1. C pocket side chain optimization in presence of NAM

Several approaches have been adopted to prepare the structures and the associated energies were compared.

Side chains selected for prediction/optimization include residue 144-180, 195, 199, 204, 207, 210, 227-234, 248, 251, 291, 294, 324 that covers all the residues in the flexible loop, residues within 7.5 Angstrom of potential NAM binding pocket suggested from xtal structures 4FVT and 4BVG.

Various calculations have been performed.

Comparison of SIRT3/INT/NAM converted from 4FVT w/ or w/o loop (res 155-178) substitution & prepared without relaxation on heavy atoms from xtal structure:

|  |  |
| --- | --- |
| **Comparison between SIRT3/INT/NAM complex** | **Prime Energy** |
| Struct B1: from 4FVT to SIRT3/INT/NAM & h-opt | -9965.2 |
| Struct B1 w/ Prime minimization | -12494.0 |
| Struct B2: Struct B1 w/ sidechain prediction | -10255.0 |
| Struct B2 w/ Prime minimization | -12498.1 |
|  |  |
| Struct B3: 4FVT to SIRT3/INT/NAM w/ loop sub for res 155-178 & h-opt | 951282.7 |
| Struct B3 w/ Prime minimization | -12502.8 |
| Struct B4: Struct B3 w/ sidechain prediction | -10062.3 |
| Struct B4 w/ Prime minimization | -12501.3 |

Comparison of SIRT3/INT/NAM converted from 4FVT w/ or w/o loop (res 155-178) substitution & prepared with relaxation on heavy atoms from xtal structure:

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| --- | --- |
| **Comparison between SIRT3/INT/NAM complex** | **Prime Energy** |
| Struct B'1: from 4FVT to SIRT3/INT/NAM & full-opt | -11687.3 |
| Struct B'1 w/ Prime minimization | -12474.0 |
| Struct B'2: Struct B1 w/ sidechain prediction | -11759.7 |
| Struct B'2 w/ Prime minimization | -12500.2 |
|  |  |
| Struct B'3: 4FVT to SIRT3/INT/NAM w/ loop sub for res 155-178 & full-opt | -11705.1 |
| Struct B'3 w/ Prime minimization | -12479.6 |
| Struct B'4: Struct B3 w/ sidechain prediction | -11740.9 |
| Struct B'4 w/ Prime minimization | -12495.1 |

Comparison of SIRT3/INT/NAM prepared from placing NAM into 4BVG w/ or w/o loop (res 155-178) substitution & prepared without relaxation on heavy atoms from xtal structure: (Note: placement of NAM is based on structural alignment between 4BVG & 4FVT prepared above - Struct B1)

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| --- | --- |
| **Comparison between SIRT3/INT/NAM complex** | **Prime Energy** |
| Struct C1: place NAM into 4BVG to form SIRT3/INT/NAM & h-opt | -9590.8 |
| Struct C2: Struct C1 w/ Prime minimization | -12614.2 |
| Struct C3: Struct C1 w/ sidechain prediction | -9909.4 |
| Struct C3 w/ Prime minimization | -12598.1 |
| Struct C4: Struct C2 w/ sidechain prediction | -12620.5 |
| Struct C4 w/ Prime minimization | -12621.8 |
|  |  |
| Struct C5: place NAM into 4BVG to form SIRT3/INT/NAM w/ loop sub for res 155-178 & h-opt | -4656.1 |
| Struct C6: Struct C5 w/ Prime minimization | -12549.3 |
| Struct C7: Struct C5 w/ sidechain prediction | -9807.1 |
| Struct C7 w/ Prime minimization | -12535.6 |
| Struct C8: Struct C6 w/ sidechain prediction | -12552.1 |
| Struct C8 w/ Prime minimization | -12577.6 |

