3D-RISM-KH approach for biomolecular modeling at nanoscale: Thermodynamics of fibril formation and beyond

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ABSTRACT

As an alternative to the conventional solvation models, we propose to use the three-dimensional molecular theory of solvation (3D-RISM-KH theory) as an essential part of a multiscale approach for nanomedical applications. Based on the rigorous statistical-mechanical foundation, this method provides a natural link between different levels of coarse-graining details in the multiscale description of the solvation structure and thermodynamics, from highly localized structural solvent and bound ligand molecules to effective desolvation potentials and self-assembling nanoarchitectures. Using 3D-RISM-KH method, we study all stages of formation of fibrillous aggregates and amyloid fibrils. We also show that the method is capable of predicting binding sites for the inhibitors of the pathological conversion and aggregation of prion proteins. Using the novel approach for fragment based drug design, we identify the binding modes in agreement with experimental data.

Keywords: 3D molecular theory of solvation, solvation structure and thermodynamics, oligomers, amyloid fibrils, protein-ligand binding

1 INTRODUCTION

Solvation effects play an essential role in all biomolecular processes. A variety of them occur on a long-time scale and involve slow conformational changes and exchange of structural solvent and/or ligand molecules between bulk solution and pockets/cavities of biomolecules. Such processes are challenging for molecular simulations, whereas continuum solvation models that rely on phenomenological parameters do not provide adequate and transferable description of non-polar effects which are crucial for these systems. As an alternative to the conventional solvation models, we propose to use the three-dimensional molecular theory of solvation (3D-RISM-KH theory [1]) as an essential part of a multiscale approach for nanomedical applications. The method is based on the statistical-mechanical integral equation theory of molecular liquids. The 3D-RISM-KH method provides direct statistical-mechanical description of solvation thermodynamics and microscopic solvation structure, skipping the sampling of equilibrium ensemble of microscopic solvent conformations. The method gives the analytical representation for the solvation free energy in terms of the solute-solvent distribution functions. The latter can be found as solutions of the integral equations of the three-dimensional (3D) molecular theory of solvation.

We use the 3D-RISM-KH method to study all stages of formation of fibrillous aggregates and amyloid fibrils. The fibrils are a hallmark of many neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases. The formation of amyloid fibrils is a particular example of self-assembly of macromolecules, and as such is governed by the general principles of self-assembly. Prior to aggregation, the amyloidogenic peptides or proteins may misfold, and these misfolded conformations are characterized by enhanced propensity for aggregation. We analyze the initial stages of misfolding of prion protein associated with the transmissible spongiform encephalopathies, based on the combined MD/3D-RISM-KH approach. To study the association thermodynamics and conformational stability of small β-sheet oligomers (fragments of amyloid fibrils), we use the MM-3D-RISM-KH approach which combines the 3D molecular theory of solvation to describe solvent degrees of freedom with the molecular mechanics to treat internal energy and conformational entropy of biomolecules [2].

The 3D molecular theory of solvation provides the distribution functions for all solvent species (including ions, co-solvents etc.) in the proximity of a biomolecule, including its internal cavities and pockets. These distributions are solutions of the integral equations of the 3D molecular theory of liquids, and thus they do not suffer from the statistical noise. We study the internal solvation of the amyloid fibrils and small β-sheet oligomers under different solvent conditions (ionic strength, level of pH). We show that amyloid fibrils can be regarded as water-filled nanotubes, as it was suggested earlier (Perutz et al., 2002), and describe the formation of water and ion channels inside the fibril core. Another important feature of the 3D-RISM-KH method is that small molecules (e.g., ligands) can be treated in the statistical-mechanical way, along with the solvent degrees of freedom. This allows one to use the method in the fragment based drug design [3], additionally replacing the empirical desolvation potentials in conventional dock-

ing approaches with the accurate statistical-mechanical description of the solvation effects. We show that the 3D-RISM-KH method is capable of predicting binding sites for the inhibitors of the pathological conversion and aggregation of prion proteins. We identified the binding modes for this system and demonstrated that the location of the most probable modes are in full agreement with the experimental data.

