

WEB320 - Docking experiments for PFOMT Paper

Benjamin Weigel

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1 Docking using AutoDockTools and autodock4 (v. 4.2.5.1)

2 141125

2.1 1112

Receptor: 3c3y from PDB, waters deleted, hydrogens added

Ligand: S-Eriodictyol

Software: Yasara using dock_runensemble.mcr Makro

good Poses (orientation):

7, 13, (15), (20), 27, 33, 41 (4'), 51, 58, 73

cluster 5 (receptor ensemble 1/5), cluster 16 (ensemble 5/5)

2.2 1455

Receptor: 3c3y from PDB, waters deleted, hydrogens added;

mutations: Y51R, N202W (chainA)

Ligand: S-Eriodictyol

Software: Yasara using dock_runensemble.mcr Makro

good Poses (orientation):

7, 13, (15), (20), 27, 33, 41 (4'), 51, 58, 73

cluster 5 (receptor ensemble 1/5), cluster 16 (ensemble 5/5)

3 Docking using Vina - 20140105

3.1 Setup

- loaded 3c3y into pymol
- removed waters from structure and saved :

Table 1: Parameters of docking

Name	Ligand	Macromolecule	Grid center (xyz)	grid dimensions (xyz)	grid spacing (Å)
taxifolin.dlg	taxifolin	PFOMT + Ca ²⁺	1.581, 5.196, 25.718	58, 54, 68	0.375
taxifolin.b.dlg	taxifolin	only PFOMT	1.581, 5.196, 25.718	58, 54, 68	0.375
taxifolin.c.dlg	taxifolin	PFOMT + SAH + Ca ²⁺	1.581, 5.196, 25.718	58, 54, 68	0.375
taxifolin.c2.dlg	taxifolin	PFOMT + SAH + Ca ²⁺	1.581, 3.196, 27.718	58, 54, 68	0.375
R-ED.a.dlg	R-eriodictyol	PFOMT + SAH + Ca ²⁺	1.581, 5.196, 25.718	58, 54, 68	0.375
S-ED.a.dlg	S-eriodictyol	PFOMT + SAH + Ca ²⁺	1.581, 5.196, 25.718	58, 54, 68	0.375

Table 2: Files associated with docking

Name (Docking-Log-File)	Ligand-File	Macromolecule-File
taxifolin.dlg	taxifolin	PFOMTChainA_noWater_addedHydrogens

```
fetch 3c3y
select H20, ////HOH
remove H20
save 20140105_3c3y_clean.pdb, 3c3y
```

- loaded clean structure into ADT
- added polar hydrogens: Edit -> Hydrogens -> Add - (polar only)
- to save: Grid -> Macromolecule -> Choose then select a filename to save

3.2 Vina 1.1.2 (May 11, 2011)

- grid center (xyz): 1.581, 5.196, 25.718
- box size (xyz, Å): 22, 20, 25
- typical configuration file for vina:

```
receptor = 20140105_3c3y_clean
ligand = S_ED

out = dock1_all
log = dock1_log

center_x = 1.581
center_y = 5.196
center_z = 25.718

size_x = 22
size_y = 20
size_z = 25

exhaustiveness = 25
```

- start calculation with: vina --config <configfile.cfg>

3.3 Mutating Y51R and N202W using Pymol Mutagenesis

Used Pymol to mutate the residues Tyr51 and Asn202 to Arg and Trp, respectively. Unfortunately, every rotamer of **Tryptophane** clashes with other residues in the active site. Used rotamer number 7, which has the least clashes to mutate.

