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Authors

Yap, Eng-Hui
Head-Gordon, Teresa

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Calculating the Bimolecular Rate of Protein-Protein Association with Interacting Crowders

Eng-Hui Yap^{1,3,†} and Teresa Head-Gordon^{1,2,3,4*}

¹UCSF and UC Berkeley Joint Graduate Group in Bioengineering, ²Department of Chemistry, ³Department of Bioengineering, ⁴Department of Chemical and Biomolecular Engineering, University of California, Berkeley, CA 94720

We have recently introduced a method termed Poisson-Boltzmann semi-analytical method (PB-SAM) for solving the linearized Poisson-Boltzmann equation for large numbers of arbitrarily shaped dielectric cavities with controlled precision. In this work we extend the applicability of the PB-SAM approach by deriving force and torque expressions that fully account for mutual polarization in both the zero and first order derivative of the surface charges, that can now be embedded into a Brownian dynamics scheme to look at electrostatic-driven mesoscale assembly and kinetics. We demonstrate the capabilities of the PB-SAM approach by simulating the protein concentration effects on the biomolecular rate of association of barnase and barstar, under periodic boundary conditions and evaluated through mean first passage times. We apply PB-SAM to the pseudo-first order reaction rate conditions in which either barnase or barstar are in great excess relative to the other protein (124:1). This can be considered a specific case in which the PB-SAM approach can be applied to crowding conditions in which crowders are not inert but can form interactions with other molecules.

[†] Current address: Department of Systems and Comp. Bio., Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461.

1. INTRODUCTION

The formation of protein-protein interactions is required for such diverse cellular processes as signaling, metabolic regulation, and motility. The ability to understand and control the mechanism of protein complex formation and the rate of association are all needed for understanding function, especially in the cellular milieu which gives rise to crowding effects¹⁻⁴. Therefore the development of new models and algorithms for protein-protein complex formation kinetics under *in vivo* conditions of crowding is a central problem in computational biology.

Since electrostatic forces often orchestrate the earliest of recognition events for biomolecular complex formation, a realistic complexation study must include an accurate treatment of bulk electrolytes. This can be modeled using continuum mean-field theory with the Poisson-Boltzmann (PB) equation, which under low field condition be further simplified to the linearized Poisson-Boltzmann equation (LPBE). Various analytical and numerical treatments can be used to solve the LPBE⁵. Analytical methods typically allow rapid solution of the LPBE equation under specialized geometries such as spheres or cylinders⁶⁻¹¹. Our group has achieved a fundamental result in deriving the first completely general analytical solution to the LPBE for computing the full *mutual* polarization and screened (salty) electrostatic interaction between arbitrary numbers of spheres of arbitrarily complex charge distributions, separated by arbitrary distance or concentration¹².

In contrast, numerical methods (see reference [5] for a recent survey) such as finite-difference (FD)¹³⁻¹⁵ and finite-element (FE)¹⁶⁻¹⁸ methods can handle arbitrary dielectric boundaries by solving for the PB potential on a 3-D grid or mesh. Recent theoretical advances have addressed some of the critical limitations in traditional FE and FD formulations: charge singularities¹⁹⁻²¹ were mitigated, and electric displacement continuity was enforced across dielectric boundaries using matched-interface techniques^{22, 23}. However, analytical formulations of forces, as derived by Gilson²⁴ and Im et. al.²⁵, are based on one single macromolecule, and therefore do not account for mutual polarization effects of the *gradient* due to movement of one macromolecule with respect to another. To correctly account for mutual gradient polarization in the FD or FE context, one could employ the ‘virtual work’ approach²⁶, whereby the electrostatic energy is recalculated after a small displacement in the x, y, and z direction respectively, and the force in each direction given by the difference in energy over the displaced distance. Even with the assumption of rigid macromolecules, this would still require four finite difference

computations for each macromolecule, making it computationally laborious. Most importantly, the requirement that the solution be solved on a grid limits its practical application to spatial domains of either two to three typical macromolecules at reasonably high resolution ($\sim 0.2\text{\AA}$), or to larger numbers of macromolecules with greatly diminished resolution ($\sim 2.0\text{-}5.0\text{\AA}$) and thus solution accuracy, rendering them unsuitable for long-lengthscale association simulations.

Boundary element (BE) methods²⁷⁻³¹ are an attractive alternative since they satisfy both the Dirichlet and von Neuman boundary conditions by construction, singular charges can be correctly treated, and most importantly the 2D solutions on the macromolecular surface removes spatial resolution limitations imposed by the 3D grid of the FD or FE solvers. However increasing the number of boundary surface element results in an increasingly large dense matrix to be solved with severe memory requirements, a problem which scales with the number of macromolecules. Acceleration of the BE approach^{28, 32} incorporating fast multipole methods have rendered BE computational times comparable to state-of-the-art software packages like the Adaptive Poisson-Boltzmann Solver (APBS)¹³ based on FD solutions. A recently available algorithm, AFMPB³³, allows calculation of electrostatics energy and forces, but it has yet to be demonstrated to be efficient enough for dynamic simulation and only includes self polarization effects.

We have previously presented a new numerical approach to solving the LPBE equation, Poisson-Boltzmann semi-analytical method (PB-SAM), that combines the advantages of both the boundary element and our analytical formalism¹², by representing a molecule as a collection of overlapping spheres³⁴. We showed that PB-SAM converges to the analytical solution with better accuracy and at greatly reduced cost relative to the readily available public domain PB solver APBS.¹³ In this work we extend our PB-SAM approach³⁴ by deriving expressions for the forces and torque that handles complete *mutual* polarization effects.

To demonstrate the power of the PB-SAM approach, we apply our new LPBE algorithm to study the bimolecular association of the ribonuclease barnase and its intracellular inhibitor, barstar under conditions of crowding. Barnase / barstar association is a classic example of electrostatically-steered diffusion-limited association. The association kinetics has been extensively characterized, both experimentally³⁵ and computationally^{36, 37} using the Northrup-Allison-McCammon (NAM) method³⁸; in the earlier computational study the electrostatics were modeled using effective charges, which accounts for self-polarization but not mutual

polarization. The NAM method is based on the Smoluchowski equation³⁸, and its underlying assumption is that the mobile molecule B only experiences isocentric forces from a stationary molecule A, i.e. concentration effects that modulate intra-species interactions A:A and B:B as per crowding are not taken into account. A potentially more powerful rate constant calculation method would take these intra-species *interaction* into account, beyond the excluded volume effect typically modeled under crowding conditions, however it demands an efficient algorithm capable of computing the electrostatic forces and torques for multiple molecules on the fly. Because the PB-SAM method enables the calculation of full mutual polarization effects in systems of hitherto inaccessible spatial dimensions, it thereby allows the calculation of kinetic parameters of rate equations which can in turn be inputs to chemical master equations or stochastic simulations.

