Supporting Information

Lou and Cheng 10.1073/pnas.0801965105

SI Methods

Methods. Four GPIbαN:A1 complexes were used as initial structures, which differ by their β-switch: (*i*) WT β-switch as β-hairpin (PDB code 1SQ0), (*ii* and *iii*) M239V and A238V β-switches as β-hairpin (modeled from 1SQ0), and (*iv*) WT β-switch in loop conformation (modeled from 1SQ0 and 1QQY). Each structure was put in a 100 × 96 × 72-Å water box with one Na^+ and four Cl^- ions to neutralize the system, which contains ≈98,000 atoms. Systems *i, ii,* and *iii* were subjected to energy minimizations with heavy atoms fixed for 10,000 steps and another 10,000 steps with all atoms free. The energy minimization process for system *iv* was concurrent with a gradual release of the constraints holding GPIbαN and A1 near the β-switch loop. The energy-minimized structures were heated gradually from 0 to 300 K in 100 ps and then equilibrated for 5–10 ns with pressure and temperature control. The temperature was held at 300 K by using Langevin dynamics, and the pressure was held at 1 atm by the Langevin piston method. The equilibrated structure was taken as the starting point for further simulations.

Free MD simulations were performed for 10–40 ns. Two additional simulations were carried out on system *i*. In the first simulation, the β-hairpin in the equilibrated structure was first disrupted while keeping its interaction with the A1 central β-sheet intact. This was done by applying a 2-nN force for 50 ps to the C_α atoms of GPIbαN residues 230–233 in the plane of the β-hairpin but perpendicular to the long axis of the β-hairpin away from A1. The C_α atoms of GPIbαN residues 235–240 were harmonically constrained to prevent their dissociation from A1. The system was then subjected to free dynamics to determine whether the β-hairpin would reform. The second simulation was similar to the first except that the 2-nN force was applied for 100 ps, and the harmonic constraints were applied to the C_α atoms of GPIbαN residues 239–241 plus A1 residues 560–567. This resulted in disruption of 2 of the 4 stable H-bonds that connect the β-switch to the A1. The system was then subjected to free dynamics. The simulations are summarized in Table S1.

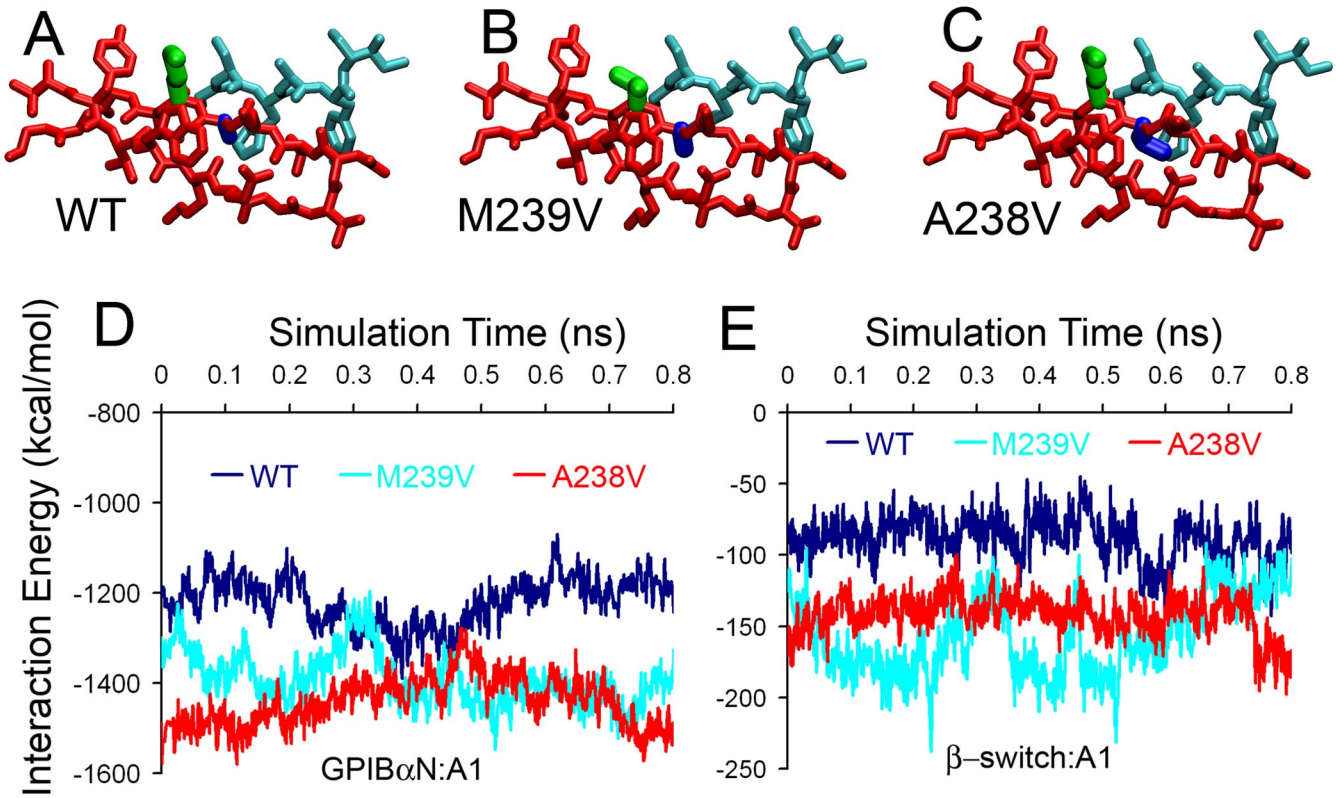


Fig. S1. Structures and interaction energies of WT and mutant GPIbαN:A1 complexes. (*A*–*C*) Zoom-in structures of the VWF-A1 central β-sheet (cyan, only the sequence that contacts the β-switch is shown) complexing with WT (*A*), M239V (*B*), or A238V (*C*) β-switch (red except residue 239, which is green, and residue 238, which is blue). WT is a crystal structure (taken from PDB code 1SQ0). M239V and A238V are structures modeled from 1SQ0. The calculated energies of interaction between A1 and the whole GPIbαN (*D*) or the β-switch alone (*E*) with a WT (blue curves), M239V (cyan curves), or A238V (red curves) sequence are plotted vs. simulation time.

