Principles of Electronic Nanobiosensors

Unit 3: Sensitivity

Lecture 3.5: Potentiometric Sensors:
Why are Biomolecules Charged?

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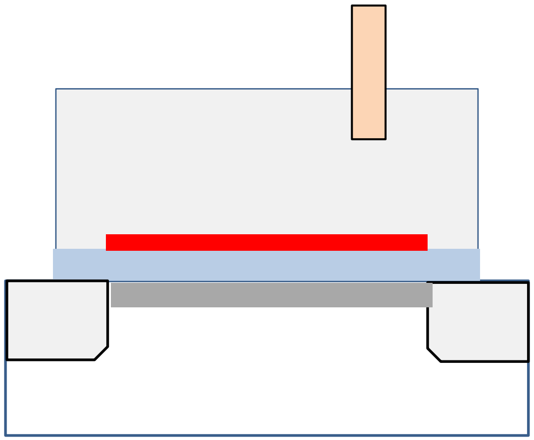
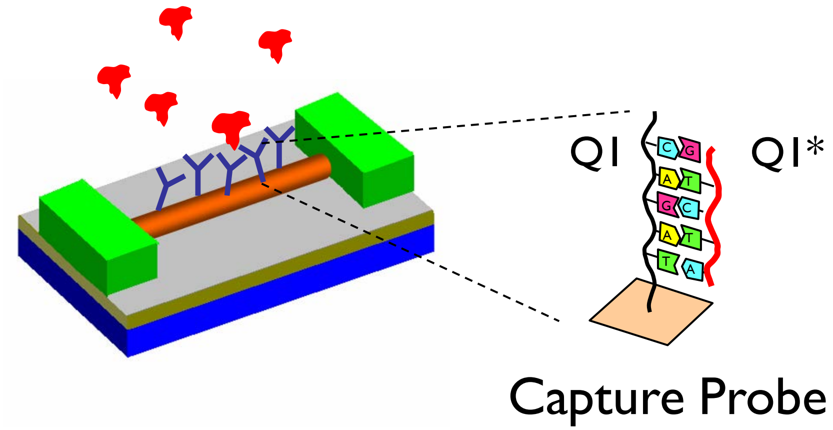
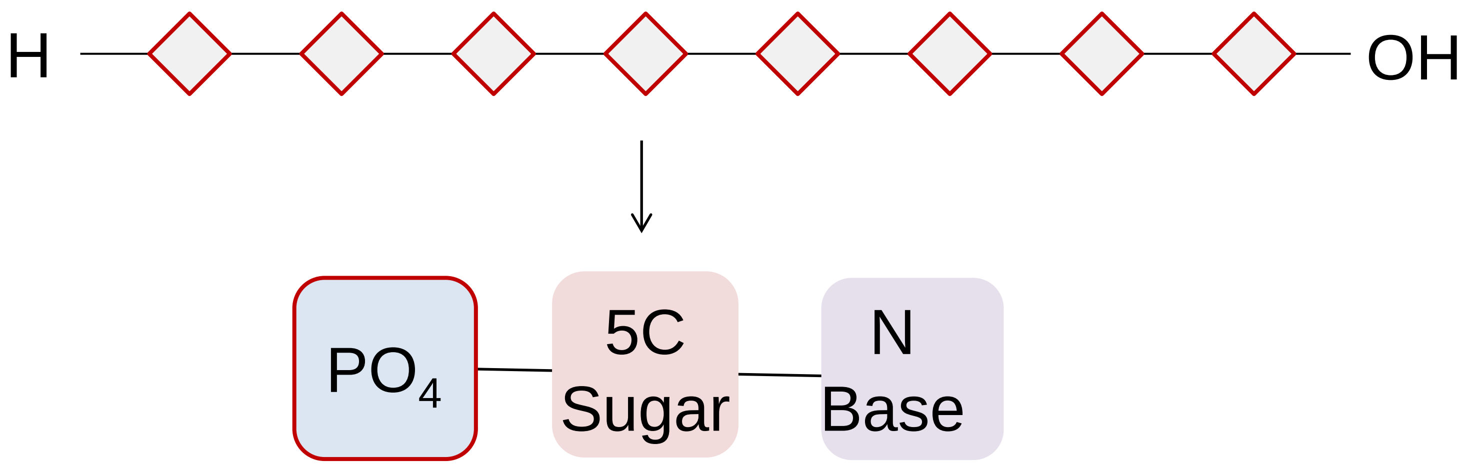
Outline

