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Effects of change-of-function mutations on helical propensity in the intrinsically disordered τ 1-core transactivation domain of the Glucocorticoid receptor

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11/18/2015 InnoMol, Zagreb, Croatia

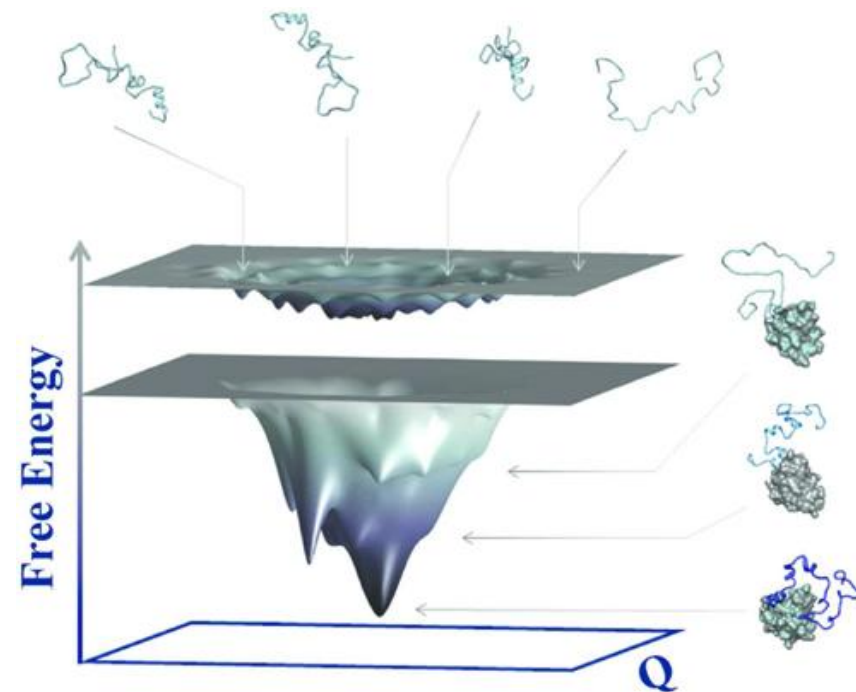
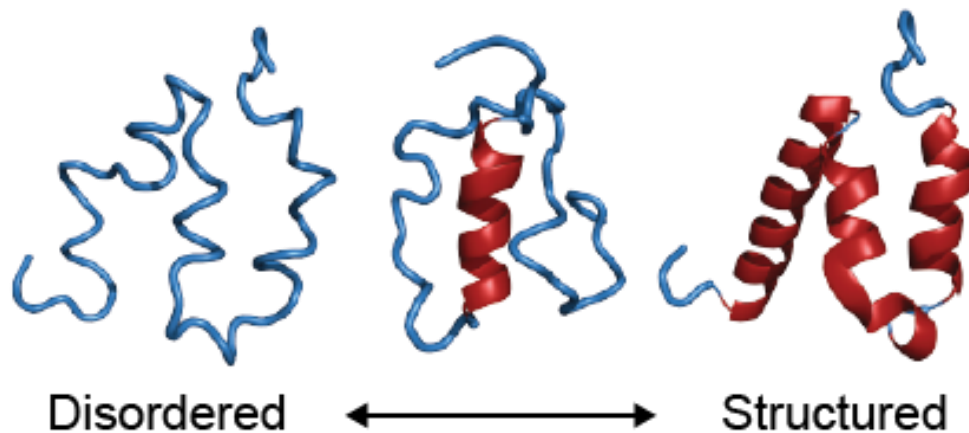
Outline

- Introduction to Intrinsically disordered proteins
- Glucocorticoid receptor(GR)- structure and function
- Experimental results
- Bioinformatics and simulation results

Intrinsically disordered proteins (IDPs)

- IDPs are proteins that either lack the 3D structure or their structure is not fixed.

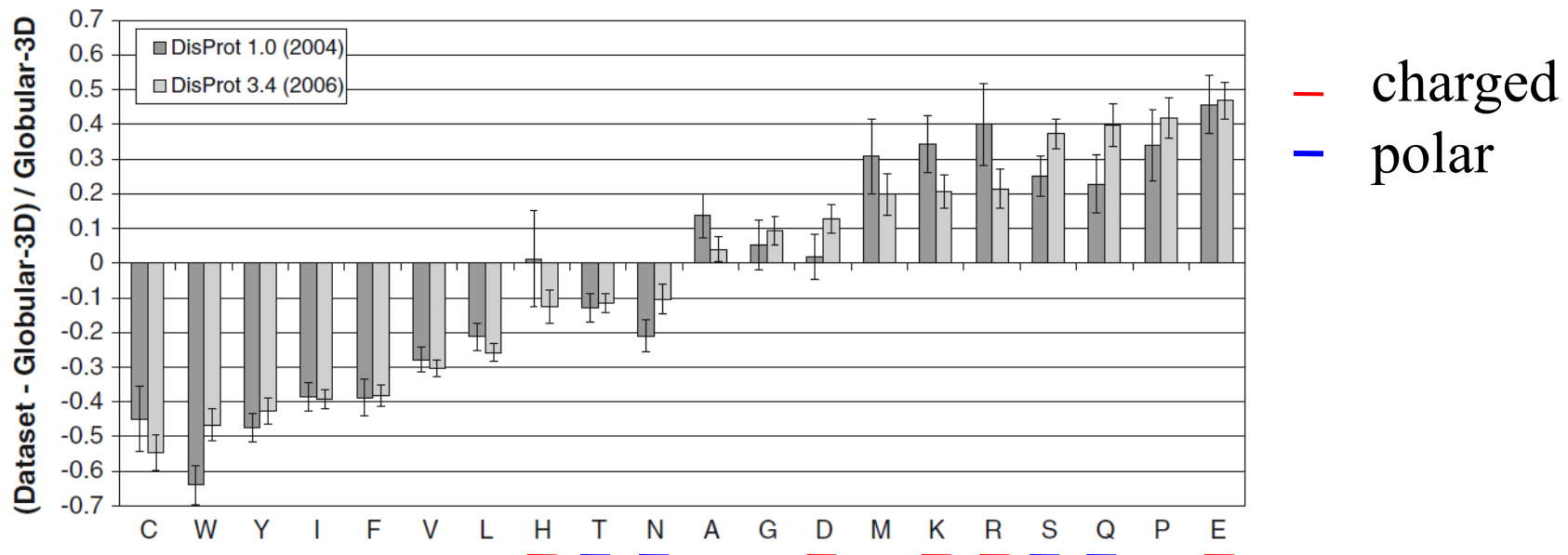
The protein disorder continuum



Dyson *et al.* Nat Rev Mol Cell Biol 2005, 6,197.
Gibbs *et al.* Biochemistry 2015, 54, 1314.

Methods for detection and amino acid content

- IDPs are more often found in eukaryotes
- IDPs cannot be resolved by X-Ray but they can be detected by NMR, CD, Fluorescence spectroscopy, Raman spectroscopy, SAXS
- Contain less bulky hydrophobic and more polar and charged residues



Uversky Protein J 2009, 28, 305.

