

Preface

The main reason behind this book was the necessity to give a proper account to the considerable interest that arose in the last ten years on the notion of minimal cell. This is broadly defined as the cell containing the minimal and sufficient number of components to be defined as alive, or at least capable of displaying some of the fundamental functions of a living cell. Obviously such a definition encompasses entire families of semi-synthetic cells, where the term semi-synthetic indicates that most of the basic components (DNA, enzymes) are extant natural components, whereas the compartments are usually ad hoc constructed vesicles (generally liposomes, i.e. vesicles made out of lipids). Today this research program is seen as part of the broader scenario of synthetic biology, and in particular of “chemical synthetic biology” (Luisi 2007), as indeed the operational procedures attending their production in the laboratory are exquisitely chemical (and not so much genetic manipulations as most of the engineering synthetic biology).

The story of the minimal cell on the basis of liposomes started in the early 1990s mostly in my laboratory at the ETH Zürich, where we set up methods to perform complex molecular biology reactions inside liposomes, for example the polymerase chain reaction (Oberholzer et al. 1995a), or the incorporation of the entire ribosome machinery inside liposomes with the production of the first polypeptide chain (Oberholzer et al. 1999). I believe the term “minimal cell”, related to the synthetic biology using liposomes, appeared in that 1995 paper with Oberholzer (Oberholzer et al. 1995b). At that time we were practically the only ones in the field, but at the turn of the century several valid groups begun to work with liposomal minimal cells, among which Yomo (Yu et al. 2001; Ishikawa et al. 2004), Noireaux and Libchaber (2004), Yoshikawa and coworkers (Nomura et al. 2003; Tsumoto et al. 2001); and well known people like David Deamer dealt with the subject (Chakrabarti et al. 1994, Pohorille and Deamer 2002). When I moved to Rome in 2003, I took with me the research projects in chemical synthetic biology, and had the good fortune to be accompanied by Cristiano Chiarabelli and Pasquale Stano, whereby the latter has been supervising in Rome all the work with liposomes, including the minimal cell work.

While progress in this field was progressing, it became clear to several of us that a number of basic concepts involving the physical chemistry of cells were necessary

to advance the work with the minimal cell/minimal life, concepts which were by no means clarified yet in the literature. The entrapment of components into a liposome was in fact re-proposing the general problem of the local concentration in biological compartments, with keywords such as molecular crowding, the state of water in a restricted volume, the anomalous internal pH, and the general question whether and to what extent the thermodynamic and kinetic patterns of the bulk solution were still valid in a small, crowded environment.

These fundamental questions relating to the physical and chemical state of components inside a living cell, and to their behaviour, form the second and complementary reason for the production of this book.

Thus, this book is formally divided into three related and partly overlapping parts. It starts with a discussion on the physical aspects, beginning with the question of the physical state of the cytoplasm (Keighron and Keating), the mechanical properties of the cell (Boal and Forde), the internal cellular crowding (Acerenza and Graña). The next question is another classic one: what are the minimal dimensions of a cell (Moore), a question which has been recently addressed in the literature with the minimal size of functional liposomes (Souza et al. 2009). The conclusive chapter of the first part deals with a discussion of confinement and crowding (Minton and Rivas).

After this physico-chemical prelude, the book moves towards biological functionality, and we will find the contribution of Lancaster and Adams where the question of the minimal physical size is seen also in terms of the minimal genome size. The two minimal sizes are related by another classic question: how can the long, rigid duplex of DNA be entrapped into a small compartment having dimensions which are an order of magnitude smaller. This question has been investigated in the literature also with reverse micelles, obtaining evidence for the super-condensed “psi” form of DNA (Osfouri et al. 2005).

And how does water present in the crowded environment play in all that? The contribution by Pollack and collaborators addresses this point.

And then the book moves towards more and more biological aspects, with the contribution by Monnard and Deamer, who tackle the question of the assembly of bio-membranes, and Gardner and Davies who address the constitution of cells, still from a chemical point of view.

And now, with the third part of the book, we are in business with the minimal cell. We start with an analysis of Yoshikawa, Nomura and collaborators on how to construct an *in vitro* model of a living cellular system, followed by a review article by our group where most of the basic concepts and experimental procedures to make a liposome minimal cell are summarized and critically evaluated. From here we move towards the most advanced operational procedure of Kumura to produce two different membrane proteins inside liposomes. With the contribution of Petra Schwille we find another classic question in the liposome field, namely, what can we do with giant vesicles, those which can reach the dimensions of real biological cells? A modelistic approach to the minimal cell is presented by Fabio Mavelli, in the very interesting case of a RNA minimal cell – no proteins, no DNA. And we

end up with a contribution by Yomo and collaborators, where we are finally taken up to the complexity of biological evolution.

It is clear, I believe, from this rich display that the research on the minimal cell(s) represents an important sector of the life science activity. Without forgetting that this project has a target of historical, almost epochal value: the construction of life in the laboratory.

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