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The clear and dark sides of water: influence on the coiled coil folding domain

Abstract: The essential role of water in extra- and intra- cellular coiled coil structures of proteins is critically evaluated, and the different protein types incorporating coiled coil units are overviewed. The following subjects are discussed: i) influence of water on the formation and degradation of the coiled coil domain together with the stability of this conformer type; ii) the water's paradox iii) design of coiled coil motifs and iv) expert opinion and outlook is presented. The clear and dark sides refer to the positive and negative aspects of the water molecule, as it may enhance or inhibit a given folding event. This duplicity can be symbolized by the Roman '*Janus*-face' which means that water may facilitate and stimulate coiled coil structure formation, however, it may contribute to the fatal processes of oligomerization and amyloidosis of the very same polypeptide chain.

Introduction

Proteins evolved in water for billions of years, a ubiquitous solvent indispensable for life. Starting from secondary and tertiary structure formation, water plays a crucial role in the most fundamental extra- and intracellular processes of proteins, thus coiled coil formation also occurs in H_2O. Understanding the real biophysical basis of the protein-water interaction, simpler *in vitro* conditions may also help to decipher the *in vivo* role of it. According to the key importance of water (1), it seems a logical concept to focus on the water and consider any protein as tightly attached to it, which means that also the protein folding is controlled by the solvent motions (2). The bulk solvent fluctuation controls the translational diffusion, intermolecular interaction and also the 3D-dynamics of proteins, while local hydration shell fluctuations are related to internal backbone and side chain dynamics (3, 4). In spite of the central role of water, most publications still neglect or underestimate the role of it mainly focusing simply on the protein itself and thus will receive an uncompleted picture of the true phenomena. Characterizing structure and dynamics, folding and interaction of proteins without considering water is similar to a symphonic orchestra playing without a conductor. This overview represents therefore a stopgap role and by this manner the aqueous reviews are of particular importance (5, 6). Considering 3D structures of proteins built up exclusively from α-helices, they still remain sufficiently versatile to produce very different classes of structures: globular, fibrous and coiled coil [Ref. (7); pp 204–205].

The hydrated forms of these different structures are represented here by myoglobin, by tropocollagen and a coiled coil trimerization motif. In most globular proteins coiled coil domains are short comprising only some ~10–30 amino acid residues. In fibrous proteins, however, the coiled coil structures can be significantly longer: ≥100 aa. Myoglobin was one of the first globular protein structure determined by X-ray some 60 years ago. The analysis of its globular fold (3) reveals that its hydration shell consists of ≈2 layers of water molecules (Figure 1). Quantum mechanics (QM) studies on the stability of the tropocollagen's hydration layer were carried out (8) and have determined that key water molecules form bridges around the [Pro-Hyp-Gly] repeats, where the stability of the water binding places range: -6.1 kcal mol^{-1} to -8.1 kcal mol^{-1} per hydrogen bond.

On the other hand, considering the formation, structure and behavior of coiled coils, excellent reviews

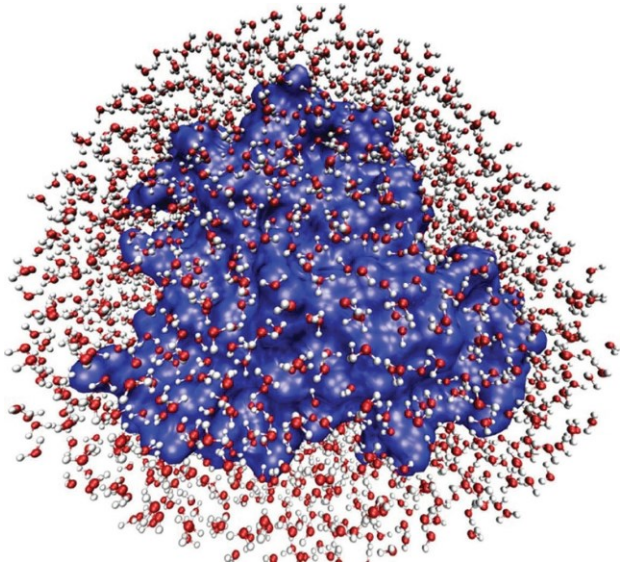

Figure 1: The hydration shell of myoglobin.

