


Chapter 1

Nanophotonics and Single Molecules

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Abstract Single emitting molecules are currently providing a new window into nanoscale systems ranging from biology to materials science. The amount of information that can be extracted from each single molecule depends upon the specific photophysical properties of the fluorophore and how these properties are affected by the nearby environment. For this reason, it is necessary to develop single-molecule emitters with as many different reporter functions as possible. The first part of this chapter describes a relatively new class of single-molecule fluorophores which offer

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tunable photophysical properties and, in turn, improved local reporting functionality on the nanometer length scale. The second part of this chapter presents metallic nanostructures which can address a second issue: the mismatch between the typical size of a single fluorophore (~ 1 nm along a long dimension) and the wavelength of light (~ 500 nm). Such nanostructures could lead to more efficient excitation of single molecules, in particular, higher excitation probability as well as reduced backgrounds, and effectively higher spatial resolution. Metallic nanostructures based on two triangles formed into a bowtie shape feature large enhancements of the local electromagnetic field and give rise to strong surface-enhanced Raman scattering of molecules. In future work, enhanced electromagnetic structures can be combined with single-molecule reporters in a variety of applications.

1.1 Introduction

In recent decades, the ability to perform optical spectroscopy and microscopy of single molecules in condensed phase samples (Moerner and Orrit 1999) has fascinated scientists in fields ranging from biophysics (Zhuang and Rief 2000; Weiss 1999; Lu 2005; Rasnik et al. 2005), to cellular biology (Moerner 2003), to materials and polymer science (Cotlet et al. 2004; Barbara et al. 2005; Lee et al. 2005), and even to quantum-mechanical single-photon sources (Moerner 2004; Lounis and Orrit 2005). Using now-standard experimental methods (Ha et al. 1999; Ha 2001; Moerner and Fromm 2003), information on local interactions can be extracted, molecule by molecule, by the measurement of excited state lifetimes, spectra, orientations, brightness, degree of energy transfer, and photon correlations, thus removing ensemble averaging and allowing exploration of heterogeneity. Single-molecule studies often reveal complex statistical fluctuations, which allow useful comparison with theoretical models in a variety of cases (Barkai et al. 2004; Watkins and Yang 2004; Hummer and Szabo 2005).

A continuing need exists for the improvement and extension of these efforts in order to increase the variety and amount of information that may be obtained from single-molecule studies. For example, at room temperature, eventual photobleaching limits the knowledge that may be extracted from each individual molecule. To compensate for this, it is necessary to continually develop new reporter functions for robust fluorophores that provide sufficiently strong signals at the single-molecule level.

The first part of this chapter describes a relatively new class of single-molecule fluorophores which offer tunable photophysical properties and, in turn, improved local reporting functionality on the nanometer length scale. While a single molecule can report on its immediate nanoenvironment, in most experiments, there is a large mismatch between the size of a single fluorophore (~ 1 nm along a long dimension) and the wavelength of light (~ 500 nm). Developing ways to overcome this would lead to more efficient excitation of single molecules, in particular, higher excitation probability as well as reduced backgrounds, and effectively higher spatial resolution.

One approach for overcoming the mismatch involves nanoscale metallic structures that concentrate light to subwavelength regions. We have explored this idea with metallic “bowtie” nanoantennas in which the enhancement of the local electromagnetic field can be directly measured. In the second part of this chapter we describe the optical properties of bowties, and illustrate their use in spectroscopy for the case of surface-enhanced Raman scattering of molecules. In future work, enhanced electromagnetic structures can be combined with single-molecule reporters in a variety of applications.

1.2 Development of Single-Molecule Fluorophores with Alternate Readout Capabilities

1.2.1 Motivations

Over the years, it is certainly true that a large number of fluorescent dyes (Tsien and Waggoner 1995) have become available for labeling applications, for example, from commercial sources such as Molecular Probes (Invitrogen Corp.), Dyomics GmbH, and many others. Of particular interest here are the fluorophores that show environmental sensitivity, that is, those which show alterations in emission spectra, fluorescence lifetimes, or fluorescence quantum yields that vary based on the polarity or the viscosity of the environment. For example, dyes such as laurdan, prodan, dansyl, NBD, merocyanines, and the recently reported di-4-ANEPPHQ have all been utilized in various reporter applications in biological systems (Parasassi et al. 1998; Maier et al. 2002; Touthkine et al. 2003; Jin et al. 2005), but this list is by no means exhaustive. For the most part, experiments using environmentally sensitive fluorophores have focused on high concentration imaging, as only a subset of the larger group of fluorophores shows the high quantum yield, weak triplet bottlenecks, and high photostability to allow detection at the single-molecule level. For this reason, it is necessary to continue research efforts to develop single-molecule emitters with as many different reporter functions as possible.

1.2.2 The DCDHF Single-Molecule Fluorophores

Recent work in the Moerner and Twieg laboratories has focused on a new class of fluorophores that meet the strict demands for single-molecule imaging and offer additional interesting properties such as a large ground state dipole moment μ_g , moderate hyperpolarizability β , and sensitivity to the rigidity of the local environment (Willets et al. 2003a, 2005). The molecules within this class all contain an amine donor and a dicyanodihydrofuran (DCDHF) acceptor linked by a conjugated unit (benzene, thiophene, styrene, etc.). These molecules are named DCDHF fluorophores after the acceptor unit (Melikian et al. 1995), which is constant among the

