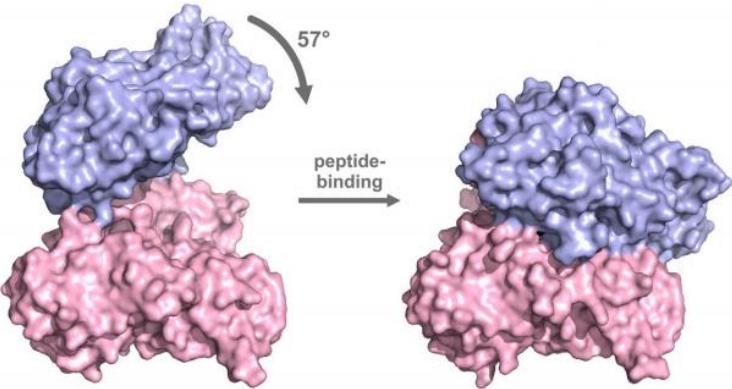


Tynorphin binding to DPP III protein homolog's analyzed by hydrogen/deuterium exchange mass spectrometry

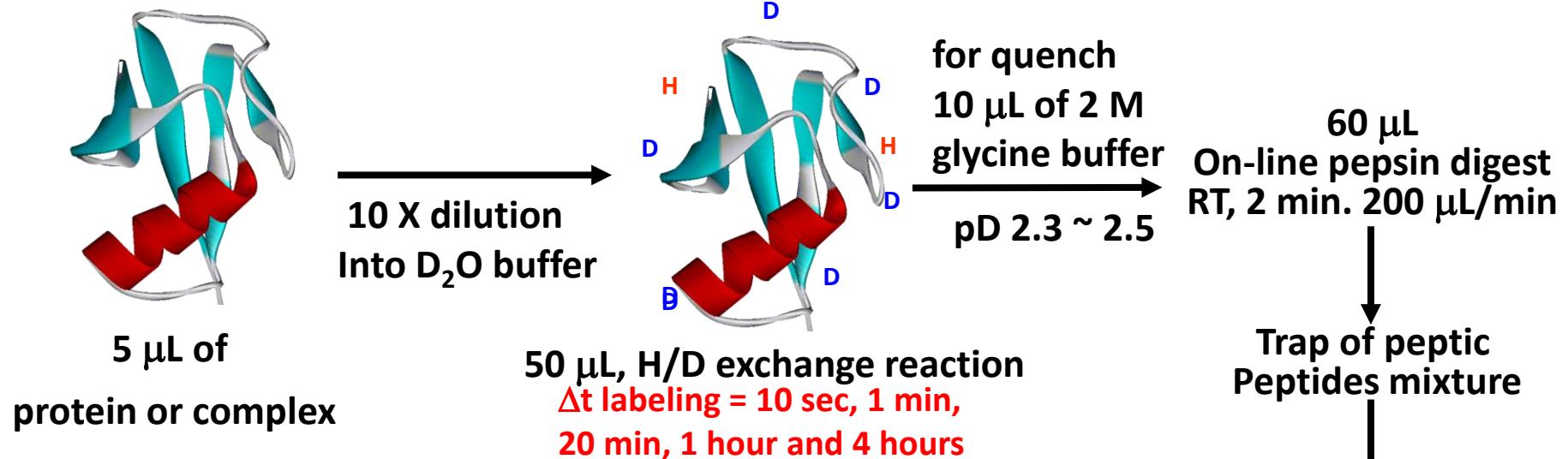


Bezerra, G. A.; Dobrovetsky, E.; Viertlmayr, R.; Dong, A.; Binter, A.; Abramić, M.; Macheroux, P.; Dhe-Paganon, S.; Gruber, K. *Proceedings of the National Academy of Sciences* **2012**, *109*, 6525.

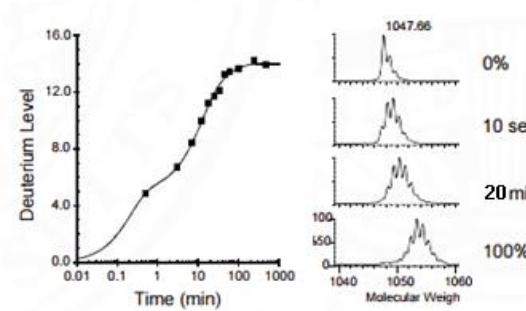
| Protein | Name | K_i tynorphin (mM) | Protein Conc. (μM) | Tynorphin Conc. (μM) | %P bound | %P bound |
|--------------------------|--|----------------------------|------------------------------------|--------------------------------------|--------------|--------------|
| | | | | | $\times 10$ | |
| hDPPIII | <i>Homo sapiens</i> | 0.03 | 35.8 | 158 | 99.98 | 99.76 |
| hDPPIII mut V412I | <i>Homo sapiens</i> <i>Mutant V412I</i> | 0.01 | 47.7 | 158 | 99.99 | 99.91 |
| yDPPIII | <i>Saccharomyces cerevisiae</i> | 0.47 | 46.8 | 500 | 99.9 | 98.97 |
| BtDPPIII | <i>Bacteroides thetaiotaomicron</i> | 0.98 | 112 | 1000 | 99.9 | 98.98 |
| PgDPPIII | <i>Porphyromonas gingivalis</i> | 0.72 | 45 | 500 | 99.84 | 98.44 |
| CaDPPIII | <i>Calditrix abyssi</i> | 16.75 | 72 | 6000 | 99.72 | 97.26 |
| PpDPPIII | <i>Physcomitrella pattens</i> | 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

