A brief history of protein crystallography in Brazil

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It is often difficult to establish exactly when a scientific discipline began. This is also true when trying to identify the moment when a particular field of research got a foothold in a new geographical region. The case of protein crystallography in Brazil is no exception and there are, therefore, several possible beginnings. For example, in 1959, Raw and Colli published a short report in *Nature* in which they briefly describe the crystallization of pig liver cytochrome b₅ (Raw and Colli 1959). It is not entirely clear from their report if this was merely fortuitous or the result of a deliberate effort. Either way, this probably represents the first venture into protein crystallization to be undertaken in Brazil and, quite possibly, the whole of South America. Curiously enough, as far as the author is aware, this structure has yet to be solved by X-ray diffraction.

Putting this brief foray aside, the first genuine attempt to establish protein crystallography in Brazil probably came with the initiative of Prof. Yvonne Mascarenhas, from the Instituto de Física de São Carlos. Already well established as a small molecule crystallographer and founder of the crystallography group in São Carlos, Yvonne was never afraid of new challenges. In 1977, she took a sabbatical and went to spend a year in the laboratory of Tom Blundell, then the occupant of the Bernal chair at Birkbeck College in London. Clearly, Yvonne had long-term plans to introduce protein crystallography into her large portfolio of projects back home. However, there was a need for someone to be specifically trained in the field and that was going to require talent—more specifically, a talented student. Someone prepared to leave behind the sunshine of the State of São Paulo in order to spend four years in the basement of Birkbeck College in central London, as Tom's PhD student. Glaucius Oliva was the ideal candidate, and after being trained

in London, he returned to Brazil in 1988. The author followed him a year later. In a sense this was the “real” beginning.

With not even distilled water in the laboratory, times were difficult at the beginning. Much ingenuity and improvisation was necessary. Nevertheless, with the help of senior colleagues, such as Yvonne herself and Eduardo Castellano, an Argentinean who had left his homeland during the military dictatorship, small projects were eventually funded enabling the acquisition of some basic equipment. In 1990 a meeting was organized at the beach resort of Guarujá, near São Paulo city, and several important structural biologists were invited to participate, including Wim Hol, Michael Rossmann, Tom Blundell, and Osnat Herzberg. Robert Huber was unable to come at the last minute. This meeting, held during torrential downpours, was a strategic move in attempting to convince the local biochemical community that it was going to be possible to do protein crystallography in Brazil. In retrospect it can probably be judged to have been a success.

At about the same time, the first Brazilian storage ring (UVX) was beginning to be assembled at the Laboratório Nacional de Luz Síncrotron (LNLS) in a provisional building near the University of Campinas. The group from São Carlos was responsible for securing the funding for a protein crystallography beamline. This required a fair number of comings and goings between the funding agency, FAPESP, and the researchers in São Carlos, before the former became convinced that the investment and associated risk were justified. Nevertheless, somebody would have to be responsible for overseeing its construction. Fortuitously, Igor Polikarpov, who had acquired considerable experience in synchrotron instrumentation during his time at Hamburg, was finishing a post-doctoral position in Edinburgh around that time and was looking for a more permanent position. He, like the author, became convinced that Brazil was a land of scientific opportunity and joined the synchrotron laboratory in 1996. The beamline MX1 finally came online together with the UVX storage ring during the following year (Polikarpov et al. 1998). The first structure solved using data from MX1 was published in 1999 (Polikarpov et al. 1999).

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While the synchrotron was still under construction, the São Carlos group was able to obtain funding for their first image-plate-based X-ray diffractometer. This was used to collect derivative data of crystals of glucosamine-6-phosphate deaminase and the structure was finally solved by multiple isomorphous replacement (Oliva et al. 1995). The native data had been collected with the help of the inimitable Zbyszek Dauter and Keith Wilson at DESY during a trip the author made in the summer of 1991. The project had been brought to São Carlos by Eduardo Horjales, a Uruguayan crystallographer who had spent many years training in Uppsala. It was the thesis project of Marcos Fontes, now a professor at the Universidade do Estado de São Paulo in Botucatu and is generally accepted to be the first protein structure solved in Brazil. It was published in 1995.

By this time, a new group had been established by Raghuvir Arni in São Jose do Rio Preto, a couple of hours drive from São Carlos. Slowly, former students from both centers began to establish groups in other parts of the country. Although, to this day, most are still to be found in the State of São Paulo, there are now about 20 different laboratories altogether which are scattered around the country.

It is undeniable that the building of the synchrotron (UVX) and the associated PX beamlines at the LNLS in Campinas was also a significant factor in the nucleation of new groups. Amongst other things, it meant that it was not necessary for the smaller and newer groups to have their own in-house X-ray equipment. The original beamline (MX1) was joined by a second one (MX2) about ten years later (Guimaraes et al.

2009). This received radiation from the first wiggler magnet allowing access to much higher energies than those previously available. The instrumentation was important but it was also necessary to grow the user community and in this respect FAPESP, the São Paulo State funding agency, played an important role. Riding the crest of the “genome wave” of the times, Rogério Meneghini, a biochemist from the LNLS, proposed an ambitious project called the Structural Molecular Biology Network (SMolBNet) which aimed to aggregate structural biologists with biochemists, molecular biologists, and cell biologists in order to train groups in the use of X-ray diffraction and NMR spectroscopy. A second call for proposals followed a few years later. As a consequence, new groups emerged including that of Chuck Farah in the Biochemistry Department of the University of São Paulo.

