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Channeling by Proximity: The Catalytic Advantages of Active Site Colocalization Using Brownian Dynamics

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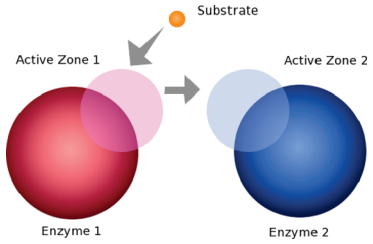
ABSTRACT Nature often colocalizes successive steps in a metabolic pathway. Such organization is predicted to increase the effective concentration of pathway intermediates near their recipient active sites and to enhance catalytic efficiency. Here, the pathway of a two-step reaction is modeled using a simple spherical approximation for the enzymes and substrate particles. Brownian dynamics are used to simulate the trajectory of a substrate particle as it diffuses between the active site zones of two different enzyme spheres. The results approximate distances for the most effective reaction pathways, indicating that the most effective reaction pathway is one in which the active sites are closely aligned. However, when the active sites are too close, the ability of the substrate to react with the first enzyme was hindered, suggesting that even the most efficient orientations can be improved for a system that is allowed to rotate or change orientation to optimize the likelihood of reaction at both sites.

SECTION Biophysical Chemistry



Nature frequently colocalizes linked catalytic functions; this is seen in single polypeptides that catalyze multiple consecutive steps in a metabolic pathway[1−3] and in large assemblies of noncovalently associated biomolecules that carry out complex cellular processes with remarkable fidelity.[4−7] A consequence of such organization is that pathway intermediates that enter solution are precisely positioned near the active center waiting to receive them. Therefore, for a period of time, the intermediate and its recipient binding site remain in close proximity, which increases the effective concentrations of these interactors and leads to enhanced binding and catalytic efficiency. Intermediates that do not enter bulk solution but remain bound to the biomolecule are channeled between binding sites via tunnels and electrostatic grooves whose conformational states are responsive to the positioning of the ligand.[8−12] It is conceivable that intermediates released from properly positioned active sites can be transferred between the sites with efficiencies that approach those of channeling systems. Whereas the effects of high local concentrations are much anticipated,[13,14] quantitative estimates of the magnitudes of these effects are lacking.

Here Brownian dynamics simulations are used to study particle trajectories using short time solutions of the Smoluchowski equation. The trajectories are used to obtain particle collision probabilities and to predict the likelihood of reaction.[15−18] The BrownDye[19] software was used to simulate trajectories and collect collision probabilities. The system has a temperature of 298 K, and the solvent has the viscosity of water. The enzyme particles were modeled as two separate spheres with either 4 or 8 Å radii, each with a spherical active site zone of 5 Å radius centered at a point on the surface of the sphere (Figure 1). The enzyme spheres were given a +1 charge located either in the center of the sphere or at the center of the active zone. The large spheres were held at a constant distance during each simulation, and the distance between the reactive zone centers was varied between 5 and 50 Å in 5 Å intervals over several simulations. Each simulation consisted of 10 000 trajectories, which provided enough successful reactions to draw conclusions while keeping the calculation time manageable. The relative orientation of the reactive zones was also varied. The original starting position (0° orientation) consisted of the reactive zones directly facing each other. The zones were rotated in opposite directions by 45, 90, 135, and 180° (Figure 3B, inset). The substrate particle was modeled as a sphere with a 1 Å radius and a −1 charge. The sizes of the enzyme and substrate spheres were chosen to give as simple a system as possible while having size ratios of 1:4 and 1:8. The substrate trajectory was modeled with BrownDye, where the translational and rotational diffusivities of the spheres follows Stokes' Law. The substrate particle started from an orientation in which its center was separated by 50 Å from the center of the first enzyme sphere's active zone. A collision was recorded whenever the substrate particle center contacted the reactive zone. It was assumed that each collision leads to a reaction because the most efficient enzymes will react with every substrate they encounter. For a reaction to be considered complete, the substrate had to first