Computer prediction programs and data banks

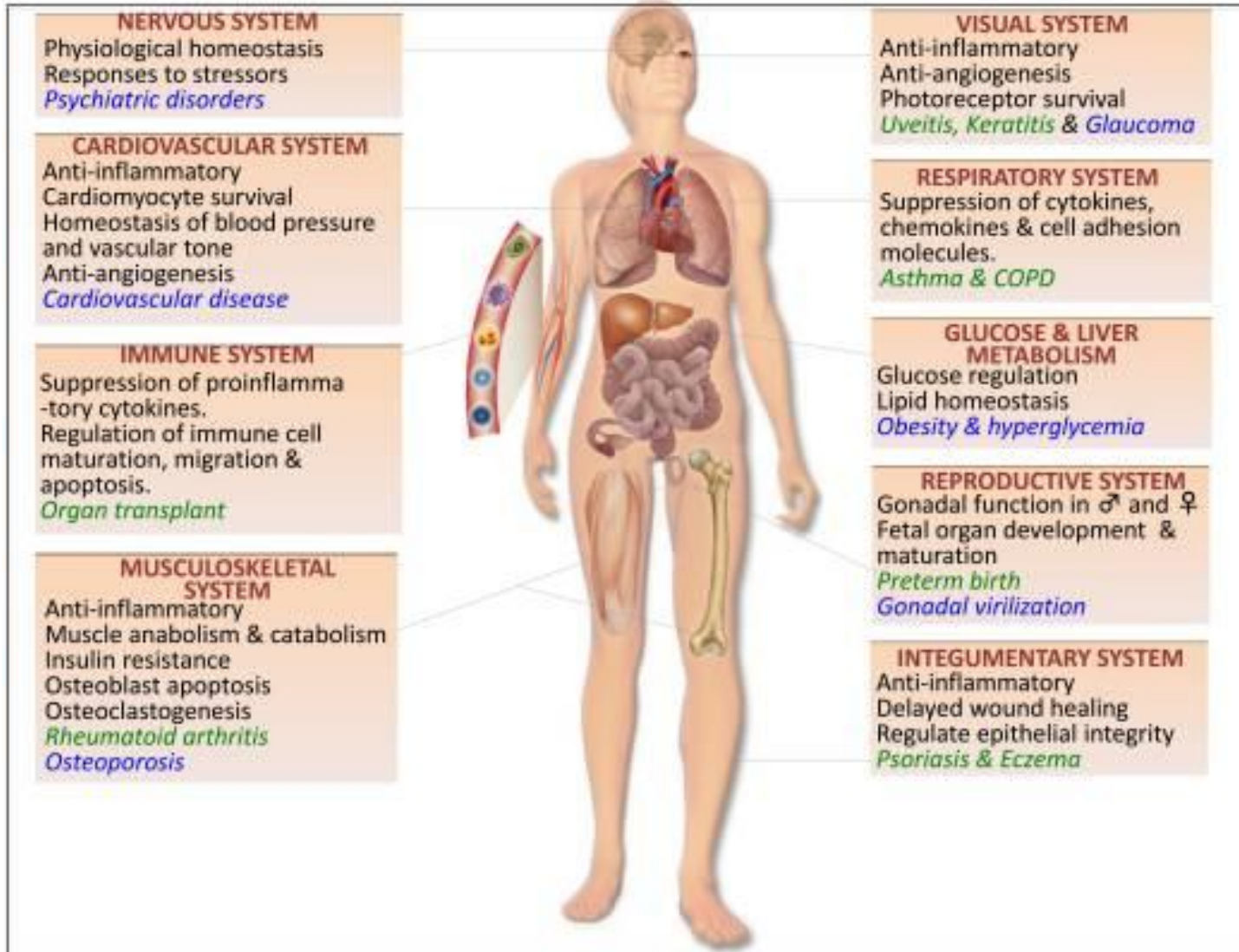
Software

- PONDR
- FoldIndex
- DisEMBL
- DISORDERED/2
- VSL
- FoldUnfold
- IUPred
- ANCHOR
- Espritz etc

Data banks:

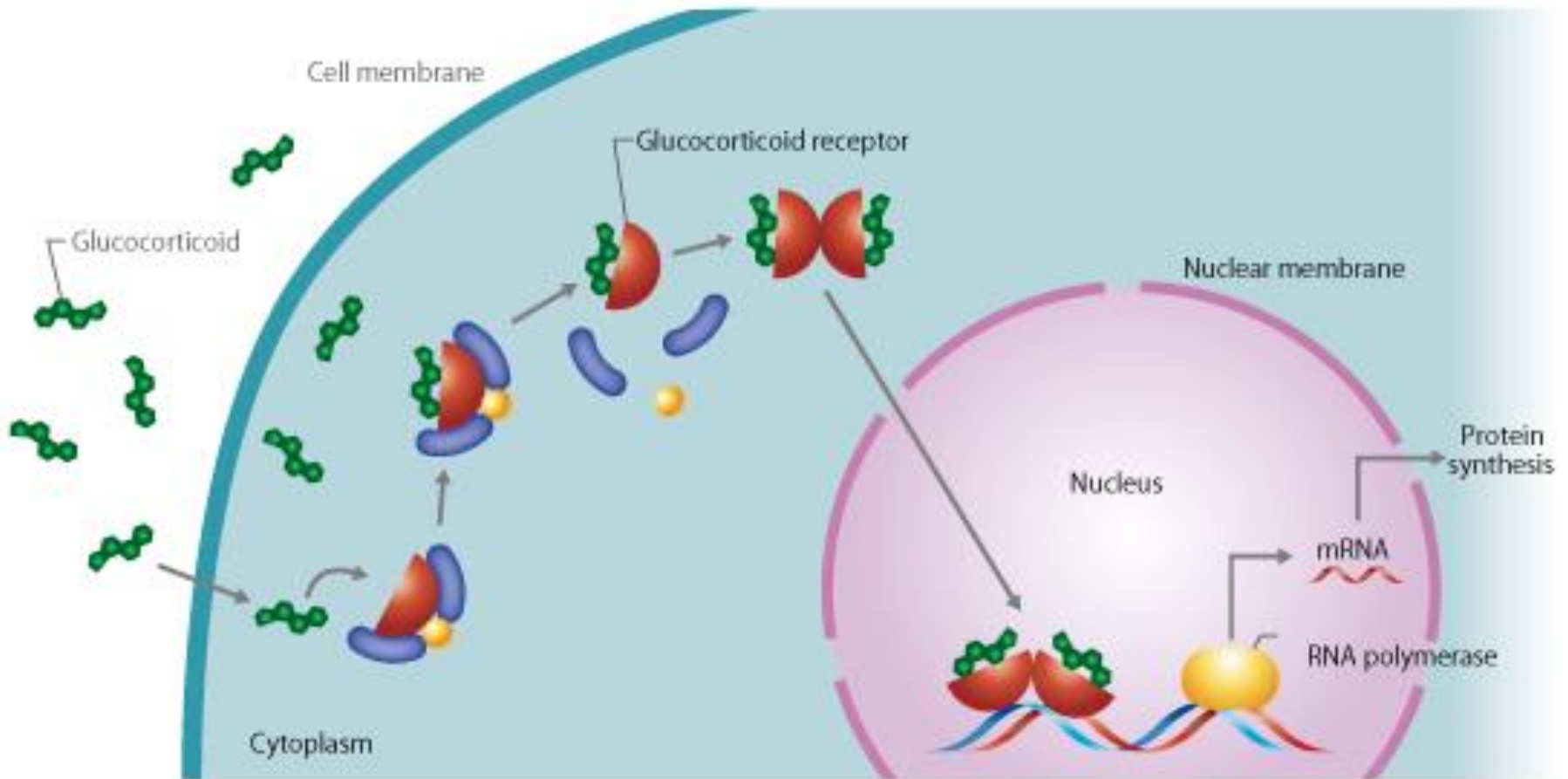
- DisProt → <http://www.disprot.org/index.php>

Glucocorticoid receptor



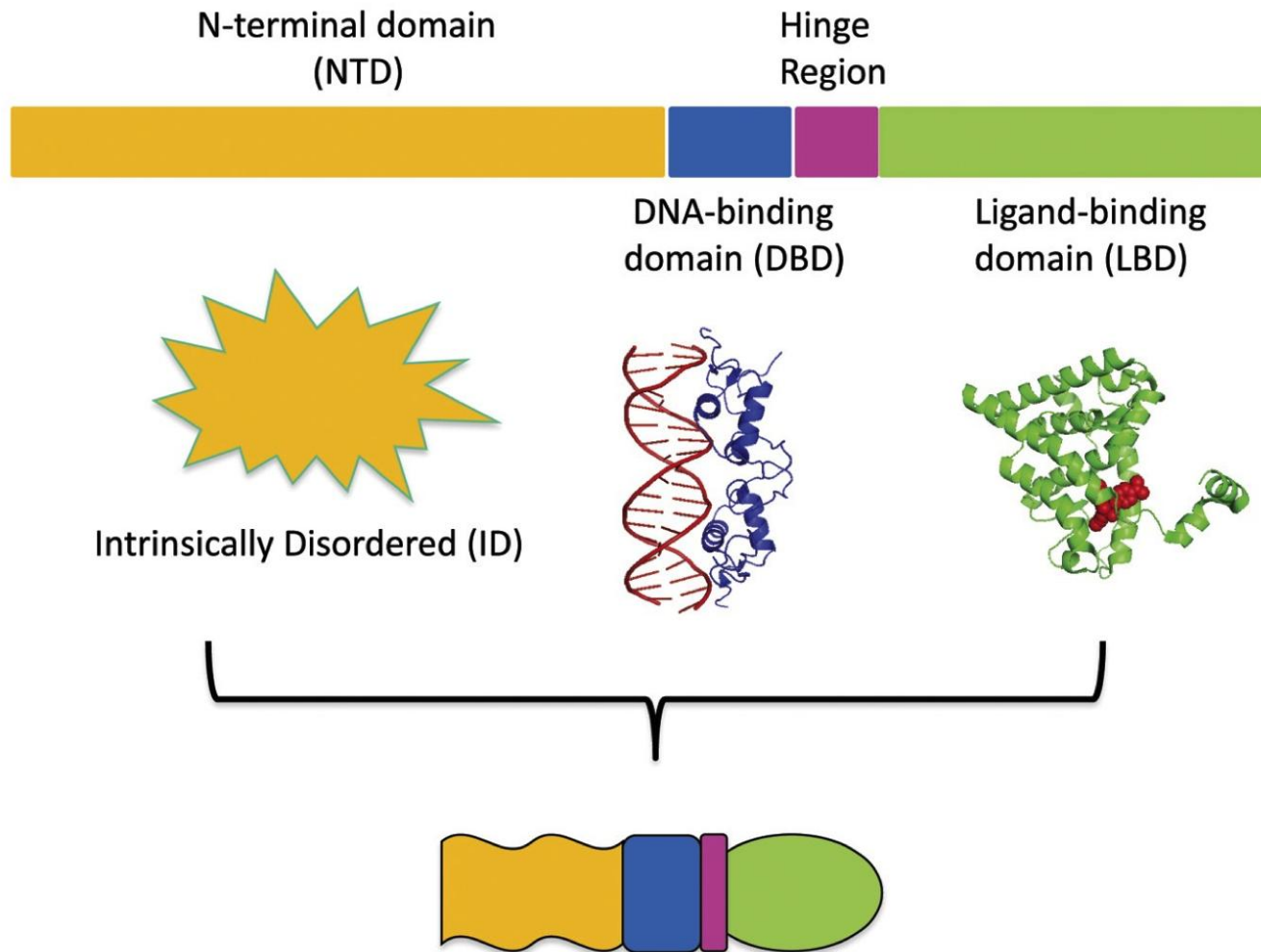
Mahita Kadmiel and John A. Cidlowski Trends Pharmacol. Sci. 2013, 34, 518.

