Well-defined, persistent, chiral phthalocyanine nanoclusters via G-quadruplex assembly

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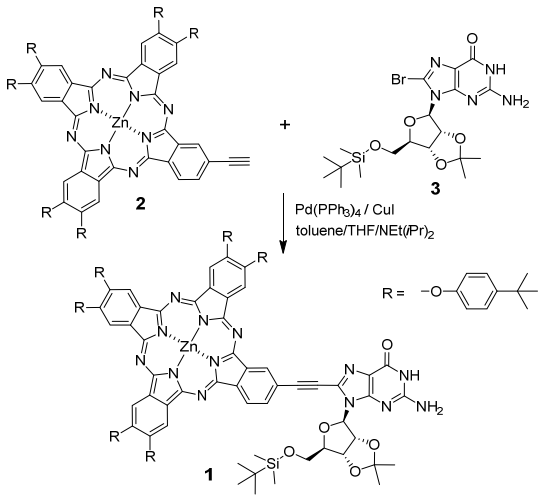
Octameric near-IR dye nanoclusters are produced by complexation of potassium cations with a phthalocyanine-guanosine conjugate. The combination of hydrogen-bonding, K^+ coordination, π–π stacking and steric interactions between the chiral side groups are responsible for defining a specific helical chromophore arrangement in the clusters, which display a high stability and maintain their supramolecular identity onto substrates.

Controlling the aggregation of dyes into organic nanoparticles (ONPs)[1] is increasingly arising scientific attention in view of the potential use of these materials in inks, cosmetics, drugs, theranostics, optoelectronics, sensing and imaging.[2] Conventional ONP production methods,[1,3] typically using emulsion and fast precipitation techniques, often allow a limited and unpredictable control on particle size (usually > 20 nm) and dispersity, as well as on the relative arrangement of the dye components. These are, however, important issues that determine their final attributes. Similar to inorganic nanoparticles, the properties of ONPs lie between those of individual molecules and bulk materials, and thus may exhibit confinement effects caused by their finite size and shape at the nanoscale.[4] These effects can tune, among other properties, absorption and emission features, due to particular stacking arrangements of the dye molecules within the ONP.[4,5]

Noncovalent synthesis[6] arises in this context as a potent tool to construct self-assembled structures in the nanometer scale in which morphology and relative molecular organization can be controlled through the supramolecular information present in the molecules. In particular, recent studies have shown the singular characteristics of guanine (G) self-assembly in providing nanostructures that are monodisperse and persistent in size and shape.[7] In the presence of alkaline salts, multiple noncovalent interactions work in concert to provide discrete and relatively stable G-quadruplexes, which can be regarded as a suitable scaffold to attach other organic functions.[5g,8a]

So far, π-conjugated dyes like porphyrin,[8b] pyrene,[8c] oligo(thiophene),[8d] oligo(phenylenevinylene)[5g] or perylenes[8e,f] have been installed within discrete G-quadruplex frameworks. Of particular relevance are the latter studies by the Wasielewski's group where a significant stabilization of photogenerated radical pairs within the G-quadruplex ensemble was demonstrated,[8e,f] which may bring possible benefits for organic photovoltaic applications. However, in order to realize the full potential of these noncovalent assemblies in functional devices, an effort must be made towards increasing their stability in order to guarantee their structural integrity when processed into thin film architectures.

Herein, we make an advance in demonstrating the viability of this approach by producing dye ONPs with phthalocyanines (Pcs),[9] discotic red-absorbing semiconductors widely used in photovoltaic applications, coupled to a lipophilic G derivative (Pc-G **1**; Scheme 1). In the presence of K^+ cations, Pc-G **1** self-assembles in chiral octameric clusters in organic solvents. The discrete ONPs formed, of about 1.3 nm high and 6.0 nm wide, exhibit an extraordinary uniformity and stability and can be transferred and studied over solid substrates.



Scheme 1. Synthesis of Pc-G **1** by Sonogashira coupling.

