Drug Discovery

Deutsche Ausgabe: DOI: 10.1002/ange.201501775
Internationale Ausgabe: DOI: 10.1002/anie.201501775

DNA-Encoded Dynamic Combinatorial Chemical Libraries**

*Francesco V. Reddavide, Weilin Lin, Sarah Lehnert, and Yixin Zhang**

Abstract: *Dynamic combinatorial chemistry (DCC) explores the thermodynamic equilibrium of reversible reactions. Its application in the discovery of protein binders is largely limited by difficulties in the analysis of complex reaction mixtures. DNA-encoded chemical library (DECL) technology allows the selection of binders from a mixture of up to billions of different compounds; however, experimental results often show low a signal-to-noise ratio and poor correlation between enrichment factor and binding affinity. Herein we describe the design and application of DNA-encoded dynamic combinatorial chemical libraries (EDCCLs). Our experiments have shown that the EDCCL approach can be used not only to convert monovalent binders into high-affinity bivalent binders, but also to cause remarkably enhanced enrichment of potent bivalent binders by driving their in situ synthesis. We also demonstrate the application of EDCCLs in DNA-templated chemical reactions.*

Dynamic combinatorial chemistry (DCC) involves the application of reversible chemical reactions between monomeric building blocks to generate a mixture of products under thermodynamic equilibrium.[1] Under selection pressure from a protein target, the high-affinity constituent(s) are stabilized, and the equilibrium will shift, thus causing an increase in the formation of the binding molecules. However, as the size of a DCC library increases, each individual binder will inevitably become a smaller fraction of the entire mixture. The lack of an efficient analytical method to identify the enriched compounds poses one of the largest challenges for large DCC libraries, as well as other drug-discovery methods based on the use of a mixture of compounds.[1a,2] In comparison, one of the most attractive features of DNA-encoded chemical library (DECL) technology is its high sensitivity in the hit-identification process, which involves DNA amplification followed by sequencing. DECL technology is compatible with mixtures of millions to billions of DNA-encoded compounds.[3] Numerous strategies have been developed.[4] In one approach, one strand of a DNA duplex is used to construct the library (single-pharmacophore libraries),[3b,c] and in another, both strands are conjugated to library members (dual-pharmacophore libraries).[3e,5] The latter approach, also known as encoded self-assembling chemical library technology, explores the combinatorial assembly of complementary sublibraries by DNA hybridization.[3e] It has advantages for the construction of large libraries of high purity. For example, it is practically impossible to purify every compound in a single-pharmacophore library with 1 million members, whereas the assembly of two 1000-membered DNA-encoded sublibraries that have been purified by HPLC and characterized by MS can also result in a library with 1 million members.

Although selection experiments with large DECLs often result in many hit compounds, one of the major challenges for the use of such libraries is to enhance the enrichment factors of potent binders relative to those of weak binders and background noise.[3b,c,6] For example, parallel selection experiments with different selection stringencies have been used to optimize selection conditions against a given protein target.[7] However, a more general method is urgently needed. In this study, we applied the concept of DCC in DECL design and selection.

The principle of a DNA-encoded dynamic combinatorial chemical library (EDCCL) is described in Figure 1. It uses the advantages of both DECL technology, that is, sensitive hit identification from a myriad of compounds, and DCC, that is, enhancement of enrichment through shifting of the equilibrium towards the generation of potent binders. To demonstrate the feasibility of an EDCCL approach to identify a bidentate protein/ligand interaction, we used iminobiotin and homotetrameric straptavidin as a model system. A DNA duplex with two iminobiotin molecules binding to streptavidin was previously used to illustrate a bidentate interaction mediated by DNA self-assembly.[8] Given that the binding of iminobiotin to streptavidin is pH-dependent, we can use this system to investigate multivalent protein/ligand interactions with a range of monovalent affinities by altering the pH value.[9] Iminobiotin binds to streptavidin with a sub-/low-micromolar K_d value at high pH values, whereas the affinity is reduced to the high-micromolar range at a neutral pH value owing to the protonation of the iminobiotin guanidinium group. Given that low- to high-micromolar affinities are expected for most de novo selection experiments with DECLs, the iminobiotin/streptavidin interaction provides not only a proof-of-principle model for the establishment of an EDCCL, but also a tunable system to cover the range of interactions most common in de novo drug-discovery campaigns.

