composition of the polymer sheets depends on the type of polymers used and the presence or absence of a capping layer. The patterns used here resulted in polymer films that can be classified either as materials or as macromolecules. More familiar systems that combine macroscopic and molecular dimensions are liposomes, SAMs, and Langmuir–Blodgett films.[22] The smallest patterns that can now be formed by μCP are approximately 0.01 μm^2; the two-dimensional polymers derived from these patterns will have $M_r \approx 100$ MDa and would begin to approach the molecular weight of very large soluble polymers such as polyacrylamide (20 MDa)[23] and λ-phage DNA (32 MDa).[24] This study represents a first step towards the fabrication (rather than synthesis) of polymers with well-defined nanosize shapes and dimensions. The combination of (nano)lithographic techniques and surface chemistry will allow the fabrication of a wide range of different shapes and chemical functionalities for these macromolecules.

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[17] The patterned substrate was placed in a 0.05 wt% solution of PEI (branched, $M_r = 750000$) in isopropyl alcohol for 20 min. The surface was briefly (< 30 s) sonicated in water and dried under a stream of nitrogen. This polymer layer was cross-linked by reacting the amine-terminated surface with a 0.05 wt% solution of POMA ($M_r = 30000$) in THF, or PEMA ($M_r = 100000–500000$) in acetone. The excess polymer was removed by briefly (< 30 s) sonicating the sample in THF or acetone and washing with CH_2Cl_2.

[18] We added 0.5% HF to remove the underlying film of Ti that was used to promote adhesion of gold to SiO_2.

[19] PEI, POMA, PEMA, and fluoresceinamine were purchased from Aldrich and used without further purification.

[20] Full characterization of patterned polymer multilayers is described in W. T. S. Huck, L. Yan, A. Stroock, R. Haag, G. M. Whitesides, *Langmuir* **1999**, *15*, 6862.

[21] The volume of a 5-nm thick 1 μm^2 film is 5×10^{-15} cm^3. The density of PEI is 1.05 $g cm^{-3}$, and the density of POMA is 0.97 $g cm^3$. Assuming an average density for the polymer film of 1.01 $g cm^3$, the molecular weight is $1.01 \times 6.02 \times 10^{23} \times 5 \times 10^{-15} \approx 3$ GDa.

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Surface-Enhanced Resonance Raman Scattering as a Novel Method of DNA Discrimination**

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Sensitive and fast analytical methods for the identification of specific DNA sequences and fragments are a prerequisite to exploit the advantages of the new understanding of DNA with the completion of the human genome map. The most frequently used analytical methods employ fluorescence spectroscopy to detect a labeled nucleic-acid probe.[1, 2] Detection of the fluorophore confirms the presence of a specific base sequence. The main disadvantage of this method for an oligomer mixture is identification of differently labeled sequences due to the inherently broad fluorescent signals. Differentiation is possible by using fluorophores with very different emission profiles[3] or by time-resolved fluorescence detection instead.[4] However, these methods require special fluorophores and complex equipment that still cannot easily

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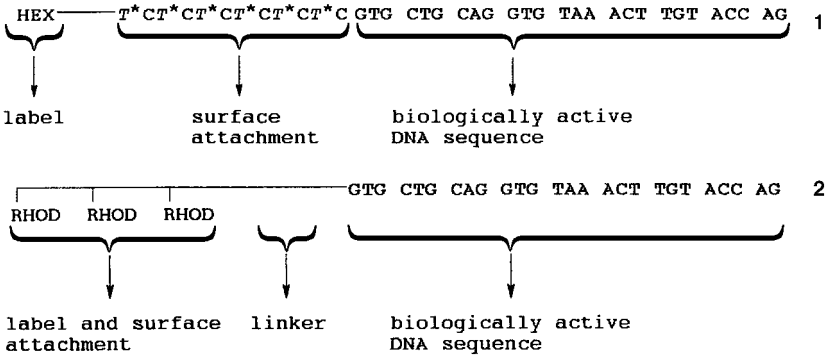
resolve mixed fluorophores in varying proportions. These factors are key limitations in the development of the fast and discriminatory assays now required. Surface-enhanced resonance Raman scattering (SERRS) has comparable sensitivity to fluorescence but also a unique advantage in that the scattered light consists of sharp, molecule-specific vibrational bands.[5–10] Thus, it has the potential to enable discrimination of a mixture of probes and to facilitate the development of more powerful DNA analysis. We report the identification of two labeled oligonucleotide probes in a mixture as an illustration of the potential power of SERRS in nucleic acid analysis. We also show how we can ue SERRS to analyse varying proportions of the oligonucleotides. Additionally, specially designed oligonucleotides are required to achieve the necessary sensitivity. We report here a new type of oligonucleotide probe design and compare it to the only previous example.

