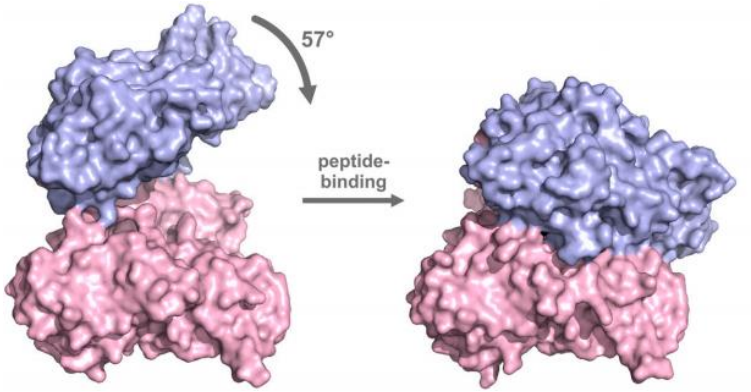
Tynorphin binding to DPP III protein homolog's analyzed by hydrogen/deuterium exchange mass spectrometry

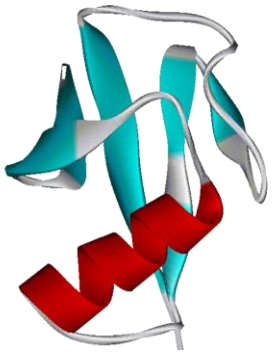


Bezerra, G. A.; Dobrovetsky, E.; Viertlmayr, R.; Dong, A.; Binter, A.; Abramić, M.; Macheroux, P.; Dhe-Paganon, S.; Gruber, K. *Proceedings of the National Academy of Sciences* **2012**, *109*, 6525.

Protein	Name	K_i tynorphin (mM)	Protein Conc. (μM)	Tynorphin Conc. (μM)	%P bound	%P bound X 10
hDPPIII	*Homo sapiens*	**0.03**	**35.8**	**158**	**99.98**	**99.76**
hDPPIII mut V412I	*Homo sapiens* *Mutant V412I*	**0.01**	**47.7**	**158**	**99.99**	**99.91**
yDPPIII	*Saccharomyces cerevisiae*	**0.47**	**46.8**	**500**	**99.9**	**98.97**
BtDPPIII	*Bacterioides thetaiotaomicron*	**0.98**	**112**	**1000**	**99.9**	**98.98**
PgDPPIII	*Porphyromonas gingivalis*	**0.72**	**45**	**500**	**99.84**	**98.44**
CaDPPIII	*Calditrix abyssi*	**16.75**	**72**	**6000**	**99.72**	**97.26**
PpDPPIII	*Physcomitrella pattens*	**0.66**	**36.7**	**500**	**99.86**	**98.60**

For each protein sample we spent 160 μL of solution in 20 mM Tris buffer, pH 7.4 with 50 μM $CoCl_2$

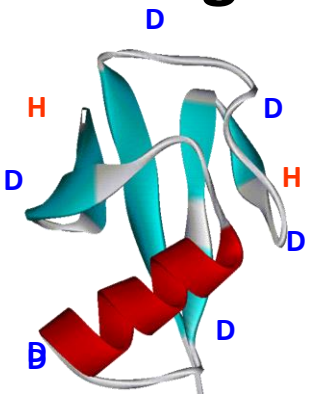
Continuous labeling experiment



5 μL of

protein or complex

10 X dilution
Into D_2O buffer



50 μL, H/D exchange reaction

Δt labeling = 10 sec, 1 min,
20 min, 1 hour and 4 hours

for quench
10 μL of 2 M
glycine buffer

pD 2.3 ~ 2.5

60 μL
On-line pepsin digest
RT, 2 min. 200 μL/min

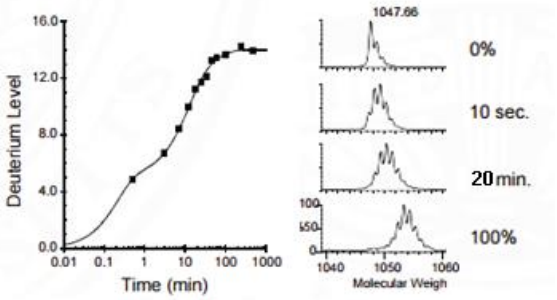
Trap of peptic
Peptides mixture

Temp
~ 0.5°C

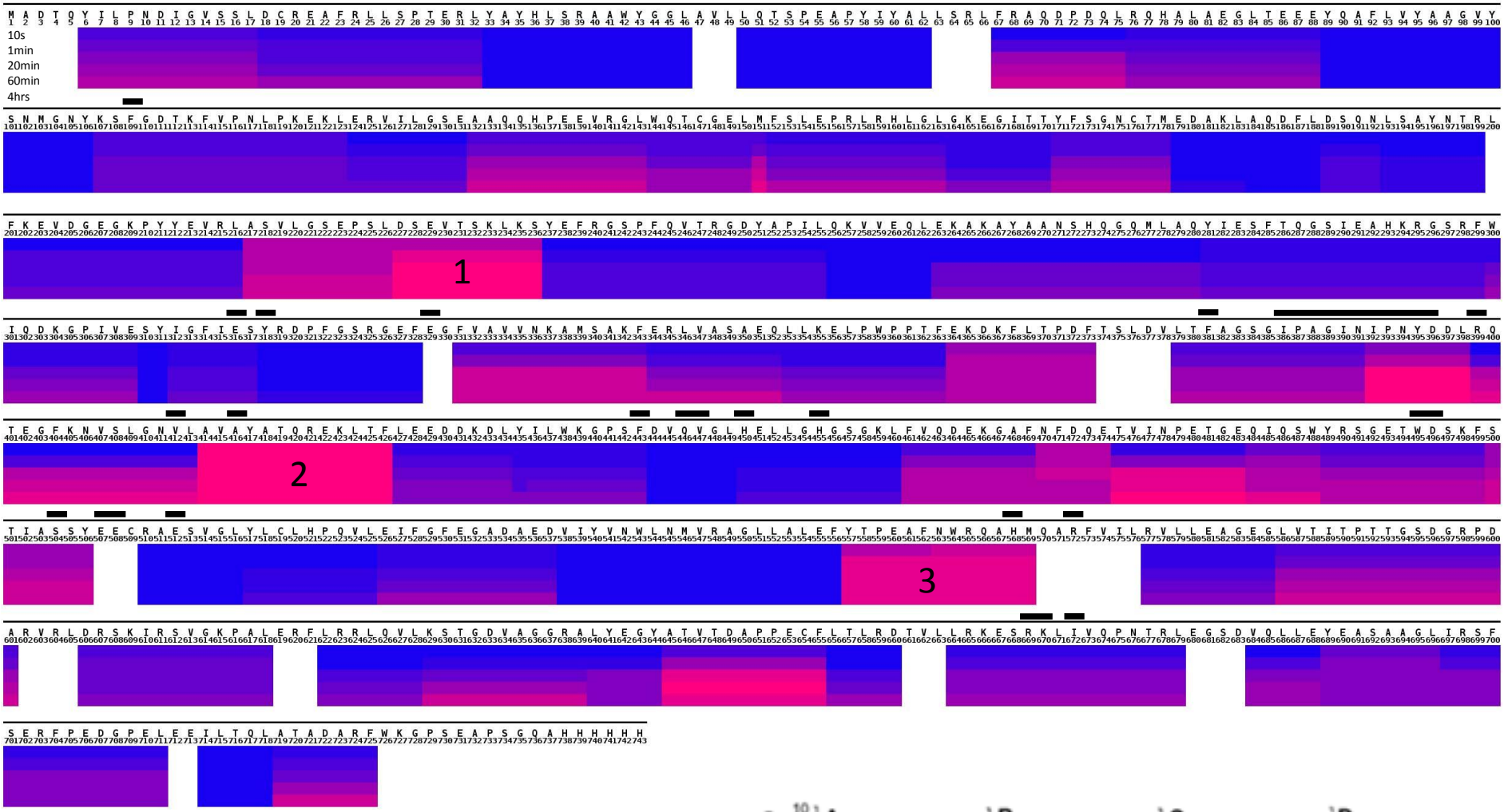
UPLC time 10 min.