The paper is organized as follows. In Section 2 we review the PB-SAM formalism, and introduce for the first time the force and torque expressions for our new LPBE solver. We also provide the necessary detail of the Brownian dynamics simulations and the molecular models of barnase and barstar. In Section 3 we compare the results of the bimolecular rate of association of a single barnase and barstar calculated using NAM and that calculated from a multi-molecular PB-SAM simulation in which there is an excess concentration of either barnase and barstar, to evaluate a pseudo-first order rate constant derived from mean first passage times of a docked complex. We ignore hydrodynamic interactions which is almost certainly important for these type of rate calculations, and could potentially limit a quantitative connection to experiments performed on barnase-barstar under crowding conditions⁴. We emphasize that the purpose of our work here is to present new PBE methodology and the ability, for example, to represent crowding agents with more complex and realistic interaction potentials. Section 4 provides our conclusions on the PB-SAM method and the outcome of our rate study which finds only a ~30% rate enhancement on barnase-barstar association rates, in agreement with experiment⁴.

2. THEORY

The method for solving mutually polarized charge distribution has been previously presented in [34]. Here we first summarize the principal result, namely the solution of the charge distribution due to mutual polarization, followed by derivation of expressions for force and torque. Further details are given in the supplementary materials.

Solution of mutually polarized charge distribution. The system of interest comprises of N_{mol} macromolecules immersed in an implicit aqueous salty solvent. Each macromolecule I is represented as a collection of $N_S^{(I)}$ overlapping spheres with dielectric constant ϵ_{in} , and embedded with $N_C^{(I)}$ fixed partial charge. The solvent is treated as a continuum with dielectric constant ϵ_{out} . Screening effects due to mobile ions is modeled via the inverse Debye length κ . The linearized PB equation (LPBE) gives the potential Φ at any point \mathbf{r} in space \mathfrak{R}^3 in e.s.u.-c.g.s. convention as

$$-\nabla[\epsilon(\mathbf{r})\nabla\Phi(\mathbf{r})] + \kappa^2\Phi(\mathbf{r}) = 4\pi\rho_{fixed}(\mathbf{r}) \quad (1)$$

where $\epsilon(\mathbf{r})$ is the relative dielectric function, ρ_{fixed} is the charge density due to the fixed protein partial charges, and $\kappa = \sqrt{8\pi\bar{n}e^2/\epsilon_{out}k_B T}$, where \bar{n} is the bulk concentration of monovalent salt in the solution, e is the fundamental electronic charge, k_B the Boltzmann constant, and T the absolute temperature.

The potentials inside and outside a molecule I are given respectively by:

$$\Phi_{out}(\mathbf{r}) = \sum_{I=1}^{N_{mol}} \left(\frac{1}{4\pi} \int_{\alpha\Omega^{(I)}} \frac{e^{-\kappa|\mathbf{r}-\mathbf{r}'|}}{|\mathbf{r}-\mathbf{r}'|} h^{(I)}(\mathbf{r}') d\mathbf{r}' \right) \quad (2a)$$

$$\Phi_{in}^{(I)}(\mathbf{r}) = \sum_{\alpha=1}^{N_C^{(I)}} \frac{1}{|\mathbf{r}-\mathbf{r}_\alpha^{(i)}|} \frac{q_\alpha^{(I)}}{\epsilon_{in}} + \frac{1}{4\pi} \int_{\alpha\Omega^{(I)}} \frac{1}{|\mathbf{r}-\mathbf{r}'|} f^{(I)}(\mathbf{r}') d\mathbf{r}' \quad (2b)$$

where $f(\mathbf{r})$ and $h(\mathbf{r})$ are the *reactive* and *effective* surface charge distributions respectively. The charge distribution on sphere k , denoted as $f^{(I,k)}(\mathbf{r})$ and $h^{(I,k)}(\mathbf{r})$, can be transformed into *reactive* and *effective* multipoles:

$$F_{nm}^{(I,k)} \equiv \frac{1}{4\pi} \int_{\alpha\Omega^{(I,k)}} f^{(I,k)}(\mathbf{r}') \left(\frac{\mathbf{a}^{(I,k)}}{r'} \right)^{n+1} \overline{Y_{nm}^{(I,k)}}(\theta', \phi') d\mathbf{r}' \quad (3a)$$

$$H_{nm}^{(I,k)} \equiv \frac{1}{4\pi} \int_{\alpha\Omega^{(I,k)}} h^{(I,k)}(\mathbf{r}') \left(\frac{r'}{\mathbf{a}^{(I,k)}} \right)^n \hat{i}_n(kr') \overline{Y_{nm}^{(I,k)}}(\theta', \phi') d\mathbf{r}' \quad (3b)$$

Coefficients of $F^{(k,I)}$ and $H^{(k,I)}$ can then be solved iteratively using

$$F_{nm}^{(I,k)} = \left\langle \mathbf{I}_{E,nm}^{(I,k)} \mathbf{W} F^{(I,k)} \right\rangle \quad (4a)$$

$$\frac{H_{nm}^{(I,k)}}{\hat{i}_n(k\mathbf{a}^{(I,k)})} = \left\langle \mathbf{I}_{E,nm}^{(I,k)} \mathbf{W} H^{(I,k)} \right\rangle \quad (4b)$$

where $\langle \rangle$ denotes an inner product, and $\mathbf{WF}^{(k,I)}$ and $\mathbf{WH}^{(k,I)}$ are scaled multipoles computed from fixed charges and polarization charges from other spheres (see Supplementary Material Eqns S1.1a-b), and $\mathbf{I}_E^{(k,I)}$ is a surface integral over the exposed surface, defined by

$$I_{E,lsnm}^{(I,k)} \equiv \frac{1}{4\pi} \int_{\phi_E} \int_{\theta_E} Y_{ls}^{(I,k)}(\theta', \phi') \overline{Y_{nm}^{(I,k)}}(\theta', \phi') \sin \theta' d\theta' d\phi' \quad (5)$$

The above surface integral is evaluated once before the simulation, using the quadrature method with each sphere discretized uniformly into 72,000 spiral grid points using the method of Rakhmanov³⁹.

The interaction energy of sphere k is the inner product of its effective multipole $\mathbf{H}^{(I,k)}$ with its local expansion of external (i.e. intermolecular) effective charges, $\mathbf{LHN}^{(I,k)}$. The total interaction energy of molecule I is in turn the sum of interaction energies of all constituent spheres

$$W^{(I)} = \sum_{k=1}^{N_S^{(I)}} \langle \mathbf{LHN}^{(I,k)}, \mathbf{H}^{(I,k)} \rangle = \sum_{k=1}^{N_S^{(I)}} \sum_{n=0}^p \sum_{m=-n}^n \mathbf{LHN}_{nm}^{(I,k)} \overline{H}_{nm}^{(I,k)} \quad (6)$$

Force on an effective charge. The surface of each sphere can be discretized into M_P grid points, of which M_E are exposed and M_B are buried. We only need to consider the force experienced at each exposed grid point P , since buried grid points have no surface charge and hence experience no force. We begin by deriving an expression for the force at P , and then summing up contributions from all exposed charges to derive the total force and torque on molecule I . We only consider forces due to external field, because forces due to intramolecular effective charges cancel out and do not contribute to the overall force and torque on molecule I .

