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PAPER

Molecular crowding and protein enzymatic dynamics

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The effects of molecular crowding on the enzymatic conformational dynamics and transport properties of adenylate kinase are investigated. This tridomain protein undergoes large scale hinge motions in the course of its enzymatic cycle and serves as prototype for the study of crowding effects on the cyclic conformational dynamics of proteins. The study is carried out at a mesoscopic level where both the protein and the solvent in which it is dissolved are treated in a coarse grained fashion. The amino acid residues in the protein are represented by a network of beads and the solvent dynamics is described by multiparticle collision dynamics that includes effects due to hydrodynamic interactions. The system is crowded by a stationary random array of hard spherical objects. Protein enzymatic dynamics is investigated as a function of the obstacle volume fraction and size. In addition, for comparison, results are presented for a modification of the dynamics that suppresses hydrodynamic interactions. Consistent with expectations, simulations of the dynamics show that the protein prefers a closed conformation for high volume fractions. This effect becomes more pronounced as the obstacle radius decreases for a given volume fraction since the average void size in the obstacle array is smaller for smaller radii. At high volume fractions for small obstacle radii, the average enzymatic cycle time and characteristic times of internal conformational motions of the protein deviate substantially from their values in solution or in systems with small density of obstacles. The transport properties of the protein are strongly affected by molecular crowding. Diffusive motion adopts a subdiffusive character and the effective diffusion coefficients can change by more than an order of magnitude. The orientational relaxation time of the protein is also significantly altered by crowding.

1 Introduction

Since the interior of a living cell is crowded with obstacles such as organelles, structural elements and numerous macromolecular species, the mechanisms by which transport and other biochemical processes take place in the cell may differ from those that operate in simple aqueous solution.^{1–5} For instance, protein folding and assembly are affected by crowding^{6–12} and diffusion of biomolecules takes place on longer time scales and is often subdiffusive in character.^{13–16} When acting as enzymes, many proteins undergo large conformational changes in the course of a complete enzymatic cycle, which involves substrate binding and ultimate product release with return of the protein to its original conformation. Crowding can alter the dynamics of enzymatic cycles by favoring certain protein conformations over others and by changing reaction rates.^{17,18}

In order to investigate the effect of molecular crowding¹⁹ on protein enzymatic kinetics, we consider a specific protein,

adenylate kinase (AKE). This protein undergoes large conformational motions during its enzymatic cycle and will serve to illustrate how enzymatic dynamics is affected by crowding. The AKE tridomain protein comprises LID, CORE and NMP domains and catalyzes the reversible reaction $\text{ATP} + \text{AMP} \rightleftharpoons 2\text{ADP}$. In the course of this reaction the LID and NMP domains undergo the large-scale hinge motions shown in Fig. 1. A proposed mechanism^{20,21} for the catalytic activity involves two additional metastable states where only one of the two domains is in the closed state, in addition to the fully open and

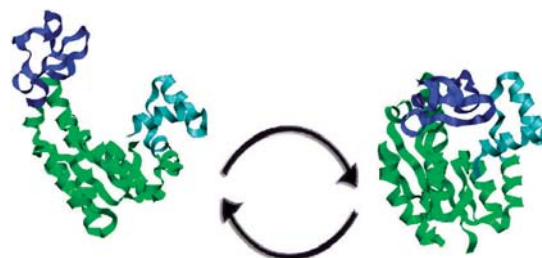


Fig. 1 Adenylate kinase domains: CORE (green), LID (blue) and NMP (light blue). The LID domain binds ATP while the NMP domain binds AMP; both domains undergo large hinge motions in the course of the catalytic cycle.

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fully closed conformations shown in the figure. A catalytic cycle involves closing of the LID domain associated with binding of ATP,²² followed by binding of AMP and closing of the NMP domain. After the phosphoryl transfer reaction with the protein in the fully closed configuration, the LID domain then opens and one of the ADP molecules is released, followed by opening of the NMP domain and release of the second ADP molecule. After these steps have taken place the protein is again in its original fully open state completing the enzymatic cycle.

In this paper we consider how the presence of obstacles that act as crowding agents affects the enzymatic cycle dynamics. We also investigate how crowding alters the diffusive motion of the protein while it is undergoing conformational changes associated with enzymatic catalysis. The crowding agents are taken to be hard stationary spherical objects with specified radii that are randomly distributed in the system. Crowding effects are studied as a function of the quenched disorder, obstacle radius and volume fraction.

The investigation of the enzymatic dynamics is carried out at a particle-based mesoscopic level where amino acid residues in the protein are represented by beads and the solvent is described by multiparticle collision dynamics.^{23,24} Substrate and product molecules are not explicitly included in the mesoscopic description; instead reactive events are modeled by probabilistic changes in the interaction potentials that give rise to specific conformational changes in the protein.²⁵

The outline of the paper is as follows. Section 2 describes the model system and presents details of the simulation method. In Section 3, we discuss the effects of molecular crowding on the steady state conformational distributions, enzymatic cycle dynamics and transport properties of this protein. The conclusions of the study are presented in Section 4.

2 Mesoscopic model

The mesoscopic model for adenylate kinase and solvent dynamics in the absence of obstacles was presented elsewhere;²⁵ consequently, our discussion of the main components of the model in this section will be brief. The way in which the crowded environment was constructed will be specified.

