A Holistic Perspective on Living Aggregate

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ABSTRACT Aggregate is one of the most extensive existing modes of matters in the world. Besides the research objectives of inanimate systems in physical science, the entities in life science can be regarded as living aggregates, which are far from being thoroughly understood despite the great advances in molecular biology. Molecular biology follows the research philosophy of reductionism that generally reduces the whole into parts to study. Although reductionism benefits the understanding on molecular behaviors, it encounters limitations when extending to aggregate level. Holism is another epistemology that is comparable important to reductionism, which claims a top-down method to explore objectives at aggregate or mesoscale level, emphasizing on the interactions and synergetic/antagonistic effects of a group of composed single entities in determining the characteristics of a whole. As a representative of holism, aggregation-induced emission (AIE) materials have made great achievements in the past two decades in both physical science and life science. In particular, the unique properties of AIE materials endow with in-situ and real-time visual methods to investigate the inconsistence between microscopic molecules and macroscopic substances, offering researchers excellent toolkits to study living aggregates. The applications of AIE materials in life science are still in its infancy and worth expanding. In this perspective, we summarize the research progresses of AIE materials in unveiling some phenomenon and processes of living systems, aiming to provide a general research approach from the viewpoint of holism. At last, insights in what we can do in the near future are also raised and discussed.

PARADIGM SHIFT: FROM REDUCTIONISM TO HOLISM

To understand the nature, scientists have viewed the world from all kinds of views. The enthusiastic efforts of scientists over the past centuries along this line of epistemology have led to the booming development of reductionism (**Figure 1**)[1]. Reductionism has been defined as "entities of a given kind are identical to, or are collections or combinations of, entities of another (often simpler or more basic) kind" according to Encyclopedia Britannica.[2] Accordingly, reductionists generally believe that "a whole is nothing but the sum of its individual parts". When a whole is too complicated to be investigated, people are used to reduce it into the elementary parts to study. Molecularism is a representative research philosophy in reductionism, which takes molecule as the core of research. According to Merriam-Webster dictionary, molecule is "the smallest particle of a substance that retains all the properties of the substance". The development of "molecularism" indeed benefits the development of modern physical science including chemistry and material science when the research targets mainly focus on molecules. However, molecularism also encounters predicaments when the research targets were extended to higher hierarchy level where aggregates beyond molecules are involved. For instance, the phenomenon of aggregation-induced emission (AIE) indicates that the aggregate is emissive but its composed molecular species could be non-luminescent.[3] Moreover, aggregation-caused quenching (ACQ) demonstrates that the emission of molecular species at isolated state could be quenched at aggregate state.[3] Both AIE and ACQ phenomenon cannot be simply understood by molecularism, which claims that molecular properties and behaviors determine the macroscopic properties of bulk materials.[4] The AIE and ACQ phenomenon as well as other inconsistent properties of microcosmic molecules and macroscopic substances suggest that molecular-level theories and rules do not necessarily applicable to macroscopic aggregates.[5]

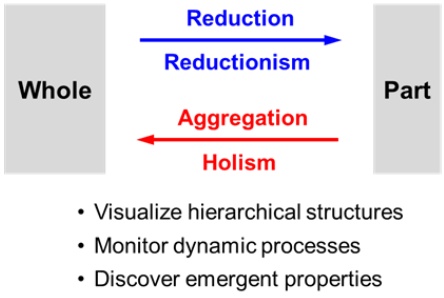

Figure 1. Paradigm shift from reductionism to holism.

Holism is another epistemology that is comparable important as reductionism, if not more important. The Holists believe that "all the properties of a given system cannot be determined or explained by its component parts alone, but the system as a whole determines in an important way how the parts behave".[6] The ancient Greek philosopher of Aristotle had realized that "the whole is bigger than the sum of its parts".[7] Karl Marx, a German philosopher in 19 century advocated that "Merely quantitative differences, beyond a certain point, pass into qualitative changes" to emphasize the significance of quantitative change in determining the characteristic of matters.[8] In the 20th century, Nobel Prize laureate Philip W. Anderson voiced his holistic opinion, claiming that the behavior of large and complex aggregates of elementary particle could not be understood by simply exploring the properties of a few particles.[9] Overall, holism claims a top-down method to investigate objects at the aggregate or mesoscale level, with a particular emphasis on the interactions and synergetic/antagonistic effect of a group of composed single entities in determining the properties or functions of a whole. Therefore, holism provides us a different view of thinking and understanding on the essence of the physical world, which benefits to overcome the limitation of reductionism and help to better understand the inconsistence between microscopic molecules and macroscopic substances. Therefore, there should be a paradigm shift from reductionism to holism in applying limited molecular-level descriptions when encountering situations involving aggregates.

To holistically understand why the macroscopic substances or aggregates exhibit different properties from molecules, a suitable toolkit that can reflect what happened when the molecules form aggregates is highly desirable. Fortunately, AIE materials could fulfill these requirements.[3, 10] Based on the mechanisms of restriction of molecular motion (RMM), twisted intramolecular charge transfer (TICT), and excited state intramolecular proton transfer (ESIPT) etc. (**Figure 2b**), various AIE molecules were designed and exhibited enriched luminescence property, with the luminescence color and/or intensity changing with the aggregation.[3, 5] These aggregation-depended properties including fluorescence, room temperature phosphorescence (RTP), reactive oxygens species (ROS), and photothermal properties could be assigned to the structure sensitivity of AIE molecules to molecular microenvironment, according to the AIE mechanisms of RMM, TICT and ESIPT etc. (**Figure 2c**). Furthermore, the change of molecular configuration, packing mode, and microenvironmental polarity within the aggregates could be visualized directly through the luminescence signals, which benefits the exploration of how these single entities interacted with each other within the aggregate to generate or influence the macroscopic function of a substance.[11-14]

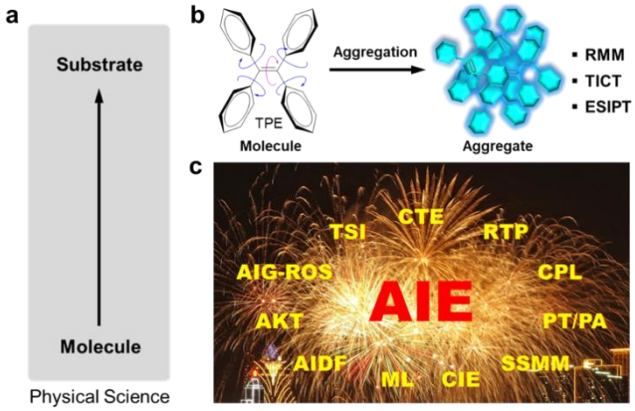

Figure 2. (a) Paradigm shift from reductionism to holism in physical science. (b) Schematic illustrations of representative aggregation processes in physical science and representative working mechanisms of aggregate. RMM: Restriction of Molecular Motion. TICT: Twisted Intramolecular Charge Transfer. ESIPT: Excited-State Intramolecular Proton Transfer. (c) Research branches based on AIE. CIE: crystallization-induced emission; RTP: room-temperature phosphorescence; AIDF: aggregation-induced delayed fluorescence; AKT: anti-Kasha transition; CTE: clusterization-triggered emission; TSI: through-space interaction; ML: mechanoluminescence; CPL: circularly polarized luminescence; AIG-ROS: aggregation-induced generation of reactive oxygen species; PT/PA: photothermal/photoacoustic phenomena; SSMM: solid-state molecular motion. [Adapted from ref 3. Copyright 2020 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.]

