Binding affinities discussion:

1. NAD+ in SIRT3 and Sir2TM in ternary complex:

|  |  |  |
| --- | --- | --- |
|  | SIRT3/Ac-CS2/ NAD+ | Sir2TM/Ac-p53/NAD+ |
| MM-PBSA | -5.35 | -23.19 |
| MM-GBSA | -65.63 | -105.48 |

* NAD+ has stronger binding affinity for Sir2TM in ternary structure, where it occupies AC binding pocket.
* The main contribution to the stronger binding affinity is due to the stronger electrostatic interaction between NAD+ and enzyme in term of EEL in the component analysis below.
* The proximity of charged groups near negatively charged pyrophosphate moiety or positively charged nicotinamde moiety favors Sir2TM, as observed from the interaction diagram between NAD+ and the receptor (including both enzyme and peptide substrate) constructed from the averaged structure of last 10ps, e.g. the ARG34 in Sir2TM and ASP156 in SIRT3.
* Other polar interactions such hydrogen bonds also favors Sir2TM/NAD+ interactions. In Sir2TM ternary structure, more hydrogen bond interactions were formed between NAD+ and receptor, and less exposure to solvents. It can also be found in the structural presentation of the binding sites.
* Also notable are the solvation energy losses. SIRT3 has less loss in solvation energy upon NAD+ binding in the ternary complex, which can be seen from the interaction diagrams that NAD+ in SIRT3 ternary complex has more exposure to solvent. However, the favorable solvation energy also leads to less favorable contacts between NAD+ and enzyme/peptide substrate, resulting in lower binding affinity.

1. Component analysis for NAD+ in ternary structures:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | SIRT3/Ac-CS2/NAD+ | | Sir2TM/Ac-p53/NAD+ | |
|  | Average | Std. Dev. | Average | Std. Dev. |
| VDWAALS\_complex | -2273.75 | 26.32 | -2117.62 | 21.42 |
| EEL\_complex | -18619.99 | 108.87 | -18522.60 | 98.20 |
| EGB\_complex | -3649.28 | 88.04 | -3632.31 | 80.44 |
| ESURF\_complex | 99.68 | 2.63 | 85.96 | 1.66 |
| EPB\_complex | -3395.79 | 89.40 | -3418.26 | 77.16 |
| ENPOLAR\_complex | 2197.40 | 13.67 | 2008.16 | 8.65 |
| EDISPER\_complex | -1263.26 | 16.80 | -1149.73 | 9.69 |
| G\_gas\_complex | -3816.40 | 108.94 | -3245.53 | 95.86 |
| G\_solv\_igb2\_complex | -3549.60 | 86.31 | -3546.35 | 79.73 |
| G\_solv\_pb\_complex | -2461.65 | 91.31 | -2559.84 | 76.73 |
| VDWAALS\_receptor | -2193.71 | 25.99 | -2028.58 | 20.52 |
| EEL\_ receptor | -18662.54 | 108.22 | -18373.64 | 97.99 |
| EGB\_ receptor | -3461.19 | 86.02 | -3601.81 | 80.87 |
| ESURF\_ receptor | 103.05 | 2.61 | 91.04 | 1.64 |
| EPB\_ receptor | -3209.19 | 87.10 | -3405.90 | 77.31 |
| ENPOLAR\_receptor | 2184.17 | 13.55 | 2002.19 | 8.56 |
| EDISPER\_receptor | -1284.01 | 16.35 | -1181.35 | 9.58 |
| G\_gas\_receptor | -3683.03 | 107.06 | -2911.08 | 95.73 |
| G\_solv\_igb2\_receptor | -3358.15 | 84.25 | -3510.77 | 80.16 |
| G\_solv\_pb\_receptor | -2309.03 | 88.76 | -2585.06 | 76.77 |
| VDWAALS\_ligand | -11.19 | 1.20 | -10.80 | 1.64 |
| EEL\_ligand | 44.70 | 10.12 | -6.08 | 8.20 |
| EGB\_ligand | -202.22 | 6.13 | -156.41 | 3.89 |
| ESURF\_ligand | 5.40 | 0.06 | 5.20 | 0.04 |
| EPB\_ligand | -211.06 | 6.28 | -164.48 | 3.54 |
| ENPOLAR\_ligand | 59.37 | 0.38 | 57.59 | 0.34 |
| EDISPER\_ligand | -66.57 | 0.45 | -65.81 | 0.36 |
| G\_gas\_ligand | -62.37 | 8.51 | -113.33 | 7.34 |
| G\_solv\_igb2\_ligand | -196.82 | 6.09 | -151.21 | 3.88 |
| G\_solv\_pb\_ligand | -218.26 | 6.26 | -172.70 | 3.60 |
| VDWAALS\_diff | -68.85 | 4.82 | -78.24 | 4.71 |
| EEL\_diff | -2.14 | 18.13 | -142.87 | 11.80 |
| EGB\_diff | 14.13 | 15.11 | 125.91 | 8.05 |
| ESURF\_diff | -8.76 | 0.25 | -10.28 | 0.16 |
| EPB\_diff | 24.47 | 15.75 | 152.11 | 8.21 |
| ENPOLAR\_diff | -46.14 | 1.10 | -51.62 | 0.66 |
| EDISPER\_diff | 87.31 | 1.58 | 97.43 | 0.98 |
| G\_gas | -70.99 | 17.71 | -221.11 | 11.07 |
| G\_solv\_igb2 | 5.36 | 15.11 | 115.64 | 8.02 |
| G\_solv\_pb | 65.64 | 16.00 | 197.92 | 8.26 |
| G\_igb2 | -65.63 | 5.91 | -105.48 | 6.48 |
| G\_pb | -5.35 | 8.37 | -23.19 | 7.27 |

