SUPPORTING INFORMATION

Unveiling the Enzymatic Pathway of UMG-SP2 Urethanase: Insights into Polyurethane Degradation at the Atomic Level

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UMG-SP2:DUE-MDA complex modeling

Table S1. List of predicted pK_a values for titratable residues estimated by PROPKA 3.5.0.

Residue	pK_a	pK_a-model
Asp40	2.6	3.8
Asp50	5.3	3.8
Asp60	3.8	3.8
Asp62	4.0	3.8
Asp66	3.8	3.8
Asp72	3.3	3.8
Asp95	5.0	3.8
Asp110	3.1	3.8
Asp116	3.0	3.8
Asp142	3.6	3.8
Asp158	2.5	3.8
Asp186	1.9	3.8
Asp208	3.3	3.8
Asp221	3.8	3.8
Asp226	5.2	3.8
Asp239	5.5	3.8
Asp245	3.4	3.8
Asp277	3.9	3.8
Asp304	4.0	3.8
Asp307	3.2	3.8
Asp314	4.2	3.8
Asp345	4.0	3.8
Asp346	3.3	3.8
Asp355	4.6	3.8
Asp362	2.9	3.8
Asp378	3.8	3.8
Asp381	3.4	3.8
Asp390	3.0	3.8
Asp421	2.9	3.8
Asp438	5.0	3.8
Asp440	4.5	3.8
Asp453	4.1	3.8
Glu18	4.0	4.5
Glu23	3.8	4.5
Glu37	3.9	4.5
Glu47	4.7	4.5
Glu74	4.1	4.5
Glu92	5.2	4.5
Glu107	4.3	4.5
Glu182	7.0	4.5

Glu213	4.7	4.5
Glu273	3.9	4.5
Glu285	4.8	4.5
Glu292	4.8	4.5
Glu330	4.5	4.5
Glu333	4.7	4.5
Glu359	4.8	4.5
Glu379	4.3	4.5
Glu450	4.7	4.5
His108	5.8	6.5
His111	6.4	6.5
His157	5.7	6.5
His159	6.8	6.5
His196	4.3	6.5
His215	1.6	6.5
His271	4.6	6.5
His376	5.4	6.5
His439	6.4	6.5
Cys39	11.3	9
Cys198	12.7	9
Cys291	11.5	9
Cys366	12.4	9
Tyr150	13.6	10
Tyr183	13.0	10
Tyr216	10.9	10
Tyr315	13.6	10
Tyr341	10.2	10
Tyr408	12.4	10
Lys69	10.3	10.5
Lys81	10.9	10.5
Lys91	6.1	10.5
Lys124	10.3	10.5
Lys133	8.4	10.5
Lys204	6.6	10.5
Lys224	8.1	10.5
Lys249	11.0	10.5
Lys384	10.6	10.5
Arg45	11.1	12.5
Arg49	12.8	12.5
Arg59	12.4	12.5
Arg63	13.6	12.5
Arg65	14.2	12.5
Arg80	12.1	12.5
Arg122	12.0	12.5

Arg152	12.0	12.5
Arg161	11.8	12.5
Arg192	13.0	12.5
Arg219	12.6	12.5
Arg235	10.8	12.5
Arg256	12.4	12.5
Arg264	13.6	12.5
Arg286	12.0	12.5
Arg317	13.1	12.5
Arg325	12.3	12.5
Arg349	13.3	12.5
Arg352	12.1	12.5
Arg356	12.5	12.5
Arg385	12.9	12.5
Arg434	12.4	12.5
Arg443	12.2	12.5
Arg454	12.5	12.5

