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Biosensing of Luminogens with Aggregation-Induced Emission Characteristics

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Biosensing of Luminogens with Aggregation-Induced Emission Characteristics

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Fluorescent biosensors are powerful analytical tools for studying biological events in living systems. Luminescent materials with aggregation-induced emission (AIE) attributes have attracted much research interest and have been identified as a novel class of luminogens to develop fluorescent turn-on biosensors with superior sensitivity. In this Tutorial Review, we present an overview of the AIE phenomenon and its mechanism. We summarize the structural design and working principle of AIE biosensors developed recently. Typical examples of AIE biosensors are presented.

Key Learning Points
1. Introduction of the aggregation-induced emission (AIE) phenomenon.
2. Mechanistic understanding of the AIE phenomenon.
3. Structural design and the working principle of AIE biosensors
4. Representative examples of AIE biosensors

1. Introduction

Development of sensitive and selective fluorescent biosensors is of great importance in the fields of biological science and technology, as they can serve as powerful analytical tools for studying biological events in living systems.[1] Thanks to the enthusiastic efforts of scientists, a wide variety of fluorophores have been developed and utilized in bio-sensory applications. Through simple modulation of their molecular structures, the behaviours and properties of organic dyes can be easily tuned to satisfy various requirements for certain purposes.[2] Conventional organic dyes, such as fluorescein, often give bright emission in dilute solution. However, their emission is weakened or quenched in concentrated solution or in the aggregated state (Figure 1A). Such a phenomenon is known as aggregation-caused quenching (ACQ) and has significantly limited the degree of labelling of dye molecules to bioanalytes and enforced researchers to use dilute solution with a compromised low sensitivity for sensory applications.[3] More importantly, the ACQ effect has compelled many fluorescent biosensors to operate in a "turn-off" mode, which is insensitive and not suitable for practical use. Although several strategies including photoinduced electron transfer and fluorescence resonance energy transfer have been applied to develop fluorescent "turn-on" biosensors, such designs are relatively complicated and involve tedious synthetic procedures.[4] Creation of new fluorogenic molecules that are free of the ACQ effect will solve the problems encountered by traditional fluorophores and provide a new platform for developing new biosensing systems.

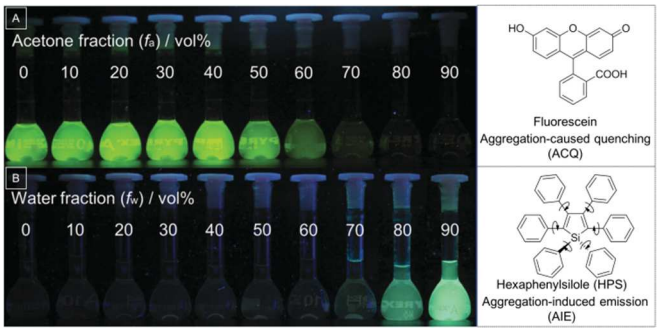

Figure 1. Fluorescent photographs of solutions and aggregates of (A) fluorescein (15 μM) in water/acetone mixtures with different fractions of acetone (f_a) and (B) hexaphenylsilole (HPS; 10 μM) in THF/water mixtures with different fractions of water (f_w). Adopted with permission from ref. 9. Copyright 2014 Wiley-VCH.

During our search for efficient luminogenic materials, we found that some propeller-shaped molecules such as siloles were non-emissive in solution but emitted intensely upon aggregate formation.[5] This unusual photophysical phenomenon is diametrically opposite to the ACQ effect as discussed above and was coined as aggregation-induced emission (AIE). This

novel AIE phenomenon is of great practical implications because it permits the use of dye solutions with any concentration for bioassays and enables the development of "turn-on" biosensors by taking the advantage of luminogenic aggregation. The "turn-on" feature of AIE biosensors offer a higher sensitivity and better accuracy than the ACQ counterparts.[6]

In this Tutorial Review, we will first give a brief introduction on the AIE phenomenon and its mechanism. Lately, we will focus our discussion on the structural design and working principle as well as the representative examples of AIE biosensors.

2. Aggregation-Induced Emission

Hexaphenylsilole (HPS) is considered as an iconic AIE luminogen (AIEgen) since it is the first molecule from which the AIE phenomenon was unearthed.[7] Unlike conventional fluorophore with ACQ effect, it shows no emission in tetrahydrofuran (THF) or in THF/water mixture with a water fraction (f_w) less than 80 vol %. Further raising the water content induces the HPS molecules to aggregate and leads to strong green fluorescence (Figure 1B). The AIE effect is not an isolated phenomenon for HPS but has been observed in other molecules such as tetraphenylethene (TPE), diphenylfumaronitrile and distyreneanthracene with common features of twisted structures and freely rotatable peripheral aromatic moieties.[8]

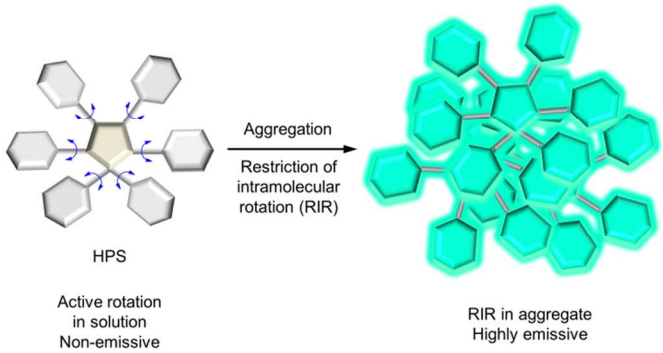

Figure 2. Propeller-shaped HPS molecule is non-emissive in solution but become highly emissive upon aggregation, due to the restriction of intramolecular rotation (RIR) of the phenyl rotors against the silole stator in the aggregated state.

