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Structural insights into the transport of small molecules across membranes

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While hydrophobic small molecules often can freely permeate a lipid bilayer, ions and other polar molecules cannot and require transporters to mediate their transport. Recently, a number of important structures have been reported which have advanced our understanding of how membrane protein transporters function to transport small molecules. Structures of TbpA/B and HmuUV provided new insight into iron uptake by pathogenic bacteria while the structures of NarK, ASBT, and *Vc*INDY revealed molecular details about the transport of nitrate, bile acids and dicarboxylates, respectively. The structure of the folate ECF transporter indicated that the S component likely undergoes a large conformational shift to mediate folate transport, while the cellulose synthase/transporter contains an elongated translocation pore for passage through the inner membrane.

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Small molecule transport systems

The transport of small molecules across membranes is essential for the import of nutrients and other energy sources into the cell and for the export of waste and other potentially harmful byproducts out of the cell [1••,2,3]. While hydrophobic molecules are permeable to membranes, ions and other small polar molecules require transport via specialized membrane transport proteins. The two major classes of membrane transport proteins are carrier proteins (transporters) and channel proteins [4] (Figure 1a). With our focus here on transporters, we will briefly highlight some recent structural biology reports that have substantially contributed to our understanding of the various mechanisms that mediate the transport of small molecules across membranes. We also discuss the structure of the cellulose synthase/transporter since its substrates can vary significantly in size. The studies discussed here represent only a fraction of the exciting structures that have been reported recently, all of which collectively advance our overall understanding of these small molecule transport systems.

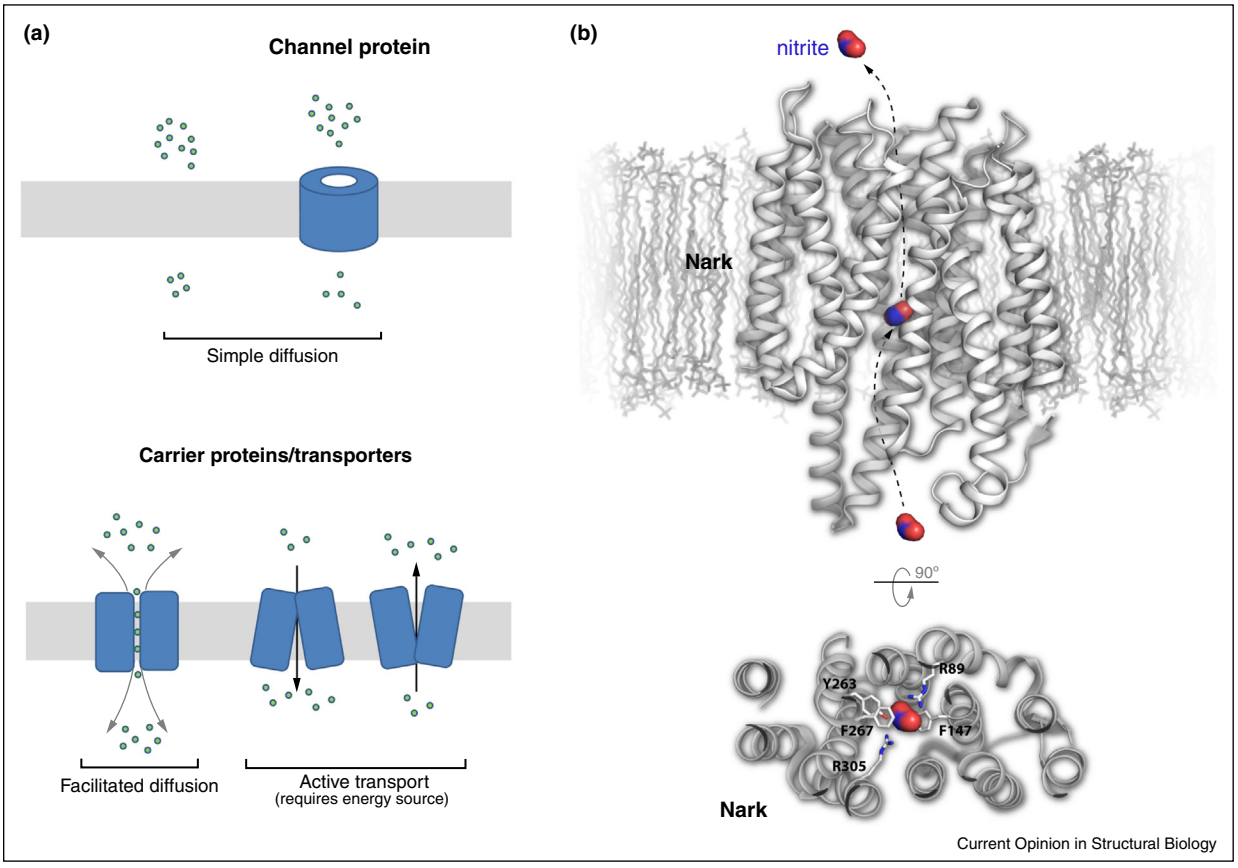
Nitrate/nitrite transport by NarK in *E. coli*

