bonds. Correct disulfide bonds can be formed in a large fragment (residues 59 to 105) of hen lysozyme (129 residues). $Trp^{62}$ is essential for the specific formation of correct S–S bonds in this fragment (*13*), suggesting that $Trp^{62}$ exerts its effect through the network of hydrophobic clusters and that this network thus has a beneficial effect on the folding in vivo, despite the fact that $Trp^{62}$ slows the lysozyme refolding reaction with preformed S–S bonds.

Could a network of hydrophobic clusters also explain the long-range order observed recently in urea-denatured staphylococcal nuclease (*14*)? It has been suggested (*15*) that steric clashes between side chains and backbone lead to the long-range structure. This model was used initially (*16*) to propose that folding begins locally at sites throughout an unfolded protein. The jury is still out on which model is correct. But the observations reported by Klein-Seetharaman *et al.* (*8*) show that accepted concepts about denatured proteins must be reexamined critically. More surprises are likely to emerge.


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PERSPECTIVES: STRUCTURE

# Close Before Opening

**Kathleen Postle**

Wherever biological membranes are found, they are accompanied by proteins that direct the trafficking of ions, nutrients, and waste products across these cellular barriers. Some proteins form simple channels or pores that allow the concentration of substrates to be equalized across the membrane without the expenditure of energy. Other proteins are active transporters that use energy to pump substrates across membranes against a concentration gradient. Interesting variations on these simple themes have arisen as our knowledge has increased. Some channels can be gated (opened or closed) by, for example, voltage changes or binding of cyclic nucleotides. One of the most complex active transporters is now described on page 1715 of this issue by Ferguson *et al.* (*1*). They report the crystal structure of FecA, a protein found in the outer membrane of the Gram-negative bacterium *Escherichia coli*. FecA, which transports ferric iron from the environment, belongs to a class of active transporters that are gated by the inner membrane protein TonB. The crystal structure of FecA yields an unexpected finding that justifies solving crystal structures for multiple members of a protein family.

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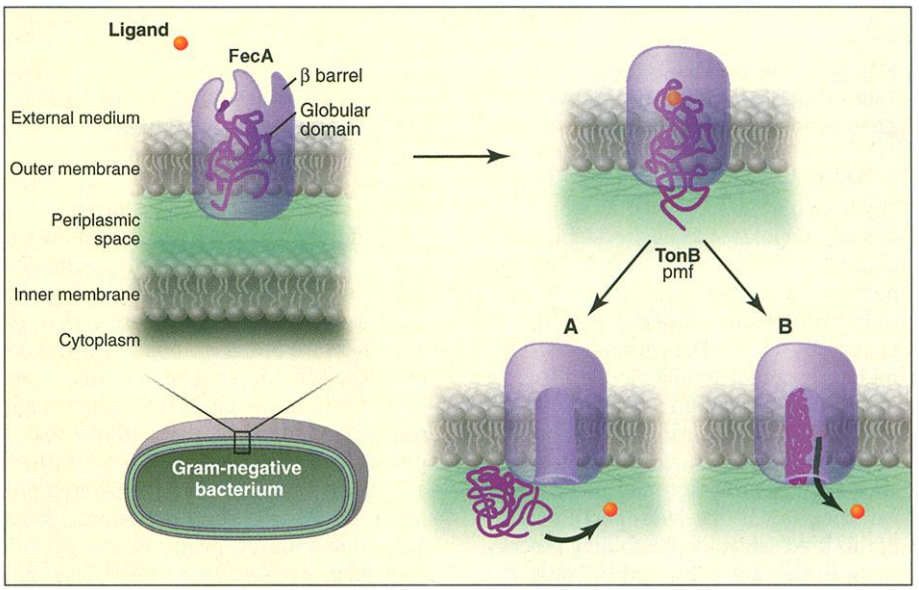
Most nutrients required by Gram-negative bacteria diffuse through outer membrane channel proteins (called porins) into the aqueous periplasmic space between the outer and inner bacterial membranes. They are then actively transported across the inner membrane into the bacterial cytoplasm (see the figure). Active transport is driven by either an ion electrochemical potential across the inner membrane or by hydrolysis of adenosine triphosphate (ATP). The acquisition of iron, an essential nutrient, presents bacteria with unique difficulties. Indeed, in vertebrate hosts, free iron levels are deliberately kept low to discourage survival of bacterial pathogens. In aerobic nonhost environments, iron is found as the virtually insoluble oxyhydroxide. Thus, being at the base of the food chain, bacteria must resort to extreme measures to obtain iron. They acquire iron by synthesizing and secreting chelator molecules called siderophores, which have an extremely high affinity for ferric iron. Iron-siderophore complexes are then bound by specific active transporter proteins in the outer membrane of Gram-negative bacteria with very high affinity (in the nanomolar range). These active iron transporters—including FhuA, FepA, and FecA and the vitamin $B_{12}$ transporter BtuB—are closely related. They




**Two gates for a TonB-gated transporter. (Left)** The TonB-gated transporter FecA (lilac) of Gram-negative bacteria, when not bound to ligand (orange spot), is in a conformation that is ready to bind to ligand. **(Right)** Binding of ligand to FecA causes the newly discovered external gate to close (*1*). Meanwhile, large conformational changes (which are likely to be signals for TonB interaction) take place at the periplasmic face of the internal globular domain (TonB-responsive gate: purple) (*3, 4, 6–9*). Activated TonB opens this gate, allowing release of ligand into the periplasmic space. This can happen in two ways, both of which require TonB and the proton-motive force (pmf) of the inner membrane (*2, 10–13*). **(A)** The globular domain leaves the β barrel completely, perhaps through denaturation of its overall structure (*14*). **(B)** The globular domain remains inside the β barrel and becomes rearranged, thus providing a passage through which ligand can exit (*14, 15*).

