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Visualizing Activation of Jak/stat Signaling in Live Cell Nanopatterning

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Class I cytokine receptors play a key role in the regulation hematopoiesis and immunity. Downstream signaling is propagated via non-covalently associated Janus kinases (JAKs), which phosphorylate signal transducer and activator of transcription (STAT) and other effector proteins. The activation of different signaling pathways is encoded by the interactions of effector and negative feedback regulators with the intracellular domain of the receptor. While some of these interactions are mediated by well-characterized interactions of SH2 domains with phosphotyrosines, detailed understanding of the cooperative interplay of effector interactions with signaling complexes in the plasma membrane is still lacking. To tackle this challenge, we have developed a novel approach for in situ dimerization and capturing of active cytokine receptor signaling complexes into live-cell nanodot arrays (NDAs). Thus, we achieved assembly of high-contrast NDAs for the model class I cytokine receptor gp130. We demonstrated efficient co-recruitment of JAKs, and confirmed JAK activation by subsequent STAT1/STAT3 recruitment and nuclear translocation. We leveraged this approach to explore activation-dependent binding of diverse effector and regulator proteins, highlighting live-cell NDAs as a powerful tool for studying protein interactions with signaling complexes at the plasma membrane of live cells.