Effects of change-of-function mutations on disordered region in the GR transactivation domain

Lennart.Nilsson@ki.se

Mika Ito, Alok Juneja, Evdokiya Salamanova, Joana Paulo

Anthony Wright, KI

Roger Strömberg, KI

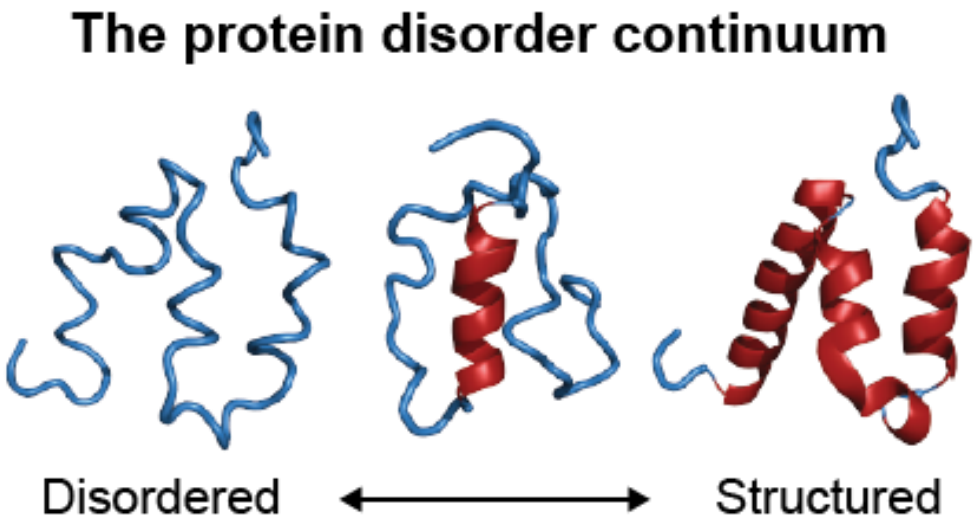
Jan Johansson, KI

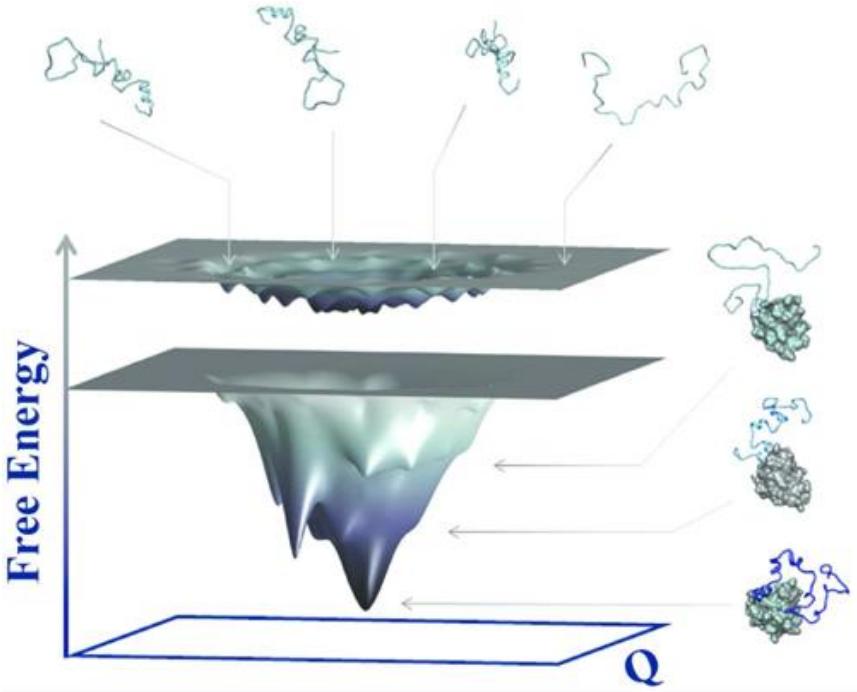
Zagreb, Feb 2016

Swedish Research Council

Karolinska Institutet Center for Biosciences

Intrinsically disordered proteins (IDPs) and regions (IDRs)



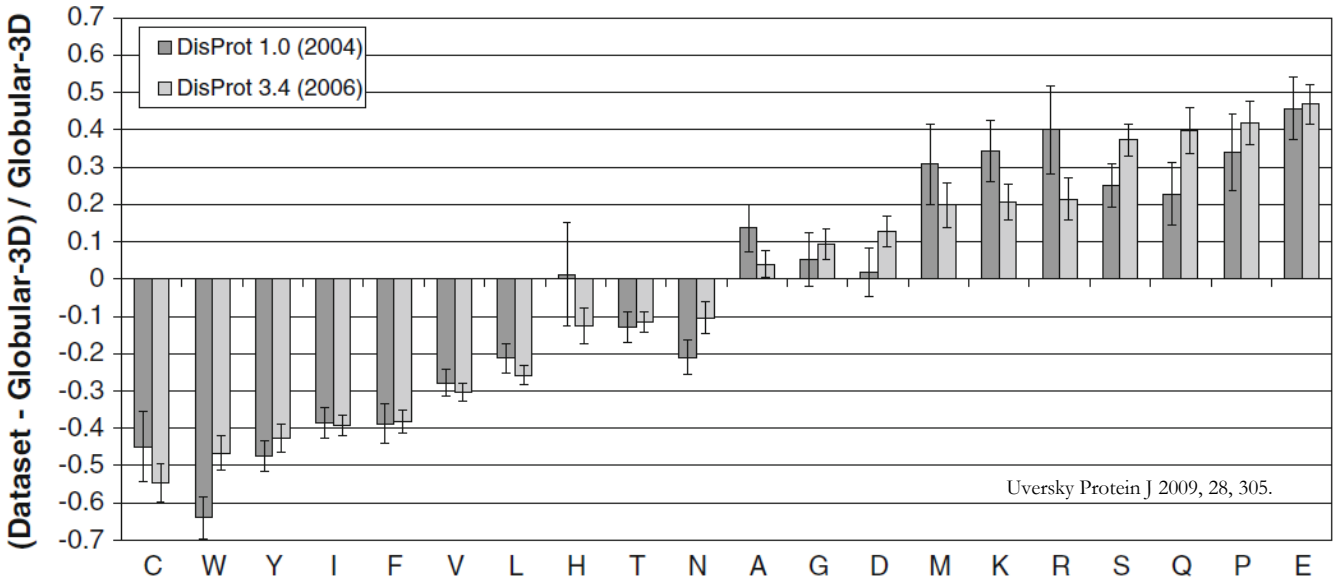


Dyson et al. Nat Rev Mol Cell Biol 2 005, 6, 197.
Gibbs et al. Biochemistry 2015, 54, 1314.

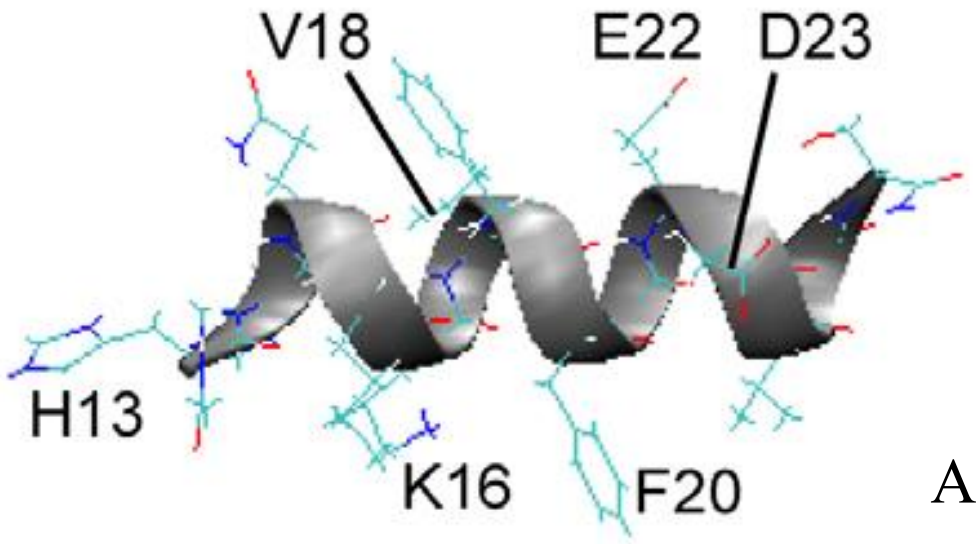
The where and why of IDPs/IDRs

- More common in eukaryotes
- Fewer bulky hydrophobic and more polar/charged residues
  aa-composition and sequence-local distribution may be as signature
- Often in transcription factors
- Phylogenetic analysis indicates IDRs in TFs are highly evolvable

Relative amino-acid abundance in IDPs *vs* globular proteins



Alzheimer's disease is a neurodegenerative disease leading to loss of cognitive function.



Aβ 39-42 aa length
Middle 5 aa (KLVFF) bind to full length peptide

Model system:

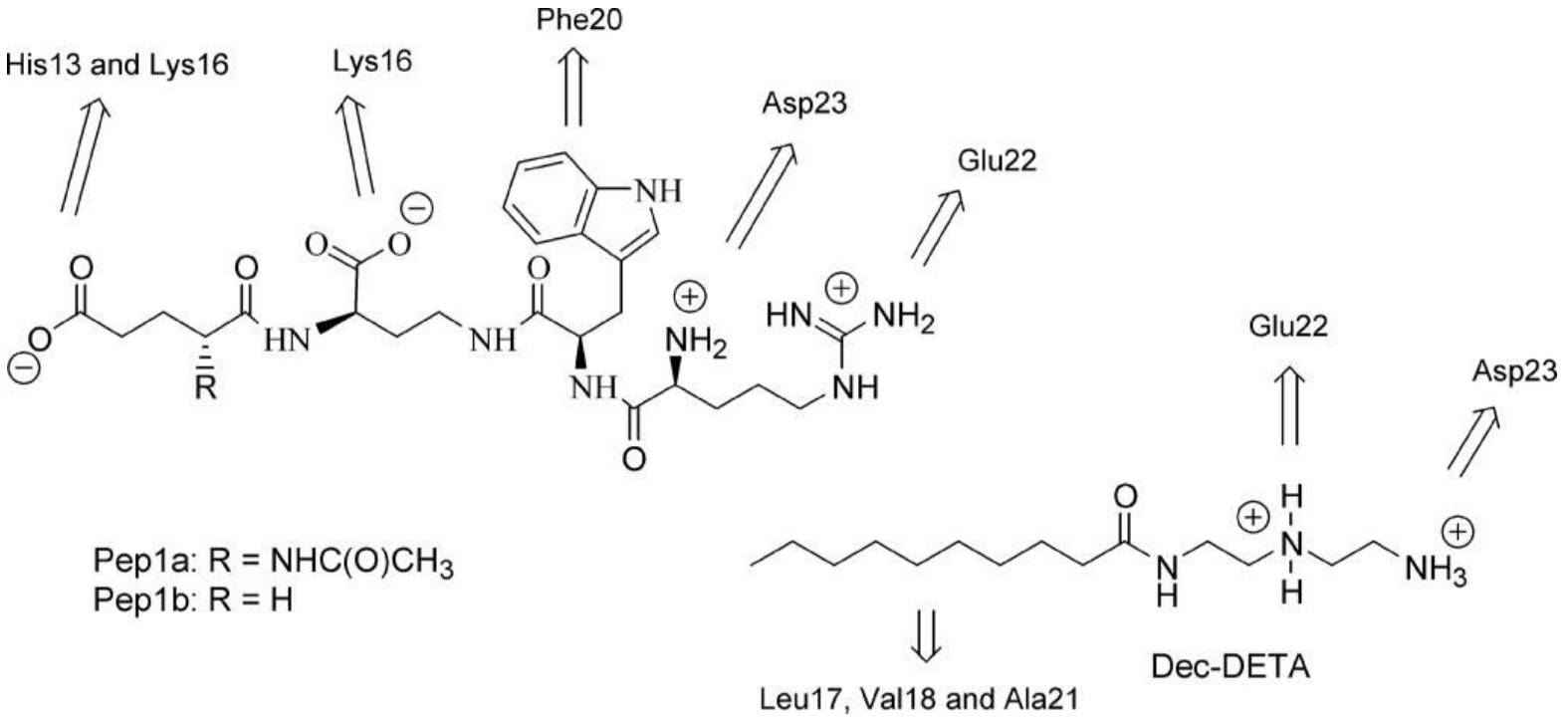
Aβ13–26; HHQ**KLVFF**AEDVGS

What state is the causative agent?

- Plaque?
- Amyloid fibrils?
- Some intermediate state(s)?

