Structure preparation:

Hydrogen atoms were added to all proteins structures, and standard protonation states at pH 7.0 were used (Asp, Glu ionized; Arg, Lys protonated). Protein assignment was performed using Maestro (Schrodinger, LLC) to optimize the positions of Ser, Thr, and Tyr hydroxyl protons, and His protonation and tautomeric states. The positions of Asn, Gln, and His side chains were also optimized via 180 degree terminal-chi flips. Waters, metals, and cofactors were removed, while any bound ligands or substrates were retained. Ligand bond orders and formal charges were set to their proper values.

Various protocols have been used to prepare the structures.

Starting from ternary complex of SIRT3, carba-NAD+ and ac-Lys peptide (4FVT), the substrate carba-NAD+ was first converted to NAD+, followed by the manual bonding forming and bond breaking conversion into SIRT3, intermediate and NAM complex. In order to investigate the effect of loop conformation on the complex structure, we substituted the residues 155-178 with the coordinates of those from SIRT3 and intermediate complex structure (4BVG) followed by minimization and/or side chain optimization.

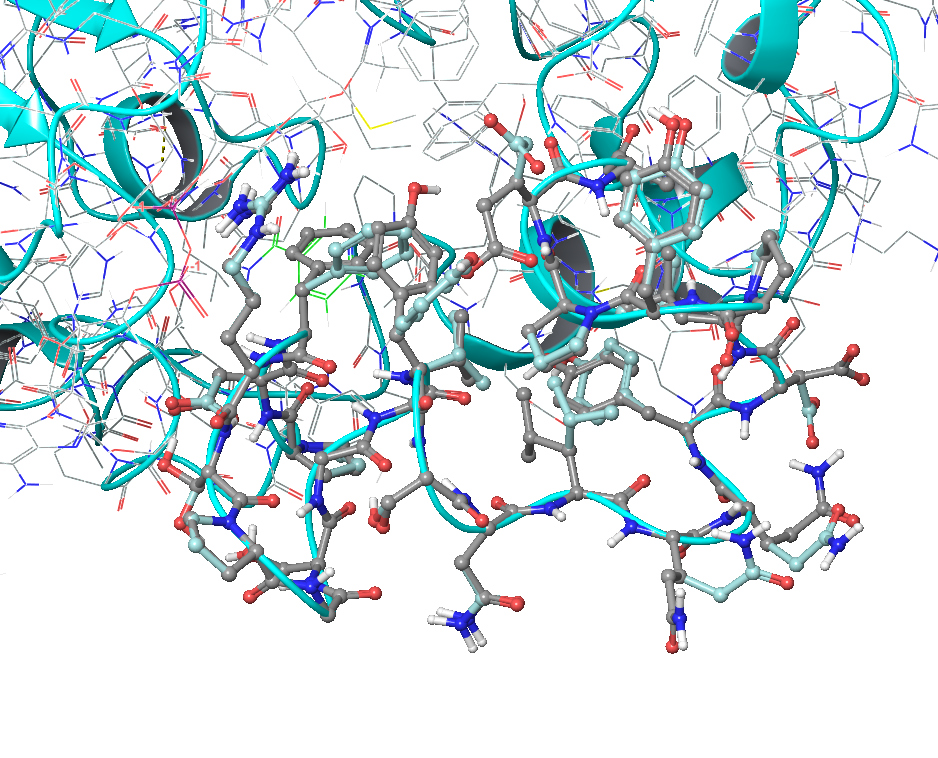
Each protein structure was subjected to relaxation using a series of restrained, partial minimizations using the OPLS-2005 force field until the average RMS deviation of the heavy atoms reached a maximum of 0.3 A˚ with respect to the starting structure. Further minimizations were performed using Prime (Schrodinger, LLC; equivalent of PLOP from Jacobson’s group) with all-atom OPLS force field and VSGB 2.0 solvation model to obtain the final energies.) We also carried out minimization without the relaxation on heavy atoms to ensure no bias was incurred using the relaxation.