Glucocorticoid receptor



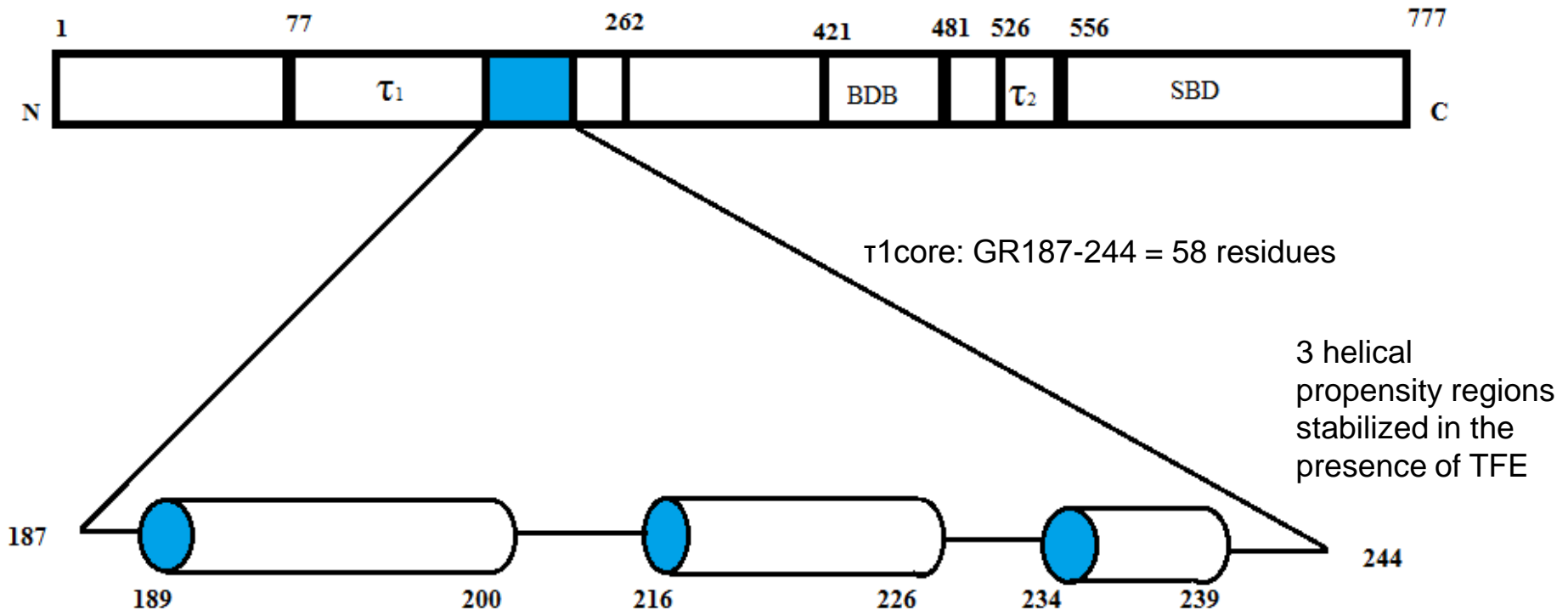
<https://lookfordiagnosis.com/>

Glucocorticoid receptor



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

Minimal activation domain (τ_1 core) in the N-terminus of the Glucocorticoid Receptor



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

Helical in TFE
wt_100
T190P_61
T190F_150
T190Y_150
F191I_38
F191V_48
F191L_57
F191A_44
F191E_29
F191D_28
D192Y_I193V_36
D192F_55
D192Y_50
D192A_45
I193F_151
I193L_42
I193A_32
I193D_27
L194V_23
L194A_19
Q195E_68
D196Y_281
L197V_42
L197E_30
E198Q_61
F199V_49
F199E_34
S200P_100
E210K_47
W213G_42
W213R_30
W213A_44
W213F_78
W213Y_117
E221F_288
C223G_60
C223R_68
L225F_174
L225I_87
L225V_83
L217V_L218V_60
N222D_L225F_48
L224V_L225V_28
L224V_L225F_36
D233Y_72
F235L_L236V_11
F235V_L236I_29
L236V_17
L236F_62
E238K_N240D_39
G239R_N240D_24

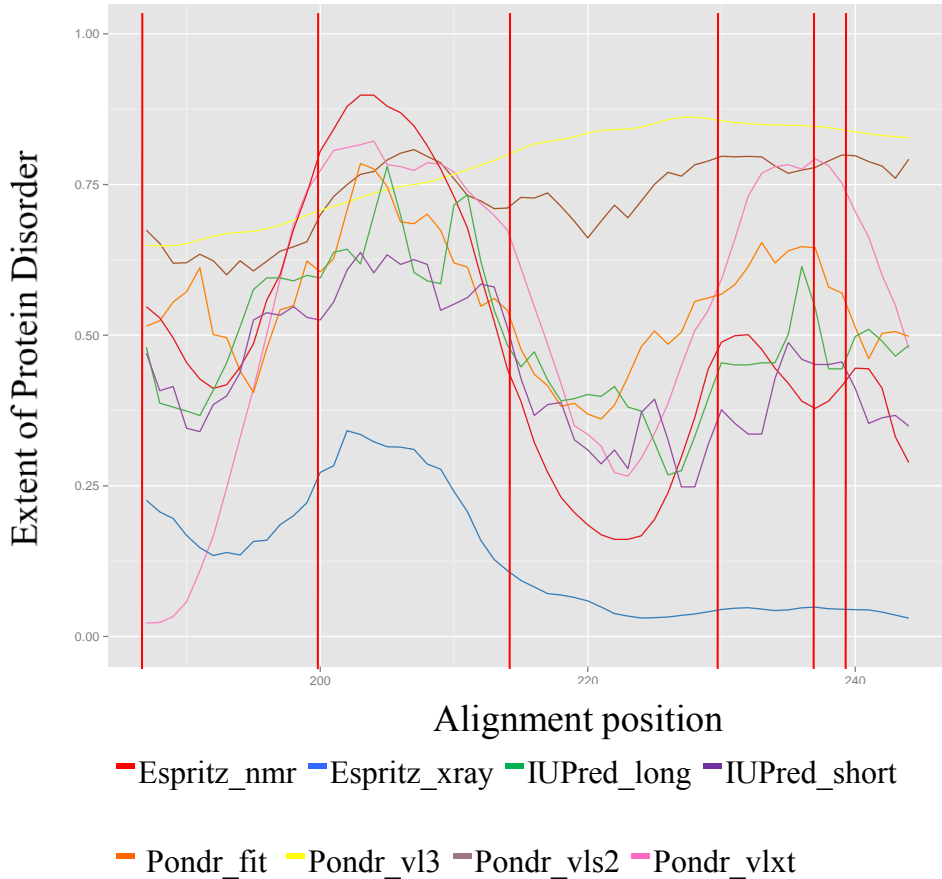
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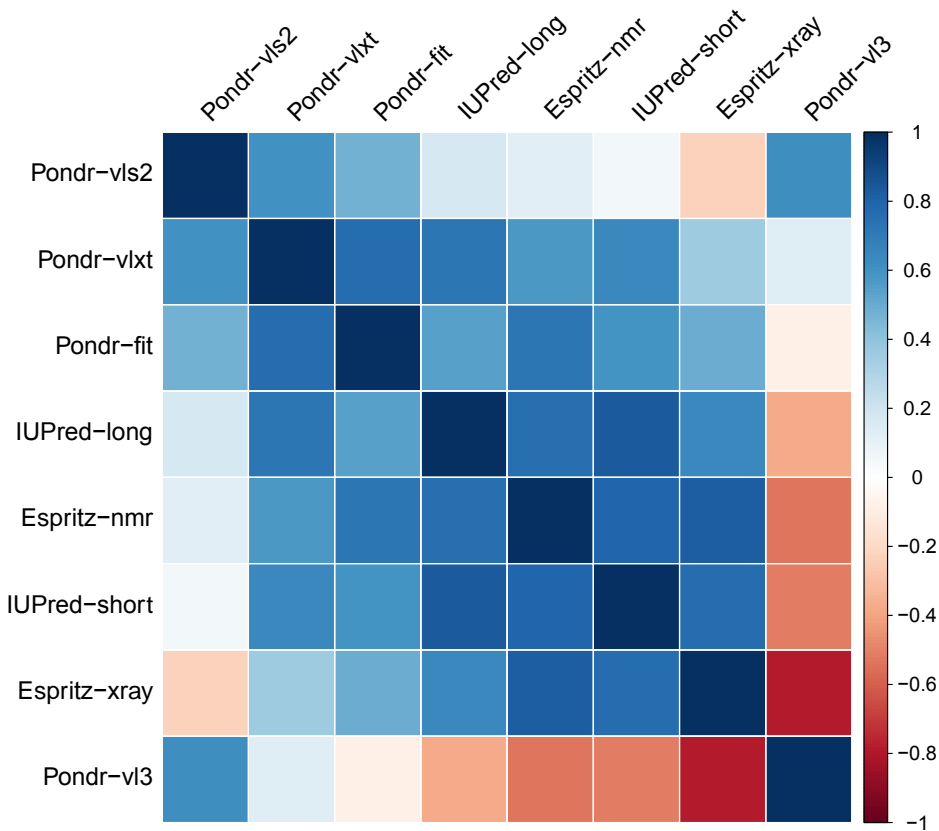
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GR-T1c IDR prediction – different methods

