Electronic Supplementary Material (ESI) for RSC Advances.

Electronic Supplementary Information: Improving the description of interactions between Ca^{2+} and protein carboxylate groups, including γ-carboxyglutamic acid: Revised CHARMM22* parameters

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Gla Force-field Summary

We created our definition of Gla in the CHARMM22* force-field (FF) based on the set-up for Glu. A schematic of the modifications made is provided in Figure S1. To summarize, the parameters for Gla can be viewed as as extension of the parameters used for Glu; in modelling Gla we kept most of the Glu parameters. We deleted one of the two Glu hydrogens attached to C_γ, and added one new carbon atom and two new oxygen atom sites in this location, labelled as C_ε, O_{i1} and O_{i2}. In this process, C_γ changed from a CT2 atom type to a CT1 atom type. The creation of these new sites required a modification of the partial atomic charges in the vicinity of this site, and the addition of new angle and proper dihedral terms.

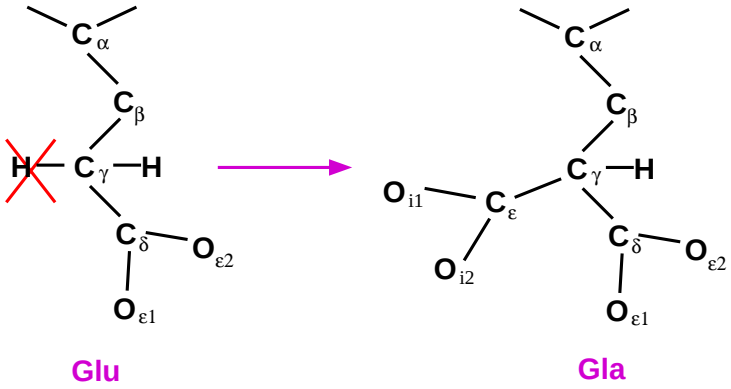

Figure S1: Schematic summarizing the changes made to the residue side-chain in generating a CHARMM22* definition for the Gla residue from the existing Glu residue.

We start with the non-bonded parameters for Gla. All homo-atomic Lennard-Jones (LJ) parameters for the new Gla site (C_ε, O_{i1} and O_{i2}) were taken from pre-existing definitions for Glu (we used the LJ parameters available for C_δ, $O_{\varepsilon 1}$ and $O_{\varepsilon 2}$). A side-by-side comparison of the partial atomic charges for the side-chains of Glu and Gla is presented in Table S1. Charge assignments for atoms on the Gla side-chain were re-purposed from existing partial charges available for the Glu side-chain, where possible. Minor modifications were made to ensure the Gla amino acid carried an overall charge of -2e.

Table S1: Summary of partial atomic charges for Glu and Gla side-chains.

Site Label	Glu	Gla
CB	−0.18	−0.18
HB1	0.09	0.09
HB2	0.09	0.09
CG	−0.28	−0.29
HG1	0.05	0.05
HG2	0.05	—
CD	0.56	0.56
OE1	−0.69	−0.72
OE2	−0.69	−0.72
CE	—	0.56
OI1	—	−0.72
OI2	—	−0.72

Next, we considered new bonded interactions in Gla that were not already present in Glu. Two new types of Urey-Bradley angle term were required to describe the C_δ–C_γ–C_ε angle and the H_γ–C_γ–C_ε angle. For these angles, we re-purposed existing Urey-Bradley angle terms from the CHARMM22* force-field; the values are provided in Table S2. Similarly, a new type of proper dihedral term was needed to describe the C_δ–C_γ–C_ε–$O_{\varepsilon 1/2}$ term. There was no closely-analogous proper dihedral term that we could re-use from the existing CHARMM22* parameter set, since the double-carboxylate motif does not appear anywhere else in the standard set of twenty amino acids. Therefore, we used quantum-chemical calculations to determine a reasonable parametrisation of this proper dihedral term.

Table S2: Summary of new Urey-Bradley angle interaction parameters for the Gla side-chain; θ_0 (degrees), k_θ and k_{UB} (kJ mol^{-1}) and r_{13} (Å).

Site Label	θ_0	k_θ	r_{13}	k_{UB}
C_δ–C_γ–C_ε	108.0	435.136	0.0	0.0
H_γ–C_γ–C_ε	109.5	276.144	2.163	25104.0

Table S3: Summary of new proper dihedral interaction parameter for the Gla side-chain; ϕ_s (degrees), k_ϕ (kJ mol^{-1}) and multiplicity (dimensionless).

