FEATURE ARTICLE

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Optical barcoding of colloidal suspensions: applications in genomics, proteomics and drug discovery

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The enormous amount of information generated through sequencing of the human genome has increased demands for more economical and flexible alternatives in genomics, proteomics and drug discovery. Many companies and institutions have recognised the potential of increasing the size and complexity of chemical libraries by producing large chemical libraries on colloidal support beads. Since colloid-based compounds in a suspension are randomly located, an encoding system such as optical barcoding is required to permit rapid elucidation of the compound structures. We describe in this article innovative methods for optical barcoding of colloids for use as support beads in both combinatorial and non-combinatorial libraries. We focus in particular on the difficult problem of barcoding extremely large libraries, which if solved, will transform the manner in which genomics, proteomics and drug discovery research is currently performed.

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1. Introduction

The design, synthesis, analysis and management of large chemical libraries have many important applications in genomics, proteomics and drug discovery.[1,2] Some of the major applications include identification of disease-related targets for therapeutic and diagnostic applications,[3] correlation of changes in gene expression or structure with disease,[4] identification of proteins for use as vaccine targets,[5] and determination of the relationship between genetic variation and disease susceptibility.[6] For example, by comparing the ways in which genes are expressed in normal and diseased tissue, the important genes and hence the associated target proteins that are part of the disease process can be identified. This information can then be used to synthesise large-scale chemical libraries in order to search for drug leads that interact with those proteins. High-throughput molecular screening plays an important and central role in each of these steps.

Depending on the specific application, chemical libraries may be comprised of different families of chemical compounds. Genomics applications require a library containing single-stranded DNA molecules (oligonucleotides or cDNAs) which are all of different sequence.[2] Proteomics studies engage a library of proteins for exploration of protein diversity, interaction, structure, and function[7] and drug discovery research requires a variety of molecular species such as polypeptides[8] and polysaccharides.[9]

With the completion of the first draft of the human genome sequence[10] in 2001, there has been a massive increase in the amount of new information to process and targets to screen. As such, the demand for economical, high-throughput and flexible molecular screening alternatives in genomics, proteomics and drug discovery research has greatly increased. The use of 'microarray' devices for solid phase genomic screening[11] is becoming more established and the technology is being transferred to other areas such as proteomics.[12] For drug discovery applications, high-density microplates coupled with sophisticated, high-throughput robotic and detection instrumentation are now commonplace.[13] Although microarrays are having an important impact on high throughput genomic and proteomic screening applications, the cost of such devices has remained relatively high because of the sequential nature of the microarray compound synthesis (required to keep track of the grid position of each compound). Also, microarrays are currently restricted to a library size of approximately 5×10^5 compounds because of their limited pixel size and two-dimensional geometry.[14] Correspondingly, the miniaturization of high-density microplate formats for drug discovery is intrinsically limited by the physical constraints of delivering

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small volumes to wells. This severely restricts library size and screening throughput rate.

Many companies and institutions are now recognising the potential of increasing the size and complexity of chemical libraries by producing libraries attached to colloidal support beads which can be rapidly screened for biological activity. The rationale is self-evident: colloids are inexpensive to produce in enormous numbers; they can be conveniently stored in small volumes of fluid; and they can be 'optically barcoded' and screened using various high-throughput detection technologies.[15] The general processes involved in producing and screening colloid-based libraries are shown in Fig. 1.

Colloid-based libraries are typically produced using either 'combinatorial' or 'non-combinatorial' procedures (Figs. 1a and 2). In a non-combinatorial method, chemical libraries are generated by sequential attachment of fully synthesised moieties (*e.g.* cDNAs, small molecules, proteins) onto aliquots of encoded colloids. Different aliquots are mixed together to form a library (see Fig. 2a).[16–18] This attachment is normally performed under aqueous conditions and may involve covalent bonding of the moiety to the colloid or physical attachment using the streptavidin–biotin interaction. Production of combinatorial libraries involves progressive synthesis of the library probes on the colloids under solvent conditions.[19,20] The use of the combinatorial split-and-mix approach allows enormous numbers of probes to be produced in a very low number of cycles (Fig. 2b).[14,19] Indeed, much larger libraries can potentially be produced *via* the combinatorial method compared with the non-combinatorial method.

Screening of a colloid-based library typically involves exposure of the library to one or more labelled target molecules (see Fig. 1b). For example, fluorescently labelled target DNA of unknown sequence can be introduced into an oligonucleotide library where hybridisation occurs between the labelled DNA and the probes that are complementary to the target sequence (Fig. 1c). This hybridisation gives rise to a bright fluorescence signal on the colloids under correct illumination, corresponding to the emission wavelength of the fluorescent label. The colloids showing the brightest fluorescence (*i.e.*, those bearing complementary sequences) are distinguished by a fluorescence detection instrument (Fig. 1d) such as a flow cytometer (Fig. 3) or fluorescence microscope.[21] Determining the structure of the hybridized probes (*i.e.*, the 'hits') would permit reconstruction of the longer target sequence (see Fig. 1e). However, one of the greatest challenges facing researchers using large colloid-based libraries is the ability to rapidly and conveniently identify the chemical structure or sequence of each probe that is found to be bioactive. The quantity of compound on a colloidal particle is usually adequate to allow detection of bioactivity (*i.e.*, to isolate the 'hits'), however, the amount is usually insufficient to permit structural elucidation by conventional analytical techniques.[22] Because colloid-based probes are randomly located in a suspension (unlike the compounds in microarrays and microplates which are in a fixed position on an array), an encoding

