HOMOLOGY MODEL RECAP

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EPA United States Environmental Protection Agency

Overview

- Look into N214A/F amino acid change with DUET protein stability and Autodock Vina for both Bayer and ITASSER protein models
- Overview ITASSER process and scoring for homology modeling
- Compare ITASSER created models visually to Bayer created models
- Dock flupyradifurone and thiacloprid to models of interest

SeqAPASS: Sequence-based results

Total Match | Partial Match | Not a Match | Susceptible Yes | Susceptible No

Common Name	Similar Susceptibility	Amino Acid 1	Amino Acid 2	Amino Acid 3	Amino Acid 4	Amino Acid 5
Apis mellifera CYP9Q3	Y	111G	214N	310S	371V	372L
Dufourea novaeangliae CYP9DL4	N	111N	214N	307T	368A	369A
Apis mellifera CYP9Q1	Y	111G	211Q	308T	370I	371L
Apis mellifera CYP9Q2	N	113S	216T	310T	371I	372A
Apis cerana CYP9Q3	Y	111G	214N	310S	371I	372L
Apis dorsata CYP9Q3	Y	111G	214N	310S	371I	372L
Apis mellifera CYP9Q3	Y	111G	214N	310S	371V	372L
Bombus impatiens CYP9Q4	N	112E	215T	308A	369V	370A
Bombus impatiens CYP9Q5	N	112E	215T	308T	369V	370A
Bombus impatiens CYP9Q6	N	111D	214F	306S	367I	368T
Eufriesa mexicana CYP9Q7	N	111D	214M	307S	368I	369A
Eufriesa mexicana CYP9Q8	N	111E	214M	307T	368I	369A
Habropoda laboriosa CYP9Q9	N	109R	212L	305T	366I	367V
Apis florea CYP9Q-like-1	N	111N	214T	306T	367I	368A
Tetragonila carbonaria CYP9Q-like-1	N	111K	215S	309S	370I	371A
Apis florea CYP9Q-like-2	N	113S	216T	309T	370V	371V
Tetragonila carbonaria CYP9Q-like-2	N	111K	214S	307S	368I	369A
Andrena vaga CYP9Q-like	N	111A	211F	304T	365I	366G
Andrena haemorrhoa CYP9Q-like	N	111A	211F	304T	365I	366G
Colletes cunicularius CYP9Q-like	N	111E	214N	307T	368I	369A
E nigrescens CYP9Q-like	N	111E	214S	307T	368I	369V
Lasioglossum xanthopus CYP9Q-like	N	111D	215N	308S	369V	370A
Macropis fulvipes CYP9Q-like	N	108K	211T	301S	362V	363A
Melitta haemorrhoidalis CYP9Q-like	N	111E	214L	306T	367I	368A
N lathburiana CYP9Q-like	N	111E	214A	307S	368I	369A
N melanderi CYP9Q-like	N	111D	214D	307S	368S	369P
Xylocopa violacea CYP9Q-like	N	111D	214T	307S	368I	369V
Melipona quadrifasciata CYP9Q-like	N	111K	214S	307T	368I	369A

Amino Acid info

ID	Name	Side Chain	Size
A	Alanine	Aliphatic	89.094
C	Cysteine	Sulfur-Containing	121.154
D	Aspartic Acid	Acidic	133.104
E	Glutamic Acid	Acidic	147.131
F	Phenylalanine	Aromatic	165.192
G	Glycine	Aliphatic	75.067
H	Histidine	Basic	155.156
I	Isoleucine	Aliphatic	131.175
K	Lysine	Basic	146.189
L	Leucine	Aliphatic	131.175
M	Methionine	Sulfur-Containing	149.208
N	Asparagine	Amidic	132.119
P	Proline	Aliphatic	115.132
Q	Glutamine	Amidic	146.146
R	Arginine	Basic	174.203
S	Serine	Hydroxylic	105.093
T	Threonine	Hydroxylic	119.119
U	Seleno-cysteine	Sulfur-Containing	168.064
V	Valine	Aliphatic	117.148
W	Tryptophan	Aromatic	204.228
X	Unknown	Unknown	-
Y	Tyrosine	Aromatic	181.191

N214F – Amidic to Aromatic AND MW diff. 33.073 g/mol
N214A – Amidic to Aliphatic AND MW diff. 43.025 g/mol

Question: Are mutations in structure destabilizing and consistent with sequence-based predictions?

