# New technologies of molecular engineering and screening for cell signaling studies

**Yingxiao Wang***
Department of Bioengineering, Institute of Engineering in Medicine, University of California, San Diego, La Jolla, CA 92093

Talks at the "New Technologies and Immuno-Signaling" Minisymposium featured the engineering and development of new technologies for cell signaling studies. Specifically, proteins have been engineered to be controlled by chemical stimulation or photostimulation for the manipulation of signaling transduction in live cells. Experimental and computational approaches have also been combined to systematically identify phosphatase substrates.

**Hui Wang** from Klaus Hahn's lab at the University of North Carolina described a novel and versatile set of new reagents termed LOVTRAP for reversible light-induced dissociation of tightly bound protein pairs. Proteins of interest are fused to a small reagent derived from high-throughput screening that binds to the LOV domain. The protein of interest is reversibly released upon irradiation of LOV, and different versions of the system are used for rapid kinetic control or long-term activation requiring only brief periodic irradiation. LOV was anchored to mitochondria, where it bound and sequestered proteins of interest in the dark. Light caused release of proteins from the mitochondria, leading to action at the plasma membrane or in the nucleus. Wang used LOVTRAP to induce precisely timed oscillations of Rho-family signaling circuits. This light-controlled activation of molecular functions can be used to regulate signaling pathways and control cellular phenotypes in a broad spectrum of biological and biomedical applications. **Patrick O'Neill** from the Gautam lab at the Washington University School of Medicine presented two complementary optogenetic approaches based on the engineering of different photosensitive proteins to reveal the dynamic contributions of G proteins to immune cell migration. The first used light-induced membrane recruitment of an RGS protein to inhibit heterotrimeric G protein signaling, and the second used light-induced membrane recruitment of a guanine nucleotide exchange factor to activate Cdc42. In cells treated with uniform chemoattractant, optically triggered inhibition of endogenous heterotrimeric G proteins created signaling gradients capable of guiding cell motility. In the absence of chemoattractant, subcellular activation of Cdc42 generated a leading edge and a myosin-dependent signaling sufficient to retract the cell rear. The molecular engineering of synthetic proteins integrated with photoactivation can allow the control of Cdc42 activation sufficient to initiate, direct, and reverse cell migration.

**Andrei Karginov** of the Department of Pharmacology at the University of Illinois, Chicago, reported on technologies for interrogation of phosphorylation-mediated signaling. Karginov's lab has developed a new method that enables transient activation of a specific protein kinase in living cells. Activation of a kinase is achieved using a rapamycin-regulated (RapR) method (Karginov *et al.*, 2010) that uses the insertion of an engineered allosteric switch, the iFKBP domain, at a specific site within the catalytic domain of a kinase. Treatment with rapamycin or its nonimmunosuppressive analogues induces interaction between iFKBP and a coexpressed FRB domain, leading to kinase activation. Inactivation of the engineered kinase can be achieved by introducing a previously reported mutation into the catalytic domain of a kinase that makes kinase sensitive to inhibition by an analogue of PP1 compound, 1NA-PP1 (Bishop *et al.*, 1998). Using this strategy, Karginov's lab successfully generated an engineered Src tyrosine kinase, RapR-Src-as2, that can be transiently activated in living cells for a defined period of time. Application of this method revealed that transient activation of Src induces PI3K/Akt signaling that continues after Src inactivation and stimulates cell spreading independently of Src. This method has also been used to regulate protein tyrosine phosphatases. A specific site within the catalytic domain of Shp2 was identified where insertion of iFKBP enables rapamycin-mediated activation of phosphatase. RapR analogues of Shp2, PTP-PEST, and PTP1B were then generated. Analysis of RapR-Shp2 activity in living cells reveals that it can stimulate endogenous Erk1/2 kinases, demonstrating that it functions similarly to wild-type Shp2. Through conjugation of FRB to a selected protein, phosphatase activation was further restricted to a complex with a specific downstream target and/or specific subcellular location. In fact, RapR-Shp2 was engineered in complex with focal adhesion kinase to down-regulate its signaling. These methods successfully provide specific and efficient control of kinase and phosphatase activities in living cells.

**Jagoree Roy** from Martha Cyert's lab in the Department of Biology at Stanford University reported a novel approach combining experimental and computational methods to systematically identify substrate peptide sequences of phosphatase calcineurin (CN). Systems-level analyses of phosphorylation-based signaling networks have transformed the understanding of kinase function, but knowledge of phosphatase signaling is limited. CN is ubiquitously expressed and critically regulates $Ca^{2+}$-dependent processes in the human immune system, heart, and brain (Roy and Cyert, 2009). CN

DOI:10.1091/mbc.E15-11-0762
*Molecular Biology of the Cell* Volume 27 Page 875
*MBoC* is pleased to publish this summary of the Minisymposium "New Technologies and Immuno-Signaling" held at the 2015 ASCB Annual Meeting, San Diego, CA, December 14, 2015.

