

Design, preparation and assessment of surface-immobilised tetraphenylethenes for biosensing applications



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ABSTRACT

Tetraphenylethene (TPE) shows a significant increase of fluorescence intensity when the rotational freedom of its phenyl groups is restricted. This special property allows the use of TPE in sensor applications, which have been previously described for the liquid phase only. However, some applications utilising arrays require the immobilisation of TPE dyes on solid surfaces. In this work, we synthesised and investigated the fluorescence behaviour of TPE derivatives on silica particles and quartz slides and suggest ways to employ the dye's properties in solid phase biosensor applications.

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1. Introduction

The intensity of fluorescence can be influenced by several factors related to concentration, such as dimerisation [1], excimer and exciplex formation [2], quenching by energy acceptors [3] or by singlet–singlet annihilation [4]. In addition to these factors, for most fluorescent dyes, the major pathway of excited state depletion is due to internal conversion followed by a radiationless vibrational cascade. For these molecules, hindering internal rotations and vibrations by a range of environmental factors, such as viscosity, polarity and steric crowding can lead to an increase in their fluorescence intensity. In the 1960s and 1970s several research groups have described the influence of the solvent's viscosity on the fluorescence intensity of *cis*-stilbene [5–7] or *trans*-1,1,4,4-tetraphenyl-2-methylbutadiene (TPMB) [8]. Similar properties were reported for tetraphenylethene (TPE), which was found to increase its fluorescence intensity by several orders of magnitude upon increasing the solvent viscosity [9,10].

When TPE dyes are excited, relaxation occurs efficiently by non-radiative torsional relaxation, for example, by rotation about the double bond and rotation of the phenyl rings. If the rotational freedom is restricted and geometric rearrangement is prohibitive, emission of light becomes the predominant pathway for relaxation [11,12]. This phenomenon was rediscovered and termed “Aggregation Induced Emission (AIE)” in the early 2000s by Tang et al. [13], which caused a renewed surge of interest in the special properties of AIE dyes. Among the potential applications of these dyes are glucose [14] and temperature [15] sensors, organic light emitting diodes (OLEDs) [16], techniques to probe the conformation of macromolecules like polyelectrolyte layers [17], or DNA [18] and protein detection assays [19]. All of these examples, however, only use solutions of the fluorophore.

AIE dyes have potential as reporters for the binding of large molecules like proteins in bioassays. Currently, many detection methods follow an approach similar to ELISA [20,21], which involves several incubation and washing steps after the binding of the analyte. These procedures are laborious, time consuming and require large volumes of reagents. In principle, immobilised tetraphenylethene fluorophores could be used to generate a fluorescence signal directly upon binding of the analyte and

