SUPPLEMENTARY INFORMATION for

Molecular dynamics simulations of Human Serum Albumin and role of disulfide bonds

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This document includes additional details about the stability and conformational fluctuations of the disulfide bonds (SS-bonds) of Human Serum Albumin (HSA). SS-bonds were classified according to their conformation and effect on structure in the absence of an explicit linkage between sulfhydryl groups. SS-bonds are formed by two cysteine residues and HSA contains 17 of them. There is a free cysteine (Cys34) that has been observed to play a role in the formation of dimers and intermolecular linkages, but the mechanism and structural changes to allow surface accessibility were not clear.[1] As described in the main manuscript, changes in conformation of the phenolic ring in Tyr84 are highly responsible for the accessibility to the sulfhydryl group from the surface. All other Cysteine residues in HSA form SS-bonds and some of them influence the local secondary structure and their absence promotes unfolding.

Fluctuations in non-bonded SS-bonds

Figure SI.1 contains the distance between the sulfhydryl groups of SS-bonds in domain I for the simulation without SS-bonds, compared to the equilibrium distance they would have when they are bonded (2.03 Å). The bond formed by Cys53-Cys62 is the only bond that does not participate in double SS-bonds. This pair of Cysteine residues is moderately stable with stronger fluctuations between 20 and 40 ns, but it reaches shorter distances of ~5 Å as the simulation continues. The first double SS-bond formed by Cys75-Cys91 and Cys90-Cys101 is stable with small fluctuations for the most part of the MD simulation, although the Sγ distance in Cys75-Cys91 samples a slightly smaller distance than Cys90-Cys101. The second double SS-bond corresponds to Cys124-Cys169 and Cys168-Cys177. In contrast to the previously described SS-

bonds, this pair does not remain in a stable conformation that allows the SS-bond to form again. . The absence of this SS-bond is responsible for the extension of the coil region and partially unfolding of alpha-helices h3 and h4 in domain IB. B-factors also revealed their influence in dynamics as discussed in the main manuscript.

