## Abstract

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 (C02), 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 video-taped 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--Rossang” 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 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
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 ¹⁸O 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*

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00am - 8:30am</td>
<td>Continental Breakfast</td>
</tr>
<tr>
<td>8:30am – 8:35am</td>
<td>Introduction/Welcome - Dr. Walter Boron, M.D., Ph.D.</td>
</tr>
</tbody>
</table>
| 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 Sharpey-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 CO\textsubscript{2} and O\textsubscript{2} 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 CO\textsubscript{2} channels in biological membranes, in combination with developing a novel method to determine the CO\textsubscript{2} permeability of cell membranes, and in the molecular mechanism of HCO\textsubscript{3}\textsuperscript{-} 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 $^{18}\text{O}$ 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 $^{18}\text{O}$ 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 in all secretory cells 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 H\textsubscript{2}S 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 Lecturer 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 focused 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

Speakers:

Walter F. Boron, MD, PhD
-Chairman, Professor, Case Western Reserve University
Case Western Reserve University
10900 Euclid Avenue
Cleveland, OH 44106

Phone: 216-368-8978
Email: wfb2@case.edu

Volker Endeward, PhD
-Assistant Professor, University of Hannover, Germany
University of Hannover
Welfengarten 1 Hannover, DE, 30167
Germany

Phone: 49-511-552711
Email: endeward.volker@mh-hannover.de

Jeffrey Garvin, PhD
-Professor, Wayne State University/Henry Ford Hospital
Case Western Reserve University
10900 Euclid Avenue
Cleveland, OH 44106

Phone: 216-368-3353
Email: jlg5@case.edu

Gerolf Gros, MD, PhD
-Professor, University of Hannover, Germany
University of Hannover
Welfengarten 1 Hannover, DE, 30167
Germany

Phone: 49-577-532-2735
Email: Gros.gerolf@mh-hannover.de

Bhanu Jena, PhD
-Professor, Wayne State University
Wayne State University
540 Canfield
5245 Gordon Scott Hall
Detroit, MI 48201

Phone: 313-577-1532
Email: bjena@med.wayne.edu

Robert Stroud, PhD
-Professor, University of California San Francisco
University of California San Francisco
600 16th Street rm. S414
San Francisco, CA 94107

Phone: (415) 476-4224
Email: stroud@msg.ucsf.edu

Emad Tajkhorshid, PhD
-Professor, University of Illinois at Urbana-Champaign
University of Illinois at Urbana-Champaign
Beckman Institute
405 N Mathews Ave
Urbana, IL 61801

Phone: 217-244-6914
Email: emad@life.illinois.edu

David Weiner, MD
-Professor, University of Florida
University of Florida
1600 SW Archer Road
Room VA A531, VA Building
PO Box 100224
Gainesville, FL 32610-0277

Phone: (352) 327-8069
Email: david.weiner@medicine.ufl.edu

Postdoctoral presenters:

Ryan Geyer, PhD
-Postdoc, Case Western Reserve University
Case Western Reserve University
10900 Euclid Avenue
Cleveland, OH 44106

Phone: 216-368-5530
Email: rrg8@case.edu

Rossana Occhipinti, PhD
-Postdoc, Case Western Reserve University
Case Western Reserve University
10900 Euclid Avenue
Xue Qin, PhD
-Postdoc, Case Western Reserve University
Case Western Reserve University
10900 Euclid Avenue
Cleveland, OH 44106

Phone: 216-368-6854
Email: xue.qin@case.edu

Additional Attendees:
CDR Matthew Swiergosz, USN
-Program Manager, Office of Naval Research
Office of Naval Research
Office of Naval Research, Code 342
875 North Randolph Street, Suite 1425
Arlington, VA 22203-1995

Phone: 703-696-0367
Email: matthew.swiergosz@navy.mil

Noah Malmstadt, PhD
-Assistant Professor, USC
University of Southern California
Mork Family Department of Chemical Engineering and Materials Science
University of Southern California
925 Bloom Walk, HED 216
Los Angeles, CA 90089-1211

Phone: 213-821-2034
Email: malmstad@usc.edu
Visualizing Gas Permeation Pathways Through Proteins at Sub-Angstrom Resolution

Emad Tajkhorshid
Computational Structural Biology and Molecular Biophysics
www.csbmb.beckman.illinois.edu

Department of Biochemistry
Center for Biophysics and Computational Biology
Beckman Institute for Advanced Science and Technology
University of Illinois at Urbana-Champaign
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

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
Large-Scale Transition of an ABC Transporter in the Membrane

![Molecular structure and graphs showing transitions in terms of \( \beta \) and \( d_{NBD} \) vs. \( \alpha \) and \( \gamma \).]

- The graph on the right shows the changes in \( \beta \) with \( \alpha \) ranging from 0 to 60.
- The graph on the bottom right illustrates the changes in \( d_{NBD} \) with \( \gamma \) ranging from 0 to 90.
- Key states are marked: OF, IF-c, and IF-o, indicating different configurations of the transporter.
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

 Pₐ-bound state

 PMF (kcal/mol)

 \( \theta_1 + \theta_7 \)
Explicit Ligand Sampling of Gas Transport

Explicit Ligand Sampling of Gas Transport

## Lipid/Water Partition Coefficients

<table>
<thead>
<tr>
<th>Simulation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CO(_2) in POPE</td>
<td>3.50</td>
</tr>
<tr>
<td>CO(_2) in POPC</td>
<td>2.74</td>
</tr>
<tr>
<td>O(_2)(P) in POPC</td>
<td>4.04</td>
</tr>
<tr>
<td>O(_2)(N) in POPC</td>
<td>3.46</td>
</tr>
<tr>
<td>O(_2)(P) in POPE</td>
<td>4.73</td>
</tr>
<tr>
<td>O(_2)(N) in POPE</td>
<td>5.79</td>
</tr>
</tbody>
</table>

### Experiment

#### CO\(_2\)
- Octanol: 1.3
- Hexadecane: 1.5
- Olive oil: 1.7

#### O\(_2\)
- Liposome: 3.9
Gas Diffusion Inside the Lipid Bilayer
Gas Diffusion Inside the Lipid Bilayer
Aquaporin Water/Gas Channels

Why Tetramers?
Implicit Ligand Sampling

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

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

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

Explicit O₂ simulation

Reddish solid: ΔΔG map of ~ -3.5 kcal/mol ; Reddish wireframe: ΔΔG map of ~ -3.0 kcal/mol

Observed Xenon binding in CcO ba₃ crystal structures

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
A Role for the Central Pore!

Central Pore

O₂

Water Pores

CO₂
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

(a) Structural comparison of AQP1 and AQP4 highlighting key residues.

(b) Graph showing pore radii comparison between AQP1 and AQP4.

(c) Detailed view of the pore region in AQP4.

