# Workshop on Gas Channels

The aforementioned grant supported a workshop on gas channels highlighting the work related to Gas Channels in Walter Boron's Laboratory. In addition, several faculty from other institutions were invited speakers at the workshop. 

### Subject Terms
Gas channels, aquaporins, Rh proteins, red blood cells, mass spectroscopy, modular dynamics, carbon dioxide (CO2), ammonia (NH3), surface-pH measurements, Xenopus oocytes.
I. **Heading**

A. **PI Name:** Walter F. Boron M.D, Ph.D.

B. **Organization:** Case Western Reserve University (CWRU)

C. **ONR Award Number:** N00014-12-1-0646

D. **Award Title:** Gas Channel Workshop
II. Scientific and Technical Objectives

The grant has two main objectives:

**Aim 1: Convene a Gas-Channel Workshop.** Topics to be discussed on Day 1 include: (A) Factors limiting gas permeable through the membrane’s lipid phase. (B) Molecular mechanism of gas permeation through AQPs and Rh proteins. (C) Physiological significance of gas channels. (D) O$_2$ channels. (E) Relevant technologies, including electrophysiology, stopped-flow techniques, mass spectrometry, x-ray crystallography, molecular dynamics and other modeling approaches. Day 2 will be devoted to assessing options for future work.

**Aim 2: Generate a Report of the Major Conclusions of the Workshop.** We will record the presentations (audio, video, as well as content of projector electronic whiteboard) and generate a written summary of the entire Workshop. We will also generate parallel multimedia presentations on our Website as well as in an iPad Textbook format.
III. Approach (N/A)
IV. Concise Accomplishments

We arranged a 2-day meeting—Thursday September 6 through Friday September 7, 2012—held in the Department of Physiology and Biophysics at Case Western Reserve University in Cleveland, Ohio. We hosted 7 outside speakers plus the PI, 3 postdoctoral participants from CWRU, and two additional attendees (see “Gas Channels Workshop Participants” in Appendix). On the evening preceding the Workshop, we held a welcoming reception at the PI’s home (funded privately). In addition, we held a dinner on Thursday evening (funded privately). Finally, several participants remained for an additional day for scientific discussions.

Aim 1 (Convene a Gas-Channel Workshop). On the first day of the Workshop (see “Gas Channels Workshop Agenda” in Appendix), the faculty participants made presentations on a range of carefully selected topics. These included a keynote lecture by Robert Stroud at 4PM that was attended by about 160. The other lectures were attended by 50-100. On the second day, three postdoctoral fellows made shorter presentations. In addition, we held a wide-ranging discussion in which we addressed specific questions on the future of gas-channel research.

Aim 2 (Generate a Report). We videotaped the lectures on Day 1 (see videos posted on the Website, described below), and recorded the more informal proceedings of Day 2. The videos are being edited and will be posted on the Website in the near future. In addition, we took notes of all Workshop activities, including the individual presentations (see “Workshop Notes on Presentations--Rossana” and “Workshop Notes on Presentations--Walter” in Appendix) and the General Discussion during the latter part of Day 2 (see “Workshop Notes--General Discussion” in Appendix). After the Workshop, we collected the presentations of all but one of the presenters. Finally, we created a site on the PI’s departmental Website (see http://physiology.case.edu/events/symposia/gas-channels-workshop-2012/), where we present the agenda, participants, notes, and links to the presentations. This site is available to all interested parties and will remain live indefinitely.

The consensus among the invited attendees as well as Clevelanders was that Workshop was a great success—in terms of the quality of the participants, the presentations, and the discussions that surrounded the formal presentations. Moreover, the participants strongly indicated that it would be most helpful for the group to get together regularly, perhaps next in 2014.
V. Expanded Accomplishments (N/A)
VI. Work Plan (N/A)
VII. Major Problems/Issues

The only negative aspect regarding the Workshop is that final permission to go forward came relatively late. Although we had tentative commitments from the participants, a firm commitment obviously depended upon the dates chosen for the Workshop and the calendars of the participants remaining open. Immediately upon getting approval for the Workshop, the ONR and the organizers at CWRU did an outstanding job to assemble a first-rate meeting. On the other hand, we could have attracted a few more outstanding speakers as well as representatives of other funding agencies if we had been able to give them more notice. Thus, I recommend that—if we do go ahead with a follow-up meeting, say in 2014—we announce the meeting 8-12 months in advance.
VIII. Technology Transfer

None.
IX. Foreign Collaborations and Supported Foreign Nationals

Two of the meeting participants, Gerolf Gros and Volker Endeward, are from Hannover in Germany.
X. Productivity (N/A)
XI. Award Participants

Military personnel: None

Walter F. Boron, M.D., Ph.D—PI

For other participants, see Gas Channels Workshop Participants in the Appendix.
Gas Channels Workshop

September 6 - 7, 2012

Case Western Reserve University
School of Medicine
Cleveland, Ohio

Sponsored by
The Office of Naval Research
Gas Channels Workshop

Thursday, September 6 & Friday, September 7, 2012

Department of Physiology and Biophysics
Case Western Reserve University
School of Medicine

Sponsored by
The Office of Naval Research
Welcome to the Workshop on Gas Channels, sponsored by the Office of Naval Research and the Department of Physiology and Biophysics in the School of Medicine at Case Western Reserve University.

The ONR handles the science and technology programs of the US Navy and Marine Corps. Divers, submariners, and individuals ascending to altitude may face a range of medical issues related to dissolved (or undissolved) gases. These include decompression illness, N₂ narcosis, O₂ toxicity, CO₂ narcosis, and hypoxia. Thus, the ONR has a strong and longstanding interest in gas transport.

The Department of Physiology and Biophysics at Case Western Reserve University is one of the few in the world that studies physiological problems from the level of the atom—through molecules, cellular organelles, whole cells, tissues, and organs—to the whole organism. We focus on the nervous, cardiovascular, and renal systems. In the past few years, we have recruited eight outstanding new faculty members. In addition, we completed a major renovation of about 40,000 gross square feet of space. We have also established three major core facilities to support our work: A Protein Expression, Purification, and Crystallization Core (PEPCC, 5th floor), a Molecular Biophysics Core (6th floor), and a Mouse Physiological Phenotyping Core (MPPC, basement).

We hope that you enjoy the Workshop. If during your visit you would like to see our facilities, we would be happy to arrange a tour.
WORKSHOP AGENDA  
Thursday, September 6, 2012

7:45 - 8:15am  Registration & Continental Breakfast

8:15am - 8:20am  Welcome/Introduction  
                   -Walter F. Boron, M.D., Ph.D.

8:20am - 9:00am  Walter Boron, M.D., Ph.D.  
                  Title: “Gas Channels”

9:00am - 9:10am:  Question/Answer Session

9:10am - 9:50am  Emad Tajkhorshid, Ph.D.  
                  Title: “Visualizing gas permeation pathways through proteins at sub-Angstrom resolution”

9:50am - 10:00am:  Question/Answer Session

10:00am - 10:25am:  Morning Break

10:25am - 11:05am  Gerolf Gros, Ph.D.  
                   Title: “Measuring cellular CO₂ permeability by $^{18}$O exchange—methodology and results on red blood cells”

11:05am - 11:15am:  Question/Answer Session

11:15am - 11:55am  Volker Endeward, Ph.D.  
                   Title: “Intrinsic CO₂ permeability of cell membranes and effect of cholesterol and aquaporin”

11:55am - 12:05pm:  Question/Answer Session
12:05pm - 1:05pm
Lunch –On your own
(Lunch provided for invited speakers in E-504)

1:05pm - 1:45pm
Bhanu Jena, Ph.D.
Title: “Involvement of elevated membrane cholesterol on G-protein regulated water and gas transport in biological membranes”

1:45pm- 1:55pm: Question/Answer Session

1:55pm - 2:35pm
Jeffrey Garvin, Ph.D.
Title: “NO transport by aquaporin 1”

2:35pm - 2:45pm: Question/Answer Session

2:45pm - 3:10pm: Afternoon Break

3:10pm - 3:50pm
David Weiner, M.D.
Title: “Role of Rh glycoproteins in gas transport — lessons from in vitro model systems”

3:50pm - 4:00pm: Question/Answer Session

4:00pm - 5:00pm
Robert Stroud, Ph.D.
Title: “What do structures of the Aquaporins, and Ammonia transporters tell us about conduction of gases?”
WORKSHOP AGENDA
Friday, September 7, 2012

8:00am - 8:30am
Continental Breakfast

8:30am – 8:35am
Introduction/Welcome
   - Dr. Walter Boron, M.D., Ph.D.

8:40am – 9:00am
Speaker: Ryan Geyer, Ph.D.
Title: “Role of membrane proteins in oxygen transport in red blood cells”

9:05am – 9:25am
Speaker: Rossana Occhipinti, Ph.D.
Title: “Mathematical modeling of gas movements in an oocyte”

9:30am - 9:50am
Speaker: Xue Qin, Ph.D.
Title: “Structure determinants for CO₂ transport of human aquaporin 5”

10:00am – 10:30am
Morning Break
(Refreshments served)

10:30am - 1:00pm
Gas Workshop Meeting

1:00pm - 2:00pm
Lunch

2:00pm - 3:30pm
Gas Workshop Meeting

3:30pm – End of Meeting
Dr. Boron is the David N. and Inez Myers/Antonio Scarpa Professor & Chairman of the Department of Physiology and Biophysics at Case Western Reserve University. He earned his AB in chemistry at Saint Louis University, and his M.D. and Ph.D. (Physiology & Biophysics) at Washington University in St. Louis. He joined Yale University as a post-doctoral fellow with Emile Boulpaep in 1978, and remained there for the next 29 years, serving as Chairman of the Department of Cellular & Molecular Physiology for three 3-year terms (1989-1998). In 2007 he returned to his hometown of Cleveland. He is the former President of the American Physiological Society (APS) and is currently Secretary-General of the International Union of Physiological Sciences (IUPS). He is the former editor-in-chief of Physiological Reviews and is the former editor-in-chief of Physiology. He and Emile Boulpaep co-edit the textbook Medical Physiology. He developed his life-long interest in acid-base transport and intracellular-pH regulation with his Ph.D. mentor Albert Roos as well as Paul De Weer, and his complementary interest in renal HCO₃⁻ transport with Boulpaep. His group currently focuses on three related areas: the molecular physiology of the Na⁺-coupled HCO₃⁻ transporters, molecular CO₂/HCO₃⁻ sensors, and gas channels. Among his previous honors are a Young Investigator Award (American Society of Nephrology/American Heart Association, 1986), the Robert F. Piits Award (IUPS, 1993), the Gottschalk Award (APS, 1998), an NIH MERIT Award (2002), the Homer Smith Award (ASN, 2005), the Sharphey-Schafer Award (The Physiological Society, 2008), and the Palade Gold Medal (shared with William Catterall and Richard Tsien, Wayne State University, 2010).
Emad Tajkhorshid received his initial training as a pharmacist from Tehran University. After attending two Ph.D. programs, one in medicinal chemistry and pharmacology at Tehran University of Medical Sciences and another one in molecular biophysics at the University of Heidelberg, he started his postdoctoral training in Computational Biophysics in the Theoretical and Computational Biophysics Group at the University of Illinois at Urbana-Champaign in 2001. In 2003 he became the assistant director of research of the NIH Center for Macromolecular Modeling and Bioinformatics at the Beckman Institute for Advanced Science and Technology. He started his independent career as an assistant professor of biophysics, biochemistry, and pharmacology in 2007 and was promoted to associate professor in 2010. The primary focus of his research is on understanding the structural and dynamical properties of membranes and membrane proteins as a basis for their biological function. Employing computational methodologies, his group investigates a wide range of membrane proteins and membrane-associated phenomena in biological systems, in particular the mechanisms of passive and active transport across the membrane.
Dr. Gros is Professor of Physiology at the Department of Physiology at the Medizinische Hochschule Hannover/Germany. He was Professor and Chairman of this Department from 1986 to 2008. He earned his MD degree in 1969 at the University of Tübingen/Germany, followed by one year of practical clinical work. In 1970 he joined Hannover Medical School as a postdoc with Waldemar Moll, and joined his mentor when he moved to the Department of Physiology at the University of Regensburg in 1972. Intermittently, he worked at the Department of Physiology with Robert E. Forster in 1973-1974. He obtained his “Habilitation” in Physiology after returning to Regensburg in 1976. From 1978-1986 he was Associate Professor of Physiology at the University of Essen, and thereafter moved to Hannover to become Full Professor and Department Chairman. He was President of the German Physiological Society in 2007, and President of the Annual Congress of Physiology held in Hannover in 2007. He developed a lifelong interest in CO₂ and O₂ transport in the body, in carbonic anhydrases and in acid-base physiology, initially stimulated by Waldemar Moll and Robert E. Forster. After his move to Hannover, he developed a second field of interest in studying the molecular mechanisms of skeletal muscle plasticity. His work was continuously supported by the Deutsche Forschungsgemeinschaft. His most recent interest is in the field of CO₂ channels in biological membranes, in combination with developing a novel method to determine the CO₂ permeability of cell membranes, and in the molecular mechanism of HCO₃⁻ transfer across the red cell membrane.
Dr. Endeward is presently Asst. Professor of Physiology in the Department of Physiology of the Medizinische Hochschule Hannover/Germany. From 1983-1995 he studied Physics at the University of Hannover and obtained his "Diplom" in 1995. Partly simultaneously, he studied Medicine at the Medizinische Hochschule Hannover from 1986-1995. From 1996 to 1997 he practiced Surgery at the Agnes-Karll hospital in Laatzen/Hannover. In 1998 he joined the Department of Physiology of Hannover Medical School and developed his research interests in CO₂ and O₂ transport and acid-base physiology in Gerolf Gros' laboratory. He has worked and published on several topics in these areas, but his main interest over the last years has been CO₂ channels in biological membranes. He has essentially contributed to the development of the mass spectrometric ¹⁸O exchange technique to measure CO₂ permeabilities of cell and vesicle membranes, including the complex mathematical description of this process and a numerical procedure to derive CO₂ and bicarbonate permeabilities from mass spectrometric measurements. He has further developed this technique by an analysis of the size and role of unstirred layers and by modelling the intracellular processes influencing the process of ¹⁸O exchange. He has presented a comprehensive experimental analysis of the role of aquaporin 1 as a CO₂ channel in the human red cell membrane, as well as the first report that the red cell Rhesus protein RhAG also acts as a CO₂ channel. Most recently he has shown that the intrinsic permeability of many biological membranes is low and identified the molecular cause of this property. In addition, he has presented a comprehensive reinvestigation of the so-called metabolon hypothesis, which proposes the existence of a functionally relevant complex of the anion exchanger 1 and carbonic anhydrase 2 in the red cell membrane. His scientific success was recognized by a special personal grant awarded to him by the Deutsche Forschungsgemeinschaft in 2009.
Dr. Bhanu Jena was born in a small town in Orissa, India, to Manju Prova and Prafulla K. Jena, a chemist. He spent his early childhood in several remote villages in India, where his grandfather practiced medicine. The dedication of his father and grandfather to science and medicine and their service to humanity greatly influenced his choice for a career in science. Dr. Jena majored in Chemistry, Zoology and Botany from BJB College in India (B.Sc., 1975) and studied Reproductive Endocrinology at Utkal University, (M.Sc., 1978). He graduated top of his class in the Masters program receiving the Prasant Ku. Memorial Prize and the Utkal University Gold Medal. In December 1988, Dr. Jena received his Ph.D. degree in Reproductive Endocrinology, and the Research Excellence Award from Iowa State University. Following postdoctoral training at Yale University, he joined Yale University as an Assistant Professor. In 2000, Dr. Jena moved to the Department of Physiology, at Wayne State University School of Medicine, as a tenured Professor, and Founder-Director of the Institute of NanoBioScience. His foray into science began 40 years ago, when he published his first scientific paper. His enquiry on how cells secrete, led to the serendipitous discovery of the “porosome” - a new cellular structure universally present at the cell plasma membrane, and involved in secretion. In early 2012, the neuronal porosome proteome was determined. The current focus of the laboratory is to further determine the structure and conformation of the neuronal porosome using cryo electron crystallography.
Jeffrey Garvin, Ph.D. is currently Professor of Physiology at Wayne State University and Division Head of the Hypertension and Vascular Research Division of Henry Ford Hospital. He received his B.S. from the University of Miami in Biology and Chemistry in 1979 and his Ph.D. from Duke University in 1984. Dr. Garvin did his postdoctoral training in the Laboratory of Kidney and Electrolyte Metabolism at the National Institutes of Health under Maurice Burg, Mark Knepper and Kenneth Spring where he was supported by a National Kidney Foundation fellowship and two National Research Service Awards. In 1988 he joined the Hypertension and Vascular Research Division of Henry Ford Hospital and became Division Head in 2009. His research deals with the regulation of transport processes in the kidney and how disregulation of these systems can contribute to hypertension. Currently he has more than 125 original publications on renal physiology. Dr. Garvin is a fellow of the Council for High Blood Pressure Research of the American Heart Association and has served on several NIH study sections. He also is an Associate Editor of The American Journal of Physiology: Renal Physiology. His research is now supported by three NIH grants, including a Program Project Grant entitled “Blood Pressure Regulation: Novel Roles for the Kidney.”
Dr. Weiner's primary research interests involve examining the mechanisms and regulation of renal ammonia metabolism and transport. Ammonia plays a central role in acid-base homeostasis, as it is the primary component of basal net acid excretion and changes in ammonia excretion comprise almost 90% of the renal response to acid-base alterations. Renal ammonia transport has traditionally been believed to involve "ammonium (NH₄⁺) trapping" and diffusive NH₃ movement.

