Stability of Proteins in Solutions: A Microscopic Investigation on the Role of Surrounding Water/Cosolvent

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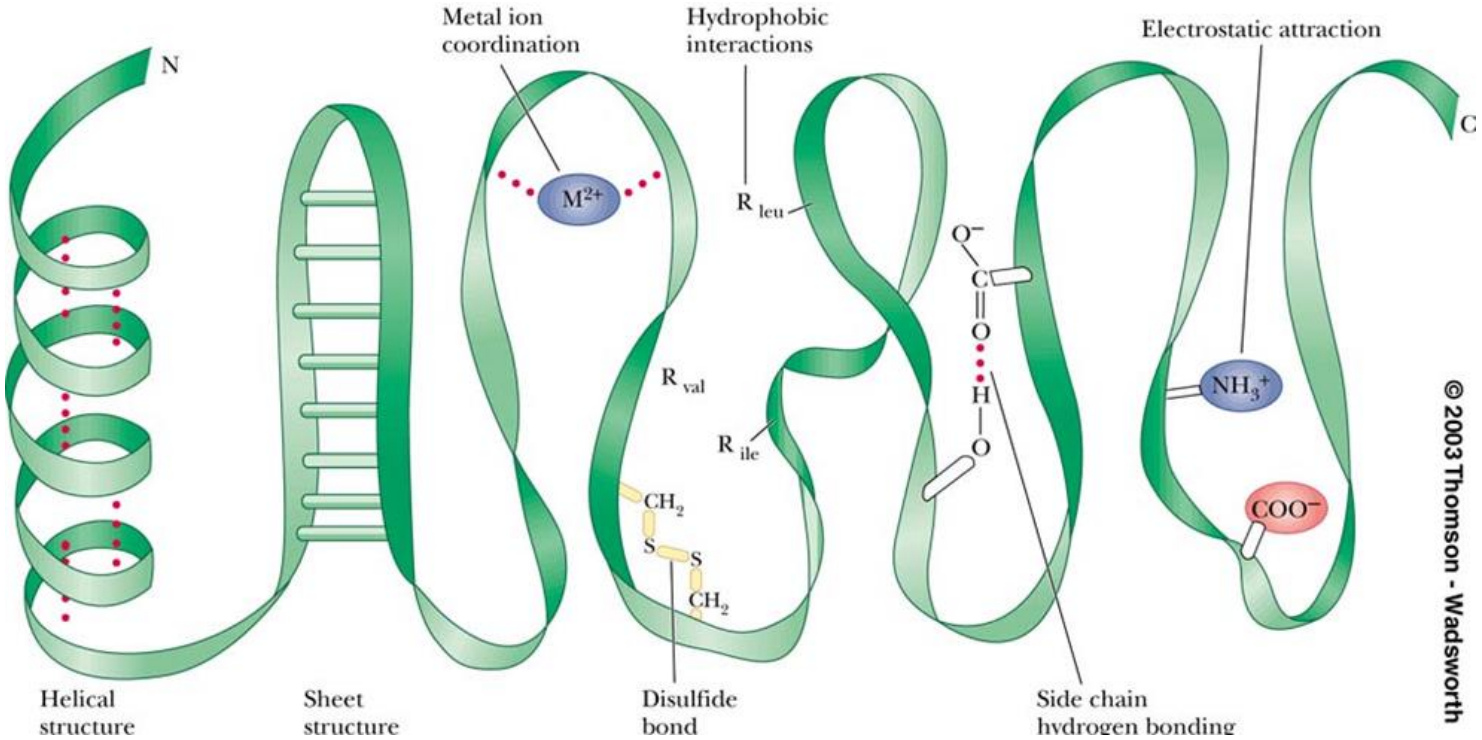
Factors that Stabilize Protein Structure



Protein and Cosolvent

- ✓ Cosolvent in experiments has become popular due to their ability to solvate biomolecular systems quite efficiently.

Protein structure is environmental sensitive. With the change in environmental conditions and solvent's physicochemical properties protein's native folded form can be disrupted.

Proteins in Alcohol Solutions

- Presence of alcohol in aqueous solution of a variety of cellular and enzymatic proteins has remarkable effect in regulating their structure and several complex biological activities, such as neuro-transmission, immune responses and catalytic activities of enzymes.

