Received: 31 July 2023 | Accepted: 12 September 2023

DOI: 10.1002/bmm2.12053

REVIEW

BMEMat WILEY

Biosensing strategies for amyloid-like protein aggregates

Yuhang Zhou[1] | **Shijun Yan**[2] | **Wanting Dong**[1] | **Chenyao Wu**[1] | **Zhen Zhao**[3] | **Renzhi Wang**[1] | **Yanhong Duo**[4] | **Yongzhi Huang**[5] | **Ding Xu**[6] | **Cheng Jiang**[1,2]

[1]School of Medicine, The Chinese University of Hong Kong (Shenzhen), Shenzhen, China

[2]Nuffield Department of Clinical Neurosciences, John Radcliffe Hospital, University of Oxford, Oxford, UK

[3]Materials Interfaces Center, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China

[4]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA, USA

[5]Academy of Medical Engineering and Translational Medicine, Tianjin University, Tianjin, China

[6]Shenzhen Hicontac Technologies Co., LTD., Shenzhen, China

Correspondence
Cheng Jiang, Yongzhi Huang and Yanhong Duo.
Email: jiangcheng@cuhk.edu.cn; cheng.jiang@ndcn.ox.ac.uk, yongzhi_huang@tju.edu.cn and yanhong.duo@ki.se

Funding information
Basic and Applied Basic Research Foundation of Guangdong Province, Grant/Award Number: 2022A1515110206; The Chinese University of Hong Kong, Shenzhen, Grant/Award Numbers: K10120220253, YXLH2218

Abstract

Protein aggregate species play a pivotal role in the pathology of various degenerative diseases. Their dynamic changes are closely correlated with disease progression, making them promising candidates as diagnostic biomarkers. Given the prevalence of degenerative diseases, growing attention is drawn to develop pragmatic and accessible protein aggregate species detection technology. However, the performance of current detection methods is far from satisfying the requirements of extensive clinical use. In this review, we focus on the design strategies, merits, and potential shortcomings of each class of detection methods. The review is organized into three major parts: native protein sensing, seed amplification, and intricate program, which embody three different but interconnected methodologies. To the best of our knowledge, no systematic review has encompassed the entire workflow, from the molecular level to the apparatus organization. This review emphasizes the feasibility of the methods instead of theoretical detection limitations. We conclude that high selectivity does play a pivotal role, while signal compilation, multilateral profiling, and other patient-oriented strategies (i.e. less invasiveness and assay speed) are also important.

KEYWORDS
biomarker, degenerative disease, protein aggregates, sensor

The first three authors (Yuhang Zhou, Shijun Yan, Wanting Dong) contributed equally to this work.

1 | INTRODUCTION

Protein aggregation is a ubiquitous phenomenon, which was initially discovered in a few neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Amyotrophic Lateral Sclerosis (ALS). However, it has since been recognized to be pervasive in other conditions, spanning from diabetes to Covid-19 (Table 1).[15,16] Recent emerging findings have also demonstrated that certain aggregates serve non-pathological functions.[17] At present, conditions where protein misfolding or aggregation plays a dominant role in pathology are categorized as protein conformational diseases. The scope of protein aggregate species is broad, including amyloid, amorphous, or native-like assemblies, etc. In this review, we primarily focus on amyloid-like aggregates and their oligomeric precursors.

It has been well-established that these aggregates exert cytotoxicity, leading to genome disruption, lysosome dysfunction, impaired energy metabolism, and obstructed intracellular transportation.[18,19] Depending on the extent of aggregation, completely isolated single proteins are referred to as monomer, aggregate with ~10 collective monomers is referred to as oligomers, and further aggregate chunks are attributed to fibrils or polymorphs.[20] Interestingly, in both neurodegenerative diseases or peripheral diseases, toxicity does not positively correlate with extend of aggregation, instead, oligomers are usually more toxic than fibrils.[4,18,19,21] Aggregation-prone proteins can undergo ordered or amorphous deposition while they interact intermolecularly, which have been increasingly linked with liquid-liquid phase separation.[22–26] Albeit a certain energy barrier must be crossed for monomers to shift into conformational ensemble, which can be significantly accelerated by existing amyloid fibrils as seeds or templates.[27] In many cases, the emergence of protein aggregates serves as a biomarker for the progression of degenerative disease.

The ominous characteristics of aggregates endow them with great potential as biomarkers.[1,2,15,28–30] Growing evidence has shown that aggregates are more relevant to disease progression compared to monomer format.[29,31] Initially, aggregates were observed only in post-mortem histology as plaques, limited to macroscopic observations. Thus, technical solutions for sensitive and selective detection of aggregate species have drawn tremendous attention.[32–34] To date, various aggregate species have been studied, and considerable sensitivity has been achieved.[35–37] However, it should be noted that most detection technologies are still primarily lab-oriented protocols, with few developed with robust practicality.

Practicality, in this context, the utility of the detection method is determined by whether it can be used to improve patient prognosis or welfare. Sensitivity is not the sole determinant; all relevant factors that may affect their performance against diverse biosamples must be considered. Judging from the invasiveness of acquiring biosamples, the most invasive method involves obtaining large tissue chunks (post-operation or post-mortem), followed by puncture biopsy or cerebral spinal fluid (CSF) collection, which is still invasive. More gentle approaches involve collecting peripheral blood, offering biomarker treasure-like protein aggregate in free floating format and their integration with extracellular vesicles format.[38–41] Their minimal-invasiveness makes them promising sources for examining aggregate species; however, this area remains largely underdeveloped due to ultra-low abundance.[36]

In this review, we critically assess in vitro optical- and electrochemical-sensing technologies/platforms for liquid

TABLE 1 Diseases that involve protein aggregate pathology.

Disease	Protein aggregate	Ref.
PD	α-synuclein	[1]
AD	Tau, amyloid β	[2]
ALS	SOD1,[a] TDP-43[b]	[3]
Diabetes	hIAPP[c]	[4]
Cancer	P53	[5]
Covid-19	NCAP[d]	[6, 7]
Huntington's disease	Huntingtin	[8]
Frontotemporal dementia	FTLD-Tau/MAPT[e]	[9]
Psychosis	DISC1[f]	[10]
Immunoglobulin Light Chain (AL) Amyloidosis	Immunoglobulin light chain	[11]
Amyloid protein A amyloidosis	SAA[g]	[12]
Transthyretin amyloidosis	Transthyretin	[13]
Fibrinogen alpha chain amyloidosis	Fibrinogen	[14]

Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; PD, Parkinson's disease.

[a]Superoxide dismutase 1.

[b]TAR DNA-binding protein-43.

[c]Human islet amyloid polypeptide, amylin.

[d]SARS-CoV-2 Nucleoprotein.

[e]Microtubule-associated protein tau.

[f]Disrupted-in-schizophrenia 1.

[g]Serum amyloid A.

biopsy roadmap against various protein aggregate species (Figure 1). We highlight their design strategies, merits and limitations. We envision protein aggregate biomarkers as pivotal grippers in combating a plethora of degenerative diseases.

2 | NATIVE PROTEIN AGGREGATES SENSING

Detecting aggregate species requires a strategy that permits the specific detection of aggregates while minimizing the interference from the common co-existing monomeric forms. This can be achieved by either exclusive recognition of aggregates or generation of distinguishable readouts for aggregates rather than monomers. To date, different receptors have been explored and integrated into either bulk instruments or portable devices to permit the specific detection of protein aggregates.

2.1 | Small molecule probes

Small molecular probes are among the earliest tools used for investigating aggregates and continue to play a significant role in aggregate detection.[42–44] Their chemical structural affinity permits the recognition of certain regions of protein aggregates, that is, confirmational specificity. These molecular probes can be designed, synthesized, and tuned case by case. Several classes of probes have gained broad acceptance, and three of them have been the prominently spotlighted. (1) Molecular Rotors: These structure-specific dye, such as Thioflavine T (ThT) and Thioflavine S (ThS), can selectively insert into β-sheets and bind to amyloid fibrillar aggregates. Thus, they can be used as both receptor and reporter for the fibrils.[44,45] ThT remains the most popular option for its low cost, high efficiency, and robustness. (2) Congo Red and derivates Congo red molecules possess a high affinity for amyloid aggregates and allow imaging of non-β sheet structures. They can serve as scaffolds for numerous fluorescent derivatives.[43,44,46] (3) Aggregation-Induced Emission (AIE): This type of unique molecule is non-emissive in aqueous solution but becomes highly emissive when gathered together.[47–49] This feature is well-suited for monitoring the aggregation process as "chemical nose" as it provides structure-dependent tunable binding affinity with synchronous signal emission. Besides these, there are promising candidates like carbon-based quantum dots (QD), cyclic peptide-based nanoparticles (c-PNPs), carbazole derivatives, or transition metal complexes that are worth considering.

The focus of this review is to discuss the prospects and roles of these probes in the future aggregate species detection. Primarily, small molecular probes can be utilized as modules within sensors to highlight the conformational features of aggregates. They are employed in the quantitative analysis of histological specimens (Figure 2a), while several quantification techniques employ them to monitor dynamic processes. However, relying solely on probes for quantifying aggregate biomarkers can be challenging due to their limited quantum yield, resulting in weak signals for trace amounts of targets and limited information entropy.