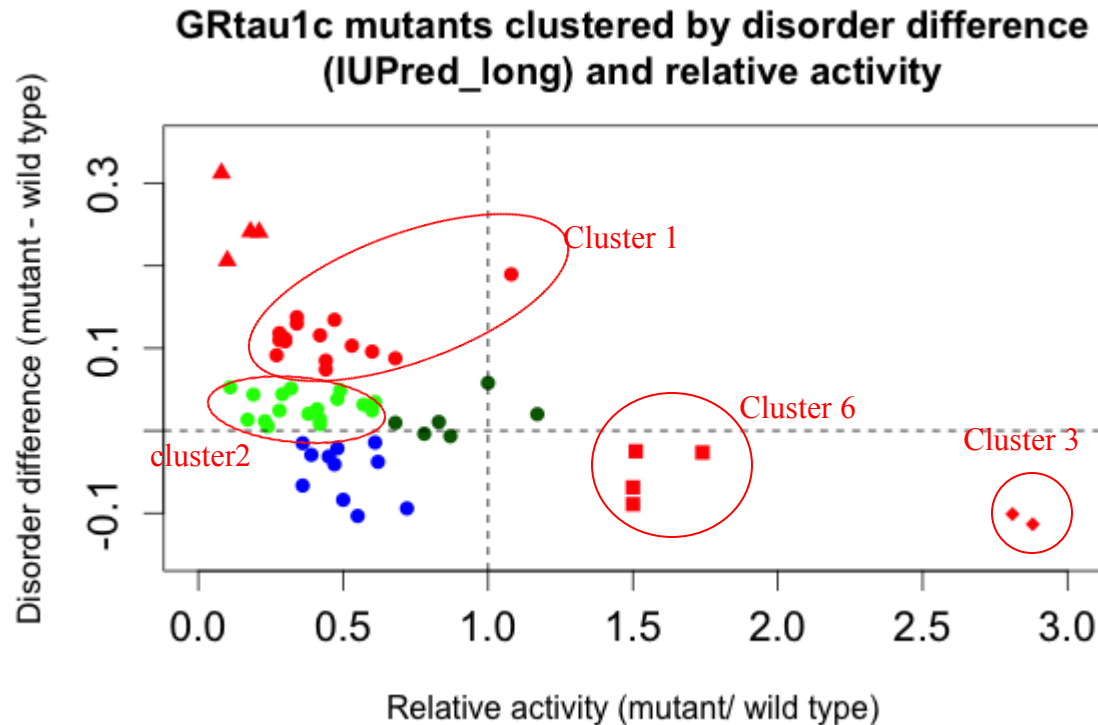
H1 H2 H3



Correlation (Spearman)



IUPred clusters considering only the energy



mutant	cluster
L197P_34	1
W213G_42	1
F199E_34	1
P3_P4_47	1
F191E_29	1
W213R_30	1
L194P_28	1
L197E_30	1
F191D_28	1
P3_219P_53	1
C223G_60	1
I193D_27	1
W213A_44	1
C223R_68	1
H3A_108	1
F191A_44	1
E221F_288	3
D196Y_281	3
T190Y_150	6
T190F_150	6
L225F_174	6
I193F_151	6

Activity and amino acid substitution differences characterise GR mutant clusters

Cluster 1*

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

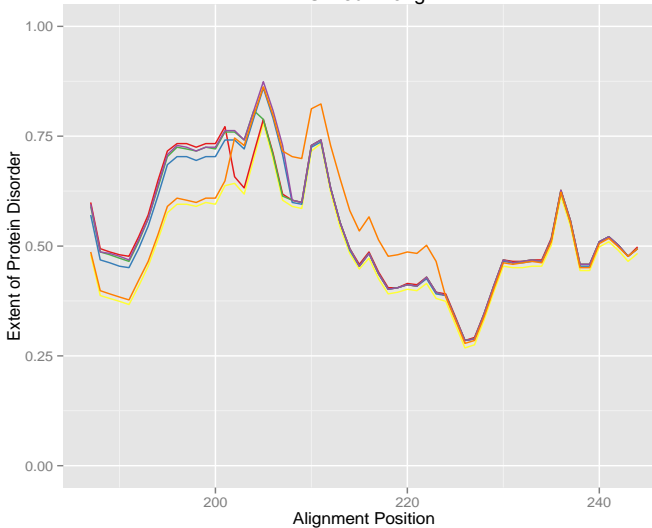
Cluster 2*

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

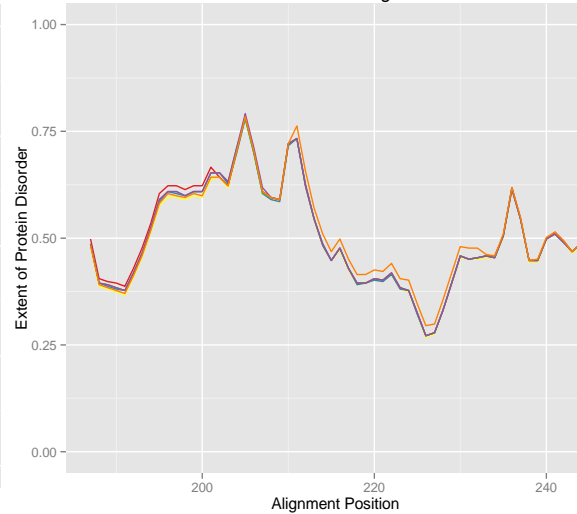
Cluster 3 and 6*

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

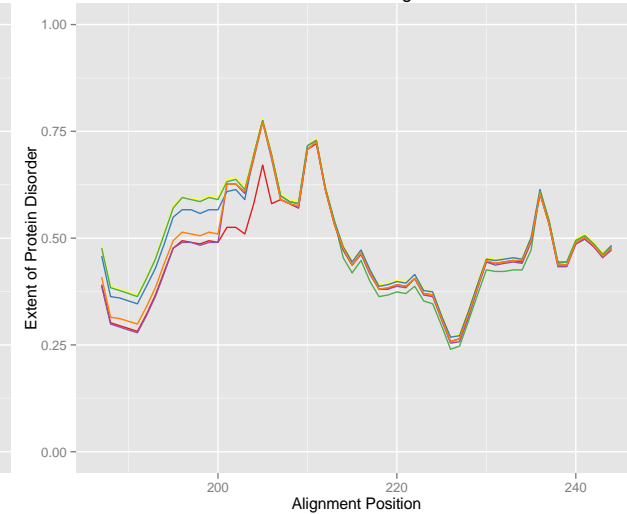
IUPred - long



IUPred - long

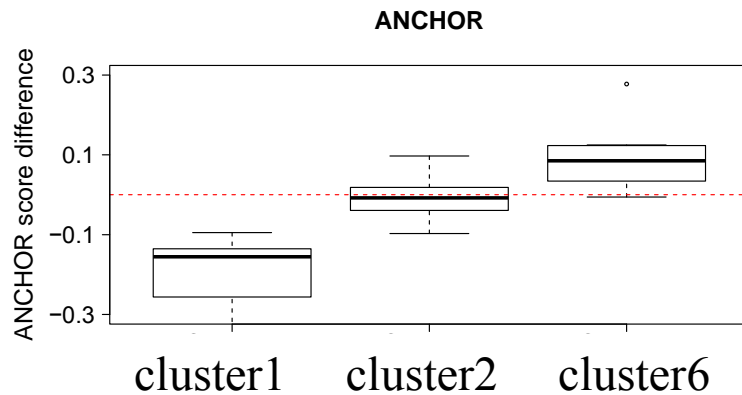
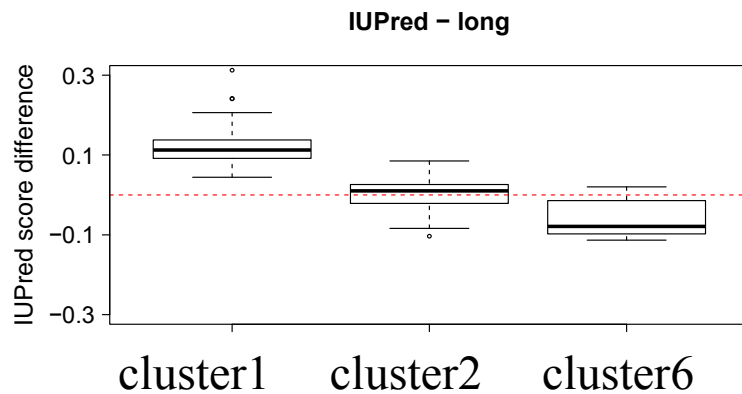


IUPred - long

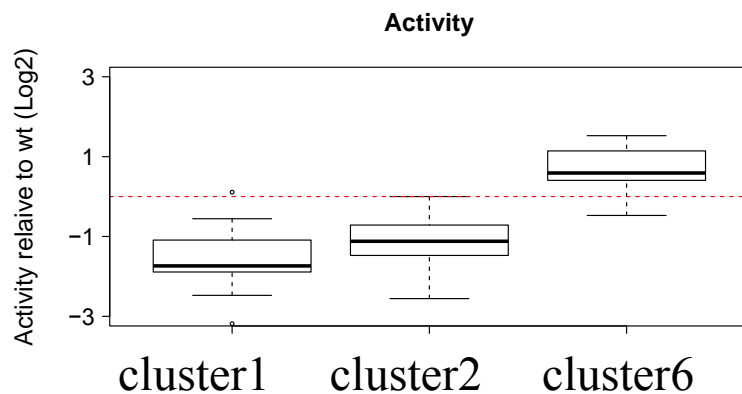


* Top five in clusters

**pairwise energy
content**



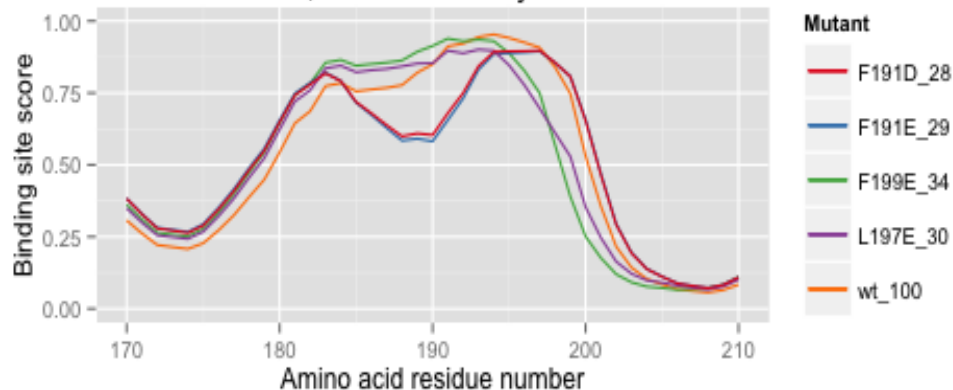
**lack of favorable
interaction to fold
on their own**



