Constructing GroEL-Streptavidin Nanoclusters to Capture Protein Folding Transient States for Drug Discovery and Folding Platform Development

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Protein misfolding is the root cause of many human diseases (e.g. Alzheimer's disease, Parkinson's disease, Cystic Fibrosis, Type II Diabetes). Proteins with folding defects are difficult to study because they aggregate very easily. In research of protein misfolding diseases, molecular chaperones are used because they play a very critical role in maintaining proteins in their proper folded states. These chaperones help proteins acquire and maintain their proper folds while preventing large-scale intracellular protein aggregation. To detect proteins in their initial disease misfolded hydrophobic states, the chaperonin GroEL (Escherichia coli) is an excellent capture platform. Since rapid separation of initial GroEL–misfolded protein complexes from folded proteins is not simple (e.g. ultrafiltration), larger GroEL complexes are constructed by biotinylating GroEL and linking other biotinylated GroEL molecules with the biotin binding protein Streptavidin to form larger GroEL nanoparticles. The formation of GroEL-Nanocluster structures were dependent on the streptavidin:GroEL ratio. Formation of GroEL-Nanoclusters was confirmed using native gel electrophoresis and electron microscopy. Larger GroEL complexes can rapidly capture "transient misfolded states" for easy separation and quantitation. The GroEL nanocluster platform can be used to rapidly test potential therapeutic small molecule stabilizers that help fold disease proteins and facilitate folding of other hard-to-fold therapeutic proteins.