EMBL-EBI Tutorial

# MSDpisa Tutorial

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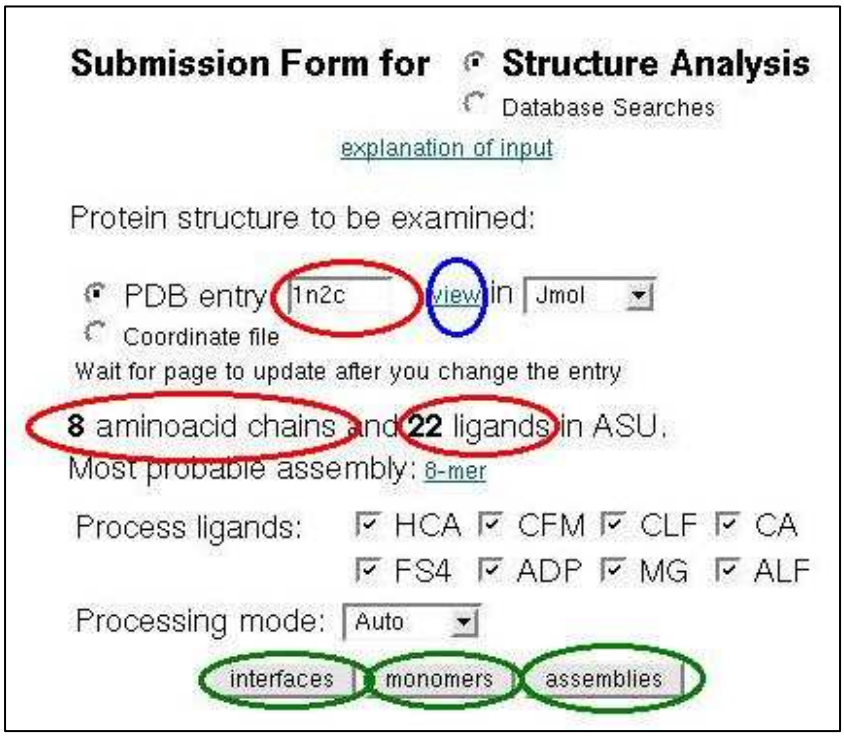
**PISA** (Protein Interfaces, Surfaces and Assemblies) is a web based interactive service offered by **MSD** to investigate stability of formation of macromolecular complexes (protein, DNA/RNA and ligand).

Stability of a macromolecular complex is governed by the following physicochemical properties:

- free energy of formation
- solvation energy gain
- interface area
- hydrogen bonds and saltbridges across the interface
- hydrophobic specificity

Go to the start page for PISA @ http://www.ebi.ac.uk/msd-srv/prot_int/cgi-bin/piserver

Type the ID code 1N2C where it asks for PDB entry




As soon as the file gets uploaded to the server, it will give you preliminary information regarding the PDB entry (number of proteins chains and bound ligands).