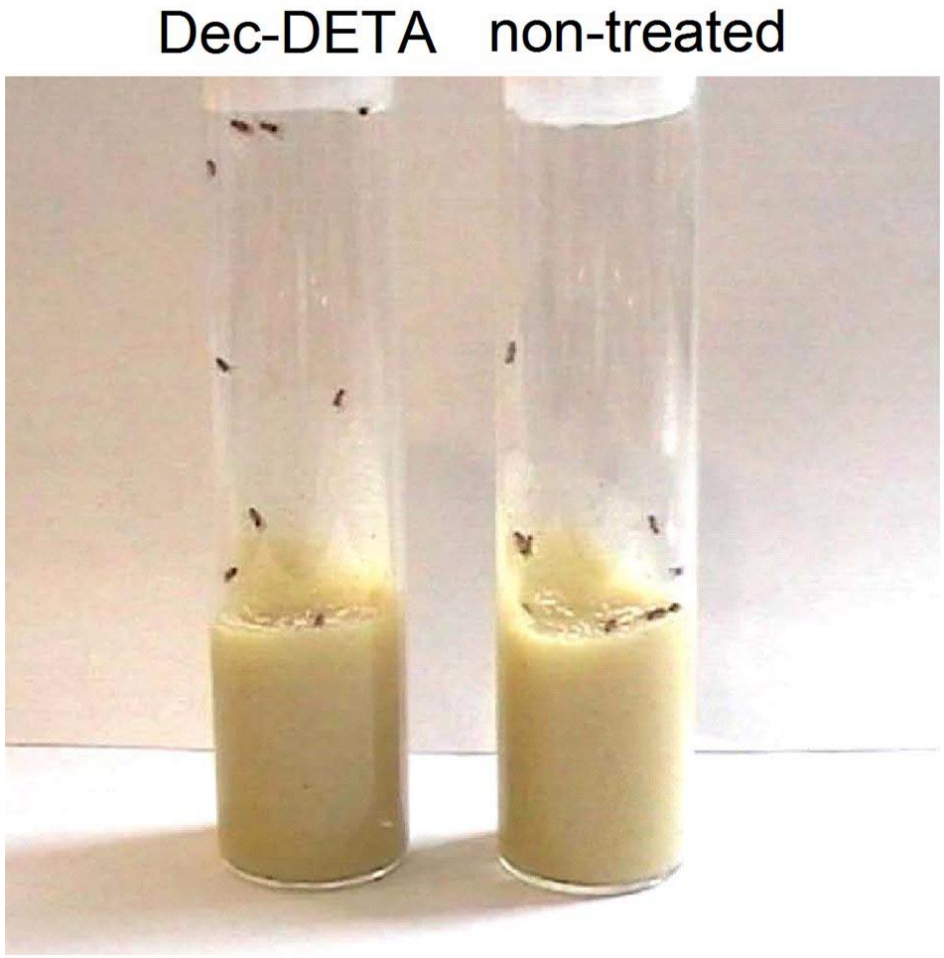
Stabilization of the membrane associated helical conformation of residues 15-24 might be a valid therapeutic strategy.

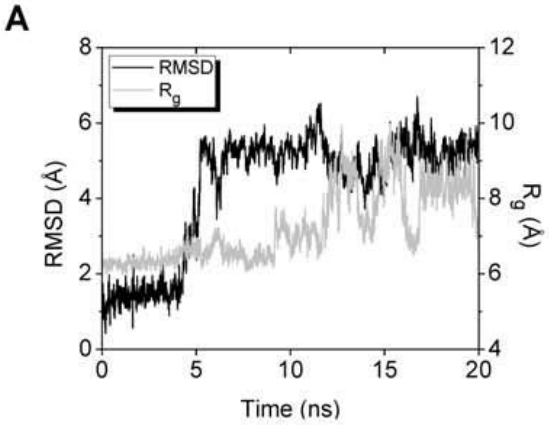
Ligands designed to stabilize helical part of the peptide

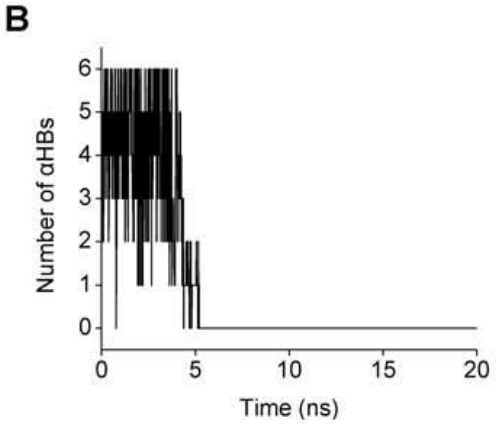


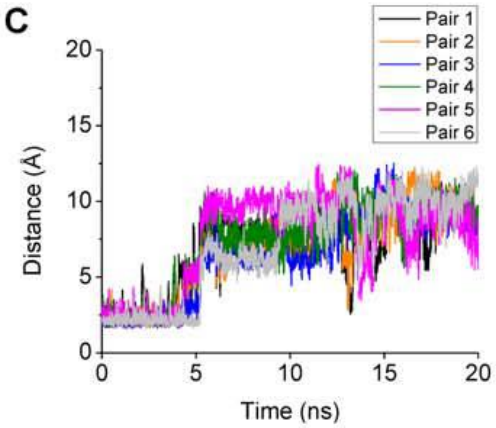
Nerelius, C., Sandegren, A., Sargsyan, H., Raunak, R., Leijonmarck, H., Chatterjee, U., Fisahn, A., Imarisio, S., Lomas, D. A., Crowther, D. C., Strömberg, R., and Johansson, J. (2009) α-Helix targeting reduces amyloid-β peptide toxicity, *PNAS 106, 9191-9196.*

Drosophila Aβ-model







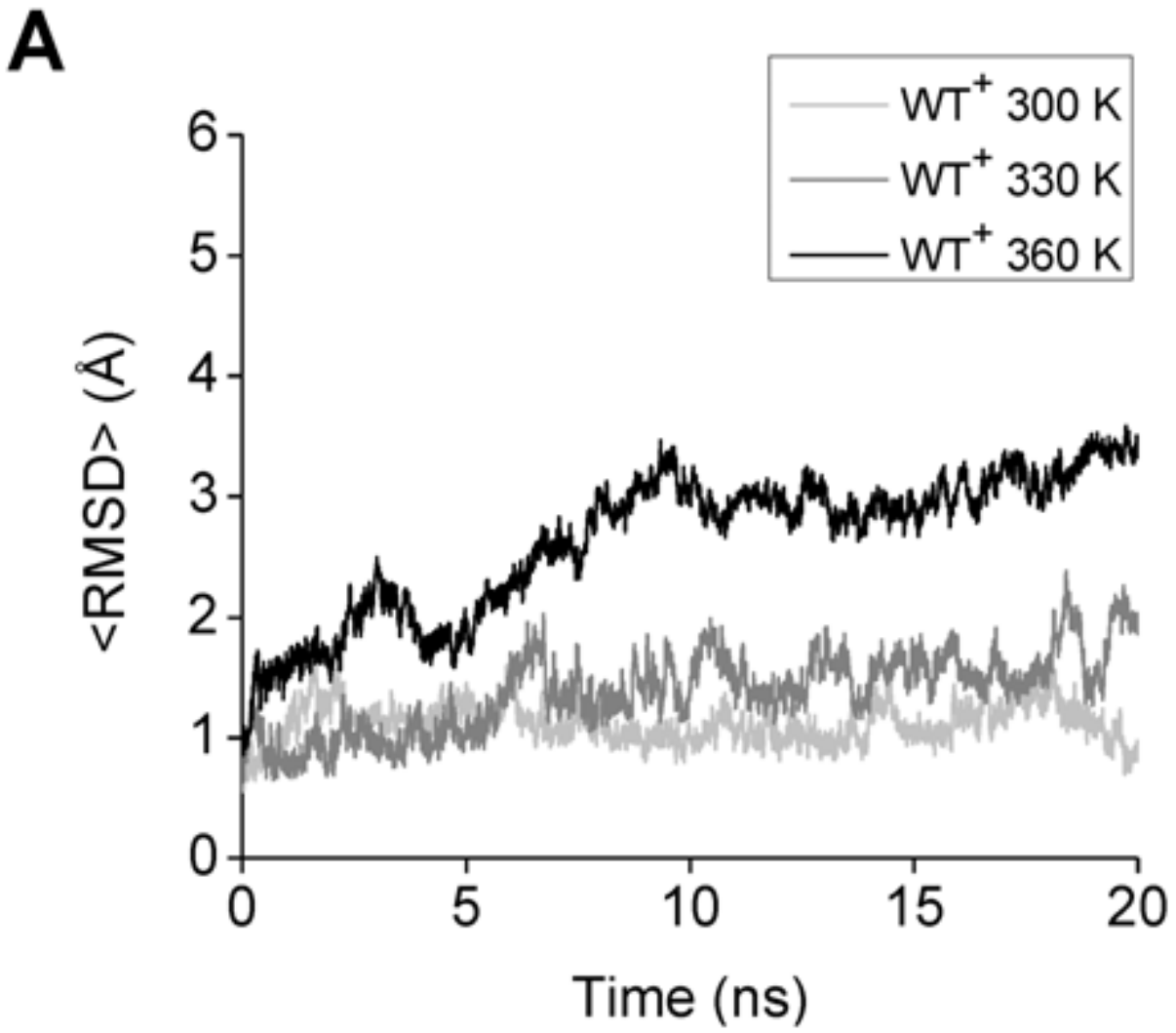


Protocol to detect Aβ-helix unfolding using 10x20ns MD simulations in explicit water

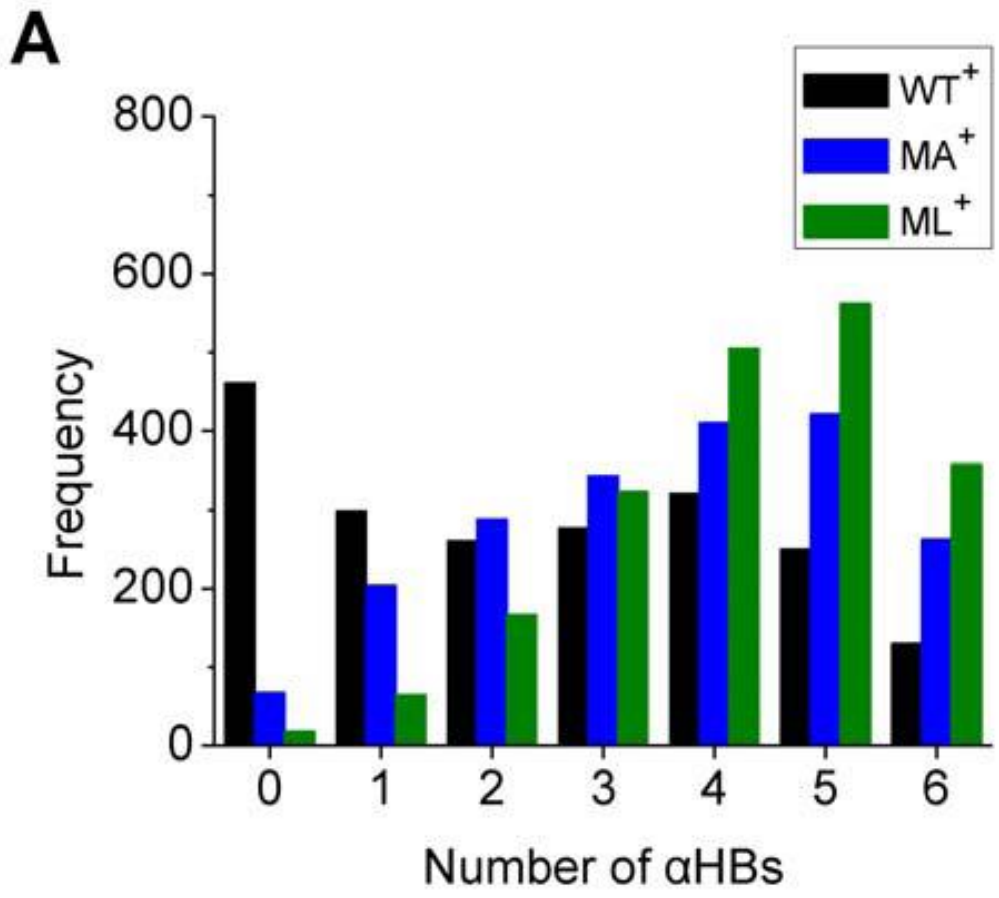
- ❑ **Structural deviation, RMSD (A)**
- ❑ **Number of backbone hydrogen bonds (B)**
- ❑ **Backbone hydrogen bond lengths (C)**

Ito M, Johansson J, Strömberg R, Nilsson L: ***PLoS ONE 2011, 6(3):e17587.***
Ito M, Johansson J, Strömberg R, Nilsson L: ***PLoS ONE 2012, 7(1):e30510.***
Juneja A, Ito M, Nilsson L: ***J Chem Theory Comp 2012, 10.1021.ct300941v***