Adenylate kinase contains 214 amino acid residues and has three mobile domains: the CORE domain with 133 residues, the NMP domain with 38 residues and the LID domain with 43 residues. This protein has been studied often, both experimentally^{26–36} and theoretically,^{20,21,37–45} making it a good candidate for additional studies of the effects of crowding on its dynamics. In our mesoscopic description AKE is modeled by a network of $N_b = 214$ beads that represent the amino acid residues in the protein. The protein potential energy functions $V_P(\mathbf{R}; \lambda)$ describe the interactions in a network where beads in the protein with coordinates $\mathbf{R} = (\mathbf{R}_1, \mathbf{R}_2, \dots, \mathbf{R}_{N_b})$ are connected by bonds. The basic conformational states that are used in the construction of this potential function are the fully open conformation with no substrates bound, and the fully closed conformation with both ATP and AMP substrates bound. The total potential energy of the AKE network is given by

$$V_P(\mathbf{R}; \lambda) = \sum_{n=1}^{n_L} V_n^P(r_n; \chi_\zeta^n), \quad (1)$$

where the sum is over all n_L bonds in the network. The protein binding state λ enters in the specification of the bond potentials in the sum in eqn (1). In particular, the following notation is used to label these protein states: let χ_ζ be a variable that takes the value $\chi_\zeta = 1$ for an open conformation and $\chi_\zeta = 0$ for a closed conformation. The index $\zeta = \text{LC}, \text{NC}$ and the variables χ_{LC} and χ_{NC} are defined for the LID–CORE and NMP–CORE conformations, respectively. Using this notation the four binding states of the protein may be labeled by $\lambda = (\chi_{\text{LC}}, \chi_{\text{NC}})$. In particular, the fully open state where both the LID–CORE and NMP–CORE domains are open may be labeled by $\lambda_1 = (1, 1)$, the fully closed state has the label $\lambda_3 = (0, 0)$ and the remaining states have the notation $\lambda_2 = (0, 1)$ and $\lambda_4 = (1, 0)$.

Substrate binding and product release are modeled by probabilistic transitions that change the binding state λ of the potential function. The transition rates between the different binding states depend on the instantaneous conformation of the protein. The distances between the centers of mass of the LID and CORE domains, $R_{\text{LC}}^{\text{cm}}$, and NMP and CORE domains, $R_{\text{NC}}^{\text{cm}}$, are monitored and these collective variables are used to determine when a binding or unbinding event occurs.²⁵ An enzymatic cycle consists of the following sequence of conformational changes:^{20,21} $\lambda_1 \rightarrow \lambda_2 \rightarrow \lambda_3 \rightarrow \lambda_4 \rightarrow \lambda_1$.⁴⁶ As a result of the continuous application of such probabilistic rules for changes in the protein binding states, the system evolves to and is maintained in a steady state where the protein stochastically executes enzymatic cycles.

The solvent is also described at a mesoscopic level using multiparticle collision (MPC) dynamics.^{23,24} In MPC dynamics, N_s point solvent particles, representing coarse grained real molecules, free stream and undergo effective collisions at discrete time intervals τ , accounting for the effects of many real collisions during this time interval. The dynamics of the protein in this solvent can be simulated by combined molecular dynamics–multiparticle collision (MD–MPC) dynamics. The protein interacts with the mesoscopic solvent by including the protein beads in the MPC collision step.⁴⁷ No protein–solvent forces need be introduced or computed. The scheme conserves mass, momentum and energy, and hydrodynamic interactions, which are important for the large-scale protein conformational motions, are properly taken into account. In order to assess the influence of hydrodynamic interactions on the conformational and diffusive dynamics one may replace the MPC collision rule with an alternative collision rule where explicit solvent particles are replaced by a heat bath and solvent correlations are destroyed.⁴⁸ More specifically, each protein bead is coupled to an effective solvent momentum, $\mathbf{P}$, which is chosen from a Maxwell–Boltzmann distribution with variance $m\gamma k_B T$, where γ is the average number of solvent particles per cell. The center of mass velocity of a cell containing a protein bead with velocity $\mathbf{v}_b$ used in the MPC collision step is then given by $\mathbf{V}_\zeta = (m_b \mathbf{v}_b + \mathbf{P})/(m\gamma + m_b)$. Momentum is not conserved locally in this collision rule and, thus, hydrodynamic interactions are destroyed. Comparisons between MPC dynamics and dynamics where hydrodynamic interactions are suppressed will be made below. Further descriptions of the MD–MPC dynamics simulation scheme along with examples of applications can be found in recent reviews.^{49,50}

The obstacles that are responsible for crowding are taken to be stationary hard spherical objects which are randomly

distributed in the system. Both the solvent molecules and protein beads interact with the obstacles by hard bounce-back collisions where the velocity of the colliding particle is reversed. The obstacle distribution was constructed in the following way. Given an obstacle radius R_O and system volume $\mathcal{V}$, the number of obstacles N_O that are needed to achieve a desired volume fraction $\phi = 4\pi N_O R_O^3 / 3\mathcal{V}$ was computed. These N_O obstacles were then placed at random positions in the volume, accounting for overlaps between the obstacles. To insert the protein in the obstacle array, a point was chosen at random inside the simulation volume and outside the obstacles and an attempt was made to insert the protein with its center of mass at the randomly chosen point. If any bead in the protein overlapped any of the N_O obstacles another random position was chosen and another attempt to insert the protein was made. This procedure was continued until there was successful insertion of the protein. Especially when ϕ is large and R_O is small it may be difficult or even impossible to find a suitable location for the protein in the finite volume. In this case, if after 10 000 attempts no suitable location for the protein was found, a new random array was generated and the process was continued until the desired number of realizations was obtained. The point solvent molecules were then placed at random positions, again accounting for overlaps with the obstacles, consistent with the desired solvent density. Because the point solvent particles interact with the protein through MPC dynamics, overlaps with the protein do not have to be taken into account.