Moreover, one major drawback of most probes is their susceptibility to aggregation-caused quenching (ACQ) effect at high concentrations, which hampers the detection of aggregate species in the concentrated milieu in bodily

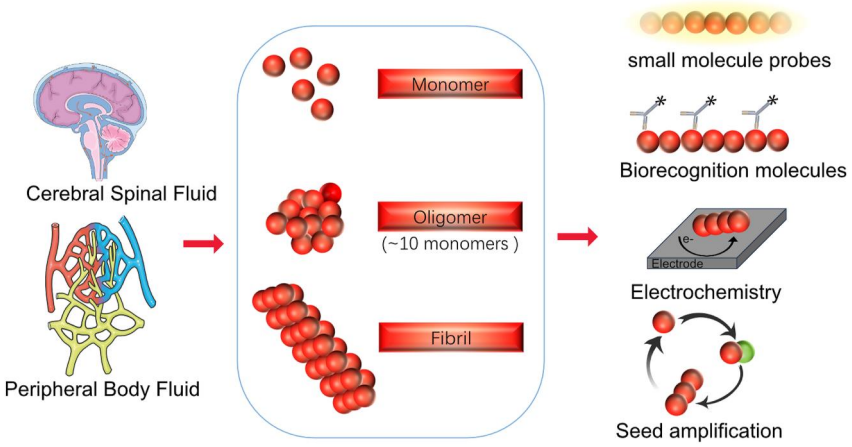

FIGURE 1 Different sensing strategies for the protein aggregate based biomarkers.

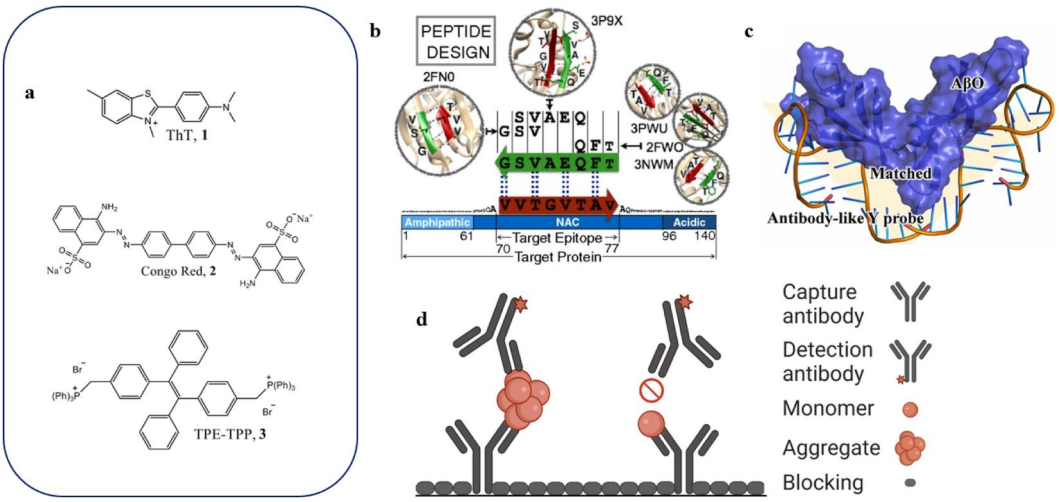

FIGURE 2 Stratification of biorecognition molecules of aggregate species. (a) Amyloid probe molecules: Thioflavine T), Congo Red, Aggregation-Induced Emission probe (TPE-TPP). Reproduced with permission.[43] Copyright © 2019 American Chemical Society. (b) Rational design of antibodies targeting protein aggregates. Reproduced under terms of the CC-BY license.[50] Copyright © 2023 National Academy of Science. (c) Design of antibody-mimic Y aptamer. Reproduced with permission.[51] Copyright © 2023 Elsevier B.V. All rights reserved. (d) Model of surface-FIDA method. Reproduced with permission.[52] Copyright © 2022 Springer Nature.

fluids.[53] Additionally, these probes tend to recognize monomers, which coexist with aggregates to some extent, leading to relatively high background noise and compromised selectivity. In contrast, AIE luminogens (AIEgens) largely evade this flaw. AIEgens are not affected by ACQ, instead, they perform better with increased aggregation. Since AIEgens emit only when aggregation occurs, they offer a clear background, making their net quantum yield against protein aggregate species more promising than traditional probes. Hence, it is speculated that AIEgens may represent the next-generation probes of aggregate species.

Furthermore, an effective probe against aggregate species should be inert to aggregate dynamics. While the aforementioned probes excel in this respect, prospective ones must be carefully evaluated based on this principle. Hegner's group proposed this principle in their microcantilever array detection design.[54] Compared to ThT, label-free detection is regarded as superior, emphasizing the importance to expel the interference that the probe brings to the aggregate. Some novel probes like transition metal complexes have been found to act as aggregation modulators but turned out to be emissive,[43] making them less suitable as probes.

Nevertheless, probes that are non-effective in fibrillation kinetics or those capable of labeling in extremely low quantities could greatly aid in the development of new assays. For instance, Fillet group reported an electrophoretic protocol to detect aggregate and monomers separately using capillary gel electrophoresis (CGE) separation without the need of confirmational specific biorecognition molecules due to their different retention times and migration rate in the column.[55] The key to establishing a robust assay relies on a highly-efficient probe that can accurately indicate target quantity. Fillet et al. adapted labeling reagents (Chromeo P503, NBD-F, etc.) that are covalently conjugated with proteins. These labeling reagents are commercialized small molecules, which have better accessibility. A similar technique was reported by Zhao et al., where self-assembling luciferase fragments probes was described.[56] Although these works achieved relatively good detection compared to traditional probes, in some cases, novel probes like AIEgens (e.g. TPE-TPP) could replace traditional fluorophores to further improve detection performance by reducing probe quantity while maintaining high emission regarding aggregate species.[48]

In essence, optical probes are molecular sensors that can bind to protein aggregates and generate optical signals. Developing novel probes with a rational design of chemical structures for functional moieties of recognition and signal emission is crucial. Additionally, it is essential to explore applicable scenarios for incorporating existing probes with other advanced modules according to their specific features.

2.2 | Biorecognition molecules

2.2.1 | Conformation-specific bioreceptor

As protein aggregation progresses, the stacked monomers adopt new conformations that can be recognized by biorecognition molecules. Antibodies and aptamers are

the typical examples of biorecognition molecules capable of recognizing specific structural characteristics, making them suitable for aggregate detection in sensory devices. In the early stages of research on PD and AD, detection of these aggregates was primarily achieved through immunohistochemistry in post-mortem brain tissue.[57] For other aggregate-associated pathologies that remain less explored, many are still reliant on commercialized but imperfect antibodies (Table 1). Despite their limitations, antibodies and aptamers remain valuable for detecting protein targets due to their conventional and straightforward approaches.

To date, numerous commercial antibodies are available, such as A11, SC-211, MJF-14-6-4-2.[58,59] Antibody development procedures generally involve rational design (third generation) and antibody screening (first and second generations).[60] Due to the complexity of the aggregation behavior, specific optimizations are required, making the third-generation technique is the most favorable nowadays. In the first stage, complementary peptides need to be identified using developed methodologies, as proposed by Sormanni et al. These selected peptides are then integrated onto antibody scaffolds, and their binding performance is evaluated.[50,60] The design of peptides is crucial, employing a cascade method to organize several complementary fragments and select promising candidates. Ideally, the peptides adopt a β-strand–like conformation (a hallmark of aggregates) and partly overlap with neighboring fragments, with overlapping regional feature, identical sequence and backbone hydrogen bond patterns (Figure 2a). Vendruscolo's group has been actively working on both theoretical and practical aspects of this field. In 2017, they proposed an antibody scanning method for the detection of Aβ42,[61] and in 2022, they presented a method to identify antibodies targeting α-synuclein oligomers.[62] The rational design (also known as third generation approach) offers advantages over in vivo (first generation) and in vitro (second generation) approaches in terms of workload and efficiency.

Regarding aptamers, these nanostructures, composed of nucleic acid molecules or small organic molecules, have been engineered to mimic the structural features of antibodies. Aptamer-based design has been widely used in both experimental treatments and assay development.[63,64] Aptamers offer attributes such as high specificity, reversibility, stability, low cost and ease of modification, making them valuable components. Therefore, aptamers are usually incorporated with transducers to generate, for example, optical-/electrochemical-aptasensors. The selection of aptamers against protein aggregates is typically accomplished through a specific in vitro method called systematic evolution of ligands by exponential enrichment (SELEX), an approach that has been widely applied and extensively reviewed by many researchers.[65]

Notably, certain aptasensors have been designed to detect α-synuclein oligomers using colorimetry, as proposed by Sun et al.[66] With aptamers tethered onto gold nanoparticles (AuNP), a concise detection protocol can be established (Figure 2b). Once the aptamer captures target oligomers, AuNP generates a colorimetric change that shifts from red to blue. By employing a linear regression equation of absorbance at 650/520 nm, a detection limit of 10 nM can be achieved. Aptasensors also function effectively with electrochemical methods (see part 2.2). Tang et al. utilized aptamers that are immobilized in microwells to capture Aβ.[67] The aptamer-Aβ complex congested the microwells, leading to electron transfer hindrance against electrochemiluminescent (ECL) reaction. Consequently, the ECL output was negatively correlated with the concentration of Aβ. This approach achieved a limit of detection (LOD) of 0.012 pM, with a dynamic range from 0.05 to 5 pM. It is noteworthy that this method is designed to detect monomers, yet it holds the potential to be converted into aggregate-oriented by replacing the current receptor with the conformation-specific one, in principle.

Another rational design case was reported by Zheng et al., where the Aβ oligomer oriented aptamer was designed in a "Y" shape to potentiate the specific molecular recognition.[51] It was previously noticed that two-correlated aptasensor Sandwich assay possesses advantages in avoiding the limitation of steric hindrance and epitope, compared to single aptasensor or aptamer-antibody Sandwich assays.[68] The "Y" shape can encompass a pair of aptamers in a unified structure and provides a rigid stem that keeps the structure more stable. When annexing to other modules, interference from the recognition site must be ruled out.

2.2.2 | Same receptor as capture and detection unit

The central challenge in employing antibody or aptamer lies in the structural resemblance between aggregate and monomers. Aggregate possesses almost all structural characteristics of monomer, and while some new structural motifs may develop in aggregates, antibodies could still respond to them.[62] Nonetheless, a concern arises that if free-monomers come in close proximity, they might also attract receptors to bind to them, leading to false positive signal. Therefore, it is unlikely to develop an antibody that solely recognize aggregates.

