





# **1    Exercise 6: In silico affinity maturation with “Physics where you want it”**

## **1.1 Objectives**

You will learn to create a “Physics where you want it” bubble about the site of a proposed mutation, locally equilibrate the mutant and wild type, and evaluate the change in binding energy. This will expose you to the following parameters and command:

- `physicsWhereYouWantIt`
- `smallGroupInertiaMultiplier`
- `globalBondTorsionScaleFactor`
- `globalBondStretchScaleFactor`
- `globalBondBendScaleFactor`
- `globalCoulombScaleFactor`
- `globalVdwScaleFactor`
- `globalAmberImproperTorsionScaleFactor`
- `includeAllResiduesWithin ..`

## **1.2 Introduction**

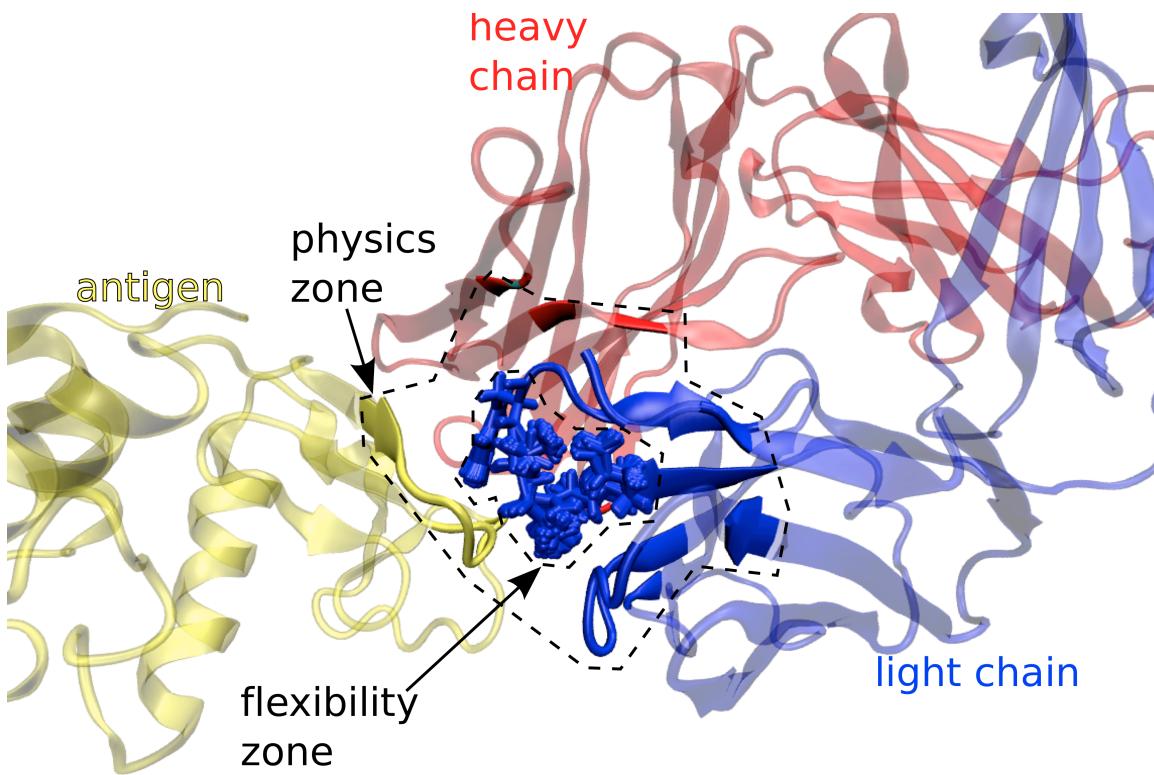
Antibodies are powerful mediators of the immune response, and synthetic monoclonal antibodies (mAbs) can potentially be used to direct the immune system’s considerable might against a pathogen. Synthetic antibodies are also used independently of the immune system, as agonists or antagonists of disease linked cell surface receptors, to deliver

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diagnostic markers, radioactive, or otherwise cytotoxic particles to specific locations in the patient, or in laboratory tests.

The simplest nontrivial design task is that of improving the affinity of an existing antibody-antigen complex of known structure by designing point mutations in the antibody. In order to do this one must (1) generate the point mutation and resolve the resulting steric clashes, (2) equilibrate the complex, and (3) evaluate the change in binding affinity. You can do the first two with MMB, and for the third you will need to download the FoldX program for your platform.

