New phosphorylated amino acid parametrization to correctly reproduce their acid/base equilibria, including in protein binding events.

Nuno F. B. Oliveira, Inês D. S. Pires, and Miguel Machuqueiro

Centro de Química e Bioquímica and BioISI: Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal

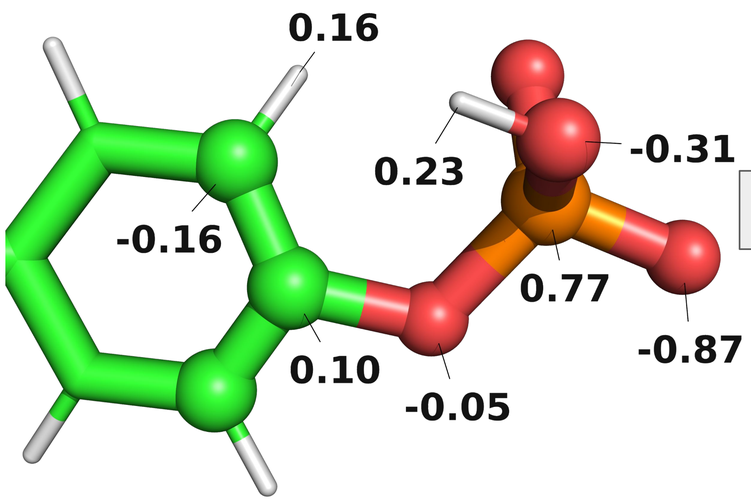
Introduction:

- Aminoacid phosphorylation is an extremely important post-translational modification since it is involved in signaling pathways, enzyme regulation, and many other cell processes.
- The phosphate group is present in many different molecules and environments, such has aminoacids, lipids, nucleotides, and several important co-factors, like ATP.
- The phosphate group in current force-fields have been parameterized mainly for lipids and, sometimes, nucleotides. However, parameters for phosphorylated amino acids, albeit their importance, are still scarce. The accurate description of such charge set will open the possibility of studying phosphorylated proteins in many important cellular processes.
- As test systems, we have used simple peptides and the well-characterized pY1021/PLC-γ1 complex. Many charge sets were tested, namely: a commonly used Hartree-Fock/RESP protocol; from the literature; and our own charge set, which was calibrated using fast Poisson-Boltzmann/Monte Carlo (PB/MC) calculations and manually curated.

Methods:

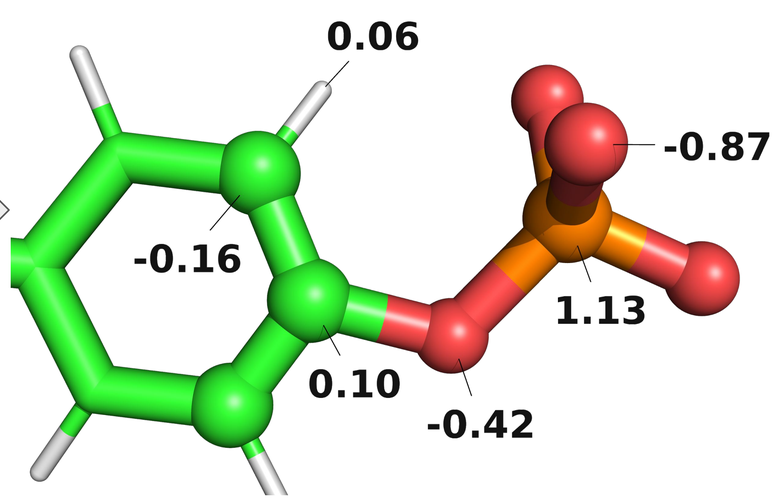
- All simulations were performed using CpHMD[1], with GROMACS v.4.0.7[2] and GROMOS 54A7[3].
- Charge sets were obtained from a commonly used HF/RESP procedure[4], from the literature[5], or manually curated and calibrated using fast rigid body PB/MC calculations.
- CpHMD simulations of Gly-Gly-X-Gly-Gly pentapeptides were used to calibrate the pK_a values of the model compounds against experimental data[6].
- In the pY1021/PLC-γ1 complex simulations, no peptide dissociation from the protein was observed at all pH values.