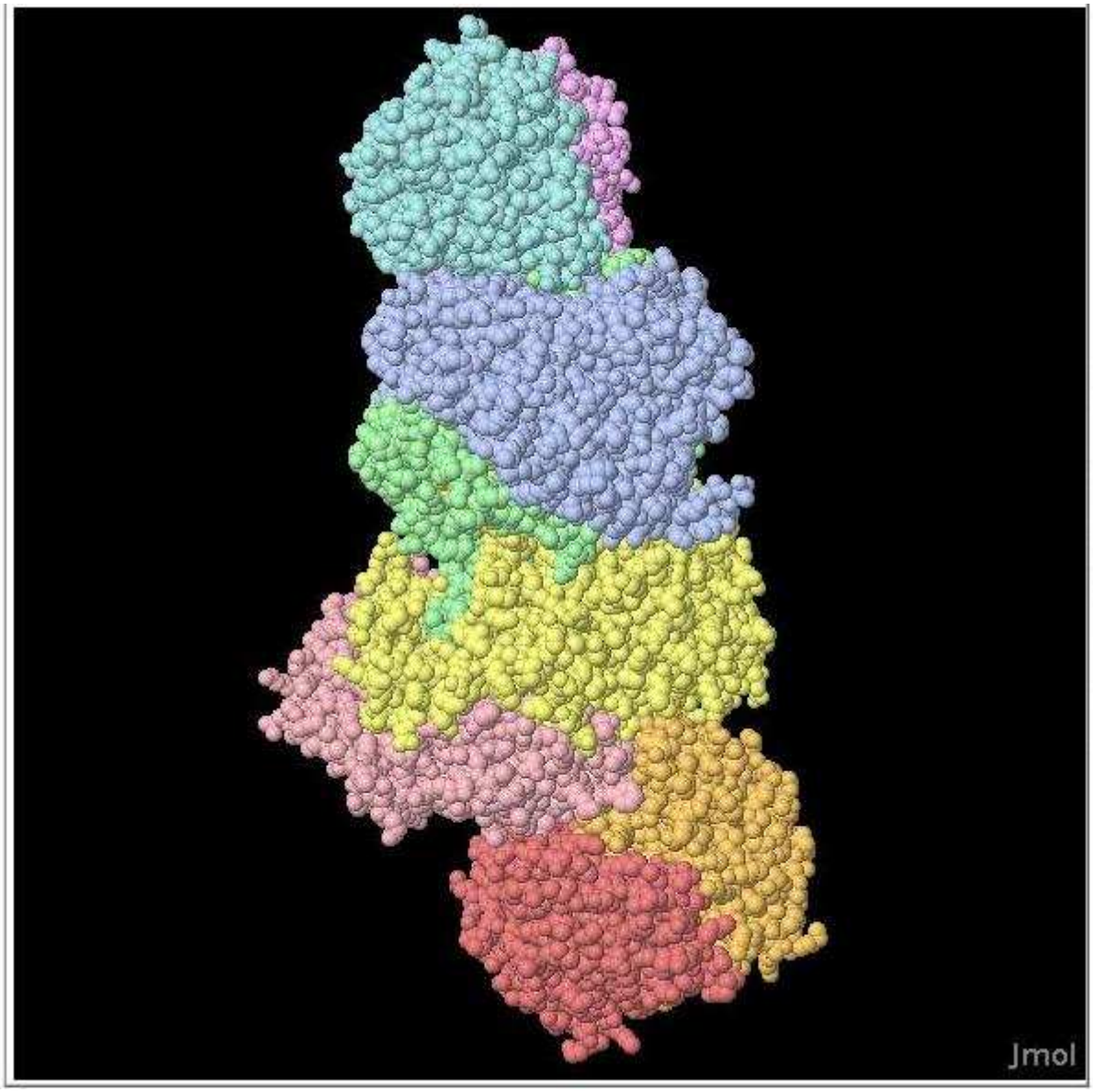
The entry 1n2c has 8 protein chains and 22 ligands.

The most probable assembly is stated as an 8-mer.

[If you want to know some more details about this PDB entry (e.g. Name of the protein, origin etc.), then go to the MSD homepage (http://www.ebi.ac.uk/msd/index.html)

and type the ID code 1n2c to view the atlas page for this entry. The atlas page for this entry gives us information that it is a nitrogenase complex structure stabilized by ADP-tetrafluoroaluminate (title). There are two different proteins, 1)NITROGENASE MOLYBDENUM-IRON PROTEIN ( Chains A, B, C and D) and 2)NITROGENASE IRON PROTEIN (chains E, F, G, H)].

You can click on the **view** button highlighted in blue to view the loaded PDB entry




There are three buttons highlighted in green at the bottom of the submission page – **interfaces**, **monomers** and **assemblies**. Each of them provide structural information

related to the protein of interest (energy of association, solvation energy, buried surface area, H-bonds and saltbridges etc.).

The **Monomers**:

Let us first start with the different monomers present in the PDB entry.

If you click on the monomers button, you get the following information about the corresponding PDB entry.

