Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information: Exponential Averaging Versus Umbrella Sampling for Computing the QM/MM Free Energy Barrier of the Initial Step of the Desuccinylation Reaction Catalyzed by Sirtuin 5

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Contents

1 Hydrogenbond between Succinyl Group and Arg105 (HB-R1)

The hydrogen bond between the succinyl group and Arg105 located in the active center of SIRT5 changes its character as the reaction progresses. This is discussed in section 4.1. In Figure S1 the distance between the oxygen of the succinyl group and the hydrogen as well as the distance between the nitrogen belonging to Arg105 and the hydrogen are shown. In the reactant state, both are neutrally charged. During the reaction, the hydrogen becomes more and more associated with the Arg105 residues, which results in higher charge separation. The cross-over takes place close to the transition state region, $\mathrm{d(C1'-O)} - \mathrm{d(N-C1')} \approx 0.25$ Å.

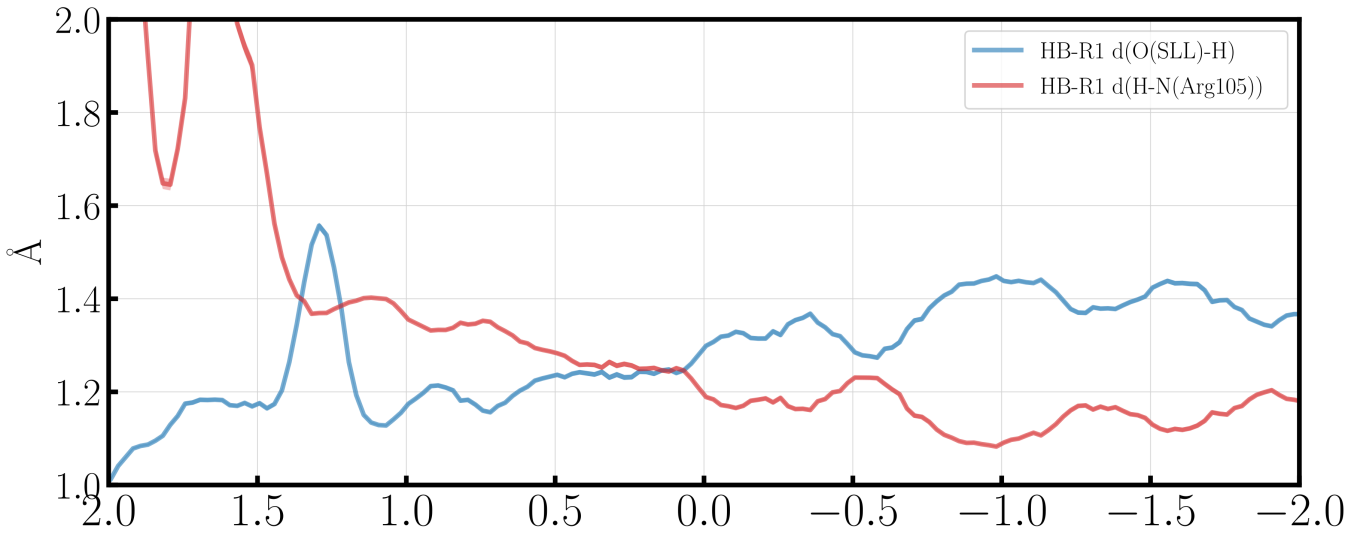

Figure S1: Change of d(O(SLL)-H) and d(H-N(Arg105)),associated with the HB-R1 interaction, during the progression of first step of the desuccinylation reaction.

2 Window Placement and Deviation of Umbrella Window Mean from Bias Potential Minimum Position

The umbrella windows have to be placed so that the space between reactant and product state is seamlessly sampled. The simulations were submitted in batches. Therefore, we were able to set our simulation widows along the becoming more and more apparent MFEP. In addition, several windows were placed at d(C-N) = 2.5, to unequivocally exclude the alternative SN1-reaction type.

