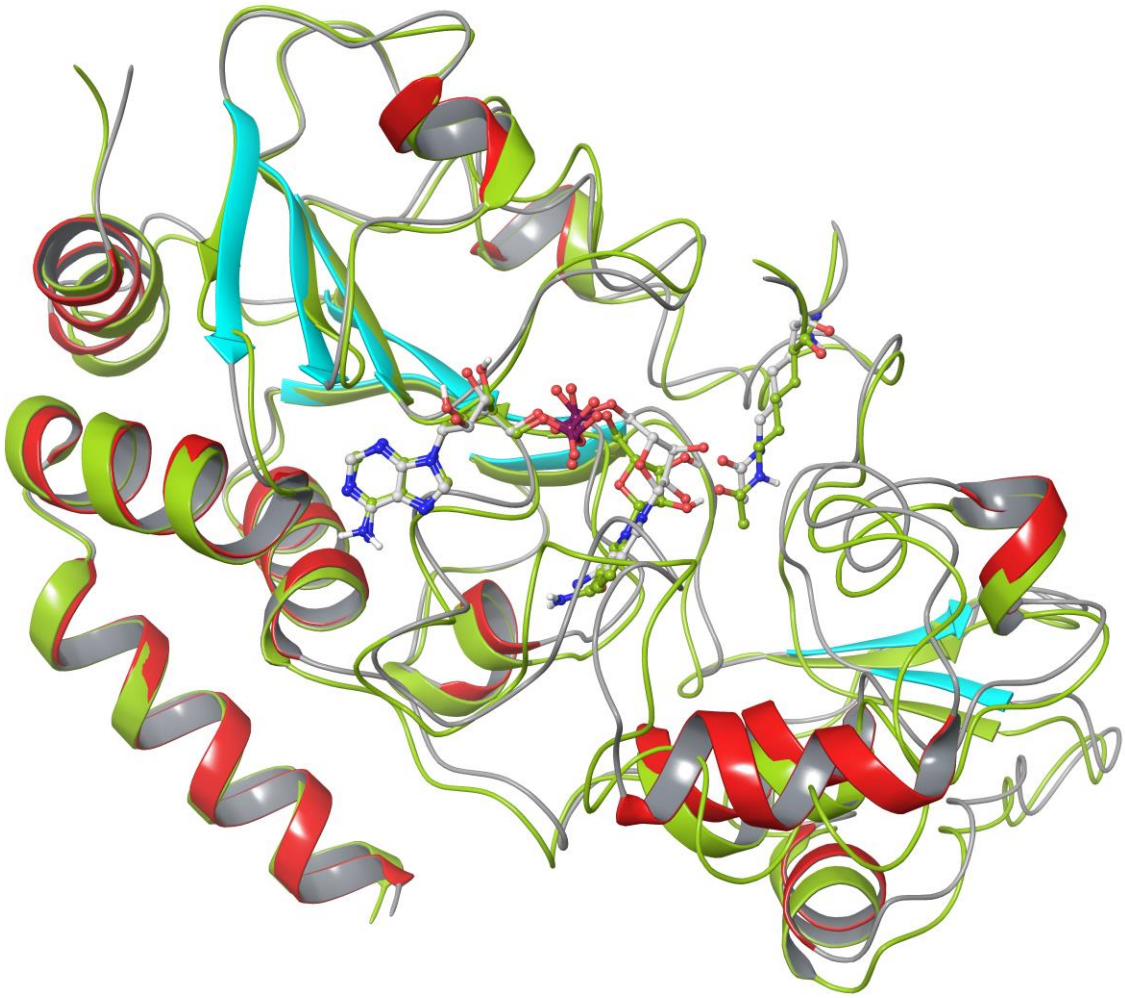


Figure 1: Alignment of ternary complex (SIRT3/Ac-ACS2/NAD+) from MD averaged structure (10 frames from last 10ps) with respect to crystal (pdbID:4FVT) structure. Ribbon representation of crystal structure is colored by secondary structure (sheets in cyan, helixes in red and coils in gray), MD averaged structure in yellow green color. Carbon atoms in crystal structure are in white, and are in yellow green for MD averaged structure. Alignment was made using residues 139-151, 313-378, which forms the stable A binding pocket in Rossmann fold domain (RMSD of backbone of these residues between crystal structure and MD averaged structure is 0.84 Angstrom). Such alignment allows better visualization of relative conformational changes. RMSD of NAD+ itself (heavy atoms only) after alignment between crystal structure and MD averaged structure is 0.56 Angstrom.

Ac-CS2: acetyl-Co-A synthase 2

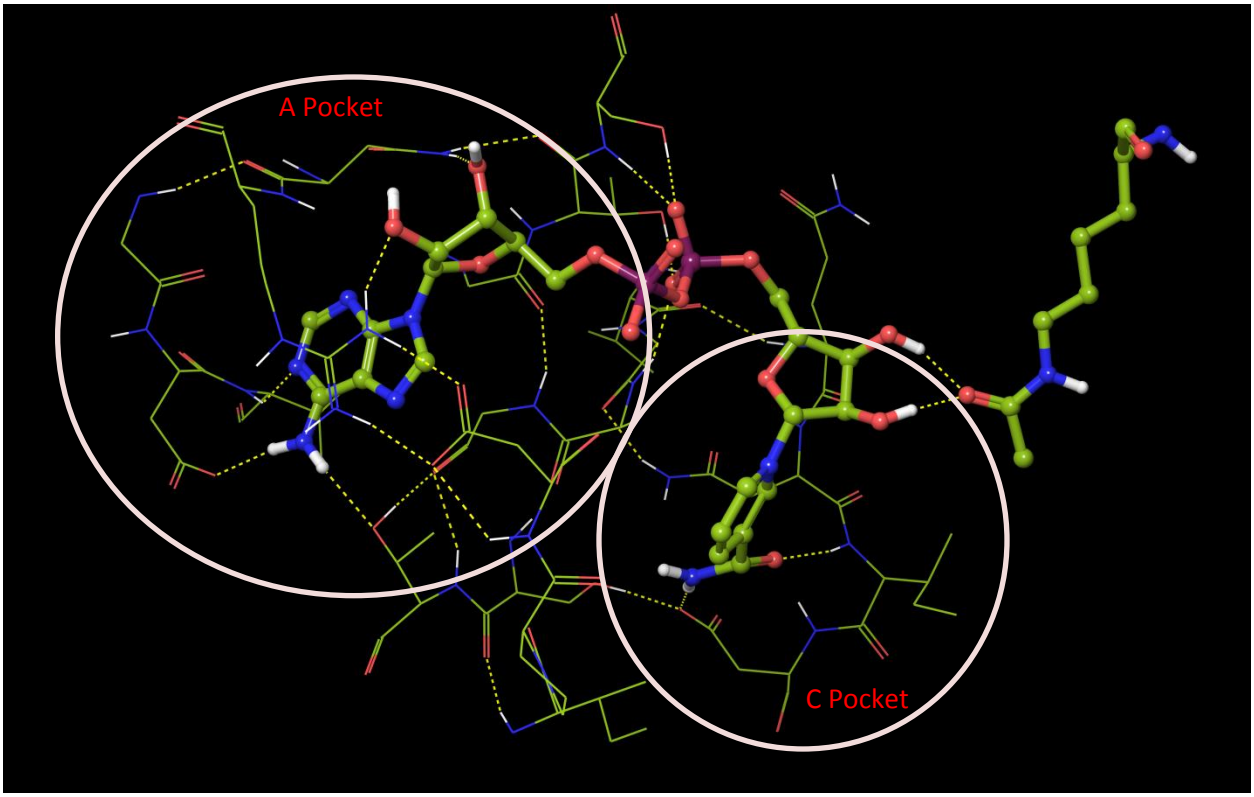
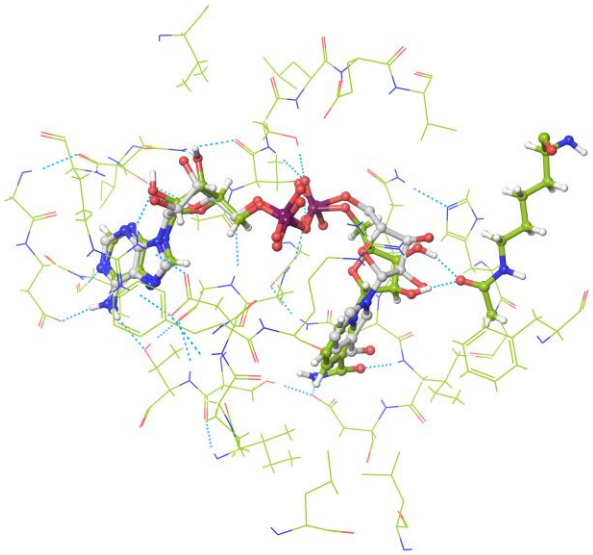



Figure 1a: Observation of A and C pockets from the MD averaged structure.



Note: white background does not work well in this case without a huge color makeover, but posted above for comparison.

