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Review Article

Advancement of Fluorescent Methods for Detection of Nitric Oxide

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Abstract

Biologically Nitric Oxide (NO) is an important inorganic compound involved in numerous signaling pathways, which has promoted the demand for analytical methods for detection of NO. Fluorescent sensing, more effective than any other detection methods, enables monitoring NO in physiological environments such as cell and blood. Fluorescent probes based detection methods have wide applications in selective and sensitive monitoring of NO production in vivo. In this review, we highlight the novel fluorescent NO sensors developed in recent years, including fabrication, analytical characteristics and biological applications.

Keywords: Nitric oxide; Fluorescent probes; Photo induced electron transfer; Reactive nitrogen and oxygen species

Abbreviations

NO: Nitric Oxide; DAFs: Diaminofluoresceins; DANs: Diaminonaphthalenes; DARs: Diaminorhodamines; SWNT: Single-Walled Carbon Nanotube; QDs: Quantum Dots; PET: Photo induced Electron Transfer; FRET: Fluorescence Resonance Energy Transfer; RNOS: Reactive Nitrogen And Oxygen Species; RH: Rhodamine B Hydrazide; RB: Rhodamine B; RBSe: Rhodamine B Selenolactone; DHA: Dehydroascorbic Acid; AA: Ascorbic Acid; iNOS: inducible NO Synthase; IFN-γ: Interferon-γ; LPS: Lipopolysaccharide; SN: Seminaphthofluorescein; CS: Chitosan; DMS: Dimethyl Sulfate; H_3TCA: Tricarboxytriphenylamine; PMOFs: Porous Metal-Organic Frameworks; NIR: Near-Infrared; HEX-DMA: 1,6-hexanedioldimethacrylate; PMMA: Poly (methyl methacrylate); mHP: Modified Hyperbranched Polyether; NTPED: N-(3-(Trimethoxysilyl) propyl) ethylenediamine; CA: Cellulose Acetate; DTC: N-(dithiocarbaxy) sarcosine

Introduction

Although NO is well known as an environmental pollutant generated from incomplete combustion of molecules containing nitrogen, it is also important in human body at the concentration ranging from sub-nanomolar to micro molar levels. Furchgott, Ignarro and Murad reported in 1987 that NO is the endothelium-derived relaxation factor and they ultimately shared the Nobel Prize in Physiology in 1998 [1]. Hereafter, many researchers have continued to explore the unknown domains of NO. NO is now understood to be active in several physiological events taking place in cardiovascular, immune, nervous systems as well as pathological processes, [2] for example, arteriolosclerosis [3] and hypertension [4] are connected with underproduction of NO while cancer [5] and diabetes [6] are related to its overproduction. NO is biosynthesized endogenously by nitric oxide Synthase which is a heme-containing enzyme and catalyzes L-arginine to L-citrulline. It is widespread in mammals, plants, bacteria and invertebrates [7]. NO released exogenously has been found to result in various biological responses such as platelet activation decrease [8] and microbial viability reduction [9]. NO diffuses rapidly with an average lifetime from milliseconds to seconds.

Great attention for NO and its biological roles have prompted the development of analytical techniques for its detection and quantification. The effect of NO depends on its widely varying concentration in human body, ranging from sub-nanomolar to micro molar levels. NO can react with oxygen, thiol, heme and so on, thus it has short half-life which is typically less than 10 seconds in biological environment [10]. Consequently, methods with adequate sensitivity and high affectivity are required in NO detection. Moreover, high selectivity for NO over interfering species is also necessary due to the complexity of biological systems.

The majority of NO detection approaches can be classified into electrochemical and spectroscopic methods. Most electrochemical NO detection methods involve electro reduction, direct electro oxidation and catalytic electro oxidation [11]. Spectroscopy methods involve either indirect detection of byproducts (i.e. Griess reaction, chemiluminescence) or direct detection of adducts (i.e. absorbance, electron paramagnetic resonance spectroscopy, fluorescence) [12,13]. However, most of these methods suffer from problems with selectivity and sensitivity as well as disadvantages for NO detection *in vivo* as a result of the toxicity and the complex processes. By contrast, fluorescent method which is commonly used for intracellular detection of NO shows many excellent characteristics such as low-cost, high selectivity and sensitivity. Fluorescent probes can respond in direct and selective manners to NO. As such, they provide a valuable approach to explore the generation, accumulation and translocation of NO in biology with both spatial and temporal resolution [14].

