Variational implicit-solvent predictions of the dry-wet transition pathways for ligand-receptor binding and unbinding kinetics

Shenggao Zhou\textsuperscript{a}, R. Gregor Weiß\textsuperscript{b}, Li-Tien Cheng\textsuperscript{c}, Joachim Dzubiella\textsuperscript{d,e,1}, J. Andrew McCammon\textsuperscript{c,1}, and Bo Li\textsuperscript{c,1}

\textsuperscript{a}Department of Mathematics and Mathematical Center for Interdisciplinary Research, Soochow University, 1 Shizi Street, Suzhou 215006, Jiangsu, China; \textsuperscript{b}Laboratory of Physical Chemistry, ETH Zürich, Vladimir-Prelog-Weg 2, CH-8093 Zürich, Switzerland; and Institut für Physik, Humboldt-Universität zu Berlin, Newtonstrasse 15, D-12489 Berlin, Germany; \textsuperscript{c}Department of Mathematics, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093-0112, USA; \textsuperscript{d}Physikalisches Institut, Albert-Ludwigs-Universität Freiburg, Hermann-Herder-Straße 3, 79104 Freiburg, Germany; and Research Group Simulations of Energy Materials (EE-GSEM), Helmholtz-Zentrum Berlin, Hahn-Meitner-Platz 1, 14109, Berlin, Germany; \textsuperscript{e}Department of Chemistry and Biochemistry, Department of Pharmacology, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093-0365, USA

This manuscript was compiled on June 27, 2019

Ligand-receptor binding and unbinding are fundamental biomolecular processes and particularly essential to drug efficacy. Environmental water fluctuations, however, impact the corresponding thermodynamics and kinetics and thereby challenge theoretical descriptions. Here, we devise a holistic, implicit-solvent, multi-method approach to predict the (un)binding kinetics for a generic ligand-pocket model. We use the variational implicit-solvent model (VISM) to calculate the solute-solvent interfacial structures and the corresponding free energies, and combine the VISM with the string method to obtain the minimum energy paths and transition states between the various metastable (“dry” and “wet”) hydration states. The resulting dry-wet transition rates are then used in a spatially-dependent multi-state continuous-time Markov chain Brownian dynamics simulations, and the related Fokker–Planck equation calculations, of the ligand stochastic motion, providing the mean first-passage times for binding and unbinding. We find the hydration transitions to significantly slow down the binding process, in semi-quantitative agreement with existing explicit-water simulations, but significantly accelerate the unbinding process. Moreover, our methods allow the characterization of non-equilibrium hydration states of pocket and ligand during the ligand movement, for which we find substantial memory and hysteresis effects for binding versus unbinding. Our study thus provides a significant step forward towards efficient, physics-based interpretation and predictions of the complex kinetics in realistic ligand-receptor systems.

Significance Statement

The kinetics of ligand-receptor (un)binding—how fast a ligand binds into and resides in a receptor—cannot be inferred solely from the binding affinity which describes the thermodynamic stability of the bound complex. A bottleneck in understanding such kinetics, which is critical to drug efficacy, lies in the modeling of the collective water fluctuations in apolar confinement. We develop a new theoretical approach that couples a variational implicit-solvent model with the string method to describe the dry-wet transition pathways, which then serve as input for the ligand multi-state Brownian dynamics. Without explicit descriptions of individual water molecule, our theory predicts the key thermodynamic and kinetic properties of unbinding and binding, the latter in quantitative agreement with explicit-water molecular dynamics simulations.

The authors declare no conflict of interest.

1 To whom correspondence should be addressed. E-mail: jmcammon@ucsd.edu or bli@math.ucsd.edu

www.pnas.org/cgi/doi/10.1073/pnas.XXXXXX

Signatures
While explicitly tracking water molecules, MD simulations are still limited to systems of relatively small sizes and events of relatively short time scales. In particular, slow and rare water fluctuations and large ligand residence times in the pocket still challenge the prediction of unbinding times.

In this work, we develop a holistic, multi-method, implicit-solvent approach to study the kinetics of ligand-receptor binding and unbinding in a generic pocket-ligand model exactly as studied previously by explicit-water MD simulations (18), focusing on the effect of solvent fluctuations and multiple hydration states on such processes.

