ATP-Based Probes for Assessment of Bacterial Histidine Kinases Activity

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Two-component systems (TCS) are the main signal transduction pathways in bacteria. They are involved in growth and cell maintenance, but also bacterial virulence, antibiotic resistance and communication, which make the TCSs an ideal target for developing antibacterial drugs. TCSs consist of a response regulator (RR) and a transmembrane protein, a histidine kinase (HK). Once a signal is received by the extracellular domain, it is transmitted to the cytoplasmic domain of the HK, initiating an autophosphorylation event. First, ATP binds to the ATP binding pocket of the catalytic domain (CA), then the γ-phosphate is transferred to the conserved histidine on the dimerization histidine phosphotransfer (DHp) domain. Next, the phosphoryl group is transferred to the RR, which binds to DNA triggering a cellular response. Some events activating the phosphorylation cascade and their TCS target are known such as temperature variation, ion concentration modification, the presence of antibiotics or host immune response to cationic antimicrobial peptides (CAMPs). To efficiently investigate the signaling molecules or environmental factors that stimulate and activate TCSs or alter their function, it is crucial to design universal ATP activity-based probes. The phosphohistidine bond is labile under typical analytical conditions to detect phosphoryl modifications. Changing the γ-phosphate to a thiphosphate or aminophosphate are potential ways to stabilize the labile phosphorus-nitrogen bond on the histidine making the subsequent analysis possible. The γ-phosphate can be further improved by adding an alkyl tail presenting a photoreactive crosslinker to covalently attach the γ-phosphate to the HK and an alkyne tag for visualization with a fluorophore or for performing pull-down assays via biotin labeling. In vivo studies require the design of a bacterial cell permeable probe. Due to its negatively charged phosphates, ATP by itself cannot cross the bacterial cell envelope. An internalizable version of ATP with a polyamine moiety is being evaluated, as well as polyamine-based delivery vehicles.