Term explanation:

VDWAALS: van der waals not including the 1-4 terms

EEL: electrostatic interactions not including the 1-4 terms

EGB: Polar contribution to solvation energy by GB method

ESURF: non-polar contribution to solvation energy using SASA (solvent accessible surface area) for GB

EPB: Polar contribution to solvation energy by PB method

ENPOLAR: non-polar contribution to solvation energy from repulsive solute-solvent interactions for PB

EDISPER: non-polar contribution to solvation energy from attractive solute-solvent interactions for PB

G\_gas: Gas phase MM energy including all bonded and non-bonded terms

G\_solv\_igb2: Total solvation energy by GB method

G\_solv\_pb: Total solvation energy by PB method

G\_igb2: Difference in total energy including gas phase MM energy and solvation energy by GB method

G\_pb: Difference in total energy including gas phase MM energy and solvation energy by PB method

1. NAD+ in SIRT3 and Sir2TM in complex with NAM and isoNAM:

|  |  |  |  |
| --- | --- | --- | --- |
|  | SIRT3/Ac-CS2/NAD+/NAM | Sir2TM/Ac-p53/NAD+/NAM | SIRT3/Ac-CS2/ NAD+/isoNAM |
| MM-PBSA | -8.10 | -16.15 | 0.33 |
| MM-GBSA | -63.12 | -81.78 | -62.70 |

* NAD+ takes AB pocket when NAM occupies the C pocket as we saw from the complexes with NAM. The NAD+ in AB poses are different in two complexes as we can see when we align them up with the NAD+ in AB pose taken from the chain A of 1YC2.
* The difference in binding affinity of NAD+ in two complexes is less than in ternary structure.
* The comparison of energetic contributions in binding free energies of NAD+ in SIRT3 complex and Sir2TM complex is similar to what we saw in ternary complex.

