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Luminescent Conjugated Polymers: Illuminating the Dark Matters of Biology and Pathology**

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Novel technologies for studying biological events are of great interest and luminescent conjugated polymers (LCPs), a material normally used in organic electronics, have proven useful for the detection of a wide range of disease-related biological processes. The conformation-sensitive optical properties of LCPs provide the ability to study the biochemical activity of biological events on the basis of a structure–function relation, rather than a molecular basis. In this Research News article, the LCP technique and its usefulness for studying protein aggregation diseases are highlighted. We also discuss the much-needed illuminating insights of the underlying pathological events regarding protein aggregation diseases. In addition, essential future basic research advances that relate to further development of LCPs as molecular probes are presented. We also confer the intriguing prospect of employing amyloid fibrils, that is, a symmetric stable nanomaterial normally associated with the dark side of horrific pathology, as a scaffold for functional polymer-protein hybrid materials.

1. Introduction

By combining the features of polymers and the electro-optical properties of conjugated molecules, conjugated polymers suitable for a wide range of applications, such as solar cells, displays, and biosensors can be created. Conjugated polymers comprise a diverse sensor platform, and can be used in numerous biomolecular recognition schemes to obtain sensory responses.[1] The application of conjugated polymers for colorimetric detection of biological targets (biochromism) was first reported by Charych et al. in 1993.[2] The technique was based on a ligand-functionalized conjugated polymer that underwent a colorimetric transition (coil-to-rod transition of the conjugated backbone) upon interaction with a receptor molecule. In that work, the detection and recognition event was a function of the nature and characteristics of the polymer side chains. Side-chain functionalization of the conjugated polymer requires advanced synthesis and extensive purification of numerous monomeric and polymeric derivatives. This first generation of sensors was also mainly using optical absorption as the source for detection, and the sensitivity of these sensors was much lower when compared with other sensing systems for biological processes.

To avoid covalent attachment of the receptor to the polymer side chain and to increase the sensitivity of the biosensors, luminescent conjugated polymers (LCPs; Fig. 1) have been utilized. These systems take advantage of the polymeric nature of the LCPs, and multivalent noncovalent interaction between a synthetic polymer, the LCP, and a natural polymer, the biomolecule, occurs. This is something quite different from what has been accomplished with many fluorescent detector dyes over the years. In addition to the covalent attachment of pointlike fluorophores to biomolecules by covalent chemistry, noncovalent environment-sensitive dyes have also been frequently employed for biomolecular recognition and can provide information on, for example, local hydrophobicity and

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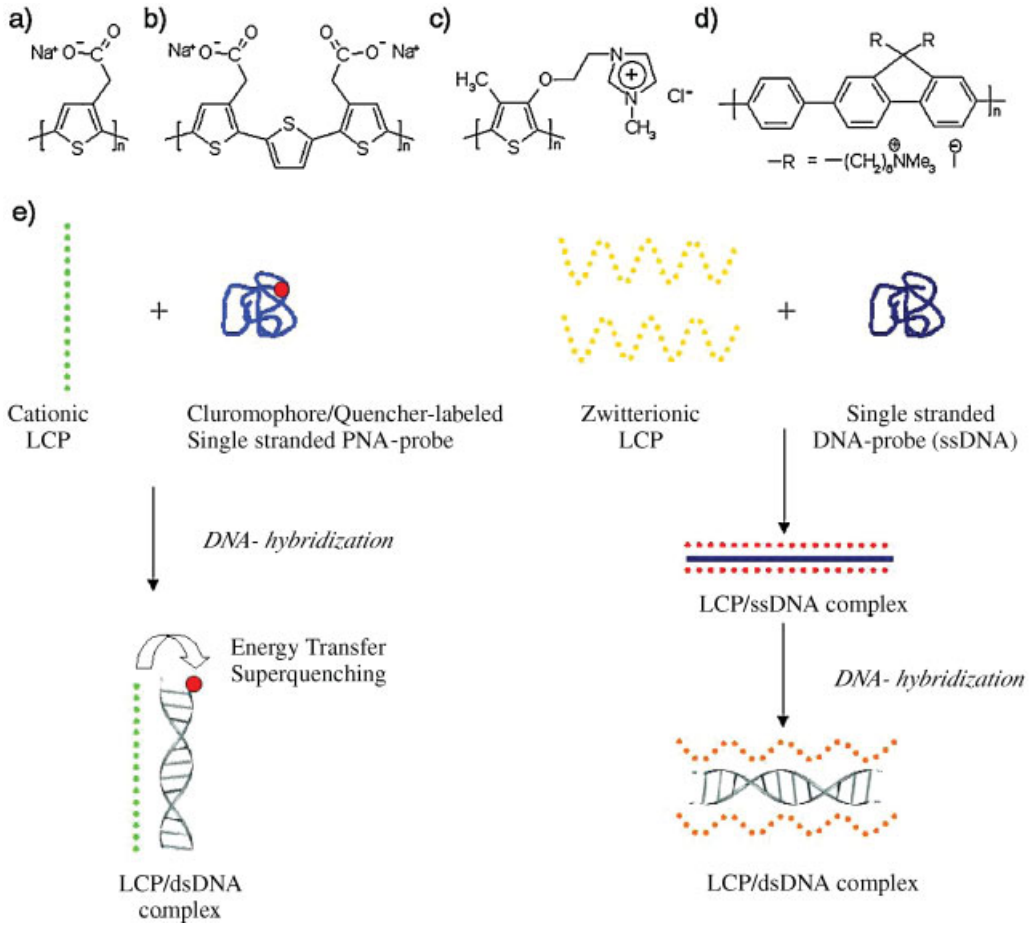