Dr. Weiner's laboratory examines the specific mechanisms of renal NH₃ movement, and has shown that, in contrast to previously thought models, that NH₃ transport involves specific proteins, namely, Rh glycoproteins. These proteins are widely expressed in ammonia transporting tissues, and Dr. Weiner's studies, using a variety of in vivo and in vitro models, including transgenic animal models utilizing cell-specific gene deletion, have shown the central role of these proteins in renal ammonia, and thereby acid-base, homeostasis.
Dr. Stroud was the first to discover fundamental mechanisms of transmembrane proteins by Aquaporins at atomic resolution. These included GlpF, AqpZ, the eye lens AQP0, the H2S channel, and the essential glycerol channel of the malaria parasite P.falciparum. He defined the structure and regulatory mechanisms of the ammonia channel AmtB and the ‘Rh factors’. He revealed the atomic basis for ‘signal sequence’ dependant membrane protein synthesis, signaling by EPO (erythropoietin) via its receptors. Stroud also determined the mechanisms of enzyme drug targets thymidylate synthase, HIV protease, HIV integrase, and KSHV protease and used these to facilitate drug discovery for human health.

He was elected to the National Academy of Sciences (of the USA) in 2003, President of the Biophysical Society (of the United States) from 1986-1987, and Founding Fellow of the Society in 2000. Dr. Stroud is a member of the Committee for the International Union of Pure and Applied Biophysics. In 1984 he was elected the DeWitt Stetten Lec- turer of the National Institutes of Health (NIH). Dr. Stroud was elected as a Fellow of the Royal Society of Medicine (United Kingdom) in 1992.
**Case Western Reserve University**

**Boron Lab Post-doctorates**

**R. Ryan Geyer, Ph.D.** is currently a postdoctoral research fellow in the Department of Physiology and Biophysics at Case Western Reserve University. He received his B.A. in biology from Earlham College in 1998 and his Ph.D. in biochemistry and molecular biology from Wright State University in 2007. In 2008, Dr. Geyer joined Dr. Boron’s laboratory and has focused his attention towards elucidating the role of membrane proteins in red blood cell oxygen transport. Dr. Geyer is currently supported by a postdoctoral fellowship from the Office of Naval Research.

**Rossana Occhipinti, Ph.D.** joined Dr. Boron’s laboratory as a postdoctoral fellow in October of 2009 shortly after obtaining her Ph.D. in Applied Mathematics from Case. During her Ph.D. studies she developed mathematical models of cellular brain metabolism and numerical methods combining optimization algorithms with Bayesian statistics. She is currently developing mathematical models to investigate the movement of acid-base equivalents across the plasma membrane. In 2009, she received the Melvin H. Knisely International Award and in 2012 the Cell & Molecular Physiology Section Research Recognition Award. Her work is currently supported by an AHA Postdoctoral Fellowship.

**Xue Qin, Ph.D.** earned her Ph.D. in Pathophysiology at Peking University in China. In 2008 she joined Case Western Reserve University in Dr. Boron’s lab. Dr. Qin’s Ph.D. work was about the signaling pathway of Nitric Oxide, cGMP and Protein Kinase G in coronary arteries. In Dr Boron’s Lab, her research has mainly focussed on gas channels. Dr. Qin uses surface pH method to study the structural functional relationships of human aquaporin 5. Her work has been supported by American Heart Association Postdoctoral Fellowship. In 2010 she won the Cell & Molecular Physiology Section Research Recognition Award.
GAS CHANNELS WORKSHOP PARTICIPANTS

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Visualizing Gas Permeation Pathways Through Proteins at Sub-Angstrom Resolution

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Molecular Dynamics Simulations

Solving the Newtonian equations of motion for all particles at every time step

Major limitations:
- Time scale / sampling
- Force field approximations

Major advantages:
- Providing a dynamical description
- Unparalleled spatial and temporal resolutions, *simultaneously*

SPEED LIMIT

1 fs

nobelprize.org/nobel_prizes/chemistry/laureates/2003/animations.html
In situ Molecular Dynamics Simulations

Atom count: 100-500k
~10 ns/day on 128-1024 processors
100-500 ns for each system
Fast Growth of Computational Power

HP 735 cluster
12 processors (1993)

SGI Origin 2000
128 processors (1997)

PSC LeMieux AlphaServer SC
3000 processors (2002)

Ranger/Kraken
~60,000 processors (2007)

Blue Waters (UIUC)
200,000+ processors (2013)

Anton/DESHAW/PSC
512 processors (2010)
Capturing Biology at sub-Å Resolution

5 ns

200 ns

75 ns

20 ns

100 ns

100 ns
Large-Scale Transition of an ABC Transporter in the Membrane
IF ↔ OF transition in an MFS Transporter in Membrane

Number of water molecules (averaged over a 1 ns window)
Chemomechanical Coupling in GlpT

- IF-OF transition requires rearrangement of periplasmic salt bridges.
- Substrate affects these salt bridges.
- Less work is required to induce the transition when the substrate is bound.

apo state

P_i-bound state

PMF (kcal/mol) vs \( \theta_1 + \theta_7 \)
Explicit Ligand Sampling of Gas Transport

Explicit Ligand Sampling of Gas Transport

Lipid/Water Partition Coefficients

<table>
<thead>
<tr>
<th></th>
<th>Simulation</th>
<th></th>
<th>Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂ in POPE</td>
<td>3.50</td>
<td>CO₂</td>
<td></td>
</tr>
<tr>
<td>CO₂ in POPC</td>
<td>2.74</td>
<td>Octanol: 1.3</td>
<td></td>
</tr>
<tr>
<td>O₂(P) in POPC</td>
<td>4.04</td>
<td>Hexadecane: 1.5</td>
<td></td>
</tr>
<tr>
<td>O₂(N) in POPC</td>
<td>3.46</td>
<td>Olive oil: 1.7</td>
<td></td>
</tr>
<tr>
<td>O₂(P) in POPE</td>
<td>4.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O₂(N) in POPE</td>
<td>5.79</td>
<td>O₂</td>
<td></td>
</tr>
</tbody>
</table>

Experiment:

CO₂
- Octanol: 1.3
- Hexadecane: 1.5
- Olive oil: 1.7

O₂
- Liposome: 3.9
Gas Diffusion Inside the Lipid Bilayer

Diff. (10^{-5} \text{ cm}^2/\text{s})

Free volume (%)

$z (\text{Å})$

$\text{CO}_2$

$\text{O}_2$

free V
Gas Diffusion Inside the Lipid Bilayer

![Graph showing pressure-membrane force (PMF) distribution for CO2 (exp), O2 (exp), and O2 (imp) across different z (Å) values.](image-url)
Aquaporin Water/Gas Channels

Why Tetramers?
Implicit Ligand Sampling

\[ \mathcal{W}(\mathbf{r}) = -k_B T \ln \left[ \frac{\rho(\mathbf{r})}{\rho_0} \right] \]

\[ F(z) = -RT \ln \sum_{x,y=0}^{L_x,L_y} \frac{e^{-F(x,y,z)/RT}}{L_xL_y} \]

Cohen, et al., 2006; Wang, et al., 2007
Oxido-reductase and Proton Pump
Rapid $O_2$ Permeation via the Hydrophobic Channel in Cytochrome C Oxidase
O₂ Pathway in Cytochrome C Oxidase

Implicit ligand sampling

Observed Xenon binding in CcO ba₃ crystal structures

Explicit O₂ simulation

All located along the hydrophobic channel

Luna VM, Chen Y., Fee JA and Stout CD (2008) Biochemistry, 47, 4657-4665 (PDB entry 3BVD)

Luna VM, Fee JA, Deniz AA and Stout CD (2012) Biochemistry, 51, 4669-4676
Simulating Membrane Gas Transport

Identical total areas

Calculating permeation rate in MD simulations

AQP1       AQP4
CO₂       O₂       NO
Gas Transport through Aquaporins

Typical permeation events (300-400 ps)
Gas Occupancy/Permeation Radial Distribution

![Graph showing the density of gas molecules and permeations over different radii, with labels A, B, C, and D on the graphs.](image)
A Role for the Central Pore!

Central Pore

O₂

CO₂

CO₂

Water Pores
## Gas Transport through Aquaporins

<table>
<thead>
<tr>
<th>SYSTEM</th>
<th>TOTAL (100x100 Å²)</th>
<th>WATER PORES (4)</th>
<th>CENTRAL PORE (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equi POPE-CO₂</td>
<td>3</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Equi POPC-CO₂</td>
<td>5</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Equi POPC-O₂(P)</td>
<td>16</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Equi POPE-O₂(P)</td>
<td>11</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Press POPE-CO₂</td>
<td>168</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Press POPC-CO₂</td>
<td>160</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Press POPE-O₂(P)</td>
<td>310</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Press POPC-O₂(P)</td>
<td>208</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Press POPE-AQP1-CO₂</td>
<td>76</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Press POPE-AQP1-O₂(P)</td>
<td>79</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>
Free Energy Profiles for $\text{O}_2$ and $\text{CO}_2$

Major Barrier Generated by Structured Water

Barrier identified and characterized through combining the implicit and explicit approaches

NO• Permeation Through AQP4

NO• Permeation Through AQP4

--- Umbrella sampling
--- Implicit sampling

50 ns equilibrium simulation

Comparison of the Central Pore in AQP1 and AQP4
Gas Transport through Aquaporins

- Computational evidence for gas transport through a membrane channel

- Central Pore in AQPs is an optimal pathway for gas diffusion
  
  Shared by other oligomeric membrane proteins?

- AQPs can be physiologically relevant gas channels in lipid bilayer with low gas permeability

- We can simulate very efficiently the process of gas diffusion, but we rely heavily on reliable initial configurations of lipids/protein
Free Energy of $O_2$ Permeation Across Charged Lipid Bilayers

DOPS/Ca$^{2+}$

DOPS / Na$^+$

PMF (kcal/mol)

$z$ (Å)
Lipid Phase and Gas Permeation

Liquid phase (30 ns)  Gel phase (30 ns)

(c) Density / 10^3 Å³

Water x 0.1  CH₂ x 0.1  Water x 0.1

CH₃ x 0.1

(d) |S CD|

- TMCL (Lα phase)
- TMCL (Lβ phase)
Lipid Phase and Gas Permeation

PMF (kcal/mol)

-40  -20   0    20   40
z (Å)

- POPE
- TMCL(L_α phase)
- TMCL(L_β phase)
Highly Mobile Membrane Mimetic (HMMM) Model for Membrane Proteins and Phenomena

Collectively more than 150 ns of biased simulations capturing a single binding event.

**HMMM model**

Highly Mobile Membrane Mimetic model

---

**Advantages**

- Increased mobility of lipids
- Retain explicit headgroups allowing for atomic details
Spontaneous and Rapid Formation of a Bilayer

60 x 60 x 120 Å
DVPSs at 3 x 3 x 6 grid points
(22 ns)
HMMM- Preserving the “Face” of the Lipid Bilayer

Perfect match in the membrane profile particularly in the head group region

Critical for proper description of lipid protein interactions
HMMM – lipids are more mobile than full-lipids
Spontaneous Insertion of FVII-GLA
Spontaneous Membrane Binding

\(n = 10\)
Spontaneous Insertion of Transmembrane Helices

$t = 0$

50 x 50 x 75 Å
Glycophorin A monomers: 2
z-constraint on 2 carbonyl carbons

12 ns
Quantitative Characterization and Optimization of HMMM

Membrane Dipolar Potential

Electrostatic potential, \( \phi(Z) \) / V

Z / Å

Electrostatic potential, \( \phi(Z) \) / V

Z / Å
Quantitative Characterization and Optimization of HMMM PMF of Amino Acid Insertion

Tail-Gro - Stepwise transformation of HMMM to full membrane representation

Step-wise insertion of P-glycoprotein

Grow tails

HMMM

Full membrane

Josh Vermaas
Highly Mobile Membrane Mimetic Model (HMMM)

Facilitating dynamical studies of membrane-associated phenomena
Computational Structural Biology and Molecular Biophysics Group (CSBMB)
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- Raif Musa-Aziz
- Xue Qin
- Robert Gennis

Yi Wang

Saher Shaikh

R01-GM086749   U54-GM087519
R01-GM101048   P41-GM104601
Measuring Cellular CO₂ Permeability by ¹⁸O Exchange – Methodology and Results on Red Blood Cells

Gerolf Gros and Volker Endeward

Zentrum Physiologie
Medizinische Hochschule Hannover
Germany
Methods Available to Measure Membrane CO$_2$ Permeability

- Surface pH transients in Xenopus oocytes
- Kinetics of cellular CO$_2$ uptake recorded by intracellular pH measurement
- pH gradients in the surface region of epithelial cell layers
- Stopped flow rapid reaction spectrophotometry
- $^{18}$O exchange between CO$_2$, HCO$_3^-$ and H$_2$O
Earlier Measurements of $\text{CO}_2$ permeability of membranes

$P_{\text{CO}_2}$ of planar phospholipid bilayers from $\text{CO}_2$ flux measurements
- 0.35 cm/s (Gutknecht et al., 1977)
- 3.2 cm/s (Missner et al., 2008)

$P_{\text{CO}_2}$ of phospholipid vesicles by stopped flow spectrophotometry
- $\sim 10^{-3}$ cm/s (Prasad et al., 1998)
- $\sim 10^{-3}$ cm/s (Yang et al., 2000)
Can the kinetics of $\text{CO}_2$ and $\text{O}_2$ uptake by red cells be reliably measured by stopped flow techniques?

