Molecular dynamics investigation of oligonucleotide-functionalized gold nanoparticles

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Abstract

CRISPR/Cas9 is a genome editing technique that targets and corrects unwanted gene mutations, with high-impact applications in many fields, including medicine and agriculture. The major limitation that occurs when implementing the CRISPR/Cas9 technology is the off-target activity. In order to improve the targeting efficiency, oligonucleotide-functionalized gold nanoparticles have been employed for successful delivery of CRISPR/Cas9 in vivo to repair errors in the dystrophin gene. An in-depth and comprehensive understanding of the CRISPR/Cas9-Gold-based delivery vector is crucial in improving efficiency and reducing off-target effects. In order to investigate the surface coverage of functionalized gold nanoparticles, we employed molecular dynamics simulations. The behavior of different surface coverage densities, used for CRISPR/Cas9 delivery application, are compared. The results show that various surface densities of oligonucleotide-functionalized gold nanoparticles lead to the formation of different packing of DNA strands on the gold surface.

Methods

- Molecular dynamics simulations used both *atomistic* (AA) and *coarse grained* (CG) models.[1,2]
- AA simulations were performed using NAMD[3] and CG simulations employing GROMACS[4] (attains 2 orders of magnitude longer simulation times).
- For AA and CG gold nanoparticles (GNPs) and DNA were used models compatible with the CHARMM[1] and MARTINI[2] force field, respectively.

Optimization of DNA loading in nanoparticle-oligonucleotide conjugates

1. Optimization of GNP surface coverage density of thiol-modified oligonucleotides
2. Molecular dynamics investigation of GNP conjugated with thiol-modified oligonucleotides

AA simulations

AA GNPs at different thiol surface coverage densities

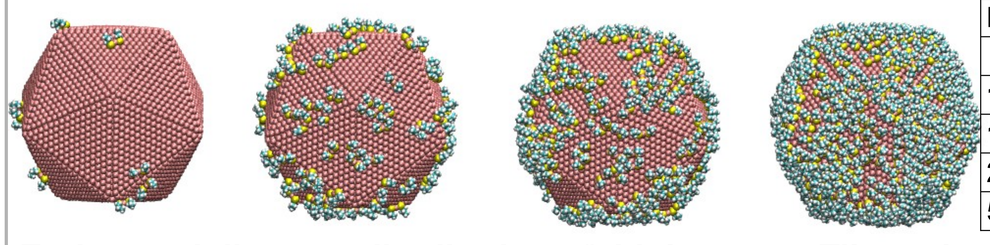
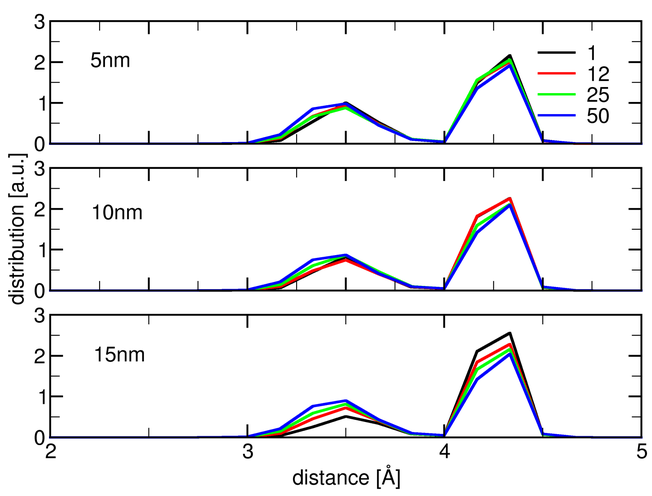


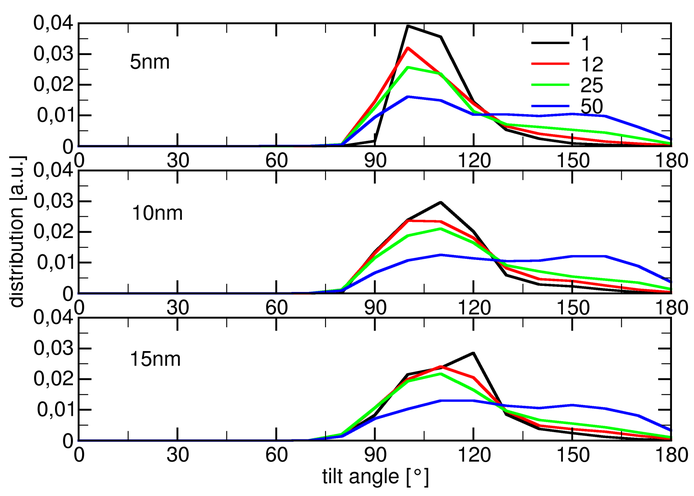
Table 1. AA Simulations

Diam.(nm)	5	10	15
Density	No. of thiols		
1%	4	18	36
12%	48	208	430
25%	100	434	894
50%	200	870	1790

