Supporting information for "Insights into the Lactonase Mechanism of Serum Paraoxonase 1 (PON1): Experimental and Quantum Mechanics/Molecular Mechanics (QM/MM) Studies"

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Table S1. Selected geometries of ES complex system 1 for lactonase of GBL after QM/MM optimization with *ab initio* Hartree-Fock theory with different basis sets

	atomic distances (Å)		
	HF/3-21G(d,p)	HF/6-31G(d,p)	Experiment[a]
O_w(WAT359)…C_4(GBL)	5.15	5.21	-
O_w(WAT359)…H_{w1}(WAT359)	0.94	0.95	-
Hw1(WAT359)…N_{E2}(H115)	3.0	3.48	-
C_4(GBL)…O_1(GBL)	1.34	1.32	-
Ca_{357}…O_2(GBL)	2.43	2.48	-
$Ca_{357...}O_w$(WAT359)	2.45	2.49	2.47
Ca_{357}…O_{E2}(Glu53)	2.52	2.52	2.41
Ca_{357}…O_{D1} (Asn168)	2.46	2.58	2.60
Ca_{357}…O_{D1}(Asn224)	2.37	2.43	2.10
Ca_{357}…O_{D1} (Asp269)	2.43	2.43	2.37
Ca_{357}…O_{D1} (Asn270)	2.39	2.44	2.25

[a]Atomic distance obtained from crystal structure of PON1 (i.e., PDB code 3srg). The name of GBL and water indicated in Scheme 1. Ca357 and WAT359 indicate the catalytic calcium and the crystallographic water interacting with Ca357.

Table S2. Selected geometries of ES complex system 2 for lactonase of GBL after QM/MM optimization at Hartree-Fock method with different basis sets

interactions[a]	atomic distances (Å)		
	HF/3-21G(d,p)	HF/6-31G(d,p)	Experiment[b]
O_w (WAT363)…C_4(GBL)	2.69	2.92	-
C_4 (GBL)…O_1 (GBL)	1.35	1.33	-
O_w(WAT363)…H_{w1}(WAT363)	0.95	0.95	-
Hw1 (WAT363)…N_{E2}(H115)	1.93	2.03	-
Ow(WAT359)…C_4 (GBL)	5.43	5.47	-
Ca_{357}…O_2 (GBL)	2.44	2.56	-
Ca_{357}…O_w (WAT359)	2.50	2.50	2.47
Ca_{357}…O_{E2}(Glu53)	2.46	2.53	2.41
Ca_{357}…O_{D1} (Asn168)	2.49	2.58	2.60
Ca_{357}-O_{D1}(Asn224)	2.37	2.43	2.10
Ca_{357}-O_{D1} (Asp269)	2.40	2.43	2.37
Ca_{357}-O_{D1} (Asn270)	2.43	2.47	2.25

[a]The name of GBL and water indicated in Scheme 1. Ca357 indicates the catalytic calcium. WAT359 and WAT363 indicate the crystallographic and the added waters, respectively; [b]Atomic distance obtained from the crystal structure of PON1 (i.e., PDB code 3srg).

Table S3. MP2/6-31G(d) Mulliken charges (in atomic units) of residue His115 in stationary points along the reaction path

atom[a]	Mulliken charges of stationary points				
	ES	TS1	TI	TS2	EP
small QM subsystem					
C_B	-0.335	-0.545	-0.548	-0.532	-0.542
H_{B1}	0.133	0.176	0.193	0.177	0.167
H_{B2}	0.146	0.199	0.206	0.201	0.185
N_{D1}	-0.830	-0.768	-0.771	-0.761	-0.787
H_{D1}	0.339	0.487	0.476	0.488	0.468
C_G	0.211	0.241	0.245	0.222	0.239
H	0.150	0.214	0.216	0.206	0.201
C_{E1}	0.513	0.363	0.391	0.353	0.290
H_{E1}	0.181	0.271	0.321	0.259	0.225
N_{E2}	-0.701	-0.758	-0.757	-0.750	-0.613
C_{D2}	0.026	-0.051	-0.047	-0.033	-0.087
H_{D2}	0.186	0.302	0.338	0.307	0.258
residue H115	0.020	0.131	0.262	0.138	0.004
large QM subsystem					
C_B	-0.557	-0.557	-0.557	-0.544	-0.553
H_{B1}	0.180	0.188	0.201	0.188	0.181
H_{B2}	0.183	0.195	0.203	0.199	0.182
N_{D1}	-0.802	-0.791	-0.791	-0.782	-0.807
H_{D1}	0.497	0.507	0.493	0.506	0.484

