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Aggregation-induced emission—fluorophores and applications

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Aggregation-induced emission—fluorophores and applications

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Abstract
Aggregation-induced emission (AIE) is a novel photophysical phenomenon found in a group of luminogens that are not fluorescent in solution but are highly emissive in the aggregate or solid state. Since the first publication of AIE luminogens in 2001, AIE has become a hot research area in which the number of research papers regarding new AIE molecules and their applications has been increasing in an exponential manner. Thomson Reuters Essential Science Indicators ranked AIE no.3 among the Top 100 Research Frontiers in the field of Chemistry and Materials Science in 2013. In this review, I will give a general introduction of the AIE phenomenon, discuss the structure-property relationship of the AIE luminogens and summarize the recent progress in the applications including as light-emitting materials in optoelectronics, as chemosensors and bioprobes, and for bioimaging (total 69 references cited).

Abbreviations

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ΔΨ_m</td>
<td>Mitochondrial membrane potential</td>
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<tr>
<td>η_edge</td>
<td>Optical edge efficiency</td>
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<td>Φ_F</td>
<td>Fluorescence quantum yield</td>
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<tr>
<td>ACQ</td>
<td>Aggregation-caused quenching</td>
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<tr>
<td>AIE</td>
<td>Aggregation-induced emission</td>
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<tr>
<td>CCCP</td>
<td>Carbonyl cyanide 3-chlorophenylhydrazone</td>
</tr>
<tr>
<td>CO_2</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>DNT</td>
<td>2,4-dinitrotoluene</td>
</tr>
<tr>
<td>FLIM</td>
<td>Fluorescence lifetime imaging microscopy</td>
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<tr>
<td>G</td>
<td>Geometry factor</td>
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<tr>
<td>GFP</td>
<td>Green fluorescent protein</td>
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<tr>
<td>HPS</td>
<td>Hexaphenylsilole</td>
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<tr>
<td>LSC</td>
<td>Luminescent solar concentrator</td>
</tr>
<tr>
<td>MOF</td>
<td>Metal-organic framework</td>
</tr>
<tr>
<td>NB</td>
<td>Nitrobenzene</td>
</tr>
<tr>
<td>OFET</td>
<td>Organic field-effect transistor</td>
</tr>
<tr>
<td>OLED</td>
<td>Organic light-emitting diode</td>
</tr>
<tr>
<td>PMMA</td>
<td>Poly(methyl methacrylate)</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TPE</td>
<td>Tetraphenylethene</td>
</tr>
<tr>
<td>TRAP</td>
<td>Telomeric repeat amplification protocol</td>
</tr>
<tr>
<td>VOC</td>
<td>Volatile organic compound</td>
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1. Introduction
It is a common observation that the aggregation of chromophores leads to the quenching of fluorescence. Conventional fluorophores, such as fluorescein, are usually fluorescent when they are in dilute solution (figure 1(A)). Their fluorescence is decreased or quenched when the concentration is increased [1, 2]. Most of these conventional fluorophores adopt flat disc-like shapes, which would easily undergo strong π stacking interactions when they are in close proximity in the concentrated solution or aggregate state [3]. In such situation, the excited states often decay via nonradiative decay pathways, which is known as the aggregation-caused quenching (ACQ) of light emission.

The ACQ effect hampers the applications of conventional fluorescent materials in many aspects [4]. For optoelectronic applications such as organic light-emitting diodes (OLEDs), organic field-effect transistors (OFETs) and luminescent solar concentrators (LSCs), the luminescent materials are used as thin solid films and crystals. Dye aggregation caused reduction of the fluorescence quantum yield in the solid state becomes one of the major obstacles in the development of efficient optoelectronic devices. Because of the ACQ effect, researchers often study and utilize fluorophores as isolated molecules in very dilute solutions [5–7]. Emissions from dilute solutions are often weak, resulting in poor sensitivity in fluorescence sensory systems, especially in bioassays of trace amounts of analytes. The sensitivity cannot be improved by simply increasing the dye to analyte concentration due to the ACQ effect [8]. The small number of the dye molecules in dilute solutions can also be quickly photobleached under the excitation of harsh light source in fluorescence imaging,
leading to poor photostability for long-term imaging and tracing [9]. Moreover, the ACQ dyes with highly planar conjugated structure also exhibit small Stokes shift and suffer from reabsorption of the dye emission, further reducing their emission efficiency [8].