What is the cause of the AIE phenomenon? Understanding its working mechanism is of crucial importance as it offers a deep insight into the luminescence processes and guides researchers to develop new AIE systems and design novel biosensors. Mechanistic studies reveal that restriction of intramolecular motion (RIM), which including rotation and vibration, in the aggregated state is the main cause for the AIE effect.[9,10] Taking HPS as an example, the six phenyl peripheries are linked to the silole core through single bonds. In dilute solution, their rotations are active and serve as a relaxation channel for the excited states to deactivate. In the aggregated state, such rotations, however, are restricted due to the physical constraint. This blocks the nonradiative pathway and thus enables the excitons to decay radiatively (Figure 2). The detailed discussion on the AIE mechanisms and different AIE systems can be found in our reviews published recently.[6–11]

3. Structural Design

The novel AIE effect offers a new platform for developing new sensing systems that operate in a "turn-on" mode. Through rational structural design, a large variety of AIE-active small molecules, bioconjugates and polymers with different skeletons, peripheries or pendants, and emission colours as well as functionalities have been developed and utilized as biosensors (Figure 3).[6–8]

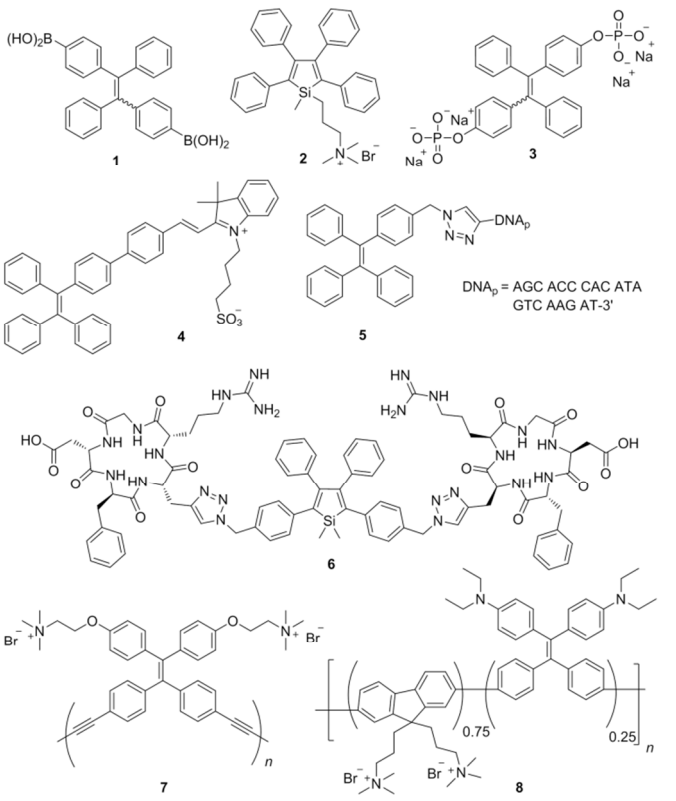

Figure 3. Examples of AIEgens for biosening. **1**–**4** are small neutral, cationic, anionic and amphiphilic AIEgens, respectively. **5** and **6** are bioconjugates. **7** and **8** are polymeric AIEgnes.

3.1 Small Molecules

Since the first report on the AIE phenomenon in 2001, various small or low molecular weight molecules with AIE attributes have been designed and prepared. The AIEgens can be decorated by different substituents to endow them with specific functional properties. Since phenylboronic acid can react with diols in aqueous media, TPE carrying two boronoic acid (**1**) units was synthesized and applied for specific detection of glucose. The oligomerization reaction of two diol units in glucose with two boronic acids in **1** formed rigid oligomers, which activated the RIM process and hence the emission of TPE.[12] Introduction of hydrophilic moieties such as quaternary

ammonium, phosphate as well as sulfonate units to the AIEgens can endow them with high water solubility. For example, cationic silole molecule **2** was nonemissive in aqueous solution, but its emission was intensified in the presence of DNA because of the formation and aggregation of **2**/DNA complex.[13] Anionic TPE fluorogen with two phosphate groups (**3**) was developed for the detection of alkaline phosphatase (ALP) and its enzymatic activity. The ALP recognizes and cleaves the phosphate groups in **3**, which gives a water-insoluble enzymatic residue that emits intense fluorescence upon aggregation.[14] Taking its chemical reactivity towards OH^-/H^+, a zwitterion TPE-cyanine derivative (**4**) was used to sense intracellular pH in a broad range by showing different emission colours and intensities: strong to moderate red emission at pH 5–7, weak to no emission at pH 7–10, and no emission to strong blue emission at pH 10–14. The emission transition is reversible and can be repeated for many cycles.[15]

3.2 Large Molecules

Selectivity is an important parameter in biosensing. It is largely dependent on the affinity between the recognition groups and the receptors. Biorecognition groups vary from saccharide to amino acid/peptide/protein and nuclei acid/RNA/DNA. They can be tethered to the AIE cores via different chemical reactions to furnish bioconjugates for diverse purposes. For example, AIE bioconjugate **5** was prepared by conjugating TPE with a single-stranded DNA via click reaction. In aqueous solution, **5** was nonfluorescent but showed a significant emission enhancement in the presence of complementary DNA strand.[16] Even when there is only a one-base or two-base mismatch in the DNA sequence, the sensing process will be greatly affected. Conjugation of cyclic arginine-glycine-aspartic acid with tetraphenylsilole furnished bioconjugate **6**, which worked as a light-up biosensor for integrin $\alpha_v\beta_3$.[17]

The practical properties and functionalities of small AIEgens are applicable in AIE polymers. Because of their polymeric nature, AIE conjugated polymers, in some cases, display a higher sensitivity than their low molecular weight counterparts in sensory applications. In particular, polyelectrolytes with AIE features are promising candidates for biosensors as they can dissolve well in aqueous solutions and their emission is intensified in the presence of targeted analytes. For example, conjugated cationic polyelectrolytes **7** and **8** were synthesized and applied in heparin assay.[18,19] The fluorescence from the probes in aqueous solutions increased gradually upon heparin addition, showing a linear response to the heparin concentration.

4. Working Principle

Since the fluorescence turn-on property of AIEgens is attributed the RIM process, different strategies including physical interactions and chemical reactions have been employed to develop various AIE biosensors. Although more than one strategy is possible to work simultaneously in some sensory systems, in the following section, we try to summarize and discuss each strategy in details.

4.1 Electrostatic Attraction

Electrostatic attraction is a sort of force that brings two species with opposite charges together. Many biomolecules such as polysaccharides, DNA and phospholipids are charged. For example, heparin is known to carry the highest negative charge density among the biological molecules. As such, various cationic fluorophores have been developed and used as heparin receptors. Tong et al. reported a cationic salicylaldehydeazine derivative (**9**) with AIE characteristic for turn-on detection of heparin based on the electrostatic attraction strategy (Figure 4).[20] In aqueous solution, **9** displayed a very weak fluorescence because of its active intramolecular motions. Upon addition of heparin, the complexation of **9** with heparin activated the RIM process and hence turned on the light emission of the dye molecule. The probe showed a high selectivity to heparin over its analogues, chondroitin sulfate (ChS) and hyaluronic acid (HA), as they possessed a lower charge density and thus interacted weakly to the probe. On the other hand, protamine is a highly cationic protein and bind tightly with heparin due to the strong electrostatic attraction. Addition of protamine into the solution of **9**/heparin disassembled the complex. Consequently, the intramolecular motions of **9** became active again, which resulted in florescence annihilation.