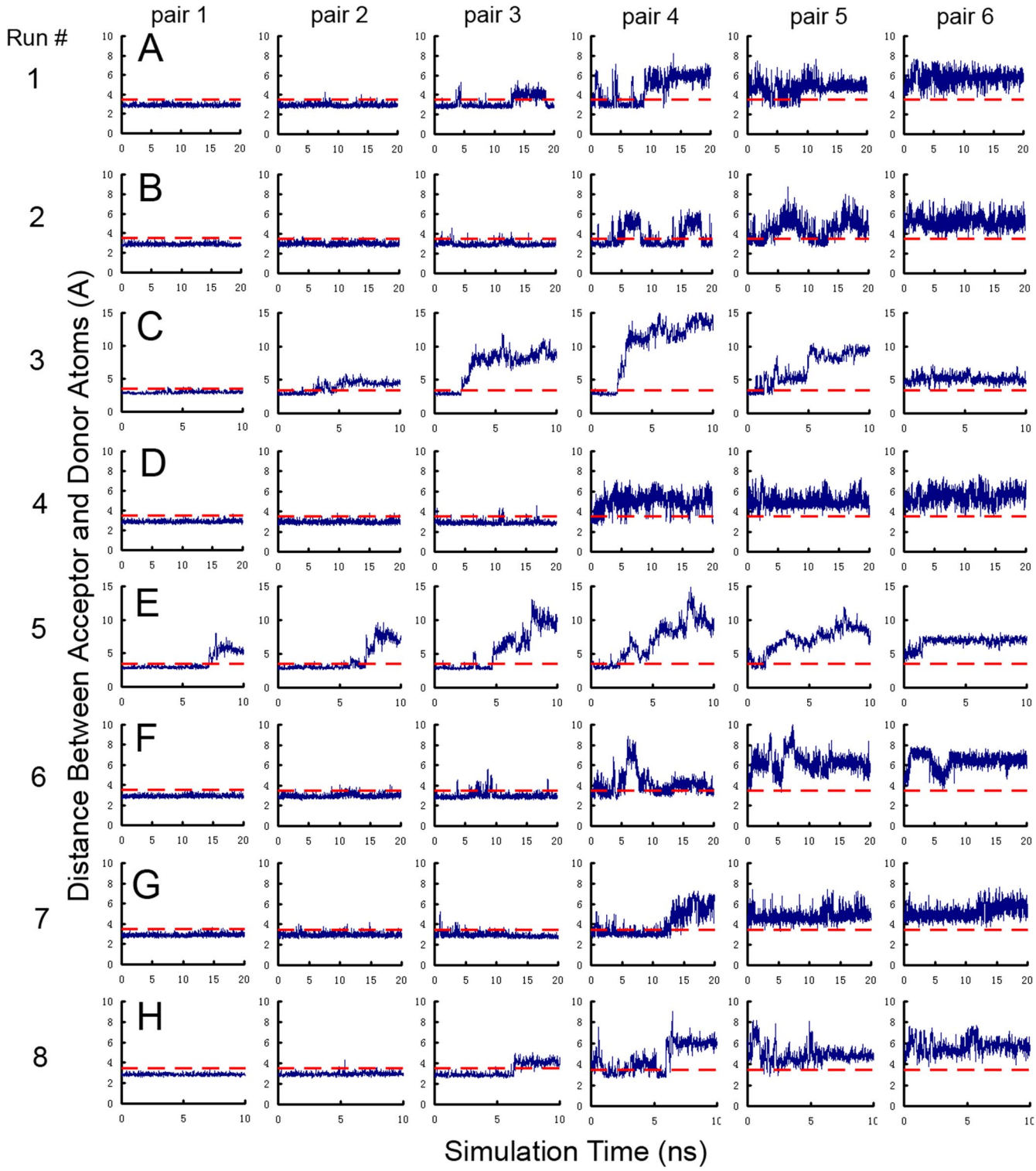


Fig. S2. Flow-induced loop-to-β-hairpin transition of isolated β-switch. The distance between each of the six H-bond donor-acceptor pairs (indicated at the top of each column) is plotted vs. simulation time for eight independent runs (indicated at the left of each row). The numbering convention for the six H-bonds is defined in Fig. 1*D* of the main article. Each simulation began (0 ns) with an equilibrated structures prepared independently. Runs 3, 5, and 8 are 10-ns simulations. The other five runs are 20-ns simulations. The red dashed lines indicate the 3.5-Å cutoff for H-bond.

