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# The effect of water dynamics on conformation changes of albumin in pre-denaturation state: photon correlation spectroscopy and simulation

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## Abstract

Water is essential for protein three-dimensional structure, conformational dynamics, and activity. Human serum albumin (HSA) is one of major blood plasma proteins, and its functioning is fundamentally determined by the dynamics of surrounding water. The goal of this study is to link the conformational dynamics of albumin to the thermal motions in water taking place in the physiological temperature range. We report the results of photon correlation spectroscopy and molecular dynamics simulations of HSA in aqueous solution. The experimental data processing produced the temperature dependence of the HSA hydrodynamic radius and its zeta potential. Molecular dynamics reproduced the results of experiments and revealed changes in the secondary structure caused by the destruction of hydrogen bonds in the macromolecule's globule.

## 1. Introduction and background

The stability of proteins macromolecules, that is the capacity to preserve their biological functions at certain conditions (pH, temperature, the structure of external environment) is an important physicochemical feature. The stability of a protein is defined by its structure as well as the interactions with surrounding molecules. The solvation properties of peptide and protein aqueous solutions have been investigated by various techniques and the fact of the ultimate connection between the dynamical properties of the environment and the protein conformational motions was supported in many experimental and simulation works [1-8]. Recent experimental studies [9-31] focused on the interactions of hydration water with solute protein molecules. These techniques allowed to investigate the static and dynamic properties of the hydration water and their relation with the properties of the hydrated molecules.

Quasi-elastic (QENS) and inelastic neutron scattering (INS), gave a possibility to study rotational and translational motions at the picosecond timescale, and the vibrational density of states of water surrounding proteins and peptides [10–12]. The fast collective dynamics of the biomolecule–solvent mutual interactions have been investigated by Brillouin neutron scattering [13]. The combination of Molecular Dynamics (MD) simulation and QENS has been performed in [14]. It was shown that water dynamics appear spatially heterogeneous in the first hydration shell. Below the critical hydration level the water translational and rotational dynamics are slow in comparison with the bulk water. Molecules of the first hydration layer form structures like a barrier for outer water molecules. The results obtained by INS [15–17] in pico- and nanosecond timescale indicate that the dynamics of the whole biomolecule are affected by the hydration water and surface protein motions are strengthened by the solvent matching those of the breaking and re-formation of hydrogen bonds.

THz spectroscopy has been used to investigate the influence of the water structure on peptides [98] and proteins [19-23]. Based on this technique, the radii of the hydration shells have been detected for such proteins as lysozyme, myoglobin, BSA [20, 23] and ubiquitin [21]. The estimations of the hydration shell thickness obtained in [23] are substantiated by NMR studies [24,25], X-ray scattering [26], and neutron scattering [27]. It was shown also [19, 22] that water molecules are arranged to form a gradient around 'active' sites and to provide reactions via the active site's 'hydration funnel'. The change in water dynamics [24, 25] at the hydration shell can be extended up to 5-8 water layers, which is longer compared to previous findings.

The stabilising effects of buried water on the protein structure have been discussed in [28-31]. Single water molecules stabilise protein by filling internal cavities. Backbone residues of loops and other polar atoms interact with the water molecules buried in the protein core. Buried water may also act as lubricant to favour loop dynamics.

Investigations carried out by FTIR spectroscopy have shown that water molecules connecting proton donor to acceptor sites or electron donor to electron acceptor sites facilitate both proton-transfer processes [32] and electron-transfer reactions [33-35]. In both cases, the transfer is faster where the linking water molecules possess stronger hydrogen bonding. Besides, a cluster structure of H-bonds in water plays a catalytic role in water oxidation in photosynthetic oxygen evolution [36].

The intra and inter-helical hydrogen bonds are important determinants of the local protein structure, dynamics, and water interactions. Molecular dynamics simulation and bioinformatics revealed that perturbation of the inter-helical hydrogen bonds can be coupled to rapid changes in water dynamics [37]. In some cases the water wire may be affected by the binding of various ions [38].

Thus the cited studies demonstrate reversible coupling of water dynamics with protein conformational stability. However it remains unsolved what initially plays the major role in conformation dynamics of proteins: surrounding molecules in the 'hydration shell' or physical properties of macromolecule. In other words "...who is the master and who is the slave in the water–macromolecule interaction..." [9].

We directed our attention to one of the aspects connected with the dynamics of surrounding water under temperature effect. As it was shown in a number of papers [39-43] the global hydrogen bonding network in water disintegrates into an ensemble of weakly interacting clusters: dimers, trimers, tetramers, *etc* in the vicinity of a specific temperature point  $T_H = 42^\circ\text{C}$ . In other words, the spatial connectivity between linear molecular chains is disrupted. The value of  $T_H$  coincides with the physiological temperature at which the thermal denaturation of many proteins takes place. As denaturation (unfolding) is a form of conformational dynamics of proteins, the dynamics of water in this region can have an effect on the protein's conformational changes. Particularly, it was shown [44] that the conformational changes of human serum albumin (HSA) in the temperature interval  $25\text{--}55^\circ\text{C}$  is a process subdivided into three sub-transitions which might be related to the links between the three structured domains of HSA. The sub-transitions are sequential and separated by a temperature interval of approximately  $9^\circ\text{C}$ . The temperature of the last sub-transition coincides with  $T_H$ , which is the maximal value of fever in human body at pathological conditions. In our study we tried to correlate the conformational dynamics of HSA (as one of major blood plasma proteins) with the dynamic properties of water in the temperature range  $25\text{--}45^\circ\text{C}$ , especially in the vicinity of  $T_H \approx 42^\circ\text{C}$  as the threshold temperature of protein conformational stability. The size and hydration were related to the structural changes of the protein molecule at different temperatures.

The structure of HSA has been determined by X-ray crystallography at high resolution [45]. Several factors like pH, temperature, etc can affect its structure and dynamics [46]. HSA plays a special role in transporting metabolites and drug molecules throughout the vascular system and also in maintaining the osmotic pressure of plasma acids, metabolites, and binding of different ligands [47–50]. HSA is a single chain protein with 585 amino-acid residues with a predominant  $\alpha$ -helical heart-shaped structure [48,51].

