9/6/12 @ 9:10 AM
Gas Channels Worksop
Emad
MD ... Sub-angstrom resolution

2016: Blue Waters → 200K processors

≠

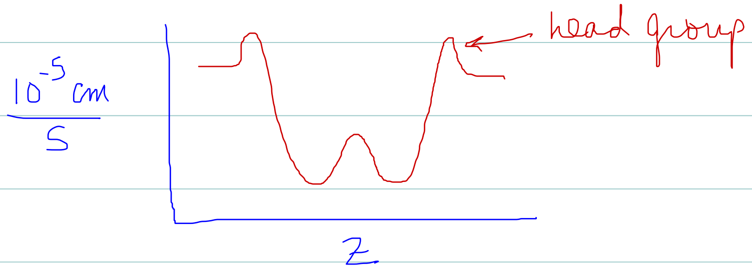
POPE 100%

Start off $\bar{c}$ 100 CO_2 near membrane

Modeling 50% Chol. is not trivial... where to place them, equilibration... have a partial solution.

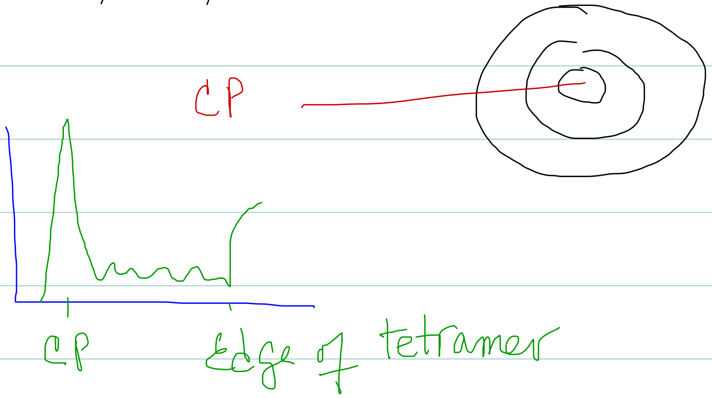
His partition coeff is ~ exp. det. values

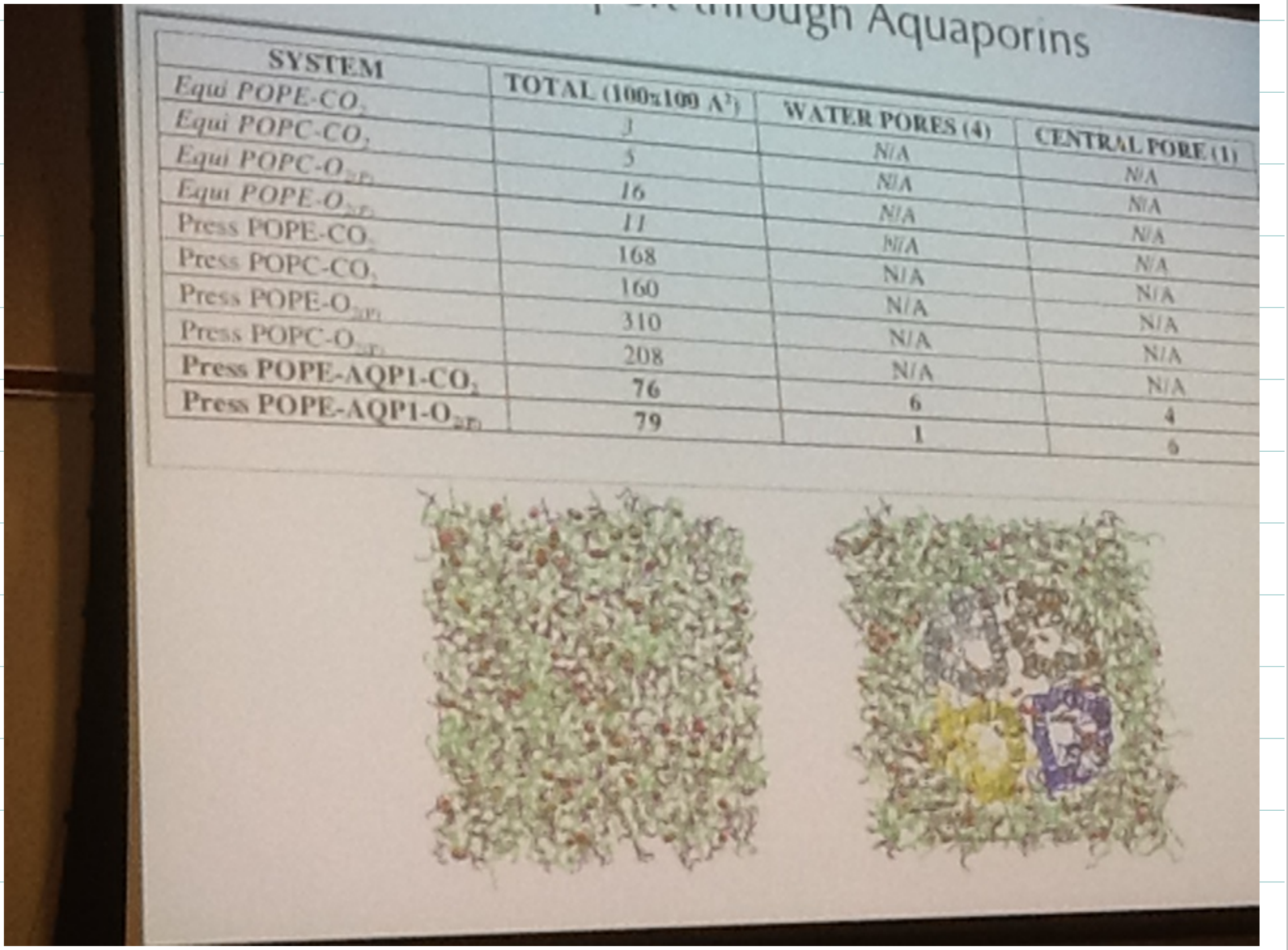
Gas reaches equil in lipid in 10-15 ns for O_2 & CO_2.



Implicit ligand sampling: works $\bar{c}$ neutral molecules (not ions, wh. perturb protein) ... results are about same as <u>explicit</u>.

{ AQP1, 4, 5
{ CO_2, O_2, NO





ough Aquaporins

SYSTEM	TOTAL (100x100 Å²)	WATER PORES (4)	CENTRAL PORE (1)
Equi POPE-CO_2	3	N/A	N/A
Equi POPC-CO_2	5	N/A	N/A
Equi POPC-$O_{2(P)}$	16	N/A	N/A
Equi POPE-$O_{2(P)}$	11	N/A	N/A
Press POPE-CO_2	168	N/A	N/A
Press POPC-CO_2	160	N/A	N/A
Press POPE-$O_{2(P)}$	310	N/A	N/A
Press POPC-$O_{2(P)}$	208	N/A	N/A
Press POPE-AQP1-CO_2	**76**	6	4
Press POPE-AQP1-$O_{2(P)}$	**79**	1	6

AQP1: D50 → H_2O coord → major barrier

NO° through AQP4
O_2 " "
(Wang, Proteins, 2010)

NB: Is $[O_2]$ in CP the same as in bulk gas phase?

AQP4 (vs. 1): diff ΔG profile

Rely heavily on init. config. of lipid

Problem: lipid molec. move v. slowly!
10^3 slower than H_2O

HMMM: highly mobile membrane mimetic
(liquid center of membrane)
Water-Oil attracts lipids to interface
Lipids are far more mobile
Even can see insertion of a peptide helix.
Chol might partition into core of bilayer,
parallel to plane of membrane.

9/6/12 @ 10:25 AM
Gas Channels Worksop
Gerolf Gros, Hanniver
18O ... CO2 permeability

Problem c̄ stopped-flow applied to vesicles

$t_{1/2}$ of CO_2 uptake by human RBC : 13 ms
O_2 80

This could be too fast, esp. if there is incomplete mixing.

But KO of AQP1 + Rh could ↑ $t_{1/2}$ by 10x ... make a Δ measurable? WB

Measure ^{18}O-labelled CO_2
46 vs 44 Mol. Mass

"HCT" : 2×10^{-4}
2×10^{-3}

3-D curve fitting → optimal P_{CO_2} & P_{HCO_3} ... no local minima

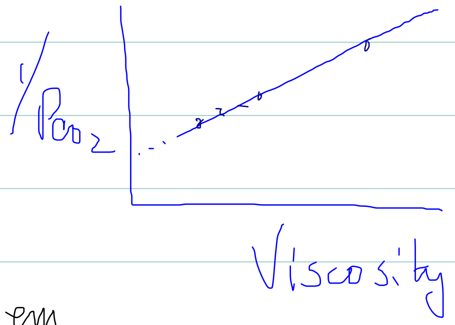
Critical params: A_i & pH_e ...
errors → big ΔP_{CO_2}
pH: ±0.01 → 20-30%

pH_i & P_{H_2O} are not important

Viscosity: $\uparrow [\text{Dextran}] \rightarrow \not\Delta P_{HCO_3}$ (wh is so small)

" $\rightarrow \downarrow P_{CO_2}$

unstirred layer



$\delta = 0.5 \mu m$

$P_M = 0.16$ cm/s

∴ UL is only a minor problem

$\delta \alpha$

P_{O_2} in human RBCs

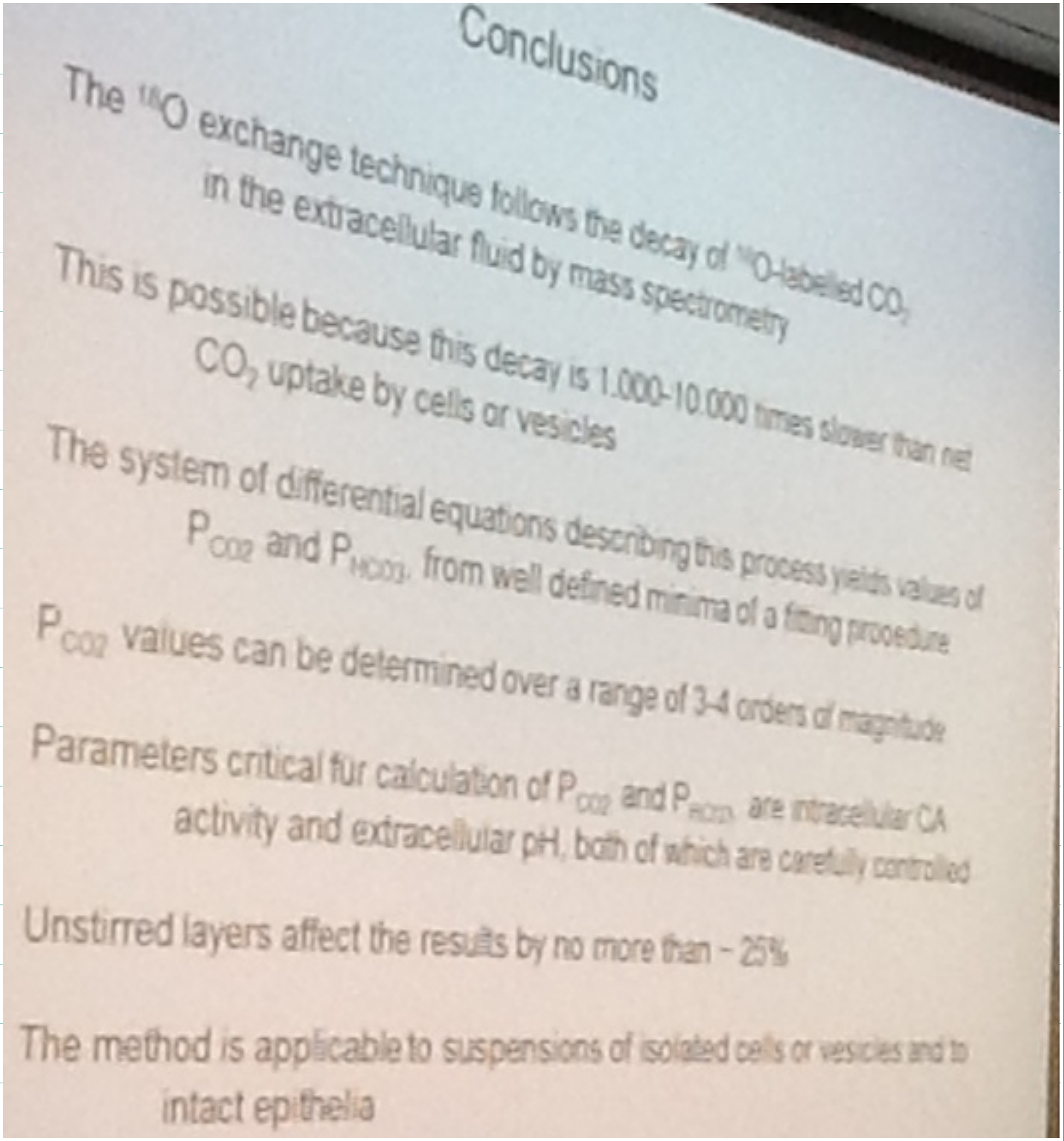
IDEAS FOR DISC.

