Supporting Material

for

Autoinhibition of Endophilin in Solution via Inter-domain Interactions

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SUPPLEMENTAL TEXT

Rotation of the H0 helix leads to secondary structure instability

In this work, we used H0/SH3 structure that we believe best hid from the solvent the large hydrophobic residues on both the H0 helix and the SH3 domain; however, it was also important that we tested the stability of other possible configurations. We generated four configurations by rotating the helix azimuthally around the helix vector by 0^o, 90^o, 180^o, and 270^o (Fig. S1). Each of the systems was initially prepared using the procedure in the Methods section describing the simulation of the H0/SH3 complex in solution. After this the systems were all simulated for at least 210 ns.

From the simulations of each of the four H0/SH3 configurations, the rotation of the H0 helix leads to secondary structure instability in both the helix and the SH3 domain. When the amphipathic helix is placed into the SH3 domain in such a way that minimizes the exposure of hydrophobic residues to the solvent, the helix and the SH3 domain maintain their secondary structure in solution. The exposure of the hydrophobic Phe10 residue to the solvent causes the N-terminal helix to become disordered. This also leads to varying amounts of disorder in the SH3 domain (Fig S2). These simulations help justify our original placement of the H0 helix in the peptide binding pocket of the SH3 domain by showing how reducing the exposure of the hydrophobic residues to the solvent helps stabilize the H0/Sh3 structure.

Metadynamics Convergence

The convergence of the metadynamics simulation was measured by calculating the energy difference between the global minimum and other significant local minima. This quantity is calculated as a function of simulation completeness and acts as a running average for the energy difference of the wells in the free energy landscape. As can be seen in Fig S3, for the last 20% of the simulation, the local minima no longer change with respect to the global minimum. During this time, the size of the added hills had decreased to nearly zero. Once the simulation has converged, the free energy surface essentially becomes a flat landscape and the simulation begins to perform a random walk in the free energy space. At this point the error can be assumed to be equivalent to the thermal energy, 0.68 kcal/mol.

SUPPLEMENTAL FIGURES

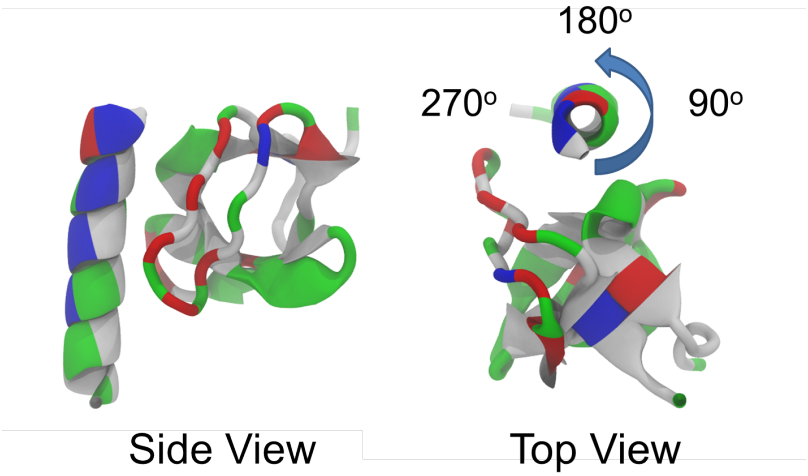

Fig. S1: Four simulations were generated by rotating the H0 helix azimuthally around the axis of the helix by 0°, 90°, 180°, and 270°. This was done to investigate whether increased exposure of the Phe10 residue on the helix would lead to structural instability in the protein. The proteins are represented here using the New Cartoon representation. The H0/SH3 complex is colored according to residue type, where hydrophobic residues are white, polar residues are green, acidic residues are red, and basic residues are blue.

