Characterization of Insulin-degrading Enzyme: Using Molecular Visualization Systems to Understand Substrate Recognition in Type 2 Diabetes and Alzheimer's

Aditi Kona

Introduction

- **Amyloid beta (Aβ)** is a biomarker for Alzheimer's Disease (AD)
  - ➢ Aβ plaques lead to AD
- **Insulin** is the principle regulator of Type 2 Diabetes (T2D)
  - ➢ Lack of insulin leads to T2D
- Both Abeta and insulin are found in endosomes
  - ➢ Internalized by receptor-mediated endocytosis
- What connect these disorders?

Insulin-degrading Enzyme (IDE)

Aβ and insulin are substrates of IDE

↙ ↘

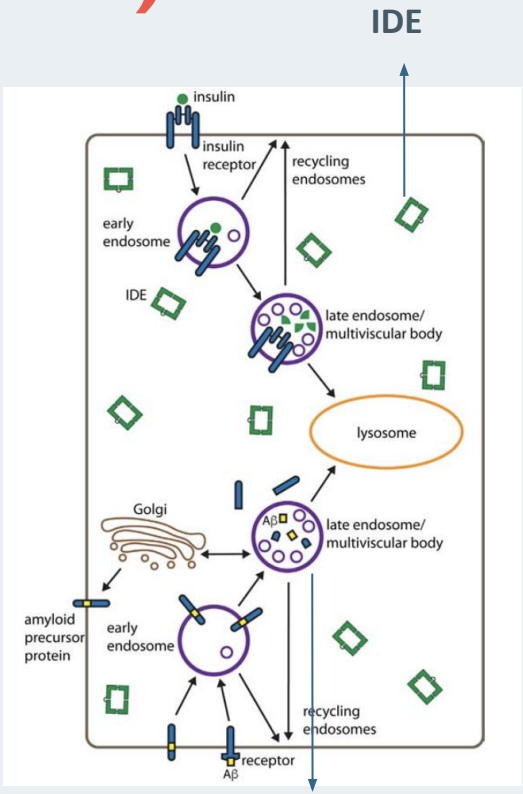
(IDE activators) **(IDE inhibitors)**

↓ ↓

Increases IDE = Aβ degradation

Inhibiting IDE = retention of insulin

How does IDE, a cytosolic enzyme, encounter Aβ and insulin in the endosome?



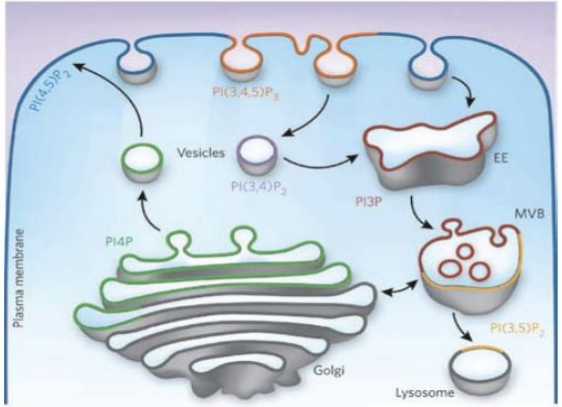
Aβ and Insulin in Endosome

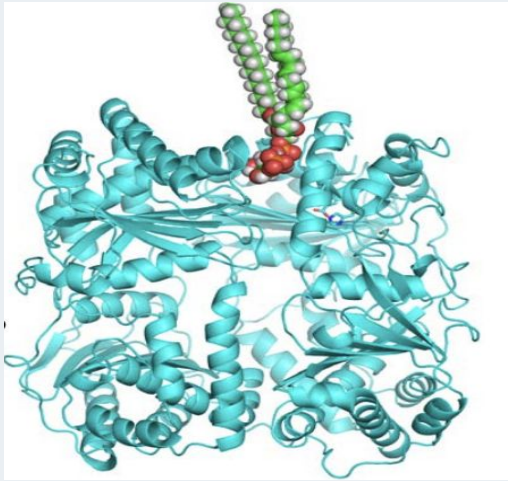
BACKGROUND

- Phosphatidylinositol phosphates (PtdInsPs) bind at the **polyanion binding site** to localize IDE to endosomes
- PtdInsPs serve as identifiers of different membranes

- Decreased localization of IDE = slowed T2D progression
- Increased localization of IDE = slowed AD progression

PtdInsPs distinguish different cell membranes.

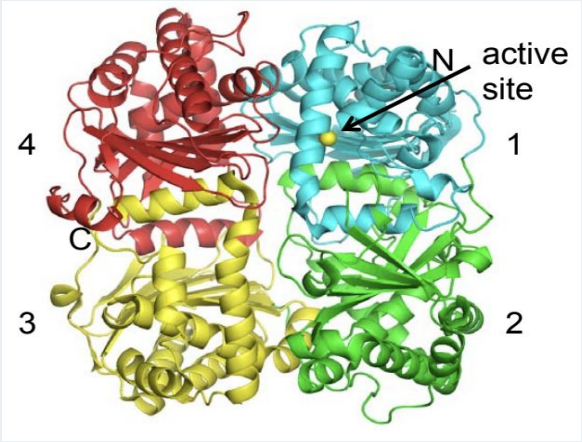


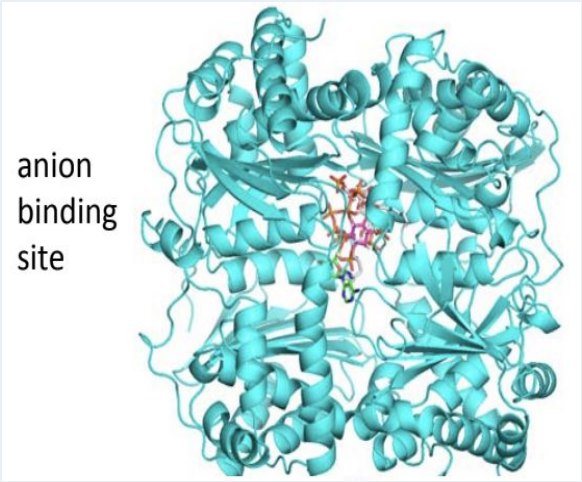


Model for interaction between IDE and membrane bound PI(3)P.

PROBLEM

- **Polyanion binding site and active site overlap**
  - Mutations in polyanion binding site could disrupt substrate degradation
- <1% of IDE is endosomal
  - High chance of off-target effects in cytosolic IDE





RESEARCH QUESTION

How can IDE be mutated such that it has a reduced ability to localize to endosomes but still retains full enzymatic activity?

HYPOTHESIS

Despite overlap between the polyanion binding site and the active site, residues/amino acids that mediate lipid headgroup binding can be mutated such that IDE's ability to bind to substrates is not disrupted.

