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| **PHASE 1** | **Target Date or Days allotted (starting 1/28)** | **Status** |
| **Tasks listed 1/28, esp validation of loop energetic scoring and sampling, Sir2 and SIRT3** |  |  |
| Sir2Tm: By reference to the by-residue RMSD figure, please indicate a) the loop residues; b) choice of residues for intermediate loop building in Sir2Tm. Also please zoom in on the loop residue numbers in the x-axis of these figures (omit more distant residues). | ~ half day | done |
| SIRT3: By reference to the by-residue RMSD figure, please indicate a) the loop residues; b) the reason for choice of 156-169 for the intermediate loop building starting from the ternary structure with constraints.  Also please zoom in on the loop residue numbers in the x-axis of these figures (omit more distant residues) so the residue numbers are clear.  need details of what us done by protein prep for methods | ~ half day | done |
| specify in structure-based sequence alignment (meetings page) where the binding loop is and indicate the residue numbers for the loop residues for SIRT3 and Sir2Tm with numbering consistent with the reports  report indicates there is a helical segment (162-170) within the binding loop for the ternary complex of SIRT3. It is not clear whether there is such a helix within the resolved loop residues of Sir2Tm and if so, where; this should be specified. highlight the helical segment in the ternary structures (e.g., 4FVT) in this alignment. please indicate whether the refined loops for 2H4F contained a helical segment (see also below) in the case that the sequence alignment suggests that the helical segment should lie within the range of missing residues (if not could specify helical constraint, if supported, to check the energy difference and validate sampling)  also indicate the residue in SIRT3 that is homologous to Phe33 in Sir2Tm. Is it Phe157 (noted from pptx below). Please do the same for Tyr40. | ~ half day | done |
| Provide a legend for notations in the alignment. Also, please indicate the helical segment of the loop (SIRT3) and the turns in the alignments so we can identify the residues involved.  In the “comparing helix” figure of Sir2 and SIRT3, please indicate which structure is which and point out the loops.  XG has prepared a table of mutations in various sirtuins that includes specification of the role of each residue (esp in Sir2 enzymes) in the catalytic mechanism of the enzyme. Please point out each of those residues in the alignments above and where possible, indicate the homologous residue in SIRT3 that corresponds to each catalytic residue in Sir2 (in the event the data pertains to Sir2 enzymes other than Sir2Tm, e.g., Hst2, you can add that alignment). This can focus on the most critical residues listed in the table (for example, residues that simply play a minor role in substrate binding can be omitted).  Note that the table also lists, in certain cases, the pocket wherein a given catalytic residue lies. If possible, the numbers of the residues in the A,B,C pockets should be listed (for any one sirtuin included in the alignment). |  | done |
| In sequence alignment pocket annotation we have indicated the residues contacting NAM. Please distinguish between contacts made in the ternary vs intermediate complexes if possible (for Sir2Tm only for now if we have xtal structure of NAM complex and NAM complex preparation for sirt3 is not finished). This need not be added directly to the sequence alignment, but can be specified at the bottom of the document. If further preparation of the NAM complex is required, this can be postponed, but should be listed as a later task.  Also, provide details regarding the steps involved in the protein preparation wizard of prime. |  | underway |
| Sir2Tm 2H59 binding loop should first be built without constraints, and by-residue RMSDs and backbone RMSDs should be reported. Apply input file preparation and prime execution from command line as needed for access to dipeptide sampling algorithms used for long loop prediction (see wiki notes).  In addition to full (~15 residue) loop, validate loop prediction on shorter loop segments until suitable RMSD is obtained.  Results for several of the highest ranking loop structures should be provided, since the highest ranking structure may not be the one with lowest RMSD.  [In particular, in each case specify the loop conformation with the lowest RMSD to native and provide its energy as well](https://webmail.pmc-group.com/exchange/raj/Inbox/For%202h59%20in%20addition%20to%2015%20residue%20loop%20make%20sure%20to%20validate%20on%20shorter%20loops%20until%20suitable%20rmsd%20is%20obtained.%20In%20each%20case%20(also%20w%20sirt3)%20make%20sure%20to%20specify%20the%20loop%20conformation%20returned%20with%20the%20lowest%20rmsd%20to%20native%20and%20provide%20its%20energy%20as%20well.EML?Cmd=open)  The energy of the native structure should be included for comparison (with full minimization).  In case of energy errors (i.e., where the native loop ranks lower - i.e., higher energy - than a modeled loop conformation), carry out the following steps for one such loop conformation: a) identify the side chains in the environment of the modeled loop conformation that were rotamer-optimized (e.g. within 7.5 A), and sample the same side chains in the native structure. Similarly, in the modeled structure, identify and sample the side chains within 7.5 A of the native loop conformation. Compare resulting energies after minimization of the above structures, which should have sampled the same degrees of freedom under the force field. | ~ 2 days |  |
