

## A Yoctoliter-Scale DNA Reactor for Small-Molecule Evolution

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**Abstract:** The center of DNA three-way junctions, constituting a yoctoliter ( $10^{-24}$  L) volume, is applied as an efficient reactor to create DNA-encoded libraries of chemical products. Amino acids and short peptides are linked to oligonucleotides via cleavable and noncleavable linkers. The oligonucleotide sequences contain two universal assembling domains at the center and a distal codon sequence specific for the attached building block. Stepwise self-assembly and chemical reactions of these conjugates in a combinatorial fashion create a library of pentapeptides in DNA three-way junctions in a single reaction vessel. We demonstrate the formation of an evenly distributed library of 100 peptides. Each library member contains a short synthetic peptide attached to a unique genetic code creating the necessary “genotype–phenotype” linkage essential to the process of *in vitro* molecular evolution. Selective enrichment of the [Leu]-enkephalin peptide from an original frequency of 1 in 10 million in a model library to a final frequency of 1.7% in only two rounds of affinity selection is described and demonstrates successful molecular evolution for a non-natural system.

### Introduction

DNA-encoded libraries of chemical compounds<sup>1–4</sup> enable the convenient manipulation of chemical libraries of up to  $10^{12}$  members since the identification of DNA-encoded compounds with desirable properties can be accomplished using the principles of molecular evolution<sup>5–8</sup> by rounds of target affinity selection, amplification of the enriched pool of DNA, and translation of the DNA sequences into the next generation of binders. Halpin and Harbury have developed a method for DNA-programmed split-and-mix synthesis of libraries of small molecule–DNA hybrids, and by iterated rounds of library selection and amplification, a known antibody epitope was isolated from a library of one million synthetic peptides.<sup>2</sup> More recently, this approach was applied for the identification of an unknown binder out of 100 million distinct compounds.<sup>3</sup>

For the formation of libraries of DNA-labeled small molecules in a single vessel, the reactions of all building blocks of the library must be controlled and all library products individually labeled. This has become possible by DNA-directed chemistry where reactants are brought together by direct or templated DNA hybridization for a chemical reaction.<sup>1,4,9</sup> In an elegant approach, Liu et al. used linear templates to synthesize a library of 65 DNA-linked peptide–fumarate macrocycles, and after one round of affinity selection and amplification, a known binder was enriched.<sup>1a</sup> Very recently, this library was extended to 13 000 members using a slightly optimized protocol; however, no selection data were reported.<sup>1b</sup>

One of the inherent problems associated with the use of linear templates is the decreasing efficiency of chemical reactions upon increased distance from the reactant on the template to the reaction center. Varying reaction efficiencies were observed due to inconsistent folding of the single-strand regions.<sup>10</sup> Furthermore, the single-strand template sequences may fold in various structures that give rise to nonspecific binding. The double-stranded (ds) DNA sequences close to the reaction centers also differ from building block to building block, and reactions may vary in efficiency due to the differences in the chemical environment.

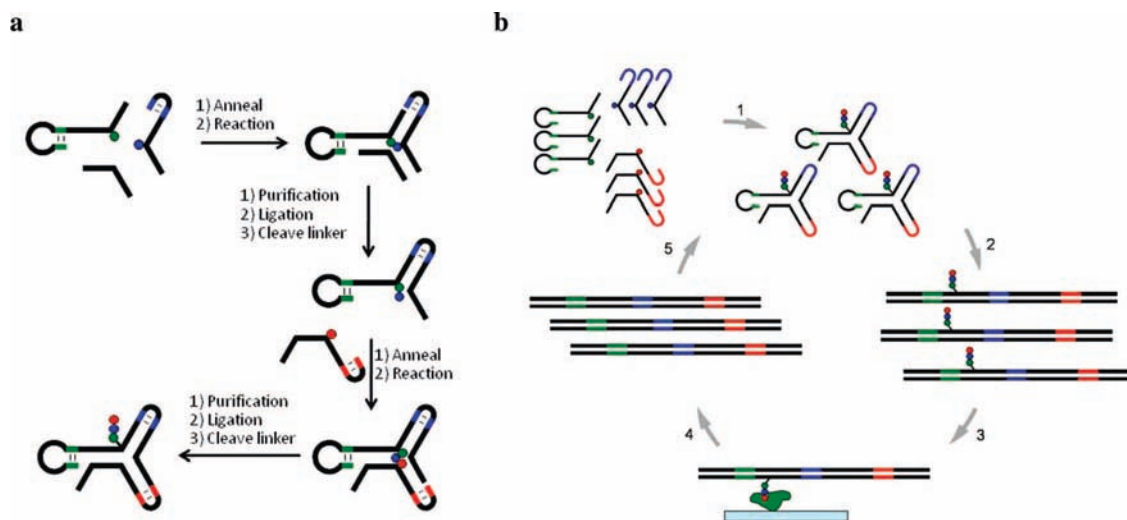
We have devised a self-assembling DNA hybrid structure that can control chemical reactions without templates and build combinatorial small-molecule chemical libraries with a unique distance independency and constant sequence environments for all building blocks, providing identical proximity in each

