intrinsically disordered

Determination of the structure and dynamics of proteins using NMR chemical shifts (CS) and CS enhanced protein data bank (CS-PDB)

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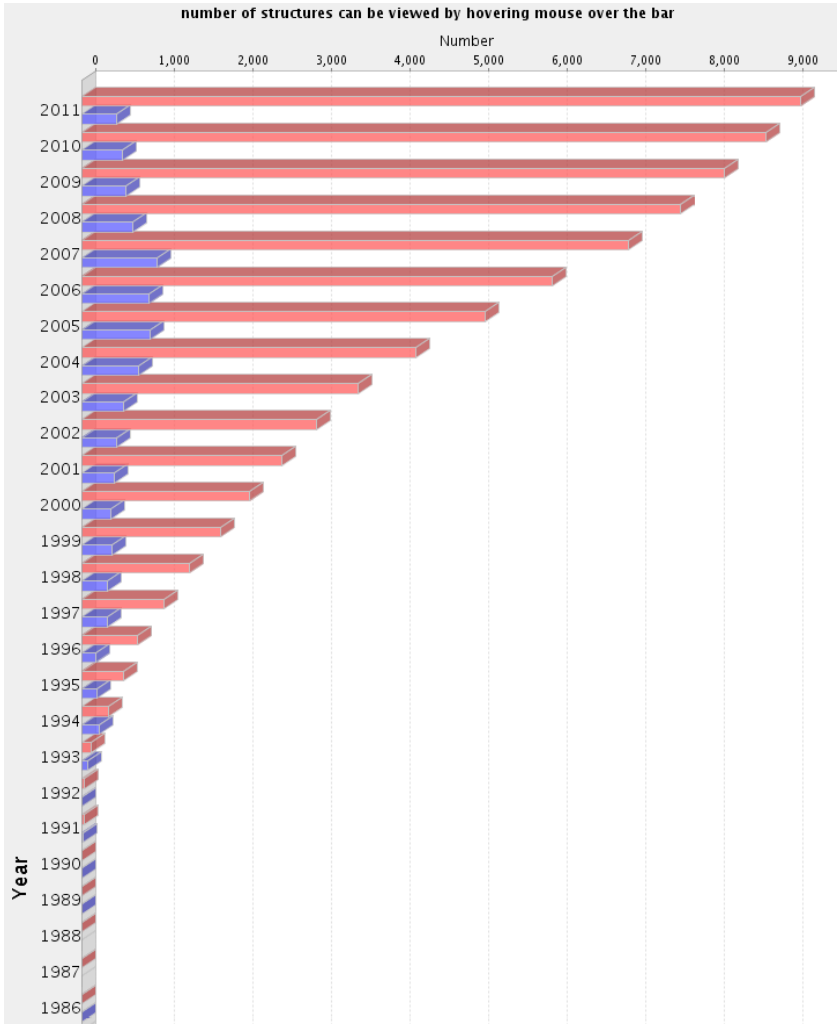
Protein NMR needs a boost!



"The rule of thumb for selecting NMR structures for inclusion in structural analysis has been the simple one of excluding them altogether!"