Temperature dependence of stability in simulations

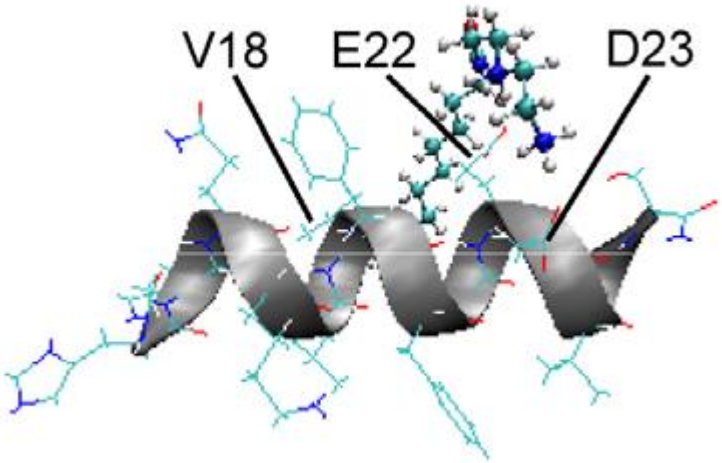


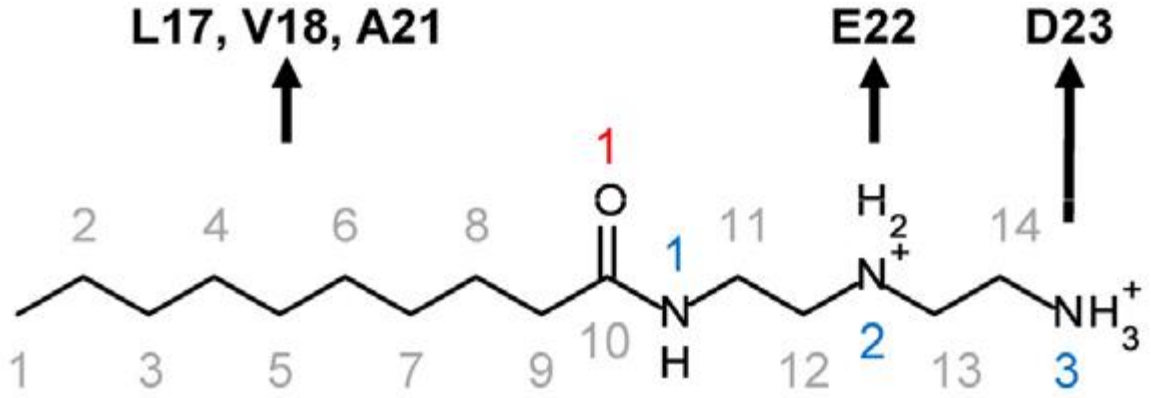
Stabilizing effect of alanine and leucine mutations with higher helix propensity



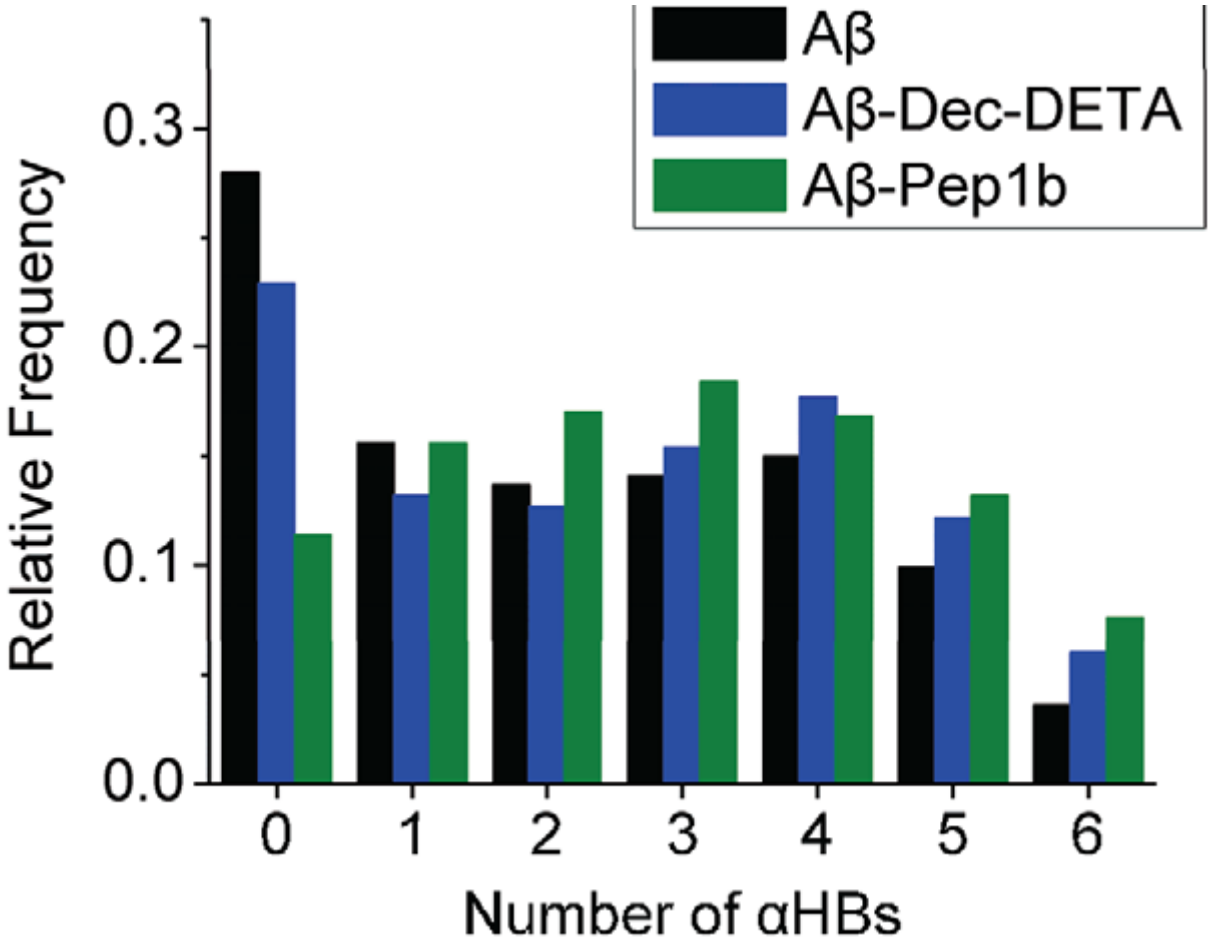
18-VFF-20→ AAA
18-VFF-20→ LLL

Ligand DEC-DETA



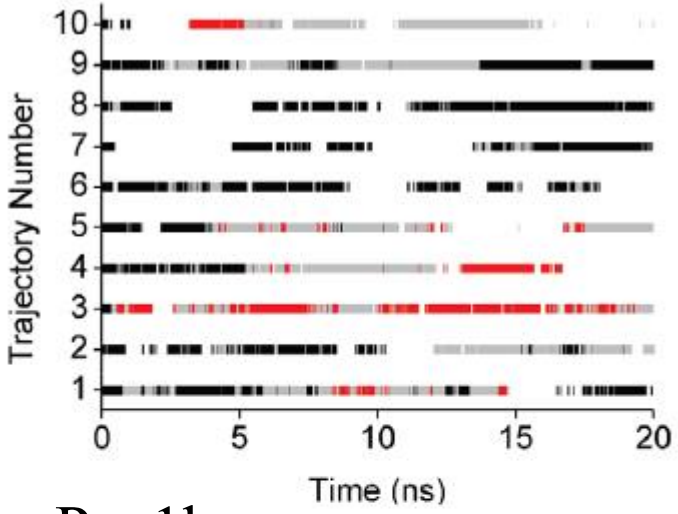


Effect of ligand on number of helical H-bonds

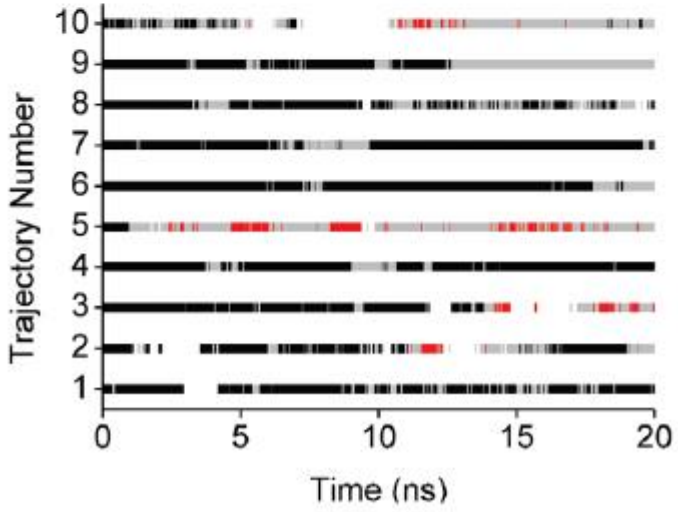


Ligand – peptide contacts

A Dec-DETA



B Pep1b

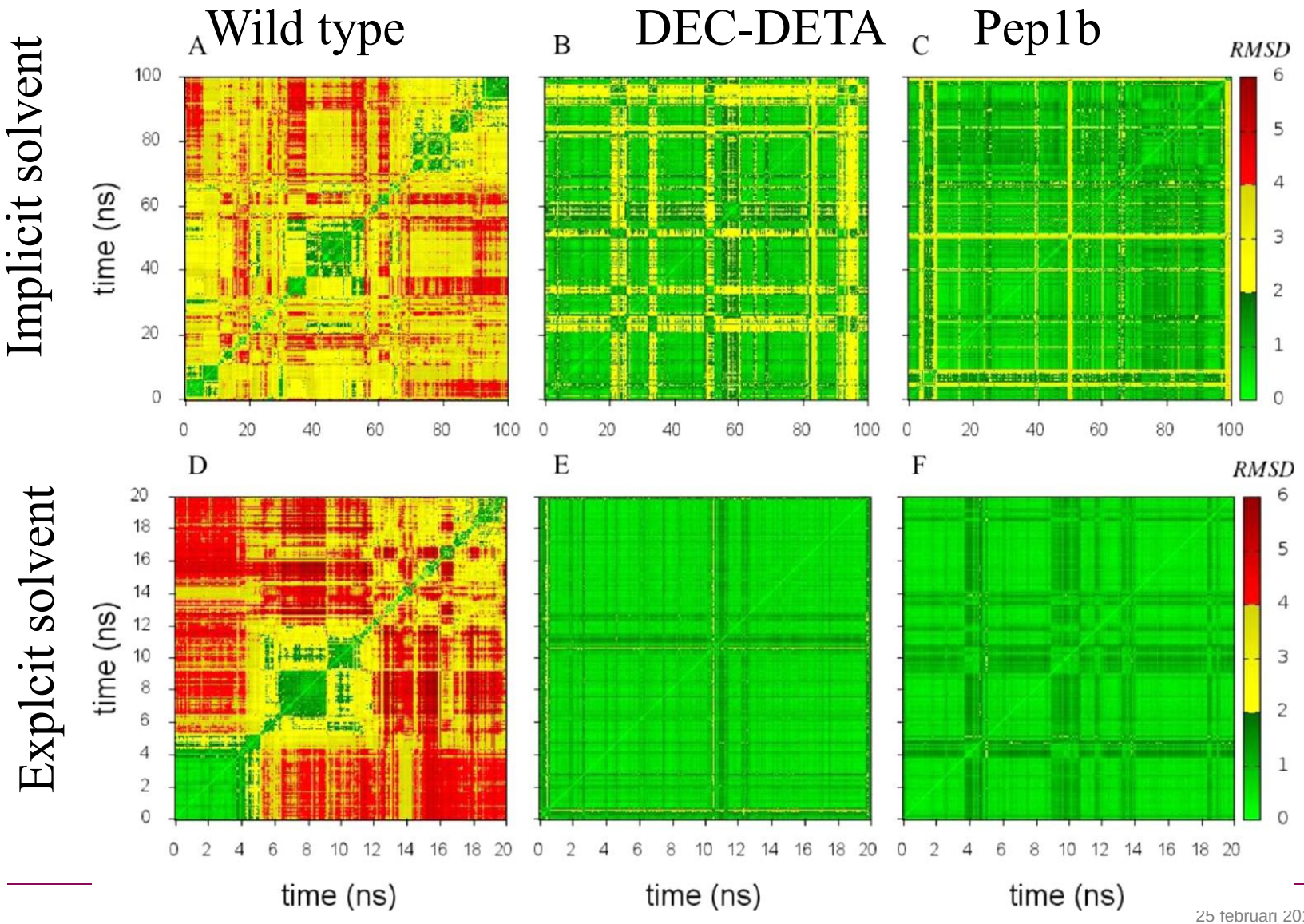


Peptide conformation classes
Class 1 (black): RMSD < 2Å
Class 2 (grey): RMSD 2-4Å
Class 3 (red): RMSD > 4Å