PURPOSE AND OBJECTIVES

Purpose: Create a Non-localizing Mutant of IDE

Objectives:

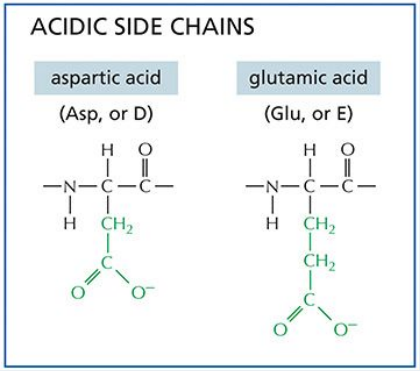
1. Identify residues vital for substrate degradation
2. Identify residues of interest to mutate in the polyanion binding site
3. Apply research to create a mutant of IDE with increased localization

METHODS

- **Used molecular visualization systems and online servers to identify which residues are important for substrate binding**
- **Advantages of online macromolecular structure analysis**
  - ➢ More efficient
  - ➢ Less expensive
  - ➢ Provides promising direction for wet-lab research

How Were Interactions Documented?

The side chain is different for every residue and thus gives the residue its properties.

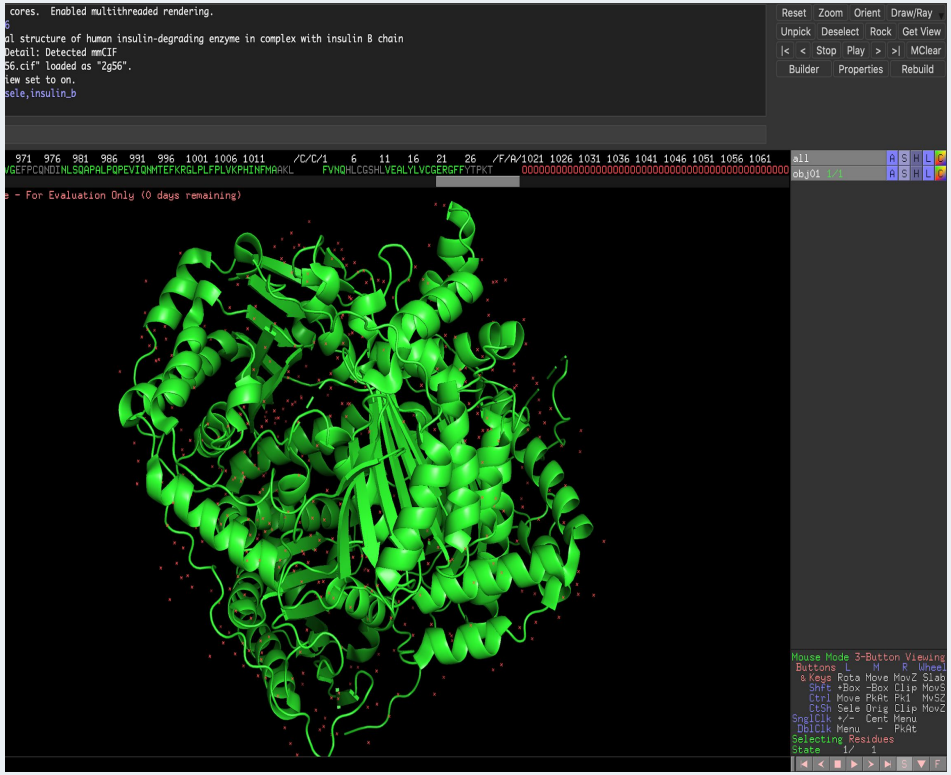


- **Side chains differentiate residues**
- **7 types of interactions between residues of substrate and IDE**
  - ➢ Main chain hydrogen bonds (H-bonds)
  - ➢ Main chain van der Waals forces (VDWs)
  - ➢ Main chain water-mediated contacts
  - ➢ Side chain hydrogen bonds
  - ➢ Side chains van der Waals forces
  - ➢ Side chain water-mediated contacts
  - ➢ Side chain salt bridges
- **Interactions were cataloged in Excel Sheet**

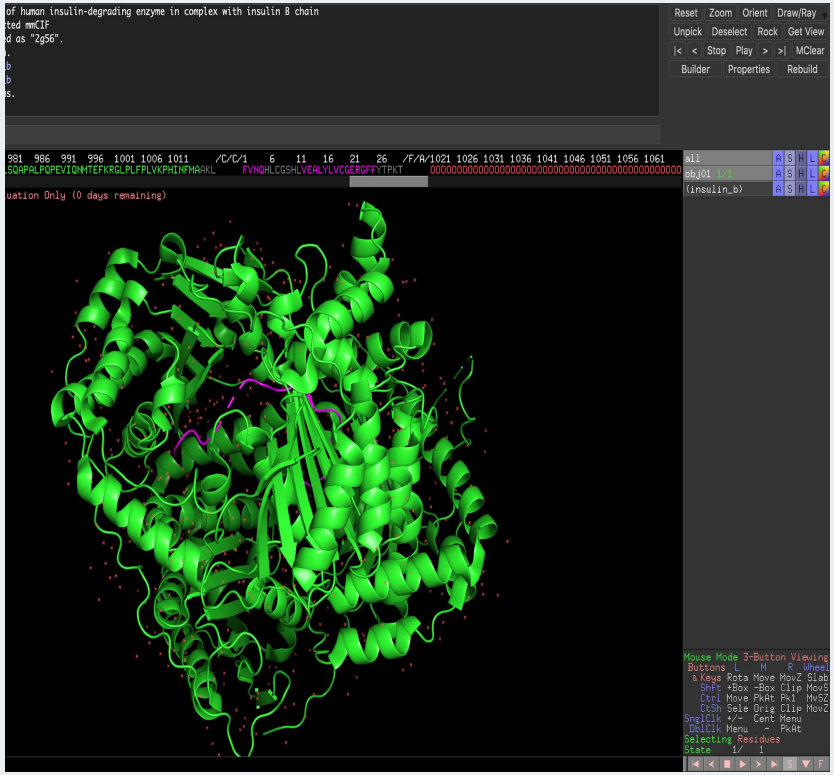
	IDE		Main_VDW	Main_hbond	Side_hbond	Side_VDW
135	A (Ala)					
136	G (Gly)					
137	S (Ser)					
138	S (Ser)					
139	N (Asn)		17 Leu	17 Leu	18 Val [two H-bonds]	17 Leu 18 Val
140	A (Ala)		16 Tyr 17 Leu	17 Leu		17 Leu
141	F (Phe)		15 Leu 16 Tyr			15 Leu 16 Tyr
142	T (Thr)		14 Ala 15 Leu	15 Leu [two H-bonds]		15 Leu
143	S (Ser)		14 Ala			14 Ala

Snippet of cataloged main chain VDWs, main chain H-bonds, side chain VDWs, and side chain H-bonds between IDE and insulin b chain.

