20180326-28, LacI input structure prep

- started with the 2p9h_REDO (LacI + IPTG) structure from PDB Redo

2p9h_REDO.pdb 370 kB

- deleted the waters that are more than 6 Å from the ligand using delete_waters.py script:

2p9hR_delwaters.pdb 331 kB

- aligned the structure with 2paf (LacI + ONPF) to get the ligands in the binding pocket, then removed all the protein from the 2paf to retain only ONPF and renamed the molecules as residues 998 + 999 / chain C + D
- also grouped and colored all the residues that don't align too well between 2paf and 2p9hR in the pymol session file - a lot of these are high-entropy sidechains like GLN and ASN

2p9hR_2paf.pse 3 MB

2paf_aligned.pdb 4 kB

- removed IPTG and waters from 2p9hR

2p9hR_noIPTG.pdb 318 kB

- added ONPF to 2p9hR and Rosetta-renumbered the new structure, still leaving out the waters, using generate_gridlig_posfile_from_PDB.py

2P9HR_NPF_clean.pdb 321 kB

- went back into 2p9hR_delwaters.pdb to further **prune the ligand binding pocket waters** (no hydrogens - only oxygens)
- 11 in chain A and 8 in chain B

2p9hR_delwaters	Chain ID	2P9HR_NPF_W	B-factor	Notes
999	A		21.01	far away
1002	A	W 541	21.21	IPTG O3 (102
1004	A		19.22	far away
1005	A	W 542	28.86	binds IPTG O
1011	A	W 543	21.54	IPTG O3, O4?
1023	A		32.76	less importar
1027	A	W 544	26.69	important O6
1028	A		20.72	less importar

1045	A	W 545	28.12	important? C
1051	A		29.20	less importar
1096	A	W 546	28.23	important? C
1003	B	W 547	22.73	IPTG O3, O4?
1025	B		27.49	less importar
1026	B	W 548	19.03	IPTG O3 (100
1029	B	W 549	25.94	important? C
1035	B	W 550	25.70	binds IPTG O
1044	B	W 551	32.57	important (O
1045	B	W 552	26.65	important? (
1051	B		30.08	less importar

- can easily get rid of 5 A waters and 2 B waters, leaving 6 in both, which are very likely the “same” 6 waters. some of the ones I’m getting rid of are in the binding pocket where the nitrophenyl ring of ONPF needs to go, anyway.
2P9H_NPF_W

2P9HR_NPF_W.pdb 322 kB

Some session files exploring hydrogen bonding networks around IPTG in 2p9hR_delwaters/2P9HR_NPF_clean

2P9HR_NPF_clean_2p9hR_delwaters_H… 2 MB

… dimer interface

2P9HR_NPF_clean_2p9hR_delwaters.pse 2 MB

… first + second shell around IPTG

- **added hydrogens** to the entire structure + ligand **by scoring** using the following command:
~/Rosetta/main/source/bin/score.macosclangrelease -database ~/Rosetta/main/database/ -s 2P9HR_NPF_W.pdb -out:output

2P9HR_NPF_W_H.pdb 826 kB

default.sc 2 kB

Scores:

SCORE: score fa_atr fa_rep fa_sol fa_intra_rep fa_intra_sol_xover4 lk_ball_wtd fa_elec pro_close hbond_sr_bb hbond_lr_bb hbond_bb_sc hbond_sc dslf_fa13 omega fa_dun p_aa_pp yhh_planarity ref rama_prepro allatom_rms gdtmm gdtmm1_1 gdtmm2_2 gdtmm3_3 gdtmm4_3 gdtmm7_4 irms maxsub maxsub2.0 rms description

SCORE: -186.568 -3527.059 895.803 2125.887 5.852 105.487 -58.518 -843.696 24.661 -215.876 -113.584 -64.929 -62.160 0.000 171.838 1087.221 -72.524 0.000 268.688 86.342 0.000 1.000 1.000 1.000 1.000 1.000 1.000 0.000 538.000 538.000 0.000 2P9HR_NPF_W_0001

- **molfile_to_params:** extracted NPF lines from 2P9HR_NPF_W_H into two separate files

NPF1.pdb 3 kB

NPF2.pdb 3 kB

- made a few corrections to the structure and valences as per previous ONPF molecule prep and saved as .mol2 files

NPF1.mol2 4 kB

NPF2.mol2 4 kB

- ran molfile_to_params using the following command:
python ~/Rosetta/main/source/scripts/python/public/molfile_to_params.py -n NPF2 molfile_to_params/NPF2.mol2
output files:

NPF1_0001.pdb 3 kB

NPF2_0001.pdb 3 kB

PF1.params 5 kB

PF2.params 5 kB

- renamed ligands PF1, PF2 to distinguish between them, replaced chains C and D in the structure and and corrected HETATM lines in PDB to have continuous atom numbers and chain IDs C, D instead of X
new PDB:

2P9HR_correctNPF.pdb 652 kB

- **relaxed with constraints** on guybrush (overnight) using the command:
~/Rosetta/main/source/bin/relax.linuxgccrelease -database ~/Rosetta/main/database/ -in:file:s 2P9HR_correctNPF.pdb -in:file:fullatom -relax:constrain_relax_to_start_coords -extra_res_fa PF1.params PF2.params

output: aligning relaxed structure with original, RMS 0.499

- movement in B-chain ligand but not too much in A-chain
- waters rotated but O did not change place (treated like BB heavy atom because of constraints)
- total score: -1804.033 (10x increase in magnitude from original structure) - smaller fa_dun, fa_rep, fa_sol, hbond terms; a little higher fa_atr.

```
SCORE: total_score dslf_fa13    fa_atr    fa_dun   fa_elec fa_intra_rep fa_intra_sol_xover4
fa_rep              fa_sol hbond_bb_sc hbond_lr_bb    hbond_sc hbond_sr_bb lk_ball_wtd       omega
p_aa_pp pro_close rama_prepro         ref yhh_planarity description
 SCORE:   -1804.033     0.000 -3423.393   539.014 -1049.184        5.696              93.707
400.822            2017.762     -93.605    -120.917    -124.349    -223.912     -81.255      80.160
-106.336     2.332       9.838     268.688         0.900 2P9HR_correctNPF_0001
```

2P9HR_correctNPF_relaxed.pdb 749 kB

score.sc 574 B

Score terms comparison between relaxed with constraints and original structures

score term	2P9H_correctNPF	2P9HR_correctNPF_relaxed
total_score	-186.568	-1804.033
fa_atr	-3527.059	-3423.393
fa_dun	1087.221	539.014
fa_elec	-843.696	-1049.184
fa_intra_rep	5.852	5.696
fa_intra_sol_xover4	105.487	93.707
fa_rep	895.803	400.822
fa_sol	2125.887	2017.762
hbond_bb_sc	-64.929	-93.605
hbond_lr_bb	-113.584	-120.917
hbond_sc	-62.160	-124.349
hbond_sr_bb	-215.876	-223.912
lk_ball_wtd	-58.518	-81.255

omega	171.838	80.160
p_aa_pp	-72.524	-106.336
pro_close	24.661	2.332
rama_prepro	86.342	9.838
ref	268.688	268.688
yhh_planarity	0.000	0.900