EMPLOYING DYNAMIC MASS REDISTRIBUTION TO IDENTIFY PHARMACOLOGICAL CHAPERONES FOR THE CELLULAR PRION PROTEIN

Saioa R. Elezgarai

The key pathogenic event underlying all forms of prion diseases is the conversion of the cellular prion protein (PrP^{C}) into an aggregated form (PrP^{Sc}) that self-propagates by imposing its abnormal conformation onto PrP^{C} molecules. Previous attempts to identify anti-prion compounds were aimed to reduce the load of PrP^{Sc} aggregates by decreasing their stability or increasing their clearance. Some of these compounds showed potent activity in vitro or in cultured cells, but little or no efficacy in vivo. Multiple pieces of evidence support the notion that PrP^{C} loses its native fold in the initial steps of the aggregation process. This concept provides a rationale for tackling PrP^{C} aggregation by stabilizing the monomeric protein precursors, instead of disrupting pre-formed PrP^{Sc} species. The underlying idea is to block aggregation by increasing the Gibbs free energy barrier (ΔG) required for the initial misfolding events. This goal could be achieved with small, high affinity ligands of PrP^{C}, capable of acting as pharmacological chaperones. In order to identify such compounds, we setup a novel screening method based on Dynamic Mass Redistribution (DMR), a label-free, fully automated biophysical technique performed on 384-well microplates, and capable of detecting molecular interactions at the equilibrium. First, we established optimal buffer composition and efficient immobilization conditions for mouse or human recombinant PrP. We then tested the interaction of a small set of previously characterized compounds to PrP^{C}. Our analyses confirmed, refined or disputed previous reports, by defining accurate binding constants for each molecule. These results demonstrate that DMR is a reliable platform for the identification of novel PrP^{C} ligands, providing a unique opportunity for future High Throughput Screening (HTS) campaigns.