Electronic Supplementary Information

Mechanism of C-terminal intein cleavage in protein splicing from QM/MM molecular dynamics simulations

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Table S1 Protein atoms within the buffer region are restrained harmonically to their crystallographic positions by applying scaled restraints. Four different slices (from 21 Å to 25 Å with respect to the system center, the C^{198} atom) and different atom types (backbone atoms (N, C_α, C and O), β atoms, γ atoms and remaining atoms) are considered in order to define the force constants of the restraints.

Buffer region	Backbone atoms	β atoms	γ atoms	Others
21-22 Å	0.27	0.24	0.23	0.22
22-23 Å	0.85	0.78	0.73	0.69
23-24 Å	1.44	1.31	1.22	1.18
24-25 Å	1.70	1.55	1.45	1.40

Table S2 Potential energy surface (in kcal/mol) for the reaction pathway illustrated in Figure 4 computed with SCCDFTB/CHARMM27 level of theory. Single point calculations were carried out treating the QM part at B3LYP/6-31+G(d) level of theory.

	SCCDFTB			B3LYP/6-31+G(d)		
	QM/MM	QM	MM	QM/MM	QM	MM
React	0.0	0.0	0.0	0.0	0.0	0.0
TS1	16.2	10.8	5.4	24.2	14.0	10.2
$React_{act}$	15.6	3.6	12.0	23.2	11.6	11.6
TS2	21.5	1.4	20.1	33.3	18.0	15.3
Int	18.2	-2.5	20.7	28.0	4.9	23.1

