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Review

Sensing Nitric Oxide in Cells: Historical Technologies and Future Outlook

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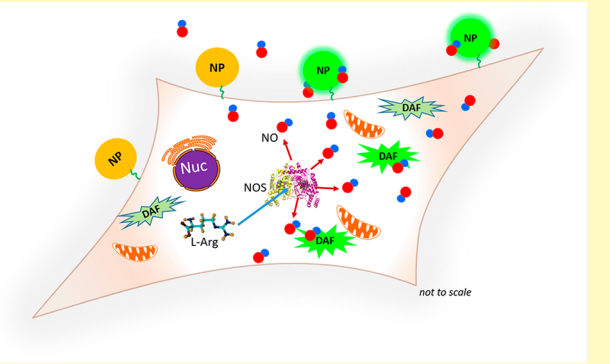
ABSTRACT: Nitric oxide (NO) is a critical cell signaling molecule with important roles in both normal cellular physiology and pathology. Over the past 20 years, multiple sensing modalities have been developed for the intracellular synthesis (endogenous) and release (exogenous) of NO. In this review, we survey the historical progression of NO sensing platforms, highlight the current state of the art, and offer a forward-looking view of how we expect the field of NO sensing to develop in the context of recent advances in bio-nanotechnology and nanoscale cellular biosensors.



KEYWORDS: *nitric oxide, biosensor, electrochemical, fluorescence, chemiluminesence, nanoparticle, sensor, mammalian cell, signaling*

Nitric oxide (NO) is a small molecule free radical that is produced by the nitric oxide synthase (NOS) family of enzymes, including endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). It has long been known to play numerous critical roles in mammalian cellular physiology and homeostasis (Figure 1).[1] Since its discovery as an endothelial cell-derived vascular relaxation factor, various investigations have established NO as an important signaling molecule in multiple physiological systems.[2] In vascular smooth muscle, NO activates soluble guanylate cyclase (sGC), which is a known receptor for NO, thereby increasing intracellular levels of cyclic guanosine 3′,5′-monophosphate and resulting in smooth muscle cell relaxation and vasodilation.[3−5] NO plays an integral role in muscular force production (excitation−contraction coupling), blood flow regulation, and myocyte differentiation in skeletal muscle.[6,2] In the nervous system, NO is a neurotransmitter involved in synaptic signaling and in the regulation of memory and neurogenesis.[7,8] For immune function, NO is a critical mediator of macrophages' reponse to invading pathogens where it elicits its cytostatic effects through induced DNA damage as well as through the abrogation of the function of enzymes involved in electron transport.[9,10] Similarly, the dysregulation of NO (under- or over-production) can result in a number of pathologies. For example, irregularly high (micromolar level) amounts of NO in tissues can lead to the formation of cytotoxic reactive nitrogen species and DNA damage.[11−13] In the bloodstream, inhibition of endogenous (intracellular) NO synthesis is associated with platelet

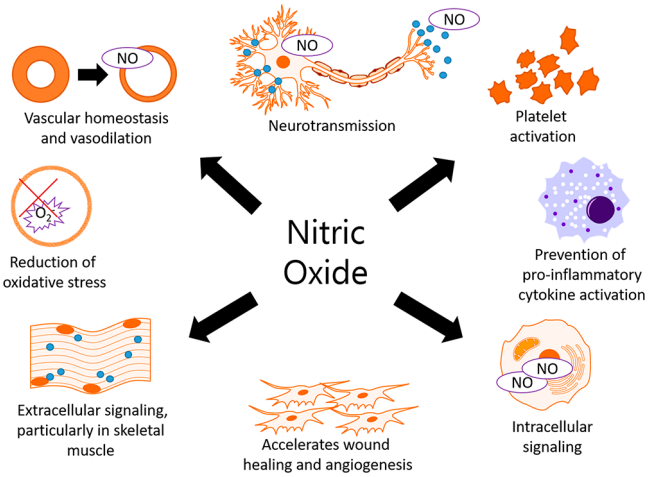


Figure 1. Roles of nitric oxide (NO) in mammalian cells. NO, which is endogenously produced by and released from cells, influences a broad range of normal physiological and disease pathologies, some of which are shown here. However, NO rapidly diffuses from the site of origin, exists in cells in the pM to nM range, and has a short lifetime, hence the need for the development of sensors capable of measuring NO both inside and outside of the cell.

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aggregation, heart failure, diabetes, hypercholesterolemia, and other diseases involving irregular platelet activation.[14] Importantly, in all of these systems, the many functions of NO are elicited in a concentration-dependent manner. For example, low concentrations of NO mediate anti-inflammatory effects by reducing T cell proliferation[15] while higher concentrations promote the pro-inflammatory response to pathogen infection.[16]

Given the important multiple roles for NO in normal cellular physiology and disease, there is clearly a need for the ability to image/sense NO *in vitro* and *in vivo* in an effort to understand both its production and function. Indeed, it is the innate physicochemical properties of NO that make its measurement a significant challenge. First, NO has a transient half-life, on the order of milliseconds to seconds (depending on the local concentration of O_2 and reactive biomolecules).[17] Second, unlike many other signaling molecules that are produced and stored in lipid vesicles, NO is synthesized in an "on-demand" fashion and diffuses readily across lipid membranes. Finally, NO elicits its function proximal to its point of synthesis, often diffusing rapidly and functioning only hundreds of micrometers from its site of origin.[18] Thus, modalities for imaging and sensing NO must be able to measure, with fidelity, cell and tissue NO concentrations under the constraints of these unique parameters. Further, it is often the NO that is released from cells to the surrounding tissues (not simply the NO that is produced intracellularly) that is biologically relevant and needs to be the target of measurement. To date, a number of sensing platforms have been developed for the real-time measurement of NO *in vitro* and *in vivo*. These include electrochemical sensors, fluorescent organic dyes or metal-based probes, and genetically encoded biosensors.

Our goal in this review is to first provide a snapshot of the current state of the art in NO sensing. We broadly divide this discussion into those technologies that sense "endogenous" or intracellular NO and those that measure "exogenous" or "extracellularly released" NO. Here, we describe exogenous NO sensors to be sensors that reside outside of the cells and thus measure NO by a group of cells or a tissue after it has diffused out of the cells. Endogenous NO sensors are those that are taken up by the cell and are incubated prior to NO stimulation such that they measure NO as it is being produced by the singular cell prior to diffusion out of the cell. Therein, we discuss the inherent advantages and limitations of these various platforms. Next, we provide a forward-looking view of how we anticipate the field of NO sensing/imaging to develop in the future. We focus particularly on the expected role of various nanoparticles (NPs) and functional bio/nanomaterials in enabling the next generation of sensing platforms for NO.

SENSING TECHNOLOGIES FOR ENDOGENOUS NITRIC OXIDE

Various technologies for sensing and imaging NO have been developed over the past three decades. These include electrochemical probes, chemiluminescent and fluorescent reporters, and genetically encoded sensors for measuring NO in bulk solution or in cells and tissues. In this section, we describe these commonly used NO sensing methodologies while highlighting their advantages for *in vitro* and *in vivo* applications.

