Fabrication and Characterization of Random Single Molecule Arrays for DNA Sequencing Chip

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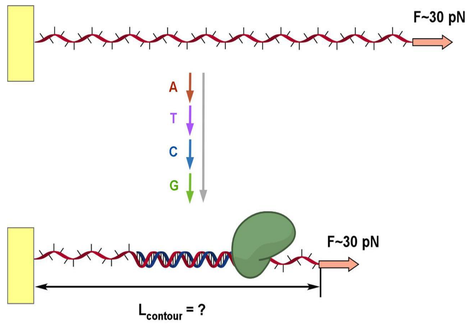
Purpose: Finding an appropriate concentration of DNA molecules on a substrate for single molecule attachment of micron sized probes.

Introduction:

- Force Spectroscopy can measure changes in the size of individual DNA molecules
- Single nucleotide addition by polymerase changes the size of DNA

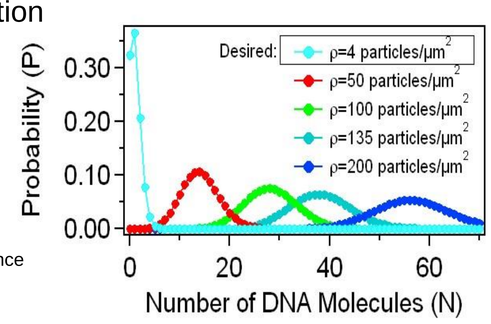


- We want a nearest neighbor distance of ~600 nm
- Well-spaced molecules bind to substrates in a Poisson distribution

$P(N) = \mu^N e^{-\mu}/N!$

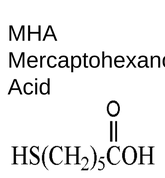
$\mu = \rho\pi D^2/4$

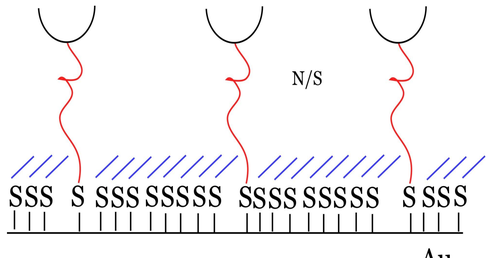
ρ ≡particle density
D≡nearest neighbor distance



Non-specific binding must be limited for single molecule detection:

MHA
Mercaptohexanoic Acid





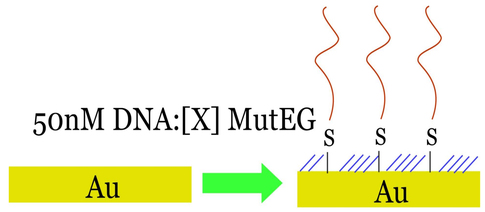
XPS Data:

Used to determine nearest neighbor distance (*D*) of our DNA

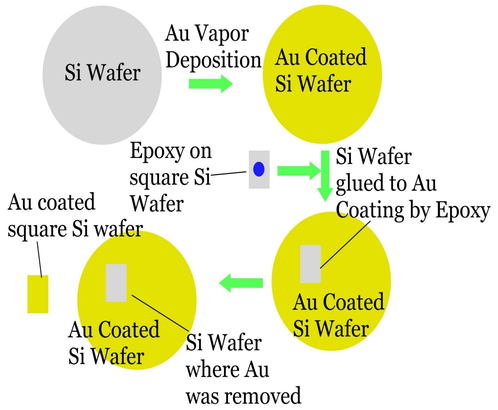
Table 1. *Composition of the surface layer formed in reactions of the end modified DNA with Au surfaces determined from the XPS spectra.*

Surface Reaction	Atomic Percent N	O	P	S S-Au	S S-H	S S-O	C	Ratio N/S	Nearest neighbor distance (nm)
100 nM ssDNA in PBS	3.4	13.6	1.2	2.8	0.7	0.0	78.4	1.2	7.7

Surface Reaction: Competitive Binding of DNA



Template-Stripped Gold:



Create an atomically smooth surface

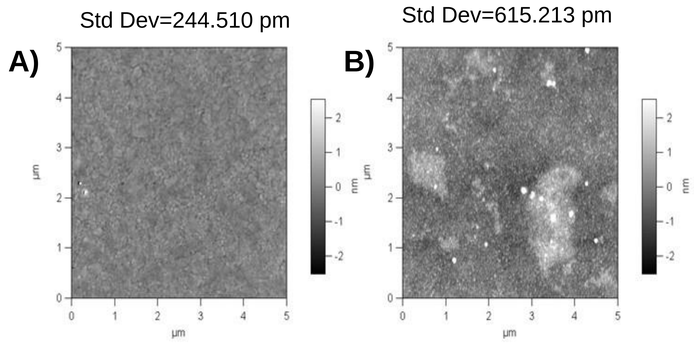


Figure 2. (A) Atomically smooth template-stripped gold surface. (B) E-beam evaporated gold (traditional gold surface). These images were taken with tapping mode on AFM.

Problem: Cannot count the number of DNA molecules on the surface compared to the MHA sample.