We shall use the shorthand h_P to denote $h(\mathbf{r}_P)$, the effective charge at space position P . The multipole coefficient $\mathbf{H}_P^{(I,k)}$ is the product of h_P and the spherical harmonic $\mathbf{Y}_P^{(I,k)} \equiv \mathbf{Y}^{(I,k)}(\theta_P, \phi_P)$. The force \mathbf{f}_P acting on the effective charge at point P is the negative gradient of the interaction energy of charge h_P with the external field:

$$\begin{aligned} \mathbf{f}_P &= -\nabla_P W_P = -\nabla_P \langle \mathbf{LHN}^{(I,k)}, \mathbf{H}_P^{(I,k)} \rangle \\ &= -\langle \nabla_P \mathbf{LHN}^{(I,k)}, \mathbf{H}_P^{(I,k)} \rangle - \langle \mathbf{LHN}^{(I,k)}, \nabla_P \mathbf{H}_P^{(I,k)} \rangle \\ &= -\langle \nabla_P \mathbf{LHN}^{(I,k)}, \mathbf{H}_P^{(I,k)} \rangle - \langle \mathbf{LHN}^{(I,k)}, \nabla_P h_P \times \mathbf{Y}_P^{(I,k)} \rangle - \langle \mathbf{LHN}^{(I,k)}, h_P \times \nabla_P \mathbf{Y}_P^{(I,k)} \rangle \end{aligned} \quad (7)$$

In rigid body dynamics, the translational force on a molecule acting through its center of mass is the sum of all forces acting on all its constituent parts. Summing up \mathbf{f}_p from Eq. (7) from all exposed points, we get the translational force \mathbf{f}_I as

$$\mathbf{f}_I = \sum_{\text{all } P} \mathbf{f}_P = \sum_k \sum_{P \in k} \mathbf{f}_P \quad (8)$$

This can be expressed in terms of effective multipoles, local expansions and their gradients (see Supplementary Material) as

$$\mathbf{f}_I = - \sum_k \langle \tilde{\nabla}_I \mathbf{LHN}^{(I,k)}, \mathbf{H}^{(I,k)} \rangle + \langle \mathbf{LHN}^{(I,k)}, \tilde{\nabla}_I \mathbf{H}^{(I,k)} \rangle \quad (9)$$

Solution of mutually polarized gradients. We now need to compute gradients $\tilde{\nabla}_I \mathbf{H}^{(I,k)}$ and $\tilde{\nabla}_I \mathbf{LHN}^{(I,k)}$, which account for how the position of molecule I changes the polarization charges. The gradient $\tilde{\nabla}_I \mathbf{LHN}^{(I,k)}$ is given by

$$\tilde{\nabla}_I \mathbf{LHN}^{(I,k)} = \sum_{J \neq I} \sum_j^{N_S^{(J)}} \tilde{\nabla}_I \mathbf{T}_\kappa^{(I,k)(J,j)} \mathbf{H}^{(J,j)} + \sum_{J \neq I} \sum_j^{N_S^{(J)}} \mathbf{T}_\kappa^{(I,k)(J,j)} \tilde{\nabla}_I \mathbf{H}^{(J,j)} \quad (10)$$

where $\mathbf{T}_\kappa^{(I,k)(J,j)}$ denotes the multipole-to-local re-expansion operator (see Supplementary Material). The first sum can be computed from the converged solutions of effective multipoles \mathbf{H} . For the second sum, we would need $\tilde{\nabla}_I \mathbf{H}^{(J,j)}$. That is, for each sphere, we need to compute the gradient of its effective multipole \mathbf{H} , with respect to *every* molecule I .

The gradient polarization step thus comprises of three nested iteration loops. The outermost loop goes over $1 \leq J \leq N_{mol}$ to compute gradients with respect to each J . The middle and innermost loops then solve for $\tilde{\nabla}_J \mathbf{H}^{(I,k)}$ of all spheres. Below we detailed the formulism for solving $\tilde{\nabla}_J \mathbf{H}^{(I,k)}$ for a sphere (I,k) .

To compute $\tilde{\nabla}_J \mathbf{H}^{(I,k)}$ with respect to molecule J , we begin by applying the gradient operator $\tilde{\nabla}_I$ to equations (4a) and (4b):

$$\tilde{\nabla}_J F_{nm}^{(I,k)} = \langle \mathbf{I}_{E,nm}^{(I,k)}, \tilde{\nabla}_J \mathbf{WF}^{(I,k)} \rangle \quad (11a)$$

$$\tilde{\nabla}_J \frac{H_{nm}^{(I,k)}}{\hat{i}_n(\kappa a^{(I,k)})} = \langle \mathbf{I}_{E,nm}^{(I,k)} \tilde{\nabla}_J \mathbf{WH}_H^{(I,k)} \rangle \quad (11b)$$

If molecule I is fixed in orientation, \mathbf{I}_E , $\mathbf{E}^{(I,k)}$, and $\mathbf{LE}^{(I,k)}$ do not depend on position of I since their positions move concertedly with I , so $\tilde{\nabla}_J \mathbf{WF}^{(I,k)}$ and $\tilde{\nabla}_J \mathbf{WH}^{(I,k)}$ can be simplified to:

$$\begin{aligned} \tilde{\nabla}_J \mathbf{WF}_{nm}^{(I,k)} &= e^{-\kappa a^{(I,k)}} \left[n \hat{\kappa}_n(\kappa a^{(I,k)}) - (2n+1) \hat{\kappa}_{n+1}(\kappa a^{(I,k)}) \right] \tilde{\nabla}_J H_{nm}^{(I,k)} + (2n+1 - n\varepsilon) \tilde{\nabla}_J F_{nm}^{(I,k)} \\ &+ a^{(I,k)} \left[n \hat{i}_n(\kappa a^{(I,k)}) + \frac{(\kappa a^{(I,k)})^2 \hat{i}_{n+1}(\kappa a^{(I,k)})}{2n+3} \right] \tilde{\nabla}_J \left(\mathbf{LH}_{nm}^{(I,k)} + \mathbf{LHN}_{nm}^{(I,k)} \right) - n\varepsilon a^{(I,k)} \left(\tilde{\nabla}_J \mathbf{LF}_{nm}^{(I,k)} \right) \end{aligned} \quad (12a)$$

$$\begin{aligned} \tilde{\nabla}_J \mathbf{WH}_{nm}^{(I,k)} &= \left(\frac{2n+1}{\hat{i}_n(\kappa a^{(I,k)})} - e^{-\kappa a^{(I,k)}} \hat{\kappa}_n(\kappa a^{(I,k)}) \right) \tilde{\nabla}_J H_{nm}^{(I,k)} + \tilde{\nabla}_J F_{nm}^{(I,k)} \\ &+ a^{(I,k)} \tilde{\nabla}_J \mathbf{LF}_{nm}^{(I,k)} - a^{(I,k)} \hat{i}_n(\kappa a^{(I,k)}) \tilde{\nabla}_J \left(\mathbf{LH}_{nm}^{(I,k)} + \mathbf{LHN}_{nm}^{(I,k)} \right) \end{aligned} \quad (12b)$$