C_G	0.246	0.240	0.243	0.223	0.238
H	0.200	0.208	0.214	0.203	0.197
C_{E1}	0.276	0.350	0.386	0.347	0.286
H_{E1}	0.214	0.262	0.313	0.257	0.221
N_{E2}	-0.596	-0.756	-0.758	-0.753	-0.617
C_{D2}	-0.106	-0.058	-0.053	-0.037	-0.086
H_{D2}	0.248	0.297	0.338	0.303	0.252
residue H115	-0.017	0.087	0.233	0.109	-0.022

[a]Atom labels according to CHARMM

Table S4. Selected geometries of ES complex system 2 for lactonase of GVL after QM/MM optimization with the HF/3-21G(d,p) level

interactions[a]	bond distances (Å)	
	HF/3-21G(d,p)	experiment[b]
O_w(WAT363)…C_4(GVL)	3.14	-
C_4(GVL)…O_1(GVL)	1.35	-
O_1(WAT363)…H_{w1}(WAT363)	0.95	-
H_{w1}(WAT363)…N_{E2}(H115)	1.97	-
Ow(WAT359)-C_4(GVL)	4.17	-
Ca_{357}-O_2(GVL)	2.46	-
Ca_{357}-O_w(WAT359)	2.49	2.47
Ca_{357}-O_{E2}(E53)	2.45	2.41
Ca_{357}-O_{D1} (N168)	2.47	2.60
Ca_{357}-O_{D1}(N224)	2.39	2.10
Ca_{357}-O_{D1} (D269)	2.41	2.37

Ca_{357}-O_{D1} (N270)	2.42	2.25

[a]The name of GVL and water as indicated in Scheme 1. Ca357 indicates for catalytic calcium. WAT359 and WAT363 indicate for crystallographic water interacting with catalytic calcium and added water; [b]Atomic distance obtained from crystal structure of paraoxone (i.e., pdb code 3srg).

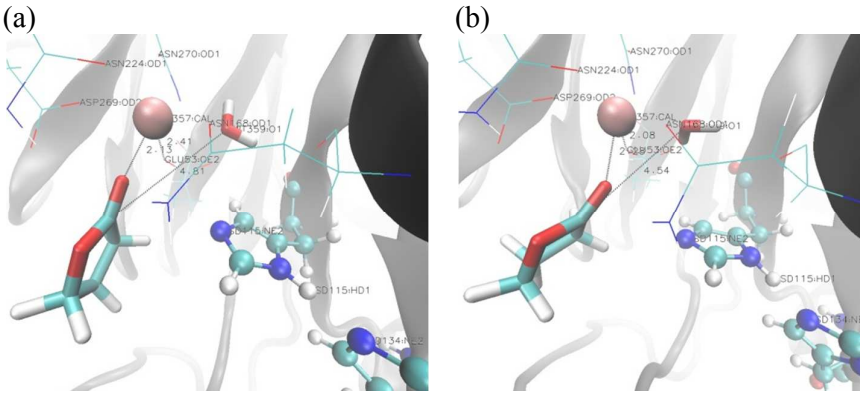

Figure S1. Snapshots of the ES complex system 1 of 3SRG docked with GBL. (A) The initial structure; (b) The structure obtained after 100 ps heating and 100 ps equilibration. GBL and the crystallographic water (licorice); His115 and His134 (CPK), catalytic calcium (CPK, pink); the ligands coordinating the catalytic calcium: Glu53, Asn168, Asn224, Asp269, and Asn270 (lines)

