Supporting Information

New Insights into the Catalytic Mechanism of Aldose Reductase: A QM/MM Study.

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Complete citations of Refs. 31, 34 and 35

Complete citation of reference 31: Alexov, E.; Mehler, E. L.; Baker, N.; Baptista, A. M.; Huang, Y.; Milletti, F.; Nielsen, J. E.; Farrell, D.; Carstensen, T.; Olsson, M. H. M.; Shen, J. K.; Warwicker, J.; Williams, S.; Word, J. M. Progress in the Prediction of pKa Values in Proteins. *Proteins* **2011**, *79* (12), 3260–3275.

Complete citation of reference 34: Bruno, I. J.; Cole, J. C.; Kessler, M.; Luo, J.; Motherwell, W. D. S.; Purkis, L. H.; Smith, B. R.; Taylor, R.; Cooper, R. I.; Harris, S. E.; Orpen, A. G. Retrieval of Crystallographically-Derived Molecular Geometry Information. *J. Chem. Inf. Comput. Sci.* **2004**, *44* (6), 2133–2144.

Complete citation of reference 35: Bowers, K. J.; Sacerdoti, F. D.; Salmon, J. K.; Shan, Y.; Shaw, D. E.; Chow, E.; Xu, H.; Dror, R. O.; Eastwood, M. P.; Gregersen, B. A.; Klepeis, J. L.; Kolossvary, I.; Moraes, M. A.; Sacerdoti, F. D.; Salmon, J. K.; Shan, Y.; Shaw, D. E. Molecular Dynamics---Scalable Algorithms for Molecular Dynamics Simulations on Commodity Clusters. In *Proceedings of the 2006 ACM/IEEE conference on Supercomputing - SC '06*; ACM Press: New York, New York, USA, 2006; p 84

Protonated HIP110: other snapshots

Snapshot 2

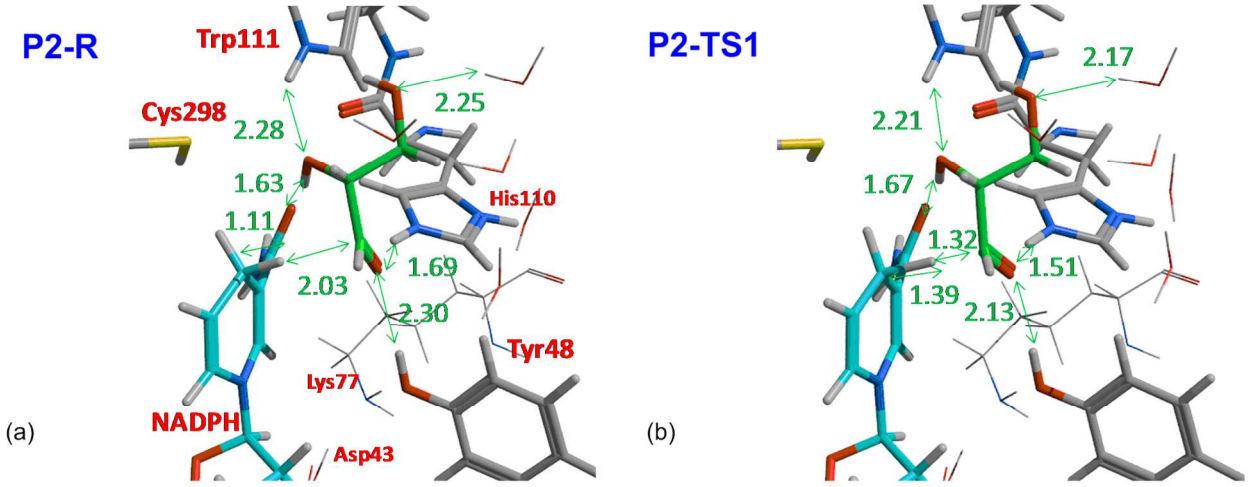


Figure S1 Reaction intermediates of the GLD reduction by AR with HIP110 as studied by the QM/MM model for snapshot 2 of HIP (a) Enzyme-substrate complex (b) Transition state (Distances shown in green, atoms numbers in brown and residues names in red).

