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Energy Transfer Assemblies Composed of Expanded Porphyrin-Oligonucleotide Conjugates

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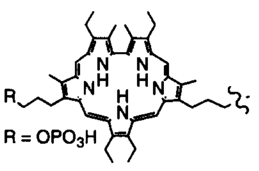
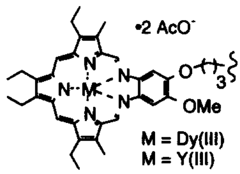
Abstract: Energy transfer assemblies composed of expanded porphyrin-oligonucleotide conjugates **1-3** are described. The donor and acceptor pair composed of the 3'-sapphyrin conjugate **1** and the 5'-Y(III) texaphyrin conjugate **2**, upon irradiation with 622 nm light, gave results consistent with energy transfer.

Considerable research effort has focused on the study of non-covalent energy transfer systems.[1-11] A primary reason for the design of such supramolecular assemblies is to mimic and study the energy- and electron-transfer processes occurring in natural photosynthetic systems.[1-4,12] In previous work in this area, we have used nucleobase and salt-bridge recognition features to provide information about energy transfer events.[2-4] While informative, this earlier approach is potentially limited in that it is predicated on the use of systems that can only be studied in organic media. In order to extend our studies of non-covalent energy phenomena into the aqueous realm, we have prepared a set of expanded porphyrin-containing conjugates, **1-3**, that can be 'mixed and matched' to form two pairs of non-covalent donor-acceptor assemblies in 10 mM bis-tris, 100 mM NaCl, pH 7.0 buffer (Figure 1). The resulting ensembles constitute novel oligonucleotide-chromophore conjugate systems. Currently, several energy transfer assemblies composed of oligonucleotide-chromophore conjugates are known. However, these latter have been primarily developed and studied in the context of preparing nucleic acid-based diagnostic probes.[5-11,13]

1 3'-sapphyrin-PO_4- AAA AAG TCG TCA TCA G-5'
2 5'-Y(III) texaphyrin-PO_4-TTT TTC AGC AGT AGT C-3'
3 5'-Dy(III) texaphyrin-PO_4-TTT TTC AGC AGT AGT C-3'



sapphyrin



texaphyrin

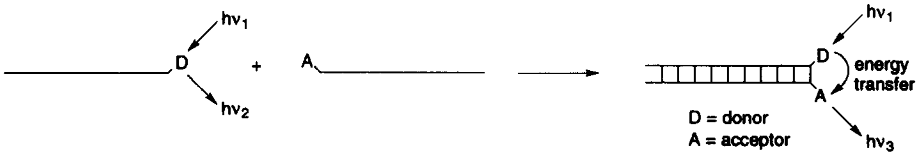


Figure 1. Schematic representation of the bimolecular oligonucleotide-based energy transfer system. As conceived, energy transfer from the donor to the acceptor is expected to occur upon binding of the donor-oligonucleotide conjugate to the acceptor-oligonucleotide conjugate and ensuing irradiation.

Conjugates **1-3** were synthesized on a commercial DNA synthesizer in which phosphoramidite derivatives of the macrocycles were attached to the appropriate sequence in place of either the first or the last nucleobase to form a 3' or 5' conjugate, respectively.[14] Standard oxidation, capping, and washing cycles, followed by removal from the solid support and deprotection using ammonium hydroxide/methylamine, gave the conjugates in solution which were then purified using preparative polyacrylamide electrophoresis.

Once in hand, the photophysical properties of conjugates **1-3** were investigated in order to assess their suitability for use in oligonucleotide-based energy transfer systems. Towards this end, the UV-visible absorption and excited singlet state fluorescence emission spectrum of each conjugate was recorded. In each case, the absorption spectra revealed peaks characteristic of both the expanded porphyrin and oligonucleotide portions of the conjugate, whereas the corresponding fluorescence spectra showed features arising from the macrocycle alone (Table 1). The fluorescence quantum yields for each of the conjugates was calculated by comparison to a standard, quinine bisulfate.[15] The Dy(III) texaphyrin conjugate **3** did not exhibit appreciable fluorescence, presumably due to the presence of the paramagnetic cation.

