Frontier luminous strategy of functional silica nanohybrids in sensing and bioimaging: From ACQ to AIE

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Abstract
Fluorescent silica organic–inorganic nanohybrids which combine designable luminescence performance of organic fluorescent dyes and various outstanding advantages of silica nanomaterials have attracted increasing research interests in these fascinating areas. Optical transparency and facile functional modification properties of silica material provide great opportunities to integrate desired fluorescent molecules for various frontier luminous applications. However, conventional organic dyes are typically subject to aggregation-caused quenching due to their aggregation in silica matrix, which could be detrimental for their performance in sensing and biomedical applications. The appearance of aggregation-induced emission lumogens (AIEgens) paves a new way for developing highly efficient fluorescent silica nanohybrids (FSNs). FSNs with intensive luminescence could be obtained due to the formation of aggregates and the restricted intramolecular motion of AIEgens in silica inorganic matrix. In this review, the reported fabrication methodologies of various FSNs based on colloidal silica nanoparticles (SNs) and mesoporous SNs including physical entrapment and covalent strategies are summarized. Especially, the AIEgens-functionalized silica hybrid nanomaterials are introduced in detail. Furthermore, chemical sensing, biosensing, and bioimaging applications of resultant FSNs are also discussed.

KEYWORDS
aggregation-induced emission, bioimaging, fluorescent, nanohybrid, sensing, silica

1 | INTRODUCTION

Nanotechnology, with the frontier advantage of “small size effect,” has shown its great potential in biosensing, drug delivery, and bioimaging. Silica as one of the most widely available natural minerals, their nanoparticles have attracted plentiful attention due to their excellent biocompatibility, facile surface modification, and scalable synthetic availability. Notably, an outstanding sol-gel process for preparing high-quality of spherical silica nanoparticles with narrow size distribution was developed in 1968, which was known as the “Stöber method.” Mesoporous silica with the pore diameter varying from 2 to 50 nm was invented in 1992 by a liquid-crystal template approach. Until now, silica nanoparticles (SNs) with various morphologies (such as spherical shaped, rod shaped, hollow, and core-shell structures) have been synthesized successfully and can be widely used through further surface modification with different functional molecules or polymeric compounds.

Fluorescent silica organic–inorganic nanohybrids have received increasing attention from researchers for sensing and imaging by virtue of their controllable morphology, excellent luminescence performance, superior photochemical stability, great biocompatibility, and multifunctional capability.
Specifically, fluorescent silica nano-hybrids (FSNs) with the advantages of great flexibility in synthesis and functionalization could overcome the disadvantages of relatively difficult synthesis, potential toxicity, non-biodegradability, and non-functionalized hydrophobic entities that widely existed in inorganic fluorescent nanoparticles.\[17–13\] Additionally, the drawbacks of fluorescent polymeric nanoparticles, such as the difficulty for modification and easy metamorphism, could also be improved by FSNs.\[14,15\] For those superior properties, FSNs that were doped with desired fluorescent molecules with the emission from blue to near-infrared (NIR) region are ideal candidates as nanoprobes and bioimaging agents.\[16,17\] FSNs are also widely used to build up multimodality nanosystems for multi-functional applications.\[18–20\] In general, colloidal SNs (CSNs) and mesoporous SNs (MSNs) are two major classes of SNs.\[21\] According to the current research, MSNs with uniform and tunable pore structure, high surface area, stable, and rigid frameworks are considered to have more versatile and wider applications than CSNs.\[22,23\]

In the past years, various fluorescent molecules with exquisite sensitivity and tunable color have been incorporated in SNs, which leads to the generation of a series of FSNs with different applications accordingly.\[24,25\] The photophysical behavior of fluorescent molecules can be precisely manipulated by changing silica architecture and tuning the distribution of the fluorophores in the silica matrix.\[26\] However, the relatively limited space in the silica matrix means the organic fluorescent dyes can easily form aggregates, which will lead to fluorescence quenching due to the strong π-π interactions between rigid planes and other nonradiative pathways. The phenomenon is known as the aggregation-caused quenching (ACQ) effect.\[27\] Owing to the ACQ effect, it is challengeable to prepare dye-doped NPs with intensive luminescence by using conventional fluorophores, which will obviously restrict their performance for optical applications. Therefore, it is necessary to improve the luminescent properties through inhibiting the aggregation of fluorophores, but the present methods are very complex. The discovery of the aggregation-induced emission (AIE) phenomenon opens up a new way for the development of fluorescence-based technology in solid state materials.\[28\] Compared with conventional organic dyes, AIE luminogens (AIEgens) possess strong fluorescence emission in aggregate formation and solid state.\[29,30\] This unique feature makes AIEgens ideal candidates for developing ultra-bright fluorescent materials.\[31\] Since the discovery of AIE phenomenon by Tang’s group, a variety of AIEgens and their corresponding nanoprobes have been designed and synthesized through diversified strategies.\[32–33\]

AIEgens have been incorporated into various nanomaterials in order to enhance their photostability, as well as improve their biocompatibility, surface characteristics, and chemical properties.\[35\] Numerous AIEgens-based nanomaterials with diverse configurations have been designed, such as polymer nanoparticles, metal-organic frameworks, carbonaceous materials, and inorganic-organic hybrids (SNs).\[36\] AIEgens-based polymer nanoparticles with excellent processability and impressive mechanical robustness have been widely studied in drug delivery, bioimaging, and therapeutics. Polymer nanoparticles can realize the fluorescence changes of AIEgens caused by the aggregation and dispersion of the materials, which makes them become a good candidate for sensor and drug carrier.\[37\] Fluorescent metal organic frameworks (MOFs), which are induced by coordination method, open a new avenue for fluorescence modulation of AIEgens. MOFs with AIEgens not only show efficient fluorescence emission due to the coordination bonding but also possess switchable signals with AIE characteristics.\[38\] However, the switch of AIE signal caused by the state change of polymers and MOFs is usually irreversible, which would lead to the problems such as cytotoxicity in practical application. Recently, AIEgens have been introduced in the graphene oxide (GO) sheet, which has great potential in developing optoelectronic devices. However, further modification and multifunctional design are often limited.\[39\]

The AIEgens-functionalized FSNs are expected to combine the advantages of both SNs and AIEgens for sensing and biological applications. Efficient fluorescence emission could be obtained for the restriction of intramolecular rotation of AIEgens in silica matrix. Meanwhile, SNs can improve the photostability and water-dispersibility of AIEgens.\[40,41\] Based on these benefits, the fluorescence improvement due to rotation limitation of fluorophores in silica frameworks has attracted extensive research. Inagaki’s group has done several efforts to study the inherent relation between the luminescent properties and intermolecular interactions of fluorescent molecules in silica matrix. The results indicate that restricted intramolecular motion of fluorophores inducing from their aggregation or immobilization effect in silica matrix can effectively enhance the fluorescence emission efficiency.\[42–44\] Specifically, an enhanced fluorescence quantum yield (QY) was founded for biphenyl-bridged silica films.\[45\] After biphenyl group was embedded into the silica framework, the biphenyl group showed higher QY (Φ = 0.45) than that of its free state in solution (Φ = 0.35).\[42\] Similarly, a tetraylnitromethylidyne-hexaphenyl-derivative (TH) with AIE characteristics also exhibited enhanced QY due to the fixation of silica matrix. The QY of TH is 0.46 in solution. However, with the increase of TH contents in silica material from 25% to 100%, the QYs of TH-silica increase from 0.52 to 0.84.\[46\] Additionally, higher QY (up to 20%) could be afforded for the fluorescent SNs which were embedded with AIE-active 9,10-distyrylanthracene.\[47\] A recent work showed that the QY of tetraarylene-derived AIEgens increased from 0.3 to 0.5 when the AIEgens were transferred from solution environment to silica matrix.\[48\]

Our group has reported several related research on the luminescence mechanism of fluorescent molecules in silica matrices. The results showed that the restriction of rotational mobility of fluorophores in silica framework could effectively reduce non-radiative rates and ensure the luminescence performance of organic fluorescent molecules when the environment of fluorophores was changed from the solution to fixed state in framework of solid materials. The above conclusions further proved that the fixation of silica skeleton could significantly increase fluorescence efficiency of AIEgens, and AIEgens-functionalized FSNs with higher fluorescence QY and better sensing efficiency can be achieved.\[46,49,50\] Intensive efforts have also been devoted to the design and synthesis of AIEgens-functionalized FSNs by Yu’s research group, and the obtained FSNs show excellent optical properties.\[51\]

With the development of FSNs, the construction strategies from ACQ to AIE system greatly improve optical
Table 1 List of various FSNs with different functional units, along with descriptions of their embedded form, structure, and their chemical sensing applications

