Supplementary

Multiplexed Detection of Human Papillomavirus Based on AzaBODIPY-Doped Silica-Coated Polystyrene Microparticles

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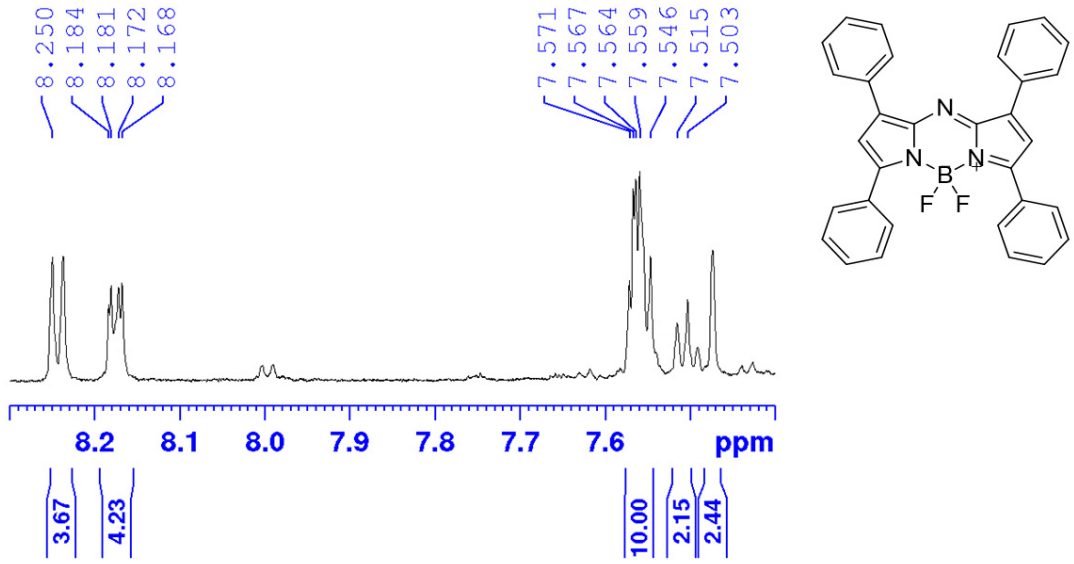

Figure S1. 600 MHz ^{1}H NMR spectrum of azaBODIPY **1** in $CDCl_3$.

