Cite this: DOI: 10.1039/c0xx00000x

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ARTICLE TYPE

The role of "disaggregation" in optical probe development

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

"Aggregation-caused signal change" is a well-established mechanism by now and has been widely used as the basis for optical probe and sensor development. Compared to aggregation, its reverse process, disaggregation, has received much less attentions and not properly discussed in the literature so far. With the less established paradigm or mechanism, although some of the reported sensors and probes seem to work through disaggregation phenomena, the proper interpretation of the results and applying the concept to novel probe development was seriously hampered. The process from aggregation to disaggregation generally causes a recovery or enhancement of fluorescence signals, and thus provides an interesting new path to design "turn-on" probes. This tutorial review will provide the balanced comparison between aggregation and disaggregation mechanism, and focus on the less explored advantages of "disaggregation" as a novel sensing mechanism and its recent applications in probe development.

Key learning points

(1) Concept, electronic and spectroscopic properties, and the exciton theory of forming H-aggregate and J-aggregate.
(2) How aggregation/disaggregation affect the properties of probes
(3) Application of the process from aggregation to disaggregation as a common mechanism in designing novel probes
(4) The conditions to form aggregation/disaggregation
(5) Technique or characteristics to confirm the formation of aggregates/disaggregation

Introduction

The need for recognizing and sensing environmentally and biologically important species has led large efforts towards useful sensor and probe development. Compared with traditional analytical methods [*e.g.*, liquid-chromatography (LC), gas-chromatography (GC) and mass spectrometry (MS)] probes exhibit distinct advantages, such as straightforward signal amplification, technical simplicity, fast processing time and low costs, which all add up to their tremendously huge impact on the sensing field. Despite the striking number of probes developed each year,[1] their sensing mechanisms are rather restricted to only a few, including photo-induced electron transfer (PET),[2] intramolecular charge transfer (ICT), resonance energy transfer (RET),[3] aggregation-induced emission (AIE), etc. These sensing mechanisms were utilized over and over in the sophisticated design of novel probes by changing their recognizing, reporting or both motifs. Compared to the efforts placed on designing novel probes, the exploration of new sensing mechanism is relatively limited.The need for novel sensing mechanism to open up new windows of probe development is ever increasing. In this tutorial review, we will discuss a novel sensing mechanism based on signal amplification caused by disaggregation from aggregated probes. Although a few research papers have already been reported utilizing this mechanism to develop novel probes, the role of "disaggregation" in probe development was never systematically discussed before. As a result of the poorly established concept, a proper interpretation of the data or an active application of disaggregation for novel probe design has been hampered. Especially, in some cases, although the mechanism of sensing process is arisen from the disaggregation of probes, the researchers may not aware it. Throughout this review we will compare the aggregation and disaggregation in a balanced way, and further discuss the design, synthesis, and performance of the probes based on disaggregation as a novel route for probe development.

Aggregation *vs.* Disaggregation

Aggregation refers to the formation of clusters of particle group from individual ones (monomers). Aggregation of probes at high concentration is a frequently encountered phenomenon, especially in aqueous solution, due to their hydrophobic nature and intermolecular van der Waals-like attractive forces among the

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molecules.[4] Although the solution may appear homogeneous, the compounds are "dispersed" in the solvent rather than "dissolved".

H-aggregate and J-aggregate

Probe aggregation in which monomers are arranged in a regular fashion are of particular interest, for example H-aggregate and J-aggregate.[5] These aggregation patterns possess unique electronic and spectroscopic properties which exhibit distinct changes in the absorption band compared with monomers. Both H-aggregate and J-aggregate are one-dimensional molecular arrangement, while the transition moments of monomers in J-aggregate are aligned parallel to the line joining their centres (end-to-end), and the transition moments of monomers in H-aggregate are aligned parallel but perpendicular to the line joining their centres (face-to-face) (Fig. 1). The characteristic feature of the absorption spectrum of J-aggregate is that it exhibits a narrow peak (J-band) bathochromic shifted (red-shifted) with respect to the monomer absorption. The absorption spectrum of H-aggregate consists of a hypsochromic shifted (blue-shifted) band with respect to the monomer absorption, which is generally not as narrow as the J-band. The energy shifts of the absorption spectra of both H-aggregate and J-aggregate has been explained by exciton theory (Fig. 1).[6] According to it, dye molecule is regarded as a dipole, and through the interaction of the two transition dipoles, the excitonic state of the dye molecules splits into two levels (S_1 and S_2). When the two dipoles are face-to-face (H-aggregate), the parallel dipoles repel each other and get the higher energy state, while the anti-parallel dipoles attract each other and lower the energy of that state. In contrast, when the two dipoles are end-to-

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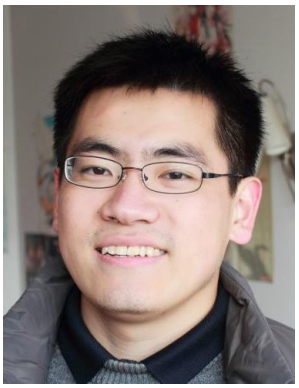
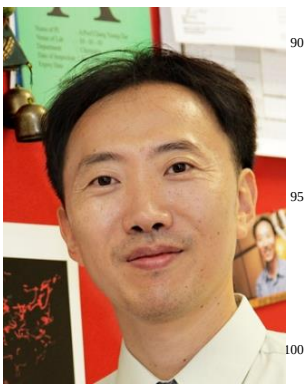


Wang Xu

Wang Xu was born in 1988 in Suzhou, a beautiful garden city of Southeast China. After receiving his Bachelor's degree in Suzhou University in 2010, he moved on to work in Prof. Young-Tae Chang's lab for Master and PhD degree, where he was introduced to the fantastic world of fluorescence. He mainly conducted water quality control using diversity-oriented fluorescence sensors and has published one paper regarding "traffic-light" fluorescent caffeine sensor. Currently he is working on development of fluorescent libraries for diversified water contaminants analysis.

Liyun Zhang

Liyun Zhang grew up in Shandong, People's Republic of China. She received her B.Sc. degree of chemistry in 2005 and her Ph.D. degree from the University of Science and Technology of China in 2009. After two years postdoctoral research at National Synchrotron Radiation Laboratory, University of Science and Technology of China, she was appointed assistant professor at Hefei Institutes of Physical Science, Chinese Academy of Sciences and promoted to associated professor in 2012. She is currently carrying out her visiting research work at Professor Chang Young-Tae group of National University of Singapore. Her research interests focus on the development of novel fluorescent sensors with disaggregation-induced emission characteristics.



