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Lab-on-a-chip platform for high throughput drug discovery with DNA-encoded chemical libraries

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ABSTRACT

The fast development of DNA-encoded chemical libraries (DECL) in the past 10 years has received great attention from pharmaceutical industries. It applies the selection approach for small molecular drug discovery. Because of the limited choices of DNA-compatible chemical reactions, most DNA-encoded chemical libraries have a narrow structural diversity and low synthetic yield. There is also a poor correlation between the ranking of compounds resulted from analyzing the sequencing data and the affinity measured through biochemical assays. By combining DECL with dynamical chemical library, the resulting DNA-encoded dynamic library (EDCCL) explores the thermodynamic equilibrium of reversible reactions as well as the advantages of DNA encoded compounds for manipulation/detection, thus leads to enhanced signal-to-noise ratio of the selection process and higher library quality. However, 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. 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. To replace the extremely laborious and repetitive manual process, a fully automated device will facilitate the use of DECL in drug discovery.

Herein we describe a novel lab-on-a-chip platform for high throughput drug discovery with hi-EDCCL. A microfluidic system with integrated actuation was designed which is able to provide a continuous sample circulation by reducing the volume to a minimum. It consists of a cooled and a heated chamber for constant circulation. The system is capable to generate stable temperatures above 75 °C in the heated chamber to melt the double strands of the DNA and less than 15 °C in the cooled chamber, to reanneal the reshuffled library. In the binding chamber (the cooled chamber) specific retaining structures are integrated. These hold back beads functionalized with the target protein, while the chamber is continuously flushed with library molecules. Afterwards the whole system can be flushed with buffer to wash out unspecific bound molecules. Finally the protein-loaded beads with attached molecules can be eluted for further investigation.

Keywords: Lab-on-a-chip, Microfluidics, DNA-encoded chemical libraries, Drug discovery, Dynamic combinatorial chemistry

1. INTRODUCTION

The discovery of specific binders for biological targets has very important applications in drug discovery, diagnostics and chemical biology research. For the last decades, high throughput screening (HTS) was the gold standard technology used by pharmaceutical companies to tackle the issue of screening a very large chemical space against a pharmaceutically relevant target. HTS can screen from few thousand to 2 million compounds one-by-one using robotics, automation and activity assays in multi-well format[1–4]. In the last decade, DNA-encoded chemical library (DECL) technology is being used more and more to complement HTS.

DECLs consist of a large collection of small organic compounds each one attached to a unique oligonucleotide working as a barcode able to identify univocally the chemical structure attached to it[5–9]. DECLs are selected through binding assay: the selection experiments (or panning) are usually carried out using an affinity chromatography with the pharmaceutically relevant target attached on a solid support (e.g. sepharose beads). After removing the unbound members of the library, the retained DNA-encoded compounds can be PCR amplified and identified via deep

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sequencing. The technology was first mentioned in 1992 with a theoretical paper from Brenner and Lerner[10–12]. In the last 25 years the technology developed enormously with the momentum from developments in solid-phase and oligonucleotide synthesis[13, 14] PCR and especially high throughput sequencing technology[15, 16].

DECLs have several advantages making it attractive for large pharmaceutical companies, small biotech companies and academic research groups. DECLs do not require a big laboratory infrastructure like robotics, automation and logistics for compound screening as HTS. The need of these investments has made it impossible for some small biotech companies and academics to access HTS technology. DECLs can now be used by them to discover lead compounds and chemical probes. Importantly, DECL selection does not require the development of an activity assay and can explore large libraries of billions of compounds[17–21]. The development of activity assay can require several months and a deep knowledge of the target protein is needed. Moreover, the activity assay requires very often a known binder and a relatively high amount of protein. It can be very interesting for researchers in the early stage of drug discovery to explore the binding feature of a new target with a wide range of chemical compounds. The peculiar feature of DECL makes it very attractive for developing inhibitors against a new target before any activity assay is available. Finally, the amount of DNA-compatible chemicals being discovered can increase the chemical diversity of DECL in the coming years[22–26].

DECLs are classified either on the base of their synthesis method (esp. DNA-recorded synthesis[18–21, 27–35], DNA-templated synthesis[36–40], DNA programmed synthesis[41–44]) or on how many covalently linked building blocks are displayed on each library member (Single[18–21, 30, 31, 34, 35, 39, 43, 45, 46] and dual pharmacophore[47–53]. In the single pharmacophore DECLs, each library member displays one covalently linked molecule generated by the reaction of 2-4 building blocks, while the dual pharmacophore libraries use the ability of DNA to form double-stranded constructs to display two building blocks on each library member. The dual pharmacophore libraries arise from the union of two independent sub libraries. Each member can be separately encoded and purified. Dual pharmacophore libraries, of which the ESACLs (DNA-encoded Self Assembly Chemical Libraries) are the most classical example, allow the generation of a large library with a very high level of purity and represent the fragment-based section of the DECL field[50]. They explore the so called chelate effects where two fragments bind in the target pocket with an affinity 10 to 1000 times higher than the single fragments[54].

One of the difficulties in DECL is the very high number of hit compounds arising from a DECL selection. In principle, the sequence counts generated by the deep sequencing analysis of the library selection represent the affinity of binding. Unfortunately, a lot of artifacts (binding on the solid support matrix, bias in the library member distribution, PCR and sequencing artifacts) make the decoding step of the library selection not straightforward and the exclusion of false positives and a fine affinity ranking must be performed afterwards[55–57].

In addition, most of the dual pharmacophore library selection in the ESAC format was performed to explore the affinity maturation setup[48, 50, 58]. In this setup, a known lead compound is combinatorically paired with all the library members to evaluate an increment in the binding affinity for the target. However, since the known binder has already some affinity for the target, it was necessary to finely tune the selection stringency both in the avidity and density of the target on the solid support and in the number and nature of washing steps[48].

Recently, the DECL field met the Dynamic Combinatorial Chemistry (DCC)[59, 60] and Dynamic Combinatorial Library (DCL)[61] concepts to help generating fewer, but more reliable hits. DCC uses reversible reactions (esp. imine formation, disulphide formation) under thermodynamic conditions to generate new molecules. All the DCL members are in a distribution driven by their thermodynamic stability and the building blocks interchange their pairing continuously. If this equilibrium is perturbed, for example by the presence of a protein in the mixture, this distribution is going to change and the building blocks binding to the protein are stabilized together causing their enrichment. The limiting factor preventing a wider spread of this brilliant technology is the relatively low sensitivity and resolution of the analytical methods used to identify the binders. Indeed, liquid chromatography, mass spectroscopy, X-Ray crystallography and NMR spectrometry are the most used methods applied to analyze the DCL. The inability to perform signal amplification (each members need to be at a detectable concentration causing solubility issues in large library) and to resolve compounds with similar chemo-physical properties have limited the library size of DCLs[62, 63].

On the other hand DECL compounds identification uses a completely different tool box of methods. The signal can be amplified via PCR, thus reducing the amount necessary of each member and the barcoding methods is not based on any chemo-physical feature of the compound. Moreover, DNA assembly can also take place under a thermodynamic equilibrium. The two technologies were joined in the DNA-encoded combinatorial chemical library (EDCCL) technology where two sets of encoded building block coupled oligonucleotides are put in a thermodynamic

equilibrium[64]. Two setups were described depending on the temperature at which the thermodynamic interchange of building blocks was happening. In the standard EDCCL, short hybridizing regions allows the interchange to happen at room temperature, while in the heat-induced EDCCL (hi-EDCCL), the interchange of the pairs is caused by heat going to melt the DNA hybridization motif.