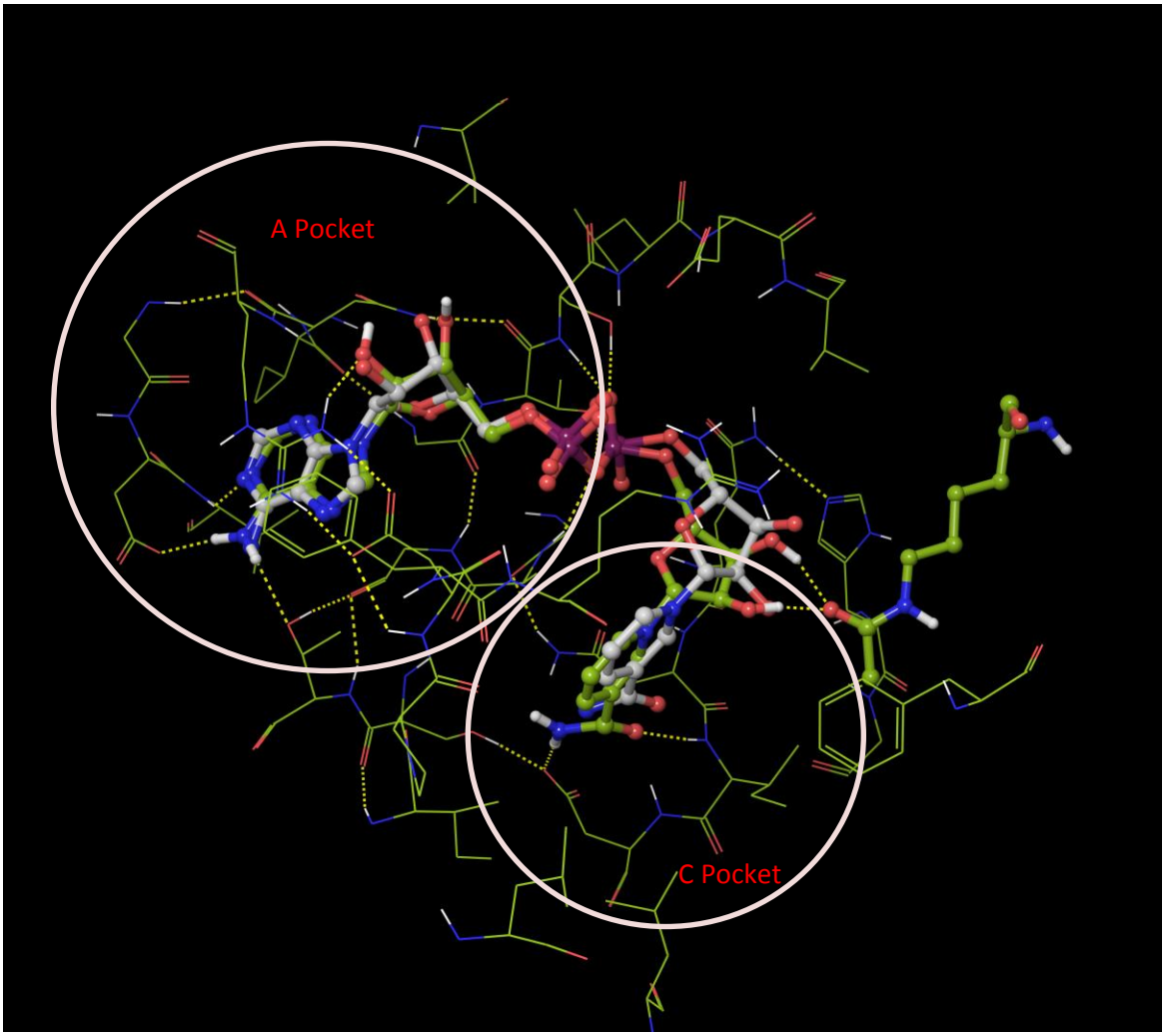



Figure 1b. Observation of A and C pockets from the MD averaged structure over last 10 ps of ternary complex (SIRT3/Ac-CS2/NAD+), reference NAD+ structure (carbon in white) taken from crystal structure 4FVT.

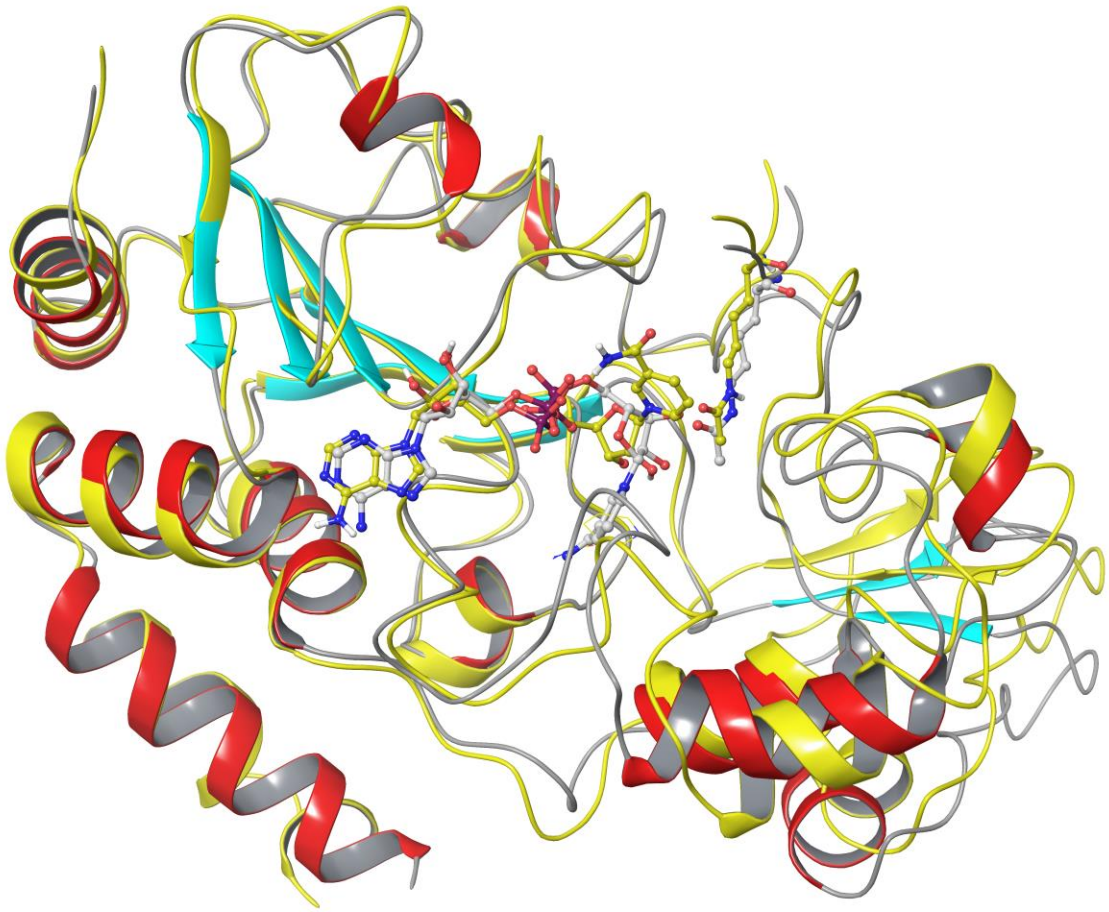


Figure 2: Alignment of complex (SIRT3/Ac-ACS2/NAD+/NAM) from MD averaged structure (10 frames from last 10ps) with respect to crystal (pdbID:4FVT) structure. Ribbon representation of crystal structure is colored by secondary structure (sheets in cyan, helixes in red and coils in gray), MD averaged structure in yellow color. Carbon atoms in crystal structure are in white, and are in yellow for MD averaged structure. Alignment was made using residues 139-151, 313-378, which forms the stable A binding pocket in Rossmann fold domain (RMSD of backbone of these residues between crystal structure and MD averaged structure is 0.74 Angstrom). RMSD of NAD+ itself (heavy atoms only) after alignment of MD averaged structure with respect to NAD+ from 1YC2:A is 3.38 Angstrom.

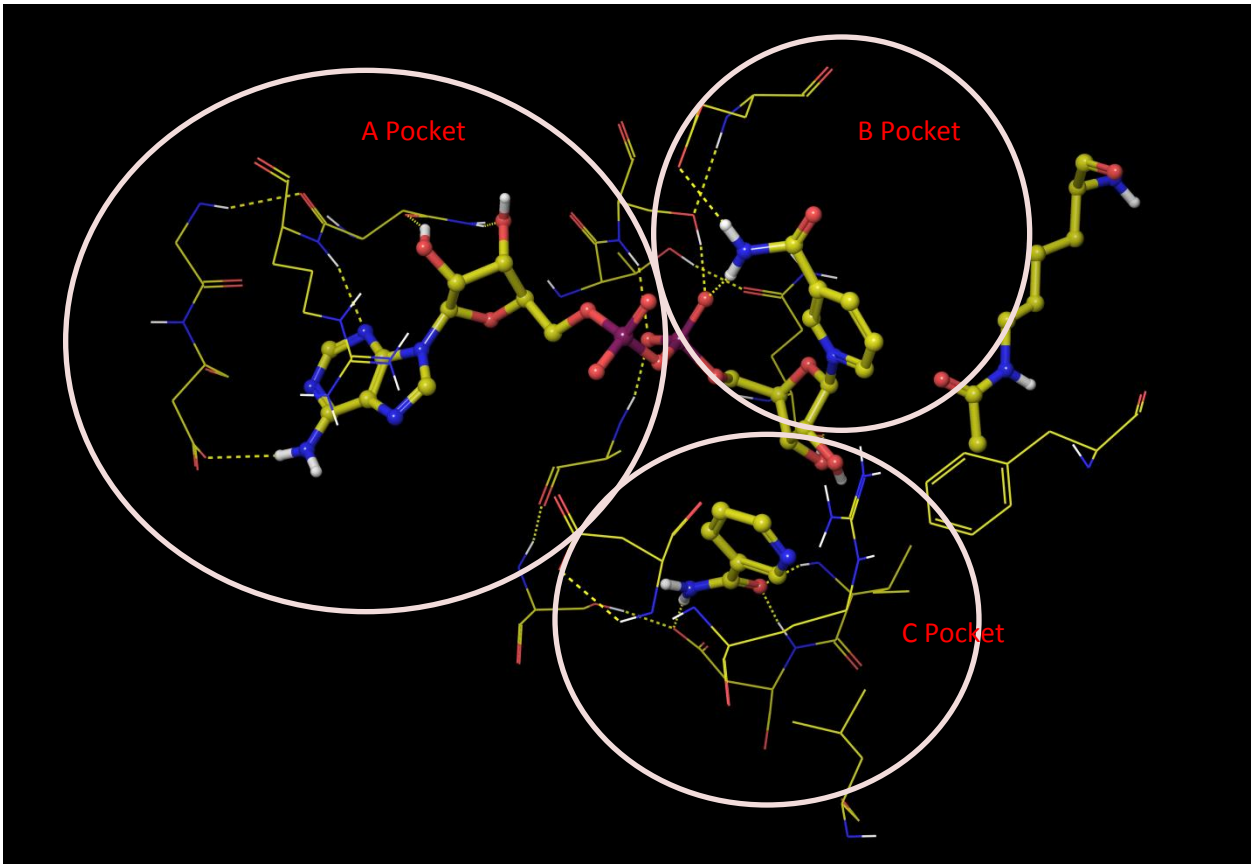



Figure 2a: Observation of A, B and C pockets from the MD averaged structure.

