Principles of Electronic Nanobiosensors

Unit 3: Sensitivity
Lecture 3.5: Potentiometric Sensors:
   Why are Biomolecules Charged?

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Outline

• Theory of DNA charges
  – DNA is an acid and acids are charged in solution

• Protein involves charged residues
  – Protein charge can be negative or positive

• Biomolecules and surface charges

• Conclusions
Transistors and Biomolecules

Capture Probe

\[ \text{Capture Probe} \]

H \rightarrow \text{5C Sugar} \rightarrow \text{N Base} \rightarrow \text{PO}_4 \rightarrow \text{OH} 

Alam, Principles of Nanobiosensors, 2013
**Charge States of DNA controlled by pH**

\[
[H_3PO_4]_0 = [H_2PO_4^-] + [HPO_4^{2-}] + [PO_4^{3-}]
\]

\[
K_{a1} = \frac{[H^+][H_2PO_4^-]}{[H_3PO_4]} \\
\approx 7.5 \times 10^{-3}
\]

\[
K_{a2} = \frac{[H^+][HPO_4^{2-}]}{[H_2PO_4^-]} \\
\approx 6.2 \times 10^{-8}
\]

\[
K_{a3} = \frac{[H^+][PO_4^{3-}]}{[HPO_4^{2-}]} \\
\approx 2.14 \times 10^{-13}
\]

This is not $H_3PO_4$ originally we put in, but $H_3PO_4$ that is still undissociated.
Average Charge per Base at pH=7

\[ \text{pH}=7 = \log[H^+] \], \quad [H^+] = 10^{-7} \text{ Molar} \]

**Q=1**

\[ \frac{[H^+][H_2PO_4^-]}{[H_3PO_4]} \approx 7.5 \times 10^{-3} \]

\[ \frac{[H_2PO_4^-]}{[H_3PO_4]} \approx 7.5 \times 10^4 \]

**Fully ionized**

**Q=2**

\[ \frac{[H^+][HPO_4^{2-}]}{[H_2PO_4^-]} \approx 6.2 \times 10^{-8} \]

\[ \frac{[HPO_4^{2-}]}{[H_2PO_4^-]} \approx 0.62 \]

**S+D=1 \ & \ D/S=0.62**

**S=0.61q \ \ D=0.39q**

**Q=1S+2D=1.38q**

**Q=3**

\[ \frac{[H^+][PO_4^{3-}]}{[HPO_4^{2-}]} \approx 2.14 \times 10^{-13} \]

\[ \frac{[PO_4^{3-}]}{[HPO_4^{2-}]} \approx 2.14 \times 10^{-6} \]

**Negligible**

Alam, Principles of Nanobiosensors, 2013
Average Charge per base at any pH

\[ x_0 = x + y_1 + y_2 + y_3 \]

\[ y_i = \frac{m_i}{x_0} \]

\[ m_i \equiv 10^{(\sum_{1}^{i} pK_i - i \times pH)} \]

\[ S + D = 1 \quad \text{and} \quad D/S = 0.62 \]

\[ S = 0.61q \quad \text{and} \quad D = 0.39q \]

\[ Q = 1S + 2D = 1.38q \]
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Biopolymers: Protein

Charge ..variable?

Enzymes
Hormones
Tissue
Transport molecules
Protein is composed of 20 Amino Acid
How to Calculate Protein Charge

• Of 20 amino-acid, only seven are charged. One should account for terminal groups (NH$_2$ and COOH).

\[ Q = q(N_{term} + \alpha K + \beta R + \gamma H + \delta D + \varepsilon E + \xi C + \eta Y + C_{term}) \]

Basic Positively Charged
Lysine(K;pK=10), arginine(R;12), histidine(H;6.5), N-terminus(NH2;8.0)
Partial Charge: \[ y/x_0 = m / (m + 1) \quad m=10^{pH-pK} \]