| Material                | Functional unit                                           | Embedded form | Structure   | Chemical sensing application | Ref. |
|------------------------|-----------------------------------------------------------|---------------|-------------|--------------------------------|------|
| MSIA                   | Anthraquinone derivative                                 | Covalent      | MSNs        | Cu^{2+} sensing               | [52] |
| RBMSiO<sub>2</sub>     | Rhodamine B derivative                                   | Covalent      | MSNs        | Al^{3+} sensing               | [53] |
| P(OEGMA-co-RhBHA) coated MSN | Rhodamine B derivative and quinoline derivative       | Covalent      | MSNs        | Hg^{2+} and Zn^{2+}            | [54] |
| NP                     | Nitrobenzoxadiazolyl derivative                          | Covalent      | MSNs        | Hg^{2+} sensing               | [55] |
| S1-Ars-3               | As(III) aptamer and rhodamine B                          | Non-covalent  | Covalent    | As(III) sensing               | [56] |
| TH-PMO                 | Tetracyantriylmethylylidene-hexaphenyl derivative        | Covalent      | MSNs        | Cu^{2+} sensing               | [46] |
| SiO<sub>2</sub>@SFNO   | safranin O derivative                                    | Covalent      | MSNs        | MnO<sub>4</sub>⁻                | [58] |
| FMH NPs-HE             | Hydroethidine derivative and fluorescein isothiocyanate  | Non-covalent  | Rattle-type CSNs | O<sub>2</sub>⁺ sensing         | [59] |
| Ru(bpy)<sub>2</sub>phen-MMS | Ruthenium(II) polypyridyl derivative                    | Covalent      | MSNs        | O<sub>2</sub> sensing          | [60] |
| BSOHS@Si               | Sulfoxide-functionalized BODIPY                          | Non-covalent  | Core-shell CSNs | H<sub>2</sub>S sensing          | [61] |
| TPEPMO                 | Tetraphenylethene derivative                            | Covalent      | MSNs        | HCl and NH<sub>3</sub> sensing | [48] |
| TPE-2NH<sub>2</sub>@SiO<sub>2</sub> NPs | Tetraphenylethene derivative                           | Non-covalent  | CSNs        | NO sensing                     | [62] |
| SNF                    | Tetraphenylethene derivative                            | Covalent      | MSNs        | Picric acid sensing           | [63] |
| Ag@TPE-SiO<sub>2</sub> NPs | Tetraphenylethene derivative                           | Non-covalent  | CSNs        | H<sub>2</sub>O₂ sensing        | [64] |
| AIE-SiO<sub>2</sub>-MnO<sub>2</sub> | 1,2-bis[4-(3-sulfonatopropoxy) phenyl]-1,2-diphenylethene | Non-covalent  | CSNs        | Organophosphorus Pesticides sensing | [65] |
| TBPE-DIMSNPs           | Tetraphenylethene derivative                            | Covalent      | MSNs        | Diethylstilbestrol sensing    | [66] |
| HBA-GMS                | 4-(2-hydroxybenzilideneamino) benzoic acid               | Non-covalent  | MSNs        | Temperature sensing           | [67] |
| Ac-100-M               | Tetraphenylethene derivative                            | Non-covalent  | MSNs        | Temperature sensing           | [68] |
| MSN@RhB@SiO<sub>2</sub>@Tpy-Cy@DBZTC@CS | 2-chloro-1,3-dibenzothiazolonecyclohexene and Tpy-Cy | Non-covalent  | MSNs        | pH sensing and O<sub>2</sub>⁺   | [69] |
| Janus SiO<sub>2</sub> NPs | Fluorescein isothiocyanate and rhodamine B             | Covalent      | CSNs        | pH sensing                    | [70] |

Abbreviations: TPEPMOs, tetraphenylethene (TPE)-bridged organosilica hybrid nanostructure
Dye molecules can be incorporated into two major classes of silica-based nanoparticles, CSNs, and MSNs, which can be divided into six structures generally according to non-covalent entrapment or covalent attachment. Non-covalent entrapment includes 1) entrapping the dyes into the growing silica matrix, 2) adsorbing dyes onto the surface of CSNs though electrostatic interaction, and 3) incorporating dyes into the pores of MSNs. Covalent linking method includes 1) encapsulating the dyes within the core and then coating a pure silica shell (core-shell CSNs), 2) anchoring dyes onto the surface of MSNs, and 3) incorporating dyes into the framework of MSNs (PMOs). Non-covalent entrapment and covalent attachment have their own advantages and disadvantages. Non-covalent entrapment is convenient and facile synthetic approach, which has fewer requirements for dye molecules modification. However, the absence of covalent linking between SNs and fluorescent dye molecules will induce dye leaching over time, which will decrease per-FSNs brightness and expose the fluorescent dye molecules to the surrounding environment. Therefore, covalent attachment is preferred because it can reduce the leakage of dyes from SNs over time, which means the resulting nano systems are afforded with intensive luminescence and high sensitivity. The method of covalent attachment is typically to synthesize derivatives of the fluorescent molecules with alkoxysilane moieties, and then they can be immobilized in the silica matrix via covalent binding. However, the limitations of covalent attachment are mainly related to the synthetic accessibility of the alkoxysilane derivatives fluorescent molecules. In this part, we will discuss the assembly strategies of fluorophores in CSNs and MSNs from the perspective of non-covalent or covalent methods.

2.1 Non-covalent entrapment

2.1.1 Colloidal silica-based fluorescent nanohybrids

Seminal works were done by Santra et al. for non-covalent entrapment of dyes into growing silica matrix, in which tris(2,2′-bipyridyl)dichlororuthenium(II) hexahydrate (Rubpy) was introduced into SNs by reverse microemulsion approach.[74,75] Leakage of Rubpy from the SNs was not observed, which means the successful entrapment of the dyes in the silica matrix. In order to eliminate self-quenching caused by dye aggregation, Rubpy concentration in SNs was investigated, and maximum luminescence intensity was observed with Rubpy concentration around 20 wt%. Photosensitivity of Rubpy in nanoparticles was greatly improved due to the protection of the silica matrix. After the surface modification of Rubpy-doped SNs, the obtained FSNs could be used as a biomarker to stain human leukemia cells. Reverse microemulsion approach is usually working well with positively charged and hydrophilic dyes, which strongly limit the synthesis of the FSNs doped with hydrophobic or negatively charged fluorophores.[72] To address this problem, some research has been done to achieve entrapment of the water insoluble fluorescent molecules into the silica matrix with this method.[76,77]

Promisingly, AIEgens in silica matrix could exhibit higher luminescent property due to the aggregation and fixation. Prasad and coworkers developed a new class of two-photon (TP) anthracene derivative (BDSA) dye with AIE properties and entrapped it into SNs with high concentration via direct micelle-assisted method.[78] The aggregation of highly loaded BDSA in SNs showed enhanced fluorescence emission, which offered an opportunity to produce highly fluorescent SNs by raising dye loading concentration. The obtained FSNs were successfully applied in TP fluorescence cell imaging. Furthermore, the authors also co-encapsulated photosensitizing drug and BDSA into SNs for TP photodynamic therapy (PDT) (Figure 1A).[79] The two works open up the door for the preparation of AIEgens-functionalized FSNs. Liang et al. reported a novel FSNs doped with AIE-active carbazole-based cyanine (CWQ-11) via Stöber method.[80] The fluorescence emission intensity of resulting nanomaterial was nearly 45.4 times higher than that of free CWQ-11. In addition, the nanomaterial also had narrow particle size distribution and excellent stability. The resulting nanomaterial was successfully applied for tumor cell imaging with strong fluorescence intensity and excellent photostability (Figure 1B).

In addition, AIEgens-functionalized FSNs can be obtained by using positively charged SNs as efficient nano-capturer to electrostatically adsorb negatively charged AIEgens.[81] Kong and coworkers reported a turn-on AIEgens-SiO₂ aptasensor for detecting prostate-specific antigen (PSA) with high sensitivity.[82] The positively charged amino-functionalized SNs were synthesized and single-stranded PSA aptamer (PA) electrostatically adsorbed on the surface to form neutral SNs-PA nanoparticles. In presence of target PSA, PSA could combine with PA and led to the separation of PA from surface of SNs. Then, the negatively charged silica matrix could be dispersed into water to form micelles which could be served as self-assembly templates to confine nucleation and growth of SNs. A large range of organic fluorescent molecules could be doped into silica through this approach which could lead to monodispersed nanoparticles with a diameter from about 5 to 90 nm.[72] Prasad and coworkers expanded on this method by using of lipophilic silica precursors such as vinyltriethoxysilane in direct micelle solution.[73]

![Scheme 1](image-url) The schematic illustrations with different structures and the applications of FSNs.

controlling the reaction parameters.[71] Direct micelle assisted method is another method through dispersing surfactants into water to form micelles which could be served as self-assembly templates to confine nucleation and growth of SNs. A large range of organic fluorescent molecules could be doped into silica through this approach which could lead to monodispersed nanoparticles with a diameter from about 5 to 90 nm.[72] Prasad and coworkers expanded on this method by using of lipophilic silica precursors such as vinyltriethoxysilane in direct micelle solution.[73]
2.1.2 Mesoporous silica-based fluorescent nanohybrids

Fluorescent MSNs with AIEgens can also be synthesized via non-covalent strategy, which is achieved by using fluorophores-based amphiphile as co-structure directing agent to participate in the construction of fluorescent MSNs. Wei and co-workers synthesized fluorescent MSNs via one-pot surfactant templated method using an AIE-active distyrylanthracene derivative (An18) and cetyltrimethyl ammonium bromide as structure-directed template for both cell imaging and cancer therapy applications (Figure 2A). Gao and co-workers developed a tetraphenylethene (TPE)-based carboxylate gemini surfactant which not only participated in the structure-directing process but also acted as anchored fluorophores for controllable fabrication of stimuli-responsive fluorescent MSNs (Figure 2B). Han et al. developed mesostructured AIEgens-silica hybrid hollow nanotubes through spontaneous chiral self-assembly of AIE-active 2-[4-(1,2,2-triphenylethynyl)phenoxy]-acetic acid (TPEA) through co-structure directing method. In this work, TPEA acted as an anionic amphiphilic compound, cationic organosilanes as co-structure directing agents, and TEOS as a silica precursor to self-assemble into chiral mesostructures under basic conditions (Figure 2C). Another method to produce luminescent MSNs by grinding multi-stimuli mono-cyclometalated Pt(II) complex with AIE properties and MSNs is reported by Laskar’s group. These molecules were successfully loaded into the pores of MSNs during this facile and dry synthetic approach.
2.2 | Covalent attachment

Covalent attachment of luminescent dyes within SNs is mainly achieved through co-assembly or post-modification methods. For the former, it is necessary to synthesize trialkoxysilane-modified fluorescent dyes which subsequently hydrolyze and condense to the growing silica matrix. For latter method, SNs are prepared firstly and then luminescent molecules are anchored on the surface of SNs via covalent bonds.