Compound **1** was prepared in 12% yield by Pd-catalyzed Sonogashira coupling between unsymmetrically ethynyl-substituted Pc **2**[10] and lipophilic 8-bromoguanosine **3**[5g,7c] (Scheme 1). Such a low-yielding coupling was partly due to the difficulties encountered during the purification of Pc-G **1**, but mainly to the well-known low reactivity 8-bromoguanines in Pd-catalyzed reactions.[11]

Despite the multiple bulky lipophilic groups attached at the periphery, Pc-G **1** turned out to be very insoluble in most solvents (DMSO-d_6, toluene-d_8, $CDCl_3$, THF-d_8) at NMR concentrations and we always observed very broad ^1H signals. This is commonly ascribed to the formation of ill-defined mixtures of H-bonded oligomeric species in dynamic exchange, which may further aggregate by stacking. The only exception was a special 2:1 THF-d_8/DMSO-d_6 mixture, in which aggregation could be prevented and each proton signal became now well-resolved (see Figs. 1 and S1).

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† Electronic Supplementary Information (ESI) available: Synthetic procedures and characterization. Complementary Figures S1-S6 See DOI: 10.1039/x0xx00000x

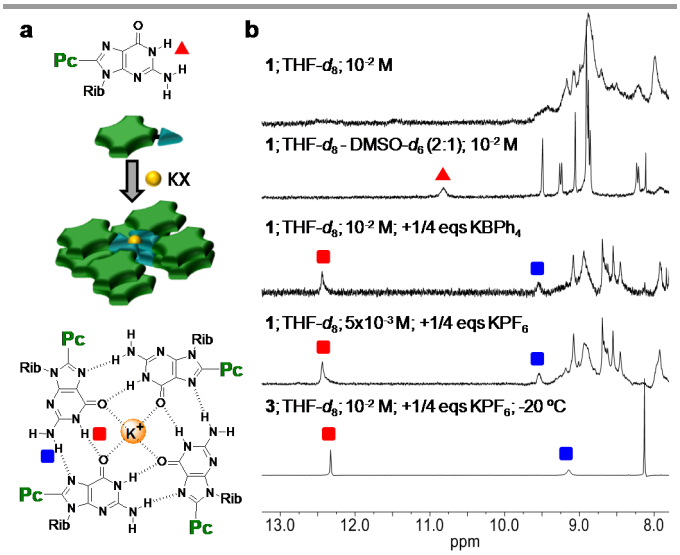

Fig. 1. (a) Pc-G **1** octamer formation in the presence of K^+ salts. The most characteristic amide and amine protons are shown at the monomer and G-quartet states. (b) Low-field region of the ^1H NMR spectrum of **1** at 25 °C in different solvents and concentrations and in the absence or presence of K^+ salts. The spectrum of $(\mathbf{3})_8{\cdot}K^+$ at -20 °C is shown below for comparison.

Then, we studied the aggregation of **1** by ^1H NMR in different solvents (acetone, THF, $CHCl_3$, toluene), in the presence of a small excess (0.25 eqs.) of K^+ or Na^+ salts with diverse anions (I^-, BPh_4^-, PF_6^- or 2,6-dinitro-4-methylphenolate$^-$) (Fig. S2). We noticed that, due to the much larger size of our system when compared to the previously studied guanosine **3**,[7c] the conditions to quantitatively detect well-defined complexes at NMR concentrations were limited to a few K^+ salts in THF. Specifically, the ^1H NMR spectra changed drastically upon addition of 0.25 equivalents of KPF_6 or $KBPh_4$. Particularly revealing are the amide and amine H-bonded signals in the G unit, which shift to 12.5 and 9.5 ppm, respectively (Fig. 1). These shifts, also shown by guanosine **3** (see Figs. 1 and S3), are characteristic of the formation of D_4-symmetric G-octamers,[7c] where two identical G-quartets stack by complexation of a potassium cation. Further confirmation about the size and nature of the assembled complex came from the determination of the relative G/K^+ molar ratio, which can be obtained by integration of the monomer and BPh_4^- signals. These values are 8.9 for Pc-G **1** and 9.4 for Br-G **3**, which supports the formation of 8^{mer} species. In all these experiments, and during the course of our initial salt-solvent screening, we did not observe any sign for the formation of higher G-quadruplexes, which is, on the other hand, reasonable, since we purposely equipped Pc-G **1** with very bulky substituents so that further stacking around the 8^{mer} would be hindered.