8-40% ACN in 8 min. pH 2,5
Temp ~ 0.5°C, flow rate 20 μL/min

Electrospray MS



Flexibility map for Human DPPIII APO



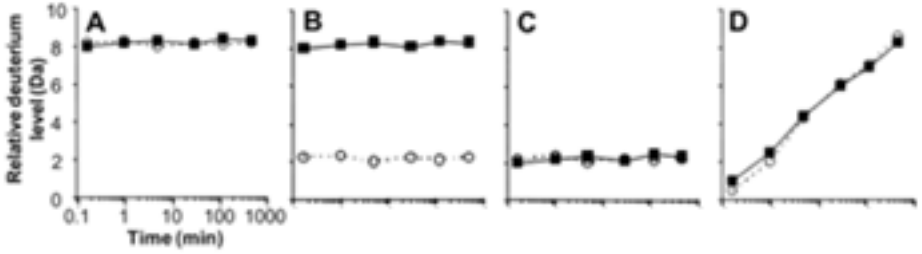
Limits:
0-10
10-20
20-30
30-40
40-50
50-60
60-70
70-80
80-90
90-100

Conditions:
pH4
pH7

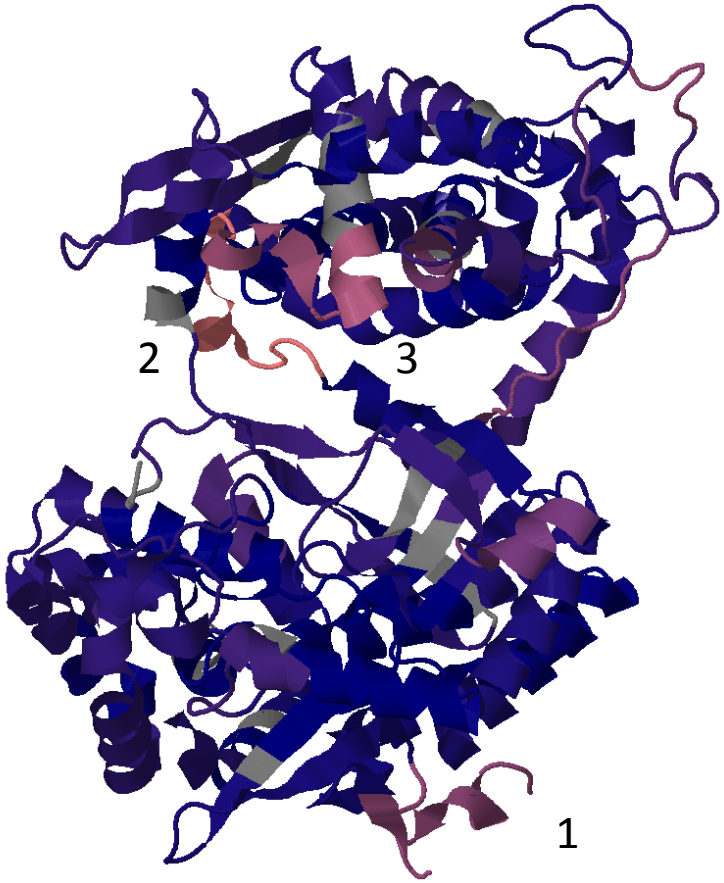
Times:
10sec
1min
20min
1h
4h

77 peptides

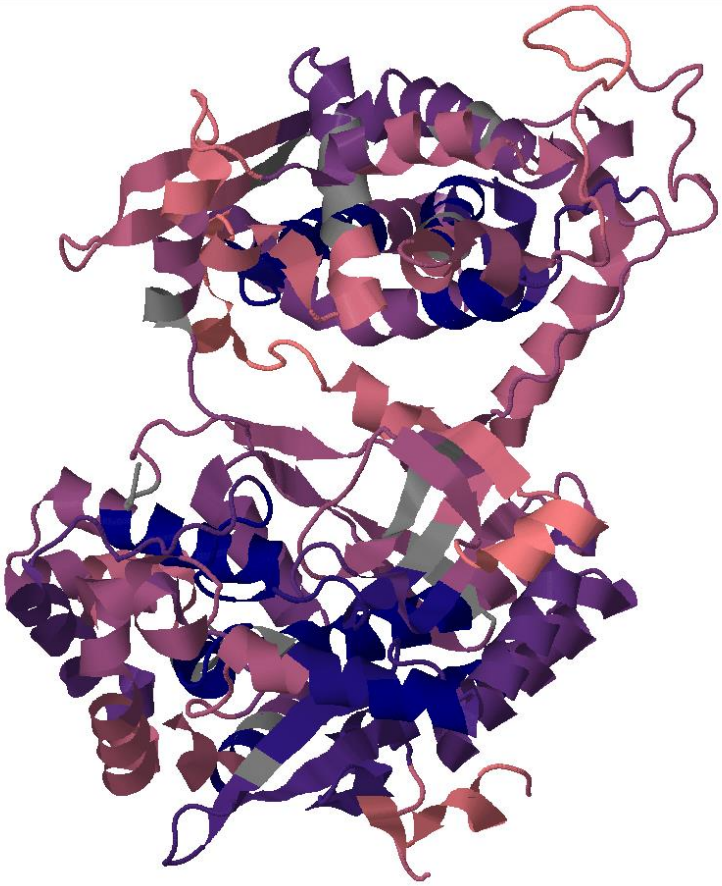
Sequence coverage: 91.4%

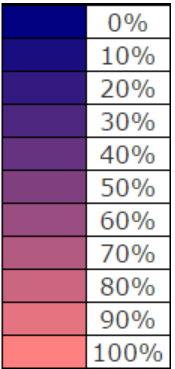


Human DPPIII APO



