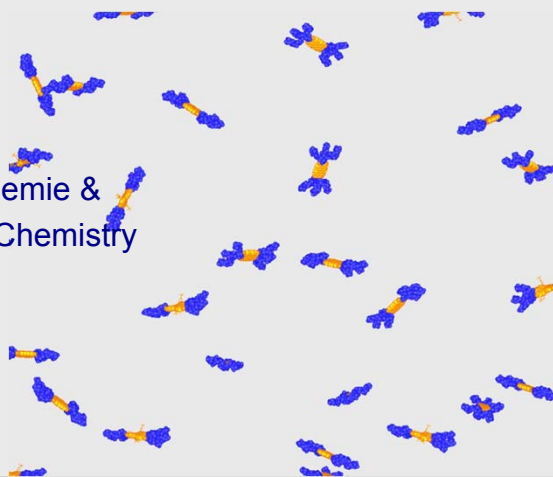


# Self-assembly of Perylene Dyes in Water and Interactions with DNA and RNA

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Center for Nanosystems Chemistry  
Universität Würzburg



InnoMol Workshop on Molecular Interactions, 1-3.6.2015, Ruđer Bošković Institute, Zagreb

## Outline

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

Basics of PBI self-assembly into  $\pi$ -stacks

PBI self-assembly into  $\pi$ -stacks in water

Micelles & vesicles: from structure to function

PBI Interactions with DNA and RNA

## Outline

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

## Basics of PBI self-assembly into $\pi$ -stacks

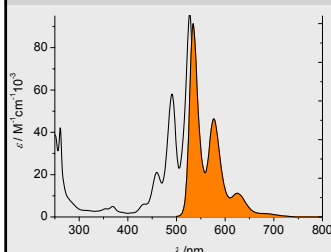
PBI self-assembly into  $\pi$ -stacks in water

Micelles & vesicles: from structure to function

PBI Interactions with DNA and RNA

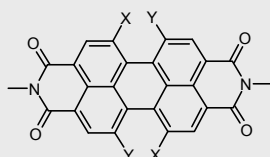
3

## Perylene Bisimides: A Versatile Class of Dyes

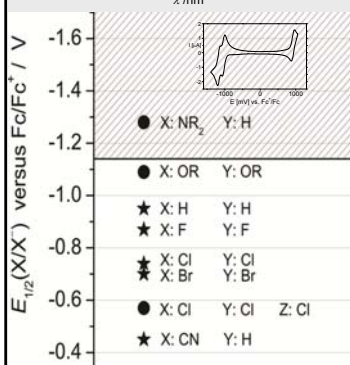


- Intense Absorption
- Fluorescence  $\Phi_f = 100\%$
- Reversible Redox Processes
- (Photo-)Stability
- n-type Semiconductivity

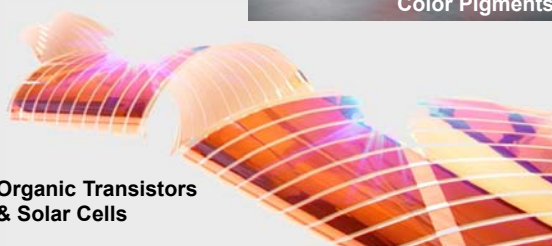
Fluorescent & Laser Dyes



Color Pigments

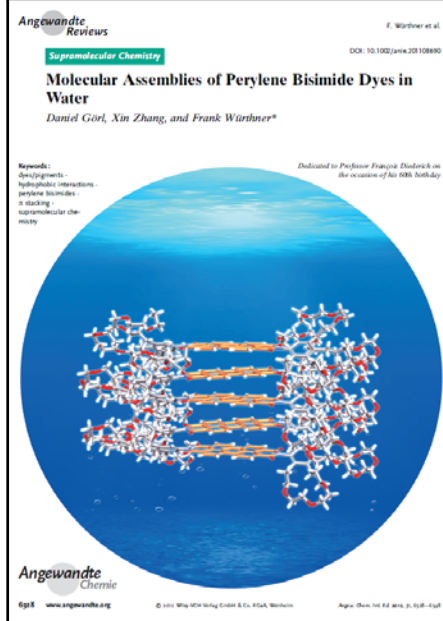


Organic Transistors & Solar Cells

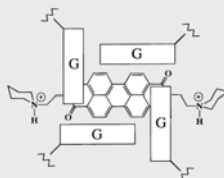


Reviews: *Chem. Commun.* **2004**, 1564 & *Chem. Commun.* **2011**, 47, 5109

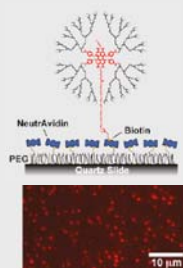
## Perylene Bisimides in Water



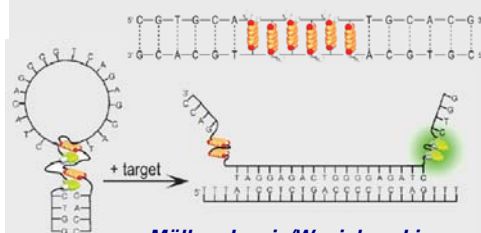
### G Quartet Binders



### Single Molecule Bioimaging



### Molecular Probes

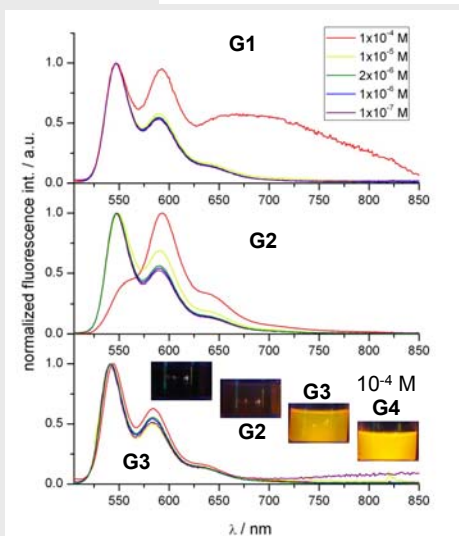
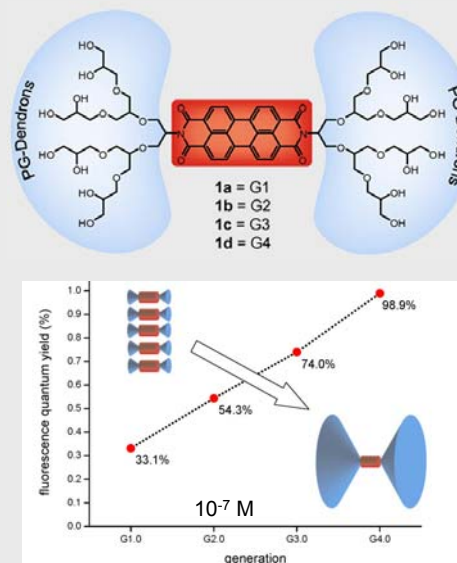


Müllen, Lewis/Wasielewski,  
Wagenknecht, Häner, Faul, Savino,  
Rybitchinski, Haag, Zimmerman, .....