Electrochemical NO Probes and Chemiluminescence/Fluorescence-Based NO Sensors. Perhaps the most commonly used methods for measuring NO released within cells are electrochemical probes and chemiluminescence or fluorescence-based sensors. The first electrochemical NO probe, described in 1992 by Malinski and Taha, was a porphyrin-decorated carbon fiber with a Nafion fluoroplymer coating to decrease noise from other molecules and improve NO detection signal. Using computer-controlled micropositioning, two microsensors were placed on porcine aortic rings, the first on the surface of an endothelial cell and the second implanted into a smooth muscle cell. Five seconds after injection of bradykinin, significant increases in NO (up to 450 nM) were measured being released from the endothelial cell.[19] Vallance *et al.* expanded on this work by using the same Nafion-coated porphyrinic microsensor to probe NO in humans.[20] The microsensor was inserted into a vein in the hand, and bradykinin was infused to realize a mean peak concentration for NO detection of 124 nM. This sensing technology has been commercialized as the amiNO sensor (Innovative Instruments Inc.) with sensitivity down to 1 nM NO and 0.01 nM resolution when measuring NO in bulk solution. Further, this technology paved the way for innovation in even more types of electrochemical probes for measuring both endogenous and exogenous NO, such as the fluorinated xerogel-derived[21] and sol−gel-derived microsensors developed by the Schoenfisch group.[22]

The limitations of electrochemical sensors for NO detection set the stage for the development of chemiluminescence and fluorescence-based molecular sensors. To date, only modest progress has been made on the chemiluminescence front. These systems measure NO indirectly through its activation of sGC, resulting in the release of pyrophosphate and the generation of ATP, which is detected by a luciferin−luciferase system with light emission at 560 nm.[23−25] While exhibiting nM sensitivity for NO, these systems have yet to be implemented *in vivo*. Much further progress has been made on fluorescence probes for NO sensing. The Nagano group developed the first generation diaminofluorescein (DAF) family of probes which remain the most commonly used probes for sensing intracellular NO.[26−28] Li *et al.* developed a photoinduced electron transfer (PeT) two-photon fluorescence probe for imaging NO specifically during endoplasmic reticulum stress (Figure 2).[29] They synthesized a fluorescence probe consisting of a naphthalimide core coupled with the functional moieties methyl sulfonamide and *o*-phenylenediamino (OPD) to target the endoplasmic reticulum (ER) and to recognize NO, respectively. In the absence of NO the probe is nonfluorescent due to eT-mediated quenching of the naphthalimide core by the OPD. Reaction of NO with the OPD induces a conformational change and inhibition of PeT, resulting in a fluorescence "on" state. When tested against the NO donor diethylamine (DEA)-NONOate, in phosphate buffered saline (PBS), a 3.3 nM detection limit was achieved. Additionally, negligible fluorescent change (response similar to a blank negative control) was seen when the sensor was tested against potential interference species such as reactive nitrogen species, reactive oxygen species, ascorbic acid, glutathione, and others; only NO resulted in a significant enhancement to fluorescent signal. In human cervical cancer (HeLa) cells, the probe localized to the cytosol and displayed a strong fluorescence signal upon addition of 20 μM DEA-NONOate. Similar results were obtained for lipopolysaccharide (LPS)-induced NO generation, and this probe was used *in vivo* for two-photon imaging of NO in mouse liver tissue sections. Among the several limitations of these fluorescent probes are

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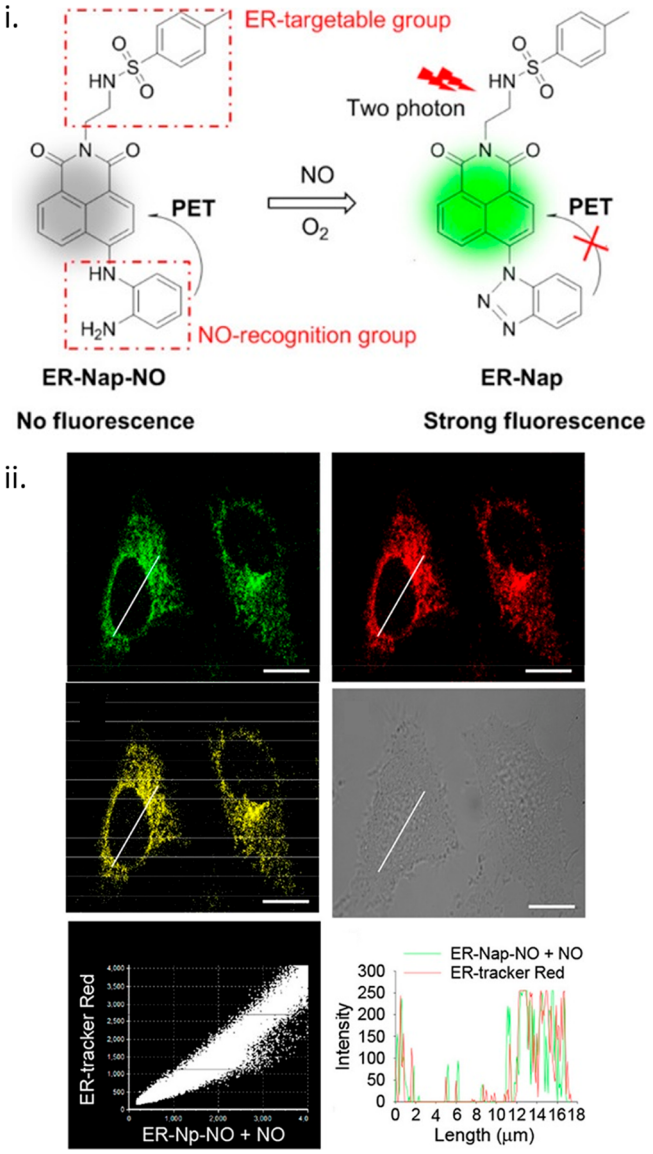

Figure 2. Current approaches for sensing endogenous nitric oxide. (i) Schematic of the ER-Nap-NO probe. (ii) Fluorescence image showing colocalization of probe in HeLa cells. Images are as follows: (top left) 10 μM ER-Nap-NO and 20 μM DEA-NONOate; (top right) ER-tracker red; (middle left) merged image of the two top images; (middle right) bright-field image; (bottom left) intensity scatter plot; (bottom right) fluorescence intensity line scan across white line shown in top images. Reprinted with permission from ref 29. Copyright 2018 American Chemical Society.

their sensitivity (typically on the order of μM NO) and the need to stimulate the cells with drugs or NO donors to induce cells to produce enough NO to be visualized. Further, these probes require high loading conditions (μM concentration of probe) which can induce cytotoxicity at longer time points. Finally, these sensors, particularly the optical fluorescent sensors, irreversibly bind with NO, thus limiting the potential for real-time, dynamic measurements of NO production.

