Electrochemical DNA Biosensors Exploiting Changes in the Charge Transfer Properties of DNA

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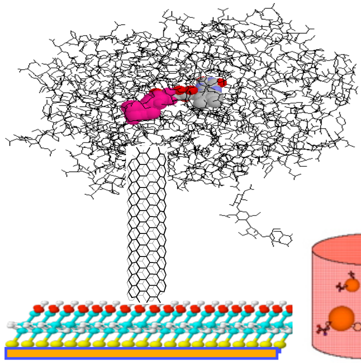
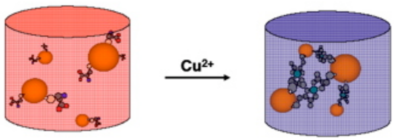
School of Chemistry

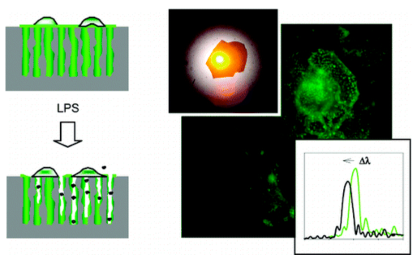
The University of New South Wales



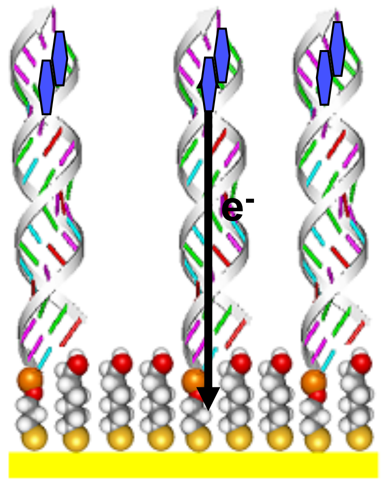
Wiring Enzymes



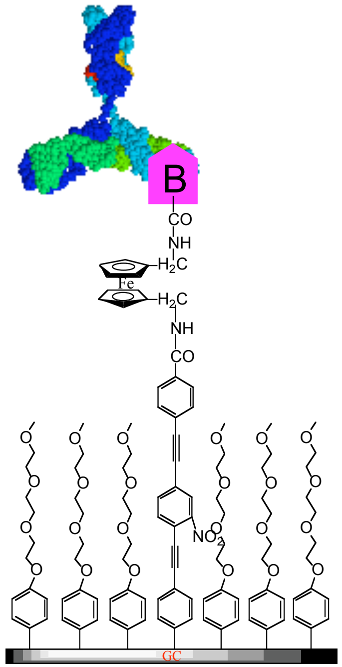
Nanoparticle Biosensors



Cell-Surface interactions



DNA-Modified Electrodes



Immunosensors

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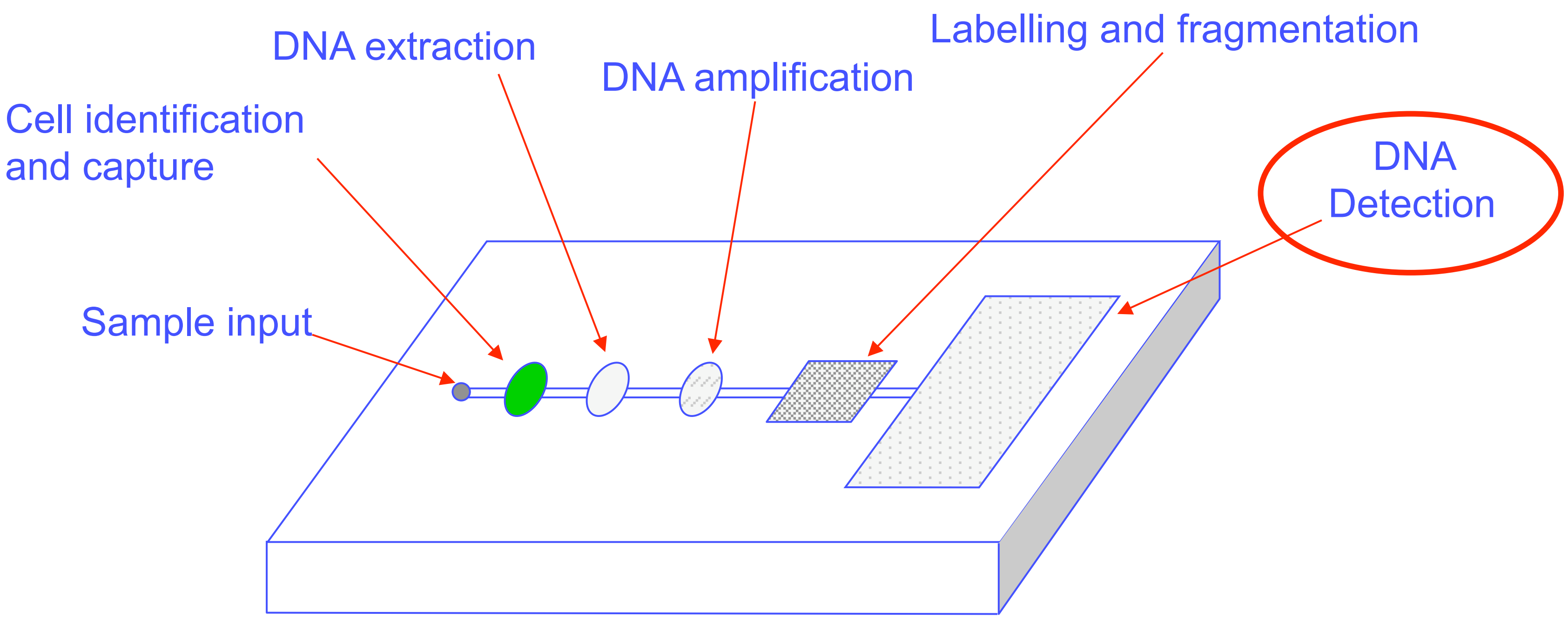
Portable DNA Biosensors



SIMPLICITY

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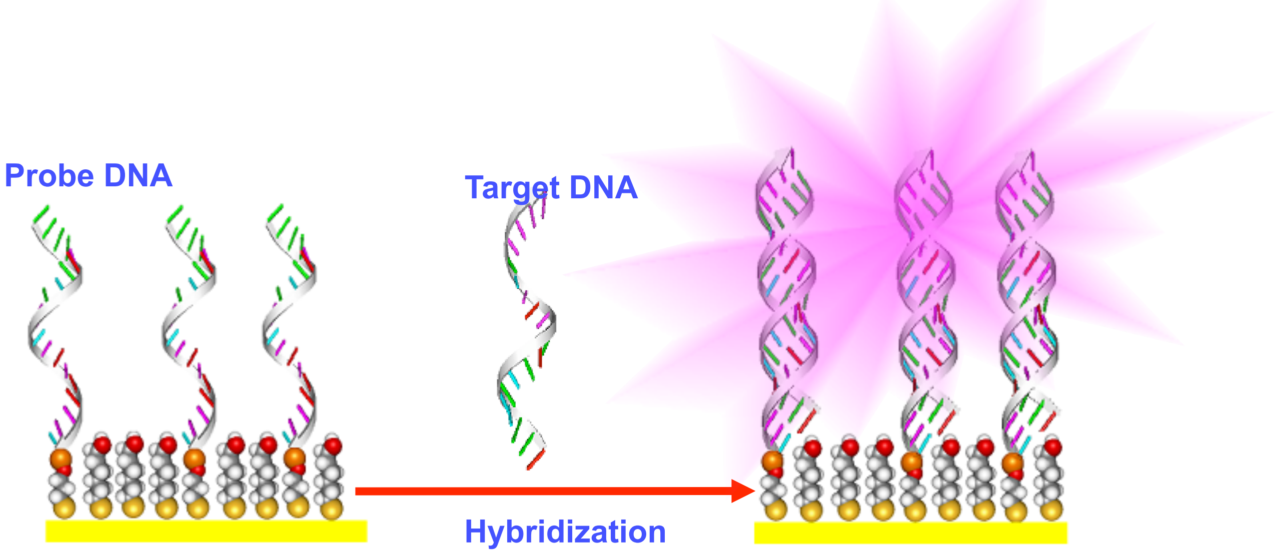
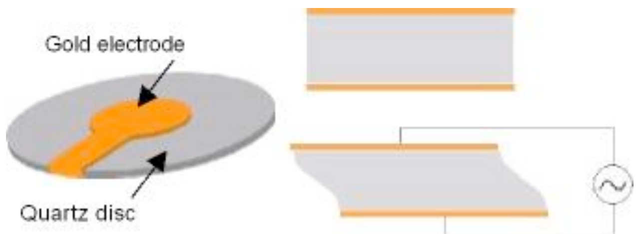
DNA Biosensors



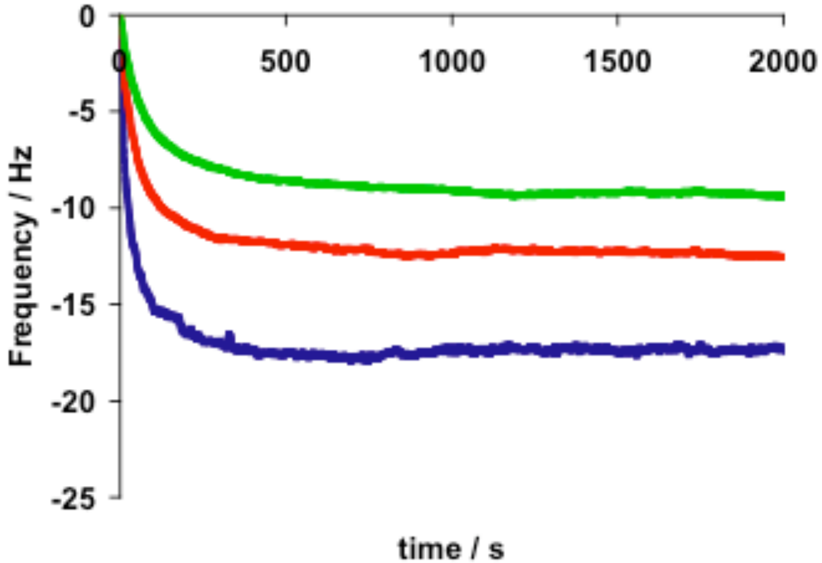


$$\Delta f = -2.3\times 10^{6} f_o^{2}\left(\frac{\Delta m}{A}\right)$$

mass

Area of quartz

Reference frequency



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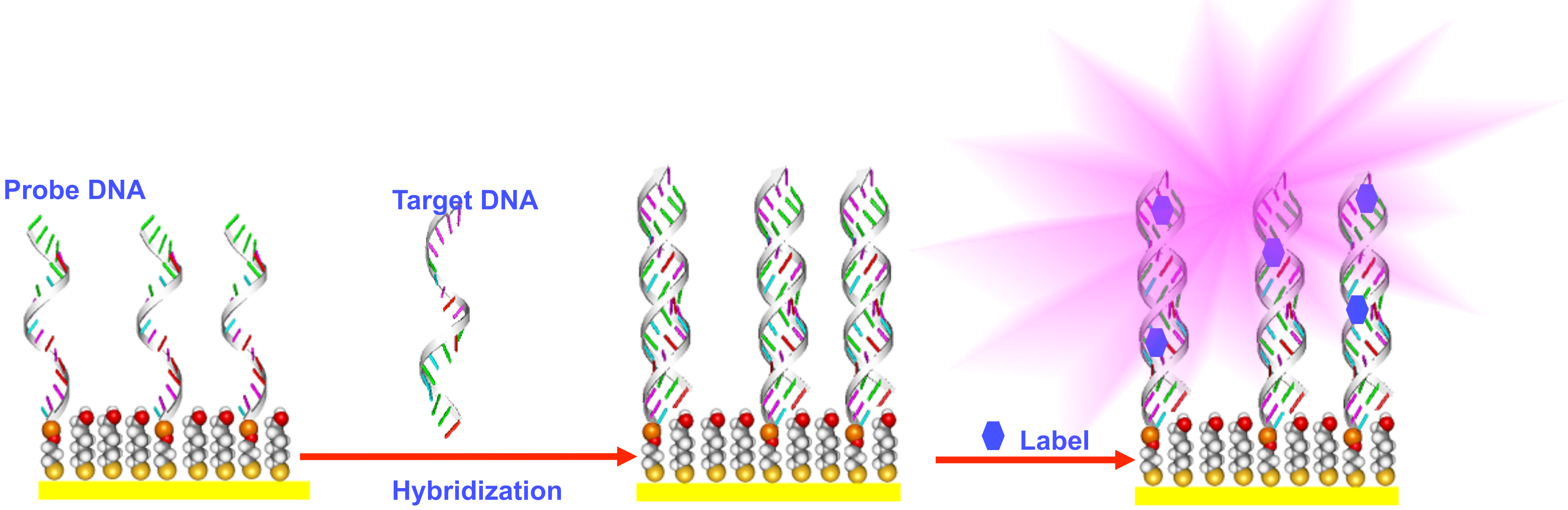
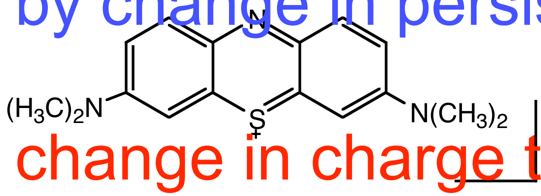
E.L.S. Wong, E. Chow, J.J. Gooding, *Langmuir* **21** 6957-6965 (2005).

DNA Biosensors



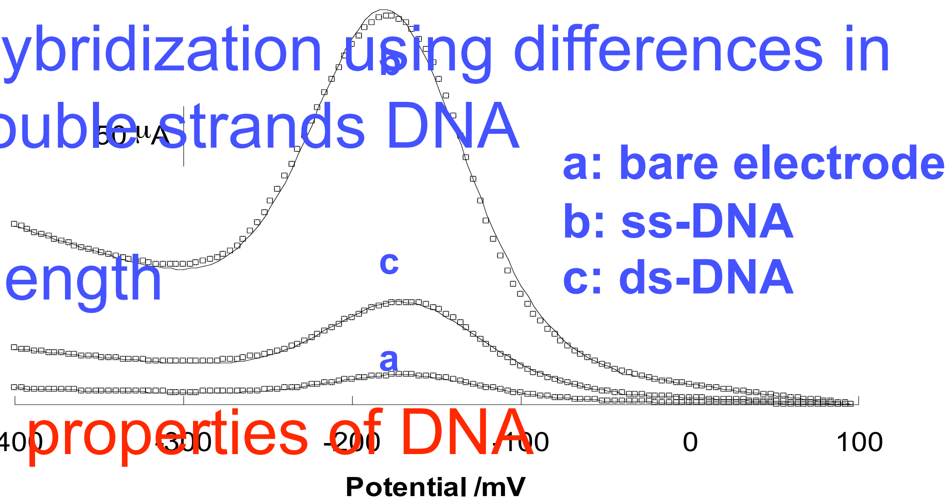
Interested in detecting DNA hybridization using differences in properties of single and double strands DNA

1) by change in persistence length



2) change in charge transfer properties of DNA

methylene blue



A. Erdem *et al Electroanalysis* **11** 586 (1999), W. Yang *et al Electroanalysis* **14** 1299 (2002)

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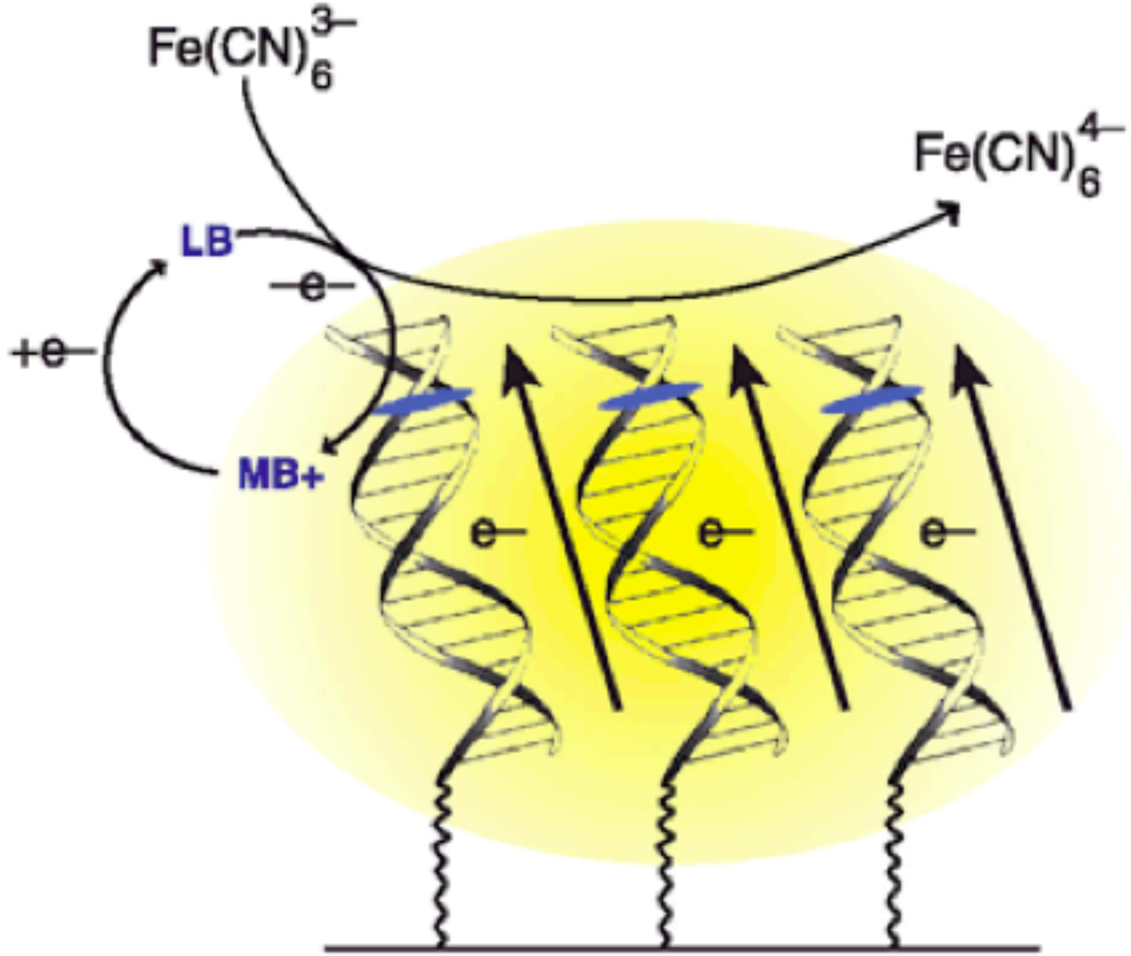
DNA Biosensors Based on Long-Range Charge Transfer



[1] S.O. Kelley *et al Nucleic Acids Res.* **24** 4830 (1999)
[2] E.M. Boon *et al Nature Biotech.* **18** 1096 (2000).

http://www.geneohm.com/

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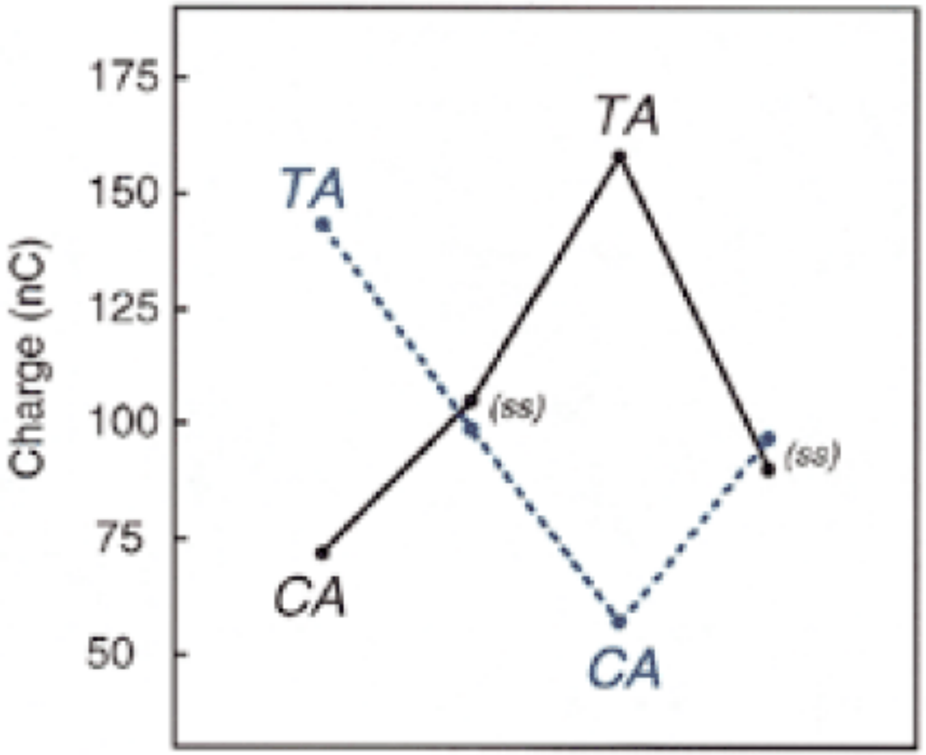
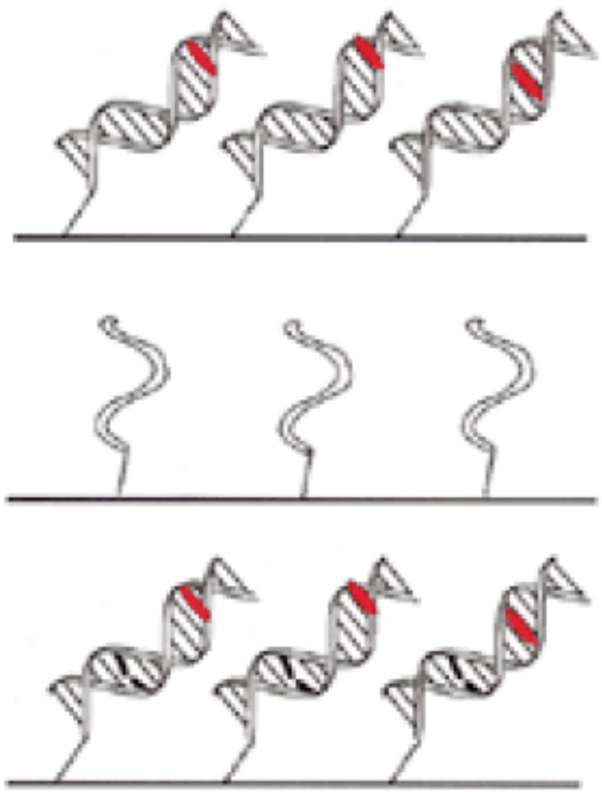
DNA Biosensors Based on Long-Range Charge Transfer

- Barton and colleagues can differentiate between ssDNA, a complete duplex and one with a mismatched.
- Needed complete surface of duplexes to achieve reliability.