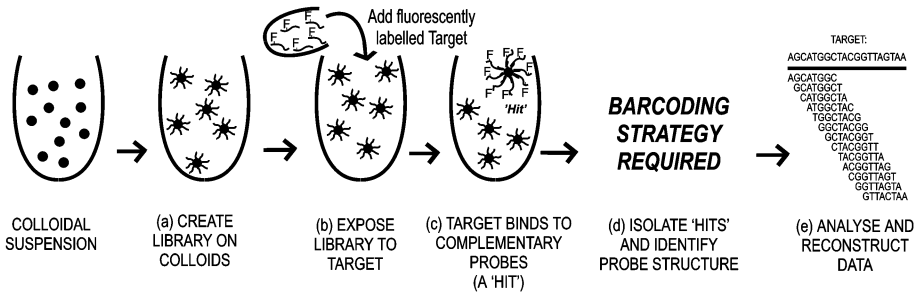

Fig. 1 The typical processes involved in producing and screening colloid-based DNA libraries. (a) A library of oligonucleotide probes is created on the colloidal particles using combinatorial or non-combinatorial methods (see Fig. 2). (b) Fluorescently labelled target DNA is mixed with the library, and those probes which are complementary will bind with the target. (c) The colloids on which the target is bound will brightly fluoresce the colour of the target label, signifying a 'hit'. (d) The 'hits' are distinguished and/or isolated using fluorescence detection instrumentation such as a flow cytometer (see Fig. 3) or fluorescence microscope. To determine the structure of a bioactive colloid-based probe (typically present in nanomolar amounts), a barcoding strategy must be in place. (e) Identification of the bioactive probes permits reconstruction of the data, thereby revealing the target sequence.

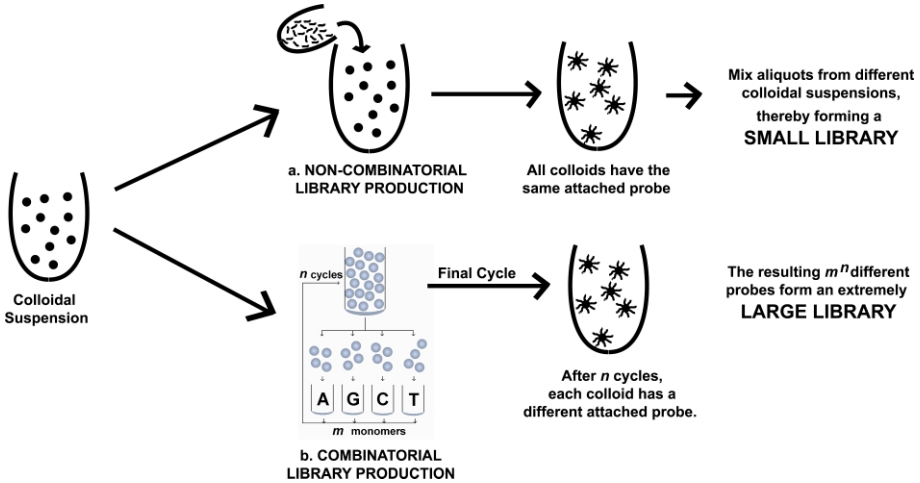

Fig. 2 Creation of a chemical library on colloidal support beads. (a) The non-combinatorial method for producing a library involves attachment of identical, fully-synthesised probes to the colloids using physical or covalent bonding. Several different suspensions are mixed together to form a small library. (b) The combinatorial method of library production involves progressive synthesis of probes on functionalised colloids over *n* cycles of a split-and-mix process. This results in an extremely large chemical library containing m^n compounds.

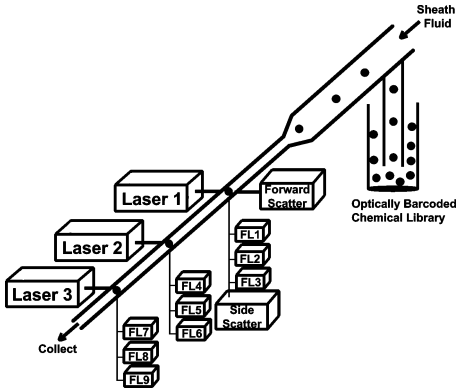

Fig. 3 Multi-parametric flow cytometers possess multiple lasers set up in parallel, with accompanying detectors that can measure a wide variety of fluorescence wavelengths as well as forward and side (90°) light scattering.

system is required to allow the rapid determination of these structures or sequences. This is particularly important in the combinatorial libraries which involve a large number of colloid-based probes. A common approach to reducing this problem is to covalently bind molecular 'identifier' tags to the colloids in parallel with the compound synthesis. These tags may be oligonucleotides,[21] electrophoretic molecular tags,[23] cleavable dialkylamine tags,[24] trityl mass-tags[25] or fluorescent tags.[26] The requirement for compatible probe and tag synthesis, however, places a considerable restriction on the molecular tagging procedure. Also, additional chemical steps are needed to synthesize the tags, and artefacts may arise as a result of interfering chemistries between the combinatorial step and the tagging step.

An alternative method of encoding colloid-based libraries, optical barcoding, has tremendous potential, and innovative methods are being developed by several companies and institutions worldwide.[14,16,17,27,28] One of the greatest advantages of using optical methods to barcode colloids is the speed and accuracy with which the structure of biologically active compounds may be determined after library screening. In this article, we give an overview of the current methods of using optically barcoded colloidal suspensions in high throughput molecular screening of non-combinatorial and combinatorial libraries. We discuss the challenge of barcoding colloidal suspensions during synthesis of large combinatorial libraries (active barcoding) and present new methods of pre-barcoding colloidal particles for combinatorial library synthesis.

