Self-assembled fluorescent nanoparticles from π -conjugated small molecules : en route to biological applications

Citation for published version (APA):

Schill, J., Schenning, A. P. H. J., & Brunsveld, L. (2015). Self-assembled fluorescent nanoparticles from π -conjugated small molecules : en route to biological applications. *Macromolecular Rapid Communications*, 36(14), 1306-1321. <https://doi.org/10.1002/marc.201500117>

Document license:
TAVERNE

DOI:
[10.1002/marc.201500117](https://doi.org/10.1002/marc.201500117)

Document status and date:
Published: 01/01/2015

Document Version:
Publisher's PDF, also known as Version of Record (includes final page, issue and volume numbers)

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

www.tue.nl/taverne

Take down policy

If you believe that this document breaches copyright please contact us at:

openaccess@tue.nl

providing details and we will investigate your claim.

Self-Assembled Fluorescent Nanoparticles from π -Conjugated Small Molecules: En Route to Biological Applications

Jurgen Schill, Albertus P. H. J. Schenning,* Luc Brunsveld*

Since the development of supramolecular chemical biology, self-organised nano-architectures have been widely explored in a variety of biomedical applications. Functionalized synthetic molecules with the ability of non-covalent assembly in an aqueous environment are typically able to interact with biological systems and are therefore especially interesting for their use in theranostics. Nanostructures based on π -conjugated oligomers are particularly promising as theranostic platforms as they bear outstanding photophysical properties as well as drug loading capabilities. This Feature Article provides an overview on the recent advances in the self-assembly of intrinsically fluorescent nanoparticles from π -conjugated small molecules such as fluorene or perylene based chromophores for biomedical applications.

1. Introduction

Extensive research efforts on self-organised architectures have led to the development of well-defined supramolecular nanostructures^[1–3] probing a variety of applications in e.g. optoelectronics,^[4–6] polyelectrolytes^[7,8] as well as biology.^[9–11] In the latter, self-assemblies have received

much attention over the last decade, especially towards various theranostic applications, in which targeted imaging and drug-delivery is combined.^[12–15] Among theranostic platforms, supramolecular assemblies have gained remarkable interest due to several advantageous properties: i) a prolonged systemic circulation; ii) an easy extravasation in tumour vasculature; iii) a large surface to volume ratio; iv) their ability to host a large variety of ligand and drug moieties via both surface functionalization and encapsulation as well as v) their adaptive and responsive properties.^[16–19] Using the powerful and elegant approach of self-assembly, many differently shaped nanoparticles have been prepared and explored by altering the preparation method as well as the chemical structure of the organic building blocks (Table 1).^[20]

Lipidic nanoparticles are supramolecular architectures self-assembled in aqueous solution into mono- or bilayers that consist of naturally occurring phospholipids or closely related synthetic amphiphiles.^[21,22] Due to their modular preparation, narrow and controllable size distribution,^[23,24] and their ability of guest encapsulation,

J. Schill, Prof. L. Brunsveld
Laboratory of Chemical Biology
Department of Biomedical Engineering, and Institute of
Complex Molecular Systems
Eindhoven University of Technology
P.O. Box 513, 5600, MB, Eindhoven, The Netherlands
E-mail: l.brunsveld@tue.nl
Prof. A. P. H. J. Schenning
Functional Organic Materials and Devices
and Institute of Complex Molecular Systems
Eindhoven University of Technology
P.O. Box 513, 5600 MB, Eindhoven, The Netherlands
E-mail: a.p.h.j.schenning@tue.nl

these nanoparticles have been widely explored and have also proven their value in various applications.^[25–27] However, functionalization with targeting ligands as well as imaging agents is essential for probing theranostic applications using lipid-based nanostructures.^[28] Closely related to lipidic nanoparticles are structures that combine the features of amphiphilic surfactants with the functions of bioactive peptides. Peptide amphiphiles consist of an alkyl chain linked to a peptidic structuring element followed by charged amino acids and finally a bioactive epitope.^[29] These amphiphiles are reported to self-assemble into a variety of nano-architectures, predominantly into cylindrical nanofibers which are explored in the field of regenerative medicine.^[30–32]

Although the aforementioned nanostructures hold great promise, the ability to detect nanoparticles via an intrinsic property instead of by functionalization with detectable ligands is often desirable. Magnetic nanoparticles have therefore been explored as they can be detected using non-optical techniques based on magnetic resonance.^[33–35] Nano-architectures composed of iron, metallic iron and copper as well as a number of different alloys have demonstrated to possess useful magnetic properties ranging from ferromagnetic nanoparticles, predominantly used in bioanalytical applications,^[36] to superparamagnetic architectures, which demonstrated to be powerful in contrast enhancement in non-invasive magnetic resonance imaging (MRI).^[37]

Another example of intrinsically detectable nanostructures are semiconducting inorganic nanoparticles, also known as quantum dots, which generally exhibit outstanding optical properties as well as good chemical stability which makes them attractive for a variety of applications.^[38–41] Their core is, however, typically composed of heavy metal atoms, which has to be coated with an inert or biocompatible layer to anticipate toxicity issues.^[42,43] Moreover, these layers are needed for ligand immobilization in order to probe biomedical applications as biomolecule recognition,^[44] *in vivo* targeted imaging,^[45,46] and theranostics.^[47]

Over the last decade, the aforementioned nano-architectures have allowed the investigation of fundamental processes in the life sciences as well as probed targeted drug-delivery applications. In this field, imaging and sensing will become even more important in the near future, especially in biomedicine where, in combination with therapeutics, multimodality allows the development of theranostics.^[48,49] Optical fluorescence is one of the major modalities used in targeted bioimaging.^[50,51] Therefore, there is a need for fluorescent nanoparticles which bear outstanding optical properties suitable for a wide range of applications. A promising strategy for generating bright fluorescent biosensors is based on the development of highly fluorescent conjugated polymers.^[52–56] The

Jurgen Schill (1989) received his Bachelor's degree in Biomedical Engineering at Eindhoven University of Technology by studying Cucurbit[8]uril-mediated protein homotetramerization. After working on genetically modified yeast during iGEM 2012 and *in vitro* mapping the interactome of TLR4-dependent immunity at the Institute for Molecular Bioscience (University of Queensland), he received his Master's degree in 2014 with his thesis on auto-fluorescent amphiphiles and their dynamic supramolecular nanoparticles in the group of Luc Brunsveld. He currently continues this work during his PhD focusing on the synthesis and biological evaluation of self-assembled supramolecular platforms for applications such as cellular imaging and targeted drug-delivery.

Albertus P. H. J. Schenning Albert Schenning (1966) is professor of functional organic materials and devices at the chemistry and chemical engineering department of the Eindhoven University of Technology. He received his PhD degree at the University of Nijmegen in 1996. In 1997, he was a post-doctoral fellow at the ETH in Zurich. From 1998 until 2002, he was a Royal Netherlands Academy of Science fellow at Eindhoven University of Technology. His research interests center on stimuli responsive and functional organic materials.

Luc Brunsveld (1975) is professor of chemical biology at the biomedical engineering department of the Eindhoven University of Technology. After obtaining a PhD in supramolecular chemistry he was a Humboldt Fellow at the Max Planck Institute of Molecular Physiology and group leader in medicinal chemistry at Organon. In 2005 became group leader at the MPI in Dortmund and subsequently moved to the TU Eindhoven. His interests include supramolecular chemistry and chemical biology with a specific focus on protein-protein interactions and interfacing supramolecular systems with biomolecules.

From left to right: A.P.H.J. Schenning, J. Schill, and L. Brunsveld.

formation of polymer nanoparticles initially developed for optoelectronics^[57,58] has attracted considerable attention in the field of biomedical applications.^[59–62] Recent research efforts have demonstrated that these so-called polymer dots exhibit excellent optical properties^[63–65] as a high absorption cross section, fluorescence brightness,

Table 1. Overview of the key properties of polymer dots, liposomes, quantum dots, and self-assembled small molecule nanoparticles as discussed in this Feature Article.