2.2.1 | Colloidal silica-based fluorescent nanohybrids

Van Blaaderen and Vrij made a preliminary attempt to incorporate fluorescent dyes into the matrix of SNs via the Stöber method in 1992. They prepared silica nanoparticles by co-condensation of tetraethyl orthosilicate (TEOS) with diethylene glycol (DEG) in the presence of surfactants like cetyltrimethylammonium bromide (CTAB). The resulting SNs were then functionalized with fluorescent dyes like fluorescein isothiocyanate (FITC) through a post-modification process, where the amino-terminated SNs were reacted with FITC in an alkaline medium. The resulting SNs were then washed and characterized for their luminescence properties.
controllably. It showed that the composition of the FSNs could be controlled via tuning the distribution of the dyes in the silica spheres. This method is later referred to as a “modified Stöber method” and provides a feasible method to covalently introduce fluorophores into silica matrix. Inspired by this method, Wiesner and co-workers developed fluorescent core-shell SNs using a two-step preparation process.[93] Firstly, the fluorescent core was formed via hydrolyzing and condensing of tetramethylrhodamine isothiocyanate (TRITC-APS) moieties, and then TRITC-rich fluorescent cores acting as seeds were surrounded by denser silica shell. The resultant core-shell SNs were referred as Cornell dots (C dots) with size from 20 to 30 nm. Per-particle brightness level was comparable to water-soluble semiconductor quantum dots due to the co-encapsulation of multiple emitters within a single particle. Thereafter, a novel type of dual-emission fluorescent core-shell FSNs which possessed TRITC fluorescent core and FITC shell was designed and successfully applied to quantitative ratiometric analysis of pH in vitro.[94] The method was later expanded to incorporate a wide range of fluorophores in SNs via covalent bonding.[95] The resultant ultrasmall FSNs referring as C dots were tested in FDA-approved human clinical trial for cancer imaging.[96]

However, the brightness of dye-rich core of Cornell dots was usually less than free dye, which indicated the cores are suffered from ACQ.[97] Park and co-workers reported a new kind of anisotropic polysilsequioxane xerogel (CNPBESi) which was prepared using cyanostilbene derivatives alkoxysilane bridges. The resulting CNPBESi exhibited green fluorescence emission, which was caused by AIE effect of the cyanostilbene derivatives bridge structure.[98] Since then, many other literatures have been published about the incorporation of AIEgens within SNs through covalent bonds.[99] Colloidal silica-based fluorescent nanohybrids with AIEgens are commonly achieved by using alkoxysilane derivatized AIEgens, and then AIEgens can be immobilized in the silica matrix via covalent binding. Chemical reactions involved for the synthesis of alkoxysilane derivatized AIE fluorophores usually include the reactions of amino groups with various groups (including, alkyl bromine,[100] propylene oxide,[101] carboxylic groups,[102] or isocyanate groups,[103] etc.), click reactions,[104] the reactions of hydroxyl groups with alkyl chloride,[105] and so on.[46]

Tang’s group reported a covalent strategy for the synthesis of core-shell FSNs with efficient light emission through Stöber method and reverse microemulsion methods (Figure 3A).[106] Firstly, alkoxysilane-derivatized TPE (TPE-alkoxysilane) and silole (silole-alkoxysilane) were facilely synthesized by click reactions. Then, TPE-silica or silole-silica fluorescent nanocores were formed via hydrolyzing and condensing of TPE-alkoxysilane or silole-alkoxysilane. Afterwards, the resulting nanocores were subjected to further reaction with TEOS to generate FSNs with core-shell structures. The size of FSNs prepared through the Stöber method was larger than reverse microemulsion methods in a controlled fashion, and the surfaces of large-sized FSNs were smoother than small nanoparticles (Figure 3B-E). The obtained FSNs showed excellent colloidal stability and emitted strong fluorescence emission at 474 and 486 nm. Particularly, The FSNs could act as fluorescent visualizers for HeLa cells imaging (Figure 3F-I). Other new TPE-alkoxysilane and silole-alkoxysilane molecules were also designed, which were used for synthesis of core-shell FSNs via Stöber method for cells imaging.[106,107] Pang and co-workers designed a silane-based AIE probe to visually monitor the process of the silica growth in modified Stöber method.[108] Yang et al. successfully fabricated two single-handed helical TPE-bridged silica nanotubes with enhanced fluorescence efficiency under basic conditions by using the TPE-bis(triethoxysilane) (BTSTPE) as the precursor and self-assembly of chiral gelators as templates. The research provides a simple method to prepare AIE-active nanomaterials possessing circularly polarized luminescence properties (Figure 4).[109]

2.2.2 Mesoporous silica-based fluorescent nanohybrids

Since the discovery of MSNs in the early 1990s,[110] it has been widely studied owing to ease of functionalization and endowed with many functionalities including luminescent properties.[111–113] The original purpose of introducing fluorescent moieties into MSNs is to study the formation mechanism of the mesoporous phase.[114,115] Subsequently, the functionalization of MSNs with abundant fluorescent molecules including ACQ and AIE system has become a hot research field for various applications including sensing, imaging, and nanomedicine.[116,117]

The incorporation of fluorescent dyes into MSNs can be achieved based on the classical cooperative assembly mechanism in three methods: 1) Subsequent modification of the surfaces of mesostructured silica phases with fluorescent dyes (grafting) (Figure 5A). 2) Simultaneous reaction of condensable inorganic silica species and trialkoxysilane-modified fluorescent dyes (co-condensation) (Figure 5B). 3) The use of bis- or multi-silylated fluorescent molecules to form periodic mesoporous organosilicas (PMOs) (Figure 5C). In particular, PMOs that are constructed by organic-bridged alkoxysilane precursors ((R0O)3Si-R-Si(OR0)3) represent a new type of multifunctional silica materials with organic–inorganic hybrid framework and ordered mesoporous structure.[118] Special structure for PMOs allows the nanoplates to be used as luminescence sensing agents and drug carriers simultaneously. Organic groups (R) are covalently incorporated into robust siloxane networks of the pore walls, which guarantees organic groups highly dispersed in framework and avoids the formation of non-emissive aggregates.[119] The emerging of PMOs greatly expands the applications of mesoporous silica materials. For luminescent applications, programmable luminescent performance of the PMOs materials can be achieved by changing the organic functionalities (R) which include a huge variety of optional fluorophores. In our previous research work, we designed a new type of four-armed AIEgens and incorporated the fluorophores into silica framework (Figure 6A). Fixation in four directions and intermolecular stacking could realize aggregation-induced fluorescence enhancement. Based on the studies of steady-state fluorescence and dynamic fluorescence for the obtained PMOs material, we found that the reduction of nonradiative decay caused by restriction of intramolecular rotational motion could effectively ensure the aggregation-induced
fluorescence emission. In addition, the more fluorophores embedded and the higher molecular density could induce stronger AIE effect.\[46\] Recently, our group designed a novel AIEgen-organosilica hybrid nanostructure (TPEPMO) by using TPE-bridged alkoxysilane precursors (TPE-Si$_4$).\[48\] TPE-Si$_4$ with AIE properties were covalently incorporated into PMO frameworks, and the obtained TPEPMO possessed high fluorescent efficiency for the restriction of intramolecular motion of TPE fluorophores occurred in PMO frameworks (Figure 6B). It is worth mentioning that more than one fluorophore can be introduced simultaneously into PMOs due to its peculiar physical-chemical properties and separate spaces, which could be used to build donor-acceptor systems based on fluorescence resonance energy transfer system (FRET) with multicolor emissions.\[120\] Generally, since AIE and ACQ systems possess the opposite luminescent properties, they can not easily integrated to construct efficient luminescent materials. The PMOs offer the possibility to couple AIE and ACQ fluorophores simultaneously by doping them in different positions for high-efficiency multicolor emission based on FRET. Yu’s group synthesized PMOs by using TPE-bridged organosilane to construct donor framework. Then, ACQ dyes as energy acceptors were introduced into mesopores of the AIE-PMOs to form ACQ@AIE-PMOs nanomaterials (Figure 6C). The resulting ACQ@AIE-PMOs showed high-efficiency multicolor emission, and the emission could be fine-tuned over the entire visible spectrum by adjusting the amount of encapsulated rhodamine B, which was achieved on the basis of FRET effect. In addition, the results of cell imaging demonstrated that the ACQ@AIE-PMOs were successfully internalized into the cells, which indicated their potential in bioimaging.\[121\]
According to the current results, AIEgens are generally introduced into MSNs covalently by post grafting and Yu’s group have done a series of research work in this field. In 2011, Yu and co-workers reported a simple method to synthesize AIEgens-functionalized MSNs (SBA-15) for drug delivery through the reaction of the TPE with the -NH2 groups modified SBA-15. Zhang and co-workers fabricated a novel kind of fluorescence MSNs through incorporating AIE-active molecule 10-phenylphenothiazine (Ph-PTZ) into MSNs via post-grafting method for controlled drug delivery (Figure 6D). Firstly, MSNs were functionalized with APS and trialkoxysilane-modified 10-phenylphenothiazine (FSCA) simultaneously. Then, copolymers were introduced on the surface of the resulting fluorescence MSNs, which was used to improve aqueous stability of the fluorescence material and endowed it with the capability of loading the anticancer drug. Shi et al. developed a new type of MSNs with AIE-active quinoline-malononitrile derivative (QM) via co-condensation method. Trialkoxysilane-modified QM was prepared firstly and subsequently subjected to the hydrolysis and co-condensing with TEOS. The resulting MSNs possessed uniform morphology and well-ordered mesoporous structure and showed excellent AIE luminescence properties in either water or ethanol, which was applied to cell imaging and drug delivery with good biocompatibility.

3 | CHEMICAL SENSING

Quantitative analysis of specific analytes is vital to various applications from environmental monitoring to biosciences. Optical sensing as an intuitive tool is an effective method to realize quantitative analysis, which is achieved by monitoring the change of optical signals in the presence of specific analytes. Fluorescence sensing as an advanced optical sensing technology has received more and more attention due to its fast response time and technical simplicity. In fluorescence sensing systems, the concentration of the targeted analyte could be achieved according to the change of fluorescence signals. The fluorescence response signals generally include the following three types: turn-on, turn-off, and ratiometric approach. Ratiometric approach has attracted great attention for this method could realize self-calibration through monitoring more than one emissions and minimize or even eliminate the interference from a variety of detection backgrounds, such as the microenvironment around the fluorescent responsive molecules, photobleaching, and instrumental parameters, etc. FSNs, as efficient luminescent materials, have been applied to detect various analytes by using fluorescence sensing with high selectivity and sensitivity, which can be attributed to the flexible incorporation of a huge variety of luminescent functional molecules. In this section, we will summarize various chemical sensing applications including ion sensing, molecular sensing, temperature sensing, and pH sensing.

