Protein disorder studied by a synergy of experiment and simulation: Molecular dynamics

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Contents

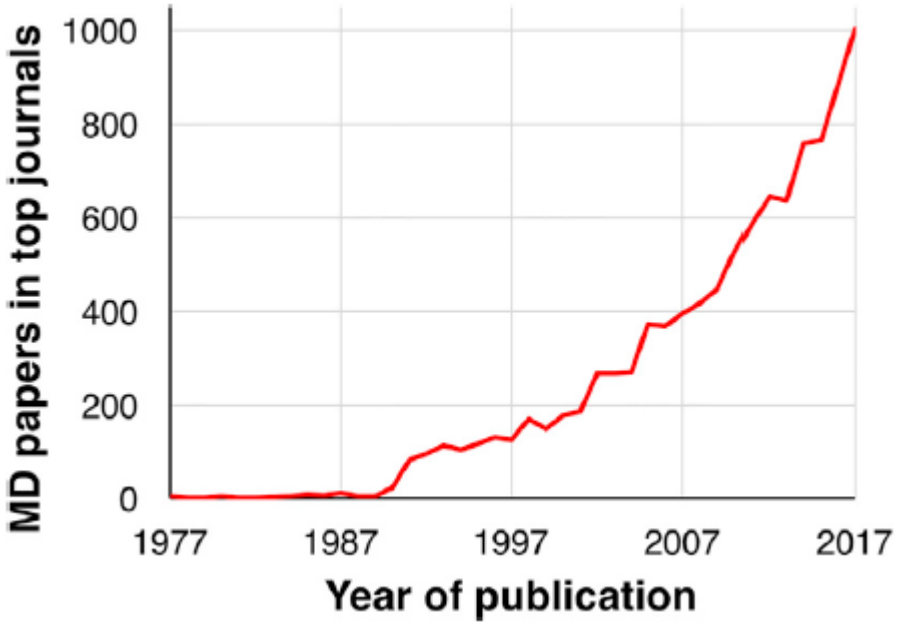
- Molecular dynamics (MD) simulations - method
- State of the art of MD with IDP linear motifs and tau protein
- Results:
  - MD simulations performed with short wild type and mutated tau peptide 218-226 (T220A)

Molecular dynamics

- Computational method - calculates the time dependent behavior of a molecular system
- Molecular dynamics simulations use a classical Newtonian representation of atoms and molecules
- The forces between them are encoded in a force-field which contains all the chemical specificity

- Provides detailed information on the fluctuations and conformational changes of proteins and nucleic acids
- Able to identify transitions and metastable states formed on 0.1 - 10 μs timescale
- Need for high performance computing

Molecular dynamics



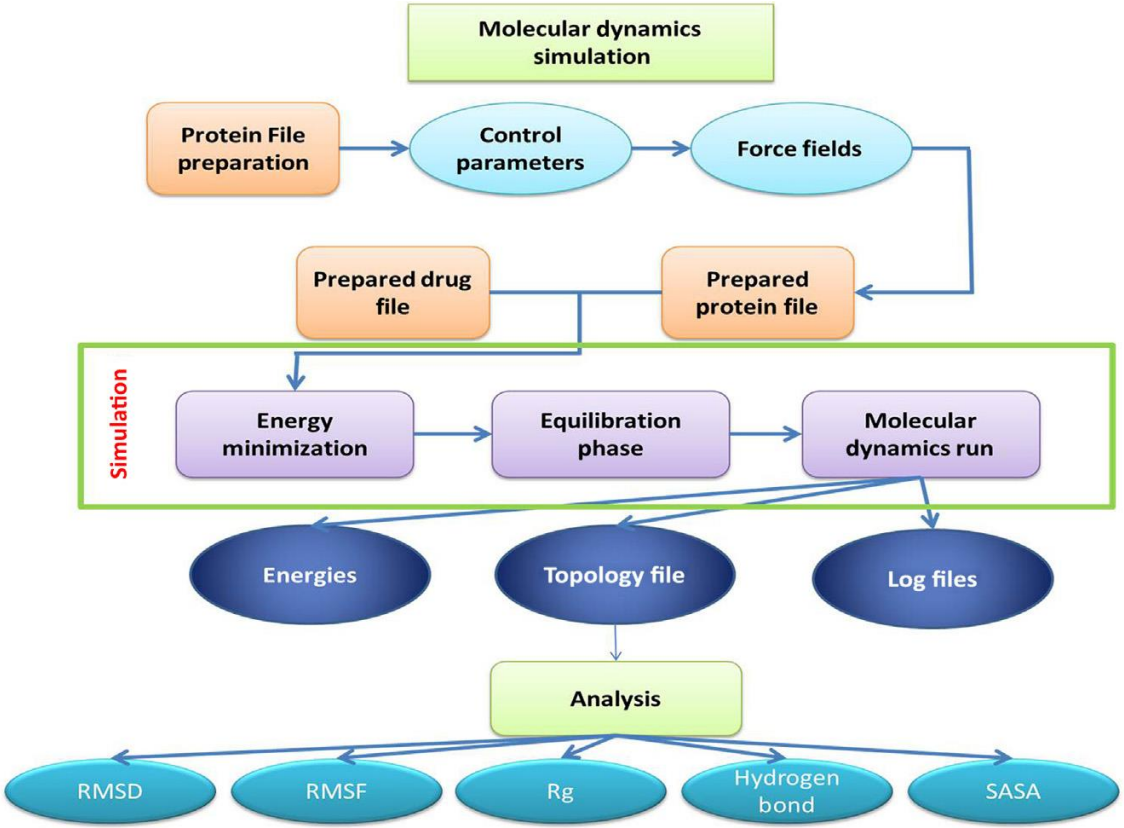
For the top 250 journals by impact factor, the number of publications per year that include the term ‘‘molecular dynamics’’ in either the title, abstract, or keywords was plotted. The analysis was performed via Web of Science in February 2018.

Hollingsworth&Dror 2018 Neuron

Molecular dynamics

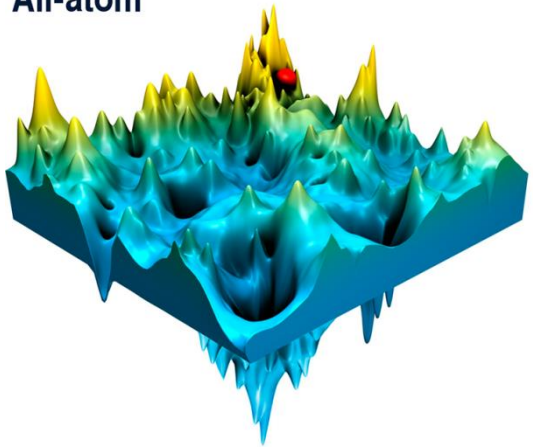
- MD program selection: GROMACS, NAMD, AMBER, CHARMM, Desmond, ...
- Method selection: clasical MD, enhanced sampling methods, coarse grained MD
- Force field selection
- Solvent modeling- explicit/implicit solvent

Molecular dynamics - workflow

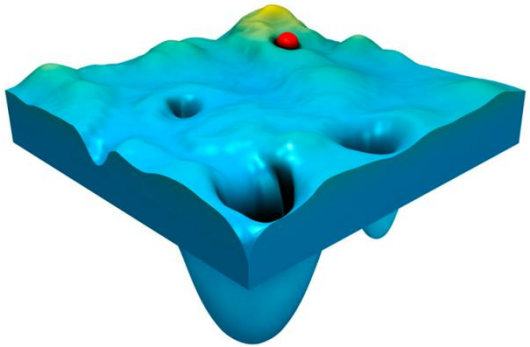


Coarse-grained molecular dynamics

All-atom



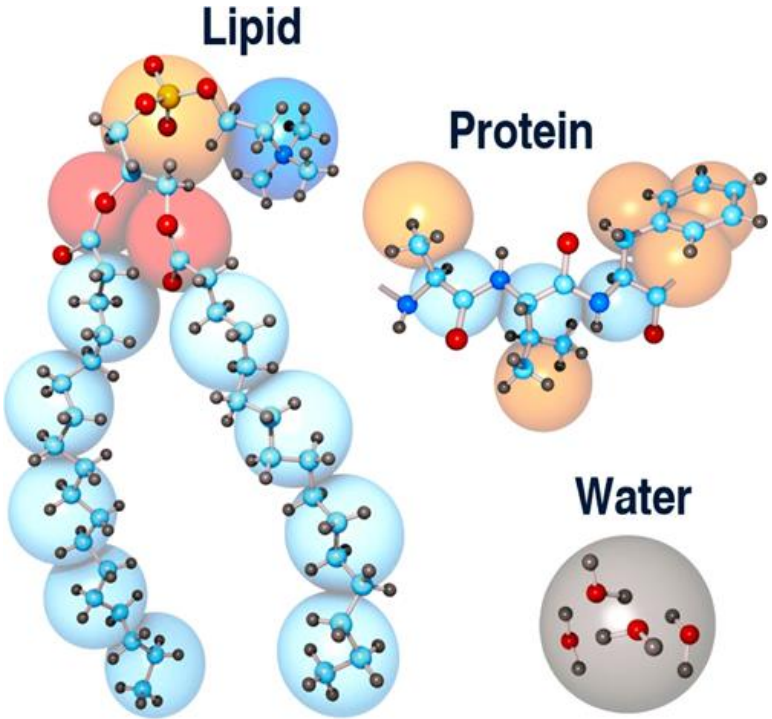
Coarse-grained



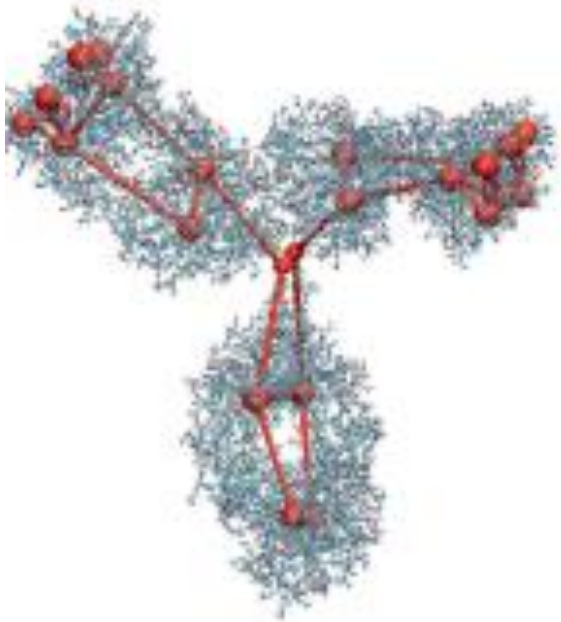
Lowering the level of protein representation from all-atom to coarse-grained opens up new possibilities for studying protein systems

Smoothening of the energy landscape in a coarse-grained model as compared to an all-atom model