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all require TonB to transport their ligands across the outer membrane, although active transport has not been demonstrated in all cases. The best example of active transport is the BtuB transporter, which can establish a vitamin $B_{12}$ concentration in the periplasmic space that is 1000 times that in the external milieu (*2*). From the periplasmic space, the substrates follow the usual path of ATP-dependent transport across the inner membrane.

By itself, the active transport of a nutrient across the outer membrane seems unremarkable, except that the outer membrane has no direct access to energy-producing pathways. Instead, this active transport step depends on activation of TonB by the proton electrochemical potential of the inner membrane. Activated TonB can then bind to the outer membrane iron transporters and transduce energy to them. In the absence of TonB or the proton gradient, ligands still bind with high affinity to their transporters but are unable to cross the outer membrane. These TonB-dependent outer membrane transporters were erroneously classed as ligand-gated porins before the crystal structure of FhuA (*3*) revealed that ligand binding did not open a pore within the transporter.

Like every protein in the outer membrane, the active transporters FhuA, FepA (*4*), and FecA (*1*) have a β-barrel skeleton; however, that structure merely reflects the requirement for their localization in the outer membrane. Outer membrane proteins cannot contain the 20–amino acid stretches of hydrophobic residues characteristic of inner membrane proteins because these sequences would prevent newly synthesized outer membrane proteins from reaching their destination. Outer membrane proteins that allow passive transport of ligands are all pores of some sort. In contrast FepA, FhuA, and now FecA have an internal globular domain (the gate) that completely occludes the β barrel. The crystal structures for both FhuA and FecA (bound and unbound to ligand) have been solved. In both cases, binding of ligand to the external face of the transporter causes a large conformational shift in the region of the protein at the periplasmic face. Intriguingly, this conformational change does not alter the location of the internal globular domain or create a passageway through which ligand can exit. Clearly, we have been confused—these outer membrane transporters do not form pores and are not gated open by their ligands, which suggests that henceforth they should be called TonB-gated transporters to reflect the way they work.

In the new study, Ferguson *et al.* (*1*) reveal that FecA is even more dynamic than other TonB-gated transporters. It has a second gate, primarily composed of external loops 7 and 8 of the β barrel. The ligand, in this case ferric citrate, binds to transporter and closes the newly discovered external gate behind it, preventing access to the external milieu. It will be interesting to learn whether this is a general feature of all TonB-gated transporters. Ferguson *et al.* propose a four-step model for iron transport by FecA: (i) The iron-siderophore complex is adsorbed by low-affinity sites on external loops of the β barrel of FecA, (ii) the complex is transferred to high-affinity sites within the external loops of FecA's internal globular domain, (iii) the external loops reposition themselves to close the external pocket of the β barrel, and (iv) a TonB-dependent conformational change in the globular domain opens the gate to allow release and transport of the ligand into the periplasmic space (see the figure).

Like any important scientific advance, this one raises interesting questions. Does the newly discovered external gate stay closed once ligand has been bound, or does it "flutter" between open and closed positions? The on-off binding rates for iron-siderophore complexes have not been measured and could be very rapid. Work on vitamin $B_{12}$ transport has shown that once in the periplasmic space, vitamin $B_{12}$ can return to the external medium (*2*). Is this reverse transport mediated by the TonB-gated transporter BtuB? Bacterial toxins (colicins) that attack *E. coli* and *E. coli*–specific bacteriophages both gain access to their bacterial hosts through TonB-gated transporters. But how? While it does not answer that question, the FecA crystal structure does explain how iron-siderophore complexes decrease bacterial susceptibility to colicins (*5*). Presumably, when the external gate closes behind the iron-siderophore complex, this completely sequesters the colicin binding site. The colicins are enormous (~60 kD) relative to iron-siderophore complexes (~1 kD), and bacteriophages are even larger. Is closing of the transporter loops a requirement for iron-siderophore complex and vitamin $B_{12}$ transport? The size of colicins and bacteriophage ϕ80 would almost certainly preclude closing of the external gate.

The crystal structure of FecA, the third member of the TonB-gated transporter family, has provided us with a new way of thinking about ligand transport. It also confirms the structural aspects of iron transport revealed by the structure of other bacterial iron transporters. Thus, crystal structures of homologous proteins continue to reveal insights that could not have been predicted from the amino acid sequence.

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PERSPECTIVES: LASER CHEMISTRY AND PHYSICS

# The Next Frontier

**Kaoru Yamanouchi**

How do atoms and molecules interact with light? One might think that sophisticated modern laser spectroscopy has already answered this fundamental question and that nothing remains but to apply our wealth of knowledge. However, the development of ultrashort, superintense pulsed lasers has led to the realization that much remains to be learned about light-matter interactions.

It is well known that ultrashort pulsed lasers allow probing of molecular processes in real time on the femtosecond time scale. The latest advances originate not, however, from the ultrashort temporal width of the laser light but from its extremely high intensity. The advent of chirped pulse amplification (CPA) has greatly increased the output energy of ultrashort pulsed lasers (*1–3*). Even in university laboratories, laser light fields as high as $10^{15}$ W/cm$^2$ (1 PW/cm$^2$) can now be generated routinely with a table-top, high-power CPA laser system. This intensity is comparable in magnitude to the Coulomb field generated by an atomic nucleus. In large-scale facilities such as JAERI in Japan and LOA in France, a laser field intensity of $10^{20}$ W/cm$^2$ (100 EW/cm$^2$) could be achieved.

At intensities well below ~$10^{12}$ W/cm$^2$ (the perturbative regime), atoms and molecules absorb one or multiple photons through a weak interaction with the light

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