Young-Tae Chang

Young-Tae Chang studied chemistry in Pohang University of Science and Technology (POSTECH, Korea) and received his B.S. in 1991 and Ph.D. in 1997 under the supervision of Prof. Sung-Kee Chung. He did his postdoctoral work with Prof. Peter Schultz at UC Berkeley and The Scripps Research Institute. In 2000, he was appointed assistant professor at New York University and promoted to associated professor in 2005. He received the NSF Career award in 2005 and his research interests have been chemical genetics, molecular evolution, and artificial tongues. In September, 2007, he moved to National University of Singapore and Singapore Bioimaging Consortium. He is running Medicinal Chemistry Program of NUS as the leader, and Lab of bioimaging Probe Development at SBIC, Biopolis. He published more than 230 scientific papers, 3 books and filed 40 patents so far.

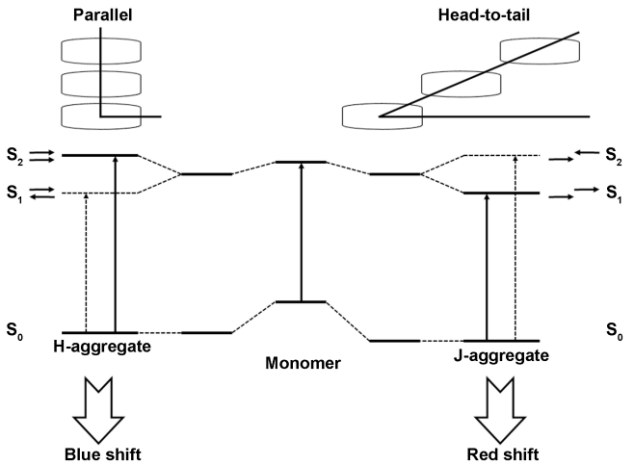


Fig. 1 Schematic presentation of the relationship between dye molecule arrangement and spectral shift of H-aggregate and J-aggregate based on the molecular exciton theory.

end (J-aggregate), the syntropic dipoles get the lower energy state, while the reversed dipoles increase the energy of that state. In addition, transition is only allowed when the transition dipole moment is greater than zero (*i.e.*, S_2 in H-aggregate and S_1 in J-aggregate). As a result, it exhibits a blue-shift in the absorption spectrum of H-aggregate, while a red-shift for J-aggregate.

Aggregation in probe development is generally unfavoured because it usually weakens or quenches the readout signal, especially for fluorescence.[7] The impact of aggregation on fluorescence is a phenomenon widely known as "concentration quenching", which is also frequently referred to as "aggregation-caused quenching" (ACQ). A structural reason for the ACQ effect is that fluorophores typically comprise of planar aromatic rings since organic fluorescence is mainly dictated by electronic conjugation and aromatic rings were introduced to increase the extent of π-conjugation. Such planar structures of fluorophores possess higher chances to form excimers or exciplexes when they are in relatively high concentration, and therefore results in ACQ phenomenon.[8] ACQ is an obstacle for the real-world application of probes, as it limits the efficiency of the emitting signal. The quenching of readout signal in probe development was also reported for other technique, such as NMR.[9]

Albeit a generally unfavoured phenomenon, in some conditions, aggregation can be smartly utilized to design probes.[10] Two major categories have been summarized: First, the aggregation of probes is induced by the detecting target, hence the resulting ACQ effect renders signal attenuation for designing quenching probes.[11] The second type arises from a less common phenomenon, aggregation-induced emission (AIE), which was first reported by Tang and co-workers.[12] AIE process refers to a series of special compounds, which is flexibly rotated in dissolved state and hence faintly emissive, but restricted with intramolecular rotation therefore highly emissive in the aggregate state. Since the first discovery of AIE phenomenon, many research groups have been working on the design and synthesis of new AIE luminogens, with a variety of novel AIE systems developed, and a number of practical applications explored.[13] However, among the probe families, compounds which possess AIE properties are still a small portion, while ACQ effect is a much common phenomenon.

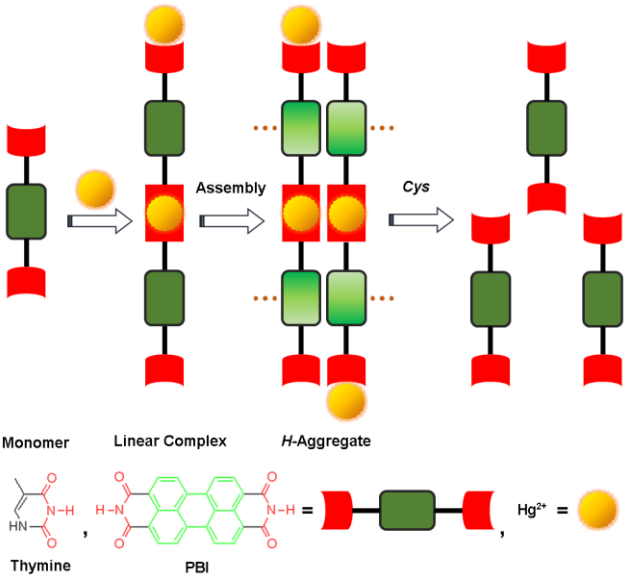


Fig. 2 Schematic presentation of Hg^{2+} induced aggregation of PBI and the disaggregation of aggregates in the presence of Cys. Adapted with permission from ref *17*. Copyright 2010 The Royal Society of Chemistry.

Aggregation is normally an irreversible process. Once aggregates have formed, they will not be easily disrupted unless their environment is dramatically changes. Disaggregation is the reverse process of aggregation, in which the aggregates dissociate to monomers. Compared to the ACQ effect, disaggregation will enhance and recover the readout signal. Unfortunately, although inducing disaggregation is a potentially interesting way to design turn-on probes, it was neglected for long time.

