Noncovalent Interactions

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Entropically Driven Self-Assembly of Bolaamphiphilic Perylene Dyes in Water

*Daniel Görl and Frank Würthner**

Dedicated to Professor Nazario Martín on the occasion of his 60th birthday

Abstract: *The specific hydrophobic effect involved in the self-assembly of a bolaamphiphilic perylene bisimide (PBI) dye bearing oligoethylene glycol (OEG) chains has been identified. In pure water, the self-assembly is entropically driven and enthalpically disfavored, as explored by optical spectroscopy and isothermal titration calorimetry studies. Besides strong π–π interactions between the PBI units that are primarily of enthalpic nature, the major contribution to the self-assembly is the gain of entropy by release of confined water molecules from the hydration shell of the hydrophilic OEG moieties. Both contributions favor self-assembly, but their countervailing thermodynamic parameters are reflected in an uncommon temperature dependence, which can be inverted upon gradual addition of an organic cosolvent that makes the π–π interaction increasingly dominant.*

The hydrophobic effect plays a crucial role in aqueous self-organization and determines the thermodynamic signature and functionality of enzymes, biological membranes, and other self-assembled systems.[1] Although the complexity and sophistication of the nano- and mesoscale architectures found in living organisms is unmatched, numerous artificial supramolecular architectures based on amphiphilic molecules have been created with various applications at the interface of chemistry, biology, and physics.[2] Among these architectures, self-assembly of amphiphilic π-conjugated molecules provides the most intriguing larger-scale structures,[3] but surprisingly, deeper thermodynamic insights into the self-assembly of these molecules by calorimetric studies have not been reported to date. Thus, the self-assembly of amphiphilic π-conjugated systems is typically attributed to strong π–π interactions that are further augmented by the hydrophobic effect in an aqueous medium. However, how hydrophobic effects exactly operate in the self-assembly of π-conjugated amphiphiles is poorly understood. Decisive aspects, particularly the complex interplay between enthalpic and entropic changes accompanying self-assembly as well as the role of water molecules mediating intermolecular interactions, still remain unclear, thus indicating a lack of fundamental knowledge.

Perylene bisimide (PBI) dyes have emerged as a workhorse in the field of functional dyes that are frequently applied to create supramolecular architectures,[4] particularly in water.[5] In our previous work, we have shown that oligoethylene glycol (OEG) appended, bolaamphiphilic PBI **2** (Figure 1) is subject to a pronounced hydrophobic effect[6] that results in hierarchical growth into extended columnar and lamellar structures in water upon increase of concentration or temperature.[7] This behavior triggered our interest towards a better understanding of the self-assembly of OEG-substituted PBIs in aqueous solution.

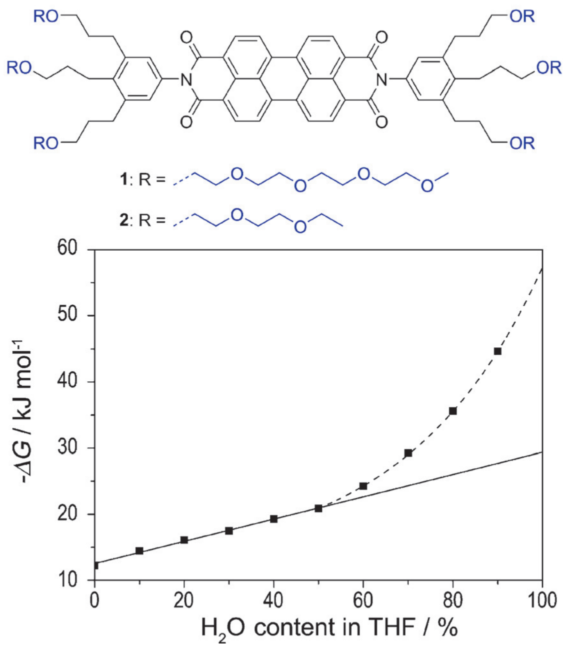

Figure 1. Chemical structures of PBIs **1** and **2** (top) and Gibbs free energies ΔG, of PBI **1** self-assembly in H_2O/THF mixtures at 22 °C (bottom). ΔG values were derived from $\Delta G = -RT\ln K$ where K is the aggregation constant according to the isodesmic model. See the Supporting Information for details.