PyMol

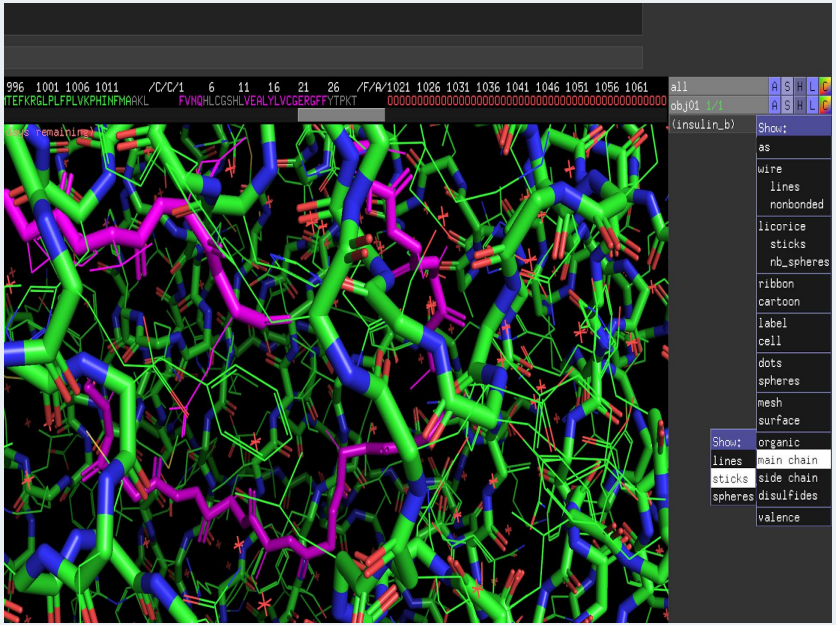


PyMol Viewer and Graphical User Interface. Pictured is upper control panel, display area, object menu panel, and mouse controls. Sequence alignment shows residues of IDE, insulin B, and water molecules.

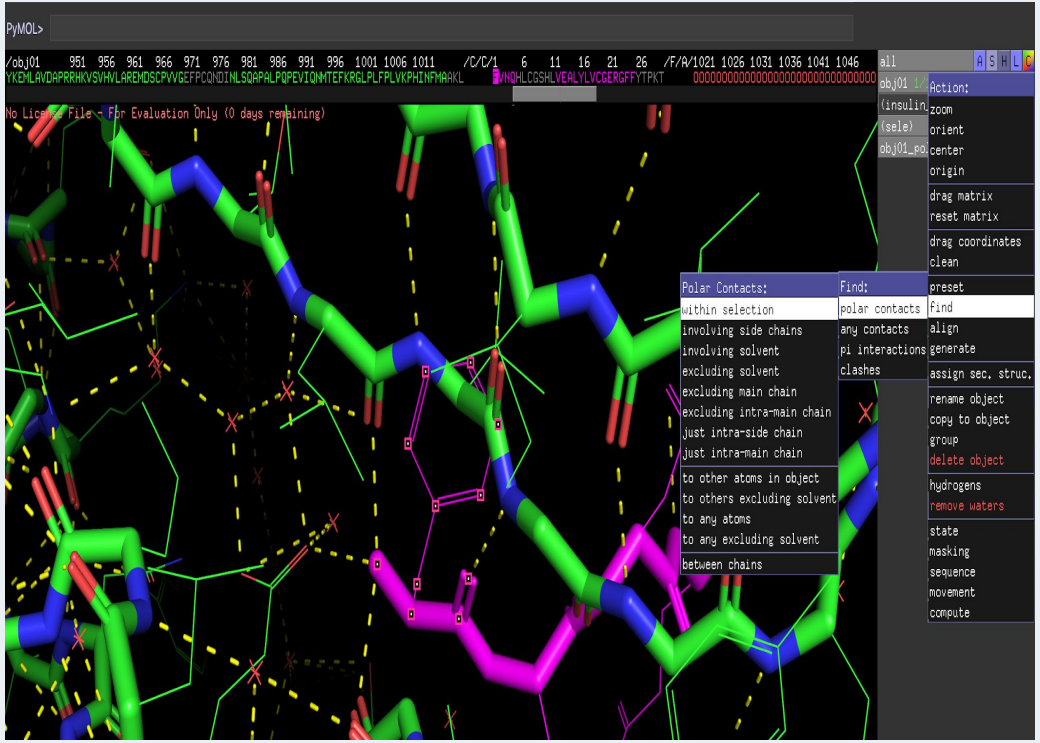


Insulin B chain residues were selected and copied into a new object titled “insulin_b.” Object was colored magenta.

PyMol

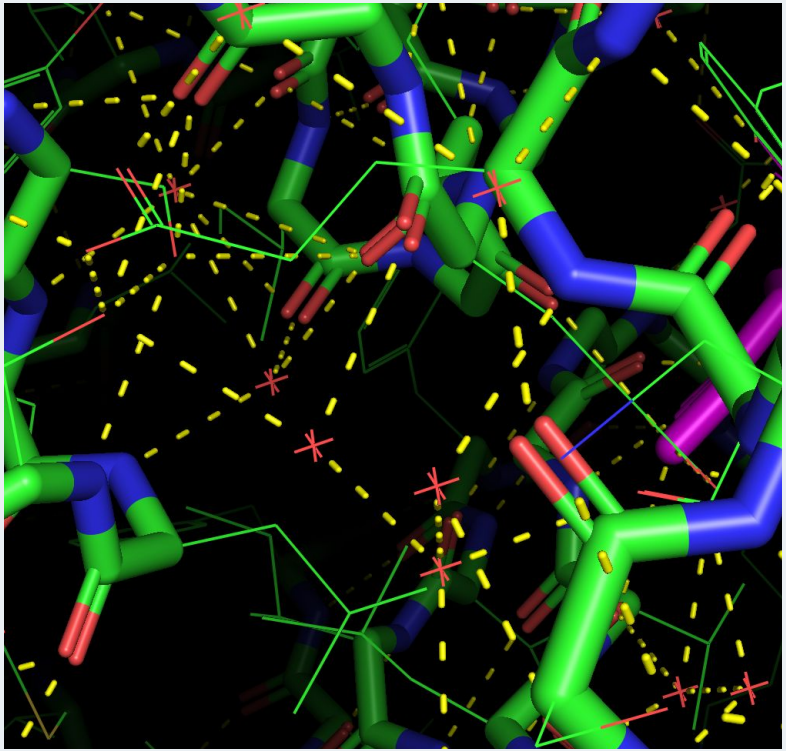


PyMol view with cartoon representation hidden. Main chains were shown as sticks and side chains were shown as lines to differentiate by thickness.

