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Cell Surface Sensors: Lightning the Cellular Environment

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Abstract

Cell surface sensors are powerful tools to elucidate cell functions including cell signaling, metabolism and cell-to-cell communication. These sensors not only facilitate our understanding in basic biology but also advance the development of effective therapeutics and diagnostics. While genetically encoded fluorescent protein/peptide sensors have been most popular, emerging cell surface sensor systems including polymer-, nanoparticle-, and nucleic acid aptamer-based sensors have largely expanded our toolkits to interrogate complex cellular signaling and micro- or nano-environments. In particular, cell surface sensors that interrogate in vivo cellular microenvironments represent an emerging trend in the development of next generation tools which biologists may routinely apply to elucidate cell biology in vivo and to develop new therapeutics and diagnostics. This review focuses on the most recent development in areas of cell-surface sensors. We will first discuss some recently reported genetically encoded sensors that were used for monitoring cellular metabolites, proteins and neurotransmitters. We will then focus on the emerging cell surface sensor systems with emphasis on the use of DNA aptamer sensors for probing cell signaling and cell-to-cell communication.

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Introduction

Monitoring cell functions, metabolism and cell-cell communication in their native cellular environment has enormous implications for cell biology and regenerative medicine.¹ Particularly, it is of great importance to be able to quantitatively measure signaling molecules at the cell surface with high spatiotemporal resolution in real time because the local target molecule concentration in the vicinity of the cell surface is often distinct from the bulk medium and fluctuates rapidly over time.^{2,3} Therefore, cell surface sensors where biosensing elements are equipped on or into the cell membrane are valuable tools to probe what cells sense and how they respond in real time to surrounding signals.^{4–7} Cell surface sensors typically incorporate a biological sensing element such as fluorescent protein and DNA aptamer modified with organic dyes that convert a biomolecular binding event or change of local environment to signals.^{4–7} The commonly used cell surface sensors are diagrammatically presented in Figure 1. Briefly, the most widely used cell surface sensor design is based on fluorescence resonance energy transfer (FRET) which relies on molecular interaction between the sensor element and the target molecule to induce conformational change or translocation of the two fluorescent species close to each other (Figure 1a, c, g, h). Fluorescent signals can also be generated by the cleavage of a substrate by an enzyme that results in separation of the fluorophore and the quencher (Figure 1b). Bioluminescent cell surface sensors comprise of luciferase that catalyzes the cleavage of a substrate using the target as a co-factor and produce strong luminescence signal (Figure 1d). There are also emerging cell surface sensors that use environmental responsive polymers and nanoparticles (e.g. gold nanoparticles (AuNPs), quantum dots (QDs)) for monitoring cell functions (Figure 1e, f, g).

In this review, we will first briefly summarize the approaches that one can use to engineer the cell surface with sensors. Secondly, we will discuss some recent examples using traditional fluorescent protein/peptide sensors to monitor cellular metabolites, proteins and neurotransmitters. We will finally focus on the most recent emerging cell surface sensor systems including DNA aptamer sensors immobilized on the stem cell surface for probing cellular environments. We believe this review will stimulate new directions for future development and use of cell surface sensors for cell function analysis, diagnostics and therapeutics.

Approaches for installation of sensors on cell surface

Engineering sensing elements on the cell surface is the first task for their subsequent applications in monitoring cell functions. Techniques to engineer the cell surface such as genetic engineering,^{4–6,8–12} covalent coupling,^{7,13–16} hydrophobic interaction,^{17,18} electrostatic interaction,^{19,20} affinity labeling (antibody/antigen),²¹ metabolic labeling,^{22–26} and enzymatic ligation^{27–31} have allowed researchers to readily modify live cell surfaces with a variety of sensing elements. The cell surface engineering approaches have been elaborately reviewed elsewhere.³² Herein, a summarized schematic diagram of the commonly used cell surface engineering approaches for sensor installation is presented in Figure 2. Traditionally, genetic approach has been widely used to display a sensor probe on the cell surface for monitoring metabolites, neurotransmitters, pH, ions, phospholipids and enzymes.^{4–6,8–12,33–36} This approach allows researchers to examine the biochemical processes within specific compartment in complex cellular contexts. More recently, chemistry and materials science approaches have emerged as simple and versatile alternatives to engineer cell membrane with sensors. For instance, Bertozzi and colleagues have established an approach where they metabolically engineered functional groups on the cell surface which can be further chemically coupled to signaling agents.^{22–26,37,38} Simple

covalent coupling has also been utilized to conjugate exogenous species such as DNA aptamers,^{7,13–15} polymers^{17,39} and nanoparticles⁴⁰ on the cell surface.