AQP1 ~ 50%
Rh ~ 50%
} ~100%

$\neq$

Colon:

Competition of H_2O vs. NH_3 or CO_2 through aquapore



Conclusions

The ^{18}O exchange technique follows the decay of ^{18}O-labelled CO_2 in the extracellular fluid by mass spectrometry

This is possible because this decay is 1.000-10.000 times slower than net CO_2 uptake by cells or vesicles

The system of differential equations describing this process yields values of P_{CO2} and P_{HCO3^-} from well defined minima of a fitting procedure

P_{CO2} values can be determined over a range of 3-4 orders of magnitude

Parameters critical für calculation of P_{CO2} and P_{HCO3^-} are intracellular CA activity and extracellular pH, both of which are carefully controlled

Unstirred layers affect the results by no more than ~ 25%

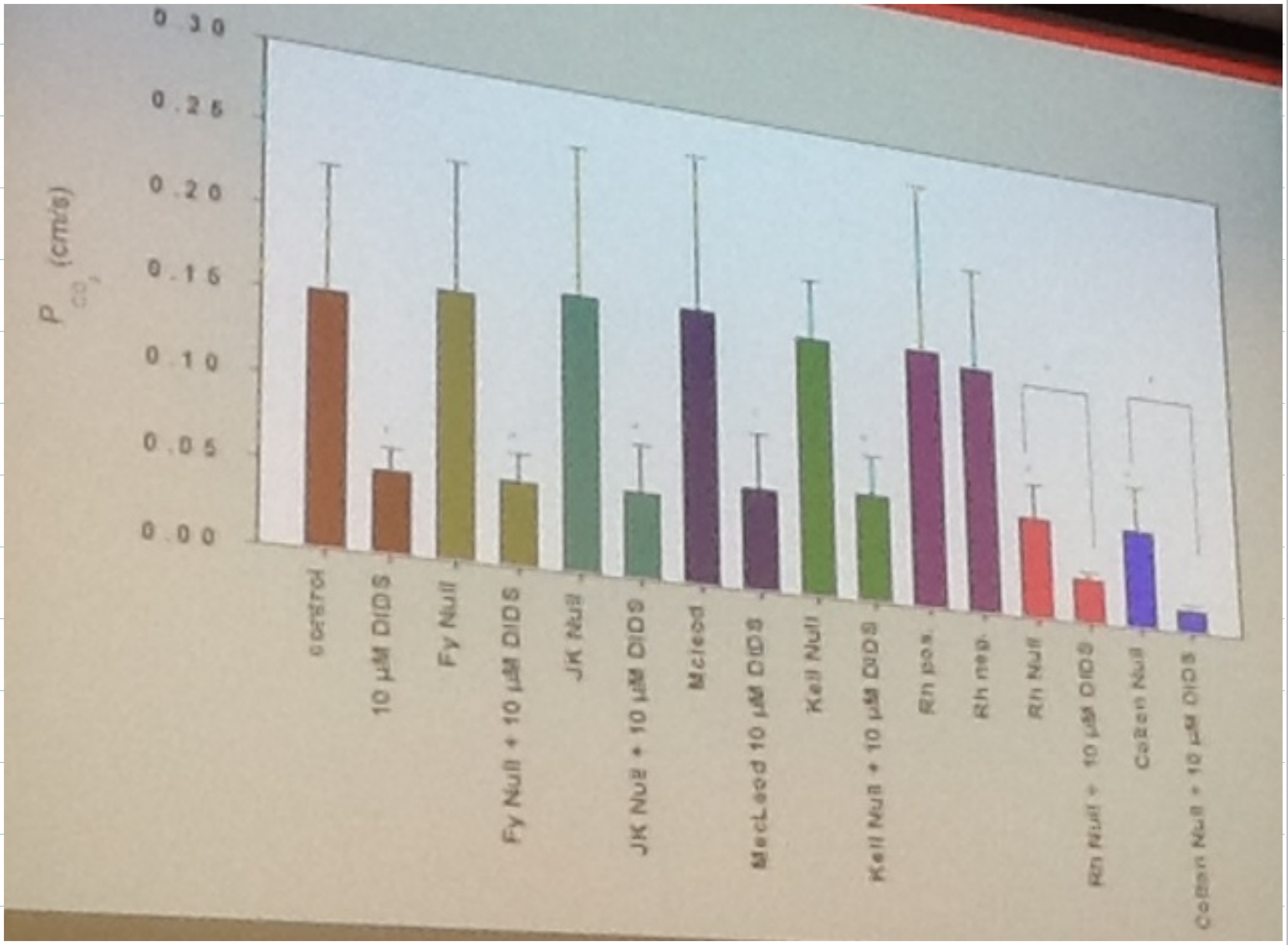
The method is applicable to suspensions of isolated cells or vesicles and to intact epithelia

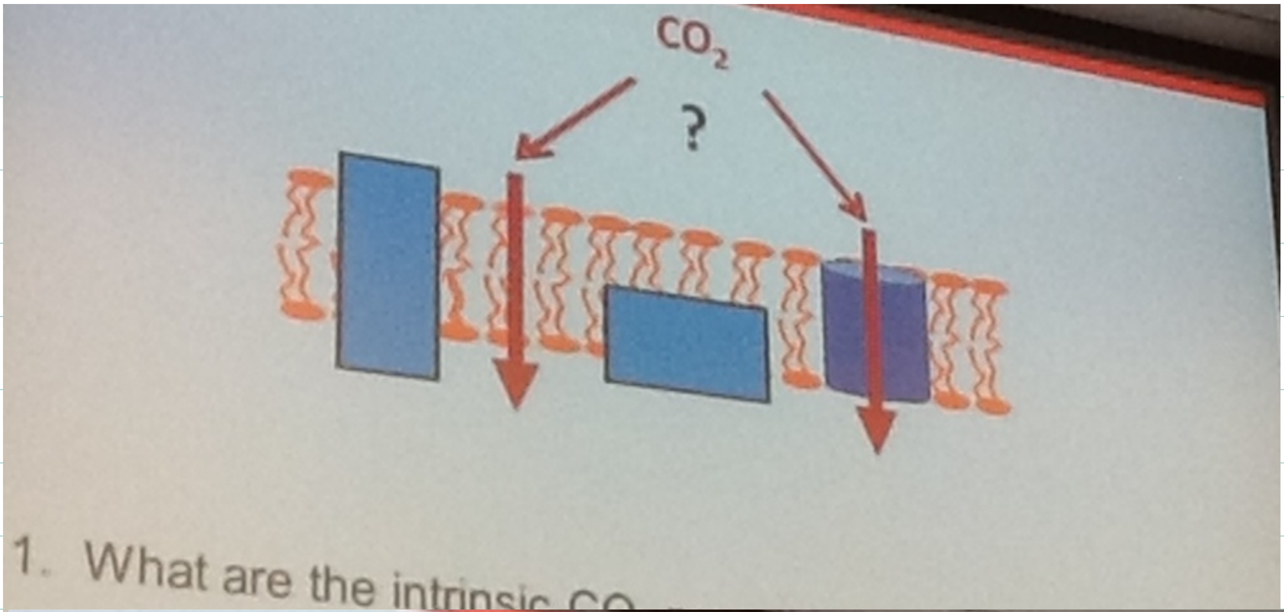
$$[X]_W = S_W P_X$$

$$[X]_L = \frac{S_L}{S_W} \underbrace{[X]_W}_{} = \frac{S_L}{S_W} \cdot \underbrace{S_W \cdot P_X}_{}$$

