Notes on the Discussion following Xue Qin's talk:

Clustering of AQPs: 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
- NH_3: general medicine. Agriculture. Microbiology (Army: Infectious diseases).
- O_2: Optical/Hb. Phosphorescence (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. ^{13}N-NMR (not very sensitive … talk to NMR guys)
- H_2S: Purple bacteria. H_2S electrode. $H_2S \rightleftarrows H^+ + HS^-$ … could one use pH? Hibernation gas (LaManna). Signaling gas. Optical/Hb (650-ish nm).
- 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 ≥ 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_2O???
- 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 CO_2 retention, reduced NO flux (less exercise-induced vasodilation)? Treadmill. RhAG-null. If we ever find the O_2 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 O_2 permeability? … but downside is susceptibility.
- Cerebral edema. Stroke, TBI, AMS … Aeromics has a drug that blocks the aquapores 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 O_2 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 O_2 requirement, would be most likely to have gas channels. Optimizes human performance.
- Exclude gas:
- Transport gas directionally.
- Wound healing, bone-fracture healing.
- COPD: CO_2 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): ↓ N_2 permeability on the way down (would also solve N_2 narcosis) … increase it on the way up.
- O_2 toxicity:
- CO_2 narcosis:
- N_2 narcosis:
- Submarine escape … DCI.
- Acute mountain sickness … hypoxia
- Increase O_2 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 aquapores, 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 CO_2, O_2, 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/: NH_3 via Rh and AQPs. 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: N_2 fixation (must be done in the absence of O_2)

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
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