A plausible solution involves applying the pigeonhole principle: If n objects are distributed across m places, and if $n > m$, then at least one place receives two objects. In

this context, if one antibody occupies the epitope of a monomer unit, it becomes improbable to be bound by the same antibody again. This principle has found widespread applications, since it can be simply adapted to general enzyme-linked immunosorbent assay (ELISA) protocols.[69–71]

The Willbold group applied this concept in a surface fluorescence intensity distribution analysis (sFIDA).[72] sFIDA can be regarded as a variant format of conventional ELISA, differing in its use of the same capture and detection antibody. When the analyte is applied to the capture antibodies, the epitope of the monomer is masked by the capture antibodies, preventing it from being re-recognized (Figure 2c). However, aggregates can still be recognized, resulting in high selectivity against aggregates.[73]

sFIDA presents several advantages, as it exclusively counts the numbers of aggregated proteins through fluorescence correlation spectroscopy scanning (FCS) across the entire surface, thus avoiding the superimposition of diffusion and thus achieving accurate readouts. By using laser scanning microscopy, the performance of the assay can be improved further.[72] Additionally, sFIDA can be incorporated with different signal reporting modules such as AIEgen particles to minimize errors. The utilization of red and green fluorescence beacons aids in reducing random errors resulting from ultrasensitivity of the instrument.[74] sFIDA has been applied to prominent aggregate species such as α-Synuclein and Tau oligomers,[52] exhibiting high accuracy with anti-interference potential in real samples such as stool.[75–77]

Although this technology offer distinct advantages, it requires highly specific linear epitope antibody, which may not always be available. Additionally, there is a possibility that once binding and capture happens, monomers fuse on the spot due to spatial proximity. Furthermore, ultrasensitive instruments are required, which could lead to potential cost concerns. There is also a risk of steric hindrance amongst epitopes interfering with binding events, potentially resulting in detection failures. Therefore, the optimization of designs incorporating this principle may be the solution for the future.

2.3 | Electrochemical methods

Depending on the platforms used to monitor the binding events of protein aggregates to the sensing interface and output signal, electrochemical sensing is one of the most commonly used methods for aggregate detection (Table 2).[35] A prominent advantage of electrochemical methods is their tunable label-free or labeled detection format coupled with decent sensitivity, direct electrical communication between protein aggregate with underlying working electrode or indirect communication with redox probe as mediator have been intensively explored. Earlier studies relied on the difference in characteristic redox potential of monomer and aggregates at the underlying electrode to differentiate and detect aggregates. Lopes et al. proposed an electrochemical monitoring method by sensing oxidizing tyrosine (Tyr) residues.[93] Since Tyr residues are surface-exposed, aggregation would affect their electrochemical accessibility, hence affecting the degree of electrooxidation. The whole detection process is straightforward but the apparent limitation of the electrochemical label-free sensing for aggregate lies in its poor specificity, since there are coexisting species in bodily fluids with similar redox properties, which can undoubtedly lead to background noise.

To address this, electrochemical methods can also be integrated with biorecognition molecules to achieve selective detection, and they can be label-free without the need of any redox tag. Electrochemical impedance spectroscopy (EIS) or differential pulse voltammetry (DPV) are frequently adapted methodologies. For example, Sun et al. designed an aptamer-based EIS detection against Aβ oligomers.[66] The aptamer-coated electrode exhibited certain impedance, and the conjugation of oligomers can increase the electron transfer resistance (R_{et}), while the conjugation of AuNPs can "switch on" electron transfer with a decrease in R_{et} (Figure 3a). By performing an aptamer-target conjugation, the change in R_{et} (ΔR_{et}) reflects the concentration of the target. AuNPs serve as an amplifier, as the occupation of the target aptamer-binding site hinders the aptamer-AuNP conjugation, leading to an enlarged ΔR_{et}. This work also compared EIS and surface plasmon resonance (SPR) protocols, showing that EIS demonstrated superior performance with a detection limit of 1–8 pM.

Additionally, Gong et al. reported a DPV-based method[94] (Figure 3b). In this design, the redox reaction was linked to aptamer-oligomer recognition, while tripedal DNA walkers were introduced to increase the efficiency of the assay. Using this approach, a LOD of 0.46 fM and a broad linear range of 1 fM to 10 pM were achieved. Several studies have employed an aptamer conjugated with redox probes as interpreters to construct electrochemical analyses, and their success greatly depends on the mechanism of electron transfer potency, indicating that a solid amplification strategy can significantly improve DPV performance.

While electrochemical assays have unique advantages, they also come with limitations. For instance, they heavily rely on nano-/micro-fabrication in case of nano-/micro-electrodes, which can introduce inconsistencies.

TABLE 2 Protein aggregate detection classification.

Sensor construction	Target	Detection method	Disease	Sample	Dynamic range	LoD	Ref
Apt-poly(T)-CuNPs/AβO/PrP[C]/AuNPs-VG-CC	AβO	DPV[a]	AD	serum	10–2200 pM	3.5 pM	[78]
Apt/AuNPs-MOF/ITO	AβO	ECL[b]	AD	serum	0.1−10 pM	71 fM	[79]
Ferrocene/Zn-MOF/AuNPs	AβO	EIS[c]	AD	CSF[d]	$0.5–10^{2}$ μM	10^{-8} nM	[80]
Aptamer-Au-Th/AβO/BSA-Ab/electrode	AβO	DPV	AD	CSF	0.5–30 nM	100 pM	[81]
AuNPs/QDs	AβO	Fluorescence	AD	N.A.	2×10^{-4} to 0.1 μM	2×10^{-4} μM	[82]
Biotinylated PrPC (95–110)/polymer-functionalized gold screen-printed electrodes	AβO	EIS	AD	Cell culture medium	N.A.	0.5 pM	[83]
SCE/platinum wire	Aβ 1-42 and TNF-α[d]	SWV[e]	AD and other	Cell culture medium	N.A.	0.048 nM	[84]
Water-in-oil nanodroplets	Aβ42	THz-TDS[f]	AD	Artificial buffer	0.5–2.0 nM	1 nM	[85]
CFP/AuPt	AβO	DPV	AD	Serum	0.5–10,000 pg/mL	0.16 pg/mL	[86]
MCH/Aptamer/MSM[g]/AuNPs/ITO/luminol probe	AβO	ECL	AD	Blood	0.05–5 pM	0.0120 pM	[67]
Exo I/TdT/methylene blue	α-synuclein oligomer	AFM[h]/SEM[i]	PD/AD	Serum	60 pM to 150 nM	10 pM	[87]
Fc-CS/AE/TAP[j]	AβO	DPV	AD	Artificial CSF	1 fM to 10 pM	0.47 fM	[88]
DesAbs[k]	a-synuclein oligomers	Fluorescence	PD	Serum/CSF	N.A.	40 mM	[62]

(Continues)

TABLE 2 (Continued)

Sensor construction	Target	Detection method	Disease	Sample	Dynamic range	LoD	Ref
CYS/SAM[l]/FTO electrode	α-synuclein	DPV/EIS	PD	Serum	10–1000 ng/mL	1.13 ng/mL	[89]
CuO	α-synuclein oligomer	Fluorescence/circular dichroism spectroscopy	PD	N.A.	N.A.	N.A.	[90]
SiNPs-CH	EGFR[m]	UV–visible spectroscopy, EDS[n]/NTA[o]	Breast cancer	Plasma	0–50 ng mL^{-1}	1.37 pg mL^{-1}	[91]
MJF14-6-4-2 antibody	α-synuclein	ELISA	PD	N.A.	0.25–20.0 ng/mL	0.25 ng/mL	[92]

Abbreviations: AD, Alzheimer's disease; CSF, cerebral spinal fluid; DPV, differential pulse voltammetry; ECL, electrochemiluminescent; EIS, electrochemical impedance spectroscopy; ELISA, enzyme-linked immunosorbent assay; LoD, limit of detection; PD, Parkinson's disease.

[a]Differential pulse voltammetry.

[b]Electrochemiluminescence.

[c]Electrochemical impedance spectroscopy.

[d]Cerebrospinal fluid.

[e]Tumor necrosis factor-α.

[f]Square-wave voltammogram.

[g]Time-domain terahertz spectroscopy.

[h]Mesoporous silica membrane.

[i]Atomic force microscopy.

[j]Scanning electron microscopy.

[k]Designed antibodies.

[l]Self-assembled monolayer.

[m]Epithermal growth factor receptor.

[n]Energy dispersive spectrometry.

[o]Nanoparticle tracking analysis.