Genetically encoded protein biosensors

Genetically encoded biosensors are generated using the cellular machinery to transcribe and translate an exogenously introduced DNA sequence that encodes the desired peptide/protein sensor (i.e. green fluorescent protein (GFP), and its derivatives including the analyte binding peptide).^{4–6,8–12} The discovery of fluorescent proteins (FPs) has revolutionized how we study cell functions. Thus far, several variants of FPs including GFP, blue fluorescent protein (BFP), yellow fluorescent protein (YFP) and red fluorescent protein (RFP) have been developed and many FRET based protein sensors have been developed accordingly. For instance, GFP variants have been employed to monitor cellular pH and membrane potential by measuring fluorescence intensity changes.^{4–6,10,41} Additionally, protease sensors have been constructed with FRET pair proteins, BFP and GFP, that are linked to a peptide sensitive to protease.⁴² Cleavage of the peptide by protease separated the FPs from each other disrupting the FRET resulting in a signal change (Figure 1b). Other examples of using FRET based sensors include detection of metabolites, neurotransmitters, signalling molecules, enzymes and other cellular events particularly inside of the cell.^{6,9,10,33–36} For instance, to measure calcium concentration changes, genetically encoded sensors for calcium were designed by fusing two FPs with different emission spectra to a recognition element consisting of calmodulin and the M13 peptide (Fluorescent indicators for Ca^{2+} based on GFPs and calmodulin).⁴³ Binding of calcium to calmodulin causes a global structural rearrangement resulting in a significant change in FRET intensity. For further readings, interested readers are recommended for some recent excellent reviews.^{4–6,10,33,44–47}

Here we only highlight some recent examples of genetically engineered cell surface sensors for detection of metabolites, hormones, enzymes and neurotransmitters in their native extracellular compartment. These molecules typically play essential roles in regulating cell signalling, cell-cell communication and neurotransmission under both physiological and diseased conditions.^{1,7,45,48} Importantly, the concentration of these molecules can fluctuate very rapidly (e.g. milliseconds) in the confined cell surface compartment which tightly regulates downstream cell signalling.³ Therefore, the ability to determine the concentration of these signalling molecules with both high spatial and temporal resolution in the extremely dynamic cell surface microenvironment is critically important for understanding of various biological processes.

One excellent example of such sensors is the glutamate sensor developed by Frommer and co-workers.¹¹ Glutamate is a key excitatory neurotransmitter in the mammalian brain and it influences essentially all forms of behaviours including consciousness, sensory perception, motor control and mood. While its prominent role in normal and disease physiology has been clearly recognized, it has been a major challenge to detect its rapid local concentration change around single synapses in high spatial and temporal resolution. In their study, the glutamate sensor, called FLIPE, consisted of the glutamate binding protein, ybeJ flanked with two FPs: enhanced cyan FP (ECFP) and Venus (YFP). They reasoned that when YbeJ binds to glutamate, the hinge-bending motion would be allosterically transduced into a FRET efficiency change between two attached FPs (Figure 3a, b). Frommer et al. engineered hippocampal neurons to display these sensors at the extracellular domain of the cell surface that enables the detection of glutamate in high spatiotemporal manner surrounding synapses (Figure 3c, d). They then demonstrated that their cell surface sensor responded to extracellular glutamate with a reversible concentration-dependent decrease in FRET efficiency (Figure 3e). Depolarization of neurons by electrical or chemical stimuli led

to reduction in FRET efficiency which is proportionally related to the concentration of released glutamate at the cell surface (Figure 1f). Tsien and co-workers have subsequently improved the performance of the genetically engineered glutamate sensors by modulating linker sequences and glutamate binding affinities.¹² The new generation sensors are able to monitor the time course of synaptic glutamate release, spillover, and reuptake with centisecond temporal and spine-sized spatial resolution.¹²

