530d An Engineered Genetic Approach to Isolating Functional Proteins Expressed from De Novo Libraries

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One of the cornerstones of biotechnology is the ability to heterologously express functional proteins. However, many important target proteins are expressed inefficiently due to the complexity of the folding process and the limited solubility of many folded domains. Accordingly, we have developed a novel cell-based genetic selection for determining the folding status, stability and solubility of an expressed protein. The basis for our technology lies in the "proofreading" mechanism inherent to the twin-arginine translocation (Tat) machinery of bacteria which naturally monitors the folding status of its substrate proteins. This system is ideally suited for screening combinatorial libraries in order to isolate correctly folded, heterologously expressed proteins. We have applied this genetic selection strategy to a designed binary patterned library in order to isolate entirely de novo proteins that assume a stable conformation when expressed in bacteria. Since the final destination of all correctly folded de novo proteins in our selection is the periplasmic space, we are able to screen the resulting folded proteins for enzymatic activity (e.g. beta-lactam hydrolysis, oxidase activity, phosphatase activity). To our knowledge, this represents the first two-dimensional genetic assay for simultaneously identifying correctly folded de novo proteins that exhibit biological activity. In essence, isolating proteins based on structure and function.