S.O. Kelley *et al Nucleic Acids Res.* **24** 4830 (1999)
E.M. Boon *et al Nature Biotech.* **18** 1096 (2000).

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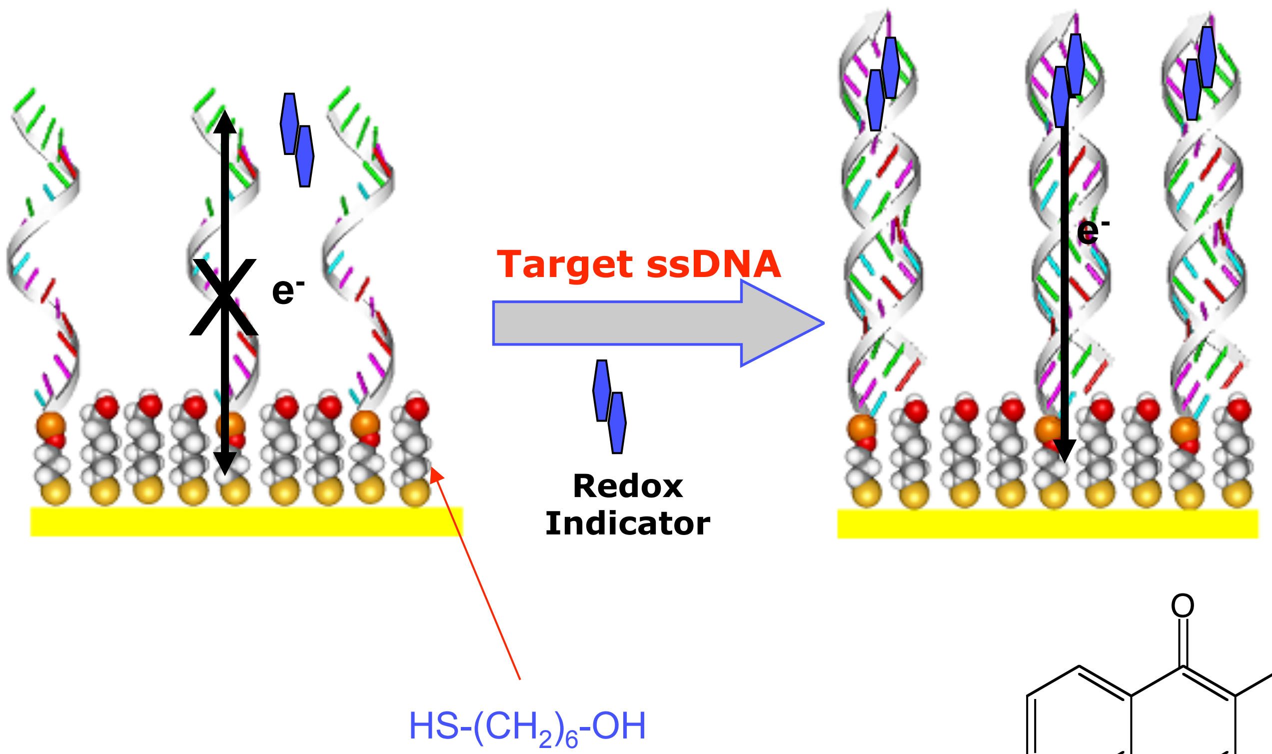
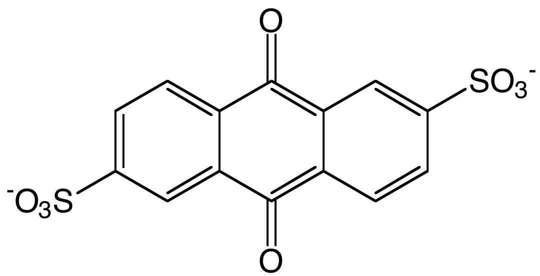
Further Interfacial Considerations





Redox Intercalator
2,6-disulfonic acid anthraquinone

Levicky *et al J. Am. Chem. Soc.* **119** 8916 (1997)
E. Wong, J.J. Gooding, *Anal. Chem.* **75** 3845-3852 (2003).

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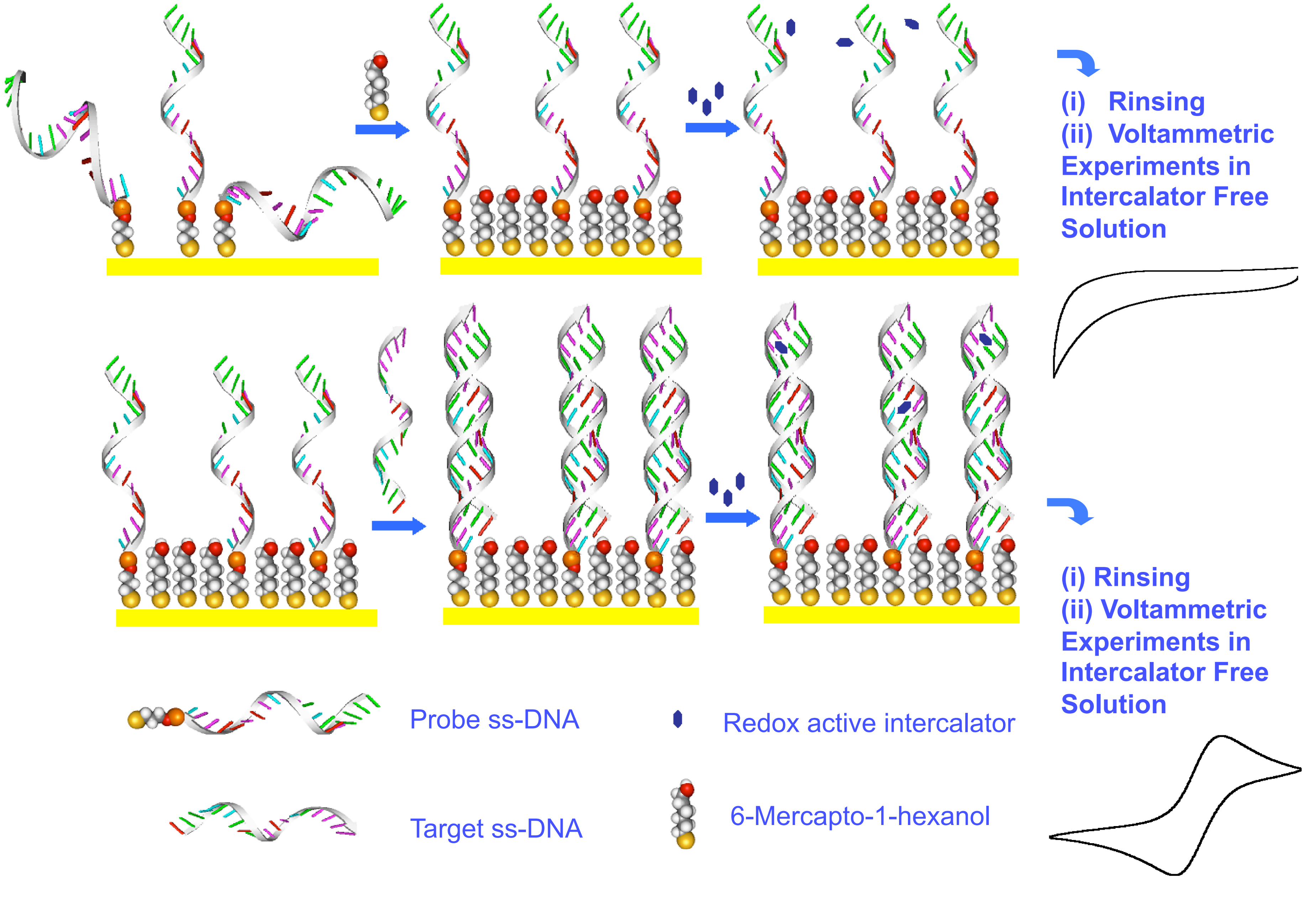
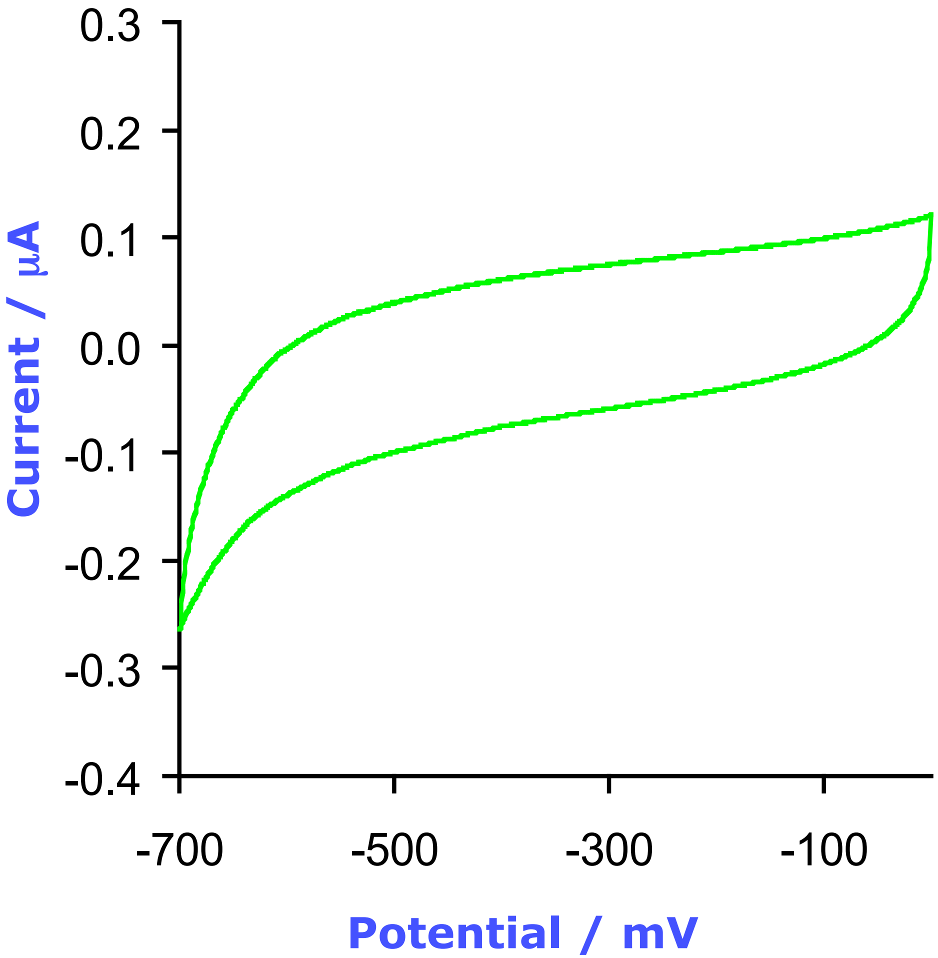
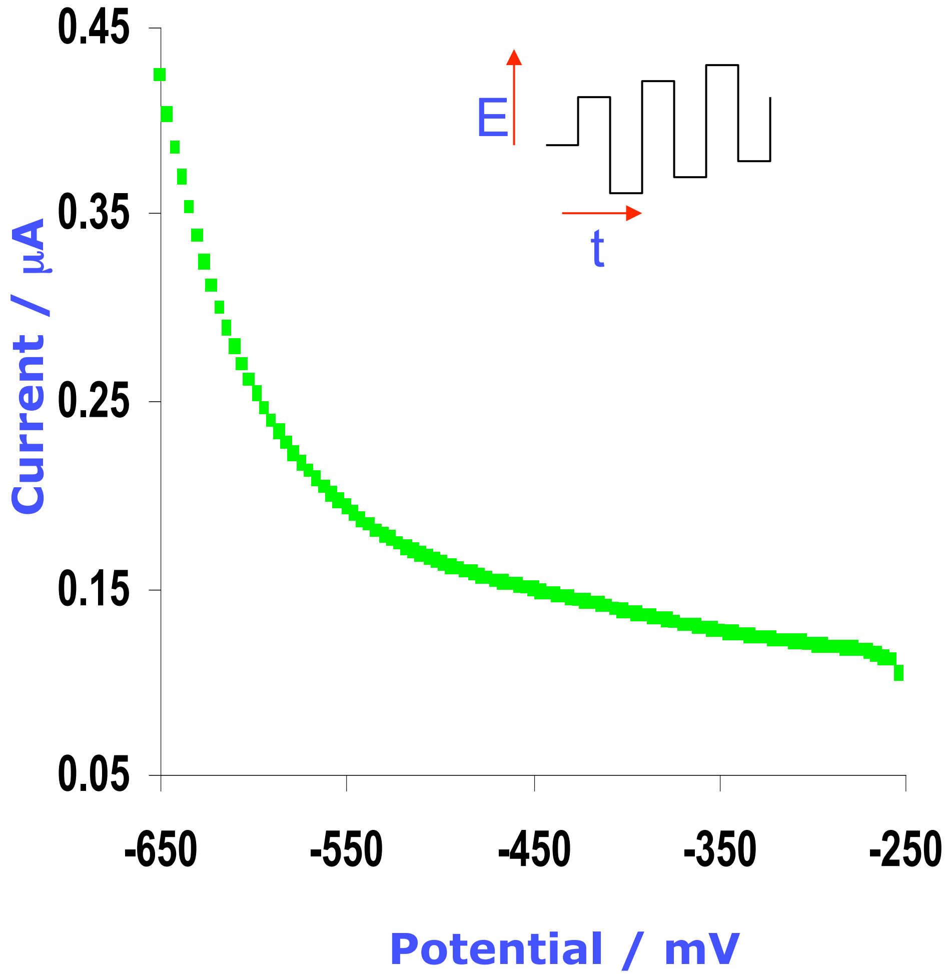


E. Wong, J.J. Gooding, *Anal. Chem.* **75** 3845-3852 (2003).

Exposure of DNA Recognition Interface to Target ssDNA



CV



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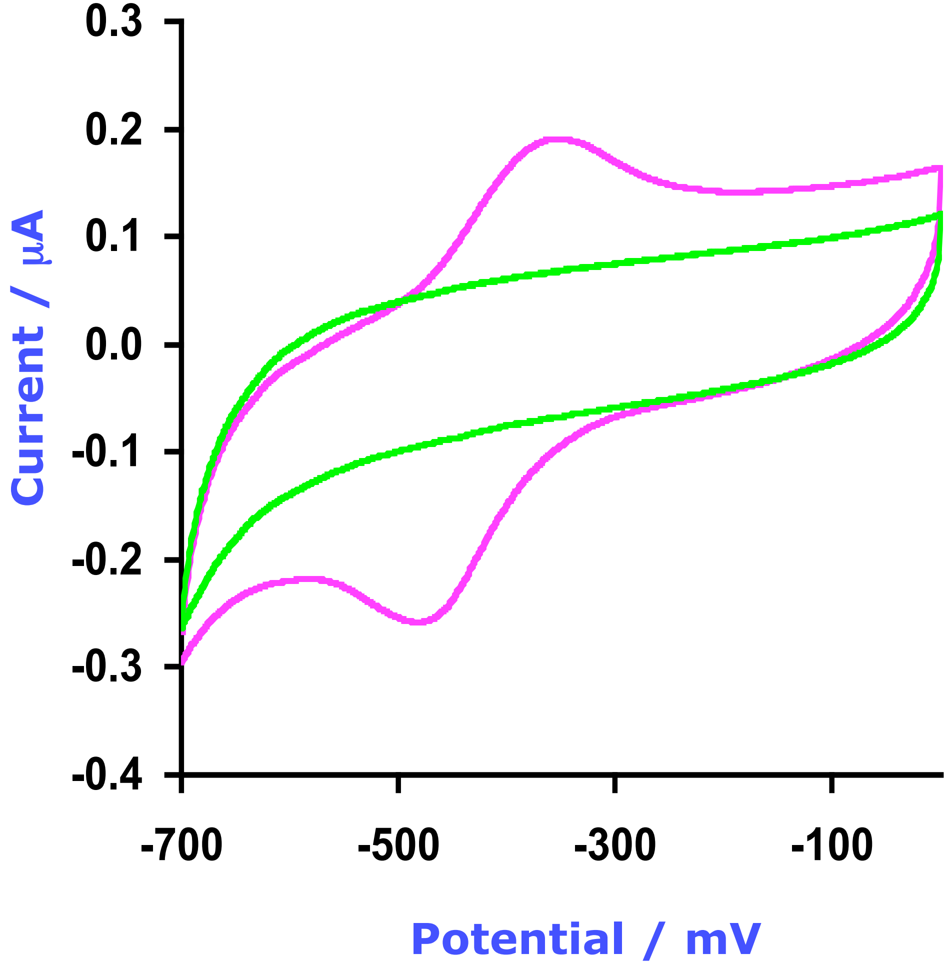
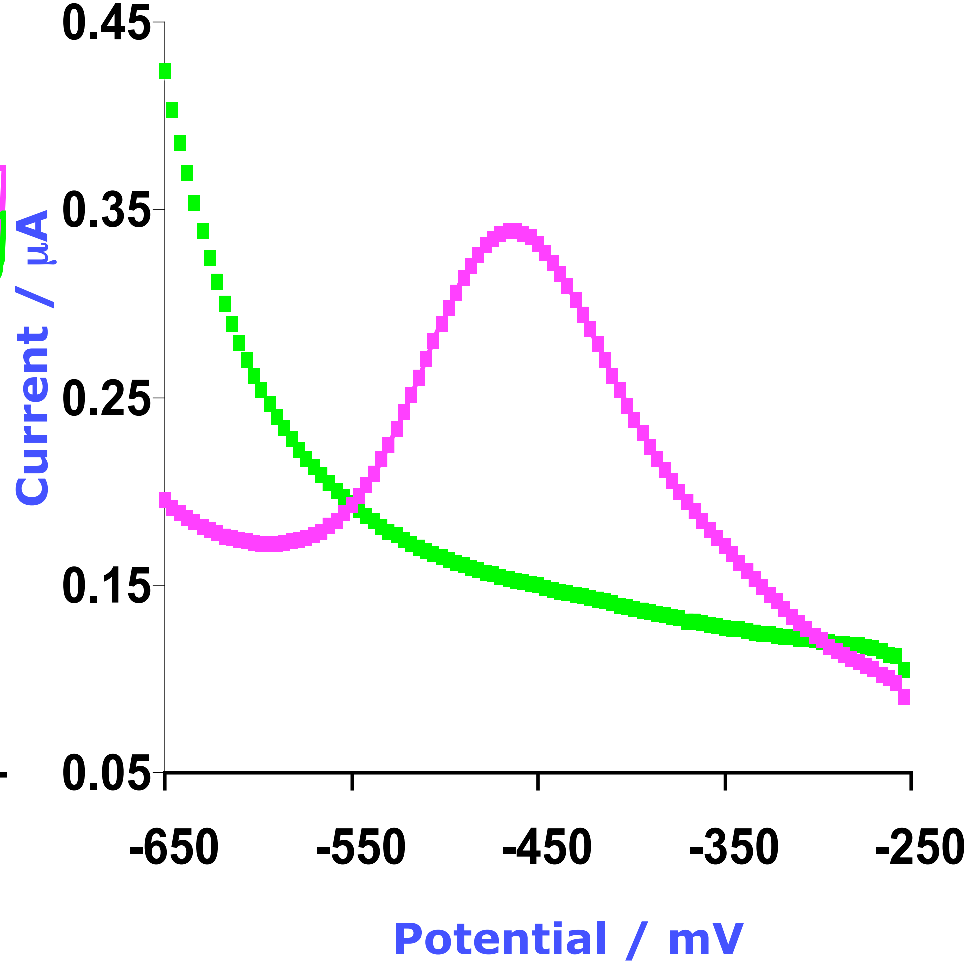
Exposure of DNA Recognition Interface to Target ssDNA



