# coupled moves, LacI + ONPF vs LacI + IPTG

**full results:**

ONPF: https://www.dropbox.com/sh/cx1ton7r6trgp26/AABwNCukxLfxFZJ22H7OKdcqa?dl=0 (20170504_CM_LacI_ONPF/20170506_with_hydrogens)

IPTG: https://www.dropbox.com/sh/kjjf80b002q0rmz/AAA0C7zp0HqFxT7GifhaOryta?dl=0 (20170510_LacI_IPTG/20170511_with_hydrogens)

**structure/input files prep:** (for IPTG, see note from 5/5 for preparing ONPF)

1. remove IPTG, chain B, waters from 2p9h for monomer w/o ligand

   2p9h_IPTG_A.pdb 204 kB

2. prepare new pdb file for IPTG ligand by adding hydrogens using babel, and save as mol2 file in Avogadro

   ```
   > babel -h IPTG.pdb IPTG_withH.sdf
   ```

   IPTG_withH.pdb 6 kB

   IPTG_withH.mol2 3 kB

3. ran molfile_to_params to generate params file

   IPTG.params 5 kB

4. renamed chain to X in the pdb file and added back into protein structure

   IPTG_chainX.pdb 3 kB

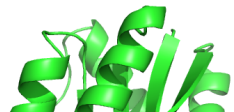
   2p9h_IPTG_AX.pdb 166 kB

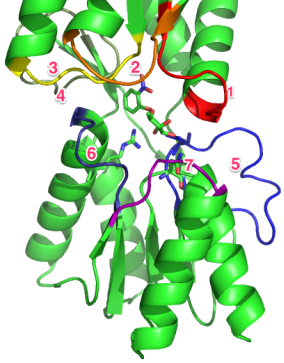
5. protonated 2p9h_IPTG_AX by scoring *(see notes below, scoring log)* and copied to local host. this removes the ligand again, so put the hydrogenated ligand back in and checked to make sure it was in the right place by superimposing 2p9h_IPTG_AX.pdb with 2p9h_IPTG_AX_H_IPTG.pdb

   2p9h_IPTG_AX_H.pdb 395 kB

   2p9h_IPTG_AX_H_IPTG.pdb 399 kB

**writing resfiles:**






1. annotated all LacI loops near the ligand, including +1 residue on either side of the loop for maximum backrub coverage
   loop1: 67-75
   loop2: 98-104
   loop3: 125-130
   loop4: 147-158
   loop5: 272-288
   loop6: 186-192
   loop7: 127-222
   *note: loops 1, 2, 5 are partially contact the dimerization interface.*

   LacI_ONPF_loops.pse 668 kB

2. wrote similar resfiles for each loop *(see notes below, resfiles)*. restricted all residues except for those on the loop to native rotamers.

**running coupled moves:**

1. in lacI/ folder: IPTG.params, input pdb file (2p9h_IPTG_AX_H_IPTG.pdb), and seven loop folders
2. in each loop folder: resfile (loopx.res), and python script to run CM on iqint, on that loop (run_coupledmoves_loopx.py)
   arguments: `['/netapp/home/anum/Rosetta/main/source/bin/coupled_moves.linuxgccrelease', '-s /netapp/home/anum/lacI/2p9h_IPTG_AX_H_IPTG.pdb', '-resfile /netapp/home/anum/lacI/loop1/loop1.res', '-database /netapp/home/anum/Rosetta/main/database', '-extra_res_fa /netapp/home/anum/lacI/IPTG.params', '-mute protocols.backrub.BackrubMover', '-ex1', '-ex2', '-overwrite', '-extrachi_cutoff 0', '-nstruct 20', '-coupled_moves::mc_kt 0.6', '-coupled_moves::ntrials 1000', '-coupled_moves::initial_repack false', '-coupled_moves::ligand_mode true', '-coupled_moves::ligand_weight 2.0', '-ignore_unrecognized_res']` (link to run script)
3. copied output files to local host.

**notes:**

scoring log for protonating protein input file

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```
[anum@iq218 lacI]$ ~/Rosetta/main/source/bin/score.linuxgccrelease -database ~/Rosetta/main/database/ -s 2p9h_IPTG_AX.pdb -ignore_unrecognized_res
```

```
-out:output
core.init: Rosetta version unknown:exported  from http://www.rosettacommons.org
core.init: command: /netapp/home/anum/Rosetta/main/source/bin/score.linuxgccrelease -database /netapp/home/anum/Rosetta/main/database/ -s
2p9h_IPTG_AX.pdb -ignore_unrecognized_res -out:output
core.init: 'RNG device' seed mode, using '/dev/urandom', seed=538322729 seed_offset=0 real_seed=538322729
core.init.random: RandomGenerator:init: Normal mode, seed=538322729 RG_type=mt19937