Our approach is based on the variational implicit-solvent model (VISM) that we have developed in recent years (34–38). In VISM, one minimizes a solvation free-energy functional of solute-solvent interfaces to determine a stable, equilibrium conformation, and to provide an approximation of the solvation free energy. The functional couples the solute surface energy, solute-solvent van der Waals (vdW) dispersive interactions, and electrostatics. This theory resembles that of Lum–Chandler–Weeks (39) [cf. also (40, 41)], and is different from the existing SAS (solvent-accessible surface) type models. We have designed and implemented a robust level-set method to numerically minimize the VISM functional with arbitrary 3D geometry (36–38, 42).

Here, for our model ligand-pocket system, we use our level-set VISM to obtain different hydration states and their solvation free energies, and use the VISM-string method (43, 44) to find the minimum energy paths connecting such states and the corresponding transition rates. Such rates are then used in our continuous-time Markov chain Brownian dynamics simulations, and the related Fokker–Planck equation calculations, of the ligand stochastic motion to obtain the mean first-passage times for the ligand binding and unbinding. We compare our results with existing explicit-water MD simulations.

The model ligand-receptor system. The generic pocket-ligand model (45) consists of a hemispherical pocket and a methane-like molecule; cf. Fig. 1 (A). The pocket, with the radius \( R = 8 \) Å and centered at \((0, 0, 0)\), is embedded in a rectangular wall, composed of apolar atoms aligned in a hexagonal-close-packed grid of lattice constant 1.25 Å. The wall surface is oriented in \( xy \)-plane. The ligand, a single neutral Lennard-Jones (LJ) sphere, is placed along the pocket symmetry axis, the \( z \)-axis, which is taken to be the reaction coordinate. Fig. 1 (B)-(D) depict the cross sections of all the possible VISM surfaces, i.e., the stable solute-solvent interfaces separating the solute region \( \Omega_s \) and solvent region \( \Omega_w \), representing different hydration states for a fixed position of ligand.

Results and Analysis

Multiple hydration states and the potential of mean force (PMF). We use our level-set method to minimize the VISM solvation free-energy functional (cf. Eq. [2] in Theory and Methods) and obtain a VISM surface. By choosing different initial solute-solvent interfaces, we obtain different VISM surfaces describing different hydration states; cf. Fig. 1.

Fig. 2 (A) shows the solvation free energies for different VISM surfaces against the reaction coordinate \( z \). For \( z < -0.5 \) Å, there is only one VISM surface, 1s-dry; cf. Fig. 1 (B). In addition to 1s-dry, a second VISM surface, 2s-wet, appears for \(-0.5 < z < 5 \) Å; cf. Fig. 1 (D). For \( 5 < z < 8 \) Å, there are three VISM surfaces. In addition to 1s-dry and 2s-wet, the third one is 2s-dry; cf. Fig. 1 (C). Once the ligand is away from the pocket with \( z > 8 \) Å, there are only two VISM surfaces: 2s-dry and 2s-wet.

Dry-wet transition paths and energy barriers. At a fixed reaction coordinate \( z \) with multiple hydration states, we use our level-set VISM coupled with the string method to calculate the minimum energy paths (MEPs) that connect these states, and the corresponding transition states, energy barriers, and ultimately the transition rates. A string or path here consists of a family of solute-solvent interfaces, and each point of a string, which is an interface in our case, is called an image.

In Fig. 3, we display the solvation free energies of images on MEPs that connect the three hydration states, 1s-dry, 2s-dry, and 2s-wet, at \( z = 6 \) Å. There are two MEPs connecting 1s-dry (marked (I)) and 2s-dry (marked (IV)). One of them passes...
through the axisymmetric transition state marked (III), and the other passes through the axisymmetric transition state marked (II). Here, symmetry or asymmetry refers to that of the 3D conformation of the VISM surface. Energy barriers in the transition from the state 1s-dry to 2s-dry along the two transition paths are estimated to be $1.09 \kT$ and $0.52 \kT$, respectively. Only one MEP is found to connect 2s-dry (marked (IV)) and 2s-wet (marked (VI)), and the corresponding transition state (marked (V)) is also found. The MEP from 1s-dry to 2s-wet always passes through the state 2s-dry. The dewetting barrier first increases as $z$ approaches the pocket, the solute-solvent interfacial energy states 1s-dry and 2s-dry; cf. Fig. 3. Note that, as the ligand is close to the pocket, since contributions of solute-solvent vdW interaction are lost during the pocket dewetting, the attractive solute-solvent vdW interaction decreases. It then reaches a plateau after the distance is greater than 7 Å. The pocket dewetting barrier (marked blue) is slightly larger when the ligand is close to the pocket, since contributions of solute-solvent vdW interaction are lost during the pocket dewetting.