More recently, Johnsson and co-workers reported a FRET-based semisynthetic sensor called Snifits (SNAP-tag based indicator proteins with a fluorescent intramolecular tether) that is able to measure metabolites on the cell surface of mammalian cells.³ The sensor, SNAP-CLIP-BP, was assembled on the surface of HEK293 cell as a fusion by genetic expression (Figure 4a). SNAP was labelled with a polyethylene glycol (PEG) linker that contains a fluorophore dye (red rectangle) in the middle and a ligand (purple ball) for specific binding to the binding protein (BP). A second fluorophore (green rectangle) was conjugated to CLIP (Figure 4a, left). Binding of the ligand to BP brings both fluorophore molecules close to each other generating a FRET signal. However, recognition of the target molecule (e.g., glutamate⁴⁹) by the BP releases the ligand and the fluorophore molecules depart away rendering fluorescent signal change. Using this sensor system, the change of fluorescence intensity ratio on living cells was observed significantly higher than the previously reported cell-surface targeted fluorescent sensor protein. These generally applicable cell-surface sensors have several advantages including independence from binding proteins that undergo a conformational change, the use of cell-impermeable and red-shifted fluorophores, and the high signal ratio change.

Umezawa and co-workers have developed a genetically encoded cell surface fluorescent indicator that can allow for high spatio-temporal examination of Phosphatidylinositol-3,4,5-trisphosphate (PtdInsP3) on a live single cell.⁴⁸ PtdInsP3 is a cell membrane glycolipid that is expressed by phosphatidylinositol-3-OH kinase (PI3K) activation and regulates diverse cellular functions including cell proliferation and apoptosis.⁵⁰ Furthermore, it has been found to play roles in diabetes and cancer.^{51,52} They constructed the PtdInsP3 cell surface sensor using a pleckstrin homology (PH) domain from GRP1⁵³ that selectively binds PtdInsP3. The PH domain was fused between cyan (CFP) and yellow (YFP) fluorescent protein variants, through rigid α -helical linkers consisting of repeated EAAAR sequences (Figure 5a). The sensor was displayed on the surface of HEK 293 cells through genetic expression using a membrane localization sequence (MLS) through the rigid α -helical linker (Figure 5b). PI(3)K activation promotes PtdInsP3 expression that binds to PH domain causing a significant conformational change of the indicator protein and brings CFP and YFP close to each other generating FRET (Figure 5c). They applied this system for investigating the dimerization of platelet derived growth factor receptor (PDGFR) on the cells surface of PDGFR over-expressing CHO cell. Treatment of PDGF promoted the dimerization of PDGFR monomers resulting in phosphorylation of multiple tyrosine residues of PDGFR through PI(3)K recruitment with the consequence of immediate decline of CFP:YFP ratio (Figure 5d).

Protease biosensors have also been developed using the same FRET phenomenon. For instance, Luo and co-workers have developed a peptide cleavage based fluorescent sensor to monitor extracellular matrix metalloproteases (MMPs) on the cell surface.⁵⁴ MMPs are a family of secretory endopeptidases that hydrolyzes the extracellular proteins promoting tumour invasion.^{55,56} Several MMPs are over-expressed in cancerous tissue compared to normal ones, and the extent of expression has been shown to be associated with tumour stage, invasiveness, metastasis and angiogenesis.⁵⁷ Therefore, monitoring cellular level of MMPs is significant. The MMP biosensor in this study was constructed by encoding YFP and CFP that were linked with a 12-amino acid MMP cleavable peptide substrate. The

encoded sensor system was displayed on the extracellular membrane. The construct exhibits FRET due to the close proximity of YFP and CFP in absence of MMP. However, the cleavage of the peptide substrate by MMP separates the FPs from each other resulting FRET signal change (Figure 1b). The system was also evaluated for monitoring the effect of MMP inhibitor in live cells, which reveals the potential of this cell surface sensor for anticancer drug screening.

