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PREFACE

Macromolecular crowding: chemistry and physics meet biology (Ascona, Switzerland, 10–14 June 2012)

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More than 60 years of biochemical and biophysical studies have accustomed us to think of proteins as highly purified entities that act in isolation, more or less freely diffusing until they find their cognate partner to bind to. While *in vitro* experiments that reproduce these conditions largely remain the only way to investigate the intrinsic properties of molecules, this approach ignores an important factor: in their natural *milieu*, proteins are surrounded by several other molecules of different chemical nature, and this crowded environment can considerably modify their behaviour.

About 40% of the cellular volume on average is occupied by all sorts of molecules. Furthermore, biological macromolecules live and operate in an extremely structured and complex environment within the cell (endoplasmic reticulum, Golgi apparatus, cytoskeletal structures, etc). Hence, to further complicate the picture, the interior of the cell is by no means a *simply crowded* medium, rather, a most crowded and *confining* one. In recent times, several approaches have been developed in the attempt to take into account important factors such as the ones mentioned above, at both theoretical and experimental levels, so that this field of research is now emerging as one of the most thriving in molecular and cell biology (see figure 1).

To promote the importance of molecular crowding and confinement and provide researchers active in this field an interdisciplinary forum for meeting and exchanging ideas, we recently organized an international conference held in Ascona from 10 to 14 June 2012. In the unique scenario of the Maggiore lake and absorbed in the magic atmosphere of the Centro Stefano Franscini (CSF) at Monte Verità, we enjoyed three-and-a-half days of intense and inspiring activity, where not only many of the most prominent scientists working on macromolecular crowding, but also experts in closely related fields such as colloids and soft matter presented their work. The meeting was intended and has been organized to bring theoreticians and experimentalists together in the attempt to promote an active dialogue. Moreover, we wanted different disciplines to be represented, notably physics and chemistry, besides biology, as cross-fertilization is proving an increasingly fundamental source of inspiration and advancement.

This issue of *Physical Biology* (PB) features a selection of the oral contributions presented at the conference, expanded in the form of research or review articles. PB, one of the scientific journals of the Institute of Physics (IOP), is one of the most dynamic and lively *forums* active at the interface between biology on one side, and physics and mathematics on the other. As its mission is stated by IOP, PB 'focuses on research in which physics-based approaches lead to new insights into biological systems at all scales of space and time, and all levels of complexity'. For these reasons, and also in view of its high reputation and broad readership, PB appears to be the ideal place for disseminating the thriving pieces of research presented at the conference. We are extremely grateful to PB and its kind and efficient editorial staff who helped make this issue a great scientific follow-up to the conference.

The opening lecture of the conference, the first of four day-opening keynote lectures, was given by Allen P Minton from NIH (USA), possibly the most influential among the pioneers in the field. He provided a lucid and well-thought-out overview of the concept of macromolecular crowding through an exhaustive chronological account of the major milestones. It is clear that the concept of excluded volume as a key factor remains central to the concept of molecular crowding. As a consequence, simple descriptive paradigms