**Fig. 3.** Solvation free energies of images on MEPs that connect the hydration states 1s-dry (I), 2s-dry (IV), and 2s-wet (VI) (shown in the bottom) with transition states (II), (III), and (V) (shown on top) and the transition energy barriers for $z = 6$ Å. In the middle plots, the horizontal axis is the string parameter $\alpha$.

**Kinetics of binding and unbinding.** We perform continuous-time Markov chain (CTMC) Brownian dynamics (BD) simulations and solve the related Fokker–Planck equation (FPE) calculations for the ligand stochastic motion with the pocket dry-wet fluctuations; see Theory and Methods. For comparison, we also perform the usual BD simulations and FPE calculations without including such fluctuations.

**Fig. 4** shows the mean first-passage times (MFPTs) for the binding and unbinding, respectively. Note that the BD simulations and FPE calculations agree with each other perfectly for both binding and unbinding, without and with the pocket dry-wet fluctuations, respectively. This validates mutually the accuracy of our numerical schemes. Note also that the binding/unbinding MFPT increases/decreases monotonically as the ligand-pocket distance increases, due to elongated/shortened ligand travel.

In Fig. 5 (A), we see that the MFPT for binding is very small if $z < -0.5$ Å. This is because the ligand diffusion constant $D_{\text{in}}$ inside the pocket is large and the PMF is highly attractive; cf. Fig. 2 (B). As the initial position $z$ increases from 0 Å to 5 Å, the difference between the two MFPTs with and without the pocket dry-wet fluctuations increases from nearly 0 ps to 100 ps. Such an increasing difference results from the existence of the hydration state 2s-wet in this range, and the solvation free energy of this state increases as the ligand moves from $z = 5$ Å to $z = 0$ Å; cf. Fig. 2 (A). The pocket dry-wet fluctuations thus decelerate considerably the ligand-pocket association. Such deceleration has been explained by the reduced diffusivity of the ligand in the vicinity of pocket entrance due to the slow solvent fluctuations (18).

Our predictions of the MFPT for binding, with the dry-wet fluctuations included, agree very well with the explicit-water MD simulations (18), improving significantly over those without such fluctuations. Note that our model predicts somewhat shorter binding times than the MD simulations for $1 < z < 6$ Å.
When the ligand is far away, there are only two VISM surfaces, 2s-dry and 2s-wet, cf. Fig. 2 (A). For such a case, our BD simulations predict the probability 32% of a wet pocket (i.e., $\chi_p = 0.32$ for large $z$) in the binding and unbinding processes. This is perfectly consistent with the equilibrium probability $e^{-G(z)_{2s-dry}/k_B T}/(e^{-G(z)_{2s-dry}/k_B T} + e^{-G(z)_{2s-wet}/k_B T})$ predicted by our VISM theory. We observe that the pocket hydration peaks at the entrance of the pocket in binding, agreeing well with MD simulations (17, 18), where it was argued that stronger pocket hydration is induced by the penetration of the ligand solvation shell. When the ligand enters the pocket the latter becomes dry as anticipated.

In comparison, the maximum pocket hydration for unbinding is shifted a bit away from the pocket. This kinetic asymmetry or “translational mismatch” can be explained as well by the asymmetric hydration states of the ligand, see Fig. 5 (E), which exits the pocket without a complete solvation shell. This behavior is reminiscent of a hysteresis, that is, the hydration states during the ligand passage depend on the history of the ligand, i.e., where it comes from.

The standard deviations of pocket hydration shown in Fig. 5 (D) depict that the dry-wet fluctuations have local maxima close to the pocket entrance ($z \simeq 3 - 5 \, \text{Å}$) and behave also significantly different for binding and unbinding. The corresponding standard deviations of ligand hydration shown in Fig. 5 (F) show massively unstable hydration (i.e., large peaks) close to the pocket entrance, while inside and far away from the pocket the fluctuations are zero, indicating a very stable (de)hydration state. Again the peaks are at different locations for binding versus unbinding, reflecting the hysteresis and memory of dry-wet transitions during ligand passage.