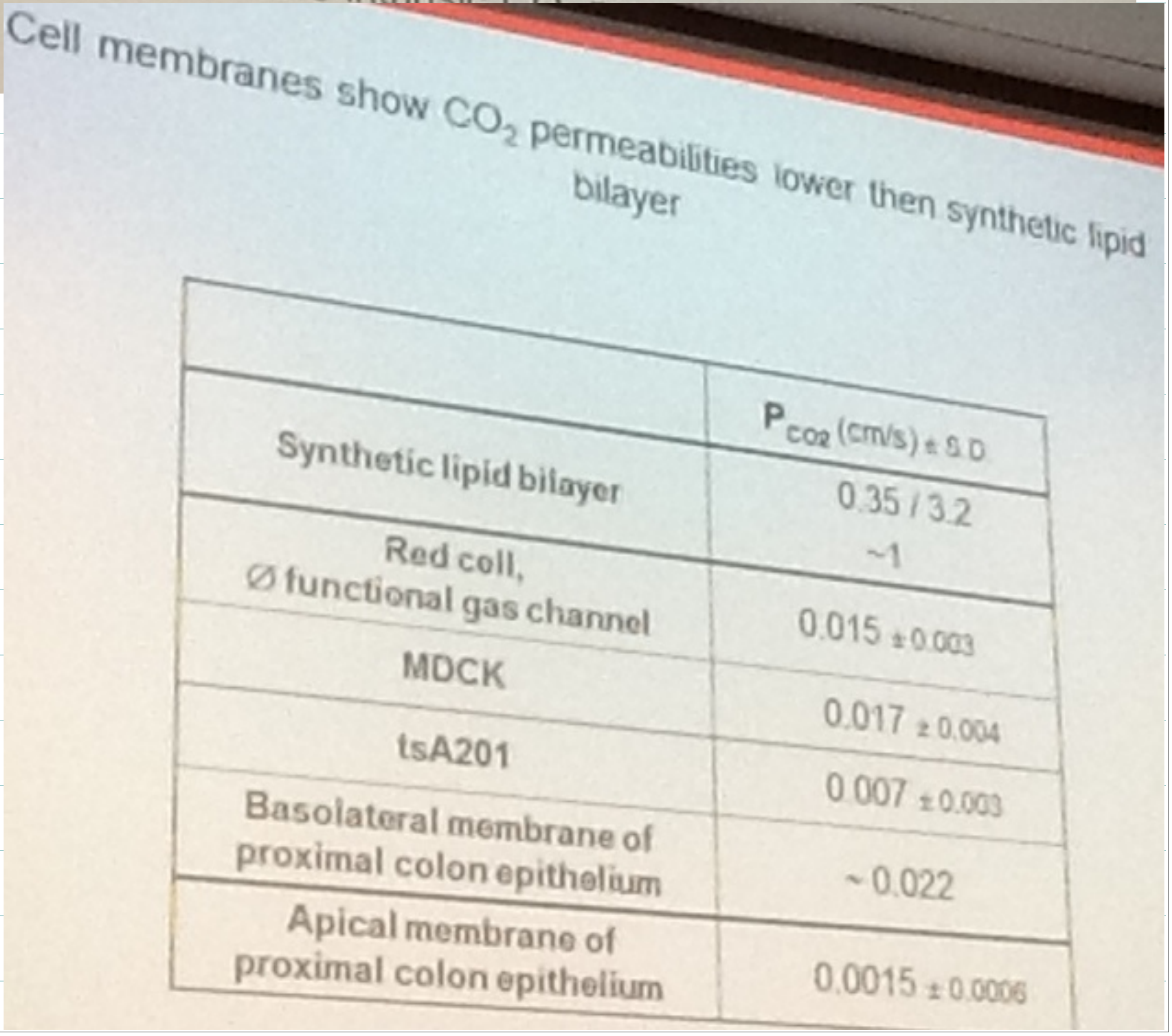
$$= S_L \cdot P_X$$

9/6/12 @ 11:15 AM
Gas Channels Worksop
Volker Endeward, Hannover
Background CO2 permeability





1. What are the intrinsic CO



Cell membranes show CO_2 permeabilities lower then synthetic lipid bilayer

	P_{CO2} (cm/s) ± S.D
Synthetic lipid bilayer	0.35 / 3.2 ~1
Red cell, Ø functional gas channel	0.015 ± 0.003
MDCK	0.017 ± 0.004
tsA201	0.007 ± 0.003
Basolateral membrane of proximal colon epithelium	~ 0.022
Apical membrane of proximal colon epithelium	0.0015 ± 0.0006

P_{CO_2} (art. lipid bilayer) >> naked mammalian membrane

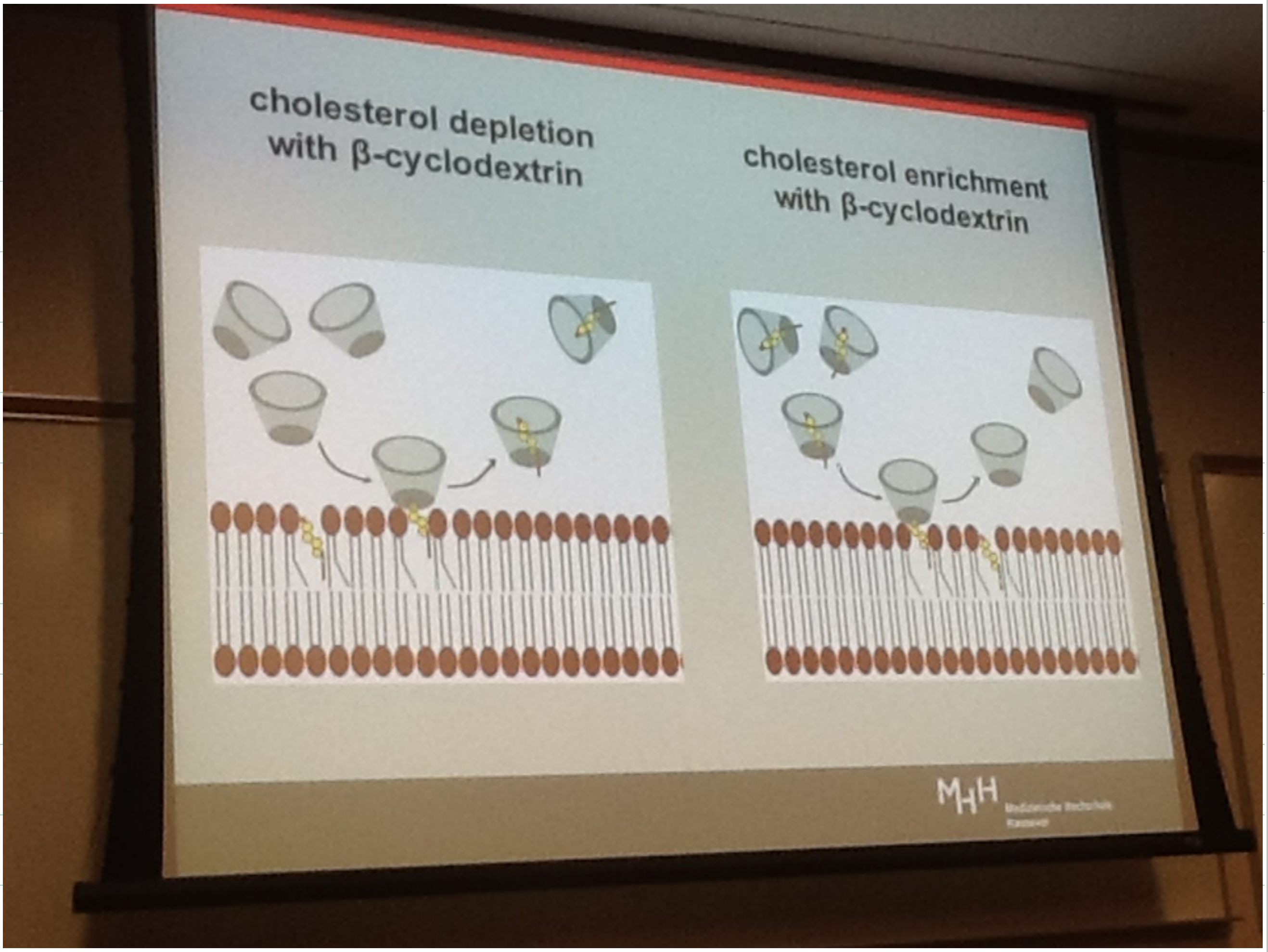
Cholesterol: 98% ↓ in P_f.
$\neq$

150 nm: mean vesicle diam... contain CA II

Chol: 0-20% → $\not\Delta$ measurable
30-70% → log-linear ↓ P_{CO_2} by ~ 10^2

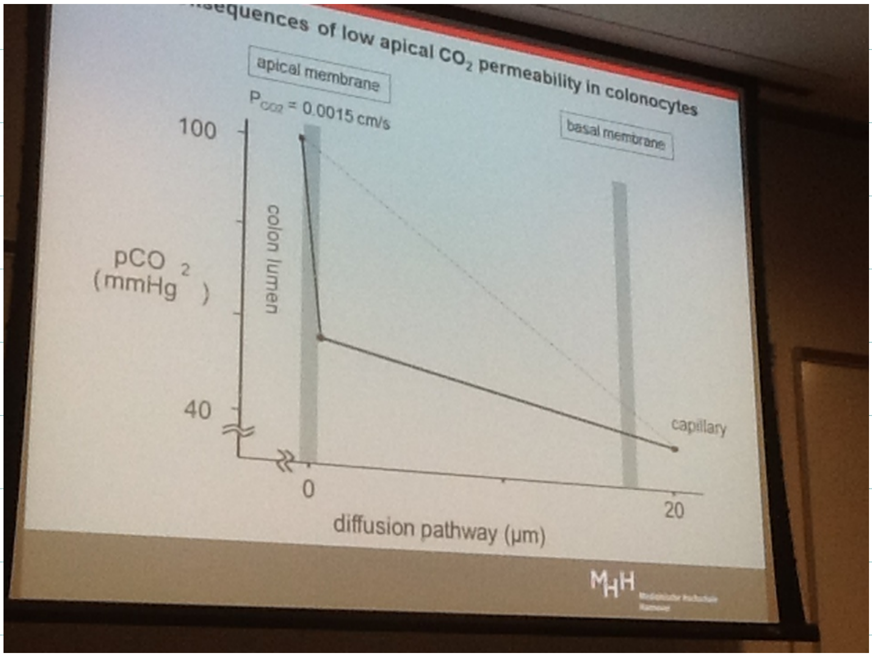
Apical colonic membrane: 70% cholesterol

β-cyclodextrin



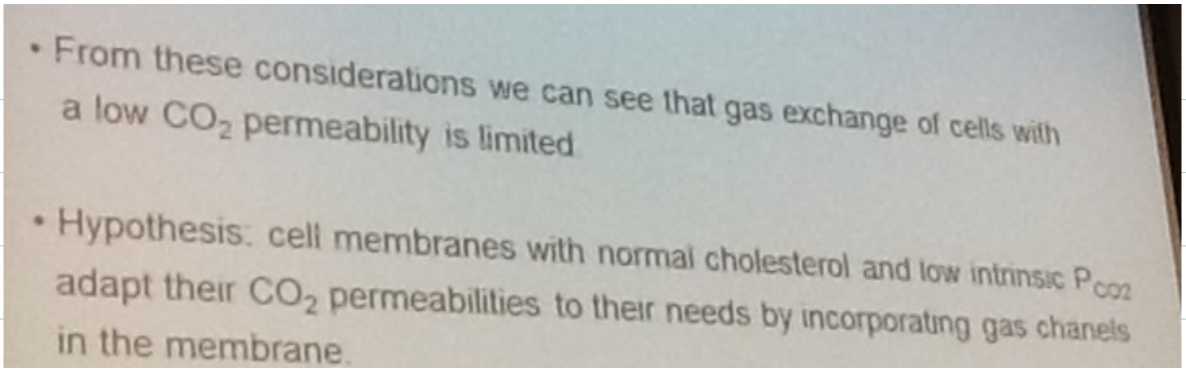
cholesterol depletion with β-cyclodextrin

cholesterol enrichment with β-cyclodextrin



…equences of low apical CO_2 permeability in colonocytes

Could we ↓ P_{CO_2}
↑ Xo by ↑
↑ Chol &
vice versa.

