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





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## 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)  $\alpha$ -Helix targeting reduces amyloid- $\beta$  peptide toxicity, *PNAS 106, 9191-9196*.

# Drosophila Aβ-model



#### Dec-DETA non-treated





20

Pair 1 Pair 2

Pair 6

20

15

15

в

Number of aHBs

С

Distance (Å)

0

20

15

0

5

5

10

Time (ns)

10

Time (ns)



# 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





## Ligand DEC-DETA







Effect of ligand on number of helical H-bonds











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<sup>a</sup>.

polar contacts <sup>b</sup> nonpolar contacts <sup>c</sup>	
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	<#a-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



3 helical propensity regions stabilized in the presence of TFE

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

Residue no.

Helical in TFE	հիհիհիհիհի	իրիրիրիս իրիրի
wt 100	DOSTEDILODIEESS	SPGKETNESPWRSDLITDENCLISPLAGEDDSFLIEGNSNED
T190P 61	DOSPEDILODLEESS	SPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
T190F 150	DOSFFDILODLEFSS	SPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
T190Y 150	DOSYFDILODLEFSS	SPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
F1911 38	DOSTIDILODLEFSS	SPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
F191V 48	DOSTVDILODLEFSS	SPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
F191L 57	DOSTIDILODLEFSS	SPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
F191A 44	DOSTADILODLEFSS	SPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
F191E 29	DOSTEDILODLEFSS	SPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
F1910 28	DQSTDDILQDLEFSS	SSPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
D192Y I193V 36	DQSTFYVLQDLEFSS(	SSPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
D192F 55	DQSTFFILQDLEFSS(	SSPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
D192Y 50	DQSTFYILQDLEFSS(	SSPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
D192A_45	DQSTFAILQDLEFSS(	SSPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
I193F_ <b>151</b>	DQSTFDFLQDLEFSS(	SSPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
1193L_42	DQSTFDLLQDLEFSS(	SSPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
1193A_32	DQSTFDALQDLEFSS(	SSPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
1193D_ <b>27</b>	DQSTFDDLQDLEFSS(	SSPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
L194V_23	DQSTFDIVQDLEFSS(	SSPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
L194A_ <b>19</b>	DQSTFDIAQDLEFSS(	SSPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
Q195E_68	DQSTFDILEDLEFSS	SSPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
D196Y_281	DQSTFDILQ <mark>Y</mark> LEFSS(	SSPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
