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Dynamic combinatorial chemistry with diselenides and disulfides in water†

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Diselenide exchange is introduced as a reversible reaction in dynamic combinatorial chemistry in water. At neutral pH, diselenides are found to mix with disulfides and form dynamic combinatorial libraries of diselenides, disulfides, and selenenylsulfides.

Dynamic combinatorial chemistry is a methodology that harnesses the power of reversible chemical reactions to form dynamic combinatorial libraries (DCLs).[1] At the heart of a DCL is the reversible reaction that connects the building blocks, and thus allows the DCLs to form and to equilibrate. A particularly important challenge in dynamic combinatorial chemistry is to identify reversible reactions that equilibrate in water at physiological pH. Of the many reactions introduced for dynamic combinatorial chemistry only a few have found practical use in DCLs that enable recognition of biologically important molecules in water. These include boronic ester exchange,[2] imine exchange,[3] hydrazone exchange,[4] and disulfide exchange,[5] which is the most widely used. Disulfide exchange comes closest to being of practical use at physiological pH, but even this exchange has some drawbacks; one of them being that the disulfide exchange reaction is most effective at slightly alkaline pH.

Selenols are more acidic than their corresponding thiols (pK_a (Cys) = 8.18, pK_a (Sec) = 5.24)[6] and higher concentrations of highly nucleophilic selenolates are present at lower pH than their thiolate counterparts.[7] At neutral pH, kinetic studies using cysteamine and selenocysteamine have shown that the rate constants for diselenide exchange are up to seven orders of magnitude higher than the corresponding rate constants for the disulfide exchange.[8]

Herein we introduce diselenide exchange as a reversible reaction for dynamic combinatorial chemistry in water that works at physiological pH. Additionally, we show how diselenide exchange can be combined with disulfide exchange to give DCLs where disulfides, diselenides and mixed selenenylsulfides coexist (Fig. 1), and moreover that simple diselenides can be used as catalysts for formation of disulfide based DCLs at neutral pH with only 1 mol% diselenide catalyst.

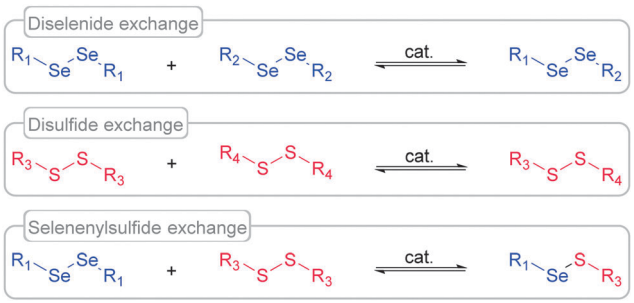

Fig. 1 Three exchange reactions for dynamic combinatorial chemistry in water that work simultaneously: diselenide exchange (top), disulfide exchange (middle), and selenenylsulfide exchange (bottom).

Disulfide based DCLs are set up by dissolving appropriate thiol-containing building blocks in buffered aqueous solutions. Air oxidation of the thiols to disulfides and simultaneous disulfide equilibration take place during DCL formation. This convenient protocol is not directly applicable to diselenide based DCLs because selenols are oxidised to diselenides too quickly to be isolated. Instead the starting point for diselenide based DCLs are mixtures of diselenides.

To study how to conveniently initiate the exchange process and reach equilibrium, we prepared DCLs from the diselenides $(\mathbf{1})_2$, $(\mathbf{2})_2$, and $(\mathbf{3})_2$. The diselenides were mixed in different orders in the presence of 20 mol% exchange initiator 4-mercaptobenzoic acid (Fig. 2 and Fig. S16–S19, ESI†). Within a day the three libraries reached the same composition of exchanged diselenides. These reactions were carried out both in ammonium acetate buffer at pH 7.6 and in phosphate buffer at pH 7.9 and in both buffer systems the libraries remained stable for at least 40 days.