where $\varepsilon = \varepsilon_{in}/\varepsilon_{out}$. During each middle-loop iteration, we consider one sphere (I,k) , and compute the local expansions $\tilde{\nabla}_J \mathbf{LF}^{(I,k)}$, $\tilde{\nabla}_J \mathbf{LH}^{(I,k)}$, and $\tilde{\nabla}_J \mathbf{LHN}^{(I,k)}$ from outer spheres' polarized gradients. The local expansions are defined below:

$$\tilde{\nabla}_J \mathbf{LF}^{(I,k)} = \sum_{j \neq k}^{N_S^{(I)}} \mathbf{T}_0^{(I,k)(I,j)} \tilde{\nabla}_j \mathbf{F}^{(I,j)} \quad ; \quad \tilde{\nabla}_J \mathbf{LH}^{(I,k)} = \sum_{j \neq k}^{N_S^{(I)}} \mathbf{T}_k^{(I,k)(I,j)} \tilde{\nabla}_j \mathbf{H}^{(I,j)} \quad (13)$$

$$\tilde{\nabla}_J \mathbf{LHN}^{(I,k)} = \sum_{M \neq I} \sum_m^{N_S^{(M)}} \tilde{\nabla}_J \mathbf{T}_k^{(I,k)(M,m)} \mathbf{H}^{(M,m)} + \sum_{M \neq I} \sum_m^{N_S^{(M)}} \mathbf{T}_k^{(I,k)(M,m)} \tilde{\nabla}_J \mathbf{H}^{(M,m)} \quad (14)$$

Note that intramolecular re-expansions (within same molecule I) does not have a $\tilde{\nabla}_J \mathbf{T} \times \mathbf{H}$ component, since for intramolecular re-expansion the operation \mathbf{T} does not change with position of I . Finally, with all local expansions computed, we can enter the innermost loop to solve for $\tilde{\nabla}_J \mathbf{F}^{(I,k)} \tilde{\nabla}_J \mathbf{H}^{(I,k)}$ using Eqs. (11a-b) and (12a-b). Since mutual polarization is a short-range effect, a cutoff r_{cut} can be used during charge and gradient polarization to simplify the computations. *Intermolecular* spheres whose surface-to-surface distances are greater than r_{cut}

will not be included in each other's external field. The validity of cutoffs for intramolecular spheres needs to be further investigated.

Expressions for force and torque. The translational force on molecule I is given

$$\mathbf{f}_I = - \sum_k^{Nk^{(I)}} \mathbf{f}_{I,k} \quad (15)$$

where

$$\mathbf{f}_{I,k} = - \langle \tilde{\nabla}_I \mathbf{LHN}^{(I,k)}, \mathbf{H}^{(I,k)} \rangle + \langle \mathbf{LHN}^{(I,k)}, \tilde{\nabla}_I \mathbf{H}^{(I,k)} \rangle \quad (16)$$

The torque on a charge at position P about the molecule I 's center of mass $c(I)$ is given by the cross product of its position $\mathbf{r}_P(I)$ with respect to $c(I)$ and the force it experienced, \mathbf{f}_P . The total torque on molecule I is then the sum of all torques:

$$\boldsymbol{\tau}_I = \sum_k^{Nk^{(I)}} \sum_{P \in k} \boldsymbol{\tau}_P = \sum_k^{Nk^{(I)}} \sum_{P \in k} \mathbf{r}_P^{(I)} \times \mathbf{f}_P \quad (17)$$

We can re-express $\mathbf{r}_P(I)$ as the sum of vectors from center of molecule I to center of sphere k ($c(I,k)$), and from center of sphere k to point P ($\mathbf{r}_P(I,k)$). The total torque about the center of molecule I is then:

$$\boldsymbol{\tau}_I = \sum_k^{Nk^{(I)}} \mathbf{c}^{(I,k)} \times \mathbf{f}_k + \sum_k^{Nk^{(I)}} \sum_{P \in k} \mathbf{r}_P^{(I,k)} \times \mathbf{f}_P \quad (18)$$

where

$$\mathbf{f}_P = - \sum_k^{Nk^{(I)}} \langle \tilde{\nabla}_I \mathbf{LHN}^{(I,k)}, \mathbf{H}_P^{(I,k)} \rangle + \langle \mathbf{LHN}^{(I,k)}, \tilde{\nabla}_I \mathbf{H}_P^{(I,k)} \rangle \quad (19)$$

and

$$\mathbf{H}_P^{(I,k)} = h(\theta_P, \phi_P) \mathbf{Y}_{nm}^{(I,k)}(\theta_P, \phi_P), \quad \alpha = X, Y, Z \quad (20a)$$

$$\tilde{\nabla}_j \mathbf{H}_{P,\alpha}^{(I,k)} = \left(\tilde{\nabla}_j h(\theta_P, \phi_P) \right)_\alpha \mathbf{Y}_{nm}^{(I,k)}(\theta_P, \phi_P) \quad (20b)$$

Equations (15-16) and equations (18-20b) will be used to compute the force and torque respectively.

Brownian dynamics. We have adopted the Brownian dynamics simulation protocol developed by Ermak and McCammon⁴⁰. Each molecule I is treated as a Brownian particle experiencing a conservative force \mathbf{f}_I and torque $\boldsymbol{\tau}_I$, in addition to friction and random force due to

the solvent. Assuming no hydrodynamic interaction between the macromolecules, the displacement $\Delta \mathbf{r}_I$ and angular rotation $\Delta \vartheta_I$ per timestep Δt are given by

$$\Delta \mathbf{r}_I = \frac{D_{I,trans} \Delta t}{k_B T} \mathbf{f}_I + \mathbf{S}_I(\Delta t) \quad (21a)$$

$$\Delta \vartheta_I = \frac{D_{I,rot} \Delta t}{k_B T} \boldsymbol{\tau}_I + \Theta_I(\Delta t) \quad (21b)$$

where the stochastic displacement (\mathbf{S}) and rotation (Θ) have the properties

$$\langle \mathbf{S}_\alpha \rangle = 0, \quad \langle \mathbf{S}_\alpha^2 \rangle = 2D_{I,trans} \Delta t \quad (22a)$$

$$\langle \Theta_\alpha \rangle = 0, \quad \langle \Theta_\alpha^2 \rangle = 2D_{I,rot} \Delta t \quad \alpha = x, y, z \quad (22b)$$

where the index I runs over molecule $1 \leq I \leq N$, and index $1 \leq i \leq 3N$ run over x , y , and z particle coordinates, F_i is the sum of systematic interparticle and external forces acting in direction i . The translational and rotational diffusion constants of each molecule I are set to the values used in [36], with $D_{trans} = 0.015 \text{ \AA}^2 \text{ ps}^{-1}$ for both barnase and barstar, and $D_{rot} = 4.5 \times 10^{-5}$ for barnase and 4×10^{-5} for barstar. Each molecule I is moved in turn, first undergoing displacement $\Delta \mathbf{r}_I$, followed by rotation $\Delta \vartheta_I$. Both displacement and rotation moves are checked for a collision, defined as when any sphere of molecule I overlaps with spheres of other molecules. If a collision occurs, a new stochastic displacement or rotation move is generated and checked, until a valid move is obtained.