**Interfacing monomers**

| ## | | | Range | Class | Structure | | Surface | | | ΔG, kcal/M |
|---|---|---|---|---|---|---|---|---|---|---|
| Id | NN | «» | | | $N_{at}$ | $N_{res}$ | $^{s}N_{at}$ | $^{s}N_{res}$ | Area, Å$^2$ | |
| 1 | 1 | ◉ | A | Protein | 3793 | 478 | 1886 | 416 | 19473.3 | -441.1 |
| | 2 | ○ | C | Protein | 3793 | 478 | 1876 | 416 | 19438.5 | -441.3 |
| | | | | *Average:* | 3793 | 478 | 1881 | 416 | 19455.9 | -441.2 |
| 2 | 3 | ○ | B | Protein | 4170 | 522 | 2233 | 465 | 23880.1 | -498.8 |
| | 4 | ○ | D | Protein | 4170 | 522 | 2240 | 466 | 23907.4 | -498.7 |
| | | | | *Average:* | 4170 | 522 | 2236 | 465 | 23893.8 | -498.7 |
| 3 | 5 | ○ | E | Protein | 2066 | 274 | 1067 | 235 | 11703.4 | -268.0 |
| | 6 | ○ | F | Protein | 2066 | 274 | 1055 | 237 | 11702.7 | -268.2 |
| | 7 | ○ | G | Protein | 2066 | 274 | 1064 | 237 | 11722.0 | -268.0 |
| | 8 | ○ | H | Protein | 2066 | 274 | 1064 | 237 | 11714.4 | -268.0 |
| | | | | *Average:* | 2066 | 274 | 1062 | 236 | 11710.6 | -268.1 |
| 4 | 9 | ○ | [HCA]A:494 | Ligand | 14 | 1 | 13 | 1 | 351.3 | |
| | 10 | ○ | [HCA]C:494 | Ligand | 14 | 1 | 13 | 1 | 351.6 | |
| | | | | *Average:* | 14 | 1 | 13 | 1 | 351.5 | |
| 5 | 11 | ○ | [CFM]A:496 | Ligand | 17 | 1 | 17 | 1 | 490.6 | |
| | 12 | ○ | [CFM]C:496 | Ligand | 17 | 1 | 17 | 1 | 489.6 | |
| | | | | *Average:* | 17 | 1 | 17 | 1 | 490.1 | |
| 6 | 13 | ○ | [CLF]A:498 | Ligand | 15 | 1 | 15 | 1 | 454.0 | |
| | 14 | ○ | [CLF]C:498 | Ligand | 15 | 1 | 15 | 1 | 453.9 | |
| | | | | *Average:* | 15 | 1 | 15 | 1 | 453.9 | |
| 7 | 15 | ○ | [CA]A:499 | Ligand | 1 | 1 | 1 | 1 | 84.9 | |
| | 16 | ○ | [CA]C:499 | Ligand | 1 | 1 | 1 | 1 | 84.9 | |
| | | | | *Average:* | 1 | 1 | 1 | 1 | 84.9 | |
| 8 | 17 | ○ | [FS4]E:290 | Ligand | 8 | 1 | 8 | 1 | 304.4 | |
| | 18 | ○ | [FS4]G:290 | Ligand | 8 | 1 | 8 | 1 | 304.4 | |

For chain A, which represents the Molybdenum-iron protein, there are total 478 amino acids in the protein chain and 416 of them are surface exposed residues.

The solvent accessible area for this protein 19473.3 Å$^2$ and the solvation energy for folding (ΔG) is -441.1 Kcal/M.

Similarly for chain E which represents the iron protein, there are total 274 amino acids and out of those 235 amino acids are present on the surface of the protein. The solvent accessible surface area 11703.4Å$^2$ and energy of solvation (ΔG) for this structure is -268 kcal/M.

You can also view the individual protein chain by click on the letter (A, B, C, D, E, F, G, H) corresponding to the protein chain.

**Identifying the amino acid residues involved in interaction:**

Click on the link which is represented as a number in the results page. In our example it is **1** for chain **A** and **5** for chain **E**.

Click on link 1 for chain A. This will take you to the following page, where you get residue by residue solvent accessibility information.