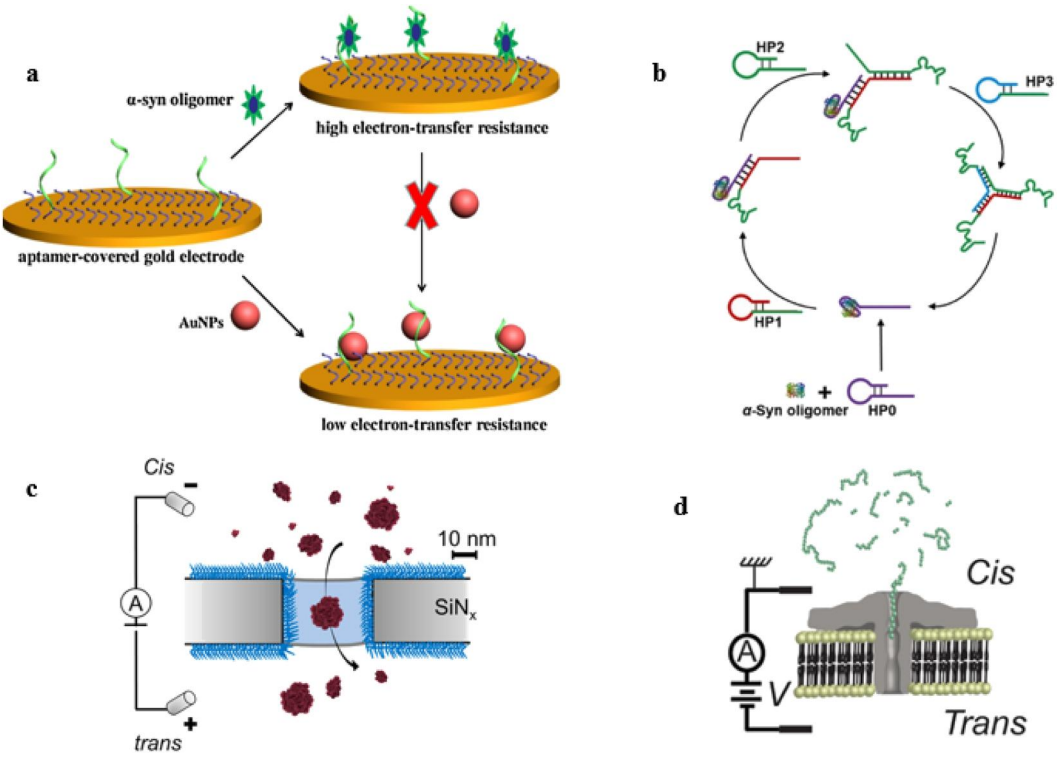

FIGURE 3 Electrochemical methods of aggregate species detection. (a) Aptamer-based electrochemical impedance spectroscopy detection of a-syn oligomers. Reproduced with permission.[66] Copyright © 2017 Elsevier B.V. (b) DPV-based tripodal aptamer oligomer assay. Reproduced with permission.[94] Copyright © 2023 Elsevier B.V. (c) Nanopore (mechanical) detecting Synuclein Oligomers in Solution. Reproduced under terms of the CC-BY license.[95] Copyright © 2023 American Chemical Society. (d) Biological nanopore as peptide chain detector. Reproduced with permission.[96] Copyright © 2023 American Chemical Society.

Additionally, well-trained personnel are needed, making them less suitable for routine use in clinics or at home. Moreover, nonspecific adsorption from complex sample matrix may block electron transfer and lead to measurement failures, making an antifouling surface design would be favorable.[33,97,98] Nevertheless, the fine quality of screen-printed electrodes, with the capability of mass production in respect of aggregate species detection, is highly valued and would be good option for future point-of-care testing using validated protein aggregate biomarker.

Moving forward, one promising electrochemical method for aggregate profiling is the nanopore technology. Due to the prominent differences (morphology, molecular weight, charge, etc.) between aggregates and monomers, as well as between oligomers and fibrils, the most straightforward idea is to distinguish and monitor their mass, which may facilitate understanding of disease evolution in terms of molecular pathology. The basic structure of a nanopore gadget involves using a film to two separate electrolyte compartments, with a pore locate on the film. When a potential difference is applied on two chambers, aggregate species translocate through the pore, resulting in a transient resistive and/or current change.[99] These characteristic resistive pulses contain information about the physical properties of the translocating particles, including their volume and shape.[100] Awasthi et al. proposed an oligomer-oriented nanopore methodology. Since the nanopores do not require biorecognition receptors, their major contribution lies in solving the challenge of distinguishing oligomers of different sizes, as there are no qualified antibodies available for this purpose[95] (Figure 3c). There are two main types of nanopore designs: mechanical (i.e., solid-state) and biological. Biological nanopores typically employ lipid bilayers to replace films made of polymers.[101] The advantage of biological nanopores lies in their dynamic nature, which allows for monitoring molecular behavior with single-molecule precision, even distinguishing amino-acid residues[96] (Figure 3d). However, the clear drawback is that the current nanopore design relies solely on electrical current fingerprint to distinguish size, meaning it cannot selectively detect desired aggregate species. This pattern works only in the sole target detection, for which real samples are far from readily available. Every non-target molecule in the sample would likely interfere with the detection. Therefore, nanopores should be integrated with other pretreatment measurements or collaborate with biorecognition modules, which is a challenge in translating the assays to products.

3 | SEED AMPLIFICATION

In addition to the detection of the aggregate in situ without pre-treatment discussed above, there is a typical concern about the detection sensitivity for protein aggregates, especially when they are present in ultra-low abundance. For example, the quantity of α-synuclein aggregates on the membrane of or within the cargo of exosomal subpopulations, such as neuronal exosomes, which represents only ~10% of total exosome populations.[40,102]

Benefiting from the fact that aggregates attract normal aggregate-prone protein monomers to join them, a self-amplification process known as seeding occurs.[103] During this self-amplification, the mass (or size) of aggregate species increases. By iteratively breaking down grown aggregates into smaller sizes, seeding can be used for massive amplification of aggregate species, resembling a repetitive cycle similar to polymerase chain reaction (PCR) (Figure 4a). Based on this concept, several sensitive aggregate detection paradigms have been developed.[36]

The seminal amplification protocol, known as protein-misfolding cyclic amplification (PMCA), was first described by Claudio Soto and colleagues in 2001 as a method to amplify prions in vitro, establishing the foundation of amplification-based protein aggregate detection technology.[107] Initially, researchers manually shattered fibrils using a microtip sonicator and incubated them at 37°C, repeating this process 5–6 times, which was arduous and limited. Later, automated apparatus was introduced, extending the cycles to days.[108] The final readout of product amount was examined by Western blotting (WB). After 2 decades of development, this cumbersome protocol has been abandoned, with the latest report using PMCA ceasing in 2013.[109]

PMCA became obsolete due to the emergence of its successor, real-time quaking-induced conversion (RT-QuIC); RT-QuIC was developed as an improvement and extension of the original PMCA.[110] The principle of autocatalytic self-amplification remains the same, but the arduous sonication is replaced by vigorous intermittent shaking, and WB readout is substituted with ThT fluorescence reading using a plate reader. Both shaking and fluorescence detection can be easily achieved in fluorometers with high-throughput, making RT-QuIC soon widely accepted. Nowadays, most medical facilities use this method to detect the presence of protein misfolding or aggregate species in patients.[111]

However, the primary drawback of RT-QuIC is that its elevated sensitivity could lead to increased false-positives. The spontaneous aggregation of the first few monomers marks the commencement of the aggregate cascade, and it is inevitable in artificial seeding amplification that monomers become seeds themselves. Additionally, the time-consuming sharking-detection process hinders its translation into the clinical practice and daily care management. To address this, it is essential to confine the amplification amplitude within a moderate range to avoid skewed results: cut-off values are set, but the determination of the cutoff is subject to the response threshold of the photoelectric sensor. Therefore, albeit the RT-QuIC readout has surpassed PMCA, the development of a better sensor for continuous readout remains a top priority. In this regard, the quartz-crystal-microbalance (QCM) method for on-surface seeding aggregation might be a viable option.[105] QCM measures real-time changes in frequency and energy dissipation of the system composed of the surface or thin film of interest, acquiring information from both mass changes and viscoelastic properties. Ogi et al. proposed a QCM design to study the amyloid-fibril network of α-synuclein, which delineated the mechanical properties of the aggregate product, showing significant differences across disease phenotypes.[105] Although this device was not tailored for aggregate detection, it demonstrated the possibility of performing seed amplification on a QCM

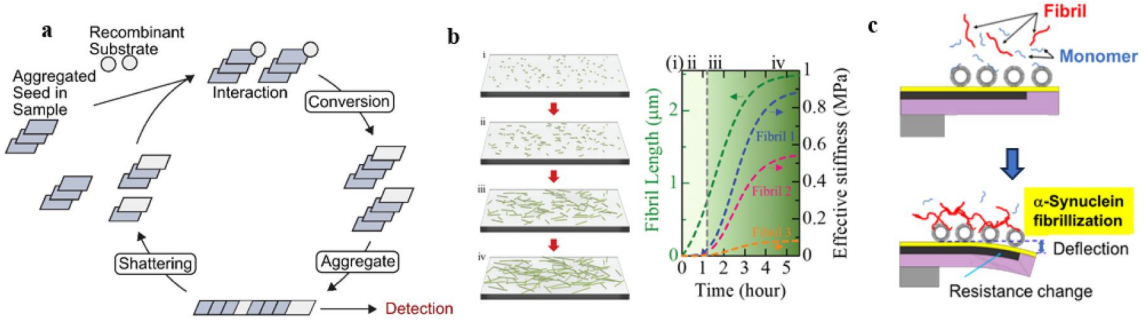

FIGURE 4 Diagram of seed amplification methods. (a) General principle of seed amplification. Reproduced with permission.[104] Copyright ©202 Elsevier Inc. (b) Quartz-crystal-microbalance biosensor for aggregate species. Reproduced with permission.[105] Copyright © 2023 American Chemical Society. (c) Liposome-immobilized cantilever sensor. Reproduced under terms of the CC-BY license.[106] Copyright © 2023 Institute of Electrical and Electronics Engineers.

(Figure 4b). The QCM-based sensor might be sensitive enough (0.057 Hz cm^2 ng^{-1}) to reduce the number of amplification cycles sufficiently to expel false-positive.[112]

Additionally, Kobayashi et al. proposed a liposome-anchored amplification strategy.[106] Liposomes were immobilized on a cantilever sensor, where the aggregation occurs, causing liposome deformation and mass changes. These changes numerically contributed to cantilever sensor mechanical deflection, which was then detected (Figure 4c). The amplification can be carried out only once, achieving a LOD of 10 pg/mL within 100–120 min, which completely outperformed RT-QuIC.

Despite the trade-off between amplitude and accuracy, at least two other inevitable flaws in this class of methods have been noticed. First, they can hardly depict the profile of the patient aggregate species, particularly distinguishing oligomers from fibrils, even though growing evidence shows that oligomers and protofibrils contribute significantly to cytotoxicity.[57] Second, unlike PCR, aggregate seed amplification progresses rather slowly, with currently used protocols taking for 5 h (QCM, one round) to weeks (PMCA), posing a challenge to the operator stamina and sample-in & result out concept. With a strikingly new understanding of the seeding-amplification process, novel analysis methodologies are being conceived.[113] It is expected that the acceleration of seeding progress using minimized device may resolve these aforementioned shortcomings.

