Supporting Information

The Role of Electrostatics in Enzymes: Do Biomolecular Force Fields Reflect Protein Electric Fields?

Richard T. Bradshaw[1,†§*] Jacek Dziedzic[1, 2,§] Chris-Kriton Skylaris[1] & Jonathan W. Essex[1*]

1. School of Chemistry, University of Southampton, Highfield Campus, Southampton, SO17 1BJ, UK

2. Faculty of Applied Physics and Mathematics, Gdańsk University of Technology, 80-233 Gdańsk, Poland

§ Authors contributed equally

* To whom correspondence should be addressed

† Current address: Department of Chemistry, King's College London, 7 Trinity Street, London, SE1 1DB, UK

* J.W.Essex@soton.ac.uk

* R.T.Bradshaw@soton.ac.uk

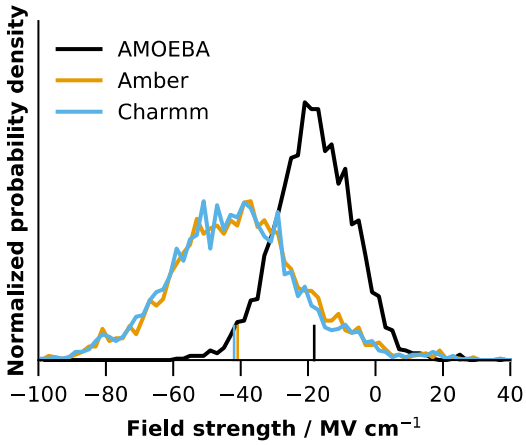

Figure S1 – Electric field strengths perpendicular to the proline ring observed in a 25 ns simulation of the WT, cis-proline CypA complex generated with the Amber ff14SB force field. Field strengths recalculated with AMOEBA (*black*) are smaller and show a narrower distribution than those calculated with either Amber (*orange*) or Charmm (*blue*) force fields. Ticks on the *x*-axis denote the mean of each distribution

