Protein Interactions: Computational Techniques

BE21B037 - Assignment Part B

1. Consider the protein-protein complex, human growth hormone receptor-human growth hormone (HGH) analyze the following:

a. Find the complex structure and functional importance from PDB

PDB ID: 1A22

Functional Importance of 1A22:

- Plays an important role in growth control.
- Its major role in stimulating body growth is to stimulate the liver and other tissues to secrete IGF-1. It stimulates both the differentiation and proliferation of myoblasts.
- It also stimulates amino acid uptake and protein synthesis in muscle and other tissues.

b. What is the structural type of the complex (homo/hetero/dimer/trimer etc.)

1A22 is a hetero dimer complex

c. Obtain binding affinity data for the complex from SKEMPI, PROXiMATE and PDBBind database

SKEMPI

Browse the database

Search by PDB code or protein name:

Records per page: 10 PDB Database to use: PDBe RCSB PDBj

Search Clear

251 records found

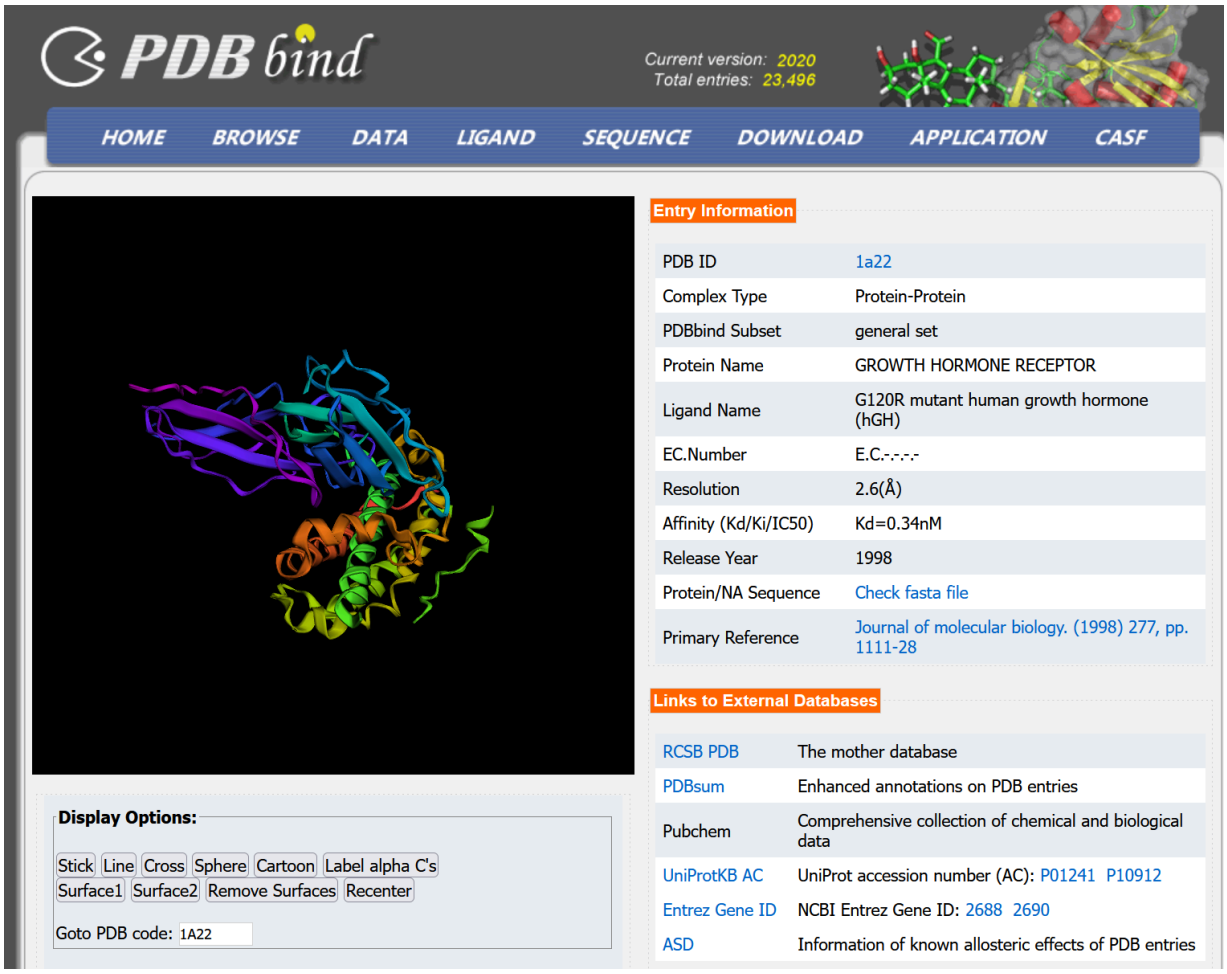
PDB Code	Mutation Pdb	Mutation Cleaned	Location	Hold Out Type	Hold Out Proteins	Affinity Mut	Affinity Wt	Ref.	Protein 1	Protein 2	T (°K)	Kon Mut	Kon Wt	Koff Mut	Koff Wt	Dh Mut	Dh Wt	Ds Mut	Ds Wt	Method
1A22_A_B	MA14A	MA14A	INT		1A22_A_B,1BP3_A_B	9E-10	9E-10		Human growth hormone	hGH binding protein	298	3E+05	3E+05	3.24E-04	2.7E-04	None	None	None	None	SPR
1A22_A_B	HA18A	HA18A	COR		1A22_A_B,1BP3_A_B	3.96E-10	9E-10		Human growth hormone	hGH binding protein	298	2.73E+05	3E+05	1.11E-04	2.7E-04	None	None	None	None	SPR
1A22_A_B	HA21A	HA21A	SUP		1A22_A_B,1BP3_A_B	1.17E-09	9E-10		Human growth hormone	hGH binding protein	298	3E+05	3E+05	3.51E-04	2.7E-04	None	None	None	None	SPR
1A22_A_B	QA22A	QA22A	RIM		1A22_A_B,1BP3_A_B	6.21E-10	9E-10		Human growth hormone	hGH binding protein	298	2.73E+05	3E+05	1.67E-04	2.7E-04	None	None	None	None	SPR
1A22_A_B	FA25A	FA25A	COR		1A22_A_B,1BP3_A_B	4.23E-10	9E-10		Human growth hormone	hGH binding protein	298	3E+05	3E+05	1.27E-04	2.7E-04	None	None	None	None	SPR
1A22_A_B	DA26A	DA26A	SUR		1A22_A_B,1BP3_A_B	6.3E-10	9E-10		Human growth hormone	hGH binding protein	298	3.37E+05	3E+05	2.13E-04	2.7E-04	None	None	None	None	SPR
1A22_A_B	QA29A	QA29A	SUR		1A22_A_B,1BP3_A_B	3.33E-10	9E-10		Human growth hormone	hGH binding protein	298	3.09E+05	3E+05	1.03E-04	2.7E-04	None	None	None	None	SPR
1A22_A_B	YA42A	YA42A	COR		1A22_A_B,1BP3_A_B	1.26E-09	9E-10		Human growth hormone	hGH binding protein	298	2.5E+05	3E+05	3.24E-04	2.7E-04	None	None	None	None	SPR
1A22_A_B	LA45A	LA45A	COR		1A22_A_B,1BP3_A_B	7.11E-09	9E-10		Human growth hormone	hGH binding protein	298	1.67E+05	3E+05	1.16E-03	2.7E-04	None	None	None	None	SPR
1A22_A_B	QA46A	QA46A	RIM		1A22_A_B,1BP3_A_B	1.08E-09	9E-10		Human growth hormone	hGH binding protein	298	2.14E+05	3E+05	2.43E-04	2.7E-04	None	None	None	None	SPR