Selective and sensitive methods based on fluorescent probes have great use in monitoring production of NO *in vivo* and explaining its biological functions. The special characteristics of NO, such as limited water solubility, gaseous property, complex red ox reaction and various concentrations in biology, challenges the selectivity of fluorescent probes for *in vivo* detection [15,16]. In

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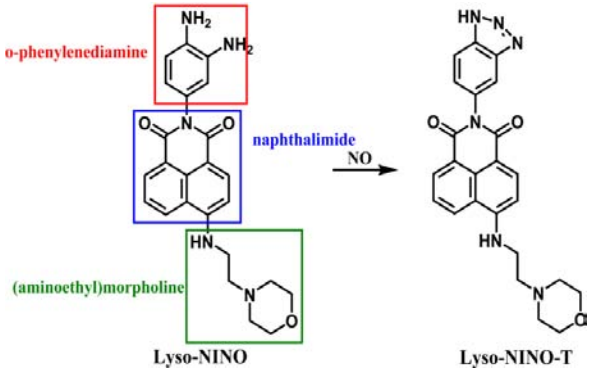

Figure 1: Chemical structure of Lyso-NINO and reaction between Lyso-NINO and NO.

the past decades, numerous fluorescent probes for effective DNA detection have been explored and the commonly used probes can be classified into organic probes (i.e., Diaminofluoresceins (DAFs), [17-21] Diaminonaphthalenes (DANs), [22-24] Diaminorhodamines (DARs), [25] DAMBO-P^{H}. [26]), metal complex-based probes (i.e., copper, [27,28] ferrum, [29] cobalt, [30] ruthenium, [31] dirhodium [32] complexes), Single-Walled Carbon Nanotube (SWNT) -based probes [33] and Quantum Dots (QDs) -based probes [33]. These fluorescent probes have excellent selectivity, high sensitivity and low toxicity for NO sensing *in vivo* [34-38]. The team led by Lippard has focused on several kinds of these fluorescent probes and reviewed all the commonly used probes before [31,33,39]. This review highlights the novel fluorescent NO sensors in recent years.

Fluorescent methods for detection of NO

In the past few years, the fluorescent methods for detection of NO are mainly based on Photo induced Electron Transfer (PET), Fluorescence Resonance Energy Transfer (FRET) and fluorescence response to ring-opened or ring-closed reaction. On the other hand, the fluorescent methods can also be classified into organic probes-based methods, metal complex probes-based methods, SWNT probes-based methods and QD probes-based methods. These fluorescent methods have excellent selectivity and sensitivity for NO and are available for different applications. In addition, these new-developed methods are more advantageous than the methods commonly used before in various aspects.

Organic probes-based methods

Numerous fluorescent methods for NO detection employ the probes with electron-rich o-phenylenediamine fraction. The introduction of o-phenylenediamine fraction in a fluorophore results in PET from the lone-pair electrons of amine to the fluorophore to quench the fluorescence. Conversion of the o-phenylenediamine to electron-poor aryltriazole in the presence of NO decreases the energy of lone-pair electrons and turns off the PET to restore fluorescence [39].

A typical example is the method employing Lyso-NINO, a lysosome-specific and two-photon fluorescent probe which has lower cytotoxicity, excellent lysosomal localization, high selectivity and sensitivity for monitoring endogenous and exogenous NO in lysosomes of macrophage cells [40]. Lyso-NINO based on PET is integration of lysosome-targeting (aminoethyl)morpholine, two-photon fluorophore naphthalimide and NO-capturing o-phenylenediamine. o-Phenylenediamine is employed not only as NO-captor but also as a fluorescence quencher for naphthalimide. In the lysosomal pH ranging from 4.5 to 5.5, Lyso-NINO exhibits weak fluorescence while the reaction conduct Lyso-NINO-T of Lyso-NINO with NO shows strong fluorescence (Figure 1). In addition, the photo properties of Lyso-NINO are not interfered by the byproducts of NO in lysosomes. Flow cytometry can be used for quantitative analysis of endogenous NO and iNOS inducers can effectively increase endogenous NO. Another example is a quinoline derivative QNO, a two-photon fluorescent probe composed of a glycinamide linker, an o-phenylenediamine and a quinoline derivative [41]. It also has characteristics such as good photo stability, low cytotoxicity and pH insensitivity. QNO itself exhibit very weak fluorescence because of PET. A triazine fraction is formed after the rapid reaction with NO, the PET is inhibited and the fluorescence is restored without any shift in wavelength (Figure 2). QNO exhibits high selectivity for NO over other biologically Reactive Nitrogen and Oxygen Species (RNOS). DANPBO-H [42] and DANPBO-M [43]

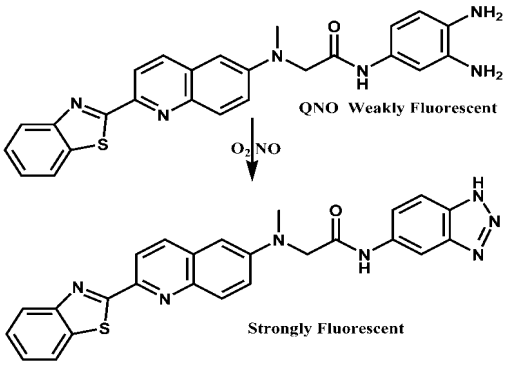

Figure 2: Chemical structure of QNO and the fluorescence response to NO.

