Expanding diversity in dynamic combinatorial libraries: simultaneous exchange of disulfide and thioester linkages†

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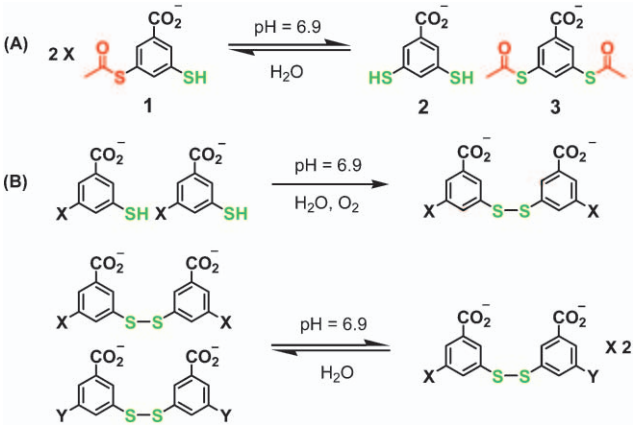
Dynamic combinatorial libraries have been prepared which feature two simultaneous covalent exchange reactions in aqueous solution at neutral pH. This allows for diversity, not only of the subunits that are linked, but also of the linkage itself.

In the past decade, dynamic combinatorial chemistry has proven to be a useful tool for the rapid identification of new molecular receptors,[1] ligands and inhibitors for biomacromolecules,[2] and a macrocyclic catalyst.[3] It is also a convenient way of tuning and probing the stabilisation induced by non-covalent interactions within macromolecular architectures.[4] Within a dynamic combinatorial library (DCL), individual members are constantly interconverting through reversible bond exchange, the mixture generated being able to respond to external influences that affect the stability of its constituents. Although many chemical reactions involving covalent bond formation can potentially be carried out under reversible conditions,[5] the few exploited to date include imine, hydrazone, disulfide and, more recently, thioester[2b,6] exchange. If dynamic combinatorial chemistry is to reach its full potential, then further development of exchange chemistry is required.

Eliseev and Lehn have reported the elegant use of double-level "orthogonal" libraries where two different exchange processes can be activated and deactivated independently.[7] We now show that structural diversity within a DCL can be expanded by maintaining two communicating exchange processes simultaneously. We have generated a double-level "communicating" library based on one-pot exchange of disulfide and thioester linkages in aqueous solution. Such libraries should not only allow for selection based on the recognition features of the building blocks, but also on the basis of their connectivity.[8]

The simple building block **1** carrying a single thiol and a single thioester functionality was chosen as a synthetic target[9] and as a single precursor for the double-level library (Scheme 1). This starting material contains two exchangeable units: a monotopic acetyl group (Scheme 1, red) acting as a labile capping group (through thioester exchange) and a ditopic dithiol monomer (Scheme 1, green) being potentially involved either in disulfide (chain elongation) or thioester (termination) linkages. Apart from the increased linkage diversity, this combination of mono- and ditopic labile fragments should, in principle, enlarge the scope of potential topologies accessible in such a library.



Scheme 1 Building blocks **1**, **2** and **3** involved in thioester exchange (**A**) and further disulfide oligomerization and exchange (**B**).

As disulfide exchange requires thiol oxidation by atmospheric oxygen, the thioester exchange (Scheme 1A) and disulfide exchange (Scheme 1B) can be addressed sequentially. After dissolving the bifunctional precursor **1** in the absence of oxygen in an aqueous solution (ammonium acetate buffer 20 mM, pH 6.9, H_2O–CD_3OD 90:10) at millimolar concentrations only thioester exchange took place. ^{1}H-NMR spectroscopy of the equilibrated solution using a water suppression sequence revealed the expected statistical distribution in equilibrium (**A**) of monomers **1**, **2** and **3** in a 0.5:0.25:0.25 ratio (see supporting information†). A solution initially containing an equimolar amount of **2** and **3** under the same conditions led to the same composition. In both cases, the equilibrium was reached within minutes and the product distribution remained unchanged when the mixture was left stirring for days in the absence of oxygen. Thiol oxidation and simultaneous disulfide exchange (Scheme 1B) were then activated by exposing the mixture to atmospheric oxygen.[10] Qualitative kinetic studies using HPLC/ESI-MS analysis revealed that a second equilibrium was reached in 36 h, after which the composition remained unchanged. The much slower rate of oxidation of the thiols as compared to the rate of exchange of the thioesters allows the sequential study of the two processes in one-pot, in the presence of oxygen, leading to the same final composition.‡ Thus, thioester and disulfide exchanges (**A**) and (**B**) can be studied sequentially: the former by adjusting the pH from mild acidic to neutral values, the latter by allowing contact with atmospheric oxygen at neutral pH.