- 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

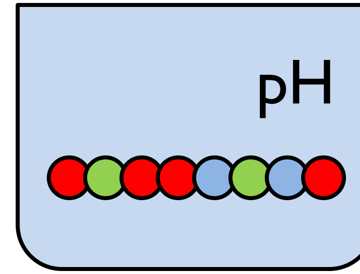
Transistors and Biomolecules



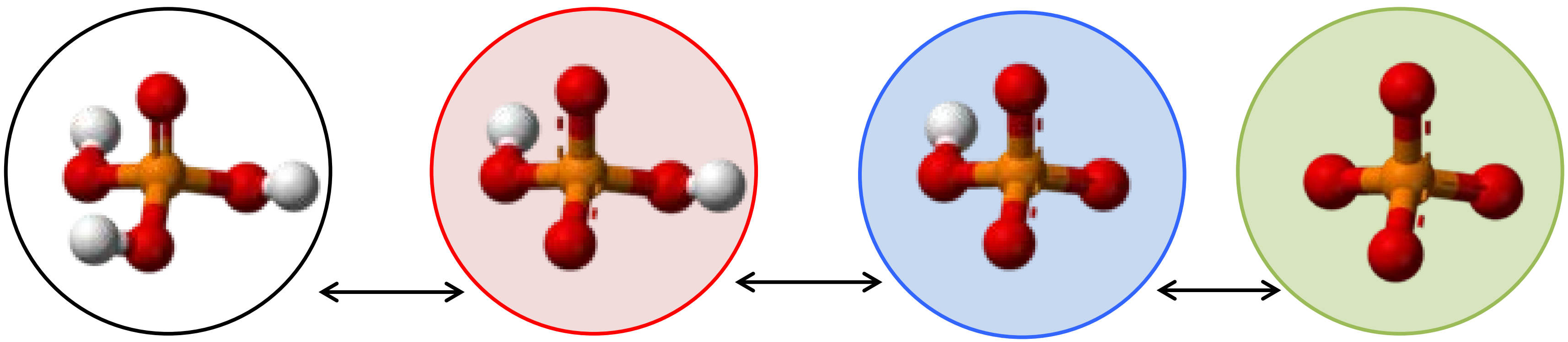




Charge States of DNA controlled by pH



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



$$K_{a1} = \frac{[H^+][H_2PO_4^{-}]}{[H_3PO_4]} \simeq 7.5*10^{-3}$$

$$K_{a2} = \frac{[H^+][HPO_4^{2-}]}{[H_2PO_4^{-}]} \simeq 6.2*10^{-8}$$

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

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

pH=7=log[H⁺], [H⁺]=10⁻⁷ Molar

Fully ionized

Q=1

$$\frac{[H^+][H_2PO_4^-]}{[H_3PO_4]} \simeq 7.5*10^{-3} \qquad \frac{[H_2PO_4^-]}{[H_3PO_4]} \simeq 7.5*10^{4}$$

Q=2

$$\frac{[H^+][HPO_4^{2-}]}{[H_2PO_4^-]} \simeq 6.2*10^{-8} \qquad \frac{[HPO_4^{2-}]}{[H_2PO_4^-]} \simeq 0.62$$

Q=3

$$\frac{[H^+][PO_4^{3-}]}{[HPO_4^{2-}]} \simeq 2.14*10^{-13} \qquad \frac{[PO_4^{3-}]}{[HPO_4^{2-}]} \simeq 2.14*10^{-6}$$