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

## Perylene Bisimides: Fluorescence in Water

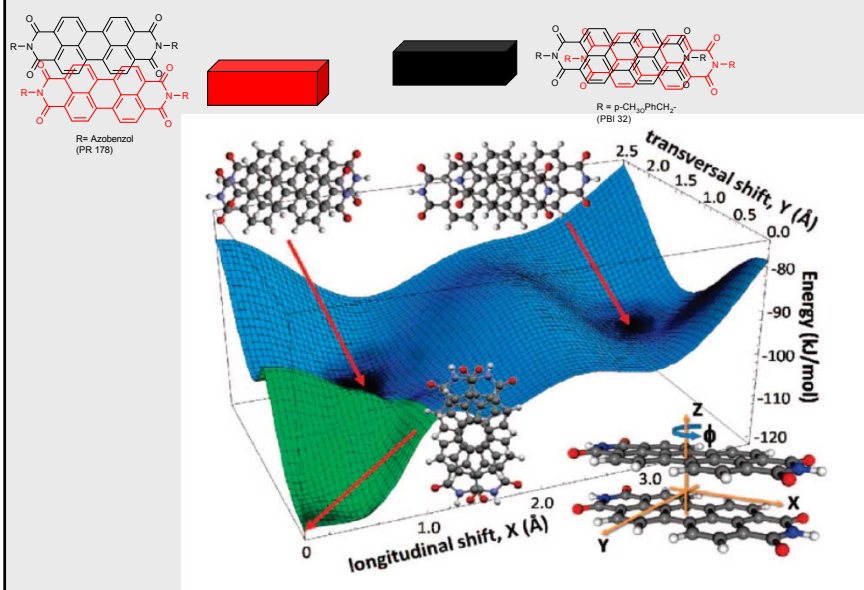
with Rainer Haag, FU Berlin



Chem. Commun. 2010, 46, 1884

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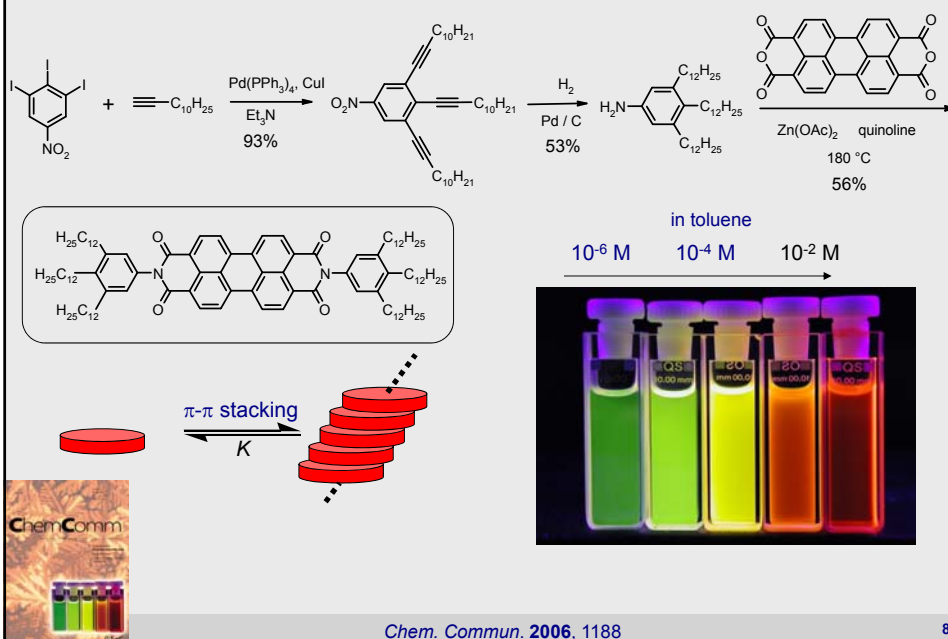
## Ground State Potential Energy Surfaces (DFT-D)



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

7

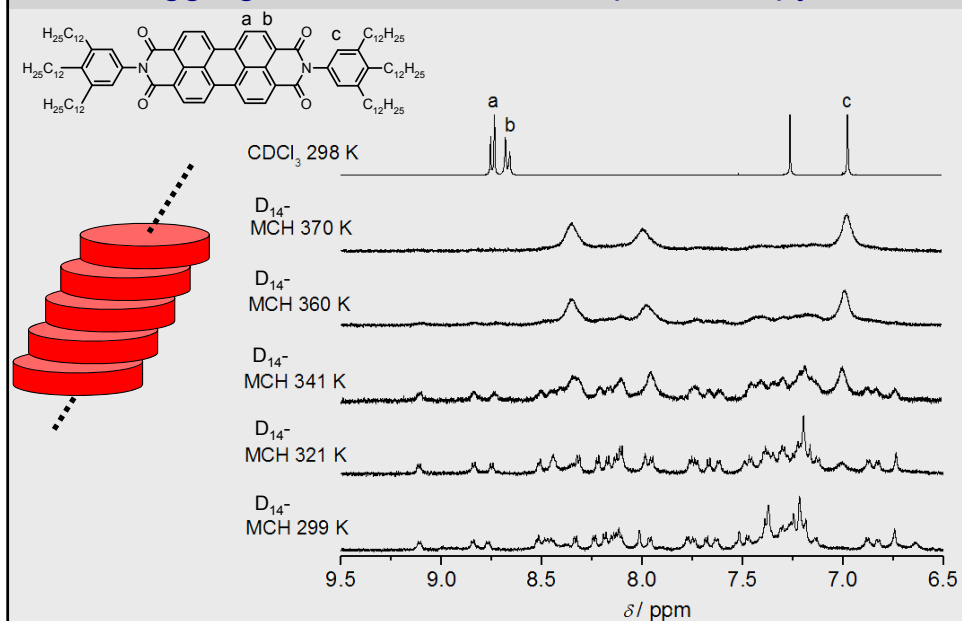
## Fluorescent Perylene Dye Aggregates



*Chem. Commun.* **2006**, 1188

8

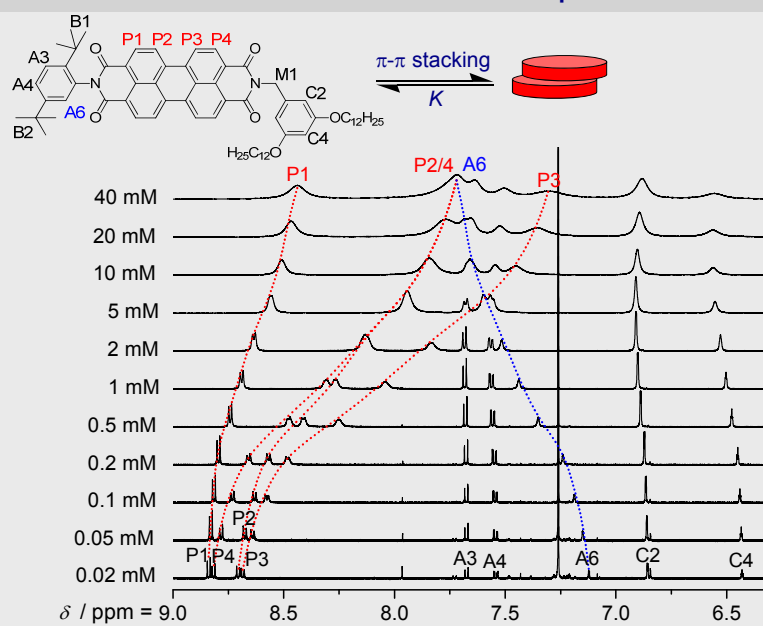
## Aggregate Structure: NMR Spectroscopy?



Chem. Eur. J. 2007, 13, 443

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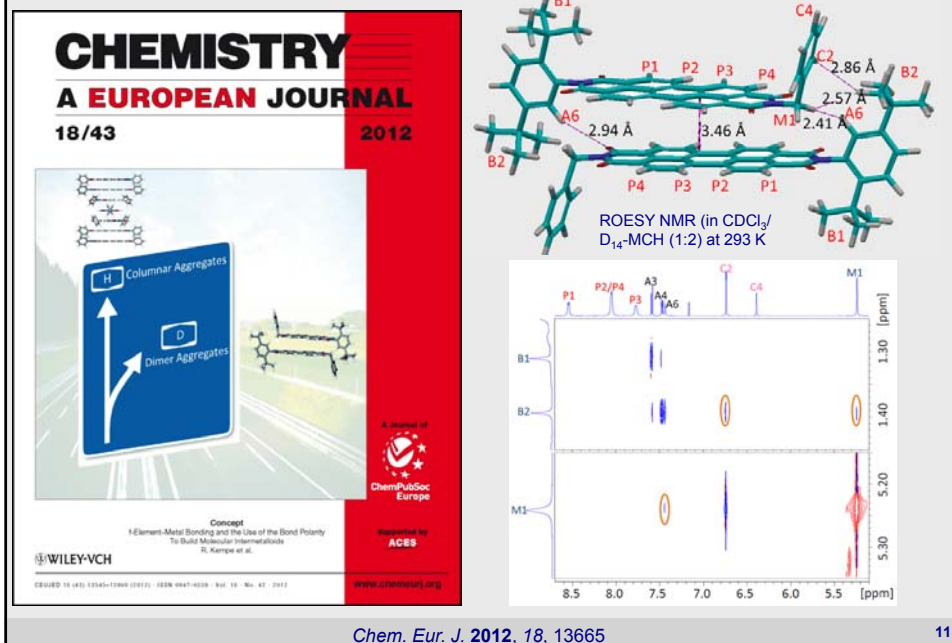
## A Dimer Model Compound