**Solvent accessibility** (¤ interface engaged in PQS[1])

Inaccessible residues  Solvent-accessible residues  Interfa

| ## | Monomer A | ASA, Å$^2$ | 2¤ | 7¤ | 10¤ |
|---|---|---|---|---|---|
| 1 | A:MET 4 | 82.86 | 0.00 | 0.00 | 0.00 |
| 2 | A:SER 5 | 42.94 | 0.00 | 0.00 | 0.00 |
| 3 | A:ARG 6 | 68.41 | 0.00 | 0.00 | 0.00 |
| 4 | A:GLU 7 | 111.20 | 0.00 | 0.00 | 0.00 |
| 5 | A:GLU 8 | 94.55 | 0.00 | 0.00 | 0.00 |
| 6 | A:VAL 9 | 3.52 | 0.00 | 0.00 | 0.00 |
| 7 | A:GLU 10 | 80.74 | 0.00 | 0.00 | 0.00 |
| 8 | A:SER 11 | 55.73 | 0.00 | 0.00 | 0.00 |
| 9 | A:LEU 12 | 9.52 | 0.00 | 0.00 | 0.00 |
| 10 | A:ILE 13 | 5.36 | 0.00 | 0.00 | 0.00 |
| 11 | A:GLN 14 | 81.73 | 0.00 | 0.00 | 0.00 |
| 12 | A:GLU 15 | 94.21 | 0.00 | 0.00 | 0.00 |
| 13 | A:VAL 16 | 5.02 | 0.00 | 0.00 | 0.00 |
| 14 | A:LEU 17 | 2.01 | 0.00 | 0.00 | 0.00 |
| 15 | A:GLU 18 | 133.14 | 0.00 | 0.00 | 0.00 |
| 16 | A:VAL 19 | 86.65 | 47.95 | 0.00 | 0.00 |
| 17 | A:TYR 20 | 15.29 | 6.45 | 0.00 | 0.00 |
| 18 | A:PRO 21 | 95.12 | 68.33 | 0.00 | 0.00 |
| 19 | A:GLU 22 | 113.74 | 0.00 | 0.00 | 0.00 |
| 20 | A:LYS 23 | 165.23 | 41.89 | 0.00 | 0.00 |
| 21 | A:ALA 24 | 11.01 | 10.86 | 0.00 | 0.00 |
| 22 | A:ARG 25 | 73.71 | 0.00 | 0.00 | 0.00 |
| 23 | A:LYS 26 | 121.28 | 0.00 | 0.00 | 0.00 |
| 24 | A:ASP 27 | 26.03 | 0.00 | 0.00 | 0.00 |
| 25 | A:ARG 28 | 3.87 | 0.00 | 0.00 | 0.00 |
| 26 | A:ASN 29 | 44.60 | 0.00 | 0.00 | 0.00 |
| 27 | A:LYS 30 | 84.90 | 0.00 | 0.00 | 0.00 |
| 28 | A:HIS 31 | 2.79 | 0.00 | 0.00 | 0.00 |
| 29 | A:LEU 32 | 1.97 | 0.00 | 0.00 | 0.00 |
| 30 | A:ALA 33 | 0.33 | 0.00 | 0.00 | 0.00 |
| 31 | A:VAL 34 | 6.26 | 0.00 | 0.00 | 0.00 |
| 32 | A:ASN 35 | 0.00 | 0.00 | 0.00 | 0.00 |
| 33 | A:ASP 36 | 44.68 | 0.00 | 0.00 | 0.00 |

All the residues are colour coded depending on their solvent accessibility. The solvent exposed residues are coloured grey, the interface residues are coloured blue and the buried residues are coloured black.