Disaggregation from template-aggregates

The formation of aggregation is usually due to high concentration. However, in some cases, molecules can be deliberately brought together by a template (induced aggregation). Such a template can serve as an assembling core (*e.g.*, metal ion) or backbone (*e.g.*, nucleobases in DNA), and its disaggregation requires the break of the interaction between probes and templates in order to release monomers.

Disaggregation from metal-mediated aggregation

Some organic groups can form strong non-covalent interactions with metal ions, for example crown ethers, polyamines, macrocyclic amines, pyridines and acetates.[14] These groups are considered as recognizing motifs for metal ions and can be adapted to sense metal ions by incorporating with a reporter.[15] Adding metal ions to the well dissolved solutions of such probes can induce the formation of complexes, which results in aggregation and signal quenching. In these cases, metal ions behave as a template for aggregation. One example was reported by Shangguan and co-workers,[16] in which they described a highly selective phthalocyaninethymine conjugate sensor, tetra-(thymin-1-yl-acetamido)-phthalocyanine Zn(II), for Hg^{2+} detection based on target induced aggregation. This compound shows a UV-absorption spectrum with a split Q-band and strong fluorescence emission in DMF/water = 7:3 (v/v). Upon the addition of Hg^{2+},

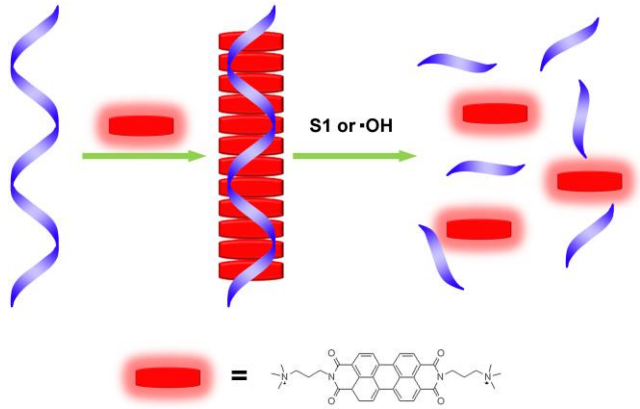


Fig. 3 Schematic presentation of the assay for ssDNA cleavage by S1 nuclease or ssDNA damage by hydroxyl radical based on the disaggregation from DNA-templated aggregate. Adapted with permission from ref *21*. Copyright 2011 The Royal Society of Chemistry.

the formation of thymine-Hg^{2+}-thymine (T-Hg^{2+}-T) complex induced aggregation of the sensor, resulting in decrease of the Q-band and quenching of fluorescence.

Target induced disaggregation from such "metal-coordinated aggregates" can be a novel sensing mechanism to design "turn-on" sensors. Jiang and co-workers reported a specific Hg^{2+}-coordinated perylene bisimide (PBI) sensor for the detection of cysteine (Cys) (Fig. 2).[17] Cys plays a critical rule in biological systems as an important structural and functional component of many proteins and enzymes.[18] Similar to the above-mensioned sensor, Hg^{2+} was found to be able to induce the aggregation of PBI, where monomers was arranged in a face-to-face fashion, forming the H-aggregates. Experimentally, 0.33 μM PBI in DMF/H_2O (9:1 v/v) showed a fluorescence band at 532 nm, which corresponds to the monomer emission. When Hg^{2+} was added, this emission band decreased due to the aggregation of PBI like a T-Hg^{2+}-T structure. Formation of H-aggregate was confirmed by the changes in the absorption and fluorescence spectra. Upon the addition of Cys or other thiol species, the Hg-imide interaction was replaced by the stronger Hg-S bond, resulting in the disaggregation of "PBI-Hg" aggregates and fluorescence recovery. There was a linear response of fluorescence intensity with respect to the concentration of Cys from 0.05 to 0.3 μM range, with a detection limit reaching as low as 9.6 nM. This sensor exhibited a high selectivity for thiol-containing amino acids over others.

Disaggregation from nucleic acid-mediated aggregation

Many fluorescent molecules are positively charged, such as rhodamine, cyanine and styryl, etc. They tend to aggregate through self-assembly mechanism in the presence of polyanion, (*e.g.*, nucleic acids) due to electrostatic interaction. In contrast, when the interaction is disturbed or weakened, the aggregates will undergo the process of disaggregation. Utilizing such mechanism, Ren and co-workers reported a DNA-templated small molecule ensemble for the label-free and real-time fluorescence turn-on detection of single-stranded DNA (ssDNA) cleavage by S1[19] nuclease or damage by hydroxyl radical[20] (Fig. 3). Both of the reactions are critically important in biological system. Especially, S1 nuclease is a widespread multifunctional endonuclease that

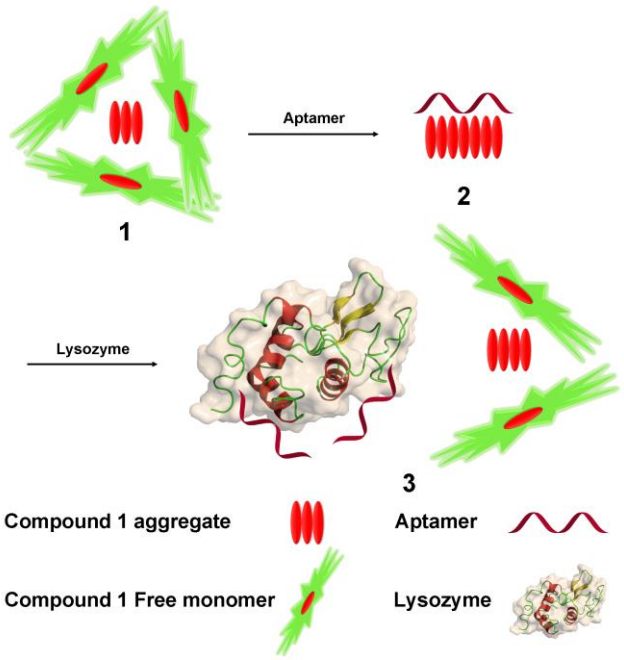


Fig. 4 Schematic presentation of the strategy for selective lysozyme sensing based on the aggregation of PTCDI mediated by DNA aptamer and disaggregation in the presence of lysozyme. Adapted from ref *22*. Copyright 2010 Wiley-VCH.

selectively digests ssDNA or RNA.[21] The authors prepared a perylene derivative which exhibited strong fluorescence in aqueous solution but was virtually nonfluorescent in the presence of nucleic acid due to the aggregation of compound induced by electrostatic interactions between the two ammonium cations and the backbone phosphate anions of DNA. When the ssDNA is cleaved by S1 nuclease or hydroxyl radical into small fragments, the electrostatic interactions between the perylene derivative and the fragmented DNA is weakened. Therefore, the compound undergoes the disaggregation process to release monomers into the solution, resulting in the recovery of fluorescence intensity. This assay provides a simple, sensitive, real-time and label free way for monitoring the cleavage of ssDNA by S1 nuclease and hydroxyl radical with a detection limit of 0.092 U mL^{-1} and 5.7 nM, respectively.