Negligible

S+D=1 & D/S=0.62

S=0.61q D=0.39q

Q=1S+2D=1.38q

Average Charge per base at any pH

$$x_0 = x + y_1 + y_2 + y_3$$

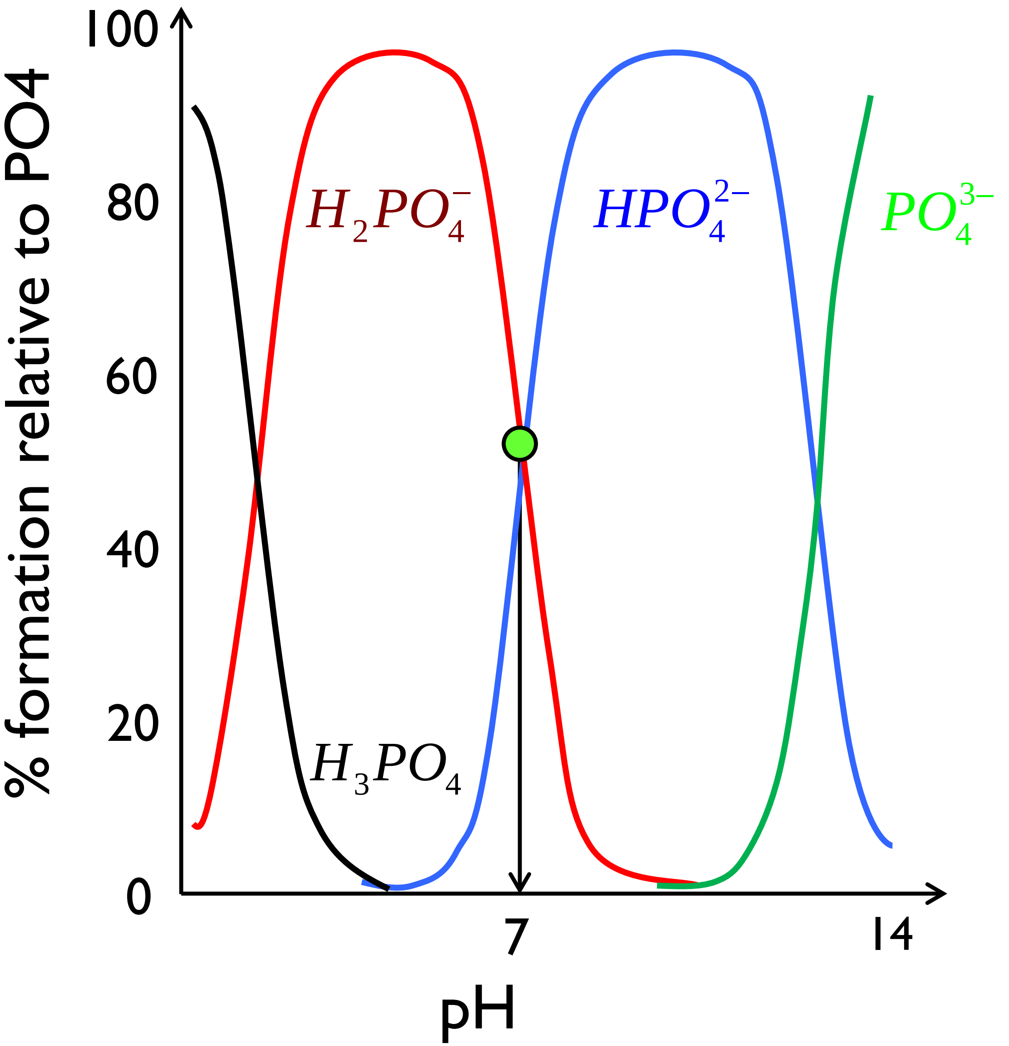
$$\frac{y_i}{x_0} = \frac{m_i}{1+m_i}$$

$$m_i \equiv 10^{+\left(\sum_1^i pK_i\right) - i \times pH}$$

S+D=1 & D/S=0.62

S=0.61q   D=0.39q

Q=1S+2D=1.38q



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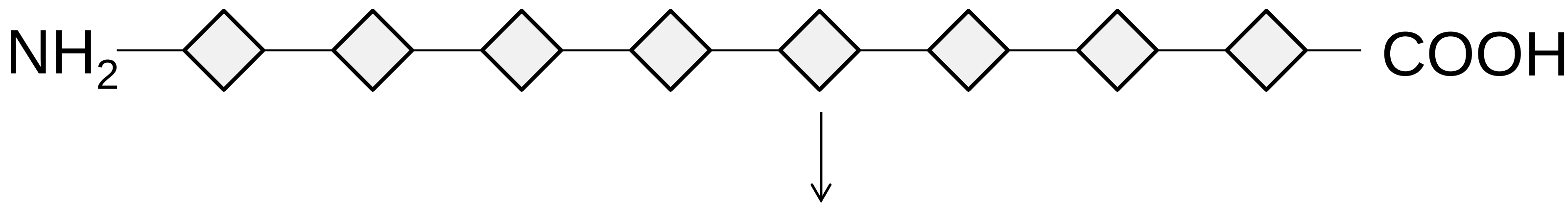
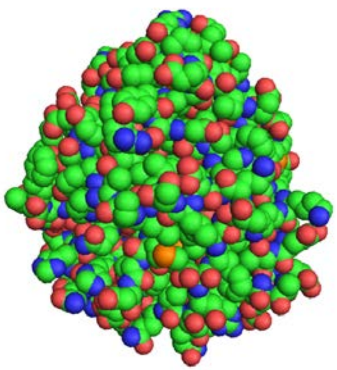
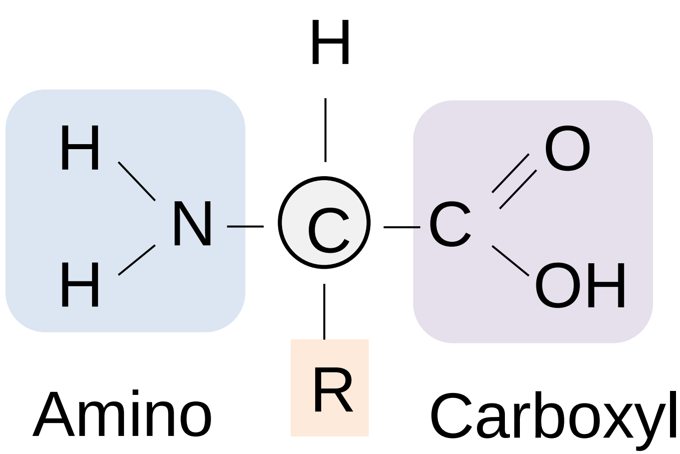
Biopolymers: Protein



