Supporting Information:

A Simple QM/MM Approach for Capturing Polarization Effects in Protein-Ligand Binding Free Energy Calculations

*Frank R. Beierlein,[1] Julien Michel,[2] and Jonathan W. Essex**

School of Chemistry, University of Southampton, Highfield, Southampton SO17 1BJ, United Kingdom

* To whom correspondence should be addressed. Tel. +44 238059 2794; Fax +44 238059 3781; E-mail: j.w.essex@soton.ac.uk

[1] Current address: a) Computer-Chemie-Centrum, Friedrich-Alexander-Universität Erlangen-Nürnberg, Nägelsbachstraße 25, 91052 Erlangen, Germany, b) Excellence Cluster "Engineering of Advanced Materials", Friedrich-Alexander-Universität Erlangen-Nürnberg, Nägelsbachstraße 49b, 91052 Erlangen, Germany

[2] Current address: School of Chemistry, University of Edinburgh, Joseph Black Building, The King's Buildings, West Mains Road, Edinburgh EH9 3JJ, United Kingdom

Brief Literature Review: Using QM(/MM) to Correct Free Energy Calculations

While the focus of the current work is on the calculation of relative protein-ligand binding free energies and solvation free energies, this problem is conceptually related to the calculation of reaction free energies (potentials of mean force, PMF). Therefore, a brief overview of the literature is given here for both problems, based on the excellent overviews given in Refs[1, 2]. Ref[1] focuses on reaction mechanisms, Ref[2] on solvation/binding free energies. Ref[3] also discusses related issues, such as pK_a calculations and redox reactions. Many interesting approaches that combine QM/MM (or QM) with free energy calculations exist;[1-47] we concentrate here on the promising idea of using a fast, but less accurate method to sample phase space and to subsequently correct the simulation results using a modest number of high level QM/MM energy calculations.[1-16, 20-29, 48]

Warshel and co-workers have developed and applied a range of approaches[3-12] that use an approximate reference Hamiltonian like a molecular mechanics (MM)[6, 9] or an empirical valence bond (EVB)[8, 10-12] potential to sample phase space and to obtain an estimate for the free energy change, which is subsequently corrected by calculating the free energies required to change from the approximate Hamiltonian to the QM/MM Hamiltonian. Correction free energies are calculated by using free energy perturbation (FEP)[6, 8] or a linear response approximation (LRA)[10, 12] approach. These approaches were used to study chemical reactions in enzymes[11, 12] and in solution,[4, 8, 10] solvation free energies, [6, 7] reduction potentials of proteins,[9] and pK_a calculations of protein residue side chains.[3]

Warshel and co-workers have also shown that extensive sampling of the configuration space is essential for a meaningful determination of the energetics of enzymatic reactions, while simple energy minimization approaches can lead to major errors in calculations of activation free energies and binding free energies.[49] Interestingly, it was also found that the use of the linear response approximation (LRA) instead of the Zwanzig equation[50] can help avoid convergence problems which are frequently observed if the reference potential surface and the QM/MM surface are too different.[10, 12]

Similar to the approaches developed by Warshel et al., Wood and co-workers use FEP in their ab initio/classical free energy perturbation approach (ABC-FEP).[24-29] Gibbs free energies differences between a classical interaction potential and a QM solute-solvent interaction are calculated by FEP. The approach is used to calculate hydration free energies of Na^+ and Cl^-,[26] water,[27-29] water dimer dissociation kinetics,[24] and solute-solvent structural properties.[25]

Rod and Ryde have developed a method which they call quantum mechanical thermodynamic cycle perturbation (QTCP).[1, 16] They optimized a reaction pathway using QM/MM and selected a number of configurations for the QM region along the reaction pathway. Based on calculated point charges for the QM region, they performed classical MD sampling and calculated classical MM-QM interaction free energy changes between subsequent fixed QM configurations along the reaction pathway. Similar to the approach by Warshel et al. (see above), they then calculated the MM $\rightarrow$ QM free energy change for each QM configuration along the reaction pathway, and, in this way, a high-level QM/MM PMF was obtained. With this approach, a converged PMF for the methyl transfer reaction in catechol *O*-methyltransferase (COMT) was obtained.[1, 16] As the QM region was fixed in the implementation of the QTCP method, well converged free energy barriers were observed.[1] The approach was compared to the QM/MM-FE approach developed by Yang et al.,[31-33] which also performed well for the problem studied.[1] The latter method was also used to compare different solvent representations (generalized Born molecular volume and TIP3P) for the methyl transfer reaction in COMT and in solution.[15] Recently, the QTCP method was used to study proton transfer at metal sites in proteins[13] and to study the protonation of a histidine ligand in heme peroxidases.[14]

A slightly different approach to the ones explained above makes use of the so-called Metropolis-Hastings algorithm.[51] The idea is to speed up sampling by doing the majority of Monte Carlo simulation steps using a cheap reference potential (e.g. classical MM) and to perform QM or QM/MM calculations on a subset of configurations only. Application of a Metropolis test leads to the acceptance or rejection

of the set of configurations last generated into the rigorous QM/MM ensemble. The method was developed and applied by Schofield and co-workers[20-22] and Bandyopadhyay[23] and is frequently referred to as molecular mechanics based importance function (MMBIF).

