Digest Journal of Nanomaterials and Biostructures Vol. 7, No. 3, July - September 2012, p. 907 - 915

Modeling the slaving of structural fluctuations in bio-molecules to those of nearby water

C. Mihai[a], A. Velea[b], N. Roman[c], L. Tugulea[a], N. I. Moldovan[d*]
[a]*Department of Electricity, Solid State and Biophysics, Faculty of Physics, University of Bucharest, Romania;*
[b]*National Institute of Materials Physics, Bucharest - Magurele, Romania;*
[c]*Department of Computer Science and Engineering, The Ohio State University - Lima, Ohio, USA*
[d]*Department of Internal Medicine, Davis Heart and Lung Research Institute, The Ohio State University - Columbus, Ohio, USA*

The functions of bio-molecules depend on their structural fluctuations, which are thought to be 'slaved' (i.e. coupled) to those of nearby bulk water. Slaving was suggested to explain the universal pattern of propagation of mechanical excitations through concentrated water-containing solutions of polymers, such as the cytoplasm. To obtain a simplified model of this phenomenon, we propose to use a cellular automaton of water-based solutions. During iterations, the model computes the compactness of both the solvent and the solute (equivalent of their density), as the average number of neighbors of each class of particles. As a surrogate of slaving, we studied the temporal co-variation of these variables, using the Pearson correlation coefficient (S_{av}). We found that S_{av} depends in a biochemically-meaningful manner on the concentration of solute, on its hydrophatic character and on molecular flexibility. The simulations also show that S_{av} is robust to mild hypothermia. In conclusion, our cellular automaton is capable to generate a slaving-like behavior of solutes in water, as an emergent phenomenon occurring in dissolved molecular systems.

(Received June 15, 2012; Accepted June 28, 2012)

Keywords: Water, Cellular automata, Complexity, Slaving, Hydrophilic, Hydrophobic, Biomechanical, Crowding, Temperature

1. Background

Structural fluctuations and the role of water dynamics are fundamental phenomena for understanding the functioning of bio-molecules. This universal solvent is intimately involved as mediator of bio-molecular self-assembly, folding, binding and catalysis [1-4]. In a unified model of protein dynamics, Frauenfelder *et al.* [5] have suggested that bio-molecules provide the structure for the biological function, but is the bulk solvent that controls their large-scale motions and conformational changes in a diffusive manner [6-8], while the hydration shell determines their internal motions [9, 10]. In this paradigm, bio-molecular motions can be non slaved or slaved [6, 11]. Non slaved motions are local and independent of the solvent fluctuations. Slaved motions are non local, tightly coupled to the solvent, and their rates are proportional to the fluctuation rate of the solvent, but are slower. The pervasiveness of slaved motions advocates for the importance of the environment in the functioning of bio-molecules.

Recently, slaving became relevant for cellular biomechanics also, being suggested to explain the dependence of cytoplasm stiffness and its power-law rheology upon water substitution

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[*] Corresponding Author: nicanor.moldovan@osumc.edu

with deuterium oxide [20]. This is known to form stronger hydrogen bonds than water [21], which naturally leads to a stiffer network and enhanced hydrophobic interactions [22-24].

Despite the theoretical [8], experimental [12, 13] and modeling [14] efforts, a general theory of solvent-slaved motions of molecules does not exist yet. To unravel the nature of dynamical coupling between bio-molecules and their aqueous environment, we propose to use a model of aqueous solutions that we have previously implemented and described [15]. The collective behavior of water molecules, each one possibly interacting with four other neighbors, gives rise to an extended network. While constantly subjected to fluctuations in local density and to molecular reconfigurations that break and form hydrogen bonds, this network proves to be a good model of the fluid water, and of basic processes such as hydrophobic self-assembly [16-19].

The cellular automaton model used here revealed that the slaved motions are dependent on the hydrophatic character of the solute, on the molecular flexibility, and on the reduction in mobility of water molecules. These findings show that slaving is an emergent, non-linear phenomenon which can be captured and studied using cellular automata.

2. Model and methods

As an abstract model of water-based solutions, we used the cellular automaton proposed by Kier *et al.* [16], with our modifications described in [15]. In this model, the sites on the grid can be empty or occupied with different types of particles, which may move in 4 directions. The particles change their position one site at a time with predetermined probabilities, and interact only through local stochastic rules. However, the system shows complex, emergent and/or cooperative behavior [25].