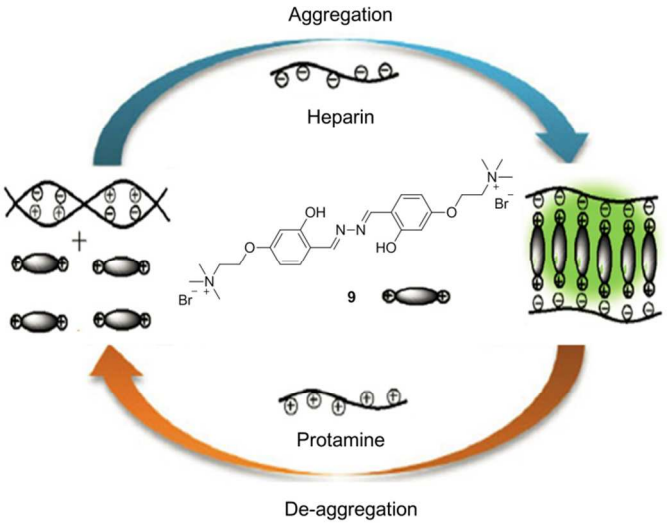

Figure 4. The design principle of fluorescent turn-on sensor for heparin and turn-off sensor for protamine based on aggregation and deaggregation of **9** *via* electrostatic attraction. Adopted with permission from ref. 20. Copyright 2013 Elsevier B.V..

4.2 Hydrogen Bonding

Hydrogen bonding is a kind of dipole-dipole interaction observed in compounds with hydrogen atom attracted to an electronegative atom such as nitrogen or oxygen. Such bonding is generally stronger than van der Waals interaction but weaker than covalent or ionic bonds. A typical example of hydrogen bonding can be found in the base pairs of DNA, where the adenine (A) unit forms two hydrogen bonds with the thymine (T) group, and the cytosine (C) and guanine (G) units interact with each other via three hydrogen bonds. Similar to A–T and

C–G base pairs, melamine and cyanuric acid are complementary each other and they can form a stable adduct through multivalent hydrogen bonds. Based on the state-dependent fluorescence, Sanji and his coworkers designed a cyanuric acid-functionalized TPE derivative (**10**) for fluorescence sensing of melamine (Figure 5).[21] The acetonitrile solution of **10** was practically nonluminescent. Upon addition of melamine into the solution, the mixture exhibited intense emission. The fluorescence enhancement is attributed to the aggregation of TPE triggered by multivalent complexation of melamine with **10**. Other relevant analytes such as ammeline, ammelide, uracil, cytosine, or thymine exerted no effect on the fluorescence of **10**. The high selectivity of the probe was endowed by the multivalent hydrogen bonding between cyanuric acid and melamine.

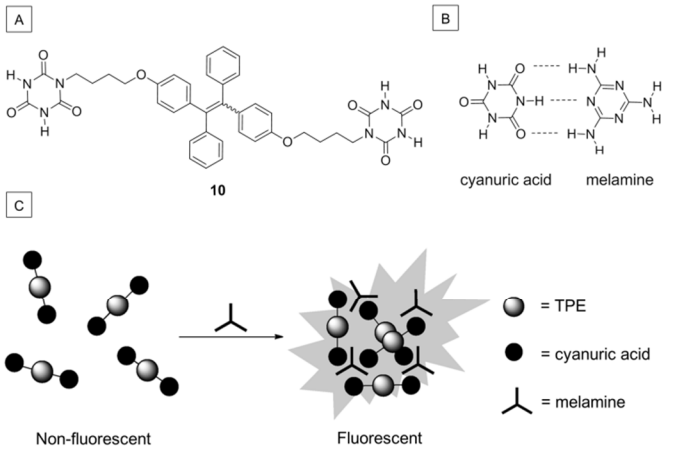

Figure 5. (A) The chemical structure of **10**. (B) Melamine–cyanuric acid adduct formed through multivalent hydrogen bonding. (C) The design principle of fluorescent "turn-on" sensor for melamine based on the formation of hydrogen bonds between melamine and **10**. Adopted with permission from ref. 21. Copyright 2012 Wiley-VCH.

4.3 Hydrophobic Effect

Hydrophobic effect refers to the tendency of nonpolar molecules to form aggregates in aqueous solution in order to minimize the exposure of their surface area to the polar water molecules. Due to the hydrophobic effect, amphiphilic organic fluorophores are driven to entry the hydrophobic pockets or cavities in the folding structures of proteins. Because of the small volume inside the pockets or cavities, the organic molecules tend to form aggregates, which restrict their intramolecular motions. As such, AIEgens are promising fluorescent turn-on biosensors for detecting proteins with abundant hydrophobic pockets and monitoring their conformation. An representative of such proteins is given by human serum albumin (HSA), which is the most abundant serum protein present in human blood plasma. We and Ouyang et al. synthesized and utilized water-soluble sulfonated AIEgens (**11** and **12**) as fluorescent probes for quantification of HSA and monitoring its conformational transitions.[22,23] Due to the present of the sulfonate groups, both **11** and **12** dissolve readily in water and show no emission in buffer solution due to the active intramolecular motions. When the dye molecules were docked and aggregated on the hydrophobic region of HSA, their RIM process was activated, which turned them into strong emitters. On the other hand, their emission was weakened or quenched when denaturants such as guanidine hydrochloride (GndHCl) were added. The GndHCl induces HSA to unfold, which releases the AIEgens to the aqueous solution. Based on the fluorescence change, the denaturation process of HSA involves a three-step transition process (Figure 6). At [GndHCl] <1.2 M, HSA remains its native conformation. Between 1.2M and 2.2 M, GdnHCl induces the domain separation process and triggers the release of AIEgens. Increasing the concentration of GdnHCl to ~2.0 M transforms HSA to molten globin state, which may bring about additional hydrophobic region and lead to more accommodation of AIEgens. Further increment of the GdnHCl concentration leads to the complete denaturation of HSA and release of AIEgens.