Coarse-grained molecular dynamics



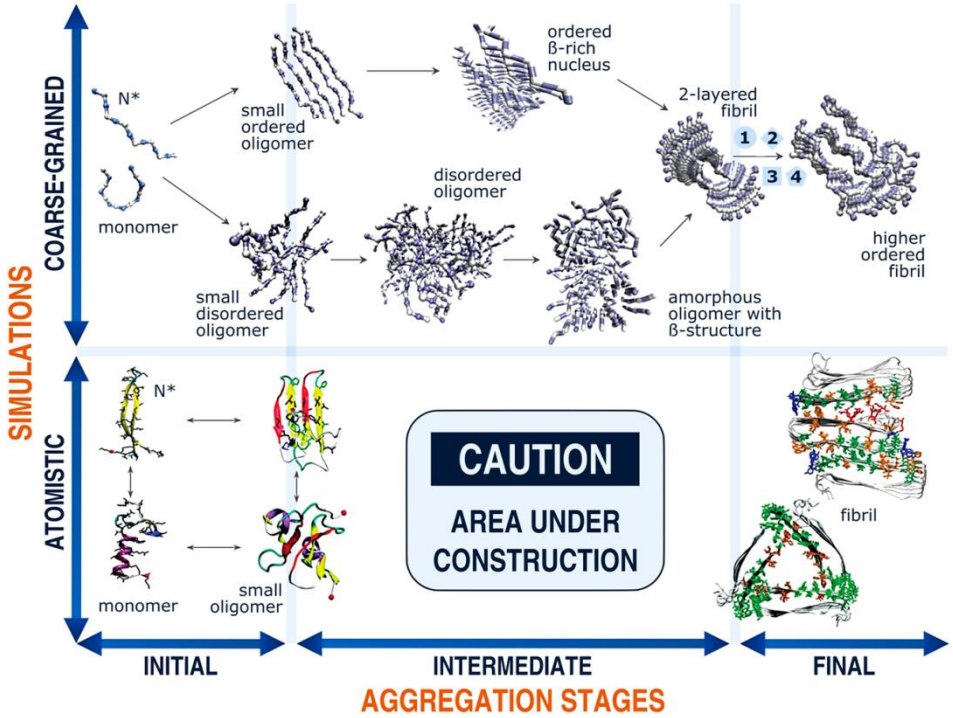
All-atom versus coarse-grained representation in the MARTINI model



Chaudhri 2013 *J. Phys. Chem. B*

Kmiecik et al. 2016 *Chem Reviews*

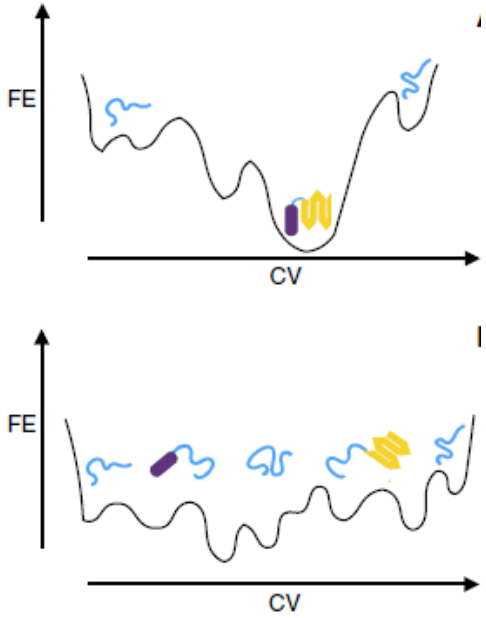
Coarse-grained molecular dynamics



Enhanced sampling methods

Obtaining adequate sampling remains an issue in atomistic simulations in explicit solvent

Particularly true for IDPs - high conformational heterogeneity.

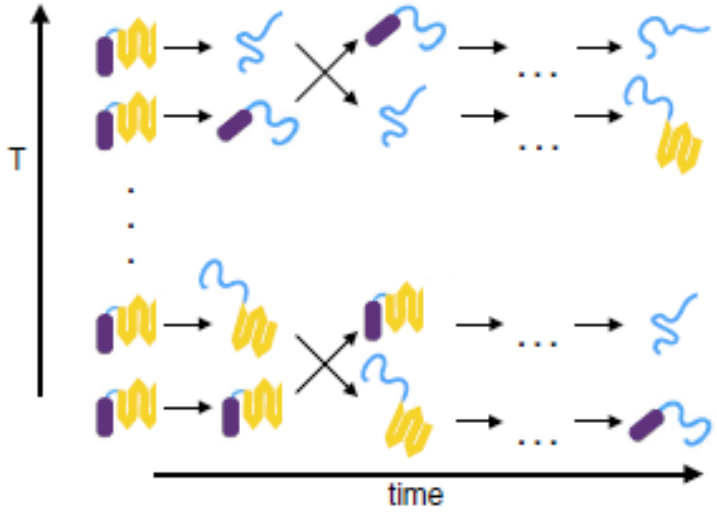


Schor et al. 2016 Biophys Rev

Replica exchange molecular dynamics

Temperature REMD- multiple copies (replicas) of the system are simulated in parallel, all at different temperatures

Exchanges between replicas adjacent in temperature take place

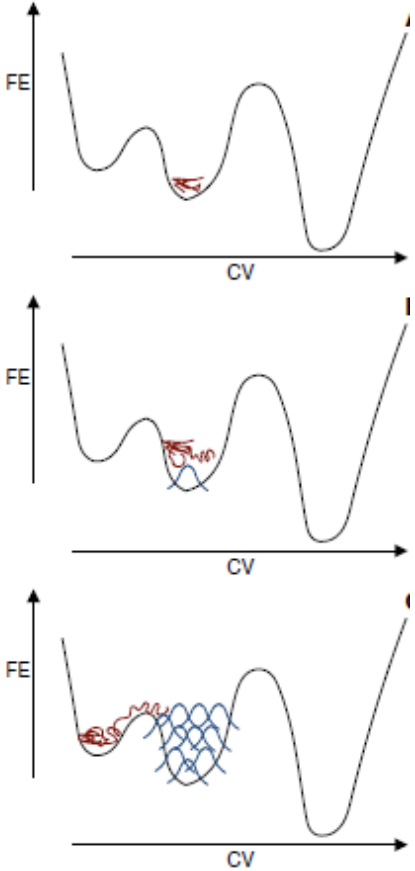


Schor et al. 2016 Biophys Rev

Metadynamics

The system is discouraged from visiting previously explored regions by a biasing potential.
This history-dependent biasing potential is built by periodically depositing Gaussians along the trajectory of the collective variable (CV)
Adaptations:

- Well tempered metadynamics – Gaussian hight is scaled
- Bias-exchange metadynamics – replicas biased in independent CVs



Schor et al. 2016 Biophys Rev

Force field

$$U(\vec{R}) = \sum_{\text{bonds}} K_b(b - b_0)^2 \tag{1a}$$

$$+ \sum_{\text{angles}} K_\theta(\theta - \theta_0)^2 \tag{1b}$$

$$+ \sum_{\text{dihedrals}} K_\chi(1 + \cos(n\chi - \delta)) \tag{1c}$$

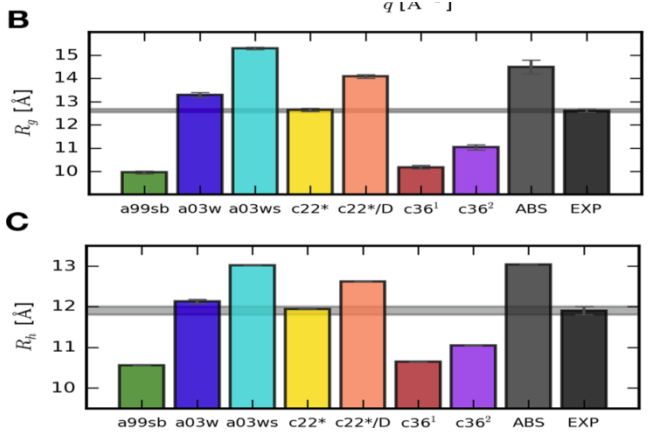
$$+ \sum_{\substack{\text{improper}\\ \text{dihedrals}}} K_{\text{imp}}(\varphi - \varphi_0)^2 \tag{1d}$$

$$+ \sum_{\substack{\text{nonbonded}\\ \text{pairs } i,j}} \left(\varepsilon_{ij} \left[\left(\frac{R_{\min_{ij}}}{r_{ij}} \right)^{12} - \left(\frac{R_{\min_{ij}}}{r_{ij}} \right)^{6} \right] \right) \tag{1e}$$

$$+ \sum_{\substack{\text{nonbonded}\\ \text{pairs } i,j}} \frac{q_i q_j}{4\pi\varepsilon_0 \varepsilon r_{ij}} \tag{1f}$$

Testing of force field accuracy

Structural ensembles of intrinsically disordered proteins depend strongly on force field

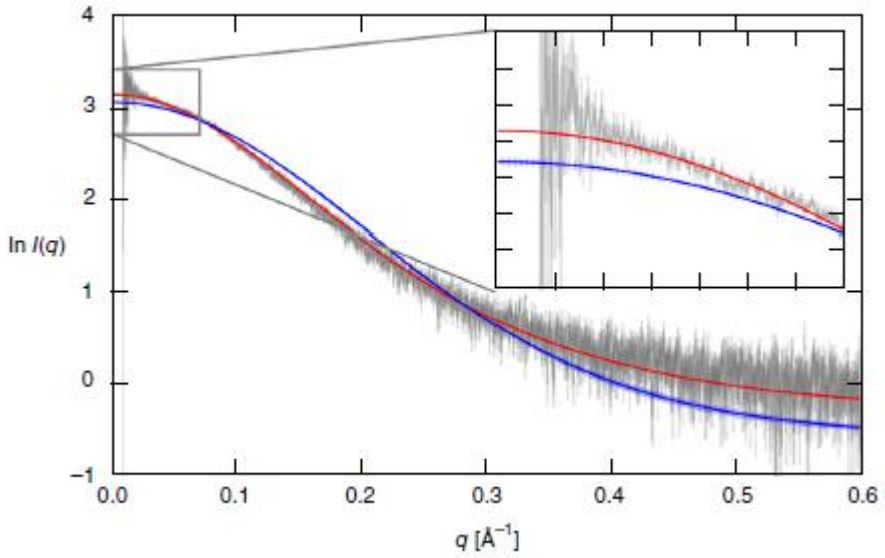


Rauscher et al. (2015) J. Chem. Theor. & Comp.

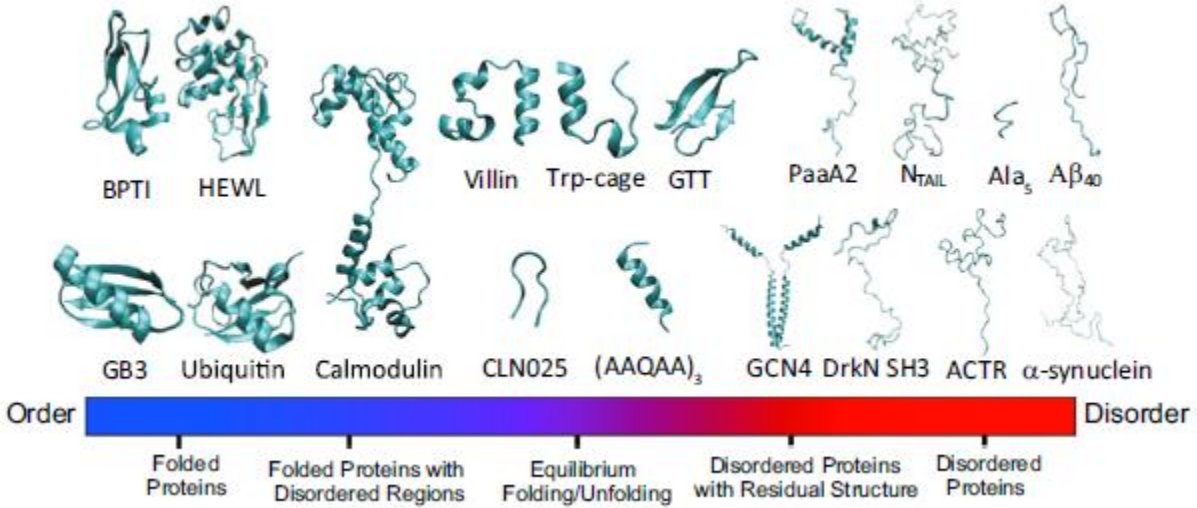
CHARMM36m: an improved force field for folded and intrinsically disordered proteins

CHARMM36 (C36) protein FF was found to generate a high population of left-handed α-helix