The grid has a dimension of 55×55 sites. We use toroidal boundary conditions in all directions, because the torus effectively simulates a small segment of a larger, unrestricted system. The system investigated contains solvent-like particles and solute-like particles, and the degree of lattice occupancy was set to 69 % according to [17].

Four parameters describing the rules of particle movement were varied in this study (see Table 1). In addition, the concentration of the solute in the system has been varied also, from very diluted (10 %), to diluted (25 %), equilibrated (50 %) and crowded (80 %).

Table 1.Simulation parameters.

Parameter	Description	Range
C	Solute concentration in the system	10, 25, 50, 80%
P_B(WW)	The break probability between two solvent molecules	0.17, 0.20
P_B(WS)	The break probability between solvent and solute molecules	0.1, 0.5, 0.9
P_B(SS)	The break probability between two solute molecules	0.1, 0.5, 0.9
J(SS)	Propensity of a solute molecule to move toward another solute molecule	0.5, 1.0, 2.0

By tuning the breaking probability between solvent particles, P_B(WW), one can simulate the behavior of the system at different virtual 'temperatures' [17]. As a preliminary investigation of the sensitivity of slaving on the mobility of water molecules, we sought to determine the effect of mild hypothermia, represented as a 15 % decrease in temperature (e.g. from 37 ^{o}C to 30 ^{o}C) which is compatible with – and even beneficial for- cellular activities *in vitro* [26, 27]. Thus, to compare the influence of temperature on the slaving patterns, we performed calculations of S_{av} for P_B(WW) differing by 15 % (0.17 and 0.20, respectively).

Another fundamental behavior of solutes in the presence of water-like solvents is expressed in their hydrophatic character, which in our model is controlled through the breaking probability between solvent and solute, P_B(WS). The lowest value of P_B(WS) used in this study was 0.1, to indicate a strong hydrophilic solute. The intermediate values describe a neutral solute which is neither hydrophilic nor hydrophobic. Finally, high values of this parameter (e.g. 0.9) were used to describe a strongly hydrophobic solute.

An important property of solutes is their ability to move away from other solute particles, which we interpret as their abstract molecular 'rigidity'. In our model, we can manipulate this parameter through the breaking probability between two solute particles, $P_B(SS)$. When $P_B(SS)$ is low, for example 0.1, it indicates a very 'stiff' solute whereas when it is around 0.5, it models a solute with intermediate flexibility. For high values of $P_B(SS)$ (e.g. 0.9), we have a very flexible solute.

The structuring tendency reflects the propensity of a particle to move toward another particle, when the site in between them is empty [17]. This parameter can take non-negative real values. When $J < 1$, the particle moves with more difficulty in that direction (suggestive of mutual repulsion), whereas for $J > 1$, the particle moves easier (indicating a short-range attraction). $J(SS) = 1$ is the case when the particles ignore each other. For water it was shown that an empirical relation holds between $P_B(WW)$ and $J(WW)$ [17].

In order to model slaving, here understood as the coupling between the fluctuations of solute and solvent compactness, we defined the spatially-averaged number of neighbors of each particle (maximum 8 neighbors, in the so-called 'Moore neighborhood'), as representing the abstract equivalent of their respective densities. To this end, we computed K_{av}, the compactness coefficients of solvent and solute, by averaging to the number of particles of a given type on the grid, the sum of all their neighbors. Then, we determined the slaving coefficient, S, defined as the Pearson linear correlation coefficient between the time functions, K_{av}, of solvent and of solute. To obtain an average slaving coefficient (S_{av}), the S values were averaged over six independent runs (replicates), performed for each set of parameters.

3. Results

The first major finding of our study is that the fluctuations of the compactness of water and that of a co-dissolved solute can be strongly coupled (see Fig. 1) in a manner evocative of the slaving determined using empirical means [5]. The values of slaving coefficient, S_{av}, can take values as high as 0.7. Also remarkable and first-time reported here, is that this parameter may also have *negative* values, suggesting an anti-correlation of these fluctuations. This predicted phenomenon awaits an experimental confirmation.