Comparison of SIRT3/INT/NAM prepared from placing NAM into 4BVG w/ or w/o loop (res 155-178) substitution & prepared with relaxation on heavy atoms from xtal structure: (Note: placement of NAM is based on structural alignment between 4BVG & 4FVT prepared above - Struct B1)

|  |  |
| --- | --- |
| **Comparison between SIRT3/INT/NAM complex** | **Prime Energy** |
| Struct C'1: place NAM into 4BVG to form SIRT3/INT/NAM & full-opt | -11818.3 |
| Struct C'2: Struct C1 w/ Prime minimization | -12555.6 |
| Struct C'3: Struct C1 w/ sidechain prediction | -11846.2 |
| Struct C'3 w/ Prime minimization | -12577.2 |
| Struct C'4: Struct C2 w/ sidechain prediction | -12550.4 |
| Struct C'4 w/ Prime minimization | -12576.8 |
|  |  |
| Struct C'5: place NAM into 4BVG to form SIRT3/INT/NAM w/ loop sub for res 155-178 & full-opt | -11757.4 |
| Struct C'6: Struct C5 w/ Prime minimization | -12569.8 |
| Struct C'7: Struct C5 w/ sidechain prediction | -11823.0 |
| Struct C'7 w/ Prime minimization | -12628.1 |
| Struct C'8: Struct C6 w/ sidechain prediction | -12562.7 |
| Struct C'8 w/ Prime minimization | -12602.3 |

Comparison of SIRT3/INT/NAM prepared from docking NAM into 4BVG using Glide XP, only rank 1 is used in further calculation:

|  |  |
| --- | --- |
| **Comparison between SIRT3/INT/NAM complex** | **Prime Energy** |
| Struct D1: docking NAM into 4BVG (Struct A1)to form SIRT3/INT/NAM | -9705.9 |
| Struct D2: Struct D1 w/ Prime minimization | -12583.5 |
| Struct D3: Struct D1 w/ sidechain prediction | -9948.7 |
| Struct D3 w/ Prime minimization | -12534.7 |
| Struct D4: Struct D2 w/ sidechain prediction | -12591.3 |
| Struct D4 w/ Prime minimization | -12599.4 |

In induced fit, residues nearby are relaxed/temporary reduced to explore and accommodate more ligand poses.

Induced Fit with standard option has been carried out.

|  |  |
| --- | --- |
| **Comparison between SIRT3/INT/NAM complex** | **Prime Energy** |
| Struct E1: Induced Fit docking of NAM to 4BVG (Struct A1) w/ standard method, rank 1 | -10110.2 |
| Struct E2: Induced Fit docking of NAM to 4BVG (Struct A1) w/ standard method, rank 2 | -10109.2 |
| Struct E3: Induced Fit docking of NAM to 4BVG (Struct A1) w/ standard method, rank 3 | -10107.8 |
| Struct E4: Induced Fit docking of NAM to 4BVG (Struct A1) w/ standard method, rank 4 | -10106.6 |
| Struct E5: Induced Fit docking of NAM to 4BVG (Struct A1) w/ standard method, rank 5 | -10102.9 |
| Struct E6: Induced Fit docking of NAM to 4BVG (Struct A1) w/ standard method, rank 6 | -10105.4 |
| Struct E7: Induced Fit docking of NAM to 4BVG (Struct A1) w/ standard method, rank 7 | -10103.1 |
| Struct E8: Induced Fit docking of NAM to 4BVG (Struct A1) w/ standard method, rank 8 | -10096.1 |
| Struct E9: Induced Fit docking of NAM to 4BVG (Struct A1) w/ standard method, rank 9 | -10093.0 |
| Struct E10: Induced Fit docking of NAM to 4BVG (Struct A1) w/ standard method, rank 10 | -10095.6 |
| Struct E11: Induced Fit docking of NAM to 4BVG (Struct A1) w/ standard method, rank 11 | -10094.5 |
| Struct E12: Induced Fit docking of NAM to 4BVG (Struct A1) w/ standard method, rank 12 | -10099.5 |
| Struct E13: Induced Fit docking of NAM to 4BVG (Struct A1) w/ standard method, rank 13 | -10093.5 |
| Struct E14: Induced Fit docking of NAM to 4BVG (Struct A1) w/ standard method, rank 14 | -10098.3 |
| Struct E15: Induced Fit docking of NAM to 4BVG (Struct A1) w/ standard method, rank 15 | -10085.1 |
| Struct E1 w/ Prime minimization | -12588.0 |
| Struct E2 w/ Prime minimization | -12604.7 |
| Struct E3 w/ Prime minimization | -12600.8 |
| Struct E4 w/ Prime minimization | -12592.8 |
| Struct E14 w/ Prime minimization | -12595.2 |
| Struct E1 w/ Prime minimization followed by another Prime minimization | -12594.6 |