2 THEORY

The statistical-mechanical 3D-RISM-KH theory [1] is based on the 3D generalization [1], [4] of the Ornstein-Zernike integral equation for a mixture of atoms/ions subject to constraints representing chemical bonds [5], [6]. The theory describes the solvation structure around a biomolecule in terms of the 3D distribution functions $g_\gamma^{uv}(\mathbf{r})$ for each solvent site γ.

The integral equation of the three-dimensional reference interaction site model (3D-RISM) for the total correlation function $h_\gamma^{uv}(\mathbf{r}) = g_\gamma^{uv}\mathbf{r}) - 1$ can be written as

$$\begin{aligned} h_\gamma^{uv}(\mathbf{r}_\gamma) &= \sum_\alpha \int d\mathbf{r}_{\alpha\gamma} c_\alpha^{uv}(\mathbf{r}_\alpha)\chi_{\alpha\gamma}^{vv}(r_{\alpha\gamma}) \\ &= \sum_\alpha \int d\mathbf{r}_{\alpha\gamma}\, c_\alpha^{uv}(\mathbf{r}_\alpha)\left[w_{\alpha\gamma}^{vv}(r_{\alpha\gamma}) + \rho_\alpha^v h_{\alpha\gamma}^{vv}(r_{\alpha\gamma})\right], \end{aligned}$$

where $c_\alpha^{uv}(\mathbf{r}_\alpha)$ is the direct correlation function, $h_{\alpha\gamma}^{vv}(r_{\alpha\gamma})$ is the site-site total correlation function of solvent, $r_{\alpha\gamma}$ is the separation between solvent sites α and γ, ρ_α^v is the bulk solvent number density, and $\chi_{\alpha\gamma}^{vv}(r_{\alpha\gamma})$ is the solvent susceptibility. The site-site intramolecular correlation matrix $w_{\alpha\gamma}^{vv}(r_{\alpha\gamma})$ describes the molecular geometry of solvent molecules. The solvent-solvent correlation function $h_{\alpha\gamma}^{vv}$ can be obtained from the 1D dielectrically consistent RISM theory [7].

The 3D-RISM equation involves two functions, $c_\gamma^{uv}(\mathbf{r})$ and $h_\gamma^{uv}(\mathbf{r})$, and thus needs to be complemented by an additional equation called a closure relation. The HNC closure has been most used to describe the solvation effects in polar liquids based on the 1D-RISM approach. In practical biomolecular simulations, the 3D Kovalenko-Hirata (3D-KH) closure [1], turns out to be superior and have been recently employed in numerous biophysical and biochemical applications (see, for example, recent studies [2], [3], [8]). The 3D-KH closure involves a partial linearization of the 3D-HNC closure and is given by

$$\begin{aligned} h_\gamma^{uv}(\mathbf{r}) + 1 &= \begin{cases} \exp\left(d_\gamma^{uv}(\mathbf{r})\right) & \text{for} \quad d_\gamma^{uv}(\mathbf{r}) \le 0\,, \\ 1 + d_\gamma^{uv}(\mathbf{r}) & \text{for} \quad d_\gamma^{uv}(\mathbf{r}) > 0\,, \end{cases} \\ d_\gamma^{uv}(\mathbf{r}) &= -\beta u_\gamma^{uv}(\mathbf{r}) + h_\gamma^{uv}(\mathbf{r}) - c_\gamma^{uv}(\mathbf{r})\,. \end{aligned}$$

This relation combines in non-trivial way the advantages of two known closures. The MSA closure is applied to the high association peaks and long-range electrostatic tails for $h_\gamma^{uv}(\mathbf{r}) > 0$, and the HNC closure is used in the core repulsion and other depletion regions for $h_\gamma^{uv}(\mathbf{r}) \le 0$. The KH closure can also be used in combination with the 1D dielectrically consistent RISM theory [7] to obtain the solvent-solvent correlation function $h_{\alpha\gamma}^{vv}$.