3.1 | Ion sensing

Chemical pollution has been one of the most serious problems in modern time along with rapidly developing of human society. Some major pollutants especially toxic ions seriously threaten environment and human health, even at extremely low concentrations. Therefore, it is urgent in need of
developing highly sensitive and selective chemosensors for
detecting various ions with fast response time. In recent
years, various luminescence silica nanomaterials have been
developed for toxic metal ions and anions to meet the require-
ments of the above application fields. Kim et al. reported
anthraquinone containing hybrid MSNs (MSIA) which was utilized as a turn-on fluorescence probe for Cu$^{2+}$
sensing with ultra-high selectivity. In the absence of Cu$^{2+}$,
suspension of the MSIA in acetonitrile showed weak fluo-
rescence emission and red in color (Figure 7A). However,
when Cu$^{2+}$ was introduced into the above suspension, it
showed an enhanced fluorescence emission at 560 nm and
a color change from red to yellow. However, there were no
significant changes in the spectra upon the addition of other
metal ions, which could be ascribed to Cu$^{2+}$-induced desul-
furization. In 2016, we designed a turn-on solid chemosensor
for Al$^{3+}$ sensing through covalently introducing rhodamine
B derivative in MSNs. The resulting chemosensor showed
excellent recognition capability for Al$^{3+}$ which could induce
non-fluorescent spirolactam form of rhodamine B to strongly
fluorescent ring-opened form. The chemsensor showed high
sensitivity toward Al$^{3+}$ with detection limit reaching 1.3 ×
10$^{-7}$ M. In addition, photo-stability of rhodamine B deriva-
tive in this hybrid MSNs material was significantly improved,
which makes it have great potential in Al$^{3+}$ sensing in bio-
logical fields. Yang and co-workers synthesized a turn-on
pH-activated rhodamine fluorescent chemosensor for Cu$^{2+}$
sensing, and the chemosensor was then loaded in nanopores
of MSNs to obtain nanosensor (Rhod-SP@MSNs) (Figure
7B). β-cyclodextrin as a gatekeeper and c(RGDyK) peptide
conjugated adamantane for recognizing αvβ3 and αvβ5
integrin receptors overexpressed on the membrane of most
cancer cells were modified on surface of the nanoprobe.
The resulting novel nanoprobe could not only be activated
by lysosomal acidic pH environment but also prevent the
macromolecules including proteins and enzymes from diff-
fusing into the nanopores to interfere the probe. The above
results indicated that the nanosensor could monitor the lysos-
omal Cu$^{2+}$ in a location-specific manner with high spatial
resolution.

Yao et al designed a novel kind of multifunctional MSNs
for detecting Hg$^{2+}$ and Zn$^{2+}$ simultaneously through site-
specific incorporation of rhodamine B derivatives (for Hg$^{2+}$
sensing) and quinoline derivatives (for Zn$^{2+}$ sensing) to
the outer and inner surfaces of MSNs, respectively (Figure
7C). Spatial separation of the two fluorophores can
effectively eliminate the FRET effect between them, which
ensured the independence for sensing of the two chromo-
phores. In the presence of Hg$^{2+}$, ring-opening process
of the spirolactam structure in rhodamine B was induced
FIGURE 6  (A) Schematic drawing of four-armed AIEgens-functionalized PMO material (TH-PMO) and fluorescence spectra of TH-PMO materials with the increase of four-armed AIEgens (TH) content. Copyright 2016, American Chemical Society. (B) Schematic illustration of the preparation process of tetraphenylethene (TPE)-bridged organosilica hybrid nanostructure (TPEPMOs). Copyright 2021, American Chemical Society. (C) Illustration scheme for the fabrication process of ACQ@AIE-PMO. Copyright 2015, Royal Society of Chemistry. (D) Illustration scheme for the synthesis of PTH@MSNs-poly(PEGMA-co-IA). Copyright 2020, Elsevier
by Hg$^{2+}$, which showed turn-on fluorescence properties. As for quinoline-based fluorophores, fluorescence enhancement could also be quickly achieved upon the addition of Zn$^{2+}$. The hybrid MSNs exhibited high sensitivity with a relatively low detection limit toward Hg$^{2+}$ ($1.8 \times 10^{-8}$ M) and Zn$^{2+}$ ($9.8 \times 10^{-8}$ M). This work firstly reported the simultaneous detection of two different heavy metals via the regional functionalization of MSNs, which provided a new preparation method of multifunctional hybrid MSNs to detect multiple metal ions.

Wu et al. synthesized an FRET-based ratiometric multilayered FSNs for Hg$^{2+}$ sensing. A nitrobenzoxadiazolyl derivative (NBD) that located in particles acted as donor and rhodamine derivative (SRhB) that was on particle surface served as Hg$^{2+}$ probe. In sensing applications, Hg$^{2+}$ could induce ring-opening reaction of rhodamine, and an efficient FRET occurred from the excited state of NBD to ring-opened SRhB. In the absence of Hg$^{2+}$, only one emission peak of NBD at 528 nm could be observed. A new emission profile at about 590 nm of ring-opened SRhB emerged upon the addition of Hg$^{2+}$. With increase of Hg$^{2+}$ concentration, emission band at 590 nm increased steadily while emission peak of NBD at 528 nm decreased gradually, which could establish a working curve of the ratio of the emission intensities at 590 nm and 528 nm ($I_{590}/I_{528}$) versus Hg$^{2+}$ concentration. The detection limit for the system could be obtained through the above working curve and reach 500 nM. Oroval and co-workers developed a novel fluorescent nanoprobe for As(III) sensing based on gated silica mesoporous materials. The obtained sensor showed high sensitivity and selectivity, which made the sensor promising in As(III) monitoring assays.

Kim’s group prepared a turn-on probe (TSBA) based on MSNs for fluoride anion sensing through anchoring silyl-ether protected FITC on amino-silane modified SBA-15 by post-grafting method. The obtained FSNs showed highly selective toward F$^-$ in totally aqueous media, and detection limit could reach 0.02 μM (Figure 8A). Recently, Yang and co-workers reported a safranin O-functionalized fluorescent cuboid MSNs (SiO$_2$@SFNO) by covalent incorporation of alkoxy silane derivatized Safranin O (SFNO) for toxic permanganate (MnO$_4^-$) sensing and adsorption. -NHCONH- groups of SFNO acted as recognition sites and coordinated with MnO$_4^-$ selectively, which endowed SiO$_2$@SFNO with attractive recognizing ability for MnO$_4^-$.

Fluorescence emission of aqueous suspension of SiO$_2$@SFNO showed a turn-off phenomenon when MnO$_4^-$ ions were added, and the emission intensity gradually decreased with
increasing the concentration of MnO$_4^{−}$−. The detection limit of SiO$_2$@SFNO for MnO$_4^{−}$ was $5.58 \times 10^{−7}$ M (67 ppb), and the material also possessed high adsorption ability toward MnO$_4^{−}$ (Figure 8B).

As one kind of reactive oxygen species, superoxide anion O$_2^{•−}$ has highly oxidative activity, and excessive accumulation of O$_2^{•−}$ in cells can lead to oxidative stress and cause damage to proteins, DNA, and other cellular components. Therefore, the detection of O$_2^{•−}$ is essential to early diagnosis and prevent related diseases. Zhu et al. developed a fluorescein doped rattle-type CSNs-based ratiometric probe for O$_2^{•−}$ sensing and imaging. FITC, which was covalently anchored in the core, acted as reference fluorophore and hydroethidine (HE) that was located in void of rattle-type SNs served as fluorescent probe for O$_2^{•−}$ (Figure 8C). In the presence of O$_2^{•−}$, the released HE was oxidized by O$_2^{•−}$, and the resulting oxidation product could emit red fluorescence with maximum emission at 570 nm. Emission intensity of oxidation product linearly enhanced with increasing of the concentration of O$_2^{•−}$. Accordingly, ratiometric determination of O$_2^{•−}$ was achieved with high selectivity and sensitivity with detection limit reaching 80 nM. This nanosensor was successfully used for ratiometric imaging of O$_2^{•−}$ in HeLa cells under normal conditions or oxidative stress.

Tang’s group designed a dual-ratiometric fluorescent nanosensor for monitoring the changes of O$_2^{•−}$ and pH in HeLa cells (Figure 8D). MSN@RhB was prepared by loading rhodamine B (Rhb) as the reference and dibenzothiazio derivative (DBZTC) for O$_2^{•−}$ and Tpy-Cy for pH sensing were incorporated simultaneously in SiO$_2$ outer shell. Subsequently, chitosan for avoiding the leakage of probe molecules and triphenylphosphonium for mitochondria targeting were modified on this nanosensor. The resulting nanoprobe exhibited good biocompatibility, specific mitochondria targeting and accurate quantitative dynamic sensing of O$_2^{•−}$ and pH.

Although a series of ACQ type silica sensing materials for various ions have been synthesized up to now, AIEgens-based silica materials for ion sensing are scarce. In order to avoid fluorescence quenching, we designed a turn-off AIEgens-based probe (TH) and embedded it into silica framework. After the modification, the obtained AIEgens-functionalized
PMOs (TH-PMO) possessed higher fluorescence quantum and stronger fluorescence emission due to AIE effect, which could produce better sensing efficiency. The detection limit of the AIEgens-PMOs-based sensor for Cu$^{2+}$ could reach $4.0 \times 10^{-8}$ M, which was much higher than fluorescence molecules in solution (Figure 9A). In addition, TH-PMOs hybrid material also exhibited excellent adsorption capacity toward Cu$^{2+}$ (Figure 9B). In 2019, Han's group successfully synthesized bis-benzimidazole PMO (BBM-PMO) ratiometric fluorescence sensor for Cu$^{2+}$. Bis-benzimidazole in BBM-PMO framework exhibited AIE properties and excited-state intramolecular proton transfer. The above properties made the hybrid material be ratiometric fluorescence sensor for Cu$^{2+}$ with ultra-high sensitivity. The detection limit of BBM-PMO material could be as low as $7.15 \times 10^{-9}$ M. Recently, this group designed an ultrasensitive fluorescence chemosensor for Cu$^{2+}$ with detection limit reaching $3.26 \times 10^{-9}$ M through incorporating a novel organosilica precursors with AIE properties into silica material (Figure 9C). The obtained PMOs material showed excellent selectivity toward Cu$^{2+}$ even in presence of other interfering metal ions (Figure 9D).

### 3.2 Molecule sensing

Silica-based fluorescent nanosensors for various molecules from gas, liquid, to solid have been widely constructed through introducing various organic fluorescent molecules into SNs. Song et al. developed a novel multifunctional nanocomposites (Ru(bpy)$_2$phen-MMS) with Fe$_3$O$_4$ cores for turn-off oxygen sensing, which was simply constructed through incorporating the ruthenium(II) polypyridyl compounds into MSNs (Figure 10A). The obtained nanocomposites showed high sensitivity and excellent reversible response toward O$_2$. Wolfbeis et al. reported an optical nanosensor for simultaneous imaging intracellular O$_2$ and pH through introducing different fluorescence molecules in separated space. The nanosensor had a core/shell nanos-structure with poly(ethylene glycol) outer shell and silica core. Probe for O$_2$ and reference dye were located at silica core, while fluorescein for pH sensing was covalently linked to poly(ethylene glycol) shell. This nanosensor realized sensing and imaging of pH and O$_2$ at same site with high spatial res-olution.

