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Multiparallel Organic Synthesis

A Three-Dimensional Array for Multiparallel Synthesis**

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Multiparallel organic synthesis combined with high-content screening has emerged as a valuable approach for identification of new molecular function. Emulating basic principles of natural selection, this strategy has proven particularly successful for discovery and optimization of modulators of metabolic pathways,[1] identification of novel catalysts,[2] and development of unique functional materials.[3] Solid-phase synthesis[4] has provided a foundation for the development of several innovative methods for the synthesis of chemical libraries.[5–11] Among these formats, light-directed synthesis[8] and split synthesis are particularly noteworthy.[9] While the former provides complete positional encoding of individual library members,[8] the latter features high throughput and operational simplicity,[9] although it requires additional split–pool steps and encoding-decoding operations.[10,11]

We describe herein a conceptually novel format for the synthesis of chemical libraries based on three-dimensional arrays of interconnected reaction wells. This method is unique and differs significantly from the existing formats of generating chemical diversity,[5–11] combining the high efficiency of the split synthesis with a spatially addressable format.

The strategy entails layer-selective functionalization of a three-dimensional matrix, consisting of interconnected reaction wells. Each well is designed to encapsulate an individual library member by attachment to a physically localized solid support. Figure 1 provides a conceptual representation of the synthesis of an ABC library randomized with two building blocks at each position (A^1 and A^2, B^1 and B^2, C^1 and C^2). The first functionalization step entails simultaneous addition of building blocks A^1 and A^2 to two parallel layers of the array. Following a washing cycle and a 90° rotation, the synthesis process is repeated with building blocks B^1 and B^2. The third set of reactants—C^1 and C^2—is introduced in a similar fashion following the final 90° rotation. This process affords a library of spatially separated compounds. The identity of each compound is established by its physical location within the array.

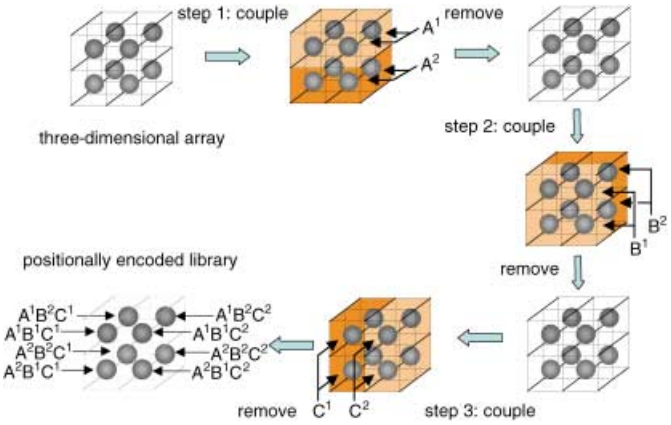

Figure 1. Conceptual representation of the synthesis of a chemical library in a three-dimensional array. The synthesis entails a layer-selective addition of reagents in each of the three dimensions. Three sets of building blocks (A^1 and A^2, B^1 and B^2, C^1 and C^2) are used sequentially. Each coupling step is followed by the appropriate washing and deprotection operations. The array is rotated by 90° prior to the addition of the next set of reagents. This process affords a positionally encoded library containing all the possible combinations.

The design of an individual reactor is depicted in Figure 2 A. Encapsulating a suitable polymeric support, each microwell is equipped with four openings, connecting it to four adjacent wells. Insertion of the appropriate sealing elements (i.e. taper pins) seals the walls of the reactors. Removal of pins allows addition and withdrawal of reagents. This simple concept provides a general method for layer-selective multiparallel chemical synthesis in each of the three dimensions without cross-contamination. The synthesis cycle begins with removal of all the pins from one side of the cube, while the perpendicular pins remain inserted (Figure 2 B). The first set of reagents (A^1 and A^2) is added, allowing all the coupling steps to be performed simultaneously. Following the washing cycle, and rotation by 90°, the second coupling step is performed with B^1 and B^2. The originally removed set of pins is reinserted, followed by withdrawal of the pins from the perpendicular side of the cube. The third coupling step is performed with C^1 and C^2, as shown. A noteworthy feature of

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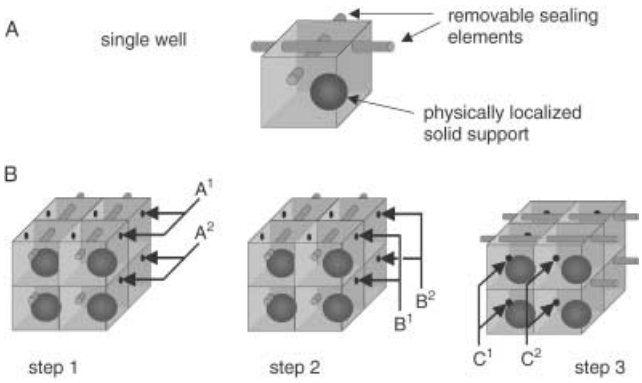