## 2. Methodology, results, and discussion

### 2.1 Photon correlation spectroscopy

We have applied photon correlation spectroscopy (PCS) [52] to obtain the temperature dependences of the hydrodynamic radius and zeta potential. This technique has much research interest due to its wide use in studies of proteins and DNA aqueous solutions [53], aqueous solutions of poly(ethylene glycol) [54], trehalose aqueous solutions [55-57], alcohols and semidiluted polymer solutions [58–60], cluster growth in aqueous sugars and glass-forming aqueous glucose solutions [61,62]. The comparative studies of PCS and incoherent quasi-elastic neutron scattering (IQENS) on hydrogenous systems such as polymeric aqueous solutions, alcohols and homologous disaccharide aqueous solutions have been presented in [63, 64]. PCS technique showed that water behaved as a good solvent and furnished the temperature evolution of the hydration number. IQENS revealed the presence of entangled water and evidenced the effects of H-bond on the diffusive motions. The comparison of the rotational relaxation times, obtained by IQENS and PCS separated the self particle contribution from the collective one.

In the process of measurement we obtain the second order correlation function or so called normalised autocorrelation function 'intensity-intensity', defined as

$$G^{(2)}(\tau) = \langle I(0)I(\tau) \rangle / \langle I \rangle^2, \quad (1)$$

where,  $I$  is the total scattered intensity. In the case of non-interacting particles the relationship between the second order and the first order correlation functions takes place

$$G^{(2)}(\tau) = 1 + \alpha |G^1(\tau)|^2, \quad (2)$$

For diffusing monodisperse spherical particles, the normalised field autocorrelation function takes a simple exponential form

$$I(\theta) = I_0 \exp\left(-\frac{2\tau}{D}\right) \quad (3)$$

where,  $D$  is the diffusion coefficient,  $\tau$  is time,  $q = 4\pi n/\lambda \sin\left(\frac{\theta}{2}\right)$ ,  $\lambda$  is the vacuum wavelength of the laser,  $n$  is the refractive index of the solution,  $\theta$  is the scattering angle.

**Hydrodynamic radius.** As it was noticed in [63,64] at low concentrations of proteins the diffusion coefficient can be connected to the macromolecular hydrodynamic radius by the Einstein-Stokes relation

$$R_H = kT/6\pi\eta D, \quad (4)$$

where,  $\eta$  is the dispersant viscosity,  $T$  is temperature,  $k$  is the Boltzmann constant. However, polymeric solutions with macromolecules at high molecular weight can be considered as polydisperse, in this case a standard cumulant analysis is more suitable for processing the data [52,60]. This technique used in the spectrometer Zetasizer Nano ZS (ZEN3600) [52]. Aqueous solutions of HSA at concentrations 1mg/ml with NaCl ions (0,075mol/l, 0,15mol/l, 0,225mol/l) were investigated. The measurements were performed in the temperature interval 25–50°C with the 1°C step. The set of measurements were carried out using commercial spectrometer Zetasizer Nano ZS (ZEN3600).

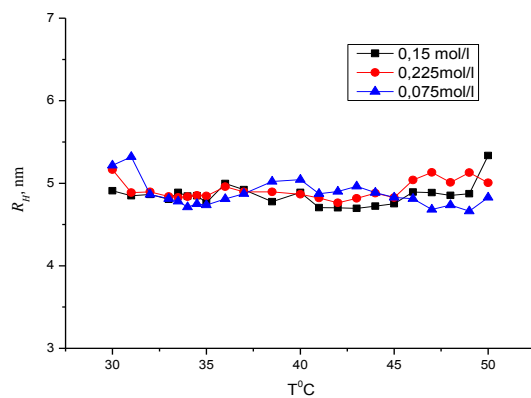


Fig.1. Temperature dependence of the macromolecular hydrodynamic radius  $R_H$  at different concentrations of NaCl

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The cumulant analysis gives a good description of the radius that is comparable with other methods for spherical, reasonably narrow monomodal samples (monomodal sample contains only particles of one size), that is with polydispersity below the value of 0.1. For samples with a slightly increased distribution width, the Z-average size and polydispersity gives values that can be used for comparative purposes. For broader distributions, where the polydispersity is over 0.5, it is unwise to rely on the Z-average mean, and a distribution analysis should be used to determine the peak positions. In our study the index of polydispersity was  $0.1 \pm 0.05$ . The values of the hydrodynamic radii obtained from the Z-average diffusion coefficient (formula (4)) in the temperature interval  $30 - 50^{\circ}\text{C}$  are presented in Fig.1. The value of the hydrodynamic radius is unchanged in the investigated temperature range. The concentration of NaCl ions does not affect  $R_H$ , and the value equals approximately  $5 \pm 0.3$  nm, that does not exceed the experimental precision of Zetasizer (0.5nm).

**Zeta potential (ZP).** In order to correlate water dynamic in the ‘hydration shell’ with conformational changes of a macromolecule we investigated the temperature influence on ZP of an aqueous solution. The ZP can be considered as a key parameter that describes the surface charge on protein and characterises its hydration layer [65-69]. The charge distribution at the macromolecule surface affects the dynamics of ions in the surrounding interfacial region. By convention the liquid layer surrounding the macromolecule may be subdivided into two parts: an inner region (the Stern layer), where surrounding molecules and the ions are strongly bound with the protein macromolecule and an outer, diffuse, region where they are less firmly attached. An electrical double layer surrounds each macromolecule. In the Stern layer the protein macromolecule move together with the surrounding molecules and the ions, beyond the inner layer, in the diffusive region, the surrounding molecules do not drift together with the macromolecule. There is a boundary between the Stern and diffusive layers. This boundary is the surface of hydrodynamic shear or so called slipping plane. The potential which exists at this boundary is the ZP.

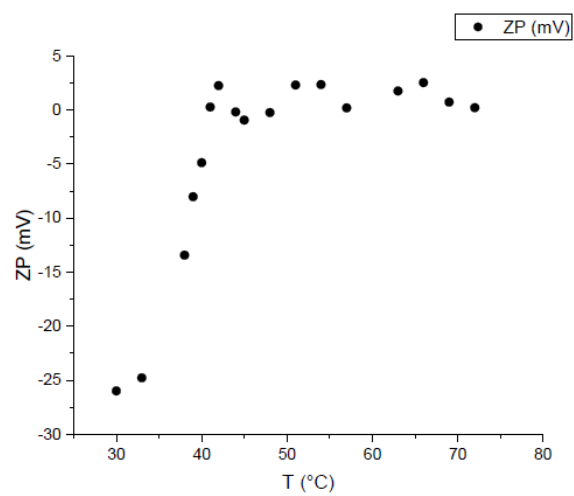


Fig.2. Temperature dependences of the zeta potential (ZP) for albumin aqueous solution

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The ZP was obtained using electrophoretic light scattering technique based on the measurement of electrophoretic mobility. The ZP of the particle can be calculated by the application of the Henry equation

$$\mu_r = \frac{2\varepsilon_r\varepsilon_0\zeta f(Ka)}{3\eta},$$

where,  $\mu_r$  is the electrophoretic mobility,  $\varepsilon_r$  is the relative permittivity/dielectric constant,  $\varepsilon_0$  is the permittivity of vacuum,  $\zeta$  is the zeta potential,  $f(Ka)$  is the Henry's function and  $\eta$  is viscosity at experimental temperature. Electrophoretic determinations of the zeta potential are most commonly done in aqueous media and moderate electrolyte concentration and two values of  $f(Ka)$  are generally accepted as approximations, either 1.5 or 1.0  $f(Ka)$ . The accuracy in defining the electrophoretic mobility is  $0.12 \cdot 10^{-8} \text{m}^2/\text{V}\cdot\text{s}$  (for particles diameter in measurements range: 3.8nm – 100 microns), hence the error for the zeta potential does not exceed approximately 5%.