Woods, Manby and Mulholland have used a combination of the Metropolis-Hastings[51] method and Warshel cycle method to investigate the relative hydration free energies of water and methane.[2] Their method was also used to check the compatibility of QM methods and MM water models in QM/MM simulations.[48] They supplement a classical thermodynamic cycle approach with additional QM/MM legs, which provide QM/MM correction free energies to the classical perturbation free energy provided by replica exchange thermodynamic integration (RETI).[52, 53] QM/MM correction free energies are calculated by performing RETI with intermediate (λ) states between an MM and a QM/MM representation of the system. The Hamiltonians for intermediate λ states are calculated according to a dual topology approach, i.e. by linearly interpolating between $H_{QM/MM}$ and H_{MM}. QM/MM sampling is done using a classical reference potential, and an ensemble that is correct for the desired QM/MM Hamiltonian (MP2/aug-cc-pVDZ) is achieved by periodically performing QM/MM single point calculations followed by a Metropolis acceptance test, in a fashion similar to the "molecular mechanics based importance function" proposed by Schofield and co-workers.[20-22]

Reddy and co-workers report a promising QM/MM based free energy perturbation method for the calculation of relative solvation free energies and protein-inhibitor binding free energies which treats the solute completely by a QM potential (AM1, HF/3-21G*) and therefore avoids the need for appropriate MM parameters for the solute.[17-19] For a perturbation of molecule A into B, QM forces and energies are computed and scaled linearly according to the λ coordinate for intermediate states.[19]

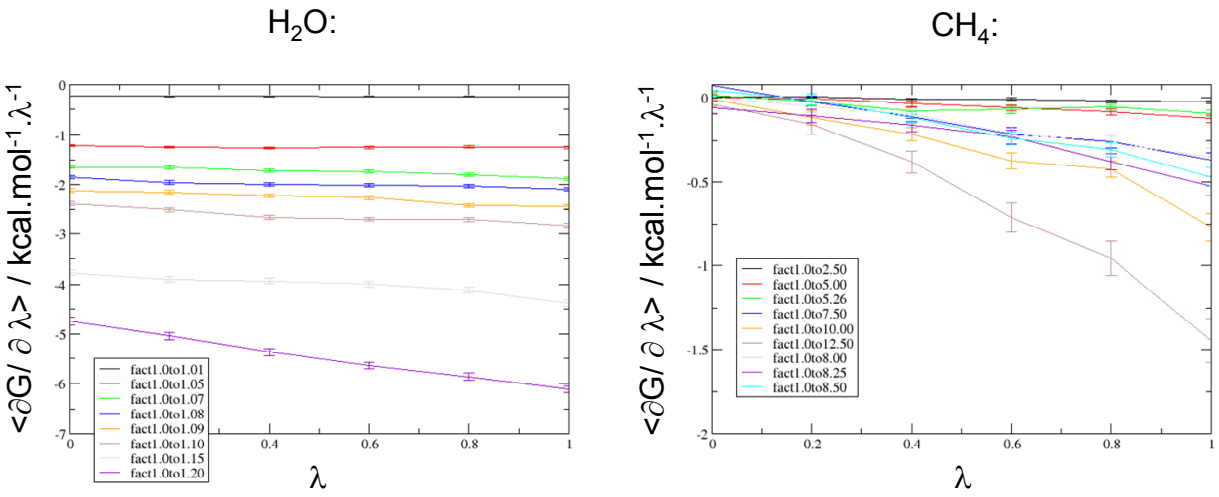

Figure S1. MM gradients for the charge perturbations of water and methane.

