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# In Silico Investigation of pH-Dependence of Prolactin and Human Growth Hormone Binding to Human Prolactin Receptor

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**Abstract.** Experimental data shows that the binding of human prolactin (hPRL) to human prolactin receptor (hPRLr-ECD) is strongly pH-dependent, while the binding of the same receptor to human growth hormone (hGH) is pH-independent. Here we carry *in silico* analysis of the molecular effects causing such a difference and reveal the role of individual amino acids. It is shown that the computational modeling correctly predicts experimentally determined pKa's of histidine residues in an unbound state in the majority of the cases and the pH-dependence of the binding free energy. Structural analysis carried in conjunction with calculated pH-dependence of the binding revealed that the main reason for pH-dependence of the binding of hPRL-hPRLr-ECD is a number of salt-bridges across the interface of the complex, while no salt-bridges are formed in the hGH-hPRlr-ECD. Specifically, most of the salt-bridges involve histidine residues and this is the reason for the pH-dependence across a physiological range of pH. The analysis not only revealed the molecular mechanism of the pH-dependence of the hPRL-hPRLr-ECD, but also provided critical insight into the underlying physic-chemical mechanism.

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**Key words**: Human prolactin, human prolactin receptor, human growth hormone, pKa calculations, pH-dependence, electrostatics.

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# 1 Introduction

Most biomolecules, including proteins, perform their function by interacting with large or small ligands and ultimately, during this process, undergo conformational and/or ionization changes [1–5]. Virtually every association event is pH-dependent and thus involves proton uptake/release at a particular pH [6–8]. The ionization changes are strongly coupled to the energetics of binding and thus contribute to its specificity [3]. Understanding the details of molecular recognition, which form the foundation of protein-protein interaction networks, requires better understanding of protonation events induced by protein-ligand association and their relation to the characteristic pH of the subcellular compartment where the unliganded proteins exist [3, 6, 9] and binding occurs [7, 8].

The importance of proton uptake/release in receptor-ligand interactions is demonstrated by the experimental observation that practically all known receptor-ligand interactions are pH-dependent [10–14]. Frequently, the variation of several pHs results in binding free energy changes of several kcal/mol [14, 15] or can even change the ligand binding preferences [16]. Moreover, different binding interactions can occur at different pHs; for example, as found in the case of beta-lactoglobulin, which is a dimer at low pH but forms a tetramer at high pH [17]. A similar phenomena was found in the case of calmodulin, whose domains adopt a compact arrangement at low pH while at high pH form a "dumbbell" shaped structure [18–20]. From a practical perspective, the ability to re-engineer enzymatic pH-activity profiles is important for the industrial application of enzymes [21]. This possibility has been theoretically and experimentally explored to re-engineer enzymatic pH-activity profiles and pH-dependence of kinetic parameters by changing active site pKa values using point mutations [22–26].

Frequently, experiments on measuring binding constants are done at a particular pH, which may not correspond to the physiological *in vivo* pH of the corresponding protein-protein complex. Not correcting the experimental value for the difference in pH may lead to serious error of assessing the physiological binding constant. However, if the pH-dependence of proton uptake/release is available, then this correction can easily be made [27]. Even more, one can use the 3D structure of the corresponding protein complex to predict the proton uptake/release [3, 7].

The overall proton uptake/release induced by protein-ligand association originates from individual pKa shifts of titratable groups induced by the complex formation [28–30]. Therefore successful pKa calculations on the pKa's of the titratable groups before and after the binding would be sufficient to determine the proton uptake/release as a function of the pH of the solution and to obtain the pH-dependence of the binding free energy [27,31]. These pKa's can be either experimentally measured or predicted *in silico* and thus the contributions of the individual amino acids to the pH-dependence can be revealed. In reverse, one can find the pH-dependence of the binding, but will not be able to pinpoint the residues contributing to it or predict effects of mutations. In the last case, the experiments on the pH-dependence of the affinity should be complemented with either