Conclusions

We have developed an implicit-solvent approach, coupling our VISM, the string method, and multi-state CTMC BD simulations, for studying the kinetics of ligand-receptor binding and unbinding, particularly the influence of collective solvent fluctuations on such processes. Without any explicit descriptions of individual water molecules, our predictions of the MFPT for the binding process, which is decelerated by the solvent fluctuations around the pocket, agree very well with the less efficient explicit-water MD simulations. Moreover, we find surprisingly that the solvent fluctuations accelerate the ligand unbinding from the pocket, which involves a much larger timescale and is thus more challenging for explicit-water MD simulations (26, 30). Importantly, our implicit-solvent approach indicates that the water effects are controlled by a few key physical parameters and mechanisms, such as polycondom nano-capillarity based on surface tension of the solute-solvent interface and the coupling of the random interface forces to the ligand’s diffusive motion.

Our approach provides a promising new direction in efficiently probing the kinetics, and thermodynamics, of the association and dissociation of complex ligand-receptor systems, which have been studied mostly using enhanced sampling techniques (18, 25, 26, 28, 30, 32). Our next step is to extend our approach for more realistic systems with general reaction coordinates and different techniques for sampling transition paths (48, 49). Our VISM can treat efficiently the electrostatic interactions using the Poisson–Boltzmann theory (38). To account for the flexibility of the ligand and receptor in their
binding and unbinding, we shall expand our solvation model to include the solute molecular mechanical interactions (50).

Theory and Methods

Variational implicit-solvent model (VISM). We consider the solvation of solute molecules, with all the solute atomic positions \( r_1, \ldots, r_N \), in an aqueous solvent that is treated implicitly as a continuum. (For our model ligand-pocket system, the solute atoms include those of the concave wall and the single atom of the ligand; cf. Fig. 1.) A solute-solvent interface \( \Gamma \) is a closed surface that encloses all the solute atoms but no solvent molecules. The interior and exterior of \( \Gamma \) are the solute and solvent regions, denoted \( \Omega_m \) and \( \Omega_s \), respectively. We introduce the VISM solvation free-energy functional (34, 35):

\[
G[\Gamma] = \Delta P \text{vol}(\Omega_m) + \int_{\Gamma} \gamma dS + \rho_0 \int_{\Omega_m} U(r) dV + G_e[\Gamma]. \tag{2}
\]

Here, \( \Delta P \) is the difference of pressures across the interface \( \Gamma \), \( \gamma \) is the solute-solvent interface surface tension, \( \rho_0 \) is the bulk solvent (i.e., water) density, and \( U(r) = \sum_{i=1}^{N} U_i(|r-r_i|) \) with each \( U_i \) a standard 12-6 LJ potential. We take \( \gamma = \gamma_0(1 - 2\pi H) \), where \( \gamma_0 \) is the surface tension for a planar interface, \( \tau \) is the curvature correction coefficient often known as the Tolman length (51), and \( H \) is the local mean curvature. The last term \( G_e[\Gamma] \) is the electrostatic part of the solvation free energy, which we will not include in this study.

Minimizing the functional Eq. [2] among all the solute-solvent interfaces \( \Gamma \) determines a stable, equilibrium, solute-solvent interface, called a VISM surface, and the corresponding solvation free energy. A VISM surface is termed dry, representing a dry hydration state, if it loosely wraps up all the solute atoms with enough space for a few solvent molecules, or wet, representing a wet hydration state, if it tightly wraps up all the solute atoms without extra space for a solvent molecule.

Implementation by the level-set method. Beginning with an initially guessed solute-solvent interface, our level-set method exploits the interface step by step in the steepest descent direction until a VISM surface is reached. Different initial surfaces may lead to different final VISM surfaces. See Supporting Information (SI) for more details of implementation.