To obtain effective SERRS, an analyte containing a chromophore must adsorb onto a suitably roughened metal surface, usually silver or gold.[6] The surface reported herein is an aggregated silver colloid, since it gives good sensitivity and has been used previously with DNA.[11] The aggregation is required to obtain maximum enhancement from the surface plasmons. The oligonucleotides are negatively charged and will not adsorb onto the silver colloid unless additional moieties, allowing adsorption, are attached.[11] Also, to provide a resonance contribution, a chromophore compatible with the exciting laser must exist. Thus, a positive charge for attachment to the surface and a suitable chromophore are required in the oligonucleotide.

Two 26mer oligonucleotides were synthesized. Oligonucleotide **1** used the fluorophore 2,5,2′,4′,5′,7′-hexachloro-6-carboxyfluorescein (HEX) as the visible chromophore. HEX is negatively charged and does not have a strong affinity for the metal surface. The surface attachment was achieved by



incorporation of six modified nucleobases, 5-(3-aminopropynyl)-2′-deoxyuridine (*T**), at the 5′-terminus next to the HEX label. Oligonucleotide **2** is labeled with positively charged rhodamine 6G (RHOD) that also adsorbs onto the metal surface. Further, to assist the detection of **2**, three RHOD labels were used. The three RHOD labels were attached at the 5′-terminus through aminohexyl-2′-deoxyribose groups separated from the rest of the oligonucleotide by a hexaethylene glycol spacer.

The two oligonucleotide probes gave efficient Raman scattering from the surface and distinctly different signal patterns (Figure 1). Both labeled oligonucleotides were

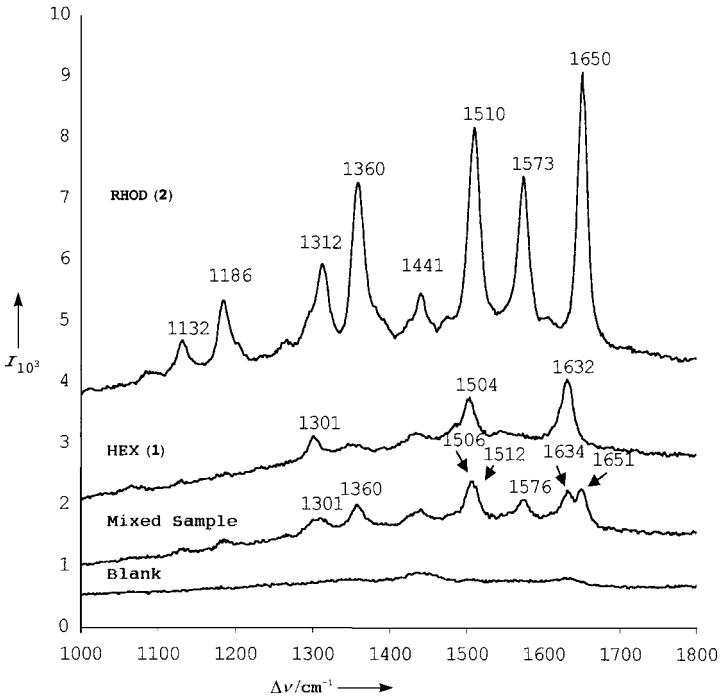


Figure 1. SERRS spectra **1** and **2** separately and mixed in a 5:1 ratio of **1**:**2**.

present at 8×10^{-13} moles, illustrating the sensitive nature of detection by this method. The spectrum from a 5:1 (**1**:**2**) mixed sample indicates that the two labels are easily discriminated. Figure 2 illustrates this for different molar ratios, selecting for illustration the quadrant aromatic stretches of both dyes at 1632 cm^{-1} for **1** and 1650 cm^{-1} for **2**. The oligonucleotides produce approximately equal scattering intensity in this region at a ratio of 5:1 (**1**:**2**).