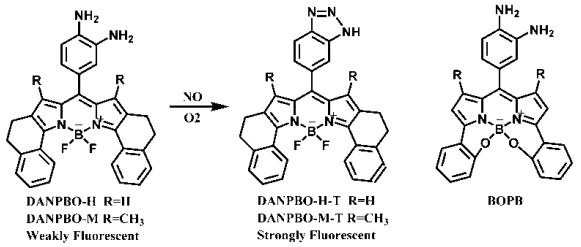

Figure 3: Chemical structures of DANPBOs and BOPB as well as reaction of DANPBOs with NO.

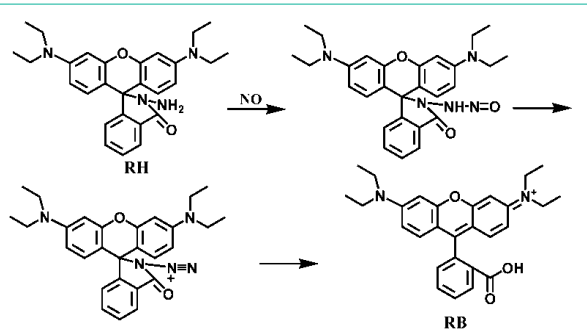

Figure 4: Sensing mechanism of RH with NO.

are also o-phenylenediamine-based probes. The incorporation of two tetrahydronaphthalene with the pyrrole moiety of BODIPY [44] has extended the excitation and emission wavelengths as well as increased the lipophilicity. The probes have excellent intracellular retention because of their strong lipophilicity. Even under the irradiation of xenon lamp over 24h, the fluorescence intensity of the probes remains unchangeable. Their good photo stability is ensured by that the probes have no heavy atoms and withdrawing groups. DANPBOs themselves have very weak fluorescence at pH above 4, however, they react with NO to generate triazoles DANPBO-Ts with high fluorescence. Other biologically RNOS have no obvious interference with NO detection. A similar probe BOPB [45] has been also reported to be utilized for NO detection (Figure 3). Such kind of probes with o-phenylenediamine fraction react with NO indirectly, thus these methods are possibly irreversible and inaccurate.

Another kind of NO detection methods apply rhodamine-based probes, in which ring-closed rhodamine moiety is non-fluorescent while the fluorescence is restored when the ring is opened in the presence of NO [46]. For example, Rhodamine B Hydrazide (RH) is a colorless and non-fluorescent probe for exogenous and endogenous NO detection [47]. NO reacts with its oxidized product NO_2 to generate N_2O_3 and then N_2O_3 nitrosylates the amino group of RH to form diazonium group. An azide intermediate is generated and converted into Rhodamine B (RB) due to the ring-opened reaction (Figure 4). The probe exhibits excellent stability under pH above 4 and shows high selectivity to NO over other biologically RNOS. The fast reaction of Rhodamine B Selenolactone (RBSe) with NO is also ring-opened reaction [48]. RBSe itself has very weak fluorescence, while pink color and strong fluorescence are immediately generated with the addition of NO (Figure 5). The fluorescence intensity is hardly affected by pH ranging from 6.8 to 7.8, thus RBSe is suitable for detection of NO in physiological environment. Furthermore, though RBSe can react with Ag (I) and Hg(II), the concentration of the two ions in normal physiological environment do not interfere the detection of NO.

Cou-Rho-NO is designed based on a FRET dyad, coumarin and rhodamine [49]. A piperazine moiety between coumarin and rhodamine is the linker to avoid fluorescence quenching in aqueous environment. In addition, an o-phenylenediamine moiety is the reaction site for NO. The FRET does not occur when the rhodamine acceptor is ring-closed in the absence of NO. In other words, when Cou-Rho-NO reacts with NO, the FRET is generated and the fluorescence intensity of the rhodamine moiety increases (Figure 6). Cou-Rho-NO reacts with NO under the solution of pH 3-10. Cou-Rho-NO probe is suitable for fluorescent imaging of endogenously intracellular NO. Furthermore, though Dehydroascorbic Acid (DHA) and Ascorbic Acid (AA) interfere with detection of NO in o-phenylenediamine-based probes, Cou-Rho-NO with o-phenylenediamine moiety essentially does not show ratiometric response to DHA or AA. In addition, a probe FP-H_2O_2-NO with a boronate-based moiety attached on the coumarin side of Cou-Rho-NO has been reported to be applied for simultaneous detection of NO and H_2O_2 which are both important biomolecules in signal transduction and oxidative pathways [50].

Especially, a method is developed based on N-nitrosation and formation of diazo ring. NO_{550} is a particular easily synthesized probe which presents selective and sensitive response to NO with the occurrence of red-shifted signal and the generation of diazo ring system (AZO_{550}, Figure 7). NO_{550} reacts with NO rapidly in neutral or even alkalescence solution. The cyano and dimethylamino groups allow internal charge transfer on photo excitation. The ring closure reaction occurs at the para to the dimethylamino group because of steric hindrance and the stability of six-membered ring [51]. The color of NO_{550} solution changes from light-yellow to deep-red and the fluorescence intensity enhances with the increased concentration of NO. NO_{550} can get across cell membranes but cannot cross nuclear membranes thus it is suitable for both extra- and intracellular NO detection. Good characteristics of NO_{550} such as high specificity and sensitivity, low-dependence of pH and facile synthesis make it an excellent fluorescent probe for NO detection.