Protein models. The structure of barnase and barstar were taken from the PDB (code 1BRS⁴¹). Chain A was used for barnase, with the missing residues 1 and 2 modeled using CE alignment⁴² of chain B onto chain A. Chain D was used for barstar. The pdb chains were converted into PQR format using pdb2pqr^{43, 44}, which assigned partial atomic charges using the AMBER 99 force field⁴⁵ and calculated protonation states at pH = 8.0 (the pH of the original experiment [35]) using PROPKA⁴⁶, giving net charges of 2.0 and -5.0 for barnase and barstar respectively.

We then generated the solvent excluded molecular surface (SES) for each protein using MSMS⁴⁷, with probe radius = 1.5 Å and a density of 3.0. The SES and PQR files are inputted into an in-house program to discretized the protein into spheres. At each iteration, the program uses a greedy Monte Carlo algorithm to search for a sphere center that encompasses the largest

number of fixed partial charges. Charges within this sphere center were then removed, and the search repeated with the remaining charges. A stipulated tolerance controls how far from the SES can the sphere surface protrude. We experimented with two sets of tolerance: (i) 0.5 Å at the binding interface and 2.0 Å otherwise ('s2i0.5'); and (ii) 1.0 Å at the interface and 5.0 Å otherwise ('s5i1'). Figure 1 shows the barnase-barstar docked complex under the s5i1 and s2i0.5 representations. We find that both representations permit docking to occur under similar docking criteria as that specified by Wade, hence the 's5i1' representation is sufficient for docking and was used in subsequent association studies.

The docking criteria we used was adopted from that specified by Gabdoulline and Wade³⁶, whereby the encounter complex is defined in terms of residue contact pairs whose docking distances were fitted to reproduce Fersht's experimental results for wild-type and mutants of the barnase and barstar system. Given the list of eight hydrogen-bond forming atoms from [36] we identified the smallest spheres in the s5i1 representation that encompasses them ('contact-spheres', see Table 1). To be considered docked i.e. forming the reactive encounter complex, at least two contact-sphere pairs must be have surface-to-surface distances within 3.5 Å. This distance translate approximately to an atom-to-atom distance of 6.25 Å used by Wade. Figure 2 shows the docked complex of 1BRS rendered in our s5i1 discretized sphere presentation (Figure 2(a)), and representative snapshots of encounter complexes satisfying our docking criteria (Figures 2b-2d). We note that our adapted docking definition using s5i1 will not be exactly the same as an all-atom representation of barnase and barstar.

Simulation Parameters. All simulations were performed at temperature $T = 298.15$ K, the solvent and protein dielectric constants were $\epsilon_s = 78$ and $\epsilon_p = 4$ respectively. We used a salt concentration of 50 mM as per ³⁶, which correspond to an inverse Debye length $\kappa = 0.07374$. A variable timestep with minimum of 2 ps is used. At each time step, the system is solved with a polarization cutoff $r_{polcut} = 10$ Å, and a force cutoff of $r_{fcut} = 100$ Å. All spheres within a separation distance of r_{polcut} from an external sphere undergo polarization of $\mathbf{H}^{(l,k)}$ and $\tilde{\mathbf{V}}_l H_{nm}^{(l,k)}$ for two iterative cycles. All multipole and gradient polarization are performed at pole order $p = 10$. Each sphere surface is discretized into $M_p = 1000$ points. At each time step, all molecules are propagated using the same Brownian dynamics algorithm described. All simulations were

performed on a single processor on an Intel(R) Xeon(R) CPU 2.27GHz processor with 24GB of physical memory.

3. RESULTS

Northrup-Alison-McCammon (NAM) Methodology. We first compute the bimolecular rate of association of barnase and barstar using the NAM method under the PB-SAM model. Prior to simulation, each protein's self-polarization multipoles are pre-computed. During the actual simulation, barnase is fixed at the origin, while barstar is positioned 100 Å away, and allowed to move with respect to barnase. If the docking criteria are met, the trajectory is considered docked and then terminated. If the center of mobile barstar moves beyond the truncation sphere of radius $q = 500$ Å from barnase, the docking event fails and the trajectory is again terminated. The docking frequency δ is then the ratio of successful docking events against the total number of trajectories, and the intrinsic bimolecular collision rate is then calculated as

$$k = \frac{k(b)\delta}{1 - (1 - \delta)k(b)/k(q)} \quad (23)$$

where $k(b)$ and $k(q)$ are rates at which a molecule B starting at infinity reaches $r = b$ and $r = q$ respectively, and which can be evaluated analytically from the Smoluchowski rate equation

$$k(R) = \left[\frac{1}{4\pi(D_{trans})} \int_R^{\infty} \frac{\exp(U(r)/k_B T)}{r^2} dr \right]^{-1} \quad (24)$$

At a screening length of $\kappa = 0.0737$, the electrostatic interaction $U(r)$ is attenuated to less than 0.01 at the distance $b = 100$ Å and effectively zero at $q = 500$ Å, so the rates can be evaluated as

$$\begin{aligned} k(b) &= 4\pi(D_{trans})b \\ k(q) &= 4\pi(D_{trans})q \end{aligned} \quad (25)$$

We simulated 10,051 trajectories and monitored the number of trajectories that docked according to our docking criteria. We recorded 22 docked trajectories, corresponding to a collision frequency of $2.189 \pm 0.099 \times 10^{-3}$, with error bounds estimated by adding or removing one docking trajectory and recomputing the collision frequency. Using Eq. (23), we obtained a rate constant of $k = 6.21 \pm 0.28 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$. Our NAM rate is comparable to the experimentally determined value³⁵ of $k = 2.86 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$, and the value calculated by Gabdoulline and Wade³⁷ of $k = 3.88 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$ using NAM and atomistic docking criteria. Our slower NAM rate

constant compared to the previous theoretical value is likely due differences in the molecular representations. Regardless, the exact docking definition does not impact our question at hand, which is: given the *same docking criterion*, will the NAM method and the multi-molecular simulation produce the same kinetic rate constants.