Since the protein is inserted in the random obstacle array, its initial state may determine where it is inserted. If the protein is in the fully closed $\lambda_3 \equiv \lambda_{cl}$ conformation, its volume is smaller than if it is in the fully open $\lambda_1 \equiv \lambda_{op}$ conformation. (Since we shall primarily be concerned with these two configurations we adopt the more obvious notations, λ_{op} and λ_{cl} , for the fully open and fully closed conformations in the remainder of the paper.) In this case the protein could be inserted into voids in the obstacle array that were too small to accommodate the protein when it is fully open. This may then have an effect on the subsequent cyclic enzymatic dynamics where the protein attempts to visit all conformational states in the cycle. The obstacle radius as well as the volume fraction control the distribution of void sizes in the obstacle array, so the dependence on both of these factors can lead to differences in the initial states for open and closed proteins. One may also draw the initial protein conformations from the set of all conformations encountered as the protein executes enzymatic cycles. The results are intermediate between the two extremes of fully open and fully closed initial conformations; thus, we have chosen to contrast results for these two limiting cases. Realizations of the system constructed when the fully open or fully closed protein is inserted in the random obstacle array will be labeled as $\mathcal{P}_{op}$ or $\mathcal{P}_{cl}$ realizations, respectively.

Of course, a crowded system where the crowding elements are identical spherical particles forming a frozen obstacle distribution is a highly idealized version of most real crowded systems. In a biological cell the crowding elements have a diverse range of geometries, ranging from nearly spherical to long filaments, some of which are anchored and others are mobile. There have been Brownian dynamics simulations, including hydrodynamic interactions, of more realistic crowded systems.⁵¹

Nevertheless, studies of frozen obstacle arrays do allow one to obtain useful information on crowding and have been used frequently for this purpose.^{52–55} They also serve to model the dynamics on intermediate but long time scales of systems where the obstacles are sufficiently massive that the time scales of their motion are far longer than characteristic times for enzymatic conformational, translational and orientational dynamics.

The simulations of AKE dynamics were carried out in a cubic box with periodic boundary conditions containing the protein, solvent and obstacles. For the multiparticle collision events, the simulation box was divided into $(42)^3$ cubic cells with side a . In the MD-MPC algorithm, velocities were rotated about randomly chosen axes by angles $\pm\pi/2$ at time intervals τ . Grid shifting was implemented in the MPC step of the dynamics.^{56,57} For MD the portions of the dynamics Newton's equations were integrated using the velocity Verlet algorithm with a time step of $\Delta t = 0.002\tau$. Dimensionless units were used in the calculations: lengths were measured in units of a , energy in units of ε and mass in units of m . The dimensionless mass of a solvent molecule was taken to be $m = 1$ while the mass of a protein bead was $m_b = 5$. The solvent average number density was $n_s = 7$ and the reduced temperature was $k_B T/\varepsilon = 1/3$. The solvent transport properties can be computed analytically for MPC dynamics.^{49,50}

3 Effects of crowding on AKE dynamics

An example of a trajectory of the protein in the field of obstacles using MD-MPC dynamics is given in Fig. 2. The trajectory of the center of mass of the enzyme is shown as a green line, while the obstacles are depicted as white spheres. The solvent molecules are not shown for clarity. The protein is represented as a network of beads (light blue) and the four metastable configurations that the protein adopts as enzymatic cycles are executed along the trajectory are explicitly shown. The random nature of the center of mass trajectory is evident and collisions of the protein with the obstacles can be seen in this figure. The transport and steady state properties reported

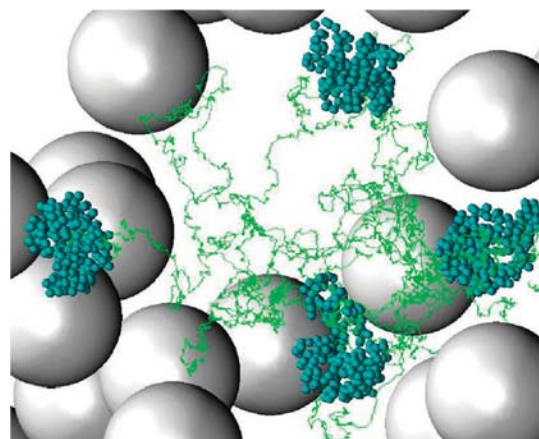


Fig. 2 A random configuration of obstacles with radius $R_O = 5.0$. The volume fraction of obstacles in the system is $\phi = 0.1$. The trajectory of the center of mass of the enzyme is shown as an irregular green line. The conformations of the protein at four different times along this trajectory are also shown. The solvent molecules are not shown in the figure.

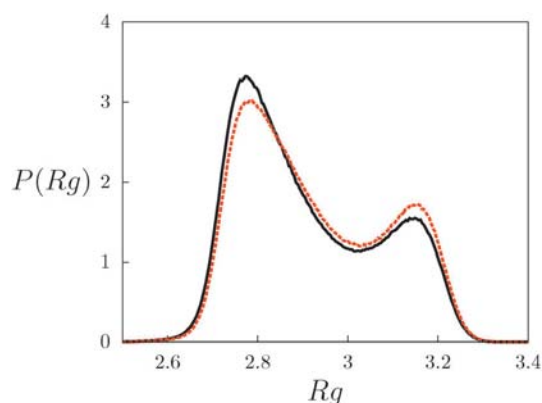


Fig. 3 Plot of the probability density $P(Rg)$ for $\phi = 0.3$ with $R_O = 5.0$ (black solid line) and for the system without obstacles (brown broken line). The histograms were constructed from averages over 200 $\mathcal{P}_{op}$ realizations.

below were obtained from time averages of such trajectories as well as averages over many realizations of the random distributions of obstacles.