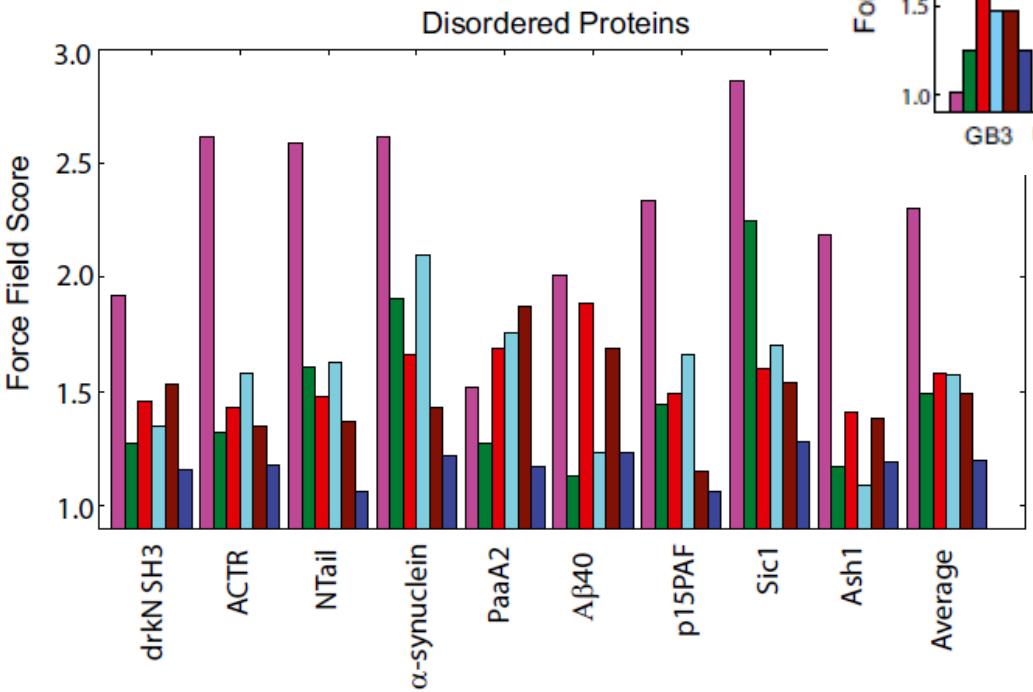
System	Simulation	α_L probability (%)	α_L propensity (%)	Max. α_L length
FG-nucleoporin peptide	C36	32 ± 6	22 ± 2	14 aa
	C36m	1.1 ± 0.3	6.2 ± 0.2	5 aa
RS peptide	C36	80 ± 2	41 ± 1	17 aa
	C36m	1.8 ± 0.5	5.5 ± 0.2	5 aa
IN	C36	64 ± 18	14 ± 2	7 aa
	C36m	3 ± 2	5.6 ± 0.5	4 aa
HEWL19 peptide	C36	11 ± 7	12 ± 2	8 aa
	C36m	0.5 ± 0.4	6.1 ± 0.7	3 aa

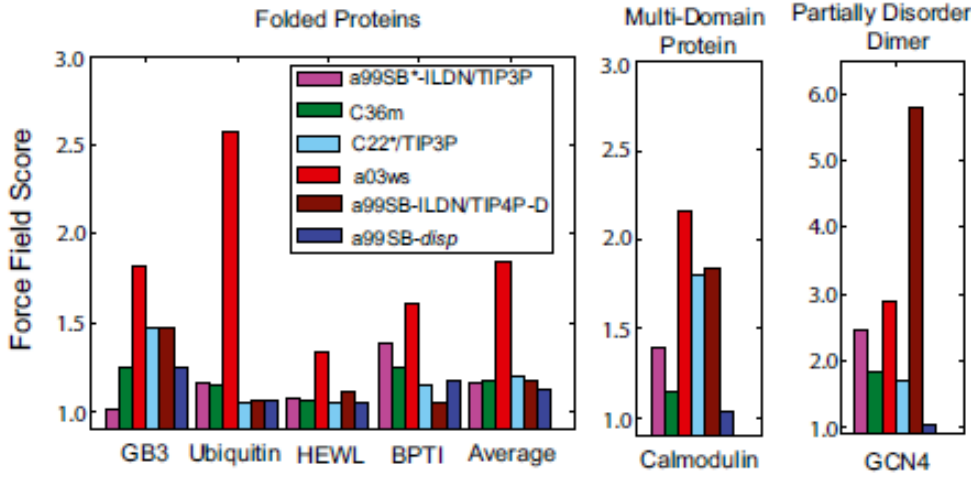


Developing a molecular dynamics force field for both folded and disordered protein states



Robustelli, Piana and D. E. Shaw 2018 PNAS



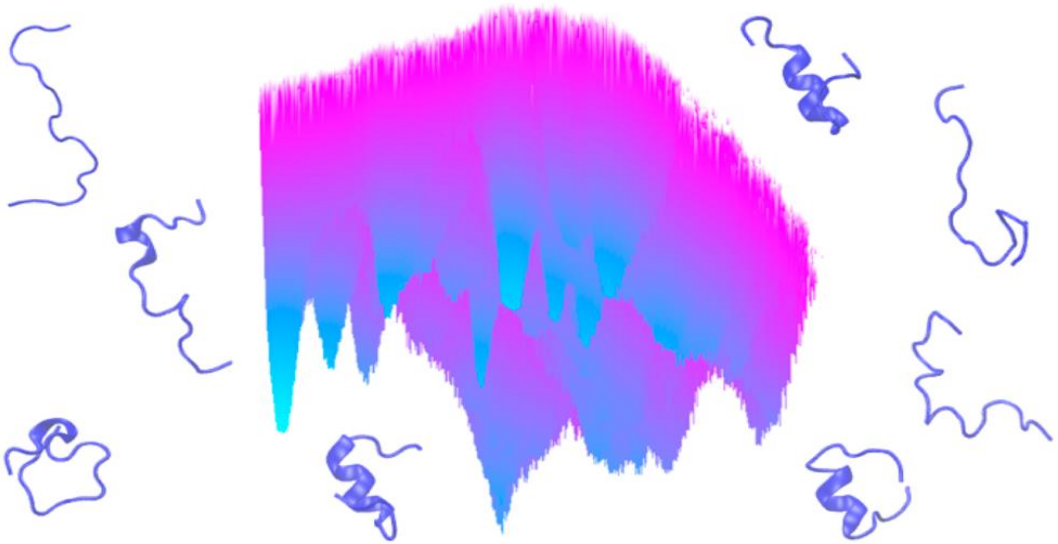


None of these previously existing force fields produce accurate dimensions and residual secondary structure propensities for disordered proteins while simultaneously providing accurate descriptions of folded proteins

a99SB-disp.: Optimized torsion parameters and introduced small changes in the protein and water vdW interaction terms

Linear motifs

- Interactions of well folded proteins involve larger surfaces discontinuous in sequence
- IDPs typically bind to targets using short (~6 residues) consecutive stretches of amino acid residues - **linear motifs (LMs)**
  - ➢ Increased hydrophobic content - promoting local structure formation
  - ➢ Structural and conformational propensities



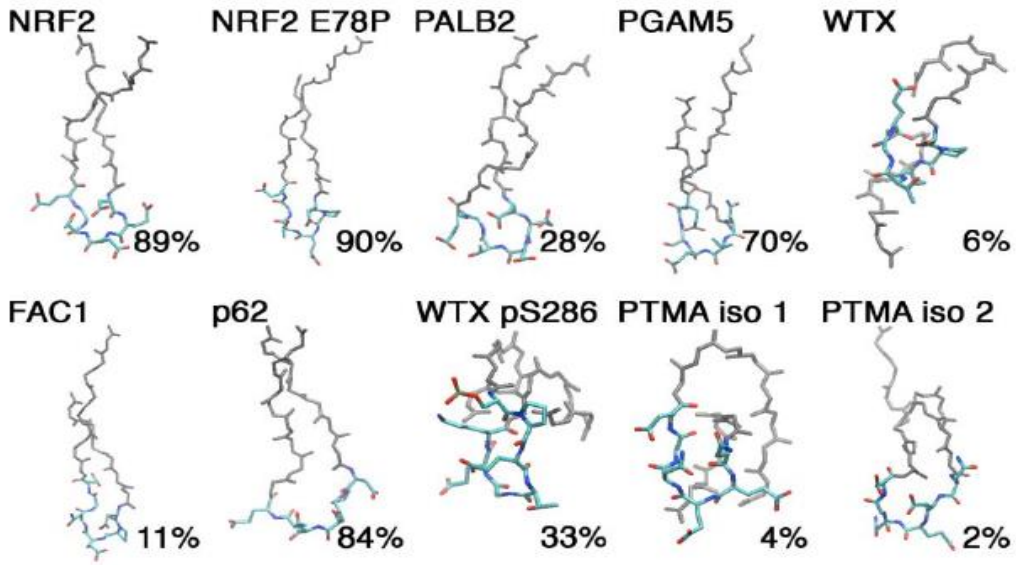
Cino et al. (2016) JPCB

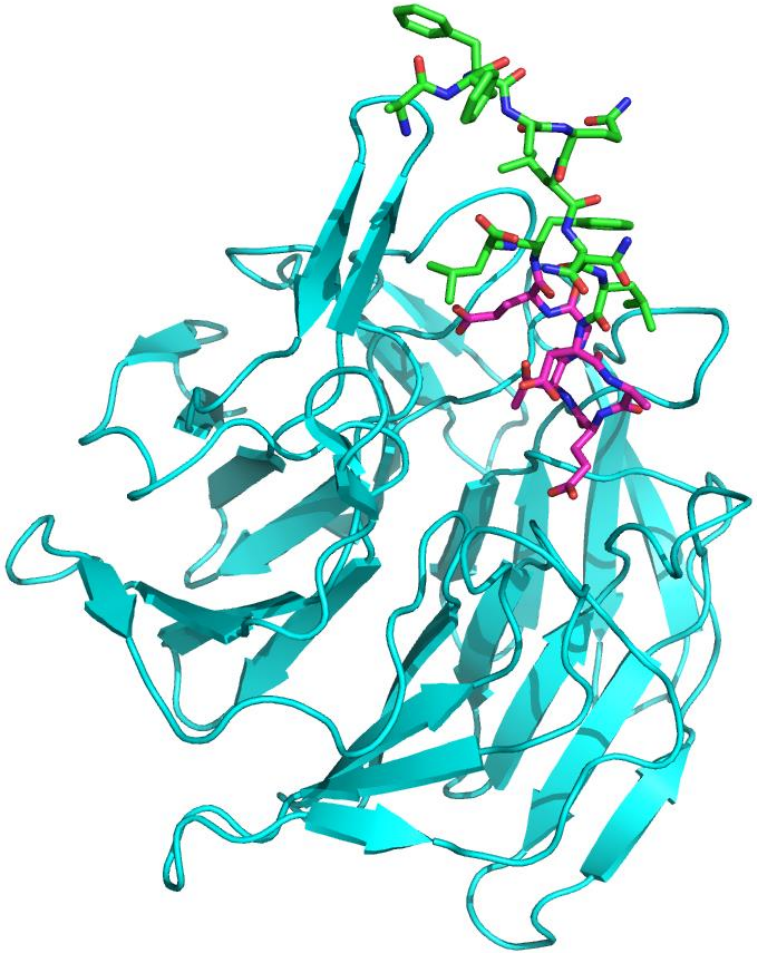
Binding of disordered proteins to a protein hub

Studied system:

- Keap 1 – well folded Kelch domain hub
- All of its identified disordered partners