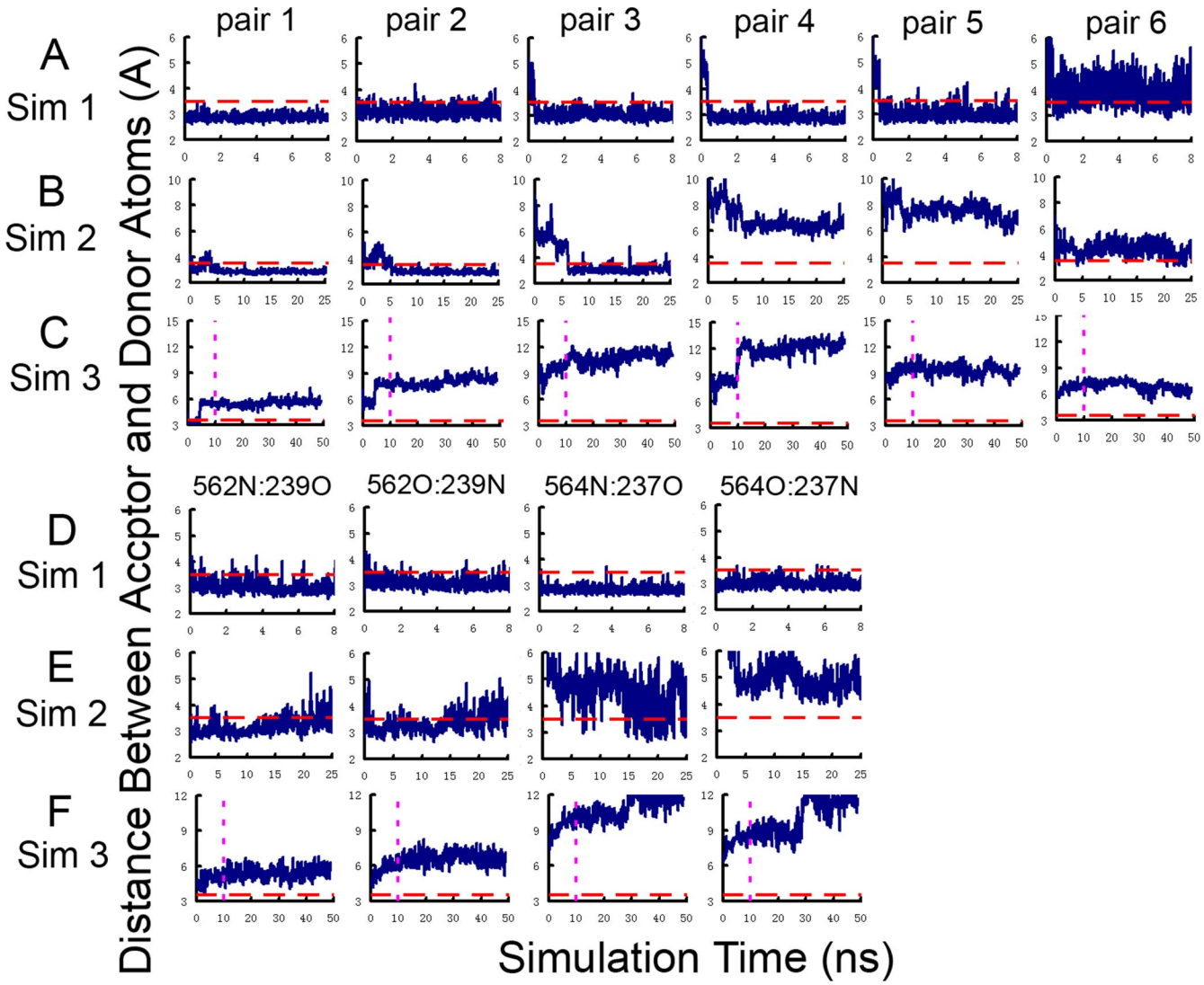


Fig. S3. Test of binding-induced fit hypothesis. The distance between each of the six donor-acceptor pairs (indicated at the top of each column for rows A–C) of H-bonds that connect the two legs of the β-switch or between each of the four donor-acceptor pairs (indicated at the top of each column for rows D–F) of H-bonds that connect the β-switch to A1 is plotted vs. simulation time for 3 simulations. (*A* and *D*) Five of the six H-bonds holding the β-hairpin together in the GPIbαN:A1 cocrystal structure were disrupted while keeping its interaction with the A1 central β-sheet intact; the system was then subjected to free dynamics for 8 ns. (*B* and *E)* A force was applied to disrupt the H-bonds between the two legs of the β-hairpin in the GPIbαN:A1 cocrystal structure as before but keeping its interaction with the A1 only partially intact; the system was then subjected to free dynamics for 25 ns. (*C* and *F*) The β-hairpin structure in the GPIbαN:A1 cocrystal was replaced by the loop structure of the isolated GPIbαN crystal. The system was equilibrated for 10 ns (dotted vertical pink lines in rows *C* and *F*) and then subjected to free dynamics for 40 ns. The red dashed lines indicate the 3.5-Å cutoff for H-bond.