$t_{1/2}$ of $\text{CO}_2$ uptake by human red cells: 13 ms
(Holland and Forster, 1975)
continuous-flow rapid reaction apparatus

$t_{1/2}$ of $\text{CO}_2$ uptake by red cells by theory: ~ 12 ms
(Endeward et al., 2008)

$t_{1/2}$ of $\text{O}_2$ uptake by human red cells: ~ 80 ms
(Vandegriff and Olson, 1984)
Determining Membrane Permeabilities of CO$_2$ and HCO$_3^-$

by the $^{18}$O Exchange Technique

Has been applied to

- Isolated cells in suspension: red blood cells, MDCK and tsA201 cells
- Phospholipid vesicles in suspension
- Intact colon epithelium
Mass spectrometer

\[ \text{HC}^{18}\text{O}^{16}\text{O}^- + \text{H}^+ \rightarrow \frac{2}{3} \text{H}_2^{16}\text{O} + \frac{1}{3} \text{C}^{18}\text{O}^{16}\text{O} \]

\[ \text{H}_2^{18}\text{O} + \text{C}^{16}\text{O}_2 \]
\[
\frac{d[C^{18}O^{16}O]_{\text{ex}}(t)}{dt} = -k_u A_{\text{ex}} [C^{18}O^{16}O]_{\text{ex}}(t) + \frac{2k_u}{3K_1} A_{\text{ex}} [H^+]_{\text{ex}} [HC^{18}O^{16}O_2]_{\text{ex}}(t) + P_{CO_2} a \frac{v}{1-v} \left( [C^{18}O^{16}O]_{\text{in}}(t) - [C^{18}O^{16}O]_{\text{ex}}(t) \right)
\]

\[
\frac{d[C^{18}O^{16}O]_{\text{in}}(t)}{dt} = -k_u A_{\text{in}} [C^{18}O^{16}O]_{\text{in}}(t) + \frac{2k_u}{3K_1} A_{\text{in}} [H^+]_{\text{in}} [HC^{18}O^{16}O_2]_{\text{in}}(t) - P_{CO_2} a \left( [C^{18}O^{16}O]_{\text{in}}(t) - [C^{18}O^{16}O]_{\text{ex}}(t) \right)
\]

\[
\frac{d[HC^{18}O^{16}O_2]_{\text{ex}}(t)}{dt} = k_u A_{\text{ex}} \left( [C^{18}O^{16}O]_{\text{ex}}(t) + [CO_2] \frac{[H_2^{18}O]_{\text{ex}}(t)}{[H_2O]} \right) - \frac{k_u}{K_1} A_{\text{ex}} [H^+]_{\text{ex}} [HC^{18}O^{16}O_2]_{\text{ex}}(t) - P_{HCO_3^-} a \frac{v}{1-v} \left( [H^+]_{\text{ex}} [HC^{18}O^{16}O_2]_{\text{ex}}(t) - [HC^{18}O^{16}O_2]_{\text{in}}(t) \right)
\]

\[
\frac{d[HC^{18}O^{16}O_2]_{\text{in}}(t)}{dt} = k_u A_{\text{in}} \left( [C^{18}O^{16}O]_{\text{in}}(t) + [CO_2] \frac{[H_2^{18}O]_{\text{in}}(t)}{[H_2O]} \right) - \frac{k_u}{K_1} A_{\text{in}} [H^+]_{\text{in}} [HC^{18}O^{16}O_2]_{\text{in}}(t) - P_{HCO_3^-} a \left( [H^+]_{\text{in}} [HC^{18}O^{16}O_2]_{\text{in}}(t) - [HC^{18}O^{16}O_2]_{\text{in}}(t) \right)
\]

\[
\frac{d[H_2^{18}O]_{\text{ex}}(t)}{dt} = \frac{1}{3K_1} \left( k_u A_{\text{ex}} [H^+]_{\text{ex}} [HC^{18}O^{16}O_2]_{\text{ex}}(t) - k_u A_{\text{ex}} \frac{[CO_2]}{[H_2O]} [H_2^{18}O]_{\text{ex}}(t) + P_{H_2O} a \frac{v}{1-v} \left( [H_2^{18}O]_{\text{in}}(t) - [H_2^{18}O]_{\text{ex}}(t) \right) \right)
\]

\[
\frac{d[H_2^{18}O]_{\text{in}}(t)}{dt} = \frac{1}{3K_1} \left( k_u A_{\text{in}} [H^+]_{\text{in}} [HC^{18}O^{16}O_2]_{\text{in}}(t) - k_u A_{\text{in}} \frac{[CO_2]}{[H_2O]} [H_2^{18}O]_{\text{in}}(t) - P_{H_2O} a \left( [H_2^{18}O]_{\text{in}}(t) - [H_2^{18}O]_{\text{ex}}(t) \right) \right)
\]

**Fig.3**
Why can we observe fast processes on such a slow time scale, allowing us to follow these processes by mass spectrometry?
Kinetics of CO₂ hydration reaction vs. that of ¹⁸O exchange
It was shown here that a time course of the decay of $[C^{18}O^{16}O]$ that is measurable by mass spectrometry, is observed when the volume fraction of human red cells is extremely small, i.e. $2 \times 10^{-4}$. Raising this volume fraction by a factor of 10, to 0.002, renders the signal already too fast compared to the time resolution of the mass spectrometer in combination with the inlet system.

It is concluded that the process of $^{18}$O exchange can be slowed down by orders of magnitude, because it is possible to use extremely small amounts of red cells and still obtain a well-defined and clear signal.

Also for this reason, the $^{18}$O exchange technique allows us to observe fast processes such as the uptake of CO$_2$ by red cells on a very slow time scale.
How well are $P_{CO2}$ and $P_{HCO_3-}$ defined by the experimental curves of decay of $[C^{18}O^{16}O]$?
It was shown here that a well-defined minimum exists for both $P_{\text{HCO}_3}\text{-}$ and $P_{\text{CO}_2}$ in the sum of squares of deviations between the experimental data of $[\text{C}^{18}\text{O}^{16}\text{O}]$ and those obtained from the best-fit calculation.

When $P_{\text{HCO}_3}$- and $P_{\text{CO}_2}$ are varied over a wide range of values, clearly only one well-defined minimum is apparent and no local minima whatsoever are visible.
The graph shows the change in the concentration of $[C^{18}O^{16}O]$ over time $t$ (in seconds). The graph demonstrates a linear decrease in concentration, indicating an uncatalysed reaction. The rate constant $A = 4$ is labeled on the graph.
$P_{HCO_3^-} = 0.0015 \text{ cm/s}$

$[C^{18}O^{16}O] - [C^{18}O^{16}O]$ over time $t$ with different $P_{CO_2}$ values:

- $P_{CO_2} = 0.15 \text{ cm/s}$
- $P_{CO_2} = 0.015 \text{ cm/s}$
- $P_{CO_2} = 0.0015 \text{ cm/s}$

$A_e = 4$
Sensitivity of calculated $P_{CO_2}$ to parameter values
The diagram illustrates the percent change in $P_{CO_2}$ for various factors. The $x$-axis represents the factors: $a + 10\%$, $a - 10\%$, $K'1 + 1\%$, $K'1 - 1\%$, $Ai + 5\%$, $Ai - 5\%$, $pHe + 0.01$, $pHe - 0.01$, $pHi + 0.1$, $pHi - 0.1$, $PH2O + 10\%$, and $PH2O - 10\%$. The $y$-axis represents the percent change in $P_{CO_2}$ ranging from -40 to 30.
To what extent do unstirred layers around cells affect the permeability determinations?
Fig. 1
thickness of unstirred layer $\delta \sim$

kinematic viscosity $\nu \times \sqrt{\text{cell diameter } \ell}$

\[ \frac{1}{P_{CO_2,\text{app}}} = \frac{1}{P_{CO_2,\text{mem}}} + \frac{\delta_{UL_e}}{D_{CO_2,\text{solution}}} \]
Viscosity ($10^{-6} \text{ m}^2/\text{s}$)

<table>
<thead>
<tr>
<th>Temperature</th>
<th>$P_{\text{app}}$ in saline (cm/s)</th>
<th>$P_M$ (cm/s)</th>
<th>$\delta$ in saline (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>37 °C</td>
<td>0.12</td>
<td>0.16</td>
<td>0.5</td>
</tr>
</tbody>
</table>
$\delta \sim \nu$

37°C

Unstirred layer (µm)

Viscosity ($10^{-6}$ m²/s)
CO$_2$ Permeability of Normal and Deficient Human Red Blood Cells
$P_{CO_2}$ of control and AQP1 deficient (Colton null) human red blood cells

Endeward et al., FASEB J, 2006
Endeward et al., 2006
$P_{CO_2}$ of control and Rhesus null human red blood cells

Endeward et al., FASEB J, 2008
Human Red Blood Cell

Endeward et al.,
2006, 2008
Applying the $^{18}$O technique to measure the CO$_2$ permeability of the apical membrane of intact colon epithelium
thermostatted water jacket

pH electrode
tepon plug

colon mucosa on teflon cylinder

magnetic stirrer

stirring bar

teflon membrane on sintered glass disc

to mass spectrometer
Intact Proximal Epithelium
Apical Side

![Graph showing the change in [C^{18}O^{16}O] (10^{-5} M) over time (s) for intact proximal epithelium on the apical side. The graph shows a decrease in [C^{18}O^{16}O] with time, indicating a decrease in CA activity. An arrow labeled 'Epithelium + extracellular CA inhibitor' is present, indicating the addition of an extracellular CA inhibitor to the system.]
### CO$_2$ and HCO$_3^-$ Permeability of the Apical Membrane of Intact Guinea Pig Colon

<table>
<thead>
<tr>
<th></th>
<th>$P_{CO2}$ ± SD (cm/s)</th>
<th>$P_{HCO3^-}$ (cm/s)</th>
<th>$A_{in}$</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact Proximal Colon</td>
<td>0.0015 ± 0.0007</td>
<td>6.3 · 10$^{-4}$ ± 4.0 · 10$^{-4}$</td>
<td>41 000</td>
<td>40</td>
</tr>
<tr>
<td>Intact Distal Colon</td>
<td>0.00077 ± 0.00021</td>
<td>0.87 · 10$^{-4}$ ± 0.56 · 10$^{-4}$</td>
<td>900</td>
<td>23</td>
</tr>
</tbody>
</table>

Endeward & Gros, 2005
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 $P_{CO_2}$ and $P_{HCO_3^-}$ from well defined minima of a fitting procedure.

$P_{CO_2}$ values can be determined over a range of 3-4 orders of magnitude.

Parameters critical for calculation of $P_{CO_2}$ and $P_{HCO_3^-}$ are intracellular CA activity and extracellular pH, both of which are carefully controlled.

Unstirred layers affect the results by no more than ~ 20%.

The method is applicable to suspensions of isolated cells or vesicles and to intact epithelia.
Intrinsic CO$_2$ permeability of cell membranes and role of CO$_2$ channels

Volker Endeward, Fabian Itel, Samer Al-Samir, Mohamed Chami, Fredrik Öberg, Kristina Hedfalk, Gerolf Gros

MH Medizinische Hochschule Hannover
Intrinsic CO₂ permeability of a red cell membrane

<table>
<thead>
<tr>
<th></th>
<th>( P_{CO2} \text{(cm/s)} )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Red cell</strong></td>
<td>0.15 ± 0.08</td>
</tr>
<tr>
<td><strong>Red cell</strong></td>
<td></td>
</tr>
<tr>
<td>( \varnothing ) AQP1,</td>
<td></td>
</tr>
<tr>
<td>( \varnothing ) functional Rh</td>
<td>0.015 ± 0.003</td>
</tr>
</tbody>
</table>
Gas permeability of synthetic phospholipid bilayers

**IONS**
- $H^+$, $Na^+$
- $HCO_3^-$, $K^+$
- $Ca^{2+}$, $Cl^-$
- $Mg^{2+}$

**LARGE UNCHARGED POLAR MOLECULES**
- glucose
- sucrose

**SMALL UNCHARGED POLAR MOLECULES**
- $H_2O$
- urea
- glycerol

**HYDROPHOBIC MOLECULES**
- $O_2$
- $CO_2$
- $N_2$
- benzene

Alberts et al.
Molecular Biology Of The Cell, 4th Edition
1. What are the intrinsic $\text{CO}_2$ permeabilities of cell membranes?

2. Which mechanisms are responsible for the given intrinsic permeabilities of cell membranes?
1. What are the intrinsic CO$_2$ permeabilities of cell membranes?
$\text{Cell} \xrightarrow{\text{HC}^{18}\text{O}^{16}\text{O}_2^- + \text{H}^+} \xrightarrow{2/3} \text{H}_2^{16}\text{O} + \text{C}^{18}\text{O}^{16}\text{O}$

$\xrightarrow{1/3} \text{H}_2^{18}\text{O} + \text{C}^{16}\text{O}_2$

$\xrightarrow{1/3} \text{H}_2^{18}\text{O} + \text{C}^{16}\text{O}_2$

$\xrightarrow{2/3} \text{H}_2^{16}\text{O} + \text{C}^{18}\text{O}^{16}\text{O}$

$\text{Mass spectrometer}$
Cell membranes show CO$_2$ permeabilities lower than synthetic lipid bilayer

<table>
<thead>
<tr>
<th></th>
<th>$P_{CO_2}$ (cm/s) ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetic lipid bilayer</td>
<td>0.35 - 3.2</td>
</tr>
<tr>
<td>Red cell, Ø functional gas channel</td>
<td>0.015 ± 0.003</td>
</tr>
<tr>
<td>MDCK</td>
<td>0.017 ± 0.004</td>
</tr>
<tr>
<td>tsA201</td>
<td>0.007 ± 0.003</td>
</tr>
<tr>
<td>Basolateral membrane of proximal colon epithelium</td>
<td>~ 0.022</td>
</tr>
<tr>
<td>Apical membrane of proximal colon epithelium</td>
<td>0.0015 ± 0.0006</td>
</tr>
</tbody>
</table>
2. Which mechanisms are responsible for the given intrinsic permeabilities of cell membranes?
<table>
<thead>
<tr>
<th>Parameter studied</th>
<th>Cholesterol fraction of total bilayer lipids (mol %)</th>
<th>Ratio of parameter w over w/o cholesterol</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{\text{NH3}}$</td>
<td>30 %</td>
<td>0.31</td>
<td>Antonenko et al. 1997</td>
</tr>
<tr>
<td>$P_{\text{NH3}}$</td>
<td>52 %</td>
<td>0.012</td>
<td>Hill &amp; Zeidel 2000</td>
</tr>
<tr>
<td>$P_{\text{H2O (f)}}$</td>
<td>40 %</td>
<td>0.18</td>
<td>Lande et al. 1995</td>
</tr>
<tr>
<td>$P_{\text{H2O (f)}}$</td>
<td>52 %</td>
<td>0.026</td>
<td>Hill &amp; Zeidel 2000</td>
</tr>
<tr>
<td>$P_{\text{H2O (d)}}$</td>
<td>66 % (L+Chol)</td>
<td>0.26</td>
<td>Finkelstein 1976</td>
</tr>
<tr>
<td>&quot;</td>
<td>66 % (SM + Chol)</td>
<td>0.037</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

PC:PS:Chol – vesicles with different cholesterol content

PC = Phosphatidylcholine
PS = Phosphatidylserine
Chol = Cholesterol (0 – 70%)

Ø = ~ 150 nm
\[ \text{[C}^{18}\text{O}_{16}\text{O}]_0 - \text{[C}^{18}\text{O}_{16}\text{O}]_\infty \] (µM)

70% chol.

30% chol.

time (s)
Effect of cholesterol on lipid vesicle CO$_2$ permeability

PC:PS = 8:2
Comparison of cell membranes and cholesterol-containing vesicles

<table>
<thead>
<tr>
<th></th>
<th>$P_{CO2}$ (cm/s) ± S.D</th>
<th>Cholesterol content (Mol%)</th>
<th>$P_{CO2}$ predicted from cholesterol effect in vesicles (cm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid bilayer</td>
<td>0.35 / 3.2</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Red cell: ∅ AQP1, ∅ functional Rh</td>
<td>0.015 ± 0.003</td>
<td>45</td>
<td>0.010</td>
</tr>
<tr>
<td>MDCK</td>
<td>0.017 ± 0.004</td>
<td>37</td>
<td>0.015</td>
</tr>
<tr>
<td>tsA201</td>
<td>0.007 ± 0.003</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Basolateral membrane prox colon epithelium</td>
<td>~ 0.022</td>
<td>42</td>
<td>0.011</td>
</tr>
<tr>
<td>Apical membrane of prox colon epithelium</td>
<td>0.0015 ± 0.0006</td>
<td>77</td>
<td>0.0016</td>
</tr>
</tbody>
</table>
CO$_2$ permeabilities of cell membranes appear to be essentially determined by their cholesterol content
cholesterol depletion with β-cyclodextrin

cholesterol enrichment with β-cyclodextrin
Is cholesterol the cause of the low CO$_2$ permeability of MDCK cells?

Reduction of cholesterol with cyclodextrin raises P$_{\text{CO}_2}$.

Enrichment with cholesterol lowers P$_{\text{CO}_2}$ compared to normal cells.
• We show that cell membranes possess a low intrinsic CO$_2$ permeability, often in the range of 0.01 cm/s.

• This permeability is 2, and in one case 3, orders of magnitude lower than the CO$_2$ permeability of pure artificial phospholipid bilayers.

