Lecture, Reading & PSets:

Chap 2: E-fields -- sources, "kinetics"

- What are E and H fields .....in BioSystems….
- Concepts: (1) *QuasiStatics*; (2) *Charge Relaxation*
- Some important & useful applications

Chap 3: Transport & Electrochemical Interactions

Effects of Molecular Charge on:

- Donnan Partitioning into tissues, gels, cells, ECM
- Electrostatics ↔ Binding (to ECM / ICM, receptors)
- Osmotic Pressure in tissues/gels ⇐ **Term Paper**
- Diffusion (D_{eff}): effects of electrostatic interactions

(Come back to this at end: "Integrative Case Studies)

Spatial Configuration and Composition of Charge Modulates Transport into a Mucin Hydrogel Barrier

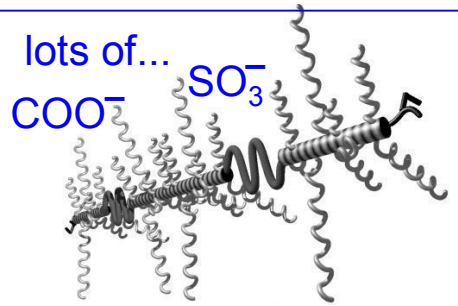
Biophysical J 2013

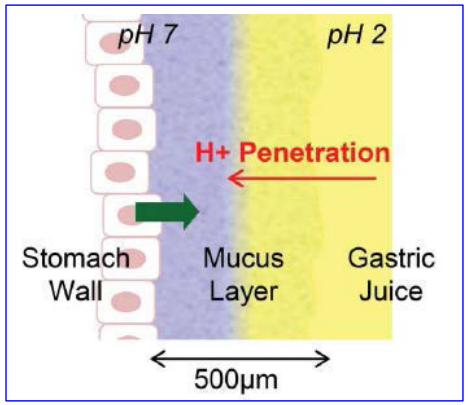
Leon D. Li,[†‡§] Thomas Crouzier,[‡] Aniruddh Sarkar,[§] Laura Dunphy,[‡] Jongyoon Han,[‡§] and Katharina Ribbeck[‡*]
[†]Harvard-MIT Division of Health Sciences and Technology, Cambridge, Massachusetts; [‡]Departments of Biological Engineering and [§]Electrical Engineering and Computer Science, Massachusetts Institute of Technology, Cambridge, Massachusetts

ABSTRACT: The mucus barrier is a **glycoprotein gel** that coats all wet surfaces in the human body, including the respiratory, gastrointestinal, and urogenital tracts.

- Criteria that govern transport through mucus barrier are unknown.
- **Charge distribution of solutes** is a critical parameter to modulate transport through mucin-based barriers: **implications for drug delivery**
- **Ionic strength** within the mucin barrier strongly influences transport specificity

Gastric mucin glycoprotein (MUC5AC 641 kDa)





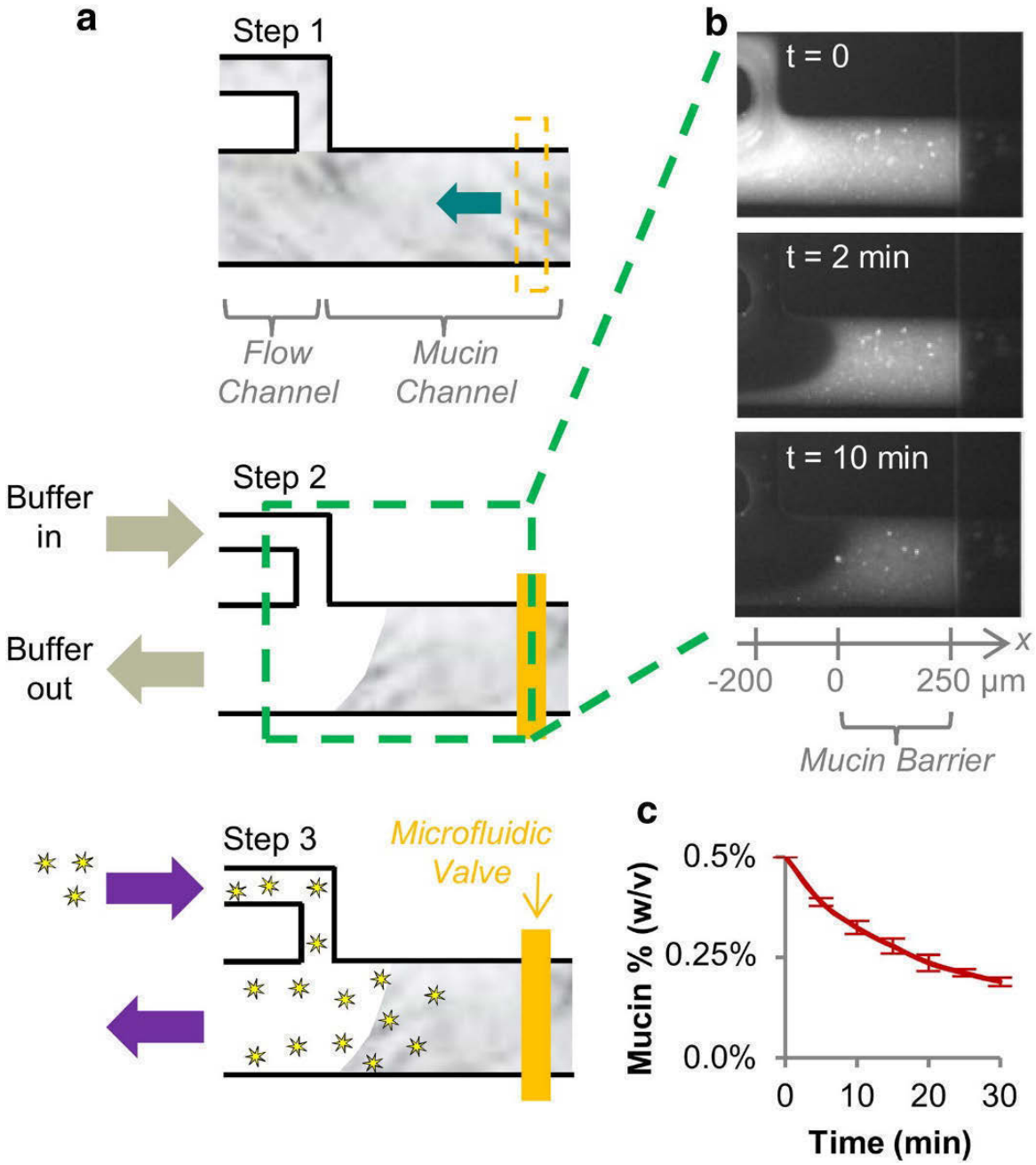
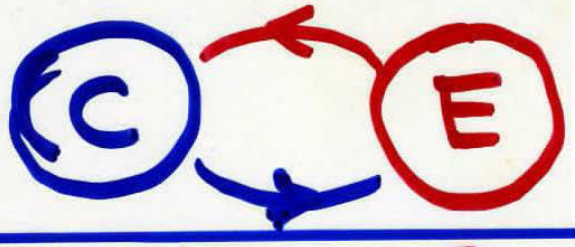


Fig. 1 Microfluidic device enables mucin barrier formation on-chip.

(a) A mucin sample initially filling both the flow and mucin channels (step 1, *top-down view*) is shaped into a layer of fixed width between a buffer flow and a microfluidic valve inside the mucin channel (step 2). **Fluorescent peptides flushed into the device** arrive at the mucin barrier surface and transport into the mucin barrier over time. (step 3)

(b) Formation and stability of the mucin barrier on-chip is assessed using fluorescently labeled mucins, showing that the mucin barrier surface interface is stable over time.

(c) Mucins are gradually lost from the mucin barrier over time, likely due to surface fluid shearing. We limit the duration of permeability measurements to 10 min to ensure that a majority of the initial mucin quantity remains inside the mucin barrier during the experiment. n = 3 devices.



(1) $\underline{N}_i = -D_i \nabla c_i + \frac{z_i}{|z_i|} u_i c_i \underline{E}$

(2) $\frac{\partial c_i}{\partial t} = -\nabla \cdot \underline{N}_i + R_i$

(3) $\nabla \cdot \epsilon \underline{E} = \rho_e^{TOT} = \left(\sum_i z_i F c_i \right) + \bar{\rho}_m$

(4) $\nabla \times \underline{E} = 0 \rightarrow \underline{E} = -\nabla \Phi$

(5) $\nabla \cdot \underline{J} = -\partial \rho_e / \partial t$

(6) $\underline{J} = \sum z_i F \underline{N}_i = \sigma \underline{E} + (\quad) \nabla c_i$

mobile charges e.g., ions

fixed charge in molecular matrix

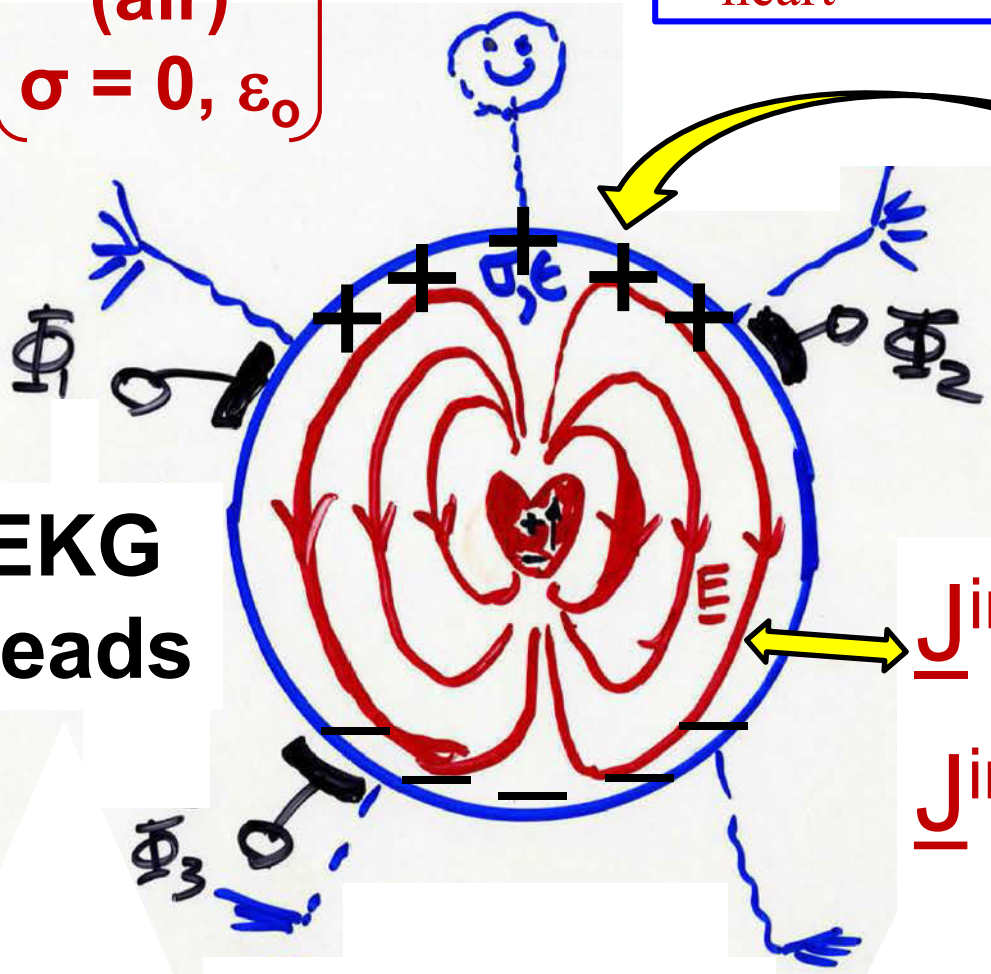
"Complete Description of Electrochemical Coupling & Transport"