2. Optical barcoding of colloids for non-combinatorial library screening

There have been a number of reports recently on the use of optically barcoded colloidal suspensions for screening small non-combinatorial libraries.[16–18,27–29] Current innovative methods of optical barcoding include the use of fluorescent dyes, nanocrystals and metals. These methods are discussed below.

The technique of using multiple intensities and multiple emission wavelengths (*i.e.*, multiplexed encoding) to barcode colloids (3–6 μm in diameter) for small library applications has been employed by a number of groups.[16–18] By entrapping various ratios of two fluorescent dyes or lanthanide complexes in the interior of colloidal particles, up to 100 different colloidal suspensions have been produced.[16–18] For each suspension, the polymeric colloids are swollen in a solvent–dye mixture containing a certain ratio of the two dyes/complexes. Rapid contraction of the colloids occurs upon exposure to an aqueous or alcoholic solution,[18] thereby entrapping the fluorescent dyes/complexes within the colloids. Typical solvents used are dimethylformamide or tetrahydrofuran. Decoding the colloids is achieved by a variety of methods including flow cytometry[16] and optical fibre microarrays.[17,18]

An alternative method of optical barcoding involves the incorporation of zinc sulfide-capped cadmium selenide nanocrystals into 1.2 μm polymer colloids in controlled ratios.[27] Many sizes of nanocrystals can be excited at a single wavelength, resulting in several emission wavelengths (colours) that can be detected simultaneously.[30] Nie and colleagues reported a DNA hybridisation experiment which involved four oligonucleotide probes and four colloidal suspensions barcoded with nanocrystals.[27] Barcoding was performed by swelling polymer colloids (0.1–5.0 μm) in a propanol (or butanol)–chloroform mixture and adding a controlled ratio of three nanocrystal colours (sizes) to the mixture. The colloids are sealed with a thin polysilane layer which fastens in the nanocrystals and improves their stability in aqueous conditions.[27]

Another barcoding technique that was reported recently adopts suspensions of colloidal rods which are encoded by complex striping patterns.[28,29] The patterned rods (15 nm–12 μm in width and 1–50 μm in length) are prepared by using sequential electrochemical deposition of metal ions into templates with uniformly sized pores. Analysis of the differential reflectivity of adjacent stripes in a conventional light microscope permits decoding of the striped patterns. To date, two colloidal rod suspensions with different barcodes have been used to demonstrate that these rods can be used as supports for biological screening. The barcoded rods were successfully used to distinguish between human and rabbit immunoglobulin (IgG) in a 'sandwich' type hybridisation assay.[28]

3. Optical barcoding in combinatorial libraries

Colloidal particles for combinatorial library synthesis differ markedly from those required in non-combinatorial library preparation (described above in Section 2) in that they must be solvent-resistant. Many optically barcoded colloids are stable in aqueous conditions only and will lose their barcode should they be placed in solvent. The colloidal supports which are used in combinatorial library synthesis must themselves withstand harsh solvents and reagents (*e.g.* acetonitrile, dimethylformamide, tetrahydrofuran) and their barcodes must be impervious to these conditions. Optical barcoding of colloid-based combinatorial libraries has been attempted by several groups,[26] however the size of these libraries is surprisingly small. Optical barcoding strategies, which have been designed to encode extremely large libraries, will have considerable advantages over the techniques currently used for genomics, proteomics and drug discovery research.

Our group at the University of Queensland (Brisbane, Australia) is tackling the optical barcoding of large combinatorial libraries in two ways. Our methods exploit the optical properties of individual colloids to encode information on support beads used for combinatorial synthesis. In one strategy, small multi-fluorescent colloids are used to generate an information-rich barcode on the solid support bead during library synthesis (a method we term 'active barcoding').[31,32] The colloidal barcode can be read in a fluorescence microscope to determine the structure of the compound synthesised on the bead. A second method uses optically unique colloids as supports for combinatorial library synthesis, with the reaction history of each colloid being recorded by a flow cytometer.[14,33] Both of these methods have the potential to solve the barcoding problem associated with the use of extremely large colloid-based libraries. These strategies are described in more detail below.

3.1 Active barcoding during combinatorial synthesis

Active optical barcoding is a simple, rapid and efficient method for encoding support beads *during* combinatorial library synthesis.[31,32] As shown in Fig. 4a, the support beads are apportioned during the 'split' step of the synthesis and each portion is encoded with one batch of fluorescently-encoded 'reporters' (*i.e.*, colloidal particles 0.5–2.5 μm in diameter) (Figs. 4b and c). A single batch of reporters containing a unique combination of dyes encodes each reaction. Reporters adhere to every support bead in multiple, but controllable, numbers. The support beads are then reacted with a different monomer (*e.g.* nucleic acid, amino acid, carbohydrate), after which the bead suspensions are recombined to complete the cycle. This process is repeated for a number of cycles (n), with a unique batch of reporters encoding each reaction. The identity of the monomer attached during each cycle, and the position of the monomer within the growing compound (known from the cycle number), is unambiguously recorded on the bead. Decoding involves visualizing the colour combination of each reporter particle, as well as its location, through different filters in a fluorescence imaging device such as an optical microscope. Determination of the set of reporters comprising the colloidal barcode enables structural elucidation of the probe synthesised on the support bead.

In order to increase the number of distinguishable batches of reporters that can be used during the split-and-mix synthesis, more than one dye is often incorporated into the individual reporters. As shown by eqn. (1), a simple combinatorial analysis of this process reveals that the number of compounds which can be encoded using this method, **M**, becomes explosive with increasing 'c', the number of fluorescent dyes used. This is due to the fact that eqn. (1) contains a power raised to a power

$$\mathbf{M} = \left(\frac{2^c}{n}\right)^n \text{ when } 2^c > n \qquad (1)$$

where c is the number of dyes, n is the number of cycles, **M** is the maximum number of uniquely encoded compounds.