**The Interfaces:**

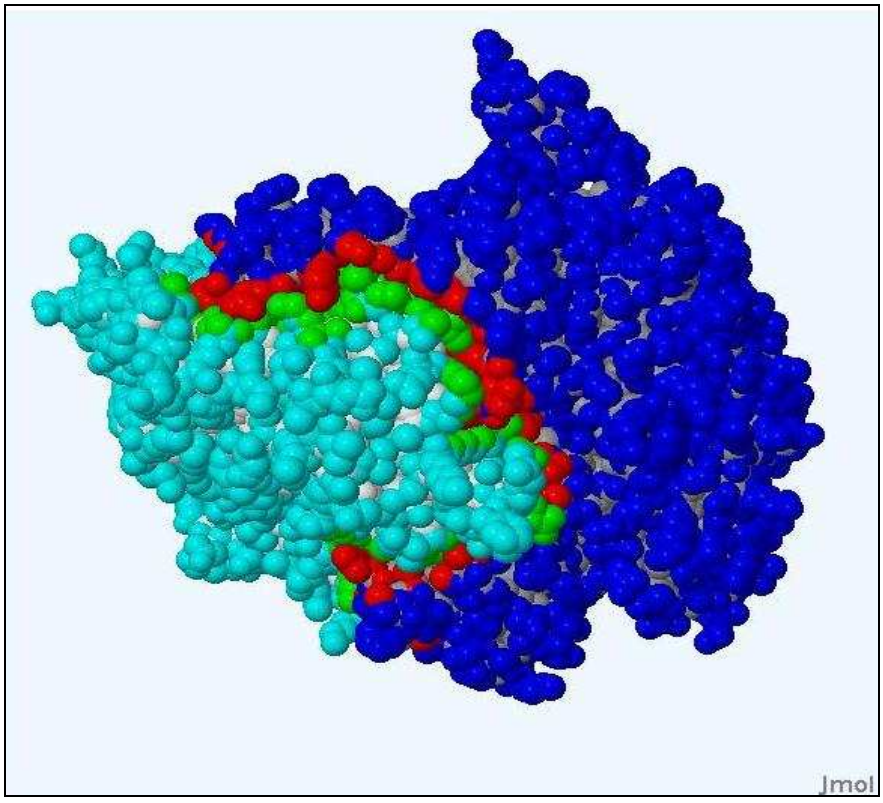
Let us now click at the interface button for this PDB entry 1n2c.

The results page will give us detailed information regarding the interface between two protein chains present in the complex structure.

**Found interfaces**

| ## | | | Structure 1 | | | × | | Interface area, Å$^2$ | $\Delta^iG$ kcal/M | $\Delta^iG$ P-value | $N_{HB}$ | $N_{SB}$ | $N_{DS}$ | CSS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Id | NN | «» | Range | $^iN_{at}$ | $^iN_{res}$ | | Range | | | | | | | |
| 1 | 1 | ◉ | D | 469 | 119 | ◊ | C | 4367.5 | -54.3 | 0.011 | 56 | 18 | 0 | 0.551 |
| | 2 | ○ | B | 474 | 120 | ◊ | A | 4360.2 | -54.4 | 0.010 | 55 | 17 | 0 | 0.551 |
| | | | | | | | | 4363.9 | -54.3 | 0.011 | 56 | 18 | 0 | 0.551 |
| 2 | 3 | ○ | D | 322 | 77 | ◊ | B | 2872.0 | -21.6 | 0.430 | 46 | 32 | 0 | 0.724 |
| 3 | 4 | ○ | H | 251 | 66 | ◊ | G | 2335.1 | -12.4 | 0.400 | 31 | 15 | 0 | 0.302 |
| | 5 | ○ | F | 248 | 66 | ◊ | E | 2321.0 | -12.4 | 0.398 | 31 | 14 | 0 | 0.302 |
| | | | | | | | | 2328.0 | -12.4 | 0.399 | 31 | 15 | 0 | 0.302 |
| 4 | 6 | ○ | B | 140 | 37 | ◊ | C | 1288.3 | -15.5 | 0.177 | 17 | 6 | 0 | 0.306 |
| | 7 | ○ | D | 137 | 36 | ◊ | A | 1283.3 | -15.3 | 0.181 | 16 | 7 | 0 | 0.306 |
| | | | | | | | | 1285.8 | -15.4 | 0.179 | 17 | 7 | 0 | 0.306 |
| 5 | 8 | ○ | G | 69 | 19 | ◊ | D | 554.0 | 1.5 | 0.724 | 5 | 3 | 0 | 0.004 |
| | 9 | ○ | E | 71 | 19 | ◊ | B | 549.2 | 1.4 | 0.703 | 5 | 4 | 0 | 0.004 |
| | | | | | | | | 551.6 | 1.4 | 0.713 | 5 | 4 | 0 | 0.004 |
| 6 | 10 | ○ | F | 61 | 19 | ◊ | A | 514.0 | -1.5 | 0.599 | 4 | 3 | 0 | 0.027 |
| | 11 | ○ | H | 60 | 19 | ◊ | C | 506.8 | -1.8 | 0.576 | 4 | 5 | 0 | 0.027 |
| | | | | | | | | 510.4 | -1.7 | 0.588 | 4 | 4 | 0 | 0.027 |
| 7 | 12 | ○ | F | 52 | 16 | ◊ | B | 417.6 | -7.8 | 0.088 | 4 | 2 | 0 | 0.030 |
| | 13 | ○ | H | 51 | 17 | ◊ | D | 405.0 | -7.5 | 0.095 | 5 | 1 | 0 | 0.030 |
| | | | | | | | | 411.3 | -7.6 | 0.091 | 5 | 2 | 0 | 0.030 |
| 8 | 14 | ○ | G | 49 | 15 | ◊ | C | 392.9 | -6.7 | 0.112 | 4 | 2 | 0 | 0.056 |
| | 15 | ○ | E | 48 | 15 | ◊ | A | 390.0 | -6.7 | 0.109 | 4 | 2 | 0 | 0.056 |

