Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

Guiding Cells with Light and Chemicals: A Toolbox for Dynamic Studies of Cell Migration

Abhijit Deb Roy,[1,2,3] Elmer Rho,[1] and Takanari Inoue[1]

[1]Department of Cell Biology and Center for Cell Dynamics, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA

[2]Center for Cell Analysis and Modeling, University of Connecticut School of Medicine, Farmington, Connecticut 06030, USA

[3]Department of Cell Biology, University of Connecticut School of Medicine, Farmington, Connecticut 06030, USA

Correspondence: abdebroy@uchc.edu; jctinoue@jhmi.edu

Cell migration is a fundamental biological process central to a number of physiological and pathophysiological events. Traditional genetic and pharmacological approaches have identified crucial molecular regulators of migration, yet they often lack the spatial and temporal resolution required to interrogate the highly dynamic signaling events that govern cell locomotion. Chemogenetic and optogenetic platforms—genetically encoded inducible systems activated by chemical or light stimuli, respectively—have emerged as powerful tools for achieving precise, on-demand control over protein function in living cells. These systems enable researchers to dissect molecular signaling pathways in real time and with subcellular precision, even as cells are actively migrating. Together with advances in de novo protein design, biosensors, and live-cell imaging, inducible molecular tools are transforming our ability to manipulate and elucidate the intricate mechanisms underlying cell motility. Looking forward, the application of these technologies in animal models will be crucial for gaining deeper physiological and pathophysiological insights.

Cell motility is one of the most dynamic physiological processes in the body. It allows cells to move from one location to another to carry out essential functions, including the elimination of pathogens and toxic agents, as well as the development of tissues and organs. Its highly dynamic nature has inspired decades of research. These studies have revealed that coordinated signal transduction and cytoskeletal action is critical for guiding cell migration. Effective migration—whether random or directional—depends on establishing and maintaining spatial asymmetry inside cells; this intracellular polarity involves the uneven distribution or activity of molecular components, including signaling molecules, cytoskeletal elements, and switch-like molecules.

Approaches such as ribonucleic acid (RNA) interference, genetic knockouts, and pharmacological perturbations have greatly advanced the identification of molecular players involved in cell migration. Visualization techniques applied

Editors: Michelle Starz-Gaiano and Peter N. Devreotes
Additional Perspectives on Cell Migration available at www.cshperspectives.org

Advanced Online Article. Cite this article as *Cold Spring Harb Perspect Biol* doi: 10.1101/cshperspect.a041754

to these players have further revealed how dynamically they behave inside migrating cells. However, these perturbation methods often lack the spatial and/or temporal precision needed to manipulate the rapid and localized molecular events that are proposed to drive motility.

To address these limitations, researchers have developed custom-designed molecular tools. These tools enable acute and specific manipulation of molecular activities or localizations, providing unprecedented control over cellular behavior. Inducible molecular actuators, in particular, allow rapid initiation or inhibition of signaling events with high temporal resolution. In doing so, they bypass the constraints of conventional genetic approaches. Although transcriptional and translational regulation has been widely and effectively used in migration studies, this review will focus on strategies that operate at the posttranslational level, directly targeting proteins. We will first outline the design principles of these tools and then highlight how they have been applied to uncover molecular mechanisms of cell motility that would otherwise remain intractable.

OVERVIEW OF INDUCIBLE MOLECULAR TOOLS

The ideal tools for dynamic molecular actions inside actively migrating cells require manipulation of target molecules with a temporal and spatial scale similar to what native molecules are regulated with. This means that, often, second timescale and submicron length scale are required. These needs were met by chemically and light-inducible tools, which can trigger intended molecular actions in response to external stimuli such as chemical ligands or light illumination. One of the first inducible chemogenetic systems was developed in the early 1990s, when Spencer et al. (1993) engineered the synthetic bivalent ligand FK1012 to induce oligomerization of chimeric T-cell receptors, thereby triggering their activation. Although not originally applied to cell motility, this system demonstrated the utility of such a synthetic tool in probing spatial-temporal dynamics of signaling events. This laid the groundwork for the field of chemically induced dimerization (CID), which leverages synthetic ligands to bring proteins into close proximity, induces protein–protein interactions, and thereby modulates cellular functions (Stanton et al. 2018). In addition, this early work inspired the development of a series of CID systems that use synthetic ligands, as well as light-inducible systems, to control protein functions and cell behaviors. Many of these inducible tools rely on the following broad strategies (Fig. 1) for their actions: (A) chemically induced heterodimerization, (B) light-induced heterodimerization, (C) homodimerization, (D) homo-oligomerization, (E) protein conformational change exposing a previously cryptic domain, (F) disassembly of a preformed protein complex, and (G) translocation from one intracellular compartment to another. We will discuss some of these inducible tools in the following segment.

CHEMICALLY INDUCIBLE DIMERIZATION (CID) AND MULTIMERIZATION SYSTEMS

One of the first CID systems, and arguably the most widely used, is the rapamycin-inducible dimerization of two protein domains: FK506- and rapamycin-binding protein (FKBP) and FKBP-rapamycin-binding domain (FRB). Upon rapamycin addition, FKBP and FRB rapidly dimerize with nanomolar affinity. Therefore, rapamycin triggers recruitment of proteins of interest (POIs) tagged with a dimerizing protein (FRB) to an intended subcellular location where the dimerization partner (FKBP) is anchored via a targeting sequence (DeRose et al. 2013). This approach has been employed to trigger localized signaling events with high temporal precision. The system's flexibility allows enhanced signaling through tandem repeats of FKBP or FRB domains, which can promote clustering and mimic nucleation-like processes (Inoue et al. 2005; DeRose et al. 2013; Nakamura et al. 2019). A modified FKBP (iFKBP) has been developed in rapamycin-based CID systems to allosterically control protein kinase activation (Karginov et al. 2010). This approach was further refined by development of the tool RapTRAP, which enabled kinase action on a specific substrate (Kar-

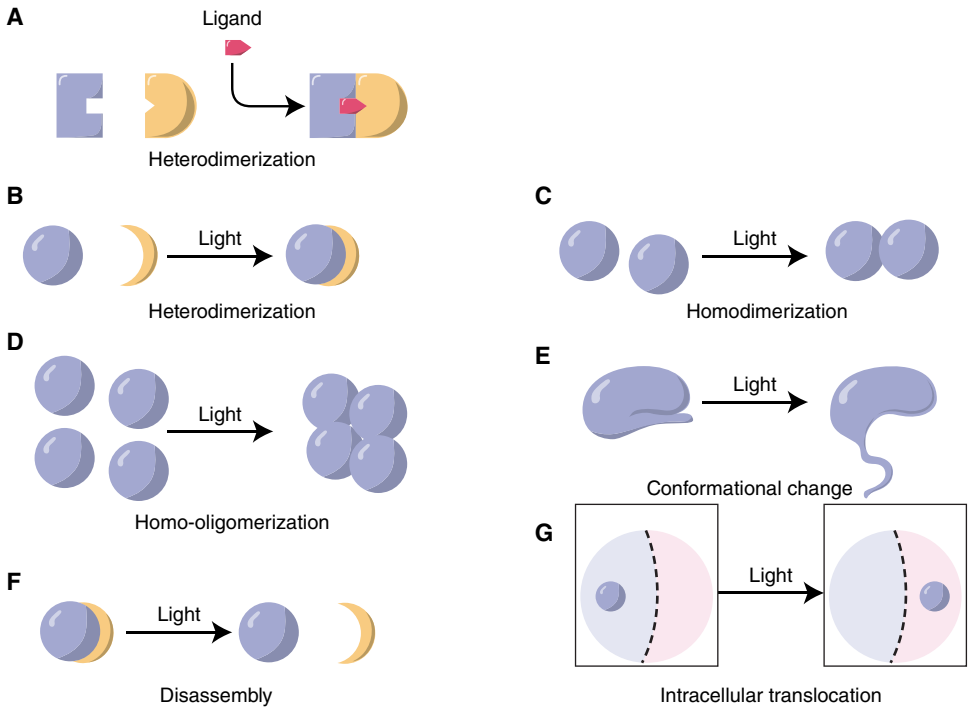

Figure 1. Strategies employed by inducible tools. (*A*) Chemically induced heterodimerization on addition of a synthetic ligand, (*B*) light-induced heterodimerization, (*C*) light-induced homodimerization, (*D*) light-induced homo-oligomerization, (*E*) light-induced conformational change, (*F*) light-induced disassembly, and (*G*) light-induced intracellular translocation.

ginov et al. 2014). Variants of the system, such as split FKBP/FRB constructs, have enabled the interrogation of three-component protein interactions with high temporal resolution (Wu et al. 2020). Additional modifications of the FKBP-FRB dimerization system have enabled inducible higher-order oligomerization of proteins.

In addition to the FKBP-FRB-rapamycin system, additional CID systems have been developed that allow multiplexed, orthogonal stimulations to control many signaling pathways and cell behaviors. Liang et al. (2011) used the plant hormone abscisic acid (ABA) to induce dimerization of the proteins PYR1-like protein 1 (PYL1, ABA receptor) and ABA-insensitive 1 (ABI1, protein phosphatase 2C 56). Miyamoto et al. (2012) used the plant hormone gibberellin to induce dimerization of gibberellin acid-insensitive (GAI) and gibberellin-insensitive dwarf 1 (GID1) proteins. Both systems function independently of rapamycin in mammalian cells, and the gibberellin-based CID was used to develop AND and OR logic gates focused on cytoskeletal dynamics in mammalian cells (Miyamoto et al. 2012).