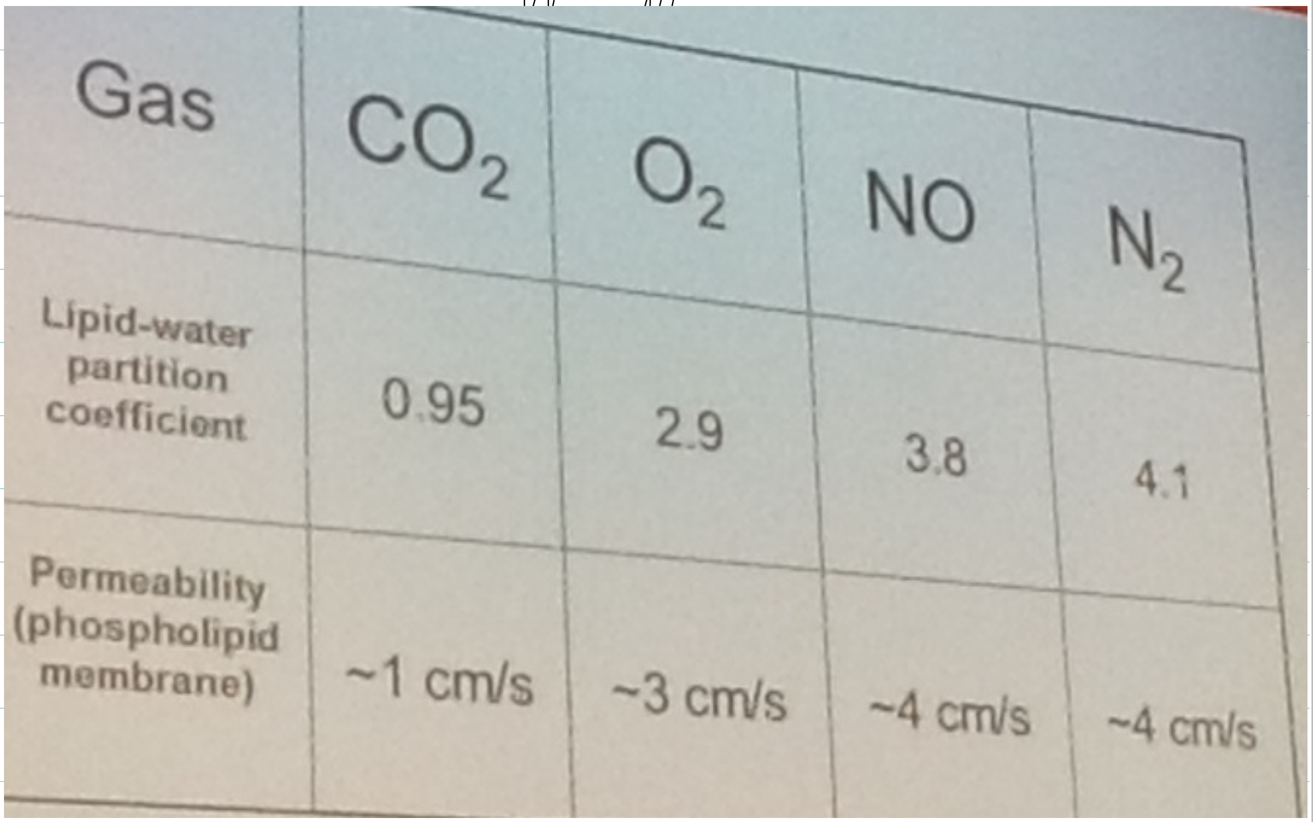


- From these considerations we can see that gas exchange of cells with a low CO_2 permeability is limited
- Hypothesis: cell membranes with normal cholesterol and low intrinsic P_{CO2} adapt their CO_2 permeabilities to their needs by incorporating gas chanels in the membrane.

AQP1 vs. AqpZ in liposomes

$\uparrow\uparrow P_{CO_2}$ ↖ $\not\Delta P_{CO_2}$

He sees a much bigger effect (? ~80%) than we do.



Gas	CO_2	O_2	NO	N_2
Lipid-water partition coefficient	0.95	2.9	3.8	4.1
Permeability (phospholipid membrane)	~1 cm/s	~3 cm/s	~4 cm/s	~4 cm/s

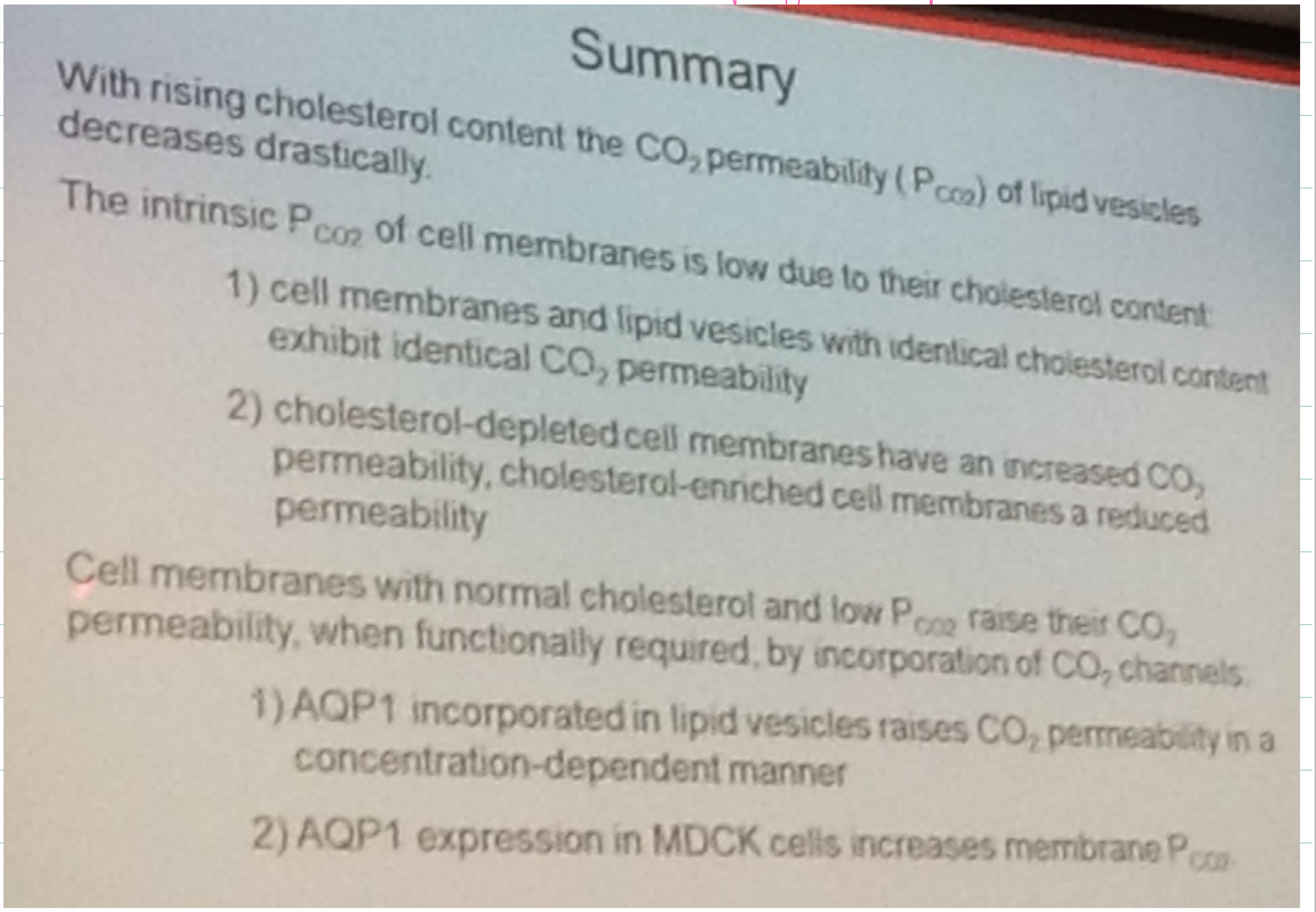
O_2 : PL membrane 3 cm/s

Chol : 1/100

PL + Chol : 0.03 ?

Heart m : $\Delta P_{CO_2} = 40$ mm Hg

way too high to be physiol. possible.



Summary

With rising cholesterol content the CO_2 permeability (P_{CO2}) of lipid vesicles decreases drastically.

The intrinsic P_{CO2} of cell membranes is low due to their cholesterol content

1) cell membranes and lipid vesicles with identical cholesterol content exhibit identical CO_2 permeability
2) cholesterol-depleted cell membranes have an increased CO_2 permeability, cholesterol-enriched cell membranes a reduced permeability

Cell membranes with normal cholesterol and low P_{CO2} raise their CO_2 permeability, when functionally required, by incorporation of CO_2 channels.

1) AQP1 incorporated in lipid vesicles raises CO_2 permeability in a concentration-dependent manner
2) AQP1 expression in MDCK cells increases membrane P_{CO2}.

$P_{CO_2} \propto [AQP1]^n$?

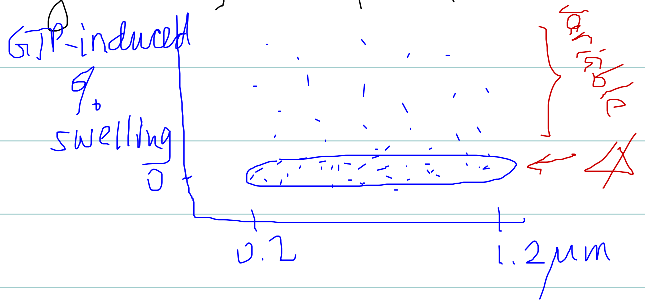
9/6/12 @ 1:05 PM
Gas Channels Worksop
Bhanu Jena, Wayne State
Cholesterol

Got interested in AQPs because of their involvement in vesicle fusion.

H_2O must enter the vesicle

$G\alpha_{i3}$: assoc. $\bar{c}$ ZGM   Jena et al, PNAS, 1997

GTP → ↑ water by volume (AFM) & ^3H_2O.

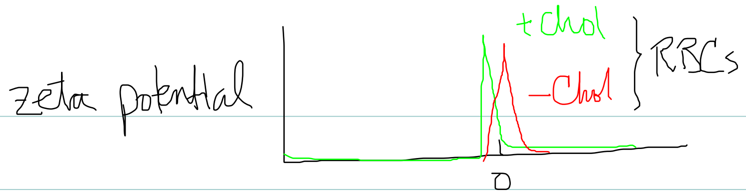


AQP1: Swelling complex } not 2,3,4,5, 7,8,9
" 6: "

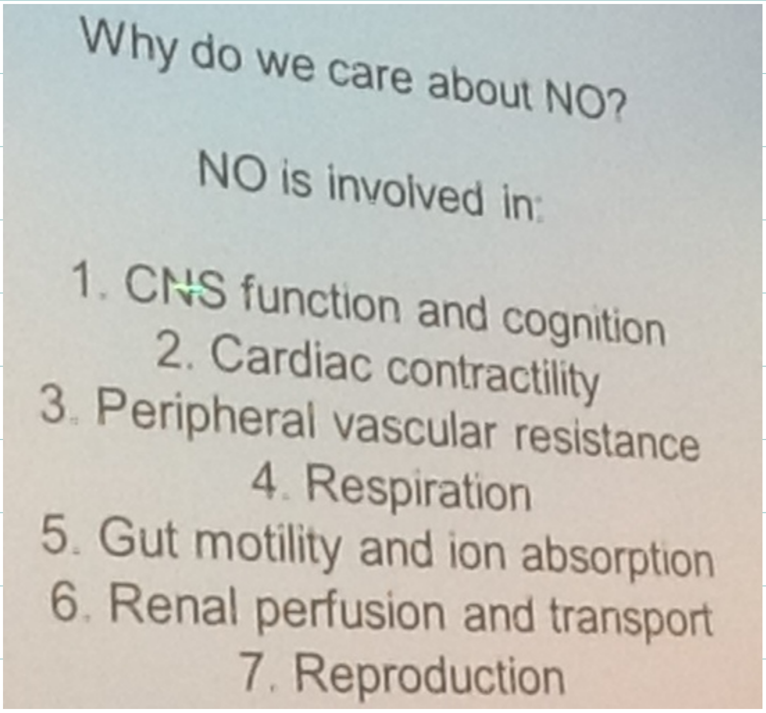
Mast (mastoparan)