3.1 Steady state conformational properties

Molecular crowding can change the steady state probability densities of the different conformational states of the protein. The major conformational changes of the protein along the trajectory can be identified by monitoring the value of the instantaneous radius of gyration $Rg(t) \equiv (N_b^{-1} \sum_{i=1}^{N_b} |\mathbf{R}_i(t) - \mathbf{R}_p(t)|^2)^{1/2}$, where $\mathbf{R}_i(t)$ is the position of bead i and $\mathbf{R}_p(t)$ is the center of mass position of the protein at time t . The probabilities of the open and closed conformational states can then be determined from the probability density, $P(Rg)$, constructed from histograms of Rg generated from an ensemble of trajectories for different realizations of the obstacle distributions. The probability densities for a system with $\phi = 0.3$ and obstacles with radius $R_O = 5.0$ (black solid line) and for a system without obstacles (brown broken line) are compared in Fig. 3. As might be expected,^{58,59} the probability of observing the protein in the closed conformation is larger and the probability of the open conformation is smaller when the system is crowded by obstacles. Let Rg^m denote the position of the central minimum in $P(Rg)$, the probability of observing the closed conformations is $P_{cl} = \int_0^{Rg^m} dRg P(Rg)$, while the probability of the open conformation is $P_{op} = 1 - P_{cl}$. The ratio P_{cl}/P_{op} is plotted in Fig. 4 as a function of ϕ for $R_O = 5.0$. We see that up to volume fractions of approximately $\phi = 0.2$ this ratio is constant within statistical uncertainty, but then strongly increases as the volume fraction increases.

The relative probabilities of open and closed conformations depend not only on the obstacle volume fraction but also on the obstacle radius, since both of these factors determine the average sizes of the voids in the obstacle array in which the protein resides. The size of the protein in the closed and open conformational states can be gauged from the maxima in the probability distribution of Rg , which lie at $Rg \approx 2.8$ and 3.2 , respectively. We consider obstacles with radii that are comparable to or larger than the protein. The effects of obstacle size on the ratio P_{cl}/P_{op} are quantified in Table 1 where this ratio is

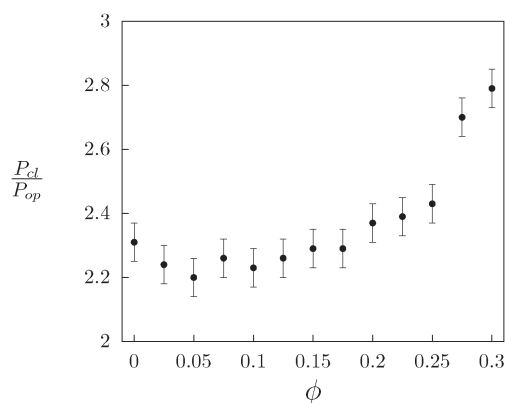


Fig. 4 Plot of P_{cl}/P_{op} as a function of ϕ for $R_O = 5.0$. The error bars denote $\pm$ one standard deviation. The results were computed from averages over 200 $\mathcal{P}_{op}$ realizations of the obstacle distributions.

Table 1 The ratio P_{cl}/P_{op} for $\phi = 0.3$ as a function of the obstacle radius R_O . The two columns show results for the $\mathcal{P}_{op}$ and $\mathcal{P}_{cl}$ realizations

R_O	$P_{cl}/P_{op} (\mathcal{P}_{op})$	$P_{cl}/P_{op} (\mathcal{P}_{cl})$
0.0	2.40 ± 0.04	—
6.0	2.60 ± 0.06	2.58 ± 0.05
5.0	2.75 ± 0.04	2.79 ± 0.05
4.0	2.72 ± 0.05	2.86 ± 0.04
3.5	2.73 ± 0.05	3.01 ± 0.06
3.0	2.27 ± 0.07	3.00 ± 0.07

given as a function of R_O for $\phi = 0.30$ for both $\mathcal{P}_{op}$ and $\mathcal{P}_{cl}$ realizations. For comparison, the first entry in this table (denoted by $R_O = 0.0$) is for the protein in the absence of obstacles. For $R_O \geq 3.5$ the ratio is roughly constant for $\mathcal{P}_{op}$ realizations, while the ratio increases slowly with decreasing R_O for $\mathcal{P}_{cl}$ realizations. Significant changes occur for $R_O = 3$. For $R_O = 3.0$ and $\mathcal{P}_{op}$ realizations, configurations can arise where the obstacles are in the region between the LID and NMP domains. In Fig. 5 we show instantaneous conformations of the protein in its open state for $\phi = 0.3$ and $R_O = 3.5$ (left) and 3.0 (right). In the right panel of the figure where $\phi = 0.3$ and the voids are small on average, the LID domain is bounded by four obstacles, accounting for the low value in Table 1. This makes conformational changes more difficult during the enzymatic cycles (see right, Fig. 5). Consequently, P_{cl}/P_{op} is less than that for

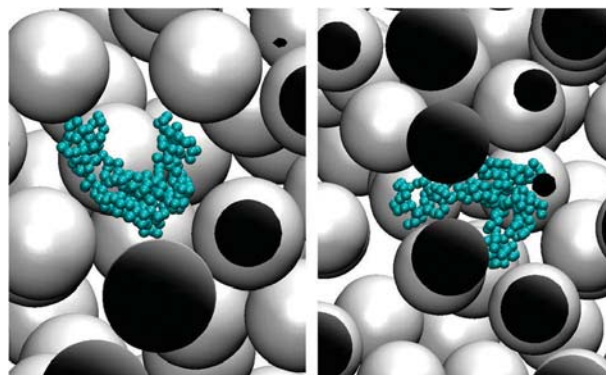


Fig. 5 Instantaneous conformations of the protein in the open state for $\phi = 0.3$ and $R_O = 3.5$ (left), and $R_O = 3.0$ (right).

