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Kinetically controlled simplification of a multiresponsive [10 × 10] dynamic imine library†

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Kinetically controlled self-sorting processes in complex synthetic mixtures represent an important model for behaviours of biological networks, which operate far from equilibrium and without interference among simultaneous metabolic pathways. However, most of the previously reported kinetic self-sorting protocols dealt with small dynamic libraries and a single external stimulus. Here, we report the iterative simplification of a large imine dynamic combinatorial library (DCL) constructed from 10 aldehydes and 10 anilines, under the sequential influence of an oxidant, an adsorbent, and an increase in temperature. Six components of this initial DCL are mechanically isolated and amplified at least three-fold relative to their equilibrium distributions at the outset of the sorting process.

The growing field of systems chemistry[1] deals with the behaviors of complex chemical systems, focusing on the properties that cannot be traced back to an individual component of the mixture but are instead only observed in the complex mixture. Some of these properties are unexpected, and often referred to as emergent.[2] Many of the emergent phenomena are observed in living systems, and behaviors of synthetic systems are often likened to their living counterparts; chief among these is the adaptability to external stimuli. In the context of response to external stimuli, dynamic combinatorial libraries (DCLs)[3]—that is, collections of molecules that can interconvert through a reversible chemical reaction—are particularly well suited for this study, as the formalism of the Le Châtelier principle allows for stimuli responsiveness even at the level of a simple reaction.

To truly begin approaching living systems, the complexity of synthetic dynamic libraries needs to be increased both in terms of a number of components (biological cells have thousands of chemicals) and their multi-responsiveness—that is, the ability to be addressed by a number of different stimuli. Such an increase in complexity of response could also advance the field of smart materials, including multiresponsive polymers and gels,[4] and low-symmetry self-assembled structures.[5]

We[6] and others[7] have studied kinetically controlled sorting processes in DCLs. Our protocols utilized chemical oxidation,[8] as well as physical processes such as distillation,[9] precipitation,[10] or adsorption,[11] to simplify complex mixtures with as many as 25 members into a handful (five or less) of pure products. In all of these protocols, an irreversible stimulus amplified the imine component of the DCL that best responded to it; that imine subsequently extracted its constituent aldehyde and amine from all other DCL members that shared them. Iterative application of these stimuli resulted in the sorting of DCLs with n^2 members into just n final products.

In this contribution, we report a dramatic increase in complexity of such a sorting methodology: sequential fractionation of a large imine DCL[12]—constructed from 10 aldehyde and 10 aniline precursors—into three discrete imine subsets, during the operation of three external stimuli. Notably, as two of the three stimuli are physical in nature, sorting is coupled with separation techniques, resulting in mechanical isolation of the amplified imines.

We constructed a [10 × 10] imine DCL by heating (65 °C, 12 h) equimolar amounts (0.56 mmol each) of substituted benzaldehydes **A–J** (Scheme 1) and anilines **1–10** in PhMe solution, with H_2O removal. After its equilibration,[13] the composition of this initial library was assessed using a combination of gas chromatography/mass spectrometry (GC/MS) and ^{1}H NMR spectroscopy. In the gas chromatogram, 53 peaks attributed to imines could be identified, while the ^{1}H NMR spectrum of the initial mixture revealed 64 imine-diagnostic N=C–H resonances. While the existence of all 100 theoretically possible imines could not be confirmed, it is reasonable to assume that the number of formed species is higher than 64, as extensive peak overlapping (in both GC and NMR) likely concealed many imines.

The initial irreversible stimulus applied to the mixture of **A1–J10** was oxidation. In our 2012 protocol,[8] iodine was used as a weak oxidant that selectively oxidized electron-rich imines derived from anilines which had an –OH or –NH_2 group in the

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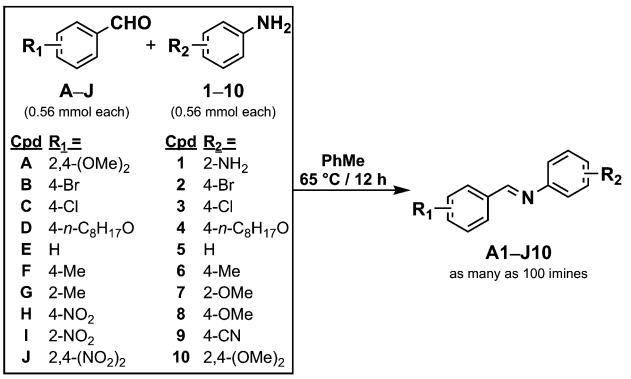
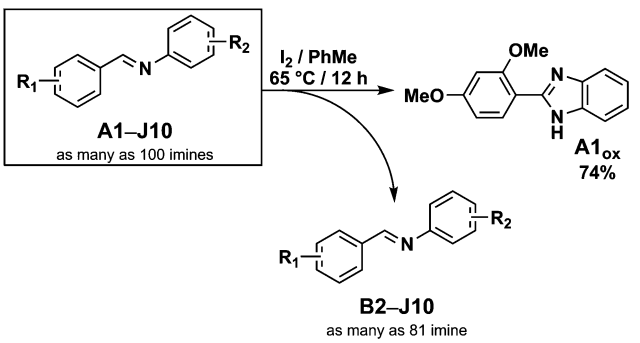