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- (1) (a) Gartner, Z. J.; Tse, B. N.; Grubina, R.; Doyon, J. B.; Snyder, T. M.; Liu, D. R. *Science* **2004**, *305*, 1601–1605. (b) Tse, B. N.; Snyder, T. M.; Shen, Y.; Liu, D. R. *J. Am. Chem. Soc.* **2008**, *130*, 15611–15626.
- (2) (a) Halpin, D. R.; Harbury, P. B. *PLoS Biol.* **2004**, *2*, 1015. (b) Halpin, D. R.; Harbury, P. B. *PLoS Biol.* **2004**, *2*, 1022. (c) Halpin, D. R.; Lee, J. A.; Wrenn, S. J.; Harbury, P. B. *PLoS Biol.* **2004**, *2*, 1031.
- (3) Wrenn, S. J.; Weisinger, R. M.; Halpin, D. R.; Harbury, P. B. *J. Am. Chem. Soc.* **2007**, *129*, 13137–13143.
- (4) (a) Gartner, Z. J.; Liu, D. R. *J. Am. Chem. Soc.* **2001**, *123*, 6961–6963. (b) Li, X.; Liu, D. R. *Angew. Chem., Int. Ed.* **2004**, *43*, 4848–4870.
- (5) Smith, G. P. *Science* **1985**, *228*, 1315–1317.
- (6) Matheakis, L. C.; Bhatt, R. R.; Dower, W. J. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 9022–9026.
- (7) Hanes, J.; Pluckthun, A. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 4937–4942.
- (8) Wilson, D. S.; Keefe, A. D.; Szosak, J. W. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 3750–3755.

- (9) Gartner, Z. J.; Grubina, R.; Calderone, C. T.; Liu, D. R. *Angew. Chem., Int. Ed.* **2003**, *42*, 1370–1375.
- (10) Snyder, T. M.; Tse, B. N.; Liu, D. R. *J. Am. Chem. Soc.* **2008**, *130*, 1392–1401.



**Figure 1.** Principles of the yoctoreactor. The black moieties represent constant assembly sequences, and the colored moieties represent codons unique for the individual BBs (colored circles). (a) Stepwise assembly and reactions forming the yoctoreactor library. (b) Key elements of the yoctoreactor molecular evolution cycle: step 1, combinatorial library assembly (annealing, chemical reactions, and DNA ligations); step 2, reactor dismantling by primer extension; step 3, affinity selection by target binding and washing; step 4, elution and PCR amplification of enriched DNA library (optionally, the composition of the library is monitored by mass DNA sequencing); step 5, enriched display library formation by rolling translation.

chemical step. The structure is built up entirely of dsDNA, thereby eliminating the problems associated with inconsistent folding of single-stranded (ss) DNA. Diverging from the concept of DNA as a linear template,<sup>1,4,9,10</sup> three-dimensional (3D) DNA junctions are applied to generate these libraries of chemical compounds. DNA junctions containing three or four arms around a single point are seen as important transient structures in the biological processes of replication and recombination.<sup>11–15</sup> We exploit such self-assembling DNA junctions both as the physical scaffold within which chemical reactions of attached building blocks (BBs) can take place and as a genetic code for BBs attached to each arm of the DNA junction (Figure 1). Highly stable 3D junctions are formed, and the BBs are spatially positioned to react at the center of the junction. Chemical reactions can be controlled by DNA by confining attached chemical BBs in space and thereby modulating their effective concentration. The center of such DNA junctions constitutes a yoctoliter ( $10^{-24}$  L) volume, and hence we call it a yoctoreactor.

