



STRUCTURE AND DYNAMICS OF SS-BOND STABILIZED MINIPROTEINS



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Introduction

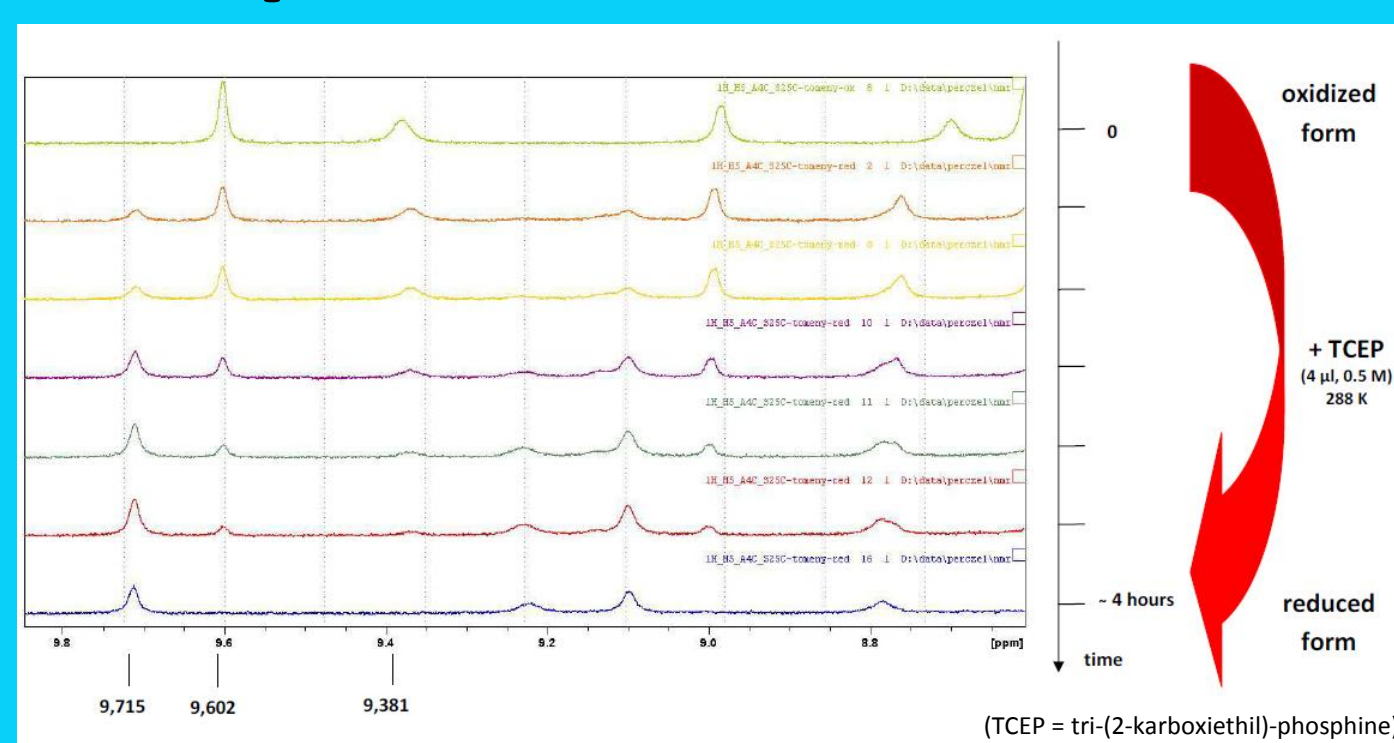
Exendin-4 (Ex-4) is a short (39 res.) but folded polypeptide used recently to treat Type 2 diabetes [1][2]. The C-terminus of Ex-4 (res. 20-39) is called as the Trp-cage foldamer supposed to stabilize the central α -helix (res. 10-28) of the macromolecule, which secondary structural element plays a key role in binding to GLP1 receptor at a nanomolar affinity.