• The main cause of this low CO$_2$ permeability is the cholesterol content of the cell membrane. With increasing cholesterol content P$_{CO2}$ decreases in artificial vesicles as well as in intact cells.
Physiological consequences of low CO₂ membrane permeabilities

1. Consequences of the extremely low CO₂ permeability of the apical membrane of colon epithelium

2. Effect of low CO₂ membrane permeability on red blood cell gas exchange
Consequences of low apical CO$_2$ permeability in colonocytes

\[ P_{CO_2} = 0.0015 \text{ cm/s} \]
Example of a cell with a high gas exchange: red blood cell

<table>
<thead>
<tr>
<th></th>
<th>$P_{CO_2}$ (cm/s)</th>
<th>$t_{95}$ (ms)</th>
<th>transit time lung capillary (ms)</th>
<th>transit time heavy exercise (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal membrane resistance</td>
<td>0.15</td>
<td>110</td>
<td>700</td>
<td>350</td>
</tr>
<tr>
<td>permeability $\varnothing$ functional gas channel</td>
<td>0.01</td>
<td>1000</td>
<td>700</td>
<td>350</td>
</tr>
</tbody>
</table>
• 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 channels in the membrane.
Aquaporin 1 as a CO$_2$ channel in cholesterol-containing lipid vesicles

Incorporation of AQP1 into vesicles causes a rise in P$_{CO2}$

Change of P$_{CO2}$ in vesicles with decreasing Lipid-Protein-Ratios (LPR)
DIDS reduces the $\text{CO}_2$ permeability of AQP1 containing vesicles
Aquaporin 1 as a CO$_2$ channel in MDCK cells

Expression of AQP1 in MDCK cells raises P$_{CO2}$
• We conclude that in a membrane of normal cholesterol content and low \( \text{CO}_2 \) permeability, incorporation of AQP1 into the membrane significantly increases the \( \text{CO}_2 \) permeability in a concentration dependent manner.

• AQP1 acts as a DIDS-sensitive \( \text{CO}_2 \) channel.
<table>
<thead>
<tr>
<th>Gas</th>
<th>CO₂</th>
<th>O₂</th>
<th>NO</th>
<th>N₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid-water partition coefficient</td>
<td>0.95</td>
<td>2.9</td>
<td>3.8</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>CO$_2$</td>
<td>O$_2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>--------------</td>
<td>-------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipid-water partition coefficient</td>
<td>0.95</td>
<td>2.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduction of membrane permeability by cholesterol</td>
<td>1/100</td>
<td>(1/100) ?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Membrane permeability</td>
<td>0.01 cm/s</td>
<td>(0.03 cm/s) ?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart muscle under heavy exercise: partial pressure difference across the membrane $\Delta P$</td>
<td>5 mmHg</td>
<td>(40 mmHg) ?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Summary

With rising cholesterol content the $\text{CO}_2$ permeability ($P_{\text{CO}_2}$) of lipid vesicles decreases drastically.

The intrinsic $P_{\text{CO}_2}$ of cell membranes is low due to their cholesterol content:

1) cell membranes and lipid vesicles with identical cholesterol content exhibit identical $\text{CO}_2$ permeability

2) cholesterol-depleted cell membranes have an increased $\text{CO}_2$ permeability, cholesterol-enriched cell membranes a reduced permeability

Cell membranes with normal cholesterol raise their $\text{CO}_2$ permeability, when functionally required, by incorporation of $\text{CO}_2$ channels:

1) AQP1 incorporated in lipid vesicles raises $\text{CO}_2$ permeability in a concentration-dependent manner

2) AQP1 expression in MDCK cells increases membrane $P_{\text{CO}_2}$. 
Involvement of elevated membrane cholesterol on G-protein regulated water and gas transport in biological membranes

Gas Channels Workshop, Case Western Reserve University, OH; Sept. 5-7, 2012

Bhanu P. Jena
Dept. of Physiology
Wayne State University School of Medicine
Dept. of Chemical Engineering & Material Science
School of Engineering
Wayne State University
Detroit, MI 48201

Porosome: The Universal Secretory Portal in Cells
Hypothetical model

EM & AFM micrographs of Zymogen Granules

Association of $G_{\alpha i3}$ with ZGM

Vesicle Size Increase After Exposure to GTP

Kelly et. al. 2004 Cell Biol. Int. 28:709-16
Kelly et. al. 2004 Cell Biol. Int. 28:709-16
Water-Channel AQP1 at ZG Membrane

Tritiated Water Entry into ZG

Introduction of AQP1 antibody into ZG

Regulation of water entry into ZG

Jena et. al. 1997 PNAS Vol 94. 13317-22
Cho et. al. 2002 PNAS Vol 99. 4720-24
AQP1 Immunoisolated Complex

Zymogene granules

Red blood cells

Abu-Hamdah et al. 2004 Cell Biol. Int. 28:7-17
ZG volume changes measured by AFM

Abu-Hamdah et. al. 2004 Cell Biol. Int. 28:7-17
Electrophysiological properties of AQP1-immunocomplex reconstituted in PC:PS bilayer

Abu-Hamdah et. al. 2004 Cell Biol. Int. 28:7-17
Amanda F., Jena B.P. (unpublished observation)
The truth about the movement of NO across cell membranes

Jeffrey Garvin

Hypertension and Vascular Research Division
Department of Internal Medicine
Henry Ford Hospital
Acetylcholine-induced EDRF release

intact

rubbed

Furchgott et al., Nature 1980
NO synthesis

\[
\begin{align*}
\text{COOH} & \quad \text{HCNH}_2 \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{CH}_2 & \quad \text{NH} \\
\text{NH} & \quad \text{CNOH} \\
\text{CNH} & \quad \text{NH}_2 \\
\text{NH}_2 & \quad \text{L-arginine}
\end{align*}
\]

\[
\begin{align*}
\text{COOH} & \quad \text{HCNH}_2 \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{CH}_2 & \quad \text{NH} \\
\text{NH} & \quad \text{CO} \\
\text{CNOH} & \quad \text{NH}_2 \\
\text{NH}_2 & \quad \text{L-citrulline}
\end{align*}
\]

\[
\begin{align*}
\text{COOH} & \quad \text{HCNH}_2 \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{CH}_2 & \quad \text{NH} \\
\text{NH} & \quad \text{NO}
\end{align*}
\]
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
Properties of NO

1. It is small.

2. It is non-polar.

3. It is RELATIVELY lipophilic with a partition coefficient of about 5.

4. It is a gas.

5. Its reactive (different from $O_2$ and $CO_2$).
How many think NO diffuses through two bilayers
Energy profile of NO with distance based on partition coefficient
A slightly more “realistic” model of NO diffusion through bilayers

endothelial cell

vascular smooth muscle cell

soluble guanylate cyclase

NOS 3
There have been no direct measurements of the NO permeability of any cell membrane!!!

There has been one calculation which is widely cited. This value of 76 cm/s was calculated based on steady-state measurements of NO within an artificial membrane using 2 mM NO.
Free diffusion creates several problems:
1. Free diffusion is relatively slow;
2. The amount of NO trapped in the membrane is relatively large;
3. If NO is only around transiently, the membrane could act as a trap;
4. There is no control over where NO goes;
5. There is no way to regulate NO release;
6. There is little control over NO entry.

As you have heard today “gas channels” including aquaporin-1 (AQP-1) has been shown to transport CO$_2$ and other gases.
Organs where AQP-1 and NO synthase are expressed
AQP-1 expression by aortic EC and VSMC isolated from CD1 mice

EC        VSMC
(15 µg)    (5 µg)

- Gly-AQP-1
- AQP-1
Hypothesis

endothelial cell

NOS 3

NO

AQP-1

vascular smooth muscle cell

soluble guanylate cyclase
If our hypothesis is correct:
1. NO permeability ($P_{NO}$) should correlate with water permeability ($P_f$).

2. Increasing AQP-1 expression 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.
Measuring NO with DAF in cultured cells

Arc Lamp

excitation 488 nm

dichroic mirror

mirror

emission 535±50 nm

Camera & Image Analysis
Correlation of $P_{NO}$ and $P_f$ in stably transfected CHO cells

$[y = 0.64x + 20.33]; r = 0.70$
1. NO permeability \( (P_{\text{NO}}) \) correlates with water permeability \( (P_f) \).

2. Increasing AQP-1 expression 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.
Effect of transiently transfecting CHO cells with aquaporin-1 (AQP-1) on NO influx

NO gradient by SPM (5 µM NO)
1. NO permeability ($P_{NO}$) correlates with water permeability ($P_f$).

2. Increasing AQP-1 expression increases NO flux.

3. Inhibitors of AQP-1 should reduce NO flux.

4. NO flux should be saturable.

5. Purified AQP-1 should transport NO.
Effect of DMSO, an AQP-1 inhibitor, on NO influx into transiently transfected CHO cells

NO gradient by SPM (5 µM NO)
Effect of 20 µM HgCl₂, an AQP-1 inhibitor, on NO influx into transfected CHO cells

NO influx
[fluorescence units/sec]

$\bar{p} < 0.005$

NO gradient by SPM (5 µM NO)
Effect of DMSO on NO influx into transiently transfected CHO cells

NO gradient by gas (5 \( \mu \text{M NO} \))

\[ p < 0.03 \]
1. NO permeability ($P_{NO}$) correlates with water permeability ($P_f$).

2. Increasing AQP-1 expression increases NO flux.

3. Inhibitors of AQP-1 reduce NO flux.

4. NO flux should be saturable.

5. Purified AQP-1 should transport NO.
Concentration-dependent NO flux using NO gas

$K_{1/2} = 0.54 \mu M$
1. NO permeability ($P_{NO}$) correlates with water permeability ($P_f$).

2. Increasing AQP-1 expression increases NO flux.

3. Inhibitors of AQP-1 reduce NO flux.

4. NO flux is saturable.

5. Purified AQP-1 should transport NO.
NO flux into proteoliposomes made with purified AQP-1

![Graph showing DAF2 fluorescence and NO influx over time.](image_url)

- **DAF2 fluorescence [f.u.]**: 0 to 6
- **Time [sec]**: -20 to 40

**Graph 1:**
- **X-axis**: Time [sec]
- **Y-axis**: DAF2 fluorescence [f.u.]
- **Legend**:
  - Control
  - AQP-1
  - 1 µM NO

**Graph 2:**
- **X-axis**: Time [sec]
- **Y-axis**: NO influx [Fluorescence units/sec]

**Statistical Analysis:**
- **p < 0.024**

- **Control**
- **AQP-1**
1. NO permeability ($P_{NO}$) correlates with water permeability ($P_f$).

2. Increasing AQP-1 expression increases NO flux.

3. Inhibitors of AQP-1 reduce NO flux.

4. NO flux is saturable.

5. Purified AQP-1 increases NO transport.
Do other aquaporins transport NO?
Partial aquaporin family tree

Adapted from Agre et al. J Physiol 542:3-16, 2002
Effect of transiently transfecting CHO cells with AQP-3 on NO influx
Effect of transiently transfecting CHO cells with AQP-4 on NO influx
AQP-3 and AQP-4 may transport NO. More data are required.
How does NO transport by AQP-1 compare to diffusion through the bilayer in “real” cells?

Is it physiologically significant?
Acetylcholine-dependent relaxation of aortic rings from wild type and AQP-1 -/- mice

WT

AQP-1 -/-

Log [Ach] concentration

% contraction to PE

$\ p < 0.0001$
The reduction in Ach-induced relaxation in AQP-1 -/- mice is NOT due to:

1. Less NOS 3. There is more in AQP-1 -/- mice than WT.

2. Defective signaling down-stream of NO. Donors that release NO inside VSMCs and cGMP relax rings from AQP-1 -/- mice the same as WT.
The reduction in Ach-induced relaxation in AQP-1 -/- mice could be due to:

1. Reduced NO efflux out of endothelial cells; and/or

2. Reduced NO influx into vascular smooth muscle cells.
Effect of inhibiting AQP-1 on NO release by pancreatic endothelial cells
NO release by cultured aortic endothelial cells from wild type and AQP-1 -/- mice

![Bar graph showing NO release from WT and AQP-1 -/- cells]

- WT: 100 [pmol/mg]
- AQP-1: 40 [pmol/mg]

* p < 0.04
Relaxation of denuded aortic rings to spermine NONOate, an NO donor that releases NO into the bathing media.

\[ p < 0.0001 \]
NO influx into isolated aortic vascular smooth muscle cells from wild type and AQP-1 -/- mice

WT         AQP-1

NO influx [fluorescence units/sec]

5 μM NO

WT

AQP-1 KO

NO flux [fluorescence units/sec]

WT

AQP-1

$p < 0.002$
We are trying to show that the reduction in NO transport by AQP-1 is physiologically relevant in vivo by showing that total peripheral resistance does not decrease in response to acetylcholine in these mice as much as wild type mice.

**BUT**

it seems that these mice have compensation mechanism including increased prostaglandin production and NOS expression that has frustrated our attempts thus far.
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.
Role of Rh glycoproteins in NH₃ gas transport – lessons from in vivo model systems

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University of Florida College of Medicine and NF/SGVHS
Thanks ... 

Jill W. Verlander, D.V.M.  
Mary E. Handlogten, M.Sc.  
Hyun-Wook Lee, Ph.D. 

Jesse Bishop, B.Sc.  
Melanie N. Cash, B.Sc.  
Amy E. Frank, B.Sc.  
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Sharon W. Matthews, Ph.D.  
Kavya Mekala, M.D.  
Manisa Sahni, M.D.  
Sriram Seshadri, M.D.  
Marshall E. Steinbaum, B.Sc.  
Arthur J. Weiner  
Florence Whitehill, B.A.  
Li Zhang, M.D.  

Cre-LoxP studies  
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Young-Hee Kim, M.D.  
Janet Klein, Ph.D.  
Shelley Kozlowski  
Jeff Sands, M.D.  
Tekla Smith  

Funding support: NIH (DK045788, NS047624), Department of Veterans Affairs, American Heart Association, International Society of Nephrology, Korea Science and Engineering Foundation, Gatorade Research Foundation and NF/SGVHS Research Service
Our studies assessing the role of Rh glycoproteins in NH$_3$ gas transport

Is renal collecting duct NH$_3$ transport diffusive or transporter-mediated?

Are Rh glycoproteins present in cells that transport NH$_3$ gas?

Does expression parallel changes in NH$_3$ gas transport?

Does Rh glycoprotein inhibition alter NH$_3$ gas transport?
How can we determine whether collecting duct NH$_3$ transport is diffusive or transporter-mediated?

- Inhibitors
- Gene knock-down
- Functional tests
  - Diffusive
    - Transport proportional to concentration gradient
How can we determine whether collecting duct NH₃ transport is diffusive or transporter-mediated?

- Inhibitors
- Gene knock-down
- Functional tests
  - Diffusive
    - Transport proportional to concentration gradient
  - Transporter-mediated
    - Saturable
Measurement of collecting duct cell (mIMCD-3) basolateral total ammonia transport

Both saturable and diffusive components

\[ J_{\text{total}} = J_{\text{transporter}} \cdot \left( \frac{[\text{MA}]}{[\text{MA}] + K_m} \right) + J_{\text{diffusive}} \cdot [\text{MA}] \]

Characteristics of collecting duct cell (mIMCD-3) basolateral membrane total ammonia transport

- **Functional characteristics**
  - Electroneutral
  - Na$^+$ and K$^+$-independent
  - Not inhibited by K$^+$ transporter or NHE inhibitors
  - Extracellular and intracellular pH dependent
  - NH$_3$ transport

- Similar findings when studying apical transport
- Similar findings in gastric, hepatic, small intestinal and colonic epithelial cells

Our studies assessing the role of Rh glycoproteins in NH₃ gas transport

Renal collecting duct NH₃ transport is both diffusive and saturable

Are Rh glycoproteins present in cells that transport NH₃ gas?

Does expression parallel changes in NH₃ gas transport?

Does Rh glycoprotein inhibition alter NH₃ gas transport?
Where is RhAG/Rhag expressed?

- SLC42A2/RhBG-2
- SLC42A2/RhBG
- SLC42A2/RhBG (Rat)
- SLC42A2/RhBG (Mouse)
- SLC42A2/RhBG (Rabbit)
- RhBG

- SLC42A3/RhCG
- SLC42A3/RhCG (Rabbit)
- SLC42A3/RhCG (Rat)
- SLC42A3/RhCG (Mouse)
- RhCG

- SLC42A1/RhAG
- SLC42A1/RhAG (Mouse)
- SLC42A1/RhAG (Rat)
- RhAG

- Cr Rh1
- Cr Rh2
- Rh

- Sc Mep1
- Sc Mep3
- Sc Mep2
- Mep1/3
- Mep2/Amt2
- Mep2/Amt2

- At AMT2
- Le AMT1.2
- At AMT1.2
- At AMT1.3
- At AMT1.1
- Le AMT1.1
- Amt1
Where is Rhbg expressed?

