Supplementary Information: Effects of a disulfide bridge prior to amyloid formation of the ABRI peptide

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1 Supplementary Text

Bias Exchange Metadynamics for initial contacting Cys residues. To obtain initial configurations where residues 5Cys and 22Cys were in contact, we performed a short all-atom molecular dynamics simulation with bias exchange metadynamics (BEM)[1]. MD parameters, such as temperature and time step, were the same as those described in Methods (see Main Text). We used only four collective variables (CV). The first CV counted the number of contacting C_α residues within the protein, with reference distance of 6.5Å. The second CV counted the number of hydrophobic residues that are in contact, with reference distance of 5Å between C_γ atoms. The last two CVs counted the number of residues in secondary structure elements α and β, by considering the Ψ dihedral angle relative to a reference angle Ψ_0, with $\Psi_0 = -45^\circ$ for α and $\Psi_0 = 130^\circ$ for β. This CV setup is defined thoroughly in Ref.[2] (as coordination number and dihedral distance) and was used previously in Ref.[3]. The biasing Gaussians were deposited every steps of 10 ps, with height of 1.5 kJ and width equal to the standard deviation of the CV's value in an unbiased MD run. Biasing potentials were exchanged every 40ps. The simulation was halted after 30ns per replica. A configuration with contacting 5Cys-22Cys was selected randomly from the conformational sample, and was the starting configuration of our two main BEM studies (see Main Text).

Convergent cluster free energies. In Supplementary Figure 1, we present the free energy of each cluster defined in a 9-dimensional CV space (see Methods for details), estimated with a weighted-histogram approach[3,4] at two different filling times: 20 and 35 ns, for the simulation with the SS-bond. We find that the data are quite correlated for the two different filling times. This correlation gives evidence of the convergence of the clustering and free energy calculations. Analogous results are found for the simulation without the disulfide bond.

Comparison of Ramachandran plot distributions for oxidized and reduced ABRI. The two-dimensional (ϕ,Ψ) dihedral angle probability distribution (Ramachandran plot) for oxidized and reduced ABRI are presented in Main Text Figure 3. To compare these two distributions, with a simple statistical measure, we calculated the Z-score with respect to the mean difference per dihedral angle pair. This measure indicates, in units of standard deviations, how far away is the difference in probability values at a given point (ϕ,Ψ) from the mean difference calculated over the full set. In Supplementary Figure 2 (top), the Z-scores for each dihedral angle pair are shown. For this data, p-values were calculated with the Wilcoxon rank-sum test, and we controlled for multiple comparisons using Bonferroni correction. P-values are shown in Supplementary Figure 2 (bottom), countour lines define a threshold at 5% (two-tail) significance. For the statistics, points were re-sampled within a 5° radius. Distinct features are found between the preferential α-helical state of oxidized ABRI $(-65^\circ,-45^\circ)$, compared to the favored β character $(-70^\circ,130^\circ)$ in the reduced form.

Local stability of the lowest free energy structure of oxidized ABRI. We studied the stability in the thermodynamic basin of the α-helical folded state found for oxidized ABRI with and without the SS-bond. We performed several unbiased MD simulations (no BEM) starting from the most probable conformation of oxidized ABRI (left column Main Text Figure 2). All MD parameters are the same as those described in the Methods section of Main Text. Three simulations starting with random velocities were ran both with and without the SS-bond. The total simulation time for each was 20ns. The root-mean-square-deviation (RMSD) from the initial configuration is shown in Supplementary Figure 3A as a function of time. If the disulfide bond is broken, we find that after $\sim$ 8ns the configurations have a larger RMSD ($\sim 4.5\dot{A}$, see red lines Suppl. Figure 3A) than the conformations in the simulation with the SS-bond, indicating that the most probable state of oxidized ABRI is less stable without the SS-bond. The probability distributions, after 20ns, of the RMSD for both peptide forms are shown in Supplementary Figure 3B. Structures in the simulation without the SS-bond differ more from the initial configuration. Overall, these results suggest that in the local thermodynamic basin, the lowest free energy conformation of oxidized ABRI is less stable if no SS-bond is present. One can speculate, that the breakage of the bond could generate and α-to-β transition from the most probable conformation of oxidized ABRI to that in the reduced form. However, with the current computational resources, this can only be obtained with sophisticated enhanced sampling calculations and not just simple MD simulations.