Some progresses have been achieved in the exploration of the new properties generated in aggregates. For example, the non-conjugated poly(ethylene glycol) (PEG) and polypeptide could show bright emission in solid state although their dilute solution are totally non-emissive.[15] It is hard to understand according to traditional molecular fluorescence theory since a conjugated chromophore is generally thought necessary.[3, 16] However, this phenomenon could be understood if we consider the contribution of intrachain through-space O···C and O···O interactions in creating a new chromophore rather than just focus on the non-conjugated single molecular chain.[17] This unique luminescence property of non-conjugated system has been coined as cluterization-triggered emission (CTE) and has become a hot research area.[3, 15] Some other aggregation generated properties like RTP, ROS and circularly polarized luminescence (CPL) could also be well explained in the view-point of holism since aggregation helps to promote effective intersystem crossing, stabilize the triplet state, as well as induce the formation of helical structures.[3]

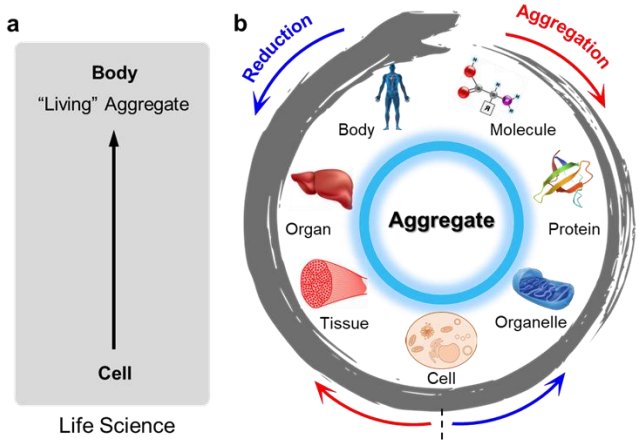

Figure 3. (a) Paradigm shift from reductionism to holism in life science. (b) Molecules aggregate into living species.

Being different from the non-living substances in physical science, the research objects of life science (from cell to body) look more like "living aggregates", which generally hold some unique characteristic of hierarchical structures, dynamic processes and emergent properties. However, the research philosophy of reductionism always fails to unveil the essence of life science despite the profound advances in molecular biology over the past half-century.[18] Indeed, a given living system could be continuously reduced to their composed molecules (e.g. amino acids, lipids and saccharides) through reductionism, but we still do not understand how these compounds interacted to constitute living things based on our understanding on the molecular biology. As descripted by Addy Pross, "it is true that in this modern era we know unequivocally that there is no *elan vital*, that living things are made up of the same 'dead' molecules as non-living ones".[18] If we can think beyond the limits of reductionism and visualize living things as a mutually interacted aggregate other than focus only on the constituted molecules; i.e., an aggregate of a systematic combination of the component units at different hierarchical levels, we may open a new window and be getting closer to the mystery of the Promise Land of life science.

Although holism provides a new viewpoint to investigate the complex living aggregate, it is still a big challenge to unveil the key factors that dominate the aggregation of constitutional units to form higher hierarchical level entities and discern the aggregation process.[18] For example, it is still difficult to illustrate how stem cells differentiate into different cells to build different tissues and organs, which are usually accompanied with the formation of different hierarchical structures, emergent properties, and dynamic processes. The differentiated cells will aggregate to form tissues, tissues further assembly to form organs, and different organs combine together and mutually support with each other to constitute the whole body (**Figure 3**). During the different hierarchical level aggregation, normal aggregation evolves into normal structures with normal properties while abnormal aggregation processes may result in dysplasia or diseases. Unfortunately, either at higher or lower hierarchical level, our understanding on these aggregation processes is limited and there are too many unsettled mysteries need to be further explored. Powerful technologies or suitable tools that could help in-situ and real-time visualize or monitor the aggregation process within living aggregate thus are urgently required.

Despite the rapid development of modern characterization methods, technologies that can really in-site, real-time and visually present the complex processes of living system are still lacking.[19] Fluorescence technology is one of the few to achieve the task, however, the luminescence of traditional luminescent probes mostly will be quenched upon aggregation due to the ACQ effect, which limit their application in monitoring or tracing the aggregation process of living aggregate. In this regard, luminescent probes with AIE property enable the in-situ and real-time imaging and monitoring various aggregation processes due to its sensitivity to molecular microenvironments.[20] Over the past 20 years, AIE probes have been widely applied in physical science as sensors or probes to detect the change of luminescence signal following the variation of pH, morphology, intermolecular interactions and aggregation.[21] Furthermore, it could also be used to trace or monitor the whole process change of biological events due to its superior stimuli-responsive property, biocompatibility, and photostability. Therefore, it is anticipated that AIE materials can also work as suitable toolkit to explore the mystery of living system. Fortunately, some exploration has been implemented with some exciting achievements.[20] In spite of this, more efforts still need to be devoted since our understanding on complex life science is only a drop in the bucket. In this perspective, we will summarize the recent research progresses of AIE probes in life science and raise insights in what we can do in the near future, aiming to further promote the applications of AIE materials in studying living aggregates.

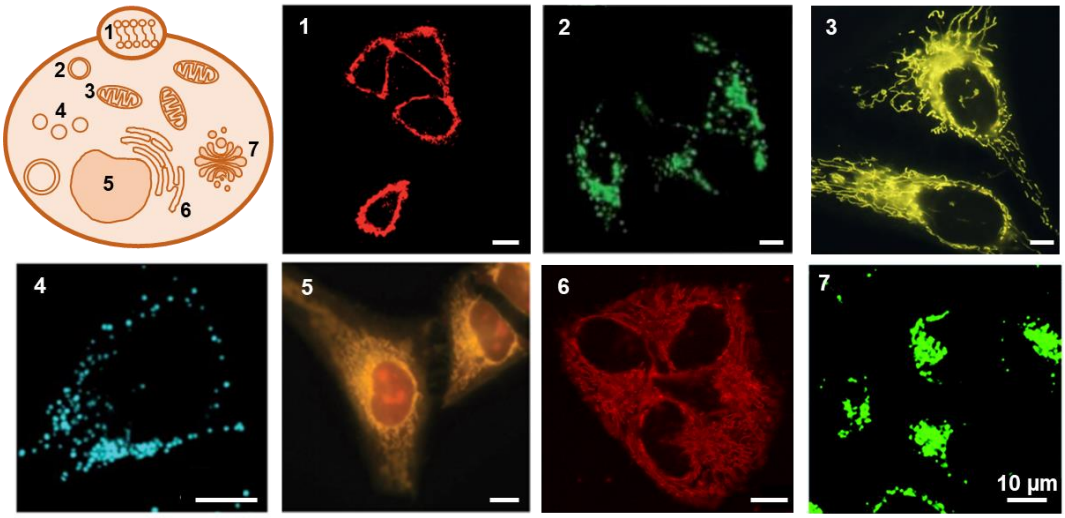

Figure 4. Visualizing hierarchical structures. Representative membrane-bounded subcellular structure imaging results for AIEgens. 1. cell membrane [Adapted with permission from ref. 29. Copyright 2018, licensed under Creative Commons Attribution 3.0 Unported Licence.]; 2. Lysosome [Adapted from ref 13. Copyright 2014 Royal Society of Chemistry.]; 3. Mitochondria [Adapted from 47. Copyright 2013 the Royal Society of Chemistry.]; 4. lipid droplet [Adapted with permission from ref. 48. Copyright 2017, licensed under Creative Commons Attribution 3.0 Unported Licence.]; 5. nucleolus [Adapted from ref. 52. Copyright 2016 Royal Society of Chemistry.]; 6. endoplasmic reticulum [Adapted from ref 55. Copyright 2020 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.]; 7. Golgi apparatus [Adapted with permission from ref. 57. Copyright 2022, licensed under a Creative Commons Attribution 4.0 International License.]. Scale bar: 10 μm.

VISUALIZING HIERACHICAL STRUCTURES

Cells are building units of living systems and they can also be regarded as living aggregates from subcellular structures such as cell membrane, lysosomes, mitochondria, lipid droplets, nucleolus, endoplasmic reticulum and Golgi apparatus (**Figure 4**).[22-24] These cellular and subcellular structures are critical in supporting the living system because their structures, locations and functions could determine the fate of living species. Dysfunction of these aggregates usually cause the occurrence of lesions and diseases, e. g., mitochondrial dysfunction has been thought related with several important diseases including cardiovascular disease, neurodegenerative disease, and cancers etc.[25-26] Therefore, imaging and monitoring the dynamic change of the structures, morphologies, and microenvironment of these organelles in a real-time manner and identifying how do they influence the organelle function benefit a lot to understand organelle dysfunction-related diseases.