1. side chain optimization validation:

Using xtal structures 4BVG and 4FVT, we explore the sidechain optimization options on the impact of final energies.

From 4BVG, we only considered the case without heavy atom relaxation during the protein preparation.

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| --- | --- |
| **Comparison between SIRT3/INT complex** | **Prime Energy** |
| Struct A1: 4BVG with protein preparation & h-opt | -9620.0 |
| Struct A2: Struct A1 w/ Prime minimization | -12500.3 |
| Struct A3: Struct A1 w/ sidechain prediciton | -9875.7 |
| Struct A3 w/ Prime minimization | -12530.8 |
| Struct A4: Struct A2 w/ sidechain prediciton | -12479.2 |
| Struct A4 w/ Prime minimization | -12542.2 |
|  |  |
| Struct A5: 4BVG w/ loop sub for res 155-178 & h-opt | -9317.1 |
| Stuct A5 w/ Prime miminization | -12430.6 |
| Struct A6: Struct A5 w/ sidechain prediction | -9749.8 |
| Struct A6 w/ Prime minimization | -12527.2 |

Additionally, a limited set of sidechain prediction using only residue 155-178 were carried out for 4BVG, and it shows much less improvement in overall energy.

|  |  |
| --- | --- |
| Struct A'3: Struct A1 w/ loop 155-178 sidechain prediciton | -9827.0 |
| Struct A'3 w/ Prime minimization | -12487.8 |

From 4FVT, we carried out sidechain predictions for SIRT3/carba-NAD+/ac-LYS complex using only the loop residues (155-178) or larger selection specified above. For results of 4FVT prepared without relaxation on heavy atoms from xtal structure:

|  |  |
| --- | --- |
| **Comparison between SIRT3/carba-NAD+/ac-LYS complex** | **Prime Energy** |
| Struct F1: 4FVT with protein preparation & h-opt | -10055.6 |
| Struct F2: Struct F1 w/ Prime minimization | -12479.0 |
| Struct F3: Struct F1 w/ loop 155-178 sidechain prediciton | -10224.3 |
| Struct F3 w/ Prime minimization | -12515.7 |
| Struct F4: Struct F1 w/ sidechain prediciton | -10342.2 |
| Struct F4 w/ Prime minimization | -12485.2 |

From 4FVT, we carried out sidechain predictions for SIRT3/carba-NAD+/ac-LYS complex using only the loop residues (155-178) or larger selection specified above. For results of 4FVT prepared with relaxation on heavy atoms from xtal structure:

|  |  |
| --- | --- |
| **Comparison between SIRT3/carba-NAD+/ac-LYS complex** | **Prime Energy** |
| Struct F'1: 4FVT with protein preparation & full-opt | -11667.0 |
| Struct F'2: Struct F'1 w/ Prime minimization | -12431.2 |
| Struct F'3: Struct F'1 w/ loop 155-178 sidechain prediciton | -11711.4 |
| Struct F'3 w/ Prime minimization | -12492.8 |
| Struct F'4: Struct F'1 w/ sidechain prediciton | -11747.6 |
| Struct F'4 w/ Prime minimization | -12491.6 |

The values obtained vary significantly. It is hard to draw any solid conclusion as to what is the method of choice in such preparation.

I have also done some MD simulations and MM-GB(PB)SA calculations, which provide some clues to the loop conformation & energies. The result will be presented in a separate report currently under preparation.