(d) Close-up view of the pore in AQP1.
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 $\text{O}_2$ Permeation Across Charged Lipid Bilayers

DOPS/Ca$^{2+}$

DOPS/Na$^+$

![Graph showing PMF (kcal/mol) vs. z (Å) for different lipid bilayer compositions.](image)
Lipid Phase and Gas Permeation

Liquid phase (30 ns)  Gel phase (30 ns)
Lipid Phase and Gas Permeation

PMF (kcal/mol)

- POPE
- TMCL($L_\alpha$ phase)
- TMCL($L_\beta$ phase)

$z$ (Å)
Highly Mobile Membrane Mimetic (HMMM) Model for Membrane Proteins and Phenomena

Ohkubo, Pogorelov, Arcario, Christensen, Tajkhoshid, Biophysical J. May 2012.
MD Simulation with Full Membrane Representation

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

Z. Ohkubo and E. Tajkhorshid, Structure 2008
**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 \times 60 \times 120 \text{ Å}
DVPSs at 3 \times 3 \times 6 grid points
(22 \text{ ns})

Zenmei Ohkubo
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
HMM – lipids are more mobile than full-lipids
Spontaneous Insertion of FVII-GLA
Spontaneous Membrane Binding

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

$t = 0$

12 ns

50 x 50 x 75 Å
Glycophorin A monomers: 2
z-constraint on 2 carbonyl carbons
Quantitative Characterization and Optimization of HMMM Membrane Dipolar Potential

- 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
Highly Mobile Membrane Mimetic Model (HMMM)

Facilitating dynamical studies of membrane-associated phenomena
Computational Structural Biology and Molecular Biophysics Group (CSBMB)
csbmb.beckman.illinois.edu

Transporter Team
Zhijian Huang                Saher Shaikh
Jing Li                      Paween Mahin
Giray Enkavi                 Po-Chao Wen
Mahmoud Moradi               Wenxun Gan
Wei Han

HMMM Team
Zenmei Ohkubo               Taras Pogorelov
Mark Arcario                Javier Baylon
Joshua Vermaas

Collaborators:
- Walter Boron
- Raif Musa-Aziz
- Xue Qin
- Robert Gennis

R01-GM086749   U54-GM087519
R01-GM101048   P41-GM104601
Measuring Cellular CO₂ Permeability by $^{18}$O 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 CO$_2$ permeability of membranes

P$_{\text{CO}_2}$ of planar phospholipid bilayers from 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 CO\textsubscript{2} and O\textsubscript{2} uptake by red cells be reliably measured by stopped flow techniques?

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

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

\( t_{1/2} \) of O\textsubscript{2} uptake by human red cells: \( \sim \) 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*
\[ \text{Mass spectrometer} \]

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

\[ \text{H}_2^{18}\text{O} + \text{C}^{16}\text{O}_2 \]

\[ \text{time (s)} \]

\[ [\text{C}^{18}\text{O}^{16}\text{O}] \text{ (µM)} \]
pH meter
Blood cells
Stirring bar
Mass spectrometer
Teflon membrane
Water 37°C
mass46/mass 44
Mass spectrometer

\[ \text{Red Cell} \]

\[ \text{HC}^{18}O^{16}O_2^- + H^+ \]

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

\[ \text{P}_{\text{H}_2O} \]

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

\[ \text{H}_2^{18}O + C^{18}O^{16}O \]

\[ \text{H}_2^{16}O + C^{16}O_2 \]

\[ \text{H}_2^{16}O + C^{16}O \]

\[ \text{H}_2O + C^{16}O_2 \]

\[ \text{H}_2O + C^{16}O \]

\[ \text{data} \]

\[ \text{fit} \]

\[ \text{red cells} \]

\[ \text{CA} \]

\[ \text{Time (s)} \]

\[ \text{[C}^{18}O^{16}O]\text{(µM)} \]

\[ 0 \quad 100 \quad 200 \quad 300 \quad 400 \quad 500 \]
\[
\begin{align*}
\frac{d[C^{18}O^{16}O]_{\text{ex}}(t)}{dt} &= -k_u A_{\text{ex}} [C^{18}O^{16}O]_{\text{ex}}(t) + \frac{2k_u}{3K_1} A_{\text{ex}} [H^+]_{\text{ex}} [H^{18}O^{16}O_2]_{\text{ex}}(t) + P_{CO_2} a \frac{v}{1-v} \left\{ [C^{18}O^{16}O]_{\text{in}}(t) - [C^{18}O^{16}O]_{\text{ex}}(t) \right\} \\
\frac{d[C^{18}O^{16}O]_{\text{in}}(t)}{dt} &= -k_u A_{\text{in}} [C^{18}O^{16}O]_{\text{in}}(t) + \frac{2k_u}{3K_1} A_{\text{in}} [H^+]_{\text{in}} [H^{18}O^{16}O_2]_{\text{in}}(t) - P_{CO_2} a \left\{ [C^{18}O^{16}O]_{\text{in}}(t) - [C^{18}O^{16}O]_{\text{ex}}(t) \right\} \\
\frac{d[H^{18}O^{16}O_2]_{\text{ex}}(t)}{dt} &= k_u A_{\text{ex}} \left\{ [C^{18}O^{16}O]_{\text{ex}}(t) + [CO_2] \frac{H^{18}O}{[H_2O]}(t) \right\} - \frac{k_u}{K_1} A_{\text{ex}} [H^+]_{\text{ex}} [H^{18}O^{16}O_2]_{\text{ex}}(t) - P_{HCO_3} a \frac{v}{1-v} \left\{ [H^+]_{\text{ex}} [H^{18}O^{16}O_2]_{\text{in}}(t) - [H^{18}O^{16}O_2]_{\text{ex}}(t) \right\} \\
\frac{d[H^{18}O^{16}O_2]_{\text{in}}(t)}{dt} &= k_u A_{\text{in}} \left\{ [C^{18}O^{16}O]_{\text{in}}(t) + [CO_2] \frac{H^{18}O}{[H_2O]}(t) \right\} - \frac{k_u}{K_1} A_{\text{in}} [H^+]_{\text{in}} [H^{18}O^{16}O_2]_{\text{in}}(t) - P_{HCO_3} a \left\{ [H^+]_{\text{in}} [H^{18}O^{16}O_2]_{\text{ex}}(t) - [H^{18}O^{16}O_2]_{\text{in}}(t) \right\} \\
\frac{d[H^{18}O]}{dt} &= \frac{1}{3K_1} k_u A_{\text{ex}} [H^+]_{\text{ex}} [H^{18}O^{16}O_2]_{\text{ex}}(t) - k_u A_{\text{ex}} \frac{CO_2}{[H_2O]} [H^{18}O]_{\text{ex}}(t) + P_{H_2O} a \frac{v}{1-v} \left\{ [H^{18}O]_{\text{in}}(t) - [H^{18}O]_{\text{ex}}(t) \right\} \\
\frac{d[H^{18}O]_{\text{in}}(t)}{dt} &= \frac{1}{3K_1} k_u A_{\text{in}} [H^+]_{\text{in}} [H^{18}O^{16}O_2]_{\text{in}}(t) - k_u A_{\text{in}} \frac{CO_2}{[H_2O]} [H^{18}O]_{\text{in}}(t) - P_{H_2O} a \left\{ [H^{18}O]_{\text{in}}(t) - [H^{18}O]_{\text{ex}}(t) \right\}
\end{align*}
\]
red cells

[\text{C}^{18}\text{O}^{16}\text{O}] \text{ (\textmu M)}

CA

data fit

Time (s)
Why can we observe fast processes on such a slow time scale, allowing us to follow these processes by mass spectrometry?
\[
\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{HCO}_3^- + \text{H}^+ \\
\text{HC}\text{O}^{18}\text{O}^{16}\text{O}^- + \text{H}^+ \leftrightarrow \text{H}_2^{18}\text{O} + \text{C}^{18}\text{O}^{16}\text{O} \\
\text{H}_2^{18}\text{O} + \text{C}^{16}\text{O}_2
\]

\[
t_{1/2} = 5 \text{ s} \\
t_{1/2} = 250 \text{ s}
\]

Kinetics of CO\textsubscript{2} hydration reaction vs. that of \textsuperscript{18}O exchange
It was shown here that a time course of the decay of $[\text{C}^{18}\text{O}^{16}\text{O}]$ 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 $P_{CO_2}$ 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 $P_{HCO_3^{-}}$ and $P_{CO_2}$ in the sum of squares of deviations between the experimental data of $[C^{18}O^{16}O]$ and those obtained from the best-fit calculation.