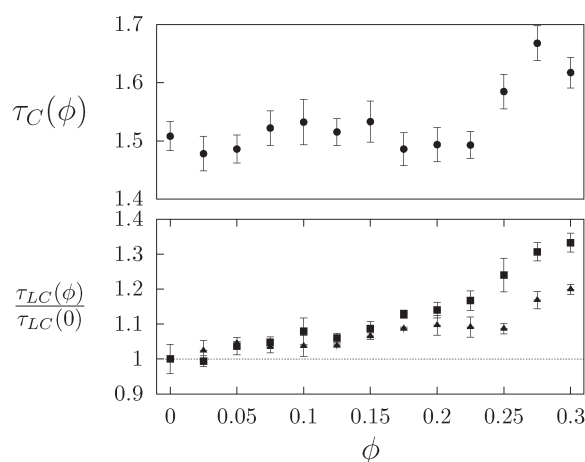


Fig. 6 (top) The average cycle time τ_C ($\times 10^{-4}$) as a function of ϕ (full circles). (bottom) Plot of the ratio $\tau_{LC}(\phi)/\tau_{LC}(0)$ versus ϕ . Results for $\mathcal{P}_{op}$ (full squares) and $\mathcal{P}_{cl}$ (full triangles) realizations are shown. The error bars denote $\pm$ one standard deviation over 200 realizations.

the protein in solution without obstacles. If instead $\mathcal{P}_{cl}$ realizations are considered, the ratio P_{cl}/P_{op} follows the increasing trend with decreasing R_O . If the obstacle radius is large, the average void size increases and the differences that depend on the type of realization become smaller.

3.2 Conformational dynamics

Several characteristic times can be used to monitor the effect of crowding on the enzymatic dynamics. The average time τ_C it takes to complete a full enzymatic cycle can be computed from averages over many realizations of long simulations involving many enzymatic cycles. Fig. 6 (top) plots τ_C versus ϕ . The average cycle time is approximately constant for most of the ϕ range but increases for the highest values, $\phi > 0.225$. The value of the time τ_C depends on the residence times of the protein in the open and closed metastable states and the times to make transitions between these states. Since transitions between the metastable states are rapid, the average cycle time mainly depends on the average times $\langle\tau_{op}\rangle$ and $\langle\tau_{cl}\rangle$ spent in the open and closed conformations, respectively. Fig. 7 shows the histograms of τ_{op} (top) and τ_{cl} (bottom) for systems with $\phi = 0.3$ and $R_O = 3.0$. Both $\mathcal{P}_{op}$ (blue bars) and $\mathcal{P}_{cl}$ (black bars) realizations were considered. We see that the distribution of open times τ_{op} is shifted to larger values for $\mathcal{P}_{op}$ realizations when compared to $\mathcal{P}_{cl}$ realizations, while the distribution of closed times τ_{cl} shows the opposite trend. The average residence time is $\langle\tau_{op}\rangle = 4100 \pm 100$ and 3500 ± 100 for $\mathcal{P}_{op}$ and $\mathcal{P}_{cl}$ realizations, respectively, while $\langle\tau_{cl}\rangle = 10300 \pm 300$ and 12300 ± 400 for these two realizations. These trends are in accord with the variations in probabilities of the open and closed conformations determined from the radius of gyration probability density, $P(Rg)$, discussed earlier. We also note that simulations show that for a given ϕ , the average cycle time is constant, within small statistical uncertainty, for all $3 \leq R_O \leq 6$ considered in this investigation.

More detailed information on the conformational dynamics can be obtained by considering the decay of the autocorrelation of the LID–CORE or NMP–CORE fluctuations. The LID–CORE correlation function is defined by,²⁵

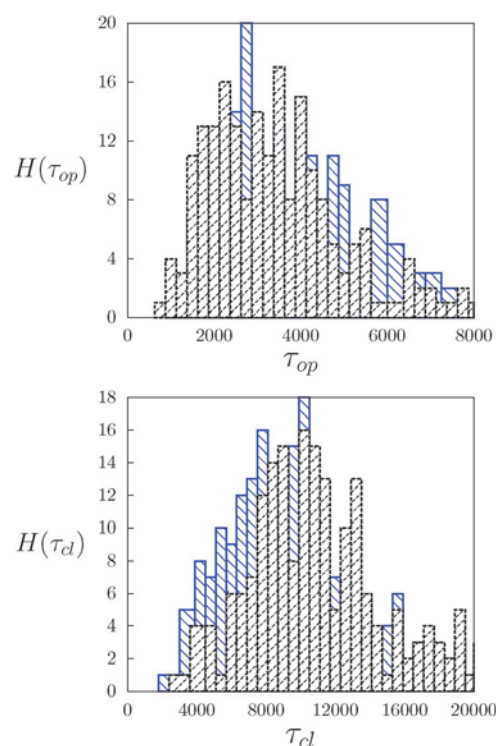


Fig. 7 The histograms of the times spent in the open conformation, τ_{op} (top), and the closed conformation, τ_{cl} (bottom), for systems with $\phi = 0.3$ and $R_O = 3.0$. Results for $\mathcal{P}_{op}$ realizations (blue bars) and $\mathcal{P}_{cl}$ realizations (black bars) are plotted. The histograms were determined from 200 realizations of the enzymatic cyclic dynamics.

$$C_{LC}(t) = \langle \delta R_{LC}^{cm}(t) \delta R_{LC}^{cm}(0) \rangle \approx \langle (\delta R_{LC}^{cm})^2 \rangle e^{-t/\tau_{LC}}, \quad (2)$$

where R_{LC}^{cm} is the distance between the centers of mass of the LID and CORE domains, $\delta R_{LC}^{cm} = R_{LC}^{cm} - \langle R_{LC}^{cm} \rangle$ and the angle brackets signify a time average and an average over realizations of the obstacle distributions. Since this correlation function focuses explicitly on the time scale of the domain motions which occur during the catalytic cycle, τ_{LC} provides a sensitive probe of crowding of conformational motions. The effect of crowding on the LID–CORE distance fluctuations can be seen in Fig. 6 (bottom) where the ratio $\tau_{LC}(\phi)/\tau_{LC}(0)$ is plotted as a function of ϕ . Results are presented for the two types of realizations. In the figure we see that the characteristic time τ_{LC} smoothly deviates from its value in the absence of obstacles and it reaches 1.2–1.3 times its value at $\phi = 0$. The deviations are somewhat larger for $\mathcal{P}_{op}$ realizations.