Figure 1. Examples of different LCPs and their use as biosensors. Chemical structure of a) polythiophene acetic acid, PTAA, b) trimer based PTAA, tPTAA, c) polythiophene methylated imidazole, PTMI and d) poly-(9,9-bis(6¢-*N*,*N*,*N*-trimethylammonium)-hexyl)-fluorene phenylene). e) Schematic drawing of biosensing schemes for the detection of DNA-hybridiazation using LCPs. Detection using superquenching or energy transfer (left) or detection based on conformational transitions of the LCP chains (right).

proximity within a complex. In contrast to stiff small molecular dye binding, complexation between a flexible polymer, the LCP, and a biological polymer cause changes in the geometry of the LCP. As the absorption and emission characteristics of the LCP are largely determined by the local electronic structure, geometrical changes of this complex can then be followed in situ, by optical absorption and emission. In addition, fluorescence is a widely used method in biochemical sensing and, aside from inherent sensitivity, this method offers diverse transduction schemes based upon changes in intensity, energy transfer, wavelength (excitation and emission), polarization, and lifetime.

2. Optical Sensors Based on LCPs

LCPs can be used to create biosensors to observe global geometrical changes that occur as biomolecules recognize their kind or when individual biomolecules change conformation. Optical sensors, based on LCPs, can mainly be divided into two different types, depending on which method of detection is utilized. As an example, schematic drawings of the two methods for detection of DNA-hybridization are shown in Figure 1. In the first approach, superquenching of the fluorescence from the LCP chain is used, in which a single site of quenching causes loss of fluorescence from the complete chain.[3–7] The quenching may be due to fluorescence resonance energy transfer (FRET) or excitation quenching. If a biomolecule labeled with a quencher is coordinated to the LCP chain by multiple non-covalent interactions (electrostatic or hydrophobic interactions), it is possible to detect the presence of a certain biomolecule in a sample by quenching of the light emitted from the LCP. A wide range of LCPs have been used as detecting elements for biological molecules in an aqueous environment, and biosensors for DNA-hybridization, as well as ligand-receptor interactions and enzymatic activity have been reported.[8–13]

The second type of biosensors is based on detection of biological processes through their impact on the conformation and the geometry of the LCP chains.[14–21] The conformational flexibility of LCPs allows direct correlation between the geometry of chains and the resulting electronic structure and optical processes such as absorption and emission. If conformational changes of biomolecules or other biomolecular events

can lead to different conformations the associated polymer backbone, an alteration of the absorption and emission properties from the polymer will be observed. Hence, this phenomenon can be used as a sensing element for a wide range of applications appropriate for making novel biosensors. The technique has been used to detect DNA hybridization, ligand–receptor interactions, conformational changes in synthetic peptides, calcium-induced conformational changes in calmodulin, and the formation of protein amyloid fibrils.[14–21]

