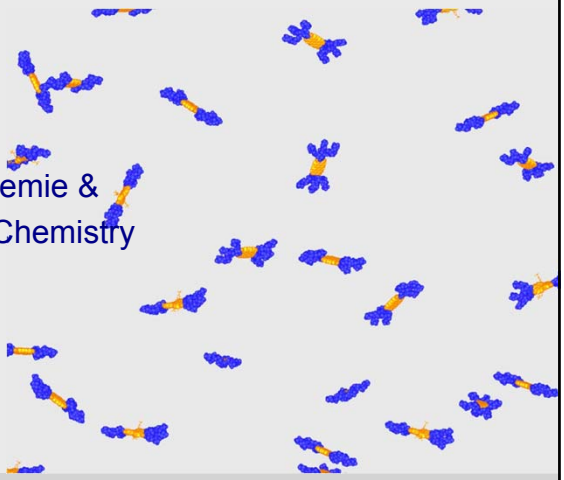
Julius-Maximilians-
UNIVERSITÄT
WÜRZBURG

Center for Nanosystems
Chemistry

Self-assembly of Perylene Dyes in Water and Interactions with DNA and RNA

Frank Würthner
Institut für Organische Chemie &
Center for Nanosystems Chemistry
Universität Würzburg



Outline

Self-assembly of Perylene Dyes in Water and Interactions with DNA and RNA

Basics of PBI self-assembly into π-stacks

PBI self-assembly into π-stacks in water

Micelles & vesicles: from structure to function

PBI Interactions with DNA and RNA

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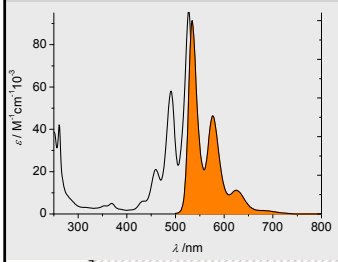
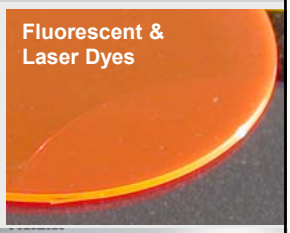
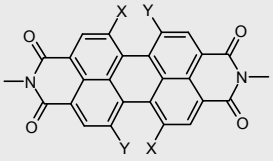
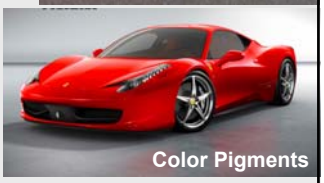
Perylene Bisimides: A Versatile Class of Dyes

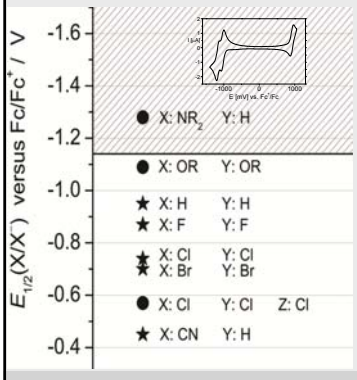


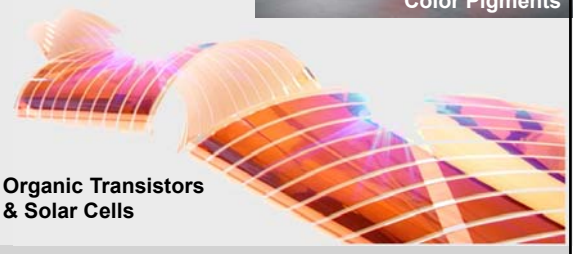
- **Intense Absorption**
- **Fluorescence Φ_f = 100 %**
- **Reversible Redox Processes**
- **(Photo-)Stability**
- **n-type Semiconductivity**







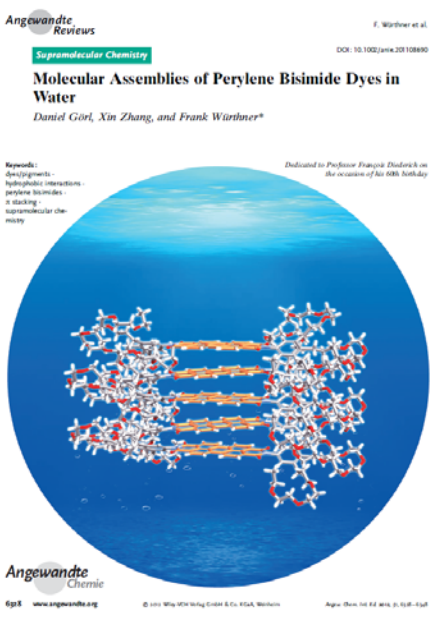




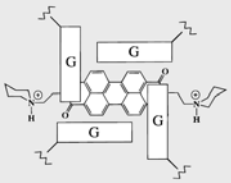
Organic Transistors & Solar Cells

Reviews: *Chem. Commun.* **2004**, 1564 & *Chem. Commun.* **2011**, *47*, 5109

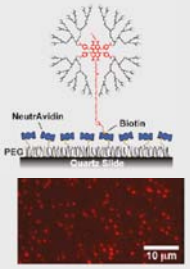
Perylene Bisimides in Water



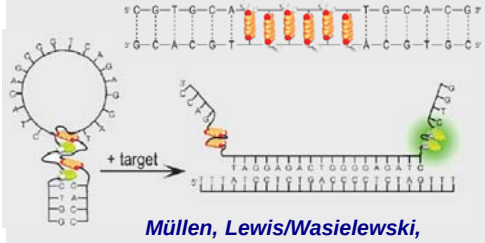
G Quartet Binders



Single Molecule Bioimaging



Molecular Probes

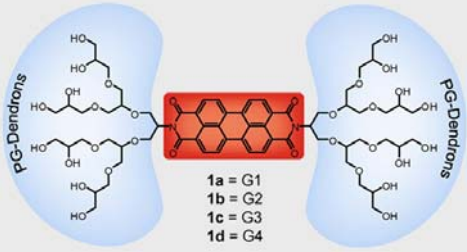


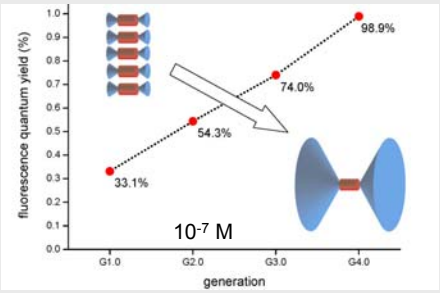
Müllen, Lewis/Wasielewski, Wagenknecht, Häner, Faul, Savino, Rybtchinski, Haag, Zimmerman, ...

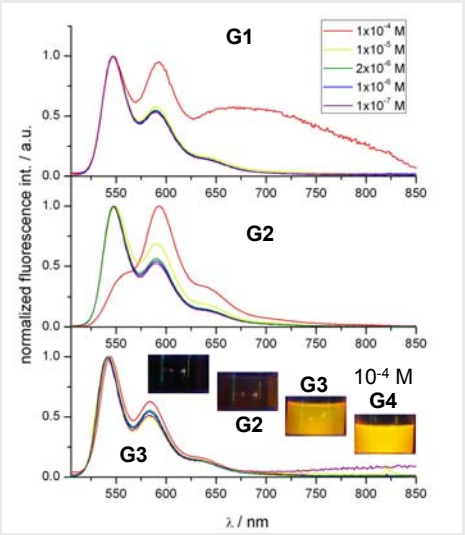
Reviews: *Angew. Chem. Int. Ed.* **2012**, *51*, 6328