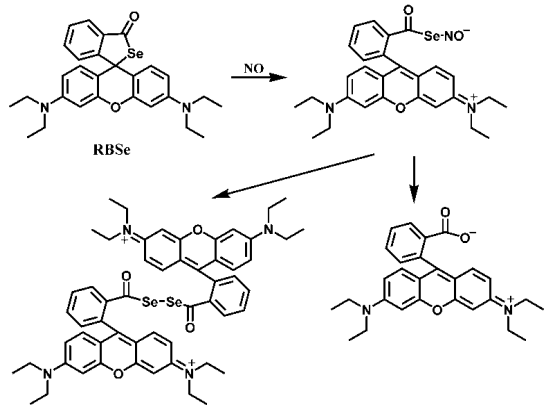

Figure 5: Chemical structure of RBSe and fluorescence response to NO.

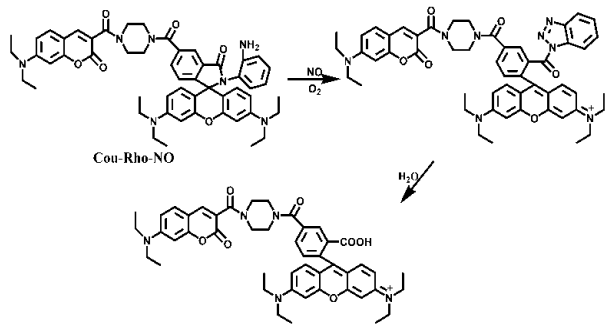

Figure 6: Reaction of Cou-Rho-NO with NO and the occurrence of FRET.

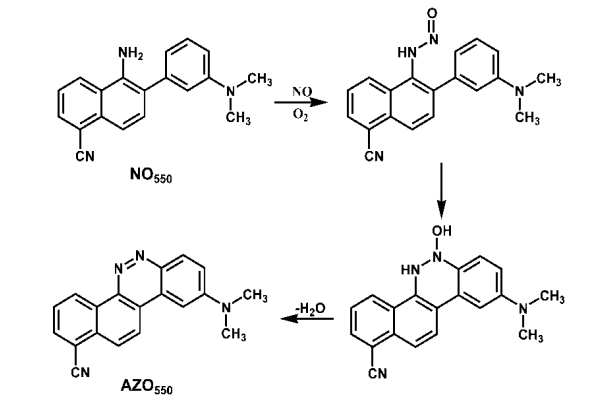

Figure 7: Reaction of NO_{550} with NO and the generation of AZO_{550}.

Cu^{2+}/Cu(II) complex probes-based methods

A widespread method for NO fluorescent detection is based on metal complex scaffold, such as cobalt, ruthenium, dirhodium complexes. However, these probes exhibit water-incompatibility and low sensitivity for *in vivo* applications. Cu (II) complexes have been developed widely to overcome these limitations. In the system, fluorescent ligand carrying a secondary amine is quenched on coordination to paramagnetic Cu(II) center, while NO reduces the paramagnetic Cu(II) to antimagnetic Cu^{+}/Cu(II) with concomitant nitrosation (Nitrosation is a process of converting organic compounds into nitro so derivatives, i.e. compounds containing the R-NO functionality) and deprotonation of the secondary amine, which decreases the energy of lone-pair electrons on the nitrogen atom as well as turns off PET in the N-nitrosated product [39]. The majority of metal complexes are only used in organic solvents because the fluorescent ligands can be replaced by H_2O molecules from the metal center in aqueous environments and the fluorescence can also restore even in the absence of NO. Though other radicals such as NO_2 and H_2O_2 interfere with the detection, such probes exhibit high selectivity for NO detection over other biologically RNOS. Unlike the o-phenylenediamine-based probes, oxygen is not required when employing Cu (II) complex-based probes, which is beneficial for NO imaging in hypoxic environments. In addition, such probes can be conjugated to Fe_3O_4 nanoparticles to form magnetism-targeted probes [52].

Several Cu(II) complexes of N-donor ligands have been reported to be highly selective and sensitive for sensing NO in a physiologically relevant media (Figure 8) [53-55]. Furthermore, Cu (bpq)(OAc)(H_2O) is utilized for direct detection of NO in which N-(8-quinolyl) pyridine-2-carboxamide) (Hbpq) is the ligand for Cu(II) [56]. The Cu (II) is stabilized by interaction with carbonyl oxygen atoms to form a dimeric compound. The fluorescence of the probe is quenched by Cu(II) acetate while the fluorescence is increased with the reduction of Cu(II) to Cu(II) in the presence of NO. The fluorescence quenching and increasing processes are reversible, in other words, the fluorescence is quenched again when NO is released (Figure 9). In addition, the fluorescence of a naphthalene-sulfonamino-quinoline based Cu (II) complex Cu $(NSQ)_2$ is completely quenched and the addition of NO can restore the fluorescence [57] (Figure 10). Cu$(NSQ)_2$ can also exhibit excellent fluorescence responding to NO in organic thin film such as polycaprolactone based film. CuQNE with naphthalimide fractions as the fluorophore is a highly selective and sensitive probe for intracellular NO detection [58] (Figure 11). CuQNE keeps a stable fluorescence under pH 6-10, facilitating NO detection in physiological conditions.