CV



SWV

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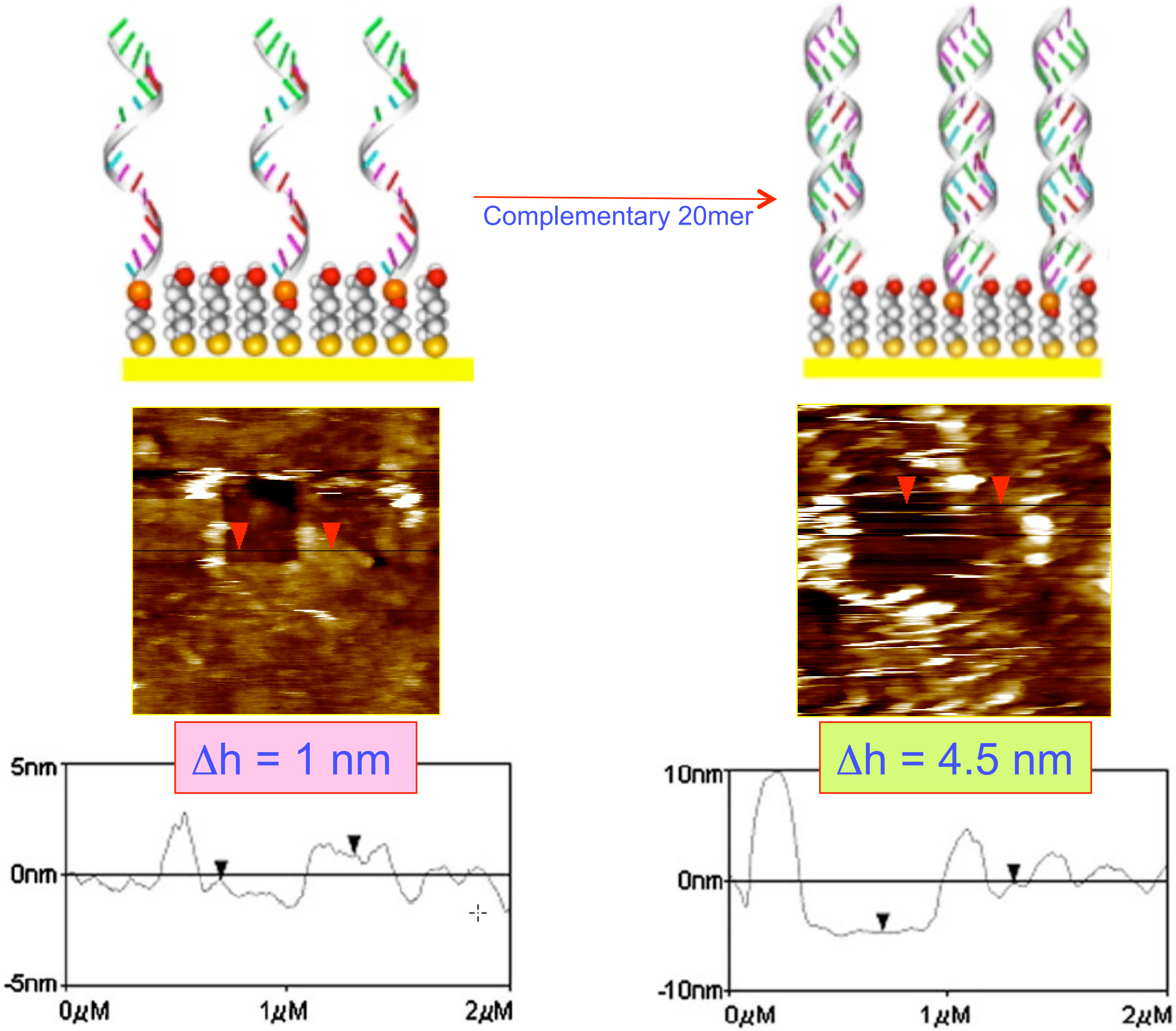
Changes in Spatial Relationships



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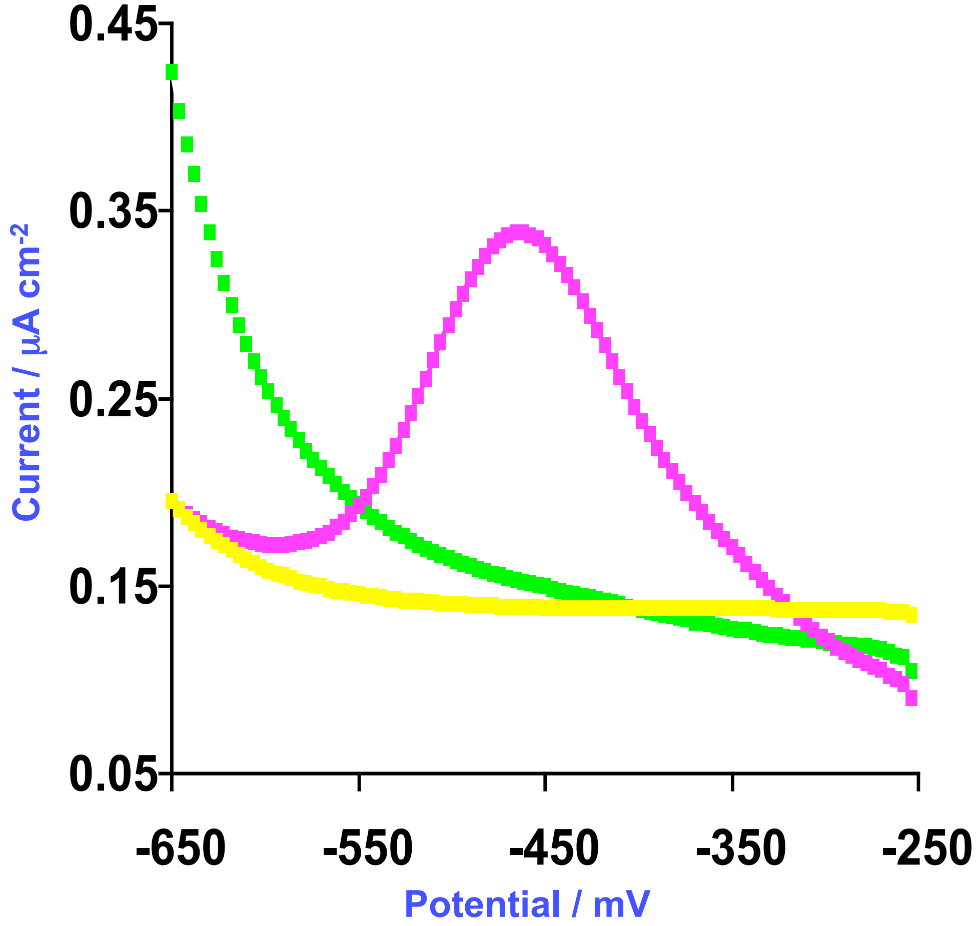
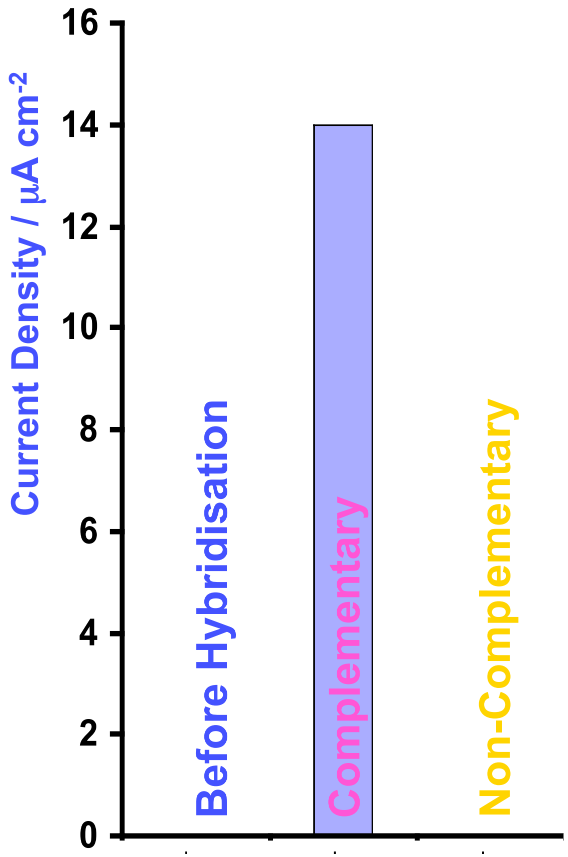
Exposure of DNA Recognition Layer to different target ssDNA





E.L.S. Wong, J.J. Gooding, *Anal. Chem.* **75** 3845-3852 (2003).

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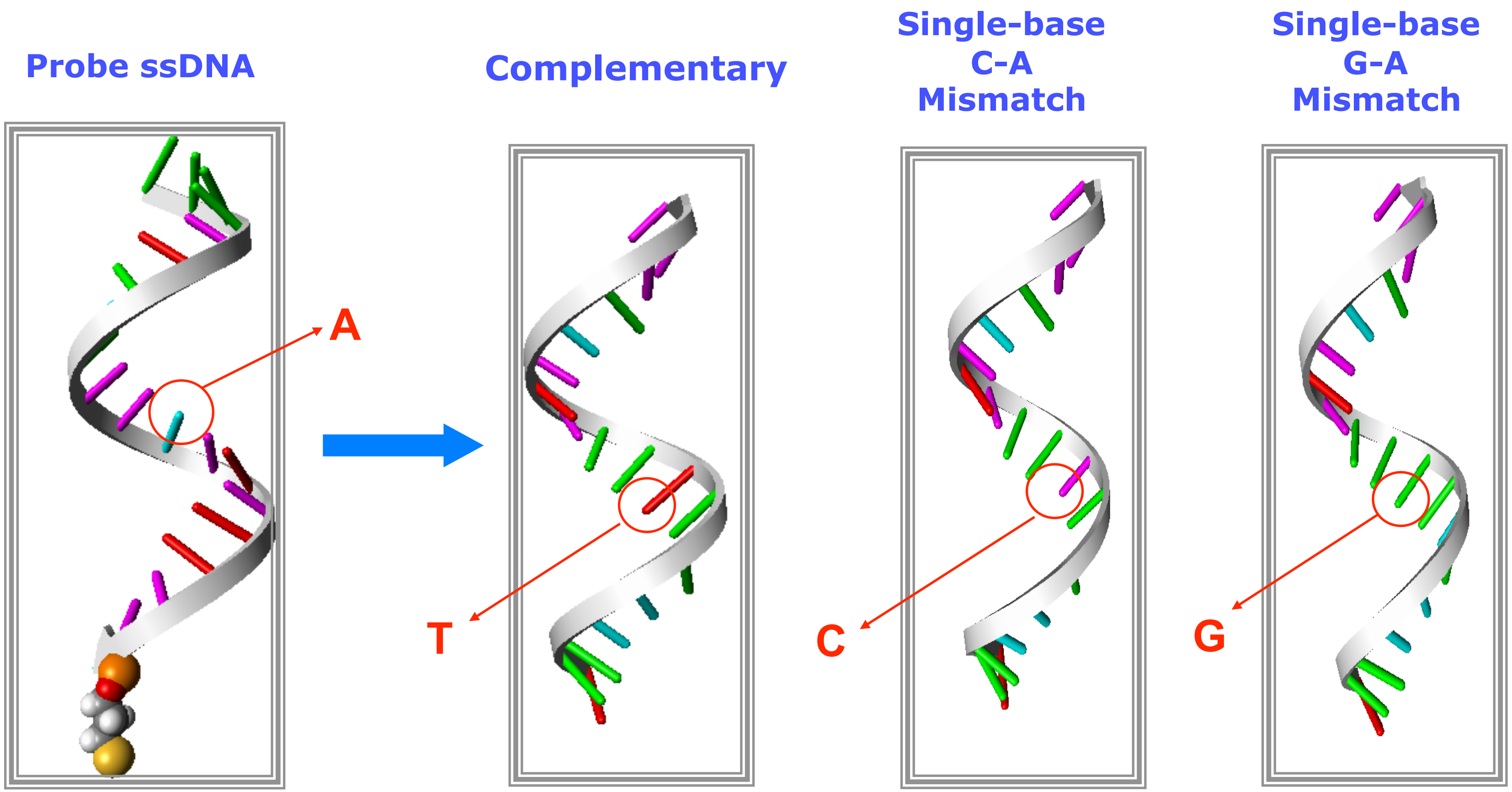
Single-Base Mismatch



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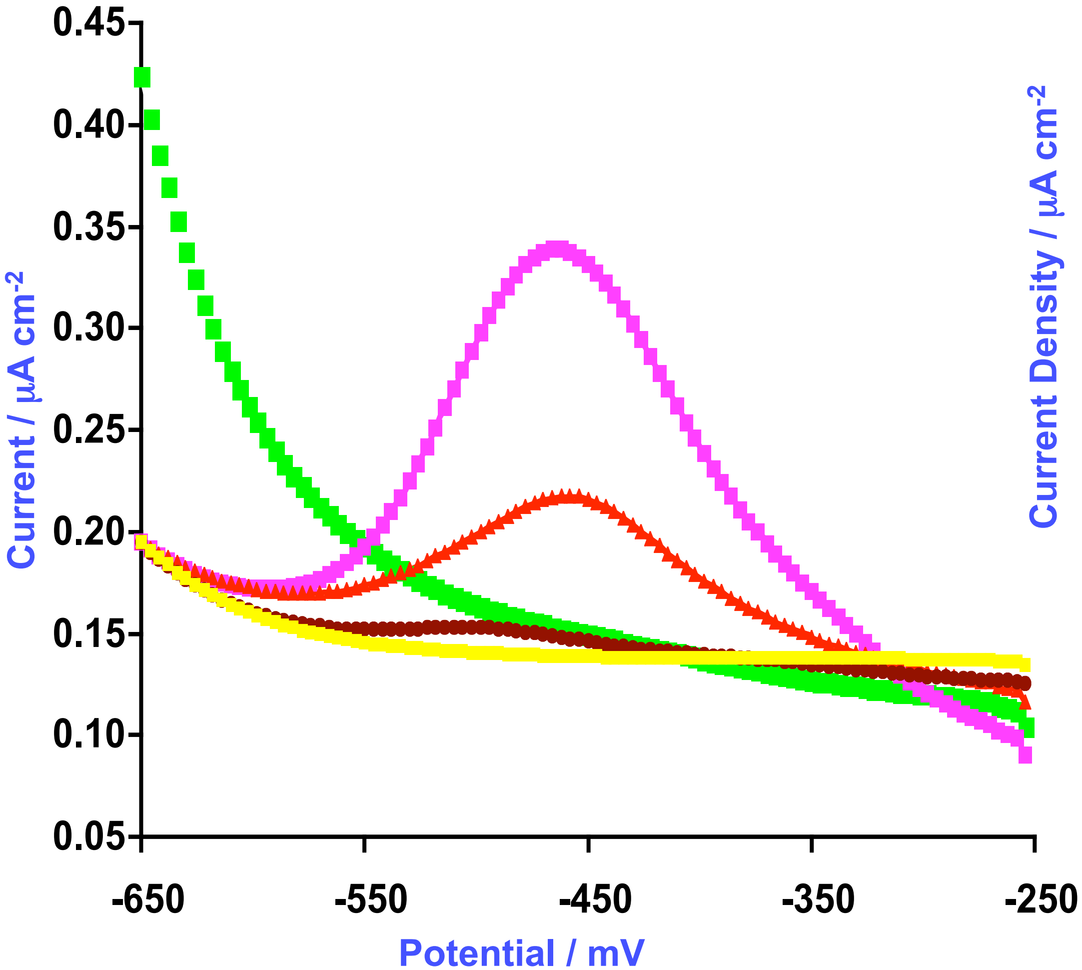
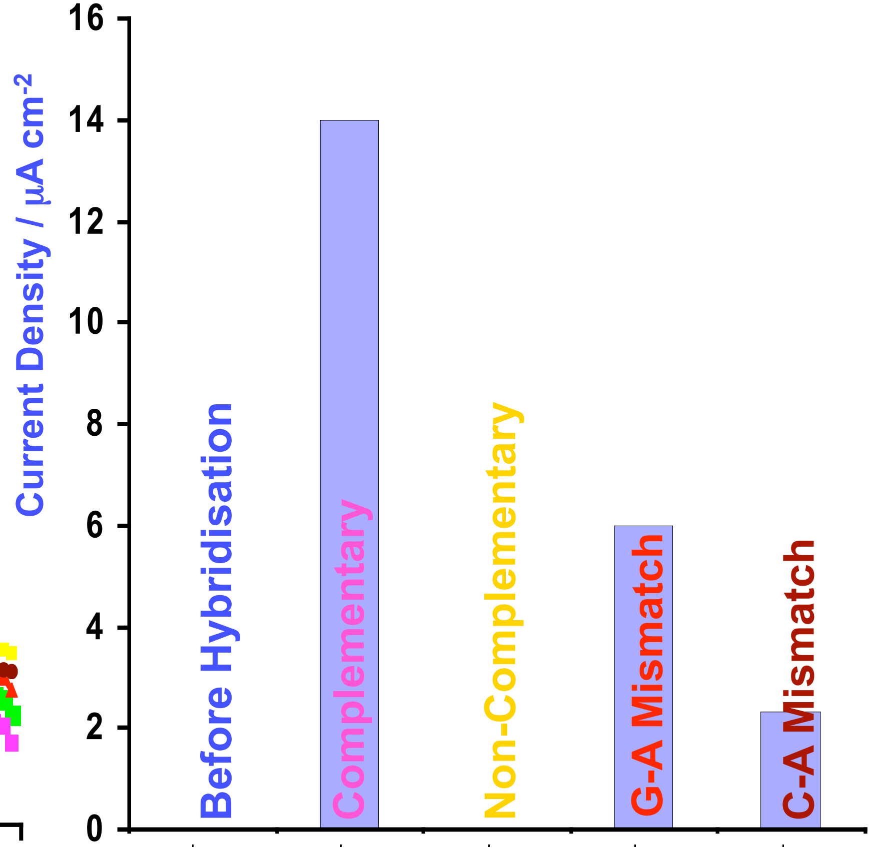
Exposure of DNA Recognition Layer to different target ssDNA