An approach similar to the EDCCL where the DNA conjugated fragments were locked together upon binding via a photo-crosslinking reaction was also publish by Li and co-authors[65].

The EDCCL library can be incubated with the solid support functionalized target protein allowing the best binders to assembly in the binding pockets. The energy of the binding causes their enrichment and slows their interchanges, while the low- and no-affinity members continue to interact and form new pairs. The main advantage of EDCCL is the ability to generate new pairs starting from the same pool of building blocks, in this way the signal-to-noise ratio increases. In a static library, indeed, the occurrence of each library pair member is only one on the total combinatorial different pairs, while in a dynamic library this value increases.

A limitation of EDCCL is the reduction of the total affinity of the paired construct for the target. Indeed, the gain of affinity of the two building blocks for the target must be high enough to compensate for the weak DNA interaction. In a static library, each member exhibits the highest affinity possible since the energy of DNA pairing is maximized being stable at room temperature. The introduction of hi-EDCCL aims to complement EDCCL separating the binding event from the dynamic reshuffling event.

The binding event of the library members is performed at room temperature or at a cooler temperature to guarantee the stability of the protein immobilized on the solid support. A stable hybridization domain ensures the maximal binding potential for each member. The mixture contains high affinity members mainly bound to the protein, and low- and no-affinity members mainly in the supernatant. The supernatant can be physically separated from the protein and heated up to a temperature where the DNA melts and new sets of pairs are formed when the temperature cools down to room temperature. The new pairs arise from a random interchange of the unbound members in the supernatant fraction. The low affinity members have another chance to form the high affinity pair. When this reshuffled supernatant is re-exposed to the protein, the high affinity members will bind to the protein while the other is ready for another cycle of reshuffling. Through several rounds of reshuffling, the high affinity pairs will accumulate on the solid support causing an increase of the signal to noise ratio. Obviously, to perform such tasks of liquid handling, supernatant and solid support compartmentalization and temperature control requires the development of automation.

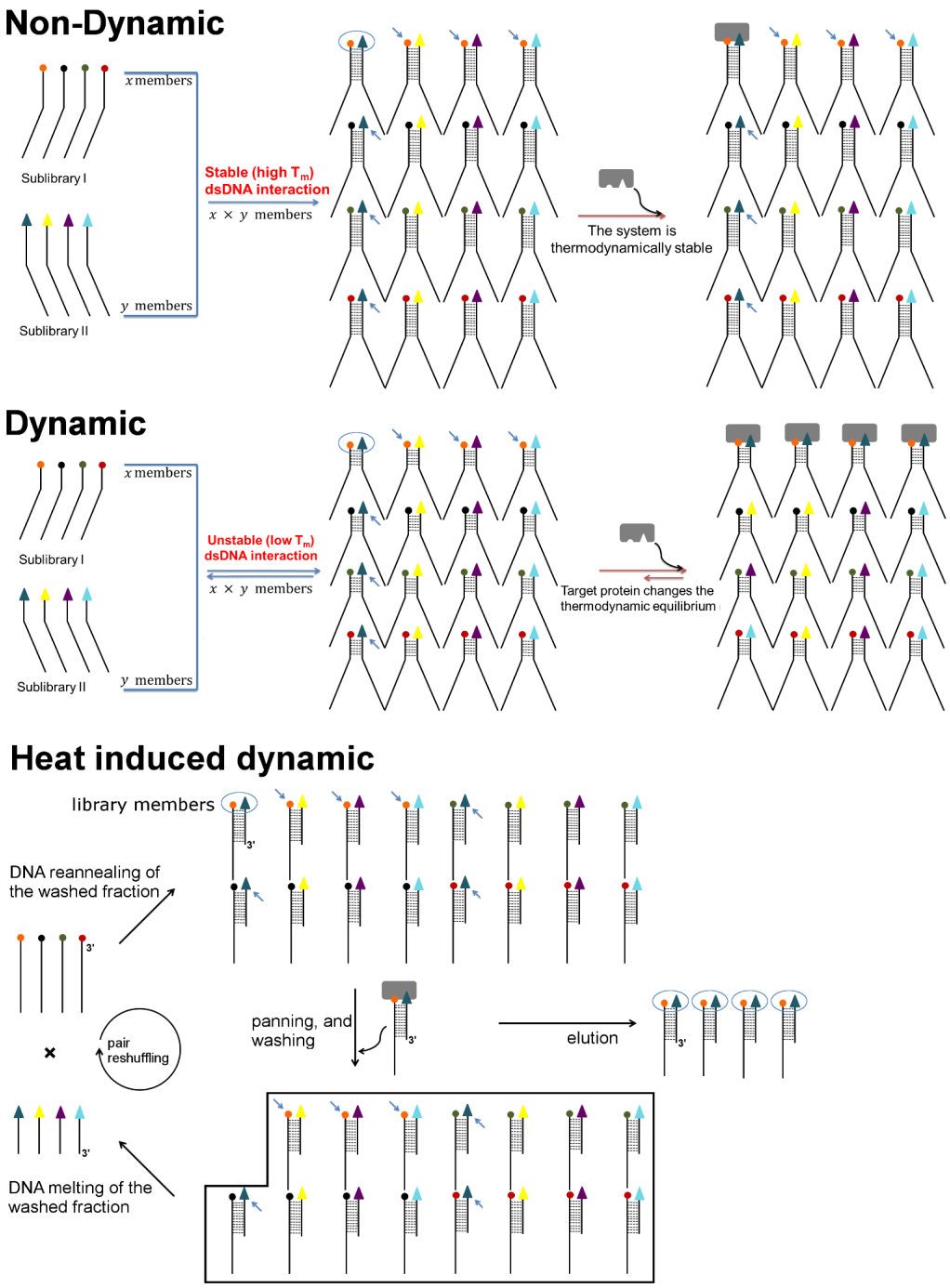

Figure 1. The principles of DNA-encoded dynamic combinatorial chemical library (EDCCL) technology and heat-induced DNA-encoded dynamic combinatorial chemical library (hi-EDCCL) technology. In the EDCCL method, thermodynamic instability causes pair reshuffling. Under selection pressure from a protein 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 from the oligonucleotide stability. When the oligonucleotides in the supernatant are melted, the two sub libraries are reformed, and the building blocks are free to self-assemble again to give rise to the high-affinity binders. Pair reshuffling and resin incubation are space- and time-separated. 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. Circles indicate the "correct pairs" (potent binders) of pharmacophores in the library, whereas arrows indicate pharmacophores "wrongly paired" in the library.