Table 2. Fractions of polar and nonpolar contacts between Aβ and Dec-DETA or Pep1b for each peptide-conformation class[a].

	polar contacts[b]			nonpolar contacts[c]		
ligand	class 1	class 2	class 3	class 1	class 2	class 3
Dec-DETA	0.71	0.75	0.71	0.70	0.62	0.38
Pep1b	0.94	0.87	0.67	0.78	0.53	0.36

Structure variation in simulations
pairwise RMSD matrices (Å)



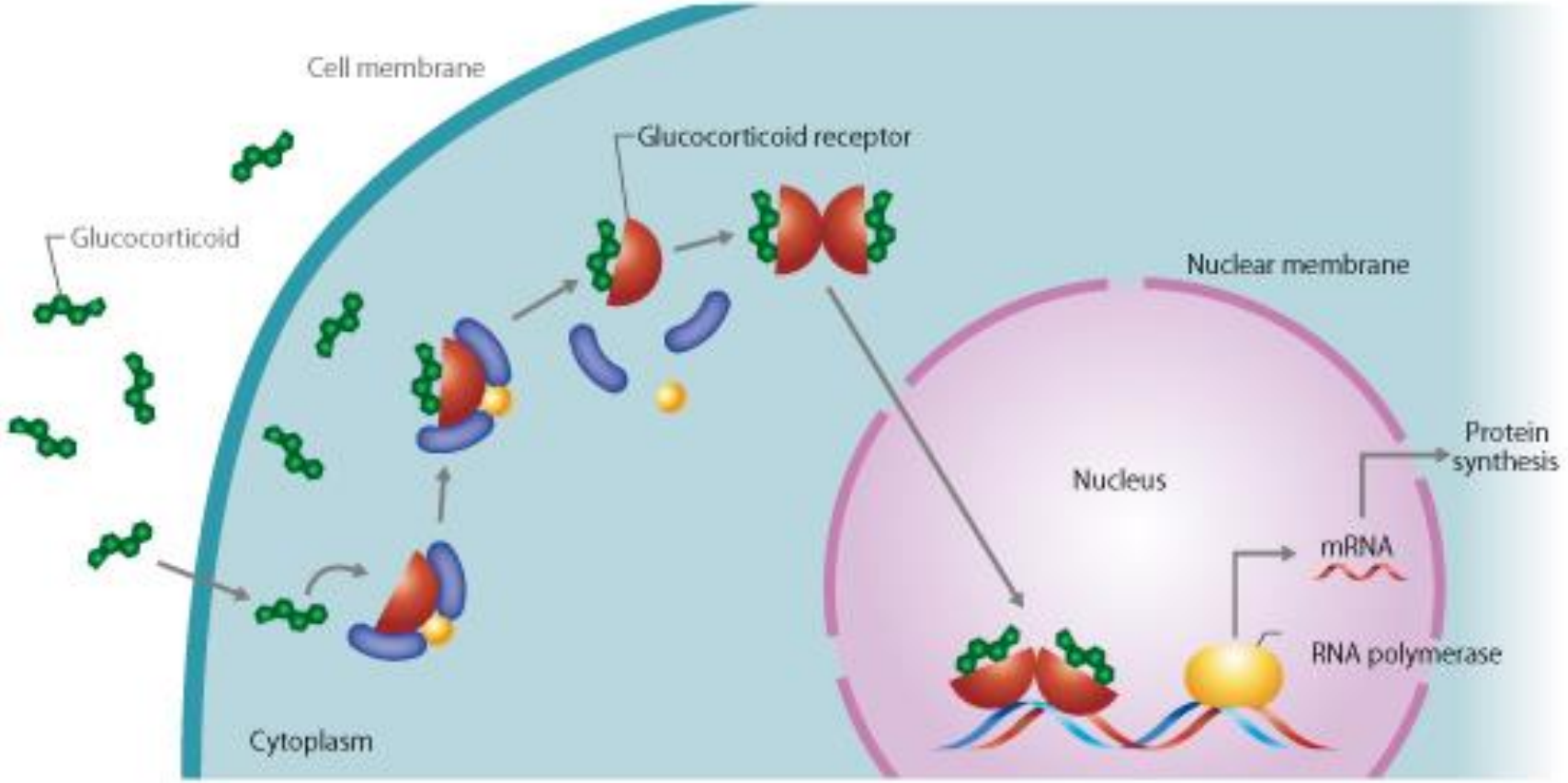
Ranking ligand variants, in progress

Ligand	<#α-Hbonds>
AL_Ac4NdiAEDabpBp	5,1
AL_MD_AEDabW_LDab_dE	5,0
AL_DH18_cff	5,0
AL_DecAEDabWDabdE	4,8
AL_8NAEDab_1	4,6
AL_8NAEDab_2	4,5
AL_acG4NdiAEDabDmn	4,5
AL_DH20_amber1	4,5
AL_DmnDab	4,5
AL_4NAEDab	4,4
AL_RdWDabdEnew3	4,3
pep1b	4,1
AL_RO13_Pep3	3,9
AL_pBpDab	3,8
AL_6NAEDab	3,4
AL_DecAEDab	2,2

SUMMARY

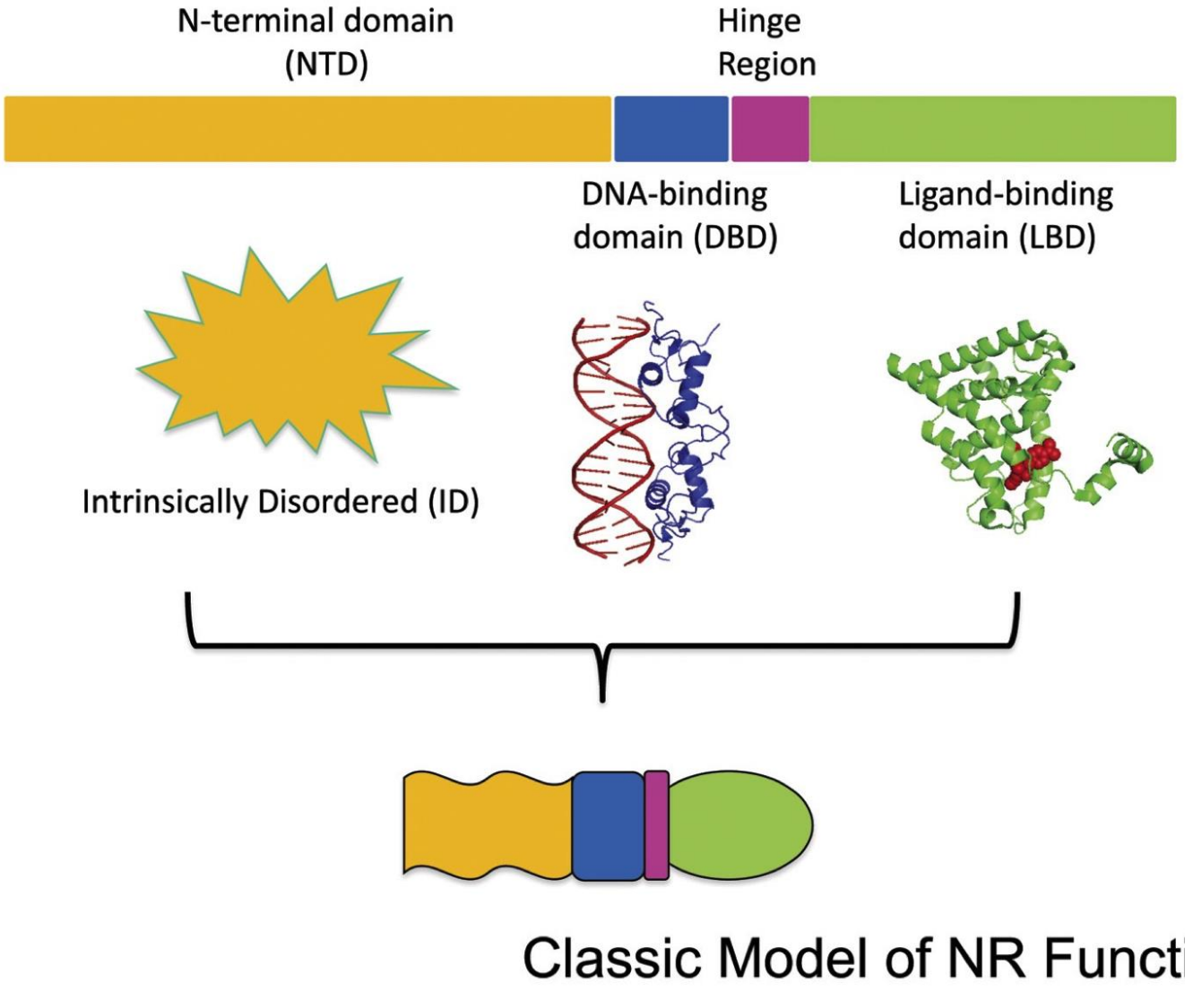
- wt Aβ unfolds more readily than the Ala and Leu mutants, in agreement with experiment.
- Protonation state of histidines does not seem to matter.
- The two ligands do stabilize the helix, and they do bind according to design. Pep1b slightly more efficient than Dec-DETA.
- A set of improved(?) ligands designed, based on details of the interaction patterns seen in first round of simulatons.

Glucocorticoid receptor



https://lookfordiagnosis.com/

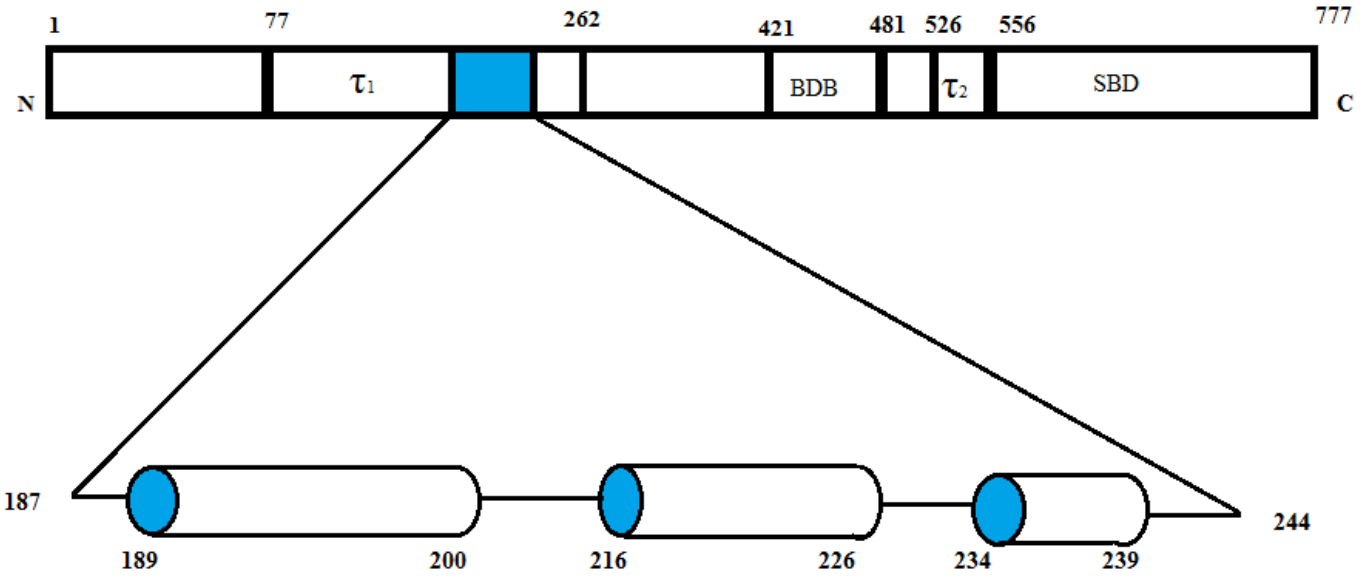
Glucocorticoid receptor



Vincent J. Hilser and E. Brad Thompson J. Biol. Chem. 2011, 286, 39675.