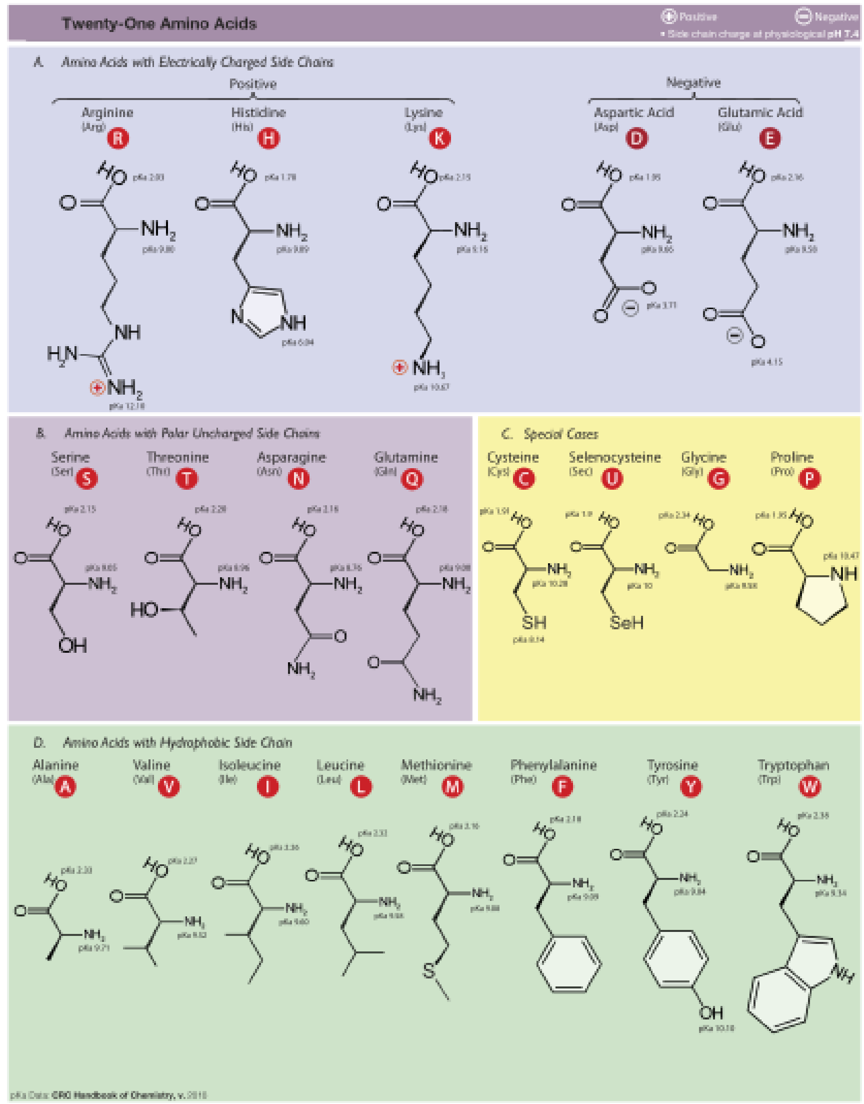




Enzymes
Hormones
Tissue
Transport molecules

Charge ..variable?

