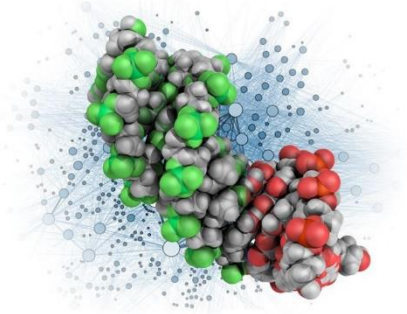

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In silico studies on the pH induced membrane insertion of pHLIP peptides

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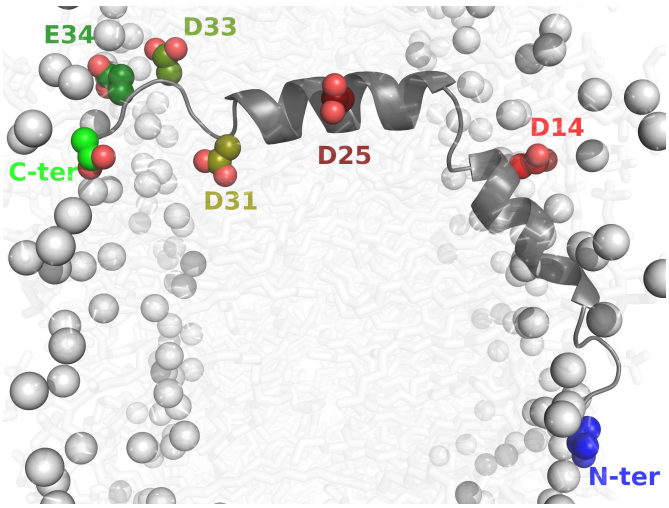
Graphical Abstract *(mandatory)*



Abstract. *(mandatory)*

The pH (low) insertion peptide (pHLIP) belongs to a family of peptides originated from a segment of the transmembranar C helix of bacteriorhodopsin. The peptide has three major states: state I - soluble and unstructured; state II - adsorbed at the membrane surface and unstructured; state III - inserted in the bilayer as an α-helix at low pH values. One of the major applications of pHLIP befalls on its ability to insert into membrane cells with an acidic vicinity, such as tumoral cells, thus working as an efficient tumor-specific biomarker[1]. However, *wt*-pHLIP has a significant limitation, since it accumulates in the kidneys in considerable amounts due to their naturally acidic extracellular pH. This limitation led to a need for increased pHLIP specificity by delimiting the pH range of insertion, further strengthening its application as a biomarker and possible drug-delivery system for inflammatory tissues.

The stochastic titration constant-pH molecular

	dynamics (CpHMD) method has been successfully used to sample protonation behaviour of titrable amino acids inserted in a lipid bilayer, presenting, however, an insufficient amount of data to extensively describe pK_a profiles[2]. The newly developed pH-replica exchange (pHRE) method, allows the exchange of pH values between replicas within a certain probability. This approach enhances the transitions between energy minima, improving the sampling of non-favorable protonation states, which leads to a better description of the pK_a profiles. This new method was applied to simulations of *wt*-pHLIP and L16H variants. The pHRE simulations led to more detailed, accurate and consistent pK_a profiles and allowed the identification of Asp14 as the key residue whose protonation state triggers the insertion process. The calculated insertion pK_a value of this residue is in good agreement with the experimental insertion pK value for the *wt* sequence. Moreover, the simulations of L16H showed that this variant exhibits a second insertion pK_a, at lower pH, indicating that, below this value, the peptide would exit the membrane. These results were corroborated by new experimental data performed by our collaborators, Prof. Oleg Andreev in Rhode Island, USA. We acknowledge financial support from FCT through projects UID/MULTI/00612/2013, PTDC/QEQ-COM/5904/2014 and grant SFRH/BPD/110491/2015.

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