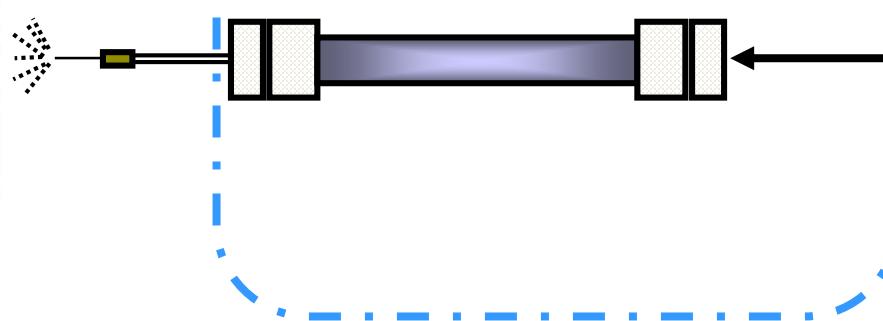
Continuous labeling experiment



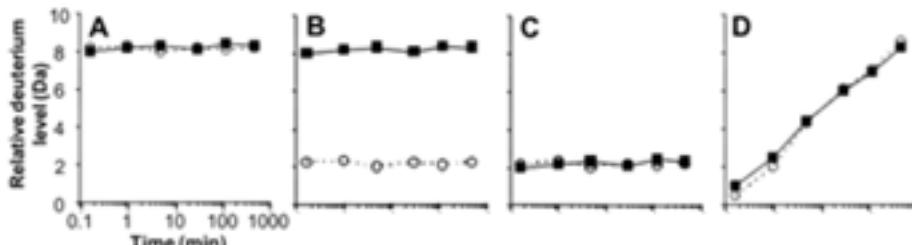
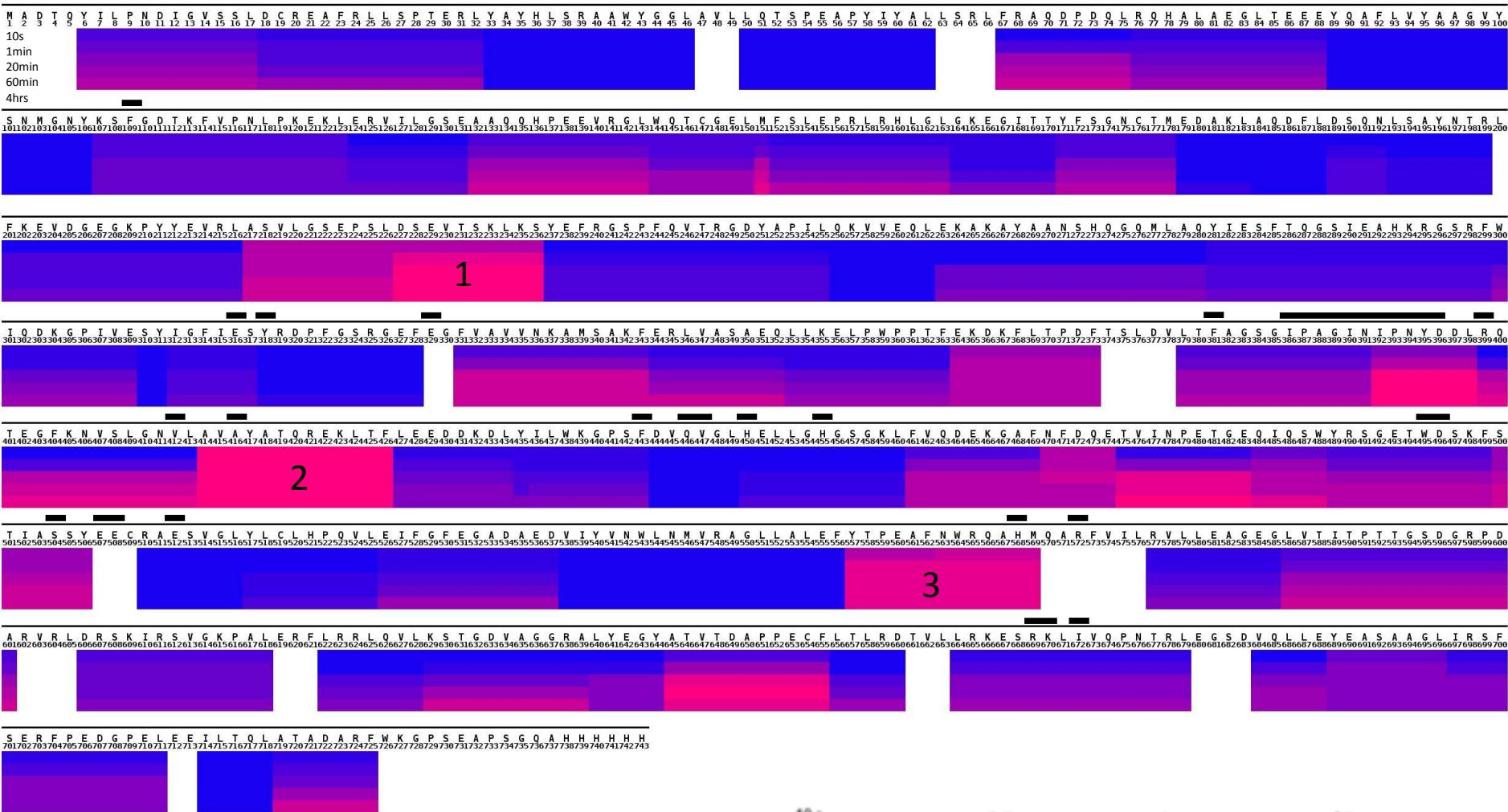
Electrospray MS



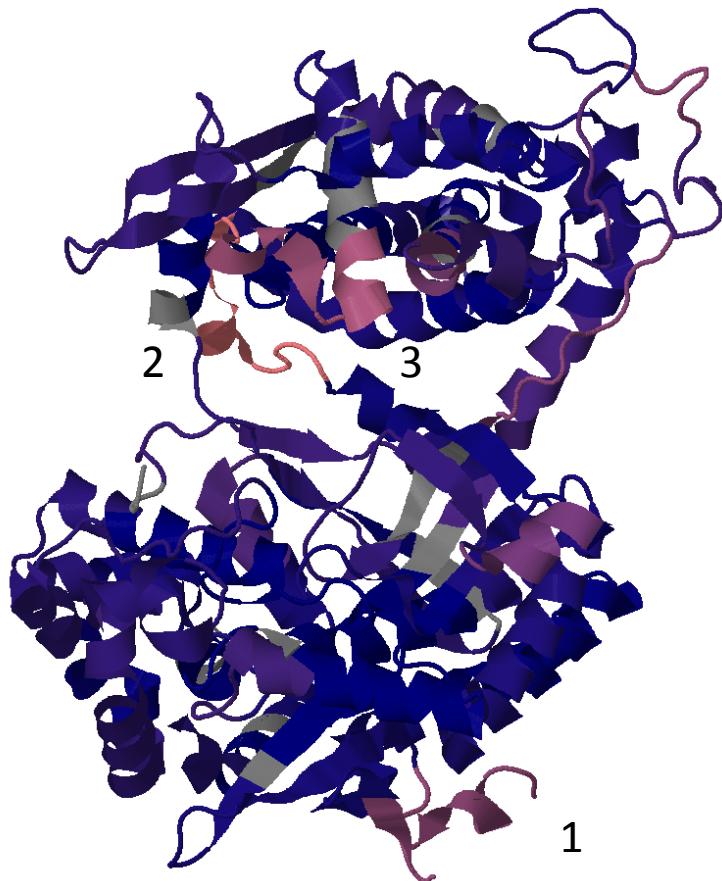
UPLC time 10 min.
8-40% ACN in 8 min. pH 2,5
Temp $\sim 0.5^\circ\text{C}$, flow rate 20 $\mu\text{L}/\text{min}$.