Rate constant from multi-molecular simulation. The second order rate equation describing the bimolecular association of barnase (A) and barstar (B) is given by

$$\frac{d[AB]}{dt} = k[A][B] \quad (26a)$$

where [A], [B], and [AB] are the concentrations of barnase, barstar and the docked encounter complex, in units of M, respectively, and k is the intrinsic bimolecular rate constant with unit M⁻¹s⁻¹. We can rewrite the rate equation as

$$\frac{d[AB]}{dt} = k'[B] \quad (26b)$$

where $k' = k[A]$, in units s⁻¹. In the limit of small t , concentrations $[A] \approx [A]_{t=0}$, the initial barnase concentration, so Eq.(26b) assumes the form of a pseudo-first order rate equation. The pseudo first-order rate constant k' can be obtained using the first passage time approach. We simulated N independent systems of N_A barnase and N_B barstar molecules, and recorded the time taken to form the encounter complex for the first time, t_i , for each system. The fraction of trajectories docked at time t is given by

$$P_{docked}(t) = (\text{No. of systems with } t_i \leq t) / N \quad (27)$$

The pseudo-first order rate constant k' can be determined from fitting on the plotted data of $P_{docked}(t)$ against t , using

$$P_{docked} = 1 - \exp(-k'(t - \tau_d)) \quad (28)$$

where τ_d is the dead time required for the system to equilibrate. Finally, the bimolecular rate constant was computed from

$$k = k' / [A]_{t=0} \quad (29)$$

For the multi-molecular method, a cubic simulation box with periodic boundaries was used. The box length is chosen such that electrostatic forces are sufficiently attenuated after 1 box length, so the minimum image convention can be used, which was 320 Å. Table 2 lists the number of barnase and barstar monomers (N_A and N_B respectively), in which one simulation $N_A=124$ and $N_B=1$ and another simulation in which $N_A=1$ and $N_B=124$. For each combination ~ 500 initial configurations were generated by placing the molecules' geometric centers on a grid at equal separation and randomly assigned the type of molecule (barnase or barstar) based on N_A and N_B ; then randomly rotated each molecule. Figure 3 illustrates a representative initial system configuration.

The fitted values of k' and τ_d are also presented in Table 2. Correlation coefficients for fitting the data to the pseudo-first order rate equation (Eq. 28) are in the range of 0.977-0.989, providing support that the encounter complex formation can indeed be approximated as a first-order reaction for small t . Standard errors for multi-molecular rates were computed using a jack-knife approach: a single docking event was removed, the remaining data was refitted to Eq. 28 to obtain k'_i , and the process repeated for all N_d docking events. The resulting standard errors in k and k' , given by

$$\sigma_{j\text{mean}}^2 = \sqrt{(N_d - 1) \sum_{j=1}^{N_d} (k'_{j_i} - k')^2 / N_d} \quad (30)$$

are reported in Table 2. To assess convergence, we monitored the fit quality (through correlation coefficients R^2) and k' as we include successively longer docking times into the fit (Supplementary figures S1(a)-(b)). We confirmed convergence by observing (i) final R^2 values have converged to 0.38% (for $N_A=124:N_B=1$) and 1.00% (for $N_A=1:N_B=124$), and (ii) final k' values have converged to 3.52% (for $N_A=124:N_B=1$) and 0.87% (for $N_A=1:N_B=124$). The rates calculated, $7.45 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$ and $8.82 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$, are still within the same order of magnitude compared to experimental and previous *in silico* NAM rates, however the rate constants from the multi-molecular simulation are clearly 15-30% faster. Since both rate calculation methods used exactly the same protein models, force field, and docking definition, the difference in rate constant can only arise from the differences in protein concentration.

4. CONCLUSION

We have extended our semi-analytical PB-SAM method for solving the linearized Poisson-Boltzmann equation by presenting expressions for computing the forces and torques on the fly that account for both self and full mutual polarization effects. We have applied the PB-SAM method to model the systematic force in a Brownian dynamics simulation to study the diffusion-limited association of barnase and barstar under crowding conditions.

We demonstrated that the traditional method of computing bimolecular rate constants using the Smolouchowski-based NAM method³⁸, which neglects intra-species interaction, underestimates the rate constant by ~15-30%. Nonetheless, our association rate constants show modest enhancement under crowding conditions that are in line with experiments that have used both large agents such as PEG or small crowders such as ethylene glycol in which influence on barnase and barstar association rates were also found to be small^{3,4}. The common explanation for the marginally faster rate constant using the multi-molecular method is that the slowed diffusion of protein molecules in general is balanced against the intra-species electrostatic repulsion and steric hindrance that effectively reduces the available diffusion space to speed up the inter-species encounter. In this particular case of barnase and barstar under pseudo-first order rate conditions, the interacting protein in excess is behaving no differently than a steric crowder that are represented by excluded volume spheres².

The PB-SAM approach combined with Brownian dynamics simulated under periodic boundary conditions provides a new methodological approach to systematically studying the effects of complex *interactions* between crowders and target proteins on kinetics and mechanism of protein-protein association. Future improvements would include improved parallelization, hydrodynamic forces, and incorporation into a community simulation code.

SUPPLEMENTARY INFORMATION. We have supplied a summary of the analytical and numerical re-expansion operations and convergence of the rate equations with longer simulation times and docking events. This information is available free of charge via the Internet at <http://pubs.acs.org/>.

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TABLES

Table 1. *Contact sphere pairs for docking criteria.* Formation of encounter complex is defined as two or more contact sphere pairs having surface-to-surface distances within 3.5 Å.

Barnase				Barstar				Corresponding barnase:barstar contact-pair in Wade[]
Sphere Center			Sphere radius	Sphere Center			Sphere radius	
x	y	z		x	y	z		
28.30 1	44.68 9	7.816	1.824	23.55 6	36.17 6	1.911	1.661	NH2 Arg-59 : OE1 Glu-76
22.93 1	39.92 6	16.517	7.382	38.50 1	41.571	-3.117	8.670	O His-102 : ND2 Asn-33
40.82 8	43.274	15.283	1.721	41.59 0	30.319	10.515	6.539	OE2 Glu-60 : N Leu-34
25.16 6	27.428	7.651	2.827	30.87 6	43.257	-3.615	2.920	N Arg-59 : OD1 Asp-35
30.21 9	46.39 7	8.927	1.487	29.51 4	34.511	-1.557	3.946	NZ Lys-27 : OG1 Thr-42
26.67 1	32.09 2	10.255	1.487	30.99 1	44.349	3.364	3.917	O Arg-83 : OH Tyr-29
37.15 8	42.422	20.865	5.717	24.71 6	37.671	2.256	2.769	NH2 Arg-83 : OD1 Asp-39
37.15 8	42.422	20.865	5.717	28.56 1	43.73 0	4.147	1.824	NH1 Arg-83 : O Gly-43

Table 2. *Bimolecular rates of association for barnase and barstar.* The first row is the kinetic rate calculated using the NAM method³⁸ in which inter-protein interactions are not represented. The next two entries correspond to the evaluation of a pseudo-first order rate constant in which either barnase or barstar is in excess of the other protein. δ is the ratio of the number of successful docking events relative to the number of trajectories simulated. The rate constant using NAM is evaluated from Eq. 23 while the pseudo-first order rate constant is determined from the fit to mean first passage docking data using Eqs. 27-29. R^2 is the measure of quality of fit. The experimental rate value is $k = 2.86 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$, and the value calculated by Gabdoulline and Wade³⁷ is $k = 3.88 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$

