CLD 411

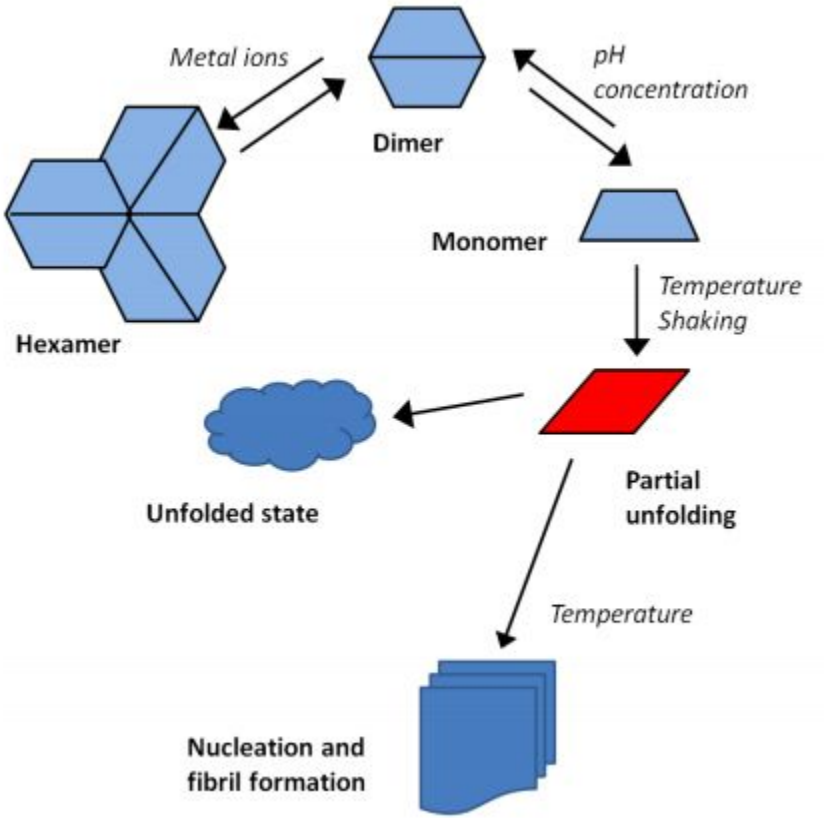
Supervisor: Prof GAURAV GOEL

By AKHIL KUMAR

About the Project

Undesirable aggregation of aqueous insulin solutions remains a serious obstacle in the development of alternative methods of diabetes therapy.

"Design of small peptides to inhibit insulin aggregation"



Insulin oligomers are nature's way for storing insulin.

Insulin can be found in the form of monomers, dimers and hexamers.

The relative concentration of each oligomer can be chemically controlled, by adjusting pH, concentration and by addition of metal ions in solution.

Aggregation prone: Low ph, High temp

Reference: http://pcwww.liv.ac.uk/~arnolds/publications/Mauri_thesis.pdf

Why Insulin

Small size
Easier to study
Of commercial importance

Lots of available experimental studies on insulin aggregation

Easier validation of model

What ligands

Ligands interact to either:
Native/PFI/Fibrils

BSPOTPE binds to PFI by hydrophobic interactions

Polyoxometalates(POMs) binds to monomer, lowers the concentration of free monomer and shifts the equilibrium away from fibrillation