Bioluminescent cell surface sensors

As an alternative to FRET system, bioluminescent sensors using luciferases have been engineered on the cell surface for monitoring ATP and protein expression on the cell membrane.^{58,59} Luciferase is a highly sensitive enzyme that catalyzes the oxidation of a substrate called luciferin using ATP as a co-factor to produce light. The intensity of the light is directly related to the amount of ATP. Cell surface immobilized-luciferase has been demonstrated to monitor ATP on the vicinity of the cell surface (Figure 1d).⁵⁹ Di Vigrilio and co-workers have genetically engineered a firefly luciferase-folate receptor chimeric protein on the cell surface that retains the N-terminal leader sequence and the C-terminal GPI anchor of the folate receptor.⁶⁰ This chimeric protein, named plasma membrane luciferase (pmeLUC), was able to sensitively detect ATP in the low micromolar to millimolar level. Using this sensor they were able to identify the pathways for nonlytic ATP release. Furthermore, in a subsequent study they have shown that this sensor can be applied in vivo to monitor extracellular ATP in tumor site.⁶¹ Bioluminescent resonance energy transfer (BRET) systems have also been developed using luciferase to analyze GPCR (G-protein coupled receptor) dimerization and oligomerization.⁶² Luciferase sensors are particularly attractive because of their high sensitivity in comparison to the fluorescent sensors, low background and higher dynamic range.

Polymer nanopatches as cell surface sensors

Polymers are great candidates in a wide variety of applications including drug delivery, cell and tissue engineering, bioseparation, and biodetection. Recently several polymers have found important applications in monitoring cellular processing. One excellent example is chromatic polydiacetylene (PDA).⁶³ Jelinek and co-workers have used PDA nanopatches to monitor the biophysical processes of cell membrane.^{64,65} The conjugated PDA nanopatches they fused on the cell membrane exhibited an intense blue color. When cell membrane is perturbed, PDA nanopatches underwent a blue-to-red color change due to conformational transitions in the conjugated (ene-yne) polymer backbone (Figure 1e).⁶⁴ During this blue-to-red color transition, the initial non-fluorescent PDA also emitted fluorescence at 560 nm and 640 nm. The PDA nanopatches can therefore be used to detect cell bilayer perturbing agents including lidocaine, polymyxin-B, cholesterol, and oleic acid, and to screen toxic pesticides and other environmentally sensitive small molecules.^{64–66}

Nanoparticle-based cell surface sensors

With their unique optical and biophysical properties, nanoparticles particularly gold nanoparticles (AuNPs) and quantum dots (QDs) have become another class of attractive materials in the biomedical research field. These materials have shown great potential for practical applications in therapeutics, diagnostics and in vivo imaging.^{67–71} These nanoparticles have also been employed on cell surfaces for studying cellular processes and cell functions. For instance, Tan and co-workers have developed a surface energy transfer (SET) “nanoruler” using aptamer immobilized-AuNP to measure the distance of the ligand binding sites of PKT7 receptor on live cells.⁷² One binding site was captured with antibody-coupled fluorophore and the other with aptamer-AuNP conjugate. The distance between the fluorophore and the AuNP was measured based on the SET signal. They employed different

AuNPs with varying sizes to obtain different signals that provided information about the distance between the antibody binding sites and the aptamer binding sites on the same protein. The ability to resolve distances between identical molecules such as receptors on the cell surface at nanometer scale as a function of time is of great interest for a broad range of cellular processes. Plasmonic coupling property of AuNPs can provide valuable information about distance between two individual particles and thus can be useful to monitor interactions or oligomerization of cell surface receptors (Figure 1f). Based on this property, Reinhard and co-workers have applied AuNPs for monitoring interactions of integrin receptors during co-localization on live HeLa cells.⁷³ Fibronectin-functionalized AuNPs were immobilized on the cell surface through fibronectin-integrin complex formations. The co-localization of receptors and their dynamic fluctuation could be effectively monitored using this system.

