Supporting Information

Destabilization of Insulin Hexamer in Water-Ethanol Binary Mixture

Saumyak Mukherjee[†], Ashish A. Deshmukh[‡#], Sayantan Mondal[†#], Balasubramanian Gopal[‡*], Biman Bagchi[†*]

[†]*Solid State and Structural Chemistry Unit, Indian Institute of Science, Bengaluru-560012, India*

[‡]*Molecular Biophysics Unit, Indian Institute of Science, Bengaluru-560012, India*

S1. Insulin hexamer and its central cavity

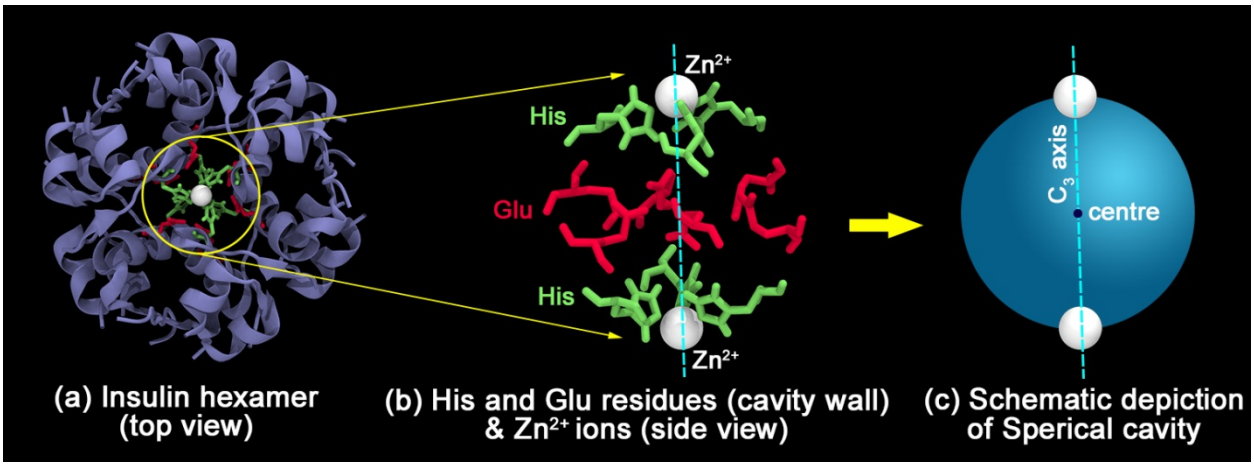

Figure S1. (a) Top view of the insulin hexamer assembly. (b) The cavity region surrounded by 6 glutamate (GLU) and 6 histidine (HIS) residues. The HIS side-chains are coordinated to the 2 Zn^{2+} ions at the two ends of the cavity. (c) Schematic representation of the spherical cavity considered in our analyses. The centre of the sphere lies on the C_3 axis of rotation, which passes through the two Zn^{2+} ions.

S2. Ethanol molecules inside the hexamer cavity

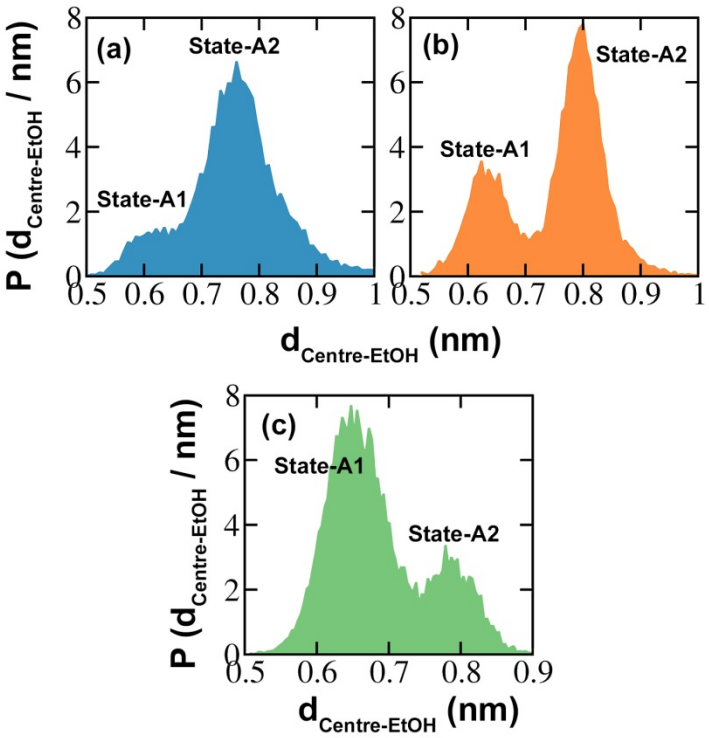

Figure S2. Distributions of the distance between ethanol molecules inside the cavity and the centre of the cavity. As discussed in the main manuscript, the state-A2 is often found to be more probable than state-A1 for ethanol stabilization. Here we show two more examples (a and b). In (c) we show a case where state-A1 is more probable than stat-A2. However, the former scenario is more prevalent.