*Address correspondence to: Yingxiao Wang (yiw015@eng.ucsd.edu).

acts on phosphosites with little primary sequence similarity; thus specificity is not encoded within regions contiguous to the phosphosite. Instead, the enzyme binds to short linear motifs (SLiMs) PxIxIT and LxVP, which can occur hundreds of residues away from dephosphorylation sites (Grigoriu *et al.*, 2013). In fact, SLiMs are a growing class of sequences that localize within intrinsically disordered regions, that is, flexible protein domains that lack a defined structure. SLiMs mediate the majority of protein–protein interactions in cells and evolve rapidly to rewire signaling networks, including that of CN (Goldman *et al.*, 2014). However, degenerate sequences and low-affinity interactions make SLiMs challenging to identify. Roy and Cyert combined experimental and computational approaches to identify CN-binding SLiMs systematically in the human proteome. Structure-based approaches were first used for PxIxIT-site prediction, incorporating the structural features of experimentally verified PxIxIT motifs, including intrinsic disorder, hydrophobicity, secondary structure content, and a newly derived PxIxIT score (PS) reflecting the binding energy of any 6-mer peptide for the PxIxIT docking pocket, as defined by published CN:PxIxIT structures (Li *et al.*, 2007, 2012; Grigoriu *et al.*, 2013). This method successfully identified nine of 12 verified PxIxIT motifs and also predicted new PxIxIT fragments in eight other proteins that were previously identified CN interactors. Two of these novel sequences, found in the CN substrates KSR2 and amphiphysin, were confirmed to bind CN in vitro. This computational strategy has been applied to the entire human proteome to identify the spectrum of human CN-interacting proteins that contain PxIxIT-like sequences. In parallel, proteome peptide phage display (ProP-PD) was used to experimentally select CN-interacting sequences from all predicted disordered regions of the human proteome (Ivarsson *et al.*, 2014). Many of the novel sequences identified by ProP-PD were confirmed to bind CN in vitro. Sequences that were identified both in the Pro-PD screen and by the computational approach constitute strong candidates for new CN substrates. Altogether, 50 novel CN targets, including ion channels, kinases, and receptors, were identified. These methods not only can be used to expand our understanding of phosphatase substrates and signaling networks, but they also can be applied to systematically identify any SLiM-mediated interaction network.

## REFERENCES


Bishop AC, Shah K, Liu Y, Witucki L, Kung C, Shokat KM (1998). Design of allele-specific inhibitors to probe protein kinase signaling. Curr Biol 8, 257–266.

Goldman A, Roy J, Bodenmiller B, Wanka S, Landry CR, Aebersold R, Cyert MS (2014). The calcineurin signaling network evolves via conserved kinase-phosphatase modules that transcend substrate identity. Mol Cell 55, 422–435.

Grigoriu S, Bond R, Cossio P, Chen JA, Ly N, Hummer G, Page R, Cyert MS, Peti W (2013). The molecular mechanism of substrate engagement and immunosuppressant inhibition of calcineurin. PLoS Biol 11, e1001492.

Ivarsson Y, Arnold R, McLaughlin M, Nim S, Joshi R, Ray D, Liu B, Teyra J, Pawson T, Moffat J, *et al.* (2014). Large-scale interaction profiling of PDZ domains through proteomic peptide-phage display using human and viral phage peptidomes. Proc Natl Acad Sci USA 111, 2542–2547.

Karginov AV, Ding F, Kota P, Dokholyan NV, Hahn KM (2010). Engineered allosteric activation of kinases in living cells. Nat Biotechnol 28, 743–747.

Li H, Pink MD, Murphy JG, Stein A, Dell'Acqua ML, Hogan PG (2012). Balanced interactions of calcineurin with AKAP79 regulate Ca2+-calcineurin-NFAT signaling. Nat Struct Mol Biol 19, 337–345.

Li H, Zhang L, Rao A, Harrison SC, Hogan PG (2007). Structure of calcineurin in complex with PVIVIT peptide: portrait of a low-affinity signaling interaction. J Mol Biol 369, 1296–1306.

Roy J, Cyert MS (2009). Cracking the phosphatase code: docking interactions determine substrate specificity. Sci Signal 2, re9.