Department of Biochemistry

THESIS DEFENSE

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“Adapting DARPin-Based Rigid Fusions for Crystallography”

The major bottleneck in macromolecular X-ray crystallography is the poor packing of protein molecules, frequently resulting in low quality crystals, and the ensuing problems in phase determination, especially with poorly diffracting crystals. DARpins [1] have been widely used as crystallization aides and are well known for their stability, high expression yield, and their ability to crystallize under various conditions. Despite all advantages, DARpins sometimes fail to provide the necessary polar surface to form crystal contacts due to their small size in comparison to the target protein. To extend the range of potential applications of the DARpins as crystallization chaperones, we have developed rigid fusion proteins linked through shared helices, either with another well crystallizing protein (β-lactamase) [2] or, more versatile, with one or more additional DARpins. As these can all be added in a series of different orientations, we have thereby introduced molecular geometry as an additional screening parameter beyond the traditional methods. Thus, a “tool box” of DARPin-based co-crystallization chaperones with a variety of rigid fusion proteins with different geometries was applied to assist the crystallization of other proteins in a simple ‘cut-and-paste’ manner of the desired DARPin fusion. This protein engineering tool box has already helped in obtaining several protein structures. Moreover, this project has shed light on the fundamental question of how to connect proteins rigidly. Rigid DARPin-DARPin fusions also have great potential in other applications, as the constructs can be used to organize supramolecular complexes of different geometries and offer new insights into the geometric constraints and mechanism of receptor activity.

References:

Date & time: Thursday, February 2, 2017 at 04:00 pm
Location: Lecture Hall Y44-H-11, UZH Irchel

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