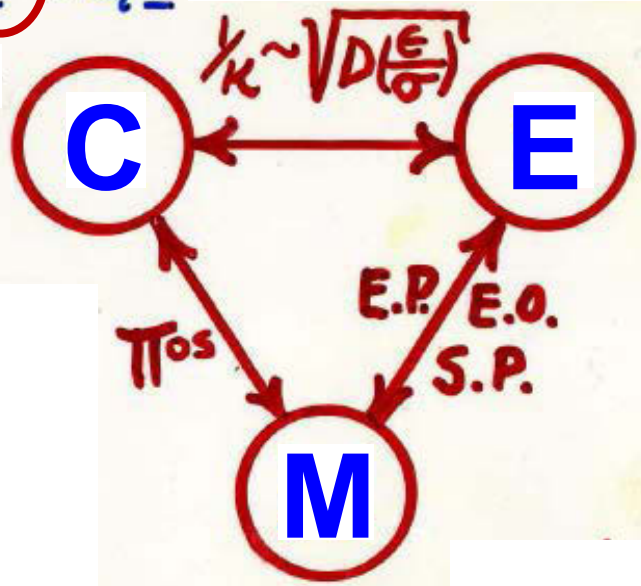
FFF: Complete Description of Coupled Transport and Biomolecular Interactions

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

$$\frac{\partial c_i}{\partial t} = -\nabla \cdot \underline{N}_i + R_{vi}$$

Diffusion-Reaction



$$\nabla \cdot \epsilon \underline{E} = \rho_e = \sum_i z_i F c_i$$

$$(\underline{E} = -\nabla \Phi)$$

$$\nabla \cdot \underline{J} = -\frac{\partial \rho_e}{\partial t}$$

$$\underline{J} = \sum_i z_i F \underline{N}_i$$

"E.Q.S."

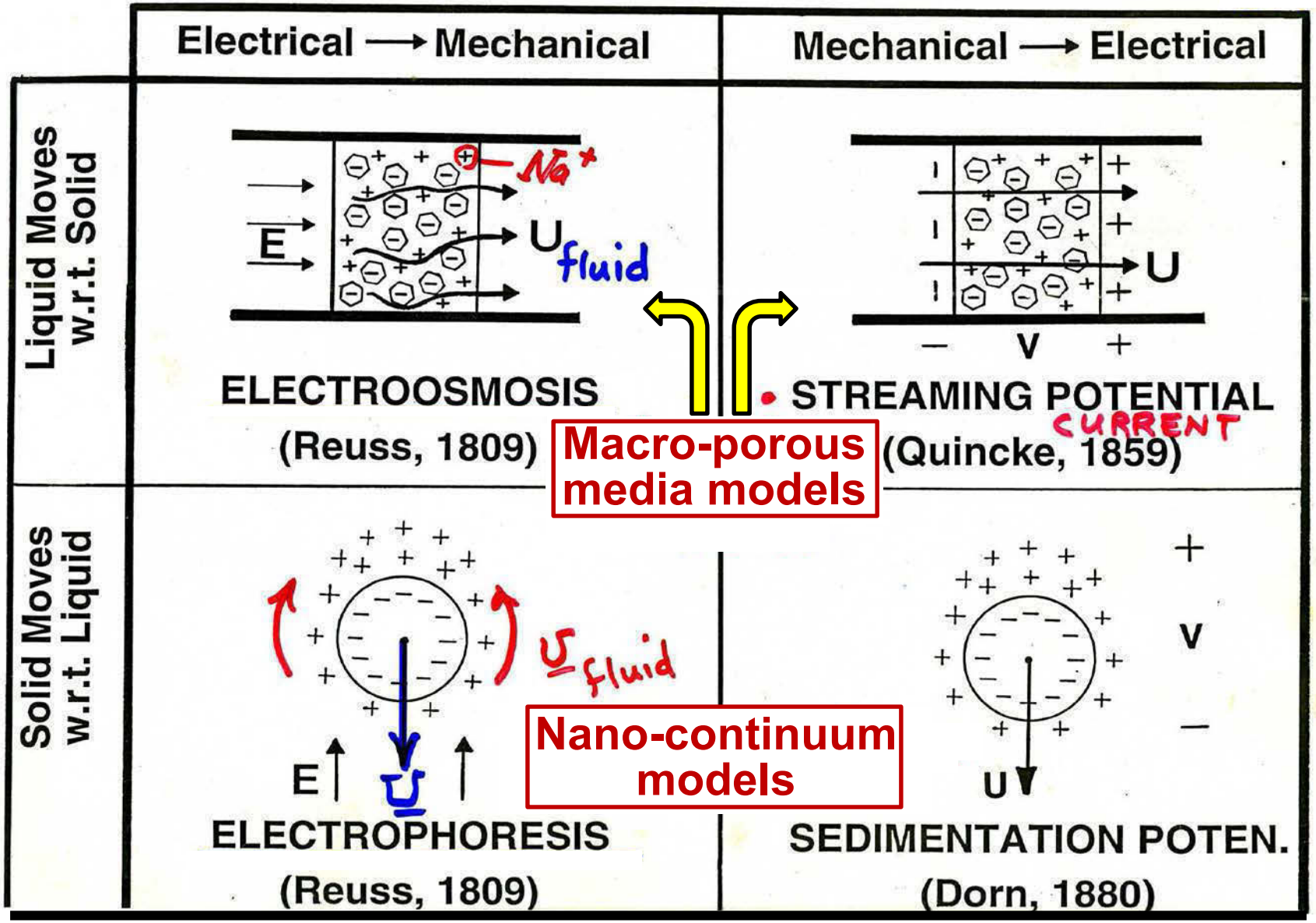
Navier Stokes

$$\rho \frac{D\underline{v}}{Dt} = -\nabla p + \mu \nabla^2 \underline{v} + \rho_e \underline{E} + \text{other}$$

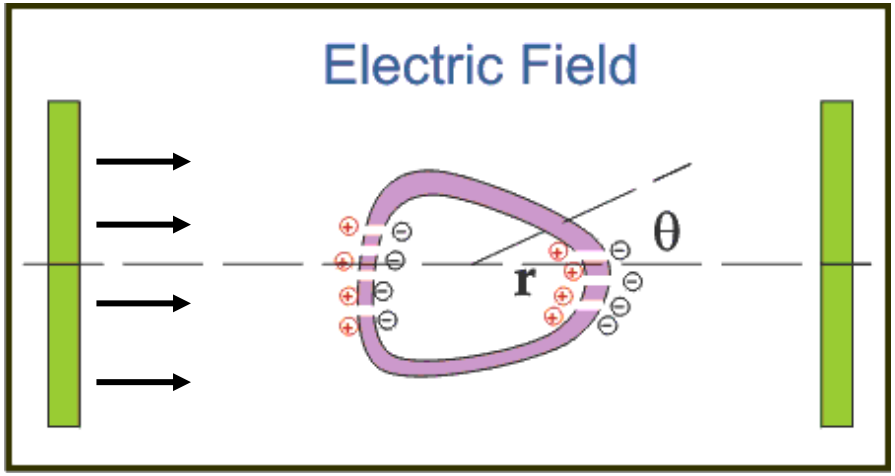
$$\nabla \cdot \underline{v} = 0 \quad \text{(incompressible fluid)}$$

