Sampling more rotamers at "hot" spots

Why do we need "hot" spots?

In some cases, we are insterested 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 machanism 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 the enzymatic fuction of lysozymen 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_noHOH.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 alse 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).

Aother 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 statitics:
   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
```

ASNA0037	1	1	2	2	2	4	2	2	2	2	2
PHEA0038	1	1	1	1	1	1	1	1	1	1	1
ASNA0039	1	1	2	2	2	2	2	2	2	2	2
THRA0040	1	1	1	1	1	1	1	1	2	2	2
GLNA0041	1	1	2	2	2	2	2	2	2	2	2
ALAA0042	1	1	1	1	1	1	1	1	1	1	1
THRA0043	1	1	1	1	1	2	1	1	2	2	2
ASNA0044	1	1	2	146	71	84	50	20	16	16	16
ARGA0045	1	1	1	1	1	2	1	4	4	4	4
ASNA0046	1	1	2	145	55	64	56	20	17	17	17
THRA0047	1	1	1	1	1	1	1	1	3	3	2
ASPA0048	1	1	1	1	1	3	3	9	15	15	15
GLYA0049	0	0	0	0	0	0	0	0	0	0	0
SERA0050	1	1	1	1	1	4	4	4	12	12	12
THRA0051	1	1	1	1	1	1	1	1	2	2	2
ASPA0052	1	1	1	73	20	26	17	51	84	84	84
TYRA0053	1	1	1	1	1	2	1	2	3	3	3
GLYA0054	0	0	0	0	0	0	0	0	0	0	0
ILEA0055	1	1	1	1	1	1	1	1	1	1	1
LEUA0056	1	1	1	1	1	1	1	1	1	1	1
GLNA0057	1	1	2	1730	190	193	24	20	20	20	20
ILEA0058	1	1	1	1	1	1	1	1	1	1	1
ASNA0059	1	1	2	145	90	115	54	20	20	20	20
SERA0060	1	1	1	1	1	2	2	2	5	5	5
ARGA0061	1	1	1	1	1	1	1	4	5	5	5
TRPA0062	1	1	1	1	1	2	1	1	1	1	1
TRPA0063	1	1	1	1	1	1	1	1	1	1	1
CYDA0064	1	1	1	1	1	1	1	1	1	1	1
ASNA0065	1	1	2	2	2	2	2	2	2	2	2
ASPA0066	1	1	1	1	1	3	2	6	10	10	10
GLYA0067	0	0	0	0	0	0	0	0	0	0	0
ARGA0068	1	1	1	1	1	1	1	4	4	4	4
THRA0069	1	1	1	1	1	2	1	1	3	3	3
PROA0070	1	1	1	1	1	1	1	1	1	1	1
GLYA0071	0	0	0	0	0	0	0	0	0	0	0
SERA0072	1	1	1	1	1	1	1	1	2	2	1
ARGA0073	1	1	1	1	1	1	1	4	4	4	4
ASNA0074	1	1	2	2	2	2	2	2	2	2	2
LEUA0075	1	1	1	1	1	1	1	1	1	1	1
CYDA0076	1	1	1	1	1	1	1	1	1	1	1

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 complehensive 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

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