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Technology Offer

Nanographene-based dyes as high performance probes for super-resolution microscopy

Ref.-Nos.: MI 0903-5658-LC; MI 0903-5696-LC

A group of scientists at the MPI for Polymer Research in Mainz developed nano-graphene structures that can be used as a new class of fluorophores for super-resolution microscopy (SMLM and STED).

They perform like hybrids of organic dyes and QDs: they have excellent environment-independent blinking properties, emit high photon numbers, display high photo-stability, possess low toxicity, have a molecular size below 2 nm, and have narrow excitation and emission spectra.

These nanographene structures are large polycyclic aromatic hydrocarbons obtained by means of organic "bottom-up" synthesis methods, which enables us to modify them with biolinking moieties or with hydrophilic moieties to enhance the water solubility of the dyes.

Background

Super-resolution microscopy critically relies on the availability of appropriate fluorophores. For single-molecule localization microscopy (SMLM), fluorophores are needed that stochastically switch between bright (on) and dark (off) states. This phenomenon is also called fluorescence blinking. For stimulated emission depletion microscopy (STED) the dyes must be photo-switchable.

Nowadays, four classes of fluorophores are used in such applications: small molecule organic dyes, photo-activatable/switchable fluorescent protein, inorganic quantum dots (QDs) and carbon dots (CDs).

Small molecule organic dyes, e.g., ThermoFisher Scientific Alexa Fluor® 647, have excellent blinking properties and emit high number of photons, but require specialized buffers for their use. This restricts their use to the imaging of fixed biological samples.

Photo-activatable/switchable fluorescent proteins have good excellent target molecule specificity but have the issue of low photon numbers resulting in a lower localization precision compared to organic dyes.

The size of QDs (~10 nm) and their unfavorable blinking properties limit their function as reliable molecular probes. Furthermore, QDs contain heavy metals, which limits their applicability in biomedicine due to toxicity.

CDs show environment-independent blinking properties, but their undefined amorphous carbon structure makes the specific coupling to biological samples difficult and leads to unfavorable broad emission spectra.

Recently graphene quantum dots (GQDs) obtained by means of "top-down" synthesis methods were proposed as another class of superior photo-switchable fluorophores due to their small size (<10 nm) and low toxicity. However, the emission spectra of GQDs are typically very broad due to their undefined chemical structures, and their blinking properties are also not ideal for SMLM applications.

Technology

Here nanographene structures are introduced as a new class of fluorophores for super-resolution fluorescent microscopy that overcome the limitations described above. These nanographene structures are large polycyclic aromatic hydrocarbons obtained by means of organic "bottom-up" synthesis methods. They perform like hybrids of organic dyes and QDs. They have excellent environment-independent blinking properties, emit high photon numbers, display high photostability, possess low toxicity, have a molecular size below 2 nm, and have

Max-Planck-Innovation GmbH
Amalienstr. 33
80799 München
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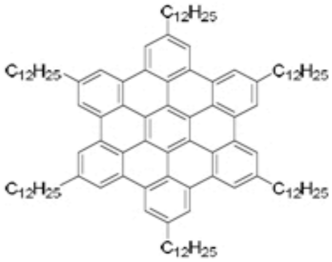
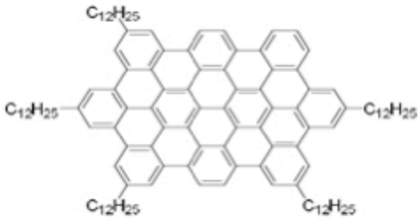
narrow excitation and emission spectra. These features make nanographenes ideal candidates for SMLM. but also for STED microscopy.

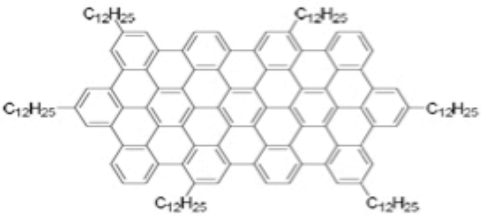


DBOV



C60

DMEP



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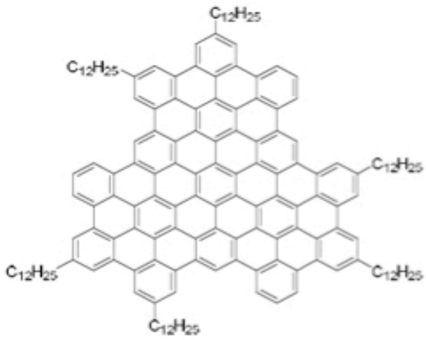