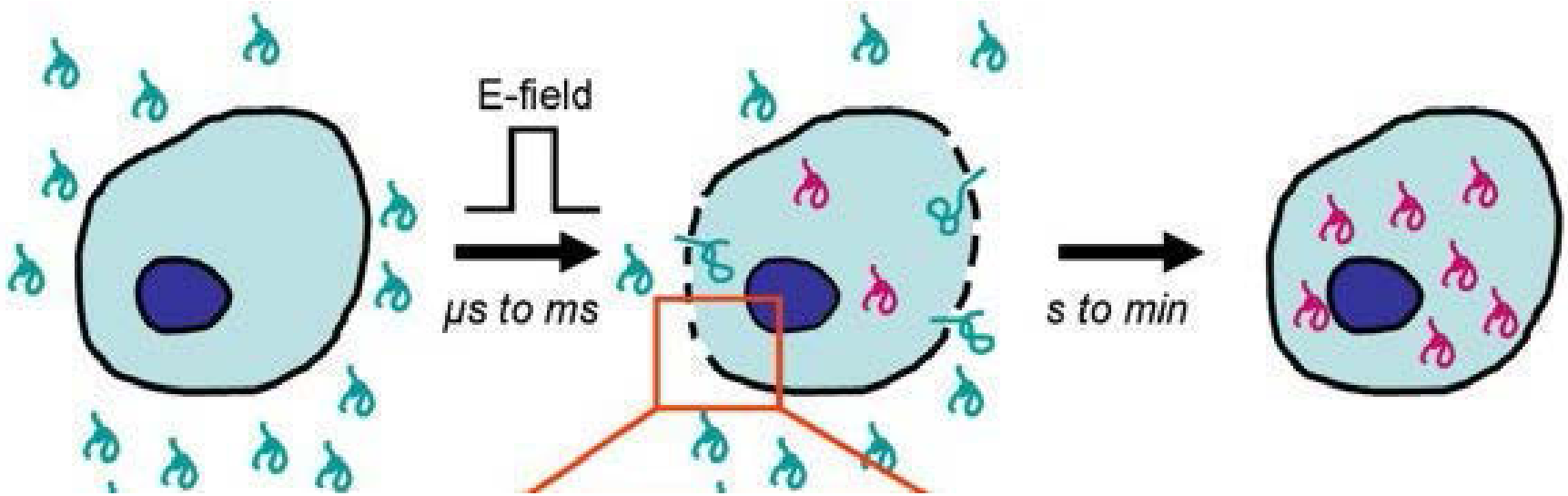
TODAY: start "fully coupled" examples

ELECTROKINETIC PHENOMENA



Electroporation: transient permeabilization of cell membrane for gene transfection/therapy; drug delivery; tumor treatment, and cell-based therapy





Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Electrokinetic transport through the nanopores in cell membrane during electroporation

2012

Saeid Movahed, Dongqing Li *

- It is shown that, in the presence of an **electric pulse**, electrokinetic effects (electroosmosis & electrophoresis) significantly influence ionic mass transfer through the nanopores, while the effect of diffusion is negligible....

- Increasing the pore radius intensifies the effect of convection (electroosmosis) on ionic flux compared to electrophoresis.

Journal of Colloid and Interface Science
www.elsevier.com/locate/jcis
2012

Electrokinetic transport through the nanopores in cell membrane during electroporation

Saeid Movahed, Dongqing Li *

The Nernst–Planck equation (Eq. (7)) is used to describe the mass transfer in the computation domain.

E.O. Diff E.P. 0

$$\nabla \cdot (\vec{u}[c_i]) - \nabla \cdot (D_i \vec{\nabla}[c_i]) - \nabla \cdot (z_i \mu_i [c_i] \nabla \phi) = 0 = \nabla \cdot N_i = -\partial c / \partial t \quad (7)$$

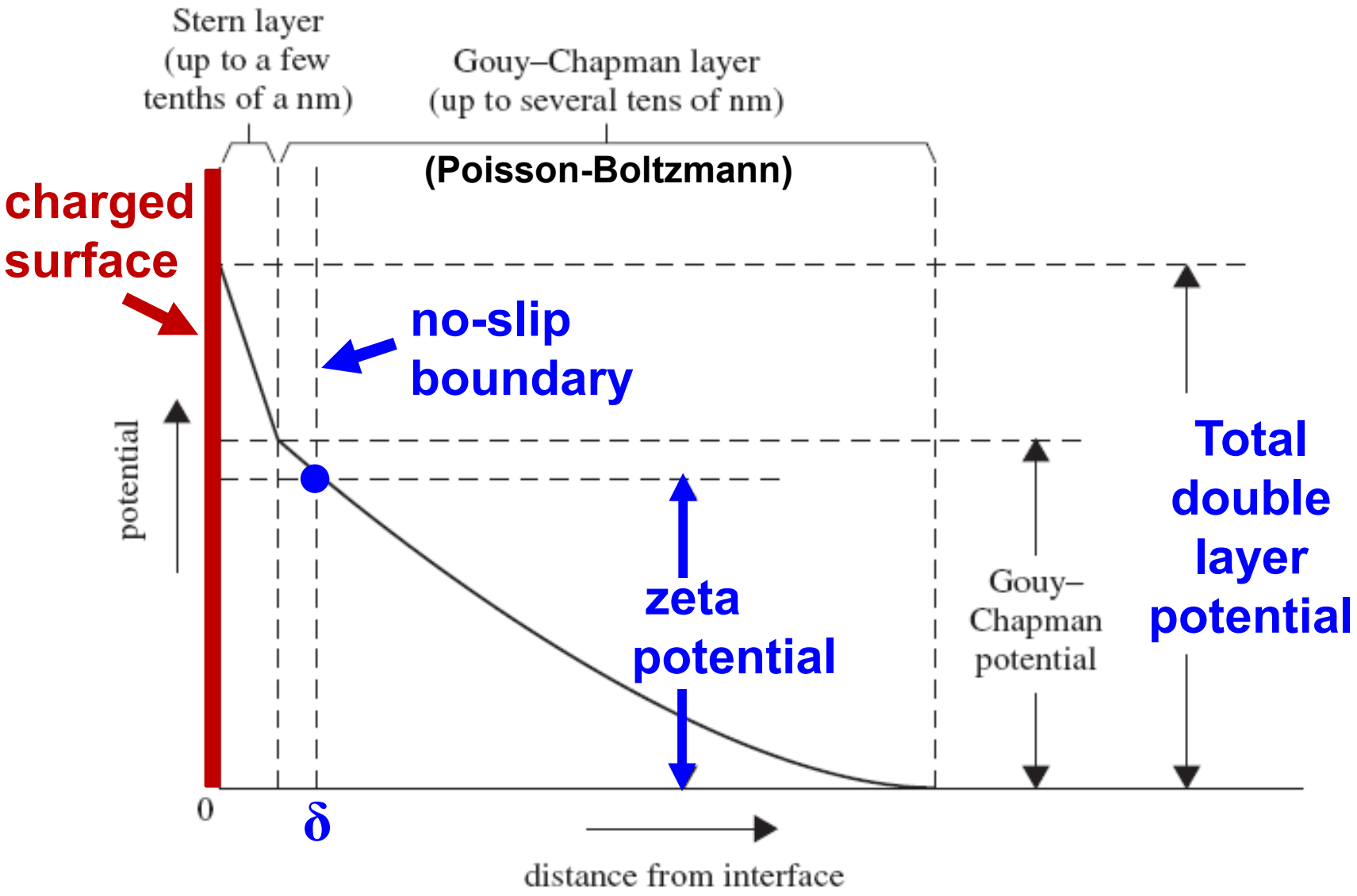
In this equation, the first term is the effect of electroosmosis (convection) on ionic mass transfer. The second and the third terms present the influences of diffusion and electrophoresis on ionic mass transfer, respectively.

$$v_z(r) = \left(\text{parabolic profile}\right)\frac{\Delta p}{L} + \left(\text{plug profile}\right)\frac{\Delta V}{L}$$

"E_{oz}"

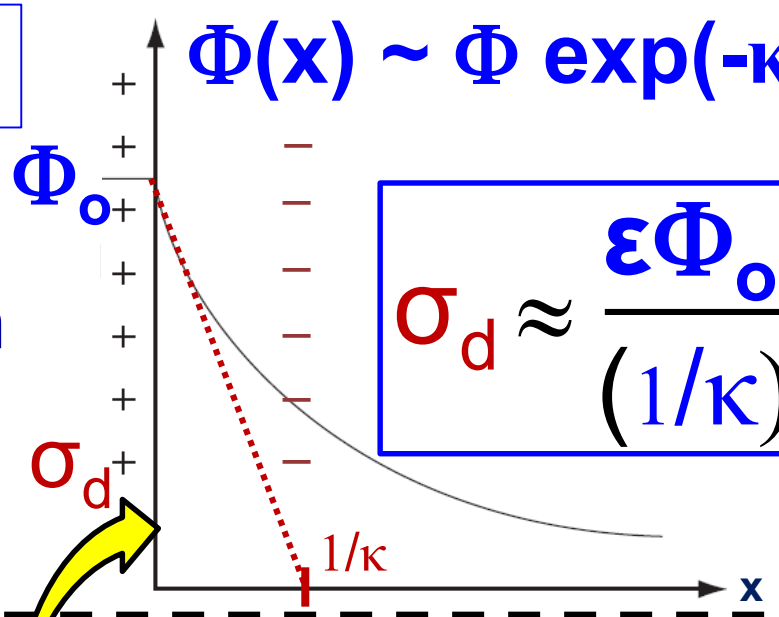
$$0 = -\nabla p + \mu \nabla^2 \underline{v} \qquad 0 = \mu \nabla^2 \underline{v} + \rho_e \underline{E}$$