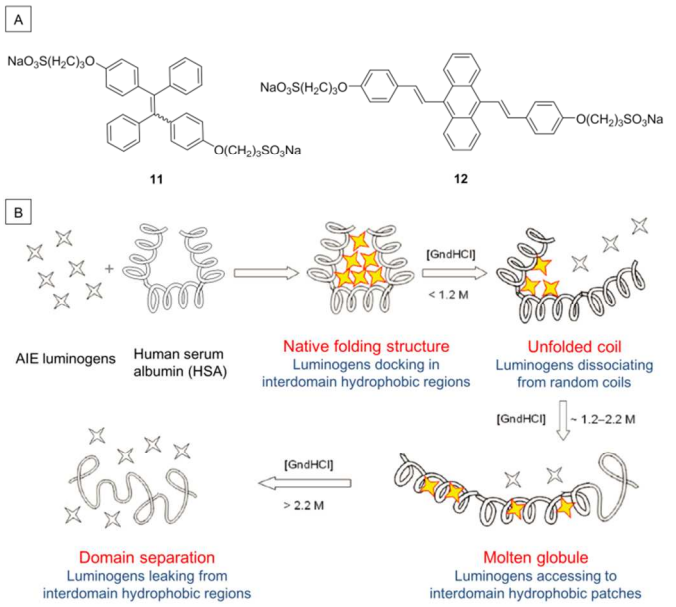

Figure 6. (A) Chemical structures of sulfonated AIEgens **11** and **12**. (B) The design principle of fluorescent turn-on sensor for human serum albumin (HSA) through hydrophobic interaction and fluorescence tracking of the GndHCl-induced unfolding process by using AIEgens. Adopted with permission from ref. 22. Copyright 2010, American Chemical Society.

4.4 Solubility Change

The fluorescence of AIEgens is state-dependence: they emit faintly in the solution state or single molecular state but brightly in the aggregated state. Therefore, a fluorescent turn-on biosensor can be readily developed if a bioanalyte can decrease the solubility of AIEgen in the tested solution and induce the formation of AIE aggregates. Based on this solubility change strategy, Liu et al. successfully applied an AIE bioconjugate (**13**) for caspase-3 activity assay (Figure 7).[24] The bioconjugate is comprised of a hydrophilic caspase-specific Asp-Glu-Val-Asp (DEVD) peptide and a hydrophobic TPE unit. Because of the high hydrophilicity of the DEVD peptide, **13** shows a very weak fluorescence in buffer solution. However, the probe

displays a significant fluorescence enhancement in the presence of caspase-3. The enzyme cleaves the DEVD peptide, generating a hydrophobic residue (TPE-K). Since TPE-K shows a poor solubility in buffer solution, it tends to form aggregates and hence turns on the emission of its TPE unit. In this way, **13** enables light-up monitoring of caspase-3 activity in both solution and cells with high sensitivity and excellent selectivity.

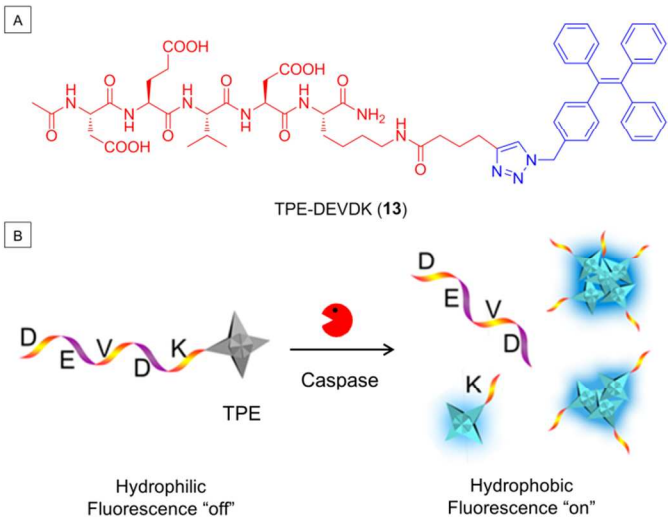

Figure 7. Schematic illustration of TPE-DEVDK (**13**) for caspase activity study based on the change in hydrophilic-hydrophobic property. Adopted with permission from ref. 24. Copyright 2012, American Chemical Society.

4.5 Photoinduced Electron Transfer

Photoinduced electron transfer (PET) has been widely utilized as mechanism for developing fluorescent turn-on biosensors, which fluoresce only in the presence of targeted analytes. The rational design of a PET biosensor comprise of a fluorophore, a spacer and a receptor. In the absence of analyte, photoexcitation of the probe leads to an electron transfer from the receptor to the fluorophore, which consumes the energy of the excitons through nonradiative relaxtion channels and hence renders the probe non-emissive. When a specific analyte binds to the receptor, excitation of the probe generates fluorescence because the energy level of the receptor is now lower than the fluorophore. This shuts down the electron transfer process and thus makes the probe emissive. Most of the PET turn-on biosensors developed so far are based on conventional fluorophores and therefore operate in the single molecular state due to the ACQ effect. On the contrary, the efficient light emission of AIEgens in the solid state makes them promising to be sensitive solid-state PET biosensors. A pioneer work of a PET sensor that operates in both AIE and PET mechanisms is illustrated in Figure 8. A maleimide-functionalized TPE derivative **14** shows no emission in both solution and aggregated states due to the PET quenching from the maleimide unit.[25] When the thiol group in cysteine reacts with the maleimide functionality through the thiol-ene click reaction, the PET process between the TPE and maleimide units is blocked. As a consequence, the emission of TPE is recovered. Taking advantage of the AIE effect, the detection can be performed in solid plates with high sensitivity. Except cysteine, other amino acids without thiol group exert no influence on the fluorescence of **14**, which is indicative of its high selectivity.

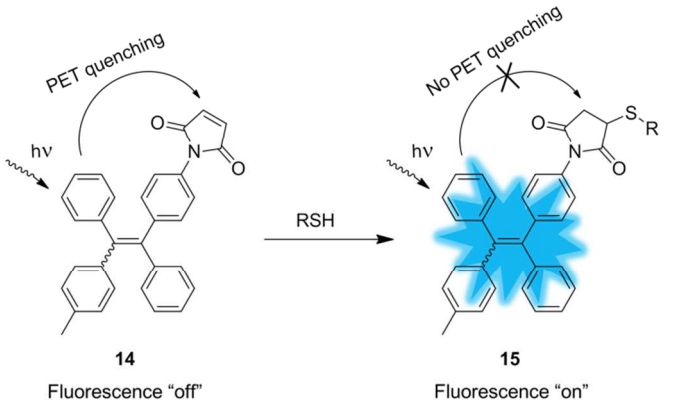

Figure 8. The design principle of fluorescent "turn on" biosensor **14** for thiol detection based on photoinduced electron transfer (PET) process. Adopted with permission from ref. 25. Copyright 2010 Wiley-VCH.