3. LCPs for Studying Protein Aggregation Diseases

Simple, sensitive, and versatile tools that report on conformational changes in proteins and on the structure of protein aggregates are of great importance, as many diseases are associated with misfolded proteins. The importance of conformational changes of proteins leading to pathogenic states, such as Alzheimer's disease (AD), Parkinson's disease, Type 2 diabetes, systemic amyloidoses, and the infectious prion diseases, have been well-documented.[22,23] Especially under conditions that destabilize the native state, proteins can self-assemble into aggregated β-sheet-rich fibrillar assemblies, known as amyloid fibrils which are unbranched 3–10 nm wide and unusually stable biological materials (Fig. 2). There are many fundamental questions regarding this aggregation process that remain unanswered.[23] Most recently, a class of LCPs has proven useful as a tool for the specific staining of protein aggregates in tissue samples.[26–28] These LCPs contain a flexible backbone built-up from repetitive thiophene rings (Fig. 1a–c) and upon binding to protein aggregates the rotational freedom of the thiophene rings and the geometry of the backbone are restricted, leading to an altered fluorescence from the LCP (Fig. 2). This is a unique property seen for LCPs that cannot be achieved by sterically rigid conventional amyloidotropic dyes such as thioflavin T (ThT) and Congo red. Thus, LCPs offer the possibility to obtain a specific spectroscopic signature for individual protein aggregates. The underlying mechanism of amyloid or protein aggregate formation is poorly understood and many lines of evidence support the existence of smaller heterogenic oligomeric species as intermediates on the pathway from misfolded protein to amyloid. In this regard, the technique using LCPs, which provides a direct link between spectral signal and protein conformation, can be used to gain more information concerning the morphology of the protein deposits and facilitate a greater understanding of the conformational phenotype encoded in the native protein aggregates. Instead of looking at the total amount of protein aggregates, the heterogenic population of specific protein aggregates can be studied and novel findings regarding toxic species and the molecular mechanism of these diseases can be obtained with the LCP technique (Fig. 3).

Upon application of LCPs to transgenic mouse models having AD pathology, we were able to identify a striking heterogenicity in the characteristic plaques composed of the A-beta peptide.[27] This phenomenon cannot readily be observed by other amyloidotrophic dyes and instead of just observing the amount of plaques, the LCPs can in more detail reveal different subpopulations of plaques and enable mapping of their architecture by resolving spectral features from the lesions (Fig. 3). Hopefully, these findings can lead to novel ways of diagnosing AD and also provide a new method for studying the pathology of the disease in a more refined manner. Especially, the LCP technique might be valuable for identifying distinct toxic entities giving rise to cell death and loss of neurons or for establishing a correlation between the type of plaque and the severity of Alzheimer's disease.

Heterogenic protein aggregates can also be found in other protein aggregation disorders, such as the infectious prion diseases. According to the ''protein-only'' hypothesis, prion disease is caused by a proteinaceous agent called PrP^{Sc}, a misfolded and aggregated version of the normal prion protein (PrP^{C}). In addition prions can occur as different strains, and the prion strain phenomenon is most likely encoded in the tertiary or quaternary structure of the prion aggregates. However, methods for mapping the structure of individual prion aggregates are not available. We have reported the use of LCPs for histological labeling of protein aggregates in brain sections from mice infected with distinct prion strains.[28] The LCPs bind specifically to the prion deposits and different prion strains can be separated because of alternative staining patterns of LCPs with distinct ionic side chains. Furthermore, the anionic LCP (polythiophene acetic acid; PTAA) emits light of different wavelengths when bound to distinct protein deposits associated with a specific prion strain. These conformation-dependent spectral characteristics can only be afforded by LCPs and provide the opportunity to get an optical finger print for protein aggregates correlating to a distinct prion strain. The LCPs also identify noncongophilic prion protein aggregates that are positive for PrP immunohistochemistry. Hence, the LCPs provide structural insights regarding the morphology of individual protein deposits and can be used as a complementary technique to conventional staining protocols for the characterization of protein deposits associated with individual prion strains.

Methods for the detection and quantification of diverse aggregated states of proteins are also of great importance with respect to the long-term stability and production of peptide pharmaceuticals in commercial pharmaceutical formulations used for the treatment of various diseases, for example, insulin for diabetes. Although, it is rare to observe iatrogenic (i.e., treatment-induced) protein aggregates due to administration of peptide pharmaceuticals, the therapeutic effect of the peptide drug becomes limited if the peptide has been converted into amyloid fibrils.