A great benefit of the molecular theory of solvation is the analytical representation for the solvation free energy in terms of the solute-solvent distribution functions. In the case of 3D-RISM-KH theory, the solvation free energy can be expressed in a closed form as [1]:

$$\begin{aligned} \Delta G_{\text{solv}} &= k_{_B}T \sum_\gamma \rho_\gamma^v \int \left[\frac{1}{2}\left(h_\gamma^{uv}(\mathbf{r}_\gamma)\right)^2 \Theta(-h_\gamma^{uv}(\mathbf{r}_\gamma)) \right. \\ &- \left. \frac{1}{2}\, h_\gamma^{uv}(\mathbf{r}_\gamma) c_\gamma^{uv}(\mathbf{r}_\gamma) - c_\gamma^{uv}(\mathbf{r}_\gamma)\right] d\mathbf{r}_\gamma, \end{aligned}$$

where $\Theta(x)$ is the Heaviside step function which puts $h_\gamma^{uv}(\mathbf{r}_\gamma)^2$ term in effect only in the regions of the solvent density depletion.

3 THERMODYNAMICS AND SOLVATION STRUCTURE

We study misfolding of the human prion proteins with the combined MD/MM-3D-RISM-KH approach. MD/MM are used for conformational sampling, for calculations of internal energy and conformational entropy of proteins. The solvation free energy is obtained by solving the 3D-RISM-KH equations. There is an overall trend of increase of the free energy upon unfolding. The solvation effects favor stability of the native fold, while the intra-protein interactions favor unfolding. We decompose the solvation free energy into its energetic and entropic contributions (Fig. 1). The increase of the solvation free energy is related to the solvation energetic effects mostly defined by the electrostatic interactions. The non-polar part of the entropic component of the solvation free energy, directly related to the hydrophobic interactions, contributes to stability of the native structure. The electrostatic contribution to the solvation free energy overcompensates the unfavorable intra-protein electrostatic part of the free energy, resulting in the overall favorable role of the electrostatic interactions in stabilization of the native fold of the human prion protein at neutral pH.

The overall trend of the increase in the free energy coincides with the increase of the solvent accessible surface area of the protein. There are competitive trends of exposure to the solvent of the non-polar residues and burying the charged ones. Misfolding may be accompanied by the optimization of the free energy through the aggregation, rather by re-folding of monomers. Based on the thermodynamic analysis, we identify the parts of misfolded prion protein with high propensity for aggregation.

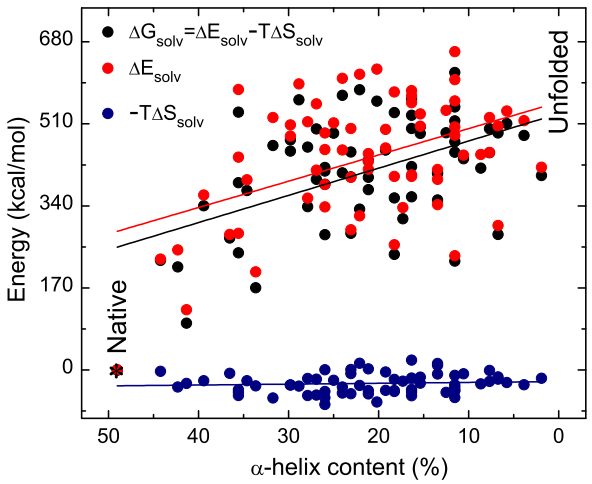

Figure 1: Solvation free energy of human prion protein, $\Delta G_{\rm solv}$, and its energetic, $\Delta E_{\rm solv}$, and entropic, $-T\Delta S_{\rm solv}$, components shown as a function of (decreasing upon unfolding) α-helical content