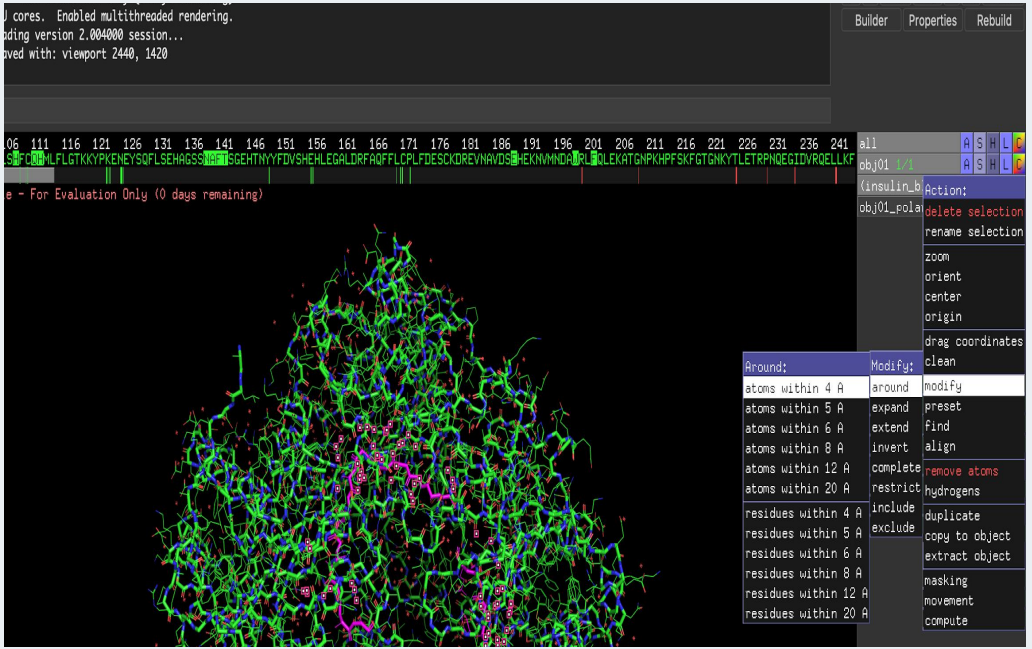


Hydrogen bonds, represented by dashed yellow lines, were found in the object. PyMol view highlighting residue 1 of insulin and the three main chain H-bonds and 1 side chain H-bonds it makes with IDE residues.

PyMol



PyMol view showing water-mediated contacts.



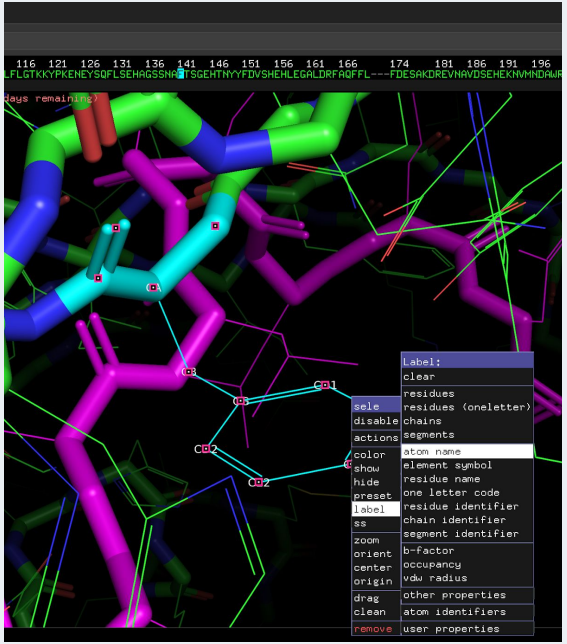
PyMol view showing residues within 4 Å of the insulin substrate. Sequence alignment highlights selected residues of interest for salt bridges.

PDBSum

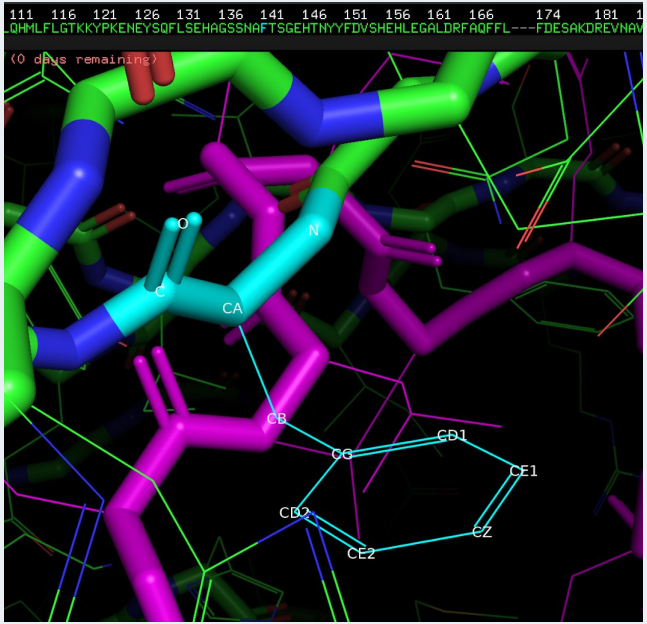
Non-bonded contacts

	Atom no.	Atom name	Res name	Res no.	Chain		Atom no.	Atom name	Res name	Res no.	Chain	Distance
1.	516	NE2	GLN	111	A	<-->	61949	O	SER	12	a	3.51
2.	523	ND1	HIS	112	A	<-->	61966	CD1	TYR	14	a	3.21
3.	523	ND1	HIS	112	A	<-->	61968	CE1	TYR	14	a	3.18
4.	525	CE1	HIS	112	A	<-->	61966	CD1	TYR	14	a	3.33
5.	525	CE1	HIS	112	A	<-->	61968	CE1	TYR	14	a	3.62
6.	733	C	SER	138	A	<-->	61980	NE2	GLN	15	a	3.64
7.	734	O	SER	138	A	<-->	61978	CD	GLN	15	a	3.67
8.	734	O	SER	138	A	<-->	61980	NE2	GLN	15	a	2.46
9.	741	CB	ASN	139	A	<-->	61980	NE2	GLN	15	a	3.69
10.	742	CG	ASN	139	A	<-->	61956	CB	LEU	13	a	3.47
11.	742	CG	ASN	139	A	<-->	61960	N	TYR	14	a	3.45
12.	742	CG	ASN	139	A	<-->	61972	N	GLN	15	a	3.84
13.	742	CG	ASN	139	A	<-->	61975	O	GLN	15	a	3.57
14.	742	CG	ASN	139	A	<-->	61980	NE2	GLN	15	a	3.30
15.	743	OD1	ASN	139	A	<-->	61953	CA	LEU	13	a	3.40
16.	743	OD1	ASN	139	A	<-->	61954	C	LEU	13	a	3.13
17.	743	OD1	ASN	139	A	<-->	61956	CB	LEU	13	a	3.38
18.	743	OD1	ASN	139	A	<-->	61960	N	TYR	14	a	2.37
19.	743	OD1	ASN	139	A	<-->	61961	CA	TYR	14	a	3.22
20.	743	OD1	ASN	139	A	<-->	61962	C	TYR	14	a	3.78
21.	743	OD1	ASN	139	A	<-->	61964	CB	TYR	14	a	3.25
22.	743	OD1	ASN	139	A	<-->	61972	N	GLN	15	a	3.34
23.	743	OD1	ASN	139	A	<-->	61980	NE2	GLN	15	a	3.88
24.	744	ND2	ASN	139	A	<-->	61956	CB	LEU	13	a	3.37
25.	744	ND2	ASN	139	A	<-->	61957	CG	LEU	13	a	3.40
26.	744	ND2	ASN	139	A	<-->	61958	CD1	LEU	13	a	3.30
27.	744	ND2	ASN	139	A	<-->	61972	N	GLN	15	a	3.62
28.	744	ND2	ASN	139	A	<-->	61974	C	GLN	15	a	3.59
29.	744	ND2	ASN	139	A	<-->	61975	O	GLN	15	a	2.47
30.	744	ND2	ASN	139	A	<-->	61978	CD	GLN	15	a	3.80
31.	744	ND2	ASN	139	A	<-->	61980	NE2	GLN	15	a	3.04
32.	748	O	ALA	140	A	<-->	61949	O	SER	12	a	3.15
33.	748	O	ALA	140	A	<-->	61953	CA	LEU	13	a	3.16
34.	748	O	ALA	140	A	<-->	61954	C	LEU	13	a	3.49
35.	748	O	ALA	140	A	<-->	61960	N	TYR	14	a	2.97
36.	751	CA	PHE	141	A	<-->	61949	O	SER	12	a	3.37
37.	754	CB	PHE	141	A	<-->	61946	N	SER	12	a	3.79
38.	754	CB	PHE	141	A	<-->	61948	C	SER	12	a	3.77
39.	754	CB	PHE	141	A	<-->	61949	O	SER	12	a	3.58
40.	769	CA	SER	143	A	<-->	61938	CG2	ILE	10	a	3.64
41.	772	CB	SER	143	A	<-->	61938	CG2	ILE	10	a	3.40
42.	835	OH	TYR	150	A	<-->	61956	CB	LEU	13	a	3.79

