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Resin-Bound Dynamic Combinatorial Chemistry

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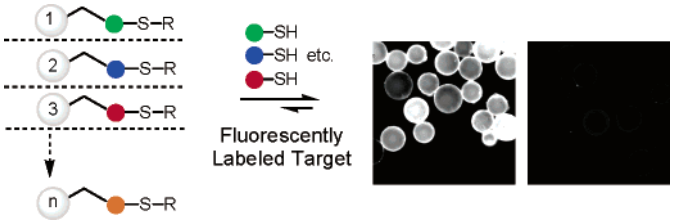
ABSTRACT



Dynamic combinatorial chemistry (DCC) is a promising technique for receptor-aided selection of high-affinity ligands from equilibrating combinatorial libraries. Identification of the specific ligand(s) selected is often challenging, however, due to difficulties associated with chromatographic separation and/or mass degeneracy within the library. Herein, we describe proof-of-concept experiments demonstrating a new technique termed resin-bound DCC (RB-DCC), which provides a solution to this problem.

Dynamic combinatorial chemistry (DCC) makes use of reversible bond-forming reactions to create thermodynamically controlled dynamic combinatorial libraries (DCLs).[1] Upon addition of a target, the mole fractions of individual library members are perturbed as a function of their affinity for that target, following the dictates of LeChatelier's principle. If the experimental system is designed effectively, this provides an efficient selection and enhancement of the highest-affinity binder.[2] Over the past decade, DCC has emerged as a promising technique for the in situ generation and screening of compounds with a broad range of desirable properties.[3] However, the size of DCLs employed has been somewhat limited thus far due to analytical challenges inherent in the methodology. For example, DCLs are frequently analyzed by HPLC or HPLC−MS. Since this requires a difference in mass for all library members, as well as (ideally) the ability to separate individual library members on a chromatography column, this task increases in difficulty as a function of library size.

Phase separation has been a useful tool in many DCC experiments. Frequently, resin-immobilized targets have been employed,[4] and extraction of DCC products into a different solution[5] or gel phase[6] as part of the selection process has also been reported. However, phase-tagging the library *components* by immobilization on a solid phase has not previously been described. In some respects inspired by Pirrung's concept of the indexed combinatorial library,[7] and various authors' development of "libraries from libraries",[8] this resin-bound DCC (RB-DCC) simplifies identification

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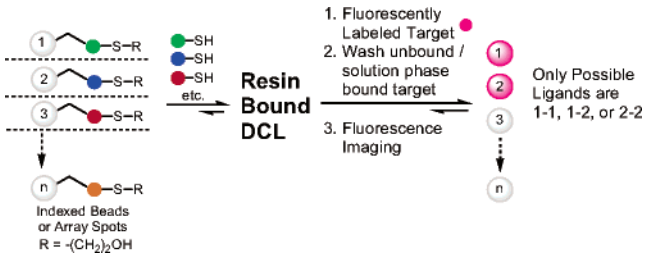
of selected compounds via spatial localization. Thus, as shown in Scheme 1 (using disulfide exchange as an example),

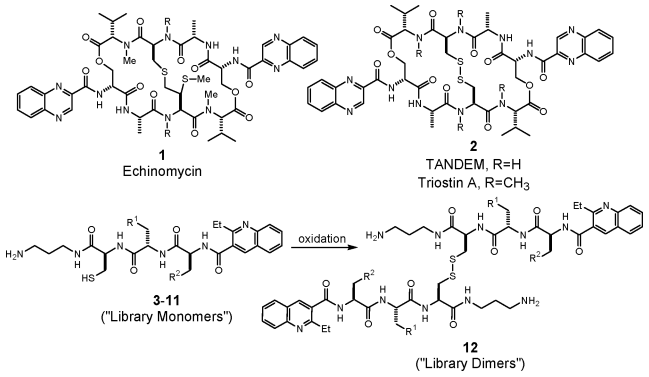
Scheme 1. Resin-Bound Dynamic Combinatorial Chemistry



a library of resin-bound "monomers" is combined with an identical library of monomers in solution and allowed to equilibrate in the presence of a fluorescently tagged target. After washing, examination of the beads by fluorescence microscopy allows ready identification of the selected library member. Spatial segregation or chemical tagging of resin identifies the monomeric units present in selected compounds. Following screening, this information greatly simplifies the process of identifying the selected ligand. For example, consider any dimeric RB-DCC libray of *n* monomeric building blocks. If beads bearing monomers 1 and 2 are found to bind the target, one could conclude that dimers 1−1, 1−2, or 2−2 are potential binders. While this process becomes increasingly complex as the size of the library increases, it nevertheless represents a significant simplification over current methodology.

