Recent trends in molecular aggregates: An exploration of biomedicine

Jeongyun Heo1 | Dhiraj P. Murale2 | Hey Young Yoon3 | V. Arun4 | Sangkee Choi4 | Eunha Kim4 | Jun-Seok Lee3 | Sehoon Kim1,5

1 Center for Theragnosis, Korea Institute of Science and Technology (KIST), Seoul 02792, South Korea
2 Molecular Recognition Research Center, Korea Institute of Science and Technology (KIST), Seoul 02792, South Korea
3 Department of Pharmacology, Korea University College of Medicine, Seoul 02841, South Korea
4 Department of Molecular Science and Technology, Ajou University, Suwon 16499, South Korea
5 KU-KIST Graduate School of Converging Science and Technology, Korea University, Seoul 02841, Republic of Korea

Correspondence
Eunha Kim, Department of Molecular Science and Technology, Ajou University, Suwon 16499, South Korea.
Jun-Seok Lee, Department of Pharmacology, Korea University College of Medicine, Seoul 02841, South Korea.
Sehoon Kim, Center for Thereagnosis, Korea Institute of Science and Technology (KIST), Seoul 02792, South Korea.
Email: ehkim01@ajou.ac.kr, junseoklee@korea.ac.kr, sehoonkim@kist.re.kr

Jeongyun Heo, Dhiraj P. Murale and V. Arun equally contributed to this work.

Funding information
Korea Institute of Science and Technology, Grant/Award Numbers: 2E31093, Korea University, Grant/Award Number: K2110571, National Research Foundation of Korea, Grant/Award Numbers: 2017M3A9D8029942, 2018M3A9B4079286, 2019M3D1A1078941, 2019R1A6A1A11051471, 2020R1A2C20304022, 2020R1C1B1010044, 2021R1A2C2005418

Abstract
Molecular aggregates are receiving tremendous attention, demonstrating immense potential for biomedical applications in vitro and in vivo. For instance, the molecular aggregates of conventional fluorophores influence the electronic excitation states of the aggregates, causing characteristic photophysical property changes. A fundamental understanding of this classical relationship between molecular aggregate structures and photophysics has allowed for innovative biological applications. The chemical characteristics of drug molecules generally trigger the formation of colloidal aggregates, and this is considered detrimental to the drug discovery process. Furthermore, nano-sized supramolecular aggregates have been used in biomedical imaging and therapy owing to their optimal properties for in vivo utility, including enhanced cell permeability, passive tumor targeting, and convenient surface engineering. Herein, we provide an overview of the recent trends in molecular aggregates for biomedical applications. The changes in photophysical properties of conventional fluorophores and their biological applications are discussed, followed by the effects of conventional drug molecule-aggregates on drug discovery and therapeutics development. Recent trends in the investigation of biologically important analytes with aggregation-induced emission are discussed for conventional and unconventional fluorophores. Lastly, we discuss nano-sized supramolecular aggregates used in imaging and therapeutic purposes, with a focus on in vivo utilization.

KEYWORDS
aggregation-induced emission, molecular aggregate, nanomedicine

1 | INTRODUCTION

In the past decade, molecular aggregates have been highlighted by the biomedical science community as a molecular tool to develop fluorescent sensors, bioimaging probes, bioactive compounds, and therapeutics.[1] Molecular aggregates of organic π-conjugated systems can exhibit significant changes in photophysical properties,[2] thus demonstrating excellent biosensing and bioimaging potential. In addition, small molecules and supramolecular aggregates exhibit sophisticated biological activity in vitro and therapeutic activity in vivo.

For instance, most conventional fluorophores, which have organic π-conjugated systems, can form molecular aggregates, and the photophysical properties of the aggregates can be largely influenced by aggregation structures and molecular stacking models.[3] Michael Kasha gave one of the earliest descriptions of the phospholuminescent properties of molecular aggregates almost six decades ago.[4] In 1958, McRae and Kasha introduced a model for the linear
molecular aggregate and the theoretical concept of Coulomb coupling between neighboring chromophores, reflecting the interactions between molecular transition dipole moments in aggregates. When the Coulomb coupling is positive (known as H-aggregate), fluorescence is suppressed, and phosphorescence is strengthened. In contrast, when the Coulomb coupling is negative (known as J-aggregate), the oscillator strength is focused on the lowest energy exciton, and the enhancement of phosphorescence or absence of fluorescence suppression is expected. Later, conventional H- and J-aggregates of fluorophores, including cyanine, BODIPY, squarine, rhodamine, and other organic \( \pi \)-conjugated materials, have been attracting increasing interest owing to their biomedical applications, such as biomolecular analyte sensing, biomedical imaging, and therapeutic capability.\(^{[13]}\) In 2001, the distinctive phenomenon of aggregation-induced emission (AIE) was reported by Ben Zhong Tang in the research on 1-methyl-1,2,3,4,5-pentaphenylsilole.\(^{[6]}\) Most conventional fluorophores are highly fluorescent in a single molecular state, but weakly fluorescent or nonemissive in the aggregated state. This phenomenon is generally referred to as aggregation-caused quenching (ACQ), which is a relatively common phenomenon wherein fluorophores undergo strong intermolecular \( \pi-\pi \) stacking interactions in the aggregated state.\(^{[7]}\) In contrast to conventional fluorophores, certain molecules show enhanced emissions in their aggregated states.\(^{[8]}\) and this nonconventional AIE phenomenon is widely applicable to renowned fluorophores, including boron-containing fluorophores, stilbene-type fluorophores, naphthalimide, dansyl, acedan, and malononitrile, among others. Fluorophores exhibiting AIE are generally referred to as AIE luminogens (AIEgens) due to their advantageous features, such as a minimized background, enhanced signal fidelity, and an increased signal-to-noise (S/N) ratio, which is ideal for biosensing and bioimaging. AIEgens have encouraged tremendous advances in the fields of biomedical research and life science.\(^{[9]}\)

On the other hand, the pharmaceutical industry has recognized that many drug-like molecules can self-aggregate in aqueous media. The drug discovery process generally begins with the screening of chemical libraries and these high-throughput screening campaigns are dominated by false-positive “hits.”\(^{[10]}\) The phenomenon of colloidal aggregation of small molecules, which was discovered 15 years ago, is now generally accepted to cause false-positive hits in high-throughput screening.\(^{[11]}\) The resulting colloidal aggregates inhibit the catalytic activity of the enzymes via the local unfolding of the protein due to nonspecific binding.\(^{[12]}\) Therefore, the process of colloidal aggregation is generally regarded as a nuisance during the drug discovery process.\(^{[13]}\) However, recent studies have indicated the therapeutic potential of colloidal small-molecule aggregates.\(^{[14]}\) Not only is the inhibition of specific enzymes possible with colloidal small molecule aggregates, but also the inhibition of receptor proteins and protein–protein interactions.\(^{[15]}\) Colloidal drug aggregates are also broadly applicable to delivery formulations.\(^{[13]}\) Additionally, nano-sized supramolecular aggregates have been considered as an innovative solution for the limitations of conventional molecular probes and therapeutics, especially for in vivo use. Nano-sized supramolecular aggregates have attractive capabilities for in vivo utility, including enhanced cell permeability, passive tumor targeting, convenient surface engineering, and delivery of therapeutic payloads. Several nanoadgregates have been reported to enhance the drug efficacy by changing the pharmacokinetics and biodistribution of the drug.

In this review, we first focus on the conventional photophysical property changes of classical fluorophores. The relationship between the different aggregation structures and aggregation-dependent luminescent properties is introduced. Then, the biological applications of regulating the photophysical properties of the fluorophore aggregates are discussed. In addition, recent trends investigating biologically important analytes with AIE and the unconventional photophysical property changes of fluorophore aggregates are discussed, depending on the types of fluorophores. Lastly, we discuss how aggregates of drug molecules and nano-sized supramolecular aggregates can be used in imaging and therapeutic purposes in vitro and in vivo. Throughout the review, we have mainly focused on molecular aggregates capable of biological analyte sensing, biological activity, biomedical imaging, and therapeutic action. However, where appropriate, we have included examples from other disciplines.

## 2 | PHOTOPHYSICAL PROPERTY CHANGES OF CONVENTIONAL FLUOROPHORE AGGREGATES FOR BIOLOGICAL APPLICATIONS

More than 80 years ago, Sheibe et al. and Jelley independently observed the unusual behavior of pseudoisocyanine chloride (PIC) in aqueous solutions, which appears as a new sharp absorption band not only with a small value for the full width at half maximum, but also with a very high absorption coefficient.\(^{[14]}\) Currently, J-aggregates (J denotes Jelley) or Sheibe aggregates indicate dye aggregates with a bathochromically shifted narrow absorption band with respect to the monomer absorption band. In addition, the J-aggregate usually has a nearly resonant fluorescence (very small Stokes shift) with a narrow band. On the other hand, H-aggregates indicate aggregates with hypsochromically shifted absorption bands (H denotes hypsochromic) with respect to the monomer band. These aggregates, in most cases, exhibit low or no fluorescence.

The spectroscopic properties of the J-aggregates of cyanine dyes are characterized by a sharp absorption band, namely the J-band, which is red-shifted from the monomer band, and resonance fluorescence with a short radiative lifetime. These features have been interpreted in the context of delocalization of a molecular exciton over chromophores that are well arranged in a one- or two-dimensional structure.\(^{[15]}\) In this section, the recent studies about relationship between the different aggregation structures and aggregation-dependent luminescent properties of conventional fluorophores are introduced. Then, the biological applications of regulating the photophysical properties of the fluorophore aggregates are discussed.

### 2.1 | Aggregates of cyanine fluorophores

Cyanine dye is a well-known NIR dye comprised of two nitrogen atoms linked through a polymethine bridge. As
we know that J- and H-aggregates form will behave different from their corresponding monomer. Therefore, research groups have installed various photoresponsive groups in cyanine moiety and extracted their new updated features, such as NIR-II emission, improved solubility, and viable photothermal property, for biomedical applications. In line with this, a few interesting recent works of J- and H-aggregates of the cyanine dyes are discussed below.

In 2018, Sletten and Cao synthesized the first cyanine-based dye (1), which easily undergoes J-aggregation in fluorous media (Figure 1A). Generally, cyanine dyes are known to aggregate well in aqueous media, but their aggregation in nonaqueous media has not yet been explored. Sletten and Cao achieved this behavior by leveraging the orthogonality of perfluorocarbons and hydrocarbons to generate nonpolar cyanine amphiphiles. The developed protocol may be compatible with different chromophore classes with amphiphilicity-induced J-aggregation. The aggregation of fluorous–lipophilic 5,5,6,6-tetrachlorobenzimidacarbocyanine dye 1 can be tuned by varying the solvent and temperature (Figure 1A). The fluorous phase J-aggregates of the dye 1 exhibit elevated photostability and are smoothly transferred to the surfaces. The fluorous phase may facilitate the printing and patterning of aggregates. Overall, the fluoruous cyanine J-aggregate dye 1 allows for improved photostability and processability.

Naritaka et al. reported a well-dispersed and stable J-aggregate liposome. It is well known that merocyanine dye (MD) forms J-aggregates because of its amphiphilic nature. The interesting characteristics of J-aggregate formation in MD are as follows: (1) the J-band wavelength sensitivity depends on the counterion species for the carboxymethyl group, and (2) the MD J-aggregates can transform through ion-exchange and change their J-band wavelength. In this study, the authors found that sample preparation at an MD to DMPC ratio between 0.10 and 0.20 ratio result in a stable J-aggregate dispersion of MD molecules (DMPC as a disper-
two different types of J-aggregates having different J-bands around 635 and 600 nm.

Yamaguchi et al. reported a mixed J-aggregate of oxacyanine dye and thiacyanine dye. Using the Langmuir–Blodgett technique, the authors fabricated monolayer assemblies of N,N’-dioctadecylthiacyanine perchlorate (S11). The mole fraction X of S11, X = [S11]/([S9] + [S11]), was varied from 0 to 1. Through the steady-state measurement of absorption spectra, fluorescence spectra, and picosecond fluorescence decay curves of monolayer assemblies, the authors found distinct changes in S9(J) and S11(J) assemblies compared to the corresponding photophysical properties of S9 and S11 J-aggregates. Considering this observation, the authors claimed a homogeneous aggregate of the persistence type (HP-aggregate), which is distinguished from the M-aggregate (mosaic-type mixed J-aggregate).

Ma investigated the deactivation dynamics of aqueous 1,1’-diethyl-2,2’-carbocyanine (PIC) J-aggregate under magic angle excitation conditions. The excitation fluence I was tuned from 5 × 10^{11} to 8.2 × 10^{13} photons/cm² per pulse. When I was 4.1 × 10^{13} photons/cm², excited-state absorption was observed at 570 nm, and a weak negative band at 615 nm was observed. When I was 8.2 × 10^{12} photons/cm², very weak positive broad band centered at 665 nm becomes visible. Notably, the exciton−exciton annihilation (EEA) phenomenon in aqueous PIC J-aggregates was extensively studied by comparing a series of excitation fluence-dependent transient absorption spectra. The resulting dual regimes were different, diffusion-limited, and coherent EEA. The coherent EEA influenced a much higher phonon−exciton coupling and opened a trapped pathway. With the help of adaptive coherent control, both types of EEA were successfully suppressed. Exciton concentration was a determining factor for the simultaneous rate of the two EEA’s. The reduced exciton concentration with optimal pulse excitation was justified by the vibronic mechanism that drives the S₁ → S₀ transition. Moreover, the results showed that in the coherent EEA regime, the formation of EST was hampered by reducing the potential strength of the exciton–phonon coupling. The outcome of this work provides a new and comprehensive understanding of the exciton deactivation dynamics in aqueous PIC J-aggregates, and demonstrates how shaped pulses affect EEA dynamics.

Cyanine-derived dyes have received immense attention in the development of novel cancer therapies with clinical applications; however, the dyes have poor stability, low photothermal efficiency, and inadequate accumulation at tumor sites in molecular forms. The existing limitations can be resolved by converting small-molecule dyes into supramolecular assemblies; this is achieved by tuning the molecular organization of monomeric structures to obtain higher-order structures. Very recently, Li et al., using this self-assembly (H-aggregates) approach, hypothesized a new therapeutic application. They found that among the various kinds of self-assembly, H-aggregates of dyes display strong face-to-face overlap, which leads to fluorescence quenching in most cases. However, they may emit energy in non-radiative forms, such as heat for photothermal cancer therapy. Therefore, the collective effect resulting of the self-assembly of cyanine dyes into H-aggregates is used to create a novel supramolecular strategy for the fabrication of small-molecule-based photothermal nanomaterials. Considering the free cyanine dyes, the H-aggregates formed by conjugating cyanine with pyrene or tetraphenylethene (TPE) (3) displayed an absorption spectrum with blue shift and fluorescence self-quenching, but distinctive photothermal properties. The H-aggregates obtained are saucer-shaped nanoparticles that show passive tumor-targeting properties that facilitate imaging-guided photothermal tumor surgery under irradiation (Figure 1C). The developed supramolecular approach paves the way for new avenues for building next-generation small molecule-based self-assembly nanomaterials for PTT cancer therapy in clinics.

4-Hydroxystyrylindolium derivatives were employed for the selective sensing of HSO₄⁻ anions by Zheng et al. The identified sensing phenomenon is based on the anion-induced rotation-displaced H-aggregates of styrylindolium dyes. The structural features of the styrylindolium dyes have the following merits: (1) the 4-hydroxy group of the dye provides an anion-binding site via hydrogen bonding, and (2) the charged nanoparticles have been identified to enhance the bioimaging around 1500 nm; however, achieving organic molecules with absorption and emission in this region is highly challenging. In 2019, Zhang et al. revealed a J-aggregate molecule with an absorption and emission of 1360 and 1370 nm, respectively, which was achieved by the self-assembly of the amphiphilic cyanine dye (2) and 1,2-dimyrystoyl-sn-glycero-3-phosphocholine (Figure 1B). The self-assembly process has been explained by molecular dynamics simulations. Superior spatial resolution and high signal-to-background ratio of J-aggregates were validated for noninvasive brain and hindlimb vasculature bioimaging beyond 1500 nm. The efficacy of the clinically used hypotensor was successfully estimated by high-resolution in vivo dynamic vascular imaging using J-aggregates. Molecular dynamics simulations were used to exemplify the self-assembly process of FD-1080 in the presence of DMPC. FD-1080 J-aggregates showed bioimaging with a higher signal-to-background ratio and spatial resolution in the NIR-II optical window to monitor dynamic vascular changes during the hypotensive process in rats (Figure 1B). These research insights afforded a novel route for the synthesis of NIR-II J-aggregates. This could be broadened to other NIR molecular dyes to form J-aggregates and accomplish remarkable bioimaging at longer wavelengths.

Cyanine-derived dyes have received immense attention in the development of novel cancer therapies with clinical applications; however, the dyes have poor stability, low photothermal efficiency, and inadequate accumulation at tumor sites in molecular forms. The existing limitations can be resolved by converting small-molecule dyes into supramolecular assemblies; this is achieved by tuning the molecular organization of monomeric structures to obtain higher-order structures. Very recently, Li et al., using this self-assembly (H-aggregates) approach, hypothesized a new therapeutic application. They found that among the various kinds of self-assembly, H-aggregates of dyes display strong face-to-face overlap, which leads to fluorescence quenching in most cases. However, they may emit energy in non-radiative forms, such as heat for photothermal cancer therapy. Therefore, the collective effect resulting of the self-assembly of cyanine dyes into H-aggregates is used to create a novel supramolecular strategy for the fabrication of small-molecule-based photothermal nanomaterials. Considering the free cyanine dyes, the H-aggregates formed by conjugating cyanine with pyrene or tetraphenylethene (TPE) (3) displayed an absorption spectrum with blue shift and fluorescence self-quenching, but distinctive photothermal properties. The H-aggregates obtained are saucer-shaped nanoparticles that show passive tumor-targeting properties that facilitate imaging-guided photothermal tumor surgery under irradiation (Figure 1C). The developed supramolecular approach paves the way for new avenues for building next-generation small molecule-based self-assembly nanomaterials for PTT cancer therapy in clinics.
indolinium scaffold affords a positive charge for electrostatic interaction with the anionic guest. Notably, no significant color and absorption changes were detected for various anions (PO₄³⁻, HPO₄²⁻, H₂PO₄⁻, NO₃⁻, SO₄²⁻, Cl⁻, F⁻, CO₃²⁻, and Ac⁻). Moreover, by increasing the temperature, the aggregates were transformed to the monomeric species, which indicates the reversibility of the anion-induced aggregation process. Based on the experimental results, they proposed that initially the anion HSO₄⁻ forms a hydrogen bond (HB) with the styryl moiety, followed by head-to-tail aggregation via intermolecular π–π interactions to form a favorable H-aggregate. Overall, it is a simple and elegant method for the selective recognition of HSO₄⁻, an exciting tetrahedral anion that has immense biological and industrial applications.

### 2.2 Aggregates of BODIPY fluorophore

BODIPY (4,4-difluoro-4-bora-3a,4a-diazas-indacene) is one of the potential candidates for plethora of bioimaging and sensing applications. Despite that, in the recent years, with the help of J- and H-aggregates, phenomenon scientists have boosted the photophysical properties of BODIPY to the next level. In below part, we discussed about how the modification at meso-position of the BODIPY accomplishes diverse appealing properties and applications.

