IDENTIFICATION OF PHARMACOLOGICAL CHAPERONES FOR THE CELLULAR PRION PROTEIN BY DYNAMIC MASS REDISTRIBUTION SCREENING

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INTRODUCTION

Genetic prion diseases are fatal dominantly inherited neurodegenerative disorders linked to mutations in the *PRNP* gene encoding the prion protein (PrP). No treatment is currently available. One of these mutations, D178N, is likely to be causal[1] and may trigger the conversion of the cellular prion protein (PrP^C) into an aggregated form (PrP^{Sc}) that self-propagates in the brain by imposing its abnormal conformation[2,3]. This concept provides a rationale for tackling aggregation by stabilizing the PrP^C monomeric protein precursors by increasing the Gibbs free energy barrier (ΔG) required for the initial misfolding events. This goal could potentially be achieved with ligands of PrP^C that act as pharmacological chaperones stabilizing PrP^C protein (Fig. 1).

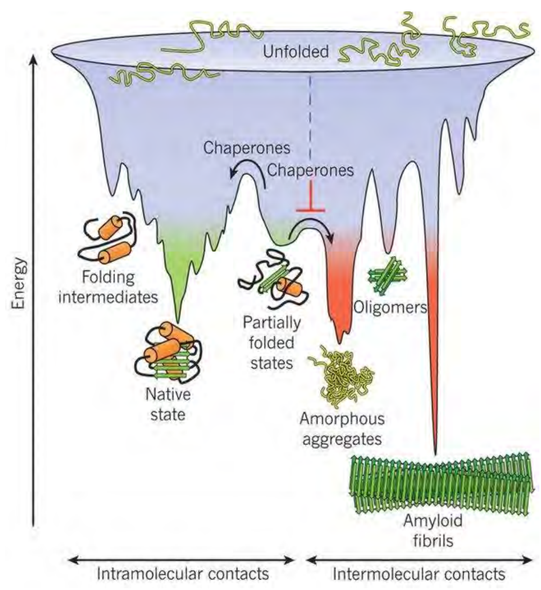

Fig.1. Scheme of the funnel-shaped free-energy surface that proteins explore as they move towards the native state by forming intramolecular contacts[3]. The free-energy landscape results in the accumulation of kinetically trapped conformations that need to traverse free-energy barriers and these steps may be accelerated by chaperones. When several molecules fold simultaneously in the same compartment, the free-energy surface of folding may overlap with that of intermolecular aggregation, resulting in the formation of amorphous aggregates, toxic oligomers or ordered amyloid fibrils.

OBJECTIVE

To develop a novel screening method based on Dynamic Mass Redistribution (DMR), a label-free, fully automated biophysical technique, capable of detecting small molecule-prion protein interactions.

MATERIAL AND METHODS

Recombinant Human PrP23-230 (recHuPrP23-30) was expressed in E. coli. Inclusion bodies were solubilized in 2M guanidine-containing buffer and the protein attached to an NTA affinity column. The protein, which attaches to the column through its histidine-containing octarepeat sequence, was allowed to fold while attached by gradual elimination of the guanidine. Folded recHuPrP23-230 was then eluted by application of 500 mM imidazole, and dialyzed against 50 mM sodium acetate, pH = 5. Protein concentration was determined from its A_{280} and proper folding assessed by CD spectroscopy. DMR assay starts with the plate activation LFB 384-well microplates (PerkinElmer 6057468) with 15 µl activation buffer (400 mM EDC + 100 mM Sulfo-NHS) and incubate 30 min R.T. After washing plate, immobilization of $HuPrP^C$ protein (20 µg/ml) in 10 mM Sodium acetate buffer pH=6 and incubating overnight at 4°C. Second day, Prestwick® chemical library was screened at 10 µM, incubating the compounds for 30 min. Light refraction signal of tested compounds was measured in PerkinElmer EnSpire® Multimode Plate Reader (1 read /2 min) (Fig. 2). Active compounds (average + 3 SD) were selected for cherry picking and triaged by Protein Misfolding Cyclic Amplification (PMCA) assay[4]. PMCA is a technique that mimics prion replication *in vitro* with similar efficiency to the *in vivo* process. It is based on cycles of sonication/incubations. After each cycle, the number of prions are amplified in an exponential fashion. The rationale to use this technique to evaluate anti-prion compounds is based on the high impact that any kind of inhibition of the *in vitro* prion replication process will have in the amplification of the final amount of prions.