Zeta Potential versus Total Double Layer Potential



Double Layer Models

(1) Poisson-Boltzmann
(expon.-like decay)



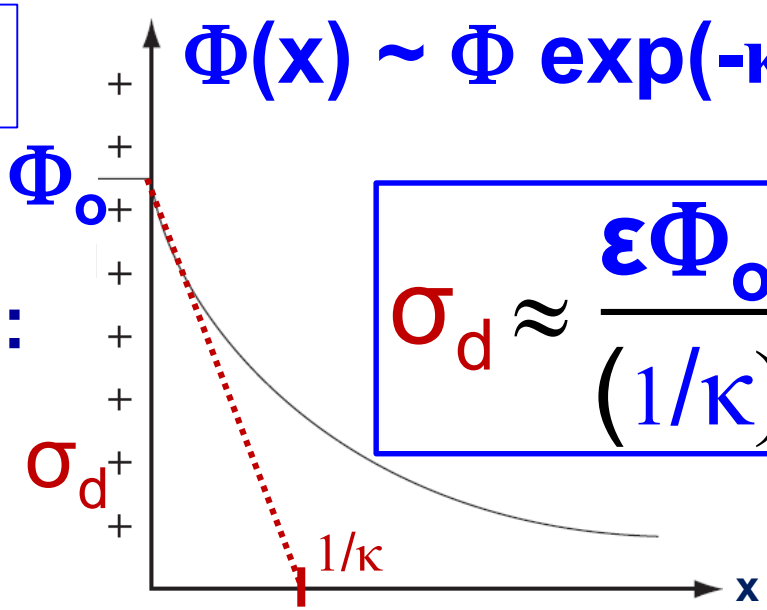
$$\sigma_d \approx \frac{\varepsilon \Phi_o}{(1/\kappa)}$$

B.C. at x = 0:

$$\sigma_d = -\epsilon \left. \frac{\partial \Phi(x)}{\partial x} \right|_{x=0}$$

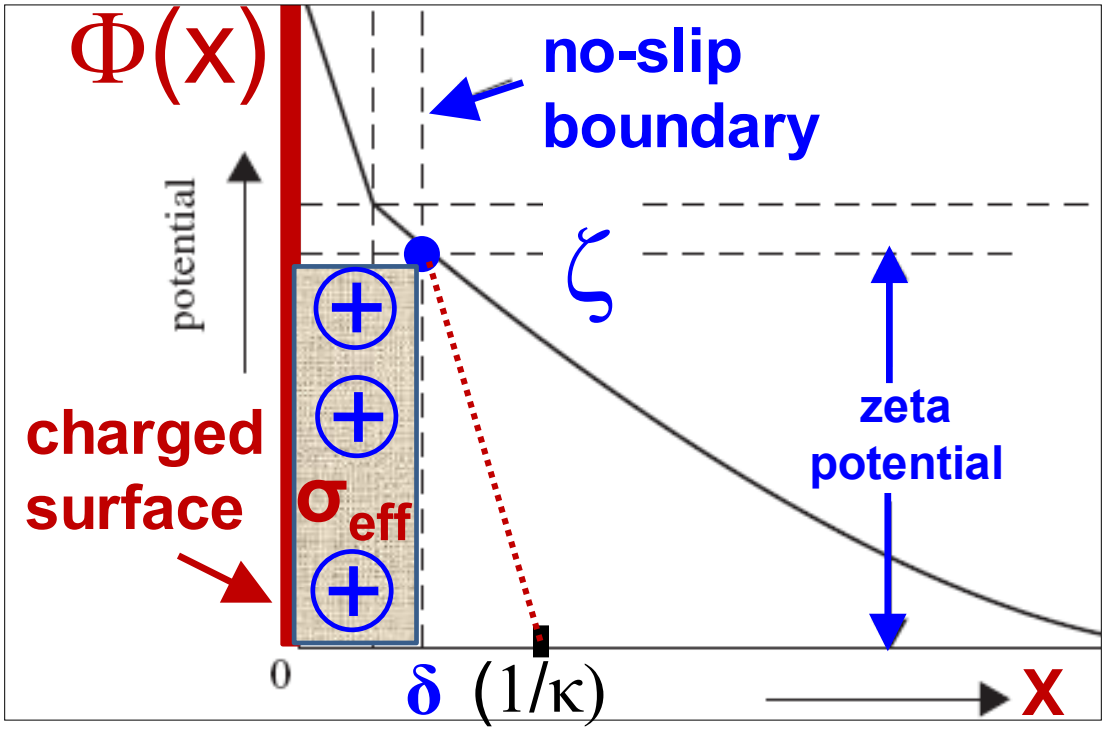
Double Layer Models

$\Phi(x) \sim \Phi \exp(-\kappa x)$



(1) Poisson-Boltzmann:

$$\sigma_d \approx \frac{\varepsilon \Phi_o}{(1/\kappa)}$$



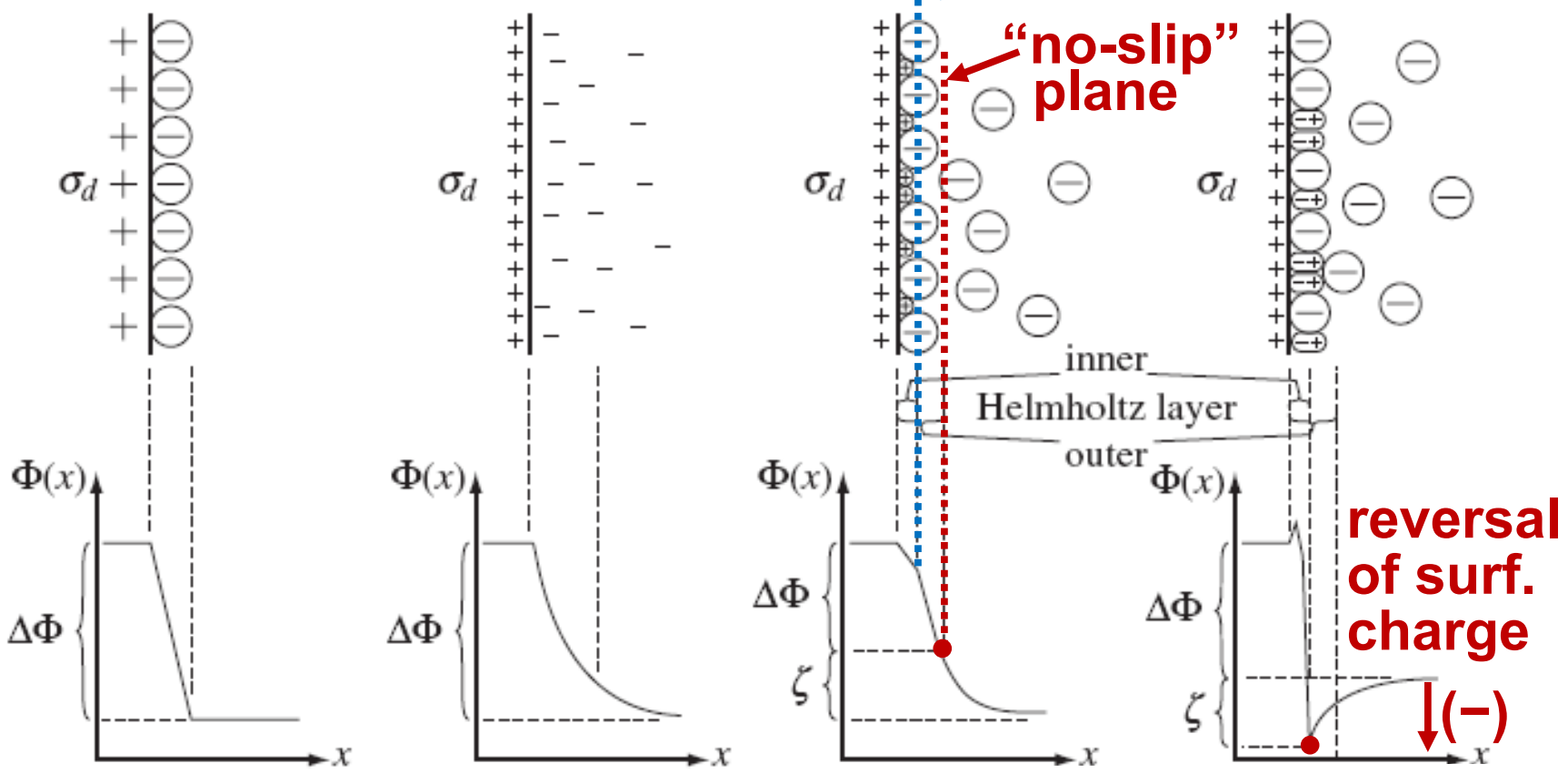
(2) Electrokinetics:

$$\sigma_d \rightarrow \text{"}\sigma_{eff}\text{"} \approx \frac{\varepsilon \zeta}{(1/\kappa)}$$