Disulphide-bond stabilized Trp-cage analogues

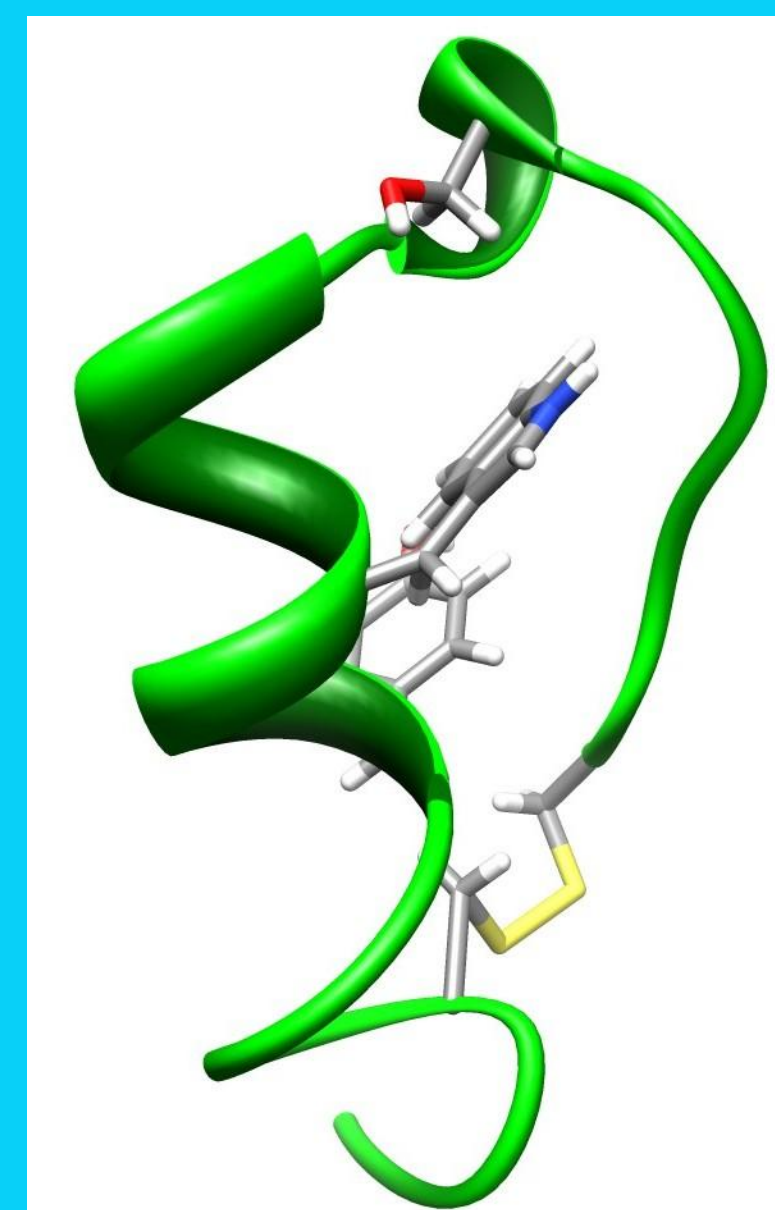
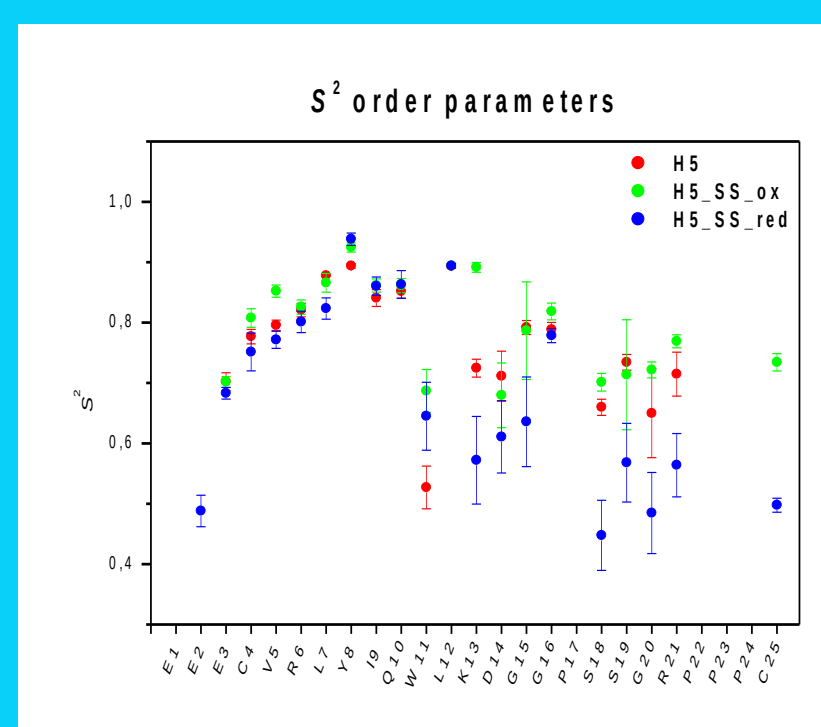
We have recently designed and synthesized new disulphide-bond stabilized Trp-cage analogues to reduce their internal mobility and to promote their crystallization. [3]