Nitrate (NO_3^-) is a key source of mineral nitrogen uptake for bacteria. However, nitrogen metabolism leads to an abundance of cellular nitrite (NO_2^-), which can eventually lead to the accumulation of nitric oxide, a cytotoxic free radial that can lead to DNA damage and degradation of iron-sulfur centers [5,6]. The exact mechanism for how bacteria manage nitrate import versus nitrite export has remained elusive until recently with the report of the crystal structure of the nitrate/nitrite transporter NarK from *E. coli* [7]s. NarK belongs to the major facilitator superfamily (MFS) of secondary transporters, containing a conserved twelve transmembrane helices structure consisting of two structurally conserved N- and C-terminal subdomains of six transmembrane helices each related by a pseudo twofold symmetry. The NarK structure was solved in apo form and in complex with sodium nitrite, with both structures found in the inward-facing conformation. The putative substrate transport pathway is formed along the interface between the two subdomains (Figure 1b). On the basis of the electrostatics of the substrate translocation pathway and the lack of classical proton transporting residues (Glu, Asp, and/or His), it was concluded that NarK is in fact a nitrate/nitrite exchanger which relies on a cyclic rocker switch mechanism, allowing nitrate binding (and nitrite release) in the outward facing conformation and nitrate release (and nitrite binding) in the inward facing conformation [7].

Iron transport in *Neisseria meningitidis*

Iron is essential for survival and therefore pathogenic bacteria have developed complex machineries to scavenge iron from the host environment or even steal iron directly from host proteins [8]. For example, *Neisseria meningitidis* has specialized surface receptors that have evolved to acquire iron from specific host sources, such as heme, lactoferrin, and transferrin (Tf) [9–12]. Recently, the crystal structures of the Neisserial Tf-binding proteins A (TbpA, a TonB-dependent transporter) and B (TbpB, a lipoprotein co-receptor) were each reported in complex with human Tf, providing molecular insight into how *Neisseria* are able to steal iron from Tf and import it across the outer membrane for survival and virulence [10,11]. TbpA, which binds both apo and diferric Tf, consists of a large N-terminal plug domain tucked inside a

Figure 1



Small molecule transport systems and the structure of NarK. **(a)** The two major classes of membrane transport proteins are carrier proteins (transporters) and channel proteins. The focus of this review is on the protein transporters which require energy for function. **(b)** The structure of NarK, a nitrate/nitrite exchanger, is shown in gray within a membrane bilayer (*top*). A cutaway view from the periplasmic side is also shown (*bottom*) illustrating the interactions of NarK residues with the bound nitrite molecule, which is shown in red/blue surface. The proposed exchange pathway is indicated by dashed arrows (nitrate import is in the opposite direction as shown for nitrite export).

22-stranded β-barrel transmembrane domain, while TbpB, which only bind to diferric Tf, is anchored to the surface of the cell via an N-terminal lipid anchor and consists of two structurally similar domains, each containing an eight stranded β-barrel with an adjacent four stranded β-rich handle domain. Tf binding to TbpA and TbpB was mediated exclusively along the C-lobe of Tf at non-overlapping binding sites (Figure 2a). Identification of a conserved lysine residue found on the helix finger in loop 3 of TbpA provided a clue suggesting that TbpA itself may be hijacking the pH sensor of Tf, which is typically utilized to mediate iron release at low pH within the human host, to catalytically trigger iron release for import through the barrel domain of TbpA.

The structure of HmuUV, a heme transporter from *Y. pestis*

As discussed earlier, iron is essential for survival and therefore pathogenic bacteria have evolved systems to acquire iron from the host environment. Another such iron source that is targeted is heme, which is the most abundant source of iron in the human body [13••]. While numerous studies have described how heme may be captured and imported across the outer membrane by TonB-dependent transporters [14], less is known about how heme makes its way across the inner membrane into the cytoplasm. Recently, the crystal structure of the heme transporter HmuUV from *Yersinia pestis* was reported and found to belong to the type II ABC transporters/importers, consisting of two copies of each component HmuU (transmembrane domain) and HmuV (nucleotide-binding domain) ($HmuU_2V_2$) and related by a twofold symmetry axis [15] (Figure 2b). Each HmuU subunit contained 10 transmembrane helices with HmuV interacting non-covalently via a conserved cytoplasmic coupling helix. Unlike type I ABC transporters, an outward facing conformation was observed in the absence of nucleotide [16,17]. A cavity, which is open to the periplasm but closed to the cytoplasm, was found at the interface between the two HmuU subunits, revealing the site where HmuT, the