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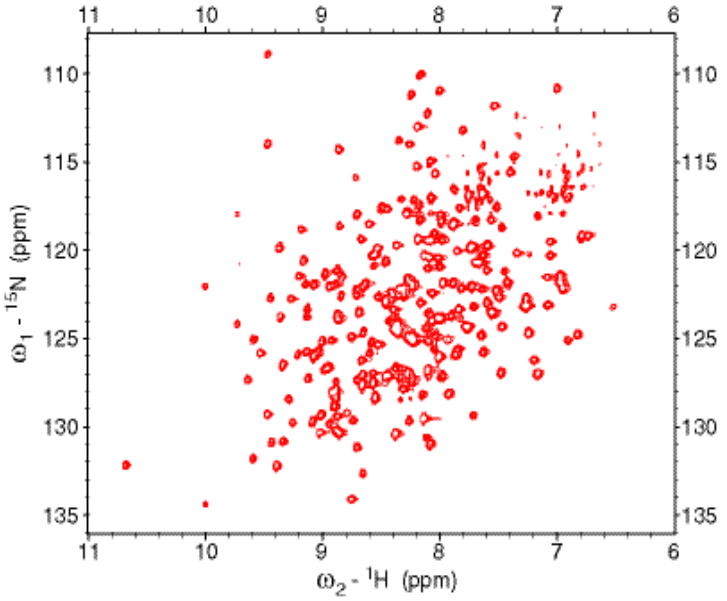
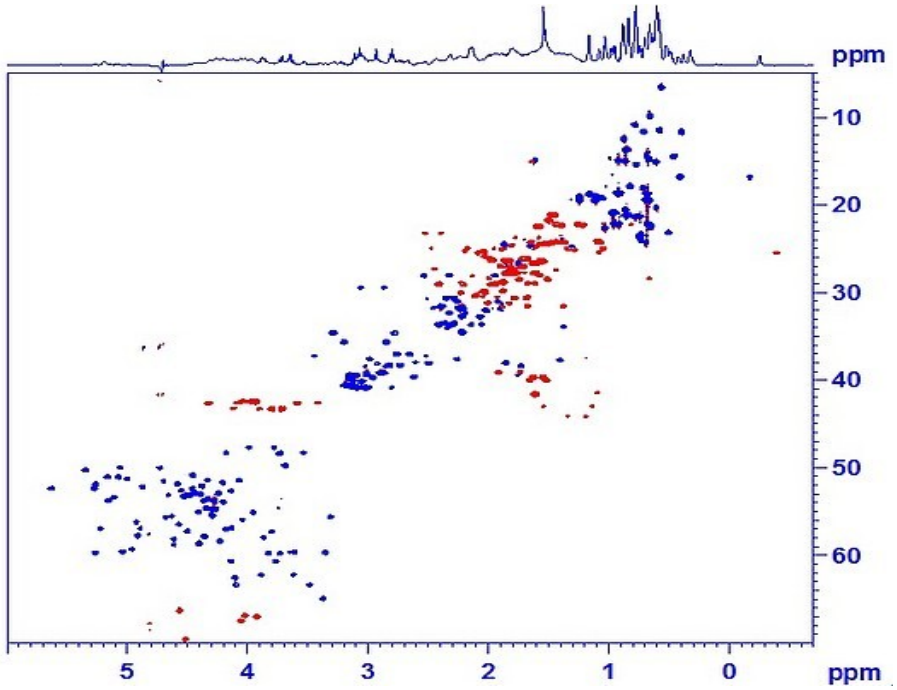
Roman Laskowski (Author of PROCHECK)

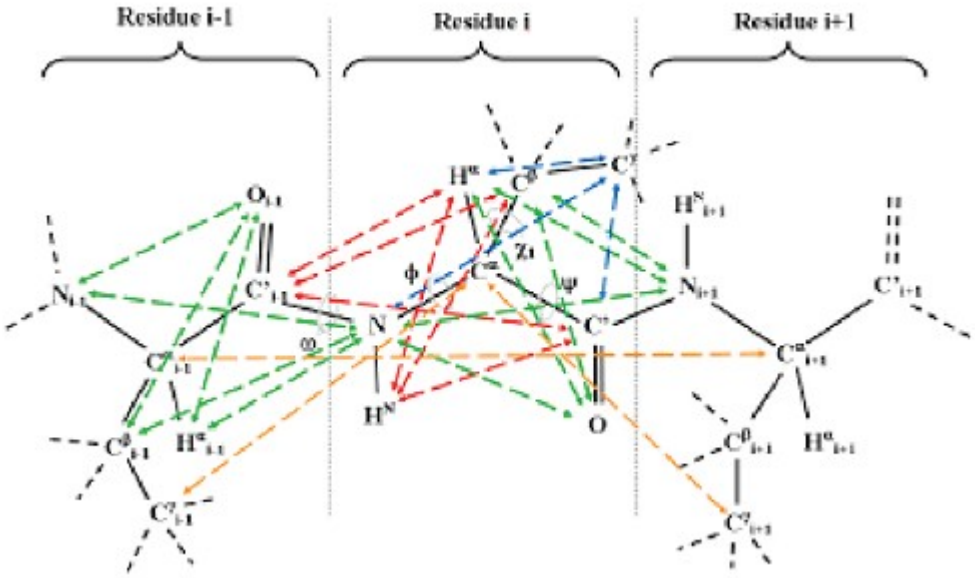
NMR chemical shifts are valuable





Chemical shifts are
1) sensitive probes of protein conformations
2) the most accessible NMR observable

Making use of the chemical shifts 1) chemical shift prediction (method of CamShift)



$$\delta_a^{pred} = \delta_a^{rc} + \sum_{b,c} \alpha_{bc} d_{bc}^{\beta_{bc}}$$

Pros:

1) Less prone to the pitfall of database bias
2) The chemical shifts are differentiable with respect to the atomic coordinates
3) It is a rapid method of comparable accuracy to the best performing protocol