H2	AV RLYIQ WLKEG GPSSG RPPPS
H2_SS	CV RLYIQ WLKDG GPSSG RPPPC
H5	EEEAV RLYIQ WLKEG GPSSG RPPPS
H5_SS	EEECV RLYIQ WLKDG GPSSG RPPPC

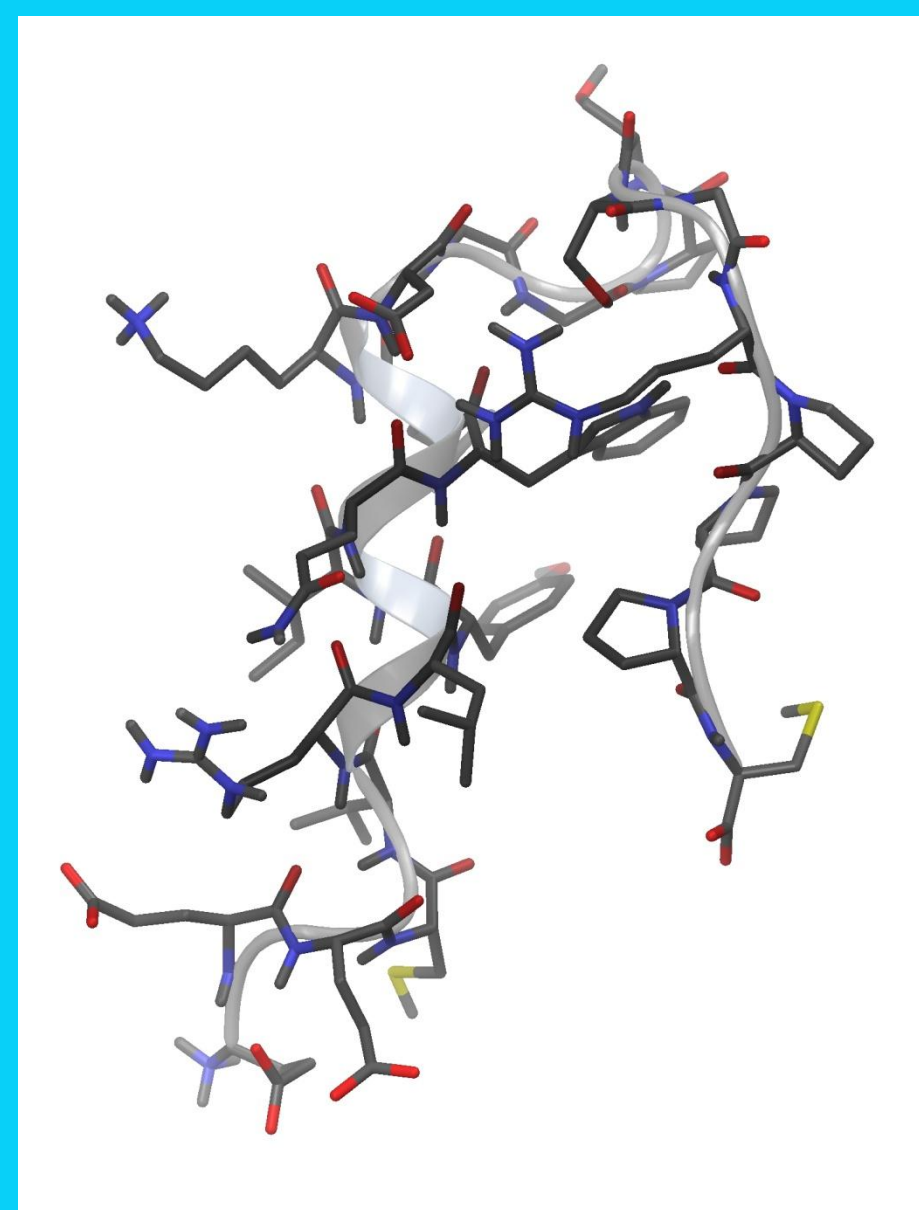
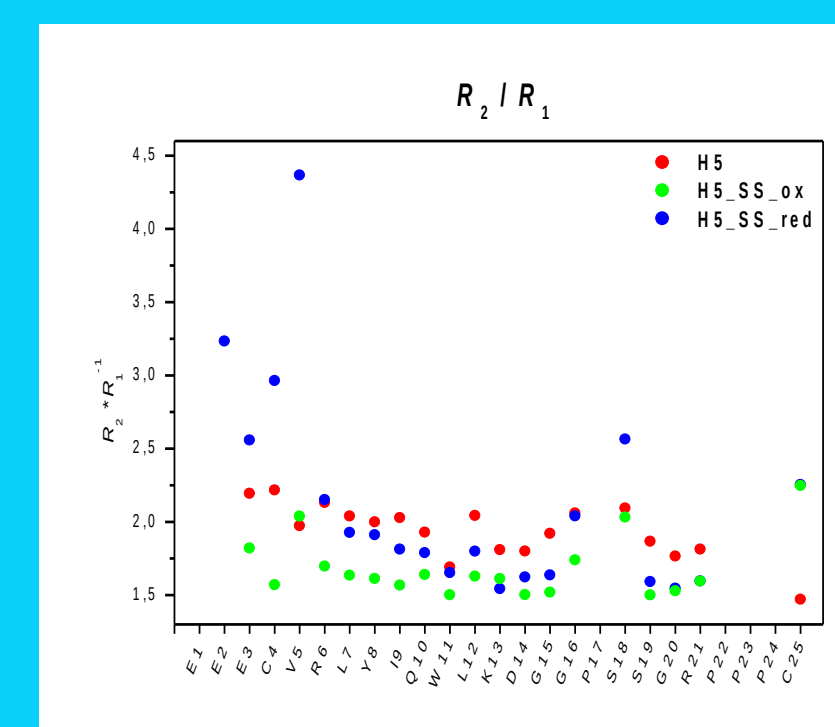
NMR-detected *in situ* reduction of disulphide-bond



