Gas channels Workshop

September 6-7 2012

Lecture 1 : Walter Boron – Gas Channels

- Solubity theory
  $P \propto S_L / S_W$

  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 : 1fs)

Force field approximations

atomistic resolution

Implicit Ligand sampling $W(r) = -k_B T \ln \left[\frac{p(r)}{p_0} \right]$

$$F(z) = -RT \ln \frac{\sum e^{-F(x,y,z)/RT}}{\dots}$$

Lecture 3: Gerolf Gros – Measuring CO_2 permeability by ^{18}O Exchange

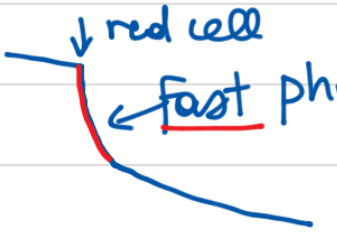
Techniques:

pH gradients in the surface of lipid bilayer

$t_{1/2}$ of CO_2 uptake ~ 12 ms (Endeward et al 2008)
In the case of CO_2 kinetics, stopped flow is not good

we have chemical eq but not isotopic equilibrium ⇒ take advantage of this in ^{18}O technique

$P_{HCO_3^-}$
P_{CO_2}
CA activity
} are the 3 main parameters



phase where P_{CO_2} dominates

monitor pHs continuously

how do extract P_{CO_2} ?

6 ODEs

Estimate P_{CO_2}, P_{HCO_3}, A_{in}, A_{out}

A_{in}, A_{out} — estimate first

fitting procedure

excellent fit

Phase 1

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

$t_{1/2} = 250s$ (isotopic exchange)

Phase 2

volume fraction of RBC is very critical (↑v => 6 time faster)

trick = use small v to reduce the time resolution for mass spectrom

P_{CO_2} = 0.15 cm/sec by RBC

Sensitivity

Keq is important

Ai is very critical parameter

ptle is also " " "

$\Rightarrow$ Ai and ptle need to be controlled

ptli is not critical

P_{H_2O} " "

How about ULs?

theoretical hydrodynamics

$\delta \sim$ viscosity $\upsilon \times \sqrt{\text{cell diameter } l} \Rightarrow$