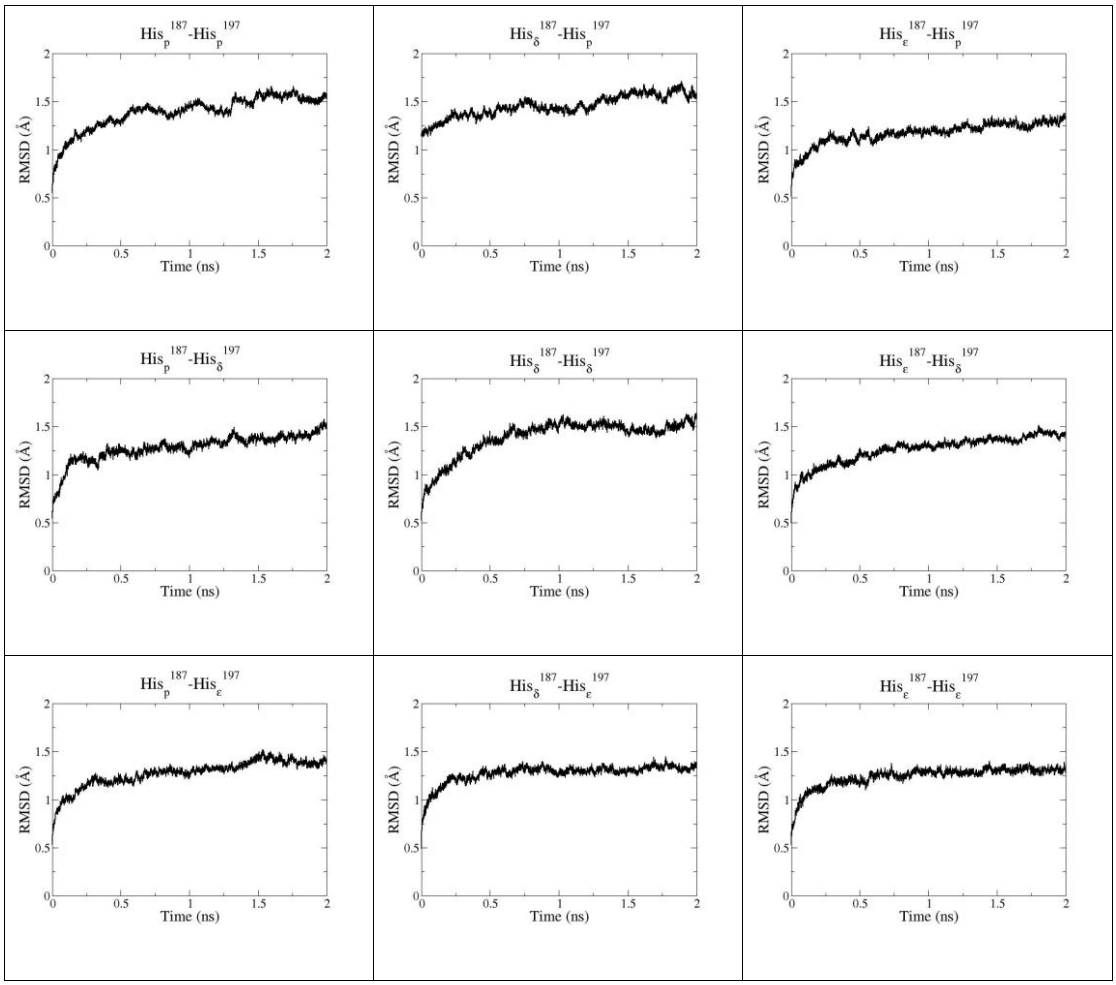

Figure S1 Root mean squared deviation (RMSD) along the 2ns production of the nine simulations. The RMSD was computed as the deviation of all intein atoms from their position in the 1AM2 crystal structure.