4. Basic Research Needs

The ability to utilize the photophysical properties of LCPs for detecting biological events and the importance of this phenomenon for development of a number of different biosensor platforms for studying a variety of diseases, points

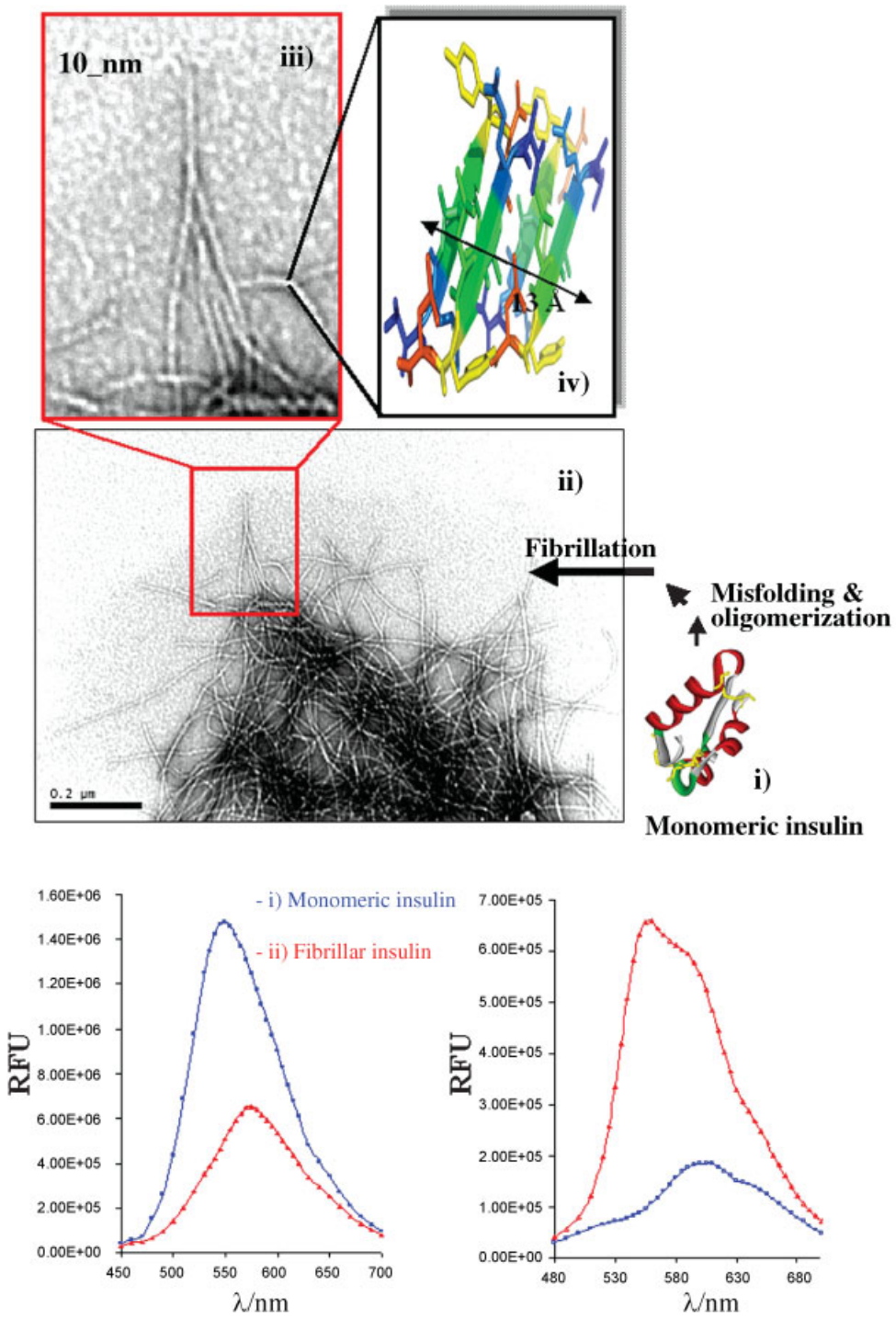

Figure 2. Amyloid fibril structure and LCP fluorescence signatures of amyloid and native protein. Monomeric native insulin (i) misfolds and self assembles into amyloid fibrils that form a carpet of intertwined fibrils shown in the central electron micrograph (ii). A close up of the micrograph illustrates the 8 nm thickness of individual fibrils (iii). The symmetrical β–sheet structure is shown in the high resolution crystal structure of a partial insulin sequence (peptide VEALYL corresponding to residues 12–17 from the B-chain) (iv). The fluorescence spectra in the lower part of the figure show the dramatic chromogenic shifts of LCP bound to the monomeric native protein (i) in blue compared to the fibrillar form composed of polymerized misfolded protein in red (ii) under neutral (pH 7) (left spectra) and acidic conditions (pH 2) (right spectra) of two different LCPs. Some LCPs forms interchain stacking in the presence of amyloid fibrils, leading to quenching of the fluorescence and a red-shift of the emission spectrum (left red spectrum), whereas the interaction between some LCPs and the amyloid fibrils will lead to a blue-shifted spectrum with increase intensity from the LCPs (right red spectrum). The molecular structures were generated from pdb code 2omq [24] for the fibrillar peptide structure and of native insulin from the pdb code 1aph [25].

