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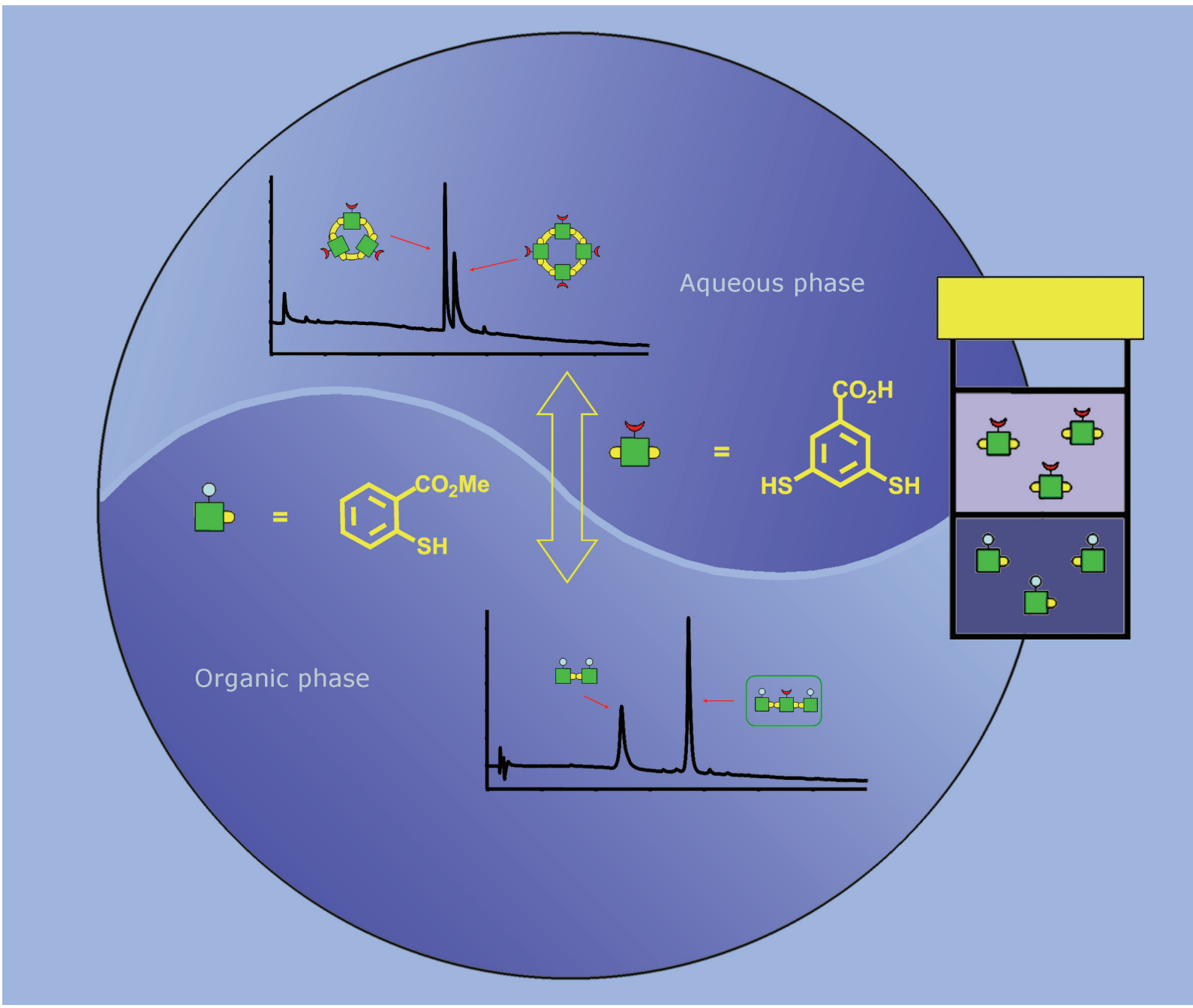

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Phase-transfer dynamic combinatorial chemistry†

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A two-phase approach to dynamic combinatorial chemistry is described using disulfide exchange chemistry; the use of two phases significantly increases the possibilities and the scope of dynamic combinatorial chemistry by facilitating the combination of otherwise incompatible building blocks.

Dynamic combinatorial chemistry is a powerful approach for finding new receptors in supramolecular chemistry: many new and sometimes surprising receptors have been discovered by allowing molecular recognition to direct their synthesis by using reversible bond formation under thermodynamic control.[1–3] We now show that creating dynamic combinatorial libraries (DCLs) using disulfide exchange as the reversible chemistry in two-phase systems allows the combination of mutually incompatible hydrophobic and hydrophilic building blocks.[4] Naturally occurring molecular receptors incorporate, to a great extent, non-polar moieties (*e.g.* non-polar amino acids in proteins) into largely polar structures, thus making the synthesis and studies of amphiphilic compounds as receptors an important goal in supramolecular chemistry.