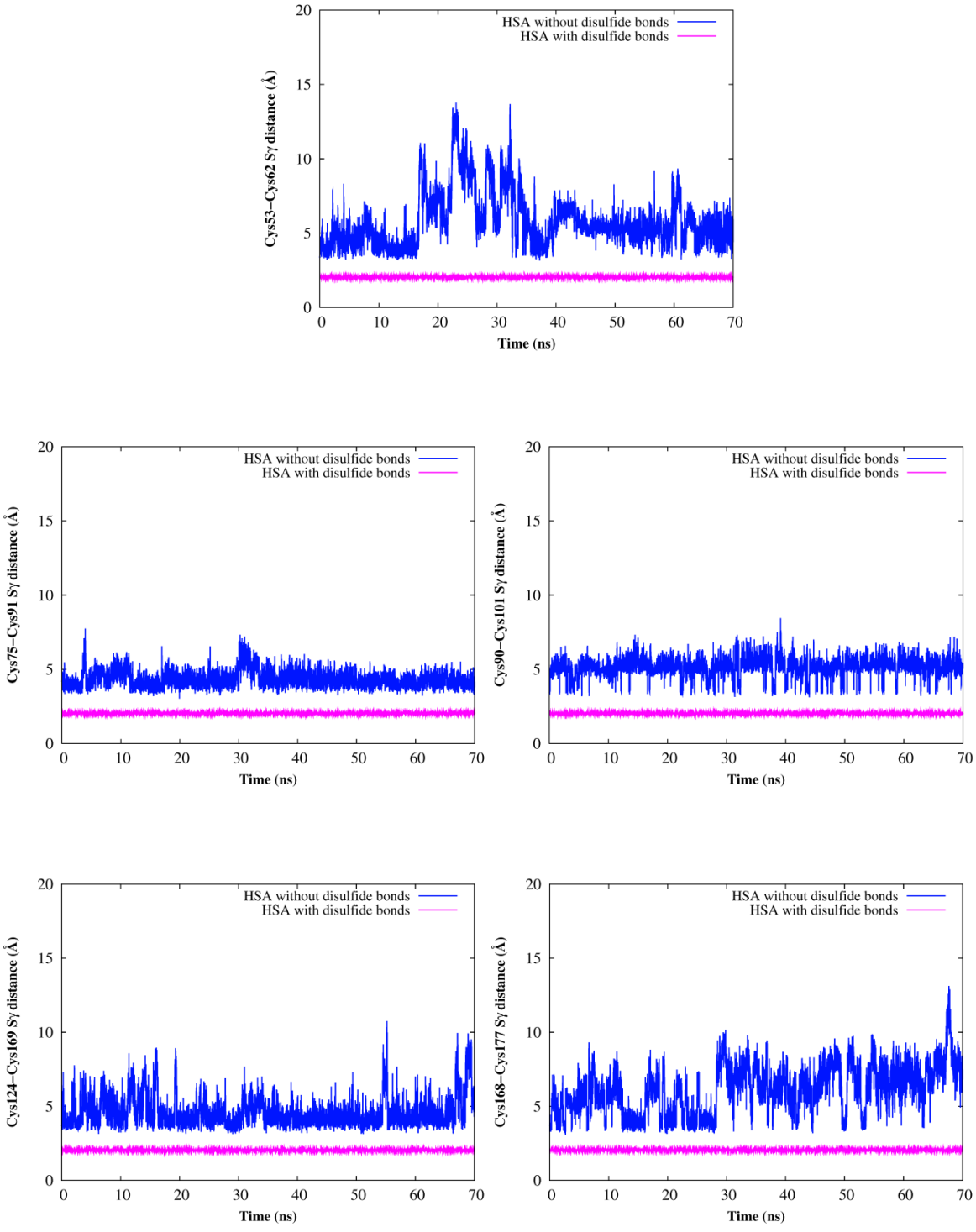

Figure SI.1. Distance between the sulfhydryl groups of SS-bonds in domain I for the simulation without SS-bonds (blue). For comparison purposes, the figure also includes the distance for the

bonded case (pink, 2.03 Å). In particular, the double SS-bond between Cys124-Cys169 and Cys168-Cys177 plays an important role in maintaining the secondary structure of alpha helices in the local neighborhood.

Similarly Figure SI.2 presents the distances between Sγ atoms in Cysteine groups for domain II including the four SS-bonds in the main binding site I. The third double SS-bond is formed by Cys200-Cys246 and Cys245-Cys253. Fluctuations in these residues are not correlated: the group Cys200-Cys246 is very stable during the entire simulation and does not affect the structure, whereas the group Cys245-Cys253 was observed to be responsible for the change in secondary structure of alpha-helices in site I, affecting the particular pocket-like structure. Besides having low B-factors, the region enclosed by Cys200 and Cys246 is rich in hydrogen bonds with high occupancies even in the absence of SS-bonds. The double bond between Cys265-Cys279 and Cys278-Cys289 has correlated motion although Cys278-Cys289 has a preferred distance that extends beyond 5 Å and shows more significant fluctuations. The double SS-bond formed by Cys316-Cys361 and Cys360-Cys369 has medium fluctuations. Remarkably, the SS-bond Cys360-Cys369 has two main preferred conformations at 3.7 and 5.0 Å of separation.

