CONFINEMENT, DYNAMICS, AND ORIENTATION OF DNA AT NANO-INTERFACES

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Abstract: The main objective of this collaborative project is to study effects of nano-confinement on dynamics, orientation, and conformations of DNA upon electrostatic binding to nanostructures of convex shapes – cationic MPCs. We aim at integrating experimental and theoretical modeling efforts. Specifically, we are fabricating a series of cationic monolayer-protected Au nanoclusters (MPCs), characterizing electrostatics of MPC interface, structure and dynamics of the ligands, and conformations of DNA with spin-labeling EPR and other methods (CD, electrophoresis, zeta-potential etc.). Yingling group is carrying out atomistic-level molecular dynamics (MD) simulations of the organic ligand shell upon interactions with DNA to predict various binding modes (second order phase transitions) that will be verified experimentally. Taken together, these studies will provide for fundamental knowledge of DNA-nano-interface and nano-confinement effects, thus, enabling further development of nano-biosensors, DNA-based materials, and novel approaches for gene therapy.

Background

Compaction of DNA by cationic proteins

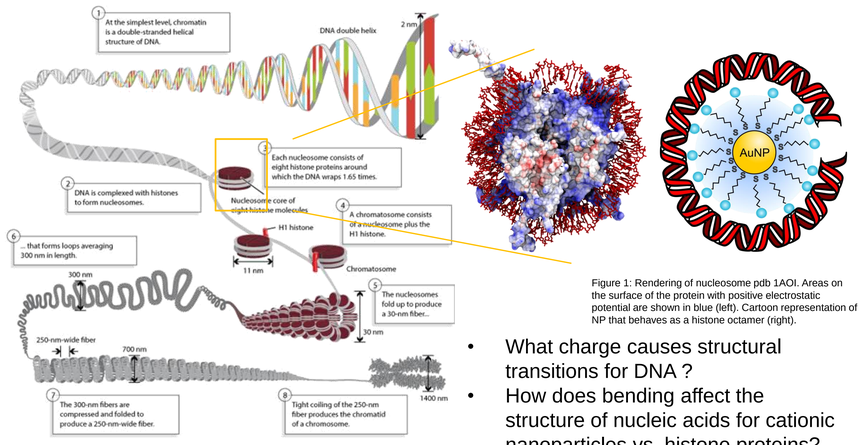


Figure 1: Rendering of nucleosome pdb 1AOI. Areas on the surface of the protein with positive electrostatic potential are shown in blue (left). Cartoon representation of NP that behaves as a histone octamer (right).

- What charge causes structural transitions for DNA ?
- How does bending affect the structure of nucleic acids for cationic nanoparticles vs. histone proteins?

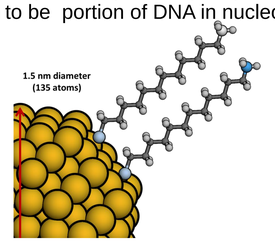
Annunziato, A. ; DNA packaging: Nucleosomes and chromatin. *Nature Education* 2008, 1(1):26

Materials and Methods

Molecular Dynamics Simulations: Atomistic molecular dynamics simulations were performed using the Amber12 software package with the ff12SB force field for nucleic acids. All systems have explicit TIP3P water with NaCl counter ions. For some systems, NaCl was added to a concentration of 0.1 M. Simulations presented are run for a minimum of 120 nanoseconds of simulation time.

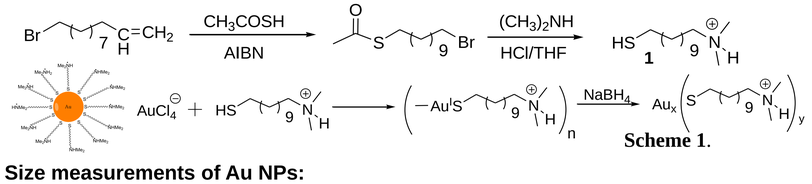
DNA/NP Details: Short sequences (30 basepairs) of DNA were used in this study. Sequence was chosen to be portion of DNA in nucleosome PDB 1AOI.pdb

Figure 2: Part of the nanoparticle core with the two types of ligands used in this study shown. The red represents nitrogen in R-NH_3^+ and the white represents carbon in R-CH_3. NP charge was varied by changed the ratio of these types of ligands.



Nanoparticles consisted of 135 gold (Au) atoms with 60 alkane thiol ligands. The charge of the nanoparticle was adjusted by varying the number of methyl vs. ammonium cation end groups on the surface of the nanoparticle.