Flexibility map for Human DPPIII APO

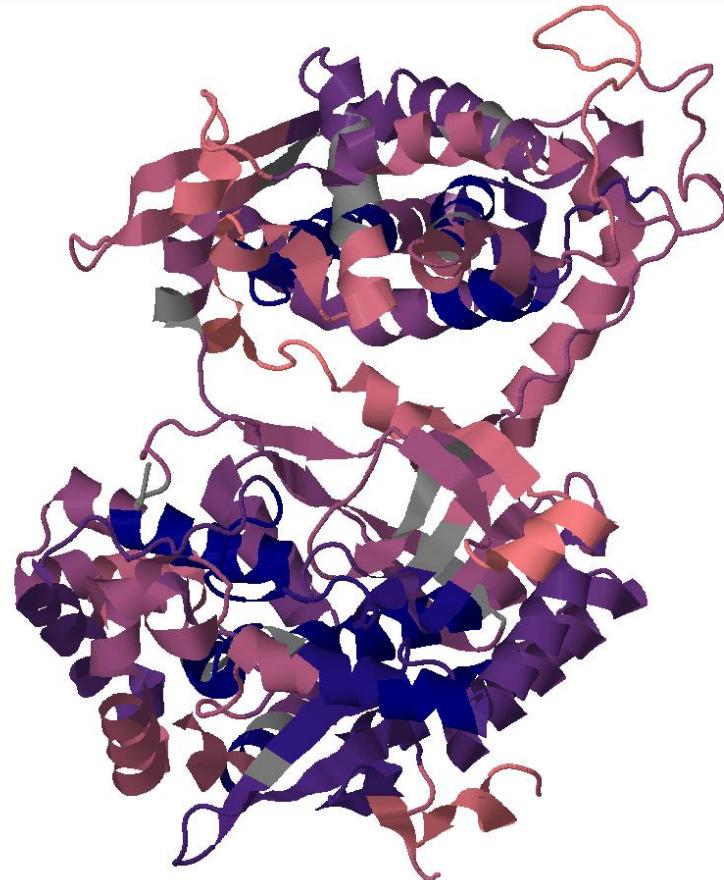


Human DPPII APO



After 10 sec

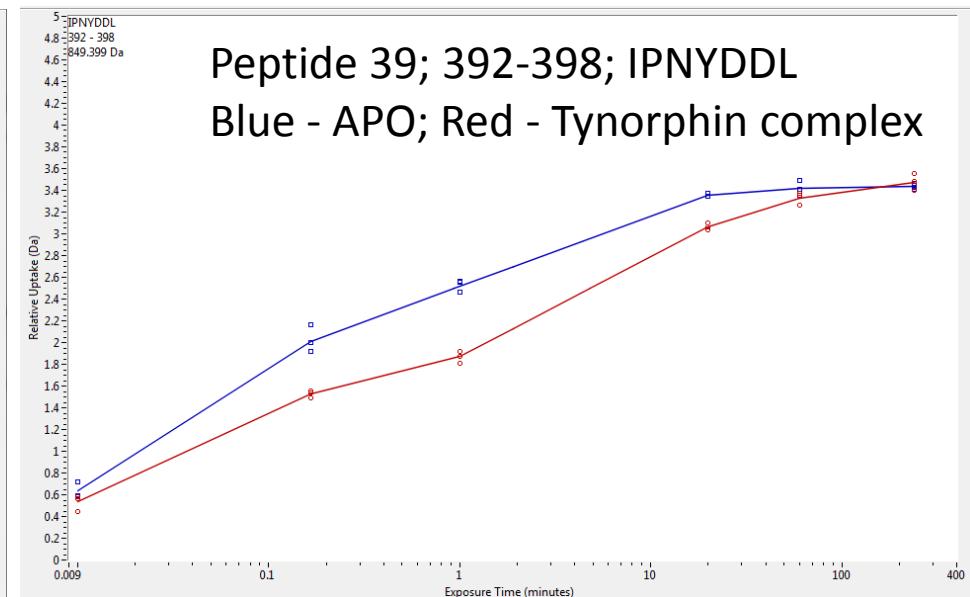
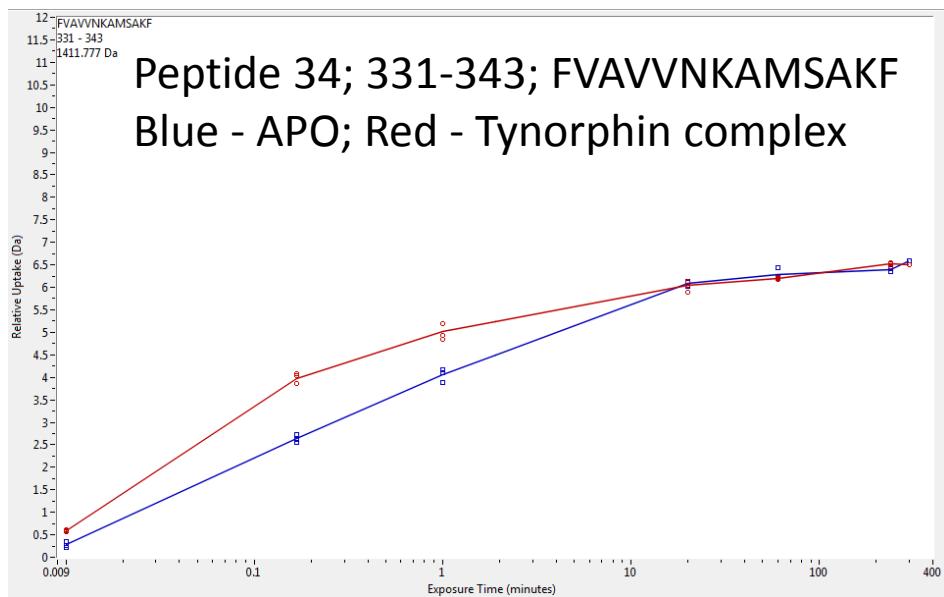
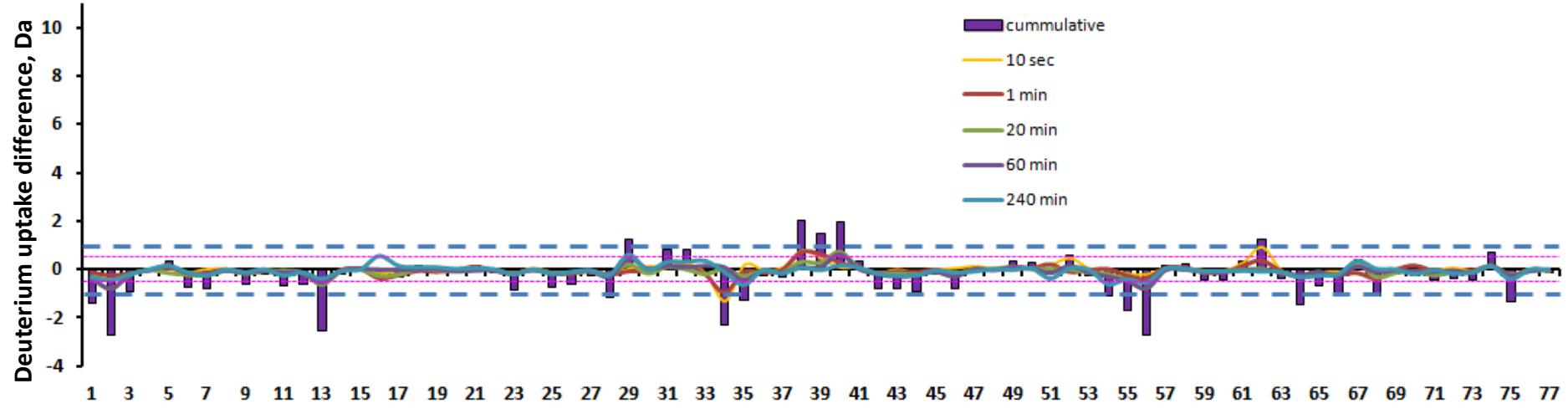
PDB 3fvy