	Polymer Dots	Liposomes	Quantum Dots	Small molecule NPs
High Quantum Yield	+	–	+	+
High Absorption	+	–	+	+
NIR-emission	+	+	+	n.d.*
Non-blinking	+	+	–	+
Good Stability	+	–	+	+
Non-Photobleaching	+	–	+	n.d.*
Cytotoxicity	+	+	–	+
Modular	–	+	–	+
Adaptive	–	+	–	+

* not determined

photostability, and non-blinking behaviour as well as feature non-toxicity,^[66] nano-encapsulation^[67] and ligand ligation abilities^[68,69] showing their potential in biology, particularly in bio-imaging^[70] and theranostics.^[71] However, despite many research advances, control over their surface chemistry still remains a challenge which has prevented their widespread usage in various applications.^[62,72] Additionally, the rigidity of polymer dots may be disadvantageous regarding the interaction with highly dynamic systems present in biology and tuning of properties requires the synthesis of new polymers for each desired modification.

The development of supramolecular chemistry has brought forward the design of dynamic and functional materials, based on self-assembly of well-defined π -conjugated small molecule or oligomeric chemical structures.^[73] The non-covalent assemblies provide tunable strength and directionality amongst molecular units while probing well-defined, though dynamic, structures that still bear the key properties of covalent polymers.^[73,74] The field of material sciences developing supramolecular nanomaterials extensively explored π -conjugated segments due to their outstanding optical properties as well as their ability of multiple interactions and hence collective response.^[59] In biology, research towards supramolecular fluorescent architectures has especially been focused on the development of amphiphilic π -conjugated systems.

2. Nanoparticles Based on Self-Assembled π -Conjugated Small Molecules

In the last decade, fluorescent π -conjugated systems have received considerable attention in targeted imaging, sensing applications, and drug-delivery.^[59,62,75,76] This is mostly due to their well-defined size and shape as well as their attractive photophysical properties. Moreover, the rigidity of the aromatic segment enhances aggregation stability in water during the self-assembling process, most likely due to secondary interactions as π - π stacking and the hydrophobic effect.^[20] Since the aromatic core of π -conjugated building blocks is hydrophobic, often hydrophilic side chains are introduced in order to obtain amphiphilic characteristics and hence confer self-assembly in aqueous solution. The vast majority of self-assembled fluorescent π -conjugated nanostructures for biomedical applications are either based on fluorene or perylene moieties, which have, besides an obvious structural difference, both very distinct properties in terms of size, shape, stability, and photophysics. Moreover, the use of these moieties as chromophores have shown to be advantageous as the emission colour of fluorene derivatives can be readily tuned and the synthesis of perylene derivatives has shown to be rather straightforward. While key properties are typically applicable to all π -conjugated organic nanoparticles, self-assembled fluorescent nanoparticles from chromophores based on other building

■ Figure 1. Molecular structure of the fluorene moiety.

blocks^[11,77] such as boron-dipyrromethenes (BODIPY)s^[78–80] squaraines^[81,82] are outside the scope of this Feature Article.^[59,60]

2.1. Fluorene Based Architectures

The fluorene moiety consists of two benzene rings which are connected by a direct carbon-carbon bond and an adjacent methylene bridge (**1**, Figure 1). This moiety is extremely favourable for fluorescence applications, due to its high fluorescence quantum yield, high photostability, and good solubility in organic solvents.^[83] However, for applications concerning aqueous solutions, good or partial solubility has to be obtained by attaching hydrophilic side chains.^[84]

Tagawa et al. were the first reporting a fluorene based oligomer functionalized with alkyl and ethylene glycol side chains which was able to self-assemble in aqueous solution (**2**, Figure 2).^[85] By simply adding the oligofluorene dissolved in THF to water, spherical nanoparticles with radii of 20–100 nm were observed by AFM. Moreover, the nanoparticle size could be controlled by the oligofluorene concentration of the organic stock solution. Interestingly, intrinsic blue emission around 400 nm in THF was quenched in aqueous solution, while a green emission band around 540 nm appeared attributed to the excitation of steady state molecular aggregates.^[84]

Based on these oligofluorene moieties, Yang et al.^[86] substituted the short ethylene glycol side chain by a substituted aniline (**3**, Figure 2) resulting in a red-shifted emission peak in THF. Self-assembly of this material in aqueous solution resulted in an aggregation-induced blue shift of the emission, while similar particle sizes were observed. These results bring forward that the spectral properties are frequently not only dependent on the aggregation state of the oligomers but that there is also a key influence of the side-chain functionalization on the resulting materials properties.

■ Figure 2. Molecular structure of the oligofluorene **2** by Tagawa et al.^[85] and **3** by Yang et al.,^[86] which are able to self-assemble into nanoparticles in aqueous solution.

Schenning et al.^[87] synthesized fluorene based oligomers in order to prepare multicomponent nanoparticles which could be used for imaging applications. Four different bola-amphiphilic fluorene-based oligomers were prepared which could co-assemble into spherical nanoparticles in aqueous solution. The π -conjugated core consists of two fluorene units linked by a central aromatic moiety. This aromatic moiety is varied by introducing naphthalene (**4**, blue), quinoxaline (**5**, green), benzothiadiazole (**6**, yellow) or thienopyrazine (**7**, red) in order to obtain different emission colours (Figure 3a). The nanoparticles fabricated by re-precipitation showed excellent quantum yields in aqueous solution, up to 70% for **6**, the benzothiadiazole core containing oligomer. The photoemission spectra of these different bola-amphiphilic oligomers span the entire visible range (Figure 3b) and the fluorescence emission can be fine-tuned, including even white, by careful mixing of these fluorene-based oligomers with only minor changes in the shape and size.^[87,88] Moreover, it was shown that the particles self-assembled from these bola-amphiphilic oligomers did not exchange molecules upon time or heating and are thus highly stable. Separate particles could be observed by fluorescence microscopy while efficient energy transfer of the acceptor oligomer dispersed in the donor matrix was shown by appropriate mixing of bola-amphiphiles containing different cores.^[89]

Using a comparable approach of mixing π -conjugated compounds bearing different colours, Wong et al.^[90] drop casted the oligofluorenes **8–10** from THF solution to vesicle-like nanospheres of 200–600 nm in diameter (Figure 4). The aromatic core can undergo π - π -stacking as the hydrophilic side chains can undergo complementary hydrogen bonding resulting in nanospheres. By mixing the three different oligomers in different ratios, supramolecular structures fluorescing in a large spectral window (including pure white) could be obtained.

Another approach to fabricate white-emitting nanoparticles has been explored by Takeuchi et al.^[91] Oligofluorenes bearing a variety of hydrogen bonding side chains (Figure 5) were intermixed with a red-orange emitting dye (DCM) in THF and then injected into water, creating spherical nanostructures of 70–180 nm in diameter. Interestingly, due to energy transfer from the intrinsically blue emitting oligofluorenes to the intermixed dye, white light emissive nanoparticles were generated.

Thus, the importance of supramolecular self-assembly on fluorescence energy transfer and hence colour tuning has been shown. Here, increasing hydrogen bonding sites in the oligomer were shown to decrease the nanoparticle size and to increase the energy transfer

Figure 3. a) Chemical structure of fluorene-based bola-amphiphiles by Schenning et al. containing different central aromatic moieties (**4–7**) and b) their corresponding emission spectra. Reproduced by permission.^[87] Copyright 2009, The Royal Society of Chemistry.

efficiency.^[92] This shows the importance of the chemical structure of the oligomers on the supramolecular properties.