The multi-parameter system described by our simulations can be represented in various ways. We chose as an independent variable the concentration of solute, in order to highlight the impact of molecular crowding, a defining overall property of the cytoplasm [32]. Our results show that the dependence of slaving on crowding is in general non-linear, with attained maxima dependent on concentration, hydropahtic character and flexibility of solute.

Thus, very hydrophilic solutes display an increasingly stronger slaving to water (Fig. 1 a, b, c). This begins with almost no such stochastic solute-solvent coupling in very diluted media, but increases with concentration until a maximum is reached in equilibrated environments. Then, it decreases again, almost to null slaving, in very crowded solutions. The slaving of these strongly hydrophilic solutes is not affected by the self-structuring tendency of solute molecules (Fig. 1 a, b, c) reflected by the parameter, J. An exception is in the case of very stiff self-attractive solutes in a crowded environment, where the slaving increases with J (Figure 1 c). Also, for very flexible self-attractive solutes in a diluted environment, the slaving decreases (Fig. 1 c).

Intermediate and very flexible neutral solutes in Fig. 1 d, e, f (green and blue curves), have a similar slaving pattern as the hydrophilic solutes, except the curves are shifted to higher slaving values. On the other hand, the very stiff intermediate solutes (Fig. 1 d, e, f - red curves) are not slaved and the system is very unstable, considering the higher values of standard deviation, SD.

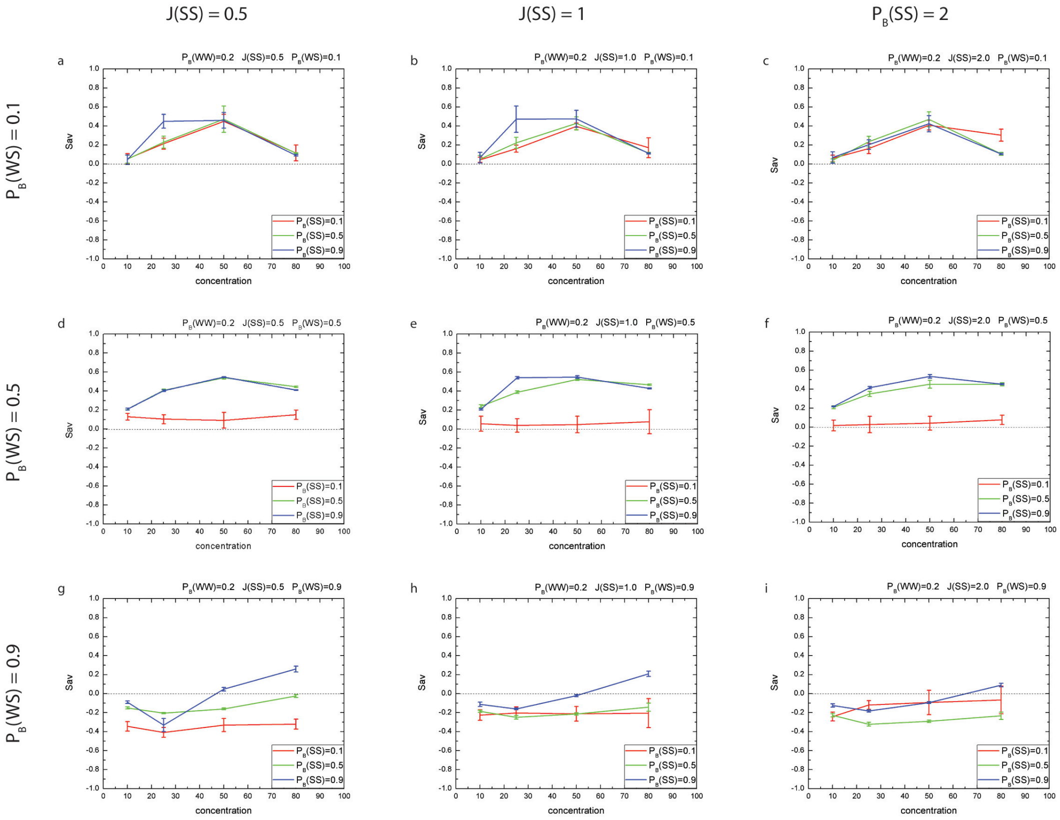

Fig. 1. Slaving patterns of solutes to H_2O and their dependence on the hydrophatic character of the solute, on its molecular flexibility, on the structuring tendency of solute molecules, and on solute concentration

Regarding the structuring tendency of solute molecules, the intermediate neutral solutes (Fig. 1 d, e, f) present a slight decrease in slaving as J(SS) changes from 0.5 to 2.0.