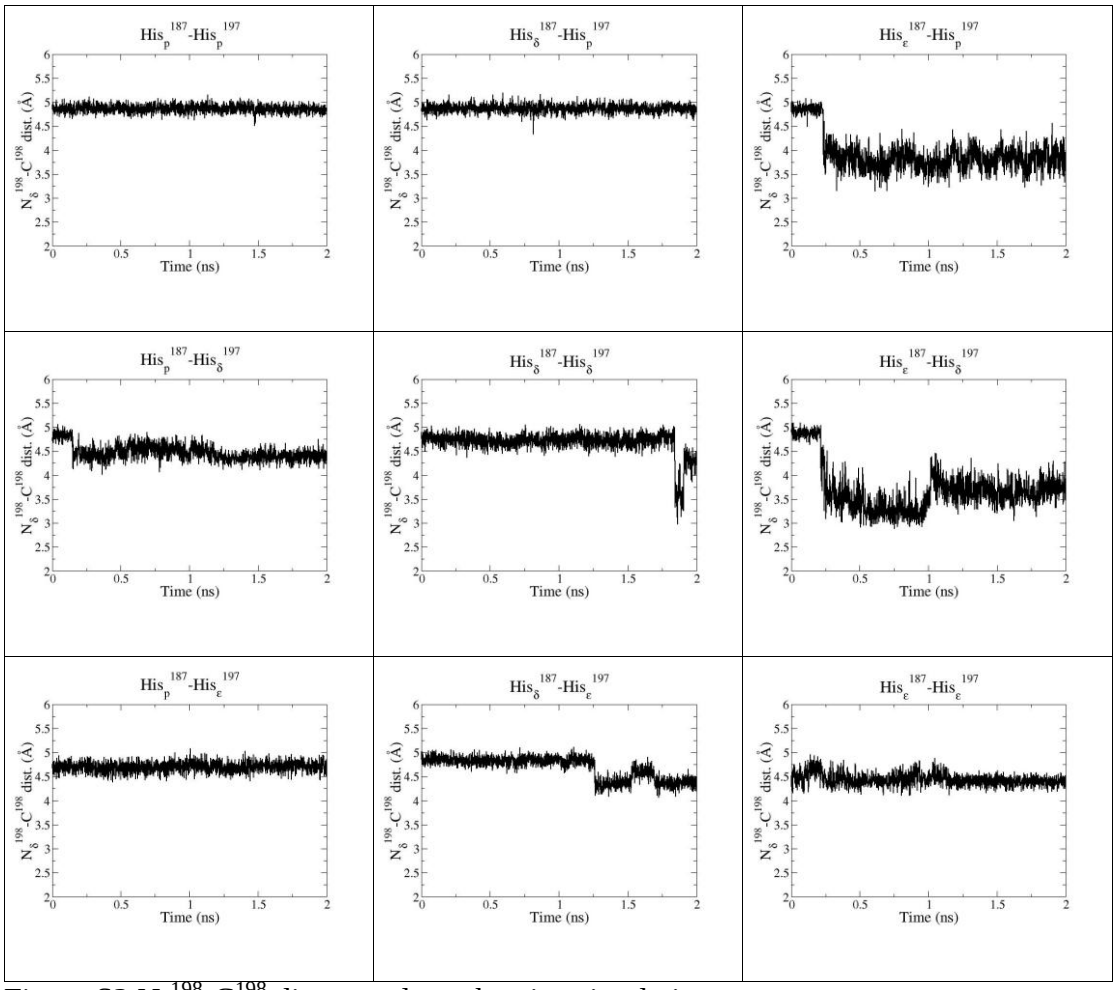

Figure S2 N_δ^{198}-C^{198} distance along the nine simulations.