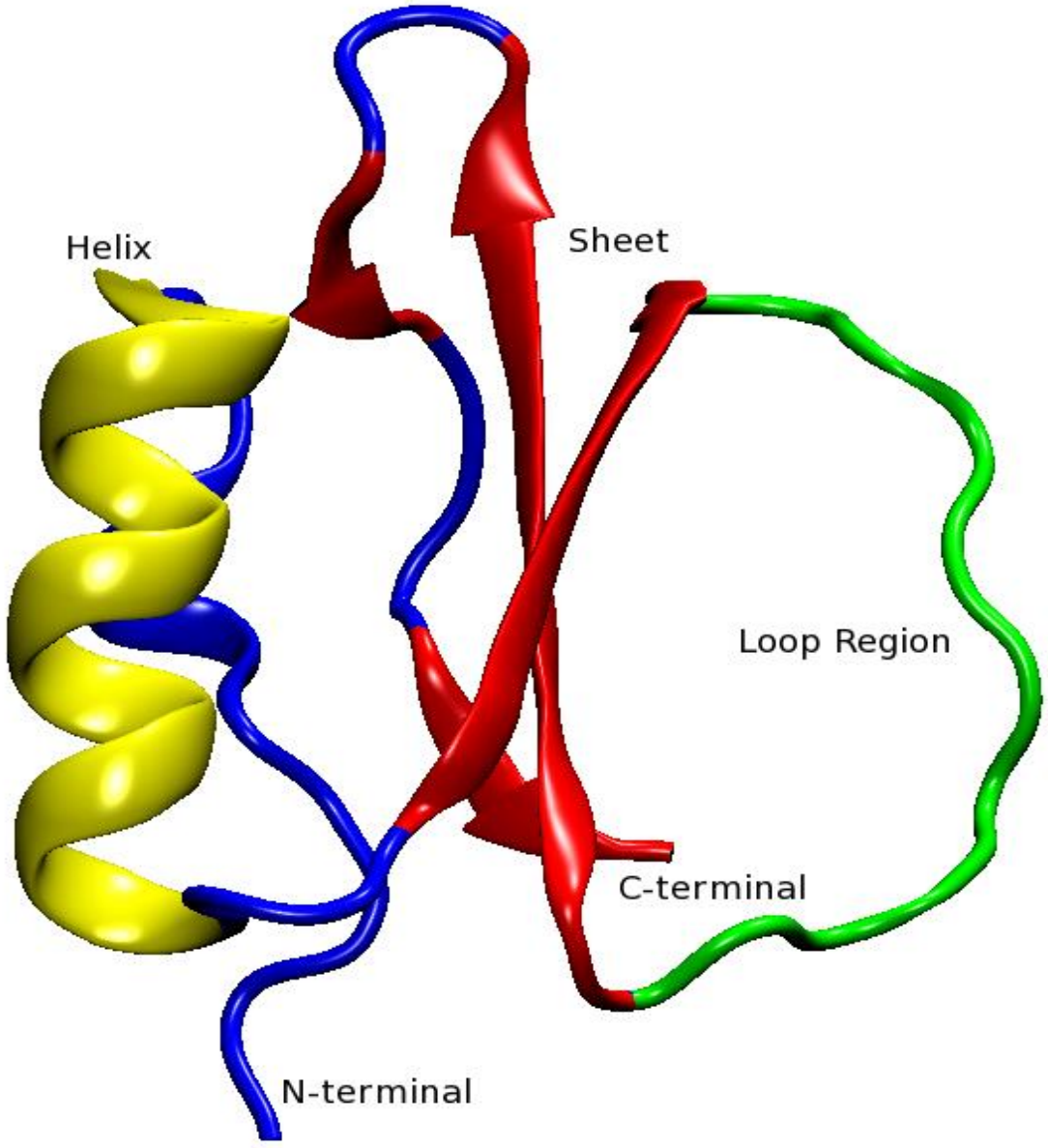
- Several proteins interact with the membrane surface and the binary mixture of water-alcohol can be a good model system that can represent hydrophobic-hydrophilic character of a membrane surface (*Bychkova. et.al.. Biochemistry*, ***1996***, *35, 6058-6063*).

- Monohydric alcohols, including halogen-substituted one disrupt the tertiary structures and stabilize the secondary structures of proteins/peptides. (*Miura, J. Pept. Sci.*, ***2011***, *17, 798-804*).

- Alcohol effects are concentration dependent (*Bhattacharyya and coworkers, JCP,* ***2014***, *140, 115105; Bagchi and coworkers, JPCB,* ***2013***, *117, 15625*)

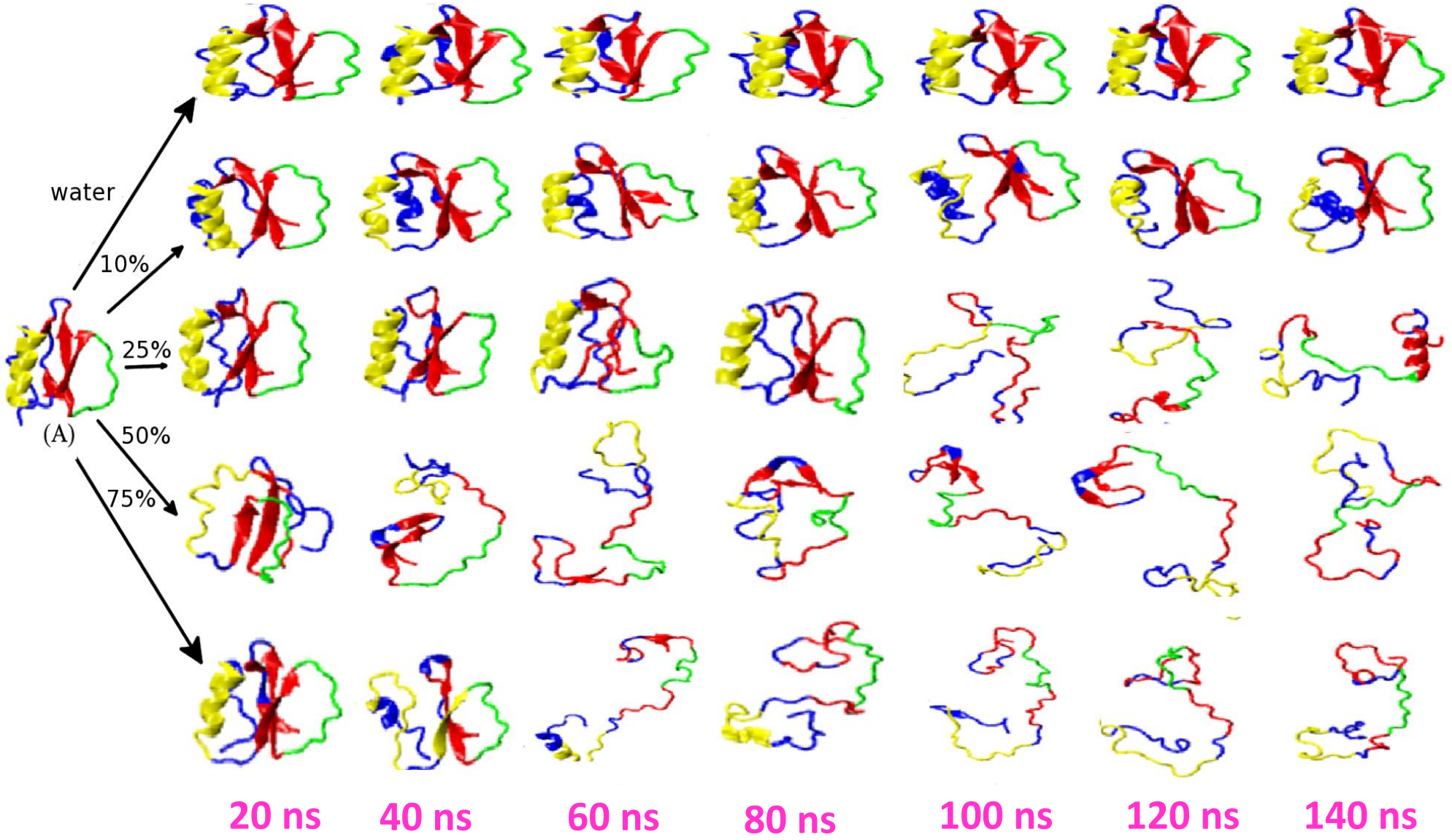
Chymotrypsin Inhibitor 2 (CI2)

