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# What history tells us XLII. A 'new' view of proteins

Michel Morange

*Centre Cavaillès, République des Savoirs: Lettres, Sciences, Philosophie USR 3608, Ecole Normale Supérieure, 29 Rue d'Ulm, 75230, Paris Cedex 05, France*

*(Email, morange@biologie.ens.fr)*

## 1. Introduction

Circa 2000, many biologists were discussing new views on proteins: on protein folding, allosteric regulation, catalysis. There was a debate, some arguing that the new views were not so new.

In a previous contribution, I discussed the new view of allostery, and some of the steps that led to it (Morange 2012). Here, I will briefly return to the global debate and show that one major shared component of these new views was the increasing importance given to the evolutionary history of proteins for explaining their characteristics.

## 2. The debate

The debate was multiform and not focused on a single issue. It first concerned the mechanism of protein folding, and the new view resulting from the models proposed by specialists of statistical mechanics (Leopold *et al.* 1992; Bryngelson *et al.* 1995). This new view was popularized through two intermingled metaphors, the energy landscape and the folding funnel: proteins no longer followed a unique folding pathway, but moved at different positions in an energy landscape resembling a funnel of irregular shape (Leopold *et al.* 1992; Bryngelson *et al.* 1995; Dill and Chan 1997).

The discovery of partially unfolded proteins opened a second debate on a new view of the way enzymes recognize their substrates and catalyse their reactions. There was a shift in the models from lock-and-key recognition by a protein with a well-defined structure to recognition and catalysis by an ensemble of different protein conformations in equilibrium (Elsenmesser *et al.* 2005).

In parallel, there emerged also a new view of allostery (Gunasekaran *et al.* 2004; Morange 2012). A similar interpretation of catalysis and allostery was justified by the hypothesis that both 'emerge via a common route' generated by the intrinsic dynamics of the proteins (Goodey and Benkovic 2008, 474).

What was initially considered as new and common to these different revolutions was that proteins (and eventually RNAs) were considered as an ensemble of different conformations and not as a unique average structure. But was this really new? Martin Karplus argued that this 'new' view was already there in the 1960s and 1970s (see, for instance, Austin *et al.* 1975, McCammon *et al.* 1977 and, for a brief historical description, Levitt 2001), but that its importance was limited by computational power and the absence of techniques able to reveal directly this dynamic behaviour of proteins (Cui and Karplus 2008). Constant progress in computational biology (molecular dynamics) and methodological advances in NMR (Mittermaier and Kay 2006) have recently convinced most protein specialists of the existence of this dynamic behaviour.

However, there is more in the new view than a simple confirmation of previous expectations. To say that a protein is not rigid and behaves dynamically is not the same as stating that the origin of its catalytic power and regulatory properties has to be looked for in its intrinsic dynamics, and that the latter emerged from the evolutionary history of these macromolecules. The 'new' view of proteins is not only a

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(re)discovery of their dynamics, but also the result of increasing interest in the way that these properties emerged in evolution. I will give in part 3 of this article four examples of this wedding between structural studies of proteins and evolutionary studies. Not only was this dovetailing of structural and evolutionary questions one of the pillars of the 'new' view of proteins, but also it will, I am convinced, play an increasing role in the future.

## 3. A 'new evolutionary' view of proteins

### 3.1 *Emergence from protein engineering*

It is quite remarkable that interest in the evolutionary history of proteins as a way to explain their structural and functional characteristics emerged in a field of research, protein engineering, which was apparently focused on fast, practical applications of the new technology of genetic engineering. Positive results were rapidly obtained: a limited number of amino acid substitutions could stabilize a protein and change the nature of its substrate or of the reaction that it catalyses.

But difficulties soon emerged. Some of the proteins newly engineered to have a different catalytic activity could not be used because they were too unstable (see, for instance, Quéméneur *et al.* 1998). There are constraints in the evolution of proteins that lead to a compromise between protein stability and protein activity (Meiering *et al.* 1992; Schreiber *et al.* 1994). These early observations were amply confirmed when site-directed mutagenesis was progressively complemented by *in vitro* evolution, a step necessary to optimize the newly engineered systems (Griffiths and Tawfik 2000). Observation of this 'trade-off' as it was soon named, using an expression familiar to evolutionary biologists when speaking of the balance between stability and activity, was not limited to the work of protein engineers, but could also be observed in nature: evolution of an antibiotic resistance enzyme was also shown to be constrained by the same trade-off (Wang *et al.* 2002).

### 3.2 *Evidence for conserved amino acid networks responsible for protein dynamics*

The work of protein engineers also revealed another limit to protein modifications: the intrinsic dynamics of proteins. Relatively rigid proteins as antibodies could be only converted to inefficient enzymes (Tramontano *et al.* 1986): the high catalytic power of a protein depends on its complex intrinsic dynamics. The network of interacting amino acids generating this dynamic behaviour has been conserved during evolution.