Comparison with Circular Dichroism (CD) experiments. We used the DichroCalc[5] program for calculating the theoretical CD spectra from the most probable ABRI configurations. We compared the results to several structural classes of α-helical (PDB code: 1W5I) , β-I (PDB code:1K43) and random-coil (PDB codes: 3WOF and 2M48) protein structures available in the Protein Data Bank (PDB). Theoretical CD spectra calculated for these structural classes, and for oxidized and reduced ABRI are shown in Supplementary Figure 4. Average CD profiles with error-bars are shown for structures having multiple models, such as random-coil PDBs from NMR and the most probable cluster obtained from simulations. From Suppl. Figure 4 we find that both ABRI forms present similar theoretical CD spectra as those of random-coil proteins, and differ significantly from those of α-helical or β proteins.

We compared these results to the available experimental CD data obtained under similar conditions as those of the simulations: low concentration (<10μM), short incubation times ($\sim$min) and close to neutral pH (6-8). Not many experimental studies are available in these conditions. Due to a high aggregation state of the dry ABRI peptide before dissolution, the purified ABRI peptide has limited solubility in aqueous solution at neutral pH. Nonetheless, CD spectra of oxidized ABRI at 5μM concentration, pH 6.9 and 7.8, after 5min of incubation time are presented in Ref.[6]. Both experimental spectra show a sharp minimum around 198nm indicating a random-coil conformation (see black and red points Suppl. Figure 4). According to Refs.[7,8] the theoretical parameters for calculating the CD spectra of random-coil proteins are not yet completely optimized and the sign of the peak is wrong (see Suppl. Figure 4, similar to Figure 4 in Ref.[7]), and thus we are limited to only qualitative comparison with theoretical and experimental CD results elucidating a predominantly unordered structure to ABRI under the conditions of the MD simulations.

References

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2 Supplementary Figures

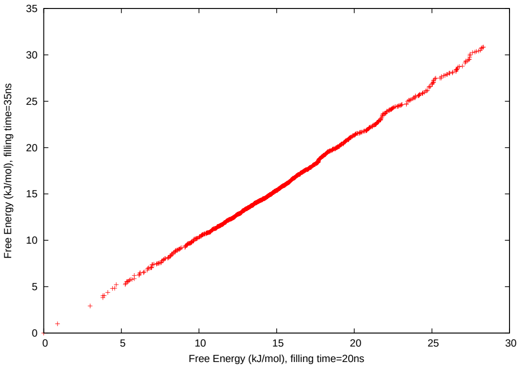

Figure 1 Convergent cluster free energies. Free energy of clusters in a 9 dimensional collective variable space estimated by a weighted-histogram approach[3,4] for two different filling times: 20 and 35 ns. Results are shown for the simulation with the SS-bond. Correlated free energies indicate convergent results.

