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Protein–water interactions in a dynamic world

Carla Mattos

Protein–water interactions are key to biological function. They have an underlying dynamic component that pervades the functional roles associated both with particular systems and with the properties of proteins in general. This article focuses on the specific ways in which the dynamics of water are important to protein structure, motion and adaptability to changes in the protein environment.

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The size, geometry, orientational flexibility and unique physical properties of water molecules have made water a ubiquitous solvent indispensable to life processes in general and to the structure and function of proteins in particular. Studies of water–protein interactions from various perspectives have generated a large amount of information that was initially hard to reconcile into one cohesive picture. Water has been observed to play a functional role in enzyme catalysis [1], protein folding [2], protein architecture [3], conformational stability [4], protein dynamics [5], protein plasticity [6], ligand binding [7] and the selectivity of specific interactions [8].

The exciting developments of recent years have provided some answers to questions of how these functional roles are achieved, and a unified picture of protein–water interactions is beginning to emerge. This picture has been continuously clarified through the lenses of X-ray and neutron crystallography, and nuclear magnetic resonance (NMR) spectroscopy, and has, in more recent years, gained new perspectives from other biophysical methods such as solution X-ray and neutron scattering [9], neutron diffraction of protein powders [10], infrared spectroscopy [11–13], Raman spectroscopy [14], fluorescence spectroscopy [15], microwave impedance dispersion [16] and osmotic stress [17]. Furthermore, computational methods are now sufficiently developed so that reliable simulations can be performed in the nanosecond timescale, providing alternative checks to experimental results and additional information not accessible by any other means [18].

Although X-ray crystallography has, for the past three decades, provided an exciting view of protein–water interactions at the molecular level, it is clear that any one method offers a skewed and incomplete picture of protein hydration. With the increase in diversity of biophysical techniques available to study water at the protein interface, the crystallographic data can today be interpreted in light of the contributions from other methods, whereas the crystal structure was, until recently, the universal standard in light of which all other results were interpreted. One of my aims in this article is to convey this important shift in perspective. It is now not only possible to talk about where water molecules are found on protein surfaces but also to discuss in quantitative terms their thermodynamic contribution to protein conformational stability [19], their electrostatic properties on the protein surface [20] and their residence times at different locations in the protein–water interface [21].

The most direct evidence of the importance of water to protein function is that, in its absence, proteins cannot move and enzymatic activity is negligible. Minimal levels of hydration have been associated with important physical and biological properties of proteins, and dehydration studies show that at least a monolayer of water molecules is required for the protein to be fully functional [22]. Lack of motion and activity can also be observed when proteins are transferred to neat organic solvents [23], making it clear not only that the presence of water is essential but also that other solvents cannot serve as substitutes. Finally, it has been known for many years that proteins undergo the so-called 'glass transition' at ~200 K; at this point, functionally important motions become frozen and biological activity is annihilated. Ultra-high-resolution crystallographic studies above and below 200 K have recently shown that both the atomic position and the dynamics of the plant-seed protein crambin are coupled to those of solvent water molecules [24]. Below the glass-transition temperature, these water molecules form highly organized rings near the protein surface, with deleterious consequences to the atomic fluctuations that are important for function [24]. A series of

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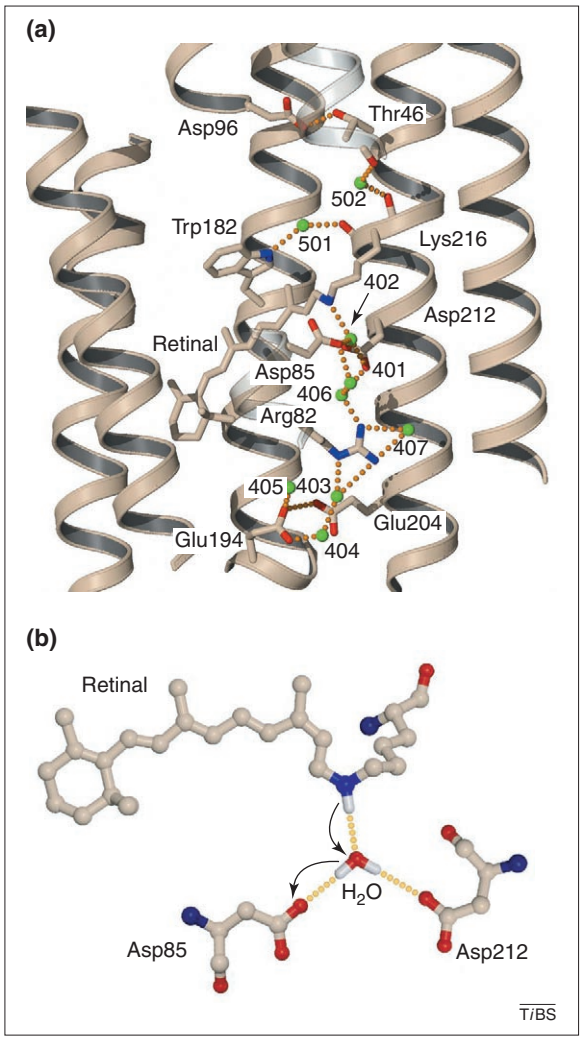