% ΔVolume of granule (swelling): GTP + Mast
+ 20–40 μM MβCD (cyclodextrin)

AQP6: $G\alpha_o$, V-H^+ ATPase complex (stability) req. Chol.
Remove Chol → complex falls apart.


zeta potential
+Chol
−Chol
RBCs
0

9/6/12 @ 1:55 PM
Gas Channels Worksop
Jeff Garvin, HFH
The truth about the movement of NO across cell membranes

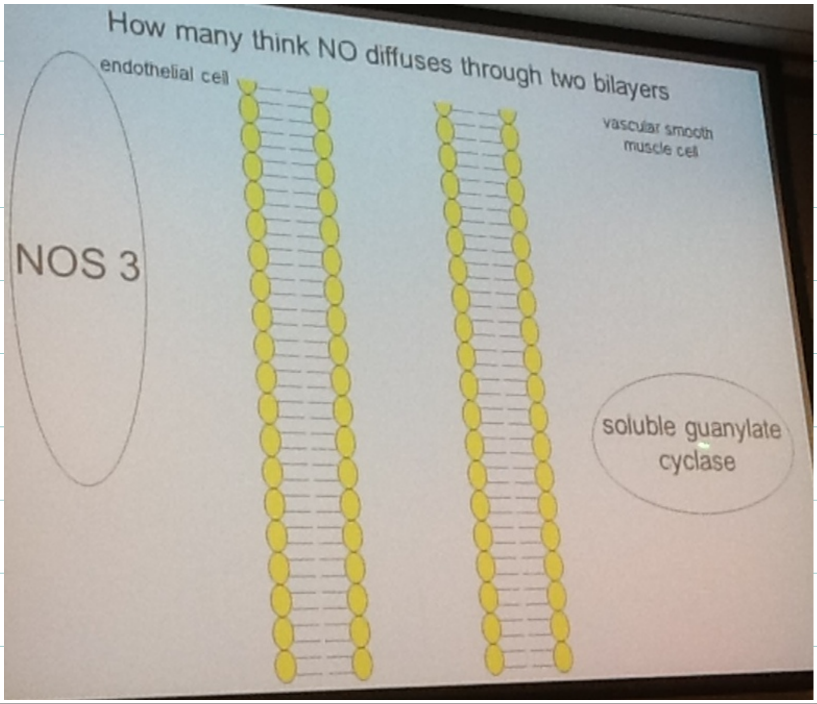


Why do we care about NO?

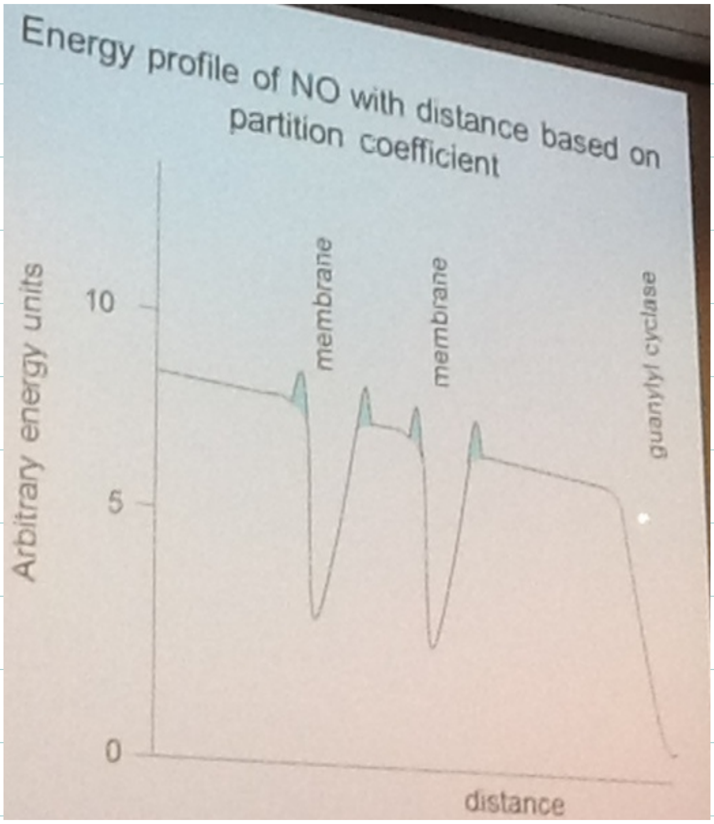
NO is involved in:

1. CNS function and cognition
2. Cardiac contractility
3. Peripheral vascular resistance
4. Respiration
5. Gut motility and ion absorption
6. Renal perfusion and transport
7. Reproduction

Partition coef: 3-5
$t_{1/2}$ ~30 s



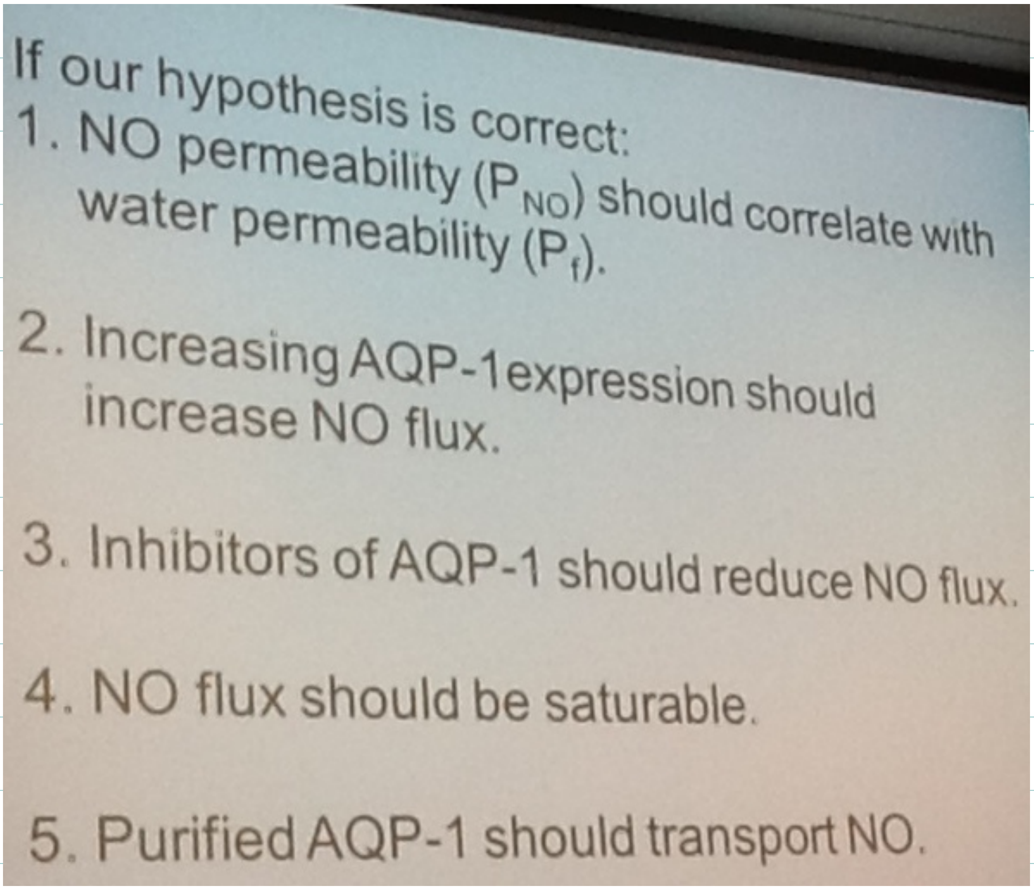
How many think NO diffuses through two bilayers



Energy profile of NO with distance based on partition coefficient

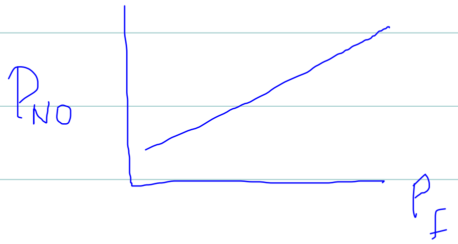
"Partition coeff say nothing about rates" ... his PhD mentor beat into him that s/s is an equilibrium parameter.

What is the chemistry of NO in lipid? More or less stable than in H_2O?



If our hypothesis is correct:

1. NO permeability (P_{NO}) should correlate with water permeability (P_f).
2. Increasing AQP-1expression should increase NO flux.
3. Inhibitors of AQP-1 should reduce NO flux.
4. NO flux should be saturable.
5. Purified AQP-1 should transport NO.



P_{NO}: fluorescent probe DAF2

NO influx → CHO cells, transiently transfected.
NO: NO donor or gas.

Hg interacts c̄ NO gas?

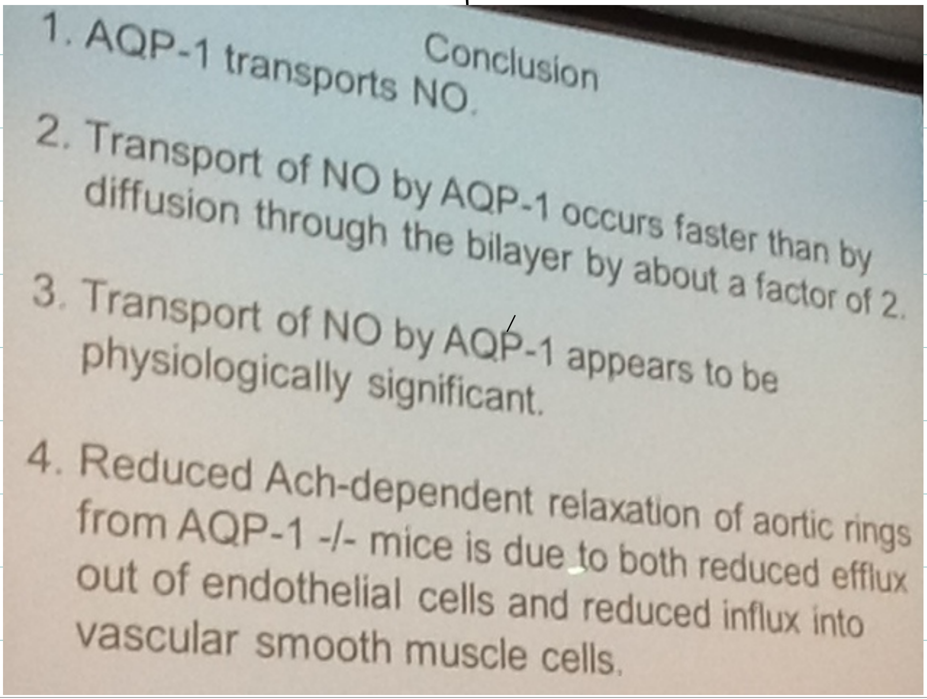
$K_{1/2}$: 0.54 μM   Physiol [NO] ≅ 0.2 μM

AQP1 reconst. into vesicles → ↑ J_{NO}

CHO cells:
AQP3: 25% ↑ over mock << AQP1. Did not ✓ expression.
" 4: 30% ↑

Aortic ring: isometric force
PE = Phenylephrine → contr.
Vary [Ach] to relax. AQP1 KO: ↓ Ach response
? ↓ NO efflux from EC or ↓ influx into VSMC

KO: ↓ NO release from EC
↓ " uptake into VSMC



Conclusion

1. AQP-1 transports NO.
2. Transport of NO by AQP-1 occurs faster than by diffusion through the bilayer by about a factor of 2.
3. Transport of NO by AQP-1 appears to be physiologically significant.
4. Reduced Ach-dependent relaxation of aortic rings from AQP-1 -/- mice is due to both reduced efflux out of endothelial cells and reduced influx into vascular smooth muscle cells.