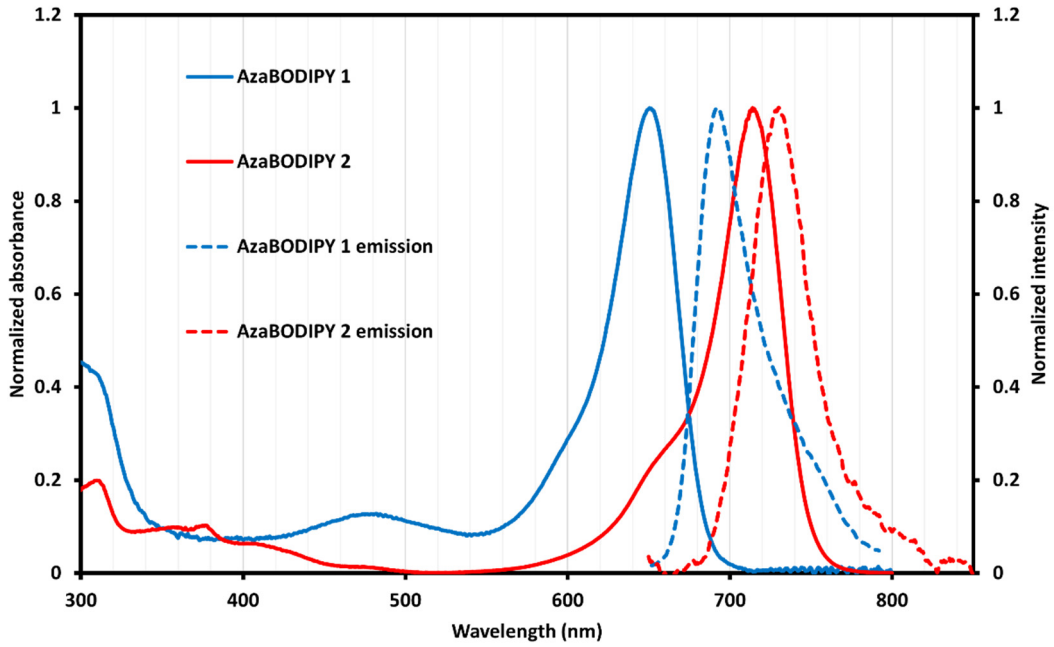

Figure S2. Normalized absorption spectra and emission spectra of azaBODIPYs **1** and **2** in EtOH.

Table S1. Photophysical properties of **1** and **2** in different solvents.

	Solvent	λ_{abs}[nm]	Log ε	λ_{em}[nm]	Stokes shift (nm)	Φ_F	τ_F (ns)
1	DMSO	658	4.5	699	401	0.36	1.2
	AcCN	643	4.7	680	37	0.22	0.69
	[a]EtOH	**647**	**4.2**	**684**	**38**	**0.31**	**0.88**
	THF	**651**	**4.9**	**693**	**42**	**0.34**	**0.99**
	Benzene	642	5.0	695	53	0.38	1.8
2	DMSO	716	5.2	741	25	0.06	1.6
	EtOH	**711**	**5.2**	**732**	**21**	**0.030**	**1.6**
	THF	**710**	**5.1**	**736**	**26**	**0.008**	**1.6**
	Toluene	714	5.0	740	26	0.10	1.97
	[b]DCM	710	5.0	733	23	0.14	1.99
	[b]EtOAc	705	5.1	731	26	0.18	1.7
	Benzene	715	5.0	738	23	0.15	2.1

[a]The photophysical properties have been reported in [1], [b]The photophysical properties have been reported in [2].

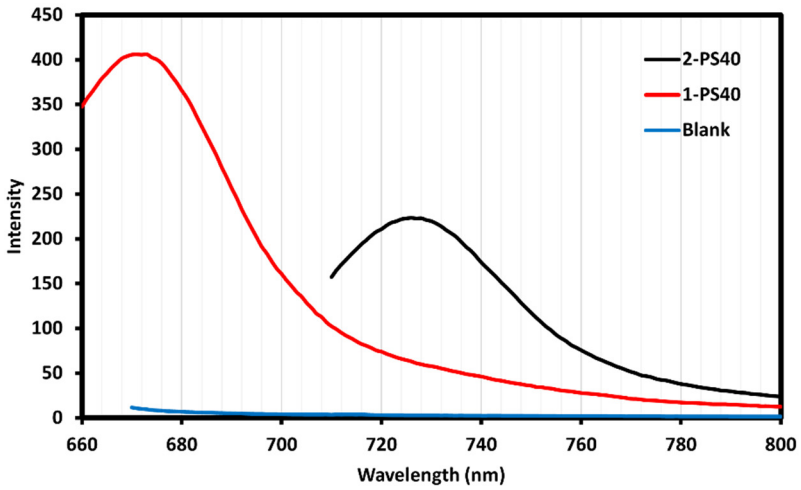

Figure S3. Emission spectra of **Blank**, **1-PS40** and **2-PS40** with excitation wavelengths at 650 and 700 nm in EtOH.