Fig. 1. The bacteriorhodopsin proton pump. The seven-helix protein is inserted into the membrane (the cytoplasmic side is towards the top and the extracellular side towards the bottom). (a) The interior of the pump, showing retinal as a Schiff base with Lys216. The buried water molecules thought to be involved in the proton translocation mechanism are shown in bright green, with water molecule 402 indicated by an arrow. (b) Close up of water molecule 402, showing a possible mechanism for its role in the deprotonation of the Schiff base. This deprotonation step is key in the photocycle, and MRD experiments suggest that water is not rate-limiting in this reaction. Reproduced, with permission, from Ref. [39].

molecular dynamics simulations of myoglobin were conducted in which the temperature of the protein and water were controlled separately. The results were consistent with those from the crambin experiment, and showed that solvent mobility is the major component determining protein atomic fluctuations above the glass-transition temperature [25]. Neutron-scattering experiments [26] and other molecular dynamics simulation studies [27] have also contributed to the view that water plays a crucial functional role in mediating protein dynamics at the molecular level. In summary, the absolute requirement for water is exemplified here in three different ways: dehydration studies, studies of proteins in organic solvents, and cryogenic experiments in which water motions essential to protein dynamics become frozen. From all three perspectives, it is clear that dynamics are linked to protein function at the molecular level. What, then, are the new insights into the dynamics of protein–water interactions and into why these interactions are so crucial to protein function?

NMR view of dynamics

The early static picture of proteins has long been replaced in the world of structural biology by one in which these macromolecules and their interactions are part of highly dynamic processes. Thermal motion is an essential component of the environment in which proteins evolved, and nature has shaped these motions so that they are optimally incorporated into protein function. It is, therefore, not surprising that protein–water interactions have evolved to be inherently dynamic. NMR spectroscopy is the most powerful experimental method for studying the motions of proteins and their interacting water molecules [28–31]. Of particular interest are the average time that a water molecule spends bound at a protein site (the residence time) and the motional freedom of the water molecule within the site, given by the mean squared order parameter S^2 (a quantity that varies from 0 to 1 as order increases) [32]. These two properties are intimately linked to the functional roles of water molecules on protein surfaces.

New improvements are continually expanding the limits of what can be observed by NMR [33,34]. In particular, nuclear magnetic relaxation dispersion (MRD) experiments [31] have provided unprecedented details about the dynamics of protein–water interactions. MRD data have shown that buried water molecules in globular proteins exchange with bulk solvent on a nanosecond to microsecond timescale [35]. These water molecules are usually extensively hydrogen bonded to the protein and play a role in stabilizing its secondary or tertiary structure [36]. The more exposed water molecules on protein surfaces have a broad distribution of residence times, centred around tens of picoseconds and extending to several nanoseconds for a few water molecules in deep surface pockets [21].

A molecular dynamics simulations study of hydration sites in myoglobin found that the residence times of surface water molecules are determined primarily by the shape of the protein surface at the water binding site, with those in deep crevices exhibiting the longer residence times [37]. Interestingly, the same study revealed no simple correlation between water binding sites with maximum water density (equivalent to the well-ordered water molecules observed in crystal structures) and the residence times of water molecules on the protein surface, in full agreement with what has been observed by NMR [37]. Regardless of location, residence time or how well-ordered water molecules are at the protein surface, it is clear that all water molecules associated with proteins are in constant dynamic exchange with the bulk solvent. An important consequence of the fast exchange of water molecules on protein surfaces is that hydration processes are unlikely to be rate limiting for protein folding or intermolecular recognition events, both of which occur in a longer time frame than the residence times of most water molecules [28].

