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Controlled aggregation of peptide-substituted perylene-bisimides†

Joseph K. Gallaher,[ab] Emma J. Aitken,[ac] Robert A. Keyzers[ac] and Justin M. Hodgkiss*[ab]

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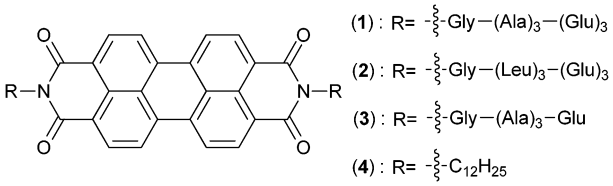
Controlled aggregation of perylene bisimides in multiple modes has been achieved *via* symmetric substitution with peptides. Using optical probes of aggregation, the balance of hydrophobic and electrostatic forces are found to play a key role in directing self assembly and are exploited *via* solvent, pH, and specific extrinsic ion effects.

Molecular semiconductors provide electronically versatile building blocks that must be assembled in order to be exploited in electronic devices.[1,2] The electronic responses of such devices are sensitive to the local geometric configurations of collections of molecular units since conduction relies on weak electronic coupling between out-of-plane π orbitals of neighbouring molecules.[3] Their optical properties also reflect a local electronic environment, making optical spectroscopy an excellent probe of the nature of assembly.[4]

Inspired by the remarkable configurational specificity of proteins, synthetic peptides have emerged as a powerful new class of components to template molecular self-assembly.[5,6] Tethered peptides have been shown to induce the formation of well-defined fibres,[7] sheets[8] and vesicles[9] in solution *via* the balance of electrostatic, hydrogen bonding, and solvophobic/philic interactions under various conditions.[10] Peptides therefore offer a promising means to direct the specific assembly of molecular semiconductors for electronic device applications.[11]

Here, we report our investigations of the assembly of peptide-functionalized perylene bisimides **1–3** using optical spectroscopy. Depending on the solvent, we find that aggregation is induced by the peptide or PBI core and strongly modulated by electrostatic repulsions between glutamate residues on the terminus of the peptides. We apply the weakly-coupled H-aggregate model to UV-visible absorption spectra to show that the specific geometric configuration between neighbouring molecules can be tuned through addition of specific ions that simultaneously bind neighbouring peptides.



Peptides were synthesized using standard Fmoc mediated solid-phase peptide synthesis procedures.[12] The symmetrically substituted PBI peptide derivatives **1–3** were delivered in 71–84% yield by reacting perylene tetracarboxylic dianhydride with the amine terminus of respective peptides.[13,14] The resulting products were purified by reversed-phase column chromatography, and their structure identities confirmed by MS, NMR, and FT-IR (see ESI). This modular and simple procedure can be applied to generate a wide variety of symmetrical peptide substituted PBIs.

The peptide sequence in **1** was selected to engender a range of properties that can be used to manipulate its self assembly under different conditions. Compound **1** features; (i) a hydrophobic PBI core adjacent to a glycine linker, (ii) a series of hydrophobic alanine residues capable of forming a hydrogen-bonded *β*-sheet and imparting chirality, and (iii) glutamic acid residues that are hydrophilic, pH sensitive, can be ionized, and can act as ligands.

Fig. 1 illustrates the strong sensitivity of **1** to its solvent environment *via* a series of UV-visible absorption spectra. In the polar aprotic solvent dimethylsulfoxide (DMSO),

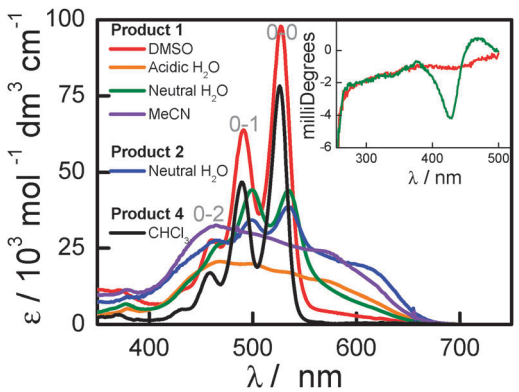


Fig. 1 UV-vis absorption spectra of **1** (3.0×10^{-6} M) in DMSO, MeCN, and H_2O (pH 3, 7), **2** (3.0×10^{-6} M) in neutral H_2O, and **4** (2.0×10^{-6} M) in chloroform, with vibronic bands labelled. Inset: Circular dichroism spectra of **1** in DMSO and in neutral H_2O.

