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IMPACT OF SOLUTE MOLECULAR PROPERTIES ON THE ORGANIZATION OF NEARBY WATER: A CELLULAR AUTOMATA MODEL

C. MIHAI[a], A. VELEA[b,e], 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, 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*
[e]*"Horia Hulubei" Foundation, Bucharest-Magurele, Romania*

The goal of this study was the creation of a model to understand how solute properties influence the structure of nearby water. To this end, we used a two-dimensional cellular automaton model of aqueous solutions. The probabilities of translocation of water and solute molecules to occupy nearby sites, and their momentary distributions (including that of vacancies), are considered indicative of solute molecular mechanics and hydrophatic character, and are reflected in water molecules packing, i.e. ‘organization’. We found that in the presence of hydrophilic solutes the fraction of water molecules with fewer neighbors was dominant, and inverse-proportionally dependent on their relative concentration. Hydrophobic molecules induced water organization, but this effect was countered by their own flexibility. These results show the emergence of cooperative effects in the manner the molecular milieu affects local organization of water, and suggests a mechanism through which molecular mechanics and crowding add a defining contribution to the way the solute impacts on nearby water.

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1. Background

Individual and collective mobility of water molecules are instrumental for many chemical and biological phenomena. For example, water splitting in photosynthesis critically depends on its accessibility to the reaction center II, which it is embedded in the lipid phase of thylakoid membranes [1]. Plasma membrane, on the other side, manifests selective filtration properties for water, displaying both controlled channel-mediated transport (through aquaporins) and a direct trans-membrane flux, larger than expected from the hydrophobic effect [2]. These and many other phenomena make the understanding of cooperative effects in water solutions an enduring but unmet goal of physical (bio)chemistry.

Solutions are complex systems of molecules, studied most often from the point of view of the solute. However, although the solvent is seemingly simpler in molecular structure and properties, its role in defining the properties of these systems is no less important, as increasingly shown for water, the universal biological solvent. In this regard, the notion of 'slaving' is notorious, describing the driving role of density fluctuations in nearby bulk water for controlling the large-scale stochastic movements of dissolved biomolecules [3]. This one-directional view of solvent influencing the solute was aptly criticized, even in the case of slaving [4], because there is a strong

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

reciprocal influence between solute and solvent. For example, the molecules often have 'kosmotropic' (water organizing) or 'chaotropic' (water disorganizing) effects on water structure [5]. An example is urea, the molecular 'denaturant' used to alter solubility of proteins, yet acting on the solvent. Therefore, solutes may be found to influence in subtle ways the cooperative nature of inter-molecular interactions in the solvent. The properties of dissolved molecules could thus inversely influence those of water producing complex, non-linear causal loops.

Molecular dynamic simulations with atomic resolution remain powerful tools for the study of such reciprocal effects of solutes and water. However, due to the immense computational demand (for programming the exquisitely heterogeneous biological systems, and at the stage of execution), their applicability to charting the laws that govern the bio-molecular solutions remains limited [6]. In search for a more efficient, but still reliable computational method to address these questions, we chose cellular automata. These are well known for their ability to capture the essence of emergent phenomena in complex stochastic and/or chaotic systems, while their results are comparable with those obtained by Monte Carlo simulations (see for example [7]).

Two dimensional models of water are useful [8-10], although their degree of sophistication depends on the study goal. One of the simplest but powerful versions is the cellular automata model of aqueous solutions developed by Kier *et al.* [11-15]. This model was largely validated against the main physico-chemical properties of water and of simple aqueous solutions, including the ability to reproduce the hydrophobic effect [11,12,15]. Kier *et al.* verified that the molar fraction (f) of water molecules, hydrogen-bonded from none to four other molecules, satisfies the known distribution in the actual fluid [13]. In other two-dimensional models of water, up to 6 neighbors have been considered [9,10].

For our work, we implemented a version of Kier *et al.* [13], with modified neighborhoods. It should be stressed that this modeling does not seek a realistic representation of water or of biomolecules (better approached by molecular dynamics and other forms of simulations). This model is designed to capture instead the general phenomena emerging in complex systems, with a new focus on molecular biomechanics, an aspect of biological systems largely neglected so far in favor of their biochemical properties.