Up to now, disulfide exchange has been investigated in just one unique phase per experiment.[5] Usually, the exchange has been performed in water, offering the possibility of targeting biomolecules,[6–8] although there have been some examples in organic solvents such as chloroform.[9,10]

The approach described here involves the use of carboxylate building blocks which are insoluble in the organic phase but which can generate a strong ion-pair with an appropriate organic base, thus promoting interactions with hydrophobic building blocks in the organic phase.

It has been reported previously that catalysis in a biphasic system can be enhanced by the use of a ligand which is soluble in an organic phase, with a strong affinity to a metal complex catalyst.[11] The ligand bound to the catalyst increases its concentration close to the interface where it can access the reagents present in the organic phase. We present here a dynamic combinatorial approach in a two-phase system using an organic base as a promoter of building block exchange, and in some cases as a phase transfer vehicle, enhancing the accessible diversity of the system.

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Several DCLs were set up using building blocks **1**[5] and **2**. Building block **1** is an aromatic dithiol equipped with a carboxylate group and is therefore soluble in water at neutral pH, whereas the monothiol building block **2** bearing a methyl ester group is soluble in chloroform. Neither building block was detectably soluble in the other phase.

The thiol functionality allows the building blocks to be oxidised to the disulfide in the presence of air; thiolate anions then induce disulfide exchange under thermodynamic control. Equimolar amounts of building block **1** (dissolved in water) and **2** (dissolved in chloroform) were allowed to oxidise and equilibrate after addition of *N*-methylmorpholine (NMM) as a base. The analysis of the composition of the library was done by LC-MS, injecting each phase separately. Control experiments in which building blocks **1** and **2** were oxidised independently in a biphasic system using NMM as a base were performed to confirm that no migration occurs of the single building blocks to the other phase under library conditions. Neither, in libraries with only one building block, did any of the resulting linear or cyclic oligomers migrate to the other phase.

The selection of the organic base NMM was based (a) on its solubility properties, which allow deprotonation of the thiol not only in the aqueous phase, but also in the organic phase, and (b) its ability to form a strong ion-pair with the carboxylate groups of building block **1**. This ion pair complex of NMM and **1** is more hydrophobic than **1** alone, thus enabling reaction with building block **2** in the organic phase (Fig. 1). As shown in Fig. 1, before mixing building blocks **1** and **2** after the addition of NMM, only cyclic oligomers in the aqueous phase (containing building block **1**) and linear dimer (homodimer of **2**) in the organic phase were observed. The two other phases only contained trace amounts of the building blocks and oligomers. Once the two building blocks were mixed a linear trimer composed of both building blocks was detected in the organic phase. The monothiol building block **2** acts as an end capping group enabling the formation of linear oligomers.

To demonstrate the reversibility of the process in this biphasic model system an equimolar amount of another monothiol (building block **3** isomeric with **2**) was added to the library after equilibration. After oxidation of the library was complete a mixture of the six possible linear oligomers was identified (Fig. 1). The equilibrium composition of the library matched that obtained by the building blocks being mixed together from the start. This confirms that the system is under thermodynamic control. The proportion of cyclic oligomers of **1** in the aqueous phase stayed constant during this experiment, and roughly 33% of **1** was transferred to the organic phase.