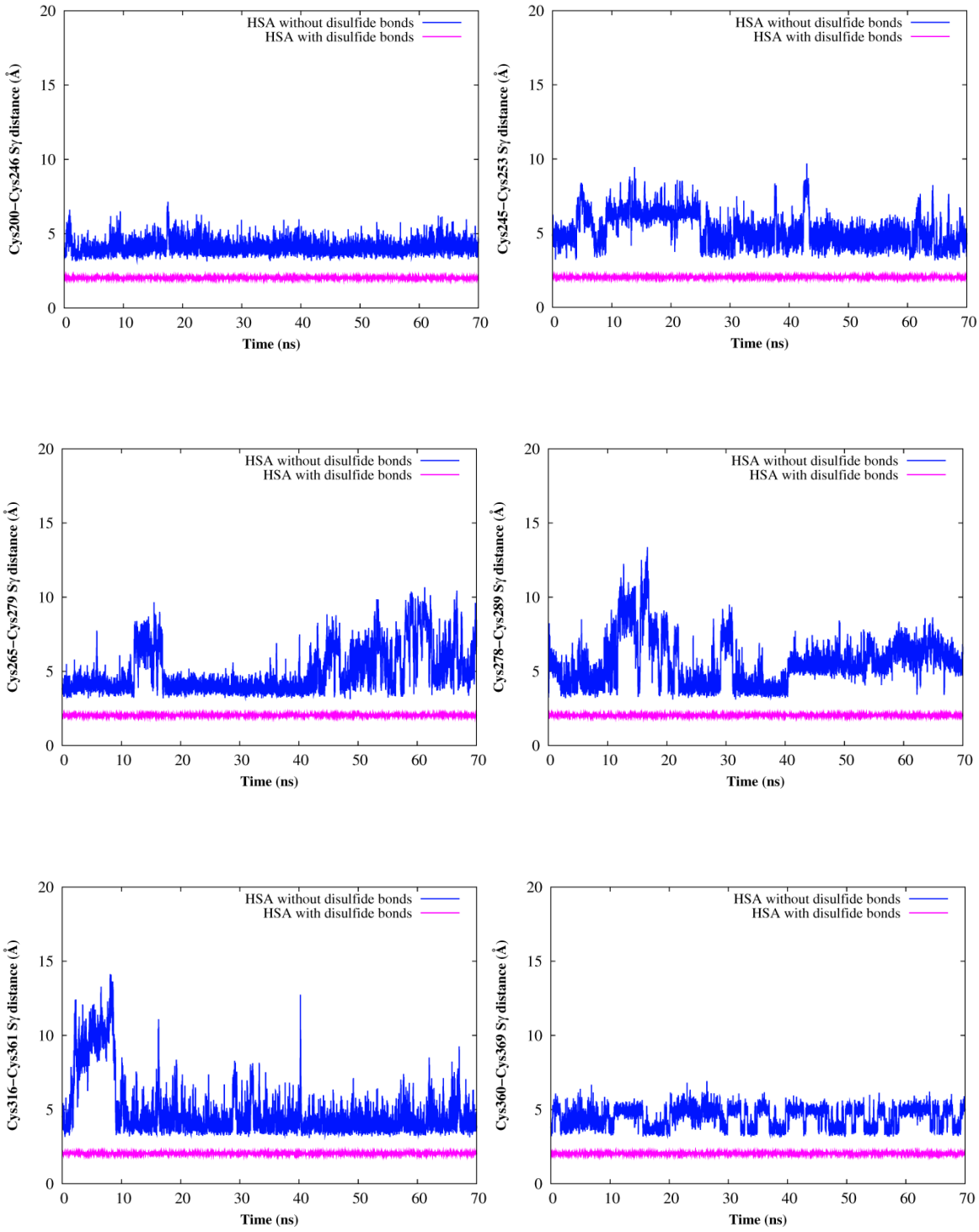

Figure SI.2. Distance between the sulfhydryl groups of SS-bonds in domain II for the simulation without SS-bonds (blue). For comparison purposes, the figure also includes the distance for the

bonded case (pink, 2.03 Å). Whereas most SS-bonds in binding site I are stable, the strong fluctuations produced by the absence of the bond between Cys245-Cys253 promotes unfolding and disrupt the particular structure of binding site I. Stable conformations are observed in non-bonded disulfide groups that prevent the propagation of coils.