We may also consider the effects of hydrodynamic interactions on these characteristic times since the hydrodynamic interactions can be suppressed in the simulation as discussed earlier. Enzymatic cycle times are longer when hydrodynamic interactions are not taken into account, leading to more rapid domain motions in solution. The effects are significant but not very large since the protein resides in its metastable states for long periods of time in a complete cycle. The hinge motions associated with substrate binding and product release are more strongly influenced by hydrodynamic interactions but these motions comprise only a small part of the full cycle. For example, in solution without obstacles $\tau_C = 15000 \pm 300$, while $\tau_C^{NH} = 18300 \pm 400$ when hydrodynamic

interactions are suppressed.²⁵ Thus, there is a modest increase in the cycle time when hydrodynamic interactions are suppressed ($\tau_C^{\text{NH}}/\tau_C \approx 1.2$).

3.3 Translational and rotational motion

The transport properties of macromolecules are strongly affected by both hydrodynamic interactions and molecular crowding. Hydrodynamic interactions couple the motions of distant parts of a macromolecule through collective solvent viscous modes. Multiparticle collision dynamics has been used to study the effects of hydrodynamic interactions on colloidal suspensions,⁶⁰ polymer transport and collapse,^{61–64} protein dynamics²⁵ and the dynamics of molecular machines.^{65–67}

Both the character and magnitude of translational diffusive motions of biomolecules are changed in a crowded environment.¹⁵ Experiment and simulation have shown that the characteristic diffusion times of large biomolecules may be orders of magnitude smaller in densely crowded systems than in aqueous solution.^{14–16,68} In addition, diffusion is often observed to be subdiffusive in character on intermediate but long time scales. Subdiffusive dynamics of proteins and finite-size probe molecules has been observed in crowded cellular systems^{69–72} and membranes.^{73–75} In systems with randomly distributed stationary obstacles, such anomalous diffusion is commonly seen on spatial scales that depend on the obstacle volume fraction and size, as well as the size of the diffusing particle. For short times that probe distances less than the characteristic spatial scale, normal diffusive behavior is seen, while subdiffusive behavior is observed on time scales sufficiently long that the particle can explore the spatial scales that characterize the obstacle distribution. As long as the particle does not become trapped, normal diffusion will again be seen on long time scales.

The character of the diffusive motion can be determined from the form of the mean square displacement (MSD) versus time,

$$\Delta L^2(t) = \langle |\mathbf{R}_p(t) - \mathbf{R}_p(0)|^2 \rangle = 6\Gamma t^\alpha, \quad (3)$$

where $\mathbf{R}_p$ is again the center of mass of the protein, Γ is a constant that does not depend on time and α is an exponent that characterizes the nature of the diffusive motion; $\alpha = 1$ for normal diffusion, $\alpha > 1$ for superdiffusive motion and $\alpha < 1$ for subdiffusive motion. Fig. 8 plots the MSD versus time for a few representative values of the obstacle volume fraction and radius. In the figure the colored solid lines are the simulated values of the MSD while the dotted lines are the best fits to the data using eqn (3). The straight dashed lines are linear fits to the data for long times. The subdiffusive character is evident in these plots and one can see the crossover into the regime of normal diffusion. When diffusion is anomalous Γ can be associated with the instantaneous diffusion coefficient⁷⁶ at time t by $D(\phi; t) = \alpha\Gamma t^{\alpha-1}$. When the time is long enough the diffusion coefficient can be obtained from the linear long-time fit. In view of the fits to the data shown in Fig. 8 we find that the values of $D(\phi, t = 10^5)$ and $D(\phi)$ extracted from the long time decay are indistinguishable within statistical errors.

Fig. 9 plots the effective diffusion coefficient $D(\phi)$ as a function of ϕ for obstacles with a radius $R_O = 5.0$. Results are shown for

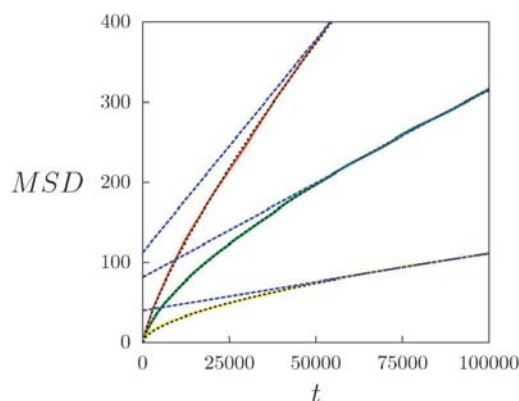


Fig. 8 Plots of the mean square displacement MSD versus time t for the protein undergoing cyclic enzymatic dynamics: (yellow line) $\phi = 0.3$ and $R_O = 4$, (green line) $\phi = 0.3$ and $R_O = 5$, and (brown line) $\phi = 0.25$ and $R_O = 5$. The fits of the data to eqn (3) for the three different cases are shown as black dotted lines. These are indistinguishable from the simulation data on the scale of the figure. In addition, linear fits to the data for $t > 5 \times 10^5$ are also shown as blue dashed lines.

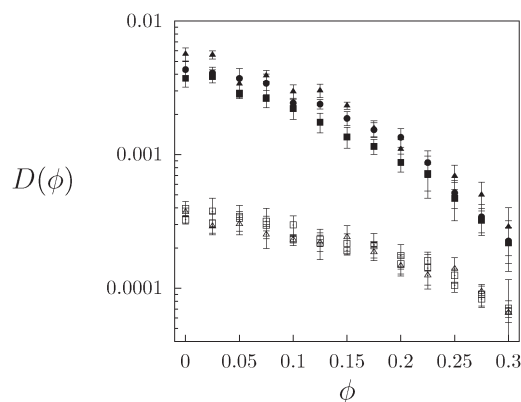


Fig. 9 The instantaneous diffusion coefficient $D(\phi)$ at $t = 100\,000$ as a function of ϕ on a semi-logarithmic scale. Results for MPC dynamics that include hydrodynamic interactions for several situations: protein in the open λ_{op} conformation (full squares), protein in the closed λ_{cl} conformation (full triangles) and for the protein undergoing enzymatic cyclic dynamics (full circles). Results without hydrodynamic coupling for the same conditions: open λ_{op} conformation (open squares), closed λ_{cl} conformation (open triangles) and for the cyclic dynamics (open circles). In all cases $\mathcal{P}_{op}$ realizations were used to obtain the results. The error bars denote $\pm$ one standard deviation over 200 realizations.