Cu(II) complexes based on ring-opened reaction of rhodamine moiety are commonly used probes for NO fluorescent detection. For example, CuRBT with a ring-closed rhodamine-containing tridentate N-donor is a Cu(II) complex-based probe for intracellular NO monitoring [59]. Tris (2-aminoethyl) amine moiety as the efficient chelator of Cu(II) is incorporated to rhodamine B moiety. Initially,

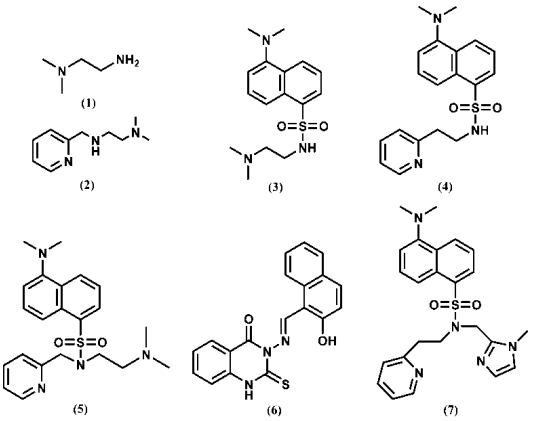

Figure 8: Structure of the N-donor ligands of Cu (II) complexes for NO detection.

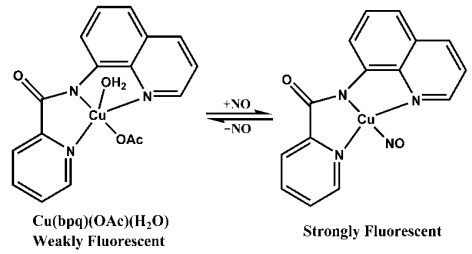

Figure 9: Fluorescence response of Cu(bpq)(OAc)(H2O) to NO.

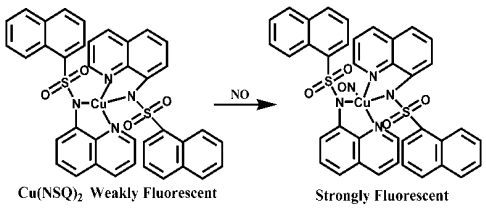

Figure 10: Chemical structure of Cu$(NSQ)_2$ and its fluorescence response to NO.

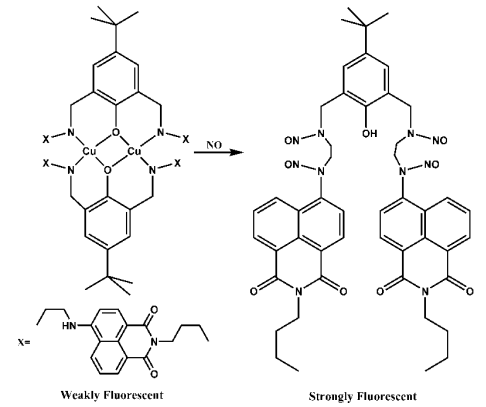

Figure 11: Chemical structure of CuQNE and the reaction of CuQNE with NO.

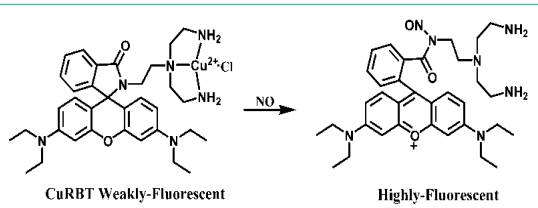

Figure 12: Chemical structure of CuRBT and reaction of CuRBT with NO.

NO coordination to Cu(II) followed by formation of NO^+, then NO^+ incorporates to the nitrogen of amide to open the spirolactam and Cu (II) is reduced to Cu(II), resulting in nitrosation and fluorescence response (Figure 12). Cu(II) in CuRBT is easily reduced to Cu(II) in the pH 6.5-9.0 only with a little variation of fluorescence intensity. RB-TP-Cu(II) and RB-Py-Cu(II) are both similar probes for intracellular NO imaging [60] (Figure 13).