EKG: L >> (1/κ) ⇒ elec. migration dominates diffusion

$\tau_{heart} \sim 1$ sec $\ll \tau_{relax} \sim 10^{-9}$ sec

(air)
$\sigma = 0, \varepsilon_o$

$\nabla^2\Phi = 0$



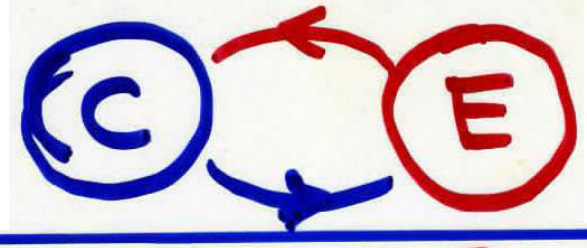
Surface charges relax to interfaces in nano-sec's

EKG Leads

$\underline{J}^{in} = \sigma \underline{E}^{in}$

$$\underline{J}^{in} = \left(\sum_i |z_i| F u_i c_i \right) \underline{E}$$

(NOTE: $\mathbf{v}_{ion}$ w.r.t. fluid $= \left(u_i \mathbf{E} \right)$ = 10's of μm/s)



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fixed charge in molecular matrix

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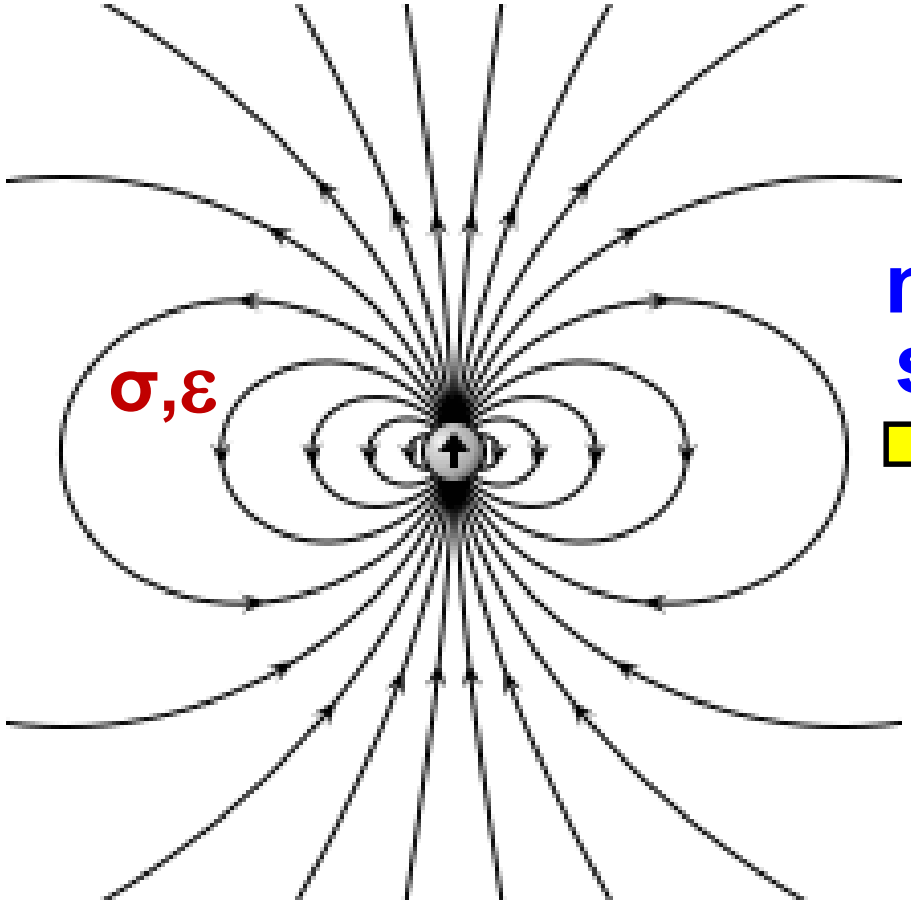
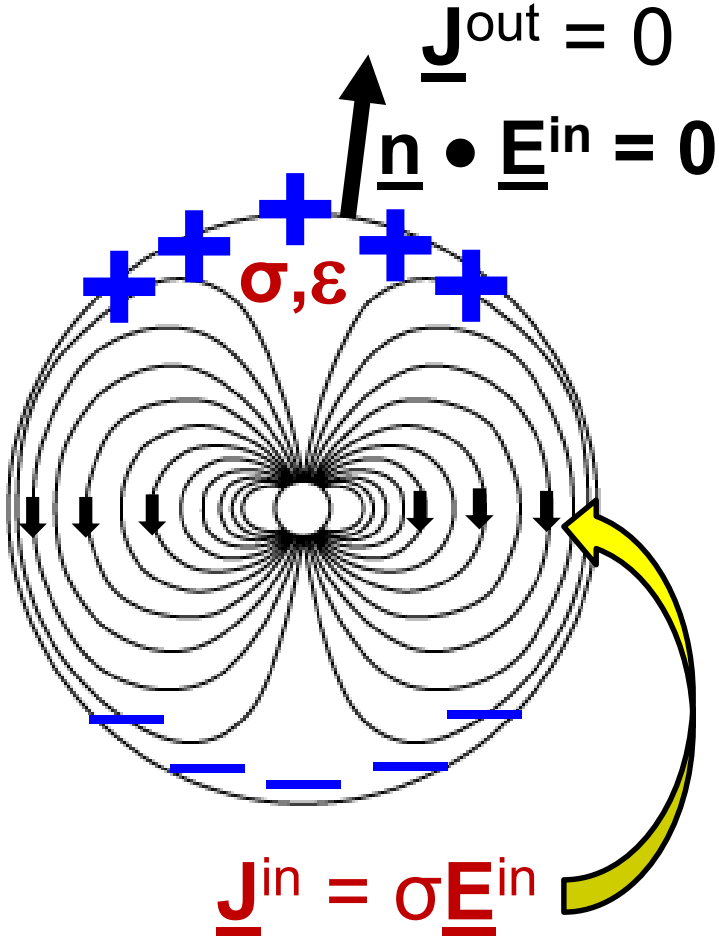
t = 0+
(after heart beats)

(air) $\sigma = 0, \varepsilon_o$



nano-sec's later



$\underline{E}^{in}$ = dipole + uniform field to match BC at r=R

EKG: L >> (1/κ) ⇒ elec. migration dominates diffusion

$\tau_{heart} \sim 1$ sec $\ll \tau_{relax} \sim 10^{-9}$ sec

(air)
$\sigma = 0, \varepsilon_o$

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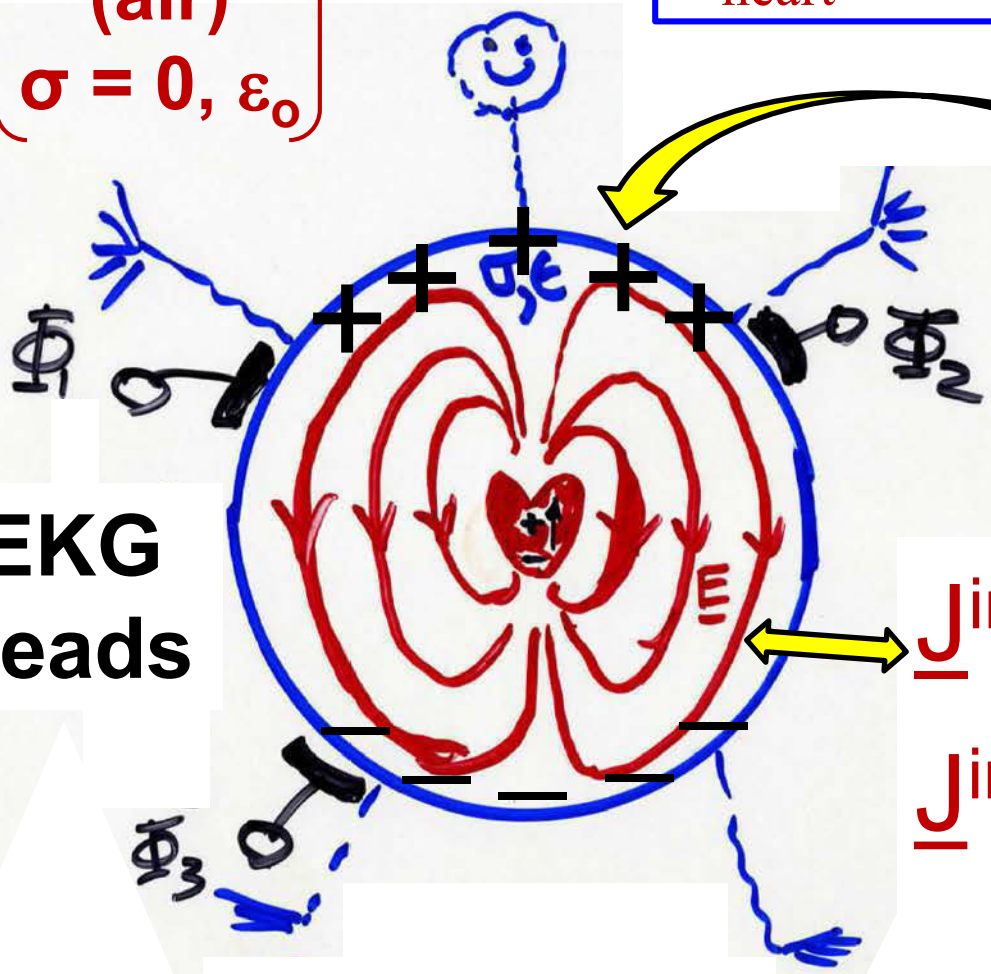