SLC42A2/RhBG-2
SLC42A2/RhBG
SLC42A2/RhBG (Rat)
SLC42A2/RhBG (Mouse)
SLC42A2/RhBG (Rabbit)

RhBG

SLC42A3/RhCG
SLC42A3/RhCG (Rabbit)
SLC42A3/RhCG (Rat)
SLC42A3/RhCG (Mouse)

RhCG

SLC42A1/RhAG
SLC42A1/RhAG (Mouse)
SLC42A1/RhAG (Rat)

RhAG

Cr Rh1
Cr Rh2

Rh

Sc Mep1
Sc Mep3
Sc Mep2

Mep1/3

Mep2/Amt2

Le AMT1.2
At AMT1.2
At AMT1.1

Le AMT1.1

At AMT1.3
At Amt1.3
At Amt1.1

Le AMT1.1

At Amt1.2

0.2
Where is Rhcg expressed?

- SLC42A2/RhBG-2
- SLC42A2/RhBG
- SLC42A2/RhBG (Rat)
- SLC42A2/RhBG (Mouse)
- SLC42A2/RhBG (Rabbit)
- RhBG

- SLC42A3/RhCG
- SLC42A3/RhCG (Rabbit)
- SLC42A3/RhCG (Rat)
- SLC42A3/RhCG (Mouse)

- RhCG

- SLC42A1/RhAG
- SLC42A1/RhAG (Mouse)
- SLC42A1/RhAG (Rat)
- RhAG

- Cr Rh1
- Cr Rh2

- Rh

- Sc Mep1
- Sc Mep3

- Mep1/3

- Sc Mep2

- Mep2/Amt2

- Sc Mep2

- At AMT2

- Le AMT1.2

- At AMT1.2

- At Amt1.3

- At AMT1.1

- Le AMT1.1

- Amt1
Renal Rh glycoprotein expression

RhCG expression in human kidney

KH Han, et al. JASN 17:2670-9, 2006.
Our studies assessing the role of Rh glycoproteins in NH₃ gas transport

Renal collecting duct NH₃ transport is both diffusive and saturable

Rh glycoproteins are present specifically in cells that transport NH₃

Does expression parallel changes in NH₃ gas transport?

Does Rh glycoprotein inhibition alter NH₃ gas transport?
Metabolic acidosis increases apical plasma membrane Rhcg expression

At least two modes of Rhcg regulation:
• Changes in steady-state protein expression
  • Post-translational regulation
  • Trafficking to and from plasma membrane
Relative roles of each are cell-specific

Metabolic acidosis increases Rhbg expression.

Relative Expression (Control = 100%)

Cortex

Control Diet
3 Days HCl
5 Days HCl

Outer Medulla

Conditions where Rhbg and/or Rhcg expression parallels ammonia transport

- **Metabolic acidosis**

- **Reduced renal mass**

- **Ischemia-reperfusion injury**

- **Cyclosporine A-induced renal tubular acidosis**

- **Hypokalemia**

- **Adaptive response to deletion of other acid-base transporters**
  - Pendrin
  - Collecting duct Rhcg
  - Intercalated cell-specific Rhcg
  - Intercalated cell-specific Rhbg
Our studies assessing the role of Rh glycoproteins in NH₃ gas transport

Renal collecting duct NH₃ transport is both diffusive and saturable

Rh glycoproteins are present specifically in cells that transport NH₃

Rh glycoprotein expression parallels NH₃ gas transport

Does Rh glycoprotein inhibition alter NH₃ gas transport?
Collecting duct-specific Rhcg deletion

Floxed Rhcg Ksp-cadherin-Cre Negative

Floxed Rhcg Ksp-cadherin-Cre Positive

Cortex

Outer Medulla

Collecting duct-specific Rhcg deletion - basal effects

**Urinary Ammonia**
-,+ / +: 86 µmol per day
-,- / -: 63 µmol per day

**Serum HCO₃⁻**
-,+ / +: 19.4 mmol per L
-,- / -: 19.9 mmol per L

P < 0.05

P = NS

Collecting duct-specific Rhcg deletion - acid loading

- **Traditional**
  - Add NH$_4$Cl to drinking water
  - Not well tolerated
    - Add glucose
  - Hard to quantify
- **“New”**
  - Add HCl directly to food
  - Use powdered food
  - Easily quantified

![Graph showing serum [HCO$_3^-$], mmol per L](image)

Control: 20.5 mmol per L
Collecting duct knock-out: 16.2 mmol per L

Collecting duct-specific Rhcg deletion - acid loading

Urine ammonia (µmol d\(^{-1}\))

Normal expression (Cre-negative)

Collecting duct deletion (Cre-positive)

Ammonia excretion different, P<0.05, at each day

Development of intercalated cell-specific Rhbg knock-out mouse

Effect of intercalated cell-specific Rhbg deletion on response to metabolic acidosis

Floxed Rhbg, B1-Cre-negative  Floxed Rhbg, B1-Cre-positive

<table>
<thead>
<tr>
<th>Day of acid-loading</th>
<th>Urinary Ammonia (µmol d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td></td>
</tr>
</tbody>
</table>

Control

IC-Rhbg-KO

P < 0.05 days 2-4

Another Rhbg gene deletion study

![Graph showing urinary ammonia (µmol d⁻¹) vs. day for NH₄Cl, Global KO and NH₄Cl, Wild-type.](chart)

Comparison of the two Rhbg gene deletion studies

- **HCl, Control**

- **HCl, IC-Rhbg-KO**

- **NH₄Cl, Global KO**

- **NH₄Cl, Wild-type**

**Graph Details**
- **Y-axis:** Urinary Ammonia (μmol d⁻¹)
- **X-axis:** Day
- The graph shows the comparison of ammonia levels in different conditions over time.

Does the role of Rhbg and Rhcg differ in different conditions?

- Hypokalemia
  - Increased urinary ammonia excretion
  - Urine alkalinization
    - Increased urine acidification cannot be the primary driving force
  - Development of metabolic alkalosis
Effect of K⁺-free diet on mouse urinary electrolytes

Our studies assessing the role of Rh glycoproteins in NH₃ gas transport

Renal collecting duct NH₃ transport is both diffusive and saturable

Rh glycoproteins are present specifically in cells that transport NH₃

Rh glycoprotein expression parallels NH₃ gas transport

Rh glycoprotein gene deletion alters NH₃ gas transport

**Rh glycoprotein-mediated NH₃ transport is central to renal ammonia metabolism and transport**
‘Role of Membrane Proteins in Oxygen Transport in Red Blood Cells’

R. Ryan Geyer, Ph.D.

PI: Walter F. Boron M.D., Ph.D.
Dept of Physiology & Biophysics
Case Western Reserve University School of Medicine
Cleveland, Ohio, USA

Gas Channel Workshop
September 7th, 2012 Cleveland, OH
Evidence for Gas Channels

The Boron Lab identified the first gas channel—the water channel AQP1—which exhibits permeability to CO₂.

DIDS—the anion transport inhibitor—not only reduces HCO₃⁻ permeability, but the CO₂ permeability in human RBCs.

Later it was shown that NH₃ also passes through AQP1.

RhAG (a component of the Rh complex in human RBC) conducts NH₃.

Lipid vesicles containing AQP1 increased Nitric Oxide influx by about 300%.

Endeward et al. found that the lipid of the RBC membrane has an extremely low permeability to CO₂.

Musa-Aziz & Boron showed for the first time that gas channels—like ion channels—can exhibit selectivity for one gas over another (CO₂ vs. NH₃).
Gas Exchange

Boron, W.F. & Boulpaep, E.L. Medical Physiology
Significance

Due to the lipid and protein composition it is likely that these red blood cells (RBCs) have a low intrinsic permeability to gases.

Therefore, it would make physiological sense to have gas channel(s) to increase the $O_2$ flux, and that such a protein would be highly expressed in the RBC membrane.
**Key Membrane Proteins Present at High Levels in the RBC**

- **AE1** (1 million copies per cell)
  - Cl⁻/HCO₃⁻ co-transporter, Band 3
  - 25% of the cell membrane surface
  - Inhibited by DIDS

- **GLUT1 (humans), GLUT4 (mouse)** (600,000 copies per cell)
  - Glucose transporter
  - In mice there is a switch at about day 5
  - Inhibited by pCMBS and phloretin

- **AQP1 (Aquaporin 1)** (200,000 copies per cell)
  - H₂O, NH₃, and some CO₂ transport inhibited by pCMBS
  - Major CO₂ pathway inhibited by DIDS

- **Rh-complex (Rhesus)** (100,000 copies)
  - Transports NH₃
  - CO₂ transport blocked by DIDS

- **MCT-1 (Monocarboxylate Transporter 1)** (80,000 copies per cell)
  - Inhibited by DIDS and pCMBS

- **UT-B (Urea Transporter)** (15,000 copies per cell)
  - Urea transport inhibited by pCMBS and phloretin
  - We have shown that UT-B can transport H₂O & NH₃, but not CO₂

All of these proteins form homo-oligomers of dimers, trimers, or tetramers, which could form additional pores.
AQP1 is a **homotetrameric** protein with 4 water channels … ‘aquapore’, which is lined by hydrophilic & hydrophobic residues.

There is an **additional pore** … the ‘**central pore**’, which is lined by hydrophobic residues.

Our Laboratory showed that AQP1 can transport both CO₂ and NH₃.
RhCG is a **homotrimeric** protein – each monomer contains 12 TMs and works as a functional **ammonia pore** ...

... playing an essential role in the secretion of $\text{NH}_3$ in the kidney, which is critical to systemic acid-base homeostasis.

Hydrophobic ‘**central pore**’ is formed at the threefold axis of symmetry.
The bacterial homolog Urea Transporter (UT-B) was crystallized as a homotrimer.

Each bundle of helices forms a monomeric urea channel.

Urea transport can be inhibited by phloretin, HgCl$_2^+$, and pCMBS.
Can we quantitate the O₂ efflux and/or influx rate of intact wild-type RBCs?

If so, is it possible to determine the contribution of the CO₂ channels (AQP1 and Rh-complex) to the O₂ efflux?

Are there other RBC membrane proteins that could also contribute to the O₂ efflux?

Can known inhibitors of transporters block O₂ efflux from the RBC?

**pCMBS** – mercurial agent that covalently reacts with cysteine thiol groups. Blocks water transport via AQPs and a variety of other transport processes.

**DIDS** – amino-reactive agent known to inhibit the anion transporter (AE1 or Band 3) activity. The interaction can be reversible and irreversible (covalent). Also shown to reduce the CO₂ permeability of AQP1 and RhAG.

**Phloretin** – known to inhibit the glucose an urea transporters.
Hemoglobin Absorbance Changes

Molar Extinction Coefficient (cm\(^{-1}\)/M)

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
</tr>
<tr>
<td>300</td>
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<tr>
<td>350</td>
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<td>700</td>
</tr>
<tr>
<td>750</td>
</tr>
<tr>
<td>800</td>
</tr>
</tbody>
</table>

HbO\(_2\) → Hb
Stopped Flow Technique

Data from multiple wavelengths ($\lambda$) can be collected and compiled into a 3-D graph (Abs. vs. Time vs. $\lambda$).

Rapidly mixes two solutions (on a millisecond timescale)

From this data we can calculate the rate of the chemical reaction.
Effect of knocking out AQP1, RhAG, GLUT4, and AQP1/RhAG on O2 efflux in mouse RBCs

The major CO2 channels of the RBC—AQP1 and RhAG—also function as modest O2 channels.
Western Blot Analysis of Membrane Proteins from wild-type and AQP1-KO

<table>
<thead>
<tr>
<th></th>
<th>anti-AE1</th>
<th>anti-GLUT4</th>
<th>anti-AQP1</th>
<th>anti-RhAG</th>
<th>anti-MCT1</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild-type</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
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<tr>
<td>KO</td>
<td>KO</td>
<td>KO</td>
<td>KO</td>
<td>KO</td>
<td>KO</td>
</tr>
</tbody>
</table>
Effect of pCMBS, Phloretin, or DIDS on Oxygen Efflux from Mouse Red Blood Cells

- pCMBS reduces the O₂ efflux by 50%.
- Phloretin reduces the O₂ efflux by 40%.
- DIDS reduces the O₂ efflux by 25%.
- pCMBS + Phloretin reduce the O₂ efflux by 70%.

Inhibitor efficacy: pCMBS + phloretin > pCMBS > phloretin > DIDS
As shown previously, pCMBS reduces the O₂ efflux rate by ~50%.

The inhibitory effect of pCMBS can be reversed with the addition of the reducing agent DTT.
The inhibition of O₂ efflux observed with DIDS appears to be non-covalent and reversible, the inhibition can be reversed when the RBCs are washed with 0.2% Bovine Serum Albumin (BSA).
Conclusions

• The data suggest that the DIDS-sensitive CO$_2$ channels (AQP1 and RhAG) of the RBC appear to modestly transport O$_2$ out of the cell.

  ... because this transport process is not greatly effected by DIDS the O$_2$ must utilize an alternative pathway(s).

• Knockouts of GLUT4 and AQP1/RhAG have O$_2$ efflux rates greater (10-25%) than the wild-type controls.

• Inhibitor efficacy:

  pCMBS + phloretin > pCMBS > phloretin > DIDS

  ... because the pCMBS/Phloretin inhibition was not totally additive, O$_2$ transport likely occurs by two or more channels.

• There must be a pathway (channel) that is sensitive to both pCMBS and phloretin and another pathway that is insensitive to both inhibitors.
Future Directions

• Compile inhibitor profiles for the knockout mice.
• Perform western blots on RBCs from knockout mice.
• Develop assays for monitoring nitric oxide (NO) and hydrogen sulfide (H₂S) transport in red blood cells.
• Investigate the effect of hypoxia on protein expression (XQ) and O₂ transport activity (RRG).
• Investigate the effect of adding and removing cholesterol from the RBC membranes on O₂ transport activity.
Acknowledgments

Principal Investigator
Walter F. Boron, M.D., Ph.D.

Collaborator
Raif Musa-Aziz, Ph.D. (Univ. of Sao Paulo)

Animal Technician
Thomas Radford (CWRU)
Talks & Posters


Musa-Aziz R, **RR Geyer** & WF Boron. Relative CO₂/NH₃ permeabilities of several members of the mammalian Aquaporin family: bAQP0, hAQP1, hAQP2, rAQP3, rAQP4-M1, rAPQ4-M23, and hAQP8. Experimental Biology, Washington, DC, *FASEB J* 25:1040.5, 2011.

Geyer RR & WF Boron. Gas transport through channels. Undersea & Hyperbaric Medicine Society Scientific Meeting in conjunction with The Office of Naval Research, Fort Worth, TX, June 15-18, 2011.

Geyer RR, R Musa-Aziz, & WF Boron. Movement of NH₃ through Human Urea Transporter B (UT-B)—a new member of gas channels. ASN Kidney Week, 2011.

Musa-Aziz R, **RR Geyer**, X Qin, & WF Boron. The CO₂/NH₃ selectivities and inhibitor sensitivities of mammalian Aquaporins. ASN Kidney Week, 2011.

Postdoctoral Fellowship for ‘Gas Transport through Channels’

Background: Red blood cells (RBCs) function to carry oxygen (O₂) to tissues and transport carbon dioxide (CO₂) away from tissues. The traditional view had been that these gases dissolve in the membrane and diffuse into or out of the cell. Our laboratory and others have shown that RBC membrane proteins can function as gas channels for the transport of CO₂ and/or NH₃. It is our hypothesis that membrane proteins can also facilitate the transport of O₂ across the RBC membrane. This research could represent a major paradigm shift, and totally reorganize our thinking of how O₂ crosses cell membranes.

Naval and Scientific Benefits: If we understand the molecular mechanism of gas transport—we could design pharmacological agents that—by inhibiting or activating gas channels—can prevent or treat decompression illness and O₂ toxicity.