Unprotonated HIE110: other snapshots

Snapshot 2

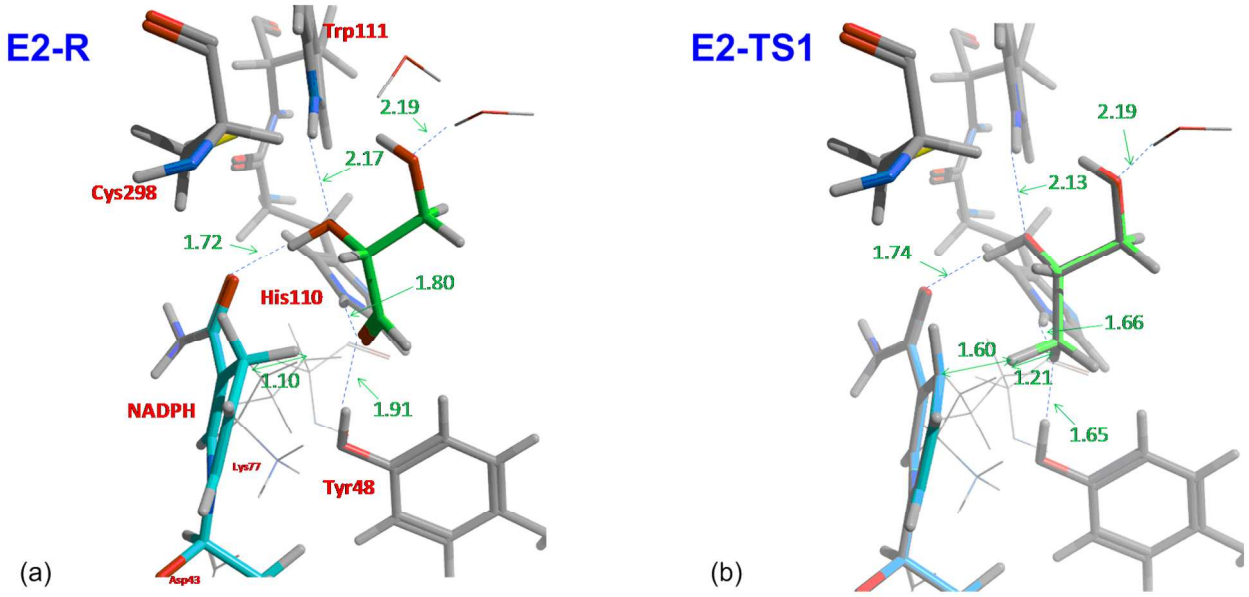


Figure S2 Reaction intermediates of the GLD reduction by AR with HIE110 as studied by the QM/MM model for snapshot 2 of HIE. (a) Enzyme-substrate complex (b) Transition state 1 (Distances shown in green, atoms numbers in brown and residues names in red).

Snapshot 3

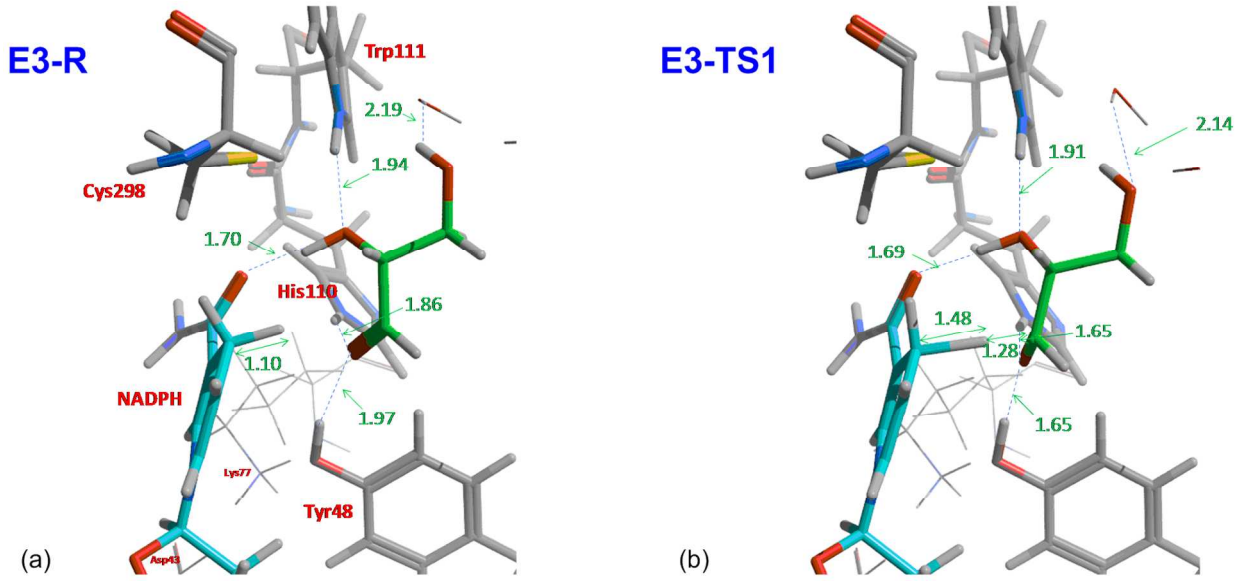
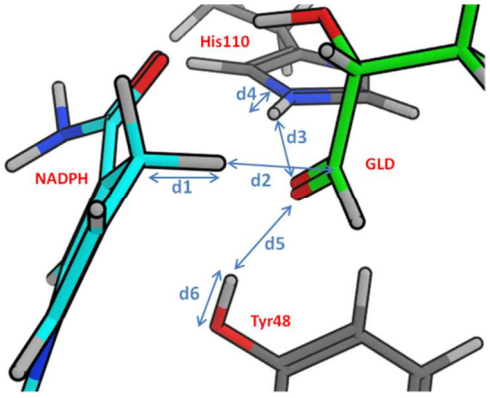