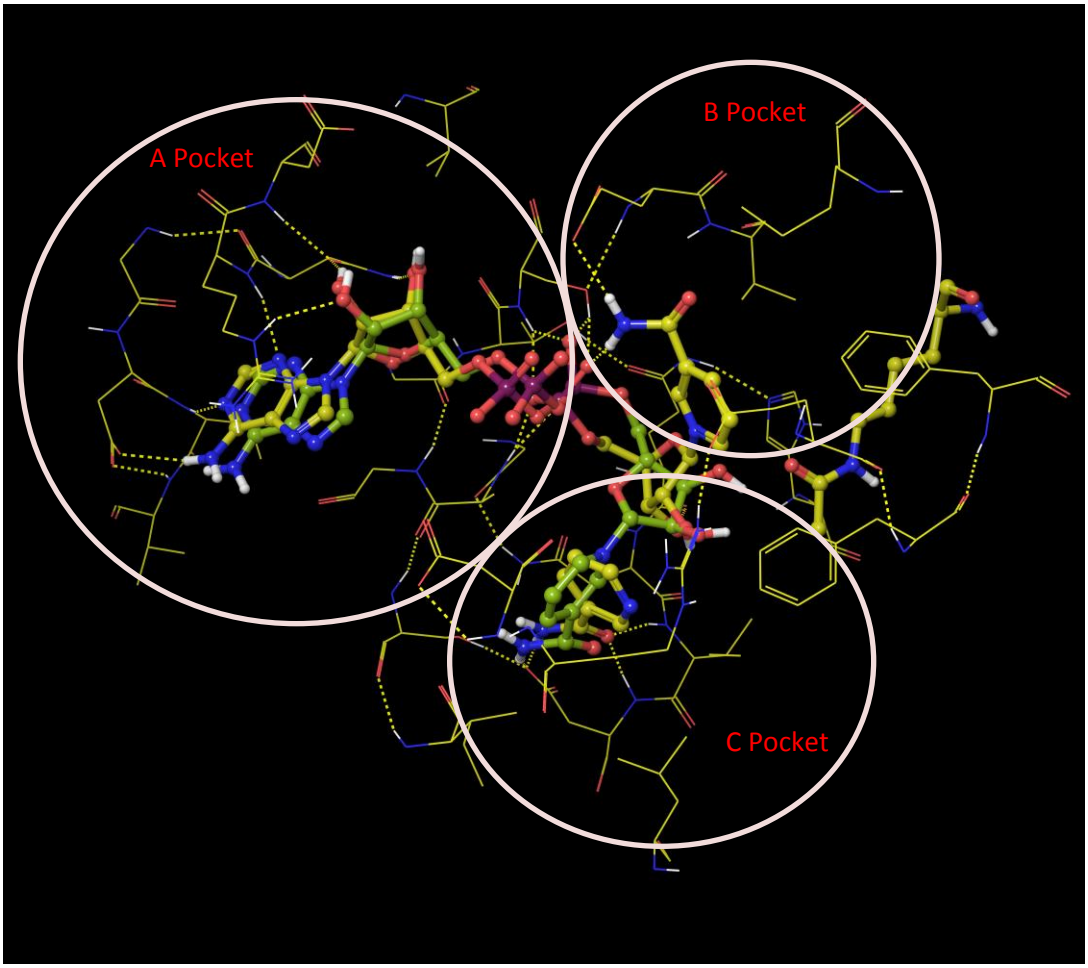



Figure 2b: Observation of A, B and C pockets from the MD averaged structure over last 10 ps of complex with NAM (SIRT3/Ac-CS2/NAD+/NAM), reference NAD+ structure (in yellow green) taken from averaged structure over last 10 ps of ternary complex.

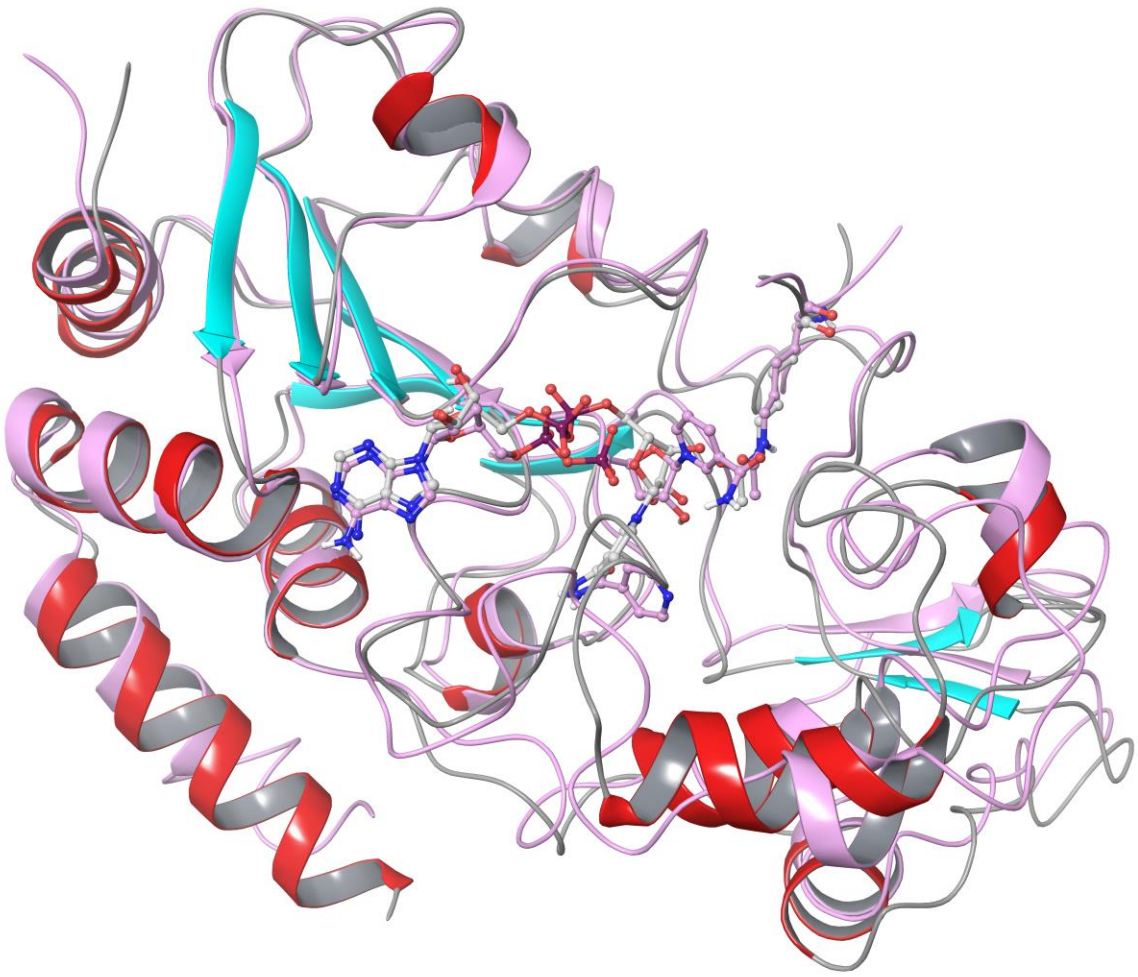


Figure 3: Alignment of complex (SIRT3/Ac-ACS2/NAD+/isoNAM) from MD averaged structure (10 frames from last 10ps) with respect to crystal (pdbID:4FVT) structure. Ribbon representation of crystal structure is colored by secondary structure (sheets in cyan, helixes in red and coils in gray), MD averaged structure in plum color. Carbon atoms in crystal structure are in white, and are in plum for MD averaged structure. Alignment was made using residues 139-151, 313-378, which forms the stable A binding pocket in Rossmann fold domain (RMSD of backbone of these residues between crystal structure and MD averaged structure is 0.70 Angstrom). RMSD of NAD+ itself (heavy atoms only) after alignment of MD averaged structure with respect to NAD+ from 1YC2:A is 2.88 Angstrom.

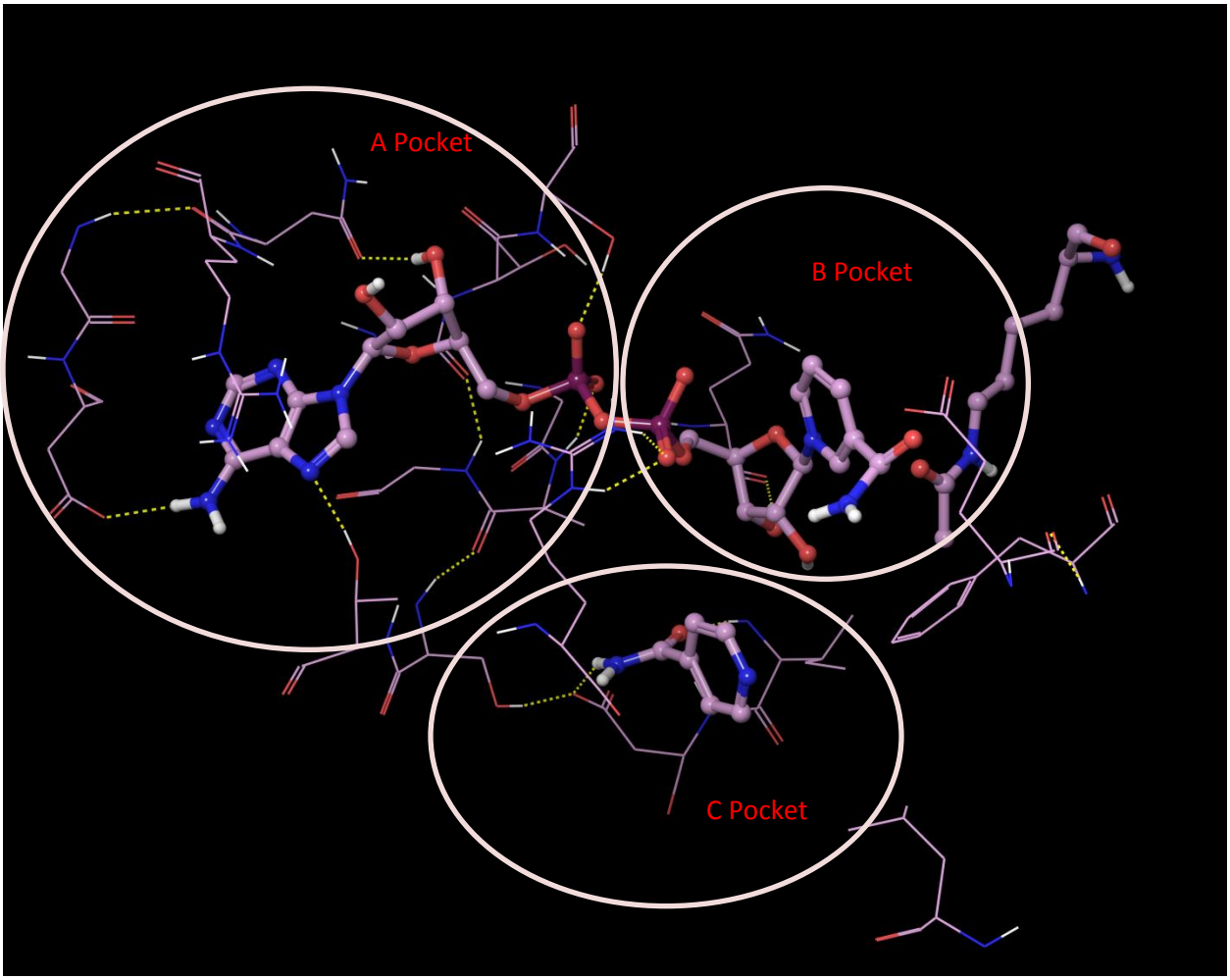



Figure 3a: Observation of A, B and C pockets from the MD averaged structure.