- Member of the potato inhibitor 1 family of serine proteinase inhibitors



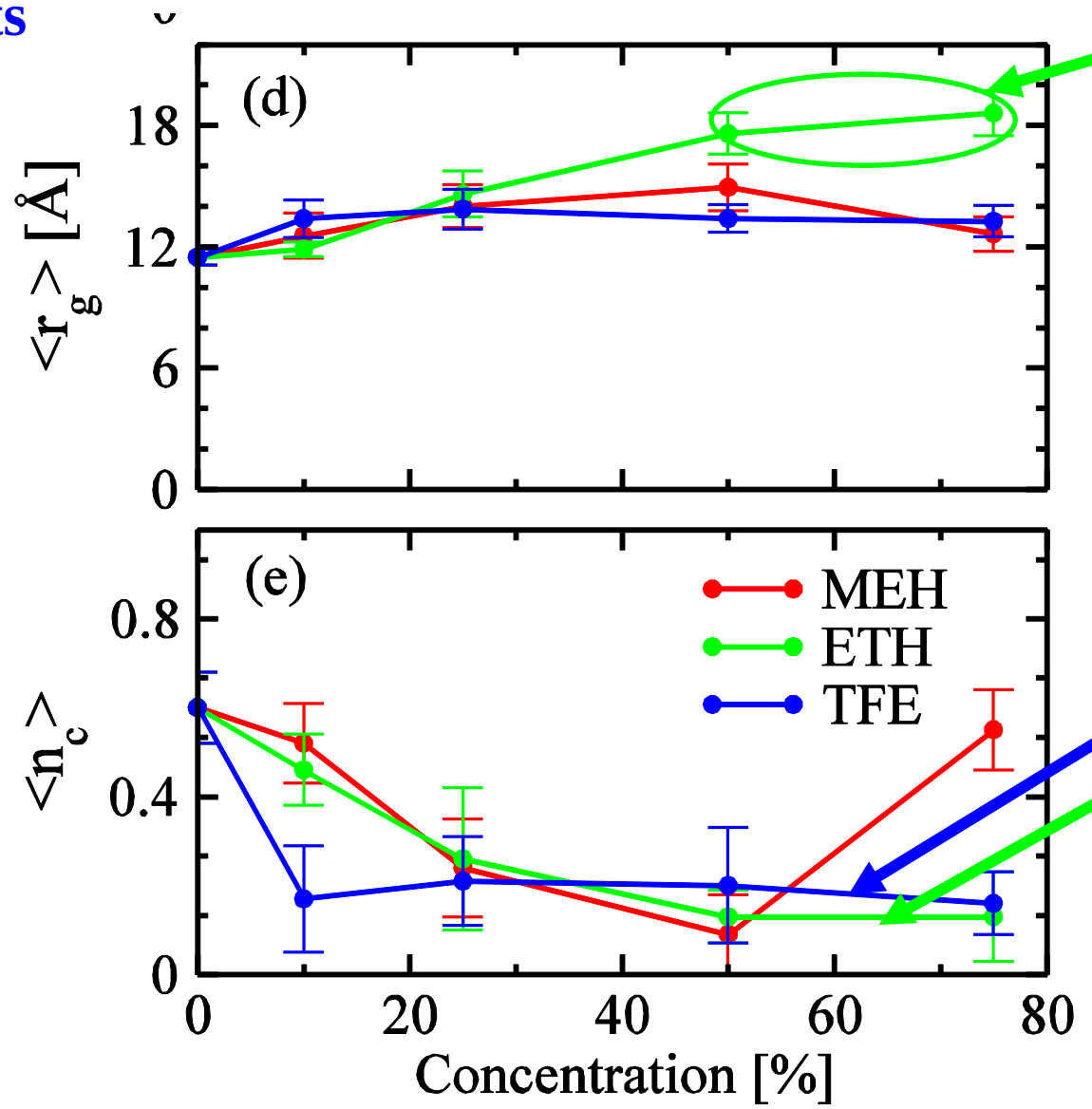
Ref: McPhalen, C. A. et.al., *Biochemistry*, **1987**, 26, 261.
Day, R., et.al., *J. Mol. Biol*,. **2002,** 322, 189.