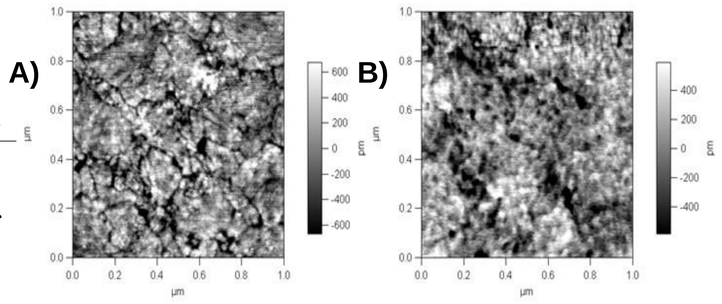
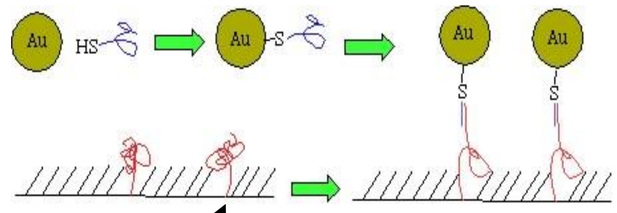
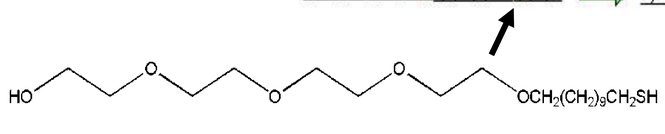


Figure 1. (A) Template stripped gold with 0.1 nM DNA solution and MHA. (B) MHA on a template stripped gold surface. These images were taken with AFM on tapping mode.

Gold Nanoparticle (AuNP) Hybridization: AuNP has complement to surface bound DNA





≡ (1-Mercaptoundec-11-yl)tetra(ethylene glycol) (MutEG)

MutEG is used to reduce non-specific binding and compete with DNA attachment. Increasing MutEG concentrations acts to lower DNA surface density

Gold Nanoparticle Hybridization Results:

50 nM DNA with [MutEG]=1 μM-1 mM

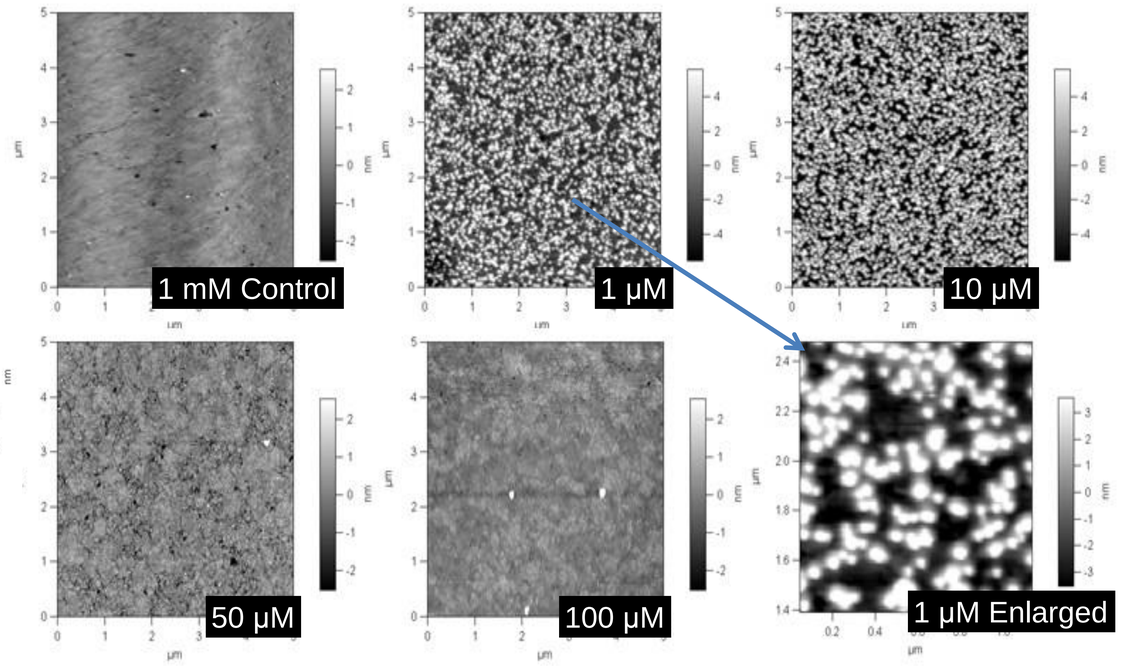


Figure 3. AuNPs bound to DNA on a template-stripped gold surface taken using tapping mode on AFM. The heights of the AuNP's are approximately 12 nm.

Nearest Neighbor Distance:

- 1 uM MutEG sample has a nearest neighbor distance of ~86 nm
- 10 uM MutEG sample has a nearest neighbor distance of ~87 nm

Conclusions and Future Works:

a) Unlabeled DNA single molecule counting is not an effective way of determining single molecule concentrations.
b) Template-stripped gold is significantly flatter than e-beam evaporated gold.
c) AuNP hybridization shows a cutoff threshold for MutEG concentrations between 10uM and 50uM.
d) More samples with lower DNA concentrations will be prepared to make further conclusions