CPK diagram (blue surface) with 1911 water molecules. The waters form a shell ≈5 Å thick around the protein. Approximately 200 water molecules are distinguishable from background with high-resolution X-ray crystallography. Reproduced from Frauenfelder et al. (3).

summarize (7, 9, 10) that these domains of the poly- peptide chain are amongst the most versatile protein folding motifs, forming ~10% of all eukaryotic proteins and < 5% of the prokaryotic ones [(7); p 203]. The classical coiled coil structure consists of two right-handed α-helices wrapped around each other into a left-handed super coil, covered with a hydrate shell. There are also varieties of three- to five-stranded super coil architectures (11), however, the parallel two-stranded ones are the most common in living organisms. The simplicity and regularity of this conformational motif have made a system to explore some of the principles of protein folding and stability. A hydrated parallel three-stranded coiled coil structure of a 17-residue-long peptide (ccβ-p) was studied by MD simulations (11) at two temperatures (278 and 330 K). The rate of exchange of water molecules in the first hydration shell ranges from 0.11 to 5.2 ps^{-1} at 278 K and from 0.09 to 5.5 ps^{-1} at 330 K. According to these findings, the MD-calculated residence times show fast exchange between surface water molecules and the bulk water phase. It has been shown (11) that a water bridge between residues Arg 8 and Glu 13 of the neighboring helices might also have an important contribution in stabilizing a trimeric coiled coil structure. Although the residues mutated, these key waters are structurally conserved and together with Arg-Glu residues they determine the three-stranded coiled coil structure. This 'water-bridge-concept' is enforced by most X-ray crystallographic data, too (11).

In classical coiled coils, seven residues form a 'heptad', labeled as *abcdefg* [(7); pp 199–200] a pattern repeated at every second turn of the helix. It is worth noting that the parallel two-stranded α-helical coiled coil, characterized by the 'heptad'-repeat pattern is the most frequently encountered oligomerization motif in proteins. Both *a* and *d* residues of the 'heptad' are mostly hydrophobic (Ile, Leu even Met) and form an interface layer, the central element of this secondary structure. The burial of these hydrophobic spots/residues in a water- filled environment is mandatory, and thus pairing both *a* and *d* residues is the thermodynamic driving force of di- or oligomerization of the polypeptide chains. On the other hand, once buried from water, dispersive interaction operative among them becomes a dominant contribution to overall stability. In fact, most stable coiled coils have a high content of these carefully positioned *a* and *d* hydrophobic residues (7).

On the other hand, residue *b*, *c* and *f* remain in a solvent exposed position even after the coiled coil is formed, while both *e* and *g* remains flanking. As a consequence of this, α-helices are wrapped around each other, with side chains

packed in a 'knobs-into-holes' manner (7), a 3D-topology characteristic to most coiled coils. Interestingly enough in two-stranded coiled coils, one of the residues forming the 'hole' also becomes a 'knob'. It should be noted that according to the 'hydropathy index' the most hydrophobic amino acids are: Ile 4.5, Val 4.2 and Leu 3.8 (12).

Despite the frequent occurrence of □-helices in globu- lar proteins, even the matured α-helixes become unstable in aqueous solution, and thus unfold (13). However, there are ways to stabilize dissected α-helices in water by packing them together through hydrophobic side chain interaction, concluding in a coiled coil. While the simplest way is to form a two-stranded coiled coil structure described above, an additional option based on strict primary sequence restriction (charged/ionic residues primarily) is to form a charged single α-helix: CSAH (14). In fact, this self-standing secondary structural element was discovered by bioinformatics, by assigning residue patches of complementary charges in a physiological environment: e.g.: EEEEKKKKEEEE or - - - - + + + + - - - -. Prediction methods revealing CSAH domains operate by primary sequence analysis looking for consecutive complementary charged residues (15). Although in the latter secondary structure type there are no hydrophobic residues to 'hid', hydration of the salt-bridges still occurs and contributes to overall stability.