Infer

Measure

Models of Elec. Double Layer



"Helmholtz" double layer P6.1; analytical model

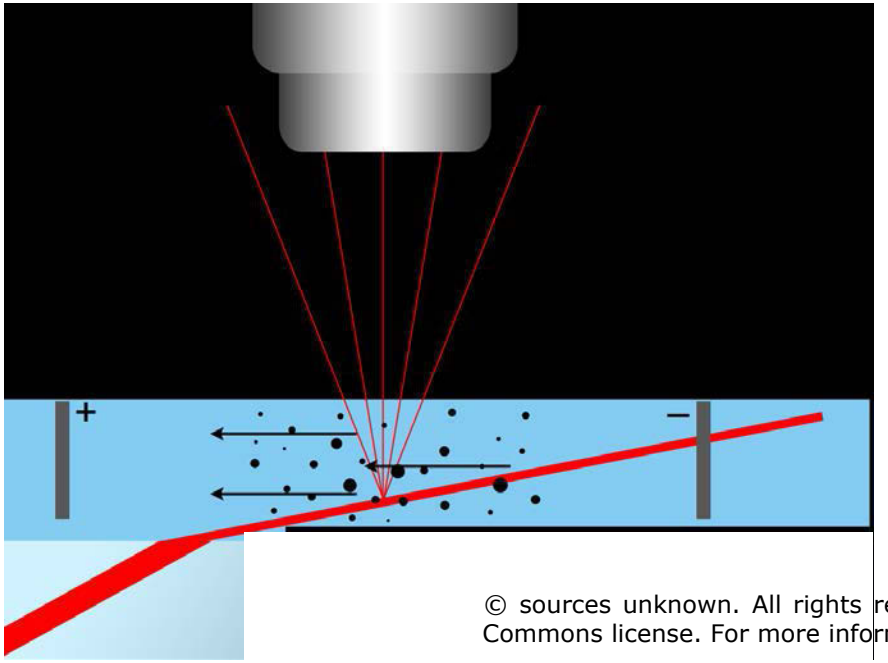
"Diffuse" P-B double layer model

with ion adsorption; ζ potential

with molec. adsorption; ζ potential

Electrokinetics

Zeta Potential (particle charge) Instruments



Measure ζ → infer "σ_{eff}"

Electrophoresis: Techniques & Methods

I. "Free Electrophoresis" (i.e., in free solution)

- 1930's "Moving Boundary" (Tiselius)
- 1970's "Micro-electrophoresis" (cells, etc.)
- 1980's "Capillary Electrophoresis"
- 1990's MEMS; channels; (E.O. & E.P)
- 2000+ NEMS

II. "Zone E.P. (w. medium to suppress convection)

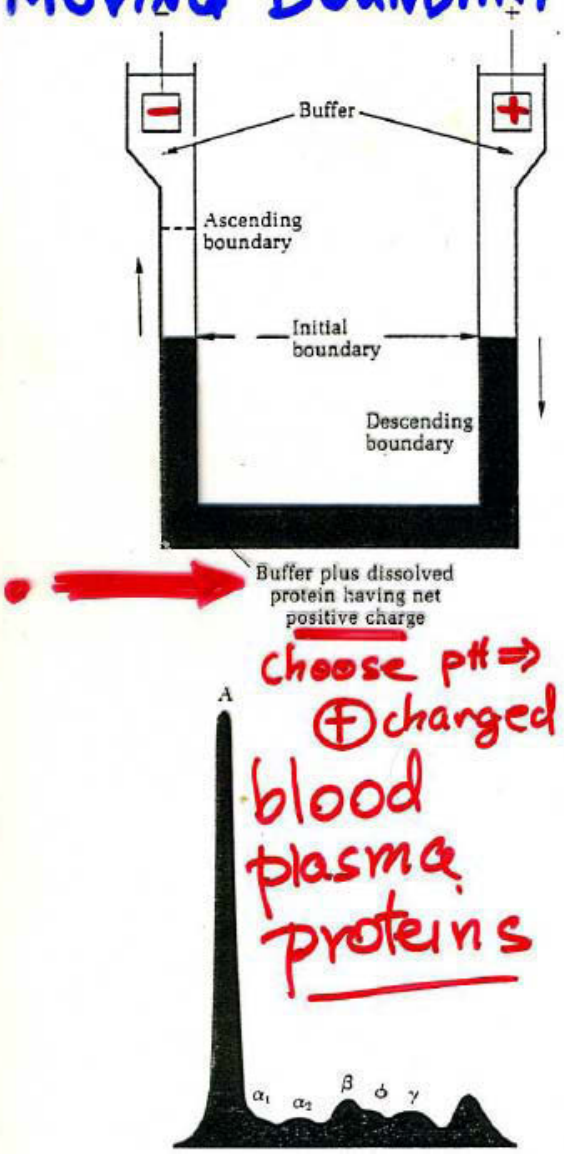
- paper; gel (SDS-PAGE)
- 1980's "Capillary "zone" (gel in pore)
- 1990's MEMS channels (with gel in channels)
- 1980s to 1990s: "Pulsed Field"; "Rotary Field"
- 2000+ Lab-on-a Chip

Tiselius, 1931

in Lehninger, Biochemistry

various globulins.

"MOVING BOUNDARY"



Electrophoresis

It is possible to separate mixtures of globular proteins in solution on the basis of their different rates of migration in an electrical field at a given pH. Such separations were first carried out in a refined way by Arne Tiselius in 1937. The electrical mobility μ of a molecule is given by the ratio of the velocity of migration v to the field strength E in cm² per volt-sec:

mobility $\mu = \frac{v}{E} \cong \frac{\Delta v}{E_0}$

For small ions, such as chloride, μ is between 4 and 9×10^{-4} cm² per volt-sec (25°C). For proteins, it is about 0.1 to 1.0×10^{-4} cm² per volt-sec at 25°C. Proteins therefore migrate much more slowly in an electrical field than do simple ions such as Na^+ or Cl^-; in general, they have a smaller ratio of charge to mass.

There are two general methods by which electrophoresis of protein mixtures is carried out. In free, or moving boundary, electrophoresis, a buffered solution of the protein mixture is placed in a U-shaped observation cell and columns of pure buffer are carefully layered over the protein solution (Figure 7-2). An electrical field is applied at constant temperature and under vibration-free conditions. The pH of the buffer is chosen to yield maximum separation of the proteins present. As each protein migrates from the solution into the zone of protein-free buffer, a front, or boundary, is formed and moves to the electrode. The refractive index of the solution changes sharply at this boundary because the protein molecules have an index of refraction different from that of the pure buffer alone. From measurements of the refractive-index changes along the cell, which are made by an optical technique called the schlieren method, electrophoretic patterns are constructed which show the direction and relative rate of migration of each protein species (Figure 7-2). Each peak in the pattern corresponds to the position of the moving boundary of a