Chemically inducible systems, broadly, do not easily provide a robust means to activate signaling pathways in a spatially restricted manner. To achieve spatially restricted control of signaling pathways, researchers have applied chemical gradients using microchannels and attained localized release of rapamycin using micropipettes (Lin et al. 2012, 2015; Razavi et al. 2024). A different approach to achieving spatially restricted CID was light-inducible uncaging of rapamycin in a spatially restricted manner. Karginov et al. (2011) modified rapamycin with a bulky acyl group, which sterically blocked its binding to iFKBP and FRB. This steric hindrance was relieved by ultraviolet (UV) light mediated uncaging of rapamycin, leading to dimerization of iFKBP and FRB. Umeda et al. (2011) applied a different strategy, modifying rapamycin with

a bulky biotin–streptavidin conjugate, which blocked its entry into cells. On UV stimulation, rapamycin was released from the biotin–streptavidin conjugate and could freely diffuse into cells and induce FKBP-FRB dimerization.

LIGHT-INDUCIBLE SYSTEMS

More recently, several genetically encoded light-inducible optogenetic systems have been developed, which have enabled researchers to manipulate specific signaling molecules and examine their impact on signaling pathways and cell behavior. Although the first optogenetic tools were developed based on ion channels to control the membrane potential in excitable cells such as neurons, here we will mainly discuss tools that have been used to study cytoskeleton and cell motility. To our knowledge, the first use of optogenetics in cell migration studies used the light, oxygen, voltage-sensing domain 2 (LOV2) from *Avena sativa* phototropin1 protein, which underwent a conformational change on blue-light stimulation. This conformational change was harnessed to control the signaling pathways downstream from small GTPases (GTP hydrolyzing enzymes) in migrating cells (Wu et al. 2009).

The first light-inducible dimerization system was developed by Kennedy et al. using the blue-light-mediated dimerization of the *Arabidopsis thaliana* photoreceptor cryptochrome 2 (Cry2) PHR domain with the cryptochrome-interacting basic helix–loop–helix protein (CIB1) (Kennedy et al. 2010). Based on the observation that Cry2 also underwent homo-oligomerization on blue-light stimulation, mutational screening identified a Cry2-olig variant, which underwent robust homo-oligomerization on blue-light stimulation (Taslimi et al. 2014). The LOV2 from *A. sativa*, discussed earlier, was used to develop a light-inducible dimerization system named improved light-induced dimer (iLID) (Guntas et al. 2015). Briefly, the Jα helix of the LOV2 domain was fused to the bacterial protein SsrA (SsrA tag peptide from bacteria), such that in the dark, SsrA was sterically blocked from interacting with its binding partner: stringent starvation protein B (SspB). Blue-light stimulation relieved this steric hindrance, enabling heterodimerization of SsrA and SspB. Various mutations to modulate the strength of SsrA and SspB binding were further exploited to develop additional iLID variants with different dynamic ranges. The As-LOV2 domain was used to develop additional optogenetic tools, namely, light-inducible nuclear export system (LEXY) and light-inducible nuclear localization signal (LINuS), to respectively control nuclear export and import of proteins (Niopek et al. 2014, 2016). A different approach employing an engineered PDZ (PSD-95, Dlg1, and ZO-1) domain fused to the LOV2 Jα helix was used to develop a different dimerization system called tunable light-controlled interacting protein (TULIP) tags (Strickland et al. 2012). In contrast to light-inducible protein–protein interaction, the Zdk-LOV2 system was developed where the Zdk-LOV2 exists as a heterodimer in the dark and is unbound on blue-light stimulation. This light-inducible dissociation was further made use of to develop the LOVTRAP module to control protein activity (Wang et al. 2016).

In addition to these plant photoreceptor-based tools, fungal photoreceptors have also been used to develop optogenetic tools. The fungal photoreceptor vivid (VVD) from *Neurospora crassa* undergoes homodimerization on blue-light stimulation (Nihongaki et al. 2014). VVD was engineered to introduce positive or negative charges on the dimerization surface to create a heterodimerization system named Magnets (Kawano et al. 2015). LOV4 photoreceptor domain from *Botrytis cinerea* (BcLov4) was engineered to undergo cytoplasm to plasma membrane translocation on blue-light stimulation without a membrane tag as well as undergo oligomerization (Glantz et al. 2018). Unlike the previously described systems, BcLOV4 did not require an additional membrane target for translocation. The original BcLOV4 was further modified to abrogate the membrane translocation, while maintaining its light-inducible clustering in cytoplasm (Huang Dennis et al. 2024).

The optogenetic systems described thus far require blue-light stimulation. Levskaya et al. (2009) developed a red-light-inducible dimerization system using the phytochrome B (PhyB) and

phytochrome interaction factor 3 (PIF3). Zhou et al. (2022) developed a red-light-inducible dimerization system called red/far-red-light-mediated and miniaturized Δphytochrome A–based photoswitch system (REDMAP) based on the plant photoreceptor phytochrome A (PhyA) and far-red elongated hypocotyl 1 (FHY1). Both the PhyB-PIF3 and the PhyA-FHY1 dimers could be dissociated by stimulating with far-red light. A far-red-light-stimulated dimerization system was also developed using the bacterial bathy phytochrome from *Rhodopseudomonas palustris* (RpBphP1) (Kaberniuk et al. 2016) and its natural binding partner RpPpsR2 (photosynthesis gene expression repressor from *R. palustris*), as well as an engineered binding partner quantum perception and signal 1 (QPAS1) (Redchuk et al. 2018). These red- and far-red light-inducible systems allow for optogenetic stimulation deeper into animal tissue, as well as multiplexing with the blue-light-stimulated optogenetic modules, thus enabling complex manipulations of cell behavior.

There are many more chemically inducible and light-inducible systems that we have not covered here. An exhaustive discussion on all inducible systems is beyond the scope of this paper. We have listed some of them in Tables 1 and 2.

APPLICATION OF INDUCIBLE SYSTEMS TO STUDY CELL MOTILITY

Directional cell migration requires precise spatial and temporal coordination of dynamic remodeling across multiple cytoskeletal systems—including actin, microtubules, and the plasma membrane—to sustain persistent front–back polarity. This polarity emerges through localized regulation of the biochemical composition, stability, and biophysical properties of these cytoskeletal components by a remarkably large network of signaling pathways and molecules, including ligand–receptor complexes, intracellular enzymes, and adaptor proteins (some of which are illustrated in Fig. 2).

Chemical and light-inducible systems have been implemented in cell migration and cytoskeleton studies for more than 30 years. These approaches enable manipulation of protein localization and activation with spatial and temporal control, which is particularly attractive in studying a highly dynamic and spatially regulated phenomenon such as cell motility. Many such tools have been developed and used to study the role of various proteins in cytoskeletal dynamics and cell motility. We will discuss some of these examples below.

INDUCIBLE SYSTEMS TARGETING THE ACTIN CYTOSKELETON AND SMALL GTPASES

The actin cytoskeleton is the fundamental regulator of eukaryotic cell motility, and the majority of cell migratory behavior is accomplished through a myriad of actin-based cellular processes, including lamellipodia, filopodia, and invadopodia. The actin cytoskeleton also engages with integrin-based adhesions to facilitate cell attachment to their extracellular matrix and with myosin motor proteins to control cell contractility. Small GTPases are critical regulators of cell motility through remodeling of the actin cytoskeleton. Small GTPases are often considered molecular switches which exist in an inactive guanosine diphosphate (GDP)-bound or an active guanosine triphosphate (GTP)-bound form. The activation of small GTPases is facilitated by a family of enzymes called guanine nucleotide exchange factors (GEFs), whereas the inactivation is mediated by another family of enzymes named GTPase-activating proteins (GAPs). In particular, Ras-related C3 botulinum toxin substrate 1 (Rac1), transforming protein RhoA, and cell division control protein 42 homolog (Cdc42) small GTPases have been extensively studied for their roles in actin dynamics, cell morphology, and motility. Holsinger et al. (1995) developed one of the very first inducible systems by using FKBP12 and FK1012 to recruit the Ras GEF Son of Sevenless (Sos) to the plasma membrane and examine the downstream signaling pathways. The first application of inducible systems to study actin dynamics employed the FKBP–FRB dimerization system to recruit a constitutively active Cdc42 mutant to the plasma membrane. The constitutively active Cdc42

Table 1. Summary of commonly used chemically inducible dimerization (CID) systems

System (ligand)	Protein domains	Key features	Common applications
Rapamycin (Inoue et al. 2005)	FKBP and FRB	High affinity (nM), rapid, modular, widely used	Signal transduction, cytoskeletal dynamics, chemotaxis
ABA (Liang et al. 2011)	PYL1 and ABI1	Orthogonal in mammalian cells, reversible	Synthetic signaling circuits, cytoskeletal control
GA_3 (Miyamoto et al. 2012)	GID1 and GAI	Orthogonal in mammalian cells, fast dimerization	Inducible subcellular targeting
Auxin (indole-3-acetic-acid) (Nishimura et al. 2009)	TIR1 and AID	Promotes degradation	Conditional and reversible protein depletion
Shield-1 (Banaszynski et al. 2006)	FKBP-derived destabilizing domain	Ligand stabilizes otherwise unstable proteins	Conditional protein expression and activation
TMP (Iwamoto et al. 2010)	DHFR	Small molecule–regulated stabilization	Transient modulation of protein levels
Methotrexate (Volpato et al. 2007, 2009; Fossati et al. 2008)	DHFR variants	Specific and tunable binding	Chemical control of protein–protein interactions
SNAP/HaloTag (synthetic ligands) (Zimmermann et al. 2014)	SNAP-tag, HaloTag, CLIP	Covalent, photocleavable to achieve reversibility	Protein labeling, subcellular localization, synthetic scaffolds

(ABA) Abscisic acid, (TMP) trimethoprim, (GA_3) gibberellin, (FKBP) FK506- and rapamycin-binding protein, (FRB) FKBP–rapamycin binding domain, (PYL1) PYR1-like protein 1, (ABI1) ABA-insensitive 1, (TIR1) transport inhibitor response 1, (AID) auxin-inducible degron, (DHFR) dihydrofolate reductase.

was targeted to the intracellular domain of the cluster of differentiation 25 (Cd25) receptor (interleukin-2 receptor α chain), which was clustered with antibody-coated beads to trigger localized actin polymerization and filopodia formation (Castellano et al. 1999). They further used this approach to recruit constitutively active Rac1 to demonstrate a role of localized actin polymerization in phagocytosis (Castellano et al. 2000).