Formation of coiled coil domain mediated by water

Formation of the hydrated coiled coil conformation in proteins is one of the fundamental examples of the bio- logical self-assembly, as both the spatial distribution and strength of the contacts are effecting their thermostability as well as their folding kinetics. Residues in coiled coils have a polar/nonpolar periodicity and it is this amphipathic nature of the assembly that drives them to associate at their hydrophobic interface (local hydrophobic col- lapse). The fully or partly helical single polypeptide-chain coated by water (the dark side) is the coiled coil's 'building brick'. However, its hydrated chain is dehydrated (or loosely hydrated) at some positions (the clear side) a necessary condition for domain formation. In general: molecular interactions of proteins have to fulfill changes in their hydrate layer for physical contact of the proteins. The large number of polar and charged residues within the poly- peptide chain involves the presence of several, strongly structured water molecules and *vice versa* the abundance of hydrophobic patches, manifested by apolar side chains together make possible coiled coil formation. However, polar and charged residues located on the surface of the domain are responsible for the aqueous solubility of the overall nanosystem and thus, the ratio of the hydro- phobic residues gluing polypeptide chains together and hydrophilic ones determines whether a coiled coil domain could be formed. The water's energy landscape of such a nanostructure is governed by thermodynamic and kinetic factors, in which the relative enthalpy (ΔH) and entropy (ΔS) terms associated with water should be considered. From this viewpoint, three consecutive events are to be taken into account*:* (i) dehydration of the single polypeptide chain, (ii) accompanied by the chains' fusion and (iii) concluded by the hydration of the two- or multi-stranded coiled coil domain. The first and unfavorable dehydration step has an enthalpy/entropy cost caused by the great 'energy consumption' of the leaving first and second layer water shells from the surface of the helix. However, this can be compensated for by the gain of the water's association and favorable enthalpy/entropy terms. The increase of entropy means the spatial randomization of the water molecules, i.e. the ordered solvent molecules of the hydrate shell are moving to the more disordered bulky water as medium. In this context an important review of particular interest was published by Kinoshita (16), on the role of translational water entropy in self-assembly processes. Although, the fusion of two or more polypeptide chains is an entropically unfavorable process as 'ordering' occurs, however, there is also a positive term, enthalpy gained from the emerging hydrophobic interfaces. These interactions are combined with the exclusion of water from these apolar surfaces and by this manner maximization of water's entropy will occur. The latter favorable effect is to be added to gains from fusion's enthalpy (heat). The third process, the stabilization of the coiled coils by hydration means again an entropy loss paid by

the water binding energy. Because the dehydration of helices can be considered as a key step in the folding of coiled coil structures, the folding intermediate has been investigated on the *C*-terminal of the 14-residue-long truncated part of the GCN4 peptide. Two relaxations were revealed with 0.2 and 15 μs time constants, as has been detected by micro- second melting of the coiled coil peptide. These constants are suggested to reflect the melting times of hydrated and non-hydrated helices. These microsecond times were monitored successfully by time-resolved T-jump/UV Raman spectroscopy (17).

Coiled coil stability

Length of coiled coils is a decisive factor on their stability: long coiled coils are usually unstable. Coiled coil stability in water was calculated using molecular dynamics (MD) (18) as a difference of the appropriate free energies, ΔG, derived for values of the coiled coil and α-helices determined separately, in an explicit solvent model of a 72 residues long domain. The residue based coiled coil stability of about -1.2 kcal/mol, $\Delta G_{\text{residue}}$, is a good indicator of the sum of electrostatic and dispersive interactions operative between residues, with an entropy term of about -0.3 kcal/mol per residue. The coiled coil stability is inversely proportional to the polypeptide chain length and directly to the side chain salt-bridges of residues at positioning *e* and *g* (electrostatic interaction between *e* of one 'heptad' and *g*' of the following 'heptad' on the other helix are operative) (11, 19). For the electrostatic interaction part of the above shown -1.2 kcal/mol sum, the following values are given in the literature: ~-0.37 kcal/mol (9, 19). For the most hydrophilic amino acids the 'hydropathy index' gives about -4.5 for Arg and -3.9 for Lys (12).