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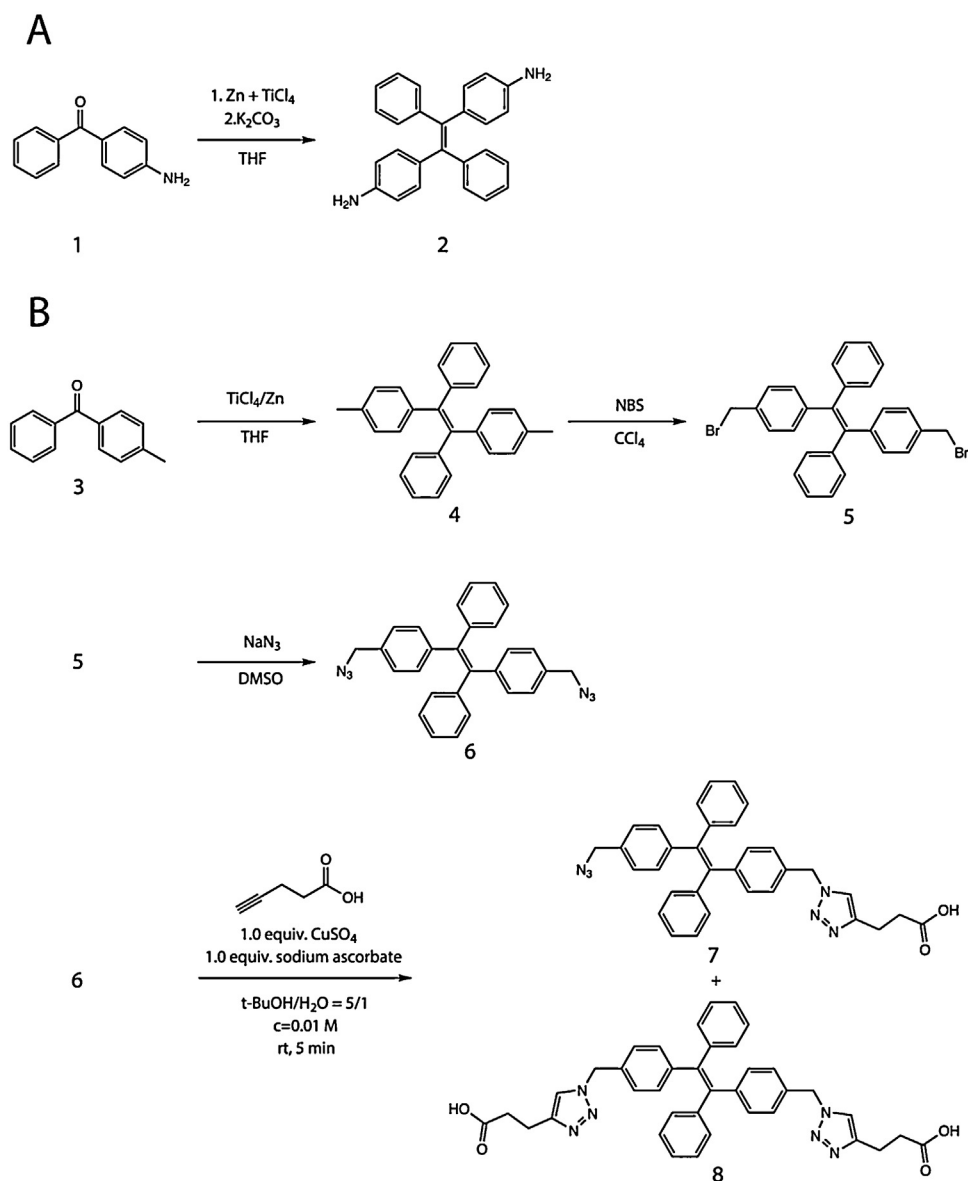


Fig. 1. Synthesis of diamine and monocarboxyl TPE.

reduce the necessary detection time significantly. Despite the clear advantages over ELISA type assays and many classical fluorophores, AIE dyes have not been used in surface-based assays to date.

In this article, we describe the fluorescence behaviour of TPE derivatives covalently bound to solid surfaces. We investigated their ability to respond to changes in both the polarity of the solvent and the steric crowding of their environment, as triggered, for example, by the binding of large molecules. Due to the possible application in bead based bioassays we used functionalised silica particles as the solid-phase substrate. Additional experiments on quartz slides were performed to access additional analytical methodologies. We found that immobilised TPE fluorophores responded to an increase in steric crowding. However, the effect was relatively weak and was only observed upon strong restriction of the rotational freedom. Immobilised dyes were also observed to fluoresce in the absence of other molecules and in a wide range of solvents. We attribute this to steric repulsion between the TPE molecules and the substrate surface, which further restricts the rotational freedoms considerably.

2. Experimental

2.1. Materials

All chemicals were purchased from Sigma–Aldrich (USA), MP Biomedicals (USA) and 1st Base (Singapore). SiO₂-COOH particles were purchased from microParticles GmbH (Germany). The particle stock solution had a concentration of 400 × 10⁶ particles/ml with an average particle size of 5.56 μm and a density of 1.9 g/cm³. The carboxylate group density was indicated by the manufacturer as >30 μmol/g.

2.1.1. Synthesis of amine TPE (2) – see Fig. 1(A)

4-Aminobenzophenone (**1**, 400 mg, 2.03 mmol) was dissolved in THF (15 ml). The solution was cooled to 0 °C and Zn dust (332 mg, 5.07 mmol, 2.5 equiv.) and TiCl₄ (1 M in toluene, 4.06 ml, 4.06 mmol, 2 equiv.) were added. After refluxing for 4 h, 50 ml of a 10% K₂CO₃ solution was added and stirred overnight at room temperature. The white precipitate **2** was extracted three times with dichloromethane (DCM).

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