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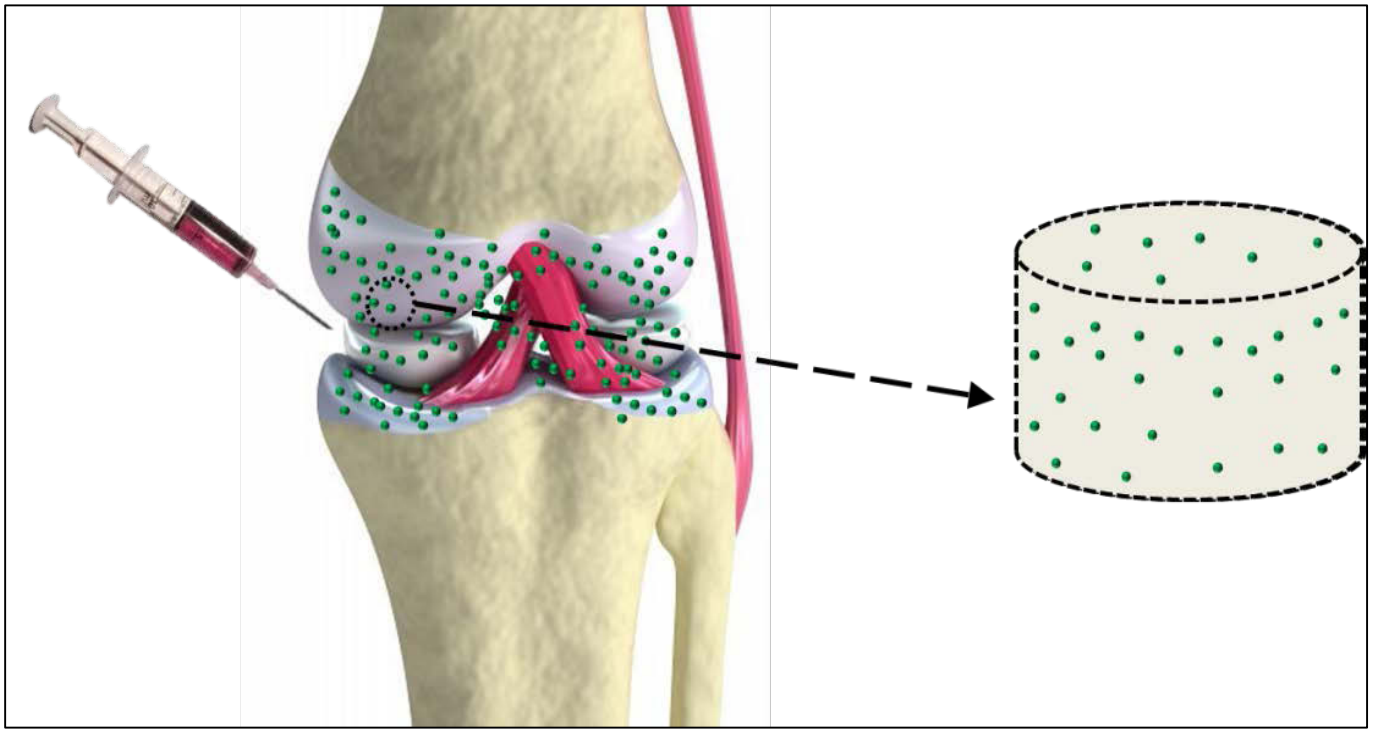
Equilibrium Upteake of Pfizer Mystery Drug:

760 Da; pI ~11 (peptide; basic)

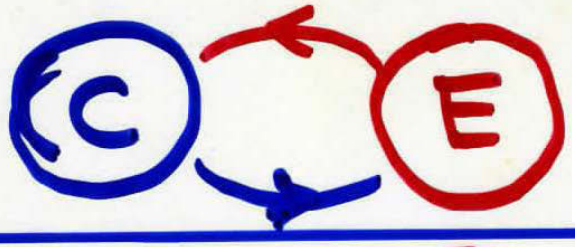
? ? ? ? ?



Courtesy of Alan Grodzinsky. Used with permission.

Can drug charge increase penetration and retention of drug into desired tissue (tumor…)

Equilibrium



- (1) $\underline{N}_i = \left(-D_i \nabla c_i + \frac{z_i}{|z_i|} u_i c_i \underline{E} \right) = 0$

(2) $\frac{\partial c_i}{\partial t} = -\nabla \cdot \underline{N}_i + R_i$ → 0

mobile charges e.g., ions

(3) $\nabla \cdot \epsilon \underline{E} = \rho_e = \left(\sum_i z_i F c_i \; (+\bar{\rho}_m) \right)^{TOT}$

(4) $\nabla \times \underline{E} = 0 \rightarrow \underline{E} = -\nabla \Phi$

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(6) $\underline{J} = \sum z_i F \underline{N}_i = \sigma \underline{E} + (\quad) \nabla c_i$

fixed charge in molecular matrix

"Complete Description of Electrochemical Coupling & Transport"

"Donnan Equilibrium"

(1) $\underline{N_i} = \left(-D_i \nabla c_i + \frac{z_i}{|z_i|} u_i c_i \underline{E} \right) = 0$, with $\underline{E} = -\nabla \Phi$

Boltzmann: $c_i = c_{i0} e^{-z_i F \Phi(x)/RT}$

(for all species)

$$\left(\frac{\bar{c}_+}{c_+}\right)^{1/z_+} = \left(\frac{\bar{c}_-}{c_-}\right)^{1/z_-} = \text{const} = e^{-F\Delta\Phi_D/RT}$$

---

(3) $\nabla \cdot \epsilon \underline{E} = \rho_e = \left(\sum_i z_i F c_i \; (+\bar{\rho}_m) \right) = 0$ (TOT)

Electro-neutrality

$$\bar{\rho}_m(x) + \sum_i z_{i+} F \bar{c}_{i+}(x) + \sum_j z_{j-} F \bar{c}_{j-}(x) = 0$$

(1) Boltzmann (for all species)

$$\left(\frac{\overline{C_i}}{C_i^{bath}}\right)^{\frac{1}{z_i}} = \text{const} = \left(\frac{\overline{C}_{Na}}{C_{Na}^{bath}}\right) = \left(\frac{\overline{C}_{H}}{C_{H}^{bath}}\right) = \underbrace{\left(\frac{\overline{C}_{drug}}{C_{drug}^{bath}}\right)^{\frac{1}{z_i}}}_{\textbf{drug}} = \left(\frac{C_{Cl}^{bath}}{\overline{C}_{Cl}}\right) = \left(\frac{C_{OH}^{bath}}{\overline{C}_{OH}}\right)$$

(3) Electroneutrality (with approximations):

$$\overline{\rho}_m + F(\overline{C}_{Na} - \overline{C}_{Cl}) = 0$$

Can You Use Donnan to Find: Charge of the Drug ??

Given

- $\bar{\rho}_m^{tissue} = \frac{\text{GAG}}{\text{water content}} \cdot \frac{2}{\text{Molecular weight}_{\text{chodroitin sulfate}}}$ — Tissue fixed charge density
- $C_{Na^+} = C_{Cl^-} = C_0 = 0.15\,\text{M}$ (bath conc. given)

- $\frac{\bar{C}_{Na^+}}{C_0} = \frac{C_0}{\bar{C}_{Cl^-}}$ (4) — Boltzmann Na^+, Cl^- partitioning
- $\rho_m + \bar{C}_{Na^+} - \frac{C_0^{\,2}}{\bar{C}_{Na^+}} = 0$ — Electroneutrality

---

$$\left(\frac{\bar{C}_{pep}}{C_{pep}}\right)^{\frac{1}{Z}} = \left(\frac{\bar{C}_{Na^+}}{C_{Na^+}}\right)$$

measured

bath

Calculated (Donnan)

bath

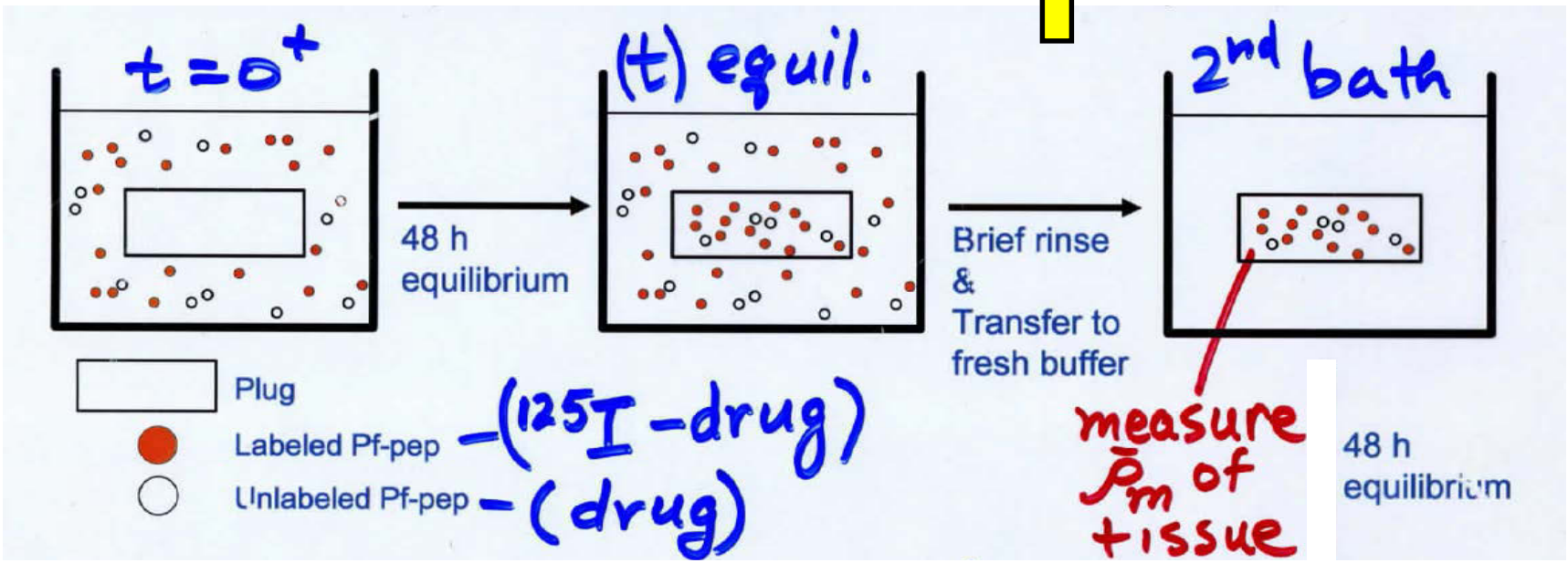
Find drug valence Z

Pfizer Mystery Drug: "Pf-Pep"