When $P_{HCO_3^{-}}$ and $P_{CO_2}$ are varied over a wide range of values, clearly only one well-defined minimum is apparent and no local minima whatsoever are visible.
$[\text{C}^{18}\text{O}^{16}\text{O}] - [\text{C}^{18}\text{O}^{16}\text{O}]$

uncatalysed

$A = 4$

$t$ (s)
$P_{HCO_3^-} = 0.0015 \text{ cm/s}$

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

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

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

$A_e = 4$
Sensitivity of calculated $P_{\text{CO}_2}$ to parameter values
To what extent do unstirred layers around cells affect the permeability determinations?
thickened of unstirred layer $\delta \sim$

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

\[
\frac{1}{P_{CO_2,app}} = \frac{1}{P_{CO_2,mem}} + \frac{\delta_{UL_e}}{D_{CO_2,solution}}
\]
<table>
<thead>
<tr>
<th></th>
<th>$P_{app}$ 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>
\[ \delta \sim \nu \]
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

PCO₂ (cm/s)

* * *

# #

Endeward et al., 2006, 2008
Applying the $^{18}$O technique to measure the CO$_2$ permeability of the apical membrane of intact colon epithelium
Intact Proximal Epithelium
Apical Side

![Graph showing time (s) vs. [C^{18}O^{16}O] (10^{-5} M) with a decrease over time, marked with an arrow indicating Epithelium + extracellular CA inhibitor.]
### CO₂ and HCO₃⁻ 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 $^{18}\text{O}$ exchange technique follows the decay of $^{18}\text{O}$-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 für 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 ~ 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>$P_{CO2}$ (cm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red cell</td>
<td>0.15 ± 0.08</td>
</tr>
<tr>
<td>Red cell Ø AQP1, Ø 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 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?
Cell membranes show CO$_2$ permeabilities lower than synthetic lipid bilayer

<table>
<thead>
<tr>
<th></th>
<th>$P_{CO2}$ (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, ∅ 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>$P_{NH3}$</td>
<td>30 %</td>
<td>0.31</td>
<td>Antonenko et al. 1997</td>
</tr>
<tr>
<td>$P_{NH3}$</td>
<td>52 %</td>
<td>0.012</td>
<td>Hill &amp; Zeidel 2000</td>
</tr>
<tr>
<td>$P_{H2O (f)}$</td>
<td>40 %</td>
<td>0.18</td>
<td>Lande et al. 1995</td>
</tr>
<tr>
<td>$P_{H2O (f)}$</td>
<td>52 %</td>
<td>0.026</td>
<td>Hill &amp; Zeidel 2000</td>
</tr>
<tr>
<td>$P_{H2O (d)}$</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
\[ \left[ C^{18}O^{16}O \right]_\infty - \left[ C^{18}O^{16}O \right] \] (µM)

- 70% chol.
- 30% chol.

\( \text{time (s)} \)
Effect of cholesterol on lipid vesicle CO$_2$ permeability

PC:PS = 8:2

$P_{CO_2}$ (cm/s) vs. % Cholesterol

$>0.16$ cm/s
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: Ø AQP1, Ø 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>
CO₂ permeabilities of cell membranes appear to be essentially determined by their cholesterol content
cholesterol depletion with $\beta$-cyclodextrin

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

Reduction of cholesterol with cyclodextrin raises $P_{CO_2}$.

Enrichment with cholesterol lowers $P_{CO_2}$ 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 $P_{CO_2}$ 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

$P_{CO2} = 0.0015$ 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 $∅$ 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 \( \text{CO}_2 \) permeability is limited.

• Hypothesis: cell membranes with normal cholesterol and low intrinsic \( \text{P}_{\text{CO}_2} \) adapt their \( \text{CO}_2 \) permeabilities to their needs by incorporating gas channels in the membrane.
Aquaporin 1 as a CO₂ 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 CO₂ permeability of AQP1 containing vesicles
Aquaporin 1 as a CO₂ 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 CO\textsubscript{2} permeability, incorporation of AQP1 into the membrane significantly increases the CO\textsubscript{2} permeability in a concentration dependent manner.

• AQP1 acts as a DIDS-sensitive CO\textsubscript{2} channel.
<table>
<thead>
<tr>
<th>Gas</th>
<th>CO$_2$</th>
<th>O$_2$</th>
<th>NO</th>
<th>N$_2$</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>CO₂</td>
<td>O₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------------</td>
<td>--------</td>
<td>--------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lipid-water partition coefficient</strong></td>
<td>0.95</td>
<td>2.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Reduction of membrane permeability by cholesterol</strong></td>
<td>1/100</td>
<td>(1/100) ?</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Membrane permeability</strong></td>
<td>0.01 cm/s</td>
<td>(0.03 cm/s) ?</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Heart muscle under heavy exercise: partial pressure difference across the membrane ΔP</strong></td>
<td>5 mmHg</td>
<td>(40 mmHg) ?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Summary

With rising cholesterol content the CO$_2$ permeability ($P_{CO2}$) of lipid vesicles decreases drastically.

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

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

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

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

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

2) AQP1 expression in MDCK cells increases membrane $P_{CO2}$. 
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
Jena Research Group

Porosome: The Universal Secretory Portal in Cells
Lee et. al. 2012 J. Proteomics 75:3952-62
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

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
ZG Swelling after Cholesterol Incubation

% Change LSI from Baseline

- Control
- Chol 10uM
- Chol 20uM
- Chol 40uM
- Chol 80uM

Amanda F., Jena B.P. (unpublished observation)
Kelly et al., 2005 Pancreatology 5:443-49
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

Furchgott et al., Nature 1980
NO synthesis

L-arginine → L-citrulline + NO
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 \( \text{O}_2 \) and \( \text{CO}_2 \)).
How many think NO diffuses through two bilayers

endothelial cell

vascular smooth muscle cell

soluble guanylate cyclase

NOS3

soluble guanylate cyclase
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
AQP-1
endothelial cell
vascular smooth muscle cell
soluble guanylate cyclase

Hypothesis

NOS 3
NO
If our hypothesis is correct:
1. NO permeability ($P_{\text{NO}}$) 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

objective lens

dichroic mirror

mirror

emission 535±50 nm

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

$[y = 0.64 \times + 20.33]; r = 0.70$
1. NO permeability ($P_{NO}$) 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 influx
[fluorescence units/sec]