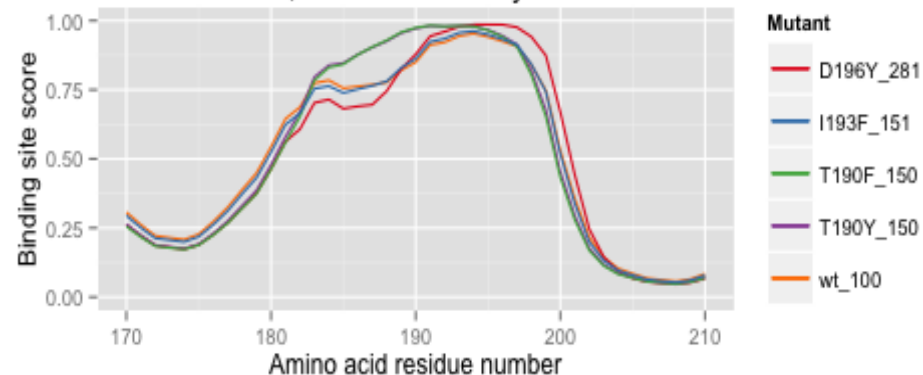
activity

ANCHOR binding site predictions

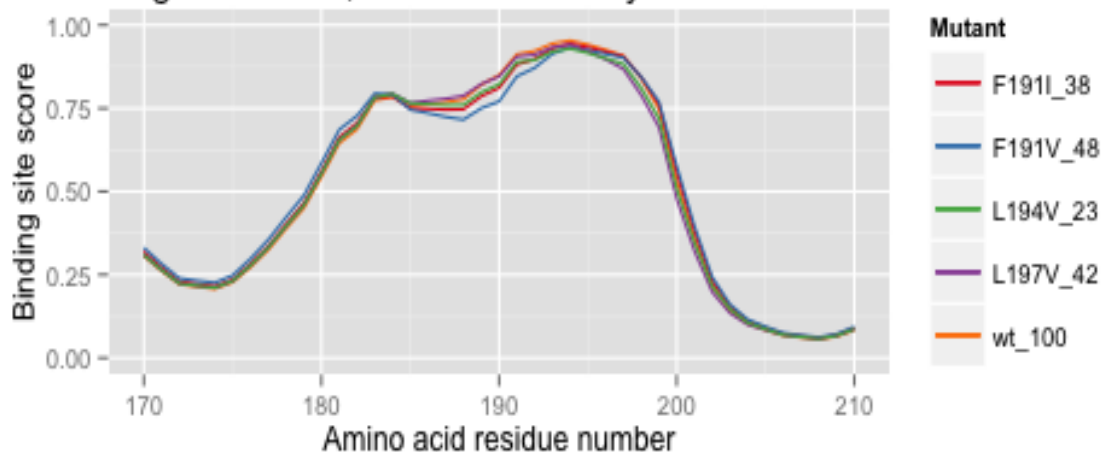
Binding site (ANCHOR) prediction for increased disorder, reduced activity mutants in GRtau1c



Binding site (ANCHOR) prediction for decreased disorder, increased activity mutants in GRtau1c

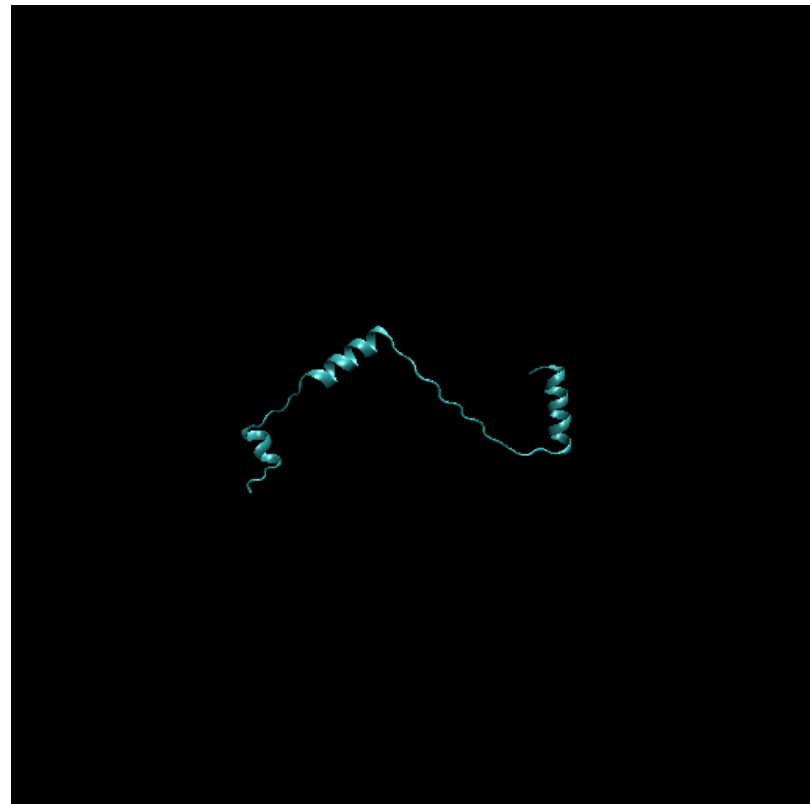


Binding site (ANCHOR) prediction for unchanged disorder, decreased activity mutants in GRtau1c



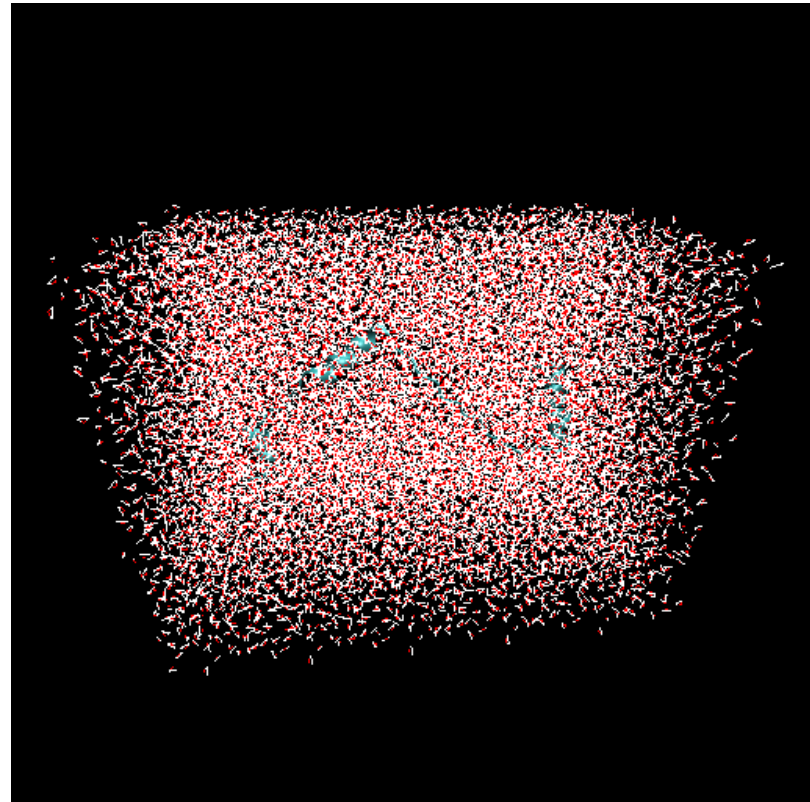
Simulation protocol

- Building the structure-atomistic representation
- CHARMM with CHARMM36ff
- GPU in cubic box
- 10 runs per mutant