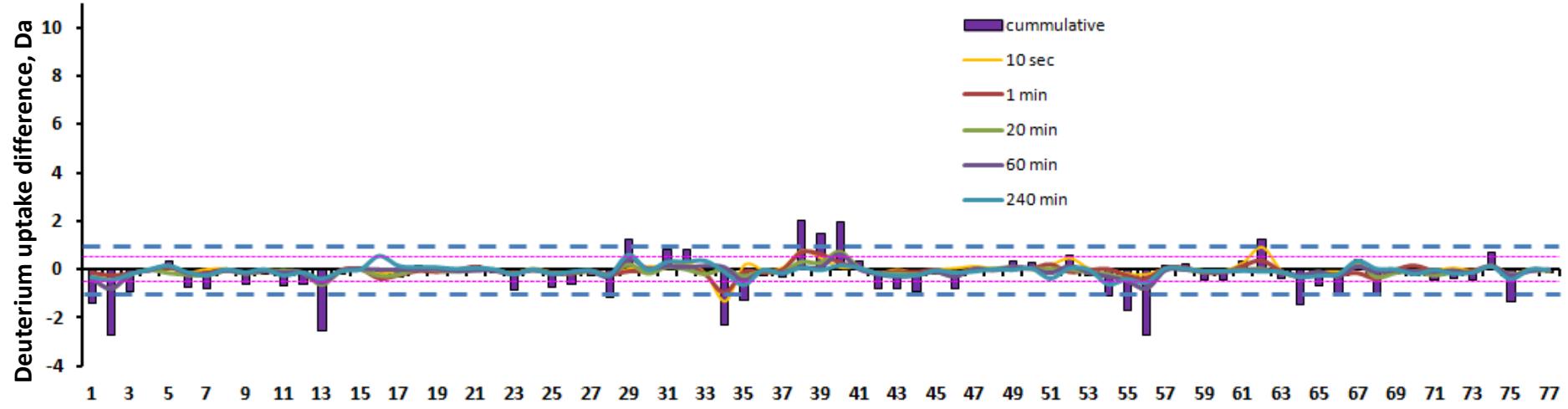
After 4 hours



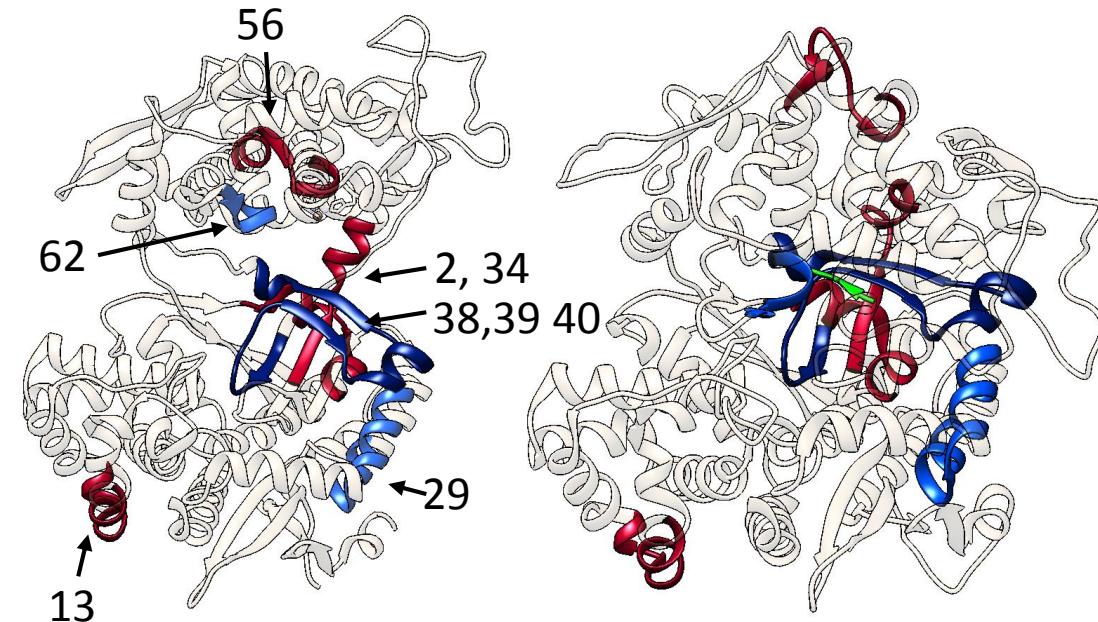
Difference between Human DPPIII APO and Tynorphin complex



Difference between Human DPPIII APO and Tynorphin complex



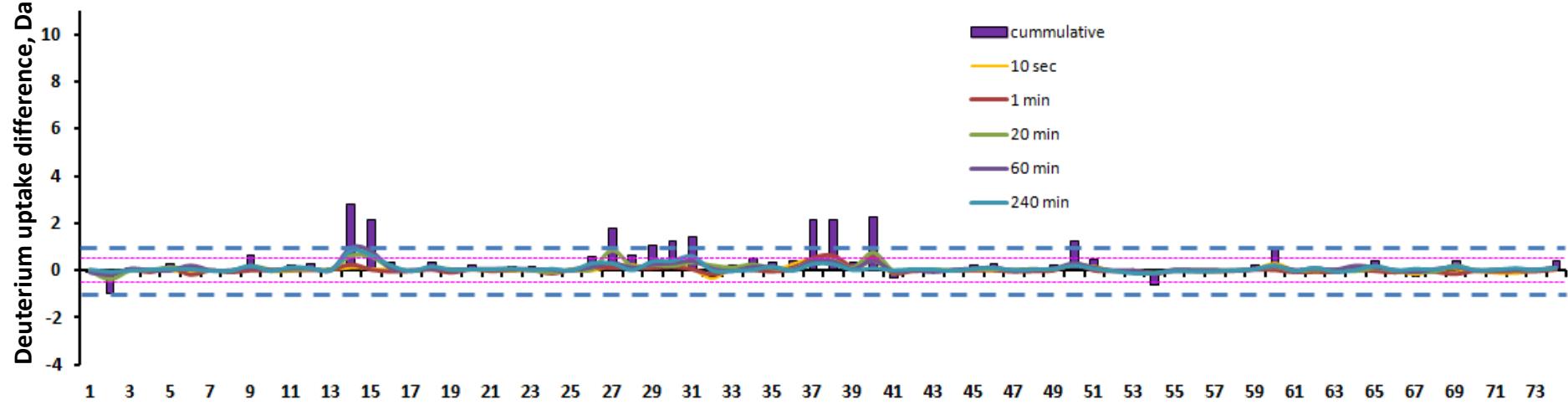
PDB 3fvy



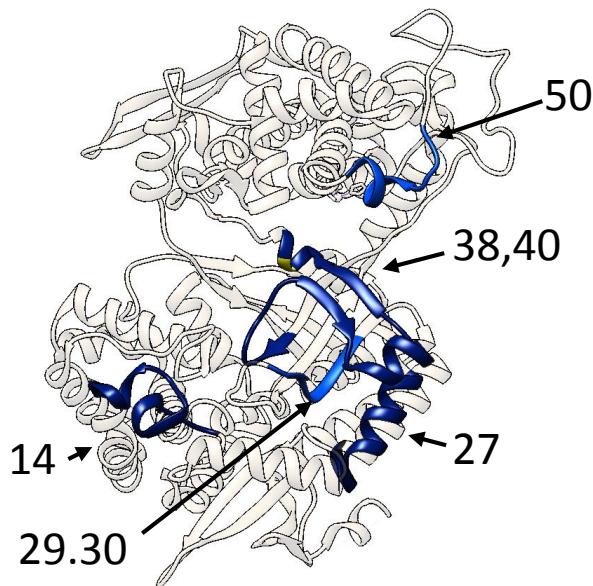
PDB 3t6j

Pep 2, 18-32 DCREAFLRLSPTERL
Pep 13, 132-143 AAQQHPEEVVRGL
Pep 29, 281-299 YIESFTQGSIEAHKRGSRF
Pep 34, 331-343 FVAVVNKAMSAKF
Pep 38, 379-398 LT_FA_GS_G**I**PAGINIPNYDDL
Pep 39, 392-398 **I**PNYDDL
Pep 40, 399-413 RQTEGFKNVSLGNVL
Pep 56, 526-537 EIFGFEGADAED
Pep 62, 563-569 NWRQAHM