PDBSum list of Non-bonded contacts.

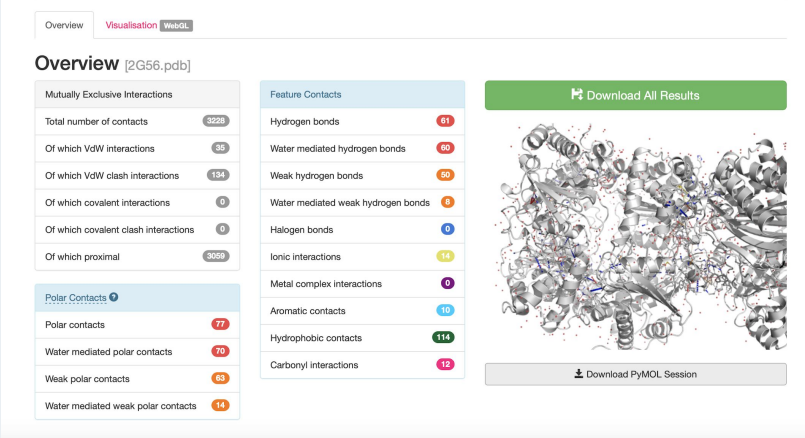


PyMol view of IDE residue 141, which was colored cyan.

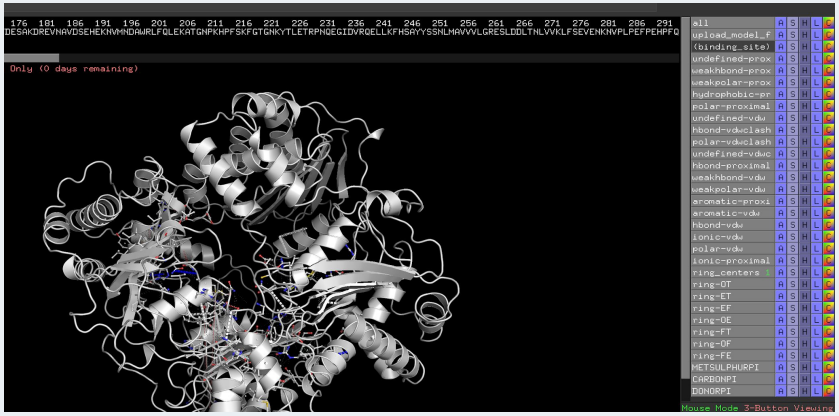


Residue 141 is labeled with atom names.

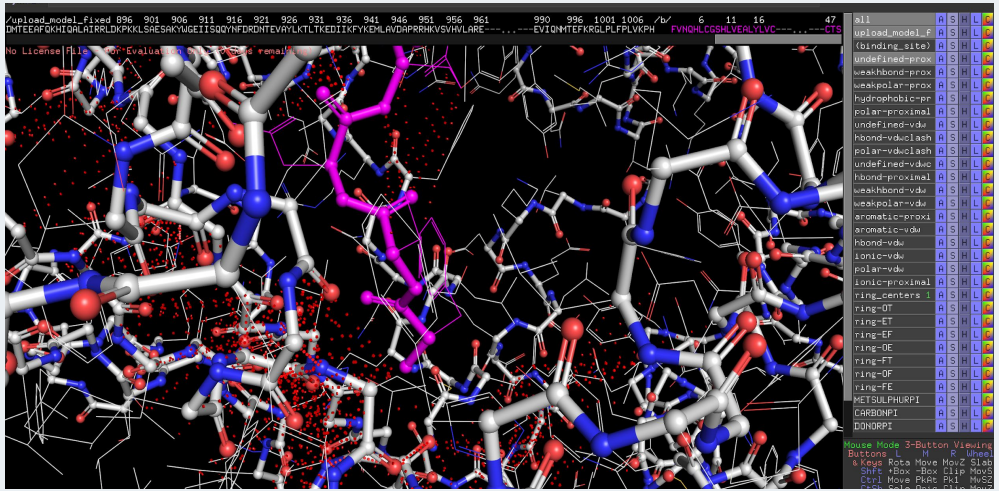
Arpeggio



Arpeggio overview. Each type of interaction is broken down.

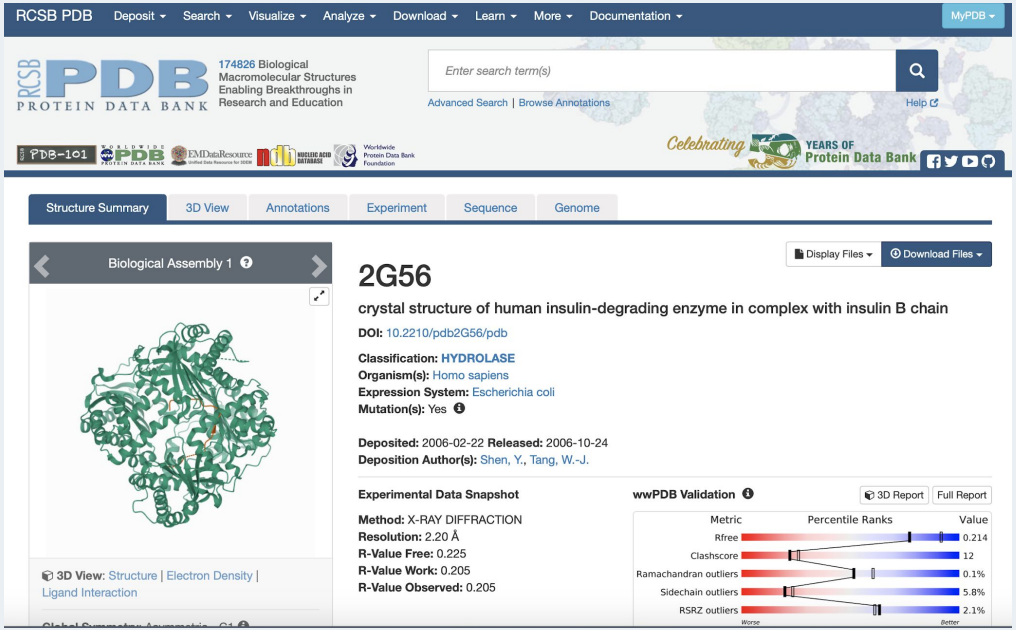


Arpeggio PyMol output with breakdown of each interaction to the right.