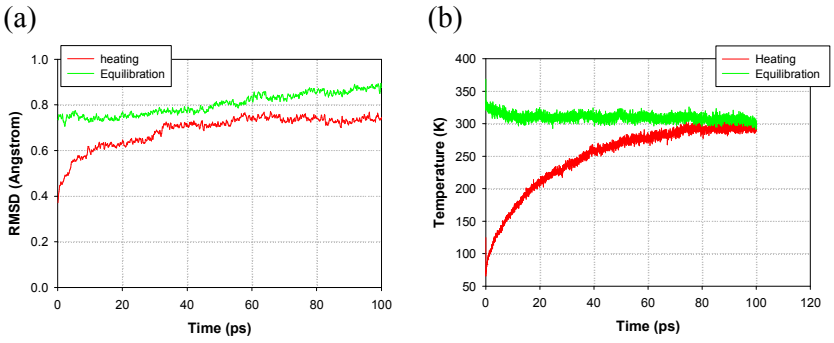

Figure S2. The ES complex system 1 of GBL-docked 3SRG during the heating phase (red) and the equilibration period (green). (a): The RMSD; (b): the system temperature.

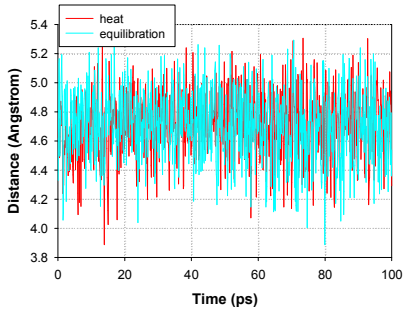

Figure S3. The atomic distance of C_4(GBL) and O_w(the crystallographic water) during the heating phase (red) and the equilibration period (cyan).

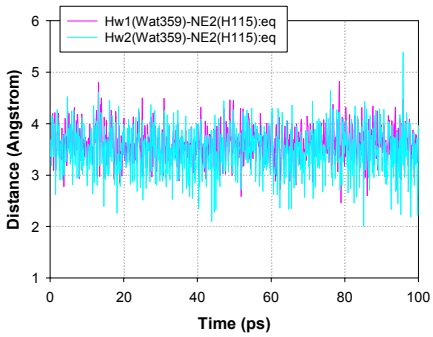

Figure S4. The atomic distances of H_w(the crystallographic water) and N_{e2}(His115) during the equilibration period. The distance of H_{w1}(the crystallographic water) and N_{e2}(His115) (purple); the distance of H_{w2} (the crystallographic water) and N_{e2} (His115) (cyan).

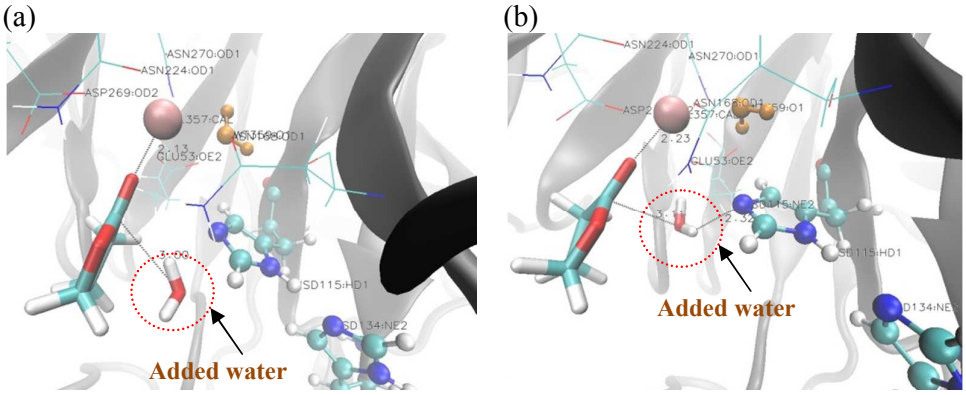

Figure S5. Snapshots of the ES complex system 2 of 3SRG docked with GBL and one added water. (a) The initial structure; (b) The structure obtained after 100 ps of heating and a 100 ps equilibration period. GBL and the added water (licorice); the crystallographic water (CPK,

organe); His115 and His134 (CPK); catalytic calcium (CPK, pink); the ligands coordinating the catalytic calcium: Glu53, Asn168, Asn224, Asp269, and Asn270 (lines).