Next, the efficiency of the exchange reaction at different pH values was studied by carrying out the same type of exchange

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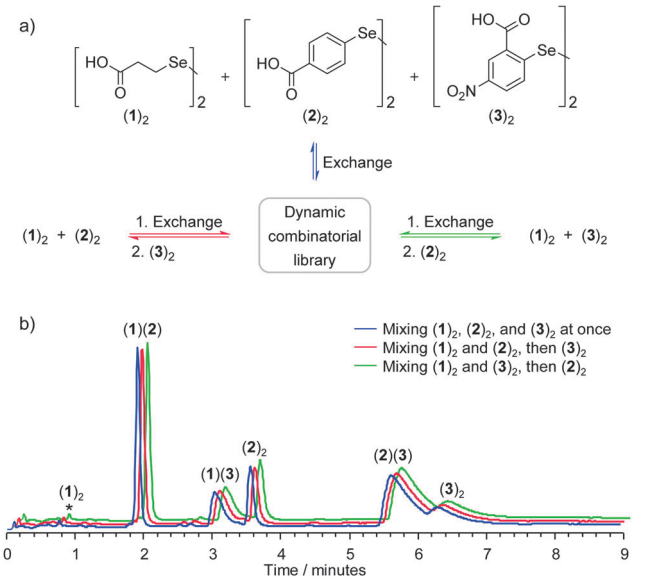

Fig. 2 (a) Setup of diselenide DCLs from different starting points with 20 mol% 4-mercaptobenzoic acid as an initiator in water at pH 7.6. (b) HPLC chromatograms (monitored at 290 nm) of the DCLs after equilibration for four days.

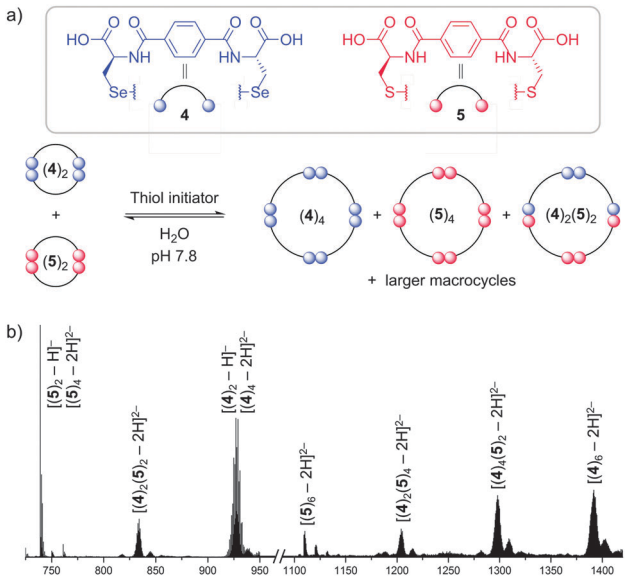

Fig. 3 Structures of the building blocks (top). The combined diselenide, disulfide, and selenenylsulfide based DCL from bis-diselenide macrocycle $(\mathbf{4})_2$ and bis-disulfide macrocycle $(\mathbf{5})_2$ (middle). The partial ESI mass spectrum showing the presence of different sized macrocycles in the DCL (bottom). The intensities of the high mass signals are scaled with a factor three relative to the low mass signals.

studies as above at pH 7.0, 6.1, 5.1, 4.0, and 2.9. It was found that the exchange reaction stayed efficient down to pH 5.0 whereas no exchange was observed at pH 4.0 and 2.9. Collectively, these findings show that diselenide based libraries (i) can be conveniently set up from diselenides, (ii) are efficiently exchanging over a broad pH range including physiological pH, (iii) are stable for extended time, and (iv) are easily tameable by lowering the pH.

After these proof-of-concept studies, more complex DCLs were prepared. For this, oxidised dimeric structure $(\mathbf{4})_2$, equipped with two diselenide functionalities, was used giving the possibility of DCLs consisting of oligo-diselenide macrocycles. When the dimer was dissolved in ammonium acetate buffer together with diselenide $(\mathbf{1})_2$ and 5 mol% 4-mercaptobenzoic acid as an initiator, diselenide exchange was observed. In the formed DCL, significant quantities of exchange products between $(\mathbf{1})_2$ and $(\mathbf{4})_2$ were identified together with mass peaks corresponding to higher diselenide macrocycles (Fig. S20 and S21, ESI†).

To form DCLs with even higher diversity, $(\mathbf{4})_2$ was mixed with the bis-disulfide macrocycle $(\mathbf{5})_2$ in phosphate buffer (pH 7.8) in the presence of 5-mercaptoisophthalic acid (Fig. 3). The resulting DCL consisted of two homotetramers, $(\mathbf{4})_4$ and $(\mathbf{5})_4$, and hexamers, $(\mathbf{4})_6$ and $(\mathbf{5})_6$, along with a mixed selenenyl based tetramer, $(\mathbf{4})_2(\mathbf{5})_2$, and two mixed hexamers, $(\mathbf{4})_4(\mathbf{5})_2$ and $(\mathbf{4})_2(\mathbf{5})_4$. The amount of the different library members varied over time showing how the diselenide exchange took place alongside both disulfide and selenenylsulfide exchanges on the way to equilibrium. Analysis of this complex dynamic mixture was aided by the characteristic isotope distribution in selenium containing molecules to unambiguously determine the structure of the formed library members (Fig. S23–S27, ESI†).