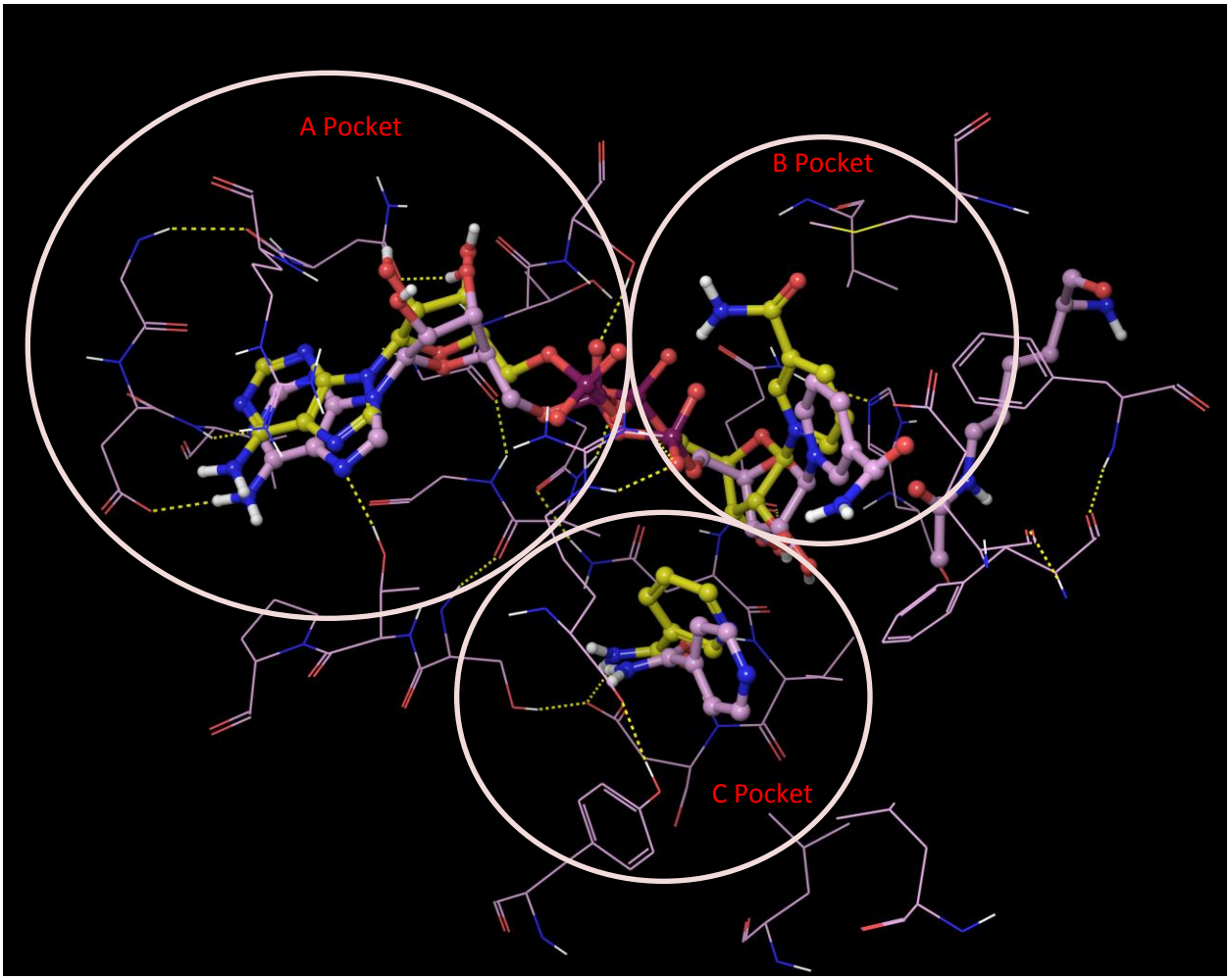



Figure 3b: Observation of A, B and C pockets from the MD averaged structure over last 10 ps of complex with isoNAM (SIRT3/Ac-CS2/NAD+/isoNAM), reference NAD+ structure (in yellow) taken from averaged structure over last 10 ps of complex with NAM.

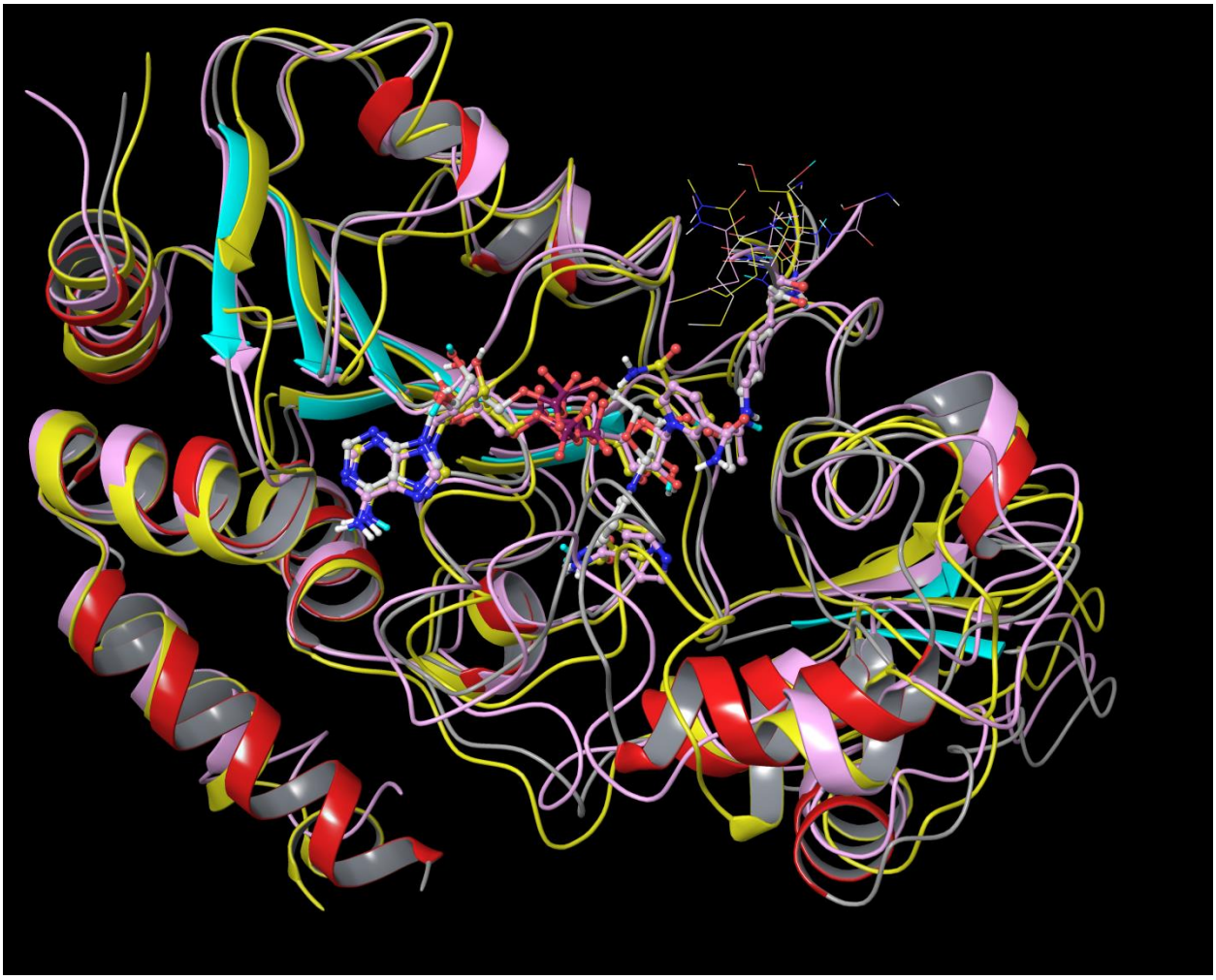


Figure 4: Alignment of complex with NAM (SIRT3/Ac-ACS2/NAD+/NAM, yellow) and isoNAM(SIRT3/Ac-ACS2/NAD+/isoNAM, plum) from MD averaged structures (10 frames from last 10ps) with respect to crystal (pdbID:4FVT) structure.