Other NO Sensing Methods. Other sensing methods that have been implemented include those based on genetically encoded NO sensors,[30−32] plasmonics,[33,34] electron spin resonance,[35] and metal−organic framework luminescence.[35−37] For example, Sato *et al.* realized an amplifier-coupled fluorescent NO indicator with nM sensitivity (0.1 nM detection limit).[30] Plasmids encoding both subunits of sGC were developed as fusions to fluorescent guanosine 3′,5′-cyclic monophosphate (cGMP) indicators. In the presence of NO, the dimers engaged in Förster resonance energy transfer (FRET). This FRET response was tested in the presence of carbon monoxide, which is known to potentially interfere with the FRET response of the sensor; however, 100 μM carbon monoxide only resulted in ∼15% emission ratio change compared to 100% change with 5 nM NO, suggesting the high selectivity of this probe. In vascular endothelial cells transfected with these plasmids, NO sensing was achieved with ∼1 nM sensitivity. Further, these sensors were found to be reversible as NO binding could be removed by oxidation and endogenous phosphodiesterases hydrolyzed the cGMP molecules following this NO removal. Similarly, the Malli group developed a genetically encoded, multicolored fluorescent, quenching-based NO probe called geNOps.[31] A bacterial NO binding domain was conjugated with different fluorescent proteins, and HeLa cells were transfected with this probe. Upon binding of the probe to NO, the fluorescence signal of the fluorescent proteins changes as a result of electron transfer. A recent review by Eroglu *et al.* describes more progress in genetically encoded fluorescent probes for measuring endogenous NO.[32]

Using a different approach, the Li group took advantage of the unique optical properties of upconversion nanoparticles (UCNPs) to develop a nanoprobe for ratiometric measurement of NO in cells.[38] The nanoprobe consists of an UCNP core (with luminescence emission peaks at 540 and 656 nm) encapsulated within a mesoporous SiO_2 shell loaded with rhodamine B. NO sensing was achieved *via* luminescence resonance energy transfer (LRET) between the rhodamine B and the UCNP upon reaction of the rhodamine B with NO. In HeLa cells, after treatment with up to 0.4 mM DEA-NONOate, emission in the 540 nm channel decreased with increasing NO concentration. The UCNPs could similarly measure NO in deep tissue slices by using emission at 625 nm.

Other systems have used reaction-based surface-enhanced Raman scattering for measuring NO at nM−μM levels.[39,40] Cui *et al.* developed nanoprobes consisting of OPD-modified gold nanoparticles (AuNPs/OPD) for endogenous NO detection within murine macrophages (RAW 264.7) (Figure 3).[39] Here, NO sensing was achieved by tracking the Raman shift as a result of NO and OPD interactions on the AuNP surface. After stimulation with 5 μg/mL LPS, the characteristic NO peak at 789 cm^{-1} appeared and strengthened over 6 h, confirming increasing concentrations of NO. Although the researchers did not quantify the NO concentration within the cells, they had found that the limit of detection of their probe in solution was 170 nM. Furthermore, the sensor was found to be highly selective when compared to a variety of potential interference species; the researchers found that only NO caused a significant shift in the Raman spectra.

Despite their high sensitivity, selectivity, and response rate in these alternative methods, they remain limited in many of the same ways as electrochemical, fluorescent, and genetically encoded sensors in that they measure only endogenously generated NO (not NO released from cells) and the probes are often sequestered within the endolysosomal pathway.

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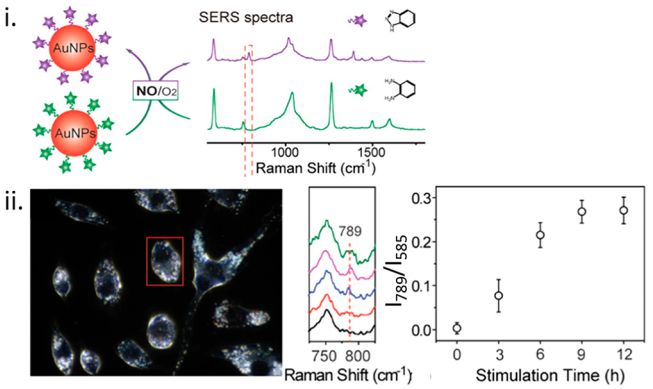

Figure 3. Current approaches for sensing endogenous nitric oxide. (i) Schematic of nanoprobes with representative SERS spectra showing the Raman shift upon interaction with NO. (ii) Dark-field microscopy image of RAW 264.7 cells incubated with nanoprobes (left), SERS spectra at 789 cm^{-1} after LPS stimulation over time (middle; black, 0 h; red, 3 h; blue, 6 h; purple, 9 h; green, 12 h), and ratiometric peak intensities over time after LPS stimulation on the basis of the SERS spectra (right). Reprinted with permission from ref 39. Copyright 2016 Elsevier B.V.

SENSING TECHNOLOGIES FOR EXOGENOUS NITRIC OXIDE

Given the important biological role of exogenous NO, many sensors and probes have been developed to sense NO released from cells. Whereas traditional methods measure exogenous NO from large groups of cells or tissue, more recent work has focused on the application of the same techniques described in the previous section to sense NO released from single cells or small subsets of cells.

Electrochemical NO Probes. Electrochemical NO probes were among the first types of probes/sensors used to measure NO production, whether endogenously or exogenously released. One such example of an electrochemical NO probe to measure exogenous NO by Deng *et al.* is composed of antimony tetroxide nanoflowers (Sb_2O_4) and reduced graphene oxide (rGO) coated onto a glassy carbon electrode.[41] In the culture medium of human umbilical vein endothelial cells (HUVECs) or adenocarcinomic human alveolar basal epithelial cells (A549), when induced with acetylcholine (ACh), there was a noticeable increase in sensor response, indicating increasing NO concentrations. Using a different approach, Kim *et al.* developed a semiconductor surface comprised of porous ZnO NPs immobilized onto a Nafion-coated, poly(terthiophene-rGO) (poly(TTBA-rGO)) probe with a glassy carbon electrode base.[42] ZnO and poly(TTBA-rGO) express synergistic electrocatalytic activity, and the porous nature of the ZnO provides a high surface area for increased ion−electron transport rates to catalyze NO. The probe was tested in a solution containing trypsinized rat adrenal medulla pheochromocytoma (PC-12) cells or normal human embryonic kidney (HEK293) cells. After stimulation with 50 μM LPS, the researchers quantified the amperometric response of the electrode, finding that PC-12 cancer cells had a greater NO response compared with HEK293 cells, demonstrating the promise of this sensor for diagnosis of cancer diseases.

Despite these successes, electrochemical NO sensors still face considerable challenges. First, these systems are bulky (10 μm to 2 mm probe size with accompanying hardware) and have yet to be miniaturized to the point that they interrogate single cells. Second, these sensors do not provide long-term monitoring capability that lends itself to passive *in vivo* monitoring.

Measuring Exogenous NO from Single Cells. Advances in graphene nanotechnology have spurred the development of electrochemical sensors that simultaneously serve as the culture substrate for adherent cells while measuring NO as it is released from the attached cells.[43−46] A summary of these four studies can be found in Table 1. Here, we describe in detail two of these studies: to address the limited diffusion distance, rapid metabolization, and degradation rates of NO, Guo *et al.* developed RGD peptide-functionalized graphene sensors wherein the RGD was covalently bound to the graphene surface to enhance cellular adhesion in close proxmity to the sensor elements (Figure 4).[43] They confirmed that the sensor measured NO released from HUVECs stimulated with 1 mM ACh with a limit of detection of 90