As illustrated in **Figure 4**, various AIE probes have been developed to achieve the targeting and imaging of specific organelles from cell membrane to Golgi apparatus.[27] Typically, plasma membrane that consists of the phospholipid bilayer is a protective two-dimensional boundary between a living cell and its surroundings.[28] The different polarity inside and outside the membrane produced by the phospholipid bilayer could be identified by AIEgen through a wash-free method due to its microenvironment sensitivity (**Figure 4-1**).[29] Furthermore, as a fluorescence tracer, AIEgen could be used to visualize the plasma membrane destroy induced cell necrosis and differentiate the tiny difference of plasma membranes of cancer cells and normal cells.[30-34] Lysosomes are membrane-bounded organelles which are responsible for intracellular degradation of materials, and also participates in recycling metabolites and ions to maintain the homeostasis of cells.[35-37] Therefore, detection, visualization, and monitoring of lysosomes could reflect the status of cells. A lot of lysosome-target AIEgens have been developed, which helped to detect the endogenous Lysosomal β-N-acetylhexosaminidase activity in live HCT116 cells, visualize the drug-escaping process from lysosomes to cell nuclei, sense lysosomal viscosity changes induced by lipopolysaccharide, nystatin, low temperature, and dexamethasone etc.(**Figure 4-2**).[27, 38-42] Mitochondria serve the cells as "energy factories" and one of their features is negatively charged membranes. AIEgens with positive charges and suitable hydrophilic-to-hydrophobic ratios have been successfully developed for mitochondrial imaging (**Figure 4-3**).[43-44] By employing mitochondria specific AIE probes, mitochondrial destabilization induced mitophagy and mitophagic flux inhibition at the autophagosome stage and the resulted cancer cell apoptosis could be observed.[45] Moreover, cell damage and mitochondria membrane potential decrease could also be presented.[46] Lipid droplets (LDs) contain mainly diverse neutral lipids, triacylglycerol and cholesteryl ester are widely found in adipocytes, hepatocytes and the adrenal cortex. Hydrophobic AIEgens tend to accumulate in lipid droplets and thus they are widely utilized for lipid droplet imaging (**Figure 4-4**).[48] LD–mitochondrion interactions during ferroptosis had been revealed by AIEgen that could target both LD and mitochondrial, which helped to unveil the LD–mitochondrion interactions in modulating cell death.[49] Furthermore, the decrease of the number of LD-mitochondria contact sites under lipopolysaccharide treatment induced inflammatory environment could also

be clearly shown by fluorescence.[50] And for the first time, signaling lipid, namely diacylglycerol (DAG), enhanced the formation of the nuclear LDs was presented by AIEgen based fluorescence imaging.[50-51] Nucleolus is the key structure in the nucleus. It serves as the site for ribosome synthesis and RNA assembly, and is thus closely related to the cell growth and proliferation. AIEgens could cross the membrane systems (plasma membrane, karyothecas) and interact with nucleic acids, the influence of ROS on the permeation of nuclear membrane and mitochondria–nucleus migration had been investigated by AIEgen based fluorescence imaging (**Figure. 4-5**).[52-54] Endoplasmic reticulum (ER) is a complicated network that communicate with other organelles all the time. Taking advantage of the lipids composition difference in endoplasmic reticulum from other organelles, AIEgens that could interact with choline phosphate cytidylyltransferase (CCT enzyme) were successfully developed for ER labeling (**Figure. 4-6**).[55] With the help of ER and LD specific AIEgens, the attachment of nascent LDs on the ER, which makes the ER an intermediate network connecting proximal and distal LDs could be supported.[56] Complexity of Golgi apparatus (GA) hindered the development of efficient fluorescent probes, ascribing to their mazy structures and functions in the classification of newly synthesized and recycled proteins and lipids to their final destinations. Fortunately, AIEgen that can effectively target the Golgi apparatus *via* caveolin/raft mediated endocytosis was developed (**Figure. 4-7**).[57] With GA targeting AIEgen, ROS caused GA fragmentation and cleavage of GA proteins are observed which enabled GA targeting PS better PDT effect. As the fundamental living aggregates, subcellular structures have been well labelled and investigated by AIEgens. Based on these achievements, more complex structures of living aggregates could be investigated by AIE materials.

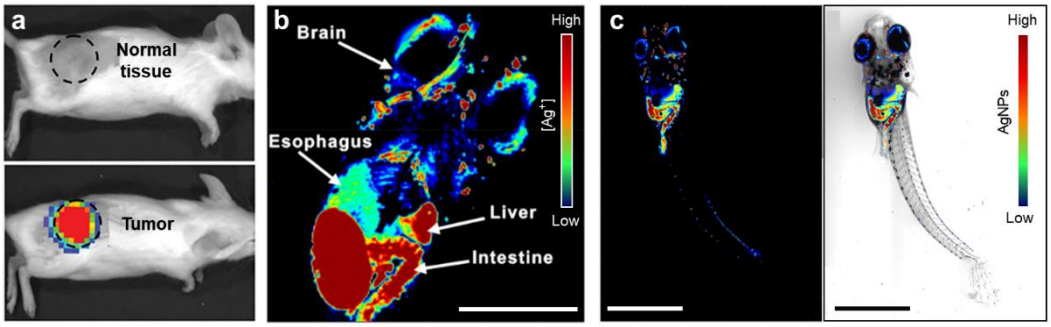

Figure 5. Visualizing hierarchical structures. (a) Tumor tissue detected and visualized by chemiluminescent AIE system. [Adapted from ref. 61. Copyright 2020 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.] (b) The distribution of Ag ions in different organs of medaka larvae. Scale bar = 0.5 mm. (c) Confocal images showing the distribution of AIE-AgNPs in medaka larvae (whole body scale). Scale Bar = 1 mm [Adapted from ref 64. Copyright 2019 American Chemical Society.].

Cells aggregate to form tissues, and organs could be regarded as living aggregates of tissues. Ultimately, organs aggregate to build whole body. The structures of tissues, organs and bodies are important to their physiological functions. Abnormal aggregation usually leads to disease, such as tumor. Furthermore, vascular malformation in tumor result in intrinsic tumor microenvironment (TME), such as high level of ROS, enhanced permeability and retention (EPR) effect, and etc.[58-60] Therefore, the detection and imaging of abnormal high level of ROS in organisms play an important role in cancer diagnosis. EPR effect in TME usually facilitate the accumulation of imaging agents in tumor. Visualization of TME could provide surgeons the detailed structures of tumor, such as the margins, which are hard to identify directly by naked eyes. In **Figure 5a**, a ROS-reactive chemiluminescent AIEgen, TBL, with near-infrared (NIR) emission, was designed for the precise imaging of tumor tissues *in vivo*. The NIR chemiluminescence could penetrate through tissues with a total thickness of 3 cm with high contrast, indicating the chemiluminescent AIE system could provide more detailed information about tumor including the location and margin of tumor to doctors.[61]

Once the organs and bodies were shaped and matured, the most frequent threats came from the outside, i.e., external factors.[62-63] For example, there is a growing concern over nanometals release into the natural environment and subsequent toxic impact towards living systems. Visualization and monitoring the distribution of pollutants in living system is ideal for fundamental research and practical application. Although numerous studies have been conducted on the toxicity and biodistribution of AgNPs and corresponding ionic counterparts, it is still debatable whether the toxicity originates from the accumulation of particles within specific organs or is mediated by the dissolved Ag ions. Yan et al employed two AIEgens, AIEgens-coated AgNPs and a fluorogenic Ag+ sensor, to visualize and quantitative analysis of distribution patterns of AIE-AgNPs and corresponding Ag ions in medaka larvae at organ (**Figure 5b**) and body (**Figure 5c**) levels, respectively.[64] The results indicated that AIE-AgNPs and Ag ions showed distinct distribution patterns, in which AIE-AgNPs were concentrated in intestine and liver. In contrast, Ag ions were accumulated mainly in the intestine of medaka larvae. Quantitative distribution patterns of AIE-AgNPs and corresponding Ag ions in different organs of medaka larvae were simultaneously analyzed by AIEgens, indicating that hierarchical structures of living aggregates could be well visualized.

