Combinatorial synthesis of a triphenylmethine library and their application in the development of Surface Enhanced Raman Scattering (SERS) probes†

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The first synthesis of a triphenylmethine (TM) library of compounds and screening of their Surface Enhanced Raman Scattering (SERS) capability was carried out to identify novel Raman reporters with high sensitivity. We identified three novel SERS reporters (B2, B7, and C7) with higher signal intensity than that of commonly used crystal violet (CV). These reporters may find potential applications in developing sensitive SERS based biosensors.

In surface enhanced Raman scattering (SERS), the intensity of the vibrational spectra of a molecule is enhanced by several orders of magnitude when the molecule is in close proximity to metallic nanoparticles such as gold or silver. SERS has been successfully applied for labeling biological systems even in cells and tissues to sense multiplexed biomarkers.[1] Nanoparticle tags that use SERS to generate detectable Raman signals have been shown to be a successful alternative to fluorescence labeling, which has the drawbacks of photobleaching, peaks overlapping in multiplexed experiments, and inability to function in some extreme environments in biological systems.[2] SERS nanotags have been used for molecular, cellular and in several *in vivo* bioimaging techniques by bioconjugating with multiple diagnostic and therapeutic agents.[1–3] Recently Qian *et al.*[4] and Keren *et al.*[5] reported the use of SERS nanotags for imaging on a rodent model. These nanotags were developed by immobilizing a Raman active dye (Raman reporter) on a metal colloid followed by bioconjugation to target specific locations. Such a nanoparticle–Raman reporter assembly is called a Raman tag in analogy with quantum dots and can provide a platform for multiplexing, targeting and tracking in bio-imaging and sensing applications.[6,7]

The reporter molecule is a major factor to enhance the sensitivity of a Raman tag, as well as metal nanoparticles. Among the different reporter molecules, triphenylmethine (TM) compounds exhibit absorption at visible ranges that enable the compounds to be a useful Raman reporter in visible-NIR excitation.[8a–c] Although a few TM compounds, *i.e.* malachite green isothiocyanate (MGITC) and crystal violet (CV), have been used as reporters,[4–9] there is a growing need for reporter molecules that are easily identifiable within a multiplexed analysis platform which can also generate higher SERS intensity. However, a systematic study for diverse SERS reporter generation and screening has not been carried out. In this context, we report the first combinatorial synthesis of a TM dye library and the screening of their SERS properties for development of highly sensitive SERS nanotags. The conventional syntheses of the TM dyes were mostly based on electrophilic aromatic substitutions and the common electrophilic reagents are phosgene, formaldehyde, chloroform, and carbon tetrachloride.[10] Our diversity oriented library approach incorporates[11,12] solid-phase chemistry to generate the final product TM, avoiding the use of toxic reagents and time-consuming purification steps. A general approach to the synthesis of the TM library is outlined in Scheme 1. Building block **A** is commercially available (Aldrich) and **B–D** were synthesized as described in ESI.† Each intermediate **A–D** was then loaded on 2-chlorotrityl chloride resin, and reacted with 29 different Grignard reagents (R^2 building block) for diversity. An acidic cleavage from the resin resulted in the dehydration of the corresponding tertiary alcohols, giving the fully conjugated TM derivatives. The choice of 4-aminophenyl groups as the R^1 building block (S1) has the advantages of providing a linker group to the resin and also of providing a reactive group to the metal surface in the final TM library compound. All the library compounds were characterized by LCMS and 52 relatively pure compounds were selected for further studies with the purity and full characterization in ESI.†

The primary screening was carried out by incubating 60 nm citrate stabilized gold colloidal solution with each dye compound to measure SERS spectra under a confocal Raman microscope. The highest SERS intensity from each spectrum was identified and compared with that of CV as a reference in Fig. 1. The result clearly shows that the SERS signal varies significantly across the different TM library compounds, and at least 13 compounds exhibited a stronger SERS signal than CV. In general, compounds with building blocks **B** and **C** show higher SERS intensity than those with building blocks **A** and **D**.

We resynthesized the best five compounds from the initial screening (**B2**, **B7**, **C3**, **C7**, and **C9**) and carried out the SERS

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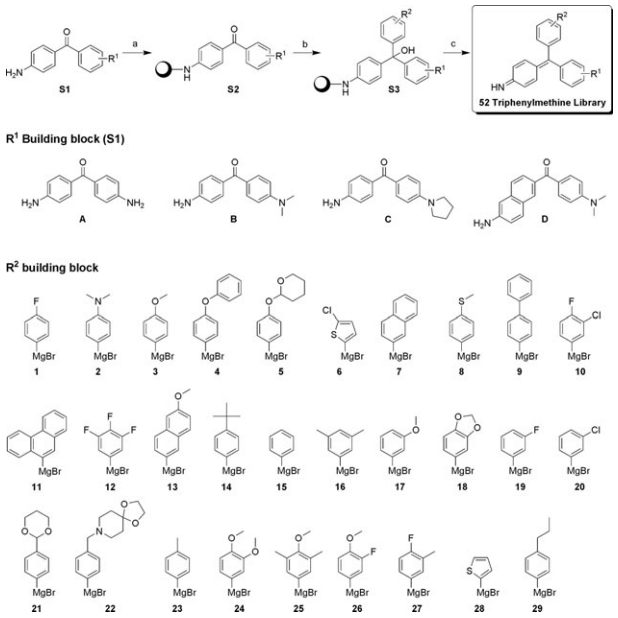

Scheme 1 Synthesis of triphenylmethine library. *Reagents and conditions*: (a) 2-chlorotrityl chloride resin (1.37 mmol g^{-1}), pyridine, CH_2Cl_2–DMF; (b) Grignard reagents, THF, 62 °C; (c) 1% TFA, CH_2Cl_2.