QDs have also been used as nanoprobes for cell surface sensing. For instance, Vu and co-workers have demonstrated the accurate measurement of the internalization and endosomal trafficking of serotonin (5-hydroxytryptamine, 5-HT) receptors using single QD probe and quantitative colocalization.⁷⁴ Their study indicated that the presence of the QD tag did not interfere with 5-HT receptor internalization or endosomal recycling. Direct measurements revealed simultaneous trafficking of the 5-HT_{1A} receptor in two distinct endosomal recycling pathways. An electrochemiluminescence energy transfer (ECL-RET) system has been generated using antibody conjugated QDs and fluorophore modified cell to detect the expression on the cell surface (Figure 1g).⁷⁵ Some other examples of using QDs include monitoring epidermal growth factors (EGF) erbB/HER mediated-signal transduction on live cells, receptor EGFR dimerization and/or oligomerization and analyzing lateral dynamics of glycine receptors on live cells.^{76–78}

Cell surface sensors on exogenously transplanted stem cells to monitor cellular niche environments

As mentioned above, the ability to explore cell function and cell-cell communication within the in vivo microenvironments (i.e., stem cell niche, tumor microenvironment and inflammatory sites) is essential to understand complex physiology, cell and molecular biology, and to develop effective therapeutics.¹ Additionally, for cell therapy there is significant interest to better understand and track the fate of transplanted cells.^{79–81} Unfortunately, probing what cells sense and how they respond to surrounding signals particularly in vivo in real-time is still an unmet challenge. We have recently introduced a simple platform approach to study and monitor the cellular micro- and nano-environment and intercellular communication at a single cell level in real-time in vitro and potentially in living animals with unprecedented spatiotemporal resolution (Figure 6a).⁷

Specifically, we have immobilized fluorescent nanosensors to the surface of cells which produce real-time signal output when target molecule(s) contact the cell surface. Our sensors are constructed using DNA aptamers, which are specific, sensitive, versatile, and can be generated for almost any signalling molecules by an in vitro selection process called Systematic Evolution of Ligands by EXponential enrichment (SELEX).^{82,83} In our proof-of-concept, we focused on developing an approach to sense the mesenchymal stem cell (MSC) microenvironment following systemic cell delivery. MSCs naturally home to both healthy and inflamed tissues including the bone marrow and ear pinna, both of which can be visualized in real-time via intravital microscopy (IVM).^{7,84} Therefore, these cells can be used to deliver sensors to tissues that allow us not only to assess/characterize the performance of our sensor platform but also to utilize it to elucidate important MSC biology. We focused specifically on aptamer sensors that detect platelet derived growth factor

(PDGF) which plays a critical role in cell trafficking/homing, angiogenesis, inflammation and tumor growth.^{85,86}

This PDGF sensor harnesses conformational changes of aptamer that occur when binding to PDGF, which brings two attached fluorescent dyes within close proximity; the crosstalk between the two dyes, which can be fluorophore/quencher (Figure 6b) or FRET pairs, yields a fluorescence signal (Figure 6c).⁷ The aptamer sensors are immobilized on the MSC surface via a well-established chemistry approach (Figure 6d) which is simple and versatile yet does not impact important cell phenotype.¹⁴

Taking a quench sensor as an example, we have demonstrated that our cell surface sensors functioned well on the cell surface: the addition of PDGF resulted in a decrease in fluorescence, which quantitatively correlates with the concentration of PDGF added into the cell solution (Figure 7a, b).⁷

We next demonstrated that our surface sensors were able to produce a real-time signal at a single cell level. This allows us to monitor cell-cell communication at high spatiotemporal resolution, which is critical to elucidate mechanisms of intercellular communication and signaling. Specifically, in a microwell assay system that allowed us to control the number of cells/well, we demonstrated that our sensor MSCs could detect PDGF secreted by neighbouring MDA-MB-231 cells in presence of 15% serum at 37 °C. As shown in Figure 7c, where the signal was imaged continuously over time (6 h), sensors on the MSC surface produced a fluorescence signal that correlated directly with the number of MDA-MB-231 cells in the same microwell with the sensor MSC.

Our ultimate goal is to use naturally homing cells (i.e., MSCs) to deliver dynamically functional sensors for visualization of cellular communication in real-time in their native in vivo microenvironment. Towards that end, we have demonstrated that sensor-MSCs can home to the bone marrow niches and can be monitored in real-time, at a single cell level in living animals by IVM. Significantly, sensor modification did not impair MSC's homing ability and transendothelial migration to an inflamed site (Figure 7d).⁷ Our ongoing research focuses on the development of robust sensor system that can function under physiological conditions as well as PDGF-producing in vivo models, which together will ultimately allow us to examine our sensor performance in vivo. We anticipate this work will lead to a new generation of biological sensing tools that function in vivo at unprecedented resolution and will be routinely apply to elucidate cell biology in vivo and to develop new therapeutics and diagnostics.