S3. Crystal structures at various ethanol concentrations

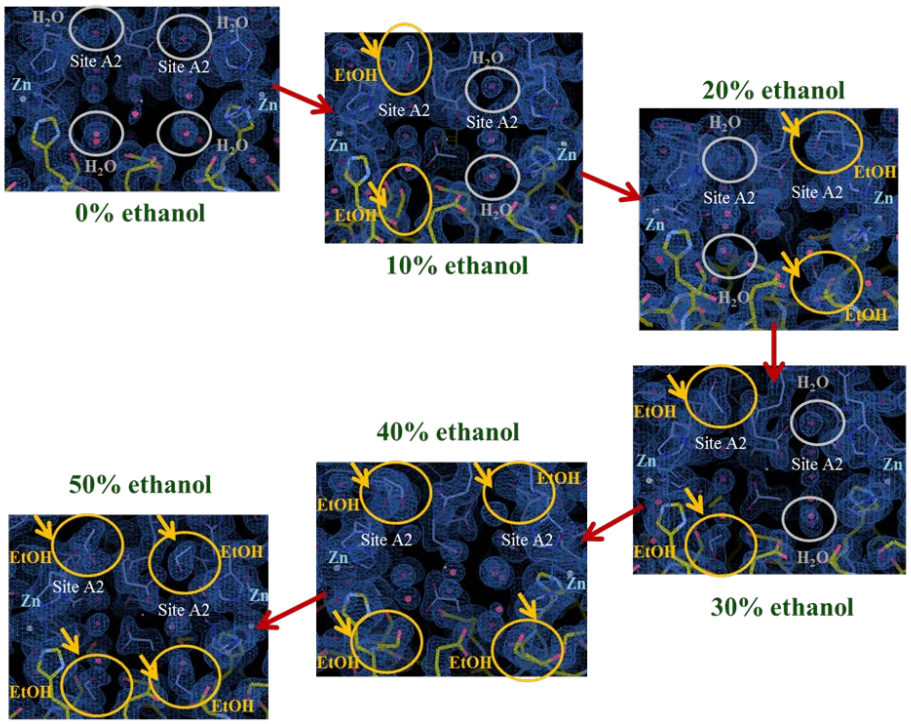

Figure S3. In the main text we have shown that ethanol molecules displace water inside insulin hexamer cavity at 30% (v/v) ethanol concentration. We find that this is true for other concentrations of ethanol as well. Here crystal structures of hexamer cavity are shown corresponding to ethanol concentrations of 10%, 20%, 30%, 40% and 5 % (v/v). Displacement probability is greater in the higher concentrations (40% and 50%).

Table S1. Data collection and refinement statistics of insulin hexamer crystal soaked in 30% (v/v) ethanol.

Parameters	Ethanol soaked crystal structure of insulin hexamer
Wavelength (Å)	1.5418 Å
Resolution (Å)	24.37-1.78 (1.87-1.78)
Space group	P1
Cell dimensions	a=33.75Å, b=48.26Å, c=48.26Å, α=114.75°, β=103.46°, γ=103.47°
R_{sym} (%)	2.2 (31.0)
Total number of reflection S	88481 (12389)
Total number of unique reflections	22493 (3150)
Mean I/σI	41.0 (28.9)
Completeness (%)	93.5 (89.5)
Anomalous completeness (%)	93.2 (89.0)
CC1/2	0.999 (0.998)

Average B factor (Å^2)	14
Wilson B-factor (Å^2)	11.9
Solvent content (%)	33.45
Moisaicity (°)	0.49
R_{free}	0.129
R_{work}	0.177
Ramachandran outliers (%)	1.08
Ramachandran preferred (%)	97.49
Ramachandran allowed (%)	1.43