Molecular docking

Interaction of 2 or more molecules to give the stable adduct

Targeted docking

HEX, Flexpepdock

MD Simulations

For refining structures obtained from targeted docking

GROMACS

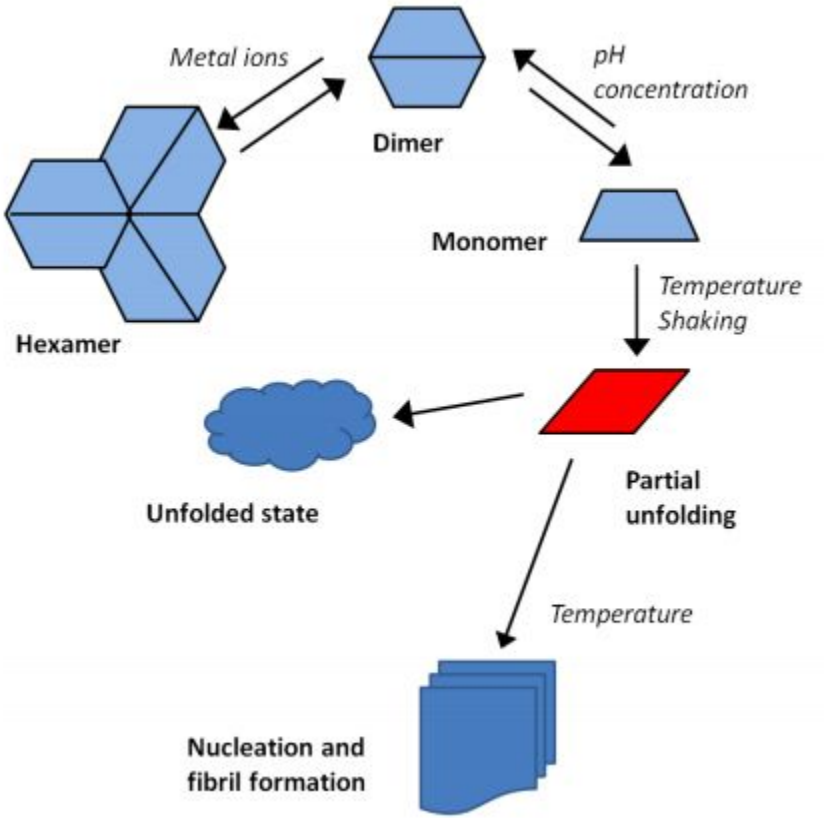
MM-PBSA Simulations

Scoring function to rank the structures

Based on interaction free energies

GROMACS

Computational Techniques to be used



Correctly folded proteins- long term stability

Under physiological conditions,
Native states- most thermodynamically stable

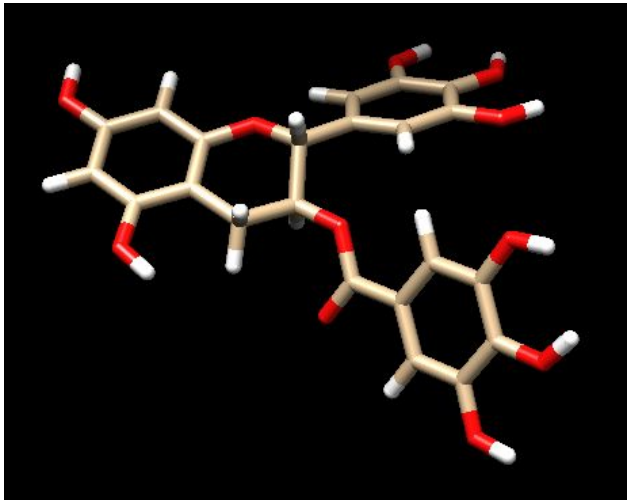
Aggregation prone: Low ph, High temp

Small size, easy to study, commercial importance

Lots of available experimental data

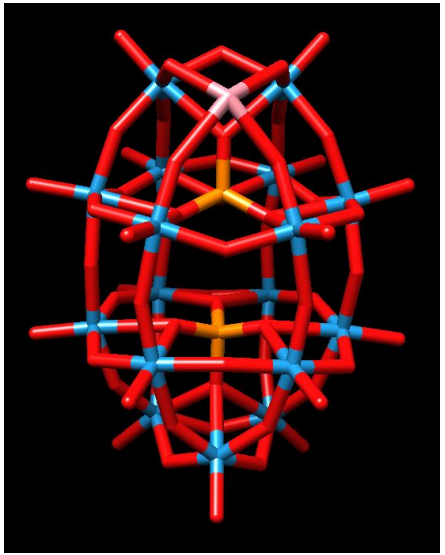
Reference: http://pcwww.liv.ac.uk/~arnolds/publications/Mauri_thesis.pdf