|  |  |  |
| --- | --- | --- |
| **without relaxation** | **Prime** | **MM-PBSA** |
| SIRT3/INT/NAM complex from 4FVT | -12494.0 | -23528.705 |
| SIRT3/INT/NAM complex from 4FVT with sidechain opt | -12509.8 | -23518.557 |
| SIRT3/INT/NAM complex with loop sub 155-178 | -12502.8 | -23649.142 |
| SIRT3/INT/NAM complex with loop sub 155-178 & sidechain opt | -12493.5 | -23566.810 |
| SIRT3/INT/NAM complex with loop sub 155-178 & MC sidechain opt | -12508.4 | -23651.898 |

|  |  |  |
| --- | --- | --- |
| **with relaxation** | **Prime** | **MM-PBSA** |
| SIRT3/INT/NAM complex from 4FVT | -12474.0 | -23505.324 |
| SIRT3/INT/NAM complex from 4FVT with sidechain opt | -12514.4 | -23505.032 |
| SIRT3/INT/NAM complex with loop sub 155-178 | -12479.6 | -23592.131 |
| SIRT3/INT/NAM complex with loop sub 155-178 & sidechain opt | -12491.1 | -23578.256 |
| SIRT3/INT/NAM complex with loop sub 155-178 & MC sidechain opt | -12489.8 | -23580.947 |

Almost all the calculations suggested that the changing the loop conformation from original ternary complex (4FVT) to that of SIRT3/INT complex (4BVG) help stabilize the immediate first step reaction products of SIRT3, intermediate and NAM, except in the case of Prime energy of SIRT3/INT/NAM complex with loop 155-178 substitution and side chain optimization, which is very close that of SIRT3/INT/NAM complex prepared directly from 4FVT. The rank ordering of PBSA energies are not always consistent with the Prime energies for loop substituted structures.

We also prepared the complex of the reverse first step reaction by placement of NAM into the C pocket of SIRT3 and intermediate complex (4BVG). Further investigations on loop conformation effect were carried out by substituting the loop 155-178 using those from ternary complex (4FVT) after alignment. The Prime side chain optimization on residues 155-178 preserved many native side chain conformations, but failed to capture the global minimum with an energy 2.4 kcal/mol higher than the native structure. Sidechain of some key residues are significantly different, e.g. PHE157, TYR165, LEU168, GLU 177, etc. (Native: carbon in grey; Sidechain opt: carbon in light blue.)



|  |  |  |
| --- | --- | --- |
| **without relaxation** | **Prime** | **MM-PBSA** |
| SIRT3/INT/NAM complex from 4BVG | -12614.2 | -23439.038 |
| SIRT3/INT/NAM complex from 4BVG & sidechain opt | -12553.2 | -23377.078 |
| SIRT3/INT/NAM complex with loop sub 155-178 | -12549.3 | -23395.338 |
| SIRT3/INT/NAM complex with loop sub 155-178 & sidechain opt | -12549.7 | -23333.988 |

|  |  |  |
| --- | --- | --- |
| **with relaxation** | **Prime** | **MM-PBSA** |
| SIRT3/INT/NAM complex from 4BVG | -12555.6 | -23416.979 |
| SIRT3/INT/NAM complex with loop sub 155-178 | -12569.8 | -23370.478 |

The Prime energies suggest that native loop conformation is favorable in energy compared to the loop conformation from 4FVT. However, the MM-PBSA energies have mixed results. The possible cause of the difference may be the single point MM-PBSA calculations that are not optimized at the same level of theory.

Another test of loop conformation was carried out using SIRT3 and intermediate complex. As can be seen in the table below, changing loop conformation destabilizes complex structure with or without further sidechain optimization.

|  |  |
| --- | --- |
| **without relaxation** | **Prime** |
| SIRT3/INT from 4BVG | -12500.3 |
| SIRT3/INT from 4BVG & sidechain opt (res 155-178) | -12487.8 |
| SIRT3/INT complex with loop sub 155-178 | -12430.6 |
| SIRT3/INT complex with loop sub 155-178 & sidechain opt | -12447.3 |

For references, SIRT3/carba-NAD+/ac-Lys ternary complex shows that sidechain optimization can improve the overall energies.