Using this model, we explored the impact of three essential properties of the biomolecules on the structure of bulk nearby water: a) the concentration (equivalent of the ‘crowding’ of cytoplasm), b) the hydrophatic character (i.e hydrophobic vs. hydrophilic) of solute molecules, and c) the molecular flexibility (*ad-hoc* defined in this study, see below).

Our data show a surprisingly strong impact of solute concentration and of its ‘stiffness’ on the packing of nearby water. These results recapitulate the main known features of water solutions studied with more refined methods, but in a more general and more comprehensive way. This effort lays out the foundation for application of our modeling in subsequent studies to other complex phenomena in aqueous biomolecular solutions (such as ‘slaving’ and stochastic molecular mechanics).

2. Model and methods

The model used throughout this study is an extension of the work of Kier *et al.* [11-15]. It is a two - dimensional cellular automaton, where the computing objects (or particles, representing water and solute) occupy discrete states on the grid sites. The squared sites of the grid are well suited for the investigation of water-related phenomena, because water molecules can participate in up to four hydrogen bonds with the neighbors. Each object interacts only with its immediate neighbor, so the rules that govern their behavior are local.

There are several modes to consider an object neighborhood used in cellular automata (Fig. 1). The most common type is the so called ‘von Neumann neighborhood’. This neighborhood of a site (*i*) is composed of the four *j* sites placed crosswise. The ‘extended von Neumann neighborhood’ is represented by the second-order *k* sites, with the same type of placement. Another version, used in this model, is the ‘Moore neighborhood’ (with eight places), that includes the first-order sites *m* found in the corners of the *i*-centered site.

Here we computed the fractions of molecules with none to eight neighbors in the final state, as derived from a Moore-type neighborhood (unlike Kier *et al.* model, where all molecules were treated in von Neumann variant [15]). This was preferred because water molecules may crowd closer to each other than in ice (the ideal distribution along the tetrahedral orbitals of oxygen, projected onto the von Neumann lattice), to form, at room temperature, clusters with up to 8 water molecules [16]. Moreover, there are situations where water is biochemically accepted for processing in even larger clusters by enzymes [17], or by the photosystem II [1]. In addition, this approach allows a more uniform treatment of both water and solutes with larger degrees of engagement with the nearby molecules (for many biomolecules, obviously higher than four neighbors).

				k				
			m	j	m			
		k	j	i	j	k		
			m	j	m			
				k				

Fig. 1: Types of neighborhoods of site i, used in cellular automata [11].

Following [15], we consider two main parameters for this model:

a) The breaking probability $P_B(XY)$ is the probability of a particle, *X*, in site *i*, to break away from a particle *Y*, in a site *j*, when there is exactly one occupied *j* site. $P_B(XY)$ can take values between 0 and 1. Low values of $P_B(XY)$ indicate a strong cohesion between *X* and *Y*, while high values reflect little interaction.

b) The joining parameter J(XY) is the propensity of particle *X* in site *i*, to move toward the particle *Y* in site *k*, when the intermediate site *j* is vacant. This parameter can take positive real values. When J(XY) = 1, the particle is not influenced by its second order neighbors. The case J(XY) > 1 simulates a short-range (i.e. involving only the adjacent sites) attraction. Situation J(XY) < 1 can be considered as a mutual repulsion. When J(XY) = 0, the particle in site *i*, cannot move toward the particle in site *k*.

The studies of Kier *et al.*[11-15] have shown that for the model to behave appropriately, P_B should be set to 0.2 for pure water. Importantly, it was established that J can be related to P_B in the case of water, through the following relationship (and thus we can use one rule for particle movement to model the system, the other being automatically derived from it):

$$\log J = -1.5P_B + 0.6 \qquad (1)$$

Specifically, for water where $P_B(WW) = 0.2$ this means J(WW) = 2. However, for solutes another relationship may govern these parameters. In order to cover the main possibilities, we made calculations for J(SS) with values of 0.5 (repulsive), 1 (neutral) and 2 (attractive).

The size of the grid was 55 × 55 sites, with toroidal boundary conditions. The degree of lattice occupation was set to 69 %, as suggested in [15]. The initial configuration is a random distribution of sites having any of the possible particle types. In Table 1 there is a complete list of values of the parameters used in simulations. The quantities determined from simulations were the molar fractions ‘f’ with 0 up to 8 neighbors in the final state (computed in Moore neighborhood). The values range over the unit interval.