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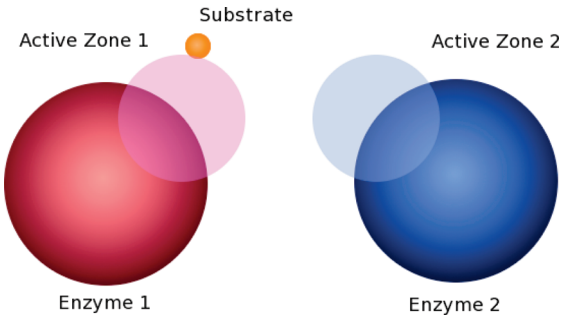

Figure 1. Schematic of experimental setup. The substrate sphere (orange) must diffuse to the active zone of the first enzyme (pink) and then to the active zone of the second enzyme (blue). Shown are the enzyme spheres with the 8 Å radius and the active zones in the 45° orientation. The center of the substrate sphere must encounter the active zone for a reaction to occur.

diffuse to and react with the first enzyme sphere and then diffuse from that position to react with the second enzyme sphere. The number of collisions for the first and second interactions was recorded separately, so the reaction probability for each interaction can be calculated independently as well as the total probability for the overall reaction pathway.

After running all of the simulations, the reaction probabilities were compared. The reaction of the substrate with the first reactive zone should not depend strongly on the location of the active zone or the distance between the zones, and so similar reaction probabilities would be expected (Figure 2A, Figure 3A). The only difference is the localization of the charge in the active zone or at the center of the enzyme sphere. Therefore, the average from the four sets of simulations was calculated; the reaction with the first zone has an average probability of reaction of 0.0713 ± 0.0063 for 4 Å spheres with charged active zones, 0.0632 ± 0.0076 for 4 Å spheres where the charge is centered in the enzyme, 0.0536 ± 0.0076 for 8 Å spheres with charged active zones, and 0.0403 ± 0.0090 for 8 Å spheres where the charge is centered in the enzyme. The results indicate that spheres with less buried active zones are more likely to have initial reactions. In addition, the spheres with the charged active zones are slightly more likely to have an initial reaction than those with the charge centered in the large sphere, which can be explained by the charge acting to guide the substrate sphere to the specific location of reaction, rather than just generally toward any point on the large sphere. Interestingly, when the reactive zones are in the 0° orientation (facing each other) and the large spheres are at a 5 Å distance from each other, the initial probability of reaction for the 4 Å spheres is 0.0650 for the charged active zones and 0.0481 for the charged enzyme spheres and for the 8 Å spheres is 0.0344 for the charged active zones and 0.0167 for the charged enzyme spheres (Figure 2A). These are all significantly lower (more than one standard deviation) than the average, although the effect is more pronounced in the 8 Å spheres. This may be because the enzyme spheres shield each other to some extent. Similarly, when the active zones are in the 45° orientation and 5 Å distance, the probabilities of the first reactions are slightly lower than the average probability for the first reaction. All of