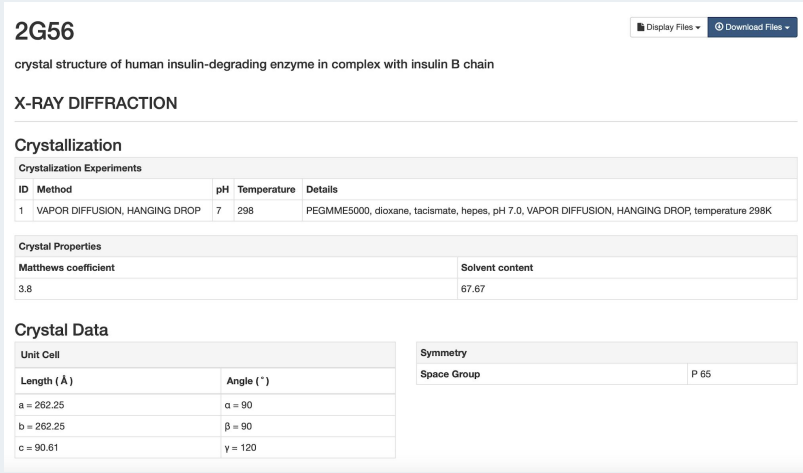


Undefined-proximity set is depicted by red dashed lines between residues.

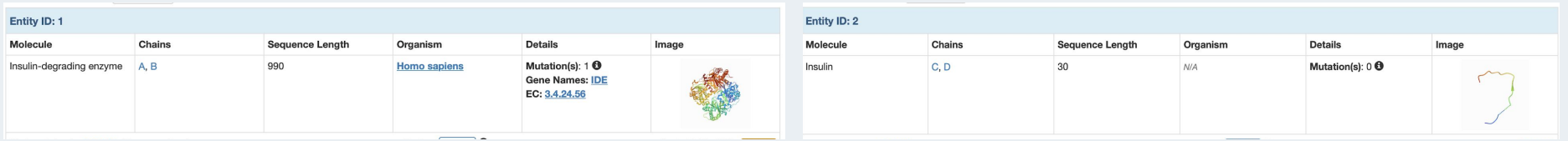
Worldwide Protein Data Bank (wwPDB)



wwPDB summary of structure 2g56.

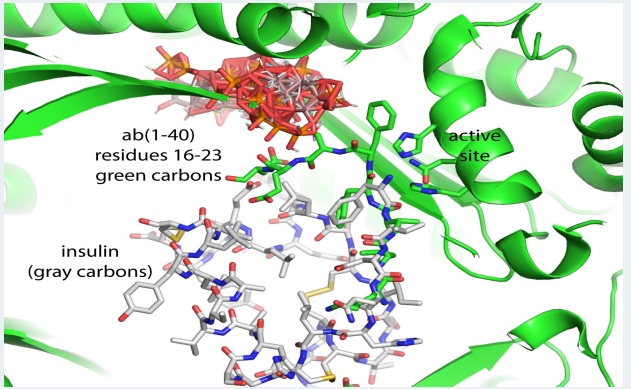


Experimental data for structure 2g56, which was determined using x-ray diffraction.



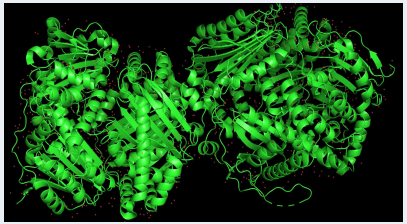
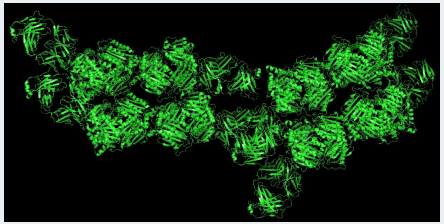
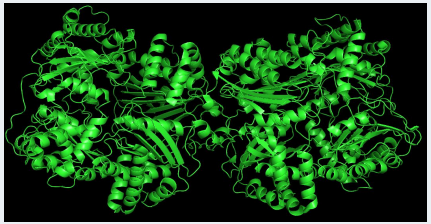
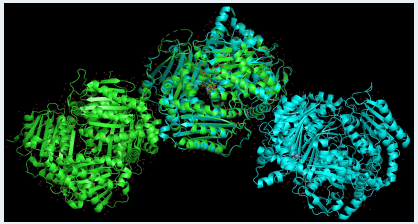
Breakdown of proteins in structure. In structure 2G56, there are two IDE chains and two insulin chains.

DATA/RESULTS



Protein Data Bank structures I analyzed:

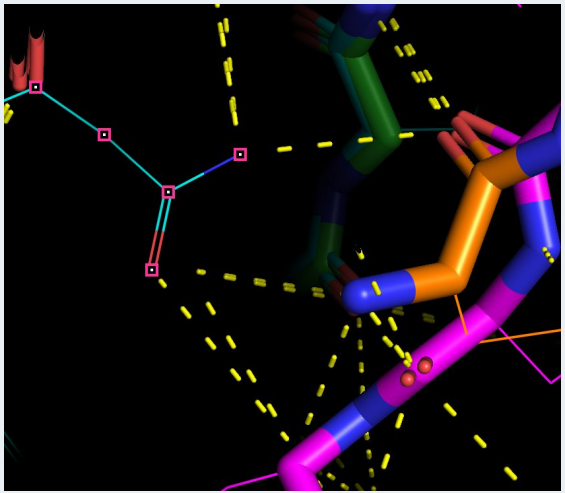
2G56	5WOB	6BFC	2G47_2WBY
Crystal structure of IDE in complex with insulin B chain	Crystal Structure Analysis of Fab1-Bound IDE in Complex with insulin A	Cryo-EM structure of IDE in complex with insulin B	Crystal structure of IDE in complex with amyloid-beta and insulin A and B chain