We noted, however, a big stability difference in the assemblies of **1** and **3** in the presence of K^+ (see Figs. S4 and S5). Whereas G-quadruplex $(\mathbf{3})_8{\cdot}K^+$ is very sensitive to temperature and concentration changes in THF-d_8 and full dissociation occurs below 10^{-3} M, $(\mathbf{1})_8{\cdot}K^+$ was found to persist along all the temperature (up to 328 K) and concentration range (down to 5 x 10^{-5} M) studied by NMR. We believe that the peripheral Pc units must provide an important additional stabilizing contribution by establishing strong stacking interactions between planar π-surfaces. Despite this stability increase, the very high molecular weight of our complex precluded its characterization by MS (ESI Q-TOF or MALDI-TOF).

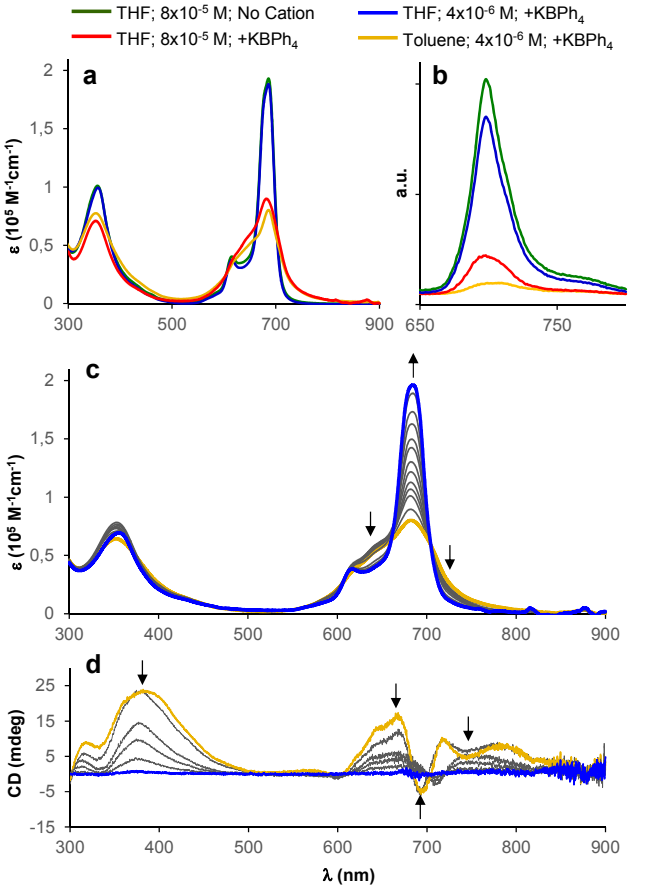

Fig. 2. (a) Absorption and (b) normalized emission spectra of **1** in different solvents and concentrations and in the absence or presence of $KBPh_4$ salts. Changes in the (c) absorption and (d) CD spectra of $(\mathbf{1})_8{\cdot}KBPh_4$ by increasing the THF content of toluene-THF $4x10^{-6}$ M solutions from 0% to 50% v/v.

Our attention was then turned to optical spectroscopy experiments, namely absorption, CD and emission spectroscopy. We first studied relatively concentrated ($8x10^{-5}$ M) THF solutions of $(\mathbf{1})_8{\cdot}KBPh_4$ in order to overlap ^1H NMR and optical spectroscopy information. The spectral characteristics monitored (Fig. 2a) – a broad Pc absorption *Q*-band, the appearance of CD Cotton effects,[12] and a quenched emission with respect to monomeric **1** in the same conditions – provided clear signs of strong Pc stacking and were thus ascribed to the Pc-G **1** complexes observed in NMR. However, further dilution in THF down to $4x10^{-6}$ M resulted in the recovery of the monomer spectroscopic features (Fig. 2a), which indicated that the octameric clusters are dissociated at very low concentrations in this solvent, as it was observed for $(\mathbf{3})_8{\cdot}K^+$ in the 10^{-2}-10^{-3} M range.[7c] These observations underline again the superior stability of $(\mathbf{1})_8{\cdot}K^+$ due to strong stacking interactions between Pc cores.

Further stabilization can be achieved in apolar solvents. Dilution experiments down to $4x10^{-6}$ M in toluene resulted now in the conservation of the spectroscopic features attributed to $(\mathbf{1})_8{\cdot}K^+$. Temperature-dependent studies revealed that the Pc nanoclusters are not dissociated in this solvent within the 293-363 K range (Fig. S6). However, increasing the THF content of toluene-THF $4x10^{-6}$ M solutions of $(\mathbf{1})_8{\cdot}KBPh_4$ led progressively to complex dissociation,

monitored by the recovery of the monomer sharp absorption *Q*-band and the disappearance of the CD signals (Fig. 2b).

