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Construction and Screening of Peptide and Nonpeptide Libraries Based on One-bead-One-compound Principle

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Introduction

The one-bead-one-compound library technique is composed of three main parts: (i) synthesizing of the library in which each solid phase particle carries a unique structure, (ii) identifying particles that interact with a given macromolecular target, and (iii) determining the structure responsible for the observed effect [1, 2]. There are several issues to be solved to successfully apply this technique. We will address only some of them: (i) manual and automated synthesis of peptide libraries and compounds identified in library screening; (ii) screening of solid phase libraries with both soluble and insoluble targets; (iii) synthesis of complete libraries; and (iv) synthesis of libraries with higher diversity than possible in peptide libraries. The one-bead-one-compound library technique was recently reviewed [2].

Results and Discussion

Synthesis of the library can be automated. A compound identified in library screening must be resynthesized for verification of its activity. The resynthesis of hits may become a bottleneck of the screening technology. It is necessary to use the capability of a multiple synthesizer, which can deliver the required number of compounds (peptides) for testing. However, all commercially available multiple synthesizers operate in a batch mode; *i.e.,* they cannot accept new sequences or prioritize syntheses once operation has commenced. The optimal reactor for solid phase peptide synthesis is a polypropylene syringe equipped with teflon frit [3], which we have used for synthesis of several thousand peptides. We have designed the MARS (multiple automatic robotic synthesizer) apparatus which synthesizes peptides in plastic syringes [4]. This machine can accept any number of synthetic requests and prioritize them immediately. The capacity is the simultaneous synthesis of twelve peptides. Overall throughput depends on the length of peptides to be synthesized and coupling time. For example, (considering two hours coupling time) it can synthesize approximately 12 octapeptides or 24 tetrapeptides a day. The robot operates continuously, and can synthesize peptides of any length or sequence in parallel. We have synthesized HIV protease (99 amino acids) together with a number of short peptides. Therefore, one hundred peptides per week is not an unusually high load.

Table 1. *Efficiency and success of on-bead-binding screening.*

Project	Time to first hit	No. libraries screened	No. compounds screened	No. hits identified	% hits confirmed
IIb/IIIa	2 wk	3	2,000,000	40	80
Thrombin	2 wk	20	20,000,000	>1000	95
Her-2	8 wk	18	43,000,000	47	27
Xa	8 wk	37	37,000,000	>800	75
VIIa	20 wk	36	36,000,000	200	3
RNase-H	4 wk	20	20,000,000	500	30
Oxidase	5 wk	9	2,800,000	84	3
Grb-2	4 wk	3	9,000,000	15	66
gp120/CD4	14 wk	5	16,000,000	17	43

Screening of a one-bead-one-compound library can be performed either by a solid phase binding assay, or by solution tests in a high-throughput mode. The practical performance of these tests has been described in detail [5, 6]. It is very important to include appropriate controls during the screening of a bead-bound library to eliminate nonspecific binding. The test is usually performed both in the presence and in the absence of a competitor several times, and only beads selected in repeated cycles of staining and decolorization are submitted for sequencing and resynthesis. An alternative to multiple rounds of screening is a technique using different colors in each step of bead staining [7]. Specificity of binding can be determined by the evaluation of bead color. Only compounds in which the biological activity has been verified in solution can be qualified as hits. Table 1 illustrates the time-lines and success rate in this type of screening. It ranges from more than 95% in the case of thrombin to 3% in the case of factor VIIa. On the other hand, structures identified in solution screening were found to be real hits in all cases. This advantage of solution screening is counterbalanced by its relatively low throughput; only several hundred thousand beads can be screened in one day, in comparison to millions screened by bead-binding assay.

Synthesis of libraries by the split and mix technique generates random mixtures of all possible structures. Therefore, there is always uncertainty about the completeness of the library [8]. This issue is especially important in the case of small libraries and in situations requiring the most economical use of reagents. We have designed a technique allowing the synthesis of all members of the particular library with only one representation of each structure. This technique is based on the principle of continually dividable carrier (membrane, thread), the synthetic "fate" of which can be easily traced based on its size or shape in the particular stage of the synthesis. Figure 1 illustrates the principle of this technique on the example of a library of eight compounds generated by three steps of randomization using two building blocks in each step. Each piece of solid carrier was cut in half in three consecutive steps and pieces were transferred to the next synthetic step, not randomly but in an organized way. We have prepared a peptide library of several thousand members and screened it both by binding and in a solution assay [9].

To increase the diversity achievable with linear peptides, we have synthesized cyclic as well as branched libraries in which not only alpha amino acids but also compounds

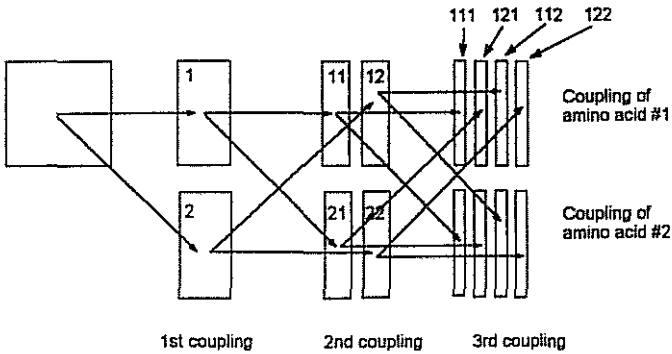

Figure 1. *General scheme for the synthesis of nonrandom libraries.*

with other combinations of amino and carboxy groups are part of the "backbone" [10]. Modifications of side chains of trifunctional amino acids in peptides is another way to increase diversity. We have also applied other reactions, such as Wittig or Mitsunobu reaction in library building (see elsewhere in this volume). In all these cases, as well as in the libraries based on a molecular "scaffold", we had to apply "coding" (see *e.g.* [11]) for elucidation of the structure, when structures in the library were not easily detectable by mass spectroscopy.

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