Table 1. Examples of Sensors for Exogenous NO from Single Cells/Small Subsets of Cells[a]

method	sensing configuration	selectivity	NO LOD	cells tested	ref
electrochemical	RGD-peptide and pyrenebutyric acid-functionalized graphene film	NO_2^-, K^+, Na^+, NO_3^-, SO_4^-, AA	80 nM	HUVEC	43
	metalloporphyrin and (3-aminophenyl)boronic acid-functionalized reduced graphene oxide film	AA, DA, UA, NO_2^-, 5-HT, H_2O_2, L-Arg, ACh	90 pM	HUVEC	44
	iron phthaolocyanine-functionalized, nitrogen-doped graphene nanocomposite film	KCl, Na_2SO_4, $NaNO_2$, glu, $Ca(NO_3)_2$, K^+, Cl^-, Na^+, Ca^{2+}, SO_4^{2-}, NO_2^-, NO_3^-	180 nM	HUVEC	45
	graphene paper loaded with Au/Pt core/shell nanoparticles	UA, AA, DA, Cl^-, NO_3^-, SO_3^{2-}, SO_4^{2-}, CA^{2+}, K^+, Na^+, Mg^{2+}, Cd^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Hg^{2+}, Pb^{2+}, Zn^{2+}, Fe^{3+}, NO_2^-	100 nM	HUVEC	46
genetically encoded	transfected cell line expressing FRET-induced, amplifier-coupled fluorescent indicator	CO, ANP, CNP, L-Glu	20 pM	neurons, VEC	47
fluorescence	amphiphilic fluorescent probe bound to hydrophobic alkyl chain	NO_3^-, NO_2^-, HNO, H_2O_2, $ONOO^-$, ClO^-,.OH, $^1O_2$, GSNO, SNAP, AA, DHA, MGO	830 pM	RAW 264.7, ECV-304	48

[a]Abbreviations: 5-HT, 5-hydroxytryptamine; AA, ascorbic acid; ACh, acetylcholine; ANP, atrial natriuretic peptide; CNP, C-type natriuretic peptide; CO, carbon monoxide; DA, dopamine; DHA, dehydroascorbic acid; ECV-304, human vascular endothelial cells; glu, glucose; GSNO, *S*-nitrosoglutathione; H_2O_2, hydrogen peroxide; HUVEC, human umbilical vein endothelial cell; L-Arg, L-arginine; L-Glu, L-glutamate; LOD, limit of detection; MGO, methylglyoxal; NO, nitric oxide; RAW 264.7, murine macrophages; SNAP, S-nitroso-N-acetylpenicillamine; UA, uric acid; VEC, vascular endothelial cells.

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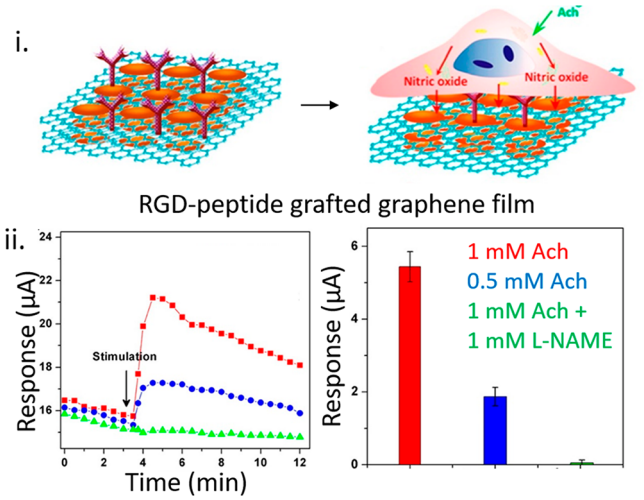

Figure 4. Current approaches for sensing exogenous/released nitric oxide. (i) Schematic of RGD-peptide grafted graphene film with seeded HUVEC cells. (ii) Real-time response of sensor to NO released from attached HUVEC cells when treated with 1 mM acetylcholine (red), 0.5 mM acetylcholine (blue), and 1 mM acetylcholine with 1 mM L-NAME (green) showing concentration differences and NO specificity as a result of signal inhibition when treated with a NO inhibitor (L-NAME). Adapted with permission from ref 43. Copyright 2012 American Chemical Society.

nM. As a result of the surface carboxyl groups, potential interference species such as nitrite and ascorbic acid were repelled, resulting in a high selectivity of the sensor for NO. Further, the researchers demonstrated the reversibility of their system by showing that the sensor could be used repeatedly without any changes to its function. Similarly, the Liu group improved the limit of NO detection by decorating nitrogen-doped graphene sensors with iron phthalocyanine (FePc) and layers of poly-L-lysine and Nafion to improve the signal-to-noise ratio.[45] HUVECs stimulated with 0.25 nM L-arginine resulted in enhanced sensor response with a limit of detection near 90 pM that could also be used repeatedly with up to 80% of its initial response retained after 3 cycled uses. Further, these sensors were shown to have <10% signal response to interfering species, demonstrating the high selectivity to NO.

Sato *et al.* built upon their dual plasmid approach (*vide supra*) to develop a cell-based indicator that would be placed outside of the NO-releasing cell (Figure 5).[47] A summary of this study is also provided in Table 1. In their previous approach, the cells of interest were transfected. However, here, porcine kidney cells (PK15) were stably transfected to express a sGC fluorescent protein indicator for cGMP that fluoresces upon NO binding to the sGC through FRET, which they named Piccell. Piccell was cocultured with vascular endothelial cells, and, upon release of NO, Piccell took up the released NO, engaged in FRET, and visualized this release. The researchers were able to measure basal NO levels of 40 pM, which increased to 100 pM NO following stimulation with 1 μM ATP. Piccell was shown to have no significant response to interfering species such as carbon monoxide, atrial natriuretic peptide, and L-glutamate, among others, up to 100 μM, and the sensor demonstrated the ability to visualize spatial NO diffusion as it is released from the cell. Additionally, Piccell was also found to be reusable due to the reversible binding of NO to sGC due to oxidation and the subsequent hydrolization of cGMP by phosphodiesterases.

Yao *et al.* sought to use a simpler approach wherein a fluorescent probe (disodium 2,6-disulfonate-1,3-dimethyl-5-hexadecyl-8-(3,4-diaminophenyl)-4,4′-difluoro-4-bora-3*a*,4*a*-diaza-*s*-indacene (DSDMHDAB)) was covalently attached to C_{16} alkyl chains to realize amphiphilic fluorescent probes that