Iminobiotin was coupled to the 5′-amino DNA strand 1′ and 3′-amino DNA strand 1, which also possessed a 5′ modification with the dye Cy5, thus resulting in Im-1′ and Cy5-1-Im, respectively. Fluorescently labeled Cy5-1-Im was

[*] F. V. Reddavide, W. Lin, S. Lehnert, Dr. Y. Zhang
B CUBE Center for Molecular Bioengineering
Technische Universität Dresden
Dresden 01307 (Germany)
E-mail: yixin.zhang@bcube-dresden.de

[**] Bundesministerium für Bildung und Forschung (BMBF; Grant Nos. 03Z2EN12 and 03Z2E511). We thank Peggy Berg and Ulrike Hofmann for technical support. We thank Andreas Hartmann for help with the Matlab enrichment simulation. We thank Elisa Mele for help with the design of the microfluidic setup. We thank Michael Thompson for critical reading of the manuscript.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201501775.

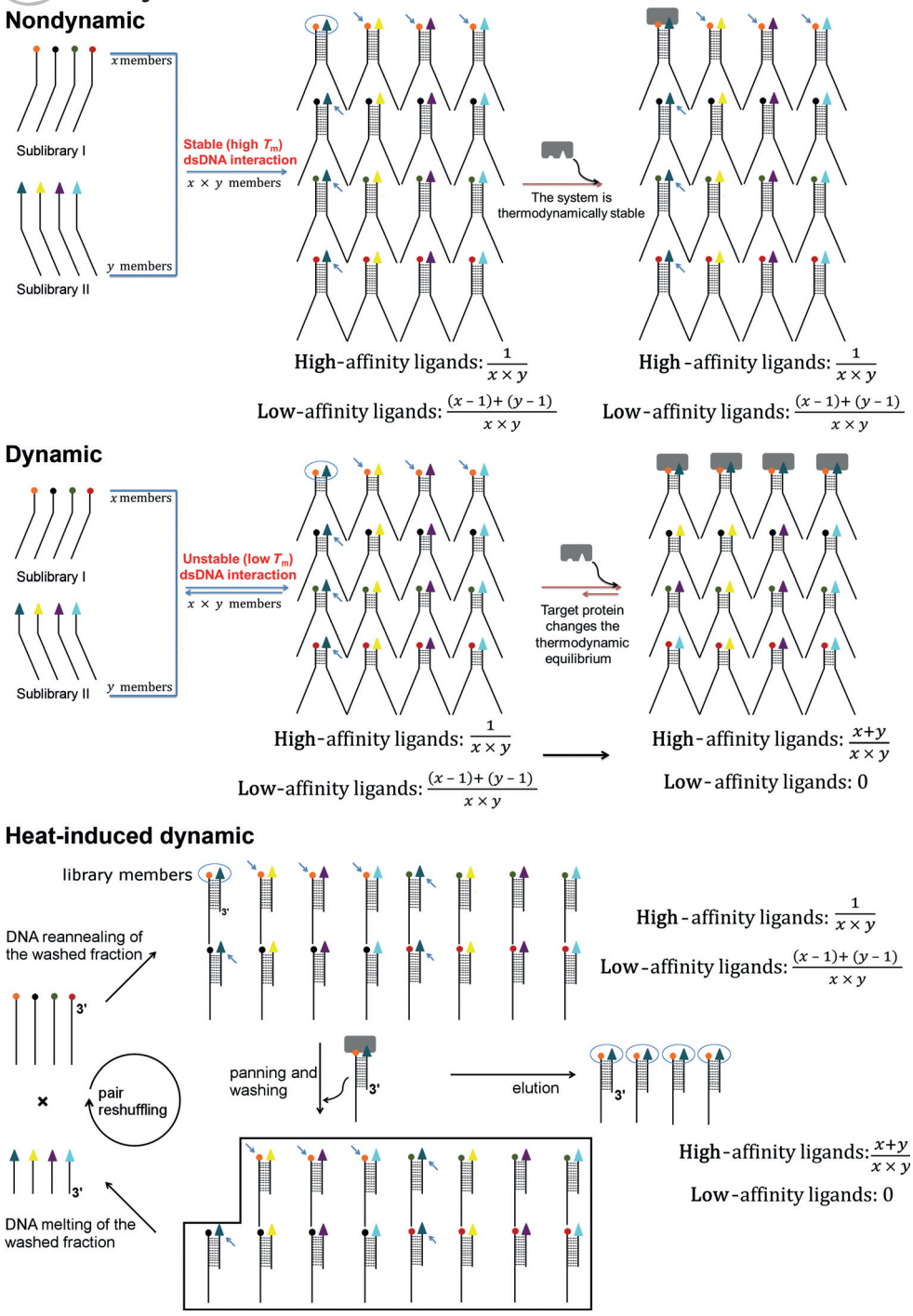

Figure 1. The principles of DNA-encoded dynamic combinatorial chemical library (EDCCL) technology and heat-induced DNA-encoded dynamic combinatorial chemical library (hi-EDCCL) technology. In the EDCCL method, thermodynamic instability causes pair reshuffling. Under selection pressure from a protein target, the constituents are shifted to the generation of potent bidentate binders. This shift results in an increase in the high-affinity-ligand signal and a decrease in noise from low-affinity ligands. In the hi-EDCCL method, full benefit is made of the oligonucleotide stability. The principle comes from the presence in the supernatant of low-affinity binders containing one of the building blocks of the high-affinity binders. When the oligonucleotides in the supernatant are melted, the two sublibraries are reformed, and the building blocks are free to self-assemble again to give the high-affinity binders. We defined this concept as pair reshuffling. After some cycles of reshuffling and incubation in the resin, the high-affinity binders can be eluted and give a higher signal as compared to that after a single cycle of incubation as in the standard dual-pharmacophore