2. MATERIALS AND METHODS

2.1 Lab-on-a-chip platform

Lab-on-a-chip (LOC) technology is already implemented for environmental analysis[66] and medical diagnostics[67], as well as to model organs to replace animal experiments for testing substances in the pharmaceutical and cosmetics industries[68]. During the last years the Fraunhofer IWS developed a multilayer based LOC-platform for perfused assays including micro pumps[69], valves, channels, reservoirs, and customized on-top modules[70]. By stacking structured foils complex three-dimensional LOC can be realized. One of the major advantages of the established LOC-platform is the possibility to integrate active components. On-chip actuation offers the possibility to create micro circulation systems with a minimum of dead volume. Moreover the effect of dilution appearing in flow through LOC with external actuation is negligible. Therefore the developed LOC comprises pneumatically driven displacement pumps consisting of three chambers in a row. The first and the last chamber are designed as seat valves. By sequentially deflecting the silicone membrane above the chambers fluid can be flushed through the system[69]. Initially the pump chamber is actuated while the inlet valve is closed and the outlet valve opened which results in the main pump pulse. Afterwards both valve states are switched which causes a smaller pulse. Then the pump chamber is filled by lifting the membrane with vacuum. Since the outlet valve is closed no fluid movement can be observed in pumping direction. In the last phase both valves are switched again which results in a back-flow because fluid fills the outlet valve chamber.
The LOC is composed of a fluidic and a pneumatic part separated by a flexible silicone membrane (SILPURAN® FILM 2030, Wacker Chemie AG) which is integrated by means of chemical surface treatment with (3-Aminopropyl)triethoxysilane (APTES)[71]. The pneumatic part acts as a linkage between the LOC device and an external pneumatic controller. It accommodates eight air pressure fittings and fluidic ports for liquid exchange. Both parts are built-up by individually micro structured polycarbonate foils with a thickness of 0.25 mm (Makrofol DE 1-1, Bayer MaterialScience AG) which are stacked, precisely aligned and laminated by thermal diffusion bonding in a hot press. The processing of the polycarbonate layers is done by laser-induced material ablation performed on a laser micro structuring system (3D MICROMAC, microSTRUCT vario) with a picoseconds laser (TIME-BANDWIDTH Products, Fuego). Direct structuring of the substrate supersedes the use of time consuming and expensive replication tools are needed. This enables a rapid, flexible and cost efficient adaption of microfluidic designs.

2.2 Microfluidic controller

To operate the LOC a complex and freely programmable controlling device based on an embedded Linux system (BeagleBone Black Mini-PC Rev C) was developed. It provides compressed air with the necessary pressure rates to operate the micro pump and valves on the LOC and switches up to 24 pneumatic outputs. The embedded system provides several interfaces e.g. ETHERNET, USB, CAN, I^2C to couple different actuators, sensors, data storages, networks, etc. This allows the remote control of the device and gives the opportunity to communicate with laboratory automation and information systems. User interaction takes place on a 7” touch screen and the possibility to run the application on external PC´s, tablets or smartphones.

3. RESULTS

3.1 Lab-on-a-chip design

A LOC has to be developed which provides a continuous media circulation through a cooled and a heated reaction chamber. The heated chamber (melting site) has to be tempered to over 75 °C for melting the DNA double strands and the cooled chamber (binding site) to less than 15 °C, for reannealing of the reshuffled library and for avoiding protein denaturing through the affinity-based selection procedure. The binding site includes specific retaining structures to hold back the beads functionalized with target protein modified solid support, while the chamber is permanently flushed with library encoded molecules.

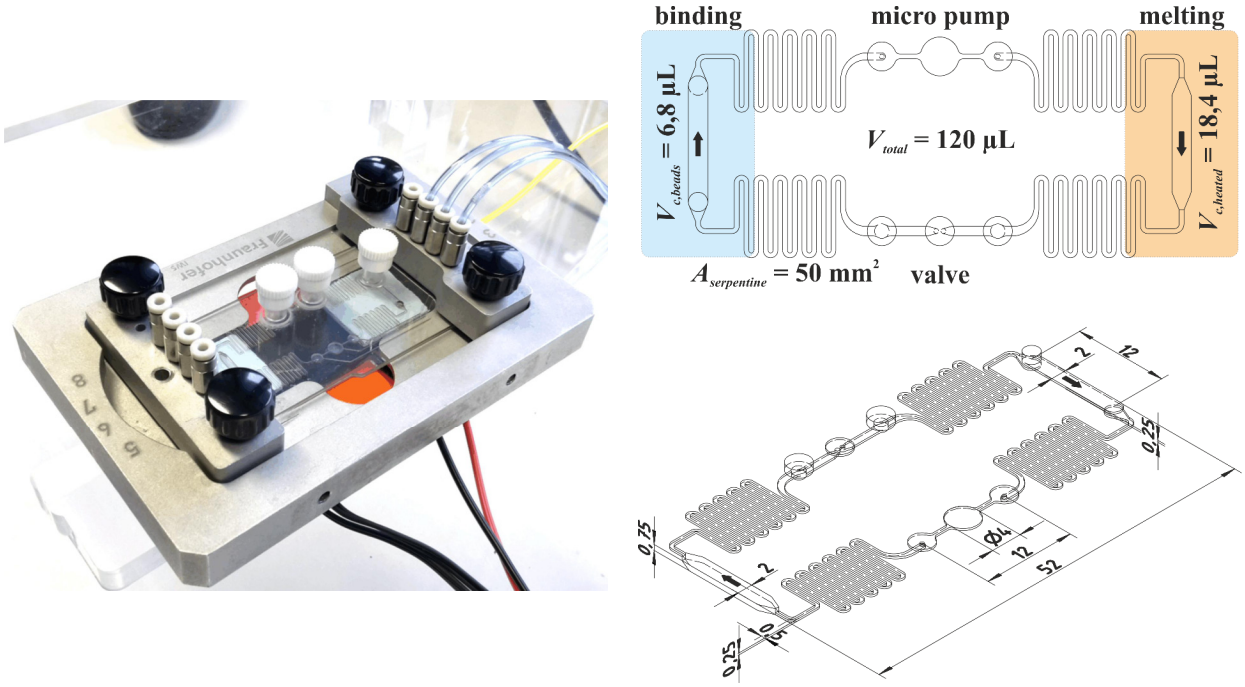

Figure 2. Left: Photograph of the LOC device mounted in a chip holder , Right: scheme of the fluidic design with relevant dimensions and volumes, arrows indicating flow direction

Moreover the dead volume needs to be reduced to prevent dilution of the used libraries. Therefore the microfluidic layout depicted in Figure 2 (Right) was designed which is able to provide a continuous circulation by reducing the volume to a minimum of 120 µL. For fluid movement the described pneumatically driven three chamber peristaltic pump is integrated. The binding of high affinity pairs to the solid support is done in a flow through chamber with a volume of about 6.8 µL. As solid support for the target proteins sepharose beads (Streptavidin-Sepharose Beads, BioVision) with a diameter of 90 µm are used. Approximately 2000 beads fit into the binding chamber so an exposed area of 200 mm^2 is realized. To hold the beads in place and separate them from the melting site retaining structures are integrated at the inlet and outlet of the binding chamber. These retaining structures are realized by 100 laser drilled through holes each in distance of 200 µm with an averaged diameter of 39 ± 6µm. These are large enough for not being blocked but not adding a too high fluidic resistance to system. The respective entirety of drilled holes at the inlet or outlet of the binding chamber has a cross-section of about 0.1 mm^2 which is comparable to the size of the valve seats typically used in these devices. To allow an adequate annealing of the shuffled library members, gradual cooling of the supernatant is needed. Therefore the surface facing ambiance is increased to 100 mm^2 by a serpentine shaped channel allowing heat dissipation. For a sufficient bubble removal the channel level is increasing in flow direction until the fluid outlet, which also acts as additional bubble trap is reached.