Lee's group developed biocompatible azidocoumarin-functionalised SNs (SiO$_2$NPs@Cy-N3) for monitoring trace H$_2$S and visualization of endogenous H$_2$S in cancer cells. Azide in azidocoumarin-4-acetic acid served as recognition site and fluorescent emission peak at 456 nm of
azidocoumarin-4-acetic acid molecules increased linearly with the increase of H$_2$S concentration. The resulting nanoprobe showed high sensitivity and selectivity even in the present of excess of other biothiols and ions, and detection limit could reach 6 nM. SiO$_2$NPs@Cy-N3 could be used not only in environmental H$_2$S monitoring but also in intracellular H$_2$S vapor sensing as a sensitive and selective approach. Zhao et al. reported a novel ratiometric nanoprobe for H$_2$S sensing in estrogen-induced cardiomyocytes and living mouse model based on core-shell SNs and NIR fluorescent molecules (Figure 10B).[61]

The gas fluorescence sensing often depends on highly sensitive solid-state materials-based fluorescence sensors. AIEgens ensure its advantages in the design of solid-state sensors. Therefore, the FSNs functionalized with AIEgens have made great progress in the field of gas sensing. Yu and co-workers reported an AIEgens-functionalized MSNs for efficient sensing toward volatile acidic gas. AIEgens were covalently bonded into the channels of MSNs, and the obtained hybrid nanomaterial (MSNF) was then made into films.[139] Fluorescence of MSNF film decreased with red-shifts immediately when it was exposed to HCl gas along.
with fluorescent emission changing from green to yellow. The visible color change from white to yellow correspondingly, which indicated that the MSNF film could act as an excellent sensor for the dual-channel HCl gas sensing. Furthermore, the film exhibited excellent reversible response under periodically switching between HCl and NH₃ vapor. Recently, our group designed an electrospinning superassembled mesoporous AIEgen-Organosilica probe (TPEPMO) for naked eye detection of NH₃ and HCl vapor (Figure 10C).[48] TPEPMO was prepared by a two-step process in which organosilica precursors with AIE features were covalently linked into frameworks of MSNs, and then the obtained TPEPMO nanospheres were dispersed in hybrid fibrous matrix (TPEPMO-CFs) using the electrospinning superassembly technique. TPEPMO-CFs that possessed diversified forms and superstability could be used as wearable and washable solid-state fluorescence smart sensors for NH₃ and HCl vapor. The stable fixation provided by superassembly method and strong fluorescence emission induced from the fixation of AIEgens ensure the stability of the flexible gas sensor, which provides a fresh perspective for the flexible wearable sensor. Tian et al. reported a fluorescence sensor-based TPE derivative (TPE-2NH₂)-functionalized SNs (TPE-2NH₂@SiO₂), which could be used for ratiometric sensing of NO gas molecules produced in the living cells.[62] In the presence of NO, fluorescence emission of the sensor significantly changed from green to red. This was because NO molecules could react with o-phenylenediamine in TPE-2NH₂, accompanied by the generation of a new molecule of TPEBZT with triazole ring. When MCF-7 cancer cells were treated with lipopolysaccharide to produce NO, strong red emission signal of the nanosenor could be observed. The above results indicated that the sensor could be applied for effective monitoring of the intracellular NO changes.

Explosive detection was also achieved by using AIEgens-functionalized silica nanomaterial. In 2012, Tang and co-workers designed a recyclable efficient fluorescent turn-off sensor (SNF) for supersensitively detecting picric acid (PA) in water solution by combining mesoporous SBA-15 with AIEgens.[63] The large pore volume and surface area of the obtained fluorescence MSNs could increase mass transport and enhance interactions of the anchored AIEgens with the PA molecules. In the presence of PA, a very rapid fluorescence quenching could be observed, which was attributed to the efficient photoinduced electron transfer and/or energy transfer between PA and AIEgens. In particular, the sensor could be recycled, which made it to be an environmentally-friendly detectors for practical explosive sensing. Yu’s group synthesized AIEgens-functionalised silica (FMSNs) by postgrafting TPE derivatives on MSNs, which could acted as an effective fluorescent probe for detecting explosives, including PA, 4-nitrotoluene, nitrobenzene in water (Figure 11A).[106] In addition, the obtained materials also exhibited excellent sensing performance toward antibiotics furazolidone and nitrofurazone with low detection limit.

Yu and co-workers reported a novel turn-on sensor (Ag@TPE-SiO₂ NPs) for H₂O₂ sensing (Figure 11B).[104] AgNPs that was on surface of TPE-SiO₂ NPs acted as a nanoquencher for quenching TPE emission efficiently. However, in the presence of H₂O₂ molecules, AgNPs could be oxidized to Ag⁺, which made the fluorescence recover and color fade for the sensor. Above results demonstrated that the Ag@TPE-SiO₂ NPs could serve as an excellent sensor for the dual-readout H₂O₂ sensing, and the detection limit could reach 0.28 and 2.1 μM for the fluorometric and colorimetric method, respectively. Tang’s group developed a mitochondria-targeted fluorescent nanoprobe based on MSNs for real-time monitoring changes of H₂O₂ and pH in living cells. The nanoprobe was constructed by loading two probes, Cy-O-SeH for H₂O₂ and fluorescein for pH in pores of MSNs.[141]

Xue et al reported a type of AIE-SiO₂-MnO₂ sandwich nanocomposites for facile and visual detection of organophosphorus pesticides (OPs) (Figure 11C).[105] BSPOTPE-SiO₂ nanocomposites formed through electrostatic attraction of negatively charged AIE molecules (BSPOTPE) on positively charged SNs. MnO₂ nanosheets covered the surface of BSPOTPE-SiO₂ nanocomposites and served as a nanoquencher for quenching BSPOTPE emission efficiently. Thiocacholine (TCh) that generated from acetylthiocholine (ATCh) by enzyme catalysis of acetylcholinesterase (AChE) could decompose MnO₂ nanosheets and thus recover the fluorescence of BSPOTPE-SiO₂ accordingly. However, the introduction of OPs which could irreversibly inhibit the catalytic activity of AChE would reduce fluorescence emission of ACh-E-AChE-system. According to this principle, AIEgens-based silica fluorescent sensor for OPs was developed. In addition, a convenient fluorescence strip for rapid visual detection of OPs was also fabricated, which indicated the turn-off fluorescent sensor was expected to be used for on-site detection.

Molecular imprinting methodology which is considered as an efficient way to produce recognition site has been widely used in the design of chemical sensors and biosensors. In 2017, Chang and co-workers designed a TPE derivative (TBPE)-grafted molecularly imprinted MSNs-based turn-off fluorescent sensor (TBPE-DIMSNP) for diethylstilbestrol (DES) sensing (Figure 11D).[106] Target molecules of DES were modified with two triethoxysilane groups and then incorporated into MSNs via thermally reversible urethane bonds. DES imprinted MSNs (DIMSNP) were achieved by removal of DES, and subsequently, TBPE that served as fluorescent probe was covalently introduced into the pores of MSNs. The obtained sensor displayed excellent sensitivity and selectivity for DES sensing through its fluorescence quenching effect of DES toward the sensor. In addition, TBPE-DIMSNP also showed excellent recyclability via the removal of the adsorbed DES, which made it to be an environmentally-friendly detector for practical DES sensing.

### 3.3 Temperature sensing

Fluorescence sensor based on functional silica nanohybrids for temperature sensing was developed by incorporating temperature sensitive fluorescent molecules. Wolosiuk and coworkers reported a temperature sensor based on luminescent tris(bipyridine)ruthenium(II)-doped SNs through Stöber method.[112] The fluorescence emission intensity of the sensor decreased linearly with increasing temperature between 20 and 60°C. In 2015, Yang et al designed a low temperature ratiometric sensor based on salicylideneanilines (HBA)-dopeded MSNs with embedded gold nanoparticles in the silica walls (HBA-GMS).[67] HBA molecules with the
thermochromism and photochromism properties were encapsulated in the channel of MSNs for temperature response and gold nanoparticles embedded in the pore wall as reference fluorophores. The resulting sensor could achieve accurate temperature sensing in the range of 100–298 K (Figure 12A).

Tian and co-workers reported a novel smart core-shell thermo-sensitive fluorescent MSNs for detection intracellular temperature and temperature-controlled drug release (Figure 12B). Luminescent carbon dot-based MSNs (CD-MSNs) as the core and then 3-(trimethoxysilyl) propyl methacrylate was anchored on surface of CD-MSNs for further grafting of the thermosensitive polymer. The obtained nanohybrid could be used as sensitive thermometers by loading signal dye (BBD) into mesopores as temperature indicator. This novel thermometer showed fast temperature responses toward fluctuation down to 0.2°C with high sensitivity and fluorescence ratio intensity (F_{BBD}/F_{CD}) increased linearly from 36.5°C to 39°C, which covered the biological temperature range. The temperature nanosensor was successful applied for accurate temperature monitoring at single cell level. In addition, this nanosensor could also be used as temperature-controlled drug delivery system for cancer therapy, which was simply induced by the thermal effects of cancer cells themselves.

Wolfbei’s group developed a novel of luminescent Eu(III) chelates-doped (Eu-DT) SNs for temperature sensing and imaging in the physiological range. The fluorescence intensity of Eu-DT in SNs seriously reduced due to
concentration quenching. In order to reduce self-quenching, the ratio of Eu-DT was reduced and poly(methylmethacrylate) (PMMA) was chosen as a co-matrix to compensate the reduction of Eu-DT. Sensitivity of this sensor toward temperature was investigated through changes of fluorescence intensity and lifetime. Fluorescence emission intensity and lifetime of the nanosensor decreased with increasing temperature from 10 to 50°C.

Gao et al. synthesized AIEgens-functionalised fluorescent MSNs by the encapsulation of AIEgens-based gemini surfactant (C_{TPE-C_{6}}-C_{TPE}).[68] The fluorescence emission intensity of the resulting nanoparticles decreased linearly to almost disappear as the temperature increased from 0 to 80 °C, which indicated the excellent sensitivity and promising potential of the AIEgens-based fluorescent hybrid materials in temperature sensing.