selection. Pair reshuffling and resin incubation are space- and time-separated. The relative abundances of high- and low-affinity bivalent ligands, under dynamic and nondynamic conditions, are shown as the probability of their occurrence in the library before and after the addition of the target protein. Circles indicate the "correct pairs" (potent binders) of pharmacophores in the library, whereas arrows indicate pharmacophores "wrongly paired" in the library.

used to evaluate the capture and enrichment of the iminobiotin-modified DNA strand. The enrichment factor is defined by the ratio between the amounts of the bound fraction (eluted by biotin) and the unbound fraction (supernatant), both in picomols (Figure 2; see also the Supporting Information).

An EDCCL is a thermodynamic system controlled by individual protein/ligand interactions, the stability of DNA duplexes, and the relative abundance of strong-, weak-, and nonbinding molecules. We started with an affinity maturation library,[3e] with 1 equivalent of Cy5-1-Im, 1 equivalent of Im-1′, and N equivalents of strand 1. A large excess of unmodified strand 1 was used to mimic the nonbinding compounds in a library (Figure 2, $N=300$, $N=10000$). The presence of a limited amount of one strand is the prerequisite for establishing a dynamic affinity maturation library, which enables the recombination of strands to be driven to the formation of high-affinity bidentate binders. To find the optimal conditions for generating a dynamic library, we investigated different numbers of hybridization base pairs between sequences 1 and 1′ ($m=4$, 5, 6, 8, or 21; Figure 2a). When the value of m is high, the formation of a stabilized duplex can result in a strong bidentate interaction between two iminobiotin groups and streptavidin, whereas the rate of chain recombination is low. In contrast, when the value of m is low, no stable duplex can be formed, and thus the bidentate effect may be diminished, although such systems could possess a high rate of recombination. Under nondynamic conditions ($m=$