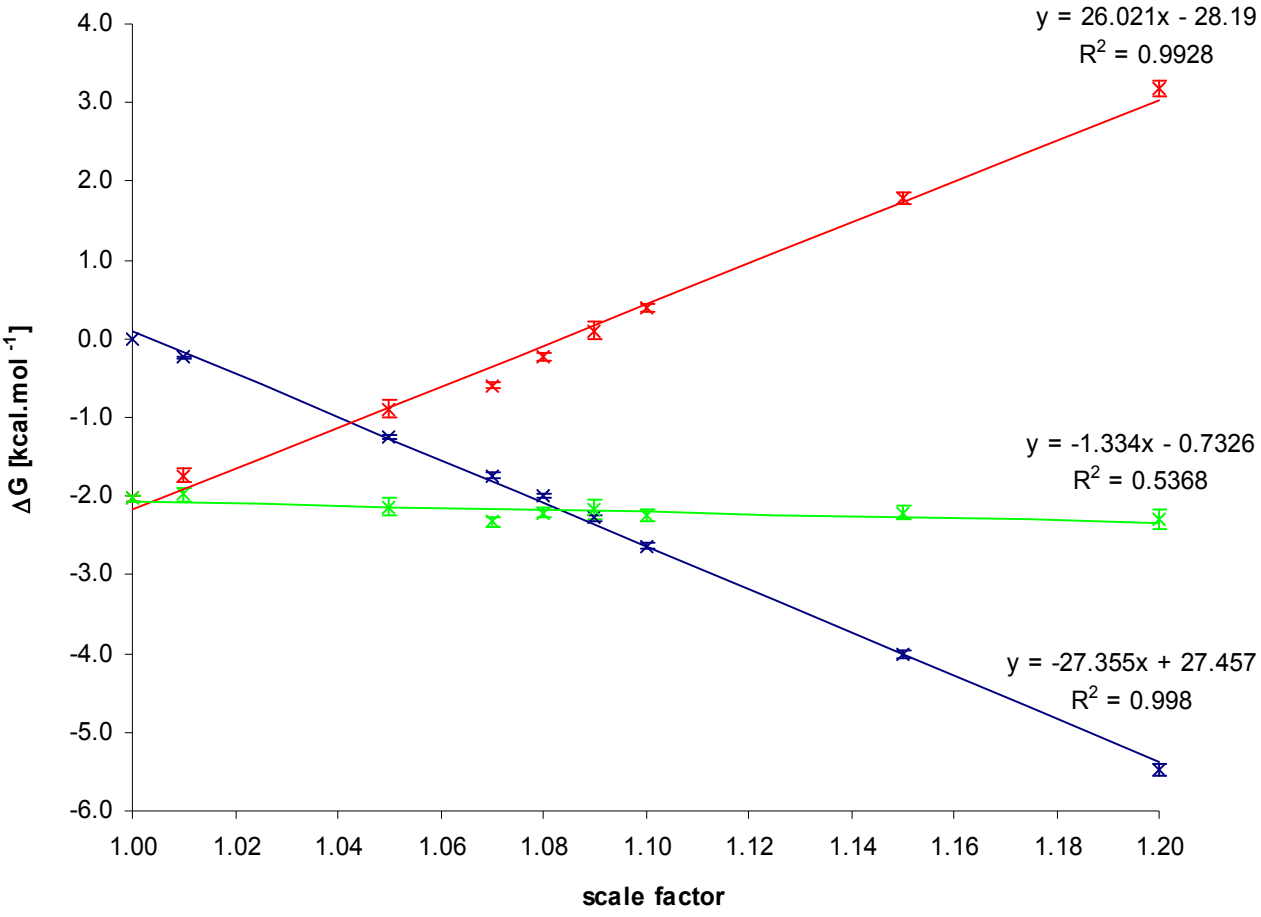

Figure S2. Free energies obtained from charge perturbations for water (B3LYP/6-31G*). Blue: classical RETI energies (ΔG_{32}). Red: QM/MM-FEP energies calculated by Eq. 2 in the main text (ΔG_{33}). Green: Sum of classical and QM/MM-FEP energies (thermodynamic cycle closure). Mean value over all charge scale factors $\langle \Delta G_{32} + \Delta G_{33} \rangle$ = -2.20 ± 0.04 kcal.mol^{-1}; ΔG_3 = -2.03 ± 0.04 kcal.mol^{-1}.

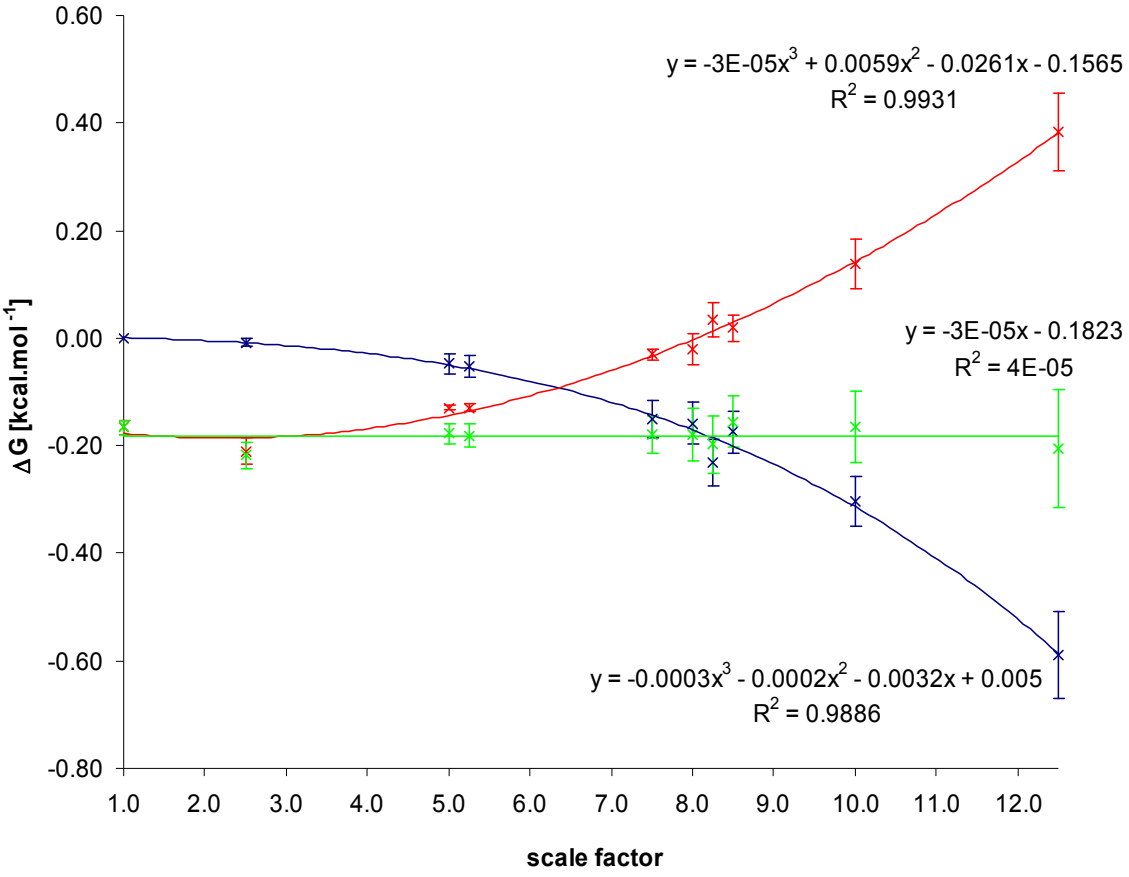

Figure S3. Free energies obtained from charge perturbations for methane (B3LYP/6-31G*). Blue: classical RETI energies (ΔG_{12}). Red: QM/MM-FEP energies calculated by Eq. 2 in the main text (ΔG_{13}). Green: Sum of classical and QM/MM-FEP energies (thermodynamic cycle closure). Mean value over all charge scale factors $\langle \Delta G_{12} + \Delta G_{13} \rangle$ = -0.18 ± 0.01 kcal.mol^{-1}; ΔG_1 = -0.17 ± 0.01 kcal.mol^{-1}.

Table S1. Free energies and standard errors as defined in Scheme 3 (main text) for the system methane-water [kcal.mol^{-1}]. For the alternative pathways ("charge perturbations") $\Delta G_{12} + \Delta G_{13}$ and $\Delta G_{32} + \Delta G_{33}$, mean values of all pathways (charge scale factors) investigated are given.