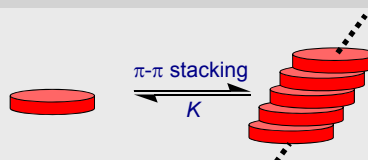
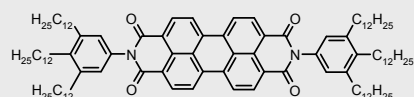
Chem. Eur. J. 2012, 18, 13665

10

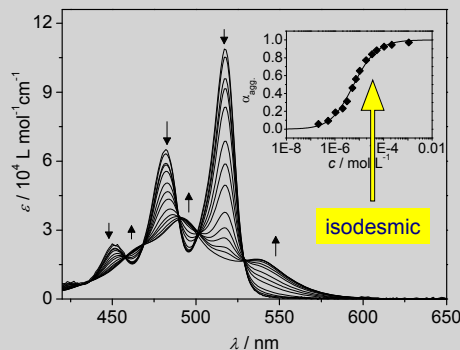
## A Dimer Model Compound



## Spectral Changes upon Perylene Bisimide Aggregation



### Concentration dependent UV/Vis spectra in methylcyclohexane



$$K = 10^6 \text{ M}^{-1} (n\text{-hexane})$$

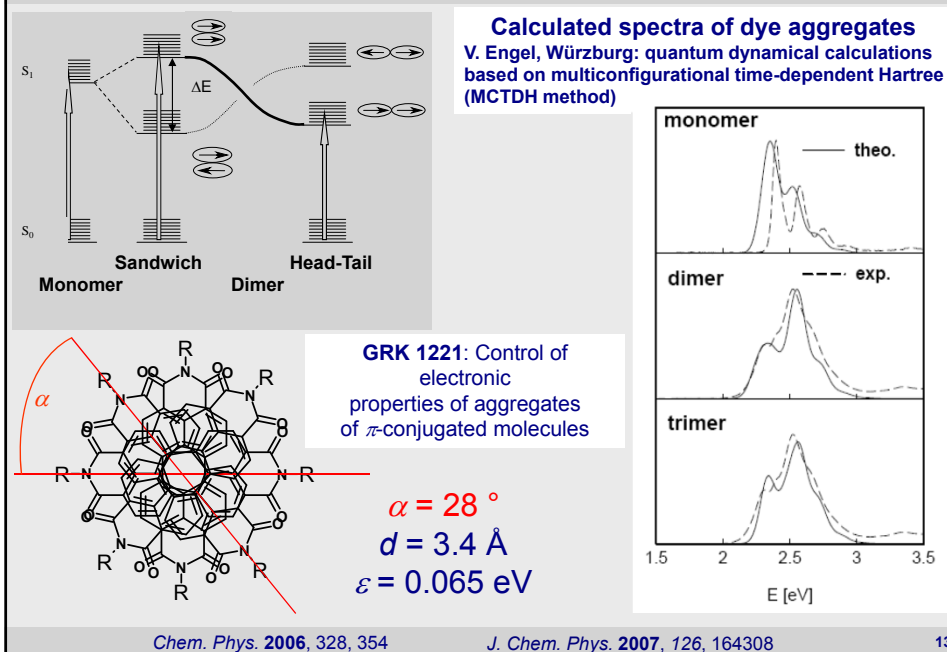
$$K = 10^5 \text{ M}^{-1} (\text{methylcyclohexane})$$

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

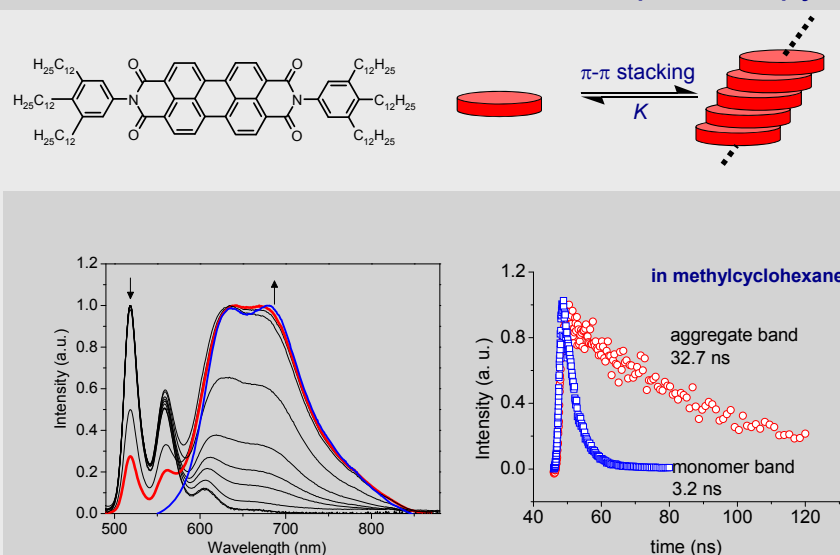
Average molecular weight & aggregation number at different temperatures from VPO measurements.

(MCH $5 \times 10^{-3} \text{ M}$ )	40 °C	50 °C	60 °C
$M / \text{g mol}^{-1}$	14280	11490	8690
$N$	9	7	6

## Theoretical Description of Spectral Changes



## Time-Resolved Fluorescence Spectroscopy

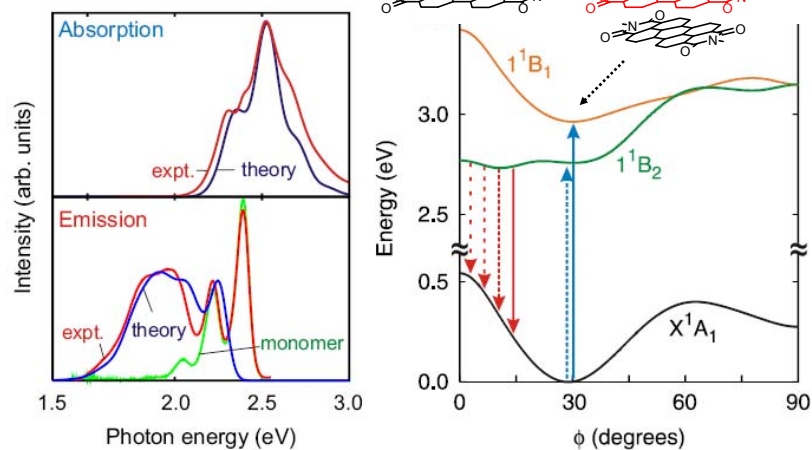




## Theoretical Description of Spectral Changes

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

TD-HFT calculation of excited state potential energy surface\*

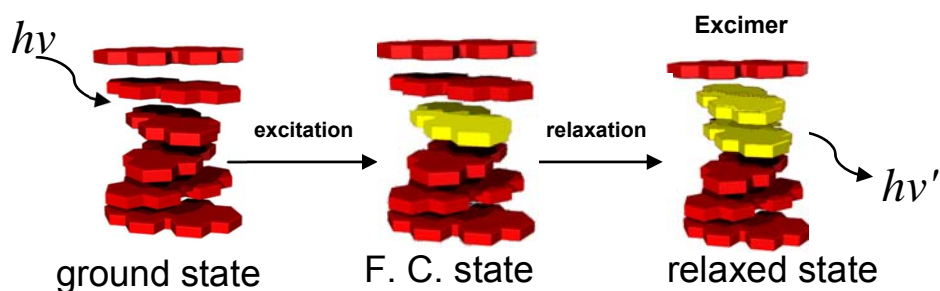


\*) Dispersion interactions embedded as ground state interaction

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

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## Consequence for Fluorescence & Exciton Transport