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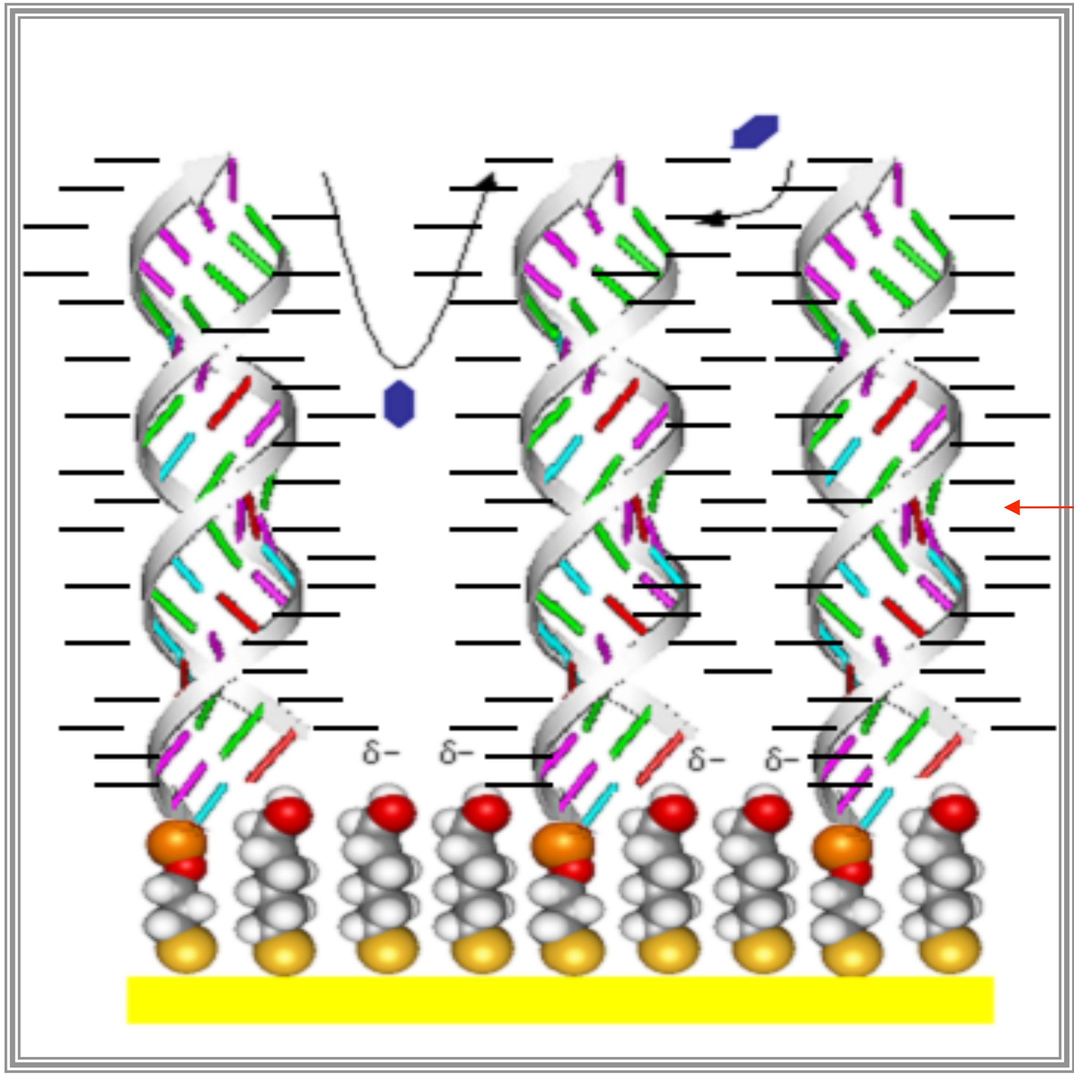
E.L.S. Wong, J.J. Gooding, *Anal. Chem.* **75** 3845-3852 (2003).

Why can we detect mismatches?



Mismatch about here

E.L.S. Wong, J.J. Gooding, *Anal. Chem.* **75** 3845-3852 (2003).

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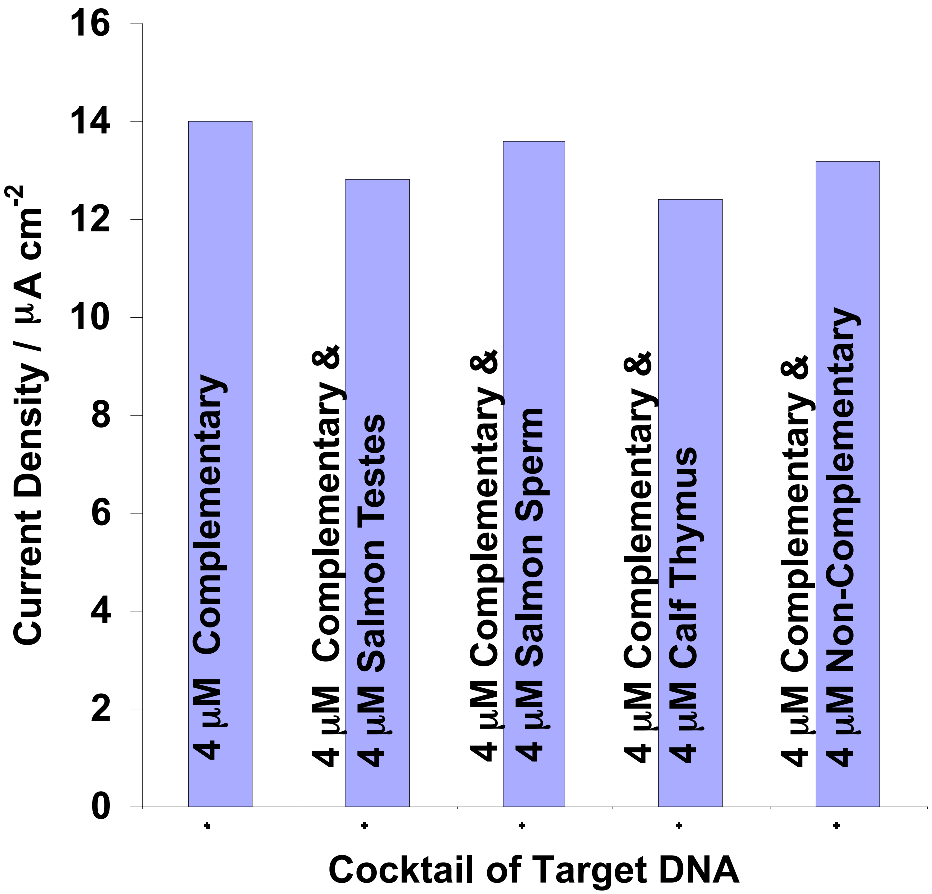
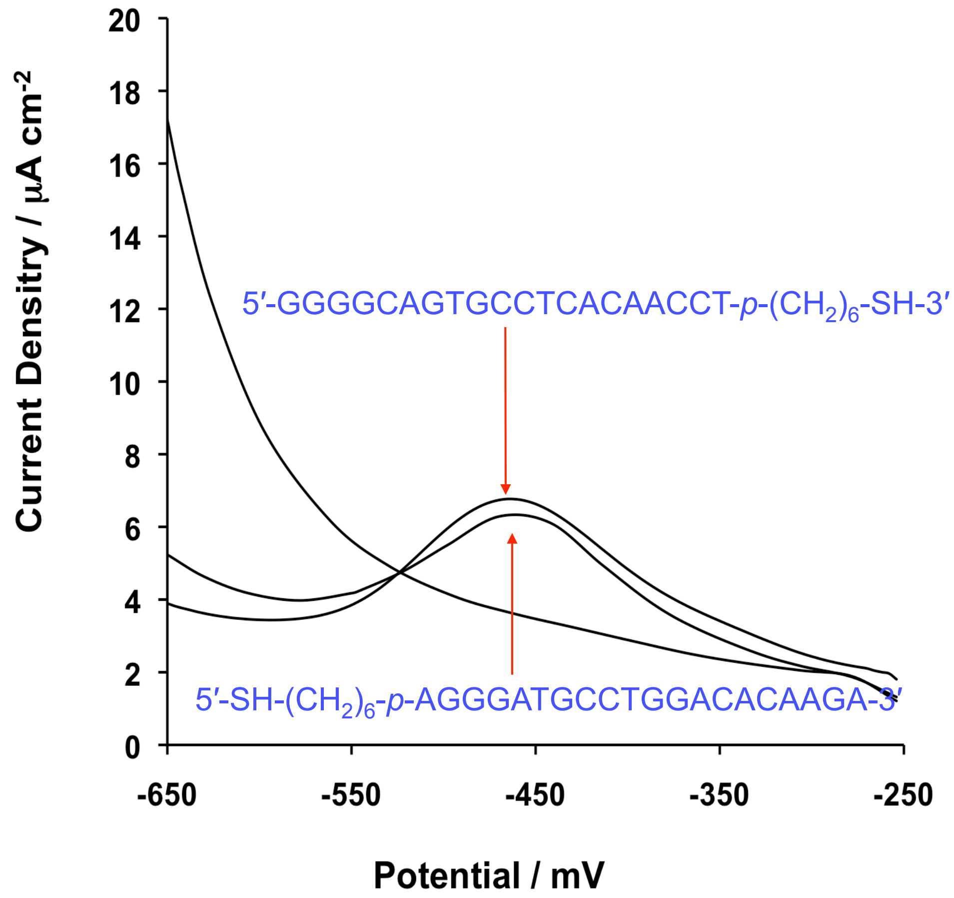
Further evaluation





E.L.S. Wong, F.J. Mearns, J.J. Gooding, *Sensors Actuators B* **111-112** 515-521 (2005).

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Pros and Cons

1) Excellent selectivity
   - **Can detect single base pair mismatches without stringency washings**
   - **Can detect complementary sequence from a DNA cocktail without loss in sensitivity**
2) Current assay time:
   - **Hybridisation ~ 120 min;**
   - **Detection (intercalation) ~180 min;**
   - **Total time 5 hours TOO LONG!!**
3) Detection limit:
   - **With best interface LOD = 100 nM**
4) Too complicated
   - **Three steps in the measurement**

E.L.S. Wong, P. Erokhin, J.J. Gooding, *Electrochem. Comm.* **6** 648-654 (2004)
E.L.S. Wong, F.J. Mearns, J.J. Gooding, *Sensors Actuators B* **111-112** 515-521 (2005).

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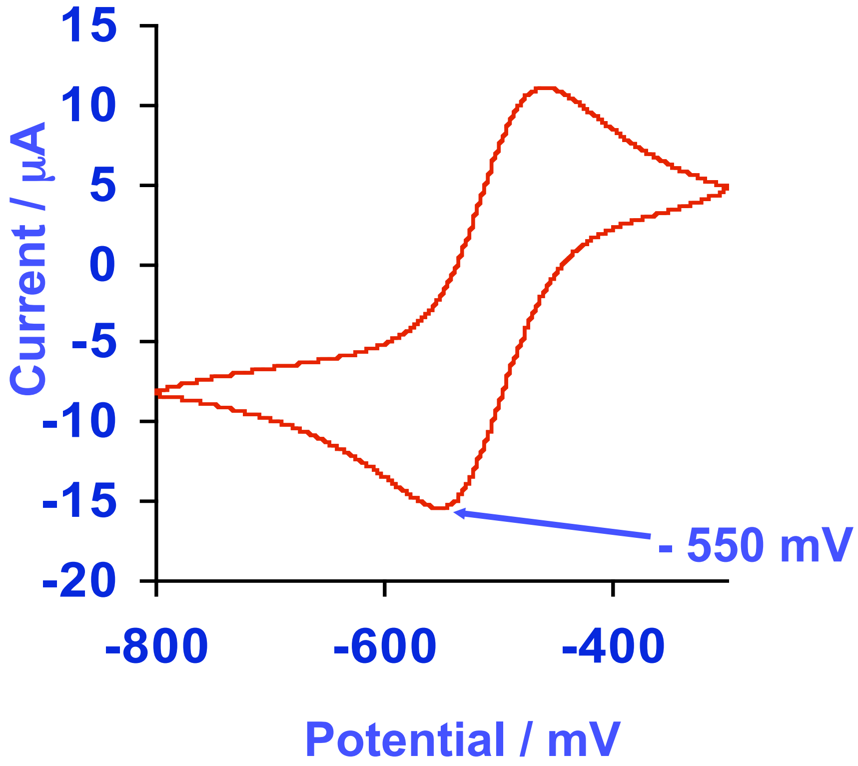
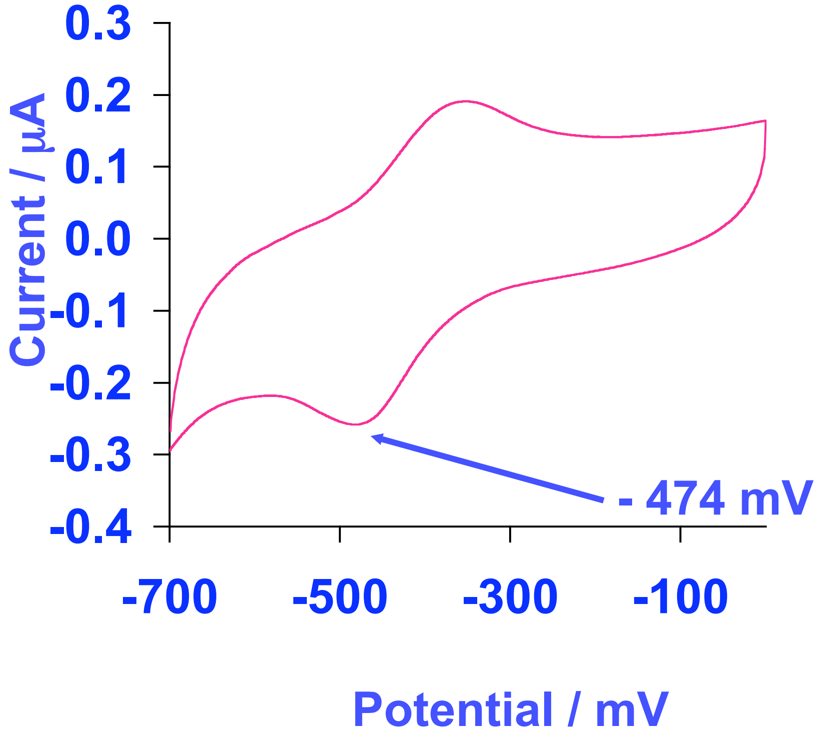
In situ experiment

AQDS in Solution



Intercalated AQDS



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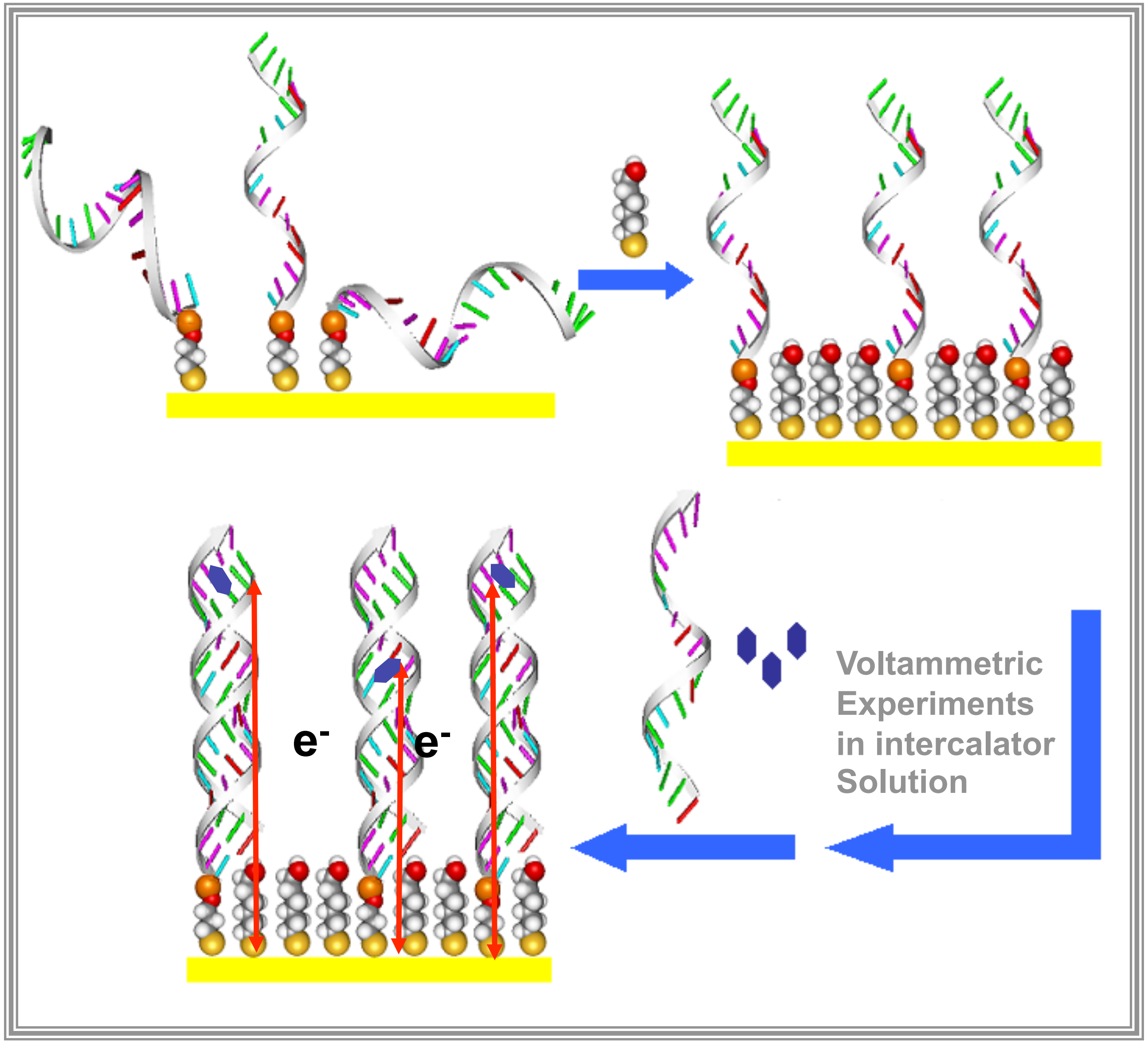
In Situ Detection



E.L.S. Wong, J.J. Gooding, *Anal. Chem.* **78** 2138-2144 (2006).