Inoue et al. (2005) developed a significantly simplified and robust approach to control small GTPase function by recruiting constitutively active or dominant-negative mutants of Rac1, Rac2, RhoA, and Cdc42 to the plasma membrane using a Lyn kinase amino-terminal sequence as a plasma membrane targeting signal. This simplified approach of inducibly targeting proteins to specific cellular compartments would inspire many new molecular actuators (Komatsu et al. 2010). Inoue and Meyer (2008) refined this technique by recruiting the GEF domain of T-cell lymphoma invasion and metastasis 1 (Tiam1) to activate endogenous Rac1, leading to robust lamellipodia formation, and implemented this inducible activation of Tiam1 to examine the molecular cross talk between phosphoinositide 3-kinase (PI3K) and Rac1 signaling in neutrophil polarization in what is arguably the first study implementing inducible tools to specifically study cell migration. This was followed by localized photo-uncaging of rapamycin to recruit FKBP–Tiam1 GEF domain within subcellular regions, which was sufficient to induce lamellipodia in a localized manner (Umeda et al. 2011). Implementation of microfluidic channels to generate linear gradients of rapamycin and recruiting the FKBP–Tiam1 GEF domain induced cell polarization and chemotaxis-like directional migrating in the absence of any chemoattractants (Lin et al. 2012). Miao et al. (2019) used plasma membrane recruitment of FKBP–RacGEF to examine wave patterns of molecular signaling pathways that mediate protrusion formation in *Dictyostelium*. Inducible recruitment of GEF or GAP domains to the plasma membrane to control small GTPase function would eventually

Table 2. Summary of commonly used light-inducible (optogenetic) systems

System (protein domains)	Light color	Reversibility	Mechanism of action	Common applications
PA-Rac1, PA-Cdc42 (LOV2) (Wu et al. 2009)	Blue	Reversible	Conformational change	Directional cell motility
CRY2–CIB1 (Kennedy et al. 2010)	Blue	Reversible	Heterodimerization	Subcellular or organelle targeting, transcriptional regulation
Cry2-Olig (Taslimi et al. 2014)	Blue	Slower reversibility compared to CRY2–CIB1	Homo-oligomerization	Protein clustering, receptor tyrosine kinase activation
iLID–SspB (Guntas et al. 2015)	Blue	Fast reversibility	Heterodimerization	Subcellular or organelle targeting
LOVTRAP (LOV2, Zdk) (Wang et al. 2016)	Blue	Reversible	Light-induced dissociation	Sequestration and release of proteins
TULIP (LOV2, ePDZ, ligand) (Strickland et al. 2012)	Blue	Reversible	Heterodimerization	Subcellular or organelle targeting
Magnets (Kawano et al. 2015)	Blue	Reversible	Heterodimerization	Subcellular or organelle targeting
PixD–PixE (Yuan and Bauer 2008)	Blue	Reversible	Oligomerization	Oscillators, synthetic signaling circuits
PhyB–PIF (Levskaya et al. 2009)	Red/ far-red	Reversible with far-red stimulation	Heterodimerization	Subcellular or organelle targeting
BphP1–QPAS1 (Kaberniuk et al. 2016)	Near IR/red	Slow reversibility in dark, fast reversibility with red-light stimulation	Heterodimerization	Subcellular or organelle targeting
UVR8–COP1 (Crefcoeur et al. 2013)	UV-B	Reversible	Dissociation of UVR8 homodimer and heterodimerization with COP1	Subcellular or organelle targeting
LEXY (Niopek et al. 2016)	Blue	Reversible	Nuclear export	Nuclear export
LiNUS (Niopek et al. 2014)	Blue	Reversible	Nuclear import	Nuclear import
BcLOV4 (Glantz et al. 2018)	Blue	Reversible	Oligomerization, membrane association	Receptor tyrosine kinase activation

(CRY2) Cryptochrome 2, (LOV2) light, oxygen, voltage-sensing domain 2, (UV) ultraviolet, (TULIP) tunable light-controlled interacting protein, (PhyB) phytochrome B, (PIF3) phytochrome interaction factor 3, (LEXY) light-inducible nuclear export system, (LINuS) light-inducible nuclear localization signal (LINuS), (iLID) improved light-induced dimer, (SspB) stringent starvation protein B, (PA-Rac1) photoactivatable-Ras-related C3 botulinum toxin substrate 1, (PA-Cdc42) photoactivatable-cell division control protein 42 homolog.

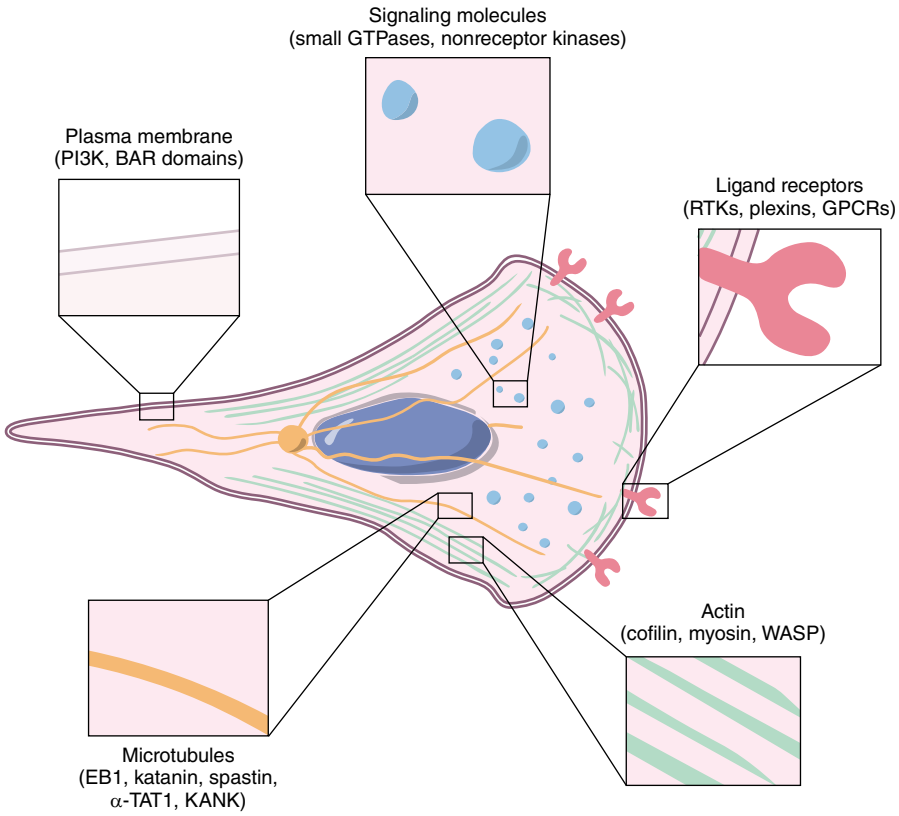

Figure 2. Examples of cellular components targeted by inducible tools to study cell motility.

become a widely used approach to manipulate actin dynamics in living cells.

The first use of optogenetics in cell migration studies was the development of photoactivatable-Rac1 (PA-Rac1) and photoactivatable-Cdc42 (PA-Cdc42). Wu et al. (2009) fused different Rac1 mutants to the LOV2 domain from *A. sativa* phototropin1 protein, which sterically blocked interaction of Rac1 or Cdc42 with its downstream effectors. On blue-light stimulation, the Jα helix of the LOV2 domain underwent a conformational change, relieving the steric hindrance and allowing interactions between Rac1 or Cdc42 and their effectors. These were also the first optogenetic tools engineered to control small GTPase activities. Subcellular stimulation of Rac1 signaling enabled localized lamellipodia formation and directional motility in HeLa cells and demonstrated negative regulation of RhoA activation by Rac1 signaling in a spatially restricted manner (Wu et al. 2009). Wang et al. (2010) applied PA-Rac1 to examine collective migration behavior in border cells in the *Drosophila* ovary. They observed that Rac1 activation or inactivation in one cell in the border cell cluster was sufficient to alter the direction of the entire group, thereby demonstrating a collective directional sensing based on Rac1 activity levels. Yoo et al. (2010) used the PA-Rac1 system to control neutrophil migration in zebrafish embryos.