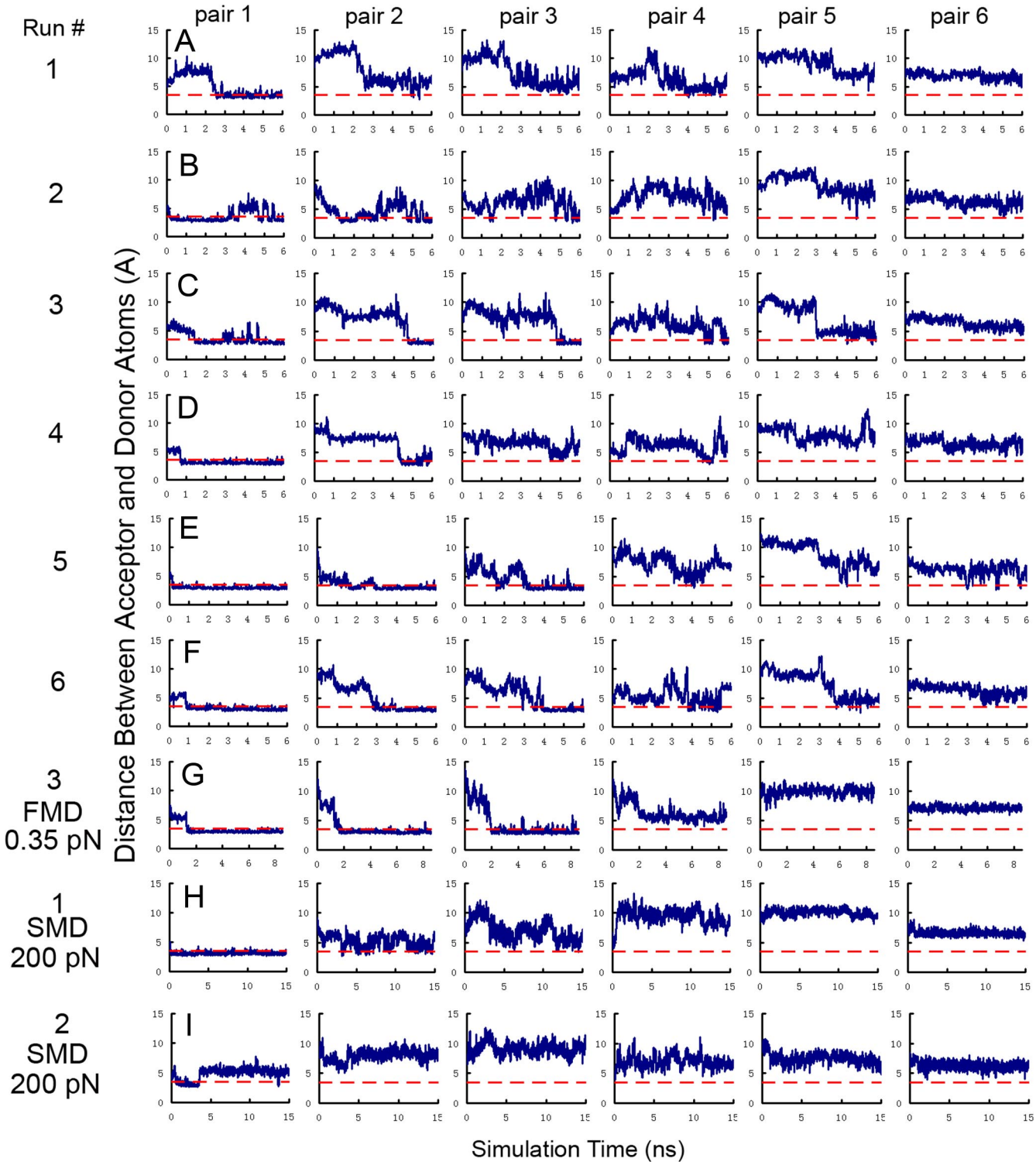


Fig. S4. Flow-induced loop-to-β-hairpin transition of WT β-switch. The distance between each of the six H-bond donor-acceptor pairs (indicated at the top of each column) is plotted vs. simulation time for six FMD runs with independently prepared initial structures and a 0.7 pN force applied to each of ~300 atoms in a 2-Å water layer (see Fig. S7*A*), an FMD run that used half of that force (0.35 pN with the same initial structure as run 3) and two SMD runs that applied 200-pN force to two C_α atoms at the far end of the β-switch (with the same initial structures as runs 1 and 2, respectively) (indicated on left of each row). The red dashed lines indicate the 3.5-Å cutoff for H-bond.

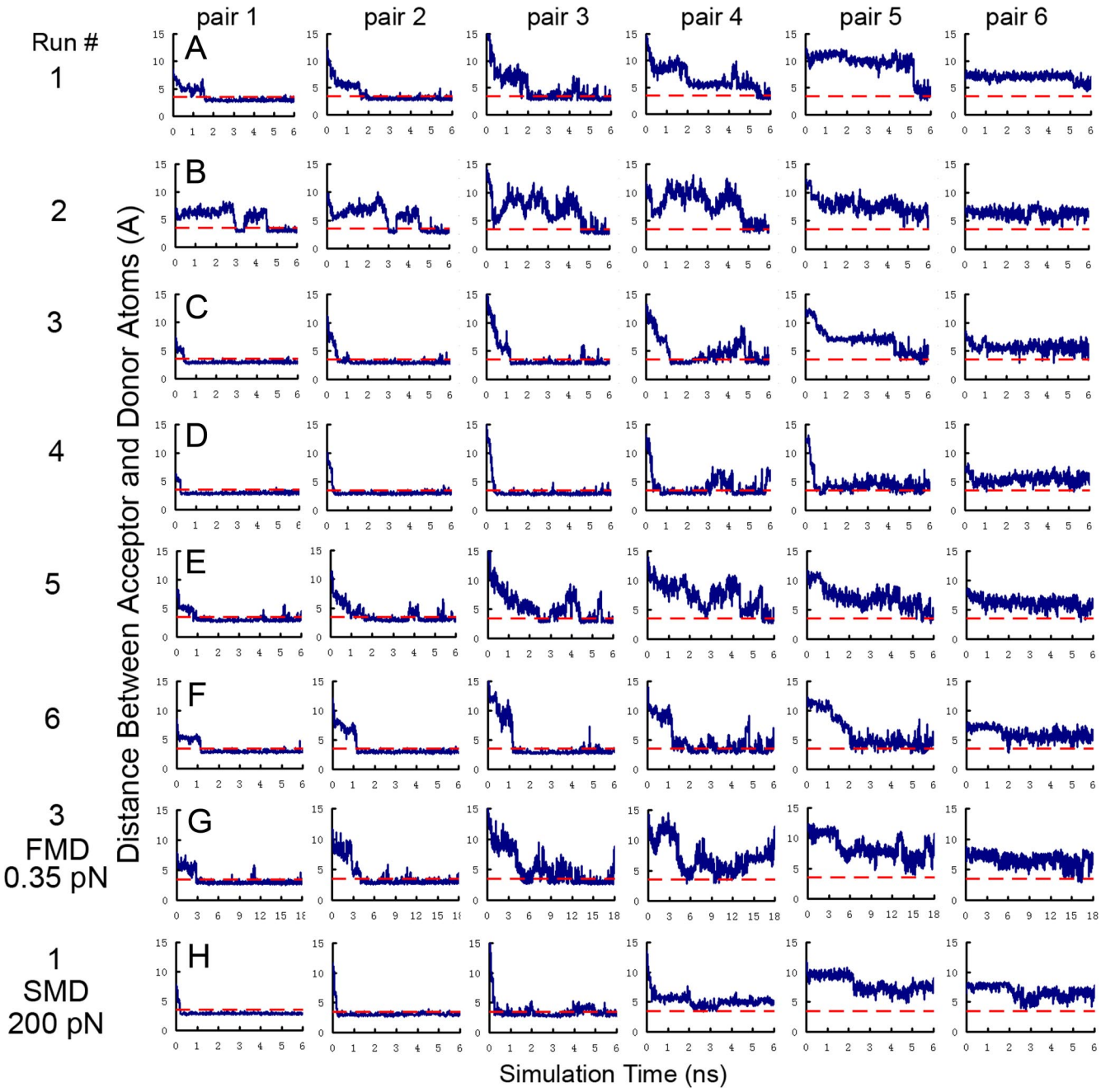


Fig. S5. Flow-induced loop-to-β-hairpin transition of M239V β-switch. The distance between each of the six H-bond donor-acceptor pairs (indicated at the top of each column) is plotted vs. simulation time for six FMD runs with independently prepared initial structures and a 0.7-pN force applied to each of ~300 atoms in a 2-Å water layer (see Fig. S7*A*), an FMD run that used half of that force (0.35 pN with the same initial structure as run 3), and an SMD run that applied 200-pN force to two C_α atoms at the far end of the β-switch (with the same initial structure as run 1) (indicated on left of each row). The red dashed lines indicate the 3.5-Å cutoff for H-bond.