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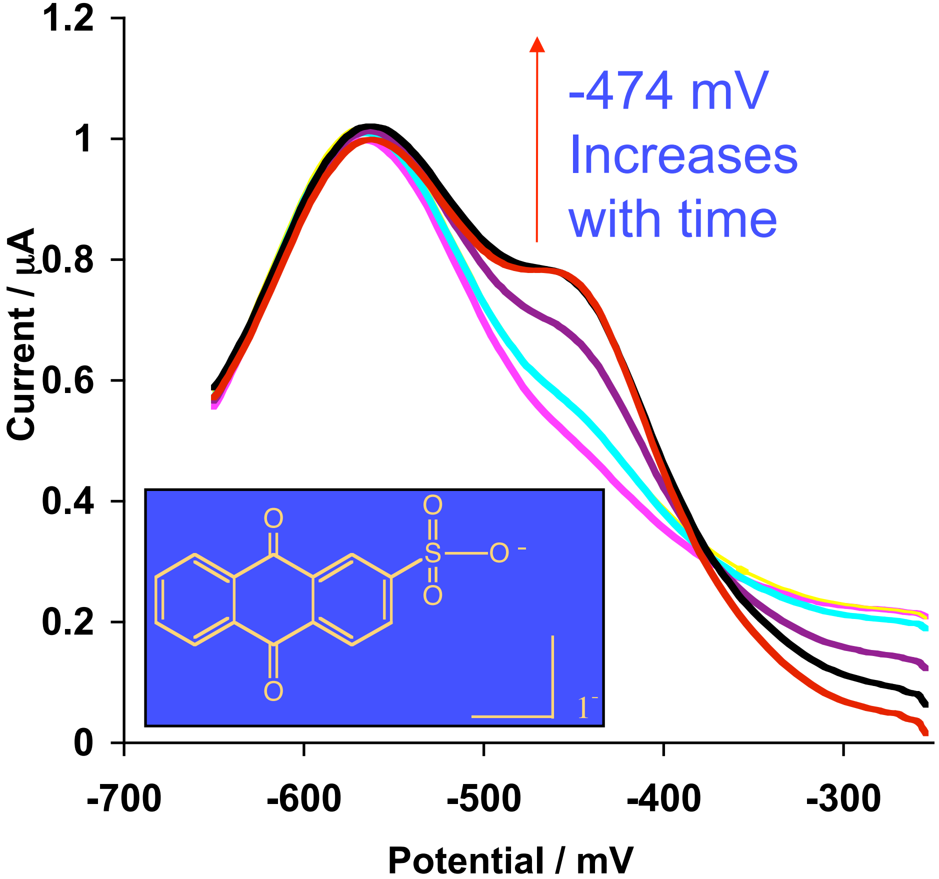
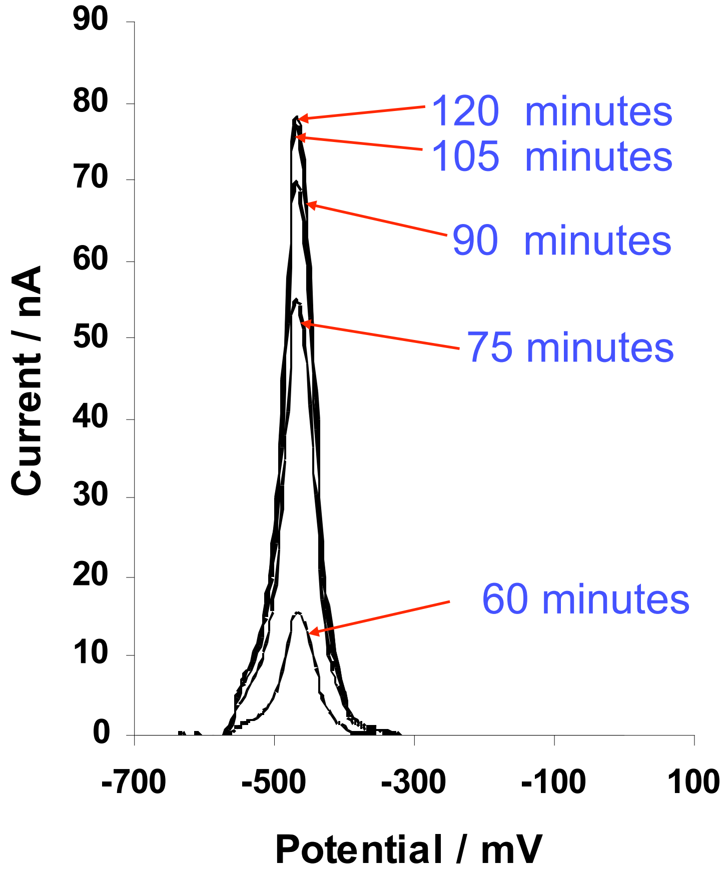
In Situ Assay





DNA Modified electrode
25 µM AQMS
4 µM complementary target ssDNA.

E.L.S. Wong, J.J. Gooding, *Anal. Chem.* **78** 2138-2144 (2006).

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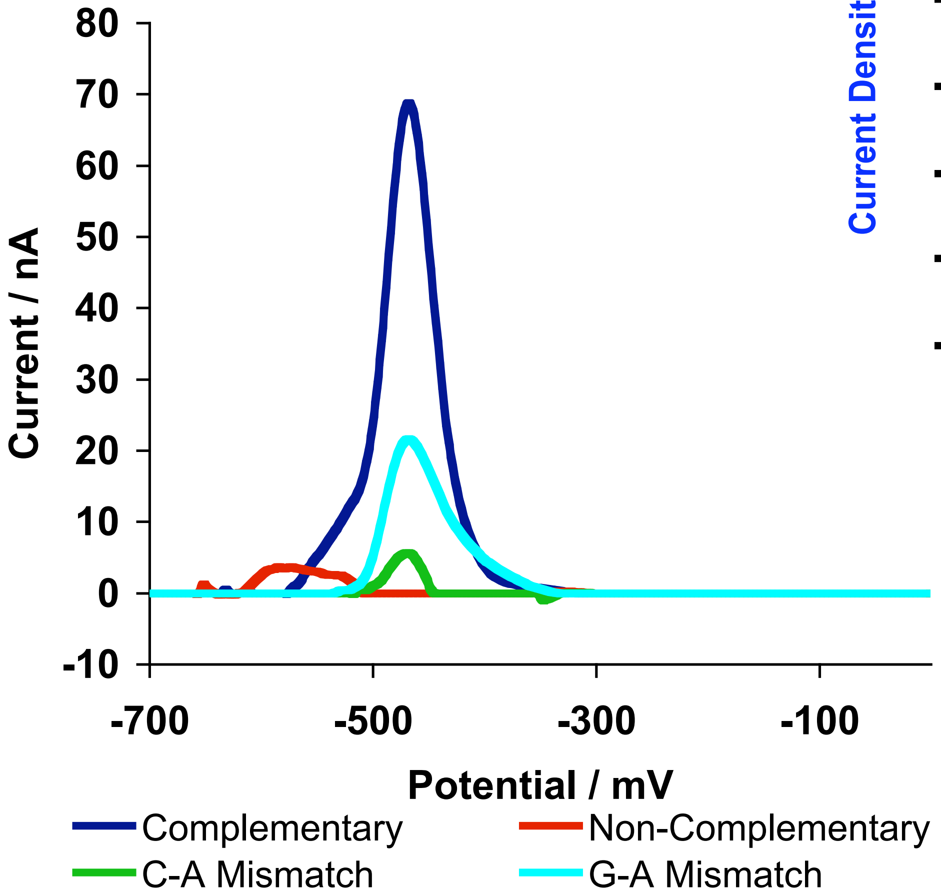
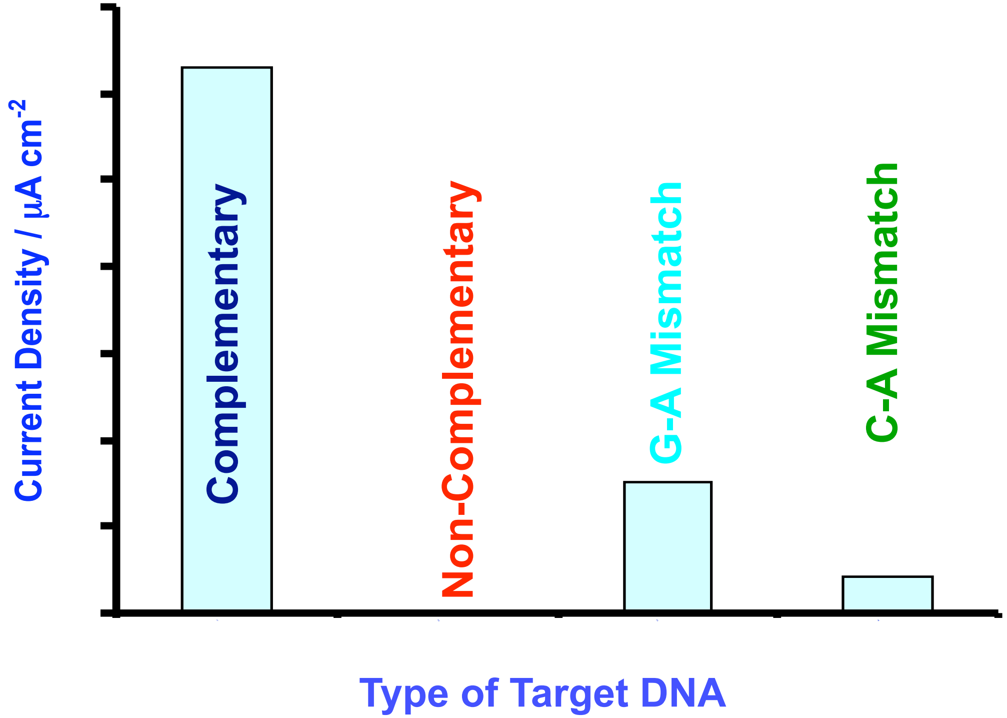
In Situ Detection – Single Base Mismatch Detection

After Subtraction





Target DNA	Sequential Current Ratio	In Situ Current Ratio
Comp : G-A	2.33 : 1	4.2 : 1
Comp : C-A	6.00 : 1	15 : 1

E.L.S. Wong, J.J. Gooding, *Anal. Chem.* **78** 2138-2144 (2006).

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Performance of the *in situ* assay

1) Current assay time one hour
   - **Hybridisation and detection (intercalation) ~60 min**
2) Detection limit:
   - **With best interface LOD = 0.5 nM (1 pmol)**
3) Simplicity, requires only on handling step, addition of the sample plus intercalator
4) *In situ* assay can also monitor hybridization in real time and be used to probe any event that affects DNA base pairing

E.L.S. Wong, J.J. Gooding, *Anal. Chem.* **78** 2138-2144 (2006).

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Where we are going

1) Detecting pathogens (cholera)
2) Improving the system performance

- Reduce detection limit
  - Smaller electrodes
  - Electrodes with lower background capacitance
  - Better surface chemistry
- More stable surface chemistry
- Make more compatible with microfabrication

Silicon

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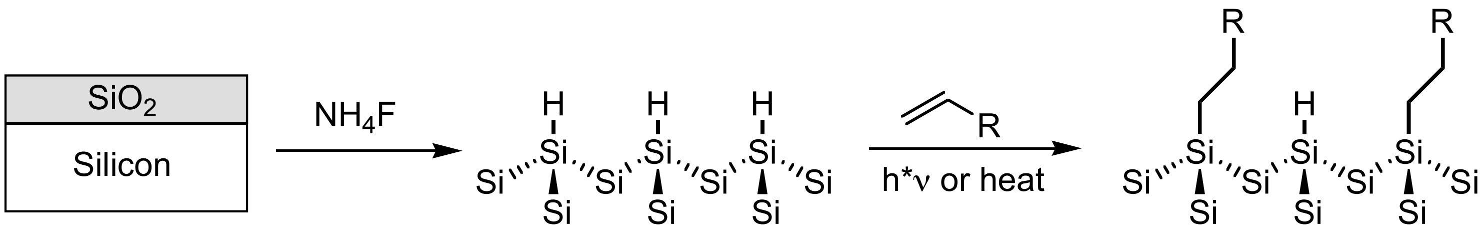
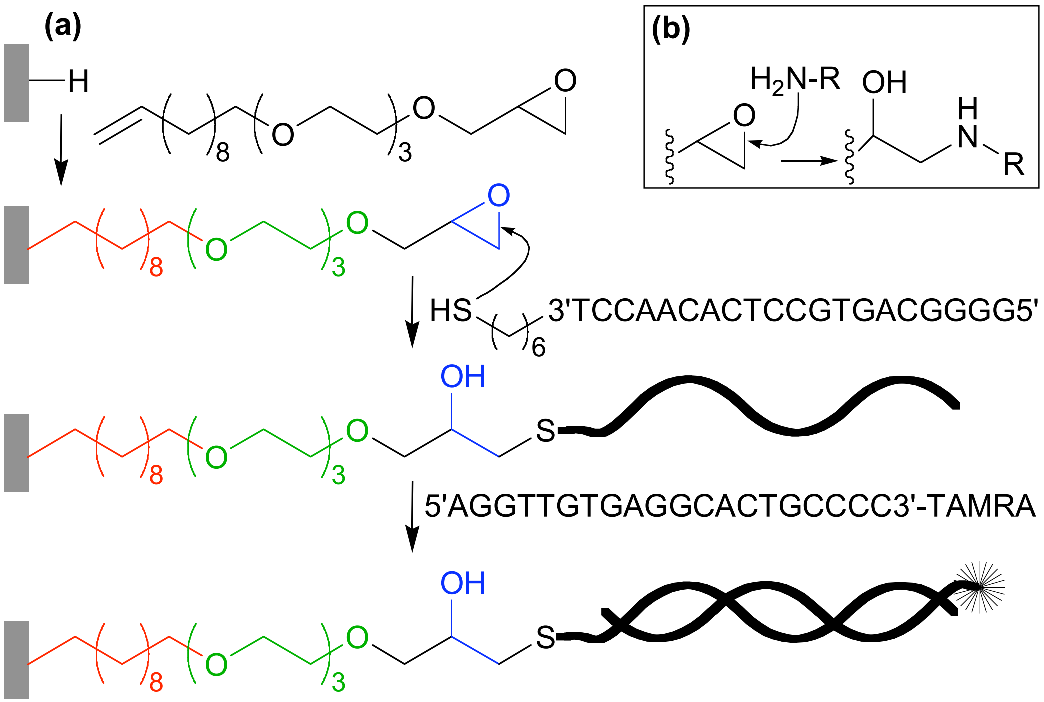
Single step fabrication of DNA chips



Linford *et al. J. Am. Chem. Soc.* **1995**, *117* 3145.



T. Böcking, K.A. Kilian, K. Gaus, J.J. Gooding, *Langmuir,* **22**, 3494-3496 (2006).

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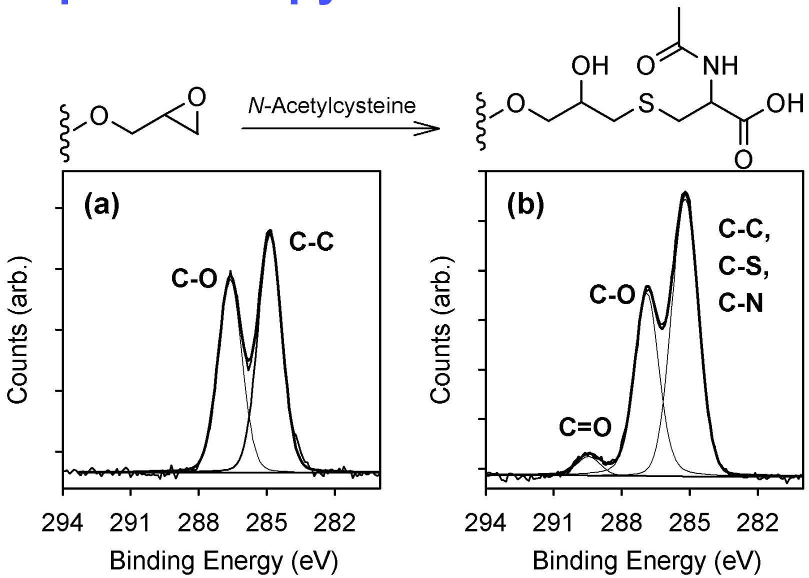
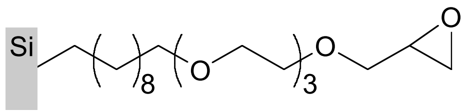
X-ray photoelectron spectroscopy



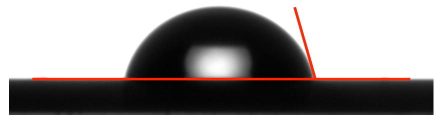
Contact angle measurements

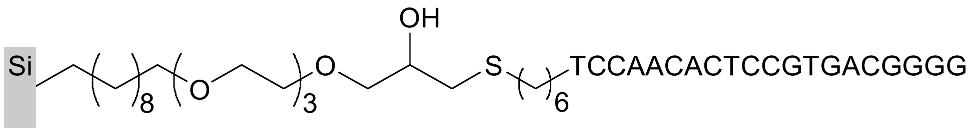
Surface modification

contact angle



74º





<10º

T. Böcking, K.A. Kilian, K. Gaus, J.J. Gooding, *Langmuir,* **22**, 3494-3496 (2006).

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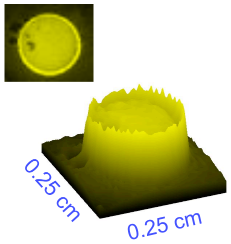
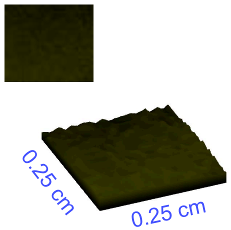
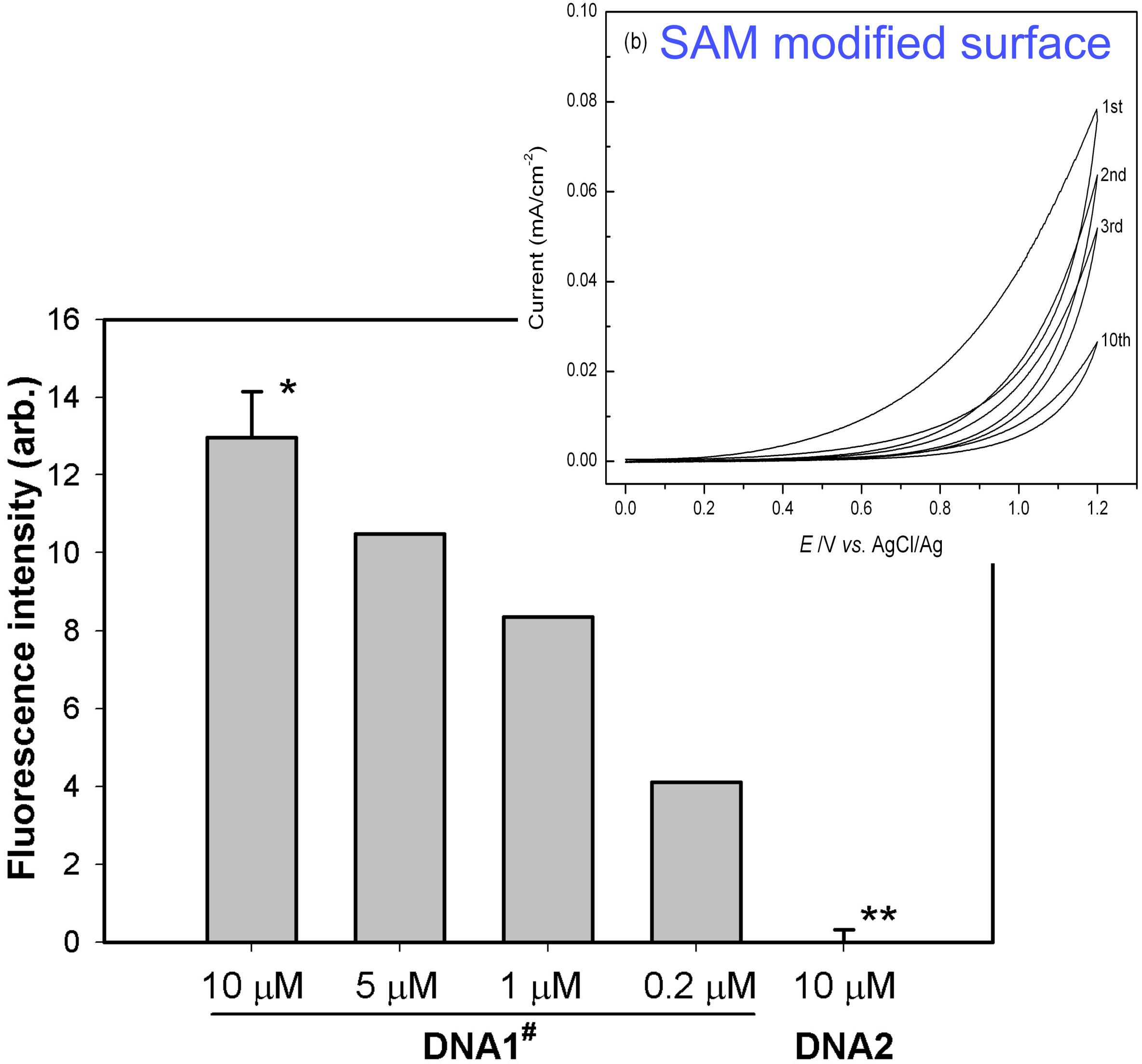


complementary DNA1



non-complementary DNA2



Fluorescence intensity as a function of target concentration of DNA1 for **n*=4, ***n*=3 independently prepared samples.

