

STRUCTURAL BIOLOGY

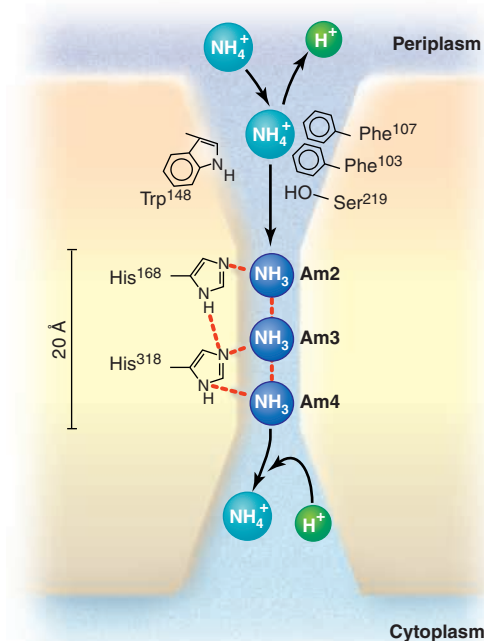
The Atomic Architecture of a Gas Channel

Mark A. Knepper and Peter Agre

“Form ever follows function.” Penned in 1896 by the renowned architect Louis Henri Sullivan in reference to the first tall office buildings, this sentence also applies to the structure of cell membrane proteins. Although high-resolution structures of protein channels that allow passage of ions, uncharged solutes, and even water have been solved, the precise mechanisms by which gases cross biological membranes have remained enigmatic. On page 1587 of this issue, Khademi *et al.* (1) provide a quantum leap forward in our understanding of gas transport. They resolve the crystallographic structure of a bacterial ammonia transport channel, AmtB, to 1.35 Å—an unprecedented resolution for an integral membrane protein.

Ammonia (NH_3) is a gas, but when dissolved in water it exists predominantly as the ammonium ion (NH_4^+) with a pK_a of about 9 under physiological conditions. For a bacterium, NH_3 is an important nutrient that must be taken up from the surroundings to provide a source of nitrogen for amino acid synthesis. AmtB is a transport protein present in the bacterial inner membrane between the cytoplasmic and periplasmic spaces that facilitates NH_3 uptake (see the figure). Interestingly, AmtB proteins are genetically related to the structural components of the Rh blood group antigens of mammalian red blood cells. The Rh-related proteins are a family of membrane proteins reported to facilitate the transport of ammonia (2) and carbon dioxide across eukaryotic cell membranes (3). Human Rh-related proteins are thought to be important in critical physiological processes and, when defective, may result in impairment of systemic pH regulation or central nervous system dysfunction due to ammonium toxicity. The structure of Rh antigens has long been pondered. Now, the trimeric structure of AmtB revealed by Khademi and colleagues suggests a simple

M. A. Knepper is in the Laboratory of Kidney and Electrolyte Metabolism, National Institutes of Health, Bethesda, MD 20892, USA. E-mail: kneperm@nhlbi.nih.gov P. Agre is in the Departments of Biological Chemistry and Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA. E-mail: pagre@jhmi.edu



The AmtB ammonia channel of *E. coli*. Resolution of the structure of the bacterial integral protein AmtB reveals a wider vestibule at the top and bottom of the channel. The amino acid residues that line the pore of the outer vestibule—Trp¹⁴⁸, Phe¹⁰⁷, Phe¹⁰³, and Ser²¹⁹—stabilize NH_4^+ (Am1). Midway through the membrane, the channel narrows over a 20 Å span. Here, two pore-lining residues, His¹⁶⁸ and His³¹⁸, stabilize three NH_3 molecules (Am2, Am3, and Am4) through hydrogen bonding (red dashed lines). The molecules return to equilibrium as NH_4^+ in the inner vestibule.

explanation for how the three Rh polypeptides of red blood cells—RhAG, RhD, and RhCE—form the Rh antigen complex in the erythrocyte plasma membrane (4). In addition, the Khademi *et al.* study reveals a mechanism of ammonia permeation in bacteria that is likely to be similar in eukaryotic cells.

Databases of solved protein structures are burgeoning with structural maps of both intracellular and extracellular proteins. However, structures of integral proteins with their many membrane-spanning loops are just now beginning to emerge. A common strategy, and one adopted by Khademi *et al.*, is to express paralogs

genes from multiple bacterial species, prepare three-dimensional crystals of the proteins they encode, and select the crystal producing the highest resolution x-ray diffraction pattern for analysis. The structures of a few eukaryotic integral proteins have been determined by cryo-electron microscopy of membrane crystals or by molecular modeling using coordinates determined from x-ray analysis of prokaryotic paralogs. Khademi *et al.*'s success with AmtB, an integral membrane protein from *Escherichia coli* with 11 membrane-spanning α helices, foreshadows continued progress with other integral membrane proteins whose structures have been elusive.

Elements of the AmtB structure reveal how this protein channel transports ammonia (see the figure). AmtB has the same structure when crystallized in both the absence and presence of ammonia, leading the authors to conclude that it is a channel rather than a transporter that would be expected to have flexible elements involved in translocation of the substrate. At the two ends of the pore, broader vestibules contain NH_3 in equilibrium with NH_4^+ . AmtB has at its center a narrow hydrophobic pore element about 20 Å in length, which allows the passage of NH_3 but not the monovalent ion NH_4^+ . This distinction is important because the structure must prevent ions such as K^+ from crossing the inner membrane. Thus, AmtB is an NH_3 channel that does not mediate the net transfer of protons and does not directly alter the membrane potential. Although these conclusions appear contrary to those of prior studies in which biophysical techniques indicated that ammonia translocation is affected by pH and voltage gradients (5), Khademi and colleagues argue persuasively that their model (see the figure) is compatible with most of the biophysical data reported so far.

Simple membrane bilayers have moderate intrinsic NH_3 permeability (6), so the necessity of ammonia channels could be questioned. Ammonia channels, however, may serve to accelerate ammonia transport at sites where the diffusion of NH_3 through the lipid bilayer is too slow for physiological needs, or may provide a molecular target for regulating the passage of NH_3 . Both functions may be important in the mammalian kidney collecting duct where two