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), cyseine(C;8.5), tyrosine(Y;10), C-terminus (COOH;3.1)

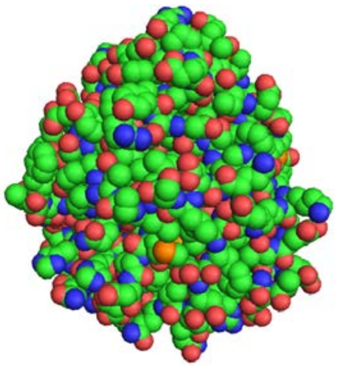
Partial Charge: $y/x_0 = m/(m+1) \quad m=10^{pK-pH}$

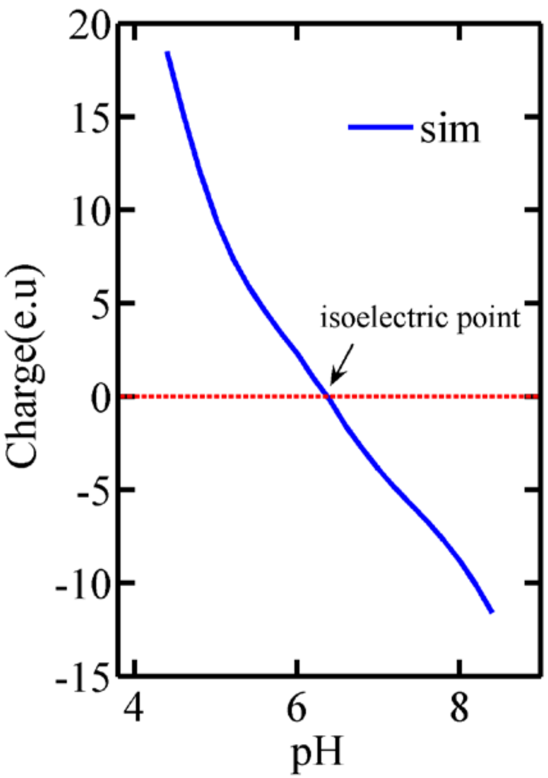
- Henderson–Hasselbalch equation
  http://isoelectric.ovh.org/files/isoelectric-point-theory.html

Example: Prostate Specific Antigen

261 AA (net charge 10-15)

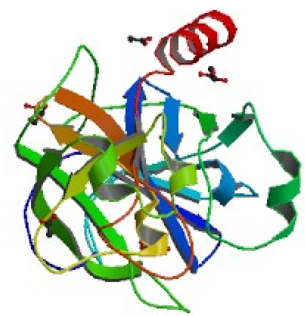
MWVPVVFLTL SVTWIGAAPL
ILSRIVGGWE CEKHSQPWQV
LVASRGRAVC GGVLVHPQWV
LTAAHCIRNK SVILLGRHSL
FHPE**D**TGQVF QVSHSFPHPL
Y**D**MSLLKNRF LRPG**DD**SSH**D**
LMLLRLSEPA ELT**D**AVKVM**D**
LPTQEPALGT TCYASGWGSI
EPEEFLTPKK
LQCV**D**LHVISN**D**VCAQVHPQ
KVTKFMLCAG RWTGGKSTCS
G**D**SGGPLVCN GVLQGITSWG
SEPCALPERP SLYTKVVHYR
KWIK**D**TIVAN P



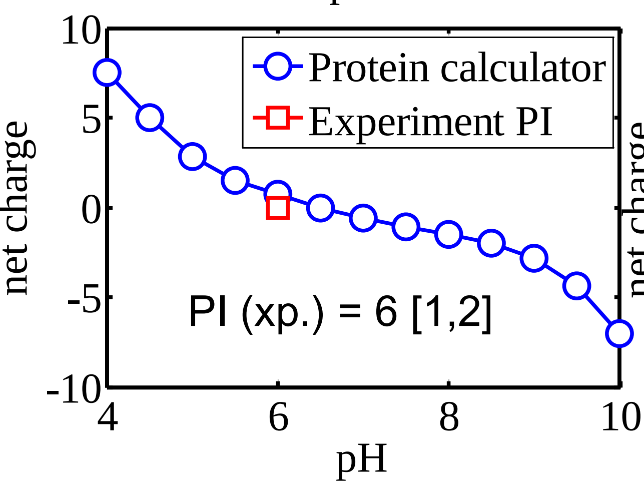


- Protein detection should be done at a pH away its isoelectric point
- Modification of OH on sensor surface due to pH