In the above example, between chains C and D there are 18 saltbridges and 56 H-bonding interactions.




If you click on the link highlighted in red, you can visualize the interface region between the protein chains.

The interface region is highlighted in red and green in the above picture.

For information regarding the specific residues involved in complex formation click on the link under column name **NN** (highlighted in green in the above figure).

**Hydrogen bonds**

| ## | Structure 1 | Dist. [Å] | Structure 2 |
|---|---|---|---|
| 1 | D:ASN 137[ ND2] | 2.90 | C:PRO 54[ O ] |
| 2 | D:TYR 142[ OH ] | 2.81 | C:LEU 56[ O ] |
| 3 | D:TYR 142[ OH ] | 3.81 | C:MET 57[ O ] |
| 4 | D:ARG 100[ NH1] | 3.90 | C:THR 58[ O ] |
| 5 | D:GLN 93[ NE2] | 3.24 | C:GLY 61[ O ] |
| 6 | D:TYR 447[ OH ] | 3.30 | C:GLN 90[ O ] |
| 7 | D:CYS 70[ SG ] | 2.79 | C:TYR 91[ OH ] |
| 8 | D:CYS 70[ N ] | 3.21 | C:TYR 91[ OH ] |
| 9 | D:LYS 34[ NZ ] | 3.73 | C:ILE 101[ O ] |
| 10 | D:ARG 453[ NH2] | 3.46 | C:THR 104[ O ] |
| 11 | D:ASN 65[ N ] | 2.83 | C:ASN 113[ O ] |
| 12 | D:THR 63[ N ] | 2.99 | C:THR 115[ O ] |
| 13 | D:LYS 68[ NZ ] | 3.02 | C:ASP 117[ OD1] |
| 14 | D:LYS 68[ NZ ] | 2.90 | C:ASP 117[ OD2] |
| 15 | D:HIS 396[ NE2] | 3.80 | C:ASP 117[ OD2] |
| 16 | D:LEU 62[ N ] | 2.95 | C:GLU 137[ OE1] |
| 17 | D:ALA 61[ N ] | 3.23 | C:GLU 137[ OE1] |
| 18 | D:ALA 61[ N ] | 3.10 | C:GLU 137[ OE2] |
| 19 | D:GLU 60[ N ] | 2.93 | C:GLU 137[ OE2] |
| 20 | D:TYR 52[ OH ] | 3.02 | C:LEU 141[ O ] |
| 21 | D:SER 92[ OG ] | 3.18 | C:CYS 154[ SG ] |
| 22 | D:GLU 120[ N ] | 3.04 | C:PHE 186[ O ] |
| 23 | D:GLN 93[ NE2] | 2.79 | C:VAL 189[ O ] |
| 24 | D:LYS 27[ NZ ] | 2.90 | C:GLU 261[ OE1] |
| 25 | D:LYS 27[ NZ ] | 2.98 | C:GLU 261[ OE2] |
| 26 | D:SER 2[ N ] | 3.85 | C:TYR 331[ OH ] |
| 27 | D:GLN 3[ N ] | 3.14 | C:GLU 334[ OE1] |
| 28 | D:SER 2[ OG ] | 3.17 | C:GLU 334[ OE2] |
| 29 | D:ARG 100[ NH2] | 3.70 | C:LYS 426[ O ] |
| 30 | D:ASP 266[ N ] | 3.70 | C:LYS 433[ O ] |
| 31 | D:GLN 268[ N ] | 3.03 | C:LYS 433[ O ] |

