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Identifying Ionizable Residues Involved in the Ph-dependent Switching Mechanism of Myristoylated Hisactophilin

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Hisactophilin is a histidine-rich, myristoylated protein that binds membranes and actin in a pH-dependent manner. Its function involves a reversible switch of its myristoyl (myr) group between two states: sequestered in a hydrophobic pocket or exposed to the solvent. This switching is thought to be regulated by an allosteric mechanism triggered by protonation. Previous studies suggested that histidines in the 88–91 turn, along with aspartic acid D57, are key regulators. Here, we designed several mutants and used NMR and stability analysis to study this mechanism. We found that a major effect on switching occurs only when all histidines in the turn are mutated. Replacing D57 restored pH sensitivity, suggesting a correlation with local net charge. Modifying these ionizable residues generally shifted the pH range for myr exposure rather than abolishing switching, indicating a more global mechanism rather than a single-site trigger. Based on NMR and molecular dynamics data, we propose that local charge repulsion in the surface leads to restructuring within the protein core. This slight reorganization might reduce the probability of the myr group to enter its pocket, favouring the accessible state as a result. Our findings highlight how tuning surface electrostatics can modulate protein core dynamics, which could be essential for potential biotechnological applications.