4 | COMPUTER-AIDED PROGRAMS

Obtaining a comprehensive picture of aggregate species is valuable for diagnostic purpose, owing to their diverse character, a single technology can hardly assay at once. In coping with this, intricate programs are proposed, which are hallmarks of the meshing imaging technique with computing technology. Photoelectrical sensing or imaging acquires sophisticated information of aggregated species, of which the data amount could be enormous; thus, a proper algorithm shall be in company. Here we briefly mention measurement technology but emphasis on how information technology exerts its power.[114]

Ultra-sensitive, high-throughput microscopy-based assays hold the potential for sophisticated measurements. There are two major variants: liposome-based evaluation, and aptamer-based DNA point accumulation in nanoscale tomography (ADpaint). Liposome-based protocols exploit different permeabilities against lipid bilayers of oligomers compared to fibrils (Figure 5a).[115–117] ADpaint relies on short complementary aptamer stands to dynamically image aggregates on a super-resolution (Figure 5b).[118,121] These sophisticated detection methods frequently exhibit a "dot by dot" pattern.[117,122–124] Individually analyzing each dot is essential for well-round measurement against aggregate species. Responding to this general need, Xia et al., constructed a computational suite for the characterization of aggregates.[119] The suite is Python-based and segments bright spots on a dark background via open-source algorithms PyStar and ComDet. (Figure 5c) Real sample validation demonstrated its fine accuracy, accessibility, applicability, and high-speed.

The trending topic of AI could further aid in to achieve well-round measurement. Kavungal et al., proposed an AI-assisted coupled plasmonic infrared sensor for aggregates.[120] Unlike the abovementioned image-based technologies, it adapts infrared spectroscopy (IR) to extract structural information (Figure 5d). The differentiation between monomers, oligomers and fibrils relies on the analysis of amide I band data. With time-resolved spectral analysis generating a colossal amount of data to process, a trained deep neural network AI swiftly predicts their ratio distribution and shows strong anti-interference ability. AI also potentiated multiplexing capacity, such as detecting α−Synuclein and tau from a single sample. It is obvious that AI is a more powerful tool than conventional algorithms in terms of highly complexed aggregate detection.

5 | CONCLUSION & PERSPECTIVE

As of now, aggregate species screening is not yet a standard service in most healthcare systems worldwide. Despite the plethora of methods developed and their importance, the establishment of aggregate species screening as a routine service may be hindered due to various factors, as we infer from our perspective. The reasons for this challenge could be multifaceted, and we hereby discuss some potential inferences.

Notably, all the assay methodologies mentioned above have considerable drawbacks. The heterogeneity of protein aggregates is enormous, and the specificity of detection still falls short of market needs. Developing new detection receptors or supporting measurements to optimize current receptor performance will be essential. Additionally, the cost of these assays should be affordable to both individuals and the medical system. Currently, both mainstream RT-QuIC and advanced assays remain expensive. Economically, only very few institutes provide these services at very high prices. Therefore, future detection protocols should be more robust and cost-efficient. Moreover, current methods often require specific samples, such as CSF or brain tissue, which involves highly invasive procedures for the collection. The

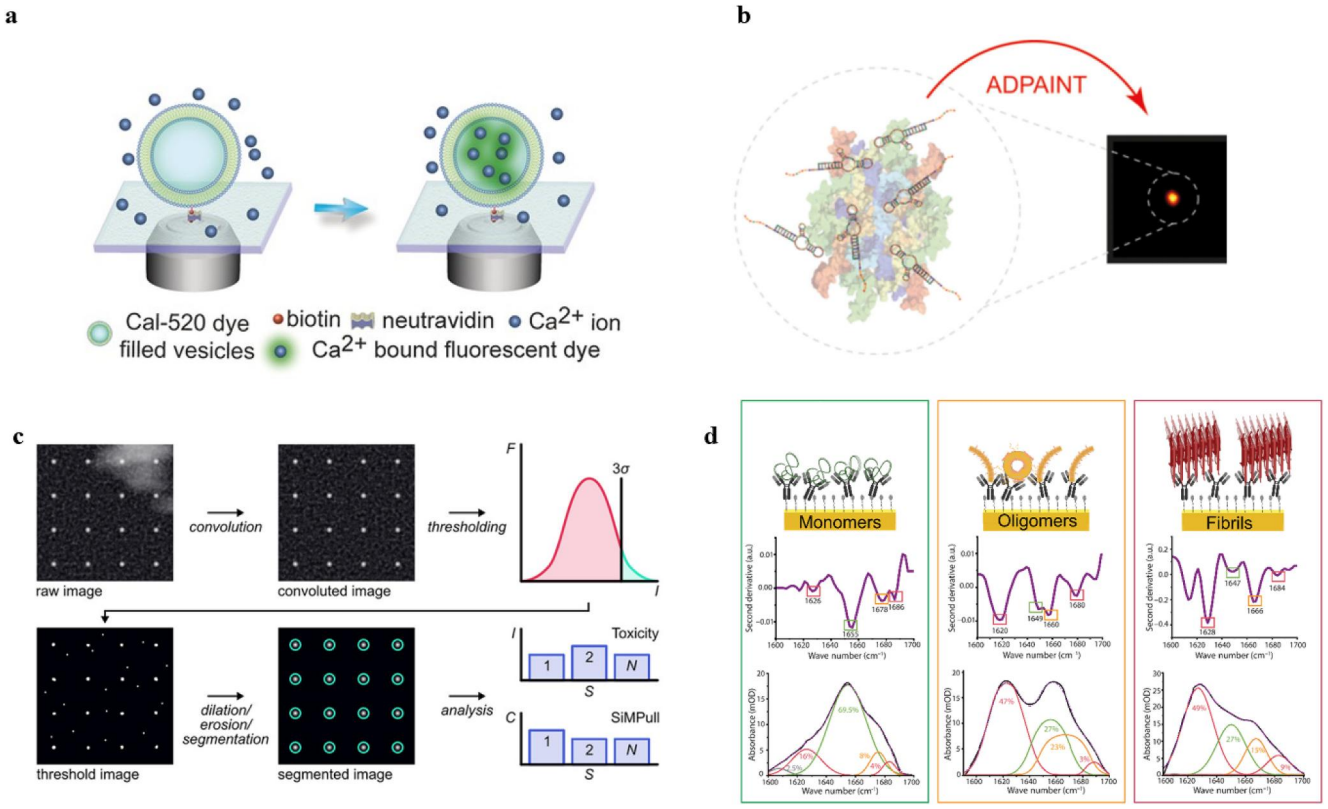

FIGURE 5 Integrated detection programs. (a) Measurement of Ca^{2+} influx into lipid vesicles induced by protein aggregates. Reproduced under terms of the CC-BY license.[117] Copyright © 2017 German Chemical Society. (b) Principle of ADpaint super resolution detection of protein aggregates. Reproduced under terms of the CC-BY license.[118] Copyright © 1999–2023 John Wiley & Sons, Inc. (c) Computational suite for the structural and functional characterization of amyloid aggregates. Reproduced under terms of the CC-BY license.[119] Copyright ©2023 Elsevier Inc. (d) Analysis of amide I band data to differentiate aggregate species. Reproduced under terms of the CC-BY license.[120] Copyright ©2023 American Association for the Advancement of Science.

development of biomarker analytes from less or non-invasive sources, such as peripheral blood and interstitial fluid, may become a trend in the field.

The use of protein aggregates as a biomarker is still a subject of debate. Although decades of work have proved that aggregates contain valuable information, interpreting this information remains challenging. Therefore, we hereby propose a conceptual goal to achieve always depict a patient aggregate species from two angles—the quantity and the degree of aggregation. In other words, understanding the proportion of monomers transforming into aggregates and distinguishing between oligomers and fibrils. Additionally, evaluating the overall cytotoxicity of the aggregates and their connections with other biomarkers is crucial. Given the highly diverse nature of aggregate species, obtaining the full composition picture is essential to harness their potential as valuable biomarkers. Although this review focused on amyloid aggregates, other types of aggregate species are also worth exploring since they are even less studied. Hong's group reported a Tetraphenylethene probe that monitors protein folding-unfolding.[125] Liu's group further proposed a tailored probe for amorphous protein aggregates.[126,127] Understanding the panorama of various aggregate species remains largely uncharted.

To achieve this goal, apart from the aforementioned directions, it is vital to promote multidisciplinary collaboration. The involvement of imaging and information technology has strikingly improved assay performance. With the recent ratification of the groundbreaking medicine Lecanemab, which highlights the growing importance of protein aggregates in both diagnosis and treatment,[128] developing more accurate, swift, panoramic detection technology with multidisciplinary effort is of utmost importance.

