High throughput Synchrotron Radiation Circular Dichroism (HT-SRCD) and Protein Folding Quality Control (PFQC)

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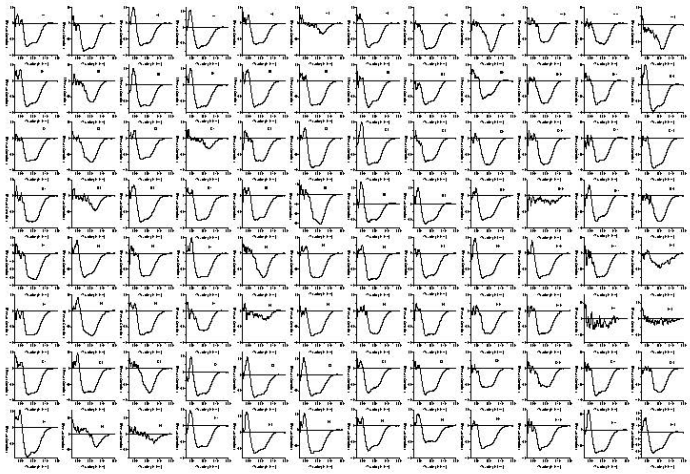
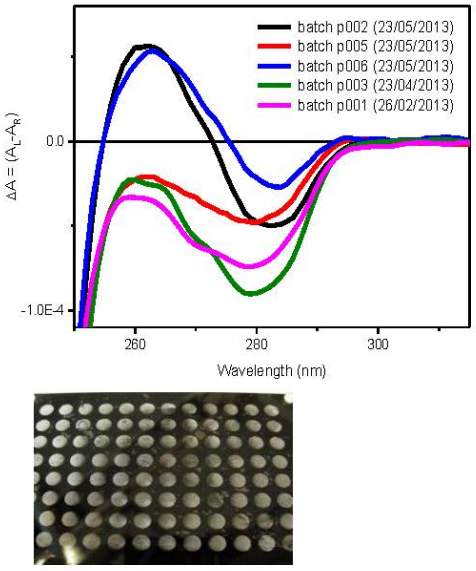
The production of diffraction quality protein crystals for X-ray crystallography has been greatly accelerated by the development of high throughput protein (HTP) methods, which enable a large number of crystallisation conditions to be rapidly investigated. Monitoring sample quality and the effect of crystallisation buffers on protein behaviour in solution should be considered as part of the crystallization experiment. Synchrotron Radiation Circular Dichroism (SRCD) spectroscopy is the ideal technique for these tasks as it can be operated in high throughput (HT) mode using 96- and 384-well multiplates otherwise unattainable with bench-top CD instruments. Ligand binding interaction screening by HT- SRCD can reveal whether the protein function/activity is retained altered or lost under different crystallisation conditions. Also, examples of batch-to-batch variation in the local tertiary structure of aromatic side-chain residues will be discussed. The fact that ligand binding properties were affected by conformational changes illustrates that the characterisation of the folding of recombinant protein batches should not be ignored but on the contrary implemented as an important part of protein quality control.





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