Very recently, Liu et al. discovered a new BODIPY dye based on J-aggregation-induced emission in the NIR-II window region. Most of the existing NIR dyes are highly dependent on the polyethylene framework, but J-aggregate formation is a complementary approach to attain the NIR-II fluorescence property. In the J-aggregate phenomenon, the transition dipole moments of individual molecules are in a slip-stacked alignment; therefore, they exhibit promising photophysical properties, such as fluorescence spectra, red-shifted absorption, and enhanced quantum yields, compared to their monomers. Inspired by the salient features of J-aggregates, the authors envisioned the creation of the NIR-II window via J-aggregation. In order to induce J-aggregation in the BODIPY molecule, the [2,2]paracyclophane (PCP) group was introduced to the meso position of BODIPY to generate 4 (Figure 2A). Remarkably, the PCP group plays a major role in the inhibition of π–π interactions between the indacene plane and promotes J-aggregation tuning, which leads to a favorable red-shifted NIR-II emission. The newly synthesized BODIPY molecules display both NIR-I (J₁-band, 900 nm) and NIR-II (J₂-band, 1010 nm) emission in the aggregated state, and only NIR-II emission in the crystalline powder state. Through the coprecipitation of PCP-BDP2 with Pluronic F-127, the J-aggregates were stabilized in the assembled NPs and showed bright NIR-II emission with a high fluorescence quantum yield (Φf) of 6.4%. Additionally, they demonstrated their potential clinical application in lymph node imaging and fluorescence-guided surgery in nude mice (Figure 2A). The outcomes of this research effort provide new avenues for controlling the optical properties of luminescent dyes for creative applications in biological imaging and sensing.

Eosinophils are granulocytic leukocytes that play a significant role in host protection against infections that are involved in the pathogenesis of asthma and allergic diseases. Eosinophil peroxidase (EPO) selectively oxidizes Br⁻ to hypobromorous acid in a pool of chloride ions. The strong oxidizing and halogenating ability of HOBr is responsible for the inflammation and tissue damage caused by eosinophilic malfunctions. In 2018, Kim et al. elegantly utilized the concept of electrophilic halogenation of an electron-rich species to discriminate between HOBr and HOCI. In this study, the BODIPY probe (5) (Figure 2B) reacts immediately with EPO-generated HOBr to furnish a dibrominated adduct with significant kinetic selectivity (≥1200:1) compared with HOCI. The main highlight of this work is that the dibrominated adduct does not suffer from poor photophysical properties, as dibromination is prevalent among brominated derivatives due to the heavy-atom effects. Unprecedentedly, it self-assembles into highly emissive red-shifted J-aggregates, furnishing an excellent turn-on fluorescence signal. The selective detection, assaying, and imaging of HOBr generation by EPO, without interference from MPO-generated HOCI, was established for the first time using this probe 5 (Figure 2B). Moreover, the identified fluorogen has been effectively used in various applications, such as EPO activity assays, dip-stick sensors, fluorescence imaging of EPO activity, assays of oxidative stress in cancer cells, and immune response detection in live mice.

Recently, Kim et al. successfully developed a new meso ester BODIPY dye (6) and studied their photophysical and self-assembling properties (Figure 2C). The team elegantly used these emissive J-aggregate fluorescent “light-up” probes for the selective detection of heparin. The molecular recognition event between heparin and BODIPY generated emissive J-aggregates, which were well validated by their narrow linewidths, improved emission rates, red-shifted bands, and efficiency (Figure 2C). The synergistic fluorometric and colorimetric “turn-on” response is relatively rapid and extremely sensitive. Moreover, it is utilized to monitor the heparin levels in human serum samples and estimate the presence of the contaminant, oversulfated chondroitin sulfate, in heparin. J-aggregation has been studied for many years, but the troublesome influence of small structural modifications over molecular stacking in the aggregate state has blocked its application as an AIE-based turn-on mechanism in designing fluorogenic probes. Therefore, the developed strategy proved the potential of J-aggregate-forming meso-esters of BOD-IPY dyes as a robust, operationally simple platform for the selective detection of heparin over other glycosaminoglycan analogs. Finally, the distinctive and promising spectroscopic properties are quite different from those of the more extensively studied TPE, silole, and pyrene excimer AIE sensing tools.

Kobayashi and coworkers designed and synthesized a new BODIPY dye (7) (Figure 2D), which is influenced by biological conditions and an appropriate interaction between Cu²⁺ and the –OH and –NH₂ substituents of the neighboring BOD-IPY molecules resulted in J-aggregation. J-aggregation, being an important mechanism of molecular self-assembly, plays an essential role in many biological processes. Therefore, cupric ions are essential trace elements that exhibit crucial roles in living systems, functioning as a catalytic cofactor in various physiological processes, namely iron uptake, mitochondrial respiration, and vesicular oxygen-processing enzymes. The high response of the J-aggregate formation of [Cu²⁺(1)₂], with a color change that is perfectly visible to the naked eye, could lead to BODIPY being used as a Cu²⁺ indicator and in colorimetric analysis. The precise elevation
FIGURE 2 Chemical structure, photophysical property changes, and biological applications of BODIPY J-aggregates. (A) Bioluminescence and NIR-II fluorescence signals of the resected nodules of unguided and PCP-BDP2 NPs-guided groups were showed. (Reprinted with permission.[24] Copyright 2021, Springer Nature). (B) Spectra of the self-assembled 5 (2.5 μM) before (black) and immediately (≤2 s) after (red) addition of HOBr (10 μM). (Reprinted with permission.[25] Copyright 2018, American Chemical Society). (C) Fluorescence spectra of the BODIPY-dye 6 in the presence and absence of Heparin were displayed. (Reprinted with permission.[26] Copyright 2020, Wiley-VCH). (D) Fluorescence spectra of 7 resulted by the addition of various metal ions to the compound were depicted. (Reprinted with permission.[27] Copyright 2010, Royal Society of Chemistry). (E) Absorption (dotted line) and emission (solid line) spectra of the compound 8. (Reprinted with permission.[28] Copyright 2014, Royal Society of Chemistry)

of fluorescence in the presence of Hg^{2+} is also promising (Figure 2D). The selective detection of Cu^{2+} in the presence of Hg^{2+} under physiological conditions could be used as a simple protocol to differentiate between these cations, which normally hinders the analysis.

One of the major challenges associated with the BODIPY dyes is their poor emission in the solid state due to aggregation-induced quenching. To overcome this issue, the packing nature of the aggregated solid state was manipulated to accomplish favorable emissive J-aggregates (head-to-tail). In this case, the transition dipoles of the monomeric dyes were aligned in a coplanar inclined fashion with a slip angle ω < 54.7°. Kim et al. prepared a new meso-trifluoromethyl-substituted dye CF₃-BODIPY (8) (Figure 2E), which showed distinctive red-shifted absorption and emission maxima (λ_{max, abs} = 553 nm, λ_{max, em} = 622 nm) in CHCl₃ solution along with broad absorption and fluorescence bands, and a large Stokes shift. Importantly, the molecular packing responsible for the formation of these aggregates was unequivocally confirmed by X-ray diffraction (XRD).[28] Moreover, it is one of the absolute examples to date of a solid-state structure in which the transition dipoles of neighboring dyes adopt a coplanar inclined arrangement, with a slip angle inferior to 54.7°, which is a characteristic of a J-type packing and shows agreement with the excitonic coupling theory.

Kim et al. reported an emissive meso-ester BODIPY dye that induced J-aggregation, using heparin, through charge-pairing interactions. The fast and highly sensitive formation of J-aggregates enhanced emission rates and efficiency as well as red-shifted bands and narrow linewidths. The BODIPY derivatives in solutions show a greenish emission resulting from a loss of planarity in the excited state, but in condensed states, they show intense fluorescence by forming J-aggregates. In this study, meso-ester BODIPYs are usually tethered through an ester side chain and self-assemble into nonemissive spherical aggregated nanostructures in aqueous solutions. The interaction with heparin, however, disrupted these spherical aggregates to J-aggregated supramolecular assemblies, and it associated a high turn-on response with specific interactions.[26]
Cheng et al. have reported an aza-BODIPY-lipid building block that can self-assemble into a liposomal nanoparticle called BODIPYsome.[29] BODIPYsomes are highly biocompatible nanocarriers that assemble into aggregates and are both optically and colloidal stable. The enhanced colloidal stability and J-dimers provide a stable platform with highly efficient NIR fluorescence properties. This BODIPYsome was integrated into the nanostructure of the J-dimer and showed a red shift and enhanced absorption for optical imaging. It improves the stability of the colloid in a cholesterol-dependent manner to maintain its J-aggregation and turn-on the fluorescent properties in prostate cancer imaging. Cholesterol plays a strategic role in liposomal formulation and confers decreased membrane permeability, enhanced in vivo stability, and improved structural rigidity. Therefore, the existence of cholesterol and BODIPYsome allows the J-dimer aggregate to maintain its colloidal structure with high stability and show high NIR fluorescence properties.

Very recently, Zhang et al. discovered a new diBODIPY-based AIE fluorescent probe (9) that displayed an impressively high molar extinction coefficient and strong absorption and emission in the J-aggregated state (970/1010 nm) in the NIR-II region (Figure 3A).[30] Using high brightness, imaging with a high frame rate (34 frames per second) at a deep “valid penetration depth” of up to 6 mm can be attained. Owing to its rigid planar structure, the dye exhibited much greater photostability and chemical stability than the well-known FDA-approved NIR bioimaging ICG/protein combinations. Due to its salient features, such as brightness and the long emission wavelength in the NIR-II region, the deeply located viscera could be easily shown, providing a contact-free and effective optical method to reveal the pulmonary and systemic circulation in living mice (Figure 3A). The developed dye could easily discriminate between acute-lung-injured mice and healthy mice by dynamically monitoring the respiratory rate. Moreover, the collateral circulation process can be monitored perfectly with a high frame rate.

### 2.3 Aggregates of squaraine dye

Squaraine dyes are unique class of organic dye constitute of zwitterionic planar structures and exhibit strong emission and absorption in the NIR region. In this section, we provide an example for tuning the J-aggregation of squaraine dye by host–guest interactions.

Liu et al. investigated various squaraine dyes that can be tailored for the far-red to NIR region. These dyes have found application in nonlinear optics, fluorescence bioimaging, dye-sensitized solar cells, and photosensitizers for photodynamic therapy (PDT). In general, squaraine dyes restrict their optical applications because of the strong quenching of the fluorescence emission by H-J-aggregation. The environmental effects on the optical properties of squaraine dyes,
such as solvent polarity and H-bonding interactions, should be carefully considered. Therefore, strong host–guest interactions between squaraine dye and beta-cyclodextrin suppress J-aggregation and restore fluorescence emission. The compound forms HBs with polar protic solvents (such as methanol). This prevents flexibility in the molecular structure and increases the torsional strain to distort the coplanarity of the structure (donor–acceptor–donor), which induces the nonradiative decay of the excited state. The introduction of beta-cyclodextrin with squaraine dye interrupted the aggregation propensity in aqueous media, which enhanced the fluorescence intensity.

### 2.4 Aggregates of Rhodamine fluorophores

Rhodamines are a significant class of fluorophore extensively used in fluorescence microscopy. In order to enhance its emission toward red-shift, diverse structural manipulations have been explored, which are summarized below.

Shirakawa et al. reported that the molecular aggregation of Rhodamine 6G and Pseudoisocyanine is affected by the light force. The aggregates can be formed by the molecule substituents that affect the intermolecular HB. When the concentration of a molecule increases or the solution conditions change, molecules form aggregates through intermolecular van der Waals interactions, HBs, or electrostatic interactions. The aggregates produced are J- or H-aggregates depending on whether the energy shift is negative or positive, respectively. The energy shift can appear as an absorbance change in the relevant spectral changes. In this study, Shirakawa et al. controlled the aggregation of laser dyes to change the absorbance spectra. Thus, Rhodamine 6G, which has a hypsochromic shift, shows H-aggregation, and PIC, which has a bathochromic shift, shows J-aggregation, when they aggregate in aqueous solution.

Fluorophore aggregation is an important phenomenon in the design of bio-related fluorescent probes. According to the exciton theory, in general, two interacting molecules can form either a nonfluorescent dimer (H-aggregates) or a fluorescent dimer (J-aggregates). Inspired by the important aspects of J-aggregates, Majima et al. developed a protocol to expedite the J-aggregate formation by incorporating a 9-phenylanthracenyl moiety into the rhodamine derivative (10) (Figure 3B). Results derived from the X-ray crystallography are well corroborated with the properties of J-aggregates, that is (1) the angle of coplanar inclined transition dipoles (Θ) is in the range of 0° < Θ < 54.7°, and (2) there is a sharp red-shifted absorption band with 2–3 nm Stokes shift. In addition, a significant red-shift in the absorption band (nearly 100 nm) was acquired by the cooperative slipped stacking of rhodamine and anthracene molecules (Figure 3B). The authors suggested that the 9-phenylanthracenyl group of 10 is a versatile intramolecular motif that can align with the targeted fluorophores depending on the presence of the organic solvent and counterions. The fluorescence properties can be easily tuned by alternating the fluorophore and anthracene functionalities. Additionally, 1O₂ is a reactive oxygen species in PDT and can react with anthracene derivatives to form endoperoxide, which exhibits good cell permeability. These promising features paved a new pathway for the detection of biologically important 1O₂ at the cellular level.

#### 2.5 Aggregates of miscellaneous fluorophores

So far, we have seen the J- and H-aggregates formation in familiar fluorescent molecules (cyanine, BODIPY, squaraine, and rhodamine) and their significance. In this section, few miscellaneous fluorophores, namely, diazapentaceneum dye, porphyrin, and benzoxazole, and respective J- and H-aggregates are discussed.

Conventionally, J-aggregates or Sheibe aggregates are molecular aggregates with a narrow absorption band bathochromically shifted and increased fluorescence intensity relative to the monomer. HAs are molecular aggregates with absorption bands shifted to shorter wavelengths with respect to the monomer band and coupled occurrence of low intensity or absorbance of fluorescence. Rodrigues et al. have reported an acene-based cationic diazapentaceneum dye to form J-aggregates in quaternary 5,12-diazapentaceneum salts to obtain the AIE property. Depending on the solubility of the solvent (or thickness, in the case of films), the photophysical properties, such as absorption, fluorescence excitation, and emission, are different. In the solvent with poor solubility (such as acetonitrile and dichloromethane), the long-wavelength absorption bands (620–680 nm) are vibrationally resolved and red-shifted compared to the spectra (550–590 nm) at the same concentration in the solvent with good solubility (such as methanol and tetrahydrofuran [THF]). The formation of J-aggregates in the solvent with poor solubility causes the aggregates to emit fluorescence, whereas in methanol and thin films, isolated molecules coexist with aggregates. In acetonitrile and dichloromethane, which dominate the J-aggregates, the fluorescence decay changes from single exponential to triexponential decay, reflecting the coexistence of isolated molecules, J-aggregates, and other disordered aggregate species, especially at higher concentrations.

Morisue et al. reported porphyrin aggregates in different conformations depending on their interactions. Electron-rich compound porphyrin rings are expected to be sandwiched in a dimer as a stacked cofacial configuration. Morisue et al. added two pentafluorophenylethynyl groups to the porphyrin at the diagonal meso positions and two 3,4,5-tri-(S)-3,7-dimethoxytoluoyl)phenyl groups at other meso positions. These additional substituents induced J-aggregation of the compound in the needle-shaped aggregates. Aggregation patterns are defined by the solvent and evaporation time that control the aggregation behavior. These compounds show nucleation growth in a mechanism similar to that of crystal growth. The geometric self-complementarity of the slipped cofacial dimer of the compound would fulfill the requirement for positive binding cooperativity for the quadrupolar interaction, and guarantee enhanced binding strength for dimer formation. The densely packed J-aggregates were composed of a stacked system that developed deep NIR-luminescent porphyrin aggregates.

Enzyme-activatable optical probes are valuable tools for the specific identification of various diseases, especially in the early diagnosis of cancer. However, they mostly suffer from low signal-to-background ratios when detecting pathological tissues. To overcome this issue, a targeted probe with efficient fluorescent modulation is required. In accordance with this, Hennig et al. developed a new bioprobe containing a
self-immolative linker and chiral J-aggregate-forming dyes.\cite{f135} Detailed characterization of the newly identified probes using fluorescence, absorption, CD, and time-resolved fluorescence spectroscopy revealed that the dye–dye and dye–protein interactions were not detected for the free dyes in the solution and enzyme, respectively. From the experimental results, it is clear that the lifetime of the probe is longer than that of the corresponding free dyes, which is probably due to the closer proximity of the self-immolative probe system and the hydrophobic microenvironment generated by the second dye. Importantly, these findings suggest that J-aggregate formation is challenging to attain with the current probe design, and interactions of the dyes with the enzyme probably influence the high signal-to-background ratios.

Encouraged by the interesting fluorescent properties of benzoxazole, Zhou et al. prepared a new functionalized 2-arylbenzoxazole derivative (11) (Figure 3C) with enhanced emission.\cite{f36} Ironically, they observed enhanced fluorescence emission in H-aggregate formation instead of a regular quenching effect. The specific slip stacking and the dimer formed by multiple intermolecular interactions circumvent the usual strong H-type coupling and are responsible for unprecedented highly emissive H-type aggregate events. Using X-ray crystallography, they proposed that the synergetic effects of intra and intermolecular interactions and intramolecular motions are restricted, and the molecular conformations are more rigid and planar. Therefore, the physical restriction of intramolecular motions (RIMs) and aggregation-induced planarization induced by molecular aggregation could be the reason for the enhanced emission phenomena. This distinctive enhanced emission with color changes could be highly beneficial for the development of new fluorescent probes and OLEDs.

3 | NONCONVENTIONAL AIE FLUOROPHORES FOR BIOANALYTE DETECTION

When a fluorophore aggregates, it shows unchanged emission, reduced emission (ACQ), or enhanced emission (AIE). The ACQ is a relatively common phenomenon, wherein fluorophores show strong emission as free molecules; however, when aggregated, the aromatic rings of the fluorophore undergo strong intermolecular π−π stacking interactions. When excited, these aggregates tend to decay or return to the ground state via nonradiative channels, resulting in emission quenching of the fluorophores. Perylene, when dissolved in THF, is a classic example of ACQ, which shows a strong emission signal and tends to quench upon increasing water content up to 80%. When the fraction of water further increased to 90%, there was strong aggregate formation and complete quenching of the emission.\cite{f11} The aromatic structures in perylene form π−π stacking interactions to promote the ACQ phenomenon.