PDB 3fvy

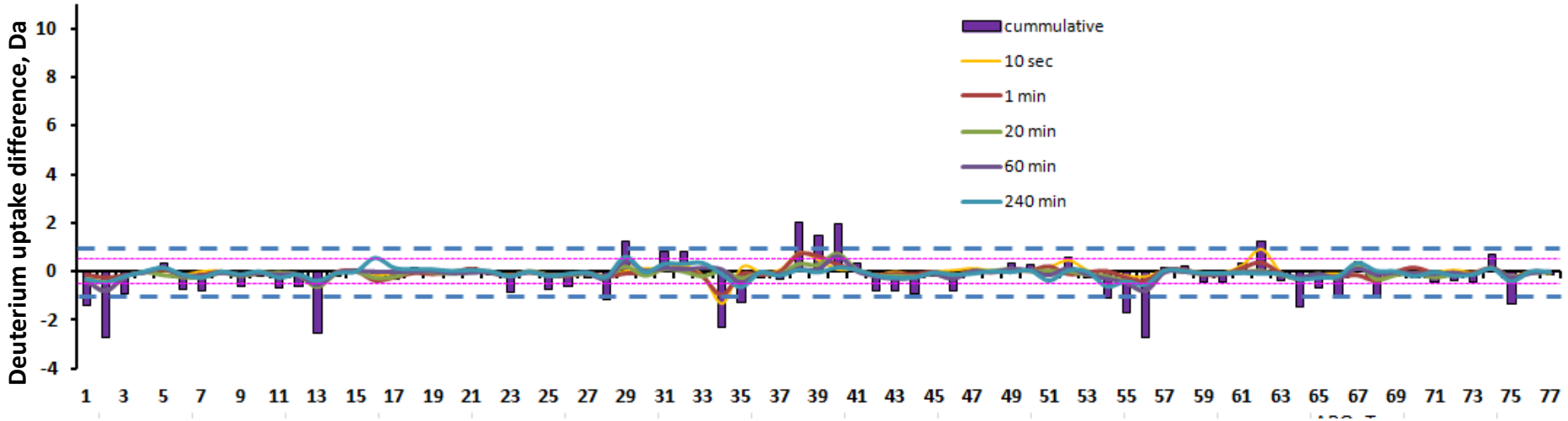


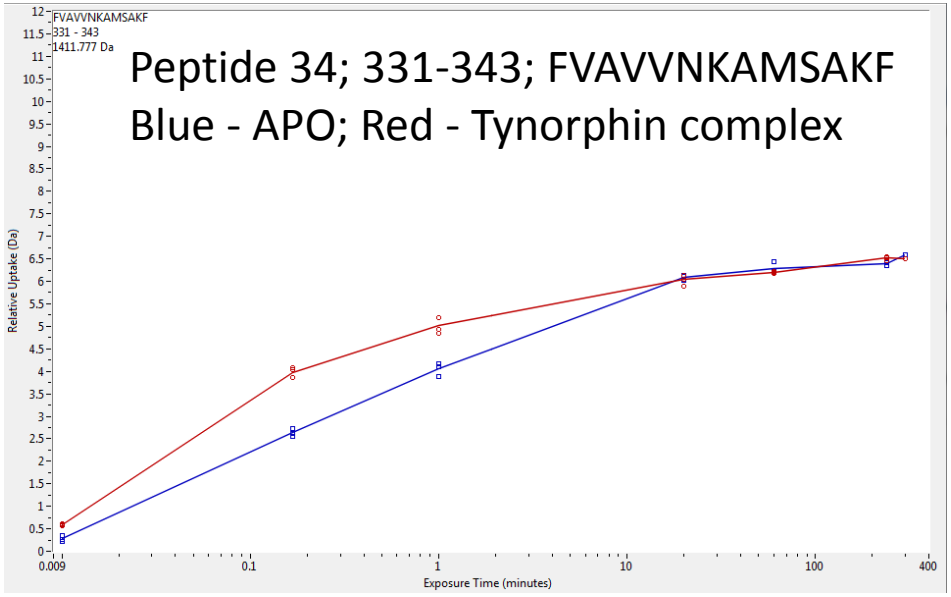


After 10 sec

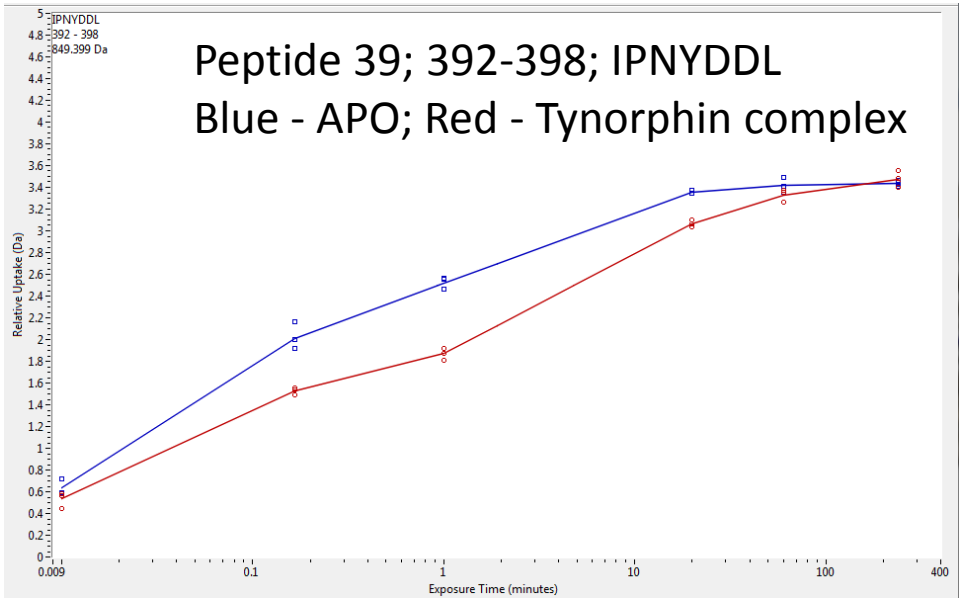
After 4 hours

Difference between Human DPPIII APO and Tynorphin complex



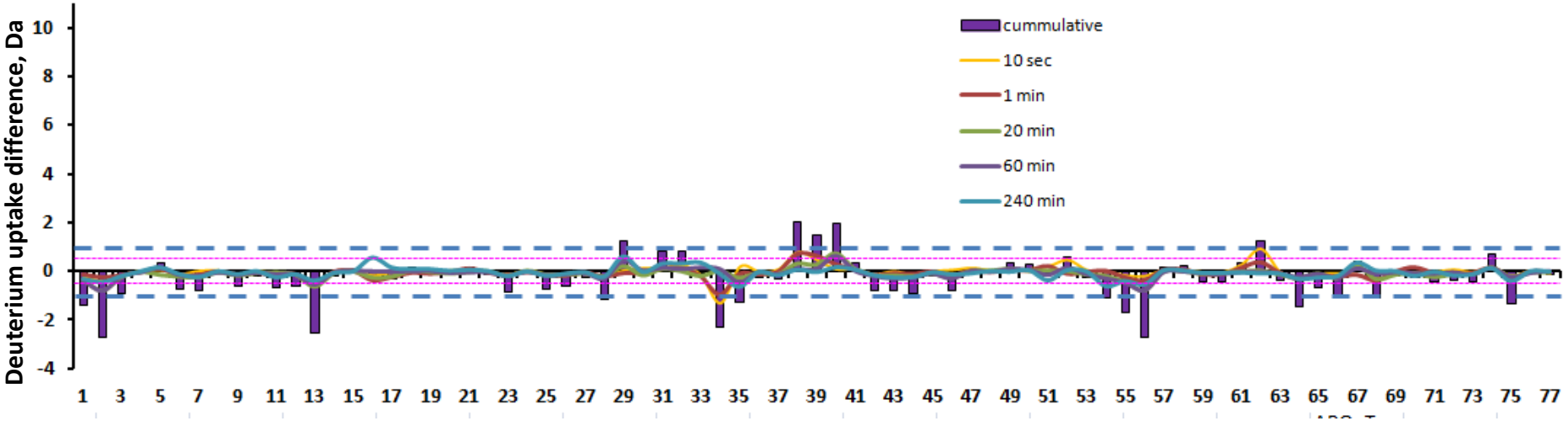


Peptide 34; 331-343; FVAVVNKAMSAKF
Blue - APO; Red - Tynorphin complex

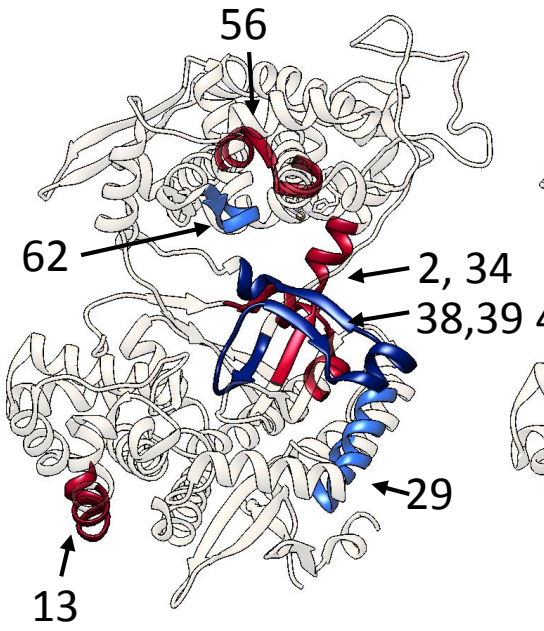


Peptide 39; 392-398; IPNYDDL
Blue - APO; Red - Tynorphin complex

