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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 | 18thAug |
| 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. | 18thAug1A&1B1 day |
| 2A | Estimation of energy error from side chain prediction. | 1.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 loop2.The second approach would be validation studies acrossnative xtal structures 4FVT and 4BVG as “detailed by Dr.Raj. Includes analysis of exposed vs buried, polar vs nonpolar RMSD vs delta energy3. 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  | 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* | 19th 22ndAugand23rdAug3 days*It may extend by a day* |
| 2B | Compute global co-factor loop and local (per-residue) loop residues RMSD | Ternary vs INT/NAM open loop conformation using the first frame (Structurally reason out why energies are drastically different here, in spite of starting with a similar conformation?) | To identify the influence of substrate/product binding (induced fit effects?) | 24thAug |
| 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. |  | 25tnAug |
| 3 | Apply by-component and by-residue MM interaction energy scoring to identify why this inconsistency arises | 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 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.*  | To identify key residues that contributes to substrate/product binding.Can be used to correlated MD findings with experimental mutagenesis data | 26thand29thAug |
| 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. | 30th Aug&31st Aug |
| 5 | Prime based loop prediction | Carry out loop refinement using Prime ( starting with 4FVT and 4BVG loops) and check if Prime based loop prediction performs better that Side chain prediction using a grafted loop | To identify if there could be analternate loop conformation different for the 4FVT(Open) and 4BVG(Closed) loop conformation | 1st and2ndSep |
| 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.***