Selection experiments with dynamic combinatorial libraries: the importance of the target concentration

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Experimental data are presented which demonstrate that the target concentration has a strong influence on the outcome of selection experiments with a dynamic mixture of self-assembled receptors.

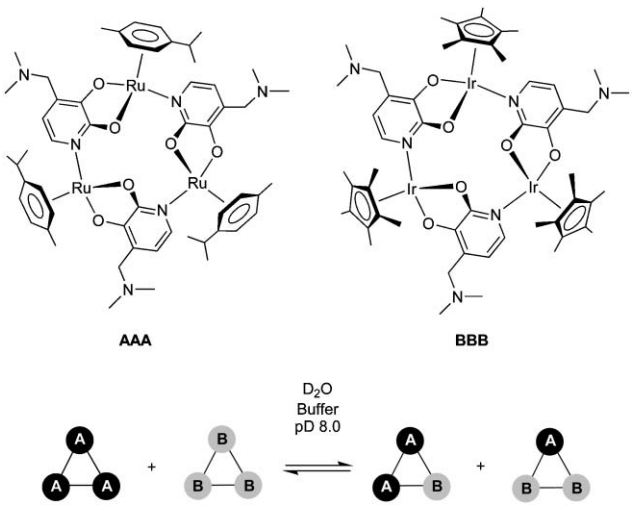
In recent years, dynamic combinatorial chemistry has received considerable attention because it has been successfully employed to identify new receptors, ligands and catalysts, among others.[1] In a typical experiment, a target is added to a dynamic combinatorial library (DCL) of molecular aggregates. If the target shows a differential affinity for the library members, a re-equilibration may occur. During this process, library members with a high affinity for the target are amplified, which allows their identification. It has been proposed that the library member, which binds most tightly to the target is the one that is amplified the most. Recent theoretical[2] and experimental[3] investigations have shown, however, that this is not necessarily the case. Among the various parameters which influence the outcome of a selection experiment with a DCL, the target concentration was found to be of special importance. Computer simulations have shown that a high target concentration may reduce the correlation between binding affinity and amplification factor.[2] In the following we demonstrate for a small model DCL that such a behaviour can indeed be observed.[4]

The experiments were based on the trinuclear metallamacrocyclic complexes AAA and BBB, which were recently described by our group.[5,6] These complexes can be obtained by self-assembly in nearly quantitative yield when the organometallic complexes $[(p\text{-cymene})RuCl_2]_2$ or $[Cp^*IrCl_2]_2$ are dissolved together with the ligand 3-hydroxy-4-dimethylaminomethyl-2-(1*H*)-pyridone in phosphate buffer at pH $\geqslant$ 7. An interesting feature of these complexes is that they represent potent and selective receptors for lithium ions.[5] In aqueous solution, macrocycle AAA displays a binding constant of $K \sim 10^3$ M^{-1} for Li^+ with the exact value being dependent on the pH. The iridium-containing receptor BBB, on the other hand, has a significantly lower affinity for Li^+ ($K \sim 0.1$ M^{-1}), most likely due to the sterically demanding Cp* π-ligands, which block the Li^+ binding site.

In order to use these macrocycles in selection experiments, we have first investigated the scrambling reaction between AAA and BBB (Scheme 1).† If an aqueous solution of AAA (5 mM, D_2O, pD 8.0) was mixed with an aqueous solution of BBB (5 mM, D_2O, pD 8.0), the formation of the mixed species AAB and ABB was observed by ^1H NMR after a few minutes. A stable equilibrium was reached after 20 h. A mixture of identical composition was obtained when the macrocycles were synthesised directly from equimolar amounts of $[(p\text{-cymene})RuCl_2]_2$ and $[Cp^*IrCl_2]_2$. Analysis of this mixture by ^1H NMR spectroscopy revealed a nearly statistical distribution with AAA : AAB : ABB : BBB ~ 1 : 3 : 3 : 1.[7] Accordingly, the ESI mass spectrum showed isotope-resolved peaks corresponding to the four mono-protonated macrocycles with the peaks of the mixed aggregates AAB and ABB being the dominant ones.