T. Böcking, K.A. Kilian, K. Gaus, J.J. Gooding, *Langmuir,* **22**, 3494-3496 (2006).

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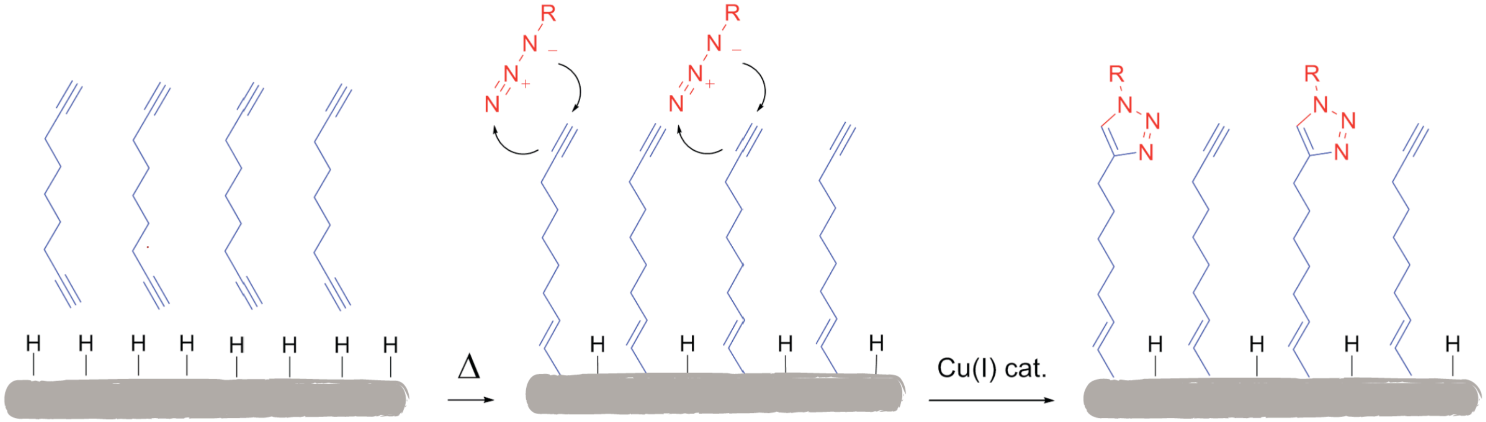
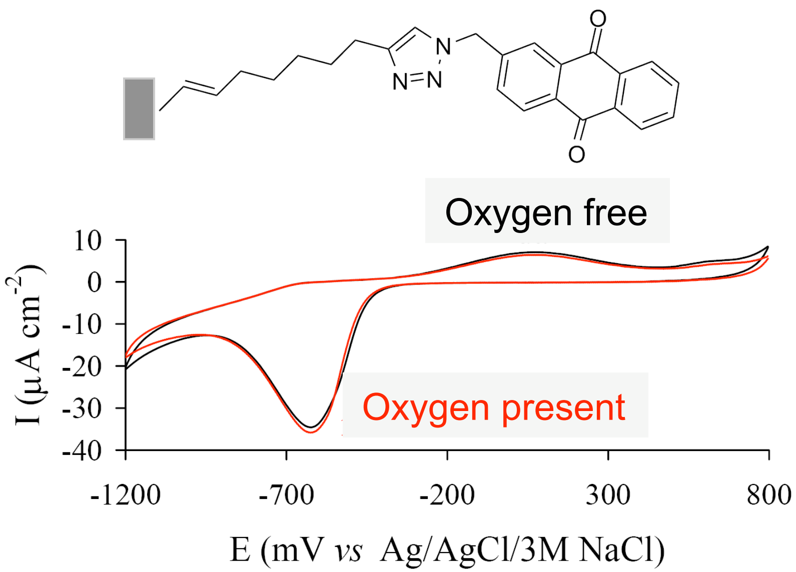
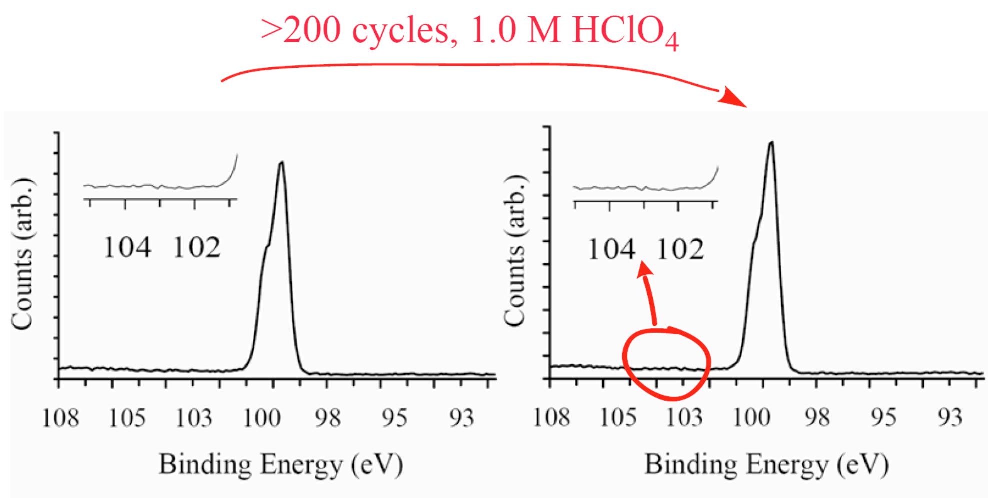
Click Chemistry on Silicon

Huisgen 1,3-dipolar cycloaddition of terminal alkynes to azides



S. Ciampi, T. Böcking, K.A. Kilian, M James, J.B. Harper, J.J. Gooding, *Langmuir* **23** 9320-9329 (2007).





S. Ciampi, P.K. Eggers, G. Le Saux, M. James, J.B. Harper, J.J. Gooding, *Langmuir* **25** 2530-2539 (2009).

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Where we are going

1) Detecting pathogens (cholera)
2) Improving the system performance
   - Reduce detection limit
     - Smaller electrodes
     - Electrodes with lower background capacitance
     - Better surface chemistry
   - More stable surface chemistry
   - Make more compatible with microfabrication
3) Using the system to understand DNA surface chemistry and disruption of DNA base pairing

Silicon

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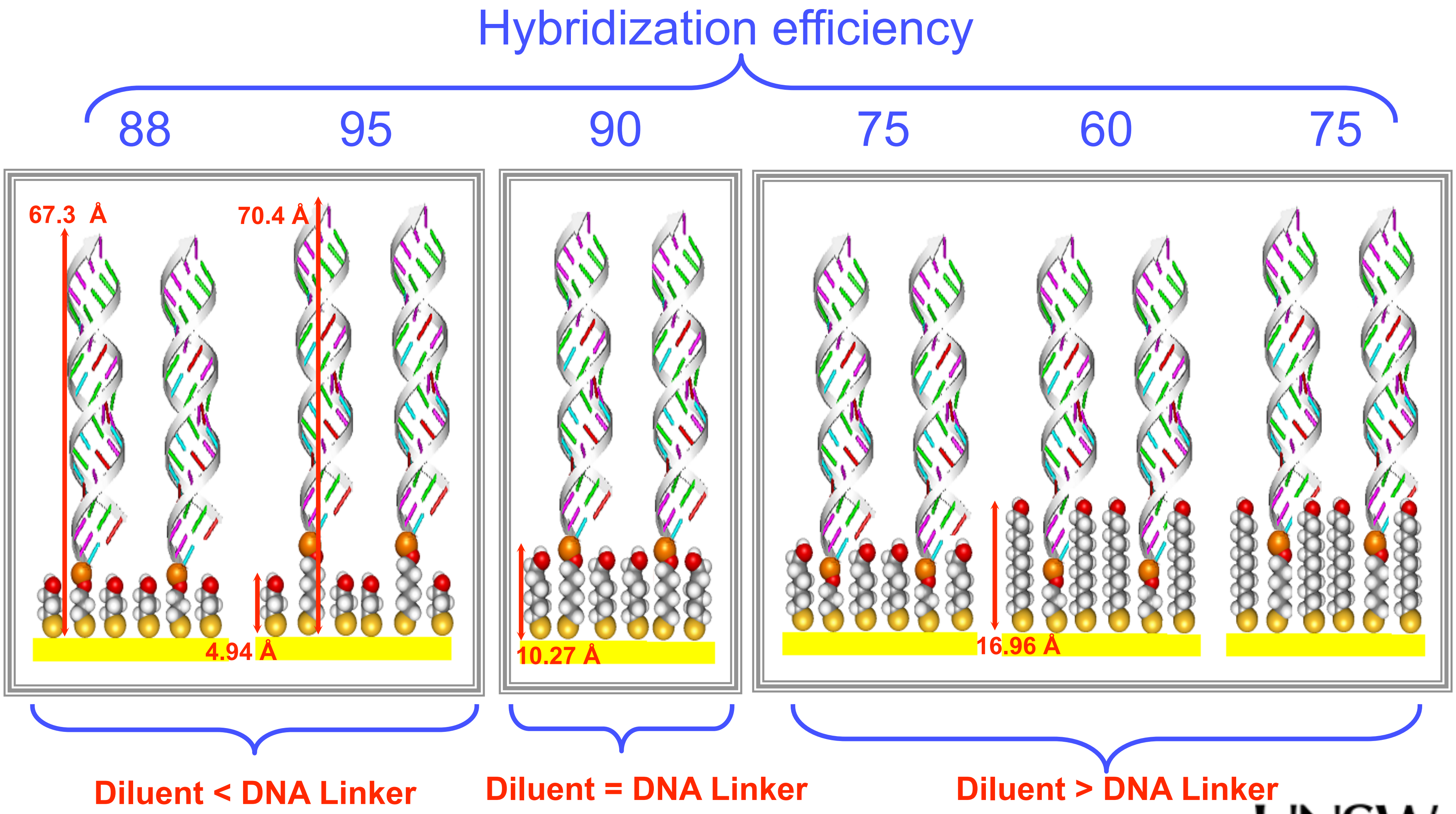
Using the surface structure to influence the performance



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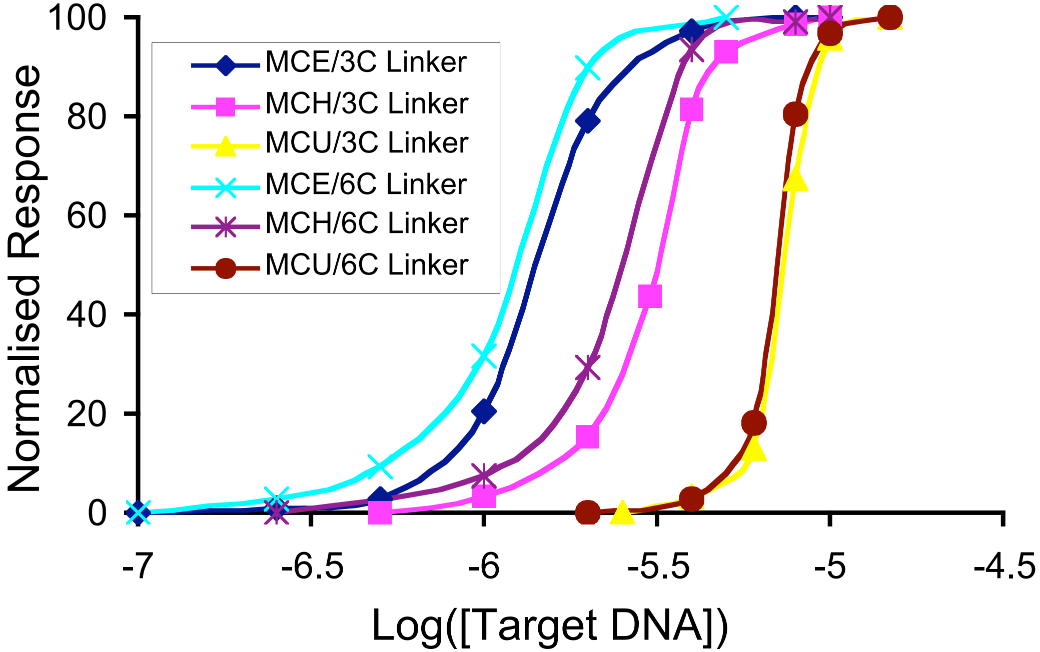
E.L.S. Wong, E. Chow, J.J. Gooding, *Langmuir* **21** 6957-6965 (2005).

Calibration Curve



$K_{a(\text{MCE/6C Linker})} = 8.32 \times 10^6$

$K_{a(\text{MCE/3C Linker})} = 7.08 \times 10^6$

$K_{a(\text{MCH/6C Linker})} = 3.98 \times 10^6$

$K_{a(\text{MCH/3C Linker})} = 3.16 \times 10^6$

$K_{a(\text{MCU/6C Linker})} = 1.41 \times 10^6$

$K_{a(\text{MCU/3C Linker})} = 1.38 \times 10^6$

K_a

E.L.S. Wong, E. Chow, J.J. Gooding, *Langmuir* **21** 6957-6965 (2005).

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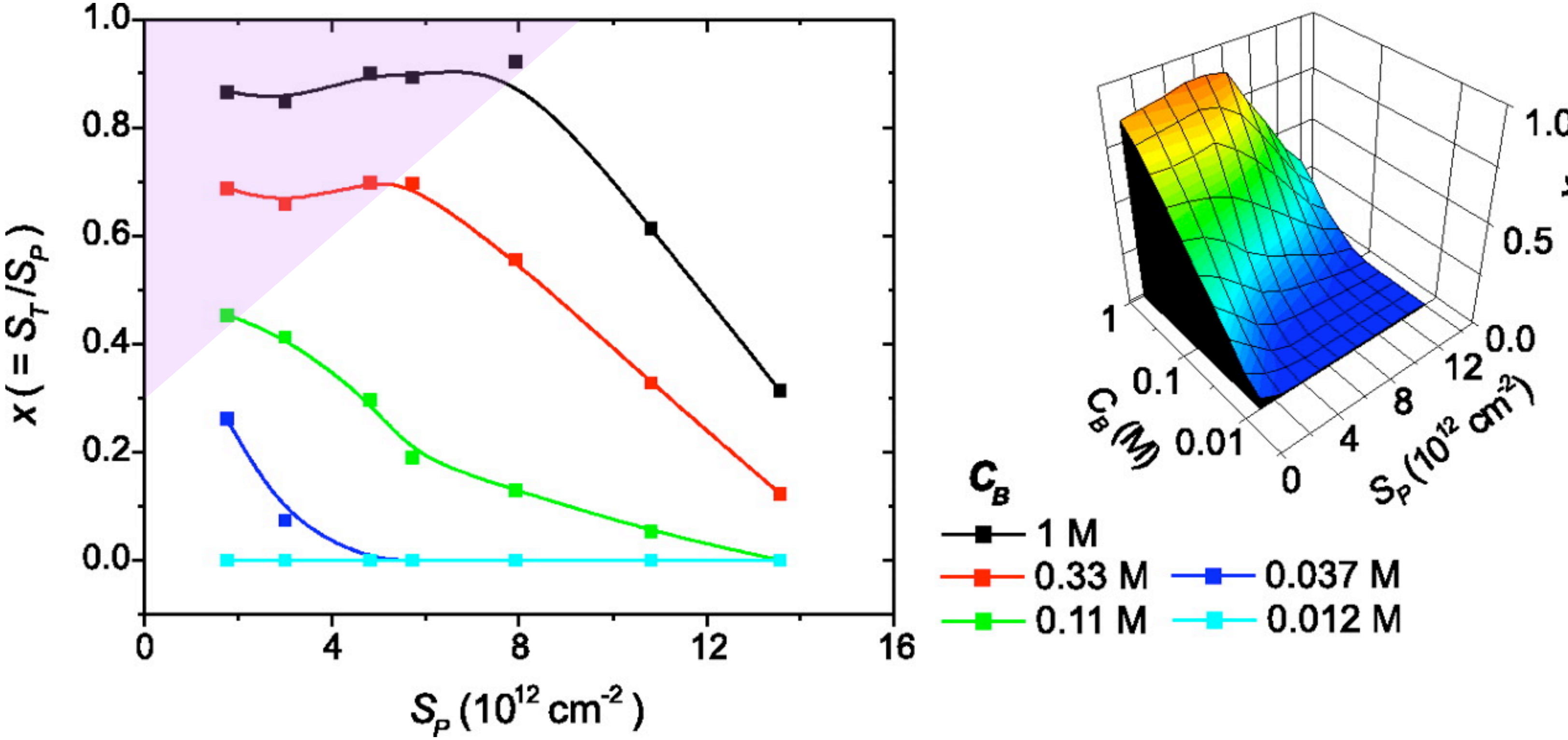
Hybridization conversion x = ST/SP as a function of probe coverage SP (probes per cm2) and ionic strength CB (moles of phosphate per liter)



Gong P., Levicky R. PNAS 2008;105:5301-5306

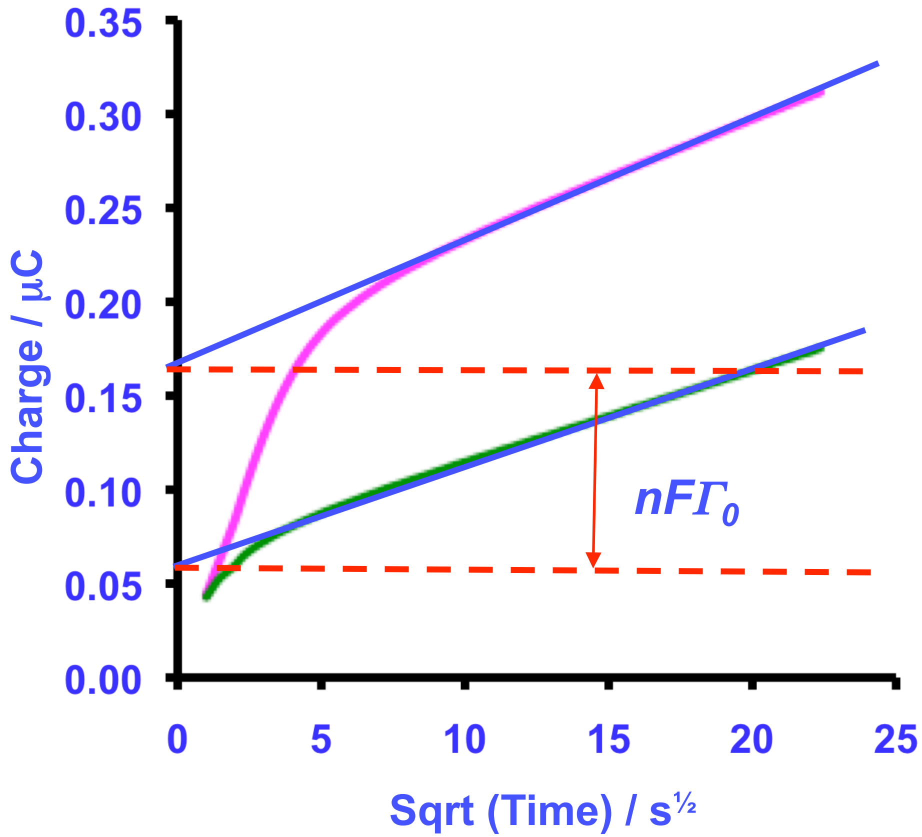
Quantification of amount of probe ssDNA

- The saturated surface $Ru(NH_2)^{3+}$ is converted to DNA probe surface density with the relationship,

$$\Gamma_{DNA} = \Gamma_0 (z/m)(N_A)$$

$\Gamma_{(probe)}$

(9.5 ± 3.2) x 10^{12} molecules cm^{-2} (n=10)



Steel *et. al*. *Analytical Chemistry*, 1998, *70,* 4670.