Figure 2. A) A single reaction well, equipped with four side openings, which allow insertion and removal of the sealing elements. B) Delivery of reagents in each of the three dimensions in a representative $2\times2\times2$ array. Teflon gaskets are used to ensure solvent-tight seals between the layers (not shown). Each well is equipped with a single 12.5-mm SynPhase Lantern (18 mol/lantern loading). Stainless-steel 4-mm taper pins are used to achieve the desired sealing.

this design is that the bottom of each well remains impermeable, allowing direct cleavage of the compound from the solid support at the end of the synthesis cycle.

To validate the method described above, we conducted a synthesis of a model peptide library. The library was generated by randomization of the tripeptide at each position with five amino acids (Gly, Ala, Phe, Val, Leu) expected to produce 125 spatially addressed compounds (5^3). Three-dimensional arraying of the five amino acids in a $5\times5\times5$ array is schematically illustrated in Figure 3. We employed a conventional Fmoc solid-phase peptide synthesis by using a HOBt/DIC coupling protocol with commercially available SynPhase D-series PA lanterns[12,13] equipped with the Rink linker.[14] The synthesis was carried out within the expected 8-h period to afford 8–10 mg of target peptides (90–95% yield). Examination of 25 peptides, randomly selected from the final array, by means of ^{1}H NMR spectroscopy, HPLC, and APCI-MS confirmed the predicted identity of each product, as well as the high chemical purity (Figure 3). Importantly, no cross-contamination between neighboring library members was observed.

In conclusion, we have presented a novel format for multiparallel synthesis, featuring a three-dimensional delivery of reactants to an array of interconnected reaction wells. Generation of a model library of positionally encoded peptides validated this strategy, highlighting the high efficiency and throughput of our approach. Ideally suited for the preparation of trimeric libraries, this technology can be further adapted to the introduction of additional variables (i.e., by employing multiple copies of interconnected arrays). The noteworthy feature of this method is the ability to conduct *simultaneously* all the coupling, deprotection, and