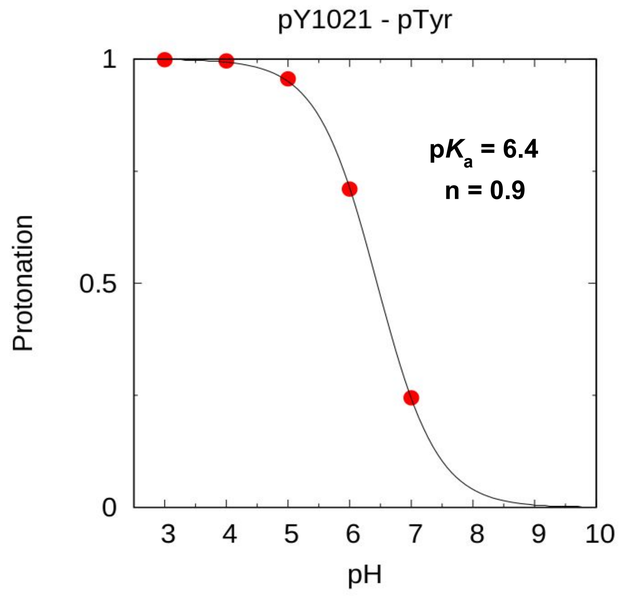
Protonated pTyr Charge set



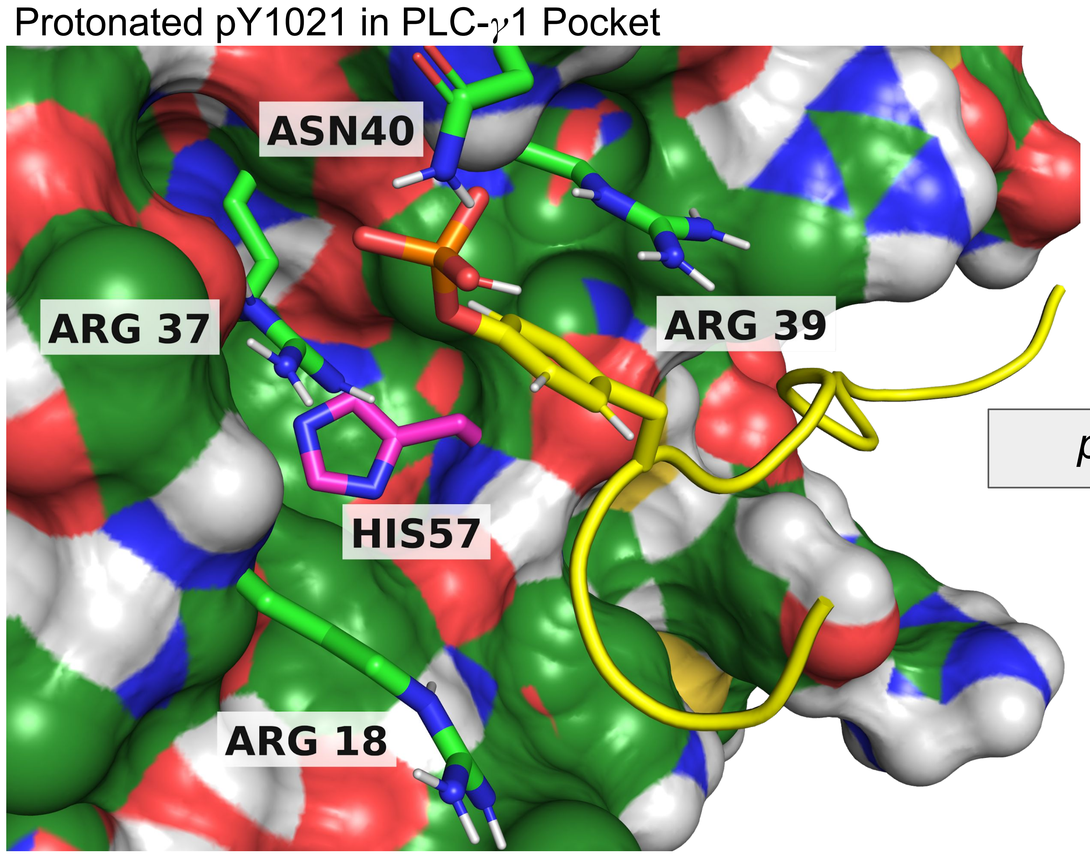
pK_a = 6.1

Deprotonated pTyr Charge set



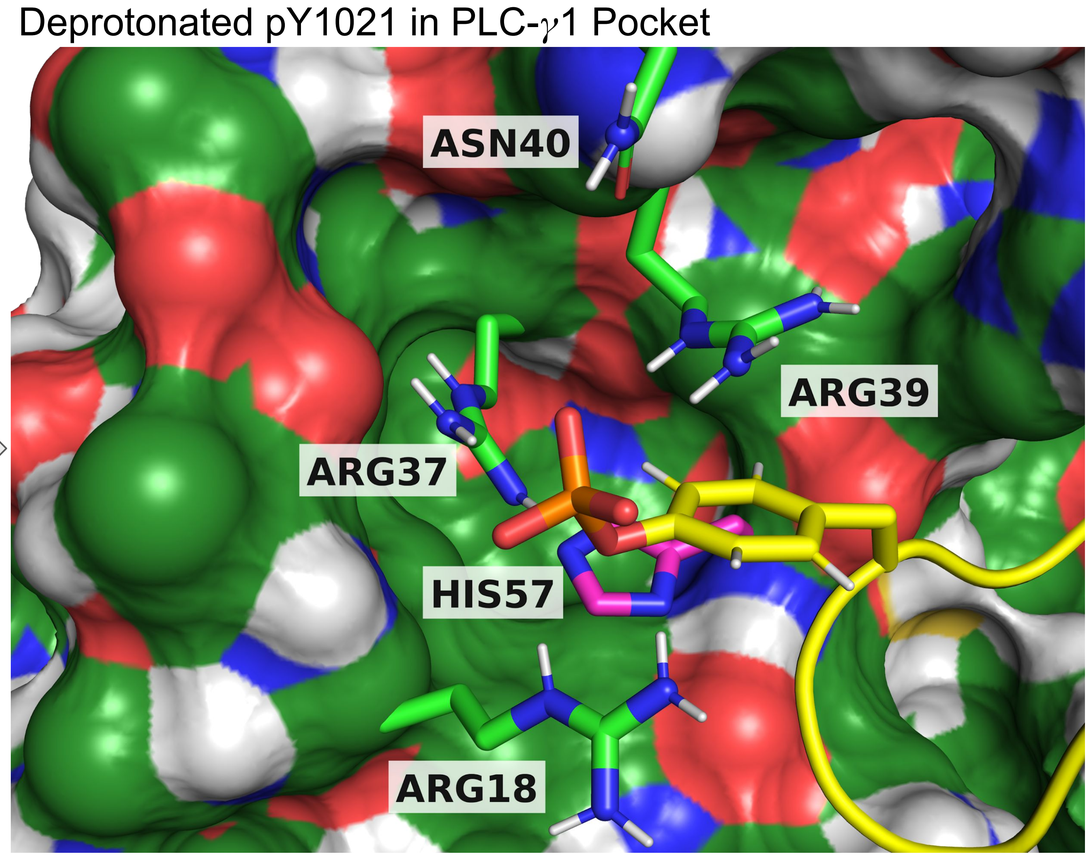


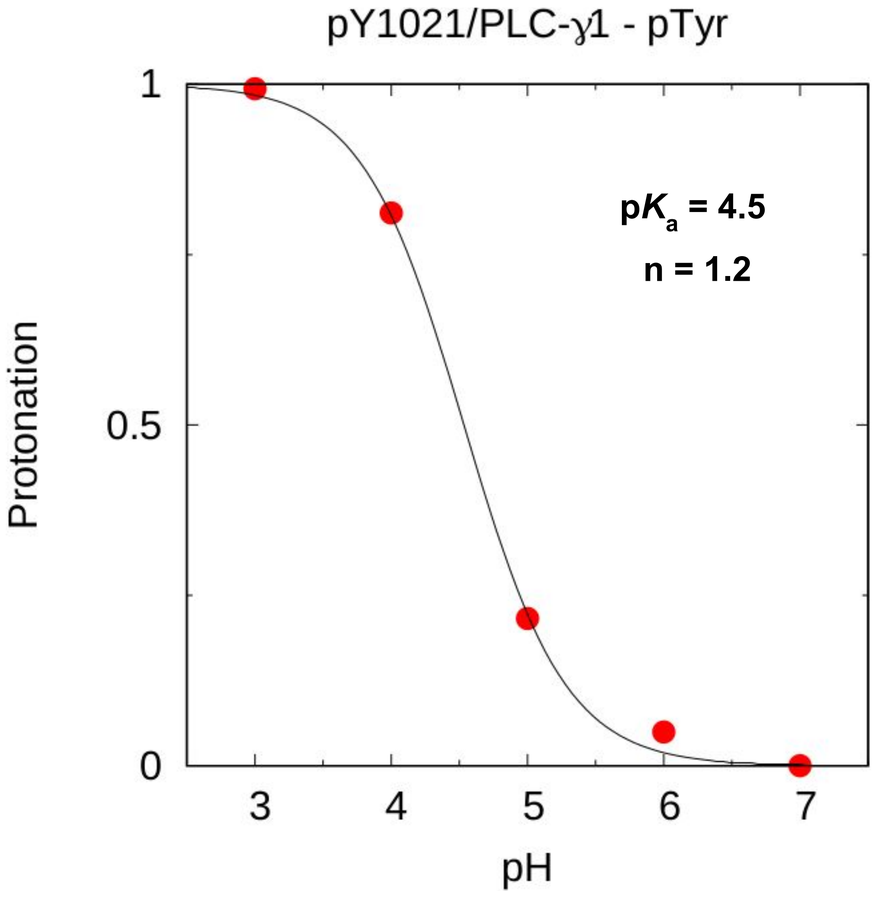
Protonated pY1021 in PLC-γ1 Pocket



pK_a = 4.0

Deprotonated pY1021 in PLC-γ1 Pocket





pK_a values obtained from CpHMD simulations

	GG-X-A			pY1021 (Free)		PLC-γ1	
	pSer	pThr	pTyr	PB/MC	CpHMD	PB/MC	CpHMD
RESP Calculation	6.0	6.4	5.9	6.0	5.6	6.7	6.4
Wojciechowski et al.	--	--	--	5.3	--	5.2	--
Manually Curated	6.1	6.5	6.5	6.0	6.4	3.3	4.5
Experimental	6.1[8]	6.1[8]	5.9[8]	6.1[7]		4.0[7]	

Conclusions:

- Charge sets obtained from QM/RESP calculations, including the ones form literature, did not capture correctly the pTyr pK_a shift in pY1021/PLC-γ1.
- Using PB/MC, to manually curate the charge set, led to an improved pK_a-shift for the phosphorylated tyrosine in the complex.

Future Work:

- Characterize the molecular interactions that are key in stabilizing the charged pTyr.
- Estimate the pH-dependent binding energies of the pY1021/PLC-γ1 system.
- Extend our studies to other systems with phosphorylated residues.

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FCT Fundação para a Ciência e a Tecnologia
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