Since its emergence in 2001, AIE has attracted immense attention for the study of bioactive species and key biological processes.\cite{f37} AIEgens are weakly emissive or nonemissive as free molecules, but when aggregated, they emit strong fluorescence. Hexaphenylisilole (HPS)\cite{f38} and TPE\cite{f39} are classical AIEgens that consist of π-conjugated core moieties and molecular rotors. Unlike perylene, when the water fraction is increased, the AIEgen aggregates produce strong emissions. The free HPS and TPE molecules in dilute solutions are almost nonemissive owing to free vibrational rotation. The AIE of HPS and TPE is caused by the synergetic effects of the restricted intramolecular rotation (RIR), which hampers intermolecular π−π stacking interactions. Thus, the RIM, comprising RIR and the restriction of intramolecular vibrations (RIV), is considered the basic mechanism behind AIE.\cite{f19,f40} AIEgens are proven fluorophores in biomedicine, owing to their inherent properties, such as high brightness, large Stokes shift, excellent photostability, and good biocompatibility.

3.1 | Boron-containing fluorophores

Among organic fluorophores, organoboron complexes, such as BODIPY, boron diketones, BOAHY, BODIHY, and BOIMPY, have been widely explored for their diverse photophysical properties, convenient synthetic methods, and high photostability. Recently, some studies have explored organoboron complexes as AIEgens. However, some organoboron complexes suffer from ACQ, which limits their bioapplication. However, some new AIE organoboron complexes have shown great utility. In this section, we highlight some of the recent organoboron complex-based AIEgens. In general, these boron-containing AIEgens have found to acquire excellent cell permeability and relatively low cytotoxicity; hence, these AIEgens have been used extensively for bioimaging and viscosity change monitoring probes (viscosimeters). The detailed bioimaging study revealed localization of these probes in lipid droplets (LDs). Some of the probes with excited-state double-bond reorganization (ESDBR) properties have been used to treat multidrug-resistant bacteria in mouse models as well. Erker et al. introduced dihydro-1,3-azaboroles, produced via a convenient three-component reaction, as a novel AIE organoboron class.\cite{f41} Here, the (Mes)BMe2-SMe2 reagent reacts with acetylene and xylylisocyanitride sequentially to give a series of unprecedented dihydro-1,3-azaborole derivatives. When the photophysical properties were checked, they found high quantum yields and AIE properties. The tetra-substitution of the dihydro-1,3-azaborole core (12) with various bulky substituents is the key for AIE via RIR. The addition of water might increase the chances of segregation owing to the improved hydrophobic interactions between the compounds, which have a good solubility in THF. Sterically hindered phenyl substituents at the 1- and 2-positions provided strong emission enhancement in water. However, less bulky substituents result in emission quenching or red-shifted emission enhancement via ACQ or excimer formation. The fluorescence quantum yield (Φ_F) of these AIEgens in water was found to be 65–70%.

Belkaya et al. reported a bright green-yellow fluorescent boron complex via the chelation of aryl(hetaryl) amino acryloyl thiopeptide scaffolds with a BF2 fragment (13).\cite{f42} The photophysical properties of the dyes depend on the nature and position of the electron-donating and electron-withdrawing substituents. When they compared solid-state emission with solution-state emission of these dyes, a remarkable bathochromic shift (43–90 nm) in the emission at 610 nm was found. The bright emission in the
solid state was attributed to AIE. The J-aggregates in the crystal stacking patterns could be the reason for the AIE properties of these dyes. These dyes are good candidates for bioimaging probes because of their good cell permeability and relatively low cytotoxicity. Imaging was performed in HeLa cells using CLSM, and flow cytometry was used in HeLa, human fibroblast, and rhabdomyosarcoma cells.

Lee et al. developed BOIMPY as a fluorescent boron complex from N-aryl iminopyrrolide ligands. The photophysical properties in the solution state are dependent on the steric and electronic factors of the substituents. Figure 4 shows the boron-containing fluorophores as AIEgens. Compound 14a in MeCN showed strong emission ($\lambda_{\text{max,em}} = 536$ nm, $\Phi = 38\%$), whereas compound 14b showed a significant red shift ($\Delta \lambda = 114$ nm) ($\lambda_{\text{max,em}} = 536$ nm, $\Phi = 2\%$). Similarly, compound 14c had an emission band ($\lambda_{\text{max,em}} = 468$ nm, $\Phi = 15\%$), whereas 14d was essentially nonfluorescent. A similar trend was also observed in the solid-state emission of these dyes. The notable difference was that the complex 14d showed increased quantum yield in the solid state compared with the solution state. The reason could be a decrease in the local dielectric, restricted molecular motions, and orthogonal arrangement of the phenyl rings. Complex 3e was designed with a water-soluble group for use as a bioimaging probe. When performing live imaging with this probe, the authors observed the localization of the probe into LDs.

Peshkov et al. described boron complexes of glyoxal-derivatives (15) as a novel class of AIEgens. These complexes were synthesized via a four-component Ugi reaction, followed by complexation with boron trifluoride diethyl etherate of the resulting 1,3-dicarbonyl compounds. The photophysical properties of these complexes reveal noticeable AIE features. These complexes showed weak emission in THF solutions with low quantum yields ($\sim 0.05$). On the other hand, the photoluminescence (PL) efficiency was improved in the solid state, with high quantum yields (0.80–1.00). The AIE properties are believed to be dependent on the hydrogen-bonding network in the crystal packing. When the hydrogen-bonding network is polymeric, the quantum yields are low in the solid state. However, if the hydrogen-binding donor substituents are absent (only $\pi-\pi$ stacking interactions) or if the network is dimeric, the quantum yields are enhanced. In addition, these dimeric networks restrict intramolecular motions and do not hamper the dissipation of the excitation energy, which could lead to AIE. When the AIE properties were checked in THF/water, there was an increase in emission intensity with white turbidity in THF/water (9:1), which served as a direct confirmation of AIE. The Ugi reaction is known to introduce bulky molecular architectures, which is key to the AIE properties of these complexes.

Xu et al. reported fluorescent bisboron complexes with emission in solution and solid state, and their application for LD imaging in living cells. Due to steric hindrance by the phenyl rings, the 17a-c probe showed a strong emission signal in solution, although these complexes have a propeller-shaped structure. When the AIE properties in the binary solvent THF-water for probes (17a-c) and their starting precursors (16a, b) were checked, 17a-c showed ACQ, and 16a and b showed AIE behavior. Probes 16a and 16b showed red-shifted emission, which is attributed to the different excitation coupling in the aggregation state. This behavior of the probes could arise from their rigid $\pi$-conjugation structure results in the formation of large aggregates, which hindered the acquisition of the emission spectra and resulted in a continuous ACQ effect with $f_{\text{p}}$ increased. The number of phenyl rings restricted free intramolecular rotations; ACQ is preferred for 17a-c. In the case of the hexane-water binary solvent, 17a-c showed better AIE properties due to the formation of relatively small aggregates that stabilize the emission in the aggregation state than in the THF-water solvent. All the probes are emissive in the solid state owing to restricted free rotations of the propeller-shaped structure. In addition, the solid-state quantum yields of 17a-c are higher than those of 16a, b, owing more twisted structure of 17a-c that prevents the $\pi-\pi$ stacking interactions in the aggregation state.

Tang et al. explored planar boron complexes to study AIE (18a-c). They mentioned that the ESDBR (restriction of intramolecular double-bond rotation in the excited state) mechanism is behind the AIE properties, even though these compounds are planar, unlike the twisted structures of traditional AIEgens. The UV-vis spectrum in THF showed 18c was red-shifted in absorbance and emission compared...
with 18a and 18b, possibly due to the enhancement of the intramolecular charge transfer (ICT) effect. 18c further showed evident solvatochromism effect, the emission was found to be red-shifted emission from nonpolar (465 nm, Hexane) to polar solvent (606 nm, DMF). The AIE properties were explored in THF/water mixtures with varying water fractions; in THF, 18b showed weak blue-green fluorescence in THF at 473 nm, and the emission intensity increased gradually as the water fraction increased from 0 to 90 vol%. As water fraction was increased beyond 90 vol%, the relative intensity dropped due to the formation of large particles proving the AIE effect. In the case of 18c, emissions become weaker upon adding water into the THF solution due to a stronger ICT effect, but increased marginally at high water fractions, proving the AIE effect. The AIE effect was further proved by time-resolved fluorescence spectroscopy, single XRD, and theoretical calculations. The ESDBR mechanism was also studied in detail using a simple mechanism-like model. These AIEgens have also been proven to act as biological probes for imaging LDs and demonstrated better photostability and target specificity toward LDs compared to commercial Nile red. This ESDBR-based strategy nullified the structural limit for AIEgen design and facilitated the creation of new coplanar AIEgens for bioapplications.

Li et al. have also reported organoboron planar AIEgens (19a and 19b) based on the ESDBR strategy for AIE luminogens [47]. In this study, they developed fluorosubstituent AIEgens for stronger intermolecular H-bonding interactions to restrict molecular motions. In addition, these probes have increased crystal density, which leads to a decrease in the nonradiative decay rate by one order of magnitude. Typically, planar luminophores suffer serious ACQ effects because of backbone rigidity, which favors the overlapping of molecular orbitals between the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO). This results in an increased radiative process. Owing to a change in the position of the substituent, the photophysical properties must differ dramatically. Owing to the weaker ICT and stronger ESDBR for 19a, the emission was blue-shifted but weaker than that of 19b in the THF solution. The AIE performance of these planar fluorophores has increased dramatically due to fluorine substitution, which strengthens the intermolecular H-bonding to restrict the intramolecular motions, including ESDBR, in the aggregation and solid states. This strategy could be an alternative way of designing new AIEgens, devoid of the bulky substituents that were used in the traditional AIEgen designs.

Wong et al. have reported AIE-active difluoroboronated acylhydrazono dyes (20a-1) (BOAHY) as GFP chromophore analogs. [48] The UV-vis absorption and emission spectra of some common organic solvents were recorded. Most of them display a strong absorption band in the 300–600 nm region. Most of these dyes are poorly emissive except 20f and 20h-1, which possess relatively higher fluorescence quantum yields of 0.11–0.30. In addition, the electron-donating and electron-withdrawing groups on the aldehyde and benzoylhydrazide segments have been studied. The solid samples of these probes showed strong fluorescence emission that could be visualized under a 365 nm hand-held UV lamp irradiation, with absolute fluorescence quantum yields up to 0.60. These probes cover the ultraviolet and entire visible region fluorescence emission spectra. The great solid-state emission properties arise from multiple intermolecular interactions, hydrogen bonding, and crystal-packing structures with varying degrees, restricting the conformational free motion, which decreases the nonradiative rate of energy loss. Encouraged by the above optical properties, the AIE properties were studied by UV-vis and fluorescence spectroscopy under a gradual increase in the water fraction (fw) from 0% to 99%. The absorption spectra of 20a and 20c–l broadened at fw values above 80%, indicating the formation of nanoparticles. By increasing the fraction of fw above 80% or 90%, the fluorescence intensity was intensified and red-shifted, proving the AIE behavior. Strong dipole–dipole interactions and rigidified molecular structures lead to large red shifts in the emission spectra in the aggregated state. Probes without strong D–A effects of the two-aryl substituent (20a and 20b) exhibit weak AIE-active phenomena. Viscosity was further studied in the glycerol-methanol binary solvent from viscosity 0.6 to 360 cP, the emission intensity of 20j was found to continuously increase with a blue-shift in the emission wavelength (618 nm at 0.6 cp to 597 nm at 64 cp to 587 nm at 109 cp to 571 nm at 360 cp).

Chuo et al. have reported boron pyridinomine composites with (21b) and without (21a) a chemical bond for studying the luminescence properties between fluorescence ACQ and AIE. [49] The photophysical properties were studied in CHCl3, owing to the smaller bandgap energy, compared to acetonitrile, wherein 21a showed a larger absorption band with a longer wavelength region than 21b. Due to structural rigidity, the emission spectra of 21b showed a smaller Stokes shift than 21a, with an excitation at 453 nm. The luminescence quantum yield was recorded as 0.01 for 21a and 0.68 for 21b. The AIE properties were investigated in acetonitrile by adding water as a poor solvent at a higher concentration. When the water content was increased to 80%, both samples showed white turbidity, wherein 21a showed significant emission enhancement (AIE-active), and 21b showed a drastic decrease in emission intensity (ACQ). Similar trend-like solution state was observed in crystalline-state optical properties. However, 21b showed a considerably decreased quantum yield from 0.68 to 0.07 in the aggregate state compared to in the solution state. The single crystals showed dimer formation for both compounds. Face-to-face π–π interactions were observed for both complexes, whereas additional CH–F interactions were found in the case of 21a. The crystal packing showed π-stacking for 21b, resulting in ACQ. In contrast, because of the small overlap in the dimer, 21a can avoid ACQ. The overall presence or absence of a chemical bond at a single site in the boron complex gave AIE-active and ACQ boron complexes.

Pandey et al. have reported novel AIEgens based on a BODIHY scaffold (22 a-c) with σ-spacers (–O, –S, and –SO2) [50]. The photophysical properties were checked in THF, which showed intense absorptions at 399 (22a), 400 (22b), and 394 nm (22c) due to ICT. The emission maxima were found to be at 521, 532, and 500 nm with fluorescence quantum yields of 0.031, 0.012, and 0.29, respectively. The blue shift in absorption and emission for 22c because of the electron-withdrawing σ-spacer (–SO2) weakened the ICT character. Similar emission properties were observed in a polyethylene methylacrylate matrix. The solid-state emission spectra for 22a and 22b upon excitation at ∼400 nm showed broad bands (450–650 nm) with maxima centered at 521 and 574 nm, respectively. In contrast, 22c showed dual emission at ∼484 and 550 nm upon excitation at 394 nm. The AIE properties were investigated in THF/water binary mixtures
with different concentrations. In a large excess of water ($f_w$ 99%), a batochromic shift in the absorption bands was seen relative to the THF solution. The emission spectra at $f_w$ 90% for probes 22a and 22b found to be at 524 and 556 nm, proving that 22a and 22b are AIEgens due to restrictions in the rotation. In contrast, 22c showed dual emission with quenching under similar conditions at $f_w$ 90–99%. This was further confirmed in methanol/glycerol mixtures with varying glycerol fractions. Scanning electron microscopy (SEM) analysis was carried out to study the morphology of the aggregates at $f_w$ of 99%. With $f_w$ 99%, H–bonding interactions are maximized by the hydrophilic BF$_2$ unit with water and the phenyl rings, which favor uniform spherical aggregates.

Wong et al. reported a novel family of AIE-active meso-2-ketopyrrolyl BODIPYs (23 a-d).[51] The in-solution photophysical properties were examined in various common organic solvents with different polarities, such as cyclohexane, toluene (TL), dichloromethane, THF, acetonitrile, and methanol. All probes showed a strong absorption band at 450–550 nm. These BODIPYs are not highly emissive in organic solvents except for 23a, which is relatively fluorescent with quantum yields of 0.10–0.21. 23b and 23c showed quantum yields of 0.03 and 0.01, respectively, in acetonitrile, possibly due to rotations of the meso-2-ketopyrrolyl group, which resulted in internal conversion through a nonradiative process. Probes 23a and 23b showed bright fluorescence emission in glycerol with fluorescence quantum yields of 0.43 and 0.15, respectively, due to the restricted rotations of the meso-2-ketopyrrolyl group. Interestingly, these probes, 23 a-d, exhibit relatively strong emission in the solid state with a redshift at 662, 620, 653, and 644 nm, respectively, with fluorescence quantum yields ranging from 0.13 to 0.25. The reason behind this solid-state fluorescence is the head-to-head or head-to-tail dimer and J-aggregating crystal packing, which restricts the free rotation of the meso-2-ketopyrrolyl group and reduces the rate of nonradiative energy loss. The AIE behavior was studied by a gradual increase in the water fraction from 0% to 99% in the acetonitrile–water system. With a water content of over 80%, the absorption band became broader with dual peaks, which is a characteristic of the J-type packing and possibly nanoparticle formation. However, when $f_w$ was increased to 90%, the probes showed enhancement in fluorescence intensity with a redshift in emission maximum, indicating that these BODIPYs are new AIEgens. This enhancement and redshift in emission in the aggregated state might be attributed to the rigidified molecular structures and strong dipole–dipole interactions. These AIE properties were demonstrated by SEM and transmission electron microscopy (TEM). The restriction of the rotation of the meso-ketopyrrolyl group in viscous media with fluorescence enhancement and the lengthened fluorescence lifetime from 0.8 to 3.9 ns made these probes novel viscosimeters. These probes are used for the real-time tracing of viscosity changes during cell apoptosis events.

3.2 Cyanostilbene and stilbene-based fluorophores

Cyanostilbene and stilbene-based derivatives, such as cyanostilbene and its derivatives, are the most studied AIEgens owing to their simple structure. Among these derivatives, most are 4-cyanostilbenes and α-cyanostilbene depending on the cyanostilbene position. The 4-cyanostilbene has a cyano group at the para-position of one aromatic ring, whereas α-cyanostilbene has a cyano group at the α-position of the ethylenic bond. Irrespective of its small size, the cyanostilbene can induce steric effects in the adjacent groups, causing a twisted conformation. In addition, the electron-withdrawing nature of the cyano group enables the design of donor–acceptor-type π-conjugated compounds with desired optical properties to cover the entire visible region. In α-cyanostilbene AIEgens, ICT is generally observed due to the π-conjugated system of the donor–acceptor-type π-conjugated systems. 4-cyanostilbene showed TICT due to the location of the cyanostilbene on the opposite side of the donor–acceptor-type π-conjugated systems. Another important characteristic of cyano-containing AIEgens is in the crystalline structure the capability of the cyano group of establishing hydrogen bonding with neighboring molecules via C–H⋯N interactions. This hydrogen bonding allows the chromophore to create a network in its crystalline structure, which tends to increase the rigidity of the crystal to prevent free movement of the free movable groups in the molecule. This rigidity of the molecules prevents π–π stacking interactions, making them better AIEgens.[52]

Simple hydrocarbons, such as stilbenes, are also used as AIEgens, which contain only carbon and hydrogen atoms in their chemical structures. The lack of any heterotatom in their structure makes the structural properties and interactions between molecules very straightforward to provide insight into the guiding mechanisms behind the AIE effect. Due to their great AIE properties, these stilbenes have great potential in organelle (mitochondria, LDs, and lysosomes) and bacterial imaging. The AIEgens with purine and phospholipid scaffold were used for wash-free plasma membrane (PM) labeling and visualization of erythrocytes in the complex brain.

Figure 5 shows cyanostilbene and stilbene-based fluorophores as AIEgens. Sánchez et al. reported three cyanostilbene-containing color-tunable divinylene π-gelators (24a-c). The long-side chains and four amide functional groups enable the formation of supramolecular polymers that help to form organogels.[53] Compounds 24a-c showed weak emission in polar solvents, such as 1,2-dichloroethane, and exhibited enhanced emission in a nonpolar solvent, such as methylcyclohexane (MCH), by forming aggregates with yellowish or bluish emission. Compound 24c was not completely soluble in MCH, but orange-emitting aggregates formed in a 9/1 mixture of MCH/DCE. Compounds 24a-c formed gels in MCH. The formation of the gel also gives rise to highly emissive, soft materials with color-tunable features. The position of the cyanostilbene group in the donor–acceptor interaction is a vital factor behind the fluorescence color changes. SEM images of the organogels formed in TL showed a characteristic dense network of fibers. The networks of compound 24a showed a long homogeneous network of fibers with a diameter of 100 nm. Compound 24b showed thick but nonhomogeneous fibers (Figure 6); however, a diluted sample of these aggregates, when studied using atomic force microscopy (AFM), showed a self-assembly of aligned thin filaments (width of approximately 5 nm). Compound 24c with thiophene showed thick domains of filaments that ended with thinner fibers (Figure 6C). The aggregated states of the molecules showed...
an H-type arrangement; the H-type dimer aggregate and higher lifetimes could be the reasons for this AIE phenomenon.