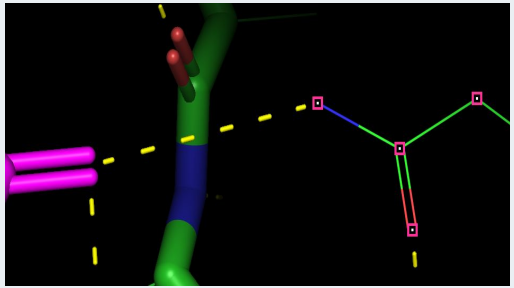
RESULTS

	4 structures	3 structures	2 structures	1 structure
IDE residues	111 Gln 139 Asn 140 Ala 141 Phe 199 Trp 824 Arg 831 Tyr	108 His 112 His 142 Thr 150 Tyr 198 Ala 202 Phe 339 Gly 359 Leu 361 Gly 363 Gln 436 Lys 683 Met 820 Phe	115 Phe 143 Ser 182 Glu 189 Glu 220 Thr 332 His 360 Val 362 Gly 374 Ile 431 Arg 609 Tyr 679 His 680 Gln 834 Phe	47 Ile 48 Lys 49 Arg 138 Ser 192 Lys 331 Gly 335 Gly 336 His 341 Glu 364 Lys 429 Arg 432 Gly 677 Gln 816 Ser 849 Ile

Residue 111 Glutamine



In structure 2G47_2WBY, residue 111 of IDE (shown in cyan) forms a side chain H-bond with residue 20 of Aβ and residue 1 of insulin.



In structure 5WOB, residue 111 forms a side chain H-bond with residue 12 of insulin.

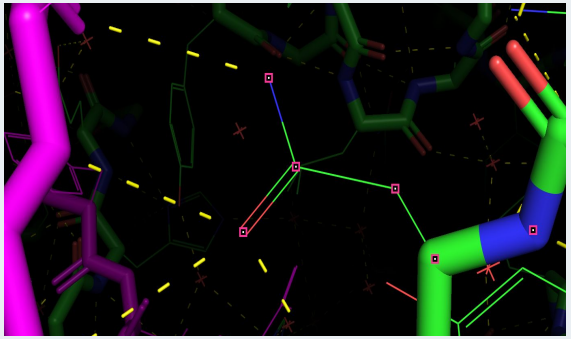
	Atom no.	Atom name	Res name	Res no.	Chain		Atom no.	Atom name	Res name	Res no.	Chain	Distance
1.	8203	ND1	HIS	108	B	<-->	15671	CB	HIS	10	b	3.74
2.	8205	CE1	HIS	108	B	<-->	15671	CB	HIS	10	b	3.62
3.	8233	OE1	GLU	111	B	<-->	15691	CG2	VAL	12	b	3.27
4.	8268	CD2	PHE	115	B	<-->	15690	CG1	VAL	12	b	3.75

In structure 6BFC, residue 111 makes a side chain VDW contact with residue 12 of the insulin B chain.

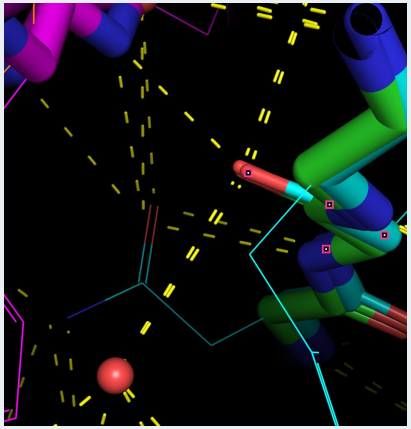
	Atom no.	Atom name	Res name	Res no.	Chain		Atom no.	Atom name	Res name	Res no.	Chain	Distance
1.	478	CD2	HIS	108	A	<-->	15760	CB	LEU	15	C	3.81
2.	479	CE1	HIS	108	A	<-->	15760	CB	LEU	15	C	3.71
3.	480	NE2	HIS	108	A	<-->	15760	CB	LEU	15	C	3.43
4.	480	NE2	HIS	108	A	<-->	15764	O	TYR	16	C	3.75
5.	505	OE1	GLN	111	A	<-->	15759	O	LEU	15	C	3.39
6.	506	NE2	GLN	111	A	<-->	15766	N	LEU	17	C	3.51
7.	506	NE2	GLN	111	A	<-->	15767	CA	LEU	17	C	3.42
8.	506	NE2	GLN	111	A	<-->	15770	CB	LEU	17	C	3.24
9.	515	CE1	HIS	112	A	<-->	15769	O	LEU	17	C	3.84
10.	516	NE2	HIS	112	A	<-->	15764	O	TYR	16	C	3.77
11.	732	CG	ASN	139	A	<-->	15771	N	VAL	18	C	3.89

In structure 2G56, residue 111 forms a side chain VDW contact with residues 15 and 17 of insulin.

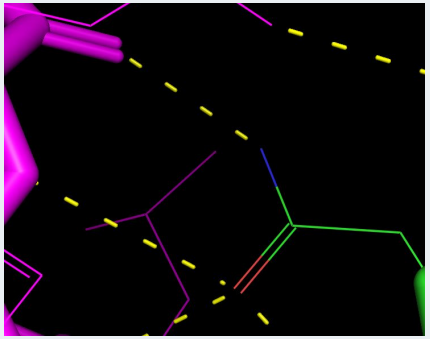
Residue 139 Asparagine



In structure 2G56, residue 139 of IDE (shown in green) forms two side chain H-bonds with residue 18.



In structure 2G47_2WBY, residue 139 of IDE forms a main chain H-bond with residue 20 of Aβ and a water-mediated contact.



In structure 5WOB, residue 139 forms a side chain H-bond with residue 14 and residue 15 of insulin.

	Atom no.	Atom name	Res name	Res no.	Chain		Atom no.	Atom name	Res name	Res no.	Chain	Distance
1.	8203	ND1	HIS	108	B	<-->	15671	CB	HIS	10	b	3.74
2.	8205	CE1	HIS	108	B	<-->	15671	CB	HIS	10	b	3.62
3.	8233	OE1	GLU	111	B	<-->	15691	CG2	VAL	12	b	3.27
4.	8268	CD2	PHE	115	B	<-->	15690	CG1	VAL	12	b	3.75
5.	8270	CE2	PHE	115	B	<-->	15690	CG1	VAL	12	b	3.66
6.	8270	CE2	PHE	115	B	<-->	15691	CG2	VAL	12	b	3.84
7.	8460	CG	ASN	139	B	<-->	15684	CD2	LEU	11	b	3.86
8.	8460	CG	ASN	139	B	<-->	15685	N	VAL	12	b	3.86
9.	8460	CG	ASN	139	B	<-->	15692	N	GLU	13	b	3.73
10.	8460	CG	ASN	139	B	<-->	15695	O	GLU	13	b	3.70
11.	8461	OD1	ASN	139	B	<-->	15678	CA	LEU	11	b	3.83
12.	8461	OD1	ASN	139	B	<-->	15679	C	LEU	11	b	3.79
13.	8461	OD1	ASN	139	B	<-->	15681	CB	LEU	11	b	3.53
14.	8461	OD1	ASN	139	B	<-->	15684	CD2	LEU	11	b	3.76
15.	8461	OD1	ASN	139	B	<-->	15685	N	VAL	12	b	2.81
16.	8461	OD1	ASN	139	B	<-->	15686	CA	VAL	12	b	3.53
17.	8461	OD1	ASN	139	B	<-->	15689	CB	VAL	12	b	3.44
18.	8461	OD1	ASN	139	B	<-->	15691	CG2	VAL	12	b	3.86
19.	8461	OD1	ASN	139	B	<-->	15692	N	GLU	13	b	3.39
20.	8462	ND2	ASN	139	B	<-->	15681	CB	LEU	11	b	3.57
21.	8462	ND2	ASN	139	B	<-->	15682	CG	LEU	11	b	3.84
22.	8462	ND2	ASN	139	B	<-->	15683	CD1	LEU	11	b	3.80
23.	8462	ND2	ASN	139	B	<-->	15684	CD2	LEU	11	b	3.58
24.	8462	ND2	ASN	139	B	<-->	15692	N	GLU	13	b	3.70
25.	8462	ND2	ASN	139	B	<-->	15694	C	GLU	13	b	3.72
26.	8462	ND2	ASN	139	B	<-->	15695	O	GLU	13	b	3.72
27.	8466	O	ALA	140	B	<-->	15670	O	HIS	10	b	3.37