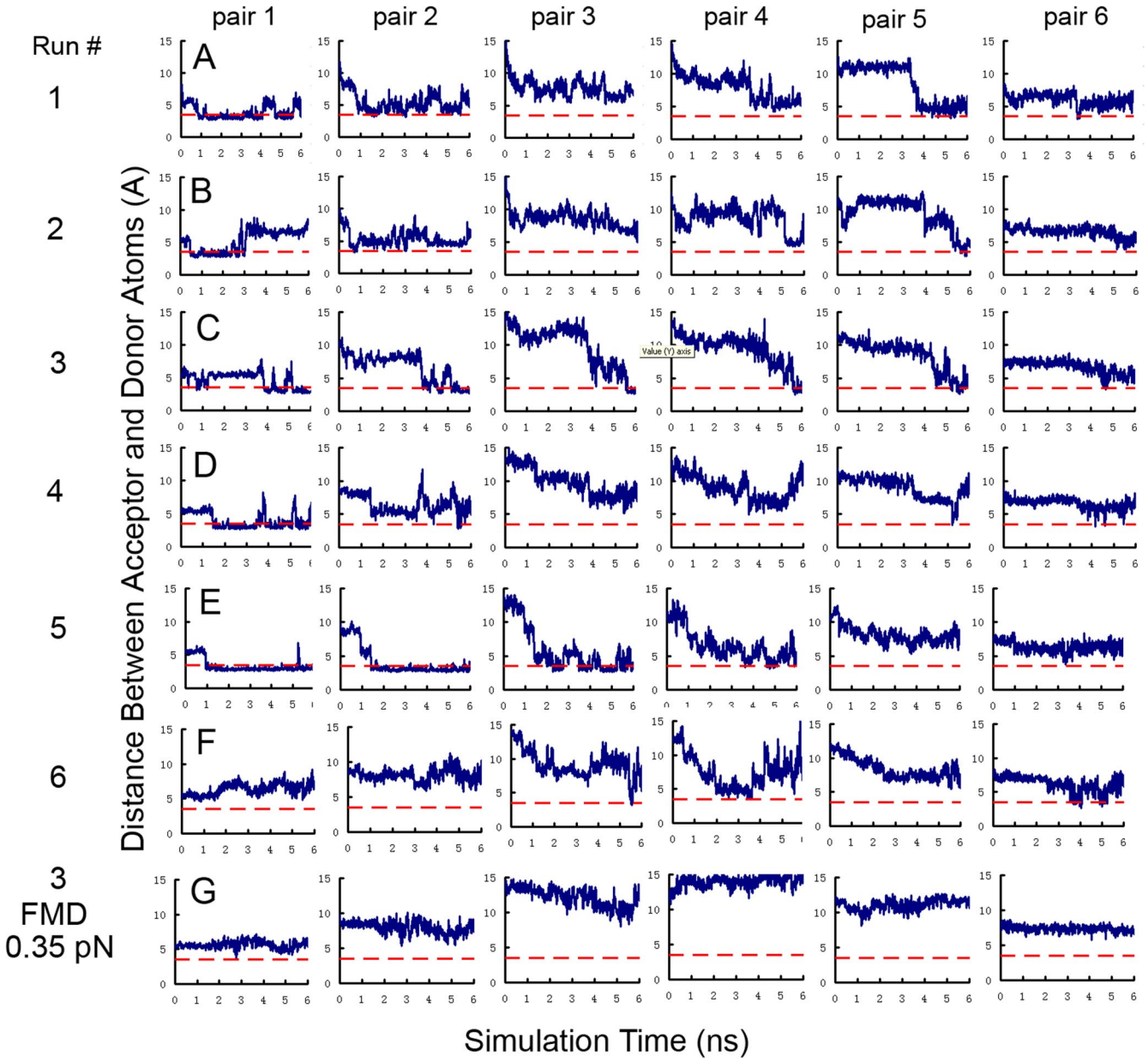


Fig. S6. Flow-induced loop-to-β-hairpin transition of A238V β-switch. The distance between each of the six H-bond donor-acceptor atom pairs (indicated at the top of each column) is plotted vs. simulation time for six FMD runs with independently prepared initial structures and a 0.7-pN force applied to each of ~300 atoms in a 2-Å water layer (see Fig. S7*A*) and an FMD run that used half of that force (0.35 pN with the same initial structure as run 3) (indicated on left of each row). The red dashed lines indicate the 3.5-Å cutoff for H-bond.