|  |  |
| --- | --- |
| **without relaxation** | **Prime** |
| SIRT3/carbaNAD/ac-Lys complex from 4FVT | -12479.0 |
| SIRT3/carbaNAD/ac-Lys complex & sidechain opt (res 155-178) | -12515.7 |

|  |  |
| --- | --- |
| **with relaxation** | **Prime** |
| SIRT3/carbaNAD/ac-Lys complex from 4FVT | -12431.2 |
| SIRT3/carbaNAD/ac-Lys complex & sidechain opt (res155-178) | -12492.8 |

MD simulations have been carried out on SIRT3/INT/NAM complex prepared from 4FVT with and without loop replacement that is taken from residue 155-178 of 4BVG. The trajectories were further analyzed using MM-GB(PB)SA calculations using NAM as the ligand. Although the energies fluctuate over the course of the MD trajectory, the average energy shows clearly that the with the loop substitution, the overall energy is lower and the binding affinity increases. The loop structures are well maintained with respect to the starting structures in both cases.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | SIRT3/INT/NAM prepared from 4FVT | | | SIRT3/INT/NAM prepared from 4FVT w/ loop (res 155-178) replacement from 4BVG | | |
| **Column1** | **Column2** | **Average** | **Std. Dev.** | **Std. Err. of Mean** | **Average4** | **Std. Dev.5** | **Std. Err. of Mean6** |
| 2-4 ns | MM-GBSA(Complex) | -7145.44 | 50.57 | 3.57 | -7230.03 | 50.98 | 3.60 |
| 2-4 ns | MM-GBSA(receptor) | -7049.49 | 50.55 | 3.57 | -7131.85 | 50.92 | 3.59 |
| 2-4 ns | MM-GBSA(ligand) | -76.00 | 2.36 | 0.17 | -75.65 | 2.54 | 0.18 |
| 2-4 ns | MM-GBSA(binding) | -19.95 | 1.83 | 0.13 | -22.53 | 1.95 | 0.14 |
| 2-4 ns | MM-PBSA(Complex) | -5870.75 | 55.89 | 3.94 | -5914.04 | 54.19 | 3.82 |
| 2-4 ns | MM-PBSA(receptor) | -5794.44 | 56.35 | 3.97 | -5833.11 | 53.73 | 3.79 |
| 2-4 ns | MM-PBSA(ligand) | -73.05 | 2.35 | 0.17 | -72.80 | 2.53 | 0.18 |
| 2-4 ns | MM-PBSA(binding) | -3.25 | 3.92 | 0.28 | -8.12 | 3.57 | 0.25 |
| 4-6 ns | MM-GBSA(Complex) | -7149.60 | 49.39 | 3.48 | -7201.11 | 45.91 | 3.24 |
| 4-6 ns | MM-GBSA(receptor) | -7052.94 | 49.52 | 3.49 | -7103.24 | 46.11 | 3.25 |
| 4-6 ns | MM-GBSA(ligand) | -76.20 | 2.65 | 0.19 | -75.81 | 2.58 | 0.18 |
| 4-6 ns | MM-GBSA(binding) | -20.45 | 2.11 | 0.15 | -22.06 | 2.06 | 0.15 |
| 4-6 ns | MM-PBSA(Complex) | -5884.68 | 51.98 | 3.67 | -5895.39 | 51.61 | 3.64 |
| 4-6 ns | MM-PBSA(receptor) | -5806.29 | 52.11 | 3.68 | -5815.61 | 51.58 | 3.64 |
| 4-6 ns | MM-PBSA(ligand) | -73.25 | 2.67 | 0.19 | -72.91 | 2.55 | 0.18 |
| 4-6 ns | MM-PBSA(binding) | -5.14 | 3.40 | 0.24 | -6.88 | 4.01 | 0.28 |
