The tumor cells were derived from primary mouse medulloblastomas, which are cerebellar tumors that, in humans, occur primarily between 5 and 10 years of age[7]. Because the tumors spread through the CNS, treatment consists of surgical resection, radiation to the entire brain and spine, and chemotherapy and, as a consequence, leaves survivors with debilitating learning and neuroendocrine hormonal deficiencies. A potential implication of this work is that neurotransmitter signaling might also sustain replicative potency of a medulloblastoma stem or progenitor cell.

Is neurotransmitter signaling the relevant target of the hits identified by the screen (as opposed to off-target effects)? On the one hand, there are some indications that neuromodulatory pathways are involved, including chemical diversity within the active compound classes, which suggests that common off-target effects are unlikely, and inhibition of the antiproliferative effect of the D2/3 dopamine receptor agonist bromocriptine by coadministration of the D2 receptor antagonist (±)-sulpride. On the other hand, physiologic evidence for the suspected neurotransmitter signaling needs to be documented. Moreover, if neurotransmitters are involved, do they act directly on the stem cells (**Fig. 1a**) or on more differentiated cells, which, as compared to stem cells, are more likely to have functional receptors (**Fig. 1b**)? It has been postulated that developmental neurotransmitter function on immature cells, but not necessarily stem cells, is important for establishing proper connectivity in the brain[8], and the neuromodulatory compounds could be interfering with a developmental function of neurotransmitter signaling recapitulated in the neurosphere culture. These simple models, however, must be distinguished from more complex alternatives, including the possibility that an antiproliferative or death response might be initiated in response to aberrant signaling caused by inappropriate binding of the neuromodulatory hits to cellular proteins. Some of these mechanistic issues could be addressed through the use of selective molecular genetic tools, which would corroborate the basic finding while potentially addressing whether cell-autonomous versus cell-non-autonomous function is involved.

Caveats aside, it is intriguing to consider that neurotransmitter regulation of NSCs is a normal homeostatic process in the ventricular subependyma, where forebrain NSCs reside, and that this process might be required for the proper wiring of the fetal and adult brain[9]. This model could have profound implications for current therapeutic application of drugs that modulate neurotransmitter function, and would causally link anti-Parkinsonian drugs to the recent finding of reduced prevalence of brain tumors in patients with Parkinson disease[10]. Finally, the possibility that neurotransmitters are involved in cancer stem cell self-renewal opens the door to the evaluation of approved neuroactive drugs for antitumor efficacy.

COMPETING INTERESTS STATEMENT
The author declares no competing financial interests.

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# High-throughput screening flows along

Hicham Fenniri & Ramon Alvarez-Puebla

**Simultaneous measurement of multiple analytes in high-throughput assays requires the design of integrated sensory elements. The latest development in this field is an engineering masterpiece based on microfluidics, photolithography and polymer science.**

The ideal clinical diagnostic tool would allow users to simultaneously investigate a sample for a large number of disease markers and draw a rapid and accurate conclusion on the health condition of a patient. Such a tool should also be affordable and amenable to implementation in a standard clinical setting. Current high-throughput screening (HTS) methods used in diagnostic approaches rely on PCR, ELISA and microarray technologies that can be costly and complicated to implement. Pregibon *et al.* have introduced an efficient approach to encode and functionalize millions of microparticles in a one-step process[1]. This method has the potential to greatly simplify HTS and biomedical diagnostics.

The search for faster, cheaper, general and noninvasive diagnostic tools is a never-ending battle. Since the advent of combinatorial chemistry in the mid 1980s[2], the notion of testing millions of molecules in a few steps was the bait that attracted hundreds of investigators in academic, government and industrial laboratories to invest in this field. However, it did not take long before this community realized that the chemical and structural identification of active members of a library is clearly the bottleneck, and their success relies entirely on the sensitivity and specificity of the assay and HTS methods.

