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A process for the rational crystallization of biological macromolecules

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A crystallization method, a microplate and an instrument were developed, allowing for the first time the application of an objective and contact-free evaluation of clear drops as well as a robust evaluation of precipitates within crystallization experiments. Laser light scattering and polarization microscopy are used for an optical characterization of solute or precipitated proteins. Both approaches form the basis of an automated, rational and adaptive crystallization process. The implementation of well-documented and unbiased information derived from thermodynamic solution parameters such as the second virial coefficient (crystallization window) and the early and objective identification of microcrystalline precipitates will probably cause a paradigm shift in the way protein crystallization is approached. The new method is automated and performed using a new microplate and 200 nL droplets of the protein solution.

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Microfluidic chips for parallelized analytical crystallization

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Macromolecular crystallization has traditionally relied upon the selection of a limited set of reagent formulations from within the vast potential reagent space that could result in the growth of diffraction-quality crystals. The number of experiments and resulting reagent space is typically limited by the amount of sample available and the number of reagent formulations that can be prepared for the experiment. Structural biologists increasingly explore variations in the sample (alternative constructs, ligands, substrates, etc.) as a method for increasing the success rates for crystallization of any particular target. Decisions on which of these samples to pursue often rely on the correlation between orthogonal biophysical techniques and the propensity of a sample to crystallize.

The TOPAZ® family of microfluidic screening chips have been designed for multiple samples to be run in parallel against the same 96-solution reagent set using a total of 1.4 ul per sample. The limited sample volume requirements allow TOPAZ chips to be used to generate analytical crystallization data for each of the samples prepared in parallel. Crystallization data from TOPAZ® chips provides a direct readout of the propensity of any given sample or construct to crystallize. When employed early on in the purification process, this information can be used to decide which sample variant to pursue. It also facilitates parallel processing of samples for crystallization early in the structure determination pipeline.

Data will also be presented from studies demonstrating the use of microfluidic liquid diffusion-based crystallization in a more traditional crystallization pipeline. Follow-on translation strategies from initial screening hits will also be described. Data will be presented from projects which have led to the successful determination of structures from TOPAZ® screening hits.