| 6-8 ns | MM-GBSA(Complex) | -7151.85 | 54.34 | 3.83 | -7183.75 | 47.38 | 3.34 |
| 6-8 ns | MM-GBSA(receptor) | -7055.55 | 54.51 | 3.84 | -7085.51 | 47.20 | 3.33 |
| 6-8 ns | MM-GBSA(ligand) | -75.92 | 2.61 | 0.18 | -75.99 | 2.42 | 0.17 |
| 6-8 ns | MM-GBSA(binding) | -20.37 | 1.96 | 0.14 | -22.25 | 1.71 | 0.12 |
| 6-8 ns | MM-PBSA(Complex) | -5870.18 | 59.74 | 4.21 | -5875.60 | 51.64 | 3.64 |
| 6-8 ns | MM-PBSA(receptor) | -5794.27 | 59.69 | 4.21 | -5795.97 | 51.49 | 3.63 |
| 6-8 ns | MM-PBSA(ligand) | -72.98 | 2.58 | 0.18 | -73.02 | 2.39 | 0.17 |
| 6-8 ns | MM-PBSA(binding) | -2.93 | 3.20 | 0.23 | -6.62 | 3.66 | 0.26 |
| 8-10 ns | MM-GBSA(Complex) | -7153.39 | 48.88 | 3.45 | -7195.55 | 48.90 | 3.45 |
| 8-10 ns | MM-GBSA(receptor) | -7056.99 | 48.80 | 3.44 | -7097.05 | 48.84 | 3.44 |
| 8-10 ns | MM-GBSA(ligand) | -75.98 | 2.49 | 0.18 | -76.07 | 2.55 | 0.18 |
| 8-10 ns | MM-GBSA(binding) | -20.42 | 1.66 | 0.12 | -22.43 | 1.87 | 0.13 |
| 8-10 ns | MM-PBSA(Complex) | -5885.39 | 50.81 | 3.58 | -5908.01 | 54.54 | 3.85 |
| 8-10 ns | MM-PBSA(receptor) | -5807.87 | 51.04 | 3.60 | -5827.36 | 54.18 | 3.82 |
| 8-10 ns | MM-PBSA(ligand) | -72.98 | 2.46 | 0.17 | -73.05 | 2.54 | 0.18 |
| 8-10 ns | MM-PBSA(binding) | -4.54 | 3.54 | 0.25 | -7.60 | 3.63 | 0.26 |
| 10-12 ns | MM-GBSA(Complex) | -7132.15 | 48.38 | 3.42 | -7207.47 | 50.48 | 3.57 |
| 10-12 ns | MM-GBSA(receptor) | -7035.85 | 48.39 | 3.42 | -7107.99 | 50.05 | 3.54 |
| 10-12 ns | MM-GBSA(ligand) | -75.83 | 2.57 | 0.18 | -76.25 | 2.48 | 0.18 |
| 10-12 ns | MM-GBSA(binding) | -20.47 | 1.75 | 0.12 | -23.23 | 1.76 | 0.12 |
| 10-12 ns | MM-PBSA(Complex) | -5857.45 | 56.12 | 3.97 | -5913.08 | 54.60 | 3.86 |
| 10-12 ns | MM-PBSA(receptor) | -5780.62 | 56.18 | 3.97 | -5830.31 | 53.83 | 3.81 |
| 10-12 ns | MM-PBSA(ligand) | -72.90 | 2.59 | 0.18 | -73.32 | 2.45 | 0.17 |
| 10-12 ns | MM-PBSA(binding) | -3.93 | 3.48 | 0.25 | -9.45 | 3.48 | 0.25 |
|  |  |  |  |  |  |  |  |
| 2-12 ns | MM-GBSA(Complex) | -7146.48 | 50.31 | 3.55 | -7203.58 | 48.73 | 3.44 |
| 2-12 ns | MM-GBSA(receptor) | -7050.17 | 50.35 | 3.55 | -7105.13 | 48.62 | 3.43 |
| 2-12 ns | MM-GBSA(ligand) | -75.99 | 2.54 | 0.18 | -75.95 | 2.51 | 0.18 |
| 2-12 ns | MM-GBSA(binding) | -20.33 | 1.86 | 0.13 | -22.50 | 1.87 | 0.13 |
| 2-12 ns | MM-PBSA(Complex) | -5873.69 | 54.91 | 3.87 | -5901.23 | 53.32 | 3.76 |
| 2-12 ns | MM-PBSA(receptor) | -5796.70 | 55.08 | 3.89 | -5820.47 | 52.96 | 3.74 |
| 2-12 ns | MM-PBSA(ligand) | -73.03 | 2.53 | 0.18 | -73.02 | 2.49 | 0.18 |
| 2-12 ns | MM-PBSA(binding) | -3.96 | 3.51 | 0.25 | -7.73 | 3.67 | 0.26 |