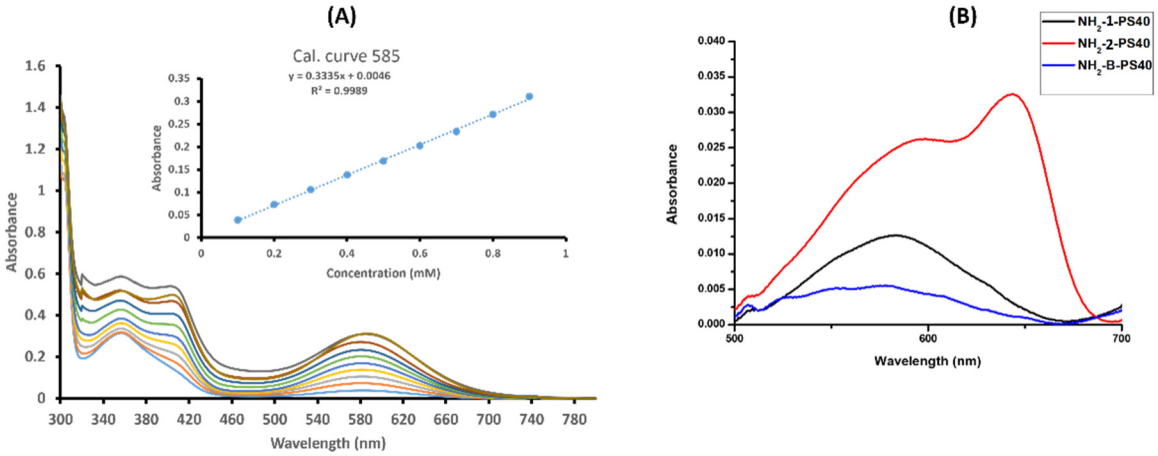

Figure S4. (A) UV-visible absorption spectra and a calibration curve for pentylamine at 580 nm, and (B) absorption spectra of the Ruhemann's purple solution obtained when the amino groups in the particles reacts with the ninhydrin reagent in EtOH.

Table S2. Absorption and concentration of COOH functionalized **Si-PS40** particles. The dye concentration in the particles was also determined using the Beer-Lambert law in EtOH.

	Absorbance	ε ($M^{-1}cm^{-1}$)	Initial concentration (M)	Final concentration (M)
COOH-1-PS40	0.2896	82100 ± 10%	0.014	3.53×10^{-6}
	0.167		0.007	2.03×10^{-6}
	0.0942		0.0035	1.15×10^{-6}
	0.0511		0.00175	6.22×10^{-7}
COOH-2-PS40	0.0905	104000 ± 10%	0.014	8.70×10^{-7}
	0.0495		0.007	4.76×10^{-7}
	0.0440		0.0035	4.23×10^{-7}
	0.0111		0.00175	1.07×10^{-7}

