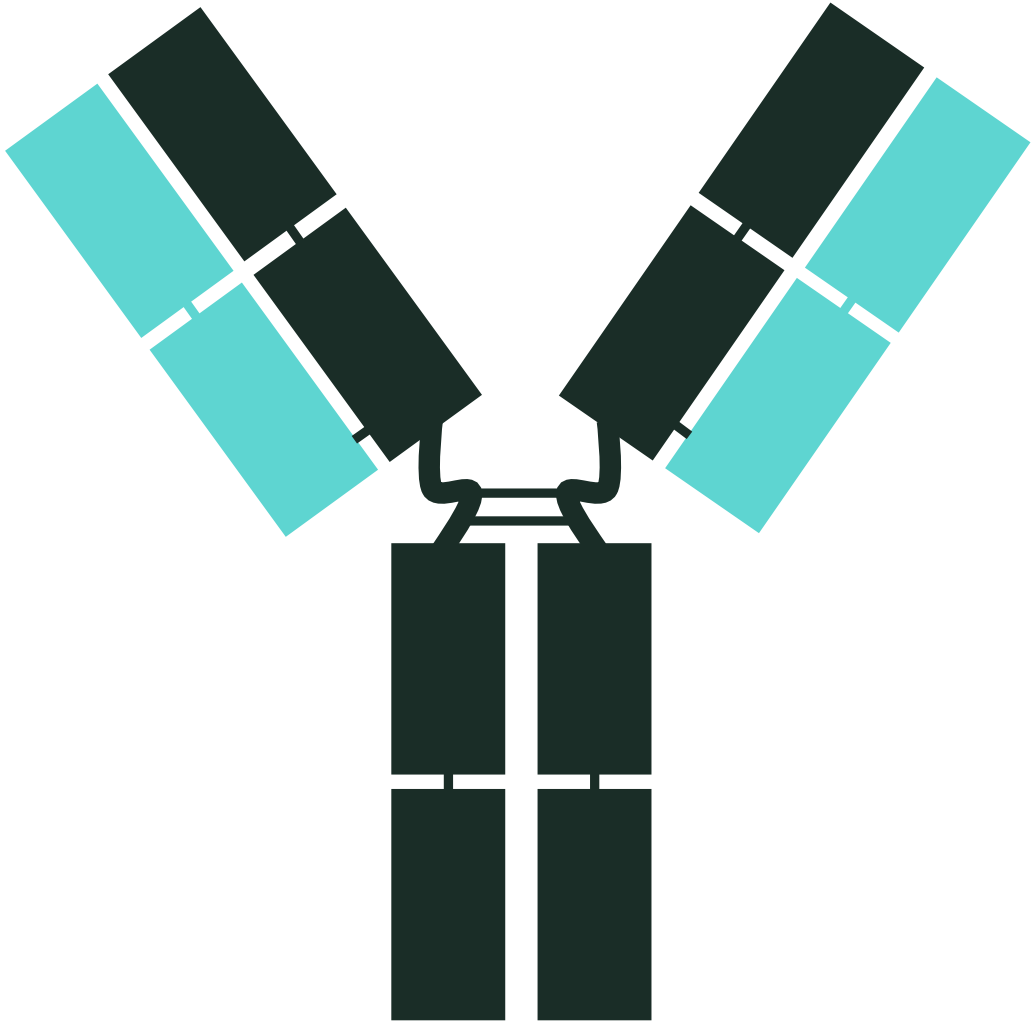
Goal:

To perform EOM analysis on SAXS data using the strategy published in Tian et al., IUCrJ. 2015 Jan 1;2(Pt 1):9-18.