N_A	Barnase [M]	N_B	Barstar [M]	δ	Pseudo 1 st order values from fitting			$k [\text{M}^{-1}\text{s}^{-1}]$
					$k' [\text{ns}^{-1}]$	$t_d [\text{ns}]$	R^2	
1	5.068×10^{-5}	1	5.068×10^{-5}	22/10531	N/A	N/A	N/A	$6.21 \pm 0.28 \times 10^7$
124	6.284×10^{-3}	1	5.068×10^{-5}	8/100	$5.540 \pm 0.271 \times 10^{-4}$	3.524	0.989	$8.82 \pm 0.43 \times 10^7$
1	5.068×10^{-5}	124	6.284×10^{-3}	9/500	$4.679 \pm 0.248 \times 10^{-4}$	2.027	0.977	$7.45 \pm 0.39 \times 10^7$

FIGURES

Figure 1. *Surface representation of barnase and barstar under the PB-SAM model.* (a) barnase and (b) barstar using discretization tolerance of 1.0 Å at the interface and 5.0 Å otherwise ('s5i1'); (c) barnase and (d) barstar using tolerance of 0.5 Å at the binding interface and 2 Å otherwise ('s2i0.5').

Figure 2. *Docked barnase and barstar encounter complexes.* (a) the docked complex (1BRS) of barnase (red) and barstar (orange) under the s5i1 representation. (b-d) representative encounter complexes under our docking criteria.

Figure 3. A representative initial configuration for the multi-molecular method, showing 124 barnase molecules (red/white/blue spectrum) and 1 barstar (green) placed in a periodic cubic box of length $L = 320\text{Å}$. The starting configuration is generated by placing the geometric center of each molecule on a 5 x 5 x 5 grid, and the molecule type (barnase or barstar) and orientation are then randomly assigned.

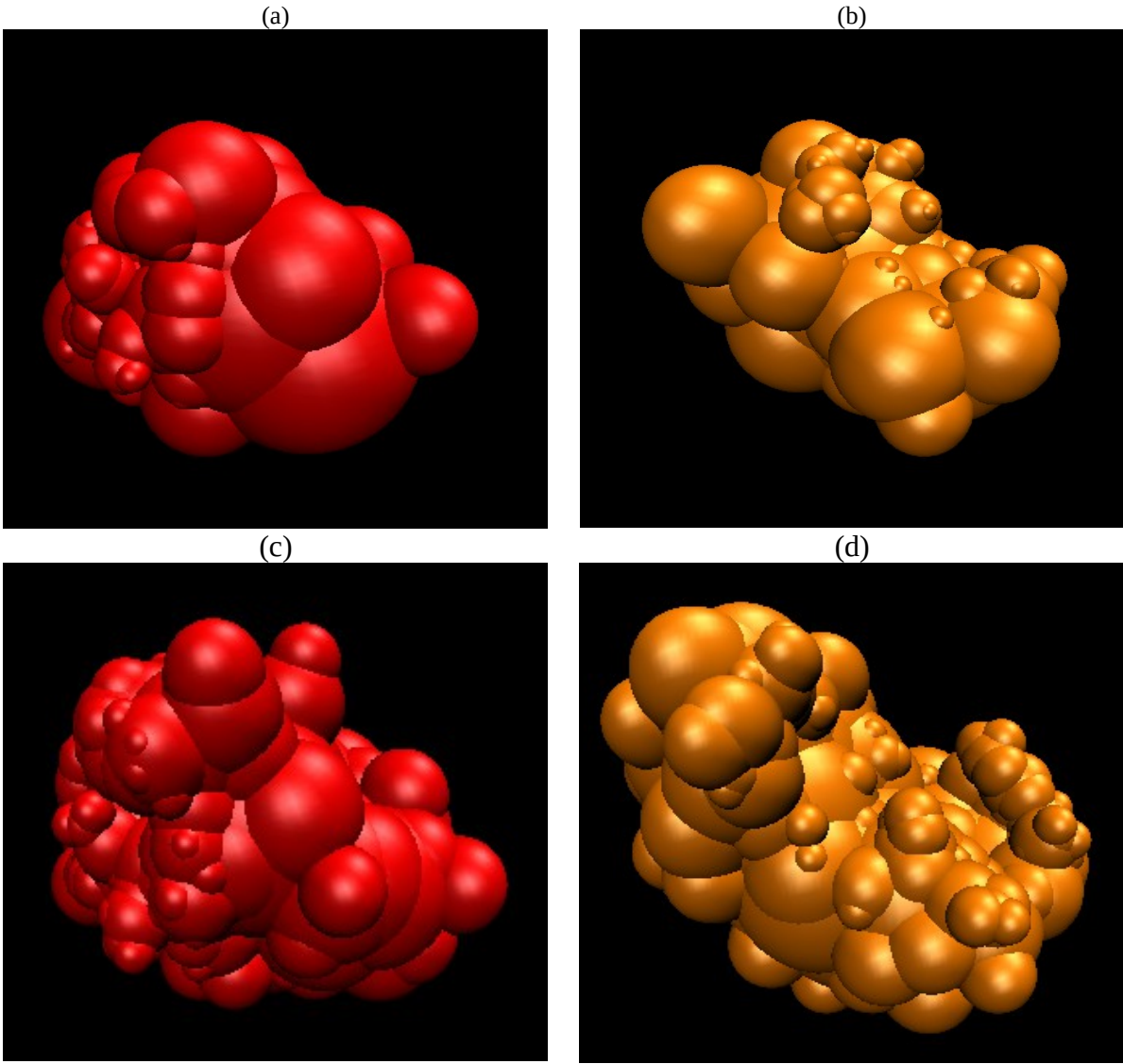
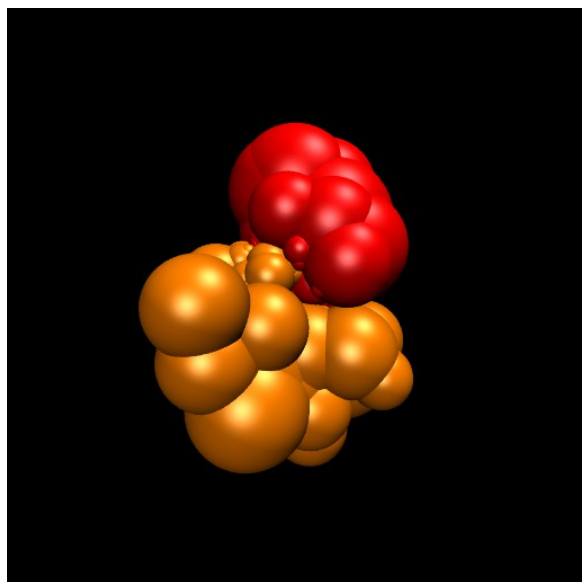