4.6 Cyclization or Coordination

According to the RIM mechanism, any perturbation that can rigidify the conformation of AIEgens can turn on their emission. With such regard, conformational locking the rotatory motors of AIEgens can be used as a strategy for turn-on sensing of analytes. For example, we have developed a fluorescent turn-on sensor from **1** for glucose detection based on the cyclization and oligomerization reactions of two diol units in glucose with two boronic acids in **1**.[12] Shinkai et al. utilized a zinc–dipicolylamine functionalized TPE derivative (**16**) to realize dicarboxylate sensing (Figure 9).[26] The zinc-dipicolylamine groups endow **16** with good water solubility and driving force to bind with carboxylates. In aqueous buffer, **16** shows no fluorescence owing to the complete consumption of the energy of the excitons by the active rotation of the phenyl rings. It, however, radiates strongly upon addition of dicarboxylates but remains nonemissive in the presence of monocarboxylates. The fluorescence enhancement is attributed to the cyclization of **16** with dicarboxylates, which rigidifies its conformation and activates its RIM process. The probe is also capable of differentiating dicarboxylate stereoisomers. For example, the adducts of **16** with L-tartaric acid and D-tartaric acid emit circularly polarized lights with opposite directions.

5. Applications

In principle, the AIE effect can be utilized to develop useful biosensors whenever the RIM process is involved, with possibilities limited only by our imagination. Thanks to the enthusiastic efforts of the researchers, many new AIEgens have been developed and utilized as fluorescent biosensors with high sensitivity and selectivity. In this session, we summarize the recent work on AIE biosensors in different categories.

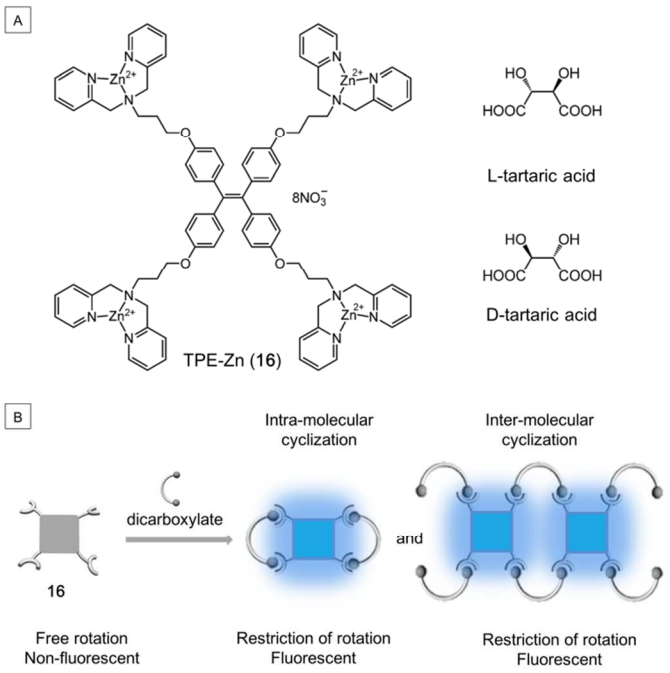

Figure 9. (A) Chemical structures of TPE-Zn (**16**) and chiral tartaric acids. (B) The design principle of fluorescent turn-on sensor for dicarboxylate detection based on cyclization mechanism. Adopted with permission from ref. 26. Copyright 2014 Wiley-VCH.

5.1 Carbohydrates

D-glucose is a basic necessity for living organisms and a main energy source for biological processes. Its level in body fluids such as blood and urine has been considered as important biomarkers for several diseases. Zhang et al. established a selective fluorescent turn-on sensor for the detection of D-glucose in aqueous solution by using arylboronic pinacoester-functionalized TPE with the aid of glucose oxidase (GOx).[27] The GOx oxidized the glucose to generate hydrogen peroxide, which subsequently oxidized the arylboronic pinacolester group of the probe to give an insoluble but emissive TPE derivative.

Heparin is a negatively-charged polysaccharide and has been used as anticoagulant. Monitoring its level during surgery is of important to prevent thromobosis or hemorrhage. Recently, our group has developed a water-soluble cationic fluorene-based AIE probe (**17**) for heparin detection (Figure 10).[28] The probe exhibits no emission in aqueous solution. It, however, becomes a strong emitter in solution with heparin, while it gives no response to the presence of chondroitin-4-sulfate (ChS) and hyaluronic acid (HA). The sensitivity and selectivity of the probe in heparin sensing can be further improved by integration of graphene oxide (GO). Addition of GO into the solution of **17**/ChS and **17**/HA disassembles the complexes and subsequently weakens the light emission. Since heparin shows a stronger affinity to bind with **17** than GO, the emission of the complex is preserved. Since the emission of **17**/GO turns on only in the presence of heparin, this allows light-up visual discrimination of heparin from its analogues such as ChS and HA. The linear light-up response enables heparin quantification in a broad dynamic range with a low detection limit.

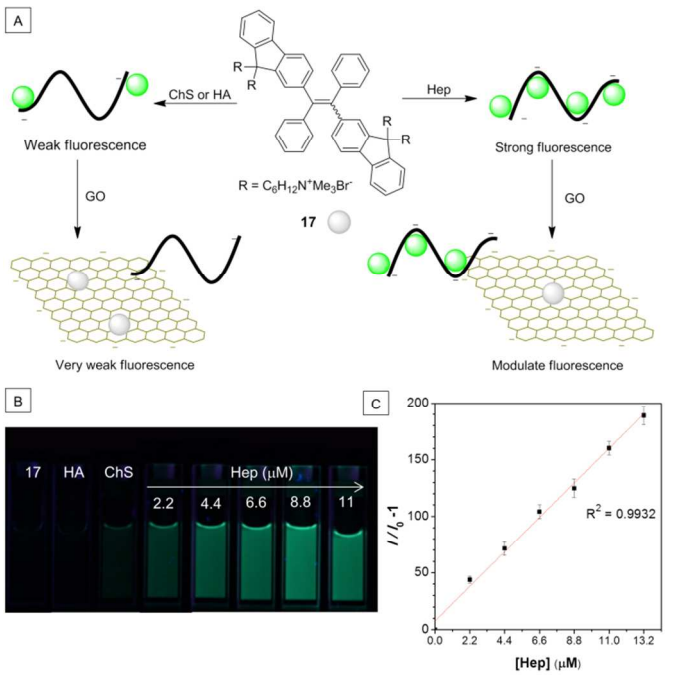

Figure 10. (A) Schematic illustration of heparin (Hep) and its analogues, chondroitin-4-sulfate (ChS) and hyaluronic acid (HA) detection with **17** and graphene oxide (GO). (B) Fluorescent photos of **17**/GO in PBS buffer in the absence and presence of HA, ChS or Hep taken under 365 nm UV illumination from a hand-held UV lamp. Concentration: 12 μM (**17**), 11 μM (HA and ChS), 2.2–11 μM (Hep), 48 μg/mL (GO). (C) Calibration curve for Hep quantitation. Concentration: 12 μM (**17**), 48 μg/mL (GO); λ_{ex}: 365 nm.