Objectives: (1.) To quantitate O₂ efflux of intact RBCs using stopped-flow absorbance spectroscopy. (2.) Determine the contribution of the CO₂ channels (AQP1 and RhAG) to the O₂ efflux, as well as other highly expressed RBC membrane proteins (AE1, GLUT4, MCT1, and UT-B). (3.) Assess the effect on O₂ efflux rate when wild-type RBCs have been treated with compounds known to inhibit: H₂O permeability (pCMBS), glucose and urea transport (phloretin), and CO₂ transport (DIDS).

FY12 Accomplishments, Discoveries, & Inventions
- Determined O₂ efflux rate of intact, wild-type RBCs.
- Completed inhibitor profile of O₂ efflux from intact RBCs.
- Investigated O₂ efflux rate of intact RBCs from AQP1-null, RhAG null, and GLUT-4 null mice.

FY13 Goals
- Quantitate O₂ efflux rate of intact RBCs from AE1 null, GLUT-4 null, and UT-B null mice.
- Investigate the transport of nitric oxide (NO) and hydrogen sulfide (H₂S) in intact RBCs.

Principle Investigator: Dr. Walter F. Boron, 216-368-3400
walter.boron@case.edu
Structure determinants for CO$_2$ transport of human aquaporin5

Xue Qin
PI: Walter F Boron

Department of Physiology and Biophysics
Case Western Reserve University
Background

Aquaporin 5 is a water channel highly expressed in salivary glands, eye, lung and trachea.

Aquaporins are composed of 6 transmembrane domains, with N- and C- terminus on the cytoplasmic side of the membrane.
Background

H$_2$O permeability
2 filter regions is important for H$_2$O transport
• Selectivity filter: ar/R region
• NPA region

CO$_2$ permeability
Not well defined

Horsefield et al, 2008 PNAS
Methods

H₂O permeability--P_f

Volumetric assay to measure osmotic water permeability
How fast the volume of oocytes change with time

CO₂ permeability--ΔpH_S

Microelectrode to measure pH on the surface of the oocytes
Outline

I  Amino acids at the mouth of the central pore
II Amino acids lining the central pore
Outline

I  Amino acids at the mouth of the central pore
II Amino acids lining the central pore
Exploring gas permeability of cellular membranes and membrane channels with molecular dynamics

Yi Wang a, Jordi Cohen a,d, Walter F. Boron b, Klaus Schulten a,d, Emad Tajkhorshid a,c,*

a Theoretical and Computational Biophysics Group, Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA
b Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT 06520, USA
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Received 12 August 2006; received in revised form 28 October 2006; accepted 3 November 2006
Available online 4 January 2007

Abstract

Aquaporins are a family of membrane proteins specialized in rapid water conduction across biological membranes. Whether these channels also conduct gas molecules and the physiological significance of this potential function have not been well understood. Here we report 140 ns of molecular dynamics simulations of membrane-embedded AQP1 and of a pure POPE bilayer addressing these questions. The permeability of AQP1 to two types of gas molecules, O2 and CO2, was investigated using two complementary methods, namely, explicit gas diffusion simulation and implicit ligand sampling. The simulations show that the central (tetrameric) pore of AQP1 can be readily used by either gas molecule to permeate the channel. The two approaches produced similar free energy profiles associated with gas permeation through the central pore: a −0.4 to −1.7 kcal/mol energy well in the middle, and a 3.6–4.6 kcal/mol energy barrier in the periplasmic vestibule. The barrier appears to be mainly due to a dense cluster of water molecules anchored in the periplasmic mouth of the central pore by four aspartate residues. Water pores show a very low permeability to O2, but may contribute to the overall permeation of CO2 due to its more hydrophilic nature. Although the central pore of AQP1 is found to be gas permeable, the pure POPE bilayer provides a much larger cross-sectional area, thus exhibiting a much lower free energy barrier for CO2 and O2 permeation. As such, gas conduction through AQP1 may only be physiologically relevant either in membranes of low gas permeability, or in cells where a major fraction of the cellular membrane is occupied by AQP1.

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Keywords: Aquaporin; AQP1; Gas permeability; O2; CO2; Free energy profile; Gas channels
According to our PMFs, the major barrier of the central pore to gas permeation is about 3.6-4.6 kcal/mol, located at the periplasmic side of the central pore (13 Å ≤ z ≤ 19 Å). This barrier, consistently found by both our approaches, as well as by another study (Hub and de Groot, 2006), surprisingly, does not correspond to a region that is sterically blocked directly by the protein. As shown in Fig. 6a, this barrier is located above the region of maximum protein contraction where the four hydrophobic residues Val52 reside; rather, it corresponds to a region that is populated solely by water. We have created a volumetric map of the local occupancy of water, as shown in Fig. 6b. It is clear that the barrier corresponds to a dense layer of water molecules surrounded by four aspartate (Asp50) residues (Fig. 6c). With a higher density than the bulk water, this water layer reduces the chance of gas molecules to access the central pore. If these aspartate residues will be mutated to neutral residues, e.g., alanines or asparagines, the strong electrostatic effects of the quadruplets may be eliminated and a less dense water structure could be expected, which might result in a better gas-conductive central pore of AQP1. Simulations of these mutants are currently underway.
### Protein sequence alignment

<table>
<thead>
<tr>
<th>bAQP1.PRO</th>
<th>hAQP5.PRO</th>
</tr>
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<tbody>
<tr>
<td>MASEFKKLFWRRAVVAEFLAMILFIFISIGSALGFHYPIKSNQTGAVQDNKVSLAFGL 60</td>
<td>MKKEVCSVAFLKAVFAEFLATLIFVFFGLGSALKWPS--------ALPITLQIALAFGL 51</td>
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<td>LPDNSLGLNALAPGVSQGLIGIEIGTQLVLCVLATTDRRRDLGGSGPLAIGFSVAL 180</td>
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<td>LSERVAIKGYEPDEDEWEEQREERKKTMELTTR 271</td>
<td>LSERVAIKGYEPDEDEWEEQREERKKTMELTTR 265</td>
</tr>
</tbody>
</table>

**Alignment Details**

- **D50** and **V52** denote key positions in the alignment.
- **T41** and **L43** indicate additional alignments.
- The alignment highlights conservation of amino acids across the sequences.

**Note:** The alignment matrix is color-coded to indicate conservation levels, with darker colors representing higher conservation.
\[ P_f (\text{cm/s}) \]

- **H\textsubscript{2}O**
- hAQP5
- T41Y
- T41W
- T41F
- T41G
Relative channel-dependent CO₂ permeability

- hAQP5 T41Y
- hAQP5 T41W
- hAQP5 T41F
- hAQP5 T41G
Conclusion I

I. Amino acids at the mouth of the central pore

Changes of $\Delta pH_S$ ($CO_2$ permeability) is more sensitive than $P_f$ ($H_2O$ permeability).
T41 is more important than L43.

II. Amino acids lining the central pore
Outline

I  Amino acids at the mouth of the central pore

II  Amino acids lining the central pore
Amino acids lining the central pore
### Sequence alignment of AQPs

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<th>hAQP9.PRO</th>
<th>GCGCVAQAILSR--------GRFGGVITINVGSAMAIYVAGGVSGGHINPAVSLAMCL 93</th>
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<td>hAQP3.PRO</td>
<td>GCGSVAQVVLRS--------GTHGFLTINLAFGPAVTGLILAGQVSAGHLNPAVTFCMF 92</td>
</tr>
<tr>
<td>hAQP7.PRO</td>
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<tr>
<td>hAQP2.PRO</td>
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<td>hAQP5.PRO</td>
<td>GLGSAKWPQ--------ALFTLQIALAFGLAIGTLAQALPGVSAGHNPAILTALL    78</td>
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<td>hAQP6.PRO</td>
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<td>hAQP10.PRO</td>
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<tr>
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**TM2**

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<td>hAQP5.PRO</td>
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<tr>
<td>hAQP6.PRO</td>
<td>VVRNSVSTQGAVAVELLTLQVLCVFASTD--SRQTS--GSPATMIGISVALGHLIGHF 190</td>
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<tr>
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<td>hAQP4-M1.PRO</td>
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<td>DLADGVNSQGQLGIEIGTLQVLCVLATTD--RRRDLLGSGGPAIGFSVALGHLLAIIDY 186</td>
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<td>hAQP8.PRO</td>
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<td>hAQP11.PRO</td>
<td>RSFACKNPIRVDLKVITEAVCSFLFHSALLHFQEVTRLRIHILLALITFLVYAGGL 210</td>
</tr>
</tbody>
</table>
If mutate to Asp (D)
Aspartic Acid (Asp)

Channel-dependent $\Delta p\text{H}_S$ for CO$_2$

LXXD – $\Delta p\text{H}_S^*$

$P_f^*$
Aspartic Acid (Asp)

\[ \text{LXXD} - P_f^* \]

Channel-dependent \[ P_f \text{ (cm/s)} \]

- \( H_2O \)
- hAQP5 T41D
- hAQP5 L43D
- hAQP5 L47D
- hAQP5 L51D
- hAQP5 L167D
If mutate to Arg (R)
Arginine (Arg)

Channel-dependent \( \Delta p\text{H}_S \) for CO\(_2\)

LXXR – \( \Delta p\text{H}_S^* \)

- hAQP5 T41R
- hAQP5 L43R
- hAQP5 L47R
- hAQP5 L51R
- hAQP5 L167R

\( P_f^* \)
If mutate to Thr (T)
Threonine (Thr)

\[
\text{LXXT} - \Delta \text{pH}_S^* \]

Channel-dependent \( \Delta \text{pH}_S \) for CO₂

- hAQP5
- hAQP5 T41
- hAQP5 L43T
- hAQP5 L47T
- hAQP5 L51T
- hAQP5 L167T

\( \Delta \text{pH}_S^* \) calculated as:

\[ \Delta pK_a \times \Delta pK_a^* \times \Delta \text{pH}_S^* \]

Thr**: 
- Thr (pKₐ 2.20)
- pKₐ Servers (6.36)
Threonine (Thr)

LXXT – $P_f^*$

Channel-dependent $P_f$ (cm/s)

[H$_2$O, hAQP5 T41T, hAQP5 L43T, hAQP5 L47T, hAQP5 L51T, hAQP5 L167T, L163T]
Conclusion II

I  Amino acids at the mouth of the central pore
Changes of $\Delta pH_S$ (CO$_2$ permeability) is more sensitive than $P_f$ (H$_2$O permeability)
T41 is more important than L43.

II  Amino acids lining the central pore
Changes of $P_f$ (H$_2$O permeability) is more sensitive than $\Delta pH_S$ (CO$_2$ permeability)
Of all the amino acids lining the central pore, L51 is most sensitive to determine $P_f$ and $\Delta pH_S$. 
Acknowledgement

Collaborator
Emad Tajkhorshid

Lab
Walter F Boron
Raif Musa-Aziz
Mark D Parker
Gas Channels Workshop

September 7, 2012
Cleveland, Ohio

Mathematical Modeling of Gas Movements in an Oocyte

Rossana Occhipinti, Ph.D.

Department of Physiology & Biophysics
Case Western Reserve University School of Medicine
10900 Euclid Avenue
Cleveland, OH 44106-4906
Xenopus oocyte: pH Changes Caused by CO₂ Influx

Bulk Extracellular Fluid (BECF)

(data kindly provided by Dr. Musa-Aziz)
An appropriate mathematical model should include:

- A spherical cell
- Transport of CO₂ across the plasma membrane
- Reactions of a multitude of extra- and intracellular buffers
- Diffusion of solutes through the extra- and intracellular spaces
- Temporal and spatial variations of solute concentrations
- Carbonic anhydrase (CA) activity at specific loci
The Mathematical Model

Extracellular Unconveected Fluid (EUF)

Bulk Extracellular Fluid (BECF)

Intracellular Fluid (ICF)

The Key Components of the Model

Bulk extracellular fluid (BECF)
- Infinite reservoir where convection could occur but not reaction or diffusion

Extracellular unconvected fluid (EUF)
- Thin layer adjacent to the surface of the oocyte where no convection occurs, but reactions and diffusion do occur

Plasma membrane
- Infinitely thin and permeable only to CO₂

In both EUF and intracellular fluid (ICF)
- Slow equilibration of the CO₂ hydration/dehydration reactions
- Competing equilibria among the CO₂/HCO₃⁻ and a multitude of non-CO₂/HCO₃⁻ buffers
Intracellular Fluid (ICF)

\[ \text{HCO}_3^- + \text{A}^- \rightarrow \text{HA}_\ell \]

Extracellular Unconvected Fluid (EUF)

\[ \text{H}_2\text{CO}_3 \rightarrow \text{HCO}_3^- + \text{H}^+ \]

\[ \text{HCO}_3^- \rightarrow \text{CO}_2 + \text{H}_2\text{O} \]

\[ \text{A}^- \rightarrow \text{H}_2\text{A} \]

Bulk Extracellular Fluid (BECF)

\[ \text{CO}_2 \rightarrow \text{H}_2\text{CO}_3 \rightarrow \text{HCO}_3^- + \text{H}^+ \]

\[ \text{H}_2\text{CO}_3 \rightarrow \text{H}_2\text{O} + \text{CO}_2 \]

\[ \text{H}_2\text{O} \rightarrow \text{H}^+ + \text{OH}^- \]

Intracellular Fluid (ICF)

\[ \text{HCO}_3^- + \text{A}^- \rightarrow \text{HA}_\ell \]

Extracellular Unconvected Fluid (EUF)

\[ \text{H}_2\text{CO}_3 \rightarrow \text{HCO}_3^- + \text{H}^+ \]

\[ \text{HCO}_3^- \rightarrow \text{CO}_2 + \text{H}_2\text{O} \]

\[ \text{A}^- \rightarrow \text{H}_2\text{A} \]

Bulk Extracellular Fluid (BECF)

\[ \text{CO}_2 \rightarrow \text{H}_2\text{CO}_3 \rightarrow \text{HCO}_3^- + \text{H}^+ \]

\[ \text{H}_2\text{CO}_3 \rightarrow \text{H}_2\text{O} + \text{CO}_2 \]

\[ \text{H}_2\text{O} \rightarrow \text{H}^+ + \text{OH}^- \]
Assuming spherical symmetry, we write a reaction-diffusion equation for each species $j$,}

$$
\frac{\partial}{\partial t} C_j(r, t) = \frac{1}{r^2} \frac{\partial}{\partial r} \left( D_j r^2 \frac{\partial}{\partial r} C_j(r, t) \right) + \sum_{\ell=-L}^{L} S_{j, \ell} \Phi_\ell(r, t), \quad 0 \leq r \leq R \leq R_\infty,
$$

with $r$ distance from the center of the oocyte.
Method of Lines

Intracellular fluid (ICF)
Extracellular Unconvected Fluid (EUF)

Somersalo, Occhipinti, Boron, Calvetti, J Theor Biol, 2012
Numerical Experiments

Assumptions

• The BECF, EUF, ICF and plasma membrane have same properties as water

• The EUF has thickness $d = 100 \, \mu m$

• Small CA-like activity uniformly distributed inside the oocyte and on the surface of the plasma membrane

• The BECF and EUF
  - contain $1.5\%$ CO$_2$/9.9 mM HCO$_3^-$ / pH 7.50
  - have a single *mobile* non-CO$_2$/HCO$_3^-$ buffer with pK = 7.5 (e.g., HEPES) and [TA] = 5mM

• The ICF
  - has initial pH$_i = 7.20$
  - $[CO_2] = [H_2CO_3] = [HCO_3^-] = 0$ mM
  - has a single *mobile* non-CO$_2$/HCO$_3^-$ buffer with pK = 7.10 and [TA] ≈ 27.31mM
Results

Extracellular concentration-time profiles for solutes

\( \text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3 \rightarrow \text{HCO}_3^- + \text{H}^+ \)  

(A) \( \text{CO}_2 \)  
(B) \( \text{H}_2\text{CO}_3 \)  
(C) \( \text{HCO}_3^- \)  

\( \text{HA}_1 \rightarrow \text{A}_1^- + \text{H}^+ \)  

(D) \( \text{pH} \)  
(E) \( \text{HA}_1 \)  
(F) \( \text{A}_1^- \)
Intracellular concentration-time profiles for solutes

\[ \text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3 \rightarrow \text{HCO}_3^- + \text{H}^+ \]

\[ \text{HA}_1 \rightarrow \text{A}_1^- + \text{H}^+ \]
Effects of Decreasing CO\textsubscript{2} Membrane Permeability

(A)

\[ P_{M,CO_2} = 34.2 \text{ cm/sec} \]

(B)

\[ (\Delta pH_S)_{\text{max}} \]

(C)

\[ -\frac{dpH_i}{dt} \]

(D)

\[ P_{M,CO_2} (\text{cm/sec}) \]

\[ P_{M,CO_2} / 10^1 \]

\[ P_{M,CO_2} / 10^2 \]

\[ P_{M,CO_2} / 10^3 \]

\[ P_{M,CO_2} / 10^4 \]

\[ P_{M,CO_2} / 2.5 \times 10^4 \]

\[ P_{M,CO_2} / 5.0 \times 10^4 \]

\[ P_{M,CO_2} / 7.5 \times 10^4 \]

\[ P_{M,CO_2} / 10^5 \]
The background permeability of the membrane (i.e., in the absence of gas channels) must be very low.