Acidic -- Negatively Charged:
Asparte (D; pK=4.4), glutamate(E; 4.4), cysteine(C;8.5),
tyrosine(Y;10), C-terminus (COOH;3.1)
Partial Charge: \[ y/x_0 = m / (m + 1) \quad m=10^{pK-pH} \]

• Henderson–Hasselbalch equation
http://isoelectric.ovh.org/files/isoelectric-point-theory.html
Example: Prostate Specific Antigen

261 AA (net charge 10-15)

MWVPVVFLTL SVTWIGAAPL
ILSRIVGGWE CEKHSQPWQV
LVASRGRAVC GGVLVHPQWV
LTAAHCIIRNK SVILLGRHSL
FHPEDTGQVF QVSHSFPHPL
YDMSLLKNRF LRPGDDSSHDD
LMLRLSEPA ELTDAVKVD
LPTQEPALGT TCYASGWGSI
EPEEFLTPKK
LQCVDLHVISNDVCAQVHPQ
KVTKFMLCAG RWTGGKSTCS
GDSGGPLVCN GVLQGITSWG
SEPCALPERP SLYTKVHYR
KWIKDTIVAN P

- Protein detection should be done at a pH away its isoelectric point
- Modification of OH on sensor surface due to pH
Isoelectric Points and Protein detection

Protein Calculator: [http://www.scripps.edu/~cdputnam/protcalc.html](http://www.scripps.edu/~cdputnam/protcalc.html)

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Recall: Biomolecules and surface charges

Charge from biomolecule...

\[
\frac{Q_{DNA}}{q} = \sum_{i=1,3} iy_i = x_0 \frac{m_i}{1 + m_i}, \quad m_i \equiv 10^{+\left(\sum_{i}^{i} pK_i\right)^{-i \times pH_B}}
\]

Surface charge...

\[
Q_s = q \left[ [\text{SiOH}^+] - [\text{SiO}^-] \right] = qN_0 \left\{ \frac{[\text{H}^+]_S / K_a - K_b / [\text{H}^+]_S}{1 + K_b / [\text{H}^+]_S + [\text{H}^+]_S / K_a} \right\}
\]

\[
[H^+]_S = [H^+]_B e^{-q\psi_0 / k_BT}
\]
Charges must be calculated self-consistently

\[
\frac{Q_{DNA}}{q} = \sum_{i=1,3} iy_i = x_0 \frac{m_i}{1 + m_i} \quad m_i \equiv 10^{+\left(\sum_i pK_i\right) - i \times pH_s}
\]

\[
Q_{SiO_2} = qN_0 \left\{ N_1 \frac{[H^+]_s/K_c}{1 + [H^+]_s/K_c} - N_2 \frac{K_b/[H^+]_s}{1 + K_b/[H^+]_s} \right\}
\]

\[
\psi_0 = \frac{k_B T_L}{q} \ln \sum_{all} Q_{bio} + Q_{SiO_2} + \alpha V_{FG}
\]

\[
[H^+]_s = [H^+]_B e^{-q\psi_0/k_B T} \quad Q_{MOS} = C_{ox} \psi_0 = C_{ox} \frac{k_B T_L}{zq} \ln \frac{Q_{SiO_2} + \sum_{all} Q_{bio}}{Q_0}
\]
Theory and Experiment: PSA

Zheng et al., Nat. Biotech., 2005

Alam, Principles of Nanobiosensors, 2013
Biasing at the Point of Zero Charge

Maximum sensitivity is obtained if surface is neutral

$$S \propto Q_{MOS} \propto \ln \frac{Q_{SiO2} + \sum_{all} Q_{bio}}{Q_0}$$
Conclusions

- Many biomolecules are charged in solution and potentiometric sensors can detect these molecules.
- DNA charge ~ 1-2, always negative.
- Protein charge could be positive or negative, depending on the pH of the environment.
- The pH at which the protein charge vanishes is called an isoelectric point.
- The charge calculations are very accurate and one can compare the theoretical results with experiments with reasonable accuracy.