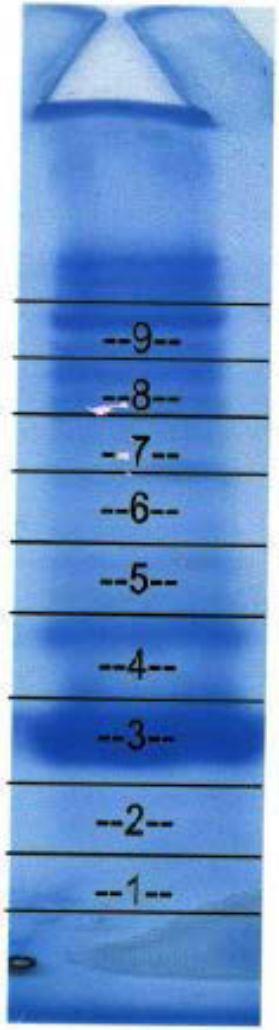
HARD

SDS-PAGE of the Medium

IL-1 = interleukin-1
TNFα = tumor necrosis factor α

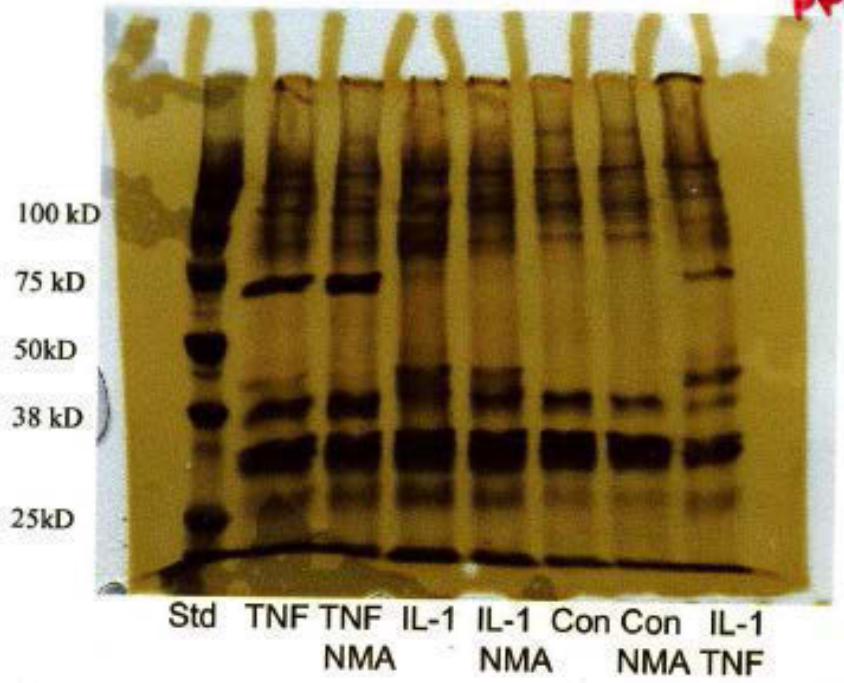
cytokines involved in inflammation → upregulate Proteases

(C.V. M.S. disease)



CONTROL

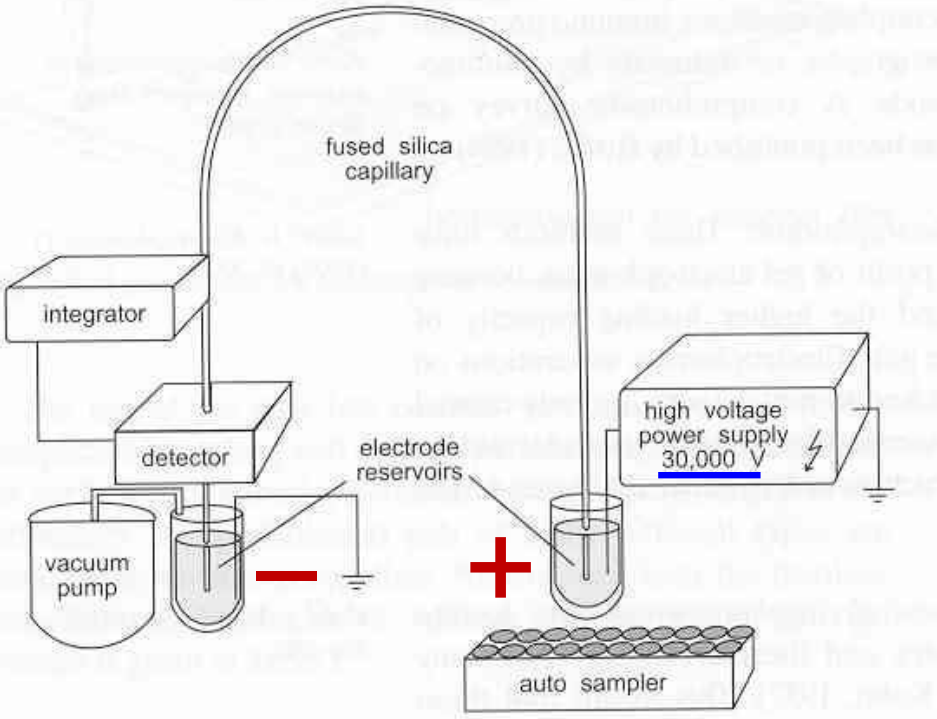
IL-1β



The pictures on the left represent the gels taken and The slices made for protein identification by mass Spectrometry. The gel above is a 1DE comparison Of medium taken from each of the treatment groups

Capillary Electrophoresis

← $E_{oz} \sim 10^5$ V/m



Electrophoresis w.r.t. moving fluid driven by electroosmosis

PAPER

E- flow thru porous media

Integrated polymer chip for two-dimensional capillary gel electrophoresis

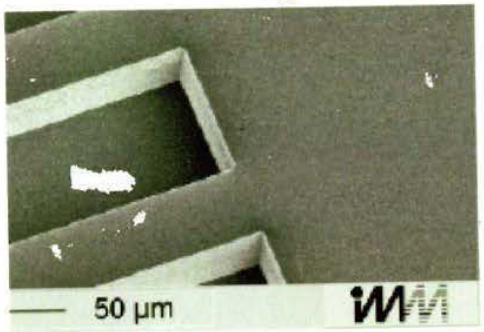
2004

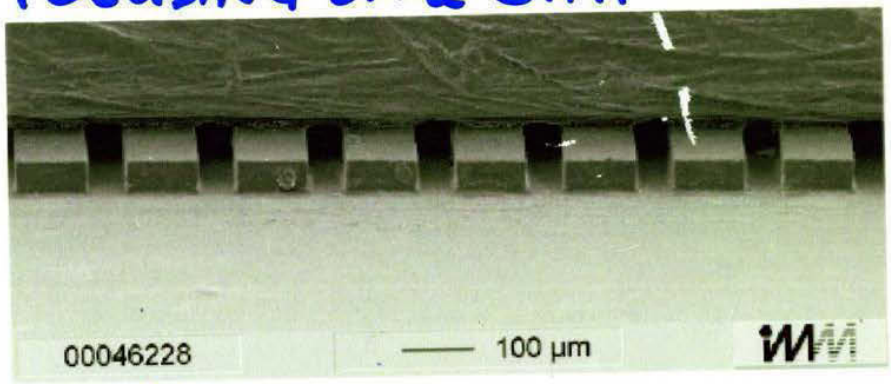
Anja Griebel, Sabine Rund†, Friedhelm Schönfeld, Wolfgang Dörner‡, Renate Konrad§ and Steffen Hardt*

Fluidics & Simulation Department, Institut für Mikrotechnik Mainz GmbH – IMM, Carl-Zeiss-Str. 18-20, 55129 Mainz, Germany. E-mail: hardt@imm-mainz.de; Fax: +49 (0) 6131 990 205

www.rsc.org/loc

"ISOELECTRIC FOCUSING on a CHIP"





h microstructures. A silicon structure

40 mm

⊕ pH 4

pH 9 ⊖

Fig. 5 IEF of Alexa 488 labeled myoglobin. The IPG strip (pH 4–9) was rehydrated for 17 h at room temperature with 5 µg protein in 140 µl

radius of a coiled molecule replaces the molecular weight. Once a gel system has been calibrated. V_h can be obtained. and then it can be combined with sedimentation data to yield the true molecular weight, just as described earlier for gel partition chromatography.

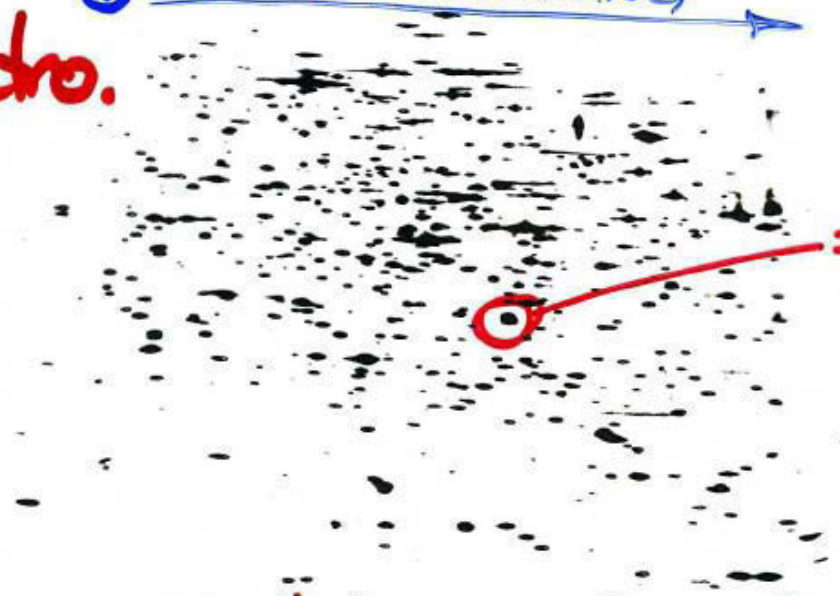
The major advantage of gel electrophoresis or chromatography is that very sharp bands are maintained. because there is no external volume to allow for rapid diffusion. The separation power of SDS electrophoresis is based totally on molecular weight, and that of isoelectric focusing is based totally on charge. It is logical that, if the two could be combined into a single separation scheme. its resolution should be quite impressive. This can be done by two-dimensional electrophoresis. in which one dimension is SDS and the other is isoelectric focusing. Figure 12-19 shows an example of this from the work of Patrick O'Farrell. More than 1.000 different proteins from *E. coli* can be distinguished in a single analysis.