Design of small peptides to inhibit insulin aggregation



EGCG

Binds to the natively unfolded polypeptides



POM

Binds to monomer

A. K7[PTi2W10O40,
B. α-Na9H[SiW9O34],
C. K8[β-SiW11O39],
D. K8[P2CoW17O61]

Highest inhibition

MarvinSketch

Avogadro Molecular Builder

Identification and Generation of Ligands

.str file from CGenFF server

```
* Toppar stream file generated by
* CHARMM General Force Field (CGenFF) program version 2.2.0
* For use with CGenFF version 4.0
*

read rtf card append
* Topologies generated by
* CHARMM General Force Field (CGenFF) program version 2.2.0
*
36 1

! "penalty" is the highest penalty score of the associated parameters.
! Penalties lower than 10 indicate the analogy is fair; penalties between 10
! and 50 mean some basic validation is recommended; penalties higher than
! 50 indicate poor analogy and mandate extensive validation/optimization.

RESI /tmp/php       0.000 ! param penalty=  54.600 ; charge penalty=  23.057
GROUP            ! CHARGE   CH_PENALTY
ATOM C1      CG2R61  0.111 !     0.000
ATOM C2      CG2R61 -0.110 !     0.000
ATOM C3      CG2R61  0.108 !     0.190
ATOM C4      CG2R61 -0.009 !     6.703
ATOM C5      CG2R61  0.147 !    19.030
ATOM C6      CG2R61 -0.108 !     2.500
ATOM C7      CG311   0.091 !    20.155
ATOM C8      CG311   0.142 !    22.956
ATOM C9      CG321  -0.177 !     6.852
ATOM H1      HGR61   0.115 !     0.000
ATOM H2      HGR61   0.115 !     0.000
ATOM H3      HGA2    0.090 !     0.000
ATOM H4      HGA2    0.090 !     0.000
ATOM O1      OG3R60 -0.343 !    23.057
ATOM O2      OG302  -0.301 !    22.627
ATOM H5      HGA1    0.090 !     0.702
ATOM C10     CG2R61  0.003 !    13.402
ATOM C11     CG2R61 -0.110 !     7.454
ATOM C12     CG2R61  0.109 !     0.000
ATOM C13     CG2R61  0.106 !     0.000
ATOM C14     CG2R61  0.109 !     0.000
ATOM C15     CG2R61 -0.110 !     7.454
ATOM H6      HGA1    0.090 !     1.559
ATOM C16     CG2O2   0.471 !     0.565
ATOM C17     CG2R61  0.087 !     0.474
ATOM C18     CG2R61 -0.109 !     0.000
ATOM C19     CG2R61  0.109 !     0.000
ATOM C20     CG2R61  0.106 !     0.000
ATOM C21     CG2R61  0.109 !     0.000
ATOM C22     CG2R61 -0.109 !     0.000
ATOM O3      OG2D1  -0.493 !     0.000
ATOM O4      OG311  -0.530 !     0.000
ATOM H7      HGP1    0.420 !     0.000
```