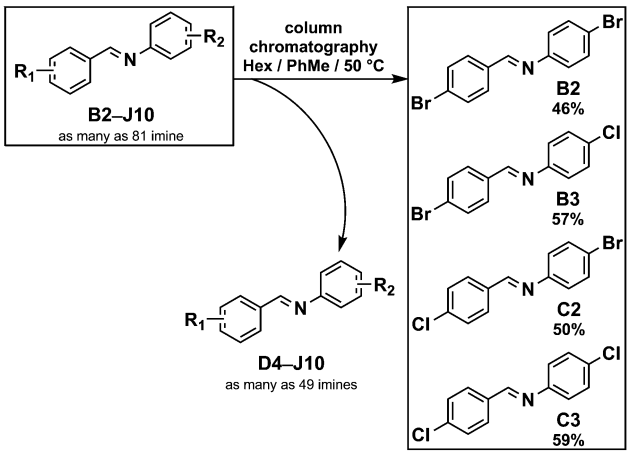
Scheme 1 Synthesis and composition of the starting [10 × 10] imine DCL.

ortho-position relative to the imine linkage. In the hundred-imine DCL studied here, this protocol was expected to selectively target imine **A1**, which was derived from the electron-richest aldehyde **A** and phenylenediamine (**1**). Indeed, upon the slow addition of a 0.05 M solution of I_2 in PhMe (12 h, addition rate 1 mL h^{-1}, 65 °C), benzimidazole $\mathbf{A1_{ox}}$ precipitated as the exclusive product in 74% yield (0.41 mmol, Scheme 2). This finding was remarkable as the ^{1}H NMR spectrum and GC/MS analysis of the initial mixture suggested that the concentration of **A1** was below the detection limit of either technique (presumably on account of the weak electrophilicity of the electron-rich aldehyde **A** and low resonance stabilization of the doubly donor-substituted imine **A1**). Thus, this reaction generated a product whose immediate precursor virtually did not exist in the starting DCL! Removal of **A1** from the DCL also resulted in the disappearance of imines **A2–A10** (which shared the aldehyde component with **A1**) and **B1–J1**, which shared the phenylenediamine component with **A1**. This first oxidation step removed 19 imines from the DCL, resulting in a smaller 81-component DCL.[14]

After the completion of this first oxidation step, the residual imines (composed of aldehydes **B–J** and anilines **2–10**) were re-equilibrated by heating in PhMe for 12 h. The new **B2–J10** DCL was shown by ^{1}H NMR spectroscopy to contain at least 61 imines,[14] and was next subjected to sorting on silica gel based on its members' affinity for this adsorbent (which correlates with polarity). The crude



Scheme 2 Slow addition of iodine into the DCL composed of **A1–J10** leads to the isolation of $\mathbf{A1_{ox}}$ in 74% yield and formation of a simplified 81-imine DCL composed of **B2–J10**.



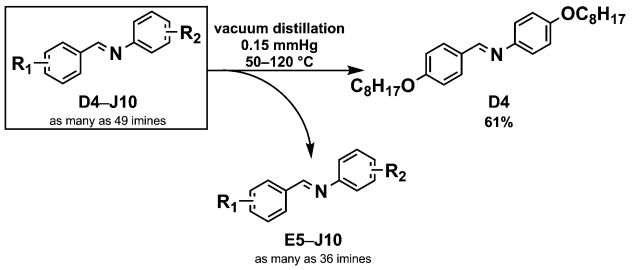
Scheme 3 Column chromatography of DCL composed of **B2–J10** on silica gel amplifies imines **B2–C3**.

mixture was pre-adsorbed onto oven-dried silica gel (2.2 g), and the solid mixture was carefully placed on top of a chromatography column (inner diameter: 12.7 mm), which was previously loaded with a 20 cm-high layer of oven-dried silica gel. The column was wrapped with heating tape and heated to ~50 °C. The imine mixture was eluted first with a 10 : 1 mixture of hexane and PhMe (v/v), and then with an 8 : 1 mixture of the same solvents (Scheme 3).