Previously discussed CID-based approaches had demonstrated that plasma membrane recruitment of constitutively active or dominant-negative small GTPases, or GEF and GAP domains, was sufficient to manipulate specific small GTPase signaling pathways. Taking advantage of this experimental strategy, numerous optogenetic dimerization systems have enabled precise, light-controlled modulation of small

GTPase activity, advancing our understanding of cell motility, contractility, and morphogenesis across diverse cellular contexts. Plasma membrane recruitment of dominant-negative RhoA inhibited actomyosin contractility and when applied to examine mesoderm invagination during *Drosophila* embryogenesis demonstrated a critical role of actomyosin contractility in preventing premature tissue relaxation but not in the actual folding of the mesoderm (Guo et al. 2022). The AsLOV2-based iLID system was used for localized recruitment of Tiam1 or intersectin GEF domain to, respectively, activate Rac1 or Cdc42 and induce localized directional motility in cells to examine differential roles of extracellular matrices in stabilizing protrusions induced by Rac1 or Cdc42, demonstrating that Rac1 required exogenous fibronectin for stable protrusions, whereas Cdc42 did not (Guntas et al. 2015; Zimmerman et al. 2017). Patterned illumination enabled a graduated recruitment of these GEF domains to establish a gradient of Rac1 and Cdc42 activity and demonstrated that a sharp Rac1 activity gradient promotes cell speed, whereas a sharp Cdc42 gradient governs the direction of cell migration (de Beco et al. 2018). The robust and rapid cell morphological changes induced by recruitment of Rac1 or Cdc42 GEFs were used by different groups to characterize new optogenetic modules (Levskaya et al. 2009; Zhou et al. 2012). Plasma membrane recruitment of the GEF domain of PDZ-RhoGEF (Rho guanine nucleotide exchange factor 11) led to RhoA activation in epithelial cells, along with increased intercellular contractility, tissue compaction, and nuclear translocation of yes-associated protein (YAP). In contrast, recruitment of this GEF domain to mitochondria led to an overall decrease in cell contractility (Valon et al. 2017). Plasma membrane recruitment of various RhoA GEF domains demonstrated that the GEF concentration determines the changes in cell morphology: Low concentrations induce retractions, whereas high basal concentrations induce protrusions through sequestration of RhoA by the GEF-PH domain and activation of Cdc42 (Seze et al. 2025). Dagliyan et al. (2016) developed a series of photo-inactivated (PI) small GTPases (PI-Rac1, PI-Cdc42, and PI-RhoA) and PI GEFs (PI-Vav2, PI-Intersectin1, and PI-GEF-H1) using LOV2 insertion into these specific proteins (GEF-H1: Rho guanine nucleotide exchange factor 2; Vav2: guanine nucleotide exchange factor VAV2). Beyond the RhoA family of small GTPases, plasma membrane recruitment of the RasGEF RasGRF1 using the FKBP-FRB system induced membrane ruffling (Komatsu et al. 2010). More recently, Pal et al. (2023b,c) used Cry2-CIB-based optogenetic activation or inhibition of Ras by using the RasGEF, RAS guanyl-releasing protein 4 (RasGRP4), and RasGAP-activating-like protein 1 (RASAL), respectively, in *Dictyostelium* or neutrophils to demonstrate a critical role of localized activation of the Ras-mechanistic target of rapamycin complex 3-protein kinase B (Ras-mTORC3-Akt) signaling pathway in chemotaxis.

Aside from small GTPase-mediated actin remodeling, various groups have used other regulatory proteins with inducible systems to manipulate actin dynamics. Plasma membrane recruitment of Wiskott–Aldrich syndrome protein (WASP) triggered filopodia formation (Castellano et al. 1999), whereas clustering of the *Listeria* actin assembly (ActA)-inducing protein induced actin comet formation (Nakamura et al. 2023). An optogenetic myosin phosphatase based on myosin phosphatase target subunit 1 (MYPT1) inhibited nonmuscle myosin II activity, leading to protrusion formation and reduction in traction forces in migrating cells (Yamamoto et al. 2021). Plasma membrane recruitment of myosin heavy chain kinase C using FKBP-FRB CID inhibited bipolar thick filament assembly of myosin, leading to a rapid increase in Ras activity, revealing an inhibitory role of myosin on Ras signaling pathways (Kuhn et al. 2025). Multiple approaches of optogenetic activation of Cofilin demonstrated that cofilin activation was sufficient to induce actin remodeling and produce protrusions and invadopodia formation in cells (Hughes and Lawrence 2014; Stone et al. 2019).

INDUCIBLE SYSTEMS TARGETING THE PLASMA MEMBRANE

Both the phospholipid composition and the morphology of the plasma membrane have been implicated in initiating various signaling

pathways relevant to cell motility. Manipulation of the phospholipid composition of the plasma membrane using inducible systems has been implemented by many groups to examine the role of phospholipids in cell motility. Plasma membrane recruitment of inositol polyphosphate 5-phosphatase (Inp54p) and PI3K using the FKBP-FRB dimerization system was used to interrogate the role of phospholipids in regulating KCNQ, potassium voltage-gated channel (Suh et al. 2006). The FKBP-FRB dimerization system was used to recruit a type IV 5-phosphatase domain to the plasma membrane to examine how rapid depletion of phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) affected diverse cellular functions (Varnai et al. 2006). Inoue and Meyer (2008) used the FKBP-FRB system to recruit the iSH domain of p85 (phosphatidylinositol 3-kinase regulatory subunit) to the plasma membrane to activate PI3K and demonstrated that uniform activation of PI3K in HL-60 neutrophils is sufficient to induce polarization and motility. Recently, a noncatalytic role of PI3K in mesenchymal cell migration was revealed by plasma membrane recruitment of the p110-inter-proto-oncogene tyrosine-protein kinase Src domain homology 2 (iSH2) domain (p110: phosphatidylinositol 3-kinase catalytic subunit) (Matsubayashi et al. 2024). CID-based plasma membrane recruitment of Inp54b phosphatase was used to reveal how phosphatase and tensin homolog (PTEN) localization is regulated by the lipid composition of the plasma membrane (Rahdar et al. 2009), and cross talk between phospholipids and Ras/Rap signaling pathways in the diverse migratory modes in *Dictyostelium* (Miao et al. 2017). Ueno et al. (2011) used the FKBP-FRB system to elegantly alter the $PI(4,5)P_2$ levels through either creation of new $PI(4,5)P_2$ or liberation of previously bound $PI(4,5)P_2$ to demonstrate different actin remodeling phenotypes. Similar strategies using light-inducible optogenetic systems were implemented by many groups to further characterize the role of phospholipids in cell migration. Optogenetic recruitment of OCRL phosphatase (inositol polyphosphate 5-phosphatase) or PI3K to the plasma membrane using the Cry2-CIB1 system was sufficient to manipulate phospholipid content in subcellular regions, leading to localized retractions or protrusions (Idevall-Hagren et al. 2012). Optogenetic stimulation of PI3K using other optogenetic systems also induced localized protrusions and revealed a role of phosphatidylinositol 3,4,5-trisphosphate-dependent Rac exchanger 1 (P-Rex1) and ARHGAP15 in transient activation of Rac1 downstream from PI3K signaling (Toettcher et al. 2011; Kawano et al. 2015; Graziano et al. 2017). Optogenetic stimulation of the PI3K signaling could also induce epithelial-to-mesenchymal transformation in cancer cells (Zhou et al. 2018). Recently, optogenetic PI3K activation was implemented to demonstrate a critical cross talk between actin cortex and plasma membrane for long-range propagation of membrane tension (De Belly et al. 2023).

Beyond manipulating the phospholipid composition, researchers have also used inducible systems to alter the biophysical properties of the plasma membrane. FKBP-FRB-mediated recruitment of Bin-Amphiphysin-Rvs (BAR) domains to the plasma membrane, including N-BAR, F-BAR, and I-BAR domains rapidly induced curvature in plasma membrane and other organelles (Suarez et al. 2014). This was followed up by Takada et al. (2018) to demonstrate a synergistic interaction between BAR domain proteins and phospholipid flippases to remodel plasma membrane architecture. Optogenetic recruitment of charged peptides to the plasma membrane altered the electrostatic properties of the inner leaflet of the plasma membrane, leading to increased or decreased protrusions, demonstrating a role of electrostatic surface charges of the plasma membrane in mediating front–back polarity and directional migration (Banerjee et al. 2022).

INDUCIBLE SYSTEMS TARGETING MICROTUBULES

Microtubules, which are dynamic polymers of α-tubulin and β-tubulin, mediate directional cell motility through various avenues including molecular cross talk and structural interactions with other cytoskeletons. One of the first adoptions of inducible systems to control microtubule dynamics was using the LOV2-Zdk system to

control microtubule stability. The LOVTRAP optogenetic module was used to develop a PI end-binding protein 1 (EB1), which dissociated from microtubule growing tips on blue-light stimulation (van Haren et al. 2018). This dissociation of EB1 from microtubule growing tips induced microtubule disassembly, leading to a change in direction of cell migration. iLID-SspB-mediated cross-linking of microtubule growing tip with F-actin stalled microtubule entry into the lamellipodial areas resulting in a microtubule-free zone in cell periphery (Adikes et al. 2018). Optogenetic recruitment of kinesin-13 led to microtubule disassembly, which was used to reveal kinesin-1 and myosin-V competition in *Drosophila* development (Lu et al. 2020). In addition to this, different chemogenetic and optogenetic systems were used by different groups to control the microtubule-severing proteins spastin and katanin to engineer disruption of microtubule network using the spastin protein (Liu et al. 2022; Meiring et al. 2022). In addition to these genetically encoded systems, there have also been a number of photoinducible chemical reagents that have been developed to control microtubule dynamics (Müller-Deku et al. 2020; Gao et al. 2021, 2022; Sailer et al. 2021). The role of posttranslational modifications of microtubules has intrigued researchers for decades. Recently, two optogenetic tools, Z-lock-α-tubulin acetyl transferase 1 (TAT1) and optoTAT were developed to control microtubule acetylation (Stone et al. 2019; Deb Roy et al. 2024). OptoTAT was used to examine the role of microtubule acetylation in directional motility and elucidate the underlying molecular cross talk with the actin cytoskeleton. Aureille et al. (2024) recently developed optoKANK using the iLID–SspB system to examine the cross talk between microtubules and focal adhesions in migrating cells (KN motif and ankyrin repeat domain-containing protein [KANK]).