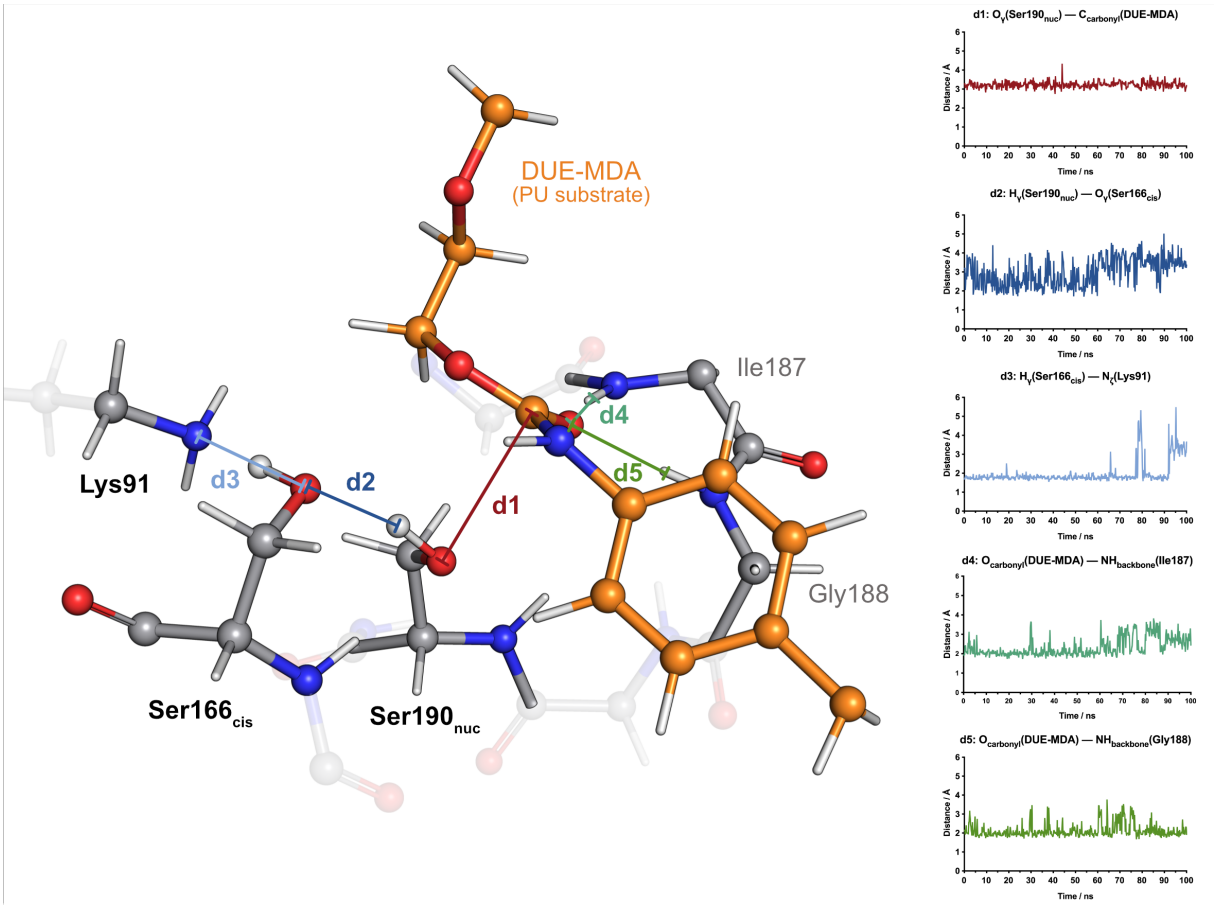

Figure S1. Catalytically relevant interatomic distances that were followed throughout the 100 ns-long production phase.