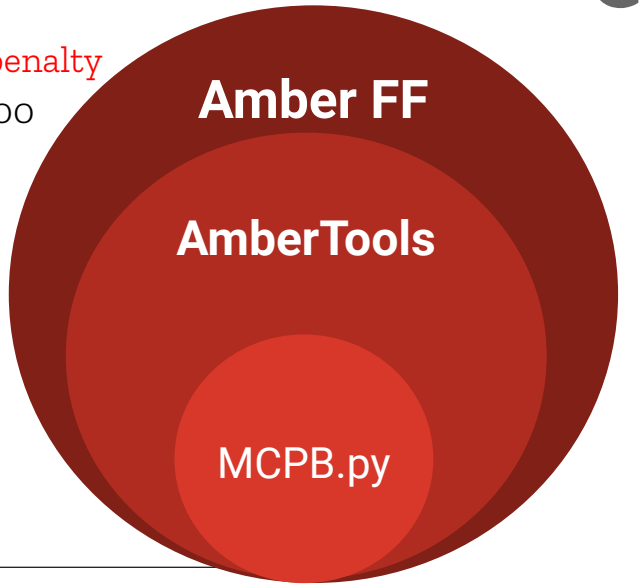
Moderate penalty; can ignore

23.057

Very high penalty

54.600

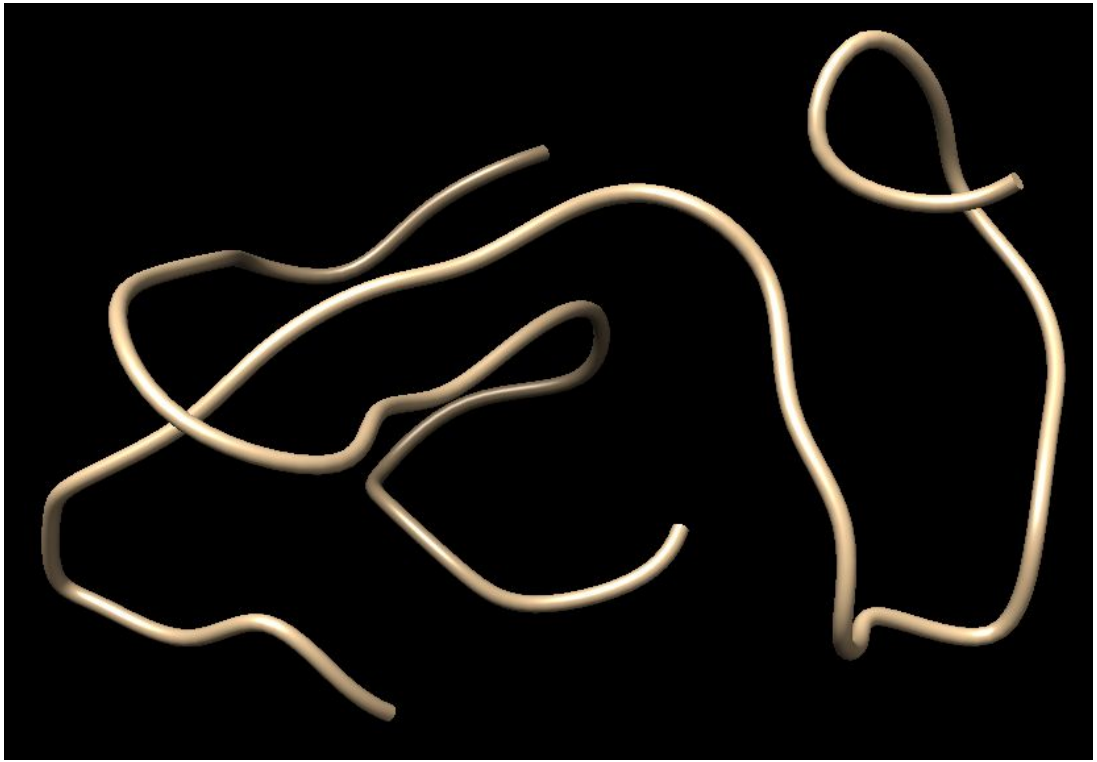
Topology Generation



ParmEd 27

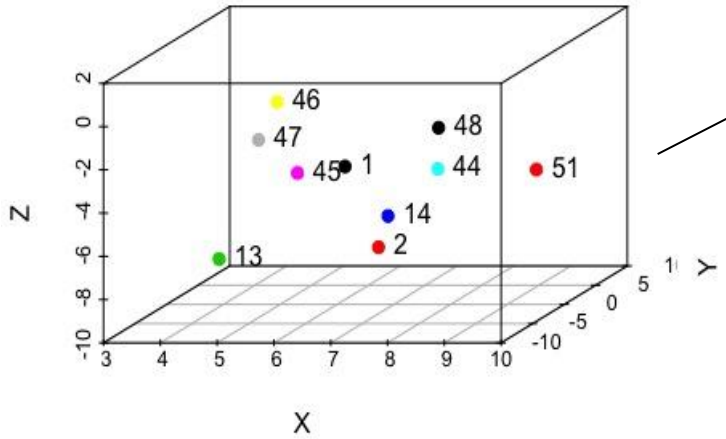
.top file in Charmm

Molecular Docking



N-PFI complex dominant during insulin aggregation

Targeted docking



only ten residues (#1, #2, #13, #14, #44, #45, #46, #47, #48, #51) at which the ligands bind with highest probability during the aggregation process.