As an initial test of the RB-DCC concept, we applied it to the selection of DNA-binding compounds using a design based loosely on the octadepsipeptide family of bis-intercalating DNA binding agents. This family includes natural products such as echinomycin (**1**, Figure 1), triostin A (**2**, R = Me), and synthetic analogues such as TANDEM (**2**, R = H). These molecules,[9] as well as synthetic variants,[10] have been extensively studied and are reported to bind DNA in the minor groove through bis-intercalation of the quinoxaline moiety. Triostin A has been shown to prefer GC-rich sequences, while TANDEM preferentially binds AT-rich sequences. Therefore, we screened our DCL against a sequence reported to be preferably bound by triostin A[11] (**DNA_1**: 5′-TCTAGACGTC-3′) and a sequence reported to be preferentially bound by its synthetic analogue TANDEM[12] (**DNA_2**: 5′-CCATGATATC-3′).

Our library design builds on a facile synthesis of quinolines we recently developed and in particular on 2-ethylquinoline-3-carboxylic acid (Quin).[13] We reasoned that a library of peptidic quinolines with the generic sequence C-R^1-R^2-Quin (**3**−**11**), when subjected to oxidative conditions which facilitate reversible disulfide exchange, would create a DCL of potential DNA binders (**12**, Figure 1). To that end, nine quinoline tripeptides (**3**−**11**) were synthesized on solid support using the amino acids serine (S), glutamine (Q), and histidine (H) (Supporting Information). In combination, these afford a DCL of 45 unique disulfides and 54 total library members.



compound	amino acid 1 (R^1)	amino acid 2 (R^2)
3	Q	Q
4	Q	S
5	Q	H
6	S	Q
7	S	S
8	S	H
9	H	Q
10	H	S
11	H	H

Figure 1. Natural product bis-intercalators echinomycin (**1**), TANDEM (**2**, R = H), and triostin A (**2**, R = CH_3). Disulfide-mediated DCL of natural product bis-intercalator mimics (**3**−**11**, **12**).

For comparison to RB-DCC, DCL selections were first carried out in a traditional solution-phase format. Library members **3**−**11** were added to vessels and allowed to equilibrate in phosphate buffer at a total concentration of 90 μM. While one solution was allowed to equilibrate in the absence of target as a control, the other equilibrated in the presence of 25 μM DNA for a time period ranging from 24 h to 7 days. Equilibration was then halted by acidification of the solution, and the library was immediately analyzed by HPLC. The results of these experiments demonstrate both the power of DCC and its inherent analytical challenges: library composition was clearly perturbed by the presence of **DNA_2**, while **DNA_1** had little obvious effect on the chromatogram. However, chromatographic overlap and mass degeneracy prevented HPLC or LC−MS identification of the specific compound undergoing enhancement.

To test the RB-DCC concept, we carried out three primary experiments (Figure 2). First, a control experiment was