Finally, conformations adopted by SS-bonds in domain III are presented in Figure SI.3. In general, these SS-bonds show changes in conformation that go beyond 5 Å and in some cases with no correlated motion. The SS-bond between Cys392-Cys477 shows a preferred conformation that is maintained at distances above 5 Å. A distance of ~3 to 4 Å or less is required for the formation of a SS-bond,[2,3,4] suggesting that once this SS-bond is broken, there is a high probability this bond does not form again, if it reaches the preferred distance of ~6 Å. Similar fluctuations are observed in its pair Cys437-Cys448. Cys461-Cys477 and Cys476-Cys487 are fairly stable during the simulation with minor fluctuations. Pronounced fluctuations are observed for the pair Cys514-Cys559 and Cys558-Cys567. Cys514-Cys559 shows two preferred conformational states, and Cys558-Cys567 shows stronger fluctuations with distances that go beyond to 10 Å. These results suggest that the new sampled conformation does not allow sulfhydryl groups to return to a closer distance and form a SS-bond.

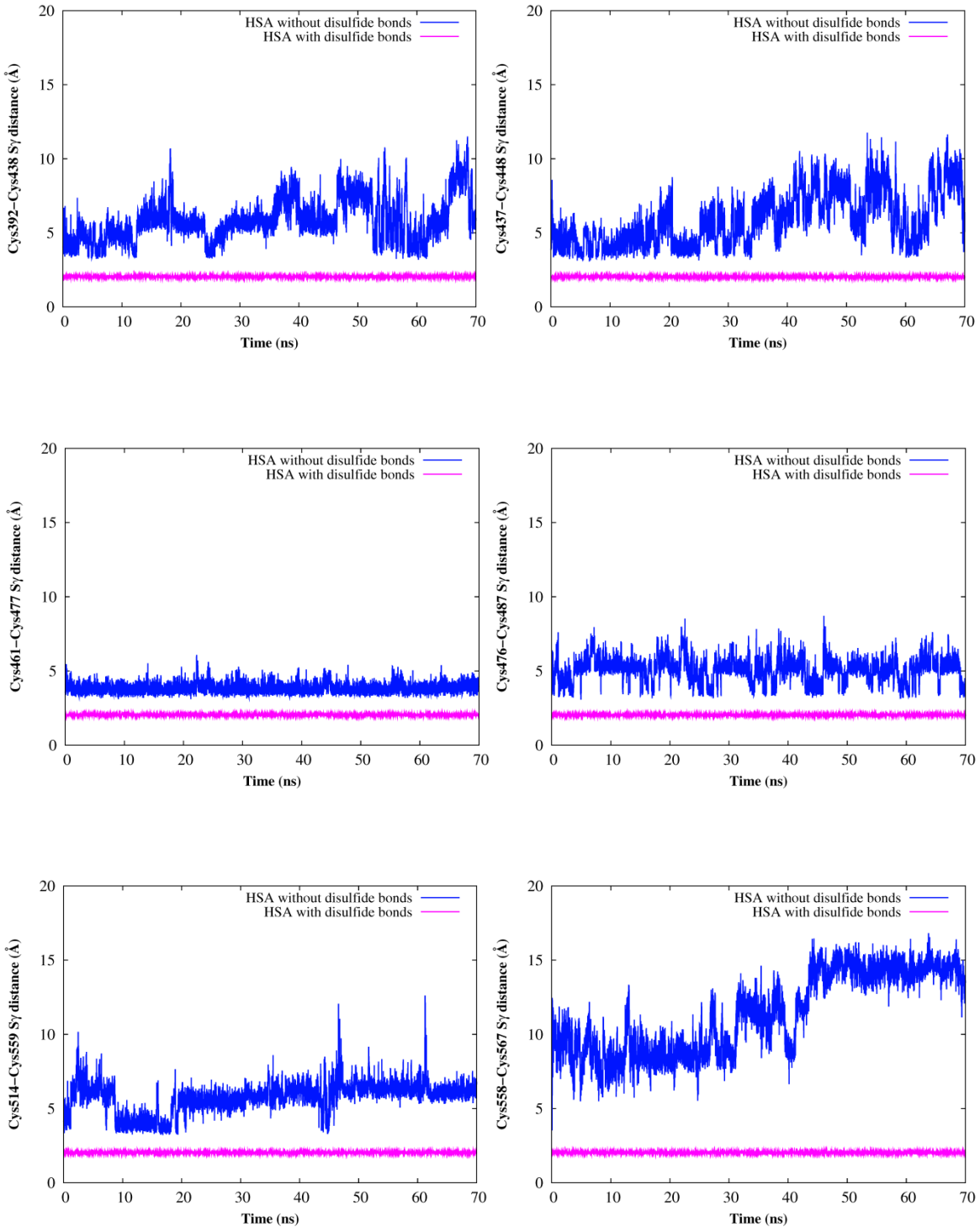

Figure SI.3. Distance between the sulfhydryl groups of SS-bonds in domain III for the simulation without SS-bonds (blue). For comparison purposes, the figure also includes the distance for the bonded case (pink, 2.03 Å). In general, conformations of SS-bonds in this

domain show from small to medium fluctuations. In particular, Cys461-Cys477 and Cys476-Cys487 may form a bond again, unless Cys476-Cys487 reaches first a stable conformation above 5 Å.

RMSD of the equilibrated structure with the other conformations

For comparison purposes and to determine similarities with the equilibrated MD structure of the native HSA, Figure SI.4 includes the RMSD as the simulation proceeds with respect to: a. the crystal structure 1AO6,[1] b. the crystal structure 1N5U,[5] and c. the last structure obtained from MD simulations. The RMSD is a measure of the structural drift between two conformations of the same molecule. The RMSD is below ~5 Å when referenced to the initial structure (1AO6) and the final simulated structure (70 ns). When referenced to the structure 1N5U, the RMSD is ~2 Å higher than the previous cases, suggesting that each structure represents a different conformation that the native HSA could adopt in solution, taking into account that the simulated structure has lower energy than the initial crystal structure 1AO6.