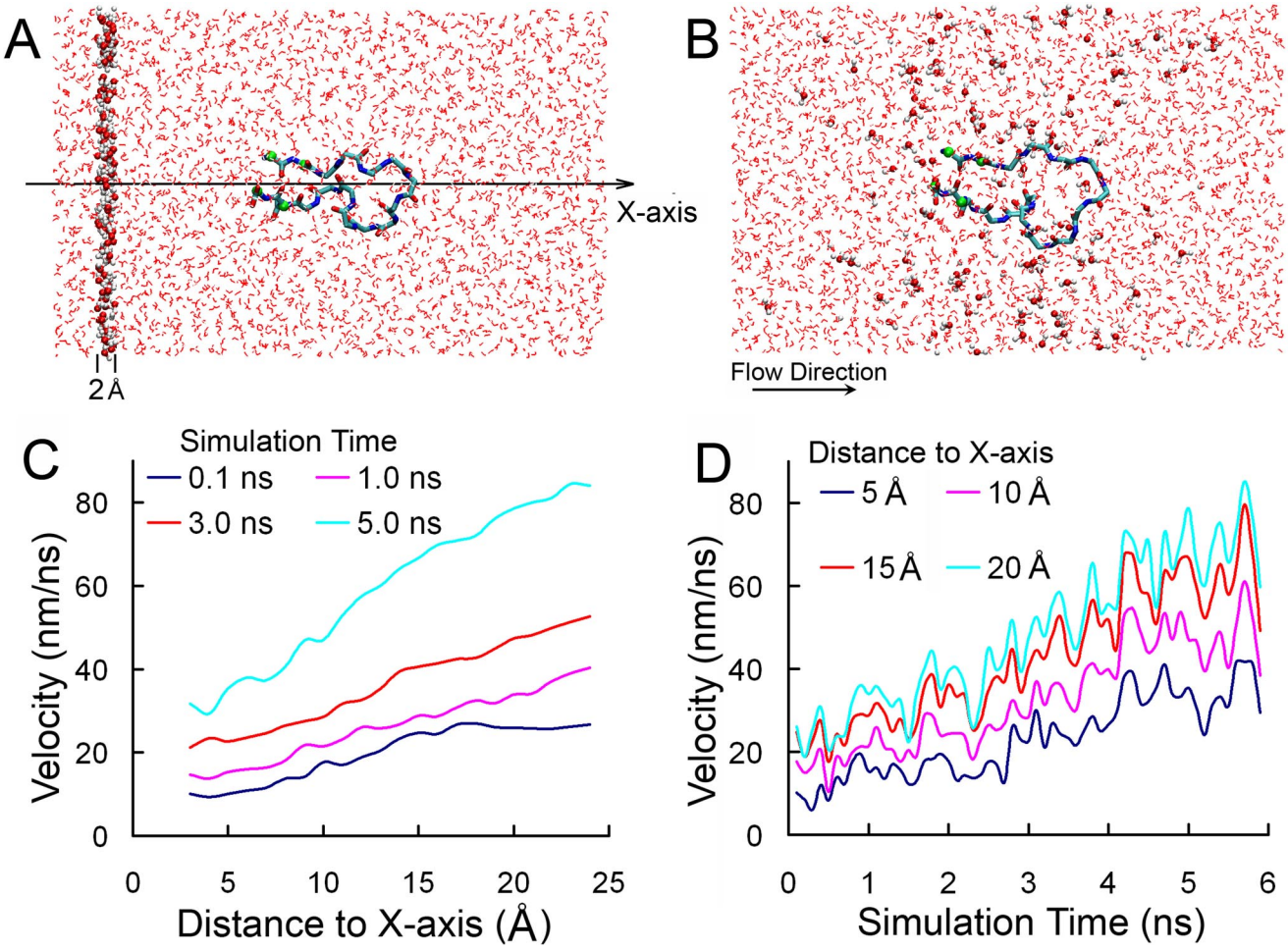
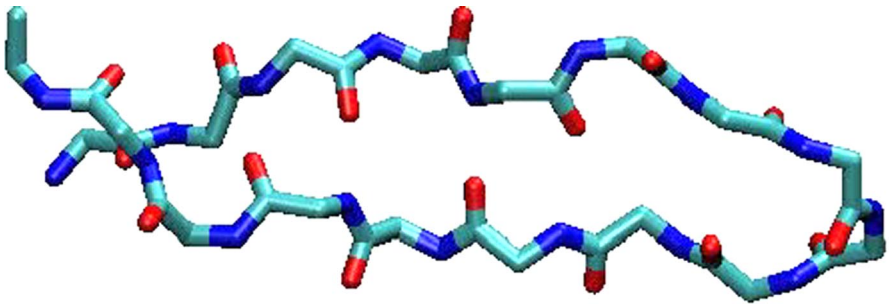
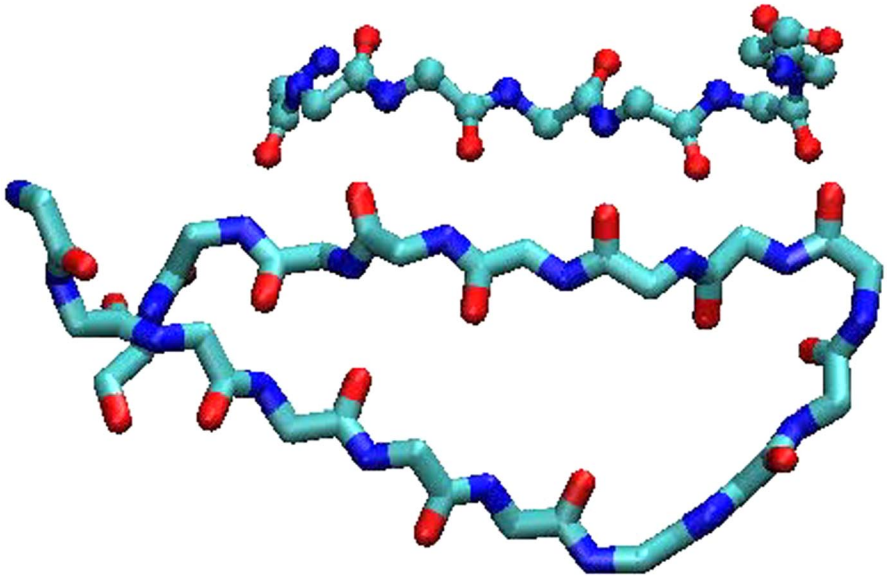


Fig. S7. FMD simulations setup. (*A*) The β-switch is soaked in the center of a water box where the *x*-axis is defined, which is the flow direction. Flow is generated by applying a 0.7 or 0.35 pN force along the *x*-direction to each of ~300 atoms in a 2-Å layer (indicated) of water molecules (large spheres) far upstream from the protein sequence. (*B*) A snapshot at 1 ns showing the locations of the water molecules (large spheres) originally from the 2-Å layer at 0 ns. (*C*) Velocity of the water molecules (averaged along *x*-direction) is plotted vs. the vertical distance from the *x*-axis at indicated time. (*D*) Velocity of the water molecules (averaged along *x*-direction) is plotted vs. simulation time for the indicated distances away from the *x*-axis.