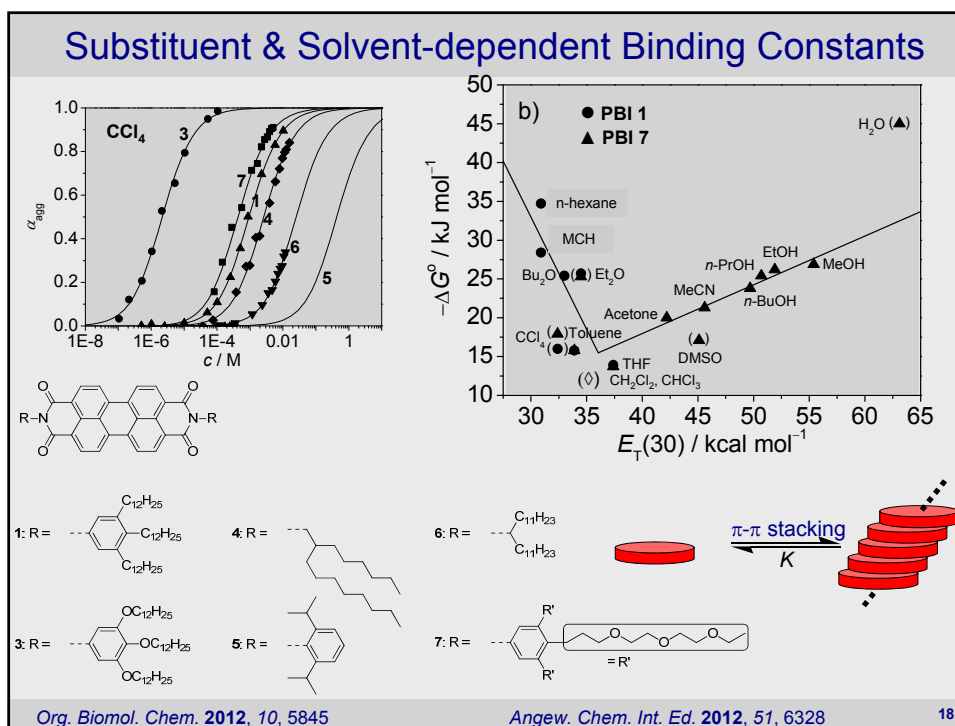
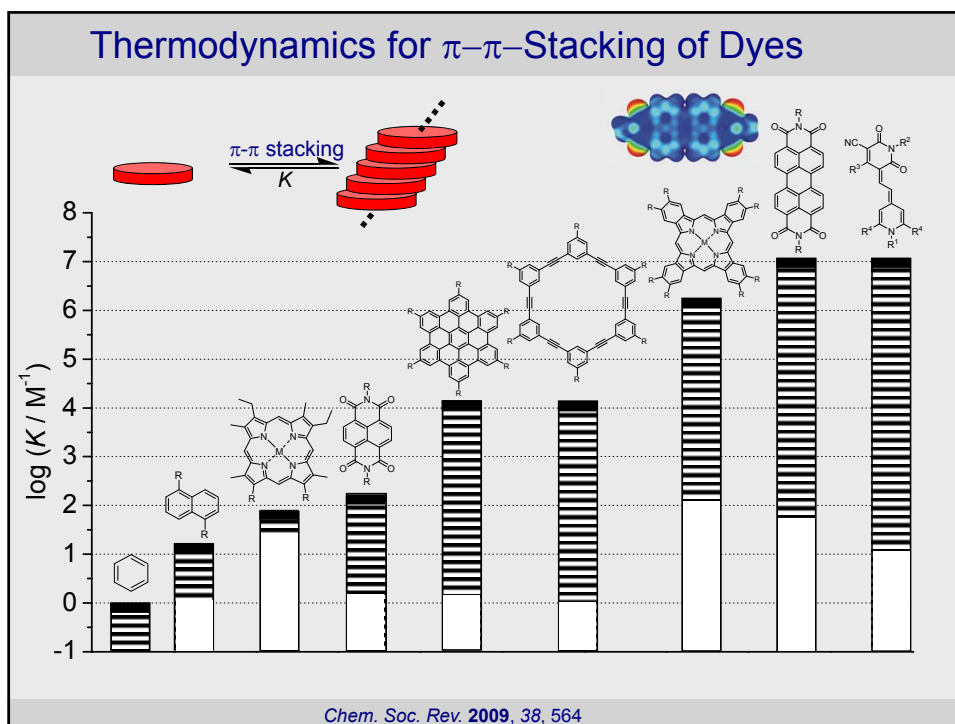
- localization of exciton
- unfavorable for applications in OLEDs or solar cells („exciton trap“)
- fluorescence lifetime increased, but quantum yield decreased

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

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

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## Outline

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Basics of PBI self-assembly into  $\pi$ -stacks

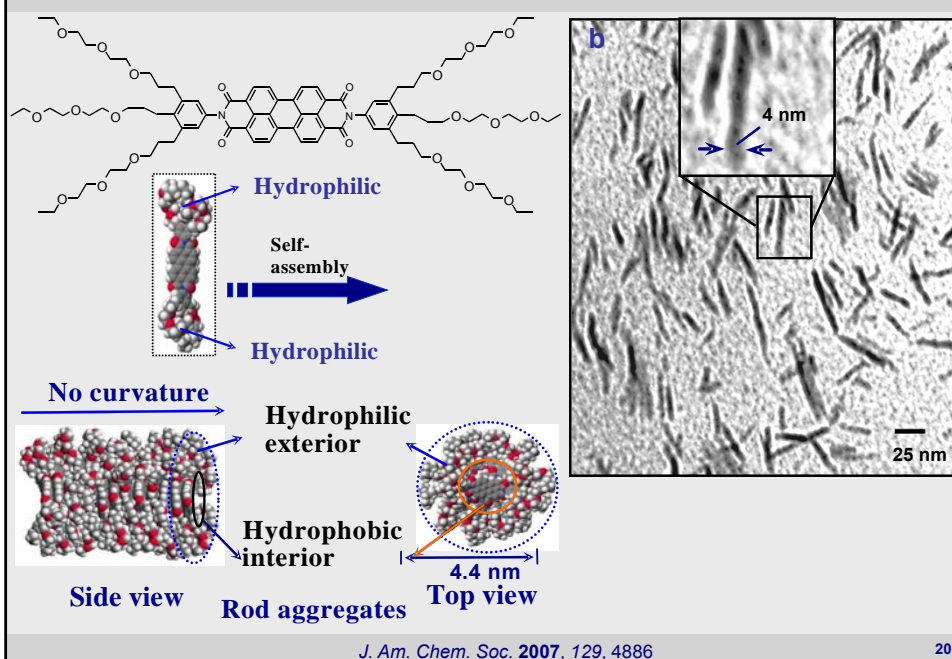
**PBI self-assembly into  $\pi$ -stacks in water**

Micelles & vesicles: from structure to function

PBI Interactions with DNA and RNA

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## Perylene Bisimide Aggregation in Water



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R =  $\text{---CH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2\text{CH}_3$

Figure 1 consists of several panels. The top left panel is a large TEM image showing a dense field of nanorods. A scale bar of 50 nm is at the bottom left. The top right panel is a 5 nm scale bar inset showing a single nanorod. The middle right panel is a 4 nm scale bar inset showing a single nanorod. The bottom section shows a 2.2 nm scale bar inset, a 4 nm scale bar inset, and a schematic of the PBI 1 molecule. The schematic shows the molecule's structure and the 4 nm scale bar. The label 'Planar PBI 1' is at the bottom left.

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# Hierarchical Growth by Nanorod Fusion

The figure displays two plots showing fluorescence intensity (y-axis, 0 to 30) versus wavelength (x-axis, 500 to 800 nm) for the hierarchical growth of PBI 1.

**Top Plot: Nanorods**  
[PBI 1] = 0.077 mg/mL  
 $\Phi = 1.7\%$   
The plot shows a broad fluorescence peak centered around 650 nm. An inset image shows a single nanorod structure.

**Bottom Plot: Nanoribbons**  
[PBI 1] = 1.0 mg/mL  
 $\Phi = 6.8\%$   
The plot shows a broad fluorescence peak centered around 650 nm, with a small shoulder around 820 nm. An inset image shows a single nanoribbon structure.

Wavelength / nm  $\longrightarrow$

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

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# Core-twisted Perylene Bisimide Amphiphiles

Chemical structure of the perylene bisimide core, showing the central perylene moiety and the imide groups. The structure is labeled with R and R' substituents.

Chemical structure of the perylene bisimide core, showing the central perylene moiety and the imide groups. The structure is labeled with R and R' substituents.

Chemical structure of the perylene bisimide core, showing the central perylene moiety and the imide groups. The structure is labeled with R and R' substituents.