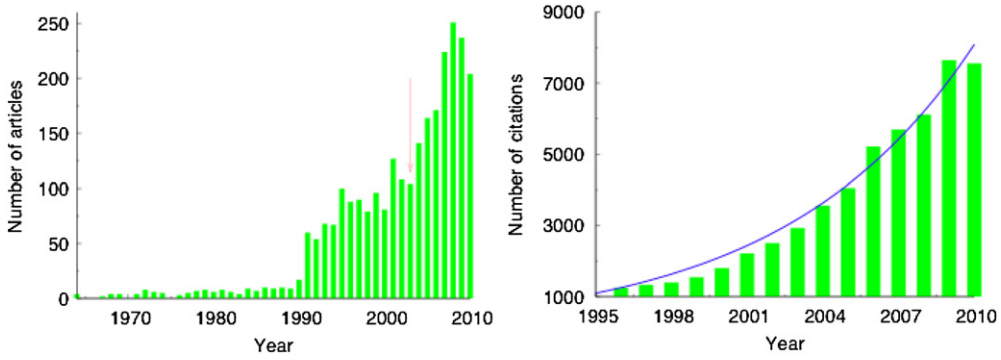

Figure 1. Left: number of articles containing the word 'crowding' as a keyword limited to the biological and chemical science domains (source: ISI Web of Science). The arrow flags the 2003 'EMBO Workshop on Biological Implications of Macromolecular Crowding' (Embo, 2012). Right: number of citations to articles containing the word 'crowding' limited to the same domains (bars) and an exponential regression curve (source: Elsevier Scopus).

borrowed essentially from colloid physics may still provide useful tools to understand the subtle effects of crowding and confinement in living matter.

The contiguity between crowding, colloids and soft matter further emerged as an important concept in the course of the conference in several theoretical lectures and a few experimental ones.

Dave Thirumalai, from the University of Maryland (USA), one of the most active theoreticians in the field of theoretical biophysics, outlined scaling theories, concepts from colloid literature and different simulation techniques to describe scenarios for crowding-induced changes in the structure and dynamics of proteins and RNA. In particular, he showed the importance of the *shape* of crowding particles in affecting folding oligomerization of amyloidogenic peptides.

Johannes Schöneberg, from IMPRS, Mathematics Institute (Germany), illustrated *ReaDDy*, a newly developed particle-based simulation software tool for reaction–diffusion dynamics, developed in the group of Frank Noe at EMPRS. He showed that ReaDDy makes it possible to bridge the gap between soft matter and molecular dynamics (MD) simulations on the one hand and particle-based stochastic reaction–diffusion simulations on the other. We asked Johannes to organize a tutorial session to lead interested participants into the package and 'get their hands wet' under the guidance of the developers. The tutorial session was indeed successful and the broad possibilities offered by the simulation toolkit appeared to be clear to the participants.

Paolo De Los Rios, from the Ecole Polytechnique Fédérale de Lausanne (EPFL, Switzerland), examined the complexity of the effects caused by crowding conditions from the point of view of statistical physics. Starting from a modification of the well-known Smoluchowski approach to calculate the encounter rate of diffusion-limited reactions, he showed how more realistic situations accounting for crowding effects could be treated equally well on the same theoretical grounds. This talk marked an important point in the conference as it reinforced the idea that simple models of theoretical physics still have the power to provide inspiring results in spite of the intrinsic simplifications of such theoretical approaches. Along the same lines, Nicolas Dorsaz, from the University of Cambridge (UK), proposed an extension of the Smoluchowski framework that incorporates repulsive and attracting interactions between the reactants. This approach was illustrated by reaction rates obtained from event-driven Brownian dynamics and dynamical Monte Carlo simulations.

Another striking example of the physical subtleties associated with modelling crowding effects was provided by Jeffrey Skolnick, from the Georgia Institute of Technology (USA). He examined the role of hydrodynamic interactions in the self-organization of biological assemblies in the presence of crowding. His results strongly suggest that hydrodynamic interactions greatly affect the kinetics of self-assembly reactions, so that including them in the picture appears crucial for understanding the dynamics of biological systems *in vivo*.

Margareth Cheung, from the University of Houston (USA), emphasized that how the crowded environment inside a cell affects the structural conformation of a protein with a spherical shape is a vital question because the geometry of proteins and protein–protein complexes are far from globules *in vivo*. Her work demonstrates the malleability of 'native' proteins and implies that crowding-induced shape changes may be important for protein function and malfunction *in vivo*.

Huan-Xiang Zhou, from the Florida State University (USA), focused on atomistic simulations of protein folding and binding under crowding conditions. His lab has developed a post-processing method that allows the atomistic representation of proteins in folding and binding processes under crowding. A comparison with experimental results was also presented.