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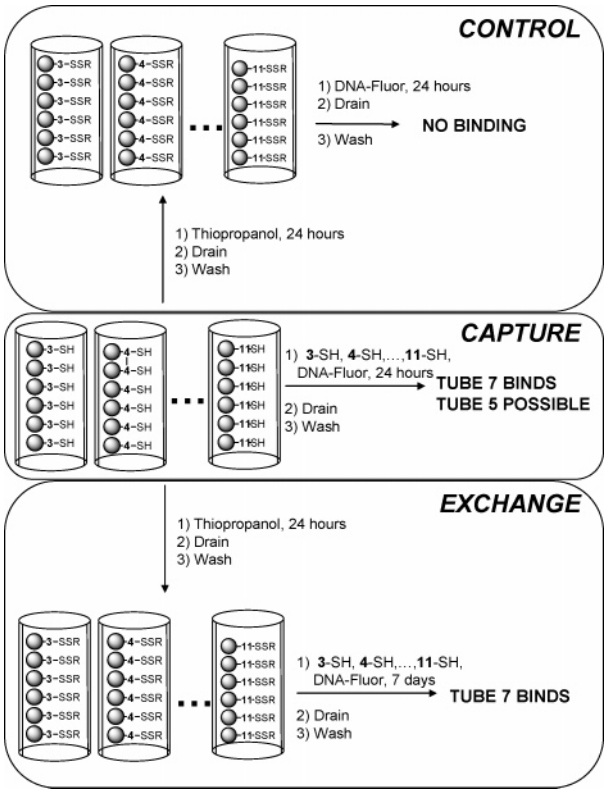

Figure 2. Resin-bound library experiments.

conducted to verify that resin-bound library monomers **3**−**11** did not possess any affinity for target DNA under the detection limits. Nine reaction vessels, each containing Tentagel-S resin bearing a single species of library monomer, were first exposed to an excess of thiopropanol. The resulting resin-bound disulfides were then exposed to a solution of fluorescently labeled **DNA_2**[14] (referred to herein as **DNA_2***) for 24 h. Each vessel was then evacuated by vacuum and agitated in 1 mL of buffer for 5 min. The resin was drained by vacuum and washed with an additional 1 mL of buffer. An aliquot of resin was then positioned on a microscope slide and viewed via epifluorescence microscopy.[15] No increase in fluorescence over background was observed.

Next, the library was screened in a "resin capture" mode. This entailed subjecting nine reaction vessels, each containing Tentagel-S resin bearing a single species of library monomer, to a solution of library monomers and **DNA_2***. After 24 h, the solution was drained from each tube, and beads were washed and imaged via fluorescence microscopy. While all beads exhibited fluorescence above baseline values, those bearing monomer **7** exhibited the highest fluorescence. Beads bearing monomer **5** were also fluorescent at levels higher than the remaining monomers, but significantly less than the beads bearing monomer **7**. These results suggested that dimer **7**-**7** was the primary "hit" in the screen, although **5**-**5** and **7**-**5** would also need to be considered as possibilities.

Finally, we screened the library using the RB-DCC protocol ("Exchange", Figure 2). After trapping the resin-bound monomer thiols with thiopropanol as in the "Control" experiment, each tube was treated with a solution of monomer thiols and **DNA_2*** at room temperature as in the "Capture" experiment. After the system was allowed to equilibrate for 7 days (a period of time chosen based on literature reports of disulfide exchange-based DCLs), vessels were drained, washed, and imaged as before. The results, as shown in Figure 3, clearly demonstrate the ability of the RB-DCC protocol to generate a single best "hit".

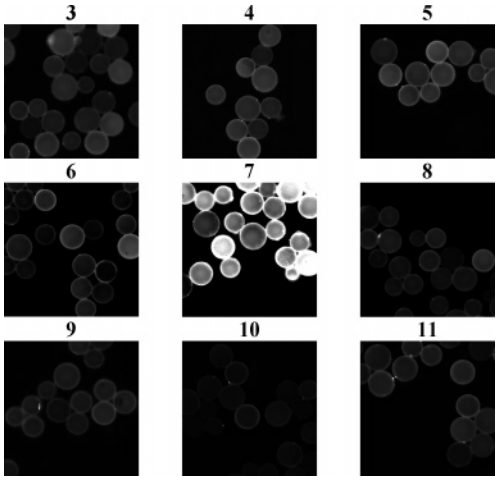

Figure 3. Fluorescence images of RB-DCC screening ("Exchange" experiment).

As in the "Capture" experiment, once again beads bearing monomer **7** gave the strongest fluorescence. Unlike the "Capture" experiment, however, resin beads bearing monomer **5** were only weakly fluorescent. We attribute this difference to the dynamic nature of the "Exchange" experiment. As confirmation, a second library experiment employing only **5** and **7** verified that only **7**-**7** was selected. It is noteworthy that while in this example only one resin-bound monomer exhibited fluorescence, the RB-DCC approach is applicable in screening large libraries where multiple "hits" may be generated. While this may require additional deconvolution steps, RB-DCC nonetheless greatly simplifies the identification process.