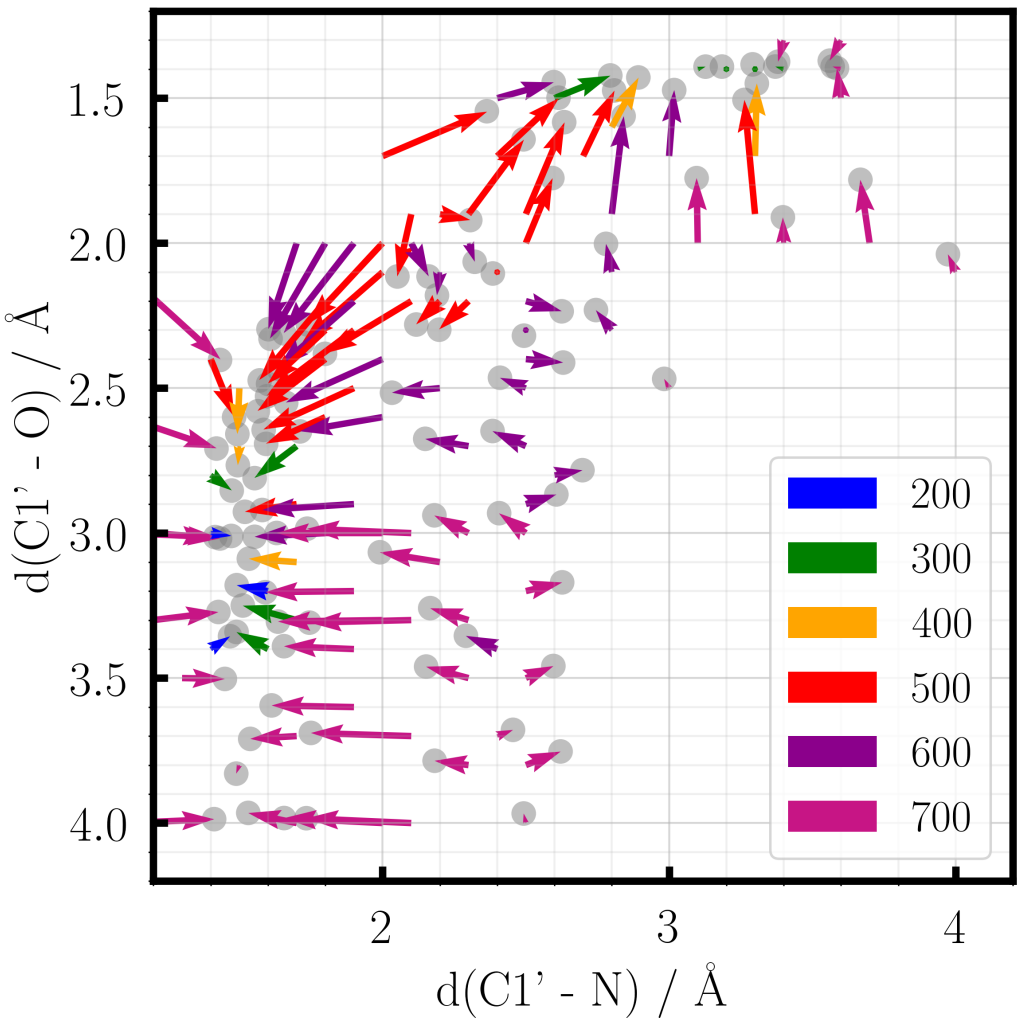

Figure S2: The origin of each arrow indicates the original window placement, and therefore the center of each biasing potential $d_{j,i}$. The arrow's color corresponds to the force constant in kJ mol^{-1} Å^{-2}. The arrow head points to the mean d(C1'-O)/d(C1'-N) sampled in each umbrella simulation.

Figure S2 visualizes the deviation of the mean along $d(\mathrm{C1'} - \mathrm{N})$ and $d(\mathrm{O} - \mathrm{C1'})$ within each umbrella window and the minimum of the biasing potential. Windows placed near the high energy transition state region or in one of the basins (either reactant or intermediate) show very little deviations between intended window mean (arrow base) and the computed mean (arrow tip). Windows placed in regions, where the free-energy profile changes rapidly, deviate more strongly even if large force constants have been used. This is due to the overestimation of the transition barrier energy by HF-3c and corresponding large forces. In contrast, much lower force constants (160 kJ mol^{-1} Å^{-2}) were used by Hu et al. [**Hu2008**] for the one-dimensional umbrella simulations (24 umbrella windows) of Sir2Tm, where they employed B3LYP/6-31G* and calculated a free energy barrier of only 65.8 kJ/mol for the deacetylation.

Table S 1: List of the exact biasing potential parameters used in the different Umbrella windows. The equilibrium distances d_0 are given in Å and the force constants in kJ/mol Å^2