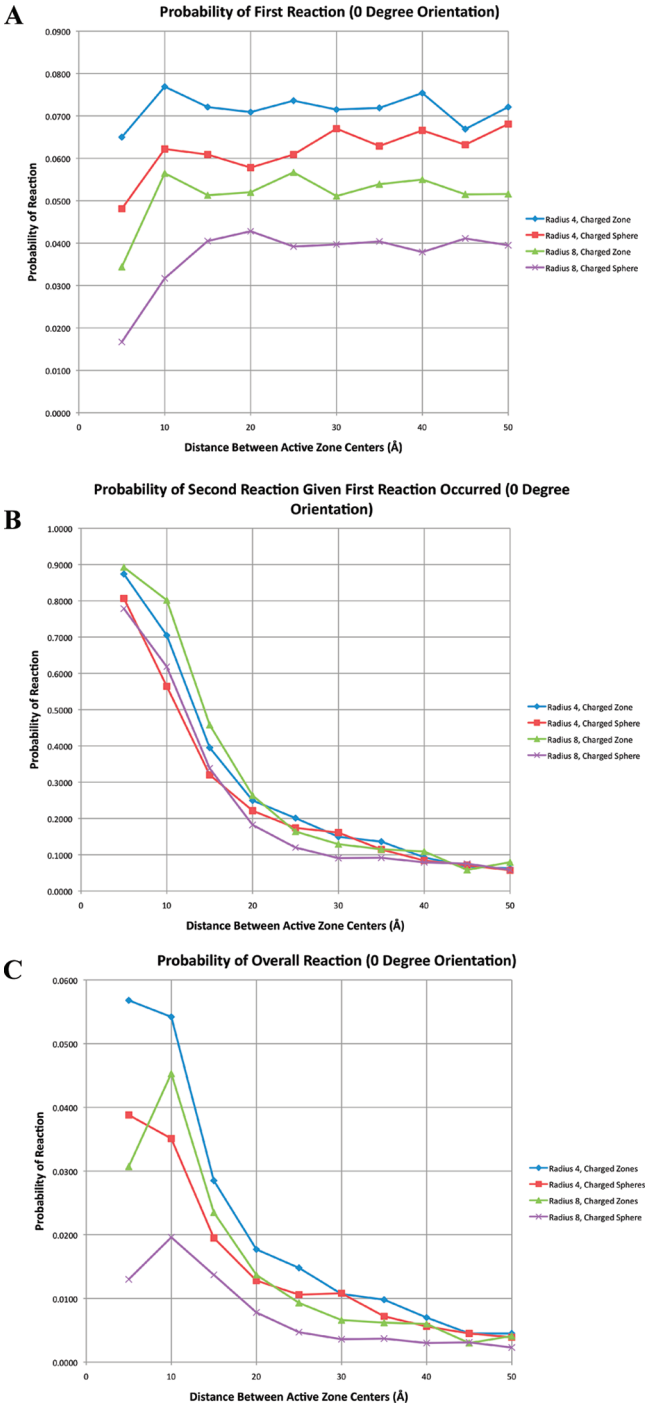

Figure 2. Probabilities of reactions at changing distances when active zones are in 0° orientation. (A) Probability of the first reaction occurring. The probability is fairly constant except when the active zone centers are only 5 Å apart. The close proximity of the enzyme spheres may hinder the substrate ability to encounter the active site. (B) Probability of second reaction given that first reaction occurred. Close proximity of the active zones leads to effective reaction pathway, with the charged active zones being more efficient until ~25 Å separation. (C) Probability of second (overall) reaction. The effect of shielding the first active zone can clearly be seen. In general, the closer active zones lead to a more effective pathway, with the charged active zones being significantly more efficient at the closer distances.

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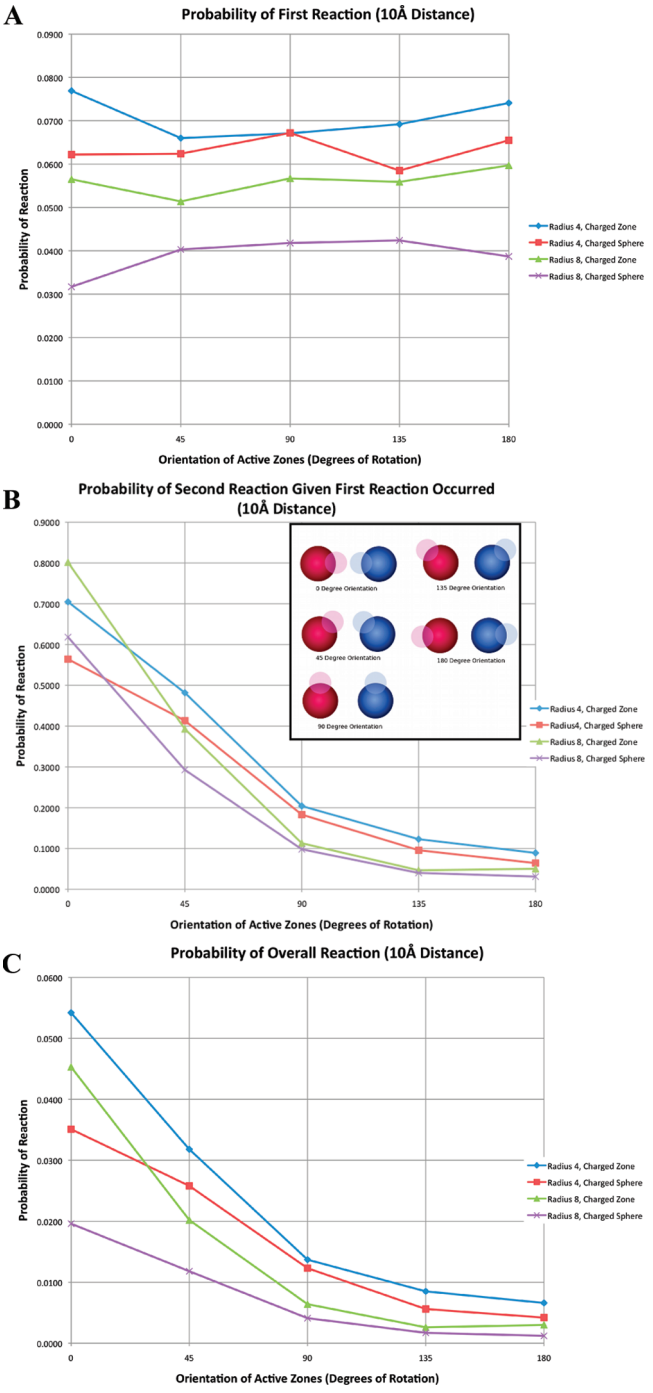