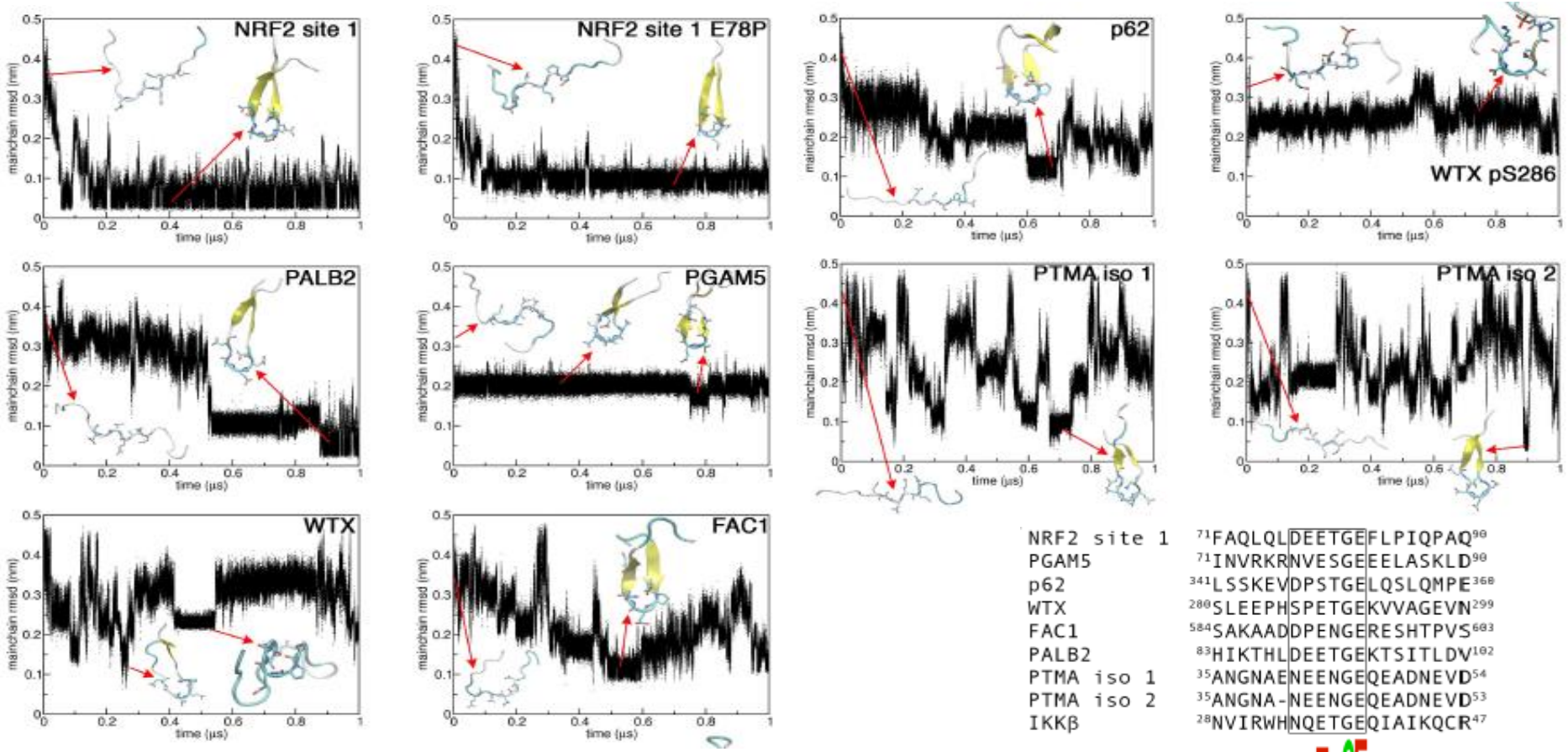
Cluster analysis of peptide structures





Cino et al. (2013) Scientific Reports

Binding of disordered proteins to a protein hub



NRF2 site 1	^{71}FAQLQLDEETGEFLPIQPAQ^{90}
PGAM5	^{71}INVRKRNVESGEEELASKLD^{90}
p62	^{341}LSSKEVDPSTGELQSLQMPE^{368}
WTX	^{280}SLEEPHSPETGEKVVAGEVN^{299}
FAC1	^{584}SAKAADDPENGERESHTPVS^{603}
PALB2	^{83}HIKTHLDEETGEKTSITLDV^{102}
PTMA iso 1	^{35}ANGNAENEENGEQEADNEVD^{54}
PTMA iso 2	^{35}ANGNA-NEENGEQEADNEVD^{53}
IKKβ	^{28}NVIRWHNQETGEQIAIKQCR^{47}

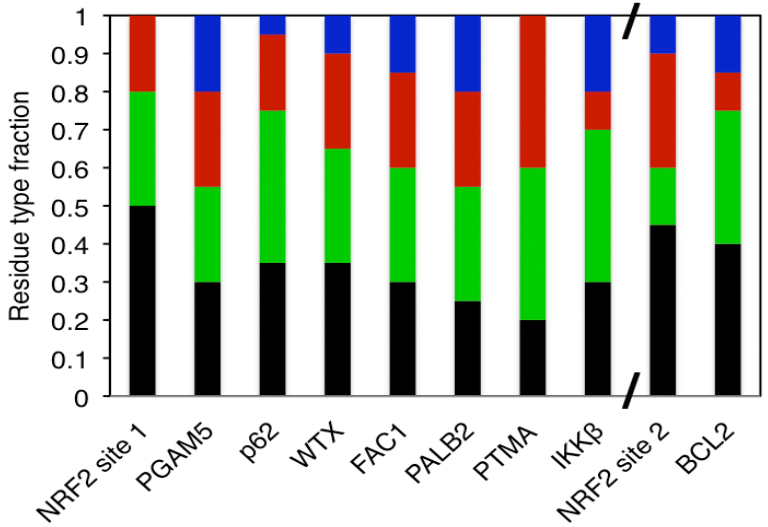
Elio A. Cino, Ryan C. Killoran, Mikko Karttunen & Wing-Yiu Choy (2013) Scientific Reports

Binding of disordered proteins to a protein hub

90% populated bound conformation throughout the simulation

Table 1 | Thermodynamic parameters for the binding of the peptides to the human Kelch domain[a]

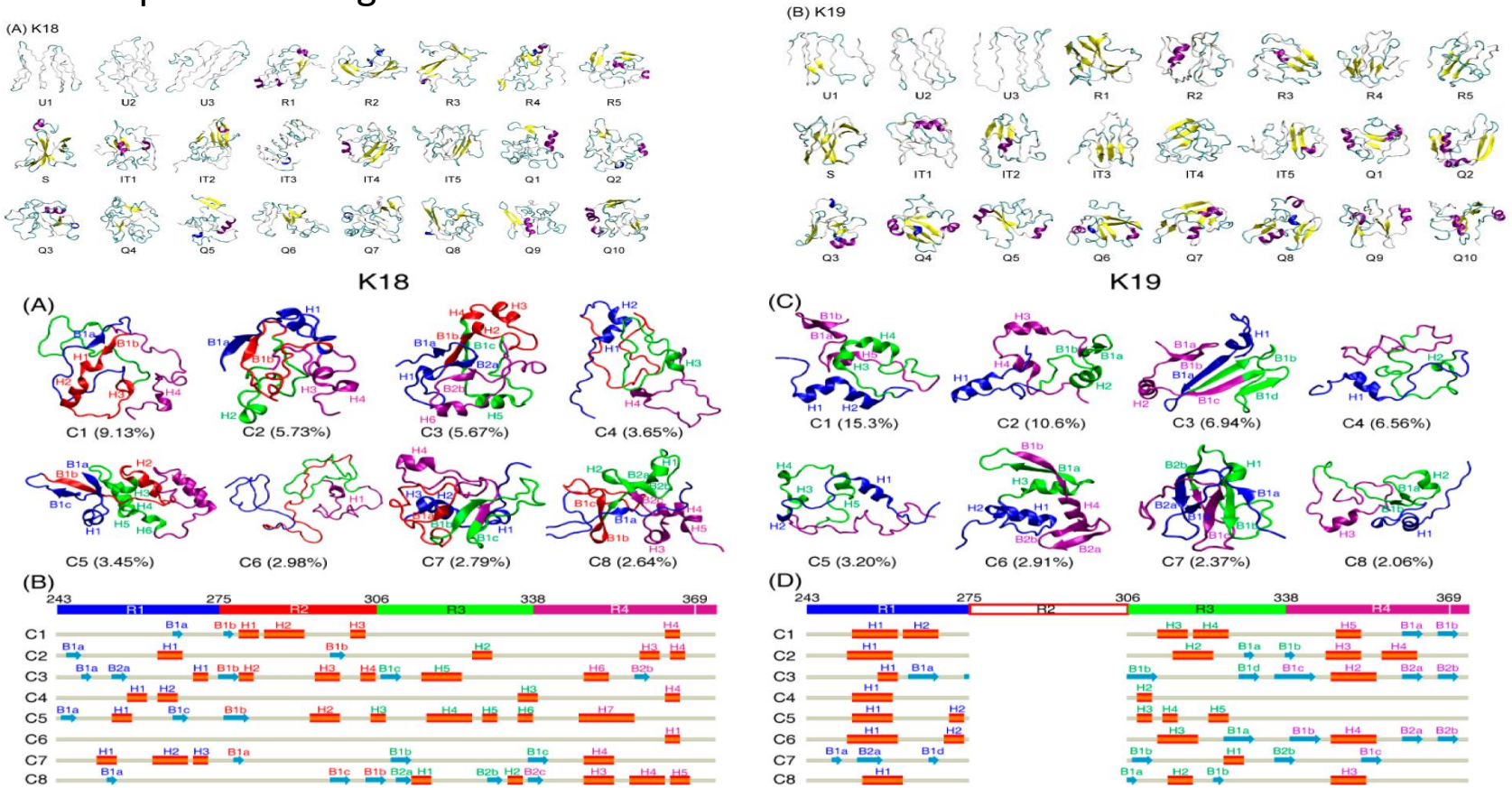
Protein	n^b	K_d^c (10^{-6} M)	ΔH^c (kcal/mol)	$T\Delta S^c$ (kcal/mol)	ΔG^c (kcal/mol)
NRF2 site 1	1.08	0.023 ± 0.002	−16.96 ± 0.05	−6.56	−10.40 ± 0.03
NRF2 site 1 E78P	0.99	0.007 ± 0.001	−16.76 ± 0.05	−5.64	−11.12 ± 0.03
PALB2	1.01	0.087 ± 0.007	−19.29 ± 0.11	−9.66	−9.63 ± 0.05
PGAM5	1.07	0.23 ± 0			
WTX	1.04	0.25 ± 0			
FAC1	0.99	1.1 ± 0			
p62	0.97	1.3 ± 0			
WTX pS286	0.98	1.5 ± 0			
PTMA iso 2	1.05	2.62 ± 0			
PTMA iso 1	1.07	11.6 ± 0			



Elio A. Cino, Ryan C. Killoran, Mikko Karttunen & Wing-Yiu Choy (2013) Scientific Reports