Aggregation-induced emission (AIE) is a unique photophysical phenomenon associated with chromophore aggregation [10]. Instead of fluorescence quenching, the emission of luminogens with AIE characteristics is turned on when they are aggregated. Tetraphenylethene (TPE) is one of the archetypes of AIE luminogens [11]. In contrast to most ACQ molecules, TPE is in propeller shape with four peripheral phenyl rings (rotors) twisted out of the central olefin (stator) [12]. In solution state, the phenyl rings undergo active intramolecular rotational/twisting motions alongside the C–C bonds against the stator upon excitation. Such dynamic motions would serve as a nonradiative decay pathway for the excited species and as a result, almost no fluorescence can be discerned for the isolated TPE molecules in a dilute solution (figure 1(B)). The addition of large amount of poor solvent, e.g. water, into the solution would force the hydrophobic molecules to aggregate. Once aggregated, the intramolecular motions would be restricted owing to the physical constraint. Meanwhile, the highly twisted structure also prevents strong π–π interactions in the aggregates [13]. Collectively, the TPE molecules exhibit strong fluorescence upon aggregate formation and in the solid state. In addition to aggregate formation, the restriction of intramolecular motion process could also be realized when an individual AIE molecule is immobilized upon binding to certain substrates. This process could be utilized in the design of chemosensors and bioprobes [14].

Although a few molecules with similar characteristics have been reported sporadically, the concept of AIE was first proposed by Tang et al in 2001 [15] and systematic investigation on the mechanistic understanding and applications of the AIE luminogens has been carried out since then. The intriguing photophysical behaviors and the practical implications of AIE materials have attracted many people in different fundamental and applied research foci to engage in the AIE area. As a result, a large amount of publications have been emerging on the design of new AIE luminogens, decipherment of the mechanism and expansion of their applications [10, 16–18]. A couple of review articles have been published in the past few years that focus on different aspects of AIE research, including a comprehensive review recently published in Chemical Reviews [16]. This topical review will provide a general introduction of fluorophores with AIE feature, decipher the underlying mechanism through interpretation of the structure-property relationship and demonstration of their advanced applications in optoelectronics, as chemosensors and for bioimaging. Due to the limit of article length, this review will mainly focus on the most recent examples that have not been covered in the previous reviews.

2. **Fluorophores**

The discovery of the AIE phenomenon was based on the observation of silole derivatives [15]. Hexaphenylsilole (HPS) is the most ‘historic’ and well-studied AIE molecule [18]. HPS is hydrophobic and soluble in most organic solvents. The THF solution of HPS is faintly fluorescent with the fluorescence quantum yield ($\Phi_F$) as low as 0.22%. The solution remains weakly emissive even in the presence of up to 70% of water in the solvent mixtures (figure 2). When the water fraction increases to 80%, the HPS molecules start to aggregate and become fluorescent. The $\Phi_F$ value boosts to ~56% in 90% water fraction and reaches as high as 78% in the
solid thin film. Crystal structure analysis reveals that HPS is a propeller-shaped non-planar molecule with the dihedral angles of ~30° for the phenyl rings on the 2,5-positions and ~70° for the phenyl plates on the 3,4-positions of the central silole core. Such twisted conformation leads to large interplane (~10 Å) and intermolecular (~7.6 Å) distances and thus prevents strong π interactions that result in nonradiative relaxation of the excited states [19].

Similar as HPS, hexaphenylbenzene (1) [20], pentaphenylpyrrole (2) [21], tetraphenylthiophene (3) [22], and pentaphenylphosphole oxide (4) [23] all adopt propeller shaped conformation and are found to be AIE-active (figure 3). In the solution state, the phenyl rings tethered at the central aromatic core of these molecules could undergo intermolecular rotational/torsional motions and thus deactivate the excited state of the molecule via nonradiative pathway. To verify this hypothesis, a series of experiments and theoretical calculation have been conducted. In the case of HPS, the emission can be enhanced through lowering the temperature, increasing the viscosity, or applying external pressure to alleviate the intramolecular motions [4, 19]. Structural modification by attaching bulky groups on the phenyl rings to hamper their free rotation would also increase the fluorescence intensity of the molecule. Computation simulation indicates a large portion of the low-frequency modes in the reorganization energy of the excited state that correspond to the intramolecular rotational motion [10]. In the aggregate state, these low frequency modes would be restricted because of the steric constraint and enhanced fluorescence could thus be observed.

Typical AIE luminogens usually consist of a stator and one or a few rotors attached to the stator. If the rotors are in the same planar as the stator, the molecule enjoys a maximal electronic conjugation. The electronic delocalization prevents the rotors to undergo free rotational motions. This is the case for most of the ACQ fluorophores [10, 16]. For AIE luminogens, the rotors are twisted against the stator because of the steric congestion. The less constraint from the electronic conjugation allows the rotational or torsional motions of the rotors, which deactivate the excited species non-radiatively. The relationships among conformational planarity, structural flexibility, intramolecular motions and the emission efficiency are depicted in figure 4. For example, although the phenyl rings in 1,4-distyrylbenzene (5) appear to be rotatable, 5 adopts a planar conformation and experiences strong π stacking in the aggregate state (figure 5). Changing the central phenyl rings with a large aromatic ring anthracene induces steric congestion and forces the styryl moieties in 6 to be twisted about 75° against the anthracene core [24]. 6 is a typical AIE molecule with ΦF increasing from 0.4% in solution to 50.8% in crystalline state. Similar phenomenon has been observed when two or four
phenyl rings (7 and 8, respectively) are attached to the central phenyl core to induce steric hindrance [25].

