Published in final edited form as:
J Phys Chem Lett. ; 3(8): 1063–1071. doi:10.1021/jz201654k.

Autotransporters: The Cellular Environment Reshapes a Folding Mechanism to Promote Protein Transport

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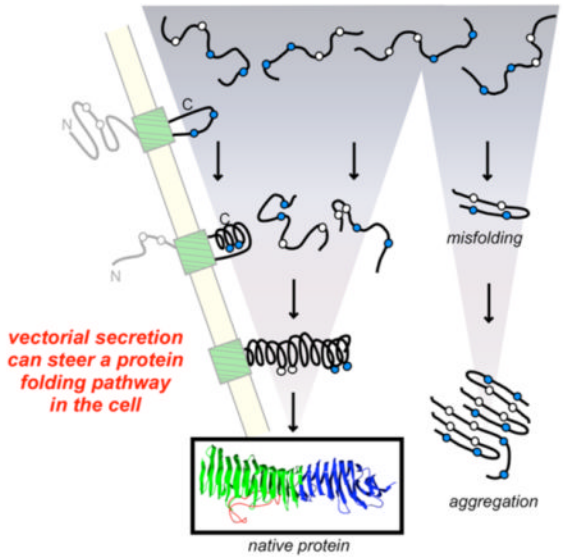
Abstract

We know very little about how the cellular environment affects protein folding mechanisms. Here, we focus on one unique aspect of that environment that is difficult to recapitulate in the test tube: the effect of a folding vector. When protein folding is initiated at one end of the polypeptide chain, folding starts from a much smaller ensemble of conformations than during refolding of a full-length polypeptide chain. But to what extent can vectorial folding affect protein folding kinetics and the conformations of folding intermediates? We focus on recent studies of autotransporter proteins, the largest class of virulence proteins from pathogenic Gram-negative bacteria. Autotransporter proteins are secreted across the bacterial inner membrane from N→C-terminus, which, like refolding *in vitro*, retards folding. But in contrast, upon C→N-terminal secretion across the outer membrane autotransporter folding proceeds orders of magnitude faster. The potential impact of vectorial folding on the folding mechanisms of other proteins is also discussed.



Keywords

kinetic mechanisms; vectorial; secretion; membrane; translation; β-helix; Gram-negative

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In 1963, Christian Anfinsen postulated that all of the information required to specify the native three dimensional, functional structure of a protein is provided by its amino acid sequence.[1, 2] Since then, tremendous efforts have been made to understand the mechanisms by which proteins fold to their native conformations.[3–7] How, for example, does a polypeptide chain "find" its native structure among all other possible conformations of the chain, on a time scale fast enough to be compatible with other cellular processes?[8] Similar to Anfinsen's classic experiment, most of these protein folding studies are based on ensemble measurements of a dilute solution of a purified protein. The model protein is first unfolded, usually by incubating it in a high concentration of a chemical denaturant such as urea or guanidine hydrochloride, which disrupts the non-covalent interactions that stabilize the folded structure.[9, 10] To initiate refolding, the unfolded protein is diluted to reduce the concentration of denaturant. To minimize aggregation, protein concentration is kept low (nM - μM range), and the reaction is performed at low temperatures (4°C). Refolding can be monitored by observing changes in protein-specific signals detected by optical or NMR spectroscopy, mass spectrometry, or enzyme activity assays.

Studying the refolding of a purified protein in a test tube enables one to control many experimental parameters, including protein concentration, buffer composition, pH, temperature and salt concentrations. And because the protein of interest is the only biomolecule present in the test tube, *in vitro* experiments facilitate quantitative descriptions of folding processes, free from complicating signals from the cellular milieu. However, in order to understand how proteins fold in their natural context, we must understand to what extent, if any, the complex cellular environment affects the folding process.

The cellular environment for protein folding

When compared to classic *in vitro* refolding conditions, protein folding in the cell has several significant differences. The optimal growth temperature for many cells (30–37°C) is significantly higher than the temperatures used for *in vitro* experiments. The cellular environment consists of a highly concentrated (>400 mg/mL) and complex mixture of proteins, sugars, and lipids,[11] and these large biomolecules introduce excluded volume effects[12, 13] that are challenging to recapitulate in test tube experiments. The crowded intracellular environment also facilitates intermolecular interactions between partner and/or regulatory proteins or other biomolecules. Of course, some aspects of these interactions can be investigated in the test tube by adding components to the refolding reaction. For example, *in vivo* a subset of newly synthesized proteins interact with molecular chaperone proteins, which can suppress off-pathway interactions that lead to aggregation and/or directly catalyze the formation of folded structure.[14–16] Great insights into the mechanistic details of chaperone action have been gained from *in vitro* experiments using purified proteins refolded in the presence or absence of a particular molecular chaperone and/or its co-chaperone partners.[17–22] However, many *in vivo* folding mechanisms rely on temporally and spatially regulated interactions with other biomolecules, including the insertion of transmembrane proteins into a lipid bilayer, the assembly of ribosomal proteins and ribosomal RNA to form the two subunits of the ribosome, and the incorporation of cofactors such as heme, NAD^+ or metal ions during protein folding. Such regulation typically cannot be captured during *in vitro* refolding reactions.