1 2 3 4 5 > >>

PROXiMATE

S.no.	PDB	Chains	Protein 1	Protein 2	Functional Class	Experimental Technique	Ionic Conditions	Temperature	pH	Pubmed/ Location	Dissociation constant (M)	ΔG (kcal/ mol)	Notes
125	1A22	A:B	Human growth hormone/ somatotropin (hGH)	Human growth hormone receptor (hGHR)	HR	Competitive displacement of ^{125}I-labeled hGH	50 mM Tris-HCl, 10 mM $CaCl_2$, 0.1% BSA, 0.02% NaN_3	298	7.4	2014261 (Table 2, p. 3409)	4.60E-10	-12.73	
126	1A22	A:B	Human growth hormone/ somatotropin (hGH)	Human growth hormone receptor (hGHR)	HR	Competitive displacement of ^{125}I-labeled hGH	50 mM Tris-HCl, 10 mM $CaCl_2$, 0.1% BSA, 0.02% NaN_3, 50 µM $ZnCl_2$, 10 mM $MgCl_2$	298	7.4	2014261 (Table 4, p. 3409)	4.40E-10	-12.76	
127	1A22	A:B	Human growth hormone/ somatotropin (hGH)	Human growth hormone receptor (hGHR)	HR	Competitive displacement of ^{125}I-labeled hGH	50 mM Tris-HCl, 10 mM $CaCl_2$, 1 g/L BSA, 0.2 g/L NaN_3	298	7.4	2034689 (Table 1, p. 4499)	4.00E-10	-12.81	
128	1A22	A:B	Human growth hormone/ somatotropin (hGH)	Human growth hormone receptor (hGHR)	HR	Competitive displacement of ^{125}I-labeled hGH	50 mM Tris-HCl, 10 mM $CaCl_2$, 1 g/L BSA, 0.2 g/L NaN_3	298	7.4	2471267 (Table 1, p. 1082)	3.40E-10	-12.91	
129	1A22	A:B	Human growth hormone/ somatotropin (hGH)	Human growth hormone receptor (hGHR)	HR	Surface Plasmon Resonance	150 mM NaCl, 10 mM sodium phosphate, 0.02% Tween 20	298	7.4	7504735 (Tables 1 and 2, pp. 556-7)	9.00E-10	-12.33	
130	1A22	A:B	Human growth hormone/ somatotropin (hGH)	Human growth hormone receptor (hGHR)	HR	Competitive Binding	50mM Tris-HCl, 10mM $CaCl_2$, 1g/L bovine albumin, 0.2 g/L NaN_3	298	7.4	7529940 (Figure 2, p. 384)	9.63E-10	-12.29	
131	1A22	A:B	Human growth hormone/ somatotropin (hGH)	Human growth hormone receptor (hGHR)	HR	Surface Plasmon Resonance	PBS with 10mM sodium phosphate, 137mM NaCl, 2.7mM KCl, 0.05% Tween 20	298	7.2	8756685 (Table 2, p. 10303)	2.90E-09	-11.64	
132	1A22	A:B	Human growth hormone/ somatotropin (hGH)	Human growth hormone receptor (hGHR)	HR	competitive displacement of hGH from hGHbp, using ^{125}I-labeled hGH as a tracer	PBS with 0.02% (v/v) Tween 20	298		9571026 (Table 1, p. 1118)	3.40E-10	-12.91	

PDBBind



d. Access PROXiMATE database and check the number of mutations for the complex with experimentally known binding free energy (ΔΔG).