Molecular Docking


```
> dist(coordinatefile)
          1         2         3         4         5         6         7         8
2   3.905005
3   5.653133  5.108282
4   5.296852  6.034744  3.863783
5  14.135466 15.621839 12.256535  9.594858
6  11.185230 12.830596  9.031591  6.909811  3.795126
7   9.744772 12.424561  9.432478  7.154793  6.392387  3.792436
8   8.119815  8.762441  6.075156  4.309774  8.925093  5.603085  3.833334
9   4.323232  7.279311  7.057586  4.247306 10.600262  8.187884  6.392647  3.870392
10  8.933668 10.413232  8.554457  4.860767  6.014266  5.071515  5.608304  5.644853
          9
2
3
4
5
6
7
8
9
10  5.339289
```

~15.62 Å

Search radius was chosen appropriately

Hex

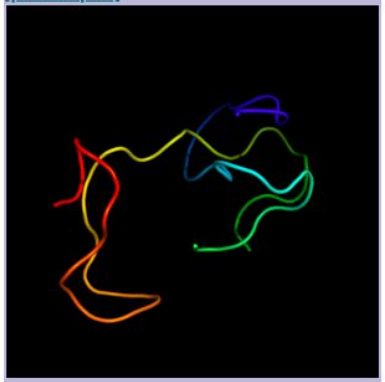
Rosetta Commons

The hub for Rosetta modeling software

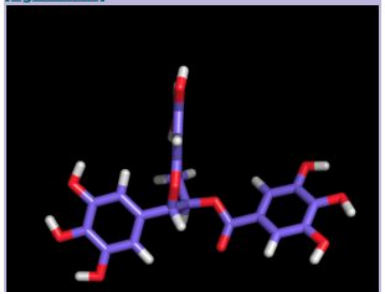
Ligand Docking Job protein residue geometric mean with ligand EGCG 「№76393」 Details

Inputs

[protein.pdb]



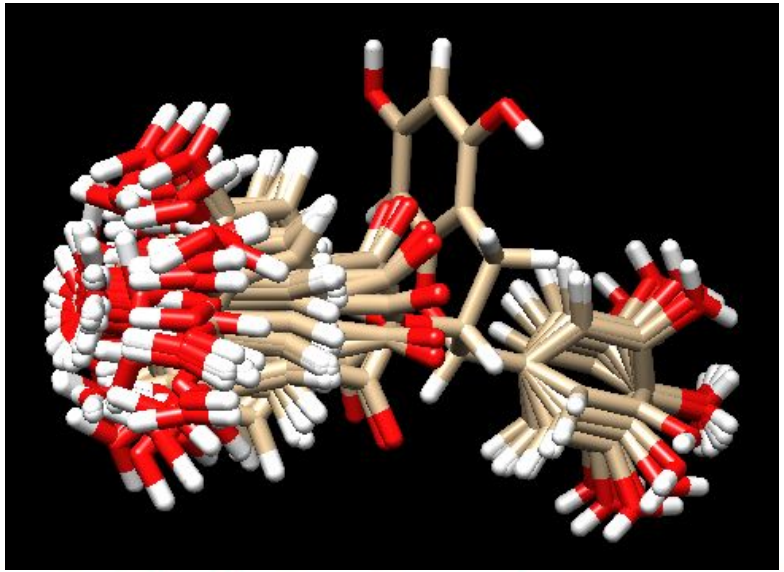
[ligand.sdf]



Status

Job ID	76393
Job Name	protein residue geometric mean with ligand EGCG
Visibility	**PUBLIC** (you can share this job)
Protocol	Ligand Docking
CPU hours used	0.1
user	iamakhilverma
Status	**Failed**
Daemon	GrayLab.Jazz-4
Description	protein residue geometric mean with ligand EGCG with radius of 7 angstrom
angle_step	5.0
chain	X
gen_conformers	True
grid_width	15.0
highres_cycles	6
highres_repack_cycles	3
initial_perturb	3.0
move_step	0.1
n_ligand_conformers	200
nstruct	1000
pocket_width	7.0
transform_cycles	500
use_input_position	False
x_start	6.48
y_start	-1.046
z_start	-3.923
Submitted time	2019-11-14 05:56
Start time	2019-11-18 22:54
End time	2019-11-18 22:58

Molecular Docking



Job 「№76393」 has failed with the message:

rosetta_scripts failed to produce score_pre file, please double check your input files!

1 MCPB.py for POM

2 Docking in HEX

3 Energy Minimization

4 Ranking with MM-PBSA

Thank you!

AKHIL KUMAR
2016CH10096