$\upsilon = 0 \Rightarrow \delta = 0$

↑ dextran $\Rightarrow$ ↑ δ for CO_2

Extrapolate to $\upsilon = 0$

P_{m,CO_2} = 0.16 cm/sec

$P_{CO_2}^{app}$ in saline = 0.12 cm/sec

$\Rightarrow \delta$ = 0.5 μm in saline

$P_{CO_2} = 0.15$ cm/sec → 50% due to AQP1

↘ 50% due to Rh proteins

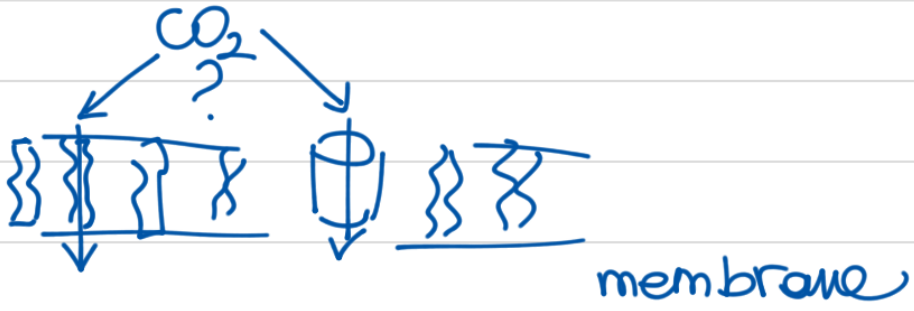
Endeward et al, 2008

2 channels

$P_{CO_2} \approx 100\, P_{HCO_3^-}$

Lecture 4: Endeward – Intrinsic CO_2 permeability of cell membrane

P_{CO_2} = 0.015 cm/sec in RBC AQP1 & Rh null



Vesicles with ≠ cholesterol content

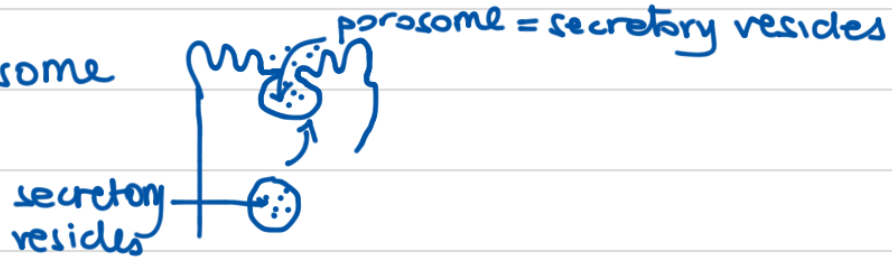
↑

contains CA II

Afternoon Sessions

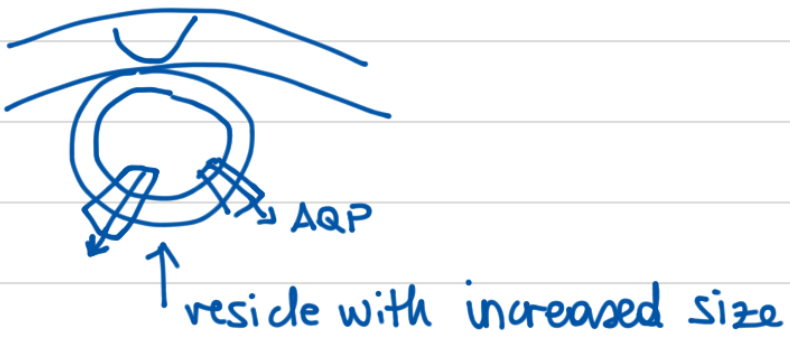
Lecture 5: Bhanu P. Jena - Involvement of elevated membrane cholesterol on G-protein regulated H_2O and gas transport in biological membranes

Porosome



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 → L-citrulline + NO

Why do we care about NO? – involved in brain CNS
– mitochondrial respiration
– . . . .

NO
↑ small, non-polar
reactive
is a gas

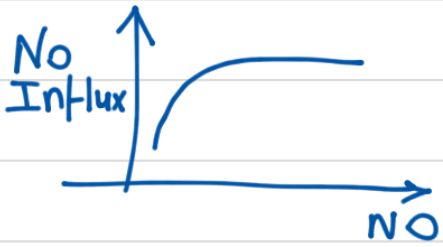
partition coefficients are measured @ equilibrium
" " say nothing about rates

Why does the heart have AQP1? It doesn't need H_2O so why?
Hp: AQP1 transports NO

Measurements: cultured cells & fluorescence
① P_{NO} correlates with P_F

② ↑ AQP1 ⇒ ↑ NO expression

③ Inhibitors of AQP1 reduce NO fluxes

④ 

⇒ NO Influx is saturable

⑤ Purified AQP-1 increases NO transport

Conclusion:
⇒ AQP1 transport 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

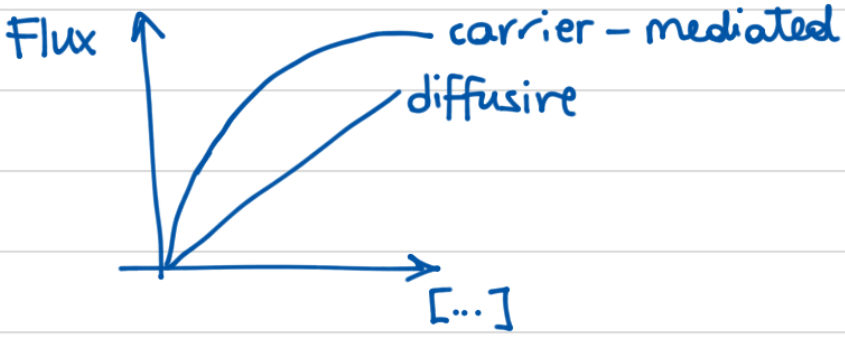
Ach

Not been able to calculate P_{NO}

Q/A:
NO electrode probably measures changes in blood flow

Lecture 7: David Weiner - Role of Rh proteins in NH_3 gas transport

Is collecting duct NH_3 diffusive or transporter-mediated ?



Data show both saturable & diffusive

$$J_{Tot} = \underbrace{J_{trans}\left([MA]/([MA]+Km)\right)}_{\text{saturable component}} + \underbrace{J_{diff}\,[MA]}_{\text{linear component}}$$

