Overview of the 13th International Conference on the Crystallization of Biological Macromolecules

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Overview of the 13th International Conference on the Crystallization of Biological Macromolecules

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ABSTRACT: The 13th International Conference on the Crystallization of Biological Macromolecules, held at Trinity College Dublin, brought together leading experts, experienced researchers, and newcomers to the field of macromolecular crystallization. Challenges pertaining to the crystallization of a wide variety of biological macromolecules were discussed in depth, with a special focus on the strategies undertaken to tackle membrane proteins. A crystallization workshop preceded the event and allowed 36 participants hands-on experience with current methodologies used in macromolecular crystallization. This article serves as an overview of the workshop and conference.

INTRODUCTION

From September 10−16, 2010, Trinity College Dublin (TCD) was the host for the 13th International Conference on the Crystallization of Biological Macromolecules (ICCBM13, http://www.iccbm13.ie). This is a biannual meeting organized on behalf of the International Organization for Biological Crystallization (IOBCr, http://www.iobcr.org), which focuses on one of the most challenging steps in structural biology: the production of structure-grade crystals of macromolecules for X-ray diffraction studies. ICCBM13 explored old and new crystallization methods, covered the recent success of brute force approaches, pipeline methodologies, crystal identification, and state-of-the-art data collection at synchrotron radiation facilities. Recent advances in the crystallization of macromolecular complexes and membrane proteins received particular attention. The four-day conference consisted of 49 talks, with four morning and three afternoon sessions each comprising of 7 talks. The 49 speakers included two Nobel laureates, world leaders in the field of structural biology and crystallization, and a selection of graduate students and postdoctoral research fellows. Poster presentations were an important part of the meeting, and 136 posters were available for viewing throughout the conference. Speaker sessions were short, allowing for longer intermissions to facilitate interaction, networking, and discussion as well as more leisurely poster and exhibit viewing. Daily lunchtime bites included PDBe (http://www.ebi.ac.uk/pdbe) presentations by Gaurav Sahni[1,2] and demonstrations of laboratory information management systems, PiMS-LIMS by Chris Morris (http://www.pims-lims.org),[3] and LIMS (Labdb) by Wladek Minor.[4,5]

There were 319 registered delegates and the major groups were senior academics (33%), postdoctoral researchers (18%), and students (25%). Industry participation was high, totalling 40 participants; additionally there were 37 exhibitor delegates. Contributions from Science Foundation Ireland (SFI) and primary industry sponsor, Formulatrix, allowed funding for 18 scholarship awards.

For those not able to attend ICCBM13, Ireland's National Education and Research Network, HEAnet (http://www.heanet.ie), provided equipment and training to enable a live webcast of the workshop and conference presentations. The three-day workshop morning talks logged a total of 243 unique online viewers with an average of 10 viewers per talk and the four-day conference talks had a total of 1,113 unique online viewers with an average of 22 viewers per talk and peaked, at times, at 50 viewers. Most overseas viewers were from the UK (18%), USA (14%), and Germany (13.5%).

WORKSHOP

ICCBM13 was preceded by a three-day crystallization workshop that consisted of lectures and hands-on demonstrations where participants were exposed to both the theoretical and practical considerations in protein crystallization and crystal handling.[6−8] The workshop was held in the Membrane Structural and Functional Biology Group laboratories at TCD (http://www.caffreylabs.tcd.ie) and was primarily sponsored by Rigaku with donations, financial and/or materials, from

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Anachem, Anachem Instruments, Carl Zeiss, Centeo Biosciences, Emerald Biosciences, Hampton Research, Molecular Dimensions, Triana Science and Technology, and TTP Labtech.

The workshop was limited to 36 students and taught by instructors who themselves are leading practitioners in their respective fields. Among the participants, eight scholarships were awarded by the ICCBM13 organizing committee. These bursaries provided gratis conference registration with select candidates also receiving gratis workshop registration and/or travel expenses via financial support of our academic, nonprofit, and industrial sponsors.