We employ a three-way yoctoreactor to generate a combinatorial library of peptides linked to encoding DNA (Figure 1a). Before library assembly, the BBs are linked via cleavable or noncleavable linkers to three types of DNA oligonucleotides, representing each arm of the reactor. The oligonucleotides are designed to spontaneously self-assemble into a three-way junction due to constant regions of DNA sequence, independent of the BBs. The distal end of the oligonucleotides, shown as the colored part of the DNA sequence in Figure 1, contains sequences (the codons) specific for the attached BBs, which ultimately permits the composition of each library compound as well as its synthetic route to be deciphered.

Chemical reactions between the BBs take place via a stepwise procedure to form the combinatorial library (Figure 1a). After

each chemical reaction, the coupling products are purified by polyacrylamide gel electrophoresis and the DNA sequences ligated. This produces a continuous strand of ssDNA still in the folded 3D reactor format with the synthesized compound buried at the center. Chemically cleavable linkers are used for all but one of the positions, yielding a library of small molecules with a single covalent connection to the DNA.

Next, the encoded library is applied to molecular evolution by target affinity selection, amplification of the enriched pool of DNA sequences, and translation of the DNA sequences into the next-generation library of enriched small-molecule compounds linked to encoding DNA (Figure 1b). The yoctoreactor is dismantled by enzymatically synthesizing the cDNA strand in a primer extension reaction. The resulting linear, dsDNA exposes the covalently attached chemical product in a way that makes it accessible for target binding. This linear double-stranded form is called the *display product*.

Selection for binding with high affinity and specificity to an immobilized target (protein of interest) is then performed using a library of display products. Nonbinders are washed away, whereas the chemical compounds binding to the target are retained together with their cognate genetic codes, which are subsequently amplified by the polymerase chain reaction (PCR). In this way the pool of DNA sequences is enriched for sequences encoding compounds that bind to the target of interest. Conveniently, all manipulations are performed in a single-pot format, and the progression in the selection is followed by mass DNA sequencing.

The amplified DNA does not carry chemical compounds and must be translated into the display products by a process we call rolling translation. Rolling translation is accomplished by a process of stepwise exchange of the sequences for the three positions in the amplified DNA with their cognate BB-linked oligonucleotides. This process is guided by codon/anticodon annealing to reintroduce the encoded BBs into the reactor. During the rolling translation process, the BBs react to form an enriched library of chemical products linked to encoding DNA. The net effect of the PCR amplification and rolling translation is to produce a sufficient quantity of the enriched library for a

- (11) Liu, Y.; West, S. C. *Nature Rev. Mol. Cell Biol.* **2004**, *5*, 937–944.
- (12) Holliday, R. A. *Genet. Res. Camb.* **1964**, *5*, 282–304.
- (13) Okazaki, R.; Okazaki, T.; Sakabe, K.; Sugimoto, K.; Sugino, A. *Proc. Natl. Acad. Sci. U.S.A.* **1968**, *59*, 598–605.
- (14) Kallenbach, N. R.; Ma, R. I.; Seeman, N. C. *Nature* **1983**, *305*, 829–831.
- (15) Ma, R. I.; Kallenbach, N. R.; Sheardy, R. D.; Petrillo, M. L.; Seeman, N. C. *Nucleic Acids Res.* **1986**, *14*, 9745–9753.

second round of selection, whereby an even stronger enrichment for the target binding compounds is achieved (*vide infra*).

## Results and Discussion

The importance of the 3D arrangement of the yoctoreactor was systematically tested (Figure 2). Control of the reaction between a carboxylic acid and an amine, leading to the formation of an amide bond, was demonstrated for both a three-way and a four-way reactor. It has previously been shown that chemical reactions can be controlled by linear annealing of two cDNA sequences linked to the BBs by bringing them into proximity.<sup>1,4,8</sup> Such reactions are, however, very dependent on distance and DNA sequence, and they often proceed in low yields if the reactants are placed more than a few bases apart on a linear template.<sup>10</sup> We observe that reactions performed by annealing only two arms of the yoctoreactor proceed in a significantly lower yield than reactions performed in the presence of helper oligonucleotide(s) which help(s) to fully assemble the 3D yoctoreactor. This demonstrates that the center of a DNA junction offers a highly favorable environment since the BBs are concentrated and spatially positioned for the chemical reaction.