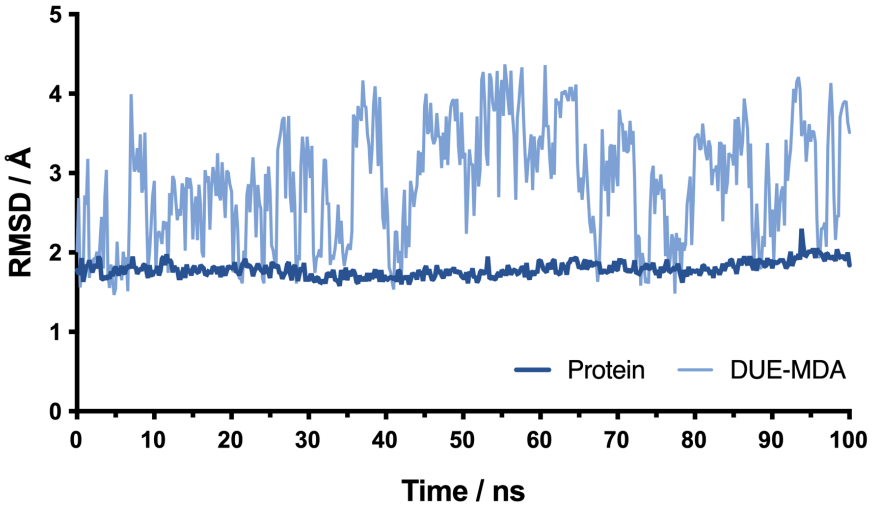

Figure S2. RMSD fluctuation of the protein (dark blue) and the DUE-MDA substrate (light blue) during the 100 ns-long production phase.

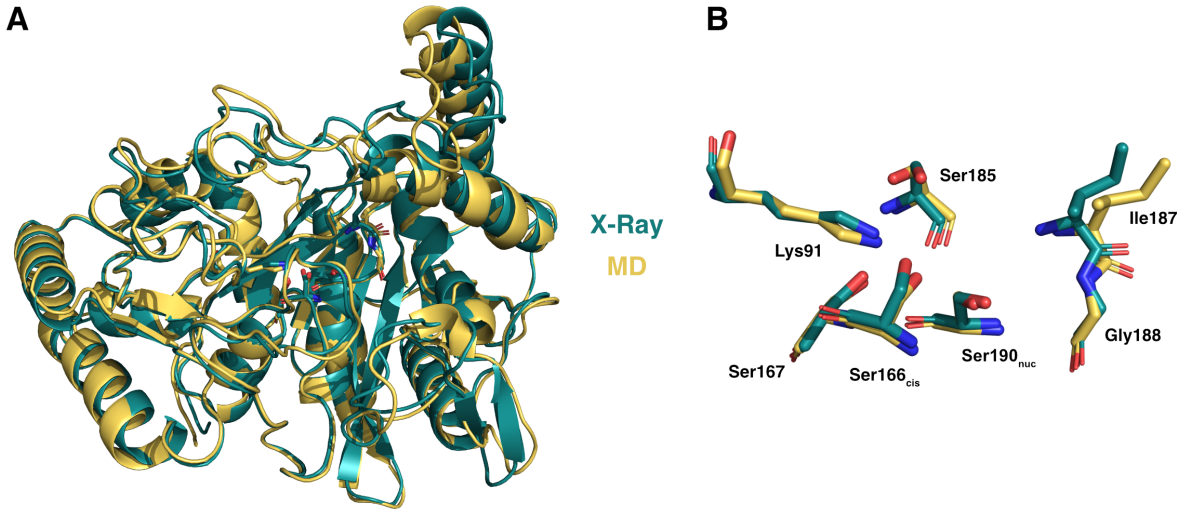

Figure S3. Structural alignment of the X-ray structure of UMG-SP2 complexed with the inhibitor phenylmethanesulfonyl fluoride (PDB ID: 8WDW) and the UMG-SP2's MD-gathered structure that was used in the QM/MM mechanistic study. Left: cartoon representation of the X-ray (teal) and MD-derived (gold) UMG-SP2 structures. The active site residues are represented as thick sticks. The phenylmethanesulfonyl fluoride inhibitor is not shown for clarity purposes. Right: close-up of the active site region, in which a selection of residues is shown as teal (x-ray) or gold (MD) sticks.