3.4 | pH sensing

Fluorescent nanoprobes for pH sensing based on silica have been widely synthesized and applied in vitro and in vivo. The non-covalent encapsulation or covalent attachment of fluorescent pH indicators with silica nanomaterials have become a widespread and well-examined approach to develop pH-responsive FSNs. Ratiometric fluorescent probe based on silica material for pH has also constructed by introducing additional pH-insensitive reference fluorophores. In one of the preliminary reports,[94] Wiesner’s group designed a novel type of dual-emission fluorescent SNs possessing TRITC fluorescent core and FITC shell, and the obtain dual-emission FSNs were successfully applied to quantitative ratiometric analysis of pH in vitro. Liu and co-workers fabricated fluorescent pH-sensing multifunctional hybrid MSNs, which could also regulate the redox reaction release of embedded guest molecules.[145] Random copolymers including pH sensitive monomer were anchored at the surface of MSNs. The resulting sensor showed good water dispersibility and could be used for pH (from 4 to 8) sensing with high sensitivity. In addition, the pH nanosensor was further loaded rhodamine B as model drug molecules, and then polymer brushes on MSNs were cross-linked with cystamine to block nanopore. The redox-responsive release of rhodamine B from the above multifunctional hybrid MSNs could be regulated through changing the amount of dithiothreitol.

Corrie’s group reported core/shell SNs containing fluorescein (for pH sensing) in shell and pH-insensitive fluorophores (Cyamine5) in core, which were employed for stable pH sensing in biological media.[146] Porosity of shell was induced with tannic acid aiming to enhance local mass transport and reduce nanosensor response time. Ratiometric fluorescence signals of the nanosensor were highly sensitive to pH in physiological range (4.5–8) with a fast response time. Silane-modified polyethylene glycol (PEG) that was modified on surface of the nanosensor is to minimize particle aggregation and enhance stability of the nanosensor.
in aqueous solutions. Tang’s group developed a ratiometric nanoprobe based on MSNs for organelles pH imaging in living cells, which was constructed by covalently incorporating pH-sensitive aminofluorescein (AF) and pH-insensitive ethidium bromide (EB) as reference fluorophore in MSNs and then the resulting nanoparticles were coated by amino-functionalized silica shell (Figure 13A).\cite{147} Finally, the organelles-targeted molecules for lysosomes and mitochondria were anchored on the nanoprobe respectively for specifically targeting organelles pH imaging. Living cell imaging indicated the resulting nanoprobe could achieve accurately monitoring pH changes of cytoplasm, lysosomes, and mitochondria in the range of 5.0–8.3. Shuang et al. reported a nanosensor for intracellular pH sensing based on Janus SNs that was hemispherically functionalized with fluorescein isothiocyanate (FITC) on one side and modified with rhodamine B (RB) on the opposite side (Figure 13B).\cite{70} The resulting ratiometric fluorescent nanosensor was successfully applied to pH sensing and imaging in cancer and normal cells with high selectivity and sensitivity. However, although ACQ-active fluorophores-based silica nanosensors for pH sensing have been widely studied, AIEgens-based fluorescence silica sensors for pH are rarely reported.

4 | BIOSENSING AND BIOIMAGING

FSNs, as efficient luminescent materials, have been widely applied to biosensing and bioimaging.\cite{18} Silica can reduce the potential toxicity and improve water-dispersibility, photostability, and fluorescence QY of these fluorophores. In particular, the surface of silica nanomaterials is easy to be functionalized, and efficient surface modification of FSNs can help to control the functionality of the nanomaterials.\cite{148} Surface modification of FSNs with permeation enhancers and targeting agents not only increases their cell permeability and biocompatibility but also favors the targeted imaging.\cite{149} These features enable the FSNs to exhibit great potential in biosensing and bioimaging. Meanwhile, the biosecurity assessment of FSNs could be extremely significant at molecular, cellular, and histological levels.\cite{150} The major parameters, such as size, shape, and surface charge distribution of SNs, can affect properties of the biocompatibility of FSNs.\cite{5} The low concentrations of FSNs have good biocompatibility and the materials always display non-cytotoxic effects at concentrations below 200 $\mu$g/mL.\cite{151} FSNs have excellent biological application performance with non-toxicity and good biocompatibility.

4.1 | Biosensing

The effective, sensitive, and selective detection of biologically relevant analytes in their native environment by nanosensors holds much promise for real-time biosensing, which requires long-term stability of nanosensors in complex bioenvironments.\cite{152,153} Because of photophysical/chemical stability and negligible cytotoxicity of silica nanomaterials,
the FSNs show their superiority in intracellular and in vivo sensing. Liu’s group designed a sensor based on AIEgens (BTPEBT)-doped SNs to detect toxins or bacteria. Green BTPEBT-F127-SiO₂ NPs were obtained by encapsulating BTPEBT with F127 and silica shell, which were subsequently modified with ricin binding aptamer with the assistance of GO. GO that was an effective quencher for AIEgens was used for quenching fluorescence of BTPEBT-F127-SiO₂ NPs to provide a turn-on approach to achieve specific detection of ricin and bacteria (Figure 14A).

The fluorescent sensor based on fluorescence silica nanohybrid materials was also synthesized for detection of biomolecules. Gai et al. developed a multiple dyes-doped SNs (MD-SCMNPs) for sensing of cysteine (Cys) in living cells by encapsulating fluorescein derivative (FCD), coumarin derivative (HCE), and Rhodamine b (RhB) in cores of SNs (Figure 14B). The resulting nanosensor showed excellent biocompatibility and could quantitatively detect Cys through the visualized color change with low detection limit. Chang et al. designed a TP fluorescence turn-on nanosensor (TPNPs) for ascorbic acid sensing and imaging in cells or tissues. Negative-charged TPNPs could adsorb positively charged cobalt oxyhydroxide (CoOOH) nanoflakes through electrostatic force to form CoOOH-Modified TPNPs. The introduction of CoOOH nanoflakes could cause significant decrease of fluorescence intensity for TPNPs. However, the addition of ascorbic acid could decompose CoOOH nanoflakes into Co²⁺ and thereby results in remarkable fluorescence enhancement of the nanosystem. The nanoprobe exhibited a high selectivity over interfering species and high sensitivity toward ascorbic acid with detection limit reaching 170 nM. In addition, the nanosensor was successfully applied to TP imaging of ascorbic acid in Hela cells and rat liver tissues with excellent membrane-permeability and biocompatibility. Zhang et al. synthesized a TP nanosensor (TP-MSNs) for glutathione (GSH) sensing in cells or tissue. In this sensing system, negatively charged MnO₂ nanosheets as a nanoquencher were adsorbed on positively charged MSNs and could quench the fluorescence of TP probes in MSNs. However, the fluorescence recovery of the nanosensor could be observed in presence of GSH. This could be attributed to that GSH as reducing agent could decompose MnO₂ nanosheets into Mn²⁺ (Figure 14C).

Ma et al. developed AIEgens-functionalized SNs (TSiO₂) for detecting human epididymis protein 4 (HE4). TSiO₂ that exhibited AIE characteristic could be obtained through doping positive charged TPE-C₄₋₄ molecules into SNs via Stöber method, and the antibody was covalently anchored onto the surface of TSiO₂ to achieve antibody immobilized nanoparticles (a-TSiO₂). In addition, magnetic beads (MBs) were modified with antibody (a-MB) which were associated with a-TSiO₂ to form sandwich immunoassay for HE4 protein sensing. The assay showed good sensitivity and selectivity for HE4 sensing, and the detection limit could reach 40 pM. Zhang and co-workers designed TPE functionalized SNs with core-shell structure for acetylcholinesterase activity and inhibitor screening. Ouyang’s group developed a plasmon-enhanced fluorescent sensor (PEF-AIE) to monitor conformational changes in protein (Figure 15). An anthracene derivative (BSNV A) which had good AIE properties was used to construct PEF-AIE sensor, and prion protein (PrP) was selected as the model. Almost no fluorescence emission could be observed when the sensor was mixed with prion protein (PrP). However, this PEF-AIE sensor showed...
strong fluorescence emission when it was mixed with disease-associated prion protein (PrPSc). The above results indicated the PEF-AIE sensor could effectively distinguish PrPSc from PrPC. In addition, this PEF-AIE sensor was successfully used for monitoring kinetic process of conformational conversion through fluorescence changes of PEF-AIE sensor. Detection limit for PEF-AIE sensor could reach 10 pM due to the effect of fluorescence signal amplification. The strategy provided a new method for protein sensing and conformational monitoring, which was hopeful to promote the search on protein-conformational diseases.

### 4.2 Bioimaging

Fluorescence imaging techniques which have a series of advantages such as fast-response, non-invasion, high sensitivity, in situ and in real-time monitoring have been widely used in living cells and in vivo. Dye-doped SNs are a superior and flexible nanoplatform to develop fluorescence imaging techniques for bioimaging applications, which have been extensively and deeply investigated in many studies. In general, fluorescent dyes with long-wavelength emission for bioimaging are more desired as they could significantly improve the signal to noise ratio and tissue penetration depth. In addition, strong fluorescence signal is the effective guarantee of the fluorescence imaging system. However, it is challenging to prepare high-bright dye-doped SNs by using conventional fluorophores due to the ACQ effect, which will obviously affect their performance for bioimaging. The AIEgens which possess the opposite luminescent properties to ACQ fluorophores could just make up for this shortage. In this part, we will review several representative biological imaging applications of ACQ fluorophores-based FSNs and mainly introduce the application of AIEgens-silica hybrid materials in biological imaging.

With the development of fluorescent biological imaging system from in vitro to in vivo, fluorescent nanoparticles with NIR imaging performance have become an important research field because of their strong tissue penetration and small tissue damage. NIR fluorescence imaging as a promising technique could achieve deep tissue and clear image in vivo due to the decrease of light scattering, absorption, and tissue autofluorescence. At present, the ACQ fluorophores-based FSNs have achieved remarkable results in this field. Hayashi and co-workers developed two kinds of NIR fluorescent silica/porphyrin nanohybrids through introducing tetrakis(4-carboxyphenyl)porphyrin (TCPP) in silica nanomaterial via covalent bonding (denoted as HNRs) and simple non-covalent encapsulation (denoted as NSs). HNRs contain more TCPP content than NSs and PEG-HNRs had longer blood circulation time than PEG-NSs, which indicated HNRs was significantly more effective than NSs in bioimaging. TCPP in silica matrix could form J...
aggregates through π-π stacking, which resulted in redshift of the absorption bands of TCPP to the NIR region. The results of tumor imaging experiment demonstrated that PEG-HNRs tended to accumulate in the tumor and could achieve tumorclear imaging and detection through NIR fluorescence imaging. Sletten and co-workers synthesized a novel NIR fluorophore IR-140-doped hollow MSNs for high resolution shortwave infrared (SWIR, 1000~2000 nm) imaging in vivo. IR-140 existed in the form of J-aggregates that could emit SWIR light inside MSNs cavity, which could endow the resulting nanomaterials with superior resolution for imaging in vivo.

