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Libraries of Composite Polyfluors Built from Fluorescent Deoxyribosides

Jianmin Gao,[†] Christoph Strässler,[†] Deborah Tahmassebi,[‡] and Eric T. Kool*,[†]

Department of Chemistry, Stanford University, Stanford, California 94305-5080, and Department of Chemistry, University of San Diego, San Diego, California 92110

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Fluorescent labels are universally applied in biology, biotechnology, and medicine.[1] In addition, fluorophores are becoming increasingly useful in combinatorial chemistry and biology, both as encoders of library members and as reporters of chemical reactions.[2] As a result of this greatly expanding use of fluorescence, there is a corresponding increase in the need for new fluorophores having a wider range of spectral characteristics.[3] One way to generate new fluorescence properties is to encourage two dyes to interact photophysically. A prominent and useful example of this is FRET dye pairs,[4] which have been instrumental in decoding the human genome. Electronically interacting fluorophores can display new properties that individual dyes do not have, including increased Stokes shifts, specifically tuned excitations, or increased brightness.

Here we report on a new class of fluorescent molecules that are composed of multiple individual fluorophores. To encourage various forms of energy transfer, including the possibilities of FRET, excimer, exciplex, and charge transfer, we encourage close interaction by mimicking the structure of natural DNA. The individual fluorophores are constructed as deoxyribosides, with the flat aromatic fluorophores replacing a DNA base (Figure 1).[5] These individually fluorescent molecules are assembled into oligofluor strings that resemble single-stranded DNA. This strategy not only allows for ready preparation on a synthesizer, but it also encourages the closest possible interactions by allowing them to stack, as do nucleobases in DNA. Finally, the DNA backbone negative charges maintain water solubility in what would otherwise be insoluble dyes.

To test whether this potentially stacked oligofluor design would result in useful fluorophore interactions, we prepared four fluorescent deoxyribosides ("fluorosides") as monomeric components of a combinatorial set. We chose pyrene (Y, a violet fluorophore), oxoperylene (E, green), dimethylaminostilbene (D, blue), and quinacridone (Q, yellow) as a simple set of test dyes (Figure 1). Their synthesis is described in the Supporting Information. We prepared them as 5′-dimethoxytrityl-3′-phosphoramidite derivatives for automated incorporation into DNA-like strings on a commercial synthesizer. We also prepared a set of 12 binary encoding compounds,[6] so that individual polyfluorophores could later be decoded after selection.

We characterized the fluorescence properties of the individual monomer fluorosides Y, E, D, and Q (see Supporting Information). They display absorbance maxima ranging from 342 to 509 nm and emission maxima from 375 to 541 nm. Molar absorptivities vary from 8.8×10^3 to 4.7×10^4 L mol^{-1} cm^{-1}. Stokes shifts range from 17 to 93 nm, and quantum yields (in air-saturated methanol) vary from 0.055 to 0.81. The properties are close to those of the parent fluorophores.[7]

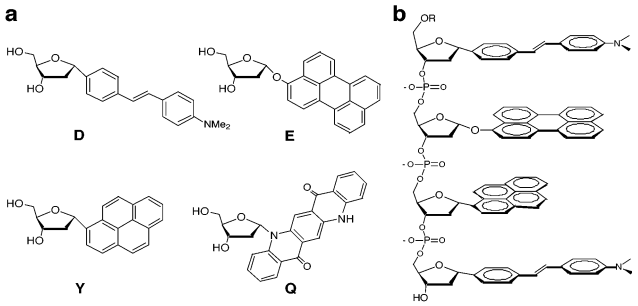

Figure 1. Structures of "fluoroside" monomers in this study (a) and of a selected tetrafluor oligomer (b) having the sequence 5′-DEYD. R = H or dimethoxytrityl.

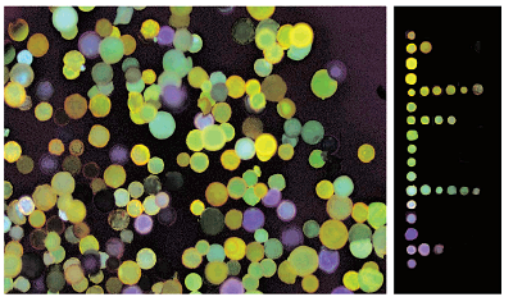

Figure 2. Fluorescence microscope image from a 256-member tetrafluor library composed of all possible tetrameric combinations of monomers D, E, Y, Q. Excitation was at 340−380 nm. Beads are PEG-polystyrene and are dry during image acquisition. At right is a representative spectrum of bead colors and intensities from the library.

We then prepared an initial small (16-member) test library comprised of these four monomers two units in length, on 130 μm PEG-polystyrene beads, using standard split-and-mix methods, and on a 1 μmol scale. Trityl cation monitoring demonstrated coupling yields that were acceptably high (at >93.6%) for each of the four monomers. We imaged the bead-based library by epifluorescence microscope, with excitation at 340−380 nm. Approximately 8−10 variations of intensity and hue were distinguishable by eye. This suggested that neither strong radiationless quenching mechanisms, nor overwhelming of apparent library color by a single bright dye, were dominant outcomes for this molecular design.