INDUCIBLE SYSTEMS TARGETING OTHER SIGNALING PATHWAYS

Consistent with the wide array of molecules involved in cell motility, researchers have applied inducible systems to address questions pertinent to many other molecules of interest. CID-based recruitment of the nonreceptor tyrosine kinase ZAP70 (tyrosine-protein kinase ZAP-70) was used to demonstrate the necessity of plasma membrane recruitment and conformational flexibility in its activation (Graef et al. 1997). Insertion of iFKBP to inhibit the catalytic activity of focal adhesion kinase (FAK), Src, and p38 (mitogen-activated protein kinase 11) kinases, which could be rescued by addition of rapamycin (Karginov et al. 2010). Cry-olig optogenetic system was used to cluster and activate fibroblast growth factor (FGF) receptor tyrosine kinase, which demonstrated its role in cell polarization and directional migration (Kim et al. 2014). A similar approach was to develop an optogenetic ephrin type-B receptor 2 (EphB2) tyrosine kinase, which demonstrated its role in inducing cell retraction (Locke et al. 2017). The BcLOV4 photoreceptor was used to develop a series of optogenetic tools to activate all the epidermal growth factor (EGF) receptor tyrosine kinase family members (Pal et al. 2023a). The Cry2–CIB system was used to develop an optogenetic Plexin-B1 to demonstrate a role of Plexin-B1 signaling in contact inhibition of locomotion between osteoblasts and osteoclasts (Deb Roy et al. 2017). Optogenetic approaches to both activate and inhibit G-protein-coupled receptors were used to induce directional migration in immune cells (Karunarathne et al. 2013; O'Neill and Gautam 2014). Various chemogenetic and optogenetic dimerization approaches have been implemented to control integrin activation (Baaske et al. 2019), integrin-kindlin association (Liao et al. 2017), plasma membrane recruitment of talin to promote integrin association (Liao et al. 2021), and vimentin localization in cells (Pasolli et al. 2025). Recently, CID and optogenetic approaches were used to reveal a critical role of plasma membrane proximal glycolysis in cell migration (Zhan et al. 2025). The approaches discussed so far use exogenous expression of the molecules of interest, which may suffer from higher background signaling even in an unstimulated state. Various groups have reported use of nanobodies coupled with optogenetics to control the function of endogenous proteins, thereby inducing signaling pathways at scales closer to endogenous levels (Yu et al. 2019; Gil et al. 2020; Redchuk et al. 2020).

PERSPECTIVES

Inducible molecular tools have transformed biological research by enabling precise control over molecular events in cells. These technologies allow researchers to modulate protein activity, interactions, and localization with fine spatial and temporal resolution while maintaining molecular specificity. However, their use requires certain precautions. First, one or more genes must be introduced into the target sample. Second, expression of these tools may influence cellular functions of interest even before stimulation by light or chemical signals. Despite these limitations, the modular and orthogonal nature of many inducible systems makes them broadly applicable. This versatility is valuable not only for probing diverse molecules in migrating cells but also for investigating biological processes beyond cell migration.

Applying these tools to study cell migration in vivo is an attractive next step, as it enables examination of migratory behavior in the native environment. The main challenge lies in delivering stimulation: Light often fails to penetrate skin, and chemicals may not readily reach target tissues. To address this, red- and far-red-activated optogenetic systems hold promise for manipulating signaling pathways in deeper mammalian tissues, as well as in other commonly used model systems such as *Drosophila* and *Caenorhabditis elegans*. Red- and far-red-activated optogenetic systems could be especially helpful for studying cell migration in *C. elegans*, which exhibits blue-light avoidance (Sawin-McCormack et al. 1995; Kniazeva and Ruvkun 2025). However, *C. elegans* does not produce the cofactor biliverdin required for the red-light-stimulated PHYB-PIF3 and BphP1-QPAS1 optogenetic modules (Zhang et al. 2023), making these optogenetic modules incompatible with the model organism without additional modifications. Therefore, development of new inducible systems or modifying existing ones to avoid these limitations may greatly advance in vivo investigations of cell migration pathways.

Recent advances have expanded inducible systems beyond chemical or light stimulation. For example, Benman et al. (2025) engineered the BcLOV4 protein to develop "Melt"—a thermogenetic molecular tool that underwent oligomerization and membrane translocation in response to change in temperature. They used Melt to develop a series of thermogenetic tools to control EGF receptor (EGFR), Ras, and Cdc42 signaling. Chemicals and light do not always reach deep tissues, and controlling temperature in tissues is technically challenging and may have off-target effects. Therefore, targeted application of inducible tools using these stimuli is complicated by the properties of the tissues involved. Discovery of various proteins that are responsive to other external stimuli such as electric or magnetic field (Maeda et al. 2012; Xu et al. 2021; Kawasaki et al. 2023; Chen and Chen 2024; Belliveau et al. 2025) may pave the way for development of newer inducible systems orthogonal to chemical and light-inducible ones.

The development of predictive tools for de novo protein and peptide design, when integrated with inducible systems, has the potential to further empower studies of cell migration. Combined with advances in biosensors, live cell, and intravital microscopy approaches, inducible molecular actuators can significantly advance our understanding of the biochemical and biophysical pathways that guide more complete understanding of cell motility. Although inducible molecular actuators often painstakingly require careful design, validation, and calibration, they provide a powerful means to dissect such complex and dynamic biological behavior such as cell motility. Overall, inducible systems have become indispensable for uncovering mechanistic insights in cell migration, and we expect that their application will continue to expand the boundaries of experimental manipulation in cell migration studies.

ACKNOWLEDGMENTS

We thank Dr. Bernard L. Cook for assistance with the figures and Dr. Yuchuan Miao for thoughtful comments on the content.

REFERENCES

Adikes RC, Hallett RA, Saway BF, Kuhlman B, Slep KC. 2018. Control of microtubule dynamics using an optoge-

netic microtubule plus end-F-actin cross-linker. *J Cell Biol* **217:** 779–793. doi:10.1083/jcb.201705190

Aureille J, Prabhu SS, Barnett SF, Farrugia AJ, Arnal I, Lafanechère L, Low BC, Kanchanawong P, Mogilner A, Bershadsky AD. 2024. Focal adhesions are controlled by microtubules through local contractility regulation. *EMBO J* **43:** 2715–2732. doi:10.1038/s44318-024-00114-4

Baaske J, Mühlhäuser WWD, Yousefi OS, Zanner S, Radziwill G, Hörner M, Schamel WWA, Weber W. 2019. Optogenetic control of integrin-matrix interaction. *Commun Biol* **2:** 15. doi:10.1038/s42003-018-0264-7

Banaszynski LA, Chen L, Maynard-Smith LA, Ooi AGL, Wandless TJ. 2006. A rapid, reversible, and tunable method to regulate protein function in living cells using synthetic small molecules. *Cell* **126:** 995–1004. doi:10.1016/j.cell.2006.07.025

Banerjee T, Biswas D, Pal DS, Miao Y, Iglesias PA, Devreotes PN. 2022. Spatiotemporal dynamics of membrane surface charge regulates cell polarity and migration. *Nat Cell Biol* **24:** 1499–1515. doi:10.1038/s41556-022-00997-7

Belliveau NM, Footer MJ, Platenkamp A, Rodriguez C, Kim H, Prinz CK, van Loon AP, Lin Y, Eustis TE, et al. 2025. Galvanin (TMEM154) is an electric-field sensor for directed cell migration. bioRxiv doi:10.1101/2024.09.23.614580

Benman W, Huang Z, Iyengar P, Wilde D, Mumford TR, Bugaj LJ. 2025. A temperature-inducible protein module for control of mammalian cell fate. *Nat Methods* **22:** 539–549. doi:10.1038/s41592-024-02572-4

Castellano F, Montcourrier P, Guillemot JC, Gouin E, Machesky L, Cossart P, Chavrier P. 1999. Inducible recruitment of Cdc42 or WASP to a cell-surface receptor triggers actin polymerization and filopodium formation. *Curr Biol* **9:** 351–360. doi:10.1016/S0960-9822(99)80161-4

Castellano F, Montcourrier P, Chavrier P. 2000. Membrane recruitment of Rac1 triggers phagocytosis. *J Cell Sci* **113:** 2955–2961. doi:10.1242/jcs.113.17.2955

Chen J, Chen Y. 2024. Controlling cell behavior with engineered magnetosensitive proteins. *Biophys J* **123:** 20a. doi:10.1016/j.bpj.2023.11.229

