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HIGH THROUGHPUT PROTEIN CRYSTALLOGRAPHY AND CURRENT APPLICATIONS

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During the past few years, progress has been made in developing high throughput technologies for protein cloning, expression, purification, crystallization, crystal imaging, and synchrotron beamline data collection. Recently, we have been able to miniaturize and parrallelize the structural biology processes significantly using nanoliter volume technologies.
Accordingly, significantly smaller amounts of materials can be used at all steps, and more parallel experiments can be engineered (genetic and mechanical) within the same space and time constraints. A description of these technology developments and the current status of throughput will be described. Applications of the technologies towards genomes, pathways, and drug discovery will be included.

Keywords: STRUCTURAL GENOMICS, HIGH-THROUGHPUT

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AUTOMATED STRUCTURE SOLUTION, DENSITY MODIFICATION, AND MODEL BUILDING FOR MAD, SAD AND MIR

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The software SOLVE and RESOLVE can carry out in an automated fashion all the steps in structure solution through model-building in the MAD, SAD, and MIR cases. The use of a scoring scheme to convert the decision-making in macromolecular structure solution to an optimization problem has proven very useful and in many cases a single clear heavy-atom solution can be obtained and used for phasing. Maximum-likelihood density modification is well suited to an automated approach to structure solution because the method is relatively insensitive to choices of numbers of cycles and solvent content. The detection of NCS in heavy-atom sites and checking of potential NCS operations against the electron-density map has proven to be a reliable method for identification of NCS in most cases. Automated model-building beginning with an FFT-based search for helices and sheets has been successful in automated model building for maps with resolution as low as 3 Å.

Keywords: STRUCTURE SOLUTION AUTOMATION MODEL BUILDING

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HIGH-THROUPUT CELL-FREE PROTEIN EXPRESSION SYSTEMS IN STRUCTURAL GENOMICS/PROTEOMICS

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The RIKEN Institute has started the Structural Genomics/Proteomics Initiative (RSGI) (http://www.rsgi.riken.go.jp) at the Genomic Sciences Center (GSC) in Yokohama and the Harima Institute at SPring-8. We are determining the three-dimensional structures of proteins of new sequence families and analyzing molecular and cellular functions of these proteins in order to establish the structure-function relationships. Efficient approaches in related key technologies have made possible to conduct the research in a high-throughput way. Cell-free protein expression is highly suitable for making protein samples.
The system may produce proteins directly from PCR-amplified linear DNA fragments, requiring no cloning procedures. Hundreds of proteins and protein domains can be expressed from cDNA clones within a day. By establishing the dialysis method, production of proteins has been further improved to the level of milligram quantities. The system is useful to assess the solubility and structural stability of proteins and thus practical for selecting protein samples for larger scale production. In addition, it is successful in preparing labeled protein samples, the uniformly [13C, 15N]-labeled proteins for NMR structure determination and selenomethionine substituted proteins for X-ray crystallography. The robotic system has been adapted to advance the automated protein production.
The system accelerates high-throughput structure determinations by both X-ray crystallography and NMR spectroscopy. The NMR facility of GSC has 16 NMR instruments (600 and 800 MHz). High-throughput Factory directed by M. Miyano has been newly established and two beam lines dedicated to structural genomics are being constructed at SPring-8 in Harima.

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VALIDATION FROM AN EDITORIAL VIEWPOINT

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Structure- and data-validation are now well-established processes for small molecule structures published in the IUCr journals; full details of what is involved are available at
http://journals.iucr.org/services/cif/datavalidation.html. The validation process should not be seen as a nuisance or publication stopper but rather as a way of ensuring rapid progress of a structure analysis through the publication path. All submissions to Acta Crystallographica C and E must be prechecked by the authors before submission. Examples of some of the most common problems that are detected at this stage will be shown and discussed. No other journals require such prechecking of crystal structure data before submission. Should we as a community try to ensure that CIFs from crystal structure analyses be put through some form of structure- and data-validation processes before submission? Should this be mandatory for all crystal structure analyses prior to publication? If so, how best to achieve this? Should we be asking all journals to require that the output from data-validation software such as in PLATON (A.L. Spek; http://www.cryst.chem.uu.nl/platon) be included with a submission for referee perusal? Alternatively, should we be building data- and structure-validation software into the data collection and refinement programs so that authors are made aware of potential (and previously undetected) problems well before the submission stage?

Keywords: STRUCTURE VALIDATION DATA VALIDATION CIF