Since the molecular structure of the π -conjugated amphiphilic building blocks showed to be of great importance for the size and shape of the corresponding self-assembled nanostructures, the nature of the side chains of oligofluorenes was systematically changed.^[84] The influence on stability, dynamics, fluorescence, and self-sorting properties of nano-sized architectures, self-assembled from fluorene co-oligomer derivatives in which the ratio of hydrophilic ethylene glycol and hydrophobic alkyl side chains are altered, was studied. Fluorene-based oligomers containing wedges and tails ranging from all nonpolar to all polar (Figure 6a) were synthesized and studied. It was concluded that all fluorene-based oligomers formed self-assembled fluorescent nanoparticles in water. However, nanostructures self-assembled from oligomers containing polar side chains were more dynamic and larger in size compared to particles formed by oligomers containing nonpolar side chains. Moreover, nonpolar oligomers generally showed a higher fluorescence quantum yield. It was also shown that fluorene based oligomers containing a high degree of polar side chains were able to dynamically

exchange between nanoparticles. Interestingly, oligomers containing the same side chains were shown to have affinity for each other over derivatives bearing other side chains, providing opportunities for sensing and diagnostics. This study elaborately shows the dynamics between nanostructures, within a single assembly and between the molecularly dissolved and the self-assembled state of the monomers (Figure 6b).

In order to enable π -conjugated fluorescent nanoparticles to actively target specific receptors on the cell surface and thus creating probes or delivery vehicles for imaging and drug-delivery, oligomer functionalization is a prerequisite. Nano-sized architectures typically require functionalization with ligands on the nanoparticle surface. Fluorene-based oligomers were pre-functionalized with either an azide or a mannose functionality (**25** and **26**, respectively, Figure 7).^[93] In order to ensure an efficient exposure of the ligands to the aqueous environment, they were introduced at the periphery of the hydrophilic ethylene glycol chains decorating the π -conjugated oligomers. Nanoparticles self-assembled from these functionalized oligomers showed to have similar sizes and optical properties as non-functionalized π -conjugated oligomers reported earlier. These findings suggest that

Figure 4. Chemical structure of the π -conjugated oligomers **8–10** by Wong et al.,^[90] which are able to self-assemble into vesicle-like supramolecular architectures.

Figure 5. Chemical structure of oligofluorene derivatives **11–14** by Takeuchi et al.,^[92] which highlights the importance of the chemical structure on the nanostructure properties.

the self-assembly of the π -conjugated oligomers and optical properties of the resulting nanoparticles are dominated by the core structure of the oligomers, and that the introduction of the ligands at the periphery of the PEG chains do not strongly perturb these properties. Both the mannose functionality, by binding *E. coli* bacteria's FimH receptor and lectin concanavalin A (ConA), and the azide functionality, by post-functionalization via the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC), were shown to be exposed to the aqueous solution and available for binding. The accessibility of the ligand at the periphery of the nanostructure was also validated by nanoparticles formed from biotin functionalized oligomers which could be extracted from solution using magnetic streptavidin coated beads. Interestingly, multi-valent nanoparticles bearing two different ligands were prepared by combining pre- and post-functionalization in order to enable dual targeting. Comparable binding behaviour proved effective introduction and availability of both ligands via both functionalization approaches.

Bard et al.^[94] similarly reported stable nanoparticles with a diameter of 16 nm bearing azide functionalities, however positioned at the center of the molecule and connected via hydrophobic spacers. Two benzothiadiazole units, flanked with triphenylamine groups, were bridged by a fluorene unit, which was substituted by two alkyl linkers bearing an azide moiety (**27**, Figure 8). While only the optoelectronic properties were explored until now, these azide bearing oligofluorenes may be useful for further functionalization with bioactive ligands and hence be explored in the fields of diagnostics and imaging.

The cellular uptake and localization properties as well as potential toxicity of fluorene-based π -conjugated oligomers were evaluated for fluorene-based oligomers as well.^[95] Since it is known that amines facilitate cellular uptake, oligomers were pre-functionalized with a single amino group (Figure 9a). These oligomers showed similar behaviour as the ones functionalized with mannose and azide in terms of optical properties. However, the size of these co-assembled nanostructures was strongly dependent on the percentage of amine-functionalized oligomer in the nanoparticle. It was discussed that the amino group might be protonated in water, which then

Figure 6. a) Molecular structures of fluorene based co-oligomers used to study the influence of hydrophobicity of the side chains on stability, dynamics, fluorescence, and self-sorting properties of nanoparticles. The colours of the side chains refer to polarity as red represents hydrophobic and blue hydrophilic residues. b) Different states of nanostructures showing their dynamics. Reproduced with permission.^[84] Copyright 2012, American Chemical Society.

Figure 7. Chemical structures of oligofluorenes used to study ligand functionalization. (i) Generation of nanoparticles pre-functionalized with mannose. (ii) Generation of nanoparticles pre-functionalized with azides and subsequent post-functionalization via copper catalyzed azide alkyne cycloaddition with alkyne derivatives of either mannose or biotin. (iii) Generation of bifunctional nanoparticles containing azides and mannose and subsequent post-functionalization via copper catalyzed azide-alkyne cycloaddition with alkyne derived biotin yielding mannose and biotin labelled nanoparticles. Adapted with permission.^[93] Copyright 2011, American Chemical Society.

affects the hydrophilic/hydrophobic ratio and hence the nanoparticle size. Remarkably, by premixing oligomers **18** and **28** it was shown that only 10% of amine decoration was sufficient to promote efficient cellular uptake (Figure 9b). In contrast to covalent assemblies,^[96] these findings indicate that the dynamic nature of supramolecular structures allows efficient clustering surface functionalities highlighting the importance of supramolecular effects of self-assembly. While this charge mediated cellular uptake was shown to be rapid, it was also shown that none of the amine-bearing nanoparticles affected the cell viability up to a concentration of 0.9 μM . Rapid cellular internalization and biocompatibility was also shown for other cationic side chain bearing oligomers.^[97] Moreover, the preparation of nanoparticles with a

different composition in terms of surface functionalization by simple intermixing of differently functionalized oligomers hold great promise in the field of multivalent theranostics.

In order to explore the encapsulation properties of fluorene-based nanoparticles, release studies were performed on guest molecule loaded nanostructures (Figure 10a).^[98] Nile Red was chosen as hydrophobic guest as its absorption spectrum overlaps with the emission spectrum of the nanoparticles, and thus fluorescence energy transfer could be used to monitor the release by dividing the emission intensity of the nanoparticle (at 510 nm) and Nile red (at 625 nm), respectively. While up to 10 mol% of Nile Red was encapsulated, moderate energy transfer was observed. However, slow release of the guest molecule was observed over time as a fast release profile was shown at elevated temperatures (Figure 10b,c). These results suggest that these fluorene-based nanostructures could be used as encapsulating vehicles that allow release of the guest molecule which might be interesting in the field of drug-delivery.

Combining fluorescence imaging, encapsulation of hydrophobic guest molecules, and drug delivery, Tuncel et al.^[99] reported on fluorene-based nanoparticles which

Figure 8. Chemical structure of an oligofluorene derivative **27** by Bard et al.,^[94] which may be useful for further functionalization.

