

Sampling more rotamers at "hot" spots

Why do we need "hot" spots?

In some cases, we are interested in what's going on in some particular sites rather than the whole structure. MCCE provides a mechanism to define these spots and sample more rotamers subsequently. These sites are called "hot" spots.

How do we do it?

The mechanism is to define a hot spot file named "list_rot.gold" in the working directory. Hot spots are at residue level. One can put any one "ATOM" line of the hot residue in the original pdb file to list_rot.gold. The step 1 of mcce will use list_rot.gold to generate a rotamer making instruction file template head1.lst, then step 2 is able to use this head1.lst to make rotamers.

A working Example

Prepare the working directory

```
mkdir 4lzt  
cd 4lzt  
getpdb 4lzt
```

This should give you a pdb file 4lzt.pdb. We will use continuum water, so remove explicit water molecules:

```
grep -v HOH 4lzt.pdb > 4lzt_noHOH.pdb
```

Prepare list_rot.gold file

We know the enzymatic function of lysozymes comes from active sites GLU35 and ASP52, so we will treat these two residues as hot spots. Pick an atom record from each of these two residues and put them in file list_rot.gold.

```
$ cat list_rot.gold
```

ATOM	273	N	GLU A	35	6.492	16.383	22.526	1.00	7.03	N
ATOM	407	N	ASP A	52	5.550	6.804	30.040	1.00	5.90	N

Run step 1

```
step1.py 4lzt_noH0H.pdb
```

This will generate a file head1.lst. Check this file to see if residue GLU35 and residue ASP52 have more rotations. Some other residues with contact to these two "hot" residues will also be marked as more rotations. These extensive rotations are marked as "R t 12" which means Rotation is true and each bond will have 12 rotation steps.

```
$ grep "R t 12" head1.lst
```

GLU A0035_	R t 12	S f	0.0	H f	00	M 000
ASN A0044_	R t 12	S f	0.0	H f	00	M 000
ASN A0046_	R t 12	S f	0.0	H f	00	M 000
ASP A0052_	R t 12	S f	0.0	H f	00	M 000
GLN A0057_	R t 12	S f	0.0	H f	00	M 000
ASN A0059_	R t 12	S f	0.0	H f	00	M 000
TRP A0108_	R t 12	S f	0.0	H f	00	M 000
ALA A0110_	R t 12	S f	0.0	H f	00	M 000

You can manually modify the flags and rotation steps at this point (after step 1 and before step 2).

Another conformer making rule in head1.lst is S (swing). When set to "t", you can give it an angle in degrees. 15 degrees are recommended. H and M flags can be ignored.

Run step 2 using "head1.lst"

By default, MCCE ignores head1.lst. To use head1.lst as rotamer making guide, we will use command:

```
step2.py -u ROT_SPECIF=t,PACK=t
```

The two user custom options are defined in "-u" switch, separated by ",". ROT_SPECIFIC=t indicates we will use head1.lst as the rotamer making instruction, and PACK=t indicates we will make rotamers regardless of rotamer making level.

If we inspect rotamer making statistics file rot_stat after step 2, we will see something like this:

\$ cat rot_stat

Rotamer making statistics:

Residue	Start	Clean	Swap	Rotate	Self	Hbond	Repack	Ioni.	TorH	OH	Elect
NTRA0001	1	1	1	1	1	1	1	2	2	2	2
LYSA0001	1	1	1	1	1	1	1	2	2	2	2
VALA0002	1	1	1	1	1	1	1	1	1	1	1
PHEA0003	1	1	1	1	1	1	1	1	1	1	1
GLYA0004	0	0	0	0	0	0	0	0	0	0	0
ARGA0005	1	1	1	1	1	1	1	4	5	5	5
CYDA0006	1	1	1	1	1	1	1	1	1	1	1
GLUA0007	1	1	1	1	1	1	1	3	5	5	5
LEUA0008	1	1	1	1	1	1	1	1	1	1	1
ALAA0009	1	1	1	1	1	1	1	1	1	1	1
ALAA0010	1	1	1	1	1	1	1	1	1	1	1
ALAA0011	1	1	1	1	1	1	1	1	1	1	1
META0012	1	1	1	1	1	1	1	1	1	1	1
LYSA0013	1	1	1	1	1	2	1	2	2	2	2
ARGA0014	1	1	1	1	1	1	1	4	4	4	4
HISA0015	1	1	2	2	1	2	2	6	6	6	6
GLYA0016	0	0	0	0	0	0	0	0	0	0	0
LEUA0017	1	1	1	1	1	1	1	1	1	1	1
ASPA0018	1	1	1	1	1	1	1	3	5	5	5
ASNA0019	1	1	2	2	2	2	1	1	1	1	1
TYRA0020	1	1	1	1	1	2	1	2	3	3	3
ARGA0021	1	1	1	1	1	1	1	4	4	4	4
GLYA0022	0	0	0	0	0	0	0	0	0	0	0
TYRA0023	1	1	1	1	1	1	1	2	2	2	2
SERA0024	1	1	1	1	1	2	2	2	3	3	3
LEUA0025	1	1	1	1	1	1	1	1	1	1	1
GLYA0026	0	0	0	0	0	0	0	0	0	0	0
ASNA0027	1	1	2	2	2	4	2	2	2	2	2
TRPA0028	1	1	1	1	1	1	1	1	1	1	1
VALA0029	1	1	1	1	1	1	1	1	1	1	1
CYDA0030	1	1	1	1	1	1	1	1	1	1	1
ALAA0031	1	1	1	1	1	1	1	1	1	1	1
ALAA0032	1	1	1	1	1	1	1	1	1	1	1
LYSA0033	1	1	1	1	1	1	1	2	2	2	2
PHEA0034	1	1	1	1	1	1	1	1	1	1	1
GLUA0035	1	1	1	1729	242	245	15	45	73	73	73
SERA0036	1	1	1	1	1	1	1	1	2	2	2