Tonga reported the control of the AIE color of cyanostilbene luminogens by modifying the different substituents on the amino donor group (e.g., fused, aryl/alkyl, and dialkyl). These amino-donor-modified probes (25a-c) furnish AIE properties with emission colors ranging from green-to-yellow-to-red.[54] In solution, the optical properties were studied in THF, and all the probes showed a weak emission in THF. The AIE properties were checked in THF/water binary systems, which showed enhanced emission upon addition of water by forming emissive aggregates. The alkyl chain substituents in 25a and 25b resulted in a twisted conformation that allowed the molecules to exhibit AIE properties in the aggregate state. The high planarity of 25c compared to 25a and 25b showed AIE properties that may arise from an E/Z transformation upon excitation.

In another report, Tonga demonstrated the AIE properties of extended viologens by exploring the effect of π-conjugation as shown: 26a < 26b < 26c. The PL spectra showed a redshift in the PL spectra with an increase in π-conjugation. The AIE characteristics were demonstrated in MeCN/TL binary systems. These probes showed tunable emissions of blue (26a), green (26b), and red colors (26c) at the aggregate states by changing the concentration of the nonpolar solvent (TL). The reason behind these AIE effects could be the restriction of rotational and vibrational motions, and E/Z isomerization of the vinylene-pyridinium units in the solid state.

Konishi et al. explored bridged stilbenes (27a-l) as AIEgens via a simple strategy to control the nonradiative decay pathway.[56] Short- and long-chained bridged stilbenes have been studied; strong fluorescence emission in solution and solid state were observed with short alkyl chains, and long alkyl chains exhibited AIE. They discovered that the length of the alkyl chain affects the mechanical control of the
conical intersection (CI) and control of the Franck–Condon state (FC) via distortion of the π-conjugation plane. These AIEgens are based on the control of the conical intersection accessibility (CCIA) mechanism. Probes with this mechanism should follow three critical points: (1) the quest for π-conjugated systems with known nonradiative decay pathways as the molecular core, (2) lower CI of molecule, and (3) nonradiative decay pathway of the molecule with molecular motion of large amplitude. The photophysical properties were studied thoroughly in bridged stilbenes (27b–d) and bridged phenyl-stilbenes (27g–i). Based on the calculations of nonradiative decay pathways and quantum yields of these probes, they demonstrated that long-alkyl-chained stilbenes showed better AIE phenomenon, which fulfills the conditions for control of the CCIA mechanism.

Hong et al. studied the effect of molecular rotors on azo-cyanostilbene (28-30) on the AIE properties.[57] Large AIE properties with a large Stokes shift can be achieved by tuning the rotor on the fluorophore. In this study, cationic azo-cyanostilbene 29 and derivatives of the reported compound 28 with a phenyl ring bridge were used, which showed enhanced AIE properties. The molecule 28 and its derivatives (28a-d) and 29 showed similar absorption and emission in different solvents. Red-shifted spectra in a solvent like dimethyl sulfoxide (DMSO), which was polar and had a higher dielectric constant, were observed for 28 and derivatives (28a-d) when compared with nonpolar solvents, such as chloroform. Due to the poor solubility of 28 and its derivatives (28a-d) in chloroform, strong fluorescence intensity was attributed to AIE effects. Viscosity was studied by increasing the glycerol percentage for all the dyes to check the effect of the molecular rotor and to induce the RIMs. All dyes showed an increase in fluorescence intensity. This result shows that the rigidification of dyes results in enhanced FL intensity based on the molecular rotor. The self-aggregation of dyes was further studied in a DMSO/chloroform binary system with an increased fraction of chloroform. The 28 series showed an increase in FL intensity beyond 70% of its maximum value in chloroform, with a 25–82 fold increase obtained with 99% chloroform. A small increase in FL intensity (2.6-fold) was observed with 99% chloroform for 29. These results proved that the more twisted structure of 28 reduces the strength of the intermolecular interaction (π–π stacking); hence, it exhibits a stronger AIE property than 29. The pyridinium units of the dyes were used for cell imaging. Among all the dyes, 28b had a hexyl chain and stained a mitochondrion-like pattern more strongly. Other dyes that failed in cell imaging may be due to poor dye penetration or low cellular retention of the dye upon fixation.

Yu et al. reported stilbene-based AIEgen 31 for rapid and ultrasensitive imaging of PMs in biosystems.[53] The probe was based on stilbene, where purine was used as the skeleton, which is known for its large Stokes shifts, good biocompatibility, and an adjustable alkyl chain. The pentyli group was chosen as the hydrophobic part for hydrophobic interactions with the PM. The ((trimethylammonium)propyl) pyridine group was selected for electrostatic interactions with the PM. DMSO/TL binary systems were selected to study the AIE behavior. The probe was nonemissive in DMSO, and the aggregates formed at above 80% TL were highly emissive and could be recognized by the aggregation-activation RIM process. In TL 99.9%, the fluorescence intensity of the probe showed 8.24-fold increase compared to that in pure DMSO. In addition, the solid-state luminescence was red-shifted compared to that of the DMSO/TL mixtures. The quantum yields were calculated in the solid state (7.2%). The value of quantum yields in the aggregation state (15.1%) was considerably higher than that in solution (lower than 0.1%), demonstrating substantial AIE property. Water solubility, AIE characteristics, and electrostatic and hydrophobic interactions made this probe an easy wash-free labeling probe for the PM both in vitro and in vivo. This is the first fluorescent probe for visualizing erythrocytes in complex brains.

Tang et al. developed a novel class of natural rosin-derived luminogens (32a–c) as AIEgens. These AIEgens showed good biocompatibility, organelle imaging, and photochromic behavior in the solid state.[59] They found that the alicyclic moiety plays a vital role in biocompatible AIEgens, which helps to suppress the excited-state molecular motion to enhance solid-state emission. The AIE phenomenon was studied in MeCN/water binary systems. The derivatives 32a and 32b were nonemissive in acetonitrile solutions, but at increased f_w, enhanced yellow and dull orange emission was observed, demonstrating their typical AIE properties. In contrast, 32c is emissive in both the pure ACN solution and aggregates (f_w = 90%), with green and greenish yellow light, respectively, indicating aggregation-enhanced emission properties. DLS also supports the formation of nanoaggregates at high f_w (80%), and the enhanced emission in this binary system is derived from their aggregation in poor solvents. The AIE mechanism could arise from the introduction of an alicyclic moiety, which made the probes rigid and suppressed the excited-state molecular motion. The probes are based on a natural product that supports BioAIEgens biocompatibility and further confirmed their localization by cell imaging. The results showed that 32a and 32c can specifically stain LDs and lysosomes, respectively. Furthermore, bacterial imaging showed that 32a and 32c can stain both gram-positive (S. epidermidis) and gram-negative (E. coli) bacteria. These results prove the universality of BioAIEgens in bioapplications.

3.3 AIEgens based on different fluorophores

Recently, few AIEgens have been reported that are derived from fluorophores based on naphthalimide, dansyl, acedan, and malononitrile. The major class of fluorophores utilized as AIEgens is based on naphthalimide (Figure 7). Strong intermolecular π–π stacking interactions were observed on the planar aromatic core of naphthalimide, which often quenches fluorescence. Thus, the proper incorporation of a naphthalene moiety can hypothetically weaken the intermolecular π–π stacking interactions and form AIEgens. Chemical modifications of the naphthalimide moiety with AIE active units, such as TPE and o xoaryl, showed aggregation or disaggregation upon reaction with the analytes.

Iyer et al. have studied the blue shifted naphthalene-containing naphthalimide derivative 33 AIEgen for 4-nitroaniline (4-NA) detection in water using the “receptor-free” inner filter effect (IFE) mechanism.[60] The AIE properties were studied in DMF/water binary systems. The insolvibility of the probe in water resulted in an increase in f_w. Upon increasing f_w to 60%, the emission intensity showed a drastic seven-fold decrease with some red shift. However, when
exceeded 60–99.8%, aggregation became prominent and blue-shifted, with a seven-fold increase in its emission intensity. This AIE property could arise from unique intermolecular π–π stacking orientations in 33 and TICT. Fluorescence quenching was observed in the aqueous solution of the probe when reacted with a DMF solution of 4-NA. The possible mechanism of the receptor-free IFE mechanism was studied further. The morphological changes in the aggregates, upon reaction with 4-NA, were further demonstrated by DLS and FESEM images (Figure 8), confirming the surface interaction of 33 with 4-NA. This is the first AIEgenic probe that detects 4-NA in water via the IFE mechanism.

Nagarajan et al. reported the impact of linkers and solvent polarity on tuning the emission of 1,8-naphthalimide aggregates. The aromatic ring of naphthalimide was modified with vinyl and ethynyl, and a direct link was introduced via Pd-catalyzed cross-coupling reactions (34a-c). The self-assembling properties of the probes were checked in different binary solvent systems, such as MeCN/H$_2$O, DOX/H$_2$O, and THF/H$_2$O. In the presence of water, the emission shifted noticeably, suggesting the formation of aggregates. The THF/H$_2$O system showed a higher quantum yield than the other systems, proving that a moderately polar environment is responsible for aggregate formation. Among all the probes with an ethynyl linker, the emission was substantially enhanced due to RIR. The morphology of the aggregates was studied using FESEM and DLS.

Govindaraju et al. reported cyclic dipeptide (CDP)-conjugated naphthalimide derivatives (35a-i) as AIEgens and their role in the detection of phenolic drugs. The introduction of CDP to naphthalic anhydride promotes intermolecular hydrogen bonding in aqueous media, leading to the formation of AIE-active aggregates, as CDP is well known for intermolecular hydrogen bonding. DMSO/water binary solvent
systems were used to study AIE behavior. Probes 35a and 35d showed considerably enhanced fluorescence intensity in aqueous media with 98% $f_{ca}$. Structure-activity studies revealed the role of an amino acid side chain and a substituent at the naphthalimide moiety in regulating the molecular assembly and efficiency of AIE. Owing to its excellent AIE properties, 35a was checked against a series of organic analytes with distinct electronic properties. The results showed that the electron-deficient phenolic compounds exhibited distinct fluorescence quenching. Among nitroaromatics, picric acid (PA) exhibited instant fluorescence quenching, indicating the vital role of the phenolic group. Based on these results, electron-deficient phenolic drugs, doxorubicin, and rifampicin were also detected with nanomolar sensitivity.

Shinokubo et al. studied the nitrogen-bridged naphthalene monoimide dimers as AIEgens. Four 4-aminonaphthalene monoimide derivatives (36a-d) were synthesized, and their AIE properties were examined. In the solid state, these dimers exhibit bright green emission with a high quantum yield. The AIE behavior was investigated in a THF/water binary solvent system. The results showed that probes 36b and 36c formed emissive aggregates with the increasing water fraction, and the emission of dimer 36b in the aggregates was higher than that of 36c. The reason behind this AIE property may be the suppression of TICT in conformationally restricted aggregated states. In addition, the XRD analysis suggests that the CH−⋯π interaction, lacking fπ−π stacking in the crystal packing, is the key behind this AIE phenomenon. The late-stage cyanation of the probe 36b affords an orange-emissive AIE-gen.

Hawes et al. studied the supramolecular aggregation properties of two morpholinosubstituted naphthalimide ligands (37a and 37b).[64] First, AIE behavior was studied in a CH$_3$CN/H$_2$O binary solvent system with an increased water fraction to induce aggregation. No enhancement of fluorescence was observed with increased H$_2$O percentage; instead, emission intensity was decreased, suggesting that aggregation is not the reason behind emission enhancement. When the effect of pH on the emission intensity was checked, the solution-state probes underwent a pH- and anion-dependent aggregation process, which led to a strong enhancement in fluorescence emission. This suggests that the change in the absorption and fluorescence is not only attributable to the protonation and deprotonation processes, but also to the supramolecular organization. This was further confirmed by DLS and SEM measurements, which suggested the formation of micrometer-scale aggregates, which helped in the formation of microparticles. Moreover, metallogels with first-row divalent transition metals were also studied using 37a with either Mn$^{2+}$ or Co$^{2+}$.

Lu et al. reported two simple N-substituted naphthalimides (38a and 38b) with an aromatic rotor (phenyl group) and an aliphatic rotor (cyclohexyl group), and their AIE, multiplexor (MCs), and self-organization were studied.[65] The AIE properties were studied in a THF/water binary solvent system. With increasing water content ($f_{ca}$), both 38a and 38b showed strong fluorescence, and the aggregates became brighter with increasing water content, exhibiting typical AIE effects. This self-assembly is caused by intermolecular π−π stacking. The same was further validated by XRD, DSC, SEM, and single-crystal X-ray structural analyses. 38a showed different photophysical properties upon different external stimuli, indicating the presence of MC effects, whereas 38b did not show such an effect. Even though both have the same fluorescence core of naphthalimide, they exhibit different photophysical properties because of the different stacking modes of the substituents under external stimuli. These MCs for the 38a samples resulted from their different intermolecular structures and regulated thermodynamic/kinetic processes. These results imply that intramolecular (RIM) and intermolecular (JXAF) structures are the deciding factors behind this AIE effect.

Han et al. reported two acetic carboxylic acids containing a 1,8-naphthalimide probe (39) for the AIE-based detection of casein micelles. Owing to the strong TICT character, the probe showed quenched fluorescence in the solution state.[66] Casein, with 20 necessary amino acids, is one of the important nutrients in milk, with the largest share of milk proteins (on average 80%). Casein has a robust tendency to self-assemble into casein micelles because of its inherent amphiphilic properties in aqueous solutions. This probe, when checked with casein micelles, showed strong blue emission with AIE properties. The AIE behavior with casein is caused by the docking of probe 39 in the hydrophobic cavity between submicelles and binds with Tyr and Trp residues forming the casein micelle–dye complex. Based on this good selectivity for casein, a novel casein assay method was established.

Some researchers have explored the use of dansyl chloride fluorophores as AIEgens. It is suspected that the dansyl fluorophore might have self-aggregation properties that induce fluorescence quenching in aqueous conditions due to its neutral and hydrophobic properties.

Lee et al. reported a self-aggregated 48-membered dansyl (DS) library for the selective detection of progesterone. The selective detection of steroids is a great challenge due to their high structural similarity.[67] Here, for the first time, they demonstrated selective fluorescence sensing for steroids by aggregation or disaggregation phenomena. This DS library was screened for 29 kinds of metabolites, including amino acids, oxido-redox-related molecules, and steroids, to check the fluorescence emission response. Due to the TICT mechanism, these probes showed quenching in the solution state, but interestingly, 40a (PG-1) and 40b (PG-2) showed fluorescence turn-on for progesterone among other steroids. To examine the possible mechanism of assembly and disassembly, various techniques have been investigated to check aggregation. DLS analysis revealed aggregate formation in the solution with the probe and progesterone. The particle size was found to increase in a dose-dependent manner, and bulk aggregates were observed within 5 min, which was distinct from the self-aggregates of PGS. Later, a similar type of bulk aggregate was observed using TEM. This selective turn-on phenomenon is based on the self-assembly-induced quenching effect of the DS fluorophore in aqueous conditions and the bulk aggregation due to the reaction between progesterone and the PG probes. Finally, the same turn-on mechanism was confirmed in blood and amniotic fluid samples from two pregnant women to prove the utility of these probes to detect progesterone in real biological samples. This is the first class of fluorescence probes that allows for the simple, selective, and fast detection of progesterone from different steroids.

Shi et al. developed a novel AIE probe based on quinolone-malononitrile (QM) fluorophore (41) for the sensitive
detection of alkaline phosphatase (ALP). QM is known to exhibit outstanding self-assembly properties by modifying the probe structures to obtain different aggregated states. Owing to its good water solubility, the probe was initially nonfluorescent in an aqueous solution. However, when reacted with ALP, the probe was activated, resulting in strong NIR fluorescence that facilitated the specific detection of ALP activity. Furthermore, this probe was used to detect endogenous ALP activity in cancer cells and drug-treated zebrafish larvae. The AIE phenomenon was studied in DMSO/water binary solvent systems. The probe was nonfluorescent in DMSO; however, when the water percentage was increased to 70%, a substantial enhancement of fluorescence with a redshift was observed due to solvent polarity, demonstrating the existence of AIE characteristics.

Another example of QM-based AIEgens (42) was reported by Zhu et al. for Aβ plaque mapping. The probe showed AIE-active NIR emission for in vivo detection of Aβ plaques with high binding affinity and blood–brain barrier permeability, and could serve as an alternative to the commercial probes ThT or ThS. The aggregation properties were studied in a water/ethanol binary solvent. The probe was nonfluorescent in an aqueous solution, but with an increase in the ethanol fraction to 95%, a strong NIR emission at 720 nm was observed. This non emissive nature of 42 in aqueous solution and enhanced NIR fluorescence in the aggregated state made this probe a perfect candidate for mapping protein fibrillogenesis, similar to Aβ aggregates. While checking the fluorescence response of the probe with Aβ aggregates, considerable NIR fluorescence enhancement was observed within ~40 min, reaching maxima. The FL intensity was blue-shifted, which could be due to the entry of the probe into the hydrophobic pockets and binding of N, N-dimethylamino units with aggregated amyloid fibrils. The SEM images of the probe with and without Aβ aggregates confirmed the binding process.

Liu et al. reported a detailed study of the TICT mechanism of 14 types (naphthalimide, phthalimide, rhodamine, rhodol, acridine, oxazine, NBD, dansyl, coumarin, and BODIPY) of popular organic fluorophores using the time-dependent density functional theory (TD-DFT). The accurate prediction of TICT is critical in designing fluorophores and AIEgens because the fluorescent properties of the probe depend upon TICT. Based on their detailed TD-DFT study, they predicted that substituting the dalkylamino group with the azetidinyl moiety considerably weakens the TICT states, resulting in quenching. In contrast, a smaller five-membered ring improved the structural planarity and rigidity, which reduced the nonradiative decay and offered good emission in solution. However, in the solid state, the planar conformation exhibits strong π–π intermolecular interactions, resulting in severe emission quenching. By simply altering the ring size of the aliphatic ring, it is feasible to have AIE and ACQ effects without affecting the electronic environment of the conjugated framework.

3.4 Excited-state intramolecular proton transfer-based AIEgens

Excited-state intramolecular proton transfer (ESIPT) is a phototautomerization (enol-keto tautomerism) process that proceeds extremely fast with the formation of intramolecular hydrogen bonding and a large Stokes shift. The mechanism behind the AIEgen design with the ESIPT process is based on the introduction of intramolecular HBs and linking different aromatic moieties by rotatable C–C/C–N/N–N single bonds. Combining AIE with ESIPT has great advantages, such as using new properties for the detection of biologically important analytes with large Stokes shift. The quenching of emission in the isolated state should be associated with dynamic intramolecular motions, indicating that the AIE properties of the ESIPT probes depend on the RIM processes.