Use DUET to explore mutations in protein structure

- **Need structural models**
  - **PDB**
  - **Point mutation**

Protein stability of N214A/F of Apis mellifera CYP9Q3

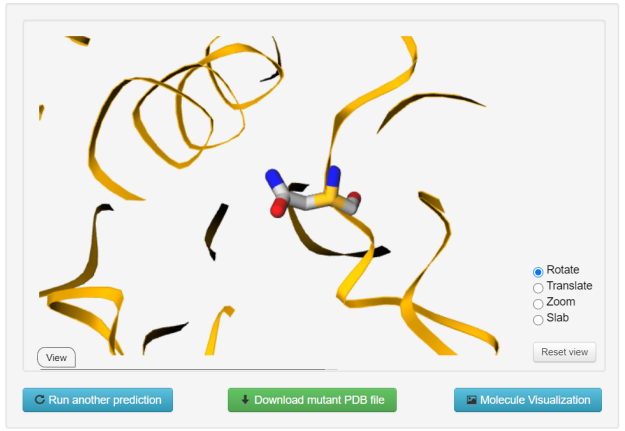
I-TASSER
Protein Structure & Function Predictions

mCSM Predicted Stability Change (ΔΔG):
-0.643 Kcal/mol (*Destabilizing*)

SDM Predicted Stability Change (ΔΔG):
0.92 Kcal/mol (*Stabilizing*)

DUET Predicted Stability Change (ΔΔG):
-0.181 Kcal/mol (*Destabilizing*)

Mutation:
Wild-type: **ASN**
Position: **214**
Mutant-type: **ALA**
Chain: **A**
Secondary structure: **Loop or irregular**



mCSM Predicted Stability Change (ΔΔG):
-0.761 Kcal/mol (*Destabilizing*)

SDM Predicted Stability Change (ΔΔG):
0.74 Kcal/mol (*Stabilizing*)

DUET Predicted Stability Change (ΔΔG):
-0.5 Kcal/mol (*Destabilizing*)

Mutation:
Wild-type: **ASN**
Position: **214**
Mutant-type: **PHE**
Chain: **A**
Secondary structure: **Loop or irregular**

BAYER

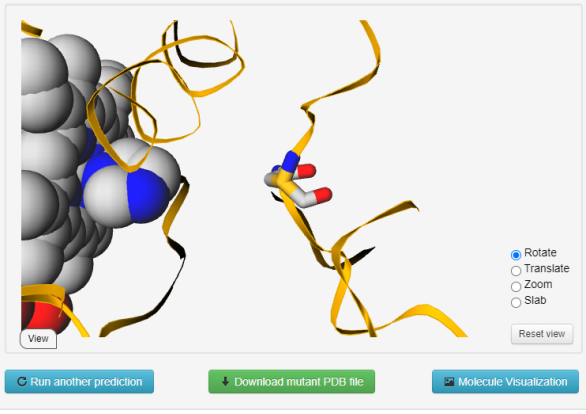
Both AM CYP9Q3 raw model

mCSM Predicted Stability Change (ΔΔG):
-0.259 kcal/mol (*Destabilizing*)

SDM Predicted Stability Change (ΔΔG):
1.29 kcal/mol (*Stabilizing*)

DUET Predicted Stability Change (ΔΔG):
0.293 kcal/mol (*Stabilizing*)

Mutation:
Wild-type: **ASN**
Position: **214**
Mutant-type: **ALA**
Chain: **A**
Secondary structure: **Loop or irregular**

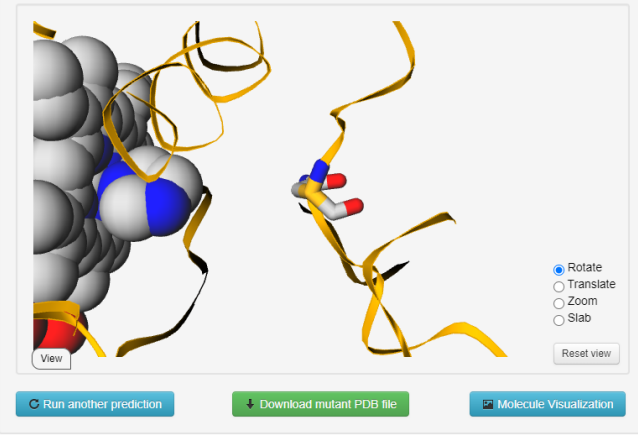


mCSM Predicted Stability Change (ΔΔG):
-0.625 kcal/mol (*Destabilizing*)

SDM Predicted Stability Change (ΔΔG):
0.85 kcal/mol (*Stabilizing*)