A similar strategy was employed by Yu and co-workers, utilizing the disaggregation of a perylene tetracaoboxylic acid diimide (PTCDI) for the lable-free and turn-on detection of lysozyme (Fig. 4).[22] In an aqueous solution, PTCDI, which contains two cations, exists in both the monomeric and the aggregated forms because of the intermolecular repulsive electrostatic interactions. Strong fluorescence can be detected due to the existence of the free monomer of dye. Similarly, aggregation of PTCDI can be induced by a nucleic acid aptamer, since nucleic acid contains multiple negatively charged phosphate functional groups in its backbone, strong electrostatic interactions between the dye and the nucleic acid cause the rapid binding. Here, anti-lysozyme aptamer (5'-ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG-3') was used as the aggregation template. The enhanced dye aggregation results in a significant decrease of fluorescence intensity. Upon the addition of lysozyme, specific binding of lysozyme to the anti-lysozyme nucleic acid

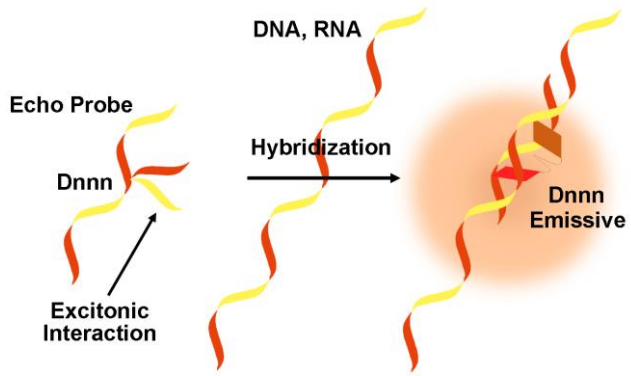


Fig. 5 Schematic presentation of an exciton-controlled hybridization-sensitive fluorescent oligonucleotide (ECHO) probe based on the disaggregation induced by hybridization. Adapted with permission from ref *24*. Copyright 2011 The Royal Society of Chemistry.

aptamer weakens the binding between PTCDI and the aptamer. As a result, the monomer of PTCDI was released, and a restoring of the fluorescence signal was observed. This method achieved a 70 pM detection limit for lysozyme with high selectivity.

In addition to electrostatic interactions, aggregation from monomers can also be designed by closely attaching two dye molecules together through covalent bond. Okamoto and co-workers reported the development of a series of NIR fluorescent probes to monitor mRNA in human cells based on an exciton-controlled hybridization-sensitive fluorescent oligonucleotide[23] (ECHO) (Fig. 5).[24] In this approach, two NIR fluorescent dye were covalently attached to a thymine base in a DNA strand. The emission from the probe was suppressed in the unhybridized state due to the formation of H-aggregate of the two fluorophores. After hybridization with the complementary strand, the formation of H-aggregate was disrupted, fluorescence signal enhancement from the separated monomeric probe therefore can be detected. This concept provides the first NIR probes possessing the function that the absorption and emission were controlled at various NIR wavelengths and facilitating the imaging of intracellular mRNA.

Disaggregation from self-aggregates

Other than the template-aggregates, in which probes are originally dissociated in the solution, and the aggregation is induced by additional components (*i.e.,* metal ion or nucleic acid), there is another big category of self-aggregates. As we addressed in the introduction part, many dye molecules tend to form aggregates in concentrated solutions. This certain solution, after intricate designing, can be used as a specific embryo to develop probes.

Disaggregation of squaraine dyes

Squaraines are a class of organic dyes with resonance-stabilized zwitterionic structures, which possess a strong absorption ($\varepsilon > 10^5$ L mol^{-1} cm^{-1}) with good photostability.[25] They exhibit strong absorption bands from visible to NIR region due to donor-acceptor-donor (D-A-D) type of charge transfer, and their photoproperties can be finely tuned by the substituents attached to the anilino nitrogen and the phenyl ring. These

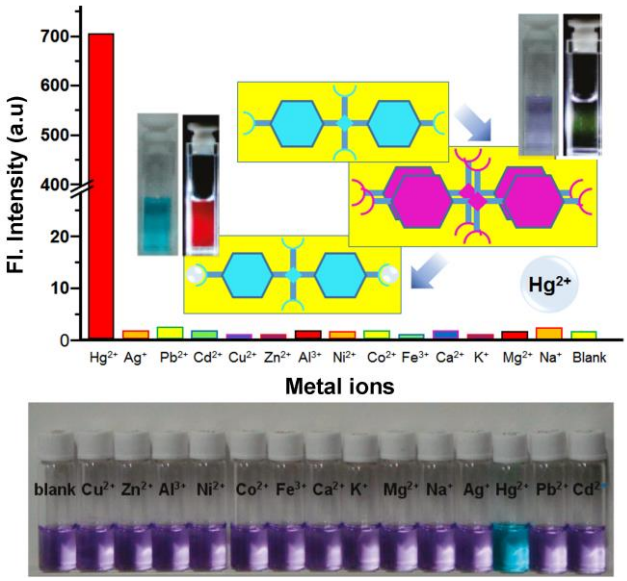


Fig. 6 Schematic presentation of Hg^{2+} detection based on the disaggregation of H-aggregate of SQ-1 (upper). Selectivity of SQ-1 to Hg^{2+} over other metal ions (lower). Adapted with permission from ref *26*. Copyright 2011 American Chemical Society.

favorable optical properties as well as their good sensitivities to environment make squaraines highly suitable as chemosensors. Especially, squaraine dyes have a high proclivity to form aggregates due to their extensively conjugated structures.