ACKNOWLEDGMENTS

The work is supported by the Chinese University of Hong Kong (Shenzhen) startup funding (K10120220253), Affiliated Hospital and University Joint Fund (YXLH2218),

GuangDong Basic and Applied Basic Research Foundation (2022A1515110206).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ORCID

Yuhang Zhou https://orcid.org/0009-0005-1470-5524

REFERENCES

1. N. K. Majbour, N. N. Vaikath, K. D. van Dijk, M. T. Ardah, S. Varghese, L. B. Vesterager, L. P. Montezinho, S. Poole, B. Safieh-Garabedian, T. Tokuda, C. E. Teunissen, H. W. Berendse, W. D. J. van de Berg, O. M. A. El-Agnaf, *Mol. Neurodegener.* **2016**, *11*, 7.
2. U. Sengupta, E. Portelius, O. Hansson, K. Farmer, D. Castillo-Carranza, R. Woltjer, H. Zetterberg, D. Galasko, K. Blennow, R. Kayed, *Ann. Clin. Transl. Neurol.* **2017**, *4*, 226.
3. M. Neumann, D. M. Sampathu, L. K. Kwong, A. C. Truax, M. C. Micsenyi, T. T. Chou, J. Bruce, T. Schuck, M. Grossman, C. M. Clark, L. F. McCluskey, B. L. Miller, E. Masliah, I. R. Mackenzie, H. Feldman, W. Feiden, H. A. Kretzschmar, J. Q. Trojanowski, V. M.-Y. Lee, *Science* **2006**, *314*, 130.
4. M. M. Altamirano-Bustamante, N. F. Altamirano-Bustamante, M. Larralde-Laborde, R. Lara-Martínez, E. Leyva-García, E. Garrido-Magaña, G. Rojas, L. F. Jiménez-García, C. Revilla-Monsalve, P. Altamirano, R. Calzada-León, *Sci. Rep.* **2019**, *9*, 18465.
5. S. Ghosh, S. Salot, S. Sengupta, A. Navalkar, D. Ghosh, R. Jacob, S. Das, R. Kumar, N. N. Jha, S. Sahay, S. Mehra, G. M. Mohite, S. K. Ghosh, M. Kombrabail, G. Krishnamoorthy, P. Chaudhari, S. K. Maji, *Cell Death Differ.* **2017**, *24*, 1784.
6. S. P. Somasekharan, M. Gleave, *FEBS Lett.* **2021**, *595*, 2872.
7. R. J. Eberle, M. A. Coronado, I. Gering, K. Korostov, A. Stefanski, K. Stühler, V. Kraemer-Schulien, L. Blömeke, O. Bannach, D. Willbold, (Preprint) *bioRxiv* **2023**, 2023.03.04.531078.
8. P. Venkatraman, R. Wetzel, M. Tanaka, N. Nukina, A. L. Goldberg, *Mol. Cell* **2004**, *14*, 95.
9. X.-M. Wang, P. Zeng, Y.-Y. Fang, T. Zhang, Q. Tian, *J. Neurochem.* **2021**, *158*, 119.
10. M. Pils, J. Rutsch, F. Eren, G. Engberg, F. Piehl, S. Cervenka, C. Sellgren, S. Troßbach, D. Willbold, S. Erhardt, O. Bannach, C. Korth, (Preprint) *medRxiv* **2023**, 2023.04.17.23288687.
11. D. Bhutani, S. Leng, S. Lentzsch, *Clin. Lymphoma, Myeloma Leuk.* **2019**, *19*, 555.
12. T. Murakami, Y. Inoshima, K.-I. Watanabe, Y. Kobayashi, T. Matsui, H. Kurazono, N. Ishiguro, *Amyloid* **2011**, *18*, 112.
13. J. H. Pinney, C. J. Whelan, A. Petrie, J. Dungu, S. M. Banypersad, P. Sattianayagam, A. Wechalekar, S. D. J. Gibbs, C. P. Venner, N. Wassef, C. A. McCarthy, J. A. Gilbertson, D. Rowczenio, P. N. Hawkins, J. D. Gillmore, H. J. Lachmann, *J. Am. Heart Assoc.* **2013**, *2*, e000098.
14. J. Chapman, A. Dogan, *Expert Rev. Proteomics* **2019**, *16*, 783.
15. F. Chiti, C. M. Dobson, *Annu. Rev. Biochem.* **2017**, *86*, 27.
16. Q. Xu, Y. Ma, Y. Sun, D. Li, X. Zhang, C. Liu, *Aggregate* **2023**, *4*, e333.
17. J. Li, T. McQuade, A. B. Siemer, J. Napetschnig, K. Moriwaki, Y.-S. Hsiao, E. Damko, D. Moquin, T. Walz, A. McDermott, F. K.-M. Chan, H. Wu, *Cell* **2012**, *150*, 339.
18. G. Niewiadomska, W. Niewiadomski, M. Steczkowska, A. Gasiorowska, *Life* **2021**, *11*, 28.
19. N. Bengoa-Vergniory, R. F. Roberts, R. Wade-Martins, J. Alegre-Abarrategui, *Acta Neuropathol.* **2017**, *134*, 819.
20. D. Zenko, J. Marsh, A. R. Castle, R. Lewin, R. Fischer, G. K. Tofaris, *Sci. Adv.* **2023**, *9*, eadd8910.
21. N. F. Altamirano-Bustamante, E. Garrido-Magaña, E. Morán, A. Calderón, K. Pasten-Hidalgo, R. A. Castillo-Rodríguez, G. Rojas, R. Lara-Martínez, E. Leyva-García, M. Larralde-Laborde, G. Domíguez, C. Murata, Y. Margarita-Vazquez, R. Payro, M. Barbosa, A. Valderrama, H. Montesinos, A. Domínguez-Camacho, V. H. García-Olmos, R. Ferrer, P. G. Medina-Bravo, F. Santoscoy, C. Revilla-Monsalve, L. F. Jiménez-García, J. Morán, J. Villalobos-Alva, M. J. Villalobos, R. Calzada-León, P. Altamirano, M. M. Altamirano-Bustamante, *PLoS One* **2020**, *15*, e0237667.
22. J. A. Riback, C. D. Katanski, J. L. Kear-Scott, E. V. Pilipenko, A. E. Rojek, T. R. Sosnick, D. A. Drummond, *Cell* **2017**, *168*, 1028.
23. A. Patel, H. O. Lee, L. Jawerth, S. Maharana, M. Jahnel, M. Y. Hein, S. Stoynov, J. Mahamid, S. Saha, T. M. Franzmann, A. Pozniakovski, I. Poser, N. Maghelli, L. A. Royer, M. Weigert, E. W. Myers, S. Grill, D. Drechsel, A. A. Hyman, S. Alberti, *Cell* **2015**, *162*, 1066.
24. S. T. Dada, M. C. Hardenberg, Z. Toprakcioglu, L. K. Mrugalla, M. P. Cali, M. O. McKeon, E. Klimont, T. C. T. Michaels, T. P. J. Knowles, M. Vendruscolo, *Proc. Natl. Acad. Sci.* **2023**, *120*, e2208792120.
25. T. C. T. Michaels, D. Qian, A. Šarić, M. Vendruscolo, S. Linse, T. P. J. Knowles, *Nat. Rev. Phys.* **2023**, *5*, 379.
26. S. Mukherjee, A. Sakunthala, L. Gadhe, M. Poudyal, A. S. Sawner, P. Kadu, S. K. Maji, *J. Mol. Biol.* **2023**, *435*, 167713.
27. S. I. A. Cohen, S. Linse, L. M. Luheshi, E. Hellstrand, D. A. White, L. Rajah, D. E. Otzen, M. Vendruscolo, C. M. Dobson, T. P. J. Knowles, *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 9758.
28. T. Lashley, J. M. Schott, P. Weston, C. E. Murray, H. Wellington, A. Keshavan, S. C. Foti, M. Foiani, J. Toombs, J. D. Rohrer, A. Heslegrave, H. Zetterberg, *Dis. Models Mech.* **2018**, *11*, dmm031781.
29. C. Agliardi, F. R. Guerini, M. Meloni, M. Clerici, *Neural Regener. Res.* **2021**, *17*, 1503.
30. N. K. Majbour, I. Y. Abdi, M. Dakna, T. Wicke, E. Lang, H. Y. Ali Moussa, M. A. Thomas, C. Trenkwalder, B. Safieh-Garabedian, T. Tokuda, B. Mollenhauer, O. El-Agnaf, *Mov. Disord.* **2021**, *36*, 2048.
31. A. S. L. Ng, Y. J. Tan, Z. Lu, E. Y. L. Ng, S. Y. E. Ng, N. S. Y. Chia, F. Setiawan, Z. Xu, K. Y. Tay, K. M. Prakash, W. L. Au, E.-K. Tan, L. C. S. Tan, *Ann. Clin. Transl. Neurol.* **2019**, *6*, 615.
32. *Neurodegeneration Methods Protocols* (Eds: H.-X. Deng, E. H. Bigio, T. Siddique, G. Manfredi, H. Kawamata), Humana Press, Totowa, NJ **2011**.
33. C. Jiang, G. Wang, R. Hein, N. Liu, X. Luo, J. J. Davis, *Chem. Rev.* **2020**, *120*, 3852.
34. C. Jiang, F. Hopfner, D. Berg, M. T. Hu, A. Pilotto, B. Borroni, J. J. Davis, G. K. Tofaris, *Mov. Disord.* **2021**, *36*, 2663.
35. P. Carneiro, S. Morais, M. do C. Pereira, *TrAC, Trends Anal. Chem.* **2023**, *166*, 117150.
36. S. Paciotti, G. Bellomo, L. Gatticchi, L. Parnetti, *Front. Neurol.* **2018**, *9*, 415.