The excited state energy levels were calculated from the intersection of the absorption and fluorescence spectra (Table 1). For each energy transfer system, the donor was selected as the higher energy member of the given complementary conjugate pair. Using this criterion, two possible energy transfer "pairs" were identified among the set of permutations defined by conjugates **1-3**, namely the complexes formed between the sapphyrin conjugate **1** and the texaphyrin conjugates **2** and **3**, respectively.

Table 1. Photophysical properties of the macrocycle-oligonucleotide conjugates.

	absorbance maxima (nm)	fluorescence maxima (nm)	excited state energy level (eV)	fluorescence quantum yield (ϕ_f)
3'-sapphyrin **1**	258, 426, 453, 622, 678	688, 765	1.95	0.0260
5'-Y(III) texaphyrin **2**	258, 423, 480, 735	742	1.75	0.1170
5'-Dy(III) texaphyrin **3**	258, 429, 480, 736	744	1.72	0.0004

Within pairs **1-2** and **1-3**, even after hybridization, both the donor and acceptor chromophores could retain considerable rotational freedom as the result of the flexible tethers used to link the expanded porphyrin subunits to the oligonucleotide "backbones". Therefore, a random orientation was assumed for the chromophores and an average value of 2/3 was used for the orientation factor. Using this value and the overlap integral, J, calculated from the overlap of the emission spectrum of the donor and the absorption spectrum of the acceptor, it proved possible to estimate the so-called Förster distance (R_o), the point at which the energy transfer process is predicted to be 50% efficient. For the 3'-sapphyrin **1**/5'-Y(III) texaphyrin **2** conjugate pair and the 3'-sapphyrin **1**/5'-Dy(III) texaphyrin **3** conjugate pair, the overlap integrals were calculated to be 4.45×10^{-13} and 4.69×10^{-13}, respectively. On this basis, the critical Förster distances for both pairs were estimated to be *ca.* 36 Å. This corresponds to a chromophore-to-chromophore separation that is larger than that which is predicted to pertain in the hybridized conjugate-conjugate pairs. Thus, these calculations served to support the notion that long-range donor-to-acceptor energy transfer should be possible within pairs **1-2** and **1-3**.

Steady state fluorescence titrations were carried out with the donor-acceptor conjugate pairs to evaluate the extent to which (if at all) energy transfer takes place between the chromophores. In each case, the donor conjugate was irradiated at a wavelength where it displayed strong absorbance and where the acceptor conjugate exhibited much less absorbance (i.e., 622 nm in the case of pairs **1-2** and **1-3**). In terms of actual experiment, the putative energy transfer process was monitored by looking at the extent to which the fluorescence of the donor was quenched upon the addition of the acceptor. In the case of both pairs, namely **1-2** and **1-3**, *ca.* 65% quenching of the sapphyrin emission was observed. In the case of the first of these pairs, the decrease in sapphyrin emission intensity was also reflected in terms of an enhanced fluorescence emission signal, ascribable to the Y(III) texaphyrin acceptor subunit (Figure 2(a)). This system, by virtue of possessing a fluorescent acceptor chromophore, thus provides more definitive support for the proposed non-covalent energy transfer process.[16]

Control studies in which the appropriate unmodified complementary oligonucleotide or the oligonucleotide-free water-soluble chromophore acceptor was added to the donor conjugate in place of the acceptor conjugate were carried out for both systems. Although the emission of the donor conjugate was unaffected upon addition of the unmodified complementary oligonucleotide, modest quenching was observed when the unconjugated chromophore acceptors were added (e.g., Figure 2(b)). Thus, although oligonucleotide-free Y(III) texaphyrin can mediate some quenching, it does so at a level that is insufficient to explain the dramatic quenching results observed within the hybridized donor-acceptor conjugate pairs.[17]