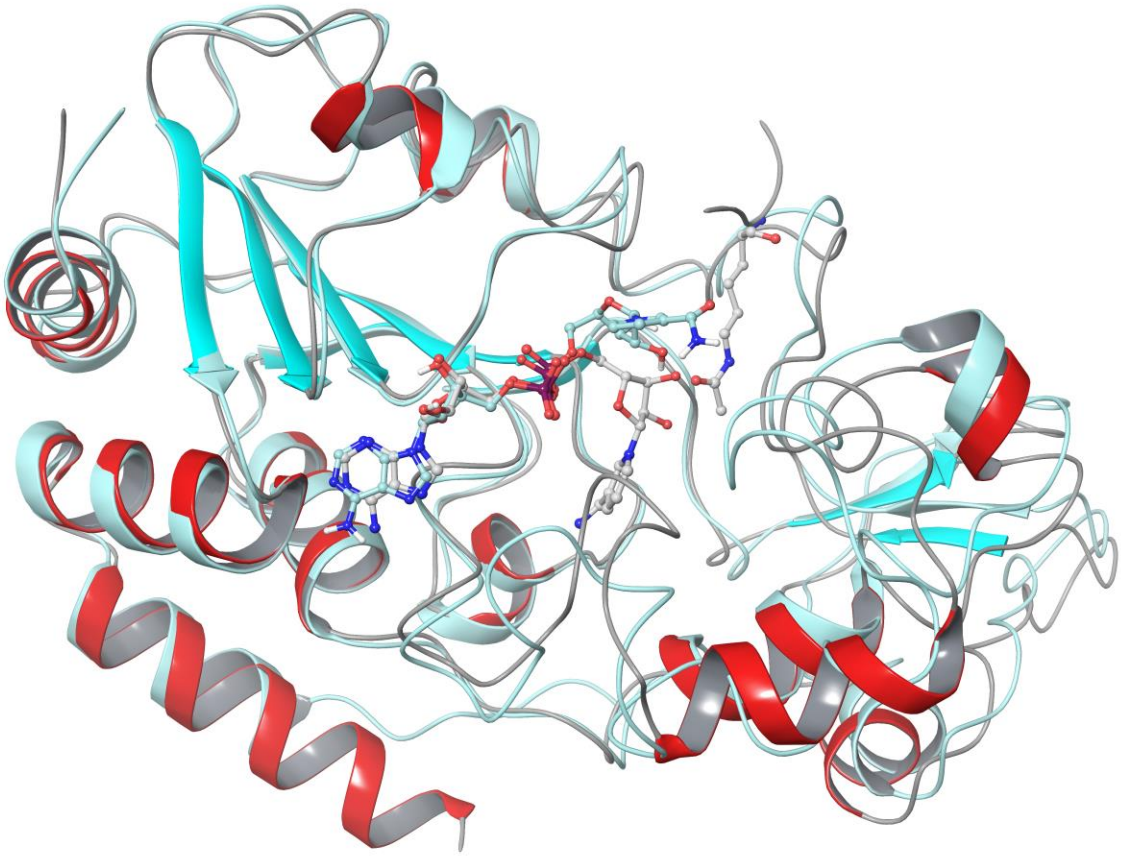


Figure 5: Alignment of binary complex (SIRT3 /NAD+) from MD averaged structure (10 frames from last 10ps) with respect to crystal (pdbID:4FVT) structure. Ribbon representation of crystal structure is colored by secondary structure (sheets in cyan, helixes in red and coils in gray), MD averaged structure in light blue color. Carbon atoms in crystal structure are in white, and are in light blue for MD averaged structure. Alignment was made using residues 139-151, 313-378, which forms the stable A binding pocket in Rossmann fold domain (RMSD of backbone of these residues between crystal structure and MD averaged structure is 0.73 Angstrom). RMSD of NAD+ itself (heavy atoms only) after alignment of MD averaged structure with respect to NAD+ from 1YC2:A is 2.71 Angstrom.

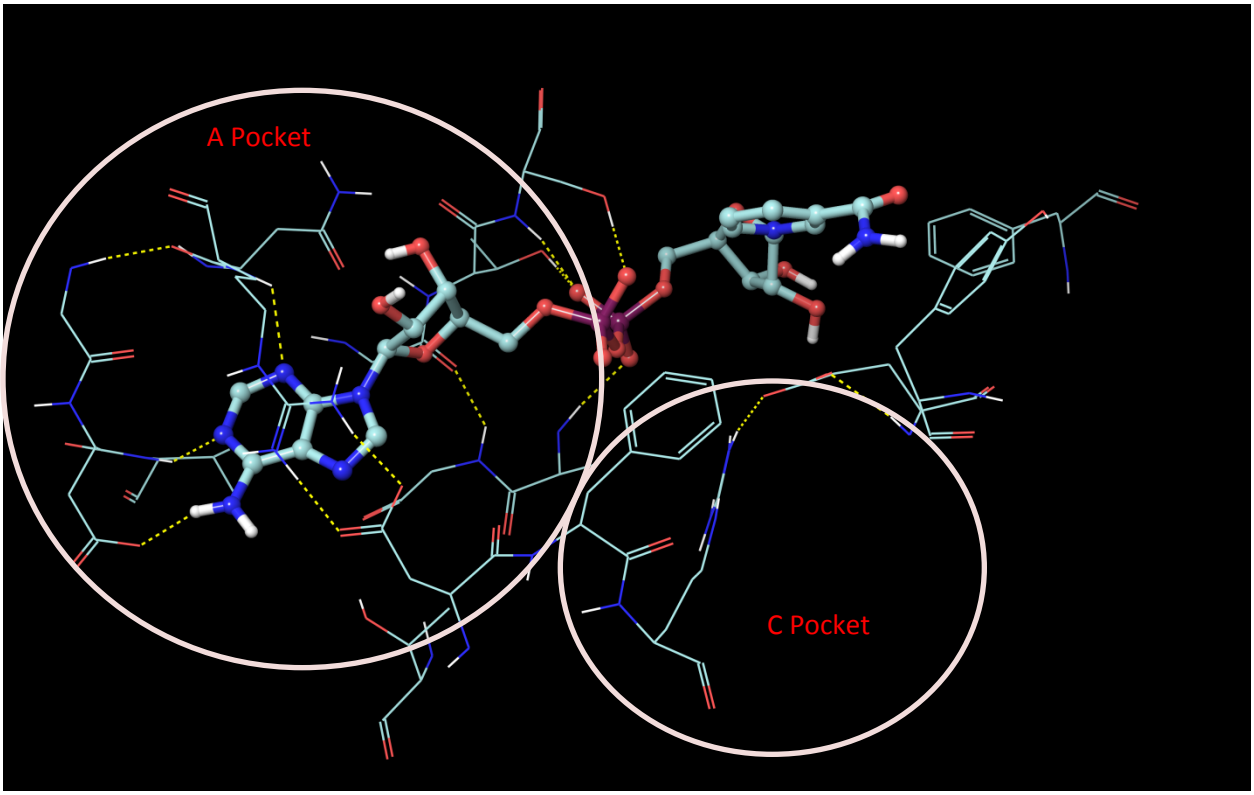



Figure 5a: Presentation of A and C pockets from the MD averaged structure.

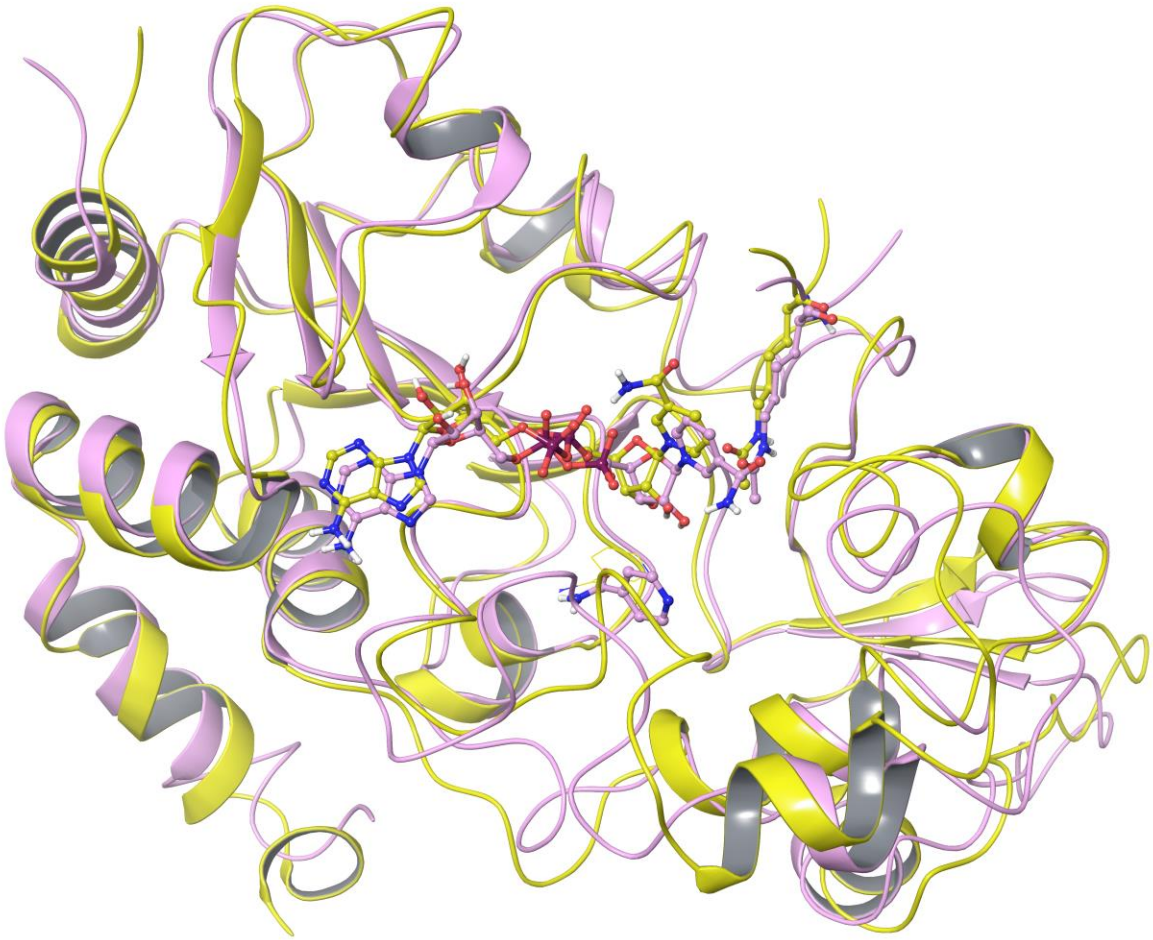


Figure 6: Alignment of complex with NAM (SIRT3/Ac-ACS2/NAD+/NAM, yellow) and isoNAM(SIRT3/Ac-ACS2/NAD+/isoNAM, plum) from MD averaged structures (10 frames from last 10ps). Alignment was made using residues 139-151, 313-378, which forms the stable A binding pocket in Rossmann fold domain with respect to crystal (pdbID:4FVT) structure.