Difference between Human DPP3 V412I APO and Tynorphin complex



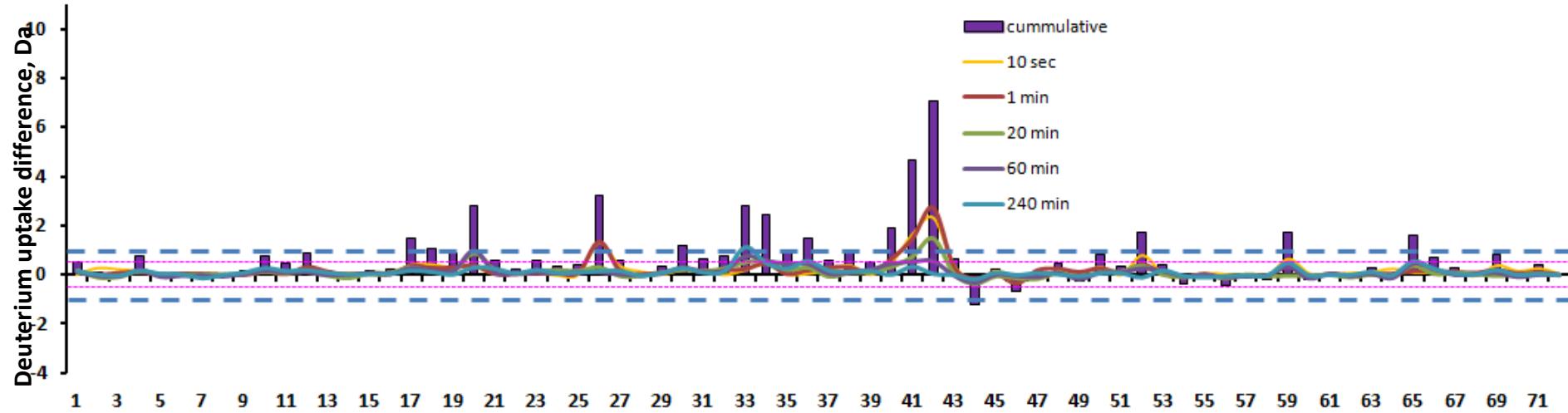
PDB 3fvv



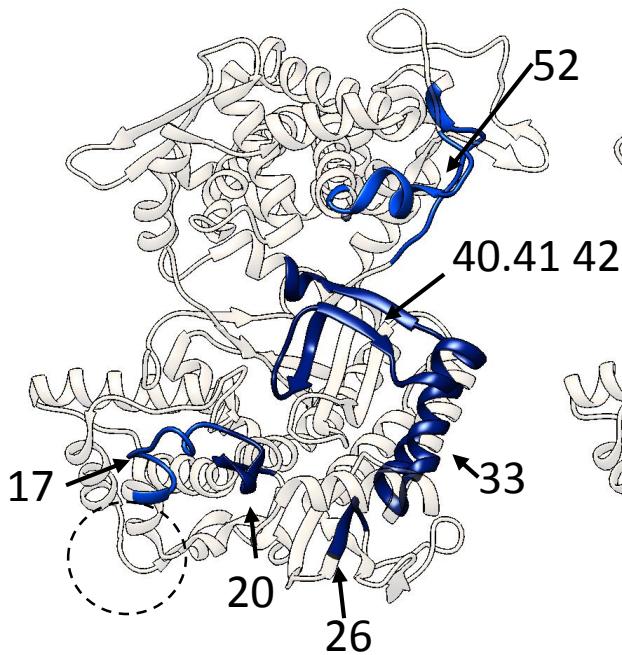
Peptide 14; 152-170 FSLEPRLRHLGLGKEGITT
 Peptide 27; 285-299 FTQGSIEAHKRGSRF
 Peptide 29; 310-314 SYIGF
 Peptide 30; 312-317 IGFIES
 Peptide 38; 381-398 FAGSS*IPAGINIPNYDDL*
 Peptide 40; 399-413 RQTEGFKNVSLGNIL
 Peptide 50; 489-499 YRSGETWDSKF

403 GFKNVSLGNIL 413 V412I mutant
 412 GFKNVSLGNIL 422 yeast

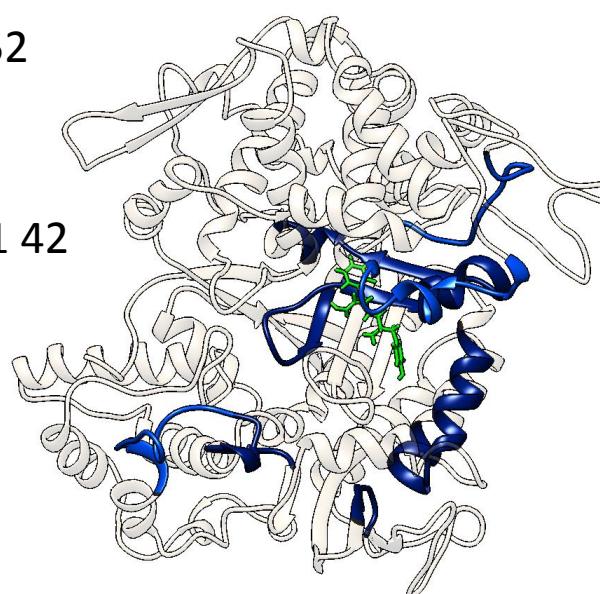
Difference between Yeast DPP3 APO and Tynorphin complex