	Methane		Water	
	$\langle \Delta G_{12} + \Delta G_{13} \rangle$	ΔG_1	$\langle \Delta G_{32} + \Delta G_{33} \rangle$	ΔG_3
B3LYP/cc-pVTZ	-0.22 ± 0.01	-0.20 ± 0.02	-1.58 ± 0.04	-1.51 ± 0.05
B3LYP/6-31G*	-0.18 ± 0.01	-0.17 ± 0.01	-2.20 ± 0.04	-2.03 ± 0.04

Detailed Protocol for the MM Free Energy Calculations (Protein-Ligand System)

In the MM part of the protocol for the investigation of the protein-ligand complex, replica exchange thermodynamic integration (RETI)[52, 53] simulations were performed according to a similar protocol as published previously.[54, 55] For the sake of completeness, only a brief overview will be given here. PDB[56-58]-code 1CX2[59] was used as a starting structure. As discussed previously, [54, 60, 61] the conformation of the sulfonamide moiety in the polar pocket is probably wrong in the X-ray structure. Therefore, the corrected conformation from Refs [54, 60] was used for this study.

Monte Carlo simulations were performed with ProtoMS 2.2[62] The protein was represented by a scoop containing protein residues within a radius of 15 Å from any solute atom (155 residues), which was solvated by a TIP4P[63] watercap of 22 Å radius, centered at the geometric center of the ligand. Water molecules present in the X-ray structure were retained. Protein sidechains within a radius of 10 Å from any solute atom (77 residues) were treated flexibly. The solutes were also treated flexibly, with the exception of rings, which were not sampled. The Amber99[64] force field was used for the protein, Gaff[65] with AM1-BCC[66, 67] charges was used for the ligand. Antechamber from the Amber 9 suite[68] was used for parameter/charge derivation. For non-bonded interactions, a 10 Å cutoff was used, which was "feathered" over the last 0.5 Å. The solvent cap was equilibrated for 20M Monte Carlo moves, after that the whole system was equilibrated for 20M moves. All simulations were performed at 298.15 K. A λ coordinate of 12 λ windows (0.00, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80, 0.90, 0.95, 1.00) was used to allow a smooth transition of ligand **OH** into ligand **CH_3**. A single topology approach with dummy atoms was used; the latter were retracted into the van der Waals radii of neighboring atoms to avoid endpoint singularity problems in the case of vanishing atoms. Each λ window was equilibrated for further 10M moves and data were collected during 30M simulation moves consisting of 300 blocks with 100,000 Monte Carlo moves each, which were then repeated 10 times (10 runs). Two different sampling schemes were compared, see main text for further information.

Table S2. Free energies and standard errors for different pathways as defined in Scheme 7 (main text) for the perturbation of ligand **OH** to **CH_3**, solvated in water ("free"), and bound to Cox-2 ("bound") [kcal.mol^{-1}]. For the alternative pathways ("charge perturbations") $\Delta G_{12} + \Delta G_{13}$, $\Delta G_{32} + \Delta G_{33}$, $\Delta G_{72} + \Delta G_{73}$ and $\Delta G_{52} + \Delta G_{53}$, mean values over all pathways investigated are given. [A]"repeated sampling": 10 independent runs were performed for the non-charge perturbation calculations, 1 run for the charge perturbations; [B]"extended sampling": 10 runs were performed for the non-charge perturbation calculations, each of them using the last configuration of the previous run as starting structure, charge perturbations: 5 runs, each of them using the last configuration of the previous run as starting structure.

		Repeated sampling[A]	Extended sampling[B]
OH free	$\langle \Delta G_{72} + \Delta G_{73} \rangle$	-1.05 ± 0.27	-
	ΔG_7	-1.18 ± 0.27	-
CH_3 free	$\langle \Delta G_{52} + \Delta G_{53} \rangle$	-0.13 ± 0.31	-1.15 ± 0.23
	ΔG_5	-0.71 ± 0.26	-1.04 ± 0.28
OH bound	$\langle \Delta G_{12} + \Delta G_{13} \rangle$	-0.39 ± 0.14	-
	ΔG_1	0.28 ± 0.23	-
CH_3 bound	$\langle \Delta G_{32} + \Delta G_{33} \rangle$	2.23 ± 0.31	1.56 ± 0.40
	ΔG_3	-0.64 ± 0.28	1.02 ± 0.41

