Journal of Fluorescence, Vol. 12, No. 2, June 2002 (© 2002)

Dramatic Increases in Resonance Energy Transfer Have Been Observed Between Fluorophores Bound to DNA Above Metallic Silver Islands: Opportunities for Long-Range Immunoassays and New DNA Arrays

Received March 20, 2002; accepted March 21, 2002

KEY WORDS: Enhanced resonance energy transfer (RET); DNA arrays; immunoassays.

In nearly all cases of resonance energy transfer (RET), its usefulness as a spectroscopic ruler is determined by the magnitude of the Förster distance, R_O. For the most favorable case of high spectral overlap and high quantum yield, the maximum value of R_O is near 55 Å for organic fluorophores and up to 90 Å for lanthanide donors [1,2]. Hence, because of this rather short upper limit of R_O, it has been difficult to use RET in immunosensing, where donor (D) and acceptor (A) molecules may be at least 100 Å apart, and in DNA, where the donors and acceptors must be spaced more closely than 30 base pairs.

However, these rather short free-space Förster distances may be substantially increased by placing the donor and acceptors pairs above silver island films. Subsequently, we have observed a dramatic increase in RET between donors and acceptors bound to double-helical DNA located above metallic silver islands (Fig. 1). Our results suggest the presence of at least two populations of D-A pairs, with the pairs close to the silver islands displaying at least a 5-fold increase (about 165 Å) of the Förster distance compared to the observed free-space condition value of 37.4 Å.

Double-helical calf thymus DNA was labeled with DAPI (4′,6-diamidino-2-phenylindole) as the donor and propidium iodide (PI) as the acceptor, where the extent of energy transfer, seen from the decrease in the DAPI donor intensity at ≈ 460 nm, was about 20%, Fig. 2. This is consistent with the R_O value of 35.7 Å for this D-A pair [3].

When placed between the silver island films, the DAPI-DNA showed no increase in fluorescence intensity, compared to unsilvered quartz slides as the control sample, whereas in the case of PI-DNA there was an approximate 2-fold increase in the PI intensity. This is consistent with our reports that large fluorescence enhancements are observed for low quantum yield fluorophores [4], (Q = 0.15 PI-DNA compared to 0.53 for DAPI-DNA) with a maximum enhancement of 1/Q, where Q is the quantum yield in the absence of metal.

However, for D-A labeled DNA a remarkable increase in the PI acceptor emission was found for the DNA sample between the silver island films, compared to the control sample (Fig. 3). Given that silver island films had only a modest effect on PI-DNA fluorescence (ca 2-fold), these results suggest an increase in the efficiency of RET from DAPI to PI because of the close proximity to the silver islands. (Scattered light was investigated and rejected).

An increase in resonance energy transfer from DAPI to PI also resulted in a decrease in the DAPI decay time, as expected, changing from 2.26 ns for the control sample to 1.67 ns between the silvered plates. Subsequently, we analysed our frequency-domain donor decays for RET in one dimension to obtain an *apparent Förster distance*[5].

Our results clearly show the presence of two populations of D-A pairs, one a value similar to the free-space R_O value and the second showing a 5-fold increase in R_O, i.e., 165.7 Å, which we attribute to D-A pairs directly above silver islands. Although this increase is truly remarkable, it is important to recognize that this value may be a conservative minimum estimate. This is because the recovered value may represent some weighted average and not the highest possible R_O value.

Such dramatic increases in RET between donors and acceptors using metallic silver particles is likely to result

1053-0509/02/0600-0131/0

Fig. 1. Experimental geometry.

in further applications of RET in biochemical and biomedical research, which may include:

- A resurgence of RET-based immunosensing, which has languished because of the relatively small D-A R_O value compared to the size of relevant antibodies.
- Metal enhanced energy transfer with DNA arrays or gene chips. At present, DNA arrays are read by measuring the amount of two fluorophores hybridized to the target DNA; even though the two probes may be a good D-A pair, energy transfer does not normally occur [6]. We visualize a new type of DNA arrays on metallic surfaces or particles based on RET between donors and acceptors positioned at long distances.
- Increasing the efficiency of light-harvesting assemblies based on RET [7].
- An increase in the extent of RET between donors and acceptors within cells but close to metallic particles [8].