Owing to the fact that minimal volume fluidic circuits with integrated displacement pumps need pressure-compensating volumes, an elastic membrane on top of the heated chamber is integrated featuring a displacement of 12.5 µL.

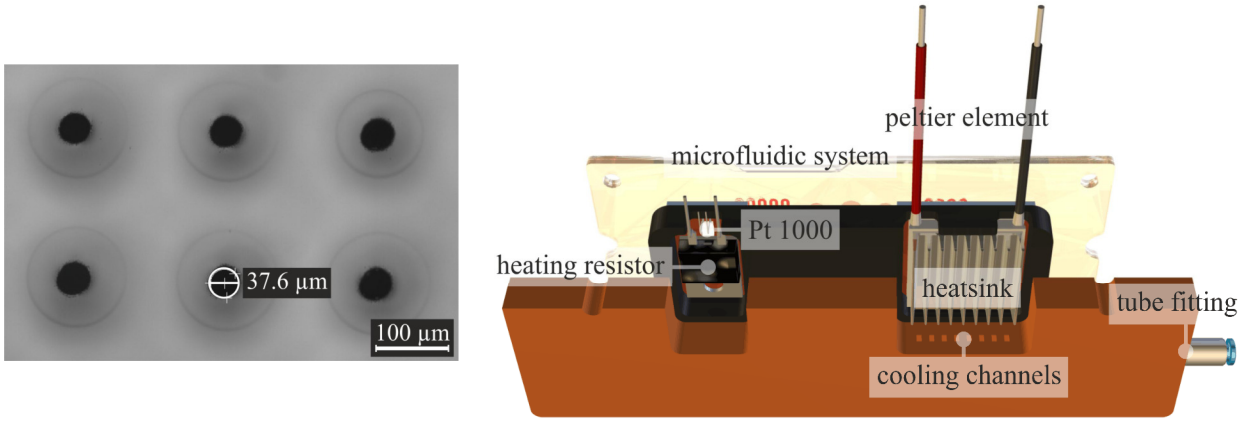

Figure 3. Left: laser drilled holes for retaining of the modified sepharose beads, Right: module for thermal control

3.2 Lab-on-a-chip periphery

For the thermal control of the process a removable module for heating and cooling mounted on the chip holder was developed. It consists of two 3D printed parts manufactured by fused deposition modeling of acrylonitrile butadiene styrene (ABS) with a renkforce RF1000 (Conrad Electronic SE). The first part incorporates two copper plates for heat distribution one connected to a PT-1000 temperature sensor and a heating resistor and one including a Peltier element as well as a second PT-1000 for cooling. The second part can be mounted on the chip holder slightly pressing the first part to the microfluidic system to create a good thermal connection. Moreover pneumatic channels for active cooling of the heat sink mounted on the Peltier element are implemented to enhance the heat transport of the element. The thermal resistance is further decreased with gap filling thermal pads (ARCTIC GmbH) between the copper plates and the microfluidic system. For the herein presented system the temperature control module of the microfluidic controller was refined to generate and regulate stable temperatures with a PID controller

3.3 Lab-on-a-chip validation

To validate the hi-EDCCL technology in the LOC, a model system was chosen. In Figure 4 a cross-sectional view of the LOC is shown to clarify the experimental setup. The design of the library is shown in figure 5. The Y-shaped construct allows joining the members of two sub-libraries after the selection process via T4 DNA ligase, when the unbound members are removed. Each library member has a specific primer region allowing its unique identification via qPCR. In particular, each pair can be identified and quantified using a specific couple of primers.

The proof-of-principle of the hi-EDCCL approach to identify bidentate protein/ligand interaction in the LOC was carried out with a model system of iminobiotin and homotetrameric streptavidin (Figure 5dA DNA duplex with two iminobiotins binding to streptavidin has previously been studied to illustrate the bidentate interaction mediated by DNA self-assembling[54]. Given that low to high µM affinities are expected for most de novo selection experiments using DECLs, the iminobiotin/streptavidin interaction provides a very nice proof-of-principle model to evaluate hi-EDCCL.

As first experiment, a library size of around 1,000 pair member was simulated. Therefore one equivalent of iminobiotin (10 nM) 5’ modified oligonucleotide was mixed with 32 fold excess of unmodified oligonucleotide to generate sub library 5L (Figure 5a). Then, similarly, sub library 3L was generated mixing one equivalent of 3’ iminobiotin modified oligonucleotide with 32 fold excess of unmodified oligonucleotides (Figure 5b).

Sub libraries 5L and 3L have a complementary hybridization domain causing the assembly of the library in the y-shaped structure depicted in Figure 5c. The model library size of 1.089 members was given by the combinatorial assembly of all the sub library members. The pairing of the two iminobiotin modified sub library members represent the high affinity members of the library, while the single iminobiotin constructs represent the low affinity members and the unmodified constructs represent the excess of no affinity binders in the library.