O'Farrell JBC, 1975

2-D Gel Electro.

① ISOELECTRIC FOCUSING

② SDS-PAGE



= X ?

Figure 12-19 can display several 1000's proteins

Two-dimensional SDS–isoelectric focusing gel electrophoresis. First the sample is run in a one-dimensional pH gradient gel (isoelectric focusing). Then this gel is placed alongside a square SDS gel, into which the sample moves at right angles to its motion in the first gel. The sample shown is total *E. coli* protein; it is visualized by autoradiography. For details of the technique, see P. O'Farrell, *J. Biol. Chem.* 250:4007 (1975). [Autoradiograph courtesy of Patrick O'Farrell.]

⇒ proteomics . . . .

Physical Biochemistry

D. Freifelder, Freeman, 1982

Chap 9 Electrophoresis

Theory of Electrophoresis

The detailed theory of electrophoresis is highly complicated and at present incomplete; a simple description of the electrophoretic principle is sufficient for an understanding of how the technique is used for most purposes.

In many ways, electrophoresis is like sedimentation (Chapter 11): a force is applied and countered by viscous drag. If a particle with charge q, suspended in an insulating medium, is in an electric field, E, the particle will move at a constant velocity, v, determined by the balance between the electrical force, Eq, and the viscous drag, fv, in which f is the frictional coefficient, that is,

$$Eq = fv \qquad (1)$$

ouch!

276

To date, the theory of electrophoresis has failed to account adequately for these complications, as well as several others, so that electrophoresis has not turned out to be very useful in supplying *detailed* information about macromolecular structure. It is, however, enormously useful as both an

Protein Purification: Principles and Practice

R. Scopes, Springer Verlag, 1982

"POINT CHARGE MODEL"

Electrophoretic Principles

A protein molecule in solution at any pH other than its isoelectric point has a net average charge. This causes it to move in an applied electric field. The force is given by E, the electric field ($V\ m^{-1}$) times z, the net number of charges on the molecule. This force is opposed by viscous forces in the medium (just as in centrifugation; cf. section 1.2), proportional to the viscosity η, particle radius r (Stokes radius), and the velocity v, in a steady state:

$$Ez = 6\pi\eta rv \qquad (5.1)$$

NO WAY!

The specific mobility $u = v/E$ is given by

$$u = \frac{z}{6\pi\eta r} \qquad (5.2)$$

It is sometimes stated that electrophoresis in free solution separates molecules according to charge alone, independent of size. But from Eq. (5.2) it can be seen that the mobility u is inversely related to the Stokes radius. So a spher-

(ouch)

Methods in Molecular Biophysics:

Serdyuk, Zaccai and Zaccai, Cambridge Univ Press, 2007

Sec D.5.4.1 Charge and Electrophoretic Mobility

ation coefficient, μ is the ratio of the particle's velocity to the strength
ving field (compare Eq. (D4.16)). If the particle happens to be spherical,
we can write

$$\mu = Ze/6\pi\eta_0 R_0 \qquad \text{(D5.7)}$$

, is the particle's radius and η_0 is the viscosity of the solvent.
nciple, Eq. (D5.7) could be generalised to take into account hydration
ations from a spherical shape (as in the case of sedimentation and dif-
However, for a charged particle undergoing steady-state motion in a
electric field, it is also necessary to account for the distortion of the
sphere from its equilibrium state (Comment D5.2). This 'ion relaxation'
important unless the macromolecule is weakly charged. The transport
ged particle is a substantially more difficult problem than the transport
charged particle. Until recently, the classical theory was applied to only
lest of model structures.

Textbook cover removed due to copyright restrictions.
Source: Waigh, Thomas Andrew. The Physics of Living Processes: A Mesoscopic Approach. John Wiley & Sons, 2014.

2014

Dr. Thomas Waigh

School of Physics and Astronomy

Photon Science Institute

University of Manchester, UK

19.14 Electrophoresis

Electrophoresis is a cheap, powerful tool for the analysis and separation of charged biological molecules such as proteins and nucleic acids (**Section 2.12**). Electrophoresis can be used to measure the size of biopolymer molecules and deduce the chemical sequence of the chains. The force experienced by a particle (F) in an electric field (E) is given by Coulomb's law,

$$F = ZeE \tag{19.91}$$

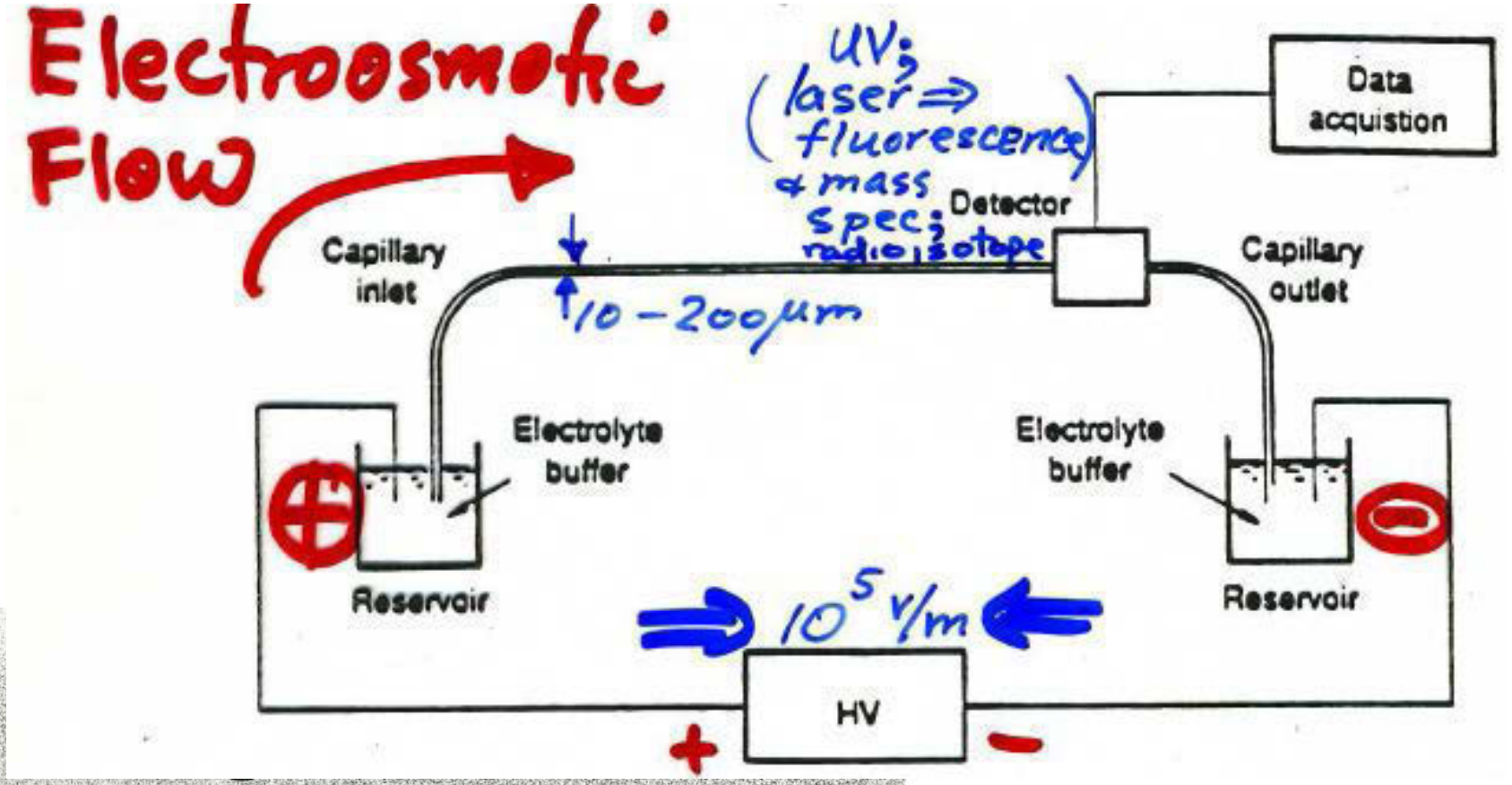
where Z is the number of charges on the particle, and e is the electronic charge. The mobility of a charged particle in an electric field is proportional to the ratio of the net charge on the particle (which provides a Coulombic force) to its frictional coefficient. Electrophoresis can be used to obtain information about either the relative charge or the relative size of charged molecules. For steady-state electrophoretic motion the frictional force (the frictional coefficient (μ) multiplied by the velocity (v), μv) is balanced by the force due to the electric field. The electrophoretic mobility (U) with colloids is defined as