This new concept of metal enhanced RET provides a unique opportunity for using the proximity of D-A pairs to modify the rates of transfer. Such effects are unique because the metal particles or surfaces, rather than the traditional solution composition, e.g., pH etc., can be used to modify the spectral properties of the probes.

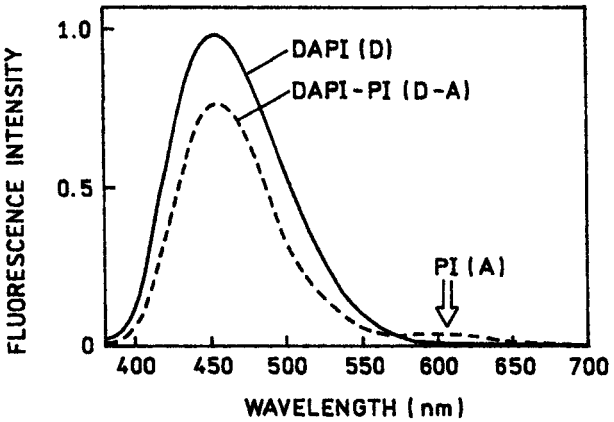

Fig. 2. Emission spectra of DNA labeled with DAPI and with DAPI and PI. The PI acceptor makes a very small contribution to the emission seen at 610 nm. **D**: Donor; **A**: acceptor molecule.

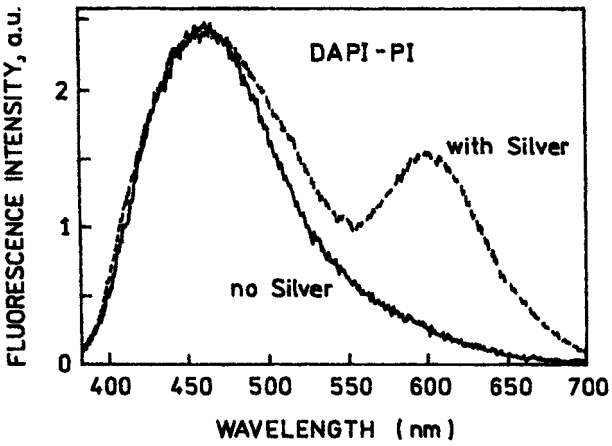

Fig. 3. Emission spectra of DNA labeled with both DAPI and PI between quartz plates (the control sample) and between silver island films. The emission spectra are normalized to the DAPI donor emission.

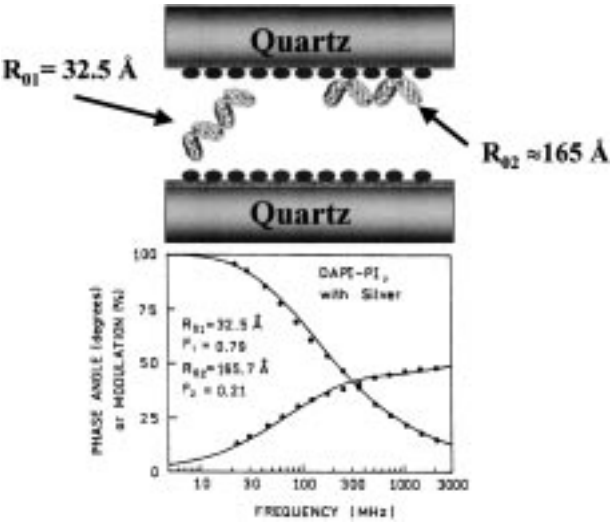

Fig. 4. Energy transfer analysis of the frequency-domain data in terms of two populations of DAPI-PI labelled DNA with different R_O values. (Top)—Graphical representation of two DAPI-PI labelled DNA populations.

In conclusion, the rapidly emerging literature on the modified properties of fluorescence by metallic surfaces or particles finds a new addition, namely, metal enhanced RET, where to the best of our knowledge there have been no other experimental demonstrations.

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