Another aspect of protein folding in the cell that is challenging to recapitulate *in vitro* is the effect of a folding vector, where one end of the polypeptide chain can start to fold before the other end can participate in the folding mechanism (Fig. 1). Vectorial folding occurs when a protein folds co-translationally, during N-to-C-terminal synthesis of the polypeptide chain by the ribosome.[23–26] Due to the technical complications of studying co-translational protein folding, this process was initially approximated simply by making C-terminal truncations of

a purified protein.[27–29] However, many of these purified C-terminal truncations were highly aggregation-prone, suggesting the attachment of the chain to the ribosome, and/or its vectorial appearance, affect inter-molecular interactions between partially-synthesized polypeptide chains. More recently, advancements in our ability to control the mechanism of translation,[25, 30] and detect the conformations of new proteins while they are being translated by the ribosome[24, 31–34] have revealed significant differences between co-translational folding and *in vitro* refolding mechanisms. Indeed, during translation by the ribosome the nascent polypeptide populates folding intermediates that are more likely to fold successfully to the native structure rather than misfold and aggregate (Fig. 1).[24, 26, 35] Moreover, several of the proteins that have been observed to follow altered co-translational folding pathways are kinetically trapped,[24, 26] meaning that once they fold to their native state they are unlikely to unfold and refold over the lifetime of the cell.[36] Co-translational folding has been the subject of several excellent recent reviews.[37–39]

Vectorial folding also occurs during the secretion (transport) of an unfolded protein across a lipid membrane. Much effort has been devoted to understanding the initiation of protein folding after transport from the cytoplasm to a subcellular organelle, including folding after passage across the membrane of the endoplasmatic reticulum;[40] and after import into mitochondria.[41, 42] Moreover, a variation of vectorial folding is vectorial *un*folding, for example prior to protein import into mitochondria,[43] or in preparation for degradation by the proteasome.[44]

Here, we focus on a related process: the vectorial folding of the largest class of secreted virulence proteins from Gram-negative bacteria. Vectorial folding not only changes the folding mechanism of these proteins, but influences the efficiency with which they are secreted across the outer membrane, defining a novel class of ATP- and proton gradient-independent molecular transporters.

Introduction to autotransporter (AT) secretion and folding

Gram-negative bacterial infections can lead to a number of potentially deadly human diseases, including bacterial meningitis, dysentery, whooping cough, peptic ulcers, pneumonia, peritonitis, and cholera.[46] For virulence, these bacteria must secrete proteins across both the inner and outer membranes of the bacteria to the outside surface of the cell. The most common secretion mechanism for virulence proteins in Gram-negative bacteria is the autotransporter (AT) system. Each monomeric AT protein is synthesized as a tripartite precursor in the bacterial cytoplasm with an N-terminal signal sequence, central passenger, and a ~300 aa C-terminal translocator domain. ATs are transported through the inner membrane (IM) co-translationally via the Sec protein complex, which uses the free energy released upon ATP hydrolysis to drive transport (Fig. 2). After signal sequence cleavage, the C-terminal translocator domain inserts into the outer membrane (OM) in a β-barrel structure, and the passenger crosses the OM (Fig. 2). After OM secretion, the passenger is typically cleaved from its translocator domain in an intramolecular cleavage reaction within the β-barrel.[47, 48] Some AT passengers remain non-covalently attached to the outside of the cell (i.e., pertactin from *B. pertussis*, Fig. 3C), while others are released from the cell surface (i.e., Pet from *E. coli* 042,[49] Fig. 3A).

AT passengers are large (>500 aa on average; Fig. 3D), and hence differ from “typical” models for protein folding. Crystal structures of two well studied AT passengers, pertactin from *B. pertussis*[52] and EspP, a homolog of Pet from *E. coli* 042[53] are shown in Fig. 3. AT passenger vary greatly in function, sequence, and length, yet the functional domain or loop for all AT crystal structures determined to date is found within non β-helical structure at the N-terminal half of the passenger. In pertactin the functional portion corresponds to a long

integrin binding loop; in Pet it resides within an N-terminal protease domain (Fig. 3). Surprisingly, despite the diversity of structures and functions of these functional regions, almost all AT passengers include right-handed parallel β-helix structure (Fig. 3).[51, 54] The prevalence of the β-helix motif in almost all AT passenger led us to hypothesize that the β-helix and its folding properties might play a more general role in AT biogenesis, such as successful secretion across the OM.