Other lecturers pointed out that there are still aspects not entirely explored in the effects of both crowding and confinement. As suggested in the talk by Gary Pielak, from the University of North Carolina (USA), the currently used synthetic crowding agents are far from being satisfactory in replicating naturally occurring effects associated with crowded environments. For example, non-specific binding seems to play a subtle role in the cell, as natural macromolecules can induce both stabilization and destabilization when used as crowders. It is indeed possible to fine-tune the effect of proteins, as crowders, on the stability of other proteins.

Another aspect that became clear is that new, more powerful methods need to be developed to study the effect of crowding, but even more to compare crowding and confinement. Indeed, it appeared clear from the lecture by Pierandrea Temussi, from the University of Naples (Italy), that a reliable comparison of the effects of crowding and confinement on the stability of proteins can only be based on the measurement of the whole stability curve of the same protein.

Controversial aspects do not pertain only to the influence of crowding on protein stability, but also to aggregation phenomena in natural fluids.

Domenico Sanfelice, from NIMR (London, UK), reported an interesting case of the apparent influence of crowding on aggregation. Hen egg white, a possible natural medium to study macromolecules in crowded conditions can dramatically increase the aggregation kinetics of proteins with an inbuilt tendency to associate. By carefully dissecting the phenomenology, it was shown that only part of this effect is due to crowding, while another factor playing an important role is the interaction with proteins from the *milieu*. In other words, high-molecular-weight glycoproteins can act as efficient molecular seeds for aggregation.

A special topic of great relevance in the conference appeared to be the direct study of crowding in living systems. Alan Verkman, from the University of California, San Francisco (USA), one of the world's leading scientific personalities in the field of experimental investigation of crowding and confinement, was invited to give the second plenary lecture devoted to the experimental study of crowding effects *in vivo*. In his keynote lecture, Dr Verkman led us on a wide and compelling tour, exploring the main experimental approaches to study molecular crowding in and around cells. After a thorough examination of methods such as fluorescence recovery after photo-bleaching, fluorescence correlation spectroscopy, photo-activation localization microscopy and stochastic reconstruction microscopy, he concluded that the general consensus emerging from experimental studies is that the notion of universally anomalous diffusion in and around cells as a consequence of molecular crowding may not be correct, and that the slowing of diffusion in cells is less marked than has been widely assumed and can be simply described through a five- to sixfold reduction of the normal diffusion coefficient. A Soranno, from the University of Zürich (Switzerland), described how, by employing FRET measurements, it is possible to quantify the effect of molecular crowding on the dimensions of the highly charged, intrinsically disordered protein human prothymosin alpha. For a large variety of polymeric crowders (PEG, PVP, Ficoll, Dextran, PVA, PAA), a collapse of the polypeptide chain is observed with increasing polymer size and polymer concentration. The largest extent of collapse is observed for polymer radii comparable to the dimensions of the protein, in agreement with theoretical considerations. For his contribution, A Soranno was awarded the CSF Award for the best contributed talk.

In his most inspiring talk, Clifford Brangwynne, from Princeton University (USA), drew attention to very important objects, namely Ribonucleoprotein (RNP) bodies. These are

non-membrane-bound macromolecular assemblies that form from the dynamic interactions of RNA and proteins. The assembly of RNP bodies may sensitively depend on the biophysical features of the surrounding cytoplasm, including the degree of crowding, transport coefficients and mechanical properties. This dependency may have important implications for the RNA processing reactions involved in fundamental biological processes such as developmental cell growth. Remarkably, Brangwynne showed how RNPs behave in the cell as liquid droplets, pointing to a possible entirely new means that the cell could use to control and fine-tune its internal processes, in fact, more than that, a completely unexplored, new state of organization of living matter, and a functional one.