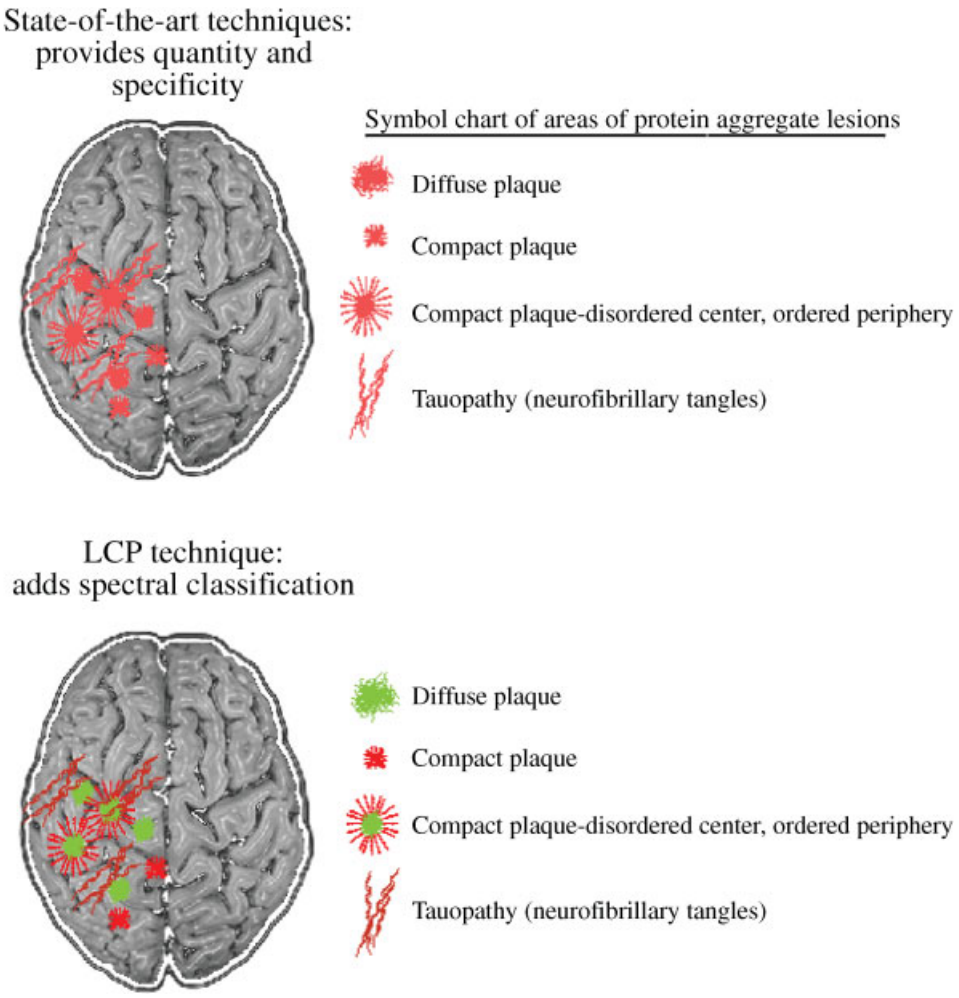

Figure 3. Schematic illustration of the complexity of brain lesions of protein aggregates in Alzheimer's disease. Top illustration: Current state-of-the-art techniques used for diagnosis. Immunohistochemistry can reveal the identity of the protein components and exposed protein epitopes and amyloidotropic dyes Congo red and Thioflavins can reveal the presence of amyloid fibril structure. Bottom illustration: LCP staining adds conformational signatures of the protein deposits from chromogenic resolution dictated by conformational heterogeneities of the analyte.

to a large unexplored potential in the development of both new applications and novel LCPs. The ability of LCPs to noncovalently interact with individual biomolecules and afford an optical finger print corresponding to a distinct conformational state of this biomolecule sets these molecules apart from conventional immunochemical techniques, potentially enabling novel technologies for studying biological processes in a more refined manner. Immunohistochemical techniques are limited by their reliance on antibodies specific for a certain protein, whereas the LCPs are identifying a specific structural motif (Fig. 3). Hence, the LCPs offers a possibility to monitor the biochemical activity of biological events on the basis of a structure–function relationship rather than on a molecular basis.