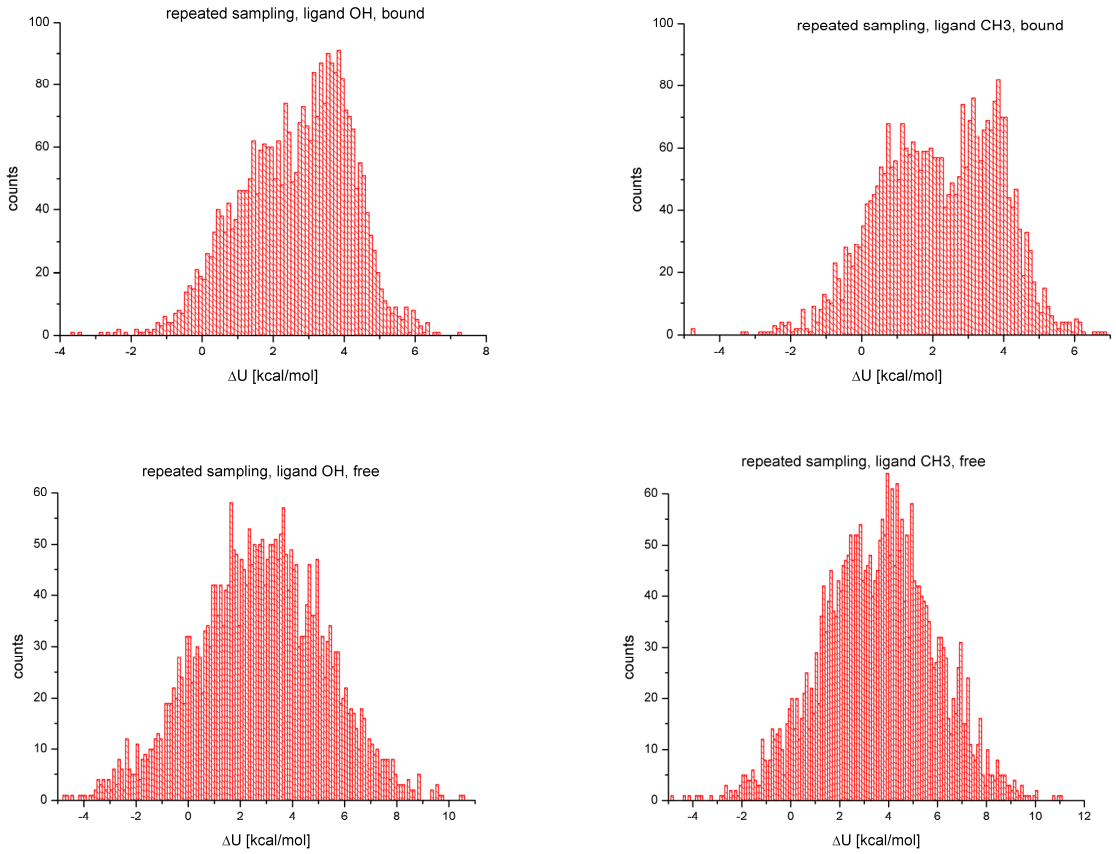

Figure S4. Histograms of the difference of solute-protein/solvent interaction energies in the QM/MM case and in the MM case, as defined in Eq. 8 of the main text ($\Delta U = U_{QM/MM}-U_{QM,vac}-U_{charges,MM}-U_{Coul,solute-solv/protein,MM}$) for the sampling scheme "repeated sampling"(see main text). **Left**: ligand **OH**, **right**: ligand **CH_3**, bound to Cox-2 (**above**), and solvated in water (**below**). Bin size 0.1 $kcal.mol^{-1}$.

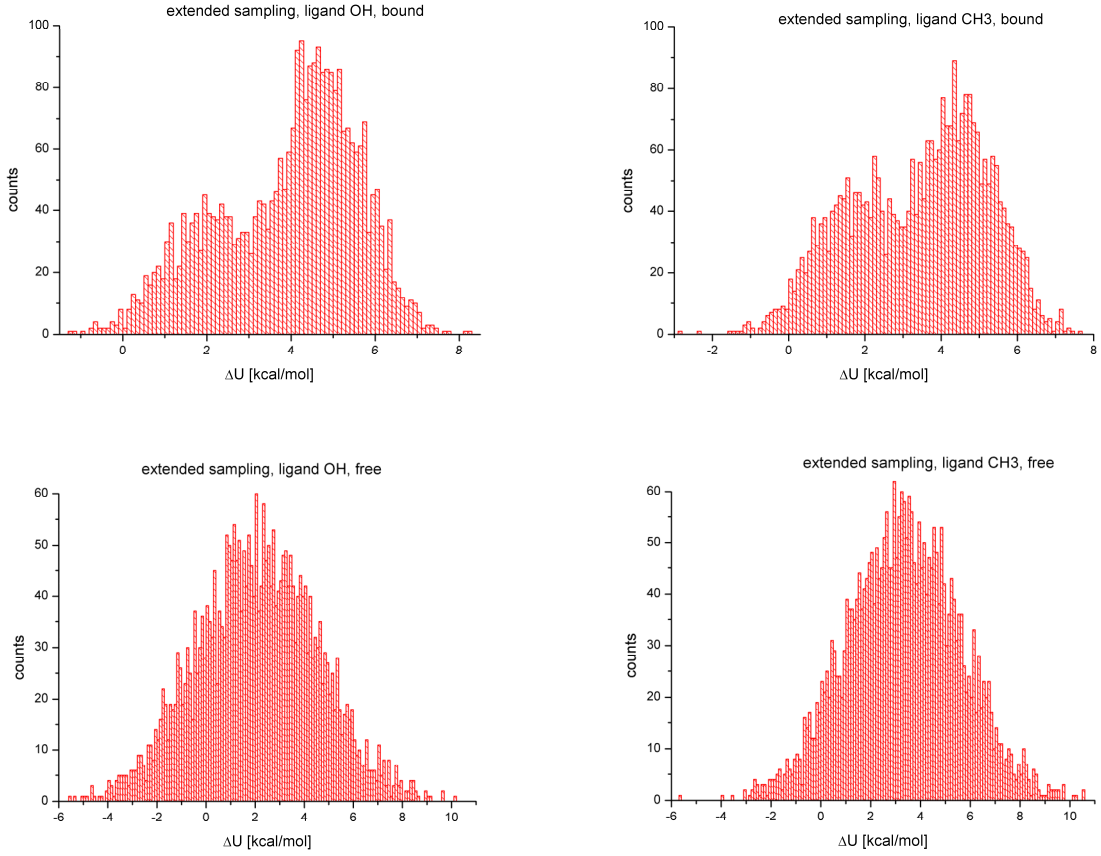

Figure S5. Histograms of the difference of solute-protein/solvent interaction energies in the QM/MM case and in the MM case, as defined in Eq. 8 of the main text ($\Delta U = U_{QM/MM}-U_{QM,vac}-U_{charges,MM}-U_{Coul,solute-solv/protein,MM}$) for the sampling scheme "extended sampling"(see main text). **Left**: ligand **OH**, **right**: ligand **CH_3**, bound to Cox-2 (**above**), and solvated in water (**below**). Bin size 0.1 kcal.mol^{-1}.