32 in audience

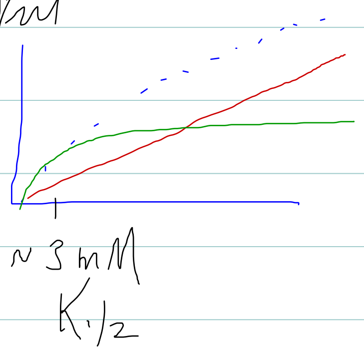
9/6/12 @ 3:10 PM
Gas Channels Worksop
David Weiner (wee)
Assessing roles of Rh glycoproteins in NH3 gas transport?

Is NH_3 transport "diffusive" or protein mediated?

Inhibitors: none

siRNA: unsuccessful

Saturation?



Saturable ✓
component +
diffusive comp.

Diffusion may dominate in inner medulla, where $[NH_3]$ is high & Rh levels are v. low.

$$RhAG + Rh30 < \begin{matrix} CE \\ D \end{matrix}$$

Cannot find RhAG by Ab anywhere but RBC.

1st cloned: RhAG

#2 " BG: Perivenous cells in hepatocytes
Hair follicles. $[NH_3]$ is
100x > plasma. Goes ↑
c̄ exercise: 2 → 10 mM.
Urine: 200-300 mM
(highest NH_3 conc)

GI → $\overset{NH_3}{250}$ mmole/day. SI > Colon

Lungs: RhBG → not in alveolar cells but in Bronchial epith cells.

RhCG: ? Glu neurotransmission.

Liver: $[NH_3]$ is ↑ in bile

Muscle: Exercise ⇒ 4-5x NH_3 ... produced by sk.m. At rest, Sk.m. is a NH_3 sink.

Kidney: 1-2% of excreted NH_3 from GFR.

≠

CD: RhBG: BLM

BalbC

CG: AM + some BLM

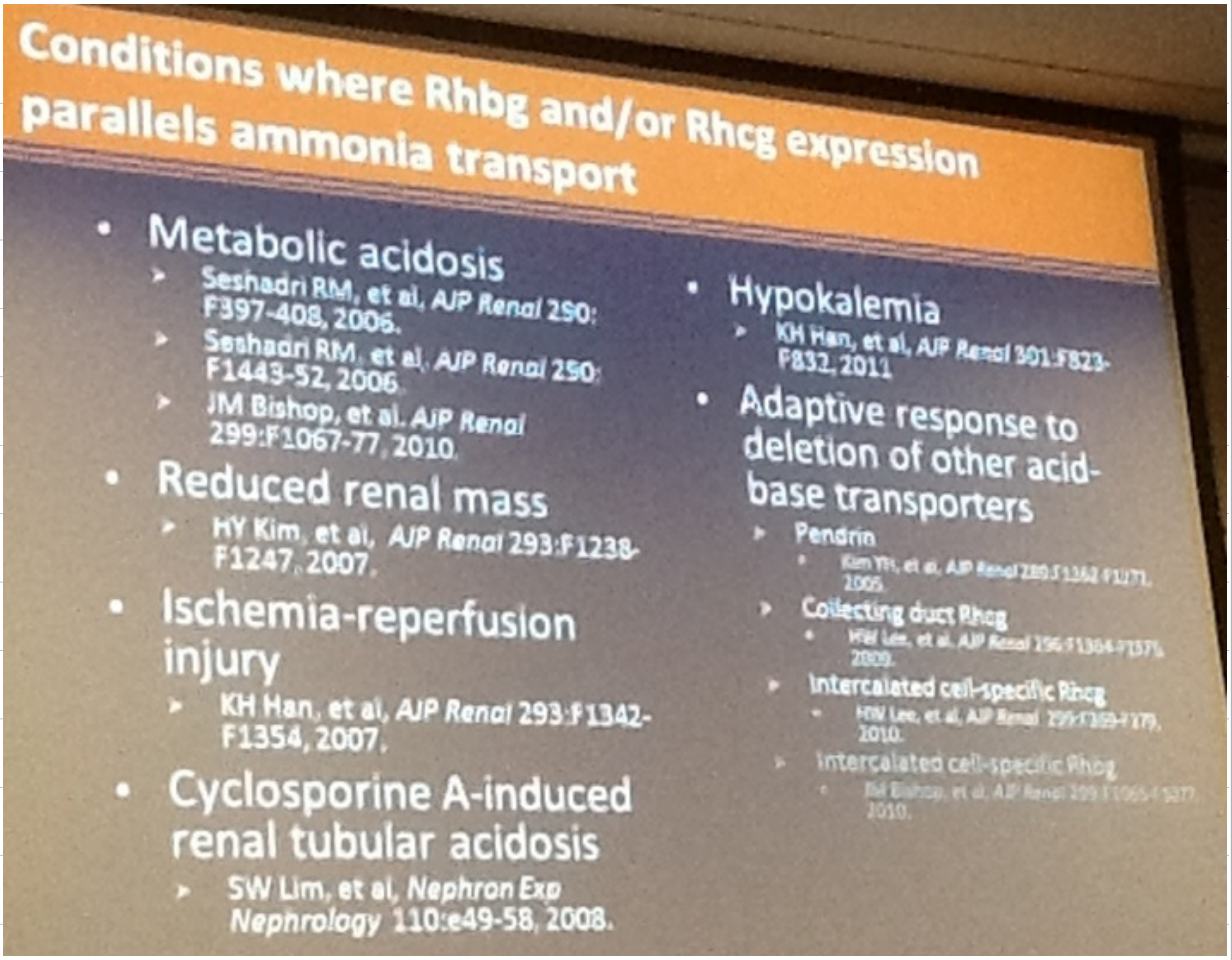
C57 Bl6: much higher

MAc → ↑ RhBG expression

Does Tenidap ↓ the "CO_2 permeability" attributable to NBC? Does it speed up pH_i ↓?

Grant: Mutant NBCe1

- Cond. KO?
- Mutations

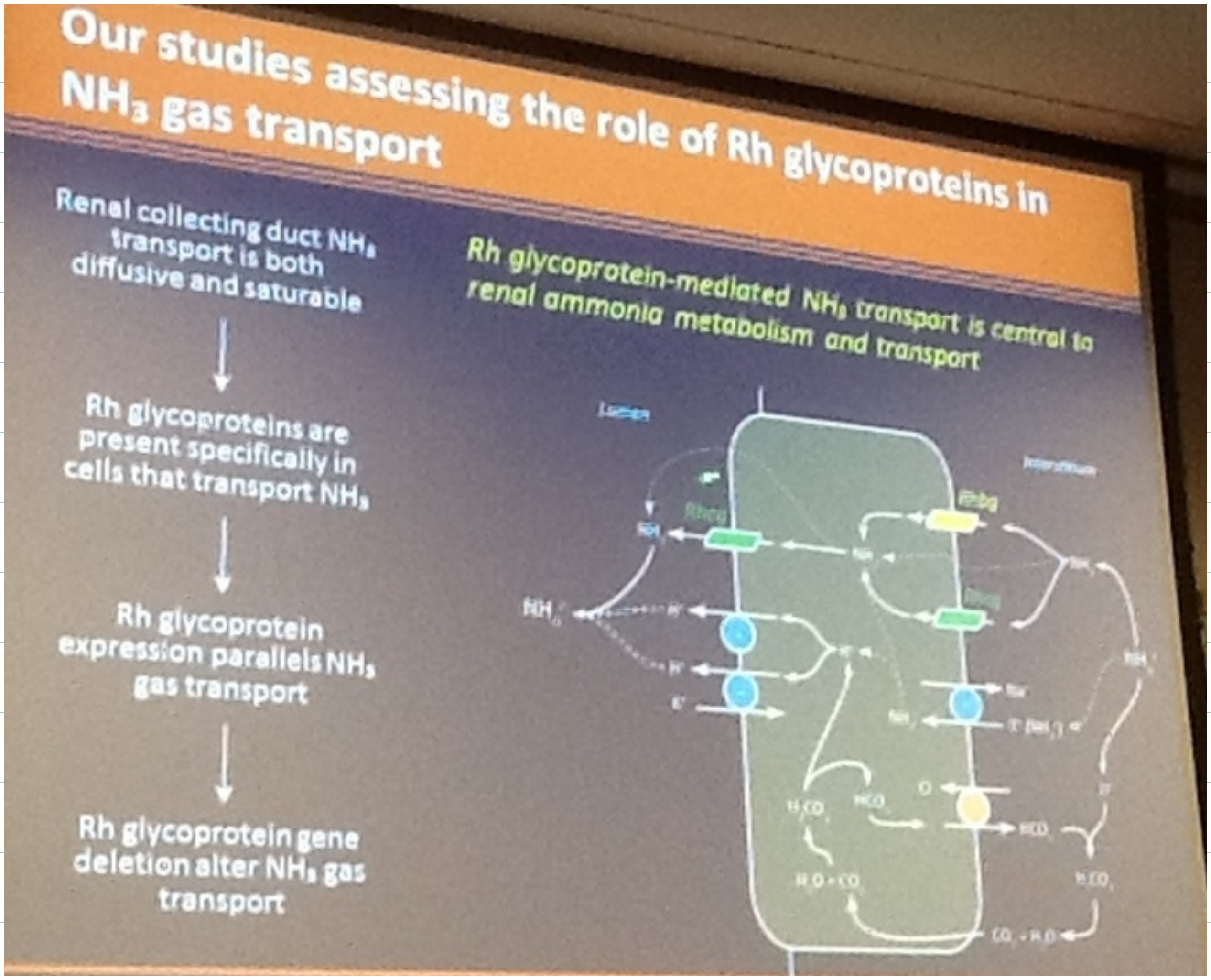


Conditions where Rhbg and/or Rhcg expression parallels ammonia transport

- Metabolic acidosis
  - Seshadri RM, et al, *AJP Renal* 290: F397-408, 2006.
  - Seshadri RM, et al, *AJP Renal* 290: F1443-52, 2006.
  - JM Bishop, et al. *AJP Renal* 299:F1067-77, 2010.
- Reduced renal mass
  - HY Kim, et al, *AJP Renal* 293:F1238-F1247, 2007.
- Ischemia-reperfusion injury
  - KH Han, et al, *AJP Renal* 293:F1342-F1354, 2007.
- Cyclosporine A-induced renal tubular acidosis
  - SW Lim, et al, *Nephron Exp Nephrology* 110:e49-58, 2008.
- Hypokalemia
  - KH Han, et al, *AJP Renal* 301:F823-F832, 2011
- Adaptive response to deletion of other acid-base transporters
  - Pendrin
    - Kim YH, et al, *AJP Renal* 289:F1262-F1271, 2005.
  - Collecting duct Rhcg
    - HW Lee, et al. *AJP Renal* 296:F1364-F1375, 2009.
  - Intercalated cell-specific Rhcg
    - HW Lee, et al, *AJP Renal* 299:F369-F379, 2010.
  - Intercalated cell-specific Rhbg
    - JM Bishop, et al. *AJP Renal* 299:F1065-F1077, 2010.