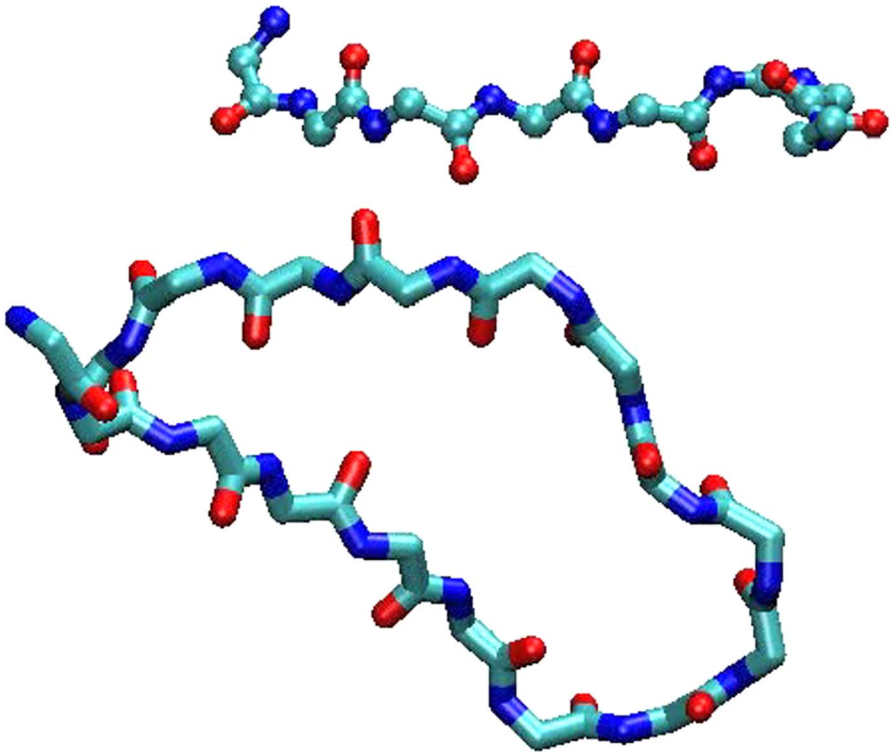
Movie S1. Free MD dynamics simulated β-hairpin-to-loop transition of an isolated β-switch in the absence of flow (run 5).

Movie S1 (MPG)



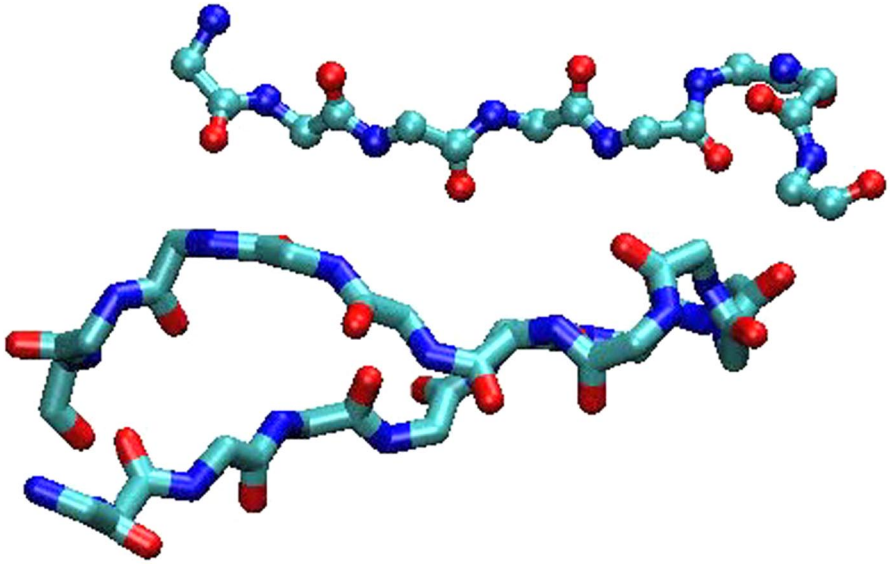
Movie S2. Free MD simulation of GPIbαN:A1 complex. The β-hairpin in the GPIbαN:A1 cocrystal structure was first disrupted by a force but its interaction with the A1 central β-sheet intact was kept intact.

Movie S2 (MPG)



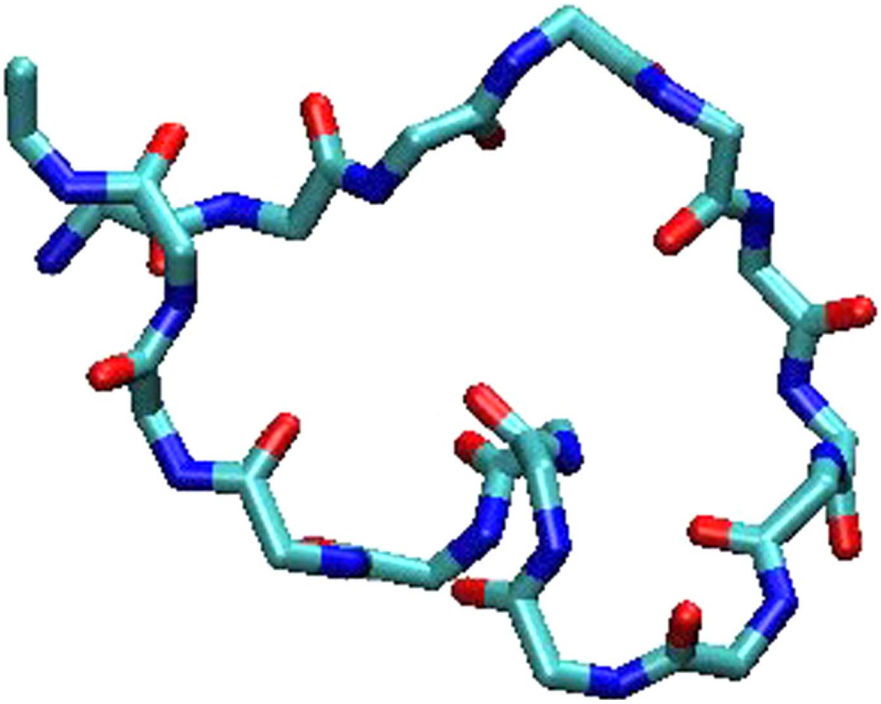
Movie S3. Free MD simulation of GPIbαN:A1 complex. The β-hairpin in the GPIbαN:A1 cocrystal structure was first disrupted by a force and two of the four stable H-bonds that connect the β-switch to the A1 central β-sheet were also disrupted.

