A quick summary of MD simulations analyzed so far.

All the files below are posted under /Dropbox/PMC-AT PLIN/Drug\_Discovery.

Movies are produced 25 frame/second, 100ps step size between each frame.

1. Compare between various SIRT3 complexes:
2. From previous MD simulations:

SIRT3/NAD+/ac-ACS2 from 4FVT crystal structure: [SIRT3\_tern\_from\_4FVT\_traj0.mp4, 20 ns]

SIRT3/NAD+/ac-ACS2/NAM from 4FVT and superimposing NAD+ in AB pose from 1YC2: [SIRT3\_NAM\_from\_4FVT\_superimpose\_traj0.mp4, 25 ns]

SIRT3/INT from 4BVG crystal structure: [SIRT3\_INT\_from\_4BVG\_traj0.mp4, 10 ns]

1. From current new constructions:

SIRT3/INT from 4FVT and superimposing Intermediate structure from 4BVG: [SIRT3\_Int\_from\_4FVT\_superimpose\_traj.mp4, 52ns]

SIRT3/INT/NAM from 4FVT by cleaving C-N bond and formation of C-O bond: [SIRT3\_INT\_NAM\_from\_4FVT\_conversion\_traj0.mp4, 35 ns] **NAM escaped from the C pocket!**

b-factors of loop from some simulations were available under Flexible\_Loop\_Bfactor\_Summary.xlsx.

**Some observation**: the b-factors for the loop region are found to be large for structures constructed from 4FVT, but relatively small for structures constructed from 4BVG/4BV3/4BVH. By analyzing the loop structure , I found that the loop structure is probably more stable in 4BVG/4BV3/4BVH as the hairpin structure creates more hydrogen bonds [HBond\_4BVG\_loop.png] than in 4FVT [HBond\_4FVT\_loop.png]. Also the former structure promotes the salt bridge and hydrogen bonds between Arg158 and phosphate group on ADP. One likely possibility of the structure change observed in 4FVT is the use of Carba-NAD+ instead of NAD+.

1. Comparison of Sir2TM ternary complexes:

Sir2TM/NAD+/ac-p53 from 2H4F (loop was added in protein preparation wizard): [Sir2TM\_from\_2H4F\_simple\_fix\_tern\_traj0.mp4, 22ns]

Sir2TM/NAD+/ac-p53 from 2H4F (loop was refined with Prime Loop refinement protocol with extended sampling]: [Sir2TM\_tern\_from\_2H4F\_loop\_refined\_traj0.mp4, 5ns] The simulation is still going.

**Some observation**: The loop refinement reproduces the hairpin loop structure. The b-factors for the loop region are found to be smaller for the second simulation, suggesting it is much stable structure compared to structure without loop refinement.