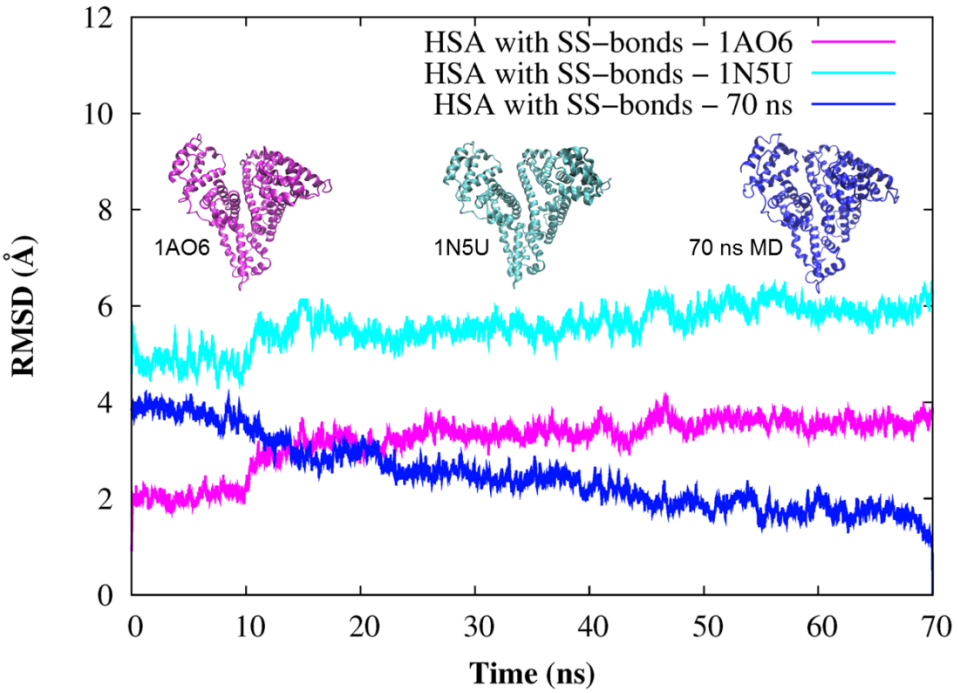

Figure SI.4. RMSD of the HSA structure with SS-bonds obtained from MD simulations, using the following structures as reference: a. the crystal structure 1AO6,[1] b. the crystal structure 1N5U,[5] and c. the last structure obtained from MD simulations. A snapshot of these structures is also presented. The RMSD plot shows that each structure corresponds to a different conformation that the native HSA can adopt, and the RMSD between each pair of structures varies from 4 to ~6 Å.

Dynamic Cross Correlation

The main manuscript includes changes in the cross-correlated motions after the removal of SS-bonds. A detailed list of the most significant changes is presented below, where the cross-correlations are compared by subtracting the cross-correlated motion in the absence of SS-bonds from the cross-correlated motion when SS-bonds are present.

Res. A	Res. B	diff
6	56	0.52
6	57	0.61
7	57	0.57
8	53	0.55
8	54	0.69
8	55	0.56
8	57	0.52
9	53	0.58
9	54	0.66
9	55	0.58
9	56	0.52
9	57	0.53
13	54	0.51
59	511	0.52
119	169	0.55
120	169	0.60
121	169	0.51
124	169	0.54
131	504	0.50
131	505	0.59
131	506	0.56
131	564	0.56
131	565	0.52
133	507	0.51
133	564	0.55
133	565	0.58
149	241	0.56
149	242	0.52
150	240	0.53
150	241	0.65
150	242	0.60
151	241	0.53
241	253	0.64
242	253	0.53
265	282	0.52
265	283	0.55
267	505	0.50
270	507	0.58
270	508	0.52
270	577	0.50
371	375	0.53
410	428	0.56
411	428	0.51
503	579	0.51
504	579	0.54
505	579	0.56
505	580	0.52
542	572	0.50
542	575	0.57
543	575	0.56
547	572	0.63
547	575	0.68
548	567	0.56
548	571	0.53
548	572	0.53
549	567	0.56
550	566	0.56
550	567	0.57
551	565	0.51
551	566	0.63
551	567	0.62
552	566	0.52
552	567	0.59
553	566	0.59
553	567	0.59
554	565	0.56
554	566	0.80
554	567	0.69
554	581	0.50
555	566	0.69
555	567	0.62
556	566	0.64
556	567	0.57
557	566	0.80
557	567	0.65
558	565	0.60
558	566	0.90
558	567	0.67
559	566	0.64
571	577	0.56
571	578	0.55
574	581	0.55

Table SI.1. Difference between the cross-correlation with SS-bonds and in the absence of SS-bonds for residue "A" (Res. A) and residue "B" (Res. B). The difference is calculated as: $DCCM_{SS\text{-}bonds}$- $DCCM_{no\_SS\text{-}bonds}$, for interactions between residues "A" and "B". A positive value (> 0.5) indicates that removing SS-bonds weakens the interaction between the corresponding residues.