Currently, the precise mechanism for OM secretion of AT passenger remains unclear, despite its apparent simplicity. Does the passenger cross the OM in a folded conformation, or must it remain unfolded? A few studies have suggested that passenger folding within the periplasm is compatible with OM secretion.[55–57] More recently, however, crystal structures of AT C-terminal translocator domains[48, 58–60] have shown that the diameter of the central hole within this domain is too narrow (1–2 nm) to permit transport of a folded passenger. What drives transport of the passenger across the OM? In this environment, neither ATP nor a proton gradient is available as an external energy source to drive secretion.[61] Here, we summarize recent results demonstrating that an AT passenger folds vectorially from C- to N-terminus as it is secreted across the OM,[62] and that the anisotropic distribution of stability within passenger domains positively contributes to efficient secretion (Fig. 2).[63] We propose that this unusual distribution of folding free energy ($\Delta G_{folding}$) provides a driving force for efficient secretion, either alone or in concert with other physiochemical differences between the periplasm and extracellular space, such as macromolecular crowding.[64]

Pertactin folding occurs extracellularly, after OM secretion

In the cell, where does AT passenger folding occur? The AT precursor is secreted across the inner membrane co-translationally, but must also cross the periplasmic space and the OM in order to execute its function as a secreted virulence protein (Fig. 2B). Does the passenger fold into its native conformation only on the outside surface of the cell, or within the periplasm?

To address the mechanism by which the AT passenger is transported across the OM, we engineered pertactin mutants bearing pairs of cysteine residues along the passenger. In Gram-negative bacteria, disulfide bond formation occurs primarily in the periplasm, catalyzed by the Dsb family of proteins.[65] We and others have shown that introducing a pair of widely spaced Cys residues into an AT passenger produces a disulfide bonded loop that is too large to be compatible with OM secretion (Fig. 4).[57, 62, 66] Stalling pertactin OM secretion via an N-terminal Cys-pair produced stable secretion intermediates in which the C-terminus of the passenger is exposed on the surface of the bacterial cell,[67] providing strong —albeit indirect— evidence that premature folding of the passenger in the periplasm is– sufficient to block OM secretion. Surprisingly, even though the entire passenger was not available for folding, the extracellular C-terminal portion was able to adopt a stable conformation that closely resembles a stable passenger fragment observed during refolding *in vitro* (see below).[51, 62] Taken together, these results demonstrate that pertactin is secreted vectorially from C- to N-terminus across the OM, and that the C-terminus of the pertactin passenger can begin to fold before OM secretion is complete, while its N-terminus still resides within the periplasm.

Mechanistic differences between pertactin folding in vitro and in vivo

In contrast to the C- to N-terminal vectorial folding of the pertactin AT passenger during its OM secretion, pertactin passenger refolding *in vitro* follows a significantly different mechanism. *In vitro* refolding is surprisingly slow, on the time scale of several hours (Fig. 5).[51, 68] By comparison, the doubling time of the host bacterium is much faster (~20 min),[69] and AT biogenesis (synthesis and processing after secretion) occurs on a similar time scale

(a few minutes for the AT EspP[56, 70]). The disparity between these time scales suggests that the cellular environment accelerates the rate of AT passenger folding.

Denaturation titration experiments revealed that the C-terminal portion of both the pertactin and Pet passengers are markedly more stable than the N-terminus (Fig. 3, blue).[50, 51] Yet the boundaries of the pertactin and Pet C-terminal stable cores could not be deduced from the crystal structures of the native passengers: both are contained within the larger hydrogen bonding network of the passenger domain β-helix structure. And the size and sequence of the stable core differs significantly between pertactin and Pet. What are the properties of the isolated N- and C-terminal halves of the passenger? In isolation, the more stable C-terminus of the pertactin passenger refolds to high yield, but slowly, at rates similar to that of the full length passenger.[68] In contrast, the N-terminus under folding conditions remains disordered, suggesting that folding of the N-terminus of the passenger requires the presence of the more stable C-terminus.[63] Yet *in vitro*, while the passenger N-terminus clearly requires the presence of the C-terminus in order to fold, we have shown that the pre-folded passenger C-terminus does not represent a kinetic intermediate for formation of the native pertactin passenger structure.[68] Moreover, the extremely slow *in vitro* refolding kinetics demonstrate that while the identification of the passenger domain C-terminal stable core provided some initial hints of the C- to N-terminal vectorial OM secretion/folding mechanism described above, the folding of AT passenger domains *in vivo* cannot be completely understood from *in vitro* refolding studies alone, motivating us to investigate further how AT proteins fold in the cell.

Reconciling pertactin folding properties in vivo and in vitro

The discoveries that the pertactin passenger is secreted vectorially from its C- to N-terminus across the OM and that the C-terminus can adopt a stable, native conformation while OM secretion is underway have interesting implications for the understanding of folding kinetics of pertactin *in vitro* (slow), versus in the cell (faster). Folding of the C-terminus of the passenger β-helix on the outside of the cell could provide a template of folded β-strands for vectorial folding of the remainder of the passenger domain, as it appears vectorially across the OM. Perhaps therefore the key for folding the passenger N-terminus is not a pre-folded, native-like C-terminus, but a C-terminal folding intermediate. In this scenario, the rate of the C- to N-terminal vectorial secretion of the passenger domain across the OM could be the key mechanistic difference for accelerating secretion and folding *in vivo* (Fig. 5). Moreover, secretion into the periplasm proceeds in the opposite direction, from N- to C-terminus. This different folding vector might favor a folding pathway that introduces a kinetic barrier, akin to the slow refolding rate observed for the entire passenger domain *in vitro*.[51, 68]