Site Label	ϕ_s	k_ϕ	multiplcity
C_δ–C_γ–C_ε–$O_{\varepsilon 1/2}$	75.0	15.0	2

To do this, we constructed an analogue molecule of the Gla side-chain that contains the double-carboxylate motif, as shown in Figure S2a). We then optimised the structure of this molecule at the HF/6-31G* level of theory. The resultant structure suggested the optimal O-C-C-C dihedral angle was at 127.7°. Using this structure as a basis, we then performed a potential energy scan of the O-C-C-C dihedral at the MP2/6-31G* level of theory, in intervals of 15°. Our calculated potential energy profile for this dihedral is provided in Figure S2b). All quantum chemistry calculations were carried out using the GAUSSIAN09 software package.

We then constructed a test molecule for testing the CHARMM22* parameter for the C_δ–C_γ–C_ε–$O_{\varepsilon 1/2}$ dihedral, in order to obtain the best match with our quantum chemical data (shown in Figure S2). Using the TINKER software package, we built a tri-peptide, Gly-Gla-Gly, as shown in Figure S2c). Using the Gromacs 4.5.4 software package, we optimised the structure of this molecule. Using this structure as a basis, we performed a similar potential energy scan, in steps of 15°. The potential energy differences calculated during both scans included contributions arising from the electrostatic and vdW interactions. The final parameter set that best matched the MP2 profile is given in Table S3. The resulting potential energy profile is shown in Figure S2d). This CHARMM22* profile captures the broad features of the MP2 profile, notably the position of the potential energy minimum, and the approximate positions of the maxima. We note that the CHARMM22* maxima are much greater in potential energy than the corresponding MP2

maxima; however, both profiles exhibit maximum peak heights $>>k_BT$ at room temperature. In summary, these Gla parameters provide an acceptable compromise between simplicity and veracity.

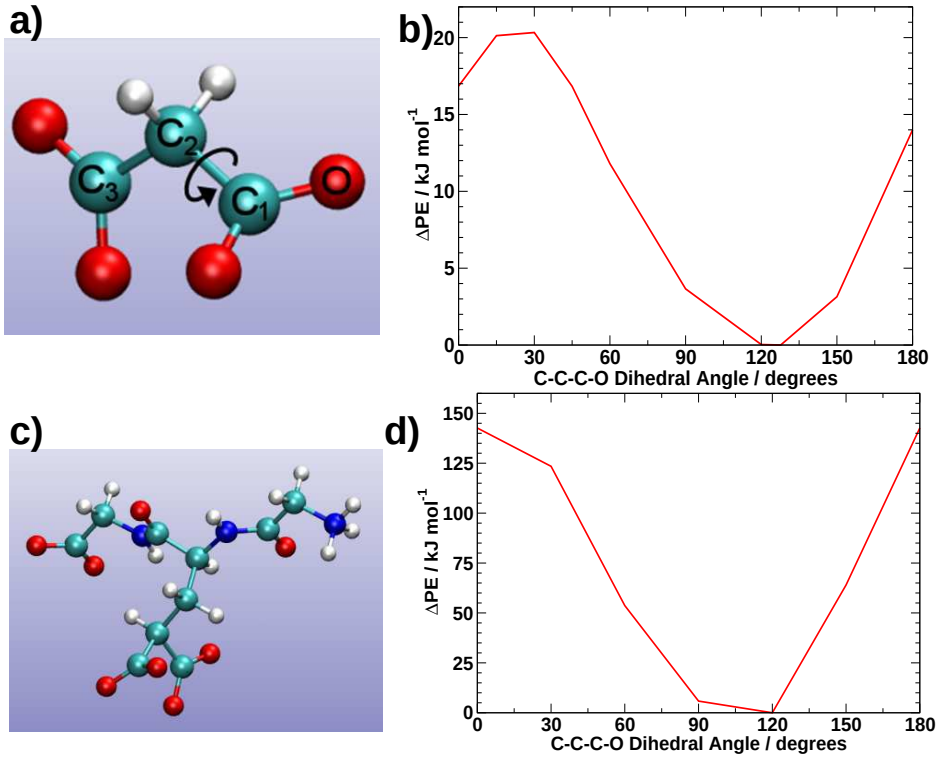

Figure S2: **a)** Structure of the Gla side-chain analogue molecule used in our quantum-chemical calculations; **b)** potential energy as a function of the O-C-C-C dihedral, calculated at the MP2/6-31G* level of theory; **c)** structure of the Gly-Gla-Gly system used to test our CHARMM22* parameter for the C_δ–C_γ–C_ε–$O_{\varepsilon 1/2}$ dihedral term; **d)** potential energy as a function of the C_δ–C_γ–C_ε–$O_{\varepsilon 1/2}$ dihedral of the Gly-Gla-Gly tri-peptide, calculated using the parameters reported in Table S3.