† Electronic supplementary information (ESI) available: NMR and HPLC/ESI-MS analyses of the libraries. See http://www.rsc.org/suppdata/cc/b5/b500638d/

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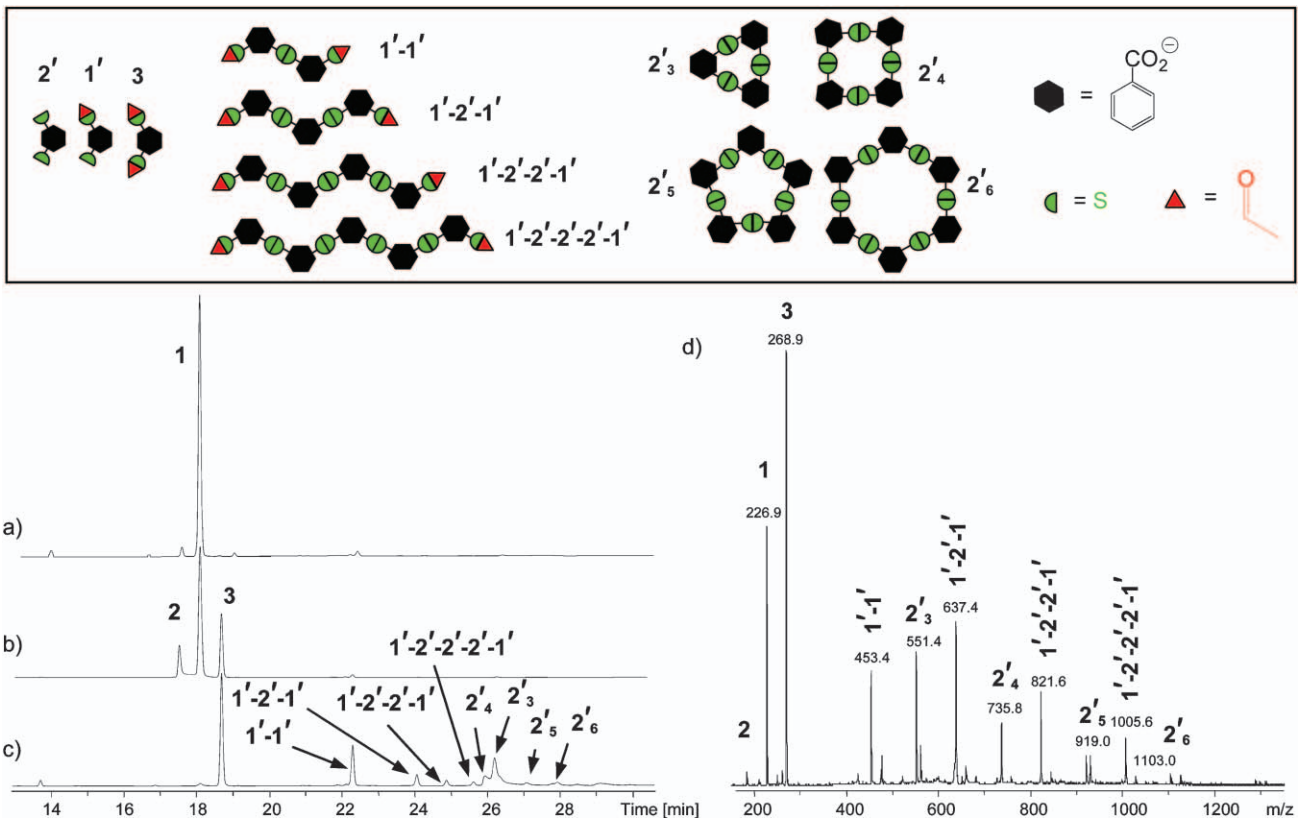

Fig. 1 HPLC analysis (λ = 290 nm) of: a) starting building block **1** (5 mM) at pH = 3, b) statistical mixture obtained after thioester exchange (**A**) by raising the pH to 6.9 and c) final equilibrium of oligomers from combined exchange (**A**) and (**B**) after 36 h in the presence of atmospheric oxygen. d) ESI-MS spectrum (negative ion mode) of the mixture corresponding to HPLC trace c). Signals corresponding to dimeric species associated with a sodium ion ($2M^- + Na^+$) were also observed.

From the intermediate library (**A**) consisting of the statistical mixture of precursors **1** to **3** (being itself generated from the single building block **1**) up to eight oligomers combining disulfide and/or thioester functionalities were unambiguously identified by HPLC/ESI-MS in the final library (**B**) (Fig. 1).