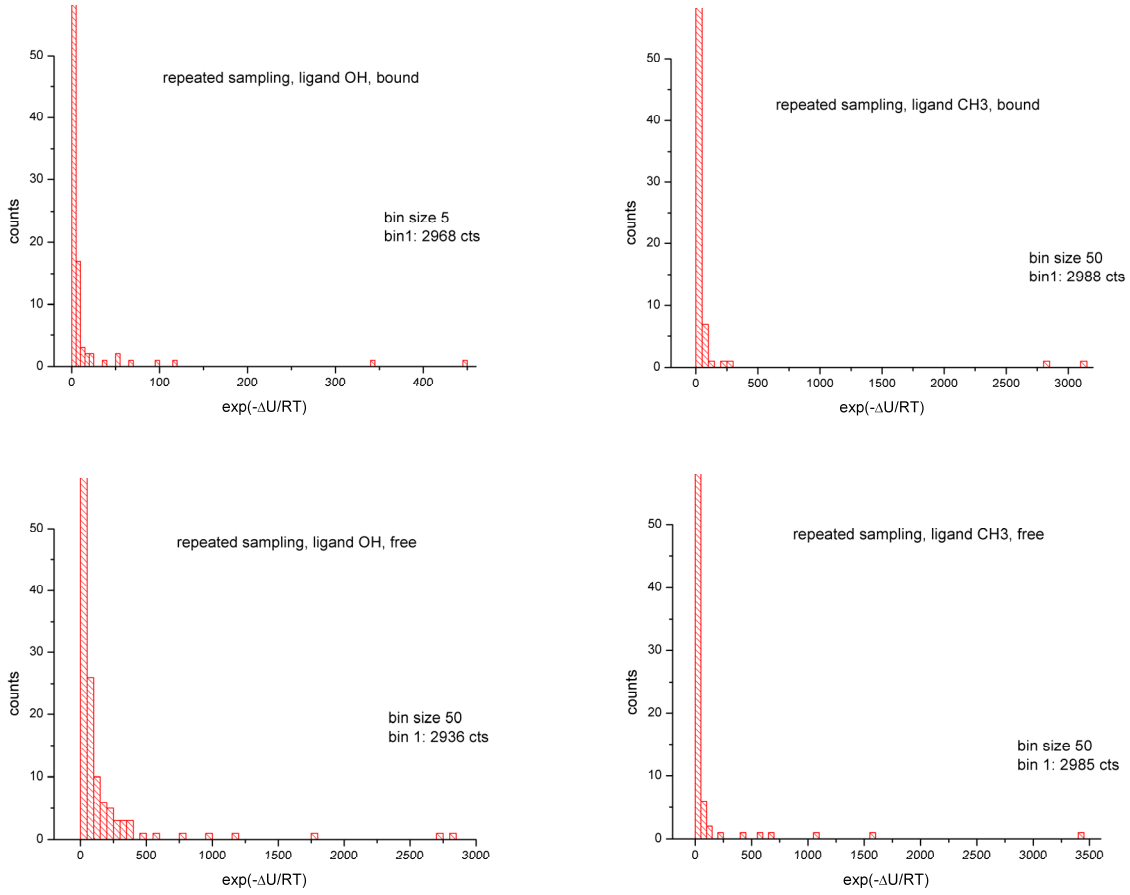

Figure S6. Histograms of the exponential term in Eq. 8 of the main text (exp[-($U_{QM/MM}$-$U_{QM,vac}$-$U_{charges,MM}$-$U_{Coul,solute-solv/protein,MM}$)/RT]) for the sampling scheme "repeated sampling" (see main text). **Left**: ligand **OH**, **right**: ligand **CH_3**, bound to Cox-2 (**above**), and solvated in water (**below**). Bin sizes are given for each plot. The first bin, which contains about 3,000 counts, was truncated for clarity (actual population given for each plot).