| Further analysis of results from preliminary studies already carried out on SIRT3 ternary and intermediate loop building in native environments:   1. Report by-residue RMSDs. Results for several of the highest ranking loop structures can be provided, since the highest ranking structure may not be the one with lowest RMSD. 2. [Proper comparison of energy of native vs predicted loops w full minimization for all the predictions completed to date](https://webmail.pmc-group.com/exchange/raj/Inbox/Fwd:%20Proper%20assessment%20of%20energy%20if%20native%20vs%20predicted%20loops%20w%20full%20minimization%20in%20our%20predictions%20to%20date.EML?Cmd=open). 3. Proceed as in above task in case of energy errors. |  |  |
| For SIRT3 4BVG, the distance constraint matrix should be applied to rebuilding of loop in the native 4BVG environment and analogous data should be provided. | ~ 2 days | This task may be postponed/modified based on results from the previous loop building tasks; will advise. |
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| Carry out additional refinement of Sir2 and SIRT3 ternary and intermediate loops as needed based on results(/constraints) from 2H59 and 4BVG. Apply dipeptide sampling algorithms from command line as above.  Fill in by-residue and backbone RMSDs in report pt 4 for missing loop residues of sir2 once we are comfortable with refinements, prior to intermediate loop building in ternary environment | ~ 2 days | This task may be postponed/modified based on results from the previous loop building tasks. |
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| **Building of intermediate loops starting from ternary loop conformation** |  |  |
| Providing more details on results obtained to date with residue substitution approach to intermediate loop building including dihedral comparison after MD  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.  --Detailed results from prior studies mentioned above should be provided. Feedback will be provided before moving on to next task. | **~ half day** |  |
| Completion of residue substitution approach to intermediate loop building Report the MM-GBSA/PBSA energies and compare to the low energy loop conformations produced by ab initio loop prediction in prime. | ~ half day |  |
| Set up calculations of ensemble average energies for intermediate loops in Sir2 and SIRT3 (MD/MM-GBSA); provide ensemble average energies for intermediate:NAM complex after equilibration  Also provide residue-by-residue B factors and NAM B factors from these simulations | ~ 1 day |  |
| Specification of constraint matrix for Sir2 intermediate loop building starting from ternary complex receptor, given results from SIRT3 and ab initio refinement of missing loop segment in Sir2  RC: Here I was referring to the constraints for building the intermediate loop starting from the ternary structure, as we did for SIRT3. I am referring to the step 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. |  |  |
| Building based on constrained loop prediction  Sir2  Single point energies |  |  |
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| **C pocket binding affinity** |  |  |
| Sir2  proper assessment of effect of Phe33 (Sir2) on binding affinity. Provide MD simulation statistics as well as prepare for IFD docking of NAM. RC to provide follow up notes for Phase 2 afterwards.  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).  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.  --Receptor preparation for NAM/Phe 33 study needs to be completed based on modeling of missing loop in Sir2Tm/intermediate/NAM complex, and used for binding affinity calculations vis-à-vis specific comments above; comparison to be made to simulation methods in paper 1 | MD is done. Analysis will be carried out. ~ 1 day. |  |
| SIRT3 – application of analogous methods | ~ 1.5 days |  |
| Compare C pocket interactions of NAM in SIRT3:peptide:NAD+ to NAM in SIRT3:intermediate:NAM | does this refer to the MD results? |  |
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| Workshop notes |  |  |
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| **PHASE II** |  |  |
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| See previous comments regarding some follow up tasks to Phase I |  |  |
| List of tasks has been prepared and details to be posted after more progress on Phase I |  |  |
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