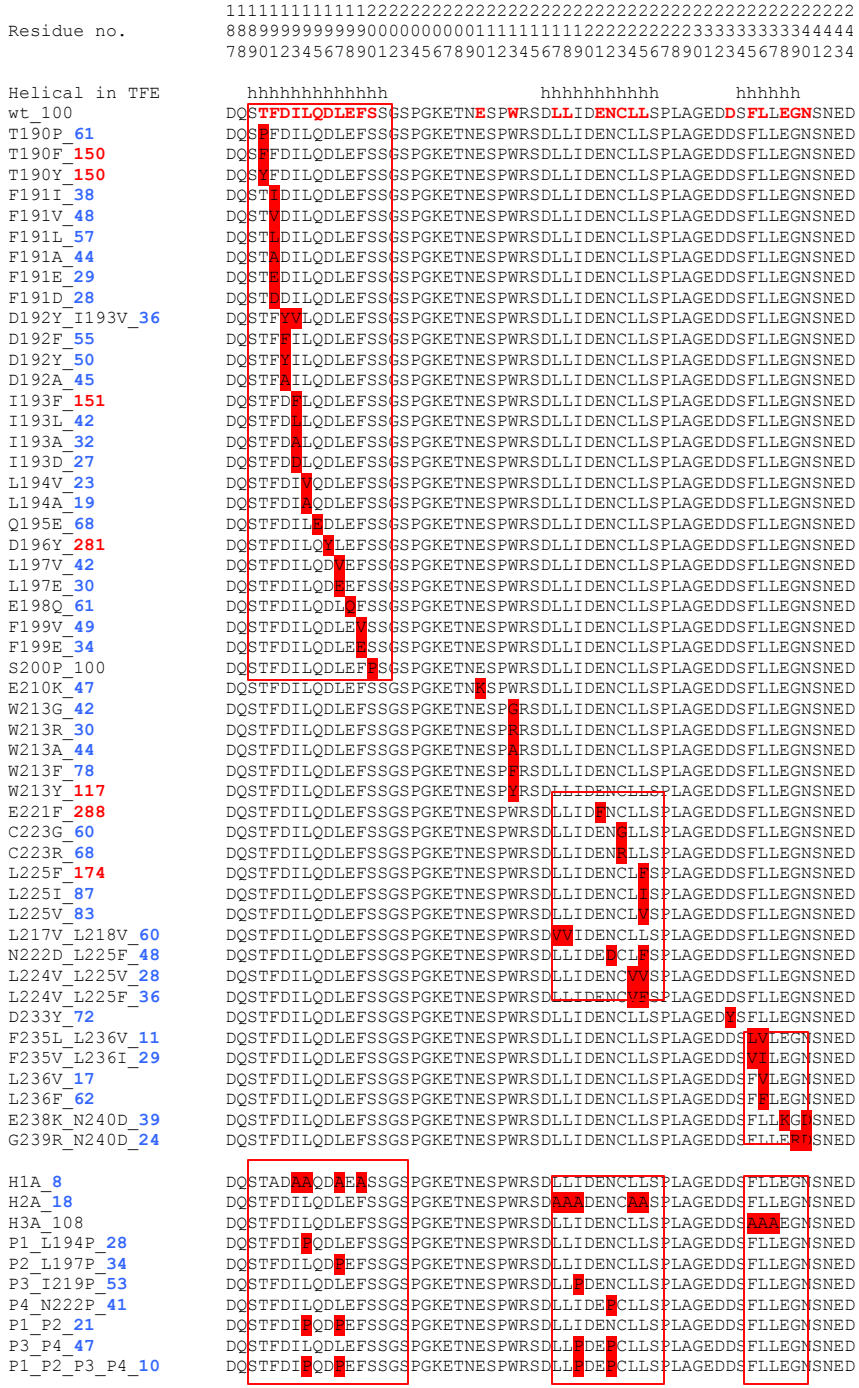
Minimal activation domain (tau1 core) in the N-terminus of the Glucocorticoid Receptor

Tau1core: GR187-244 = 58 residues



3 helical propensity regions stabilized in the presence of TFE

Dahlman-Wright et al. 1994 PNAS 91, 1619.



Change-of-function (increased or decreased activity) mutants are mainly found in three putative helices

TABLE 1. Relative β-galactosidase activities of τ1-core mutants

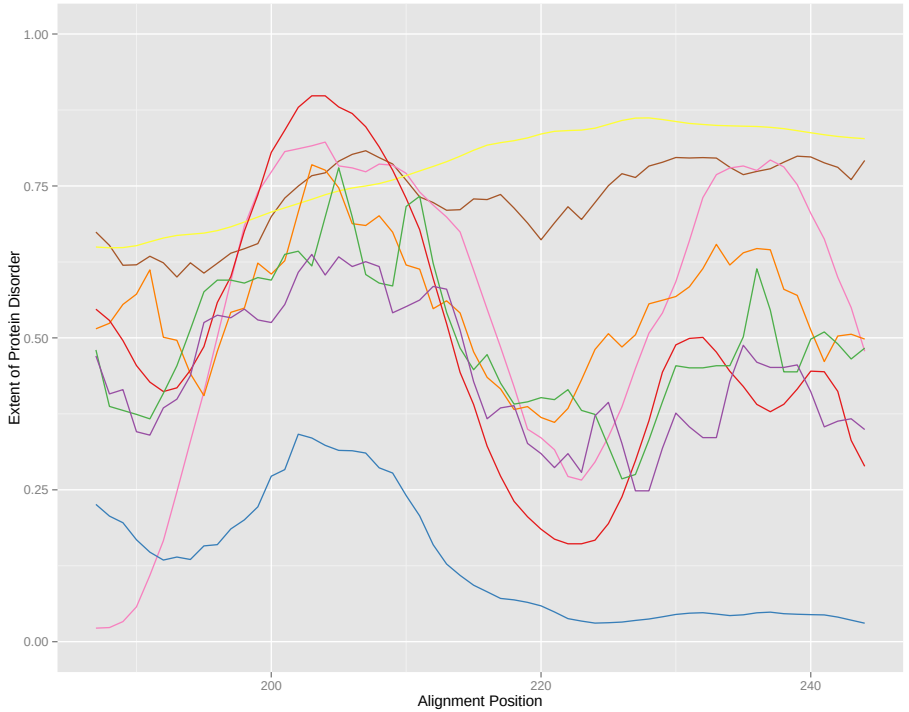
Mutant	Activity[a]	Mutant	Activity[a]
Helical region I		Helical region II	
T190P[b]	61 ± 4	E221F[c]	288 ± 15
F191I	38 ± 10	C223G	60 ± 3
F191V	48 ± 6	C223R	68 ± 5
F191L	57 ± 11	L225F[c]	174 ± 17
F191A[c]	44 ± 3	L225I	87 ± 6
F191E[c]	29 ± 3	L225V	83 ± 10
F191D[c]	28 ± 3	L218V/L219V[c]	60 ± 8
D192Y/I193V	36 ± 8	N222D/L225F	48 ± 7
I193F	151 ± 9	L224V/L225V	28 ± 3
I193L	42 ± 7	L224V/L225F	36 ± 8
I193A[c]	32 ± 2		
I193D[c]	27 ± 8	Helical region III	
L194V	23 ± 2	D233Y	72 ± 11
L194A[c]	19 ± 6	F235L/L236V	11 ± 2
Q195E[c]	68 ± 5	F235V/L236I	29 ± 9
D196Y	281 ± 26	L236V	17 ± 2
L197V	42 ± 7	L236F	62 ± 1
L197E[c]	30 ± 1	E238K/N240D	39 ± 2
E198Q	61 ± 1	G239R/N240D	24 ± 5
F199V	49 ± 3		
F199E[c]	34 ± 8		
S200P[b]	100 ± 10		
Loop			
E211K	47 ± 17		
W213G	42 ± 1		
W213R	30 ± 2		
W213A[c]	44 ± 5		
W213F[c]	78 ± 5		
W213Y[c]	117 ± 25		

[a] Mean relative β-galactosidase activity (percentage of wild-type level ± standard deviation (n = 3).
[b] τ1-core mutant found by sequencing the mutant pool prior to screening.
[c] τ1-core mutant made by site-directed mutagenesis.

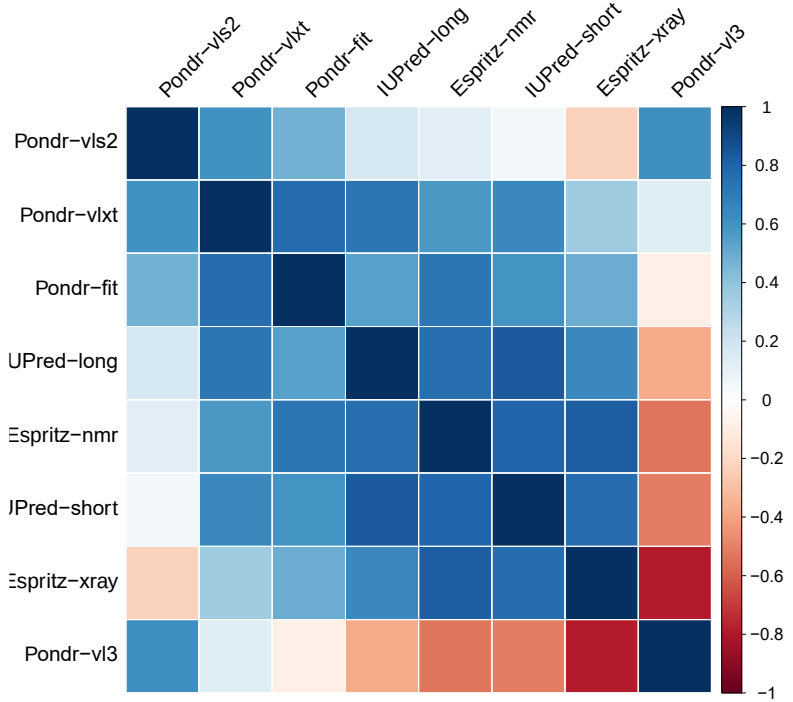
A collection of 60 functionally characterised mutants in the GR-tau1core activation domain

Almlöf et al. 1997 Mol. Cell. Biol. 17, 934.
Almlöf et al. 1998 Biochemistry 37, 9586.

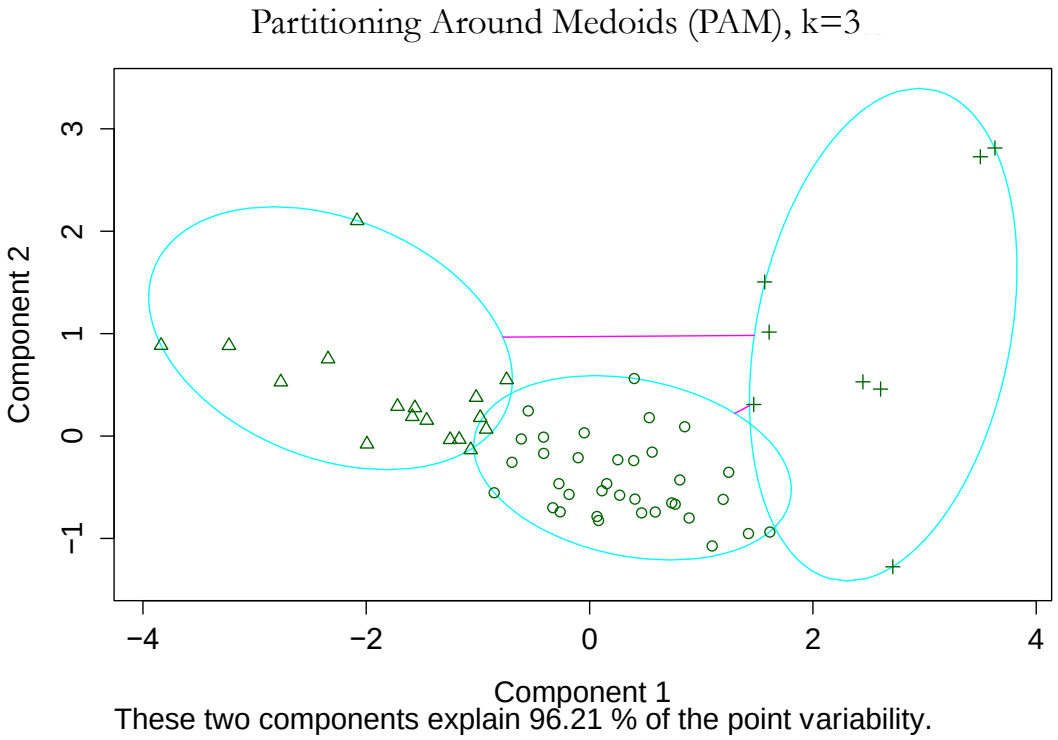
GR-tau1c IDR prediction – different methods agree well



Correlation between predictors



GR mutants can be partitioned into three main clusters



Partitioned data = IUPred-long, ANCHOR, Activity

Activity and amino acid substitution differences characterise GR mutant clusters

Cluster 1*
Low activity
High disorder

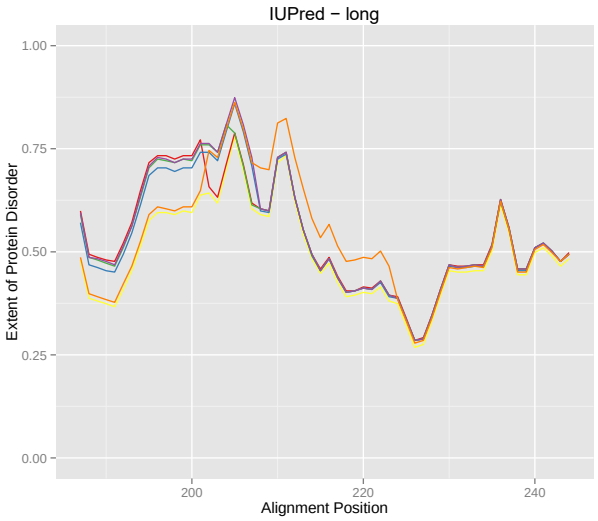
Mutant	Relative activity (%)
L197P	34
L197E	30
L194P	28
W213R	30
F191D	28