Figure 9. a) Molecular structure of oligofluorene **28** pre-functionalized with an amino group. b) Nanoparticles with different composition by premixing of oligomers **18** and **28**, generating unfunctionalized nanoparticles of **18** alone (NP) and amine-functionalized nanoparticles with 10% (amine-10-NP), 50% (amine-50-NP) and 90% (amine-90-NP) of **28**. Two-photon micrograph of live HeLa cells incubated for 2 h with the aforementioned nanoparticles. Adapted with permission.^[95]

encapsulate a drug and emit fluorescence in the red region tailing to infrared (Figure 11). The two fluorene units, both bearing two amine substituted side chains, are bridged by a central divinyl benzothiadiazole moiety, resulting in an oligomer bearing a hydrophobic backbone and hydrophilic side chains. In water, **29** self-assembles

into spherical nanostructures of 50 nm in diameter. Due to the hydrophobic nature of the nanoparticle core, Camptothecin, an anticancer drug, could be encapsulated. In an acidic environment, disassembly of nanoparticles due to repulsive forces of the formed ammonium ion resulted in the release of Camptothecin. In order to avoid nonspe-

Figure 10. a) Overview of the encapsulation of Nile Red into fluorene-based nanostructures and the resulting fluorescence energy transfer. The time dependent change in fluorescence energy transfer of the nanostructure to 1 mol% of encapsulated Nile Red at b) 20 °C and c) 30 °C and (b, inset) the fluorescence spectrum of nanostructures encapsulating 1 mol% Nile Red in time show a slow time dependent and fast temperature dependent release profile. Adapted with permission.^[98]

Figure 11. Overview of CB-capped drug loaded nanoparticles, which enable a pH-triggered drug release mechanism. Adapted with permission.^[99] Copyright 2014, American Chemical Society.

cific absorption of biomolecules to the amine functionalized surface, the amines were capped with cucurbit[7]uril (CB7). Besides preventing nonspecific absorption of proteins, CB7-capped nanoparticles also showed higher stability in physiological pH compared to bare nanoparticles. These stable pH-responsive CB7-capped nanoparticles enable a sustained drug release at physiological pH and a fast release at low pH. Together with efficient cellular uptake and a reduced cytotoxicity due to CB7-capping, these fluorene based architectures are envisioned to have great potential in theranostic applications.

Self-assembly in aqueous solution, tuneable emission colours spanning the entire visible range, the capability to encapsulate hydrophobic guest molecules, and the ability to functionalize the nanoparticle surface without affecting its properties makes fluorene-based nanoparticles appealing for imaging and drug-delivery applications.

2.2. Perylene Based Architectures

The perylene moiety consists of two naphthalene subunits which are connected by a carbon-carbon bond at position 1 and 8 on both molecules (**30**, Figure 12). For biological applications, most often perylene bisimides (PBI **31**, Figure 12) are used. It has already been known for decades that water-soluble PBIs with substituents at the imide position show excellent photophysical properties with extremely high quantum yields as well as stability.^[100] PBIs have extensively been studied for their potential in protein labelling and as probes for single-molecule spectroscopy.^[101] For these applications, especially substituting the bay position has been explored as this introduction effectively prevents aggregation through electrostatic shielding

Figure 12. Chemical structure of the perylene moiety (**30**) as well as of perylene bisimide (PBI) (**31**).

and steric hindrance resulting in strongly emitting water-soluble probes.^[102,103] Despite a lot of work on PBIs is committed to water-soluble probes, only recently the potential of perylene-based supramolecular architectures for biomedical purposes has been evaluated. The introduction of hydrophilic substituents at the imide moiety in combination with a strong π - π -stacking of the PBIs core typically results in the formation of supramolecular aggregates in water.^[104,105]

In order to study the aggregation behaviour of imide substituted perylene derivatives, Haag et al.^[106] synthesized dendron-functionalized perylene bisimides. Generation 1 (G1) to 4 (G4) hydrophilic polyglycerol dendrons (**32** and **33**, respectively) were attached to the imide nitrogen prior to injection into water (Figure 13). Photophysical measurements revealed that G1-dendronized PBIs aggregate in water over a large concentration range and as a result broadening of the absorption spectrum occurs as well as a drop to 33% quantum yield. However, dendrons of the third and fourth generation do not show aggregation as the hydrophobic PBI core seemed to be sufficiently shielded and hence 100% quantum yield is regained. Spectral changes such as a broader and less structured absorption spectrum as well as a reduced absorption coefficient upon aggregation are due to strong excitonic interactions between the PBI chromophores.^[107] Suppressing aggregation of perylene derivatives by shielding the PBI chromophore, however, can result in comparable fluorescence quantum yields in water and organic solvents.^[108,109] Similar behaviour was observed for Newkome dendronized perylene derivatives (**34**) by Hirsch et al.^[110]; first generation Newkome dendronized PBIs aggregated into irregular architectures and second and larger generation PBIs dissolved molecularly. Moreover, chiral alanine and lysine residues were used as linker between the PBI core and the Newkome dendrons to direct the aggregation to helical architectures.^[111] These dendronized perylene bisimide derivatives are also used to disperse poorly soluble materials as carbon nanotubes through π - π -interactions and are envisioned to have potential in the field of medicine as solubilizing agent.^[112,113]

Figure 13. Chemical structure of polyglycerol dendronized PBI **32** and **33** by Haag et al.^[106] and Newkome dendronized PBI **34** by Hirsch et al.^[110] which aggregate or molecularly dissolve in water depending on the dendron generation.

ionic PBIs also decreases upon aggregation, they may be suitable as pH sensors. Interestingly, cationic PBIs have also shown to have potential for the stabilization of G-quadruplex DNA by stacking in and with DNA,^[117,118] which have eventually led to self-organised PBIs as DNA/RNA sensitive probes.^[119] Recently, Hirsch et al.^[120] reported EDTA functionalized PBI **38**, which bears a high affinity for heavy trivalent metal ions as well as lanthanide cations and is envisioned as heavy metal imaging agent in aqueous environment.

PBI substituents as well as solvent

Sun et al.^[114] prepared symmetrically trialkylammonium substituted PBIs (**35**, Figure 14) which self-assembled into one-dimensional nanotubes and nanorods depending on the solvent the oligomer was dissolved in. Interestingly, as these structures were explored as amine sensors, hollow nanotubes showed to be more effective for the detection of amines than rod-like structures, showing that the supramolecular architecture is indeed a key feature for its application. The aggregation behaviour of bolaamphiphiles has also been explored using PBIs substituted with multiply charged ionic side chains (**36**, Figure 14).^[115] PBI **36** is molecularly dissolved in water at low concentrations and bears a high quantum yield at a nearly neutral pH. However, upon increasing concentration, the formation of aggregates is accompanied by a decreasing pH. Protons from the side chain, which cause a positive charge and hence repulsion at low concentrations, may be transferred to the surrounding water leading to neutral PBI derivatives, which are able to aggregate into rod-like structures. Positively charged ammonium groups and neutral amines might even facilitate π - π -stacking of the perylene core. Similar behaviour has been observed for PBIs substituted with β -cyclodextrin (**37**, Figure 14).^[116] As the fluorescence quantum yield for these charged

interactions have a strong influence on various properties of the supramolecular architecture. To study the aggregation behaviour in more detail, Liu et al.^[121] extended the work on cyclodextrin substituted perylene derivatives by synthesizing PBI **39** bearing an amphiphilic character (Figure 15a). Although the supramolecular architecture is still dependent on the solvent composition, PBI **39** is more prone to aggregate in aqueous solution than PBI **36** due to the hydrophobic alkyl residues. Starting from nanorods in methanol in which PBI **39** is rather randomly stacked, PBI **39** self-assembles into well-defined vesicular structures in a 9:1 mixture of water and methanol (Figure 15b). Faul et al.^[122] simplified the latter PBI design and reported galactosyl substituted PBI **40**, which aggregates into right-handed superhelical architectures of 100 nm in diameter in an aqueous THF mixture (1:1 v/v ratio). This superhelix is composed of multiple helical fibres that are self-assembled via hydrogen bonding of the galactosyl group. In order to create transport vehicles as well as membrane labels, Hirsch et al.^[110] explored amphiphilic Newkome dendronized PBI **41**. Compared to the aforementioned double Newkome dendronized PBI **34** derivative, PBI **41** bears besides a single Newkome dendron, a dodecyl chain, which makes this PBI derivative more prone to aggregate into spherical nanostructures in water. Second generation amphiphiles showed a pronounced aggregation behaviour into regularly shaped micelles of 16 nm in diameter, as the peripheral Newkome dendrons interact with the aqueous environment and the dodecyl chains form the hydrophobic interior.