Figure 7 shows AIEgens based on different fluorophores and ESIPT mechanisms. Horak et al. reported five AIE- and ESIPT-based benzimidazole derivatives as a novel class of solid-state fluorophores. All the probes possessed a D–π–A structure with bulky aromatic groups that could undergo a twisted molecular conformational change. The hydroxy group was responsible for ESIPT. The emission was tuned from blue to red based on the substituents on the π-conjugated backbone. The solid-state emission in ethanol showed strong absorbance in the violet-blue region due to charge transfer. While they are weakly emissive in ethanolic solutions, they show strong fluorescence under 365 nm illumination. The solid-state emission showed a lower wavelength for the weak electron-withdrawing group (methyl), whereas a red shift in emission occurred with a strong electron-withdrawing group (–CN and –NO2). Ethanol/water and DMSO/water binary systems were used to study the solid-state emission. Upon increasing the water fraction (f_w), the probes showed a new fluorescence band, suggesting the formation of aggregates in water. Further, aggregate formation was confirmed by the DLS method, and the morphology of the crystal formation was examined by powder X-ray diffraction and SEM. Reversible mechanochromic luminescence was observed with wavelength shifts ranging from 16 to 48 nm by disrupting the AIE using external stimuli (grinding and exposure to vapors of organic solvents).

Zhang et al. reported AIEgens based on seven-membered free-base BOPYINs (NBs, 46a–d) synthesized from benzoin-dole and β-substituted pyrroles. The ESIPT phenomenon
arises from the seven-membered ring HB and steric hindrance. The reason behind the AIE activity was found to be a limitation of the cis-trans conformation process in the excited state and weak interaction in the aggregate state. The AIE properties were checked in binary solvent systems, such as DMF/water, DMSO/water, and ACN/water. The results showed that with increasing \( f_{se} \) from 0% to 99.5%, the fluorescence intensity was found to be increased by 2.8- to 11.1-fold, suggesting the AIE nature of these dyes. Solid-state properties were also found to be bathochromically shifted when compared to the solution-state properties. Further, the AIE behavior was checked by single-crystal XRD for 46a-c. The crystal structures showed cis-diarylethene, induced by intramolecular HBs and steric hindrance due to planar conformation HBs with a seven-membered ring for both crystals. The crystal packing structures showed a slipped-stacked alternating arrangement and J-aggregation (\( \pi-\pi \) stacking interactions). Thus, the enhancement of the crystal solid may be due to the restriction of the excited-state cis-trans conformation by multiple N–H⋯N, C–H⋯\( \pi \), and C–H⋯O hydrogen bonds with a J-aggregate. This AIE-ESIPT probe with intramolecular HBs showed a strong long-wavelength emission and an extremely large Stokes shift over 140 nm, making these probes advantageous compared with commonly used AIEgens.

### 3.5 Heterocycles as AIEgens

Heteroatoms on the \( \pi \)-system could influence the photophysical properties by changing the electron density, hydrogen-bonding properties, and dipole–dipole interactions. Moreover, some heterocyclic compounds have been extracted from natural sources or are known pharmcophores. Owing to these potential characteristics, heterocyclic compounds, especially AIEgens, have been considered as newly emerging luminophores. The representative heteroaromatic AIEgens are indolizine derivatives that feature an indolizine core ring and substituents to control the electron distribution. In addition, the substituents functioned as rotors and recognition sites.

Figure 9 shows a few heteroatomic AIEgens. Kaleidolizines (47) are a type of compound that contain indolizine derivatives as AIEgens.[77] The derivatives 47a-e enabled the tuning of the emission wavelength systematically through various substituents at the R1, R2, and R3 positions on the indolizine core. Substitution at R1 and R3 induced effective fluorescence changes, and 47b, which contains a trifluoromethyl group at the R1, R2, and R3 positions, showed a 120-fold fluorescence enhancement in the high water fraction solution. The AIE characteristics of 47a–e were attributed to the synergistic effect of ICT and RIR. Moreover, 47e, which contains triphenylphosphonium as a targeting moiety for mitochondria, was shown to facilitate the visualization of innate mitochondria in live cells. Despite some facile synthetic strategies that have been reported, new synthetic strategies that favor the use of indolizine as AIEgens have been developed. Recently, one-pot chemoselective domino condensation was exploited to obtain diverse indolizine-based AIEgens (48a–u) with yellow emission in the aggregate state and a large Stokes shift (~170 nm).[78] The substituents (R1 and R2) of 48 could function as rotors and 48a exhibited the highest quantum yield; it was demonstrated to be nontoxic against the HUVEC and C126 cell lines at concentrations of up to 100 \( \mu \)g/ml. Indolizine-based probes, with attached heterocyclic moieties, were used for the detection of metals. The attached heterocyclic moieties acted as rotors and metal-binding sites. The anthracene-conjugated imidazol[1,5-1]pyridine (49) with ICT and AIE characteristics was studied as an AIEgen to detect Cu\(^{2+}\).[79] In a 90% water mixture, anthracene can stack with each other and four intermolecular H-bonds between the N atoms of the indolizine derivatives and H atoms of the anthracene could stabilize the stacked form to generate nanoaggregates that emit at 510 nm. Upon increasing the Cu\(^{2+}\) concentration, the emission intensity decreased due to disaggregation of the nanoaggregates. The interaction between Cu\(^{2+}\) and N atoms on the pyridyl and imidazole groups disrupts the intermolecular H-bonds. Using an IoT-embedded 3D printed portable device with 49, the detection of Cu\(^{2+}\) in a real water sample was achieved. Unlike 49, 50 exhibited fluorescence enhancement upon the addition of Fe\(^{3+}\).[80] The derivative 50 containing extended indolizine and thiophene could generate nanoaggregates (~150 nm) with concomitant increased emission in solutions that have a high water fraction and high concentrations. The inter and intramolecular H-bonds on the O and N atoms would contribute to the formation of nanoaggregates, and after the addition of Fe\(^{3+}\), it could interact with the S atom of thiophene and O atoms to restrict the rotation of thiophene and rigidify the aggregates of 50, resulting in fluorescence enhancement. The detection of autophagosome-lysosome fusion using autophagy was possible using 50.

Berberine chloride (51), a natural isoquinoline alkaloid isolated from herbal plants and its derivatives, quinolizinium substances, are popular DNA intercalators.[81] Quinolizinium and 51 are heterocyclic compounds that bear an inherent positive charge on the N atom of the aromatic ring. The strong fluorescence intensity of quinolizinium is attributed to the absence of significant structural changes in the excited state and well-delocalized positive charge and frontier orbitals.[82] Recently, their AIE properties with multisubstituted forms and easy synthesis procedures have been explored.[83] This type of AIEgen contains positively charged aromatic rings and counter anions, which induce anion–\( \pi \)–\( \pi \) interactions. The anion is located between positively charged aromatic rings in the aggregate state to impede \( \pi-\pi \) stacking and ACQ. The compound 51 has a nonplanar conformation due to the twisted electron-donating phenyl group. Its intermolecular distance in a parallel manner is 3.851 and 4.090 Å,[84] which is longer than the \( \pi-\pi \) stacking distance (3.5 Å). Through the emission evaluation of 51 in a viscous environment using the macromolecule cucurbit[7]uril as a host, it was demonstrated that the AIE phenomenon was caused by the suppression of the intermolecular vibration and the TICT effect. In addition, 51 selectively visualized LDs in the cells and liver tissue. Owing to its photophysical properties and positive charge, the potential of 51 as a theranostic agent was also described through the selective staining of mitochondria and ROS generation from 51 under light irradiation.[85] Reduced berberine (52), which has neutral tertiary nitrogen instead of positively charged isoquinolinum, is known to be a metabolite of 51. The compound 52 showed fluorescence enhancement under viscous conditions and crystal states and exhibited dramatic emission wavelength changes from 454 to...
511 nm in the pH range of 7–10. Considering the conformational similarity of \( \text{52} \) to \( \text{51} \), the AIE performance was also caused by the RIV. The ion trapping phenomenon was visualized in live cells using the pH-dependent ratiometric fluorescence changes of \( \text{52} \).

The aforementioned AIEgens (\( \text{51, 52} \)) possessed no rotors, whereas \( \text{53} \) featured two rotative phenyl rings linked to benzoquinolizinium salt with CF3COO– to achieve dramatic AIE performance. Typical AIE characteristics were exhibited by \( \text{53} \) in THF/iPrOH solution, and it enabled the visualization of the chromosome periphery (CP) by restricting the rotation of phenyl rings when \( \text{53} \) binds to the CP protein, resulting in fluorescence enhancement.

The expanded scaffold \( \text{54} \) that has an inherent positive charge on the \( \pi \)-system and four rotative phenyl rings was developed by a one-pot reaction with the aim of PDT. Crystal structure studies revealed that the F of PF6– interacted with the benzoquinoline cores, blocking \( \pi \)-\( \pi \) stacking. Intermolecular and intramolecular F⋯H interactions and CH⋯π interactions in the aggregates constrained the rotation of the phenyl rings. Moreover, it was shown that the aggregates of \( \text{54} \) generate more singlet oxygen than \( \text{54} \) in solution because \( \text{54} \) aggregates promote intersystem crossing (ISC). This is explained by the energy splitting of the excited state and minimizing the competitive nonradiative decay. The compound \( \text{54} \) was shown to be feasible for use as an aggregation-induced singlet oxygen generator.

Thienopyrimidine derivatives (\( \text{55, 56} \)) are bioactive heterocyclic AIEgens. Thienopyrimidine was reported to bind to the ATP-binding pocket of MAP kinase signal integrating kinases (MNKs). AIE and solid-state emission in the range of 400–560 nm was achieved through the incorporation of various rotatable aryl moieties to thienopyrimidine. Based on DFT calculations, \( \text{55a-c} \) were stabilized by intermolecular H-bonds, such as NH⋯O and CH⋯O in the dimer state, rather than \( \pi \)-\( \pi \) interactions. In solution, the emission of \( \text{55b} \) increased at high concentrations, and \( \text{55a} \) and \( \text{55c} \) showed an enhancement of fluorescence intensity with an increase in the poor solvent (benzene) fraction in DMSO. The thienopyrimidine scaffold of \( \text{55a-c} \) exhibited antibacterial and antifungal activity. Among them, \( \text{55b} \) showed high inhibitory potency, which might be caused by the different rotors influencing the binding efficiency to MNKs. This means that the introduction of diverse rotative groups to thienopyrimidine could provide plausible candidates for AIEgens with high antibacterial and antifungal potency.

In addition to the heterocyclic bioactive AIEgens, some efforts to develop novel luminophores containing heterocyclic skeletons have been reported. For easy synthesis of compounds with tailored photophysical properties, a small library of (thio)aryl-maleimide fluorophores (\( \text{56a-h} \)) bearing push-pull characteristics was prepared. The thioaryl groups are twisted out of the planarity of the maleimide, and the torsion angles could affect the \( \pi \)-communication. In the solid state, ACQ was observed in most derivatives, but \( \text{56f} \) exhibited a 400-fold increase in fluorescence compared to that in the solution state. The torsion angle of \( \text{56f} \) (57.3°) and the bulkiness of the thioaryl groups would restrict the \( \pi \)-communication in solution. However, increased intermolecular interactions in the solid state caused the torsion angle to decrease to 42.56°–47.90°, which allows \( \pi \)-communication between thioaryl groups and the maleimide, resulting in increased fluorescence.

Using nitrile transformation and palladium-catalyzed multicomponent reactions, 1H-isoindole and its derivatives (\( \text{57a–d} \)) were prepared, and their optical properties were studied. Most of the compounds showed very weak fluorescence (quantum yield = 0.2–0.9%) in solution; however, their quantum yields increased up to 1.9–26.3% in the range of 408–438 nm in the solid film. Significant AIE was exhibited by \( \text{57d} \), which is composed of bulky alkyl substituents to suppress close \( \pi \)-\( \pi \) packing and a thiophene to extend the conjugation system and strengthen electron donor–acceptor interactions. The lipophilic properties of \( \text{57d} \) and \( \text{57a} \) allowed
visualization of the location of LDsin HeLa cells with excellent LD-targeting specificity.

The aryl S,N-ketene acetal derivatives (58) were explored as a new class of AIEgens.\(^\text{92}\) Through the diversity-oriented one-pot reaction with benzoazolium salts and acid chlorides, which was performed to access the π-conjugation extended aryl S,N-ketene acetals, a library of 35 compounds was obtained. The introduction of various substituents from electron-donating groups to the electron-withdrawing groups to the aryl S,N-ketene acetal scaffold, could tune the solid-state emission color from deep blue to red. Except for 58a, b, and k, the remaining 58 derivatives showed solid-state emission and AIE characteristics. The absence of AIE in 58k, which has a methyl substituent at the R2 position, indicated that phenyl substituents at the R2 position played a crucial role in the AIE characteristics.

In addition to the azaheterocyclic scaffolds, sulfur-heterocyclic compounds and 2,3-disubstituted-thiochromenones (59) were obtained through the N-catalyzed cycloaddition of sulfobenzoic anhydride with 3-hexyne.\(^\text{93}\) Because of the TPA rotors, 59a and b exhibited AIE characteristics at 540 and 720 nm, respectively, and the red-shifted emission of 59b was attributed to the much stronger electron-withdrawing ability of sulfone compared to that of sulfur.

### 3.6 Atom(sp\(^3\))-bridged AIEgens

To achieve successful AIE characteristics and avoid ACQ, the aggregated structure may require an appropriate intermolecular distance or torsion between π-systems as well as the reduction of nonradiative decay. The TICT character is generally used for AIEgens to reduce the nonplanar conformation-induced π–π stacking. In addition to TICT, atom(sp3)-bridged π-conjugated donor and acceptor systems forming bent conformations could be an alternative strategy to prevent ACQ in aggregates. In addition to carbon-bridged π-conjugates, heteroatom-bridged aromatic rings can achieve AIE characteristics owing to their lone pair electrons, electron density, and polarity.

Figure 10 shows a few atom(sp3)-bridged AIEgens. The compounds 60a and 60b were developed by the combination of an electron-deficient boryl group and phenothiazine, which is a strong electron donor containing two aromatic rings bridged by N and S.\(^\text{94}\) The phenothiazine core in 60a and b was significantly folded along the N-S axis and the nonplanar conformation was able to flip and flap in THF, inducing weak emission. In the solid state and water/THF mixture, the fluorescence intensities of 60a and b increased, and 60a exhibited stronger emission than 60b. The absence of additional methyl groups on the aryl rings contributed to strong intermolecular interactions, generating densely packed structures in the solid state. Another N-bridged benzoazepine oxide (61) was synthesized using Tf\(_2\)O to generate a phosphonium cation intermediate and 2,6-lutidine to remove the acid.\(^\text{95}\) Charge transfer characteristics were observed in 61a and 61b that could induce emission. 61b was emitted in the solution and solid states with a quantum yield higher than that of 61a, but the intensity decreased in the solid state compared to that in the solution state. On the contrary, 61a showed a six-fold emission enhancement in the solid state.

The typical AIE characteristics were ascribed to the TICT from N to the phosphacycle π-conjugation system, and the rotative N-phenyl group.

Some aggregates of organic solids exhibit phosphorescence instead of fluorescence. This phenomenon, termed as room-temperature phosphorescence (RTP), is attributed to the fact that the nonradiative loss of the triplet excitons was minimized by the rigidification of the molecular conformations.\(^\text{96}\) To achieve RTP, singlet-triplet ISC should occur efficiently, which is related to spin–orbit coupling (SOC). Although ISC and SOC are affected by halogen bonding, H-aggregation, n–π transition, and active intramolecular motions, their performance is unpredictable in solution. Tang et al. suggested that crystallization can contribute to strong RTP through the rigidification of the conformation to suppress nonradiative decay from the triplet state. Moreover, the intra and intermolecular charge transfer from an electron donor to an acceptor was reported to boost the efficiency of ISC and increase RTP. In spite of that RTP compounds are now on the developing stage and have a few examples, they could play numerous roles for bioapplications, due to their extreme sensitivity, large Stoke shifts, and zero background from gated emission.

σ-Bonds provide molecular rotations and a tetrahedron-like geometry, reducing π–π stacking. A methylene linker (sp\(^3\)) was used for donor–acceptor AIE–RTP systems 62a and 62b, bearing AIE properties.\(^\text{97}\) Both 62a and b emitted at 445 nm at 77 K, but no fluorescence was observed at 293 K. In the solid state, 62b showed a stronger and delayed emission than 62a at 550 nm, and the same emission wavelength confirmed the localized triplet state on phthalimide. Their tetrahedron-like geometry hindered strong π–π stacking. Instead, 62a formed a partial π stacking between the phthalimides, and 62b could generate alternative stacking between the methoxy phenyl rings. The lower interaction of phthalimides in 62b resulted in a strong RTP.

The compound 62c showed no emission in solution, but displayed bright green-blue emission at 505 nm with a lifetime of 0.49 µs in solid state.\(^\text{98}\) On the other hand, 62d exhibited strong emission in solution, solid state, and at 77 K, but its lifetime was in nanoseconds, indicating weak triplet-state activity. When 62c was blended with 1% 62d, the blended sample exhibited strong emission at 565 nm with a shoulder band at 506 nm and a lifetime of 45.3 ms. Based on XRD calculations, the two σ\(^*\) orbitals of C-Br and the non-bonding orbital of NMe\(_2\) communicated with each other to enhance the SOC and resulted in an intensive RTP of 62d in the 62c matrix.

In addition to carbon as an sp\(^3\) bridge, the tetrahedron-like geometry was exploited using sulfur as the tethering atom of the donor and acceptor. Sulfur is a larger and more polar atom than carbon, inducing stronger intermolecular interactions through the bridged atom.

The incorporation of proton-activated acceptors, such as pyridine and quinolone, enabled the detection of versatile acidic vapors in solid states with RTP.\(^\text{99}\) Although all the derivatives of 63 exhibited weak emission in solution, 63c featured extreme charge transfer characteristics and showed blue emission at 483 nm. It also displayed strong afterglow emission with yellow color (524 nm), with a lifetime of 82.5 ms in solid states, indicating that it also has AIE and RTP properties. After exposure to acid gases, the protonated
63d and 63e increased the RTP at 507 nm with 5.7% quantum yield and a lifetime of 58 μs, and at 529 nm with 10.7% quantum yield and a lifetime of 10.2 μs. This RTP enhancement was attributed to the charge transfer characteristics, which could accelerate the ISC rate as well as the sulfur linker of 63, triggering the twisted conformation to reduce π–π stacking.

Considering persulfated benzenes as luminogens with aggregation-induced phosphorescence, sulfurated benzene-based molecules with nitriles (64) were explored. The compounds 64a and 64b have similar structures, but 64c possesses a more rigid conformation due to ether and thioether linkers. The compounds 64a and 64b exhibited stronger emission at red-shifted wavelength in the solid state with a lifetime of 0.4 μs compared to that in the solution state. They contained an electron donor and acceptor on the twisted structures, which led to phosphorescence enhancement because of the significant separation of HOMO and LUMO. The charge transfer could enable access to the ISC process. On the other hand, 64c emitted bright green fluorescence at 516 nm in THF and yellow fluorescence at 576 nm in the solid state with a lifetime of nanoseconds. This was considered to be the nonplanar conformation to impede the π–π stacking inducing ACQ in the solid state.