Conclusion and perspective

Cell surface sensors are powerful tools for elucidating cell functions and their interaction with cellular environment and for developing new therapeutics and diagnostics. An ideal cell surface sensor should be able to reside on the surface for desirable period of time and provide information about cell signalling at a relevant spatiotemporal resolution without interfering with cellular biology. However, challenges remain in the development and use cell surface sensors: 1) engineering sensors on the cell surface may potentially alter the native biology of cells, 2) cell surface sensors are typically unstable on the cell surface due to native cellular membrane turnover, internalization and enzyme degradation (particularly for in vivo studies), 3) most of sensors are irreversible which may present a challenge when one wants to monitor dynamic changes (i.e. increase and decrease) of target molecules, and 4) imaging cell-surface sensors in vivo often is interfered by the complex tissue environment (e.g., autofluorescence). Therefore, sensors need to be robust and have a high signal/noise ratio (in our experience, signal/noise ratio > 4 is required for imaging in living animals).

Future endeavours need to address the above-mentioned challenges. For instance, the metabolic approach, pioneered by Bertozzi and colleagues, has advantage of using the cellular machinery to introduce functional groups on the cell surface with minimal impact on cell biology.^{22–26,37,38} Additionally, modulation of the size and shape of sensing elements (i.e. nanoparticles) and the choice of immobilization pathway can be used to create relatively long-lived cells surface sensors.³² Furthermore, to address the sensor irreversibility issue, a “structure switching” aptamer sensor system has been developed.^{87,88} In this system, a part of the aptamer domain is initially blocked by a short complementary DNA strand that is connected by a PEG linker. When target is present at a certain concentration, target molecule will bind to the aptamer and replace the short complementary DNA strand that changes the distance between FRET dyes (which are labelled separately on aptamer and complementary strands) and therefore produces a FRET signal.^{87,88} A key feature of this system is its reversibility, i.e. when target concentration drops below the threshold, the complementary DNA will be restored to the initial signal state of the sensor.

An emerging trend in the cell surface sensor field is to develop and utilize robust, stable, dynamically functional sensors to monitor cell signalling and functions in their native environment in living animals. For instance, our ongoing work aims to develop a large panel of cell surface aptamer sensors that can be widely used in the future. The versatility of SELEX makes it possible to obtain aptamer sensors directly via in vitro selection for many targets under a given condition. Of particular interest is to build multifunctional systems that, for example, deliver drugs to a target tissue where sensors monitor drug releasing profile and how cells respond to drugs during therapy. One potential problem of using nucleic acid sensors in vivo is their rapid degradation by nucleases in the biological system (i.e., half-life of few minutes). However, it has been well-established that one can improve the bioavailability and nuclease resistance of nucleic acids by modifications including PEG, phosphorothioates, and nanomaterials.^{89,90} Transplantable cells equipped with sensors may also be valuable tools to continuously monitor biomarkers, metabolites, cytokines and toxins in vivo during disease, infection or therapy. Finally, a particularly exciting future direction is to engineer cell surface sensors directly in vivo, which may bypass the complex ex vivo manipulation. For instance, Bertozzi and colleagues have employed click chemistry to metabolically label surface glycans in live animals.^{24,38} This may serve as a gateway to further generate cell surface sensors in situ in living animals which can dynamically monitor cellular functions.