Movie S3 (MPG)



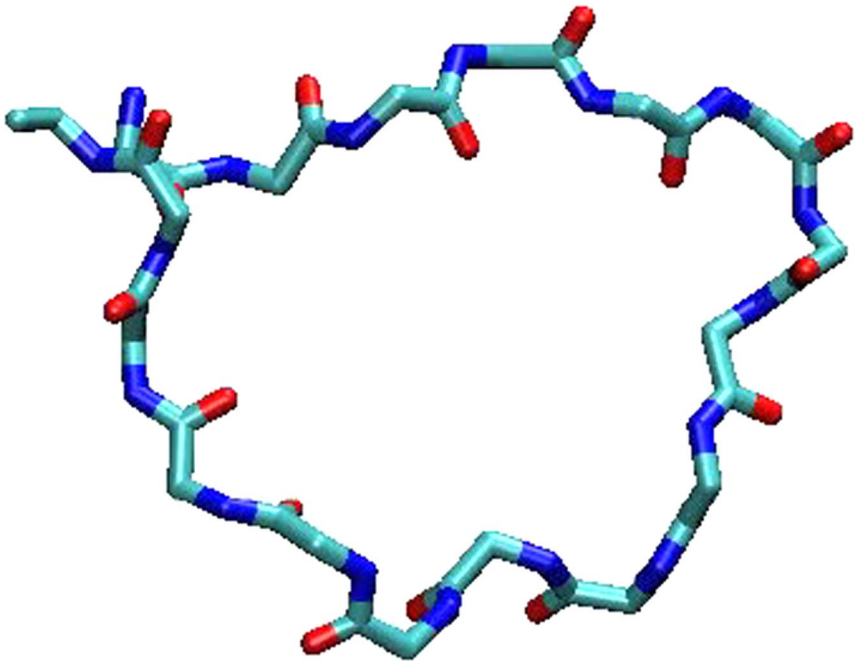
Movie S4. Free MD simulation of GPIbαN/A1 complex. The β-hairpin in the GPIbαN:A1 cocrystal was replaced by the loop of the isolated GPIbαN crystal.

Movie S4 (MPG)



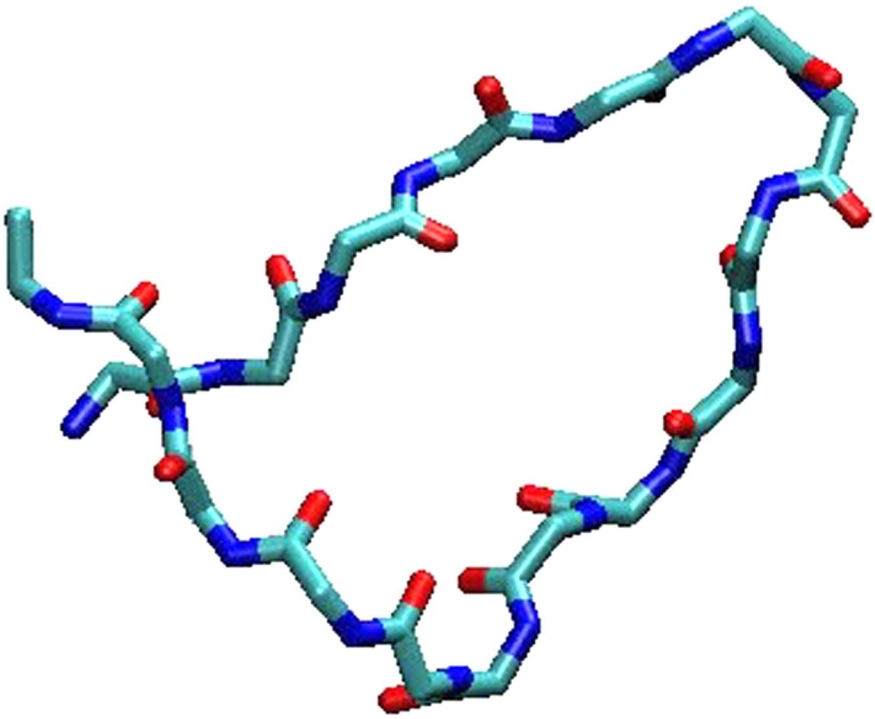
Movie S5. FMD simulated loop-to-β-hairpin transition of an isolated wild-type β-switch (Run 6).

Movie S5 (MPG)



Movie S6. FMD simulated loop-to-β-hairpin transition of an isolated gain-of-function mutant (M239V) β-switch (Run 3).

Movie S6 (MPG)



Movie S7. FMD simulated loop-to-β-hairpin transition of an isolated loss-of-function mutant (A238V) β-switch (Run 3).

Movie S7 (MPG)

Table S1. Summary of Simulations

System	Type of simulation	No. of runs	Simulation time for each run, ns
β-switch WT (hairpin)	Free dynamics	5	20
	Free dynamics	3	10
β-switch WT (loop)	FMD, $f = 0.7$ pN	6	6
	FMD, $f = 0.35$ pN	1	8.2
	SMD, $F = 200$ pN	2	15
β-switch M239V (loop)	FMD, $f = 0.7$ pN	6	6
	FMD, $f = 0.35$ pN	1	18
	SMD, $F = 200$ pN	1	6
β-switch A238V (loop)	FMD, $f = 0.7$ pN	6	6
	FMD, $f = 0.35$ pN	1	6
GPIbαN:A1 WT	Free dynamics	1	20
	Free dynamics (β-hairpin disrupted, interaction with A1 intact)	1	20
	Free dynamics (β-hairpin disrupted, interaction with A1 partially disrupted)	1	25
	Free dynamics (β-hairpin replaced by loop)	1	40
GPIbαN:A1 M239V	Free dynamics	1	10
GPIbαN:A1 A238V	Free dynamics	1	10