To study association thermodynamics of biomolecular complexes, we proposed to use a novel approach which combines explicit solvent MD for conformational sampling, MM for calculations of the internal energy and conformational entropy of biomolecules, and the 3D-RISM-KH method for calculations of the solvation free energy. We employ this approach for analysis of the association thermodynamics of small β-sheet oligomers, fragments of amyloid (A) β fibrils [2]. We found that the charge reduction of Aβ peptides due to mutations or protonation of the solvent exposed glutamic acids results in a significant shift of the balance between different physical components of the association free energy. At the same time, the overall change in the association free energy for the E22Q mutants is relatively small compared to the change of the individual components, which is a consequence of the complementarity and the resulting partial compensation between the direct and the solvation electrostatic effects. Our results suggest that the difference in the oligomerization propensity may be defined by the early stages of aggregation, namely, by the conversion of Aβ monomers from the random coil to the β-sheet conformation (Grant et al., 2007). We show that the solvation entropic contribution always favors the thermodynamic stability of β-sheet oligomers. The dispersion interactions also contributes to their stability. The latter is a consequence of the high degree of shape complementarity and tight packing of the Aβ peptides in the β-sheet oligomers and amyloid fibrils.

Additionally to the solvation thermodynamics, the 3D-RISM-KH method provides comprehensive information on the microscopic solvation structure of biomolecular complexes. The water distribution in the proximity of β-sheet fragments of amyloid fibrils (e.g., Aβ oligomers) is characterized by the complementarity between density distributions for oxygen and hydrogen sites at the edge of a fragment. This provides the possibility for efficient docking of amyloidogenic peptides at the edge of the fibril allowing the fibril to grow by attaching the monomers to its edge. At larger distances from the fibril, the complementary between oxygen and hydrogen (or any other solvent sites with the opposite charges) can be seen around the entire fragment. The observed microscopic structure of the solvation shells is a result of interplay between solute-solvent and solvent-solvent interactions, including the hydrogen bonds formation between water molecules from the one hand and water molecules and solute, from the other.

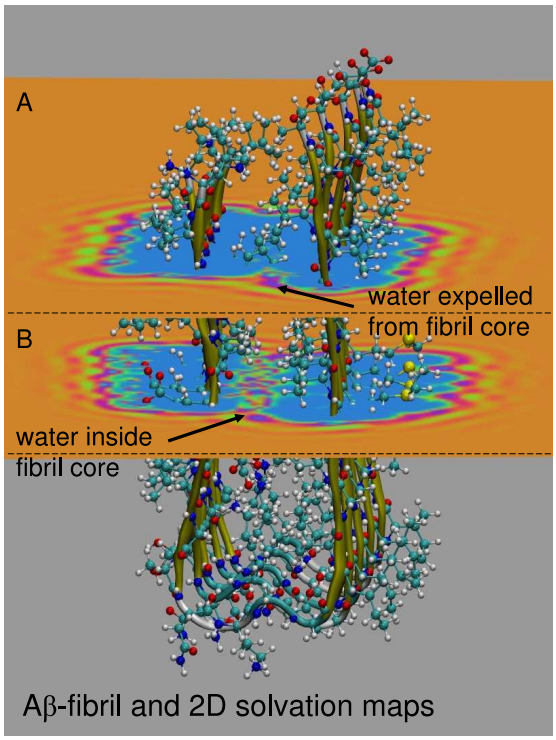

Figure 2: Solvation shells of the 5-mer fragment of the Aβ fibril (Lührs et al., 2005) represented by the cross-sections of the water oxygen distribution by planes parallel to the fibril axis.

