Engineering surface epitopes for improving protein crystallization

Technology #m08-082

High-quality protein crystal structures are an invaluable tool for drug-discovery and protein engineering projects. However, attempts to prepare high-quality protein crystals suitable for structure determination are expensive, time-consuming, and often unsuccessful. The low success rate is rooted in the inability to predict the crystallization behavior of a protein based on its amino acid sequence alone and in the lack of efficient methods to engineer proteins to improve this behavior. This technology describes methods that facilitate the process of obtaining high-quality protein crystals by identifying mutations that improve crystallization behavior. The technology introduces established “crystallization epitopes” into proteins at sites identified exclusively by the analysis of amino acid sequences. These epitopes were identified via a comprehensive topological analysis of the crystal structures in the Protein Data Bank (PDB), which was used as a data mine of information. Based on this analysis, software was developed to identify point mutations that introduce such epitopes into proteins without compromising their function. Introducing such mutations into test proteins yielded soluble proteins that produced high-quality crystal structures for the vast majority of crystallization-resistant proteins evaluated. As such, the technology provides a platform capable of engineering more efficient protein crystallization than other methods, and can expedite the generation of crystal structures for drug-discovery and structural biology research.

Protein database and software algorithm for improved success rate of protein crystallization

This technology comprises a method of probabilistically engineering protein surfaces to improve their propensity to form crystals useful for high-resolution X-ray crystallographic structure determination. The method is based on using point mutations to introduce local, low-entropy surface epitopes with high inter-protein interaction potential. In addition, the technology can also identify whole epitopes to be replaced rather than individual amino acid residues. These suggested epitope modifications do not alter thermodynamic stability or thermodynamic solubility relative to the amorphously precipitated state. Thus, unlike previously reported methods, wherein improved crystallization comes at the cost of impaired solubility, less than 5% of mutant proteins developed by this method show reduced stability or solubility as compared to the wild-type
protein. This method improves protein crystallization properties with substantially greater efficiency than any previously proposed mutational strategy.

This technology has been used to introduce mutations into a large panel of test proteins, and has yielded soluble protein preparations suitable for crystallization screening in 95% of experiments.

**Lead Inventor:**

John F. Hunt, Ph.D.

**Applications:**

- Design mutations that improve protein crystallization for x-ray structure determination
- Better understand the mechanism of protein crystallization
- Enhanced efficiency of structure-based drug-development projects
- Facilitate production of high-quality crystal structures for drug-discovery and protein engineering

**Advantages:**

- Epitope modifications reduce the time and cost of protein production and structure determination
- Modifications increase the efficiency and quality of structure-determination efforts.
- Improved protein solubility

**Patent Information:**


Patent Pending ([US/20150269308](https://www.uspto.gov/patents-application-status))

Tech Ventures Reference: IR M08-082, IR M10-014

**Related Publications:**


**Inventors**

John F. Hunt