Temperature and pH are most important factors affecting ZP. In our study we have been carried out the measurements at fixed pH 7 and concentration of NaCl (0,075mol/l). This value of pH corresponds to the value of the zeta potential approximately -27mV at the temperature 30°C. The values of the ZP in the temperature range 25-70°C are presented in the Fig. 2.

Based on the presented data we can conclude that the change of the ZP with temperature is a two-stage process. On the first stage we observe the decrease of the ZP magnitude: starting from -27mV and temperature 35°C up to zero value of the ZP in the vicinity of the temperature point 42°C. Thus, zero value of the ZP or the isoelectric point (IEP) is located in the close proximity of 42°C. It should be noted that the system is not particularly stable in a region surrounding the condition of IEP. Then the value of the ZP varies from -2.5mV to 2.5mV up to the denaturation temperature.

## 2.2 Simulation

The dynamics of HSA macromolecule in aqueous solution at different temperatures was simulated using the multifunction package HARLEM, which combines the methods of molecular and Brownian dynamics. The crystallographic structure (PDB entry 1AO6) [45] of albumin at the resolution of 2.5 Å was used. The albumin macromolecule consists of 585 amino-acid residues, 17 pairs of disulfide bridges, and one free cysteine (Fig. 3).

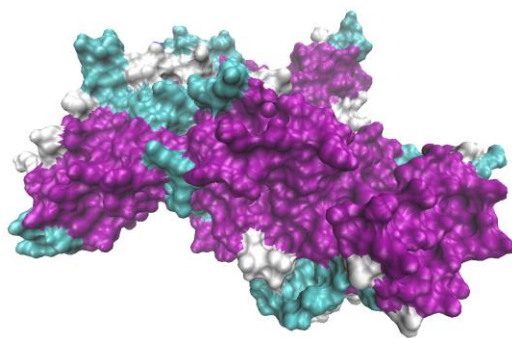


Fig.3. Visualization of the albumin crystallographic structure from PDB [45].  
Components of the regular secondary structure are shown:  
 $\alpha$ -helix – purple, turn – cyan, coil – white

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The initial structure for the simulation was prepared by solvating the protein macromolecule (5672 atoms) with 118722 water molecules of the fixed density in a cubic cell with the edge of 20 nm. To avoid additional stresses, structure energy minimisation in vacuum was implemented over 10000 steps for each model. Minimisation of protein with water was carried out over 20000 steps using the descent method and the conjugate gradients scheme. The Langevin thermostat was used to fix momenta with a collision frequency of 2:0. The initial temperature was set 4K and then increased by 20K at every simulation step up to 304K. The preparatory simulation duration was 50 ps.

At the next stage, the obtained optimised model was investigated by molecular dynamics (MD). MD simulations were performed for 50 ns for every model with given temperature. The data were saved every 10 ps. Periodic boundary conditions were applied with a constant pressure of 1 atm, using the Amber 99 force field, the cutoff for non-bonded interactions was set to 12 Å. The TIP3P water model with unfixed inner degree of freedom was used. Electrostatic interactions were calculated using the particle mesh Ewald molecular dynamics (PMEMD) [70–72]. Intramolecular bonds were constrained using the SHAKE algorithm [73]. The Berendsen thermostat with the time constant (relaxation time) of 2 ps was applied in order to maintain temperature.

**Gyration radius.** The values of the gyration radius was calculated in the above mentioned temperature range (Fig. 4). The radius of gyration was defined by the formula given below on the basis of the crystallographic data [45] for atom coordinates and their changes under temperature effect

$$R_g^2 = \sum m_i r_i^2 / \sum m_i, \quad (5)$$

where,  $m_i$  is the mass of the  $i$ -th atom in the macromolecule and  $r_i$  is the distance from the centre of mass to the  $i$ -th atom.

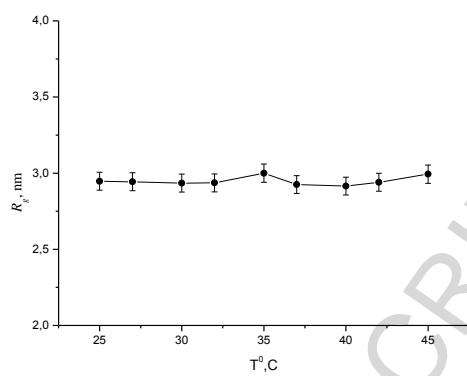


Fig.4. Temperature dependence of the gyration radius calculated from the simulation data

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Both hydrodynamic and gyration radii remain unchanged within random fluctuations in the temperature range 25-50<sup>0</sup>C. As one can see they approximately differ from each other by 1.5 nm, which coincide with the values of the hydration shell reported in [20,23]. This difference has natural explanation. By definition the hydrodynamic radius measured by PCS is the radius of a hypothetical hard sphere that diffuses with the same speed as the macromolecule. In reality, such hard sphere does not exist. Macromolecules in solution are non-spherical, dynamic, and solvated. The hydrodynamic radius calculated from the diffusional properties of the macromolecule is indicative of the *apparent* size of the dynamic hydrated/solvated particle. Whereas, as it was mentioned above, the gyration radius is the average distance from the center of mass to the macromolecular surface [74].

At the next stage we have analysed the temperature effect in the vicinity of 42<sup>0</sup>C on the distances between the sulfur atoms (S $\gamma$ ) in pairs of cysteines and COO-groups of asparaginic or glutamic acids and NH<sub>3</sub>-groups of lysine or arginine (salt bridges).

**Disulfide bonds (bridges)** or SS bonds are covalent bonds formed from two thiol groups [75,76]. The effect of SS bonds on the protein structural stability, dynamics, correlated motion, and flexibility of local residues has been investigated experimentally [77-80] and computationally [81-87]. Three necessary conditions were formulated [88] for cycteins pairs to be involved in SS bonds: a bond length of 2.05 $\pm$ 0.3 $\text{\AA}$ , an angle between the sulfur atoms and  $\beta$ -carbon atom of 103<sup>0</sup>, and an orientation of the two  $\beta$ -carbon atoms corresponding to a rotation of  $\pm$ 90<sup>0</sup> about the SS bonds. In [87] atomistic MD simulation of HSA with and without SS bonds have been carried out. The authors have shown that the removal of SS bonds mainly destabilises the tertiary structure, and the removal of some of them affected the secondary structure of the macromolecule.