In contrast with the hydrophilic and neutral solutes, the hydrophobic solutes (Fig. 1 g, h, i) display *negative* slaving coefficients. There is a small maximum slaving reached in diluted media, then these values decrease. Also, in crowded solutions, the very flexible solutes (Fig. 1 g, h, i - blue curves) have positive slaving values. Very stiff solutes (Fig. 1 g, h, i - red curves) are part of unstable systems, as can be noted from the high SD values.

The structuring tendency has a larger impact on the slaving patterns of strongly hydrophobic solutes (Fig. 1 g, h, i). Very stiff solutes (Fig. 1 g, h, i - red curves) present a decrease of the slaving values as J(SS) goes from self-repulsive to self-attractive solutes. In the same time, there is a significant increase in the system instability, as we can see from the high values of SD. On the other hand, intermediate solutes show an increase in their slaving values which is more prominent at higher concentrations (Fig. 1 g, h, i - green curves). The slaving pattern for very flexible solutes is shifted to higher negative values, showing higher slaving coefficients at low concentrations and lower slaving at high concentrations (Fig. 1 g, h, i - blue curves).

In the second part of this study, using the same parameters as in Figure 1, we computed the corresponding curves for a lower temperature and for comparison we superimposed them. This was done in order to better extract the information regarding the influence of physiological temperature shifts [26, 27] on the patterns of solute-to-solvent 'slaving'. The differences in slaving coefficients found between the two conditions are in general very small, although often statistically significant, as expected given the slight difference (from 0.20 to 0.17) in the solvent mobility embodied in $P_B(WW)$ (Figs. 2-4).

In the case of strongly hydrophilic solutes (Fig. 2), the lower temperature determines slightly higher slaving values for very stiff and intermediate solutes, in diluted and equilibrated environments, for indifferent (J(SS) = 1) and self-attractive (J(SS) = 2) solute particles (shown with dashed circles). From Figure 2 we can see that the occurrence frequency of this effect increases from self-repulsive solutes, where it appears only once, towards self-attractive solute particles, where it is present across all rigidity cases.

