

Seminar biokemijska informatika

Preučuješ rakave celice in pri tem opaziš, da se celice hitro delijo, kljub temu da protein p53 ni bil mutiran. Med branjem raziskav naletiš, da obstaja sorodni protein p73, ki ima podobno zgradbo in lahko aktivira iste gene za apoptozo, vendar se v rakavih celicah obnaša različno kot p53. Proteina p53 in p73 primerjaj.

Kateri protein je večkrat omenjen v znanstvenih preglednih člankih in koliko takih člankov obstaja? V koliko znanstvenih člankih sta omenjena oba proteina hkrati.

P53, saj ima p53 13601 zadetkov, p73 pa samo 384 (V obeh primerih označimo še abstract).

Oba proteina sta omenjena v 343 znanstvenih člankih.

Primerjaj dolžini človeške mRNA za oba proteina. Navedi tudi število aminokislin, ki sestavljata proteina. Zakaj pride do razlike v dolžini proteina? Ali ima kateri od proteinov kakšno dodatno domeno?

P53: 2451 nukleotidov, 393 aminokislinskih ostankov

P73: 2083 nukleotidov, 636 aminokislinskih ostankov

P73 ima dodatno domeno SAM na C-koncu, zato je daljši. (Vidimo na uniprot – Family and domains)

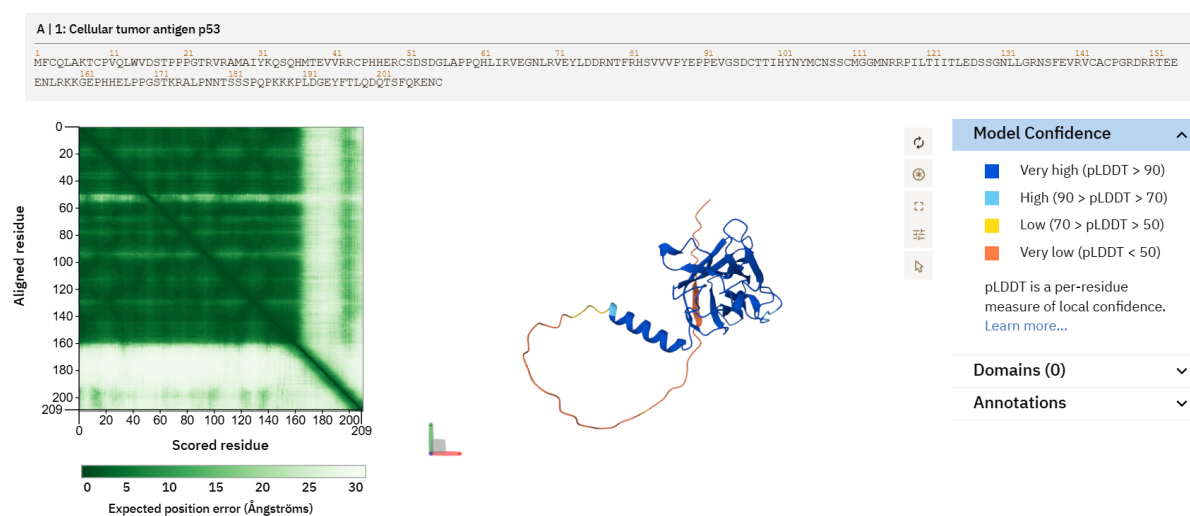
P53 in p73 spadata v isto družino. Ali imata še kakšnega paraloga?

Da, p63. (Uniprot- homologs)

Poišči strukturi obeh proteinov pridobljene iz človeka in napiši accession code. Kaj opaziš? Razloži svoja opaženja. (Pomagaj si z AlphaFold)

P73: 2wtt

Strukture p53 pridobljene iz človeka ni na pdb. Strukturno p53 poiščemo na AlphaFold in opazimo, da struktura ni natančno določena, ker ima fleksibilen C-konec.



Poišči strukturo s kodo 2mw4 in odgovori na vprašanja. Kako se protein ureja? Kaj je bil izvorni organizem za določitev te strukture in s katero metodo so strukturo določili? Kako se p53 razlikuje iz ostalih proteinov iz iste družine glede na sekundarno strukturo.

V tetramer. Morska vaza (*Ciona intestinalis*), NMR v raztopini (solution NMR).

P53 nima druge alfa vijačnice.



2MW4 | pdb_00002mw4

Tetramerization domain of the *Ciona intestinalis* p53/p73-b transcription factor protein

PDB DOI: <https://doi.org/10.2210/pdb2MW4/pdb>
BMRB: 25298

Classification: TRANSCRIPTION
Organism(s): *Ciona intestinalis*
Expression System: *Escherichia coli*
Mutation(s): No

Deposited: 2014-10-27 Released: 2015-10-28
Deposition Author(s): Heering, J.P., Jonker, H.R.A., Loehr, F., Schwalbe, H., Doetsch, V.