37. E. V. Suprun, S. P. Radko, S. A. Kozin, V. A. Mitkevich, A. A. Makarov, *Biochem. Mosc.* **2023**, *88*, S88.
38. C. Jiang, Y. Fu, G. Liu, B. Shu, J. Davis, G. K. Tofaris, *Nano-Micro Lett.* **2021**, *14*, 3.
39. C. Jiang, F. Hopfner, A. Katsikoudi, R. Hein, C. Catli, S. Evetts, Y. Huang, H. Wang, J. W. Ryder, G. Kuhlenbaeumer, G. Deuschl, A. Padovani, D. Berg, B. Borroni, M. T. Hu, J. J. Davis, G. K. Tofaris, *J. Neurol., Neurosurg. Psychiatry* **2020**, *91*, 720.
40. Y. Fu, C. Jiang, G. K. Tofaris, J. J. Davis, *Anal. Chem.* **2020**, *92*, 13647.
41. I. Martinez-Valbuena, G. G. Kovacs, A. E. Lang, *Brain* **2022**, *145*, 2946.
42. M. Cingolani, L. Mummolo, F. Lugli, M. Zaffagnini, D. Genovese, *New J. Chem.* **2021**, *45*, 14259.
43. A. Aliyan, N. P. Cook, A. A. Martí, *Chem. Rev.* **2019**, *119*, 11819.
44. C. W. Bertoncini, M. Soledad Celej, *Curr. Protein Pept. Sci.* **2011**, *12*, 206.
45. L. M. Young, A. E. Ashcroft, S. E. Radford, *Curr. Opin. Chem. Biol.* **2017**, *39*, 90.
46. C. Yan, J. Dai, Y. Yao, W. Fu, H. Tian, W.-H. Zhu, Z. Guo, *Nat. Protoc.* **2023**, *18*, 1316.
47. Y. Tang, D. Zhang, X. Gong, J. Zheng, *Adv. Funct. Mater.* **2022**, *32*, 2208022.
48. C. W. T. Leung, F. Guo, Y. Hong, E. Zhao, R. T. K. Kwok, N. L. C. Leung, S. Chen, N. N. Vaikath, O. M. El-Agnaf, Y. Tang, W.-P. Gai, B. Z. Tang, *Chem. Commun.* **2015**, *51*, 1866.
49. Y. Ji, W. Zhang, J. Zhang, X. Li, F. Yu, C. Li, G. Liu, G. Xing, *J. Mater. Chem. B* **2022**, *10*, 5602.
50. P. Sormanni, F. A. Aprile, M. Vendruscolo, *Proc. Natl. Acad. Sci.* **2015**, *112*, 9902.
51. Y. Zheng, J. Zhao, L. Zhang, W. Wang, *Sens. Actuators, B* **2023**, *381*, 133418.
52. L. Blömeke, M. Pils, V. Kraemer-Schulien, A. Dybala, A. Schaffrath, A. Kulawik, F. Rehn, A. Cousin, V. Nischwitz, J. Willbold, R. Zack, T. F. Tropea, T. Bujnicki, G. Tamgüney, D. Weintraub, D. Irwin, M. Grossman, D. A. Wolk, J. Q. Trojanowski, O. Bannach, A. Chen-Plotkin, D. Willbold, *npj Parkinson's Dis.* **2022**, *8*, 1.
53. D. Su, W. Diao, J. Li, L. Pan, X. Zhang, X. Wu, W. Mao, *ACS Chem. Neurosci.* **2022**, *13*, 540.
54. J. Jensen, M. Farina, G. Zuccheri, W. Grange, M. Hegner, *J. Sens.* **2011**, *2012*, e874086.
55. A. Napp, V. Houbart, A. Demelenne, M.-P. Merville, J. Crommen, M. Dumoulin, G. Garraux, A.-C. Servais, M. Fillet, *Electrophoresis* **2018**, *39*, 3022.
56. J. Zhao, T. J. Nelson, Q. Vu, T. Truong, C. I. Stains, *ACS Chem. Biol.* **2016**, *11*, 132.
57. D. J. Selkoe, J. Hardy, *EMBO Mol. Med.* **2016**, *8*, 595.
58. R. Kayed, E. Head, J. L. Thompson, T. M. McIntire, S. C. Milton, C. W. Cotman, C. G. Glabe, *Science* **2003**, *300*, 486.
59. T. R. Sampson, J. W. Debelius, T. Thron, S. Janssen, G. G. Shastri, Z. E. Ilhan, C. Challis, C. E. Schretter, S. Rocha, V. Gradinaru, M.-F. Chesselet, A. Keshavarzian, K. M. Shannon, R. Krajmalnik-Brown, P. Wittung-Stafshede, R. Knight, S. K. Mazmanian, *Cell* **2016**, *167*, 1469.
60. P. Sormanni, F. A. Aprile, M. Vendruscolo, *Chem. Soc. Rev.* **2018**, *47*, 9137.
61. F. A. Aprile, P. Sormanni, M. Perni, P. Arosio, S. Linse, T. P. J. Knowles, C. M. Dobson, M. Vendruscolo, *Sci. Adv.* **2017**, *3*, e1700488.
62. K. Kulenkampff, D. Emin, R. Staats, Y. P. Zhang, L. Sakhnini, A. Kouli, O. Rimon, E. Lobanova, C. H. Williams-Gray, F. A. Aprile, P. Sormanni, D. Klenerman, M. Vendruscolo, *Chem. Sci.* **2022**, *13*, 13815.
63. Y. J. Chung, C. H. Lee, J. Lim, J. Jang, H. Kang, C. B. Park, *ACS Nano* **2020**, *14*, 16973.
64. X. Ren, Y. Zhao, F. Xue, Y. Zheng, H. Huang, W. Wang, Y. Chang, H. Yang, J. Zhang, *Mol. Ther. Nucleic Acids* **2019**, *17*, 726.
65. C. Erkmen, G. Aydoğdu Tığ, G. Marrazza, B. Uslu, *TrAC Trends Anal. Chem.* **2022**, *154*, 116675.
66. K. Sun, N. Xia, L. Zhao, K. Liu, W. Hou, L. Liu, *Sens. Actuators, B* **2017**, *245*, 87.
67. R. Tan, Y. Wang, X. Mi, H. Li, Y. Tu, *Sens. Actuators, B* **2022**, *352*, 131065.
68. Y. Zheng, X. Geng, X. Yang, S. Li, Y. Liu, X. Liu, Q. Wang, K. Wang, R. Jia, Y. Xu, *Anal. Chem.* **2020**, *92*, 2853.
69. O. M. A. El-Agnaf, S. A. Salem, K. E. Paleologou, M. D. Curran, M. J. Gibson, J. A. Court, M. G. Schlossmacher, D. Allsop, *FASEB J.* **2006**, *20*, 419.
70. X. Wang, S. Yu, F. Li, T. Feng, *Neurosci. Lett.* **2015**, *599*, 115.
71. M. J. Wang, S. Yi, J. Han, S. Y. Park, J.-W. Jang, I. K. Chun, S. E. Kim, B. S. Lee, G. J. Kim, J. S. Yu, K. Lim, S. M. Kang, Y. H. Park, Y. C. Youn, S. S. A. An, S. Kim, *Alzheimer's Res. Ther.* **2017**, *9*, 98.
72. E. Birkmann, F. Henke, N. Weinmann, C. Dumpitak, M. Groschup, A. Funke, D. Willbold, D. Riesner, *Vet. Microbiol.* **2007**, *123*, 294.
73. O. Bannach, E. Birkmann, E. Reinartz, K.-E. Jaeger, J. P. M. Langeveld, R. G. Rohwer, L. Gregori, L. A. Terry, D. Willbold, D. Riesner, *PLoS One* **2012**, *7*, e36620.
74. S. A. Funke, L. Wang, E. Birkmann, D. Willbold, *Rejuvenation Res.* **2010**, *13*, 206.
75. K. Kühbach, M. Hülsemann, Y. Herrmann, K. Kravchenko, A. Kulawik, C. Linnartz, L. Peters, K. Wang, J. Willbold, D. Willbold, O. Bannach, *Front. Neurosci.* **2016**, *10*.
76. M. Hülsemann, C. Zafiu, K. Kühbach, N. Lühmann, Y. Herrmann, L. Peters, C. Linnartz, J. Willbold, K. Kravchenko, A. Kulawik, S. Willbold, O. Bannach, D. Willbold, *J. Alzheimer's Dis.* **2016**, *54*, 79.
77. A. Schaffrath, S. Schleyken, A. Seger, H. Jergas, P. Özdüzenciler, M. Pils, L. Blömeke, A. Cousin, J. Willbold, T. Bujnicki, O. Bannach, G. R. Fink, D. Willbold, M. Sommeraauer, M. T. Barbe, G. Tamgüney, *npj Parkinson's Dis.* **2023**, *9*, 1.
78. Y. Zhou, Y. Lv, H. Dong, L. Liu, G. Mao, Y. Zhang, M. Xu, *Sens. Actuators, B* **2021**, *331*, 129429.
79. L. Yin, Y. Wang, R. Tan, H. Li, Y. Tu, *Microchim. Acta* **2021**, *188*, 53.
80. J. Qin, M. Cho, Y. Lee, *ACS Appl. Mater. Interfaces* **2019**, *11*, 11743.
81. Y. Zhou, H. Zhang, L. Liu, C. Li, Z. Chang, X. Zhu, B. Ye, M. Xu, *Sci. Rep.* **2016**, *6*, 35186.
82. N. Xia, B. Zhou, N. Huang, M. Jiang, J. Zhang, L. Liu, *Biosens. Bioelectron.* **2016**, *85*, 625.
83. J. V. Rushworth, A. Ahmed, H. H. Griffiths, N. M. Pollock, N. M. Hooper, P. A. Millner, *Biosens. Bioelectron.* **2014**, *56*, 83.
84. H. Li, H. Xie, Y. Cao, X. Ding, Y. Yin, G. Li, *Anal. Chem.* **2013**, *85*, 1047.
85. C. Tang, J. Yang, Y. Wang, J. Cheng, X. Li, C. Chang, J. Hu, J. Lü, *Sens. Actuators, B* **2021**, *329*, 129113.