Table 3: Parameters of *vina* docking

Output (*.pdbsqt)	Log	Config (*.cfg)	Ligand	Macromolecule	minimization FF	comment
dock1_all	dock1_log	dock1	S-ED	PFOMT	-	rigid receptor
dock1_flex1_all	dock1_flex1_log	dock_flex1	S-ED	PFOMT	-	flexible Y51 and N202
dock_flex2_all	dock_flex2_log	dock_flex2	S-ED	PFOMT	amber03	flexible resis: 51, 157, 184, 202
dock_flex3_all	dock_flex3_log	dock_flex3	S-ED	PFOMT	yasara	flexible resis: 51, 157, 202
dock_flex4_all	dock_flex4_log	dock_flex4	S-ED	PFOMT	yasara	yasara for mutation; flexible resis: 51, 157, 184, 202
dock_flex5_all	dock_flex5_log	dock_flex5	S-ED	PFOMT	-	no minimization; flexible resis: 51, 157
dock_flex6_all	dock_flex6_log	dock_flex6	R-ED	PFOMT	-	flexible resis: 51, 202
dock_flex7_all	dock_flex7_log	dock_flex7	S-ED	PFOMT	-	flexible resis: 51, 157, 184, 202
dock8_all	dock8_log	dock8	S-ED	R51W202	amber03	rigid
dock9_all	dock9_log	dock9	S-ED	R51W202	yasara	rigid
dock10_all	dock10_log	dock10	S-ED	R51W202	yasara	yasara for mutation, rigid
dock11_all	dock11_log	dock11	S-ED	PFOMT	-	rigid receptor

View that was used below (Figure 1):

```
set_view (\n    0.387520641,    0.813636184,   -0.433348358,\n    -0.865751505,    0.482709885,   0.132122964,\n    0.316683739,    0.323972791,   0.891478777,\n    0.000000000,    0.000000000,  -84.650573730,\n    4.299249649,    -5.553125381,   28.580125809,\n    66.064750671,   103.236396790,  -20.000000000 )
```

Minimized the energy of the structure using amber03 and yasara forcefields to prevent clashing. The minimized structures (Figure 2) align equally well. The most movement is in the residue W202. Most notably, the tryptophane residue adopts another conformation, when using Yasara to mutate the N202.

- used Yasara to do an energy minimization.
- the forcefields used for minimization were *amber03* and *yasara*

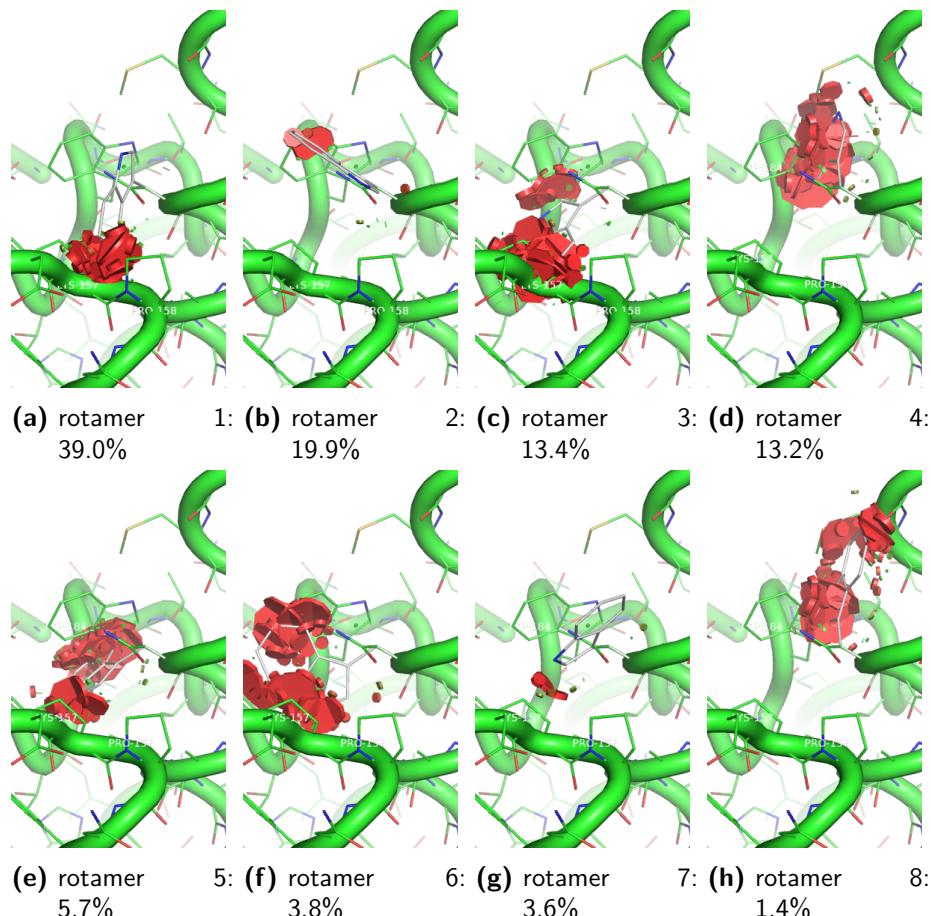
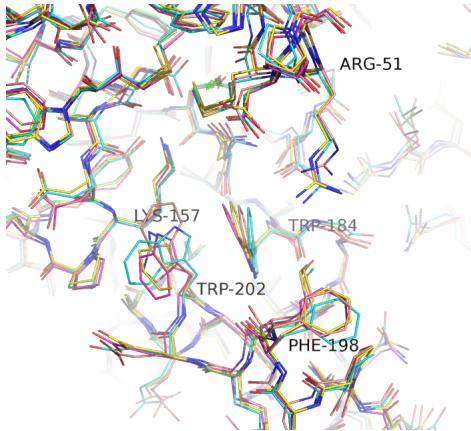


