

Reprogramming the bacterial Tat system for monitoring protein folding directly in cells

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Abstract:

Protein folding is a process fundamental to all of life. When proteins do not fold correctly, there can be severe consequences including many well-known diseases such as Alzheimer's, Huntington's and cystic fibrosis. As though that weren't enough, many of the unexpected difficulties biotechnology companies encounter when trying to produce human protein drugs in bacteria also result from something amiss when proteins fold. For these reasons, a reporter system enabling rapid and efficient characterization of the folding state of proteins could have a broad and significant impact on human health. Accordingly, we have developed a novel cell-based system for high-throughput assessment of protein folding and solubility, which we term Twin-ARGinine translocation of Expressed Target Sequences (TARGETS). The basis for TARGETS is the proofreading mechanism inherent to the twin-arginine translocation (Tat) pathway of bacteria which naturally monitors the folding status of its substrate proteins. Since the machinery selectively transports only those proteins which are soluble in the cytoplasm, we have exploited this proofreading capacity to screen target sequences for their ability to fold properly in the aqueous cytoplasmic environment. This system is thermally and chemically robust, protein-based and genetically encodable. Finally, the application of this system to combinatorial library screening for: i) the engineering of soluble proteins; and ii) the discovery of well-ordered protein structures derived from entirely random sequences will be discussed.