Conditional KO of Rhcg: ↓ Urinary excretion but $\not\Delta$ pH_a

Slowest renal response: NH_3 transport ... req. 4-5 days



Our studies assessing the role of Rh glycoproteins in NH_3 gas transport

Renal collecting duct NH_3 transport is both diffusive and saturable

↓

Rh glycoproteins are present specifically in cells that transport NH_3

↓

Rh glycoprotein expression parallels NH_3 gas transport

↓

Rh glycoprotein gene deletion alter NH_3 gas transport

Rh glycoprotein-mediated NH_3 transport is central to renal ammonia metabolism and transport

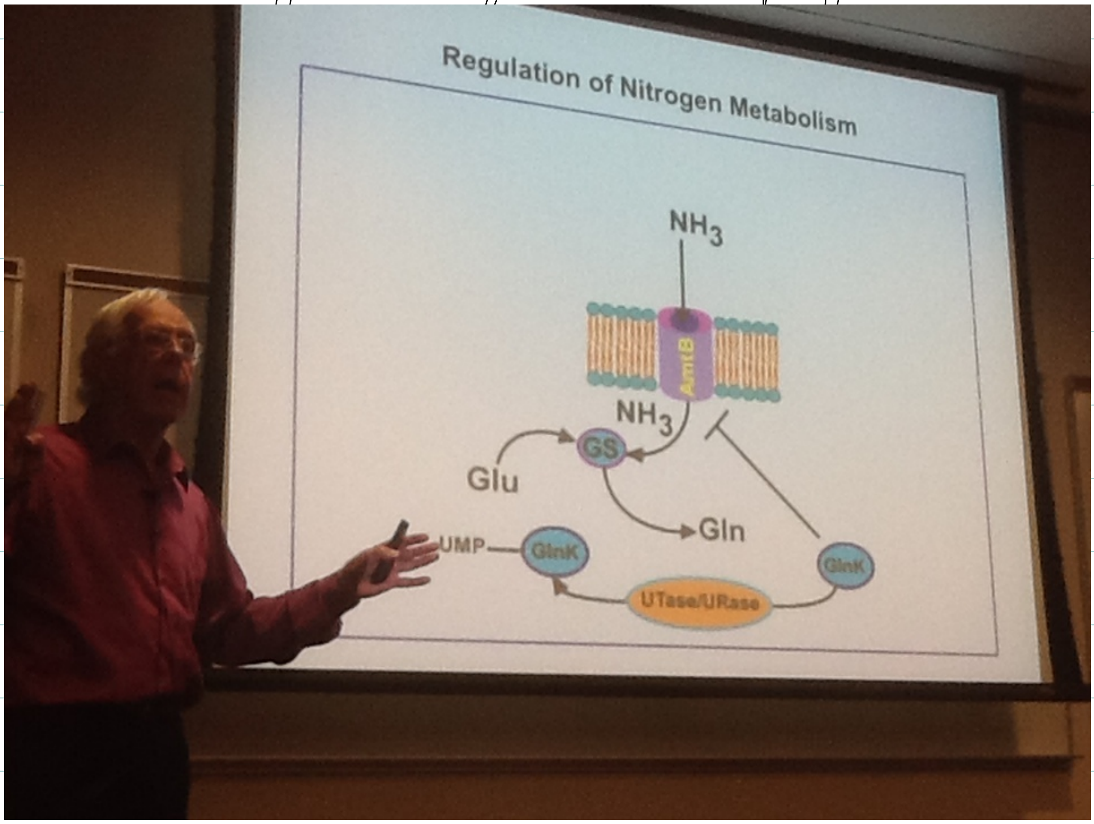
9/6/12 @ 4:00 PM
Gas Channels Worksop
Robert Stroud, UCSF
What do structures tell us about Gas Channels? QED!

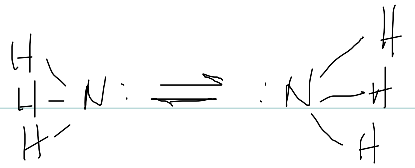
Much harder to discover channels for neutral substances (H_2O, 1990 ... gases only now).

QED: Quantum Electrodynamics

I. Rh Family (Amt / MEP)
Bact. Yeast

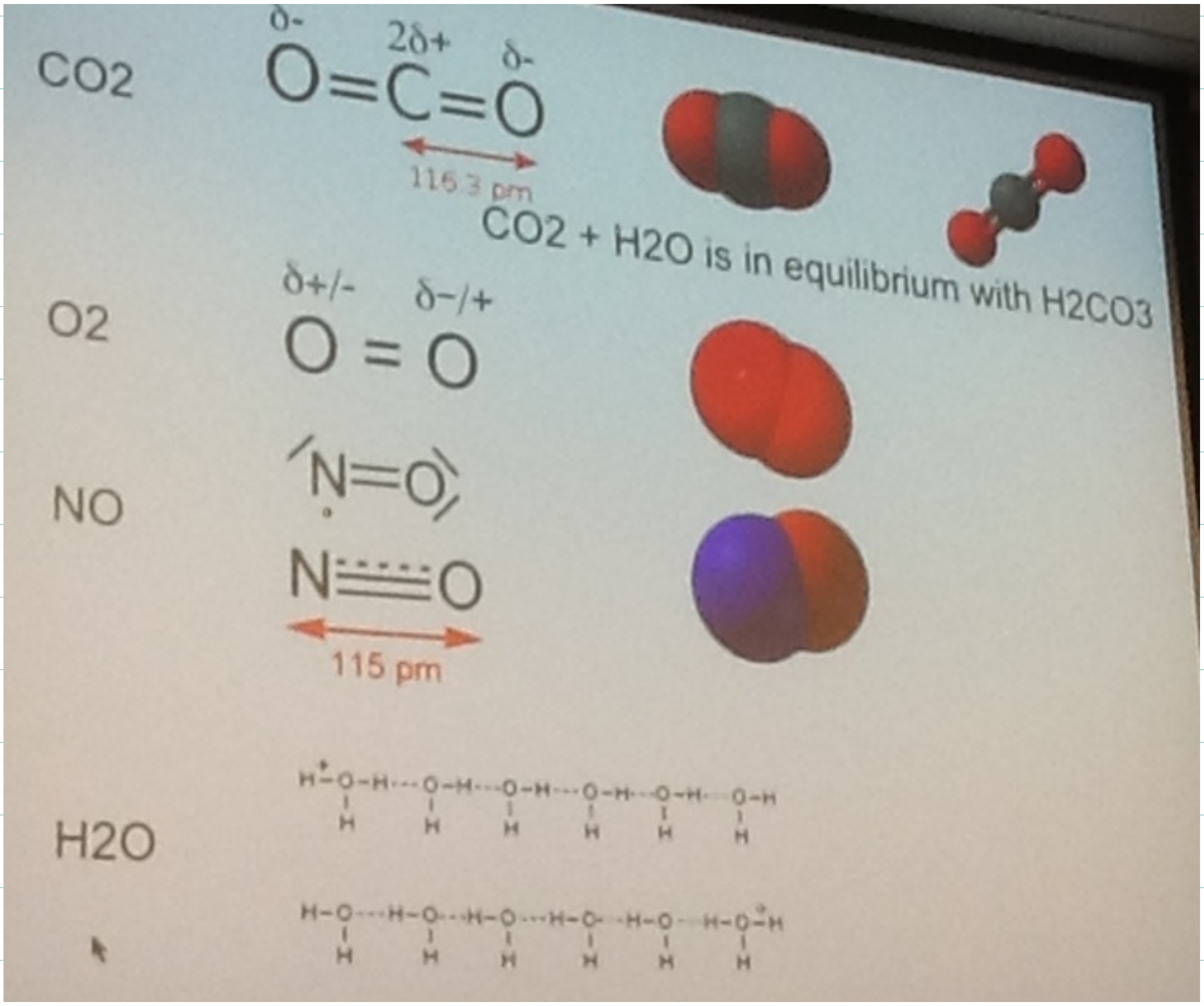
Bact: req. N as food source, pref. as NH_3


Regulation of Nitrogen Metabolism



Rapid inversion

Dipole moment ~ H_2O



CO2

CO2 + H2O is in equilibrium with H2CO3

O2

NO

H2O

Why is H_2O not a gas ... H-bonding

But H_2O can be a gas! WB

Some still think that Rh prot. are NH_4^+ channels!

AmtB : 1.35 Å

11 TMs

inverted repeat

NH_3: black hole of crystallography

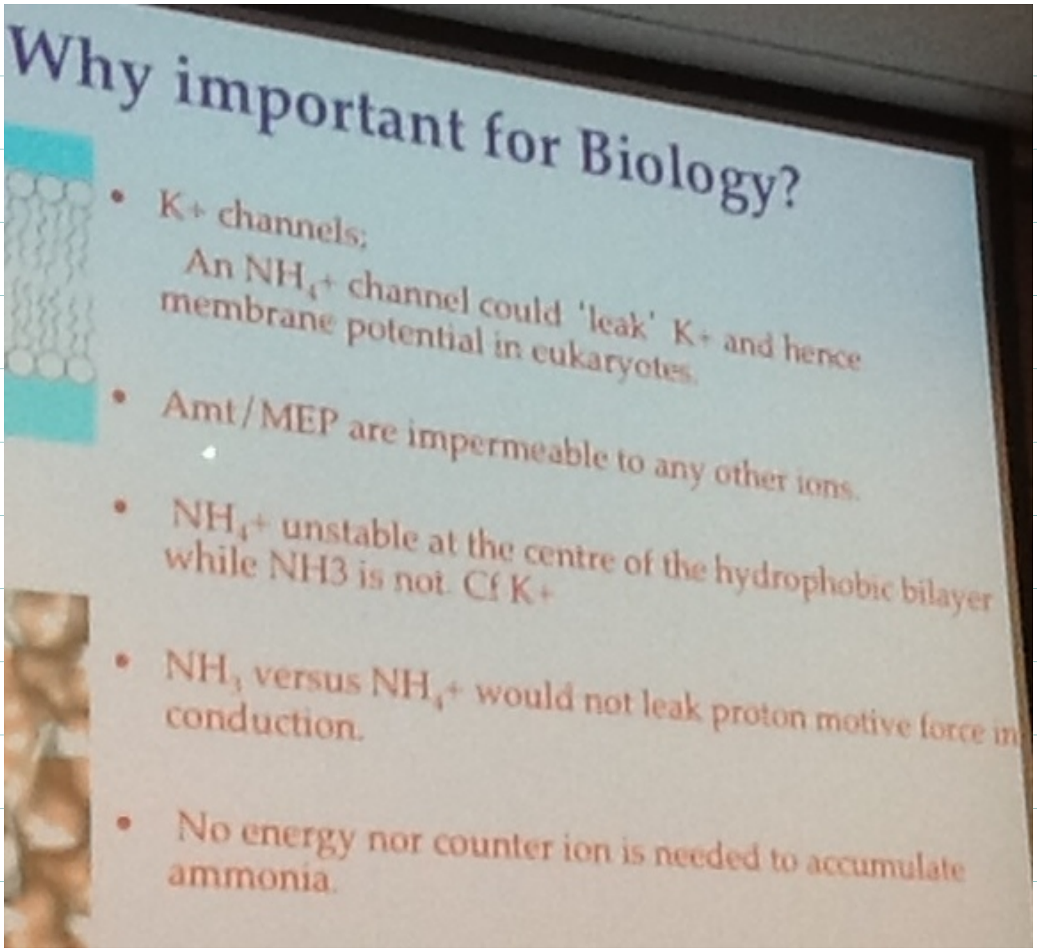
10 e^- also Na^+. Mg^{++}

Can also use $MeNH_3$

Channel : No H_2O. 1/7 occupancy by NH_3 @ 3 sites.
pore

$$\underbrace{pKa\ 9.6 \longrightarrow < 7}_{\text{binding of } NH_4^+}$$

No water, no ions



Why important for Biology?