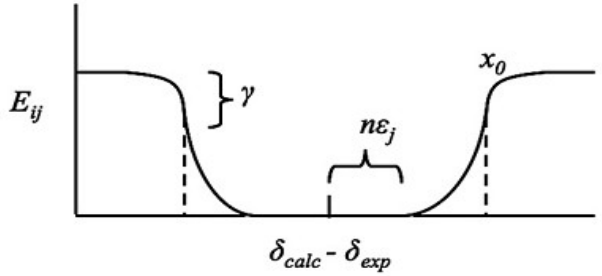
Kohlhoff, K. J. , Robustelli P., Cavalli A., Salvatella X., Vendruscolo M. (2009). Fast and Accurate Predictions of Protein NMR Chemical Shifts from Interatomic Distances. J. AM. CHEM. SOC. 131, 13894–13895

Making use of the chemical shifts
2) Applying the chemical shift restraints

The use of the chemical shifts is obtained by adding an E_{cs} energy term to the force field:

$$E_{cs} = \lambda \sum_{i=1}^{N} \sum_{j=1}^{6} E_{ij} \left(\delta_{ij}^{calc} - \delta_{ij}^{exp} \right)$$

The functional form of the chemical shift penalty function is:



Robustelli P.,Kai Kohlhoff K., Andrea Cavalli A., Vendruscolo M. (2010). Using NMR Chemical Shifts as Structural Restraints in Molecular Dynamics Simulations of Proteins. Structure 18: 923-933

Camilloni C., Robustelli P., Simone A. D., Cavalli A., Vendruscolo M. (2012). Characterization of the Conformational Equilibrium between the Two Major Substates of RNase A Using NMR Chemical Shifts. JACS. 134(9): 3968-3971

Making use of chemical shifts
3) Restraining the MD simulations

The MUMO (minimal under-restraining minimal over-restraining) method:
1) When the protein structure fluctuates between multiple well-defined conformers, it is improper to force the restraints as a single harmonic term (over-restraining)

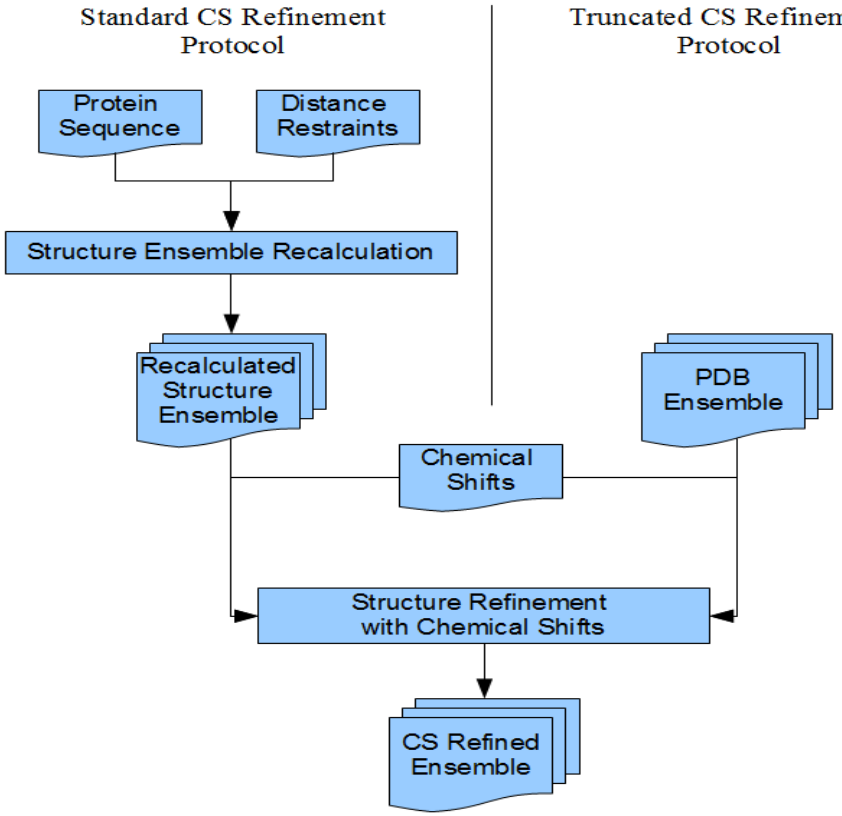
One solution is to use multiple replica and apply replica averaged restraints

$$\delta_{ij}^{calc} = \frac{1}{M}\sum_{k=1}^{M}\delta_{calc}^{ij,k}$$

The optimal value of *M* was found by the strategy of "test of reference ensemble"