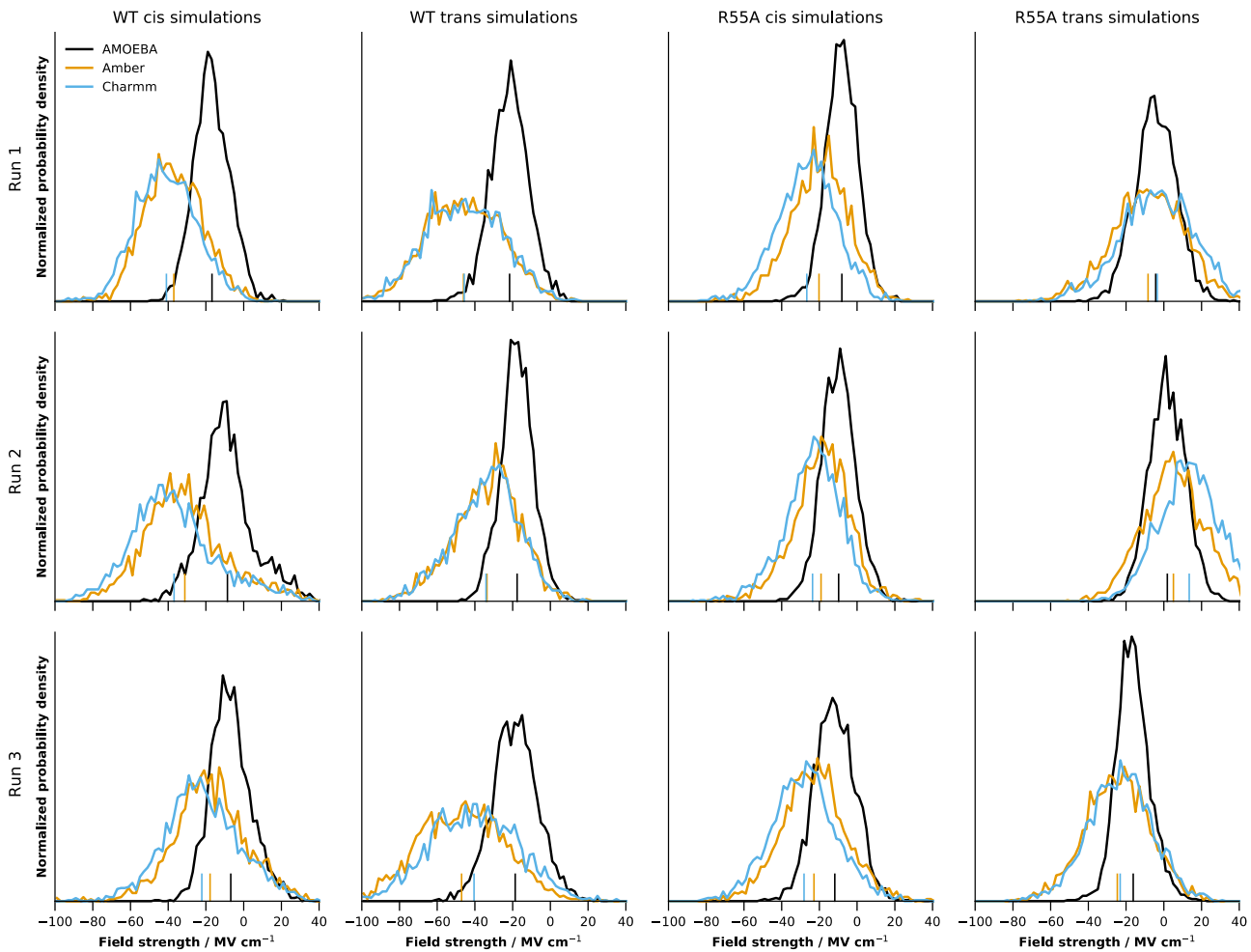

Figure S2 – Electric field strengths perpendicular to the proline ring observed in all CypA simulations generated with the AMOEBA force field. Columns correspond to results observed from alternative CypA systems (wild type (WT) cis-proline, WT trans-proline, R55A cis-proline and R55A trans-proline) and rows correspond to results from three independent simulations. In almost all simulations, fields calculated with AMOEBA (*black*) were smaller and showed a narrower distribution than those calculated with either Amber (*orange*) or Charmm (*blue*) force fields. Ticks on the *x*-axis denote the mean of each distribution

Table S1 – Simulations performed in this study

Force field	CypA system	Proline isomer	Initial PDB	Substrate sequence	Simulation length / ns	Simulation replicates
AMOEBApro13	WT	Cis	1M9Y	AAAPIA	25	3
AMOEBApro13	WT	Trans	1M9C	HAGPIA	25	3
AMOEBApro13	R55A	Cis	1M9Y	AAAPIA	25	3
AMOEBApro13	R55A	Trans	1M9C	HAGPIA	25	3
Amber ff14SB	WT	Cis	1M9Y	AAAPIA	25	1

Table S2 – Mean fields projected perpendicular to the proline ring, and uncertainties, observed in all CypA simulations generated with the AMOEBA force field. Fields were calculated at the proline N atom, the C atom of the preceding peptide, and at the bond midpoint, taken as the linear average of the field strength at the two atoms. Fields for each simulation frame were calculated separately with the AMOEBA, Amber and Charmm force fields. Uncertainties were estimated as the standard error in the mean of 2500 simulation frames.

