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Optical sensors to gain mechanistic insights into signaling assemblies

Brian Tenner[1,2], Sohum Mehta[1] and Jin Zhang[1,3]

Protein complexes play a major role in transducing information from outside the cell into instructions for growth and survival, and understanding how these complexes relay and shape intracellular signals has been a central question in signaling biology. Fluorescent proteins have proven paramount in opening windows for researchers to peer into the architecture and inner workings of signaling assemblies within the living cell and in real-time. In this review, we will provide readers with a current perspective on the development and use of genetically encoded optical probes to dissect the function of signaling complexes.

Addresses
[1] Department of Pharmacology, University of California, San Diego, La Jolla, CA, United States
[2] Department of Biophysics and Biophysical Chemistry, Johns Hopkins School of Medicine, Baltimore, MD, United States
[3] Department of Pharmacology and Molecular Sciences, Johns Hopkins School of Medicine, Baltimore, MD, United States

Corresponding author: Zhang, Jin (jzhang32@ucsd.edu)

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Introduction

Signaling networks are essential for cells to sense their environment and rapidly translate external information into decisions that promote growth and sustainability. Due to the complexity of the intracellular space, these networks have evolved multimolecular assemblies to facilitate signal transduction by specifically positioning enzymes and substrates for interaction [1]. Extensive biochemical characterization over the last few decades has provided researchers a glimpse into the assembly of signaling complexes and the mechanisms through which they shape signals. However, obtaining a more complete understanding of intracellular communication requires tools that are capable of capturing the spatiotemporal dynamics of these processes within individual living cells.

Recent technological advances in live-cell imaging and the landmark discovery of fluorescent proteins (FPs) have revolutionized the signaling field and led to the development of genetically encoded biosensors for interrogating signaling in the native biological context as opposed to in the test tube [2]. In addition, these tools have opened up the possibility of monitoring individual cells at the time scale at which signaling events occur, whereas bulk measurement techniques often fall short of accurately describing the cell-to-cell heterogeneity of signaling, i.e., an average response can mask important dynamics, such as oscillations and transient spiking [3]. Further adaptations of these tools over the last ten years have promoted the design of fluorescence-based biosensors that can actually provide mechanistic descriptions of signaling assemblies by measuring localized protein-protein interactions (PPIs), second messenger concentrations, and enzymatic activities [4].

In this review, we will briefly cover some of the optical-based tools that are currently utilized to probe multiprotein signaling complexes. The technologies covered here are not exhaustive, but will rather focus on a few important techniques and the present state of probe and sensor development.

Assembly architecture

Signaling complexes can be assembled from many different enzymes, adaptors, and scaffolds, forming a localized, information-rich protein interaction network. The components within the assembly can exist in many physiologically relevant molecular states, such as being bound to the complex, being chemically modified, or adopting a specific conformation [1]. Perturbations to the composition of an assembly, for example, by mutations, often transform signaling pathways and can cause detrimental effects such as cancer and Alzheimer's disease [5,6]. To understand the signal-shaping mechanisms of these multiprotein machines, it is important to characterize the dynamic PPIs in the local context of the living cell. Below, we outline a few technologies that have assisted researchers in dissecting the architecture of signaling complexes and highlight current developments that push the limits of measurement.

