Supporting Information

For

Molecular Dynamic Simulation on the Role of CL5D in Accelerate the Product Dissociation of SIRT6

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Supplementary Method

Binding free energy analysis

The binding free energies between SIRT6 and ADPr were calculated using the MM/GBSA approach[1, 2], focusing on comparing how the presence of a 9-mer peptide or the agonist CL5D affects the binding energy between SIRT6 and ADPr. The systems studied included four complexes: SIRT6, SIRT6_peptide, SIRT6-CL5D, and SIRT6-CL5D_peptide, each bound to ADPr. For each system, 500 snapshots were evenly extracted from the last 200 ns of the molecular dynamics (MD) trajectory, and these snapshots were used to perform the MM/GBSA calculations.

The binding free energy ΔG_{bind} for each complex was computed as the sum of several components:

$$\Delta G_{bind} = \Delta E_{ele} + \Delta E_{vdw} + \Delta E_{int} + \Delta G_{GB} + \Delta G_{SA} \quad (1)$$

where ΔEele is the change in electrostatic energy, ΔEvdw is the change in van der Waals energy, ΔEint is the internal energy of the complex, ΔGGB is the Generalized Born polar solvation energy, and ΔGSA is the nonpolar solvation energy, calculated based on the solvent-accessible surface area.

Energy decomposition analysis was performed to determine the individual contributions of each residue to the overall binding free energy, using the appropriate tools for per-residue energy calculation.

The MMGBSA calculations were performed using the Generalized Born model (igb=5) for calculating the polar solvation energy, with a salt concentration set to 0.15 M to mimic physiological conditions.

Supplementary Figures

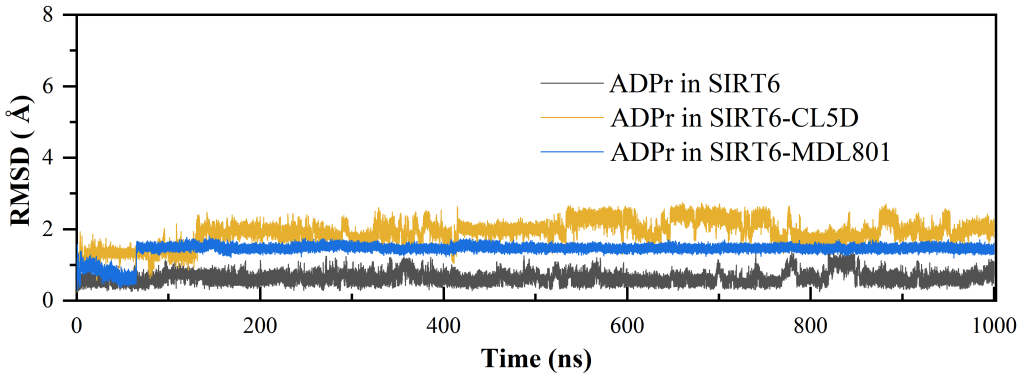

Figure S1. The RMSD of the ADPr in the SIRT6 (black), SIRT6-CL5D (orange) and SIRT6-MDL801 (blue) system.

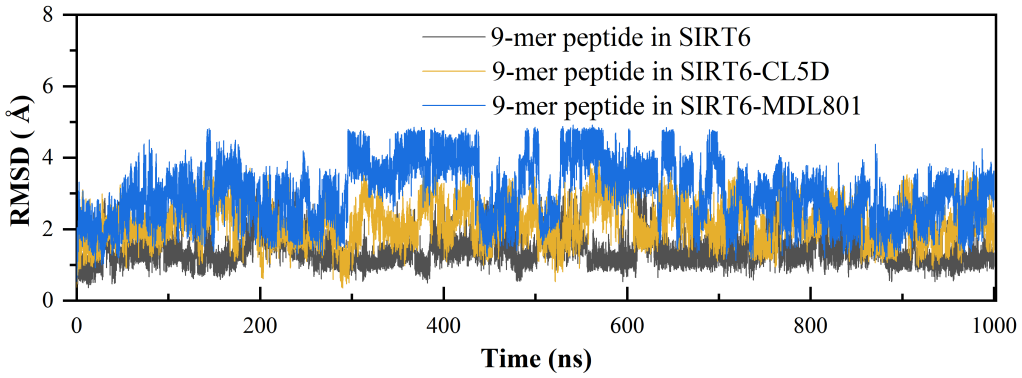

Figure S2. The RMSD of the protein backbone of 9-mer peptide in the SIRT6 (black), SIRT6-CL5D (orange) and SIRT6-MDL801 (blue) system.