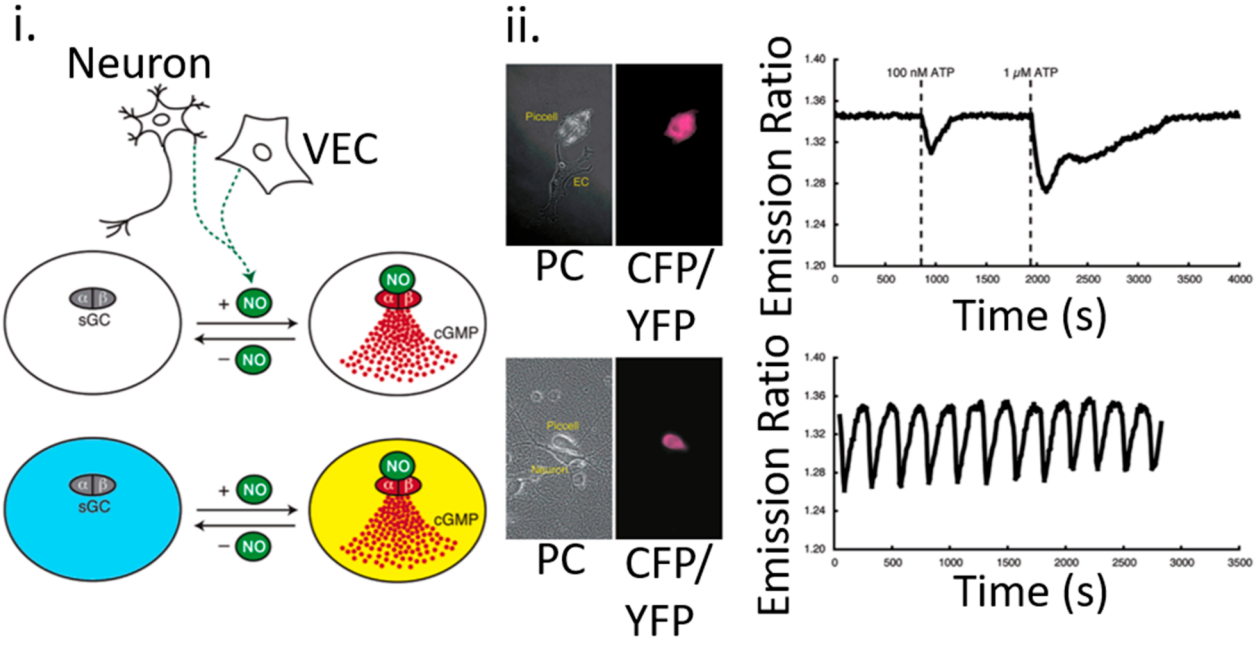

Figure 5. Current approaches for sensing exogenous/released nitric oxide. (i) Schematic demonstrating fabrication of the cell-based NO sensor, Piccell, *via* plasmid transfection. (ii) Phase contrast and pseudocolor image of CFP/YFP emission ratio for VEC cocultured with Piccell (top left) and corresponding FRET time course after stimulation with ATP (top right). Phase contrast and pseudocolor image of CFP/YFP emission ratio for hippocampal neurons cocultured with Piccell (bottom left) and corresponding, oscillatory FRET time course with no stimulation (bottom right). Reprinted with permission from ref 47. Copyright 2006 American Chemical Society.

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could insert into the plasma membrane of the cell and measure released NO at the cell surface (Figure 6).[48] A summary of this

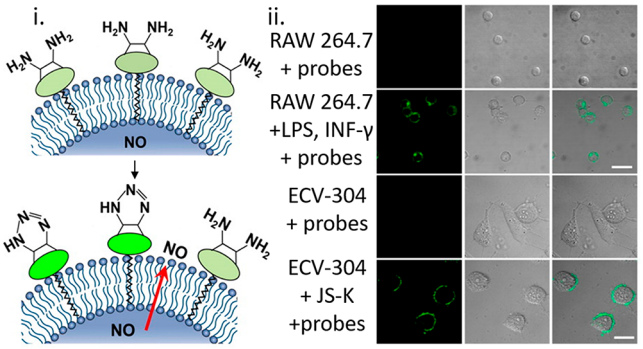

Figure 6. Current approaches for sensing exogenous/released nitric oxide. (i) Schematic of fluorescent sensor appended to the cellular plasma membrane. (ii) Confocal fluorescence images of RAW 264.7 cells (top) and ECV-304 cells (bottom) with and without probes and stimulants showing response (fluorescent signal) at the edge of the cells to released NO. Adapted with permission from ref 48. Copyright 2016 American Chemical Society.

study is also provided in Table 1. The sensor exhibited high selectivity against multiple interference species, even when the interference species were tested at mM concentrations and the NO was at 8 μM. Both RAW 264.7 macrophages and human vascular endothelial (ECV-304) cells demonstrated elevated fluorescent signals at the plasma membrane after incubation with stimulants, resulting in NO concentrations ranging from 0 to 20 μM.

FUTURE OUTLOOK AND CHALLENGES

Clearly, significant progress has been made over the past 20 years in the development of cellular sensing platforms for both endogenous and exogenous NO. These technologies have facilitated the measurement of biologically relevant NO concentrations with spatiotemporal resolution. So, going forward, what can we expect? Despite the good sensitivity, selectivity, and biocompatibility of the sensors discussed here, there remain various limitations that we must seek to address in our design of the next generation of NO sensors and probes. While biocompatibility of the probes is high, many of these tests are conducted at short time scales and maintaining cellular viability over long time scales can be challenging, especially when translated to an *in vivo* model with a host immune system. Further, sensitivity and selectivity remain a concern due to the very low (pM) concentrations of NO naturally. In many of these studies, NO production by the cell is artificially stimulated prior to measurement. Thus, reducing noise to sense NO without external stimulation is another key aspect future studies should consider. Finally, NO diffuses through cell membranes very quickly and has a short half-life, as previously described. Thus, the kinetics of these sensors must be improved, as these measurements must be conducted quickly without much lag time between measurements.

Additionally, given the critical nature of exogenous/released NO in cellular physiology and the important role played by the plasma membrane in regulating its release,[49] there still exists ample opportunity for the realization of sensing technologies that interface directly with the membrane bilayer to measure NO release in real time. Such a membrane-tethered NO sensing capability would, for example, enable the measurement of NO release from large populations of cells (hundreds to thousands) with single cell resolution. With the dawn of personalized cell-based therapies (*e.g.*, chimeric antigen receptor T cell therapy for cancer[50]) and cellular engineering programs (*e.g.*, DARPA's Biological Control Program (https://www.darpa.mil/program/biological-control)), which are aimed at genetically engineering intracellular controllers that intimately self-modulate the cellular production of desired molecules, there is an ever-increasing need for cell-based sensors that can measure the output from single cells for screening, identification, selection, and optimization.

It is in this context that we expect a variety of functional bio/nanomaterials such as semiconductor quantum dots (QDs), AuNPs, and organic dye−lipid conjugates to play a central role. NP bioconjugates (NPs interfaced with various biological moieties) offer a number of distinct advantageous over other materials for sensing at the plasma membrane. First, their small size (<10 nm) makes them ideal for interfacing non-invasively with the 5 nm expanse of the membrane bilayer, and a number of moieties including peptides,[51−53] cholesterol derivatives,[54,55] and purified membrane fragments[56] have recently been reported that provide persistent tethering to the exofacial leaflet of the membrane bilayer. Second, NPs possess a variety of optoelectronic properties (FRET, charge transfer, and redox) that can serve as the basis for sensing schemes for NO at the membrane. Finally, for *in vivo* applications, NPs' circulation time and clearance properties can be tailored by iteratively tuning the nature of the NP surface.[57]

Multiple examples have already detailed NP-based approaches for NO sensing (Table 2), and QDs have played a major role in these platforms. The Huang group developed

Table 2. Nanoparticle-Based NO Sensors[a]

nanoparticle type	composition	selectivity	NO LOD	cells tested	ref
QD	CdSe/ZnS QDs with surface-bound Fe(III) dithiocarbamate ligands	$ONOO^-$, NO_2^-, NO_3^-, ClO^-, OH^-, TBHP, H_2O_2, O_2^-, CO, CN^-	3 μM	n/a	58
	CdSe QDs encapsulated in mHP nanospheres	NO, NO_2^-, NO_3^-, H_2O_2, ClO^-, $ONOO^-$	25 nM	n/a	59
	CdTe/CdS/ZnS QDs functionalized with DAN-1	N_3^-, NO_3^-, NO_2^-, ClO^-, SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$, CO_3^{2-}, GSH, Vc, Cys, H_2O_2,.OH	51 nM	n/a	60
C-dot	aminoguanidine/citric acid C-dots	H_2O_2, NO_3^-, NO_2^-, KO_2, O_2, HNO_4	80 nM	RAW 264.7	61
AuNP	AuNP-alkyne or AuNP-azide	SO_2, CO_2, NO_2, O_2, CO	1.4 ppm (v/v)	n/a	62

[a]Abbreviations: AuNP, gold nanoparticle; C-dot, carbon dot; CdS, cadmium sulfide; CdSe, cadmium selenide; CdTe, cadmium telluride; CO, carbon monoxide; Cys, cysteine; DAN-1, 4-(((3-aminonaphthalen-2-yl)amino)methyl)benzoic acid; GSH, glutathione; H_2O_2, hydrogen peroxide; LOD, limit of detection; mHP, modified hyperbranched polyether; n/a, not applicable; NO, nitric oxide; ppm, parts per million; QD, quantum dot; RAW 264.7, murine macrophages; TBHP, *tert*-butyl hydroperoxide; v/v, volume ratio; Vc, vinyl chloride; ZnS, zinc sulfide.