Res. A	Res. B	diff
116	573	-0.51
156	290	-0.54
199	210	-0.51
199	211	-0.58
200	211	-0.51
202	211	-0.59
203	210	-0.52
203	211	-0.62
204	210	-0.52
204	211	-0.56
238	302	-0.52
238	307	-0.51

Res. A	Res. B	diff
239	506	-0.50
239	564	-0.53
239	565	-0.51
408	543	-0.51
409	504	-0.52
409	541	-0.58
409	542	-0.57
409	543	-0.56
410	538	-0.54
410	539	-0.55
410	540	-0.58
410	541	-0.66

Res. A	Res. B	diff
410	542	-0.73
410	543	-0.68
411	541	-0.53
411	542	-0.59
411	543	-0.60
412	504	-0.51
412	505	-0.54
412	506	-0.52
423	504	-0.54
438	566	-0.51
505	540	-0.54
506	529	-0.51

Res. A	Res. B	diff
506	530	-0.61
506	531	-0.51
506	533	-0.64
506	534	-0.51
506	537	-0.52
506	540	-0.57
506	541	-0.57
507	533	-0.54
509	555	-0.56
509	556	-0.53
510	555	-0.50

Table SI.2. Difference between the cross-correlation with SS-bonds and in the absence of SS-bonds for residue "A" (Res. A) and residue "B" (Res. B). The difference (diff) is calculated as: $DCCM_{SS\text{-}bonds}$ - $DCCM_{no\_SS\text{-}bonds}$, for interactions between residues "A" and "B". A negative value (< -0.5) indicates that removing SS-bonds strengths the interaction between the corresponding residues.

Hydrogen bonds analysis

The removal of SS-bonds also affects the occupancy of other important intramolecular interactions such as hydrogen bonds. Some hydrogen bonds were observed to be stable even when SS-bonds were removed, whereas others vanished or increased their occupancy. Figure SI.5 shows the region in which hydrogen bonds are no longer present after disulfide bonds are removed (pink) during the MD trajectory between 60 to 70 ns. Specific regions can be identified where many hydrogen bonds are maintained, such as those involving the main binding sites (subdomain IIA and IIIA). Table SI.3 presents in detail the list of hydrogen bonds between a donor and acceptor residue with occupancies above 80%, for the MD simulations with and without SS-bonds. The hydrogen bond formed by the only free cysteine (Cys34) and Tyr140 has been highlighted (see main manuscript, section 3.4. for discussion).

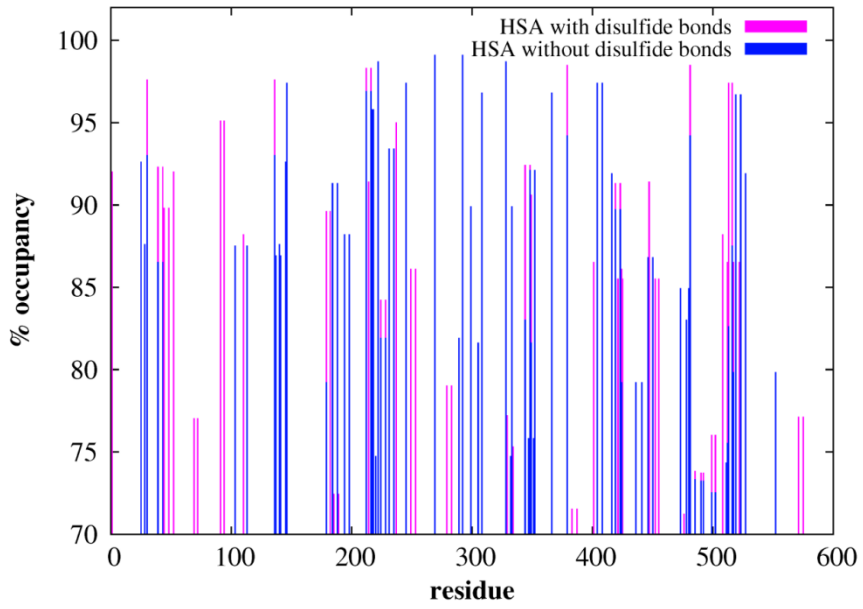

Figure SI.5. Percentage of occupancy of hydrogen bonds after the removal of SS-bonds. Pink bars indicate the residues for which the hydrogen bonds vanished in the absence of SS-bonds. Many of the hydrogen bond interactions prevailed (blue bars) in the absence of SS-bonds in specific regions such as those involving the main binding sites.