1. Component analysis for NAD+ in complexes with NAM and isoNAM:

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | SIRT3/Ac-CS2/NAD+/NAM | | Sir2TM/Ac-p53/NAD+/NAM | | | SIRT3/Ac-CS2/ NAD+/isoNAM | | |
|  | Average | Std. Dev. | | Average | Std. Dev. | | Average | Std. Dev. | |
| VDWAALS\_complex | -2292.54 | 22.24 | | -2128.33 | 21.19 | | -2291.36 | 26.33 | |
| EEL\_complex | -18607.05 | 79.63 | | -18440.98 | 115.51 | | -18848.86 | 94.11 | |
| EGB\_complex | -3628.77 | 63.85 | | -3643.52 | 104.17 | | -3549.13 | 75.36 | |
| ESURF\_complex | 97.99 | 1.71 | | 85.03 | 1.61 | | 99.39 | 2.24 | |
| EPB\_complex | -3364.63 | 60.02 | | -3421.00 | 101.88 | | -3283.26 | 78.97 | |
| ENPOLAR\_complex | 2194.97 | 9.05 | | 2011.84 | 8.04 | | 2201.98 | 11.31 | |
| EDISPER\_complex | -1250.40 | 10.93 | | -1144.61 | 9.22 | | -1260.10 | 13.26 | |
| G\_gas\_complex | -3935.93 | 86.17 | | -3291.05 | 120.15 | | -3968.19 | 98.67 | |
| G\_solv\_igb2\_complex | -3530.79 | 63.31 | | -3558.48 | 103.55 | | -3449.73 | 74.09 | |
| G\_solv\_pb\_complex | -2420.06 | 59.71 | | -2553.77 | 101.12 | | -2341.38 | 79.66 | |
| VDWAALS\_receptor | -2209.64 | 21.52 | | -2044.26 | 20.49 | | -2206.88 | 25.30 | |
| EEL\_receptor | -18637.48 | 77.84 | | -18350.63 | 116.14 | | -18844.00 | 90.31 | |
| EGB\_receptor | -3441.90 | 62.07 | | -3570.51 | 105.92 | | -3406.37 | 72.00 | |
| ESURF\_receptor | 101.74 | 1.65 | | 88.84 | 1.59 | | 102.88 | 2.25 | |
| EPB\_receptor | -3177.13 | 58.46 | | -3356.23 | 104.72 | | -3146.28 | 74.75 | |
| ENPOLAR\_receptor | 2182.62 | 8.87 | | 2000.84 | 7.98 | | 2189.29 | 11.23 | |
| EDISPER\_receptor | -1269.82 | 10.65 | | -1168.49 | 8.82 | | -1280.68 | 13.15 | |
| G\_gas\_receptor | -3794.12 | 84.24 | | -3020.55 | 122.24 | | -3787.60 | 94.57 | |
| G\_solv\_igb2\_receptor | -3340.16 | 61.50 | | -3481.67 | 105.25 | | -3303.49 | 70.67 | |
| G\_solv\_pb\_receptor | -2264.33 | 58.56 | | -2523.87 | 103.67 | | -2237.67 | 75.53 | |
| VDWAALS\_ligand | -9.96 | 1.70 | | -11.67 | 1.34 | | -10.28 | 1.61 | |
| EEL\_ligand | -22.14 | 7.69 | | -8.14 | 8.15 | | -13.45 | 9.76 | |
| EGB\_ligand | -152.93 | 3.73 | | -154.86 | 4.27 | | -154.30 | 5.87 | |
| ESURF\_ligand | 5.06 | 0.04 | | 5.22 | 0.05 | | 5.14 | 0.04 | |
| EPB\_ligand | -159.70 | 3.27 | | -160.81 | 3.91 | | -161.15 | 5.92 | |
| ENPOLAR\_ligand | 57.28 | 0.34 | | 58.08 | 0.37 | | 57.58 | 0.34 | |
| EDISPER\_ligand | -65.58 | 0.35 | | -65.61 | 0.43 | | -66.06 | 0.43 | |
| G\_gas\_ligand | -121.45 | 6.48 | | -115.90 | 7.24 | | -114.98 | 8.35 | |
| G\_solv\_igb2\_ligand | -147.87 | 3.71 | | -149.63 | 4.25 | | -149.16 | 5.85 | |
| G\_solv\_pb\_ligand | -168.00 | 3.24 | | -168.34 | 3.88 | | -169.64 | 5.92 | |
| VDWAALS\_diff | -72.94 | 4.35 | | -72.40 | 4.57 | | -74.20 | 4.24 | |
| EEL\_diff | 52.57 | 13.53 | | -82.21 | 20.85 | | 8.59 | 16.28 | |
| EGB\_diff | -33.94 | 12.02 | | 81.85 | 15.75 | | 11.54 | 12.75 | |
| ESURF\_diff | -8.81 | 0.28 | | -9.03 | 0.33 | | -8.63 | 0.23 | |
| EPB\_diff | -27.80 | 12.52 | | 96.05 | 18.91 | | 24.18 | 14.07 | |
| ENPOLAR\_diff | -44.93 | 1.14 | | -47.08 | 1.44 | | -44.89 | 0.98 | |
| EDISPER\_diff | 85.00 | 1.46 | | 89.48 | 1.83 | | 86.64 | 1.26 | |
| G\_gas | -20.37 | 13.27 | | -154.60 | 21.64 | | -65.61 | 15.43 | |
| G\_solv\_igb2 | -42.76 | 12.10 | | 72.82 | 15.62 | | 2.91 | 12.68 | |
| G\_solv\_pb | 12.27 | 12.58 | | 138.45 | 19.06 | | 65.93 | 14.02 | |
| G\_igb2 | -63.12 | 4.37 | | -81.78 | 8.01 | | -62.70 | 5.27 | |
| G\_pb | -8.10 | 5.71 | | -16.15 | 8.35 | | 0.33 | 6.52 | |