Perylene Bisimides: Fluorescence in Water

with Rainer Haag, FU Berlin







Chem. Commun. **2010**, *46*, 1884

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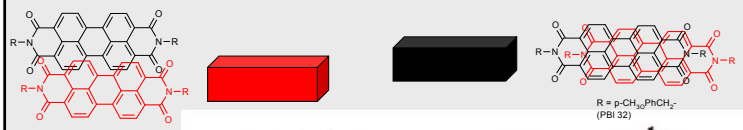
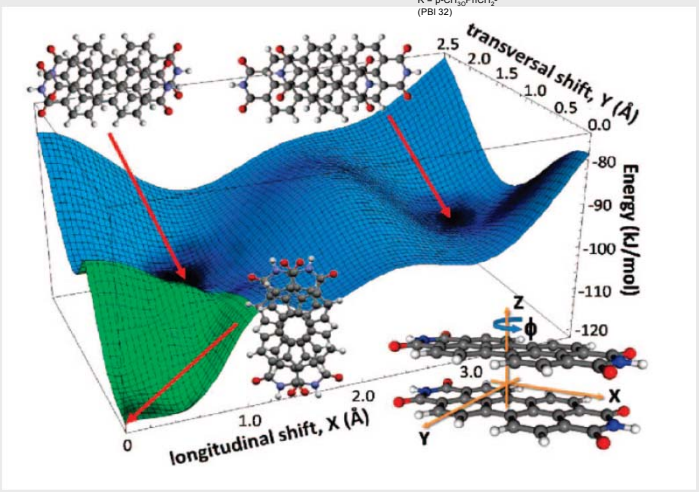
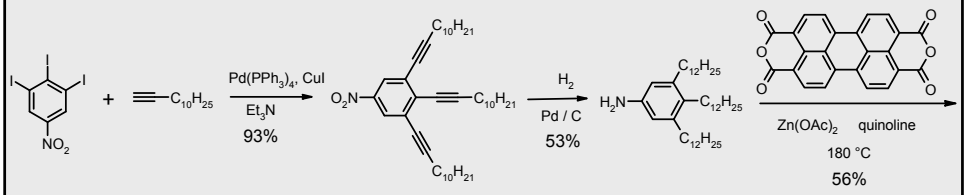
Ground State Potential Energy Surfaces (DFT-D)

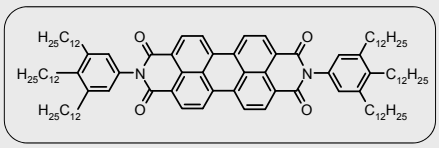


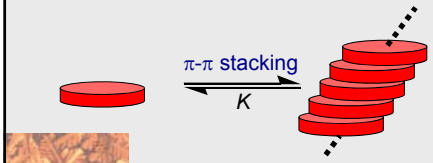


J. Am. Chem. Soc. **2008**, *130*, 12858

Fluorescent Perylene Dye Aggregates





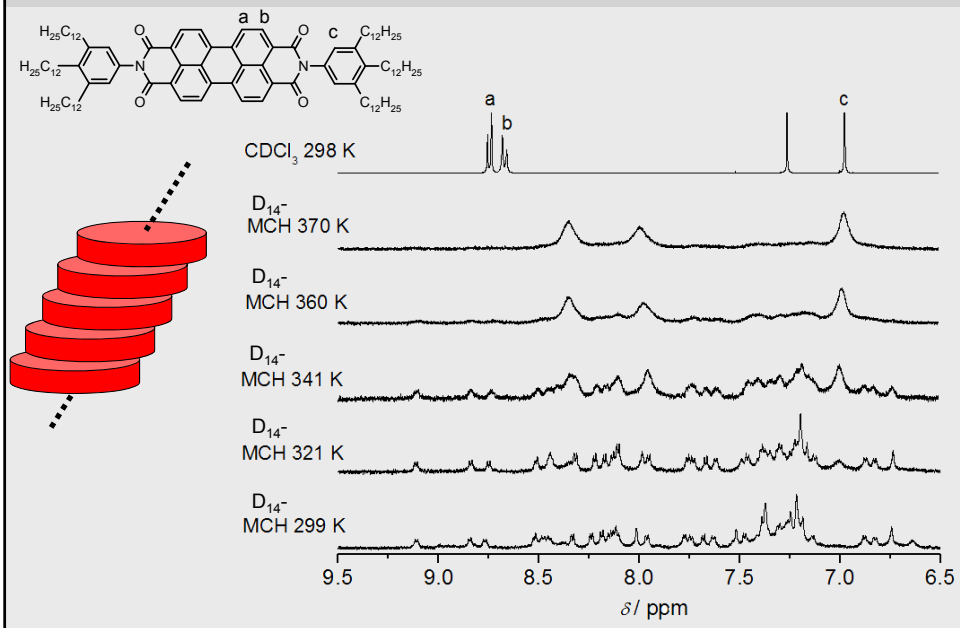


in toluene

10^{-6} M 10^{-4} M 10^{-2} M

Chem. Commun. **2006**, 1188

Aggregate Structure: NMR Spectroscopy?



Chem. Eur. J. **2007**, *13*, 443

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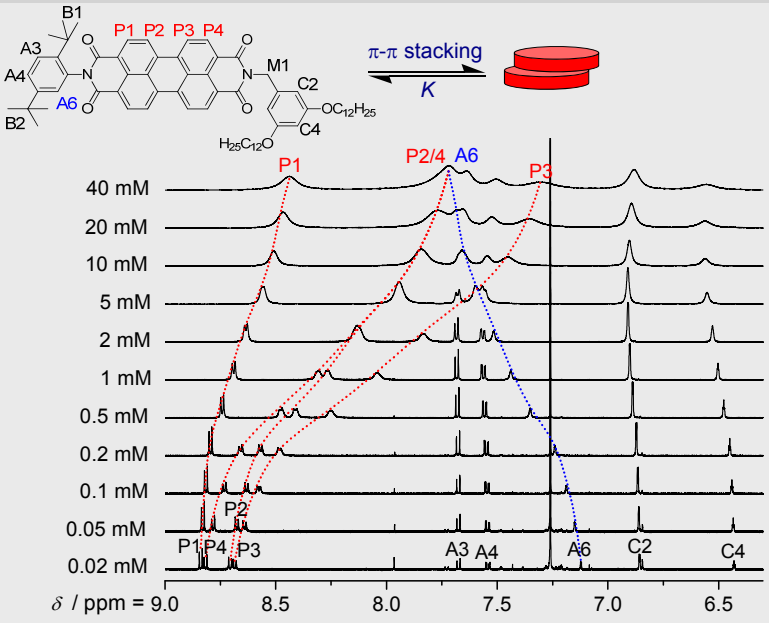
A Dimer Model Compound



Chem. Eur. J. **2012**, *18*, 13665

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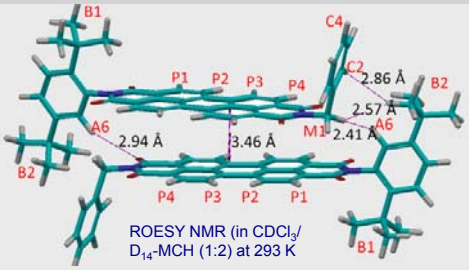
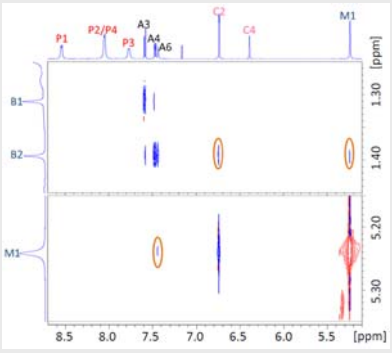
A Dimer Model Compound



ROESY NMR (in $CDCl_3$/ D_{14}-MCH (1:2) at 293 K



Chem. Eur. J. **2012**, *18*, 13665

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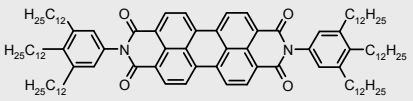
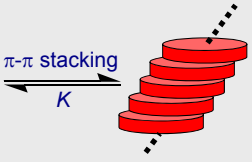
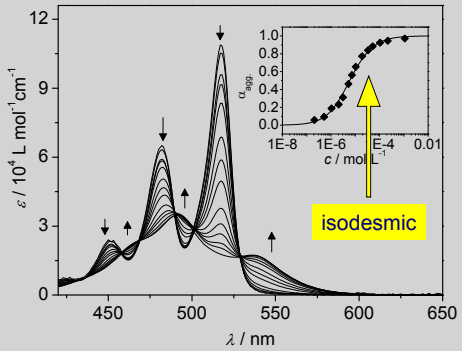
Spectral Changes upon Perylene Bisimide Aggregation





Concentration dependent UV/Vis spectra in methylcyclohexane



K = 10^6 M^{-1} (*n*-hexane)

K = 10^5 M^{-1} (methylcyclohexane)

K = 600 M^{-1} (toluene)

Average molecular weight & aggregation number at different temperatures from VPO measurements.