Synthesis of Au NPs decorated with ligands terminated with ionizable groups:



Scheme 1.

Size measurements of Au NPs:

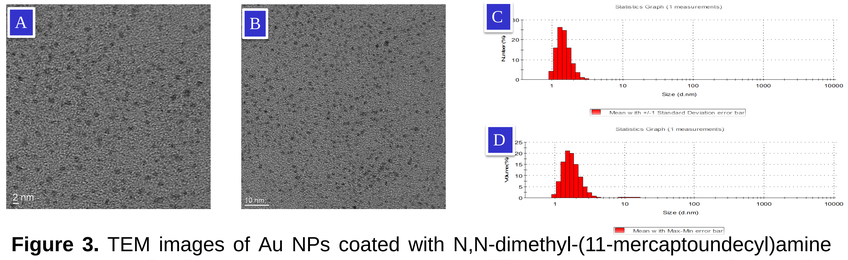


Figure 3. TEM images of Au NPs coated with N,N-dimethyl-(11-mercaptoundecyl)amine ligand (A and B). Particle size measurement from TEM yielded d≈1.5 nm. (C) and (D): Dynamic Light Scattering measurements for Au NPs coated with N,N-dimethyl-(11-mercaptoundecyl)amine yielded average nanoparticle diameter of d ≈1.5 nm.

Results

Theoretical Studies of DNA Bending

The ability of a nanoparticle to bend DNA is correlated with both the nanoparticle charge (Figure 4) and the shape of the nanoparticle ligand shell (Figure 5)

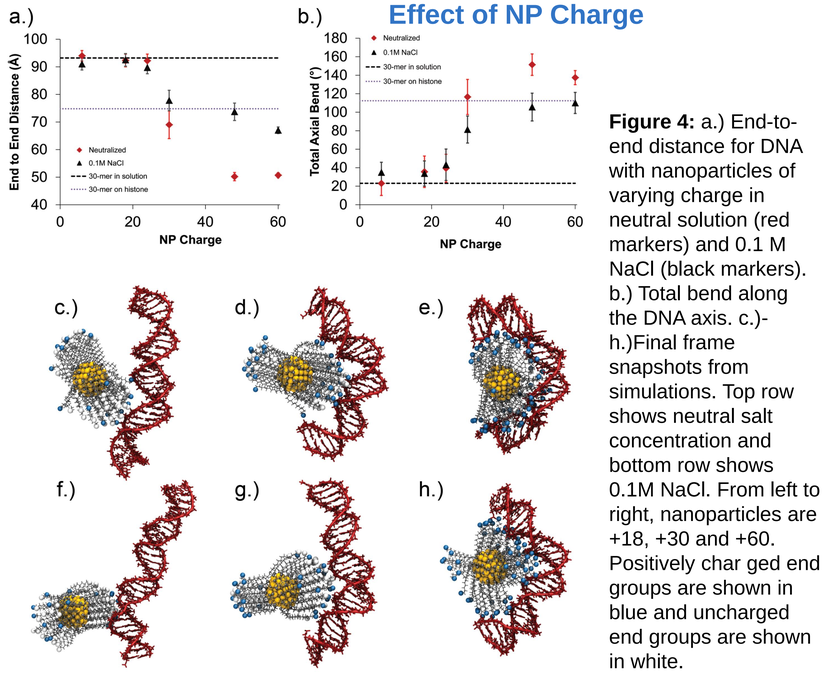
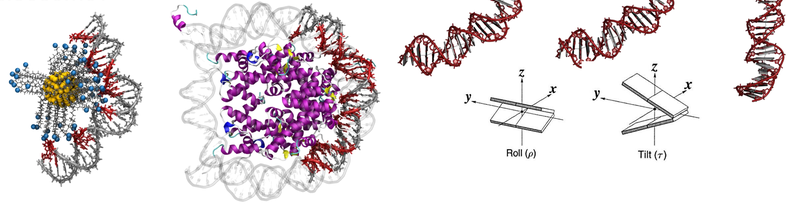


Figure 4: a.) End-to-end distance for DNA with nanoparticles of varying charge in neutral solution (red markers) and 0.1 M NaCl (black markers). b.) Total bend along the DNA axis. c.)-h.)Final frame snapshots from simulations. Top row shows neutral salt concentration and bottom row shows 0.1M NaCl. From left to right, nanoparticles are +18, +30 and +60. Positively char ged end groups are shown in blue and uncharged end groups are shown in white.