Herein we report on the surprising outcome of our studies that self-assembly of OEG-appended PBI **1** in pure water is entropically driven and enthalpically even disfavored, which rationalizes the counterintuitive observation of aggregate growth upon heating. As we will further show, successive

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addition of an organic cosolvent results in inversion towards an enthalpy driven self-assembly with aggregate growth upon cooling. Both processes could be characterized by UV/Vis spectroscopy and isothermal titration calorimetry (ITC). MMFF calculations revealed the decisive role of the hydrophilic moiety in determining the thermodynamic signature of the self-assembly of π-conjugated molecules.

In our earlier work,[6] we showed a linear free-energy relationship for the Gibbs free energy ΔG for the self-assembly of OEG-appended PBI **2** with the microscopic solvent polarity parameter $E_T(30)$ for protic and dipolar aprotic solvents but an exceptionally strong aggregation in pure H_2O. We now utilized THF/water mixtures to elucidate the origin of the previously not understood, most pronounced aggregation in H_2O. Notably, whilst our previous work utilized PBI **2** with only three ethoxy repeat units per OEG chain, for the current study we synthesized the new PBI derivative **1** (Figure 1; for synthetic details see the Supporting Information) that contains five ethoxy repeating units per OEG chain to overcome the solubility problems encountered for PBI **2** at higher water contents (Figures S1, and S2).

π–π stacking of PBI **1** is weak in THF, similar to the behavior of PBI **2**. Thus in dilute THF solution (2×10^{-6} M), PBI **1** is monomerically dissolved, as revealed by an absorption profile with well-defined vibronic fine structure, whereas at the same concentration in pure water, PBI **1** forms H-aggregates (Figure S3). To experimentally address this strong aggregation in pure water, we first determined binding constants and related Gibbs free aggregation energies of PBI **1** in various THF/H_2O mixtures by concentration-dependent UV/Vis spectroscopy. In mixtures with water contents up to 70%, a concentration increase revealed the transition from monomeric into aggregated species accompanied by the presence of well-defined isosbestic points (Figure S4a–h). The corresponding data points could be satisfactorily fitted by the isodesmic aggregation model[8,9] (see the Supporting Information for the description of the model, and also Figure S5a), which is consistent with our previous studies on PBIs with shorter OEG chains in organic solvents.[6] For higher water contents, the increased aggregation strength prevents the generation of pure monomeric PBI **1** but the data could still be analyzed by taking into consideration the absorption coefficients obtained for monomeric dyes in more THF-enriched solutions. In pure water, aggregation becomes so strong that only minor spectral changes are observed (Figure S4k), thus only a rough estimation for the Gibbs free binding energy could be derived ($\Delta G < -50\ \mathrm{kJ\,mol^{-1}}$).

The plot of the ΔG values for PBI **1** aggregation versus solvent composition reveals a monotonic increase of the Gibbs free binding energy with increasing H_2O content (Figure 1, bottom). However, a rather linear relationship is observed up to 50% H_2O content, whereas further H_2O addition leads to a drastic increase of the binding strength, thus suggesting the presence of a pronounced hydrophobic effect. In order to unravel the hydrophobic effect that is governing PBI **1** self-assembly, we next conducted temperature-dependent experiments. Upon temperature increase hypochromism is observed (Figure 2a), which is indicative of the growth of the aggregates into larger structures.[9] Concomitantly, the excimer-type emission, indicative of larger aggregate species,[7] is remarkably enhanced upon temperature increase (Figure S6a). The plot of the normalized fluorescence intensity of the maximum (652 nm) versus temperature reveals a nonlinear relationship between fluorescence quantum yield and temperature (Figure S6b), which is due to a rigidification of the aggregate at higher temperatures (Figure S7). We would like to emphasize that these temperature-dependent measurements are highly reversible, thus suggesting that PBI **1** forms thermodynamically equilibrated aggregates. These experiments demonstrate that aggregation in water is enhanced at elevated temperatures, which has rarely been reported for π-conjugated molecules[10,11] and is even atypical for PBIs[12] whose aggregates commonly break upon temperature increase.[13]

Indeed, this specific behavior can be inverted into the more familiar temperature response by the addition of small amounts of an organic cosolvent (Figure 2b,c). Thus, in H_2O/THF 9:1 only minor spectral changes are observed within a broad temperature interval (Figure 2b), suggesting that in this solvent mixture aggregation is almost independent of temperature. Already in H_2O/THF 8:2 a raise of temperature results in breaking of PBI **1** aggregates, illustrated by the partial upcoming of the monomeric absorption band at 529 nm (Figure 2c), which is in agreement with the common temperature response of self-assembled π-systems in organic solvents. It is important to note that a broad temperature interval is still necessary to obtain significant absorption changes.