[a] *School of Chemical and Physical Sciences, Victoria University of Wellington, PO Box 600, Wellington, New Zealand. E-mail: Justin.Hodgkiss@vuw.ac.nz*
[b] *MacDiarmid Institute for Advanced Materials and Nanotechnology, New Zealand*
[c] *Centre for Biodiscovery, Victoria University of Wellington, PO Box 600, Wellington, New Zealand*

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the pronounced vibronic progression ($Abs_{0-0} > Abs_{0-1} > Abs_{0-2}$) reflects that of PBI monomers in solution, as shown by the comparison with a dilute solution of alkyl substituted PBI derivative **4** in a good solvent (chloroform). DMSO is therefore considered a good solvent for **1**. On the other hand, the vibronic structure is completely absent from the absorption spectrum of **1** in acetonitrile (MeCN) and the broadened spectrum extends 0.3 eV lower in energy. The pronounced spectral broadening is reminiscent of spectra found in liquid crystalline phases of PBI derivatives[13,15] and indicates long range order with many chromophores contributing to the formation of an exciton band. The peptide precursor from **1** forms a gel in MeCN (see ESI), suggesting that the fibre-forming tendencies of the peptide in MeCN may drive the formation of extended semicrystalline aggregates of **1**.

The UV-visible absorption spectrum of **1** is strongly dependent on pH in water, highlighting the role of the ionizable glutamic acid residues in controlling aggregation. In acidic water (<pH 4), the glutamic acid residues remain protonated (pK_a ~4.2) and the corresponding UV-visible absorption spectrum strongly resembles the aggregates observed in MeCN. The significantly higher pK_a of carboxylic acids in MeCN (pK_a acetic acid ~24) ensures that they will remain protonated. On the other hand, in neutral or basic water where the glutamate form prevails, the UV-visible absorption spectrum of **1** recovers vibronic peaks in addition to an underlying broad absorption from an extended aggregate. The solvent and pH dependence of the absorption spectrum of **1** confirms that its design allows for environment dependent aggregation. Unlike in DMSO, aggregation in water is confirmed by the observation of ~20 nm particles in dynamic light scattering measurements and the emergence of a visible circular dichroism signal corresponding to a PBI aggregate with chirality imparted by the alanine residues.

In order to gain greater control over the extent and nature of aggregation, spectra were recorded as a function of solvent composition. Fig. 2a shows spectra of **1** in solvent mixtures varying from neat MeCN to neat H_2O. Vibronic peaks emerge with the addition of just a few% (by volume) of H_2O to the MeCN solution, suggesting that H_2O induces the reorganization of a significant fraction of **1**. On the basis that oscillator strength is quantitatively redistributed from the broad aggregate band into a more structured vibronic progression, the spectra can be well fit as a linear combination of these components. Fig. 2b shows how the fraction of the vibronically structured component varies with solvent composition, revealing three distinct regions; (1) A strongly aggregated form with broad absorption in <5% H_2O, (2) A rapidly increasing fraction of the chromophores whose spectra resemble monomers with water addition, and (3) formation of a different type of aggregate with vibronic structure at high (>80%) water content. This classification is supported by noting that weak fluorescence observed in region 1 is red-shifted to λ_{max} ~655 nm (see ESI), however the strong fluorescence throughout region 2 (λ_{max} ~544 nm) matches that of a monomer (*e.g.*, dilute 4, see ESI) and its intensity closely follows the population of monomers extracted from the series of absorption spectra (Fig. 2b). Fig. 2b also shows the