Snapshots of CI2 in Water-EtOH



D. Mohanta and M. Jana ***J. Chem. Phys.* 2016**, 144, 165101.

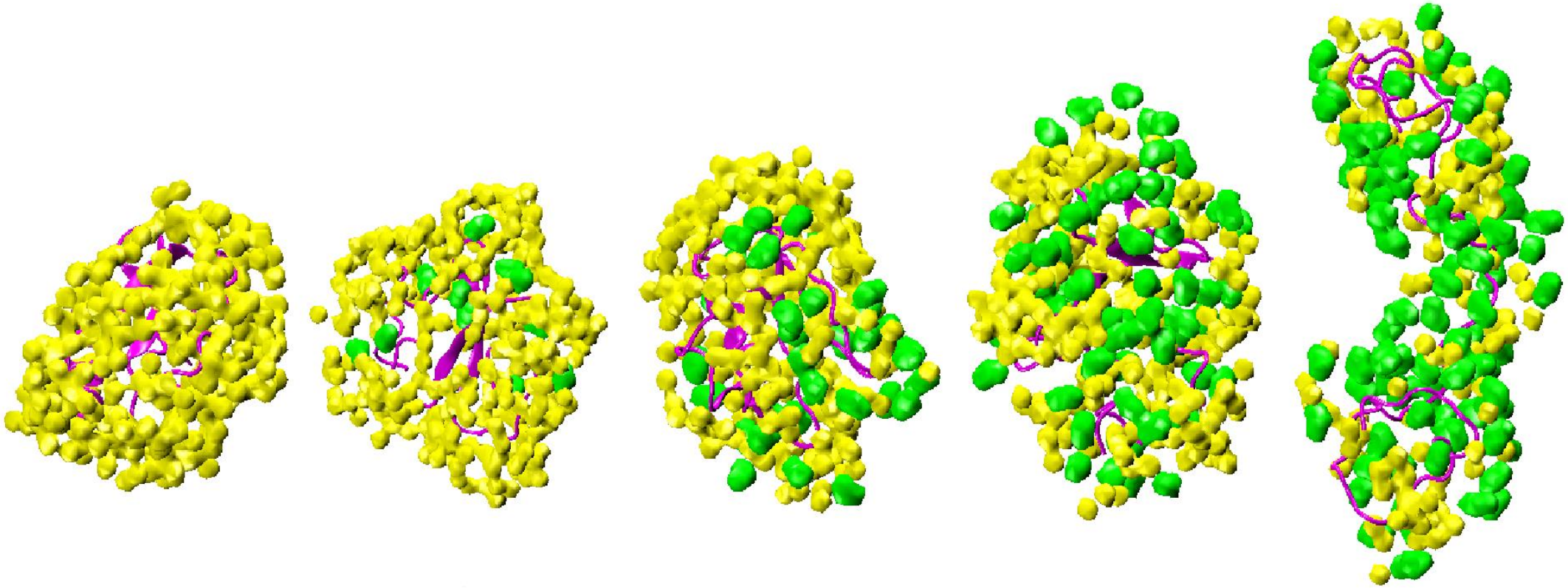
Rg and Native contacts



Lose in structural compactness

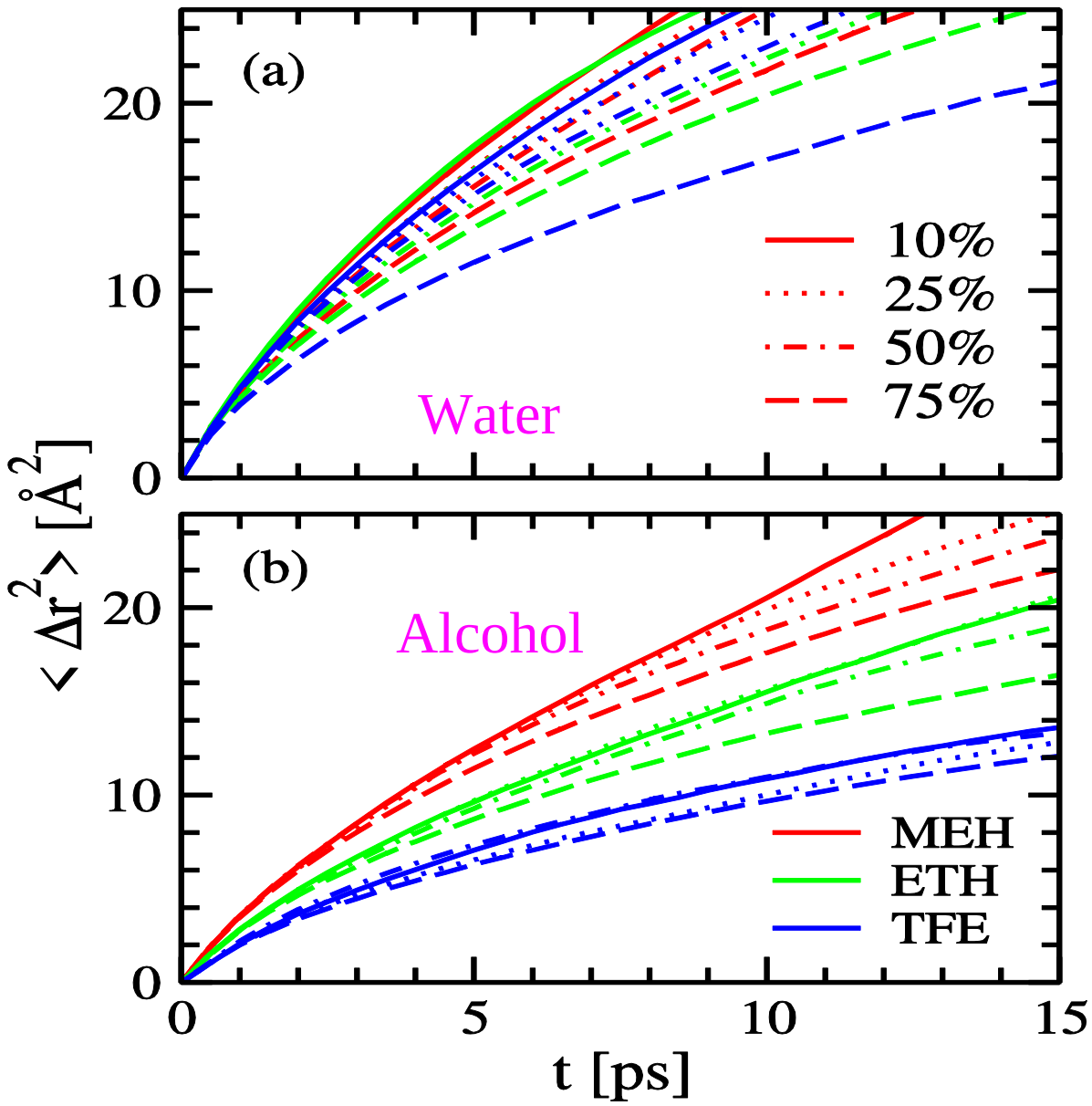
80 – 90 % contacts are broken

Solvation Layer: CI2 in Water-Ethanol Mixture



D. Mohanta and M. Jana ***J. Chem. Phys.* 2016**, 144, 165101.

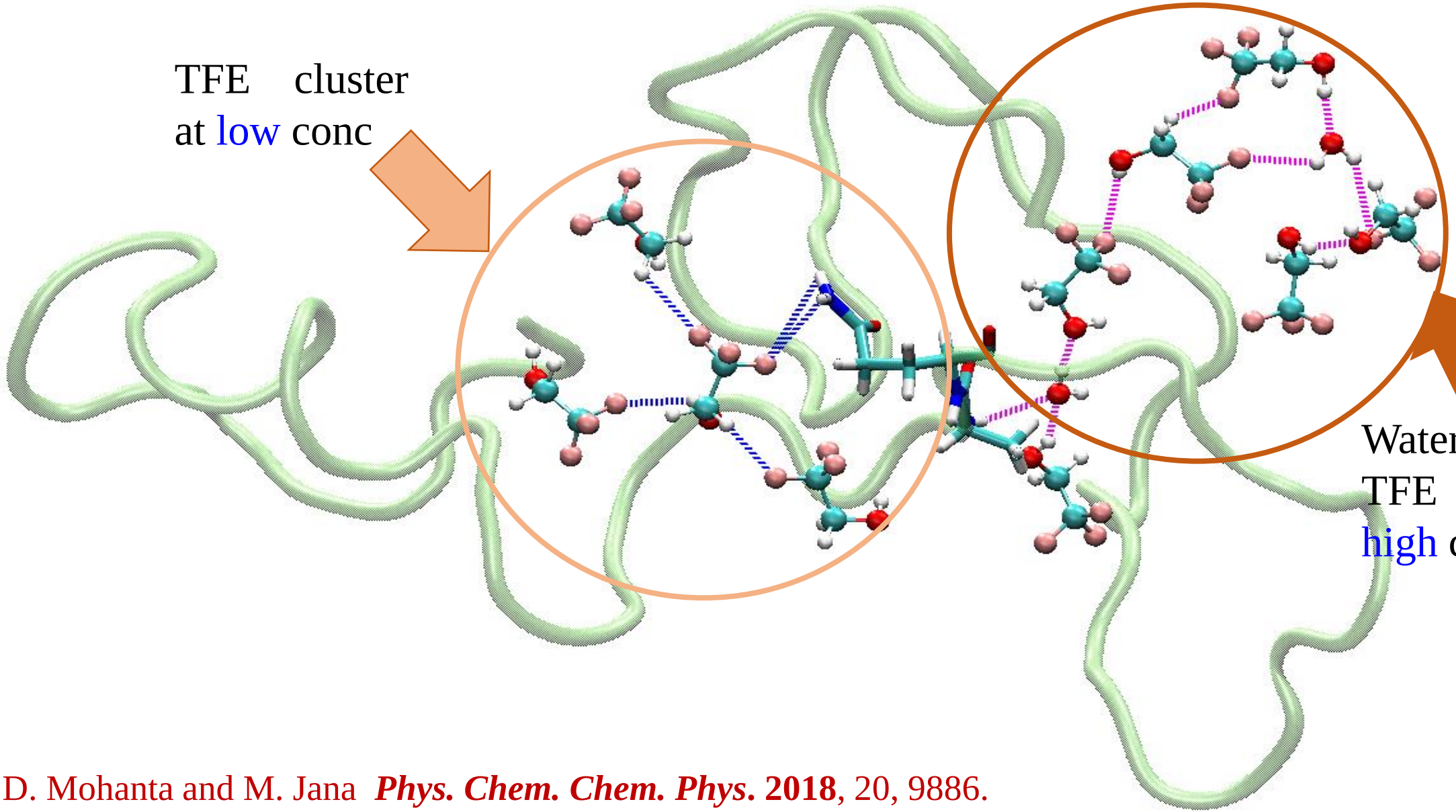
Translational Mobility of Solvent



The average relaxation and life-times of different HBs

		S1				S2			
System	Conc	$\langle\tau_C^{P-W}\rangle$	$\langle\tau_C^{P-A}\rangle$	$\langle\tau_S^{P-W}\rangle$	$\langle\tau_S^{P-A}\rangle$	$\langle\tau_C^{P-W}\rangle$	$\langle\tau_C^{P-A}\rangle$	$\langle\tau_S^{P-W}\rangle$	$\langle\tau_S^{P-A}\rangle$