C96

Dye	Excitation maximum (nm)	Emission maximum (nm)	Extinction ($M^{-1}cm^{-1}$)	Quantum yield	Detected photons/ switching event	Duty cycle (x 10^{-4})	Blinking time (ms)
Alexa 647	650	665	239,000	0.33	3438	2.1	65
DBOV	610	614	70, 000	0.79	5570[a]	4.7[a]	108[a]
C60	412	701	22,000	0.1	4960[a]	1.2[a]	79[a]
DMEP	466	518	51,000		6164		102
C78	310	513	54,000	0.02	5740[b]	2.7[b]	83[b]
C96	491	650	61,000	0.01	5020[b]	1.7[b]	94[b]

[a] in air; [b] in polystyrene

The “bottom-up” method allows the synthesis of nanographenes with well-defined absorption and emission spectra due to their precise chemical structure, enables the introduction of various functional groups for binding to specific ligands, reacting to specific target molecules or allowing water solubility.

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80799 München
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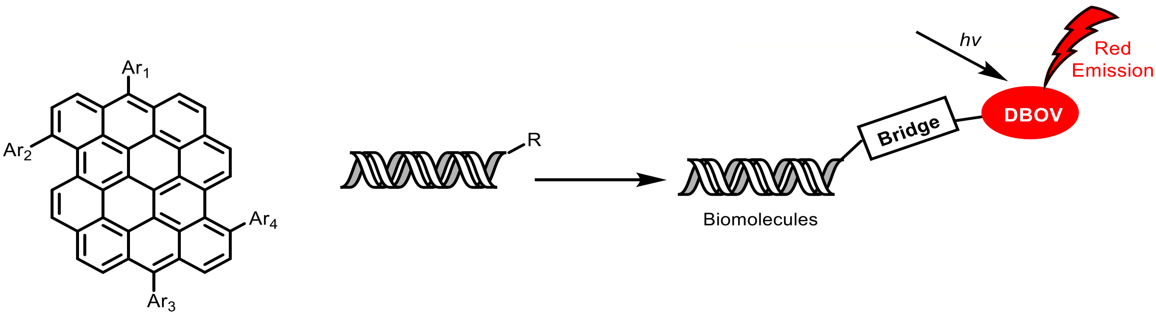

Figure 2.: Biolinkable GQDs - one Ar-group is a clickable functional group, the others can be water-soluble functional groups.

In particular DBOV-Mes has very narrow absorption and emission spectra compared to commonly used organic dyes while being water soluble, making this nanographene ideal for multi-color imaging
COSEM can naturally be adapted to alternative target organisms based on their tRNA pools for exploratory analyses. The full algorithm can then be trained and validated for these new organisms with a dataset of measured protein expression levels.

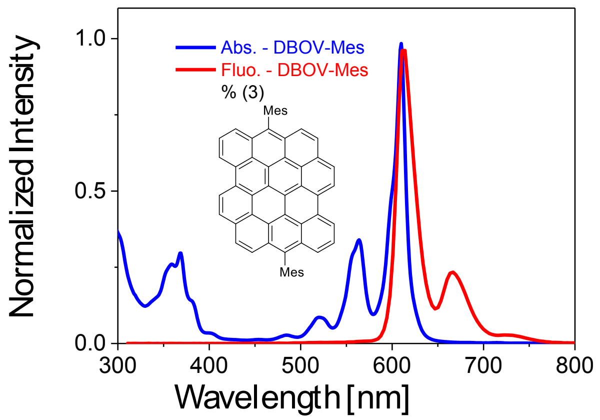

Figure 3. Absorption and emission and chemical structure of DBVO-Mes

Patent Information

- MI0903-5658: "Use of a substituted or unsubstituted polycyclic aromatic hydro-carbon compounds for high-resolution microscopy", EP priority application filed in October 2018, PCT application filed in October 2019.
- MI0903-5696: "Hydrophilic and particularly water soluble DBOV-derivatives", EP priority application filed in October 2018, PCT application filed in October 2019.

Literature

X. Liu, S-Y Chen, Q. Chen, X. Yao, M. Gelléri, S. Ritz, S. Kumar, C. Cremer, K. Landfester, K. Müllen, S. Parekh, A. Narita, M. Bonn: "Nanographenes: ultrastable, switchable, and bright probes for super-resolution microscopy", Angewandte Chemie, Published online 28 October 2019, https://doi.org/10.1002/ange.201909220

Contact

Dr Lars Cuypers
Senior Patent- & License Manager
Chemist

Phone: +49 (0)89 / 29 09 19 - 21
eMail: cuypers@max-planck-innovation.de

Max-Planck-Innovation GmbH
Amalienstr. 33
80799 München
www.max-planck-innovation.de