The realization that large is not necessarily desirable led the community to abandon the grand idea of tracking millions to billions of compounds and resort to more focused design approaches intended to minimize structural, conformational and electronic redundancies[2]. Smaller libraries featuring a fine balance between rigidity and flexibility, high density of functional groups, and exhaustive coverage of the universe of chemical diversity, shapes, functional group distribution and electrostatic surfaces were pursued. The result of this exercise is small libraries of a few thousand compounds synthesized in parallel, using encoded combinatorial chemistry[2,3] or spatially resolved two-dimensional arrays[4,5]. The advantages of these approaches are that (i) each library member is well characterized and present in sufficient quantities, (ii) artifacts resulting from nonspecific interactions with low-affinity ligands are minimized, (iii) quantitative analysis is possible

*Hicham Fenniri and Ramon Alvarez-Puebla are at the National Institute for Nanotechnology, University of Alberta, 11421 Saskatchewan Drive, Edmonton, Alberta T6G 2M9, Canada*
*e-mail: hicham.fenniri@ualberta.ca*

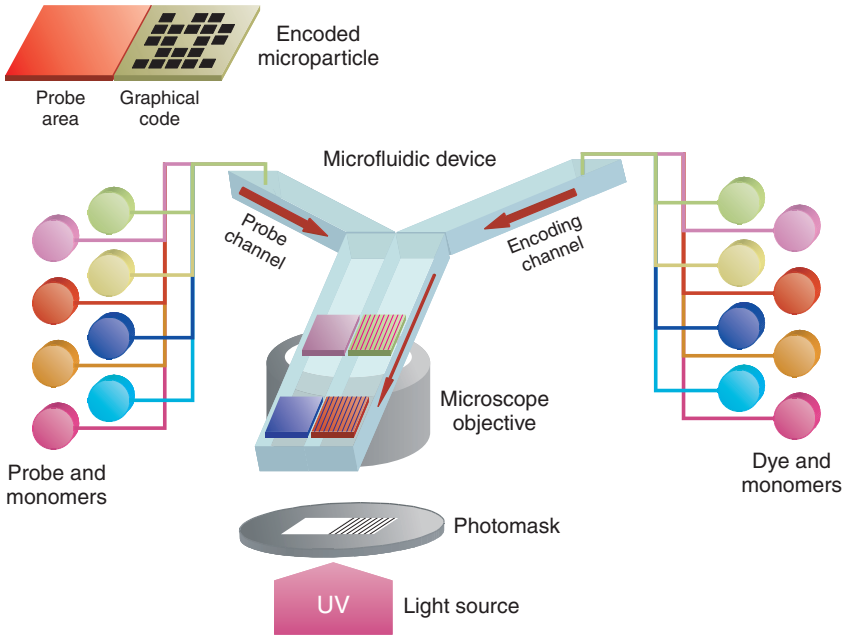


**Figure 1** Schematic diagram of encoded microparticle synthesis for multiplexed HTS. The colored wells on the right represent a light-activated polymerizable monomer that may or may not contain a probe molecule (dye and monomers). The colored wells on the left represent a probe molecule co-mixed with a light-activated polymerizable monomer (probe and monomers). In the top left corner is a schematic representation of a dot-encoded microparticle with a probe section (left) and a two-dimensional binary dot code allowing for more than a million codes to be readily prepared (right).

and qualitative analysis is more reliable and (iv) active components are more readily identified via their spatial location or encoding element.

Despite this coming to terms with the limitations of current screening technologies, the community did not lose sight of the potential power of HTS, and a number of strategies belonging to two general approaches were devised. The first relies on spatially resolved two-dimensional arrays in which each element of the library is identified via its (*x*,*y*) coordinates[4,5]. The second relies on suspensions of encoded microparticles, in which each element of the library is conjugated to a microparticle with a unique addressable code[6,7]. Though in both cases ultra-high-density DNA and protein arrays can be generated and screened, these methods have their limitations. Notably, their implementation in a research or clinical setting requires extensive investment in equipment and expertise.

Pregibon *et al.*[1] have introduced an encoding method that could dramatically simplify HTS. The method uses laminar flow in a microfluidic system (**Fig. 1**) to generate particles with a probe component (for example, DNA or protein) and a two-dimensional graphical code, similar to the barcodes found in supermarkets, that serves to identify the particle. Two fluids are flowed without intermixing into tiny channels (laminar flow). One fluid serves to generate the graphical code and the other contains the probe molecule that is used to detect the analyte of interest. A key property of these fluids is that they can be turned into solid pieces of plastic in a fraction of a second upon exposure to a flash of ultraviolet light. Thus, as the fluid passes over a microscope objective it gets exposed through a photomask to an ultraviolet flash that turns the exposed area into solid microparticles whose shape and dimensions are defined by the photomask. This photolithographic process leads to the generation of microparticles with built-in probe and the corresponding graphical codes (**Fig. 1**). The process looks simple, and it is. In principle, the method could generate millions of encoded particles in a short period of time in different formats and shapes.