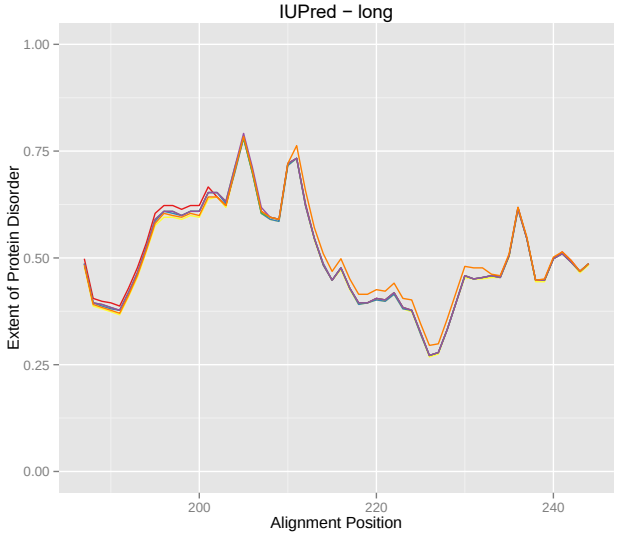
Cluster 2*
Low activity
Unchanged disorder

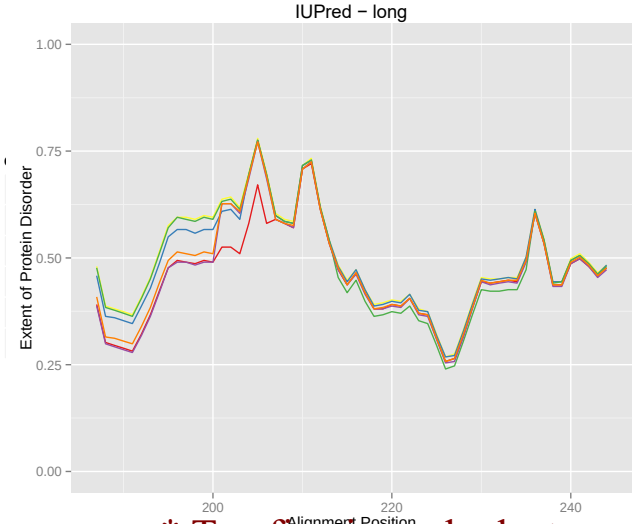
Mutant	Relative activity (%)
I193L	42
L197V	42
F191I	38
N222P	41
L194V	23

Cluster 3*
High activity
Low disorder

Mutant	Relative activity (%)
T190Y	150
T190F	150
L193F	151
L225F	174
D196Y	281

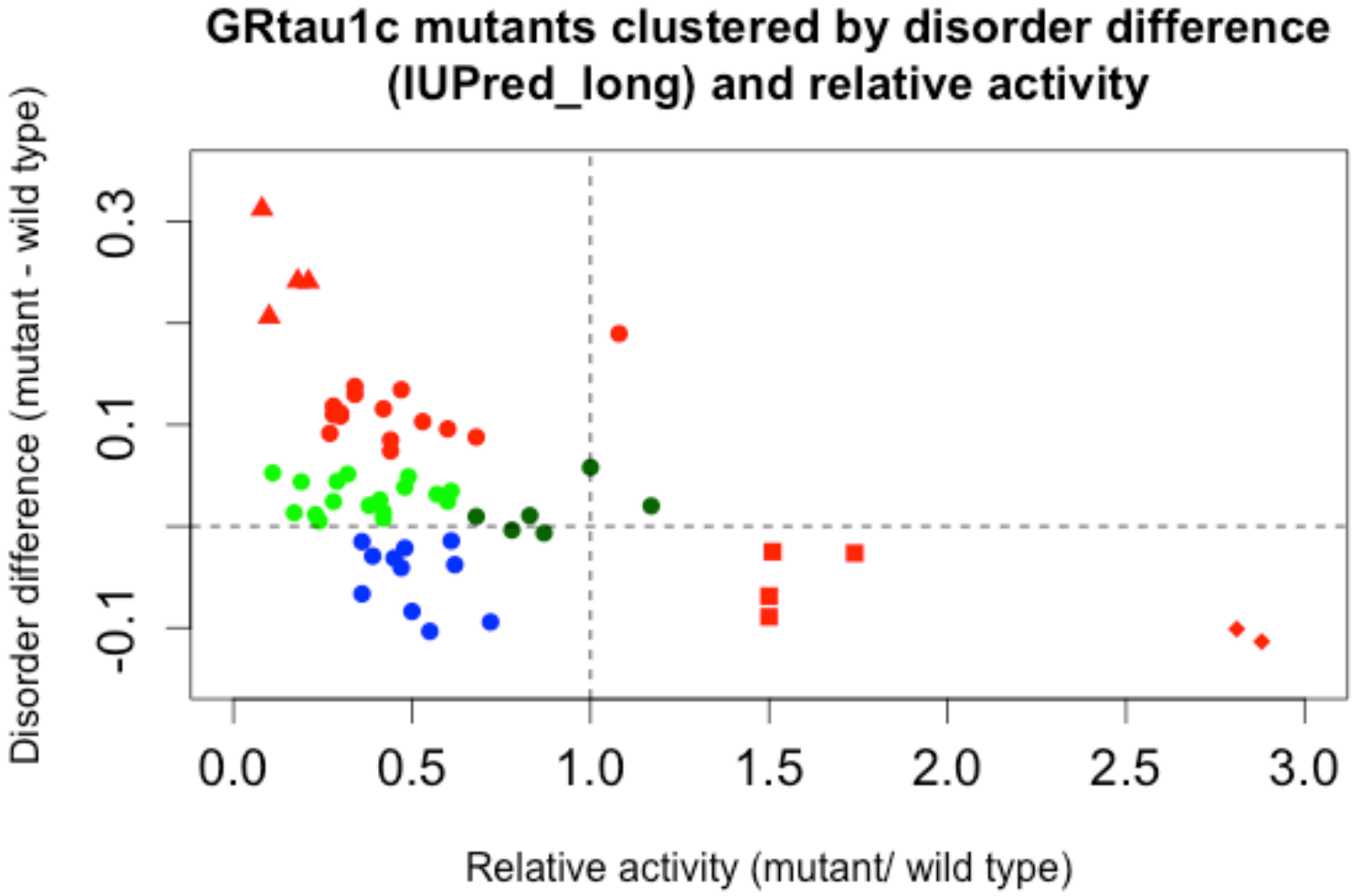




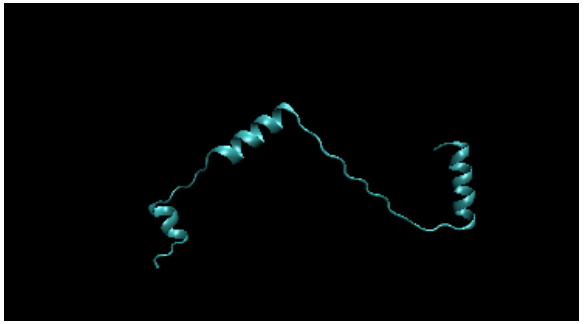


* Top five in each cluster

Predicted disorder *vs* relative activity

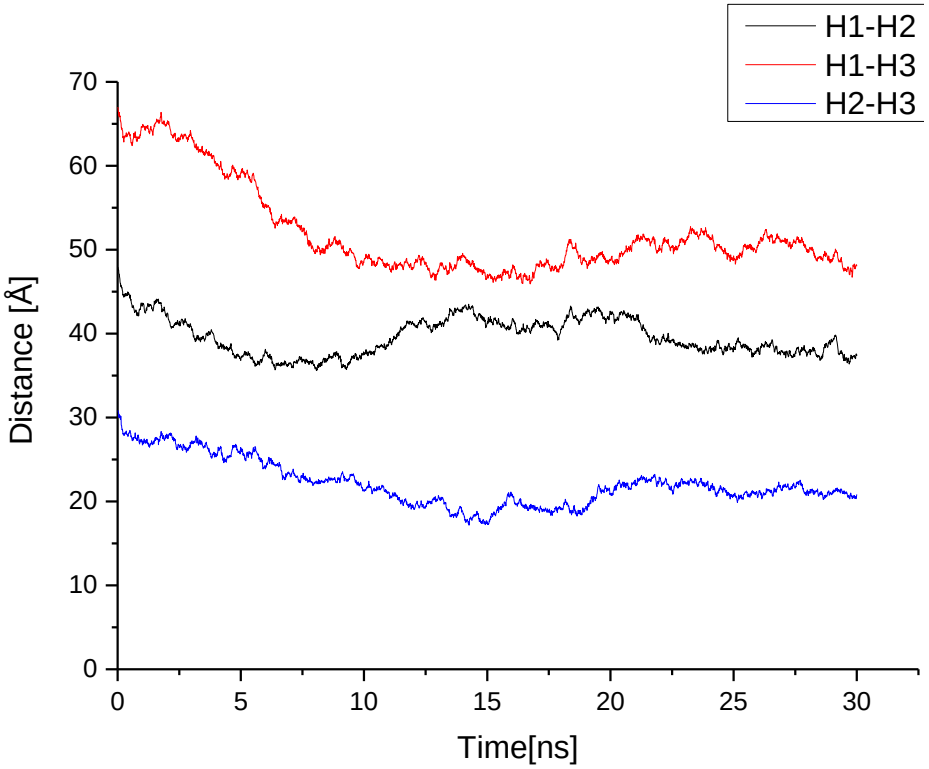


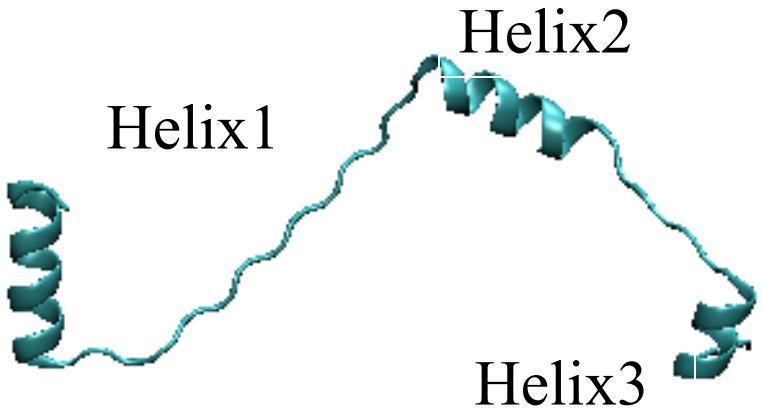
Simulation protocol



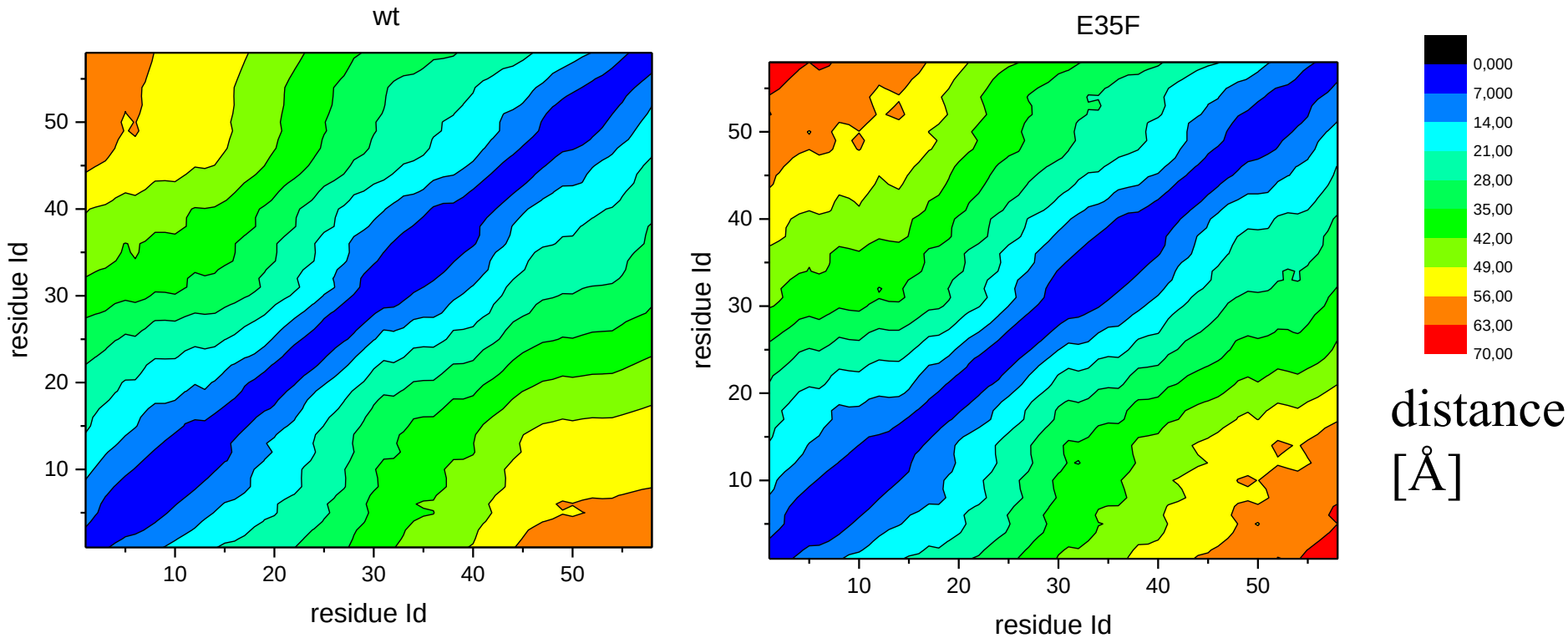
- Start with α-helices separated by extended linkers
- CHARMM36 FF, explicit solvent; 360K and 400K
- 10 x 100 ns for each system
- Measure first passage time to fraction α helix = 0.5