760 Da; pI ~ 11 (5 amino acids; basic)

Measure Equil Uptake of ^{125}I-Pf-pep @ pH 7:

$$R_U = K_{part}\left(1 + \frac{n}{K_d + c_F}\right)$$

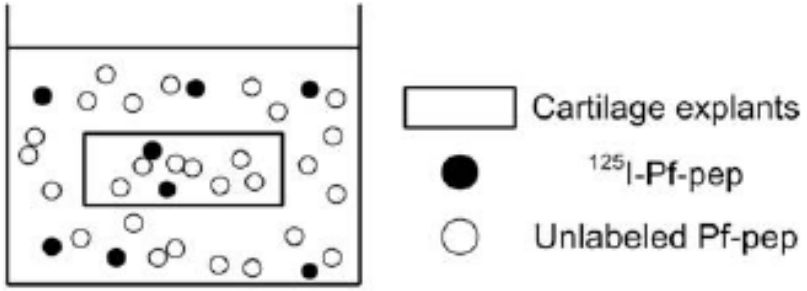


Is "Pf-pep" $\stackrel{?}{=}$ Arg-Tyr-Lys-Arg-Thr

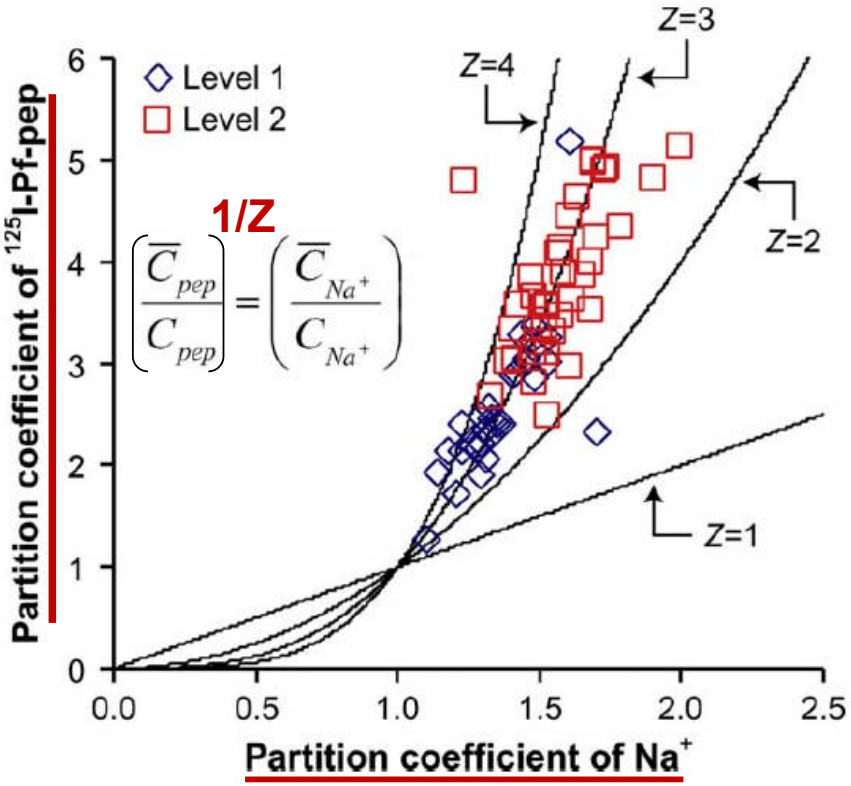
- small (760 Da)
- basic (pI ~ 11)