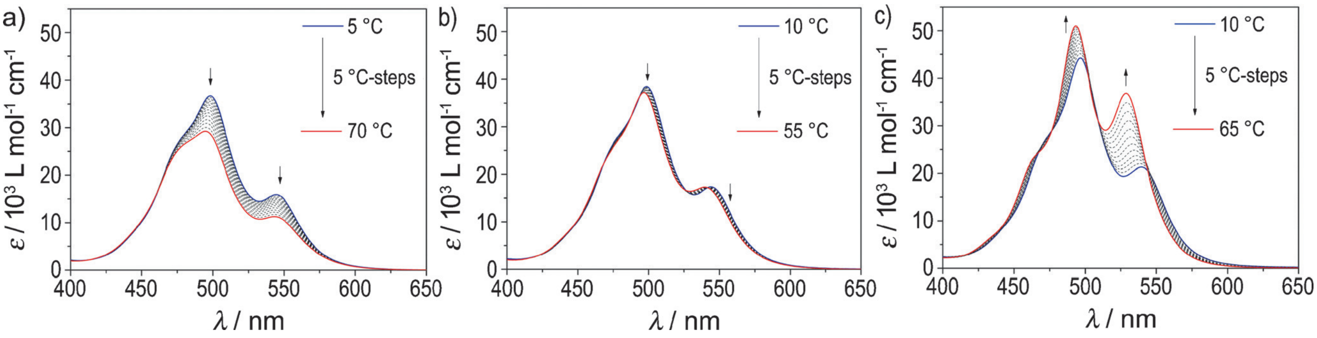

Figure 2. Temperature-dependent UV/Vis spectra (density corrected) of PBI **1** in a) H_2O ($c = 3.3\times10^{-5}$ M), b) H_2O/THF 9:1 ($c = 2.3\times10^{-5}$ M) and c) H_2O/THF 8:2 ($c = 2.2\times10^{-5}$ M). Arrows indicate spectral changes upon heating.

As the temperature responsiveness of a process is deliberately encoded in its thermodynamic signature, these experiments are suggestive of an entropic driving force of PBI **1** self-assembly in pure water and an enthalpic driving force in organic media. To get an insight into the thermodynamic signature, we next conducted ITC dilution experiments at temperatures of 22 °C and 30 °C (Figures 3, S8, and S9). In pure water, dissociation of PBI **1** aggregates induced by the injection of a highly concentrated solution of PBI **1** into the respective solvent produced an exothermic heat flow. Thus, self-assembly of PBI **1** is not only entropically driven but even enthalpically disfavored, a surprising fact, since strong π–π interactions[14] are known to be operative in PBI self-assembly as demonstrated by the H-aggregate UV/Vis absorption bands (Figure 2 and S3). Like the temperature responsiveness, the signature of heat release is inverted upon gradual addition of THF. Whereas in H_2O/THF 9:1 no heat flow could be detected, the inversion is again observed in H_2O/THF 8:2 (Figure S9). Although rather weak signals are observed, the endothermic dissociation is clearly displayed in Figure S9 b. Self-assembly in this solvent mixture is thus enthalpically favored as usual for π-conjugated molecules in organic media.