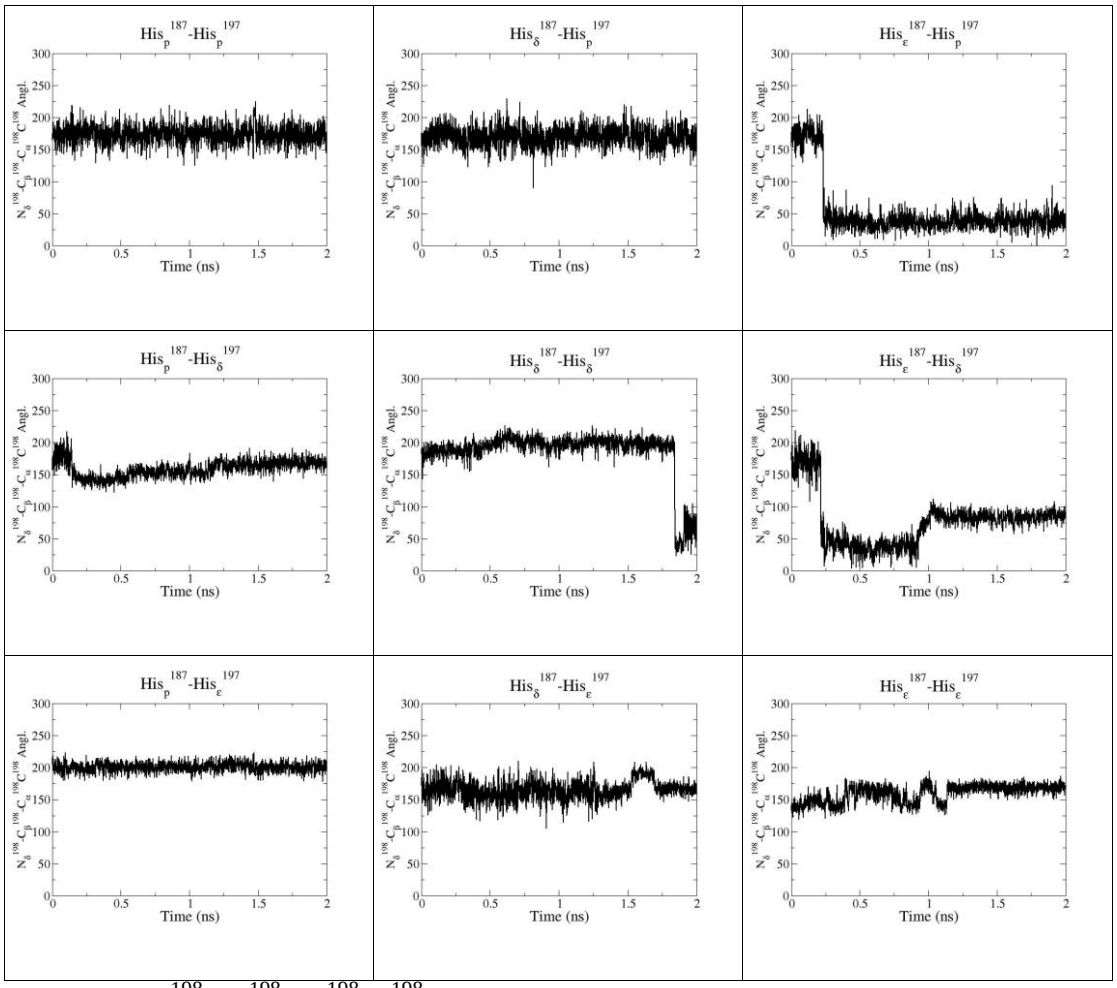

Figure S3 N_δ^{198}-C_β^{198}-C_α^{198}-C^{198} dihedral angle along the nine simulations.