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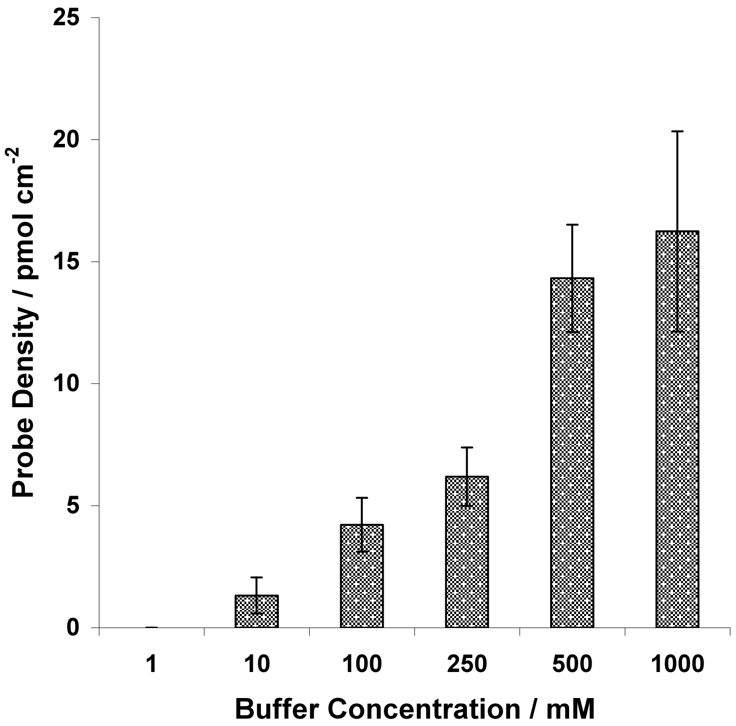
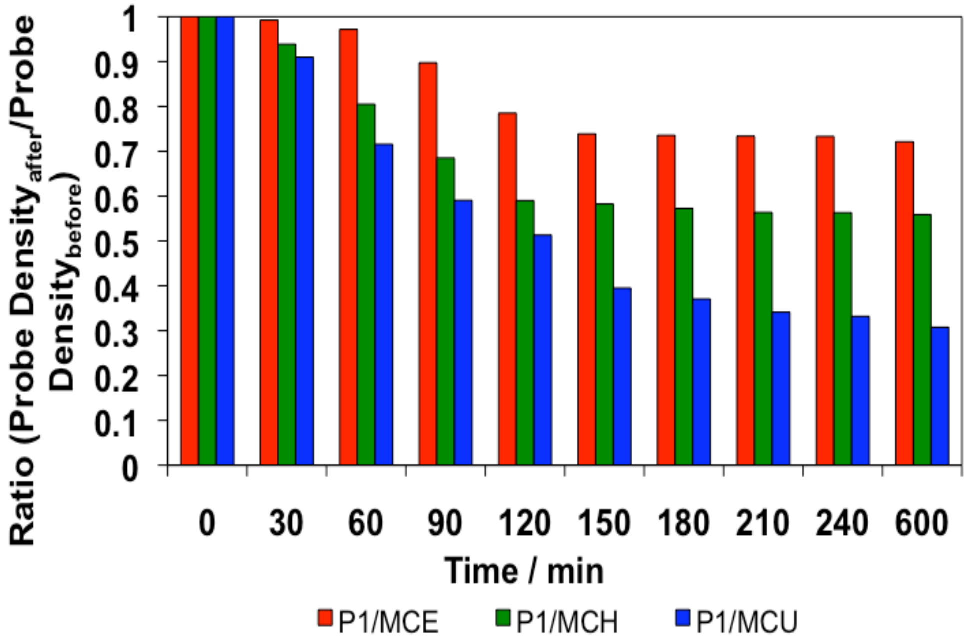
DNA Surface Coverage





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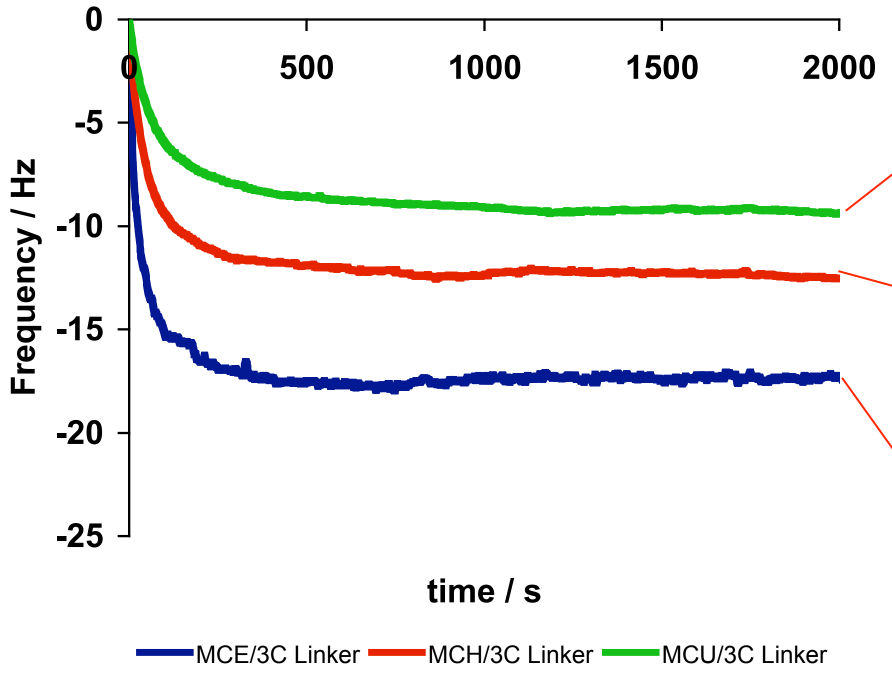
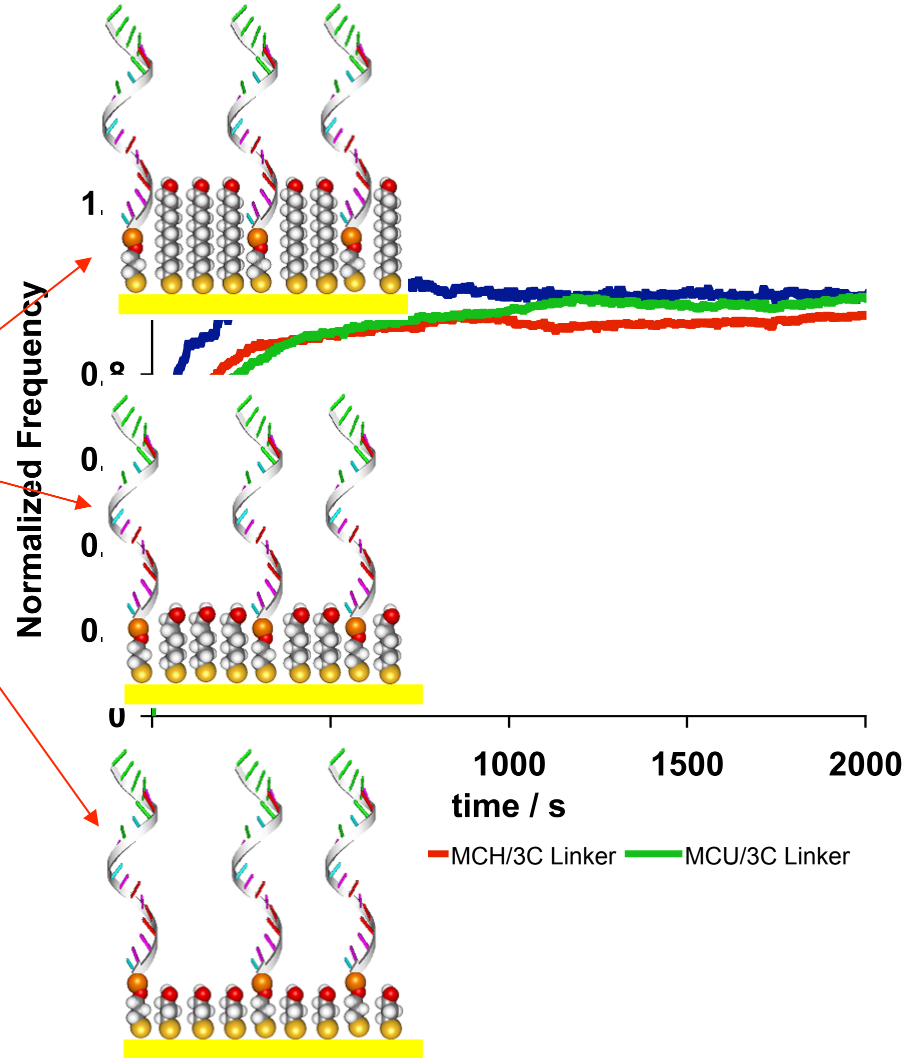
Hybridization Kinetics





E.L.S. Wong, E. Chow, J.J. Gooding, *Langmuir* **21** 6957-6965 (2005).

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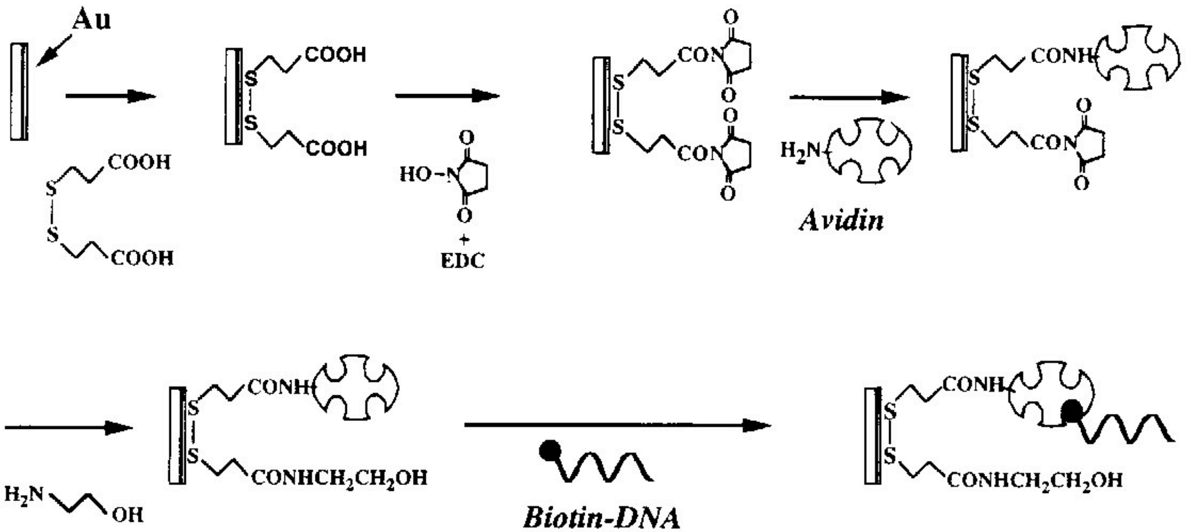
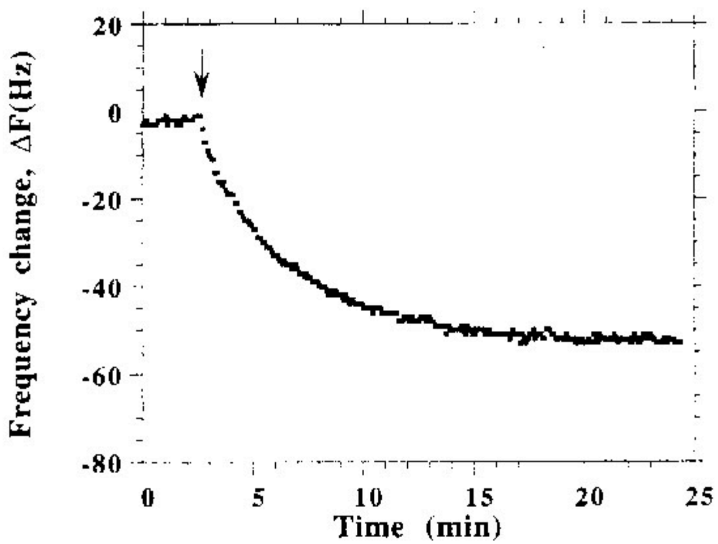
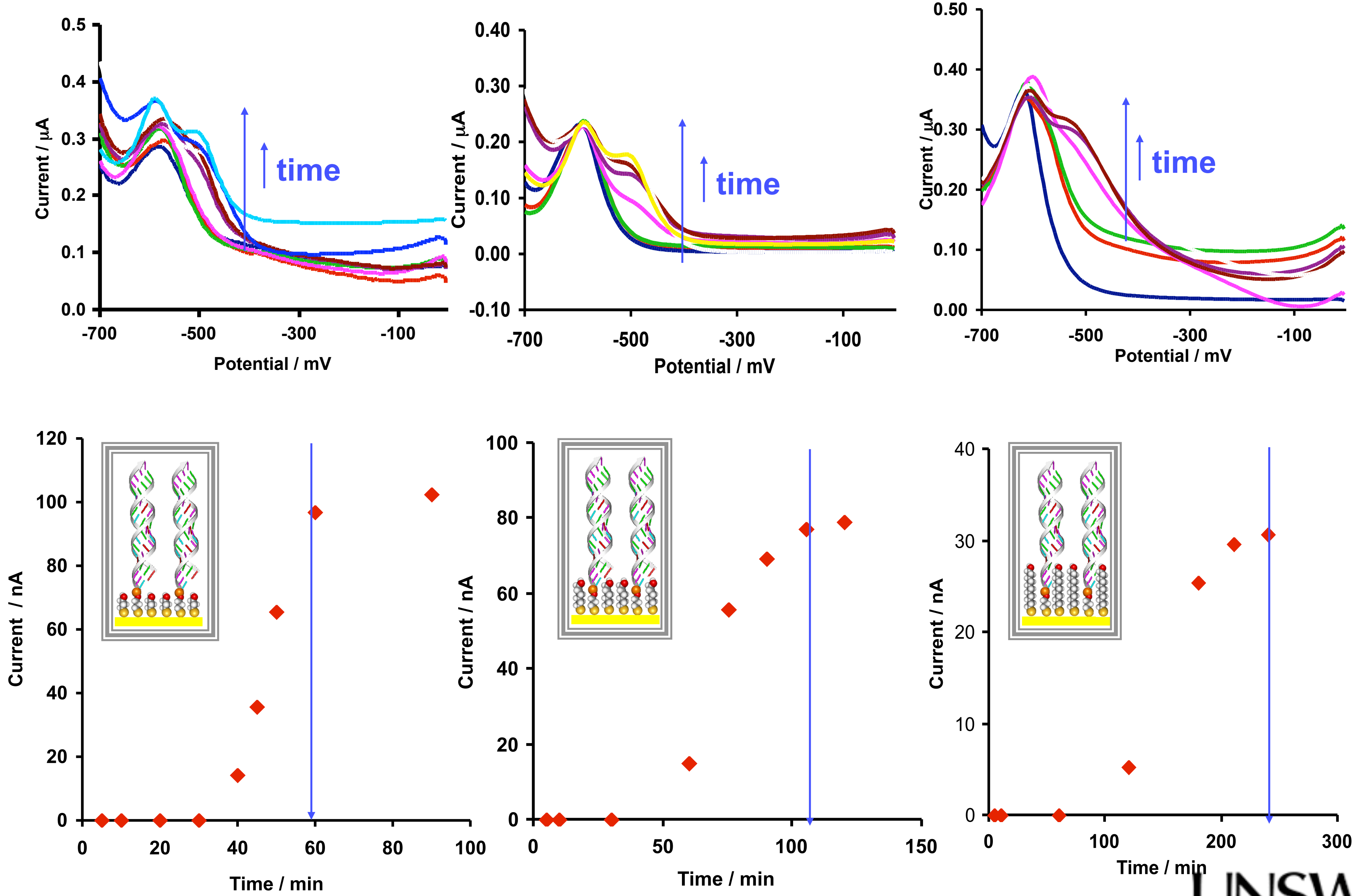
DNA Hybridisation Biosensor





F. Caruso *et al* Anal. Chem. 69 2043-2049 (1997)

In Situ Analysis on Hybridisation Kinetics



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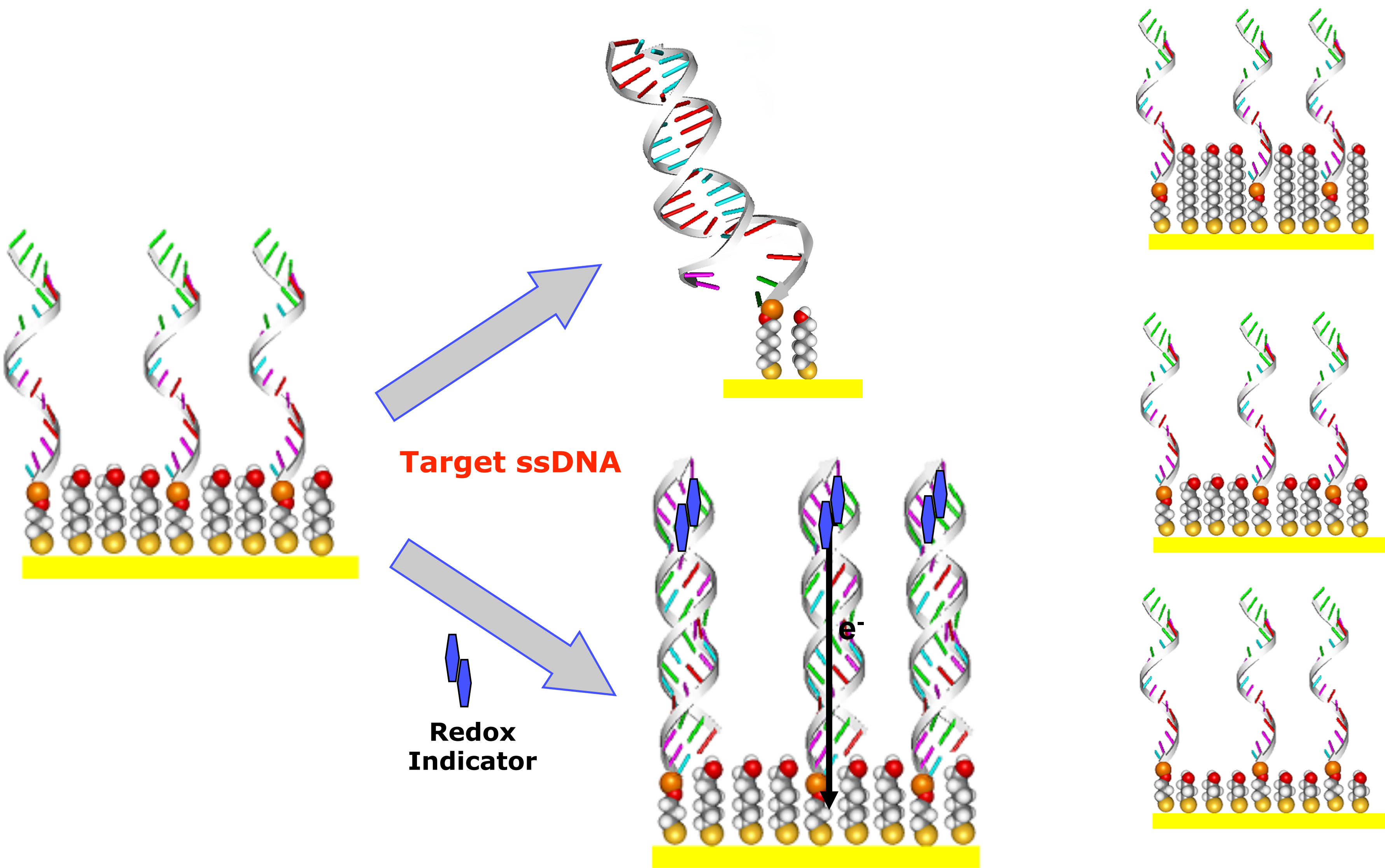
E.L.S. Wong, E. Chow, J.J. Gooding, *Langmuir* **21** 6957-6965 (2005).