MD simulations on SIRT3/INT/NAM complex prepared from 4BVG with and without loop replacement that is taken from residue 155-178 of 4FVT show mixed results. The trajectories were analyzed using the same MM-GB(PB)SA calculations as above. While the MM-GBSA results show clearly that the with the loop substitution, the overall energy is higher and the binding affinity decreases, the MM-PBSA results show that the loop substitution actually lower the overall energy, and the binding affinity is lower as well.

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| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | 4BVG w/NAM and loop replacement | | | 4BVG with NAM placement | | |
| time | Eng | Average | Std. Dev. | Std. Err. of Mean | Average | Std. Dev. | Std. Err. of Mean |
| 2-4 ns | MM-GBSA(Complex) | -7432.84 | 50.68 | 3.57 | -7464.74 | 51.08 | 3.60 |
| 2-4 ns | MM-GBSA(receptor) | -7339.14 | 50.98 | 3.60 | -7370.14 | 51.00 | 3.60 |
| 2-4 ns | MM-GBSA(ligand) | -75.67 | 2.54 | 0.18 | -75.67 | 2.40 | 0.17 |
| 2-4 ns | MM-GBSA(binding) | -18.03 | 1.81 | 0.13 | -18.93 | 2.20 | 0.16 |
| 2-4 ns | MM-PBSA(Complex) | -6127.56 | 57.23 | 4.04 | -6128.43 | 52.74 | 3.72 |
| 2-4 ns | MM-PBSA(receptor) | -6054.95 | 56.71 | 4.00 | -6048.63 | 53.02 | 3.74 |
| 2-4 ns | MM-PBSA(ligand) | -72.60 | 2.50 | 0.18 | -72.90 |  |  |
| 2-4 ns | MM-PBSA(binding) | -0.02 | 4.55 | 0.32 | -6.90 |  |  |
| 4-6 ns | MM-GBSA(Complex) | -7417.88 | 49.62 | 3.50 | -7419.88 | 50.52 | 3.56 |
| 4-6 ns | MM-GBSA(receptor) | -7324.78 | 49.76 | 3.51 | -7323.77 | 50.32 | 3.55 |
| 4-6 ns | MM-GBSA(ligand) | -75.65 | 2.51 | 0.18 | -75.92 | 2.44 | 0.17 |
| 4-6 ns | MM-GBSA(binding) | -17.44 | 1.85 | 0.13 | -20.18 | 1.96 | 0.14 |
| 4-6 ns | MM-PBSA(Complex) | -6116.08 | 60.73 | 4.28 | -6085.57 | 55.37 | 3.91 |
| 4-6 ns | MM-PBSA(receptor) | -6044.37 | 60.22 | 4.25 | -6008.62 | 55.26 | 3.90 |
| 4-6 ns | MM-PBSA(ligand) | -72.67 | 2.50 | 0.18 | -72.89 | 2.42 | 0.17 |
| 4-6 ns | MM-PBSA(binding) | 0.96 | 4.00 | 0.28 | -4.06 | 3.54 | 0.25 |
| 6-8 ns | MM-GBSA(Complex) | -7431.85 | 52.10 | 3.67 | -7485.77 | 50.38 | 3.55 |
| 6-8 ns | MM-GBSA(receptor) | -7338.70 | 51.76 | 3.65 | -7388.92 | 50.21 | 3.54 |
| 6-8 ns | MM-GBSA(ligand) | -75.65 | 2.50 | 0.18 | -75.81 | 2.47 | 0.17 |