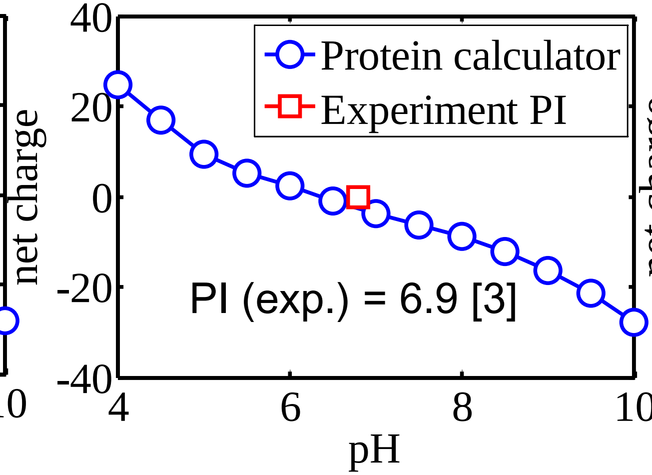
Isoelectric Points and Protein detection



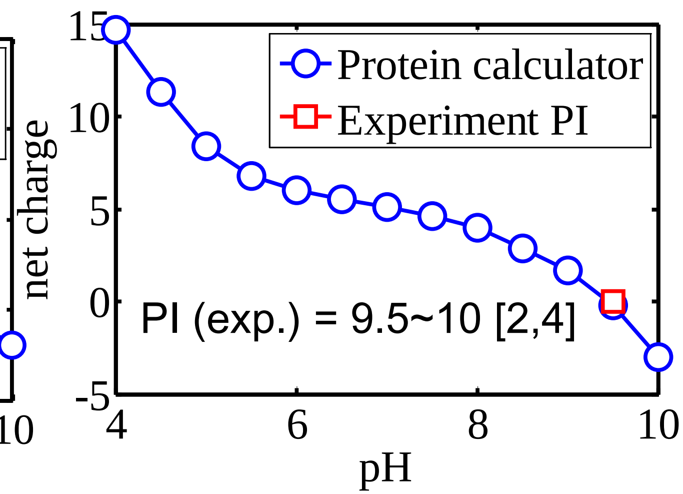
Streptavidin



PSA



Avidin



Protein Calculator: http://www.scripps.edu/~cdputnam/protcalc.html

1.D. E. Leckband, F.-J. Schmitt, J. N. Israelachvili, and W. Knoll, Direct Force Measurements of Specific and Nonspecific Protein Interactions, *Biochemistry* **33**, 4611-4624 (1994) [link] 2. N. Michael Green**,** Avidin and streptavidin, *Meth. Enzym.* **184**, 51-67 (1990) [link] 3.Armbruster D. A. Prostate-specific antigen: Biochemistry, analytical methods, and clinical application. *Clin. Chem.* **39**(2):181–195 (1993) [link] 4.Yao Z. *et al.* The Relationship of Glycosylation and Isoelectric Point with Tumor Accumulation of Avidin *J. Nucl. Med.* **40**(3) 479–483 (1999) [link]

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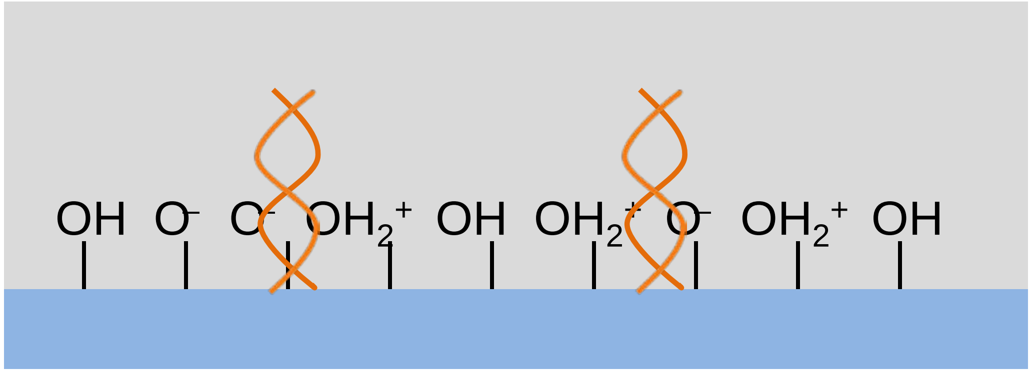
Recall: Biomolecules and surface charges



Charge from biomolecule…

$$\frac{Q_{DNA}}{q} = \sum_{i=1,3} i y_i = x_0 \frac{m_i}{1+m_i} \qquad m_i \equiv 10^{+\left(\sum_1^i pK_i\right) - i \times pH_B}$$