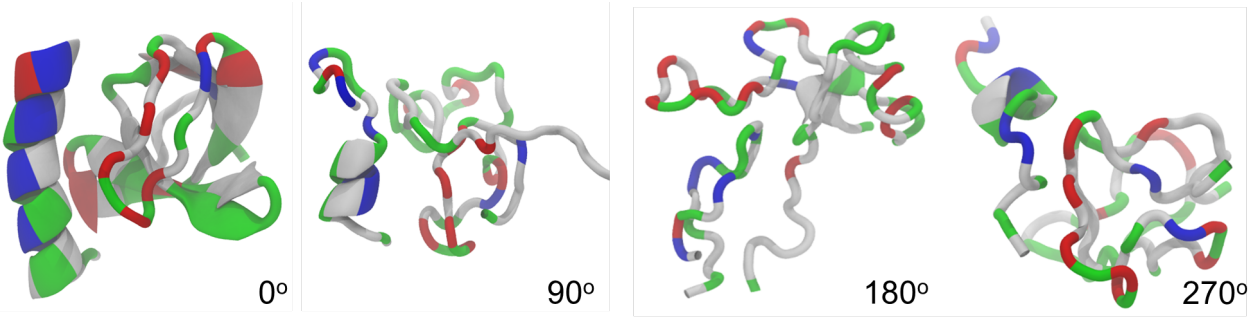

Fig. S2: The four simulations generated by rotating the H0 helix azimuthally around the axis of the helix by 0°, 90°, 180°, and 270°, shown from the side, the top, the bottom, and the side, respectively. The simulated structures how that the exposure of the Phe10 to the solvent leads to the destabilization of both the helix and the SH3 domain. The original structure, by hiding the hydrophobic residues from the solvent, leads to a stabilization of the H0/SH3 complex. When the helix is rotated by 90°, both the helix and the SH3 domain become disordered. This is especially true when the helix is rotated by 180° and the hydrophobic residues are fully exposed to the solvent. The H0/SH3 complex is colored according to residue type, where hydrophobic residues are white, polar residues are green, acidic residues are red, and basic residues are blue.

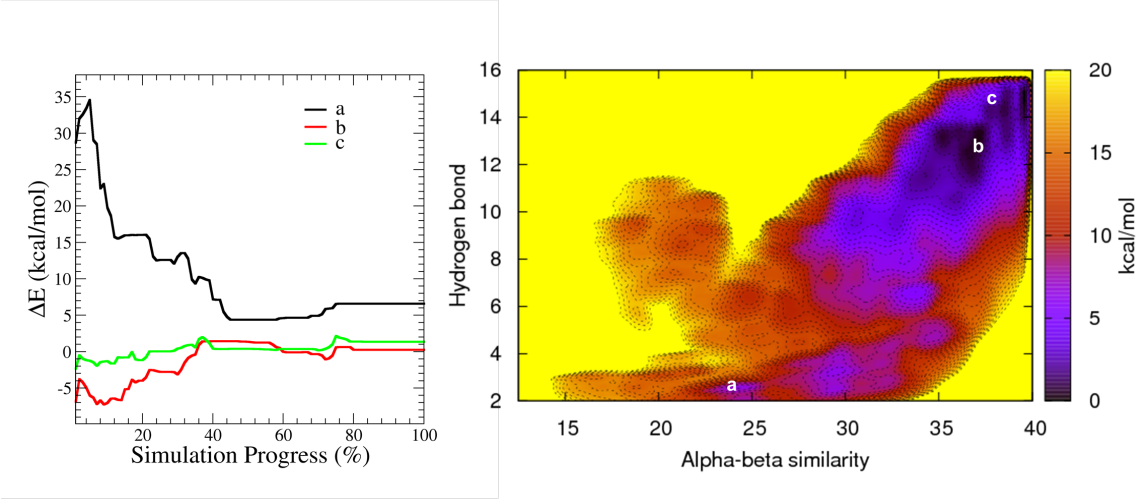

Fig. S3: The convergence of the metadynamics simulation was measured by calculating the energy difference between the global minimum and the important local minima (a, b, and c). This energy difference was plotted as a function of simulation completeness. For approximately the last 20% of the simulation, the well depths of the free energy minima remained constant, showing that the metadynamics are converged.