In one of the most recent advances using MRD, residence times were determined for internal water

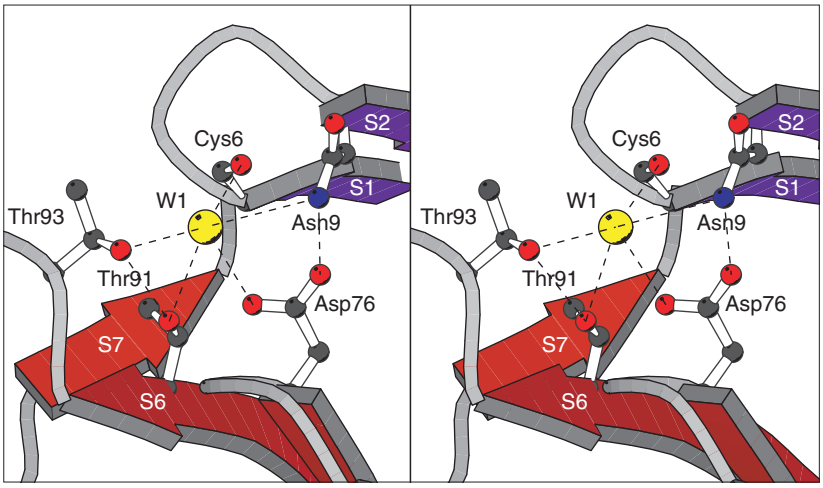

Fig. 2. Stereoscopic view of water molecule W1 in ribonuclease (RNAse) T1. This water molecule is conserved in all the available crystallographic models of microbial RNases, including distant family members. W1 exhibits considerable rotational entropy despite its extensive hydrogen-bonding interactions with protein atoms. Reproduced, with permission, from Ref. [3].

molecules in bacteriorhodopsin solubilized in micelles of octyl glucoside [38]. Bacteriorhodopsin is a proton pump driven by the photoisomerization of a retinal chromophore that is covalently attached by Schiff base to a Lys residue of this membrane protein. Crystallographic studies determined that the number and location of buried water molecules vary significantly throughout the photocycle and that these water molecules are probably important in the proton translocation mechanism of the reaction [39] (Fig. 1a). The MRD experiments determined that the deeply buried water molecules exchange with bulk on a microsecond timescale, much faster than the rate-limiting deprotonation of the Schiff base, which occurs in ~2 msec [38] (Fig. 1b). Here is a specific example in which water provides a crucial functional role without being rate limiting, just as is expected in folding and recognition events. A picture is emerging in which, owing to its fast exchange with the bulk, water is not rate limiting in biological events in general. This would be an ideal situation, in which nature can take advantage of the unique properties of water without being burdened by its presence. Thus, situations in which water is rate limiting would either have evolved to be particularly advantageous or would be intrinsic to a catalytic reaction in which the mechanism itself dictates the rate limiting step.

One of the ways in which functionally important water-binding sites can be identified is through retention across evolutionarily related proteins. For example, a study comparing many crystal structures of microbial ribonucleases has revealed that a single water molecule is conserved across the entire family, including distantly related members [3]. This water molecule (W1 in Fig. 2) makes four strong hydrogen bonds to protein atoms, is part of a larger network of highly conserved water binding sites and is linked to the catalytic activity of ribonuclease T1, probably by affecting the dynamics of the active site [40]. MRD experiments determined that W1 has a mean residence time of 7 ±3 ns at 27°C and substantial rotational freedom, as indicated by the S^2 value (0.45 ±0.08) [40]. Molecular dynamics simulations of RNase T1 are consistent with NMR experiments and, in general, show significant water translational and rotational motions on the picosecond timescale in a fully hydrated system [41]. Relatively low order parameters were also observed for three of the four buried water molecules in bovine pancreatic trypsin inhibitor, which is consistent with significant rotational freedom within these sites [35]. The combination of strong water hydrogen-bonding interactions with protein atoms and high rotational entropy favours the existence of spatially localized water binding sites, diminishing the commonly assumed entropic cost that is associated with tightly bound structural water molecules [35,40]. The ramifications of the high entropy associated with most bound water molecules, which results from low residence times and order parameters, can be profound and might particularly affect our current understanding of the balance of energy involved in release of water molecules upon ligand binding [35,42].