Position isomers refer to the compounds with same functional group(s)/substituent(s) but attached in different position(s) of the parent structure. Such differences can affect the conformation of the molecules and thus their photophysical properties. Li et al. reported the intriguing photophysical behaviors of the (9-anthryl) vinyl(9-phenanthryl)vinylbenzene position isomers [26]. As shown in figure 6, the two substituents are in ortho- (9), meta- (10), and para-position (11) of the phenyl ring of the molecule. 9 and 11 are weakly fluorescent in organic solvents. Their fluorescence is intensified dramatically when the water fraction in the solvent mixture is over 50%, showing the AIE characteristics (figure 6). 10 behaves in an opposite way. When dissolved in organic solvent, 10 emits brightly. The emission, however, is quenched at high water fractions (>50%). The difference in their fluorescence behavior can be attributed to their distinct molecular configurations. From crystal structure analyses, neither the anthryl nor the phenanthryl ring is coplanar with the central benzene ring in 9 and 11 owing to the steric hindrance, while for 10, the phenanthryl moiety is almost coplanar with the benzene core. Strong \( \pi \) interaction between adjacent parallel phenanthrylnvinylbenzene moieties are observed for 10 with the interplane distance of \( \sim 3.4 \) Å. Therefore, the fluorescence of 10 in the crystalline state becomes less efficient when compared to that in the solution state. On the other hand, the intermolecular distance of 9 between adjacent anthracenes or phenanthrenes is \( \sim 6.5 \) Å, which is too far for intermolecular interactions. Multiple C–H…\( \pi \) interactions are found in the crystal packing of 9, which further rigidify the molecules and inhibit the intramolecular motions of 9.

Recent study by Zhao and Tang revealed that the high-frequency stretching motion of fragments in the stator also plays an important role in the deactivation of the excited species of the AIE molecules [27]. Whether the low-frequency rotational motion or the high-frequency stretching motion is predominant is determined by the structural rigidity of the central stators and the rotation ability of the rotors. Due to the steric hindrance between the rotors in 4, the stretching motion of the butadiene moiety is the main nonradiative decay pathway and the rotational motion becomes secondary in deactivating the excited state (figure 7(a)). The high-frequency modes, however, can hardly be hampered by aggregate formation. To further enhance the emission
Topical Review

efficiency in the aggregate state, a phenyl ring is fused to the phosphole to form a phosphindole core (12) to lower the stretching motion of the butadiene fragment. Similar as 4, 12 also adopts a highly twisted structure and experiences weak intermolecular interactions in the crystal lattice. As shown in figure 7(b), the portion of the high-frequency modes is reduced as compared to that of 4. As a result, the solid-state quantum yield of 12 can reach 68% while the one of 4 is 33%. Fusing another phenyl ring to 12 to further increase the rigidity, however, makes the molecule ACQ-active instead. Therefore, the balance between the rigidity of the stator and flexibility of the rotors is essential in the rational design of efficient AIE luminogens.

Based on the mechanistic understanding, more AIE luminogens whose AIE phenomenon is operated by restriction of intramolecular motions have been reported. Shankarling et al have observed the AIE activity of the β-ketoiminate based organoboron complexes (13 and 14) [28]. Both compounds are weakly fluorescent in solution but highly emissive in the solid state (figure 8). Addition of water into the THF solution of 14 could force the dye molecules to aggregate and the fluorescence is increased along with the aggregate formation. Structural optimization reveals that the boron chelate with benzoxyazolyl/benzthiazolyl part is planar while the two aryl rings are twisted for about 30°. In solution, the two phenyl rings are rotatable and deactivate the excited state through nonradiative decay pathway, while in aggregate state, the twisted conformation of the aryl rings prevents compact packing of the molecules, which eliminate self-quenching and enhanced solid-state fluorescence is observed.

Naka et al have reported new AIE luminogens based on maleimide derivatives (15 and 16 given in figure 9) [29]. Theoretical study shows that the luminescent center is localized at the maleimide ring conjugated with the secondary aryl amine. Owing to the free rotational motion of the flexible C–N and/or C–C bonds in the luminogens, 15 and 16 show no emission when dissolved in common organic solvents. In the solid state, both dyes become fluorescent. 16 also exhibits mechanochromism. Upon mechanical stimulus, the emission color of 16 is altered from green to yellow because of the change in the molecular packing in the crystal. Such process is reversible by treatment with dichloromethane.