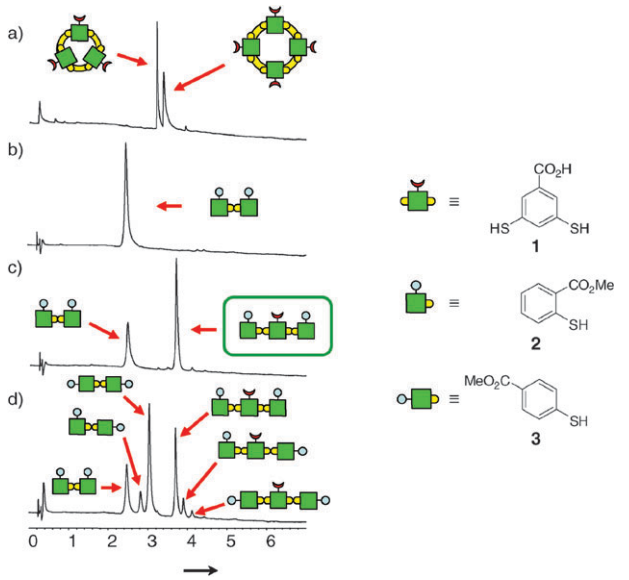

Fig. 1 HPLC analyses (260 nm) of libraries made from thiols **1**, **2** and **3** (5 mM each) using NMM as a base: (a) aqueous phase injection of building block **1** only; the organic phase only contained trace amounts of **1** and its oligomers; (b) organic phase injection of building block **2** only; the aqueous phase only contained trace amounts of **2** and its disulfide linked dimer; (c) organic phase injection of building blocks **1** and **2**; the proportion of cyclic species in the aqueous phase stays constant, and roughly 33% of **1** has transferred to the organic phase; (d) organic phase injection of building blocks **1**, **2** after addition of **3**; the proportion of cyclic species in the aqueous phase stays constant, and roughly 34% of **1** has transferred to the organic phase.

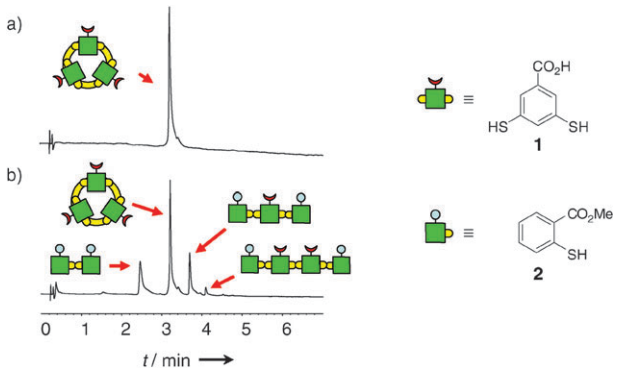

Fig. 2 HPLC analyses (260 nm) of the organic phase after addition of tributylamine to libraries made from (a) building block **1** (5 mM); (b) building blocks **1** and **2** (5 mM each). Building block **1** or its oligomers are not detected in the aqueous phase after library equilibration.

To confirm the importance of the organic solubility properties of the base, experiments were performed with an inorganic base. For example, building block **1** was dissolved in an aqueous NaOH solution at a pH of 7.4 while building block **2** was dissolved in pure chloroform. The two-phase library was oxidised and equilibrated as above. In the absence of an organic base no mixed oligomers were detected in the organic phase; only cyclic oligomers (of building block **1**) in the aqueous phase and linear dimers (of building block **2**) in the organic phase were observed. In the presence of a borate buffer solution at pH 7 as aqueous phase the same results were obtained. However, the use of different organic bases such as triethylamine, tetramethylethylenediamine, Tris buffer HCl (1 M, pH 7.4) and dimethylpiperazine resulted in the formation of mixed oligomers between building blocks **1** and **2**. These results were comparable to those obtained with NMM. The transfer of building block **1** into the organic phase was monitored by HPLC at 260 nm: it was between 4% with tetramethylethylenediamine used as a base and 33% when NMM was the base.

Quantitative transfer of building block **1** into the organic phase was achieved by using the more lipophilic tributylamine. Consequently, linear tetramer **2**·**1**·**1**·**2** was detected in the organic phase, together with the linear dimer of building block **2**, the linear trimer combining both building blocks **2**·**1**·**2** and, somewhat unexpectedly, the cyclic trimer of building block **1**. In this experiment building block **1** was dissolved in water using Tris buffer. Then, building block **2** was dissolved in chloroform and tributylamine added and the library was allowed to oxidise and equilibrate (Fig. 2).

Experiments with building blocks **1** and **2** were also performed in monophasic systems (in $CHCl_3$). A suspension of **1** and **2** in chloroform with tributylamine was sonicated until building block **1** was dissolved, presumably as the ion pair. The library was left to equilibrate as described above. The formation of some of the same mixed components was indeed observed, but there are a number of advantages associated with the two-phase system. Firstly, the oxidation of the thiols to disulfides is much slower in $CHCl_3$ than in the two-phase systems, at least in this particular case. Secondly, in the monophasic experiment using Bu_3N as the base, fewer components were observed by LC-MS analysis, *e.g.* the cyclic tetramer of **1** was not observed. Thirdly, the biphasic system has the advantage that templates which are insoluble in one phase can nevertheless be recognised in the other phase or at the interface.