HSA with SS-bonds					HSA without SS-bonds			
Donor	Acceptor	%occupied	distance (Å)		Donor	Acceptor	%occupied	distance (Å)
383	485	98.5	2.741		296	273	99.1	2.647
216	220	98.3	2.687		226	332	98.7	2.692
34	140	97.6	2.711		408	412	97.4	2.712
520	517	97.4	2.648		249	150	97.4	2.642
296	273	97.3	2.631		216	220	96.9	2.728
95	98	95.1	2.77		312	370	96.8	2.713
241	150	95	2.737		523	527	96.7	2.727
408	412	93.31	2.738		221	222	95.8	2.787
226	332	92.71	2.737		383	485	94.21	2.78
348	352	92.41	2.733		383	485	93.81	2.781
43	47	92.31	2.762		235	239	93.41	2.752
56	5	92.01	2.739		34	140	93.01	2.744
451	218	91.41	2.804		149	29	92.61	2.814
423	427	91.31	2.753		352	356	92.11	2.74
370	353	90.61	2.751		531	420	91.91	2.713
48	52	89.81	2.718		188	192	91.31	2.733
183	186	89.61	2.801		303	337	89.91	2.809
235	239	89.41	2.759		423	427	89.71	2.766
383	485	88.91	2.803		198	202	88.21	2.752
512	114	88.21	2.792		144	32	87.61	2.836
95	98	87.61	2.809		520	117	87.51	2.773
451	218	87.51	2.813		107	32	87.51	2.837
516	521	86.51	2.81		141	145	86.91	2.822
405	526	86.51	2.826		450	454	86.81	2.75
526	428	86.11	2.804		43	47	86.51	2.775
253	257	86.11	2.827		477	484	84.92	2.8
303	337	86.01	2.819		482	348	83.02	2.844
512	114	85.51	2.798		520	517	82.62	2.73
456	459	85.51	2.838		293	222	81.92	2.798
425	429	85.51	2.823		228	232	81.92	2.757
141	145	85.41	2.827		309	353	81.62	2.778
228	232	84.22	2.767		556	521	79.82	2.782
144	32	84.22	2.838		440	445	79.22	2.829
450	485	82.62	2.742		183	428	79.22	2.778
198	202	81.32	2.737		351	355	75.82	2.761
450	348	80.62	2.78		516	521	75.52	2.829

Table SI.3. Hydrogen bond analysis for the MD simulations with and without SS-bonds. Only hydrogen bonds with occupancies above 80% are presented. Analysis was performed for time frame between 60 and 70 ns. Repeated donor and acceptor residues could be observed if two or more hydrogen bonds are formed by the same residue but distinct hydrogen atoms. The highlighted hydrogen bond corresponds to the interaction between Tyr140 and Cys34, the latter being the only free cysteine residue of HSA.

In conclusion, the breakage of SS-bonds could significantly influence the conformational stability and correlated motion of the protein and residues in the neighborhood, but this is not always the case. Some SS-bonds promote unfolding of alpha helices and dynamical changes in HSA (*e.g.*, Cys168-Cys177, Cys278-Cys289). Some SS-bonds have medium fluctuations and partially influence local motion and structural changes (*e.g.*, Cys245-Cys253 and Cys514-Cys559). Cys90-Cys101, Cys278-Cys289, and Cys392-Cys438 reach highly stable conformations at distances that SS-bonds do not likely form again. SS-bonds such as Cys75-Cys91, Cys200-Cys246 andCys461-Cys477 have very stable conformations during the entire simulation at average distances between 3.7 and 4.3 Å suggesting that these SS-bonds are likely to form again and do not influence changes in structure and dynamics of the local residues.

References

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