MEH	10	105.75	268.09	1.45	2.18	93.97	201.94	1.16	2.86
	25	127.53	257.54	1.43	2.17	106.13	183.05	1.29	2.26
	50	132.72	217.96	2.02	1.14	109.02	176.77	1.60	0.92
	75	129.41	203.53	2.53	0.78	119.11	178.78	2.10	1.02
ETH	10	113.75	246.51	1.31	2.16	90.76	219.69	1.15	2.2
	25	134.97	249.11	1.14	1.16	95.42	201.62	1.15	1.59
	50	140.79	243.31	1.52	1.85	98.83	187.12	1.51	0.99
	75	150.02	240.94	2.58	0.80	104.52	188.19	2.56	0.70
TFE	10	120.59	255.29	1.30	1.48	93.52	214.77	1.16	2.80
	25	122.17	242.73	1.99	1.66	96.77	201.33	1.24	1.36
	50	145.11	240.73	1.77	1.80	99.35	200.03	1.64	0.97
	75	150.28	232.41	2.92	0.94	109.32	198.27	2.31	1.64

TFE cluster at low conc



Water mediated TFE cluster at high conc.

D. Mohanta and M. Jana ***Phys. Chem. Chem. Phys.* 2018**, 20, 9886.

Correlation Between BE and Nature of Residue

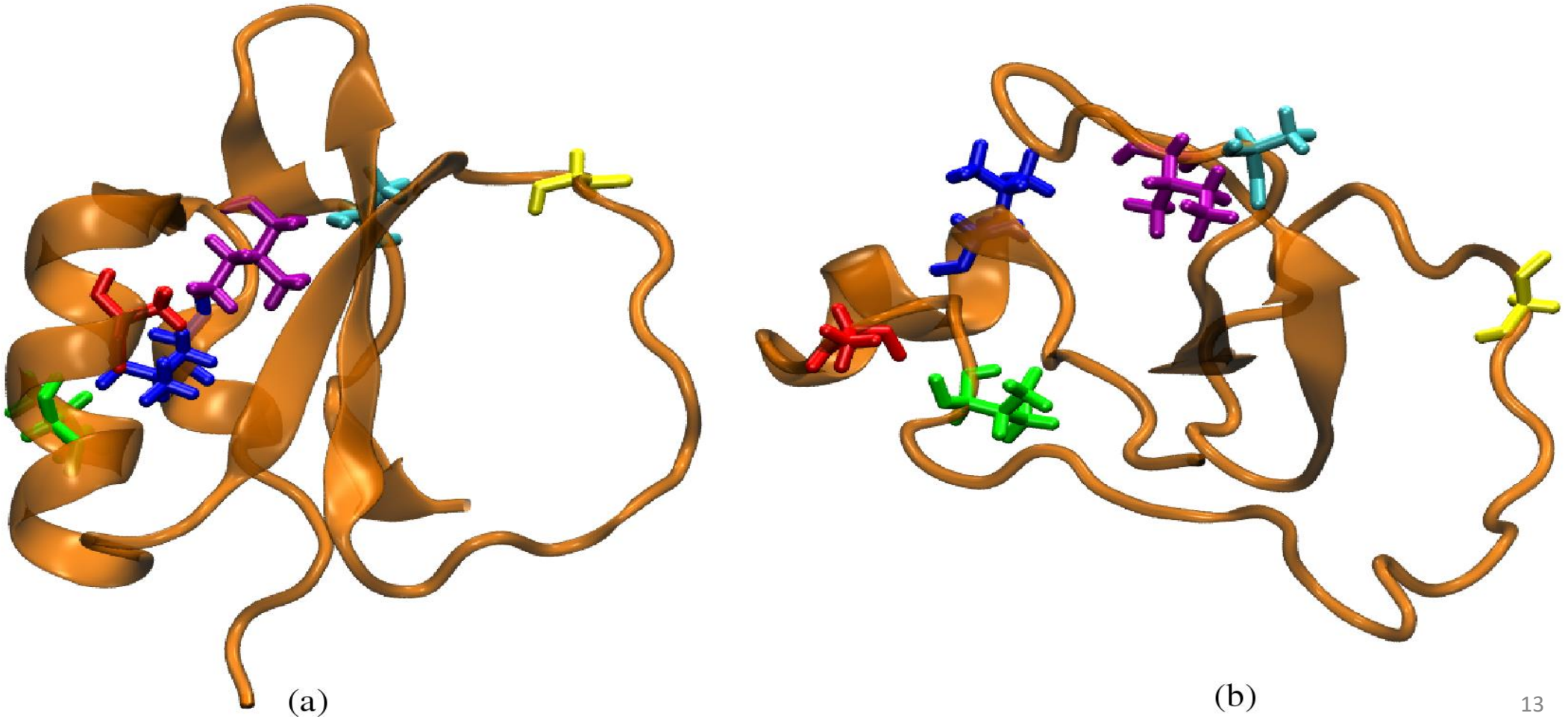
Correlation exists between the hydrophobicity (*wimley and white, Nat. Struct. Mol. Biol.* ***1996****, 3, 842−8.*) of the amino acids with the BEret / BErw for the unfolded protein complexes whereas these are found to be anti-correlated for the folded protein

System	r^2	
	Folded	Unfolded
Water	-0.54	0.56
alcohol	-0.42	0.76

D.Mohanta, and M. Jana et al. ***J. Phys. Chem. A*** **2017**, 121, 6172-6186.

