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Catalyst Screening Using an Array of Thermistors**

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The use of combinatorial techniques to discover and optimize chemical and biological catalysts is becoming increasingly widespread.[1] Methodology for the production of catalyst libraries is now advanced, particularly in the case of biological catalysts with the advent of DNA shuffling and error-prone PCR.[2] Efficient screening methodology is less advanced; although the literature abounds with ingenious screens contrived to suit a particular reaction there are few general screens. Recently IR thermography has emerged as a potentially general technique to monitor reactions associated with a temperature change.[3] Several groups, notably those of Reetz[3a, 3b] and Morken,[3c] have demonstrated the power of IR thermography in catalyst screening, but the method suffers from a number of problems including relatively low resolution of temperature change (± 10 mK), the requirement for visualization to be effected through IR-transparent materials, and inconvenient data analysis techniques. In pioneering work Danielsson and co-workers have demonstrated the use of thermistors to monitor temperature changes downstream of an immobilized enzyme in a continuous flow of substrate.[4] We now report that a multiplexed array of thermistors can be used as an alternative to IR thermography for chemical and biochemical catalyst screening.

The apparatus we constructed is shown in Figure 1. A commercially available 96-well plate dispenser (Multi-

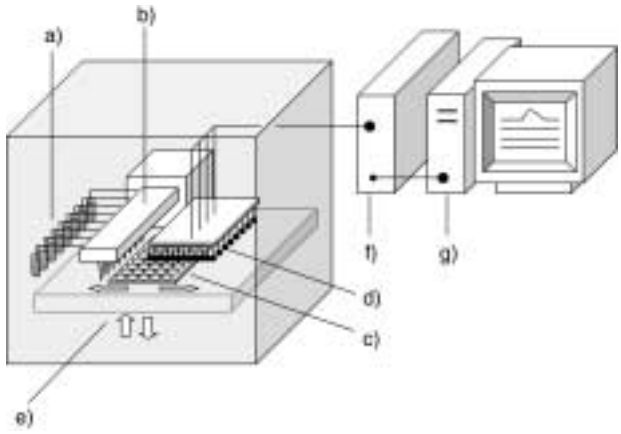

Figure 1. Thermistor array apparatus comprising: a) 8 reagent/substrate reservoirs, b) an 8-channel dispensing head, c) a 96 well plate and holder (movable in *x*-dimension), d) an 8×12 thermistor array, e) a base plate (movable in *z*-dimension), f) a multiplexing apparatus, g) a microcomputer.

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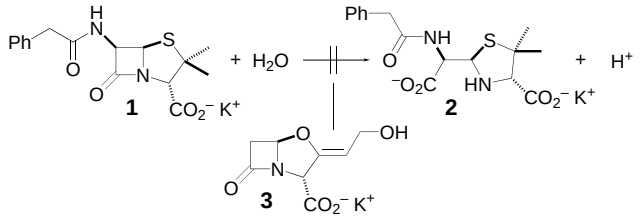
drop 384 from Labsystems) is used to dispense up to eight different solutions into predetermined wells of a plate containing the other reaction components. After shaking the plate briefly to effect mixing, the plate is moved horizontally to position it under the thermistor array. Mechanical raising of the plate then causes immersion of the thermistors into the centers of all the wells and the temperature changes are detected as resistance changes in the multiplexed thermistors. The dispensing apparatus and thermistor array are housed in an incubator to enable thermal pre-equilibration of reaction components prior to mixing. We have found it convenient to monitor the temperature prior to the addition and mixing (a process, which typically takes of the order of 10 s) as well as during the subsequent reaction. Data are recorded as numerical files for each channel and can be displayed simultaneously on-screen during the reaction. Application of a zeroing procedure to the data just before mixing makes it easiest to detect active catalysts by eye; alternatively an electronic management system can be used to select those channels where temperature exceeds a pre-set threshold. These features taken with the capacity for plates to be changed robotically suggest that the method could easily be adapted to high-throughput. As a representative biological reaction we chose the β-lactamase-catalyzed hydrolysis of penicillin G (**1**) to pencilloate (**2**), a reaction known to be moderately exothermic (Scheme 1).[5] Six wells of a microtiter



