Supplementary Information for SEEKR: Simulation Enabled Estimation of Kinetic Rates, A Computational Tool to Estimate Molecular Kinetics and Its Application to Trypsin-Benzamidine Binding

Convergence of k_{on} and k_{off} values:

The k_{on} calculation is fairly well converged in the 47000 umbrella sampling frames. Although k_{off} is not as well converged within that span of time, fluctuations are less than one order of magnitude.

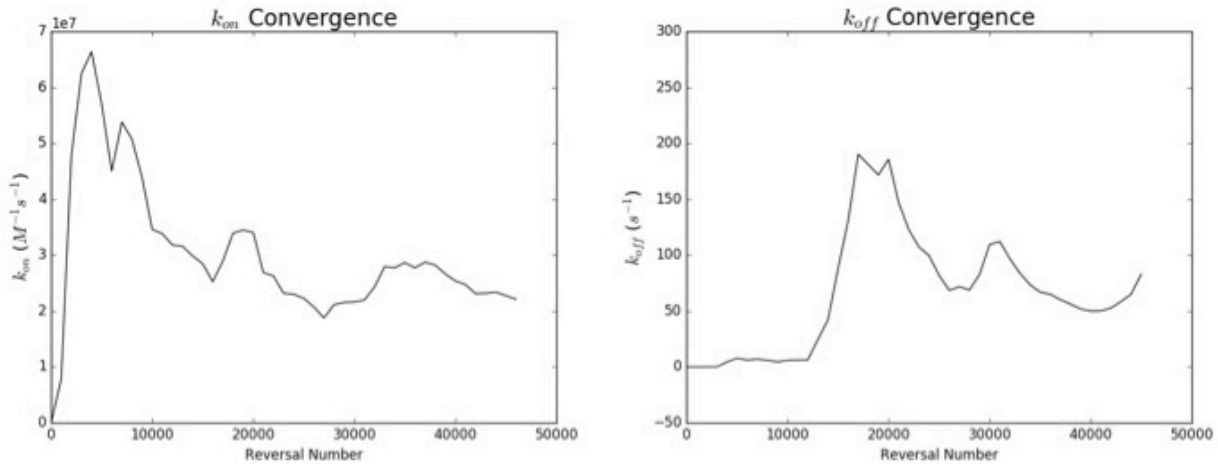

Figure S1. Convergence of rate constants as a function of umbrella sampling length. Fluctuations in the rate constant for the k_{on} are fairly well converged. In contrast, the k_{off} is likely to require additional umbrella sampling to fully converge, but both kinetic values seem to have converged to within one order of magnitude given the amount of umbrella sampling.

Sensitivity of the system to ionic concentration:

In order to investigate the sensitivity of this system to the ionic concentrations within the BD stage simulations, we also computed the k_{on} at various ionic concentrations. The dependences of concentration on the k_{on} and k_{off} are listed in table S1. If ionic strength is neglected, then the k_{on} decreases by about a factor of three.

Ion strength factor	TrisHCl concentration (M)	$CaCl_2$ concentration (M)	Debye Length (Å)	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	ΔG_{bind} (kcal/mol)
0	0	0	∞	$6.3\pm0.8\bullet10^{6}$	83±14	-6.7±0.1
0.5	0.03	0.01	12	$2.1\pm0.3\bullet10^{7}$	83±14	-7.4±0.1
1	0.06	0.02	8	$2.1\pm0.3\bullet10^{7}$	83±14	-7.4±0.1
2	0.12	0.04	6	$2.2\pm0.3\bullet10^{7}$	83±14	-7.4±0.1

Table S1. The effect of ion concentration on the computed k_{on} and k_{off}.

The reason that ionic strength must be accounted for so carefully in this system is that the k_{on} between trypsin and benzamidine shows high dependence on ionic strength in our BD simulations (table S1). This is likely because the k_{on} is close to the diffusion limit, and electrostatic forces are screened by the dissolved ions. One surprising observation, however, is that increased ionic strength in the BD simulations (smaller Debye length) actually increases the computed k_{on}. This result suggests that the benzamidine experiences repulsive forces during its

approach to binding with trypsin, likely contributing to the low free energy barrier to entry observed in the profile in figure 4 of the main text, and that these repulsive forces are shielded by higher ionic strength, and are thus electrostatic in nature. Another possibility is that the high ionic strength is shielding attractive hot spots on the surface of trypsin that compete with the active site for binding. Electrostatic maps show large regions of positive electrostatic fields surrounding the majority of trypsin. These positive fields likely repel the positively charged benzamidine (figure S2). It is clear, however, that ion concentration in kinetics calculations of this system must be carefully chosen in order to properly reproduce experimentally observed values.

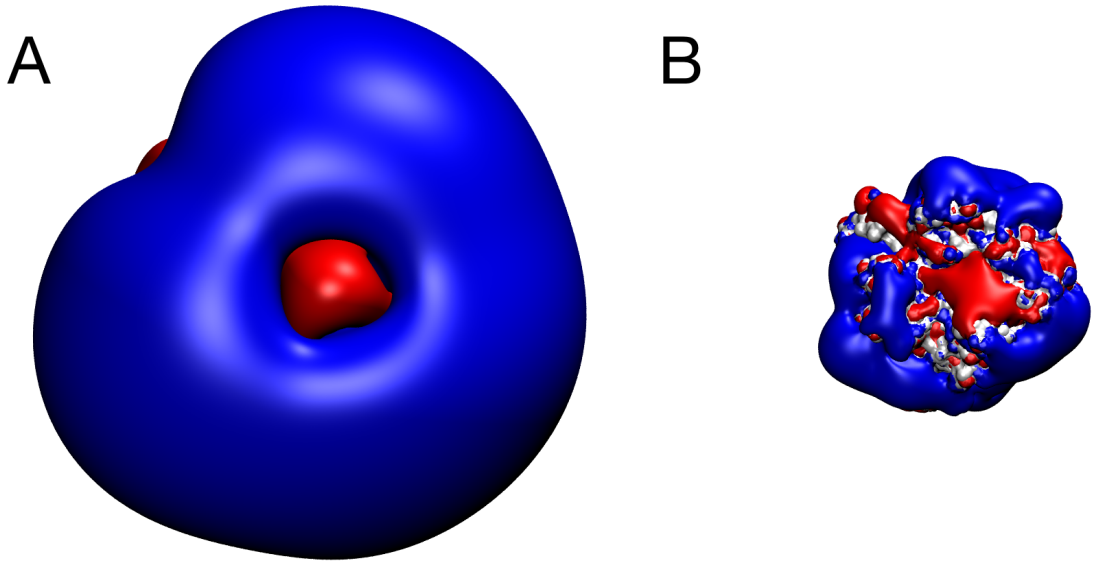

Figure S2. The electrostatic potentials around trypsin. In panel A, the isosurface is drawn in blue at 0.01 kT/e, and in red at -0.01 kT/e. In panel B, the isosurface is drawn in blue at 1 kT/e, and in red at -1 kT/e. The large positive field surrounding trypsin provides a reasonable explanation for why we observe a faster k_{on} at higher salt concentrations.