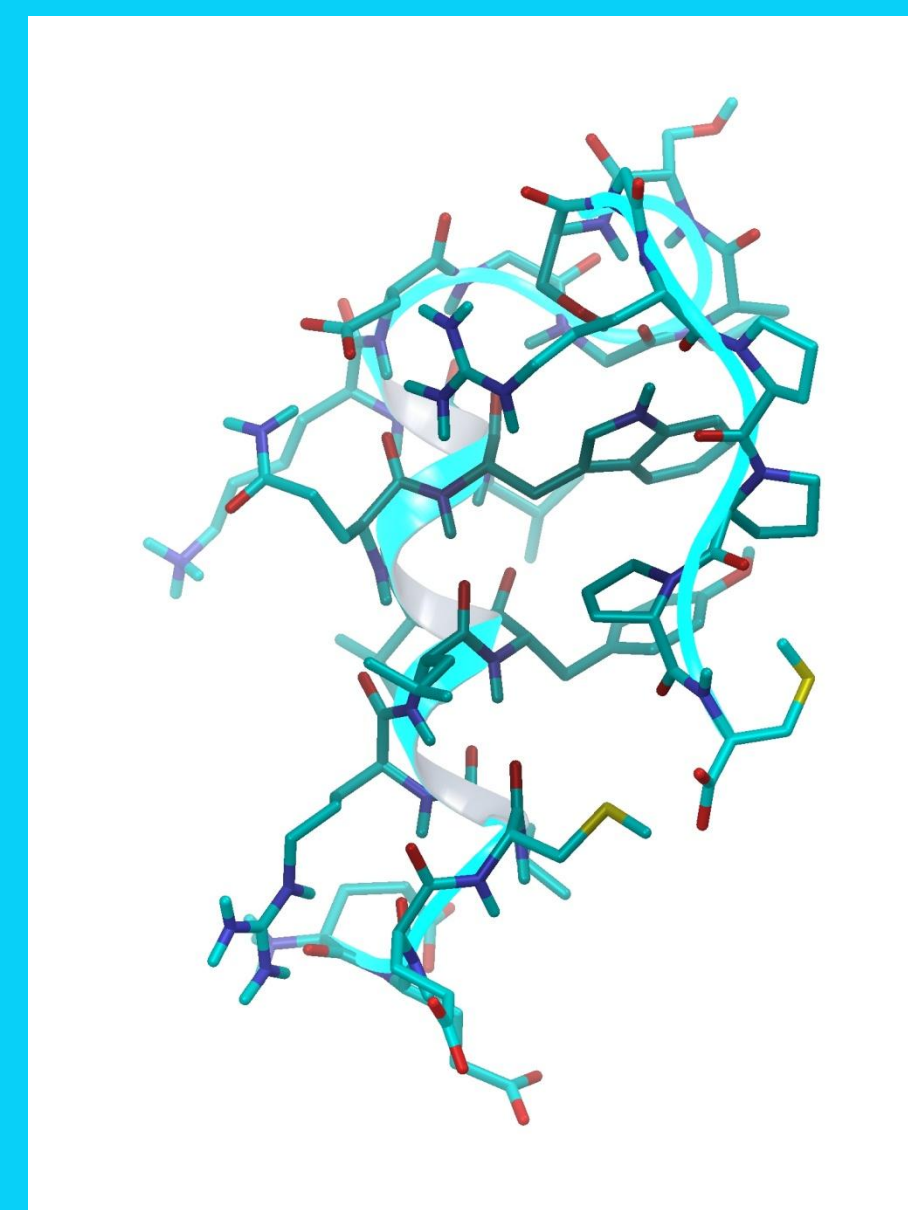
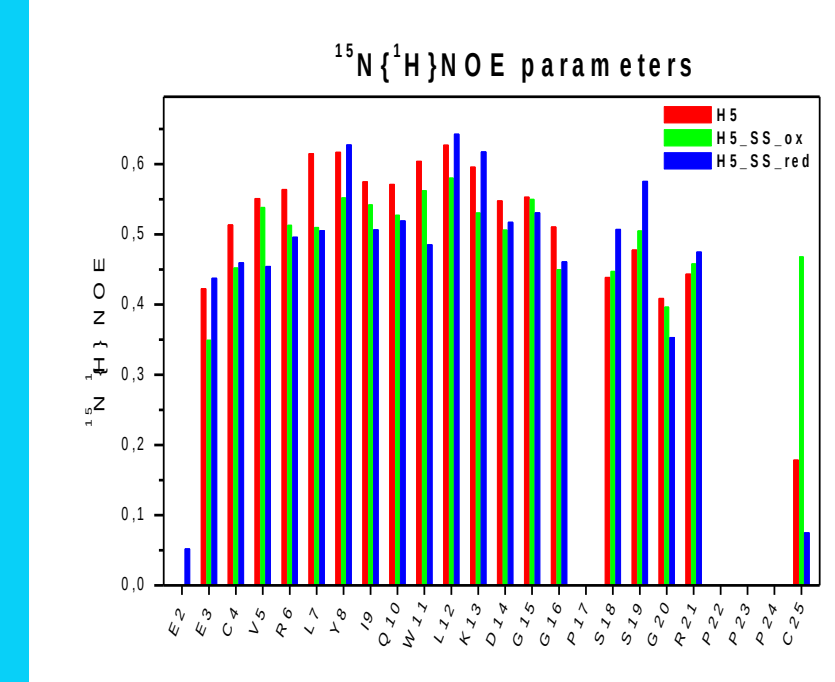
Dynamical study