Stabilizing and destabilizing clusters

Series of two-stranded coiled coils were designed and synthesized to determine the nature of the effects specifying a stabilizing or destabilizing cluster in the hydrophobic core (20). The results showed destabilization already caused by a single Leu to Ala mutation in the hydropho-bic core, on formation of a three-residue-long cluster (ΔT_m of 17–21°C). This Leu to Ala substitution contributes to $\Delta\Delta G$ 2.7–3.5 kcal/mol destabilization energy. Nevertheless, these results cannot be considered as a big surprise, if we take into account the difference of the 'hydropathy index': Leu 3.8 and Ala 1.8, respectively (12), which means also that Ala is the least hydrophobic one of the nonpolar amino acids. Beside this hydrophobic decrease, the large stereochemical difference between the Leu vs. Ala side chains should also be considered. In the formation of clusters, the role of water is also important. This means that the small Ala residue also left enough space for hydrating the coiled coil chains, i.e. for destabilizing the clusters (dark water's side). However, in the case of Leu there is an entropy gain (16) by moving the ordered water molecules from the hydrophobic environment to the more disordered bulky water phase and in this manner stabilizing the system (clear water's side). Any further Leu substitution with Ala, which is increasing the size of the destabilizing cluster to five or seven core residues, has little more effect on stability (ΔT_m of 1.4–2.8°C). These data show that Leu contribution to protein stability is context-dependent on whether the hydrophobic moiety is in the neighborhood of a stabilizing cluster. A 3-membered cluster is a good example for such a context- dependency which was designed from two Leu and one Ala residues (21). Although, the Leu-Ala-Leu cluster interspersed by Ala did not produce any gain in stability, the Leu-Leu-Ala or Ala-Leu-Leu structures did show a stability gain of 0.9 kcal/mol. Also native coiled coils of long chains: the tropomyosin of 284 residues and the coiled coil domain of the myosin rod of 1084 residues were studied for the as above (20). In the hydrophobic core of both proteins three types of clusters were present; namely stabilizing, destabilizing ones and intervening regions including both stabilizing and destabilizing

residues, as well. In the native coiled coils discussed, Leu is the most abundant residue in the hydrophobic core of stabilizing clusters and also in the intervening regions. Similarly, Ala is the most predominant residue in the destabilizing clus- ters. In all cases Leu or Ala residues are evenly distributed between the positions *a* and *d*.

For the modeling the stabilizing and destabilizing clusters, the cortexillin I protein's dimerization rod domain was used (Figure 2). The 18-heptad-repeat-long α-helical coiled coil (22) domain of cortexillin I from *Dictyostelium discoideum* is a tightly packed parallel two- stranded coiled coil.

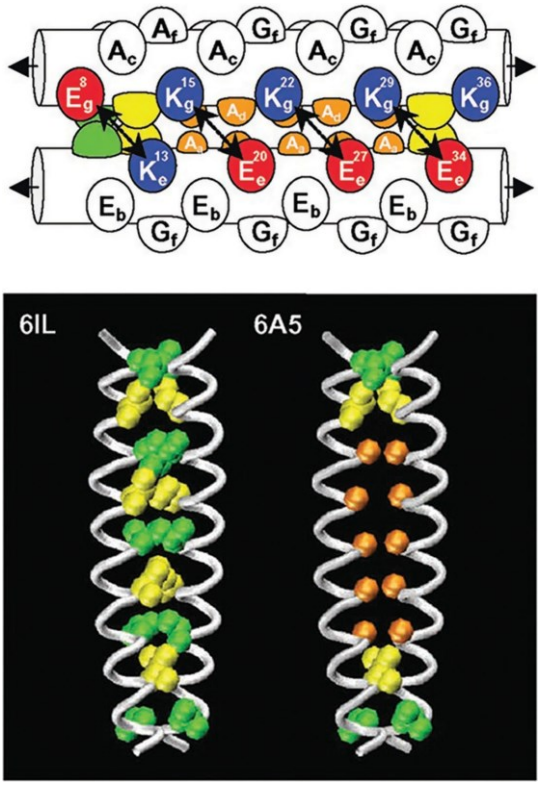

Figure 2: Models of coiled coils with either a stabilizing cluster (Ile, Leu) or a destabilizing cluster (Ala) in the hydrophobic core, which are also the clear and dark sides of water.
Top panel, a schematic model of a 5-alanine residue cluster located at the center of peptide 6A5 (residues 8–36). The five small consecutive alanine residues (brown) on the non-polar surface of an amphipathic helix pack 'knobs-into-holes' onto the alanines (brown) on the other amphipathic helix. The alanine residues are smaller compared with the other large hydrophobic residues (Ile, green, and Leu, yellow). Inter-chain electrostatic interactions (*i* to *i'* + 5) between Lys (blue) at position ***g*** and Glu (red) at position ***e***' are shown by the double-headed arrow. Bottom panel, a side view of space-filling models depicting the different side chain packing interactions in a stabilizing cluster of Ile and Leu residues in the hydrophobic core (6IL) and a destabilizing cluster of five Ala residues (6A5) in the hydrophobic core, at positions ***a*** and ***d,*** of a model two-stranded parallel coiled-coil (the cortexillin dimerization domain, 1D7M was used to build the model). Side chains of Ile (green), Leu (yellow), and Ala (brown). Reproduced from Ref. (20).

