Lecture 1: Walter Boron - Gas Channels

- Solubility theory
  \[ P = \frac{s_l}{s_w} \]
  Note: Henry's law is true at steady-state
- Solubility-Diffusion theory
- Access-Solubility-Diffusion-Egress theory
Lecture 2: Emad

Newtonian equations

Major limitation -> time scale (speed limit: 1 fs)

Force field approximations

Atomistic resolution

Implicit Ligand Sampling

\[ W(r) = -k_T \ln \left[ \frac{P(r)}{P_0} \right] \]

\[ F(z) = -RT \ln \sum e^{-F(x, y, z)}/RT \]

\[ \ldots \]
Lecture 3: Gerolf Gros – Measuring CO₂ permeability by \(^{18}O\) Exchange

Techniques:

pH gradients in the surface of lipid bilayer

\( t_{1/2} \) of CO₂ uptake \( \sim 12 \text{ ms} \) (Endeward et al 2008)
In the case of CO₂ kinetics, stopped flow is not good

we have chemical eq but not isotopic equilibrium \( \Rightarrow \) take advantage of this in \(^{18}O\) technique

\[ \text{Pito}_2, \text{Paco}_2, \text{CA activity} \]

\( \downarrow \) red cell
\( \rightarrow \) fast phase where \( \text{Paco}_2 \) dominates

monitor pts continuously
How do extract $P_{O_2}$?

6 ODEs
Estimate $P_{CO_2}, P_{HCO_3},$ A$in, A_{out}$
\[\text{estimate first}\]

fitting procedure
excellent fit

Phase 1

\[t_{1/2} = 5 \text{ s for } CO_2 \leftrightarrow HCO_3^- + H^+\]

\[t_{1/2} = 250 \text{ s (exchange)}\]

iso.

Phase 2
volume fraction of RBC is very critical ($\uparrow v \Rightarrow$ time faster)

trick = use small $v$ to reduce the time resolution for mass spectrom
\( P_{CO_2} = 0.15 \text{ cm/sec by RBC} \)

**Sensitivity**

- \( K_{eq} \) is important
- \( A_i \) is very critical parameter \( \Rightarrow \) \( A_i \) and pH need to be controlled
- pH is not critical

\( P_{H_2O} \)

- How about ULs?

**Theoretical hydrodynamics**

\[ \delta = \text{viscosity} \times \sqrt{\text{cell diameter}} \]

\[ \nu = 0 \Rightarrow \delta = 0 \]

↑ dextran \( \Rightarrow \) ↑ \( \delta \) for \( CO_2 \)

- Extrapolate to \( \nu = 0 \)

\[ P_{m, CO_2} = 0.16 \text{ cm/sec} \]

\[ \delta < 0.5 \mu m \text{ in saline} \]

\[ P_{CO_2} \text{ in saline} = 0.12 \text{ cm/sec} \]
P_{CO_2} = 0.15 \text{ cm/sec} \quad \Rightarrow 50\% \text{ due to AQPI} \\
\downarrow 50\% \text{ due to Rh protein} \\

Endeward et al, 2008

2 channels

P_{CO_2} = \sim 100 \cdot \text{P}_{HC_3O^-}
Lecture 4: Endoward - Intrinsic \( CO_2 \) permeability of cell membrane

\[ P_{CO_2} = 0.015 \text{ cm/sec in RBC, AQP4 & Rh null} \]

\[ \text{ Vesicles with } \neq \text{ cholesterol content} \]

\[ \text{ contains GALT} \]
Lecture 5: Bhanu P. Jena - Involvement of elevated membrane cholesterol on G-protein regulated H2O and gas transport in biological membranes

Porosome = secretory vesicles

We will focus on the porosome plasma membrane in synaptic vesicles

Jena et al. 1997, PNAS
Lecture 6: Jeff Garvin – Movement of NO across cell membr.

First described by Furchgott in 1980
L-arginine $\rightarrow$ L-citrulline + NO

Why do we care about NO?
- Involved in brain CNS
- Mitochondrial respiration
- ....

NO
$\uparrow$
Small, non-polar, reactive
Is a gas

Partition coefficients are measured @ equilibrium
"""say nothing about rates"

Why does the heart have AQP1? It doesn't need H2O so why?
Hyp: AQP1 transports NO

Measurements: cultured cells & fluorescence
1. $p_{NO}$ correlates with $P$
2. $\uparrow$ AQP1 $\Rightarrow$ $\uparrow$ NO expression
Inhibitors of AQP1 reduce NO fluxes

\[ \text{No Influx} \rightarrow \text{NO Influx is saturable} \]

Purified AQP-1 increases NO transport

Conclusion:
\[ \Rightarrow \text{AQP1 transports NO} \]

How about other AQPs?
AQP3 transports NO but not as rapidly as AQP1. Same for AQP4

Is it physiologically relevant?
Use Aortic ring preparation

Ach

Not been able to calculate PNO

Q/A:
NO electrode probably measures change in blood flow
Is collecting duct NH3 diffusive or transporter-mediated?