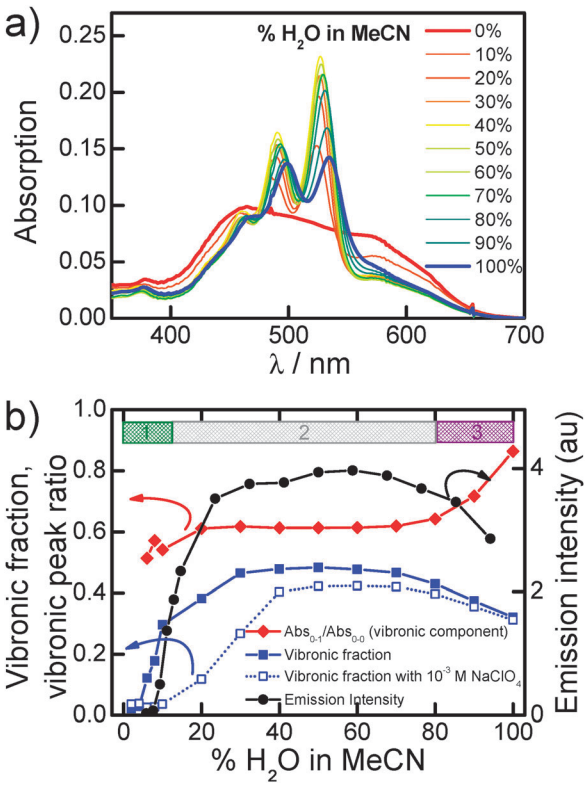


Fig. 2 (a) UV-vis absorption spectra of **1** in mixtures of MeCN and H_2O. (b) Fraction of chromophores with resolved vibronic peaks extracted from the spectral series in (a) and the same series with added salt. Also shown are the vibronic peak ratios and fluorescence intensity as a function of solvent composition with no added salts.

ratio of 0–1 : 0–0 vibronic peak intensities from the vibronically structured component. Up to 80% H_2O, the constant ratio of ~0.6 reflects a PBI core that is not coupled to other chromophores (comparable to that of dilute **4**). However, with higher water content, the vibronic peak intensities are almost equal. The redistribution of oscillator strength to higher energy and relative weakening of 0–0 vibronic peaks is a signature of an H-aggregate because the lowest energy transition for a pair of cofacially stacked chromophores is dipole forbidden, as illustrated in model bridged PBI dimers.[16,17] Unlike other vibronic peaks in the dipole forbidden electronic transition, the zero vibration transition cannot gain oscillator strength by coupling to a vibrational mode.[4] A weakly-coupled H-aggregate in water-rich solutions is rationalized based on the minimization of hydrophobic interactions in a cofacial arrangement. When the alanine residues are replaced with more sterically demanding leucines in **2**, the vibronic signature of a cofacial H-aggregate is lost and a new peak emerges beyond 600 nm that might indicate sterically induced J-aggregate formation.

The pH dependent spectra in Fig. 1 suggest that reorganization of monomers with increasing water content (*i.e.*, the transition from region 1 to region 2) is driven by electrostatic repulsion between ionized glutamic acid residues. To confirm this, we repeated the solvent composition dependence in the presence of an electrolyte to screen electrostatic repulsions. Fig. 2b confirms that the transition is suppressed in the

