Chemical Probes for *In Vitro* and *In Vivo* Assessment of Bacterial Histidine Kinase 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, 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 CA domain, then the γ-phosphate is transferred to the conserved histidine. 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. ATP molecules bearing an alkyne tag or biotin are potential probes for labeling studies. Also, modifying the γ-phosphate by a thiophosphate or aminophosphate are potential ways to stabilize the labile phosphorus-nitrogen bond on the histidine and for ease of study. For *in vivo* studies, a bacterial cell permeable probe is needed. 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. ATPγS is recognized as a substrate by HKs and the transferred thiophosphate stabilizes the phosphorus-nitrogen bond on the histidine. Our lab developed the first non-radioactive activity based probe BODIPY-FL-ATPγS (B-ATPγS), which allows for fast and quantitative read out of the autophosphorylation event using fluorescence. Nevertheless, pull-downs were not successful with this probe, leading to the hypothesis that the turnover for B-ATPγS is quite low. To investigate this, kinetics parameters of ATPγS and ATP are being evaluated by using [γ-^{32}P]ATP or [γ-^{35}S]ATP.