From the above, it is evident that the financial support of FAPESP has been an essential component over the course of the years. However, FAPESP was not the only player in the game. CNPq (the Brazilian National Research Foundation) was also important, but in a slightly different guise. In 2007, a group of structural biologists including Glaucius Oliva together with colleagues from Uruguay (Alejandro Buschiazzi) and Argentina (Alejandro Vila and Fernando Goldbaum) proposed founding a regional network. This seemed to make sense, given the limited number of groups active in the field at the time. By joining forces, they hoped to achieve more. This network came to be known as CeBEM and was supported initially by a unilateral initiative under the auspices of CNPq. Over the years, the network has slowly grown to 12 laboratories including the

Fig. 1 A timeline for protein crystallography in Brazil

incorporation of one each from Paraguay and Venezuela. This ad hoc institution has worked hard over the last ten years to maintain funding (mostly from Brazil, Argentina and Uruguay), all of which is invested in advanced training courses in structural biology (many in collaboration with CCP4) or in exchange visits between the nodes which comprise the network. Many of these nodes now have an international agreement giving access to the cutting-edge infra-structure provided by INSTRUCT-ERIC in Europe. It is probably not an exaggeration to say that CeBEM has significantly contributed to changing the structural biology landscape in the region, including within Brazil itself.

You may be beginning to ask yourself what this all has to do with Structural Biology in South Africa! Given that both Brazil and South Africa are still classified as developing nations, there are several parallels to be drawn and maybe some take-home messages which can be benefitted from.

Firstly, protein crystallography in Brazil grew from a well-established nucleus of small molecule crystallographers (Fig. 1). Yvonne Mascarenhas in particular had battled for many years to build the infra-structure and critical mass. This created a solid foundation on which to build and finally to venture out into the world of biological crystallography. South Africa also has a strong tradition in physical crystallography with R.W. James perhaps its most famous “son.”

Secondly, Yvonne was tireless in her efforts to attract talent from abroad and to bring world-renowned crystallographers to give lectures and courses. These included Paul Ewald, Herb Hauptman, Jerome Karle, Tom Blundell, and many others. This tradition has been maintained over the years and the workshops run by CeBEM have been important in training young protein crystallographers to a high standard. The initiative of the “Biophysics and Structural Biology at Synchrotrons” workshop, run by Trevor Sewell in Cape Town earlier this year, which included the participation of many specialists from around the world, is an excellent example of how to impact the community and cultivate the formation of new research groups.

Thirdly, the building of the UVX synchrotron in Campinas was undoubtedly an essential component in accelerating the growth of structural biology in Brazil. This central facility, together with the SMolBNet program, acted as a hub to increase awareness of the impact that structural techniques can have on biological research. Statewide or nationwide initiatives of this kind can be extremely useful but rely on large-scale investment on the part of funding bodies. In Brazil, the future looks bright as a new fourth generation synchrotron is currently under construction. Without doubt, the confidence necessary for such an enterprise was partly derived from the success of the UVX machine and the science which came out of it, including the innumerable protein structures that were solved. Furthermore, the crystallographic community in Brazil now feels confident to tackle more

challenging problems in structural biology, compared with 30 years ago, when most of the effort was concentrated on readily available proteins extracted from their natural sources. The discussions to build a synchrotron facility in Africa are still in their early days but are a clear sign of the desire to move forward and the necessity to address an unmet need on a continent which has still to realize its full scientific potential.

Finally, regional organization with colleagues can prove to be extremely effective and relatively inexpensive. CeBEM, which is currently coordinated by the author, is an example of this type of approach. More than anything else, it requires the desire of a group of like-minded individuals to pull together in the direction of a common cause. The remote data collection at Diamond which took place during the Cape Town workshop is a perfect example of such an attitude. This required the local coordination of sample collection from all over South Africa and subsequent shipping to the UK. Several new structures were solved as a result.

It is hoped that this short report will be a catalyst for reflecting on the best possible options for moving structural biology forward in South Africa and the African continent as a whole. Times have changed and there is no reason to believe that other developing countries should necessarily follow a similar route to that taken by Brazil. Local boundary conditions will naturally apply!

Compliance with ethical standards

Conflict of interest Richard C. Garratt declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by the author.

References

- Guimaraes BG, Stanfelici L, Neuenschwander RT, Rodrigues F, Grizolli WC, Raulik MA, Piton JR, Meyer BCX, Nascimento AS, Polikarpov I (2009) The MX2 macromolecular crystallography beamline: a wWiggler X-ray source at the LNLS. *J Synchrotron Radiat* 16:69–75
- Oliva G, Fontes MR, Garratt RC, Altamirano MM, Calcagno ML, Horjales E (1995) Structure and catalytic mechanism of glucosamine 6-phosphate deaminase from *Escherichia coli* at 2.1 Å resolution. *Structure* 3:1323–1332
- Polikarpov I, Perles LA, de Oliveira RT, Oliva G, Castellano EE, Garratt RC, Craievich A (1998) Set-up and experimental parameters of the protein crystallography beamline at the Brazilian National Synchrotron Laboratory. *J Synchrotron Radiat* 5:72–76
- Polikarpov I, Junior MS, Marangoni S, Toyama MH, Teplyakov A (1999) Crystal structure of neurotoxin Ts1 from *Tityus serrulatus* provides insights into the specificity and toxicity of scorpion toxins. *J Mol Biol* 290:175–184
- Raw I, Colli W (1959) Crystalline cytochrome b_5 . *Nature* 184:1798–1799

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