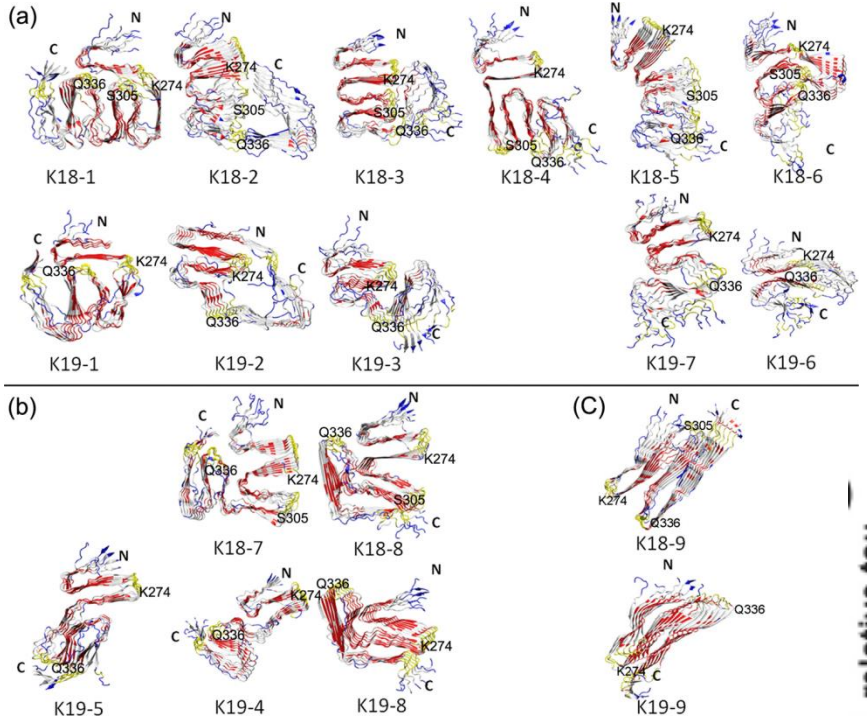
REMD of tau repeat regions

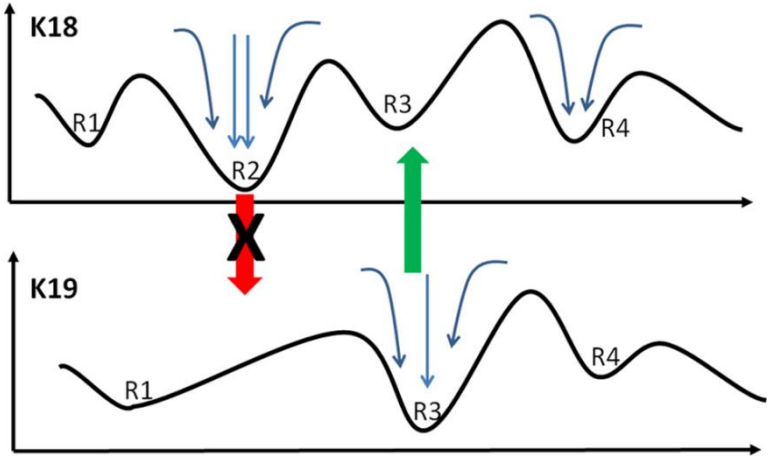
- Replica-exchange MD with K18 and K19

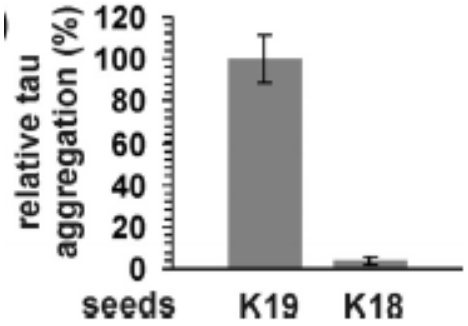


Luo et al. (2014) J. Phys. Chem. Lett.

Cross-seeding barrier between Three- and Four-repeat Human Tau Proteins

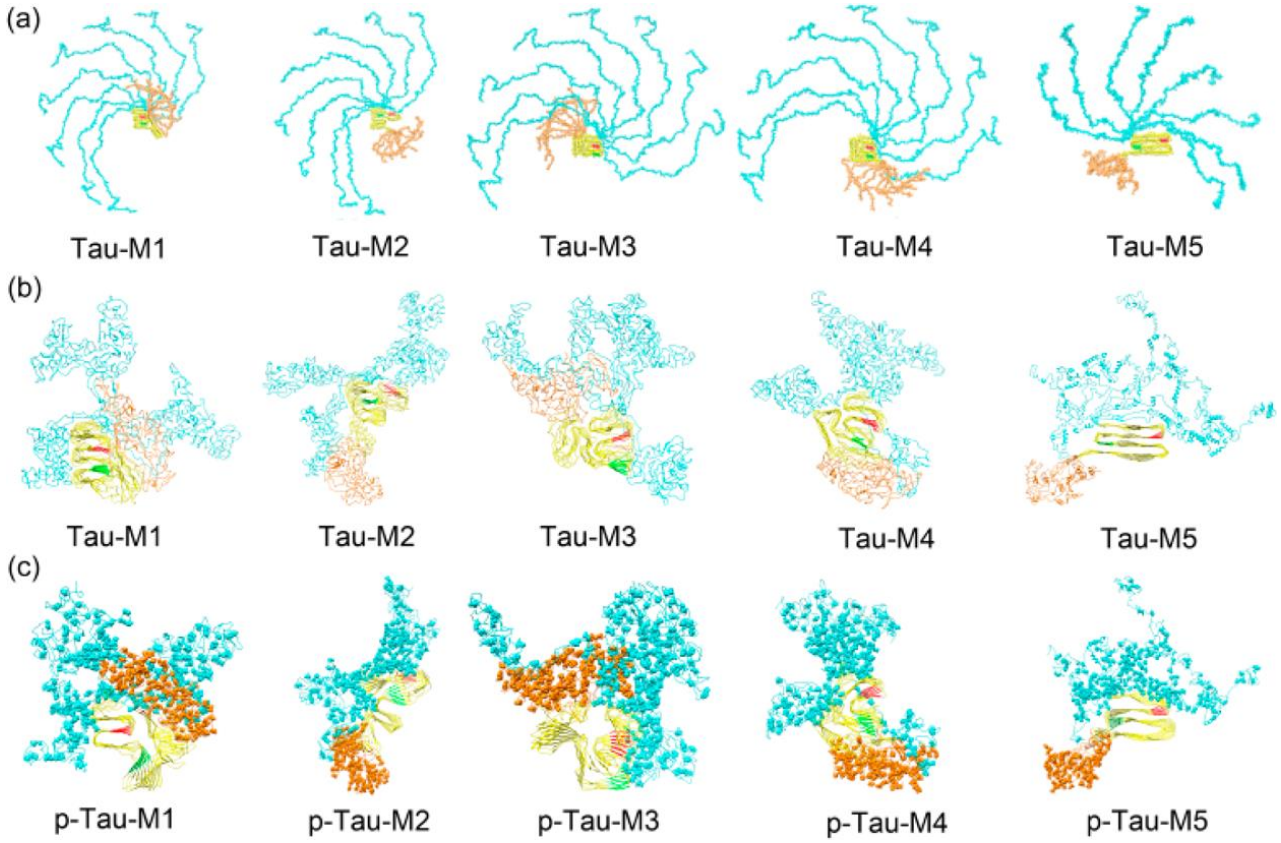






Yu 2012 *J. Biol. Chem.*

How Does Hyperphopsphorylation Modulate Filament Structure and Stability?

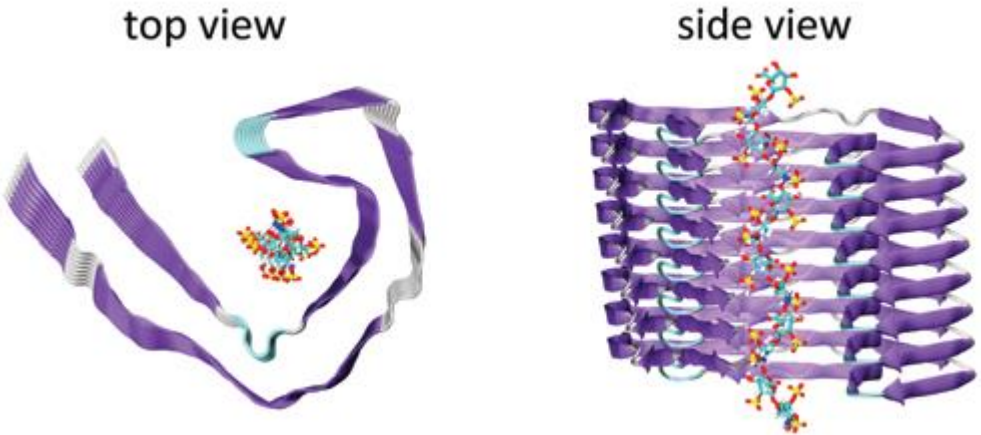


Hyperphosphorylation of the two terminal domains decreases the attractive interactions among the N and C-terminus and repeat domain.

Xu et al. 2016 *ACS Chem. Neurosci*

The distinct structural preferences of tau protein repeat domains

C-shaped motif is only stable for R3–R4, while R1–R2 tends to be linear in shape
Heparin can further stabilize the C-shaped R3–R4 motif, but not other repeats



Li 2018 *Chem. Commun.*

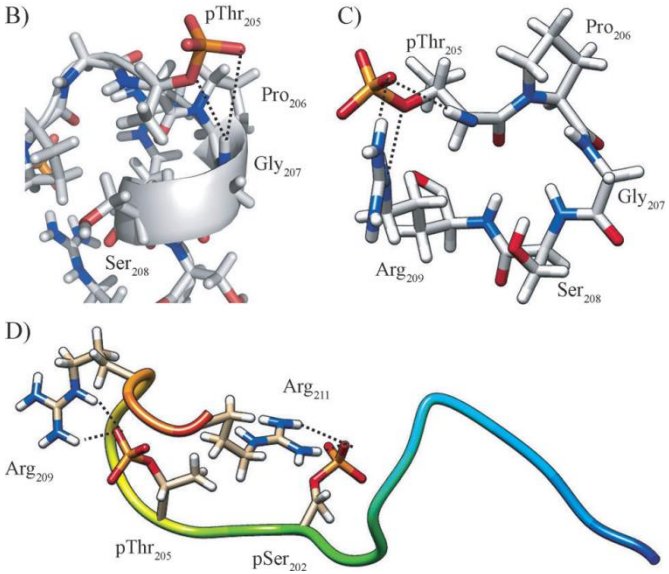
Tau protein phosphoepitope – AT8 antobody