Crefcoeur RP, Yin R, Ulm R, Halazonetis TD. 2013. Ultraviolet-B-mediated induction of protein–protein interactions in mammalian cells. *Nat Commun* **4:** 1779. doi:10.1038/ncomms2800

Dagliyan O, Tarnawski M, Chu P-H, Shirvanyants D, Schlichting I, Dokholyan NV, Hahn KM. 2016. Engineering extrinsic disorder to control protein activity in living cells. *Science* **354:** 1441–1444. doi:10.1126/science.aah3404

de Beco S, Vaidžiulytė K, Manzi J, Dalier F, di Federico F, Cornilleau G, Dahan M, Coppey M. 2018. Optogenetic dissection of Rac1 and Cdc42 gradient shaping. *Nat Commun* **9:** 4816. doi:10.1038/s41467-018-07286-8

De Belly H, Yan S, Borja da Rocha H, Ichbiah S, Town JP, Zager PJ, Estrada DC, Meyer K, Turlier H, Bustamante C, et al. 2023. Cell protrusions and contractions generate long-range membrane tension propagation. *Cell* **186:** 3049–3061.e15. doi:10.1016/j.cell.2023.05.014

Deb Roy A, Yin T, Choudhary S, Rodionov V, Pilbeam CC, Wu YI. 2017. Optogenetic activation of Plexin-B1 reveals contact repulsion between osteoclasts and osteoblasts. *Nat Commun* **8:** 15831. doi:10.1038/ncomms15831

Deb Roy A, Gonzalez CS, Shahid F, Yadav E, Inoue T. 2024. Optogenetically induced microtubule acetylation unveils the molecular dynamics of actin-microtubule crosstalk in directed cell migration. bioRxiv doi:10.1101/2024.12.01.626286

DeRose R, Miyamoto T, Inoue T. 2013. Manipulating signaling at will: chemically inducible dimerization (CID) techniques resolve problems in cell biology. *Pflüg Arch* **465:** 409–417. doi:10.1007/s00424-012-1208-6

Fossati E, Volpato JP, Poulin L, Guerrero V, Dugas DA, Pelletier JN. 2008. 2-Tier bacterial and in vitro selection of active and methotrexate-resistant variants of human dihydrofolate reductase. *J Biomol Screen* **13:** 504–514. doi:10.1177/1087057108318783

Gao L, Meiring JCM, Kraus Y, Wranik M, Weinert T, Pritzl SD, Bingham R, Ntouliou E, Jansen KI, Olieric N, et al. 2021. A robust, GFP-orthogonal photoswitchable inhibitor scaffold extends optical control over the microtubule cytoskeleton. *Cell Chem Biol* **28:** 228–241.e6. doi:10.1016/j.chembiol.2020.11.007

Gao L, Meiring JCM, Heise C, Rai A, Müller-Deku A, Akhmanova A, Thorn-Seshold J, Thorn-Seshold O. 2022. Photoswitchable epothilone-based microtubule stabilisers allow GFP-imaging-compatible, optical control over the microtubule cytoskeleton. *Angew Chem Int Ed* **61:** e202114614. doi:10.1002/anie.202114614

Gil AA, Carrasco-López C, Zhu L, Zhao EM, Ravindran PT, Wilson MZ, Goglia AG, Avalos JL, Toettcher JE. 2020. Optogenetic control of protein binding using light-switchable nanobodies. *Nat Commun* **11:** 4044. doi:10.1038/s41467-020-17836-8

Glantz ST, Berlew EE, Jaber Z, Schuster BS, Gardner KH, Chow BY. 2018. Directly light-regulated binding of RGS-LOV photoreceptors to anionic membrane phospholipids. *Proc Natl Acad Sci* **115:** E7720–E7727. doi:10.1073/pnas.1802832115

Graef IA, Holsinger LJ, Diver S, Schreiber SL, Crabtree GR. 1997. Proximity and orientation underlie signaling by the non-receptor tyrosine kinase ZAP70. *EMBO J* **16:** 5618–5628. doi:10.1093/emboj/16.18.5618

Graziano BR, Gong D, Anderson KE, Pipathsouk A, Goldberg AR, Weiner OD. 2017. A module for Rac temporal signal integration revealed with optogenetics. *J Cell Biol* **216:** 2515–2531. doi:10.1083/jcb.201604113

Guntas G, Hallett RA, Zimmerman SP, Williams T, Yumerefendi H, Bear JE, Kuhlman B. 2015. Engineering an improved light-induced dimer (iLID) for controlling the localization and activity of signaling proteins. *Proc Natl Acad Sci* **112:** 112–117. doi:10.1073/pnas.1417910112

Guo H, Swan M, He B. 2022. Optogenetic inhibition of actomyosin reveals mechanical bistability of the mesoderm epithelium during *Drosophila* mesoderm invagination. *eLife* **11:** e69082. doi:10.7554/eLife.69082

Holsinger LJ, Spencer DM, Austin DJ, Schreiber SL, Crabtree GR. 1995. Signal transduction in T lymphocytes using a conditional allele of Sos. *Proc Natl Acad Sci* **92:** 9810–9814. doi:10.1073/pnas.92.21.9810

Huang Dennis Z, Benman W, Dong L, Bugaj LJ. 2024. Rapid optogenetic clustering in the cytoplasm with BcLOVclust. *J Mol Biol* **436:** 168452. doi:10.1016/j.jmb.2024.168452

Hughes RM, Lawrence DS. 2014. Optogenetic engineering: light-directed cell motility. *Angew Chem Int Ed* **53:** 10904–10907. doi:10.1002/anie.201404198

Idevall-Hagren O, Dickson EJ, Hille B, Toomre DK, De Camilli P. 2012. Optogenetic control of phosphoinositide metabolism. *Proc Natl Acad Sci* **109:** E2316–E2323. doi:10.1073/pnas.1211305109

Inoue T, Meyer T. 2008. Synthetic activation of endogenous PI3K and Rac identifies an AND-gate switch for cell polarization and migration. *PLoS One* **3:** e3068. doi:10.1371/journal.pone.0003068

Inoue T, Heo WD, Grimley JS, Wandless TJ, Meyer T. 2005. An inducible translocation strategy to rapidly activate and inhibit small GTPase signaling pathways. *Nat Methods* **2:** 415–418. doi:10.1038/nmeth763

Iwamoto M, Björklund T, Lundberg C, Kirik D, Wandless TJ. 2010. A general chemical method to regulate protein stability in the mammalian central nervous system. *Chem Biol* **17:** 981–988. doi:10.1016/j.chembiol.2010.07.009

Kaberniuk AA, Shemetov AA, Verkhusha VV. 2016. A bacterial phytochrome-based optogenetic system controllable with near-infrared light. *Nat Methods* **13:** 591–597. doi:10.1038/nmeth.3864

Karginov AV, Ding F, Kota P, Dokholyan NV, Hahn KM. 2010. Engineered allosteric activation of kinases in living cells. *Nat Biotechnol* **28:** 743–747. doi:10.1038/nbt.1639

Karginov AV, Zou Y, Shirvanyants D, Kota P, Dokholyan NV, Young DD, Hahn KM, Deiters A. 2011. Light regulation of protein dimerization and kinase activity in living cells using photocaged rapamycin and engineered FKBP. *J Am Chem Soc* **133:** 420–423. doi:10.1021/ja109630v

Karginov AV, Tsygankov D, Berginski M, Chu PH, Trudeau ED, Yi JJ, Gomez S, Elston TC, Hahn KM. 2014. Dissecting motility signaling through activation of specific Src-effector complexes. *Nat Chem Biol* **10:** 286–290. doi:10.1038/nchembio.1477

Karunarathne WKA, Giri L, Patel AK, Venkatesh KV, Gautam N. 2013. Optical control demonstrates switch-like PIP_3 dynamics underlying the initiation of immune cell migration. *Proc Natl Acad Sci* **110:** E1575–1583. doi:10.1073/pnas.1220755110

Kawano F, Suzuki H, Furuya A, Sato M. 2015. Engineered pairs of distinct photoswitches for optogenetic control of cellular proteins. *Nat Commun* **6:** 6256. doi:10.1038/ncomms7256

Kawasaki H, Okano H, Ishiwatari H, Kishi T, Ishida N. 2023. A role of cryptochrome for magnetic field-dependent improvement of sleep quality, lifespan, and motor function in *Drosophila*. *Genes Cells* **28:** 496–502. doi:10.1111/gtc.13030

Kennedy MJ, Hughes RM, Peteya LA, Schwartz JW, Ehlers MD, Tucker CL. 2010. Rapid blue-light–mediated induction of protein interactions in living cells. *Nat Methods* **7:** 973–975. doi:10.1038/nmeth.1524

Kim N, Kim JM, Lee M, Kim CY, Chang KY, Heo WD. 2014. Spatiotemporal control of fibroblast growth factor receptor signals by blue light. *Chem Biol* **21:** 903–912. doi:10.1016/j.chembiol.2014.05.013

Kniazeva M, Ruvkun G. 2025. The effects of intense light on *Caenorhabditis elegans*. bioRxiv doi:10.1101/2025.06.05.658132

Komatsu T, Kukelyansky I, McCaffery JM, Ueno T, Varela LC, Inoue T. 2010. Organelle-specific, rapid induction of molecular activities and membrane tethering. *Nat Methods* **7:** 206–208. doi:10.1038/nmeth.1428