Crystal structures in the light of dynamics

Most of what is known about the average location of water molecules on protein surfaces has been obtained by X-ray crystallography. The number of water molecules observed in a protein crystal structure varies primarily with the resolution of the diffraction data. At 2 Å resolution, about one water molecule per amino acid residue is expected to be visible in the electron density map [43]. This represents only a portion of the water molecules on the protein surface and is skewed towards those that make strong polar interactions with protein atoms. Nevertheless, the accumulation of structures in the Protein Data Bank [44] has made possible several studies that extract statistically significant trends about the distribution of water on protein surfaces. Furthermore, different types of non-random protein–water interactions have been inferred from the functional roles of water that influence protein architecture, complex formation and the catalytic mechanisms of biological processes [36]. In these exciting times, as many biophysical techniques advance at an unprecedented pace [45], crystallographic studies continue to be extremely powerful in the study of protein–water interactions. During the past three years, such studies have contributed significantly to the literature in this area [1,46–50].

What do the crystallographic data reveal about hydration sites in which water molecules can be found in the perpetually dynamic world of biological systems? In any crystal structure, the main indication of motion is given by the atomic temperature factor, which is highly correlated with occupancy in all but the ultra-high-resolution protein crystallographic models [51]. Even when the atomic temperature factor and occupancy can be deconvoluted, a high atomic temperature factor indicates disorder but gives limited information about

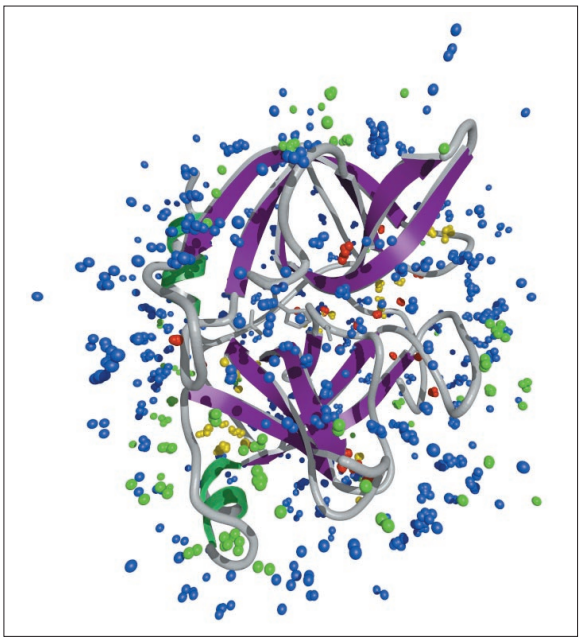

Fig. 3. Water molecules from 11 structures of porcine pancreatic elastase solved in different solvent conditions are superimposed on a ribbon diagram of the protein. The elastase α helices are shown in green, the β sheets in purple and the coils in grey. The catalytic triad (Asp108, His60 and Ser203) is shown explicitly in the cleft between the two domains. There are 1661 water molecules that occupy 426 unique hydration sites. Buried water molecules are shown in red, channel water molecules in yellow and surface water molecules in blue and green; the green water molecules represent those that are in crystal contacts. Reproduced, with permission, from Ref. [36]; copyright (2001) International Union of Crystallography.

the spatial dynamics of the protein and its solvent. Multiple crystal structures of a given protein or a group of structurally related proteins can be used to broaden the dynamic perspective of X-ray diffraction studies. Recent multiple-structure studies of hydration include the structures of RNase A at different humidity levels [52], an analysis of the water structure of trypsin in three different crystal forms at 100 K and 293 K [53], a comparative analysis of water molecules across the large and diverse family of microbial ribonucleases [3], a study of RNase A at six different pH values [54], and an analysis of water in 11 crystal structures of elastase solved in different organic-solvent–water mixtures [36].