Here we apply the yoctoreactor principle to demonstrate its ability to enrich for a known binder in a model system where the known binder initially represents 1 in 10 million in a spiked library. Specific enrichment is demonstrated for [Leu]-enkephalin (ENK, TyrGlyGlyPheLeu), the display product from a 100-member library spiked 1:100 000-fold into scrambled ENK (sENK, same amino acids in different order). The enrichment is obtained after two rounds of affinity selection toward the anti-ENK antibody 3-E7.<sup>16</sup>

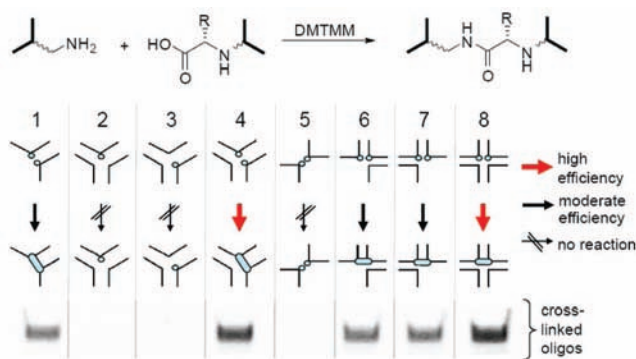
The first steps in creating a yoctoreactor library involve conjugation of the BBs to the oligonucleotides. For convenience we name the arms in the three-way junction A, B, and C. In position A, four different tripeptides were synthesized directly on the amine-modified position A oligos via a noncleavable PEG linker according to Halpin and Harbury.<sup>2</sup> Amino acids were linked to B and C via a cleavable linker (cl), succinimidyl 2-(vinylsulfonyl)ethyl carbonate, by a procedure developed in our laboratories (Figure 3a and Figure S2, Supporting Information).<sup>17</sup> The linker is readily cleaved at elevated pH to release a new terminal amino group. It is crucial that the residue of the

cleavable linker (cl) left behind on the oligo (here B) after cleavage does not interfere with the subsequent steps in the synthesis (see Supporting Information for further information on the DNA-conjugation chemistry).

The coupling between the four position A tripeptides and five position B amino acid BBs was performed as shown in Figure 3a. A helper sequence (oligo C) was applied for the formation of the three-way junction before the coupling reagent was added. The products were purified by denaturing PAGE, which eliminated all unsuccessful reactions. The subsequent ligation and elimination of the cleavable linker led to a significant shift in the mobility of the intermediate in the denaturing PAGE gel (Figure 3a–c, lanes 1–2). The third BBs were introduced by coupling of the A-B intermediate with five amino acid BBs on position C, yielding a library of 100 different pentapeptides. Despite the increasing length of the amino acid chain, no significant difference in the efficiency of the two coupling reactions was observed. After the second chemical reaction, the library of successfully cross-linked products was purified by denaturing PAGE, gel-extracted, and ligated, and the cl was cleaved. These three last steps were monitored by denaturing PAGE (Figure 3b,c, lanes 3–5). The formation of the trimer product causes a significant retardation of the product mobility (lane 3), whereas the subsequent ligation only leads to a minor shift (lane 4). The final cleavage of the C oligonucleotide linker causes a significant shift in the mobility of the product (lane 5). A weaker band with a mobility identical to that of the band in lane 4 is also observed in lane 5. This is due to incomplete cleavage of the cl; however, this minor byproduct is removed. In this manner a library containing 100 pentapeptide products was obtained from which all unsuccessful reactions had been eliminated by the denaturing PAGE purifications. Finally, the library of display products was prepared by primer extension (for more detailed information, see the Supporting Information).

The library members were identified by PCR amplification of the library and subsequent mass DNA sequencing.<sup>18,19</sup> The relative abundance of the library members resulting from the sequencing data is shown in Figure 3d. All 100 sequences were present between 0.4 and 2.4%. The successful formation of a library of 100 peptides with very low variation in abundance demonstrates that, in the case of the applied  $\alpha$ -amino acids, chemical reactions within the yoctoreactor occur with very limited influence of the structure of the BBs and the individual codons on the reaction yields. The creation of the same reactor in every step assures similar effective concentration in each step, which facilitates the formation of a homogeneous library.