Encouraged by this, we prepared a larger 256-member binary-encoded tetrafluor library composed of all combinations of fluorosides Y, E, D, and Q. On the basis of the scale of the library, each member was represented with an ca. 40-fold redundancy. This new set of tetrafluors exhibited at least 50 different hues and intensities distinguishable by eye (Figure 2), ranging from violet to yellow-orange in color, and from relatively bright to nearly completely dark. As an initial sample, we picked and sequenced a set of tetrafluors based on bright beads of various hues as well as a few especially dark examples. Approximately 90% of 50 selected

* To whom correspondence should be addressed. E-mail: kool@stanford.edu.
[†] Stanford University.
[‡] University of San Diego.

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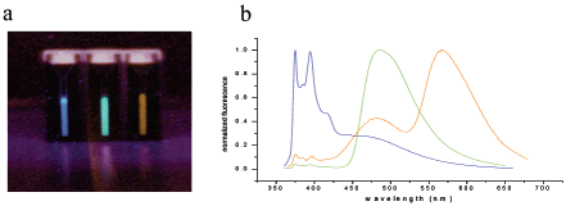

Figure 3. Fluorescence of selected library members. (a) Aqueous solutions of three tetrafluors having sequence 5′-YDDD (left), 5′-YEEY (center), and 5′-QYYY (right), under UV excitation. (b) Normalized emission spectra of the same three tetrafluors (excitation 345 nm).

beads yielded a decodable sequence; a full analysis of properties of some of these tetrafluors is underway.

Screening of molecules on solid supports does not guarantee that they will retain the same properties in solution. In the present case, it is possible that effects arising from the solid-supported methods might in some cases affect the outcome. As a test, we synthesized and purified three tetrafluors, identified under the microscope as bright blue (5′-YDDD), green (5′-YEEY), and yellow dyes (5′-QYYY) on the bead support. These were conveniently purified by gel electrophoresis, as if they were oligonucleotides, and were characterized by MS and spectroscopically. Results showed that the new tetrafluors exhibited the same apparent color in solution as on the solid support (Figure 3). This does not, of course, guarantee that other members will not exhibit such methodology-related effects (we have observed a few cases that do[8]). However, the findings establish that a major fraction of the selected beads in such a library will yield comparable results in solution.

Importantly, these three selected tetrafluors display properties markedly different than any of the monomeric fluorophores that make them up. Their colors differ in nontrivial ways from the monomers that compose them; for example, QYYY is yellow-orange despite being composed of three violet dyes and only one yellow dye. Stokes shifts are often much larger for these tetrafluors (40−221 nm) than for the monomers, and the three display their varied emission colors with a single excitation wavelength. Ordinarily the observation of three differently colored commercial monomeric dyes under the microscope requires three different excitations with three different filter sets, and they could only be observed simultaneously by artificially overlaying three digital images. Thus, the preliminary results quite successfully demonstrate new and useful fluorescence properties.

Future work will be needed to study in detail the photophysical properties of a broader set of tetrafluors. We expect that such compounds might exhibit properties that individual commercially available fluorophores cannot. One example is the aforementioned large effective Stokes shifts of greater than 200 nm in some cases. In addition, the high total molar absorptivity of four chromophores implies the possibility of light-harvesting effects,[9] allowing for brighter emission than a single dye might offer on a per-molecule basis. Finally, the closeness of the monomers in the library, and their potentially stacked arrangement, offer the possibility of forms of energy transfer beyond simple FRET, to include excimer, exciplex, and charge-transfer mechanisms. Indeed, we have observed multiple examples of non-FRET forms of energy transfer in this library (J. Gao, work in progress).

A few recent studies have applied up to three commercial dyes in DNA-based FRET libraries, generating up to eight different molecules spaced by 5−10 nucleotides.[10] The present approach offers a number of potentially useful differences from previous strategies. First, it allows for a broader array of energy transfer mechanisms, resulting in greater diversity in photophysical outcomes. Second, the present library is considerably larger than those prepared earlier. This is because a greater number of different fluorophores can be introduced into a smaller molecule and because we are not limited by commercially available phosphoramidite-derivatized dyes. Third, the present tetrafluors are much smaller, simpler, and less expensive to prepare (previous triple-fluorophore molecules are a total of 13 nt in length[10]). The relatively small size of the current molecules makes them good candidates for conjugation to proteins and DNA.

In summary, we have described a new strategy for the construction and screening of a wide variety of discrete, water-soluble fluorescent species from a small set of monomer "fluoroside" dyes. Future work will be aimed at characterizing properties of individual tetrafluors from this library, at constructing a wider variety of monomer dyes and larger libraries that contain them, and at developing methods to conjugate them to other molecules of interest. We expect that oligofluorosides may find use as labels having new characteristics for applications in the biomedical sciences and in combinatorial encoding and discovery.

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Supporting Information Available: Experimental details of monomer synthesis, library preparation, and fluorescence measurements (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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