1. NAM and isoNAM in C pocket of SIRT3 and Sir2TM:

|  |  |  |  |
| --- | --- | --- | --- |
|  | SIRT3/Ac-CS2/NAD+/NAM | Sir2TM/Ac-p53/NAD+/NAM | SIRT3/Ac-CS2/NAD+/isoNAM |
| MM-PBSA | 0.26 | -5.78 | 3.89 |
| MM-GBSA | -19.82 | -20.74 | -15.68 |

* NAM in the C pocket again favors Sir2TM over SIRT3. The difference is not very significant in MM-GBSA values. isoNAM in C pocket is less favorable than NAM in SIRT3.

1. Component analysis for NAM and isoNAM in C pocket in SIRT3 and Sir2TM systems:

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | SIRT3/Ac-CS2/NAD+/NAM | | | Sir2TM/Ac-p53/NAD+/NAM | | | SIRT3/Ac-CS2/ NAD+/isoNAM | | | |
|  | | Average | Std. Dev. | | Average | Std. Dev. | | Average | Std. Dev. |
| VDWAALS\_complex | | -2292.54 | 22.24 | | -2128.33 | 21.19 | | -2291.36 | 26.33 |
| EEL\_complex | | -18607.05 | 79.63 | | -18440.98 | 115.51 | | -18848.86 | 94.11 |
| EGB\_complex | | -3628.77 | 63.85 | | -3643.52 | 104.17 | | -3549.13 | 75.36 |
| ESURF\_complex | | 97.99 | 1.71 | | 85.03 | 1.61 | | 99.39 | 2.24 |
| EPB\_complex | | -3364.63 | 60.02 | | -3421.00 | 101.88 | | -3283.26 | 78.97 |
| ENPOLAR\_complex | | 2194.97 | 9.05 | | 2011.84 | 8.04 | | 2201.98 | 11.31 |
| EDISPER\_complex | | -1250.40 | 10.93 | | -1144.61 | 9.22 | | -1260.10 | 13.26 |
| G\_gas\_complex | | -3935.93 | 86.17 | | -3291.05 | 120.15 | | -3968.19 | 98.67 |
| G\_solv\_igb2\_complex | | -3530.79 | 63.31 | | -3558.48 | 103.55 | | -3449.73 | 74.09 |
| G\_solv\_pb\_complex | | -2420.06 | 59.71 | | -2553.77 | 101.12 | | -2341.38 | 79.66 |
| VDWAALS\_receptor | | -2271.60 | 22.11 | | -2107.89 | 21.10 | | -2271.30 | 25.59 |
| EEL\_receptor | | -18638.66 | 79.45 | | -18479.81 | 115.47 | | -18756.56 | 94.44 |
| EGB\_receptor | | -3639.47 | 63.75 | | -3648.08 | 104.03 | | -3551.26 | 75.50 |
| ESURF\_receptor | | 99.15 | 1.70 | | 86.18 | 1.61 | | 100.41 | 2.16 |
| EPB\_receptor | | -3384.01 | 59.90 | | -3429.62 | 102.12 | | -3293.48 | 78.59 |