Data show both saturable & diffusive

\[ J_{\text{tot}} = J_{\text{trans}} \left( \frac{[MA]}{[MA] + K_m} \right) + J_{\text{diff}} [MA] \]

saturable component

linear component

Handlogren et al., *AJP Renal*, 2004

Are Rh proteins present in cells with NH3 transport?

*RhAG* in RBC....

*RhbG* in liver, kidney, sweat glands, intestine, lungs

When you sweat, NH3 ↑

*RhCG* in kidney, brain, testis, intestine, liver, skeletal muscle
Rh are present in cells that transport NH₃.

MAC increases Rhcg expression.

Rhcg & Rhbc expression increase in:
1) MAC
2) Ischemia-reperfusion injury
3) Metabolic acidosis
4) etc...
Keynote speaker: Robert Stroud

What do structures tell us about gas channels? QED!

2 families of membrane proteins that can move gases; gases are uncharged
- Rh Family
- AQP Family

Ammonia Transport: Amt/MEP/Rh Family in bacteria

Nitrogen Metabolism in bacteria

\[
\begin{align*}
\text{low pH } & \quad \text{pk}=9.25 \quad \text{high pH} \\
\text{NH}_4^+ & \quad \leftrightarrow \quad \text{NH}_3^+ + \text{H}^+ \\
\text{H} & \quad \text{H} \quad \text{N} \quad \text{H} \\
\end{align*}
\]

\[
\text{Dipole moment} \quad \delta^- \quad 2\delta^+ \quad \delta^- \\
0 = C = 0 \quad \delta^+/- \quad \delta^+/- \\
0 = 0
\]
$\text{NH}_3^+ \leftrightarrow \text{NH}_3 + \text{H}^+$

$\text{NH}_3$ channel

$\text{Am+B Crystalllography}$

$\uparrow$ trimer

lyphosome
$\text{Am+B conducts NH}_3$ but not $\text{H}_2\text{O}$

Wed 28th November: http://rmii.2012.org/ San Francisco
Xue Qin: AQP5

\[ \Delta pHe^* = (\Delta pHe)_{AQP} - (\Delta pHe)_{H_2O \text{ control}} \text{ daily matched} \]

T41 in AQP5

L43

No significant change w/ L43 mutations.

Interesting changes for T41

Movement of ions in the central pore

In order to see what happens we need the crystal structure

The central pore — what is the best molecule to see what goes through the central pore

AQP6 carries very little H2O or none

Do something to the CO2 permeability without affecting the H2O permeability.

crystal structure ~ difficult

O2 diffusion through cavities
nice packing between the helices
partition coefficient of water to octanol $\rightarrow$ hydrophobic channel

DIDS has no significant effect on the water permeability in AQP5 (and probably to all AQPs)

AQP4 in astrocytic endfeet
$\uparrow$
Pf is insensitive to DIDS
$\uparrow$
non specific
you get specificity by making mutations (in NBCs)
but for AQPS we do not know where the binding site is.

glycosylation
reaction that is covalent

Wisdom: cystines within the central pore. To do: add mercury
L43C mutant: CO2 permeability is normal
ND96 solution
reacts those cystines with other things

AQP5 has the biggest spike
DDR doesn't do anything...

expose to a solution to be oxidized
T41C is probably misfolded
1. Which other families of gas channels might be there? So far we have looked at:
   - CO₂
   - NH₃ (general medicine)
   - O₂: EPR, Optical/Hb; we want to measure fluxes of O₂ and we want to do it faster
   - NO: Hb
   - CO: Hb
   - CH₄: swamp bacteria
   - H₂S: purple bacteria
   - N₂: nitrogenase - Raman Spectroscopy (fast but not sensitive)
   - Ethylene: plants
   - H₂

   How do we measure N₂ fluxes?
   - ¹³N - NMR (not very sensitive, slow)

   H₂S ⇌ H⁺ + HS⁻

   pH measurements

   Signaling gas
   Optical/Hb

2. What other families of gas channels might be there?
• AE1, GLUT1/4, AQP1, Rh, MCT-1

  RBCs protein

• Endothelial cells in capillaries
• BBB, BRotina-B
• BTB, B0B
• Lungs: AQP5
• Striated Muscles... myoglobin
• Mitochondrion: CO₂ is formed into the matrix. AQP8, AQP9
  cytochrome oxidase

③ Physiological implications?
• Exercise
• Size scaling; Allometry: might expect to see a lot of gas channels in mice, but not in elephants
• Fish gills
• Zebrafish (swim bladder)

Effects of pressure on gas permeability

Pharmacological Intervention
TETRAMER

COE experiments (Jing Lu)

NBC as a CO₂ channel

ONR global Funding Opportunities

Director's initiative: point of contact