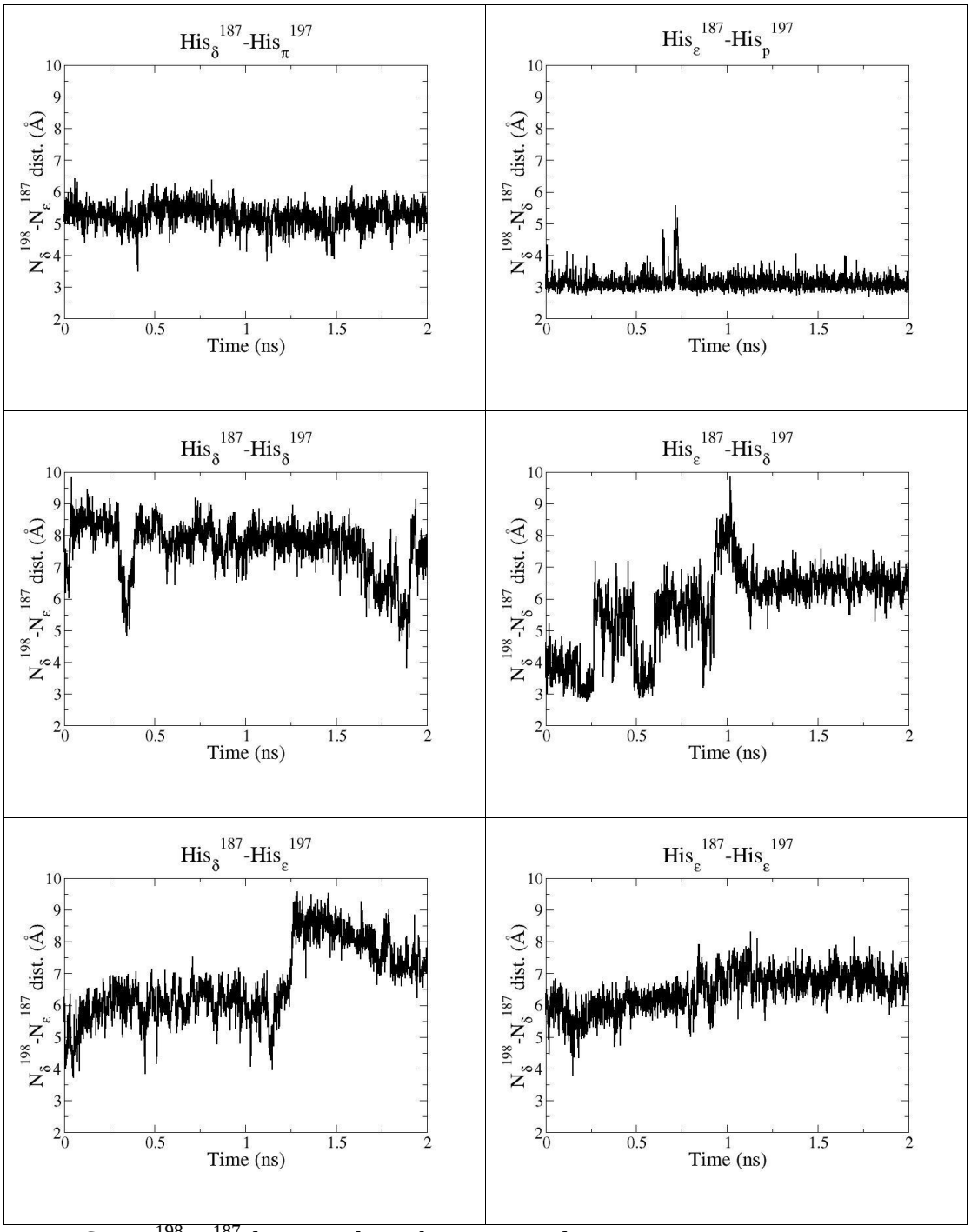

Figure S4 N_δ^{198}-N^{187} distance along the nine simulations.