Table 1. Parameters used in simulations.

Parameter	Description	Range
c	Concentration of solute in the system	20, 45, 75%
P_B(WW)	Probability of a water molecule to break away from another water molecule	0.2
P_B(WS)	Probability of a water/solute molecule to break away from a solute/water molecule	0.1, 0.3, 0.5, 0.7, 0.9
P_B(SS)	Probability of a solute molecule to break away from another solute molecule	0.2, 0.5, 0.9
J(SS)	Propensity of a solute molecule to move toward another solute molecule	0.5, 1.0, 2.0

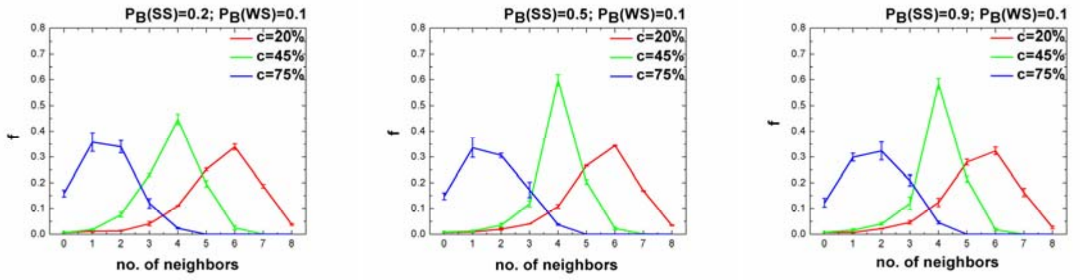
We analyzed the distribution of water-like objects in the cellular automaton model of aqueous solutions, as grouped in classes based on the number of neighbors. The model variables were three molecular properties of the solute: concentration, hydropathic (i.e. hydrophobic or hydrophilic) character, and molecular ‘stiffness’ (P_B(SS)).

Molecular concentration is known to essentially affect biochemical reactions *in vivo* [18], but its impact on local water structure was not systematically explored, in spite of the conspicuous impact of confinement on water structure [19].

The abstract property of ‘flexibility’ (P_B(SS)), reflects the probability of separation between two adjacent solutes, and is meaningful for the propensity of solute structural units to ‘make room’ for the incoming water molecules to occupy that space. The model does not specify what type of interactions take place between the unit components of the solute, which can be covalent bonds or other types of interactions. Also the relative scale of solvent and that of water does not make a difference here (except that of relative concentration, which is taken as an explicit variable). In fact, we may say that the model treats the water and the solute structural unit as both being mass- and size-normalized. Thus, the correspondent of solute in the biochemical reality can be the model unit object, a cluster thereof, or all of a given class in the system. This generality allows the model to treat all circumstances where water may reside simultaneously, whether intra-molecular, superficial or intermolecular.

3. Results and discussions

A first general observation is that solute concentration, impacting on the local water molar proportion, is a strong determinant of water aggregation but only for the hydrophilic solutes (Fig. 2a-c). Remarkably, in this case a large proportion of single water molecules can be observed in the system, indicating a gas-like phase. This phase was previously found experimentally, and also shown by molecular dynamics simulations to occur in liquid water, but associated mainly with strongly hydrophobic cavities [20,21].

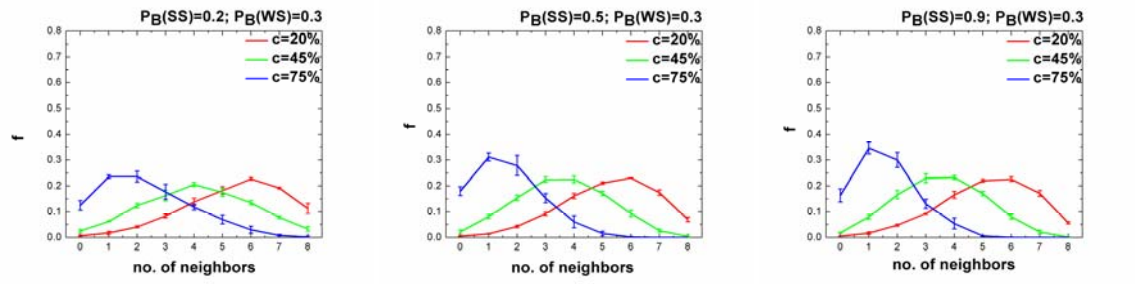


a **b** **c**

Fig. 2. Distribution of water in the presence of strongly hydrophilic (P_B(WS)=0.1) solutes, at three solute concentrations. Increasing the crowding of the system leads to water unstructuring, with little influence of solute flexibility (P_B(SS)).