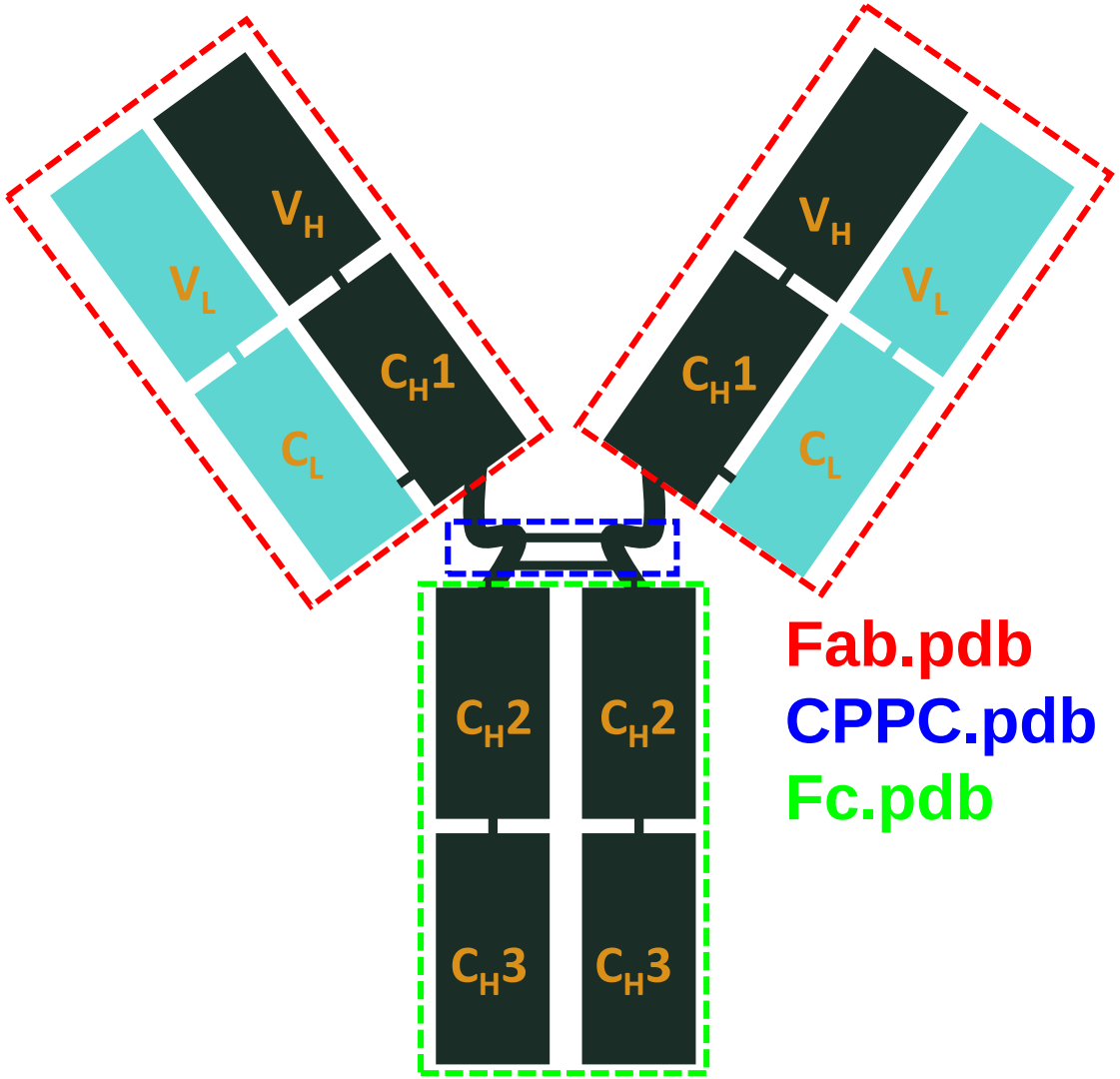
Problem:

1, When Fab + CPPC + Fc domains are all used for EOM, I got ERROR message "ERROR: the pool cannot be constructed".

2, When only Fab + Fc domains are used, the "CPPC" contact constrain is not defined.



Each IgG is a homodimer consisting two H (black) and two L (teal) chains



4 Domains: 2 Fab, 1 CPPC (inter-H disulfide bonds), 1 Fc

Inputs for EOM

Sequence file: IgG.seq (1 L chain followed by 1 H chain)