Assessing the effect of different theoretical methods on the free energy profile

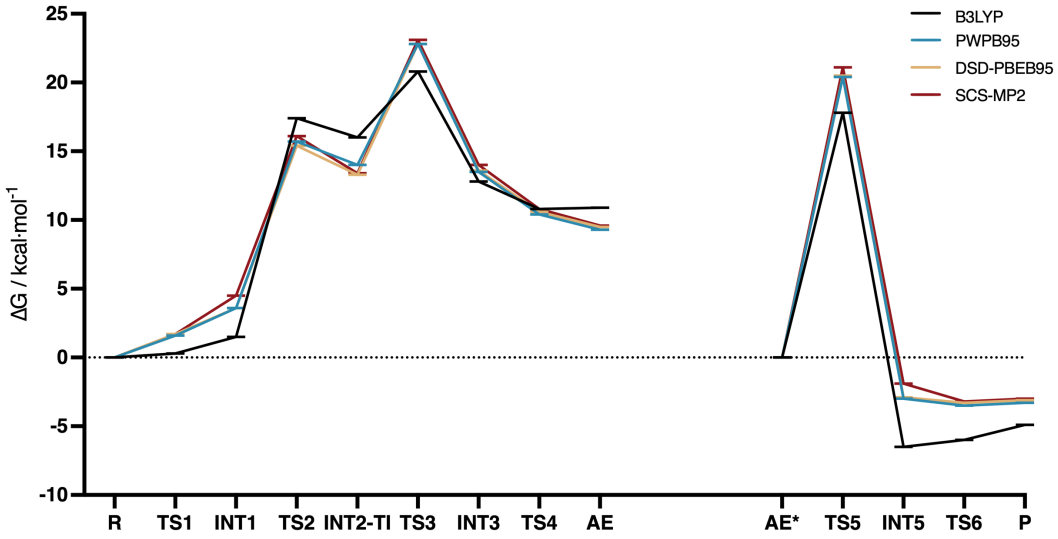

Figure S4. Free energy profiles for the UMG-SP2-catalyzed reaction, determined at different theoretical levels. The ΔG values were determined using the B3LYP/6-31G(d)-D3(BJ):*ff*14SB-optimized geometries of each stationary state. The B3LYP single-point calculations used the 6-311+G(2d,2p) basis set and the D3(BJ) dispersion correction. The single-point calculations performed with the remaining theoretical methods were conducted with the def2-TZVPP basis set. The D3(BJ) dispersion correction was contemplated in the calculations performed with the PWPB95 and DSD-PBEB95 density functionals. The values are presented in kcal·mol^{-1}.

Table S2. ΔG values, determined at different theoretical levels. The calculations were performed using the B3LYP/6-31G(d)-D3(BJ):*ff*14SB-optimized geometries of each stationary state. The B3LYP single-point calculations used the 6-311+G(2d,2p) basis set and the D3(BJ) dispersion correction. The single-point calculations performed with the remaining theoretical methods were conducted with the def2-TZVPP basis set. The D3(BJ) dispersion correction was contemplated in the calculations performed with the PWPB95 and DSD-PBEB95 density functionals. The values are presented in kcal·mol^{-1}.

Stationary State	B3LYP	PWPB95	DSD-PBEB95	SCS-MP2
R	0.0	0.0	0.0	0.0
TS1	0.3	1.6	1.7	1.7
INT1	1.5	3.6	3.6	4.5
TS2	17.4	15.7	15.4	16.1
INT2-TI	16.0	14.0	13.3	13.4
TS3	20.8	22.8	22.8	23.1
INT3	12.8	13.5	13.6	14.0
TS4	10.8	10.4	10.6	10.8
AE	10.9	9.3	9.5	9.6

AE*	0.0	0.0	0.0	0.0
TS5	17.8	20.4	20.5	21.1
INT5	-6.5	-3.0	-2.9	-1.9
TS6	-6.0	-3.5	-3.3	-3.2
P	-4.9	-3.3	-3.1	-3.0

We conducted single-point calculations using three additional theoretical methods to determine whether the calculated Gibbs free energy profile is independent of the density functional used (B3LYP): the double-hybrid-meta-GGA functional PWPB95, which has 50 % Hartree-Fock exchange; the double-hybrid-meta-GGA functional DSD-PBEB95, which has 66 % Hartree-Fock exchange; and the resolution identity spin-component-scaled second-order Møller–Plesset perturbation theory (SCS-MP2).