Indeed, a library containing more than 4.3×10^9 oligonucleotides (4^{16} single stranded DNA probes) can potentially be encoded with just 6 fluorescent dyes.[14] Thus, this encoding method is extremely powerful owing to the efficient use of relatively few fluorescent dyes to record an extremely large amount of information on each solid support bead.

3.2 Pre-barcoded colloidal supports for combinatorial synthesis

In addition to the active barcoding strategy described in Section 3.1, our group at the University of Queensland has developed a second strategy,[13,14,33] which encompasses the combinatorial synthesis of libraries on pre-encoded colloids and the use of these libraries in high-throughput molecular screening. Crucial to this strategy are the pre-barcoded colloids which are designed to possess a distinguishable 'optical signature'. The optical signature of a colloidal particle is the composition of multiple fluorescence and light scattering attributes which can be detected by an instrument such as a flow cytometer (Fig. 3). Colloidal suspensions containing particles which display a diverse range of optical signatures are termed 'optodiverse'. Optodiversity can be introduced into the particles (i) through incorporation of dyes of various fluorescence excitation and emission wavelengths, (ii) through incorporation of dyes in various concentrations, (iii) by controlling the internal structure thereby giving the colloids a unique refractive index profile and (iv) by permitting fluorescence resonance energy transfer processes to occur. Fig. 5 shows a three-dimensional flow cytometry plot where each dot represents the optical signature of one colloid. The optodiversity in the colloidal suspension is clearly visible, with colloids possessing optical signatures across almost the entire three-dimensional space. Naturally, the optodiversity is not limited to just three parameters. Indeed, up to nine different fluorescence parameters and two light scattering parameters can currently be used to distinguish the optical signature of a colloid.[33] The strategy for preparing optodiverse colloidal suspensions is presented later, following further discussion of the complete encoding method.

After synthesis of an optodiverse colloidal suspension, the colloids possessing a unique optical signature are removed from the suspension using a specially modified flow cytometer. Several fluorescence and light scattering detectors in the flow cytometer segregate the optically unique colloids from the optodiverse colloidal suspension. By observing the optical

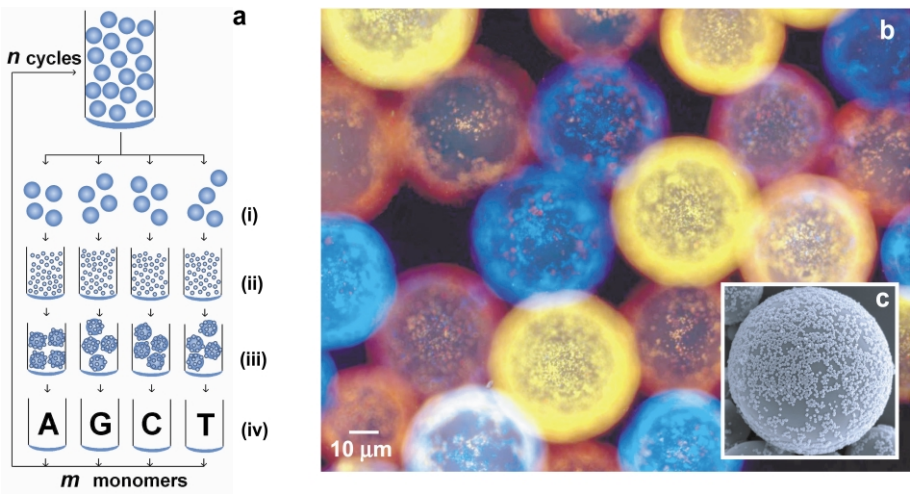

Fig. 4 Active optical barcoding of combinatorial libraries. (a) A schematic diagram of a split-and-mix DNA library synthesis on support beads: (i) a large number of colloids is equally partitioned into $m = 4$ vessels; (ii) each portion of beads is mixed with a unique type of 'reporter' that contains a distinct combination of fluorescent dyes; (iii) fluorescent silica reporters become attached to each colloid and (iv) a different monomer (*i.e.*, one of 4 nucleic acids, A, G, C or T) is reacted with each portion, and the beads are recombined to complete the cycle. The split-and-mix process is repeated for a chosen number of cycles, n, resulting in a large DNA library consisting of all combinations of oligonucleotides of length 'n'. The number of oligonucleotides in the library is given by m^n. (b) Fluorescence microscopy image of multiple support beads (PEGA resin) with various colloidal barcodes. Identification of the set of silica reporters comprising the colloidal barcode enables structural elucidation of the probe synthesised on the support bead. (c) A scanning electron microscope image of 1 μm reporters attached to a ceramic support bead.

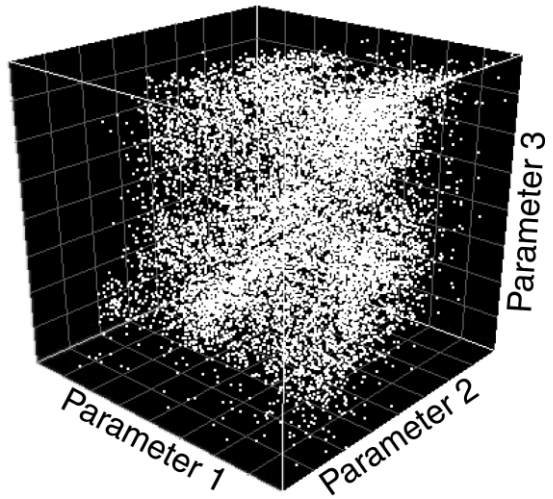

Fig. 5 A three-dimensional flow cytometry plot showing optodiversity in a suspension of multi-fluorescent silica colloids (3 μm in diameter). Each dot on the plot represents the optical signature of one colloid.