SEARCH RESULTS

You have searched for: 1A22
Found **261 entries** matching your search criteria. Click here to download all results.

1 2

Entry	PDB	Mutation(s)	Protein 1	Protein 2	Pubmed/ Reference	Wild-type K_D (M)	Mutant K_D (M)	Wild-type ΔG (kcal/ mol)	ΔΔG (kcal/ mol)	Secondary Structure	Relative Accessibility
3964	1A22	A:F10A	Human growth hormone/ somatotropin (hGH) P01241	Human growth hormone receptor (hGHR) P10912	2014261 (Table 2, p. 3409)	4.60E-10	2.71E-09	-12.73	1.05	A:F10A=Alpha-helix	A:F10A=0.00
3965	1A22	A:N12A	Human growth hormone/ somatotropin (hGH) P01241	Human growth hormone receptor (hGHR) P10912	2014261 (Table 2, p. 3409)	4.60E-10	5.52E-10	-12.73	0.11	A:N12A=Alpha-helix	A:N12A=0.29
3966	1A22	A:L15A	Human growth hormone/ somatotropin (hGH) P01241	Human growth hormone receptor (hGHR) P10912	2014261 (Table 2, p. 3409)	4.60E-10	5.98E-10	-12.73	0.16	A:L15A=Alpha-helix	A:L15A=0.68
3967	1A22	A:R16A	Human growth hormone/ somatotropin (hGH) P01241	Human growth hormone receptor (hGHR) P10912	2014261 (Table 2, p. 3409)	4.60E-10	6.90E-10	-12.73	0.24	A:R16A=Alpha-helix	A:R16A=0.21
3968	1A22	A:H18A	Human growth hormone/ somatotropin (hGH) P01241	Human growth hormone receptor (hGHR) P10912	2014261 (Table 2, p. 3409)	4.60E-10	7.36E-10	-12.73	0.28	A:H18A=Alpha-helix	A:H18A=0.12
3969	1A22	A:R19A	Human growth hormone/ somatotropin (hGH) P01241	Human growth hormone receptor (hGHR) P10912	2014261 (Table 2, p. 3409)	4.60E-10	3.22E-10	-12.73	-0.21	A:R19A=Alpha-helix	A:R19A=0.30
3970	1A22	A:H21A	Human growth hormone/ somatotropin (hGH) P01241	Human growth hormone receptor (hGHR) P10912	2014261 (Table 2, p. 3409)	4.60E-10	1.52E-10	-12.73	-0.66	A:H21A=Alpha-helix	A:H21A=0.01
3971	1A22	A:F25A	Human growth hormone/ somatotropin (hGH) P01241	Human growth hormone receptor (hGHR) P10912	2014261 (Table 2, p. 3409)	4.60E-10	2.90E-10	-12.73	-0.27	A:F25A=Alpha-helix	A:F25A=0.14

261 entries with known binding free energies

e. Identify the mutation, which has the most favorable (highest negative value) and last favorable (highest positive energy value) binding energy

Copying the database and sorting based on the binding energy gives us the most negative value of -0.92 kcal/mol

Entry	PDB	Mutation(s)	Protein 1	Protein 2	Pubmed/ Reference	Wild-type K_D (M)	Mutant K_D (M)	Wild-type ΔG (kcal/ mol)	ΔΔG (kcal/ mol)	Secondary Structure	Relative Accessibility
4558	1A22	A:E174A	Human growth hormone/ somatotropin (hGH) P01241	Human growth hormone receptor (hGHR) P10912	7504735 (Tables 1 and 2, pp. 556-7)	9.00E-10	1.89E-10	-12.33	-0.92	A:E174A=Alpha-helix	A:E174A=0.11

And the least favorable value as 3.78 kcal/mol

Entry	PDB	Mutation(s)	Protein 1	Protein 2	Pubmed/ Reference	Wild-type K_D (M)	Mutant K_D (M)	Wild-type ΔG (kcal/ mol)	ΔΔG (kcal/ mol)	Secondary Structure	Relative Accessibility
4066	1A22	A:K172A, A:F176A	Human growth hormone/ somatotropin (hGH) P01241	Human growth hormone receptor (hGHR) P10912	2014261 (Table 4, p. 3409)	4.40E-10	2.60E-07	-12.76	3.78	A:K172A=Alpha-helix A:F176A=Alpha-helix	A:K172A=0.04 A:F176A=0.00

f. Group the mutations into (i) nonpolar to nonpolar, (ii) nonpolar to polar and (iii) polar to polar and obtain average binding energy [nonpolar: A, C, F, G, I, L, M, V, W, Y]

