

A rational approach to crystallising proteins in the pharmaceutical industry, the impact of micro seed matrix seeding

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Crystallization is often referred to as a bottleneck in protein structure determination. However applying rational, knowledge based strategies and attention to detail, has proved to be very successful in obtaining crystallization systems for studying protein/inhibitor complexes in drug discovery.

This seminar will describe the rationale and the methods that have been successfully used to crystallize medically relevant proteins [1] in four different pharmaceutical companies (Hoffmann-la Roche, Morphochem, Novartis and Actelion). The first part will deal with protein characterization prior to initiating crystallization trials [2], the different methods and strategies of screening and examples of protein modification for crystallization.

The second part will focus on seeding and describe general seeding methods as an introduction to the most important subject of the talk, **Microseed Matrix Seeding (MMS)** [3-4]. One of the most powerful methods introduced into protein crystallization in the past 10 years in particular for establishing suitable cocrystallisation or soaking systems for obtaining X-ray structures of inhibitors complexes.

Examples will be given of increased hit rates, elimination of twinning, improved diffraction and promoting different space groups.

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2. The protein as a variable in protein crystallization. D'Arcy A, Mac Sweeney A, Haber A. *Acta Crystallogr D Biol Crystallogr*. 2003 Jul;59(Pt 7):1343-6. Dale GE, Oefner C, D'Arcy A. *J. Struct Biol*. 2003 Apr;142(1):88-97. Review.1;50(Pt 4):469-71.1
3. An automated microseed matrix-screening method for protein crystallization. D'Arcy A, Villard F, Marsh M. *Acta Crystallogr D Biol Crystallogr*. 2007 Apr;63(Pt 4):550-4.
4. Microseed matrix screening for optimization in protein crystallization: what have we learned? D'Arcy A, Bergfors T, Cowan-Jacob SW, Marsh M. *Acta Crystallogr F Struct Biol Commun*. 2014 Sep;70(Pt 9):1117-26.

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