Richter, B., Gsponer, J., Varnai, P., Salvatella, X., Vendruscolo, M. (2007). The MUMO (minimal under-restraining minimal over-restraining) method for the determination of native state ensembles of proteins. J Biomol NMR. 37:117–135

Results: CS-Refine Pipeline

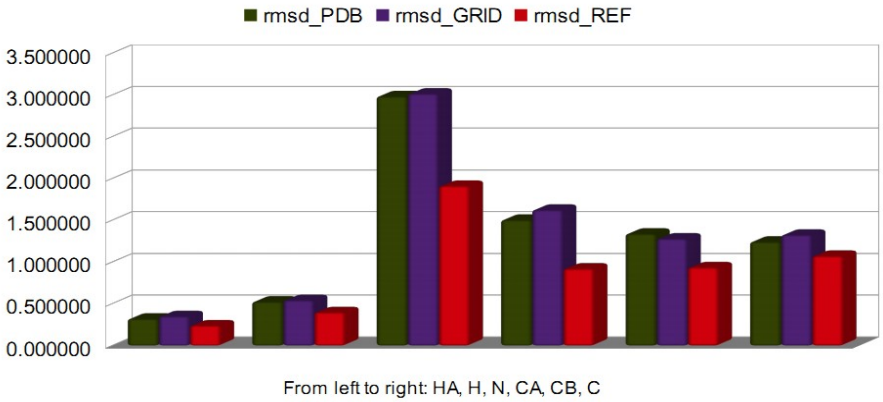


With the current version of Almost, the chemical shift of Hα, Cα, Cβ, C', HN, and N atoms can be restrained for NMR structure refinement.

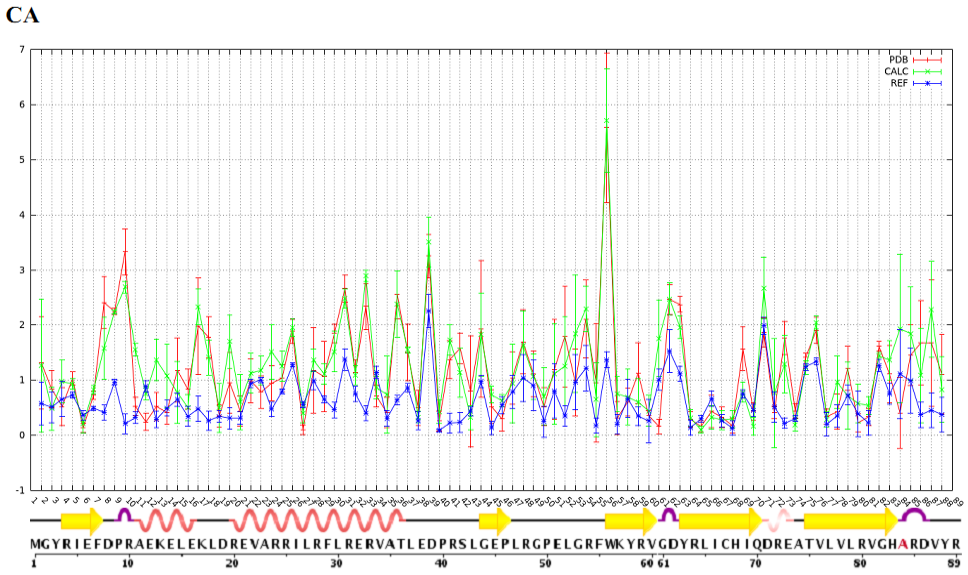
The data fetching, structure calculation and refinement processes are automated with CS-Refine pipeline

Fu B., Camilloni C., Vendruscolo M. and Cavalli A. (2012). ALMOST: an open source framework for structure determination. Manuscript in preparation. Http://open-almost.org

Results: CS-Refine Pipeline



From left to right: HA, H, N, CA, CB, C



The agreement between experimental and theoretical chemical shifts was significant improved.

Results: CS-Refine Pipeline

The distance-based restraints and the chemical shift restraints can be simultaneously refined.