(MCH 5 x10^{-3} M)	40 °C	50 °C	60 °C
M / g mol^{-1}	14280	11490	8690
N	9	7	6

Chem. Eur. J. **2007**, *13*, 443

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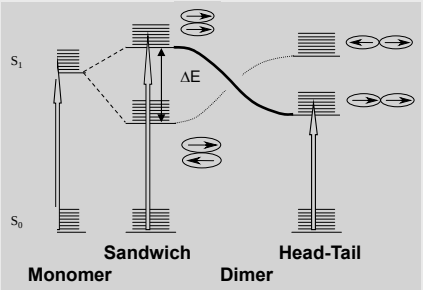
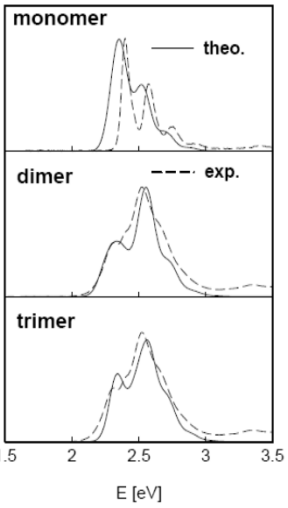
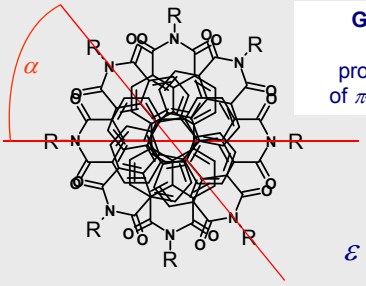
Theoretical Description of Spectral Changes



Calculated spectra of dye aggregates

V. Engel, Würzburg: quantum dynamical calculations based on multiconfigurational time-dependent Hartree (MCTDH method)





GRK 1221: Control of electronic properties of aggregates of π-conjugated molecules

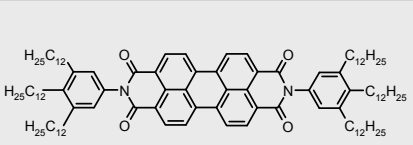
$\alpha = 28\ °$

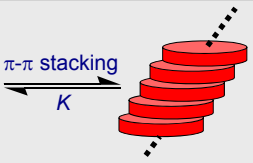
$d = 3.4$ Å

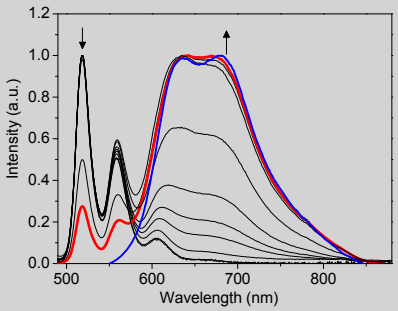
$\varepsilon = 0.065$ eV

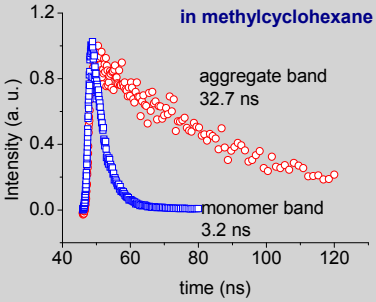
Chem. Phys. **2006**, 328, 354 *J. Chem. Phys.* **2007**, *126*, 164308

Time-Resolved Fluorescence Spectrocopy







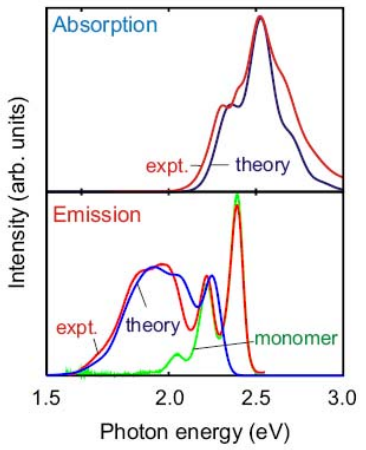


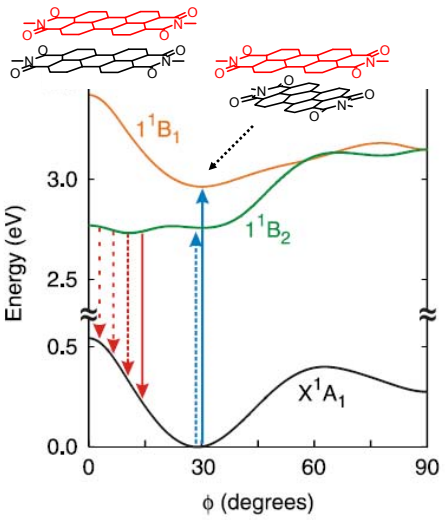
Chem. Eur. J. **2007**, *13*, 443

Theoretical Description of Spectral Changes

Collaboration in GK 1221 with B. Engels & V. Engel

TD-HFT calculation of excited state potential energy surface*





*) Dispersion interactions embedded as ground state interaction

J. Am. Chem. Soc. **2008**, *130*, 12858

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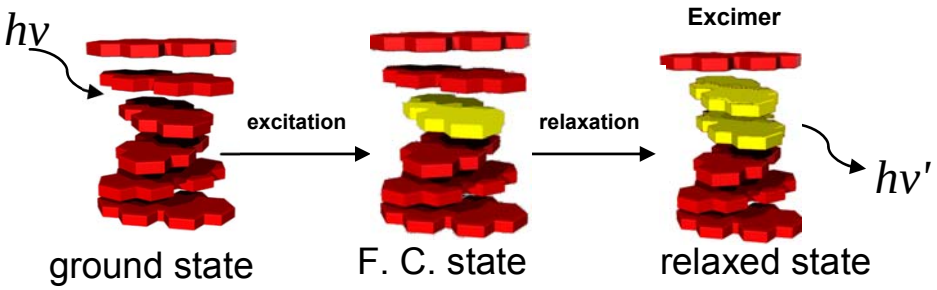
Consequence for Fluorescence & Exciton Transport



- **localization of exciton**
- **unfavorable for applications in OLEDs or solar cells („exciton trap“)**
- **fluorescence lifetime increased, but quantum yield decreased**

Chem. Eur. J. **2007**, *13*, 443

J. Am. Chem. Soc. **2008**, *130*, 12858

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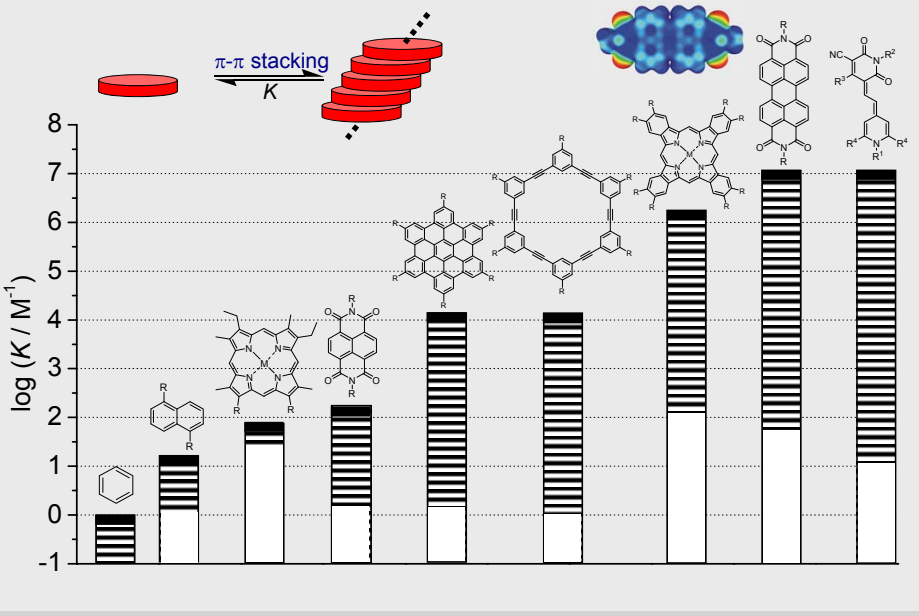
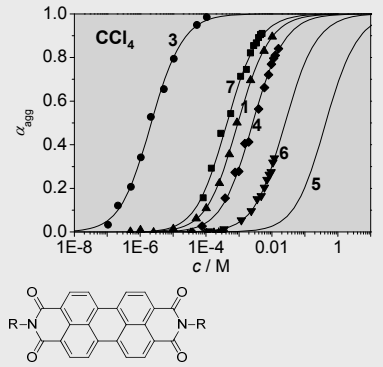
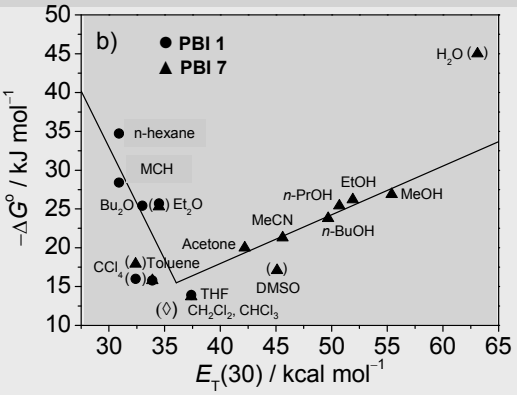
Thermodynamics for π–π–Stacking of Dyes