5.2 Lipids

Cardiolipin (CL) is a unique phospholipid exclusively found in inner membrane of mitochondrion. CL consists of four unsaturated acyl chains and a polar head with two negative charges. It plays important roles in regulating the function of mitochondrion such as respiration and apoptosis. The depletion of CL is critically indicative of aging, Barth syndrome, and a number of diseases associated with the mitochondrial respiratory functions.

Our group has synthesized a water-soluble cationic AIEgen (**18**) for CL detection and quantification (Figure 11).[29] With the aid of the four quaternary ammonium substituents, **18** is completely soluble in water and is almost nonfluorescent in aqueous solution. Its emission was enhanced dramatically when incubated in the CL-containing vesicle (TOCL), but remains weak in the CL-free vesicle (DOPC) as well as other major lipids found in the mitochondrial membrane. With an increase in the ionic strength of the solution, no fluorescence signals are recorded, indicating that the fluorescence enhancement is mainly attributed to the electrostatic attraction between **18** and the CL-containing vesicles rather than the hydrophobic effect. The fluorescence intensity is proportional to the concentration or fraction of CL, which enables quantitative analysis and visualization of isolated mitochondria. Compared with 10-nonyl acridine orange, which is a commercially available probe

for CL sensing, **18** offers much higher sensitivity and selectively as well as simple operation.

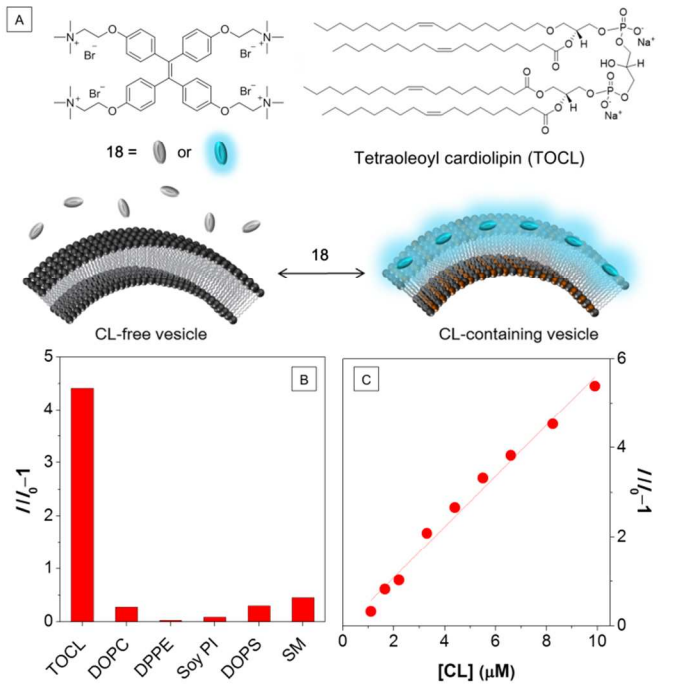

Figure 11. (A) Schematic illustration of detection of cardiolipin (CL) in lipid vesicle with **16**. (B) Change in fluorescence intensity of **18** with vesicles of different lipid contents (left to right: 20% tetraoleoyl cardiolipin (TOCL), 100% 1,2-dioleoyl-sn-glycero-4-phosphocholine (DOPC), 40% 1,2-dipalmitoleoylsn-glycero-3-phosphoethanolamine (DPPE), 2% L-α-phosphatidylinositol (soy PI), 1% 1,2-dioleoyl-sn-glycero-3-phospho-L-serine (DOPS), and 2% N-hexanoyl-D-sphingomyelin (SM); the rest of each type of vesicle is filled by DOPC. (C) Linear region of the ($I/I_0 - 1$) value vs CL concentration. [**18**] = 10 μM; λ_{ex} = 350 nm. Adopted with permission from ref. 29. Copyright 2014, American Chemical Society.

5.3 Amino acids

Amino acids are the structural units of proteins. Each of them is essential and indispensable as they play different roles in biological processes. It is important but challenging to develop fluorescent biosensors for the detection and recognition of individual amino acid as they show a high similarity in structure. Cysteine (Cys) is the only amino acid with a thiol functional group and serves as a unique unit in protein construction, enzyme active sites and cofactors.[30] Many fluorescent sensors have been explored to detect Cys based on the cyclization of Cys with the aldehyde functionality to form thiazolidine, which changes the electronic structures of the probes and thereby alters their fluorescence and color. However, this strategy provides little discrimination and selective on Cys over its analogue, homocysteine (Hcy) because they possess a similar structure and show almost the same reactivity towards aldehyde.

Recently, we have developed a fluorescent biosensor that is capable of discriminating Cys from Hcy by taking advantage of aggregate formation of reaction-dependent product. An aldehyde-functionalized silole **19** can selectively react with Cys and Hcy to form thiazolidine and thiazinane derivatives in the presence of diverse amino acids (Figure 12).[31] Remarkably, the reaction rate of **19** with Cys is significantly faster than with Hcy. Strong fluorescence was recorded when **19** was incubated in Cys solution for one hour, while only a weak fluorescence was emitted from Hcy solution. Owing to the difference in the reaction kinetics, Cys can be discriminated from the fluorescence response.

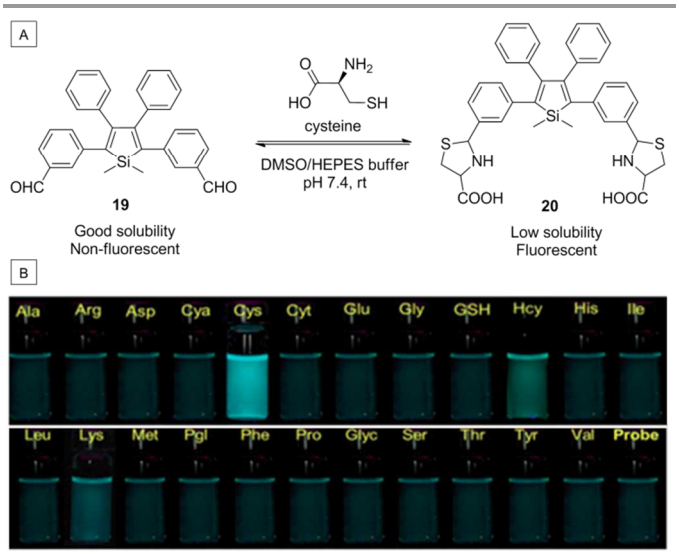

Figure 12. (A) Mechanistic representation of the detection of cysteine (Cys) by **19**. (B) Fluorescent images of **19** to diverse amino acids (Ala, Arg, Asp, Cys, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Ser, Thr, Tyr, Val, Pro, Hcy, Pgl and GSH), glucose or Glyc and protected Cys [Fmoc-Cys(Trt)-OH, or Cyt and N-acetyl-L-Cys, or Cya] in the mixture of DMSO and 10 mM HEPES buffer (6/4 by volume, pH 7.4). [**19**] = 25 mM, [analyte] = 2.5 mM; λ_{ex} = 356 nm.