Given a sufficiently small $P_{M,CO_2}$, gas channels could contribute to CO$_2$ permeability even in the presence of a large $d$ (in our numerical experiments $d = 100\mu m$).

With additional refinements to the model, we ought to be able to estimate absolute permeabilities.
Effects of Changing the Width of the EUF

The EUF is a generalization of the concept of unstirred layer (UL)

ULs are thin, diffuse layers of fluid, always present near the surface of solid bodies immersed in a fluid, where molecules move predominantly via diffusion (Dainty and House, *J Physiol*, 1966; Korjamo et al, *J Pharm Sci*, 2009)

For a particular solute, the width of the UL ($\delta$) is defined as

$$\delta = \frac{D}{P}$$

where $D$ is the diffusion constant and $P$ is the empirically measured permeability

The width of the UL:
1. A steady-state concept
2. Solute-dependent
3. Ignores the effects of chemical reactions

It is because our system is dynamic, involves multiple solutes, and solutes can react in the “UL”, that we decided to define the EUF
Implications

There is competition between diffusion and reaction in replenishing the lost $\text{CO}_2$ near the outer surface of the oocyte.

We quantify this competition by introducing the diffusion reaction ratio (DRR)

$$\text{DRR} = \frac{\text{rate of } \text{CO}_2 \text{ replenisced by diffusion}}{\text{rate of } \text{CO}_2 \text{ produced by reaction}}$$

DRR rises as the width $d$ of the EUF decreases.
The Vitelline Membrane: $pH_s$ Spike

Additional diffusion barrier to the movement of solutes

Implemented by reducing the mobility $D$ of each solute near the outer surface of the oocyte by the same factor $\gamma$, i.e., $D_* = D/\gamma$
As we increase $\gamma$, the maximal height of the $pH_S$ spike, $(\Delta pH_S)_{\text{max}}$, increases.

Implementation of the vitelline membrane reduces the contribution of diffusion and enhances the contribution of reaction at the surface.
Implications

Implementation of the vitelline membrane – which reduces the contribution of diffusion and enhances the contribution of the reaction – can explain the height of the $pH_S$ spike

Because the $pH_S$ electrode creates a special environment with restricted diffusion, our implementation of the vitelline membrane somehow mimics this environment
Conclusions

The model can reproduce the pH transients observed experimentally.

The simulations predict that:

1. The background permeability of the oocyte membrane must be very low.
2. Given a sufficiently small $P_{M,CO_2}$, gas channels could contribute to CO$_2$ permeability even with a large EUF.

The model provides new insights into the competition between diffusion and reaction processes near the outer surface of the plasma membrane.
Future Directions

Apply the model to investigate the movements of ammonia and ammonium across the plasma membrane.

Model the pH$_S$ electrode’s touching on the oocyte surface to explore the special environment underneath the pH$_S$ electrode.
Acknowledgments

Principal Investigator
Walter F. Boron, M.D., Ph.D.

Collaborators
Erkki Somersalo, Ph. D. (CWRU)
Daniela Calvetti, Ph. D. (CWRU)
Raif Musa-Aziz, Ph.D. (University of Sao Paulo)
2016: Blue Waters $\rightarrow$ 200K processors

Pope 100%.

Start off 100 CO$_2$ near membrane
Modeling 50% Chl. 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$_2$.

\[ \frac{10^{-3} \text{cm}}{s} \]

Implicit ligand sampling: Works for neutral molecules (not ions, which perturb protein)... results are about same as explicit.
\[ \{ \text{AQP}1, 4, 5 \} \]
\[ \text{CO}_2, \text{O}_2, \text{NO} \]

![Diagram with text: CP edge of tetramer]

**Table: HOLE THROUGH AQUaporins**

<table>
<thead>
<tr>
<th>SYSTEM</th>
<th>TOTAL (100x100 Å²)</th>
<th>WATER PORES (4)</th>
<th>CENTRAL PORE (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equi POPE-CO$_2$</td>
<td>7</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Equi POPC-CO$_2$</td>
<td>5</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Equi POPC-CO$<em>2$$</em>{np}$</td>
<td>16</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Equi POPE-O$<em>2$$</em>{np}$</td>
<td>11</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Press POPE-CO$_2$</td>
<td>168</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Press POPC-CO$_2$</td>
<td>160</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Press POPE-O$<em>2$$</em>{np}$</td>
<td>310</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Press POPC-O$<em>2$$</em>{np}$</td>
<td>208</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Press POPE-AQP1-CO$_2$</td>
<td>76</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Press POPE-AQP1-O$<em>2$$</em>{np}$</td>
<td>79</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>
ADP1: $D_{50} \rightarrow H_2O$ coord $\rightarrow$ major barrier

No through ADP4

$O_2$ “”

(Wang, Proteins, 2010)

WP: Is $O_2$ in CP the same as in bulk gas phase?

ADP4 (vs. 1): diff $\Delta G$ profile

Rely heavily on init. config. of lipid

$\xrightarrow{\text{diff. head group}}$

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.
Problem is stopped-flow applied to vesicles

$\frac{1}{2}$ of CO$_2$ uptake by human RBC: 13 ms

Meas 18O-labelled CO$_2$

HCT: $2 \times 10^{-4}$

2 $\times 10^{-3}$ 180

3-D curve fitting

optimal $P_{CO_2}$ & $P_{HCO_3}^{-}$... no local minima

Critical parameter: $A_i$ & pH$_e$...

errors $\rightarrow$ big $\Delta P_{CO_2}$

pH: 0.01 $\rightarrow$ 20-30%

pHi & $P_{HCO_3}^{-}$ are not important

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

But KO of AQP1 + RH could $\uparrow$ $t/2$

by 10x... make a $\Delta$ measurable?
Viscosity: \[ \frac{[\text{Dextran}]}{[\text{Dextran}]} \rightarrow \frac{P_{CO_2}}{P_{CO_2}} \] (wh is so small)

\[ \frac{\partial}{\partial t} \frac{P_{O_2}}{P_{O_2}} \]

\[ S = 0.5 \mu m \]

\[ P_M = 0.16 \text{ cm/s} \]

...UL is only a minor problem

\[ \alpha \]

\[ P_{O_2} \text{ in human RBCs} \]

ADP \( \sim 50\% \)

Rh \( \sim 50\% \) \( \sim 100\% \)

Colonic: Competition of \( CO_2 \) vs. \( NH_3 \) or \( CO_2 \) through aquaporin.
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 $P_{\text{CO}_2}$ and $P_{\text{HCO}_3^-}$ from well defined minima of a fitting procedure.

$P_{\text{CO}_2}$ values can be determined over a range of 3-4 orders of magnitude.

Parameters critical for calculation of $P_{\text{CO}_2}$ and $P_{\text{HCO}_3^-}$ 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.
\[ [x]_w = S_w P_x \]

\[ [x]_l = \frac{S_L}{S_w} [x]_w = \frac{S_L}{S_w} S_w P_x = S_L P_x \]
Volker Endeward, Hannover
Background CO2 permeability
1. What are the intrinsic CO₂ permeabilities?

Cell membranes show CO₂ permeabilities lower than synthetic lipid bilayer.

<table>
<thead>
<tr>
<th></th>
<th>( P_{CO_2} ) (cm/s) ± 9.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetic lipid bilayer</td>
<td>0.35 / 3.2</td>
</tr>
<tr>
<td>Red cell, ( \emptyset ) functional gas channel</td>
<td>~1</td>
</tr>
<tr>
<td>MDCK</td>
<td>0.015 ± 0.003</td>
</tr>
<tr>
<td>tsA201</td>
<td>0.017 ± 0.004</td>
</tr>
<tr>
<td>Basolateral membrane of proximal colon epithelium</td>
<td>~ 0.022</td>
</tr>
<tr>
<td>Apical membrane of proximal colon epithelium</td>
<td>0.0015 ± 0.0006</td>
</tr>
</tbody>
</table>
P_{O_2} (avg. lipid bilayer) \gg \text{naked mammalian membrane}

Cholesterol: 98.7% in Pf.

\neq

150 \text{ nm}: \text{mean vesicle diam... contain CA II}

Chol: 0-20% \rightarrow \delta\text{ measurable}

30-70% \rightarrow \log-linear \delta P_{O_2} \text{ by } \sim 10^2

Apical colomic membrane: 70% cholesterol

\beta\text{-cyclodextrin}
Could we raise $X_{O_2}$ by ↑ Chol & vice versa.
• From these considerations we can see that gas exchange of cells with a low CO₂ permeability is limited.

• Hypothesis: cell membranes with normal cholesterol and low intrinsic P_{CO₂} adapt their CO₂ permeabilities to their needs by incorporating gas channels in the membrane.

AQP1 vs. AQP2 in liposomes

↑↑P_{CO₂} × P_{CO₂}

He sees a much bigger effect (~20%) than we do.

<table>
<thead>
<tr>
<th>Gas</th>
<th>CO₂</th>
<th>O₂</th>
<th>NO</th>
<th>N₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid-water partition coefficient</td>
<td>0.95</td>
<td>2.9</td>
<td>3.8</td>
<td>4.1</td>
</tr>
<tr>
<td>Permeability (phospholipid membrane)</td>
<td>~1 cm/s</td>
<td>~3 cm/s</td>
<td>~4 cm/s</td>
<td>~4 cm/s</td>
</tr>
</tbody>
</table>
$O_2$: PL membrane 3 cm/s

End: 1/100

PL + Chol: 0.03 ?

Heard m: $\Delta p_{CO_2} = 40$ mm Hg

Way too high to be physical possible.

Summary

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

The intrinsic $P_{CO_2}$ 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 $P_{CO_2}$ 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 $P_{CO_2}$

$P_{CO_2} \propto [AQP1]^n$ ?
Got interested in AQP5s because of their involvement in vesicle fusion. H₂O must enter the vesicle.

Golgi: acce. = Z5M

GTP → 9 water by volume (AFM) + 3 H₂O.

Jena et al., PNAS, 1997

GTP-induced swelling

AQP1: Swelling complex  

"6":

AQP6: Go⁺, V⁺, ATPase complex req. Chol.

Mast (mastoparan)

swelling

ΔVolume of granule: GTP + Mast + 20–40 μM MBCD (cycloextrin)

stability

Remove Chol → complex falls apart.
zeta potential

\{ RRCs \}

+Chol
-Chol
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 coeff: 3-5

$t/2 \sim 30 \text{ s}$

How many think NO diffuses through two bilayers

NOS 3

endothelial cell

vascular smooth muscle cell

soluble guanylate cyclase
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 H₂O?
If our hypothesis is correct:
1. NO permeability ($P_{NO}$) should correlate with water permeability ($P_f$).

2. Increasing AQP-1 expression 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.

\[
\frac{P_{NO}}{P_f} \quad \text{fluorescent probe DAF2}
\]

NO influx → CHO cells, transiently transfected.

$NO$: NO donor or gas.

Hg interacts with NO gas?

$K_{1/2}: 0.54 \mu M$ \hspace{1cm} \text{Physiol } [NO] = 0.2 \mu M$
AQP1 reconst. into vessels ⇒ ↑ J_{NO}

CHD cells:

AQP3: 25% ↑ over mock < AQP1. Did not ↑ expression.

"4: 30% ↑

Aortic ring: isometric force
PE = Phenylephrine ⇒ contr.
Varied [ACH] to relax. AQP1 KO: ↓ ACh response
NO efflux from EC or ↑ influx into VSMC

KO: ↓ NO release from EC

" uptake into VSMC

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.

3.2 in Audience
Assessing roles of Rh glycoproteins in NH3 gas transport?

\[ \text{NH}_3 \]

Is transport "diffusive" or protein mediated?

- Inhibitors: none
- RNA: unsuccessful
- Saturation?

\[ \begin{align*}
\text{Me-NH}_2 & \\
\text{Baggot uptake} & \\
\text{Saturation curve} & \\
\text{Saturation component} & \text{diffusive comp.}
\end{align*} \]

Diffusion may dominate in inner medulla

where \([\text{NH}_3]\) is high & Rh levels are low.

\[ \text{RhAG} + \text{RhN3} \xrightleftharpoons{C} \]

by Ab

Cannot find RhAG anywhere but RBC.

1st cloned: RhAG

#2 "BG": Perivenous cells in hepatocytes

Hair follicles: \([\text{NH}_3]\) is 100x > plasma. Goes up

Exercise: 2 \(\rightarrow\) 10 mM.

Urine: 200-300 mM (highest NH3 concentration)
$\text{NH}_3$

GI $\rightarrow$ 250 mmole/day. SI > Colon

Lungs: RhBG $\rightarrow$ not in alveolar cells but in
bronchial epithelial cells.

RhCG: ? Glu neurotransmission.

Liver: $[\text{NH}_3]$ is ↑ in bile

Muscle: Exercise $\rightarrow$ 4-5x $\text{NH}_3$...produced
by sk.m. At rest, sk.m. is a
$\text{NH}_3$ sink. excreted

Kidney: 1-2% of $\text{NH}_3$ from GFR.

CD: RhBG $\rightarrow$ BLM $\leftarrow$ Balbc

CG: AM + some BLM $\leftarrow$
CS7 B16: much higher

M4c $\rightarrow$ ↑ RhBG expression

Does Tenidap if the
"CO$_2$ permeability"
attributable to NBC?

Does it speed up pHi? h?

Grant: Mutant NBCel

Cond. KO?

Mutations
Conditions where Rhbg and/or Rhcg expression parallels ammonia transport

- Metabolic acidosis

- Reduced renal mass

- Ischemia-reperfusion injury

- Cyclosporine A-induced renal tubular acidosis

- Hypokalemia

- Adaptive response to deletion of other acid-base transporters
  - Pendrin
  - Collecting duct Rhbg
  - Intercalated cell-specific Rhbg
  - Intercalated cell-specific Rhbg

---

Conditional KO of Rhcg: proinflammatory effects and pI6A

Slowest renal response: NH₃ transport... req. 4-5 days
Our studies assessing the role of Rh glycoproteins in NH₃ gas transport

Renal collecting duct NH₃ transport is both diffusive and saturable.

Rh glycoproteins are present specifically in cells that transport NH₃.

Rh glycoprotein expression parallels NH₃ gas transport.

Rh glycoprotein gene deletion alters NH₃ gas transport.

Rh glycoprotein-mediated NH₃ 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 (H₂O, 1990 ... gases only now).

QED: Quantum Electrodynamics

J. Rh Family (Amt/MEP)
Back: Yeah
Back: reg. N as food source, pref. as NH₃
Rapid inversion
Dipole moment ~ H₂O

CO₂
O = C = O
116.3 pm

CO₂ + H₂O is in equilibrium with H₂CO₃

O₂
O = O
δ+/− δ+/−

NO
N = O
115 pm

H₂O

H₂O \cdots H₂O \cdots H₂O \cdots H₂O

H \cdots H \cdots H \cdots H \cdots H

Why is H₂O not a gas... H-bonding

Some still think they're protein... are N₄⁺ channels!