Figure 1: Mutagenesis of Asn202 to tryptophane using Pymol. Clashes with Trp184, Pro158 and Lys157 are common.



```

set_view (\n
    0.817390680, -0.485194504, 0.310517728,\n
    0.283943981, 0.808350325, 0.515652537,\n
    -0.501217663, -0.333326846, 0.798540056,\n
    0.000000000, 0.000000000, -109.694801331,\n
    4.772169590, -4.331441402, 31.016996384,\n
    95.474258423, 123.915359497, -20.000000000 )

```

Figure 2: Alignment of differently energy minimized structures of the mutated Y51R N202W PFOMT. **yellow** - unminimized (mutated using PyMol), **magenta** - minimized using yasara FF, **cyan** - minimized using amber03 FF, **orangeish** - mutated using yasara, minimized using yasara FF.

All docking poses were examined by eye using pymol. Poses, that were chemically sensible, were flagged with a `chemical_sense` tag in a table for later analysis using R (`chemical_sense = TRUE`). The distances of the 3' and 4' oxygen atoms to the sulfur atom in SAH were calculated for all poses using a Pymol script using the formula for the *euclidean distance*:

$$d(p, q) = d(q, p) = \sqrt{\sum_{i=1}^n (q_i - p_i)^2} \quad (1)$$

```

# — StateGetDistances.py
#
# from pymol import cmd, stored
import math
import numpy

def state_get_dist( sel1 , sel2 , writeout ):
    """
DESCRIPTION
Brief description what this function does goes here
@sel1 — selector for the receptor
@sel2 — selector for the ligand
@writeout — filename to write the data to
"""

# to store coordinates
stored.sel1 = []
stored.sel2 = []
# for the distances
dist = []

cmd.iterate_state(0, selector.process(sel1), 'stored.sel1.append((x,y,z))')
cmd.iterate_state(0, selector.process(sel2), 'stored.sel2.append((x,y,z))')

```

```

# calculate the distances between sel1 and sel2
if (len(stored.sel1)) == 1:
    L = len(stored.sel2)
    assert( L > 0 )
    for x in range(L):
        dist.append( math.sqrt ( (stored.sel1[0][0] - stored.sel2[x][0])**2 +
(stored.sel1[0][1] - stored.sel2[x][1])**2 +
(stored.sel1[0][2] - stored.sel2[x][2])**2 ) )
    else:
        assert( len(stored.sel1) == len(stored.sel2) )
        L = len(stored.sel2)
        assert( L > 0 )
        for x in range(L):
            dist.append( math.sqrt ( (stored.sel1[x][0] - stored.sel2[x][0])**2 +
(stored.sel1[x][1] - stored.sel2[x][1])**2 +
(stored.sel1[x][2] - stored.sel2[x][2])**2 ) )

id1 = cmd.identify(sel1, mode = 1)
id2 = cmd.identify(sel2, mode = 1)

print "Distances between %s and %s:" % (id1, id2)
print dist

with open(writeout, 'w') as f:
    f.write('Distance between ID1 = ' + str(id1) + ' and ID2 = ' + str(id2) + ':\n')
    f.write('\n'.join( map(str, dist) ) )

cmd.extend( "state_get_dist", state_get_dist );

```

If a pose had a greater distance between 3'O-S than between 4'O-S and was chemically sensible, it was also flagged with a switched = TRUE.