C       AQP-1     C      AQP-1

DMSO

$\ p < 0.005$

NO gradient by SPM (5 $\mu$M NO)

C  AQP-1  C  AQP-1

DMSO
Effect of 20 µM HgCl₂, an AQP-1 inhibitor, on NO influx into transfected CHO cells

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

NO gradient by gas (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 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
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?
Aortic ring preparation

- Force transducer
- Aortic ring
- Bath solution
- Inlet
- Outlet
- Gas
Acetylcholine-dependent relaxation of aortic rings from wild type and AQP-1 -/- mice

Log [Ach] concentration

% contraction to PE

WT

AQP-1 -/-

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

- GAPDH siRNA: NO release (pAmps/µg) = 2.5
- AQP-1 siRNA: NO release (pAmps/µg) = 1.5
  - $p < 0.05$

- Control: NO release (pAmps/µg) = 6.0
- HgCl$_2$: NO release (pAmps/µg) = 0.5
  - $p < 0.005$
NO release by cultured aortic endothelial cells from wild type and AQP-1 -/- mice

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

![Graph showing relaxation of denuded aortic rings to spermine NONOate.](image)

- **Log [SPM] concentration**
- **% contraction to PE**
- **p < 0.0001**
- **AQP-1 -/-**
- **WT**
NO influx into isolated aortic vascular smooth muscle cells from wild type and AQP-1 -/- mice

**Graph 1:**
- **Y-axis:** NO flux [fluorescence units/sec]
- **X-axis:** Time [sec]
- **Legend:**
  - WT
  - AQP-1 KO
- **Data Points:**
  - 5 µM NO

**Graph 2:**
- **Y-axis:** NO flux [fluorescence units/sec]
- **X-axis:**
  - WT
  - AQP-1
- **Statistical Test:** $p < 0.002$
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

David Weiner, M.D.
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.
Hui Guo, Ph.D.
Seong-Pyo Hong, M.D., Ph.D.
George Kasnic, B.S.
Hye-Young Kim, M.D., Ph.D.
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
Donald Kohan, M.D., Ph.D.
Peter Igarashi, M.D.
Raoul Nelson, M.D., Ph.D.
Lance Miller, Ph.D.

University of Florida Collaborators
Bill Clapp, M.D.
Byron Croker, M.D., Ph.D.
Kirsten M. Madsen, M.D.
R. Tyler Miller, M.D.

Clinical fellows:
Allen Vander, M.D.
Tom DelGiorno, M.D.
Kevin Schroeder, M.D.
Ian Steele, M.D.
Dietrich Werner, M.D.

New Zealand
Rob Walker, M.D.
Jennifer Bedford, Ph.D.
John Leader, Ph.D.

Ewha Womans University, Seoul, Korea
Ki-Hwan Han, M.D., Ph.D.

University of Pennsylvania
Connie M. Westhoff, Ph.D.
Emory University
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 NH₃ gas transport

1. Is renal collecting duct NH₃ transport diffusive or transporter-mediated?
2. Are Rh glycoproteins present in cells that transport NH₃ gas?
3. Does expression parallel changes in NH₃ gas transport?
4. Does Rh glycoprotein inhibition alter NH₃ gas transport?
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
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
  - Transporter-mediated
    - Saturable

Initial Uptake Rate

[Solute]
Measurement of collecting duct cell (mIMCD-3) basolateral total ammonia transport

Both saturable and diffusive components

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

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

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

- **Cr Rh1**
- **Cr Rh2**

- **Sc Mep1**
- **Sc Mep3**
- **Sc Mep2**
- **At AMT2**

- **Le AMT1.2**
- **At AMT1.2**
- **At AMT1.3**
- **At AMT1.1**
- **Le AMT1.1**

**RhBG**

**RhCG**

**RhAG**

**Rh**

**Mep1/3**

**Mep2/Amt2**

**Amt1**

**Amt2**

**Le**

**At**

**Sc**

**Cr**

**Atm1.3**

**Atm1.1**

**Amt1.2**

**Atm1.2**

**Atm1.1**

**Le AMT1.1**

**At AMT1.1**

**At AMT1.2**

**At AMT1.3**

**Le AMT1.2**

**At AMT1.2**

**At AMT1.3**

**At AMT1.1**

**Le AMT1.1**
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
- Mep1/3
- Sc Mep2
- Mep2/Amt2
- At AMT2
- Le AMT1.2
- At AMT1.2
- At Amt1.3
- At AMT1.1
- Le AMT1.1
- Amt1
Where is Rhcg expressed?

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

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

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

Cr Rh1
Cr Rh2

Sc Mep1
Sc Mep3
Sc Mep2

At AMT2
Le AMT1.2
At AMT1.2
At Amt1.3
At AMT1.1
Le AMT1.1

RhbG
RhCG
RhAG
Rh
Mep1/3
Mep2/Amt2
Mep2/Amt2
Amt1

Liver
Portal vein
Hepatic vein
Hepatic artery
Cava vein

0.2
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%)

- Control Diet
- 3 Days HCl
- 5 Days HCl

Cortex
- Control Diet
- 3 Days HCl
- 5 Days HCl

Outer Medulla
- Control Diet
- 3 Days HCl
- 5 Days HCl

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

Collecting duct-specific Rhcg deletion - basal effects

**Urinary Ammonia**

- **+/+**: 86 µmol per day
- **-/-**: 63 µmol per day

P < 0.05

**Serum HCO$_3^-$**

- **+/+**: 19.4 mmol per L
- **-/-**: 19.9 mmol per L

P = NS

Collecting duct-specific Rhcg deletion - acid loading

- **Traditional**
  - Add NH₄Cl to drinking water
  - Not well tolerated
    - Add glucose
  - Hard to quantify
- **“New”**
  - Add HCl directly to food
  - Use powdered food
  - Easily quantified
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

Urinary Ammonia (µmol d⁻¹)

Day of acid-loading

Control

IC-Rhbg-KO

P < 0.05 days 2-4

Another Rhbg gene deletion study

- NH₄Cl, Global KO
- NH₄Cl, Wild-type


**Graph:**
- X-axis: Day
- Y-axis: Urinary Ammonia (µmol d⁻¹)
- Dashed line: NH₄Cl, Global KO
- Solid line: NH₄Cl, Wild-type
Comparison of the two Rhbg gene deletion studies

- HCl, Control

- HCl, IC-Rhbg-KO

- NH₄Cl, Global KO

- NH₄Cl, Wild-type

Graph showing the comparison of urinary ammonia (μmol d⁻¹) levels over days for different conditions.
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 CO$_2$.

DIDS—the anion transport inhibitor—not only reduces HCO$_3^-$ permeability, but the CO$_2$ permeability in human RBCs.

Later it was shown that NH$_3$ also passes through AQP1.

RhAG (a component of the Rh complex in human RBC) conducts NH$_3$.

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 CO$_2$.

Musa-Aziz & Boron showed for the first time that gas channels—like ion channels—can exhibit selectivity for one gas over another (CO$_2$ vs. NH$_3$).
Gas Exchange

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

Hemoglobin saturation (%)

O₂ content (ml O₂/dL blood)

Oxygen partial pressure (pO₂, mmHg)

Red blood cell

Oxygen from lungs

Oxygen released to tissue cells

Hemoglobin molecules

Oxygen bonded with hemoglobin molecules

http://www.infobarrel.com/media/image/96184.jpg
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.