d_0(C1'-O)	k_{CO}	d_0(C1'-N)	k_{CN}
1.3	700.0	3.4	700.0
1.3	700.0	3.6	700.0
1.4	300.0	3.1	300.0
1.4	300.0	3.2	300.0
1.4	300.0	3.3	300.0
1.4	300.0	3.4	300.0
1.4	700.0	3.6	700.0
1.5	600.0	2.4	600.0
1.5	300.0	2.6	300.0
1.5	700.0	3.6	700.0
1.6	400.0	2.8	400.0
1.7	500.0	2.0	500.0
1.7	500.0	2.4	500.0
1.7	500.0	2.7	500.0
1.7	600.0	3.0	600.0
1.7	400.0	3.3	400.0
1.9	500.0	2.1	500.0
1.9	500.0	2.2	500.0
1.9	500.0	2.3	500.0
1.9	500.0	2.5	500.0
1.9	600.0	2.8	600.0
1.9	500.0	3.3	500.0
2.0	600.0	1.7	600.0
2.0	600.0	1.8	600.0
2.0	600.0	1.9	600.0
2.0	500.0	2.0	500.0
2.0	600.0	2.1	600.0
2.0	600.0	2.3	600.0
2.0	500.0	2.5	500.0
2.0	700.0	3.1	700.0
2.0	700.0	3.4	700.0
2.0	700.0	3.7	700.0
2.1	700.0	1.1	700.0
2.1	500.0	2.0	500.0
2.1	600.0	2.2	600.0
2.1	500.0	2.4	500.0
2.1	600.0	2.8	600.0
2.1	700.0	4.0	700.0
2.2	500.0	1.8	500.0
2.2	600.0	1.9	600.0
2.2	500.0	2.1	500.0
2.2	500.0	2.2	500.0
2.2	500.0	2.3	500.0
2.2	600.0	2.5	600.0
2.3	500.0	1.8	500.0
2.3	500.0	1.9	500.0
2.3	600.0	2.5	600.0

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Table S1 – *Continued from previous page*

d_0(C1'-O)	k_{CO}	d_0(C1'-N)	k_{CN}
2.3	600.0	2.8	600.0
2.4	500.0	1.4	500.0
2.4	500.0	1.8	500.0
2.4	600.0	2.0	600.0
2.4	600.0	2.5	600.0
2.5	400.0	1.5	400.0
2.5	500.0	1.9	500.0
2.5	600.0	2.2	600.0
2.5	600.0	2.5	600.0
2.5	700.0	3.0	700.0
2.6	700.0	1.1	700.0
2.6	500.0	1.8	500.0
2.6	600.0	2.0	600.0
2.7	400.0	1.5	400.0
2.7	300.0	1.7	300.0
2.7	600.0	2.3	600.0
2.7	600.0	2.5	600.0
2.8	300.0	1.4	300.0
2.8	600.0	2.6	600.0
2.9	500.0	1.7	500.0
2.9	600.0	1.9	600.0
2.9	600.0	2.5	600.0
3.0	700.0	1.1	700.0
3.0	700.0	1.2	700.0
3.0	200.0	1.4	200.0
3.0	600.0	1.8	600.0
3.0	700.0	2.0	700.0
3.0	700.0	2.1	700.0
3.0	700.0	2.3	700.0
3.0	700.0	2.5	700.0
3.1	400.0	1.7	700.0
3.1	700.0	2.2	700.0
3.2	200.0	1.6	200.0
3.2	700.0	1.9	700.0
3.2	700.0	2.5	700.0
3.3	700.0	1.2	700.0
3.3	300.0	1.7	500.0
3.3	700.0	2.0	700.0
3.3	700.0	2.1	700.0
3.3	700.0	2.3	700.0
3.4	200.0	1.4	200.0
3.4	300.0	1.6	400.0
3.4	700.0	1.9	700.0
3.4	600.0	2.4	600.0
3.5	700.0	1.3	700.0
3.5	700.0	2.3	700.0
3.5	700.0	2.5	700.0
3.6	700.0	1.9	700.0
3.7	700.0	1.7	700.0
3.7	700.0	2.1	700.0

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Table S1 – *Continued from previous page*

d_0(C1'-O)	k_{CO}	d_0(C1'-N)	k_{CN}
3.7	700.0	2.4	700.0
3.8	700.0	1.5	700.0
3.8	700.0	2.3	700.0
3.8	700.0	2.5	700.0
4.0	700.0	1.1	700.0
4.0	700.0	1.7	700.0
4.0	700.0	2.0	700.0
4.0	700.0	2.1	700.0
4.0	700.0	2.5	700.0