Chen *et al.* synthesized a novel squaraine-based chemosensor, SQ-1, by selecting (phenylazanediyl)-bis-(ethane-2,1-diyl)-bis-diethyl-carbamodithioate as a binding arm for Hg^{2+} (Fig. 6).[26] SQ-1 can form H-aggregate in AcOH-H_2O (40:60, v/v) resulting in a purple colour solution. The formation of H-aggregate was confirmed by the blue-shift in absorption spectrum from 644 nm to 548 nm. Upon the addition of Hg^{2+}, a visual colour change from purple to blue was observed, arising from the disaggregation of the H-aggregate. Job's plot study suggested the formation of SQ-1-Hg^{2+} complex with a 1 : 2 stoichiometry. The coordination of Hg^{2+} with the side dithiocarbamate arms induced steric hindrance and rendered disaggregation of SQ-1, which also resulted in a 700-fold fluorescence enhancement with a detection limit of 7.1 nM. This probe also possesses good selectivity over other metal ions (e.g., Pb^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, Al^{3+}, Ni^{2+}, Co^{2+}, Fe^{3+}, Ca^{2+}, K^+, Mg^{2+}, Na^+, and Ag^+) and high sensitivity allowing detection of Hg^{2+} in drinking water.

Subsequently, Chen *et al.* reported a more soluble unsymmetrical squaraine probe USQ-1 by replacing one of the binding arm of SQ-1 with an *N*,*N*-dibutylanilino group for Hg^{2+} detection in aqueous media based on the similar metal mediated disaggregation.[27] Differently, the monomer of USQ-1 exhibited an absorption peak at 644 nm, while the absorption of its aggregate appeared in the region from 550 to 750 nm in AcOH-H_2O (10 : 90, v/v), suggesting the formation of both H-aggregate and J-aggregation due to the unsymmetrical structure. The addition of Hg^{2+} caused a vivid color change from lilac to brilliant blue of USQ-1 solution, as well as a 10-fold fluorescence enhancement. Job's plot revealed the binding between USQ-1 and Hg^{2+} in a 1 : 2 stoichiometry. This probe also showed a good

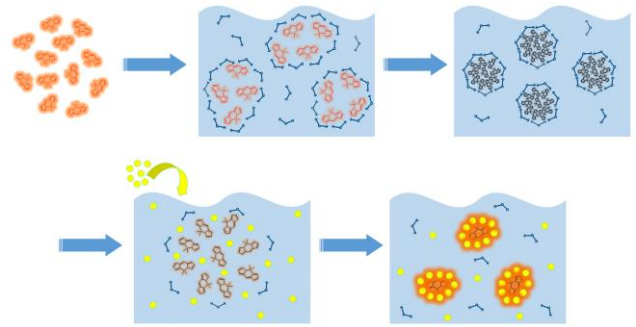


Fig. 7 Schematic presentation of GBL detection based on the disaggregation of Green Date in aqueous solution. Adapted with permission from ref *31*. Copyright 2013 The Royal Society of Chemistry.

selectivity over Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Al^{3+}, Mn^{2+}, Pb^{2+}, Cd^{2+}, Ag^+, Cu^+, and Zn^{2+} with a detection limit of 45 nM.

Disaggregation of BODIPY dyes

BODIPY dyes have superior photophysical properties, such as good photostability, high quantum yield, high extinction coefficient, narrow emission bandwidth and environmental insensitivity.[28] Additionally, the emission wavelength of BODIPY dyes is tunable through appropriate substitution, which makes them become surrogates for some traditional dyes, such as fluorescein, tetramethylrhodamine, Texas red and many others.[29] Another important property of BODIPY dyes is the overall lipophilicity and electrically neutral character, which allow them to be incorporated into lipophilic probes and induce minimal perturbation of functional properties of conjugates.[30] Most BODIPY dyes intend to form aggregation in polar solution due to their relatively hydrophobic nature.

Recently, Zhai *et al.* reported the development of a fluorescent probe for illicit date rape drug-gamma-butyrolactone (GBL) (Fig. 7).[31] GBL is the pro-drug of gamma-hydroxybutyric acid (GHB), and overdose of GBL may lead to dangerously low respiratory rates, unconsciousness, seizures, bradycardia and even death. Through a high throughput screening system, the authors discovered a BODIPY active ester (Green Date),[32] which exhibit fluorescence enhancement towards GBL. Green Date formed aggregate in aqueous solution, which was confirmed by light scattering experiment and ^{19}F NMR, resulting in fluorescence quenching. When the aqueous solution of Green Date was excited by a green laser pointer, green light can directly pass through instead of turning on the fluorescence of dye. In the presence of GBL, GBL molecule can insert into the aggregates of Green Date due to their relatively similar hydrophobicity, reducing the aggregation of Green Date. With the increasing concentration of GBL, eventually Green Date disaggregated into monomer with a fully turn-on of fluorescence. As a result, the aqueous solution of Green Date emitted orange fluorescence when it is excited with a green laser pointer. This approach provides a convenient detection way for GBL, which will help to prevent the drug-facilitated sexual assault problems.

Another example of utilizing the aggregation property of BODIPY compound in aqueous solution is the development of a fluorescent caffeine "traffic light" detector, reported by Xu *et al* (Fig. 8).[33] Caffeine has attracted abundant attention due to its extensive existence in beverages and medicines. The authors developed a novel aqueous phase fluorescent caffeine sensor, based on BODIPY structure and named it Caffeine Orange, which exhibits a 250-fold fluorescence enhancement upon caffeine activation and high selectivity. Caffeine Orange formed aggregate in aqueous solution, which resulted in the fluorescence quenching. In the presence of caffeine, the aggregation of Caffeine Orange was attenuated due to their π-stacking and hydrogen-bonding interaction, which was confirmed by NMR, fourier transform IR, light scattering and transmission electron microscopy (TEM) experiments. Based on TEM figures, they proposed that Caffeine Orange served as a core that attracted caffeine molecules to destroy their intrinsic aggregates. With the increasing amount of caffeine, the color of Caffeine Orange solution turned from green to deep orange under the irradiation of a green laser pointer, which is similar to the color changes of traffic lights. This property was further expanded to develop a real-life caffeine traffic light detector, in which "red" color represents a stop sign for non-caffeine users, "yellow" color represents a warning sign and "green" color indicates a safe zone.