Different tissue samples having varying $\overline{\rho}_m^{tissue}$

Measure Pf-pep partitioning

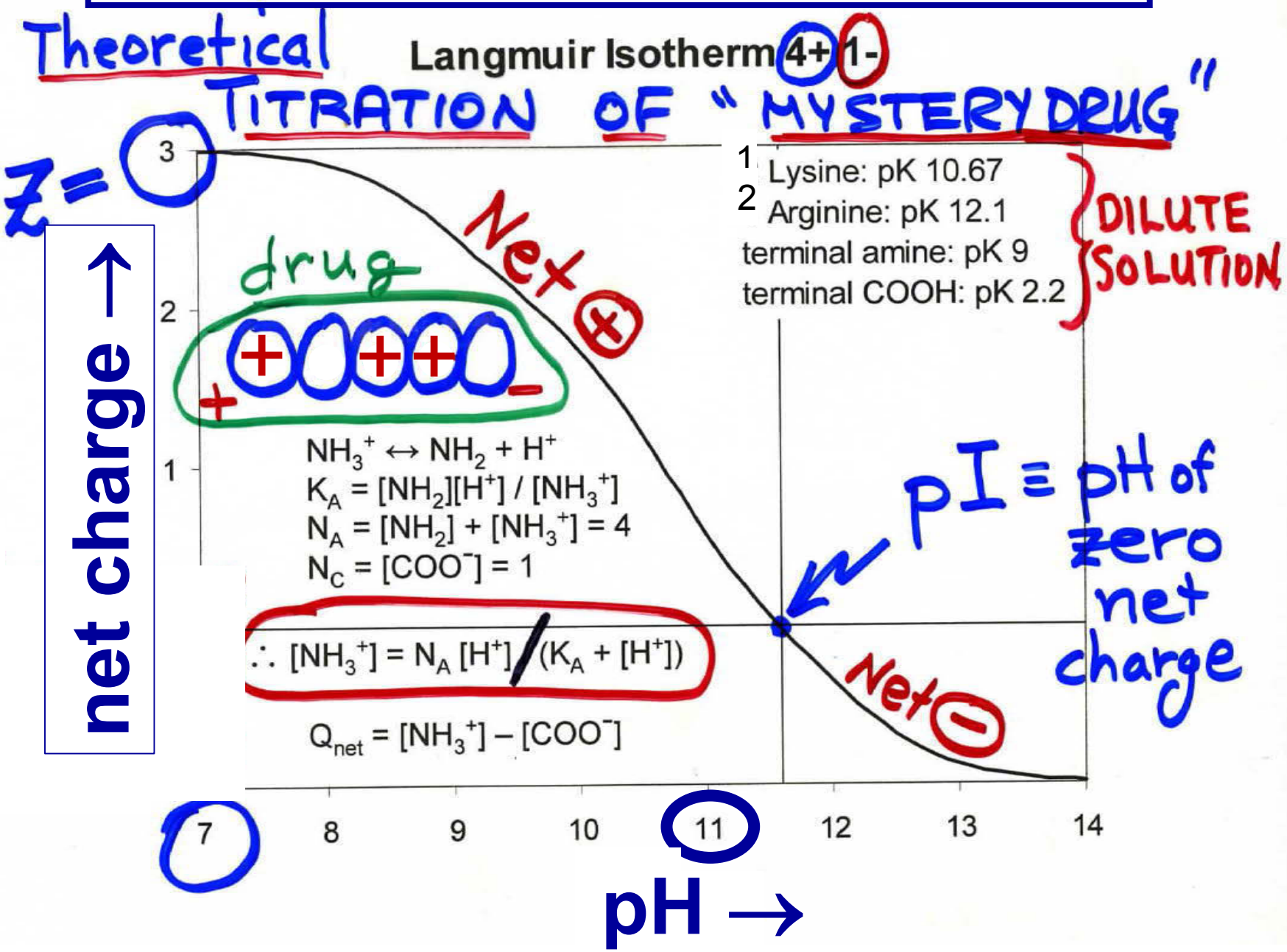


Donnan partitioning experiment

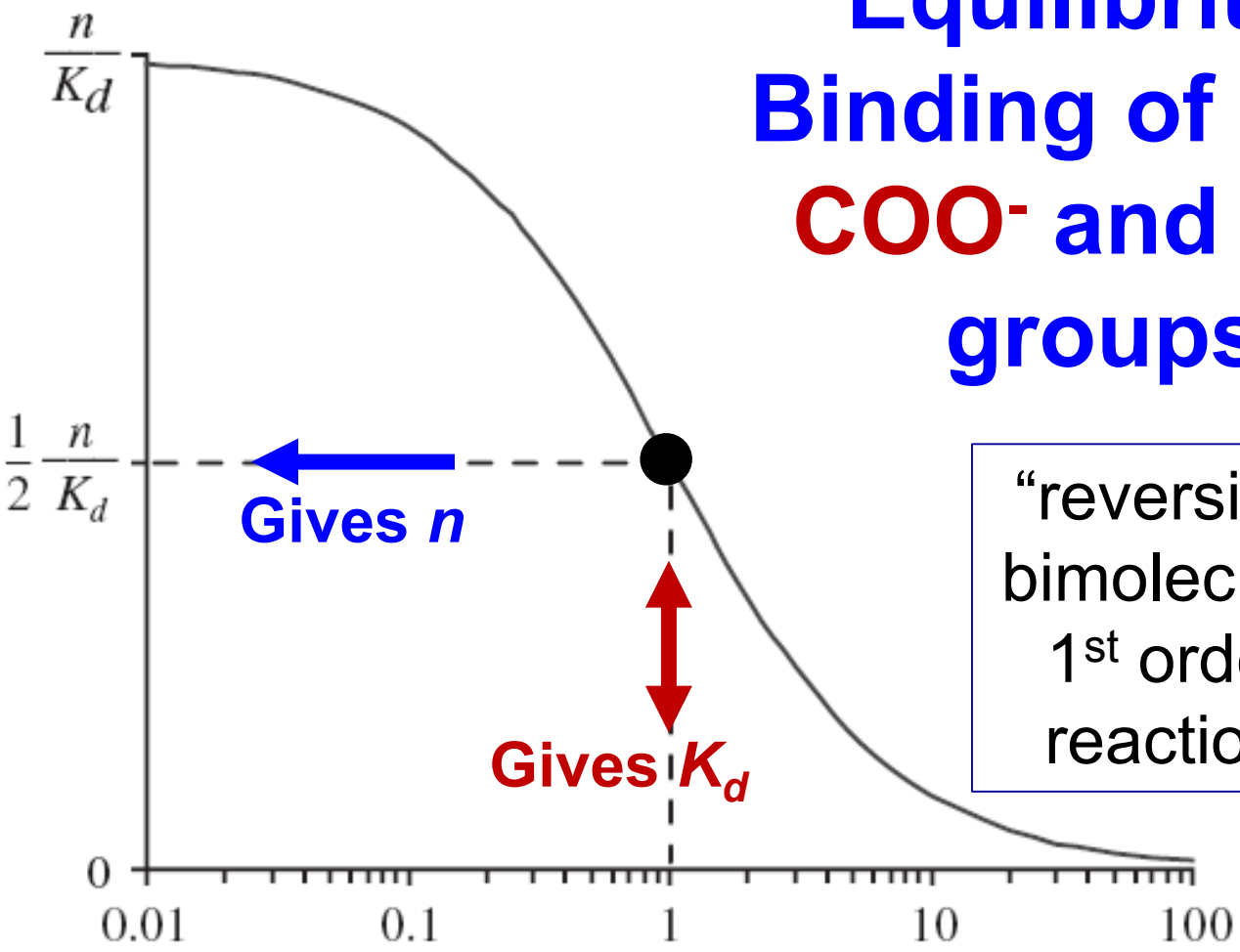


Calculate from Donnan

Is "Pf-pep" = Arg-Tyr-Lys-Arg-Thr



Equilibrium Binding of H^+ to COO^- and NH_2 groups



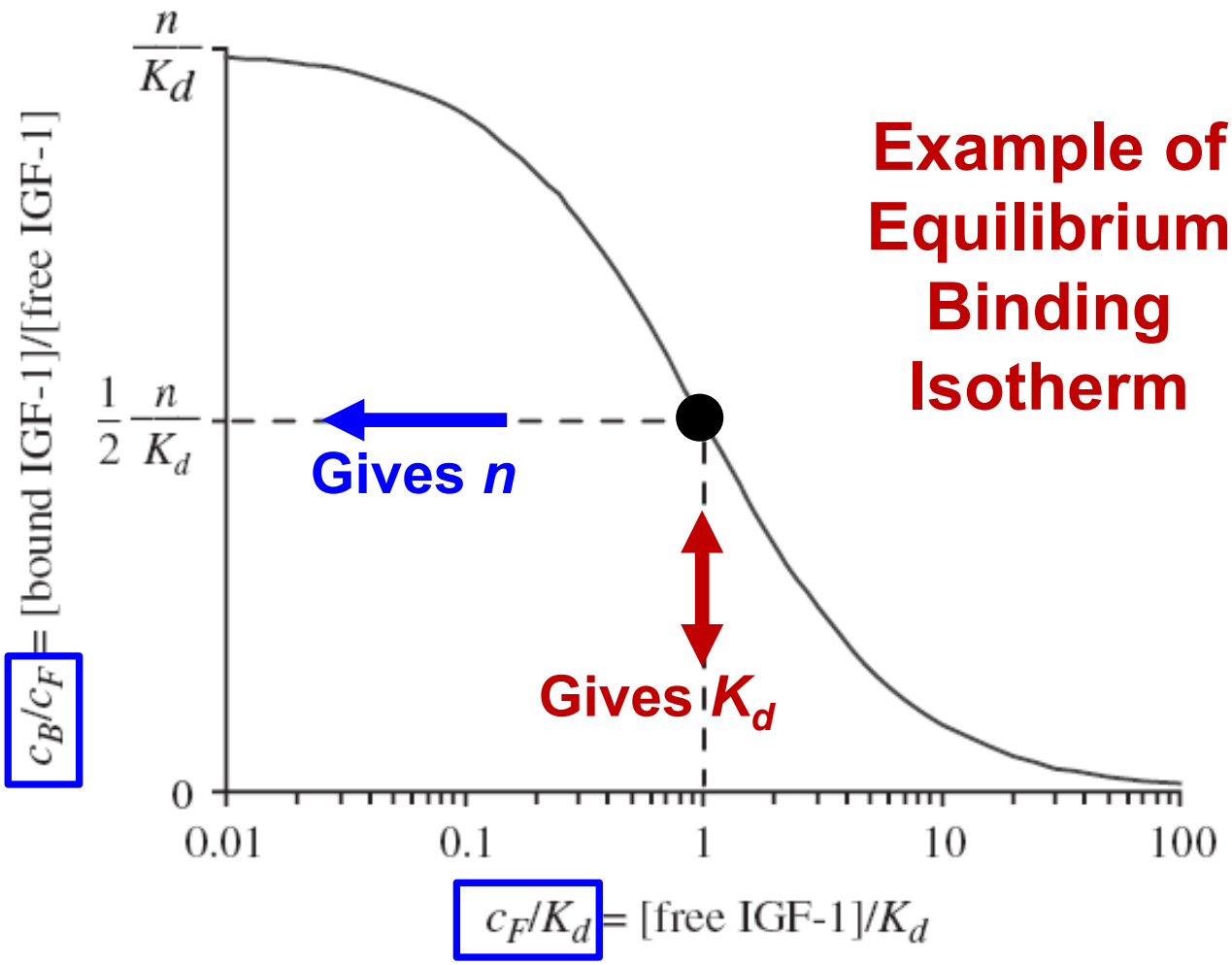
"reversible, bimolecular, 1st order" reactions

Nobel Prize in Chemistry, Oct 9, 2013..... for the development of multiscale models for complex chemical systems.... The computer simulations combine classical physics, which is able to track a multitude of atoms, and quantum mechanics..... including electrostatic interactions....

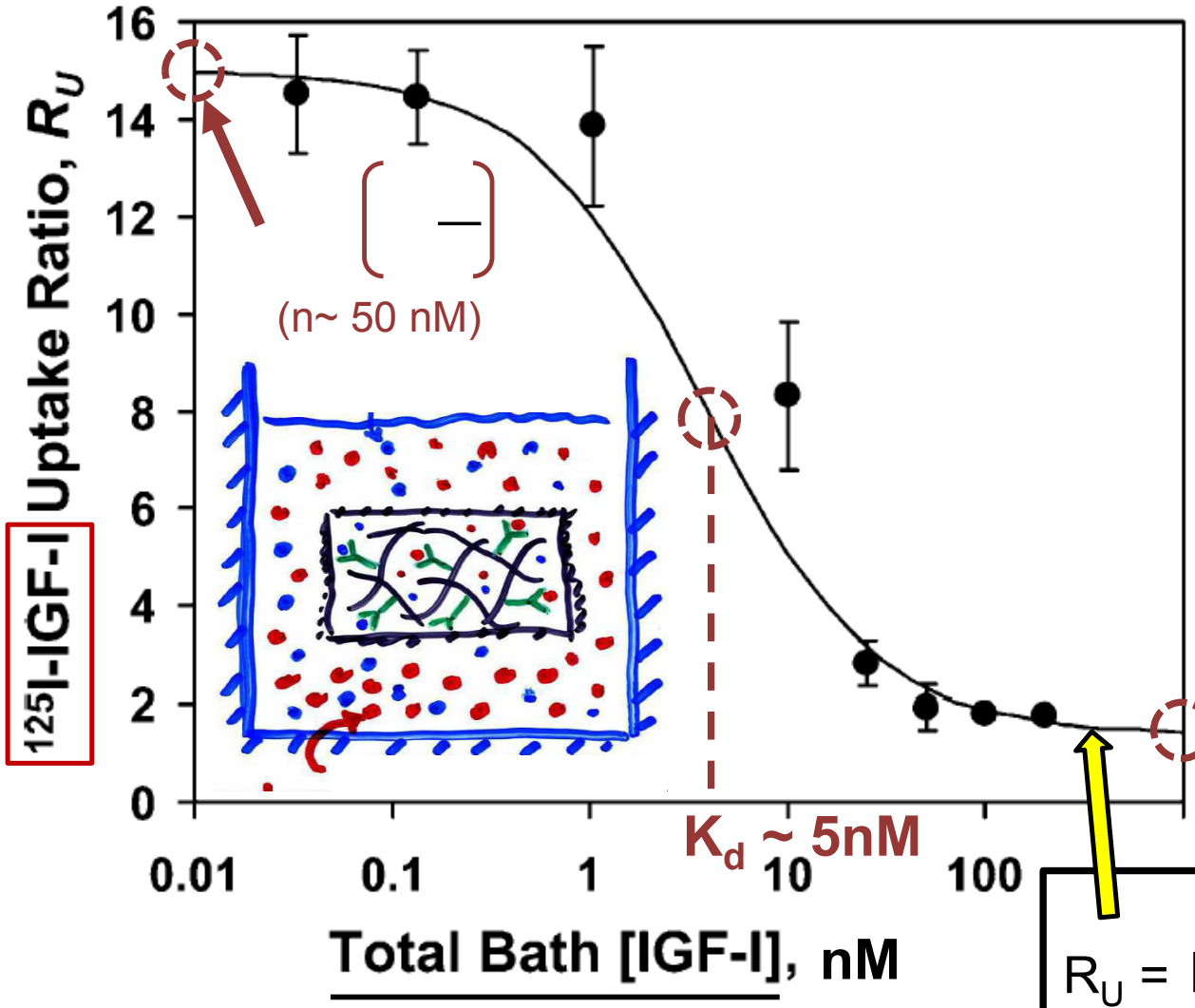
CHARMM (**Chemistry at HARvard Macromolecular Mechanics**) is the name of a widely used set of force fields for molecular dynamics as well as the name for the molecular dynamics simulation and analysis package associated with them. The CHARMM Development Project involves a network of developers throughout the world working with Martin Karplus and his group at Harvard to develop and maintain the CHARMM program.

Photographs of scientists removed due to copyright restrictions.

Does Pfizer "Pf-pep" bind to charge groups inside cartilage (tumor ECM, etc?)



Equilibrium Binding



$$R_U = \left(\frac{c_B^{tiss} + c_F^{tiss}}{c_{bath}} \right)$$

$$K_{part} = \frac{c_F^{tiss}}{c_{bath}}$$

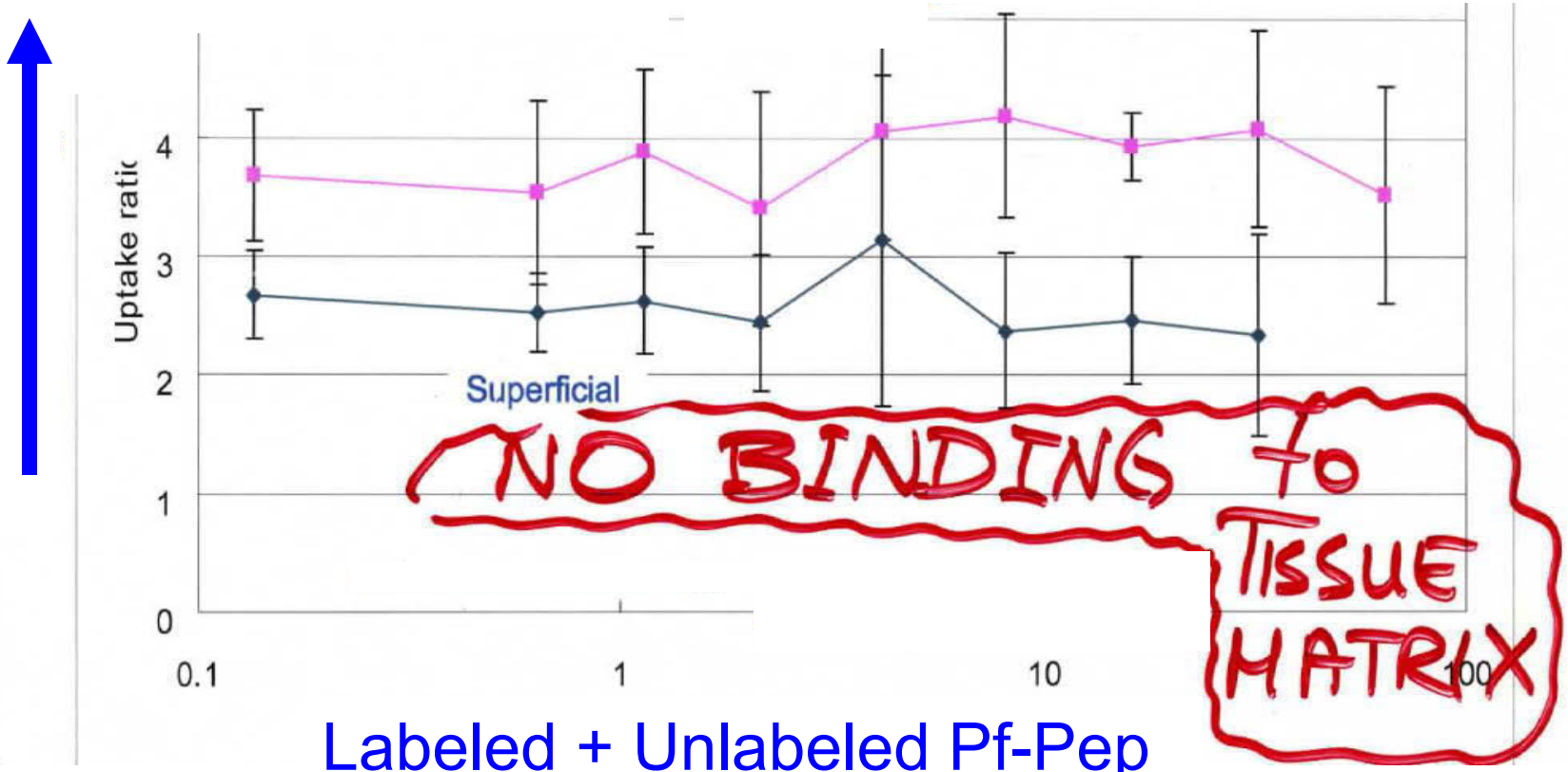
$$K_{part} = \frac{^{125}c_F}{^{125}c_{bath}}$$