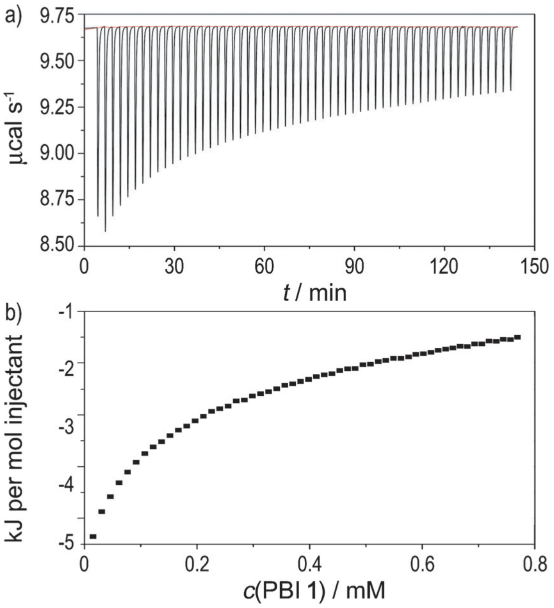

Figure 3. ITC dilution experiment of PBI **1** in H_2O. a) Heat release per injection of an aqueous solution of PBI **1** ($c = 5.1 \times 10^{-3}$ M) into pure water at 22 °C. b) Corresponding enthalpogram.

In order to understand the specific hydrophobic effects involved in the self-assembly of PBI **1**, it is inevitable that solvation of the amphiphilic solute has to be taken into account. Whether the self-assembly of π-surfaces is enthalpically dominated or not has not been addressed in detail to date. However, the seminal work of Diederich and coworkers on the inclusion of pyrene into water-soluble cyclophane cavities[15] as well as complexation studies on cationic porphyrins[1d,16] disclosed that assembly of π-surfaces follows common solvophobic effects. Importantly, such processes are of an enthalpic nature, originating from dispersion interactions that are enhanced in water.[17] The vast majority of reported PBI assemblies in water accordingly shows temperature-dependencies that correspond to enthalpically driven processes, which are indicated by dissociation of dye assemblies upon increasing temperature.[13] Hence, the entropic driving force observed for PBI **1** self-assembly originates from the hydrophilic moiety and countervails the enthalpic contribution from the π–π interaction.

Addressing the contribution of hydrophilic head groups to the overall self-assembly of amphiphilic molecules constitutes a major challenge in aqueous self-assembly. However, the fact that OEG–PBI bolaamphiphiles show clouding at particular temperatures upon heating[7b] is indeed strong evidence that the OEG groups are surrounded by a well-defined hydration shell. For shorter OEG chains these clouding points, associated with the lower critical solution temperature (LCST), can be as low as 26 °C,[7b] whilst for PBI **1** the LCST is raised to 69 °C (at $c = 4.9 \times 10^{-4}$ M) because of the larger number of hydrophilic OEG units (Figure S10). The crucial impact of the OEG chains is related to highly directional hydrogen bonds between OEG oxygen atoms and the surrounding water molecules that are known to be orientationally restricted.[11] As such the hydrated OEG head group opposes the self-assembly tendency of the PBI cores because of its steric demand. Thus, self-assembly enforces release of water molecules into the bulk, that is an entropically favored process (Figure 4 a). Consequently, the loss in interaction energies attributed to the breakage of hydrogen bonds between OEG and water overwhelms the enthalpic contribution arising from the strong π–π interaction, and results in an overall enthalpically disfavored self-assembly of PBI **1** in water.

An increase in temperature results in a weakening of the interaction between OEG units and water molecules of the hydration shell and hence the release of water affords a decreasing spatial demand of the hydrophilic moiety (Figure 4 b). As a consequence, π–π stacking occurs and eventually leads to a critical temperature (LCST) at which complete phase separation between π-stacked PBI molecules and water is observed, resulting in precipitation.

For further insight into how water mediates the self-assembly of PBI **1**, we next simulated the interplay between one hydrophilic head group of PBI **1** and different amounts of water molecules by MMFF geometry optimization (Figure 4 b, left; Figure S11). These simulations point to the fact that the presence of water in close proximity to the hydrophilic moiety results in a stretching of the OEG chains. This is reasonable, as the water molecules (or water clusters) likely bridge OEG units because of chelating effects. Thus, a well-hydrated state predominantly comprises conformations of the hydrophilic moiety that impede self-assembly. The release of such confined interchain-bound water molecules upon self-assembly should then contribute to the entropic driving force of PBI **1** self-assembly along with the positive net change of enthalpy.