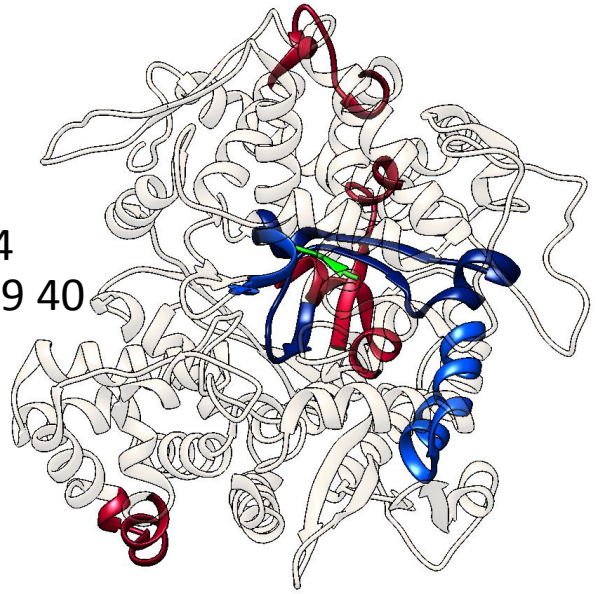
Difference between Human DPPIII APO and Tynorphin complex



PDB 3fvy

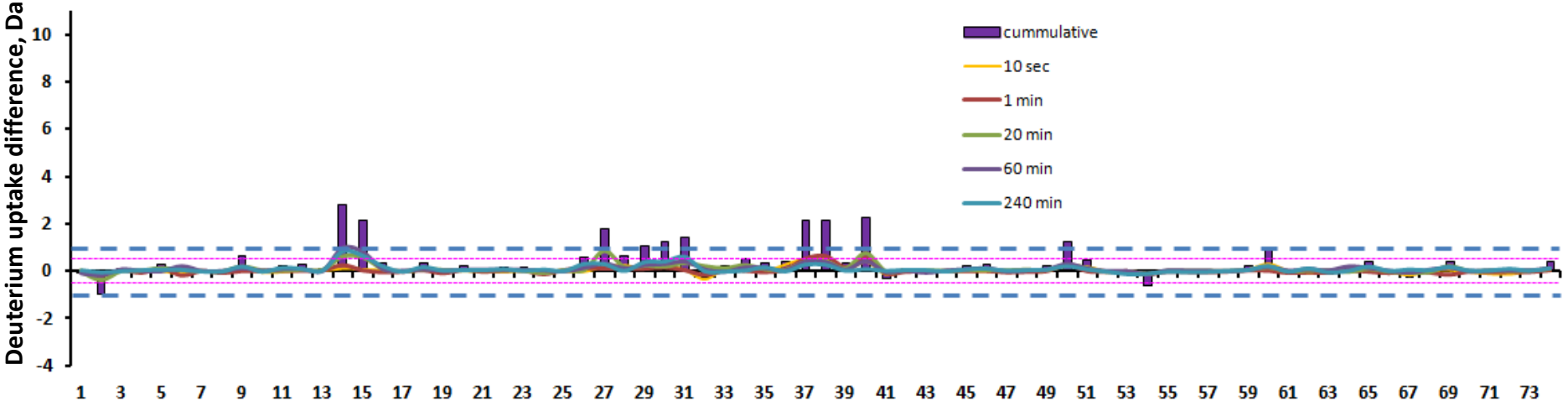


PDB 3t6j

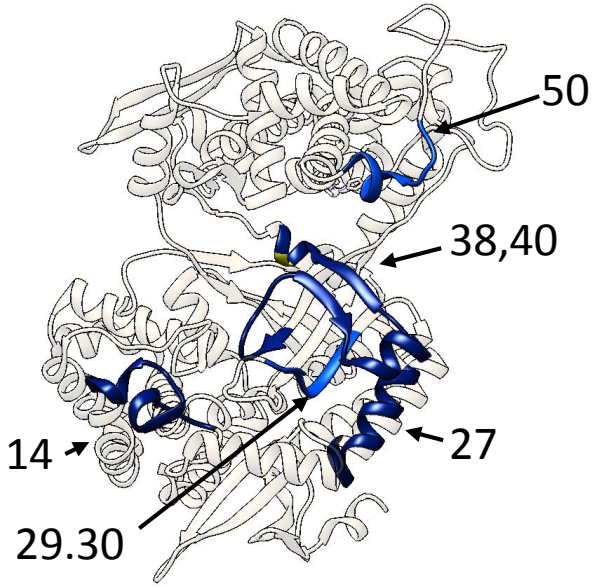


Pep 2, 18-32 DCREAFRLLSPTERL
Pep 13, 132-143 AAQQHPEEVRGL
Pep 29, 281-299 YIESFTQGSIEAHKRGSRF
Pep 34, 331-343 FVAVVNKAMSAKF
Pep 38, 379-398 LTFAGSGIPAGINIPNYDDL
Pep 39, 392-398 IPNYDDL
Pep 40, 399-413 RQTEGFKNVSLGNVL
Pep 56, 526-537 EIFGFEGADAED
Pep 62, 563-569 NWRQAHM