DTVLTQSPALAVSPGERVTISCRASDSVSTLMHWYQQKPGQQPKLLIYLASHLESGVPARFSGSGSGTDFTLTIDPVEADDTATY

YCQQSWNDPWTFGGGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS

TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECEVQLVESDGGLVQPGRSLKLPCAASGFTFSDYYMAWVRQAPT

KGLEWVASISYDGSSTYYRDSVKGRFTISRDNAKSTLYLQMDSLRSEDTATYYCGRHSSYFDYWGQGVMVTVSSATTKGPSVFPL

APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK

RVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS

TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES

NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

PDB files: Fab.pdb, CPPC.pdb, Fc.pdb

SAXS data file: IgG.dat

Terminal screenshots (Fab + CPPC + Fc domains → error)

```
fubin@ubuntu:~/Desktop/Shared/H/EOM/simple$ eom
*******  ------------------------------------------------------  *******
*******     Advanced Ensemble Optimization Method                *******
*******     EOM - version 2.1 - (r10552)                      *******
*******     Copyright (c) ATSAS Team                             *******
*******     EMBL, Hamburg Outstation, 2007 - 2014                *******
*******                                                          *******
*******     For doubts/questions please visit SAXIER forum:      *******
*******     http://www.saxier.org/forum/viewforum.php?f=10       *******
*******                                                          *******
*******     In case of bugs please refer to:                     *******
*******     G. Tria, D.I. Svergun, EMBL BioSAXS group            *******
*******     atsas@embl-hamburg.de                                *******
*******  ------------------------------------------------------  *******
Core symmetry? Select one of: (1) p1, (2) p2, (3) p3, (4) p4, (5) p5,
(6) p6, (7) p7, (8) p8, (9) p9, (10) p10, (11) p11, (12) p12, (13)
p13, (14) p14, (15) p15, (16) p16, (17) p17, (18) p18, (19) p19, (20)
p22, (21) p32, (22) p42, (23) p52, (24) p62, (25) p72, (26) p82, (27)
p92, (28) p102, (29) p112, (30) p122, (31) p222 (default: p1) ........ : 2
Overall symmetry? Select one of: (s) Symmetry, (a) Asymmetry, (m) Mix
(default: Mix) ..................................................... :
Percentage of Symmetric structures? (default: 50) ................... :
Chain type to generate? Select one of: (c) compact-chain, (n)
native-like, (r) random-coil (default: random-coil) ................. :
Sequence file name? ................................................ : IgG.seq
Number of domains? (max. 50) (default: 0) .......................... : 3
------ Domain  1 ------
PDB file name for the domain ....................................... : Fab.pdb
Does this PDB contain multiple chains (i.e. a predefined interface)?
(default: no) ...................................................... :
Fix the subunit in original position/orientation? (default: no) ..... :
PDB file for D/RNA bound to the subunit (CR for none) ............... :
------ Domain  2 ------
PDB file name for the domain ....................................... : CPPC.pdb
Does this PDB contain multiple chains (i.e. a predefined interface)?
(default: no) ...................................................... : yes
Fix the subunit in original position/orientation? (default: no) ..... : yes
PDB file for D/RNA bound to the subunit (CR for none) ............... :
------ Domain  3 ------
PDB file name for the domain ....................................... : Fc.pdb
Does this PDB contain multiple chains (i.e. a predefined interface)?
(default: no) ...................................................... : yes
Fix the subunit in original position/orientation? (default: no) ..... : yes
PDB file for D/RNA bound to the subunit (CR for none) ............... :
Total number of models to generate (default: 10000) ................. :
```

```
------ Domain  2 ------
PDB file name for the domain ....................................... : CPPC.pdb
Does this PDB contain multiple chains (i.e. a predefined interface)?
(default: no) ...................................................... : yes
Fix the subunit in original position/orientation? (default: no) ..... : yes
PDB file for D/RNA bound to the subunit (CR for none) ............... :
------ Domain  3 ------
PDB file name for the domain ....................................... : Fc.pdb
Does this PDB contain multiple chains (i.e. a predefined interface)?
(default: no) ...................................................... : yes
Fix the subunit in original position/orientation? (default: no) ..... : yes
PDB file for D/RNA bound to the subunit (CR for none) ............... :
Total number of models to generate (default: 10000) ................. :
File enumeration starting from? (default: 1) ........................ :
Save the generated PDB files? (default: no) ......................... :
Suffix of generated pdb files? (default: eom) ....................... : IgG
Calculate Intensity? (default: yes) ................................. :
Number of harmonics (min. 10, max. 50)? (default: 15) ............... :
Maximum s value, sm (min. 0.1, max. 0.5)? (default: 0.500) ........... :
Number of points (min. 10, max. 201)? (default: 51) ................. :
Run the Genetic Algorithm? (default: yes) ........................... :
How many times (min. 1)? (default: 1) ............................... :
Number of experimental curves to fit? (default: 1) .................. :
Experimental data file name  1? (*.dat) ............................. : IgG.dat
 Loading values and configuration ...
 Number of residues per chain:          659
 -------------------- ATTENTION! ---------------------
 - The domains specified as fixed may be too far away -
 - to be connected by the linker extracted from the   -
 - sequence. EOM 2.1 may not be able to make the pool.-
 -----------------------------------------------------
 ... starts making models ...
[  1%]
 ... completed making models

 ERROR: the pool cannot be constructed. Please check the input files
 Sequence used for pool generation (         659  residues per chain) :
DTVLTQSPALAVSPGERVTISCRASDSVSTLMHWYQQKPGQQPKLLIYLASHLESGVPARFSGSGSGTDFTLTIDPVEADDTATYY
CQQSWNDPWTFGGGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECEVQLVESDGGLVQPGRSLKLPCAASGFTFSDYYMAWVRQAPTKGL
EWVASISYDGSSTYYRDSVKGRFTISRDNAKSTLYLQMDSLRSEDTATYYCGRHSSYFDYWGQGVMVTVSSATTKGPSVFPLAPSS
KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK
SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
```