The interplay between OEG units and water is significantly altered upon addition of an organic cosolvent. In the present case, the gradually added THF molecules compete with the OEG chains for the interaction with the water

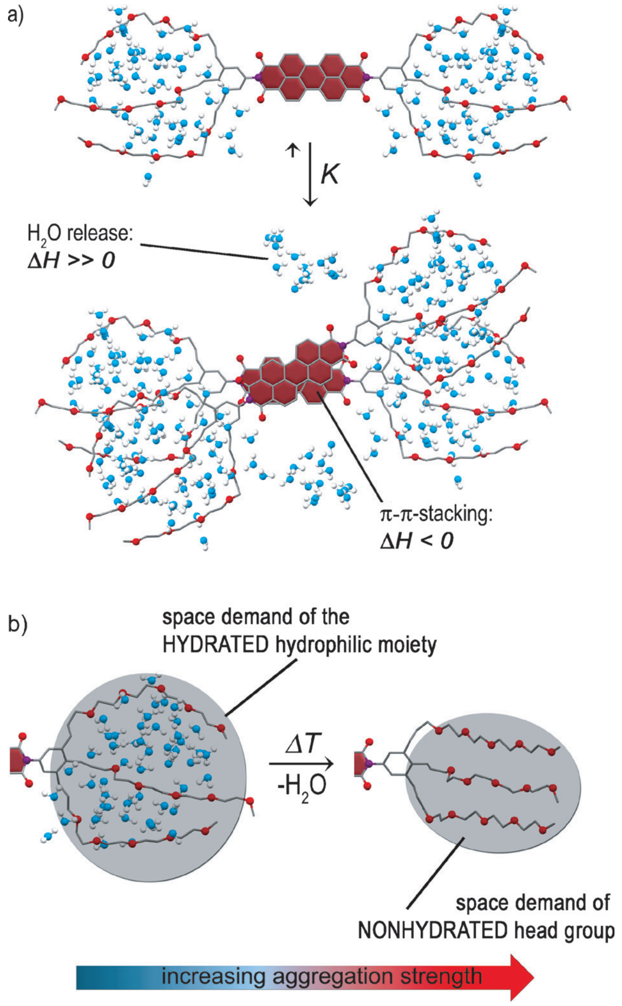

Figure 4. a) Illustration of the release of water molecules from the hydrophilic moiety as entropic driving force and decisive origin of the enthalpically disfavored self-assembly of PBI **1**. b) Illustration of the different spatial demands of the hydrophilic head group based on geometry optimization of PBI **1** in the presence and absence of 50 water molecules.

molecules, thus weakening the interaction between the latter. Therefore, the entropic driving force vanishes, thus self-assembly of PBI **1** in a specific solvent composition (H_2O/THF 9:1) does not show appreciable temperature-dependent changes, and, upon further THF addition, ultimately adopts the familiar temperature response of PBIs in organic media, that is, breakage of PBI **1** aggregates upon temperature increase. Because of the lack of specific interactions between THF and PBI **1** the π–π interaction becomes increasingly dominant in THF-rich solvent mixtures and below 50 % water content prevails entirely with no noticeable deviation from a linear relationship between binding strength and solvent composition (Figure 1). The entropic driving force is therefore solely attributed to the unique conformational arrangement of the hydrophilic OEG brush-type substituent in water.

In summary, we have demonstrated that the hydrophilic OEG moiety essentially determines the thermodynamic signature of the self-assembly of bolaamphiphilic PBI **1** in water. The entropically driven release of confined water molecules from the well-defined hydration shell into bulk water even countervails the favorable enthalpy change arising from strong π–π stacking interactions between the PBI units upon self-assembly. The thermodynamic signature is inverted upon addition of THF and at a composition H_2O/THF 9:1, PBI **1** self-assembly shows the peculiarity of temperature independency. The use of the OEG motif as water-solubility providing element appears to be a promising strategy to induce entropically driven self-assembly of amphiphilic π-conjugated molecules, giving rise to uncommon temperature dependencies. Taking the tremendous diversity of hydrophilic moieties into account, we expect that the present study will stimulate further exploration of aqueous self-assembly of amphiphilic molecules, particularly π-systems, in order to bridge the gap towards nature's fascinating molecular ensembles and to unravel the physicochemical principles that govern their formation.

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Keywords: entropy · hydrophobic effect · perylene bisimides · π–π stacking · self-assembly

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