1. Completion of residue substitution approach to intermediate loop building Report the MM-GBSA energies and compare to the low energy loop conformations produced by ab initio loop prediction in prime.

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| **SIRT3/Intermeidate/NAM complex** | **Prime Energy (kcal/mol)** |
| Struct 1: Convert from 4FVT with new procedue (restrained minimization on hydrogen only) | -9965.2 |
| Struct 2: Convert from 4FVT with new procedue (restrained minimization on all atoms) | -11687.3 |
| Prime full minimization of Struct 1 | -12494.0 |
| Prime full minimization of Struct 2 | -12474.0 |
| Struct 3: Repalce residue 155-178 of Struct 1 using those of 4BVG (restrained minimzation on hydrogen only) | 951282.7 |
| Struct 4: Repalce residue 155-178 of Struct 1 using those of 4BVG (restrained minimzation on all atoms) | -11705.1 |
| Side chain optimization (for res 155-178 only) of Struct 3 using Prime Default setting | -9937.2 |
| Side chain optimization (for res 155-178 only) of Struct 4 using Prime Default setting | -11704.1 |
| Prime full minimization of Struct 3 | -12502.8 |
| Prime full minimization of Struct 4 | -12479.6 |
| Struct 5: Side chain optimization of Struct 3 using Prime Monte Carlo Sampling (top rank) | -10168.5 |
| Struct 6: Side chain optimization of Struct 4 using Prime Monte Carlo Sampling (top rank) | -11784.6 |
| Prime full minimization of Struct 5 | -12493.5 |
| Prime full minimization of Struct 6 | -12491.1 |

A whole new procedure was applied to prepare the structure for SIRT3/INT/NAM complex starting from 4FVT ternary complex, which includes

1. Ternary complex (4FVT) was prepared using Protein Preparation Wizard (restrained minimization on hydrogen only).
2. Manual creation of C-O bond between ac-LYS and ribose and cutting of C-N bond between ribose and NAM, modifying C=N bond within ac-LYS and set appropriate formal charges on NAM and ac-LYS, carrying out minimization on selected atoms only (ribose and end group of ac-LYS).
3. Save the coordinates in PDB file, manual editing of resname for NAM, and CONECT section.
4. Re-load the PDB file, and prepared using Protein Preparation Wizard.

Loop substitution steps:

1. Aligned 4BVG (SIRT3/INT) with the 4FVT (both prepared using Protein Preparation Wizard), cut and replace the coordinates of residue 155-178 of above prepared SIRT3/INT/NAM complex PDB file using those from prepared 4BVG.
2. Re-prepare the complex with Protein Preparation Wizard.

\* In the last step of the Protein Preparation Wizard, the constrained minimization using OPLS\_2005, minimization can be done for hydrogen only (keep all heavy atoms fixed) or minimizing with constraints on heavy atom RMSD (< 0.3 Angstrom). As it turns out, the choice of minimization has an impact on the full minimization using Prime (with the inclusion of VSGB solvation model).

\* There are a few options in Prime Side chain optimization. The default one modifies only the side chain. The Monte Carlo approach can produce more structures but change backbone atoms as well.

1. Carry out loop substitution for 4BVG with loops from 4FVT and placement of NAM. Calculate the energies associated with each structure. Results with side chain optimization also included.

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| Struct 7: Placement of NAM from full minimized Struct 1 into 4BVG (without reparameterization) | -9317.1 |
| Struct 8: Placement of NAM from full minimized Struct 1 into 4BVG (with reparameterizationusing Epik) | -9283.8 |
| Struct 9: Restrained minimization of Struct 8 on hydrogen only using OPLS\_2005 | -9590.8 |
| Struct 10: Restrained minimization of Struct 8 on all atoms using OPLS\_2005 | -11818.3 |
| Prime full minimization of Struct 9 | -12614.2 |
| Prime full minimization of Struct 10 | -12555.6 |
| Struct 9a: Side chain optimization (for res 155-178 only) of Struct 9 using Prime Default setting | -9871.9 |
| Prime full minimization of Struct 9a | -12553.2 |
| Struct 11: Replace residue 155-178 of Struct 9 using those of 4FVT (restrained minimzation on hydrogen only) | -4656.1 |
| Struct 12: Replace residue 155-178 of Struct 10 using those of 4FVT (restrained minimzation on all atoms) | -11757.4 |
| Prime full minimization of Struct 11 | -12549.3 |
| Prime full minimization of Struct 12 | -12569.8 |
| Struct 11a: Side chain optimization (for res 155-178 only) of Struct 11 using Prime Default setting | -9738.2 |
| Prime full minimization of Struct 11a | -12549.7 |

1. Repeat the above calculations using method 2) proposed below (remove NAM). When substituting the 4FVT loop in 4BVG, this should require no changes to the ligands (intermediate). Results with side chain optimization also included.

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| Struct 13: 4BVG prepared using Protein Preparation Wizard (restrained minimzation on hydrogen only) | -9620.0 |
| Struct 14: Replace residue 155-178 of Struct 13 using those of 4FVT (restrained minimzation on hydrogen only) | -4577.2 |
| Struct 15: Replace residue 155-178 of Struct 13 using those of 4FVT (restrained minimzation on all atoms) | -11668.8 |
| Prime full minimization of Struct 14 | -12430.6 |
| Prime full minimization of Struct 15 | -12496.4 |
| Struct 14a: Side chain optimization (for res 155-178 only) of Struct 14 using Prime Default setting | -9659.5 |
| Prime full minimization of Struct 14a | -12447.3 |