The RTP properties of 10-phenyl-10-H-phenothiazine 5,5′-dioxide derivatives (65), which contain sulfone and nitrogen-bridged scaffolds, were explored based on diverse substituents. The derivatives 65d–f formed J-aggregates and sustained the π–π distances around 3.677–3.773 Å, stabilizing the excited triplet state. Their lifetimes and melting points increased in the order F > Cl > Br > H. The electron-donating groups could increase π–π repulsion due to high π-electron density, whereas the electron-withdrawing groups could decrease the π-electron density, resulting in strong π–π interactions. In the case of 65g, which also contained an electron-withdrawing group, the RTP appeared after a period of irradiation. This phenomenon might be induced by molecular motion under irradiation to allow for a more stable packing.

### 3.7 Miscellaneous scaffolds as AIEgens

Numerous natural substances, such as amino acids and deoxyribonucleic acids, are chiral molecules. Monitoring the behavioral or conformational changes of these chiral molecules during biological processes could provide some clues to understand the mechanisms of these processes. The combined conventional AIEgen (TPE) with axially chiral 1,1′-bi-2-naphthol (BINOL) is a representative chiral molecule that can be used to monitor conformational changes through CD intensity. In addition to chiral AIEgens, new types of chiral AIEgens without TPE have been reported. Figure 11 shows a few miscellaneous scaffolds as AIEgens. 1,4-dihydropuridine derivatives (66) incorporating BINOL with an alkyl chain generated AIE exciplexes to be applicable for chiral recognition. In viscous environments, high water fraction, and solid state, they exhibit high emission intensity at 430 nm, which is the exciplex emission wavelength. The alkyl chain between 1,4-dihydropuridine and BINOL could bend, and protons on the oxygen and nitrogen can interact.
with aromatic rings under viscous conditions, resulting in exciplex emission. In addition, aggregation in the high-water-fractioned mixture could induce a bent conformation, leading to exciplex formation. During these events, the CD signals of their R and S forms were significantly clear, indicating that these exciplex-AIE molecules could be potential probes for chiral recognition. Another example of chiral AIEgens is the conjugation of BINOL with furan and the attachment of electron-deficient phenyl derivatives.\(^{67}\)[104] The inherent distortion of the BINOL scaffold interrupts the intermolecular \(\pi-\pi\) stacking and limits the rotation, which triggers strong emission intensity in aggregates and solid states. The CD spectra proved that the chirality of \(^{67}\) was sustained, demonstrating the feasibility of the cell image. Although the detection of the viscosity changes and cell imaging were exhibited in the reports, these chiral probes have the potential to discriminate and detect chiral biomolecules in biological systems.

Molecular self-assembly is the process of forming organized supramolecular architectures based on \(\pi-\pi\) stacking, hydrogen bonding, van der Waals forces, and hydrophobic effects. Many AIEgens achieve their strong emission through RIR, and the self-assembly process can generate strong intermolecular interactions to restrain their dynamic motions. Thus, it could be a good strategy to develop light-emitting materials and biomaterials using the self-assembly process with AIEgens. Some scaffolds that incorporated TPE into long alkyl chains or amphiphiles have reported the AIE properties of self-assembled structures.\(^{105}\) Other types of scaffolds have also been reported as AIEgens, which feature tripod configurations without typical AIEgens. Tripodal structures are well-known self-assembly moieties that introduce various functional groups and assemble as diverse nanostructures.\(^{106}\) Tripods can control the size and cavity of their supramolecular structures through noncovalent forces, concentration, environmental polarity, and temperature. Some reported tripods that exhibit AIE contained a benzene moiety as a core ring with linked functional groups. Three pyridine carboxamides attached to the benzene core ring give \(^{68}\), which was used as a supramolecular monomer to detect 3-fluorobenzaldehyde(3-FB).\(^{107}\) The \(^1\)H-NMR, MS, and FT-IR results indicated that 3-FB generated intermolecular \(\pi-\pi\) bonds between the F on 3-FB and NH on \(^{67}\), and the H on aldehyde and O on \(^{68}\), resulting in a fluorescent hydrogel (\(\lambda_{em} = 470\) nm). At high temperatures, fluorescence decreased in the solution state. After cooling to room temperature, the solution was transformed to a gel with enhanced fluorescence. The compound \(^{69}\) is composed of a benzene core ring linked to nitrogen mustards (NMs).\(^{108}\) NM, which is a popular DNA alkylation agent for chemotherapy, could affect the anticancer efficiency of \(^{69}\). The fluorescence intensity of \(^{69}\) increased up to 10-fold when the water fraction increased from 50% to 90%. The hydrophobicity of the probe could induce self-assembly with an increase in the water fraction, which resulted in the restricted rotation of single bonds. The aggregates of \(^{69}\) successfully entered the HepG2 cells and accumulated in the nucleus, showing higher toxicity than free NM. Unlike the abovementioned examples, \(^{70a-e}\) contain three ethyl groups and three pyrazole derivatives on the benzene core ring.\(^{109}\) \(^{70a-e}\) showed their AIE by fluorescence enhancement in a high water fractionated mixture, but they generated nanoggregates with irregular morphology. The pyrazole units of \(^{70a-e}\) functioned as hydrogen-bond acceptors to interact with PA, which is a polynitroaromatic compound. Single crystal studies of \(^{70c}\) (PA)\(_2\) explicated all the pyrazolyl groups placed on one side from the phenyl plane to form numerous \(\pi-\pi\) interactions between the phenyl rings of the host. The host–guest complex was stabilized by strong

\[ \text{FIGURE 11} \quad \text{Chemical structures of AIEgens based on numerous miscellaneous scaffolds} \]
H-bonding between the N-atoms of pyrazoles and the phenolic proton of PA, and the π-π interaction between 70c and PA. However, as PA bound to 70a-c, the fluorescence intensity decreased due to the charge transfer from 70a-c to PAs.

In other reports, the triazine moiety, which contains protons to induce steric hindrance and molecular torsion, was used as a core ring instead of a benzene ring. Garzon-Ruiz et al. reported star-shaped molecules based on 1,3,5-tris(styryl)-benzene(70a) and tris(styryl)-s-triazine cores (71b). Compared to benzene rings, a triazine ring has no protons on the ring, which can reduce the steric hindrance and torsion. The compound 71a showed AIE in the water/ACN and water/THF binary systems with water fractions of 40% and 50%, respectively. The 71a formed X aggregates and exhibited similar absorption and emission spectra. It is known that E/Z isomerization, which can affect the emission properties, easily occurs in styril compounds. However, once the stacking aggregates were formed, E/Z isomerization could be blocked and luminescence was increased. The quantum yield of 71b was lower than 3% in organic solvents, but was increased up to 11% with a water fraction of 80%. The faint luminescence of 71b in the free molecular state in solutions was because the planarity of the structure was lost in the excited state. However, the aggregates in the mixture of water and organic solvents would interrupt their torsion to lose planarity, resulting in luminescence enhancement. Miljanic et al. confirmed that tripodal compounds (72a and 72b) generated self-assembled architectures and AIE through fluorescence changes in low temperature, high pressure, and viscous solvents. Assemblies 72a and 72b, which show emissions at 382 and 371 nm, respectively, can dissolve in solvents without decomposition. The emission of 72a increased with an increase in the water fraction, whereas 72b has a central benzene ring and its emission was already “ON.” The differences in these phenomena are ascribed to their different intramolecular dynamics in dilute solutions. The energy barrier of intramolecular rotation along the C-C bond between the central ring and three branches in 72b is 2.6 times higher than that in 72a, because of the steric repulsion between H on the central benzene ring and the fluorines. Thus, 72b has the more rigid and restricted structure, which triggered the constantly “ON” state of 72b. On the other hand, the dynamic intramolecular rotation of 72a generated fluorescence in the water-containing solution, indicating that 72a showed AIE.

Macrocyclic AIEgen is one of the new AIEgen classes, which contains size-selective cavities to control their host–guest interactions and can be applied for molecular sensing, separation, chemical storage, catalysis, and so on. Pillararenes are representative macrocyclic hosts for supramolecular chemistry and are used for host–guest assembly, owing to their facile synthesis and molecular modifications. In contrast to the reported strongly fluorescent macrocycles, such as aza/bora-cyclophanes, pillar[5]arenes show weak luminescence in solutions because they feature carbon-bridged alkoxy/hydroxy benzenes that can flip and induce nonradiative decay in solution. In 2017, pillar[5]arene, which was modified with an acrylhydrozone group and two long alkyl chains (73), was reported as a fluorescent gelator to detect iodide ions under gel conditions. Pillar[5]arene would function as a fluorescent group and self-assembly site through CH···π and π–π interactions, and two long alkyl chains interact with pillar[5]arenes with van der Waals forces. The acrylhydrozone group functioned as a self-assembly site and an anion-binding site, forming HBs. In cyclohexanol, 73 could be assembled into a supramolecule to generate a fluorescent organogel, whereas it was nonfluorescent in the hot solution. Upon addition of the iodide anion, the fluorescence intensity decreased without gel-sol phase changes. The sustained gel phase was attributed to the strong π–π interactions and van der Waals forces, despite the iodide anion binding to the acrylhydrozone moiety, destroying the H-bonds. Another modified pillar[5]arene (74) was also demonstrated to exhibit fluorescent enhancement in the aggregate and solid states. The derivative 74a obtained a high quantum yield efficiency of 19% in the solid state and 13.2% in EtOH/CH₂Cl₂ (fEtOH = 98%). Based on the XRD analysis, there is no strong π–π stacking in nonplanar macrocycles, but multiple intermolecular CH···O and CH···C interactions enabled the macrocycles to anchor to each other and restrict the intramolecular flipping of the ring structures. The aggregate and solid states of 74a could detect Fe³⁺ with a limit of detection of 24.6 µM. Fe³⁺ induced the disassembly of 74a, which was explained by a decrease in the diameter of the aggregates upon the addition of Fe³⁺. The disassembly of the aggregates as well as pronounced spectral overlap between Fe³⁺ and 74a resulted in emission quenching of the 74a aggregates and solid. Finally, pillar[5]arene derivative has been reported to be a new class of AIE genes for luminescent detection. Given that the self-assembled AIEgens and macromolecule AIEgens induced their emission based on their assembled structures through environmental forces, such as viscosity, temperature, hydrogen-bonds, or the interaction with analytes, it is certain that this type of AIEgens will be applicable to study biological system.

4 | DRUG AGGREGATES

Many classes of drug molecules are hydrophobic and have poor solubility in aqueous media. The limited water solubility of hydrophobic drug molecules results in their propensity to form aggregates in solution via a myriad of self-association reactions. Thousands of compounds have been reported to form colloidal particles. Because the aggregate interferes with the signal for various assays in the drug discovery process, colloidal drug aggregates are generally considered to hinder the drug discovery process. Therefore, many efforts have been made to combat the colloidal aggregation of small molecules during drug discovery. However, recent studies have revealed the potential advantages of colloidal drug aggregates.

In 1982, Chaires et al. reported the aggregation of daunomycin, a potent anthracycline antibiotic widely used in cancer treatment. Using visible absorbance, sedimentation equilibrium, and proton NMR measurements, the authors characterized the aggregation of the inhibitor. The concentration dependence of the visible absorbance spectrum of daunomycin convinced the authors of the self-association of daunomycin. Besides, the previous dimerization model could not explain the results of the sedimentation equilibrium experiment, indicating higher aggregation formation. The concentration dependence of the proton NMR spectrum of daunomycin in D₂O was confirmed.
High-throughput screening occasionally encounters nondrug-like screening hits, such as noncompetitiveness, minimal structure–activity relationship, and poor selectivity. To understand this peculiar behavior, McGovern et al. investigated multiple compounds. The authors screened the inhibitors from multiple high-throughput screening projects against a variety of targets and found that the inhibitors behave as micromolar inhibitors of several unrelated enzymes (including beta-lactamase, chymotrypsin, dihydrofolate reductase, and beta-galactosidase). Preincubation of the inhibitor with the model enzyme improved inhibition, which was reversible. The inhibition was minimally affected by temperature, whereas it was reduced upon denaturation and addition of bovine serum albumin (BSA). DLS experiments showed that 25 different compounds form submicron particles and the apparent diameter of the particle varies from 95 to 400 nm, which is larger than that of the enzymes (5.0–18.5 nm). Interestingly, in the case of 8-anilino-1-naphthalene-sulfonic acid (ANS), which does not form aggregates, enzyme inhibition was not observed; however, in the case of Congo Red (CR), which forms an aggregate, the nonspecific inhibition of model enzymes was observed. In the additional screening of 30 substances, approximately 20 substances showed a nonspecific inhibition effect, and it was confirmed that most of them showed light scattering at a concentration of 10–500 μM.

McGovern et al. reported on how drug aggregates inhibit their targets. By using centrifugation and gel electrophoresis, the authors found a direct interaction between the drug aggregates and target proteins. For instance, a centrifuged solution of tetraiodophenolphthalein (14PHT) exhibited reduced enzyme inhibition by more than 20-fold against beta-lactamase. In contrast, the inhibition efficacy of nonaggregating molecules, such as ANS, did not change after centrifugation. To understand the mechanism of the inhibition, the resulting pellet from the centrifugation experiment with and without drug aggregates was analyzed using SDS-PAGE. The authors found that centrifuging the protein alone or with nonaggregating compounds did not result in pellet formation, but centrifugation with drug aggregates resulted in protein concentration in the pellet. Observation using an electron microscope confirmed that the protein was adsorbed on the surface of the drug aggregate. Results from fluorescence microscopic studies of the interaction between 14PHT aggregate and green fluorescent protein (GFP) confirmed that GFP accumulated as spots. Low concentrations of nonionic detergents prevent interactions between drug aggregates and proteins, and higher concentrations of the detergent prevent drug aggregate formation.

For a deeper understanding of the inhibition mechanism of colloidal drug aggregates, Duan et al. reported a structural mechanistic study. Taking advantage of the improved homogenization of the coformulated colloidal drug aggregates, a basic structural study of colloidal drug aggregates coformulated with sorafenib and CR (ratio 25:1) was conducted using small-angle X-ray scattering (SAXS), DLS, and multilight image scattering (MALS). The average size of colloidal aggregates was ~33 nm, and the authors found a well-packed internal structure of colloidal drug aggregates with MALS and SAXS studies. Interestingly, colloidal drug aggregates preferentially bind with proteins over DNA or peptides, likely through surface interactions. In addition, colloidal drug aggregates have different binding abilities toward globular ribosomal protein L2 (L2gd), human serum albumin, malate dehydrogenase, and AmpC beta-lactamase.

Ganesh et al. reported the pharmacokinetic consequence of the stability of colloidal drug aggregates in high serum conditions. Because the DLS technique is ineffective in serum conditions due to scattering from serum proteins, the authors developed a fluorescence-based assay by employing cholesterol-modified BODIPY dyes. The hydrophobic nature causes the compound to incorporate readily during colloid formation. Because the photophysical properties of the dyes change depending on the drug aggregate formation, the authors effectively investigated the serum stability of the drug aggregate by monitoring the fluorescence intensity changes of the BODIPY fluorescence resonance energy transfer (FRET) pair. The authors used fulvestrant, a medication used to treat hormone receptor-positive metastatic breast cancer, and investigated the effect of concentration, medium composition, and excipients (ultrapure polysorbate 80, UP80) on the stability of the drug aggregate. A high percentage of the serum causes a higher level of critical aggregation concentration, above which small molecules self-assemble into liquid–liquid phase-separated particles. In addition, the presence of a low percentage of excipients greatly increased the serum stability of drug aggregates. Plasma drug concentrations in tumor-bearing mice were quantified post the intravenous administration of 6 mg/kg fulvestrant as stable colloids (with 0.03% UP80) with a diameter of 201 ± 9 nm (PDI = 0.12 ±/− 0.003). Interestingly, it resulted in a longer plasma retention time than that when a monomeric solubilized-drug solution (w/ 5% UP80) was administered. An additional PK study with the anthracycline prodrug, pentyloxy carbonyl-(p-amino-benzyl) doxazolidinylcarbamate (PPD), stabilized by coaggregation with UP80 and poly(D,L-lactide-co-2-methyl-2-carboxy trimethylene carbonate)-g-poly(ethylene glycol) (PLAC-PEG), resulted in a similar phenomenon. Consequently, this study suggests that serum-stable colloidal drug aggregates indeed influence drug fate and that they may be intentionally designed to do so. It is conceivable that such a colloidal formulation strategy may be adapted to benefit many drugs that aggregate at relevant concentrations.

Over 50% of the drug targets are membrane-bound receptors. Sassano et al. explored the effects of the colloidal aggregation of drug molecules on GPCR activity. The authors found that four different drugs, including clotrimazole, itraconazole, quercetin, and TIP, nonspecifically inhibited model GPCRs, such as CCR4, CX3CR1, and vasopressin 2 receptor (V2R), with IC50 values in the micromolar range, above known CAC values of the compound. Centrifugation of the drug solution weakened the inhibitory effects, which is consistent with the colloidal mechanism of drug aggregates. In addition, the Tween-80 detergent treatment not only disrupted colloid formation but also diminished the inhibitory activity of all four inhibitors against beta-arrestin recruitment assay and Ca2+ mobilization. The authors confirmed that when the drug colloid and peptide were incubated together, the size of the colloid increased and the inhibition effect of the drug decreased. In contrast, four drug aggregates inhibited basal GPCR activity in the absence of an agonist. Collectively, the authors proposed two mechanisms for the GPCR inhibition of drug aggregates: the sequestration of peptides and...
via nonspecific association with drug aggregates with the peptide ligand, and inverse-agonism through direct binding of the drug aggregates to the receptors.

Blevitt et al. described the X-ray structure of aggregation-based inhibition of a protein–protein interaction involving tumor necrosis factor α (TNFα). To monitor the complex formation of the soluble form of TNFα with the soluble ectodomains of TNFR1 and TNFR2, the authors used TR-FRET between donor (Tb) and acceptor (d2) fluorophore pairs on TNFα and the receptor, respectively. In the assay, the apparent IC50 values for the inhibition of complex formation by the small-molecule inhibitor (JNJ5252) were in the micromolar range, and detergents, such as Triton X-100, abrogated the inhibitory effect of the inhibitor. Further proof of aggregate formation convinced the authors that the aggregate-induced inhibition mechanism of the compound was used for the interaction between TNFα and the receptors. X-ray analysis revealed that the compound forms an aggregating conglomerate that competes for a protein subunit of the TNFα trimer and induces a change in the quaternary structure. Consequently, the aggregate formation of the inhibitor inhibits the protein–protein interaction between TNFα and its receptors by inducing a quaternary structure switch of TNFα, which is disrupted upon induction by the aggregate.