In order for the LCP technology to be realized, a focus must be turned to the fundamental underlying photophysical processes of LCPs, and the molecular details regarding the selective binding site of LCPs to specific protein aggregates. Although the achievement of obtaining certain spectroscopic LCP signatures from heterogenic populations of protein aggregates are beneficial compared to conventional amyloidothrophic dyes, correlating this spectroscopic signature to a specific form of the aggregated protein is still necessary in order to gain novel insight into the pathological process of the disease. Therefore, a general understanding of photophysical processes of LCP and design rules in the synthesis of LCPs will be important for continued progress in understanding protein aggregation disease. Until then, the LCPs can still be used for comparison of heterogenic protein aggregates in well-defined experimental systems and the technique could potentially be utilized in clinical diagnostics of protein aggregation diseases.

The photophysical processes are impacted by a variety of factors, including polymer interchain packing and intrachain conformation. Controlling these processes by forming complexes between in-vitro-produced protein aggregates with defined conformations and LCPs with distinct ionic side chain

functionalities or different chain lengths will likely be necessary in order to understand the correlation of the spectroscopic read-out from the LCP and the molecular structure of the protein aggregate. On the other hand, these hybrid complexes can also be used to study the optoelectrical properties of conjugated polymers in more detail, as the protein aggregates provides an excellent platform for aligning the conjugated polymers chain in an oriented fashion (see more below).

Future chemical design of novel LCPs will certainly realize combinatorial approaches for optimizing the thiophene core structure, which may provide more effective binders for different classes of protein aggregates. It may even be possible to develop LCPs that selectively stain oligomeric or fibrillar species. Recently, we reported a successful approach for the synthesis of more well-defined oligomeric thiophenes, and these molecules showed a higher selectivity for amyloid fibrils than their polymeric polydispersed counter parts.[29] LCPs having different and well-defined chain lengths would also be of interest in order to establish and optimize the maximum effective conjugation length necessary for selective fluorescence from LCPs being bound to a wide range of heterogenic protein aggregates.

Smaller, appropriately functionalized, but still selective LCPs may also cross the blood/brain barrier and be utilized in powerful multiphoton imaging applications for in vivo imaging that can monitor Alzheimer's pathology and disease progress upon treatment, especially in transgenic mouse models. The LCPs have been reported to have an excellent cross-section area compared to small fluorescent dyes, making these molecules suitable for multiphoton applications.[26,30] However, multiphoton imaging might not be suitable as a clinical diagnostic tool for protein aggregation diseases. In this regard, radiolabeled or fluorinated versions of the LCPs may also be advantageous for PET, SPECT or MRI imaging.

5. Can Dark Matters of Biology Provide Novel Materials for Electronics?

Clearly there is much to be gained through a multidisciplinary scientific approach; the integration of information can provide new insight and discoveries within diverse fields of research. Unexpectedly, conjugated polymer materials that originate from electronics and solar cells have provided novel insights into the biology and pathology of protein aggregation diseases. As an interesting pay-off, further development of LCPs showing a high conductivity could be beneficial for organic electronic applications. Biosensors for electronic detection of amyloid fibrils is one unexplored tantalizing possibility, and in addition the stable structurally defined amyloid fibrils can be used as a template for making well defined nanowires on which conjugated polymers can be symmetrically aligned. The high aspect ratio, unusual stability, and the symmetry of the amyloid fibrils afford an attractive avenue for functional nanodevices. Such an approach was recently presented by Herland et al., where a zwitterionic LCPs was incorporated into amyloid fibrils forming an amyloid–LCP hybrid material during the fibrillization process.[31] The lack of control over the alignment of the materials appears to be a factor affecting the performance of electronic devices based on conjugated polymers and the use of amyloid fibrils as a nanostructural motif possibly provides a solution to this problem. However, more work is needed to better characterize and understand the operating mechanism and the device architecture of such hybrid devices, as a complex set of processes are affecting the performance of electronic devices based on conjugated polymers. Nevertheless, the future for these materials appears to be as bright as ever before.

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