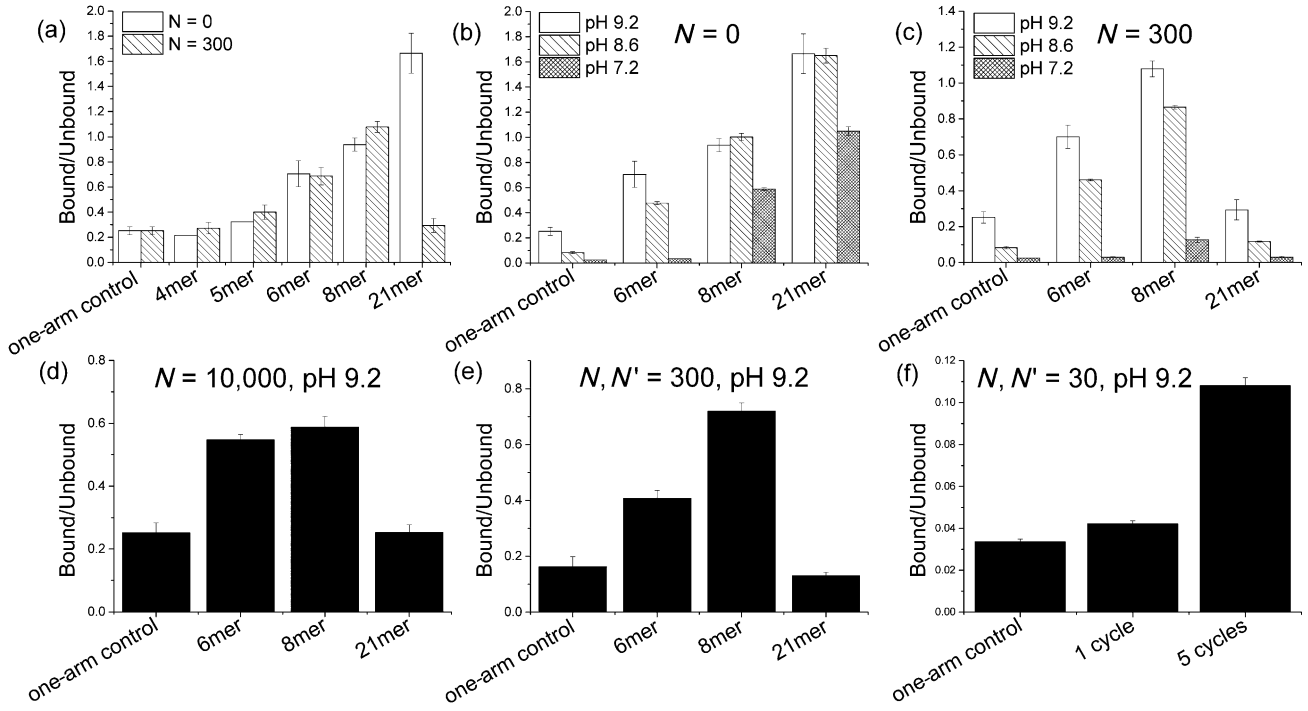

Figure 2. Selection of an EDCCL containing iminobiotin by the use of streptavidin sepharose resin under various conditions. b) Cy5-1-Im or a 1:1 mixture of Cy5-1-Im and Im-1′ with $m = 6$, 8, or 21 was incubated with streptavidin resin at pH 9.2, 8.6, or 7.2. c) Cy5-1-Im or a 1:1 mixture of Cy5-1-Im and Im-1′ with m = 6, 8, or 21 in the presence of a 300-fold excess of strand 1 was incubated with streptavidin resin at pH 9.2, 8.6, or 7.2. d) Cy5-1-Im or a 1:1 mixture of Cy5-1-Im and Im-1′ with a 10000-fold excess of strand 1 and with $m = 6$, 8, and 21 was incubated with streptavidin sepharose resin at pH 9.2. e) Cy5-1-Im or a 1:1 mixture of Cy5-1-Im and Im-1′ with a 300-fold excess of strand 1 and a 300-fold excess of strand 1′ and with $m = 6$, 8, and 21 was incubated with streptavidin sepharose resin at pH 9.2. f) Cy5-1-Im or a 1:1 mixture of Cy5-1-Im and Im-1′ with a 30-fold excess of strand 1 and a 30-fold excess of strand 1′ and with $m = 18$ was incubated with streptavidin sepharose resin at pH 9.2 and subjected to one or five cycles of heat-induced dynamics. The data in graphs (a–f) were obtained by measuring the ratio of fluorescently labeled DNA in the supernatant and in the final elution buffer containing biotin. N and N' indicate the fold excess of strands 1 and 1′, respectively. The value of m indicates the number of hybridization base pairs used to investigate the dynamic library properties. Error bars indicate the standard deviation ($n \geq 3$).