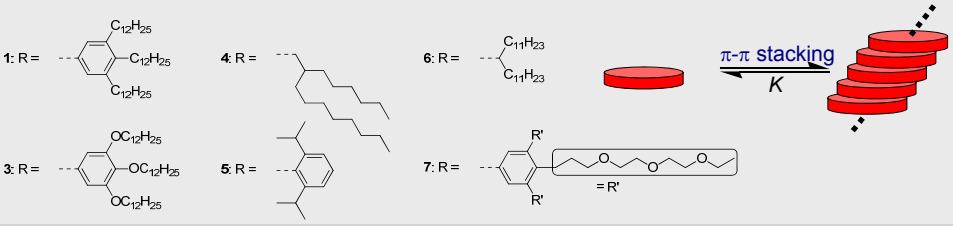


Chem. Soc. Rev. **2009**, *38*, 564

Substituent & Solvent-dependent Binding Constants







Org. Biomol. Chem. **2012**, *10*, 5845

Angew. Chem. Int. Ed. **2012**, *51*, 6328

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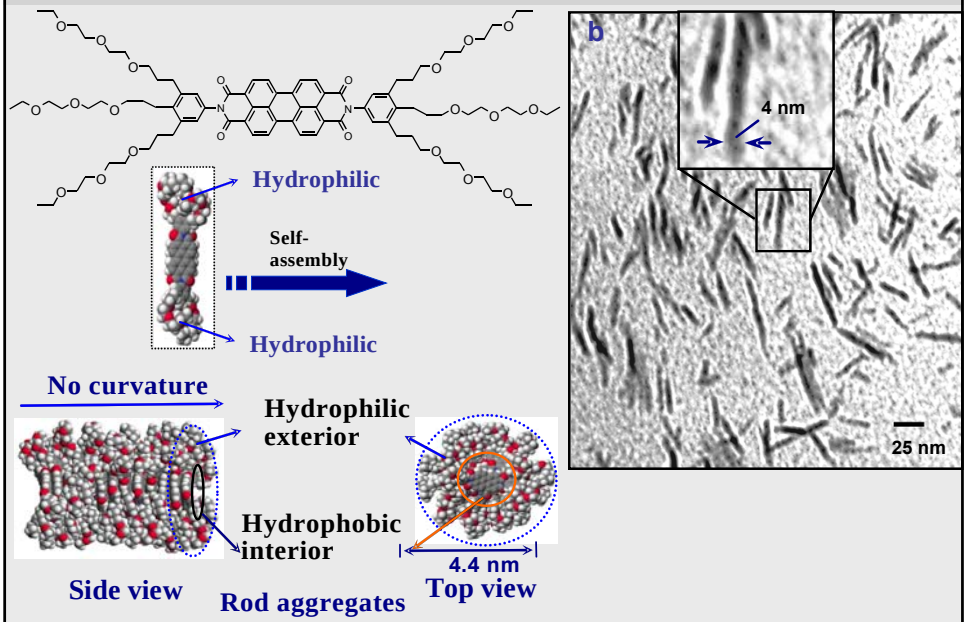
Perylene Bisimide Aggregation in Water



J. Am. Chem. Soc. **2007**, *129*, 4886

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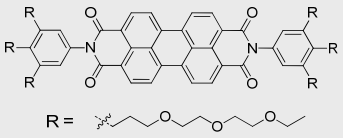
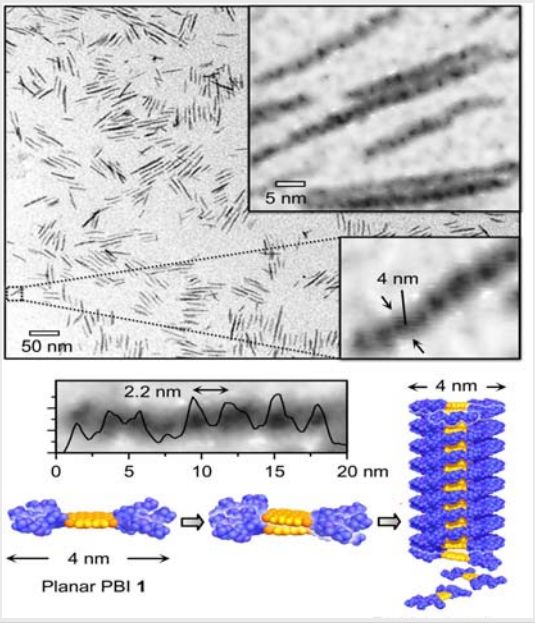
Hierarchical Growth by Nanorod Fusion



0.077 mg/mL in water



Angew. Chem. Int. Ed. **2014**, *53*, 1270

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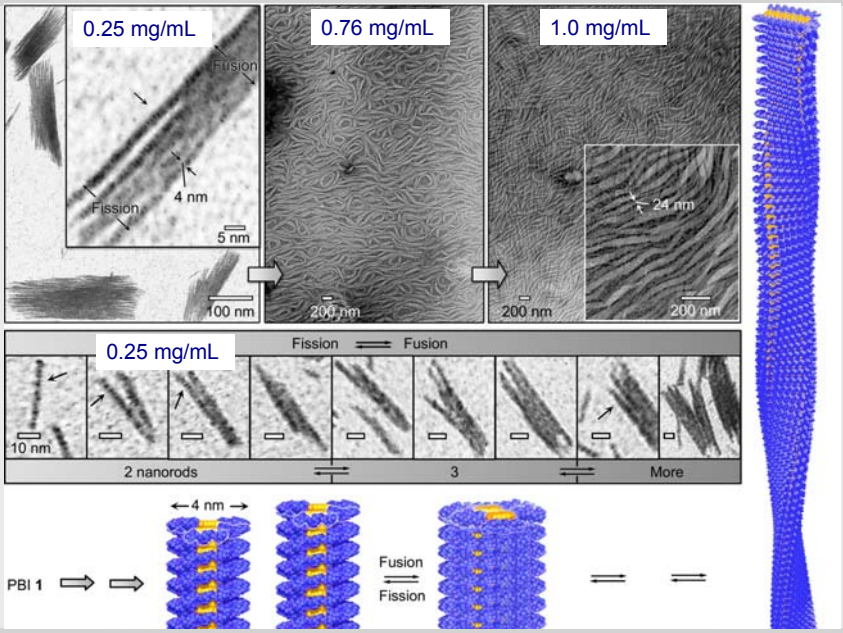
Hierarchical Growth by Nanorod Fusion



Angew. Chem. Int. Ed. **2014**, *53*, 1270

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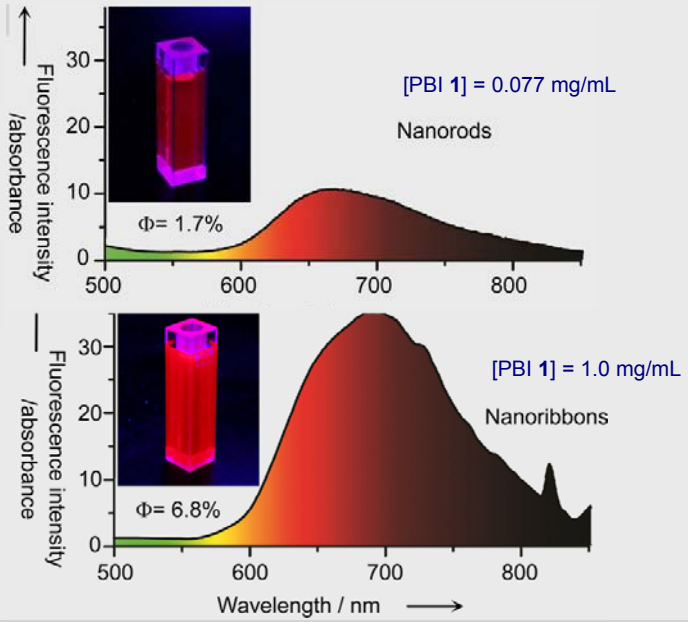
Hierarchical Growth by Nanorod Fusion