Lectures started with an introduction to the biological macromolecule's solubility curve (phase diagram) and its relevance to crystallization, before expanding to a detailed discussion of the different crystallization methodologies explained in the context of the phase diagram (Jose A. Gavira, Jeroen Mesters, Lata Govada, and Sahir Khurshid). Time and again, during the lectures, students were reminded of the importance of the quality (monodispersity, purity, activity and stability) of the biological macromolecule of interest and knowledge of its biochemical and biophysical properties (Christian Betzel). Furthermore, careful analysis and interpretation of crystallization drop phenomena, both visually and through fluorescence labeling (Terese Bergfors and Marc Pusey),[8,9] and their relationship to the target's phase diagram as tools in crystal optimization were explored. The pros and cons of the application of a variety of seeding techniques, from streak seeding, macro- and microseeding approaches, to the use of homogeneous and heterogeneous nucleants were discussed during the lectures (Terese Bergfors, http://xray.bmc.uu.se/terese/tutorials.html. Convection and its effects on crystallization were addressed during the microgravity and counter-diffusion lecture (Jose A. Gavira).[6] Introduction to methodologies designed and optimized for the crystallization of integral membrane proteins using bicelles and lipidic mesophases (Martin Caffrey) rounded out the crystallization talks.[11−13] Presentations on the cryoprotection and cooling of macromolecular crystals (Leif Hanson),[7] as well as addressing the susceptibility of biological samples to radiation damage during data collection (Elspeth Garman), concluded the workshop.[14]

The hands-on practical sessions included conventional vapor diffusion (Jeroen Mesters), microbatch (Lata Govada and Sahir Khurshid), seeding techniques (Terese Bergfors), counter-diffusion and microgravity experiments (Jose A. Gavira), and the *in meso* (lipidic cubic phase) method (Dianfan Li, Nicole Höfer, and Martin Caffrey).[11−13,15−17] Crystal handling and cryocooling practicals were led by Leif Hanson and Elspeth Garman. Demonstrations of protein characterization by dynamic light scattering (Christian Betzel and Karsten Dierks) coupled with industrial presentations on high-throughput crystallization technologies (Anachem Instruments and TTP Labtech) and temperature control systems (Centeo Biosciences) rounded out the workshop.

CONFERENCE

Historical Perspective. A small number of presentations were dedicated to historical perspectives on two challenging systems, once thought to be intractable, the ribosome and membrane proteins.

The Ribosome. Peter Moore described the huge body of work carried out to glean three-dimensional (3D) structural information on the ribosome before it was certain that a crystal structure would ever be elucidated.[18] Analytical ultracentrifugation allowed for the isolation of the ribosome and distinction between large and small subunits. Small-angle neutron scattering led to the first "balls model" of the small subunit, and negative stain electron microscopy resulted in low resolution models of the large and small ribosomal subunits. Biochemical studies allowed for an inventory or "parts list" for each subunit. It was concluded, in light of the crystal structures, that the results from some approaches were beneficial to our understanding of the ribosome, while other experiments were not terribly informative.

Ada Yonath (Nobel laureate, 2009, chemistry) described the long and challenging route to the ribosome crystal structures.[19] It was thought that ribosomes would never be crystallized due to their chemical complexity, heterogeneity, flexible, mobile and asymmetric nature, large size, and instability. However, the observation that hibernating bears pack their ribosomes in an orderly fashion on the inner side of their cell membrane, perhaps to retain integrity and function, suggested that ribosomes could be crystallized, and indeed they were. Crystals of the ribosome decayed quickly when exposed to synchrotron radiation, hampering data collection. To combat radiation sensitivity cryocrystallography was pioneered; this technique has since developed and revolutionized data collection.

Membrane Proteins. Hartmut Michel (Nobel laureate, 1988, chemistry) presented a historical account of membrane protein crystallization. From the textbook opinion in 1976 (Stryer) that it was "impossible to crystallize membrane proteins" initial encouragement was gained from the electron diffraction studies on bacteriorhodopsin.[20] Knowledge of membrane protein's polar and hydrophobic surfaces, detergent size, and the concept of adding small amphiphiles to form mixed micelles, that could promote the formation of crystal lattices, led to the first membrane protein crystals and structure.[21] A number of other membrane proteins were then pursued, and neutron scattering was used later to examine micelle packing. The use of fragments of monoclonal antibodies to promote protein−protein interactions for crystal formation led to more successes. Recent developments of note in membrane protein crystallization were highlighted and included the use of the lipidic cubic and sponge phases, and bicelles.[22,23]

Irish Crystallographers. Tribute was paid to two Irish crystallographers, Kathleen Lonsdale and John Desmond Bernal, both of whom were enormously influential in laying the foundations of crystallography.