21), the annealing temperature of the duplexes is much higher than room temperature, whereas for $m = 4$, 5, 6, and 8, libraries with different dynamic properties are created. At all pH values tested (pH 9.2, 8.6, and 7.2) and $N = 0$, nondynamic conditions ($m = 21$) led to the highest enrichment (Figure 2b). Because duplexes with $m = 6$ or 8 are not as stable as those with $m = 21$, their enrichment is not as high as that of Cy5-1-Im/Im-1′ with $m = 21$. When the number of hybridization base pairs was further decreased ($m = 4$ and 5), no significant enrichment was observed (Figure 2a). However, in a library setup with $m = 21$, for example, N is 300 (Figure 2c) or 10000 (Figure 2d), the enrichment factors were similar to those for one-arm control. Although the bidentate binding with $m = 21$ is strong, the bidentate compound is diluted by a factor of $(N+1)$ because of the formation of the stable monovalent binder Cy5-1-Im/1′. When the number of hybridization base pairs was decreased to allow dynamic recombination ($m = 6$ and 8), the equilibrium could be shifted to the formation of Cy5-1-Im/Im-1′ in the presence of streptavidin. We then performed the selection experiment with a 1:1 mixture 1-Im (without the fluorescent label) and Im-1′ ($m = 8$ or 21) at pH 9.2 in the presence of a 1000-fold excess of strand 1 ($N = 1000$). We subjected the selected compounds to qPCR to determine the threshold-cycle (C_T) values.[10] As compared to the one-arm control 1-Im, the nondynamic construct led to only a minor decrease in C_T from 13.05 to 12.20, whereas the use of the dynamic construct resulted in a significant change in the C_T value from 13.05 to 9.67 (see Tables S2 and S3).

We used the pH-dependent streptavidin–iminobiotin interaction as a model system to illustrate how these factors contribute to the enrichment of potent bidentate binders under different selection conditions. The binding of Cy5-1-Im to streptavidin decreased remarkably as the pH value decreased from 9.2 or 8.6 to 7.2 (Figure 2b; see also Table S1). With $N = 300$ at pH 9.2, a 2.8- and 4.3-fold increase in the enrichment factor relative to one-arm control was observed for $m = 6$ and 8, respectively (Figure 2c). Remarkably, at pH 8.6, a five- and tenfold increase in the enrichment factor was observed for $m = 6$ and 8, respectively (Figure 2c). Interestingly, at pH 7.2, the dynamic library with eight base pairs showed a fivefold increase in the enrichment factor, whereas the enrichment was completely diminished for $m = 6$ (Figure 2c). Therefore, when the monovalent binding is very weak, the stability of the DNA duplexes in a dynamic library becomes essential for the chelation effect. If potent monovalent binders are not expected in a de novo selection experiment, a relatively high m number should be used, although such experiments will take longer (see Figure S4 in the Supporting Information). Moreover, under dynamic conditions, a nonsignificant variation in enrichment between

$N = 0$ and $N = 300$ is observed when the bivalent interaction is strong. However, when the bivalent interaction is not very strong, a large excess of the nonbinding strands can shift the equilibrium and lead to a low degree of enrichment. For example, in the presence of a 300-fold excess of strand 1, the enrichment factor ($m = 8$) at 7.2 was only a quarter of that found with $N = 0$ (Figure 2b,c).

The EDCCL technology was further validated in dual-pharmacophore formats. We tested the system with a 90601-member (301 × 301) dual-pharmacophore library for which Cy5-1-Im and Im-1′ were premixed with a 300-fold excess of DNA strands 1 and 1′, respectively. A 2.5- and 4.5-fold increase in the enrichment factor was observed for $m = 6$ and 8, respectively (Figure 2e).

To demonstrate that the EDCCL approach can also be used for in-solution selection experiments,[10a,11] we performed exonuclease-coupled selections (see Figure S9) by using the iminobiotin/streptavidin system and a bidentate binder against alpha-1-acid glycoprotein (AGP).[5d] When the "correct" pair of compounds binds to the target protein, DNA strands would be protected from exonuclease digestion both by the stabilized double helix and by blocking of the 3′ end by a protein/ligand interaction.[11b,12] Given that the exonuclease-coupled method for in-solution selection requires an unstable DNA duplex, this method is not compatible with nondynamic dual-pharmacophore libraries. As quantified by qPCR, in the presence of 2 μM streptavidin in solution, an eightfold increase in the amount of the iminobiotin-conjugated DNA strand was detected, as compared to the experiment without the protein. Although both monovalent compounds have no detectable binding affinity for AGP,[5d] a twofold increase in the amount of the furan building block was detected when AGP (2 μM) and the sulfonyl derivative were present in the solution. Unlike previously reported in-solution selection methods, the new selection approach avoids any chemical modification of the target protein.