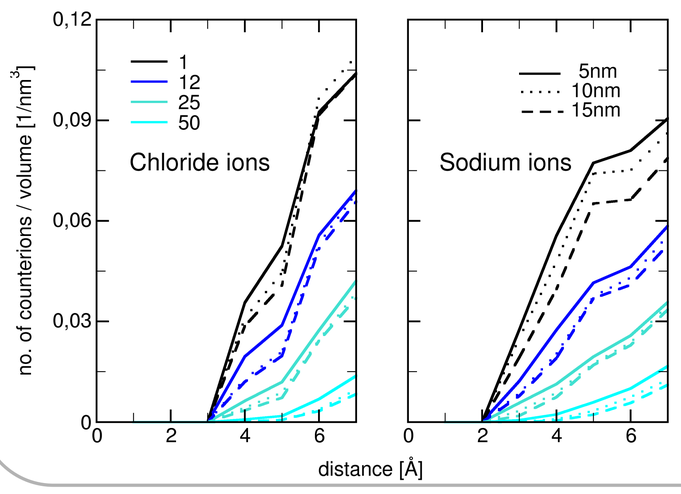
End-to-end distance distribution of thiols



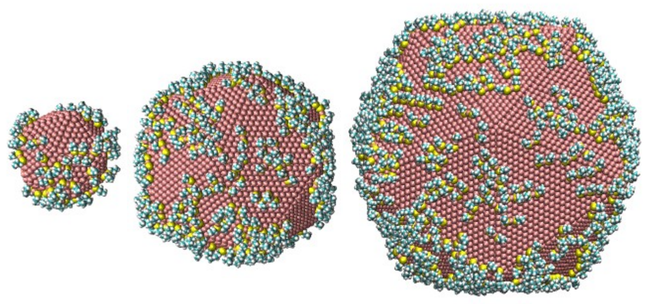
Tilt angle distribution of thiols



Charge distributions of the counterions



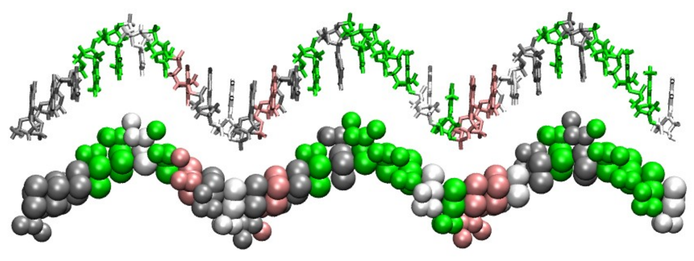
AA GNPs of diameters 5, 10 and 15 nm



CG simulations

The thiol-modified DNA (ssDNA-thiol) and donor DNA sequences were determined based on the sgRNA.
ss DNA sequence:
thiol-AAATTCTGACAGATATTTCTGGCATATTTC

AA
CG



CG GNPs at different surface coverage densities of oligonucleotides

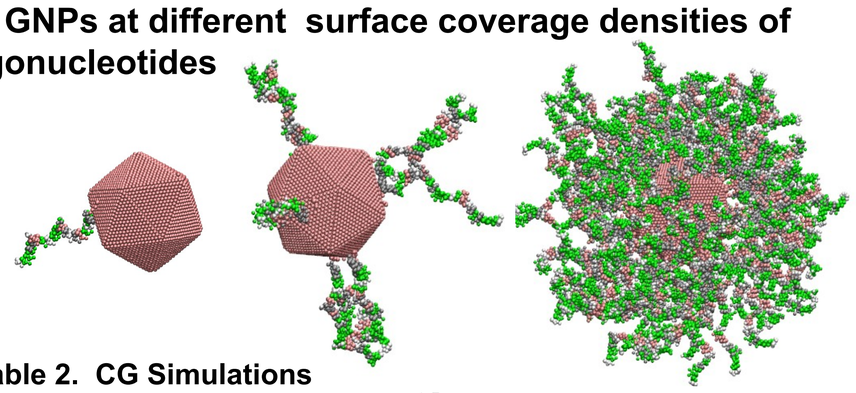
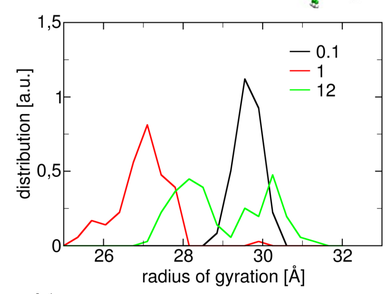
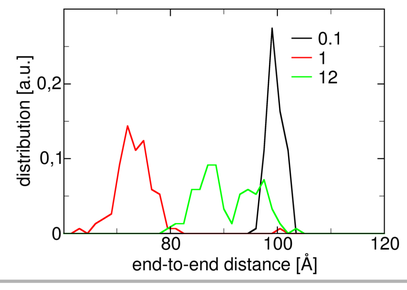
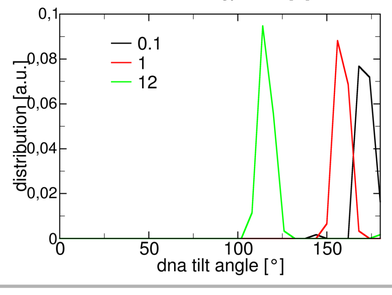


Table 2. CG Simulations

Diam.	10nm	
Density	No. of thiols	No.of DNA
0.1%	1	1
1%	9	9
12%	104	104







Conclusions

- At low and medium concentrations, the thiol chains prefer to be close to the GNP surface, in a quasi-parralel conformation
- Gold nanoparticle size and their thiol modifications' surface coverage density are key elements that should be used in manipulating the thiol-modified GNP properties (e.g. counterions attraction)
- The DNA chains of GNP-thiol-oligonucleotides systems show regimes of order separated by non-ordered transition zones
- The conformational structure of the DNA chains strongly depend on the oligonucleotides surface cover density, being a result of an interplay between the energetic interaction between the DNA chains

References

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