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(a) Conformational change (binding) **(e) Polymer patch** **(b) Cleavage by enzymes** **(f) Gold nanoparticles (AuNP)** **(c) Receptor clustering** **(g) Quantum dot (QD)** **(d) Luciferase (Luc)** **(h) Aptamer sensor** **Figure 1.**

Schematic presentation of commonly used cell surface sensor designs. (a) Fluorescent proteins undergo conformational changes upon binding to a specific target inducing FRET.^{3–5,48} (b) Fluorescence signalling cell surface sensor based on cleavage of the substrate by specific enzymes.⁵⁴ (c) FRET sensor to monitor cell surface receptors aggregation.⁶² (d) Luciferase enzyme sensor for ATP monitoring on cell surface.⁶⁰ Luciferase on the cell surface is activated by ATP which is excreted by cells and cleaves a substrate producing luminescence. (e) Polymer based colorimetric cell surface sensor to monitor cell surface perturbation that undergoes color transition.⁶⁴ (f) Gold nanoparticles with unique surface plasmon resonance properties as probes for monitoring cell surface receptor aggregation.⁷³ (g) FRET based sensor using quantum dots to monitor cell surface markers.⁷⁵ (h) Aptamer sensors on cell surface using FRET mechanism for monitoring cell signalling and cellular microenvironment.⁷

Figure 2.

Schematic illustration of representative approaches of cell surface sensor installation. (a) Genetic approach: DNA that can encode a desired protein is inserted into a plasmid DNA and introduced into the cell which is expressed by the cell and transported onto the cell. (b) Covalent coupling: There are two approaches for covalent coupling. In the first one, a biotin is covalently attached onto the cell surface protein to their amine groups using NHS (N-hydroxysuccinimide)-biotin followed by coupling with avidin/streptavidin. The surface is then labelled with biotinylated exogenous molecules of interest (i.e. aptamer). In the second one, the surface protein molecules on the cell surface are directly conjugated to NHS-activated sensing moieties. (c) Hydrophobic interaction: A fatty acid (palmitoyl derivatives)-

conjugated sensing moiety is mixed with cells. The hydrophobic fatty acid tail is incorporated into the cell membrane displaying the sensor part outside the cell membrane. (d) Affinity labeling: Specific ligands such as antibody or aptamer can recognize their targets on the cell surface. The ligand can be pre-conjugated to other materials such as nanoparticles and enzymes. (e) Enzymatic ligation: Acceptor peptide (AP)-tagged recombinant protein on the cell surface can be enzymatically biotinylated using special enzyme such as biotin ligase. The biotins are available for interacting with streptavidin modified ligands or any desired species. (f) Metabolic labelling: A functional sugar derivative such as fucose-azide (FucAz) or keto-sugar is introduced into cell culture media. The cells use the sugar moieties in their metabolic pathway to be tagged into the glycoproteins and finally transported onto the cell membrane. The functional groups are now available for coupling with other biomolecules of interest. (Adapted from ref 32).

Figure 3.

Glutamate nanosensor (FLIPE). (a) Schematic presentation of the sensor displayed on the cell surface using transmembrane spanning domain of the platelet-derived growth factor (PDGF). (b) FLIPE construct consisting of a secretion signal at the N-terminus of FLIPE and a C-terminal fusion with the PDGF membrane span. (c) Confocal images of hippocampal neurons expressing FLIPE-600n. (d) A hippocampal cell stained with FM4-64. Synapses stained in red. (e) and (f) FRET change at the surface of hippocampal neurons expressing FLIPE in response to electrical stimulation. (e) Ratiometric images and (f) corresponding quantification of a hippocampal neuron expressing FLIPE after electrical stimulation or perfusion with varying concentrations of glutamate. The presence of glutamate caused a decrease of FRET signal. (Reprinted from ref 11 with permission)

Figure 4.

Semisynthetic Snifit sensors for monitoring metabolites on cell surface. (a) Design of the sensor and fluorescent signal generation on the cell surface upon interacting with the target. The protein part of the sensor is a fusion protein of SNAP-tag, CLIP-tag, and a binding protein (BP, shown as grey). The active semisynthetic sensor protein is obtained by labelling SNAP-tag with a PEG molecule containing a fluorophore (red rectangle) and a ligand for the binding protein (purple ball) and by labelling CLIP-tag with a second fluorophore (green rectangle). In the absence of analyte, the intramolecular ligand binds to the binding protein, keeping the sensor protein in a closed conformation. Donor and acceptor fluorophores are in close proximity, resulting in a high FRET efficiency. In the presence of analyte, the

