Automation of High-Throughput Combinatorial

Protein MicroCrystallization Trials

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A major bottleneck for protein structure determination using x-ray diffraction is the difficulty in finding conditions for crystallization and optimizing those conditions for the production of high-quality crystals. Hundreds of crystallization trials are typically necessary to find optimized crystallization conditions, but these are limited by the amount of protein sample available. Our goal was to increase the number of crystallization trials while at the same time reducing the amount of protein sample required, all in a high-throughput mode. These design goals suggested automation of all key protocols.

Begun in the spring of 1998 at the request of personnel now at NIFG, the BioInstrumentation Group at LBNL undertook to increase the throughput of protein crystallization trials by an order of magnitude while reducing protein usage per trial by 90%. In order to achieve this goal, two completely automated robotic systems were designed and constructed with initial testing of the completed system in the spring of 1999. Joining a combinatorial approach with parallel processes, protein samples are screened against a coarse array of up to 480 unique solutions at a rate of eight seconds/trial. This compares with a rate of one to two minutes/trial either by commercial robots or manual methods. Along with the increased throughput, protein usage was reduced up to 50-fold where only 20 nanoliters of protein/trial were needed to grow crystals compared to current best practices using one microliter of protein. Based on sub-optimal growth conditions identified by hits from the coarse screen, a second system narrows in on the optimal conditions by synthesizing a new two-dimensional array of solutions out of 72 stock chemicals and new crystallization trials begin. By automating crystal growth trials, effort previously spent on manual trial preparation is freed to work on other aspects of proteomic research.

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