Why the different kinetic



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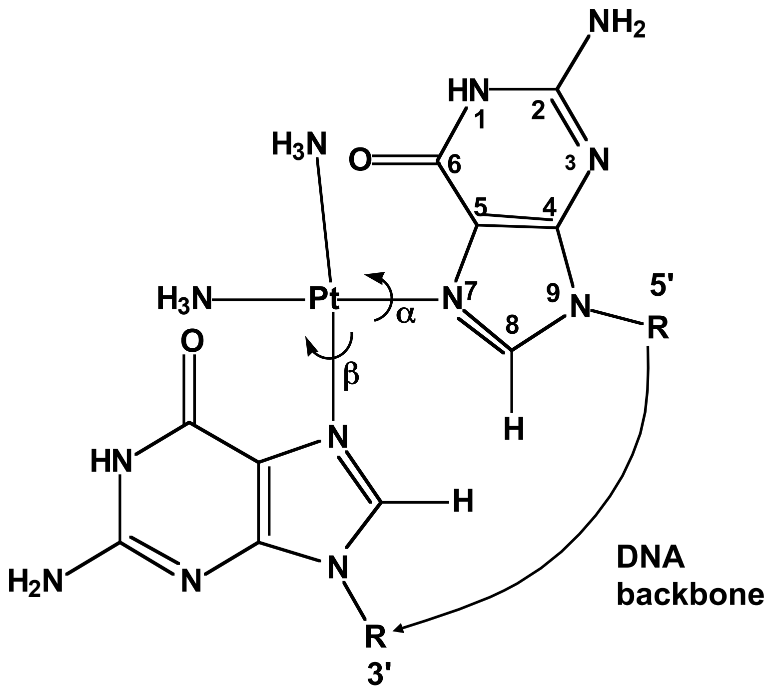
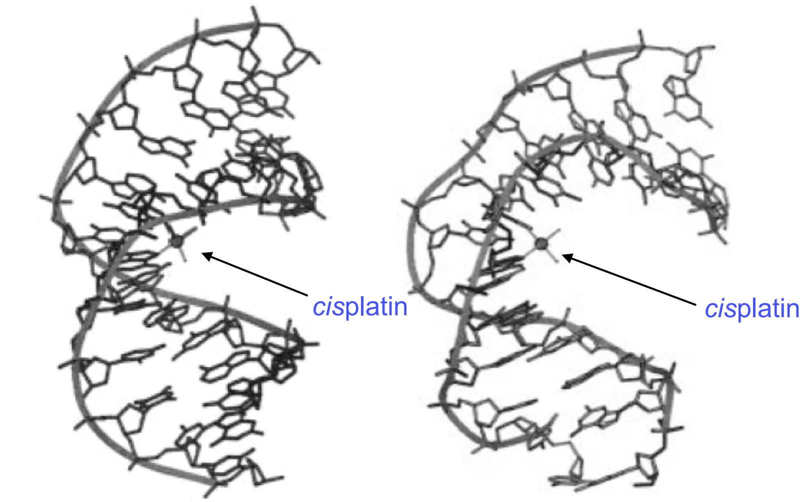
Detecting Small Compounds Binding to DNA





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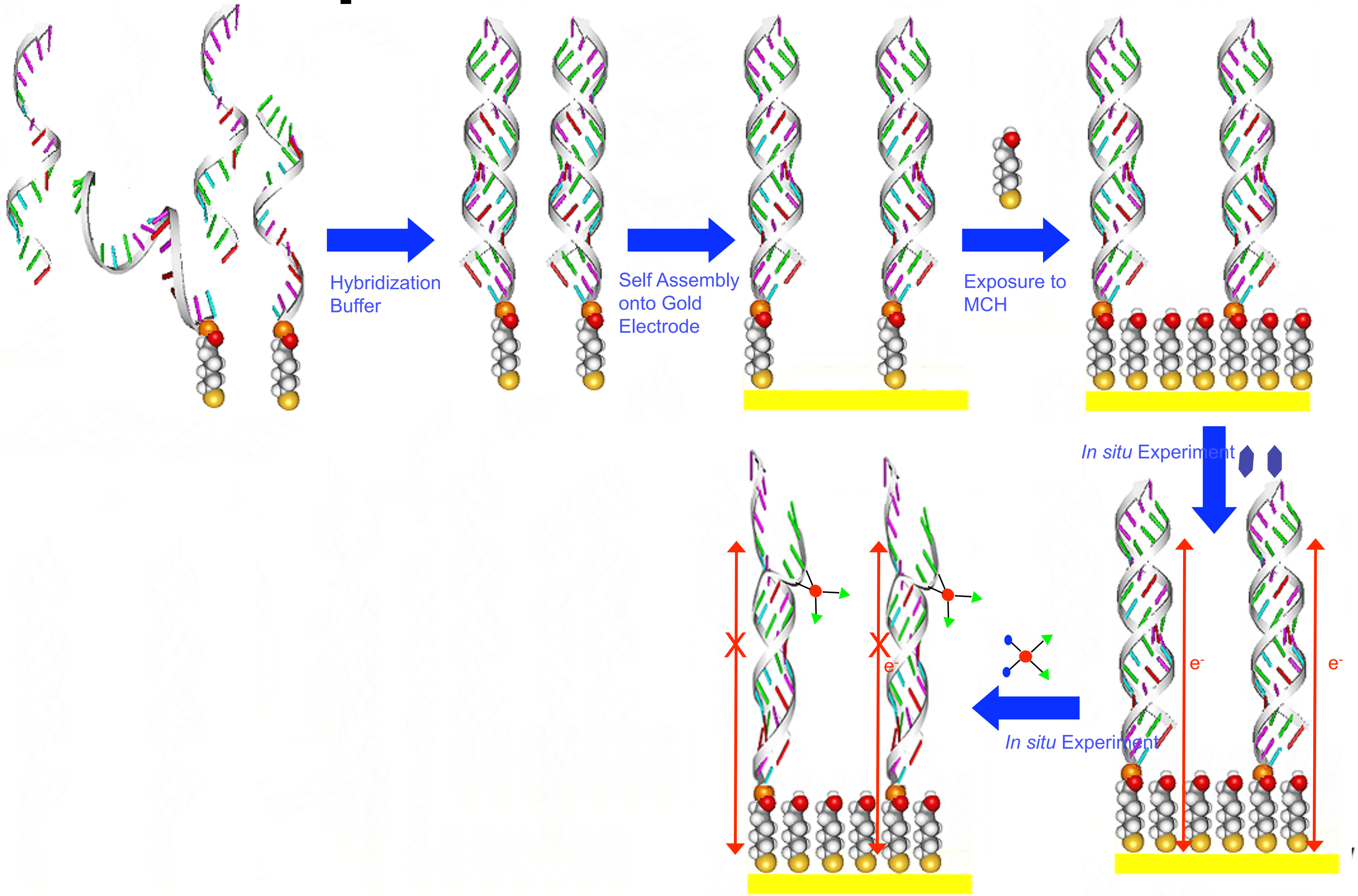
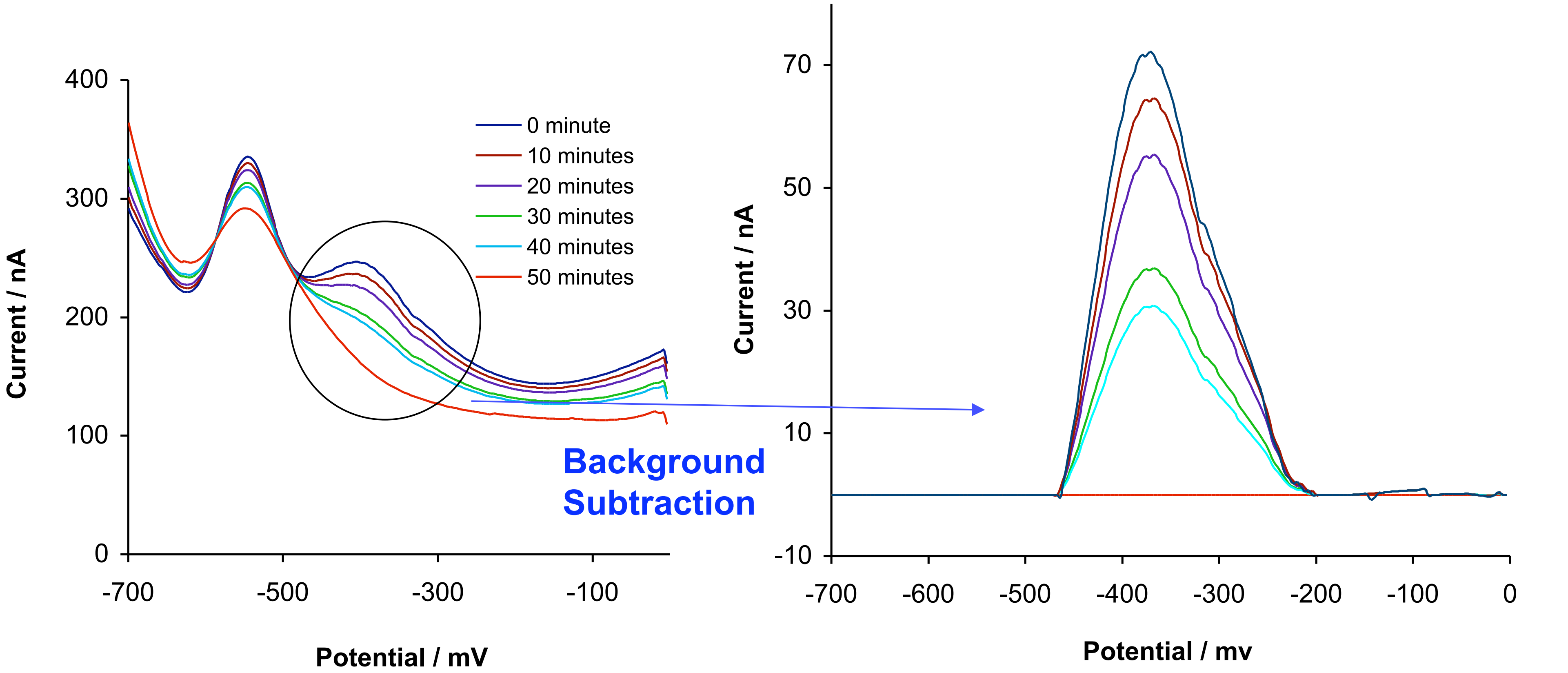
Experimental Procedure



Results



1 mM *cis*platin

E.L.S. Wong, J.J. Gooding, *J. Am. Chem. Soc.* **129** 8950-8951 (2007).

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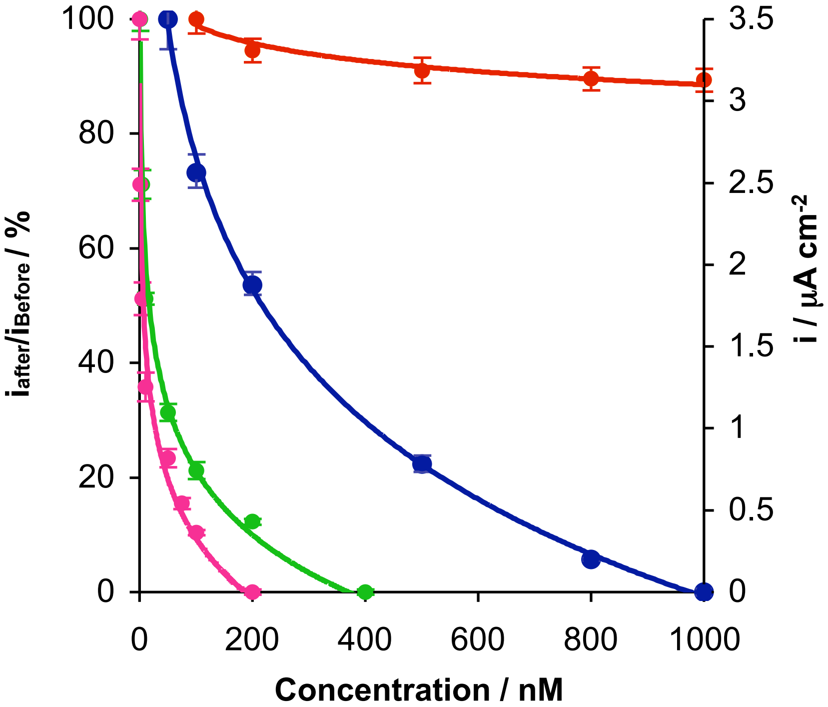
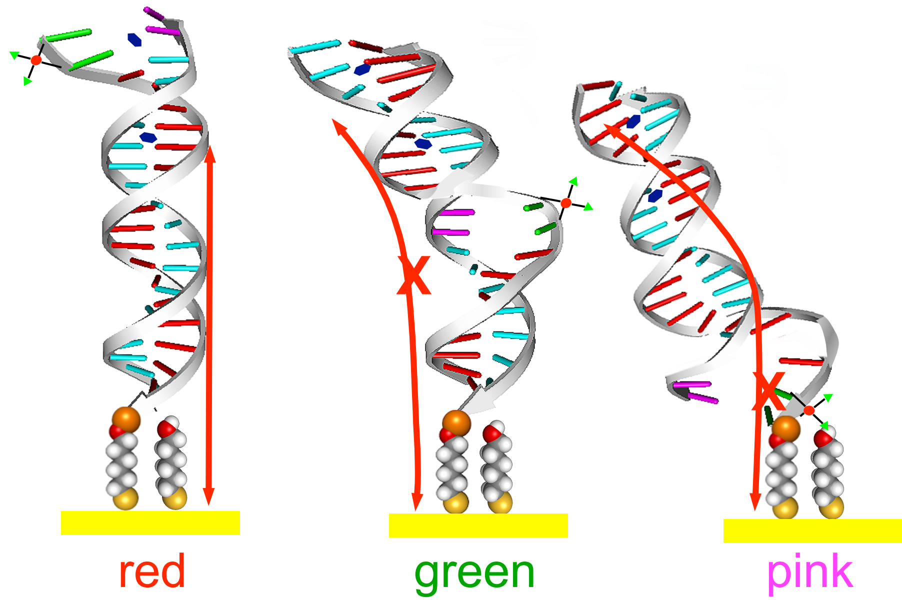
Better Defined Sequences

DNA Biosensor	DNA Sequence
p53	5'-GGGGCAGTGCCTCACAACCT-*p*-$(CH_2)_6$-SH-3'
	3'-CCCCGTCACGGAGTGTTGGA-5'
P4/8	5'-GGAAAAAAAAAAAAAAAAAA-*p*-$(CH_2)_6$-SH-3'
	3'-CCTTTTTTTTTTTTTTTTTT-5'
P5/9	5'-AAAAAAAAAAGGAAAAAAAA -*p*-$(CH_2)_6$-SH-3'
	3'-TTTTTTTTTTCCTTTTTTTT-5'
P6/10	5'-AAAAAAAAAAAAAAAAAAGG -*p*-$(CH_2)_6$-SH-3'
	3'-TTTTTTTTTTTTTTTTTTCC-5'

Influence of the GG Position





E.L.S. Wong, J.J. Gooding, *J. Am. Chem. Soc.* **129** 8950-8951 (2007).

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Summary: DNA Modified Electrodes

Using long range charge transfer can produce an DNA hybridisation biosensor with:

- excellent selectivity
- good sensitivity
- which is exceedingly simple to use and
- can monitor hybridisation to give complete duplexes in real time
- can monitor the interaction of anticancer drugs with DNA

The Future:

- Investigate small molecule binding with DNA
- Investigate DNA damage
- Apply to detecting pathogens
- Make the system more robust and more compatible with microfabricated devices

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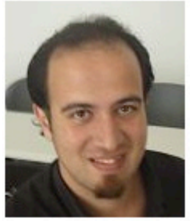
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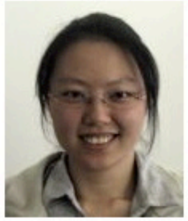
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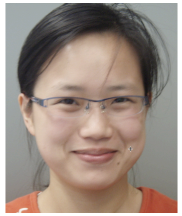
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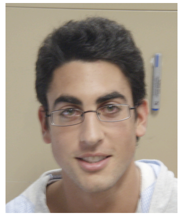
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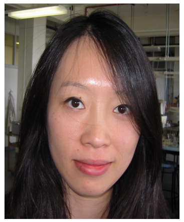
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Dr Kris Kilian

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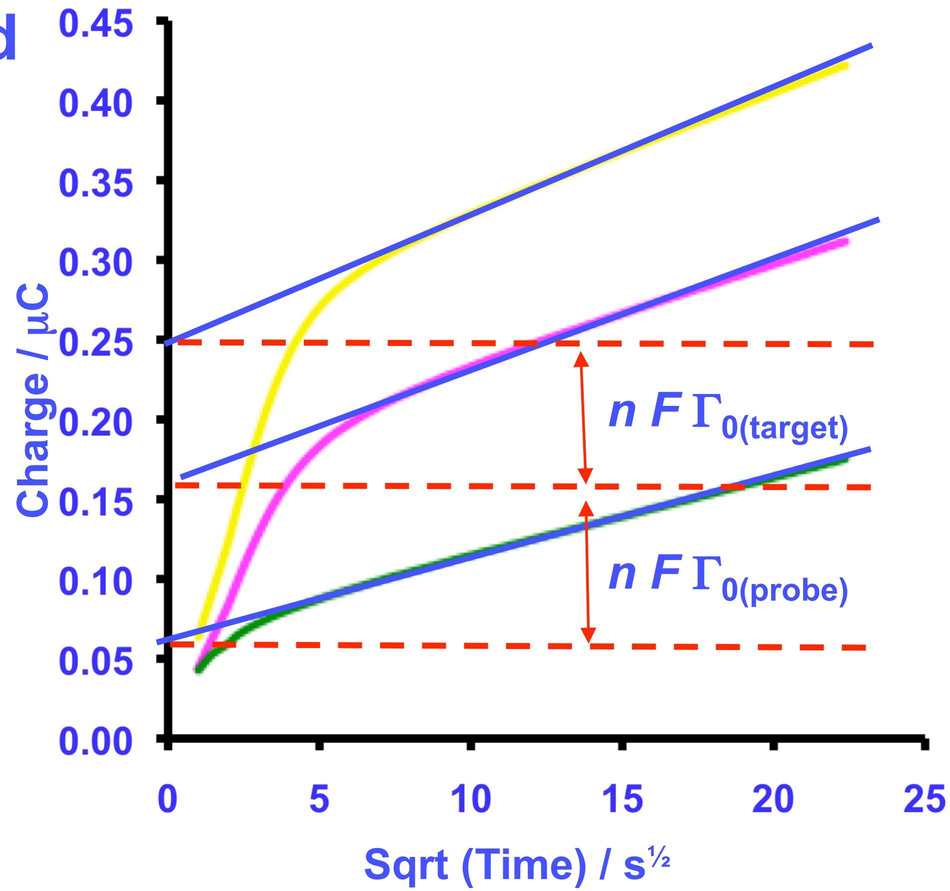
Hybridisation Efficiency

- A similar strategy was used to monitor hybridization.

Hybridisation Efficiency

$$\frac{\Gamma_{target}}{\Gamma_{probe}} = 88\%$$



Steel *et. al*. *Analytical Chemistry,* **1998,** *70,* 4670.

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