Figure 3. Probabilities of reactions at rotated active zone orientations when active zones are at 10 Å distance. (A) Probability of the first reaction occurring. The probability is fairly constant as expected. (B) Probability of second reaction, given that first reaction occurred. As the active zones rotate away from each other, the reaction probability decreases considerably, and the effect of enzyme sphere size becomes more important. Inset shows active zone orientations. (C) Probability of second (overall) reaction. The active zones that face each other demonstrate a more effective pathway. Again, other than the 0° orientation, enzyme size is more important than location of the charge.

the other active zone orientations (90, 135, and 180°) did not show this lowered probability for the closer spheres. At the larger distances (10−50 Å), the reaction probabilities are about the same for all active zone orientations.

The probability of the second reaction depends on the orientation of the active zones and the distance between the zones as well as the location of the enzyme's charge. The probability can be examined either as the probability of the second reaction given that the first reaction occurred (Figure 2B, Figure 3B), which demonstrates the efficiency of the active zone orientation and distances in the system or as the overall probability of the second reaction (Figure 2C, Figure 3C), which is the probability that the product from the complete reaction pathway will be produced. As expected, for the 4 Å spheres, the largest number of successful reactions occurs when the two active zones are facing each other in the 0° orientation at 5 Å distance with the charges localized on the active zones. However, for the 8 Å spheres, the effect of shielding the active site of the first reaction at the 5 Å distance can clearly be seen to hinder the success of the overall reaction (Figure 2C). Although this hinders the success of the overall reaction, it is still the most efficient configuration for passing the substrate sphere from the first to the second active zone, perhaps because this shielding effect prevents the substrate sphere from escaping once it has interacted with the active sites (Figure 2B). In general, the probability of reaction completion decreases as the active zones are rotated away from each other and as the distance between the zones increases. The charged zones have a higher reaction probability at the 0° orientation until the separation between the active zones reaches 25 Å, after which the size of the enzyme sphere seems to be a more important factor in determining reaction success (Figure 2B,C). For the other active zone orientations, the size of the enzyme sphere seems to determine the probability of reaction more strongly than does the position of the charge (Figure 3B,C).

Clearly, the orientation of the active zones and the distance between the zones as well as the size of the active zone relative to the enzyme sphere are important in determining the success of a reaction. For enzymes of the same size, the overall reaction probability is greater when the charge is localized on the active zone, and this effect is more significant when the two active zones are closer together. Once the zones have been rotated away from the original 0° orientation or separated by a distance of greater than 25 Å, the charge localization has much less impact than the size of the enzyme spheres. However, when the active zones are too close to each other, the enzyme spheres can hinder initial access of the substrate to the active zones. Although this provides a more efficient substrate transfer, it makes the overall reaction less effective. This behavior could potentially argue for a mechanism in which the enzymes either move or rotate to control the efficiency of both initial substrate uptake and substrate transfer. These and other more realistic models will be the subject of future studies.

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