The first eluted fraction contained a [2 × 2] sub-library of least polar imines, composed of **B2** (0.13 mmol, 46%), **B3** (0.16 mmol, 57%), **C2** (0.14 mmol, 50%), and **C3** (0.17 mmol, 59%).[15] According to the GC-based estimate of the relative distributions of imine components in the initial **A1–J10** library, imines **B2–C3** were present in the amounts of 0.044 mmol (**B2**), 0.021 mmol (**B3**), 0.020 mmol (**C2**), and 0.049 mmol (**C3**). After the column chromatography, all of these imines were amplified, with the amplification factors being 3.0 for **B2**, 7.6 for **B3**, 7.0 for **C2**, and 3.5 for **C3**. It should be noted that minor amounts of some other imines (**G2**: 0.05 mmol, **G3**: 0.06 mmol, **G5**: 0.03 mmol, **G7**: 0.003 mmol, **B6**: 0.018 mmol, and **C6**: 0.014 mmol) were also observed in the first eluted fraction, suggesting that aldehyde **G** was close in its polarity to **B** and **C**.

The dominant elution of imines **B2–C3** led to the dramatic lowering of concentrations (but not complete disappearance) of all other imines that shared either the aldehyde or the aniline component with them.

In the final step of this iterative self-sorting procedure, the mixture of residual imines was subjected to vacuum distillation. Prior to this step, the imines were left to reequilibrate, by heating for 12 h in PhMe in a flask equipped with a Dean–Stark adapter. PhMe was removed, leaving behind an oily dark brown mixture, which was set to distill *in vacuo* (50–120 °C, 0.15 mmHg). After 12 d, the light imines were distilled away, leaving as the distillation residue only the least volatile imine **D4**, which was isolated in 61% yield (0.34 mmol), along with minor amounts of **J4** (0.13 mmol) and **H4** (0.008 mmol). In the initial library, imine **D4** was present in the amount of only 0.092 mmol, corresponding to approx. 3.7-fold amplification (Scheme 4).



Scheme 4 Slow distillation of imine DCL **D4–J10** removes all but the least volatile imine **D4** from the distillation flask; **D4** is amplified in the process and isolated in 61% yield.

In conclusion, we have demonstrated that sequential application of three irreversible stimuli—chemical oxidation, chromatography on silica gel, and vacuum distillation—can result in iterative simplification of an imine DCL with one hundred components into just six preferred products. This set of experiments also allowed side-by-side comparison of the fidelity of these three self-sorting techniques. Chemical oxidation results in the highest amplification of its preferred substrate from the most complex initial library. Distillation and chromatography showed more moderate but still synthetically useful amplification factors. Our future work will explore whether multiple chemical stimuli can be applied to a high-complexity DCL. We will seek to answer the question: how many different chemical reactions can find their preferred substrate in ''messy'' precursor mixtures analogous to **A1–J10** DCL? Results of our studies will be reported in due course.

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Notes and references

1 (*a*) E. Mattia and S. Otto, *Nat. Nanotechnol.*, 2015, **10**, 111; (*b*) K. Ruiz-Mirazo, C. Briones and A. de la Escosura, *Chem. Rev.*, 2014, **114**, 285; (*c*) Web-themed issue of *Chem. Commun.*, 2014, **50**, 14924; (*d*) R. F. Ludlow and S. Otto, *Chem. Soc. Rev.*, 2008, **37**, 101; (*e*) J. J. P. Peyralans and S. Otto, *Curr. Opin. Chem. Biol.*, 2009, **13**, 705.

2 V. Darley, Emergent Phenomena and Complexity, *Artificial Life IV: Proceedings of the Fourth International Workshop on the Synthesis and Simulation of Living Systems*, 1994.

3 (*a*) M. E. Belowich and J. F. Stoddart, *Chem. Soc. Rev.*, 2012, **41**, 2003; (*b*) S. Otto, *Acc. Chem. Res.*, 2012, **45**, 2200; (*c*) J. N. H. Reek and S. Otto, *Dynamic Combinatorial Chemistry*, Wiley-VCH, Weinheim, Germany, 2010; (*d*) P. T. Corbett, J. Leclaire, L. Vial, K. R. West, J.-L. Wietor, J. K. M. Sanders and S. Otto, *Chem. Rev.*, 2006, **106**, 3652; (*e*) S. J. Rowan, S. J. Cantrill, G. R. L. Cousins, J. K. M. Sanders and J. F. Stoddart, *Angew. Chem., Int. Ed.*, 2002, **41**, 898.

4 For reviews and select examples, see: (*a*) P. Schattling, F. D. Jochum and P. Theato, *Polym. Chem.*, 2014, **5**, 24; (*b*) G. Pasparakis and M. Vamvakaki, *Polym. Chem.*, 2011, **2**, 1234; (*c*) J. B. Beck and S. J. Rowan, *J. Am. Chem. Soc.*, 2003, **125**, 13922; (*d*) K. Jie, Y. Zhou, B. Shia and Y. Yao, *Chem. Commun.*, 2015, **51**, 8461.