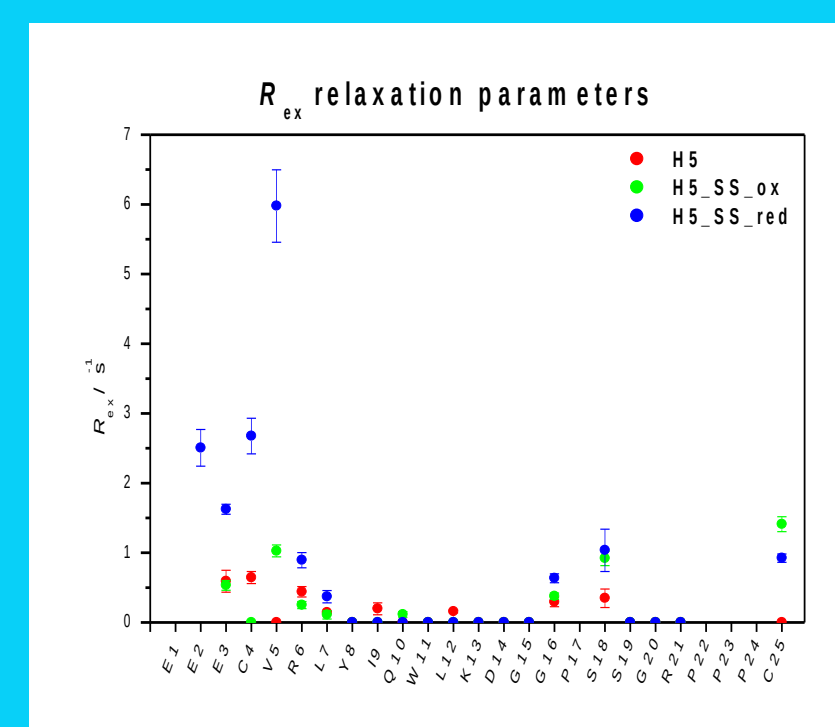
H5_SS_ox ($\tau_c=2,21$ ns)



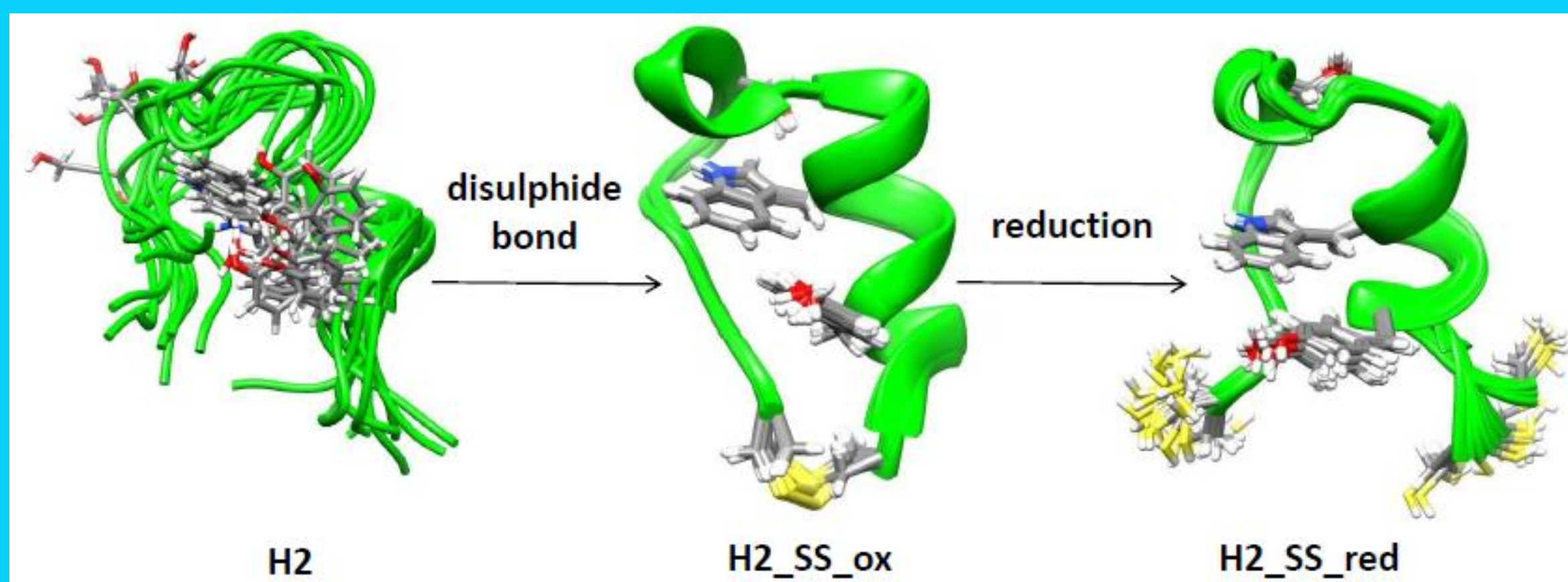
H5_SS_red ($\tau_c=2,57$ ns)
most dominant (~ 80 %) conformer