The library dynamics are caused by the weak interactions between the DNA strands, which also result in relatively low affinity of the bidentate interaction, as compared to a stable DNA duplex (Figure 2a). To take advantage of both stably assembled dual-pharmacophore libraries and EDCCLs, we extended the concept of EDCCLs to heat-induced EDCCLs (hi-EDCCLs), in which the heat-induced recombination process of stable DNA duplexes and affinity capture are carried out separately (Figure 1). The selection experiment consists of: 1) library incubation with the protein resin at room temperature; 2) after centrifugation and the elution of non-binding compounds, reannealing from 90 to 25 °C (duplex recombination); 3) repetition of steps (1) and (2) as necessary. We tested a library with 31 × 31 members by using an encoded self-assembled chemical library design and the hi-DECCL procedure. A remarkable increase in enrichment was observed after five cycles (Figure 2f), in good agreement with the simulation (see Figure S7). Similar results were also obtained by qPCR analysis (see Table S4). Importantly, this procedure is compatible with the different formats of existing encoded self-assembled chemical libraries.[5d]

DCC is a general concept in many fields of chemistry. To demonstrate that EDCCL technology can be used not only for drug discovery, but also for other applications, such as

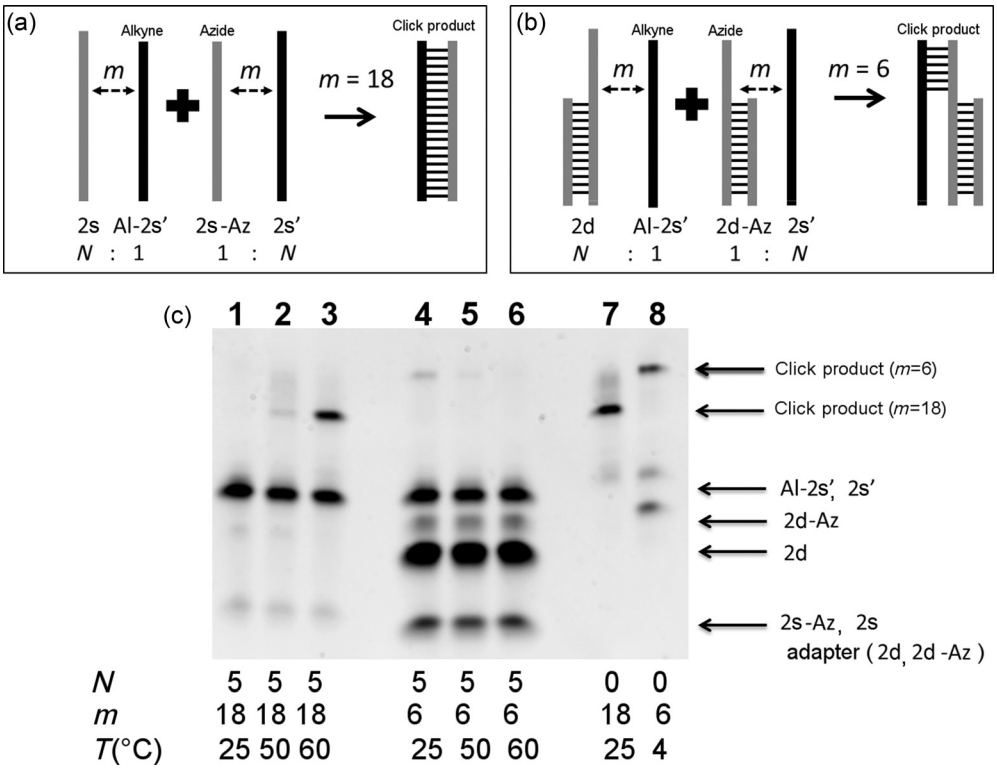

Figure 3. DNA-templated chemical reaction based on EDCC. a) The DNA–alkyne conjugate Al-2s′ premixed with 2s (fivefold excess) was mixed with the DNA–azide conjugate 2s-Az premixed with 2s′ (fivefold excess) with $m = 18$. b) Al-2s′ premixed with double-stranded 2d (fivefold excess) was mixed with double-stranded 2d-Az premixed with single-stranded 2s′ (fivefold excess) with $m = 6$. c) The resulting mixtures were incubated at 25, 50, or 60 °C for 1 h, followed by treatment with tris(2-carboxyethyl)phosphine (TCEP) to quench all reactive azide groups. The mixtures were then separated on a denaturing gel. The migration of the click product could be observed on the gel when $m = 18$ (lane 7) and $m = 6$ (lane 8) after the incubation of the reacting oligonucleotides overnight at room temperature and 4 °C, respectively, without any unreactive competing oligonucleotide. In the presence of competing species, the reaction became temperature-dependent: When $m = 18$, an increase in the temperature destabilized the double strand, thus permitting the reaction to take place (lanes 1, 2, and 3). On the other hand, when $m = 6$, the reaction was only possible at 25 °C (lane 4) because an increase in the temperature further melted the DNA duplex (lanes 5 and 6).