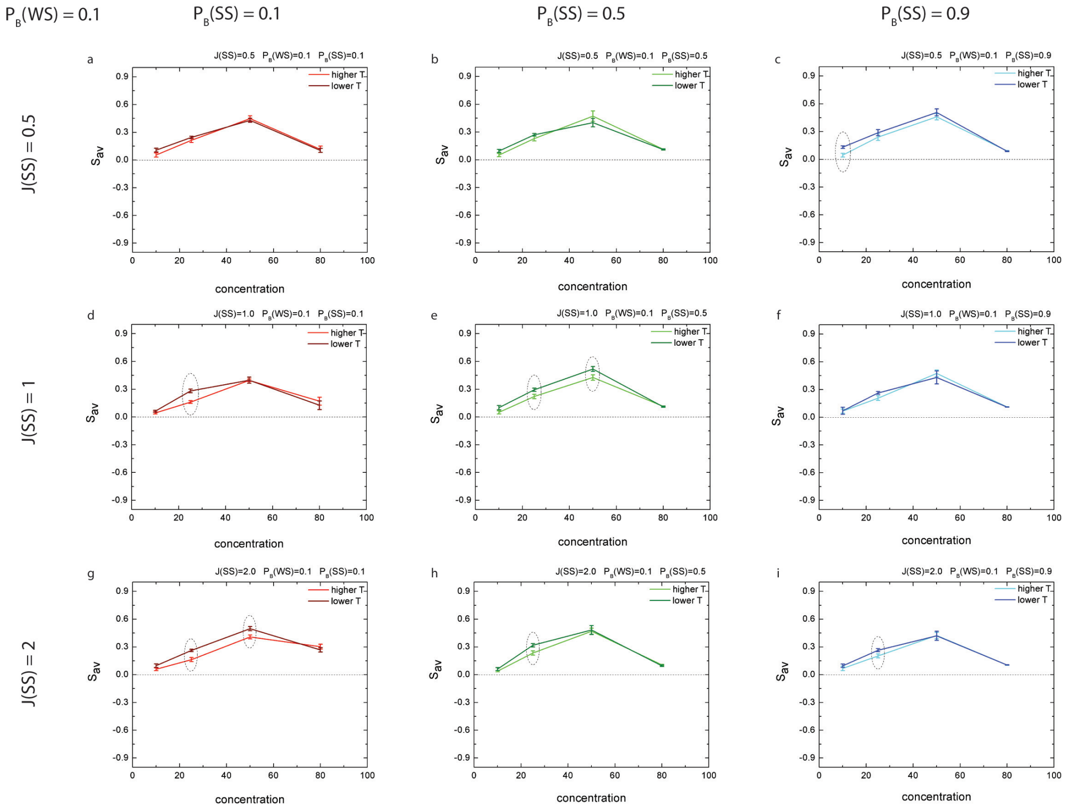

Fig. 2. Influence of physiological temperature (T) shifts on the slaving patterns of strongly hydrophilic solutes. Error bars indicate the standard errors of the mean (SEM). The dashed circles show significant differences between S_{av} values computed for the same concentration but different temperatures ($p < 0.05$), according to TukeyHSD tests.

Considering the neutral ($P_B(WS) = 0.5$) solutes (Fig. 3), one can note a new pattern: lower temperature produces higher slaving values for intermediate and very flexible self-repulsive solute particles (such as those carrying opposite electrical charges). In the case of indifferent and self-attractive solute particles (e.g. similarly charged) those that are significantly affected are the very flexible ones. Also, for stiff self-attractive solutes the effect is present in equilibrated and crowded places. As a general observation for neutral solutes, this effect is stronger in crowded media and affects more substantially the very flexible solutes.