The AIE phenomenon is not restricted to propeller-like luminogens with aromatic rotors and stators. Recently, many new luminogens with intriguing structures different from typical propeller-shaped molecules have been reported to be AIE active. Coumarin is a naturally occurring organic pigment found in many plants. As most of the ACQ dyes, the planar skeleton of coumarin tends to form strong π interactions and experiences self-quenching of fluorescence in the aggregate state, a phenyl ring is fused to the coumarin to form a coumarinophane core (17) to lower the stretching motion of the butadiene fragment. Similar as 4, 12 also adopts a highly twisted structure and experiences weak intermolecular interactions in the crystal lattice. As shown in figure 7(b), the portion of the high-frequency modes is reduced as compared to that of 4. As a result, the solid-state quantum yield of 12 can reach 68% while the one of 4 is 33%. Fusing another phenyl ring to 12 to further increase the rigidity, however, makes the molecule ACQ-active instead. Therefore, the balance between the rigidity of the stator and flexibility of the rotors is essential in the rational design of efficient AIE luminogens.
Topical Review

state. Zhao and Tang have reported a coumarin derivative modified with a seven-membered aliphatic ring (17) possesses typical AIE features, whereas its analogue with a five-membered aliphatic ring (18) shows opposite ACQ effect (figure 10) [30]. Experimental and theoretical results suggest that the large aliphatic ring in 17 induces the nonplanarity and weakens the structural rigidity, which promotes out-of-plane twisting/bending motions of the molecular backbone to dramatically populate nonradiative decay of the excited state.

Figure 7. (Left) Calculated reorganization energy versus the normal mode wavenumbers for (a) 4 and (b) 12. Top view (central) and side view (right) of two adjacent molecules of (a) 4 and (b) 12 in crystals. The numbers indicate the distances between the planes of the central rings, as determined by single-crystal x-ray crystallography. (Reprinted from [27] by permission from Wiley-VCH Verlag GmbH & Co. KGaA. Copyright © 2015 by Wiley-VCH Verlag GmbH & Co. KGaA.)

Figure 8. Change of fluorescence intensity of 14 in THF and THF/water mixtures with different water content. Photographs showing the fluorescence of 13 in solid state and 14 in solution, aggregate and solid states. (Reprinted from [28] by permission from Elsevier. Copyright © 2015 by Elsevier.)
The intramolecular rotations and vibrations can co-exist such as in 20 when it is dissolved in good solvents [32]. 20 adopts a highly contorted geometry as revealed by the crystal analysis: the central dihydroanthracene backbone bending for 132.4° and the diphenylmethene groups twisting out for 79°. The peripheral phenyl rings can undergo rotational/torsional motions as in TPE while the dihydroanthracene backbone experiences active vibrational motions. Collectively, these intramolecular motions consume energy and deactivate the excited species through heat dissipation. The highly contorted conformation of 20 results in a loose molecular packing with weak intermolecular interactions in the crystal state, which enable 20 to display reversible polymorphism-dependent emission behaviors.

Apart from the abovementioned intramolecular rotations and vibrations, flipping between two conformations or among multiple conformations could be another possibility to nonradiatively deactivate excited state energy. TPE is achiral. However, due to steric hindrance, when one of the phenyl rings twists in one direction, all the other phenyl rings will follow the same orientation. As a result, it can adopt two twisted chiral geometries (P-order and M-order refer to right-handed and left-handed propeller, respectively) [10]. Covalent linking two TPE moieties with ethynyl bonds creates an AIE macrocycle (21), whose 3D structure resembles Penrose stairs [33]. 21 possesses typical AIE characteristics. Owing to the twisted chirality (P or M) of the TPE motif and the axial chirality (R or S) of the macrocyclic linkage, 21 exhibits two distinct configurations, which exist in an equal ratio in the crystal state at room temperature (figure 11). Theoretical calculation reveals that the energy barrier for the flipping is relatively low and the interconversion between these two conformations is thus energetically favourable. Rapid transformation between these species could take place when the molecule is under photo excitation.

3. Applications

Attracted by the intriguing AIE property, tremendous research efforts have been devoted to expanding the scope of the technological applications based on AIE luminogens. This ranges from but is not limited to optoelectronic devices, chemosensing and a variety of biological applications [16]. The incorporation of the AIE materials has in turn opened new avenues of technological innovation in these areas. This
Topical Review

section will briefly discuss the most recent progresses in the area, with the emphasis on those accomplished after the publication of the comprehensive AIE review in 2015.

3.1. Optoelectronic applications

Optoelectronic devices are instruments that operate electrical-to-optical or optical-to-electrical transduction. In this area, the light-emitting materials are mostly used in the solid state and the emission efficiency of the materials plays a critical role in determining the performance of the devices. Therefore, AIE luminogens would be ideal candidates for optoelectronic applications. Previous studies have demonstrated AIE materials as efficient solid-state emitters that overcome the barricade for conventional luminescent materials in the construction of high-performance OLEDs [11, 18]. AIE luminogens can form ordered microstructures through self-assembly or self-organization, which can waveguidingly propagate light emission to realize amplified spontaneous emission and organic lasers [34, 35]. AIE luminogens with chiral pendants have been reported to form helical assembles that can generate circular polarized luminescence with large emission dissymmetry factor and high emission efficiency [36, 37]. Attaching either long alkyl/alkoxy groups or well-known mesogens to AIE cores creates AIE mesogens that can be fabricated to light-emitting liquid crystals [29, 38, 39]. Details of these examples and the design rationale have been discussed in previous review articles [16].