Surface charge …

$$[\mathrm{H}^+]_\mathrm{S} = [\mathrm{H}^+]_\mathrm{B} e^{-q\psi_0/k_B T}$$

$$Q_s = q\left[[\mathrm{SiOH}_2^+] - [\mathrm{SiO}^-]\right] = qN_0 \left\{ \frac{\left[\mathrm{H}^+\right]_S / K_a - K_b / \left[\mathrm{H}^+\right]_S}{\left[1 + K_b / \left[\mathrm{H}^+\right]_S + \left[\mathrm{H}^+\right]_S / K_a\right]} \right\}$$

Charges must be calculated self-consistently

$$\frac{Q_{DNA}}{q} = \sum_{i=1,3} i y_i = x_0 \frac{m_i}{1+m_i} \qquad m_i \equiv 10^{+\left(\sum_1^i pK_i\right) - i \times pH_s}$$

$$\downarrow$$

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

$$\downarrow$$

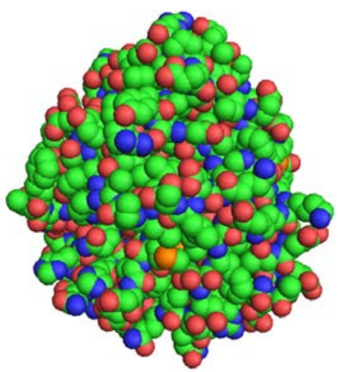
$$\psi_0 = \frac{k_B T_L}{q} \ln \frac{\sum_{all} Q_{bio} + Q_{SiO_2}}{Q_0} + \alpha V_{FG}$$

