Phosphorylation of Peptide Side Chains by DNA Catalysts

Shannon M. Walsh and Scott K. Silverman

Site-specific covalent modifications of proteins are abundant in nature. Protein phosphorylation is a common natural post-translational modification regulated by kinases and phosphatases. Phosphorylation of specific amino acid side chains can have profound effects on protein function, thus making these modifications critical within cellular signaling networks. Due to the challenges of generating homogenously phosphorylated proteins, we are developing DNA catalysts (deoxyribozymes) for the site-specific phosphorylation of proteins. Deoxyribo-zymes have been identified to catalyze many different chemical reactions, and we used in vitro selection to identify the first tyrosine and serine kinase deoxyribozymes. These DNA catalysts transfer the γ-phosphoryl group from either a 5′-triphosphorylated RNA oligonucleotide donor or nucleoside 5′-triphosphate (NTP) donor to a tyrosine or serine hydroxyl acceptor of a DNA-anchored peptide. DNA enzymes have been identified with specificity towards tyrosine or serine side chains. Separately, we found deoxyribozymes that discriminate strongly in favor of phosphorylating tyrosine in the context of specific peptide sequence motifs. With the ability to sequence-specifically phosphorylate peptides, our efforts are now focused on unanchored peptide and protein phosphorylation.