An essential property of the yoctoreactor is the capability to robustly perform molecular evolution by repeated rounds of selection, amplification, and translation. The selection depends on binding, which initially was demonstrated in an electromobility assay where the ENK yoctoreactor product synthesized analogously to the library, as described above, was bound to the mAb 3-E7 antibody (Figure S4, Supporting Information). Selection was performed on the 100-member library described above spiked 1:100 000-fold into a scrambled ENK (sENK) yoctoreactor product. The nonbinding sENK product has the



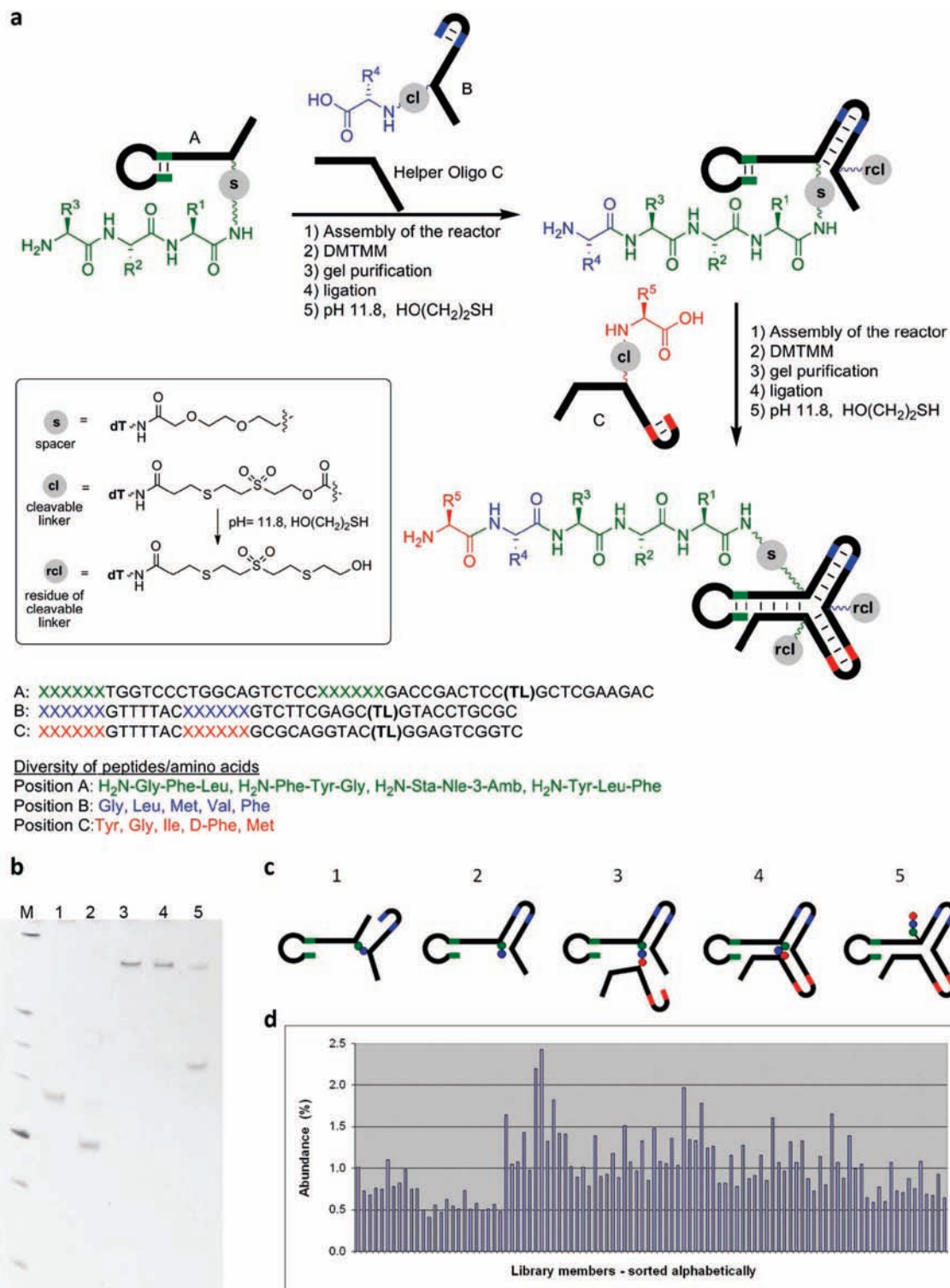
**Figure 2.** Analysis of reaction mixtures by denaturing PAGE. In lanes 4 and 8 the effect of the full assembly of the yoctoreactor in the 3- and 4-way formats is shown. This gives the most intense product bands. When the yoctoreactor is not fully assembled, as in lanes 1, 6, and 7, a weaker product band is observed. If the sequences are not complementary to one another, annealing is not achieved and no product is formed (lane 5). Lanes 2 and 3 are negative controls where one of the reactants is excluded. DMTMM, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride.