Table S4: Absolute heteroatomic Lennard-Jones σ values for the Ca^{2+}–O_{carb} pair for the unmodified CHARMM22* force-field, plus the six variants studied for the aqueous Glu–Ca^{2+} system.

Version	σ/Å
unmodified	2.7323800
σ + 2.0%	2.7870276
σ + 2.2%	2.7924923
σ + 2.4%	2.7979571
σ + 2.6%	2.8034219
σ + 2.8%	2.8034219
σ + 3.0%	2.8143514

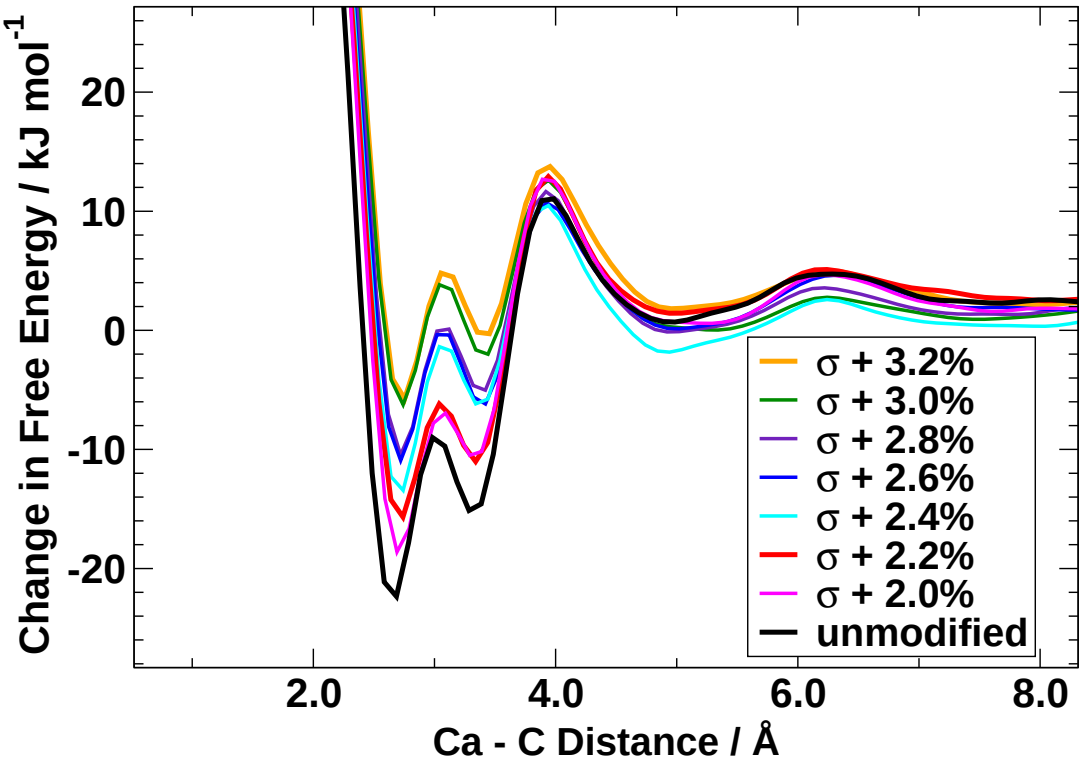

Figure S3: Predicted potential of mean force profiles for the interaction between Ca2+ and the carboxylate side chain of glutamic acid (Glu), for the unmodified force-field and for the six variants considered in this study.

Table S5: Predicted free energy of binding calculated for the aqueous Glu–Ca^{2+} system for the unmodified CHARMM22* force-field, plus the six variants studied in this work.