$$\frac{c_B^{tiss}}{c_F^{tiss}} = \left(\frac{n}{K_d + c_F} \right)$$

$$R_U = K_{part} \left(1 + \frac{n}{K_d + c_F} \right)$$

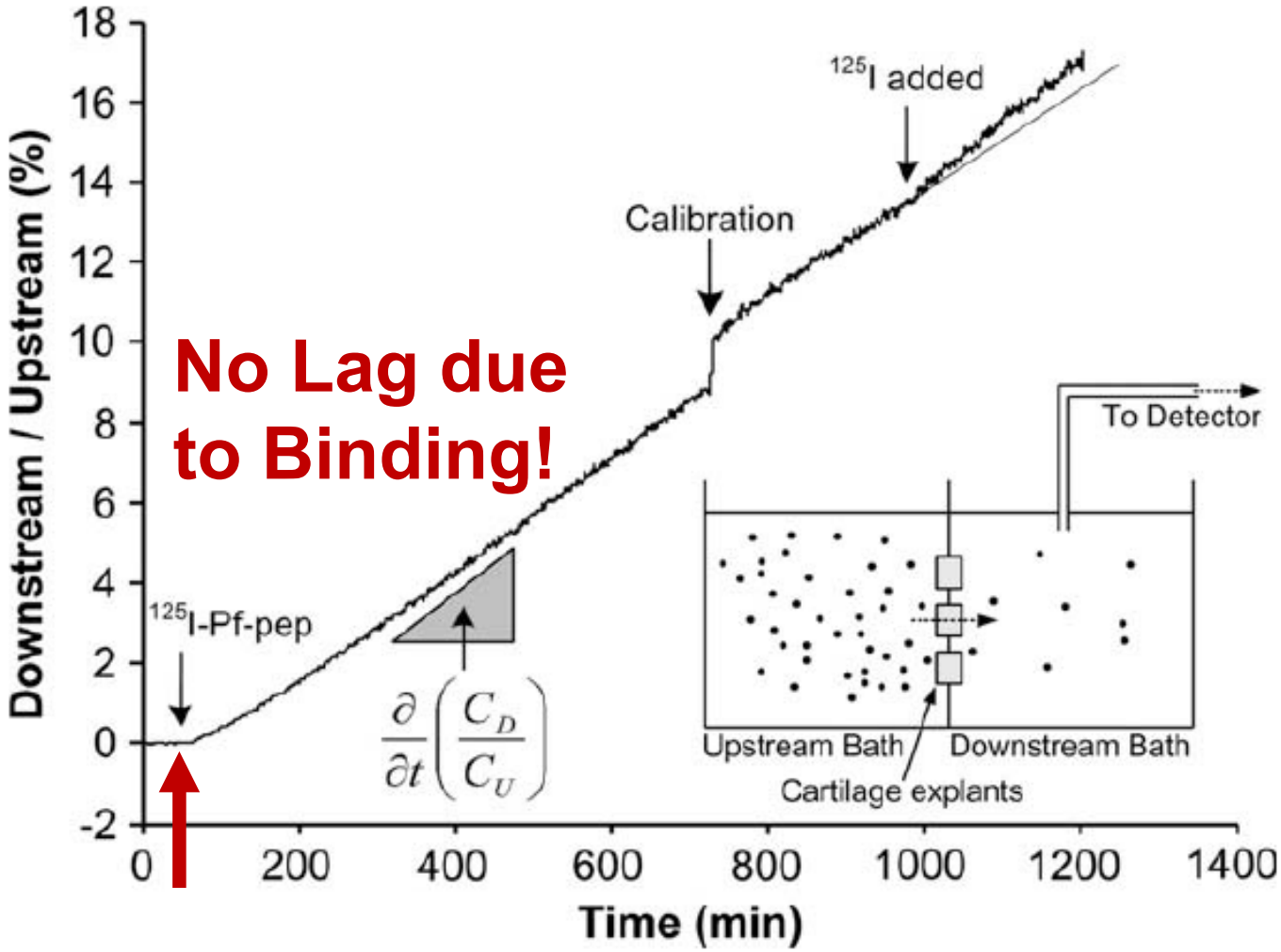
$$R_U = K_{part}\left(1 + \frac{\cancel{n \to 0}}{K_d + c_F}\right)$$

Uptake of Pf-pep Measured at pH 7



Labeled + Unlabeled Pf-Pep

Non-Equil Transport into and across tissue of "^{125}I-Pf-pep" = (Arg-Tyr-Lys-Arg-Thr)

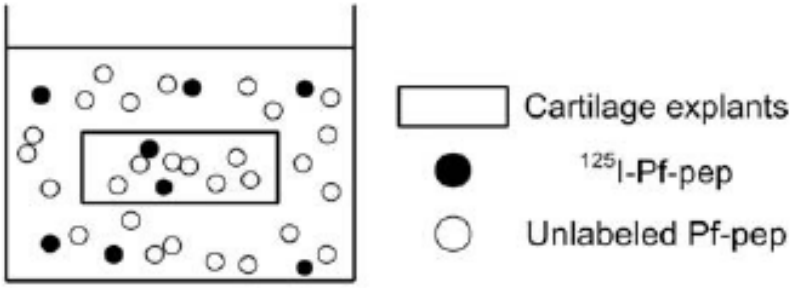


Transport and equilibrium uptake of a peptide inhibitor of PACE4 into articular cartilage is dominated by electrostatic interactions

results suggest that small positively charged therapeutics will have a higher concentration within cartilage than in the surrounding synovial fluid, a desired property for local delivery; however, such therapeutics may rapidly diffuse out of cartilage unless there is additional specific binding to intra-tissue substrates that can maintain enhanced intra-tissue concentration for local delivery.

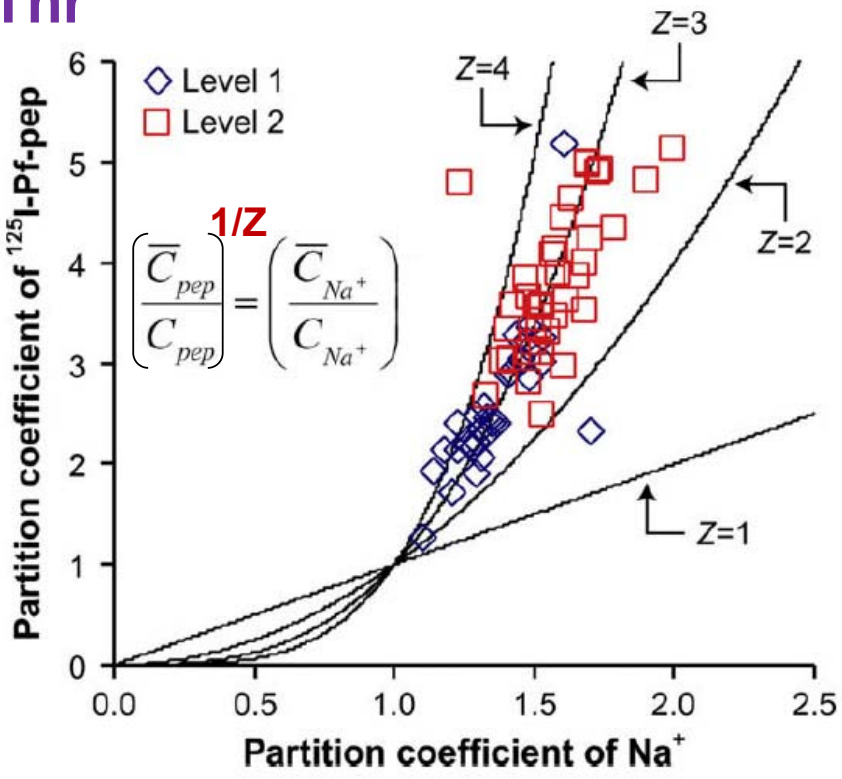
"Pf-pep" = Arg-Tyr-Lys-Arg-Thr

- small (760 Da)
- basic (pI ~ 11)



Donnan partitioning experiment

(Byun+, 2010)



Annals Rheumatic Diseases, 2012

A Role for PACE4 in Osteoarthritis Pain: Evidence from Human Genetic Association and Null Mutant Phenotype

Anne-Marie Malfait[1,*], Albert B. Seymour[2], Feng Gao[2], Micky D. Tortorella[3], Marie-Pierre Hellio Le Graverand-Gastineau[2], Linda S. Wood[2], Michael Doherty[4], Sally Doherty[4], Weiya Zhang[4], Nigel K. Arden[5], Frances L. Vaughn[6], Paul L. Leaverton[6], Tim D. Spector[7], Deborah J. Hart[7], Rose A. Maciewicz[8], Kenneth R. Muir[9], Rosalina Das[1], Robert E. Sorge[10], Susanna G. Sotocinal[10], Ara Schorscher-Petcu[10], Ana M. Valdes[7], and **Jeffrey S. Mogil[10]**

[1]Rush University Medical Center, Chicago IL

[2]Pfizer Worldwide Research and Development, Cambridge MA

[3]Guangzhou Institute for Biomedical Health, Guangzhou China

[4]University of Nottingham, UK

[5]University of Oxford UK

[6]The Arthritis Research Institute of America, Clearwater FL

[7]King's College London UK

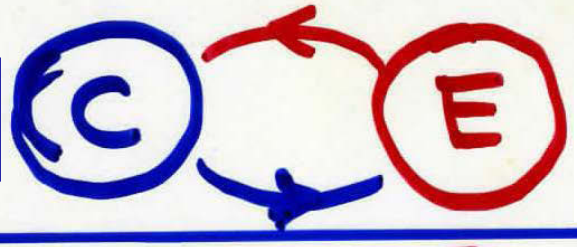
[8]AstraZeneca, Charnwood R&D, Loughborough, UK

[9]Health Sciences Research Institute, Warwick Medical School, University of Warwick UK

[10]Dept of Psychology and Alan Edwards Centre for Research on Pain, McGill University, Montreal QC Canada