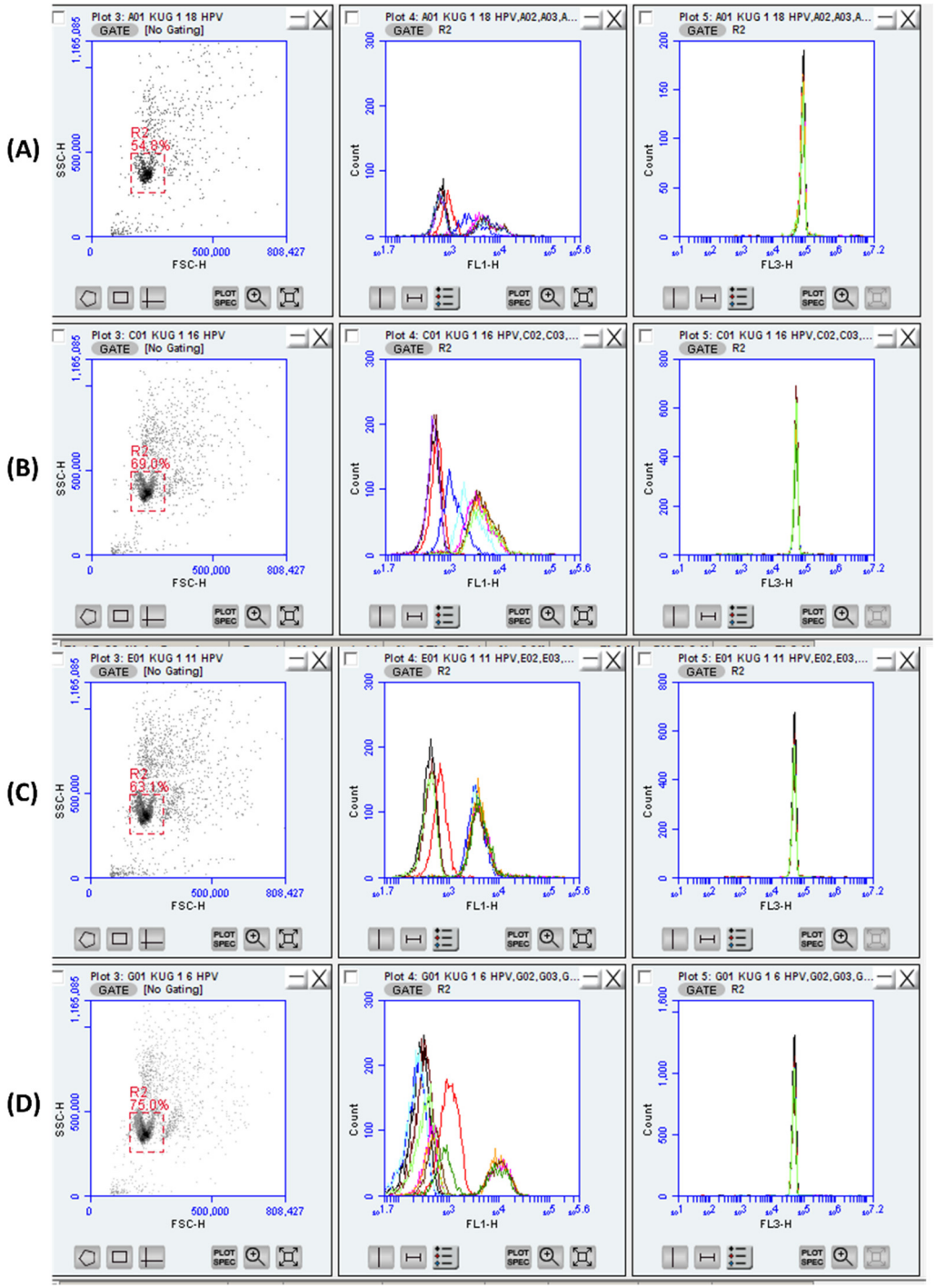

Figure S5. Flow cytometry measurements for the 1:1 hybridisation assay of complementary FAM-labeled t-DNA towards the C_1-C_4 concentrations of (A) **c-DNA18-2-PS40**, (B) **c-DNA16-2-PS40**, (C) **c-DNA11-2-PS40** and (D) **c-DNA6-2-PS40** particles at nine different concentrations of the strand complementary to the target, showing dot plots (left) and the fluorescence measured in the FL1-H (middle) and FL3-H (right) channels.