signatures with up to 11 detectors, many billions of unique colloids can possibly be collected from an optodiverse colloidal suspension.[33] These unique colloids are the optically barcoded supports on which a combinatorial library can be synthesised using the split-and-mix method (Fig. 6). With the flow

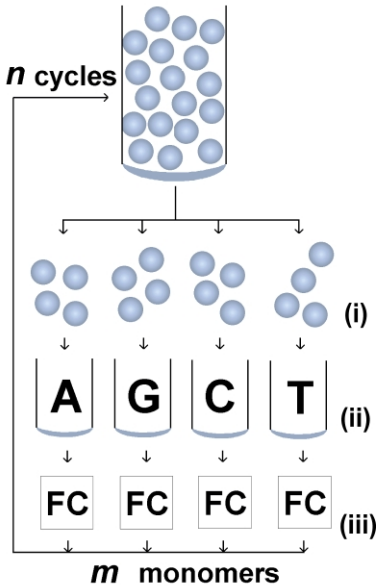

Fig. 6 Combinatorial library synthesis on optically unique colloids. In the split and mix cycle, (i) a large number of colloids are partitioned into several vessels, (ii) a different nucleic acid (A, G, C, or T) is reacted with each portion, (iii) the colloids are passed through the flow cytometer (FC) and then the particles are recombined to complete the cycle. The process is repeated for a chosen number of cycles, *n*, resulting in an oligonucleotide library consisting of all m^n combinations. The flow cytometer tracks the synthesis performed on each colloidal particle by detecting its optical signature and storing the reaction history of each particle.

cytometer tracking the combinatorial synthesis performed on each colloid and storing the reaction history, immediate identification of the colloid-based probe is possible by detecting the colloid's optical signature. Screening of the resulting large library is performed with the probes attached to the colloidal particles. Binding of a fluorescently-labelled target with any colloid-based probe in the library gives rise to a bright fluorescence signal on each colloid under correct illumination, corresponding to the emission wavelength of the target's label. The flow cytometer then selects the beads that show the brightest fluorescence and the bioactive probes are identified by decoding the optical signatures of the colloids on which they reside. This is done automatically, by recalling the data stored by the flow cytometer during library synthesis.

As well as the requirement for each particle in a colloidal suspension to display a distinguishable optical signature, the particles must also be solvent-resistant to enable their use as encoded solid supports in combinatorial library synthesis. Ceramic colloids, for example, silica-based colloids produced by the Stöber process[34] (see Fig. 7a), are resistant to a multitude

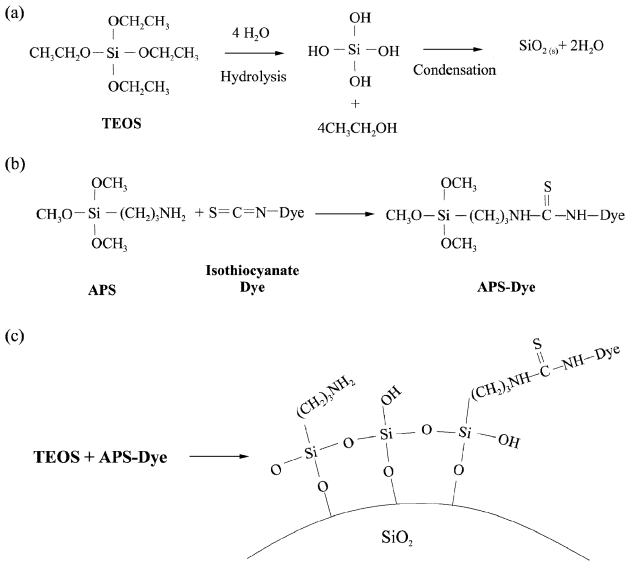

Fig. 7 Reaction scheme for synthesis of fluorescent silica layers around colloidal silica particles. (a) Solid silica is formed using the 'Stöber process'[34] which involves hydrolysis and condensation of tetraethoxysilane (TEOS) in a mixture of water, ammonia and ethanol. In order to form a fluorescent silica layer, (b) a fluorophore (*e.g.* an isothiocyanate dye) is first coupled to a silane coupling agent [*e.g.* (3-aminopropyl)trimethoxysilane (APS)] to form an APS–dye conjugate[37,38] and (c) the APS–dye conjugate is added, together with TEOS, to the colloidal suspension which results in the formation of a fluorescent layer around each colloidal particle.[14,33,37]

of organic solvent conditions. Below we describe derivatised silica ceramic particles which possess optodiverse and solvent-resistance properties.

A colloidal suspension can be optically barcoded by synthesising multiple, concentric, fluorescent silica layers around 'core' silica particles (2–5 μm in diameter).[13,14,33] The reaction scheme for preparing fluorescent silica layers is shown in Figs. 7b and c. To obtain optodiversity, a split-and-mix strategy is used, whereby the colloidal suspension is apportioned into several vials, a different fluorescent layer is synthesised onto each colloid and the colloids are recombined to complete the cycle. The process is repeated for a chosen number of cycles, resulting in a population of multi-fluorescent colloids which display a diverse range of fluoresence emissions and intensities (Fig. 8a).

Fluorescence emission is just one attribute of a colloid that contributes to its optical signature. Indeed, the forward and 90° light scattering parameters of the flow cytometer can be used to detect diversity in the refractive index profile and size between colloids. Our studies show that synthesis of up to twelve layers on a core colloid increases its diameter by up to 15%. This equates to a contribution of up to 36 nm, on average, by each synthetic layer to the overall diameter of the particle. Varying the number of layers synthesised on the core colloids diversifies colloid size, adding to optodiversity in the colloidal suspension.