Dame Louise Johnson gave an exquisite account of Kathleen Lonsdale, from her birth in 1903 in County Kildare through to her many endeavors, achievements, and esteemed colleagues, such as William H. Bragg, William Astbury, J. D. Bernal, and Arthur Lindo Patterson. Kathleen Lonsdale solved the crystal structures of hexamethylbenzene and hexachlorobenzene, made fundamental contributions to the International Tables of Crystallography in the form of mathematical crystallography and space group theory, and was one of the first women to be elected to the Royal Society (UK). In addition to her scientific prowess, Kathleen Lonsdale was a Quaker and peace activist and was concerned with the plight of women in prison.

Declan Doyle talked about J. D. Bernal, an inspirational Irish scientist, born in County Tipperary in 1901. Bernal worked with William H. Bragg and determined the structure of graphite. From there, Bernal worked on many different tasks with the common theme of "form and function". The first protein diffraction pattern by X-rays was taken by Bernal and Dorothy Crowfoot (Hodgkin) after working out how to keep the crystal hydrated. Bernal was the founder of the Crystallography Department at Birkbeck College, London, and also laid the foundations for

Social Science and Operational Research. Bernal worked tirelessly for peace and encouraged people to work on difficult problems.

Protein Crystallization. The growth of macromolecular crystals suitable for structural studies is not always a straightforward task; it usually requires extensive screening at many levels. Different approaches are often sought in parallel to achieve and optimize crystals for structural studies.

Construct Design. One of the most important variables in screening for protein crystal growth is construct design, and a number of presentations highlighted this to great effect. Annie Hassell described the various approaches used at GlaxoSmithKline to study kinases. Full length and truncated versions were screened in parallel with construct design based on homology modeling. This was generally complemented with flexible loop and surface mutations to counteract protein aggregation and promote crystal contacts. In the most difficult cases, surrogate kinases had their active sites mutated to the target sequence of interest. Ligands were screened against targets, with and without their purification tags, during protein expression and purification to promote stabilization. Investigations with heterogeneous seeding, by seeding from a mixed kinase seed stock, were also employed to generate crystals. Zygmunt Derewenda focused on what the amino acid sequence can tell us about the crystallizability of the protein, for which the server http://ffas.burnham.org/XtalPred-cgi/xtal.pl can be used.[24,25] The strategy of using a series of point mutations (generally to Ala, Ser, or Thr) in order to provide more suitable surface patches for protein−protein interactions in a crystal lattice was discussed. Suitable sites for these mutations can be predicted by the surface entropy reduction (SER) server, http://services.mbi.ucla.edu/SER. Chris Tate described strategies for working with G protein-coupled receptors (GPCRs). Focusing on protein engineering, protein thermostability was optimized by making site-directed mutations for every residue in the protein (Ala scanning). For each mutant the stability was assessed by ligand binding, temperature stability, and total expressed receptor, with subsequent combinations of mutations evaluated to make a construct that was optimally thermostable.[26−28]

Promoting Interactions for Crystal Formation. Alex McPherson gave an overview of protein crystallization and described what can be done to enhance intermolecular interactions by either introducing small molecules that can act as nucleants and allow for additional intermolecular interactions, or by modifying amino acid side chains using traditional protein chemistry.[29] Catarina Coelho described her success with using ionic liquids to produce diffraction quality crystals.[30] This work was initiated when problems were encountered in scaling up the drop volume from nanodrops, set up using a crystallization robot, to manually dispensed 2 μL drops. Catarina concluded that combinations of ions and cations, and their concentrations, can be screened and used as additives in crystallization. Markus Grütter introduced design ankyrin repeat protein (DARPin) technology, which screens DARPin libraries for their potential to act as crystallization chaperones.[31,32] DARPins are small modular proteins, exceedingly stable and cysteine-free, and can be recombinantly expressed in high yields.[31−36] DARPins are an alternative to antibody fragments, for both soluble and membrane protein targets, and the in vitro selection of several binders from the libraries takes 6−8 weeks (http://www.p-cube.eu). Vadim Klenchin talked about the use of coiled coil stoppers as an aid to protein expression and crystallization and their use to help phase the structure of interest.[37] Jie-Oh Lee described the use of leucine rich repeat (LRR) proteins to help crystallize toll-like receptors (TLR).[38] The hybrid LRR technique involved screeing of TLRs and hagfish variable lymphocyte receptors (VLR) fusions for crystallization. The use of camelid nanobodies, as an alternative to more traditional antibodies, was addressed by Brian Kobilka and is summarized below in the Crystallizing Membrane Proteins section of this overview.