Photovoltaics, also known as solar cells, convert solar energy into flows of electrons to generate electric power. The AIE luminogens are rarely used directly as photovoltaically active materials. Recent research has shown that integrating AIE luminogens as luminescent materials in photovoltaic devices could improve their performance significantly. Compared with conventional photovoltaics, luminescent solar concentrators (LSCs) have recently attracted much attention because of the advantage of simple device configuration and the feasibility of incorporation into urban environments such as windows and walls at low cost [40]. For LSCs, luminescent materials are embedded in plastic or glass substrates. Upon absorption of solar light, subsequent luminescence produced by the dyes is concentrated at the thin edges of the substrates due to the optical wave-guiding property, which can potentially improve the output of photovoltaic devices. Coumarin, perylenes and rhodamine were the first-generation dyes used in LSCs. These dyes, however, suffer from the ACQ problem in the solid state as well as the reabsorption of light emission due to small Stokes shifts, which limit the LSC efficiencies to below theoretical limits. AIE luminogens, which are free of the concentration-quenching and reabsorption problems, are promising alternatives for LSC applications.

Wong and Ghiggino have firstly demonstrated the utility of TPE dispersed in PMMA for planar concentrator [41]. The optical edge efficiency \( \eta_{\text{edge}} \) of the LSC, which refers to the ratio of the number of fluorescence photons that are waveguided to the edge to the number of incident photons absorbed, were found to be 13.2% (geometry factor \( G = 25 \)), which is comparable with that of most organic dyes and without the use of any optical accessories to enhance the trapping efficiency of the LSCs (ratio of photons emitted from the edges to the total photon emitted from the LSCs).

The emission range of TPE, however, is at the short wavelength that is not ideal for LSCs to be coupled with high efficiency silicon or GaAs photovoltaic cells. To solve this problem, the same group has reported the use of non-covalent excitation energy transfer approach with an AIE luminogen (22 shown in figure 12) with a red-shifted emission as light-harvesting donor and the acceptor, DCJTB, that emits at longer wavelength range [42]. 22-DCJTB films on glass are prepared by dissolving the two dyes and poly(methyl methacrylate) (PMMA) matrix in chloroform followed by drop-casting on a glass substrate. Figure 12(a) shows the
UV-vis absorption and emission spectra of the 22-DJCJTB films, which indicate the successful energy transfer and downshifting of the light emission. The data also imply that with the high emission efficiency of AIE donor in the solid state, such approach can minimize both transmission and reabsorption losses to achieve high light collection and concentration efficiencies. By coupling with silicon solar cell, the short-circuit current is estimated by varying the distance between a narrow excitation beam and the edge of the LSC. The results in figure 12(b) clearly show that the short-circuit current of the 22-DJCJTB LSC is substantially higher than the one with only DCJTB as chromophore in the LSC, providing additional evidence for improved performance.

3.2. Chemo- and biosensors

Mechanistic understanding of the AIE phenomenon suggests that the fluorescence of AIE luminogens can be triggered on by restriction of intramolecular motions. Based on this idea, a large variety of AIE luminogens have been developed as optical sensors implicated in different areas [10, 14, 16, 43, 44]. For example, the AIE dyes have been used as chemical sensors for the detection of metal/anionic ions, explosives, volatile organic compounds (VOCs), gas, reactive oxygen species, for pH/temperature/viscosity measurement, and as bioprobes in the detection of amino acids, proteins, nucleic acids, sugars, lipids, for monitoring conformational change of DNA or protein [16]. Compared with conventional fluorescent dyes, the AIE dyes offer advantages such as superior stability, high signal-to-noise ratio, low background noise, facile fabrication and easy functionalization. The incorporation of the AIE concept also leads to the recent advancement in the development of ‘turn-on’ probes and label-free detection systems. In this section, a couple of examples not covered in the previous review articles will be introduced.

The emission of carbon dioxide (CO₂) contributes a significant part to the global climate change that poses threat to the environment as human wellbeing. Monitoring the level of CO₂ either as dissolved CO₂ or gaseous CO₂ is thus of utmost interest. Previously Tang et al have reported the use of HPS for CO₂ sensing through measuring the viscosity change induced by the formation of carbamate ionic liquid of dipropylamine and CO₂ [45]. Recently Chartterjee and co-workers have proposed another approach for detecting dissolved CO₂ based on ion-induced self-assembly of a TPE derivate (23) [46]. Chitosan, a naturally occurring biopolymer, bearing multiple amine groups is used in the assay. In the absence of CO₂, 23 would not interact with the neutral chitosan and weakly fluorescence is observed (figure 13). The presence of dissolved CO₂ can protonate the amine groups in chitosan to make it positively charged. The negatively charged 23 can thus
aggregate on the surface of the chitosan by electrostatic interaction and generate intense blue emission. The fluorescence output of 23 is linearly correlated to the concentration of CO₂ in the range of 5 to 50 μM with a detection limit of 5 × 10⁻⁶ M (0.001 27 hPa).