PDB 3csk

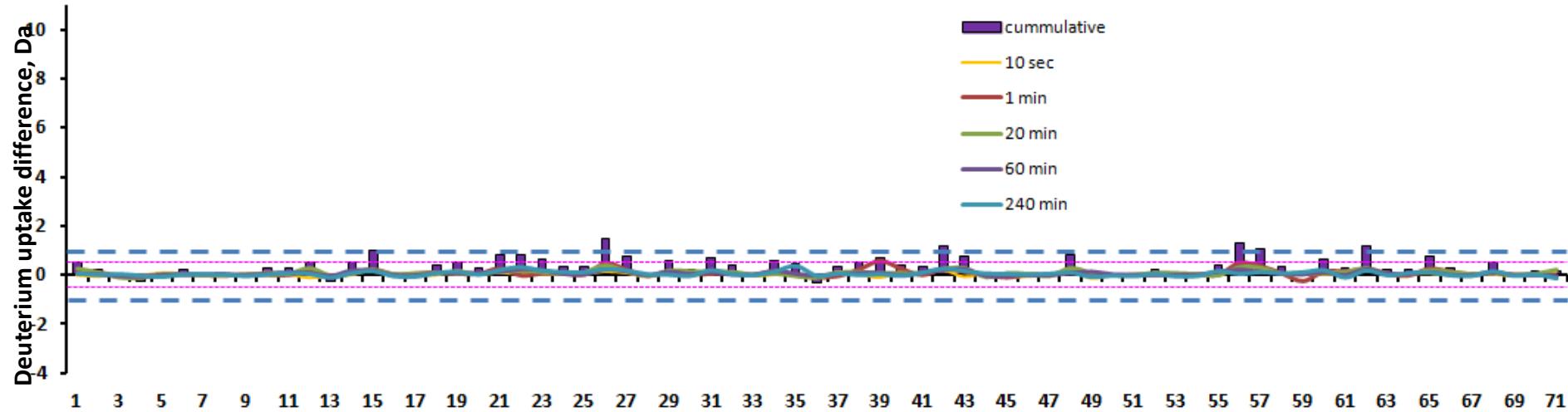


MD YFNHOH 100ns

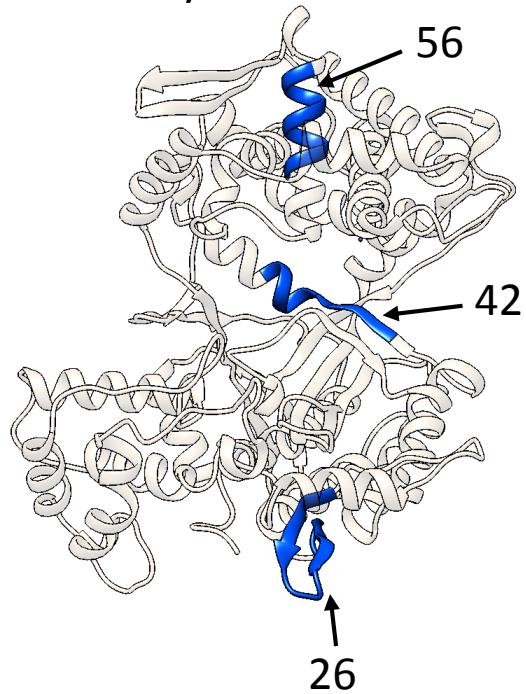


Pep 17; 160-171 IGIYHVEEKAAL
 Pep 20; 172-182 LGFPSQGYTSA
 Pep 26; 223-229 QIWVASE
 Pep 33; 290-308 YINHFVTGSSQAHKEAQKL
 Pep 40; 372-382 YEKPIFNPPDF
 Pep 41; 387-406 VLTFTGSGI**PAGINIPNYDD**
 Pep 42; 407-422 VR~~L~~KIGFKNVSLGN
 Pep 52; 498-511 YKVGETWGSKFGQL

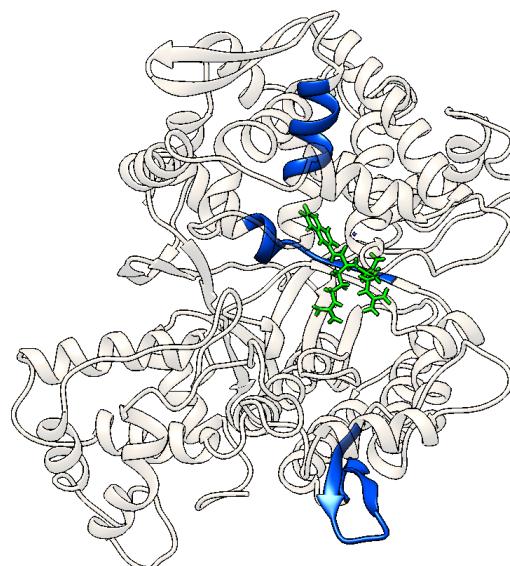
Difference between Bt DPP3 APO and Tynorphin complex