Besides affecting colloid size, increasing the number of layers also has an influence on fluorescence emission intensity,

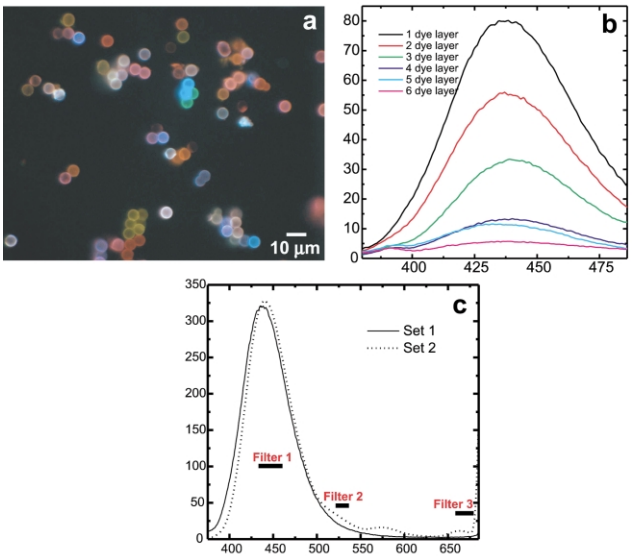

Fig. 8 Fluorescent, multilayered colloids as optodiverse supports for combinatorial library synthesis. (a) Fluorescence microscope image of colloidal particles, optically barcoded by synthesising concentric fluorescent silica layers around 'core' silica particles (5 μm in diameter). (b) Increasing the number of layers synthesised on the core particle influences the intensity of the observed fluorescence emitted from each layer. This intensity variation is a very useful strategy in obtaining optodiversity in the colloidal suspension. Synthesis of 5 dye layers on top of the first (innermost) layer, significantly decreases the emission intensity of the first layer at its emission wavelength of 437 nm. (c) In these fluorescence emission spectra, Set 1 colloids possess one dye-labelled silica layer and Set 2 colloids have six concentric fluorescent silica layers, with a different dye being used in each layer. Emission peaks evolving from dyes synthesised later in the multilayered colloids are clearly observed when the colloids are excited at 346 nm. This phenomenon of energy transfer between dyes in separate layers enhances the optodiversity of the colloidal suspension.

particularly the intensity of the innermost layers. As shown in Fig. 8b, synthesis of up to 11 concentric layers on top of the first (innermost) layer, significantly decreases the emission intensity of the first layer at its emission wavelength of 437 nm. The intensity decrease is likely to be due to the increase in scattering of emitted photons, caused by the increasing particle size and changing refractive index of the colloid as additional layers are deposited. Rather than being unfavourable, this intensity variation is a very useful strategy in obtaining optodiversity in the colloidal suspension.

Furthermore, a feature of using multiple fluorescent dyes is the ability to exploit energy transfer processes (which is usually unfavourable) to promote the optodiversity by broadening the range of optical signatures found throughout our colloidal suspensions. The fluorescence spectra in Fig. 8c show two sets of colloids, Set 1 and Set 2, each with a dye excited at 346 nm incorporated into the innermost silica layer. The Set 1 colloids possess one dye-labelled silica layer and the spectrum shows one emission peak at 437 nm when the colloids are excited at 346 nm (Fig. 8c). The colloids in Set 2 have six concentric fluorescent silica layers, with a different dye being used in each layer. Emission peaks evolving from dyes synthesised later in the multilayered colloids are clearly observed when the colloids are excited at 346 nm (Fig. 8c). By separately preparing single layer fluorescent colloids composed of the dyes in question, we confirmed that these dyes are not normally excited at the wavelength used to excite the dye in the first layer (in this case 346 nm). It is apparent, therefore, that the extra emission peaks are the result of the reabsorption of photons emitted by the fluorophores incorporated into the innermost layer. Clearly, the phenomenon of energy transfer between dyes in separate layers enhances the optodiversity of the colloidal suspension and careful choice of filters on the UV laser line on the flow cytometer can separate the beads exhibiting the spectra in Fig. 6c.

We have also developed a new class of ceramic colloid for use as pre-barcoded solid supports in combinatorial library synthesis.[35] These colloids have a smooth external surface, with a controlled internal structure (Fig. 9a). The colloids are highly

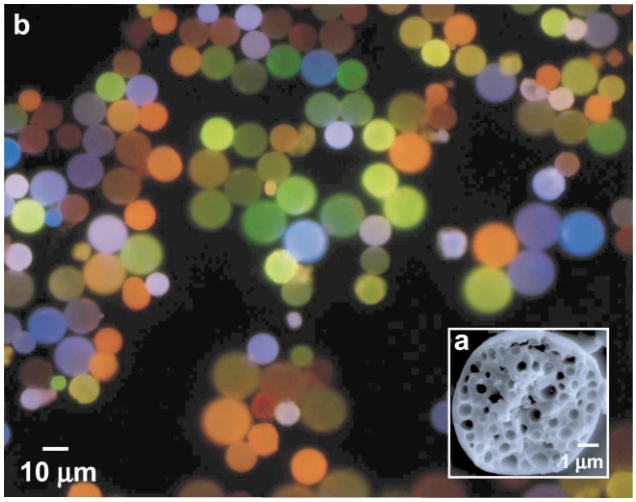

Fig. 9 Ceramic colloids for use as pre-barcoded solid supports in combinatorial library synthesis. (a) The colloids are smooth on the surface, with numerous internal pores of various sizes (0.2–1.0 μm). (b) The porous nature of the colloids permits diffusion of a wide variety of fluorescent dyes into the colloids where they covalently bind to the functionalized silica. By careful selection of dye excitation and emission properties, as well as dye concentration, an optodiverse population of particles can be produced.

functionalised and organic linkers can be coupled onto the particles, thereby making the colloids suitable for solid phase synthesis of chemical libraries.[35]

A wide variety of fluorescent dyes can be infused into the colloids where they covalently bind to the functionalized ceramic (Fig. 9b). By careful selection of dye excitation and emission properties, as well as dye concentration, an optodiverse population of particles can be produced. Fluorescence resonance energy transfer between dyes is found to be advantageous because excitation of a dye by the emission of another within the same colloid enhances the optodiversity of the colloids. Furthermore, Förster energy transfer, between fluorophores less than about 5 nm apart,[36] adds to the complexity and diversity of the optical signatures which can be generated.