VOCs are organic chemicals that are prone to evaporating at ordinary room temperature. Some VOCs are dangerous to human health and harmful to the environment. Liu and Zhao reported the use of porous metal-organic frameworks (MOFs) with TPE analogue (24) as building blocks for gas adsorption and VOC sensing [47]. With the four carboxylate chains, 24 can coordinate with zinc ions to form one-dimensional (1D) metal carboxylate chain secondary building units (figure 14(a)). The three-dimensional (3D) porous structures are constructed based on the connection of the 1D channels. The low dimensional channel and the small pore size of the MOFs allow the guest molecules to concentrate in the pore and interact with the luminescent center of 24. The Zn-coordinated 24 complex shows appreciable luminescence from the TPE-based luminogens in the framework. Drying the Zn-coordinated 24 under vacuum at 50 °C can further activate the MOFs with a more rigid framework that emits strongly at 535 nm, much more red-shifted than the as-synthesized one (figure 14(b)). The emission wavelength of the activated MOFs varies when exposed to different VOCs (figure 14(b)) and the fluorescence intensity is greatly weakened in the presence of nitrobenzene (NB) and 2,4-dinitrotoluene (DNT) (figure 14(c)), demonstrating its promising capacity in the identification and detection of toxic VOCs.

In addition to environmental sensing, the AIE dyes have also been demonstrated their applications in sensing biological substances such as biomarkers for disease diagnosis. Telomerase is an enzyme that catalyzes the elongation of the DNA strand at the end of chromosomes, which is associated with cell immortality and cancers. In most normal somatic cells, telomerase activity is suppressed, whereas in approximately 90% cancer cells, the up-regulation or reactivation of telomerase activity is observed [48]. Detecting telomerase activity could thus be useful for cancer diagnosis, screening anticancer drugs and evaluation of cancer therapy. Traditionally, telomerase activity is evaluated mainly using telomeric repeat amplification protocol (TRAP). Despite the tedious procedures, there is a high tendency of getting false positive or false negative results by using TRAP. Lou and Xia have designed a novel strategy of using AIE luminogens for sensitive detection of telomerase activity [49]. 25 is a cationic AIE dyes that is not fluorescent when dissolved in aqueous solution (figure 15). Thanks to the electrostatic attraction, 25 is able to bind to oligonucleotides but still emit faintly due to the limited physical constraint provided by the short DNA strands. The fluorescence of 25 could be greatly enhanced with the elongation of the DNA strand, which is an indication of telomerase activity. This method has been successfully employed in bladder cancer diagnosis. Urine samples were collected from bladder cancer patients. Telomerase were extracted by treated the urine samples with cell lysis buffer. The telomerase extracts was incubated with solution containing 25, oligonucleotides (primer), RNase inhibitor and deoxynucleotide mixtures (dNTPs). With active telomerase, the (TTAGGG)ₙ repeats is added to the primer and the DNA strand is thus elongated, resulting in stronger emission of 25 (figure 15(a)). The simple strategy has been demonstrated for quantification of telomerase activity in patient urine specimens (both clear or bloody urine samples) and those from normal control group (figure 15(b)). The sensitivity of the assay has been further improved by attaching a quencher group on the primer to lower the background noise and amplify the fluorescence output [50].

Figure 13. Schematic illustration of the working mechanism of using 23 coupled with chitosan for the detection of dissolved CO₂. (Reprinted from [46] by permission from American Chemical Society. Copyright ® 2015 by American Chemical Society.)
Correct protein folding is essential for proteins to exert their functions. Failure of the quality control machinery in the cells could lead to protein misfolding and aggregation. Amyloid protein fibrillation has been regarded as the pathological hallmarks for a variety of neurodegenerative diseases, diabetes and other disease conditions [51]. Efficient detection and monitoring of amyloid fibril formation could thus advance the early diagnosis of these diseases and the interpretation of their pathogenic mechanism. The AIE dyes, whose fluorescence is triggered by aggregate formation, are ideal candidates for amyloid fibril detection. The proof-of-concept studies have been carried out on bovine insulin and Parkinson’s disease related α-synuclein fibrillation [52, 53]. Recently, Ghorai and Jana have prepared an amyloid probe consisting of a fluorophore, i.e. TPE derivative, and a peptide component that selectively binds to amyloid structure (25) [54]. 25 is non-fluorescent in the presence of amyloid β-peptide (Aβ) monomers. The fluorescence is switched on when amyloid fibrils are formed (figure 16(a)). The formation of amyloid fibrils was confirmed by using transmission electron microscopy (TEM, figure 16(b)). The Aβ fibrils can be well-stained and observed under fluorescence microscopy (figure 16(c)). Compared with the ‘golden standard’ thioflavin T, the AIE probes offer high signal-to-noise ratio, absence of the self-quenching problem and compatible with other quencher ions/nanoparticles. The AIE
probes could thus be powerful alternatives to thioflavin T in the detection and monitoring amyloid fibrillation.