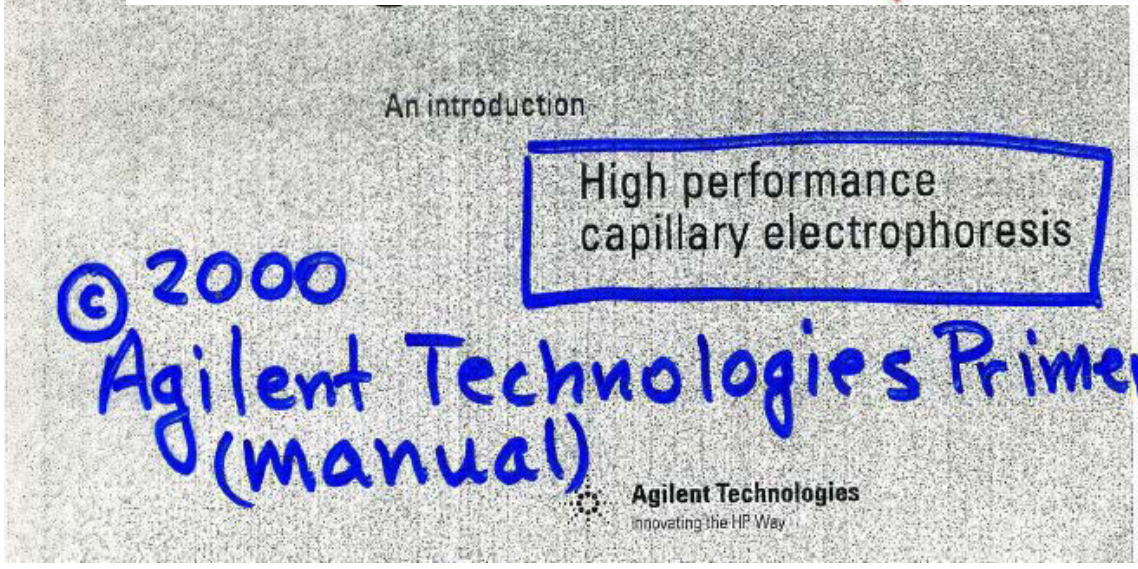
$$U = \frac{v}{E} = \frac{Ze}{\mu} \tag{19.92}$$

The Stokes law for the frictional force (equation (7.11)) can be inserted into this equation to give

$$U = \frac{Ze}{6\pi\eta R} \tag{19.93}$$

"Thus, ……the mobility can be related to the charge (Z) and the radius (R)."





1980's

Page 20

The magnitude of the EOF can be expressed in terms of velocity or mobility by

$$v_{EOF} = (\varepsilon \zeta / \eta) E \quad (7)$$

or

$$\mu_{EOF} = (\varepsilon \zeta / \eta) \quad (8)$$

correct

where:
- v_{EOF} = velocity
- μ_{EOF} = EOF "mobility"
- ζ = zeta potential
- ε = dielectric constant.

(note the independence of mobility on applied electric field)

The zeta potential is essentially determined by the surface charge on the capillary wall. Since this charge is strongly pH dependent, the magnitude of the EOF varies with pH. At high pH, where the silanol groups are predominantly deprotonated, the EOF is significantly greater than at low pH where they become protonated. Depending on the

Electrophoresis:

that ion. The mobility is determined by the electric force that the molecule experiences, balanced by its frictional drag through the medium. That is

$$\mu_e \alpha = \frac{\text{Electric force } (F_E)}{\text{Frictional force } (F_F)} \qquad (2)$$

The electric force can be given by

$$F_E = q\,E \qquad (3)$$

Electrophoresis:

that ion. The mobility is determined by the electric force that the molecule experiences, balanced by its frictional drag through the medium. That is

$$\mu_e \propto = \frac{\text{Electric force } (F_E)}{\text{Frictional force } (F_F)} \qquad (2)$$

The electric force can be given by

$$F_E = q\,E \qquad (3)$$

and the frictional force (for a spherical ion) by

$$F_F = -6\,\pi\,\eta\,r\,v \qquad (4)$$

where q = ion charge
η = solution viscosity
r = ion radius
v = ion velocity.

During electrophoresis a steady state, defined by the balance of these forces, is attained. At this point the forces are equal but opposite and

$$q\,E = 6\,\pi\,\eta\,r\,v \qquad (5)$$

Page 676

Biophysical Chemistry

Cantor and Schimmel, Freeman, 1980

12-4 ELECTROPHORESIS

If a macromolecule has a net charge q, then application of an electric field $\mathbf{E}$ will result in an applied force $\mathbf{F} = q\mathbf{E}$. This force will cause acceleration of the particle in a fluid until a steady-state velocity $\mathbf{v}$ is reached. At this velocity, frictional forces are equal and opposite to the applied force, so

$$\mathbf{v} = q\mathbf{E}/f \qquad (12\text{-}67)$$

uh oh...

For a spherical macromolecule of radius a with a charge equal to ze (where e is the charge on the electron), we have

$$\mathbf{v} = ze\mathbf{E}/6\pi\eta a \qquad (12\text{-}68)$$

not again....?

If the electrical field originates from parallel plates or the equivalent, the molecule travels in a straight line. By analogy with the definition of the sedimentation coefficient, the mobility u can be defined as the velocity per unit field, $u = \mathbf{v}/\mathbf{E}$.

Unfortunately, this description of electrophoresis (the transport of charged particles in the presence of an electric field) is completely inadequate. An immediate difficulty arises over what to call the net charge on a macromolecule.

!!!

Biophysical Chemistry

Cantor and Schimmel, Freeman, 1980

..... for a spherical molecule with radius a of

$$u = (ze/6\pi\eta a)X_1(\kappa a)/(1 + \kappa a) \qquad (12\text{-}73)$$

additional analysis . . .

