of Phosphodiester Groups'. *Langmuir* 20 (25): 11108–14. <https://doi.org/10.1021/la048597+>.
- Omoike, A., and J. Chorover. 2006. 'Adsorption to Goethite of Extracellular Polymeric Substances from *Bacillus Subtilis*'. *Geochimica et Cosmochimica Acta* 70 (4): 827–38. <https://doi.org/10.1016/j.gca.2005.10.012>.
- Pääbo, S. 1989. 'Ancient DNA: Extraction, Characterization, Molecular Cloning, and Enzymatic Amplification.' *Proceedings of the National Academy of Sciences* 86 (6): 1939–43. <https://doi.org/10.1073/pnas.86.6.1939>.

- Paget, E., L. Jocteur Monrozier, and P. Simonet. 1992. 'Adsorption of DNA on Clay Minerals: Protection against DNase I and Influence on Gene Transfer'. *FEMS Microbiology Letters* 97 (1–2): 31–39. <https://doi.org/10.1111/j.1574-6968.1992.tb05435.x>.
- Parker, K. M., V. Barragán Borrero, D. M. Van Leeuwen, M. A. Lever, B. Mateescu, and M. Sander. 2019. 'Environmental Fate of RNA Interference Pesticides: Adsorption and Degradation of Double-Stranded RNA Molecules in Agricultural Soils'. *Environmental Science & Technology* 53 (6): 3027–36. <https://doi.org/10.1021/acs.est.8b05576>.
- Pietramellara, G., J. Ascher, F. Borgogni, M. T. Ceccherini, G. Guerri, and P. Nannipieri. 2009. 'Extracellular DNA in Soil and Sediment: Fate and Ecological Relevance'. *Biology and Fertility of Soils* 45 (3): 219–35. <https://doi.org/10.1007/s00374-008-0345-8>.
- Poinar, H. N. 1999. 'DNA from Fossils: The Past and the Future'. *Acta Paediatrica* 433: 133–40. <https://doi.org/10.1111/j.1651-2227.1999.tb14423.x>.
- Poly, F., C. Chenu, P. Simonet, J. Rouiller, and L. Jocteur Monrozier. 2000. 'Differences between Linear Chromosomal and Supercoiled Plasmid DNA in Their Mechanisms and Extent of Adsorption on Clay Minerals'. *Langmuir* 16 (3): 1233–38. <https://doi.org/10.1021/la990506z>.
- Romanowski, G., M. G. Lorenz, and W. Wackernagel. 1991. 'Adsorption of Plasmid DNA to Mineral Surfaces and Protection against DNase I'. *Applied and Environmental Microbiology* 57 (4): 1057–61. <https://doi.org/10.1128/aem.57.4.1057-1061.1991>.
- Saeki, K., T. Kunito, and M. Sakai. 2010. 'Effects of pH, Ionic Strength, and Solutes on DNA Adsorption by Andosols'. *Biology and Fertility of Soils* 46 (5): 531–35. <https://doi.org/10.1007/s00374-010-0447-y>.
- Salamon, M., N. Tuross, B. Arensburg, and S. Weiner. 2005. 'Relatively Well Preserved DNA Is Present in the Crystal Aggregates of Fossil Bones'. *Proceedings of the National Academy of Sciences* 102 (39): 13783–88. <https://doi.org/10.1073/pnas.0503718102>.
- Salomo, M., K. Kegler, C. Gutsche, M. Struhalla, J. Reinmuth, W. Skokow, U. Hahn, and F. Kremer. 2006. 'The Elastic Properties of Single Double-Stranded DNA Chains of Different Lengths as Measured with Optical Tweezers'. *Colloid and Polymer Science* 284 (11): 1325–31. <https://doi.org/10.1007/s00396-006-1517-4>.
- Sarkar, J. M., A. Leonowicz, and J.-M. Bollag. 1989. 'Immobilization of Enzymes on Clays and Soils'. *Soil Biology and Biochemistry* 21 (2): 223–30. [https://doi.org/10.1016/0038-0717\(89\)90098-9](https://doi.org/10.1016/0038-0717(89)90098-9).