3 Free energy surface of the initial reaction step catalyzed by SIRT5

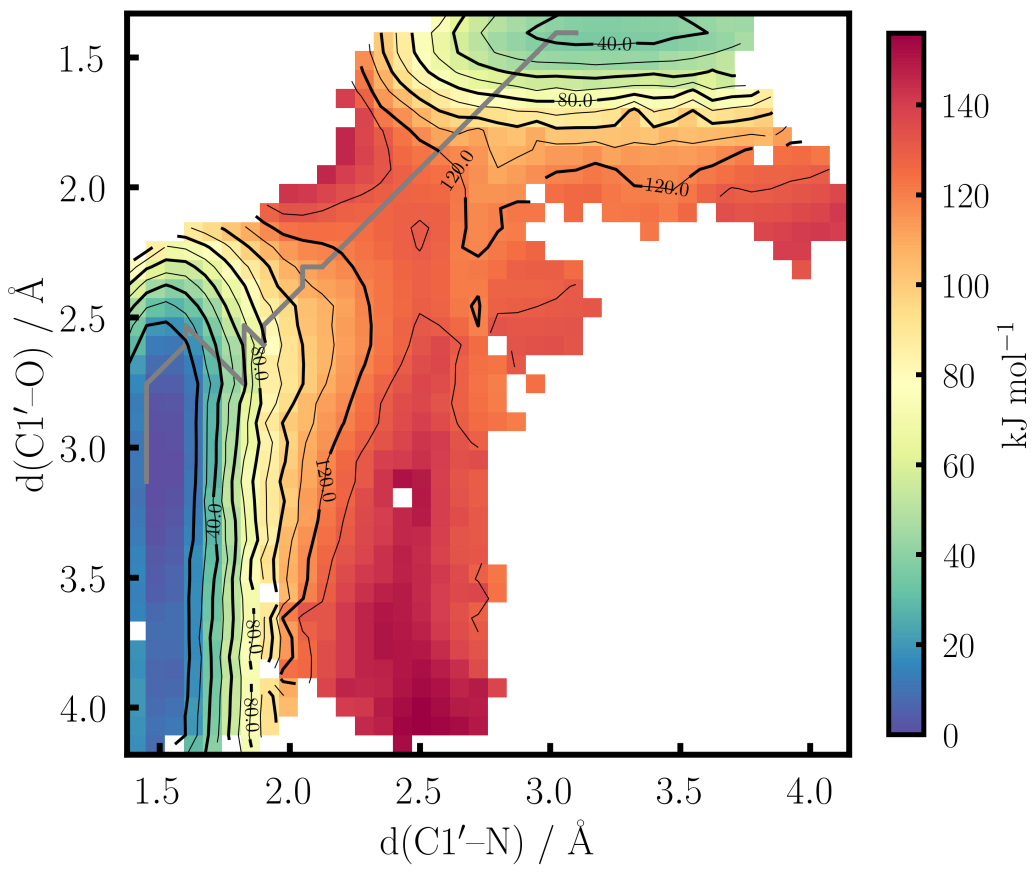

Figure S 3: Original free energy surface of the first reaction step catalyzed by SIRT5 calculated with HF-3c/MM. The minimal free energy path (MFEP) connecting the reactant and intermediate state is shown in grey. White areas were not visited during the simulations.

4 Influence of Bin Width and Sample Number

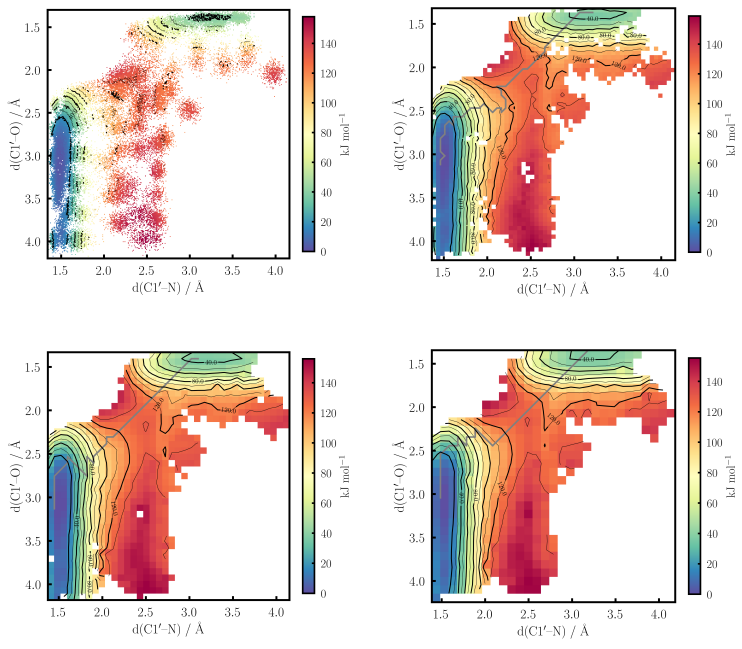

Figure S4: All plots are based on the full data set (data points are 2 fs apart). The bin sizes used for the surfaces are the same along $d(\mathrm{O}-\mathrm{C1}')$ and $d(\mathrm{C1}'-\mathrm{N})$. The sizes are from left to right and top to bottom 0.01 Å, 0.05 Å, 0.075 Å, and 0.1 Å, respectively.

The influence of bin size on the free activation energy as well as the location of the minimal free energy path connecting the two minima on the surface was tested. Figure S4 indicates that there is no significant influence.