S4. Time evolution of Lindemann ratio of insulin hexamer

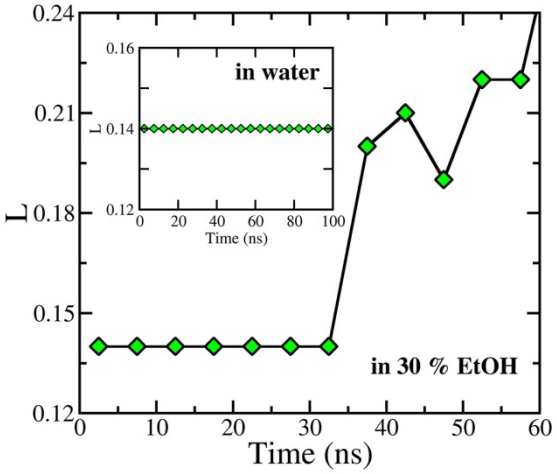

Figure S4. Lindemann ratio of insulin hexamer with respect to the geometric centres of the individual monomer units. Each point represents the midpoint of the 5 ns time windows, for which they are calculated. In water the value remains constant at 0.14, while in ethanol solution an onset of instability at ~35 ns is observed. The data for insulin hexamer in pure water is shown in the inset.

A parameter that readily shows changes in the organizational patterns in solids is the Lindemann ratio given by the following equation.

$$L = \frac{\sqrt{\langle \delta r^2 \rangle}}{a}$$

Here, $\sqrt{\langle \delta r^2 \rangle}$ is the root mean square displacement of the solid particles and a is the average nearest neighbour distance. Sudden change in the value of *L* denotes the onset of instability and consequent phase transition. Here, we consider each of the monomers as a particle in a solid lattice that defines the insulin hexamer. The behaviour of *L* is similar of that of B-factor reported in the main text.

S5. Distance between the two Zn^{2+} ions: Change in number of cavity water

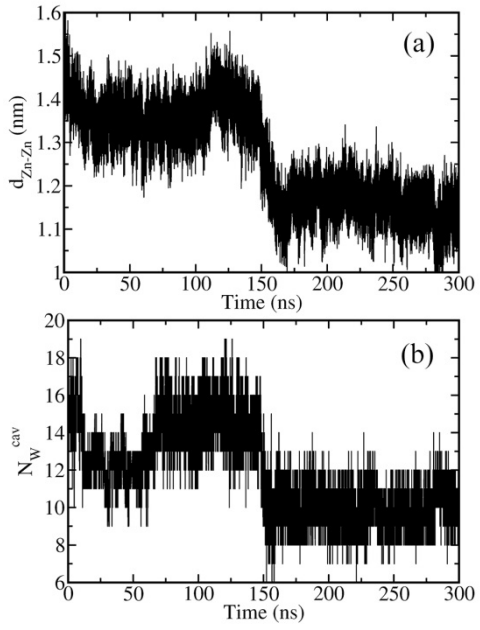

Figure S5. (a) Time evolution of the distance between the two Zn^{2+} ions ($d_{Zn\text{-}Zn}$) inside insulin cavity. (b) Number of water molecules inside the cavity as a function of time. Both the trajectories show sudden decrease at ~150 ns.

An interesting aspect of the disruption of hexamer structure is the substantial decrease in the distance between the two Zn^{2+} ions ($d_{Zn\text{-}Zn}$) (Figure S4a). This, in turn results in a sudden decrease in the cavity volume. Consequently, the capacity of the cavity to hold water molecules reduces considerably. Hence, along with the ethanol molecule, some water molecules also diffuse out into the bulk. This is represented in Figure S4b. Figure S3a shows the Zn-Zn distance trajectory, which suffers a sudden decrease at ~150 ns. This results in a decreased cavity volume causing water molecules to diffuse out into the bulk. Hence the number of water molecules in the cavity (N_W^{cav}) decreases. The correlation coefficient between these two events is 0.76. This high positive correlation denotes that these two processes are coupled and occur simultaneously.

S6. Structural perturbations of insulin hexamer in ~0.6 % v/v/ EtOH solution

As a consequence of interactions with ethanol, insulin hexamer suffers structural deformations. In the main paper, we have shown these structural manifestations in ~30 % v/v EtOH solution. Even in low ethanol concentration, signatures of certain structural changes can be observed.

These are shown in Figure S5 (distances between the geometric centres of consecutive monomers) and Figure S6 (difference in distances between atoms of consecutive monomers in the initial and the final configurations of the protein).