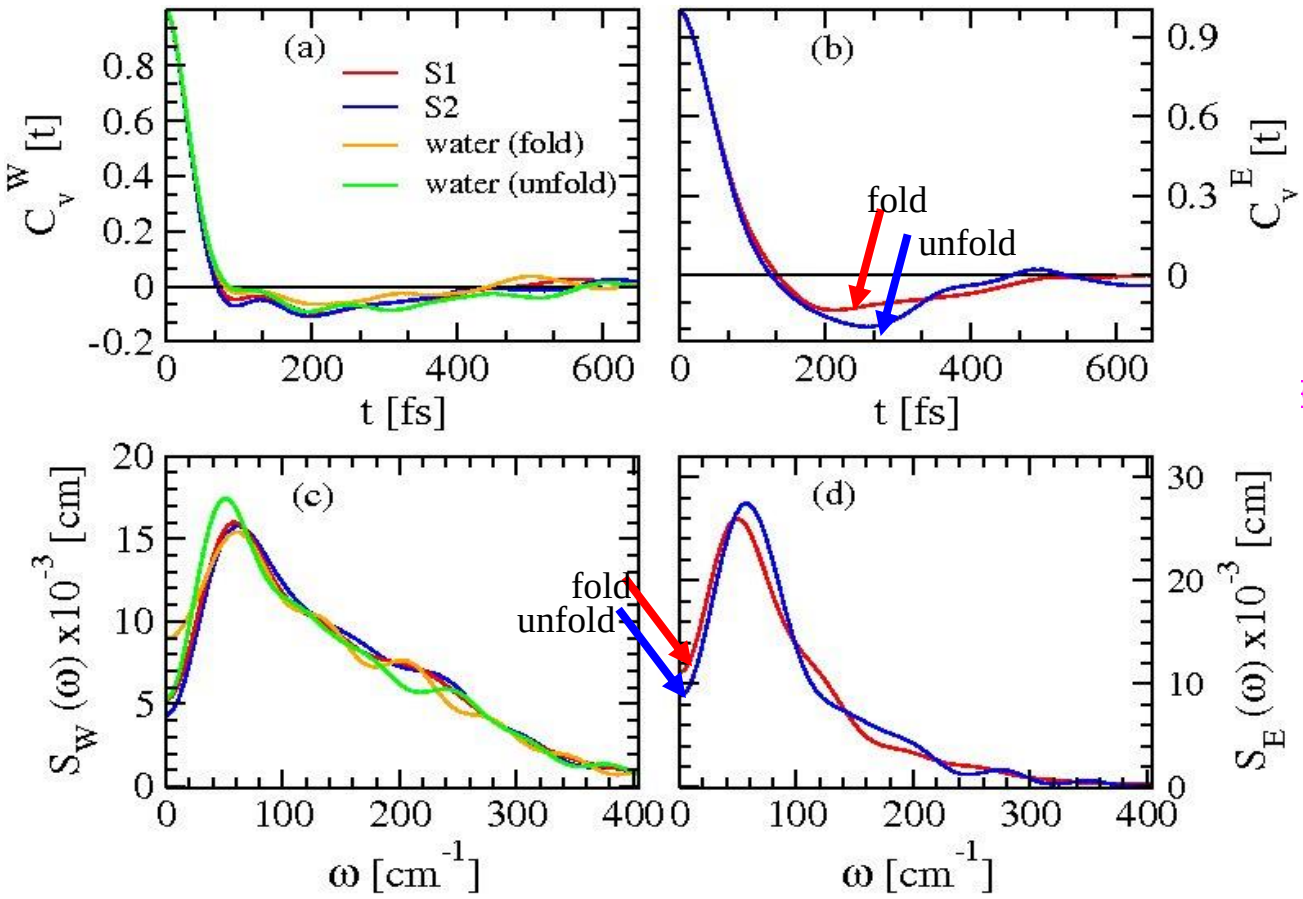
Residue Name	$\langle BE_{rw} \rangle$		$\Delta\langle BE_{rw} \rangle$	$\langle BE_{ret} \rangle$		$\Delta\langle BE_{ret} \rangle$
	Folded	Unfolded		Folded	Unfolded	
Met	-11.88	-5.61	6.26	-6.59	-8.45	-1.86
Lys	-15.36	-7.41	7.95	-12.87	-9.68	3.18
Thr	-4.65	-5.53	-0.88	-5.03	-5.64	-0.61
Glu	-13.69	-9.56	4.13	-8.60	-10.88	-2.27
Trp	-4.07	-9.71	-5.64	-3.61	-4.69	-1.08
Pro	-1.92	-4.07	-2.15	-2.92	-4.27	-1.35
Leu	-7.58	-6.47	1.10	-6.61	-3.67	2.94
Val	-6.10	-4.04	2.06	-3.66	-3.66	0.00
Gly	-7.06	-9.23	-1.63	-3.19	-5.73	-2.54
Ser	-14.04	-5.82	8.22	-5.89	-5.82	0.07
Ala	-6.47	-5.87	0.59	-2.78	-4.99	-2.21
Ile	-3.95	-5.52	-1.57	-4.73	-5.26	-0.53
Phe	-7.58	-6.47	1.10	-6.61	-3.67	2.94
Gln	-5.08	-7.64	-2.57	-3.13	-4.66	-1.53
Asp	-10.78	-15.95	-5.17	-14.46	-11.59	2.87
Tyr	-3.75	-12.22	-8.47	-0.76	-1.41	-0.65
Arg	-10.43	-7.68	2.75	-4.00	-7.28	-3.27
Asn	-4.50	-5.06	-0.56	-0.90	0.74	1.64
average			**8.90**			**-8.39**