To further confirm a binding interaction between **7**-**7** and **DNA_2**, ^{1}H NMR titrations were performed. While significant spectral overlap of DNA and ligand aromatic proton signals obscured observation of many resonances, binding was easily detected by monitoring changes in the chemical shift of the downfield singlet corresponding to the quinoline C-4 proton. At equimolar DNA−ligand concentration, a shift of Δppm = 0.47 was observed. In contrast, a control experiment using D_2O as the titrant resulted in a shift of Δppm = 0.002. Equilibrium dialysis experiments were then

(14) **DNA_2*** is TAMRA labeled at the 5′ end with a six- carbon spacer separating DNA and fluorophore.

(15) Fluorescence imaging was performed with an exposure time chosen to provide the maximum differential between resins (100 ms).

carried out to examine ligand binding to an extended analogue of **DNA_2**.[16]

We found that **7**-**7** exhibited a dissociation constant (K_D) of 2.8 μM (Figure 4), while compound **10**-**10**, not selected

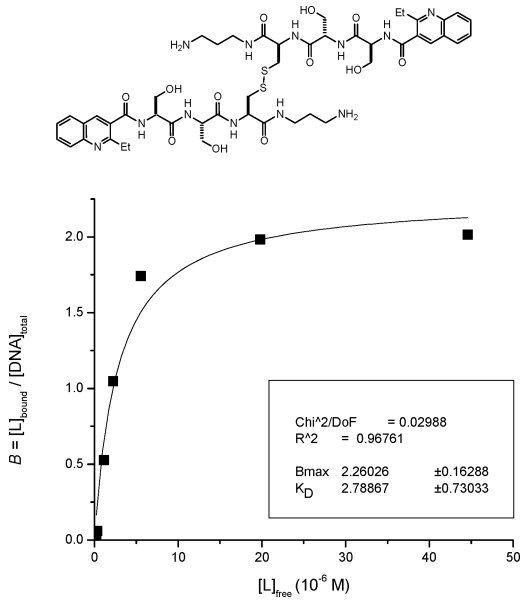

Figure 4. Binding isotherm for compound **7**-**7** to **DNA_2b**.

in the library screen, had a K_D of 10.8 μM.[17] Control experiments with independently synthesized resin-bound **7**-**7** and **10**-**10** gave fluorescence intensities consistent with Figure 3 upon incubation with **DNA_2***. To the extent that the solution K_D values reflect the relative affinities of the bead-immobilized compounds, the results indicate that the RB-DCC protocol successfully distinguished between higher and lower affinity ligands in the library.

In conclusion, we have provided the first demonstration of a dynamic combinatorial library method employing solid phase-immobilized "monomers" or resin-bound DCC (RB-DCC). Using a natural product-inspired DCL, we observed a selective enhancement of one DCL member and subsequently demonstrated that it had an affinity for the targeted DNA comparable to the natural product it was intended to model.[18] The number of compounds produced in this DCL was small enough that one could imagine synthesizing and screening them individually in an equivalent amount of time; however, evaluation of this relatively simple library was an essential initial step in validating the RB-DCC method. We anticipate that RB-DCC will be readily extendable to significantly larger libraries, particularly when implemented in a microarray format or on beads as mixtures using an encoding scheme. This will afford researchers an ability to empirically observe selection among spatially localized DCL members, alleviating potential analytical difficulties associated with solution phase DCC and opening the door to much broader use of DCC. Efforts to apply RB-DCC to a variety of targets are currently underway in our laboratories.

Acknowledgment. We thank Charles Mace and Peter Gareiss for extensive discussions regarding the analysis of equilibrium dialysis experiments. Financial support was provided by the sponsors of The Center for Future Health, University of Rochester. This paper is dedicated to the memory of Professor Nabi Magomedov.

Supporting Information Available: HPLC and full spectral analysis of compounds **3**−**11**, solution-phase DCC experiments, fluorescence images, NMR titrations, equilibrium dialysis, and full experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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