Given the lack of typical external energy sources (ATP, proton gradient) outside the inner membrane, it is possible that the free energy of vectorial folding on the outside surface of the cell is somehow harnessed to provide a driving force for secretion. Alternatively, the anisotropic distribution of passenger stability might serve as a Brownian ratchet (see below), perhaps also exploiting the difference in molecular crowding on either side of the outer membrane. A third possibility is that productive folding of C-terminal segments during the secretion process provides only assistance to secretion: after folding of the passenger C-terminus, its bulky structure would prevent it from sliding backwards into the periplasm. Interestingly, the Fernandez lab found that deleting a segment corresponding to the very C-terminal end of the passenger of BrkA, a pertactin homolog, dramatically affects productive BrkA folding and secretion *in vivo*, consistent with a role for the AT passenger C-terminus in efficient OM secretion and folding. Similarly, Peterson *et al*. studied the secretion kinetics of EspP, a homolog of Pet, and found that mutations in the EspP passenger C-terminus that

slow the rate of refolding *in vitro* also result in slower OM secretion, supporting the idea that efficient folding of the C-terminus of AT passengers is required for successful secretion.[70]

Correlation between differential passenger domain stability and secretion

To systematically test the effects of passenger N-terminal stability on secretion, we replaced the globular protease domain at the Pet N-terminus with dihydrofolate reductase (DHFR, Fig. 3B), a well-studied model for protein refolding *in vitro*.[72–74] We used this chimeric DHFR-Petβ-helix passenger to study the correlation between stability at the N-terminus of the passenger and secretion efficiency. We observed a linear correlation (R=0.83) between the stability of DHFR ($\Delta G_{folding}$) and OM secretion efficiency of the chimera (Fig. 6): Destabilization of the Pet passenger N-terminus resulted in enhanced OM secretion efficiency, whereas stabilization reduced OM secretion efficiency, compared to the chimera bearing wild type DHFR (green in Fig. 6).[63] Surprisingly, the correlation between DHFR stability and OM secretion efficiency was stronger than correlations with DHFR refolding or unfolding kinetics.[63] We also characterized point mutations that selectively destabilize either the less stable N-terminal or more stable C-terminal portions of the pertactin passenger β-helix, to directly test the mechanistic role for the passenger domain C-terminal "stable core" in AT OM secretion. Destabilizing the less-stable N-terminus of the pertactin β-helix resulted in wild type-like secretion efficiency, but two mutations that destabilize the pertactin C-terminus each resulted in a significant decrease in OM secretion efficiency (Fig. 6B).[63]

Taken together, these results demonstrate that the anisotropic distribution of stability along the AT passenger contributes to OM secretion in a predictable way: The C-terminal stable core[50, 51] is exposed on the outside of the cell first,[62] and its folding into a stable structure increases secretion efficiency. The N-terminus is secreted after the C-terminus and we have shown that its stability is inversely correlated with secretion efficiency. The correlation between $\Delta G_{folding}$ and OM secretion efficiency suggests that C- to N-terminal vectorial protein folding, enabled by regional differences in stability along the passenger domain, could drive translocation across the OM, and could function as a unique molecular motor.

Molecular motors and Brownian ratchets

"Classical" examples of molecular motors include myosin movement along actin, or DNA and RNA polymerase movement along DNA. In these cases, movement of a protein is "powered" by ATP or GTP hydrolysis. Other examples where an external energy source is used to facilitate movement in a unidirectional manner include ATP synthase and the bacterial flagellum, where ATP hydrolysis is used to power (unidirectional) rotary motors.[75] The molecular origin for directed motion in these molecular machines can be rationally explained when considering that thermal fluctuations provide constant, random movement, and that the external energy source "steers" this movement in a unidirectional fashion, hence the name Brownian ratchet.

Translocation of AT passenger proteins across the bacterial OM can be viewed as a molecular motor as well: As soon as the most C-terminal part of the passenger domain is transported across the OM, the polypeptide chain could either slide back into the periplasm or out of the cell by random Brownian motions. The anisotropic distribution of free energy of folding along the passenger domain steers this random motion, resulting in directed, C- to N- terminal translocation across the OM. Yet in contrast to classical examples of molecular motors, this ATP-independent motor represents a "one-shot" molecular machine: Each passenger molecule provides the energy to transport itself, and once it is secreted, folded, and cleaved from its C-terminal translocator domain, this energy source is no longer available.

