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Two way street – complementary methods

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The 2014 CCP4 Study Weekend, held at the University of Nottingham, covered the subject of complementary methods. While CCP4 meetings generally cycle through the main crystallography software topics of data processing, phasing, molecular replacement and refinement, memorable meetings in the past were also held on topics such as macromolecular complexes (2006) and low-resolution structure determination (2008). The topic of the 2014 meeting reflected the fact that various methods can complement the traditional crystallographic approaches in order to shed light on the dynamics, interactions and higher order structures of the macromolecules on which we work. In addition, these complementary techniques can also assist us in the process of protein structure determination.

The biological systems we study are invariably multi-component and are involved in complex dynamic processes; indeed more than half of the macromolecules deposited in the PDB are oligomeric. In recent years, the extraordinary developments that have taken place in the techniques available in molecular biophysics provide macromolecular crystallographers with a strong driving force to integrate their results into a more complete understanding of the systems under study. Whilst NMR (nuclear magnetic resonance) and EM (electron microscopy) are two of the classic complementary methods, today spectroscopic, scattering, calorimetric and numerous other techniques are commonly used in our laboratories. Likewise, synchrotron beamlines today have advanced from being simple producers of bright X-rays to experimental set-ups with multiple on-line detectors, and synchrotron facilities have become major cross-disciplinary science hubs with a multitude of methods and equipment available. CCP4 is therefore rightly engaging with developers of these important techniques, which augment our work, to fully exploit their potential as tools for the structural biologist.

The first session of the meeting was on biophysics, and was chaired and introduced by Mike Hough (Essex). A fascinating description by Huaying Zhao (NIH, Bethesda) covered new computational methods for combining various biophysical measurements in the analysis of multi-component protein complexes. This was followed by a presentation on the application of several such methods by Ehmke Pohl (Durham) and a review and presentation of software for *in crystallo* spectroscopic techniques by Florian Dworkowski (SLS). Antoine Royant (ESRF) then gave an insightful presentation on the capabilities of the ESRF Cryobench facility for various spectroscopic investigations of protein crystals.

The next session, which was chaired by Edward Snell (Hauptman–Woodward Institute), was concerned with optimal strategies for the collection of solution scattering data – measurements that allow description of protein and other macromolecular assemblies. A presentation from Javier Pérez (Soleil) described the application of these methods to membrane proteins embedded in lipid/detergent. In the subsequent two presentations, Frank Gabel (IBS Grenoble) discussed the uniqueness of model predictions obtained by SAXS techniques and Adam Round (ESRF) described a software tool for automated SAXS data collection and online feedback.

It is evident that the availability and recent developments in these methods challenge the crystal-centred way in which we work, as it is essential to understand *what's in that crystal*. It was therefore logical to address the question of how we presently bring our samples into the X-ray beam. In the session on crystal manipulation, chaired by Frank von Delft (Diamond/Oxford), Alex Soares (BNL) outlined fascinating methods for mounting crystals and simultaneous ligand-screening, involving use of acoustic droplets. Alternative approaches for fully automated crystal mounting were then described by Florent Cipriani (EMBL, Grenoble). Next, Joseph Lyons (Aarhus) described methods for automatically locating microcrystals at the synchrotron beamline and Dianfan Li (Dublin) told us about an application of serial femtosecond crystallography using the free-electron laser to study membrane proteins crystallized in the lipid cubic phase

(LCP). Finally, Robin Owen (Diamond) gave a presentation on room temperature *in situ* data collection. While the session focused on presently available techniques at synchrotron radiation sources, it is clear that free-electron laser applications will change the way in which we look at crystal mounting, as they provide the opportunity to add time-resolution to the crystallographic experiment.

The classic NMR and EM methods were presented on day two of the meeting, and progress on the respective CCPs is covered in this issue. The session on EM was chaired and introduced by Helen Saibil (Birkbeck, London) who also reported on a new facility at the Diamond Light Source, providing 24/7 access to EM modelled on the crystallography BAG system (Block Allocation Group). A fascinating account of the theory and practice of EM studies of heterogeneous macromolecular complexes was presented by Elena Orlova (also Birkbeck). Following this, Werner Kühlbrandt (MPI, Frankfurt) gave an intriguing and wide-ranging lecture on the applications of single-particle imaging, molecular tomography and high-resolution cryo-EM. In his words ('watch out!'), these herald a new phase of EM where we can expect to see a revolution in resolution because of new detector technology, similar to what has happened over the last ten years in the X-ray field with the advent of solid-state detectors and millisecond exposures. Alan Brown (MRC, Cambridge) reported on methods in ribosome structure determination for the interpretation of high-resolution EM data, as the borders between EM and X-ray structures now dissolve and X-ray crystallographic software can be used in EM refinement.

The subsequent session on protein dynamics was chaired by Arwen Pearson (Leeds/Hamburg) and included a presentation by John Christodoulou (UCL) on the unique insights provided by NMR spectroscopy on the dynamics, disorder and meta-stability of proteins. Next up, Fraser MacMillan (UEA) presented a number of applications of electron paramagnetic resonance in studying metalloproteins and spin-labelled macromolecular assemblies. Finally, the methods available to study the molecular dynamics of biomacromolecules were presented by Sarah Harris (Leeds), who demonstrated that these approaches, in the same way as experimental biophysical methods, powerfully complement structure determination.

The final session dealt with a different aspect of providing complementary structural information. Since nearly three quarters of entries in the PDB contain a ligand and indeed the atomic description for many structures could well be incomplete as these most likely contain unknown ligands, we initially thought we might address this, but instead decided to focus on the subject of drug design, which was introduced and chaired by Dave Brown (Kent). Following on from the previous topics on biophysical methods and NMR, the presentation by Glyn Williams (Astex) included the applications of NMR in drug screening. This was followed by a description of approaches for the identification of binding pockets in proteins and ligand screening by Judit Debreczeni (Astra Zeneca). The session concluded with a demonstration of some of the related features of the new CCP4 GUI by Martin Noble (Newcastle).

As organizers, we were aware of the time constraints in a two-day meeting and we can perhaps partially redress the necessary limited selection of topics by touching briefly on a number of other complementary techniques in the structural biologist's armory. One feature of X-ray diffraction is that hydrogen atoms are nearly always poorly defined in the final structure due to the low scattering factor of this element. In contrast, neutrons have a much higher cross section and thus are far more sensitive to hydrogen than X-rays, and the isotope of hydrogen, deuterium, scatters neutrons as strongly as does carbon. Deuterium can be introduced into protein crystals by vapour diffusion or soaking and non-exchangeable hydrogen atoms can be replaced by expressing the protein in deuterated growth media, using techniques which are largely routine in the NMR field. Along with improvements in detectors and pulsed sources, these techniques have allowed neutron diffraction data to be collected from much smaller crystals than ever before, thus shedding important mechanistic light on a number of enzymes. Another technique, which has allowed studies of catalytic mechanisms is the X-ray Laue method, due to the speed with which the data can be collected following initiation of the reaction. Recent advances in this field include the study of laser-triggered reactions where the light pulses can be synchronized with the synchrotron beam by use of single-bunch mode and/or high-speed X-ray beam choppers. Finally, an open question not addressed in the meeting is how we deposit all these complementary data with our structure.

We would like to wholeheartedly compliment the speakers and chairs for their excellent contributions to all sessions of the 2014 Study Weekend. We especially thank those who contributed papers to the current journal issue, which, we hope, constitutes an informative record of a very memorable meeting. We also thank the CCP4 administrative staff (Karen McIntyre, Carol Malpass and Shirley Miller) for their pivotal support in the organization of the meeting.