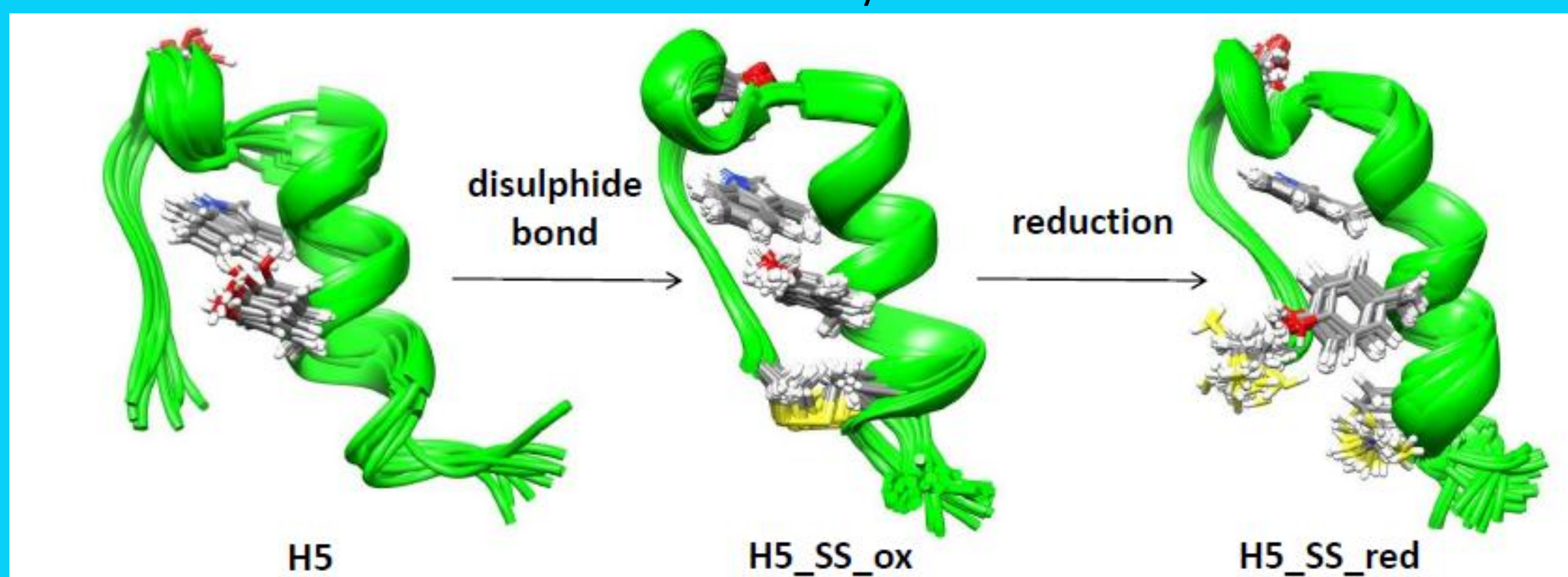
H5_SS_red ($\tau_c=2,57$ ns)
minor component with Cys 4 forming basically 2 types of associations with terminal Cys 25:
-most frequently: (Cys 4)SH----COO⁻(Cys 25) – cyan
-in some: (Cys 4)HS----HS(Cys 25) – green