L197V_42	DQSTFDILQDVEFSS	GSPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
L197E_30	DQSTFDILQDEEFSS	SPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
E1980_61	DQSTFDILQDLQFSS	SSPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
F199V_49	DQSTFDILQDLEVSS	GSPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
F199E_34	DOGLEDITODIE	SSPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
5200P_100	DOCHEDITODIEECO	SSPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
M212C 42	DOCHEDITODIEECO	SECRETINESPERSOLLIDENCLISPLAGEDDEFLIEGNENED
W213G_42 W213D_20	DOGLEDITÖDTERSO	
W213A 44	DOSTEDILODIEESS	35PGKETNESPARSDLLIDENCLLSPLAGEDDSFLLEGNSNED
W213F 78	DOSTEDILODLEESS	SSPGKETNESPERSDLUTDENCLUSPLAGEDDSFLUEGNSNED
W213Y 117	DOSTEDILODLEESS	GSPGKETNESPYRSD <del>LLIDENCLLS</del> PLAGEDDSFLLEGNSNED
E221F <b>288</b>	DOSTEDILODLEESS	GSPGKETNESPWRSDLLTD NCLLSPLAGEDDSFLLEGNSNED
C223G 60	DOSTFDILODLEFSS	GSPGKETNESPWRSDLLIDEN LLSPLAGEDDSFLLEGNSNED
C223R 68	DOSTFDILODLEFSS	GSPGKETNESPWRSDLLIDENRLLSPLAGEDDSFLLEGNSNED
L225F 174	DQSTFDILQDLEFSS	GSPGKETNESPWRSDLLIDENCLESPLAGEDDSFLLEGNSNED
L2251 87	DQSTFDILQDLEFSS	GSPGKETNESPWRSDLLIDENCL <mark>I</mark> SPLAGEDDSFLLEGNSNED
L225V 83	DQSTFDILQDLEFSS	GSPGKETNESPWRSDLLIDENCL <mark>W</mark> SPLAGEDDSFLLEGNSNED
L217V_L218V_60	DQSTFDILQDLEFSS	GSPGKETNESPWRSD <mark>VV</mark> IDENCLLSPLAGEDDSFLLEGNSNED
N222D_L225F_ <b>48</b>	DQSTFDILQDLEFSS	GSPGKETNESPWRSDLLIDEDCLESPLAGEDDSFLLEGNSNED
L224V_L225V_ <b>28</b>	DQSTFDILQDLEFSS	GSPGKETNESPWRSDLLIDENC <mark>VV</mark> SPLAGEDDSFLLEGNSNED
L224V_L225F_ <mark>36</mark>	DQSTFDILQDLEFSS	GSPGKETNESPWRSD <mark>LLIDENC<mark>VE</mark>SPLAGEDDSFLLEGNSNED</mark>
D233Y_72	DQSTFDILQDLEFSS	GSPGKETNESPWRSDLLIDENCLLSPLAGED <mark>W</mark> SFLLEGNSNED
F235L_L236V_11	DQSTFDILQDLEFSS	GSPGKETNESPWRSDLLIDENCLLSPLAGEDDSLVLEGNSNED
F235V_L2361_29	DQSTFDILQDLEFSS	GSPGKETNESPWRSDLLIDENCLLSPLAGEDDSVILEGNSNED
L236V_17	DQSTFDILQDLEFSS	GSPGKETNESPWRSDLLIDENCLLSPLAGEDDSFVLEGNSNED
L236F_62	DQSTFDILQDLEFSS	GSPGKETNESPWRSDLLIDENCLLSPLAGEDDSFELEGNSNED
E238K_N240D_39	DOCTEDILODIRECO	GSPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLL <mark>AGU</mark> SNED
G239R_N240D_24	DÖSTEDITÖDTEESS	JSPGREINESPWRSDELIDENCEESPERGEDDSELLERUSNED
H1A 8	DOSTADAAODAF	
H2A 18	DOGLEDITODIEEGO	24 BURELNES BAB SDATT DENCUTS LAGEDDO ET LEGNEND
H3A 108	DOSTEDITODITERS	SSPGKETNESPWRSDLIJDENCLISPLAGEDDGAAAFGNSNED
P1 1194P 28	DOSTEDIDODLEESS	33PGKETNESPWRSDLJIDENCLISPLAGEDDS FLLEGN SNED
P2 1197P 34	DOSTEDILODERESS	GSPGKETNESPWRSDLIJ DENCLISPLAGEDDSFLLEGN SNED
P3 1219P 53	DOSTFDILODLEESS	GSPGKETNESPWRSDLL DENCLLSPLAGEDDSFLLEGNSNED
P4 N222P 41	DOSTFDILODLEFSS	GSPGKETNESPWRSDLLIDE CLLSPLAGEDDSFLLEGN SNED
P1 P2 21	DOSTFDIPODPEFSS	GSPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLLEGNSNED
P3 P4 47	DQSTFDILQDLEFSS	GSPGKETNESPWRSDLLPDECLLSPLAGEDDSFLLEGNSNED
P1_P2_P3_P4_10	DQSTFDIPQDPEFSS(	GSPGKETNESPWRSDLLEDECLLSPLAGEDDSFLLEGNSNED