Another important feature of the microscopic solvation structure in the proximity of Aβ fibrils (β-sheet oligomers) is localization of water molecules inside the fibril core. The possibility of internal hydration and water (ion) channels in Aβ fibrils was discussed previously based on the results of the explicit solvent MD simulations (Buchete et al., 2005). We use the 3D-RISM-KH method to provide accurate statistical-mechanical picture of internal hydration of fibrils and β-sheet oligomers

(Fig. 2). Along with the water molecules, positive ions characterized by the relatively small van der Waals radii can be easily locked in the inner domains of the fibril. For Aβ fibrils such domains are adjacent to the protonatable glutamic acid residues, with the implication that the small positive ions locked inside the fibril core can mimic lowering the level of pH, thus affecting in the similar way the propensity for aggregation and thermodynamic stability of the fibril.

Theoretical description of amyloid fibrils built of a large number of peptides is a computationally challenging problem. To overcome its complexity, we employ a novel periodic approach which has been recently used to study thermodynamic stability of microtubules [8]. Accounting for the cross-β periodic motif of amyloid fibrils, the approach allows one to obtain thermodynamic properties and solvation structure of a full length fibril from simulations with just one constituting unit of the fibril in a periodic cell. We found that the solvation entropic effects (fully quantified by using the 3D-RISM-KH method), closely related to the hydrophobic interactions, and the inter-protein dispersion interactions, are two major factors contributing to stability of amyloid fibrils. The barrier in the solvation free energy as a function of the separation between peptides was identified and its origin from the desolvation effect was demonstrated.

4 BINDING MODES PREDICTION

It was recently shown that the 3D-RISM-KH method can predict binding sites of a protein-ligand complex in a good agreement with the x-ray and NMR experimental data [3]. Compared to the conventional docking algorithms, the method describes from the first principles all physical contributions to the binding energy (including chemicals specificities of hydrogen bonding, solvation entropic effects and hydrophobicity), as well as ligand concentration, solvent composition and temperature effects. The approach uses the grid representation for protein-ligand interaction potentials and solvation density maps. The grid data are processed with the efficient parallel fast Fourier transform algorithm similar to the grid-based docking algorithms.

As a validation and illustration of the approach capability for predicting binding sites for the inhibitors of the prion conversion, we predict the binding sites of 2-pyrrolidin-1-yl-N-[4-[4-(2-pyrrolidin-1-yl-acetylamino) -benzyl]-phenyl]-acetamide binding to the mouse prion protein. The compound is known to inhibit the pathological conversion of prion proteins and prolong the survival of TSE-infected mice (Kuwata et al., 2007). We illustrate the 3D-RISM-KH based docking algorithm in Fig. 3.

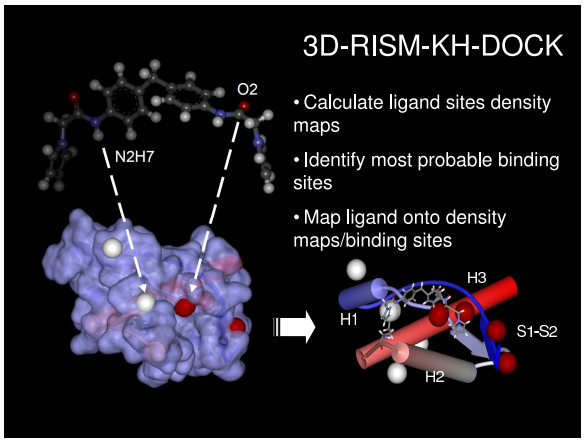

Figure 3: Most probable binding mode of 2-pyrrolidin-1-yl-N-[4-[4-(2-pyrrolidin-1-yl-acetylamino)-benzyl]-phenyl]-acetamide (Kuwata et al., 2007) at mouse PrP. Possible binding sites of ligand nitrogen, hydrogen and oxygen are shown as white (N-H) and red (O) spheres. The binding sites at Glu-196 and Asn-159 are characterized by largest affinity in agreement with the experiment.

5 ACKNOWLEDGMENTS

We acknowledge the financial support by the Alberta Prion Research Institute (APRI) and the National Research Council (NRC) of Canada.

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