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Self-selection of a single topology from dynamic combinatorial libraries in aqueous solvent through supramolecular interactions

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We describe the formation of a single product arising from the combination of building blocks with different topologies through disulphide chemistry in a Dynamic Combinatorial Library (DCL). The supramolecular interactions established by a zwitterionic cysteine moiety are responsible for the self-selection of one product among all virtual members of a large library.

Self-assembling and self-organization are inherent processes in Nature that occur by the spontaneous folding and assembling of relatively simple chemical entities.¹ For that, the delicate inter- and intramolecular interactions are established and regulated by proof-reading and self-correction.² Accordingly, the chemical information stored in a given chemical structure is decoded to render beautifully complex and fully functional biomolecular systems. For decades, supramolecular chemists have been fascinated and inspired by this biomolecular machinery. More recently, Constitutional Dynamic Chemistry (CDC) and Dynamic Combinatorial Chemistry (DCC) have proposed the implementation of complex dynamic systems formed by members able to exchange under thermodynamic control.³ These dynamic systems (Dynamic Combinatorial Libraries, DCLs) are excellent benchmark models for decoding the chemical information stored in the combination of simple building blocks towards the emergence of new assembled structures.⁴

Disulphide chemistry has been exploited widely for the generation of DCLs in aqueous media.⁵ However, most of the systems described are based on molecules with two reacting sites (bipodal). Thus, although some impressive (and somehow unexpected) geometries have been described such as catenanes and knots,⁶ the tri-dimensional space covered is relatively small, and the libraries are mostly directed towards the generation and interconversion of cyclic oligomers.⁷ The formation of cage-like organic structures has seldom been described by oxidation of tripodal building blocks or by combination of tripodal and bipodal building blocks.⁸ The stoichiometry and the presence of a template were critical in the latter. More recently Sanders has described the diverse topologies obtained by combination of tri- and monothiols in water.⁹ In order to expand the structural and topological diversity of supramolecular assemblies by DCC we decided to study the effect of combining mono-, di- and trisulphides in the same DCL. In

principle, by combining a series of building blocks of different topology, a broad structural variety is expected. We chose to combine pseudopeptidic C₂-symmetric dithiols **1a-b** described earlier by our group¹⁰ with the tripodal building block **2**⁸ and a series of monothiols **3a-g**. Thus, one may expect the formation of several compounds with different architectures some of which are schematically represented in Scheme 1b.

Scheme 1. a) Thiol building blocks used in this study. b) Schematic representation of some of the possible architectures generated by the combination of topologically different building blocks.

Figure 1 displays three XRD patterns labeled (a), (b), and (c). The x-axis represents the diffraction angle 2θ in degrees, ranging from 10 to 40. Pattern (a) compares the mixture of 1a and 2 (blue line) with the mixture of 1a, 2, and 3a (red line). Pattern (b) compares the mixture of 1b and 2 (blue line) with the mixture of 1b, 2, and 3a (red line). Pattern (c) shows the mixture of 1a, 2, 3a, 3f, and 3g (blue line). Bragg reflections are indexed for several peaks in each pattern.

(a) 1a+2 (blue) and 1a+2+3a (red):

- Blue peaks: $[1a_2]$, $[1a_2, 2]$, $[1a_2, 2]$, $[1a_2, 2]$.
- Red peaks: $[2, 3a]$, $[2]$, $[1a_2, 2, 3a]$, $[1a_2]$, $[1a_2, 2, 3a]$, $[1a_2]$, $[1a_2, 2]$, $[1a_2, 2]$.

(b) 1b+2 (blue) and 1b+2+3a (red):

- Blue peaks: $[1b_2]$, $[1b_2, 2]$, $[1b_2, 2]$, $[1b_2, 2]$.
- Red peaks: $[2, 3a]$, $[2]$, $[1b_2, 2, 3a]$, $[1b_2]$, $[1b_2, 2, 3a]$, $[1b_2]$, $[1b_2, 2]$, $[1b_2, 2]$.

(c) 1a+2+3a+3f+3g (blue):

- Blue peaks: $[1a_2, 2, 3a]$, $[1a_2, 2, 3f]$, $[1a_2, 2, 3g]$, $[1a_2, 2]$, $[1a_2, 2]$.

To our surprise, when the same experiment was repeated for **1a** and **2** in the presence of 2.5 mM of cysteine (enough to saturate all free thiols) a single product containing each of the three building blocks (**1a23a**) was predominantly formed (Fig. 1a, red trace). The other minor detectable species are the combination of **2** with 3 cysteines and a structure of the formula **1a₂23a**. Since Ser-derivative **1b** presented similar behaviour (figure 1b) we focused our studies on Asn-derivative **1a**. The distribution of products was mostly unchanged when varying the concentration of Cys from 0.5 mM (equimolar with other components) to up to 100 mM, showing the stability of the product formed.¹² In addition, we combined building blocks **1a** and **2** in different proportions (1:2 and 2:1) in the presence of 1 eq of Cys per thiol. In these experiments almost all the limiting reagent was consumed in the same product of formula **1a23a** whereas the reagent in excess formed either adducts with Cys or the corresponding homodimer (ESI+). We attribute the selection of the major product formed in the mixture to some sort of self-recognition within the building blocks during the assembly process. Thus, stabilization through supramolecular interactions would lead to the formation of a single, more stable compound. Reversibility tests confirmed the reaching of the thermodynamic equilibrium in the mixture (ESI+).

ammonium group being more critical, as a hydrogen bond donor in that position clearly enhances recognition. In fact, the experiments done with thiols **3a-e** showed the trend $\text{NH}_3^+ > \text{OH} > \text{NHAc}$ (ESI[†]) to effectively select a single product from the mixture.