(16) Herz, A.; Gramsch, C.; Holtt, V.; Meo, T.; Riethmuller, G. *Life Sci.* **1982**, *31*, 1721–1724.

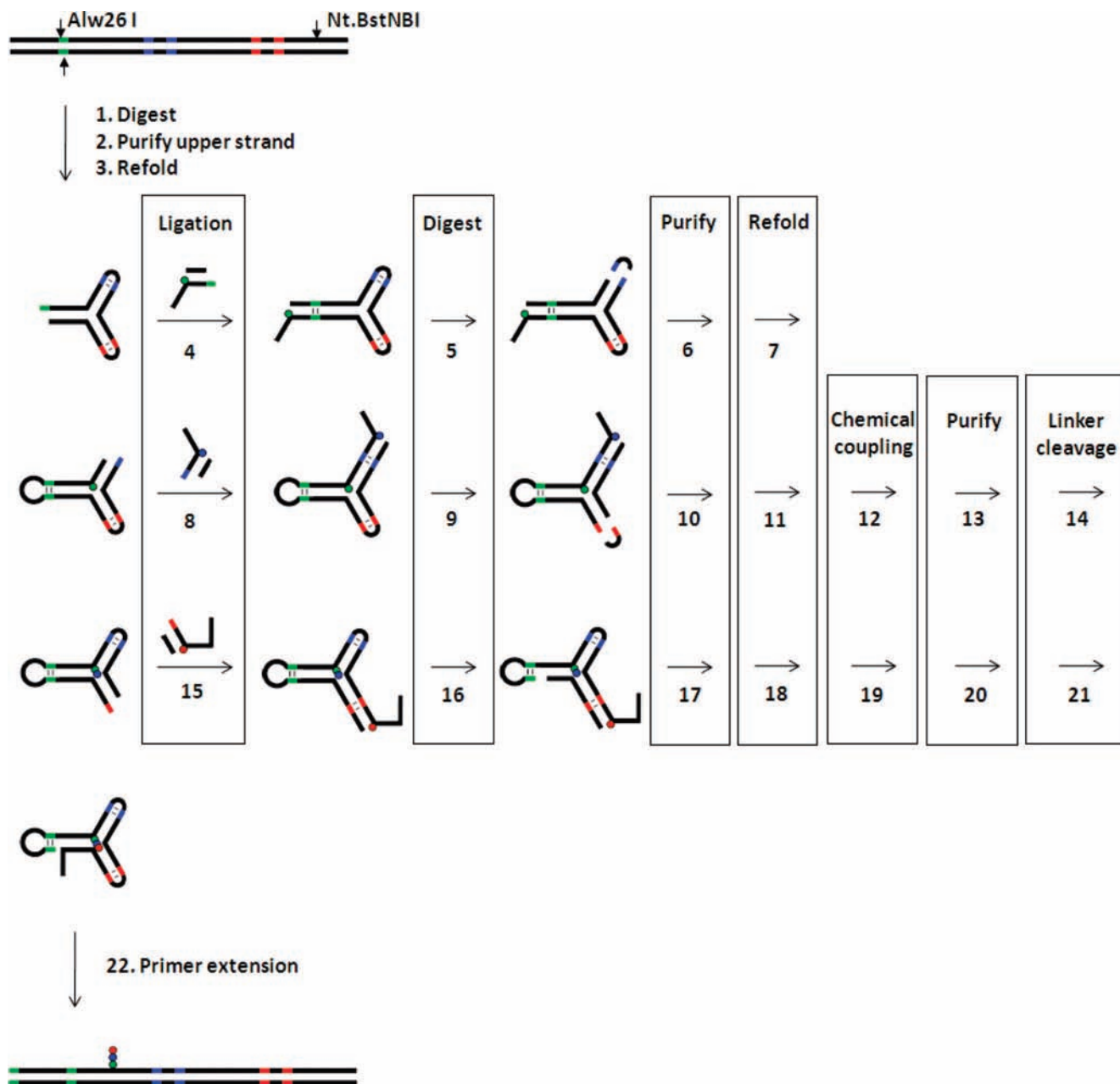
(17) Højfeldt, J. W.; Blakskjær, P.; Gothelf, K. V. *J. Org. Chem.* **2006**, *71*, 9556–9559.