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red-emitting QDs decorated with Fe(III)-bound dithiocarbamate ligands wherein exposure to NO caused a reduction of Fe(III) to Fe(II) and decreased FRET from the QD donor and subsequent increase in QD photoluminescence with increasing NO concentration.[58] Liu *et al.* reported on CdSe core QDs encapsulated within a hyperbranched polyether matrix that served as both a NO donor and sensor.[59] The QD−ligand−NO complexes release NO molecules when placed in aqueous buffer resulting in an increase in QD photoluminescence. While these two aforementioned systems are limited in their biocompatibility due to their ligand configurations, recent examples have described more biocompatible strategies. Bhattacharya *et al.* prepared carbon dots where aminoguanidine and citric acid enabled simultaneous colorimetric and fluorescence detection of NO through diazo bond formation on the NP surface.[60] The Hu group developed CdTe/CdS/ZnS QDs functionalized with an aminonaphthalene benzoic acid derivative that yielded highly fluorescent naphthotriazole in the presence of NO.[61] In a nonfluorescent scheme, the Parra group realized colorimetric AuNPs that undergo azide−alkyne click chemistry-mediated aggregation in the presence of NO.[62] On the basis of the established biocompatibility of a number of NPs and the availability of a variety of membrane-targeting moieties, we anticipate the further development and transition of these novel sensing platforms to *in vitro* and *in vivo* applications.

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Notes

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ABBREVIATIONS

ACh, acetylcholine; AuNP, gold nanoparticle; cGMP, guanosine 3′,5′-cyclic monophosphate; DAF, diaminofluorescein; DEA, diethylamine; eNOS, endothelial nitric oxide synthase; eT, electron transfer; FRET, Förster resonance energy transfer; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; LRET, luminescence resonance energy transfer; NO, nitric oxide; NOS, nitric oxide synthase; nNOS, neuronal nitric oxide synthase; NPs, nanoparticles; OPD, *o*-phenylenediamino; PeT, photoinduced electron transfer; poly(TTBA), poly(terthiophene); QDs, quantum dots; rGO, reduced graphene oxide; sGC, soluble guanylate cyclase; UCNP, upconversion nanoparticle

VOCABULARY

Cell signaling, A biological comunication process within or between cells whereby molecules act as effectors to elicit defined cellular behaviors in response to various stimuli; **Nitric oxide**, A free radical gaseous signaling molecule involved in multiple biological processes including smooth muscle relaxation, vasodilation, and inflammation; **Sensor**, An analytical device designed to detect and measure a target molecule and consisting of a signal-producing recognition element, a signal transducer, and a signal detector; **Nanoparticle**, A particle of matter that is between 1 and 100 nm in at least one dimension, increasingly being used as the basis for cellular sensors and drug delivery; **Förster resonance energy transfer (FRET)**, A biophysical mechanism involving a donor and acceptor chromophore wherein excitation of the donor results in dipole−dipole coupling and energy transfer, wherein the efficiency of the process is directly dependent on the sixth power of the separation distance between the donor and acceptor

REFERENCES

(1) Bedioui, F.; Griveau, S. Electrochemical detection of nitric oxide: assessement of twenty years of strategies. *Electroanalysis* **2013**, *25* (3), 587−600.

(2) Levine, A. B.; Punihaole, D.; Levine, T. B. Characterization of the role of nitric oxide and its clinical applications. *Cardiology* **2012**, *122* (1), 55−68.

(3) Joyner, M. J.; Dietz, N. M. Nitric oxide and vasodilation in human limbs. *J. Appl. Physiol.* **1997**, *83* (6), 1785−1796.

(4) Epstein, F. H.; Moncada, S.; Higgs, A. The L-arginine-nitric oxide pathway. *N. Engl. J. Med.* **1993**, *329* (27), 2002−2012.

(5) Roberts, C. K.; Barnard, R. J.; Jasman, A.; Balon, T. W. Acute exercise increases nitric oxide synthase activity in skeletal muscle. *Am. J. Physiol. Endocrinol.* **1999**, *277* (2), E390−E394.

(6) Stamler, J. S.; Meissner, G. Physiology of nitric oxide in skeletal muscle. *Physiol. Rev.* **2001**, *81* (1), 209−237.

(7) Garthwaite, J.; Boulton, C. Nitric oxide signaling in the central nervous system. *Annu. Rev. Physiol.* **1995**, *57* (1), 683−706.

(8) Vincent, S. R. Nitric oxide neurons and neurotransmission. *Prog. Neurobiol.* **2010**, *90* (2), 246−255.

(9) Wink, D. A.; Kasprzak, K. S.; Maragos, C. M.; Elespuru, R. K.; Misra, M.; Dunams, T. M.; Cebula, T. A.; Koch, W. H.; Andrews, A. W.; Allen, J. S.; et al. DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. *Science* **1991**, *254* (5034), 1001−3.

(10) Fehsel, K.; Jalowy, A.; Qi, S.; Burkart, V.; Hartmann, B.; Kolb, H. Islet Cell DNA Is a Target of Inflammatory Attack by Nitric Oxide. *Diabetes* **1993**, *42* (3), 496−500.

(11) Kröncke, K.-D.; Fehsel, K.; Kolb-Bachofen, V. Nitric oxide: cytotoxicity versus cytoprotection—how, why, when, and where? *Nitric Oxide* **1997**, *1* (2), 107−120.