Difference between Human DPP3 V412I APO and Tynorphin complex



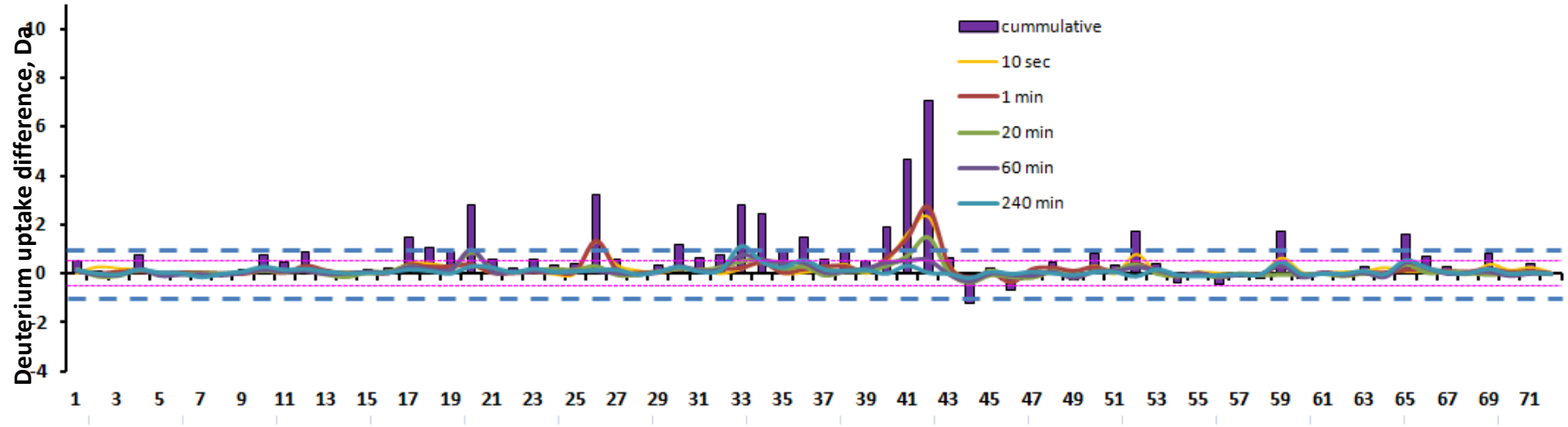
PDB 3fvy



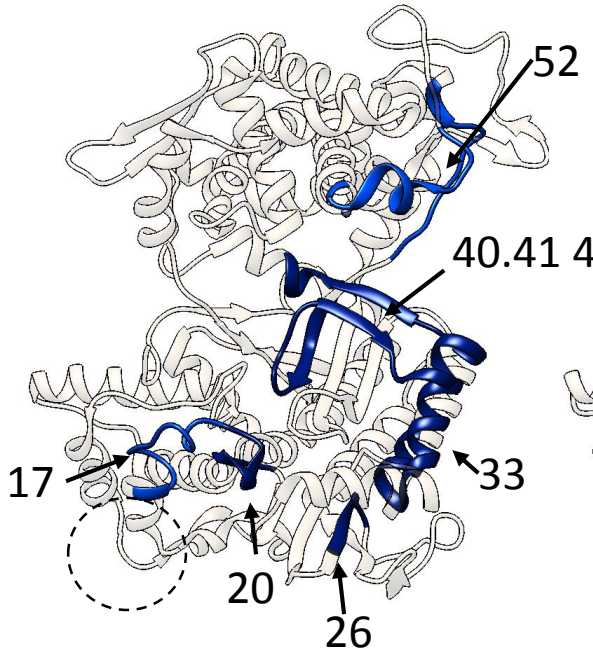
Peptide 14; 152-170 FSLEPRLRHLGLGKEGITT
Peptide 27; 285-299 FTQGSIEAHKRGSRF
Peptide 29; 310-314 SYIGF
Peptide 30; 312-317 IGFIES
Peptide 38; 381-398 FAGSGIPAGINIPNYDDL
Peptide 40; 399-413 RQTEGFKNVSLGNIL
Peptide 50; 489-499 YRSGETWDSKF

403 GFKNVSLGNIL 413 V412I mutant
412 GFKNVSLGNIL 422 yeast

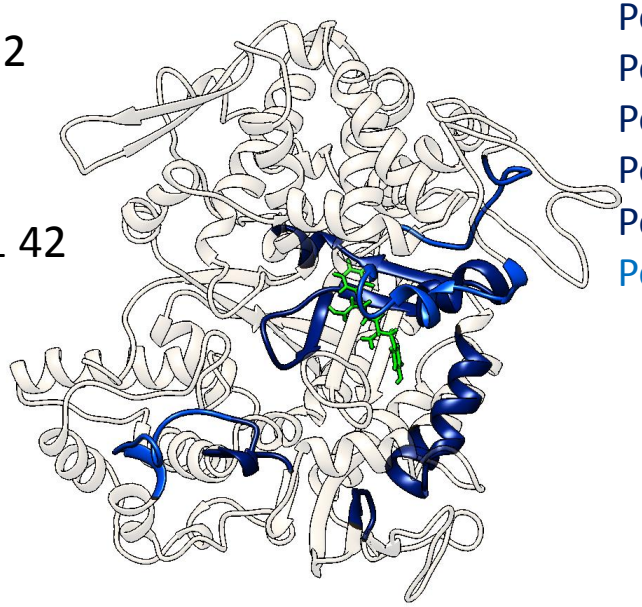
Difference between Yeast DPP3 APO and Tynorphin complex