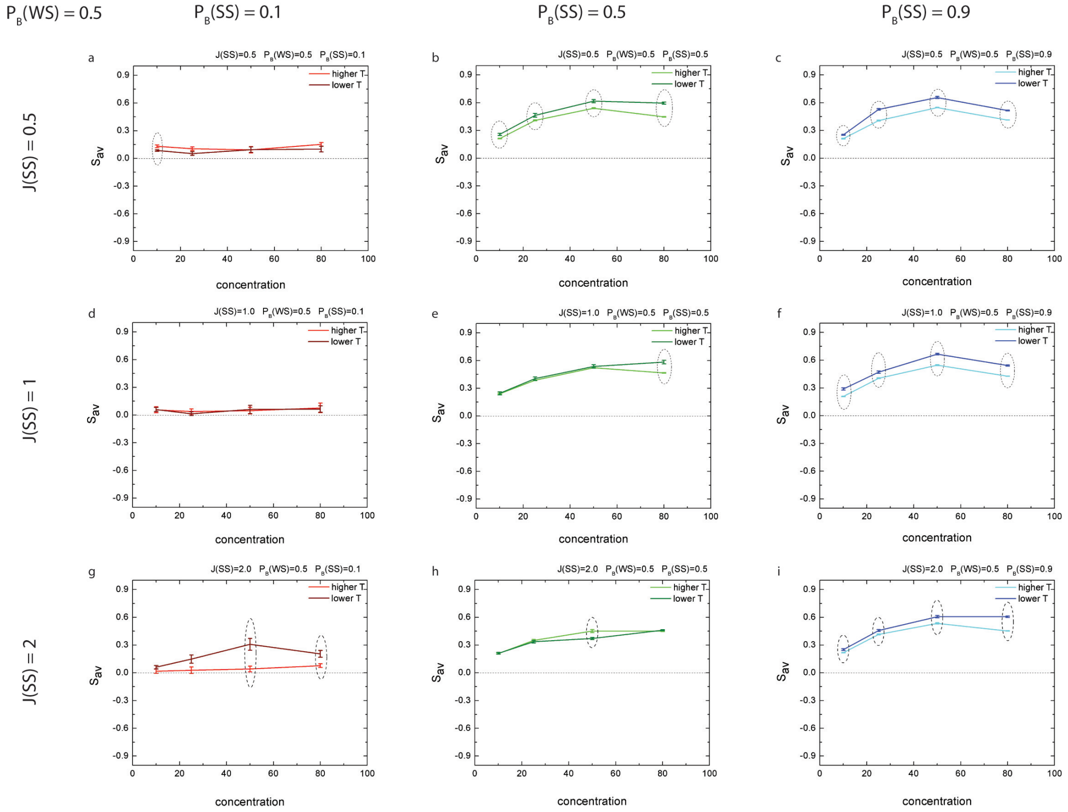

Fig. 3. Comparison between the influence of lower and higher temperature (T) on the slaving patterns of neutral solutes. Error bars indicate the standard errors of the mean (SEM). The dashed circles show significant differences between S_{av} values computed for the same concentration but different temperatures ($p < 0.05$), according to TukeyHSD tests.

As opposed to the neutral (from hydropathic point of view) case, where very flexible solute particles were the most affected by lower temperature, for very hydrophobic solutes these are much less affected. Instead, the solutes with intermediate flexibility are still affected by the change in temperature, although the magnitude of the effect is reduced. Stiff solutes are affected in very diluted media for all types of self-attractive solutes. We should notice that, again as opposed to the case of neutral solutes, where slaving was stronger in crowded media, now the effect is present mostly in diluted and very diluted environments.

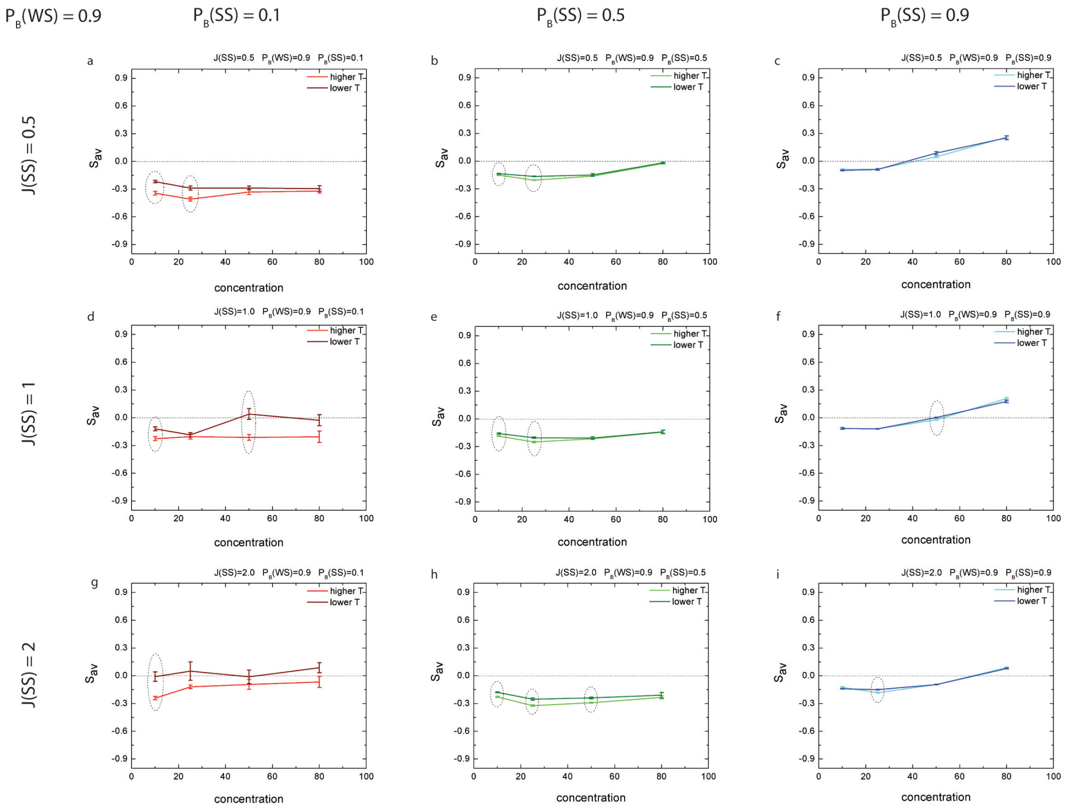

Fig. 4. Comparison between the influence of lower and higher temperatures (T) on the slaving patterns of strongly hydrophobic solutes. Error bars indicate the standard errors of the mean (SEM). The dashed circles show significant differences between S_{av} values computed for the same concentration but different temperatures ($p < 0.05$), according to TukeyHSD tests.