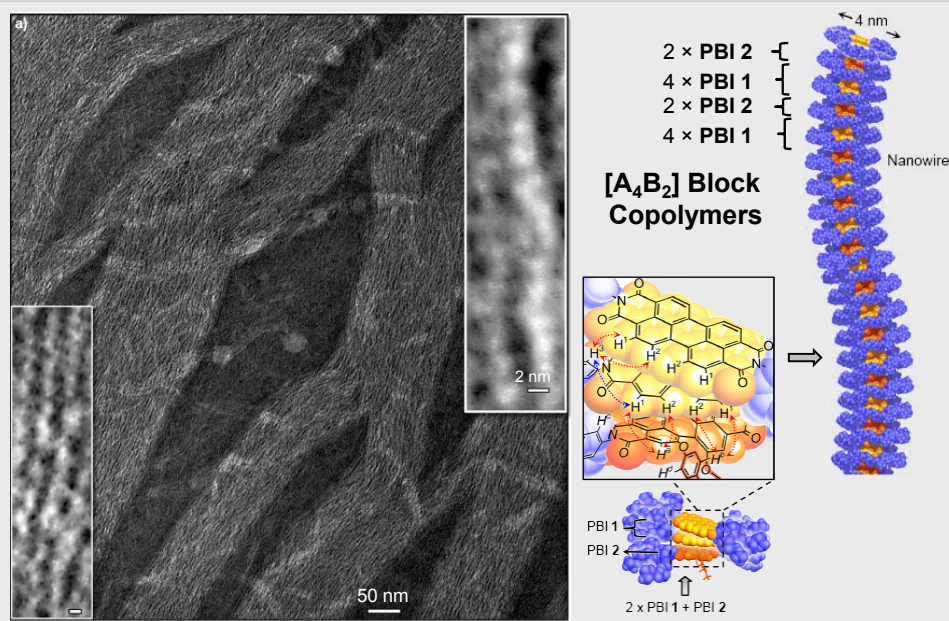
Figure 1: Absorption and fluorescence spectra of PBI 1 and PBI 2. The plot shows the molar absorptivity ( $\epsilon / 10^{-3} \text{ L mol}^{-1} \text{ cm}^{-1}$ ) and fluorescence intensity (a.u.) versus wavelength ( $\lambda / \text{nm}$ ). PBI 1 (solid blue line) shows a strong absorption peak around 520 nm and a fluorescence peak around 580 nm. PBI 2 (dashed red line) shows a strong absorption peak around 580 nm and a fluorescence peak around 650 nm. An inset shows the color of the solutions: PBI 1 is green and PBI 2 is orange.

Figure 2: Atomic force microscopy (AFM) images of PBI 1 and PBI 2. The images show the morphology of the aggregates. PBI 1 (left) forms long, thin, needle-like structures. PBI 2 (right) forms shorter, thicker, and more irregular aggregates. Scale bars are shown in the bottom left of each image.

Nature Commun. 2015, 6, 8009

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## Supramolecular Block Copolymers



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

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Basics of PBI self-assembly into  $\pi$ -stacks

PBI self-assembly into  $\pi$ -stacks in water

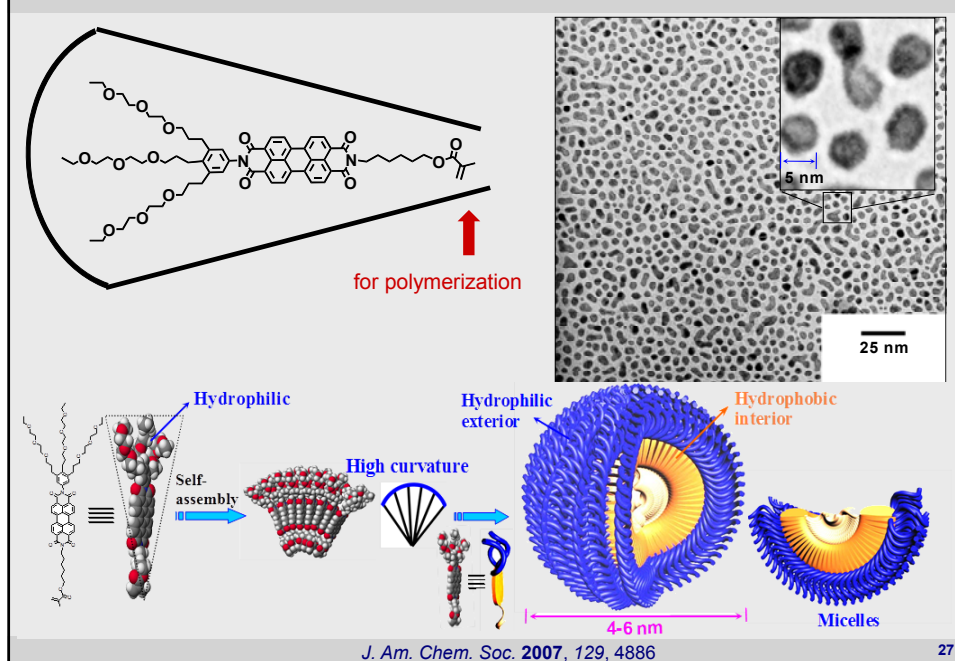
**Micelles & vesicles: from structure to function**

PBI Interactions with DNA and RNA

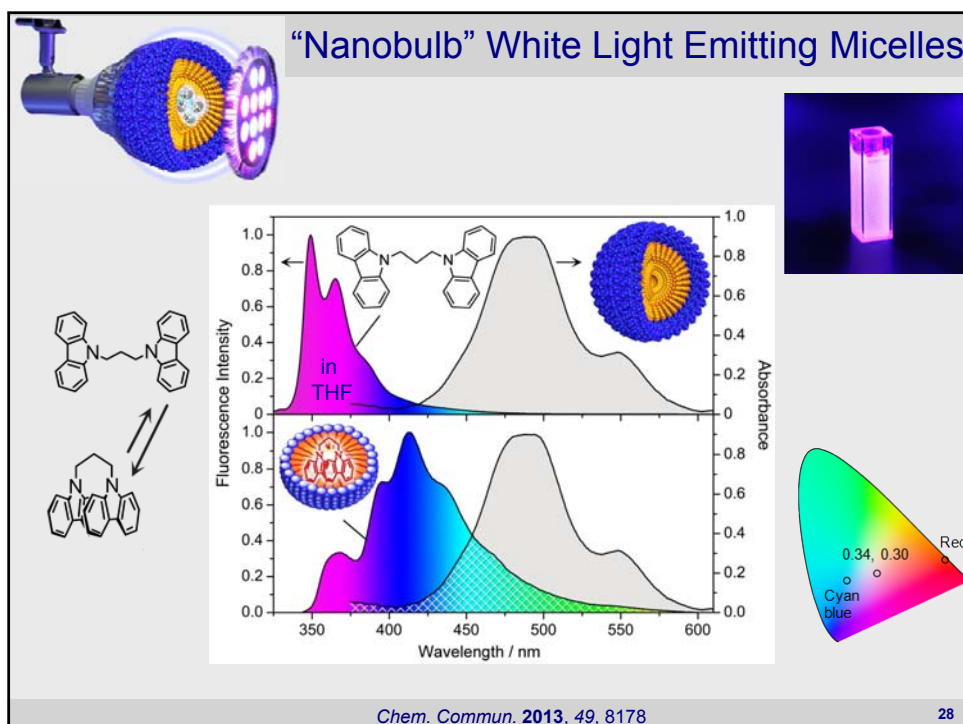
26



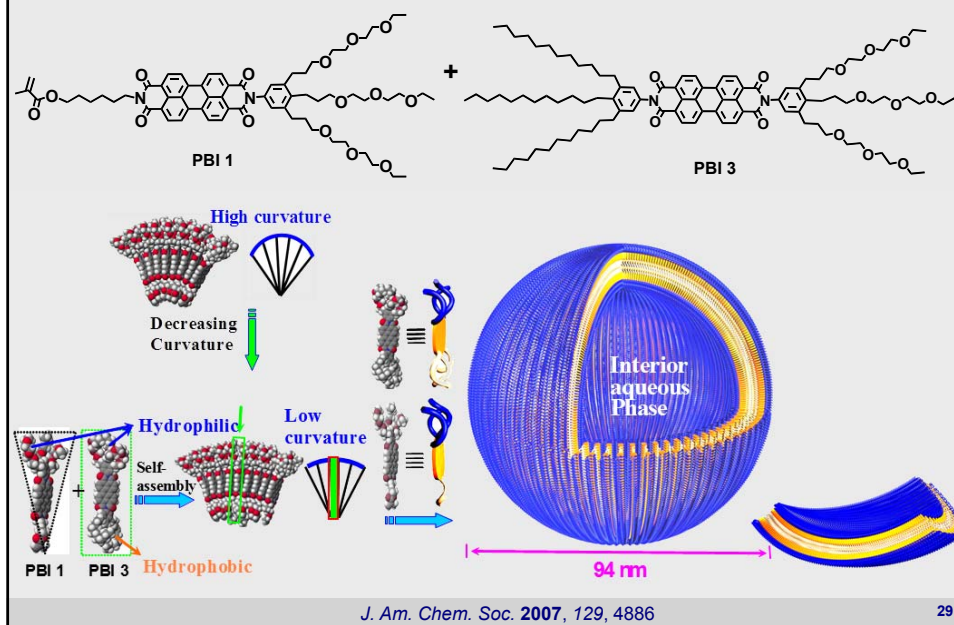
## Micelle Formation from Wedge-shaped Dyes