This domain at 344–352, is exceptionally long where the number of the *C*-terminal residues are. An interchain attractive ionic interaction, which provided some additional stabilization, is mediated by Lys and Glu residues (in the top panel of Figure 2). The side chain interactions of the Leu-Leu and Ile-Ile pairings in the coiled coil core were represented by space-filling models. A significant increase of the destabilization effect is observed in a coiled coil analog (6A5) as a destabilizing cluster of five Ala residues having poor van der Waals contact and spatial gap, leaves much more space for water (Figure 2, bottom panel).

Degradation of the coiled coil domain

It is an interesting and fundamental question which way the stability of coiled coils can be lost, leading to partial or full disassembly, i.e. degradation of this otherwise exceptionally stable protein structure. To explore this exciting question, simulations of a trimeric coiled coil molecule have been studied in explicit water solvent and extreme environments such as elevated temperatures and/or in detergent (urea, guanidinium chloride) (23). This trimeric structure contained three homo helical strands each composed of 29 amino acids. This structure is stabilized by the hydrophobic Val and Leu at positions *a* and *d* as well as by a salt bridge forming Glu^{-}.^{+}Lys at positions *e* and *g*. The results confirmed that the □-helix unfolding is the first event which helps then the coil to unzip. In the steered molecular dynamics (SMD) simulation studies, a helix unfolding revealed that the coiled coils are super- elastic protein bundles. On the other hand, the MetaD simulations with 2D sampling (24) served to define the free-energy landscapes of helix unfolding, coil unzipping, and also the coupling of these two processes. It is shown, that once the energy barrier of unfolding has been passed over and a segment is unfolded, the extra energy required for the unzipping is practically very low. Nevertheless, the final driving force of such a stepwise degradation should be the self-stabilization of the individual polypeptide strands by effective hydration. Summarizing the MetaD results; the disassembly's free-energy of a single coil from the trimer has been estimated as -28 kcal/mol (24). This value agrees fairly with the experimental unfolding free energy of a similar three-stranded coiled coil, as being -18.4 kcal/mol per helix (25). Naturally, the energy values of coiled coils may deviate from these ones, depending on the size, the number of strands and the peptide chains' sequences.

Watertight seal

Not only water soluble globular proteins, but also coiled coil structures maintain their backbone hydrogen bonds watertight to ensure their structural integrity. This protection is achieved by sealing and thus fine tuning thebackbone amide-carbonyl hydrogen bonds. The tighter the backbone structure is the more they are buried or wrapped around by nonpolar sidechain groups. This strategy efficient during molecular evolution brings in sub-nanoscale surface ruggedness and represents a tunable molecular machinery of protecting H-bonds in an otherwise H-bond weakening hydrophilic media (26). In other words, layering brings in the solution into molecular architecture, as a H-bond weak in a water is strengthened by 'moving it' into a locally hydrophobic environment by sealing layers from each other.

Designed coiled coil motifs

A great advantage of any designed coiled coil motif is its applicability for a wide variety of *in vitro* and *in vivo* bio-chemical purposes. The way the particular system was designed strongly influences the macroscopic character, thus by the ratio of polar and apolar residues within the optimized nano-construct and their hydration. The critical review (27) discusses the potential of coiled coil peptide structures for the development of responsive, self-assembling and bioactive materials. *Inter alia*, also the role of designed coiled coils is stressed in the same review article (27). Concerning the designed coiled coil motifs, Woolfson and his group developed the self-assembled cage-like nanoparticles (SAGE) concept, which offers routes to closed systems with the potential for encapsulation (28). This exciting idea means the SAGE, which can be made from short, *de novo,* α-helical coiled coil peptides. These tools can be used as vehicles for drug and biomolecular delivery, and also as frameworks for protocell development. Also

another article has been published of similar targets of coiled coil peptides with self-assembly properties (29). Obviously, they are designed and fine-tuned accordingly, by simply controlling hydrations determined by different purposes. In summary, in the era of foldamers, it can be concluded that coiled coils are one of the best subjects of protein design, where either natural or non-natural amino acid residues could be used as *Lego* elements for helix design of enforced stability.