Building on this hypothesis, Rama Ranganathan and colleagues developed a new statistical method to identify the amino acids that are functionally coupled in these networks. What is searched for in a large family of homologous proteins is not the conservation of residues, but the coevolution of pairs of residues. The validity of the method was first demonstrated in proteins possessing a PDZ domain (Lockless and Ranganathan 1999), before being extended to other proteins and confirmed by other approaches. The progressive tailoring of these regulatory networks was the result of a long evolutionary history. And the evolvability of proteins depends on the structure and modifications of these networks (Raman *et al.* 2016).

### 3.3 *A new evolutionary view of protein folding*

A third field of research progressively permeated by evolutionary biology was protein folding. The new models of the 1990s were not immediately positioned in evolutionary perspectives. Rather, they solved thermodynamic and kinetic issues that had been raised in the 1950s and 1960s.

Retrospectively, however, it is difficult not to see that the new models fitted evolutionary models perfectly. Evolutionary biologists consider that natural selection sieves the result, not the ways by which the result was reached: there are many alternative ways of increasing fitness. In the case of protein folding, what is sieved is a (sufficiently) rapid, and efficient folding process. The new models of protein folding suggested that what has been selected was not, in most cases, a unique pathway, but an ensemble of different pathways, with a different order of bond formation.

A good illustration of how viewing protein folding from an evolutionary perspective can help to understand its extant characteristics is provided by the study of chaperonins. These high-molecular-weight protein machines help a fraction of cellular proteins to fold correctly. Their mechanisms of action have been amply discussed: they provide a favourable, protected environment for folding, but their main role is probably to partially unfold imperfectly folded proteins, dropped in what constitute folding dead ends.

In 2005, Ulrich Hartl and his colleagues tried, in a proteome-wide study, to determine the structural characteristics of the proteins that are the targets of chaperonin action (Kerner *et al.* 2005). No clear picture emerged, although some structural families of proteins, such as the α/β proteins, were shown to be favoured targets of chaperonins. In discussing their findings, the authors abandoned a purely structural point of view for an evolutionary one: their study only provides a snapshot in a permanently moving landscape of protein-chaperonin interactions. Mutations occur that increase the activity of a protein, but destabilize it: the protein becomes a target for chaperonins. Additional mutations may occur that stabilize the protein, making the folding process more rapid and more efficient and thereby rendering the action of the chaperonin unnecessary.

Dan Tawfik demonstrated that overexpression of chaperonins favours genetic variation and protein evolution (Tokuriki and Tawfik 2009). In synthetic biology, protein engineers can modify chaperonin levels, and it is also possible that levels of chaperonins varied at certain stages of evolution, when rapid adaptation to a new environment was required. Whether or not such a mechanism has played a role in evolution, these studies amply demonstrate that protein structure and folding can only be understood in the light of evolution.

### 3.4 *Protein promiscuity*

From the evidence that proteins exist in different conformations, Tawfik explored another consequence of the new view of protein structure: the possibility that a protein can bind different ligands and so is functionally promiscuous (James and Tawfik 2003). As Tawfik admitted, his ideas were not radically new: a similar hypothesis was (incorrectly) proposed by Linus Pauling (Pauling 1940) to explain the extraordinary capacity of antibodies to bind any chemical molecule, but also later by Roy Jensen (Jensen 1976), and by Henrik Kacser and R. Beeby (Kacser and Beeby 1984). These hypotheses were now supported by recently acquired evidence of protein structure flexibility. It might be that early proteins were more promiscuous than extant ones: evolution would have progressively given proteins more specificity. In vitro reconstruction of ancestral proteins, such as the early form of the glucocorticoid receptor, supports this hypothesis (Dean and Thornton 2007): the ancestral form of the receptor was able to bind both glucocorticoids and mineralocorticoids, whereas the extant receptor is specific to glucocorticoids. The importance of promiscuity in early proteins, its role in evolution, and its potential use in biotechnology are now being explored through numerous studies.

## 4. Conclusions

Maybe what is most striking in the new view of proteins are the possibilities that it offers to dovetail mechanistic and evolutionary explanations. This is the true novelty of this new view.

Evolutionary explanations are often considered by functional biologists, biochemists, molecular biologists, and physiologists as something that can enrich mechanistic explanations, but not something essential. This is not the case for proteins. Evolutionary explanations are not a small addition to broadly self-sufficient mechanistic explanations, but rather are crucially required to explain the structures and functions of proteins. This field of research is a wonderful illustration of the functional synthesis that Antony Dean and Joseph Thornton saw emerging from the encounter of evolutionary models and molecular data (Dean and Thornton 2007).

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