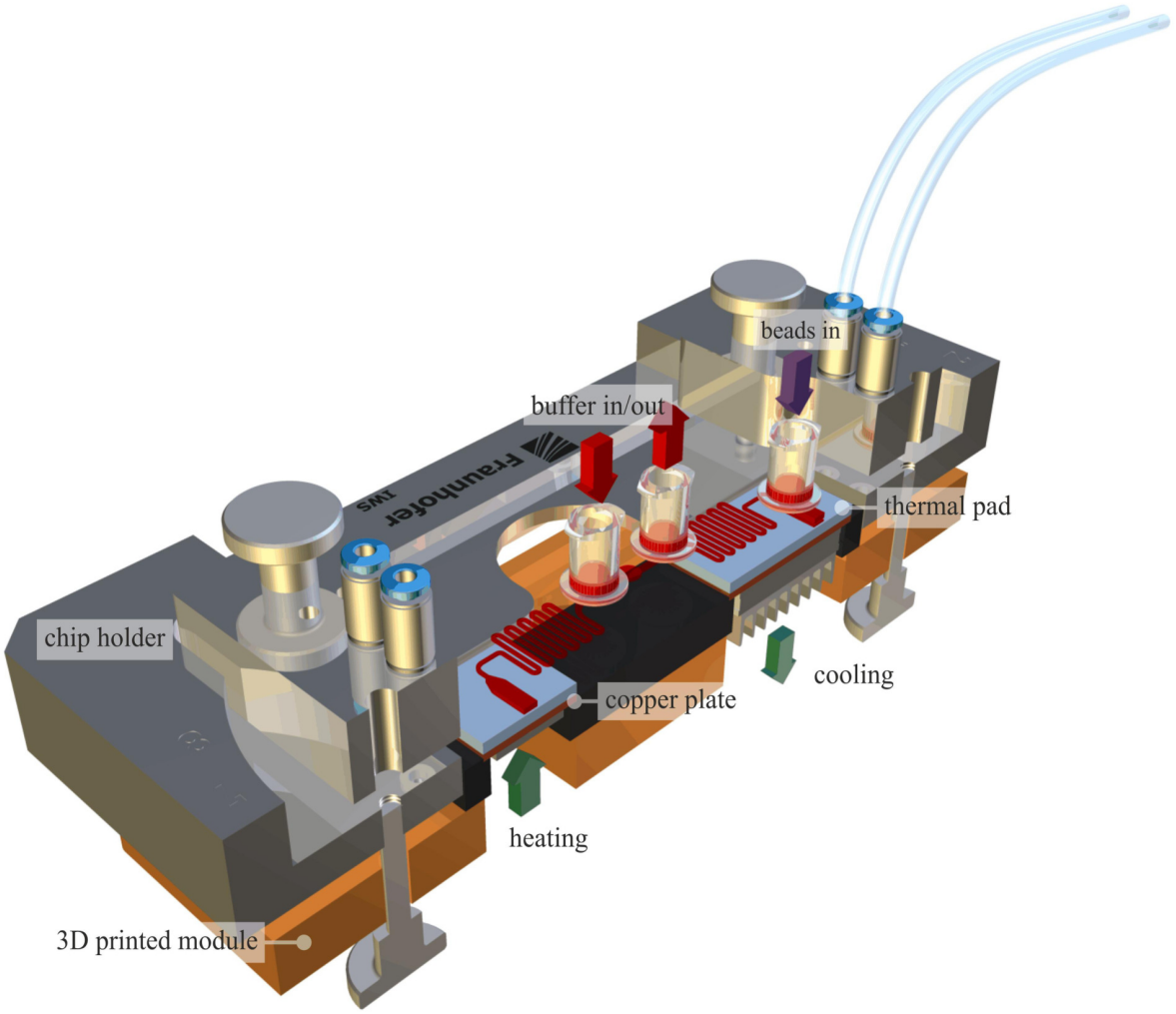

Figure 4. Cross-sectional view of the assembled LOC (for a better depiction only the fluid is shown)

Prior to the experiment the LOC was filled with binding buffer via the fluidic Luer-ports by closing the valve between inlet and outlet (Figure 4, red arrows) and pumping the buffer through the system. The binding buffer used was 25 mM $NaHCO_3$, 150 mM NaCl, 0.01% Tween20, pH9.2. The streptavidin coated sepharose beads were loaded together with the library on the device by pipetting them in a Luer-port on top of the binding site (Figure 4, purple arrow). To flush the beads and the library from the Luer-port into the device the micro pump was switched on. The beads were hosted in the binding chamber which was kept at room temperature while the pump transported the unbound part, composed by low and no affinity binders, from the binding site to the reshuffling chamber where a temperature of 70 °C was applied. Afterwards, the reshuffled library was pumped back to the binding site where the newly recreated high affinity pairs are going to bind to the streptavidin. The system was kept pumping for 345 min. With a flowrate of 0.7 µL/s determined in a reference system without beads this will result in more than 100 cycles. After that the beads were removed from the device, washed two times with 1 mL binding buffer and 100 µL of ligation mixture was added (1 mM ATP, NEB 2 buffer, T4 DNA ligase 0.4 U) and incubated over night at 16 °C. Finally the bound part was eluted adding 0.2 mg biotin in DMSO (1 µL) and heated at 95 °C for 10 min. The eluted part was analyzed via qPCR. As control, the same experiment was performed in an unheated device and the sample was processed in the same way.
The results of the qPCR analysis showed an enrichment of the high affinity binders of 2.35 folds in the heated device compared to the unheated one. The rather low enrichment may be caused by a smaller number of performed cycles than assumed. Perhaps this is due to blocked channels contrary to the assumption that the drilled through holes are big enough. Another possibility is the accumulation of beads and/or library molecules in dead volumes like the fluidic connections.

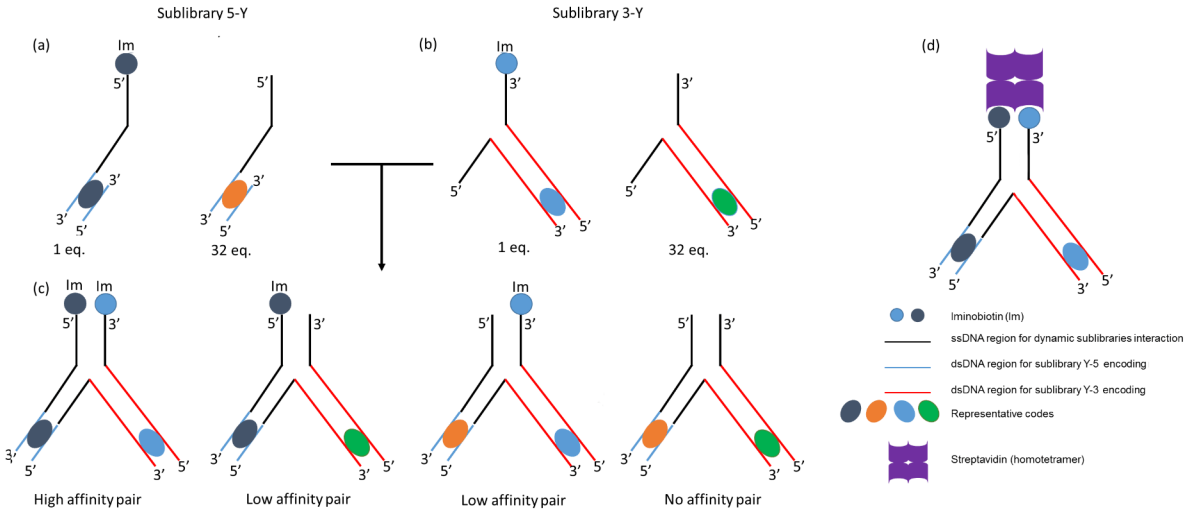

Figure 5. Model library preparation and selection. The sub library 5-Y (a) was generated mixing 1 equivalents (10 nM) of the preannealed annealed 5'-C12-Iminobiotin modified DNA with 32 equivalents (320 nM) of the unlabeled preannealed competitor, similarly the sub library 3-Y was prepared mixing 1 equivalents (10 nM) of the preanneled annealed 3'-C7-Iminobiotin modified DNA with 32 equivalents (320 nM) of the unlabeled preannealed competitor. Each DNA constructs carries a specific and unique sequence (representative codes) allowing its identification and quantification with quantitative PCR. The combinatorial assembly of all the sub library members generates a library of 1089 (33 x 33) paired members (c). The high affinity members represents the lowest population, while the no affinity pairs represent the excess of unbinding members expected in a DECL. The high affinity members bind the protein streptavidin (d). Streptavidin has four binding pockets for iminobiotin and it is very nicely suited for exploring multivalent binding and the chelate effect.

4. OUTLOOK AND CONCLUSION

With the herein presented LOC device we could show the possibility to perform drug screening with hi-EDCCL in general. Although the device was capable to perform the intended liquid handling tasks there is potential for improvement of the overall efficiency. To gain more information about the performance of the device repeated measurements are needed. To exclude the risk of blockage other retaining mechanisms may be examined. More experiments could be performed in less time when the loading of beads and library molecules is automated by directly coupling the device to a laboratory automation platform. Moreover the flushing and mixing of the beads could be improved. The improvement of the device features would improve the fold of enrichment compared with the non-dynamic libraries. Moreover, the use of larger and diverse libraries against pharmaceutically relevant target will expand the application of hi-EDCC libraries.

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5. REFERENCES

[1] Macarron, R., Banks, M. N., Bojanic, D., Burns, D. J., Cirovic, D. A., Garyantes, T., Green, D. V. S., Hertzberg, R. P., Janzen, W. P., Paslay, J. W., Schopfer, U. and Sittampalam, G. S., "Impact of high-throughput screening in biomedical research,'' Nat Rev Drug Discov 10, 188–195 (2011).