Additional Methods and Technologies. Hiroyoshi Matsumura described the practicalities and benefits of solution stirring to improve crystal growth and summarized a number of successes with soluble and membrane proteins and protein−RNA complexes for structural studies using X-ray and neutron diffraction. In addition, a floating and stirring technique (FAST) which increases the growth rate of crystals and a top seeding solution growth technique (TSSG) to prevent polycrystallization were both described.[39,40] Marc Pusey talked about the fluorescence analysis of crystallization technologies (FACTs) approach to improve interpretation of crystallization screens and allow for efficient optimization.[8] Paul Kenis presented the design, development, and fabrication of microfluidic platforms for crystallization of proteins to allow for in situ analysis (X-ray data collection and spectroscopy).[41−43] A chip developed for mixing the lipidic cubic phase with applications in membrane protein crystallization and lipid phase diagram determination was introduced. Vassiliy Lubchenko talked about the theoretical understanding of anomalous mesoscopic phases in protein solutions, with the aim of being able to control protein aggregation.[44] He used dynamic light scattering to observe mesoscopic clusters and their formation and behavior in a number of different environments. Nancy Campbell described working with G-quadruplexes. Here, the importance of high purity and choice of flanking nucleotides was highlighted.[45,46] Claude Sauter gave an overview of the strategies for the crystallization of viruses using the example of grapevine fanleaf virus and a transmission defective mutant.[47,48]

Crystallizing Membrane Proteins. The difficulties in crystallizing a membrane protein, as evident from the relatively small number of known structures in the PDB (http://www.pdb.org,[49] http://www.mpdb.tcd.ie),[50] were addressed. A historical overview of membrane protein crystallization was presented by Hartmut Michel and is summarized in the Historical Perspective section of this overview.

General Approaches. Christine Ziegler opened the conference with a talk on the challenges that needed to be overcome in solving the structure of the betaine symporter in different conformations.[51,52] She highlighted the iterative nature of the crystal optimization process that progresses through the screening of lipid, detergents, and constructs. Interesting points included a temperature-dependent effect of detergents on the oligomeric state of the protein, as monitored by size exclusion chromatography. Bob Stroud addressed the practical aspects of membrane protein expression, isolation, and purification. From his experience of working with eukaryotic membrane protein targets, he stressed the need to use an expression system that is the best match to the organism from which the target comes, such as yeast, insect, or mammalian cells. Control of the extent and nature of the post-translational modifications are often key to successful crystallization endeavors. In the context of purification, he echoed many other talks in which lipid add-back or augmentation and detergent exchange before crystallization were highlighted as being important.[53−56] J. Preben Morth spoke, in general, about membrane protein crystallization and methods to promote the generation of high quality crystals for structure determination.[57−59] By modifying approaches used in

two-dimensional (2D) crystallization, they have been successful in developing a methodology, termed HiLiDe, to systematically screen the addition of extra lipid and the amount and type of detergent(s) to achieve diffraction quality crystals.[60−62] Observations on the handling of plate-like crystals were also made.