**Salt bridges**

| ## | Structure 1 | Dist. [Å] | Structure 2 |
|---|---|---|---|
| 1 | D:LYS 68[ NZ ] | 3.02 | C:ASP 117[ OD1] |
| 2 | D:LYS 68[ NZ ] | 2.90 | C:ASP 117[ OD2] |
| 3 | D:HIS 396[ NE2] | 3.80 | C:ASP 117[ OD2] |
| 4 | D:LYS 27[ NZ ] | 2.90 | C:GLU 261[ OE1] |
| 5 | D:LYS 27[ NZ ] | 2.98 | C:GLU 261[ OE2] |
| 6 | D:SER 2[ N ] | 3.41 | C:ASP 454[ OD1] |
| 7 | D:SER 2[ N ] | 3.36 | C:ASP 454[ OD2] |
| 8 | D:GLU 32[ OE1] | 2.90 | C:LYS 76[ NZ ] |
| 9 | D:GLU 32[ OE2] | 3.01 | C:LYS 76[ NZ ] |
| 10 | D:GLU 33[ OE1] | 3.16 | C:ARG 210[ NH2] |
| 11 | D:GLU 33[ OE1] | 3.53 | C:LYS 146[ NZ ] |
| 12 | D:GLU 33[ OE2] | 2.99 | C:ARG 210[ NH1] |
| 13 | D:GLU 33[ OE2] | 3.20 | C:ARG 210[ NH2] |
| 14 | D:GLU 109[ OE1] | 2.71 | C:LYS 433[ NZ ] |
| 15 | D:GLU 109[ OE2] | 3.37 | C:LYS 433[ NZ ] |
| 16 | D:ASP 121[ OD1] | 3.49 | C:LYS 51[ NZ ] |
| 17 | D:ASP 133[ OD1] | 3.93 | C:LYS 23[ NZ ] |
| 18 | D:ASP 133[ OD2] | 3.01 | C:LYS 23[ NZ ] |

**Disulfide bonds**

| ## | Structure 1 | Dist. [Å] | Structure 2 |
|---|---|---|---|
| No disulfide bonds found | | | |

**Covalent bonds**

| ## | Structure 1 | Dist. [Å] | Structure 2 |
|---|---|---|---|
| No covalent bonds found | | | |

In addition to the saltbridge and H-bonding interactions between the residues, the results page also provides information about Buried and accessible surface areas and solvation energies of the interfacing residues.

**Interfacing residues** (not a contact table)

Display level: Residues

Inaccessible residues
Solvent-accessible residues

HSDC

**ASA** Accessible Surface Area, Å² **BSA** Buried Surface Area, Å² $\Delta^iG$

| ## | Structure 1 | HSDC | ASA | BSA | $\Delta^iG$ |
|---|---|---|---|---|---|
| 1 | D:SER 2 | HS | 109.18 | 72.80 | -0.13 |
| 2 | D:GLN 3 | H | 79.30 | 71.64 | 0.21 |
| 3 | D:GLN 4 | | 93.12 | 0.00 | 0.00 |
| 4 | D:VAL 5 | | 129.40 | 89.67 | 1.21 |
| 5 | D:ASP 6 | | 107.81 | 10.57 | 0.14 |
| 6 | D:LYS 7 | | 154.14 | 0.00 | -0.00 |
| 7 | D:ILE 8 | H | 123.53 | 101.65 | 1.34 |
| 8 | D:LYS 9 | | 54.02 | 0.74 | -0.01 |
| 9 | D:ALA 10 | | 69.36 | 45.43 | 0.69 |
| 10 | D:SER 11 | | 72.64 | 52.55 | 0.17 |
| 11 | D:TYR 12 | | 170.20 | 0.00 | -0.00 |
| 12 | D:PRO 13 | | 53.69 | 0.00 | -0.00 |
| 13 | D:LEU 14 | | 21.83 | 21.83 | 0.35 |
| 14 | D:PHE 15 | | 93.51 | 71.76 | 1.15 |
| 15 | D:LEU 16 | | 94.22 | 0.00 | 0.00 |
| 16 | D:ASP 17 | | 38.02 | 0.00 | -0.00 |
| 17 | D:GLN 18 | | 118.45 | 0.00 | -0.00 |
| 18 | D:ASP 19 | | 92.27 | 28.19 | -0.29 |
| 19 | D:TYR 20 | H | 53.43 | 53.43 | -0.01 |
| 20 | D:LYS 21 | | 102.43 | 0.00 | 0.00 |
| 21 | D:ASP 22 | | 60.06 | 0.00 | 0.00 |

All the residues in this table are colour coded depending on their solvent accessibility (grey-surface exposed, black – buried, light blue – interfacing residues). The color red represents the residues which are involved in **H**ydrogen/**D**isulphide bond, **S**alt bridge or **C**ovalent interactions.