The level-set VISM-string method for minimum energy paths (MEPs). Let us fix all the solute atomic positions and assume that \( \Gamma_0 \) and \( \Gamma_1 \) are two VISM surfaces (e.g., dry and wet surfaces). We apply the string method (43, 44) to find a MEP that connects \( \Gamma_0 \) and \( \Gamma_1 \). A string or path here is a family of solute-solvent interfaces \( \{ \Gamma_{\alpha} \}_{\alpha \in [0,1]} \) that connects the two states \( \Gamma_0 \) and \( \Gamma_1 \). Such a string is a MEP, if it is orthogonal to the level surfaces of the VISM free-energy functional. To find a MEP connecting \( \Gamma_0 \) and \( \Gamma_1 \), we select some initial images (i.e., points of a string), and then update them iteratively to reach a MEP. Different initial images may lead to different MEPs. Once a MEP is found, we can then find a saddle point on the MEP. Alternatively, we can fix one of the VISM surfaces, select some initial images, and allow the last image to climb up to reach a saddle point, and then find the MEP connecting the two VISM surfaces passing the saddle point. We refer to SI for more details on our implementation of the method.

Consider now our ligand-pocket system; cf. Fig. 1. For any reaction coordinate \( z \), we label all the three hydration states 1s-dry, 2s-dry, and 2s-wet (cf. Fig. 1) as the states 0, 1, and 2, respectively. We define for each \( i \in \{0,1,2\} \) the potential

\[
V_i(z) = G_i(z) + U_0(z),
\]

where \( G_i(z) \) is the solvation free energy of the \( i \)th state at \( z \) (cf. Fig 2 (A)) and \( U_0(z) \) is the ligand-pocket vdW interaction potential defined below Eq. [1]. We set \( V_i(z) = 0 \) if the \( i \)th state does not exist at \( z \).

With the energy barriers summarized in Fig. 4, we can calculate for each \( z \) the rate \( R_{ij} = R_{ij}(z) \) of the transition from one state \( i \) to another \( j \). If a MEP from \( i \) to \( j \) passes through another state \( k \) (cf. Fig. 3), then we set \( R_{ij}(z) = 0 \). If there is only one MEP connecting \( i \) and \( j \) (see, e.g., \( z < 4 \) in Fig. 2), then \( R_{ij} = R_0 e^{-B_{ij}(z)/k_B T} \) with \( B_{ij}(z) \) the energy barrier from \( i \) to \( j \) and \( R_0 \) a constant prefactor, describing the intrinsic time scale of water dynamics in the pocket. Finally, if there are two MEPs (axisymmetric and axiasymmetric) connecting \( i \) and \( j \), we use the same formula but with two effective barriers.

Continuous-time Markov chain (CTMC) Brownian dynamics (BD) simulations and the mean first-passage time (MFPT). To include explicitly the dry-wet fluctuations, we introduce a position-dependent, multi-state, random variable \( \eta(t) \): \( \eta(t) = i \) \( i \in \{0,1,2\} \) if the system is in the \( i \)th hydration state when the ligand is located at \( z \), with the transition rates \( R_{ij}(z) \) given above. We define the potential \( V_{\text{fluc}}(\eta,z) = V_i(z) \) (cf. Eq. [3]) if \( \eta(t) = i \). (52) The random position \( z = z(t) = z_i \) of the ligand is now determined by our CTMC BD simulations in which we solve the stochastic differential equation

\[
dz = \left[ -\frac{D(z)}{k_B T} \frac{\partial V_{\text{fluc}}(\eta,z_i)}{\partial z} + D'(z_i) \right] dt + \sqrt{2D(z_i)} d\xi_t.
\]

Here, the partial derivative of \( V_{\text{fluc}} \) is with respect to its second variable, \( D(z) \) is an effective diffusion coefficient that smoothly interpolates the diffusion coefficients \( D_{\text{in}} \) and \( D_{\text{out}} \) inside and outside the pocket, respectively, and \( \xi_t \) is the standard Brownian motion. Solutions to this equation are constrained by \( z_i \in [z_{\text{in}},z_{\text{out}}] \) for some \( z_{\text{in}} \) and \( z_{\text{out}} \). For the simulation of a binding unbinding process, we reset the value of \( z_i \) to \( 2z_{\text{out}} - z_i \) if \( z_i \geq z_{\text{out}} \), and we stop the simulation if \( z_i \leq z_{\text{in}} \). For the simulation of an unbinding process, we reset the value of \( z(t) \) to \( z_i \) if \( z_i \leq z_{\text{in}} \), and we stop the simulation if \( z_i \geq z_{\text{out}} \). The distribution of \( \eta(z_0) \) for an initial ligand position \( z_0 = z_{\text{in}} \) is based on the equilibrium probabilities \( e^{-G_i/k_B T} / \sum_{j=0}^2 e^{-G_j/k_B T} \) (\( i = 0,1,2 \)), where \( G_i \) is the solvation free energy of the \( i \)th hydration state at \( z_0 \).