RESULTS

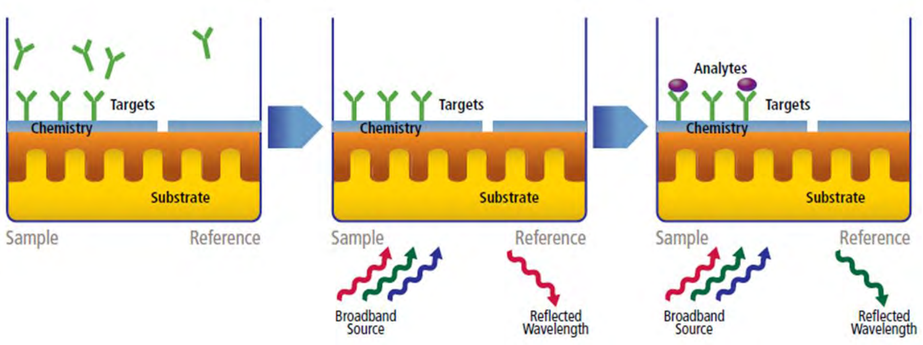

Fig.2. DMR principle. First, target is immobilized on the microplate amine-coupling surface. After incubation o/n, the plate is washed and baseline read. Finally, the compounds are added and final reading is performed.

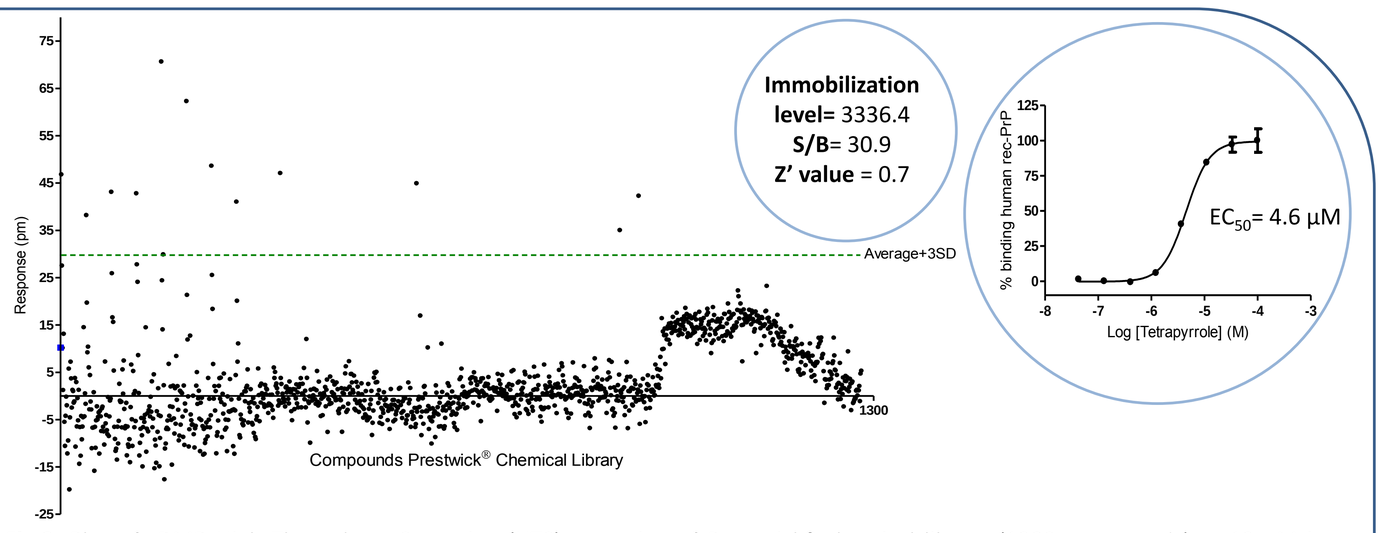

Fig.3. Plot of DMR High Throughput Screening (HTS) campaign of Prestwick® chemical library (1280 compounds) at 10 µM in human PrP^C. The hit Threshold was fixed at average + 3SD and 13 hits were identified. Black circles represents the tested compounds and blue square represents negative control (Buffer).At the top right of the figure shows the metric parameters and dose-response curve for positive control Tetrapyrrole (TMPyP-Fe(III)).

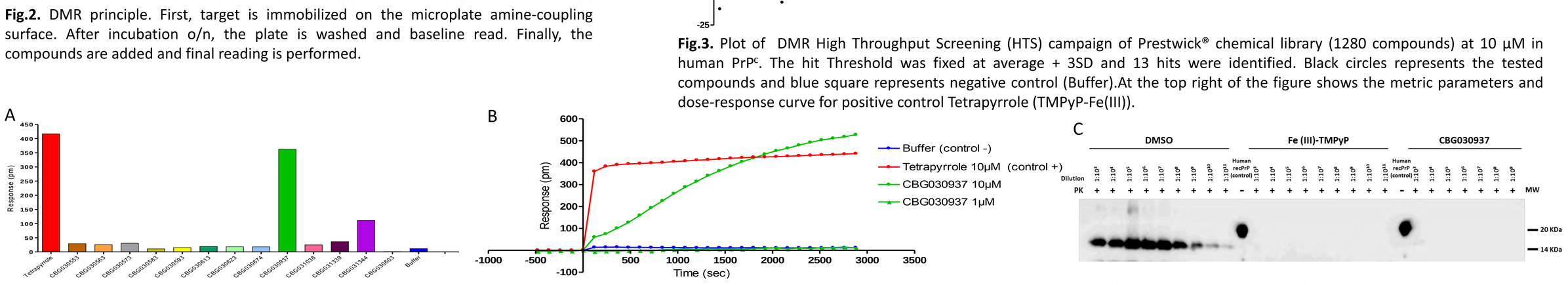

Fig.4. 13 active compounds which stabilize the conformation of human PrP^C identified on DMR-based HTS campaign were later confirmed by cherry picking assays. A) HTS hits tested at 10 µM. B) Kinetic response for the compound showed greater affinity (CBG030937). C) *In vitro* PrP^{res} amplification by rec-PMCA with or without CBG030937**.** 50 µl of human rec-PrP complemented with chicken brain homogenate, seeded with different dilutions of human recombinant prion ($1:10^3$ – $1:10^{11}$) were mixed with 0.5 µl of DMSO, TMPyP-Fe(III) (used as positive control) or CBG030937 diluted in DMSO (at final concentration of 250 µM) and subjected to a unique 48 h round of rec-PMCA. Amplified samples were digested with 50 µg/ml of proteinase K (PK) and analyzed by western blot using monoclonal antibody 3F4 (1:10,000). Fe(III)-TMPyP and CBG030937 exhibited a potent inhibitory activity toward the amplification of human recombinant prion of at least 10^6 fold. NBH: Normal brain homogenate. MW: Molecular marker.

CONCLUSIONS

- Our results demonstrated that DMR is a reliable platform for the identification of novel pharmacological chaperones targeting the cellular prion protein.
- We have identified hits that might interact with prion protein. One of them is used in neurological disorders and stands out from others for its high affinity. Moreover, it inhibits in a dose-dependent manner the conversion of PrP^C into PrP^{Sc}, the key molecular event of prion disease. A range of assays (e.g., biochemical, biophysical, cell-based), along with computational chemistry approaches are being performed to validate the hits.

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