Carola Hunte presented her work on the crystallization of multisubunit membrane protein complexes, namely, the mitochondrial Complex I and cytochrome bc_1 complex from yeast.[63−65] Strategies to promote better membrane protein crystal quality were explored with the use of antibodies, and the importance of screening lipids and detergents was noted. Michael Oldham described his work on understanding the transport cycle of the *Escherichia coli* maltose ABC transporter. In his talk, Michael brought the audience through the process of structure determination of three different conformations of this transporter.[66,67] As the protein expressed well and crystallized easily, the challenging step was to optimize the crystals to achieve diffraction beyond 7−8 Å resolution. It was important to replace the large extramembranal loops present in this protein with shorter linkers to promote stability. Important criteria for detergent selection was the attainment of a monodisperse sample, as evaluated by following the green fluorescent protein tagged target on size exclusion chromatography, and the ability to concentrate the protein solution to ≥3 mg/mL. The combination of the right detergent, at the right concentration, and shorter protein loops was key to obtaining the high resolution structures.

The title "Blood, Sweat and Tears" summarized the efforts of Jeff Abramson and his group in their adventure to elucidate the structure of the sodium/galactose symporter.[68] From the initial 8 Å diffraction images, crystals were improved through iterative cycles of small molecule and detergent screening. Challenges in the experimental phasing were overcome through screening of cysteine mutations at the predicted ends of the transmembrane regions to promote heavy atom binding, finally resulting in the initial Patterson map. Jeff talked about the hurdles that they, like so many other groups, faced in the structure solution of a membrane protein, where a single structure can take many years to bring to completion.

High Throughput Approach. Michael Malkowski gave a historical perspective on the design of membrane protein crystallization screens from 1997 to the present day. He explained how, using a hydrophobic dye added to precipitant cocktails, it was possible to identify phase separation (the hydrophobic dye going to the detergent-rich phase). These observations allowed for the visual mapping of the phase boundary for ternary mixtures of PEG, salt, and detergent. Finally, he described several test cases with high success rate where initial detergent phase boundary determination was key to obtaining crystals. Phase boundary data for more than a 1,000 precipitant cocktails is publicly available online via a Slickspot spreadsheet at http://www.hwi.buffalo.edu.[69]

In Meso Method. Recent advances and expansions in the *in meso* method, also known as the lipidic cubic phase (LCP) method, were addressed in a number of talks. Joseph Lyons highlighted the benefit of using rational design of the cubic phase microstructure through the synthesis of a short chain monoacylglycerol in his efforts to crystallize and solve the structure of the caa_3-type cytochrome *c* oxidase. This was later expanded on by Nicole Höfer, who used a number of synthetic monoacylglycerols, which screened bilayer thicknesses, in her work on the crystallization of a transmembrane peptide in the lipidic cubic phase. Wei Liu spoke about complementary techniques such as fluorescence recovery after photobleaching (LCP-FRAP), that can be used to monitor the diffusion of membrane proteins, in this case GPCRs, reconstituted into the lipid bilayer of the LCP and how diffusion characteristics can be used to guide crystallization efforts.[70−72] Two other talks about crystal detection in situ (Garth Simpson) and about the location and centering (David Aragão) of crystals grown *in meso* are described in the Data Collection, Beamlines and Crystal Detection section of this overview.

G-Protein Coupled Receptors (GPCRs). Brian Kobilka talked about the approaches his group has used to stabilize GPCRs in different states using antibody/nanobody-GPCR complexes and an engineered T4 lysozyme-receptor chimera.[73−79] He stressed the importance of employing a multitude of crystallization techniques to obtain diffraction quality crystals for this class of protein, which included classical in surfo, bicelle, and *in meso* methodologies. Chris Tate showed data suggesting that the thermostabilization approach, as described in the Protein Crystallization − Construct Design section of this overview, could be applied to other classes of membrane proteins such as transporters. Gebhard Schertler went on to describe the success of the latter strategy in receptor thermostabilization combined with both vapor diffusion and lipidic cubic phase crystallization for structure determination. The development of a new robot for in meso crystallization was also described (TTP LabTech's mosquito LCP). Gebhard ended with details of their recent advances in trapping a receptor in an active state.[80,81]