4. Conclusion

Market pressure to discover new therapies in a much shorter time is forcing pharmaceutical corporations to search for alternatives in drug discovery, genomics and proteomics. It is expected the popularity of colloid-based libraries will rise significantly over the next few years because they are versatile, inexpensive, able to be stored in extremely small volumes and they can be analysed at an extremely high rate using instruments such as flow cytometers. Optical barcoding of colloid-based libraries is already proving to be a popular encoding method, since it is fast, sensitive, non-invasive, cost-effective and safe. With the rapid advances in optical barcoding currently being made, high throughput molecular screening using large colloid-based libraries is set to be transformed.

Notes and references

1 (*a*) R. L. Affleck, *Curr. Opin. Chem. Biol.*, 2001, **5**, 257; (*b*) M. J. Page, B. Amess, C. Rohlff, C. Stubberfield and R. Parekh., *Drug Discovery Today*, 1999, **4**, 55.

2 M. J. Cunningham, *J. Pharmocol. Toxicol. Methods*, 2001, **44**, 291.
3 (*a*) S. Steiner and N. L. Anderson, *Ann. N. Y. Acad. Sci.*, 2000, **919**, 48; (*b*) F. Mehraban and J. E. Tomlinson, *Eur. J. Heart Failure*, 2001, **3**, 641; (*c*) M. R. Fannon, *Trends Biotechnol.*, 1996, **14**, 294; (*d*) J. F. Leary, L. M. Reece, P. M. D. Szaniszlo, T. W. Tarl and N. M. D. Nan, *Proc. SPIE-Int. Soc. Opt. Eng.*, 2001, **4255**, 16; (*e*) G. A. Kouzmitcheva, V. A. Petrenko and G. P. Smith, *Clin. Diagn. Lab. Immunol.*, 2001, **8**, 150; (*f*) M. A. M. Boekel, B. A. W. de Jong, J. W. Drijhout, P. E. de Koning, J. van Delft and W. J. van Venrooij, *Autoimmun., Autoantigens, Autoantibodies*, 2000, **1**, 140.
4 (*a*) D. Shiffman and J. G. Porter, *Curr. Opin. Biotechnol.*, 2000, **11**, 598; (*b*) J. Hess, H. Laumen and T. Wirth., *Curr. Opin. Immunol.*, 1998, **10**, 125; (*c*) S. Katsuma, K. Nishi, K. Tanigawara, H. Ikawa, S. Shiojima, K. Takagaki, Y. Kaminishi, Y. Suzuki, A. Hirasawa, T. Ohgi, J. Yano, Y. Murakami and G. Tsujimoto, *Biochem. Biophys. Res. Commun.*, 2001, **288**, 747; (*d*) N. L. Van Berkum and F. C. P. Holstege, *Curr. Opin. Biotechnol.*, 2001, **12**, 48.
5 (*a*) M. Lizotte-Waniewski, W. Tawe, D. B. Guiliano, W. Lu, J. Liu, S. A. Williams and S. Lustigman, *Infect. Immun.*, 2000, **68**, 3491; (*b*) M. M. Becker, B. H. Kalinna, G. J. Waine and D. P. McManus, *Gene*, 1994, **148**, 321.
6 (*a*) A. V. S. Hill, *Annu. Rev. Genomics Hum. Genet.*, 2001, **2**, 373; (*b*) V. N. Kristensen, D. Kelefiotis, T. Kristensen and A. Borresen-Dale, *BioTechniques*, 2001, **30**, 318; (*c*) J. K. Pritchard, *Am. J. Hum. Genet.*, 2001, **69**, 124.
7 D. A. Moffet and M. H. Hecht, *Chem. Rev.*, 2001, **101**, 3191.
8 P. A. Lohse and C. M. Wright, *Curr. Opin. Drug Discovery Dev.*, 2001, **4**, 198.
9 D. O. Beenhouwer, P. Valadon, R. May and M. D. Scharff, *Mol. Mimicry, Microbes, Autoimmun.*, 2001, 143.
10 J. C. Venter, M. D. Adams and E. W. Myers *et al.*, *Science*, 2001, **291**, 1304.
11 (*a*) J. A. Rininger, V. A. DiPippo and B. E. Gould-Rothberg, *Drug Discovery Today*, 2000, **5**, 560; (*b*) P. O. Brown and D. Botstein, *Nat. Genet.*, 1999, **21**(Suppl.), 33; (*c*) C. Debouck and P. N. Goodfellow, *Nat. Genet.*, 1999, **21**(Suppl.), 48; (*d*) G. Walter, K. Bussow, D. Cahill, A. Lueking and H. Lehrach, *Curr. Opin. Microbiol.*, 2000, **3**, 298; (*e*) Q. Emili and G. Cagney, *Nat. Biotechnol.*, 2000, **18**, 393; (*f*) G. MacBeath and S. L. Schreiber, *Science*, 2000, **289**, 1760; (*g*) P. J. Hergenrother, K. M. Depew and S. L. Schreiber, *J. Am. Chem. Soc.*, 2000, **122**, 7849.
12 J. P. Kiplinger, *Drug Discovery World*, 2001, **2**(3), 40.
