

**Multimode Surface Functional Group Determination: Combining Steady-State and Time-Resolved Fluorescence with X-ray Photoelectron Spectroscopy and Absorption Measurements for Absolute Quantification**

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## **I. Conditioning and washing of slides prior to and after silane deposition as well as labeling.**

The substrates were thoroughly cleaned with Hellmanex II solution under ultrasonication, followed by rinsing with water and isopropyl alcohol (IPA) and drying using an argon jet, activated with a low-pressure air plasma for 10 min and dried in vacuo (< 0.1 mbar).

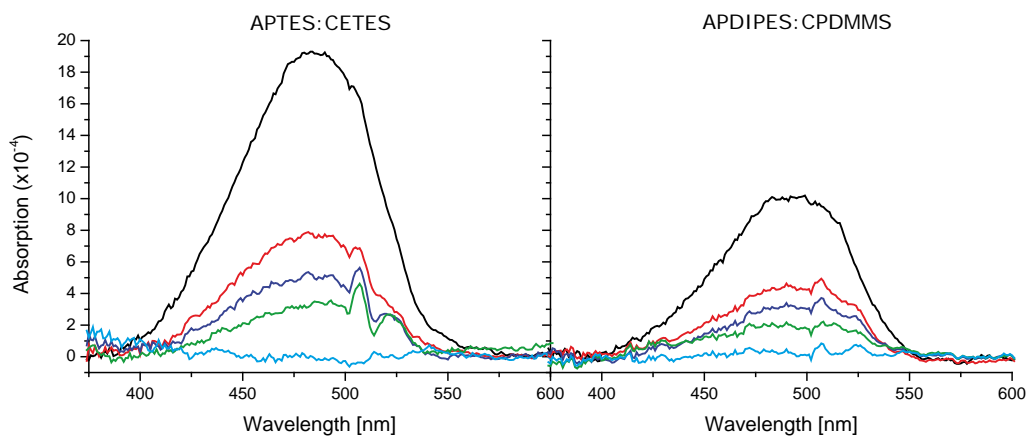
After silane deposition and removal from vacuum, the slides were rinsed with acetone and (IPA), followed by cleaning in a Soxhlet extractor with acetone overnight. After further rinsing with acetone and IPA, the slides were dried with an Ar jet and in vacuum before storage.

## **II. Solvatochromism of monoamino-substituted **1**.**

Careful comparison of the absorption maxima of **1** bound to amino surface groups (Figure 2), 485 nm, and the pentylamine adduct of **1**, **1PA**, dissolved at dilute concentrations in acetonitrile (Figure 5), 473 nm, raises the question whether indeed similar species are present. First, it has to be noted that whereas the spectrum in Figure 5 was measured in a 10 mm cell in acetonitrile solution, the other spectra in Figure 2 were measured for the dye on a slide surface most likely covered by a thin water film due to the normal laboratory conditions the measurement was performed in. We thus investigated the solvatochromism of **1PA** in various highly polar solvents in conventional 10 mm cells, yielding for instance maxima at 480 nm in dilute ethylene glycol (EG) and 497 nm in dilute aqueous solution.<sup>1</sup> Apparently, the monoamino-substituted BODIPY core undergoes rather pronounced solvatochromism in polar solvents. The value of 485 nm in Figure 2 further suggests that the actual environment of **1** grafted to the slide surface lies in between that of EG and water, either because of certain confinement effects or because the thin native water film on the slide surface possesses slightly different characteristics as bulk water does.

### III. Absorption spectroscopic analysis of APDIPES/CPDMMS slides labeled with 1.

A set of slides was prepared and characterized in analogy to ref. 2 with the two monoalkoxysilanes 3-(aminopropyl)diisopropylethoxysilane (APDIPES) and 3-(cyanopropyl)dimethylmethoxysilane (CPDMMS) and was analyzed additionally by absorption spectroscopy as detailed in here. Whereas Figure S1 compares the respective spectra of the APTES/CETES and the APDIPES/CPDMMS system, Table S1 collects the dye and amino group densities calculated for the APDIPES/CPDMMS system. Comparison of the latter with the corresponding data of Table 3 shows that the amino group density is ca. 2.5-fold higher for APTES/CETES and the dye density ca. 2.0-fold higher. These findings correlate well with earlier findings by Shricliff et al. on the DNA conjugation efficiency of amino silane mono- vs. multilayers.<sup>3</sup>



**Figure S1.** Reproduction of Figure 2 (left) and the corresponding spectra obtained in an analogous way for the APDIPES/CPDMMS system of ref. 2 (right).

**Table S1.** Functional group and label densities for the APDIPES/CPDMMS surfaces labeled with dual-mode dye **1** (given errors are only experimental); % = % APDIPES. Data are directly comparable to Table 3.

%	Calculated dye density (dyes·10 <sup>12</sup> cm <sup>-2</sup> )	Amino group density (groups·10 <sup>14</sup> cm <sup>-2</sup> )
100	11 ± 1	1.6 ± 0.1
50	4.8 ± 1	0.7 ± 0.6
25	3.5 ± 1	0.49 ± 0.6
12.5	2.6 ± 1	0.37 ± 0.6
0	0.0	0.0

#### IV. Notes and references.

1. A detailed investigation of such dyes is currently in progress, Hecht, M.; Sarma, D.; Rurack, K. *et al.*, manuscript in preparation.
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3. Shircliff, R. A.; Stradins, P.; Moutinho, H.; Fennell, J.; Ghirardi, M. L.; Cowley, S. W.; Branz, H. M.; Martin, I. T. *Langmuir* **2013**, 29, 4057-4067.