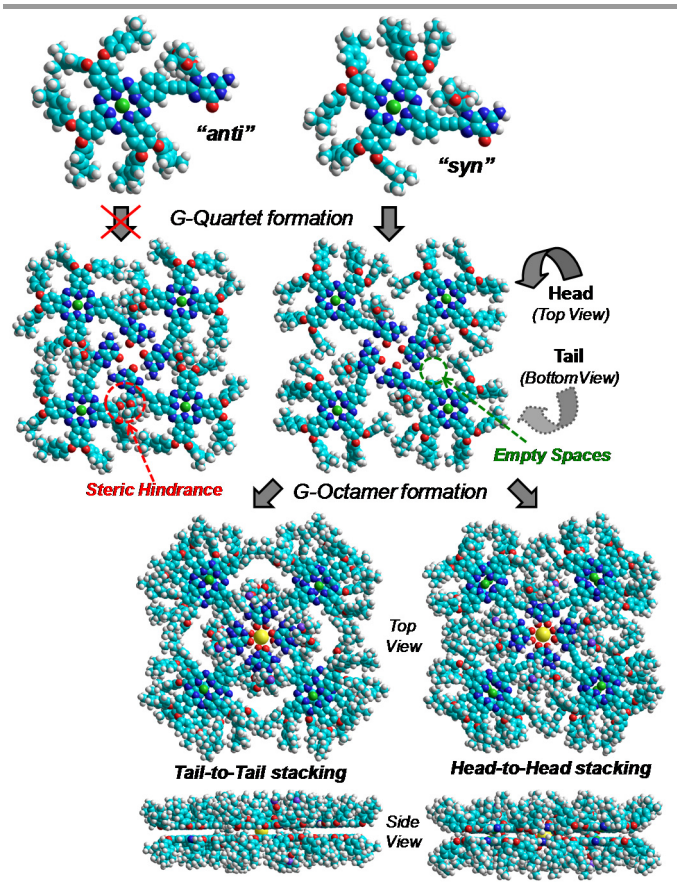

Fig. 3. Stepwise model construction from the Pc-G **1** monomer to the corresponding $(\mathbf{1})_8{\cdot}K^+$ octamers. Atom color code: C: light blue, H: white, N: dark blue, O: red, Zn: green, Si: purple, K: yellow.

In order to shed light into the internal structure and the relative chromophore arrangement in these Pc nanoclusters we performed molecular modeling studies at the PM7 level. Two main conformations were optimized for **1**, that we call *anti* and *syn*, depending whether the bulky ribose and the Pc core are on opposite or the same side, respectively, with respect to the ethynylene rotation axis. Please, do not confuse these conformations with those adopted by the ribose by rotation around the glicosidic C-N bond. When these *anti* and *syn* conformers cyclotetramerize in G-quartets, two faces are defined depending on the direction of the circular arrangement of H-bonds (taken from the Watson-Crick donor edge to the Hoogsten acceptor edge): a *head*, where this arrangement is clockwise, and a *tail*, where the direction is counter-clockwise.[7a,13] During model construction (see Fig. 3) we immediately noticed that cyclotetramerization of **1** in the *anti* conformation would be impossible due to obvious steric interactions between the ribose of one molecule and the closest Pc-isoindole group of the adjacent one. Hence, the G-quartets must be constructed by the exclusive participation of *syn* isomers.

The symmetry observed by ^{1}H NMR revealed that both quartets in $(\mathbf{1})_8{\cdot}K^+$ are identical, so the octamer must be formed by either *head*-to-*head* or *tail*-to-*tail* G-quartet stacking. We built both models (Fig. 3) and noticed that stacking can only be achieved with a specific rotation angle between tetramers around the central octacoordinated K^+ ion. This unique intermolecular arrangement is imposed by the ribose groups, which must fix their conformation so that each of the isopropylidene moieties fit in the empty space left by the adjacent quartet (see Fig. 3). The larger TBDMS groups do not fit in these empty spaces and are forced to arrange toward the upper and lower faces of the octamers. Together with the bulky *tert*-butylphenoxy groups, this arrangement should prevent further $(\mathbf{1})_8{\cdot}K^+$ stacking, a phenomenon that we did not see experimentally.

