



Threading proteins through a nanopore needle

Electrostatic traps control single-molecule interactions in a model pore system.

Sometimes the most basic questions in biology are the hardest to answer. For example, it has long been known that proteins translocate into and out of cells through protein pores against prevailing gradients and without using biological energy in the form of adenosine triphosphate (ATP). But how? Using a model bacterial pore system, Liviu Movileanu and co-workers at Syracuse University and Northwestern University have taken an important step toward finding the answer (*J. Am. Chem. Soc.* **2008**, *130*, 4081–4088).

“This is going to be an influential paper,” says Sergey Bezrukov of the National Institutes of Health. “It is a clear demonstration of the main physical interactions involved in protein translocation, and clues for understanding the mechanisms of this process have been very scarce.”

Protein translocation is important and ubiquitous in nature, Movileanu points out, “but it is based on very complex, unstable systems, so most biophysicists have stayed away from it.” To study the process, which he compares to “pulling spaghetti through the eye of a needle,” Movileanu and co-workers turned to α -hemolysin (α HL), which has become highly valued in nanopore studies because it is exceptionally robust under a wide range of harsh experimental conditions and manipulations. α HL is a β -barrel pore, and its seven subunits assemble into a tube-shaped protein complex that spans lipid bilayer membranes.

In the case of β -barrel pores such as α HL, “the protein is usually folded closed, and there’s an entropic price for it to open,” Movileanu explains. “So, the question has to be: how is it possible for a folded protein to go through a narrow pore like this in the absence of any ATP-dependent cellular factor?” Mov-

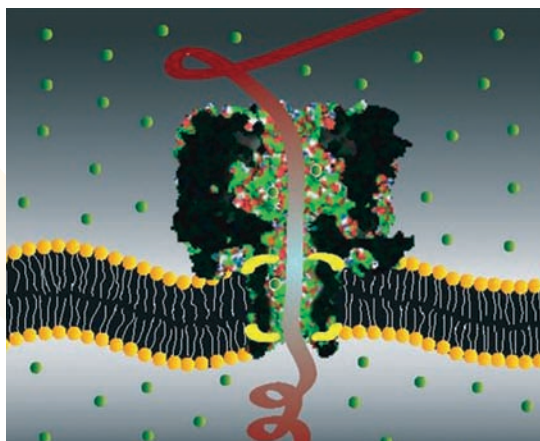


Illustration showing the α HL β -barrel pore system, with translocating pb_2 -Ba protein (red). Electrostatic traps are highlighted in yellow at the entrance and exit of the transmembrane domain.

ileanu and his team suspected that electrostatic interactions between a positively charged leading sequence on the target protein and negative charges within the pore lumen itself must play a critical role.

The researchers first engineered α HL to contain aspartate-residue electrostatic “traps” at the entrance of the pore’s transmembrane domain, at its exit, or both. They then used high-resolution, single-channel electrical recording to monitor transpore movement of a target protein engineered from the N-terminal region of precytochrome b_2 (pb_2) fused with the N-terminus of the small ribonuclease barnase (Ba). As expected, the positively charged pb_2 presequence was drawn into the α HL lumen through electrostatic attraction to the negatively charged electrostatic traps. Movileanu and colleagues found that they could tune the interaction by altering the ionic strength of the aqueous phase, the length of the pb_2 leading sequence, and the position of electrostatic traps.

“The results demonstrate [that] transport of peptides can be controlled by decorating a membrane channel’s interior with a particular pattern of

charged residues,” notes Aleksei Aksimentiev of the University of Illinois Urbana–Champaign. This suggests that cells may use related mechanisms to direct the movement of proteins around cell compartments. The research also shows that multiple electrostatic traps can work together in a synergistic manner. “They act cooperatively on the polypeptide chain,” Movileanu says. “Each one does some work, but together the effect increases dramatically.” The physics turns out to be quite simple, he says, “but the biology is much more complex. We can’t understand complex biological systems if we don’t understand their physics.”

That understanding could soon lead to practical payoffs. For example, pore modifications have the potential to speed development of single-molecule, high-throughput proteomics analysis and pharmaceutical screening. Insights gained about basic biology might also help guide disease detection and treatment. “There are many diseases of protein unfolding and translocation,” Movileanu says. “They involve many different systems, but they all start from the simple process of how proteins translocate through a hole.”

“The mechanisms of protein translocation are also crucially important in the toxic action of many pathogens,” Bezrukov points out. The anthrax bacterium, for example, causes illness by forming transmembrane pores in target cells, followed by translocation of lethal proteins. “If we don’t understand how that happens in nature,” asks Movileanu, “how can we hope to treat it?” The answers, he says, will come from the high-resolution analytical techniques made possible by the interactions of single polypeptide molecules and individual nanopores. ▀

—Thomas Hayden