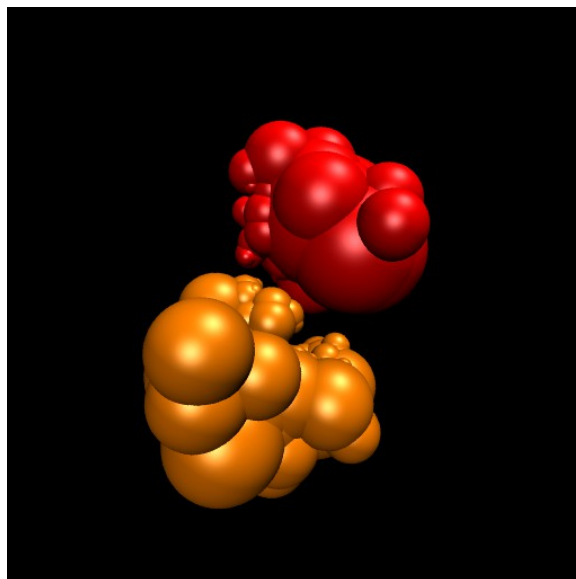
Figure 1. Yap and Head-Gordon

(a)

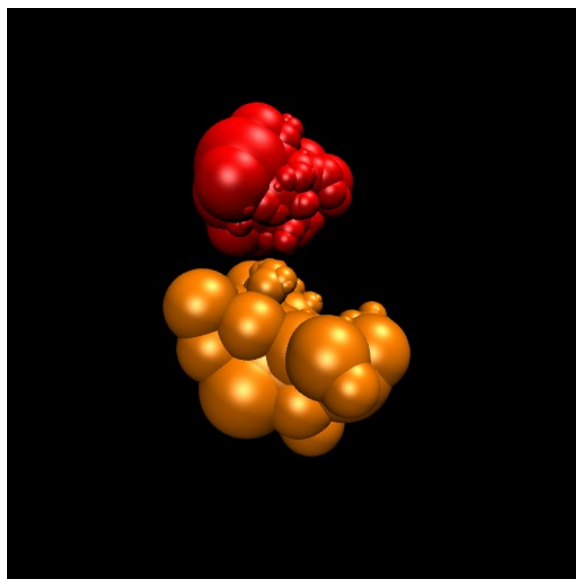


(c)

(b)



(d)



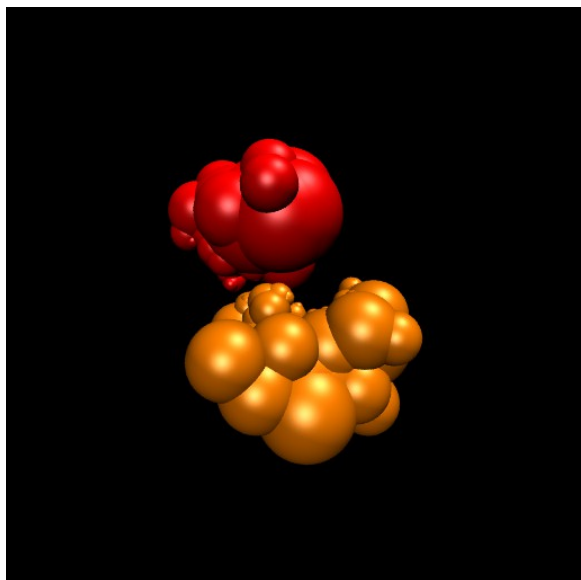


Figure 2. Yap and Head-Gordon

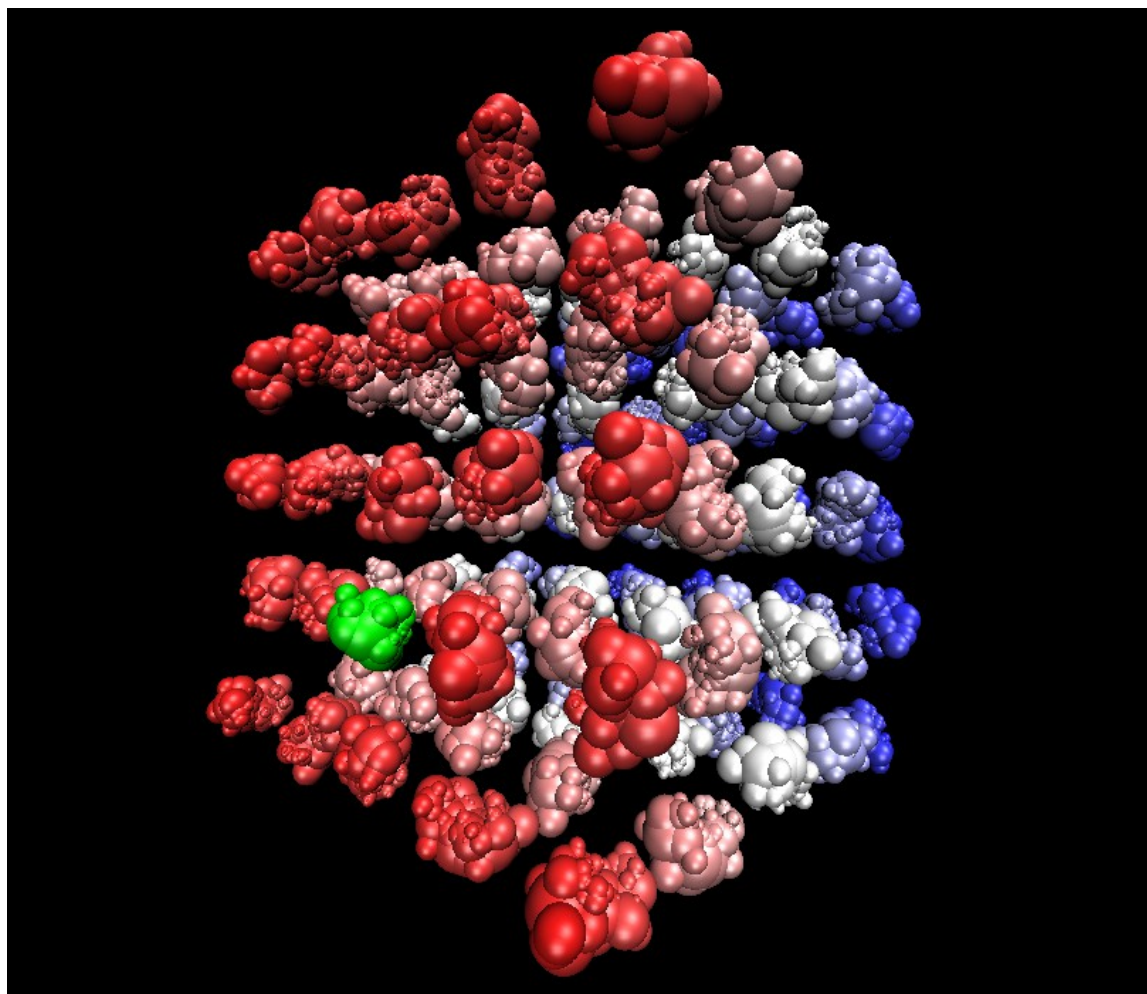


Figure 3. Yap and Head-Gordon