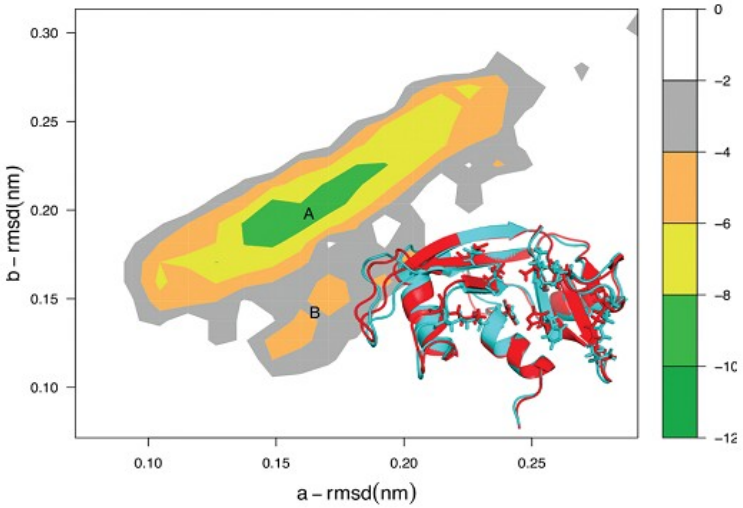
Distance Restraint Violation Statistics					
	> 0.1 Å (number of restriant)				
ID	noe_PDB	noe_DREF	noe_GRID	noe_REF	noe_REF_noCS
2ITH	466 ± 9	67 ± 3	372 ± 6	89 ± 3	78 ± 4
2LA3	363 ± 31	33 ± 4	351 ± 10	22 ± 2	19 ± 2
2KZC	132 ± 13	12 ± 2	94 ± 3	9 ± 1	8 ± 1
1R73	138 ± 12	19 ± 4	157 ± 4	14 ±1	14 ± 1
2KJW	49 ± 3	9 ± 2	98 ± 5	10 ± 3	5 ± 2
2LDK	40 ± 6	0 ± 0	40 ± 9	0 ± 0	0 ± 0

Thus the CS-based refinement protocol can be included as a standard step in refining NMR structures.

Results: CS restrained MD simulation

The number of two replicas was found optimal for applying chemical shift restraints

The free energy surface of Rnase A was correctly characterized by CS restrained MD simulation



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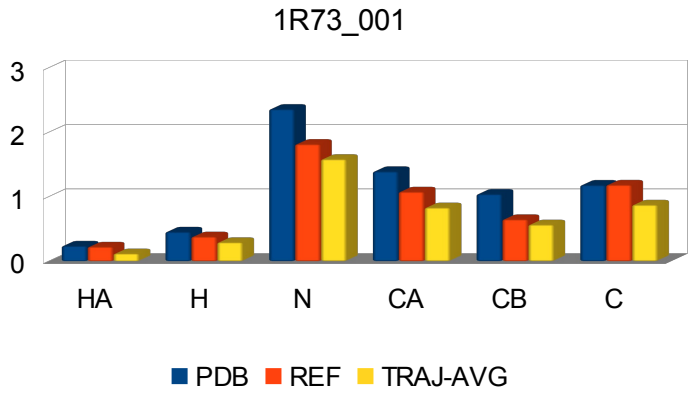
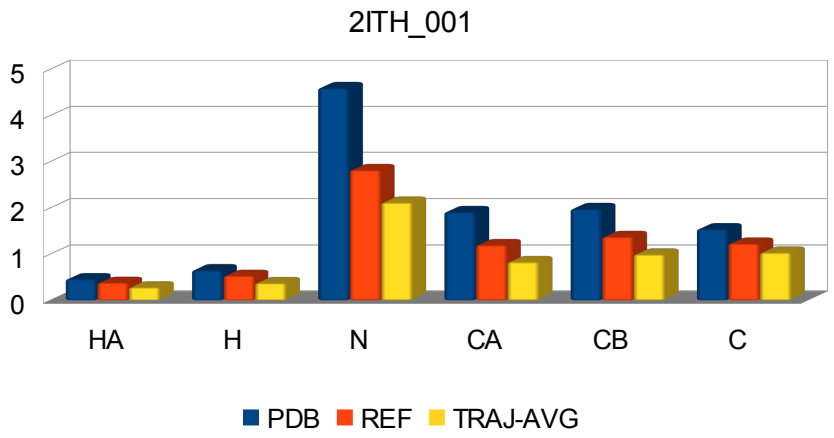
Results: CS restrained MD simulation

By averaging the chemical shift predictions along the MD trajectories, the experimental-calculated chemical shift agreements were significantly improved





The Values of CS-RMSD Improvements in Percentage

	Hα	HN	N	Cα	Cβ	C'
1R73_001	35%	19%	5%	22%	20%	22%
1R73_020	40%	24%	14%	25%	22%	22%
2ITH	25%	28%	20%	28%	26%	18%