1A22	A:E174A	-0.92	A	E	A	Polar	Nonpolar
1A22	A:E174A	-0.9	A	E	A	Polar	Nonpolar
1A22	A:E174A	-0.9	A	E	A	Polar	Nonpolar
1A22	A:K168A	-0.77	A	K	A	Polar	Nonpolar
1A22	B:V371A	-0.7	B	V	A	Nonpolar	Nonpolar
1A22	A:H21A	-0.66	A	H	A	Polar	Nonpolar
1A22	B:V371A	-0.62	B	V	A	Nonpolar	Nonpolar
1A22	A:Q29A	-0.59	A	Q	A	Polar	Nonpolar
1A22	B:Q274A	-0.58	B	Q	A	Polar	Nonpolar
1A22	B:S299A	-0.51	B	S	A	Polar	Nonpolar
1A22	A:H18A	-0.49	A	H	A	Polar	Nonpolar
1A22	A:E65A	-0.47	A	E	A	Polar	Nonpolar
1A22	A:F25A	-0.45	A	F	A	Nonpolar	Nonpolar
1A22	B:N272A	-0.44	B	N	A	Polar	Nonpolar
1A22	B:T273A	-0.44	B	T	A	Polar	Nonpolar
1A22	A:F25A	-0.43	A	F	A	Nonpolar	Nonpolar
1A22	B:Q278A	-0.41	B	Q	A	Polar	Nonpolar
1A22	B:S298A	-0.33	B	S	A	Polar	Nonpolar
1A22	A:E65A	-0.31	A	E	A	Polar	Nonpolar
1A22	A:E65A	-0.31	A	E	A	Polar	Nonpolar
1A22	A:F25A	-0.27	A	F	A	Nonpolar	Nonpolar
1A22	B:N297A	-0.26	B	N	A	Polar	Nonpolar

After Grouping the average binding energy for each of the three cases are

(i) nonpolar to nonpolar (62 mutations)

Average Binding Energy =0.602096774 kcal/mol

(ii) nonpolar to polar (2 mutations)

Average Binding Energy = -0.04 kcal/mol

(iii) polar to polar(4 mutations)

Average Binding Energy = 0.8925 kcal/mol

g. Obtain the average binding energy at different secondary structures of the mutant,helix, strand and coil

The following are the average binding energies for different secondary structures of the mutant

1) Alpha-helix - 90 entries = 00.477888889 kcal/mol
2) 3/10 - helix = 0.685 kcal/mol
3) Turn = 0.087857143 kcal/mol
4) Bend = 0.6488 kcal/mol
5) Beta-bridge = -0.05 kcal/mol
6) Loop/irregular= 0.409361702 kcal/mol
7) Strand = 0.381025641 kcal/mol

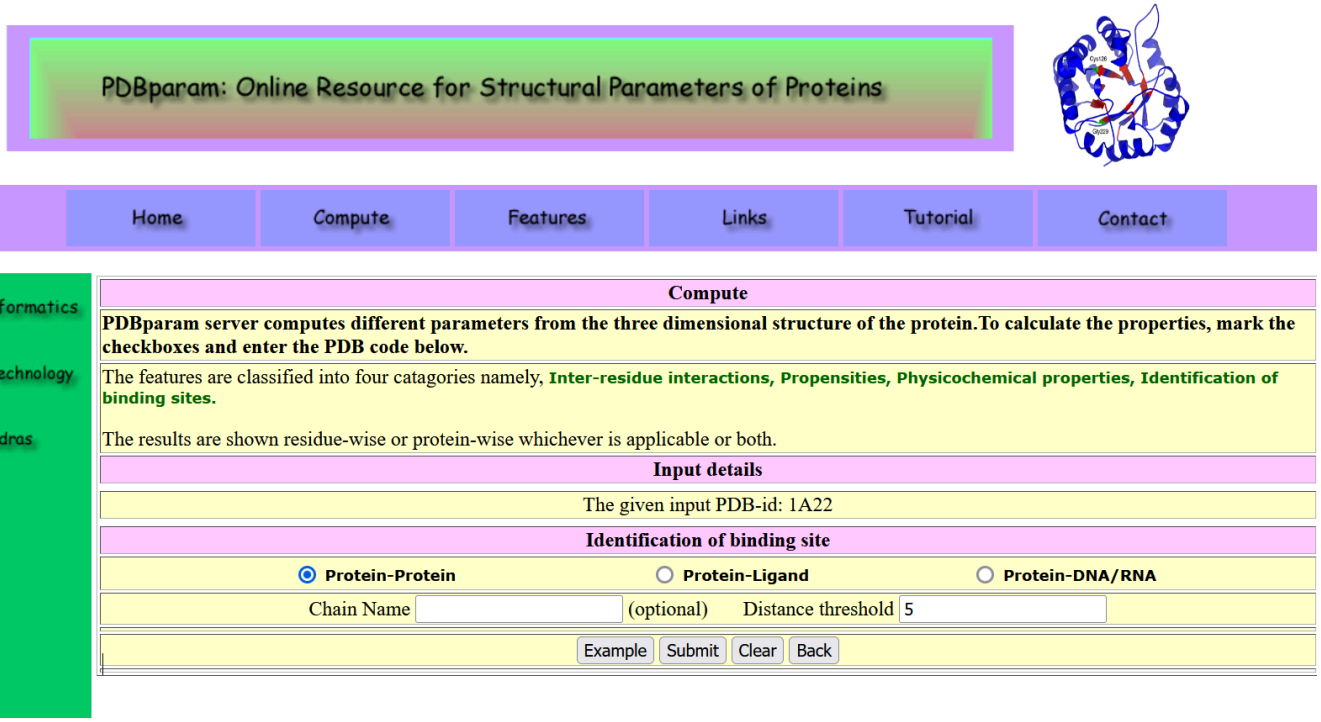
Helix = ⅓ (alpha-helix, 3/10 helix, turn(helix-turn)) = 0.477888889 + 0.685 + 0.087857143= 1.250746032 / 3 = 0.417

h. Repeat the same for different ranges of relative ASA, 0-2%, 2-50%, >50%

The following are the average binding energies for different ranges of relative ASA of the mutant