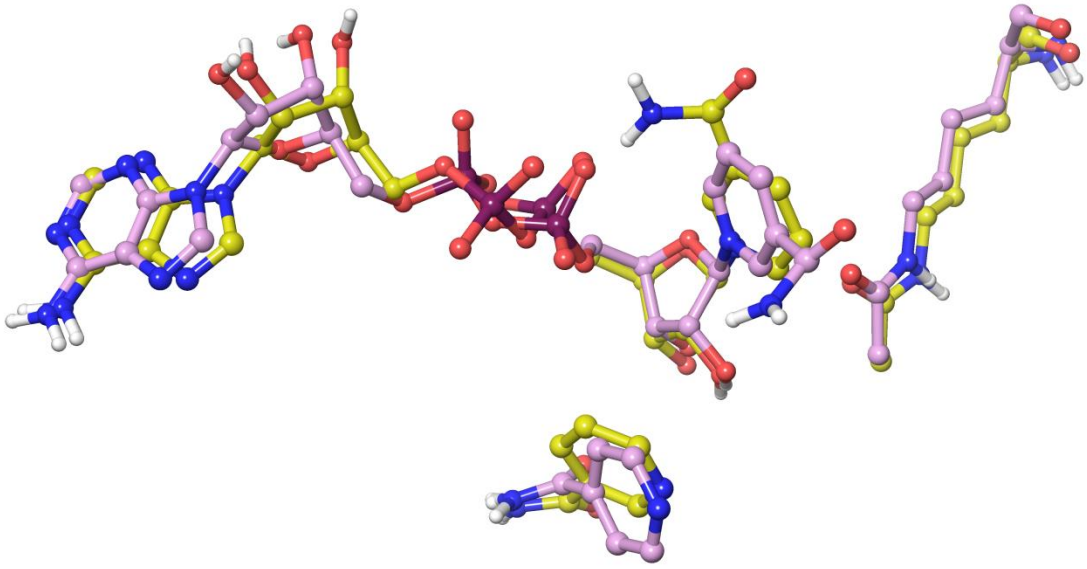
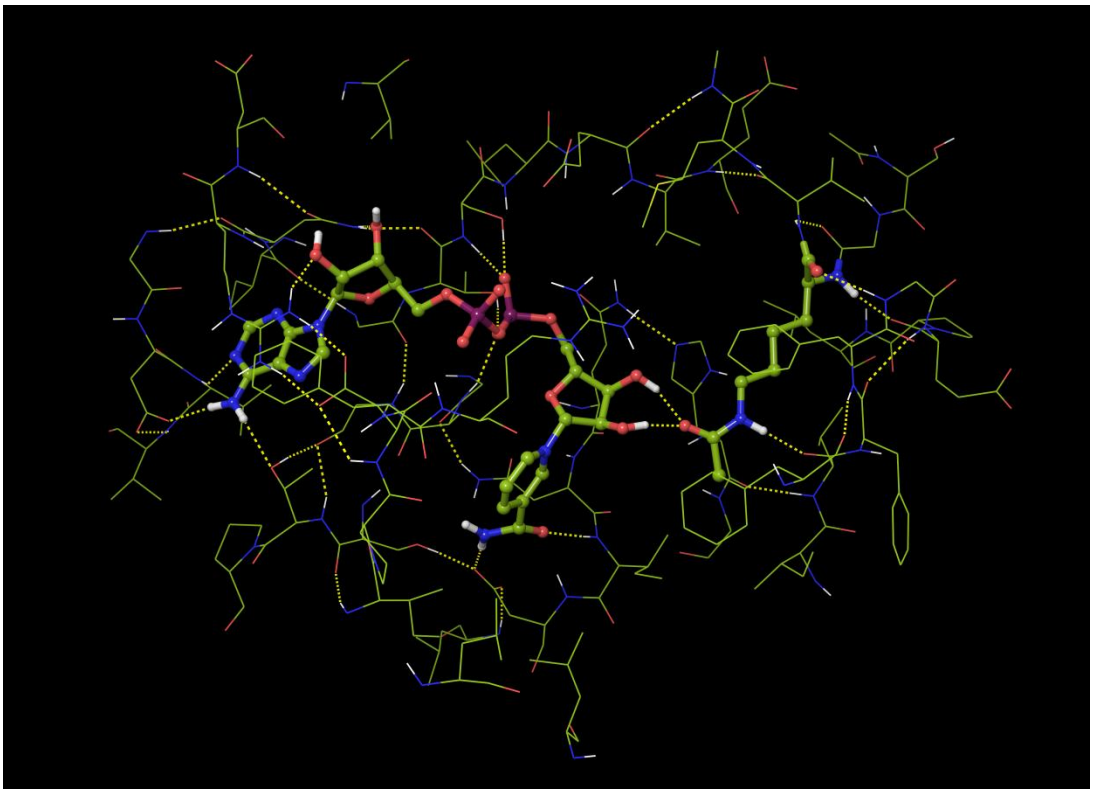
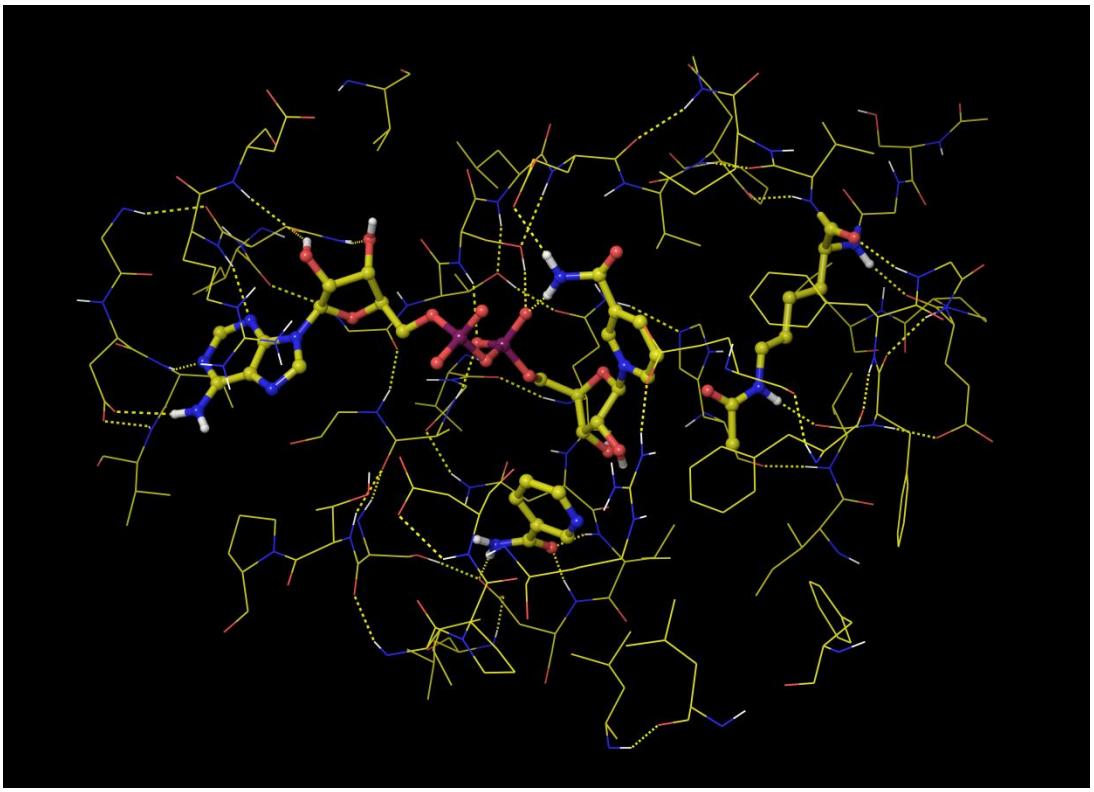


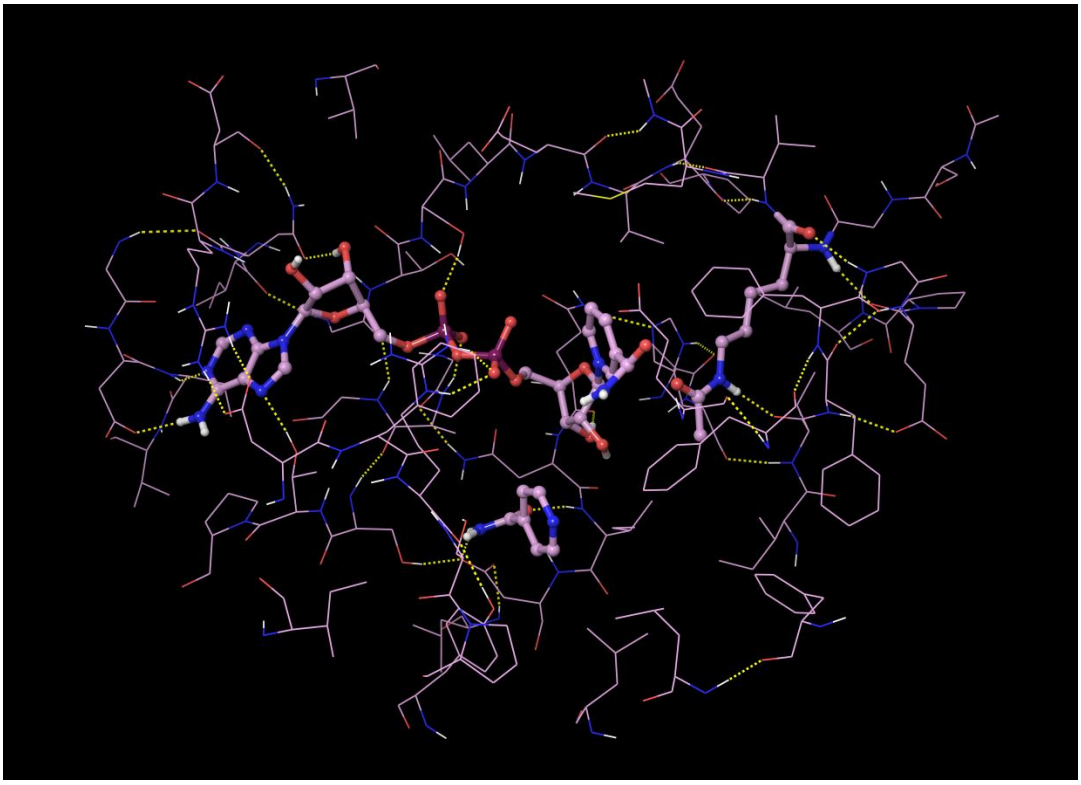
Figure 6: After alignment of residues 139-151, 313-378 in Rossmann fold domain, the display of NAD+, acetylated lysine, NAM (carbon in yellow) and isoNAM (carbon in plum) shows close resemblance between NAM and isoNAM binding from MD averaged structures (10 frames from last 10ps). The MM-GBSA calculations suggest that NAM binds slightly stronger to the C pocket than isoNAM prior to enzymatic reaction (-19.82 kcal/mol vs -15.68 kcal/mol in MM-GBSA scores).