Crystal structure



MD complex ff12sb



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

Cedric Notredame

SCORE=840

^

BAD AVG GOOD

h_DPP3 268 YAANSHQGQMLAQYIESFTQGSIEAHKRGSRFWIQDKGPIVESYIGFIESYRDP-FGSRGEFEGFVAVVNKA 338

y_DPP3 277 FAANDTQKAMILQEYINHFTVGSSQAHKEAQKLWVRD13PVIETNIGIETYREP-SGIIGEFE3LVAIQNRE 347

BtDPP3 259 VAENDAQKAVISKLQFYETGSLKDFDEYAILWVKLDLSRIDFWNGFTESYGDP-LGVKA3WE3L VNFKDLD 329

PgDPP3 243 YTENEQAAACIRLLCDYRTGDIRLYDRCPIRWENNRTRIDFINGFTEVYADP-IGIHGSWEGLVHMQIEE 313

CaDPP3 195 FADNPSSLKKYLQLRAEAFLNNND--YYESDLAWMDLNDHTIEVVI3GPYEVYEDKLFNYKAFAEAFITLRDPV 263

PpDPP3 410 SAENPSSLRLLKAKADAFLSND--YYESDIAWME-LD3PLDVITIGPYETYEDGLFGYKATFEAFIGIRDDE 477

cons 505 : * : : : : . . . * : . : : : : : : : : : : : 576

h_DPP3 339 M3AKFERLVASAEQLLKEPWPPFTFKDKFLT-PDFTSLDVLTFAGS---GIPAGINIPNYDDLRQTEGFK 405

y_DPP3 348 RTAKFSSIVNNAEF18LLPWSDYKPKIFNP-PDFTSLVLTFTG3---GIPAGINIPNYDDVRLKIGFK 414

BtDPP3 330 ATHRTEII3SNAQWFEDHSVDK3FKKERVKG-V3AKVITAA1LAGDLY-PATAIGINLNANWIRAHHGSK 399

PgDPP3 314 AGRRTRII3EHAGWFEEAHPIDARFRKKNPHG-I3ATVWNVLTIAQDSY-PATFIGINLNADWIRAEHGSK 383

CaDPP3 264 E3AKLKKFVGYLDEMEKNLPIPDAYKKNFRNGSE3RMVVVQEVF8AGDTKAGVTLAFNLPNDEKVREAKGSK 335

PpDPP3 478 ATQRRLKLFSHNLQEMEDNLPMDDEYK3KIVTF-3PIRV1QLLYNSGDVK-GPQTAVFNLPNDDRIVKERGTA 547

cons 577 : : : : * : . . . : : : : : : : : : : : : 648

h_DPP3 406 NV3LGNVLAVAAYATQ--REKLTFLE-EDDKDLYILWKGPSFDWVQGLHEL LGHGS GKL FVQD EKGAFN FDQ 473

y_DPP3 415 NV3LGNILSAAKSSS-KHPPSF18-QEDRPIFEKYQ3SDSFEVQVGIHELL GHGS GKL LTF TDG F-N FDK 482

BtDPP3 400 SVTIGNITDAYNKAAGHNGFNEEFVCNDEE RQRI DQY Q DLT GEI HEC LHGS GKL LPG----- 461

PgDPP3 384 SVTIDNITDAYNHAARGTGYLEEFIPDEEWRRHVELHADLTD S I HEC LHGS GKL LPG----- 445

CaDPP3 336 KVMLKNIHEAKFDKLL-KPIAEKVLFAQBLPLIV--TPEGF FNHTIMHEI-SHGLGPKGIV-IN----- 393

PpDPP3 548 MVMLKNI3QAKFDYIL-LPIANVCVEASQRGAV--DFDSF FTI TICHEC-CHGIGPHNIVTPD----- 606

cons 649 : * : : : : : : : : : : : : : : : : : : : 720

h_DPP3 474 ETVINPETGEQIQS WYRSGETWDSKF3TIASSYE3CRAESVGLYLCLHPQVLEIFGFEGAD-AEDV1YVNWL 544

y_DPP3 483 ENPPLGLDGKPV3TYKVGETWGS KFGQIAGPFECRAEVIAFMFLTNKKI LDIFGFHDV3QIKV1YAGYL 554

BtDPP3 462 -----VDEDALKAVG STIEEARADLFGLYVADPKLVELKLVPDAE-AYKA EYYTFL 512

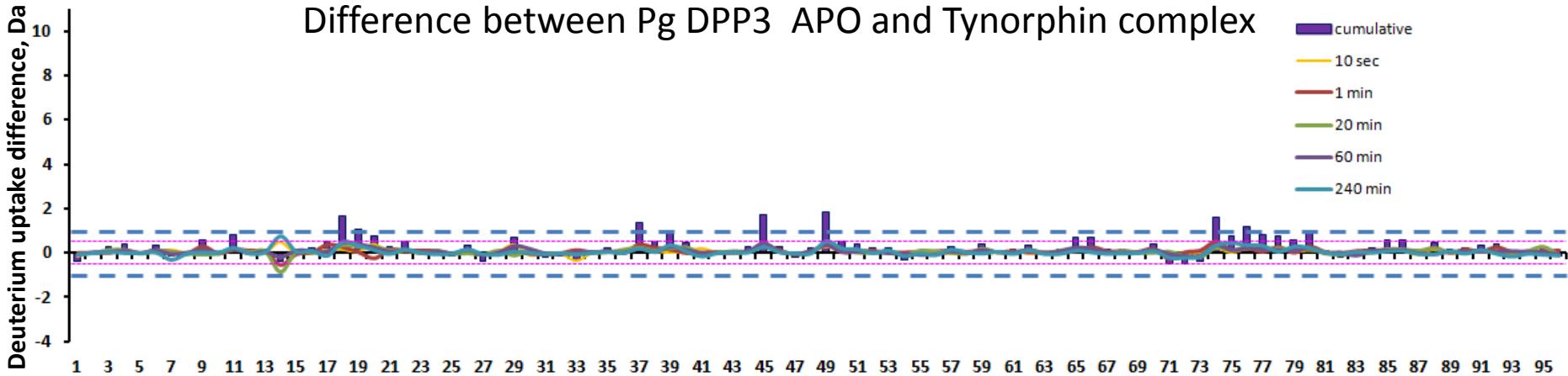
PgDPP3 446 -----VPGDALGEHA STIEETRADLFLALYFLADPRMIELGLLTD PD-AYKANYYYKYM 496

CaDPP3 394 -----GRQTEVKKEKETYSSIEECKADAVLGMYNNL--EMI EKGVYPP-E-FEKQ1YVTFL 445

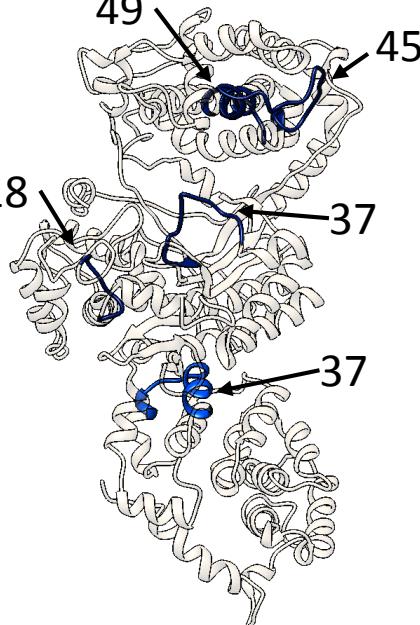
PpDPP3 607 -----GRAS T VRLELQEVYSAIEEAKADIVGLWA LH--FLVDKGLLPR-3-LENTMYV3FL 658

cons 721 : 792

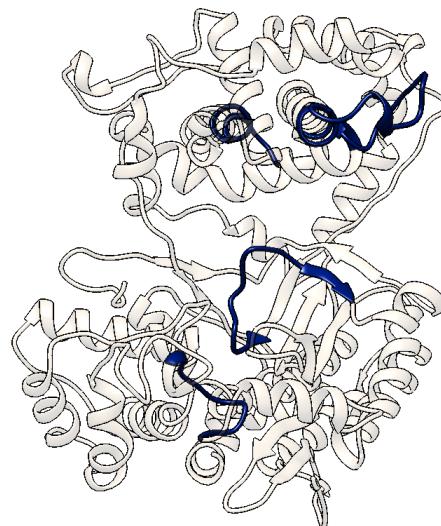
Difference between Pg DPP3 APO and Tynorphin complex