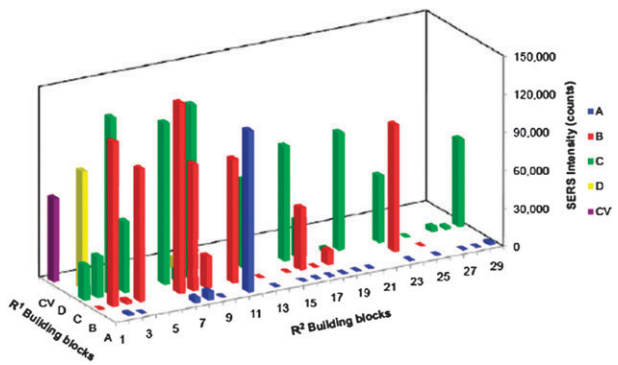

Fig. 1 Comparative SERS intensities of TM compounds from **A** to **D** building blocks with CV. SERS spectra were obtained from excitation at 633 nm with laser power of 3 mW.

study in various conditions. In many cases, we observed that the SERS signal was fluctuating over the measurement and storage time, perhaps due to the aggregation of Au nanoparticles. To stabilize the SERS signal, we modified the nanotags by encapsulating the gold surface with thiolated PEG which protected the nanotags from aggregation in aqueous media. We observed that PEG encapsulated nanotags did not show significant aggregation under ambient conditions and furnished stable SERS intensity for all five compounds. Moreover, the SERS intensities of the PEG encapsulated nanotags could be optimized by changing the dye concentration to that which gave the most stable enhanced signals as shown in Fig. 2. The novel reporters **B2**, **B7**, and **C7** showed 2.3-, 2.0-, and 1.8-fold increased signal respectively compared with CV (Fig. 2) under optimum conditions (10 μM of each dye). The SERS spectra of our best three reporters, **B2**, **B7** and **C7**, and **CV** are shown in Fig. 3. The highest intensity peak has been chosen for comparison.

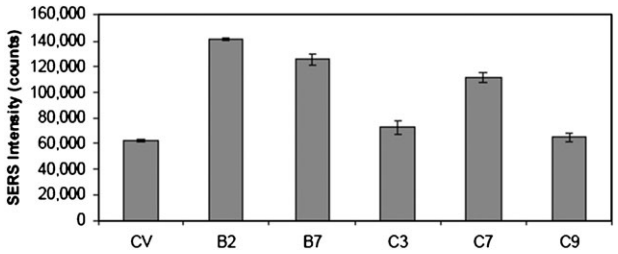

Fig. 2 SERS intensities of PEG encapsulated nanotags. The average intensities of five individual measurements with error bars denoting their standard deviation. SERS spectra were obtained from excitation at 633 nm with laser power of 3 mW.

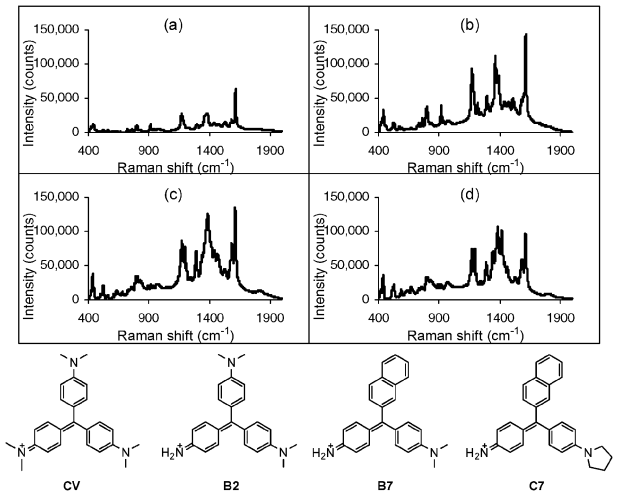

Fig. 3 SERS spectra of PEG encapsulated nanotags and structure of TM compounds: (a) **CV**, (b) **B2**, (c) **B7**, and (d) **C7**.

In summary, we have synthesized a structurally diverse triphenylmethine library by a combinatorial solid phase approach and have systematically screened their SERS intensity. By modifying the hit compounds with surface stabilization, we finally identified three highly Raman active compounds (**B2**, **B7**, and **C7**) with high potential as SERS reporters for biological applications. Further studies towards *in vivo* bioimaging and the combinatorial synthesis and screening of a wider spectrum of libraries are in progress.

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