Giuseppe Zaccai, from Institut Laue Langevin, Grenoble (France), showed that protein dynamics is more sensitive than structure to environmental factors such as crowding, solvent, temperature or pressure. Furthermore, he convincingly explained how neutron scattering provides unique experimental data to underpin MD calculations in this context. Following up on environment-induced modulations of protein functional dynamics, Ruth Nussinov, from Tel Aviv University (Israel), addressed the important problem of whether cellular signals can travel long distances in a crowded environment. She proposed a model based on the evolution of at least three properties: a modular functional organization of the cellular network, sequences in some key regions of proteins, such as linkers or loops, and compact interactions between proteins, possibly favoured by a crowded environment.

The workshop ended on a keynote lecture by Jean-Marie Lehn, from the Université de Strasbourg (France). Lehn, 1987 Nobel Laureate in chemistry, offered a 'supramolecular view' of the field of molecular interactions. *Supramolecular chemistry* explores the design of systems undergoing *self-organization*, i.e. systems capable of generating well-defined functional supramolecular architectures by self-assembling from their components, thus behaving as *programmed chemical systems*. Chemistry may therefore be considered an *information science*, the science of informed matter. Supramolecular chemistry is intrinsically a *dynamic chemistry* in view of the ability of the interactions connecting the molecular components of a supramolecular entity and the resulting ability of supramolecular species to exchange their constituents. The same holds for molecular chemistry when the molecular entity contains covalent bonds that may form and break reversibly, so as to allow a continuous change in constitution by the reorganization and exchange of building blocks. These features define a *constitutional dynamic chemistry* (CDC) on both the molecular and supramolecular levels. CDC takes advantage of dynamic constitutional diversity to allow variation and selection in response to either internal or external factors to achieve *adaptation*. The merging of the features—information and programmability, dynamics and reversibility, constitution and structural diversity—points towards the emergence of *adaptive* and *evolutive chemistry*.

The whole workshop could have not taken place without the help of the Centro Stefano Franscini. The CSF is the congress centre of the Swiss Federal Institute of Technology of Zurich (ETH Zurich) and has been situated at Monte Verità since 1989. It is an ideal meeting point for all members of the international scientific community who wish to discuss the state-of-the-art and new challenges of any field of research. The CSF supports 20–25 international conferences every year and, since 2010, up to ten winter doctoral schools[1]. The competence and professionalism of the staff were at the same level of beauty and inspiring character as that of Monte Verità.

A meeting of this sort, if successful, leaves the audience with more open questions than settled answers, and this was definitely the case for Crowding 2012. Excluded volume is clearly a fundamental concept that has allowed crowding, a very familiar concept in soft matter, to enter into the domain of biological sciences. However, the complexity of the biological *milieu* calls for more refined descriptions. What is the role of electrostatic and electrodynamic interactions? What is the role of hydrodynamics interactions? To what extent does the strong spatial inhomogeneity (clustering of molecules, cellular compartmentalization, etc) have to be taken into account? Or, more generally, what are the minimal elements that prove crucial to describe reactions within a cell? How does the diffusion proceed (diffusion, slow diffusion, sub-diffusion) given that the experimental evidences are still controversial?

[1] Source: www.csf.ethz.ch/

In conclusion, we knew that allowing scientists with very different backgrounds and ideas to mingle was a hazardous attempt. Despite that, the workshop turned out to be a very successful experiment, which was highly enjoyed both by the participants and the organizers. Discussions sparked regularly among ever-changing groups, comprising senior scientists and students, despite the rather tight schedule, adding to the sense of fulfilment ignited by the outstanding level of the presentations. Given the success of the meeting Crowding 2012, a new event has been organized and will take place on the same themes during fall 2013, this time in the beautiful scenery of the Loire valley in France. The workshop 'Macromolecular crowding effects in cell biology: models and experiments' will be held on the CNRS campus in Orléans, France, on 24–25 October 2013. More information can be found on the workshop website: http://dirac.cnrs-orleans.fr/∼piazza/.