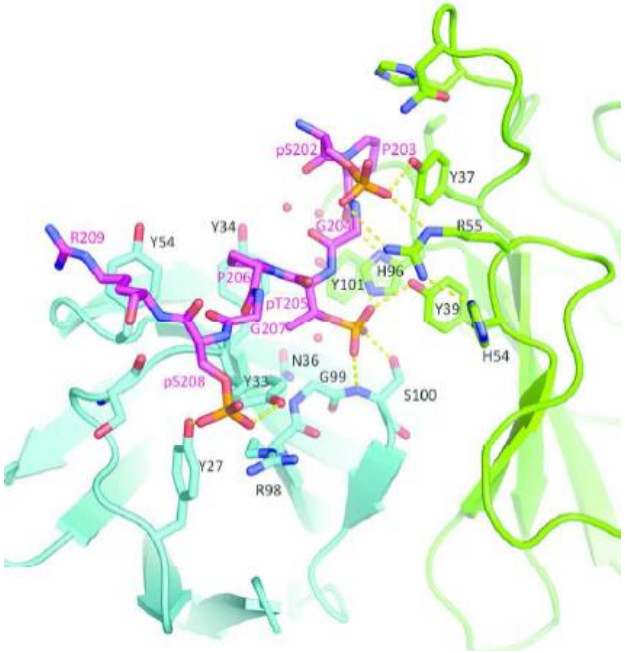
- AT8 epitope NMR, MD

vs

X-ray structure

62.5 %

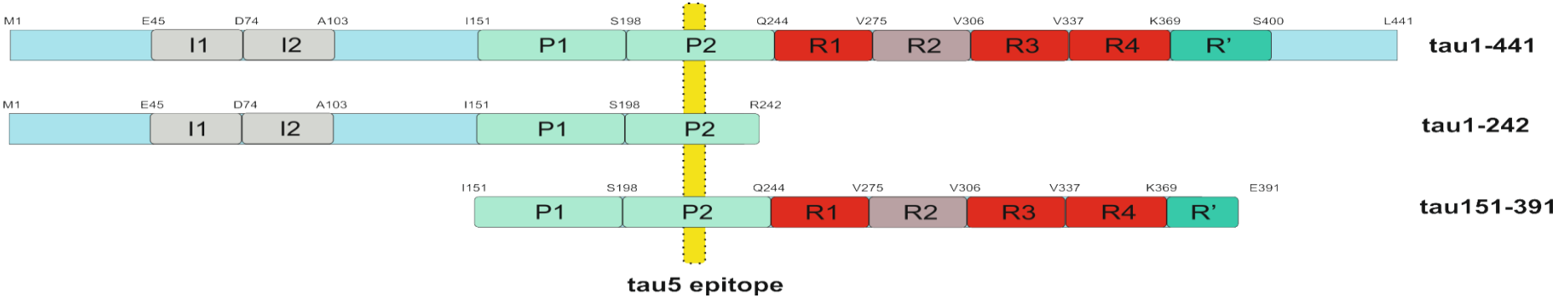




Gandhi et al. (2015) Ang. Chemie

Malia et al. (2016) Proteins

Tau5 antibody epitope in the proline rich region of tau

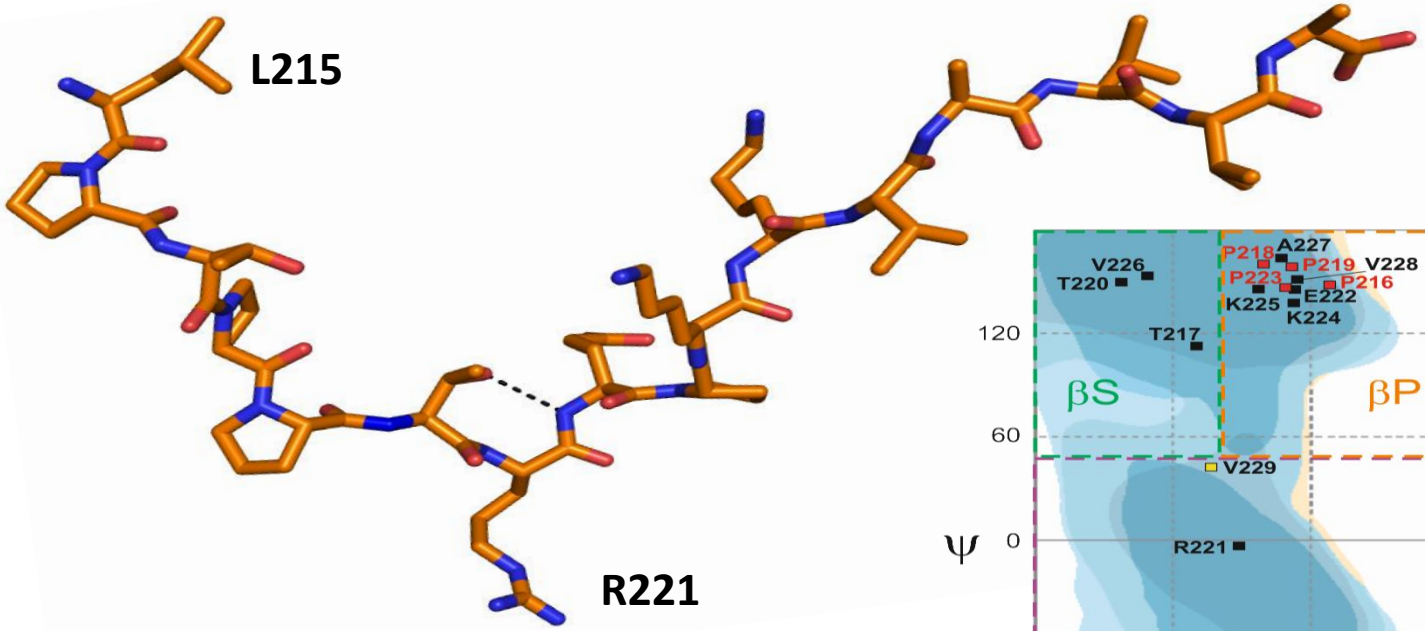


Tau5 epitope

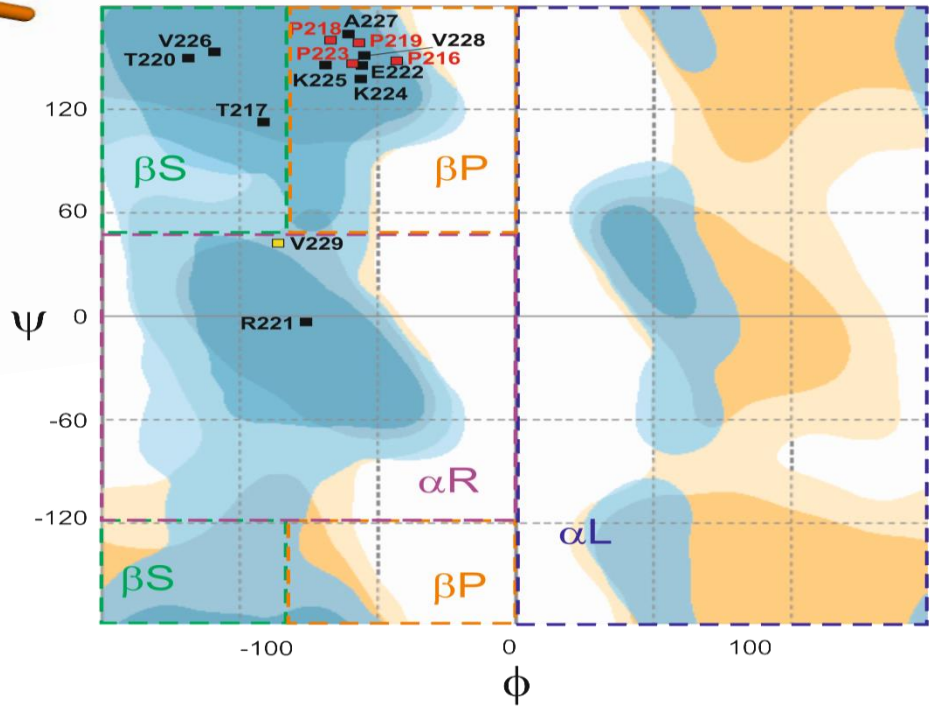
- lies in the tau-MT binding hotspot ^{214}SLPTPPTREPKKVAVVRT^{231} (Mukrasch et al, 2009)
- Flanking tau regions contain number of AD-relevant phosphorylation sites – pT231, pT235
- NMR detected significant propensity for transient secondary structures in this region (Mukrasch et al, 2009)

- The complex between Tau5 Fab and tau peptide crystallized at 1.6 Å resolution – Cehlar et al. (2012) Acta F [PDB ID: 4TQE]

Structure of tau peptide in complex with Tau5 Fab



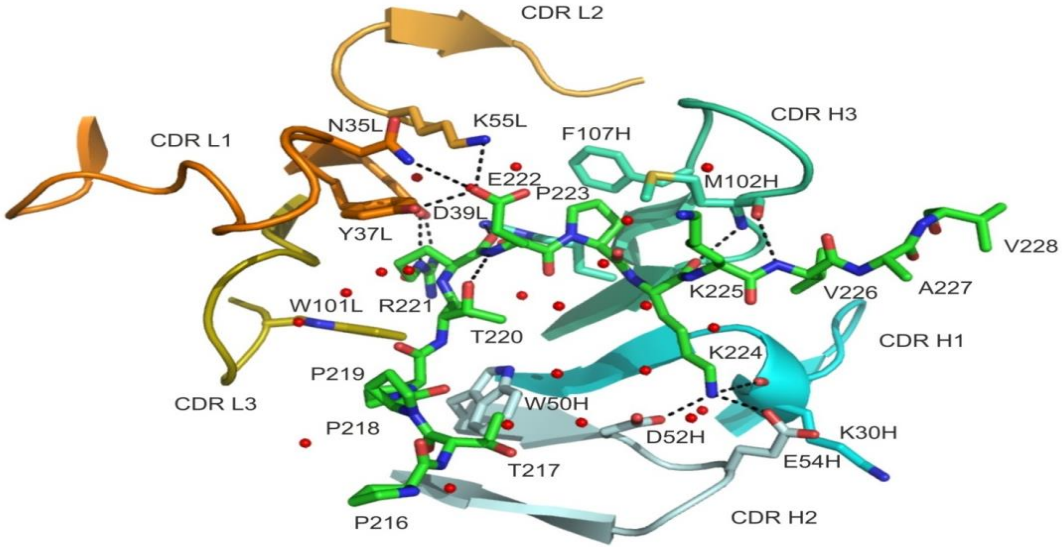
^{215}LPTPPTREPKKVAVVR^{230}



Conformation of tau peptide from proline rich region

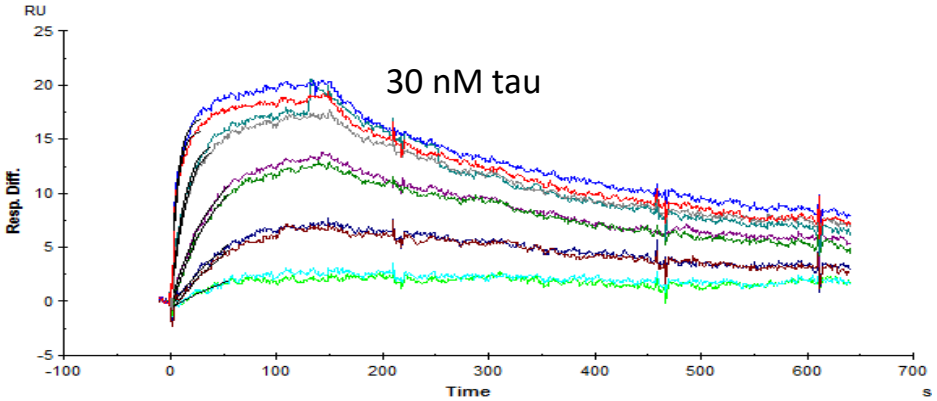
Goal:

- Probe the stability of X-ray observed tau peptide conformation in solution
- Compare the interaction of WT and T220A mutated peptide by biophysical methods
- Probe the role of intrachain hydrogen bond ($Thr^{220}OG$-$Glu^{222}N$) forming ST-turn motif on the peptide stability by comparing the simulation results for wild type and T220A mutated peptide



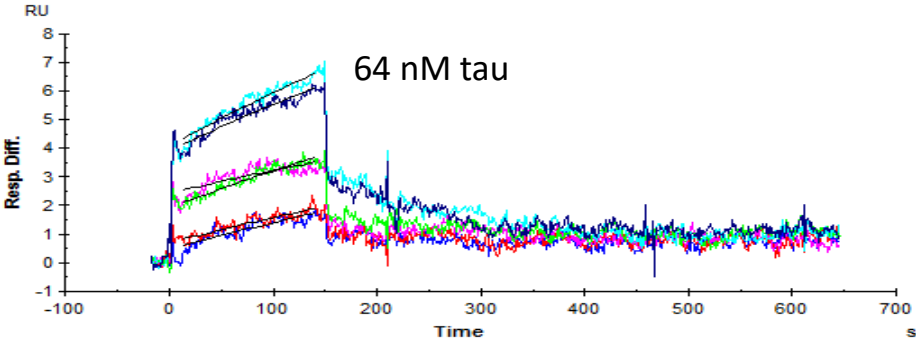
Surface Plasmon Resonance - comparison of WT and mutated tau proteins