Both *head*-to-*head* and *tail*-to-*tail* octamer models reveal similar intermolecular Zn-Zn distances (3.6 Å and 3.9 Å, respectively) and tilt angles between Pcs (68° and 59°, respectively), defined by the Zn-Zn vector and the Pc aromatic plane. According to the molecular exciton approximation developed by Kasha,[14] these calculated tilt angles should lead mostly to aggregates with blue-shifted absorption and emission bands and with lower emission intensity when compared to the monomer, which is in agreement with our measurements. This contrasts our previous results on related assemblies of oligo(phenylenevinylene),[5g] in which the π-conjugated moieties were arranged in *J*-type aggregates with enhanced emission due to their different rod-like structure.

In order to confirm the integrity and uniformity of the complexes and evaluate their dimensions, we dropcasted diluted 4×10^{-6} M toluene solutions of $(\mathbf{1})_8{\cdot}KBPh_4$ onto HOPG or mica substrates and, after slow solvent evaporation, imaged the surface by atomic force microscopy (AFM; Fig. 4). Each substrate revealed very distinct features that were rationalized by the different strength of the surface-ONP interactions, which must influence their 2D mobility upon physisorption. Isolated nanoparticles could only be observed onto HOPG, whose dimensions – 1.2 ± 0.5 nm height and 10 ± 5 nm width (considering the convolution with a tip of radius of curvature of *ca.* 15-20 nm)[15] – nicely match those estimated for $(\mathbf{1})_8{\cdot}K^+$ (1.3 nm height and 6.0 nm width). On the contrary, on the more polar mica substrate, we imaged larger and rather uniform squared-shaped objects with the expected height (1.4 ± 0.5 nm) for a sandwiched octamer, but with a larger width (*ca.* 100 nm). We ascribe these features to laterally aggregated $(\mathbf{1})_8{\cdot}K^+$ nanoclusters that are formed on the substrate during solvent evaporation. It should be remarked that these discrete particle-like structures were only detected in the presence of potassium salts, and that the formation of elongated, fiber-like structures that could have been formed by further aggregation of Pc-G **1** quartets was never observed.

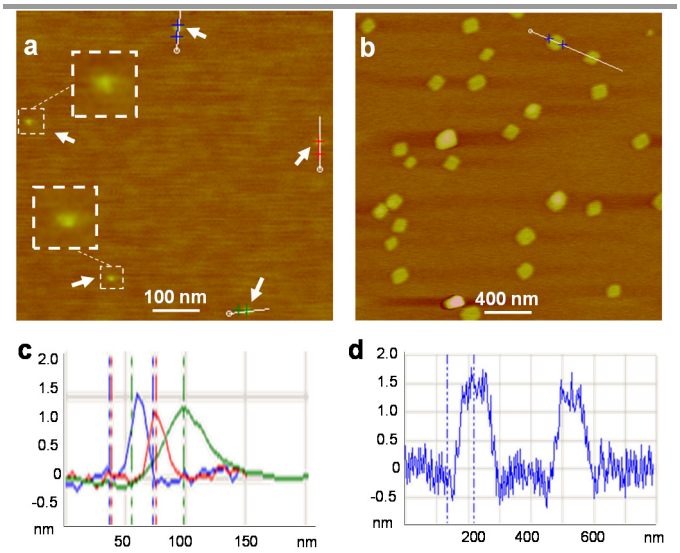

Fig. 4. (**a**,**b**) Height-mode AFM images of dropcasted $4x10^{-6}$ M toluene solutions of (**1**)$_8$·$KBPh_4$ onto freshly cleaved HOPG (**a**; the arrows indicate particle position and the insets show 2 magnified images) or mica (**b**). (**c**,**d**) Corresponding eight profiles along the lines shown in (**a**,**b**).

In conclusion, the special features of G-quadruplex self-assembly, where several supramolecular interactions work cooperatively to impart stability to the assemblies and, at the same time, limit their growth to a certain extent, have been successfully profited in this work to produce uniform, monodisperse Pc nanoclusters. These assemblies comprise exactly 8 macrocyclic red-absorbing dyes that are H-bonded in a well-defined, chiral helical arrangement, defined by the G-K^+ coordination and steric interactions between the chiral side groups within the complex. The clusters maintain their supramolecular identity when transferred onto HOPG or mica substrates and their dimensions could be resolved by AFM. The use of this self-assembly strategy appears as versatile and general, and may allow the noncovalent synthesis of a wide diversity of functional well-defined nanoclusters.

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