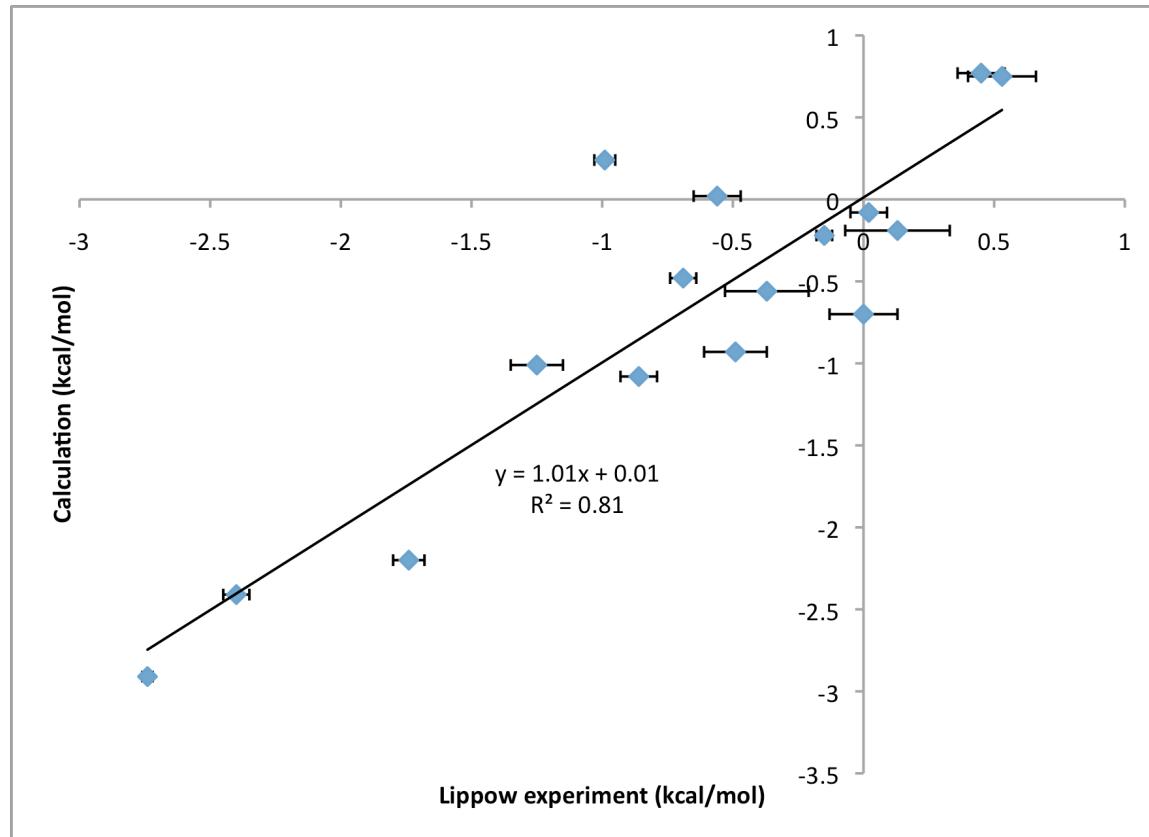
In this work we account for backbone and side chain flexibility employing an unusually small flexibility zone in internal coordinates. We do an equilibration of this zone taking into account physical interactions only within a small neighborhood of the flexible zone. We do this once for each possible mutant and once for the wild type complex, then use a knowledge-based potential as mentioned to evaluate the interaction energy of each, thereby obtaining the change in binding energy induced by each mutant.



**“Physics where you want it” equilibration for mutant L:N92A.** Five residues (90-94) about the mutated light chain residue 92 form the flexibility zone (blurred blue licorice); their backbone and side chain bond torsions can vary while the rest of the protein residues are rigid and fixed to ground. The physics zone consists of residues within 9Å of the flexibility zone (based on C $\alpha$  distances), including the flexibility zone itself. Residues in the physics zone have nonbonded (electrostatic, van der Waals, etc.) and bonded Amber99 force field terms turned on. The remainder of the protein residues are rigid, fixed, and non-interacting.

Standard error for this method is 0.4 kcal/mol. The files needed to reproduce the graph below are available from [simtk.org/home/antibodydesign](http://simtk.org/home/antibodydesign).

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**Calculated binding energy vs. (Lippow) experiment.** This includes single and multiple mutants. Note fitted line has slope of almost exactly unity, and passes through the origin. Standard error in calculation (taking experiment as true value) is 0.4 kcal/mol.

### 1.3 Preparing the command file

Open the file `commands.A_N.32.G.dat`. Look at the first two lines. These are the antibody light chain:

```
protein A 1
DIELTQSPATLSVTPGDSVSLSCRASQSISNGLHWYQQKSHESPRLLIKYVSQSSSGIPSRFSGS
GSGTDFTLSINSVETEDFGMYFCQQSNSPRTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGA
SVVCFLNNFYPKDINVWKWIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLKDEYERHNSYTCE
ATHKTSTSPIVKSFNRNEC
```

Note that in the above, residue 32 is a “G”, whereas in the wild type it would be “N.”