| 6-8 ns | MM-GBSA(binding) | -17.50 | 2.02 | 0.14 | -21.03 | 1.57 | 0.11 |
| 6-8 ns | MM-PBSA(Complex) | -6162.60 | 59.01 | 4.16 | -6139.09 | 56.28 | 3.97 |
| 6-8 ns | MM-PBSA(receptor) | -6089.22 | 58.39 | 4.12 | -6063.15 | 56.34 | 3.97 |
| 6-8 ns | MM-PBSA(ligand) | -72.59 | 2.48 | 0.18 | -72.79 | 2.48 | 0.17 |
| 6-8 ns | MM-PBSA(binding) | -0.80 | 3.11 | 0.22 | -3.15 | 3.20 | 0.23 |
| 8-10 ns | MM-GBSA(Complex) | -7434.14 | 45.86 | 3.23 | -7492.73 | 45.21 | 3.19 |
| 8-10 ns | MM-GBSA(receptor) | -7341.21 | 46.18 | 3.26 | -7395.78 | 44.79 | 3.16 |
| 8-10 ns | MM-GBSA(ligand) | -75.53 | 2.86 | 0.20 | -75.98 | 2.60 | 0.18 |
| 8-10 ns | MM-GBSA(binding) | -17.40 | 2.03 | 0.14 | -20.97 | 1.57 | 0.11 |
| 8-10 ns | MM-PBSA(Complex) | -6176.31 | 48.02 | 3.39 | -6155.15 | 48.74 | 3.44 |
| 8-10 ns | MM-PBSA(receptor) | -6104.37 | 48.35 | 3.41 | -6078.98 | 48.73 | 3.44 |
| 8-10 ns | MM-PBSA(ligand) | -72.45 | 2.80 | 0.20 | -72.97 | 2.60 | 0.18 |
| 8-10 ns | MM-PBSA(binding) | 0.51 | 3.07 | 0.22 | -3.20 | 3.51 | 0.25 |
| 10-12 ns | MM-GBSA(Complex) | -7457.50 | 50.78 | 3.59 | -7476.44 | 49.70 | 3.51 |
| 10-12 ns | MM-GBSA(receptor) | -7363.65 | 50.44 | 3.57 | -7383.50 | 49.95 | 3.53 |
| 10-12 ns | MM-GBSA(ligand) | -75.91 | 2.26 | 0.16 | -75.92 | 2.39 | 0.17 |
| 10-12 ns | MM-GBSA(binding) | -17.93 | 1.93 | 0.14 | -17.02 | 2.56 | 0.18 |
| 10-12 ns | MM-PBSA(Complex) | -6189.51 | 55.40 | 3.92 | -6152.64 | 53.05 | 3.75 |
| 10-12 ns | MM-PBSA(receptor) | -6116.29 | 55.13 | 3.90 | -6076.08 | 53.31 | 3.77 |
| 10-12 ns | MM-PBSA(ligand) | -72.83 | 2.27 | 0.16 | -72.95 | 2.36 | 0.17 |
| 10-12 ns | MM-PBSA(binding) | -0.39 | 2.95 | 0.21 | -3.61 | 4.20 | 0.30 |
|  |  |  |  |  |  |  |  |
| 2-12 ns | MM-GBSA(Complex) | -7434.84 | 49.81 | 3.51 | -7467.91 | 49.38 | 3.48 |
| 2-12 ns | MM-GBSA(receptor) | -7341.50 | 49.82 | 3.52 | -7372.42 | 49.25 | 3.48 |
| 2-12 ns | MM-GBSA(ligand) | -75.68 | 2.53 | 0.18 | -75.86 | 2.46 | 0.17 |
| 2-12 ns | MM-GBSA(binding) | -17.66 | 1.93 | 0.14 | -19.63 | 1.97 | 0.14 |
| 2-12 ns | MM-PBSA(Complex) | -6154.41 | 56.08 | 3.96 | -6132.18 | 53.24 | 3.76 |
| 2-12 ns | MM-PBSA(receptor) | -6081.84 | 55.76 | 3.93 | -6055.09 | 53.33 | 3.76 |
| 2-12 ns | MM-PBSA(ligand) | -72.63 | 2.51 | 0.18 | -72.90 | 1.97 | 0.14 |
| 2-12 ns | MM-PBSA(binding) | 0.05 | 3.54 | 0.25 | -4.18 | 2.89 | 0.20 |