Experimental Data Snapshot

Method: SOLUTION NMR
Conformers Calculated: 100
Conformers Submitted: 20
Selection Criteria: target function

wwPDB Validation

Metric	Percentile Ranks	Value
Clashscore		31
Ramachandran outliers		0.0%
Sidechain outliers		18.8%

Explore in 3D: Structure | Sequence Annotations | Validation Report

PubMed: 26473758 [Search on PubMed](#) [Search on PubMed Central](#)
DOI: <https://doi.org/10.1002/pro.2830>
Primary Citation Related Structures:
[2MW4](#)

PubMed Abstract:
Most members of the p53 family of transcription factors form tetramers. Responsible for determining the oligomeric state is a short oligomerization domain consisting of one β -strand and one α -helix. With the exception of human p53 all other family members investigated so far contain a second α -helix as part of their tetramerization domain. Here we have used nuclear magnetic resonance spectroscopy to characterize the oligomerization domains of the two p53-like proteins from the tunicate *Ciona intestinalis*, representing the closest living relative of vertebrates. Structure determination reveals for one of the two proteins a new type of packing of this second α -helix on the core domain that was not predicted based on the sequence, while the other protein does not form a second helix despite the presence of crucial residues that are conserved in all other family members that form a second helix. By mutational analysis, we identify a proline as well as large hydrophobic residues in the hinge region between both helices as the crucial determinant for the formation of a second helix.

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Kateri posttranslacijski modifikaciji opazimo pri obeh proteinih, če prezremo PTM »Modified residue (large scale data)«?

Modificiran ostanek fosfotreonin, prečni povezovalc Lys-Gly. (UniProt- PTM/Processing)

Poišči točne molekulske mase proteinov in napiši katera dva aminokislinska ostanka se največkrat pojavita v zaporedju. Kakšen tip ionskega izmenjevalca bi uporabili pri pH 7.

P53: M=43653.18Da, p73:M=69623.32, prolin in serin, anionski izmenjevalec (pI<pH)
(ProtParam)

Number of amino acids: 636
Theoretical pI: 6.47
Molecular weight: 69623.32

Amino acid composition: [CSV format](#)

Ala (A)	42	6.6%
Arg (R)	32	5.0%
Asn (N)	25	3.9%
Asp (D)	27	4.2%
Cys (C)	11	1.7%
Gln (Q)	39	6.1%
Glu (E)	34	5.3%
Gly (G)	49	7.7%
His (H)	24	3.8%
Ile (I)	24	3.8%
Leu (L)	47	7.4%
Lys (K)	23	3.6%
Met (M)	20	3.1%
Phe (F)	19	3.0%
Pro (P)	58	9.1%
Ser (S)	62	9.7%
Thr (T)	39	6.1%
Trp (W)	4	0.6%
Tyr (Y)	20	3.1%
Val (V)	37	5.8%
Pyl (O)	0	0.0%
Sec (U)	0	0.0%

(B)	0	0.0%
(Z)	0	0.0%
(X)	0	0.0%

Total number of negatively charged residues (Asp + Glu): 61
Total number of positively charged residues (Arg + Lys): 55

Number of amino acids: 393
Theoretical pI: 6.33
Molecular weight: 43653.18

Amino acid composition: [CSV format](#)

Ala (A)	24	6.1%
Arg (R)	26	6.6%
Asn (N)	14	3.6%
Asp (D)	20	5.1%
Cys (C)	10	2.5%
Gln (Q)	15	3.8%
Glu (E)	30	7.6%
Gly (G)	23	5.9%
His (H)	12	3.1%
Ile (I)	8	2.0%
Leu (L)	32	8.1%
Lys (K)	20	5.1%
Met (M)	12	3.1%
Phe (F)	11	2.8%
Pro (P)	45	11.5%
Ser (S)	38	9.7%
Thr (T)	22	5.6%
Trp (W)	4	1.0%
Tyr (Y)	9	2.3%
Val (V)	18	4.6%
Pyl (O)	0	0.0%
Sec (U)	0	0.0%

(B)	0	0.0%
(Z)	0	0.0%
(X)	0	0.0%

Total number of negatively charged residues (Asp + Glu): 50
Total number of positively charged residues (Arg + Lys): 46

Poravnaj človeška proteina p53 in p73. Katera poravnava je ustrežnejša – lokalna ali globalna?

Lokalna poravnava, saj sta si proteina podobna, le da ima p73 dodatno domeno. (Water ima višji score) (Emboss Needle/Water)

```
# Aligned_sequences: 2
# 1: P73_HUMAN
# 2: P53_HUMAN
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 380
# Identity:      160/380 (42.1%)
# Similarity:   218/380 (57.4%)
# Gaps:         59/380 (15.5%)
# Score: 729.5
#
#
```

```
"
# Aligned_sequences: 2
# 1: P73_HUMAN
# 2: P53_HUMAN
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 662
# Identity:      167/662 (25.2%)
# Similarity:   230/662 (34.7%)
# Gaps:         295/662 (44.6%)
# Score: 715.0
#
#
```