Thrombin is a trypsin-like protease that plays opposing functional roles in blood coagulation because of the interaction with numerous macromolecular substrates, receptors, and inhibitors. The protein exists as an ensemble of active (E) and inactive (E*) conformations that differ in their accessibility to the active site. Using molecular dynamics (MD) simulations, we showed that the redistribution of the E*–E equilibrium can be achieved by perturbing the electrostatic properties of the enzyme. Removal of the negative charge of the catalytic Asp102 (by mutation to Asn102) in the primary specificity site destabilizes the E form and causes a shift in the 215–217 segment that compromises substrate entrance.

In order to distinguish the electrostatic effect from a possible steric effect on the E*–E equilibrium, our computational approach consisted in performing an MD simulation while carrying out an “alchemical” mutation of the protein. Along the trajectory, the charges of the side chain atoms of Asp102 were gradually modified and once the total negative charge was quenched, we performed the change of atoms to complete the D102N mutation. A MD simulation of the wildtype protein was also carried out to be used as a “blank” experiment to which compare the properties of the “alchemical mutants”, as well as of the real D102N mutant.

Not only the MD simulations confirm the stabilization of the E* form upon mutation, documented from solution studies and existing structures of D102N. They also show that the perturbation of the electrostatics within the active site of thrombin is specifically the cause of the drastic alteration of the E*–E equilibrium, and provide a detailed mechanistic framework for the interpretation of key functional and structural features of the enzyme.

References