## “Nanobulb” White Light Emitting Micelles

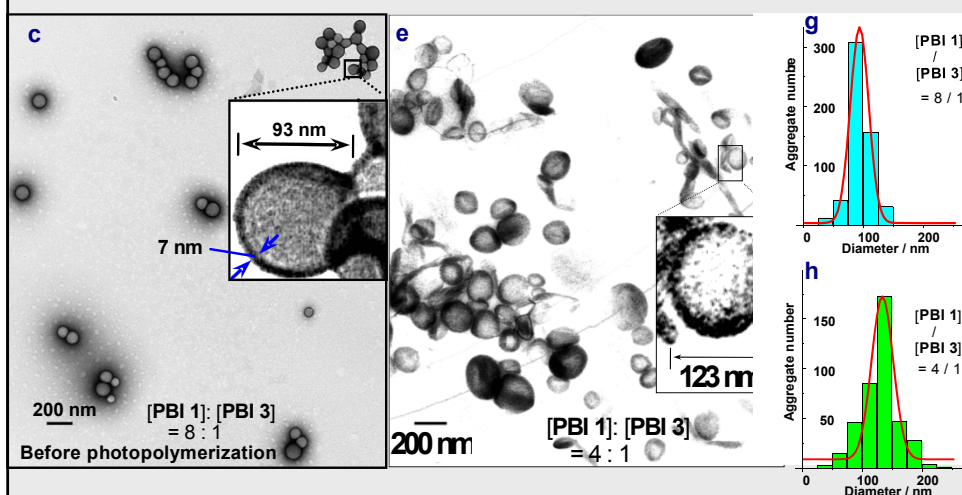


## Vesicle Formation from Wedge & Dumbbell-shaped Dyes



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## TEM Analysis and Photopolymerization

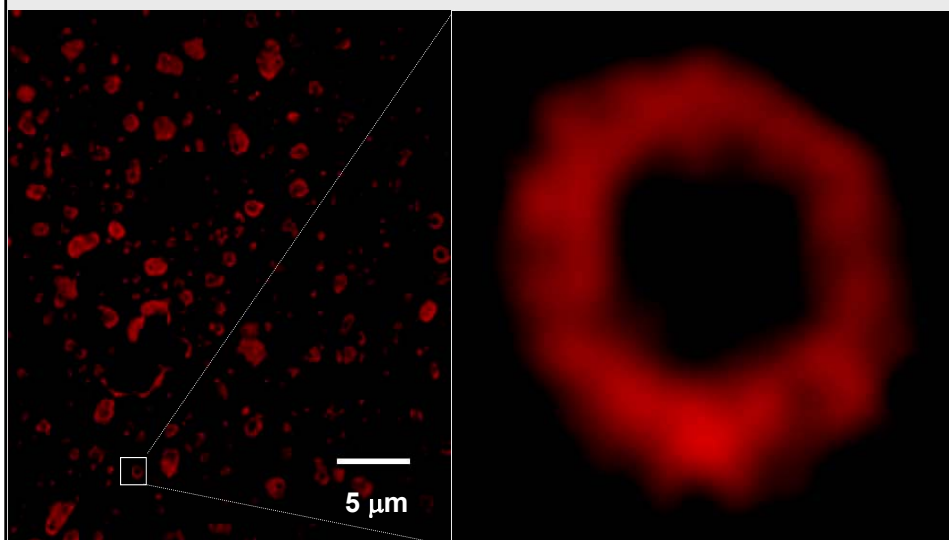


*J. Am. Chem. Soc.* **2007**, *129*, 4886

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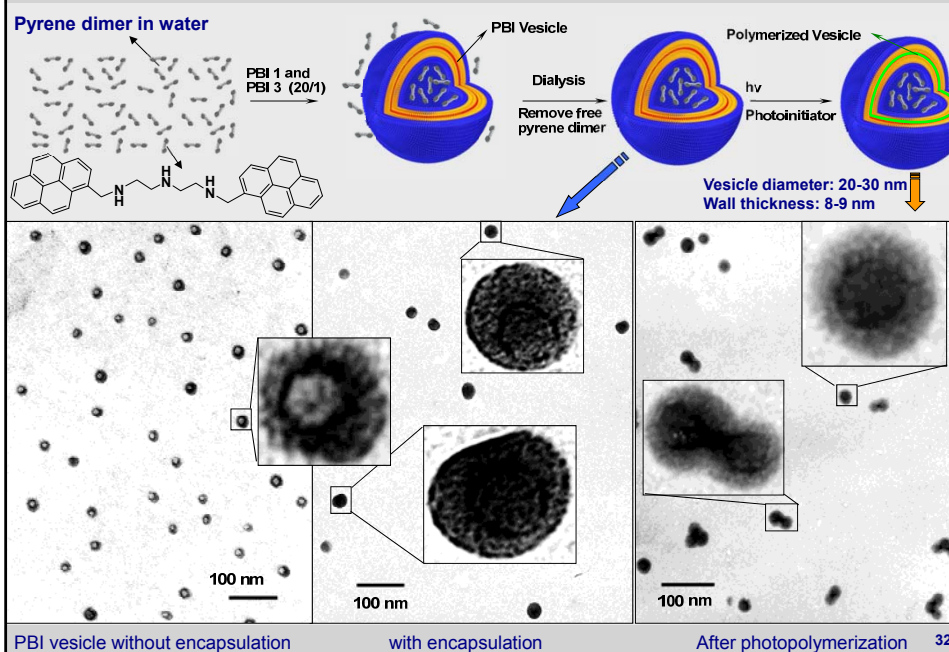
## Confocal Fluorescence Microscopy of Vesicles



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

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## Encapsulation of PBI Vesicles with Pyrene Dimer



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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:**