CuFL is widely used for NO sensing, however, it easily diffuses out of cells under continual media perfusion. To improve the detection ability, several analogue based on CuFL are developed recently. Cu_2 (FL2A) and Cu_2(FL2E) are generated by combining the ligands FL2A and FL2E with two equivalents of $CuCl_2$ [61-64] (Figure 14). Cu_2 (FL2E) employs ester extending for cell-trapping and is suitable for detection of endogenous NO in live cells. Cu_2 (FL2A) is membrane-impermeable because the negative charge of carboxyl ate prevents it from entering live cell (Figure 10). Inducible NO synthase (iNOS) such as Interferon-γ (IFN-γ) and Lipopolysaccharide (LPS) can significantly increase fluorescence. In addition, such probes are more sensitive for NO over Zn(II) and can be used to monitor NO production in olfactory bulb by fluorescence imaging. Cu (II) complex of SNFL used for NO sensing is also analogue of CuFL1 [65]. Cu(II) is bound at an 8-aminoquinaldine unit and a Seminaphthofluorescein (SN) moiety is extended to it. Fluorescence intensity increases significantly under anaerobic conditions due to the generation of fluorescent nitrosamine product (Figure 15). Such probes emit at longer wavelengths comparing to CuFL thus it is available for multidye imaging and deeper imaging. In addition, the lower wavelength excitation decreases cells and tissues damage. Though the SN and similar probes have been reported to be applied for detection of Zn(II), CuSNFL is more sensitive to NO. CuFL-CS NS diazeniumdiolates are synthesized by incorporating FL with Chitosan (CS) through electrostatic self-assembly, and then reacts with pressurized NO and Dimethyl Sulfate (DMS). The resultant FL-CS NS diazeniumdiolates are protected by methyl groups and react with $CuCl_2$. CuFL-CS NS diazeniumdiolates themselves are spherical and non-fluorescent due to the paramagnetic Cu(II) as well as the PET [66]. Under physiological conditions, the protecting methyl groups can segregate and release NO, some of which react with the CuFL fraction and reduce Cu(II) to Cu(II) (Figure 16). Consequently, such stable fluorescent probes can be utilized to monitor the released NO based on the fluorescence increase caused by NO.

Especially, Cu-TCA (H_3TCA=tricarboxytriphenylamine) has a three-dimensional porous structure steadied by the well-established $Cu_2(O_2CR)_4$ units (Figure 17) [67]. The Porous Metal-Organic Frameworks (PMOFs) can deliver gaseous NO, confirming their applications for gas storage and detection. Cu(II) quenches the fluorescence of trephenylamine and Cu-TCA only presents very weak fluorescence, however, the distinctly fluorescent enhancement is provided in the presence of NO. This probe can be used in the biological NO imaging in living cells. Another similar PMOFs Eu-TCA based on the hydrothermal reaction between europium nitrate and H_3TCA has applications on ratio metric detection of NO.

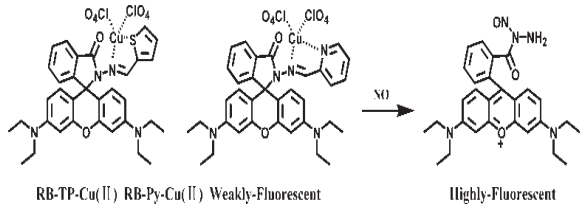

Figure 13: Fluorescent response of RB-TP- Cu(II) and RB-Py- Cu(II) to NO.

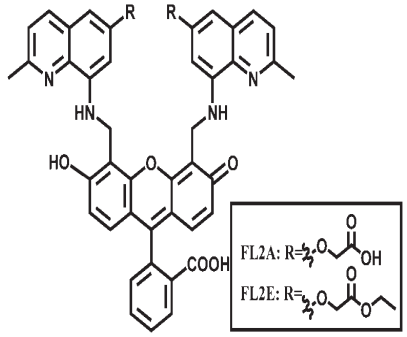

Figure 14: Chemical structures of FL2A and FL2E.

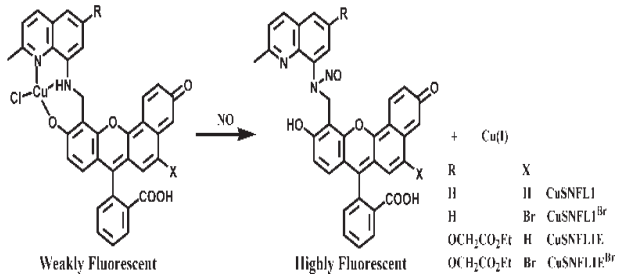

Figure 15: Chemical structure of CuSNFL family and fluorescence response to NO.

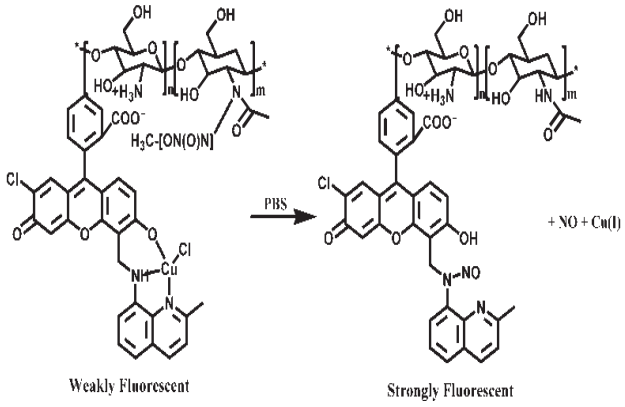

Figure 16: NO release and detection with CuFL-CS NS diazeniumdiolates.