PACE4 = a **"pro-protein convertase"**….activates matrix metalloproteases and ADAMTS-family proteases

Non-Equilibrium



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Biophysical J 2013

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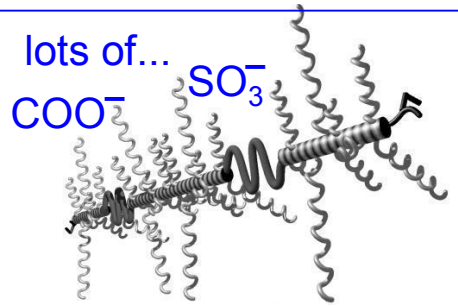
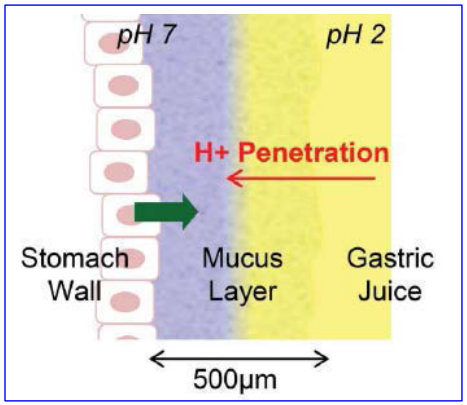




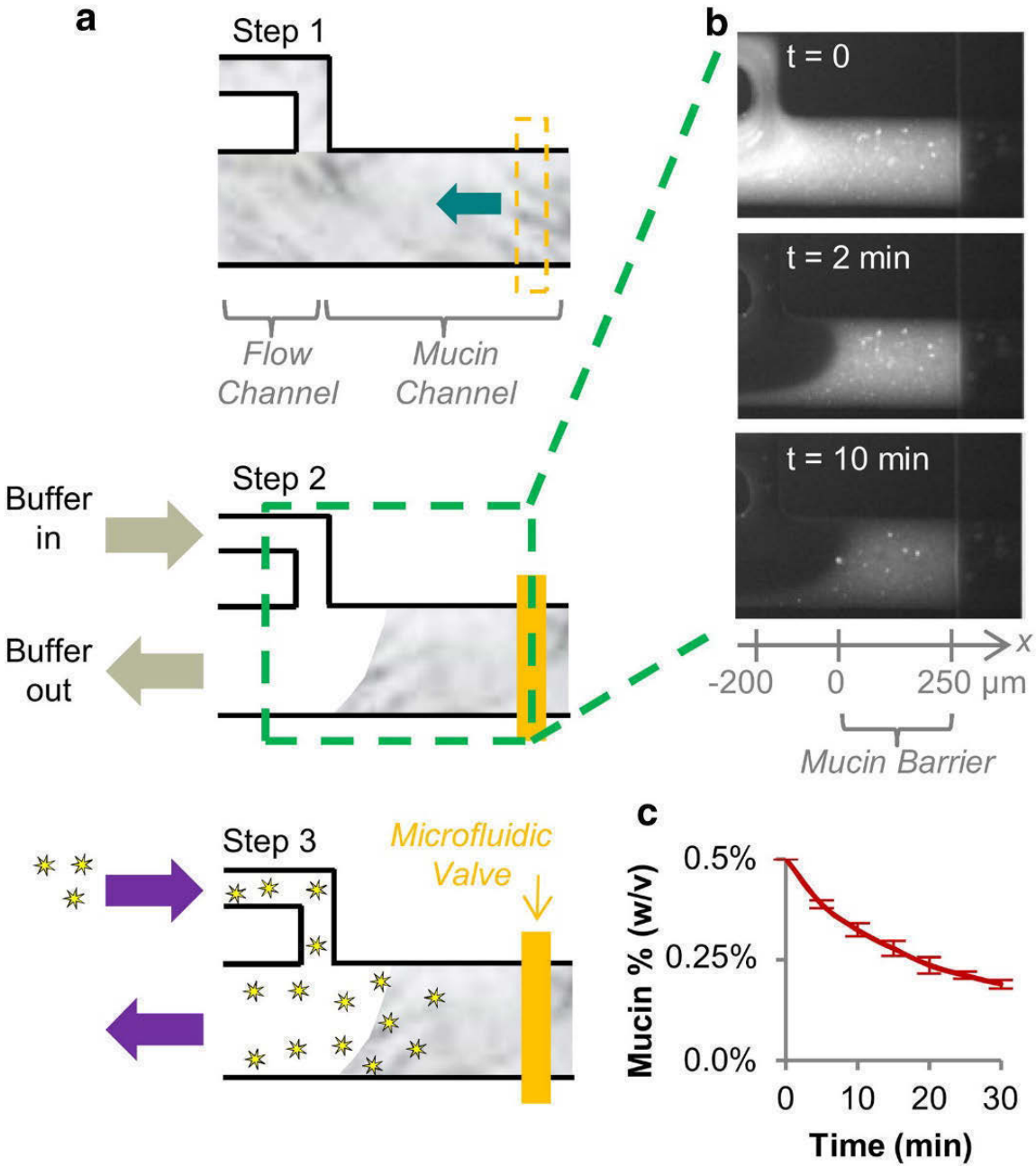


Fig. 1 Microfluidic device enables mucin barrier formation on-chip.

(a) A mucin sample initially filling both the flow and mucin channels (step 1, *top-down view*) is shaped into a layer of fixed width between a buffer flow and a microfluidic valve inside the mucin channel (step 2). **Fluorescent peptides flushed into the device** arrive at the mucin barrier surface and transport into the mucin barrier over time. (step 3)

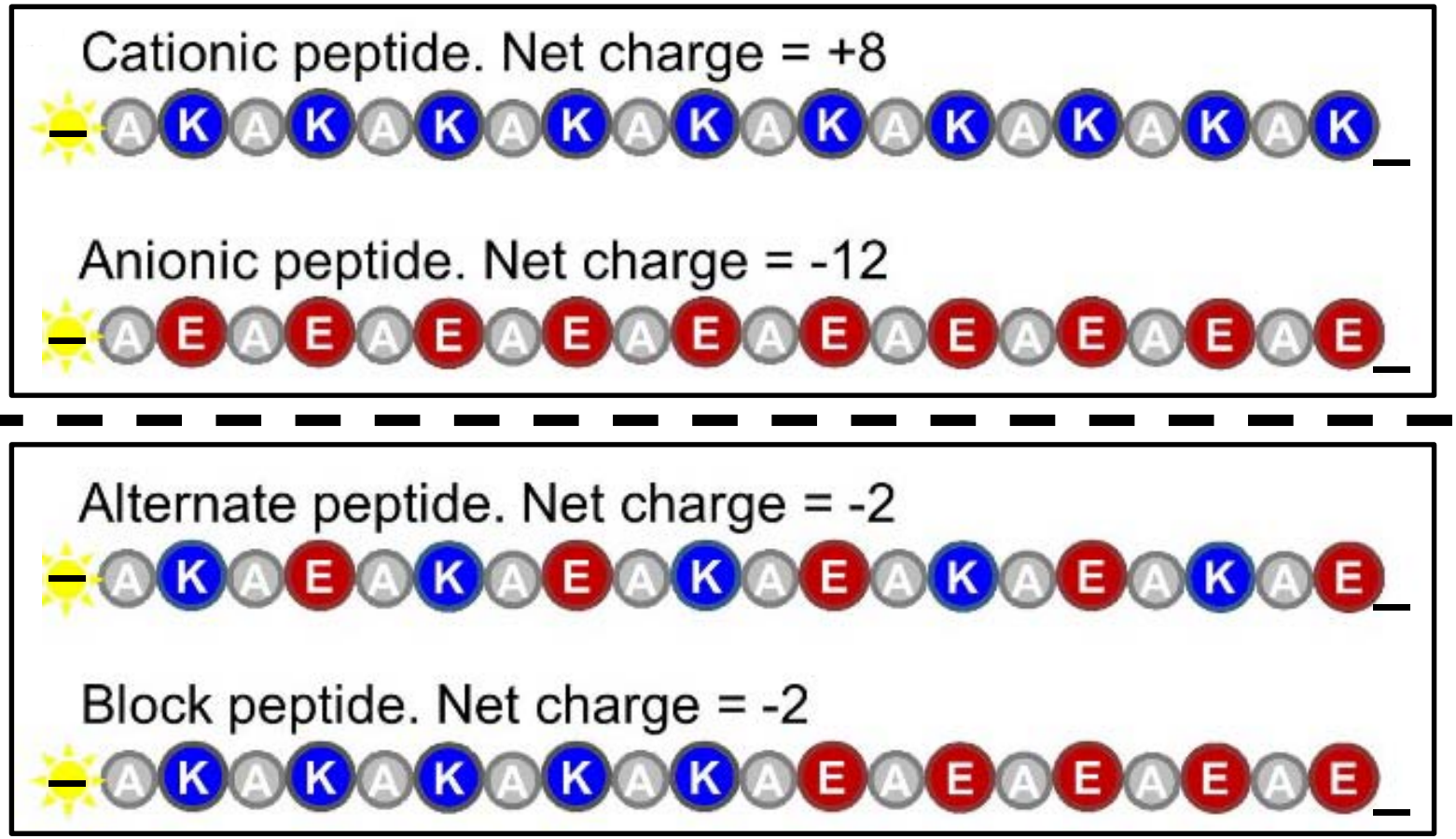
(b) Formation and stability of the mucin barrier on-chip is assessed using fluorescently labeled mucins, showing that the mucin barrier surface interface is stable over time.

(c) Mucins are gradually lost from the mucin barrier over time, likely due to surface fluid shearing. We limit the duration of permeability measurements to 10 min to ensure that a majority of the initial mucin quantity remains inside the mucin barrier during the experiment. n = 3 devices.