Structural study

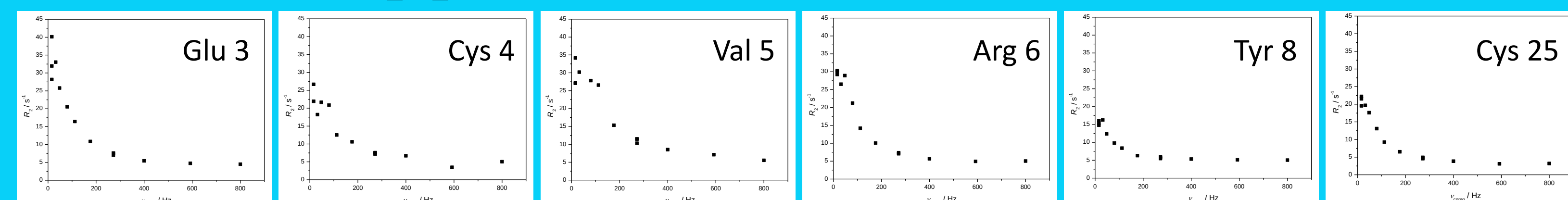


structural stability at 288 K



H2 <<< H2_SS_red $\approx$ Tc5b $\approx$ H5 < H5_SS_red < H5_SS_ox < H2_SS_ox

CPMG measurements of H5_SS_red at 300 K



Homo- and heteronuclear NMR spectroscopic methods were used to decipher structural and dynamics changes of disulphide bond containing variants as function of their chemical composition and state. Both the oxidized and the reduced forms of H2 and H5 were characterized and compared to the parent H2 and H5 miniproteins. As long as H2 is largely unfolded, presenting high internal dynamics on the ns-ps time scale of motion, H5 has a nice fold easy to characterize with a single 3D structure in a time average manner. Thus, the introduction of a smartly placed disulphide bridge could induce larger structural changes and stabilization for the less folded H2 then for the more folded H5 nanosystem

Results and discussion

Following the resonance assignment and NOE based structure characterization of both the reduced and oxidized forms of H2_SS and H5_SS we have found cysteine residues of both H2_SS and H5_SS stabilize the molecules even in their reduced forms. Thus, the interaction of Cys results in an increased structural stability even via their SH groups.

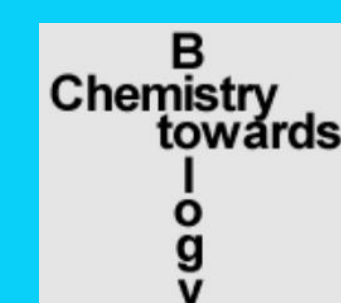
These findings are also supported by MD simulations. Local structure stabilizing effects such as H-bond between the SH and the C-terminal carboxyl group could play a role. These H-bonds with additional structure stabilizing effects could have a role in modulating backbone dynamics of H5_SS(red) so that specific slow dynamics appear. Fast (ns-ps) and slow (ms) backbone motions detected by R_1 , R_2 , hetNOE and CPMG measurements of the present nano-system will be presented.

References

- [1] Neidigh, J. W., Fesinmeyer, R. M., Prickett, K. S., Andersen, N. H., *Biochemistry*, vol. 40, pp. 13188-13200, 2001.
- [2] Neidigh, J. W., Fesinmeyer, R. M., Andersen, N. H., *Nat. Struct. Biol.*, vol. 9, pp. 425-430, 2002.
- [3] Hudák, P., Stráner, P., Farkas, V., Váradi, Gy., Tóth, G. K., Perczel, A., *Biochemistry*, vol. 47, pp. 1007-1016, 2008.

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