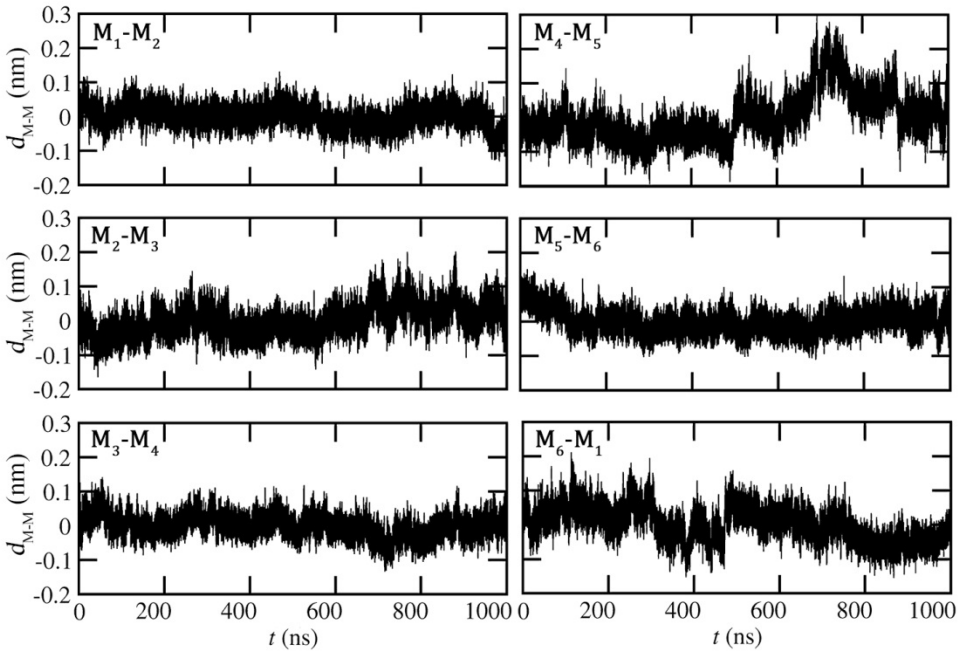

Figure S6. Time evolution of monomer-monomer distance in insulin hexamer for 1 μs in ~0.6 % v/v ethanol solution. Large magnitude fluctuations set in after a certain period of time. This is particularly visible for M_4-M_5 junction in this trajectory.

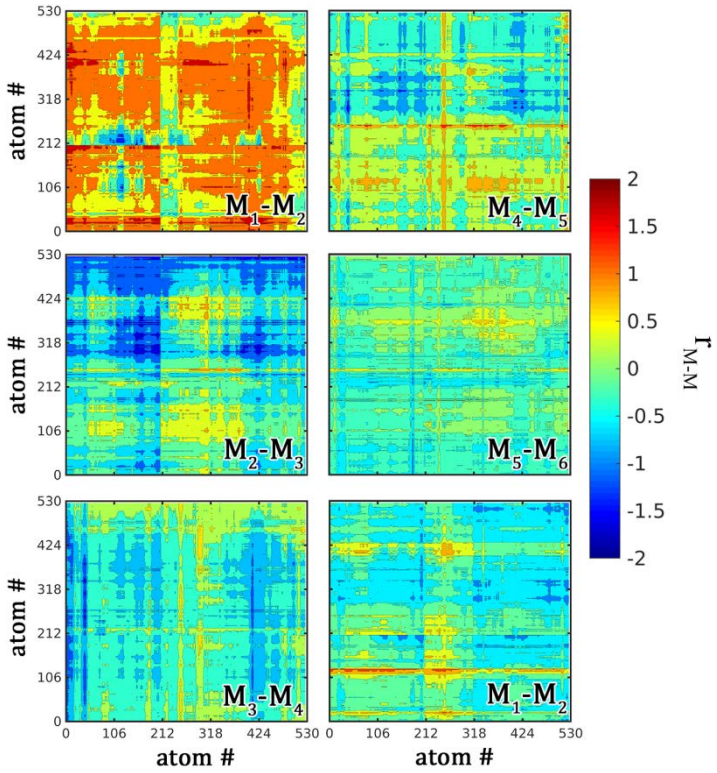

Figure S7. Colour coded distance matrix for atomic differences in the final and the initial configurations of insulin hexamer in ~0.6 % v/v ethanol. Red denotes increased distance. It is seen that the atomic distances between M_1 and M_2 show marked increase.