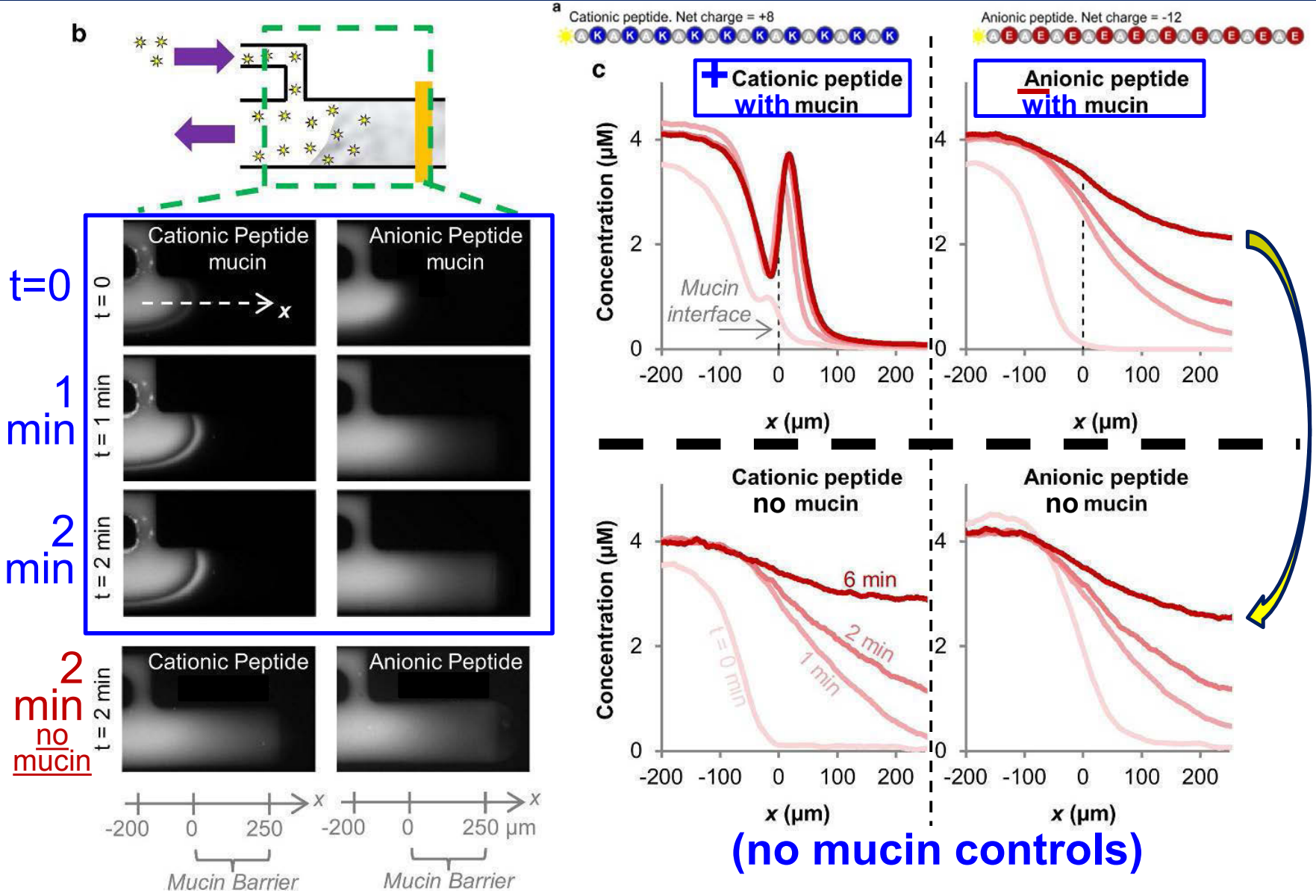
Peptide Solutes containing 20 amino acids:

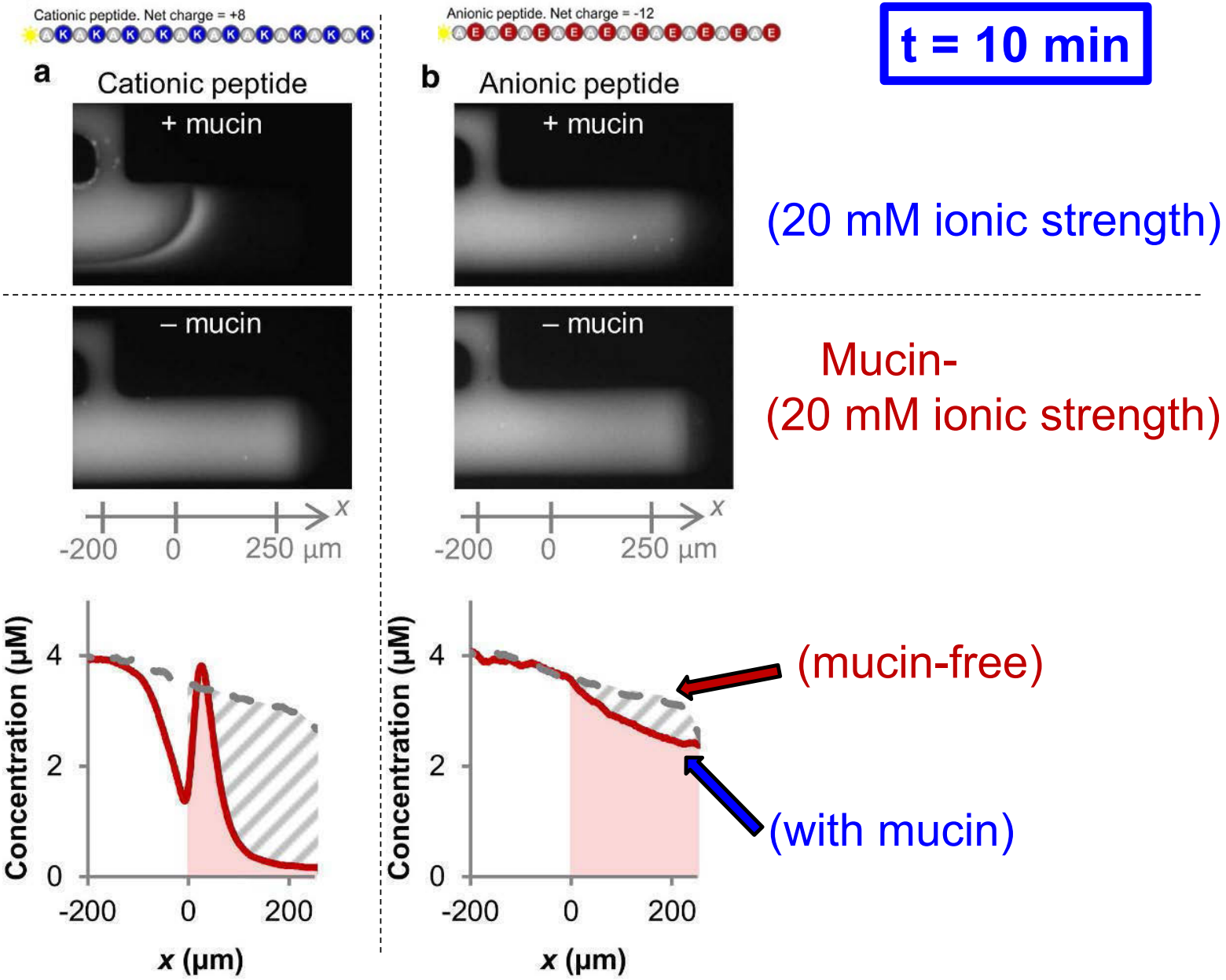
- K = lysine (10, +)
- E = glutamic acid (10, -)
- A = alanine (10, neutral)

Fluorescein tag
net charge = -1



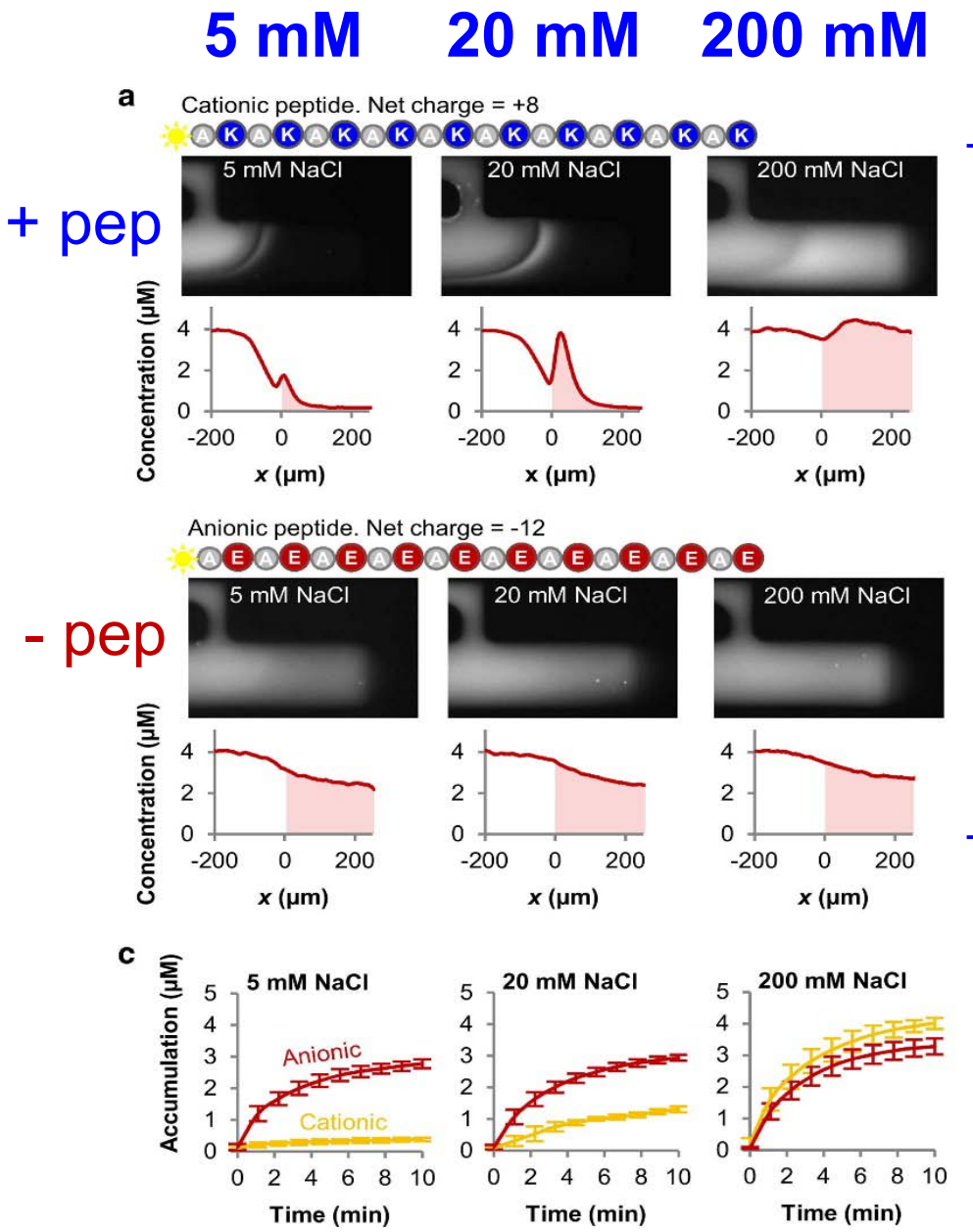
0.5% mucin in 20 mM NaCl / 20mM HEPES; peptides at 4 μM





Source: Li, Leon D. et al. "Spatial configuration and composition of charge modulates transport into a mucin hydrogel barrier." Biophysical Journal 105, no. 6 (2013): 1357-1365.

Effects of Ionic Strength: shields electrostatic binding interactions



t = 10 min

The ionic strength of the mucin barrier can regulate its selective properties.

- Increasing ionic strength within the mucin barrier significantly increases penetration and transport of the cationic (9-fold increase in accumulation from 5 to 200 mM ionic strength), but only marginally increases transport of the anionic peptide

20.430J / 2.795J / 6.561J / 10.539J Fields, Forces, and Flows in Biological Systems
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