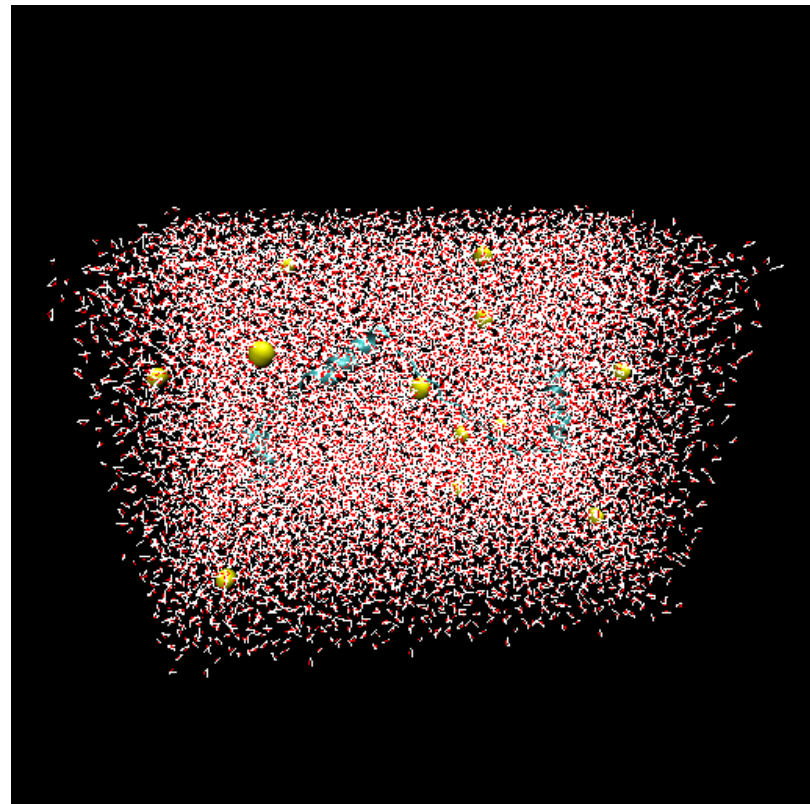
Simulation protocol

- Building the structure-atomistic representation
- CHARMM with CHARMM36ff
- GPU in cubic box
- 10 runs per mutant



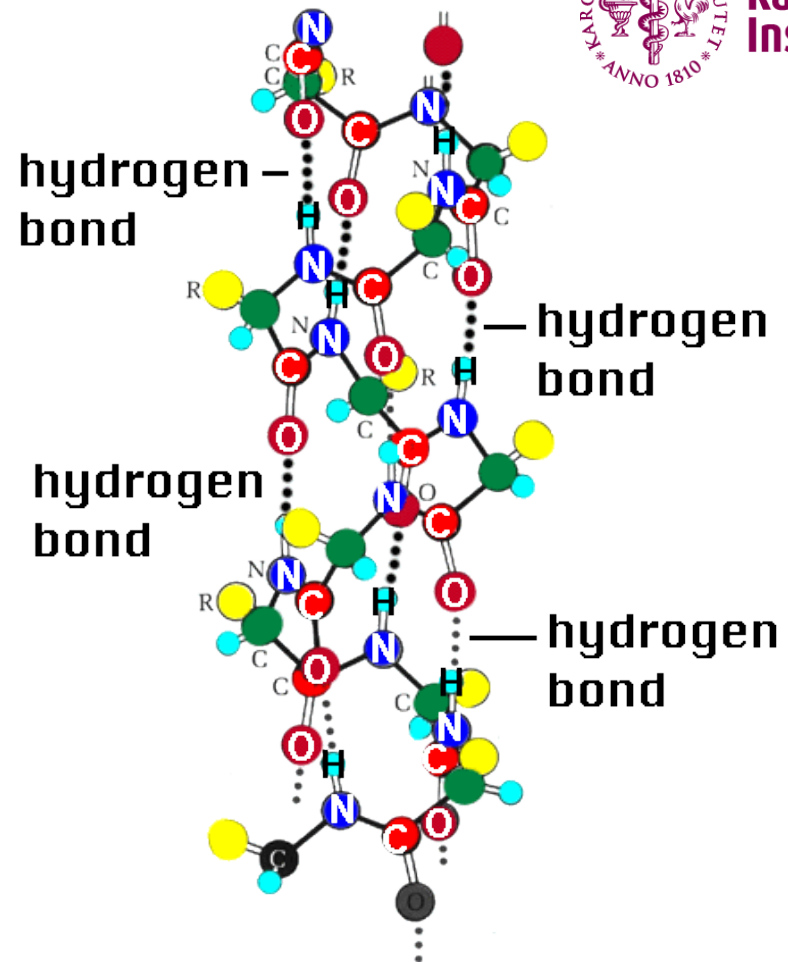
Simulation protocol

- Building the structure-atomistic representation
- CHARMM with CHARMM36ff
- GPU in cubic box
- 10 runs per mutant



Analysis

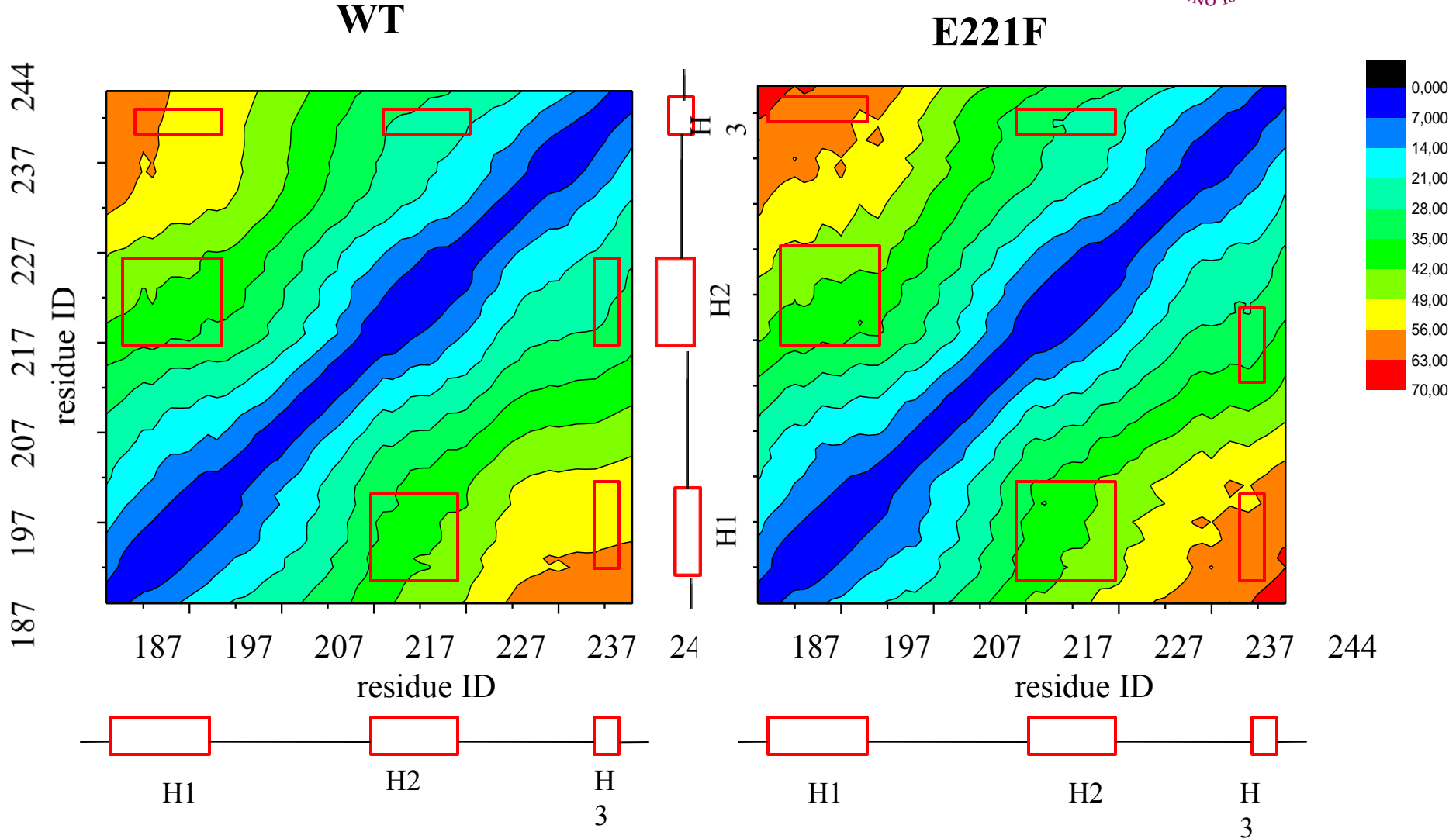
- Calculation of secondary structure using Kabsch&Sander method