(12) Kendall, H.; Marshall, R.; Bartold, P. Nitric oxide and tissue destruction. *Oral Dis.* **2001**, *7* (1), 2−10.
(13) Lim, M. H.; Lippard, S. J. Metal-Based Turn-On Fluorescent Probes for Sensing Nitric Oxide. *Acc. Chem. Res.* **2007**, *40* (1), 41−51.
(14) Schäfer, A.; Wiesmann, F.; Neubauer, S.; Eigenthaler, M.; Bauersachs, J.; Channon, K. M. Rapid regulation of platelet activation in vivo by nitric oxide. *Circulation* **2004**, *109* (15), 1819−1822.
(15) Liew, F. Y. Regulation of lymphocyte functions by nitric oxide. *Curr. Opin. Immunol.* **1995**, *7* (3), 396−9.
(16) Bogdan, C. Nitric oxide and the immune response. *Nat. Immunol.* **2001**, *2* (10), 907−916.
(17) Thomas, D. D.; Liu, X.; Kantrow, S. P.; Lancaster, J. R., Jr. The biological lifetime of nitric oxide: implications for the perivascular dynamics of NO and O2. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98* (1), 355−360.
(18) Esplugues, J. V. NO as a signalling molecule in the nervous system. *Br. J. Pharmacol.* **2002**, *135* (5), 1079−1095.
(19) Malinski, T.; Taha, Z. Nitric oxide release from a single cell measured in situ by a porphyrinic-based microsensor. *Nature* **1992**, *358* (6388), 676−678.
(20) Vallance, P.; Bhagat, K.; MacAllister, R.; Patton, S.; Malinski, T.; Radomski, M.; Moncada, S. Direct measurement of nitric oxide in human beings. *Lancet* **1995**, *346* (8968), 153−154.
(21) Shin, J. H.; Weinman, S. W.; Schoenfisch, M. H. Sol-Gel Derived Amperometric Nitric Oxide Microsensor. *Anal. Chem.* **2005**, *77*, 3494−3501.
(22) Shin, J. H.; Privett, B. J.; Kita, J. M.; Wightman, R. M.; Schoenfisch, M. H. Fluorinated Xerogel-Derived Microelectrodes for Amperometric Nitric Oxide Sensing. *Anal. Chem.* **2008**, *80* (18), 6850−6859.
(23) Zhang, R.; Ye, Z.; Wang, G.; Zhang, W.; Yuan, J. Development of a Ruthenium(II) Complex Based Luminescent Probe for Imaging Nitric Oxide Production in Living Cells. *Chem. - Eur. J.* **2010**, *16* (23), 6884−6891.
(24) Woldman, Y. Y.; Eubank, T. D.; Mock, A. J.; Stevens, N. C.; Varadharaj, S.; Turco, J.; Gavrilin, M. A.; Branchini, B. R.; Khramtsov, V. V. Detection of nitric oxide production in cell cultures by luciferin-luciferase chemiluminescence. *Biochem. Biophys. Res. Commun.* **2015**, *465* (2), 232−8.
(25) Woldman, Y. Y.; Sun, J.; Zweier, J. L.; Khramtsov, V. V. Direct chemiluminescence detection of nitric oxide in aqueous solutions using the natural nitric oxide target soluble guanylyl cyclase. *Free Radical Biol. Med.* **2009**, *47* (10), 1339−45.
(26) Kojima, H.; Sakurai, K.; Kikuchi, K.; Kawahara, S.; Kirino, Y.; Nagoshi, H.; Hirata, Y.; Akaike, T.; Maeda, H.; Nagano, T. Development of a Fluorescent Indicator for the Bioimaging of Nitric Oxide. *Biol. Pharm. Bull.* **1997**, *20* (12), 1229−1232.
(27) Kojima, H.; Sakurai, K.; Kikuchi, K.; Kawahara, S.; Kirino, Y.; Nagoshi, H.; Hirata, Y.; Nagano, T. Development of a fluorescent indicator for nitric oxide based on the fluorescein chromophore. *Chem. Pharm. Bull.* **1998**, *46* (2), 373−5.
(28) Kojima, H.; Nagano, T. Fluorescent Indicators for Nitric Oxide. *Adv. Mater.* **2000**, *12* (10), 763−765.
(29) Li, S.-J.; Zhou, D.-Y.; Li, Y.; Liu, H.-W.; Wu, P.; Ou-Yang, J.; Jiang, W.-L.; Li, C.-Y. Efficient Two-Photon Fluorescent Probe for Imaging of Nitric Oxide during Endoplasmic Reticulum Stress. *ACS Sens.* **2018**, *3* (11), 2311−2319.
(30) Sato, M.; Hida, N.; Umezawa, Y. Imaging the nanomolar range of nitric oxide with an amplifier-coupled fluorescent indicator in living cells. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102* (41), 14515−14520.
(31) Eroglu, E.; Gottschalk, B.; Charoensin, S.; Blass, S.; Bischof, H.; Rost, R.; Madreiter-Sokolowski, C. T.; Pelzmann, B.; Bernhart, E.; Sattler, W.; Hallstrom, S.; Malinski, T.; Waldeck-Weiermair, M.; Graier, W. F.; Malli, R. Development of novel FP-based probes for live-cell imaging of nitric oxide dynamics. *Nat. Commun.* **2016**, *7*, 10623.
(32) Eroglu, E.; Charoensin, S.; Bischof, H.; Ramadani, J.; Gottschalk, B.; Depaoli, M. R.; Waldeck-Weiermair, M.; Graier, W. F.; Malli, R. Genetic biosensors for imaing nitric oxide in single cells. *Free Radical Biol. Med.* **2018**, *128*, 50−58.
(33) Rivera Gil, P.; Vazquez-Vazquez, C.; Giannini, V.; Callao, M. P.; Parak, W. J.; Correa-Duarte, M. A.; Alvarez-Puebla, R. A. Plasmonic Nanoprobes for Real-Time Optical Monitoring of Nitric Oxide inside Living Cells. *Angew. Chem., Int. Ed.* **2013**, *52* (51), 13694−13698.
(34) Zhang, D.; Xiong, J.; Hu, J.; Li, Y.; Zhao, B. Improved method to detect nitric oxide in biological systems. *Appl. Magn. Reson.* **2001**, *20* (3), 345−356.
(35) Wang, J.; He, C.; Wu, P.; Wang, J.; Duan, C. An Amide-Containing Metal-Organic Tetrahedron Responding to a Spin-Trapping Reaction in a Fluorescent Enhancement Manner for Biological Imaging of NO in Living Cells. *J. Am. Chem. Soc.* **2011**, *133* (32), 12402−12405.
(36) Wu, P.; Wang, J.; He, C.; Zhang, X.; Wang, Y.; Liu, T.; Duan, C. Luminescent Metal-Organic Frameworks for Selectively Sensing Nitric Oxide in an Aqueous Solution and in Living Cells. *Adv. Funct. Mater.* **2012**, *22* (8), 1698−1703.
(37) Ye, Y.; Liu, H.; Li, Y.; Zhuang, Q.; Liu, P.; Gu, J. One-pot doping platinum porphyrin recognition centers in Zr-based MOFs for ratiometric luminescent monitoring of nitric oxide in living cells. *Talanta* **2019**, *200*, 472−479.
(38) Wang, N.; Yu, X.; Zhang, K.; Mirkin, C. A.; Li, J. Upconversion Nanoprobes for the Ratiometric Luminescent Sensing of Nitric Oxide. *J. Am. Chem. Soc.* **2017**, *139* (36), 12354−12357.
(39) Cui, J.; Hu, K.; Sun, J.-J.; Qu, L.-L.; Li, D.-W. SERS nanoprobes for the monitoring of endogenous nitric oxide in living cells. *Biosens. Bioelectron.* **2016**, *85*, 324−330.
(40) Li, L.; Si, Y.; He, B.; Li, J. Au-Ag alloy/porous-SiO2 core/shell nanoparticle-based surface-enhanced Raman scattering nanoprobe for ratiometric imaging analysis of nitric oxide in living cells. *Talanta* **2019**, *205*, 120116.
(41) Deng, X.; Zou, Z.; Zhang, Y.; Gao, J.; Liang, T.; Lu, Z.; Ming Li, C. Synthesis of merit-combined antimony tetroxide nanoflowers/reduced graphene oxide to synergistically boost real-time detection of nitric oxide released from living cells for high sensitivity. *J. Colloid Interface Sci.* **2021**, *581*, 465−474.
(42) Kim, M.-Y.; Naveen, M. H.; Gurudatt, N. G.; Shim, Y.-B. Detection of Nitric Oxide from Living Cells Using Polymeric Zinc Organic Framework-Derived Zinc Oxide Composite with Conducting Polymer. *Small* **2017**, *13* (26), 1700502.
(43) Guo, C. X.; Ng, S. R.; Khoo, S. Y.; Zheng, X.; Chen, P.; Li, C. M. RGD-Peptide Functionalized Graphene Biomimetic Live-Cell Sensor for Real-Time Detection of Nitric Oxide Molecules. *ACS Nano* **2012**, *6* (8), 6944−6951.
(44) Liu, Y.-L.; Wang, X.-Y.; Xu, J.-Q.; Xiao, C.; Liu, Y.-H.; Zhang, X.-W.; Liu, J.-T.; Huang, W.-H. Functionalized graphene-based biomimetic microsensor interfacing with living cells to sensitively monitor nitric oxide release. *Chem. Sci.* **2015**, *6* (3), 1853−1858.
(45) Xu, H.; Liao, C.; Liu, Y.; Ye, B.-C.; Liu, B. Iron Phthalocyanine Decorated Nitrogen-Doped Graphene Biosensing Platform for Real-Time Detection of Nitric Oxide Released from Living Cells. *Anal. Chem.* **2018**, *90* (7), 4438−4444.
(46) Zan, X.; Fang, Z.; Wu, J.; Xiao, F.; Huo, F.; Duan, H. Freestanding graphene paper decorated with 2D-assembly of Au@Pt nanoparticles as flexible biosensors to monitor live cell secretion of nitric oxide. *Biosens. Bioelectron.* **2013**, *49*, 71−78.
(47) Sato, M.; Nakajima, T.; Goto, M.; Umezawa, Y. Cell-Based Indicator to Visualize Picomolar Dynamics of Nitric Oxide Release from Living Cells. *Anal. Chem.* **2006**, *78* (24), 8175−8182.
(48) Yao, H.-W.; Zhu, X.-Y.; Guo, X.-F.; Wang, H. An Amphiphilic Fluorescent Probe Designed for Extracellular Visualization of Nitric Oxide Released from Living Cells. *Anal. Chem.* **2016**, *88* (18), 9014−9021.
(49) Pawloski, J. R.; Hess, D. T.; Stamler, J. S. Export by red blood cells of nitric oxide bioactivity. *Nature* **2001**, *409* (6820), 622−626.
(50) Feins, S.; Kong, W.; Williams, E. F.; Milone, M. C.; Fraietta, J. A. An introduction to chimeric antigen receptor (CAR) T-cell