DUET Predicted Stability Change (ΔΔG):
-0.346 kcal/mol (*Destabilizing*)

Mutation:
Wild-type: **ASN**
Position: **214**
Mutant-type: **PHE**
Chain: **A**
Secondary structure: **Loop or irregular**



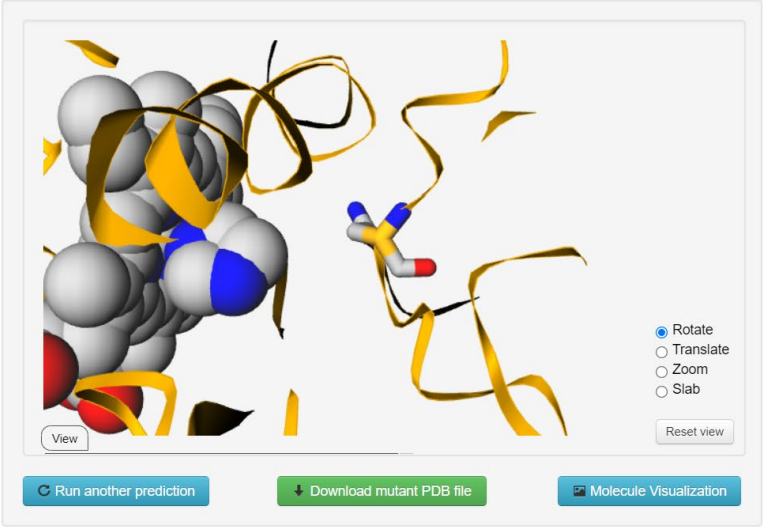
Protein Stability N214A/F of Apis mellifera CYP9Q3 cont.

mCSM Predicted Stability Change (ΔΔG):
-1.265 kcal/mol (*Destabilizing*)

SDM Predicted Stability Change (ΔΔG):
0.19 kcal/mol (*Stabilizing*)

DUET Predicted Stability Change (ΔΔG):
-1.034 kcal/mol (*Destabilizing*)

Mutation:
Wild-type: **ASN**
Position: **214**
Mutant-type: **ALA**
Chain: **A**
Secondary structure: **Loop or irregular**

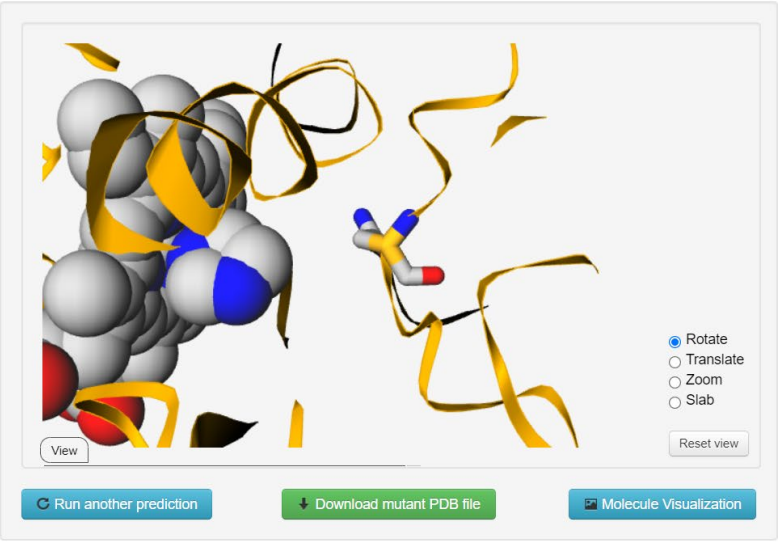


mCSM Predicted Stability Change (ΔΔG):
-1.15 kcal/mol (*Destabilizing*)

SDM Predicted Stability Change (ΔΔG):
-0.48 kcal/mol (*Destabilizing*)

DUET Predicted Stability Change (ΔΔG):
-1.267 kcal/mol (*Destabilizing*)

Mutation:
Wild-type: **ASN**
Position: **214**
Mutant-type: **PHE**
Chain: **A**
Secondary structure: **Loop or irregular**



AM CYP9Q3 Bayer's minimized model

Apis mellifera CYP9Q3 Dock prepped

mCSM Predicted Stability Change (ΔΔG):

-0.643 kcal/mol (*Destabilizing*)

SDM Predicted Stability Change (ΔΔG):

0.92 kcal/mol (*Stabilizing*)

DUET Predicted Stability Change (ΔΔG):

-0.181 kcal/mol (*Destabilizing*)