Kuhn J, Banerjee P, Haye A, Robinson DN, Iglesias PA, Devreotes PN. 2025. Complementary cytoskeletal feedback loops control signal transduction excitability and cell polarity. *Nat Commun* **16:** 7482. doi:10.1038/s41467-025-62799-3

Levskaya A, Weiner OD, Lim WA, Voigt CA. 2009. Spatiotemporal control of cell signalling using a light-switchable protein interaction. *Nature* **461:** 997–1001. doi:10.1038/nature08446

Liang FS, Ho WQ, Crabtree GR. 2011. Engineering the ABA plant stress pathway for regulation of induced proximity. *Sci Signal* **4:** rs2. doi:10.1126/scisignal.2001449

Liao Z, Kasirer-Friede A, Shattil SJ. 2017. Optogenetic interrogation of integrin αVβ3 function in endothelial cells. *J Cell Sci* **130:** 3532–3541. doi:10.1242/jcs.205203

Liao Z, Gingras AR, Lagarrigue F, Ginsberg MH, Shattil SJ. 2021. Optogenetics-based localization of talin to the plasma membrane promotes activation of β3 integrins. *J Biol Chem* **296:** 100675. doi:10.1016/j.jbc.2021.100675

Lin B, Holmes WR, Wang CJ, Ueno T, Harwell A, Edelstein-Keshet L, Inoue T, Levchenko A. 2012. Synthetic spatially graded Rac activation drives cell polarization and movement. *Proc Natl Acad Sci* **109:** E3668–E3677. doi:10.1073/pnas.1210295109

Lin B, Yin T, Wu YI, Inoue T, Levchenko A. 2015. Interplay between chemotaxis and contact inhibition of locomotion determines exploratory cell migration. *Nat Commun* **6:** 6619. doi:10.1038/ncomms7619

Liu GY, Chen S, Lee G, Shaiv K, Chen P, Cheng H, Hong S, Yang W, Huang S, Chang Y, et al. 2022. Precise control of microtubule disassembly in living cells. *EMBO J* **41:** e110472. doi:10.15252/embj.2021110472

Locke C, Machida K, Tucker CL, Wu Y, Yu J. 2017. Optogenetic activation of EphB2 receptor in dendrites induced actin polymerization by activating Arg kinase. *Biol Open* **6:** 1820–1830. doi:10.1242/bio.029900

Lu W, Lakonishok M, Liu R, Billington N, Rich A, Glotzer M, Sellers JR, Gelfand VI. 2020. Competition between kinesin-1 and myosin-V defines *Drosophila* posterior determination. *eLife* **9:** e54216. doi:10.7554/eLife.54216

Maeda K, Robinson AJ, Henbest KB, Hogben HJ, Biskup T, Ahmad M, Schleicher E, Weber S, Timmel CR, Hore PJ. 2012. Magnetically sensitive light-induced reactions in cryptochrome are consistent with its proposed role as a magnetoreceptor. *Proc Natl Acad Sci* **109:** 4774–4779. doi:10.1073/pnas.1118959109

Matsubayashi HT, Mountain J, Takahashi N, Deb Roy A, Yao T, Peterson AF, Saez Gonzalez C, Kawamata I, Inoue T. 2024. Non-catalytic role of phosphoinositide 3-kinase in mesenchymal cell migration through non-canonical induction of p85β/AP2-mediated endocytosis. *Nat Commun* **15:** 2612. doi:10.1038/s41467-024-46855-y

Meiring JCM, Grigoriev I, Nijenhuis W, Kapitein LC, Akhmanova A. 2022. Opto-katanin, an optogenetic tool for localized, microtubule disassembly. *Curr Biol* **32:** 4660–4674.e6. doi:10.1016/j.cub.2022.09.010

Miao Y, Bhattacharya S, Edwards M, Cai H, Inoue T, Iglesias PA, Devreotes PN. 2017. Altering the threshold of an

Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

excitable signal transduction network changes cell migratory modes. *Nat Cell Biol* **19:** 329–340. doi:10.1038/ncb3495

Miao Y, Bhattacharya S, Banerjee T, Abubaker-Sharif B, Long Y, Inoue T, Iglesias PA, Devreotes PN. 2019. Wave patterns organize cellular protrusions and control cortical dynamics. *Mol Syst Biol* **15:** e8585. doi:10.15252/msb.20188585

Miyamoto T, DeRose R, Suarez A, Ueno T, Chen M, Sun T, Wolfgang MJ, Mukherjee C, Meyers DJ, Inoue T. 2012. Rapid and orthogonal logic gating with a gibberellin-induced dimerization system. *Nat Chem Biol* **8:** 465–470. doi:10.1038/nchembio.922

Müller-Deku A, Meiring JCM, Loy K, Kraus Y, Heise C, Bingham R, Jansen KI, Qu X, Bartolini F, Kapitein LC, et al. 2020. Photoswitchable paclitaxel-based microtubule stabilisers allow optical control over the microtubule cytoskeleton. *Nat Commun* **11:** 4640. doi:10.1038/s41467-020-18389-6

Nakamura H, DeRose R, Inoue T. 2019. Harnessing biomolecular condensates in living cells. *J Biochem* **166:** 13–27. doi:10.1093/jb/mvz028

Nakamura H, Rho E, Lee CT, Itoh K, Deng D, Watanabe S, Razavi S, Matsubayashi HT, Zhu C, Jung E, et al. 2023. Actuator, a *Listeria*-inspired molecular tool for physical manipulation of intracellular organizations through de novo actin polymerization. *Cell Rep* **42:** 113089. doi:10.1016/j.celrep.2023.113089

Nihongaki Y, Suzuki H, Kawano F, Sato M. 2014. Genetically engineered photoinducible homodimerization system with improved dimer-forming efficiency. *ACS Chem Biol* **9:** 617–621. doi:10.1021/cb400836k

Niopek D, Benzinger D, Roensch J, Draebing T, Wehler P, Eils R, Di Ventura B. 2014. Engineering light-inducible nuclear localization signals for precise spatiotemporal control of protein dynamics in living cells. *Nat Commun* **5:** 4404. doi:10.1038/ncomms5404

Niopek D, Wehler P, Roensch J, Eils R, Di Ventura B. 2016. Optogenetic control of nuclear protein export. *Nat Commun* **7:** 10624. doi:10.1038/ncomms10624

Nishimura K, Fukagawa T, Takisawa H, Kakimoto T, Kanemaki M. 2009. An auxin-based degron system for the rapid depletion of proteins in nonplant cells. *Nat Methods* **6:** 917–922. doi:10.1038/nmeth.1401

O'Neill PR, Gautam N. 2014. Subcellular optogenetic inhibition of G proteins generates signaling gradients and cell migration. *Mol Biol Cell* **25:** 2305–2314. doi:10.1091/mbc.e14-04-0870

Pal AA, Benman W, Mumford TR, Huang Z, Chow BY, Bugaj LJ. 2023a. Optogenetic clustering and membrane translocation of the BcLOV4 photoreceptor. *Proc Natl Acad Sci* **120:** e2221615120. doi:10.1073/pnas.2221615120

Pal DS, Banerjee T, Lin Y, de Trogoff F, Borleis J, Iglesias PA, Devreotes PN. 2023b. Actuation of single downstream nodes in growth factor network steers immune cell migration. *Dev Cell* **58:** 1170–1188.e7. doi:10.1016/j.devcel.2023.04.019

Pal DS, Lin Y, Zhan H, Banerjee T, Kuhn J, Providence S, Devreotes PN. 2023c. Optogenetic modulation of guanine nucleotide exchange factors of Ras superfamily proteins directly controls cell shape and movement. *Front Cell Dev Biol* **11:** 1195806. doi:10.3389/fcell.2023.1195806

Pasolli M, Meiring JCM, Conboy JP, Koenderink GH, Akhmanova A. 2025. Optogenetic and chemical genetic tools for rapid repositioning of vimentin intermediate filaments. *J Cell Biol* **224:** e202504004. doi:10.1083/jcb.202504004

Rahdar M, Inoue T, Meyer T, Zhang J, Vazquez F, Devreotes PN. 2009. A phosphorylation-dependent intramolecular interaction regulates the membrane association and activity of the tumor suppressor PTEN. *Proc Natl Acad Sci* **106:** 480–485. doi:10.1073/pnas.0811212106

Razavi S, Wong F, Abubaker-Sharif B, Matsubayashi HT, Nakamura H, Nguyen NTH, Robinson DN, Chen B, Iglesias PA, Inoue T. 2024. Synthetic control of actin polymerization and symmetry breaking in active protocells. *Sci Adv* **10:** eadk9731. doi:10.1126/sciadv.adk9731

Redchuk TA, Kaberniuk AA, Verkhusha VV. 2018. Near-infrared light-controlled systems for gene transcription regulation, protein targeting and spectral multiplexing. *Nat Protoc* **13:** 1121–1136. doi:10.1038/nprot.2018.022

Redchuk TA, Karasev MM, Verkhusha PV, Donnelly SK, Hülsemann M, Virtanen J, Moore HM, Vartiainen MK, Hodgson L, Verkhusha VV. 2020. Optogenetic regulation of endogenous proteins. *Nat Commun* **11:** 605. doi:10.1038/s41467-020-14460-4

Sailer A, Meiring JCM, Heise C, Pettersson LN, Akhmanova A, Thorn-Seshold J, Thorn-Seshold O. 2021. Pyrrole hemithioindigo antimitotics with near-quantitative bidirectional photoswitching that photocontrol cellular microtubule dynamics with single-cell precision. *Angew Chem Int Ed* **60:** 23695–23704. doi:10.1002/anie.202104794