Wang et al. reported a novel NIR fluorophore doped fluorescent SNs with large Stokes shift (>200 nm) based on FRET by synchronously introducing Rubpy and methylene blue as donor and acceptor in SNs (Figure 16A). Compared with single NIR fluorophore-functionalized silica nanohybrids, FRET system that has relatively larger Stokes shift could significantly decrease background and crosstalk between the excitation and emitting light simultaneously. Consequently, the obtained Rubpy and MB-doped FSNs (LSS-NFSiNPs) with high resolution could achieve deep-tissue fluorescent imaging in vivo.

TP fluorescence imaging as an advanced imaging technique could also achieve deeply and clearly three-dimensional images of cells and tissues by localized excitation in NIR spectral region. Durand and co-workers designed gold@bridged silsesquioxane nanoparticles for enhanced TP fluorescence imaging and therapy of cancer cells. In this report, organic TP sensitizer molecules
with four trialkoxyxilyl groups were covalently linked within SNs to form TP probe (BSNPs). Gold-doped BSNPs were constructed by introducing gold nanospheres to enhance TP properties. The obtained nanocomposites exhibit ultra-bright fluorescence imaging and excellent therapy performance. They also developed disulfide-gated MSNs for drug release and bioimaging using the same TP sensitizing molecules.[174] Xu and co-workers synthesized a novel kind of multifunctional nanocomposites (Au NRs/mSiO$_2$-HP) with a gold nanorod core for TP imaging and porphyrin-doped mesoporous silica shell for PDT (Figure 16B). Porphyrin in nanocomposites could generate higher singlet oxygen than free porphyrin. The high TP luminescence efficiency and the increased generation efficiency of singlet oxygen of the nanocomposites made it a promising platform for TP bioimaging and real-time PDT.

Dual-modal or even multi-modal bioimaging nanoprobe is an alternative approach to achieve accurate diagnostic information, which could be achieved through introducing other imaging modalities into one fluorescence nanoparticle.[175] Zeng and co-workers successfully performed the dual-modality sentinel lymph nodes mapping simultaneously through photoacoustic and NIR fluorescence using NIR dye (Cy754) functionalized MSNs with core-shell structure.[176] Zhao et al. designed a novel fluorescence contrast system based on gold nanospheres and NIR dye IR-783 (water-soluble cyanine dye)-loaded MSNs for both computed tomography (CT) and fluorescence imaging (Figure 16C).[170]

In order to improve the fluorescence bioimaging efficiency of FSNs, enhancing fluorescence emission intensity is also an effective method. The discovery of the AIE phenomenon provides a new strategy to prepare intensive luminescent silica nanomaterials because higher concentration of AIEgens is allowed to be incorporated in silica. At present, AIEgens-functionalized silica luminescent nanohybrids have been widely reported in biological imaging applications.[199] Here, different types of AIEgens-functionalized FSNs, and their bioimaging applications are listed in Table 2.

Tang’s group designed a new type AIEgens-doped MSNs (denoted as TTF@SiO$_2$ NPs) for long-term and super-resolution fluorescence imaging of cancer cells by use of stimulated emission depletion (STED) nanoscopy.[185] TTF@SiO$_2$ NPs possessed high STED efficiency (more than 60%) and high photobleaching resistance. More importantly, STED nanoscopy imaging of cancer cells with TTF@SiO$_2$ NPs was performed, and the lateral spatial resolution could reach 30 nm.

In order to achieve targeted cell imaging and increase uptake efficiency for nanoparticles, surface of fluorescent silica materials are further functionalized with targeting ligands such as biotin, folic acid, and aptamers, etc. Since folate receptors are overexpressed on surface of tumor cells, Tian and co-workers developed core-shell folate-functionalized MSNs by using AIE-active 9,10-distyrylanthracene dyes as the core and folate-functionalized MSNs as the shell.[177] The obtained nanomaterial was successfully applied for targeted intracellular imaging of HeLa cells. Belfield et al. designed a folate receptor-targeted FSNs with AIE and NIR emitting properties for one-photon in vivo and TP ex vivo fluorescence bioimaging.[194] Yan and coworkers fabricated a novel hybrid siliceous fluorescent vesicle (HSFVs) through the ionic self-assembly of an AIEgen to form the template vesicle whose surface was then deposited with folic acid modified silica shell (Figure 17A).[195] The resulting HSFVs integrated the multiple functions of fluorescence imaging, cancer-cell targeting, and controlled drug release, which provided a promising strategy in precision medicine. Tang’s group designed biotin-decorated SNs with AIE-active silole fluorophores as core and biotin-decorated silica as shell for targeted imaging of tumor cells with overexpressed biotin-specific receptors.[178] The surface of AIEgens-functionalized SNs were also modified with DNA aptamers by Xiang’s group to achieve targeted cell imaging.[186]

Apart from cell imaging, AIEgens-functionalized silica luminescent nanohybrids have also been successfully applied in vivo bioimaging. Liu and co-workers developed a novel fluorescent hybrid nanocomposite (TPETPAFN-F127-SiO$_2$ NPs) with high fluorescence QY (50%) for TP excited fluorescence bioimaging.[179] The fluorescence emission of the obtained nanocomposite was obviously brighter than that of commercial quantum dot (QD655), and the average number of photons emitted by each TPETPAFN-F127-SiO$_2$ nanoparticle was around 3.7-fold of QD655. For the biological application, the nanocomposite was injected intravenously into mouse, and vasculature of the tibial muscle was imaged using TP-excited microscopy. The results showed that both major blood vessels and smaller capillaries of the tibial muscle could be clearly visualized due to good dispersion of the nanocomposite with no obvious aggregation. Li and co-workers designed fluorescent hybrid nanoparticles (QM-2@PNPs) through encapsulating the AIE-active quinoline-malononitrile derivative (QM-2) into the silica shell and block copolymer.[182] The imaging experiments of MCF-7 cell demonstrated that QM-2@PNPs could label cells with superior photostability and long-term tracking abilities. For in vivo bioimaging, the obtained fluorescent hybrid nanoparticles preferred accumulation in tumor site of tumor-bearing mice with high brightness while bare QM-2 aggregates showed whole body distribution of mice. The above results indicated the fluorescent hybrid nanoparticles displayed efficient tumor-targeting ability (Figure 17B).

Dual-modal bioimaging nanoprobes based on AIEgens-functionalized SNs were also developed. Wang et al. designed a dual-modal nanoprobe for both fluorescence imaging and $^{19}$F magnetic resonance imaging (MRI) ($^{19}$F-MRI) by encapsulating AIE-active tetraaniline derivatives (ETTA) with polysuccinimide derivatives and fluorinated agent (PDTEs).[184] The ETTA aggregates in the core was used for fluorescence imaging, and $^{19}$F moieties in PDTEs were for $^{19}$F MRI simultaneously with deep penetration. The aforementioned nanocomposites were further modified with a cell targeting peptide for specific targeting of cancer cell to obtain the dual-modal nanoprobe. Cell experiments demonstrated the nanoprobe-possessed specific targeting ability toward HeLa cells, and cellular uptake of the nanoprobe could be obviously observed from $^{19}$F NMR spectroscopy. For in vivo imaging, the nanoprobe could achieve tumor imaging in tumor-bearing mice and clearly distinguish tumor from the surrounding normal tissues (Figure 17C). Zhang et al. designed a novel dual-mode nanoprobe by incorporating red AIEgens (TPATBT) and Gd$^{3+}$ in MSNs for fluorescence imaging and MRI.[193] The introduction of APTES could not only encapsulate TPATBT in mesopores but also...
| Material                  | Functional unit                              | Embedded form             | Structure                      | Bioimaging application                                  | Ref. |
|--------------------------|----------------------------------------------|---------------------------|--------------------------------|----------------------------------------------------------|------|
| BDSA/ORMOSIL             | 9,10-bis(4′-(4′-aminostyryl)styryl)anthracene derivative | Non-covalent entrapment   | CSNs                           | Two-photon fluorescence cell imaging                     | [78] |
| AIE-active FSNPs         | Tetraphenylethene or silole derivative        | Covalent attachment       | Core–shell CSNs               | Cytoplasm of HeLa cells imaging                          | [106]|
| MFSNPs                   | Silole derivative                             | Covalent attachment       | Core–shell CSNs               | Cytoplasmic imaging                                      | [107]|
| FFSNPs                   | 9,10-distyrylanthracene                      | Covalent attachment       | Core–shell CSNs               | Targeted cell imaging                                    | [177]|
| FSNP s                   | Silole derivative                             | Covalent attachment       | Core–shell CSNs               | Cytoplasm of tumor cells targeted imaging                | [178]|
| FSNPs                    | Tetraphenylethene or silole                  | Covalent attachment       | Core–shell CSNs               | HeLa cells imaging                                       | [104]|
| CWQ-11@SiO2 NPs          | CWQ-11                                       | Non-covalent entrapment   | CSNs                           | Tumor cells imaging                                      | [80]  |
| AIE-F127-SiO2 NPs        | Tetraphenylethylene derivative               | Non-covalent entrapment   | CSNs                           | Two-photon fluorescence imaging of vasculature           | [179]|
| AIE-MSNs                 | 9,10-distyrylanthracene derivative           | Non-covalent entrapment   | MSNs                           | A549 cells imaging                                       | [88]  |
| FHMSNs                   | Tetraphenylethene                            | Covalent attachment       | Hollow MSNs                   | HeLa cells imaging and drug delivery                    | [180]|
| Apte-AIE-FSNPs           | Salicylaldehyde hydrazones                  | Non-covalent entrapment   | CSNs                           | Targeted MCF-7 cell imaging                              | [181]|
| QM-2@PNPs                | Quinoline-malononitrile                      | Non-covalent entrapment   | CSNs                           | Tumor-targeted bioimaging in vivo                        | [182]|
| FMBG                     | Tetraphenylethene                            | Covalent attachment       | MSNs                           | HeLa cells imaging and drug delivery                    | [183]|
| NC-RGD                   | 4,4′,4″,4‴-(ethene-1,1,2,2-tetrayl)tetraamidine | Non-covalent entrapment   | CSNs                           | Fluorescence and 19F magnetic resonance imaging of cell and in vivo | [184]|
| TTF@SiO2 NPs             | 2,3-bis(4-(phenyl(4-(1,2,2-triphenylvinyl)phenyl)amino)phenyl) furanotinurile | Non-covalent entrapment   | MSNs                           | Cancer cells imaging                                     | [185]|
| CFMSNs                   | Tetraphenylethene                            | Covalent attachment       | MSNs                           | Cells imaging and therapy                                | [186]|
| FMSNs                    | Tetraphenylethene                            | Covalent attachment       | MSNs                           | HeLa cells imaging and drug delivery                    | [187]|
| FMSN@CuS                 | Tetraphenylethene                            | Covalent attachment       | MSNs                           | Gastric cancer cells imaging and chemo-photothermal therapy | [188]|
| Pr-MSNP-E                | Bis(diphenylphosphino)methane phenyl pyridine platinum(II) chloride | Non-covalent entrapment   | MSNs                           | Cancer cell targeted imaging and therapy                 | [189]|
| DCDPP-2TPA-encapsulated silica NPs | 5,6-bis(4′(diphenylamino)-[1,1′-biphenyl]-4-y]pyrazine-2,3-dicarbonitrile | Non-covalent entrapment   | CSNs                           | Two-photon cell imaging and photodynamic therapy        | [190]|
| PhENH2-MoS2-FA MSNs      | PhENH2                                       | Covalent attachment       | MSNs                           | Targeted cellular imaging and photothermal therapy       | [191]|
| Apt-MSHNs                | poly(N,N-diphenyl-4-(4-(1,2,2-triphenylvinyl)styryl)aniline) | Non-covalent entrapment   | Hollow MSNs                   | Targeted cancer cells imaging                            | [84]  |
| SNP                      | Tetraphenylethene                            | Covalent attachment       | Core–shell CSNs               | HeLa cells imaging                                       | [192]|
| Gd-TPATBT-MSNs           | TPATBT                                        | Non-covalent entrapment   | MSNs                           | Fluorescent and magnetic resonance cells imaging         | [193]|
| PTH@MSNs-poly(PEGMA-co-IA) | 10-phenylphenothiazine                        | Covalent attachment       | MSNs                           | A375 cells imaging and drug delivery                    | [122]|