Hamachi and co-workers introduced a novel mechanism for the specific protein detection based on the aggregation property of BODIPY (Fig. 9).[34] The authors prepared a fluorescent probe consisting of a fluorophore (BODIPY), a linker part and a recognition ligand. This probe formed self-assembled J-aggregate in buffer, resulting in red-shift of the absorption maximum and

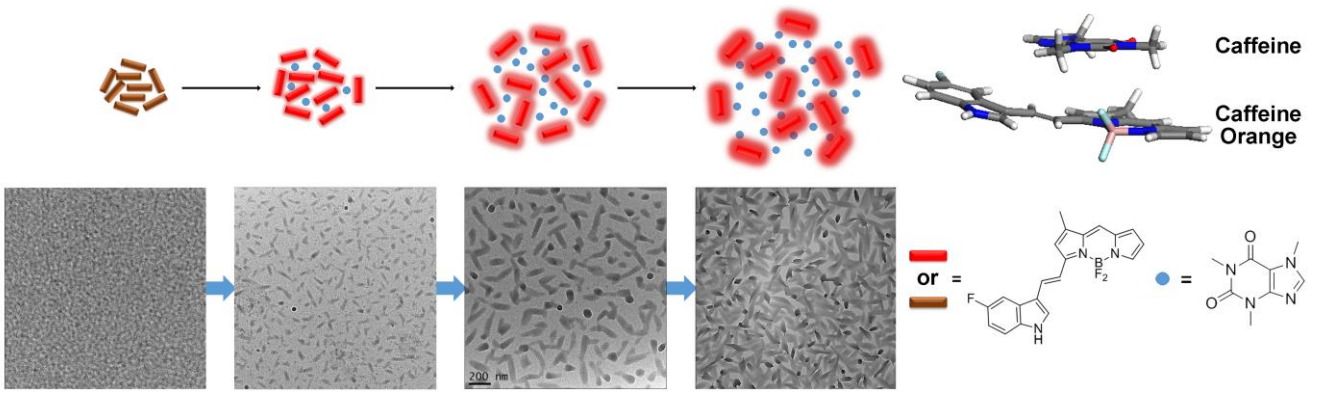


Fig. 8 Schematic presentation of caffeine detection based on the disaggregation of Caffeine Orange in aqueous solution. Adapted with permission from ref *33*. Copyright 2013 Nature Publishing Group.

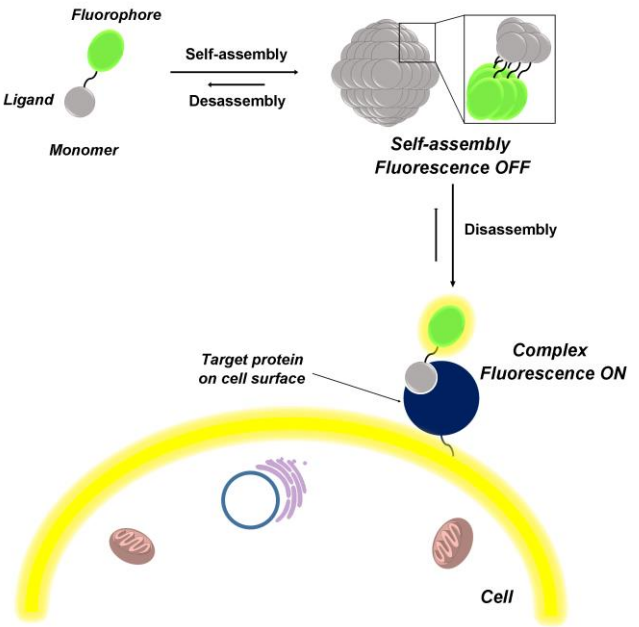


Fig. 9 Schematic presentation of recognition-driven disaggregation approach to design turn-on fluorescent probe for cell surface protein imaging. Adapted with permission from ref *35*. Copyright 2012 American Chemical Society.

quenching of fluorescnece. The formation of J-aggregate was further confirmed by atomic force microscopy (AFM) and dynamic light scattering measurements, which revealed the size of aggregates ranging from 100 to 200 nm. When the probe bind to the specific protein (human carbonic anhydrase I, hCA), a clear fluorescence turn-on signal was detected, as well as the recovery of absorption spectrum, due the recognition-driven disaggregation of the probe. This probe provides a very good selectivity to hCA with a detection limit of 70 nM. Remarkably, this system can also be expanded to other proteins by varying the ligand part of the probe (e.g., biotin ligand for avidin detection and benzamidine derivative for trypsin detection). Subsequently, the authors extended the range of applicable fluorophores to more hydrophilic ones (*i.e.*, fluorecein and rhodamine) in order to finely tune the aggregation properties of the probe, which would enhance the flexibility of the probe design.[35] Through a similar mechanism, the authors succeeded in developing probes, that allowed specific visualization of overexpressed folate receptor (FR) amd and hypoxia-inducible membrane-bound carbonic anhydrases (CA) on the surface of live cancer cells, both of which are tumor specific biomarkers.

Disaggregation of Cyanine dyes

Cyanine dyes are well known for the discovery of J-aggregates by Scheibe and Jelley. The hydrophobic effect is the main driving force for the aggregation of cyanine dyes in water. Yang *et al.* reported a novel cyanine compound, 3,3'-di(3-sulfopropyl)-4,5,4',5'-dibenzo-9-ethyl-thiacarbocyanine triethylammonium salt (ETC), for the recognition of mixed G-quadruplex in human telomeres.[36] ETC formed J-aggregate in aqueous solution with a 80 nm red-shift in its absorption spectrum. A specific antiparallel G-quadruplex without diagonal loop could strongly interact with ETC and disassemble its J-aggregates. This process consequently

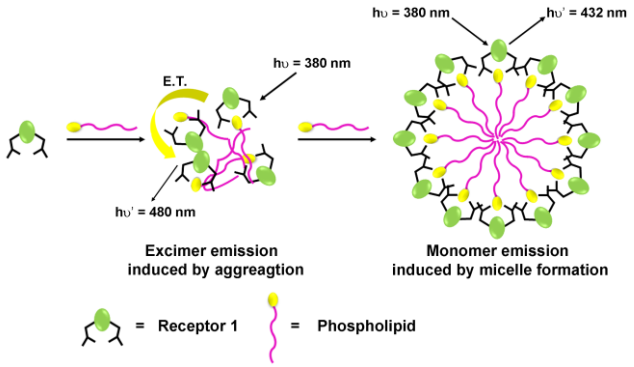


Fig. 10 Schematic presentation of aggregation/micelle formation for LysoPA detection. Adapte from ref *38*. Copyright 2008 American Chemical Society.

induced recovery of absorption peaks from 660 nm to 584 nm, as well as the strong fluorescence enhancement (70-fold).