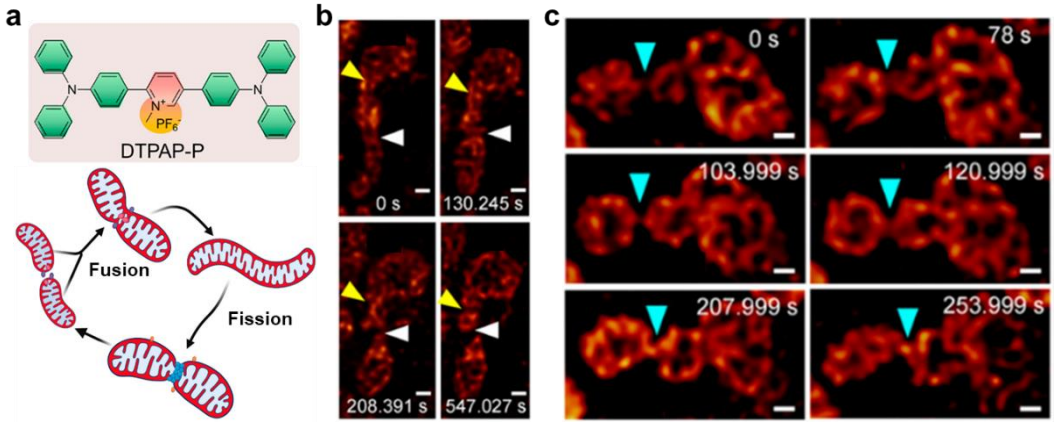

Figure 6. Monitor dynamic processes. (a) Chemical structure of DTPAP-P and the illustrative diagram of the dynamic processes of mitochondrial fusion and fission. (b) Time-lapse STED imaging of mitochondrial fission, yellow and white arrow in DTPAP-P labeled HeLa cells Scale bar = 500 nm. (c) Time-lapse STED imaging of mitochondrial fusion, indigo arrow in DTPAP-P labeled HeLa cells Scale bar = 500 nm. [Adapted from ref 54. Copyright 2022 American Chemical Society.]

MONITORING DYNAMIC PROCESSES

The nature of living system is highly dynamic, living aggregates are generated and participated in these dynamic processes. It was difficult to investigate these dynamic processes of living aggregates since the lack of efficient tools to track the processes real-time and *in situ* without interferences.[65] Observing how the organelles behave *in vivo* could boost biologists' understanding toward living systems. For example, mitochondrial fission and fusion are dynamic processes and could indicate the status of cells (**Figure 6a**). The maintenance of mitochondrial integrity and homeostasis is extremely critical, which is achieved through continual fission and fusion.[66-67] Therefore, monitoring the fission and fusion in mitochondria with the substructures of cristae at nanometer scale are required. Ascribing to the high brightness and good photostability, some AIE systems were suitable for super-resolution microscopies, thus are promising tools for live cell super-resolution imaging.[68-71] Xu and Dang et al. reported an AIEgen, DTPAP-P, with high quantum yield, favorable photostability and biocompatibility for super-resolution imaging *via* stimulated emission depletion (STED) nanoscopy.[54] In live cells, mitochondrial dynamics monitoring (fission, **Figure 6b**; fusion, **Figure 6c**) has been achieved in ultrahigh resolution with a full-width-at-half-maximum (fwhm) value of only 165 nm by STED nanoscopy. During the long-term tracking, it is noted that the photobleaching process does not occur. This work demonstrates that AIEgens are promising materials for real-time tracking organelle dynamics at nanometer scale.

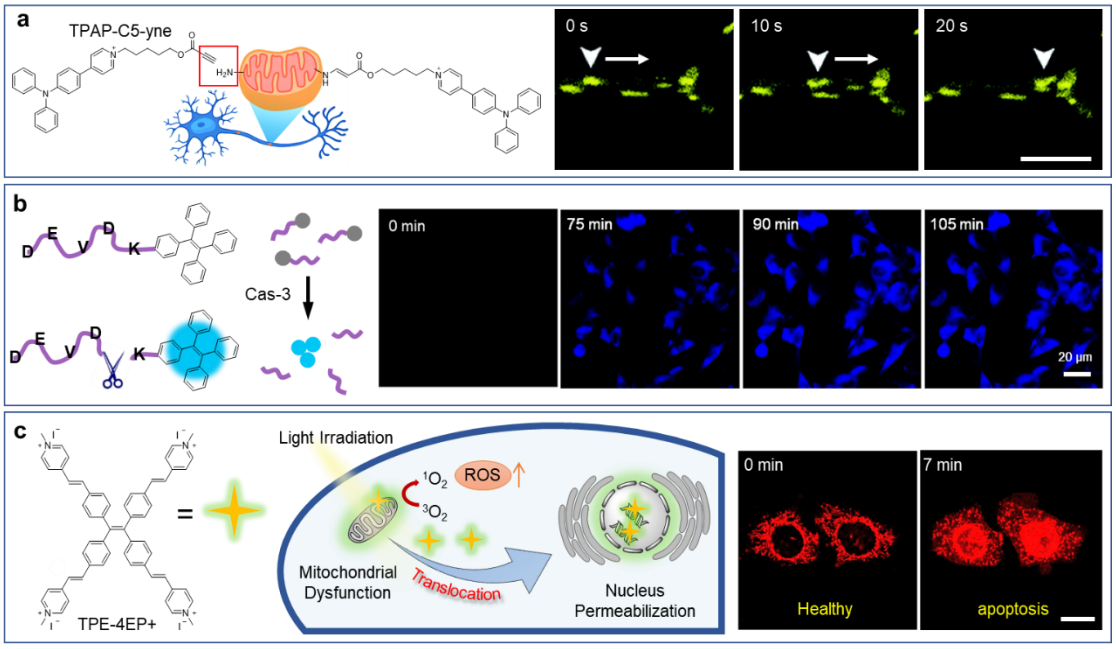

Figure 7. Monitoring dynamic processes. (a) Mitochondrial movements in neuron visualized by TPAP-C5-yne. (left) Molecular structure of TPAP-C5-yne and its bioconjugate after reacting with an amine group. (right) Confocal microscopy

images of neurons stained with TPAP-C5-yne. White arrows indicate a single mitochondrion. Scale bar = 5 μm. [Adapted with permission from ref. 73. Copyright 2022, licensed under Creative Commons Attribution 3.0 Unported Licence.] (b) Real-time visualization of cell apoptosis using Ac-DEVDK-TPE. Scale bar = 20 μm. [Adapted from ref 74. Copyright 2012 American Chemical Society.] (c) Self-reporting therapeutic AIE system. Proposed mechanism of mitochondria-to-nucleus translocation of TPE-4EP+. Real-time confocal imaging of HeLa Cells under continuous 405 laser irradiation stained with TPE-4EP+. Scale bar = 20 μm. [Adapted from ref 75. Copyright 2019 American Chemical Society.]

Likewise, more complicated cellular processes required investigations in a real-time manner. Based on the sources of stimulus, cellular processes could be classified into two major categories, spontaneous processes, and stimulus-responsive cell processes. The former includes the movements of organelles in live cells and programmed cell death. Stimulus-responsive cell processes include drug-triggered cellular stress response etc. The location regulation of mitochondria is an important property of spontaneous cellular process. In neurons, mitochondria are exceptionally important for fulfilling the extraordinarily high metabolic rate of the central nervous system.[72] Tracking mitochondrial movement in neurons is an attractive but challenging research field as dysregulation of mitochondrial motion is associated with multiple neurological diseases. As depicted in **Figure 7a**, a mitochondria-targeting and clickable AIEgen, TPAP-C5-yne, was designed to achieve long-term tracking through a bioconjugation strategy.[73] Specifically, TPAP-C5-yne contains a cationic pyridinium moiety and an activated alkyne terminus, which are responsible for mitochondria targeting and bioconjugation with amine groups on mitochondria, respectively. The stable fluorescence signal from TPAP-C5-yne guarantee the accurate analysis of the motion of a single mitochondrion in live primary hippocampal neurons and the long-term tracking of mitochondria for up to a week in live neurons has been realized.

Apoptosis is another representative dynamic cellular process. Real-time monitoring of cell apoptosis could provide valuable insights into early detection of therapy efficiency and evaluation of disease progression. Tang and Liu et al reported a live-cell-permeable, fluorescent light-up AIE probe for real-time cell apoptosis imaging (**Figure 7b**).[74] The probe was comprised of a hydrophilic caspase-specific Asp-Glu-Val-Asp (DEVD) peptide and a hydrophobic tetraphenylethene (TPE) unit. In the right panel of **Figure 7b**, apoptosis was triggered by Staurosporine. The probe was almost nonfluorescent at time 0 min but displayed significant fluorescence enhancement since 75 min, indicating that the apoptotic processes were activated and caspase-3/-7 were generated to cleave the DEVD moieties. This strategy provided an efficient platform for real-time imaging of cellular processes in live cells, further allowed *in situ* investigation of other enzyme-related cellular processes.