c1ccc2c(c1)ccc3c2ccc4c3ccc5c4ccc6c5ccc7c6ccc8c7ccc9c8ccc10c9ccc11c10ccc12c11ccc23c12ccc45c23ccc67c45ccc89c67ccc1011c89ccc1213c1011ccc1415c1213ccc1617c1415ccc1819c1617ccc2021c1819ccc2223c2021ccc2425c2223ccc2627c2425ccc2829c2627ccc3031c2829ccc3233c3031ccc3435c3233ccc3637c3435ccc3839c3637ccc4041c3839ccc4243c4041ccc4445c4243ccc4647c4445ccc4849c4647ccc5051c4849ccc5253c5051ccc5455c5253ccc5657c5455ccc5859c5657ccc6061c5859ccc6263c6061ccc6465c6263ccc6667c6465ccc6869c6667ccc7071c6869ccc7273c7071ccc7475c7273ccc7677c7475ccc7879c7677ccc8081c7879ccc8283c8081ccc8485c8283ccc8687c8485ccc8889c8687ccc9091c8889ccc9293c9091ccc9495c9293ccc9697c9495ccc9899c9697ccc100101c9899ccc102103c100101ccc104105c102103ccc106107c104105ccc108109c106107ccc110111c108109ccc112113c110111ccc114115c112113ccc116117c114115ccc118119c116117ccc120121c118119ccc122123c120121ccc124125c122123ccc126127c124125ccc128129c126127ccc130131c128129ccc132133c130131ccc134135c132133ccc136137c134135ccc138139c136137ccc140141c138139ccc142143c140141ccc144145c142143ccc146147c144145ccc148149c146147ccc150151c148149ccc152153c150151ccc154155c152153ccc156157c154155ccc158159c156157ccc160161c158159ccc162163c160161ccc164165c162163ccc166167c164165ccc168169c166167ccc170171c168169ccc172173c170171ccc174175c172173ccc176177c174175ccc178179c176177ccc180181c178179ccc182183c180181ccc184185c182183ccc186187c184185ccc188189c186187ccc190191c188189ccc192193c190191ccc194195c192193ccc196197c194195ccc198199c196197ccc200201c198199ccc202203c200201ccc204205c202203ccc206207c204205ccc208209c206207ccc210211c208209ccc212213c210211ccc214215c212213ccc216217c214215ccc218219c216217ccc220221c218219ccc222223c220221ccc224225c222223ccc226227c224225ccc228229c226227ccc230231c228229ccc232233c230231ccc234235c232233ccc236237c234235ccc238239c236237ccc240241c238239ccc242243c240241ccc244245c242243ccc246247c244245ccc248249c246247ccc250251c248249ccc252253c250251ccc254255c252253ccc256257c254255ccc258259c256257ccc260261c258259ccc262263c260261ccc264265c262263ccc266267c264265ccc268269c266267ccc270271c268269ccc272273c270271ccc274275c272273ccc276277c274275ccc278279c276277ccc280281c278279ccc282283c280281ccc284285c282283ccc286287c284285ccc288289c286287ccc290291c288289ccc292293c290291ccc294295c292293ccc296297c294295ccc298299c296297ccc300301c298299ccc302303c300301ccc304305c302303ccc306307c304305ccc308309c306307ccc310311c308309ccc312313c310311ccc314315c312313ccc316317c314315ccc318319c316317ccc320321c318319ccc322323c320321ccc324325c322323ccc326327c324325ccc328329c326327ccc330331c328329ccc332333c330331ccc334335c332333ccc336337c334335ccc338339c336337ccc340341c338339ccc342343c340341ccc344345c342343ccc346347c344345ccc348349c346347ccc350351c348349ccc352353c350351ccc354355c352353ccc356357c354355ccc358359c356357ccc360361c358359ccc362363c360361ccc364365c362363ccc366367c364365ccc368369c366367ccc370371c368369ccc372373c370371ccc374375c372373ccc376377c374375ccc378379c376377ccc380381c378379ccc382383c380381ccc384385c382383ccc386387c384385ccc388389c386387ccc390391c388389ccc392393c390391ccc394395c392393ccc396397c394395ccc398399c396397ccc400401c398399ccc402403c400401ccc404405c402403ccc406407c404405ccc408409c406407ccc410411c408409ccc412413c410411ccc414415c412413ccc416417c414415ccc418419c416417ccc420421c418419ccc422423c420421ccc424425c422423ccc426427c424425ccc428429c426427ccc430431c428429ccc432433c430431ccc434435c432433ccc436437c434435ccc438439c436437ccc440441c438439ccc442443c440441ccc444445c442443ccc446447c444445ccc448449c446447ccc450451c448449ccc452453c450451ccc454455c452453ccc456457c454455ccc458459c456457ccc460461c458459ccc462463c460461ccc464465c462463ccc466467c464465ccc468469c466467ccc470471c468469ccc472473c470471ccc474475c472473ccc476477c474475ccc478479c476477ccc480481c478479ccc482483c480481ccc484485c482483ccc486487c484485ccc488489c486487ccc490491c488489ccc492493c490491ccc494495c492493ccc496497c494495ccc498499c496497ccc500501c498499ccc502503c500501ccc504505c502503ccc506507c504505ccc508509c506507ccc510511c508509ccc512513c510511ccc514515c512513ccc516517c514515ccc518519c516517ccc520521c518519ccc522523c520521ccc524525c522523ccc526527c524525ccc528529c526527ccc530531c528529ccc532533c530531ccc534535c532533ccc536537c534535ccc538539c536537ccc540541c538539ccc542543c540541ccc544545c542543ccc546547c544545ccc548549c546547ccc550551c548549ccc552553c550551ccc554555c552553ccc556557c554555ccc558559c556557ccc560561c558559ccc562563c560561ccc564565c562563ccc566567c564565ccc568569c566567ccc570571c568569ccc572573c570571ccc574575c572573ccc576577c574575ccc578579c576577ccc580581c578579ccc582583c580581ccc584585c582583ccc586587c584585ccc588589c586587ccc590591c588589ccc592593c590591ccc594595c592593ccc596597c594595ccc598599c59659

# Ultrasensitive pH Measurement by Fluorescence Color

The diagram illustrates the mechanism of ultrasensitive pH measurement using a fluorescent probe. The probe is shown in two states: an acidic state (left) and a basic state (right). In the acidic state, the probe is in its protonated form (H<sup>+</sup>), emitting light at 380 nm (blue) upon excitation at 363 nm (blue). In the basic state, the probe is in its deprotonated form (OH<sup>-</sup>), emitting light at 670 nm (red) upon excitation at 363 nm (blue). The transition between the two states is reversible, as indicated by the equilibrium arrows.

The graph below shows the fluorescence spectra of the probe at various pH values (4, 7, 9, 10, 11). The x-axis represents the wavelength in nm, ranging from 400 to 800. The y-axis represents fluorescence intensity. The spectra show a shift in the emission peak from approximately 450 nm at pH 4 to approximately 650 nm at pH 11. A color scale on the right indicates the corresponding color change from blue to red. An inset image shows five Erlenmeyer flasks containing the probe at pH values of 4, 7, 9, 10, and 11, demonstrating the color change from blue to red.

**acidic interior**      **basic interior**

**Excitation 363 nm**      **Excitation 363 nm**

**Emission 380 nm**      **Emission 670 nm**

**OH<sup>-</sup>**      **H<sup>+</sup>**

**pH**

**pH increase from 2 to 13**

**nature chemistry**

**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  $\pi$ -stacks

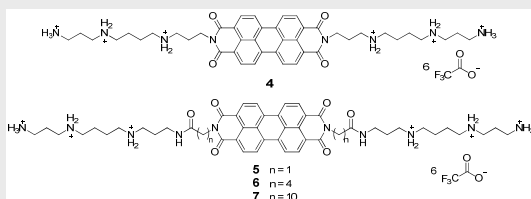
PBI self-assembly into  $\pi$ -stacks in water

Micelles & vesicles: from structure to function

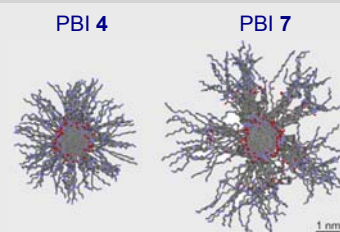
**PBI Interactions with DNA and RNA**

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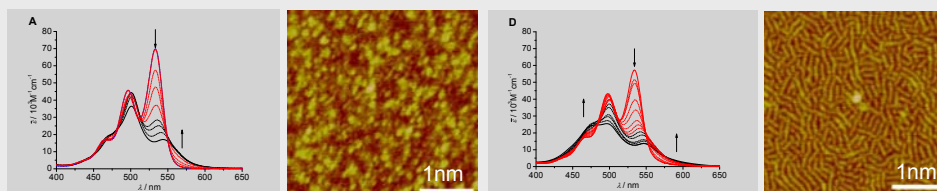
## Ionic PBI Dyes as Supramolecular Building Blocks



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



PBI	4	5	6	7
$\Phi_F$	0.90	0.78	0.72	0.39



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

Chem. Eur. J. 2010, 16, 3372

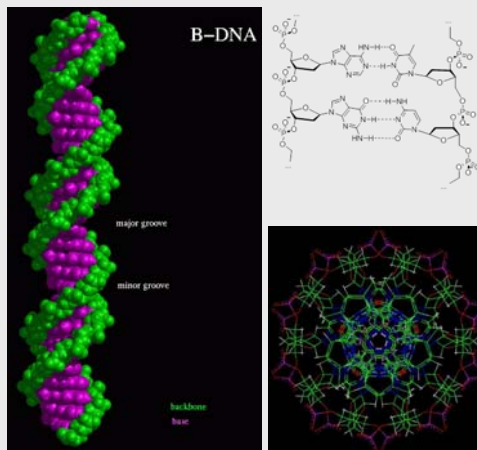
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## DNA (or RNA) as Supramolecular Building Block

- 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?