S7. Time evolution of RMSD and shape of insulin hexamer in 30 % v/v EtOH solution

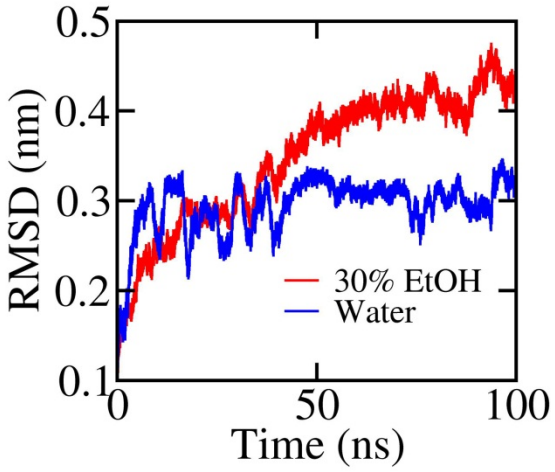

Figure S8. Time evolution of root mean square deviation of atomic distances of insulin hexamer in 30 % v/v EtOH solution (red) as compared to that in neat water (blue). RMSD shows steady increase in the former solution. In water, it reaches as equilibrium value.

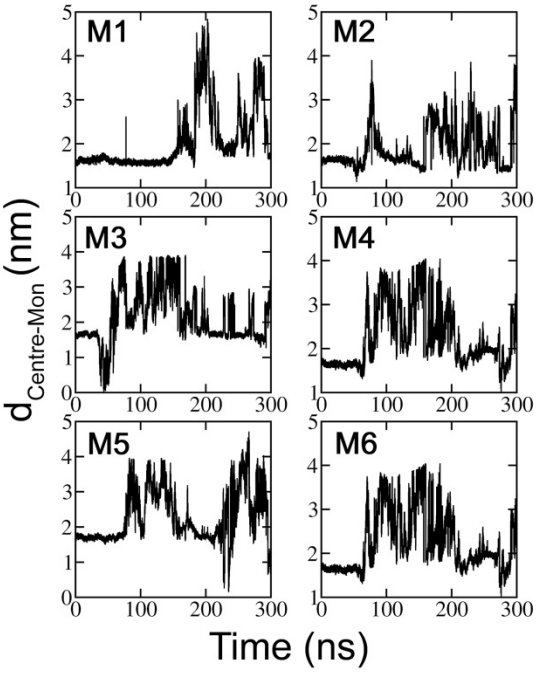

Figure S9. Time evolution of distances between the centre of the cavity and geometric centres of the six monomers of insulin hexamer in 30% v/v EtOH solution. The distances, after some period of stability, shows large fluctuations. Intermittent stable regions are also observed. Hence, we do not observe any dissociation process.

S8. Structural deformation of insulin hexamer in 30% v/v ethanol solution

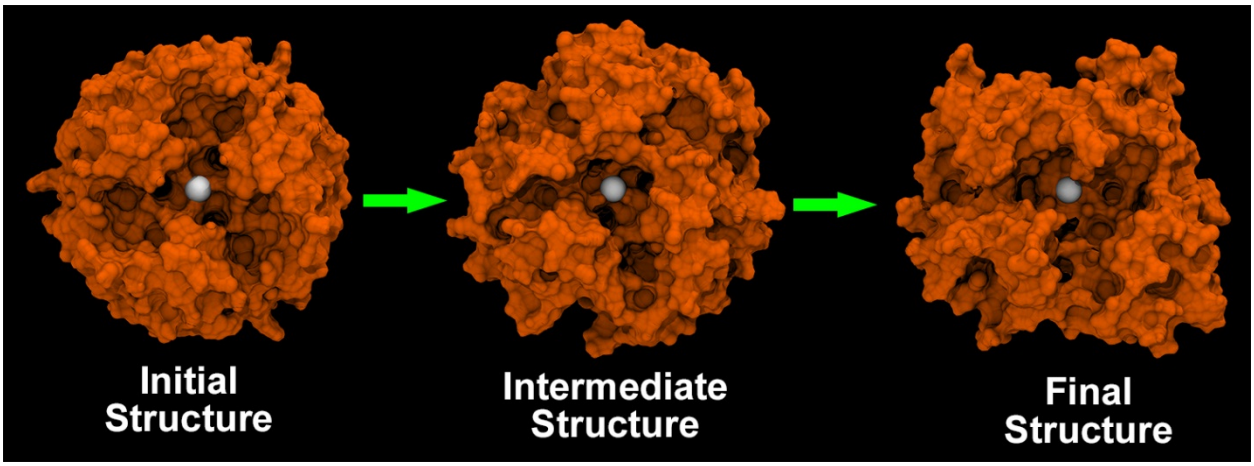

Figure S10. Snapshots of insulin hexamer at the initial, intermediate and final steps of the MD simulation in 30% v/v ethanol solution. The white sphere is the Zn^{2+} ion. Interactions with

ethanol severely damages the structural integrity of the hexamer. The C_3 symmetry is destroyed. The protein assembly does not assume any new defined symmetry group.