| ENPOLAR\_receptor | | 2193.97 | 9.05 | | 2010.82 | 8.01 | | 2199.94 | 11.04 |
| EDISPER\_receptor | | -1259.60 | 10.88 | | -1153.21 | 9.14 | | -1268.60 | 12.89 |
| G\_gas\_receptor | | -3830.47 | 85.72 | | -3191.80 | 119.99 | | -3920.32 | 98.27 |
| G\_solv\_igb2\_receptor | | -3540.32 | 63.22 | | -3561.89 | 103.40 | | -3450.85 | 74.28 |
| G\_solv\_pb\_receptor | | -2449.64 | 59.59 | | -2572.01 | 101.32 | | -2362.13 | 79.21 |
| VDWAALS\_ligand | | -0.59 | 0.24 | | -0.49 | 0.26 | | -0.60 | 0.26 |
| EEL\_ligand | | 55.71 | 0.83 | | 57.31 | 1.09 | | -80.72 | 1.00 |
| EGB\_ligand | | -16.82 | 0.51 | | -16.01 | 0.57 | | -15.97 | 0.55 |
| ESURF\_ligand | | 1.73 | 0.01 | | 1.73 | 0.01 | | 1.73 | 0.01 |
| EPB\_ligand | | -14.59 | 0.47 | | -13.86 | 0.52 | | -13.72 | 0.51 |
| ENPOLAR\_ligand | | 15.47 | 0.10 | | 15.45 | 0.10 | | 15.53 | 0.12 |
| EDISPER\_ligand | | -16.01 | 0.12 | | -16.00 | 0.12 | | -15.97 | 0.13 |
| G\_gas\_ligand | | -61.02 | 2.53 | | -60.82 | 2.47 | | -16.83 | 2.68 |
| G\_solv\_igb2\_ligand | | -15.09 | 0.51 | | -14.28 | 0.56 | | -14.24 | 0.55 |
| G\_solv\_pb\_ligand | | -15.12 | 0.50 | | -14.40 | 0.50 | | -14.17 | 0.53 |
| VDWAALS\_diff | | -20.34 | 1.90 | | -19.94 | 1.87 | | -19.46 | 2.32 |
| EEL\_diff | | -24.10 | 2.84 | | -18.48 | 2.95 | | -11.58 | 3.69 |
| EGB\_diff | | 27.51 | 1.64 | | 20.57 | 1.69 | | 18.10 | 2.02 |
| ESURF\_diff | | -2.89 | 0.08 | | -2.88 | 0.08 | | -2.75 | 0.16 |
| EPB\_diff | | 33.96 | 2.84 | | 22.49 | 2.98 | | 23.94 | 2.93 |
| ENPOLAR\_diff | | -14.47 | 0.25 | | -14.44 | 0.28 | | -13.49 | 0.59 |
| EDISPER\_diff | | 25.20 | 0.59 | | 24.60 | 0.64 | | 24.47 | 0.93 |
| DG\_gas | | -44.44 | 2.59 | | -38.42 | 2.84 | | -31.04 | 3.67 |
| DG\_solv\_igb2 | | 24.62 | 1.63 | | 17.69 | 1.67 | | 15.35 | 2.01 |
| DG\_solv\_pb | | 44.70 | 3.20 | | 32.65 | 3.15 | | 34.92 | 3.33 |
| DG\_igb2 | | -19.82 | 1.89 | | -20.74 | 2.05 | | -15.68 | 2.48 |
| DG\_pb | | 0.26 | 3.26 | | -5.78 | 3.51 | | 3.89 | 4.18 |