Würthner et al. reported substituted dumbbell-like PBIs of which the side chains were systematically changed (Figure 16a). It was shown that dumbbell-like PBI **42** bearing apolar side chains is able to self-assemble into columnar stacks in organic solvents^[123] as similar aggregation behaviour was obtained for bolaamphiphilic PBI **43** in aqueous solution.^[124] The π - π -stacking of the PBI **43** core in water was so pronounced that almost complete aggregation was observed even at

Figure 14. Charged ionic bolaamphiphilic PBI **35**, **36**, **37** and **38** by Sun et al.,^[114] Würthner et al.,^[115] Liu et al.^[116] and Hirsch et al.,^[120] respectively, which are able to aggregate into rod-like structures.

Figure 15. a) Chemical structure of amphiphilic PBI **39** by Liu et al.,^[121] PBI **40** by Faul et al.^[122] and PBI **41** by Hirsch et al.,^[110] which are able to form vesicular, helical and micellar architectures in aqueous solution, respectively. b) Self-assembly models of **39** depending on the solvent polarity. Reprinted with permission.^[121] Copyright 2010, American Chemical Society.

nanomolar concentrations.^[125] Wedge-shaped PBI **42** showed micellar aggregation in particles with a 4–6 nm diameter, which is in accordance with findings on PBI **41**. However, when PBI **44** is coaggregated with PBI **45** (8:1 molar ratio), vesicular architectures bearing a bilayer membrane are formed with a diameter of 100 nm and a membrane thickness of 7–8 nm. Moreover, the surface curvature of the bilayer bearing vesicles, and hence the diameter, can be tuned by varying the oligomer ratio. Increasing the hydrophobic ratio leads to an overall decrease in surface curvature and thus a larger particle diameter. Therefore, maximum surface curvature has been observed for particles consisting of only PBI **44** as the supramolecular architectures become larger upon increasing amount of coaggregated PBI **45** (Figure 16b). Additional stability in terms of size and shape was obtained by photopolymerization of the acrylate moieties of PBI **44** in the self-assembled state. Moreover, these spherical architectures could be loaded with dyes that show fluorescence resonance energy transfer to the photoactive exterior and hence could be explored as supramolecular sensors.^[126,127]

In another approach, Rybtchinski et al. studied the aggregation behaviour of PBIs by modifying its bay

positions. Multiple PBI designs showed that bay PEGylated PDIs are excellent building blocks for self-assembly in aqueous solution.^[128–132] However, only recently bay PEGylated PBIs were explored for biological applications. Bay position substituted PBI **46** (Figure 17) consists of two PBI units which are bridged by a bipyridyl linker and was developed into supramolecular membranes which could be used for separation of nanoparticles^[133] as well as for the immobilization and biocatalytic utilization of proteins.^[134] Moreover, large proteins such as enzymes can be deposited on the membrane which may allow the development of enzyme-based sensors.

While substantial effort has been made to prevent fluorescence quenching by shielding the PBI chromophore using various approaches, Genovese et al.^[135] reported a perylene-based imaging agent which actually makes use of fluorescence quenching. Self-assembly of PBI **47** into supramolecular nanospheres leads to almost complete fluorescence quenching (Figure 18a). However, cellular uptake causes disaggregation

of the nanospheres resulting in fluorescence recovery. Due to this dissociation of the aggregates, cells become green or red fluorescent, depending on the concentration of PBI **47**. More in particular, multicolour fluorescence imaging can be envisioned using a photo-tuning process. Specific cellular compartments can be given a specific photo-tuned emission as they respond differently to photo-activation.

Similarly, Haag et al.^[136] reported spherical micelles self-assembled from perylene imino diesters (PIDEs) bearing two alkyl chains and a polyglycerol dendron of which the fluorescence quantum yield is extremely low (Figure 18a,b). However, PIDE **48** recovers high fluorescence in an lipophilic environment. It was shown that this system could be used as a fluid phase marker for disordered domains in artificial membranes as well as a staining agent for the plasma membrane in living cells. As the dendron side is easy accessible for ligand functionalization, PIDE **48** is envisioned to anchor and track bioactive ligands in cellular membranes.

Besides imaging applications, perylene-based architectures have also been explored as single component drug delivery systems.^[137] Therefore, an anticancer drug was conjugated to the perylene moiety yielding a photocaged

Figure 16. a) Chemical structure of imide substituted dumbbell-like PBI **42** and **43**, which are able to self-assemble into columnar stacks in organic solvents^[123] and in aqueous solution,^[124] respectively, as well as amphiphilic PBI **44** and **45**, which are able to self-assemble into vesicle-like architectures in aqueous solution.^[124] The colour of the side chains refer to polarity as red represents hydrophobic and blue hydrophilic residues. Adapted with permission.^[124] Copyright 2007, American Chemical Society.

perylene-chlorambucil conjugate (Pe-Cbl **49**, Figure 18a,c), which was able to self-assemble into spherical nanostructures of 30 nm in diameter. HeLa cells incubated for 4 hours with Pe-Cbl nanospheres showed efficient cellular uptake, a uniform distribution inside the cell and no significant change in cell viability. However, upon visible light irradiation, Pe-Cbl **49** showed an increasing cytotoxicity due to the photorelease of chlorambucil inside the cell. By varying the amount of substituents the emission properties of the nanoparticles could be tuned (Pe-Cbl **50**).^[138] Moreover, the emission wavelength of the

perylene derivative shifts towards smaller wavelengths upon photorelease of the drug. Therefore, this system is not only a versatile drug carrier and a photocaged compound for the release of an anticancer drug, but also an imaging agent to monitor the drug release in real time.

3. Conclusion and Outlook

Supramolecular architectures based on π -conjugated small molecules have emerged as versatile platforms for biological applications as their size and shape are well-defined while bearing outstanding photophysical properties. Fluorene and perylene based building blocks have been widely explored in order to enable and tune the self-assembly in water as well as the interaction with biological matter. π -Conjugated small molecules bearing an amphiphilic character can form stable and well-defined supramolecular architectures in an aqueous environment. Decoration with bioactive ligands expands the functionality of the supramolecular nanoparticles, for example by providing them with the ability to induce cellular uptake and to specifically deliver cargo. Self-assembly of π -conjugated building blocks can also give rise to spectral changes. Fluorene-based oligomers have been reported to

Figure 17. Bay position substituted PBI **46** by Rybtchinski et al.,^[133,134] which is explored as building block for a supramolecular membrane.

