RC: Please make sure you have addressed all points mentioned in my recent emails so I don’t need to resend any of those.

You should refer to the inconsistencies doc posted by RC for details of each task when you work on them. This is only a brief summary for purposes of schedule. It is possible that some of the tasks have been omitted from that doc in the process of preparing this schedule.

Some of the orderings of tasks below are not consistent with a,b,c,d ordering in RC’s doc, as highlighted below.

If you are not sure that all comments will be addressed in your next revision, please post this markup to wiki as well.

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| --- | --- | --- | --- | --- |
| **Priority** | **Task** | **Sub tasks** | **Goal** | **Date** |
| 1A | Detailed draft based on an analysis of conformational energies | Verify if the conformational energies from Prime/MM-GBSA follow the same trend as 2-12 ns energies. | To identify if we could rank order the stability of the complex/loop reasonably | 19thAug |
| 1B | Analysis of binding energiesVerify if the binding energies (from all energy functions –Prime MM/PBSA, Amber MM-PBSA and Amber MM-GBSA) for open/closed loops among complexes are along expectations | Binding affinity of NAD+ is greater for open loopBinding affinity of INT is greater for closed loopBinding affinity of co-product (AADPr) is greater for closed loop.These needs to be validated.Document a report to Dr.Raj | To identify if binding energy estimates are able to recapitulate experimental findings. | 19thAug1A&1B1 day |
| 2A | Estimation of energy error from side chain predictionCont... | Based on “derived Apo energies” as estimated by Dr.Raj compare1. SIRT3/INT/NAM Open

VsSIRT3/NAD+/AC-CS2 Open loop1. SIRT3/INT/NAM Closed

VsSIRT3/NAD+/AC-CS2 Closed loop | Sirt3/INT/NAM – Open doneClosed loop needs to be done Prepare a report | Aug 22nd  |
| 2B | Extensive validation of side chain errors cont. .. | Estimate the change(Δ) in energies pre and post side chain modeling on all modelled (4FVT)/side chain repacked (4BVG loop) complex. Identify problematic residues  | Identify residues/positions that have problems during repacking/predictionAs a first step we will estimate the total energies, later on we will move forward to estimate Delta E vs RMSD | 23rdAug |
| 2C | Effect of Prime minimization post side chain prediction? This is to check the effect of global minimization and to see if there are issues with global minimization. | This is to check the effect of Prime minimization and to see if there are issues with global minimization.It will be carried out on all structured prepared by VR. |  | 24tnAug |
| 2D | Extract energies by-component to identify why this inconsistency arises | The script will produce a output equivalent to what was reported in the PLOS paper.ΔVdw, ΔELE, ΔGB, ΔEsurf, ΔEPB, ΔENPOLAR, ΔEDISPER, Δg Gas, Δg Solv(gb), Δg Solv (PB), Δg GB, Δg PB | Will help to explore which energy component contributes to the changes in conformational energies  | 25th Aug |
| 2E | Extensive validation of side chain errors  | Side chain validation studies acrossnative xtal structures 4FVT and 4BVG as “detailed by Dr.Raj. Begin with a comparison of RMSD for 4FVT vs 4BVG post side chain predictionDetailed analysis will Include analysis of exposed vs buried, polar vs nonpolar RMSD vs delta energyResidues within 7.5Å form the loop would be repacked.Residues subjected to be repacking/prediction may not be consistent (some residues tend to vary between open/closed conformations as we are using a distance based cut-off).Report global RMSD and local (loop/per-residue of loop region) RMSD | To identify the amount of energy error and the level of error propagated in each model*NB\*Each side chain prediction takes about 4 hrs.**\*We need to develop scripts or adapt existing scripts to compute per-residue RMSD* | 26th , 29th and 30th Aug*It may extend by a day* |
| 2F | Per-residue energies  | Amber per-residue interaction/binding energies can be obtained but not per residue MM based potential energies.*NB\* we need to re-run Amber MM/GBSA script on MD trajectories to extract per-residue binding energies. Each run takes about 3-4 hrs. We also need to write analysis script to extract them from the output file.* The output obtained will be the energetic contribution of each binding site residue to affinity | To identify key residues that contributes to substrate/product binding.Can be used to correlate MD findings with experimental mutagenesis data.*Per residue script will parse a .dat file, read the values corresponding to residue of interest, loop through all the log files and fetch the average energy. This will be done for all residues listed in an array*. | 31st Aug1st Sep&2nd Sep |
| 4 | Loop generated from MD | Identify loop conformations generated by MD sampling and try to rank them ( Either clustering of loop or RMSD) | Use these loop co- formations and try to see if Prime could rank-order it. | 6th Sep |
| 6 | Need for new simulation  | Based on analysis ascertain the need for new MD simulation | To be decided by Dr.Raj |  |

***NB\* The extra time available during side chain modeling runs will be used to complete the Perl script, which is almost half way through. Hence, I have not listed it as a separate task.***

***NB\* Add literature reference and compare the Prime loop prediction protocol mentioned in literatures.***