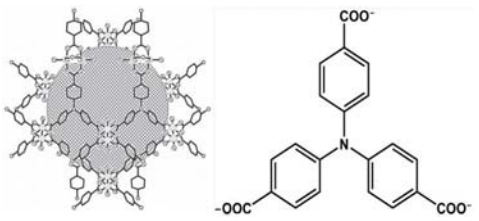

Figure 17: Schematic view of Cu-TCA crystal packing and the chemical structure of TCA. (Reprinted from reference 67. Copyright 2012 Advanced Functional Materials).

SWNT probes-based methods

Semiconducting SWNT-based methods are developed recently for intracellular NO sensing. SWNT has several unique advantages such as Near-Infrared (NIR) fluorescence, which enables photo bleaching resistance and detection in deeper tissues. SWNT is usually encased in phenylated derivative of o-phenylenediamine-functionalized dextran which is capable of adsorption onto SWNT for NO sensing [68]. However, it exhibits disadvantage of fluorescence bleaching after reaction with NO. Recently, a novel probe AT_{15}-SWNT based on specific sequence of $d(AT)_{15}$ oligonucleotides attaching on SWNT has been developed [69]. Though SWNT combined with other DNA sequences show fluorescence enhancement or quenching from analytes such as NADH, dopamine, riboflavin and L-ascorbic acid, $d(AT)_{15}$ uniquely imparts SWNT with high selectivity and sensitivity for NO. The bases of AT_{15}-SWNT stack on SWNT sidewall through π-π stacking while the phosphate and deoxyribose backbone extends away from SWNT, allowing SWNT to remain colloidal and stable (Figure 18). Fluorescence decrease is generated when the AT_{15}-SWNT is exposed to NO, indicating the dynamics of NO adsorption through SWNT exciton quenching. The electron transfer from SWNT to non-covalently adsorbed NO results in the fluorescence quenching. The wrapping AT_{15} is responsible for the selectivity of the probe because certain odor molecules can be recognized by the sequence-specific DNA attached on SWNT through electronic resistance change. The adsorption rate as well as the quenched fluorescence intensity is linearly proportional to the concentration of NO.

QDs probes-based methods

QDs are fluorescent semiconductor nanocrystals with several unique properties, such as one hundred times stronger fluorescence than organic dyes, photo bleaching resistance and stable fluorescence even exposed to long-time lighting. The QDs-based methods utilizing conjugation of CdSe-ZnS nanocrystals and tris (dithiocarbamato) iron (III) have been reported before for NO sensing [70]. Recently, QDs adsorbed or conjugated on polymers have been developed. QD adsorbed on polymethacrylate surface has been applied for time-resolved fluorescence detection of NO. For instance, 1,6-Hexanedioldimethacrylate (HEX-DMA) is used for the synthesis of polymethacrylate film [71]. The fluorescence is quenched with the increasing concentration of NO. Penetration of QDs into the polymethacrylate flims is very scarce, facilitating the contact of QDs with NO. Another kind of QD-based probe QDs-poly (methyl methacrylate) (PMMA) nanocomposites are synthesized through in situ bulk polymerization [72]. The optical properties of this probe can be tuned and stabilized by varying the molar ratio of QDs to PMMA. The fluorescence intensity does not change obviously when oxygen is introduced because most of QDs are enveloped by PMMA so that the oxygen cannot interact directly with the surface of QDs. In addition, the modified Hyper Branched Polyether (mHP) as the supporting material conjugated QDs is a spherical probe employed for NO donating and real-time detecting [73]. The HP is synthesized by cation ring-opening polymerization then the HP is modified with N-(3-(Trimethoxysilyl) propyl) ethylenediamine (NTPED) to obtain amino groups. The mHP reacts with NO to generate mHP-NO nanospheres containing diazeniumdiolates as NO donors and then conjugated with QDs. The QDs-mHP-NO release NO in physiological environment and the NO molecules diffuse to the surface of nanospheres as well as take advantage of the lone pair electrons of Cd, resulting in the decrease of fluorescence (Figure 19). Apart from these probes, Cellulose Acetate (CA) [74] and Chitosan (CS) [75] can also be utilized to embed QDs for NO detection (Figure 20). However, most of such probes present no selectivity for discrimination of biologically RNOS.

