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Homage to Prof. M.G. Replacement: A Celebration of Structural Biology at Purdue University

Meeting Review

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Summary

On a glorious spring day in the American Midwest, friends, colleagues, collaborators, and alumni of Prof. M.G. Replacement gathered together at the campus of Purdue University, West Lafayette, Indiana to celebrate 40 years of structural biology and honor the man behind it all: M.G. Rossmann. The date also corresponded approximately to MGR's 75th birthday. It was a memorable occasion for several reasons. An earlier meeting 10 years ago did also render homage to Michael (New Directions in Protein-Structure Relationships: Symposium in Honor of Professor M.G. Rossmann's 65th Birthday, Purdue University, October 21, 1995), but on this occasion the symposium was much more encompassing of structural biology and had a more global character. A large number of featured speakers presented and discussed advances in vast areas of structural biology and came from the four corners of the world to share their work with the new generations of structural biologists currently being trained at Purdue University.

Among the attendees (Figure 1) at the symposium were a large percentage of earlier alumni from MGR's laboratory and from the extended structural biology faculty at Purdue as well as long time collaborators such as Helen Berman (Director of the PDB) and the distinguished virologist Eckard Wimmer (SUNY, Stony Brook, NY), among others.

After the brief opening comments by Jeff Vitter (Dean) and Richard Kuhn (Department Chair), the symposium was organized in morning and afternoon sessions. The morning session was chaired by Jeff Bolin (Associate Dean of Research) and focused on proteins and complex assemblies.

Dale Wigley (London Research Institute, London, UK), an earlier visiting scientist in MGR's lab, discussed in detail the structure of helicases, focusing on the quaternary complex of RecBCD bound to a DNA hairpin duplex. RecBCD is a multifunctional enzyme complex that processes DNA ends resulting from a double-strand break. It is a bipolar helicase that splits the duplex into its two component strands and digests them until it encounters a recombinant hotspot or Chi site. The structure showed intriguing features that provided structural clues on how the complex is able to (1) spread open the two strands of DNA resulting in a fork by a prominent pin and (2) feed the two separate ssDNA strands into separate tunnels that emerge next to the nuclease domain of RecB. The structure also suggested mechanisms for the regulation of such a complex process (Singleton et al., 2004).

Andrew Leslie (MRC, Cambridge, UK), a former postdoc and member of the virus group in the late 1970's, reviewed the main structural and functional features of the multimeric structure of the ATPase unveiled in the last 10 years (Abrahams et al., 1994; Stock et al., 1999), combining structural data with the results of the dynamic fluorescence studies of Yoshida and coworkers in Japan, who were able to visualize the rotation of the "rotor" element by attaching a fluorescence probe (Noji et al., 1997). The structural work on this critical enzyme of the energy-converting machinery in the cell has added a new perspective to the field of nanomachines and molecular motors. He concluded by reviewing his most recent results on the vacuolar type, sodium-pumping ATPase (V-Type Na^+-ATPase) of bacteria (Murata et al., 2005).

After an animated coffee break, Anders Liljas (University of Lund, Sweden), one of the postdocs in Michael's lab during the heroic years of LDH (lactate dehydrogenase), addressed one of the fundamental questions of protein synthesis: How is translocation catalyzed in the ribosome? Structures at high resolution of elongation factors EF-G and EF-Tu complexed with GDP or analogs (GDPNP) as well as specific mutants (e.g., T84A) are providing valuable information, but not enough to provide a complete picture. He pointed out the limitations of the structures of the so-called complete ribosomes, indicating that L1 and L11 are missing from them, and showed how the ^{15}N-labeled NMR spectra of 70S *E. coli* ribosomes can help to locate the L7/L12 components in the structure of the intact ribosome. He suggested a more integrated, five-pronged approach where X-ray, NMR, cryoEM, theoretical (computational) chemistry, and physical chemistry would be needed to understand the most complex problems in molecular biology. The most complete developments in understanding biological processes at the molecular level have been discussed in a recent symposium (Von Heijne and Liljas, 2005), and Anders has reviewed the structural aspects of protein synthesis in a recent book (Liljas, 2004).

Tomitake Tsukihara (Osaka University, Japan), a former member of the group that solved southern bean mosaic virus (SBMV) in the late 1970s, presented the results that the group in Japan has obtained during the last decade on the structure and reaction mechanism of cytochrome c oxidase. This key enzyme of the aerobic metabolism in living organisms catalyzes the reduction of molecular oxygen to water with the concomitant extrusion of four protons from the intramitochondrial compartment:

$$4H^+ + 4e^- + O_2 \rightarrow 2H_2O + nH^+$$

The meticulous combination of point mutations and detailed structural work on these mutants has identified some of the most critical residues in the reaction mech-