(18) Rogers, Y. H.; Venter, J. C. *Nature* **2005**, *437*, 326–327.

(19) Binladen, J.; Gilbert, M. T. P.; Bollback, J. P.; Panitz, F.; Bendixen, C.; Nielsen, R.; Willerslev, E. *PLoS ONE* **2007**, *2*, e197.



**Figure 3.** Library creation using the three-way junction form of the yoctoreactor. (a) Stepwise library synthesis. In the first reaction, the four position A BB-linked oligonucleotides and the five position B BB-linked oligonucleotides were annealed together with a position C helper oligonucleotide (oligonucleotides lacking chemical modifications). Coupling products were purified by PAGE, and A and B oligonucleotides were ligated. Finally, the cleavable linker was cleaved. The second reaction, with five position C BB-linked oligonucleotides, proceeds in a similar manner, with C replacing the helper oligonucleotide. Middle left: Structure and chemistry of the noncleavable spacer used in A BB-linked oligonucleotides and cleavable linker used in B and C BB-linked oligonucleotides (for further details see Supporting Information). Bottom: Sequences of the oligonucleotides and the diversity of BBs attached. The position A oligonucleotides constitute, from the 5' end: a 6 nt building block specific codon, an 18 nt PCR priming site, a 6 nt anticodon, a 10 nt segment for annealing with C oligonucleotides, a chemically modified thymine (TL), and finally a 10 nt segment for annealing to B oligonucleotides. Position B BB-linked oligonucleotides constitute, from the 5' end: a 6 nt building block specific codon, a 7 nt loop forming segment, a 6 nt anticodon, 10 nt for annealing with A oligonucleotides, a chemically modified thymine, and finally 10 nt for annealing to C oligonucleotides. C BB-linked oligonucleotides were designed like B BB-linked oligonucleotides, with a 20 nt 3' PCR priming site (not shown here, see Supporting Information). (b) Analysis of the library synthesis by denaturing PAGE (DNA stained by SYBR Green I). (c) Constitution of the products shown in lanes 1–5 in (b); 1, purified cross-linked dimer; 2, ligated dimer and cl cleaved; 3, purified cross-linked trimer; 4, ligated trimer; 5, ligated trimer and cl cleaved. (d) Abundance of the 100 library members obtained by mass DNA sequencing (39 433 sequences in total). Components are listed alphabetically after amino acid sequence analysis.



**Figure 4.** Steps of the rolling translation. For simplicity the rolling translation is shown for a single molecule; however, libraries are translated in a single-pot format by using a library of PCR amplicons and repertoires of BB-linked oligonucleotides on the various positions.

sequence GlyLeuPheTyrGly and is synthesized analogously to the library as described above. Since the initial frequency of ENK in the 100-member library was approximately 1%, the resulting abundance of ENK after spiking is 1:10 million. This library was subjected to affinity selection toward immobilized anti-ENK mAb 3-E7 and an isotype-matched negative control mAb in parallel. After incubation of the library with the immobilized mAbs and washing steps, the elution was performed by adding free ENK peptide.<sup>2</sup> The DNA in the eluates was amplified by PCR and the outcome analyzed by mass DNA sequencing. As expected for the first round of selection, the sENK completely dominated the outcome of the sequencing analysis (Table 1).

The enriched and amplified DNA sequences from the first round were translated into the encoded yoctoreactor display products via the rolling translation process (Figure 4). In this process the A, B, and C arms of the amplified DNA sequences

(containing no BBs) are replaced with codon specific oligonucleotides A, B, and C containing the specific BBs by consecutive enzymatic digestions and ligations. In step 1 the ends of the PCR amplicon are trimmed by restriction enzyme digest with *Alw26I* and *Nt.BstNBI*, and in step 2 the DNA strand corresponding to the strand formed by the oligonucleotides in the library synthesis is purified by virtue of size. Step 3 consists of spontaneous refolding to the original yoctoreactor structure under native conditions, with the A anticodon protruding at the 5' end. In step 4 codon/anticodon-guided DNA ligation is performed, and in step 5 the next arm is digested with *BpiI*, which cuts exactly 5' to the B anticodon. Purification by virtue of DNA size is performed in step 6. Step 7 is a refolding as in step 3, leading to protrusion of the B anticodon at the 5' end, with the BB on A positioned in the center of the reactor. Steps 8–11 are analogous to steps 4–7, using the restriction enzyme *BveI*.