In structure 6BFC, residue 139 forms side chain VDW contacts with residues 11, 12, and 13 of insulin.

CONCLUSIONS

- Amino acids that mediate substrate degradation are not part of the polyanion binding site
- Minimal off-target effects
- Research tells us how to make mutations with minimal disruption to substrate interactions
- Hypothesis was accepted
    - **Although polyanion binding site and active site partially overlap, most of the residues that likely mediate lipid headgroup interactions are not used to bind substrates**

FUTURE DIRECTIONS

- **Protein docking**
  - Predict orientation of ligand when bound to enzyme
- Promising candidates to mutate in-lab
- If you can selectively inhibit IDE, you can slow degradation of insulin or even improve length of time over which insulin acts

Ligand Docking Job gpi4p_2g47_repeat 「№91429」 Details

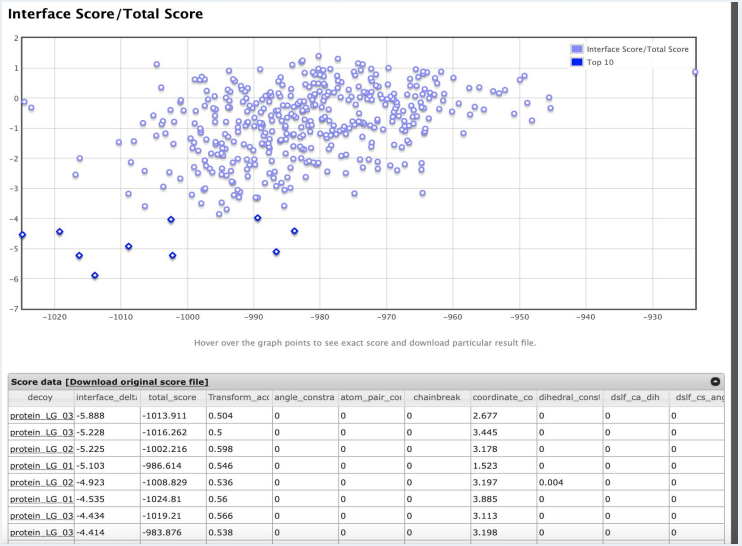
Inputs

[protein.pdb]

[ligand.sdf]

Status

Job ID	91429
Job Name	gpi4p_2g47_repeat
Visibility	**PUBLIC** (you can share this job)
Protocol	Ligand Docking
CPU hours used	20.9
user	aditikona
Status	**Finished**
Daemon	GrayLab.Rosetta-2
Description	
angle_step	5.0
chain	X
gen_conformers	True
grid_width	15.0
highres_cycles	6
highres_repack_cycles	3
initial_perturb	3.0
move_step	0.1
n_ligand_conformers	200
nstruct	400
pocket_width	7.0
transform_cycles	500
use_input_position	False
x_start	85.3908081055
y_start	71.4642181396
z_start	3.0
Submitted time	2021-02-22 14:48
Start time	2021-02-22 14:49
End time	2021-02-22 15:13



Interface Score/Total Score

Hover over the graph points to see exact score and download particular result file.

Score data [Download original score file]

decoy	interface_delt	total_score	Transform_acc	angle_constra	atom_pair_cor	chainbreak	coordinate_co	dihedral_const	dslf_ca_dih	dslf_cs_ang
protein_LG_03	-5.888	-1013.911	0.504	0	0	0	2.677	0	0	0
protein_LG_03	-5.228	-1016.262	0.5	0	0	0	3.445	0	0	0
protein_LG_02	-5.225	-1002.216	0.598	0	0	0	3.178	0	0	0
protein_LG_01	-5.103	-986.614	0.546	0	0	0	1.523	0	0	0
protein_LG_02	-4.923	-1008.829	0.536	0	0	0	3.197	0.004	0	0
protein_LG_01	-4.535	-1024.81	0.56	0	0	0	3.885	0	0	0
protein_LG_03	-4.434	-1019.21	0.566	0	0	0	3.113	0	0	0
protein_LG_03	-4.414	-983.876	0.538	0	0	0	3.198	0	0	0

REFERENCES

Abdul-Hay, S. O., Kang, D., McBride, M., Li, L., Zhao, J., & Leissring, M. A. (2011). Deletion of insulin-degrading enzyme elicits antipodal, age-dependent effects on glucose and insulin tolerance. PloS one, 6(6), e20818. https://doi.org/10.1371/journal.pone.0020818

Hulse, R. E., Ralat, L. A., & Wei-Jen, T. (2009). Structure, function, and regulation of insulin-degrading enzyme. Vitamins and hormones, 80, 635–648. https://doi.org/10.1016/S0083-6729(08)00622-5

Noinaj, N., Song, E. S., Bhasin, S., Alper, B. J., Schmidt, W. K., Hersh, L. B., & Rodgers, D. W. (2012). Anion activation site of insulin-degrading enzyme. The Journal of biological chemistry, 287(1), 48–57. https://doi.org/10.1074/jbc.M111.264614

Song, E. S., Ozbil, M., Zhang, T., Sheetz, M., Lee, D., Tran, D., Li, S., Prabhakar, R., Hersh, L. B., & Rodgers, D. W. (2015). An Extended Polyanion Activation Surface in Insulin Degrading Enzyme. PloS one, 10(7), e0133114. https://doi.org/10.1371/journal.pone.0133114

Song, E. S., Jang, H., Guo, H. F., Juliano, M. A., Juliano, L., Morris, A. J., Galperin, E., Rodgers, D. W., & Hersh, L. B. (2017). Inositol phosphates and phosphoinositides activate insulin-degrading enzyme, while phosphoinositides also mediate binding to endosomes. Proceedings of the National Academy of Sciences of the United States of America, 114(14), E2826–E2835. https://doi.org/10.1073/pnas.1613447114

ACKNOWLEDGMENTS

Thank you to Dr. David Rodgers for providing remote guidance through this project.