https://doi.org/10.1021/acssensors.1c00051
ACS Sens. XXXX, XXX, XXX−XXX

immunotherapy for human cancer. *Am. J. Hematol.* **2019**, *94* (S1), S3−S9.
(51) Boeneman, K.; Delehanty, J. B.; Blanco-Canosa, J. B.; Susumu, K.; Stewart, M. H.; Oh, E.; Huston, A. L.; Dawson, G.; Ingale, S.; Walters, R.; Domowicz, M.; Deschamps, J. R.; Algar, W. R.; DiMaggio, S.; Manono, J.; Spillmann, C. M.; Thompson, D.; Jennings, T. L.; Dawson, P. E.; Medintz, I. L. Selecting Improved Peptidyl Motifs for Cytosolic Delivery of Disparate Protein and Nanoparticle Materials. *ACS Nano* **2013**, *7* (5), 3778−3796.
(52) Park, K.; Kuo, Y.; Shvadchak, V.; Ingargiola, A.; Dai, X.; Hsiung, L.; Kim, W.; Zhou, H.; Zou, P.; Levine, A. J.; Li, J.; Weiss, S. Membrane insertion of—and membrane potential sensing by—semiconductor voltage nanosensors: Feasibility demonstration. *Sci. Adv.* **2018**, *4* (1), No. e1601453.
(53) Nag, O. K.; Stewart, M. H.; Deschamps, J. R.; Susumu, K.; Oh, E.; Tsytsarev, V.; Tang, Q.; Efros, A. L.; Vaxenburg, R.; Black, B. J.; Chen, Y.; O'Shaughnessy, T. J.; North, S. H.; Field, L. D.; Dawson, P. E.; Pancrazio, J. J.; Medintz, I. L.; Chen, Y.; Erzurumlu, R. S.; Huston, A. L.; Delehanty, J. B. Quantum Dot-Peptide-Fullerene Bioconjugates for Visualization of in Vitro and in Vivo Cellular Membrane Potential. *ACS Nano* **2017**, *11* (6), 5598−5613.
(54) Muroski, M. E.; Oh, E.; Nag, O. K.; Medintz, I. L.; Efros, A. L.; Huston, A.; Delehanty, J. B. Gold-Nanoparticle-Mediated Depolarization of Membrane Potential Is Dependent on Concentration and Tethering Distance from the Plasma Membrane. *Bioconjugate Chem.* **2020**, *31* (3), 567−576.
(55) Carvalho-De-Souza, J. L.; Nag, O. K.; Oh, E.; Huston, A. L.; Vurgaftman, I.; Pepperberg, D. R.; Bezanilla, F.; Delehanty, J. B. Cholesterol Functionalization of Gold Nanoparticles Enhances Photoactivation of Neural Activity. *ACS Chem. Neurosci.* **2019**, *10* (3), 1478−1487.
(56) Luk, B. T.; Fang, R. H.; Hu, C. M.; Copp, J. A.; Thamphiwatana, S.; Dehaini, D.; Gao, W.; Zhang, K.; Li, S.; Zhang, L. Safe and Immunocompatible Nanocarriers Cloaked in RBC Membranes for Drug Delivery to Treat Solid Tumors. *Theranostics* **2016**, *6* (7), 1004−11.
(57) Yoo, J.-W.; Chambers, E.; Mitragotri, S. Factors that Control the Circulation Time of Nanoparticles in Blood: Challenges, Solutions and Future Prospects. *Curr. Pharm. Des.* **2010**, *16* (21), 2298−2307.
(58) Wang, S.; Han, M.-Y.; Huang, D. Nitric Oxide Switches on the Photoluminescence of Molecularly Engineered Quantum Dots. *J. Am. Chem. Soc.* **2009**, *131* (33), 11692−11694.
(59) Liu, S.; Jin, L.; Chronakis, I. S.; Li, X.; Ge, M. Hyperbranched polyether hybrid nanospheres with CdSe quantum dots incorporated for selective detection of nitric oxide. *Mater. Lett.* **2014**, *123*, 104−106.
(60) Bhattacharya, S.; Sarkar, R.; Chakraborty, B.; Porgador, A.; Jelinek, R. Nitric Oxide Sensing through Azo-Dye Formation on Carbon Dots. *ACS Sens.* **2017**, *2* (8), 1215−1224.
(61) Xu, G.; Wei, F.; Cen, Y.; Cheng, X.; Hu, Q. Dual-Emissive Fluorescent Sensor Based on Functionalized Quantum Dots for the Simultaneous Determination of Nitric Oxide and Hydrogen Sulfide. *ACS Biomater. Sci. Eng.* **2020**, *6* (11), 6086−6094.
(62) Martí, A.; Costero, A. M.; Gaviña, P.; Parra, M. Selective colorimetric NO(g) detection based on the use of modified gold nanoparticles using click chemistry. *Chem. Commun.* **2015**, *51* (15), 3077−3079.

https://doi.org/10.1021/acssensors.1c00051
ACS Sens. XXXX, XXX, XXX−XXX