5.4 Proteins

Protein fibrillation or amyloidosis is associated with a variety of pathologic conditions. The excessive accumulation of protein fibrils may cause many neurodegenerative diseases such as Alzheimer's and Parkinson's diseases.[32] Because of its great health implications, there is an urgent need to develop efficient probes to detect the formation of protein fibril in the early state and monitor the protein fibrillation process. Thioflavin T (ThT) is a well-known dye for such application. However, the probing process is normally carried out in dilute solution because of the ACQ effect of the dye molecule, which leads to poor sensitivity and false-positive signal. On the contrary, the emission of AIEgens is enhanced by solution thickening, and such characteristic makes them suitable for such study.[22,23,33–37] A water-soluble sulfonated TPE derivative (**11**) was developed and applied in studying and monitoring the insulin fibrillation process (Figure 13).[32] The AIEgen **11** was nonfluorescent when admixed with the native form of insulin but it became emissive once the fibrils were formed. The emission of **11** increased linearly with increasing the fraction of fibrillar insulin, indicating that **11** can be used for quantitative assay of fibrillar insulin. Molecular dynamics simulations and docking model studies reveal that the molecules of **11** were prone to bind to the partially unfolded form of insulin, instead of its native form, via hydrophobic interaction.

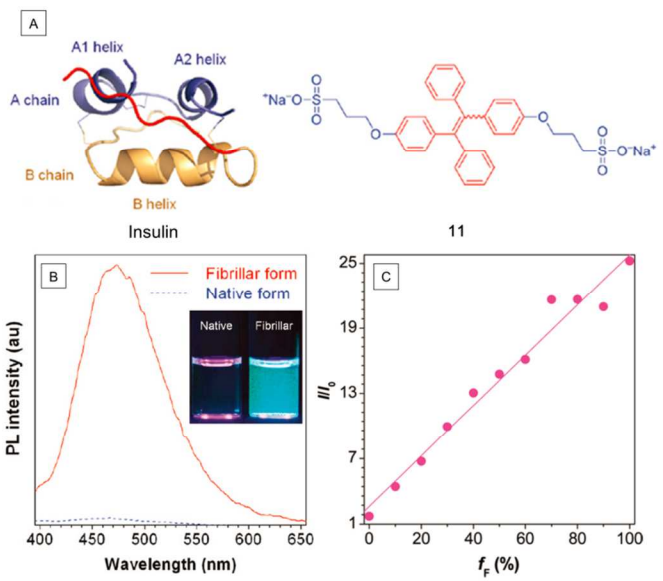

Figure 13. (A) Crystal structure of bovine insulin (PDB ID: 2ZP6) and chemical structure of **9**. (B) PL spectra of **11** in the presence of native and fibrillar forms of bovine insulin. The amyloid fibrils were obtained by heating an insulin solution in a pH 1.6 buffer at 65 °C for 20 h. PL measurements were performed in a pH 7 buffer at [**11**] = 5 μM, [insulin] = 5 μM, and λ_{ex} = 350 nm. Inset: photographs of mixtures of **11** with native and fibrillar forms of insulin taken under 365 nm UV light illumination. (C) Plot of PL intensity of **11** in insulin mixture versus the molar fraction of fibrillar insulin (f_F). The total protein concentration (5 μM) was kept constant in each run. I_0 is the PL intensity in the absence of insulin. [**11**] = 5 μM; λ_{ex} = 350 nm. Adopted with permission from ref. 33. Copyright 2012, American Chemical Society.

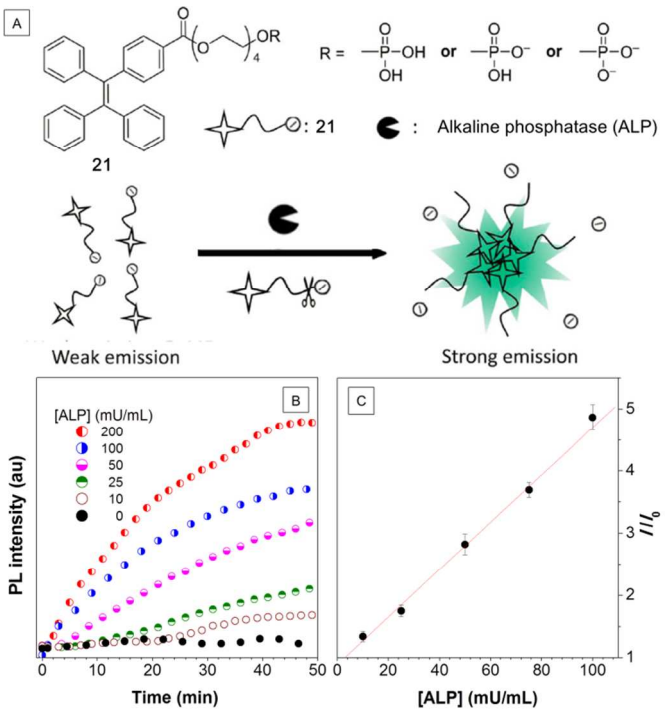

Figure 14. (A) Schematic illustration of sensing mechanisms of **21** in alkaline phosphatase (ALP) activity assay. (B) Time-dependent PL intensity of **21** in 10 mM Tris–HCl buffer solution (pH 9.6) at 492 nm versus the hydrolysis reaction time in the presence of different concentrations of ALP at 25 °C. (C) Plot of relative fluorescence intensity (I/I_0) at 492 nm after incubation with different concentrations of ALP for 30 min. [**21**] = 10 μM; λ_{ex} = 330 nm.

5.5 Enzymes

Enzymes are responsible for numerous metabolic processes. Exploring their functions and determining their activities can help researchers gain deep insights into metabolic mechanisms. Enzymes like alkaline phosphatase (ALP), caspase and acetyl cholinesterase (AChE) are key mediators of important biological processes and their abnormal levels will induce severe impair. Enzyme activity assay thus plays a critical role in diagnostics and clinic treatments. Inspired by the unique property and outstanding performances of AIEgens, many research groups have successfully applied them for various enzymatic activity assays such as ALP, caspase-3/7, histone deacetylase, AChE, trypsin, carboxylesterase and nuclease,[14,24,38–44] based on the mechanisms of electrostatic interaction and solubility change.