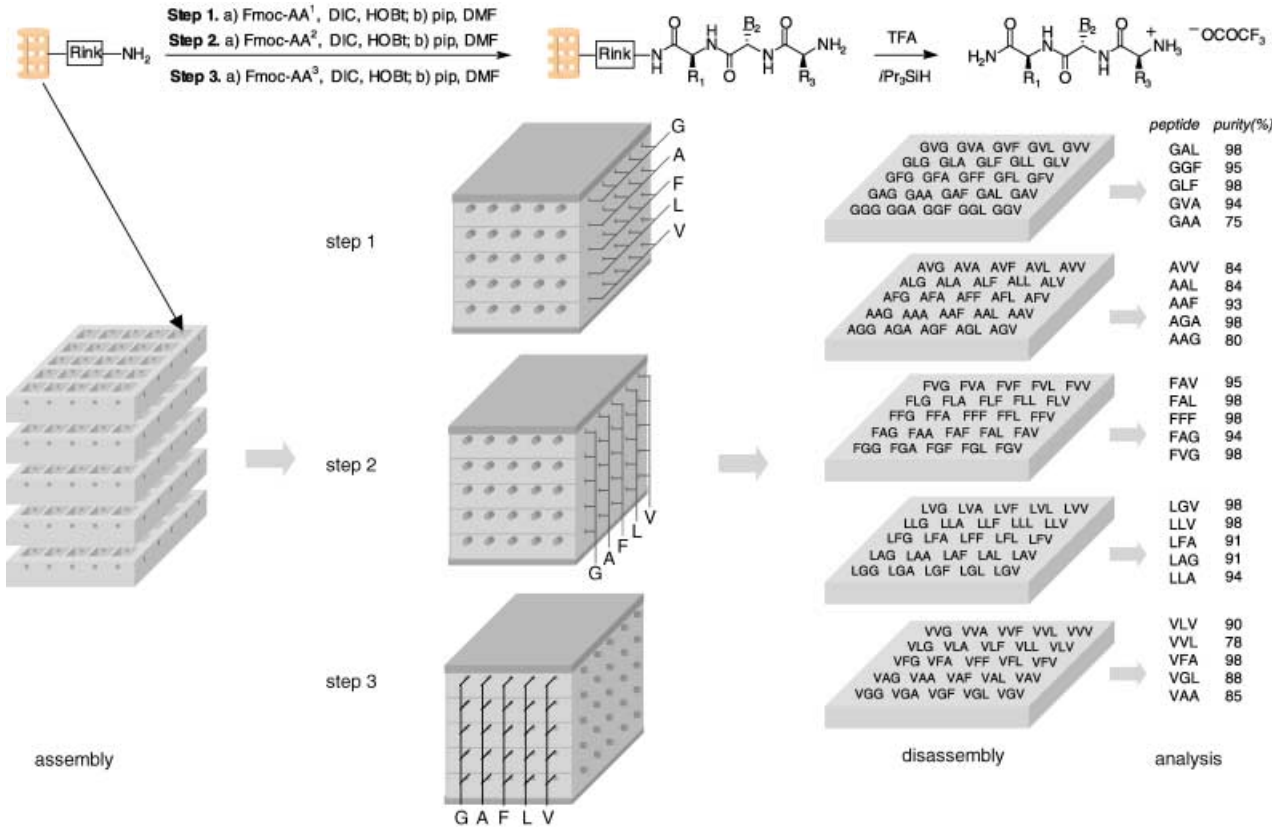

Figure 3. Generation of a 125-membered library of tripeptides in a $5\times5\times5$ array. The system represents a set of five 25-well polypropylene plates stacked to form the desired $5\times5\times5$ matrix. Each peptide coupling and Fmoc deprotection was followed by extensive washing ($4\times$DMF). Following the completion of the synthesis process, the plates were disassembled, 25 representative peptides were removed, cleaved with TFA/H_2O/iPr_3SiH (95:2.5:2.5), and analyzed as described in the text and Supporting Information. Fmoc = 9-fluorenylmethoxycarbonyl, DIC = diisopropylcarbodiimide, HOBt = 1-hydroxy-1*H*-benzotriazole, pip = piperidine, DMF = *N*,*N*-dimethylformamide, TFA = trifluoroacetic acid.

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washing manipulations in the entire array. Significantly, the efficiency of the synthesis does not depend on the number of building blocks used along each axis. This feature provides a unique advantage of this method over the use of 2D arrays, representing a direct alternative to the split synthesis.[9] Although the operational simplicity of the split synthesis exceeds that of the present method, the ability to spatially resolve and positionally encode individual library members offered by the current approach compares favorably with the split synthesis, which requires additional encoding–decoding operations.[10,11] Extension of the work presented herein to the development of suitable systems for the synthesis of 1000–10000-membered small-molecule libraries is in progress and will be reported in due course.

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DNA Structure

Hyperstranded DNA Architectures Observed by Cold-Spray Ionization Mass Spectrometry**

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Formation of duplex, triplex, and quadruplex DNA structures has been a subject of extensive investigation because of their fundamental functional roles in living organisms. The structures of these multistranded DNAs have been examined[1–3] by electrospray ionization[4] mass spectrometry (ESI-MS). However, noncovalent complexes of multiply stranded DNA are difficult to observe by conventional methods because of their low melting temperature (T_m). The heat from the desolvation chamber is thought to be necessary for ionization in the gas phase in conventional ESI. However, we recently developed a direct solution analysis method, cold-spray ionization (CSI)[5] mass spectrometry, a variant of ESI-MS operating at low temperature, and we have applied this method to study various labile solution structures.[6] Here we report the characterization of triple- and quadruple-stranded oligodeoxynucleotides by means of CSI-MS.

First, triple-stranded oligodeoxynucleotides $T_n{\cdot}A_n{\cdot}T_n$ derived from 2:1 mixtures of 5′-dT_n-3′ (T_n) and 5′-dA_n-3′ (A_n) ($n = 8$, 10, 15, 20, and 25) were analyzed. Negative CSI-MS measurements were performed with a two-sector (BE) mass spectrometer (JMS-700, JEOL) equipped with the CSI source.[7] In the case of the 8-mer (Figure 1a), comparable

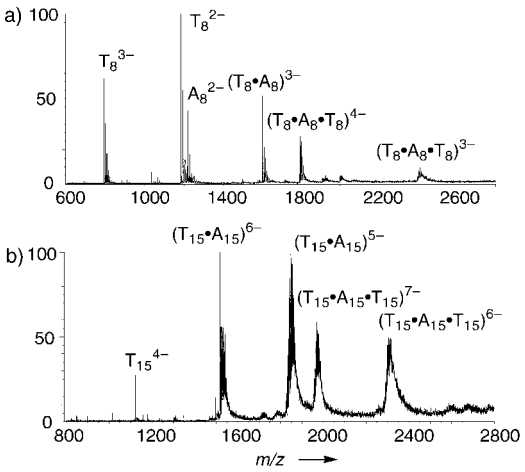

Figure 1. Negative CSI mass spectra of a) $T_8{\cdot}A_8{\cdot}T_8$ and b) $T_{15}{\cdot}A_{15}{\cdot}T_{15}$.

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