McLaughlin et al. reported a strategy to stabilize colloidal aggregates of drug molecules. The authors studied the colloid formation of seven known aggregators, including chlorotrianisene, fulvestrant, nilotinib, lapatinib, sorafenib, tetraiodophenolphthalein (TIPt), and vemurafenib, with and without the addition of two bis-azo dyes, CR and Evans Blue (EB). Interestingly, colloid formation of the known aggregators with CR or EB generally resulted in a homogenous colloid (polydispersity < 0.2) solution with smaller particles, with an average size of 100 nm. In addition, colloids of sorafenib:CR, vemurafenib:CR, and TIPt:CR formed stable small particles (<50 nm). The authors investigated the effect of concentration and drug:dye ratio on colloid size and stability, and found that a parent compound concentration of 500 μM and a 25:1 molar ratio between the drug and dye resulted in the formation of stable, coformulated colloids with small particle sizes (80 ± 8 nm). Incubation of AmpC β-lactamase (AmpC) with sorafenib:CR and sorafenib:EB inhibited the enzyme activity, but preincubation with the BSA protein reduced the enzyme inhibition ability of the colloids. The authors also found that an increase in protein concentration had a minimal effect on the stability and size of the molecules. Compared to single compound colloids, the colloid–protein complex could be purified by centrifugation and sustained enzyme inhibition after 72 h of purification. Interestingly, enzymatic activity was fully restored after the addition of Triton X-100 (TrX, 0.1%).

Shamay et al. reported on the use of indocyanine-stabilized drug nanoparticles as a targeted drug delivery system. First, the authors found that indocyanine IR783 allowed for the formation of sorafenib and paclitaxel nanoparticles at equimolar concentrations compared to other tested excipients, including sodium dodecyl sulfate, sodium dodecylbenzene sulfonate, sodium deoxycholate, poly-4- styren sulfonate, lignin sulfonate, dextran sulfate, Brilliant Blue G, CR, EB, acid green 5, phthalocyanine tetra sulfonate, RA, and chromaxane cyanine R. Absorbance spectroscopy revealed a relative increase and decrease in the IR783 λmax at 780 nm, and at the 640 nm peak, the results were consistent with the dissolution of the indocyanine H-aggregates. In addition, the bathochromic shift of the λmax 780 nm peak to 850 nm suggests the formation of J-aggregates. Using retrospective QSAR, the authors found four different molecular descriptors with a Pearson correlation coefficient greater than 0.85, which is highly correlated with the experimental data for nanoparticle formation. Two INPs encapsulating the kinase inhibitors of sorafenib and trametinib were further prepared with indocyanine IR783. The INPs were 80 and 55 nm in diameter and exhibited drug loadings of 86% and 83%, respectively. Inhibitors of caveolin-mediated endocytosis and CAV1 protein knockdown, the main protein scaffold of caveolae, significantly attenuated nanoparticle uptake in multiple cell lines; differential uptake was observed across cell types. INPs were intravenously injected into the mice, and pharmacokinetics and biodistribution were monitored using bioimaging experiments. In healthy mice, INP accumulation in the liver was observed within 20 min after the injection and an increased accumulation in the lungs was observed between 0 and 24 h. At 24 h, a low signal was observed in the liver and spleen. Interestingly, tumor tissue-specific localization was observed with sorafenib-encapsulated indocyanine nanoparticles in an MYC-driven murine hepatic tumor model, and the authors claimed that CAV1 expression mediated a specific uptake mechanism. Surprisingly, both INPs encapsulating sorafenib and trametinib exhibited striking anticancer therapeutic effects.

## 5 | BIOMEDICAL APPLICATIONS OF MOLECULAR NANOAGGREGATES

This section provides an overview of the applications of supramolecular aggregates in biomedical imaging and therapy. We discuss nano-sized aggregates used in imaging and therapeutic purposes, with a focus on in vivo utilization. In addition, we describe supramolecular aggregates with their biomedical meanings.

### 5.1 | Nanoaggregates for imaging purposes

Nano-sized supramolecular aggregates are valuable for biomedical applications, and have optimal properties for in vivo utility, including enhanced cell permeability, passive tumor targeting due to the enhanced permeability and retention (EPR) effect, convenient surface engineering for multiple bioconjugations for targeting, and delivery of therapeutic payloads. While fabricating a nanoaggregate, proper choice of the particulate matrix material is vital to achieving an appropriate balance between the hydrophilic matrix and hydrophobic molecule, producing stable and high-performing self-assembly suitable for in vitro and in vivo applications. Nanoaggregates of the AIE fluorophores have been applied to produce intense fluorescence signals for biomedical fluorescence imaging. Despite the advantages of fluorescence imaging, such as high sensitivity, low cost, multiple parameters (intensity, wavelength, and lifetime), and multiplexing possibilities for molecular imaging, it is primarily used for small-animal studies rather than clinical analyses. Photon-limiting interferences (scattering, absorption,
and autofluorescence) occur in biological media. For minimal photon-limiting interference in biological conditions, NIR absorbing and emitting fluorophores have been studied because NIR interferes less with biomolecules. In addition, efforts have been made to develop intense bright fluorophores that can overcome the autofluorescence background from biological conditions. Fluorescence brightness is given by the number of photons detected from an individual fluorophore, and is proportional to the extinction coefficient and fluorescence quantum yield. In such absorption-limited in vivo conditions, fluorescence brightness is determined by the absorption capacity of a fluorophore, which is affected by the extinction coefficient and fluorophore concentration. Most organic fluorophores lose their emission and absorption at high concentrations. However, AIEgens generally enhance adsorption in concentrated or aggregated states, resulting in intense emissions, unlike most organic fluorophores at high concentrations. Theoretically, the fluorescence signal output from individual AIE nanomaterials is proportional to the fluorescence efficiency and loading density of the embedded AIE fluorophores.

The derivatives of the AIEgen \( \pi \)-cyanostilbene, conjugated from 5 to 83 mol% on biocompatible glycol chitosan, formed densely concentrated self-assemblies that showed changes in optical properties, absorption enhancement, and emission shift to NIR in water. By increasing the loading amount of the AIEgen into a proper matrix, a sufficiently bright fluorescent signal of the AIE nanomaterials was achieved, which was a breakthrough for in vivo use.\[128a\]

In addition, AIE cyanovinylene-backboned \( \pi \)-conjugated polymer nanoparticles produced by in situ colloidal Knoevenagel polymerization showed NIR bright emission, colloidal/chemical stability, and mesoscopic size range that allows real-time sentinel lymph mapping in a mouse model.\[128b\]

Recently, it was reported that far-red/NIR-emitting AIE-encapsulated phospholipid nanomaterials were excited by the NIR-II laser.\[129\] To reduce bio-interference with light and to obtain intense fluorescence, three-photon excitation with NIR-II was applied. The lipid nanomaterials showed excellent stability in an aqueous medium with high lipid-targeting specificity, enabling efficient in vivo labeling and imaging of lipids in lipid-rich and deep tissues, such as fatty liver, atherosclerotic plaque in brain vasculature, and carotid arteries.

Chemiluminescence (CL) is the emission from a fluorophore excited by the energy generated from a chemical reaction without photoexcitation. It offers ultra-high sensitivity because the biological background does not generate autofluorescence, which causes interference, by photoexcitation. The well-known peroxalate-based CL (POCL) reaction is the oxidation of peroxalate by hydrogen peroxide (\( \text{H}_2\text{O}_2 \)) to produce an electronically excited 1,2-dioxetanediene intermediate, which subsequently transfers its energy to an emitting dye that generates fluorescence by purely chemical excitation. POCL has been adopted for the sensitive and selective in vivo imaging of \( \text{H}_2\text{O}_2 \) related to biological events, including signaling pathways and diseases.

Advanced applications of POCL-based nanoparticle systems have been reported to sense ultra-low levels of hydrogen peroxide in vivo.\[130\] The critical approach is the nanoparticulate integration of a highly condensed peroxy-oxalate fuel and NIR-emissive fluorophore. Highly concentrated fuel molecules and colocalized energy-accepting dyes in the nanoscopic space are anticipated to maximize the sensitivity of the intraparticle CL reaction toward external \( \text{H}_2\text{O}_2 \). The design was an aqueous formulation of biocompatible Pluronic (F-127) nanoparticles encapsulating bis[3,4,6-trichloro-2-(pentyloxy)carbonyl]phenyl \( \text{oxalate} \) (CPPO) as a concentrated POCL fuel with high reactivity to \( \text{H}_2\text{O}_2 \), a small amount of \( 3,3' \)-diethylthiadicarbocyanine iodide (Cy5) as an NIR dye, and poly(lactic-co-glycolic acid) (PLGA) as a biocompatible polymeric binder for tissue penetration. Stable homogeneous dispersion with nano-sized precipitates suggests that all the hydrophobic components (CPPO, Cy5, and PLGA) were embedded and aggregated in the hydrophobic interior of the self-assembled nanostructure of F-127. It achieved high contrast in vivo imaging in an early stage of the inflammation model of bilateral ankles and near the tumor region.

However, this POCL nanoreactor approach has an inherent limitation in that the local concentration of dyes coloaded in the nanoreactor is limited to prevent the typical quenching of molecular fluorescence at high concentrations or in the aggregated state. The inevitably low loading of emitting dyes in this POCL nanoreactor results in a limited emission output from the peroxalate-concentrated nanoreactor. To resolve this limitation, it was reported that an AIEgen was employed in a nanoparticulated POCL system.\[131\] Moreover, nanoparticles concentrated with AIEgen offer efficient intraparticle AIE energy transfer when codoped with a low-energy acceptor, which provides a simple way of tuning the CL color into a longer-wavelength optical window for in vivo imaging. Because the chemical structures of emitting fluorophores in the POCL reaction need to be energy matched with the chemically excited intermediate of peroxalates, their chemical structures need to be engineered to satisfy NIR emission enhancement, and energy matching is critical for molecular design. This imposes complexity on the molecular design for optimal NIR POCL performance. Nanophotonic energy relay bridges the energy gap between the peroxalates and the new emitter. The POCL energy matching is boosted by codoping another photonic molecule that relays the chemically generated energy to the polymer aggregates. The codoped "relay molecule" plays the role of a nanophotonic bridge that effectively accepts the energy from the chemically excited intermediate of peroxalates, and relays it to the polymer aggregates in the same nanoscopic space for consecutive intraparticle energy transfer.

They tested the CL brightness of nanoparticles formulated with highly condensed CPPO as a CL-emitting energy fuel and a 9,10-distryrylanthracene derivative as the AIE dye into the hydrophobic interior of Pluronic F-127 as a surfactant. The nanoparticles can be detected at a detection limit close to the normal in vivo concentration of \( \text{H}_2\text{O}_2 \) (\( \approx 10^{-7} \text{M} \)), suggesting that the NPs can detect an abnormal increase in the \( \text{H}_2\text{O}_2 \) level in biological objects. When doping 0.4 wt% of Nile Red to the nanoparticle, the CL color of the nanoparticle was readily tuned from green to red, indicating that the CL energy transfer is efficient owing to the close proximity of the donor/acceptor pair within the same nanoparticle space. A rational nanophotonic method for boosting NIR POCL signals without the complex design of the emitter structure to meet the energy-matching requirement.\[131a\]
Another example of an energy-delay-transfer CL nanosystem was reported with a low-bandgap conjugated polymer as a bright NIR emitter showing AIE and green-emissive BODIPY as an energy gap-bridging photonic molecule. The energy-relayed nanointegration showed intense NIR brightness with an H$_2$O$_2$ sensing signal boosted 50 times, down to $10^{-9}$ M and a high tissue penetration depth (>12 mm) (Figure 12A).\textsuperscript{[131b]}

A chemically modified AIE NIR CL emitter was also reported, which was synthesized by conjugating a luminol unit with triphenylamine-combined benzothiadiazole as the AIE material; subsequently, the aggregates were prepared using F127 as the surfactant. Luminol is a blue-emitting CL material that reacts with H$_2$O$_2$ and generates CL emission through many electron-rich intermediates. Electron-accepting benzothiadiazole-conjugating luminol enhanced its reaction yield. The through-bond energy transfer between activated luminol and the triphenylamine-combined benzothiadiazole AIEgen is more efficient than through-space energy transfer. It exhibited significant NIR CL emission at the penetration depth, with a total thickness of over 3 cm. It is crucial to transfer the blue emission of luminol into the NIR region to enhance the penetration depth and efficiency (Figure 12B).\textsuperscript{[132]}

5.2 | Nanoaggregate modulation photomedicine

Nanoaggregates have been considered as a new way to administer medication and overcome the limitations of conventional molecular drugs. Several types of nanoaggregates...
have been reported to enhance the drug efficacy by changing their pharmacokinetics and biodistributions; here, we discuss the nanoaggregates used in PDT and photothermal treatment (PTT). PDT and PTT have recently attracted attention due to their potential to transform cold tumors into hot ones by enhancing the immune response, and their application in non-specific cases without the specific marker. It is meaningful to overview nanoaggregates for PDT and PTT.

Photosensitizers (PSs) are key to enabling PDT and PTT. Light-activated (photon-absorbed) PS generates singlet oxygen \( (\text{O}_2) \) as a primary cytotoxic source to destroy abnormal cells, which occurs through energy transfer from the long-lived excited triplet state of PS (3PS*) to ambient oxygen \( (\text{O}_2) \). In addition, photon-absorbed PS can make the heat a cytotoxic weapon through an energy relaxation process of activated PS. The ideal PS for in vivo use can first be absorbed NIR to minimize photon-limiting interference in biological conditions and then selectively accumulate in malignant cells.

PSs, widely used and studied clinically, are primarily in the phthalocyanine family, and have a hydrophobic aromatic structure with poor solubility in physiological media. This results in a limitation to the selective accumulation of PS molecules in target cells when applied by systemic administration. PS aggregates in water-dispersible carriers, such as FDA-approved Pluronics and biocompatible glycol chitosan, have been studied. They were shown to improve the PDT and PTT efficacy by preventing undesired PS aggregation and promoting targetability by the EPR effect.[133]

However, PS aggregates at a high concentration in the nanoscopic space have a limitation in PDT application. PS aggregation causes quenching due to the strong \( \pi-\pi \) stacking of PS, resulting in reduced \( \text{O}_2 \) generation efficiency. However, when PS loading is limited to a low amount to prevent PS aggregation, it causes significant dilution of the therapeutic PS in the nanocarrier. Various AIE PSs have been studied to solve this problem. A PS nanoaggregate coencapsulated with highly concentrated iodine atoms in a particular inner matrix was developed to solve this dilemma. Because the population of \( 3\text{PS}^* \) occurs as a result of ISC from the photoexcited singlet PS (1PS*), the quantity of generated \( \text{O}_2 \), which governs the efficacy of PDT, is dependent on the efficiency of ISC (1PS* to 3PS*). The intraparticle heavy-atom effect facilitates the ISC of the photoexcited PS from the singlet state (1PS*) to the long-lived triplet state (3PS*), where the latter photoproduct sensitizes the surrounding molecular oxygen \( (\text{O}_2) \) into \( \text{O}_2 \).

The researchers utilized particulate constituents that are all organic and biocompatible, significantly enhanced \( \text{O}_2 \) generation efficiency, and improved its in vitro PDT efficacy.[134] Pluronic F-126 is a polymeric surfactant, with iodine-rich diatrizoic acid as a core-forming inner matrix, and chlorine e6 (Ce6) encapsulated therein as a PS molecule. Diatrizoic acid (3,5,-bis(acetamido)-2,4,6-triiodobenzoic acid) was chosen as an iodinated aromatic core matrix because it is safe in vivo and is being used clinically as a radiocontrast agent for X-ray imaging and computed tomography. Furthermore, the researchers designed a novel iodinated chitosan-backboned conjugate (GC-I-Ce6) that was prepared to fabricate self-assembled biopolymeric nanoparticles with heavy-atom-effected enhanced singlet oxygen generation as well as biological merits.[135] The self-assembled GC-I-Ce6 nanoparticles have enhanced the capability of singlet oxygen generation by the intraparticle heavy-atom effect, along with high tumor targetability in vitro and in vivo owing to the glycol chitosan surface exterior with biocompatible, positively charged, and tumor-homing characteristics. Actual efficacy improvement in the PDT of a human breast cancer cell line (MDA-MB-231) with any nonspecific tumor marker demonstrated the potential of photophysically and pharmacologically motivated hybrid bioconjugate approaches for nanomedicine applications.

The most common side effects of PDT and PTT are sensitivity to bright light and sunlight. These reactions caused by PDT light can appear on the skin where the drug is applied. After treatment, patients must not expose the treated areas to light for a certain period, and are required to stay in a dark room during excess PS clearance. Attempts to counter the side effects of PDT and PTT have been made.

Recent progress in AIE PS has focused on increasing the targetability of PS. For example, bacteria that hide in the host phagocytes are challenging to kill, and can cause phagocyte disorders resulting in local and systemic tissue damage. A theranostic AIE PS-based nanoprobe with hypochlorous acid (HClO) activatable PS was developed and fabricated by encapsulating DTF PSs and HClO-sensitive molecules, and FFP together using a highly biocompatible Pluronic F-127 as the matrix.[136] Macrophage primarily generates HClO as one of the cytotoxic substances against bacterial infection. Owing to the FRET between DTF and FFP, FFP can quench both fluorescence and singlet oxygen generations production in DTF; thus eliminating the phototoxicity of DTF-FFP NPs in normal cells and tissues even under irradiation conditions. Once delivered to the infection sites, DTF-FFP NPs light up with red fluorescence and efficiently generate ROS owing to FFP degradation by the stimulated release of HClO in phagocytes. The selective activation of fluorescence and photosensitization was successfully confirmed by both in vitro and in vivo results, demonstrating the effectiveness and theranostic potential of DTF-FFP NPs in precise bacterial therapy (Figure 13).

Depending on their chemical properties, AIE PS can initiate and accumulate damage in different cellular compartments, such as the mitochondria, lysosomes, endoplasmic reticulum (ER), plasma membrane (PM), and lipid droplets (LDs). However, these PSs cannot distinguish specific cells from the surrounding cells; for example, cancer cells from the surrounding immune cells in a tumor microenvironment (TME). The researchers found that LDs accumulate in various cancer cells in the TME, developing AIE PS nanoaggregates to target cytoplasmic lipid-enriched organelles. In addition, by coating with a dendritic cell membrane that T cells recognize, AIE PS nanoaggregates can cross the biological barrier and efficiently accumulate around the tumor by hitchhiking on T cells.[137]

Glioblastoma is extremely difficult to treat compared to other tumors. Recently, PTT has demonstrated advanced therapeutic efficacy; however, its application in deep-seated tumors remains challenging because laser light has relatively low tissue penetration efficiency. Researchers have designed a bradykinin (BK)-conjugated AIE nanooaggregates, which offers selective penetration through the blood–tumor barrier (BTB) and strong absorbance of NIR.[138] The BK ligand prompts the BTB adenosine receptor activation, resulting in
AIE nanoaggregate transportation and accumulation inside tumors. The nanoaggregates exhibited high photothermal conversion efficiency under 980 nm NIR laser irradiation, facilitating the treatment of deep-seated tumors. It was observed that the PTT treatment of GBM-bearing mice activated natural killer cells, CD3+ T cells, CD8+ T cells, and M1 macrophages in the GBM area increased the therapeutic efficacy.