Angew. Chem. Int. Ed. **2014**, *53*, 1270

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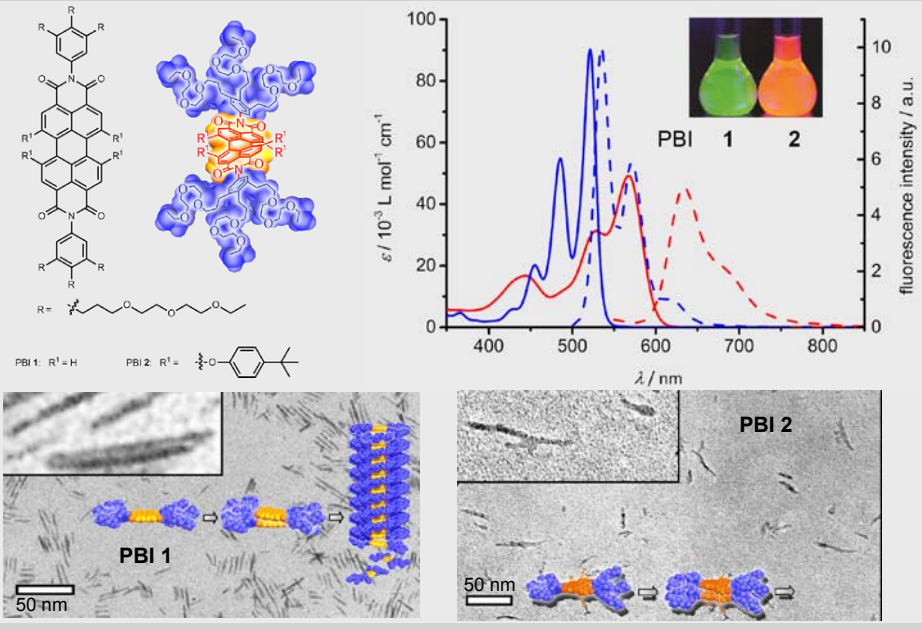
Core-twisted Perylene Bisimide Amphiphiles



Nature Commun. **2015**, *6*, 8009

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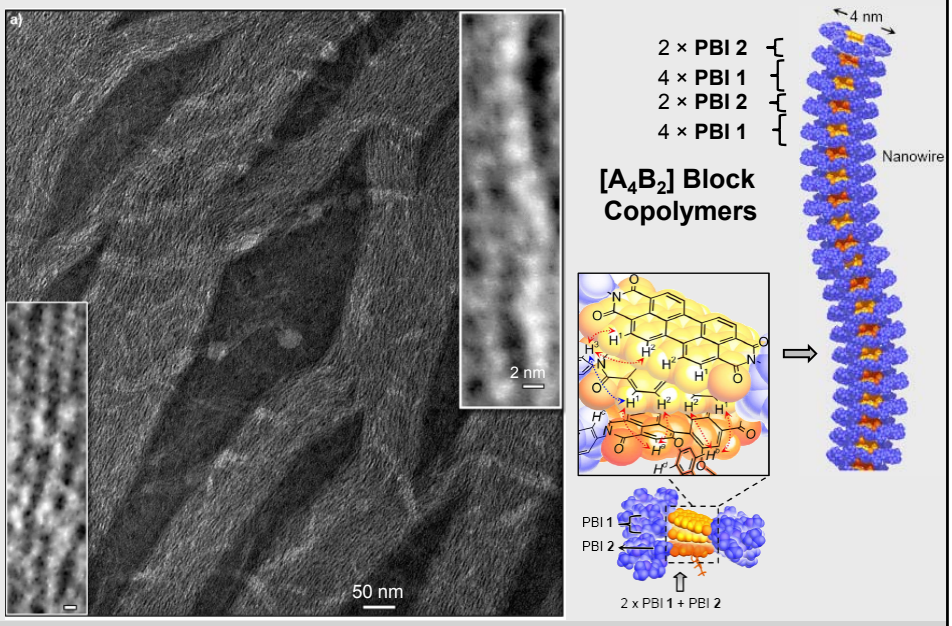
Supramolecular Block Copolymers



Nature Commun. **2015**, *6*, 8009

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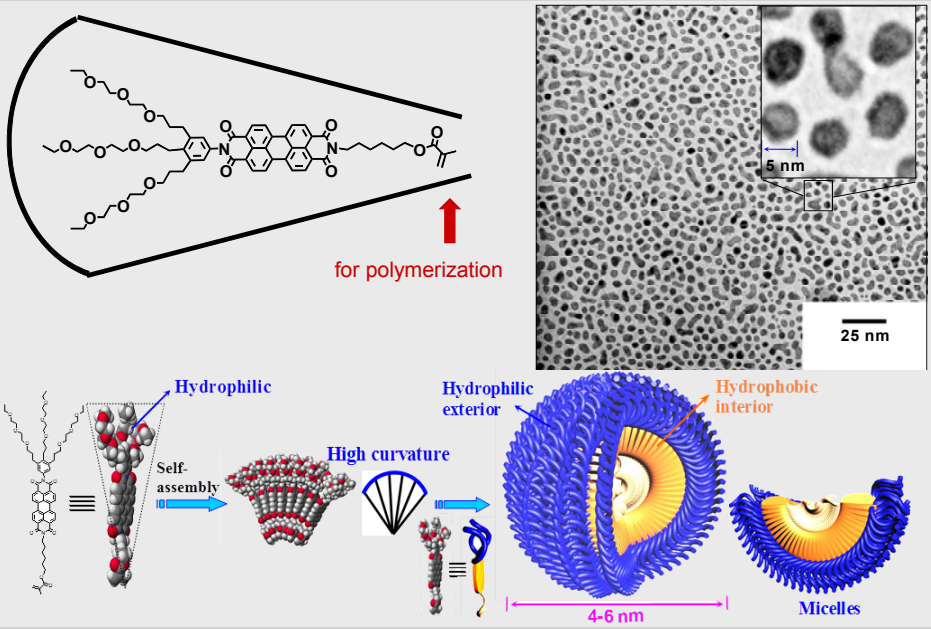
Micelle Formation from Wedge-shaped Dyes



J. Am. Chem. Soc. **2007**, *129*, 4886

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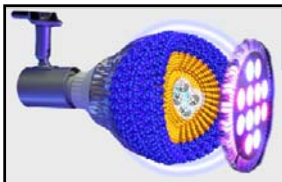
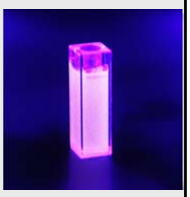
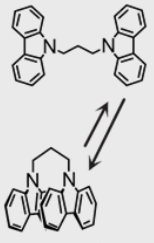
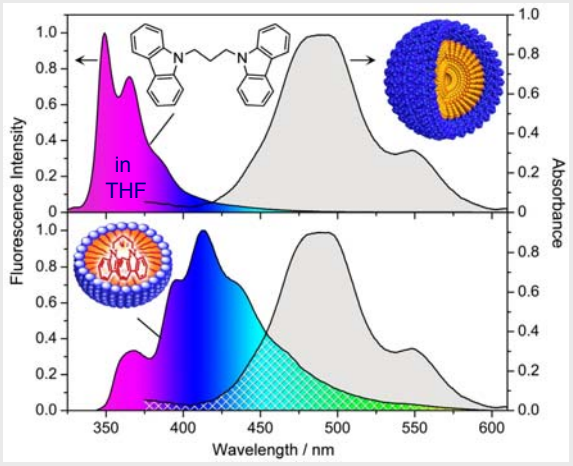
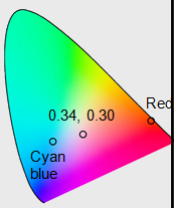
"Nanobulb" White Light Emitting Micelles