Mutation:

Wild-type: **ASN**
Position: **214**
Mutant-type: **ALA**
Chain: **A**
Secondary structure: **Loop or irregular**

mCSM Predicted Stability Change (ΔΔG):

-0.761 kcal/mol (*Destabilizing*)

SDM Predicted Stability Change (ΔΔG):

0.74 kcal/mol (*Stabilizing*)

DUET Predicted Stability Change (ΔΔG):

-0.5 kcal/mol (*Destabilizing*)

Mutation:

Wild-type: **ASN**
Position: **214**
Mutant-type: **PHE**
Chain: **A**
Secondary structure: **Loop or irregular**

ITASSER min (100 grad) and overall scoring

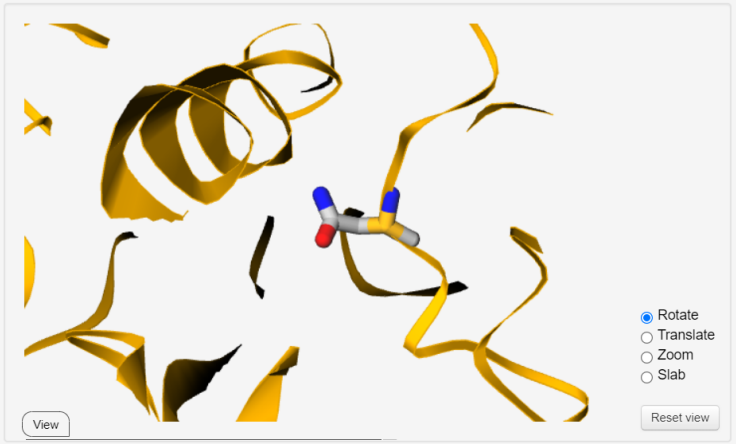
I-TASSER
Protein Structure & Function Predictions

mCSM Predicted Stability Change (ΔΔG):
-0.659 kcal/mol (*Destabilizing*)

SDM Predicted Stability Change (ΔΔG):
0.92 kcal/mol (*Stabilizing*)

DUET Predicted Stability Change (ΔΔG):
-0.197 kcal/mol (*Destabilizing*)

Mutation:
Wild-type: **ASN**
Position: **214**
Mutant-type: **ALA**
Chain: **A**
Secondary structure: **Loop or irregular**

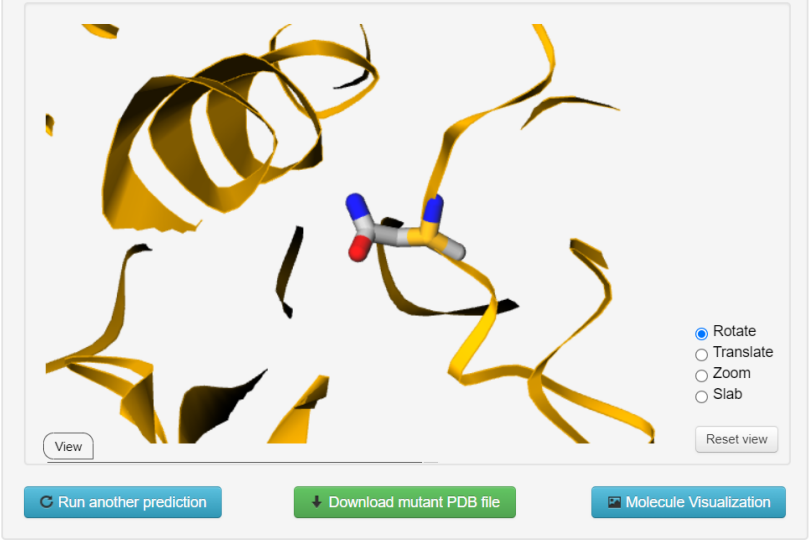


mCSM Predicted Stability Change (ΔΔG):
-0.7 kcal/mol (*Destabilizing*)

SDM Predicted Stability Change (ΔΔG):
0.74 kcal/mol (*Stabilizing*)

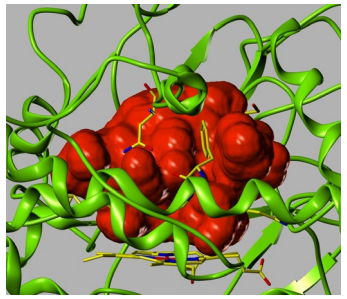
DUET Predicted Stability Change (ΔΔG):
-0.447 kcal/mol (*Destabilizing*)