The formation of the cyclic trimer of **1** in the organic phase when Bu_3N is the base, which can be described as cation-mediated phase transfer from water into chloroform, was followed by HPLC as shown in Fig. 2. The tributylamine solubilises the cyclic trimer in the organic phase by ion-pairing. Once in the organic phase, it can either exchange with building block **2** resulting in linear trimer (**2**·**1**·**2**) and tetramer (**2**·**1**·**1**·**2**), or stay as a cyclic trimer (of building block **1**) complexed with tributylammonium cations. The absence of building block **1** in the aqueous phase shows that transfer into the organic phase is quantitative. Similar results were obtained using longer alkyl chain bases such as dimethyloctyl amine and dimethyldodecyl amine. Comparable results were obtained with Bu_4NBr, a conventional phase transfer catalyst. This confirms that the phenomenon that gives the highly diverse libraries in the organic phase(s) is phase transfer of the hydrophilic building block(s) to the organic phase rather than molecular recognition of Bu_3N (or any of the other bases). Thus, this two-phase solvent system can mimic a biological water/membrane system, the cyclic trimer bearing three carboxylate groups being observed in the organic phase (Fig. 2).

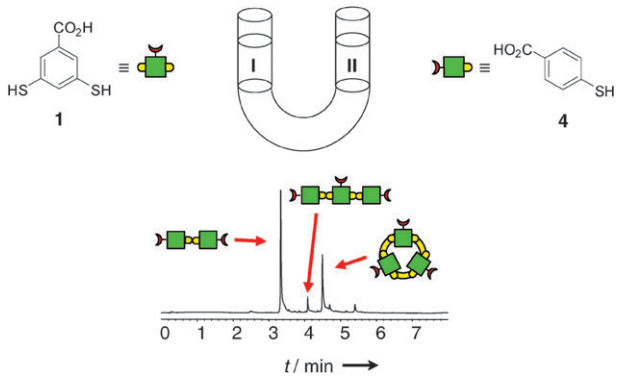

Fig. 3 Transport experiment across bulk liquid membrane; the figure illustrates the experimental setup. HPLC analysis (260 nm) of the organic phase shows the formation of mixed species.

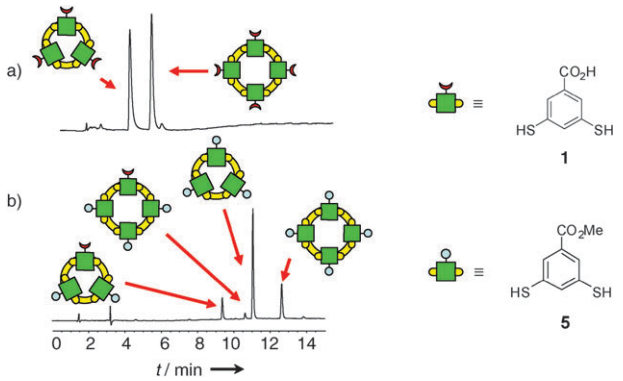

Fig. 4 HPLC analyses (260 nm) of libraries made of thiols **1** and **5** (5 mM) using NMM as organic base: (a) aqueous phase; (b) organic phase. Roughly 7% of **1** is transferred to the organic phase in this experiment.

The membrane analogy was pursued in transport experiments across bulk liquid membranes (Fig. 3). Building blocks **1** (aqueous phase **I**) and **4** (aqueous phase **II**) were separated by an organic layer (chloroform) to which tributylamine was added. As before, building block **1** was dragged into the organic phase and the same phenomenon was observed with building block **4**. Analysis of the organic layer reveals the formation of the mixed oligomer **4**·**1**·**4**. Analyses showed that most of the building blocks were in the organic phase, but the composition of the residual material in both aqueous compartments was identical. This experiment shows that hydrophilic building blocks can be transported through bulk organic solvent, but whether the exchange chemistry takes place at the interface of the two solvents or in one of the bulk phases is not clear at this point.

To illustrate the generality of the concept, and to obtain novel macrocyclic species, the behaviour of the chloroform soluble building block **5** (synthesised from **1** by treatment with H_2SO_4 in methanol) was studied. This building block (**5**) forms homo-oligomers in the organic phase (cyclic trimer and tetramer). In a similar fashion building block **1** forms cyclic oligomers (cyclic trimer and tetramer) in the aqueous phase (Fig. 4). A two-phase library of both building blocks was treated with NMM as in previous examples, and mixed cyclic species (both cyclic trimers and cyclic tetramers) were detected in the organic phase. This experiment shows that the two-phase approach is a convenient way to achieve greater diversity in dynamic combinatorial libraries.

In summary, we have shown that dynamic combinatorial chemistry using disulfide exchange as reversible chemistry is feasible in two-phase systems. This broadens the scope of dynamic combinatorial chemistry since it enables the use of a wider range of guests and building blocks, overcoming many solubility problems and thereby increasing the diversity of libraries that can be generated and explored.

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