General conclusions for understanding protein folding in the cell

While studying refolding in the test tube has provided valuable information on the kinetic and equilibrium thermodynamic properties of a wide variety of protein folding pathways and structures, it has become clear that only a small fraction of all proteins can be refolded efficiently *in vitro*, and these proteins tend to be small, monomeric, α-helical, and have only a modest energy barrier between their folded and unfolded conformations.[7, 76–78] In contrast, proteomes as a whole contain a larger percentage of proteins that are large, multimeric, β-sheet (or mixed α/β), and a significant number of these native structures are energetically trapped in their folded state due to a large energy barrier.[36, 79] We and others have shown that co-translational folding intermediates can differ significantly from the predominant intermediates formed during refolding *in vitro*,[23, 24, 26, 32, 35, 80, 81] which might provide an alternative kinetic route to the native structure, bypassing the high kinetic barrier encountered for refolding of the free polypeptide chain.[35]

Recent results with AT passenger domains have further highlighted that *in vivo* folding mechanisms can differ significantly from refolding *in vitro*. And, AT passenger folding *in vivo* follows very different kinetic routes depending on whether folding is initiated from N-to-C-terminus (as upon appearance into the periplasm), or from C-to-N-terminus (as occurs after secretion across the OM). Moreover, the unusual anisotropic distribution of stability within the passenger domain β-helix, which appears to play no role in refolding *in vitro*, provides a crucial driving force for OM secretion *in vivo*. The mechanistic differences between AT passenger folding *in vivo* and refolding *in vitro* illustrate the influence of the cellular context on protein folding pathways. Other scenarios where vectorial folding could play a role in the cell include the coupled secretion and folding of other virulence proteins. For example, toxins such as CyaA consist of an array of RTX (repeat in toxin) motif, each of which is intrinsically disordered in the absence of Ca^{2+}.[82] But Ca^{2+} is present in the extracellular medium, and therefore might facilitate vectorial folding of these domains, and hence their successful secretion.

More broadly, vectorial protein folding has the potential to play a broad role in shaping protein folding mechanisms *in vivo*: every protein is synthesized on the ribosome, and many have the potential to start folding from N-to-C-terminus during synthesis. Because many large and multi-domain proteins fail to refold reversibly *in vitro*, *in vivo* experiments might represent our best option to study the folding of these proteins. Yet currently there are few methods available that are compatible with studying folding in the complex environment of the cell. Increasing the number of methods capable of following protein folding *in vivo* will be crucial in order to understand how the cellular environment shapes the folding of these proteins, and their biological mechanisms.

Acknowledgments

We thank Jonathan Renn, Felix Vietmeyer, and Igor Drobnak and other members of the Clark laboratory for fruitful discussions. Autotransporter research in our laboratory is supported by grant R01 GM097573 from the NIH. E.B. is supported by a National Institutes of Health Ruth L. Kirschstein National Research Service Award (Notre Dame Chemistry-Biology-Biochemistry Interface Program, T32 GM075762).

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Biographies

Esther Braselmann received her Diplom in Biochemistry from the University of Bielefeld (Germany) in 2009. During her Diplom studies, she received a DAAD German Academic Exchange Program fellowship to support a research internship at the University of Notre Dame (2007). She returned to Notre Dame and the Clark laboratory to join the biochemistry PhD program, where she is currently a fellow of the Chemistry-Biochemistry-Biology-Interface Graduate Training Program.

Patricia L. Clark (http://chemistry.nd.edu/faculty/detail/pclark1/) received her B.S. in chemistry from Georgia Tech and her Ph.D. in molecular biophysics from the University of Texas Southwestern Medical Center in Dallas. She was an NIH postdoctoral fellow at MIT with Jonathan King before joining the Notre Dame faculty in 2001, where she is currently the O'Hara Associate Professor of Chemistry & Biochemistry and Concurrent Associate Professor of Chemical and Biomolecular Engineering. Her research interests include the influence of the cellular environment on protein folding mechanisms, and the folding and assembly of large, complex protein topologies.

