Answers to requested questions/tasks:

- 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.

- 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).

- are missing loop segments already built for Sir2Tm? (were these used for the final plot in report pt 4) If so, can we proceed with the splicing of loops after structure alignment without licenses?

PL: No. The analysis was done using Xtal structures in report part 4. There are some differences between the loop built using Prime loop refinement and the available loop Xtal structure in 2H59 and 2H2I.

- did Sir2 qm/mm paper describe their loop preparation methodology?

PL: No, no information was provided in their papers on how the missing residues were prepared.

- 3D81 and 2H4F are the structures most relevant. please confirm that qm/mm studies used these two structures

PL: Yes, the qm/mm studies used these two structures.

- we discussed calculating residue-by-residue rmsd for the above two structures after building missing loop segments. I believe loop building was believed to be accurate due to short length of missing loop segment.

PL: After inspecting the loop structure obtained using Prime Loop Refinement protocol and compared it with the loop structure of 2H59, I believe there are still improvement to be made for the loop structure.

b) I did not yet receive mm-gb/pbsa plots for MD trajectories (binding affinity data was provided but the aforementioned plots originally discussed at the group meeting were requested). Please provide this vis-a-vis the types of loop RMSD plots (possibly residue by residue) for the trajectories that were presented at the group meeting. (This will be helpful in sampling the potential energy landscape of modified loop conformations, which will subsequently also be studied by ab initio loop sampling once we get licenses.)

PL: I have written a script to extract the energy terms for each frame during the MM-GBSA calculations. I am now working to compile all the data and make plots of the mm-gbsa energies and corresponding loop rmsd alone the trajectories.

c) Regarding the base exchange mechanism literature analysis task, the following question was posed a couple of months ago:

The C pocket was reported to be occluded by a side chain in at least one Sir2Tm xtal structure. Check for overlap of side chains with NAM binding site. Previously, you indicated this was only the case in the absence of NAM, but in its presence the side chain was not occupying the C pocket. This may still be consistent with this side chain reducing the affinity of NAM binding. You were planning to report on the side chain conformations observed during our MD simulations. In our NAM binding affinity studies, do we ever see this side chain occupying the C pocket? See also the Steegborn plos paper literature task above in this context. This is part of a larger study of how the loop and side chain conformations can affect NAM binding.

PL: Yes. PHE33 in 3D81 was getting too close to the nicotinamide moiety in 2H4F after alignment. (the PHE33 in 3D81 was in ball-and-stick and perpendicular to ring structure as in 2H4F). I still need to check the MD simulation trajectories to answer your question regarding the effect of NAM in the C pocket. Here is the superposition of 2H4F and 3D81 around the C pocket.