Disaggregation of other fluorophore

Lysophosphatidic acid (LysoPA) is a useful biomarkers for diagnosis of ovarian cancer. A zinc complex of anthryl bis(dipicolylamine) was previously found to displays a selective fluorescence response to LysoPA.[37] Later, Fang and co-workers discovered that aggregation of the zinc complex is induced in the presence of 0-10 equiv. of LysoPA, resulting a large red shift of fluorescence maximum typical of excimer formation. As the concentration of LysoPA increases beyond 10 equiv., the fluorescence is reversed back to the emission monomer (Fig. 10).[38] The authors suggested that the unusual behavior or this process is due to the long aliphatic chain of LysoPA, which leads to aggregation at low concentration and forms micelle at high concentration.

Disaggregation of none-fluorescent compounds

The effect of aggregation on non-fluorescent sensing has also been explored. Jiang and co-workers reported the synthesis of cationic aryl triazole oligomers through " click chemistry".[39] Through circular spectroscopy, they showed that the oligomers adopted a helical conformation in water or in a mixture of water and methanol, but a random-coiled conformation in methanol. Using dynamic light scattering experiments, the authors discovered the aggregation of the cationic oligomers to form higher order architectures with a size range from 100 to 500 nm. The aggregation of cationic oligomers was influenced by the concentration and polarity of the environment. The authors also showed that the cationic oligomers were able to sense chloride and fluoride anions in aqueous solution, due to the destabilized aggregation (disaggregation) in the recognition event.

Hamachi and co-workers studied the effect of aggregation on ^{19}F NMR measurement.[9] They prepared an amphiphilic compound consisting of an ^{19}F reporter, a linker and a protein ligand for hCAI. This compound formed self-assembled aggregates in aqueous solution, resulting in the quenching of ^{19}F NMR signal. The formation of aggregate was confirmed by various techniques, including AFM, TEM and scanning electron microscopy (SEM), revealing the size of aggregate of approximately 200 nm. In the presence of target protein, hCAI,

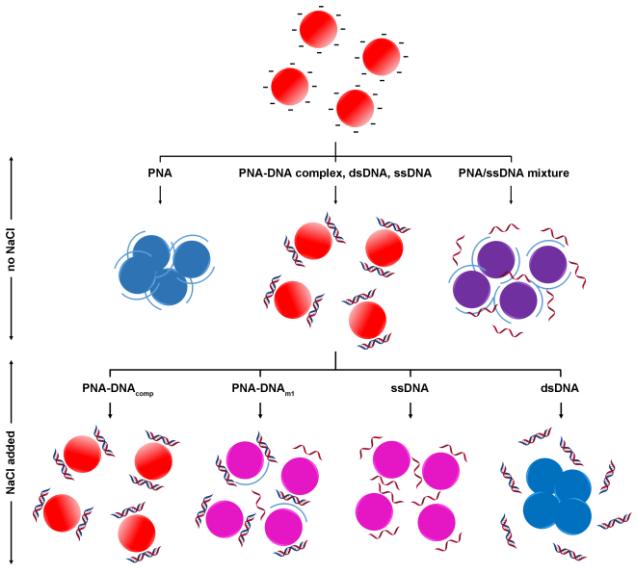


Fig. 11 Schematic presentation of how different nucleic acids (PNA, PNA_DNA complexes, ssDNA, dsDNA, and PNA/DNA mixture) affect AuNPs' intrinsic stability and aggregation. Adapted with permission from ref *42*. Copyright 2009 American Chemical Society.

the probe disaggregated to monomer through the recognition-driven disassembling with a turn-on in ^{19}F NMR signal. Based on this property, the authors were able to developed a supramolecular organic probe to detect specific proteins by ^{19}F-based MRI in an off/on mode.

Disaggregation of nanoparticles

In the last 20 years, the field of biological and chemical probes using nanomaterial has witnessed an explosion due to the unique optical properties of noble nanostructures. Aggregation is generally a challenge in large-scale nanoparticle preparation due to the nanoparticle interaction potential. On the other hand, aggregation caused signal change was also smartly utilized to design novel nanoparticle probes. For example, Mirkin and co-workers developed a colorimetric Cu^{2+} detection using DNA-modified gold-nanoparticle aggregates as probes.[40] Additionaly, similar to AIE phenomenon, aggregation sometimes can enhance the signal of nanoparticle, for example surface enhanced Raman spectroscopy (SERS). Aggregation causes the formation of hot spots which will largely enhance the SERS signal.[41] Such mechanism has been utilized in designing novel SERS probes, however, is beyond the focus of "disaggregation" of this review. Compared to the aggregation, disaggregation of nanoparticles for probe development has received much less attentions.

DNA probe

Kanjanawarut and co-workers reported an example of utilizing the disaggregation of nanoparticles to develop a probe for colorimetric DNA detection (Fig. 11).[42] The authors prepared citrate ion-coated gold and silver nanoparticles (AuNPs and AgNPs), whose aggregation can be induced by peptide nucleic acids (PNA). Upon hybridization of PNA with a specific DNA, the aggregation of NPs was disrupted, resulting in a significant color change. Utilizing this aggregation/disaggregation system, the authors were able to develop a method to detect a specific DNA sequence with single-base-mismatch resolution.

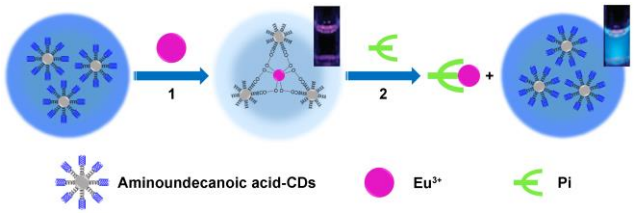


Fig. 12 Schematic representation of Pi detection based on the aggregation/disaggregation of carbon dots mediated by Eu^{3+}. Adapted with permission from ref *43*. Copyright 2011 The Royal Society of Chemistry.