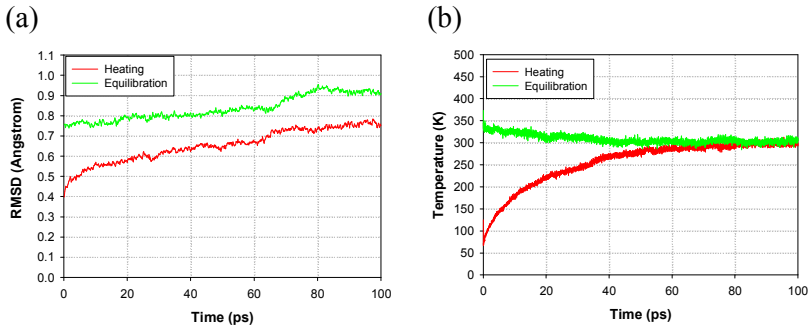

Figure S6. The ES complex system 2 of 3SRG docked with GBL and the added water during the heating phase (red) and the equilibration period (green). (a): The RMSD; (b): the system temperature

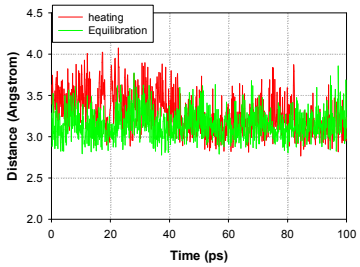

Figure S7. The distance of C_4(GBL) and O_w(added water) of the 3SRG-docked GBL complex during the heating phase (red) and the equilibration period (green).

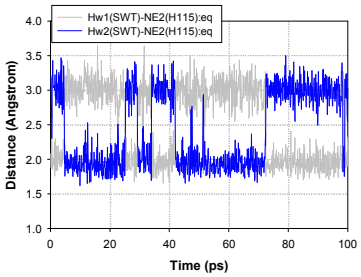

Figure S8. The atomic distances of H_w(added water) and N_{e2}(His115) of the 3SRG-GBL complex during equilibration period. The distance of H_{w1}(added water) and N_{e2}(His115) (gray); the distance of H_{w2} (added water) and N_{e2} (His115) (blue).

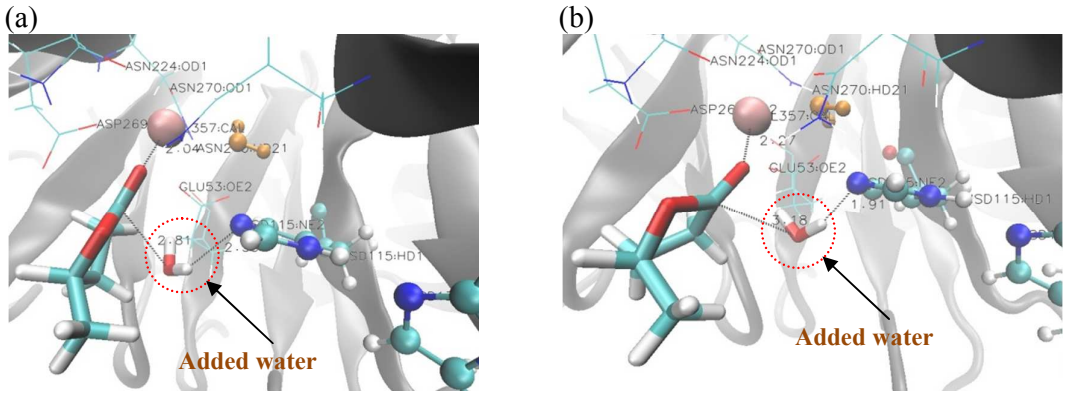

Figure S9. Snapshots of the ES complex system of 3SRG docked with GVL and one added water. (a) The initial structure; (b) The structure obtained after 100 ps of heating and a 200ps equilibration period. GVL and the added water (licorice); crystallographic water (CPK, orange); His115 and His134 (CPK); catalytic calcium (CPK, pink); the ligands coordinating the catalytic calcium: Glu53, Asn168, Asn224, Asp269, and Asn270 (lines).