MPC dynamics where hydrodynamic interactions are properly included, as well as dynamics where these interactions are suppressed. Computations have been carried out for the protein in the open λ_{op} and closed λ_{cl} conformations, as well as when the protein is executing enzymatic cyclic dynamics. The diffusion coefficient decreases as ϕ increases; in addition, the importance of hydrodynamic interactions decreases since hydrodynamic interactions are screened by the stationary obstacle array.⁶⁴ Also, in both cases, there are no discernable differences in $D(\phi)$ for $\mathcal{P}_{op}$ and $\mathcal{P}_{cl}$ realizations. The diffusion coefficient decreases by approximately a factor of ten in going from pure solvent to a crowded environment with an obstacle volume fraction of $\phi = 0.3$, consistent with that found in previous studies.⁷⁷

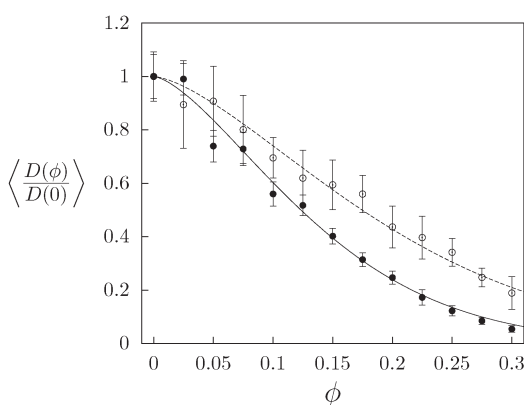


Fig. 10 Plot of the average diffusion coefficient ratio $\langle D(\phi)/D(0) \rangle$ versus ϕ . The diffusion coefficients for the protein in its open λ_{op} , closed λ_{cl} conformations and while undergoing enzymatic cyclic dynamics were averaged to obtain the results shown in the graph. The conditions are the same as in Fig. 9. The solid and dashed lines are best fits of the data to eqn (4) for MPC dynamics (full circles) and dynamics without hydrodynamic interactions (open circles), respectively.

From the measured values of $D(\phi)$ (and direct observation of the diffusive trajectories of the protein) we can estimate the average characteristic linear distance $\ell_D = \sqrt{D(\phi)t_{\text{sim}}}$ over which the protein diffuses during the total simulation time $t_{\text{sim}} = 2 \times 10^5$. We find $\ell_D \approx 10R_p$ for the system without obstacles, while $\ell_D \approx 3R_p$ for the most crowded system. Here R_p is the radius of the protein. Thus, the simulation times are long enough for the protein to diffusively explore the obstacle environment, as suggested by the MSD plots.

Analysis of experimental data on the diffusion of macromolecules has indicated that the volume fraction dependence of the diffusion coefficient could be fit with the phenomenological functional form,^{78,79}

$$D(\phi) = D(0)e^{-\beta\phi^\nu} \quad (4)$$

where β and ν are scaling parameters that depend on the molecular weight of the protein and the solvent in which it is dissolved. The solid and dashed lines in Fig. 10 are best fits of our simulation data to eqn (4) for both MPC dynamics and dynamics without hydrodynamic interactions, respectively. In both cases $\nu \approx 1.5$, but $\beta \approx 15.0$ for MPC dynamics and $\beta \approx 8.5$ when hydrodynamic interactions are suppressed. This is consistent with observations that suggest that only β depends on hydrodynamic coupling.⁷⁹

The exponent α in eqn (3) that characterizes subdiffusive dynamics in the crowded medium depends on the volume fraction. This dependence is shown in Fig. 11 which plots α versus ϕ . The magnitude of α and its dependence on ϕ are similar to that observed in experiments in crowded media with anchored obstacles.⁷⁵ Hydrodynamic interactions do not have a noticeable effect on this exponent.

The translational diffusion properties in the crowded medium depend strongly on the obstacle radius. The results in Table 2 show that $D(\phi)$, relative to its value in pure solution without obstacles, changes by approximately two orders of magnitude for R_O in the range $3 \leq R_O \leq 6$. From examination of the ratio $D(\phi)/D^{\text{NH}}(\phi)$ in this table we can see that the effects are stronger when hydrodynamic interactions are

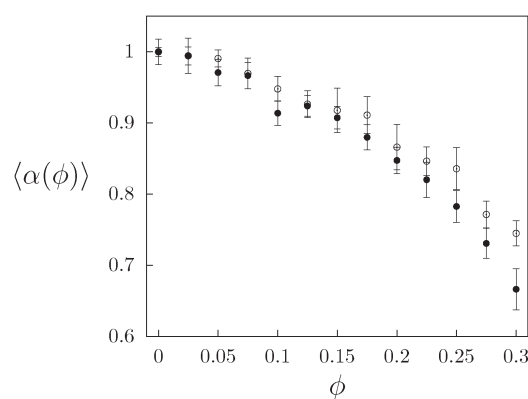


Fig. 11 The average value of α versus ϕ . The average was taken over results from fits to the expression $\Delta L^2(t) \approx t^\alpha$, when the protein was in its open λ_{op} and closed λ_{cl} conformations and as well as when it was executing cyclic enzymatic dynamics: MPC dynamics (full circles), hydrodynamics suppressed (open circles). Conditions are the same as in Fig. 9.