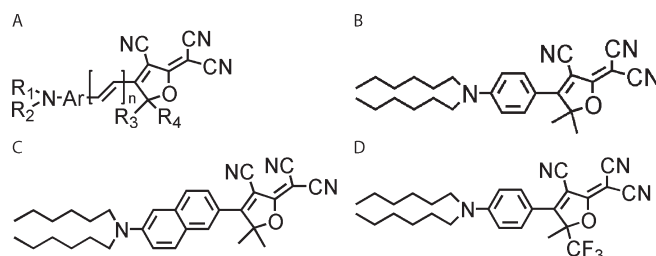


Figure 1.1 Representative structures of the DCDHF fluorophore class. In the general structure **A**, the aromatic group (Ar) may be one or more benzene or thiophene groups, and different substituents may be added at the positions R1–R4 to modify the properties of the molecule. The structure **B** shows the first molecule studied in this class, DCDHF-6. Structure **C**, DCDHF-N-6, is a long-wavelength version; structure **D**, DCDHF-CF₃, has been used in membrane labeling studies as described in the text

class. Figure 1.1A shows the general structure of these molecules, and Figure 1.1B shows the molecule DCDHF-6, the first and most studied compound in this group. The DCDHF molecules are examples of nonlinear optical chromophores possessing large second-order nonlinearity by virtue of the asymmetric donor–acceptor structure and high degree of conjugation (Nicoud and Twieg 1987; Kanis et al. 1994; Zyss 1994; Zhang et al. 2001).

Nonlinear optical chromophores at high concentrations in polymers have received much attention from the chemical community in recent decades owing to the utility of such materials for second-harmonic generation, electro-optic phase modulation, and many other potential applications (Prasad and Williams 1991; Burland et al. 1994; Nalwa and Miyata 1997; Kuzyk and Dirk 1998). The DCDHF molecules were originally designed for and utilized extensively in high-performance photorefractive polymer materials (Wright et al. 2001; He et al. 2002; Ostroverkhova et al. 2002; Ostroverkhova and Moerner 2004).

Usually, nonlinear optical chromophores are not thought to be particularly fluorescent, inasmuch as the strong charge-transfer transition producing the optical nonlinearity is often accompanied by considerable excited-state distortion of the molecule. In spite of this, the DCDHF fluorophores are surprisingly well-suited for single-molecule fluorescence detection (Willets et al. 2003). This may be a result of the fact that their earlier optimization for photorefractive applications rested more upon producing a large polarizability anisotropy and dipole moment rather than a large hyperpolarizability (Kippelen et al. 1997). Figure 1.2A shows an epifluorescence image of single molecules of DCDHF-6 in a poly(methyl methacrylate) thin film at room temperature. Observing the time-dependent emission from single spots (Figures 1.2B, 1.2C) shows clear single-step photobleaching. The blinking event for Figure 1.2C near 3 seconds is a property common to many single molecules (Moerner 1997), but in this system, 85% of the molecules observed did not show blinking on the time scale of the measurement (100 ms), itself a surprising result.

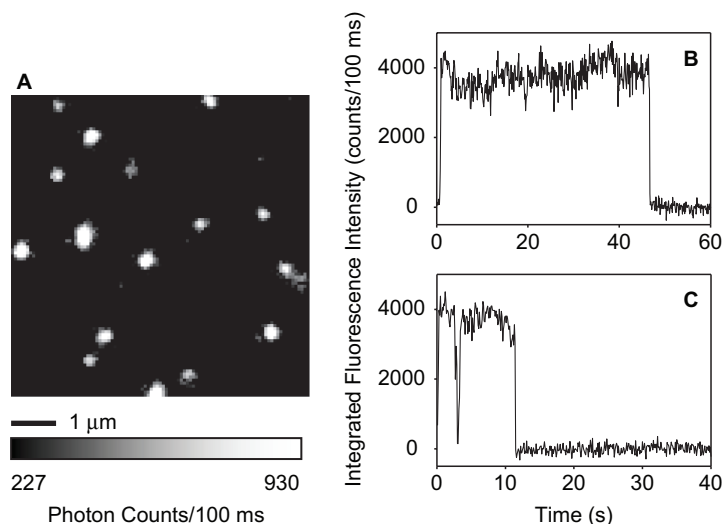


Figure 1.2 (A) Epifluorescence image of single molecules of DCDHF-6 in a thin film of PMMA at room temperature. The time traces in (B) and (C) show single-step photobleaching. (Reprinted by permission from Willets et al. (2003a))

1.2.3 Comparing DCDHF Molecules to Well-Known Single-Molecule Fluorophores

To illustrate some of the properties of molecules in the DCDHF class compared to conventional fluorophores, Table 1.1 shows the properties of three DCDHFs compared to Rhodamine 6G (R6G) and Texas Red.

Although the absorption and emission of R6G and Texas Red are at longer wavelengths than for DCDHF-6, the synthetic flexibility of the DCDHFs at the conjugated linker (Ar plus n vinyl groups; Figure 1.1A) has allowed derivatives to be produced with absorption as long as 700 nm (for a thiophene–thiophene–vinyl linker) while maintaining the advantageous characteristics of the parent molecule. The fourth entry in the table for DCDHF-N-6 (see Figure 1.1C) is an example of one of the long-wavelength emitters where the aromatic coupling group is a naphthalene (Lord et al. 2007). The fifth entry is for DCDHF-A-6, the anthracene analogue to DCDHF-N-6. Comparing the first three rows in the table, the extinction coefficients ϵ for R6G and Texas Red are larger than for DCDHF-6, but this parameter too is larger for other longer wavelength versions of the DCDHF molecules not shown here.

Surprisingly, in toluene liquid solution, the fluorescence quantum yield Φ_F for DCDHF-6 is quite small. The first clue that these molecules have strong environmental sensitivity came from the observation that in the relatively more rigid PMMA environment, the fluorescence quantum yield is much higher, comparable to R6G and Texas Red. It is important to note that the bulk photobleaching quantum yields Φ_B are

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