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anism (i.e., Asp51, the peptide bond across Tyr440-Ser441, His503) of the oxidase. Tomitake discussed the structural evidence supporting the controversial proton transfer across the peptide bond and the electron transfer path from the carbonyl of Ser441 to heme a3 and through the helical bulge H378-F377-H376 to the outside. The high-resolution structures (1.8/1.9 Å in the fully oxidized/reduced states) showed that the net positive charge created upon oxidation of the low-spin heme of the enzyme drives the active proton transport from the interior of the mitochondria to Asp51 on the enzyme surface. During the reverse reaction, enzyme reduction induces proton ejection from the aspartate to the mitochondrial exterior. Given the sheer size of the oxidase (13 subunits), and the complexity of the reaction catalyzed by this enzyme, one is constantly reminded of the subtlety of the conformational changes required to transfer protons or electrons by such a large multimeric enzyme across the mitochondrial membrane (Tsukihara et al., 1996, 2003).

In perfect consonance with the spirit and intensity of the honoree, the organizers allowed the attendees only a brief break for lunch packed with anecdotes and recollections from a few persons whose lives intersected with Prof. M.G. Replacement at critical times in their careers. Over a background of images of the French Alps and MGR in full hiking gear provided by Janet Smith, Don Bilderback (CHESS, Cornell High Energy Synchrotron Source, NY) reviewed the impact that the crystallographic work on rhino virus played in the development of synchrotron radiation for virus crystallography first, and later for other biological applications at CHESS. Alwyn Jones (Institute of Cell and Molecular Biology, Uppsala, Sweden), in his inimitable style, captured the audience with his recollections of the impact of the early days of computer graphics on macromolecular crystallography. He visited Purdue in 1980 with the special mission of adapting FRODO to the then-new MMSX hardware, which proved to be full of challenges, not the least of those was to survive MGR's anxiety about the entire project.

It was a very special moment when Sharon Wilder, MGR's personal assistant and factotum, told the story of how she found her way to Michael's office for her job interview. The rest is history. Outsiders to the laboratory will probably never realize what a unique role Sharon has played in the field. Anonymous, unassuming, but always extremely competent and knowledgeable in a myriad of tasks, she always orchestrated the routine of the Purdue laboratory with a gentle but effective harmony. She is always a bottomless source of information, such as references, history, and personal references to name a few, and also, I am most certain, of the current state of MGR's grant finances. The audience recognized her unique contribution when at the end of her brief discourse they gave her a prolonged standing ovation. By taking care of so many odd jobs for MGR, she let him concentrate on the important tasks and revolutionize the field. This is indeed a major contribution.

John E. ("Jack") Johnson (Scripps Research Institute, California), a close associate of Michael's for many years, concluded the lunch period with some candid anecdotes of his time at Purdue, from the late days of the dehydrogenases (GPDH and LDH) to his impact on the critical work on the first virus solved at Purdue, southern bean mosaic virus (SBMV). The methods developed during those years are now mainstream in the area of virus crystallography.

The afternoon session was chaired by Prof. Carol Post (Purdue University) and was devoted almost exclusively to virus work. The first speaker presented structural genomics results. Ming Luo (University of Alabama), a former graduate student in MGR's lab, presented the results obtained by the South Eastern Collaborative for Structure Genomics and its efforts to expedite the structure determination of the proteins contained within the *C. elegans* "ORFome" (i.e., set of proteins coded by the different open reading frames in the genome) using synchrotron radiation at beamline 22ID (SER-CAT) at the APS in Chicago (Luan et al., 2004) .

Roger M. Burnett (Wistar Institute and University of Pennsylvania, Philadelphia), MGR's first (surviving) graduate student, discussed the evolutionary implications of the finding that the structure of the major coat protein (P3) of bacteriophage PRD1 (Benson et al., 1999) resembles that of the human adenovirus hexon (Rux et al., 2003). As their names indicate, these two DNA viruses with pseudo-T=25 virions infect two very different hosts. PRD1 infects gram-negative bacteria while human adenoviruses do not contain a membrane and can cause pathological conditions in animals ranging from fish to humans. Based on the structural features of the viral coat proteins containing the "double-barrel trimer" motif, Roger argued that viruses infecting bacteria could be related by evolution to those infecting animal hosts. Using structure-based modeling, he presented data suggesting that other large viruses, infecting members of the three domains of life eukarya, bacteria, and archea, may have coats constructed in a

Figure 1. Photograph of Attendees

similar manner and using the same motif (Benson et al., 2004).