Handlogten et al, AJP Renal, 2004

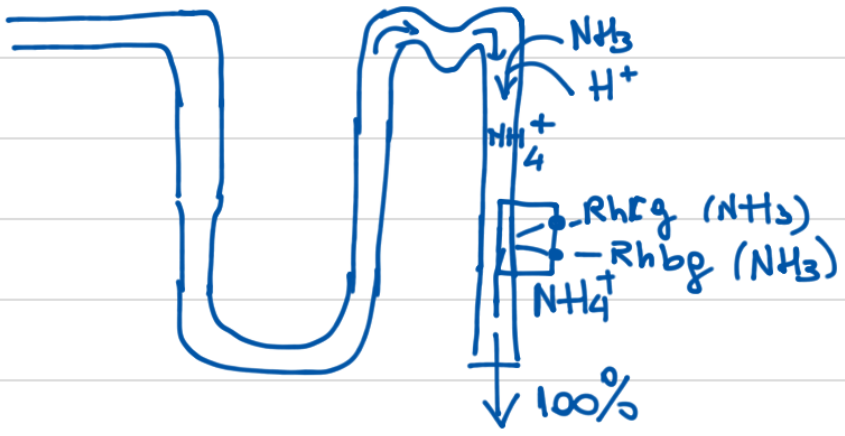
Are Rh proteins present in cells with NH_3 transport ?

RhAG  in RBC ....

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

↑ when you sweat, NH_3 ↑

RhcG in kidney, brain, testis, intestine, liver, skeletal Muscle



Rh are present in cells that transport NH_3

MAc increases Rhcg expression

Rhcg & Rhbc expression increase in :

1) MAc
2) Ischemia-reperfusion injury
3) pt acidosis
4) etc ...

Keynote speaker: Robert Stroud

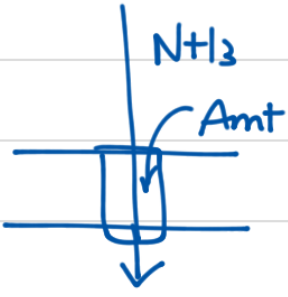
What do structures tell us about gas channels? QED!

(2) families of membrane proteins that can move gases
gases are uncharged

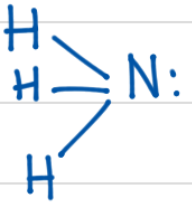
→ Rh family
AQP family

Ammonia Transport : Amt/MEP/Rh Family
↑
in bacteria

Nitrogen Metabolism in bacteria



low pH $NH_4^+ \longleftrightarrow NH_3^+ + H^+$ high pH
pK=9.25



δ^- $2\delta^+$ δ^-
O=C=O

Dipole moment

$\delta+/-$ $\delta+/-$
O=O

$NH_4^+ \longleftrightarrow NH_3 + H^+$

NH_3 channel

AmtB Crystallography

↑ trimer

lyphosome
AmtB conducts NH_3 but not H_2O

Wed 28th November : http://rmi2012.org/ San Francisco

Day 2 09/07/12

Xue Qin : AQP5

$\Delta pHs^* = (\Delta pHs)_{AQP} - (\Delta pHs)_{H_2O\ control\ /\ daily\ matched}$

T41 in AQP5
L43

No significant change in 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 H_2O or none
Do something to the CO_2 permeability without affecting the H_2O permeability

crystal structure ~ difficult

O_2 diffusion through cavities

nice packing between the helices
partition coefficient of water to octanol → hydrophobic channel

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

AQP4 in astrocytic endfeet
↑ P_f is insensitive to DIDS
↑
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 : CO_2 permeability is normal
ND96 solution
reacts those cystines with other things

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

expose to a solution to be oxidized
T41C is probably misfolded

Workshop meeting - Look at future directions

① Which other families of gas channels might be there?

So far we have looked at

CO_2

NH_3 general medicine

O_2 : EPR, Optical /Hb; we want to measure fluxes of O_2 and we want to do it faster

NO : Hb

CO : Hb

CH_4 : swamp bacteria

H_2S : purple bacteria

N_2 : nitrogenase - Raman Spectroscopy (fast but not sensitive

Ethylene : plants

H_2

How do we measure N_2 fluxes?

^{13}N - NMR (not very sensitive, slow)

$H_2S \rightleftharpoons H^+ + HS^-$

pH measurements

Signaling gas

Optical /Hb

② What other families of gas channels might be there?

- AE1; GLUT1/4; AQP1, Rh, MCT-1

RBCs proteins

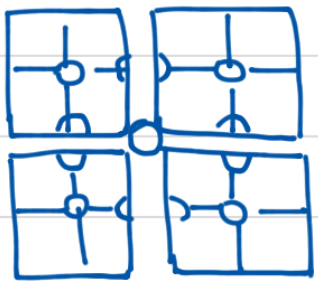
- Endothelial cells in capillaries
- BBB, BRetinaB
- BTB, BOB
- Lungs: AQP5
- Striated Muscles ... myoglobin
- Mitochondrion: CO_2 is formed into the matrix - AQP8, AQP9 cytocrome oxidase

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 Interrention



TETRAMER

DOE experiments (Jing Lu)
NBC as a CO_2 channel

ONR global Funding Opportunities

Director's initiative : point of contact