Chem. Commun. **2013**, *49*, 8178

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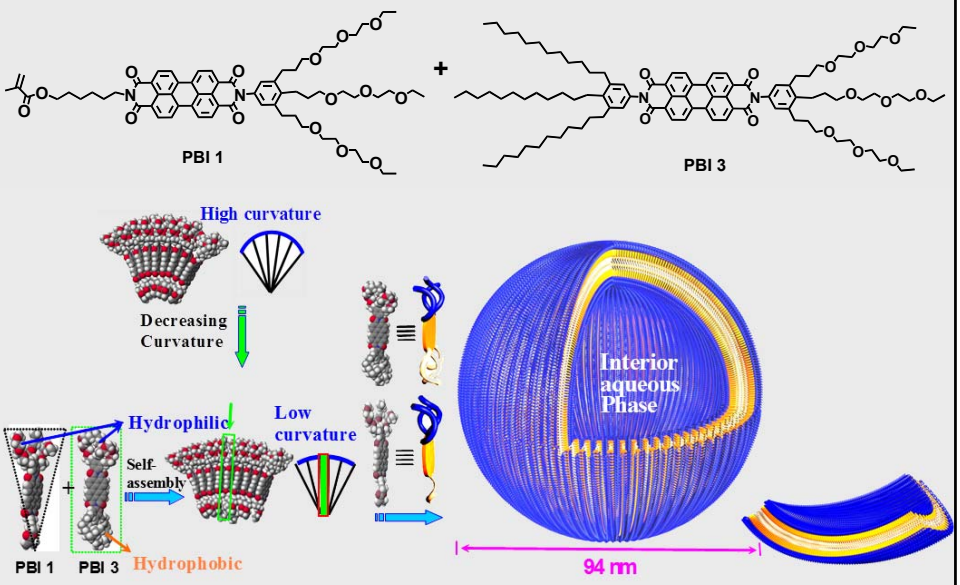
Vesicle Formation from Wedge & Dumbell-shaped Dyes



J. Am. Chem. Soc. **2007**, *129*, 4886

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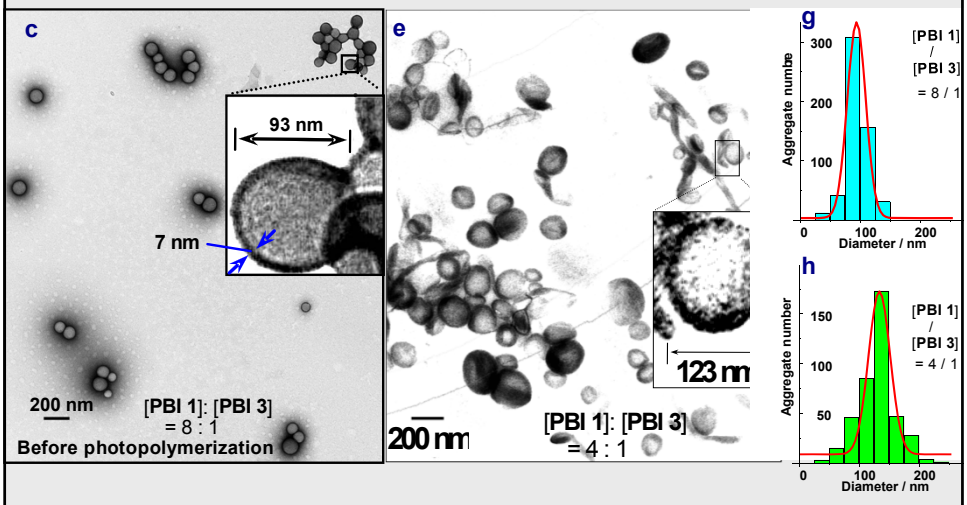
TEM Analysis and Photopolymerization



J. Am. Chem. Soc. **2007**, *129*, 4886

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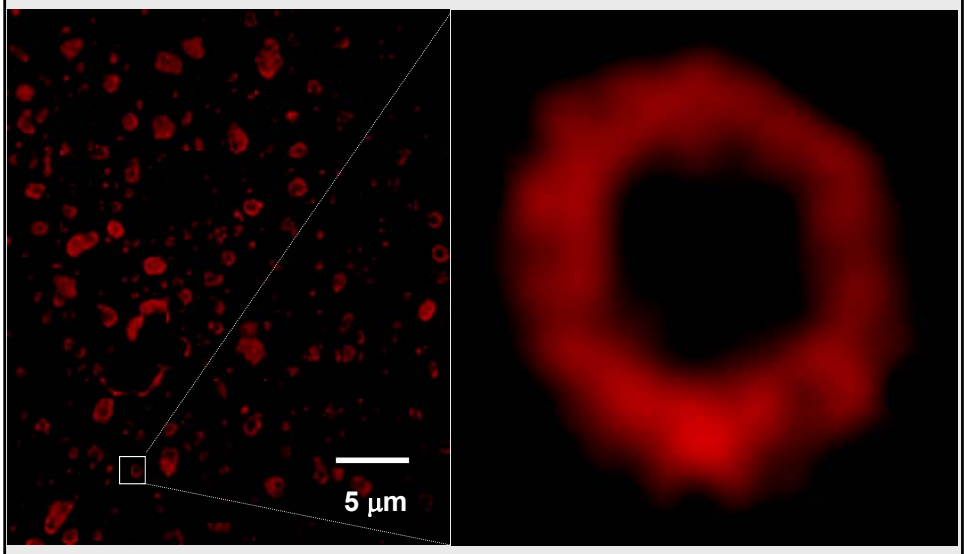
Confocal Fluorescence Microscopy of Vesicles



J. Am. Chem. Soc. **2007**, *129*, 4886

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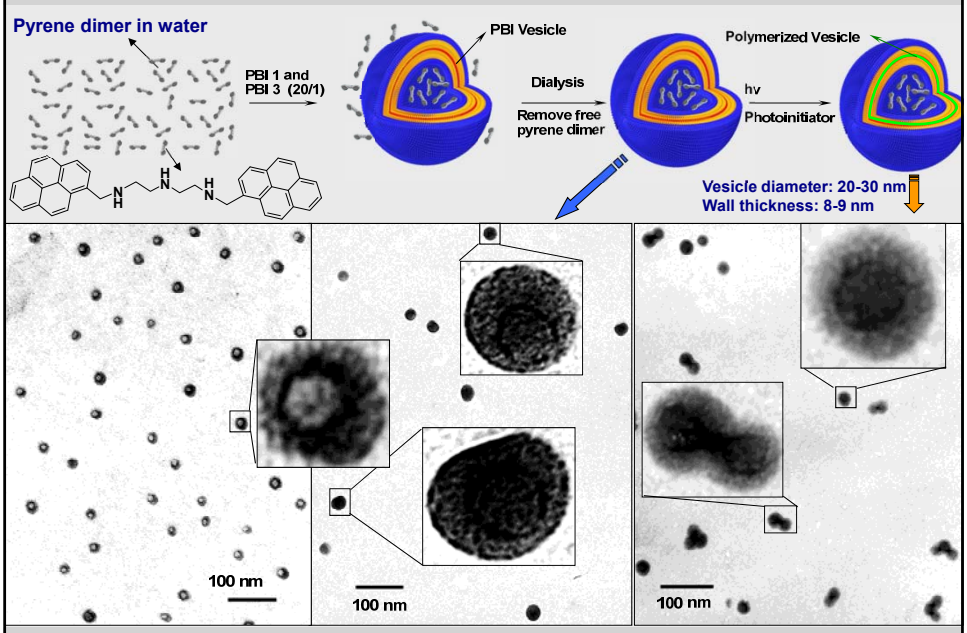
Encapsulation of PBI Vesicles with Pyrene Dimer



PBI vesicle without encapsulation | with encapsulation | After photopolymerization

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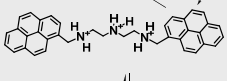
pH-dependent FRET Efficiency

Fluorescence of Pyrene dimer (Donor) in aqueous solution

Monomer emission at 375 nm

Excitation at 363 nm

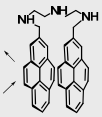
Pyrene dimer :