A correspondent for this effect of concentrated hydrophilic phase in un-structuring the water (and a conciliator of the likely disparity between the molecular dynamics simulations versus ours), can be the recently discovered ‘hyper-mobile water’ [5, 22-24]. Large molecules with high surface charge density (and thus expectedly ‘hydrophilic’) such as the polymerized actin, were found to have a dual effect on nearby water: besides a fraction displaying reduced diffusive properties, another significant percentage of water molecules (up to 80 %) of molecular volume showed increased average mobility (‘hyper-mobility’), also translated in a smaller density than the bulk water [5]. This chemically surprising and biologically important phenomenon was proposed to explain the asymmetrical interaction of actin filaments with myosin, and thus the functioning of the acto-myosin molecular motor. In support of this came the findings that myosin II itself [22], as well as the ‘high energy’ nucleotide triphosphates (including ATP) [23], have this property to produce hyper-mobile water in their proximity.

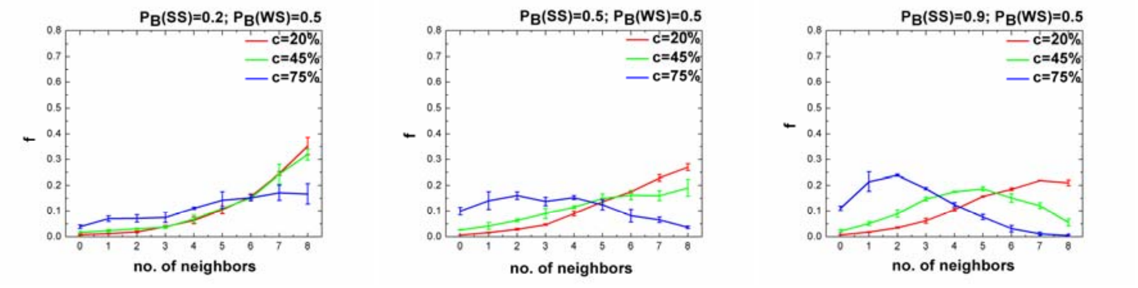
At $P_B(WS) = 0.3$, a breaking solute-water probability higher than the breaking probability between pure water molecules (0.2), the proportion of these ‘hyper-mobile’ water molecules decreases even at high solute concentrations. However, this effect is smaller in the presence of high-mobility solutes, able to compensate and thus maintain a higher proportion of ‘free’ water in the system (Fig. 3a-c).



a **b** **c**

Fig. 3. Distribution of water around hydrophilic solutes ($P_B(WS)=0.3$). Here, the increased solute flexibility favors water dispersion, mainly at higher solute crowding.

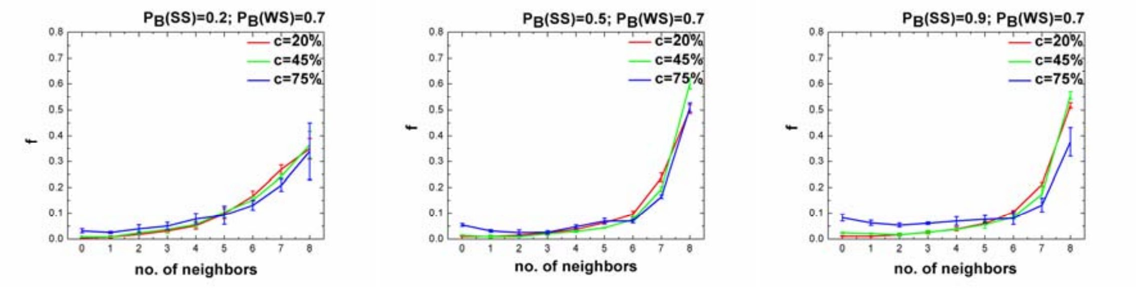
This landscape changes dramatically in the presence of rigid ‘neutral’ solutes (from the point of view of affinity for water, $P_B(WS) = 0.5$), where the majority of water molecules became clustered (Fig. 4a, b). Again, the concentrated flexible solutes better maintain the associated water dispersed in smaller aggregates (of 1-3 molecules, Fig. 4c).