3.4 Results of Vina Docking

The following shows the summary of all dockings. Generally, a pose was chemically sensible if the distance **O-S** was below 4.5 Å (Figure 3a). The type of oxygen atom (3' or 4') did not matter for this distinction. The unproductive poses (Fig. 4d) showed high distances **O-S**. The conformation **C** (Fig. 4c) almost exclusively showed a 4' productive conformation.

The docking itself performed well for the wildtype PFOMT, where about 40% of the poses were chemically sound (Table 5, Figure 5). Only 12.5% of these poses showed 4' specificity. However, in the variant only 16% of the poses made sense chemically, but 78% of these showed 4' specificity (Table 6).

3.5 Poses selected for PFOMT Paper

- selected for wildtype poses: rigid docking 1 using state 1 for pose B and state 2 for pose A
- selected for variant poses: rigid docking 9 using state 2 for pose A and state 6 for pose C
- made pictures for each pose with SAH from xtal structure and SAM from previous docking
- **viewX_wt_SAM_poseA.png** selected as **Fig. 4 for paper**

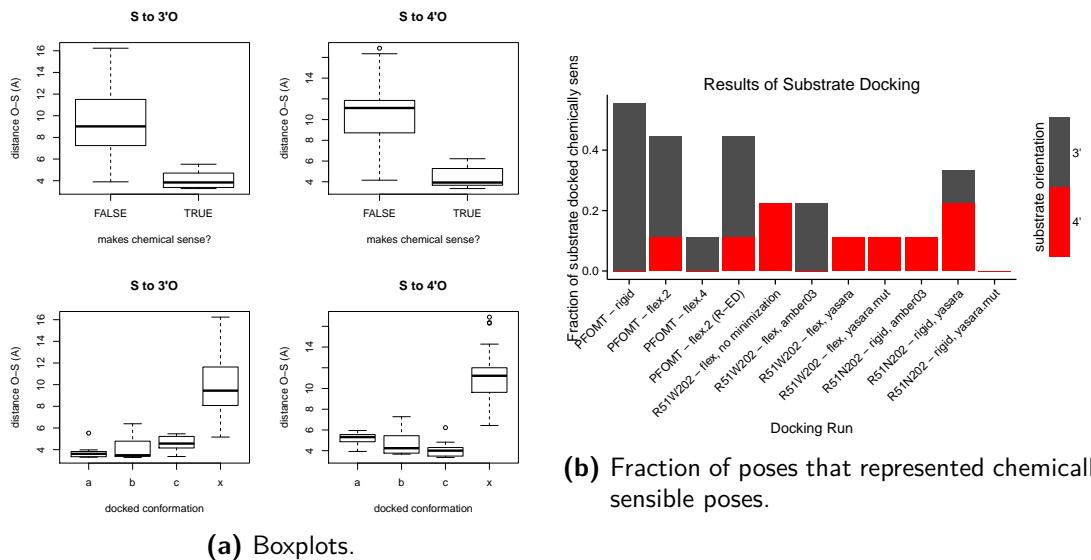


Figure 3: Graphical Summary of Dockings.