Structural Genomics Pipelines/High Throughput. There were three presentations from the Structural Genomics Consortium. Frank von Delft (Oxford) discussed the importance of protein concentration in crystallization trials.[82] Both Frank and Pär Nordlund (Stockholm) communicated the importance of changing the protein for crystallization trials,[83] either by screening different constructs, ligands/substrates, and/or different tags. Pär further described their multiconstruct strategy and the use of in situ proteolysis. Colony filtration blots were shown to be most efficient in selecting targets that expressed well from a library of constructs.[84] The use of isothermal titration calorimetry to measure the affinity between the target protein and substrates/ligands to select the best combination for crystallization was highlighted. Liz Carpenter (Oxford) focused on membrane proteins and highlighted the practical differences when working with membrane, as opposed to soluble, proteins. A broad spectrum of integral membrane proteins were being targeted, with multiple constructs for each. Purified proteins were assessed by mass spectrometry, gel filtration, SDS−PAGE, thermostability, and activity assays. The crystallization success rate was reported as 1.5% in the past 12 months.[85] Stephen Burley presented the high throughput pipeline, from cloning to structure, in operation at Eli Lilly. 977 PDB entries have been the result of this approach, with 786 of these submitted in the last 5 years. This represents a 20% success rate from the target stage.

Edward Snell gave an overview of the high-throughput crystallization screening facility at the Hauptman-Woodward Medical Institute.[86−89] Focus was placed on chemical space mapping, cryocooling characterization, UV imaging, and differential scanning fluorimetry. The success rate with their protein structure initiative (PSI) partners was 22%. Samples submitted by the PSI were also characterized by small-angle X-ray scattering in order to gain insight into the folded state and size of the protein in solution and structural information in the form of low resolution envelopes.[90] Currently, all information that has been generated in terms of image analysis and chemical conditions is being investigated in order to facilitate data and pattern mining.

Bernhard Rupp presented an overview of what has been learned from the high throughput protein crystallography initiative. The benefits include instrument and method development, remote data collection at synchrotrons, and advances in high throughput crystallization screening and monitoring.

Data Collection, Beamlines, and Crystal Detection. *Data Collection and Beamlines.* Important developments in software and instrumentation are ongoing at synchrotron radiation sources worldwide. Clemens Schulze-Briese presented the advances at the Swiss Light Source for in situ diffraction screening,[91] and the new window of opportunity that the PILATUS 6 M detector has opened with its shutter-free data acquisition modes and ultrafine phi slicing.[92−94] The self-proclaimed "MAD scientist", James Holton, gave a very educational talk about crystal quality and how this affects the data collected.[95,96] He outlined the reasons why crystals show internal disorder and how this is reflected in the diffraction pattern. The effects on data quality arising from osmotic shock and stress created during crystal harvesting were illustrated through examples. Dave Stuart and Bob Fischetti presented the recent developments at the Diamond Light Source and at GM/CA-CAT at the Advanced Photon Source (APS), respectively. Dave focused on the need for vaccines for foot and mouth disease and the contribution that microbeam stations have, and will have in the future, in understanding the molecular basis of this disease. He also presented the hardware now available at the I24 microfocus beamline which includes the PILATUS 6 M detector.[97,98] Bob described the substantial work done by the team at GM/CA-CAT in producing micrometer-sized beams using collimators.[99] He reported on a study showing that radiation damage extends to 4 μm beyond the spot of irradiation by 18.5 keV X-rays. When using smaller beams (0.84 μm compared with 15.6 μm) a 3-fold reduction in radiation damage was noted for a given dose.[100] Finally, he presented recent software developments for beamline control with an interface totally rewritten in Java and the implications on the current diffraction rastering techniques available at beamlines 23ID-B and 23ID-D.[101]

Crystal Detection. David Aragão introduced the use of X-ray diffraction and fluorescence, as implemented at GM/CA-CAT (APS), as a means to raster cryocooled mesophase containing loops in order to locate membrane protein crystals. He described practical aspects of how to center crystals that are not visible by eye.[102−104] Garth Simpson showed how second order nonlinear optical imaging can be used to locate chiral crystals (SONICC).[105] He described examples of where SONICC performed at least as well as, if not better than, standard imaging using bright-field, birefringence, UV-excited fluorescence, or trace fluorescence labeling. The importance of this method was tested in situ on GPCR crystals grown in meso. Plans for having a SONICC system available at beamlines for use in locating crystals in cryocooled loops were described. Wladek Minor highlighted the value of protein crystals by suggesting they were a more expensive entity, by volume, than the Star of Africa, one of prize possessions in the British Crown Jewels. Strategies in obtaining the best quality data from protein crystals were discussed. Wladek presented the strengths of the program HKL2000 for indexing and the implications it has on efficient data collection. He showed the novel features of the HKL3000 suite which covers all aspects of structure determination.[106]

