imaging probe accumulation in certain regions relates mainly to target expression and probe-target interaction. However, other factors—such as blood flow, extravascularization of the probes, and interstitial pressure—also need to be taken into account (Niu et al., 2009). Although ^{111}In-bevacizumab uptake in tumors decreased significantly after treatment with sorafenib, based on immunohistochemistry data, VEGF levels did not change accordingly. These results demonstrated that VEGF expression is not the major parameter that determines bevacizumab accumulation. Other factors such as tumor vessel characteristics appear to be more important (Desar et al., 2010). Small molecular peptides with a better tissue penetration will therefore be less affected by these factors than antibodies. However, a nonspecific uptake into tumor regions still exists. Indeed, although the observed increase of tumor uptake of ^{18}F-Z-3B correlated with the measured levels of hVEGF, the slope of the regression line was low, indicating that the imaging quantification still needs to be improved to accurately reflect VEGF level (Fedorova et al., 2011). Thus, along with the development of probes with optimal specificity and affinity, further improvements in sensitivity and spatial/temporal resolution of the used imaging techniques and advanced quantification algorithm and models are required to thoroughly decipher the images.

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Extending Optogenetics to a Ca^{2+}-Selective Channel

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Many cellular processes are regulated by Ca^{2+} signaling. In this issue of *Chemistry & Biology*, Pham et al. have developed a photo-activated protein, LOVS1K, which enables the generation of local or global Ca^{2+} signals through binding to the Ca^{2+}-specific membrane channel Orai.

Ca^{2+} regulates a variety of physiological processes, including secretion, contraction gene transcription, cell proliferation, and migration (Berridge et al., 2003). A primary Ca^{2+} entry pathway in nonexcitable cells is determined by the Ca^{2+} release activated Ca^{2+} channel. Their two limiting molecular components include the Ca^{2+}-sensor protein stromal interaction molecule 1 (STIM1) that is located in the endoplasmic reticulum and the Orai channel in the plasma membrane (Hogan et al., 2010). STIM1 senses the luminal Ca^{2+} content, in that store depletion induces STIM1 oligomerisation into punctate-like structures. This in turn induces the coupling of the cytosolic C-terminal domain of STIM1 with Orai1, thereby triggering channel opening and Ca^{2+} entry (Fahrner et al., 2009).

In the current issue of *Chemistry & Biology*, Pham et al. (2011) present a method that enables control of Ca^{2+} entry through Orai1 channels via a photo-activated STIM1 protein. They utilized the LOV2 domain of phototropin-1 to generate a photo-activated switch (Strickland et al., 2008; Wu et al., 2009) in a fusion protein of LOV2 that is N-terminally linked to a cytosolic fragment of STIM1 (amino acids 233–450), which they termed LOVS1K. Upon a 300 ms exposure to blue light, LOVS1K relocates within seconds from the cytosol onto Orai1. This translocation is reversible within 10–30 s in the dark, leading to LOVS1K dissociation from Orai1 in the plasma membrane, thus allowing for repeated recruitment by subsequent photo-stimulation.

The mechanism of this light-induced LOVS1K relocation, as suggested by the authors in their model, may involve a conformational rearrangement within the LOV2 domain that reduces the sterical hindrance that prevents the interaction of LOVS1K with Orai1 in the dark state. Sterical shielding has recently been also reported (Yu et al., 2011) for residues

fused to the STIM1 C terminus, which somehow interfere with the intramolecular transition that exposes the Orai1 interacting domain within the C terminus of STIM1 (Muik et al., 2011). Whether the LOV2 domain simply behaves in a passive, sterical manner or actively contributes to the intramolecular transition of STIM1 C terminus is not yet clear. Interestingly, potential dimerization of LOV2 has been reported (Nakasone et al., 2008), and the extent of dimerization may increase upon photo-activation, which in turn could contribute to the intramolecular transition coupling STIM1 C terminus to Orai1.

Close inspection of the fluorescence images and colocalization analysis of LOVS1K and Orai1 presented by Pham et al. (2011) reveals only a partial and ~30% increase in colocalization for regions of interest following light exposure. With respect to this moderate degree of colocalization, there is place for improvement, as the additional fluorescent protein labeling at the C terminus of LOVS1K and Orai1 will clearly reduce affinity of the STIM1/Orai1 interaction. Nevertheless, the amount of LOVS1K recruited was sufficient to induce local as well as global Ca^{2+} entry, depending on the number of blue light exposures applied (Pham et al., 2011). Moreover, repeated activations (80 times over 40 min) led to gradual increase in global Ca^{2+} signal.

In conclusion, the probe developed by Pham et al. (2011) represents an important step toward the optogenetics of Ca^{2+} channels, as genetically encoded LOVS1K together with endogenous Orai1 will allow for a selective modulation of local and global Ca^{2+} entry with the use of light. The classical optogenetic tool, channelrhodopsin-1, is of limited value for this purpose, despite the recent development of a modified version that shows a ~2-fold increase in permeability to Ca^{2+} (Kleinlogel, et al., 2011). LOVS1K shall be put to the test initially in nonexcitable cells, such as Jurkat T-lymphocytes and RBL mast cells, as it is expected that LOVS1K-transfected cells will show conditional Ca^{2+} entry through endogenous Orai1 channels upon blue light exposure. In excitable cells, such as neurons and muscle cells, and also possibly in in vivo models, light-mediated control of Ca^{2+} entry will allow for a better understanding of the role of Orai1. Moreover, is tempting to speculate that mutated STIM1 fragments, which interact with Orai1 without activating it, will provide valuable tools to be able to conditionally knockdown Ca^{2+} entry.

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Finding the Missing Code of RNA Recognition by PUF Proteins

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Pumilio and FBF homology (PUF) proteins represent highly promising candidates for engineering sequence-specific RNA recognition, but were only known to recognize G, A, and U, significantly limiting applications. Two groups (Filipovska et al., 2011; Dong et al., 2011) have now reported the discovery of the cytosine-recognition code for PUF proteins.

RNA-binding proteins (RBPs) play essential roles in regulating every step of RNA maturation and function. Typically, they recognize specific RNA structural features and sequences through a small number of very common RNA-binding modules (Lunde et al., 2007) and are often complemented by other enzymatic or structural domains that perform additional functional roles. It would be very useful to engineer RBPs with desired RNA-binding specificities for investigating RNA biology and for potential biomedical applications, but this task has proven to be very challenging (Mackay et al., 2011). The only exception has been the PUF domain, which has been successfully manipulated to target single-stranded RNA in a sequence-specific manner. Thus, this domain represents the most promising candidate for the routine generation of