Anstee. *VoxSanguinis* 100, 2011
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 NH₃ 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 and urea transporters.
Hemoglobin Absorbance Changes

HbO₂ → Hb

Molar Extinction Coefficient (cm⁻¹/M)

Wavelength (nm)

HbO₂
Hb
Stopped Flow Technique

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

From this data we can calculate the rate of the chemical reaction.

Rapidly mixes two solutions (on a millisecond timescale)
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>anti-AE1</th>
<th>anti-GLUT4</th>
<th>anti-AQP1</th>
<th>anti-RhAG</th>
<th>anti-MCT1</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
</tr>
<tr>
<td>KO</td>
<td>KO</td>
<td>KO</td>
<td>KO</td>
<td>KO</td>
</tr>
</tbody>
</table>

Image of Western Blot Analysis showing protein expression levels for wild-type (wt) and AQP1-KO (KO) conditions for each antibody.
Effect of pCMBS, Phloretin, or DIDS on Oxygen Efflux from Mouse Red Blood Cells

- pCMBS reduces the O$_2$ efflux by 50%.
- Phloretin reduces the O$_2$ efflux by 40%.
- DIDS reduces the O$_2$ efflux by 25%.
- pCMBS + Phloretin reduce the O$_2$ 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.

*P ≤ 10⁻⁴ vs Control
The inhibition of $O_2$ 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 CO₂ channels (AQP1 and RhAG) of the RBC appear to modestly transport O₂ out of the cell.

  ... because this transport process is not greatly effected by DIDS the O₂ must utilize an alternative pathway(s).

• Knockouts of GLUT4 and AQP1/RhAG have O₂ efflux rates greater (10-25%) than the wild-type controls.

• Inhibitor efficacy:

  \[\text{pCMBS + phloretin} > \text{pCMBS} > \text{phloretin} > \text{DIDS}\]

  ... because the pCMBS/Phloretin inhibition was not totally additive, O₂ 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 (H$_2$S) transport in red blood cells.
• Investigate the effect of hypoxia on protein expression (XQ) and O$_2$ transport activity (RRG).
• Investigate the effect of adding and removing cholesterol from the RBC membranes on O$_2$ 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)


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
Gas Channel Workshop

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

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

$\text{CO}_2$ permeability
Not well defined
Methods

$H_2O$ permeability--$P_f$

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

$CO_2$ permeability--$\Delta pH_S$

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
c Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA
d Department of Physics, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

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 AQPs.

© 2006 Elsevier Inc. All rights reserved.

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

**bAQP1.PRO**
MASEFKKLFWRAVVAEFLAMILFIFISIGSALGFHYPIKSNQTTGAVQDNKVSFLAFGL 60

**hAQP5.PRO**
MKKEVCVSAFLKAVFAEFLATLIFVFGLGSALKWPS--------ALPTLQIALAFGL 51

**bAQP1.PRO**
SIATLAQSVGHISGAHLNPATLGLLLSCQISVLRAIMYIIAQCVGAIVATAILSGITSS 120

**hAQP5.PRO**
AIGTLAQALGPVSGGHRNPALTLALLVGNQISLLRAFFYVAAQLVGAIAGAGILYGVAPL 111

**bAQP1.PRO**
LPDNSLGLNALAPGNSGQGLIEIIGTQLVLCLVATTDRRRRDLGGSGPLAIGFSVAL 180

**hAQP5.PRO**
NARGNLAVNAlNNTQGQAMVVELILTFQLALCIFASTDSRRTPVGSPAALSIGLSVTL 171

**bAQP1.PRO**
GHLLAIDYTGCINGPARSFGGSSVITHNFQ-DHWIFWVGPIGAALAVLIYDFILAPRSSD 239

**hAQP5.PRO**
GHLVGIYFTGCMSNAPARSFPGAIVMNRFSPAHWFVWGVPIVGAVLAIALYFyllFPNSLS 231

**bAQP1.PRO**
LTDKVKERL--QVEEYDLDADDINSRVEMKPK 271

**hAQP5.PRO**
LSERVAIIGTYEPDEWEEQREERKKTMELTTR 265
Relative channel-dependent CO$_2$ permeability

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

I  Amino acids at the mouth of the central pore
Changes of ΔpH₅ (CO₂ permeability) is more sensitive than Pₚ (H₂O 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

hAQP9.PRO          GCGCVAQAILSR-------GRFGGVLTINFGSNAVAMAIYVAGGSFGGHINPAVSLAMCL 93
hAQP10.PRO         TQGAVAQAVTSG-------ETKGNPFTTMLAGSIAVTIAIVYGGNVSAGHLNPAFSLAMCI 91
hAQP3.PRO          GCGSVAQVVLISR-------GTHGFLTLINAFGLPAVTGLILLAGQVSGHHLNPATFAMCF 92
hAQP7.PRO          GLGSVAHMVNL-------KKYGSYLVGNLFGFVGTMVHAGRISGAHMAAVTANCA 103
hAQP2.PRO          GLGSALNWPQ-------ALPSVLAAFGGLIIGTVQLGHLHISGHLNPATVACLPLV 77
hAQP5.PRO          GLGSALKWPIS-------ALFTLQIALAFLGIALTAGLQALPVSGHHINPAITLALLLV 78
hAQP6.PRO          GVSAMRWPT-------ALPSVQIAITFNIVTaVQTVKASAGHANPAPTALFLV 91
bAQP0.PRO          GLGASLRWAP-------GPLHVLQIALLAFGLALTAVIGHGHLHISGHLNPATVFLV 77
hAQP4-M1.PRO       SLGSTINW-------GTEKP--LPVDMVLISLCTGSAITMVCGFHSIGHINPAVTAMV 106
hAQP4-M23.PRO      SLGSTINW-------GTEKP--LPVDMVLISLCTGSAITMVCGFHSIGHINPAVTAMV 84
bAQP1.PRO          SIGSARGHYPIKSNQTVDWVQDVLSAGLSIATLQASVQVHSISGHLNPATVLGLL 87
hAQP1.PRO          SIGSARGKFYGPVGNQ----AVQDVNKLAFGLSIATAQSVQHISGHLNPATVLGLL 85
hAQP8.PRO          GCLSVIENG-------DTGGLQPAIALAAHLGALGLVIATLGNISGGHIFPAVLSAAML 101
hAQP11.PRO         LCCCTHELQLLS-------EQHPAPHTWPTLTVFVFFSVHGLTVGTSSNPCGVMQQMM 108