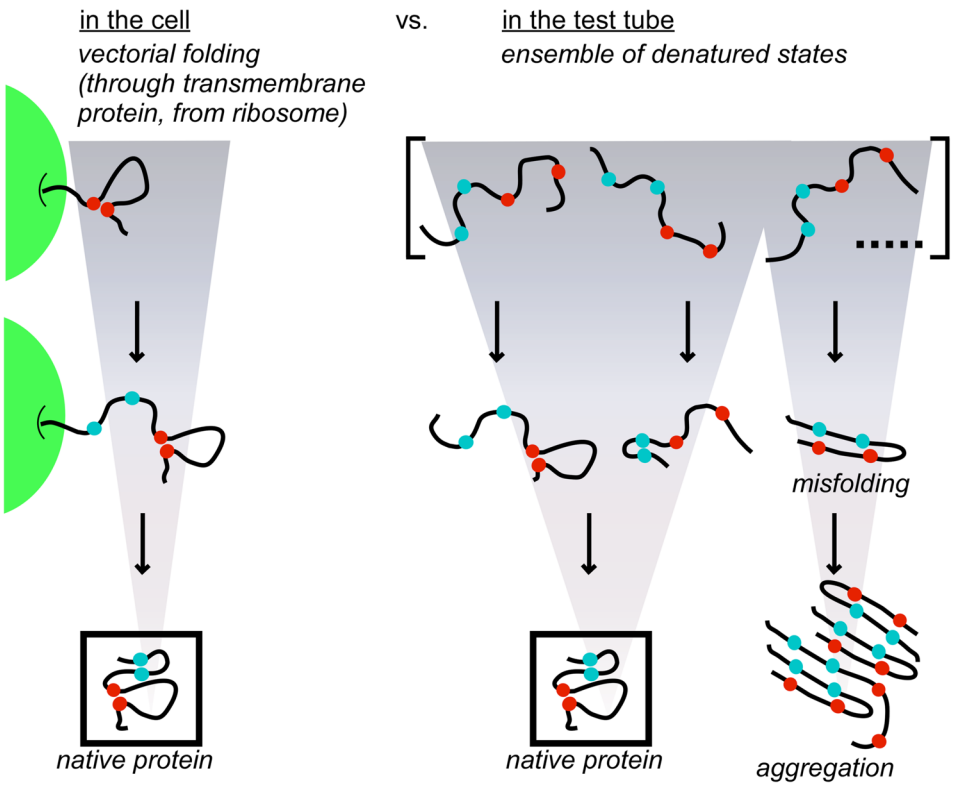

Figure 1. Protein folding *in vivo* versus in the test tube

Left: During protein synthesis and many protein secretion mechanisms, one end of a polypeptide chain can begin to fold before the remainder of the protein can participate in the folding reaction. Right: In contrast, in the test tube a full-length unfolded protein adopts an interconverting ensemble of denatured states. Upon dilution of the protein out of denaturant, all parts of the protein are simultaneously available to form interactions with other portions of the protein. Hence native contacts that might be assembled in a particular order *in vivo* will not necessarily be formed in the same order *in vitro*. Given that the protein sequence has not evolved to fold successfully after dilution out of denaturant, refolding *in vitro* might result in non-native contacts that can lead to protein misfolding and aggregation.

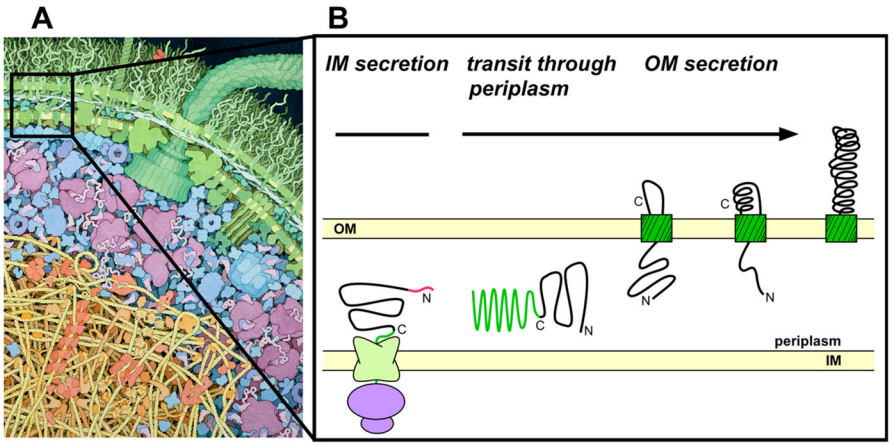

Figure 2. Overview of autotransporter protein secretion within Gram-negative bacteria

(A) Watercolor illustrating a cross-section through a corner of an *E. coli* cell, by David Goodsell (reprinted with permission).[45] Bright yellow: DNA is concentrated within the central region of the cell. Orange/Red: DNA binding proteins. Blue: Cytoplasmic proteins. Purple: Ribosomes synthesizing proteins from mRNA (in white). Pale yellow: Cell wall, consisting of the IM (lower) and OM (upper); membrane proteins are shown in dark green. The large green assembly pointing out of the top of the cell is a flagellar motor, which controls cell movement. **(B)** AT secretion across the inner membrane (IM, pale yellow) starts co-translationally, during synthesis of the AT precursor by the ribosome (purple). After IM secretion, the N-terminal signal sequence (red) is removed, and the remaining precursor, consisting of the central passenger (black) and C-terminal translocator domain (dark green), crosses the periplasm. The translocator domain folds into a 12-stranded β-barrel across the OM, followed by secretion of the passenger across the OM. Depicted here is the proposed “hairpin” mechanism passenger secretion through the C-terminal translocator domain: see text for more details.