- Schmidt, M. P., and C. E. Martínez. 2017. 'Ironing Out Genes in the Environment: An Experimental Study of the DNA–Goethite Interface'. *Langmuir* 33 (34): 8525–32. <https://doi.org/10.1021/acs.langmuir.7b01911>.
- Schwertmann, U., P. Cambier, and E. Murad. 1985. 'Properties of Goethites of Varying Crystallinity'. *Clays and Clay Minerals* 33 (5): 369–78. <https://doi.org/10.1346/CCMN.1985.0330501>.
- Schofield, R. K. 1950. 'Effect of pH on Electric Charges Carried by Clay Particles'. *European Journal of Soil Science* 1 (1): 1–8. <https://doi.org/10.1111/j.1365-2389.1950.tb00713.x>.
- Sherbet, G. V., M. S. Lakshmi, and F. Cajone. 1983. 'Isoelectric Characteristics and the Secondary Structure of Some Nucleic Acids'. *Biophysics of Structure and Mechanism* 10 (3): 121–28. <https://doi.org/10.1007/BF00537554>.
- Sidorova, N. Y., and D. C. Rau. 2001. 'Linkage of EcoRI Dissociation from Its Specific DNA Recognition Site to Water Activity, Salt Concentration, and pH: Separating Their Roles in

- Specific and Non-Specific Binding*. Journal of Molecular Biology 310 (4): 801–16. <https://doi.org/10.1006/jmbi.2001.4781>.
- Sigg, L., and W. Stumm. 1981. *'The Interaction of Anions and Weak Acids With the Hydrous Goethite (α -FeOOH) Surface'*. Colloids and Surfaces 2 (2): 101-17. [https://doi.org/10.1016/0166-6622\(81\)80001-7](https://doi.org/10.1016/0166-6622(81)80001-7).
- Slatkin, M., and F. Racimo. 2016. *'Ancient DNA and Human History'*. Proceedings of the National Academy of Sciences 113 (23): 6380–87. <https://doi.org/10.1073/pnas.1524306113>.
- Slon, V., C. Hopfe, C. L. Weiß, F. Mafessoni, M. De La Rasilla, C. Lalueza-Fox, A. Rosas, et al. 2017. *'Neandertal and Denisovan DNA from Pleistocene Sediments'*. Science 356: 605–8. <https://doi.org/10.1126/science.aam9695>.
- Sodnikar, K., K. M. Parker, S. R. Stump, L. K. ThomasArrigo, and M. Sander. 2021. *'Adsorption of Double-Stranded Ribonucleic Acids (dsRNA) to Iron (Oxyhydr-)Oxide Surfaces: Comparative Analysis of Model dsRNA Molecules and Deoxyribonucleic Acids (DNA)'*. Environmental Science: Processes & Impacts 23 (4): 605–20. <https://doi.org/10.1039/D1EM00010A>.
- Sposito, G. 1984. *'The Surface Chemistry of Soils'*. New York: Oxford University Press.
- Sposito, G. 2016. *'The Chemistry of Soils'*. Third edition. New York: Oxford University Press.
- Stein, V. M., J. P. Bond, M. W. Capp, C. F. Anderson, and M. T. Record. 1995. *'Importance of Coulombic End Effects on Cation Accumulation near Oligoelectrolyte B-DNA: A Demonstration Using ^{23}Na NMR'*. Biophysical Journal 68 (3): 1063–72. [https://doi.org/10.1016/S0006-3495\(95\)80281-X](https://doi.org/10.1016/S0006-3495(95)80281-X).
- Stotzky, G. 2000. *'Persistence and Biological Activity in Soil of Insecticidal Proteins from Bacillus Thuringiensis and of Bacterial DNA Bound on Clays and Humic Acids'*. Journal of Environmental Quality 29 (3): 691–705. <https://doi.org/10.2134/jeq2000.00472425002900030003x>.
- Strauss, R., G. W. Brümmer, and N. J. Barrow. 1997a. *'Effects of Crystallinity of Goethite: I. Preparation and Properties of Goethites of Differing Crystallinity'*. European Journal of Soil Science 48: 87–99. <https://doi.org/10.1111/j.1365-2389.1997.tb00188.x>.