Phyre2 model
full length



Phyre2 model
DPP III part



Pep 18; 164-172 IKASSVNF

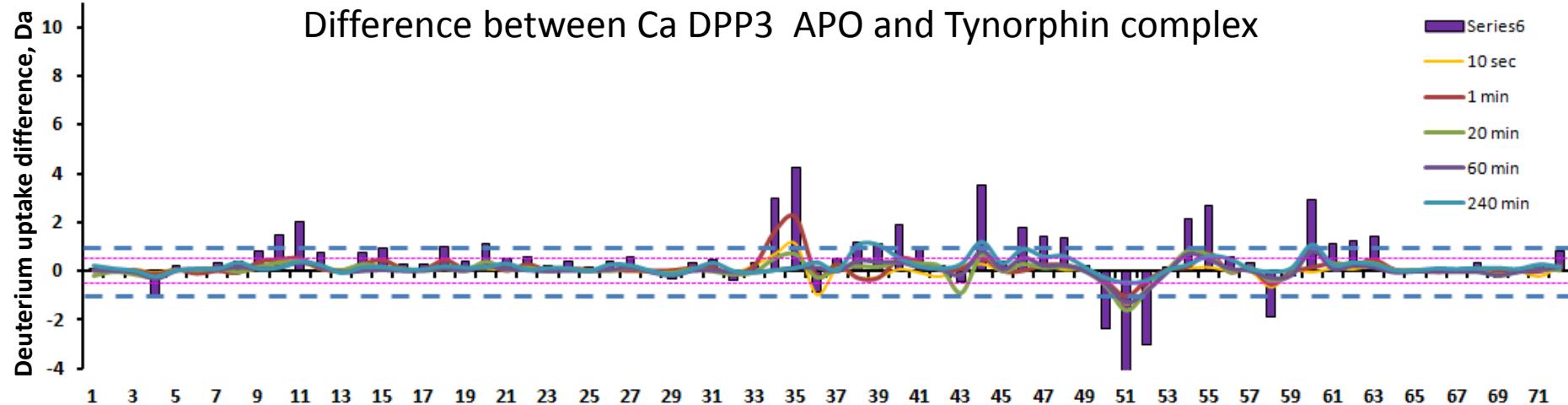
Pep 37; 357-370 LTIAGDSYPATPIG

Pep 45; 435-460 HECLGHGSGQLLPGVPGDALGEHAST

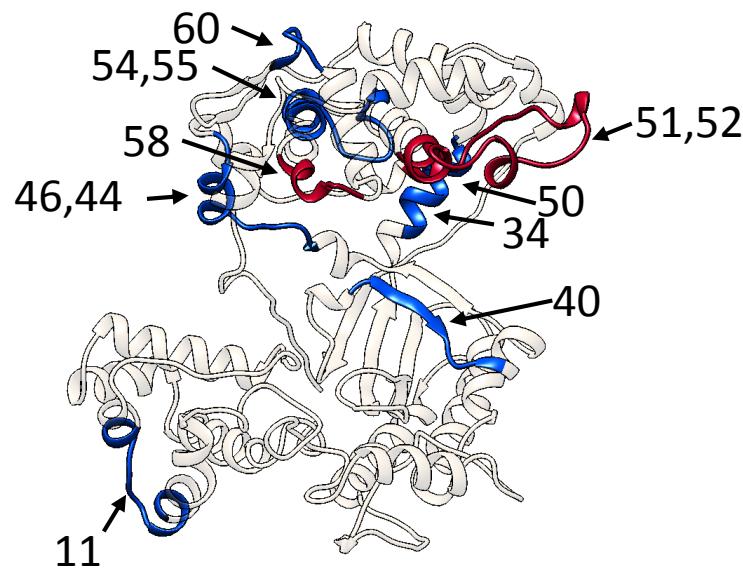
Pep 49; 482-490 LGLLTDPDA

Pep 74; 705-719 LRLARTADASAPLAD

Difference between Ca DPP3 APO and Tynorphin complex

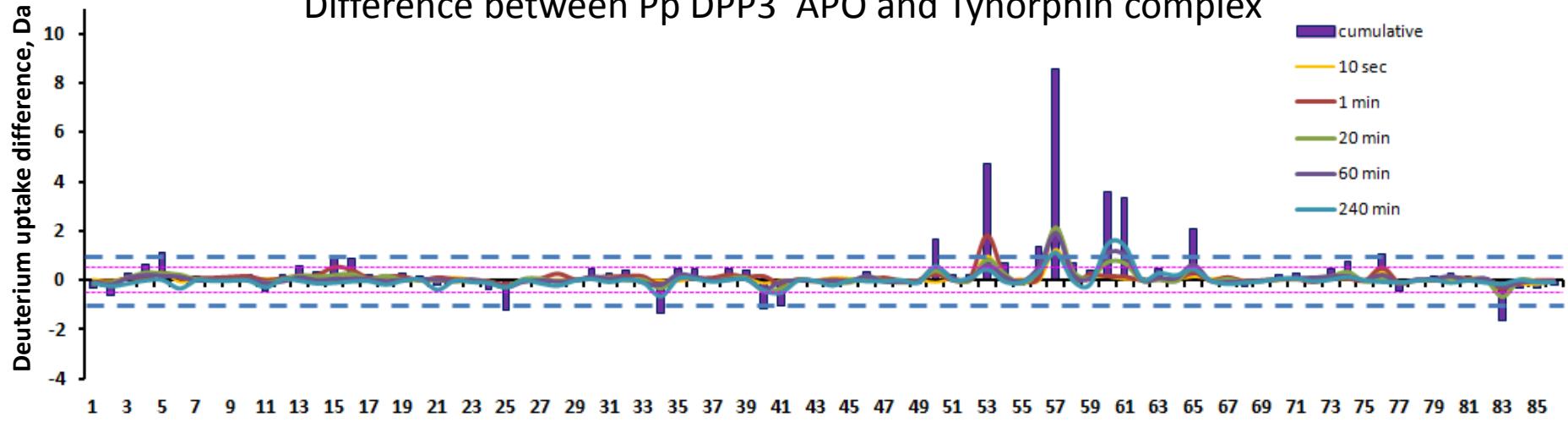


Phyre2 model

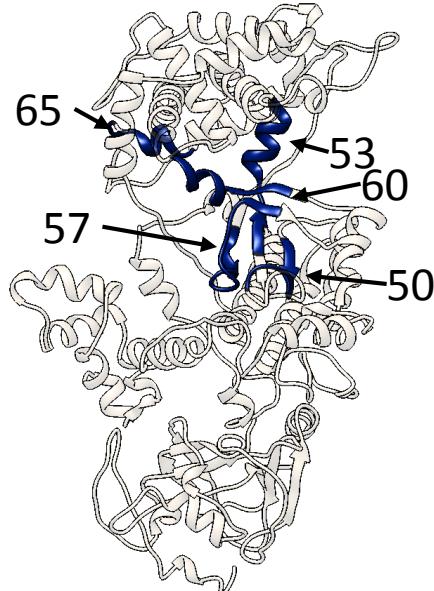


- Pep 11; 89-100 RASSDPLDQLRL
 Pep 34; 265-274 SAKLKKFVGY
 Pep 40; 318-326 LAFNLPNDE
 Pep 44; 345-356 AKFDKLLKPIAE
 Pep 46; 352-359 KPIAEKVL
 Pep 50; 379-392 HEISHGLGPGKIVL
 Pep 51; 393-412 NGRQTEVKKELKETYSSIEE
 Pep 52; 399-412 VKKELKETYSSIEE
 Pep 54; 421-425 YNNLF
 Pep 55; 425-441 FMIEKGVYPPEFEKQIY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

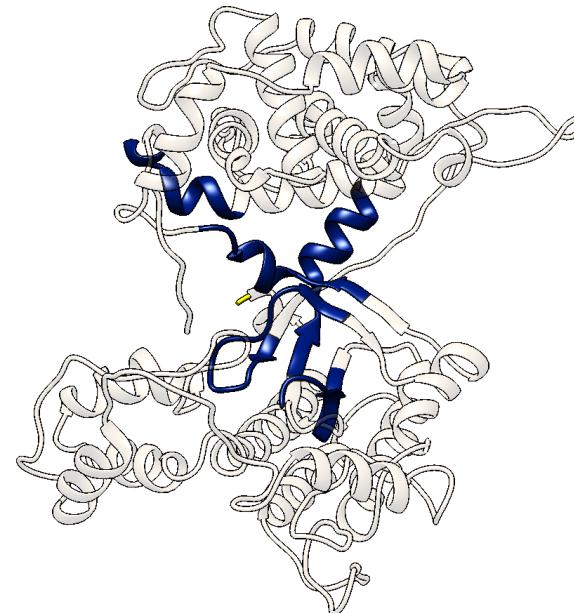
Difference between Pp DPP3 APO and Tynorphin complex



Phyre2 model
full length



Phyre2 model
DPP III part



Pep 50; 448-457 VTIGPY**E**TYE
 Pep 53; 471-484 IRDDEATQLKL
 Pep 57; 518-532 LY**N**SGDVKG**P**QTVAF
 Pep 60; 549-559 VMLKN**I**SQAKF
 Pep 65; 571-581 VEASQRGA**V**D**F**