We turned then to investigate the structure of the multicomponent molecule to see what possible interactions would lead to the formation of a single product. In principle two architectures are possible for a molecule of the formula **1a23a**: the symmetrical structure **1a** and the less symmetrical **1b** (Scheme 1). We considered **1a** more likely as it would present a more compact structure in solution.

To confirm this possible arrangement we reproduced the synthesis of **1a23a** on a preparative scale. The NMR spectra of the purified product in buffered water at pH 6.5 with 15% DMSO- d_6 were acquired using water signal suppression by excitation sculpting (Fig. 2a). All the signals were successfully assigned by combination of ^1H -NMR and 2D-TOCSY experiments. All expected protons are visible in the ^1H -NMR spectra apart from the three protons corresponding to the alpha carbons of the amino acids from bipodal and tripodal fragments, which fall in the region of water suppression. However, these protons are visible in the TOCSY experiment. Three doublets corresponding to the three amide bonds coupled to the chiral centre of the amino acids are clearly visible in a 2:2:1 ratio (H_A , H_B , H_E). In addition, protons H_C and H_{18} from the phenylenediamine fragment, and H_4 and H_2 from the trimesic acid appear as singlets. Overall, this data is consistent only with the formation of the more symmetrical product **1a**.

Figure 2. a) Partial ^1H -NMR spectrum (500 MHz, buffered H_2O with 15% DMSO-d_6 , 298 K) showing the aromatic and amide region for molecule **1a23a**. b) Proposed structure for major product **1a23a** (**1a**) with atom labelling (left) and structure obtained by molecular modelling (right).

Some other structural considerations can be done in view of the ^1H -NMR spectra. H_E displays a relatively low chemical shift compared to H_A , which could be indicative of a twisting out of

the aromatic ring plane. This also explains the large chemical shift difference between H_2 and H_4 , both from the tripodal aromatic ring. On the other hand, H_C and H_B appear in the downfield amide region, which is consistent with some kind of hydrogen bonding phenomena, most likely with any of the carboxylate anions present in the molecule. To shine some light in the possible tri-dimensional structure of the compound in solution we performed a conformational search using MMFFaq force field. Molecular modelling rendered a folded structure with several stabilizing interactions as shown in Fig. 2b.

The most stable conformer shows a structure where the pendant Cys folds over the macrocyclic cavity. The ammonium cation resides in the centre of the macrocycle and is stabilized by the three carboxylates that arise from the trimesic acid moiety. The carboxylate group of the cysteine residue would as well be stabilized by hydrogen bonding interactions with the macrocycle amide protons (H_C/H_B). Other hydrogen bonding interactions between the macrocyclic peptidic amides and the carboxylates would further stabilise the structure. Interestingly a *cis* amide bond is observed for the tripodal amide that is not part of the macrocycle. We can also observe different disposition (in/out) for the carbonyls of the amides both in the bipodal and tripodal moieties. This data is in agreement with what we observed by 2D-NOESY experiments. NOE correlations were visible for proton H_A with both H_4 and H_2 which would indicate a dynamic rotation of the amide bonds. Similar behaviour is observed for H_C and H_{18}, H_{16} showing that some degree of conformational freedom is present.

Unluckily, we could not assign unambiguous through-space correlations that supported the proposed folding, since proton H_{23} (from the pendant cysteine) overlaps with protons H_9 (within the macrocycle backbone). Besides, as stated before, protons H_6 , H_{11} and H_{19} fall in the water suppression region and thus, no NOE correlations could be assigned for these protons neither. Nevertheless, further evidence of the folded structure was obtained when the sample was acidified with TFA (10 eq), because some significant shifts of the NMR signals were observed (ESI[†]). Thus, H_E and H_A moved downfield and to a similar chemical shift while H_B and H_4 moved upfield. This is consistent with an unfolding of the structure upon protonation, which would leave all the amide or aromatic protons of the tripodal moiety in a more similar chemical environment.

Finally, to prove that small structural changes had a decisive effect on the product distribution we tested the unnatural enantiomer of cysteine (D-Cys, **3f**) and structurally related homocysteine (**3g**). D-Cys formed a major product containing all the building blocks with no major differences with L-Cys. Homocysteine, however, formed a major product **1a23g**, but other compounds were also readily detected. Strikingly, in a competition experiment L-Cys was slightly preferred over D-Cys (51:49, ESI[†]) and in a competition experiment between L-, D-, and homocysteine the tendency showed that the preference is L>D>homo (39:37:24) demonstrating that subtle structural differences have an impact on the formation of the major species (see figure 1c). This provides further evidence of a folded structure in solution as homocysteine would have a looser fit.

Conclusions

We show the importance of the synergic action of subtle recognition events within the components of a DCL. Combination of building blocks of different topology could result in a plethora of possible structures. However, cooperative

supramolecular interactions between the building blocks result in the self-recognition and the selective amplification of a single product even when it is statistically disfavoured. The structural importance of these interactions is proved by the difference observed in the chromatographic profile of the DCL when delicate changes are introduced. We expect that our findings will improve the comprehension of the behaviour of complex dynamic libraries of compounds.

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Notes and references

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