**Assemblies:**

In order to get the quaternary structure information for 1N2C click on the "assemblies" button.

Analysis of complex represented *As Is* by PDB entry is found here.

**Analysis of protein interfaces suggests that the following quaternary structures are stable in solution**

| PQS set NN | PQS set «» | mm Size | Formula | Composition | Id | Stable | Surface area, sq. Å | Buried area, sq. Å | $\Delta G^{int}$, kcal/M | $\Delta G^{diss}$, kcal/M |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ◉ | 8 | $A_2B_2C_4d_2e_2f_2g_2h_2i_4j_4k_4$ | ACBDEFGH[HCA]$_2$[CFM]$_2$[CLF]$_2$[CA]$_2$[FS4]$_2$[ADP]$_4$[MG]$_4$[ALF]$_4$ | 1 | yes | 84866.0 | 55490.3 | -450.7 | 28.1 |
| 2 | ○ | 4 | $ABC_2defghi_2j_2k_2$ | CBGH[HCA][CFM][CLF][CA][FS4][ADP]$_2$[MG]$_2$[ALF]$_2$ | 2 | yes | 56472.6 | 13690.3 | -131.9 | 1.2 |
| | ○ | 4 | $ABC_2defghi_2j_2k_2$ | ADEF[HCA][CFM][CLF][CA][FS4][ADP]$_2$[MG]$_2$[ALF]$_2$ | 2 | yes | 56529.3 | 13664.5 | -131.6 | 0.5 |
| 3 | ○ | 4 | $A_2B_2c_2d_2e_2f_2$ | ACBD[HCA]$_2$[CFM]$_2$[CLF]$_2$[CA]$_2$ | 3 | yes | 56944.1 | 32516.1 | -299.1 | 91.2 |
| | ○ | 2 | $A_2bc_2d_2e_2$ | GH[FS4][ADP]$_2$[MG]$_2$[ALF]$_2$ | 4 | yes | 17843.1 | 7621.0 | -55.9 | 25.3 |
| | ○ | 2 | $A_2bc_2d_2e_2$ | EF[FS4][ADP]$_2$[MG]$_2$[ALF]$_2$ | 4 | yes | 17839.8 | 7592.5 | -56.0 | 25.3 |
| 4 | ○ | 3 | $AB_2cdefg_2h_2i_2$ | CGH[HCA][CFM][CLF][FS4][ADP]$_2$[MG]$_2$[ALF]$_2$ | 5 | yes | 35180.3 | 11017.5 | -108.5 | 1.9 |
| | ○ | 3 | $AB_2cdefg_2h_2i_2$ | AEF[HCA][CFM][CLF][FS4][ADP]$_2$[MG]$_2$[ALF]$_2$ | 5 | yes | 35200.0 | 11001.4 | -108.2 | 1.2 |
| | ○ | 2 | $A_2b_2$ | BD[CA]$_2$ | 6 | yes | 42021.6 | 5935.8 | -39.4 | 39.4 |
| | ○ | 2 | $A_2bc_2d_2e_2$ | GH[FS4][ADP]$_2$[MG]$_2$[ALF]$_2$ | 4 | yes | 17843.1 | 7621.0 | -55.9 | 25.3 |

For this entry the proposed quaternary structure by **PISA** is a hetero-octamer which is already present as a stable assembly in the PDB file. In the **assemblies** result page **PISA** also gives information about the buried and accessible surface area, and free energy of solvation gained upon the formation of the entire complex structures.

Therefore using PISA you can get valuable information about the type of complexes that can be formed based on chemical stability and crystal contacts. The residue by residue information provided by PISA can be used to identify the amino acids that are crucial to the formation of stable complexes which can be biologically relevant.