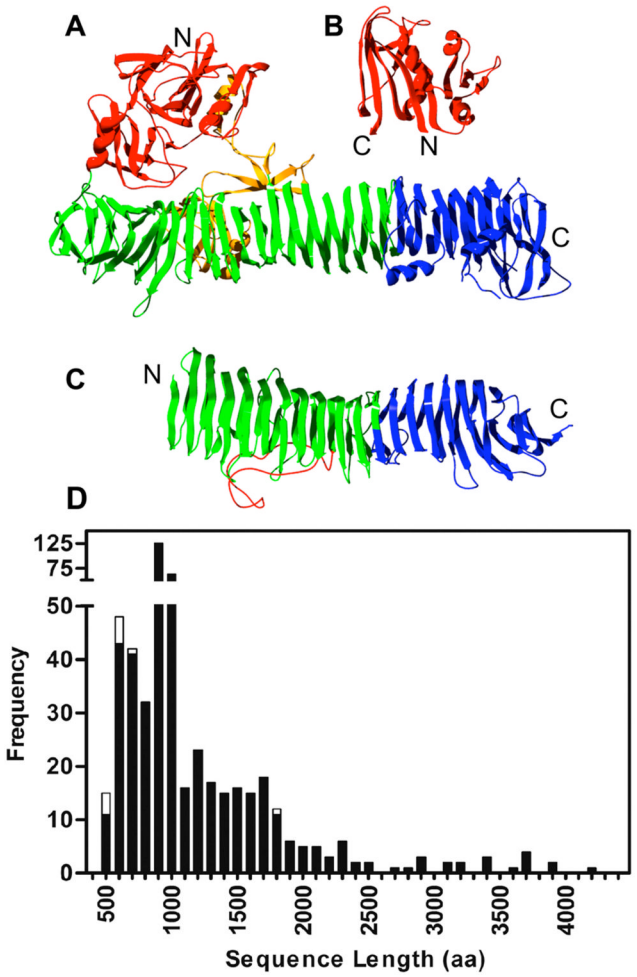

Figure 3. Domain organization of model AT passengers

Top: Crystal structure of **(A)** hemoglobin protease AT passenger (PDB ID: 1WXR), a homolog of Pet (26% identical, 43% similar), **(B)** *E. coli* dihydrofolate reductase (DHFR; PDB ID: 7DFR), and **(C)** pertactin AT passenger (PDB ID: 1DAB). Blue: C-terminal stable core identified in the Pet[50] and pertactin[51] passengers; Green: other β-helical structure; Red: domains associated with function (protease in Pet, binding loop in pertactin); Yellow: other, non β-helical structure. **(D):** Length distribution of identified AT passengers. Those predicted to contain right-handed β-helical content (>97%) are shown as filled bars. Figure reproduced from ref 63 (panels A–C) and 51 (panel D).

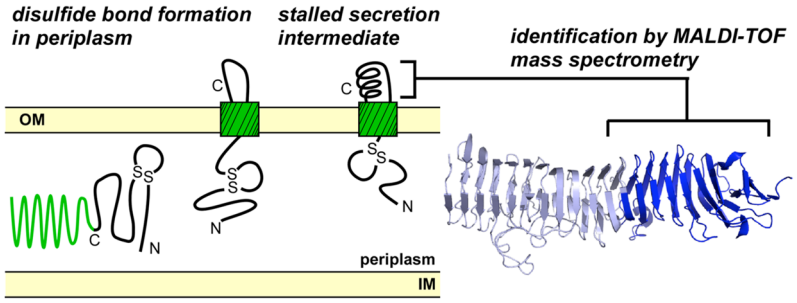

Figure 4. In the periplasm, formation of a disulfide bonded loop within the AT passenger stalls OM secretion of the passenger domain at the loop position, with the C-terminus of the passenger exposed at the bacterial cell surface

Protease digestion of intact cells expressing the stalled intermediates produced fragments that were identified by MALDI-TOF mass spectrometry as peptides from the pertactin passenger C-terminus.