The pattern that has emerged from these and previous studies is that only a few water molecules are found in conserved positions as the environment of the protein varies in diverse ways. In the comparison of five independently solved structures of the same protein in the same mother liquor and at a given temperature, most hydration sites were found to be reproduced from one structure to another [3]. By contrast, the crystal structures of proteins solved in different crystal-packing environments show that the crystal contacts strongly influence the location of surface water molecules [3,55]. The changes in temperature, hydration level, pH and solvent environments have significant but more modest effects. Decreasing the humidity level in the crystal, or increasing the temperature at which data are collected, leads to fewer crystallographically observed water molecules, but changes in the solvent environment result in crystal structures with about the same number of water molecules as in aqueous solution.

In all cases, the locations of many surface water molecules are shifted as the environment of the protein changes. For example, in elastase, there are 426 unique water-binding sites observed in the 11 superimposed models obtained in the presence of organic solvents [36] (Fig. 3). Of these, 178 sites (40%) were found in only one of the structures. Only ~20 of the remaining 248 sites were conserved in all the different solvent environments. The conserved sites are well clustered, found in deep crevices and invariably make the same three or four hydrogen bonds with the protein in each of the models (Fig. 3, red). Several water molecules are found in channels where there is a set of many possible hydrogen bonds, a few of which are made by two or three water molecules that appear in every model (Fig. 3, yellow). Collectively, the water molecules observed in the elastase channels fulfil all the available hydrogen bonds within these sites. The surface water molecules are not well conserved across the models and have positions that are largely influenced by the local environment and the properties of the solvent (Fig. 3, blue and green). Although the elastase example is based on a particular kind of change in the environment (organic solvents), these patterns are probably general and might be related to specific functional roles. For instance, the channel water molecules might be involved in facilitating low-frequency vibrations associated with large-scale protein motions, whereas the surface water molecules might be more important for higher-frequency motions that are essential for local adaptations to changes in the protein environment.

The stage is now set for a dynamic interpretation of the protein–water interactions seen in multiple crystal structures of a protein solved under different conditions. Bacteria respond to changes in the environment through response regulators that are activated upon phosphorylation, which leads to a conformational change of the protein [56]. Recent NMR experiments unambiguously showed that the phosphorylation-induced conformational change is represented by a shift in equilibrium towards the active species, which is sampled even when in the unphosphorylated inactive form [57]. This idea could be extended to the way in which surface water molecules sample the protein surface. The water-binding sites seen in any set of multiple crystal structures of a protein solved in different environments can be viewed as a representation of the space sampled by water molecules in any one structure, including the one found under physiological conditions. The equilibrium between the different water-binding sites is shifted in different environments so that the relative population in each site changes with variations in pH, salts, buffer, organic solvents, temperature, humidity level and so on.

This view reconciles the lack of consistency in hydration sites observed in independently solved crystal structures of a protein, for which the detailed data collection and crystal solvent conditions can differ from one structure to another. Differences in hydration between two isomorphous crystal

structures of a protein are probably, in part, a result of fitting water molecules into noise, subjective interpretation of the data, and inherent limitations of the methods. However, much of this difference might actually be real and a consequence of shifts in the populations within water-binding sites that are sensitive to even slight changes in the surrounding medium. This idea is consistent with a dynamic picture in which water molecules serve to mediate interactions between the protein and its environment, and in which the rapid exchange with bulk solvent is a crucial component of adaptability in a shorter timescale than most biological events.

Conclusions

When protein–water interactions were first studied by different biophysical methods such as crystallography, NMR and molecular dynamics, each of the methods offered a unique perspective through narrow, non-overlapping windows. The scopes of these windows are widening as the methods become more sophisticated and the results obtained by any one method are interpreted in light of all others. The current view of protein–water interactions is supported by consistent results from a variety of sources. Water molecules associated with proteins are involved in a variety of functional roles, some of which are specific to a given system, whereas others are general to all proteins. All protein-associated water molecules are in constant exchange with the bulk solvent and this dynamic component is crucial to their function in several ways: water is unlikely to be rate limiting in biological processes; rotational entropy contributes to the binding of highly localized water molecules; the rapid exchange of surface water molecules is key to protein motion; and water is an important mediator for protein adaptability to changes in the environment. The location, dynamics and functional roles of protein–water interactions are now being elucidated within the context of a big picture. One of the next challenges in the study of protein–water interactions is to uncover further the precise molecular mechanisms through which water exerts its influence in protein folding, in protein–protein and protein–ligand interactions, and in the biological function of proteins in general.