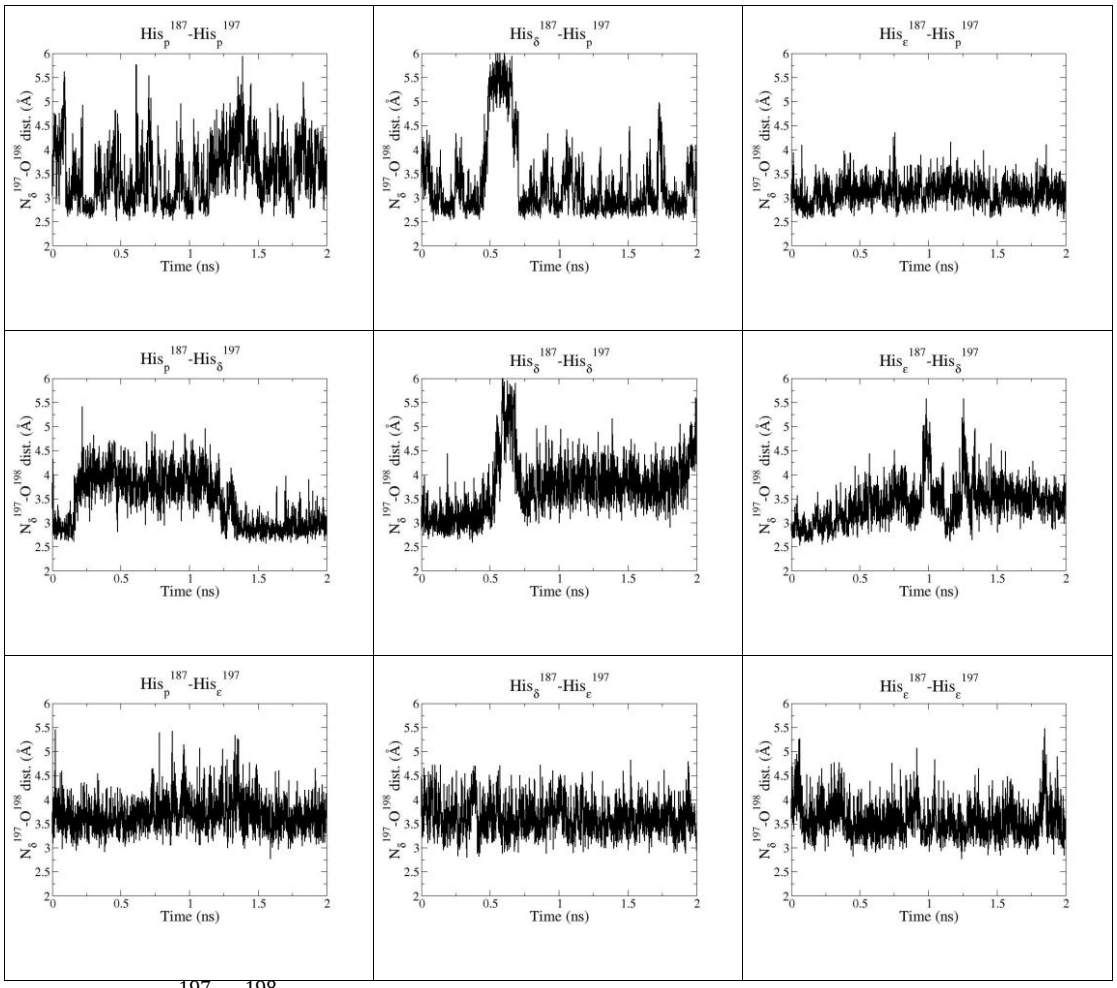

Figure S5 N_δ^{197}-O^{198} distance along the nine simulations.

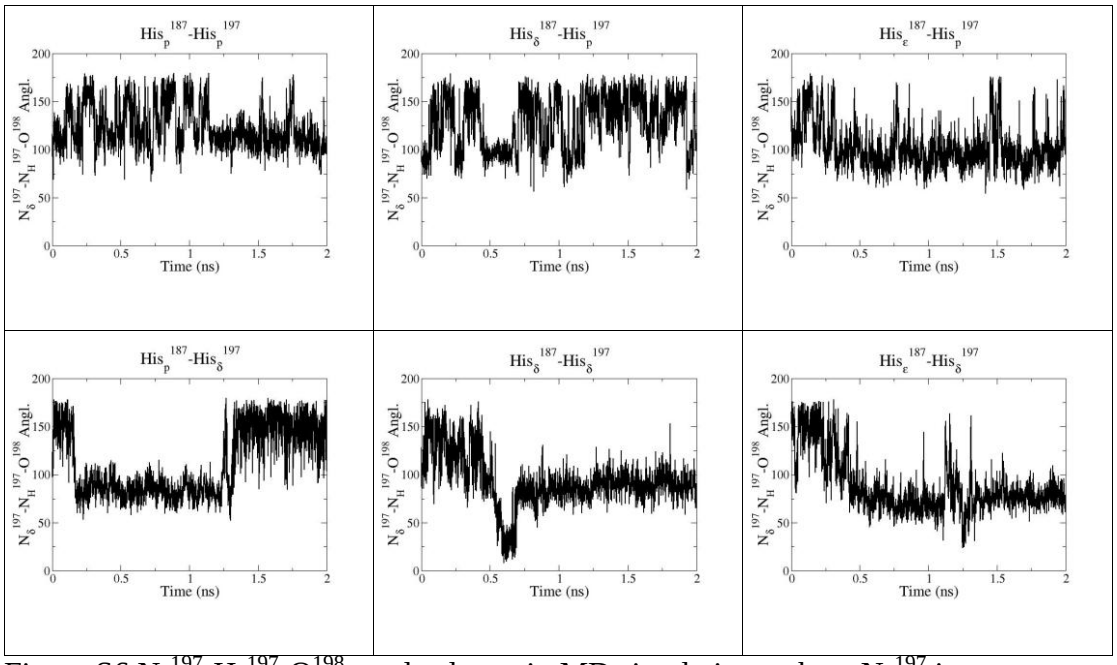

Figure S6 N_δ^{197}-H_δ^{197}-O^{198} angle along six MD simulations where N_δ^{197} is unprotonated.

