QUANTIFYING THE KINETIC STABILITY OF UNPURIFIED OVEREXPRESSED PROTEINS

IN CELL CULTURE VIA SDS-TRAPPING

By

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ABSTRACT

Protein stability can be considered as thermodynamic stability (TS) or/and kinetic stability (KS). TS means the equilibrium between a protein's native state and unfolded state is thermodynamically favored by free energy towards the folding direction. KS is characterized by a large activation energy barrier separating the native state from the unfolded state. KS endows a protein with a very slow unfolding rate and helps maintain a protein's biological function especially in crowded and harsh environments. KS is a most relevant concept since abnormal change of KS closely relates to several protein misfolding and amyloid diseases. Transthyretin amyloidosis is one of those diseases. In order to quantify KS, our lab recently developed an assay named SDS-Trapping of Proteins (S-TraP), which is simple, accessible, and overcomes the drawbacks of previous methods. Another potential advantage of S-TraP is the ability to quantify the KS of unpurified proteins. In this study, to test this feasibility, the unfolding rates of over-expressed human TTR in E. Coli lysate were determined by S-TraP without purification. The results were quite close to those yielded from S-TraP analysis of purified TTR. This demonstrates that it's suitable and reliable to use S-TraP to quantify KS of unpurified proteins. This would be extremely valuable when purification of the protein of interest is challenging or time consuming.