But H₂O can be a gas! W/B
\[ A_{\text{NH}_3}B : 1.35 \text{Å} \]

11 TMs
inverted repeat

\[ \text{NH}_3 : \text{Stack hole of crystallography} \]
\[ 10^{-2} \text{ also Na}\text{. Na}^+ \]

Can also use Me\text{NH}_3

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

\[
\begin{align*}
\text{pH} & \rightarrow 9.6 \\
\rightarrow & < 7
\end{align*}
\]

binding of NH_4^+

No water, no ions
Why important for Biology?

• K+ channels;
  An NH₄⁺ channel could ‘leak’ K⁺ and hence membrane potential in eukaryotes.

• Amt/MEP are impermeable to any other ions.

• NH₄⁺ unstable at the centre of the hydrophobic bilayer while NH₃ is not. Cf K⁺

• NH₃ versus NH₄⁺ would not leak proton motive force in conduction.

• No energy nor counter ion is needed to accumulate ammonia.

Amt B
Completely turned off when enough NH₃ is around!

GlnK: Finger points to AmtB & blocks it.

ADP bound
Prevents re protonation of entering NH₃.
Nitrosomas europaea... more similar than Amt to mammalian Rh. Has a 'stake' extending into cytosol. (They do not pay much attl to it)

RhCG: Expressed in HEK293S

"NIH Common Fund" 4th NIH Roadmap meeting
Nov. 28-30
SFO: Westin Hotel
II. Aquaporins

PfARP: Plasmodium → Glycerol

1.8 Å - Rotamers can be unambiguously determined

9 H2O molecules in a chain - Solute can be identified

H2O but not H⁺

A single file of H2O conducts H⁺ very fast
Beitz et al. PNAS, 2004

An ATP synthase transport H2O and glycerol both very well.

*Malaria* One mutation: Glu → Ser (twice up above aquaporin) — H2O permeability.

Removes 1 H bond.

Channel holds on to H2O.
Ryan: What was sampling rate?
  Dead time?
  9 wavelengths: PMT, array?
  Fumbling in details cost you control of presentation.
  Showing non-case KDs is their controls were shooting yourself in the foot.

Q10:?

What was Verkman's Q_{10} for P_{f}?

CK MCV: As there a shift 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 Δ's are not due to something other than the cell membrane.]
WB: Would be nice to have a dye to monitor pHs.

DRR: spelling error.

Jeff: Animal vs. Vegetal poles.

Bhanu: Optical tweezers $\rightarrow$ viscosity across the entire diameter.
9/7/12 @ 9:30 AM
Gas Channels Workshop
Xue Qin
CO2 permeability of AQP5

Email: Rotamer search ... what is stable?
Gas channels Workshop

September 6-7 2012

Lecture 1: Walter Boron - Gas Channels

- Solubility theory
  \[ P \propto \frac{S_L}{S_w} \]
  Note: Henry's law is true at steady-state
- Solubility - Diffusion theory
- Access - Solubility - Diffusion - Egress theory
Newtonian equations

Major limitation → time scale (speed limit: 1 fs)
Force field approximations

Atomistic resolution

Implicit Ligand Sampling $W(r) = -k_B T \ln \left[ \frac{P(r)}{P_0} \right]$

$F(z) = -RT \ln \sum e^{-F(x, y, z)}/RT$
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 \( \approx \) 12 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

\[ \text{P}_{\text{HCO}_3^-} \]

\[ \text{P}_{\text{CO}_2} \]

\[ \text{CA activity} \]

\{ are the 3 main parameters

↓ red cell

↑ fast phase where \( \text{P}_{\text{CO}_2} \) dominates

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

6 ODEs

Estimate $P_{O_2}, P_{HCO_3}, A_{in}, A_{out}$

estimate first

Fitting procedure

excellent fit

Phase 1

$\frac{t}{2} = 5s$ for $CO_2 \leftrightarrow HCO_3^- + H^+$

$\frac{t}{2} = 250s$ (exchange)

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**

- Important

- \( A_i \) is very critical parameter \( \Rightarrow \) \( A_i \) and pH need to be controlled

- pH is not critical

- \( P_{H_2O} \) is

- How about ULs?

- **Theoretical hydrodynamics**

  \[ \delta \sim \text{viscosity} \times \sqrt{\text{cell diameter}} \quad \Rightarrow \]

  \[ V = 0 \Rightarrow \delta = 0 \]

  \[ \uparrow \text{dextran} \Rightarrow \uparrow \delta \text{ for } CO_2 \]

- Extrapolate to \( V = 0 \)

- \( P_{m,CO_2} = 0.16 \text{ cm/sec} \)

- \( \delta \Rightarrow 0.5 \mu m \text{ in saline} \)

- \( P_{CO_2} \text{ in saline} = 0.12 \text{ cm/sec} \)
\[ P_{CO_2} = 0.15 \text{ cm/sec} \]

50\% due to AQP1

50\% due to Rh protein

Endeward et al, 2008

2 channels

\[ P_{CO_2} \approx 100 \cdot P_{HCO_3^-} \]
Lecture 4: Endovascular - Intrinsic CO₂ permeability of cell membrane

\[ P_{CO_2} = 0.015 \text{ cm/sec in RBC AAP 1 & Rh null} \]

\[ \rightarrow \text{CO}_2 \]

\[ \rightarrow \text{membrane} \]

Vesicles with different cholesterol content

↑ contains CAII

Gas Channels Workshop 2012 7 / 20 9/6/12
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 mem.

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

↑ 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?
Hp: AQP1 transports NO

Measurements: cultured cells & fluorescence

1. $P_{NO}$ correlates with $P$

2. ↑ AQP1 $\Rightarrow$ ↑ NO expression
③ Inhibitors of AQP1 reduce NO fluxes

④ \[ \text{No Influx} \rightarrow \text{NO Influx is saturable} \]

⑤ Purified AQP-1 increases NO transport

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

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 PNO

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

Data show both saturable & diffusive

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

\text{saturable component}

Handlogten et al, \textit{AJP Renal}, 2004

Are Rh proteins present in cells with NH₃ transport?

\text{RhAG} \quad \text{in RBC} \ldots.

\text{RhG} \quad \text{in liver, kidney, sweat glands, intestine, lungs}

\text{when you sweat, NH₃ ↑}

\text{RhCG} \quad \text{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

- 
  - Rh Family
  - AQP Family

Ammonia Transport: Amt/MEP/Rh Family

in bacteria

Nitrogen Metabolism in bacteria

\[
\begin{align*}
\text{NH}_4^+ & \leftrightarrow \text{NH}_3^+ + \text{H}^+ \\
\text{H} & \xrightarrow{\text{H}} N: \\
\text{H} & \end{align*}
\]

Dipole moment

\[
\delta^- \quad 2\delta^+ \quad \delta^{-} \\
0 = C = 0
\]

\[
\delta^{+/-} \quad \delta^{+/-} \\
O = O
\]
$\text{NH}_4^+ \leftrightarrow \text{NH}_3 + \text{H}^+$

$\text{NH}_3$ channel

Am+B Crystalslography

↑ trimer

lyphosome

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

Wed 28th November: http://rmi2012.org/ San Francisco
Xue Qin : AQP5

\[ \Delta p \text{H}2 \text{O}^* = (\Delta p \text{H}2 \text{O})_{\text{AQP}} - (\Delta p \text{H}2 \text{O})_{\text{H}2 \text{O control}} \]

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 H2O or none.
Do something to the CO2 permeability without affecting the H2O permeability.

crystal structure ≈ difficult

O2 diffusion through cavities
niche packing between the helices
partition coefficient of water to octanol \rightarrow\text{hydrophobic channel}

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

AQP4 in astrocytic endfeet
\uparrow
P_f is insensitive to DIDS
\uparrow
non specific
you get specificity by making mutations (in NBCs)
but for AQP5 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
DTR 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:
   
   - \( \text{CO}_2 \)
   - \( \text{NH}_3 \) - general medicine
   - \( \text{O}_2 \) - EPR, Optical/Hb; we want to measure fluxes of \( \text{O}_2 \) and we want to do it faster
   - \( \text{NO} \) - Hb
   - \( \text{CO} \) - Hb
   - \( \text{CH}_4 \) - swamp bacteria
   - \( \text{H}_2\text{S} \) - purple bacteria
   - \( \text{N}_2 \) - nitrogenase - Raman Spectroscopy (fast but not sensitive)
   - Ethylene - plants
   - \( \text{H}_2 \)
   
   How do we measure \( \text{N}_2 \) fluxes?
   
   - \( ^{13} \text{N} - \text{NMR} \) (not very sensitive, slow)

   \( \text{H}_2\text{S} \leftrightarrow \text{H}^+ + \text{HS}^- \)

   - \( \text{pH} \) 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, BOB
• 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
COE experiments (Jing Lu)
NRC as a CO₂ channel
ONR global Funding Opportunities

Director’s initiative: point of contact
Notes on the Discussion following Xue Qin’s talk:

Clustering of AQP5s: Does the gas permeability increase with clustering, suggesting an inter-tetramer contribution to permeability?

How to attack the central-pore hypothesis? The outermost residue is T41 in TM2. Reducing agents or Cu^{2+}? In a T41C mutant, Cu^{2+} should bind to Cys residues and block, reversibly. Zn^{2+} or Ni^{2+} could coordinate with His in a T41H mutant. Bob Stroud thinks that we should use HgCl₂ rather than pCMBS. Worries about DIDS being non-specific. Dose-response for DIDS? Others.

General Discussion

I. What gases should we be interested in?
- CO₂
- O₂: Optical/Hb. Phosphorescence (if fast). EPR.
- NO: Optical/Hb. Electrodes
- CO: Optical/Hb.
- N₂: nitrogenase to turn it into NH₃? Raman Spectroscopy (fast but not very sensitive). Surface enhancement with gold particles? Agriculture. Microbiology. ¹³N-NMR (not very sensitive … talk to NMR guys)
- CH₄: Swamp bacteria
- Ethylene: signaling in plants
- H₂: would it need a channel

II. What other families of gas channels might there be?
Any multimeric membrane protein whose monomers are functionally active (excludes ion channels)
- RBC proteins/O₂: AE1, GLUT1/4, AQP1, Rh, MCT-1 (all ≥ 100k/cell) … dozens of proteins 10k-25k copies/cell.
- Endothelial cells in capillaries, etc:
- BBB, BRetinaB (Pigment epithelial)
- Blood-testis barrier, blood-ovary barrier
- Lungs: AQP5 (no alveolar Rh proteins).
- Striated Muscle … myoglobin
- Mitochondrion: MIM. CO₂ is formed in the matrix. Perhaps O₂ as well? AQP8, AQP9 (MIM). AQP5. H₂O???
- Associations …. Proteomics.
- Connexins, pannexins, and similar proteins
- Strategies for finding new kinds of channels: (1) subjecting mice to chronic hypoxia and harvest RBC … proteomic analysis. Check mRNA levels in retics. Normalize to 18S RNA, etc … proteomics, lipidomics (↓ cholesterol), MCV (surface-volume ratio), P50 (pHᵢ, 2-3-DPG). Splice variants change?
III. What are the physiological implications of gas channels?

The gas-channel hypothesis, if true, would be a major paradigm shift … changing the way we think about all processes involving gases. Game changer. Definitive health and performance issues. Gas channels provide:

- High flux
- Selectivity
- Control by signal transduction
- **Pharmacological intervention**: block or stimulate (signal-transduction: trafficking, post-translational modification) a specific pathways for specific gases, in specific places.
- Performance → Exercise, athletics, Warfighter performance, altitude: AQP1-null mouse has a 50% voluntary exercise deficit (**performance defect**). Worse at altitude. Could be due to CO₂ retention, reduced NO flux (less exercise-induced vasodilation)? Treadmill. RhAG-null. If we ever find the O₂ channel(s) … those KOs? NO channel
- Performance → mental: AQP1-null mouse has a 50% voluntary exercise deficit. Do AQP4-null mice have ↑ cerebral capillary density to compensate for low O₂ permeability? … but downside is susceptibility.
- Cerebral edema. Stroke, TBI, AMS … Aeromics has a drug that blocks the aquaporins of AQP4 (and AQP2) … we hope not the gas. After the first 3 days of stroke, when edema is resolving … stimulate AQP4
- AQP5: gas permeability of the lungs … but downside is susceptibility. pulmonary edema. Selective drug to block P_f and stay away from gas (if it is important).
- Effect of pressure on gas permeability. In Fish … Different channels or splice variants at different depth.
- HRE (hypoxia-response elements): which proteins unexpectedly have HREs. HIF-1α.
- Shear stress: ↑ expression of NOS
- Are different splice variants used under different conditions?
- Size scaling, Allometry: Might expect to see a lot of gas channels in mice, but not in elephants. Also a lower O₂ consumption/gram.
- Fish gills. Compare tuna to a flounder.
- Horse has a wide range of performance.
- Joe LaManna: membranes with low intrinsic permeability—lots of proteins or cholesterol—and a high O₂ requirement, would be most likely to have gas channels. Optimizes human performance.
- Exclude gas:
- Transport gas directionally.
- Wound healing, bone-fracture healing.
- COPD: CO₂ retention,
- Stroke, MI … low gas permeability could contribute to the development of the problem?? Increasing gas permeability could help in recovery.
- Decompression illnesses (DCS +AGE, arterial gas embolism): ↓ N₂ permeability on the way down (would also solve N₂ narcosis) … increase it on the way up.
- O₂ toxicity:
- CO₂ narcosis:
- N₂ narcosis:
- Submarine escape … DCI.
- Acute mountain sickness … hypoxia
- Increase O₂ transport into tumors just before radiation
- Bacteria that need to transport gas/antibiotic. Helicobacter …
- Parasites … inhibit gas transport … if the organism has a sufficiently phunky gas channel
IV. **How do gases pass through the gas channel?**
- Monomeric pores: AQP1 aquaporines, Rh ammoniapores, UT urea pores
- Central pores (3- or 4-fold axes of symmetry)
- Side pockets (e.g., between the edges of 2 AQP tetramers)
- Corner pockets (e.g., at the corners of 4 AQP tetramers)
- Packing?: Emad tried to pack AQP4 monomers based on Fujioshi’s/Engel’s EM data. He thinks that the monomers did not get close enough together. The only reference they had. Could one do Atomic Force Microscopy (Jeff)?
- WFB/Jeff: Might it be possible to push the sides of tetramers together to see if the sides like to be together?
- Arrays: AQP4/M1 forms very small arrays, AQP4/M23 (BBB) form extended arrays of tetramers. Verkman found that it is aa17-22 in M1 that obstruct array formation. AV took MM23 Nt and transplanted it to AQP1 and got AQP1 to form arrays.
- Nanotubes, peptides that form channel-like structures … NSF … conduct CO₂, O₂, etc. Could be used as sensors. Cannot emphasize medical side. They fund the basic science. Plants.

V. **How can we better model the movement of gases across cell membranes?**
- More crystal structures
- More molecular dynamics

VI. **What funding mechanisms are possible?**
Early on, we have to hit at least one home run.
- ONR-BRC (Basic Research Challenge): Navy.
- ONR-MURI (Multi-Univ Research Initiative): OSD (Office of the Secretary of Defense) oversight.
- ONR-Undersea Medicine/Stress Physiology:
- ONR Young Investigator: Tenure track.
- Chief of Naval Research (CNR/2*)
- DHP (Defense Health Program): Army is the agent. Warfighter protection/performance … AMS, …
- PPG NIH-HL/Hypertension:
- PPG NIH-HL/Blood:
- PPG NIH-HL/Lung:
- PPG NIH-DK/: NH₃ via Rh and AQP. Acid-base balance.
- Director’s Initiative ????: Sept 25 … no preliminary data necessary … high risk/high impact. Up to $5M/year. Could we get: Point of Contact. List of past recipients. Also train future scientists to carry torch. Most of winners are Associate Professor.
- ONR-G (ONR Global)/Foreign Only: VSP (Visiting Scientist Program), meetings, NICOP
- NSF: nanotubes, etc.
- Dept of Agriculture: N₂ fixation (must be done in the absence of O₂)
Ignore the following:

ACh-induced ↓ in resistance … how affected by AQP1 KO?

WFB: We need to get together in-silico and stay in touch … plan grant applications. Jeff: we need to be focused … in each grant … stay out of KMBD (kiss of death) … aim for Kidney Pathobiology and Urologic Diseases, Hypertension and microcirculation.

Rose:
- Please collect notes from volunteers
- PPTs
- Set up a teleconference in 1 month
- Send out these notes for annotation