hAQP9.PRO          YPAPYLSLNAFADQVATMILLIIIVAFIIFSRNLGAPRGLPTAIAGGLIIIVIAASSLGLN 210
hAQP10.PRO         YPAPYLSLNNFGLQVLGTMGLVILLAILDRNKGVAGLEPYYGMLILALGLMGAN 208
hAQP3.PRO          YPSGHLDMINGFDQFIQASTLIVCVLATIDPYNVPRGLAEATVQVLLVLTGSMGFN 209
hAQP7.PRO          YLPDHMTLWRGFLNEAWLTMGLQCLFAITHDFQWINPAPLPSTGALVIGLVIIVGSLMN 220
hAQP2.PRO          ALSNSTTAGAVTVELTLQLVLClFASTD--ERRGENPCTPALDIGFSVALGHLLIGHY 178
hAQP5.PRO          ALNNNTOTQGAMVVELILETFQALCTIFASTD--SRRTSPVGPSPALDIGSVTLGHLVGYF 179
hAQP6.PRO          VVRNSVSTGQAVAELLTTLQLVLCVFASTD--SRQTS--GSPTMIGSISVAHGLHGJHF 190
bAQP0.PRO          TLHPGFSVGQATIVEFILTQLVQVFASTD--ERRNGRLGSVALVGFSLTLGHLLFGMY 178
hAQP4-M1.PRO       MVHGNLTAHGLLVELILETFQQLVFTIFASCD--SRRTDVTGTSIADIGSVAIGHAFAINY 207
hAQP4-M23.PRO      MVHGNLTAHGLLVELILETFQQLVFTIFASCD--SRRTDVTGTSIADIGSVAIGHAFAINY 185
bAQP1.PRO          ALAPGVNSQQLGLIEIGIQTGQLVLCLATTD--RERRDDLGSGPLOAIGSVALGHLLAIDY 188
hAQP1.PRO          DLADGVNSQQLGLIEIGIQTGQLVLCLATTD--RERRDDLGSGPLOAIGSVALGHLLAIDY 186
hAQP8.PRO          TVQEQQGVAGALVAEIIITTLALAVCMGAINEKTKP--IAPHISGAFTVFDLHAGGPV 204
hAQP11.PRO         RSFACKNHPIRVDDKAVITEAVCSFLFSALLHFGV FFTQIRHIALLALITFLVYAGSS 210
If mutate to Asp (D)
Aspartic Acid (Asp)

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

LXXD - \( \Delta p\text{H}_S^* \)

\[
\begin{align*}
\text{pK}_a & = 1.95 \\
\text{pK}_a & = 3.71 \\
\end{align*}
\]

<table>
<thead>
<tr>
<th>Protein Variant</th>
<th>( \Delta p\text{H}_S )</th>
</tr>
</thead>
<tbody>
<tr>
<td>hAQP5 T41D</td>
<td>0.09</td>
</tr>
<tr>
<td>hAQP5 L43D</td>
<td>0.06</td>
</tr>
<tr>
<td>hAQP5 L47D</td>
<td>0.03</td>
</tr>
<tr>
<td>hAQP5 L51D</td>
<td>0.00</td>
</tr>
<tr>
<td>hAQP5 L167D</td>
<td>0.06</td>
</tr>
</tbody>
</table>

\( P_f^* \)
If mutate to Arg (R)
Arginine (Arg)

Channel-dependent $\Delta p_{H_S}$ for CO$_2$

$\text{LXXR} - \Delta p_{H_S}^*$
Arginine (Arg)

LXXR – $P_f^*$

Channel-dependent $P_f$ (cm/s)

- H$_2$O
- hAQP5 T41R
- hAQP5 L43R
- hAQP5 L47R
- hAQP5 L51R
- hAQP5 L167R
If mutate to Thr (T)
Channel-dependent $\Delta p$H$_S$ for CO$_2$

Threonine (Thr)
channel-dependent $P_f$ (cm/s)

Threonine (Thr)

LXXT - $P_f^*$

- $H_2O$
- 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 $\Delta p\text{H}_S$ ($\text{CO}_2$ permeability) is more sensitive than $P_f$ ($\text{H}_2\text{O}$ permeability)
T41 is more important than L43.

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

Collaborator
Emad Tajkhorshid

Lab
Walter F Boron
Raif Musa-Aziz
Mark D Parker
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)

- CO₂ → CO₂
- H₂O → CO₂
- HCO₃⁻ → HCO₃⁻

\[ \text{[CO}_2\text{]}_S \downarrow \]

\[ \text{pH}_S \uparrow \] (data kindly provided by Dr. Musa-Aziz)
An appropriate mathematical model should include:

- A spherical cell
- Transport of CO$_2$ 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
Intracellular Fluid (ICF)

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

Extracellular Unconvedted Fluid (EUF)

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

Bulk Extracellular Fluid (BECF)

\[ \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{O} \]

The Mathematical Model

Somersalo, Occhipinti, Boron, Calvetti, J Theor Biol, 2012
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 CO$_2$

In both EUF and intracellular fluid (ICF)

Slow equilibration of the CO$_2$ hydration/dehydration reactions
Competing equilibria among the CO$_2$/HCO$_3^-$ and a multitude of non-CO$_2$/HCO$_3^-$ buffers
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.
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$_2$CO$_3$] = [HCO$_3^-$] = 0 mM
  - has a single *mobile* non-CO$_2$/HCO$_3^-$ buffer with pK = 7.10 and [TA] $\approx$ 27.31mM
Results

Extracellular concentration-time profiles for solutes

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

\[ \text{HA}_1 \xrightarrow{f} \text{A}_1^- + \text{H}^+ \]

(A) \[\text{CO}_2\]
(B) \[\text{H}_2\text{CO}_3\]
(C) \[\text{HCO}_3^-\]
(D) \[\text{pH}\]
(E) \[\text{HA}_1\]
(F) \[\text{A}_1^-\]
Intracellular concentration-time profiles for solutes

\[
\begin{align*}
CO_2 + H_2O & \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+ \\
HA_1 & \rightleftharpoons A_1^- + H^+
\end{align*}
\]
Effects of Decreasing CO₂ Membrane Permeability

(A) 

(B) 

(C) 

(D)
Implications

The background permeability of the membrane (i.e., in the absence of gas channels) must be very low.

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

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 multiples 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 CO$_2$ near the outer surface of the oocyte. We quantify this competition by introducing the diffusion reaction ratio (DRR)

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

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 $\text{pH}_S$ spike, $(\Delta\text{pH}_S)_{\text{max}}$, 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 $P_{M,\text{CO}_2}$, 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 of \textasciitilde 100 \text{CO}_2 near membrane

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

It's partition coeff is \approx \text{exp. det. values}.

Gas reaches equil in lipid in 10-15 ns for \text{O}_2 \& \text{CO}_2.

Implicit ligand sampling: Works \& neutral molecules (not ions, which perturb protein)... results are about same as explicit.
\[ \{ \text{AQP1, 4, 5} \} \quad \{ \text{CO}_2, \text{O}_2, \text{NO}\} \]

**CP**

**cp** edge of tetramer

---

<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-OH₂</td>
<td>16</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Equi POPE-OH₂</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-OH₂</td>
<td>310</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Press POPC-OH₂</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-OH₂</td>
<td>79</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>
AQP1: D50 \rightarrow H_2O accord -> major barrier

No' through AQP4

O_2 " "

(Wang, Proteins, 2010)

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

AQP4 (vs. 1): diff ΔG profile

Rely heavily on init. config. of lipid

Problem: Lipid molecules move very slowly!

10^3 slower than H_2O

HMMM: highly mobile membrane mimetic

(lipid 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 vessels

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

Measure $^{18}\text{O}$-labelled $\text{CO}_2$
$4\text{O} \leftrightarrow 4\text{H} \text{ Mol. Mass}$

$\text{HCT}: 2 \times 10^{-4}$
$2 \times 10^{-3}$ $^{18}\text{O}$

Mixing:
But $K_D$ of $\text{AQP1 + Rh}$
could $\uparrow \text{t}_\frac{1}{2}$
by $10 \times \ldots$
make a $\Delta$
measurable?