Mutation:
Wild-type: **ASN**
Position: **214**
Mutant-type: **PHE**
Chain: **A**
Secondary structure: **Loop or irregular**

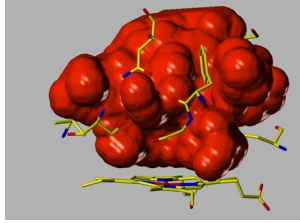


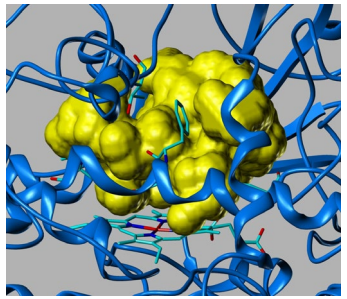
		ITASSER	100	Bayer	2500
		N214A	N214F	N214A	N214F
Duet predicted stability change	Raw model	-0.181	-0.5	0.293	-0.346
	Min model	-0.197	-0.447	-1.034	-1.267

A robust computational modeling approach predicting P450-mediated detoxification *in silico* largely fails due to the lack of respective bee P450 crystal structures

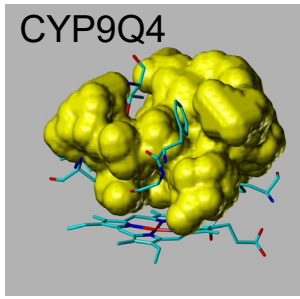


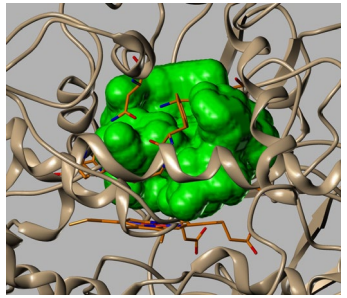
CYP9Q3



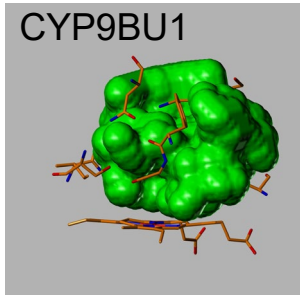


CYP9Q4

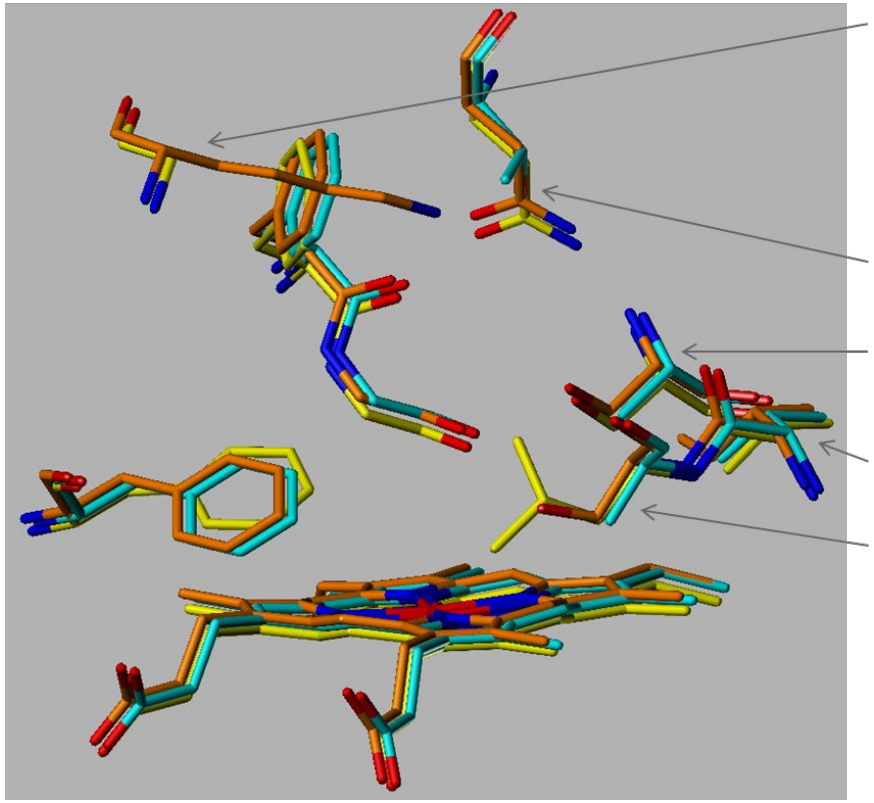




CYP9BU1



Q3 / Q4 / BU1



G111 / - / L111

The backbone loops were predicted significantly different in this region and so the equivalent residue is too far away from the catalytic oxygen