- K+ channels;
  An NH_4+ channel could 'leak' K+ and hence membrane potential in eukaryotes.
- Amt/MEP are impermeable to any other ions.
- NH_4+ unstable at the centre of the hydrophobic bilayer while NH3 is not. Cf K+
- NH_3 versus NH_4+ would not leak proton motive force in conduction.
- No energy nor counter ion is needed to accumulate ammonia.

AmtB

Completely turned off when enough NH_3 is around!

GlnK: $\underbrace{\text{Finger points}}_{\text{ADP bound}}$ to AmtB & blocks it.

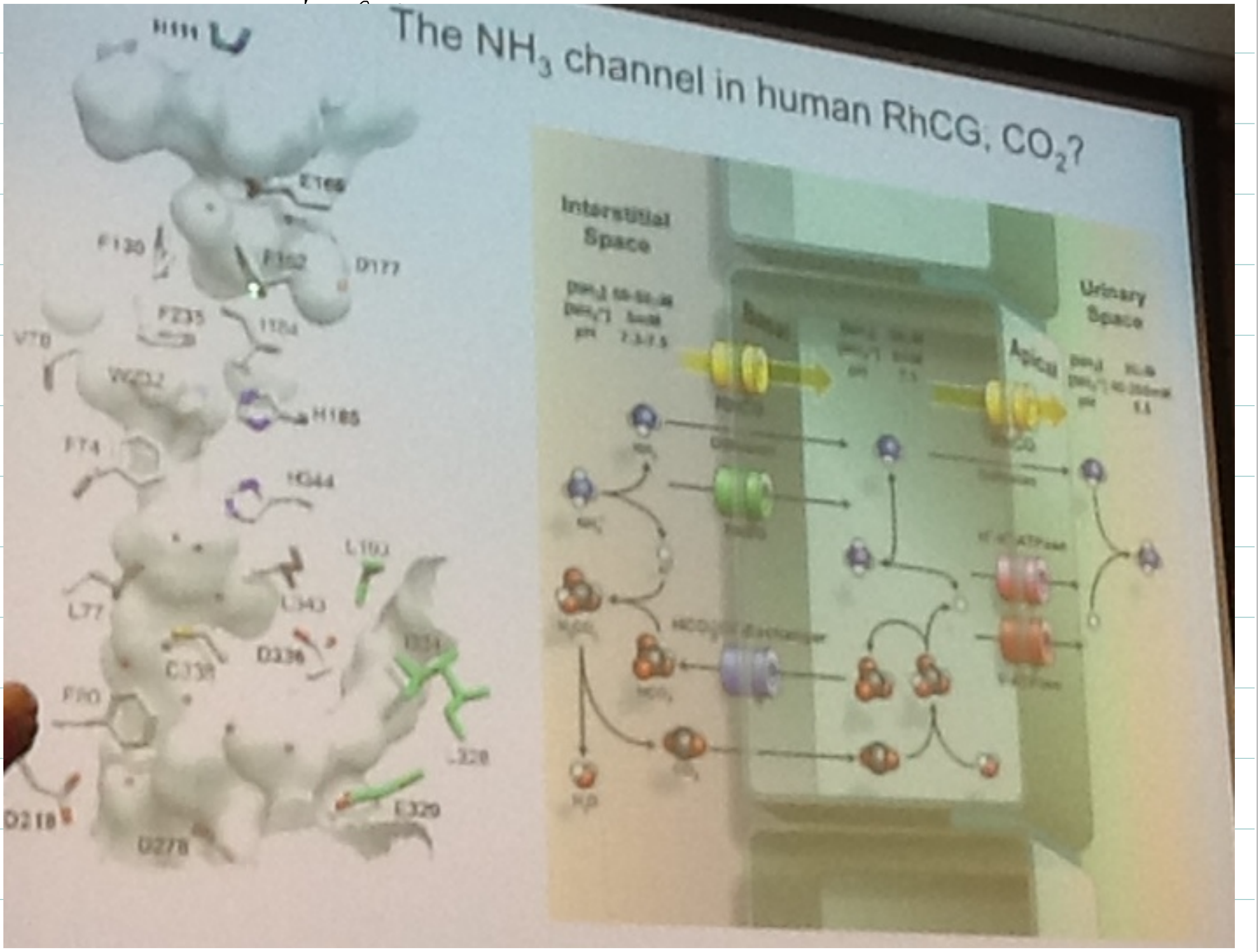
Prevents reprotonation of entering NH_3.

Nitrosomas europea ... more similar than Amt to mammalian Rh. ~~Has~~ a 'stake' extending into cytosol. (They do not pay much attn. to it)

NeRh

≠

RhCG: Expressed in HEK293 S



The NH_3 channel in human RhCG, CO_2?

"NIH Common Fund". 4th NIH Roadmap meeting

Nov. 28-30

SFO: Westin Hotel

II. Aquaporins

Glycerol is Water like

H_2C-OH
$HC-OH$
H_2C-OH

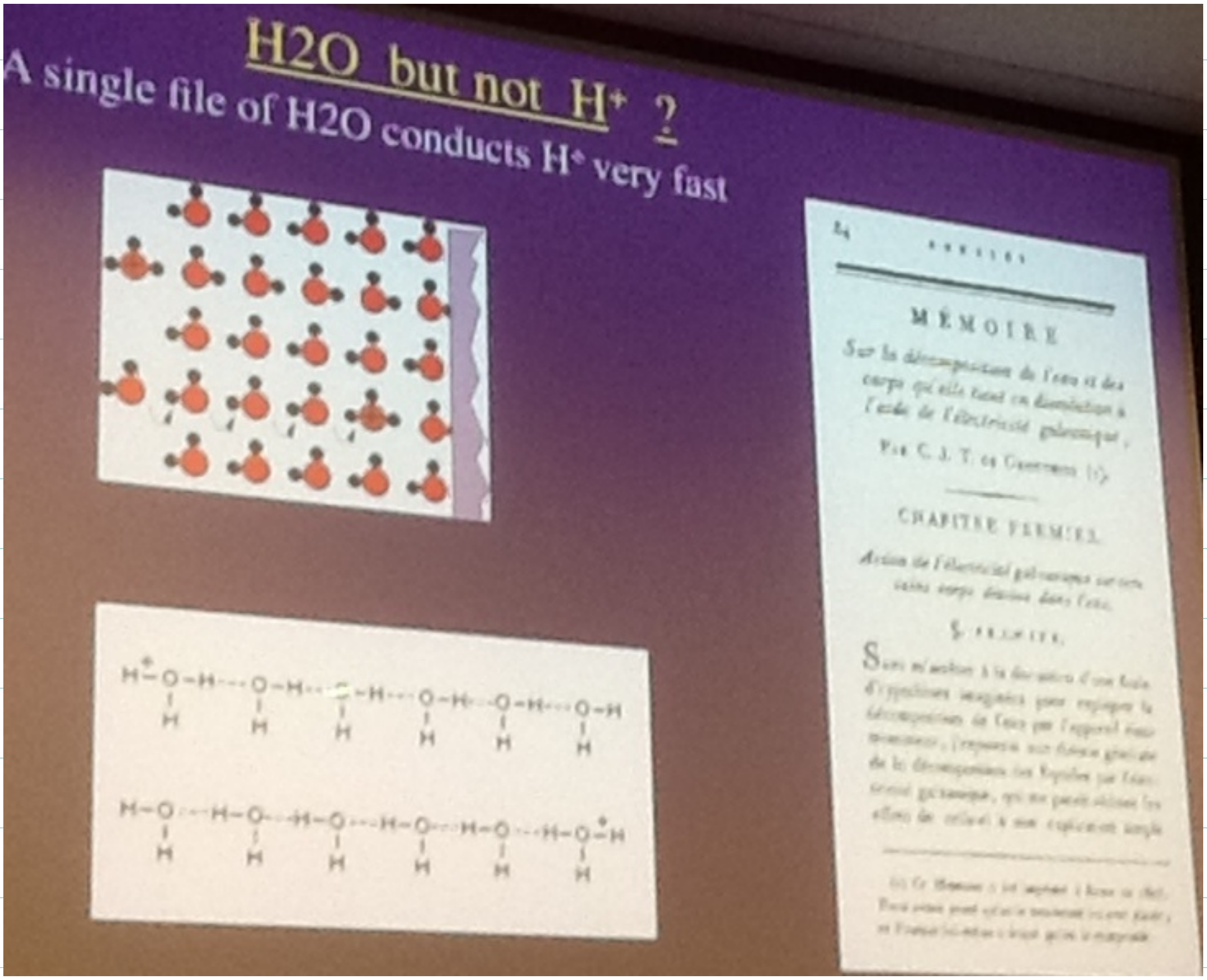
$H-OH$

PfAQP: Plasmodium → Glycerol

1.8 Å - Rotamers can be unambiguosly determined

9 H_2O molec. in a chain

- H_2O density clear
- Solutes can be identified



H2O but not H+ ?

A single file of H2O conducts H+ very fast

MÉMOIRE

CHAPITRE PREMIER.

Beitz et al PNAS, 2004

An AQP that transport H_2O & Glycerol both v. well.

↖ Malaria

One mutation: Glu → Ser (way up above aquapore) $\dashv$ H_2O permeability.

↓

Removes 1 H bond.

Channel holds on to H_2O.

9/7/12 @ 8:30 AM
Gas Channels Worksop
Ryan Geyer
O2 Transport in RBCs

Ryan: What was sampling rate?
Dead time?
9 wavelengths: PMT, array?
Fumbling c̄ details cost you control of presentation.
Showing non-case KOs s̄ their controls was shooting yourself in the foot.

{ Q_{10}: ?
{ What was Verkman's Q_{10} for P_f? 4?
Ck MCV: Is there a slv' Δ. Ck Hb.
{ Ck P_{50} in WT vs KO vs blockers.
{ 2,3 DPG, pH, etc ⤴ are addressed by P_{50}.

[WB interpretation: we'll be OK, but we have to dot i's & cross t's to be sure that your Δs are not due to something other than the cell membrane.]

9/7/12 @ 8:30 AM
Gas Channels Worksop
Rossana Occhipinti
Mathematical modeling

WB: Would be nice to have a dye to monitor pHs.

DRR: spelling error.

Jeff: Animal vs. Vegital poles
Bhanu: Optical tweezers → viscosity across the entire diameter.

9/7/12 @ 9:30 AM
Gas Channels Worksop
Xue Qin
CO2 permeability of AQP5

Emad : Rotamer search ... what is stable?