Table 2 The diffusion coefficient for $\phi = 0.3$ and several obstacle radii. The diffusion coefficient in the absence of obstacles is $D(0) = 0.0072$

R_O	$D(\phi)/D(0)$	$D(\phi)/D^{\text{NH}}(\phi)$	$\alpha(\phi)$	$\alpha^{\text{NH}}(\phi)$
6.0	0.145	7.15	0.88 ± 0.04	0.77 ± 0.03
5.0	0.050	4.08	0.68 ± 0.05	0.68 ± 0.03
4.0	0.013	2.22	0.58 ± 0.03	0.63 ± 0.04
3.5	0.0030	1.92	0.38 ± 0.02	0.41 ± 0.04
3.0	0.0009	1.95	0.28 ± 0.03	0.28 ± 0.03

taken into account. The exponent α also varies with R_O and changes by about a factor of three for systems both with and without hydrodynamic interactions over the radius range shown in the figure.

The rotational motion of the AKE protein is also strongly influenced by hydrodynamic interactions²⁵ and crowding. To examine the rotational dynamics of the protein we consider a unit vector $\hat{n}$ in the CORE domain directed between beads labeled 4 and 111. The CORE domain beads do not undergo large relative motions when the LID and NMP domains open or close, so the dynamics of this vector provides information on the overall rotational motion of the protein. The rotational dynamics may be characterized by the decay of the autocorrelation function,

$$C_\theta(t) = \langle \hat{n}(t) \cdot \hat{n}(0) \rangle = \langle \cos(\theta(t)) \rangle \approx e^{-t/\tau_R}, \quad (5)$$

where $\theta(t)$ is the angle between the $\hat{n}(t)$ and $\hat{n}(0)$ vectors and τ_R is the time that characterizes the approximate exponential decay of this function. Fig. 12 shows the ratio of $\tau_R(\phi)/\tau_R(0)$ as a function of ϕ for MPC dynamics. Trends similar to those seen in the conformational dynamics data for τ_{LC} are also apparent in the orientational correlation times.

The behavior of τ_R in a crowded medium and in a medium without obstacles for systems, with and without hydrodynamic interactions, is shown in Table 3 as a function of R_O . The results in this table indicate that the changes in τ_R in MPC dynamics are greater than those for dynamics without hydrodynamic interactions for all values of R_O considered. The difference decreases as the radius decreases.

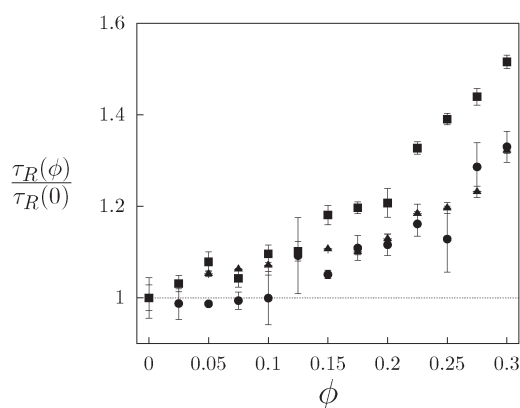


Fig. 12 Plots of $\tau_R(\phi)$ relative to its value in the absence of obstacles versus ϕ for the protein in its open λ_{op} state (full squares), its closed λ_{cl} state (full triangles) and undergoing cyclic enzymatic dynamics (full circles). The error bars denote $\pm$ one standard deviation over 200 realizations of the obstacle distribution.

Table 3 The relative values of $\tau_R(\phi)$ for $\phi = 0.3$ and for several values of R_O

R_O	$\tau_R(\phi)/\tau_R(0)$	$(\tau_R(\phi)/\tau_R(0))^{NH}$	$\tau_R(\phi)/\tau_R^{NH}(\phi)$
6.0	1.14	1.11	0.176
5.0	1.33	1.13	0.197
4.0	1.65	1.34	0.210
3.5	2.43	1.68	0.237
3.0	4.15	3.04	0.370

4 Summary and remarks

Molecular crowding is known to affect the structural and dynamical properties of proteins. Our investigations of AKE in an environment crowded by stationary hard spherical obstacles provided quantitative information on the precise forms that these crowding effects take for this protein while it is undergoing cyclic enzymatic activity. Here we highlight a few of these findings. In general, as might be expected, the closed conformation of the protein is favored over the open conformation when obstacles are present in high volume fraction, with the ratio P_{cl}/P_{op} increasing by a factor of approximately 1.4 for the highest volume fraction of $\phi = 0.3$ over its value in the absence of obstacles. There are less anticipated results as well. When the obstacle radius is comparable to or even smaller than that of the protein, the obstacles may lock the protein in the open conformation and give rise to a lower ratio.

For volume fractions $0 \leq \phi \leq 0.225$ the average cycle time is approximately constant and only increases by a small amount for the highest volume fractions. The enzymatic cycle time is large and most of that time is spent in the open and closed metastable states. Since the steady state probabilities of open and closed states do not vary greatly, neither does the average cycle time. However, there are noticeable changes in the probability distributions of the residence times in the open and closed states.

The most significant effects are seen when the transport properties of the protein are considered. Translational diffusion adopts a subdiffusive character on the long time scales of our simulations. The exponent α that characterizes this

behavior decreases with increasing volume fraction in a fashion similar to that seen in experiments. This exponent does not depend strongly on the conformational state of the protein. The effective diffusion coefficient can decrease by more than an order of magnitude depending on the obstacle volume fraction and radius. Similar trends are seen in the orientational relaxation time of the protein.

While our crowded environment was highly simplified the investigation provides insights into some of the effects to be expected on enzymatic conformational dynamics and transport in crowded media. The particle-based simulation methods used in the paper may be extended to study protein dynamics in more realistic models of crowded systems where there are a variety of different crowding agents, both mobile and immobile, with various sizes.⁵¹

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