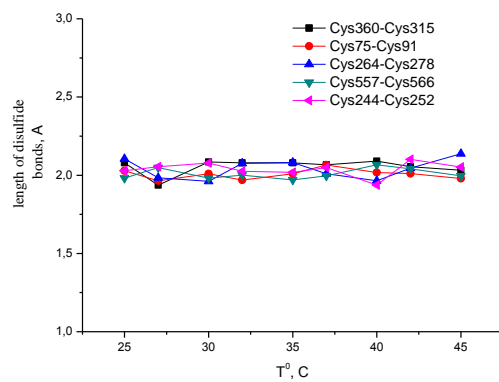


Fig.5. The temperature dependence of distances between  $S_{\gamma}$  atoms in cysteines pairs.

Initially, the equilibrium distances between  $S_\gamma$  atoms in the presence of SS bonds were defined as  $2.05\pm 0.03\text{\AA}$  (1AO6 [45]). Destabilising changes occurred after 35 ns of simulation. The distances between  $S_\gamma$  atoms reached above  $5\text{\AA}$  and mostly below  $10\text{\AA}$ , but in some pairs of cysteine distances increased up to  $15\text{\AA}$ . The results of simulation for distances between  $S_\gamma$  atoms are presented in Supplementary information and some of them in Fig.5. As one can see SS bonds are not changed by temperature as the lengths of disulfide bridges are stable and correspond to one of the Creighton requirements [88], namely the distances between  $S_\gamma$  atoms to be  $2.05\pm 0.03\text{\AA}$ .

**Salt bridges** represent a combination of two noncovalent bonds: the hydrogen bond and the electrostatic interactions. Noncovalent bonds are considered to be relatively weak interactions, but these interactions add up to make an important contribution to the overall stability of proteins [89]. The salt bridge derives from the anionic carboxylate of either aspartic acid or glutamic acid and the cationic ammonium from lysine or the guanidinium of arginine [90]. The distances between the residues participating in the salt bridge are considered important. The length of salt bridge is approximately  $4\text{\AA}$ . Lengths greater than this distance do not qualify as forming a salt bridge [91]. We have analysed the temperature effect on the salt bridge length for 40 pairs of residues. Most pairs of residues have distances of about  $4\text{\AA}$  with a standard deviation of  $0.5$  (Supplementary information).

Minor fluctuations of the distance with temperature have been observed for the pair of GLU530-LYS499, the value was changing from  $3.5\text{\AA}$  at temperature  $25^\circ\text{C}$  up to  $2.5\text{\AA}$  at temperature  $45^\circ\text{C}$ , but in general, most of distances between amino-acid residues forming the salt bridge have the constant value in the temperature range  $25\text{-}45^\circ\text{C}$ . The error at calculation of both distances between  $S_\gamma$  atom and the lengths of the salt bridges was  $0.07\text{\AA}$ .

**The dynamics of amino-acid residues** with decreasing temperature were studied by calculating of root-mean-square deviation (RMSD). Evaluation of the RMSD allows us to investigate the conformational changes of the structure by comparing the residue positions in the reference points. The RMSD values were calculated for close conformational states in specified temperature points with respect to the initial structure (PDB). The residues displacements and the values of RMSD are presented in Fig. 6.

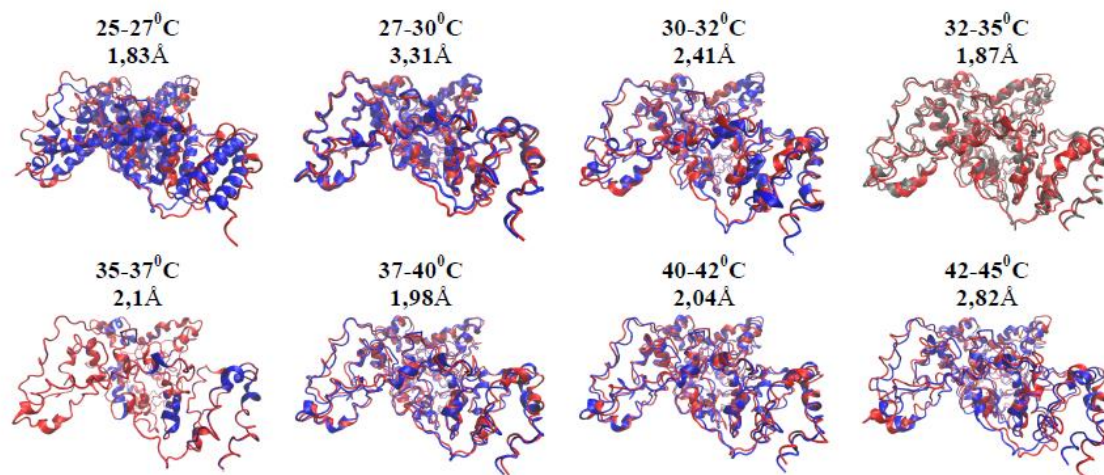


Figure 6. Cartoon representation of the amino-acids residue displacements and the RMSD values corresponding to every temperature interval. Blue and silver colours correspond to the conformational state in the first temperature point, red indicates the conformational state in the second temperature point for every snap-shot.  
The visualisation was created using Visual Molecular Dynamics (VMD)



Thus, the structure of the macromolecule does not undergo significant transformations with the temperature increase, we observe only slight deflection of the RMSD values with respect to the reference temperature in the certain temperature interval. The average value of the RMSD for all temperature range is 2.3Å. The simulation of the HSA structure [87] without SS bonds gave the RMSD value 4.5Å. Some previous MD studies [92-97] reported values ranging from 2 to 10Å, at those for stable structures from 2 to 3Å [96].