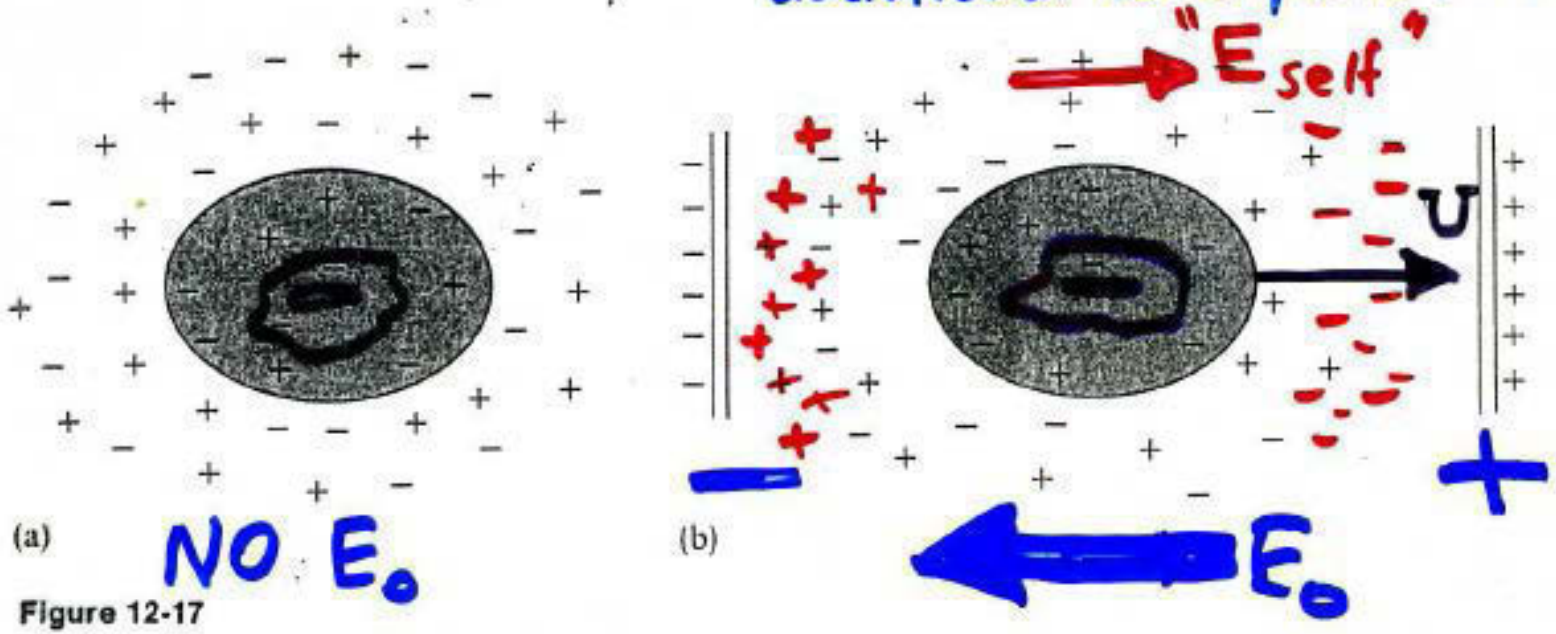


Figure 12-17

A protein with a net negative charge, and its counterion atmosphere. **(a)** In the absence of an applied electric field. **(b)** While the molecule is being transported by the applied electric field shown. Note the distortion of the ion atmosphere. A change in the charge distribution of the protein itself also is shown; this is not a necessary consequence of an applied field, but it certainly is a realistic possibility.

Biophysical Chemistry

Cantor and Schimmel, Freeman, 1980

$$u = (ze/6\pi\eta a)X_1(\kappa a)/(1 + \kappa a) \qquad (12\text{-}73)$$

where $X_1(\kappa a)$ is a function that has been tabulated by D. C. Henry (see Rice and Nagasawa, 1961). However, the applied field—and the resulting motions of the macromolecule and small ions—distorts the ion atmosphere (Fig. 12-17). This distortion alters the potential given by Equation 12-70. To calculate all of these effects is a formidable problem. The result is an equation with the same leading term as Equation 12-73, but with many additional terms. The most satisfactory treatment of this problem was worked out by F. Booth (see Rice and Nagasawa, 1961, for a detailed discussion). The simple theory of Equation 12-73 predicts that mobilities will increase linearly with increasing charge on the macromolecule. The more complete theories show that, at sufficiently high charges, the mobility is less than that expected from Equation 12-73 and can even start to decrease as z increases.

As complicated as all this is, the Booth theory does not adequately explain observed macromolecular electrophoresis results. It holds only for spheres of uniform charge density, and this model is a very poor representation of a protein or nucleic acid. Just imagine an ellipsoid with an asymmetric charge distribution. The electric field will apply torques as well as net displacement. There will be preferred orientation, and the motion of the macromolecule no longer can be described by a rotationally averaged frictional coefficient. Furthermore, all of these effects will couple into the distortion of the ion atmosphere.

(uniform double layer)

LEVICH model (& others)

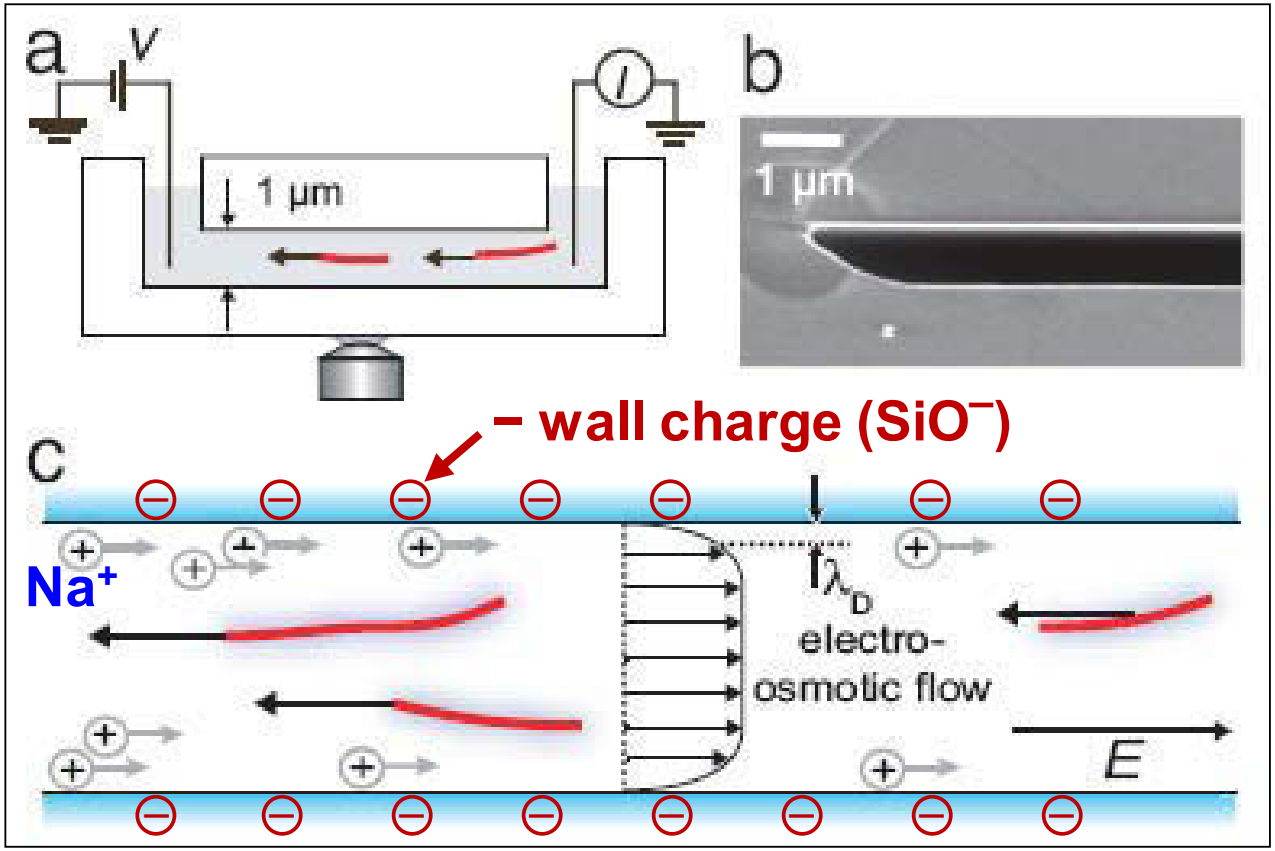
BUT

Electrophoresis of individual microtubules in microchannels

PNAS 2007

M. G. L. van den Heuvel, M. P. de Graaff, S. G. Lemay, and C. Dekker*

1980's huge bench top instrument becomes a little chip:



Electrophoresis of individual microtubules in microchannels

PNAS 2007

M. G. L. van den Heuvel, M. P. de Graaff, S. G. Lemay, and C. Dekker*

We use micrometer-sized fluidic channels to confine and measure electrophoresis of freely suspended individual microtubules. We measure orientation-dependent velocities of microtubules and the electro-osmotic flow mobility in our channels to infer the anisotropic electrophoretic mobility of microtubules under physiological conditions. We discuss the difference between electrophoresis and purely hydrodynamic motion and its implications for interpreting mobility measurements. We show that the mobility anisotropy is a factor of 0.83, clearly different from the well known anisotropy factor of 0.5 in Stokes drag coefficients for cylindrical objects. We also show that the velocity is independent of microtubule length, which would be different for hydrodynamic motion. We demonstrate that the electric force on the counterions has important consequences for the interpretation of electrophoresis experiments and that ignoring this can lead to an underestimation of the effective charge by orders of magnitude. From the electrophoresis measurements, we calculate an effective surface-charge density of -36.7 ± 0.4 mC/m^2 for microtubules. Electrophoretic measurements of subtilisin-digested microtubules, which have the negatively charged C termini on the outer surface removed, show a 24% decrease in mobility and, correspondingly, in surface charge, but no change in anisotropy.

σ_d

in conclusions → these results are often neglected in recent exp.."

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