[2] Inglese, J., Johnson, R. L., Simeonov, A., Xia, M., Zheng, W., Austin, C. P. and Auld, D. S., "High-throughput screening assays for the identification of chemical probes,'' Nat Chem Biol 3, 466–479 (2007).

[3] Lee, H., Zhu, T., Patel, K., Zhang, Y. Y., Truong, L., Hevener, K. E., Gatuz, J. L., Subramanya, G., Jeong, H. Y., Uprichard, S. L. and Johnson, M. E., "High-throughput screening (HTS) and hit validation to identify small

molecule inhibitors with activity against NS3/4A proteases from multiple hepatitis C virus genotypes,'' Plos One 8 (2013).
[4] Bleicher, K. H., Bohm, H.-J., Muller, K. and Alanine, A. I., "Hit and lead generation: beyond high-throughput screening,'' Nat Rev Drug Discov 2, 369–378 (2003).
[5] Franzini, R. M. and Randolph, C., "Chemical Space of DNA-Encoded Libraries,'' J Med Chem 59, 6629–6644 (2016).
[6] Yuen, L. H. and Franzini, R., "Achievements, Challenges, and Opportunities in DNA-Encoded Library Research: An Academic Point-of-View,'' Chembiochem (2016).
[7] Zimmermann, G. and Neri, D., "DNA-encoded chemical libraries: foundations and applications in lead discovery,'' Drug Discov Today 21, 1828–1834 (2016).
[8] Shi, B., Zhou, Y., Huang, Y., Zhang, J. and Li, X., "Recent advances on the encoding and selection methods of DNA-encoded chemical library,'' Bioorganic & medicinal chemistry letters (2016).
[9] Kleiner, R. E., Dumelin, C. E. and Liu, D. R., "Small-molecule discovery from DNA-encoded chemical libraries,'' Chem Soc Rev 40, 5707–5717 (2011).
[10] Nielsen, J., Brenner, S. and Janda, K. D., "Towards Implementation of Encoded Combinatorial Chemistry,'' Abstr Pap Am Chem S 207, 484-ORGN (1994).
[11] Needels, M. C., Jones, D. G., Tate, E. H., Heinkel, G. L., Kochersperger, L. M., Dower, W. J., Barrett, R. W. and Gallop, M. A., "Generation and Screening of an Oligonucleotide-Encoded Synthetic Peptide Library,'' Proceedings of the National Academy of Sciences of the United States of America 90, 10700–10704 (1993).
[12] Brenner, S. and Lerner, R. A., "Encoded combinatorial chemistry,'' Proc Natl Acad Sci U S A 89, 5381–5383 (1992).
[13] Venkatesan, N., Kim, S. J. and Kim, B. H., "Novel phosphoramidite building blocks in synthesis and applications toward modified oligonucleotides,'' Current medicinal chemistry 10, 1973–1991 (2003).
[14] Caruthers, M. H., Beaucage, S. L., Becker, C., Efcavitch, J. W., Fisher, E. F., Galluppi, G., Goldman, R., deHaseth, P., Matteucci, M., McBride, L. and et al, "Deoxyoligonucleotide synthesis via the phosphoramidite method,'' Gene amplification and analysis 3, 1–26 (1983).
[15] Mannocci, L., Zhang, Y. X., Scheuermann, J., Leimbacher, M., Bellis, G. de, Rizzi, E., Dumelin, C., Melkko, S. and Neri, D., "High-throughput sequencing allows the identification of binding molecules isolated from DNA-encoded chemical libraries,'' Proceedings of the National Academy of Sciences of the United States of America 105, 17670–17675 (2008).
[16] Ambardar, S., Gupta, R., Trakroo, D., Lal, R. and Vakhlu, J., "High Throughput Sequencing: An Overview of Sequencing Chemistry,'' Indian journal of microbiology 56, 394–404 (2016).
[17] Mullard, A., "DNA tags help the hunt for drugs,'' Nature 530, 367–369 (2016).
[18] Soutter, H. H., Centrella, P., Clark, M. A., Cuozzo, J. W., Dumelin, C. E., Guie, M. A., Habeshian, S., Keefe, A. D., Kennedy, K. M., Sigel, E. A., Troast, D. M., Zhang, Y., Ferguson, A. D., Davies, G., Stead, E. R., Breed, J., Madhavapeddi, P. and Read, J. A., "Discovery of cofactor-specific, bactericidal Mycobacterium tuberculosis InhA inhibitors using DNA-encoded library technology,'' Proceedings of the National Academy of Sciences of the United States of America 113, E7880-e7889 (2016).
[19] Cuozzo, J. W., Centrella, P. A., Gikunju, D., Habeshian, S., Hupp, C. D., Keefe, A. D., Sigel, E. A., Soutter, H. H., Thomson, H. A., Zhang, Y. and Clark, M. A., "Discovery of a potent BTK inhibitor with a novel binding mode using parallel selections with a DNA-encoded chemical library,'' Chembiochem (2017).
[20] Deng, H. F., Zhou, J. Y., Sundersingh, F., Messer, J. A., Somers, D. O., Ajakane, M., Arico-Muendel, C. C., Beljean, A., Belyanskaya, S. L., Bingham, R., Blazensky, E., Boullay, A. B., Boursier, E., Chai, J., Carter, P., Chung, C. W., Daugan, A., Ding, Y., Herry, K., Hobbs, C., Humphries, E., Kollmann, C., Nguyen, V. L., Nicodeme, E., Smith, S. E., Dodic, N. and Ancellin, N., "Discovery and Optimization of Potent, Selective, and in Vivo Efficacious 2-Aryl Benzimidazole BCATm Inhibitors,'' ACS medicinal chemistry letters 7, 379–384 (2016).
[21] Harris, P. A., King, B. W., Bandyopadhyay, D., Berger, S. B., Campobasso, N., Capriotti, C. A., Cox, J. A., Dare, L., Dong, X., Finger, J. N., Grady, L. C., Hoffman, S. J., Jeong, J. U., Kang, J., Kasparcova, V., Lakdawala, A. S., Lehr, R., McNulty, D. E., Nagilla, R., Ouellette, M. T., Pao, C. S., Rendina, A. R., Schaeffer, M. C., Summerfield, J. D., Swift, B. A., Totoritis, R. D., Ward, P., Zhang, A., Zhang, D., Marquis, R. W., Bertin, J. and Gough, P. J., "DNA-Encoded Library Screening Identifies Benzo[b][1,4]oxazepin-4-ones as Highly Potent and Monoselective Receptor Interacting Protein 1 Kinase Inhibitors,'' J Med Chem 59, 2163–2178 (2016).