Sawin-McCormack EP, Sokolowski MB, Campos AR. 1995. Characterization and genetic analysis of *Drosophila melanogaster* photobehavior during larval development. *J Neurogenet* **10:** 119–135. doi:10.3109/01677069509083459

Seze JD, Bongaerts M, Boulevard B, Coppey M. 2025. Optogenetic control of a GEF of RhoA uncovers a signaling switch from retraction to protrusion. *eLife* **12:** RP93180. doi:10.7554/eLife.93180

Spencer DM, Wandless TJ, Schreiber SL, Crabtree GR. 1993. Controlling signal transduction with synthetic ligands. *Science* **262:** 1019–1024. doi:10.1126/science.7694365

Stanton BZ, Chory EJ, Crabtree GR. 2018. Chemically induced proximity in biology and medicine. *Science* **359:** eaao5902. doi:10.1126/science.aao5902

Stone OJ, Pankow N, Liu B, Sharma VP, Eddy RJ, Wang H, Putz AT, Teets FD, Kuhlman B, Condeelis JS, et al. 2019. Optogenetic control of Cofilin and αTAT in living cells using Z-lock. *Nat Chem Biol* **15:** 1183–1190. doi:10.1038/s41589-019-0405-4

Strickland D, Lin Y, Wagner E, Hope CM, Zayner J, Antoniou C, Sosnick TR, Weiss EL, Glotzer M. 2012. TULIPs: tunable, light-controlled interacting protein tags for cell biology. *Nat Methods* **9:** 379–384. doi:10.1038/nmeth.1904

Suarez A, Ueno T, Huebner R, McCaffery JM, Inoue T. 2014. Bin/Amphiphysin/Rvs (BAR) family members bend membranes in cells. *Sci Rep* **4:** 4693. doi:10.1038/srep04693

Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

Suh BC, Inoue T, Meyer T, Hille B. 2006. Rapid chemically induced changes of PtdIns(4,5)P_2 gate KCNQ ion channels. *Science* **314:** 1454–1457. doi:10.1126/science.1131163

Takada N, Naito T, Inoue T, Nakayama K, Takatsu H, Shin H-W. 2018. Phospholipid-flipping activity of P4-ATPase drives membrane curvature. *EMBO J* **37:** e97705. doi:10.15252/embj.201797705

Taslimi A, Vrana JD, Chen D, Borinskaya S, Mayer BJ, Kennedy MJ, Tucker CL. 2014. An optimized optogenetic clustering tool for probing protein interaction and function. *Nat Commun* **5:** 4925. doi:10.1038/ncomms5925

Toettcher JE, Gong D, Lim WA, Weiner OD. 2011. Light control of plasma membrane recruitment using the Phy–PIF system. *Methods Enzymol* **497:** 409–423. doi:10.1016/B978-0-12-385075-1.00017-2

Ueno T, Falkenburger BH, Pohlmeyer C, Inoue T. 2011. Triggering actin comets versus membrane ruffles: distinctive effects of phosphoinositides on actin reorganization. *Sci Signal* **4:** ra87. doi:10.1126/scisignal.2002033

Umeda N, Ueno T, Pohlmeyer C, Nagano T, Inoue T. 2011. A photocleavable rapamycin conjugate for spatiotemporal control of small GTPase activity. *J Am Chem Soc* **133:** 12–14. doi:10.1021/ja108258d

Valon L, Marín-Llauradó A, Wyatt T, Charras G, Trepat X. 2017. Optogenetic control of cellular forces and mechanotransduction. *Nat Commun* **8:** 14396. doi:10.1038/ncomms14396

van Haren J, Charafeddine RA, Ettinger A, Wang H, Hahn KM, Wittmann T. 2018. Local control of intracellular microtubule dynamics by EB1 photodissociation. *Nat Cell Biol* **20:** 252–261. doi:10.1038/s41556-017-0028-5

Varnai P, Thyagarajan B, Rohacs T, Balla T. 2006. Rapidly inducible changes in phosphatidylinositol 4,5-bisphosphate levels influence multiple regulatory functions of the lipid in intact living cells. *J Cell Biol* **175:** 377–382. doi:10.1083/jcb.200607116

Volpato JP, Fossati E, Pelletier JN. 2007. Increasing methotrexate resistance by combination of active-site mutations in human dihydrofolate reductase. *J Mol Biol* **373:** 599–611. doi:10.1016/j.jmb.2007.07.076

Volpato JP, Yachnin BJ, Blanchet J, Guerrero V, Poulin L, Fossati E, Berghuis AM, Pelletier JN. 2009. Multiple conformers in active site of human dihydrofolate reductase F31R/Q35E double mutant suggest structural basis for methotrexate resistance. *J Biol Chem* **284:** 20079–20089. doi:10.1074/jbc.M109.018010

Wang X, He L, Wu YI, Hahn KM, Montell DJ. 2010. Light-mediated activation reveals a key role for Rac in collective guidance of cell movement in vivo. *Nat Cell Biol* **12:** 591–597. doi:10.1038/ncb2061

Wang H, Vilela M, Winkler A, Tarnawski M, Schlichting I, Yumerefendi H, Kuhlman B, Liu R, Danuser G, Hahn KM. 2016. LOVTRAP: an optogenetic system for photoinduced protein dissociation. *Nat Methods* **13:** 755–758. doi:10.1038/nmeth.3926

Wu YI, Frey D, Lungu OI, Jaehrig A, Schlichting I, Kuhlman B, Hahn KM. 2009. A genetically encoded photoactivatable Rac controls the motility of living cells. *Nature* **461:** 104–108. doi:10.1038/nature08241

Wu HD, Kikuchi M, Dagliyan O, Aragaki AK, Nakamura H, Dokholyan NV, Umehara T, Inoue T. 2020. Rational design and implementation of a chemically inducible heterotrimerization system. *Nat Methods* **17:** 928–936. doi:10.1038/s41592-020-0913-x

Xu J, Jarocha LE, Zollitsch T, Konowalczyk M, Henbest KB, Richert S, Golesworthy MJ, Schmidt J, Déjean V, Sowood DJC, et al. 2021. Magnetic sensitivity of cryptochrome 4 from a migratory songbird. *Nature* **594:** 535–540. doi:10.1038/s41586-021-03618-9

Yamamoto K, Miura H, Ishida M, Mii Y, Kinoshita N, Takada S, Ueno N, Sawai S, Kondo Y, Aoki K. 2021. Optogenetic relaxation of actomyosin contractility uncovers mechanistic roles of cortical tension during cytokinesis. *Nat Commun* **12:** 7145. doi:10.1038/s41467-021-27458-3

Yoo SK, Deng Q, Cavnar PJ, Wu YI, Hahn KM, Huttenlocher A. 2010. Differential regulation of protrusion and polarity by PI3K during neutrophil motility in live zebrafish. *Dev Cell* **18:** 226–236. doi:10.1016/j.devcel.2009.11.015

Yu D, Lee H, Hong J, Jung H, Jo Y, Oh BH, Park BO, Heo WD. 2019. Optogenetic activation of intracellular antibodies for direct modulation of endogenous proteins. *Nat Methods* **16:** 1095–1100. doi:10.1038/s41592-019-0592-7

Yuan H, Bauer CE. 2008. Pixe promotes dark oligomerization of the BLUF photoreceptor PixD. *Proc Natl Acad Sci* **105:** 11715–11719. doi:10.1073/pnas.0802149105

Zhan H, Pal DS, Borleis J, Deng Y, Long Y, Janetopoulos C, Huang CH, Devreotes PN. 2025. Self-organizing glycolytic waves tune cellular metabolic states and fuel cancer progression. *Nat Commun* **16:** 5563. doi:10.1038/s41467-025-60596-6

Zhang H, Papadaki S, Sun X, Wang X, Drobizhev M, Yao L, Rehbock M, Köster RW, Wu L, Namikawa K, et al. 2023. Quantitative assessment of near-infrared fluorescent proteins. *Nat Methods* **20:** 1605–1616. doi:10.1038/s41592-023-01975-z

Zhou XX, Chung HK, Lam AJ, Lin MZ. 2012. Optical control of protein activity by fluorescent protein domains. *Science* **338:** 810–814. doi:10.1126/science.1226854

Zhou X, Wang J, Chen J, Qi Y, Nan D, Jin L, Qian X, Wang X, Chen Q, Liu X, et al. 2018. Optogenetic control of epithelial-mesenchymal transition in cancer cells. *Sci Rep* **8:** 14098. doi:10.1038/s41598-018-32539-3

Zhou Y, Kong D, Wang X, Yu G, Wu X, Guan N, Weber W, Ye H. 2022. A small and highly sensitive red/far-red optogenetic switch for applications in mammals. *Nat Biotechnol* **40:** 262–272. doi:10.1038/s41587-021-01036-w

Zimmerman SP, Asokan SB, Kuhlman B, Bear JE. 2017. Cells lay their own tracks—optogenetic Cdc42 activation stimulates fibronectin deposition supporting directed migration. *J Cell Sci* **130:** 2971–2983. doi:10.1242/jcs.205948

Zimmermann M, Cal R, Janett E, Hoffmann V, Bochet CG, Constable E, Beaufils F, Wymann MP. 2014. Cell-permeant and photocleavable chemical inducer of dimerization. *Angew Chem Int Ed* **53:** 4717–4720. doi:10.1002/anie.201310969

Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org