DNA microarrays have essentially revolutionized the field of molecular biology and have had a critical role in the development of the fields of genomics and proteomics. However, the microparticle approach benefits from additional advantages such as (i) larger surface area for functionalization relative to flat, nonporous substrates, (ii) better chemical reactivity as a result of better accessibility of the analytes to the entire sample volume, and (iii) greater versatility in sample analysis and data acquisition. In addition, the authors' system offers even more advantages, such as the ease of preparation, functionalization and encoding. To demonstrate the versatility of their platform, Pregibon *et al.* used their system to detect oligonucleotide sequences. To achieve this, the process is again quite simple. Readily available oligonucleotides were co-mixed with the probe fluid and injected into the microfluidic system. The probes that came out on the other end of the device were washed and exposed to a fluorescently labeled oligonucleotide sequence that was complementary to the probe sequence. In less than 10 min, the region of the microparticle with the probe sequence turned fluorescent, thus confirming the formation of the DNA duplex.

Surface functionalization in any microarray has traditionally been a source of problems, and in some cases may even limit the practicality of a device. In the case of Pregibon *et al.*'s microparticles, this problem was overcome in quite a simple way. Rather than chemically attaching the probe to the microparticle, an acrylate derivative of the probe was generated and co-mixed with the polymer matrix before exposure to light and solidification. The ability to incorporate a probe during the preparation of the microparticles is in effect one of the key strengths of the system. However, this may also be its Achilles' heel. To earn the HTS title, the method has to be tested with millions of probes, which may pose some technical challenges. That is, to make one million microparticles each with a unique probe, one has to separately synthesize one million probes. To achieve this feat in the case of DNA detection for instance, the oligonucleotides must be synthesized one at a time in parallel using standard DNA synthesizers, purified (if necessary), chemically modified to make them compatible with the microfluidic polymerization scheme, and delivered in parallel to the microfluidic system for incorporation in the encoded microparticles. All of these steps may require another engineering masterpiece. In the case of two-dimensional DNA microarrays, this problem was overcome using light-directed spatially addressable parallel chemical synthesis[8]. In the case of Pregibon *et al.*'s microparticles it is unclear at this stage how the incorporation of a large number of probes could be achieved. Finally, despite this apparent technical limitation, the method is powerful and compatible with a wide range of probes, including proteins, peptides and antibodies. Furthermore, each microparticle can be tailored to create a gradient of probe or even multiple regions with different probes.

The strategy that Pregibon *et al.* have introduced offers opportunities for the development of new technological platforms. For instance, one may envision replacing the photolithographically generated dot

encoding with self-encoded polymers[3], in which case the polymer would report on the microparticle and serve as a support for probe conjugation. Such an approach could significantly miniaturize the size of the microparticles, as they could be interrogated with a laser beam with a spot size of less than 1 $\mu m^2$. Decreasing the size of the microparticles means increasing and accelerating the throughput—and reducing the costs.

COMPETING INTERESTS STATEMENT
The authors declare no competing financial interests.

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# Methyllysine analogs: rewriting the code

Monica Bhatia & Paul R Thompson

**Lysine methylation has been implicated in gene transcription and epigenetic control. Chemical modification of cysteine residues results in a highly similar structural and functional analog of methylated lysine and provides a means to study this important modification in nucleosomes.**