13 B. J. Battersby and M. Trau, *Trends Biotechnol.*, 2002, **20**, 167.
14 B. J. Battersby, G. A. Lawrie and M. Trau, *Drug Discovery Today*, 2001, **6**(Suppl.), 123.
15 M. B. Meza, *Drug Discovery Today: HTS Suppl.*, 2000, **1**, 38.
16 (*a*) K. G. Oliver, J. R. Kettman and R. J. Fulton, *Clin. Chem.*, 1998, **44**, 2057; (*b*) P. L. Smith, C. R. WalkerPeach, R. J. Fulton and D. B. DuBois, *Clin. Chem.*, 1998, **44**, 2054; (*c*) R. J. Fulton, R. L. McDade, P. L. Smith, L. J. Kienker and J. R. Kettman, Jr., *Clin. Chem.*, 1997, **43**, 1749; (*d*) D. A. A. Vignali, *J. Immunol. Methods*, 2000, **243**, 243.
17 (*a*) F. Szurdoki, K. L. Michael and D. R. Walt, *Anal. Biochem.*, 2001, **291**, 219; (*b*) D. R. Walt, *Science*, 2000, **287**(5452), 451; (*c*) M. Lee and D. R. Walt, *Anal. Biochem.*, 2000, **282**, 142.
18 F. J. Steemers, J. A. Ferguson and D. R. Walt, *Nat. Biotechnol.*, 2000, **18**, 91.
19 Á. Furka, F. Sebestyén, M. Asgedom and G. Dibó, *Int. J. Pept. Protein Res.*, 1991, **37**, 487.
20 K. S. Lam, M. Lebl and V. Krchnák, *Chem. Rev.*, 1997, **97**, 411.
21 M. C. Needels, D. G. Jones, E. H. Tate, G. L. Heinkel, L. M. Kochersperger, W. J. Dower, R. W. Barrett and M. A. Gallop, *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 10700.
22 Z.-J. Ni, D. MacLean, D. C. P. Holmes, M. M. Murphy, B. Ruhland, J. W. Jacobs, E. M. Gordon and M. A. Gallop, *J. Med. Chem.*, 1996, **39**, 1601.
23 M. H. J . Ohlmeyer, R. N. Swanson, L. W. Dillard, J. C. Reader, G. Asouline, R. Kobayashi, M. Wigler and W. C. Still, *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 10922.
24 W. L. Fitch, T. A. Bayer, W. Chen, F. Holden, C. P. Holmes, D. Maclean, N. Shah, E. Sullivan, M. Tang and P. Waybourn, *J. Comb. Chem.*, 1999, **1**, 188.
25 M. S. Shchepinov, R. Chalk and E. M. Southern, *Nucleic Acids Symp. Ser.*, 1999, **42**, 107.
26 (*a*) B. J. Egner, S. Rana, H. Smith, N. Bouloc, J. G. Frey, W. S. Brockelsby and M. Bradley, *Chem. Commun.*, 1997, **8**, 735; (*b*) R. H. Scott and S. Balasubramanian, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1567; (*c*) E. Campian, F. Sebestyén, F. Major and Á. Furka, *Drug Dev. Res.*, 1994, **33**, 98; (*d*) E. Campian, F. Sebestyen and Á. Furka, *Colored and fluorescent solid supports in Innovation and Perspectives in Solid Phase Synthesis and Complementary Technologies*, ed. R. Epton, Mayflower Worldwide Limited, Kingswinford, England, UK, 1994, pp. 469–472; (*e*) B. Yan, P. C. Martin and J. Lee, *J. Comb. Chem.*, 1999, **1**, 78; (*f*) A. Nanthakumar, R. T. Pon, A. Mazumder, S. Yu and A. Watson, *Bioconjugate Chem.*, 2000, **11**, 282.
27 M. Han, X. Gao, J. Z. Su and S. Nie, *Nat. Biotechnol.*, 2001, **19**, 631.
28 S. R. Nicewarner-Pena, R. G. Freeman, B. D. Reiss, L. He, D. J. Pena, I. D. Walton, R. Cromer, C. D. Keating and M. J. Natan, *Science*, 2001, **294**(5540), 137.
29 H. Zhou, S. Roy, H. Schulman and M. J. Natan, *Trends Biotechnol.*, 2001, **19**(10, Suppl.), 34.
30 M. Bruchez, Jr., M. Maronne, P. Gin, S. Weiss and A. P. Alivisatos, *Science*, 1998, **281**, 2016.
31 B. J. Battersby, D. Bryant, W. Meutermans, D. Matthews, M. L. Smythe and M. Trau, *J. Am. Chem. Soc.*, 2000, **122**, 2138.
32 L. Grøndahl, B. J. Battersby, D. Bryant and M. Trau, *Langmuir*, 2000, **16**, 9709.
33 M. Trau and B. J. Battersby, *Adv. Mater.*, 2001, **13**, 975.
34 W. Stöber, A. Fink and E. Bohn, *J. Colloid Interface Sci.*, 1968, **26**, 62.
35 A. P. R. Johnston, honours thesis, 2000.
36 J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 2nd edn., Kluwer Academic/Plenum, New York, 1999.
37 A. van Blaaderen and A. Vrij, *Langmuir*, 1992, **8**, 2921.
38 D. C. Matthews, L. Grøndahl, B. J. Battersby and M. Trau, *Aust. J. Chem.*, 2001, **54**, 649.