PDB 3csk

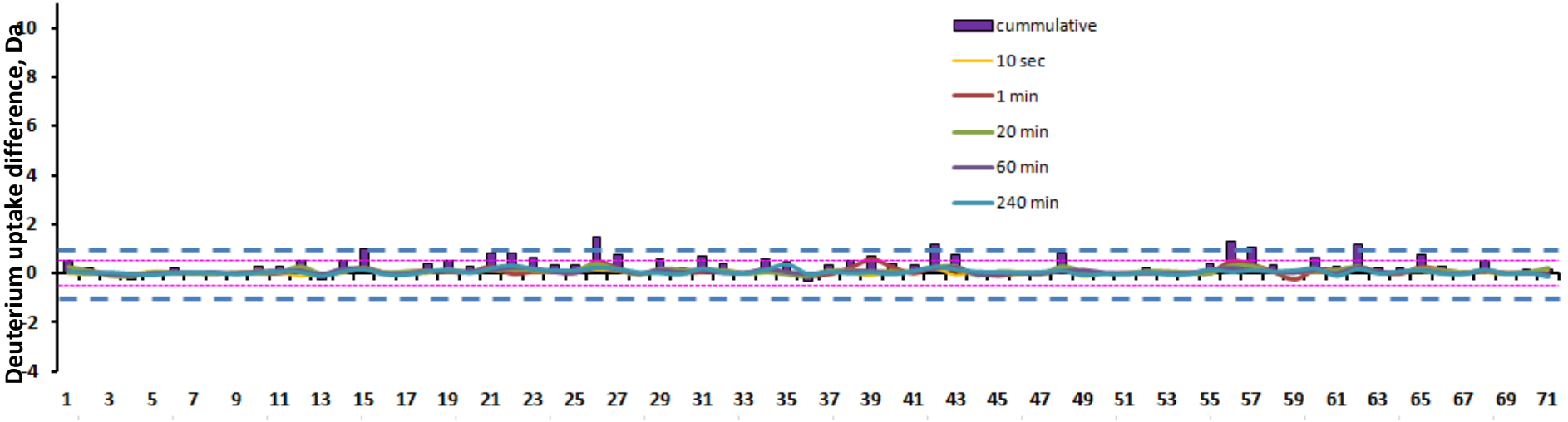


MD YFNHOH 100ns

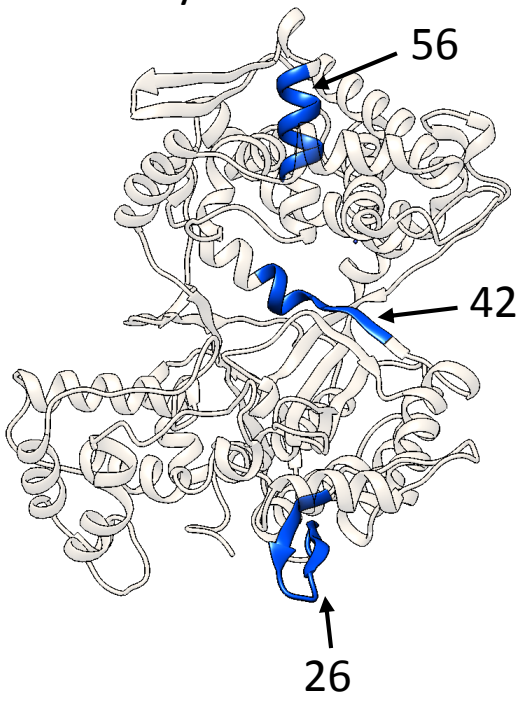


Pep 17; 160-171 IGIYHVEEKAAL
Pep 20; 172-182 LGFPSQGYTSA
Pep 26; 223-229 QIWVASE
Pep 33; 290-308 YINHFVTGSSQAHKEAQKL
Pep 40; 372-382 YEKPIFNPPDF
Pep 41; 387-406 VLTFTGSGIPAGINIPNYDD
Pep 42; 407-422 VRLKIGFKNVSLGN
Pep 52; 498-511 YKVGETWGSKFGQL

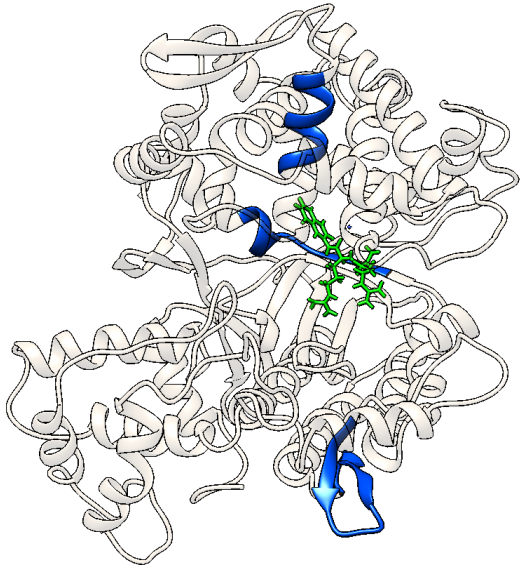
Difference between Bt DPP3 APO and Tynorphin complex