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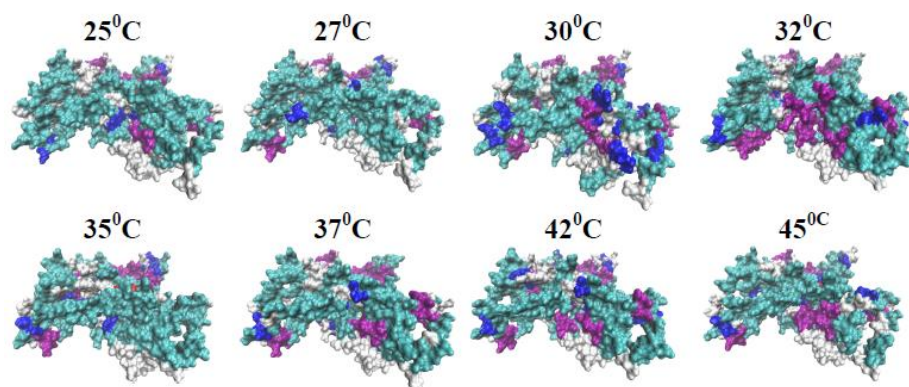


Figure 7. Visualization of conformation changes with temperature.  
Components of the regular secondary structure:  
 $\alpha$ -helix – purple,  $3_{10}$ -helix – blue, turn – cyan, coil – white,  $\pi$ -helix - red

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The simulation of the temperature effect on the conformation stability has shown structural changes in secondary structures of HSA (Fig. 7). Conformational changes take place in the components of the regular secondary structures, which are stabilised by hydrogen bonds. Most the structural rearrangements are observed in the  $\alpha$ -helix. The  $\alpha$ -helix is maintained by the hydrogen bonds between the hydrogen and oxygen atoms of peptides groups. Thus conformational changes in the  $\alpha$ -helix are governed by the hydrogen bonds destruction in the investigated temperature range 25-45<sup>0</sup>C.

### 3. Conclusions

The results presented here demonstrate that the conformation changes of albumin are ultimately correlated with water dynamics in the vicinity of the temperature point 42<sup>0</sup>C. On the one hand the experimental (hydrodynamic radius) and simulation (gyration radius, stability of disulfide bonds and salt bridges) data support the fact of the macromolecule is stable in the temperature range 25-50<sup>0</sup>C with approximately the same size. The value of the gyration radius obtained from molecular dynamics is supported by the results in [20,23]. The difference in 1.5 nm between the hydrodynamic and gyration radii is proved. Additionally, this value coincides with the estimation of the hydration layer thickness obtained by THz spectroscopy [20]. Thus, this suggests that the hydrodynamic radius includes both solvent and shape effects. We obtain, on the basis of comparison of the simulation and PCS data, a well established value of the hydration layer thickness.

On the other hand the zeta potential behaviour from temperature 35<sup>0</sup>C up to denaturation indicates that the hydration layer beyond the Stern layer (an inner region, where surrounding molecules and the ions are strongly bound with the protein macromolecule of HSA) is drastically changed in the vicinity of 42<sup>0</sup>C. The temperature dependence of the zeta potential for HSA may imply conformational changes in the macromolecule structure, surface modifications, and, as a result the initial stage of denaturation process. In this context the simulation data just revealed the conformational changes of the secondary structures, which are stabilized namely by hydrogen bonds. Thus it can mean that the temperature range 40 - 42<sup>0</sup>C is the initial stage of denaturation for HSA.

For deeper understanding of the water collective dynamics around the protein and the correlation with conformation changes we are planning to apply and develop the methods based on Linear Stochastic Estimation [98]. We reserved also, for the future work, the Molecular Dynamics simulation of the temperature effect on the retardation factor [9], which is the relation of the characteristic times in the bulk water and the hydration layer. This important parameter is used to quantify the correlations between the conformational changes of the macromolecule and the dynamics of the water shell.

## References

- [1] R. Abseher, H. Schreiber, O. Steinhauser, The influence of a protein on water dynamics in its vicinity investigated by molecular dynamics simulation, *Proteins: Struct., Funct., Bioinf.* 25 (1996) 366–378.
- [2] V. A. Makarov, B.K. Andrews, P.E. Smith, B.M. Pettitt, Residence times of water molecules in the hydration sites of myoglobin, *Biophys. J.* 79 (2000) 2966–2974.
- [3] A. Garcia, G. Hummer, Water penetration and escape in proteins, *Proteins: Struct., Funct., Genet.* 38 (2000) 261–272.
- [4] M. Tarek, D. Tobias, Role of protein-water hydrogen bond dynamics in the protein dynamical transition, *Phys. Rev. Lett.* 88 (2002) 138101-1.
- [5] F. Pizzitutti, M. Marchi, F. Sterpone, P.J. Rossky, How protein surfaces induce anomalous dynamics of hydration water, *J. Phys. Chem. B.* 111 (2007) 7584–7590.
- [6] H. Frauenfelder, G. Chen, J. Berendzen, P.W. Fenimore, H.Jansson, B. H. McMahon, I.R. Stroec, J.Swenson, R.D. Young, A unified model of protein dynamics, *PNAS.* 106 (2009) 5129–5134.
- [7] F.Sterpone, G.Stirnemann, D. Laage, Magnitude and molecular origin of water slowdown next to a protein, *J. Am. Chem. Soc.* 134 (2012) 4116–4119.
- [8] O. Rahaman, S. Melchionna, D. Laage, F. Sterpone, The effect of protein composition on hydration dynamics, *Phys. Chem. Chem. Phys.* 15 (2013) 3570–3576.
- [9] L. Comez, M. Paolantoni, P. Sassi, S. Corezzi, A. Morresi, D. Fioretto, Molecular properties of aqueous solutions: a focus on the collective dynamics of hydration water, *The Royal Society of Chemistry. Soft Matter.* 12 (2016) 5501-5514. DOI: 10.1039/c5sm03119b.
- [10] A. Frolich, F. Gabel, M. Jasnin, U. Lehnert, D. Oesterhelt, A. M. Stadler, M. Tehei, M. Weik, K. Wood and G. Zaccai, From shell to cell: neutron scattering studies of biological water dynamics and coupling to activity, *Faraday Discuss.* 141 (2009) 117–130.
- [11] D. Russo, R. K. Murarka, J. R. D. Copley, T.Head-Gordon, Molecular view of water dynamics near model peptides, *J. Phys. Chem. B.* 109 (2005) 12966-12975.
- [12] S. Khodadadi, J. H. Roh, A. Kisliuk, E. Mamontov, M. Tyagi, S. A. Woodson, R. M. Briber and A. P. Sokolov, Dynamics of biological macromolecules: not a simple slaving by hydration water, *Biophys. J.* 98 (2010) 1321–1326.
- [13] A. Orecchini, A. Paciaroni, A. D. Francesco, C. Petrillo and F. Sacchetti, Collective dynamics of protein hydration water by Brillouin neutron spectroscopy, *J. Am. Chem. Soc.* 131 (2009) 4664–4669.
- [14] D. Russo, G. Hura and T. Head-Gordon, Hydration dynamics near a model protein surface, *Biophys. J.* 86 (2004) 1852–1862.
- [15] A. Orecchini, A. Paciaroni, A. R. Bizzarri and S. Cannistraro, Dynamics of different hydrogen classes in  $\beta$ -lactoglobulin: a quasielastic neutron scattering investigation, *J. Phys. Chem. B.* 106 (2002) 7348–7354.
- [16] A. Paciaroni, A. Orecchini, E. Cornicchi, M. Marconi, C. Petrillo, M. Haertlein, M. Moulin, H. Schober, M. Tarek and F. Sacchetti, Fingerprints of amorphous icelike behavior in the vibrational density of states of protein hydration water, *Phys. Rev. Lett.* 101 (2008) 148104.
- [17] A. Paciaroni, E. Cornicchi, M. Marconi, A. Orecchini, C. Petrillo, M. Haertlein, M. Moulin and F. Sacchetti, Coupled relaxations at the protein–water interface in the picosecond time scale, *J. R. Soc., Interface.* 6 (2009) S635–S640.
- [18] B. Born, H.Weingartner, E. Brundermann and M. Havenith, Solvation Dynamics of Model Peptides Probed by Terahertz Spectroscopy. Observation of the Onset of Collective Network Motions, *J. Am. Chem. Soc.*, 131 (2009) 3752–3755.
- [19] V. C. Nibali, M. Havenith, New insights into the role of water in biological function: studying solvated biomolecules using terahertz absorption spectroscopy in conjunction with molecular dynamics simulations, *J. Am. Chem. Soc.*, 136 (2014) 12800–12807.