Figure 18. a) Chemical structure of PBI **47** by Genovese et al.^[135] and PIDE **48** used as b) a dye to stain membranes by Haag et al.,^[136] as well as the chemical structure of Pe-Cbl **49** and **50** by Singh et al.^[137] and Zhao et al.,^[138] respectively, which have been explored as c) single-component perylene-based drug delivery system for photocontrolled delivery of an anticancer drug. b) Reproduced with permission.^[136] Copyright 2013, American Chemical Society. c) Reproduced with permission.^[137] Copyright 2012, American Chemical Society.

bear an enhanced fluorescence quantum yield upon self-assembly in aqueous solution, which is in direct contrast to perylene-based oligomers which, upon aggregation, feature a broader and less structured absorption spectrum with a reduced absorption coefficient and concomitant low fluorescence quantum yield. As the fluorescence quantum yield of perylene-based architectures is strongly dependent on strong excitonic interactions of its chromophore, substantial effort has been made to shield the perylene core while simultaneously allowing the shielding substituents to also direct the morphology of the supramolecular architecture. En contraire, the large difference in fluorescence quantum yield between self-assembled and molecularly dissolved perylene-based oligomers could be of great value for sensing and imaging applications. While many research advances have already highlighted the potential of dynamic supramolecular architectures in biological applications, the number of studies showing their direct use in biomedicine is however limited. The major challenges lie in the synthesis of the small molecules as well as the functionalization and the dynamics of the supramolecular system. Although not discussed, it should be noted that in most cases the number of synthetic steps to prepare the π -conjugated building blocks is rather high. From an application point of view it would be

desirable to have a limited number of steps. At the same time that would allow readily tunable self-assembly properties. Moreover, knowledge on the functionalization of supramolecular architectures with bioactive ligands and especially their influence on the self-assembly behavior should be expanded in future studies. Deep understanding on the relationship between the amphiphilicity and the self-assembly could provide control over the dynamics of the supramolecular system and thus provide control over key properties as modularity and adaptivity. While extensive research efforts on the aforementioned issues are inevitable, we foresee that supramolecular architectures based on π -conjugated small molecules will have a broad and lasting impact on applications in the fields of i) sensing and targeted imaging, for example, by introducing different targeting ligands that might induce synergistic binding effects; ii) therapeutics, e.g. drug-delivery, and iii) theranostics when reporter systems and therapeutic cargoes are combined in a single dynamic architecture.

Acknowledgements: The research was supported by funding from HTSM through STW grant 12859-FluNanoPart and by the Ministry of Education, Culture and Science (Gravity program 024.001.035).

Received: February 24, 2015; Revised: March 26, 2015;
Published online: May 20, 2015; DOI: 10.1002/marc.201500117

Keywords: supramolecular nanoparticles; fluorescence;
 π -conjugated small molecules; self-assembly; biological applications

- [1] S. I. Stupp, V. LeBonheur, K. Walker, L. S. Li, K. E. Huggins, M. Keser, A. Amstutz, *Science* **1997**, 276, 384.
- [2] J.-M. Lehn, *Science* **2002**, 295, 2400.
- [3] L. Maggini, D. Bonifazi, *Chem. Soc. Rev.* **2012**, 41, 211.
- [4] F. Würthner, *Chem. Commun.* **2004**, 1564.
- [5] F. J. M. Hoebe, P. Jonkheijm, E. W. Meijer, A. P. H. J. Schenning, *Chem. Rev.* **2005**, 105, 1491.
- [6] A. P. H. J. Schenning, E. W. Meijer, *Chem. Commun.* **2005**, 3245.
- [7] L. An, *Chem. Asian J.* **2009**, 4, 1196.
- [8] G. Feng, D. Ding, B. Liu, *Nanoscale* **2012**, 4, 6150.
- [9] R. Subbiah, M. Veerapandian, K. S. Yun, *Curr. Med. Chem.* **2010**, 17, 4559.
- [10] H. Jin, W. Huang, X. Zhu, Y. Zhou, D. Yan, *Chem. Soc. Rev.* **2012**, 41, 5986.
- [11] K. Petkau-Milroy, L. Brunsveld, *Org. Biomol. Chem.* **2012**, 11, 219.
- [12] Z. Cheng, A. A. Zaki, J. Z. Hui, V. R. Muzykantov, A. Tsourkas, *Science* **2012**, 338, 903.
- [13] D. Mishra, J. R. Hubenak, A. B. Mathur, *J. Biomed. Mater. Res. A* **2013**, 101, 3646.
- [14] S. Jain, A. S. Doshi, A. K. Iyer, M. M. Amiji, *J. Drug Target.* **2011**, 21, 888.
- [15] W. Poon, X. Zhang, J. Nadeau, *Crit. Rev. Oncog.* **2014**, 19, 223.
- [16] O. C. Farokhzad, R. Langer, *ACS Nano* **2009**, 3, 16.
- [17] J. B. H. Parag Aggarwal, *Adv. Drug Deliv. Rev.* **2009**, 428.