H^+ OH^-

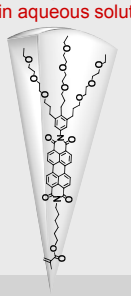
Excimer emission at 480 nm

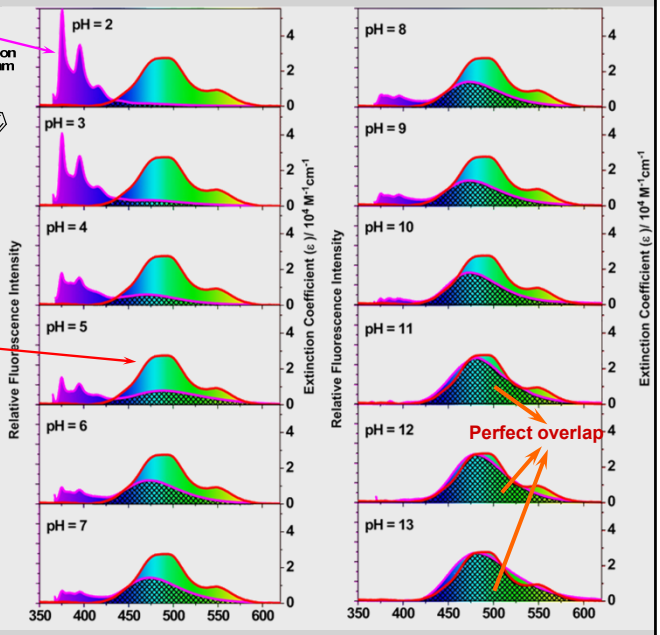
Excitation at 363 nm



Absorption of Perylene bisimides (Acceptor) in aqueous solution

Perylene bisimide :





Perfect overlap

Nature Chem. **2009**, *1*, 623

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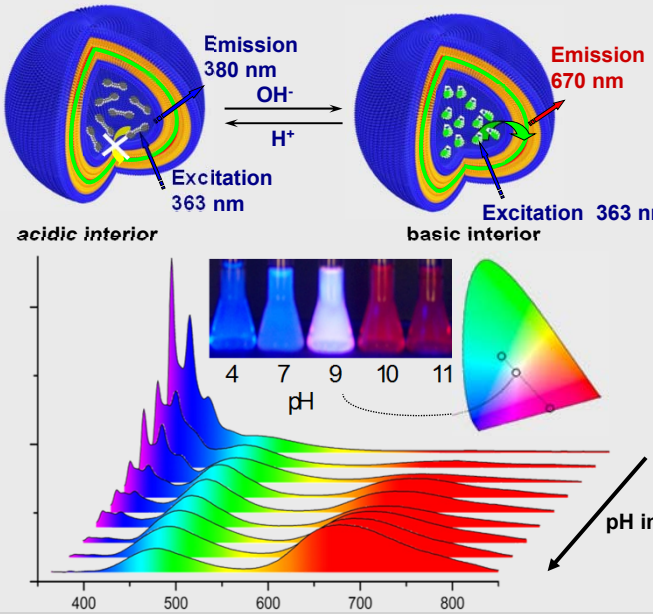
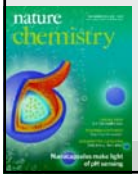
Ultrasensitive pH Measurement by Fluorescence Color





Nature Chem. **2009**, *1*, 623

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Outline

Self-assembly of Perylene Dyes in Water and Interactions with DNA and RNA

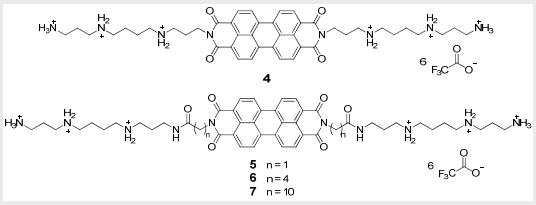
Basics of PBI self-assembly into π-stacks

PBI self-assembly into π-stacks in water

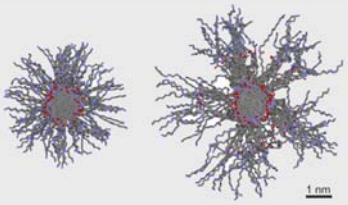
Micelles & vesicles: from structure to function

PBI Interactions with DNA and RNA

Ionic PBI Dyes as Supramolecular Building Blocks

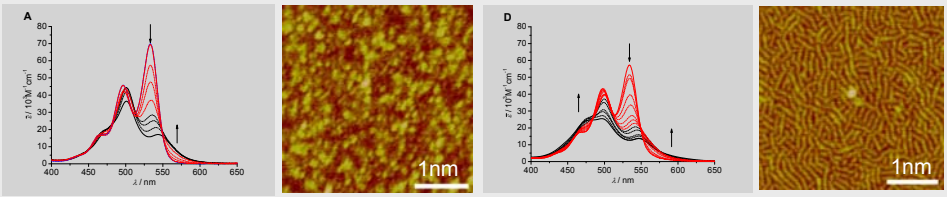


PBI **4** PBI **7**



→ Less favored aggregation due to electrostatic repulsion between positively charged side chains

PBI	4	5	6	7
Φ_{Fl}	0.90	0.78	0.72	0.39



Absorption spectra of PBIs **4** and **7** in water and AFM images on mica: A) **4**, *c* = 4 x 10^{-3} to 7 x 10^{-7} M; D) **7**, *c* = 5 x 10^{-3} to 5 x 10^{-7}M
The red curves represent the low concentration range

Chem. Eur. J. **2010**, *16*, 3372

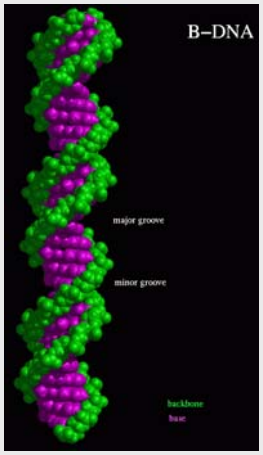
DNA (or RNA) as Supramolecular Building Block

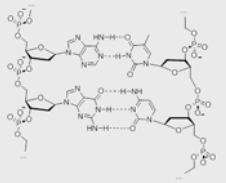
- Biomacromolecules with defined structure and binding sites for guest molecules
- Guest binding may change DNA/RNA structure and influence the functionalities

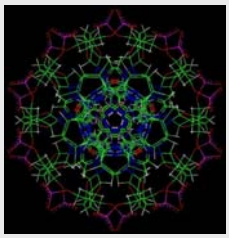
→ Research goals: high selectivity, large association constants; Applications as chemotherapeutic drugs and in detection of nucleic acids (DNA staining)

Our interest:

- How does such a biomacromolecule interact with a supramolecular nanosystem?
- Can the DNA backbone template the geometry of an associated dye aggregate?







Images: Jena Library of Biological Macromolecules http://jenalib.fli-leibniz.de/IMAGE.html; H. R. Drew, R. M. Wing, T. Takano, C. Broka, S. Tanaka, K. Itakura, R. E. Dickerson, *Proc. Natl. Acad. Sci. Usa.* 1981, *78*, 2179 – 2183; H. Ihmels, D. Otto, *Top. Curr. Chem.* Supramolecular Dye Chemistry **2005**, *258*, 161 – 204.

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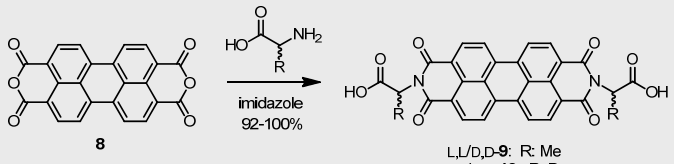
Synthesis of amino acid connected ionic PBIs

with Ivo Piantanida (Zagreb)



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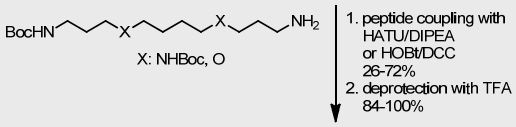
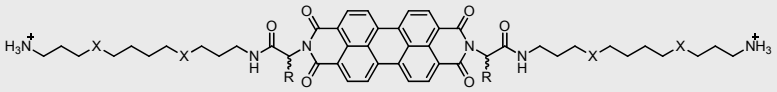
L,L/D,D-**9**: R: Me
L,L/D,D-**10**: R: Bz





L,L/D,D-**11**: R: Me, X: NH_2^+
L,L/D,D-**12**: R: Bz, X: NH_2^+
L,L/D,D-**13**: R: Me, X: O
L,L/D,D-**14**: R: Bz, X: O

Chem. Sci. **2012**, *3*, 3393 & *Chem. Eur. J.* **2015**, *21*, 7886

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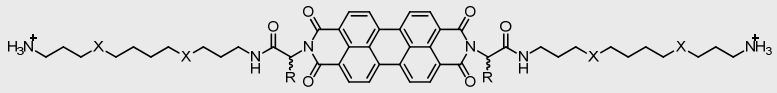
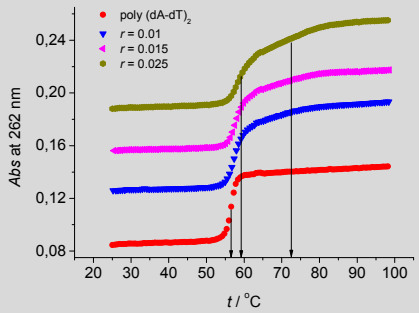
Stabilization of polynucleotides with PBIs



