Accurate and individual side chain titrations curves measured by NMR spectroscopy

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Nuclear Magnetic Resonance (NMR) spectroscopy has the unrivalled potential to quantify electrostatic interactions in proteins experimentally. However, NMR chemical shifts are affected by several sources that need to be disentangled: (i) the protonation state of a residue itself, (ii) charges developing in its direct environment, and (iii) conformational changes. We have made significant progress over the years in developing new NMR methodology that allows unambiguous access to the first term for all amino acids in proteins, using 13C and 15N chemical shifts as strategic reporters [1]. This improved methodology has allowed accurate insights into the microscopic charge states [2,3] and nature of electrostatic coupling in small proteins [4,5]. In addition, we developed an effective approach for modelling electrostatics in unfolded and intrinsically disordered proteins [6], to help in predicting pH-dependent protein stability. In recent work we have also investigated the contribution of electrostatics to backbone amide hydrogen exchange.

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