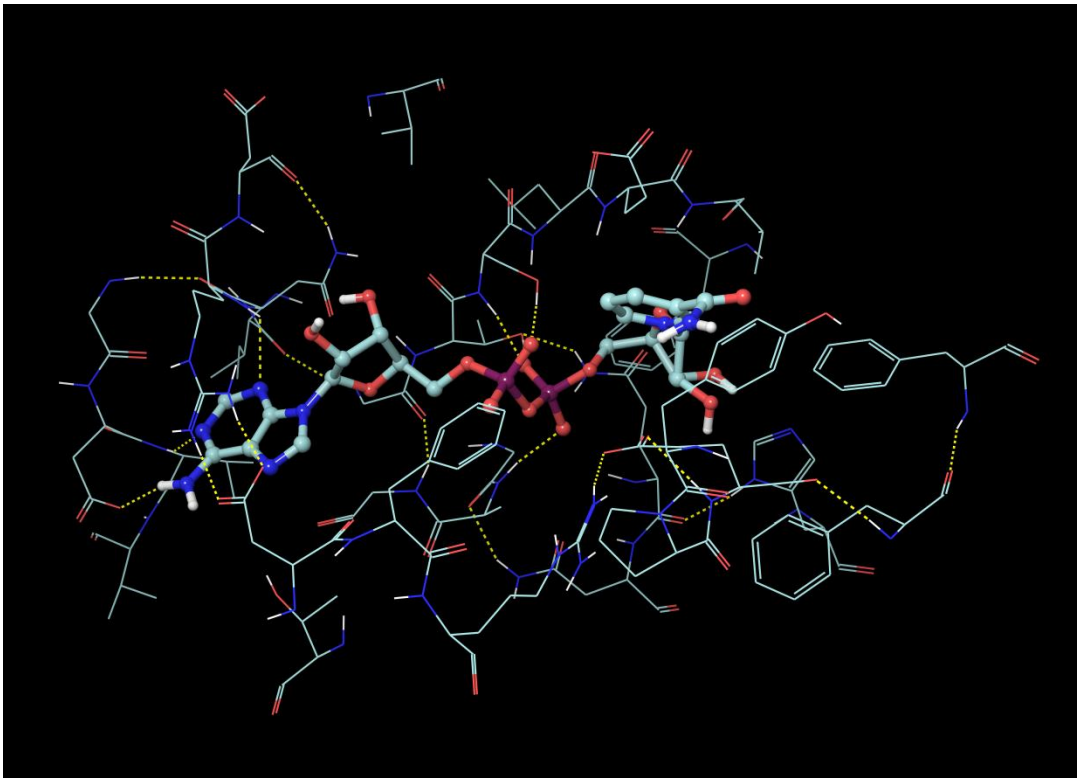
(a)



(b)



(c)



(d)

Figure 7. Binding of NAD+, NAM, isoNAM in (a) ternary complex (SIRT3/Ac-CS2/NAD+); (b) complex with NAM (SIRT3/Ac-CS2/NAD+/NAM); (c) complex with isoNAM (SIRT3/Ac-CS2/NAD+/isoNAM); (d) binary complex (SIRT3/NAD+).

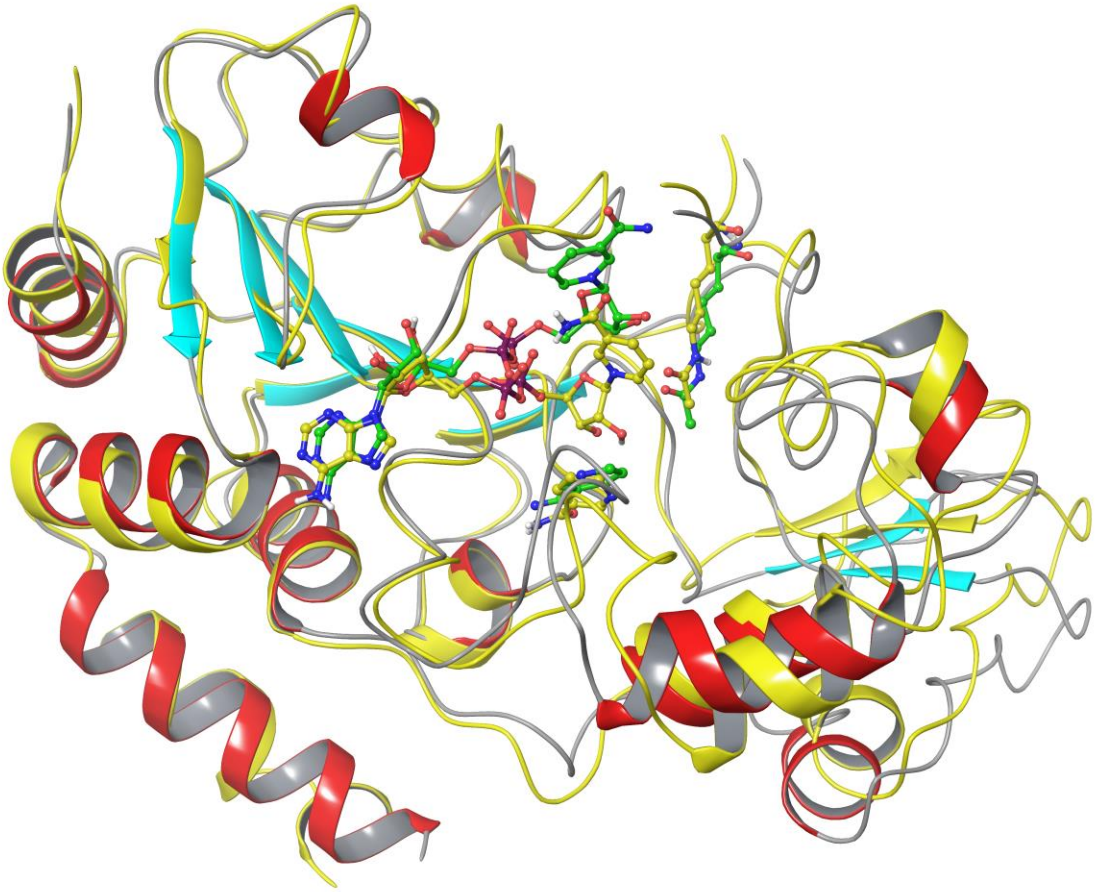


Figure 8: Alignment of complex with NAM (SIRT3/Ac-ACS2/NAD+/NAM, yellow) from MD averaged structures (10 frames from last 10ps) with respect to starting structure (SIRT3/Ac-CS2 from 4FVT, NAD+ in AB pose and NAM in C pocket taken from 1YC2:A after alignment).

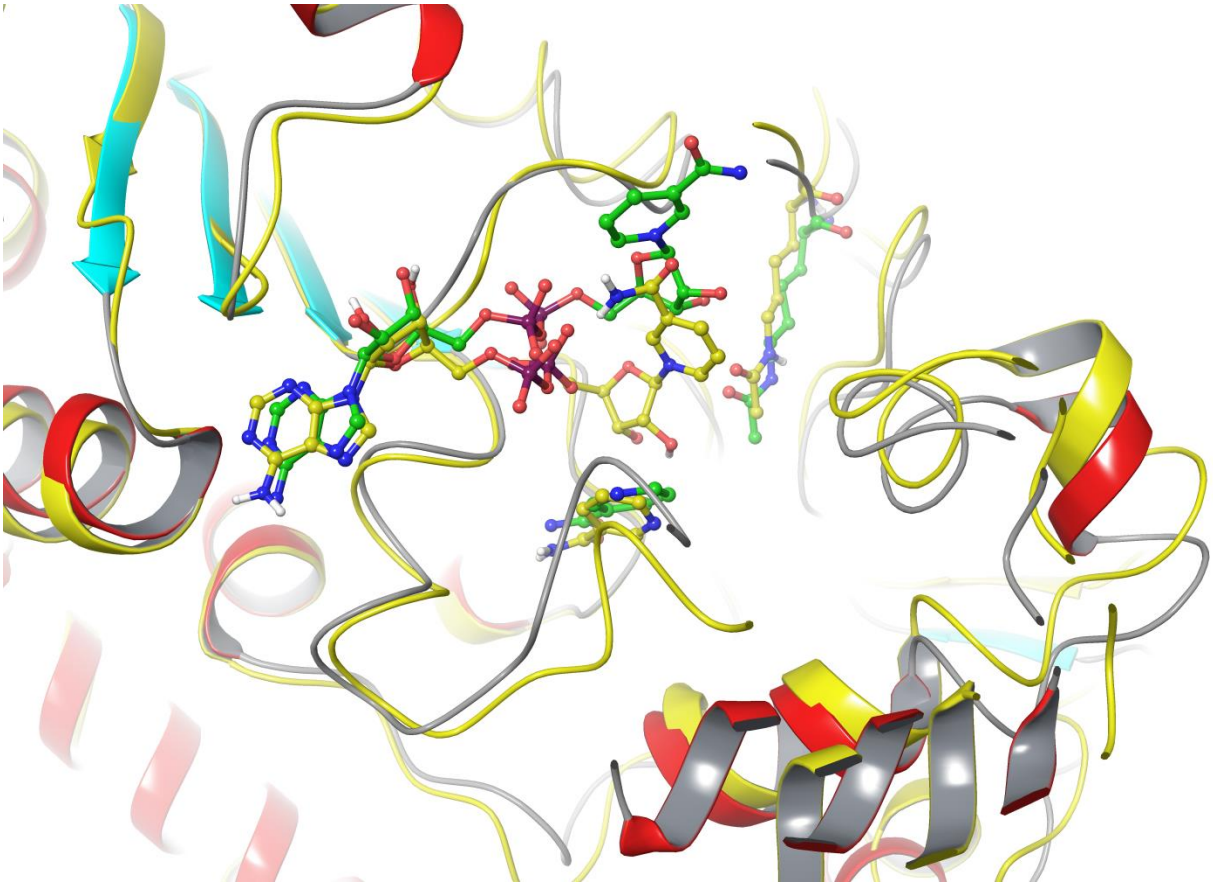
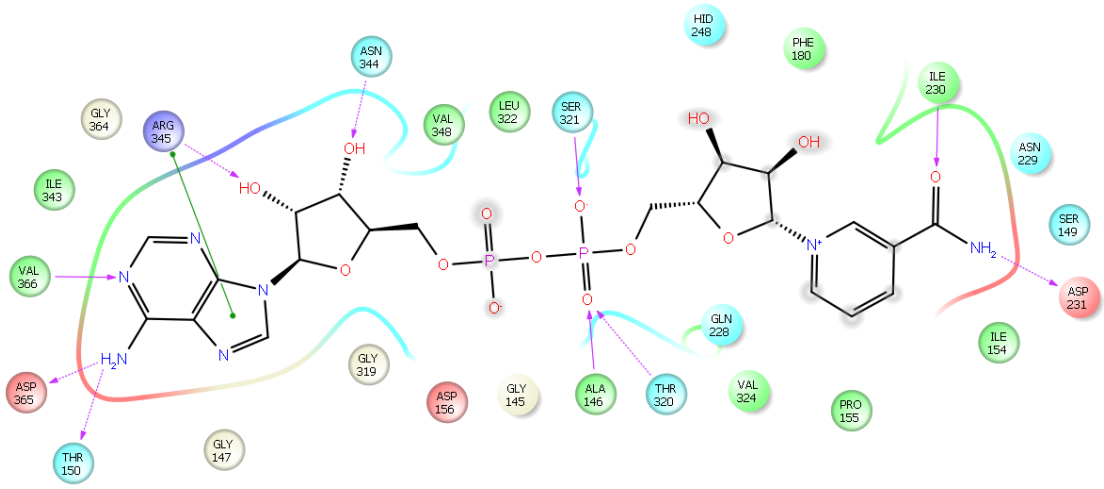