Figure S3 Reaction intermediates of the GLD reduction by AR with HIE110 as studied by the QM/MM model for snapshot 3 of HIE. (a) Enzyme-substrate complex (b) Transition state 1 (Distances shown in green, atoms numbers in brown and residues names in red).

Effect of the basis set and the QM size on selected geometrical characteristics

Although the energies differ, the geometrical characteristics were very similar to previous studies. For the HIP model, the geometries look particularly similar at the transition state (Table S1a). At the reactant state the ligand seem to be in closer proximity to the enzyme in our model as both hydrogen bonds between the oxygen carbonyl and residues His110 and Tyr48 are shorter [1.83 Å and 2.02 Å, respectively], compared to the Lee and co-workers[12] model [2.0 Å and 2.53 Å, respectively]. The hydride is also initially closer to the carbonyl carbon in our model compared to previous results [2 Å and 2.59 Å, respectively]. The same is also observed for the HIE model (Table S1b). At the reactant state the ligand seem to be closer proximity to the enzyme in our model as both hydrogen bonds between the oxygen carbonyl and residues His110 and Tyr48 are shorter [1.86 Å and 1.89 Å, respectively], compared to the Varnai and co-workers[10] model [2.22 Å and 2.64 Å, respectively]. The hydride is also initially closer to the carbonyl carbon in our model compared to previous results [2.23 Å and 2.49 Å, respectively].

Table S1 Comparison of key distances from Lee and co-workers[12] model (Lee) and Varnai and co-workers[10] model (Var.) to (a) HIP and (b) HIE models. Δ is the difference between the two results. A threshold of 0.1 Å has established in order to differentiate between small changes (green) and bigger ones(red).



a.

	reactant			TS			product		
	HIP	Lee	Δ	HIP	Lee	Δ	HIP	Lee	Δ
distances between atoms in the active site (Å)									
d1	1.11	1.09	-0.02	1.45	1.35	-0.1	3.22	NC	/
d2	2.00	2.59	0.59	1.26	1.34	0.08	1.09	NC	/
d3	1.83	2.00	0.17	1.61	1.57	-0.04	1.01	NC	/
d4	1.03	1.00	-0.03	1.07	1.06	-0.01	1.72	1.96	0.24
d5	2.02	2.53	0.51	1.83	1.75	-0.08	1.72	1.91	0.19
d6	0.98	0.96	-0.02	1.00	0.98	-0.02	1.00	0.97	-0.03

b.

	R			TS1			I			TS2			P		
	HIE	Var.	Δ	HIE	Var.	Δ	HIE	Var.	Δ	HIE	Var.	Δ	HIE	Var.	Δ
distances between qm atoms in the active site (Å)															
d1	1.10	1.13	0.03	1.54	1.75	0.21	1.96	2.37	0.41	2.22	2.38	0.16	2.49	3.42	0.93
d2	2.23	2.49	0.26	1.34	1.2	-0.14	1.13	1.14	0.01	1.11	1.14	0.03	1.10	1.12	0.02
d3	1.86	2.22	0.36	1.69	1.99	0.3	1.62	1.9	0.28	1.62	1.93	0.31	1.70	2.12	0.42
d4	1.02	1.00	-0.02	1.04	1.01	-0.03	1.06	1.02	-0.04	1.05	1.01	-0.04	1.03	1.00	-0.03
d5	1.89	2.64	0.75	1.66	1.86	-0.2	1.48	1.8	0.32	1.36	1.48	0.12	1.01	0.97	-0.04
d6	0.98	0.97	-0.01	1.02	0.98	-0.04	1.06	0.99	-0.07	1.13	1.06	-0.07	1.71	2.11	0.4

Energies of the minimised snapshots

	Protonated HIP110		Unprotonated HIE110		
Snapshot	1	2	1	2	3
Potential Energy (kJ/mol)	-154707	-126960	-126077	-155740	-128372