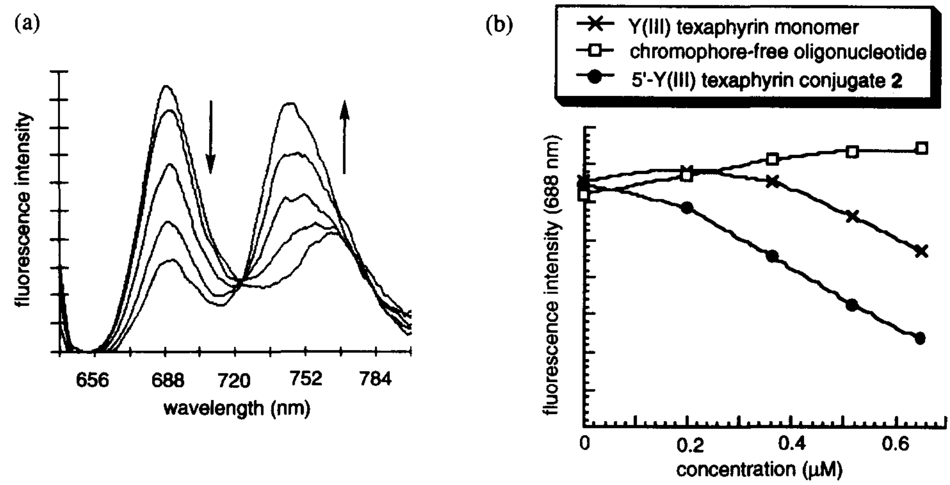


Figure 2. (a) Overlay of fluorescence emission spectra for a 0.5 μM solution of 3'-sapphyrin conjugate **1** recorded in the presence of increasing concentrations of 5'-Y(III) texaphyrin conjugate **2** (from 0 to 1.2 equiv) in 10 mM bis-tris, 100 mM NaCl buffer, pH 7.0. Upon irradiation with 622 nm light, the initial sapphyrin emission at 688 nm (0 equiv) decreases with a concomitant increase in the Y(III) texaphyrin-based emission band at 742 nm (1.2 equiv). The arrows indicate the growth or decay of the indicated emission band throughout the titration. (b) The change in fluorescence intensity at 688 nm (quenching) for 0.5 μM 3'-sapphyrin conjugate **1** observed upon the addition of 0-1.2 equiv of unconjugated Y(III) texaphyrin monomer, unmodified complementary oligonucleotide, and 5'-Y(III) texaphyrin conjugate **2**.

The first generation systems described herein provide the framework for the design of energy transfer systems which incorporate expanded porphyrins in more complex, well-defined, hydrogen bonding assemblies. Indeed, work is currently underway to prepare systems that will allow the study of energy transfer processes within DNA architectures of more optimized structure.

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13. An ancillary advantage of conjugates **1-3** is that they could define nucleic acid probes with improved, red-shifted spectral characteristics. For a discussion of the advantages that could accrue for *in vivo* applications of such properties, see: Sessler, J. L.; Sansom, P. I.; Král, V.; O'Connor, D.; Iverson, B. L. *J. Am. Chem. Soc.* **1996**, *118*, 12322-12330.
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16. The difference between the efficiency (50%) at the calculated Förster critical distance (36 Å) and the observed efficiency (65%) in the conjugate-derived ensemble reflects the fact that the overlap integrals and orientation factors used to calculate Förster critical distances are approximate. Moreover, the actual center-to-center distance between the chromophores in the duplex formed between the oligonucleotide conjugates is difficult to estimate because of the flexible attachment to the oligonucleotide. This distance is, however, expected to be less than 35 Å, which is consistent with the greater efficiency which is observed.
17. Under the concentration conditions of this control experiment, we were surprised to see quenching at all. The modest quenching observed with the 3'-sapphyrin conjugate **1** and the Y(III) texaphyrin monomer may be rationalized by the fact that lanthanide texaphyrins bind well to anionic phosphate esters: Lisowski, J.; Sessler, J. L.; Lynch, V.; Mody, T. D. *J. Am. Chem. Soc.* **1995**, *117*, 2273-2285. Thus, the "bimolecular" quenching observed may not be purely diffusional in character, but rather due in part to this association.

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