Various therapeutic methods such as photodynamic therapy (PDT) have been developed to trigger apoptosis of target cells to cure tumors. It is believed that subcellular structures and relevant processes will change during apoptosis. However, directly visualization and monitoring the treatment effects of PDT agents towards tumor cells were difficult because extra therapeutic response probes make the monitoring process complicated, *ex situ*, and delayed. Zhang et al. reported a cationic AIE-based photosensitizer, TPE-4EP+, which could serve as fluorescent tracer and PS at the same time. TPE-4EP+ underwent mitochondria-to-nucleus translocation during apoptosis induced by PDT, thus enabled the *in situ* real-time monitoring *via* fluorescence migration (**Figure. 7c**).[75] Moreover, TPE-4EP+ could induce cell apoptosis by the intrinsic high $^1O_2$ generation efficiency under irradiation. The fluorescent photosensitizer can serve as a self-reporter to monitor the subcellular processes.

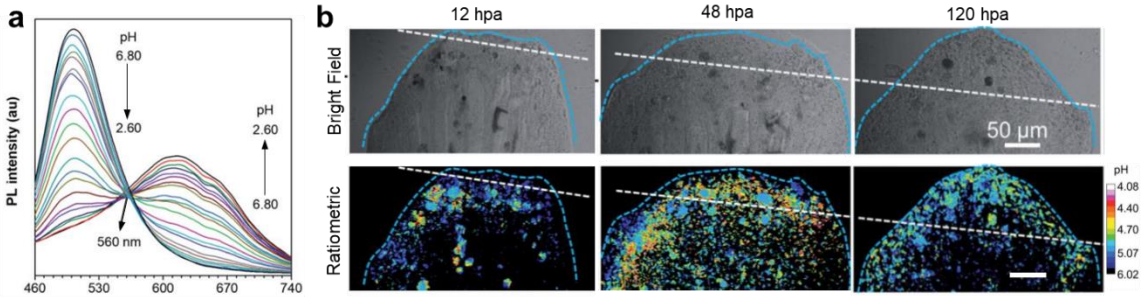

Figure 8. Monitoring dynamic processes. (a) Fluorescence spectra of CSMPP (10 μM) in buffers in the pH range of 2.60–6.80. (b) The CSLM images of the CSMPP-stained medaka larva's caudal fin before amputation and after amputation at different times. hpa: hours post-amputation. Scale bar: 50 μm. [Adapted with permission from ref. 77. Copyright 2020, licensed under Creative Commons Attribution 3.0 Unported Licence.]

Dynamic processes at tissue levels are more complicated for real-time monitoring. Tissue regeneration is a crucial self-renewal capability involving many complex biological processes.[76] However, simultaneous quantification and visualization of tissue regeneration processes is not easy to achieve. Shi et al developed a simple and quantitative

method for the real-time and non-invasive observation of the process of tissue regeneration by using a ratiometric AIEgen CSMPP with high selectivity and reversibility for lysosomal pH responses (**Figure. 8a**).[77] Lysosomes are reported to involve in autophagy, which is indispensable to tissue engineering. Lysosomal pH is an important parameter along with tissue regeneration process, i.e., wound healing (0 – 12 hpa), blastema formation (12 – 48 hpa), and regenerative outgrowth (> 48 hpa).[78] As demonstrated in **Figure 8b**, the caudal fin regeneration of a medaka larvae was monitored by tracking the lysosomal pH change using CSMPP. At 12 hpa, the mean lysosomal pH gradually decreased after amputation, which was consistent with wound healing process where wound was closed by a thin layer of epithelium. And the lowest pH was reached during 24–48 hpa, which was reported to be the stage of blastema formation. This indicated that lysosomes were most active at this stage. Then, the lysosomal pH increased after 48 hpa and returned to normal after 120 hpa, indicating that the amputated caudal fin was about to be completely regenerated into the original one. This work proved that the regeneration processes in model animals could be directly monitored by ratiometric AIE systems.

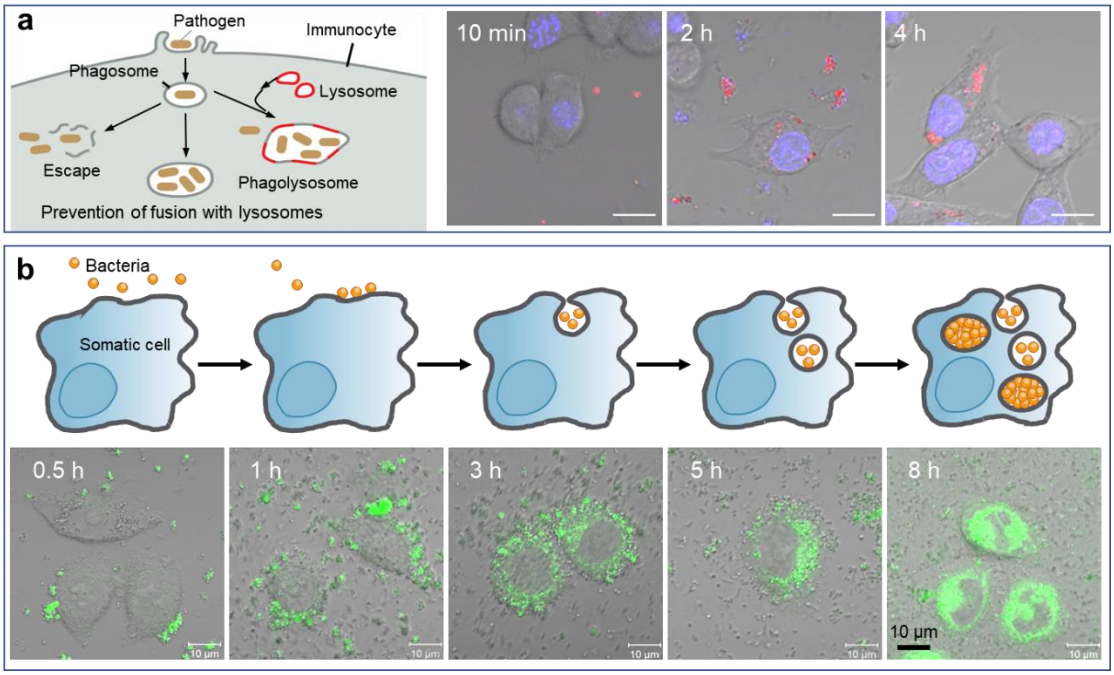

Figure 9. **Monitoring dynamic processes**. (a) Left: Illustrative diagram of phagocytosis processes between pathogens and phagocytes. Right: Visualization of phagocytosis between RAW264.7 macrophages with CDPP-NCS labeled *S. aureus*. Scale bar = 10 μm. [Adapted from ref. 19. Copyright 2022 Elsevier Ltd.] (b) Illustrative diagram of the invasion process of bacteria. And the CLSM images of HeLa cells infected by M1-DPAN-labelled living *S. aureus* for 0.5, 1, 3, 5 and 8 h. Scale bar = 10 μm. [Adapted from ref. 84. Copyright 2022 Elsevier Ltd.]

Interactions between cells and cells or cells and pathogens involve more complex dynamic processes. Visualization of immunocyte-microbe interactions is of great importance to reveal the physiological role and working mechanism of innate and adaptive immune system. Bacteria enter host cells by phagocytosis, which is a normal function of phagocytes such as neutrophils, macrophages, and dendritic cells.[79] It is well-known that these phagocytes patrol the tissues of the body and ingest and destroy microbes, but the details are rather complicated. It is reported that macrophages could kill bacteria through the interactions with bacteria, and the deeper investigations revealed that various organelles such as lysosomes, mitochondria, and even lipid droplets could interact with the engulfed bacteria (phagosomes).[80-82] These complicated processes require advanced technologies and materials to realize the in-situ visualization. Zhao and Tang et al reported a new clickable AIEgen, CDPP-NCS, for capturing the processes of macrophage-bacterium interactions with subcellular resolution.[19] As illustrated in **Figure 9a**, the formation processes of phagolysosomes were captured by this clickable AIEgen. Confocal images provided direct information of the phagocytosis process between RAW264.7 macrophages and *S. aureus*, where the intracellular red dots indicated the phagosomes. Detailed experiments revealed that the lysosomes moved towards bacteria along with time and finally overlapped with each other, demonstrating a direct way to monitor the formation process of phagolysosomes.