The high efficiency of the above diselenide based DCLs combined with their interplay with sulfur systems inspired us to study how diselenides could affect disulfide based DCLs. Previous studies have shown that selenols catalyse the disulfide exchange reaction,[9] and that diselenides catalyse the oxidation of thiols.[10] Consequently, selenium additives have found use as catalysts in protein folding studies,[11] and substitution of Cys with Sec in proteins has led to an improved control of the folding process.[12] In the search for a similar effect on the rate of library formation, we examined a simple DCL of macrocyclic disulfides formed from 3,5-dimercaptobenzoic acid, **6**. When **6** was dissolved in ammonium acetate buffer a disulfide based DCL consisting of a cyclic trimer and a cyclic tetramer was formed (Fig. 4a). The DCL was studied at pH 7.0, 7.6, 8.3, 9.4, and 10.0. The libraries were formed efficiently in a day at pH 9 and 10, whereas one week was required for reactions to complete at the lower pH values. The rates of library formation at pH 7.0, 7.6, 8.3 in the presence of 10 mol% diselenide $(\mathbf{1})_2$ were monitored over time, and it was observed that the catalyst caused accelerated library formation (Fig. 4b). This catalytic effect was observed to be general since at pH 7.0 the time required for library formation was reduced from 7 to 2 days, at pH 7.6 from 7 to 2 days, and at pH 8.3 from 7 to 1 day. The acceleration of the DCLs was still pronounced down to 1 mol% catalyst loading (Fig. S29–S34, ESI†), thereby providing this as a new approach to catalyse disulfide based DCL formation in water.[13]

Analysis of the LC/MS data gave indications of the origin of the catalytic effect. During the formation of the DCLs, a small peak corresponding to linear selenenylsulfide $(\mathbf{6})_2(\mathbf{1})_1$ was observed and after complete library formation this peak

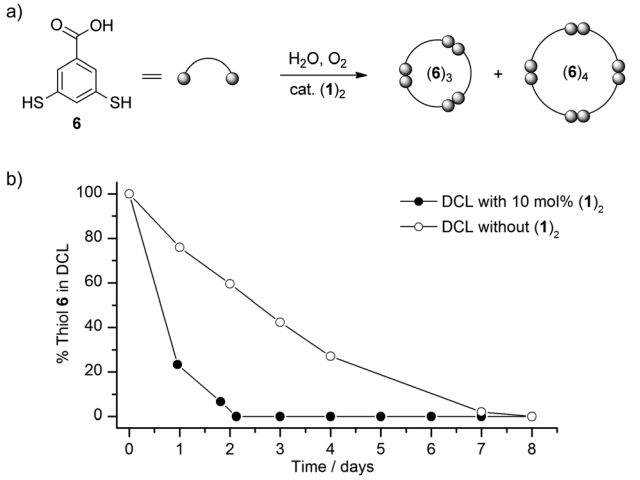

Fig. 4 (a) The studied disulfide based DCL. (b) Graph showing the effect of 10 mol% diselenide $(\mathbf{1})_2$ on the oxidation of thiol (**6**) in the disulfide DCL at pH 7.0. All libraries were studied at 0.5 mM.

disappeared as the catalyst was released. Based on these observations, it appears that the catalytic effect originated from a reaction sequence where a thiol reacts with the diselenide to generate selenenylsulfide $(\mathbf{6})_2(\mathbf{1})_1$ which subsequently reacts efficiently with another thiol to give a disulfide.

To conclude, we have demonstrated for the first time that diselenide based DCLs equilibrate under thermodynamic control at neutral pH and that combinations of diselenide and disulfide based DCLs give mixtures where disulfides, diselenides and selenenylsulfides coexist. We have also shown that diselenides catalyse the formation of disulfide based DCLs at physiological pH. These discoveries pave the way to form DCLs with diselenides and disulfides at truly physiological pH, and currently we are studying how addition of templates affects the distribution of library members under such conditions.

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