From the CS refined structure, the further improvements are on average about 20% (5%- 40%)

Future works

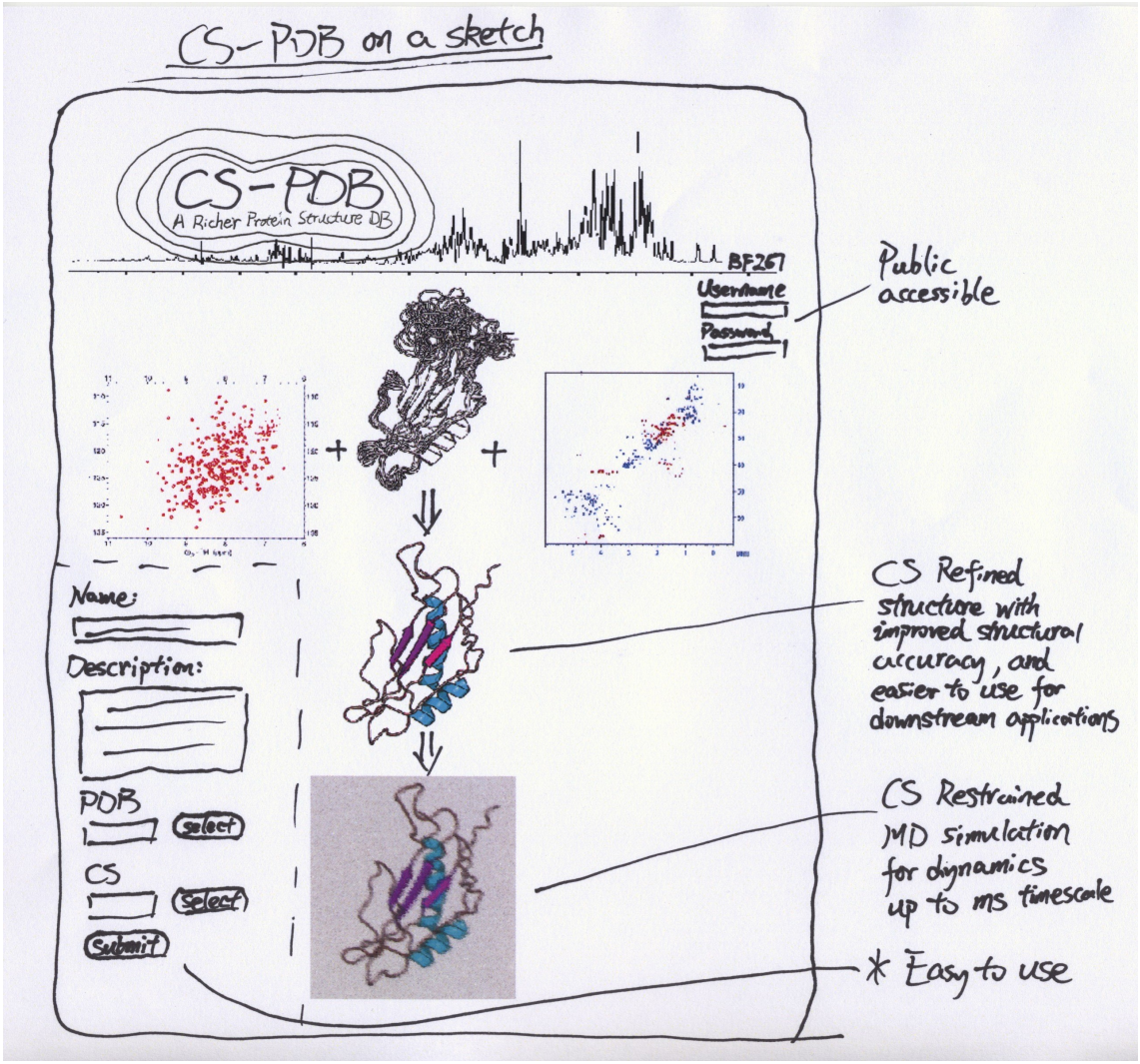
Adding support of the side chain chemical shifts (methyl and aromatic groups) into the protocol

Making plots of protein near equilibrium free energy landscapes

To resolve the PDB paradox - CS-PDB

- People want structures with higher resolutions/precisions
- Proteins are flexible

CS-PDB – A richer Protein Structure Database enhanced with Chemical Shifts!



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IDPbyNMR colleagues and fellows

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