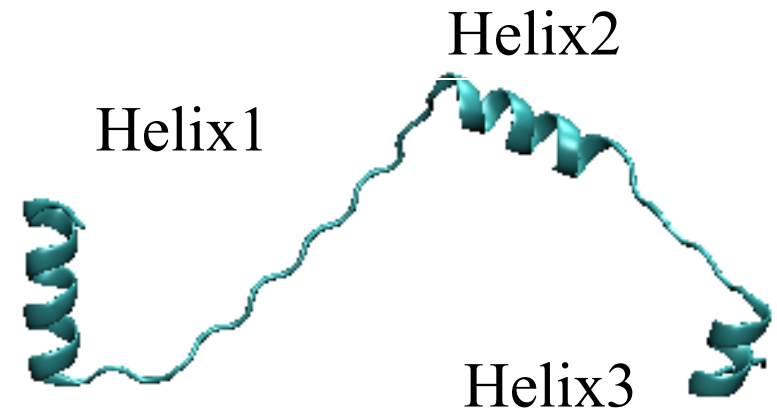
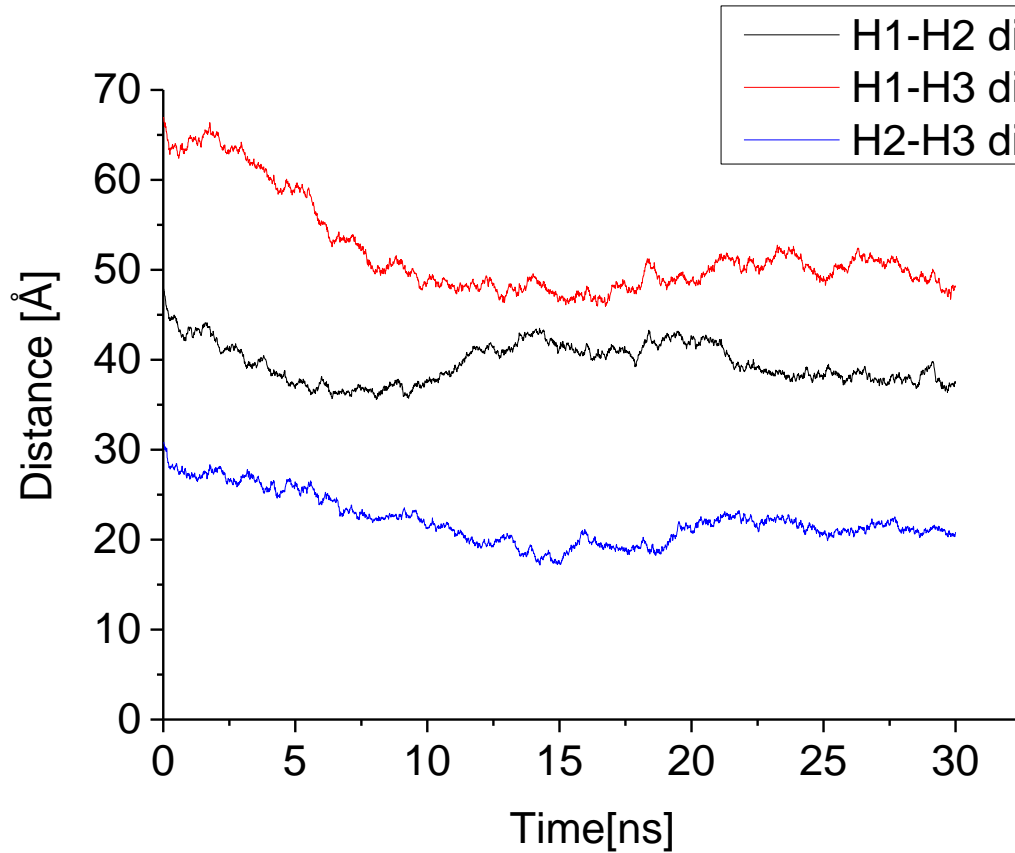
Distance matrices [\AA] of WT and E221F



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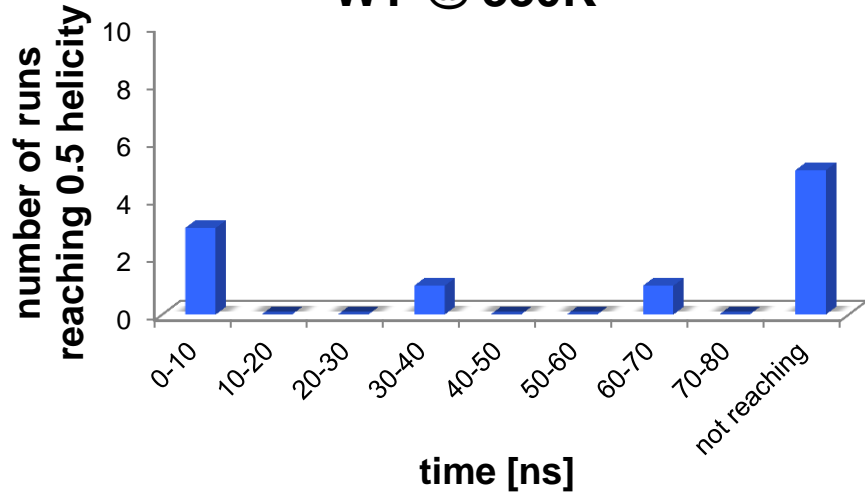