- Strauss, R. G. W. Brümmer, and N. J. Barrow. 1997b. *'Effects of Crystallinity of Goethite: II. Rates of Sorption and Desorption of Phosphate'*. European Journal of Soil Science 48: 101–14. <https://doi.org/10.1111/j.1365-2389.1997.tb00189.x>.
- Stumm, W. 1995. *'The Inner-Sphere Surface Complex: A Key to Understanding Surface Reactivity'*. In: Huang, Chin Pao, Charles R. O'Melia, and James J. Morgan, eds. 1995. *'Aquatic Chemistry: Interfacial and Interspecies Processes'*. Advances in Chemistry 244. <https://doi.org/10.1021/ba-1995-0244>.
- Tran, A. T. T., D. A. Patterson, and B. J. James. 2012. *'Investigating the Feasibility of Using Polysulfone–Montmorillonite Composite Membranes for Protein Adsorption'*. Journal of Food Engineering 112: 38–49. <https://doi.org/10.1016/j.jfoodeng.2012.03.031>.
- Valdré, G., M. Antognozzi, A. Wotherspoon, and M. J. Miles. 2004. *'Influence of Properties of Layered Silicate Minerals on Adsorbed DNA Surface Affinity, Self-Assembly and Nanopatterning'*. Philosophical Magazine Letters 84 (9): 539–45. <https://doi.org/10.1080/09500830512331325082>.

- Van de Ven, T. G. M. 1994. '*Kinetic Aspects of Polymer and Polyelectrolyte Adsorption on Surfaces*'. *Advances in Colloid and Interface Science* 48: 121-40. [https://doi.org/10.1016/0001-8686\(94\)80006-5](https://doi.org/10.1016/0001-8686(94)80006-5).
- Van Elsas, J. D., S. Turner, and M. J. Bailey. 2003. '*Horizontal Gene Transfer in the Phytosphere*'. *New Phytologist* 157 (3): 525-37. <https://doi.org/10.1046/j.1469-8137.2003.00697.x>.
- Vereecken, H., J. Maes, J. Feyen, and P. Darius. 1989. '*Estimating the Soil Moisture Retention Characteristic From Texture, Bulk Density, and Carbon Content*'. *Soil Science* 148 (6): 389-403. <https://doi.org/10.1097/00010694-198912000-00001>.
- Wang, W.-N., J. Zhou, P. Wang, T.-T. Tian, Y. Zheng, Y. Liu, W.-J. Mai, and A.-L. Wang. 2009. '*Oxidative Stress, DNA Damage and Antioxidant Enzyme Gene Expression in the Pacific White Shrimp, Litopenaeus Vannamei When Exposed to Acute pH Stress*'. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 150 (4): 428-35. <https://doi.org/10.1016/j.cbpc.2009.06.010>.
- Yu, W. H., N. Li, D. S. Tong, C. H. Zhou, C. X. (Cynthia) Lin, and C. Yun Xu. 2013. '*Adsorption of Proteins and Nucleic Acids on Clay Minerals and Their Interactions: A Review*'. *Applied Clay Science* 80-81 (August):443-52. <https://doi.org/10.1016/j.clay.2013.06.003>.
- Zeltner, W. A., and M. A. Anderson. 1988. '*Surface Charge Development at the Goethite/Aqueous Solution Interface: Effects of CO₂ Adsorption*'. *Langmuir* 4 (2): 469-74. <https://doi.org/10.1021/la00080a039>.
- Zeng, L., X. Li, and J. Liu. 2004. '*Adsorptive Removal of Phosphate from Aqueous Solutions Using Iron Oxide Tailings*'. *Water Research* 38 (5): 1318-26. <https://doi.org/10.1016/j.watres.2003.12.009>.
- Zinger, L., P. Taberlet, H. Schimann, A. Bonin, F. Boyer, M. De Barba, P. Gaucher, et al. 2019. '*Body Size Determines Soil Community Assembly in a Tropical Forest*'. *Molecular Ecology* 28 (3): 528-43. <https://doi.org/10.1111/mec.14919>.