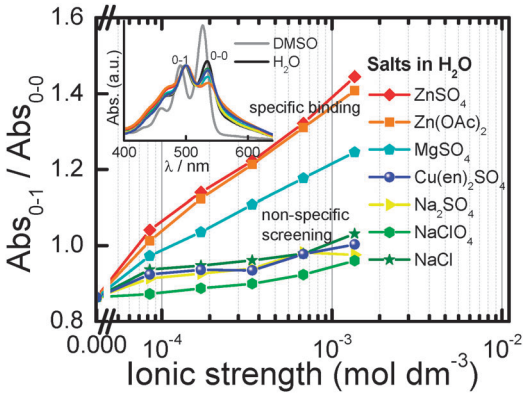
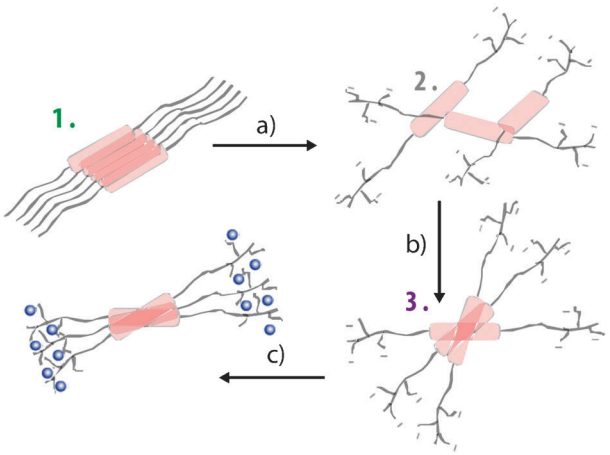


Fig. 3 Ratio of vibronic absorption peaks for **1** in H_2O as a function of ionic strength of various salts. The inset shows spectra of the same salts (Ionic strength = 6.8×10^{-4} M), as well as the spectra of **1** in distilled H_2O and DMSO for comparison.

presence of an electrolyte. Moreover, the comparison of **1** and **3** (see ESI) confirms the dominant reorganising role of electrostatic repulsions because with fewer ionisable glutamic acid residues, **3** is slower to respond to water addition and its response is virtually independent of ionic strength. The need to accommodate charge on the peptide in water may also explain the weaker coupling and limited long range order of **1** in water compared with MeCN.

In order to further interrogate the influence of the glutamic acid residues in templating the aggregation of the PBI core, we titrated **1** against a number of different salts in water. Fig. 3 shows that for most salts, the resulting effect on aggregation can be attributed to the ionic strength rather than the nature of the salt. This includes salts with monovalent ion pairs (NaCl and $NaOCl_4$), divalent anions (Na_2SO_4), and non-coordinating divalent cations (Cu(ethylenediamine)$_2SO_4$). However, salts with divalent cations capable of coordinating to glutamates ($ZnSO_4$, $Zn(OAc)_2$ or $MgSO_4$) exhibit a much more pronounced effect on the spectrum of **1**. Fig. 3 shows that the relative intensity of the 0–0 vibronic peak in the presence of 4.5×10^{-4} M Zn^{2+} salts inverts and diminishes to 0.65 of the 0–1 peak. The observed spectral change indicates increased H-aggregate character in the presence of Zn^{2+} ions but not in the presence of most other ions. We propose that the strong ability of Zn^{2+} ions to coordinate two glutamates from neighbouring molecules of **1** introduces cross-links that twist the PBI cores into a more cofacial geometry rather than merely screening the electrostatic repulsions of glutamates. The similar response of **3** (see ESI) confirms that Zn^{2+} is likely to bridge inter- rather than intramolecularly.

In summary, we have developed a peptide scaffold for driving the controlled assembly of organic semiconductor aggregates. By monitoring the shape of the absorption spectra, we are able to probe the extent and nature of aggregates for different peptide sequences and under a range of conditions. Scheme 1 summarizes the coupled influences of solvent composition, charge and specific ions on the assembly of our peptide-PBI conjugates. Upon forming weakly-coupled H-aggregates in water, the configuration can be further tuned *via* binding specific extrinsic ions to the



Scheme 1 Self-assembly transitions of **1** upon (a) moving from MeCN to a MeCN/H_2O mix, (b) going to high H_2O content, and (c) adding crosslinking dications. The regions labelled 1, 2, and 3 correspond to those in Fig. 2b and described in the text.

peptide. This work demonstrates the ability of peptides to both drive the assembly of organic semiconductor building blocks and gate the electronic coupling between them. Upon applying this understanding to the growth of thin films, we expect this concept to be exploited in electronic devices and biosensors.

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