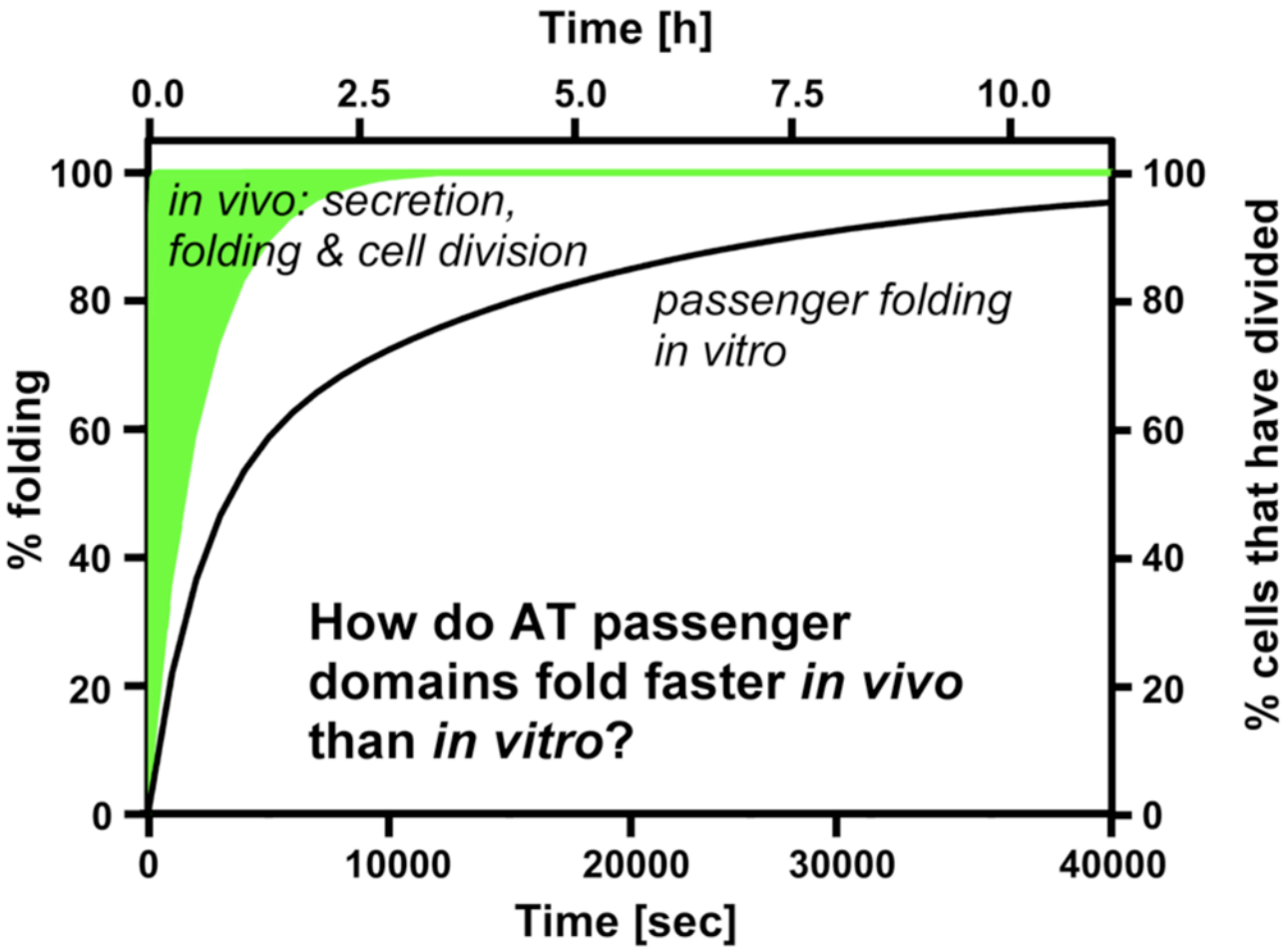

Figure 5. Schematic comparison of AT passenger folding kinetics *in vivo* and *in vitro*

Pertactin refolding *in vitro* takes several hours (black line, measured by Trp-fluorescence).[51, 68] In comparison, secretion, processing and folding (measured as the development of protease resistance) of the EspP AT[56, 70] and the doubling time of *E. coli,*[71] are much faster (green shading).

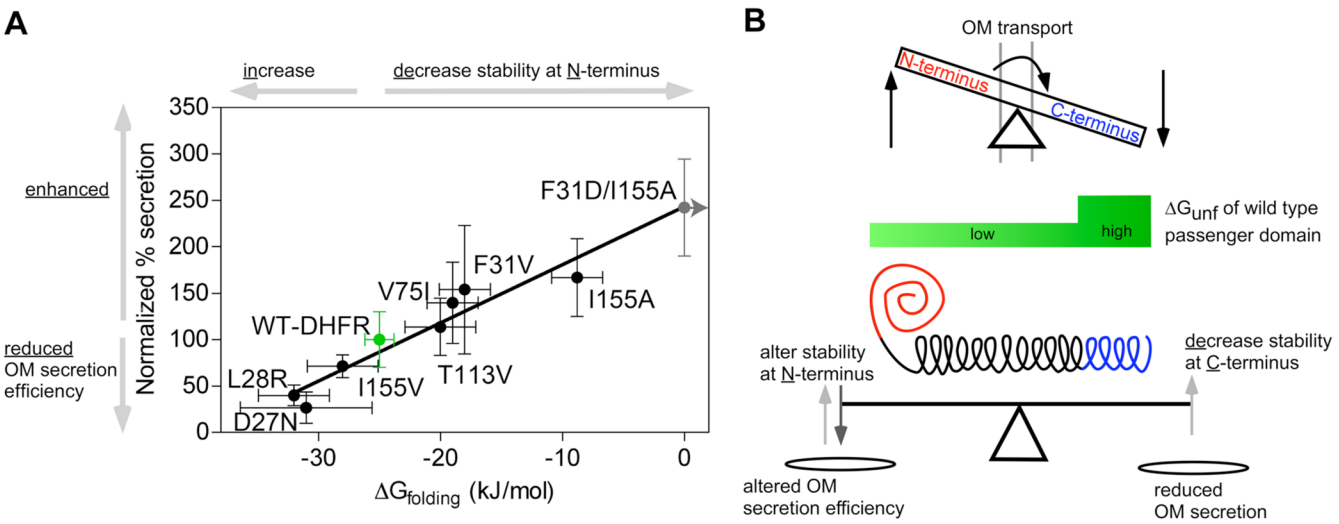

Figure 6. Regionalized stability of the passenger domain correlates with secretion efficiency

(A) We constructed a chimeric passenger, DHFR-Pet β-helix, to test the effects of the stability of the N-terminal globular domain on AT passenger OM secretion. The stability of the N-terminal DHFR (Fig. 3) is inversely correlated with AT OM secretion efficiency (normalized to 100% secretion of wild type DHFR, green). Figure reproduced from ref. 63. (B) Successful AT passenger secretion is ensured by high stability ($\Delta G_{folding}$) at the C-terminus of the passenger, and low stability at its N-terminus. Decreasing the stability of the N-terminus enhances OM secretion efficiency; inversely, increasing the stability of the N-terminus reduces OM secretion efficiency. The high stability of the C-terminus is key for efficient OM secretion, as decreasing stability selectively at the C-terminus of pertactin severely reduces OM secretion efficiency.