We run our CTMC BD simulation for the ligand starting at a position \( z_0 = z_{\text{in}} \) and record the time at which the ligand reaches \( z_i \) (or \( z_0 \)) for the first time for a binding (or unbinding) simulation. We run simulations for 3,000 times and average these times to obtain the corresponding MFPTs.
Fokker–Planck equations (FPE) and the MFPT. The probability densities $P_i = P_i(z,t)$ for the ligand at location $z$ at time $t$ with the system in the $i$-th hydration state are determined by the generalized FPEs (25, 52):

$$\frac{\partial P_i}{\partial t} = \frac{\partial}{\partial z} \left\{ D(z) \left( \frac{\partial P_i}{\partial z} + \frac{1}{k_BT} V'(z) P_i \right) \right\} + \sum_{j \neq i} R_{ij}(z) P_j - \sum_{j \neq i} R_{ji}(z) P_i,$$

for $i = 0, 1, 2$, with $V$ defined in Eq. [3]. These equations are solved for $z_L < z < z_R$, with the boundary conditions $P_i(z_L, t) = 0$ and $P_i(z_R, t) = 0$ for binding, and $\partial_t P_i(z_L, t) + (1/\kappa_BT) V'(z) P_i(z_L, t) = 0$ and $P_i(z_R, t) = 0$ for unbinding, respectively. The initial conditions are $P_i(z, 0) = \delta(z - z_{ini})$ if the ligand is initially at $z_{ini}$. We obtain the MFPT as the double integral of $\sum_{i=0}^2 P_i(z,t)$ over $(z,t) \in [z_L, z_R] \times [0, \infty].$

**Parameters.** We set the temperature $T = 298$ K, bulk water density $\rho_0 = 0.033$ Å$^{-3}$, the solute-water surface tension constant $\gamma_0 = 0.143 k_BT/\kappa_s$ ($\kappa_s$ is the Boltzmann constant), and the Tolman length $\sigma = 0.8$ Å. We set $\Delta P_{\text{vol}} (\Omega_0) = 0$ as it is relatively very small. The LJ parameters for the wall particles, ligand, and water are $\varepsilon_{\text{wall}} = 0.000967 k_BT$ and $\sigma_{\text{wall}} = 4.152$ Å, $\varepsilon_{\text{ligand}} = 0.5 k_BT$ and $\sigma_{\text{ligand}} = 3.73$ Å, and $\varepsilon_{\text{water}} = 0.26 k_BT$ and $\sigma_{\text{water}} = 3.154$ Å, respectively. The interaction LJ parameters are determined by the Lorentz–Berthelot mixing rules. The prefactor $R_0 = 0.13$ ps$^{-1}$. The diffusion constants are $D_{\text{out}} = 0.26 \text{ Å}^2/\text{ps}$ (18) and $D_{\text{in}} = 1 \text{ Å}^2/\text{ps}$. The cut-off position distinguishing the inside and outside of the pocket is $z_r = -0.5$ Å. BD simulations and FPE calculations are done for $z_L \leq z \leq z_R$ with $z_L = -4$ Å and $z_R = 15.5$ Å.

**ACKNOWLEDGMENTS.** SZ was supported in part by NSF of Jiangsu Province, China, through grant BK20160302, NSFC through grant NSFC 21773169 and NSFC 11601361, and Soochow University through a start-up grant QL17004135. RGW and JD thank the DFG for financial support. JD also acknowledges funding from the ERC within the Consolidator Grant with Project No. 640659–NANOREACTOR. Work in the McCammon group is supported in part by NIH, NCBR, and SDSC. LTC and BL were supported in part by the NSF through the grant DMS-1620487. SZ thanks Dr. Yanan Zhang for helpful discussions on the string method.