The Li^+ binding constants of the two receptors AAA and BBB were determined by ^1H NMR spectroscopy.[8] Under the conditions employed (D_2O, 100 mM K_2HPO_4/KH_2PO_4 buffer, pD 8.0), the macrocycles were found to bind Li^+ with $K_{AAA} = 4.4 (\pm 0.6) \times 10^3$ M^{-1} and $K_{BBB} = 1.1 (\pm 0.2) \times 10^{-1}$ M^{-1}. Based on this data it was expected that the mixed aggregates AAB (one Cp*Ir fragment) and ABB (two Cp*Ir fragments) show a lower Li^+ affinity than the homo-trimer AAA. Previous studies had shown, however, that electronic as well as steric effects influence the binding constants,[5b] which complicates any prediction.

To study the influence of the Li^+ concentration on our small model DCL, we have prepared equilibrated mixtures containing equal amounts of the building blocks A and B (7.5 mM each), the hydroxy-pyridone ligand (15.0 mM) and various amounts of Li_2SO_4 in degassed D_2O (100 mM K_2HPO_4/KH_2PO_4, pD = 8.0) (Scheme 2).‡ Since the ^1H NMR spectra of the resulting mixtures were too complicated to allow a quantitative analysis, we have used ^7Li NMR spectroscopy to analyse the system in a semi-quantitative fashion (Fig. 1).[9]



Scheme 1 In solution, the trinuclear metallamacrocycles AAA and BBB rapidly exchange building blocks to generate a dynamic mixture of homo- and heteroassemblies.

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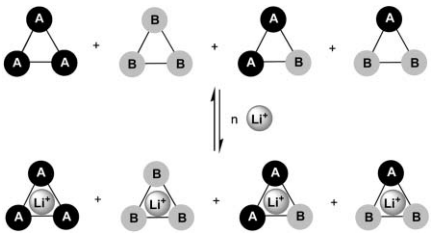

Scheme 2 Addition of Li^+ to a dynamic mixture of macrocyclic receptors AAA, BBB, AAB and ABB.

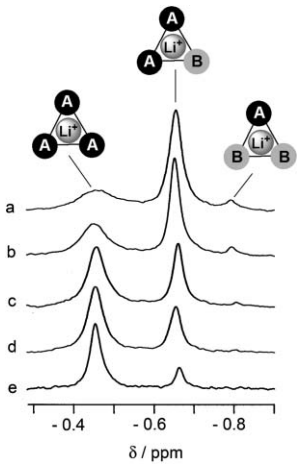

Fig. 1 ^7Li NMR spectra (D_2O) of a dynamic mixture of AAA, AAB, ABB, and BBB (total conc. 5.0 mM) after equilibration with 0.2 (e), 2.5 (d), 5.0 (c), 15 (b), and 40 equiv. of Li^+ (a).

At a low concentration of Li^+ (1.0 mM), signals of two different Li^+ adducts were detected the relative ratio of which was 1.0 : 0.2 (Fig. 1e). The dominant species was the complex [AAA·Li^+] as shown by control experiments with the pure receptor AAA. The second species was the complex [AAB·Li^+] as evidenced by ESI mass spectrometry. Since the target concentration (1.0 mM) was significantly lower than the total receptor concentration (5.0 mM), only a small re-equilibration was expected to occur. The system could thus be regarded as a 'classical' competition experiment from which the macrocycle AAA was identified as the best Li^+ receptor followed by the mixed receptor AAB.