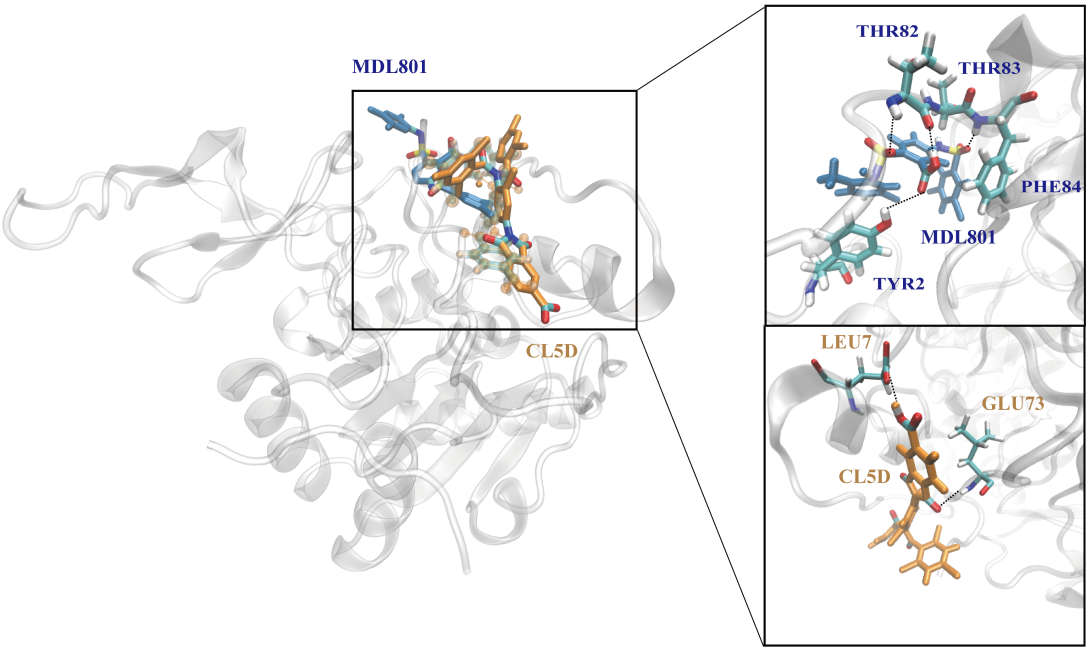

Figure S3. Hydrogen bonds between CL5D and SIRT6 in the SIRT6-CL5D system, and between MDL801 and SIRT6 in the SIRT6-MDL801 system. CL5D is depicted as an orange licorice, while MDL801 is shown as a blue licorice. The yellow transparent licorice represents the docking conformation of CL5D, whereas the solid orange licorice indicates the equilibrated conformation of CL5D.