Are the three putative helices independent? Distances between helices in WT



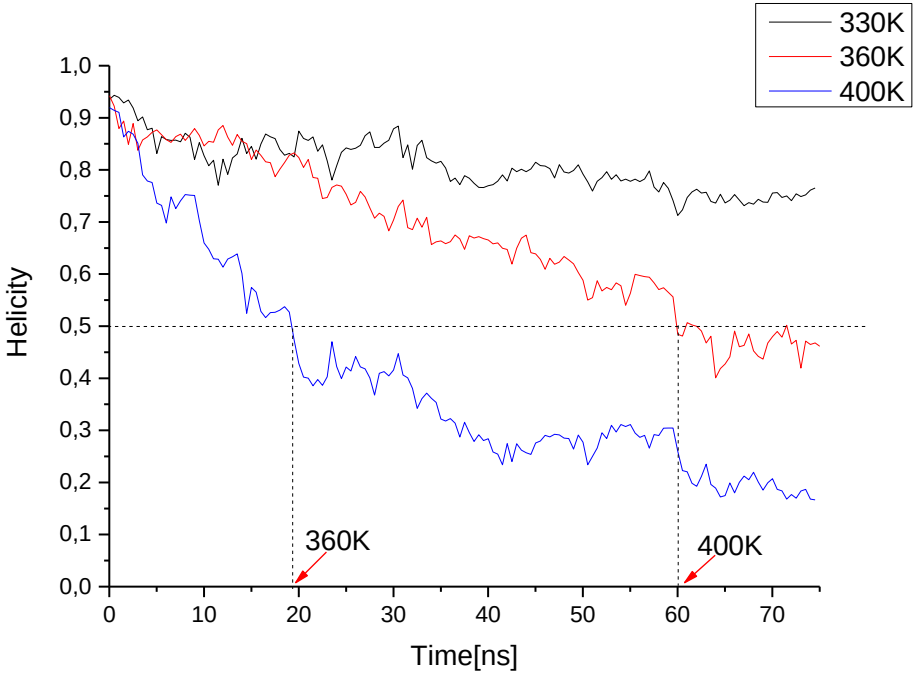


<|res(i)-res(j)|> of wt and E35F



Focus on <u>helix 1</u>: 16aa D_{187}QSTFDFILQDLEFSSG_{202}
Simulate wt + 14 mutants, peptide in $(64Å)^3$ box TIP3P
27000 atoms, ~20μs total simulation time
(65ns/day on GTX980TI GPU, CHARMM/OpenMM)

Temperature effect on the helical content of WT



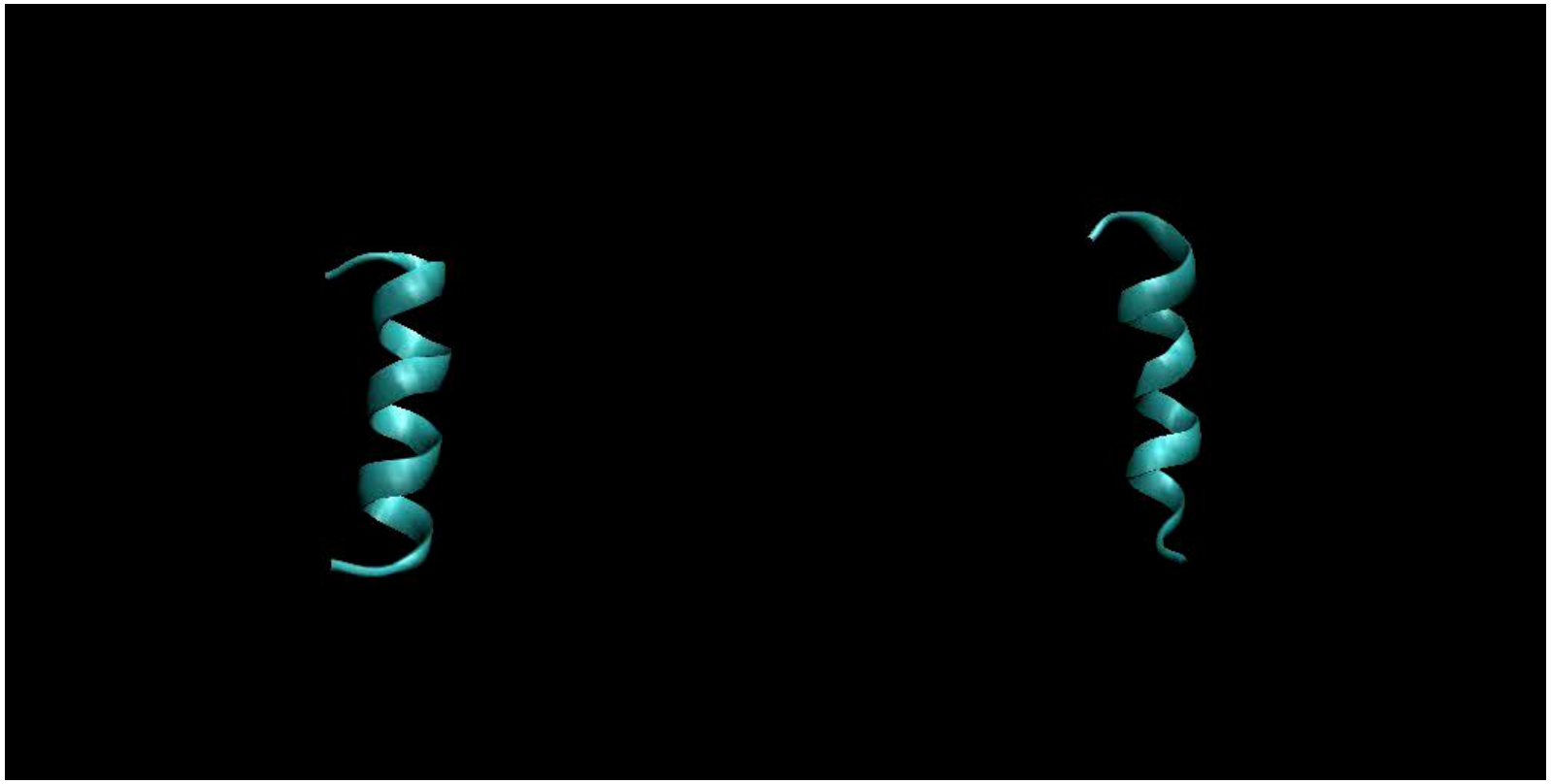
First passage time

- 330K- not reaching
- 360K=60ns
  400K=20ns

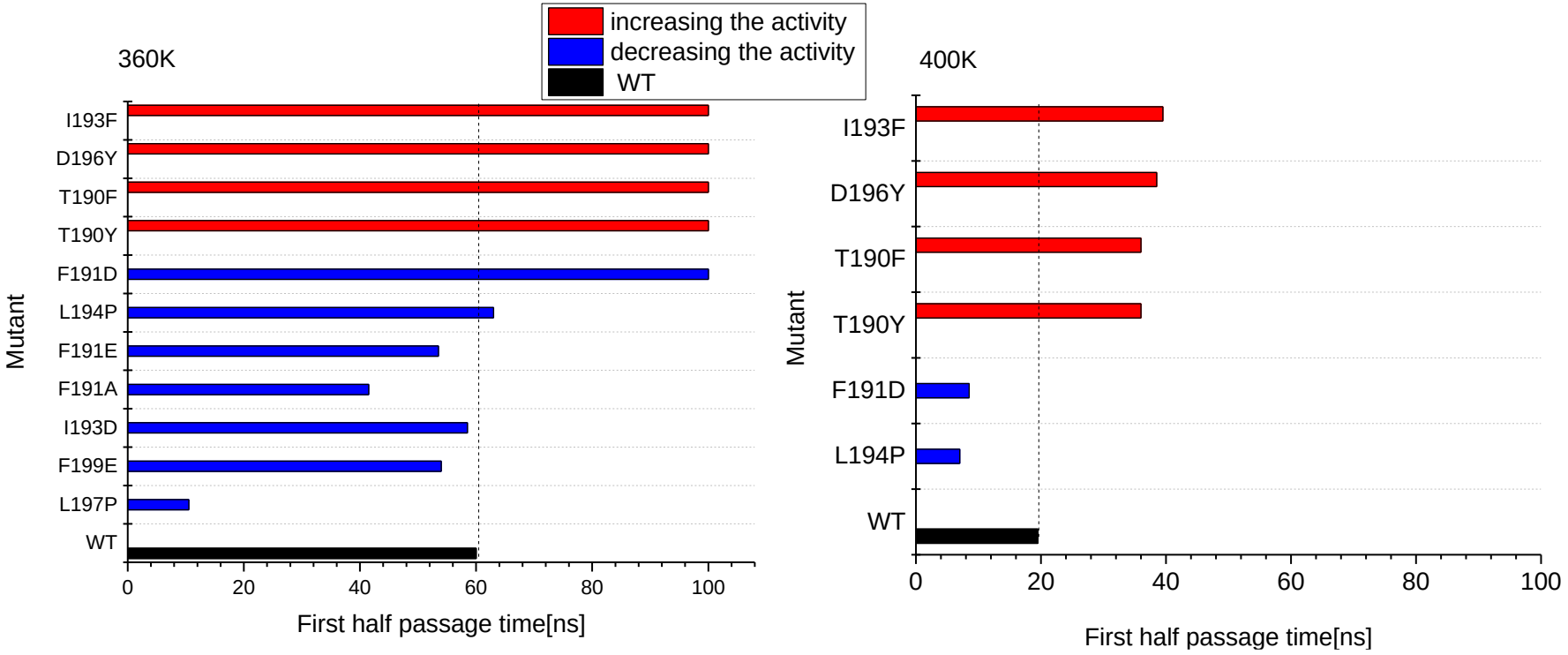
Molecular dynamics simulations
Mutants in Helix 1