Figure 8a: A close-up view of binding sites in complex with NAM (SIRT3/Ac-ACS2/NAD+/NAM, yellow) from MD averaged structures (10 frames from last 10ps) with respect to starting structure (SIRT3/Ac-CS2 from 4FVT, NAD+ in AB pose and NAM in C pocket taken from 1YC2:A after alignment).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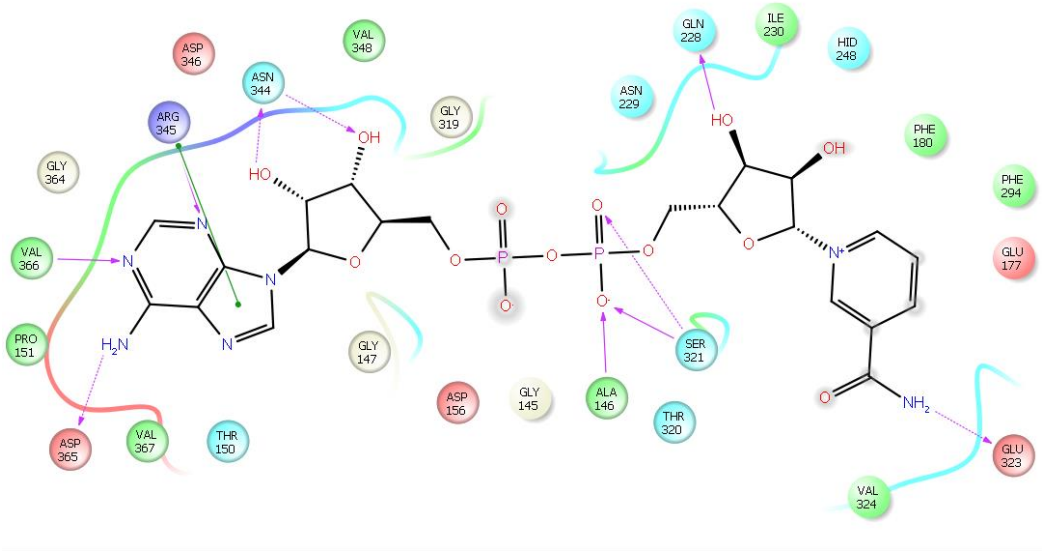
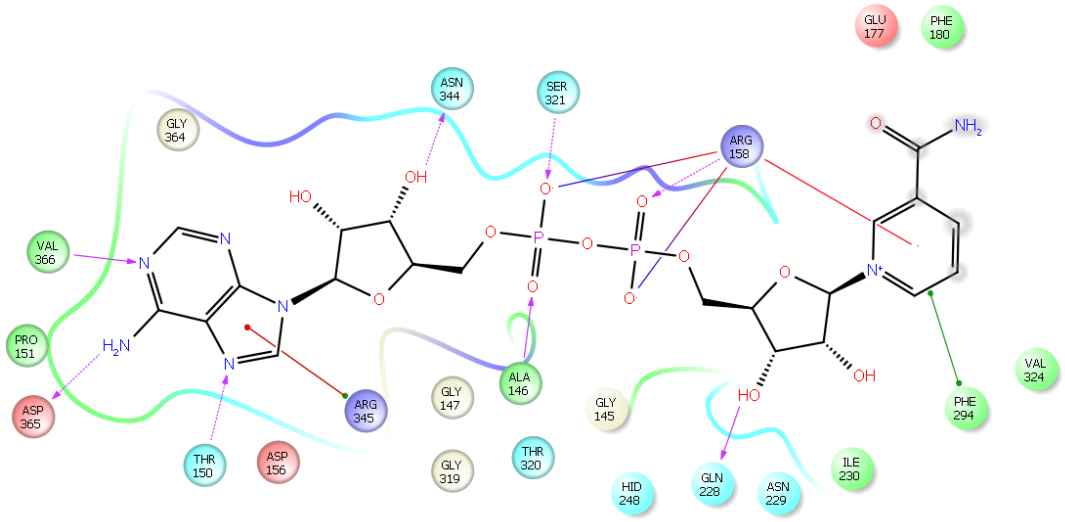
PHE 180
ASN 344
ILE 230
GLY 364
ARG 345
VAL 348
LEU 322
SER 321
HO
OH
ASN 229
ILE 343
SER 149
VAL 366
NH2
ASP 231
GLN 228
ILE 154
GLY 319
ASP 365
ASP 156
GLY 145
ALA 146
THR 320
VAL 324
PRO 155
THR 150
GLY 147

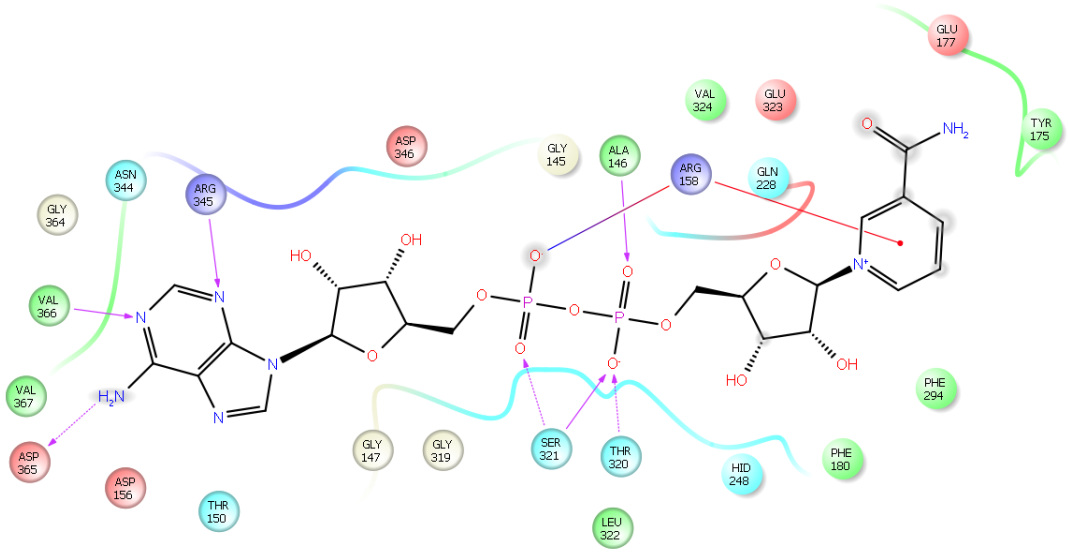
(a)


ASP 346
VAL 348
GLN 228
ILE 230
ASN 344
HID 248
ASN 229
ARG 345
GLY 319
HO
OH
PHE 180
GLY 364
PHE 294
VAL 366
GLU 177
PRO 151
SER 321
GLY 147
ASP 156
GLY 145
ALA 146
THR 320
ASP 365
VAL 367
THR 150
GLU 323
VAL 324

(b)

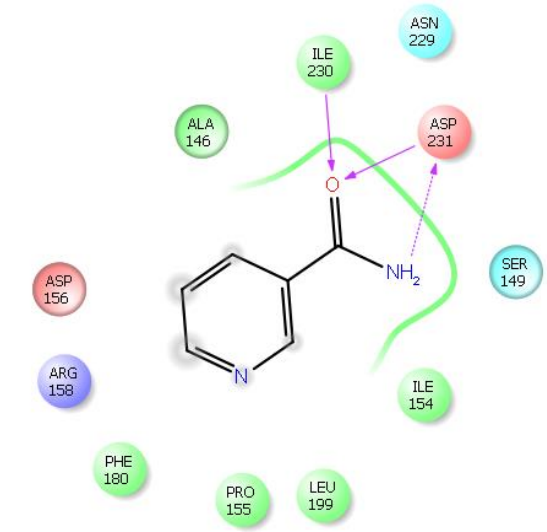



(c)

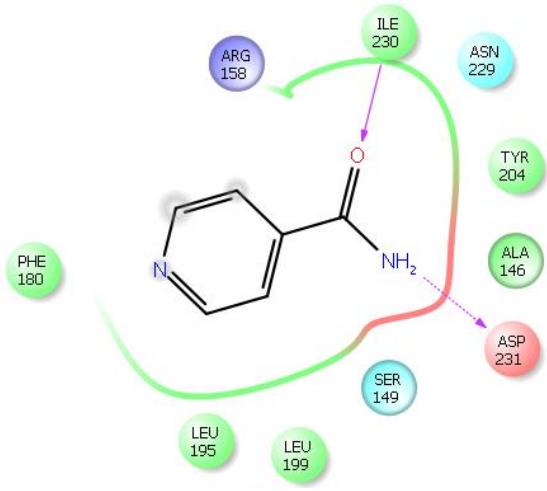



(d)

Figure 9. SIRT3-NAD+ interaction diagrams of MD averaged structures (10 frames from last 10ps) (a) ternary complex (SIRT3/Ac-CS2/NAD+); (b) complex with NAM; (c) complex with isoNAM; (d) binary complex of SIRT3 and NAD+.




(a)




(b)

Figure 10. SIRT3-NAM/isoNAM interaction diagrams of MD averaged structures (10 frames from last 10ps) (a) complex with NAM; (b) complex with isoNAM.