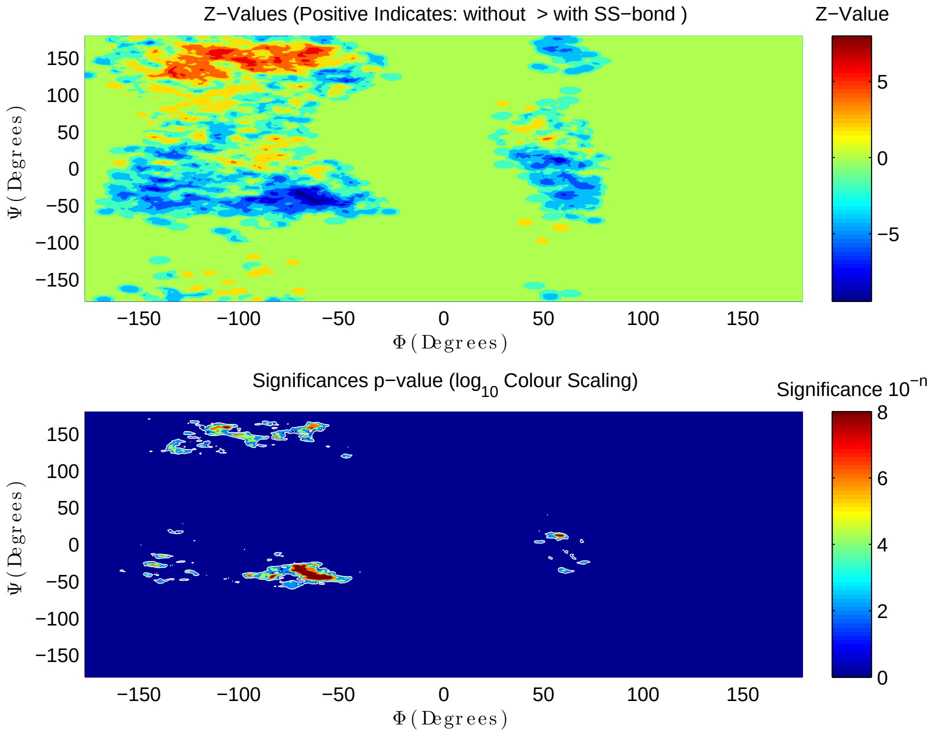

Figure 2 Comparison of Ramachandran plot distributions for oxidized and reduced ABRI. (Top) Z-score of the difference in the Ramachandran plots presented in Main Text Figure 3 with respect to the mean difference for each specific dihedral angle (ϕ,Ψ) pair. Values indicate the number of standard deviations from which the difference in distributions differs from the mean, larger |Z-score| suggests a greater difference between the two distributions. **(Bottom)** P-values for data calculated with the Wilcoxon rank-sum test, and controlled for multiple comparisons using Bonferroni correction. Countour lines define a threshold at 5% (two-tail) significance. For the statistics, points were re-sampled within a 5° radius.