…

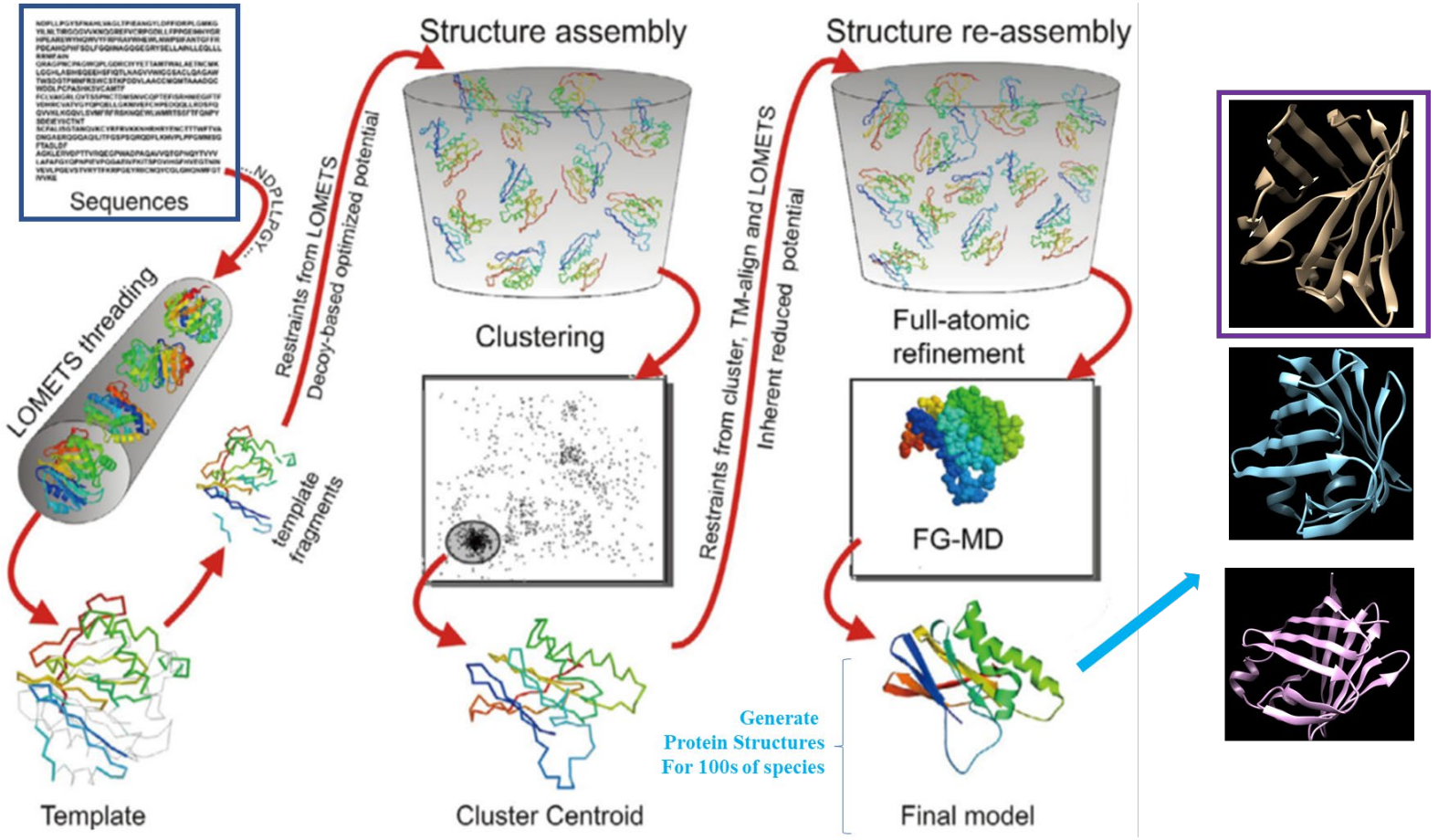
N214 / T218 / N214

S310 / A308 / S306

V371/ V369/ I367

L372 / A370 / S368

Creating homology models with the Iterative Threading ASSEmbly Refinement (ITASSER) tool