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

Mutant	Activity <sup>a</sup>	Mutant	Activity <sup>a</sup>
Helical region I		Helical region II	
Т190Р <sup>ь</sup>	$61 \pm 4$	E221F°	$288 \pm 15$
F191I	$38 \pm 10$	C223G	$60 \pm 3$
F191V	$48 \pm 6$	C223R	$68 \pm 5$
F191L	$57 \pm 11$	L225F <sup>c</sup>	$174 \pm 17$
F191A <sup>c</sup>	$44 \pm 3$	L225I	$87 \pm 6$
F191E°	$29 \pm 3$	L225V	$83 \pm 10$
F191D°	$28 \pm 3$	L218V/L219V <sup>c</sup>	$60 \pm 8$
D192Y/I193V	$36 \pm 8$	N222D/L225F	$48 \pm 7$
I193F	$151 \pm 9$	L224V/L225V	$28 \pm 3$
I193L	$42 \pm 7$	L224V/L225F	$36 \pm 8$
I193A <i>°</i>	$32 \pm 2$		
I193D <i>°</i>	$27 \pm 8$	Helical region III	
L194V	$23 \pm 2$	D233Y	$72 \pm 11$
L194A <sup>c</sup>	$19 \pm 6$	F235L/L236V	$11 \pm 2$
Q195E°	$68 \pm 5$	F235V/L236I	$29 \pm 9$
D196Y	$281 \pm 26$	L236V	$17 \pm 2$
L197V	$42 \pm 7$	L236F	$62 \pm 1$
L197E <sup>c</sup>	$30 \pm 1$	E238K/N240D	$39 \pm 2$
E198Q	$61 \pm 1$	G239R/N240D	$24 \pm 5$
F199V	$49 \pm 3$		
F199E <sup>c</sup>	$34 \pm 8$		
S200P <sup>b</sup>	$100 \pm 10$		
Loop			
E211K	$47 \pm 17$		
W213G	$42 \pm 1$		
W213R	$30 \pm 2$		
W213A <sup>c</sup>	$44 \pm 5$		
W213F <sup>c</sup>	$78 \pm 5$		
W213Y <sup>c</sup>	117 ± 25		

TABLE 1. Relative  $\beta$ -galactosidase activities of  $\tau$ 1-core mutants



# A collection of 60 functionally characterised mutants in the GRtau1core activation domain

<sup>a</sup>Mean relative  $\beta$ -galactosidase activity (percentage of wild-type level  $\pm$  standard deviation (n = 3).

 ${}^{b}\tau$ 1-core mutant found by sequencing the mutant pool prior to screening.

 $c_{\tau 1}$ -core mutant made by site-directed mutagenesis.

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



Partitioning Around Medoids (PAM), k=3



# Activity and amino acid substitution differences characterise GR mutant clusters



Cluster 3<sup>\*</sup> High activity Low disorder

Mutant	Relative activity (%)
T190Y	150
<b>T190F</b>	150
L193F	151
L225F	174
D196Y	281



Cluster 1\* Low activity

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



Cluster 2<sup>\*</sup> Low activity Unchanged disorder

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

#### IUPred – long



### Predicted disorder vs relative activity





Relative activity (mutant/ wild type)

# 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  $\alpha$  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<sub>202</sub> Simulate wt + 14 mutants, peptide in (64Å)<sup>3</sup> 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 WT WT F191D 1,0 F191D 1,0  $WT_{0.5}$ =60ns; $SD_{0.5}$ =±0,22  $WT_{0.5}$ =20ns; $SD_{0.5}$ =±0,25 F191D<sub>0.5</sub>=not reaching F191D<sub>05</sub>=9ns; SD<sub>05</sub>=±0,29 0,8 0,8 Helicity 0,6 Helicity 0,6 0,4 0,4 0,2 0,2 WT F191D WT 0,0 0,0 20 40 60 80 100 0 0 20 40 60 80 100 Time[ns] Time[ns]

### Helix stability and protein activity





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

WT at 360k Mutants at 360k WT at 400k Mutants at 400k

### Helix stability and protein activity





# Conclusions



- Both bioinformatics and MD methods show correlation between change-of-function mutations and helix stability
- Temperature affects stability of the model peptides
- Mutations of hydrophobic residues increase helical stability
- Mutations of polar/charged residues decrease helical stability
- D<sub>187</sub>QSTFDFILQDLEFSSG<sub>202</sub> is possible target for compounds designed from our peptidomimetic library