**Table 1.** DNA Sequencing Data of the Products from the *in Vitro* Selections

manipulation	target	total counts	compound	name	counts	frequency (%)
first-round selection	anti ENK mAb	16638	GlyLeuPheTyrGly	sENK	16638	100.0
	control mAb	13242	GlyLeuPheTyrGly	sENK	13242	100.0
second-round selection	anti ENK mAb	4226	GlyLeuPheTyrGly	sENK	4141	98.0
			<b>TyrGlyGlyPheLeu</b>	<b>ENK</b>	<b>71</b>	<b>1.7</b>
			GlyLeuTyrLeuPhe		8	0.2
			GlyValStaNle3-Amb		4	0.1
	control mAb	5483	GlyLeuStaNle3-Amb		2	0.0
			GlyLeuPheTyrGly	sENK	5462	99.6
			GlyLeuGlyPheLeu		14	0.3
			IleValStaNle3-Amb		4	0.1
			TyrMetTyrLeuPhe		3	0.1

In step 12 the first chemical reaction for the formation of the enriched library is performed. DM-TMM is added to induce the chemical coupling reaction between A and B BBs which by the refolding have been positioned in the center of the reactor. The successful products are purified in step 13 by denaturing PAGE, which is exploited for separation of coupled species. In step 14 the B linker is cleaved, leading to exposure of an amine on the growing chemical compound. Steps 15–21 are analogous to steps 8–14, apart from using the restriction enzyme Nt.BstNBI. In the final step 22 the yoctoreactor is dismantled by primer extension reaction using a primer reverse complement to the 3' end of C whereby the yoctoreactor display product is formed and ready for affinity selection. A more detailed description can be found in the Supporting Information.

The enriched yoctoreactor library of display products was subjected to a second round of affinity selection and amplification was performed. The progress of the *in vitro* selection was followed by mass DNA sequencing and the result is shown in Table 1. After the second round of selection toward anti-ENK mAb 3-E7, the DNA encoding the ENK was observed with a frequency of 1.7%. This constitutes an overall enrichment factor of more than 150 000 after only two rounds of selection. In contrast, the ENK encoding sequence was not observed in the selections performed using the control antibody. Consequently, a potent and specific enrichment of the known binder was observed. Encouragingly, this enrichment level is comparable to those achieved by biological selection techniques such as phage display, relying on the highly effective translation system of the cell.<sup>5</sup>

## Conclusion

As demonstrated here, the yoctoreactor represents a compelling approach to control chemical reactions and build chemical libraries for the rapid identification of high-affinity binders to a protein target of interest. Its unique features include its 3D structure and an invariable reaction site which confines BBs in high concentration to yield highly efficient and unbiased chemical reactions. Furthermore, the reactor possesses intrinsic error-control measures, ensuring high fidelity between the genetic code and the corresponding synthesized chemical product during library assembly and translation: Since the reactor is a self-assembled structure

rather than a template-based structure, the code and the BB travel intimately together via the A, B, and C oligonucleotides, eliminating the risk of mismatches. The ability to perform robust *in vitro* molecular evolution enables a single-tube method that makes it possible to work with vast chemical libraries.

The successful manipulation of a yoctoreactor library through two rounds of selection, amplification, and translation has been demonstrated by the enrichment of the [Leu]-enkephalin peptide synthesized in the library. Although the library presented herein has a low diversity, the more than 150 000-fold enrichment from an original frequency of 1 in 10 million in only two rounds of affinity selection demonstrates the power of the molecular evolution process and lays the foundation for the generation of libraries consisting of up to 10<sup>12</sup> independent members.

Here DNA-encoded assembly of three building blocks was demonstrated. The process can easily be extended to a higher number of building blocks and a more diverse set of chemical reactions. In principle, the diverse set of chemistries that have been successfully used in DNA-directed reactions are applicable to the yoctoreactor technology.<sup>4b</sup> In addition, the principle can easily be extended to higher order DNA junctions containing a higher number of building blocks. The construction of large libraries containing four DNA-encoded building blocks assembled in a four-way reactor using a multitude of chemistries is in progress. Access to such great chemical diversity has the potential to accelerate the identification of chemical compounds having high target affinity and selectivity for potential pharmaceutical use. In extension, a range of further applications can be envisioned for the yoctoreactor, such as the formation of and selection for sensors and artificial enzymes.

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**Supporting Information Available:** Materials and experimental procedures; Figures S1–S4 and Tables S1, S2. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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