Complementary Methods with Crystals. In order to make the most of your protein crystal, Arwen Pearson discussed the use of single crystal spectroscopy (UV−visible, fluorescence, and resonant Raman) as a complement to X-ray diffraction. Single crystal spectroscopy can provide information on, and allow investigation of, enzyme kinetics and redox state through freeze-trapping the crystal in discrete intermediate states.[107] The practicalities of aligning the spectrometer and the availability of this technique at a number of synchrotrons around the world were discussed.[108]

Three methods were presented with the aim of gaining structural information from nanocrystals, which would currently be deemed too small for single crystal X-ray diffraction. Irene Margiolaki described the application of powder diffraction to protein nanocrystals, from well-packed nanocrystalline samples in a capillary, in order to determine protein structure. Fifteen proteins have been characterized to date using this technique.[109−112] A combination of methods and software designed for single crystal and powder diffraction have allowed structures to be phased by molecular replacement and single/multiple isomorphous replacement techniques and refined using Rietveld refinement. Jan Pieter Abrahams discussed the use of electron diffraction with crystals that are too small for structure determination using X-ray diffraction.[113] The use of human hair as a universal nucleant was described as a way to grow nanocrystals suitable for electron diffraction.[114,115] And finally, John Spence presented the use of the world's first X-ray laser powerful enough to look at nanocrystals of membrane proteins, working toward snapshot chemistry, dynamic studies, and molecular movies.[116] This method uses femtosecond X-ray pulses on a stream of droplets containing nanocrystals, eliminating the need for cryoprotectant. New approaches to solving the phase problem using data from this technology were also discussed.[117−119]

In the absence of crystals, Charles Sanders gave an overview of the application of nuclear magnetic resonance (NMR) for protein structure determination with a focus on membrane proteins. High field magnets (700 MHz and higher) and perdeuteration of all nonexchangeable protons were required. Detergents/lysophospholipids were screened in terms of how they affected enzyme activity and the use of bicelles was discussed for solution NMR. In order to add cholesterol into the sample, two approaches were described, first by using a tetra-saccharide linked variant and second by introducing cholesterol at the protein expression stage.[120,121]

■ PRIZES

The prize for best presentation selected from submitted abstracts was awarded to Joseph Lyons. He described the expansion of the *in meso* method through lipid rational design and its use in the crystallization of a caa_3-type cytochrome *c* oxidase. There were eight poster prizes, awarded for work on producing and crystallizing an ATP synthase and its rotor ring (Doreen Matthies),[122] mutagenesis to further stabilize GPCRs (Jennifer Miller), mutagenesis and macro-seeding as tools to help crystallize tyrosine kinases (ChiehYing Chang),[123] the PEG, salt, and glycerol (PSG) system of optimizing protein crystallization (Laura Vera),[124] protein crystallization in high-concentration agarose gels (Shigery Sugiyama),[40] a study on the comparison of crystallization in microgravity environments and use of gels (E. Melero-Garcia),[125] the coupling of counter-diffusion and microseeding for crystal growth (Maria A. Hernandez),[6] and an investigation of crystal growth using microfluidic dynamic light scattering (Aaron Streets).[126] The IOBCr prize for innovation went to Sudipto Guha for his poster on X-ray compatible microfluidic platforms for *in meso* membrane protein crystallization.

FINAL REMARKS

During the meeting, the new president of the IOBCr was named as Abel Moreno Cárcamo from the Instituto de Quimica, Mexico. Abel succeeds Naomi Chayen from Imperial College London, UK. The next ICCBM meeting, ICCBM14 (http://iccbm14.org), will take place in Huntsville, Alabama, USA, where Joe Ng and Marc Pusey will be at the helm. This ICCBM13 virtual special issue of *Crystal Growth & Design* has attracted a number of contributions,[6−8,16−18,22,29,39,48,60,71,87,91,115,123,124,127−133] in addition to this overview. Lastly, there was a small "profit" from ICCBM13, which has been used to cover the first annual subscription for Ireland's affiliation with the International Union of Crystallography (IUCr).

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