- [18] K.-J. Chen, M. A. Garcia, H. Wang, H.-R. Tseng, in *Supramolecular Chemistry: Supramolecular Nanoparticles for Molecular Diagnostics and Therapeutics*, John Wiley & Sons, Ltd, **2012**.
- [19] H. Gao, Q. He, *Expert Opin. Drug Deliv.* **2014**, 11, 409.
- [20] J.-H. Ryu, D.-J. Hong, M. Lee, *Chem. Commun.* **2008**, 1043.
- [21] Willis, Forssen, *Adv. Drug Deliv. Rev.* **1998**, 29, 249.
- [22] C. R. Safinya, K. K. Ewert, *Nature* **2012**, 489, 372.
- [23] A. Jesorka, O. Orwar, *Annu. Rev. Anal. Chem. Palo Alto Calif* **2008**, 1, 801.
- [24] R. Parhi, P. Suresh, *Curr. Drug Discov. Technol.* **2012**, 9, 2.
- [25] W. T. Al-Jamal, K. Kostarelos, *Acc. Chem. Res.* **2011**, 44, 1094.
- [26] S. Langereis, T. Geelen, H. Grull, G. J. Strijkers, K. Nicolay, *NMR Biomed.* **2013**, 26, 728.
- [27] S. Grinberg, C. Linder, E. Heldman, *Crit. Rev. Oncog.* **2014**, 19, 247.
- [28] W. J. M. Mulder, G. J. Strijkers, G. A. F. van Tilborg, D. P. Cormode, Z. A. Fayad, K. Nicolay, *Acc. Chem. Res.* **2009**, 42, 904.
- [29] J. D. Hartgerink, E. Beniash, S. I. Stupp, *Science* **2001**, 294, 1684.
- [30] H. Cui, M. J. Webber, S. I. Stupp, *Biopolymers* **2010**, 94, 1.
- [31] M. J. Webber, J. A. Kessler, S. I. Stupp, *J. Intern. Med.* **2010**, 267, 71.
- [32] J. B. Matson, S. I. Stupp, *Chem. Commun.* **2012**, 48, 26.
- [33] A.-H. Lu, E. L. Salabas, F. Schuth, *Angew. Chem. Int. Ed.* **2007**, 46, 1222.
- [34] C. Sun, J. S. H. Lee, M. Zhang, *Adv. Drug Deliv. Rev.* **2008**, 60, 1252.
- [35] S. R. Dave, X. Gao, *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **2009**, 1, 583.
- [36] J. B. Haun, T.-J. Yoon, H. Lee, R. Weissleder, *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **2010**, 2, 291.
- [37] R. Jin, B. Lin, D. Li, H. Ai, *Curr. Opin. Pharmacol.* **2014**, 18, 18.
- [38] X. Michalet, F. F. Pinaud, L. A. Bentolila, J. M. Tsay, S. Doose, J. J. Li, G. Sundaresan, A. M. Wu, S. S. Gambhir, S. Weiss, *Science* **2005**, 307, 538.
- [39] P. Zrazhevskiy, M. Sena, X. Gao, *Chem. Soc. Rev.* **2010**, 39, 4326.
- [40] M. Cheki, M. Moslehi, M. Assadi, *Eur. Rev. Med. Pharmacol. Sci.* **2013**, 17, 1141.
- [41] E. Petryayeva, W. R. Algar, I. L. Medintz, *Appl. Spectrosc.* **2013**, 67, 215.
- [42] J. L. Pelley, A. S. Daar, M. A. Saner, *Toxicol. Sci.* **2009**, 112, 276.
- [43] V. Biju, T. Itoh, M. Ishikawa, *Chem. Soc. Rev.* **2010**, 39, 3031.
- [44] V. N. Mehta, S. K. Kailasa, H.-F. Wu, *J. Nanosci. Nanotechnol.* **2014**, 14, 447.
- [45] W. J. M. Mulder, G. J. Strijkers, K. Nicolay, A. W. Griffioen, *Angiogenesis* **2010**, 13, 131.
- [46] Y. Zhu, H. Hong, Z. P. Xu, Z. Li, W. Cai, *Curr. Mol. Med.* **2013**, 13, 1549.
- [47] G. Xu, S. Mahajan, I. Roy, K.-T. Yong, *Front. Pharmacol.* **2013**, 4, 140.
- [48] R. Weissleder, M. J. Pittet, *Nature* **2008**, 452, 580.
- [49] J. Key, J. F. Leary, *Int. J. Nanomedicine* **2014**, 9, 711.
- [50] X. S. Xie, J. Yu, W. Y. Yang, *Science* **2006**, 312, 228.
- [51] B. N. G. Giepmans, S. R. Adams, M. H. Ellisman, R. Y. Tsien, *Science* **2006**, 312, 217.
- [52] X. Feng, L. Liu, S. Wang, D. Zhu, *Chem. Soc. Rev.* **2010**, 39, 2411.
- [53] K.-Y. Pu, B. Liu, *Adv. Funct. Mater.* **2011**, 21, 3408.
- [54] C. Zhu, L. Liu, Q. Yang, F. Lv, S. Wang, *Chem. Rev.* **2012**, 112, 4687.
- [55] L. Feng, C. Zhu, H. Yuan, L. Liu, F. Lv, S. Wang, *Chem. Soc. Rev.* **2013**, 42, 6620.
- [56] S. Rochat, T. M. Swager, *ACS Appl. Mater. Interfaces* **2013**, 5, 4488.
- [57] T. Kietzke, D. Neher, K. Landfester, R. Montenegro, R. Güntner, U. Scherf, *Nat. Mater.* **2003**, 2, 408.
- [58] J. Pecher, S. Mecking, *Chem. Rev.* **2010**, 110, 6260.
- [59] A. Kaeser, A. P. H. J. Schenning, *Adv. Mater.* **2010**, 22, 2985.
- [60] D. Tuncel, H. V. Demir, *Nanoscale* **2010**, 2, 484.
- [61] K. Li, B. Liu, *J. Mater. Chem.* **2011**, 22, 1257.
- [62] C. Wu, D. T. Chiu, *Angew. Chem. Int. Ed.* **2013**, 52, 3086.
- [63] C. Wu, C. Szymanski, Z. Cain, J. McNeill, *J. Am. Chem. Soc.* **2007**, 129, 12904.
- [64] C. Wu, B. Bull, C. Szymanski, K. Christensen, J. McNeill, *ACS Nano* **2008**, 2, 2415.
- [65] S. Huang, S. Liu, K. Wang, C. Yang, Y. Luo, Y. Zhang, B. Cao, Y. Kang, M. Wang, *Nanoscale* **2014**, 7, 889.
- [66] C. Wu, S. J. Hansen, Q. Hou, J. Yu, M. Zeigler, Y. Jin, D. R. Burnham, J. D. McNeill, J. M. Olson, D. T. Chiu, *Angew. Chem. Int. Ed.* **2011**, 50, 3430.
- [67] S. Vignaud, J.-P. Benoit, P. Saulnier, *Biomaterials* **2011**, 32, 8593.
- [68] X. Zhang, J. Yu, C. Wu, Y. Jin, Y. Rong, F. Ye, D. T. Chiu, *ACS Nano* **2012**, 6, 5429.
- [69] P. H.-L. Tran, T. T.-D. Tran, T. V. Vo, *J. Nanosci. Nanotechnol.* **2014**, 14, 815.
- [70] F. Canfarotta, M. J. Whitcombe, S. A. Piletsky, *Biotechnol. Adv.* **2013**, 31, 1585.