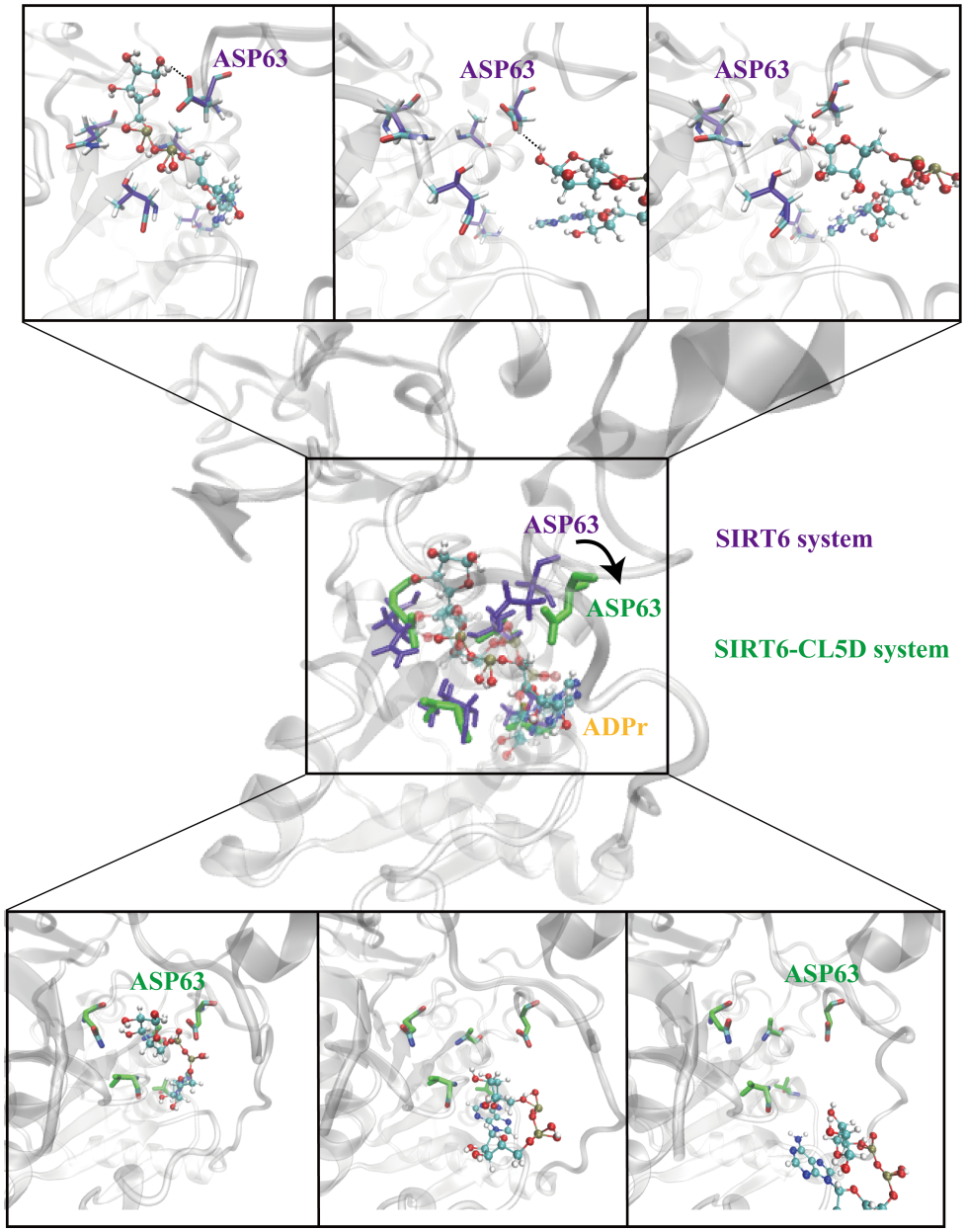

Figure S4. Snapshots of ADPr interactions with SIRT6 residues in the SIRT6 and SIRT6-CL5D systems during substrate binding. ADPr is represented by licorice in the SIRT6 system and by a transparent licorice in the SIRT6-CL5D system. The upper panels depict snapshots of the ADPr release process in the SIRT6 system, while the lower panels illustrate the ADPr release process in the SIRT6-CL5D system.

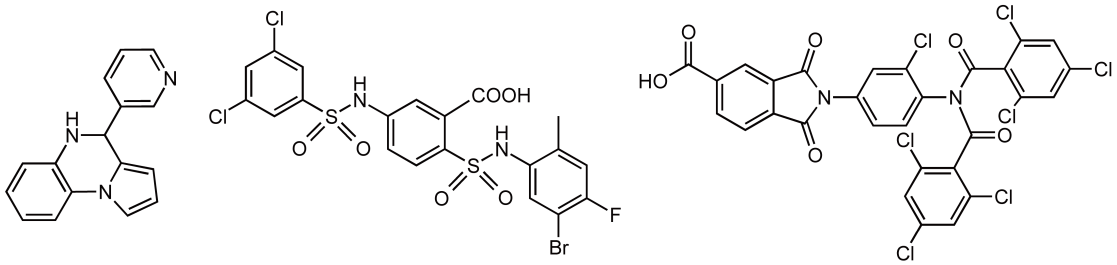

Figure S5. Chemical formulae for the SIRT6 agonists UBCS039[3], MDL801 and CL5D[4]