Cancer cells have been reported to resist PDT by overexpressing B-cell lymphoma 2 (Bcl-2), which yields a higher cellular concentration of glutathione (GSH), shifting the intracellular redox potential to a more reduced state. GSH reacts with the ROS generated from PDT, resulting in reduced PDT efficacy. Hybrid nanoaggregates containing Fe3+, AIE PS, and Bcl-2 inhibitor of sabutoclax were constructed via coordination-driven self-assembly in aqueous media. The Fenton reaction by Fe3+ increased the PDT efficacy by increasing the intracellular O2 concentration. Applying a Bcl-2 inhibitor helped to reduce intracellular GSH to facilitate cancer cell apoptosis and mitigate the PDT resistance of AIE PS. The design of the multifunctional hybrid nanospheres demonstrated a prospective nanoplatform for image-guided enhanced PDT of tumors.\[139\]

5.3 | Supramolecular therapeutics

The supramolecular assembly of peptide-based monomers into supramolecular structures offers many promising applications in advanced therapies.

The general practice of PDT comprises repeated multiple sessions, where PS is repeatedly administered before each operation of light irradiation. Consequently, potential problems arise from the total overdose of PS by repeated injections. Researchers designed an RGD peptide derivative conjugated with PS and a quencher (Ppa-iRGDC-BK01), whose solution formed a self-assembled depot by a single injection in vivo. The hydrophobic interaction of the Ppa pendants and the reorganization of the interpeptide hydrogen bonding of Ppa-iRGDC-BK01 give rise to a stable depot under biological conditions. It showed a multiple-quenching effect on photosensitivity to prevent nonspecific phototoxicity and photobleaching of PS. It is activated by the sustained release, tumor targeting, and tumor-selective proteolytic/reductive cleavage of the iRGD segment. This enables autonomous tumor photosensitization by sustained release, tumor accumulation, and intratumoral activation over time. During repeated PDT, depot-implanted normal tissues around the tumor exhibited
no phototoxic damage under laser exposure. This approach can be a safer and more precise operation of PDT through a nonconventional protocol composed of a one-time photosensitizer injection and multiple laser irradiations.\(^{[140]}\)

Chirality plays an essential role in the development of some chiral drugs. While one enantiomer of a chiral drug may be therapeutic, another enantiomer of the molecule may be inactive. In peptides, L- and D-isomers often result in different shapes of self-assembly. When L- and D-isomers are combined, they often show enhanced properties compared to their enantiomeric analogs, such as faster kinetics of formation, higher mechanical strength, and enzymatic stability. To monitor the occurrence and consequences of the heterochiral assembly of peptides in the cellular microenvironment, precisely the mitochondria of cancer cells, mitochondria penetrating tripeptides containing a diphenyl alanine building unit (Mito-FF) were designed. The short peptide amphiphile Mito-FF coassembled with its mirror pair, Mito-ff, to induce superbifibrils of approximately 100 nm in diameter and 0.5–1 \(\mu\)m in length, whereas its enantiomers formed only narrow fibers of 10 nm in diameter. The coadministration of Mito-FF and Mito-ff in the cell induced drastic mitochondrial disruption both in vitro and in vivo. Experimental and theoretical analyses revealed that pyrene capping played a major role in inducing superbifil morphology upon the coassembly of racemic peptides. This work showed the impact of chirality control on peptide self-assembly inside a biological system, thus demonstrating a potent strategy for fabricating promising peptide biomaterials by considering chirality as a design modality.\(^{[141]}\)

Chirality is considered to produce a self-assembled DNA nanostructure with precise size and shape control for tailored uses. Self-assembled mirror DNA (L-DNA) tetrahedron nanostructures have been developed to resolve the poor serum stability of natural D-DNA. The nanostructures of L-DNA show thermodynamic properties identical to those of natural D-DNA with significantly enhanced stability in serum. This unique characteristic has a significant effect on the pharmacokinetics and biodistribution of DNA nanostructures. It was demonstrated that the mirror DNA nanostructures could deliver anticancer drugs selectively to tumors with enhanced cellular and tissue penetration and show more significant anticancer effects than conventional PEGylated liposomes. This approach provides an alternative strategy for tumor-specific delivery of anticancer drugs and highlights the promising potential of mirror DNA nanostructures as a novel drug delivery platform.\(^{[142]}\)

Morelli et al. disclosed new mixed aggregates with improved properties and examined them as promising target-selective nanovectors of drugs. The new nanovector was accomplished by mixing the monomers. The two alkyl chains in both monomers induced the formation of aggregates, whereas the CCK8 peptide and the anionic DTAPGlu chelating part were exposed on the external surface of the nanovectors. Based on experimental studies, they found that the monomers are prone to form diverse aggregates with different sizes and shapes. Importantly, the occurrence of the bioactive peptide exposed on the outer surface of the aggregate allows selective targeting of nanocarriers to the cholecystokinin receptors overexpressed by the cancerous cells.\(^{[143]}\)

Li et al. reported a new and highly effective supramolecular self-assembling peptide-based anticancer agent (DBT-2FFGYSA, Figure 14), which contains a fluorophore 4,7-di(thiophen-2-yl)-2,1,3-benzothiadiazole (DBT) and two peripheral peptides FFGYSAYPDSVPMMS (FFGYSA). DBT has an electronic donor–acceptor structure, resulting in fluorescence quenching in an aqueous environment but fluorescence amplifying in less polar conditions than water. Two aromatic phenylalanines (FF) are an excellent self-assembly aiding unit to enhance the self-assembly property of peptides. YSA peptide sequence binds specifically to transmembrane receptor tyrosine kinase Eph receptor A2 (EphA2), which is overexpressed in many cancers and plays a significant role in promoting cancer malignancy. DBT-2FFGYSA was able to achieve the real-time image of EphA2 receptor and selectively killed EphA2 receptor-overexpressed cancer cells both in vitro and in vivo, but is nontoxic to normal cells and organs. DBT-2FFGYSA formed a large aggregate on the specific EphA2 receptors, and this aggregate subsequently favored activating EphA2 tyrosine phosphorylation and antitumorigenic signaling. DBT-2FFGYSA was also an effective agent to induce immunogenic cell death and T-cell infiltration. This work demonstrates that supramolecular self-assembly can potentially drive cellular signaling and change the immunological conditions of malignant tumors. This study suggests that we can take advantage of mediating and amplifying homogeneous protein–protein interactions driving cell signaling, which may facilitate the suitable therapeutic management of protein targets (Figure 14).\(^{[144]}\)

Microtubules (MTs) are polymers composed of \(\alpha/\beta\)-tubulin, which have multiple roles involved, for example, in the eukaryotic cellular cytoskeleton sustain, cell division, and intracellular transport, subsequently making attractive molecular targets for cancer therapy and molecular assembly. Zhang et al. reported that the benzylimidazolium-modified antimitic peptide (BP) recognized the MTs and concurrently formed stable inclusion complexes with avirulent cucurbit[7]uril (CB[7]) and cucurbit[8]uril (CB[8]) in different binding strength. The self-assembly morphology of MTs was changed from fibrous to nanoparticulate aggregates by extensive cross-linking between BP and CB[8], leading to significant cell apoptosis and tumor ablation in vivo. The advantage of a combinatorial strategy involving a biocompatible tubulin-targeting agent and controlled supramolecular complexation with orthogonal host–guest and polypeptide–tubulin interactions could strongly affect the biological performance of MTs and discover innovative drugs avoiding the general cytoxicity and multidrug resistance of conventional chemotherapeutic compounds.\(^{[145]}\)

6 | SUMMARY AND PERSPECTIVE

In summary, we have overviewed the recent trends in study of molecular aggregates for biomedical applications. The effect of aggregation on changes in photophysical properties of conventional fluorophores and their biological applications were discussed, followed by the recent trends in the investigation of biologically important analytes with AIE phenomena of conventional and unconventional fluorophores. In addition, effects of conventional drug molecule-aggregates on drug discovery and therapeutics development were discussed. We also discussed nano-sized supramolecular aggregates used in
FIGURE 14  Supramolecular self-assembling peptide systems in cancer theranostics. (A) Chemical structure of DBT-2FFGYSA. (B) Schematic illustration of the aggregate formation of DBT-2FFGYSA on the specific EphA2 receptor and activating cellular signals. (C) Immunogenic cell death (ICD) and T-cell infiltration induced by DBT-2FFGYSA in 4T1 breast tumor-bearing mice. (Reprinted with permission.[144] Copyright 2020, Wiley-VCH)

imaging and therapeutic purposes, with a focus on in vivo utilization.

First of all, J- and H-aggregates phenomenon are elegantly used to manipulate the emission property of the well-known fluorophores namely cyanine and BODIPY. Especially, the introduction of perfluorinated alkyl group, $\pi$-extended aromatic framework and long aliphatic chain to the appropriate position of cyanine fluorophore furnish excellent emission property in NIR-II region. Moreover, the derived cyanine analogues displayed promising applications in in vivo bioimaging and image-guided cancer surgery. Similarly, BODIPY emission property also tuned and enhanced by installing various functional groups like ester, trifluoromethyl, and aminophenol. The functionalized BODIPY is used as ratiometric probe for the selective detection of HOBr and Heparin in the biological system. From these profound interesting features and applications, it is believed that J- and H-aggregates can be easily achieved by introducing suitable functional group and also the fluorophore property will be elevated for the desired bioimaging and sensing applications. Despite the interesting J- and H-aggregates property, there is no clear thumb rule which states that the introduction of specific functional groups will furnish selective fluorescence property. It varies according to the fluorophore and anticipated bioapplications. Therefore, there is a lot of scope and spaces are available to explore this area of research. On the other hand, most of the common fluorophores grieve from ACQ, where fluorophores show strong emission as free molecules but when aggregated, the aromatic rings of the fluorophore go through strong intermolecular $\pi-\pi$ stacking interactions. But AIE strategies are centered on aggregation processes and their emissions are enhanced via aggregate formation. The restriction of their intramolecular motions (RIM) in the aggregate state is the main trigger behind the AIE process. Likewise, AIEgens have an ability to eliminate the ACQ problem without initiating some other side effects. Most of the AIEgens are conventionally established on HPS and TPE moieties but here in this comprehensive review, we attempted to encapsulate some of the nonconventional AIEgens and their potential application in biomedicine field with their working principles. These diverse nonconventional AIEgens include boron-containing stilbenes, different common fluorophores, ESIPT-based fluorophores, heterocycles, Atom(sp3)-bridged, and some miscellaneous scaffolds, which achieved the AIE properties through the various strategies to affect the RIM or stacking modes. These AIEgens can easily be chemically modified for the development of high-tech probes in biomedicine field for numerous diverse functions. These nonconventional AIEgens have attracted much consideration thanks to this structure tunability to design innovative AIEgen for biomacromolecule. However, still there are many challenges persisted in this area to develop better probes. Conquering those challenges will be a big hurdle for researchers to deepen our knowledge in the AIEgens working mechanism and efficiency.

Many of drug molecules exhibit self-aggregation to form colloidal particles. Although colloidal aggregates of drug molecules have been historically regarded as a nuisance artifact in drug discovery process, however, enthusiastic efforts have been continued to exploit their unique properties are changing this outlook. Many studies implicate that the colloidal drug aggregate not only specifically inhibited the cytosolic target protein but could inhibit the receptor proteins...
and even the protein–protein interactions. In addition, the colloidal drug aggregates could be stabilized by using excipients, such as polymers, proteins, and other small molecules, and many studies exhibited their potential use as intentional drug formulations. The stabilized colloidal drug aggregates may provide alternative formulations for high drug loadings than conventional nanoparticle formulations.

The scope of in vivo applications of conventional hydrophobic molecules has been widened through controllable molecular aggregates to enhance solubility, cell permeability, targeting capability, and delivery of therapeutic payloads. Self-assembly with a good balance between hydrophobic and hydrophilic components can achieve stable and high performance in biological conditions. The supramolecular assembly of peptide-based monomers into supramolecular structures offers many promising applications in advanced therapies.

ACKNOWLEDGMENTS

This work is supported by a Korea University Grant (K2110571), the Creative Materials Discovery Program through the National Research Foundation (2019M3D1A1078941), the National Research Foundation funded by the Ministry of Science, ICT & Future Planning (NRF-2017M3D9B29942, NRF-2018M3A9H4079286, NRF-2019R1A6A1A1051471, NRF-2020R1A2C2004422, NRF-2020R1C1C1010044, and NRF-2021R1A2C2005418), and KIST intramural funding.

ORCID

Jeongyoun Heo https://orcid.org/0000-0002-0507-9358
Dhiraj P. Murale https://orcid.org/0000-0002-5743-9415
Hey Young Yoon https://orcid.org/0000-0003-3341-531X
V. Arun https://orcid.org/0000-0003-3341-531X
Yeon Joo https://orcid.org/0000-0002-0568-965X
Eunha Kim https://orcid.org/0000-0002-0766-696X
Jun-Seok Lee https://orcid.org/0000-0003-3641-1728
Sehoon Kim https://orcid.org/0000-0002-8074-1006

REFERENCES

1. H. Zhang, Z. Zhao, A. T. Turley, L. Wang, P. R. McConigal, Y. Tu, Y. Li, Z. Wang, R. T. K. Kwok, J. W. Y. Lam, B. Z. Tang, Adv. Mater. 2020, 32, 2001457.
2. a) S.-J. Yoon, J. W. Chung, J. Gierschner, K. S. Kim, M.-G. Choi, D. Gobec, S. Pecar, A. Zega, Nature Rev. Drug Discov. 2019, 18, 1187;
   b) M. Shirakawa, T. Kobayashi, E. Tokunaga, Appl. Sci. 2020, 10, 18361.
3. J. Mei, Y. Hong, J. W. Lam, A. Qin, Y. Tang, B. Z. Tang, Angew. Chem. 2019, 131, 15328.
4. Q. Zhang, P. Yu, Y. Fan, C. Sun, H. He, X. Liu, L. Lu, M. Zhao, H. Zhang, F. Zhang, Angew. Chem. 2021, 13, 4013.
5. M. Shirakawa, T. Kobayashi, E. Tokunaga, Angew. Chem. 2020, 11, 200161.
6. J. Mei, Y. Hong, J. W. Lam, A. Qin, Y. Tang, B. Z. Tang, Angew. Chem. 2019, 131, 15328.
7. J. Mei, H. Zhang, Z. Zhao, J. W. Lam, A. Qin, Y. Tang, B. Z. Tang, Adv. Mater. 2014, 26, 5429.
8. C. Zhu, R. T. Kwok, J. W. Lam, B. Z. Tang, ACS Appl. Bio Mater. 2018, 1, 1768.
9. J. Mei, C. G. Daniliuc, K. K. Kartha, G. Fernández, G. Kehr, G. Erker, J. Am. Chem. Soc. 2020, 142, 14015.
10. K. I. Lugovik, A. K. Eltyshev, P. O. Suntsova, L. T. Smoluk, A. V. Belousova, M. V. Ulitko, A. S. Minin, P. A. Slepukhin, E. Benassi, N. P. Belskaya, Org. Biomol. Chem. 2018, 16, 5150.
11. S. Zhang, Z. Zhao, H. Zhang, Z. Xue, J. Mack, Z. Shen, X. You, N. Kobyayashi, Chem. Commun. 2016, 52, 18723.
12. J. Mei, Y. Hong, J. W. Lam, L. Cheng, H. Chen, C. Qiu, H. S. Kwok, X. Zhan, Y. Liu, D. Zhu, B. Z. Tang, Chem. Commun. 2001, 37, 7009.
13. J. Mei, Y. Hong, J. W. Lam, A. Qin, Y. Tang, B. Z. Tang, Adv. Mater. 2014, 26, 5429.
AUTHOR BIOGRAPHIES

Jeongyoon Heo received her PhD in Materials Science and Engineering in 2016, from the College of Engineering at Seoul National University (SNU). She was a postdoctoral researcher from 2016 to 2020 at KIST and the State University of New York at Buffalo (SUNY). She rejoined at KIST in 2021. Her research interests are focused on the development of stimuli-responsive nanosystems for molecular imaging, photomedicine, and drug delivery.

Dhiraj P. Murale received his PhD in 2014 from Korean Advanced Institute of Science and Technology (KAIST) Korea working with Prof. David G. Churchill on reaction-based small molecule fluorescent probes for detection of biologically important analytes. After this, he joined Prof. Jun-Seok Lee’s lab at Korea Institute of Science and Technology (KIST) Korea, as a Post-Doc. He is currently a research scientist at Korea Institute of Science and Technology (KIST) Korea. His research interests are focused on chemical biology, protein–protein interaction, and fluorescent probe development. He is the recipient of Korea Research Fellowship (KRF, 2015) from NRF Korea.

Hey Young Yoon obtained her BS degree in Chemistry Department of Korea University and her MS and PhD degrees in Chemistry from Seoul National University, Korea, under the guidance of Professor Jong-In Hong. She is now carrying out her postdoctoral research on chemical biology with Prof. Jun-Seok Lee and her research interests focus on chemical biology and luminescent molecular probe development.

V. Arun earned his PhD (2017) from BITS Pilani-Pilani campus under the guidance of Prof. Dalip Kumar. Subsequently joined as an NPDF fellow (2019) under Prof. Suman De Sarkar in IISER Kolkata. Presently, pursuing Post-doc in Ajou University, South Korea under the guidance of Prof. Eunha Kim. His research interests are developing small fluorescent molecules for bioprobe.
Sang-Kee Choi received his BS degree in 2016 from the Department of Applied Chemistry, Konkuk University, Korea. He is currently a Doctor’s candidate in the Department of Molecular Science and Technology of Ajou University, Korea. His research interests are focused on design and application of fluorescent probes based on aggregation-induced emission.

Eunha Kim is an associate professor at Ajou University. He received his PhD from the Department of Chemistry at Seoul National University in 2011. After postdoctoral work at Harvard Medical School, he began his academic career in 2015 in the Department of Molecular Science and Technology at Ajou University in Suwon. His research interests are focused on the development of novel fluorescence-based smart molecules for applications in the sensing and imaging.

Jun-Seok Lee received his PhD in 2009 from New York University working on a diversity-oriented BODIPY library for bioimaging probe discovery. After this, he joined the Korea Institute of Science and Technology, where he served as a principal research scientist. Currently, he is an associate professor at Korea University College of Medicine, where his research focuses on chemical proteomics and the host–pathogen interaction mechanism. He is the recipient of numerous awards, including TJ Park Posco Science fellowship, Young Investigators Award (KCS), and CSJ Lectureship Award (CSJ).

Sehoon Kim received his PhD in Materials Science and Engineering in 2002, from the College of Engineering at Seoul National University (SNU). After research experiences as a post-doctor at the State University of New York at Buffalo (SUNY), he joined Korea Institute of Science and Technology (KIST) in March 2008. He is currently a Principal Research Scientist and Head of Center for Theragnosis at KIST. He is also affiliated as Professor at KU-KIST Graduate School of Converging Science and Technology (Korea University) and Division of Biomedical Engineering, Korea University of Science and Technology (UST). His research interest is the development of advanced drug delivery system and biophotonic molecular nanoparticles for in vivo theranostic applications.

How to cite this article: J. Heo, D. P. Murale, H. Y. Yoon, V. Arun, S. Choi, E. Kim, J.-S. Lee, S. Kim, Aggregate 2022, 3, e159. https://doi.org/10.1002/agt2.159