Dynamic conformational ensembles: challenges of multiple time scales and multidomain proteins

Zoltán Gáspári, Nóra Epresi

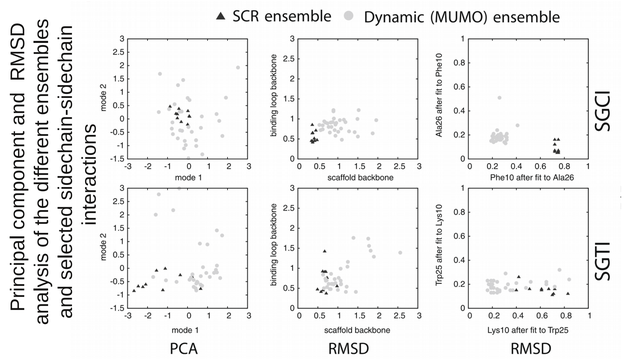
Faculty of Information Technology and Bionics,
Pázmány Péter Catholic University, Budapest, Hungary

http://itk.ppke.hu/~gaszo | gaspari.zoltan@itk.ppke.hu

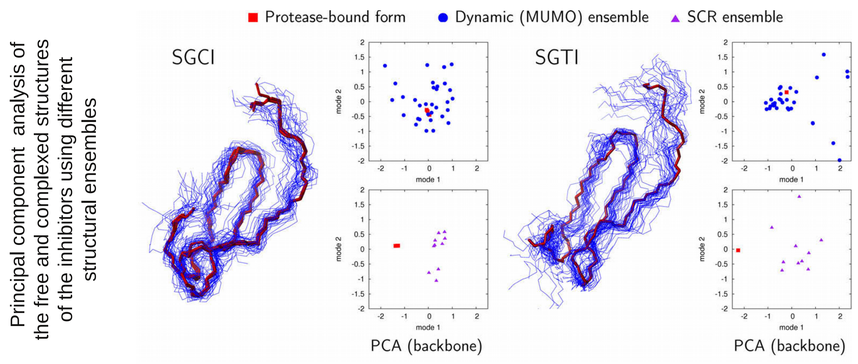
A previous application: small canonical serine protease inhibitors

- Standard mechanism, canonical inhibitors comprise multiple, unrelated protein families
- Substrate-like interaction with the target enzyme
- Common structural motif: protease binding loop
- Conventional view of efficiency [1]:
  - Rigidity of the binding loop is key
  - No conformational change upon enzyme binding
  - Based primarily on X-ray structures of free and complexed inhibitors

- NMR studies consistently reveal increased flexibility in the protase binding loop (ps-ns time scale)
- SGCI & SGTI: small inhibitors from desert locust with high efficiency and dynamic overall structure



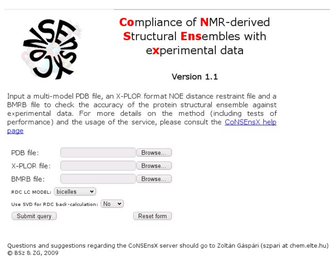
- Dynamic structural ensembles of the inhibitors are more diverse than SCR (single-conformer refinement) ones
- At the same time, they are more realistic than those calculated by conventional methods

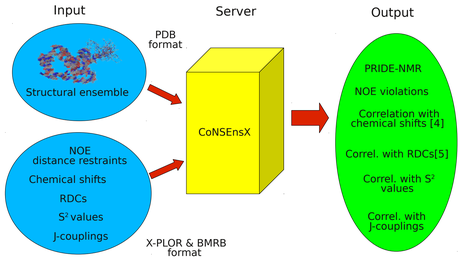


- Enzyme-bound conformers are present in the solution state
- The conformational transitions are much faster (ps-ns scale) than the association with the enzyme (ms time scale)

- No energetic cost of protease binding related to conformational changes in 'nanosecond-timescale conformer selection'
- This is in agreement with the expectations of the rigid binding loop model [2]

The CoNSEnsX web server to analyze dynamic conformational ensembles



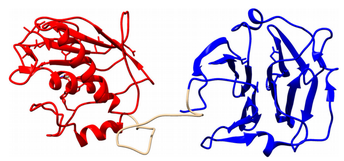


- http://consensx.chem.elte.hu [3]
- Asesses the correspondence of back-calculated observables to measured ones for the full ensemble

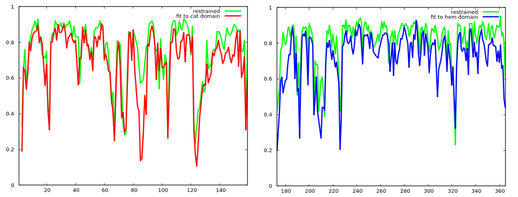
Details of the implementation

- In-house extensions for GROMACS 3.3.1 & 4.5.5, available for download on our home page
- Usage conforms to GROMACS conventions, restraints can be specified in the topology, other parameters in the run control file
- Order parameters (S^2) implemented as described in [6] and with fitting to reference group at each calculation step
  - Modification #1: (only in 3.3.1 yet): an induvidual fit group can be defined for not fully rigid molecules
  - Modification #2: can be defined for sub-ensembles for the generation of ensembles reflecting motions on multiple time scales
- NOE: pairwise averaging over replicas is implemented (similarly to the MUMO approach [7])

Exploratory calculations



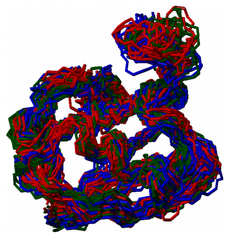
- The catalytic (red) and hemopexin-like (blue) domains of MMP12 show largely independent reorientation [7]
- Using synthetic S^2 values back-calculated from a 5 ns MD run (GROMACS 4.5.5, amber99sb FF, TIP3P water) for both domains separately
- Restraining in GROMACS 3.3.1. (0.3 ns, oplsaa FF, 4 replicas) with two fit groups corresponding to the two domains

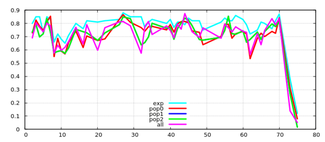


Challenges:

- Get better agrement for the flexible loops
- Can we obtain meaningful S^2 values from experimental relaxation data for such a protein?

- Ubiquitin displays motions on the submicrosecond time scale that are not present on the ps-ns time scale [9]
- Using experimental S^2 values and back-calculated RDCs from the EROS ensemble (PDB ID 3K39)
- Restraining in GROMACS 4.5.5. (1.2 ns, amber99sb FF, 3X4 replicas)





	N-H S^2			N-H RDC			Cα-C RDC		
	Pearson	Q (%)	RMSD	Pearson	Q (%)	RMSD	Pearson	Q (%)	RMSD
pop0	0.936	10.81	0.08	0.905	51.06	2.66	0.872	68.8	0.74
pop1	0.927	12.15	0.09	0.979	45.93	2.12	0.957	61.76	0.60
pop2	0.896	12.94	0.09	0.972	39.53	1.95	0.960	49.74	0.53
all	0.917	14.93	0.10	0.975	41.87	2.01	0.944	59.07	0.60

Challenges:

- Find the optimal combination of relative restraint weights as well as the number of replicas and sub-ensembles
- Find the motional modes that discriminate the motions on different time scales
- Try to understand the connections between motions by detailed analyss of residue-residue interactions

References

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Acknowledgements

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