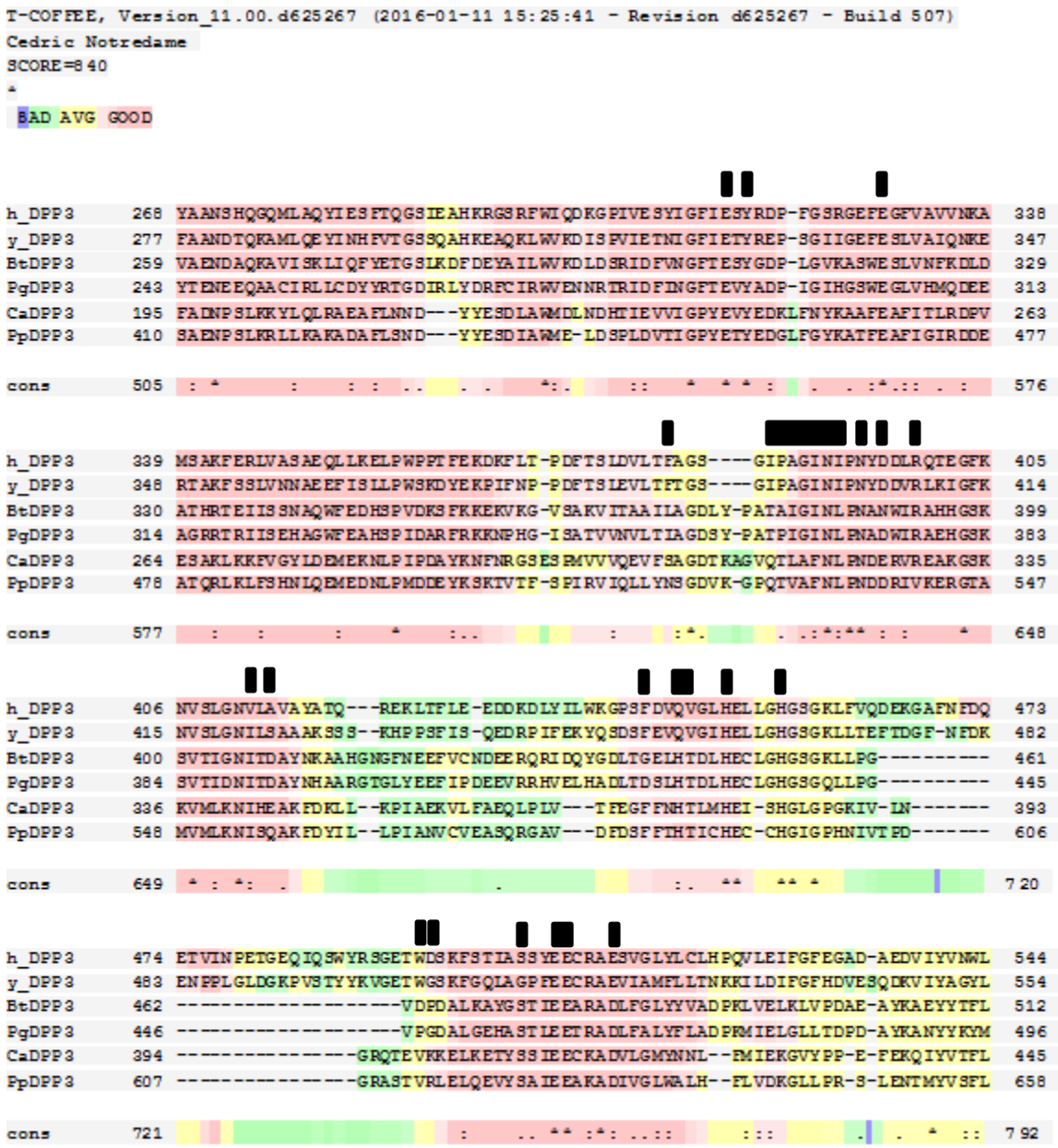
Crystal structure



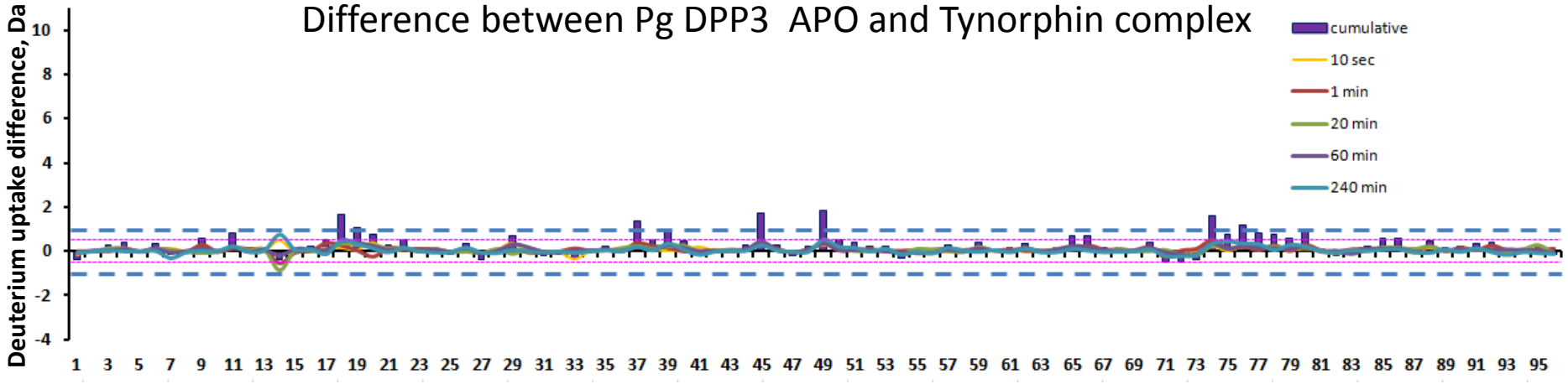
MD complex ff12sb



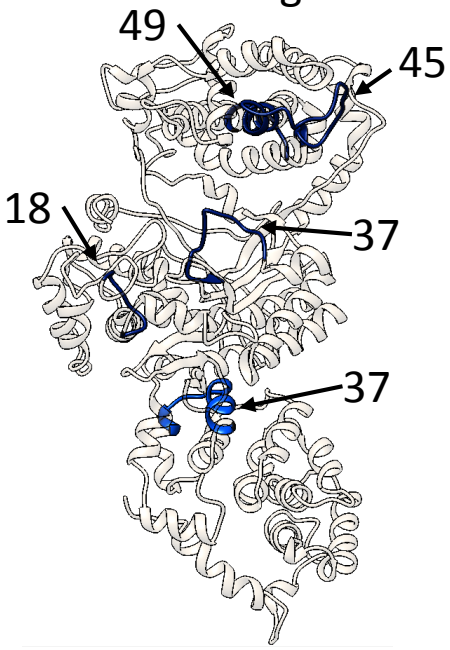
Pep 26; 207-224 YGAMKDPKDETPVSY
Pep 42; 385-393 IGINLPNAN
Pep 56; 523-531 LVRIEPGNN



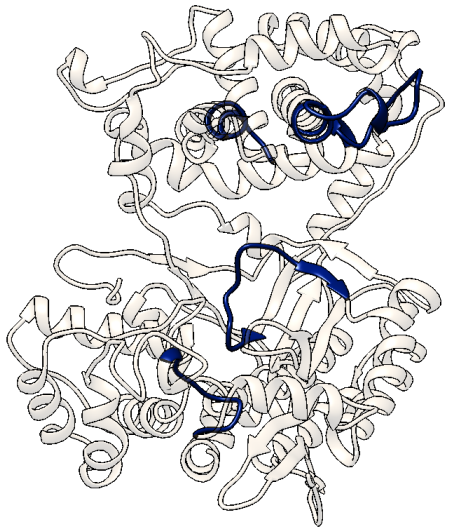
Kelley, L.A., et al., *The Phyre2 web portal for protein modeling, prediction and analysis.* Nat. Protocols, 2015. **10**(6): p. 845-858.