Version	ΔG_{ads} / kJ mol^{-1}
unmodified	−12.1
σ + 2.0%	−8.3
σ + 2.2%	−6.1
σ + 2.4%	−3.8
σ + 2.6%	−1.7
σ + 2.8%	−1.2
σ + 3.0%	0.87

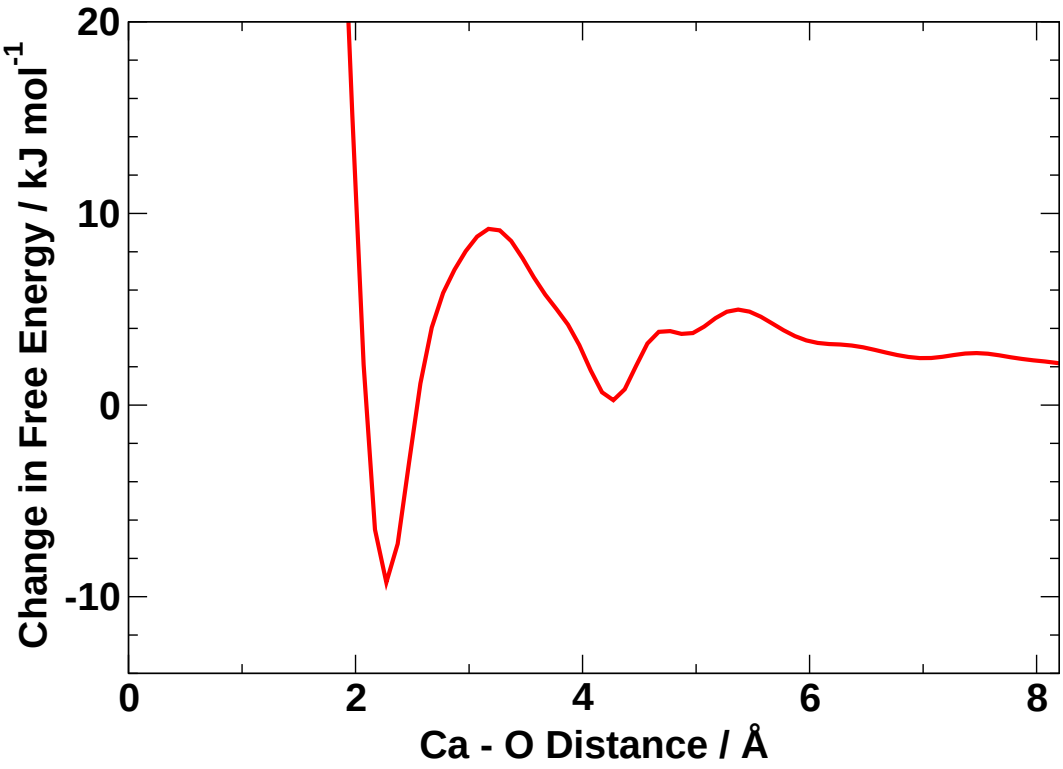

Figure S4: Predicted potential of mean force profile for Glu calculated using a different CV, namely the Ca···carboxylate-oxygen distance, calculated with the default CHARMM parameters.

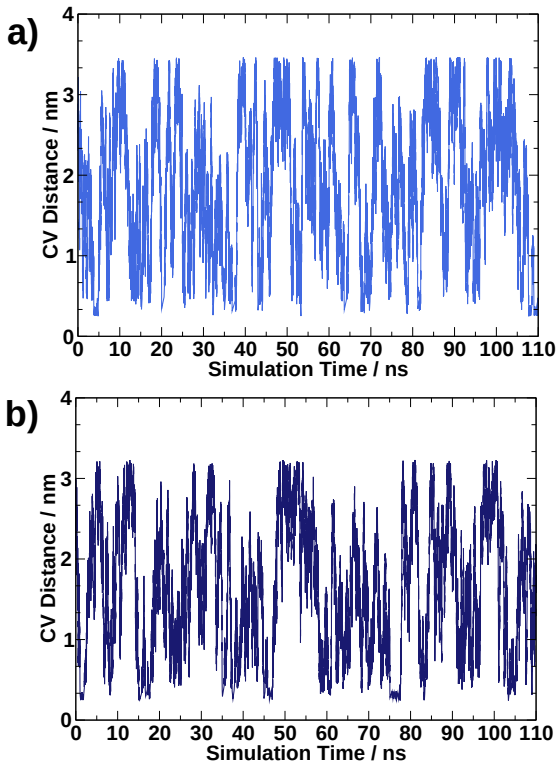

Figure S5: Evolution of the CV with simulation time for the metadynamics simulations of **a)** Glu and **b)** Gla. Both data sets are shown for the $\sigma + 2.2\%$ case.