L,L/D,D-**11**: R: Me, X: NH_2^+ L,L/D,D-**13**: R: Me, X: O
L,L/D,D-**12**: R: Bz, X: NH_2^+ L,L/D,D-**14**: R: Bz, X: O



Thermal denaturation of poly(dA-dT)$_2$ upon addition of PBI L,L-**13** as an example; *r* (PBI/poly(dA-dT)$_2$) = 0.01-0.025, pH 7.0 (buffer sodium cacodylate, *I* = 0.05 M)

→ all PBIs stabilize ds-polynucleotides

→ all spermine-substituted PBIs (L,L/D,D-**11,12**) give large ΔT_m

→ for less charged side chains L,L-**13** gives larger ΔT_m than D,D-**13** and L,L/D,D-**14**

→ often biphasic melting curves

Chem. Sci. **2012**, *3*, 3393 & *Chem. Eur. J.* **2015**, *21*, 7886

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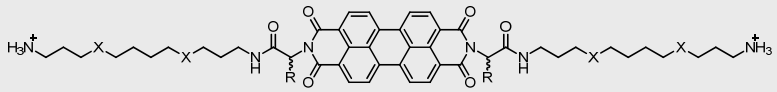
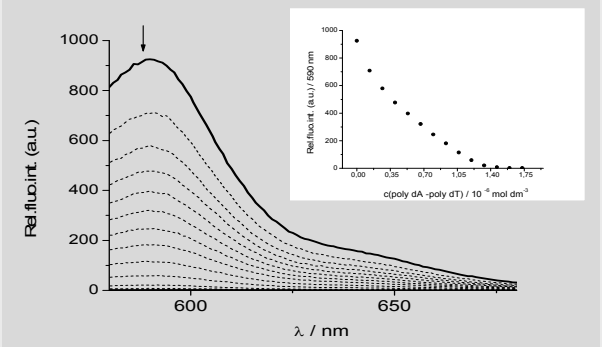
Binding Constants from Fluorometric Titrations



L,L/D,D-**11**: R: Me, X: NH_2^+ L,L/D,D-**13**: R: Me, X: O
L,L/D,D-**12**: R: Bz, X: NH_2^+ L,L/D,D-**14**: R: Bz, X: O



Fluorimetric titration of D,D-**11** (*c* = 5 x 10^{-7} M) with poly dA-poly dT, pH 7.0 (sodium cacodylate buffer, *I* = 0.05 M)

Scatchard Plots:

→ High binding constants logK_s
= 9.2-9.8 (L,L/D,D-**11** and -**12**)
= 6.5-7.9 (L,L/D,D-**13** and -**14**)

→ PBIs are among the strongest groove binders (typical logK_s-values are between 5-7)

Chem. Sci. **2012**, *3*, 3393 & *Chem. Eur. J.* **2015**, *21*, 7886

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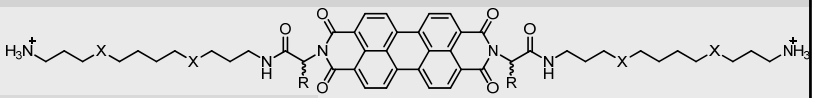
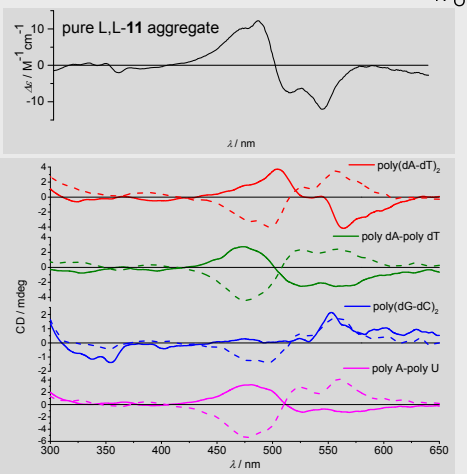
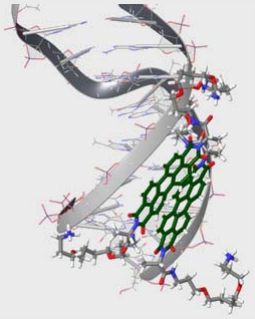
Circular Dichroism

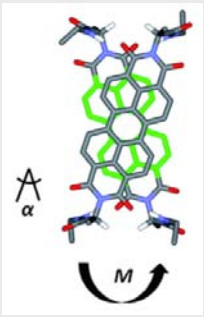




CD spectra of PBIs L,L/D,D-**11** in the absence (top) and presence (bottom) of different polynucleotides (*c* (polynucleotide) = 4 x 10^{-5} M); *r* (PBI/polynucleotide) = 0.16, pH 7.0 (buffer sodium cacodylate, *I* = 0.05 M), *L* configured (solid line), *D*-configured (dashed line).

L,L/D,D-**11**: R: Me, X: NH_2^+ L,L/D,D-**13**: R: Me, X: O
L,L/D,D-**12**: R: Bz, X: NH_2^+ L,L/D,D-**14**: R: Bz, X: O





→ bisignate CD signals indicate presence of dimers (or larger oligomers) within DNA/ RNA grooves

→ dimer geometry obviously influenced by the resp. grooves

Chem. Sci. **2012**, *3*, 3393

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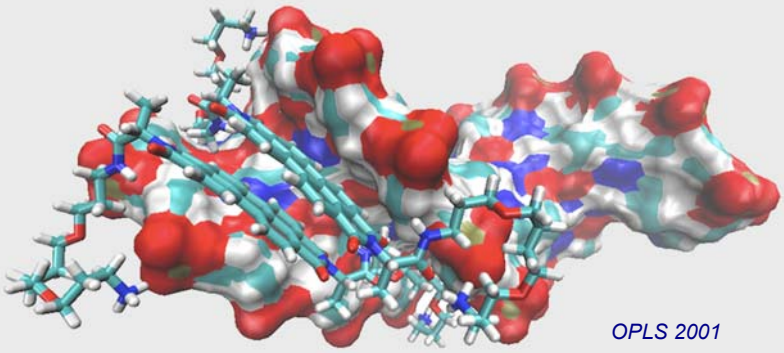
Molecular Modeling



OPLS 2001

On-going work in Zagreb: *in-vitro* experiments with cancer cells

- very fast uptake of PBIs into the cell
- spermine-functionalized PBIs show high cytotoxicity to cancer cells
- PBIs bearing oxygen in the side chains exhibit desired inhibition of proliferation

⇒ Inhibition of proliferative activity of different cancer cell lines, depending on the side chains

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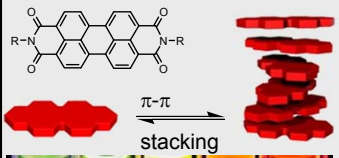
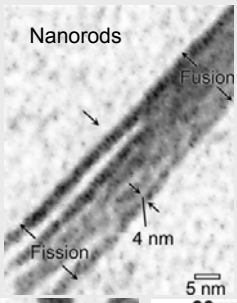
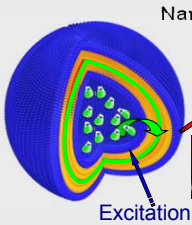
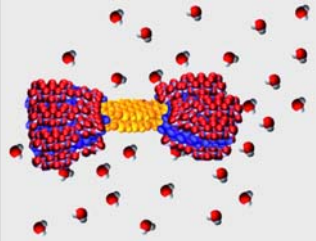
Conclusion

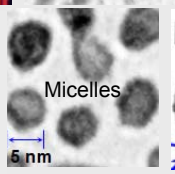
Dye Molecule → Dye Aggregate → Self-assembled Soft Matter → Functional Systems

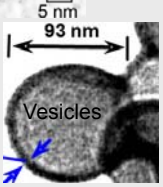


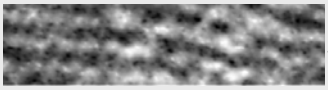


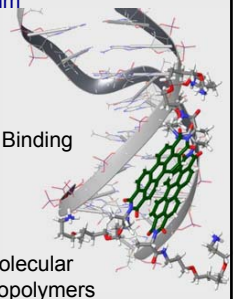












Supramolecular
Block Copolymers

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