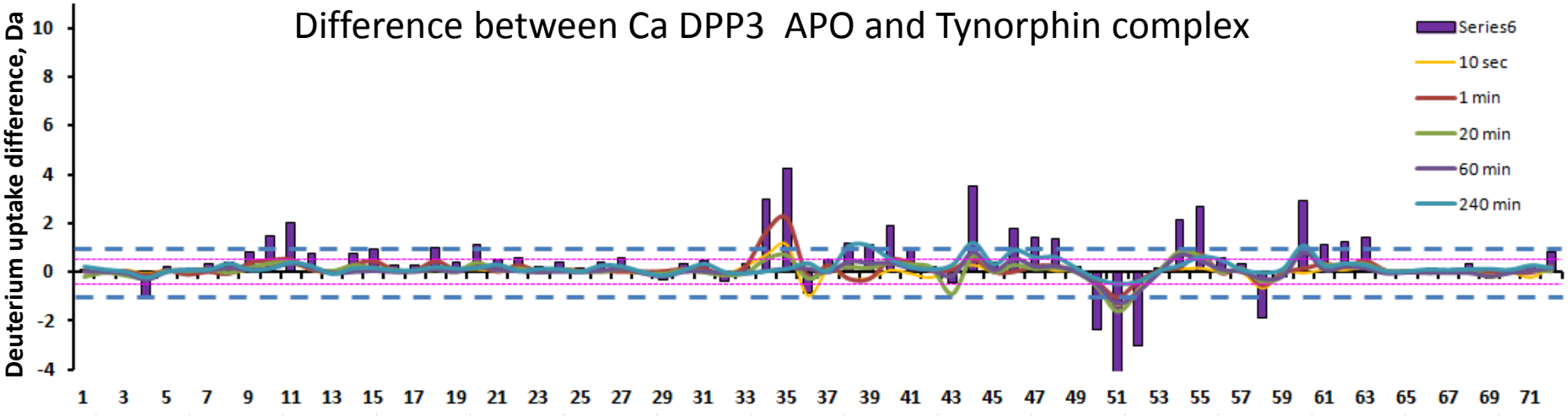
Phyre2 model
full length



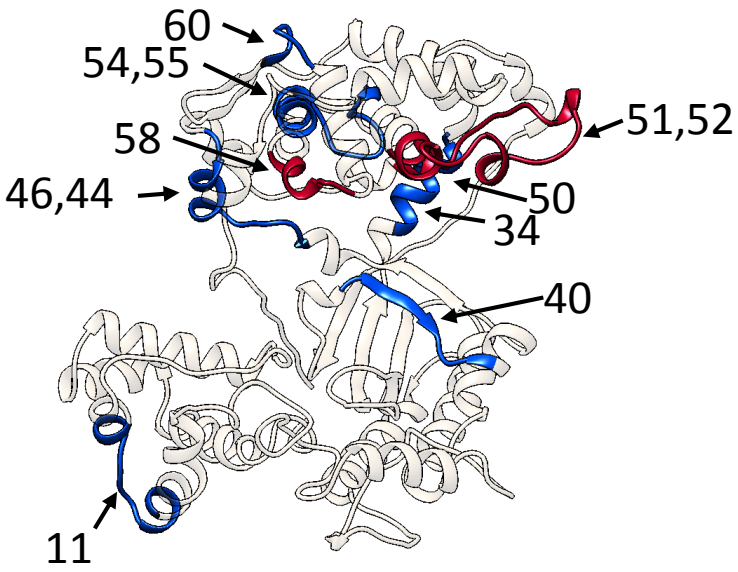
Phyre2 model
DPP III part



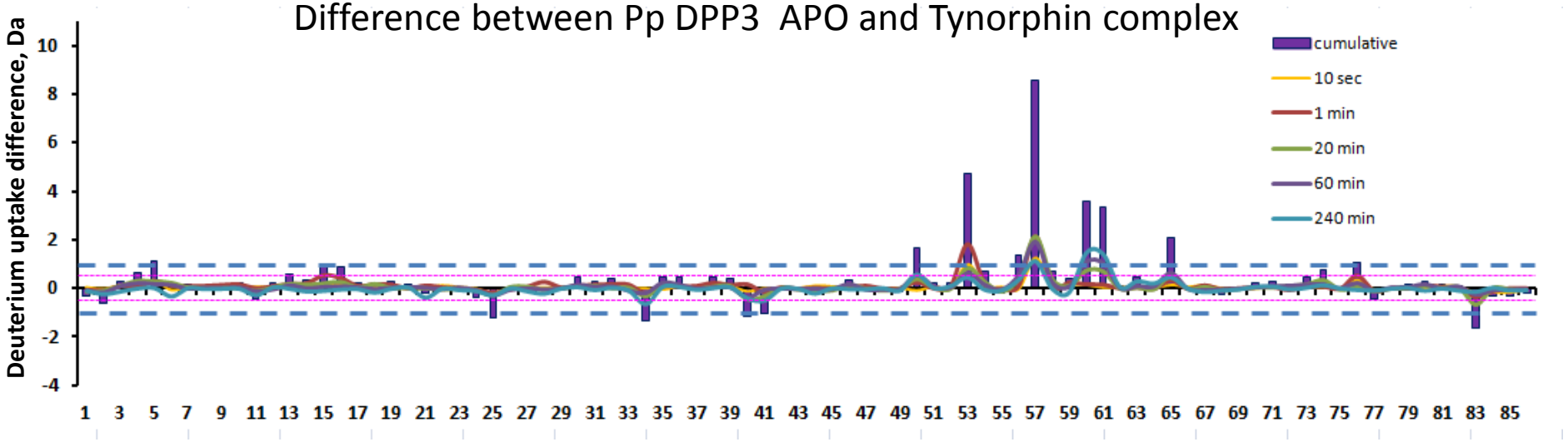
Pep 18; 164-172 IKASSVNF
Pep 37; 357-370 LTIAGDSYPATPIG
Pep 45; 435-460 HECLGHGSGQLLPGVPGDALGEHAST
Pep 49; 482-490 LGLLTDPDA
Pep 74; 705-719 LRLARTADASAPLAD



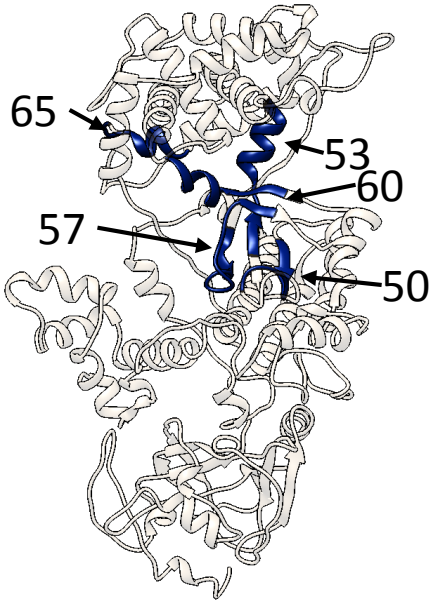
Phyre2 model



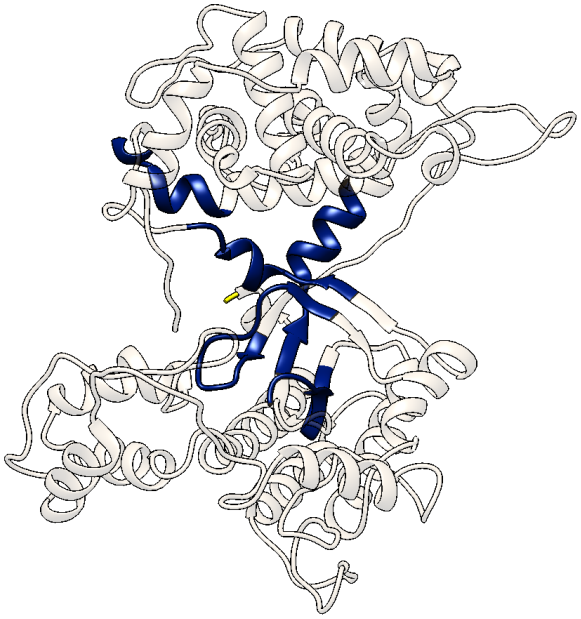
Pep 11; 89-100 RASSDPLDQLRL
Pep 34; 265-274 SAKLKKFVGY
Pep 40; 318-326 LAFNLPNDE
Pep 44; 345-356 AKFDKLLKPIAE
Pep 46; 352-359 KPIAEKVL
Pep 50; 379-392 HEISHGLGPGKIVL
Pep 51; 393-412 NGRQTEVKKELKETYSSIEE
Pep 52; 399-412 VKKELKETYSSIEE
Pep 54; 421-425 YNNLF
Pep 55; 425-441 FMIEKGVYPPEFEKQIY
Pep 58; 450-457 RTIRFGIN
Pep 60; 472-477 LEKGAY



Phyre2 model
full length



Phyre2 model
DPP III part



Pep 50; 448-457 VTIGPYETYE
Pep 53; 471-484 IRDDEATQRLKL
Pep 57; 518-532 LYNSGDVKGPQTVAF
Pep 60; 549-559 VMLKNISQAKF
Pep 65; 571-581 VEASQRGAVDF