On the other hand, some intracellular bacterial pathogens such as *M. tuberculosis* use phagocytosis as their advantage and have evolved to survive and multiply inside macrophages.[79] Moreover, some intratumoral bacteria can obviously reduce the curative effect of chemotherapy drugs according to recent studies.[83] Therefore, monitoring bacterial invading and surviving processes at cell level is charming but challenging. Hu et al reported a Gram-positive-specific AIEgen, M1-DPAN, to label *S. aureus* and

monitor their invading processes into HeLa cells.[84] As shown in **Figure 9b**, *S. aureus* could be observed to get close to and surrounded HeLa cells within 1 h. Then, invading process was observed in the following two hours. After incubation for 8 h, numerous *S. aureus* successfully crossed over the cell membrane and distributed in the cytoplasm of HeLa cells. The intensified fluorescent signals implied that the *S. aureus* had grown and reproduced rapidly after entrance into the cells. This work provided researchers a simple method to label and monitor the infection processes *in situ* and *in vivo*.

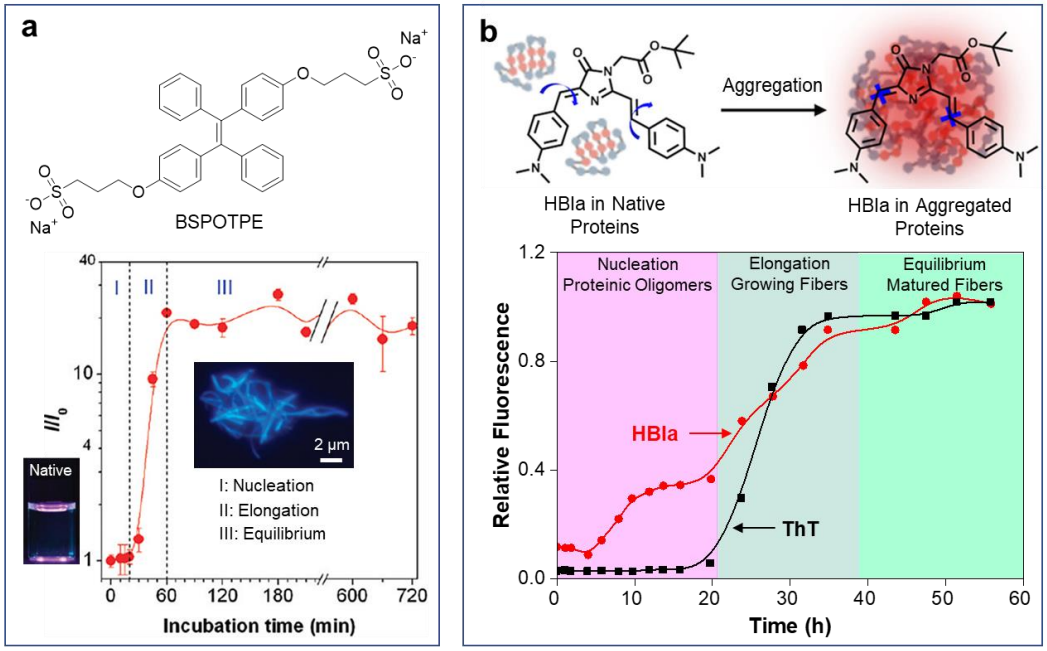

Figure 10. Monitoring dynamic processes. (a) Insulin fibrillogenesis process monitored by BSPOTPE bioprobe. [Adapted from ref 88. Copyright 2012 American Chemical Society.] (b) Protein aggregates provide a crowded environment to restrict the rotational motion of 4-hydroxybenzylidene-imidazolinone (HBI) analogues (HBIa) and turn on its fluorescence. Aggregation kinetics of α-synuclein as measured by HBIa and Thioflavin T (ThT), a probe that detects fiber formation. [Adapted from ref 89. Copyright 2018 American Chemical Society.]

Abnormal protein aggregation always leads to disease, such as Alzheimer's, Parkinson's and Huntington's diseases, spongiform encephalopathy, etc. Amyloidal fibrils are insoluble protein aggregates, an excessive accumulation of which in organs and tissues can lead to biological dysfunctions and result in pathologic symptoms.[85] Each amyloid disease involves predominantly the aggregation of a specific protein.[86] Specifically, in neurodegenerative diseases, the quantities of aggregates involved can sometimes be so small as to be almost undetectable, whereas in some systemic diseases literally kilograms of protein can be found in one or more organs.[87] Therefore, sensitive detection of the protein tangles and plaques and mechanistic understanding of the amyloid deposition processes in detail are of diagnostic importance and have therapeutic implications in neurodegenerative diseases.

AIEgens are sensitive to tiny fluctuations in microenvironment and thus are promising tools to monitor the misfolding of proteins. Hong et al. designed and synthesized a biocompatible AIEgen BSPOTPE that could serve as an *ex situ* monitor for protein fibrillation in insulin model.[88] As depicted in **Figure 10a**, BSPOTPE is non-emissive when it is dissolved with native insulin in an incubation buffer but starts to fluoresce when it is mixed with preformed insulin fibril, enabling *ex situ* monitoring of amyloidogenesis kinetics and high-contrast fluorescence imaging of protein fibrils. The data indicated that the insulin amyloidogenesis process involves three distinct steps: (I) nucleation, (II) elongation, and (III) equilibration. BSPOTPE showed promising ability to monitor the kinetic process of amyloid fibrosis of commercial insulin, especially during the elongation period.

By incorporating TICT property with 4-hydroxybenzylidene-imidazolinone (HBI, chromophore of green fluorescence protein), Zhang et al. reported a more sensitive AIEgen, named HBI analogues (HBIa), for the monitoring of the aggregation process of α-synuclein in a higher resolution.[89] The fluorescence was triggered by inhibition of TICT of HBIa in the rigid microenvironment of viscous solvent or protein aggregates. As shown in **Figure 10b**, HBIa carrying many rotatable moieties and found that the molecule was nonluminescent in folded proteins but showed red emission in aggregated proteins. α-synuclein has been reported to aggregate via a three-step process: (I) formation of soluble oligomers, (II) growth of amyloid fibers, and (III) maturation of fibers. Although

Thioflavin (ThT) could detect growth and maturation of fibers, it failed to detect soluble oligomers that are increasingly speculated to be the toxic species. Meanwhile, thanks to the AIE character, the sensitivity of HBIa was higher than ThT. Fluorescence of HBIa started to increase at 4 h that was the early stage of the first process of α-synuclein aggregates. In short, aggregation processes are highly dynamic where new structures are generated and microenvironment are fluctuated, AIE materials could provide powerful and visualizable platforms to monitor these processes *in situ* and *in vivo*.

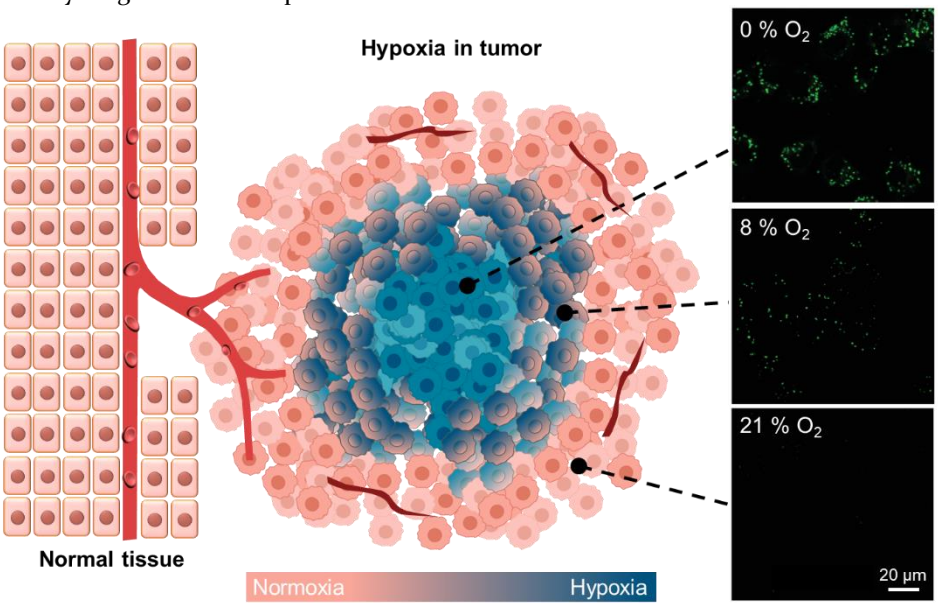

Figure 11. Discovering emergent properties. (a) Schematic illustration of hypoxia in tumor. (b) Fluorescent images of HeLa cells cultured with TPE-2E N-oxide (200 μM). The HeLa cells were incubated at various oxygen concentrations for 3 h. Scale bar = 20 μm. [Adapted from ref 91. Copyright 2019 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.]