		WT cis			**WT trans**			**R55A cis**			**R55A trans**		
Run	Atom	AMOEBA	Amber	Charmm	AMOEBA	Amber	Charmm	AMOEBA	Amber	Charmm	AMOEBA	Amber	Charmm
1	N	-22.2 ± 0.2	-44.4 ± 0.3	-49.2 ± 0.3	-25.8 ± 0.2	-53.0 ± 0.4	-54.5 ± 0.4	-15.6 ± 0.1	-27.2 ± 0.3	-34.6 ± 0.3	-12.7 ± 0.2	-18.6 ± 0.4	-14.1 ± 0.4
	C	-11.4 ± 0.3	-29.7 ± 0.4	-32.8 ± 0.4	-17.4 ± 0.3	-39.1 ± 0.4	-36.8 ± 0.4	-0.5 ± 0.2	-13.3 ± 0.3	-18.8 ± 0.3	4.1 ± 0.3	1.8 ± 0.4	7.1 ± 0.4
	Midpoint	-16.8 ± 0.2	-37.0 ± 0.3	-41.0 ± 0.3	-21.6 ± 0.2	-46.0 ± 0.4	-45.7 ± 0.4	-8.1 ± 0.2	-20.2 ± 0.3	-26.7 ± 0.3	-4.3 ± 0.2	-8.4 ± 0.4	-3.5 ± 0.4
2	N	-19.9 ± 0.2	-44.6 ± 0.4	-51.5 ± 0.4	-22.1 ± 0.1	-40.4 ± 0.3	-42.9 ± 0.3	-14.6 ± 0.1	-25.5 ± 0.3	-30.8 ± 0.3	-9.2 ± 0.1	-4.1 ± 0.3	2.8 ± 0.3
	C	2.7 ± 0.4	-17.9 ± 0.5	-22.3 ± 0.5	-13.2 ± 0.2	-26.9 ± 0.4	-25.4 ± 0.4	-4.9 ± 0.3	-12.7 ± 0.3	-16.5 ± 0.3	13.0 ± 0.3	14.3 ± 0.4	24.1 ± 0.4
	Midpoint	-8.6 ± 0.3	-31.2 ± 0.4	-36.9 ± 0.5	-17.6 ± 0.2	-33.6 ± 0.3	-34.2 ± 0.3	-9.8 ± 0.2	-19.1 ± 0.3	-23.6 ± 0.3	1.9 ± 0.2	5.1 ± 0.3	13.5 ± 0.3
3	N	-13.7 ± 0.2	-25.5 ± 0.3	-30.8 ± 0.4	-21.1 ± 0.2	-50.2 ± 0.4	-43.5 ± 0.4	-20.3 ± 0.2	-33.2 ± 0.3	-39.1 ± 0.3	-19.3 ± 0.2	-29.9 ± 0.3	-30.4 ± 0.3
	C	0.0 ± 0.3	-10.2 ± 0.4	-13.6 ± 0.4	-16.2 ± 0.3	-43.9 ± 0.5	-37.4 ± 0.5	-3.3 ± 0.3	-12.5 ± 0.4	-17.1 ± 0.4	-13.1 ± 0.3	-19.5 ± 0.4	-15.8 ± 0.4
	Midpoint	-6.8 ± 0.2	-17.8 ± 0.4	-22.2 ± 0.4	-18.7 ± 0.2	-47.1 ± 0.5	-40.5 ± 0.5	-11.8 ± 0.2	-22.8 ± 0.3	-28.1 ± 0.4	-16.2 ± 0.2	-24.7 ± 0.3	-23.1 ± 0.3

All values in MV cm^{-1}

Movie S1 – Electric fields (*indigo-red-yellow*) and vectors perpendicular to the proline ring (*blue*) at the proline N atom in the first WT cis-proline CypA simulation. Vectors for each simulation frame are shown as points on a sphere of unit radius, with the N atom positioned at the origin, and after superimposition of all frames based upon protein backbone RMSD

Movie S2 – Electric fields (*indigo-red-yellow*) and vectors perpendicular to the proline ring (*blue*) at the alanine C atom in the first WT cis-proline CypA simulation. Vectors for each simulation frame are shown as points on a sphere of unit radius, with the C atom positioned at the origin, and after superimposition of all frames based upon protein backbone RMSD