Intrinsically disordered proteins (IDPs)

Beside the above listed different structures of the protein's chain, there could be also large disordered segments of the peptide, i.e. which lack of a well-structured 3D fold [see also the IDP chapter of Ref. (6)]. These are significant fractions of proteomes, especially the eukaryotic ones. IDP domains contain particularly polar and charged, the strongly hydrated residues, mean the peptide chain's stabilization. This is just the opposite of the coiled coil structure with apolar dominance in its domain, i.e. it rep- resents a softer stabilization by the hydrate shell. Therefore, the coiled coil domain should be fine-tuned for the hydrophobic/hydrophilic chain ratio, namely, whether the coiled coil forming part or the hydration part will dominate.
An interesting method has been published, which is based on the overlaps between disorder, coiled coil and collagen predictions in complete proteomes (30). It has been pointed out that fibrillar protein motifs such as the coiled coils and collagen triple helical segments can be identified as intrinsically disordered, considering the full proteomes.

Crystal structure of short coiled coil protein (SCOC)

The crystal structure of the human short coiled coil protein (SCOC) has been determined (31). Interestingly, it can be seen as two different coiled coils in the crystal structure (Figure 3), which indicates a high conformational flexibility. This plasticity is explained by researchers (31) with the high number of polar and charged residues at the *a/d*-heptad positions. Considering also the structural water molecules (Figure 3), it can be suggested that these hydrate molecules increase the stability of both conformers.

Expert opinion and outlook

Although the simpler *in vitro* conditions may also help to understand the *in vivo* ways, the question of what is the proteins' situation in a cell should be kept in mind. First of all, the most important question is the relation- ship of proteins and water in an extremely crowded molecular environment. Namely, macromolecules also including the coiled coils are present in the crowded cell at an extremely high molar concentration, ranging from about 300 to 400 mg ml^{-1} (32). This means, that the above discussed questions are very important with respect to the cellular environment, where water is available

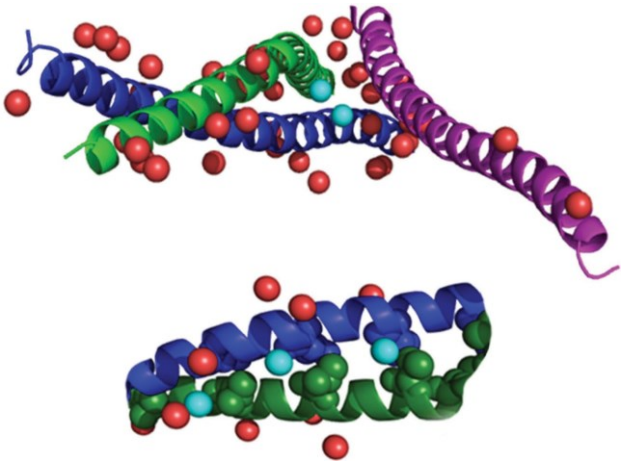

Figure 3: Crystal structure of the human short coiled coil protein (SCOC).
Top panel: the three chains form two and three stranded parallel coiled coils (blue, green and magenta) with structural water mol- ecules (red). Two water molecules (cyan) occupy the proximal side to the third coil (magenta) at the open side of dimer (blue-green). Bottom panel: magnification shows additional water molecules (cyan) at the interface of the dimer (blue-green) formed by Leu residues as 'knobs' (shown as spheres). Reproduced from PDB entry 4bwd; PMID: 24098481.

but the amount of bulk water is different from case to case. Therefore, it should be considered that the cellular functions of proteins are strongly dependent from their interactions with proteins of their neighborhood, also including their affinity to each other. Currently an important method has been published where the effective concentrations within these intra molecular inter- actions can be systematically varied (33). Namely, the tool is a modular encoded linker; the single α-helix of different lengths, e.g. ER/K [see: CSAH (Ref. (14))], which enables regulation of the protein-protein interactions. Recently some of us were involved in the discovery of the important role of an anti-parallel two-stranded coiled coil structure, an integrated part of the podocin protein, linked to a special nephrotic syndrome (34). In molecular modeling studies, this dimer was investigated by MD simulations and its water shell was also calculated. As an optimistic speculation about complicated diseases like this coiled coil based one, one could imagine a personalized, faster investigation and treatment of similar cases, in the coming 8–10 years!

Acknowledgments: We thank András Láng for fruitful discussion and the financial support of the Hungarian Scientific Research Fund (OTKA NK101072).

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