 Rosetta Tool:   score   -  rescores PDBs and silent files, extracts PDBs from silent files, assembles PDBs into silent files.
 Usage:
   PDB input:      -in:file:s *.pdb   or
                   -in:file:l  list_of_pdbs
                   -no_optH                                  Dont change positions of Hydrogen atoms! (default true, specify false if you want
optH)
   Silent input:   -in:file:silent silent.out                silent input filesname
                   -in:file:tags                             specify specific tags to be extracted, if left out all will be taken
                   -in:file:fullatom                         for full atom structures
                   -in:file:binary_silentfile                for non-ideal structures (such as from looprelax)
                   -in:file:silent_optH                      Call optH when reading silent files (useful for HisD/HisE determination)
                   -score_app:linmin                         Run a quick linmin before scoring
   Native:         -in:file:native                           native PDB (rms, maxsub and gdtm scores will be calculated)
   Scorefunction:  -score:weights  weights                   weight set or weights file
                   -score:patch  patch                       patch set
                   -score:optH_weights                       Weights file for optH (default standard.wts w/ sc12 patch)
                   -score:optH_patch                         Weights patch file for optH
                   -rescore:verbose                          display score breakdown
   Output:         -out:nooutput                             don't print PDB structures (default now)
                   -out:output                               force printing of PDB structures
                   -out:file:silent                          write silent-out file
                   -out:file:scorefile name                  write scorefile (default default.sc)
                   -out:prefix  myprefix                     prefix the output structures with a string
  Examples:
   score  -database ~/minirosetta_database -in:file:silent silent.out -in::file::binary_silentfile -in::file::fullatom -native 1a19.pdb
   Will rescore all structures in silent.out, in full atom mode and accounting for nonideal structure if present. Additionally
   it will print a PDB for every structure with -out:output flag



core.scoring.ScoreFunctionFactory: SCOREFUNCTION:
talaris2014
```

```
core.scoring.etable: Starting energy table calculation
core.scoring.etable: smooth_etable: changing atr/rep split to bottom of energy well
core.scoring.etable: smooth_etable: spline smoothing lj etables (maxdis = 6)
core.scoring.etable: smooth_etable: spline smoothing solvation etables (max_dis = 6)
core.scoring.etable: Finished calculating energy tables.
basic.io.database: Database file opened: scoring/score_functions/hbonds/sp2_elec_params/HBPoly1D.csv
basic.io.database: Database file opened: scoring/score_functions/hbonds/sp2_elec_params/HBFadeIntervals.csv
basic.io.database: Database file opened: scoring/score_functions/hbonds/sp2_elec_params/HBEval.csv
basic.io.database: Database file opened: scoring/score_functions/rama/Rama_smooth_dyn.dat_ss_6.4
basic.io.database: Database file opened: scoring/score_functions/P_AA_pp/P_AA
basic.io.database: Database file opened: scoring/score_functions/P_AA_pp/P_AA_n
basic.io.database: Database file opened: scoring/score_functions/P_AA_pp/P_AA_pp
protocols.jobdist.main: PDB Output Mode
protocols.jobdist.JobDistributors: Looking for an available job: 1 1 2p9h_IPTG_AX.pdb 1
core.chemical.GlobalResidueTypeSet: Finished initializing fa_standard residue type set.  Created 544 residue types
core.chemical.GlobalResidueTypeSet: Total time to initialize 1.03 seconds.
core.import_pose.import_pose: File '2p9h_IPTG_AX.pdb' automatically determined to be of type PDB
core.conformation.Conformation: [ WARNING ] missing heavyatom:  OXT on residue LEU:CtermProteinFull 269
protocols.jobdist.main: Starting 2p9h_IPTG_AX_0001 ...
core.import_pose.import_pose: File '2p9h_IPTG_AX.pdb' automatically determined to be of type PDB
core.conformation.Conformation: [ WARNING ] missing heavyatom:  OXT on residue LEU:CtermProteinFull 269
core.pack.dunbrack.RotamerLibrary: Using Dunbrack library binary file '/netapp/home/anum/Rosetta/main/database/rotamer/ExtendedOpt1-
5/Dunbrack10.lib.bin'.
core.pack.dunbrack.RotamerLibrary: Dunbrack 2010 library took 0.44 seconds to load from binary
protocols.jobdist.main: Finished 2p9h_IPTG_AX_0001 in 4 seconds.
protocols.jobdist.main: Finished all 1 structures in 7 seconds.
```

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## resfiles - examples

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## loop1:

```
NATRO
START
67 - 75 A ALLAA
```

## loop5 (one immovable residue, makes contact with hydroxyl group on ligand sugar ring):

```
NATRO
START
272 - 288 A ALLAA
274 A PIKAA D
```

run script - examp