D196Y **increasing** activity 50ns

L197P **decreasing** activity 25ns

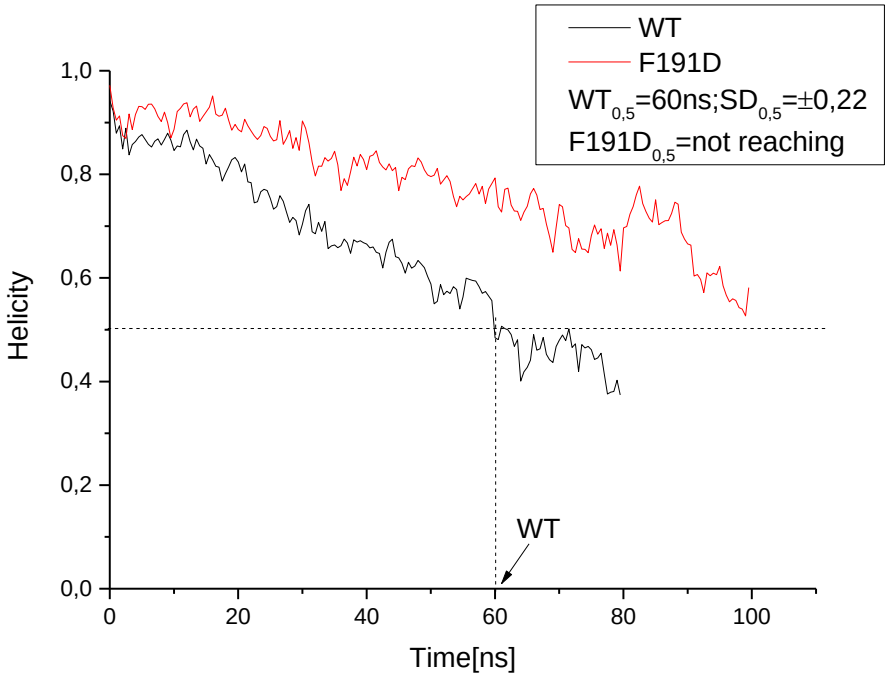


First passage times for some mutants at 360K and 400K

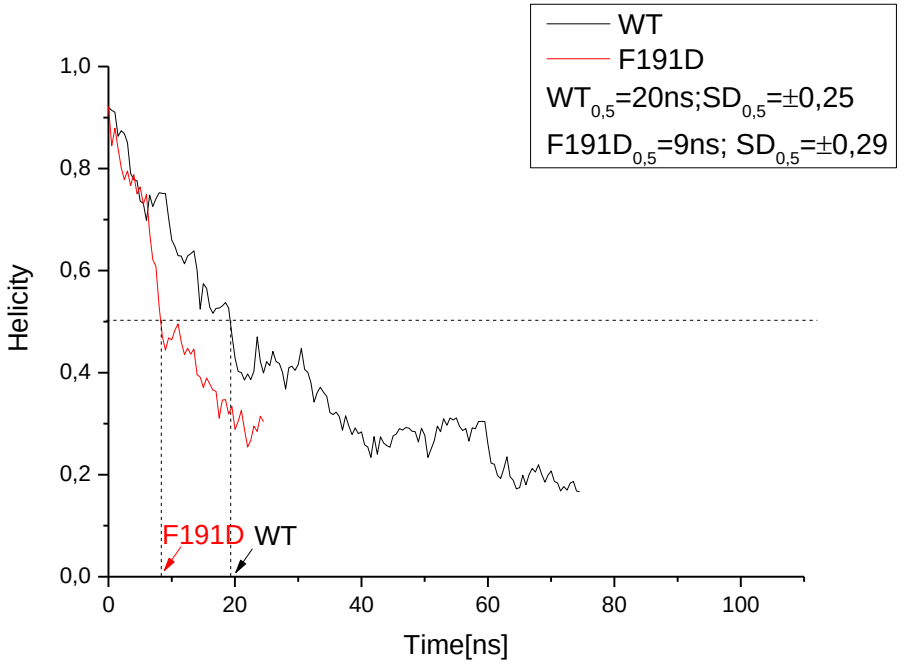


WT/F191D first passage times

- At 360K

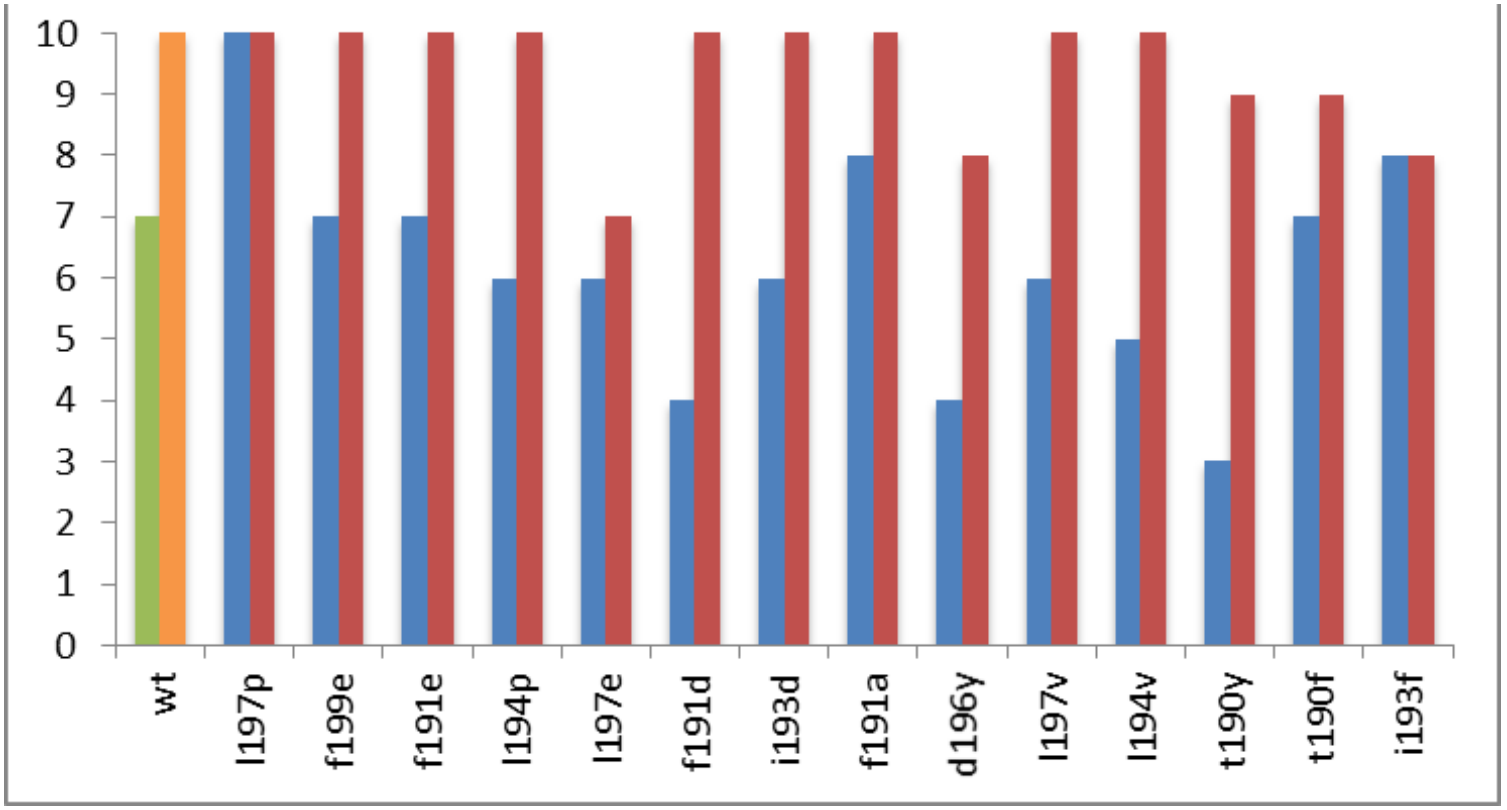


- At 400K

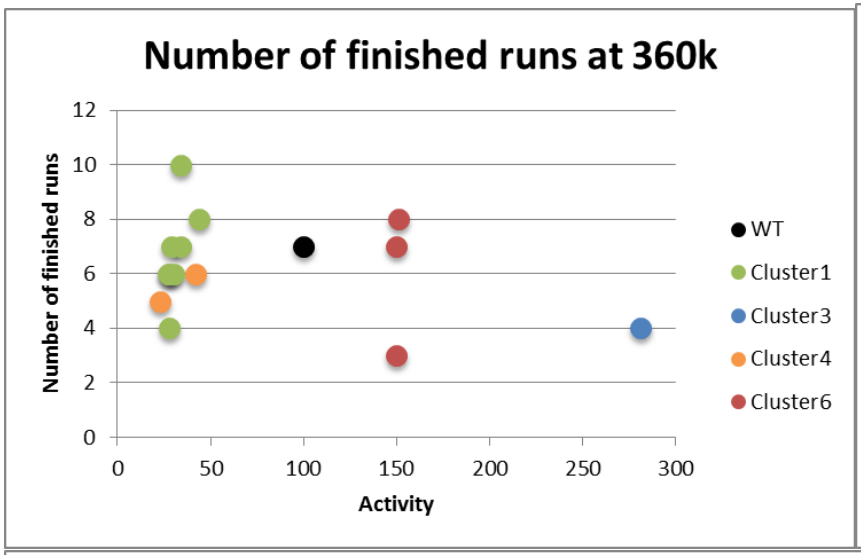


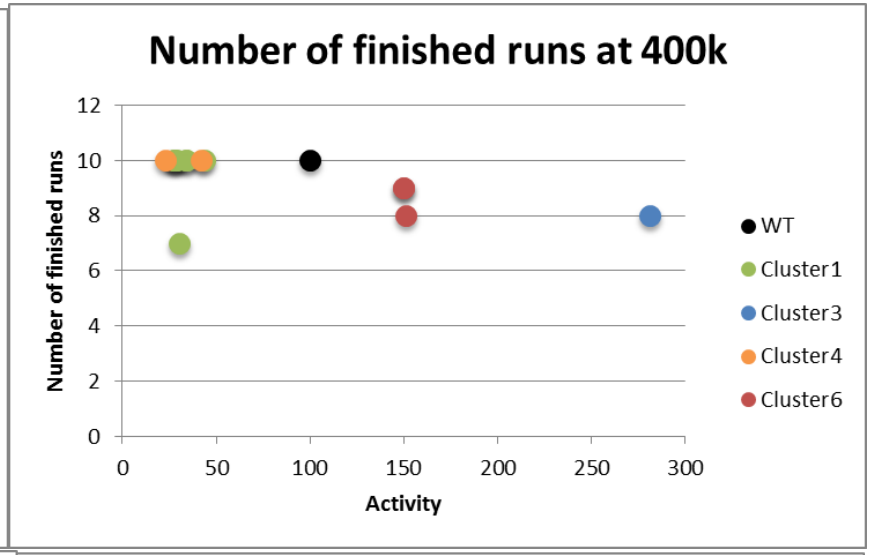
Helix stability and protein activity

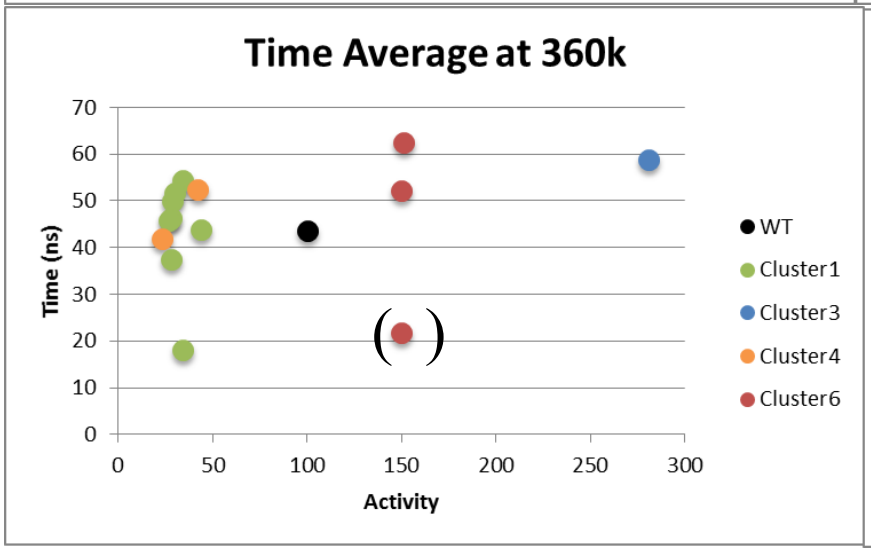
Number of runs that reach 50% helix in < 100ns

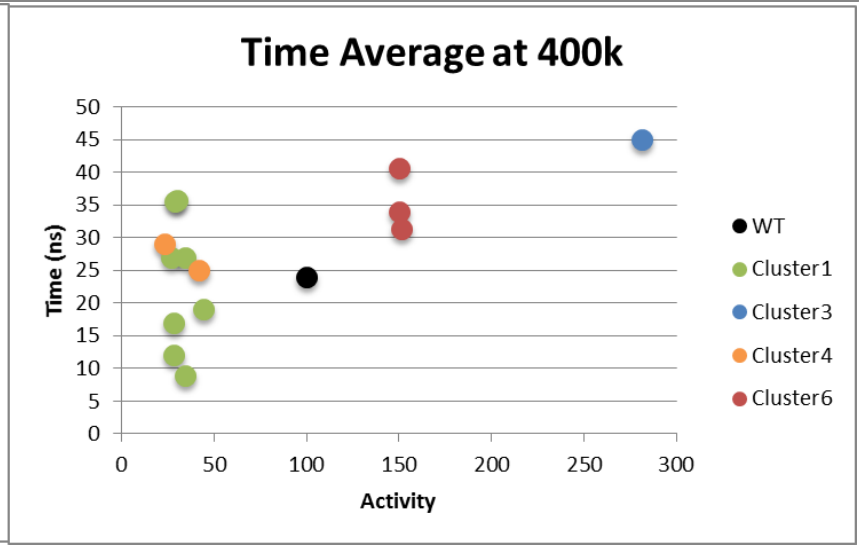


Helix stability and protein activity









Conclusions

- Both bioinformatics and MD methods show correlation between change-of-function mutations and helix stability
- Overall picture: mutations affect function through an influence on folding – more structure ⇔ higher activity
- Temperature affects stability of the model peptides
- Mutations of hydrophobic residues increase helical stability
- Mutations of polar/charged residues decrease helical stability
- $D_{187}QSTFDFILQDLEFSSG_{202}$ is possible target for compounds designed from our peptidomimetic library