To improve the selectivity over other biologically RNOS, QDs modified with ferric ammonium N-(Dithiocarbaxy) sarcosine (DTC) complexes have been designed [76]. The carboxyl of DTC is covalently bound with the amino polymer on the surface of QDs through condensation reaction. The fluorescence of QDs-Fe (III)(DTC)3 is weak as a result of the FRET from QDs to Fe(III)(DTC)3 complexes. NO can displace the DTC in Fe(III)(DTC)3 and reduce Fe(III) to Fe(II), resulting in the obstruction of FRET and greatly enhancing the fluorescence of QDs-Fe(III)(DTC)3 (Figure 21). The specific

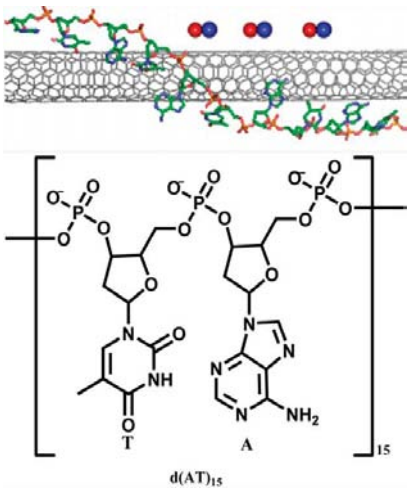

Figure 18: Schematic structure of AT15-SWNT and the unit of d $(AT)_{15}$. (Reprinted from reference 69. Copyright 2011 American Chemical Society.)

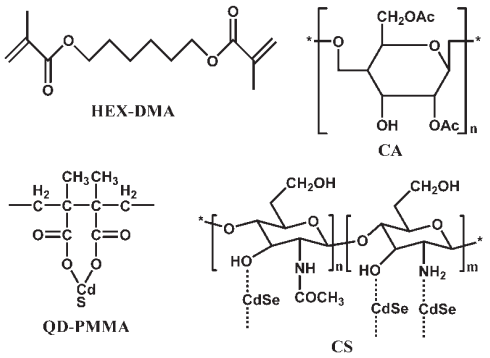

Figure 19: Chemical structure of HEX-DMA, QD-PMMA, CA and CS.

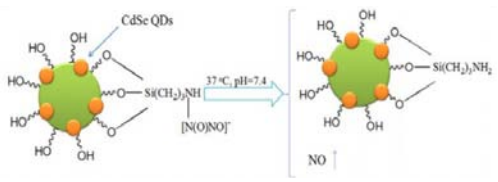

Figure 20: NO release and detection mechanism of QDs-mHP-NO nanopheres. (Reprinted from reference 73. Copyright 2014 Materials Letters.)

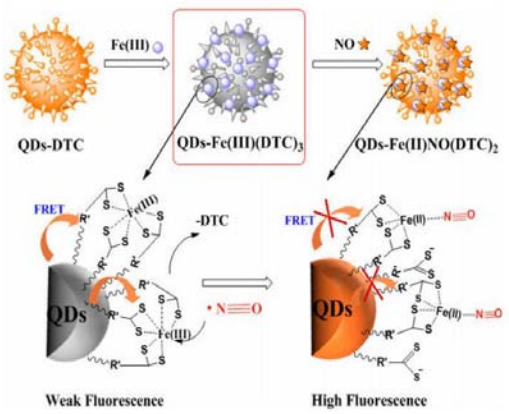

Figure 21: Schematic illustration of method for fluorescent NO detection with QDs-Fe (III)$(DTC)_3$. (Reprinted from reference 76. Copyright 2014 Analytical Chemistry).

detection is not interfered with other biologically RNOS. QDs-Fe(III)(DTC)3 is highly resistant to photo bleaching. In addition, though with the presence of Cu(II), Hg(II) and Cd(II) decrease the sensitivity of QDs-Fe(III)(DTC)3 because they quench the fluorescence induced by NO, these metal cations can be removed by simple pretreatment.

Conclusion and Future Perspectives

In this review, the recent advancement of fluorescent methods for the detection of NO based on organic probes, metal complex probes, SWNT probes and QDs probes have been discussed. Specifically, the use of clinical NO measurement as diagnostic and prognostic indicators necessitates inexpensive and convenient devices. Fluorescent NO sensors, more than any other type of NO measurement technique, are well suited to fill this role. In addition to their ease of fabrication and miniaturization, the instrumentation required to perform sensitive measurements is both affordable and portable.

However, considerable challenges remain in synthesizing ideal probes for sensitive detection or *in vivo* imaging because of the low concentration and short lifetime of NO in physiological environment. More emphasis should be placed on probes exhibiting highly selective to NO and the interaction mechanisms. Furthermore, other factors should be considered in the design of probes for biological systems, such as good solubility, low toxicity, high photo stability, good cell-permeability and high interference-resistance. Probes with NIR fluorescence have a great potential for *in vivo* NO imaging because of low background and high penetrability. These probes will be significant for understanding the physiological and pathological functions of NO in living systems. In addition, though a continued focus on improving the analytical performance of fluorescent NO sensors is important, future research also must address and improve the ability of NO sensors to resist biofouling for more reliable use in vivo. Indeed, sensors biofouling often results in diminished analytical performance and poor reproducibility. Strategies for improving biocompatibility include passive protection of the sensor through the use of sensor membranes that resist biofouling and polymers that actively release antifouling agents. A most promising approach for reducing biofouling of implantable sensors is based on NO-release from sensor membranes. Clearly, such a strategy would be problematic for NO sensors.

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