Models created for each species

- Apis mellifera
  - CYP9Q3 with restraint 1TQN (ITASSER)
  - CYP9Q3 with no restraint (ITASSER)
  - CYP9Q3 with no restraint (Phyre2)
- Bombus terrestris
  - CYP9Q4 with restraint 1TQN (ITASSER)
  - CYP9Q4 with no restraint (ITASSER)
  - CYP9Q4 with no restraint (Phyre2)
- Osmia bicornis
  - CYP9BU1 with restraint 1TQN (ITASSER)
  - CYP9BU1 with no restraint (ITASSER)
  - CYP9BU1 with no restraint (Phyre2)

Comparing model scores

ITASSER Scoring text

- Correlation coefficient of C-score of the first model with TM-score to the native structure is 0.91, while the coefficient of C-score with RMSD to the native structure is 0.75
- TM-score >0.5 indicates a model of correct topology and a TM-score<0.17 means a random similarity
- A higher cluster density means the structure occurs more often in the simulation trajectory and therefore signifies a better-quality model

Project	Protein	Template	Species	Model #	Cscore	TM-Score	RMSD	Decoys	Density
CYP9Q	CYP3A4	1TQN	Human	1 of 5	1.09	0.86+-0.07	5.0+-3.2	3316	0.556
CYP9Q	CYP9Q3	1TQN	Apis mellifera	1 of 5	1.01	0.85+-0.08	5.2+-3.3	4559	0.428
CYP9Q	CYP9Q4	1TQN	Bombus terrestris	1 of 5	0.83	0.83+-0.08	5.6+-3.5	2088	0.365
CYP9Q	CYP9BU1	1TQN	Osmia bicornis	1 of 5	0.57	0.79+-0.09	6.1+-3.8	1579	0.28
CYP9Q	CYP9Q3	None	Apis mellifera	1 of 5	1.07	0.86+-0.07	5.1+-3.3	4515	0.456
CYP9Q	CYP9Q4	None	Bombus terrestris	1 of 5	0.72	0.80+-0.09	5.9+-3.7	3153	0.326
CYP9Q	CYP9BU1	None	Osmia bicornis	1 of 5	0.54	0.79+-0.09	6.2+-3.8	1042	0.272

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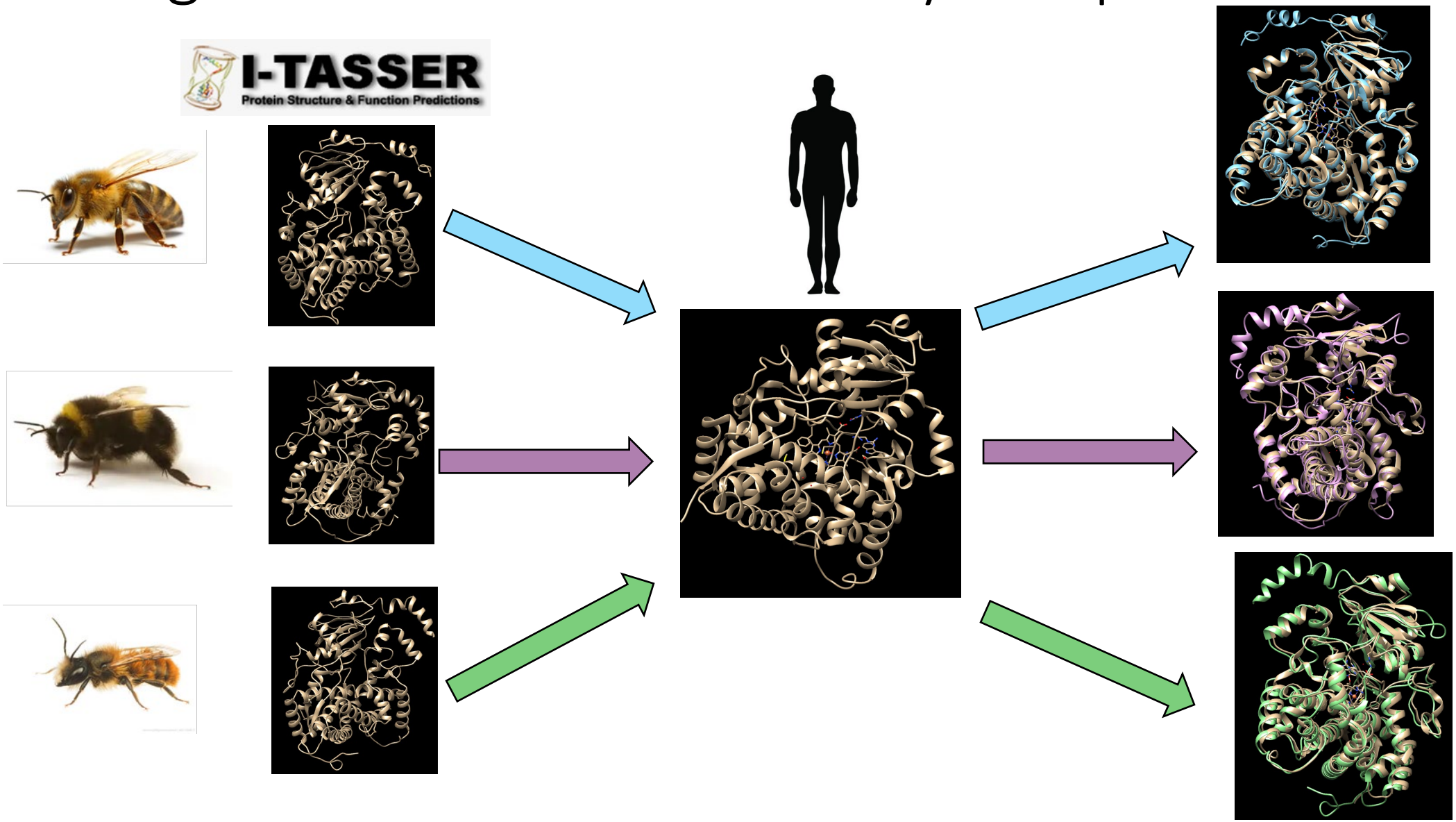
Utilizing UCSF Chimera to visually compare models