3-D curve fitting $\rightarrow \Delta$

optimal $P_{\text{CO}_2}$ & $P_{\text{HCO}_3}$ \text{... no local minima}

Critical parameter: $A_i$ & $p\text{Hi}$ \ldots
errors $\rightarrow$ big $\Delta P_{\text{CO}_2}$
$p\text{Hi} \pm 0.01 \rightarrow 20-30 \%$

$p\text{Hi}$ & $P_{\text{HCO}_3}$ are not important
Viscosity: $\uparrow$ [Dextran] $\rightarrow$ $\downarrow$ $P_{\text{HNO}_3}$ (which is so small)

unstirred layer

$P_{\text{O}_2}$

$S = 0.5 \mu m$

$P_M = 0.16 \text{ cm/s}$

: UL is only a minor problem

$\alpha$

$P_{\text{O}_2}$ in human RBC'S

$\text{AOPI } \sim 50\%$

$\text{Rh } \sim 50\%$ $\sim 100\%$

Colonic:

IDEAS FOR DISC.

$\neq$

Competition of $\text{H}_2\text{O}$ vs. $\text{NH}_3$

or $\text{CO}_2$ 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.
\[ [x]_w = S_w P_x \]

\[ [x]_L = \frac{S_L}{S_w} [x]_w = \frac{S_L}{S_w} \cdot S_w \cdot P_x = \frac{S_L}{S_w} \cdot P_x \]

\[ = S_L \cdot P_x \]
Gas Channels Workshop
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_{CO₂} (cm/s) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetic lipid bilayer</td>
<td>0.35 ± 0.32</td>
</tr>
<tr>
<td>Red cell, Ø 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) $\gg$ naked mammalian membrane

Cholesterol: 98% of Pf.

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

Chol: 0-20% $\rightarrow$ not measurable

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

Apical colonic membrane: 70% cholesterol

$\beta$-cyclodextrin
Could we consider using Chol & Xo by raising pCO₂ and 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 P₉CO₂ adapt their CO₂ permeabilities to their needs by incorporating gas channels in the membrane.

AQP1 vs. AQPx in liposomes

↑↑P₉CO₂  ↑ΔP₉CO₂

He sees a much bigger effect (≈80%) 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 ?
Heart m: $\Delta p_{CO_2} = 40 \text{ mm Hg}$

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 $P_{CO_2}$

$P_{CO_2} \propto [\text{AQP1}]^n$ ?
Got interested in AQP s because of their involvement in vesicle fusion.

H$_2$O must enter the vesicle

\[
\text{G} \rightarrow 3 : \text{acce.} \rightarrow Z \rightarrow \text{M}
\]

Jena et al., PNAS, 1997

\[
\text{GTP} \rightarrow 4 \text{ water by volume (AFM) } \rightarrow 3 \text{ H}_2\text{O}
\]

GTP-induced swelling

\[
\begin{array}{c}
\text{Go} \rightarrow 3 \text{ swollen complex} \\
\text{not 2, 3, 4, 5, 7, 8, 9}
\end{array}
\]

Mast (mastoparan)

\[
\text{swelling}
\]

\[
\% \Delta \text{Volume of granule: GTP + Mast} + 20-40 \mu \text{M MBCD (cyclodextrin)}
\]

AQP 6: Go, V$_+$, ATPase complex req. Chol.

Remove Chol \rightarrow complex falls apart.
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