- [71] N. Han, Y. Y. Yang, S. Wang, S. Zheng, W. Fan, *Curr. Drug Metab.* **2013**, *14*, 661.
- [72] C. Wu, T. Schneider, M. Zeigler, J. Yu, P. G. Schiro, D. R. Burnham, J. D. McNeill, D. T. Chiu, *J. Am. Chem. Soc.* **2010**, *132*, 15410.
- [73] T. Aida, E. W. Meijer, S. I. Stupp, *Science* **2012**, *335*, 813.
- [74] L. Albertazzi, D. van der Zwaag, C. M. A. Leenders, R. Fitzner, R. W. van der Hofstad, E. W. Meijer, *Science* **2014**, *344*, 491.
- [75] D. Görl, X. Zhang, F. Würthner, *Angew. Chem. Int. Ed.* **2012**, *51*, 6328.
- [76] I. Fischer, A. P. H. J. Schenning, in *Organic Electronics* (Eds: F. Cicoira, C. Santato), Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany **2013**, pp. 1–25.
- [77] K. Petkau-Milroy, M. H. Sonntag, L. Brunsveld, *Chem. Eur. J.* **2013**, *19*, 10786.
- [78] T. Kowada, H. Maeda, K. Kikuchi, *Chem. Soc. Rev.* **2015**, DOI: 10.1039/C5CS00030K.
- [79] N. K. Allampally, A. Florian, M. J. Mayoral, C. Rest, V. Stepanenko, G. Fernández, *Chem. Eur. J.* **2014**, *20*, 10669.
- [80] L. Yang, G. Fan, X. Ren, L. Zhao, J. Wang, Z. Chen, *Phys. Chem. Chem. Phys.* **2015**, *17*, 9167.
- [81] P. Anees, S. Sreejith, A. Ajayaghosh, *J. Am. Chem. Soc.* **2014**, *136*, 13233.
- [82] D. Zhang, Y.-X. Zhao, Z.-Y. Qiao, U. Mayerhöffer, P. Spenst, X.-J. Li, F. Würthner, H. Wang, *Bioconj. Chem.* **2014**, *25*, 2021.
- [83] R. Abbel, A. P. H. J. Schenning, E. W. Meijer, *J. Polym. Sci. Part Polym. Chem.* **2009**, *47*, 4215.
- [84] A. Kaeser, I. Fischer, R. Abbel, P. Besenius, D. Dasgupta, M. A. J. Gillisen, G. Portale, A. L. Stevens, L. M. Herz, A. P. H. J. Schenning, *ACS Nano* **2013**, *7*, 408.
- [85] Y. Koizumi, S. Seki, S. Tsukuda, S. Sakamoto, S. Tagawa, *J. Am. Chem. Soc.* **2006**, *128*, 9036.
- [86] L. Zhu, C. Yang, J. Qin, *Chem. Commun.* **2008**, 6303.
- [87] R. Abbel, R. van der Weegen, E. W. Meijer, A. P. H. J. Schenning, *Chem. Commun.* **2009**, 1697.
- [88] R. Abbel, C. Grenier, M. J. Pouderoijen, J. W. Stouwdam, P. E. L. G. Leclère, R. P. Sijbesma, E. W. Meijer, A. P. H. J. Schenning, *J. Am. Chem. Soc.* **2009**, *131*, 833.
- [89] A. L. Stevens, A. Kaeser, A. P. H. J. Schenning, L. M. Herz, *ACS Nano* **2012**, *6*, 4777.
- [90] K.-P. Tseng, F.-C. Fang, J.-J. Shyue, K.-T. Wong, G. Raffy, A. Del Guerso, D. M. Bassani, *Angew. Chem. Int. Ed.* **2011**, *50*, 7032.
- [91] C. Vijayakumar, K. Sugiyasu, M. Takeuchi, *Chem. Sci.* **2011**, *2*, 291.
- [92] B. Balan, C. Vijayakumar, S. Ogi, M. Takeuchi, *J. Mater. Chem.* **2012**, *22*, 11224.
- [93] K. Petkau, A. Kaeser, I. Fischer, L. Brunsveld, A. P. H. J. Schenning, *J. Am. Chem. Soc.* **2011**, *133*, 17063.
- [94] J. Suk, J.-Z. Cheng, K.-T. Wong, A. J. Bard, *J. Phys. Chem. C* **2011**, *115*, 14960.
- [95] I. Fischer, K. Petkau-Milroy, Y. L. Dorland, A. P. H. J. Schenning, L. Brunsveld, *Chem. Eur. J.* **2013**, *19*, 16646.
- [96] M. Kosuge, T. Takeuchi, I. Nakase, A. T. Jones, S. Futaki, *Bioconj. Chem.* **2008**, *19*, 656.
- [97] F. Tang, C. Wang, J. Wang, X. Wang, L. Li, *ACS Appl. Mater. Interfaces* **2014**, *6*, 18337.
- [98] I. Fischer, A. Kaeser, M. A. M. Peters-Gumbs, A. P. H. J. Schenning, *Chem. Eur. J.* **2013**, *19*, 10928.
- [99] J. Pennakalathil, E. Jahja, E. S. Özdemir, Ö. Konu, D. Tuncel, *Biomacromolecules* **2014**, *15*, 3366.
- [100] W. E. Ford, *J. Photochem.* **1986**, *34*, 43.
- [101] T. Weil, T. Vosch, J. Hofkens, K. Peneva, K. Müllen, *Angew. Chem. Int. Ed.* **2010**, *49*, 9068.
- [102] J. Qu, C. Kohl, M. Pottekk, K. Müllen, *Angew. Chem. Int. Ed.* **2004**, *43*, 1528.
- [103] S. K. Yang, X. Shi, S. Park, S. Doganay, T. Ha, S. C. Zimmerman, *J. Am. Chem. Soc.* **2011**, *133*, 9964.
- [104] Z. Chen, A. Lohr, C. R. Saha-Möller, F. Würthner, *Chem. Soc. Rev.* **2009**, *38*, 564.
- [105] Z. Chen, B. Fimmel, F. Würthner, *Org. Biomol. Chem.* **2012**, *10*, 5845.
- [106] T. Heek, C. Fasting, C. Rest, X. Zhang, F. Würthner, R. Haag, *Chem. Commun.* **2010**, *46*, 1884.
- [107] J. Seibt, T. Winkler, K. Renziehausen, V. Dehm, F. Würthner, H.-D. Meyer, V. Engel, *J. Phys. Chem. A* **2009**, *113*, 13475.
- [108] B. Gao, H. Li, H. Liu, L. Zhang, Q. Bai, X. Ba, *Chem. Commun.* **2011**, *47*, 3894.
- [109] H. Liu, Y. Wang, C. Liu, H. Li, B. Gao, L. Zhang, F. Bo, Q. Bai, X. Ba, *J. Mater. Chem.* **2012**, *22*, 6176.
- [110] C. D. Schmidt, C. Böttcher, A. Hirsch, *Eur. J. Org. Chem.* **2007**, *2007*, 5497.
- [111] C. D. Schmidt, C. Böttcher, A. Hirsch, *Eur. J. Org. Chem.* **2009**, *2009*, 5337.
- [112] C. Backes, C. D. Schmidt, K. Rosenlehner, F. Hauke, J. N. Coleman, A. Hirsch, *Adv. Mater.* **2010**, *22*, 788.
- [113] C. Backes, F. Hauke, A. Hirsch, *Adv. Mater.* **2011**, *23*, 2588.
- [114] Y. Huang, B. Quan, Z. Wei, G. Liu, L. Sun, *J. Phys. Chem. C* **2009**, *113*, 3929.
- [115] S. Rehm, V. Stepanenko, X. Zhang, T. H. Rehm, F. Würthner, *Chem. Eur. J.* **2010**, *16*, 3372.
- [116] K.-R. Wang, D.-S. Guo, B.-P. Jiang, Z.-H. Sun, Y. Liu, *J. Phys. Chem. B* **2010**, *114*, 101.
- [117] J. T. Kern, S. M. Kerwin, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3395.
- [118] E. Micheli, D. D'Ambrosio, M. Franceschin, M. Savino, *Mini Rev. Med. Chem.* **2009**, *9*, 1622.
- [119] T. H. Rehm, M. R. Stojković, S. Rehm, M. Škugor, I. Piantanida, F. Würthner, *Chem. Sci.* **2012**, *3*, 3393.
- [120] M. Marcia, P. Singh, F. Hauke, M. Maggini, A. Hirsch, *Org. Biomol. Chem.* **2014**, *12*, 7045.
- [121] B.-P. Jiang, D.-S. Guo, Y. Liu, *J. Org. Chem.* **2010**, *75*, 7258.
- [122] Y. Huang, J. Hu, W. Kuang, Z. Wei, C. F. J. Faul, *Chem. Commun.* **2011**, *47*, 5554.
- [123] Z. Chen, V. Stepanenko, V. Dehm, P. Prins, L. D. A. Siebbeles, J. Seibt, P. Marquetand, V. Engel, F. Würthner, *Chem. Eur. J.* **2007**, *13*, 436.
- [124] X. Zhang, Z. Chen, F. Würthner, *J. Am. Chem. Soc.* **2007**, *129*, 4886.
- [125] X. Zhang, D. Görl, V. Stepanenko, F. Würthner, *Angew. Chem. Int. Ed.* **2014**, *53*, 1270.
- [126] X. Zhang, S. Rehm, M. M. Safont-Sempere, F. Würthner, *Nat. Chem.* **2009**, *1*, 623.
- [127] X. Zhang, D. Görl, F. Würthner, *Chem. Commun.* **2013**, *49*, 8178.
- [128] J. Baram, E. Shirman, N. Ben-Shitrit, A. Ustinov, H. Weissman, I. Pinkas, S. G. Wolf, B. Rybtchinski, *J. Am. Chem. Soc.* **2008**, *130*, 14966.
- [129] E. Krieg, E. Shirman, H. Weissman, E. Shimoni, S. G. Wolf, I. Pinkas, B. Rybtchinski, *J. Am. Chem. Soc.* **2009**, *131*, 14365.
- [130] H. Weissman, A. Ustinov, E. Shimoni, S. R. Cohen, B. Rybtchinski, *Polym. Adv. Technol.* **2011**, *22*, 133.
- [131] A. Ustinov, H. Weissman, E. Shirman, I. Pinkas, X. Zuo, B. Rybtchinski, *J. Am. Chem. Soc.* **2011**, *133*, 16201.
- [132] Y. Tidhar, H. Weissman, S. G. Wolf, A. Gulino, B. Rybtchinski, *Chem. Eur. J.* **2011**, *17*, 6068.

- [133] E. Krieg, H. Weissman, E. Shirman, E. Shimoni, B. Rybtchinski, *Nat. Nanotechnol.* **2011**, *6*, 141.
- [134] E. Krieg, S. Albeck, H. Weissman, E. Shimoni, B. Rybtchinski, *PLoS ONE* **2013**, *8*, e63188.
- [135] M. Montalti, G. Battistelli, A. Cantelli, D. Genovese, *Chem. Commun.* **2014**, *50*, 5326.
- [136] T. Heek, J. Nikolaus, R. Schwarzer, C. Fasting, P. Welker, K. Licha, A. Herrmann, R. Haag, *Bioconjug. Chem.* **2013**, *24*, 153.
- [137] A. Jana, K. S. P. Devi, T. K. Maiti, N. D. P. Singh, *J. Am. Chem. Soc.* **2012**, *134*, 7656.
- [138] A. Jana, K. T. Nguyen, X. Li, P. Zhu, N. S. Tan, H. Ågren, Y. Zhao, *ACS Nano* **2014**, *8*, 5939.