[illegible]

ASNA0077	1	1	2	2	2	2	1	1	1	1	1
ILEA0078	1	1	1	1	1	1	1	1	1	1	1
PROA0079	1	1	1	1	1	1	1	1	1	1	1
CYDA0080	1	1	1	1	1	1	1	1	1	1	1
SERA0081	1	1	1	1	1	1	1	1	2	2	1
ALAA0082	1	1	1	1	1	1	1	1	1	1	1
LEUA0083	1	1	1	1	1	1	1	1	1	1	1
LEUA0084	1	1	1	1	1	1	1	1	1	1	1
SERA0085	1	1	1	1	1	1	1	1	3	3	3
SERA0086	1	1	1	1	1	1	1	1	2	2	2
ASPA0087	1	1	1	1	1	1	1	3	5	5	5
ILEA0088	1	1	1	1	1	1	1	1	1	1	1
THRA0089	1	1	1	1	1	2	1	1	3	3	3
ALAA0090	1	1	1	1	1	1	1	1	1	1	1
SERA0091	1	1	1	1	1	1	1	1	2	2	2
VALA0092	1	1	1	1	1	1	1	1	1	1	1
ASNA0093	1	1	2	2	2	2	2	2	2	2	2
CYDA0094	1	1	1	1	1	1	1	1	1	1	1
ALAA0095	1	1	1	1	1	1	1	1	1	1	1
LYSA0096	1	1	1	1	1	1	1	2	2	2	2
LYSA0097	1	1	1	1	1	1	1	2	2	2	2
ILEA0098	1	1	1	1	1	1	1	1	1	1	1
VALA0099	1	1	1	1	1	1	1	1	1	1	1
SERA0100	1	1	1	1	1	1	1	1	3	3	3
ASPA0101	1	1	1	1	1	1	1	3	5	5	5
GLYA0102	0	0	0	0	0	0	0	0	0	0	0
ASNA0103	1	1	2	2	2	2	2	2	2	2	2
GLYA0104	0	0	0	0	0	0	0	0	0	0	0
META0105	1	1	1	1	1	1	1	1	1	1	1
ASNA0106	1	1	2	2	2	2	2	2	2	2	2
ALAA0107	1	1	1	1	1	1	1	1	1	1	1
TRPA0108	1	1	1	145	10	18	2	2	2	2	2
VALA0109	1	1	1	1	1	1	1	1	1	1	1
ALAA0110	1	1	1	2	1	1	1	1	1	1	1
TRPA0111	1	1	1	1	1	2	1	1	1	1	1
ARGA0112	1	1	1	1	1	1	1	4	5	5	5
ASNA0113	1	1	2	2	2	2	2	2	2	2	2
ARGA0114	1	1	1	1	1	1	1	4	4	4	4
CYDA0115	1	1	1	1	1	1	1	1	1	1	1
LYSA0116	1	1	1	1	1	1	1	2	2	2	2

GLYA0117	0	0	0	0	0	0	0	0	0	0	0
THRA0118	1	1	1	1	1	1	1	1	2	2	2
ASPA0119	1	1	1	1	1	1	1	3	5	5	5
VALA0120	1	1	1	1	1	1	1	1	1	1	1
GLNA0121	1	1	2	2	2	2	2	2	2	2	2
ALAA0122	1	1	1	1	1	1	1	1	1	1	1
TRPA0123	1	1	1	1	1	1	1	1	1	1	1
ILEA0124	1	1	1	1	1	1	1	1	1	1	1
ARGA0125	1	1	1	1	1	1	1	4	5	5	5
GLYA0126	0	0	0	0	0	0	0	0	0	0	0
CYDA0127	1	1	1	1	1	1	1	1	1	1	1
ARGA0128	1	1	1	1	1	1	1	4	4	4	4
LEUA0129	1	1	1	1	1	1	1	1	1	1	1
CTRA0129	1	1	1	1	1	1	1	3	5	5	5
Total	119	119	137	4240	803	893	350	379	492	492	489

Certain residues at hot spots, hot residues defined in list_rot.gold and and residues in contact with the hot residues, are given more rotamers.

Step 3 and step 4

We can now proceed with step 3 and step 4 as we did in the [lysozymen pKa calculation example](#).

step3.py

step4.py

The hot spots may help relaxing structure in small regions. It is useful for big structures that a global comprehensive rotamer sampling at level 2 (with command step2.py -l 2) is too expensive.

Rotamer creation figure sourced from:

Seo, U., Kim, K.-J., & Kang, B. S. (2018). An Algorithm for Computing Side Chain Conformational Variations of a Protein Tunnel/Channel. *Molecules*, 23(10), 2459.
<https://doi.org/10.3390/molecules23102459>