5 (*a*) M. Schmittel, S. De and S. Pramanik, *Org. Biomol. Chem.*, 2015, **13**, 8937; (*b*) M. L. Saha, N. Mittal, J. W. Bats and M. Schmittel, *Chem. Commun.*, 2014, **50**, 12189; (*c*) M. L. Saha and M. Schmittel, *J. Am. Chem. Soc.*, 2013, **135**, 17743; (*d*) K. Mahata, M. L. Saha and M. Schmittel, *J. Am. Chem. Soc.*, 2010, **132**, 15933; (*e*) K. Mahata and M. Schmittel, *J. Am. Chem. Soc.*, 2009, **131**, 16544. For a recent example of a multiresponsive DCL, see: (*f*) A. G. Orrillo, A. M. Escalante and R. L. E. Furlan, *Chem. – Eur. J.*, 2016, **22**, 6746.

6 (*a*) Q. Ji, R. C. Lirag and O. Š. Miljanić, *Chem. Soc. Rev.*, 2014, **43**, 1873; (*b*) K. Osowska and O. Š. Miljanić, *Synlett*, 2011, 1643.

7 (*a*) P. Mukhopadhyay, P. Y. Zavalij and L. Isaacs, *J. Am. Chem. Soc.*, 2006, **128**, 14093; (*b*) K. L. Morris, L. Chen, J. Raeburn, O. R. Sellick, P. Cotanda, A. Paul, P. C. Griffiths, S. M. King, R. K. O'Reilly, L. C. Serpell and D. J. Adams, *Nat. Commun.*, 2012, **4**, DOI: 10.1038/ncomms2499; (*c*) J. W. Sadownik and D. Philp, *Angew. Chem., Int. Ed.*, 2008, **47**, 9965; (*d*) P. Vongvilai and O. Ramström, *J. Am. Chem. Soc.*, 2009, **131**, 14419. For reviews, see: (*e*) M. M. Safont-Sempere, G. Fernández and F. Würthner, *Chem. Rev.*, 2011, **111**, 5784; (*f*) Z. He, W. Jiang and C. A. Schalley, *Chem. Soc. Rev.*, 2015, **44**, 779; (*g*) M. L. Saha and M. Schmittel, *Org. Biomol. Chem.*, 2012, **10**, 4651. It should be noted that there are some differences in the various definitions of self-sorting presented in the literature. Sorting events described in this work may not fall under the strictest definition of self-sorting, as they occur not by themselves but instead under the influence of external stimuli. We nevertheless use the term ''self-sorting'' to describe them, as the mixtures still sort because of their inherent properties (exhibited under a given stimulus) rather than an intervention of an intelligent ''sorter''.

8 K. Osowska and O. Š. Miljanić, *J. Am. Chem. Soc.*, 2011, **133**, 724.

9 (*a*) Q. Ji and O. Š. Miljanić, *J. Org. Chem.*, 2013, **78**, 12710; (*b*) Q. Ji, N. S. El-Hamdi and O. Š. Miljanić, *J. Chem. Educ.*, 2014, **91**, 830; (*c*) K. Osowska and O. Š. Miljanić, *Angew. Chem., Int. Ed.*, 2011, **50**, 8345. See also: (*d*) A. Herrmann, *Chem. – Eur. J.*, 2012, **18**, 8568; (*e*) A. Herrmann, *Org. Biomol. Chem.*, 2009, **7**, 3195.

10 R. C. Lirag, K. Osowska and O. Š. Miljanić, *Org. Biomol. Chem.*, 2012, **10**, 4847.

11 C.-W. Hsu and O. Š. Miljanić, *Angew. Chem., Int. Ed.*, 2015, **54**, 2219.

12 For an example of a treatment of a large (but simulated) DCL, see: P. T. Corbett, S. Otto and J. K. M. Sanders, *Chem. – Eur. J.*, 2004, **10**, 3139.

13 Equilibration of this and subsequent DCLs was confirmed by following the changes in the DCLs' ^{1}H NMR spectra: once no further changes were observed, it was assumed that the mixture was at equilibrium. Typical equilibration times were 6–12 h in PhMe at elevated temperatures.

14 Same caveat applies to this DCL as to the initial one: the existence of all 81 imines could not be unambiguously confirmed, but is highly logical.

15 Yields of these compounds were determined using 1,3,5-trimethoxybenzene as an internal standard in ^{1}H NMR spectroscopy, and are reported relative to the theoretical situation in which **B2**, **B3**, **C2**, and **C3** were all in 1 : 1 : 1 : 1 ratio, with 0.28 mmol of each imine.