The calculated ΔG values for the four theoretical methods were similar, suggesting that the overall Gibbs free energy landscape remained consistent. TS3 consistently remained as the rate-limiting TS of the overall reaction, with its energy fluctuating only slightly within the range of 20.8 to 23.1 kcal·mol^{-1}.

Table S3. Most relevant interatomic distances involved in the hydrolysis of the DUE-MDA PU substrate, determined at ONIOM(B3LYP/6-31G(d)-D3(BJ):*ff*14SB level of theory. The values are presented in Å.

Stationary State	O_γ(Ser190$_{nuc}$) – C_{carb}(DUE-MDA)	H_γ (Ser190$_{nuc}$) – O_γ(Ser166$_{cis}$)	H_γ(Ser166$_{cis}$) – N_ζ(Lys91)	C_{carb}(DUE-MDA) – O_{carb}(DUE-MDA)	O_γ(Ser167) – $N_{\zeta1}$(Lys91)	O_γ(Ser185) – $N_{\zeta1}$(Lys91)	O_{carb}(DUE-MDA) – NH(Ile187)	O_{carb}(DUE-MDA) – NH(Gly188)
R	2.72	1.60	1.56	1.34	2.10	2.22	1.90	1.84
TS1	2.69	1.50	1.28	1.34	2.00	2.16	1.90	1.84
INT1	2.65	1.04	1.06	1.34	1.83	1.97	1.87	1.82
TS2	1.69	0.99	1.05	1.42	1.82	1.88	1.75	1.76
INT2-TI	1.52	0.99	1.04	1.48	1.79	1.86	1.73	1.76
TS3	1.38	1.19	1.07	2.09	1.84	1.89	1.89	1.80
INT3	1.33	1.61	1.14	2.79	1.93	1.98	2.17	1.76
TS4	1.33	1.67	1.25	2.81	1.99	2.06	2.21	1.77
AE	1.33	1.76	1.52	2.82	2.10	2.18	2.28	1.78
Stationary State	O_γ(Ser190$_{nuc}$) – C_{carb}(DUE-MDA)	H(Wat$_{cat}$) – O_γ(Ser166$_{cis}$)	H_γ(Ser166$_{cis}$) – N_ζ(Lys91)	O(Wat$_{cat}$) – C_{carb}(DUE-MDA)	O_γ(Ser167) – $N_{\zeta1}$(Lys91)	O_γ(Ser185) – $N_{\zeta1}$(Lys91)	O_{carb}(DUE-MDA) – NH(Ile187)	O_{carb}(DUE-MDA) – NH(Gly188)
AE*	1.33	1.85	1.54	3.32	2.01	2.15	2.20	1.79
TS5	1.40	1.32	1.09	1.89	1.88	1.93	1.85	1.80
INT5	2.55	1.03	1.05	1.33	1.87	1.94	1.77	1.76
TS6	2.60	1.46	1.30	1.33	2.05	2.13	1.79	1.78
P	2.62	1.55	1.54	1.33	2.13	2.19	1.80	1.78

Contribution of individual residues to the activation energy

Table S4. Reference carbon atoms. The table presents the residue-specific carbon atoms, defined as the reference atoms used to measure the distance of the given residue to the O_{ester}(DUE-MDA) and O_{γ}(Ser190$_{nuc}$).

Residue	Reference atom
Nonpolar	C_{α}
Arginine	C_{ζ}
Asparagine	C_{γ}
Aspartate	C_{γ}
Cysteine	C_{β}
Glutamine	C_{δ}
Glutamate	C_{δ}
Histidine	$C_{\delta 2}$/$C_{\varepsilon 1}$
Lysine	C_{ε}
Methionine	C_{β}
Threonine	C_{β}
Tyrosine	C_{β}