Terminal screenshots (Fab + Fc domains → running → results without CPPC disulfide bond constrain)

```
fubin@ubuntu:~/Desktop/Shared/H/EOM/simple$ eom
*******  ------------------------------------------------------  *******
*******     Advanced Ensemble Optimization Method                *******
*******     EOM - version 2.1 - (r10552)                      *******
*******     Copyright (c) ATSAS Team                             *******
*******     EMBL, Hamburg Outstation, 2007 - 2014                *******
*******                                                          *******
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*******     http://www.saxier.org/forum/viewforum.php?f=10       *******
*******                                                          *******
*******     In case of bugs please refer to:                     *******
*******     G. Tria, D.I. Svergun, EMBL BioSAXS group            *******
*******     atsas@embl-hamburg.de                                *******
*******  ------------------------------------------------------  *******
Core symmetry? Select one of: (1) p1, (2) p2, (3) p3, (4) p4, (5) p5,
(6) p6, (7) p7, (8) p8, (9) p9, (10) p10, (11) p11, (12) p12, (13)
p13, (14) p14, (15) p15, (16) p16, (17) p17, (18) p18, (19) p19, (20)
p22, (21) p32, (22) p42, (23) p52, (24) p62, (25) p72, (26) p82, (27)
p92, (28) p102, (29) p112, (30) p122, (31) p222 (default: p1) ........ : 2
Overall symmetry? Select one of: (s) Symmetry, (a) Asymmetry, (m) Mix
(default: Mix) ..................................................... :
Percentage of Symmetric structures? (default: 50) ................... :
Chain type to generate? Select one of: (c) compact-chain, (n)
native-like, (r) random-coil (default: random-coil) ................. :
Sequence file name? ................................................ : IgG.seq
Number of domains? (max. 50) (default: 0) .......................... : 2
------ Domain  1 ------
PDB file name for the domain ....................................... : Fab.pdb
Does this PDB contain multiple chains (i.e. a predefined interface)?
(default: no) ...................................................... :
Fix the subunit in original position/orientation? (default: no) ..... :
PDB file for D/RNA bound to the subunit (CR for none) ............... :
------ Domain  2 ------
PDB file name for the domain ....................................... : Fc.pdb
Does this PDB contain multiple chains (i.e. a predefined interface)?
(default: no) ...................................................... : yes
Fix the subunit in original position/orientation? (default: no) ..... : yes
PDB file for D/RNA bound to the subunit (CR for none) ............... :
```

```
------ Domain  2 ------
PDB file name for the domain ....................................... : Fc.pdb
Does this PDB contain multiple chains (i.e. a predefined interface)?
(default: no) ...................................................... : yes
Fix the subunit in original position/orientation? (default: no) ..... : yes
PDB file for D/RNA bound to the subunit (CR for none) ............... :
Total number of models to generate (default: 10000) ................. :
File enumeration starting from? (default: 1) ........................ :
Save the generated PDB files? (default: no) ......................... :
Suffix of generated pdb files? (default: eom) ....................... : IgG
Calculate Intensity? (default: yes) ................................. :
Number of harmonics (min. 10, max. 50)? (default: 15) ............... :
Maximum s value, sm (min. 0.1, max. 0.5)? (default: 0.500) ........... :
Number of points (min. 10, max. 201)? (default: 51) ................. :
Run the Genetic Algorithm? (default: yes) ........................... :
How many times (min. 1)? (default: 1) ............................... :
Number of experimental curves to fit? (default: 1) .................. :
Experimental data file name  1? (*.dat) ............................. : IgG.dat
 Loading values and configuration ...
 Number of residues per chain:          659
 ... starts making models ...
[  1%] >>>>>>>>>>>>>>>>>>>>>>>>>
```