DNA-templated chemical reactions,[13] we designed an EDCCL for the copper-free click reaction.[14] An alkyne group was conjugated to a 5′-amino DNA molecule (Al-2s′), and an azide group was conjugated to a 3′-amino DNA molecule (2s-Az). Strands 2s′ and 2s are partially complementary to each other with $m = 6$ or 18. For $m = 6$, to prevent other interactions between the two strands from influencing the dynamics, 2s-Az was annealed to a sequence complementary to the region not involved in the dynamic assembly, thus resulting in the construct 2d-Az. The DNA-templated reaction led to the conjugation of two strands (Figure 3). When Al-2s′ and 2s-Az were premixed with 2s and 2s′, respectively, in a 1:5 ratio under nondynamic conditions with $m = 18$, the conjugation reaction did not occur at room temperature (Figure 3). However, when Al-2s′ and 2d-Az were premixed with 2d and 2s′ in fivefold excess under dynamic conditions with $m = 6$, a clear conjugation product was detected at room temperature (Figure 3). The dynamic conditions at room temperature were also demonstrated by the use of a competing unreactive species in 15- and 30-fold excess (see Figure S14). Given that the dynamics are associated with the annealing temperature, we investigated the influence of the temperature on the DNA-templated chemical reaction.

Interestingly, when $m = 6$ and $N = 5$, an increase in the temperature led to a decrease in the reaction yield, because the duplex was not stable enough to allow the DNA-templated click reaction to occur. However, when $m = 18$, an increase in temperature caused the recombination of DNA strands and induced the templated reaction. When the temperature was above the annealing temperature (60 °C), a dramatically increased reaction yield was observed.

In summary, we have described a method to transform DECLs into dynamic combinatorial libraries and to generate such libraries with unprecedented sizes. Under conditions allowing the dynamic recombination of complementary DNA strands, potent bidentate binders can be evolved by shifting the thermodynamic equilibrium. Moreover, this method can also be used in applications such as DNA-templated reaction discovery. We have extended the concept of EDCCL technology to hi-EDCCL technology, which can be applied to existing dual-pharmacophore libraries. However, as the hi-EDCCL procedure is very cumbersome, it is practically impossible to carry out more than 10 cycles manually. Therefore, we have designed an automated continuous selection system based on microfluidics (see Figure S8), which will be tested with large libraries in the future.

Keywords: DNA-encoded chemical libraries · DNA-templated reactions · drug discovery · dynamic combinatorial chemistry · protein–ligand interactions

How to cite: *Angew. Chem. Int. Ed.* **2015**, *54*, 7924–7928
Angew. Chem. **2015**, *127*, 8035–8039

[1] a) P. T. Corbett, J. Leclaire, L. Vial, K. R. West, J. L. Wietor, J. K. Sanders, S. Otto, *Chem. Rev.* **2006**, *106*, 3652–3711; b) I. Huc, J. M. Lehn, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 2106–2110; c) S. Ladame, *Org. Biomol. Chem.* **2008**, *6*, 219–226.

[2] I. Muckenschnabel, R. Falchetto, L. M. Mayr, I. Filipuzzi, *Anal. Biochem.* **2004**, *324*, 241–249.

[3] a) S. Brenner, R. A. Lerner, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 5381–5383; b) M. A. Clark, R. A. Acharya, C. C. Arico-Muendel, S. L. Belyanskaya, D. R. Benjamin, N. R. Carlson, P. A. Centrella, C. H. Chiu, S. P. Creaser, J. W. Cuozzo, C. P. Davie, Y. Ding, G. J. Franklin, K. D. Franzen, M. L. Gefter, S. P. Hale, N. J. Hansen, D. I. Israel, J. Jiang, M. J. Kavarana, M. S. Kelley, C. S. Kollmann, F. Li, K. Lind, S. Mataruse, P. F. Medeiros, J. A. Messer, P. Myers, H. O'Keefe, M. C. Oliff, C. E. Rise, A. L. Satz, S. R. Skinner, J. L. Svendsen, L. Tang, K. van Vloten, R. W. Wagner, G. Yao, B. Zhao, B. A. Morgan, *Nat. Chem. Biol.* **2009**, *5*, 647–654; c) R. E. Kleiner, C. E. Dumelin, G. C. Tiu, K. Sakurai, D. R. Liu, *J. Am. Chem. Soc.* **2010**, *132*, 11779–11791; d) L. Mannocci, Y. Zhang, J. Scheuermann, M. Leimbacher, G. De Bellis, E. Rizzi, C. Dumelin, S. Melkko, D. Neri, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 17670–17675; e) S. Melkko, J. Scheuermann, C. E. Dumelin, D. Neri, *Nat. Biotechnol.* **2004**, *22*, 568–574.

