Making better enzymes and protein drugs

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Natural selection results in protein sequences that are only soluble to the level that is required to carry out its physiological function. However, in biotechnological applications, we need these proteins to survive concentrations that are up to 1000-fold higher that what naturally occurs, e.g. an antibody drug in the syringe prior to injection. Moreover, these proteins are isolated at high purity and can thus no longer count on the help of molecular chaperones that all living organisms employ to keep its proteins in shape.

As a result, biotechnological and therapeutic applications of protein are often hampered or rendered impossible by the mismatch between the natural solubility of a protein and the requirements of the application. This raises the question whether the solubility of natural protein sequences could be improved without affecting their intended function. Ashok Ganesan and Aleksandra Siekierska from the SWITCH laboratory, under the direction of Frederic Rousseau and Joost Schymkowits (VIB/KU Leuven) show that there is an anti-correlation between the number of aggregation prone regions (APRs) in a protein's sequence and its solubility, suggesting that mutational suppression of APRs could provide a simple strategy to increase protein solubility.

Joost Schymkowitz (VIB/KU Leuven): 'Aggregation is a bottleneck in the production process of proteins you want to use as biotech product, for instance as a drug. We now show that by using our state of the art software algorithms, we can improve the solubility of proteins by making very minor changes to their primary amino acid composition. This will not only significantly speed up the production and development

process of so-called biologicals and enzymes significantly, it will also empower us to employ proteins that were until now simply to insoluble to be of any practical utility.'

The VIB researchers have shown that mutations at specific positions within a protein structure can suppress protein aggregation without affecting protein stability or function. These hot spots for protein solubility are both structure and sequence dependent but can be computationally predicted. They have demonstrated this by mutationally reducing the aggregation of human a-galactosidase, which is used as a drug for Fabry's Disease, and a protein from Bacillus anthracis that is part of a vaccine against Anthrax. In combination with thorough computional analysis of a large number of protein structures, these results indicate that many proteins possess similar hot spots that will allow to improve their solubility.

Frederic Rousseau (VIB/KU Leuven): 'We already have collaborations with the pharmaceutical industry to integrate our methods in their development pipelines for new drugs, clearly showing the relevance of our findings. We hope in this way to contribute to a new class of improved protein drugs.'

More information: Ashok Ganesan et al. Structural hot spots for the solubility of globular proteins, *Nature Communications* (2016). DOI: 10.1038/ncomms10816

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