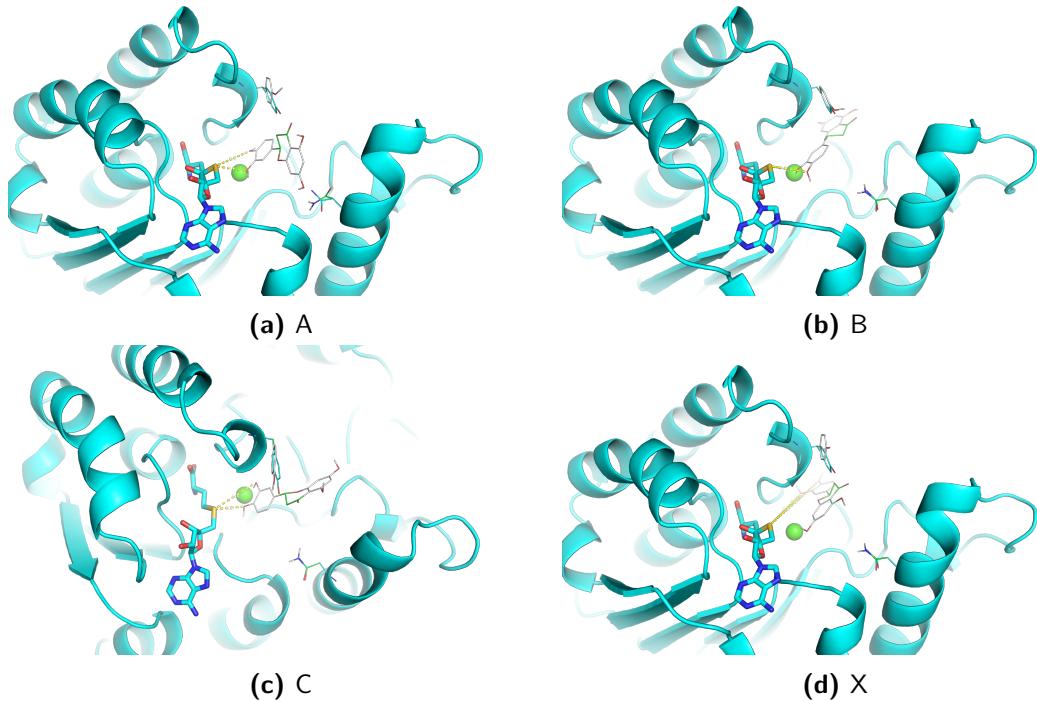


Figure 4: Different docking poses taken from flexible docking number 1. There were four main docking poses in all dockings. **a** - the pose that was also discussed in ???, **b** - another different pose, that should also lead to product, **c** - this pose usually adapted a pro-4' conformation, **d** - all other non chemically sensible poses

Table 4: Fraction of poses that were switched in specificity and that were chemically sound.

	dockrun	wt	switched	chemical_sense
1	1r	TRUE	0.00	0.56
2	1	TRUE	0.25	0.44
3	7	TRUE	0.00	0.11
4	6	TRUE	0.25	0.44
5	5	FALSE	1.00	0.22
6	2	FALSE	0.00	0.22
7	3	FALSE	1.00	0.11
8	4	FALSE	1.00	0.11
9	8	FALSE	1.00	0.11
10	9	FALSE	0.67	0.33
11	10	FALSE		0.00

Table 5: *summary* table for wildtype PFOMT (ref. Table 4).

```
##      dockrun      wt          switched      chemical_sense
## 1r      :1 Mode:logical Min.   :0.000 Min.   :0.1111
## 1       :1 TRUE:4        1st Qu.:0.000 1st Qu.:0.3611
## 7       :1 NA's:0        Median :0.125 Median :0.4444
## 6       :1             Mean   :0.125 Mean   :0.3889
## 5       :0             3rd Qu.:0.250 3rd Qu.:0.4722
## 2       :0             Max.   :0.250 Max.   :0.5556
## (Other):0
```

Table 6: *summary* table for variants (ref. Table 4).

```
##      dockrun      wt          switched      chemical_sense
## 5       :1 Mode :logical Min.   :0.0000 Min.   :0.0000
## 2       :1 FALSE:7        1st Qu.:0.7500 1st Qu.:0.1111
## 3       :1 NA's :0        Median :1.0000 Median :0.1111
## 4       :1             Mean   :0.7778 Mean   :0.1587
## 8       :1             3rd Qu.:1.0000 3rd Qu.:0.2222
## 9       :1             Max.   :1.0000 Max.   :0.3333
## (Other):1             NA's   :1
```

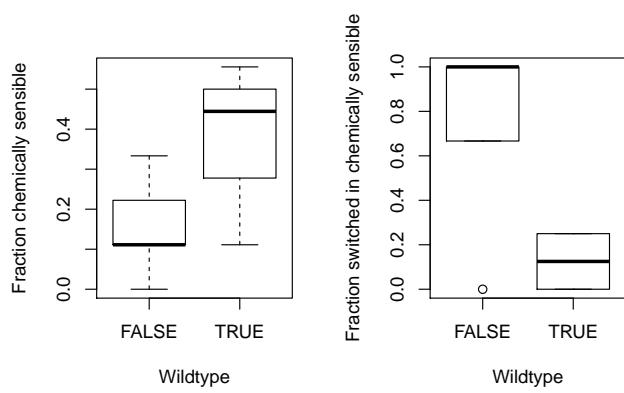


Figure 5: Graphical summary of the fraction of switched specificity (3' to 4') and the fraction that was chemically sensible.