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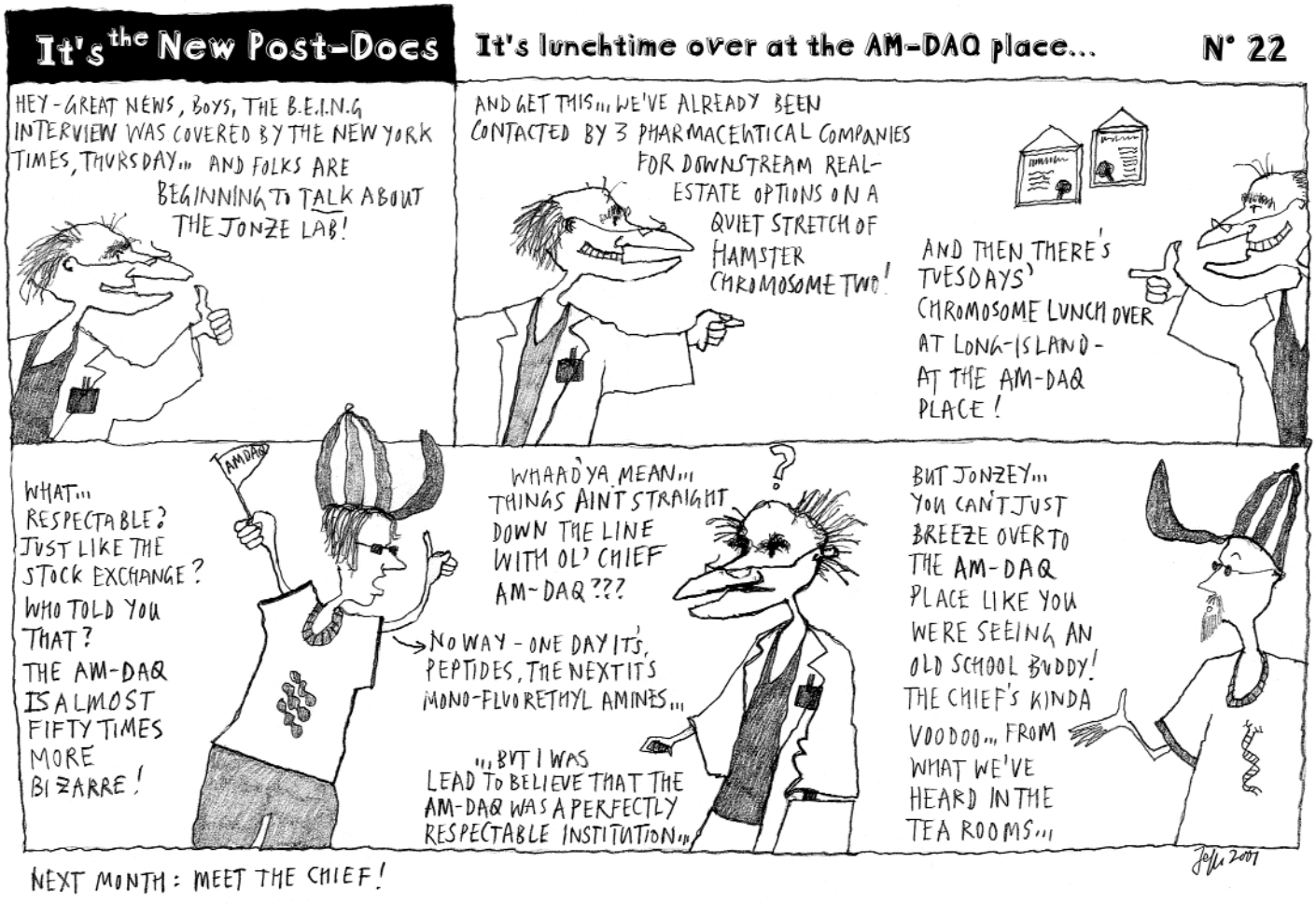

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