DISCOVERING EMERGENT PROPERTIES

According to the discussion before, in a holistic view, at each stage of a living aggregate, specific structures accompanying with relevant processes will result in emergent properties. When looking at aggregates holistically, from a top-down standpoint, starting with the higher-level properties and entities and seeing what they are composed of instead of beginning with an ontology that consists only of lower-level physical components and properties, the properties of higher-level entities are taken as part of one's ontology from the outset.

Tumors could be regarded as living abnormal aggregates originating from abnormal structures. During the formation of tumor tissues, undesirable properties are generated, such as hypoxia microenvironment, acidic microenvironment, vascular malformation, and heterogenization.[90] These emergent properties are highly dynamic due to the variational development of hierarchical structures. Hypoxia, aroused in solid tumors because of the imbalance between aberrant blood vessel formation and increased oxygen demands for tumor cell proliferation, has been regarded as a biomarker for tumor diagnosis. As indicated in **Figure 11**, from the outside part to the core part of solid tumor, the microenvironment varied from normoxia to hypoxia gradually. Based on AIE mechanism, Xu et al. designed and synthesized a kind of probe for hypoxia imaging.[91] Under normoxia environment, the AIE probe is non-emissive since the zwitterionic character endowed the probe good solubility in aqueous media and quenched the emission. Under hypoxic condition, the hydrophilic segment of the probe could be specifically cleaved by cellular reductase overexpressed under hypoxic conditions, which reduced the molecular solubility and led to the aggregation of remaining molecular backbone and thus turn on the emission. Therefore, through rationally design, AIE probe could indicate the oxygen level in HeLa cells at various oxygen concentrations.

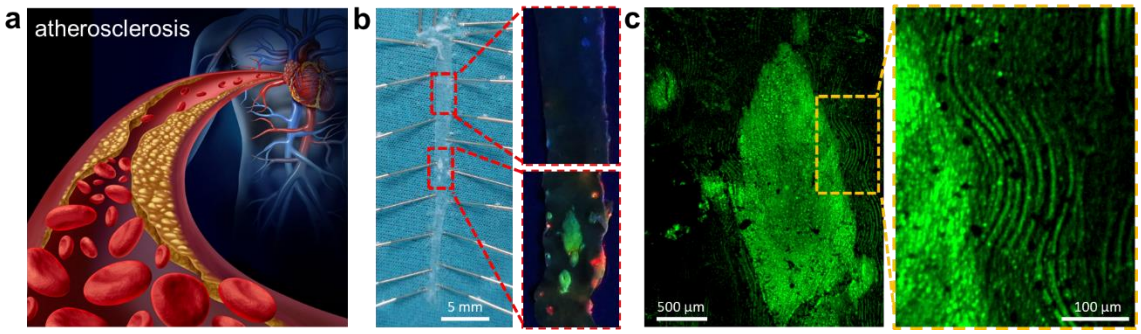

Figure 12. Discover emergent properties. (a) Schematic illustration of atherosclerosis. (b) *En face* photograph of the opened aorta taken under daylight (left). Fluorescent images at high magnification taken under 365 nm UV illumination corresponding to the upper and lower boxed areas (right). (c) Two-photon image of the atherosclerotic plaques. Zoom-in (boxed region) is shown to the right. [Adapted from ref. 93. Copyright 2019 Royal Society of Chemistry.]

Atherosclerosis refers to the accumulation of fatty and/or fibrous material in the innermost layer of intima, the aggregation of lipids thus plays a significant role for the generation of advancement of atherosclerosis.[92] (**Figure 12a**). To gain direct insights into lipid functions within atherosclerosis, it is necessary to observe their amounts, localizations, and distributions in the arteries. High-resolution imaging of lipids within the artery is thus of great value to obtain fundamental knowledge about their roles in atherosclerosis. However, ideal probes are still lacking. Situ et al. reported an AIE probe, IND, for two-photon visualization of lipids within cell lines and arteries.[93] Taking advantage of high lipid specificity, deep tissue penetrability, and excellent two-photon imaging performance, IND can achieve the high-resolution imaging of lipids and the mapping of three-dimensional lipid distributions in mouse atherosclerotic plaques without tedious histological preparations (**Figure 12b**). IND was suitable for *in-situ* two-photon imaging of atherosclerosis at tissue level. In **Figure 12c**, with two-photon excitation at 900 nm, the boundary, location and shape of each atherosclerotic plaque in the artery wall could be lighted up by IND. AIE materials provide a simple method with minimal disruptive procedures for studying atherosclerosis at the tissue level.

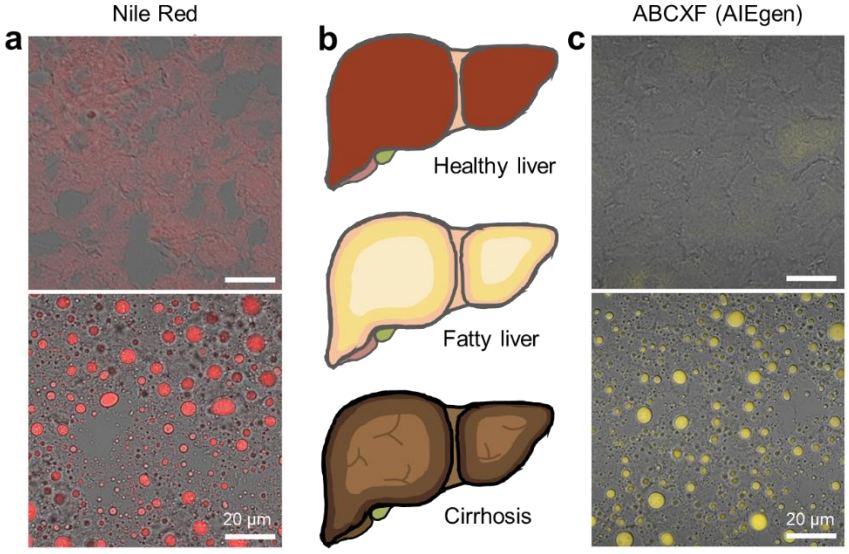

Figure 13. Discovering emergent properties. (a) Fluorescence images of normal and high-fat feeding guinea pig liver tissues stained with Nile Red (1 μM). Scale bar: 20 μm. [Adapted from ref. 96. Copyright 2021 Royal Society of Chemistry.] (b) Schematic illustrations of healthy liver, fatty liver and cirrhosis. (c) Fluorescence images of normal and high-fat feeding guinea pig liver tissues stained with ABCXF (1 μM). Areas in white dash curves indicated the weak signals from normal liver tissue. Scale bar: 20 μm. [Adapted from ref. 96. Copyright 2021 Royal Society of Chemistry.]

At organ level, emergent properties could also be visualized and monitored. Hepatic steatosis, commonly known as fatty liver disease (FLD), is a medical condition where excess lipid droplets accumulate in hepatocytes in the form of triglyceride.[94] Nonalcoholic steatohepatitis is much easier to cause progressive liver fibrosis and eventual liver-related illness and death than isolated steatosis (**Figure 13b**).[95] Therefore, early diagnosis of FLD with a reliable detection method is of paramount importance to the patients' prognostic outcome. Previous studies showed that the hallmark of FLD is excess lipid accumulation in the liver tissue. ABCXF is an AIEgen that can target lipid droplets.[96] By incubating sectioned tissues with ABCXF for 1 h before the imaging, ABCXF showed fluorescence in the

FLD tissue from the high-fat feeding guinea pig due to its intramolecular charge transfer effect and the viscous and low polar lipid environment. As a contrast, only very faint emission was collected in the control group due to the low abundance of lipid droplets in the normal tissue (**Figure 13c**). It is worthy to note that in normal feeding tissue with lower lipid content, ABCXF displayed much better performance than Nile Red (**Figure 13a**).

