⮕ SCREENING

A platform for probing protein-aggregation inhibitors

> compounds known to strongly inhibit hIAPP aggregation dose-dependently restored the resistance of β-la–hIAPP-expressing *E. coli* to ampicillin

Several diseases are characterized by the aggregation of proteins; for example, type II diabetes is associated with the formation of human islet amyloid polypeptide (hIAPP) amyloid fibrils. One strategy to prevent such aggregation is to use small molecules that bind specifically with the protein precursor before it misfolds and aggregates; however, screens to identify such small molecules are challenging and require large amounts of precursor protein. Saunders *et al.* now introduce a new *Escherichia coli*-based platform that can predict the potential of different proteins to aggregate, and use this system to identify specific small-molecule inhibitors of hIAPP aggregation.

First, the authors developed a way of testing the propensity of different test proteins to aggregate. They linked the gene sequence encoding the test protein between the sequences that encode the two domains of the bacterial β-lactamase (β-la) TEM1, which localizes in the space between the inner and outer membranes of the bacterium. If the test protein sequence folds normally here, the two β-la domains are brought together to form the functional enzyme, rendering the bacterium resistant to β-lactam antibiotics such as ampicillin. By contrast, if the test protein sequence aggregates, the β-la domains do not join, and the bacterium is susceptible to β-lactam antibiotics.

The authors confirmed the validity of this system by measuring the antibiotic resistance of *E. coli* carrying sequences encoding β-la constructs that contained test proteins known to have high aggregation propensities (hIAPP, amyloid-β_{1-40} ($A\beta_{1-40}$) and $A\beta_{1-42}$) or rat IAPP (rIAPP), which does not aggregate into amyloid fibrils. Whereas *E. coli* expressing β-la–hIAPP, β-la–$A\beta_{1-40}$ or β-la–$A\beta_{1-42}$ were all susceptible to 80 μg per mL ampicillin, bacteria expressing β-la–rIAPP or a control sequence were relatively resistant. Measures of antibiotic resistance correlated with the propensity of each protein to aggregate *in vitro* (assessed using biophysical methods).

Next, the authors tested a panel of 20 compounds known to have different abilities to prevent aggregation of hIAPP in this system. Indeed, the compounds known to strongly inhibit hIAPP aggregation dose-dependently restored the resistance of β-la–hIAPP-expressing *E. coli* to ampicillin, whereas the compounds with no ability to prevent aggregation did not.

To find new inhibitors of hIAPP aggregation, 59 additional compounds were tested in a miniaturized version of the *E. coli* system (to reduce the amount of test compound needed to ~0.07 mg). The 59 compounds included 31 compounds known to inhibit aggregation of other proteins, and 28 compounds that were computationally selected for their structural similarity to a known inhibitor of hIAPP called JCS-1. Of the 59 test compounds, 6 compounds moderately or strongly rescued the resistance of β-la–hIAPP-expressing *E. coli* to ampicillin; the ability of the strongest hit, dopamine, to limit hIAPP aggregation was confirmed using biophysical methods.

This platform could potentially be adapted to screen small molecules for inhibitors of the aggregation of many different proteins, using only small amounts of screening compounds, and without the need to produce large amounts of test protein.

Natasha Bray

ORIGINAL ARTICLE Saunders, J. C. *et al.* An *in vivo* platform for identifying inhibitors of protein aggregation. *Nat. Chem. Biol.* http://dx.doi.org/10.1038/nchembio.1988 (2015)

S.Bradbrook/NPG