### 3.3. Bioimaging

Fluorescence imaging is one of the most commonly used techniques in biological and biomedical research because of the advantages of large variety of fluorescent reagents, high temporal and spatial resolution, low cost and wide applicability [8, 55]. Fluorescent probes that can selective illuminate organelles, sense intracellular environment, and monitor biological processes are in high demand. Despite the emergence of
genetic-encoded fluorescent proteins and photostable inorganic quantum dots, fluorescent probes based on organic dyes are still of great interest because different functions of organic dyes can be easily achieved by structural modification. The use of organic dyes as cell stains is much handy, as compared to using fluorescent proteins where sophisticated transfection process is required. Because of the small size of organic dyes, the interference to the biological substances and processes could be minimized. Previous studies have demonstrated the advance of using AIE luminogens for specific imaging of intracellular organelles, measuring intracellular pH, evaluating enzymatic activity, visualizing protein receptors on cell surface, long-term cell tracing and even in vivo imaging of tumors [16].

In comparison with conventional organic dyes, these AIE probes enjoy superior photostability and low cytotoxicity. Because of their hydrophobic nature, after diffusing to the cytoplasm, the AIE molecules would accumulate at certain organelles or binding pockets. As clusters, even the outermost layer of the aggregates are damaged by excitation light, the interior in the aggregates can still emit and thus exhibit excellent photostability [9]. This will allow the use of AIE dyes for long-term observation of the biological processes inside the cells. In this part, a few examples of newly developed fluorescent imaging agents based on AIE luminogens will be introduced.

Mitochondria are the organelles existing in almost all eukaryotic cells that play a fatal role in both the life and death of the cells. The most prominent function of mitochondria is to generate ATP, the energy currency of the cell. In order to synthesize ATP, mitochondria have to continuously oxidize substrates and maintain a proton gradient across the double lipid bilayers in the respiratory electron transport chain of mitochondria with a large membrane potential ($\Delta \Psi_m$). $\Delta \Psi_m$ is a critical parameter that reflects the functional status of mitochondria and the vitality of the cells. Cationic lipophilic dyes would generally be attracted to mitochondria because of the large $\Delta \Psi_m$. Tang and coworkers have reported a red-emitting mitochondria-specific AIE luminogen (27) [56]. 27 possesses excellent specificity to mitochondria, comparable with commercial mitochondrial dyes and mitochondrial-labeling green fluorescent protein (GFP). The advantage of using small organic dyes over fluorescence proteins is revealed in the colocalization experiment. In the co-staining experiment, all the cells are homogenously labelled with 27 and give bright emission in the red channel (figure 17(a)). In the green channel of mitochondria GFP, however, several cells emit much weaker than the others in the observation window. This could be attributed to the inhomogeneous transfection efficiency and different expression levels of mitochondria GFP in cells. On the other hand, different from the other mitochondria AIE dyes which are inert to the environmental changes [9, 57], the emission of 27 is sensitive to the change of $\Delta \Psi_m$. When cells are treated with oligomycin and then carbonyl cyanide 3-chlorophenylhydrazone (CCCP), significant increase and subsequent decrease of the fluorescence signals are observed, which is correlated to the increase and decrease of $\Delta \Psi_m$ respectively (figure 17(b)).

![Figure 17.](image-url)
in high-throughput analysis of cell samples, flow cytometry is employed. Figure 17(c) presents the change of fluorescence intensity of 27-stained HeLa cells under different stimuli, which is consistent with the results obtained from confocal microscopy. Moreover, the use of flow cytometry could offer a more efficient and quantitative way to analyse the mitochondria function and cell activity in different environments.

Photoactivatable fluorophores refer to fluorophores whose fluorescence can be converted from non-emissive state to highly fluorescent state through light activation [58]. These types of fluorophores have attracted much interest because of their applications in the study of cell–cell interactions, cancer metastasis, and also thanks to the recent advance of super-resolution imaging techniques such as photoactivated localization

Figure 18. The working mechanism of the photoactivatable α-28. (a) Merged bright-field and fluorescence image of live HeLa cells stained with α-28. The cells in the white ellipse region were selected to expose to 405 nm with a power of 35% for 1 s and then the same wavelength with the power of 1.5% for imaging. Scale bar: 20 μm. (b) Change of fluorescent intensity in cells of the selected region after photoactivation. (Reprinted from [60] by permission from Wiley-VCH Verlag GmbH & Co. KGaA. Copyright © 2015 by Wiley-VCH Verlag GmbH & Co. KGaA.)