Employing both mono- and ditopic complementary labile fragments (or capping and propagating units) leads to the coexistence of linear and cyclic topologies up to hexameric species within the same thermodynamic mixture. This is, to our knowledge, the first example of a library exploring the interconversion between closed and persistent open members.[11]

In order to verify that the two different sources of exchange were communicating, *i.e.* that both equilibriums (**A**) and (**B**) could affect and displace each other, the amount of parent monomers **1′**, **2′** and **3**[12] (from which all detectable library members were formed) were summed (see supporting information†) and the total amount compared to the intermediate statistical mixture (**A**). The combined disulfide oligomerization and exchange (**B**) displaces the thioester equilibrium (**A**) from the statistical 0.5:0.25:0.25 into a 0.2:0.4:0.4 distribution of the parent monomers **1′**, **2′** and **3** (Fig. 2, entries II and III respectively). This clearly indicates that the disulfide exchange has a thermodynamic impact on the thioester equilibrium, *i.e.* that the two equilibria can chemically communicate within a dynamic library.

Inevitably, **2** and **3** are simultaneously produced in equal concentration by the disproportionation of **1** in equilibrium (**A**). Building block **2** is a polyvalent molecular ingredient for the construction of both linear and cyclic species, whereas its counterpart **3** is a molecular "dead end". Dithioester **3** acts as a

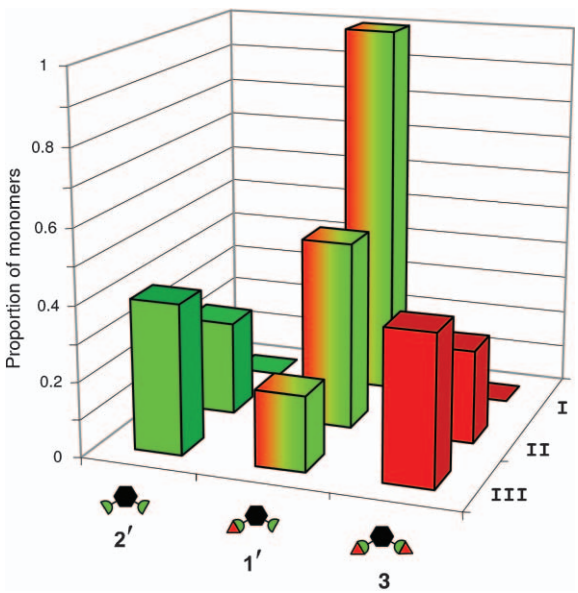

Fig. 2 Evolution of the composition of the system analysed in terms of the total amount of monomers **1′**, **2′** and **3**. From starting material **1** (entry I), *via* thioester equilibrium (**A**) (entry II), to the final library combining thioester and disulfide exchanges (entry III).

reporter[13] of the extent to which disulfide oligomerization of **2′** shifts the parent equilibrium (**A**). Since disulfides are represented in most of the members in the final dynamic library (8 out of 11), process (**B**) strongly perturbs the parent equilibrium (**A**). In most conventional DCLs, the thermodynamic differentiation of a member occurs through an additional non-covalent binding equilibrium with a additional species (the template) responsible for the equilibrium displacement (called amplification). In the present case, the 2-fold increase of the amount of **3**, due to a statistical or entropic driving force instead of the classical enthalpic differentiation, occurs through a second covalent equilibrium. Such a dynamic library, relying on two simultaneous and communicating equilibria is uniquely suited for a subsequent selection process through the non-covalent binding of a selected oligomeric member with a guest (or host) molecule. Moreover, libraries can be biased toward linear or cyclic topologies by using a non-stoichiometric starting mixture of **2** and **3** instead of the bifunctional precursor **1**.

In conclusion, our results demonstrate that two simultaneous sources of covalent exchange can be used within the same dynamic combinatorial library. A single polyfunctional building block, very easily accessible synthetically, generates a dynamic mixture of at least eight oligomers of various topologies and functionalities. This double-level communicating library can be activated and studied simultaneously or sequentially. Furthermore, this system provides, for the first time, access to dynamic mixtures where selection can take place on the basis of the nature of the bonds between monomeric units in addition to the units themselves. Whereas the present system only incorporates monotopic thioester units, extension to multitopic thioester systems should result in an enriched pool of possible receptors available for molecular recognition. Alternatively, some binding *scenarii* may require synthetic linear partners, which can potentially be amplified from such a library employing labile capping groups.

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Notes and references

‡ In a typical experiment, the building block **1** (3.42 mg, 0.015 mmol) was dissolved in a mixture of 20 mM aqueous ammonium acetate and CD_3OD (90:10, 3 ml, pH 6.9). The solution was stirred during 15 min and immediately analysed by HPLC/ESI-MS (see supporting information†). $CuCl_2$ (0.025 M in H_2O, 12 μL, 2 mol%) was then added and the mixture was allowed to oxidize and equilibrate in air for another 36 hours, followed by HPLC/ESI-MS analysis using the same conditions as above.

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