- [20] J. W. Bye, S. Meliga, D. Ferachou, G. Cinque, J. A. Zeitler and R. J. Falconer Analysis of the hydration water around bovine serum albumin using terahertz coherent synchrotron radiation, *J. Phys. Chem. A*, 118 (2014) 83-88.
- [21] B. Born, S. J. Kim, S. Ebbinghaus, M. Gruebele, M. Havenith, The terahertz dance of water with the proteins: the effect of protein flexibility on the dynamical hydration shell of ubiquitin, *The Royal Society of Chemistry, Faraday Discuss.* 141 (2009) 161–173.
- [22] Y. Xu, M. Havenith, Perspective: Watching low-frequency vibrations of water in biomolecular recognition by THz spectroscopy, *J. of Chem. Phys.* 143 (2015) 170901-1-7.
- [23] O. Sushko, R. Dubrovka and R. S. Donnan, Sub-terahertz spectroscopy reveals that proteins influence the properties of water at greater distances than previously detected, *J. Chem. Phys.* 142 (2015) 055101-1-9.
- [24] C. Mattea, J. Qvist, B. Halle, Dynamics at the protein-water Interface from  $^{17}\text{O}$  spin relaxation in deeply supercooled solutions, *Biophys. J.* 95 (2008) 2951–2963.
- [25] J. Qvist, E. Persson, C. Mattea, B. Halle, Time scales of water dynamics at biological interfaces: peptides, proteins and cells, *Faraday Discuss.* 141 (2009) 131–144.
- [26] D. I. Svergun, S. Richard, M. H. J. Koch, Z. Sayers, S. Kuprin, G. Zaccai, Protein hydration in solution: Experimental observation by X-ray and neutron scattering, *Proc. Natl. Acad. Sci. U. S. A.* 95 (1998) 2267–2272.
- [27] A. Pertsemlidis, A. M. Saxena, A. K. Soper, T. Head-Gordon, R. M. Glaeser, Direct evidence for modified solvent structure within the hydration shell of a hydrophobic amino acid, *Proc. Natl. Acad. Sci. U. S. A.* 93 (1996) 10769–10774.
- [28] K. Takano, Y. Yamagata, K. Yutani, Buried water molecules contribute to the conformational stability of a protein, *Protein Eng* 16 (2003) 5–9.
- [29] S. Park, J. G. Saven statistical and molecular dynamics studies of buried waters in globular proteins, *Proteins: Structure, Function, and Bioinformatics* 60 (2005) 450–463.
- [30] O. Carugo, Statistical survey of the buried waters in the protein data bank, *Amino Acids*. 48 (2016) 93–202. doi 10.1007/s00726-015-2064-4.
- [31] O. Carugo, Structure and function of water molecules buried in the protein core, *Curr. Protein Pept. Sci.* 16 (2015) 259-265.
- [32] F. Garczarek and K. Gerwert, Functional waters in intraprotein proton transfer monitored by FTIR difference spectroscopy, *Nature*. 439 (2005) 109-112.
- [33] J. Lin, I. A. Balabin and D. N. Beratan, The nature of aqueous tunneling pathways between electron-transfer proteins, *Science*. 310 (2005) 1311-1313.
- [34] A. de la Lande, N. S. Babcock, J. Řezáč, B. C. Sanders, D. R. Salahub, Surface residues dynamically organize water bridges to enhance electron transfer between proteins *PNAS*. 107 (2010) 11799-11804.
- [35] C. F. A. Negre, G. E. Jara, D. M. A. Vera, A. B. Pierini, C. G. Sánchez, Detailed analysis of water structure in a solvent mediated electron tunneling mechanism, *J. Phys.: Condens. Matter*. 23 (2011) 245305.
- [36] B. C. Polander and B. A. Barry, A hydrogen-bonding network plays a catalytic role in photosynthetic oxygen evolution, *PNAS*. 109 (2012) 6112-6117.
- [37] C. del Val, L. Bondar and A.-N. Bondar, Coupling between inter-helical hydrogen bonding and water dynamics in a proton transporter, *J. Struct. Biol.* 186 (2014) 95-111.
- [38] T. Jiang, W. Han, M. Maduke, E. Tajkhorshid, Molecular basis for differential anion binding and proton coupling in the  $\text{Cl}^-/\text{H}^+$  exchanger *ClC-ec1*, *J. Am. Chem. Soc.* 138 (2016) 3066-3075.
- [39] A.I. Fisenko, N.P. Malomuzh, To what extent is water responsible for the maintenance of the life for warm-blooded organisms?, *Int. J.Mol.Sc.* 10, (2009) 2383-2411.
- [40] T.V. Lokotosh, S. Magazu, G. Maisano, and N. P. Malomuzh, Nature of self-diffusion and viscosity in supercooled liquid water, *Phys. Rew. E* 62 (2000) 3572-3580.
- [41] A.I. Fisenko, N.P. Malomuzh, A.V. Oleynik, To what extent are thermodynamic properties of water argon-like?, *Chem. Phys. Lett.* 450 (2008) 297-301.