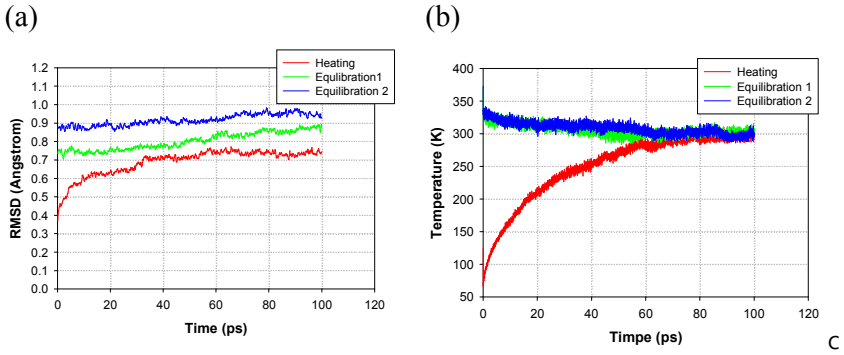

Figure S10. The ES complex of the 3SRG docked with GVL and one added water during heating phase (red), the first 100 ps equilibration period (green) and the second 100ps equilibration period (blue). (a): The RMSD; (b): the system temperature.

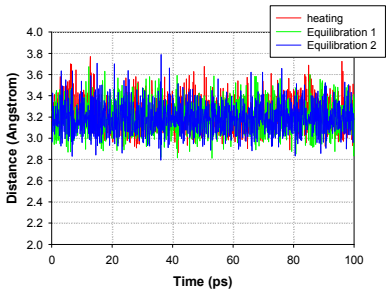

Figure S11. The distance of C_4(GVL) and O_w(added water) of the 3SRG-GVL complex during the heating phase (red), the first 100 ps equilibration period (green) and the second 100 ps equilibration period (blue).

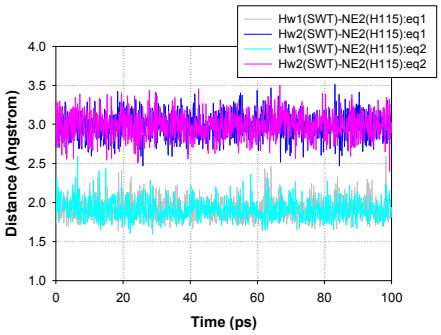

Figure S12. The distances of H_w (added water) and N_{e2} (His115) of the 3SRG-GVL complex during equilibration. The distance of H_{w1} (added water) and N_{e2} (His115) (i.e., the first equilibration (gray), the second equilibration (cyan)), and the distance of H_{w2} (added water) and N_{e2} (His115) (i.e., the first equilibration (blue), the second equilibration (pink)).

(a) (b)

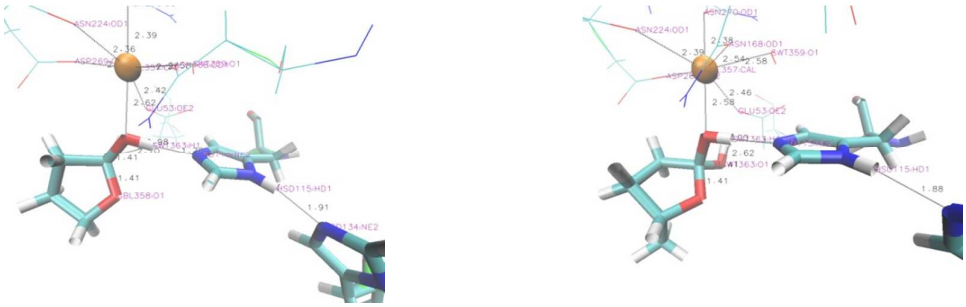

Figure S13. The structures at ξ_{step1}=1.0 and ξ_{step2}=0.0 of the PES scan in the first step of the lactonase reaction of (a): GBL; (b): GVL.