Abbreviations: BDSA, anthracene derivative dye; CSNs, colloidal silica nanoparticles; FMBG, tetraphenylethene functionalised mesoporous bioactive glass; MSNs, mesoporous silica nanoparticles; NC-RGD, bioconjugated with a cell targeting peptide (Arg-Gly-Asp, RGD); ORMOSIL, organically modified silica; SNP, silica nanoparticles.

Chelate with Gd\(^{3+}\) in MSNs. The obtained nanoprobe displayed strong red fluorescence emission from TPATBT and thus could be used for fluorescence imaging. As an effective contrast agent, Gd\(^{3+}\) which could improve the contrast of MRI allowed the obtained nanoprobe to be applied in contrast-enhanced MRI. The results of cell imaging demonstrated that the nanoprobe with low cytotoxicity and good biocompatibility displayed a strong red fluorescence response and an excellent MRI T1 enhancement. The above results indicated that the nanoprobe was a promising candidate for...
bioimaging and provided an alternative method for synthesis of dual-modal nanoprobe based on silica nanomaterials.

Multifunctional nanoprobes based on AIEgens-functionalized SNs for fluorescence imaging and therapy were also successfully designed and fabricated. Yu’s group developed two multifunctional nanotheranostic systems based on AIEgens-functionalized MSNs and CuS nanoparticles for fluorescence imaging and chemo-/photothermal therapy. In one work, AIE molecules for fluorescence imaging were incorporated into MSNs through post-grafting method to obtain fluorescence MSNs, and ultra-small CuS nanoparticles were attached on the surface of FMSNs as the photothermal agent. The resulting nanocomposites (CFMSNs) were then loaded with anticancer drugs DOX by electrostatic interaction. CFMSNs could efficiently release DOX in an acidic environment, and the release rate for DOX in lower pH could be obviously enhanced by NIR light due to thermal effect from CuS nanoparticles. More importantly, better therapeutic effect was achieved through the combination of chemotherapy and photothermal therapy compared with individual therapy. In the other work, AIEgens were introduced into MSNs through co-condensation method, and per-6-thio-β-cyclodextrin-modified ultrasmall CuS nanoparticles were assembled on the the surface of as-prepared FMSNs. The obtained nanocomposites (FMSN@CuS) were loaded with DOX and then were used for imaging guided chemotherapy and photothermal therapy (Figure 17D).

Tang’s group designed a nanotheranostic system by encapsulating pyrazine-containing AIEgens as efficient photosensitizer in SNs for TP PDT. Chen and co-workers fabricated AIEgens and folic acid-bifunctionalized MSNs with embedded MoS2 nanosheets as an excellent photothermal agents for bioimaging-targeted photothermal therapy of cancer cells.

5 CONCLUSIONS AND PERSPECTIVES

The review outlines the fabrication methodologies of inorganic-organic hybrid fluorescent silica materials and their sensing and imaging applications. The fluorescent dyes can be introduced into two major classes of SNs including CSNs and MSNs through non-covalent entrapment or covalent strategies. The main synthetic methods of FSNs are Stöber-Van Blaaderen method, reverse microemulsions, and direct micelle assisted method. The choice of synthesis method depends on the properties of the chosen dyes, such as their hydrophilicity and hydrophobicity properties, charge properties, and synthetic accessibility of the alkoxysilane derived dyes. So far, many conventional dyes and AIEgens have been incorporated into silica material through physical doping or covalent linking to integrate the advantages of both inorganic and organic components. The obtained fluorescence silica hybrid materials exhibit many advantages including tunable and strong fluorescent emission, high photostability, good biocompatibility, and ease of surface modification, which make them widely applied in chemical sensing, biosensing, and bioimaging. In contrast to ACQ system, the AIEgens provide a new strategy to overcome the ACQ effect of conventional organic fluorescent dyes for developing highly efficient luminescence silica materials. The combination of AIEgens and SNs can take full advantage of both. The FSNs that are functionalized with AIEgens possess higher fluorescence intensity, and the photostability of AIEgens is significantly

FIGURE 17 (A) Schematic drawing of fabrication of FA-HSFVs and its targeted cancer cell imaging. Copyright 2017, American Chemical Society. (B) Schematic drawing of the fabrication of QM-2@PNPs and its in vivo imaging after injection of QM-2@PNPs and bare QM-2 for 0.5, 2, and 24 h. Copyright 2016, Wiley-VCH. (C) Synthesis routes of dual-modal nanoprobe and its dual-modal imaging applications. Copyright 2017, Royal Society of Chemistry. (D) Scheme of the preparation of the DOX-loaded FMSN@CuS and its intracellular drug release. Copyright 2017, American Chemical Society.
improved by silica matrix, which greatly enhances the value of AIEgens-functionalized FSNs in practical applications. Multi-functional and multi-modal AIEgens-based FSNs can be established through effective design, which provides a potential choice for fluorescent sensing and imaging in cells, tissues, and complex biological environments.

Tremendous efforts have been made to develop conventional organic fluorescent dyes-functionalized silica hybrid materials for sensing and biological applications. However, AIEgens-functionalized SNs are still a young research field, and there are some challenges remained to be addressed. As a new class of fluorescent materials, the types of AIEgens are far less than the traditional ACQ molecules, and the most used AIE motifs are still restricted to TPE derivatives and siloles. In addition, AIEgens-functionalized SNs are rarely involved in the fields of near infrared, TP, upconversion, dual-modal bioimaging applications, and dual/multi-functional systems. These can be attributed to the need of complicated molecular design and structural modifications of AIEgens-functionalized SNs. Finally, SiO$_2$ framework can irreversibly immobilize AIE molecules, which can lead to that AIEgens could not aggregate to produce fluorescence properties change in present of analytes and limits the advantages of AIEgens in sensing.

With the development of FSNs in the optical application fields, the fluorophores embedded in SNs are constantly being improved to meet different needs. The balance between application requirements and material design is becoming more and more perfect. The future developing directions for AIEgens-functionalized SNs include but are not limited to the following points (Figure 18). Firstly, the family of AIEgens should be expanded and more brand new AIEgens should be explored to extend the analyte detection ranges. AIEgens-functionalized SNs for chemical sensing (including ions, molecules, pH and temperature) and biosensing (such as bacteria, cell, protein, enzyme, DNA, and other intracellular biomacromolecules, etc.) can be designed based on the abundant AIEgens. Secondly, reversible immobilization of AIEgens in SNs should be realized. In general, the most common sensing principle of AIEgens for specific analytes is based on fluorescence properties change of AIEgens which is caused by the interaction between AIEgens and specific analytes through the formation of aggregates or the restricted intramolecular motion of AIEgens. Although the immobilization of AIEgens in SNs can give full play to the advantages of AIEgens, it can also block the optical changes of AIEgens caused by the change of aggregation morphology. Thirdly, developing NIR fluorescence imaging, dual modal/multimodal imaging (through the incorporation of AIEgens with other imaging modalities, such as MRI, CT, ultrasound imaging, etc.), TP imaging, upconversion imaging probes that are based on AIEgens-functionalized SNs is highly desirable to achieve accurate and clear bioimaging through simple molecular design and structural modifications. Fourthly, multifunctional nanoprobes based on AIEgens-functionalized SNs are also need to be developed to realize accurate bioimaging and targeted theranostics simultaneously, which provides a promising strategy in precision medicine. In addition, incorporating AIEgens into silica through covalent attachment can avoid the leakage of fluorophores from silica matrix, which could also improve the precision of bioimaging. Moreover, to promote practical biological application of AIEgens-functionalized SNs, more studies on their toxicity assessment, uptake mechanism, circulation time, and clearance in cells and organisms are also necessary. We hope this review can attract more attention of researchers in these areas and promote the development of these fascinating materials.

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**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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