4. Discussions

Realistic molecular dynamics simulations and their various implementations have attained a high degree of refinement and sophistication. However, these come with the price of substantial computational demand, which limits their applicability to well defined cases. Charting the landscape of all combinations of variables would benefit from simpler yet more general methods. In this regard, although seemingly oversimplified, the two-dimensional lattice models of water and aqueous solutions are continuously created and used [28-31].

The cellular automata model used here is capable to reveal a slaving-like process that appears in aqueous solutions. By using the Pearson correlation coefficient as a measure of the bio-molecular structural fluctuations ‘coupled’ to those of water, we have shown that this process is dependent on the hydrophatic character and on the molecular flexibility of the solute.

An unexpected result is the finding that while hydrophilic solutes show positive slaving coefficients, the hydrophobic ones (particularly those which are more rigid) may display negative slaving, indicating an *anti-correlation* between fluctuations of their compactness and that of nearby water molecules. This predicted effect awaits experimental confirmation.

We are aware of the many limitations of this model, even in comparison with other lattice-based models of water and solutes [30, 31]. However, it harnesses the power of cellular automata to capture emergent phenomena in complex systems. For this reason, we consider it as a first sketch of what could become a new method to study these processes in solutions.

Given the abstract meaning of 'particles' in our system, as in other similar models [31], and the unspecified nature of the 'bonds' rendered by the movement probabilities, our results apply equally well from small molecular domains (where the particles may represent covalently-bound atoms) to aggregates where each particle could be a larger cluster, interacting non-covalently with the others. Of note, the whole collection of particles contributes to the coherent behavior of the system. Nevertheless, our results are informative for the behavior of real molecules or parts thereof, opening the way for further modeling and for empirical validation.

The results regarding the effects of temperature on slaving should be interpreted in this context. Although widespread, they are very small in absolute terms in the current comparison of an arbitrarily defined 'normothermia' with mild hypothermia [26, 27]. Our previous study using the cellular automaton approach demonstrated that the same molecular parameters affect the organization, and through this, the mobility of nearby water molecules [15]. Thus, a goal of our future modeling effort in this direction is to more specifically explore the dependence of slaving on water mobility.

Acknowledgements

This work was supported by the strategic grant POSDRU/88/1.5/S/56668 - *Invest in people!* European Social Fund, Human Resources Development Operational Program 2007 - 2013, Priority axis 1 - "Education and training in support of growth and development of knowledge-based society". The simulations were run at the Ohio Supercomputer Center under the start-up grant #: PAS0541-1 awarded to Dr. N. Roman.

References

[1] V. Makarov, B.M. Pettitt, Feig M. Acc Chem Res **35**, 376 (2002)
[2] T. M. Raschke, Curr Opin Struct Biol **16**, 152 (2006)
[3] Y. Levy, J.N. Onuchic, Annu Rev Biophys Biomol Struct **35**, 389 (2006).
[4] P. Ball Water as an active constituent in cell biology. Chem Rev **108**, 74 (2008)
[5] Hans Frauenfelder, Guo Chen, Joel Berendzen, Paul W. Fenimore, Helén Jansson, Benjamin H. McMahon, Izabela R. Stroe, Jan Swenson, and Robert D. Young, Proc Natl Acad Sci USA. **106**(13): 5129–5134 (2009).
[6] P. W. Fenimore, H. Frauenfelder, B. H. McMahon, and F. G. Parak, Proc. Natl. Acad. Sci. USA **99**, 16047, 2002.
[7] Frauenfelder H., Fenimore P.W., Chen G., McMahon B.H. Proc Natl Acad Sci USA **103**, 15469 (2006).
[8] Lubchenko V., Wolynes P.G., Frauenfelder H. J Phys Chem B **109**, 7488 (2005)
[9] Fenimore P.W., Frauenfelder H., McMahon B.H., Young R.D., Proc Natl Acad Sci USA **101**, 14408–14413 (2004).
[10] G. Chen, P.W. Fenimore, H. Frauenfelder, F. Mezei, J. Swenson & R.D. Young, Phil Mag 88:3877–3883 (2008).
[11] R. H. Austin, K. W. Beeson, L. Eisenstein, H. Frauenfelder, I. C. Gunsalus, Biochemistry **14**, 5355 (1975).
[12] Weihong Qiu, Ya-Ting Kao, Luyuan Zhang, Yi Yang, Lijuan Wang, Wesley E. Stites, Dongping Zhong, and Ahmed H. Zewail, Proc. Natl. Acad. Sci., **103**(38), 13979 (2006).
[13] Tanping Li, Ali A. Hassanali, Ya-Ting Kao, Dongping Zhong, Sherwin J. Singer, J. Am. Chem. Soc. **129**, 3376-3382 (2007).
[14] Natalia Shenogina, Pawel Keblinski, and Shekhar J. Chem. Phys. **129**, 155105 (2008).
[15] C. Mihai, A. Velea, N. Roman, L. Tugulea, N. I. Moldovan, Digest Journal of Nanomaterials and Biostructures, **7**(2), 469 (2012).

