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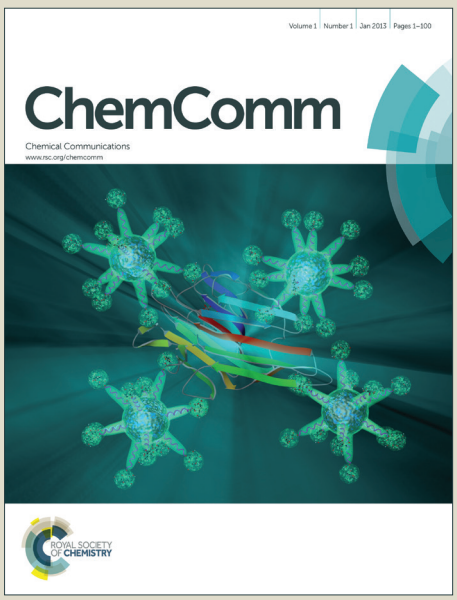





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# Dynamic Combinatorial Chemistry with Diselenides and Disulfides in Water

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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 of them 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 disulfide exchange has some drawbacks; one of them being that the disulfide exchange reaction are most effective at slightly alkaline pH.

Selenols are more acidic than their corresponding thiols,[6] and therefore highly nucleophilic selenolates are present in a broader pH range 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 disulfide exchange.[8] Hence, the diselenide exchange reaction could be an attractive reaction for dynamic combinatorial chemistry in water at physiological pH.

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 DCLs at neutral pH with only 1 mol% diselenide catalyst.

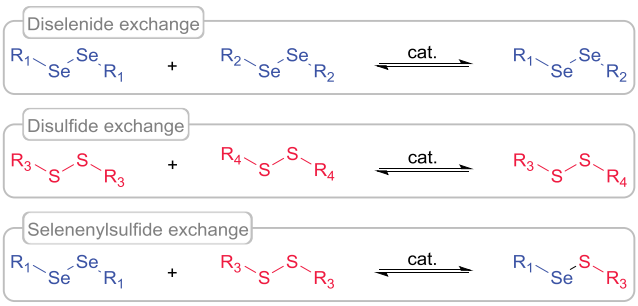


**Fig. 1** Three exchange reactions for dynamic combinatorial chemistry in water that work simultaneously; diselenide exchange (top), disulfide exchange (middle), and selenenylsulfide exchange (bottom).

Disulfide based DCLs are set up by dissolving appropriate thiol-containing building blocks in buffered aqueous solution. Air oxidation of the thiols to disulfides and simultaneous disulfide equilibration take place during DCL formation. This convenient protocol is not directly transferable to diselenide based DCLs because selenols oxidise 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% of the exchange initiator 4-mercaptobenzoic acid (Fig. 2, Fig. S16-S19). Already within a day the three libraries reached the same composition of exchanged diselenides. This study was carried out both in an ammonium acetate buffer at pH 7.6 and in a phosphate buffer at pH 7.9 and in both buffer systems the libraries remained stable for at least 40 days.

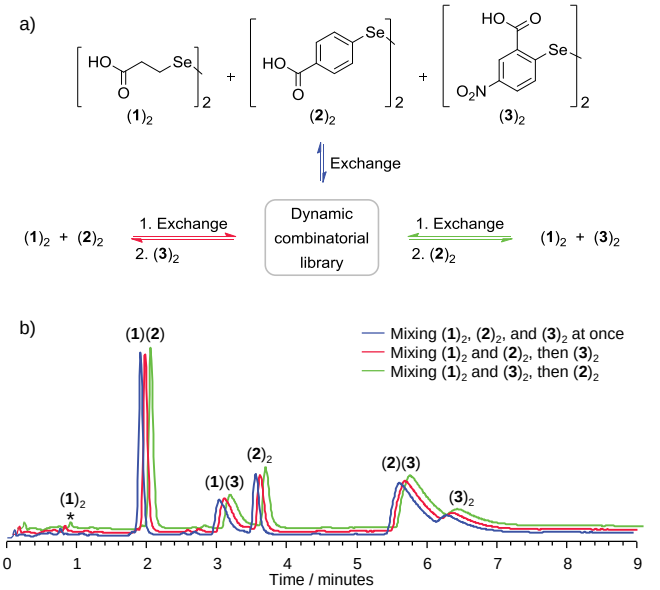


**Fig. 2** a) Setup of diselenide DCLs from different starting points with 20 mol% of 4-mercaptobenzoic acid as initiator in water at pH 7.6. b) HPLC chromatograms (monitored at 290 nm) of the DCLs after equilibration for four days.