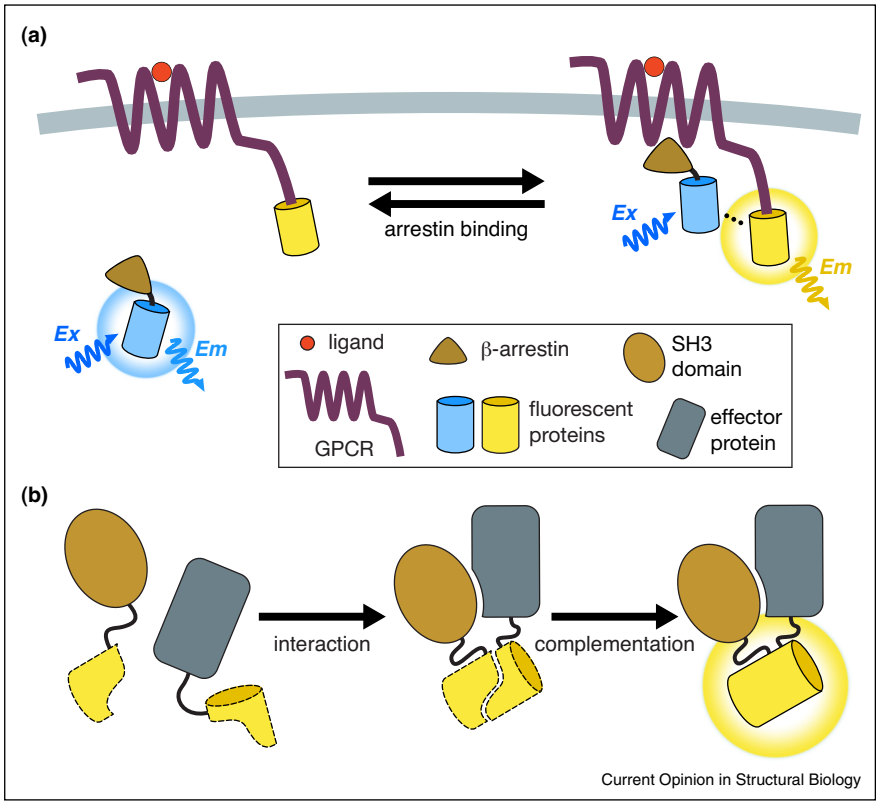
From revealing the composition of a signaling complex to measuring the dynamic changes within a signaling complex, Förster Resonance Energy Transfer (FRET) is a powerful tool for probing PPIs. FRET involves the non-radiative transfer of energy from an excited donor

fluorophore to an acceptor fluorophore. This photophysical process is immediate and functions effectively in the 1–10 nm regime with an inverse (distance)^6 dependence, making FRET a sensitive method to study interactions at the scale of macromolecules *in situ* (Figure 1a) [7]. Advancements in imaging/detection techniques and the discovery of spectral FP variants have led to the routine utilization of FRET in signaling research.

Many signaling interactions occur quickly and reversibly, and thus require dynamic, reversible probes. Non-covalent interactions can be effectively coupled to the distance-dependent FRET signal by directly fusing or tagging the proteins of interest to fluorescent proteins [8]. This methodology has been utilized to build signaling network models. For example, the G-protein signaling pathway comprises many PPI events, including receptor activation, G-protein dissociation, and receptor recycling; almost every ligand-induced event in this pathway has been monitored using FRET [9,10]. In addition, many of the players undergo conformational changes upon binding or unbinding, and FRET can be used to the measure these changes in an intramolecular fashion. For example, ligand-inducible changes of GPCRs can be monitored by inserting CFP into an intracellular loop and placing YFP at the C-terminus [11]. The G-protein pathway has also benefitted from BRET, another resonance energy transfer-based method that utilizes a bioluminescent luciferase as the energy donor, obviating the need for potentially damaging exogenous illumination and leading to less photobleaching and lower background [12].

The finite range of the visible spectrum and the broad excitation and emission spectra of FPs can make FRET a spectrally expensive technique [13]. Fortunately, alternative single-color methods can also be employed. The EGF receptor is known to dimerize and oligomerize upon stimulation, but only recently has a method for monitoring real-time changes in multimerization been reported [14]. This technique, known as homo-FRET, involves FRET between identical fluorophores and can be quantified by measuring the polarization of emitted light at a single wavelength relative to the excitation light [15,16]. Homo-FRET has been successfully applied to measure receptor clustering by labeling the EGF receptor with a single, monomeric GFP and measuring the loss of fluorescence anisotropy [17]. Live-cell imaging using this

Figure 1



A signaling complex's internal architecture can be probed by tagging components with fluorescent proteins (FPs). As schematically shown in **(a)**, two interacting proteins, such as a GPCR and β-arrestin, are tagged with FPs capable of FRET. BiFC can also be utilized to detect PPIs, as shown in **(b)**. Two nonfluorescent FP fragments are fused to an interacting protein pair, such as an SH3 domain and an effector, and complex formation triggers the reconstitution of the fluorescent signal.