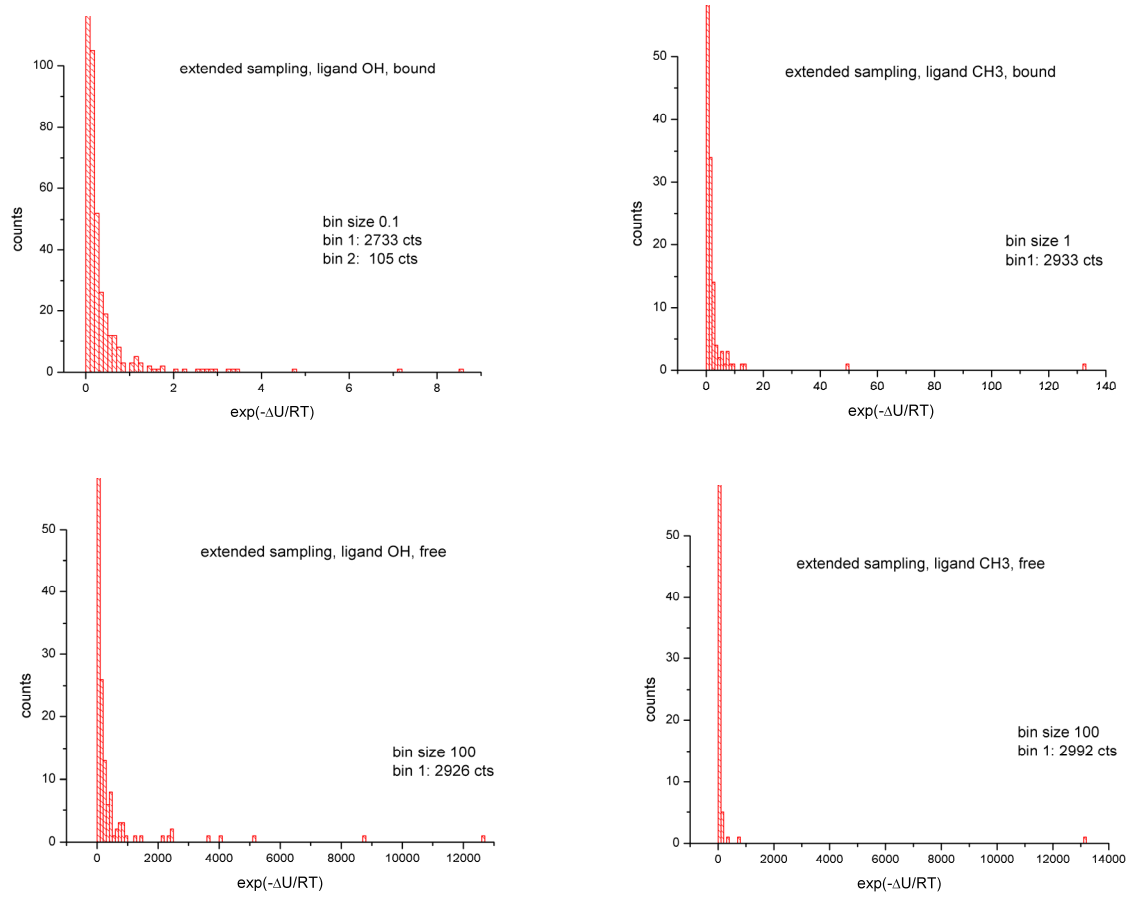

Figure S7. Histograms of the exponential term in Eq. 8 of the main text ($\exp[-(U_{QM/MM}-U_{QM,vac}-U_{charges,MM}-U_{Coul,solute-solv/protein,MM})/RT]$) for the sampling scheme "extended sampling" (see main text). **Left**: ligand **OH**, **right**: ligand **CH_3**, bound to Cox-2 (**above**), and solvated in water (**below**). Bin sizes are given for each plot. The first bin, which contains about 3,000 counts, was truncated for clarity (actual population given for each plot).

References

(1) Rod, T. H.; Ryde, U. *J. Chem. Theory Comput.* **2005,** *1*, 1240-1251.
(2) Woods, C. J.; Manby, F. R.; Mulholland, A. J. *J. Chem. Phys.* **2008,** *128*, 014109.
(3) Kamerlin, S. C. L.; Haranczyk, M.; Warshel, A. *J. Phys. Chem. B* **2009,** *113*, 1253-1272.
(4) Rosta, E.; Haranczyk, M.; Chu, Z. T.; Warshel, A. *J. Phys. Chem. B* **2008**, 5680-5692.
(5) Kamerlin, S. C. L.; Haranczyk, M.; Warshel, A. *ChemPhysChem* **2009,** *10*, 1125 -1134.
(6) Wesolowski, T.; Warshel, A. *J. Phys. Chem.* **1994,** *98*, 5183-5187.
(7) Vaidehi, N.; Wesolowski, T. A.; Warshel, A. *J. Chem. Phys.* **1992,** *97*, 4264-4271.
(8) Muller, R. P.; Warshel, A. *J. Phys. Chem.* **1995,** *99*, 17516-17524.
(9) Olsson, M. H. M.; Hong, G.; Warshel, A. *J. Am. Chem. Soc.* **2003,** *125*, 5025-5039.
(10) Štrajbl, M.; Hong, G.; Warshel, A. *J. Phys. Chem. B* **2002,** *106*, 13333-13343.
(11) Bentzien, J.; Muller, R. P.; Florián, J.; Warshel, A. *J. Phys. Chem. B* **1998,** *102*, 2293-2301.
(12) Rosta, E.; Klähn, M.; Warshel, A. *J. Phys. Chem. B* **2006,** *110*, 2934-2941.
(13) Kaukonen, M.; Söderhjelm, P.; Heimdal, J.; Ryde, U. *J. Chem. Theory Comput.* **2008,** *4*, 985-1001.
(14) Heimdal, J.; Rydberg, P.; Ryde, U. *J. Phys. Chem. B* **2008,** *112*, 2501-2510.
(15) Rod, T. H.; Rydberg, P.; Ryde, U. *J. Chem. Phys.* **2006,** *124*, 174503.
(16) Rod, T. H.; Ryde, U. *Phys. Rev. Lett.* **2005,** *94*, 138302.
(17) Reddy, M. R.; Singh, U. C.; Erion, M. D. *J. Comput. Chem.* **2007,** *28*, 491-494.
(18) Reddy, M. R.; Erion, M. D. *J. Am. Chem. Soc.* **2007,** *129*, 9296-9297.
(19) Reddy, M. R.; Singh, U. C.; Erion, M. D. *J. Am. Chem. Soc.* **2004,** *126*, 6224-6225.
(20) Iftimie, R.; Salahub, D.; Schofield, J. *J. Chem. Phys.* **2003,** *119*, 11285-11297.
(21) Iftimie, R.; Schofield, J. *J. Chem. Phys.* **2001,** *114*, 6763-6773.
(22) Iftimie, R.; Salahub, D.; Wei, D.; Schofield, J. *J. Chem. Phys.* **2000,** *113*, 4852-4862.
(23) Bandyopadhyay, P. *J. Chem. Phys.* **2005,** *122*, 091102.
(24) Ming, Y.; Lai, G.; Tong, C.; Wood, R. H.; Doren, D. J. *J. Chem. Phys.* **2004,** *121*, 773-777.
(25) Wood, R. H.; Liu, W.; Doren, D. J. *J. Phys. Chem. A* **2002,** *106*, 6689-6693.
(26) Liu, W.; Sakane, S.; Wood, R. H.; Doren, D. J. *J. Phys. Chem. A* **2002,** *106*, 1409-1418.
(27) Sakane, S.; Liu, W.; Doren, D. J.; Shock, E. L.; Wood, R. H. *Geochim. Cosmochim. Acta* **2001,** *65*, 4067-4075.
(28) Sakane, S.; Yezdimer, E. M.; Liu, W.; Barriocanal, J. A.; Doren, D. J.; Wood, R. H. *J. Chem. Phys.* **2000,** *113*, 2583-2593.
(29) Wood, R. H.; Yezdimer, E. M.; Sakane, S.; Barriocanal, J. A.; Doren, D. J. *J. Chem. Phys.* **1999,** *110*, 1329-1337.
(30) Hu, H.; Yang, W. *J. Chem. Phys.* **2005,** *123*, 041102.
(31) Cisneros, G. A.; Liu, H.; Zhang, Y.; Yang, W. *J. Am. Chem. Soc.* **2003,** *125*, 10384-10393.
(32) Zhang, Y.; Liu, H.; Yang, W. *J. Chem. Phys.* **2000,** *Vol. 112*, 3483-3492.
(33) Liu, H.; Zhang, Y.; Yang, W. *J. Am. Chem. Soc.* **2000,** *122*, 6560-6570.
(34) Kästner, J.; Senn, H. M.; Thiel, S.; Otte, N.; Thiel, W. *J. Chem. Theory Comput.* **2006,** *2*, 452-461.
(35) Benighaus, T.; Thiel, W. *J. Chem. Theory Comput.* **2009,** *5*, 3114-3128.
(36) Kollman, P. A.; Kuhn, B.; Donini, O.; Perakyla, M.; Stanton, R.; Bakowies, D. *Acc. Chem. Res.* **2001,** *34*, 72-79.
(37) Kuhn, B.; Kollman, P. A. *J. Am. Chem. Soc.* **2000,** *122*, 2586-2596.
(38) Stanton, R. V.; Perakyla, M.; Bakowies, D.; Kollman, P. A. *J. Am. Chem. Soc.* **1998,** *120*, 3448-3457.