When the Li^+ concentration was increased from 1.0 to 200 mM, the relative intensity of the signals of [AAA·Li^+] and [AAB·Li^+] inversed from 1.0 : 0.2 to 1.0 : 3.9 (Fig. 1a–e). In addition, a third small signal could be detected, which likely corresponds to [ABB·Li^+]. At a high target concentration of 200 mM, the complex [AAB·Li^+] was the dominating Li^+ adduct in solution, clearly outcompeting the homo-trimer AAA. A selection experiment performed under such conditions would therefore identify a good receptor (AAB) but not the best one (AAA). This result is in perfect agreement with recent theoretical studies, which suggest that a high target concentration reduces the correlation between binding affinity and amplification factor.[2]

Another prediction from computer simulations of DCLs is that at high target concentrations, the correlation between binding affinity and amplification factor becomes worse, the higher the average binding energies to the target are.[2] This prediction was also tested with our model DCL. In order to increase the binding constant for the complexation of Li^+, we have performed a second set of experiments using a mixture of CD_3OD and D_2O (30 : 70) as the solvent. It is well known that the binding constants for the complexation of alkali metal ions increase when methanol is used as a solvent instead on water.[10] Experiments performed in this new solvent mixture gave a similar trend as in pure D_2O: at a low Li^+ concentration of 1.0 mM, the dominant Li^+ adduct was [AAA·Li^+] and upon addition of an excess of Li^+ (25 mM), the dominant complex was [AAB·Li^+]. For the latter experiment, however, an AAA : AAB ratio of 1.0 : 1.3 was observed whereas in pure D_2O a ratio of 1.0 : 0.7 was found. This shows that upon addition of an access of Li^+, the best receptor AAA is indeed less favoured in methanol/water.

In summary, we have demonstrated for a simple model DCL comprised of four Li^+ receptors that the target concentration has a strong influence on the outcome of a selection experiment. A high Li^+ concentration was shown to favor a receptor of intermediate affinity for Li^+ and not the best one. In addition, the system was shown to depend on the overall binding constants of the receptors. The importance of these boundary conditions should be considered for the future design of selection experiments with DCLs.

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Notes and references

† Preparation of the model DCL: A degassed D_2O solution of the macrocycle AAA (5.0 mM, 100 mM phosphate buffer, pD 8.0) was mixed with a D_2O solution of the macrocycle BBB (5.0 mM, 100 mM phosphate buffer, pD 8.0) and the composition of the mixture was analysed by ^1H NMR spectroscopy. After 20 h at room temperature, a stable equilibrium was reached. A mixture with an identical composition (^1H NMR) was obtained by adding 2.0 mL degassed D_2O (100 mM phosphate buffer, pD 8.0) to 3-hydroxy-4-dimethylaminomethyl-2-(1*H*)-pyridone (5.0 mg, 30 μmol), $[(cymene)RuCl_2]_2$ (4.6 mg, 7.5 μmol) and $[Cp^*IrCl_2]_2$ (6.0 mg, 7.5 μmol) and then stirring for one night at room temperature.
‡ Selection experiments with Li^+: The experiments were performed by adding Li_2SO_4 (0.50, 6.25, 12.5, 37.5 and 100 mM) to the dynamic mixture of macrocycles described above. The mixtures were then tempered at 40 °C for 40 hours. The relative proportions of the lithium adducts were determined from ^7Li NMR spectroscopy.

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7 The ^{1}H NMR spectrum of the equilibrated mixture showed four signals for the methyl group of the cymene π-ligand ($CH_3C_6H_4{}^{i}Pr$) at $\delta = 1.78$, 1.82, 1.85 and 1.86 ppm. One signal is expected for AAA, one for ABB and two for AAB. The relative intensity of the signals was approximately equal, which points to a ratio of AAA : AAB : ABB = 1 : 3 : 3.
8 Many the signals of the free and the complexed receptor are baseline separated and the exchange of the Li^+ guest is slow on the NMR timescale. This allows us to calculate the association constant by integrating the respective signals.
9 The ^{7}Li spectra were recorded with a relaxation delay time of 500 ms. When this value was increased to 30 s, only small changes in the relative signal intensities were observed indicating similar relaxation times for the various Li^+ complexes.
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