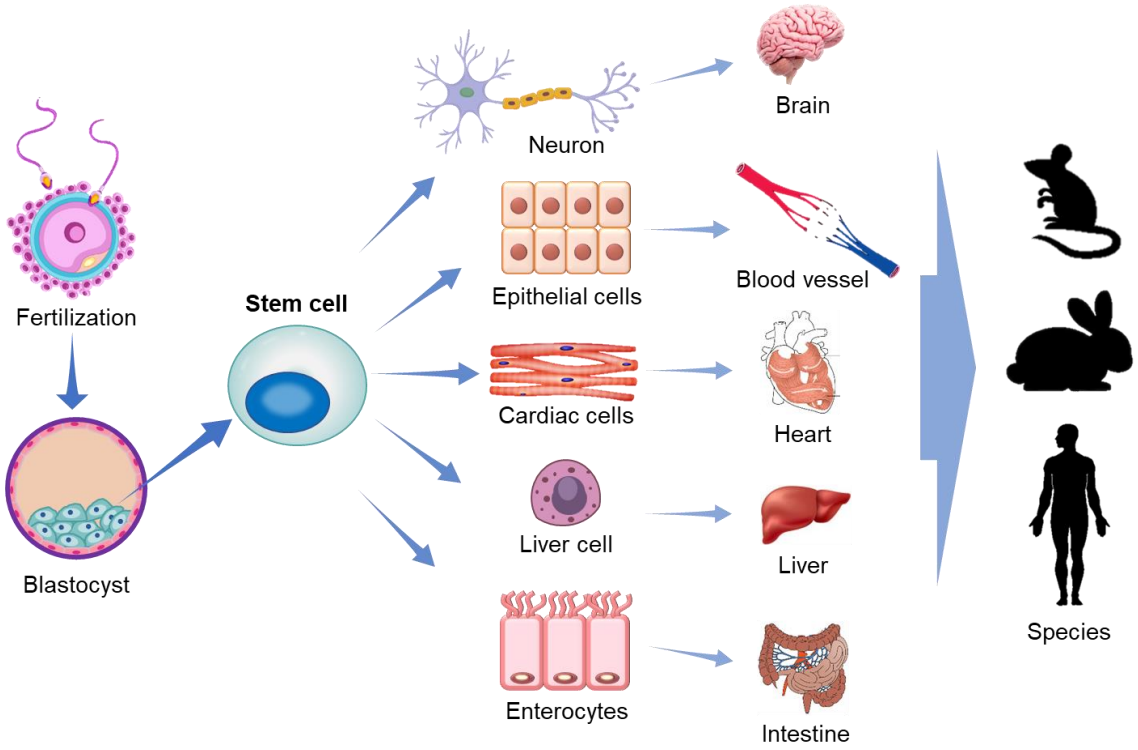

Figure 14. Schematic illustrations typical aggregation process in living systems form fertilization process to species

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PERSPECTIVE

In the former part, individual works have been discussed, in order to explore the paradigm shift from reductionism to holism by using AIE materials as useful tools. As we discussed before, living systems could be regarded as living aggregates and they possess three characteristics: hierarchical structures, dynamic processes, and emergent properties. But the relationship between life with chemistry is still mysterious. Indeed, guided by reductionism, from whole body to an individual cell, and even to proteins and nucleic acids, numerous researches have been carried out. And they worked very well and provided incredibly rich information for us to understand the nature of life. In fact, investigating life science from holistic view is as important as reductionism be. From the holistic view, normal aggregation process could create normal living systems while abnormal aggregation may lead to dysplasia, disease or even death. Figure 14 portrayed the processes from stem cells to various somatic cells are typical in the aggregation manner. During the differentiation processes, not only relevant genes activated to promote the differentiation of stem cells into specific somatic cells, but complicated factors such as cell-cell interaction, cell force, cell tension, morphology, cytokine communication, and pH variation induced by aggregation processes occurred.[97-98] These facts refute the Copernican view of man's place in the universe that "the human race just a chemical scum on a moderate-sized planet". Fortunately, with the development of omics, the complicated interactions during differentiation have been revealed inch by inch. Though there is still a "Black box" in which the intermediate structures and dynamic processes are still insufficient in research. Visualization and tracking of these intermediate structures and dynamic processes are of vital importance. From the fertilization to stem cell and then to species, the whole picture involves many aggregation processes with variations in structures, processes, and properties at each stage. By taking advantage of AIE materials rationally, these complicated processes could be monitored or visualized and are anticipated to be understood deeper in future. Two representative works regarding the research area of stem cells were chosen as demonstrations to show the great potential of AIE materials .

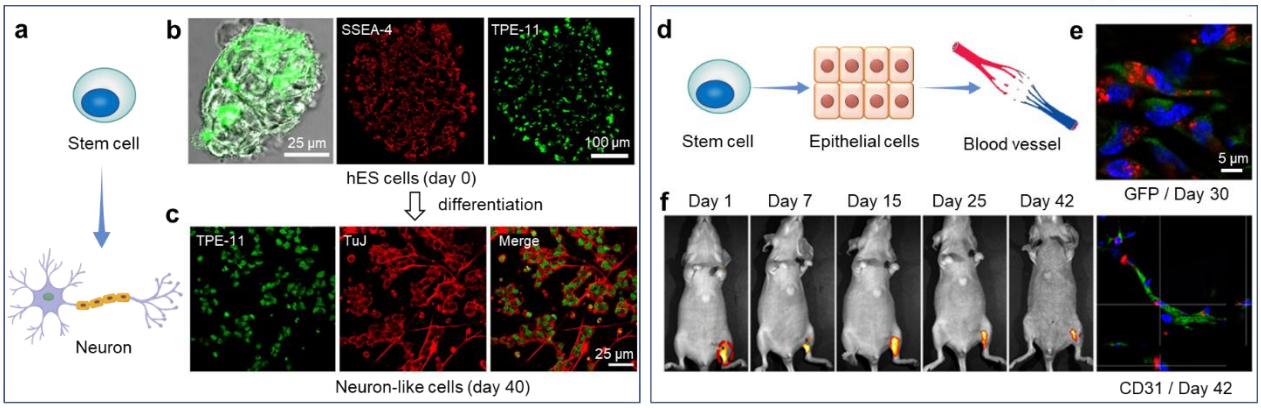

Figure 15. Applications of AIEgens in monitoring and visualization of the differentiation of stem cells to (a-c) neurons [Adapted from ref. 99. Copyright 2018 Acta Materialia Inc. Elsevier Ltd.] and (d-f) vasculature cells. [Adapted from ref 100. Copyright 2014 American Chemical Society.]

From stem cell to neuron, AIEgens have been applied for the monitoring and visualization of the differentiation process (**Figure. 15**). Compared to traditional genetic modification approaches, labeling cells with nanoparticles has advantages, especially for the additional safety they provide by avoiding genomic integration. However, it remains a challenge to determine whether nanoparticles interfere with cell traits and provide long-lasting signals in living cells. Owing to AIE effect and the ability that self-assemble could enhance the persistence of signals, an AIEgen TPE-11 was developed for labeling and tracking the differentiation of hES cells.[99] Immunostaining analysis and teratoma formation assays showed that the pluripotency remained unaltered after labeling (**Figure 15b**). Additionally, these nanoparticles allowed for long-term monitoring of hES cell differentiation into neuron-like cells lasting for 40 days (**Figure 15c**). In addition, adipose-derived stem cells (ADSCs) were chosen as model for the differentiation study. With the development of efficient stem cell therapies, it is important to track the fate and regenerative capability of the administrated stem cells in both experimental models and clinical trials over a long period of time. Ding et al. applied AIE NPs as exogenous fluorescent probes for in vitro and in vivo long-term ADSC tracking, demonstrated superior cell tracking performance to the most popular commercial cell trackers (**Figure 15d-e**).[100] Detailed experiments also revealed that the AIE NPs internalization by the ADSCs did not affect their pluripotency, secretome, and *in vivo* treatment efficacy. More importantly, the AIE NPs were demonstrated to be able to precisely track the ADSCs and their regenerative capacity, which provided us insight into understanding how ADSCs contributed to the ischemia therapy.

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Author Contributions

W. H., Z. Z. and B. Z. T. conceived the original idea for this perspective work. W. H., R. T. K. K., Z. Z. and B. Z. T. discussed the whole work and designed the figures. B. Z. T. supervised the whole process. W. H., R. T. K. K., Z. Z. and B. Z. T. discussed for the manuscript and prepared the manuscript. W. H. wrote the manuscript, and R. T. K. K., Z. Q., Z. Z. and B. Z. T. revised the manuscript. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no conflict of interest.

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