$\frac{1}{2} \approx 30 \text{ s}$
Energy profile of NO with distance based on partition coefficient

```
Partition coeff say nothing about rates" his PhD mentor beat into him that 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.

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

NO influx $\rightarrow$ CHO cells, transiently transfected.

NO: NO donor or gas.

Hg interferes $\propto$ NO gas?

$K_{1/2} = 0.54 \mu M$  Physiol $[NO]$ $\approx 0.2 \mu M$
AQP1 reconst. into vessels → ↑ J_{NO}

CHD cells:
AQP3: 25% ↑ over mock << AQP1. Did not V expression.
" 4: 30% ↑
≠

Aortic ring: isometric force
PE = Phenylephrine ⇒ contr.

Vary [ACh] to relax. AQP1 KO: ↓ ACh response
? NO efflux from EC or ↓ influx into VSMC

KO: ↓ NO release from EC

" uptake into VSMC

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.
Assessing roles of Rh glycoproteins in NH₃ gas transport?

Is transport "diffusive" or protein mediated?

Inhibitors: none

Saturation?

Diffusion may dominate in inner medulla, where [NH₃] is high & Rh levels are low.

\[ \text{RhAG} + \text{Rh30} \rightarrow_{\text{D}} \]

Cannot find RhAG anywhere but RBC.

1st cloned: RhAG

#2 " BG: Perivenuous cells in hepatocyte

Hair follicles. [NH₃]is 100X > plasma. Goes up in exercise. 2 \rightarrow 10 \text{mM. Urine: } 200-300 \text{mM (higher NH₃ core)
$NH_3$

GI: 250 mmole/day. SI > Colon
Lungs: RhBG → not in Alveolar cells but in Bronchial epithelial cells.

RhCG: (?) Glu neurotransmission.
Liver: $[NH_3]$ is ↑ in bile

Muscle: Exercise → 4-5× $NH_3$ is produced by sk.m. At rest, sk.m. is a $NH_3$ sink. excreted

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

CD: RhBG: BLM → BalbC

CG: AM + some BLM → CS7 BL6: much higher

Mac: ↑ RhB6 expression

Does Tenidap ↓ the "CO2 permeability" attributable to NBC?

Does it speed up pH? "

Grant: Mutant NBCe1

(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

Conditional KO of Rhcg: 
- Urinary excretion but
- 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 Workshop
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

I. Rh Family (Amt/MEP)

Back: Yeah

Back: reg. N as food source, pref. as NH₃

Regulation of Nitrogen Metabolism

- NH₃
- Glu
- GS
- Gln
- GlnK
- UMP
- UTase/URase
Why is H\textsubscript{2}O not a gas... H-bonding

Some still think the Rh prol.
are NH\textsubscript{4}\textsuperscript{+} channels!

But H\textsubscript{2}O can be a gas! WB
\[ A_{\text{H-B}} = 1.35 \text{ Å} \]

11 TMs
inverted repeat

\[ \text{NH}_3 : \text{Hole hole of crystallography} \]
\[ 10e^- \text{ also Na}^+ \text{, Na}^{++} \]

Can also use MeNH\(_3\)

Channel: No H\(_2\)O \(1/7\) occupancy by NH\(_3\) @ 3 sites

\[ pK_a \text{ } 9.6 \rightarrow < 7 \]

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.

\[ \text{Amt B} \]
Completely turned off when enough NH₃ is around!

\[ \text{GlnK: Finger points to Amt B \& blocks it.} \]

\[ \text{ADP bound \hspace{1cm} Prevents re-protonation \hspace{1cm} of entering NH₃.} \]
*Nitrosoma europaea*... more similar than *Amoeba* to mammalian Rh. Has a ‘stake’ extending into cytosol. (They do not pay much attn to it)

**RhCG**: Expressed in HEK293s

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

**PfARP: Plasmodium → Glyceral**

- Glyceral is water-like
- Rotamers can be unambiguously determined
- $9 \text{ H}_2\text{O} \text{ molec.}$
- $\text{H}_2\text{O}$ density clean in a chain
- Solute can be identified

A single file of $\text{H}_2\text{O}$ conducts $\text{H}^+$ very fast
Beitz et al. PNAS, 2004

An AQP that transport H2O and Glycerol both v. well.

*Malaria* One mutation: Glu -> Ser (Way 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 C details cost you control of presence.
Showing non-case KDs is their controls were shooting yourself in the foot.

[Q10:]
What was Venkman's Q_{10} for P? 4?
CK MCV: Is there a shift? 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 Δi'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. Vegital poles
Bhaw: Optical tweezers \(\rightarrow\) viscosity across the entire diameter.
Email: Rotamer search ... what is stable?
Lecture 1: Walter Boron - Gas Channels

- Solubility theory

\[ P_{oc} \cdot S_L / S_w \]

Note: Henry's law is true at steady-state

- Solubility-Diffusion theory

- Access-Solubility-Diffusion-Egress theory
Newtonian equations

Major limitation \rightarrow time scale \ (speed limit: 1 \text{fs})

Force field approximations

atomistic resolution

Implicit Ligand Sampling

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

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

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

Techniques:

pH gradients in the surface of lipid bilayer

\(t/2\) of CO₂ uptake \(\sim 12\) 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

\(P_{\text{HCO}_3^-}\) \(P_{\text{CO}_2}\) CA activity \(\{\) are the 3 main parameters

\(\downarrow\) red cell

\(\rightarrow\) fast phase where \(P_{\text{CO}_2}\) dominates

monitor pH to 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

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

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

Phase 2

volume fraction of RBC is very critical ($^1V \to 5$ time faster)

trick: use small $V$ to reduce the time resolution for mass spectrom
\( P_{\text{CO}_2} = 0.15 \text{ cm/sec} \quad \text{by RBC} \)

**Sensitivity**

- \( K_{eq} \) is important
- \( A_i \) is very critical parameter \( \Rightarrow \) \( A_i \) and pH need to be controlled
- PtFe is also important
- pH is not critical
- \( P_{H_2O} \)

**How about ULs?**

**Theoretical hydrodynamics**

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

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

\[ \uparrow \text{dextran} \Rightarrow \uparrow \delta \text{ for } \text{CO}_2 \]

Extrapolate to \( \nu = 0 \)

\[ P_{\text{m,CO}_2} = 0.16 \text{ cm/sec} \]

\[ \Rightarrow \delta^{\text{\small{app}}} = 0.5 \mu m \text{ in saline} \]

\[ \text{P}_{\text{CO}_2 \text{ in saline}} = 0.12 \text{ cm/sec} \]
\[ P_{CO_2} = 0.15 \text{ cm/sec} \rightarrow 50\% \text{ due to AQP1} \]

\[ 50\% \text{ due to Rh protein} \]

Endeward et al, 2008

2 channels

\[ P_{CO_2} \approx 100 \times P_{\text{HCO}_3^-} \]
Lecture 4: Endowment - Intrinsic CO₂ permeability of cell membrane

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

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

\[ \text{ contains \text{GATII}} \]
Afternoon Sessions

**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
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?
Hypothesis: AQP1 transports NO

Measurements: cultured cells & fluorescence

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

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

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

5. Purified AQP-1 increases NO transport

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

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

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

saturable component  
linear component

Handlogten et al., AJP Renal, 2004

Are Rh proteins present in cells with NH₃ transport?

RhAG in RBC....

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

\[ \text{when you sweat, NH}_3 \uparrow \]

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

Molecule increases Rhcg expression

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

What do structures tell us about gas channels? QED!

2 families of membrane proteins that can move gases:
gases are uncharged

- Rh Family
- AQP Family

Ammonia Transport: Amt/MEP/Rh Family in bacteria

Nitrogen Metabolism in bacteria

\[ \text{Dipole moment} \]

\[
\begin{align*}
\delta^- & \quad 2\delta^+ \quad \delta^- \\
O = C = O & \quad \delta^+ / \delta^+ \quad D = D
\end{align*}
\]
\[ \text{NH}_4^+ \leftrightarrow \text{NH}_3 + \text{H}^+ \]

\text{NH}_3 \text{ channel}

\text{AmtB Crystallography}

↑ \text{trimer}

\text{lyphosome}

\text{AmtB conducts NH}_3 \text{ but not H}_2\text{O}

\text{Wed 28th November : http://rmi.2012.org/ San Francisco}
Day 2

Xue Qin: AQP5

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

T41 in AQP5
L43

No significant change w/ L43 mutations.
Interesting changes for T41

Movement of ions in the central pore
In order to see what happens we need the crystal structure

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

AQP6 carries very little H2O or none
Do something to the CO2 permeability without affecting the H2O permeability

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

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

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

glycosylation
reaction that is covalent

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

AQPs has the biggest spike
DO doesn’t do anything...

expose to a solution to be oxidized
T41C is probably misfolded
1. Which other families of gas channels might be there?

So far we have looked at:

- CO₂
- NH₃: general medicine
- O₂: EPR, Optical / Hb; we want to measure fluxes of O₂ and
- NO: Hb; we want to do it faster
- CO: Hb

- CH₄: swamp bacteria
- H₂S: purple bacteria

- N₂: nitrogenase - Raman Spectroscopy (fast but not sensitive)
- Ethylene: plants

How do we measure N₂ fluxes?

13 N - NMR (not very sensitive, slow)

H₂S ⇌ H⁺ + HS⁻

- pH measurements

- Signaling gas
- Optical / Hb

2. What other families of gas channels might be there?
• AE1; GLUT1/4; AQP4, Rh, MCT-1
  • RBCs protein

• Endothelial cells in capillaries
• BBB, BRotina-B
• BTB, BOB
• Lungs: AQP5
• Striated Muscles... myoglobin
• Mitochondrion: \( \text{CO}_2 \) 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
CCE experiments (Jing Lu)

NBC 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 HgCl$_2$ rather than pCMBS. Worries about DIDS being non-specific. Dose-response for DIDS? Others.

**General Discussion**

I. **What gases should we be interested in?**
- CO$_2$
- O$_2$: Optical/Hb. Phorescence (if fast). EPR.
- NO: Optical/Hb. Electrodes
- CO: Optical/Hb.
- N$_2$: nitrogenase to turn it into NH$_3$? Raman Spectroscopy (fast but not very sensitive). Surface enhancement with gold particles? Agriculture. Microbiology. $^1$N-NMR (not very sensitive … talk to NMR guys)
- CH$_4$: Swamp bacteria
- Ethylene: signaling in plants
- H$_2$: 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/O$_2$: AE1, GLUT1/4, AQP1, Rh, MCT-1 (all $\geq$ 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. CO$_2$ is formed in the matrix. Perhaps O$_2$ as well? AQP8, AQP9 (MIM). AQP5. H$_2$O???
- 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 (pH$_i$, 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 aquaporines 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 aquaporins, 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