Relative ASA 0-2% - 0.815686275 kcal/mol
Relative ASA 2-50% - 0.47281768 kcal/mol
Relative ASA >50% - 0.04804878 kcal/mol

i. Identify the binding site residues (interface) using distance based criterion (use PDBparam) using a cut off of 5A



The unique interacting residues include

Residue	No	Residue	No	Residue	No
HIS	18	ARG	167	TRP	304
HIS	21	LYS	168	ILE	305
GLN	22	ASP	171	PRO	306
PHE	25	LYS	172	CYS	308
TYR	28	GLU	174	GLU	320
LYS	41	THR	175	LYS	321
TYR	42	PHE	176	CYS	322
LEU	45	ARG	178	PHE	323
GLN	46	ILE	179	SER	324
ASN	47	CYS	182	ASP	326
PRO	48	CYS	189	GLU	327
SER	51	ARG	243	ASP	364
LEU	52	GLU	244	ILE	365
GLU	56	ARG	271	GLN	366
PRO	61	THR	273	LYS	367
SER	62	GLN	274	GLY	368
ASN	63	GLU	275	TRP	369
ARG	64	TRP	276	VAL	371
GLU	65	SER	298	ARG	417
THR	67	THR	301	ASN	418
GLN	68	SER	302	SER	419
TYR	164	ILE	303	GLY	420

j. Identify the binding site residues (interface) using ASA based criterion (use GETAREA or DSSP for ASA values).

The binding sites are identified using the formula

If change in ASA before and after binding is > 0.1 $Å^2$

The following are the residues found

Residue1	No1	Residue2	No2
HIS	18	GLU	244
HIS	21	ARG	270
GLN	22	ARG	271
PHE	25	THR	273
TYR	28	GLN	274
LYS	41	GLU	275
TYR	42	TRP	276
LEU	45	TRP	280
GLN	46	SER	298
PRO	48	SER	299
GLN	49	THR	301
SER	51	SER	302
LEU	52	ILE	303
GLU	56	TRP	304
PRO	61	ILE	305
SER	62	PRO	306
ASN	63	CYS	308
ARG	64	GLU	320
GLU	65	LYS	321
THR	67	CYS	322
GLN	68	PHE	323
TYR	164	SER	324
ARG	167	ASP	326
LYS	168	GLU	327
ASP	171	ASP	364
LYS	172	ILE	365
GLU	174	GLN	366
THR	175	LYS	367
PHE	176	GLY	368
ARG	178	TRP	369
ILE	179	MET	370
CYS	182	VAL	371
ARG	183	THR	395

CYS	189	ARG	417
GLY	190	ASN	418
PHE	191	SER	419
ARG	243	GLY	420

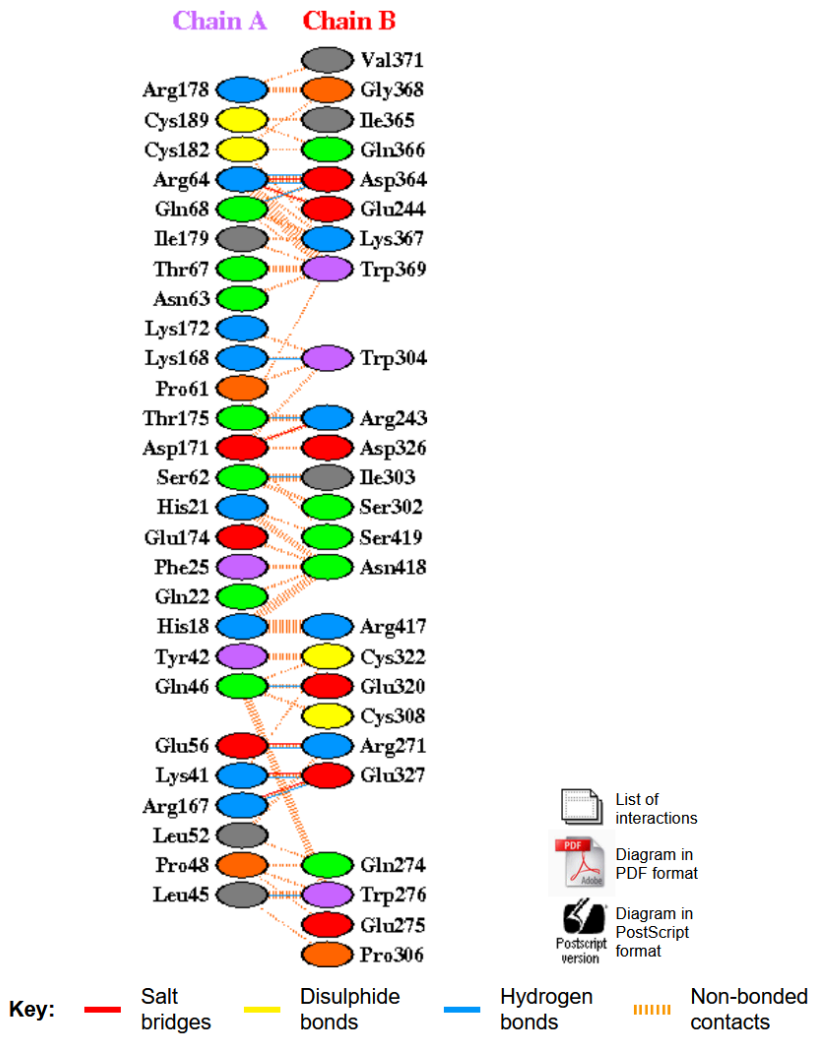
Yellow cells represent the A chain.