The figures clearly show that the rhodamine 6G-labeled oligonucleotide **2** gives a better signal-to-noise ratio than the HEX-labeled oligonucleotide **1** by an amount greater than that expected with the use of three RHOD labels as opposed to one HEX. The cross section capture of the two dyes for SERRS is not known, but in part the difference in sensitivity is due to the design of the oligonucleotides. In the case of **1**, the HEX label itself does not adhere to the silver surface but is held in proximity by the adsorption of the nucleotide to the surface. In the case of **2**, the attachment group is on the chromophore and consequently the label adsorbs directly onto the surface. This latter method is likely to provide more effective surface enhancement. Extension products generated by the action of DNA polymerase have also been detected by SERRS. Further investigation into the use of these probes for meaningful biological analysis is currently underway.

This study illustrates the ability of SERRS to discriminate different oligonucleotide probes in mixtures without requiring

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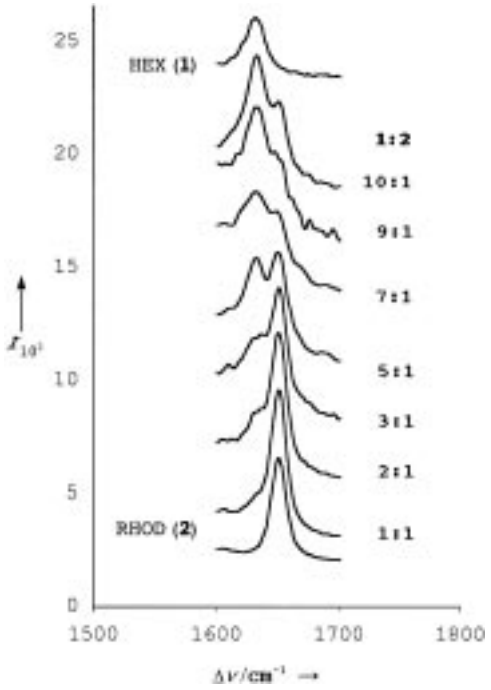

Figure 2. The signal corresponding to the quadrant aromatic stretches of the labeled oligonucleotides, as two single samples and in a mixture.

separation and in varying proportions. In addition, these initial experiments indicate that oligonucleotide probe design is crucial and there is considerable potential for further development particularly as both nonfluorescent and fluorescent chromophores can be used as SERRS labels. The modified oligonucleotide probes can also still be used as substrates for polymerases, therefore demonstrating that they remain biologically active. These results prove that, by considering the chemistry involved, SERRS can feature in the development of more effective DNA assays allowing the development of assay formats not possible by conventional methods.

Experimental Section

A solution of modified oligonucleotide (20 μL, 4×10^{-8} M) was premixed on ice with an aliquot of spermine tetrahydrochloride (20 μL, 8×10^{-2} M). Water (500 μL) and citrate-reduced silver colloid (500 μL) were added to this solution. Analysis used a Renishaw 2000 Raman Microprobe with excitation provided by a Spectra-Physics Model 2020 argon-ion laser (100 mW, $\lambda = 514.5$ nm). The detector was a charge-coupled device (CCD). Samples were analysed over 1 s in a plastic micro-titre plate using a X10 objective with the grating centred at 1400 cm^{-1}.

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α-Oxymethyl Ketone Enolates for the Asymmetric Mannich Reaction. From Acetylene and *N*-Alkoxycarbonylimines to *β*-Amino Acids**

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The reactions of enolizable carbonyl compounds with azomethine functions, usually referred to as Mannich-type reactions (also termed aza–aldol reactions), result in the formation of *β*-amino acids, ketones, or aldehydes.[1] These reactions are conceptually equivalent to the aldol reactions, but, in sharp contrast, they have been substantially less developed.[2, 3, 4] There are two reasons that can justify this situation: firstly, the poorer electrophilicity of the azomethine function relative to that of the carbonyl function, and secondly, the preference of enolizable azomethines to undergo *α*-deprotonation rather than addition.[5] To date, there are two main ways to approach these problems. One strategy lies in the use of activated forms of azomethines,[1, 2] and the other in the use of trialkylsilylenol ethers or *O*-(trialkylsilyl)ketene acetals as nucleophiles.[3, 6] The later strategy has led to outstanding advances in enantioselective Mannich reactions[7] catalyzed by chiral ruthenium,[8a–b] palladium,[8c–e] and copper[8e–g] complexes. Some highly diastereoselective methods involving chiral azomethines are also known,[6, 9] but in most

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