Distances between Helices in WT

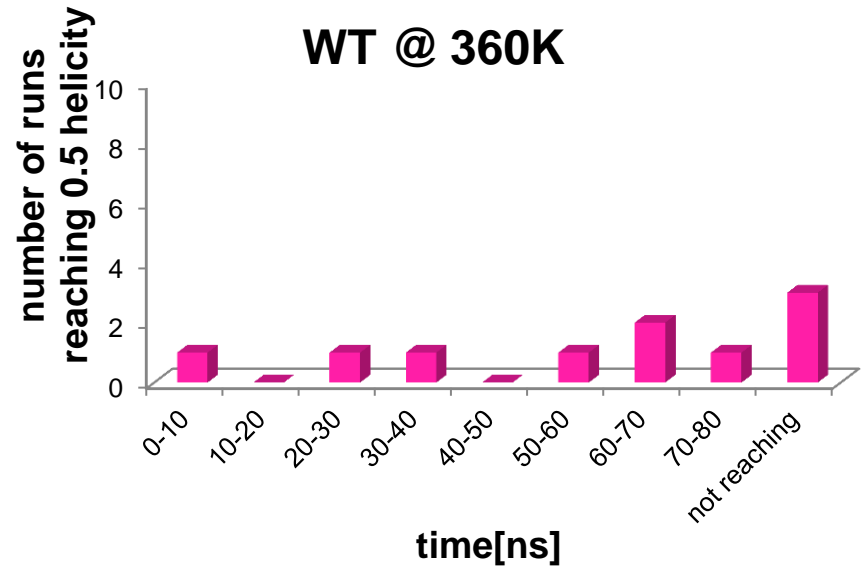


Temperature effect on the helical content of WT

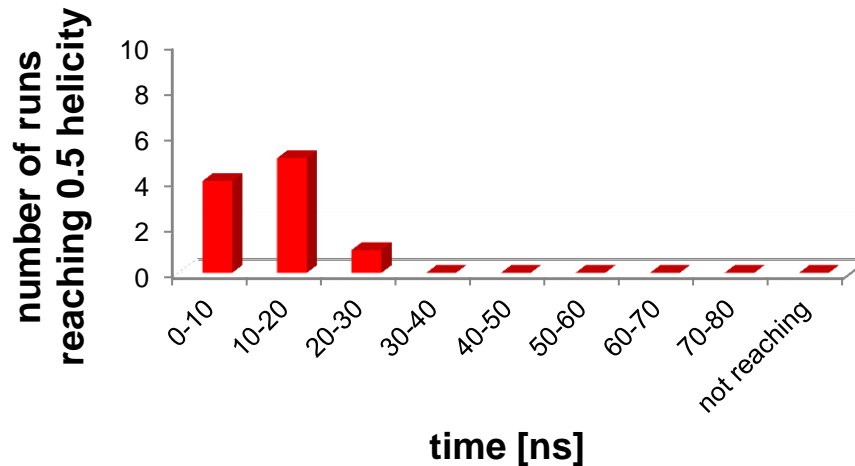
WT @ 330K



WT @ 360K

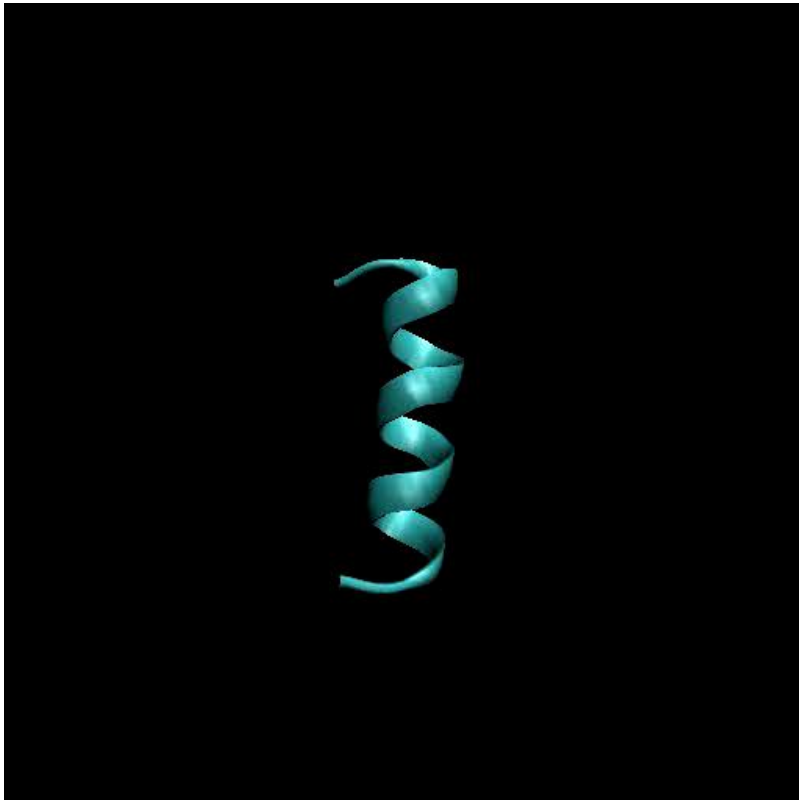


WT @ 400K

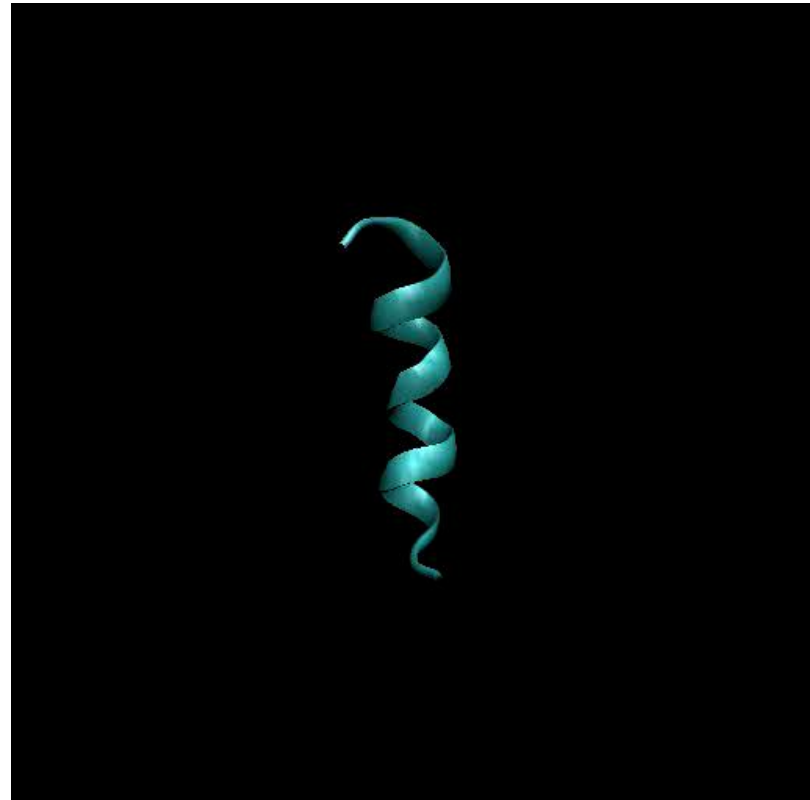


Molecular dynamics simulations on mutants in Helix 1 at 360K

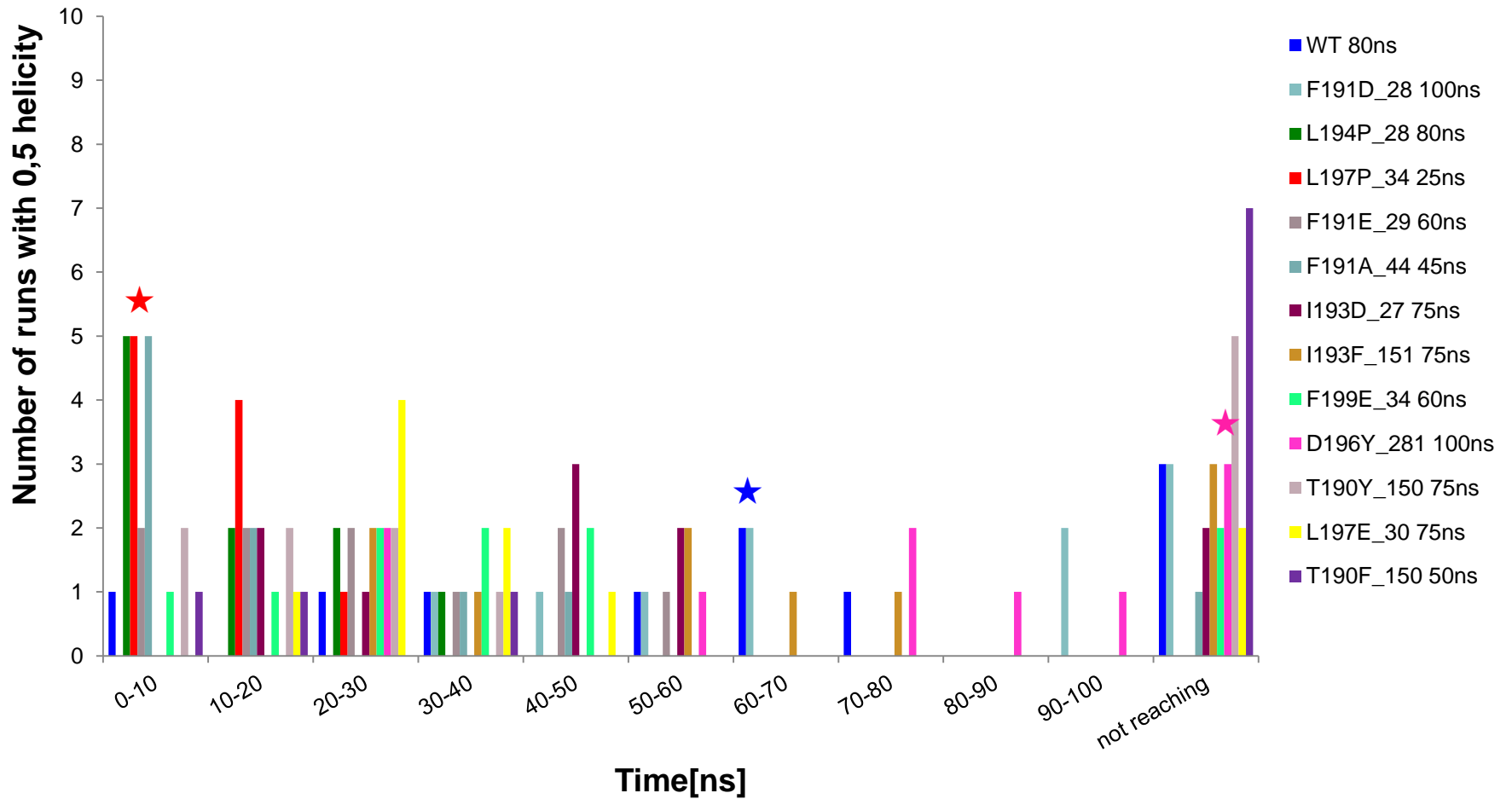
D196Y increasing activity 50ns



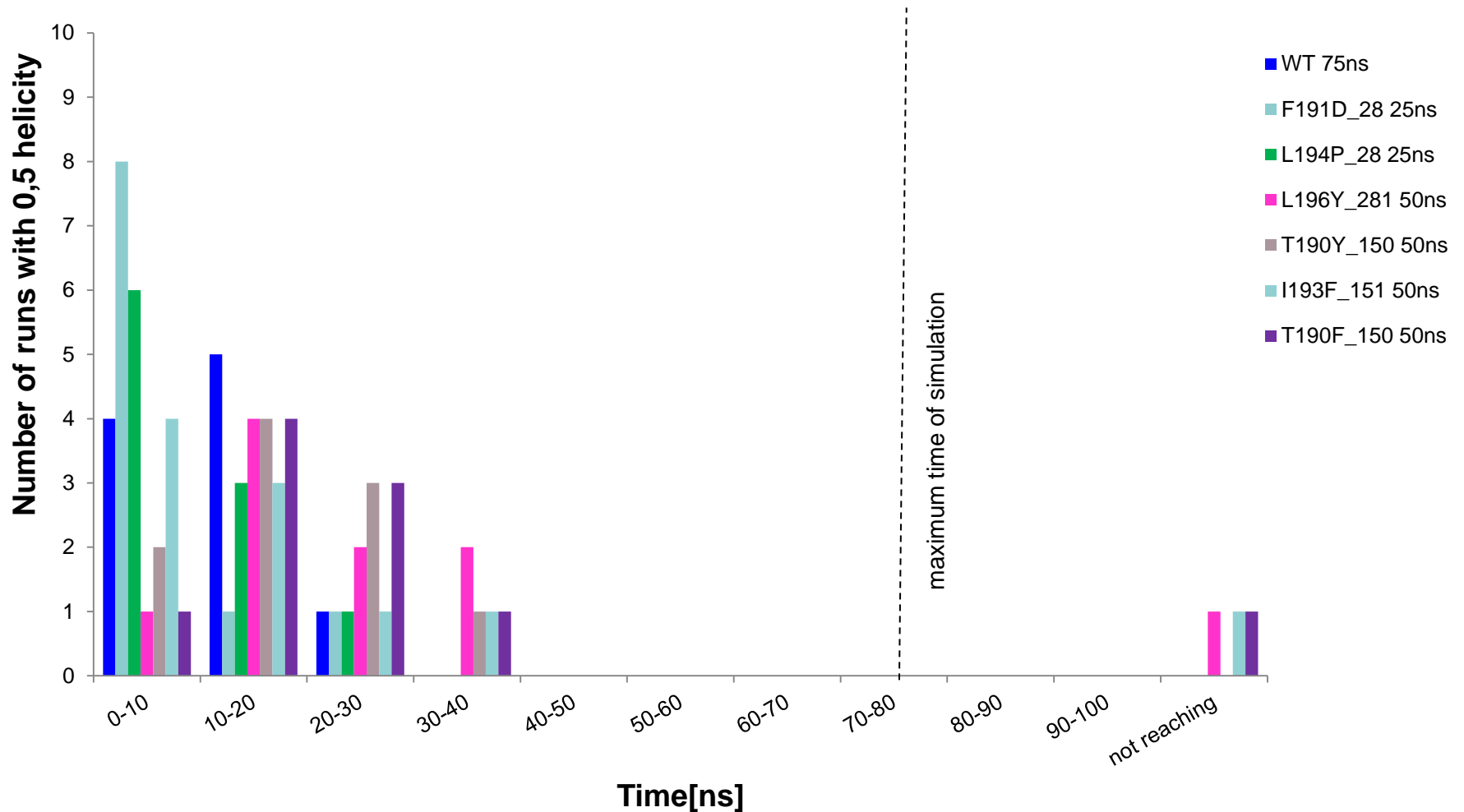
L197P decreasing activity 25ns



Helical content at 360K (distribution)



Helical content at 400K (distribution)



Conclusions

- After applying both- Bioinformatics and MD methods and comparing the results with experimental activity, we see a trend in less stable mutants tend to be less active
- The energy content and ANCHOR parameter also show visible correlation to the helical propensity
- Temperature variations affect the stability of the model peptides
- Point mutations to hydrophobic residues increase the helical propensity
- Mutations to polar or charged residues has the opposite effect (decrease the stability of the helices)

Acknowledgements

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- Joana Paulo