k. Check the common interface residues listed in PDBsum, distance and ASA based criteria
PDBsum gives the following reason

Interface statistics

Chain	No. of interface residues	Interface area (Å^2)	No. of salt bridges	No. of disulphide bonds	No. of hydrogen bonds	No. of non-bonded contacts
A	27	1308	6	-	11	155
B	25	1333				

Residue interactions across interface
Coloured by residue type



PDB code: 1a22 Chains A }{ B

Hydrogen bonds

	<----- A T O M 1 ----->					<----- A T O M 2 ----->				
	Atom no.	Atom name	Res name	Res no.	Chain		Atom no.	Atom name	Res name	Res no.	Chain	Distance
1.	351	NZ	LYS	41	A	<-->	2170	OE2	GLU	327	B	3.04
2.	384	O	LEU	45	A	<-->	1761	NE1	TRP	276	B	2.65
3.	397	NE2	GLN	46	A	<-->	2114	OE2	GLU	320	B	3.05
4.	474	OE2	GLU	56	A	<-->	1722	NH2	ARG	271	B	2.88
5.	513	O	SER	62	A	<-->	1975	N	ILE	303	B	2.71
6.	531	NE	ARG	64	A	<-->	2434	OD2	ASP	364	B	2.90
7.	534	NH2	ARG	64	A	<-->	2434	OD2	ASP	364	B	3.27
8.	568	NE2	GLN	68	A	<-->	2434	OD2	ASP	364	B	2.98
9.	1277	NH2	ARG	167	A	<-->	2170	OE2	GLU	327	B	3.04
10.	1286	NZ	LYS	168	A	<-->	1986	O	TRP	304	B	3.00
11.	1341	OG1	THR	175	A	<-->	1556	NH2	ARG	243	B	2.65

Non-bonded contacts

	<----- A T O M 1 ----->					<----- A T O M 2 ----->				
	Atom no.	Atom name	Res name	Res no.	Chain		Atom no.	Atom name	Res name	Res no.	Chain	Distance
1.	141	O	HIS	18	A	<-->	2896	OD1	ASN	418	B	3.54
2.	143	CG	HIS	18	A	<-->	2896	OD1	ASN	418	B	3.61
3.	144	ND1	HIS	18	A	<-->	2886	NE	ARG	417	B	3.80
4.	144	ND1	HIS	18	A	<-->	2887	CZ	ARG	417	B	3.73
5.	144	ND1	HIS	18	A	<-->	2889	NH2	ARG	417	B	3.60
6.	144	ND1	HIS	18	A	<-->	2895	CG	ASN	418	B	3.88
7.	144	ND1	HIS	18	A	<-->	2896	OD1	ASN	418	B	2.90
8.	145	CD2	HIS	18	A	<-->	2889	NH2	ARG	417	B	3.80
9.	146	CE1	HIS	18	A	<-->	2886	NE	ARG	417	B	3.29
10.	146	CE1	HIS	18	A	<-->	2887	CZ	ARG	417	B	3.05
11.	146	CE1	HIS	18	A	<-->	2888	NH1	ARG	417	B	3.44
12.	146	CE1	HIS	18	A	<-->	2889	NH2	ARG	417	B	3.27
13.	146	CE1	HIS	18	A	<-->	2895	CG	ASN	418	B	3.61
14.	146	CE1	HIS	18	A	<-->	2896	OD1	ASN	418	B	2.99
15.	146	CE1	HIS	18	A	<-->	2897	ND2	ASN	418	B	3.74
16.	147	NE2	HIS	18	A	<-->	2887	CZ	ARG	417	B	3.41
17.	147	NE2	HIS	18	A	<-->	2888	NH1	ARG	417	B	3.49
18.	147	NE2	HIS	18	A	<-->	2889	NH2	ARG	417	B	3.39
19.	147	NE2	HIS	18	A	<-->	2896	OD1	ASN	418	B	3.69
20.	173	ND1	HIS	21	A	<-->	2894	CB	ASN	418	B	3.50
21.	173	ND1	HIS	21	A	<-->	2896	OD1	ASN	418	B	3.88
22.	175	CE1	HIS	21	A	<-->	2893	O	ASN	418	B	3.38
23.	175	CE1	HIS	21	A	<-->	2894	CB	ASN	418	B	3.48
24.	175	CE1	HIS	21	A	<-->	2903	OG	SER	419	B	3.63
25.	176	NE2	HIS	21	A	<-->	2893	O	ASN	418	B	3.30
26.	176	NE2	HIS	21	A	<-->	2894	CB	ASN	418	B	3.88
27.	178	CA	GLN	22	A	<-->	2897	ND2	ASN	418	B	3.72
28.	181	CB	GLN	22	A	<-->	2897	ND2	ASN	418	B	3.65
29.	204	CG	PHE	25	A	<-->	2893	O	ASN	418	B	3.64
30.	206	CD2	PHE	25	A	<-->	2893	O	ASN	418	B	3.28
31.	208	CE2	PHE	25	A	<-->	2893	O	ASN	418	B	3.76
32.	350	CE	LYS	41	A	<-->	2127	O	CYS	322	B	3.84
33.	351	NZ	LYS	41	A	<-->	2168	CD	GLU	327	B	3.78
34.	351	NZ	LYS	41	A	<-->	2169	OE1	GLU	327	B	3.74