[22] Satz, A. L., Cai, J., Chen, Y., Goodnow, R., Gruber, F., Kowalczyk, A., Petersen, A., Naderi-Oboodi, G., Orzechowski, L. and Strebel, Q., "DNA Compatible Multistep Synthesis and Applications to DNA Encoded Libraries,'' Bioconjugate chemistry 26, 1623–1632 (2015).
[23] Malone, M. L. and Paegel, B. M., "What is a "DNA-Compatible" Reaction?,'' Acs Comb Sci 18, 182–187 (2016).
[24] Skopic, M. K., Bugain, O., Jung, K., Onstein, S., Brandherm, S., Kalliokoski, T. and Brunschweiger, A., "Design and synthesis of DNA-encoded libraries based on a benzodiazepine and a pyrazolopyrimidine scaffold,'' Medchemcomm 7, 1957–1965 (2016).
[25] Ding, Y., Franklin, G. J., Delorey, J. L., Centrella, P. A., Mataruse, S., Clark, M. A., Skinner, S. R. and Belyanskaya, S., "Design and Synthesis of Biaryl DNA-Encoded Libraries,'' Acs Comb Sci 18, 625–629 (2016).
[26] Ding, Y. and Clark, M. A., "Robust Suzuki–Miyaura Cross-Coupling on DNA-Linked Substrates,'' Acs Comb Sci 17, 1–4 (2015).
[27] Buller, F., Zhang, Y. X., Scheuermann, J., Schafer, J., Buhlmann, P. and Neri, D., "Discovery of TNF Inhibitors from a DNA-Encoded Chemical Library based on Diels-Alder Cycloaddition,'' Chemistry & biology 16, 1075–1086 (2009).
[28] Mannocci, L., Melkko, S., Buller, F., Molnar, I., Bianke, J. P. G., Dumelin, C. E., Scheuermann, J. and Neri, D., "Isolation of Potent and Specific Trypsin Inhibitors from a DNA-Encoded Chemical Library,'' Bioconjugate chemistry 21, 1836–1841 (2010).
[29] Buller, F., Steiner, M., Frey, K., Mircsof, D., Scheuermann, J., Kalisch, M., Buhlmann, P., Supuran, C. T. and Neri, D., "Selection of Carbonic Anhydrase IX Inhibitors from One Million DNA-Encoded Compounds,'' Acs Chem Biol 6, 336–344 (2011).
[30] Leimbacher, M., Zhang, Y., Mannocci, L., Stravs, M., Geppert, T., Scheuermann, J., Schneider, G. and Neri, D., "Discovery of small-molecule interleukin-2 inhibitors from a DNA-encoded chemical library,'' Chemistry 18, 7729–7737 (2012).
[31] Samain, F., Ekblad, T., Mikutis, G., Zhong, N., Zimmermann, M., Nauer, A., Bajic, D., Decurtins, W., Scheuermann, J., Brown, P. J., Hall, J., Graslund, S., Schuler, H., Neri, D. and Franzini, R. M., "Tankyrase 1 Inhibitors with Drug-like Properties Identified by Screening a DNA-Encoded Chemical Library,'' J Med Chem 58, 5143–5149 (2015).
[32] Concha, N., Huang, J., Bai, X., Benowitz, A., Brady, P., Grady, L. C., Kryn, L. H., Holmes, D., Ingraham, K., Jin, Q., Pothier Kaushansky, L., McCloskey, L., Messer, J. A., O'Keefe, H., Patel, A., Satz, A. L., Sinnamon, R. H., Schneck, J., Skinner, S. R., Summerfield, J., Taylor, A., Taylor, J. D., Evindar, G. and Stavenger, R. A., "Discovery and Characterization of a Class of Pyrazole Inhibitors of Bacterial Undecaprenyl Pyrophosphate Synthase,'' J Med Chem 59, 7299–7304 (2016).
[33] Clark, M. A., Acharya, R. A., Arico-Muendel, C. C., Belyanskaya, S. L., Benjamin, D. R., Carlson, N. R., Centrella, P. A., Chiu, C. H., Creaser, S. P., Cuozzo, J. W., Davie, C. P., Ding, Y., Franklin, G. J., Franzen, K. D., Gefter, M. L., Hale, S. P., Hansen, N. J. V., Israel, D. I., Jiang, J. W., Kavarana, M. J., Kelley, M. S., Kollmann, C. S., Li, F., Lind, K., Mataruse, S., Medeiros, P. F., Messer, J. A., Myers, P., O'Keefe, H., Oliff, M. C., Rise, C. E., Satz, A. L., Skinner, S. R., Svendsen, J. L., Tang, L. J., van Vloten, K., Wagner, R. W., Yao, G., Zhao, B. G. and Morgan, B. A., "Design, synthesis and selection of DNA-encoded small-molecule libraries,'' Nature chemical biology 5, 647–654 (2009).
[34] Ding, Y., O'Keefe, H., Delorey, J. L., Israel, D. I., Messer, J. A., Chiu, C. H., Skinner, S. R., Matico, R. E., Murray-Thompson, M. F., Li, F., Clark, M. A., Cuozzo, J. W., Arico-Muendel, C. and Morgan, B. A., "Discovery of Potent and Selective Inhibitors for ADAMTS-4 through DNA-Encoded Library Technology (ELT),'' ACS Med Chem Lett 6, 888–893 (2015).
[35] Yang, H., Medeiros, P. F., Raha, K., Elkins, P., Lind, K. E., Lehr, R., Adams, N. D., Burgess, J. L., Schmidt, S. J., Knight, S. D., Auger, K. R., Schaber, M. D., Franklin, G. J., Ding, Y., DeLorey, J. L., Centrella, P. A., Mataruse, S., Skinner, S. R., Clark, M. A., Cuozzo, J. W. and Evindar, G., "Discovery of a Potent Class of PI3Kalpha Inhibitors with Unique Binding Mode via Encoded Library Technology (ELT),'' ACS medicinal chemistry letters 6, 531–536 (2015).
[36] Hansen, M. H., Blakskjaer, P., Petersen, L. K., Hansen, T. H., Hojfeldt, J. W., Gothelf, K. V. and Hansen, N. J. V., "A Yoctoliter-Scale DNA Reactor for Small-Molecule Evolution,'' Journal of the American Chemical Society 131, 1322–1327 (2009).
[37] Gartner, Z. J. and Liu, D. R., "The generality of DNA-templated synthesis as a basis for evolving non-natural small molecules,'' Journal of the American Chemical Society 123, 6961–6963 (2001).