Next, the efficiency of the exchange reaction at different pH was studied by carrying out the same type of exchange 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 whereas no exchange was observed at pH 4 and 3. Collectively, these findings show that diselenide based libraries (*i*) can be conveniently set up from diselenides, (*ii*) are efficiently exchanging over at 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 that establish the diselenide exchange as a desirable exchange reaction in dynamic combinatorial chemistry, more complex DCLs were prepared. For this, the 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 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-S21).

To form DCLs with even higher diversity, $(\mathbf{4})_2$ was mixed with the bis-disulfide macrocycle $(\mathbf{5})_2$ in a phosphate buffer (pH 7.8) in the presence of 5-mercaptoisophthalic acid (Fig. 3). The resulting DCL consisted of the 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 exchange 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).

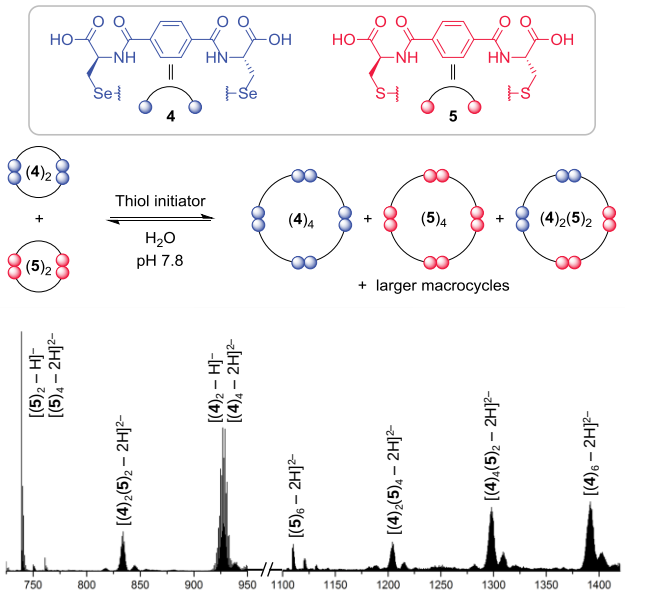


**Fig. 3** Structures of the building blocks (top). Combined diselenide, disulfide, and selenenylsulfide based DCL from the bis-diselenide macrocycle $(\mathbf{4})_2$ and the bis-disulfide macrocycle $(\mathbf{5})_2$ (middle). Partial ESI mass spectrum showing the presence of different sized macrocycles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

The high efficiency of the above diselenide DCLs combined with their interplay with sulfur systems inspired us to study how diselenides could affect disulfide 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 let to 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 formed efficiently in a day at pH 9 and 10, whereas one week of reaction was required 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) 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 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 the selenenylsulfide $(\mathbf{6})_2(\mathbf{1})_1$ which subsequently reacts efficiently with another thiol to give a disulfide.

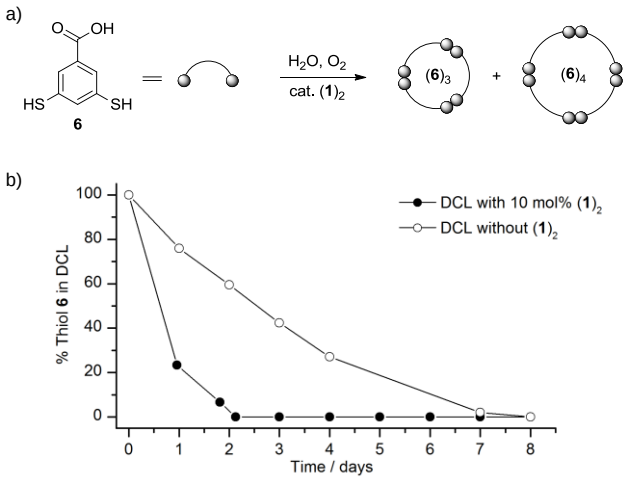


**Fig. 4** (a) The studied disulfide DCL. (b) Graph showing the effect of 10 mol% diselenide $(\mathbf{1})_2$ on the oxidation of thiol (**6**) in the disulfide DCL at pH 7.0. All libraries were studied at 0.5 mM.

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 DCLs and disulfide DCLs gives mixtures where disulfides, diselenides and selenensulfides coexist. We have also shown that diselenides catalyse the formation of disulfide based DCLs under physiological pH. These discoveries pave the way to perform 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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## Notes and references

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Electronic Supplementary Information (ESI) available: Experimental procedures, spectral characterisation data, description of the library preparations, and data from these. See DOI: 10.1039/c000000x/

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