- [42] L.A. Bulavin, A.I. Fisenko, N.P. Malomuzh, Surprising properties of the kinematic shear viscosity of water, *Chem.Phys.Lett.* 453, (2008) 183-187.
- [43] T.V. Lokotosh, N. P. Malomuzh, K. N. Pankratov, Thermal Motion in water+electrolyte solutions according to quasi-elastic incoherent neutron scattering data, *J. Chem. Eng. Data.* 55 (2010) 2021-2029.
- [44] M. Rezaei-Tavirani, S.H. Moghaddamnia, B.Ranjbar, M. Amani, S.A. Marashi, Conformational study of human serum albumin in pe-denaturation temperatures by differential scanning calorimetry, circular dichroism and UV spectroscopy, *Journal of biochemistry and molecular biology.* 39 (2006) 530-536.
- [45] S. Sugio, A. Kashima, S. Mochizuki, M. Noda, K. Kobayashi, Crystal structure of human serum albumin at 2.5 Å resolution, *Protein Eng.* 12 (1999) 439-446.
- [46] J.R. Brown, in: V.M. Rosenoer, M. Oratz, M.A. Rothschild (Eds.), *Albumin Structure, Function and Uses*, Pergamon Press, Oxford, UK, 1977.
- [47] M. A. Rothschild, M. Oratz, S.S. Schreiber, *Serum albumin*, *Hepatology.* 8 (1988) 385-401.
- [48] D.C. Carter, J.X. Ho, Structure of serum albumin, *Adv. Protein Chem.* 45 (1994) 153-203.
- [49] F. Kratz, Albumin as a drug carrier: design of prodrugs, drug conjugates and nanoparticles, *J. Controlled Release.* 132 (2008) 171- 183.
- [50] M. Rozga, W. Bal, The Cu(II)/A $\beta$ /human serum albumin model of control mechanism for copper-related amyloid neurotoxicity, *Chem. Res. Toxicol.* 23 (2010) 298-308.
- [51] D.C. Carter, X.-m He, S.H. Munson, P.D. Twigg, K.M. Gernert, M.B. Broom, T.Y. Miller, Three-Dimensional Structure of Human Serum Albumin, *Science.* 244 (1989) 1195-1198.
- [52] H.Z. Cummins, E.R. Pike, *Photon correlation and light beating spectroscopy*, Plenum press, New York and London, 1974. Published in cooperation with NATO Scientific Affairs Division.
- [53] S. Magazu, G. Maisano, F. Mallamace, N. Micali, Growth of fractal aggregates in water solutions of macromolecules by light scattering, *Physical Review A.* 39 (1989) 4195-4200.
- [54] D.I. Donato, M.P. Janelli, S. Magazu, G. Maisano, D. Majolino, P. Migliardo, R. Ponterio, Viscosity and photon correlation spectroscopy measurements in aqueous solutions of poly(ethylene glycol), *Journal of Molecular Structure* 381 (1996) 213-217.
- [55] S. Magazù, G. Maisano, P. Migliardo, and V. Villari, Experimental simulation of macromolecules in trehalose aqueous solutions: A photon correlation spectroscopy study. *Journ. of Chem. Phys.* 111 (1999) 9086-9092.
- [56] C. Branca, S. Magazù, G. Maisano, P. Migliardo, V. Villari and A. P. Sokolov, The fragile character and structure-breaker role of alpha, alpha trehalose: viscosity and Raman scattering findings, *J. Phys. Cond. Matter*, 11 (1999) 3823-3832.
- [57] S. Magazù, G. Maisano, P. Migliardo, E. Tettamanti, V. Villari, Transport phenomena and anomalous glass-forming behaviour in alpha, alpha-trehalose aqueous solutions, *Mol. Phys.*, 96 (1999) 381-387.
- [58] M.P. Jannelli, S. Magazu, P. Migliardo, U. Wanderlingh, Chain length dependence and H-bond effects on diffusive processes of alcohols by IQENS, DLS and NMR, *Physica B: Condensed Matter.* 234 (1997) 355-356.
- [59] M.P. Jannelli, S. Magazu, P. Migliardo, F. Aliotta, E. Tettamanti, Transport properties of liquid alcohols investigated by IQENS, NMR and DLS studies, *Journal of Physics:Condensed Matter.* 8 (1996) 8157-8171.
- [60] A. Faraone, S. Magazu, G. Maisano, R. Ponterio, V. Villari, Experimental evidence of slow dynamics in semidilute polymer solutions, *Macromolecules.* 32 (1999) 1128-1133.
- [61] D.L. Sidebottom, Ultraslow relaxation of hydrogen-bonded dynamic clusters in glass-forming aqueous glucose solutions: A light scattering study, *Physical Review E.* 76 (2007) 011505.
- [62] D.L. Sidebottom, Tri D. Tran, Universal patterns of equilibrium cluster growth in aqueous sugars observed by dynamic light scattering, *Physical Review E.* 82 (2010) 051904
- [63] S. Magazù, IQENS - Dynamic light scattering complementarity on hydrogenous systems *PHYSICA B.* 226 (1996) 92-106.