[16] Kier, L.B., Cheng, C.K., Testa, B., Carrupt, A Cellular Automata Model of the Hydrophobic Effect, P.A., Pharm. Res.. **12**, 615-620 (1995).

[17] Kier, L. B., Seybold, P. G., Cheng, C.-K. Modeling Chemical Systems using Cellular Automata. Springer, Amsterdam (2005).
[18] Kier, L.B., Cheng, C.K., Testa, B., Carrupt, P.A., Pharm.Res.**13,** 1419 (1996).
[19] Kier, L.B., Cheng, C.K., Nelson, J.D., Models of Solute Aggregation Using Cellular Automata Chem. Biodivers. **6**, 396 (2009).
[20] Lenormand G, Millet E, Park C.Y., Hardin CC, Butler J.P., Moldovan N.I., Fredberg J.J., Phys. Rev. E **83**, 061918 (2011).
[21] Scheiner S., Cuma M., J Am Chem.Soc **118**, 1511 (1996).
[22] Parker, M. J., A. R. Clarke. Biochemistry, **36**, 5786 (1997).
[23] Chakrabarti, G., K. Shane, L. G. Mohan, Jr., J. S. Barton, R. H. Himes. Biochemistry. **38**, 3067 (1999).
[24] Patrizia Cioni and Giovanni B. Strambini, Effect of Heavy Water on Protein Flexibility, Biophysical Journal Volume **82**, 3246 (2002).
[25] Wolfram, Stephen, A New Kind of Science. Wolfram Media, Inc., 2002.
[26] Alison Moore, Jennifer Mercer, George Dutina, Christopher J. Donahue, Kenneth D. Bauer, Jennie P. Mather, Tina Etcheverry & Thomas Ryll, Effects of temperature shift on cell cycle, apoptosis and nucleotide pools in CHO cell batch cultures**,** Cytotechnology **23,** 47 (1997).
[27] Niraj Kumar, Patrick Gammell, Paula Meleady, Michael Henry and Martin Clynes, Differential protein expression following low temperature culture of suspension CHO-K1 cells, BMC Biotechnol. **8,** 42 (2008).
[28] Kier, L. B., Cheng, C.-K., Testa B., Carrupt, P.-A. J. Pharm. Sci., **86,** 774 (1997),doi: 10.1021/js9700513
[29] Bernd Mayer, Gottfried Köhler, and Steen Rasmussen, Phys. Rev. E **55**, 4489 (1997).
[30] K. Stokely, M. G. Mazza, H. E. Stanley, and G. Franzese, Effect of Hydrogen Bond Cooperativity on the Behavior of Water, Proc. Natl. Acad. Sci. USA **107**, 1301-1306 (2010)
[31] Bryan A. Patel, Pablo G. Debenedetti, Frank H. Stillinger and Peter J. Rossky, A Water-Explicit Lattice Model of Heat-, Cold-, and Pressure-Induced Protein Unfolding, Biophysical Journal, **93**, 4116 (2007).
[32] Tadashi Ando and Jeffrey Skolnick, Crowding and hydrodynamic interactions likely dominate in vivo macromolecular motion, Proc. Natl. Acad. Sci., **107**(43), 18457 (2010).