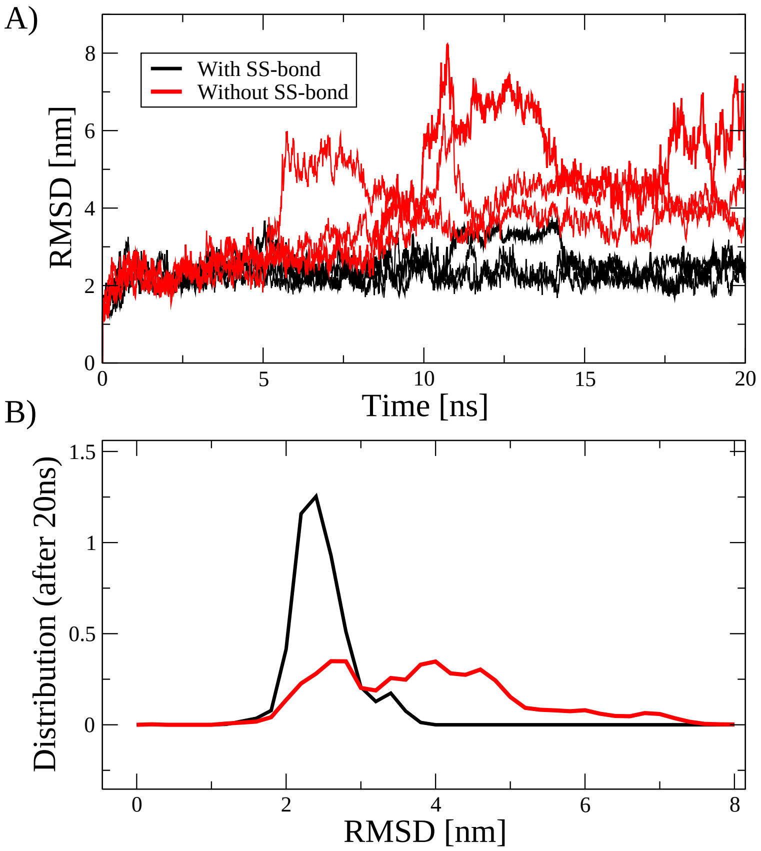

Figure 3 Local stability of the lowest free energy structure of oxidized ABRI. A) Root mean square deviation (RMSD) from the initial lowest free energy configuration of oxidized ABRI as a function of time for three unbiased MD simulations of ABRI with (black) and without (red) the disulfide bond between residues 5Cys and 22Cys. Trajectories were started with random velocities. B) RMSD distributions of the MD trajectories after 20ns for both disulfide forms. Lower RMSD indicates a more stable conformation with respect to the initial α-helical structure.

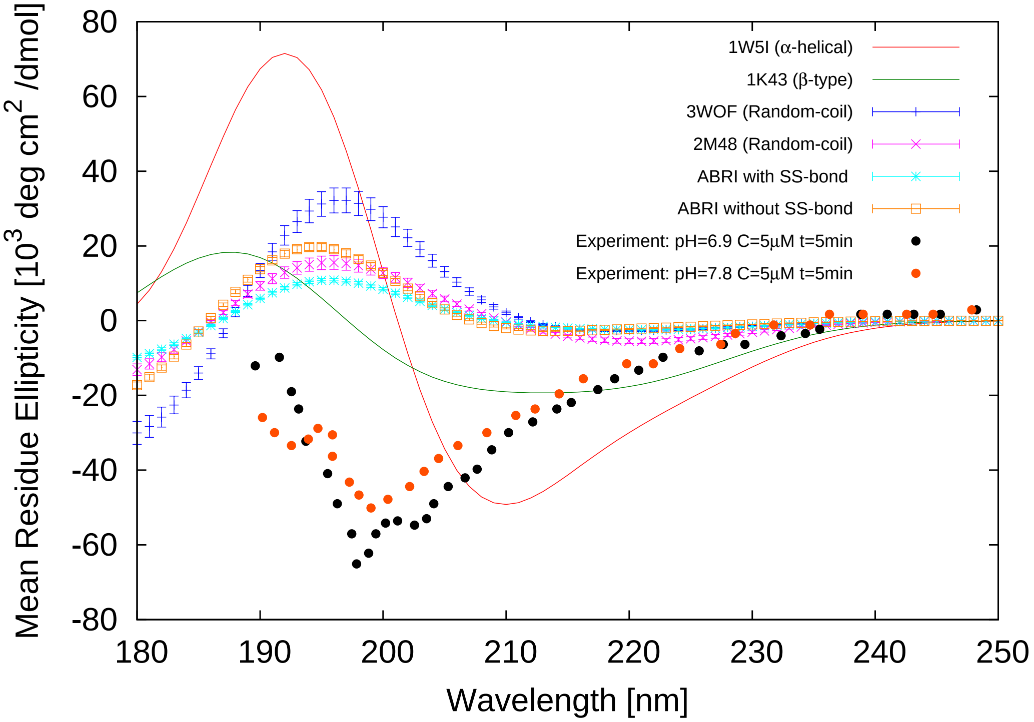

Figure 4 Comparison with Circular Dichroism (CD) experiments. Theoretical CD spectra calculated using the DichroCalc[5] program for several PDB structures belonging to different structural classes: α-helical (PDB code: 1W5I) , β-I (PDB code:1K43) and random-coil (PDB codes: 3WOF and 2M48). Shown is also the calculated CD spectra for oxidized (cyon) and reduced (orange) ABRI. For structures having multiple models, presented are average CD profiles with error-bars corresponding the standard deviation of the mean divided by the number of models. Experimental CD spectra of oxidized ABRI at 5μM concentration, pH 6.9 and 7.8, after 5min of incubation time are presented as black and red points, respectively.