Tau5 Fab vs Tau151-391 4R



K_D=2.3 x 10^{-10} M

Tau5 Fab vs Tau151-391 4R (T220A)

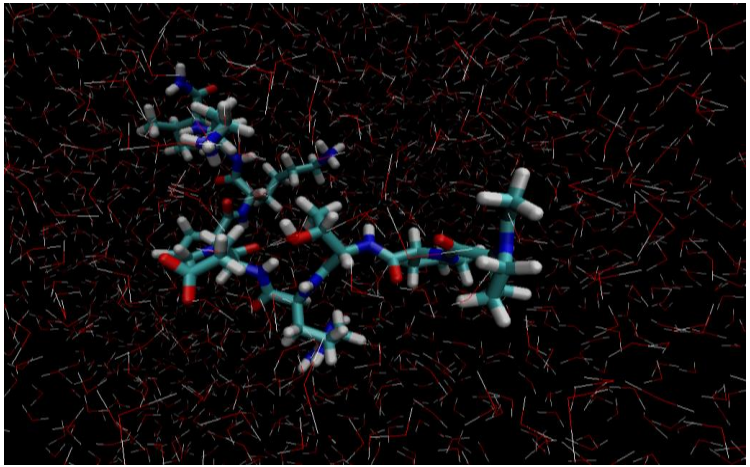


K_D=8.8 x 10^{-8} M

- T220A mutant of tau151-391 4R exhibits app. 370 times lower affinity for Tau5 than WT

Microsecond MD simulations of tau peptides

- 1 μs supercomputer simulations of WT and T220A tau peptides ^{218}Ac-PPTREPKKV^{226}-NH_2 starting from X-ray observed conformation in NAMD
- State of the art force field CHARMM36m (Huang et al. 2016 Nature Methods)
  - ➢ Improved accuracy in generating polypeptide backbone conformational ensembles for intrinsically disordered peptides and proteins
  - ➢ Sampling of α_L helical conformations in IDP ensembles is significantly lower compared to previous versions

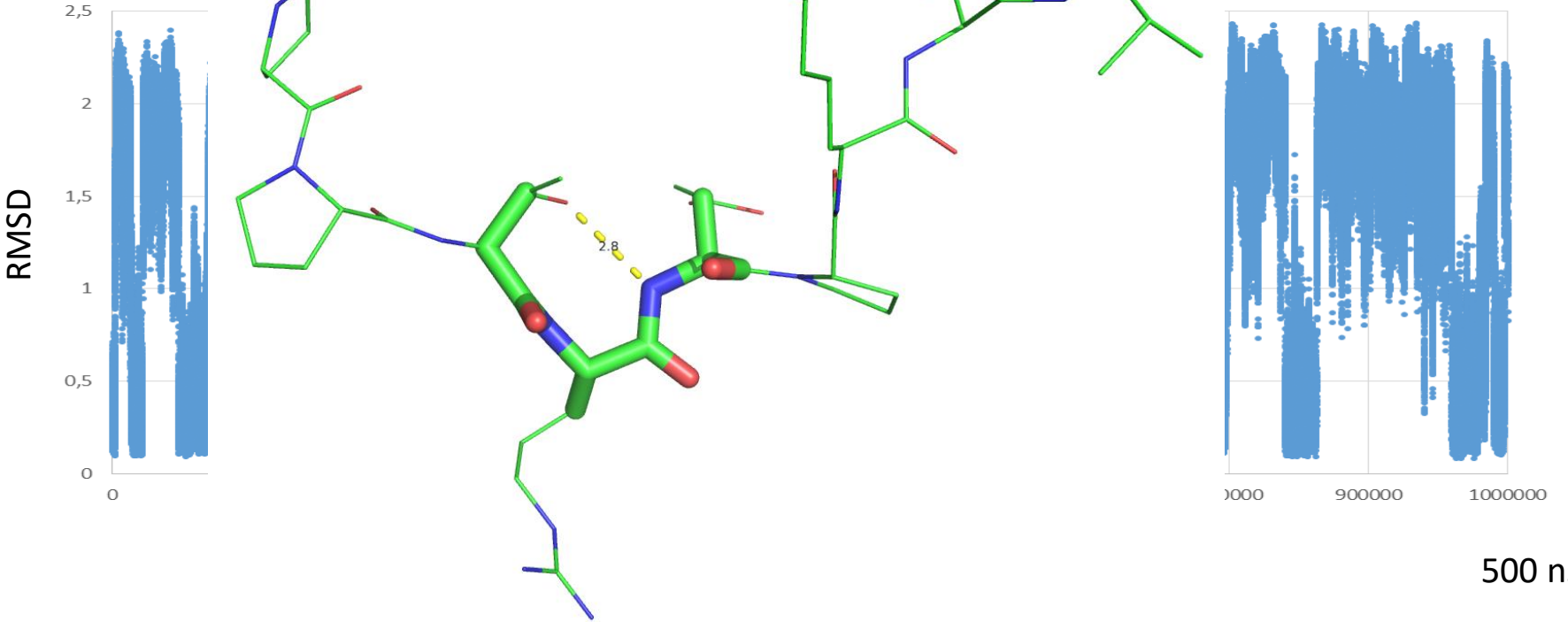


Microsecond MD simulations of tau peptides

Analysis criterion chosen:

- RMSD residues 220 to 222 & atoms Cα, Cβ, C, N, O

WT

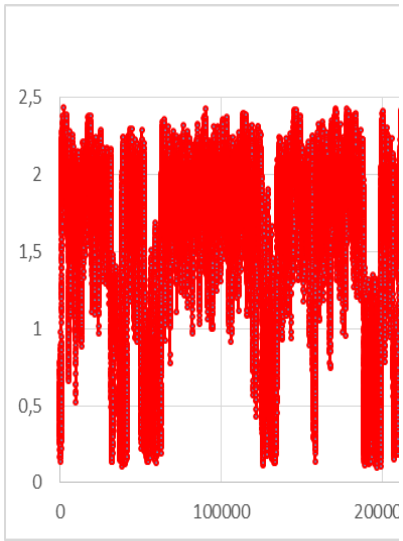


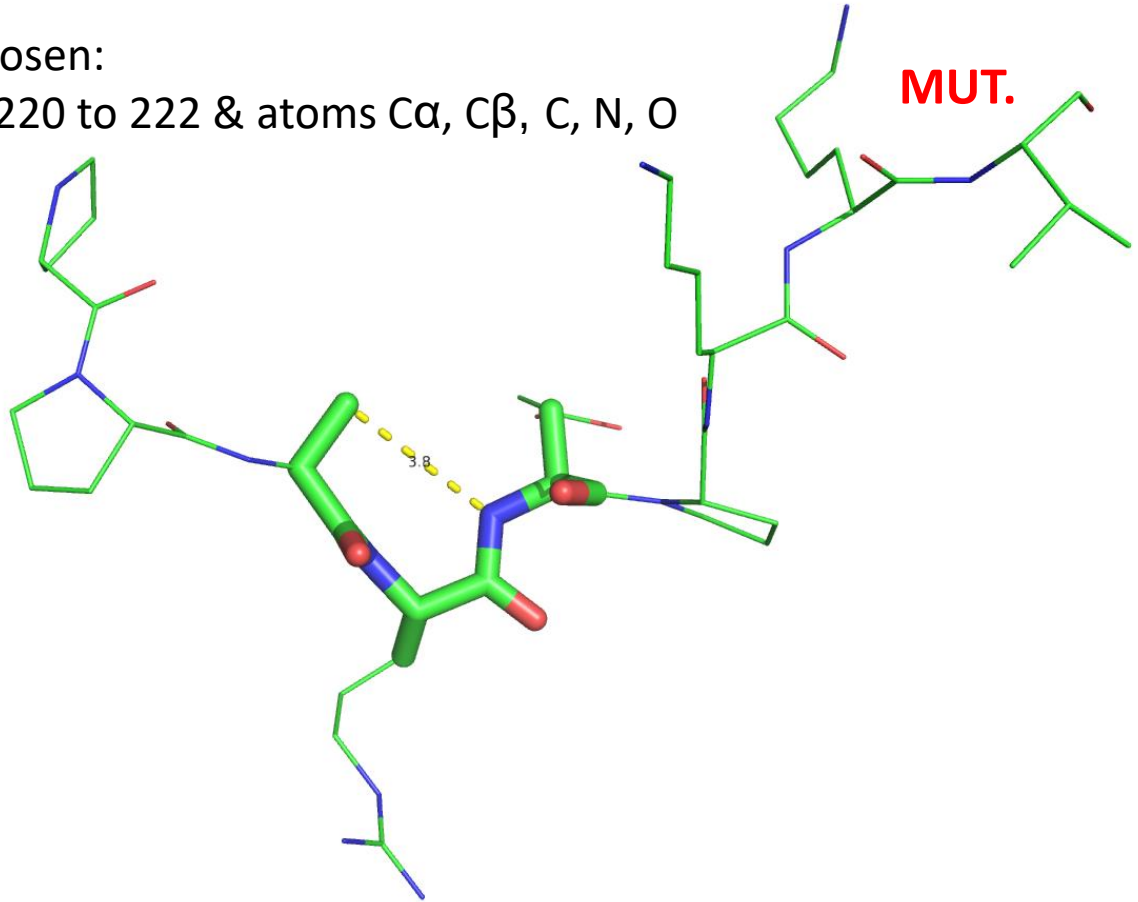
500 ns

Microsecond MD simulations of tau peptides

Analysis criterion chosen:

- RMSD residues 220 to 222 & atoms Cα, Cβ, C, N, O



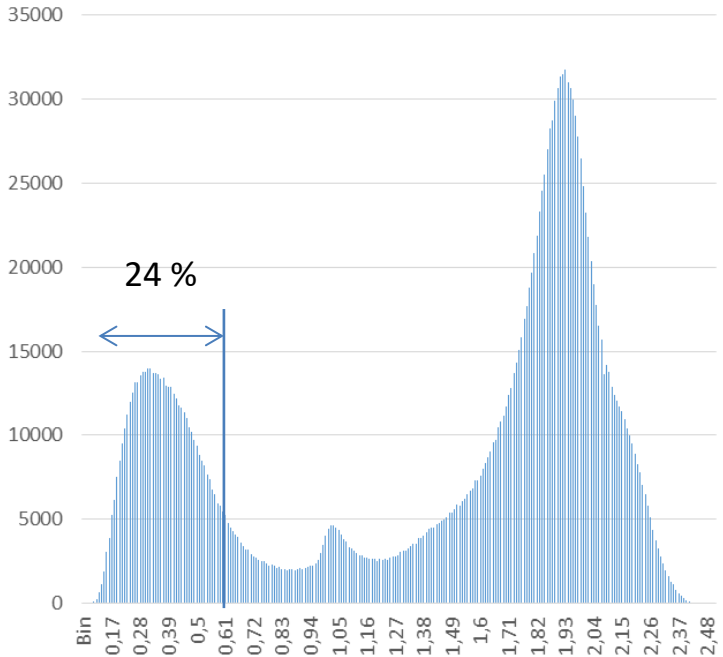


500 ns

Microsecond MD simulations of tau peptides

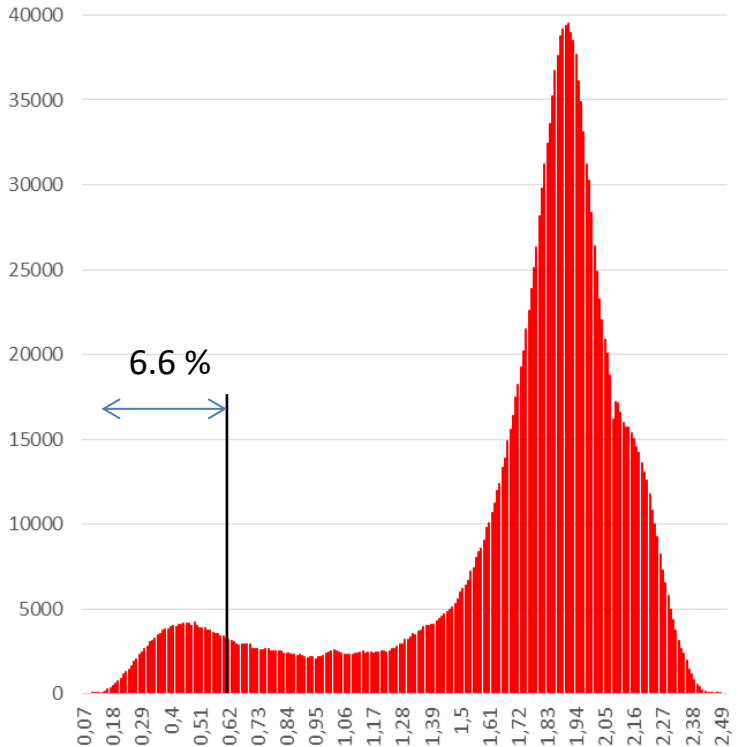
1 µs
simulation time

PPTREPKKV **WT**



RMSD

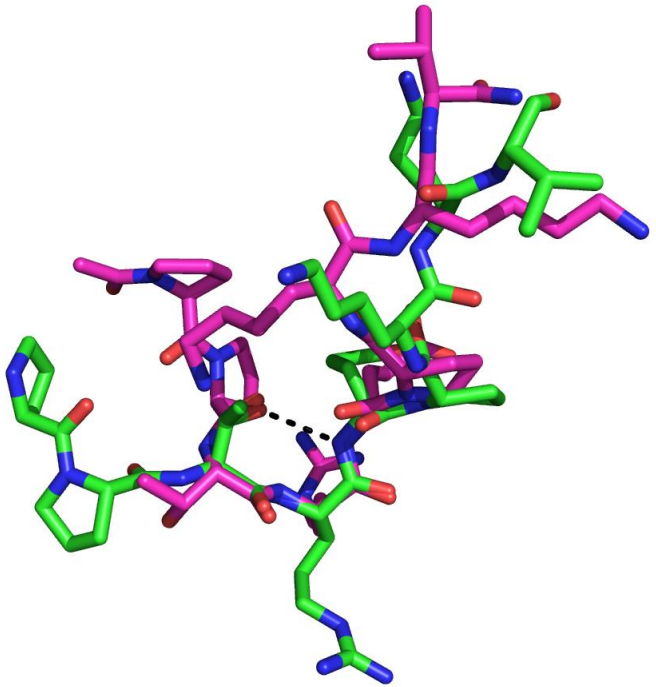
PPAREPKKV **MUT.**



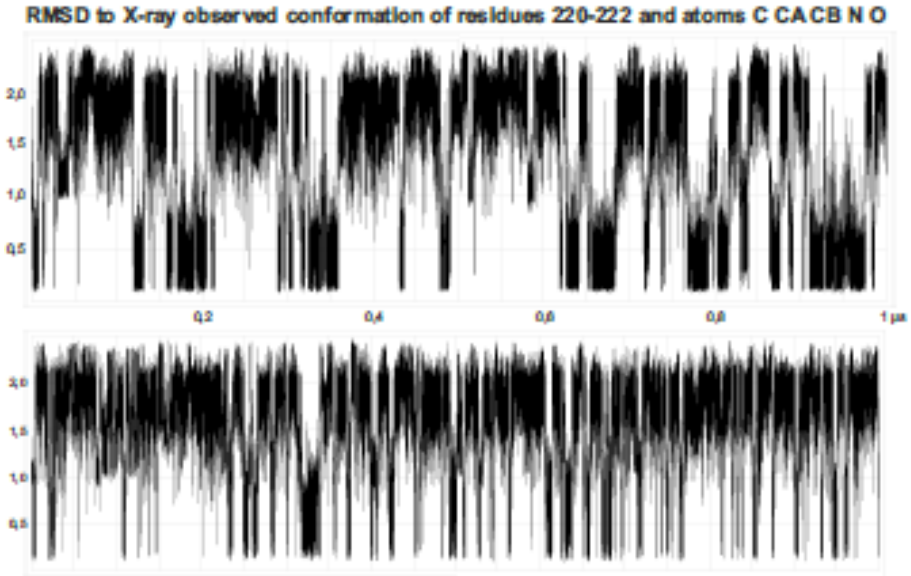
RMSD

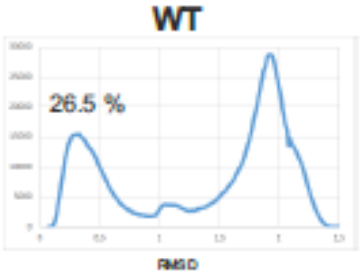
Microsecond MD simulations of tau peptides

Simulations starting from X-ray independent conformation (produced by simulated annealing protocol – 1 ns heating up, 4 ns at 450 K, 2 ns cooling down, 3 ns at 300 K



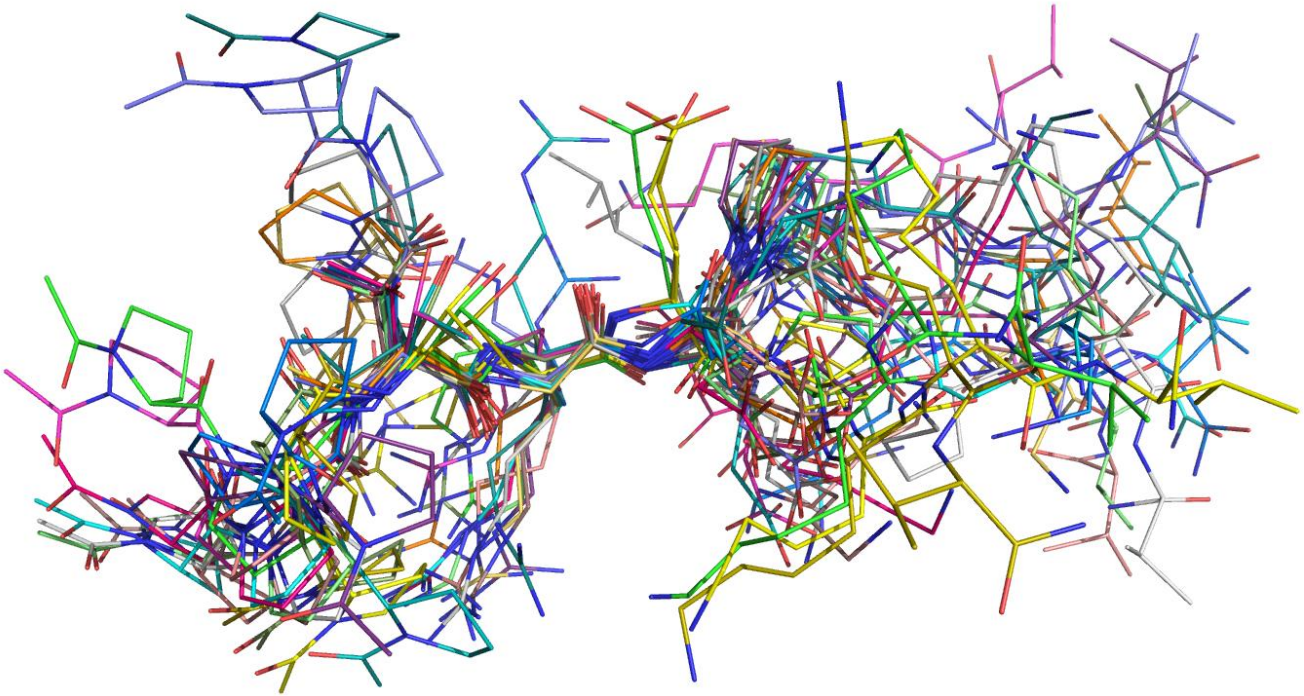
Microsecond MD simulations of tau peptides





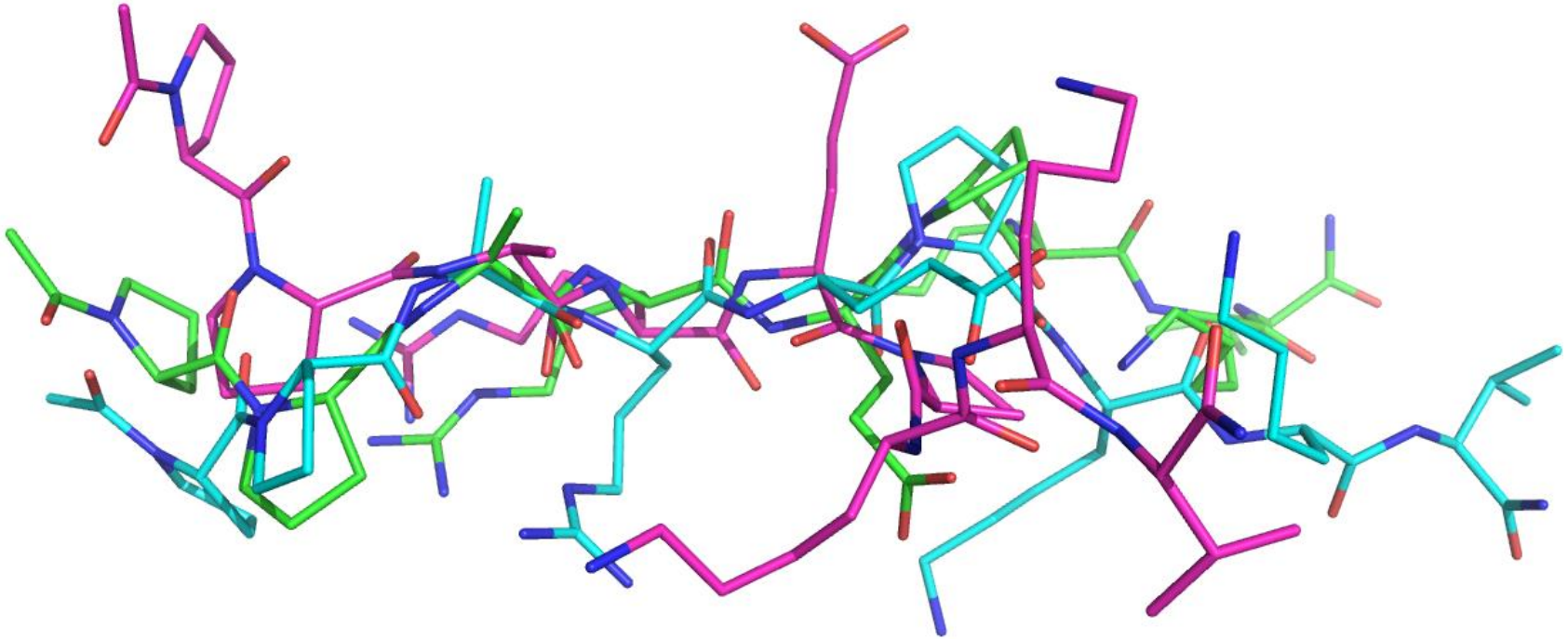
Microsecond MD simulations of tau peptides

Cluster analysis from dPCA WT



Microsecond MD simulations of tau peptides

Cluster analysis from dPCA T220A



Microsecond MD simulations of tau peptides

Conclusion:

- The conformation of tau peptide is stabilized with intrachain hydrogen bond throughout the simulation
- The WT peptide occupies the bound-like conformation (RMSD < 0.6 Å) 3.6 times more than T220A mutated tau peptide

Acknowledgement

- Slovak infrastructure for high performance computing

Thank you for attention

Rostislav Skrabana
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