And a few more

```
Salt bridges
------------

      <----- A T O M   1 ----->       <----- A T O M   2 ----->

      Atom Atom Res  Res              Atom Atom Res  Res
       no. name name no.  Chain        no. name name no.  Chain  Distance
  1.    351  NZ  LYS   41    A   <-->  2170  OE2 GLU  327    B      3.04
  2.    474  OE2 GLU   56    A   <-->  1722  NH2 ARG  271    B      2.88
  3.    533  NH1 ARG   64    A   <-->  1564  OE1 GLU  244    B      3.76
  4.    531  NE  ARG   64    A   <-->  2434  OD2 ASP  364    B      2.90
  5.   1277  NH2 ARG  167    A   <-->  2170  OE2 GLU  327    B      3.04
  6.   1310  OD2 ASP  171    A   <-->  1555  NH1 ARG  243    B      3.19

Number of salt bridges:            6

Number of hydrogen bonds:          11

Number of non-bonded contacts:    155
```

The list of unique residues are

VAL	371	HIS	21
ARG	178	SER	302
GLY	368	GLU	174
CYS	189	SER	419
ILE	365	PHE	25
CYS	182	ASN	418
GLN	366	GLN	22
ARG	64	HIS	18
GLN	68	ARG	417
ILE	179	TYR	42
THR	67	CYS	322
ASP	364	GLN	46
GLU	244	GLU	320
LYS	367	CYS	308
TRP	369	GLU	56
ASN	63	ARG	271
LYS	172	LYS	41
LYS	168	GLU	327
TRP	304	ARG	167
PRO	61	LEU	52
THR	175	PRO	48
ARG	243	GLN	274
ASP	171	LEU	45
ASP	326	TRP	276
SER	62	GLU	275

The list of common residues in all three are

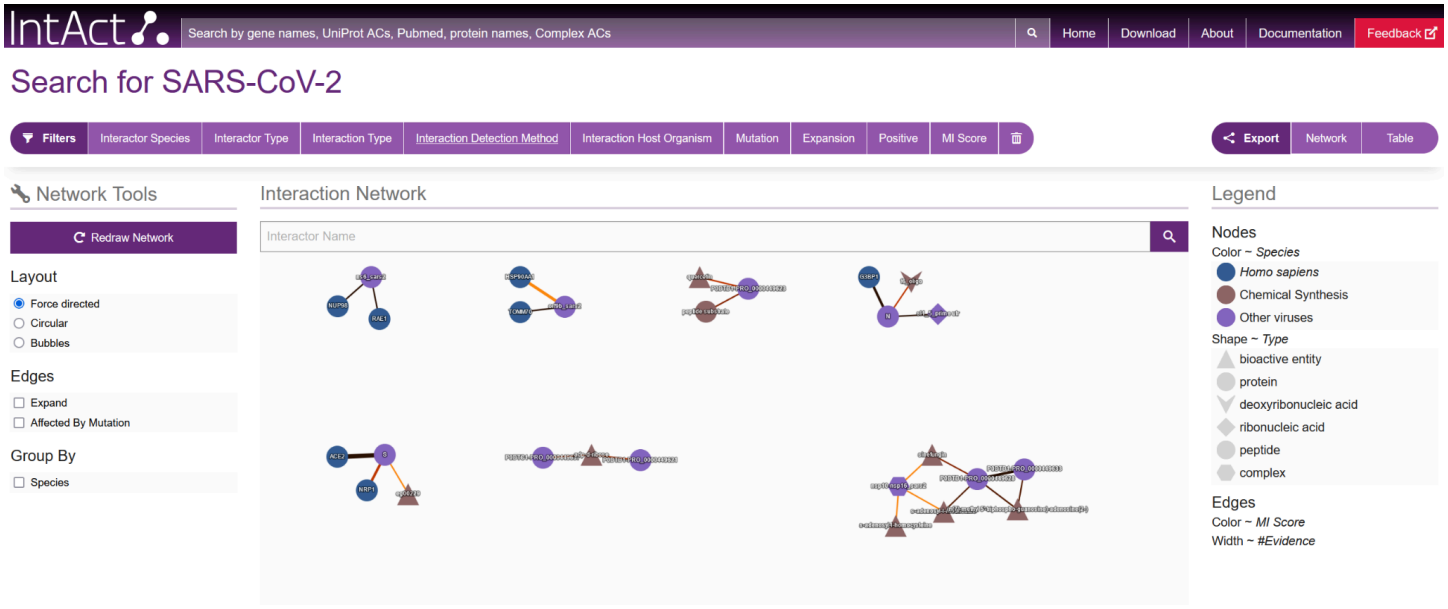
Residue	No	Residue	No	Residue	No
HIS	18	ARG	167	TRP	304
HIS	21	LYS	168	ILE	305
GLN	22	ASP	171	PRO	306
PHE	25	LYS	172	CYS	308
TYR	28	GLU	174	GLU	320
LYS	41	THR	175	LYS	321
TYR	42	PHE	176	CYS	322
LEU	45	ARG	178	PHE	323
GLN	46	ILE	179	SER	324
ASN	47	CYS	182	ASP	326
PRO	48	CYS	189	GLU	327
SER	51	ARG	243	ASP	364
LEU	52	GLU	244	ILE	365
GLU	56	ARG	271	GLN	366
PRO	61	THR	273	LYS	367
SER	62	GLN	274	GLY	368
ASN	63	GLU	275	TRP	369
ARG	64	TRP	276	VAL	371
GLU	65	SER	298	ARG	417
THR	67	THR	301	ASN	418
GLN	68	SER	302	SER	419
TYR	164	ILE	303	GLY	420