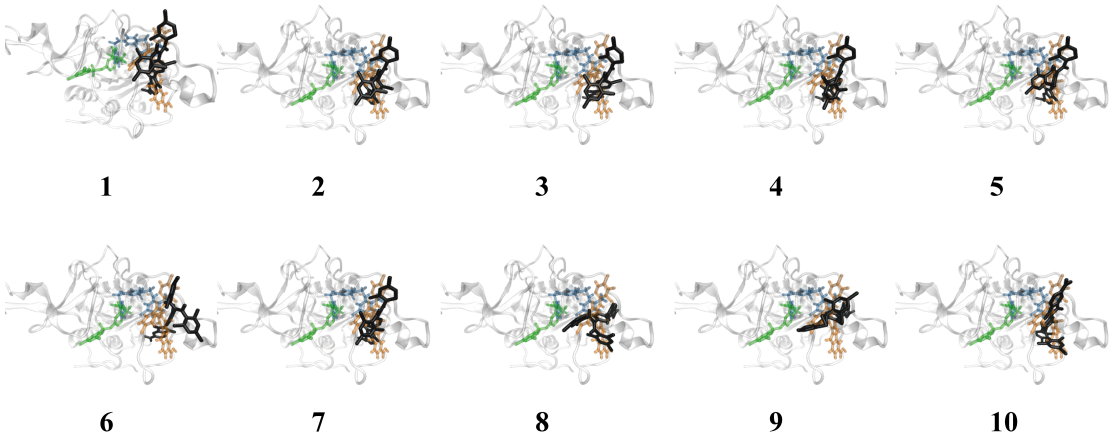

Figure S6. Snapshots of the docking structure. The CL5D molecule is represented in black licorice. The green transparent licorice represents MDL801 binding site 1 and its conformation from Huang's study,[5] while the blue transparent licorice represents the MDL801 binding site and conformation from Steegborn's study.[6] The orange transparent licorice depicts the CL5D binding site and conformation in the SIRT6-CL5D equilibrium structure from this study. Docking structures 1-10 are arranged in ascending order of energy, from lowest to highest.

Supplementary Table

Table S1. Hydrogen bonds between SIRT6 and ADPr in SIRT6 system with or without 9-mer peptide

Residue		Occupancy (%)	
donor	acceptor	SIRT6-peptide	SIRT6
ADPr@O1A	THR_215@OG1	73.31	62.96
ASP_63@OD1	ADPr@O1D	56.41	49.09
GLN_113@O	ADPr@O3D	37.72	36.50
ADPr@O5D	ALA_53@N	34.58	24.12
ASP_63@OD2	ADPr@O1D	33.15	18.22
ADPr@N1	VAL_258@N	31.02	12.93
HIE_133@ND1	ADPr@O3D	31.01	32.01

Table S2. Binding free energy of different SIRT6 and SIRT6-CL5D interfaces (kcal/mol). ΔE_{vdW}, the van der Waals interaction energy; ΔE_{ele}, the electrostatic interaction energy; ΔG_{GB}, the generalized Born electrostatic solvation energy; ΔG_{SUR}, the nonpolar solvation energy; ΔG_{bind}, the binding free energy.

Interface	ΔE_{vdW}	ΔE_{ele}	ΔG_{GB}	ΔG_{SUR}	ΔG_{bind}
SIRT6	-61.09±4.69	-142.07±12.49	83.68±20.43	-7.66±0.17	-127.13±20.43
SIRT6_peptide	-64.27±5.01	-167.92±10.36	84.92±19.10	-7.94±0.17	-155.22±18.69
SIRT6-CL5D	-64.15±4.12	-146.66±13.49	108.85±23.16	-7.40±0.19	-109.37±24.77
SIRT6-CL5D_peptide	-66.88±3.39	-96.97±7.51	53.79±19.76	-7.64±0.16	-117.70±21.11

Supplementary Results and Discussions

In the presence of substrate binding, the angle of dissociation is slightly different between the agonist and agonist-free systems(Supplementary 5), as shown by snapshots of the release processes (Supplementary 5) as well as the hydrogen bonding interactions at equilibrium (Supplementary 4), where ADPr is able to form a stable hydrogen bond with ASP63 interactions until ADPr leaves the binding site as a whole, and this interaction affects the angle of dissociation of ADPr from SIRT6.

To verify whether 9-mer peptide affected the binding affinity of ADPr to SIRT6, we

counted the hydrogen bonds between SIRT6 and ADPr in SIRT6 system with or without 9-mer peptide (Table S1), and the results showed that 9-mer peptide before and after binding, the hydrogen bond interactions between ADPr and SIRT6 did not change significantly, indicating that 9-mer peptide did not significantly affect the binding affinity of ADPr to SIRT6.

The data in Table S2 clearly demonstrate that the 9-mer peptide enhances ADP-ribose binding affinity in both the SIRT6 and SIRT6-CL5D systems. In the SIRT6 system, the binding free energy (ΔG_{bind}) decreases from -127.13 ± 20.43 kcal/mol to -155.22 ± 18.69 kcal/mol with the addition of the 9-mer peptide, indicating a significant enhancement primarily driven by more favorable electrostatic interactions (ΔE_{ele}), which decrease from -142.07 ± 12.49 kcal/mol to -167.92 ± 10.36 kcal/mol. In the SIRT6-CL5D system, the presence of the 9-mer peptide leads to a modest improvement in binding affinity, with ΔG_{bind} changing from -109.37 ± 24.77 kcal/mol to -117.70 ± 21.11 kcal/mol.These findings suggest that the 9-mer peptide enhances ADP-ribose binding affinity mainly through thermodynamic stabilization, contributing to more favorable electrostatic interactions and improved conformational stability.

Supplementary References

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