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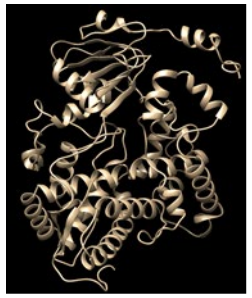
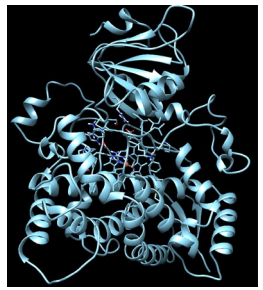
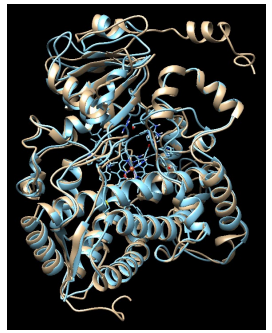
Utilizing UCSF Chimera to visually compare models

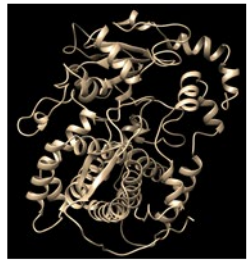
I-TASSER Protein Structure & Function Predictions

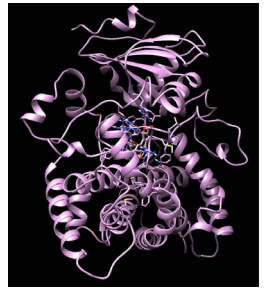
BAYER

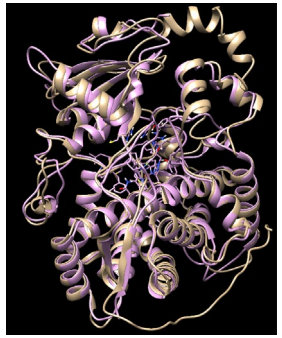


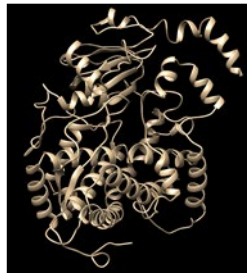


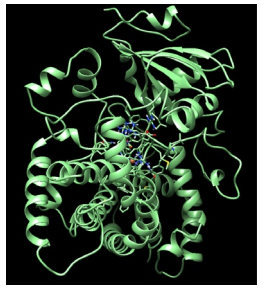


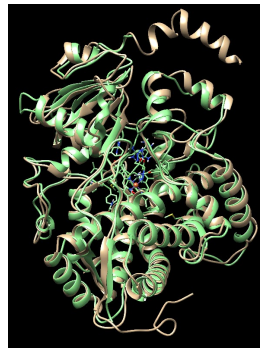












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Completed work recap and next possible steps…

- Minimize ITASSER structures to match Bayer .min structures
- Look at superimposed positions for critical amino acids
- Dock flupyradifurone and thiacloprid to structures and determine docking scores
- Point mutate Bayer models and analyze docking scores of flupyradifurone and thiacloprid
- Create homology models for other bee species of interest
  - Possible step