Phosphate probe

One example of using nanoparticle aggregate as a fluorescent turn-on probe was reported by Huang and co-workers.[43] This method is based on the induced aggregation of carbon dots (CDs) by Eu^{3+} as a probe to detect phosphate (Pi) (Fig. 12). Pi is an essential component of the nutrition chain of aquatic microorganism and a convenient tracer or indicator of organic pollution in water.[44] Eu^{3+} as an important rare earth ions exhibit a certain affinity to the oxygen-donor atoms. It can coordinate with the carboxylate groups, however, display a higher affinity to the oxygen-donor atoms from phosphates.[45] Based on these information, the authors prepared carboxylate modified CDs, which showed an intense emission at 420 nm in Tris-HCl solution. Upon the addition of Eu^{3+}, the emission band dramatically decreased arising from the induced aggregation of CDs due to the coordination of Eu^{3+} with the carboxylate moieties on the CDs surface. Addition of Pi induced the recovery of the fluorescence emission of CDs, which was ascribed to the disaggregation process because of the preferential coordination between Eu^{3+} and Pi. This approach can achieve a detection limit as low as 51 nM for HPO_4^{2-}, with a good selectivity over other anions (*i.e.*, SO_4^{2-}, SO_3^{2-}, NO_3^-, NO_2^-, ClO_3^-, ClO^-, BrO_3^-, S_2^-, Cl^-, Br^-, $S_2O_3^{2-}$, F^-, I^-, Hcy, Ser, Glu and Arg). It also allows the detection of HPO_4^{2-} in artificial wetland systems.

Li and co-workers also reported a switchable sensor for the phosphate detection based on the aggregation/disaggregation of quantum dots (QDs).[46] Ce^{3+} could induce the aggregation of mixture of cysteine-capped CdS QDs and AgNPs, which induced electron or energy transfer between CdS QDs and AgNPs and serious fluorescence quenching. Upon the addition of phosphate, the aggregation of CdS QDs and AgNPs dissociated, recovering the fluorescence of CdS QDs triggered by AgNPs. This developed method was applied in the detection of phosphate in real water samples.

Cys and metal ion probe

Kondo and co-workers reported a sensitive and selective assay to detect cysteine and Hg^{2+} in aqueous solutions using AuNPs, which was stabilized by carboxylethyl quaternized cellulose (CEQC) (Fig. 13).[47] Upon the addition of cysteine, the CEQC-stablized AuNPs formed aggregation, resulting in a obvious red-shift in absorption spectra with visible color change. On the other hand, Hg^{2+}, who is more apt to cysteine than AuNPs, can remove cysteine and trigger AuNPs disaggregation. By taking advantage

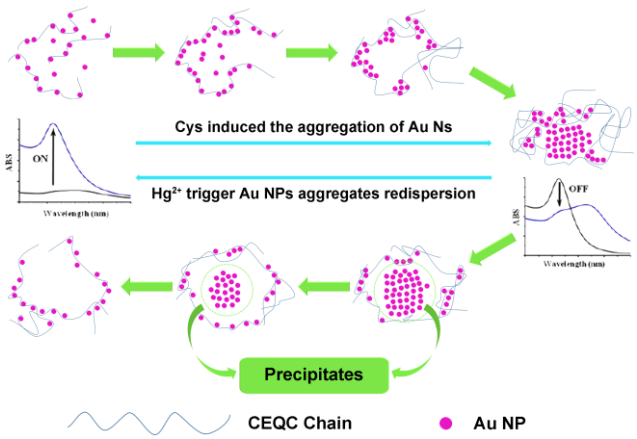


Fig. 13 Schematic representation of cysteine and Hg^{2+} detection based on the aggregation/disaggregation process of AuNPs. Adapted with permission from ref *47*. Copyright 2013 American Chemical Society.

of this mechanism, the authors developed a novel colorimetric sensor for cysteine and Hg^{2+} detection, which could selectively detect cysteine and Hg^{2+} with the detection limit as low as 20 and 40 nM in aqueous solutions, respectively.

Huang and co-workers reported a reverse way to develop cysteine and Pb^{2+} detection system.[48] Dithiocarbamate (DTC) capped AgNPs was prepared, whose aggregation can be induced by Pb^{2+}, due to the strong metal affinity of DTC, resulting in an enhaced resonance light scattering (RLS) signal. In the presence of cysteine, Pb^{2+} was removed from the surfaces of the DTC-AgNPs along with a decreased RLS signal, due to the strong binding preference of Pb^{2+}-S bond. Therefore, a simple and sensing system was developed for the detection of Pb^{2+} in water based on RLS technology, which shows high sensitivity for Pb^{2+} and was successfully used to detect Pb^{2+} in river and tap water samples.

Future perspectives

The strategy of using "disaggregation" as a sensing mechanism has several advantages. First, due to the formation of aggregate, the original signal is monomer is largely quenched, which makes this kind of probes exhibit very low background, large fold change upon binding with analytes, and high sensitivity. Second, the enhancement or the recovery of the signal by disaggregation, renders the probe with "turn-on" property. Third, ACQ is a very common phenomenon among the probe families, therefore aggregation/disaggregation process would be applicable to a variety of probes, without the requirement of specific synthetic efforts. With this review, we expect the proper growth of sensor development utilizing the less explored disaggregation mechanism.

Conclusions

"Aggregation-caused quenching" is a well-established and thus commonly conceived phenomenon in probe development. Disaggregation is a reverse process of aggregation, in which aggregate dissociates to monomers, and the mechanism has received much less attentions so far and was never systematically discussed before. This tutorial review provides the first balanced comparison between aggregation and disaggregation as a sensing mechanism, and summarized the recent examples in probe development based on disaggregation. The disaggregation of probes generally causes the enhancement or recovery of fluorescent signals, hence, provides a unique path to design novel "turn-on" probes.

Acknowledgement

This study was supported by an intramural funding from A*STAR (Agency for Science, Technology and Research, Singapore) Biomedical Research Council and a Singapore Ministry of Education Academic Research Fund Tier 2 (MOE2010-T2-2-030).

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