- [64] S. Magazù, F. Migliardo, M. T. F. Telling, Study of the dynamical properties of water in disaccharide solutions, *Eur. Biophys. J. with Biophysics Letters*. 36 (2007) 163-171.
- [65] A. Sze, D. Erickson, L. Ren, and D. Li, Zeta-potential measurement using the Smoluchowski equation and the slope of the current–time relationship in electroosmotic flow, *Journal of Colloid and Interface Science*. 261 (2003) 402–410.
- [66] Karl Fischer, Manfred Schmidt, Pitfalls and novel applications of particle sizing by dynamic light scattering, *Biomaterials*. 98 (2016) 79-91.
- [67] Sourav Bhattacharjee, DLS and zeta potential – What they are and what they are not?, *Journal of Controlled Release*. 235 (2016) 337–351.
- [68] M. Předota, M. L. Machesky, and D. J. Wesolowski, Molecular Origins of the Zeta Potential, *Langmuir*. 32 (2016) 10189–10198.
- [69] S. R. Maduar, A. V. Belyaev, V. Lobaskin, and O.I. Vinogradova, Electrohydrodynamics Near Hydrophobic Surfaces, *Phys. Rev. Lett.* 114 (2015) 118301.
- [70] T. Darden, D. York, L. Pedersen, Particle Mesh Ewald: An  $N \log(N)$  Method for Ewald sums in large systems, *J. Chem. Phys.* 98 (1993) 10089–10092.
- [71] U. Essmann, L. Perera, M.L. Berkowitz, T. Darden, H. Lee, L.G. Pedersen, A smooth particle mesh Ewald method, *J. Chem. Phys.* 103 (1995) 8577–8593.
- [72] M. Crowley, T. Darden, T. Cheatham, D. Deerfield, Adventures in improving the scaling and accuracy of a parallel molecular dynamics program. *J. Supercomput.* 11 (1997) 255–278.
- [73] J.-P. Ryckaert, G. Ciccotti, H.J.C. Berendsen, Numerical Integration of the Cartesian equations of motion of a system with constraints: molecular dynamics of n-alkanes, *J. Comput. Phys.* 23 (1977) 327–341.
- [74] C.M. Kok, A. Rudin, Relationship between the hydrodynamic radius and the radius of gyration of a polymer in solution, *Die Makromolekulare Chemie, Rapid Communications*, Wiley Online Library. 2 (1981) 655-659.
- [75] W. Wang, C.J. Roberts, *Aggregation of Protein Therapeutics*, John Wiley & Sons: Hoboken, NJ, 2010.
- [76] N.J. Bulleid, L. Ellgaard, Multiple ways to make disulfides, *Trends in Biochemical Sciences*. 36 (2011) 485-492.
- [77] A. Cooper, S.J. Eyles, S.E. Radford, C.M. Dobson, Thermodynamic consequences of the removal of a disulphide bridge from hen lysozyme, *J. Mol. Biol.* 225 (1992) 939–943.
- [78] E. Drakopoulou, J. Vizzavona, J. Neyton, V. Aniot, F. Bouet, H. Virelizier, A. Menez, C. Vita, Consequence of the removal of evolutionary conserved disulfide bridges on the structure and function of charybdotoxin and evidence that particular cysteine spacings govern specific disulfide bond formation, *Biochemistry*. 37 (1998) 1292–1301.
- [79] K.S. Siddiqui, A. Poljak, M. Guilhaus, G.D'. Feller, S. Amico, C. Gerday, R. Cavicchioli, Role of disulfide bridges in the activity and stability of a cold-active  $\alpha$ -amylase, *J. Bacteriol.* 187 (2005) 6206–6212.
- [80] D.C. Vaz, J.R. Rodrigues, W. Sebald, C.M. Dobson, R.M.M. Brito, enthalpic and entropic contributions mediate the role of disulfide bonds on the conformational stability of interleukin-4, *Protein Sci.* 15 (2006) 33–44.
- [81] M. E. Moghaddam, H. Naderi-Manesh, Role of disulfide bonds in modulating internal motions of proteins to tune their function: molecular dynamics simulation of scorpion toxin lqh-III, *Proteins: Struct., Funct., Bioinf.* 63 (2006) 188–196.
- [82] M. Qin, J. Zhang, W. Wang, W. Effects of disulfide bonds on folding behavior and mechanism of the  $\beta$ -sheet protein tendamistat, *Biophys. J.* 90 (2006) 272–286.
- [83] Y.L. Tsai, H.-W. Chen, T. Lin, W.-Z. Wang, Y.-C. Sun, Molecular dynamics simulation of folding of a short helical toxin peptide, *J. Theor. Comput. Chem.* 06 (2007) 213–221.
- [84] M. Aschi, A. Bozzi, R. Di Bartolomeo, R. Petruzzelli, The role of disulfide bonds and n-terminus in the structural properties of hepcidins: insights from molecular dynamics simulations, *Biopolymers*. 93 (2010) 917–926.

- [85] J.R. Allison, G.-P. Moll, W.F. van Gunsteren, Investigation of stability and disulfide bond shuffling of lipid transfer proteins by molecular dynamics simulation, *Biochemistry*. 49 (2010) 6916–6927.
- [86] A. Godwin, J.-W. Choi, E. Pedone, S. Balan, R. Jumnah, S. Shaunak, S. Brocchini, M. Zloh, Molecular dynamics simulations of proteins with chemically modified disulfide bonds, *Theoretical Chemistry Accounts*. 117 (2007) 259–265.
- [87] M. M. Castellanos, C.M. Colina, Molecular dynamics simulations of human serum albumin and role of disulfide bonds, *J. Phys. Chem. B*. 117 (2013) 11895–11905.
- [88] T.E. Creighton, Disulphide bonds and protein stability, *BioEssays*. 8 (1988) 57–63.
- [89] D.E. Anderson, W.J. Becktel, F.W. Dahlquist, pH-induced denaturation of proteins: a single salt bridge contributes 3-5 kcal/mol to the free energy of folding of t4 lysozyme, *Biochemistry*. 29 (1990) 2403–2408.
- [90] D. A. Dougherty, *Modern Physical Organic Chemistry*, Sausalito, CA: University Science Books. 2006.
- [91] S. Kumar, R. Nussinov, Close-range electrostatic interactions in proteins, *Chem. Bio. Chem.* 3 (2002) 604–617.
- [92] J. Li, X. Zhu, C. Yang, R. Shi, Characterization of the binding of angiotensin ii receptor blockers to human serum albumin using docking and molecular dynamics simulation, *J. Mol. Model.* 16 (2010) 789–798.
- [93] S.I. Fujiwara, T. Amisaki, Molecular dynamics study of conformational changes in human serum albumin by binding of fatty acids, *Proteins: Struct., Funct., Bioinf.* 64 (2006) 730–739.
- [94] B. Sudhamalla, M. Gokara, N. Ahalawat, D.G. Amooru, R. Subramanyam, Molecular dynamics simulation and binding studies of  $\beta$ -sitosterol with human serum albumin and its biological relevance, *J. Phys. Chem. B*. 114 (2010) 9054–9062.
- [95] M. Rueda, C. Ferrer-Costa, T. Meyer, A. Perez, J. Camps, A. Hospital, J.L Gelpi, A.M. Orozco, A consensus view of protein dynamics, *Proc. Natl. Acad. Sci. U.S.A.* 104 (2007) 796–801.
- [96] O. Deeb, M.C. Rosales-Hernández, C. Gómez-Castro, R. Garduño-Juárez, J. Correa-Basurto, Exploration of human serum albumin binding sites by docking and molecular dynamics flexible ligand–protein interactions, *Biopolymers*. 93 (2010) 161–170.
- [97] R. Artali, G. Bombieri, L. Calabi, A. Del Pra, A molecular dynamics study of human serum albumin binding sites, *II Farmaco*. 60 (2005) 485–495.
- [98] D.Nerukh, S. Karabasov, Water-peptide dynamics during conformational transitions. *J. of Phys. Chem. Let.* 4 (2013) 815-819.



## Highlights

The conformation changes of albumin are ultimately correlated with water dynamics.

The experimental and simulation data support the fact of the macromolecule is stable.

The zeta potential behaviour indicates that the hydration layer is drastically changed.

The temperature dependence of the zeta potential for HSA implies the initial stage of denaturation.

The simulation data revealed the conformational changes of the secondary structures.

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