l. Compute the reduction in accessibility upon complex formation: ASA of HGH, HGH receptor, complex and difference using GETAREA

Difference =[ASA (HGH) + ASA (HGH receptor)] - ASA (complex)

ASA (complex) = 17635.87
ASA (HGH) + ASA (HGH receptor) = 13977.59 + 68314.34 = 82291.93
Difference = 82291.93 - 17635.87 = 64656.06

2. Find the protein–protein interactions in the IntAct database for SARS-CoV-2 obtained with ITC.



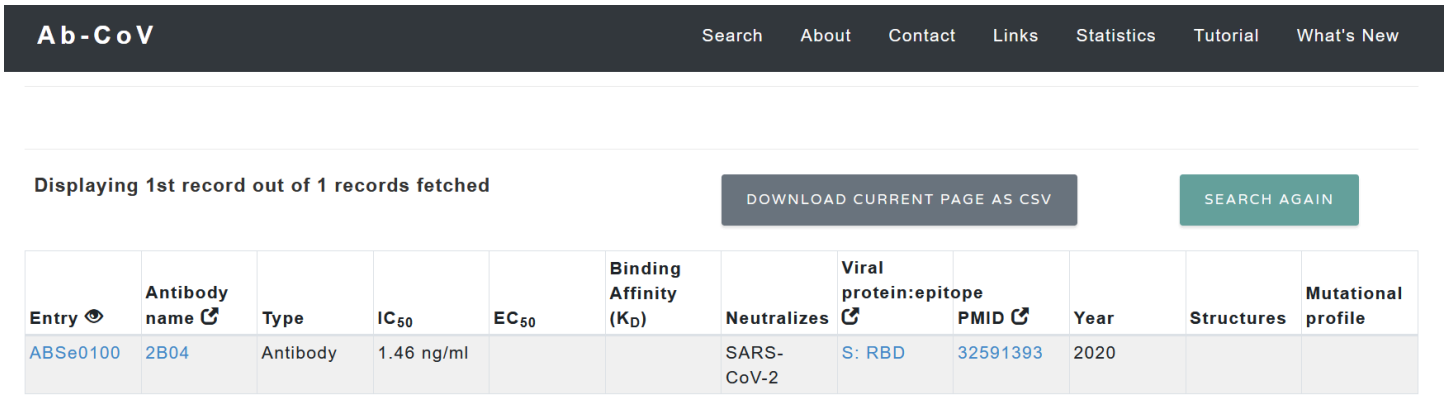
The above is the interaction networks, attached below is the list of interactions obtained with ITC

Interactions | Interactors | Column toggle

Showing 1 to 32 of 32 entries | 1 | Show 500 entries

Select	Molecule A	Molecule B	Identifier A	Identifier B	Type A	Type B	Species A	Species B	Host Organism	Positive interaction	Detection Method	Publication IDs	Interaction Type	Inte
	S	ACE2	UniProt P0DTC2	UniProt Q9BYF1	protein	protein	SARS-CoV-2	Homo sapiens	In vitro	✔	itc	32637958	direct interaction	EBI-2
	S	ACE2	UniProt P0DTC2	UniProt Q9BYF1	protein	protein	SARS-CoV-2	Homo sapiens	In vitro	✔	itc	34478710	direct interaction	EBI-2
	S	ACE2	UniProt P0DTC2	UniProt Q9BYF1	protein	protein	SARS-CoV-2	Homo sapiens	In vitro	✔	itc	34478710	direct interaction	EBI-2

3. Using the AbCov database (https://web.iitm.ac.in/bioinfo2/ab-cov/), for the monoclonal antibody 2B04, find the IC50 value. Find whether it is a neutralizing antibody or not and the source of the antibody.



IC50 value of 1.46 ng/ml

- One mAb, 2B04, neutralized wild-type SARS-CoV-2 in vitro with remarkable potency (half-maximal inhibitory concentration of <2 ng/ml).

- In a murine model of SARS-CoV-2 infection, 2B04 protected challenged animals from weight loss, reduced lung viral load, and blocked systemic dissemination

- Origin: Immunised Mouse