A phosphorylated TPE derivative **21** has been developed for ALP activity assay.[45] The designed probe is comprised of a phosphate group, a tetraethylglycol linker and a hydrophobic TPE unit. In aqueous solution, the probe is nonfluorescent but starts to emit upon addition of ALP. Such fluorescence turn-on response is ascribed to the conversion of the phosphate group to hydroxyl functionality in **21** by ALP and the aggregation of the insoluble enzymatic residue (Figure 14). With increasing the ALP concentration, more obvious fluorescence change was observed. The linear light-up response of the probe enables ALP quantitation in a broad range which covers the physiological level of ALP activity in the human serum.

5.6 Nucleic Acids

The detection of nucleic acids, such as DNA and RNA, is of great importance for genetic engineering, forensics, and bioinformatics. We have developed a versatile fluorescent probe (**18**) for nucleic acid detection and quantitation.[46,47] In aqueous solution, the nonemissive TPE derivative is induced to emit upon its binding to DNA/RNA through electrostatic attraction on account of its multiple positive charge.[49-50] Such light-up feature enables quantitation and visualization of nucleic acids in aqueous media and electrophoretic gel.

Nucleic acid hybridization with a fluorescent probe is a practical way to detect a complementary target sequence in a complex nucleic acid mixture. Some fluorescent turn-on probes based on molecular beacon and FRET techniques have been developed and demonstrated successfully for sequence specific DNA detection. However, they require a dual labelling and thus complicated probe design. Liu et al. developed a simple singly labelled DNA probe (**5**) with AIE characteristic for specific DNA hybridization detection (Figure 15).[16] The probe is comprised of an oligonucleotide sequence and a TPE fluorogen. In aqueous solution, the probe displays a weak fluorescence due to the active intramolecular motion. In the presence of complementary oligonucleotides, the probe is hybridized. The resulting double helix structure hampers the free rotation and vibration of the TPE unit, and hence turns on its emission. The fluorescence of the probe/oligonucleotide adduct is weakened with the extent of mismatch in the nucleotide sequence.

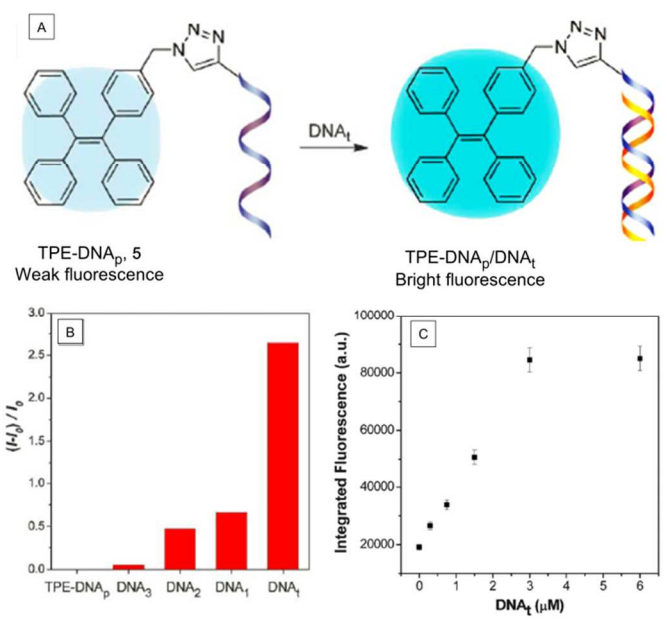

Figure 15. (A) Schematic illustration of an AIEgen in nucleic acid hybridization detection. DNA_p and DNA_t stand for the probe DNA and the target complementary DNA, respectively. (B) Comparison of the solution fluorescence intensities for the probe and that in the presence of different DNA sequences. *I* is the integrated area under the fluorescence spectrum, I_0 is the background fluorescence of **5** alone. (C) Integrated fluorescence of **5** (3 μM) in response to different amounts of DNA_t (0.3, 0.75, 1.5, 3, 6 μM) added.

Conclusions

AIEgens are a class of luminescent materials which are nonemissive in solution but are induced to emit intensely by aggregate formation. The AIE effect permits the use of dye solutions with any concentration for bioassays and enables the development of turn-on biosensors by taking the advantage of luminogenic aggregation. In this Tutorial Review, we overviewed the discovery and mechanism of AIE phenomenon. We proposed that the RIM process is the main cause for the AIE effect. Through mechanistic decipherment of the photophysical processes, a large variety of AIEgens ranged from small molecules to polymers as well as bioconjugates are designed and utilized as biosensors. The AIE biosensors enable the detection of specific bioanalyses through different working principles, such as electrostatic interaction, hydrogen bonding, hydrophobic effect, solubility change, PET and cyclization as well as coordination. In some sensory systems, there may be more than one strategy worked simultaneously. In-depth discussion of several biosensing systems for carbohydrates, lipids, amino acids, proteins, enzymes and nucleic acids are presented. Due to the space limitation, only part of AIE-based biosensors is discussed here. More examples are given in the Electronic Supplementary Information (ESI). We hope this review can stimulate new ideas and inspire new endeavors in this emerging area of research.

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Electronic Supplementary Information (ESI) available
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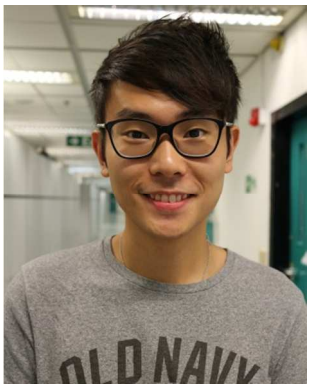

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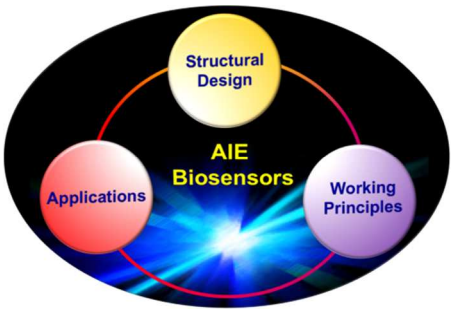
Table of contents entry

Biosensing of Luminogens with Aggregation-Induced Emission Characteristics

Ryan T. K. Kwok, Chris W. T. Leung, Jacky W. Y. Lam and Ben Zhong Tang*



This tutorial review outlines the concept of aggregation-induced emission and its utility in biosensing applications.