(39) Acevedo, O.; Jorgensen, W. L. *Acc. Chem. Res.* **2010,** *43*, 142-151.
(40) Jorgensen, W. L. *Acc. Chem. Res.* **1989,** *22*, 184-189.
(41) Chandrasekhar, J.; Smith, S. F.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1985,** *107*, 154-163.
(42) Chandrasekhar, J.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1985,** *107*, 2974-2975.
(43) Acevedo, O.; Jorgensen, W. L.; Evanseck, J. D. *J. Chem. Theory Comput.* **2007,** *3*, 132-138.
(44) Kaminski, G. A.; Jorgensen, W. L. *J. Phys. Chem. B* **1998,** *102*, 1787-1796.
(45) Li, G.; Cui, Q. *J. Phys. Chem. B* **2003,** *107*, 14521-14528.
(46) Li, G.; Zhang, X.; Cui, Q. *J. Phys. Chem. B* **2003,** *107*, 8643-8653.
(47) Kaukonen, M.; Söderhjelm, P.; Heimdal, J.; Ryde, U. *J. Phys. Chem. B* **2008,** *112*, 12537-12548.
(48) Shaw, K. E.; Woods, C. J.; Mulholland, A. J. *J. Phys. Chem. Lett.* **2009,** *1*, 219-223.
(49) Klähn, M.; Braun-Sand, S.; Rosta, E.; Warshel, A. *J. Phys. Chem. B* **2005,** *109*, 15645-15650.
(50) Zwanzig, R. W. *J. Chem. Phys.* **1954,** *22*, 1420-1426.
(51) Hastings, W. K. *Biometrika* **1970,** *57*, 97-109.
(52) Woods, C. J.; Essex, J. W.; King, M. A. *J. Phys. Chem. B* **2003,** *107*, 13703-13710.
(53) Woods, C. J.; Essex, J. W.; King, M. A. *J. Phys. Chem. B* **2003,** *107*, 13711-13718.
(54) Michel, J.; Verdonk, M. L.; Essex, J. W. *J. Med. Chem.* **2006,** *49*, 7427-7439.
(55) Michel, J.; Verdonk, M. L.; Essex, J. W. *J. Chem. Theory Comput.* **2007,** *3*, 1645-1655.
(56) Bernstein, F. C.; Koetzle, T. F.; Williams, G. J. B.; Meyer Jr., E. F.; Brice, M. D.; Rodgers, J. R.; Kennard, O.; Shimanouchi, T.; Tasumi, M. *J. Mol. Biol.* **1977,** *112*, 535-542.
(57) Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. *Nucleic Acids Res.* **2000,** *28*, 235-242.
(58) RCSB PDB web page. http://www.pdb.org
(59) Kurumbail, R. G.; Stevens, A. M.; Gierse, J. K.; McDonald, J. J.; Stegeman, R. A.; Pak, J. Y.; Gildehaus, D.; Miyashiro, J. M.; Penning, T. D.; Seibert, K.; Isakson, P. C.; Stallings, W. C. *Nature (London, U. K.)* **1996,** *384*, 644-648.
(60) Michel, J. Ph.D. thesis, University of Southampton, Southampton, UK, 2006.
(61) Plount Price, M. L.; Jorgensen, W. L. *J. Am. Chem. Soc.* **2000,** *122*, 9455-9466.
(62) Woods, C.; Michel, J. *ProtoMS 2.2*, University of Southampton: Southampton, UK, 2002-2007.
(63) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. *J. Chem. Phys.* **1983,** *79*, 926-935.
(64) Wang, J.; Cieplak, P.; Kollman, P. A. *J. Comput. Chem.* **2000,** *21*, 1049-1074.
(65) Wang, J.; Wolf, R. M.; Caldwell, J. W.; Kollman, P. A.; Case, D. A. *J. Comput. Chem.* **2004,** *25*, 1157-1174.
(66) Jakalian, A.; Bush, B. L.; Jack, D. B.; Bayly, C. I. *J. Comput. Chem.* **2000,** *21*, 132-146.
(67) Jakalian, A.; Jack, D. B.; Bayly, C. I. *J. Comput. Chem.* **2002,** *23*, 1623-1641.
(68) Case, D. A.; Darden, T. A.; Cheatham III, T. E.; Simmerling, C. L.; Wang, J.; Duke, R. E.; Luo, R.; Merz, K. M.; Pearlman, D. A.; Crowley, M.; Walker, R. C.; Zhang, W.; Wang, B.; Hayik, S.; Roitberg, A.; Seabra, G.; Wong, K. F.; Paesani, F.; Wu, X.; Brozell, S.; Tsui, V.; Gohlke, H.; Yang, L.; Tan, C.; Mongan, J.; Hornak, V.; Cui, G.; Beroza, P.; Mathews, D. H.; Schafmeister, C.; Ross, W. S.; Kollman, P. A. *AMBER 9*, University of California: San Francisco, 2006.