Then there’s the antibody heavy chain:

```
protein B 1
QVQLQESGAEVMKPGASVKISCKATGYTFSTYIEWVKQRPGHGLEWIGEILPGSGSTYYNEKFK
GKATFTADTSSNTAYMQLSSLTSEDSAVYYCARGDGNYGYWGQGTTLVSSASTTPPSVFPLAPG
SAAQTNSMVTLGCLVKGYFPEPVTVWNSGSLSSGVHTFPAVLQSDLYTLSSSVTPSSPRPSET
VTCNVAHPASSTKVDDKKIVPRDC
```

Then there's the antigen chain:

```
protein E 1
KVFGRCELAAAMKRHGLDNYRGYSLGNWVCAAKFESNFNTQATNRNTDGSTDYGILQINSRWWCN
DGRTPGSRNLCNIPCSALLSSDITASVNCACKIVSDNGMNAWVAWRNRCKGTDVQAWIRGCRL
```

We will be constraining to ground, so we need to set:

```
removeRigidBodyMomentum FALSE
```

If it is left at TRUE (the default) then MMB would try to move the system so the center of mass is at the origin, and would periodically reset the overall linear and angular momentum to zero. This is not consistent with the constrainToGround and similar commands.

Next we leave the mutation site and two residues on each side flexible, and rigidify the rest of the complex:

```
mobilizer A 1 Rigid A 29
mobilizer A 35 Rigid A 214
mobilizer B 1 Rigid B 218
mobilizer E 1 Rigid E 129
```

And fix the rigid fragments to ground:

```
constrainToGround A 1
constrainToGround A 214
constrainToGround B 1
constrainToGround E 1
```

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These are familiar:

```
firstStage 2
lastStage 2
reportingInterval .1
numReportingIntervals 150
```

This is a new one:

```
smallGroupInertiaMultiplier 11
```

It means that small chemical groups (methyl, alcohol, etc) get their inertia matrices arbitrarily multiplied by a constant, in this case 11. This is a somewhat crude way of slowing down potentially super-fast spinning of these groups, which would force the time integrator to take small time steps, making the simulation take forever.

We need to tell MMB to turn on the MD force field terms only for the residues that we will later specify:

```
physicsWhereYouWantIt TRUE
```

The default is `FALSE`, meaning the force field terms apply to all atoms in the system.

Next we need to turn on MD force field terms, for the physics zone that we are soon to define:

```
globalBondTorsionScaleFactor 1
globalBondBendScaleFactor 1
globalCoulombScaleFactor 1
globalVdwScaleFactor 1
globalAmberImproperTorsionScaleFactor 1
```

Finally, we specify what residues are in the physics zone:

```
includeAllNonBondAtomsInResidues A 2 2
includeAllNonBondAtomsInResidues A 27 34
```

In the above line, for example, we are including chain A residues 27 to 34 in the physics zone. We continue:

```
includeAllNonBondAtomsInResidues A 49 51
includeAllNonBondAtomsInResidues A 71 71
includeAllNonBondAtomsInResidues A 89 96

includeAllNonBondAtomsInResidues B 101 101
includeAllNonBondAtomsInResidues B 25 33
includeAllNonBondAtomsInResidues B 48 71
includeAllNonBondAtomsInResidues B 77 77
includeAllNonBondAtomsInResidues B 98 99

includeAllNonBondAtomsInResidues E 41 43
includeAllNonBondAtomsInResidues E 45 50
includeAllNonBondAtomsInResidues E 53 53
includeAllNonBondAtomsInResidues E 65 70
includeAllNonBondAtomsInResidues E 79 81
includeAllNonBondAtomsInResidues E 84 84
```

I have provided the antibody-antigen complex. First do `cp antibody-antigen.pdb last.1.pdb` .

Now run MMB with this command file (`commands.A_N.32.G.dat`). When you’re done move `last.2.pdb` to `A_N.32.G.pdb` .

Next run MMB using `commands.A_N.32.N.dat`. This is the wild type complex. Alternatively, just modify `commands.A_N.32.N.dat` so that the 32<sup>nd</sup> residue is “N” rather than “G”.

When you’re done move `last.2.pdb` to `A_N.32.N.pdb` .

## 1.4 Evaluating binding energy using FoldX

We can’t distribute FoldX with MMB, unfortunately. Download that from [foldx.crg.es](http://foldx.crg.es). Then create a file called `batch.txt` containing the following two lines:

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A\_N.32.N.pdb

A\_N.32.G.pdb

Save and close it. You find a file called `runfile.txt` in your MMB distribution. Run FoldX, choose option 3 (run file) and give it the file name “`runfile.txt`”. This will evaluate the binding energy for the two equilibrated structures listed. When it’s done, evaluate the difference :

(energy of `A_N.32.G.pdb`) - (energy of `A_N.32.N.pdb`)

This will give you the change in binding energy due to the mutation. You should have gotten -1.08 kcal/mol. Compare to the experimental value of -0.86 kcal/mol.

### **1.5 Challenge: find mutations that lower the binding energy even further**

The manuscript files at [simtk.org/home/antibodydesign](http://simtk.org/home/antibodydesign) should help. Try to find mutations that are even better than those reported. This will not be trivial!