In eukaryotes, DNA is packaged in the cell with the help of proteins called histones. The histone–DNA complex, called the nucleosome, not only directs the formation of chromatin and chromosomes, but also regulates processes such as gene transcription[1]. A variety of post-translational modifications occur at the N-terminal tails of histones—for example, acetylation, methylation and phosphorylation—and in a simplified model these modifications are believed to serve as 'on' and 'off' switches for transcription via the downstream recruitment of specific proteins that regulate access of the transcriptional machinery to DNA[2]. The effects of histone lysine methylation are particularly intriguing because lysines can bear multiple methyl marks (for example, mono-, di- or trimethyllysine), and specific effects on transcription are dependent on the number of methyl groups present on a particular lysine residue[3]. However, attempts to study the role of lysine methylation on the nucleosomal level have proven challenging, in part because lysine methyltransferases do not in all cases regiospecifically modify a single lysine residue, and also because the degree of methylation is heterogeneous. Thus, enzymatic methods cannot reliably be used to generate site-specifically modified nucleosomes. In contrast, native chemical ligation (NCL) has been used to generate such nucleosomes, but this method can be cumbersome and technically challenging[4,5]. A simpler and more economical solution may be to create methyllysine-like residues on histones and then assemble them into nucleosomes, as demonstrated by Simon *et al.* in a recent issue of *Cell*[6].

Simon *et al.*[6] have synthesized methyllysine analogs (MLAs) via the aminoethylation of strategically placed cysteine residues, a reaction historically used by enzymologists to study protein function[7]. Under suitable denaturing conditions that minimized side reactions with other amino acids (for example, histidine), the thiol group of cysteine was alkylated to generate a residue that is akin to lysine with the γ-methylene replaced by a sulfur atom (**Fig. 1a**). The approach, which takes advantage of a chemoselective reaction between an alkyl halide and the cysteine thiol, is able to generate novel MLA-containing histones that can be incorporated into nucleosomes. These MLAs are

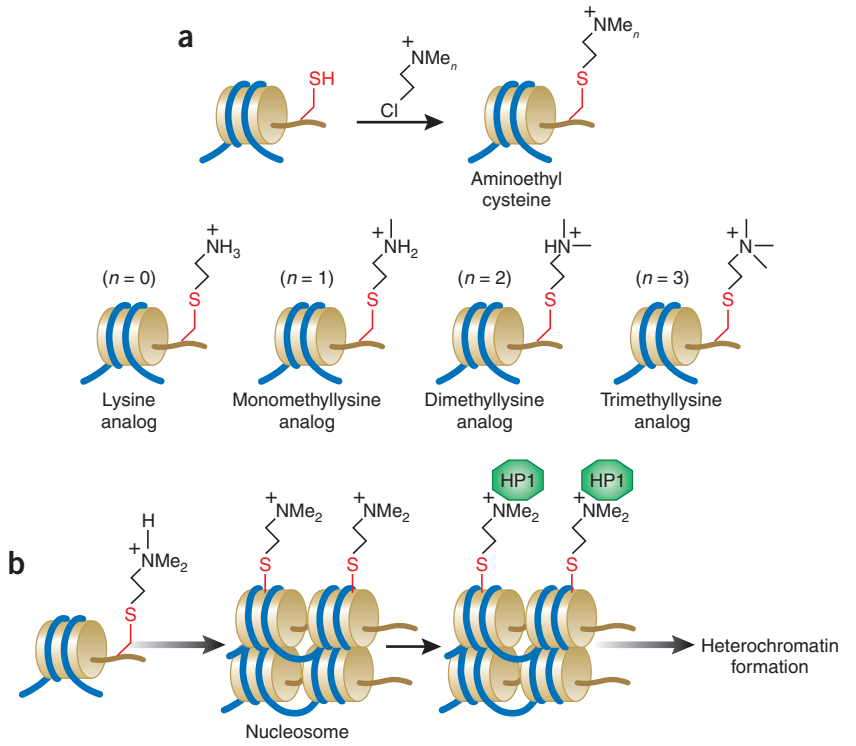


**Figure 1** Creating methyllysine analogs. (**a**) Simon *et al.*[6] demonstrate the synthesis of MLAs via aminoethylation of cysteines. The resulting residues are highly similar to lysines. (**b**) When present on histones, the MLAs can be assembled into histone octamers to form nucleosomes. The dimethylated analog of Lys9 on histone H3 is able to recruit HP1, a protein involved in heterochromatin formation.

*Monica Bhatia and Paul R. Thompson are in the Chemistry and Biochemistry Department, University of South Carolina, 631 Sumter Street, Columbia, South Carolina 29208, USA.*
*e-mail: thompson@mail.chem.sc.edu*