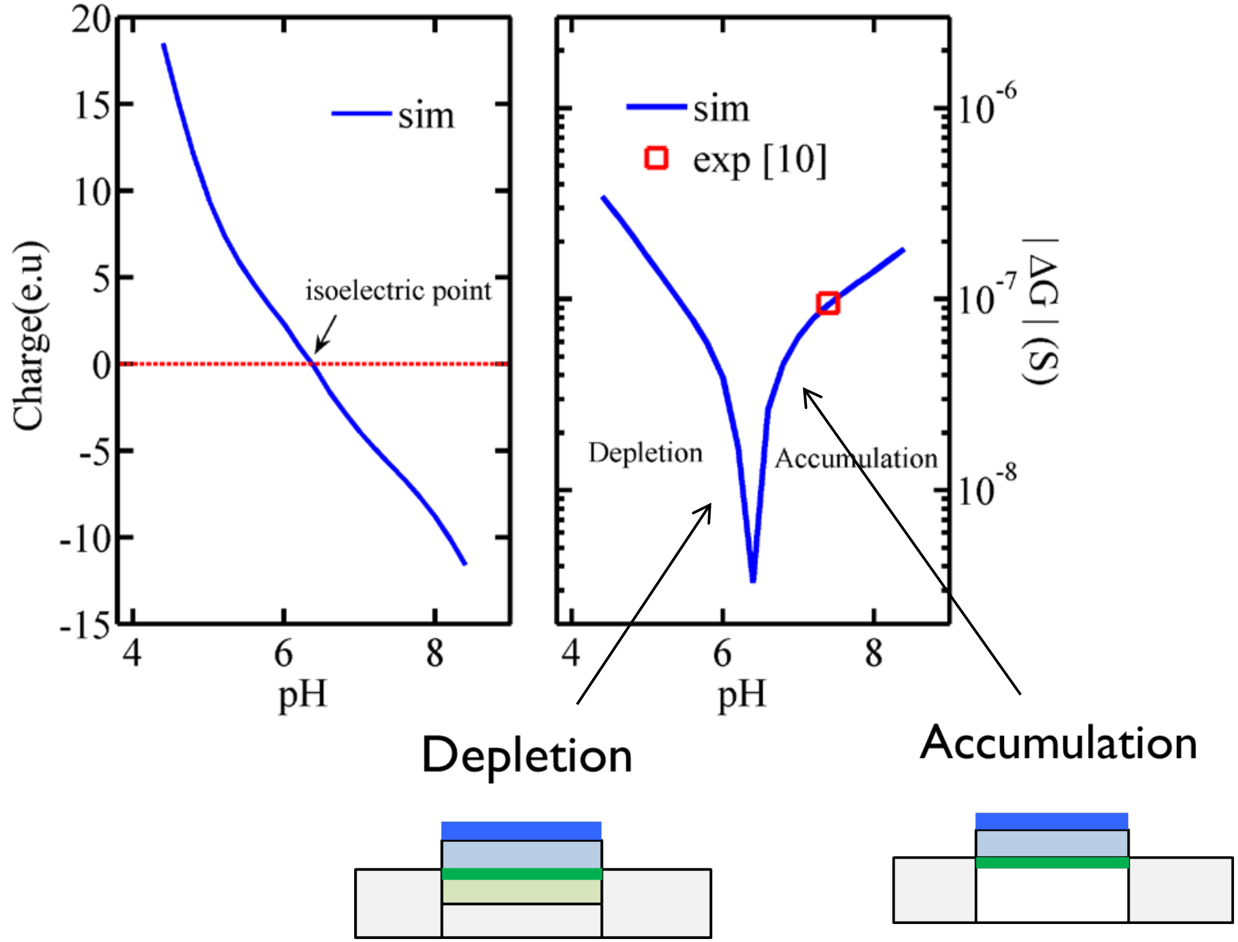
$$\downarrow$$

$$[\mathrm{H}^+]_\mathrm{S} = [\mathrm{H}^+]_\mathrm{B} e^{-q\psi_0/k_B T} \qquad Q_{MOS} = C_{ox}\psi_0 = C_{ox} \frac{k_B T_L}{zq} \ln \frac{Q_{SiO2} + \sum_{all} Q_{bio}}{Q_0}$$

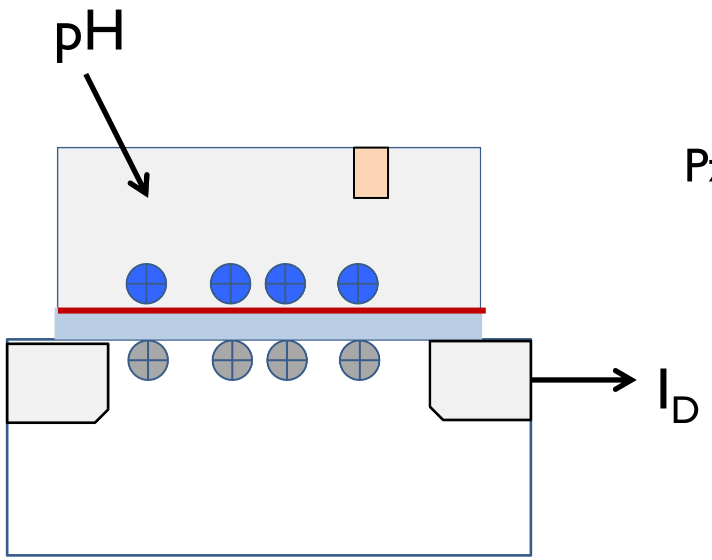
Theory and Experiment: PSA

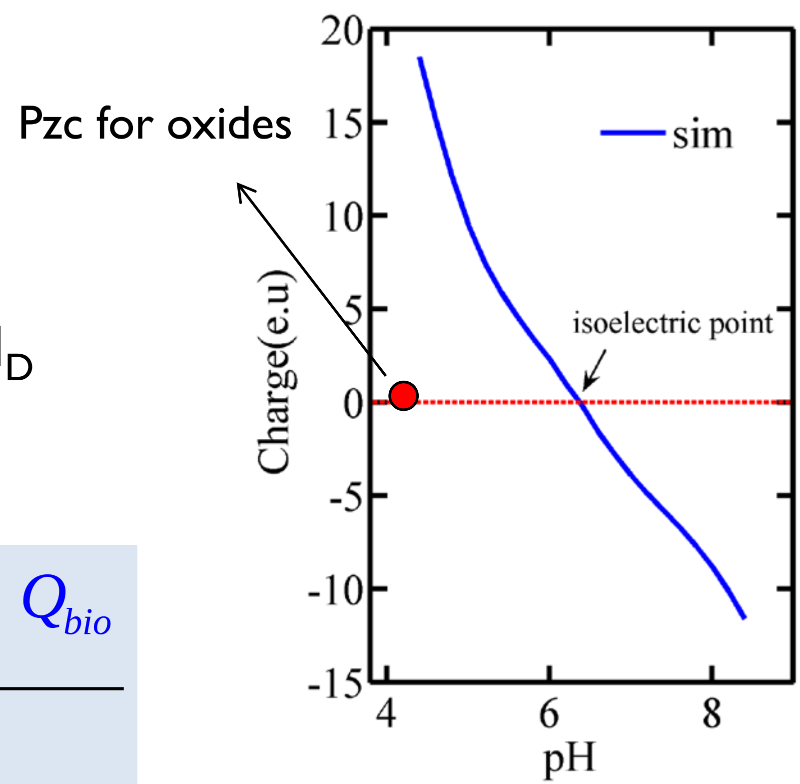
Zheng et al., Nat. Biotech., 2005





Biasing at the Point of Zero Charge





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

Maximum sensitivity is obtained if surface is neutral

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.