86. Y. Liu, Q. Xu, Y. Zhang, B. Ren, L. Huang, H. Cai, T. Xu, Q. Liu, X. Zhang, *Talanta* **2021**, *231*, 122360.
87. S. M. Taghdisi, N. M. Danesh, M. A. Nameghi, M. Ramezani, M. Alibolandi, M. Hassanzadeh-Khayat, A. S. Emrani, K. Abnous, *Biosens. Bioelectron.* **2019**, *123*, 14.
88. J. Hu, X. Chen, J. Xu, X. Wang, *J. Electrochem. Soc.* **2023**, *170*, 017507.
89. C.-Y. Ge, M. M. Rahman, W. Zhang, N. S. Lopa, L. Jin, S. Yoon, H. Jang, G.-R. Xu, W. Kim, *Sensors* **2020**, *20*, 617.
90. J. Lyu, X. Long, T. Xie, G. Jiang, J. Jiang, L. Ye, Q. Li, *J. Mol. Liq.* **2021**, *323*, 115051.
91. F. G. Ortega, S. V. Piguillem, G. A. Messina, G. R. Tortella, O. Rubilar, M. I. Jiménez Castillo, J. A. Lorente, M. J. Serrano, J. Raba, M. A. Fernández Baldo, *Talanta* **2019**, *194*, 243.
92. L. B. Lassen, E. Gregersen, A. K. Isager, C. Betzer, R. H. Kofoed, P. H. Jensen, *PLoS One* **2018**, *13*, e0196056.
93. P. Lopes, H. Dyrnesli, N. Lorenzen, D. Otzen, E. E. Ferapontova, *Analyst* **2014**, *139*, 749.
94. Y. Gong, M. Fu, L. Li, Y. Yin, Q. Tang, W. Zhou, G. Zhang, X. Liao, F. Gao, *Sens. Actuators B Chem.* **2023**, *378*, 133150.
95. S. Awasthi, C. Ying, J. Li, M. Mayer, *ACS Nano* **2023**, *17*, 12325.
96. M. Afshar Bakshloo, S. Yahiaoui, M. Bourderioux, R. Daniel, M. Pastoriza-Gallego, J. J. Kasianowicz, A. Oukhaled, *ACS Chem. Neurosci.* **2023**, *14*, 2517.
97. C. Jiang, M. T. Alam, S. G. Parker, N. Darwish, J. J. Gooding, *Langmuir* **2016**, *32*, 2509.
98. C. Jiang, M. T. Alam, S. M. Silva, S. Taufik, S. Fan, J. J. Gooding, *ACS Publ.* **2016**, *1*, 1432.
99. X. Zeng, Y. Xiang, Q. Liu, L. Wang, Q. Ma, W. Ma, D. Zeng, Y. Yin, D. Wang, *Nanomaterials* **2021**, *11*, 1942.
100. I. Abrao-Nemeir, J. Bentin, N. Meyer, J.-M. Janot, J. Torrent, F. Picaud, S. Balme, *Chem. Asian J.* **2022**, *17*, e202200726.
101. N. Subramanian, B. Watson, C.-Z. Li, M. Moss, C. Liu, *Sens. Actuators Rep.* **2023**, *6*, 100170.
102. M. Sharafeldin, S. Yan, C. Jiang, G. K. Tofaris, J. J. Davis, *Anal. Chem.* **2023**, *95*, 7906.
103. X. Wei, T. Tian, S. Jia, Z. Zhu, Y. Ma, J. Sun, Z. Lin, C. J. Yang, *Anal. Chem.* **2016**, *88*, 2345.
104. I. Zerr, *Lancet Neurol.* **2021**, *20*, 165.
105. L. Zhou, T. Hajiri, K. Nakajima, C. Aguirre, K. Ikenaka, H. Mochizuki, K. Yamaguchi, Y. Goto, H. Ogi, *ACS Sens.* **2023**, *8*, 2598.
106. R. Kobayashi, K. Kamitani, M. Sawamura, H. Yamakado, R. Takahashi, M. Sohgawa, M. Noda, *IEEE Sens. J.* **2023**, *23*, 12495.
107. C. Soto, G. P. Saborio, L. Anderes, *Trends Neurosci.* **2002**, *25*, 390.
108. P. Saá, J. Castilla, C. Soto, *Science* **2006**, *313*, 92.
109. P. Saá, L. Cervenakova, *Virus Res.* **2015**, *207*, 47.
110. R. Atarashi, K. Satoh, K. Sano, T. Fuse, N. Yamaguchi, D. Ishibashi, T. Matsubara, T. Nakagaki, H. Yamanaka, S. Shirabe, M. Yamada, H. Mizusawa, T. Kitamoto, G. Klug, A. McGlade, S. J. Collins, N. Nishida, *Nat. Med.* **2011**, *17*, 175.
111. A. Iranzo, G. Fairfoul, A. C. N. Ayudhaya, M. Serradell, E. Gelpi, I. Vilaseca, R. Sanchez-Valle, C. Gaig, J. Santamaria, E. Tolosa, R. L. Riha, A. J. E. Green, *Lancet Neurol.* **2021**, *20*, 203.
112. C. Tonda-Turo, I. Carmagnola, G. Ciardelli, *Front. Bioeng. Biotechnol.* **2018**, *6*, 158.
113. A. Sakunthala, D. Datta, A. Navalkar, L. Gadhe, P. Kadu, K. Patel, S. Mehra, R. Kumar, D. Chatterjee, J. Devi, K. Sengupta, R. Padinhateeri, S. K. Maji, *J. Phys. Chem. Lett.* **2022**, *13*, 6427.
114. W. Zhang, Y. Lu, C. Su, Y. Wang, Y.-F. Wang, B. Zhang, C. Jiang, K. Guo, C. Xu, *Innov. Med.* **2023**, *1*, 100023.
115. I. Kocsis, E. Sanna, C. A. Hunter, *Org. Lett.* **2021**, *23*, 647.
116. A. Hirano, H. Yoshikawa, S. Matsushita, Y. Yamada, K. Shiraki, *Langmuir* **2012**, *28*, 3887.
117. P. Flagmeier, S. De, D. C. Wirthensohn, S. F. Lee, C. Vincke, S. Muyldermans, T. P. J. Knowles, S. Gandhi, C. M. Dobson, D. Klenerman, *Angew. Chem., Int. Ed.* **2017**, *56*, 7750.
118. D. R. Whiten, Y. Zuo, L. Calo, M.-L. Choi, S. De, P. Flagmeier, D. C. Wirthensohn, F. Kundel, R. T. Ranasinghe, S. E. Sanchez, D. Athauda, S. F. Lee, C. M. Dobson, S. Gandhi, M.-G. Spillantini, D. Klenerman, M. H. Horrocks, *ChemBioChem* **2018**, *19*, 2033.
119. Z. Xia, Y. Wu, J. Y. L. Lam, Z. Zhang, M. Burke, E. Fertan, R. T. Ranasinghe, E. Hidari, J. S. H. Danial, D. Klenerman, *Cell Rep. Methods* **2023**, *3*, 100499.
120. D. Kavungal, P. Magalhães, S. T. Kumar, R. Kolla, H. A. Lashuel, H. Altug, *Sci. Adv.* **2023**, *9*, eadg9644.
121. D. I. Sideris, J. S. H. Danial, D. Emin, F. S. Ruggeri, Z. Xia, Y. P. Zhang, E. Lobanova, H. Dakin, S. De, A. Miller, J. C. Sang, T. P. J. Knowles, M. Vendruscolo, G. Fraser, D. Crowther, D. Klenerman, *Brain Commun.* **2021**, *3*, fcab147.
122. I. Pérez-Pi, D. A. Evans, M. H. Horrocks, N. T. Pham, K. S. Dolt, J. Koszela, T. Kunath, M. Auer, *Anal. Chem.* **2019**, *91*, 5582.
123. A. Jain, R. Liu, B. Ramani, E. Arauz, Y. Ishitsuka, K. Ragunathan, J. Park, J. Chen, Y. K. Xiang, T. Ha, *Nature* **2011**, *473*, 484.
124. A. Bhumkar, C. Magnan, D. Lau, E. S. W. Jun, N. Dzamko, Y. Gambin, E. Sierecki, *Angew. Chem.* **2021**, *133*, 11981.
125. S. Zhang, M. Liu, L. Y. F. Tan, Q. Hong, Z. L. Pow, T. C. Owyong, S. Ding, W. W. H. Wong, Y. Hong, *Chem. Asian J.* **2019**, *14*, 904.
126. D. Shen, W. Jin, Y. Bai, Y. Huang, H. Lyu, L. Zeng, M. Wang, Y. Tang, W. Wan, X. Dong, Z. Gao, H.-L. Piao, X. Liu, Y. Liu, *Angew. Chem.* **2021**, *133*, 16203.
127. D. Shen, Q. Zhao, M. Wang, B. Zhong, W. Jin, Y. Huang, H. Jin, B. Jing, W. Wan, X. Zhang, L. Zhang, Y. Liu, *Anal. Chem.* **2023**, *95*, 6358.
128. M. Shi, F. Chu, F. Zhu, J. Zhu, *Front. Aging Neurosci.* **2022**, *14*, 870517.

AUTHOR BIOGRAPHIES

Wanting Dong is a senior student at the School of Medicine at Chinese University (Shenzhen). Her research is dedicated to investigating the protein aggregate pathology associated with degenerative diseases.

Yanhong Duo received her PhD degree from Tsinghua University and Lanzhou University (joint training), and she conducted her postdoctoral study at Karolinska Institutet and completed her visiting scholar at Harvard University. She is currently a distinguished professor in the Research Institute of Tsinghua University in Shenzhen, and a guest professor in Shenzhen People's Hospital and The Eightth Affiliated Hospital, Sun Yat-Sen University, Major project partner of Peking University Shenzhen Hospital. Her research focuses on the design of AIEgens for biological applications.

Yongzhi Huang received his PhD degree from the University of Chinese Academy of Sciences in 2016. He was then joined the Nuffield Department of Surgical Sciences at the University of Oxford as a Postdoctoral fellow. He is currently an Associate Professor of biomedical engineering at Tianjin University. His main research interest is centered around biomedical signal analysis and neural engineering toward detection, diagnostics and treatment of various brain disorders.

Cheng Jiang received his PhD degree in chemistry from the University of New South Wales in 2016. He was then recruited in a joint program between the Nuffield Department of Clinical Neurosciences (NDCN) and the Department of Chemistry as a postdoctoral fellow at the University of Oxford. He is now an assistant professor of biomedical engineering at the Chinese University of Hongkong, Shenzhen. His main research interests include the screening and validation of circulating biomarkers using high-throughput multiplexed sensing platforms with algorithms toward disease diagnostics, stratification, and heath management.

How to cite this article: Y. Zhou, S. Yan, W. Dong, C. Wu, Z. Zhao, R. Wang, Y. Duo, Y. Huang, D. Xu, C. Jiang, *BMEMat* **2024**, *2*(1), e12053. https://doi.org/10.1002/bmm2.12053