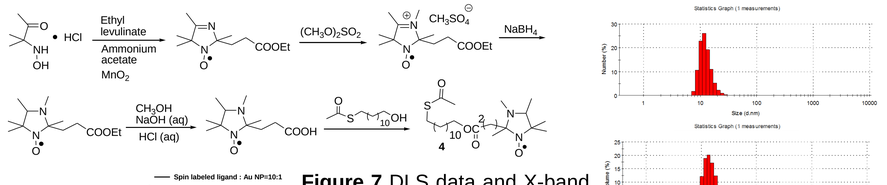
Structural Changes in DNA

How does the structure of DNA with nanoparticles compare to the structure of DNA in the nucleosome?



Experimental Studies

Scheme 2. Synthesis of the thioacetate ligand **4** modified with an protonatable nitroxide.



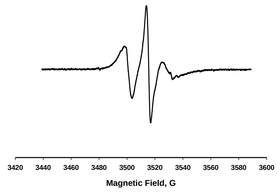
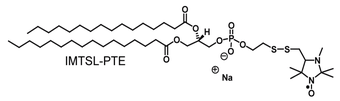


Figure 7 DLS data and X-band EPR spectrum from aqueous solution of Au NPs spin-labeled with ionizable thioacetate ligand **4** (Scheme 2) at 10:1 NP:SL ligand molar ratio. EPR spectrum was taken at pH=7.02

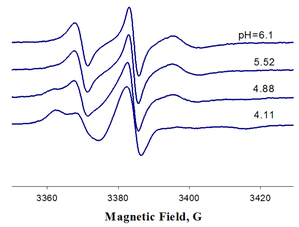
Measurements of Surface Electrostatic Potential by EPR of pH-Sensitive Lipids

To assess the surface electrostatics of the lipid membrane we employed the Electron Paramagnetic Resonance (EPR) spectroscopy of a recently introduced phospholipid with a pH-sensitive nitroxide covalently attached to the polar head group. We followed the pH-induced changes in magnetic parameters of the spin-labeled lipid to determine the pK_a of its ionizable group that depends on the surface electrostatics. Figure below shows chemical structure of a synthetic spin-labeled phospholipid IMTSL-PTE.



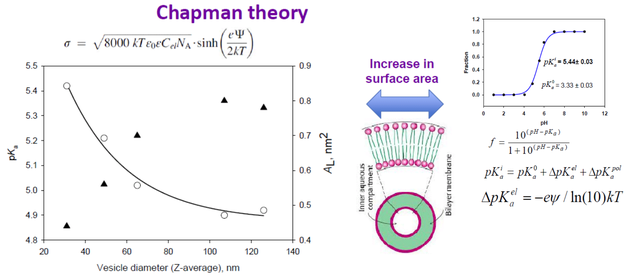
Chemical structure of a synthetic spin-labeled phospholipid (IMTSL-PTE) synthesized at NCSU.

Figure below shows a series of EPR spectra from POPG (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol)) and DMPG (1,2-dimyristoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) doped with 1 mol. % of IMTSL-PTE. The spectra were acquired at 17 ºC, which is above (fluid phase) and below (gel phase) the main phase transition temperature of POPG (T_m= -2 ºC) and DMPG (T_m= 23 ºC), respectively.

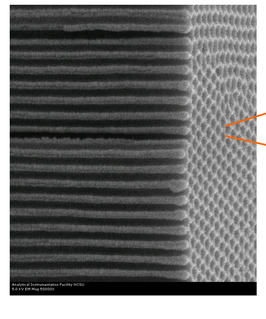


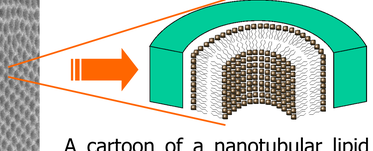
Representative X-band (9.5 GHz) EPR spectra of unilamellar phospholipid vesicles composed of POPG (left, average diameter of 106 nm) and doped with 1 mol % of IMTSL-PTE measured at various pH.

Compression of lipids upon increase in curvature? Surface area per phospholipid from the Gouy-Chapman theory



Macroscopically Aligned Lipids In Nanoporous Anodic Aluminum Oxide Substrates





A cartoon of a nanotubular lipid bilayer (top) and SEM images (left and bottom) of ordered nanoporous anodic aluminum oxide (AAO) substrate fabricated at NCSU and developed previously using DOE support.