[4] R. M. Franzini, D. Neri, J. Scheuermann, *Acc. Chem. Res.* **2014**, *47*, 1247–1255.

[5] a) J. Scheuermann, C. E. Dumelin, S. Melkko, Y. Zhang, L. Mannocci, M. Jaggi, J. Sobek, D. Neri, *Bioconjugate Chem.* **2008**, *19*, 778–785; b) C. Scheibe, A. Bujotzek, J. Dernedde, M. Weber, O. Seitz, *Chem. Sci.* **2011**, *2*, 770–775; c) M. Ciobanu, K. T. Huang, J. P. Daguer, S. Barluenga, O. Chaloin, E. Schaeffer, C. G. Mueller, D. A. Mitchell, N. Winssinger, *Chem. Commun.* **2011**, *47*, 9321–9323; d) M. Wichert, N. Krall, W. Decurtins, R. M. Franzini, F. Pretto, P. Schneider, D. Neri, J. Scheuermann, *Nat. Chem.* **2015**, DOI: 10.138/NCHEM.2158.

[6] F. Buller, M. Steiner, J. Scheuermann, L. Mannocci, I. Nissen, M. Kohler, C. Beisel, D. Neri, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4188–4192.

[7] M. Leimbacher, Y. Zhang, L. Mannocci, M. Stravs, T. Geppert, J. Scheuermann, G. Schneider, D. Neri, *Chemistry* **2012**, *18*, 7729–7737.

[8] S. Melkko, C. E. Dumelin, J. Scheuermann, D. Neri, *Chem. Biol.* **2006**, *13*, 225–231.

[9] N. M. Green, *Biochem. J.* **1966**, *101*, 774–780.

[10] a) L. M. McGregor, D. J. Gorin, C. E. Dumelin, D. R. Liu, *J. Am. Chem. Soc.* **2010**, *132*, 15522–15524; b) D. J. Gorin, A. S. Kamlet, D. R. Liu, *J. Am. Chem. Soc.* **2009**, *131*, 9189.

[11] a) L. M. McGregor, T. Jain, D. R. Liu, *J. Am. Chem. Soc.* **2014**, *136*, 3264–3270; b) P. Zhao, Z. Chen, Y. Li, D. Sun, Y. Gao, Y. Huang, X. Li, *Angew. Chem. Int. Ed.* **2014**, *53*, 10056–10059; *Angew. Chem.* **2014**, *126*, 10220–10223.

[12] Y. Cao, S. Zhu, J. Yu, X. Zhu, Y. Yin, G. Li, *Anal. Chem.* **2012**, *84*, 4314–4320.

[13] a) Y. Chen, A. S. Kamlet, J. B. Steinman, D. R. Liu, *Nat. Chem.* **2011**, *3*, 146–153; b) Z. J. Gartner, B. N. Tse, R. Grubina, J. B. Doyon, T. M. Snyder, D. R. Liu, *Science* **2004**, *305*, 1601–1605; c) M. W. Kanan, M. M. Rozenman, K. Sakurai, T. M. Snyder, D. R. Liu, *Nature* **2004**, *431*, 545–549; d) K. Gorska, N. Winssinger, *Angew. Chem. Int. Ed.* **2013**, *52*, 6820–6843; *Angew. Chem.* **2013**, *125*, 6956–6980.

[14] E. M. Sletten, C. R. Bertozzi, *Angew. Chem. Int. Ed.* **2009**, *48*, 6974–6998; *Angew. Chem.* **2009**, *121*, 7108–7133.

Received: February 24, 2015
Revised: April 21, 2015
Published online: May 26, 2015

