A field enhanced fluorescence experiment was performed to determine the association constant between a surface immobilised oligonucleotide 15mer (15 bases) and a complementary, solution phase oligo’ which either has no mismatches (White squares below) or has a single base mismatch (black squares below). Points refer to intensity measured once equilibrium has been reached following titration of specific concentrations of oligo’.

(a) Determine $K_A$ for both the no mismatch (mm0) and single base mismatch (mm1) from the titration data shown below.
(b) Why are they different?
(c) What energy difference does this correspond to hybridised duplex strand of DNA? *Hint: think about bond strength of H bonds.*
2. A potential step experiment was performed to determine the diffusion coefficient, D of ferrocene carboxylic acid. 1 mmol dm$^{-3}$ Fe(CO$_2$H) was placed in an electrochemical cell with 0.1 mol dm$^{-3}$ KCl background electrolyte. The standard reduction potential of Fe(CO$_2$H) is +0.2 V vs. Ag|AgCl. A 1 cm$^2$ carbon working electrode and Ag|AgCl reference electrode were employed. The potential was stepped from 0 V to 0.4 V vs. Ag|AgCl and the resultant current flow measured (see below). Calculate the diffusion coefficient.

<table>
<thead>
<tr>
<th>Time / seconds</th>
<th>Current /mA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.66</td>
</tr>
<tr>
<td>0.1</td>
<td>0.44</td>
</tr>
<tr>
<td>0.2</td>
<td>0.36</td>
</tr>
<tr>
<td>0.3</td>
<td>0.315</td>
</tr>
<tr>
<td>0.4</td>
<td>0.281</td>
</tr>
<tr>
<td>0.5</td>
<td>0.257</td>
</tr>
</tbody>
</table>

\[ i = \frac{nFACD^{1/2}}{\pi^{1/2}A^{1/2}} \]

*Hint: Take care about units: If A is in m$^2$ then conc. C = mol m$^{-3}$.

3. An AFM cantilever, with a force constant of 100 mN m$^{-1}$ experienced a deflection of 1 Å when in contact with a surface. Calculate the force experienced by the tip.

4. Surface Plasmon Resonance was used to measure the binding of a protein to an antibody surface. The following kinetic curves were measured for different concentrations of protein. Calculate the association and disassociation rates constants.

Fitted values on the pseudo rate constant, $k_{on}$ were:

- 76 nmol dm$^{-3}$ $k_{on} = 0.0037$ s$^{-1}$
- 303 nmol dm$^{-3}$ $k_{on} = 0.0042$ s$^{-1}$
- 760 nmol dm$^{-3}$ $k_{on} = 0.0068$ s$^{-1}$
K_D is concentration equal to R_{max} / 2 for two situations.

mm0: K_D = 1.5 \times 10^{-9} \text{ mol dm}^{-3} \quad K_A = 6.6 \times 10^8 \text{ mol}^{-1} \text{ dm}^3

mm1: K_D = 5 \times 10^{-8} \text{ mol dm}^{-3} \quad K_A = 2 \times 10^7 \text{ mol}^{-1} \text{ dm}^3

(b) On mm1 system have less H bonding keeping the two strands together. Explain G – A and T – C rules. Average 2.5 H bonds / base pair, so association energy 2 or 3 H bonds lower in mm 1 than mm 0.

(c) H bond av. = 20 kJ mol^{-1}. Say, two H bonds less = 6.6 \times 10^{-20} \text{ J / Duplex}
Q2

Plot $1/i^2$ vs $t$ to get:

From rearrangement of Cotrell.

So: $D = \frac{\pi}{n^2F^2A^2C^2gradient}$

$D = 1.5 \times 10^{-9} \text{ m}^2\text{s}^{-1}$

Question 3 Hook’s law: $F = kx$; so force experienced by tip = $1 \times 10^{-11}$ N.

Question 4 Plot $k_{on}$ vs. conc of analyte, since: $k_{on} = k_{ass}[\text{analyte}] + k_{diss}$

So $k_{ass} = 5178 \text{ M}^{-1}\text{s}^{-1}$

$k_{diss} = 0.0028 \text{ s}^{-1}$