Alexander Gorbalenya (Leiden University Medical Center, The Netherlands), a former visiting scientist to Purdue University, discussed his explorations into the classification and life cycles of various genera within the universe of RNA-containing viruses. Within the most populated class of single-stranded, positive polarity RNA viruses (ssRNA$^+$), his evolution-based informatics analysis has uncovered relationships and insights into the structure and functions of NS2-3-related proteins critical to the life cycles and pathogenesis of pestiviruses (family *Flaviviridae*). In addition, Alexander's work on the members of the order *Nidovirales*, which includes coronaviruses such as the SARS virus, has provided insights into the components and function of the viral giant replicase present in these viruses. Finally, he discussed earlier results showing the unexpected internal permutation that is conserved in the virus-encoded, RNA-dependent RNA polymerases of the double-stranded *Birnaviridae* family (dsRNA *Birnaviridae*) and the ssRNA$^+$, *Tetraviridae* family (Lackner et al., 2004; Snijder et al., 2003; Gorbalenya et al., 2002).

Ignacio Fita (Institut de Biologia Molecular de Barcelona, Spain), a former postdoc on the catalase project, presented the structure of a representative of the minor group of human rhinovirus (HRV2-V23) bound to the ligand binding repeats of its cellular receptor (VLDLR). Viruses that belong to this group do not uncoat and penetrate the cells by clathrin-mediated endocytosis. Ignacio discussed the results in the context of the more conventional and larger group that contains the serotype that causes the common cold virus (HRV14). The receptor piece bound around the 5-fold axis and away from the "canyon" site, which is the recognition site of the larger group of rhinoviruses (Verdaguer et al., 2004; Olson et al., 1993).

Terje Dokland (University of Alabama, Birmingham), a former postdoc associated with the lab, presented his results on the structures of the nucleocapsid and core proteins of two enveloped ssRNA$^+$ virus of two different families. He discussed the structure of the core protein of west nile virus (WNV, family *Flaviviridae*) and the nucleocapsid protein of the porcine reproductive respiratory syndrome virus (PRRSV, family *Arteriviridae*). Even though the proteins are about the same size and both forms dimerize, the structures differ dramatically. The WNV protein is entirely α-helical and resembles proteins of the HEAT/ARM repeat class. In contrast, the nucleocapsid protein of PRRSV consists of a β sheet floor, covered and flanked by helices, and resembles crudely the coat protein of bacteriophage MS2. A distinct solution to the disposition of the proteins in the viral capsid was not possible yet, but he presented several possibilities (Dokland et al., 2004; Doan and Dokland, 2003; Dokland, 2000).

Jack Johnson (Scripps Institute, California) presented the results of several structural techniques (i.e., crystallography, SAXS, and intrinsic capsid fluorescence) to illuminate the nature of particle maturation in the bacteriophage HK97. The structure of the capsid of this virus (T=7) was described as a "molecular balloon," because the particle diameter ranges in the icosahedral shell from 540 to 630 Å, but the thickness of the shell is only 18 Å. However, the capsule is extremely rigid due to the presence of cross-linked rings between Lys-Asn amino acid residues in the viral shell. Those characteristics implied that the virus has to undergo a multistep maturation process concomitant with structural changes, the most dramatic being the change from prohead to EI-1 (expansion intermediate I). Jack presented combined evidence suggesting that this pH-dependent transition takes place for a single virus particle within the time scale of approximately 1 s and appeared to be a stochastic process. His metaphor of a "popcorn-like," binary transition caught my attention and found its way to my notes (Wikoff et al., 2000; Lee et al., 2004).

It was most fitting to hear MGR himself at the end of the day, reviving the stories related to the myriad of scientific projects and achievements that have taken place in his laboratory since 1964 (when he moved to Purdue University) and what might still be in store for the future. In closing, he emphasized once again what he wrote in the Foreword to *Crystals and Life* (Abad-Zapatero, 2002): "It has been my privilege to host and work with many pre- and postdoctoral students with vastly different cultural backgrounds during almost 40 years at Purdue University in Indiana. We have together enjoyed the pleasures of discovery and agonized over disappointments." I think that I can speak for the majority of the attendees to the symposium when I say: "Michael, it has been our privilege to work with you and to experience the pleasures of discovery and the agonies and anguish of the temporary failures along the way. You have enriched our personal and professional lives immensely."

After the official reception at the end of the meeting, it was time to honor another anonymous contributor to the field: MGR's wife Audrey. From her early cartoons drawn to convey the meandering of the polypeptide chain in LDH, she has followed the breakthroughs of the laboratory by inviting and hosting for dinner and other social events generation upon generation of students, postdocs, associates, and collaborators. On the side, she was always producing pottery masterpieces to mark each scientific milestone of the laboratory or the departure of friends or coworkers. The attendees flocked to University Place to present their tribute, friendship, and homage to her. There were hugs and emotional exchanges with this remarkable woman who has played such an important role in the life of not only Michael, but also of every single person who has had the good fortune of interacting with her.

A beautiful sunset completed this momentous day of spectacular structural biology, excellent science, warm friendships, camaraderie, and unforgettable memories at the Purdue University campus on the occasion of honoring Prof. M.G. Replacement's contributions to structural biology. Based on what we observed during our visit, they are nowhere near finished yet.

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