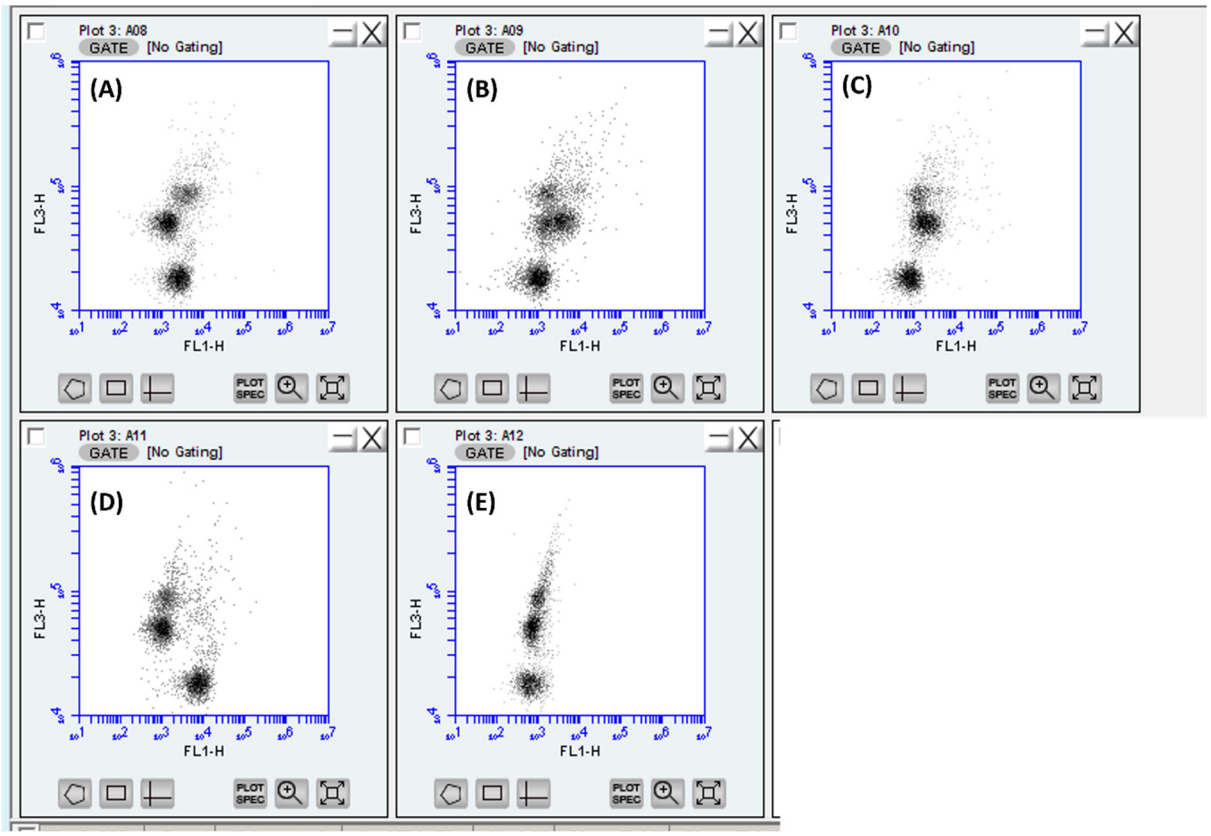

Figure S6. Flow cytometry measurements of the 4:1 multiplex assay using **DNA-2-PS40.** (A-D) The assay response in the presence of the HPV18, HPV16, HPV11 and HPV6 t-DNA strands, respectively. (E) The assay response to the particles free of HPV t-DNA strands.

Methods used to determine limits of blank (LOB), detection (LOD) and quantification (LOQ) and dynamic range

The parameters Limit of Blank (LOB), Limit of Detection (LOD), and Limit of Quantification (LOQ) describe the smallest concentration of a sample that can be reliably measured by an analytical procedure [3]. LOB is defined as the highest putative analyte concentration expected to be found when replicates of a blank sample in absence of the analyte are measured. LOD is defined as the lowest analyte concentration likely to be reliably distinguished from the LOB and at which detection is feasible, whereas the LOQ is defined as the lowest concentration at which the analyte cannot only be reliably detected but can be properly quantified. These parameters were estimated according the following equations:

LOB = mean blank + 1.645(σ blank)

LOD = LOB + 1.645(σ low concentration sample)

LOQ = LOB + 10(σ low concentration sample)

The dynamic range was defined as the concentration range between the concentrations that possessed a signal intensity between 20% (IC_{20}) and 80% (IC_{80}) of the maximum signal.

References

1. Gorman, A.; Killoran, J.; O'Shea, C.; Kenna, T.; Gallagher, W.M.; O'Shea, D.F. In vitro demonstration of the heavy-atom effect for photodynamic therapy. *J. Am. Chem. Soc.* **2004**, *126*, 10619–10631. https://doi.org/10.1021/ja047649e.
2. Gresser, R.; Hummert, M.; Hartmann, H.; Leo, K.; Riede, M. Synthesis and characterization of near-infrared absorbing benzannulated aza-BODIPY dyes. *Chem. Eur. J.* **2011**, *17*, 2939–2947. https://doi.org/10.1002/chem.201002941.
3. Armbruster, D.A.; Pry, T. Limit of blank, limit of detection and limit of quantitation. *Clin. Biochem. Rev.* **2008**, *29* (Suppl. 1), S49–S52.