a **b** **c**

Fig. 4. In vicinity of molecules of neutral hydropathic character ($P_B(WS) = 0.5$), water could be found more disorganized in molecularly-crowded regions, in a flexibility-dependent manner.

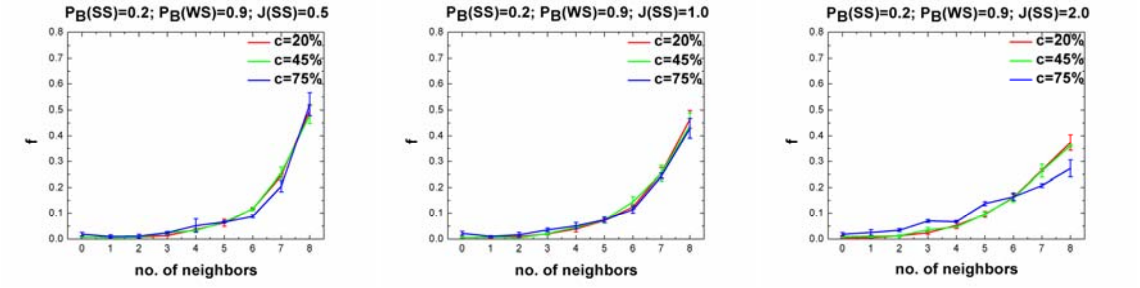
When considering hydrophobic solutes ($P_B(WS) = 0.7$)), most of nearby water is found consistently in packed structures; in this case, higher local solute mobility is of little or no effect, although increased concentrations still display some highly dispersed water (Fig. 5a-c). This is to be taken also as a support of the validity of our model, which can faithfully recapitulate the known water-organizing effect of non-polar solutes derived from hydrophobic interaction [25]. Very hydrophobic domains showed the same overall pattern, only even more accentuated (data not shown).



a **b** **c**

Fig. 5. Hydrophobic molecular domains have a strong water-organizing effect, which is only slightly reduced by crowding of very flexible solutes.

Finally, we also analyzed the role of J(SS), the ‘joining parameter’ between solute molecules (or sub-components thereof), on nearby water packing. This parameter empirically reflects the local affinity between the solutes, such as attraction (J > 1) or repulsion (J < 1) (case J = 1 is describing the neutral condition). This variable was found to have very little impact on water aggregation, irrespective of solute concentration or flexibility. The only notable difference was found for rigid hydrophobic solutes (Fig. 6), where inter- or intra-molecular repulsion (Fig. 6a) (illustrative of charged solutes, for example), shows a stronger water structuring activity than the case of repulsion (Fig. 6c), with the neutral condition placed in between (Fig 6b).



a **b** **c**

Fig. 6. Influence of J(SS) parameter on water packing around hydrophobic and rigid solutes (P_B(WS) = 0.9; P_B(SS) = 0.2): a) J(SS) = 0.5; b) J(SS) = 1; c) J(SS) = 2.

4. Conclusions

We showed that cellular automata are able to capture the essence of emergent phenomena in complex systems which are difficult to be accounted for by more traditional analytic tools, and computationally much more efficient than the molecularly-accurate dynamics simulations. Here we demonstrate that in a reverse process of determination, the solute parameters strongly influence the status of water, long known to be prone to subtle yet poorly understood ‘clustering’ effects. We found that besides the now classical hydropathic properties, molecular flexibility and crowding of the solute have concurrent effects on nearby water structure. These, in a complementary but not overlapping mode, oppose the water organizing activity of hydrophobic proximities. Molecular crowding also amplifies the water dispersing properties of hydrophilic molecules. Our study establishes a platform for further *in silico* investigations of the interplay during the emergence of biologically-specific mechanisms, between water as solvent and bio-molecular solutes.

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