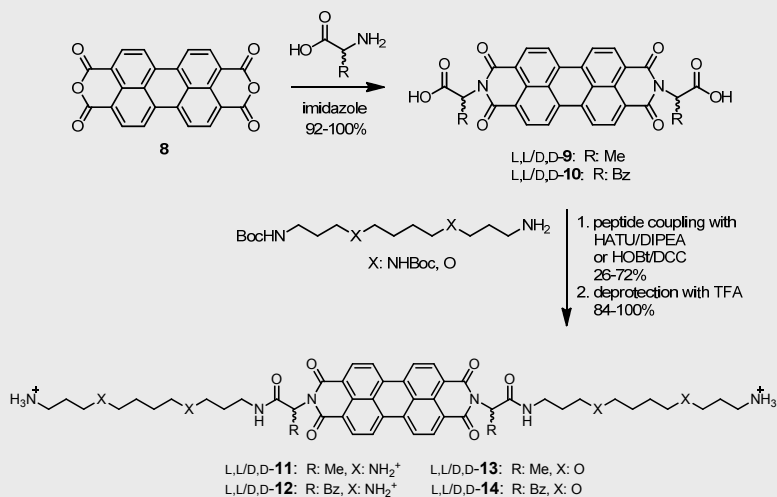


Images: Jena Library of Biological Macromolecules <http://jenalib.fli-leibniz.de/IMAGE.html>; H. R. Drew, R. M. Wing, T. Takano, C. Broka, S. Tanaka, K. Itakura, R. E. Dickerson, *Proc. Natl. Acad. Sci. USA* 1981, 78, 2179–2183; H. Ihmels, D. Otto, *Top. Curr. Chem. Supramolecular Dye Chemistry* 2005,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

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## Synthesis of amino acid connected ionic PBIs

with Ivo Piantanida (Zagreb)



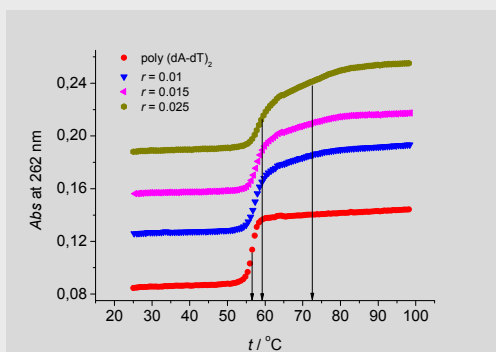
*Chem. Sci.* 2012, 3, 3393 & *Chem. Eur. J.* 2015, 21, 7886

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## Stabilization of polynucleotides with PBIs



L,L/D,D-**11**: R: Me, X: NH<sub>2</sub><sup>+</sup> L,L/D,D-**13**: R: Me, X: O  
L,L/D,D-**12**: R: Bz, X: NH<sub>2</sub><sup>+</sup> L,L/D,D-**14**: R: Bz, X: O



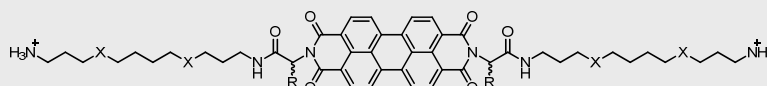
Thermal denaturation of poly(dA-dT)<sub>2</sub> upon addition of PBI L,L-**13** as an example;  $r$  (PBI/poly(dA-dT)<sub>2</sub>) = 0.01-0.025, pH 7.0 (buffer sodium cacodylate,  $I$  = 0.05 M)

- all PBIs stabilize ds-polynucleotides
- all spermine-substituted PBIs (L,L/D,D-**11,12**) give large  $\Delta T_m$
- for less charged side chains L,L-**13** gives larger  $\Delta T_m$  than D,D-**13** and L,L/D,D-**14**
- often biphasic melting curves

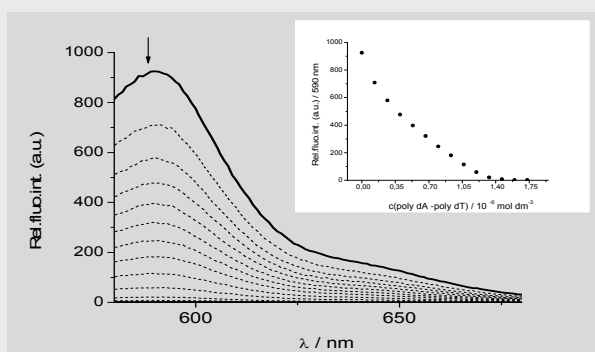
*Chem. Sci.* **2012**, 3, 3393 & *Chem. Eur. J.* **2015**, 21, 7886

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## Binding Constants from Fluorometric Titrations



L,L/D,D-**11**: R: Me, X: NH<sub>2</sub><sup>+</sup> L,L/D,D-**13**: R: Me, X: O  
L,L/D,D-**12**: R: Bz, X: NH<sub>2</sub><sup>+</sup> L,L/D,D-**14**: R: Bz, X: O



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

Scatchard Plots:

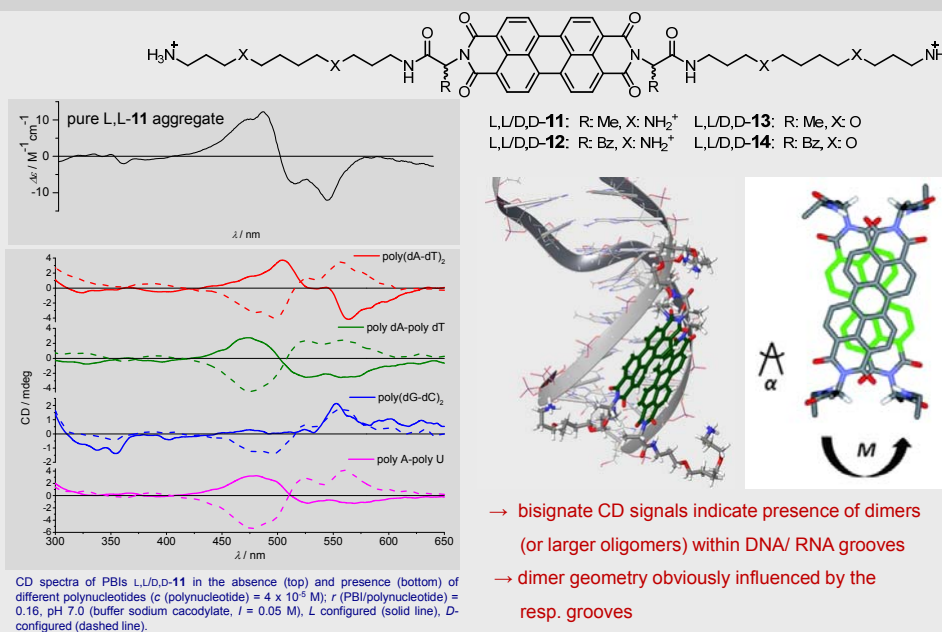
- High binding constants  $\log K_s$   
= 9.2-9.8 (L,L/D,D-**11** and -**12**)  
= 6.5-7.9 (L,L/D,D-**13** and -**14**)

- PBIs are among the strongest groove binders (typical  $\log K_s$  - values are between 5-7)

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

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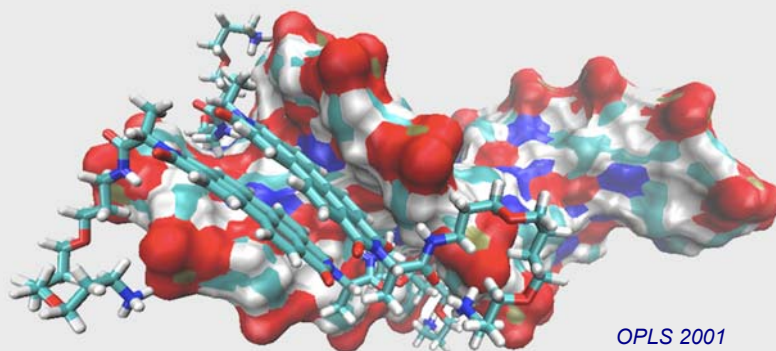
## Circular Dichroism



Chem. Sci. 2012, 3, 3393

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## Molecular Modeling



**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