Figure 19. (a) Fluorescence signals from cell autofluorescence and cells stained with 29 upon two-photon excitation at 600 nm. (b) Fluorescence lifetime distribution histogram and (c) FLIM image of HeLa cells stained with 29. Scale bar: 30 μm. (Reprinted from [61] by permission from Wiley-VCH Verlag GmbH & Co. KGaA. Copyright © 2015 by Wiley-VCH Verlag GmbH & Co. KGaA.)
microscopy and stochastic optical reconstruction microscopy. Tang et al have designed and prepared a photoactivatable AIE luminogen, o-28 for specific tracking of mitochondria [59]. The original o-28 is weakly fluorescent in buffer solution due to the twisted intramolecular charge-transfer effect. After entering the cells, the molecules are prone to accumulating in mitochondria because of the pyridinium cationic group. Upon photoactivation, o-28 undergoes photocyclodehydrogenation to form c-28. With the change of conjugation as well as restriction of intramolecular motions, c-28 becomes highly fluorescence with large signal-to-background ratio (figure 18(b)), which enables the visualization of mitochondria distribution and structures in the illuminated area (figure 18(a)). Compared with steady-state fluorescence, time-resolved fluorescence can offer valuable information that is masked in the ensemble-averaging process. We have recently reported the use of fluorescence lifetime imaging microscopy (FLIM) for mapping intracellular viscosity with the aid of the AIE dye [60]. Intracellular viscosity is one of the critical parameters that indicate the functioning status of the cells. The abnormal change of intracellular viscosity has been regarded as a major contributor or indicator for a variety of diseases including atherosclerosis, diabetes, and Alzheimer’s disease. Manipulated by the restriction of intramolecular motion process, AIE dyes should be intrinsically sensitive to environmental viscosity change. In a more viscous environment, the intramolecular motions become difficult and consequently the radiative decay would be populated, resulting in enhanced fluorescence intensity and prolonged decay lifetime. Compared with molecular rotor dyes, which predominate the viscosity sensing area, the AIE dyes, which contain more rotatable components, might be more sensitive to the subtle change in viscosity in the microenvironment. 29 is thus hired for this purpose. With the increase in viscosity, the fluorescence intensity of 29 is enhanced and the lifetime becomes longer, which has been proven by using solvent mixtures with different glycerol content and lipid vesicles with different phospholipid composition. By using two-photon excitation at 600 nm, the fluorescence signals of 29 can be well separated from the autofluorescence from cells (figure 19(a)). Inside the cells, 29 exhibits a broad range of lifetime distribution (figure 19(b)), which is indicative of the heterogeneity of the intracellular viscosity and the complexity of the intracellular environment. Careful analysis of the FLIM image reveals that the long lifetime species is mostly distributed in membrane-bound organelles such as mitochondria, while the shorter lifetime is originated from the lipid droplets. The ordered packing of the lipid bilayers of the membrane-bound organelles leads to high viscosity/low fluidity and thus long lifetime of the dye is observed in these locations. The loose packing of the lipid molecules inside lipid droplets results in a low-viscosity environment that allows the dyes to undergo free intramolecular motions and shorter lifetime is thus recorded.

Recent studies have reported the design of long-wavelength AIE luminogens for lipid droplets imaging [61, 62], for nucleus targeting [63], and for tracking autophagy process through lysosome targeting [64]. In addition, a large variety of applications based on nanoparticles with embedded AIE dyes for live cell as well as in vivo imaging have been discussed in the other reviews [2, 65] and would not be covered in this article due to space limit.

4. Summary and outlook

This topical review introduces the discovery of the AIE phenomenon and summarizes the recent progress in AIE research. The global interest on AIE research is reflected on the increasing number of publications and citations since 2001 (figure 20). AIE represents a unique photophysical process originated from a series of propeller-shaped conjugated molecules. AIE luminogens are virtually non-fluorescent in the solution state but highly emissive in the aggregate and solid states due to the restriction of intramolecular motions. In certain cases, the AIE luminogens remain
weakly fluorescent in the amorphous state and only emit strongly in the crystalline state. Such phenomenon is also called crystallization-induced emission (CIE), which is a special case of AIE. Cyclodibutenone, fulvene, and indigo derivatives have been reported to exhibit CIE behaviour [66, 67]. Most of these compounds also possess mechanochromic property [68]. Apart from the organic molecules described above, a large number of organometallic molecules have been found to be AIE-active. This includes Au-thiolate nanoclusters [69], Pt-containing compounds and Ir complexes, details of which have been covered in the comprehensive AIE review [16].

The emergence of AIE phenomenon has brought forth a revolution both conceptually and practically in many different research areas. The rationale of molecular design and the advantages of using AIE luminogens in representative applications have been touched on in this article, which is introductory to readers interested in fluorescence-related research. The current research momentum is mainly on the innovative applications of the AIE luminogens in optoelectronics, environmental sensing and bioimaging. Fundamental mechanistic understanding especially by applying advanced photophysical techniques is still scarce in the area. Meanwhile, most of the fluorescence assays are conducted in a cuvette or a microtiter plate. The strong fluorescence of AIE luminogens in solid state will also allow the design of paper-based sensors, microchips, or other continuous sensing devices, which have not yet been developed and could be a research area of interest based on AIE luminogens. Further investigation of AIE is anticipated to tackle the current limitation of AIE luminogens by emphasizing the following research directions: (a) to improve the optical performance such as pushing the excitation wavelength to the red region, which is more desirable for in vivo applications; (b) to enhance the selectivity and reversibility in the sensing process, so that these sensors can be continuously used to monitor the changes of the substrates in a long period of time; and (c) to achieve quantitative imaging in living context. This will require the careful calibration of the results obtained by using AIE luminogens with other traditional techniques.

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