- Identified key residues are: ala16/val19 from helix, ile57, ala58 from sheet, gly35 from loop and leu8 from others. – Excellent agreement with the experimental report (Fersht and coworkers *J. Mol. Biol., 1995, 254, 289-304.*)



(a) (b)

Key-Residue-water/ EtOH VACF:



Water around key-residue

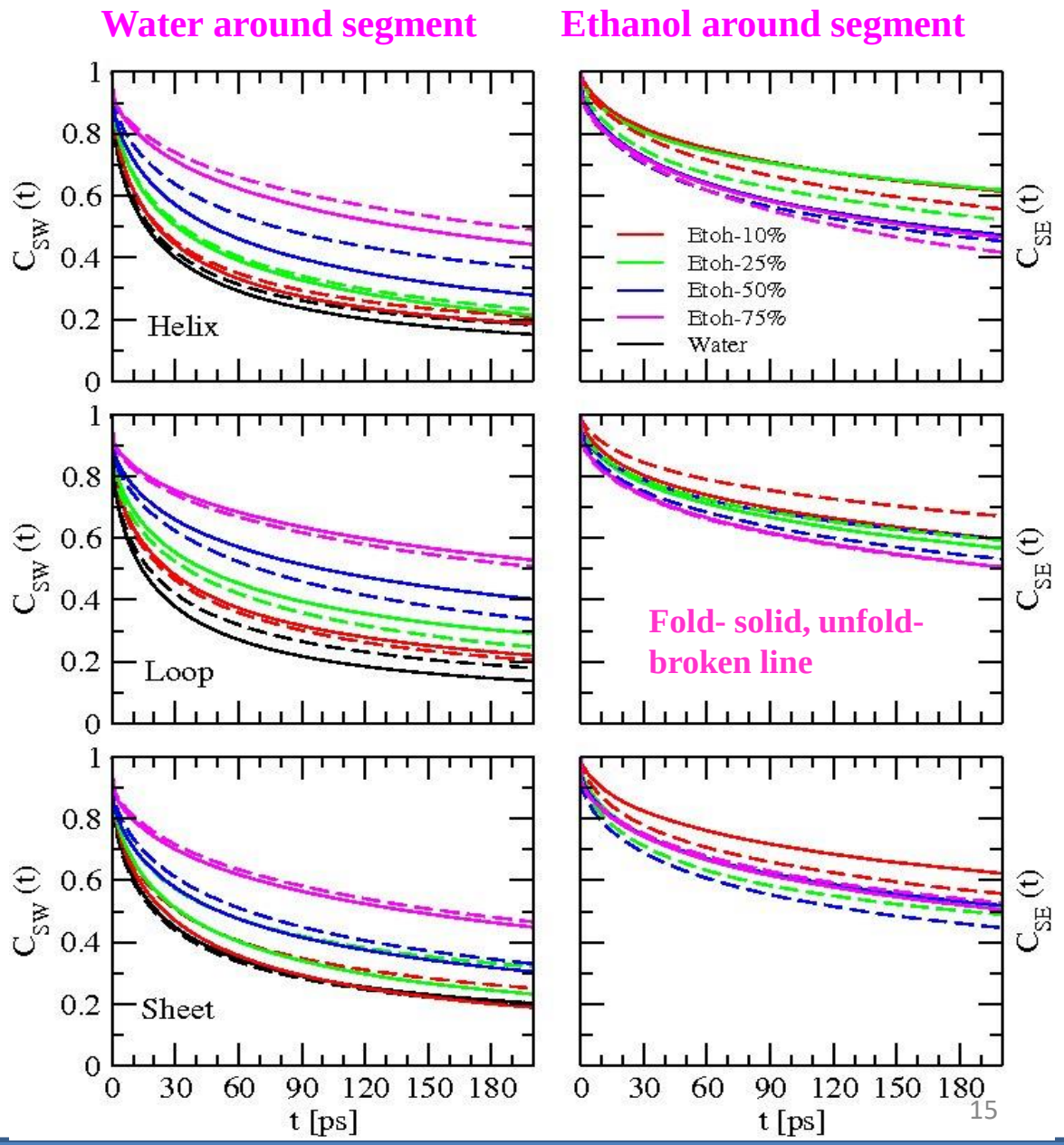
Ethanol around key-residue

- Rigid water-ethanol mixed layer around the key-residues – prominent upon unfolding
- Restricted translational motions of solvent around the unfolded Protein

D. Mohanta, S. Santra and M. Jana. ***Phys. Chem. Chem. Phys***. **2017**, 19, 32636-32646

Protein-Water vs Protein-Ethanol Hydrogen Bond Dynamics

- Restricted structural relaxation of **Protein-Water** HBs: unfolded > folded form (prominent at High Conc.)
- Faster structural relaxation of **Protein-Ethanol** HBs : unfolded > folded ➔ promotes water-unfolded protein interactions: inferring indirect mechanism



D. Mohanta, and M. Jana. ***Mol. Simul***. **2018**, 44, 1278.

Summary

- Along with methanol and ethanol, TFE is noted to be an efficient denaturants.
- Protein-water hydrogen bond dynamics shows restricted relaxation and the existence of long-lived protein-water hydrogen bonds at concentrated alcoholic solutions ➔ indirect effect of alcohol
- Binding interactions infer that residue-ethanol complexation is a favorable driving force for unfolding whereas residue-water complexation is a favorable driving force for folding.
- Direct protein-ethanol Hbond interactions and water mediated interactions play important role

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