[38] Gartner, Z. J., Kanan, M. W. and Liu, D. R., "Multistep small-molecule synthesis programmed by DNA templates,'' Journal of the American Chemical Society 124, 10304–10306 (2002).
[39] Gartner, Z. J., Tse, B. N., Grubina, R., Doyon, J. B., Snyder, T. M. and Liu, D. R., "DNA-templated organic synthesis and selection of a library of macrocycles,'' Science 305, 1601–1605 (2004).
[40] Seigal, B. A., Connors, W. H., Fraley, A., Borzilleri, R. M., Carter, P. H., Emanuel, S. L., Fargnoli, J., Kim, K., Lei, M., Naglich, J. G., Pokross, M. E., Posy, S. L., Shen, H., Surti, N., Talbott, R., Zhang, Y. and Terrett, N. K., "The discovery of macrocyclic XIAP antagonists from a DNA-programmed chemistry library, and their optimization to give lead compounds with in vivo antitumor activity,'' J Med Chem 58, 2855–2861 (2015).
[41] Halpin, D. R. and Harbury, P. B., "DNA display I. Sequence-encoded routing of DNA populations,'' Plos Biol 2, 1015–1021 (2004).
[42] Halpin, D. R. and Harbury, P. B., "DNA display II. Genetic manipulation of combinatorial chemistry libraries for small-molecule evolution,'' Plos Biol 2, 1022–1030 (2004).
[43] Halpin, D. R., Lee, J. A., Wrenn, S. J. and Harbury, P. B., "DNA display III. Solid-phase organic synthesis on unprotected DNA,'' Plos Biol 2, 1031–1038 (2004).
[44] Weisinger, R. M., Marinelli, R. J., Wrenn, S. J. and Harbury, P. B., "Mesofluidic Devices for DNA-Programmed Combinatorial Chemistry,'' Plos One 7 (2012).
[45] Buller, F., Mannocci, L., Zhang, Y. X., Dumelin, C. E., Scheuermann, J. and Neri, D., "Design and synthesis of a novel DNA-encoded chemical library using Diels-Alder cycloadditions,'' Bioorganic & medicinal chemistry letters 18, 5926–5931 (2008).
[46] Deng, H., Zhou, J., Sundersingh, F. S., Summerfield, J., Somers, D., Messer, J. A., Satz, A. L., Ancellin, N., Arico-Muendel, C. C., Sargent Bedard, K. L., Beljean, A., Belyanskaya, S. L., Bingham, R., Smith, S. E., Boursier, E., Carter, P., Centrella, P. A., Clark, M. A., Chung, C. W., Davie, C. P., Delorey, J. L., Ding, Y., Franklin, G. J., Grady, L. C., Herry, K., Hobbs, C., Kollmann, C. S., Morgan, B. A., Pothier Kaushansky, L. J. and Zhou, Q., "Discovery, SAR, and X-ray Binding Mode Study of BCATm Inhibitors from a Novel DNA-Encoded Library,'' ACS Med Chem Lett 6, 919–924 (2015).
[47] Melkko, S., Scheuermann, J., Dumelin, C. E. and Neri, D., "Encoded self-assembling chemical libraries,'' Nat Biotech 22, 568–574 (2004).
[48] Melkko, S., Zhang, Y., Dumelin, C. E., Scheuermann, J. and Neri, D., "Isolation of high-affinity trypsin inhibitors from a DNA-encoded chemical library,'' Angew Chem Int Edit 46, 4671–4674 (2007).
[49] Scheuermann, J., Dumelin, C. E., Melkko, S., Zhang, Y. X., Mannocci, L., Jaggi, M., Sobek, J. and Neri, D., "DNA-encoded chemical libraries for the discovery of MMP-3 inhibitors,'' Bioconjugate chemistry 19, 778–785 (2008).
[50] Wichert, M., Krall, N., Decurtins, W., Franzini, R. M., Pretto, F., Schneider, P., Neri, D. and Scheuermann, J., "Dual-display of small molecules enables the discovery of ligand pairs and facilitates affinity maturation,'' Nature chemistry 7, 241–249 (2015).
[51] Novoa, A., Machida, T., Barluenga, S., Imberty, A. and Winssinger, N., "PNA-Encoded Synthesis (PES) of a 10 000-Member Hetero-Glycoconjugate Library and Microarray Analysis of Diverse Lectins,'' Chembiochem 15, 2058–2065 (2014).
[52] Daguer, J. P., Zambaldo, C., Ciobanu, M., Morieux, P., Barluenga, S. and Winssinger, N., "DNA display of fragment pairs as a tool for the discovery of novel biologically active small molecules,'' Chem Sci 6, 739–744 (2015).
[53] Novoa, A. and Winssinger, N., "DNA display of glycoconjugates to emulate oligomeric interactions of glycans,'' Beilstein journal of organic chemistry 11, 707–719 (2015).
[54] Melkko, S., Dumelin, C. E., Scheuermann, J. and Neri, D., "On the magnitude of the chelate effect for the recognition of proteins by pharmacophores scaffolded by self-assembling oligonucleotides,'' Chem Biol 13, 225–231 (2006).
[55] Lin, W., Reddavide, F. V., Uzunova, V., Gur, F. N. and Zhang, Y., "Characterization of DNA-conjugated Compounds Using Regenerable Chip,'' Analytical chemistry (2014).
[56] Zimmermann, G., Li, Y., Rieder, U., Mattarella, M., Neri, D. and Scheuermann, J., "Hit validation methodologies for ligands isolated from DNA-encoded chemical libraries,'' Chembiochem (2017).
[57] Satz, A. L., "Simulated Screens of DNA Encoded Libraries: The Potential Influence of Chemical Synthesis Fidelity on Interpretation of Structure-Activity Relationships,'' Acs Comb Sci 18, 415–424 (2016).
[58] Melkko, S., Sobek, J., Guarda, G., Scheuermann, J., Dumelin, C. E. and Neri, D., "Encoded self-assembling chemical libraries,'' Chimia 59, 798–802 (2005).

[59] Otto, S., Furlan, R. L. E. and Sanders, J. K. M., “Dynamic combinatorial chemistry,‘’ Drug Discov Today 7, 117–125 (2002).
[60] Herrmann, A., “Dynamic combinatorial/covalent chemistry: a tool to read, generate and modulate the bioactivity of compounds and compound mixtures,‘’ Chem Soc Rev 43, 1899–1933 (2014).
[61] Ramström, O. and Lehn, J.-M., “Drug discovery by dynamic combinatorial libraries,‘’ Nat Rev Drug Discov 1, 26–36 (2002).
[62] Huang, R. J. and Leung, I. K. H., “Protein-Directed Dynamic Combinatorial Chemistry: A Guide to Protein Ligand and Inhibitor Discovery,‘’ Molecules 21 (2016).
[63] Mondal, M. and Hirsch, A. K., “Dynamic combinatorial chemistry: a tool to facilitate the identification of inhibitors for protein targets,‘’ Chem Soc Rev 44, 2455–2488 (2015).
[64] Reddavide, F. V., Lin, W., Lehnert, S. and Zhang, Y., “DNA-Encoded Dynamic Combinatorial Chemical Libraries,‘’ Angew Chem Int Ed Engl 54, 7924–7928 (2015).
[65] Li, G., Zheng, W., Chen, Z., Zhou, Y., Liu, Y., Yang, J., Huang, Y. and Li, X., “Design, preparation, and selection of DNA-encoded dynamic libraries,‘’ Chem Sci 6, 7097–7104 (2015).
[66] Stanley, C. E., Grossmann, G., i Solvas, X. C. and deMello, A. J., “Soil-on-a-Chip: microfluidic platforms for environmental organismal studies,‘’ Lab on a chip 16(2), 228–241 (2016).
[67] Cui, F., Rhee, M., Singh, A. and Tripathi, A., “Microfluidic Sample Preparation for Medical Diagnostics,‘’ Annual review of biomedical engineering 17, 267–286 (2015).
[68] Bhatia, S. N. and Ingber, D. E., “Microfluidic organs-on-chips,‘’ Nature biotechnology 32(8), 760–772 (2014).
[69] Busek, M., Nötzel, M., Polk, C. and Sonntag, F., “Characterization and simulation of peristaltic micropumps,‘’ J. Sens. Sens. Syst. 2(2), 165–169 (2013).
[70] Sonntag, F., Grünzner, S., Schmieder, F., Busek, M., Klotzbach, U. and Franke, V., “Multilayer based lab-on-a-chip-systems for substance testing,‘’ SPIE Proceedings, 93510C (2015).
[71] Lee, K. S. and Ram, R. J., “Plastic-PDMS bonding for high pressure hydrolytically stable active microfluidics,‘’ Lab on a chip 9(11), 1618–1624 (2009).