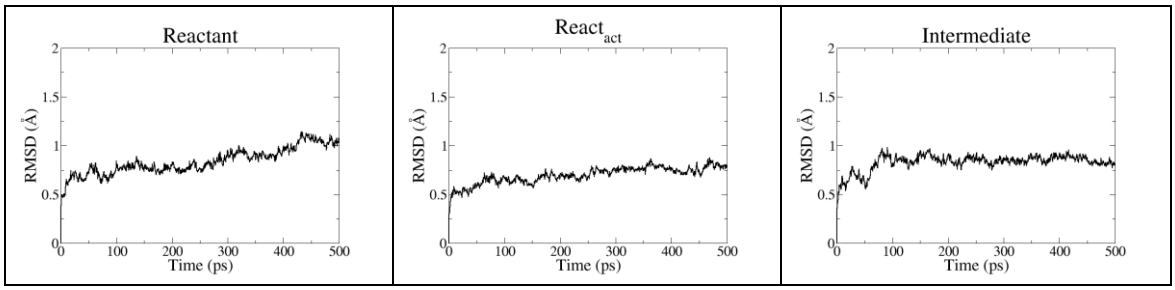

Figure S7 Root mean squared deviation (RMSD) along the 500 ps production of the SCCDFTB/CHARMM27 MD simulations for the initial reactant, activated reactant and intermediate. The RMSD was computed as the deviation of all intein atoms from the initial geometry, the last structure of the umbrella sampling window associated with the corresponding stationary point.

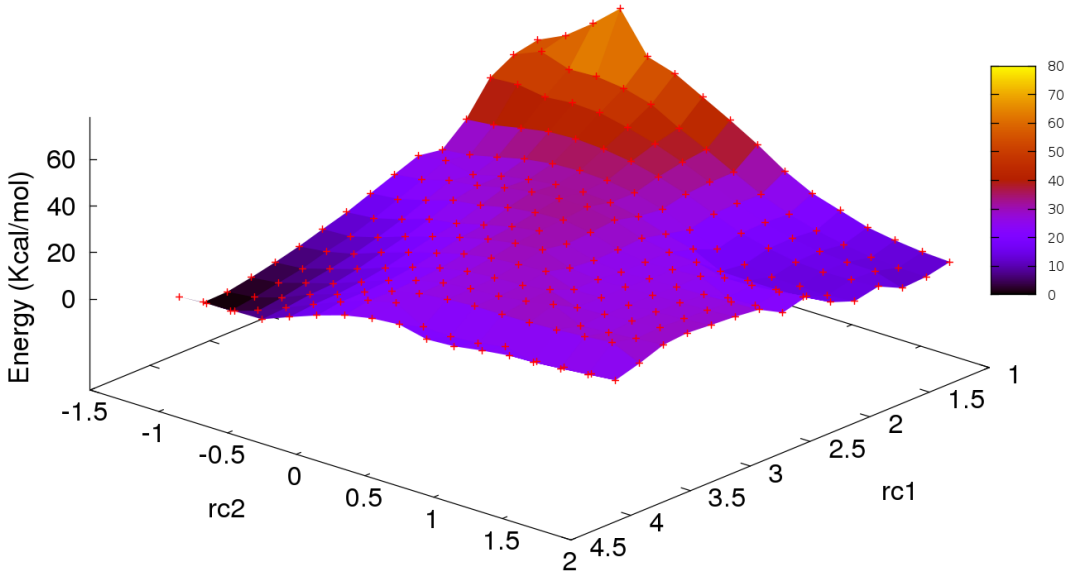

Figure S8 Potential energy surface characterized defining two reaction coordinates: i) rc_1, the distance between the N_δ^{198} and C^{198} atoms and ii) rc_2, antisymetric combination of two distances: d1 (N_δ^{198}-H_δ^{198}) and d2 (N_δ^{187}-H_δ^{198}). The minimum energy reaction pathway goes through a stepwise mechanism in which first the proton is transferred from Asn198 side chain to His187, followed by the cyclization of Asn198 to reach the intermediate. Based on these results, the free energy profile was characterized following the stepwise mechanism and defining a single reaction coordinate at each step (see main body text).