--RC: Phe in Sir2Tm C pocket: please indicate how you prepared the NAM complex structures and how the receptor energy was handled when computing binding affinities. Did you carry out separate MD simulation on the receptor or derive the receptor energies from the complex simulation? The latter may not properly account for the effect of Phe on binding affinity. For example, if you used a ternary complex to prepare the structures, removing NAD+ and docking NAM into the C pocket, Phe conformations within the C pocket found in the apo/intermediate receptor structure may not be sampled. If you carried out separate simulations for the receptor, please do check the existing MD trajectories to see if Phe conformations within the C pocket are sampled. (I noted your related comments about long MD simulations for C pocket binding affinity calculations in report pt 4, and will reply to that later.) If not, we can use side chain rotamer sampling to check the MM-GB(PB)SA energy of the conformational change (related to running IFD on this system).

Usually, we are interested in rank ordering binding affinities, and so accurate receptor energy calculation is less important. However, when comparing across sirtuins (e.g. Sir2Tm, SIRT3), which may differ in the side chains in the C pocket, this may not be sufficient for the mechanistic analysis. We should do analogous calculations for SIRT3 and check whether there are any side chains that inhibit NAM binding to the C pocket. Of course, there are limitations on achievable accuracy here, but we may be able to draw some important qualitative conclusions.

-PL: for Sir2TM complex with NAM, the MD simulation was recently completed. The simulation started with Sir2TM:NAD+:ac-p53 structure (2H4F). The missing residues in the binding loop was modeled using Prime Loop refinement module, followed by manually creation of the bonding formation and bond breaking process to form the intermediate and NAM. The receptor here is set as Sir2TM:Intermediate.

Usually, single complex MD trajectory was used to extract receptor, ligand and complex energies in the MM-GBSA calculation. There is also a more time consuming three trajectories process that run receptor, ligand MD simulation together with complexes. There are debates on how much improvement we can expect and the problem of insufficient sampling.

--RC: answer the question about loop dihedrals after residue substitution following structure alignment for SIRT3

PL: If you are referring to the loop that was built by substituting the loop in ternary structure with the residues coordinates taken from SIRT3:Intermediate (4BVG) after alignment, then there is no different in loop dihedrals.

RC: I thought you had carried out a minimization thereafter. I don't believe I received the details of results/methods for this. E.g., you had indicated there were some issues with the loop termini.

-PL: No structural minimization is done in the Schrodinger program. Instead, the minimization was carried out in NAMD followed directly by MD simulations.

-RC: provide proposed loop building constraints for Sir2 analogous to those for SIRT3.

PL: So far, I have used Prime Loop Refinement protocol to build a missing loop (less than 10 residues) to construct Sir2TM complex structures. Another possibility is to build the loop by using the coordinates taken from the resolved loop Xtal structure taken from Sir2TM(H116A):p53:ac-ADPR complex (2H59).

RC: Here I was referring to the constraints for building the intermediate loop starting from the ternary structure, as we did for SIRT3. I understand there is an additional preliminary step here for Sir2Tm, namely filling in the missing loop residues (less than 10). I am referring to the step thereafter, after the missing residues have been filled in, where you will create a table of constraints for Sir2Tm as you did for SIRT3, because the whole loop (longer than 10 residues) needs to built in an alternate conformation.

-PL: I haven’t used 3D81, the Sir2TM/thioimidate Intermediate complex structure to carry out any simulation before. 3D81 has a longer gap than 2H4F, which make it a bit tricky in using Prime for loop construction and refinement. However, after two constraints on PHE33 and ARG34, a reasonable loop structure is obtained. Details will be provided in the next report.