intramolecular ligand is displaced, and the sensor protein shifts toward an open conformation. Donor and acceptor fluorophore are more distant from each other than in the closed conformation and FRET efficiency therefore decreases. (b) Kinetic and thermodynamic characterization of the sensor protein SNAP-CLIP-BP on the surface of HEK 293T cells. Time course of the perfusion of the labelled sensor with increasing amount of the metabolite, benzenesulfonamide (a = 1 μ M, b = 10 μ M, c = 50 μ M, d = 100 μ M, e = 500 μ M, f = 1 mM, g = 10 mM). (c) Benzenesulfonamide titration curve of SNAP-CLIP-BP on the extracellular surface of HEK 293T cells. (Adapted from ref 3)

Figure 5.

Cell surface sensor for monitoring phospholipid expression on the cell membrane. (a) Design of the sensor and FRET signaling properties after binding to the targets. The sensor is composed of PH (Pleckstrin Homology) domain that binds to PtdInsP₃ and two fluorescent proteins CFP and YFP including a rigid helix (EAAAR)_n and a gly-gly hinge. The sensor is displayed on the cell surface using MLS (membrane localization sequence). (b) Diagram of the sequence design indicating different domains including the linkers. (c) Subcellular localization of the sensor (flip-pm) in CHO cells. The YFP images are shown in green. V, vertical sections. H, horizontal. (d) Color images of the CFP:YFP ratio at different time points (0, 100, 300 and 500 s) after addition of 50 ng/mL PDGF. (Adapted from ref 48).

Figure 6. Cell surface aptamer sensors. (a) Schematic representation of the concept of probing cellular niche environment and signaling using cells engineered with cell surface aptamer sensors. PDGF aptamer sensors immobilized on Mesenchymal Stem Cells (MSCs) (deep yellow indicated as Sensor-cells) recognize signaling molecules (PDGF in this case) secreted by niche cells sitting on the extracellular matrix and produces fluorescent signal. (b) Sequence and design of the PDGF aptamer sensor with fluorophore and quencher pair. 5'-end of the aptamer sensor is labelled with fluorophore (FAM). The extended 3'-termini is hybridized with a short capture oligonucleotide which is labelled with biotin for immobilization on the MSCs through streptavidin-biotin method as shown in (d). (c) The solution performance of quenching ability of the aptamer sensor upon addition of PDGF. (Adapted from ref 7)

Figure 7.

Performance of the MSC-aptamer sensor in living cells and animal. (a) Representative fluorescent microscope images of the MSC-aptamer sensor before and after adding PDGF. (b) Signal generated by MSC-aptamer sensor at different concentration of PDGF (signals were defined as the ratio of geometric means (GM) before and after the addition of PDGF). (c) Real-time sensing of PDGF secretion from neighboring MDA-MB-231 cells by sensor-engineered MSCs. Left panel: Microwells containing different numbers of PDGF-producing MDA-MB-231 cells (green) including sensor-MSC (red) at time $t = 0$ (n is the number of MSCs used in the analysis). In this set of experiments, the aptamer was labelled with a red dye (Cy5), and with Iowa Black RQ as a quencher. Right panel: fluorescence of MSC-aptamer declining during the course of PDGF production. The signal, which is defined as the percentage of MSCs that have fluorescence intensity less than 50% of their initial value at the indicated time, correlates with the number of PDGF-producing MDA-MB-231 cells in the same well as a sensor MSC. (d) Bone marrow homing and transmigration of aptamer-labelled MSCs. (d-I) Large-area map of right parietal bone marrow compartments in eight week-old Balb/c mouse 24 h after injection of native MSC and aptamer-MSC. Several image stacks were acquired in the right parietal bone $\sim 200 \mu\text{m}$ to the right of the sagittal suture. (d-II) Zoomed-in image of the area in the white box in d-I, shows a similar distribution of MSC (green) and aptamer-MSC (blue) in the vicinity of a large venule (red). (d-III) Quantification of the average number of cells per z-stack. (d-IV) Quantification of percentage of cells positioned outside blood vessels shows no difference between MSC and aptamer-MSC (P -value = 0.116). MSC, green; aptamer-MSC, blue; blood vessels, red; bone, white. Scale bar, $100 \mu\text{m}$. (Adapted from ref 7)