Scheme 1. β-Lactamase-catalyzed hydrolysis of **1** to **2** and its inhibition by **3**.

plate were charged with a solution of β-lactamase in phosphate buffer, with the remaining wells containing only buffer. The plate dispenser was primed with a solution of **1**, the plate was loaded, and the apparatus allowed to equilibrate at 37 °C before the addition of **1** and mixing. The output of the apparatus is shown in Figure 2: the coloured lines correspond

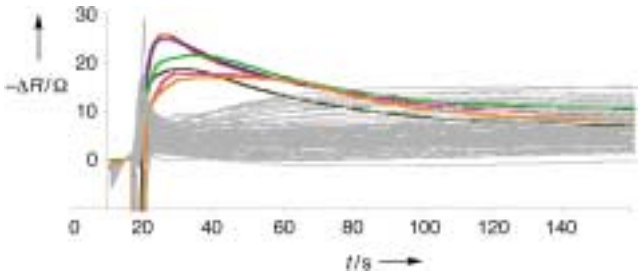


Figure 2. Time-resolved output (resistance change) of the apparatus during the screening of a plate with six wells containing β-lactamase in buffer (500 IU) and the remaining wells containing buffer alone. Reaction was initiated by the addition of **1** to all wells (to a final concentration of 0.06 M) 20 s after recording was started. After recording, the output of all channels was zeroed at a point corresponding to about 10 s before initiation.

to those wells that contain the enzyme and are clearly distinguishable from the remaining channels. Some degree of quantitation is possible by measuring the maximum temperature attained in each well. A plate containing β-lactamase at various concentrations was monitored after the addition of **1** (Figure 3). The enzyme was serially diluted left to right in

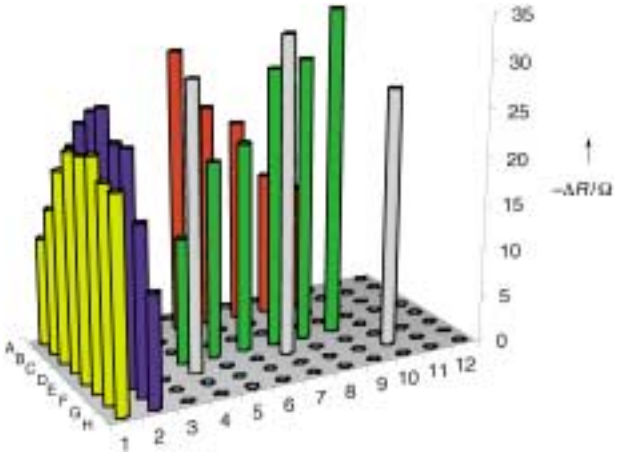


Figure 3. The maximum increase in temperature (as indicated by resistance change) for selected wells in a plate during an experiment in which the amount of β-lactamase was varied (red and green), and the effect of **3** on the hydrolysis of **1** to **2** was investigated (blue and yellow). Three positive enzyme controls are shown in gray.[8]

row B and right to left in row E to minimize the effects of temperature gradients in the incubator. As can be clearly seen the maximum temperature attained correlates with the concentration of enzyme in each well. Screening for active catalysts amidst a background of inactive or less-active catalysts is crucial to directed evolution experiments in biology using DNA shuffling. Our results suggest that screening with thermistor arrays should be a general method for those biochemical reactions associated with at least a modest temperature change.

In drug discovery there is considerable interest in general high-throughput enzyme-inhibitor screening. To demonstrate the use of thermistor arrays in this regard we examined the inhibition of β-lactamase by potassium clavulanate (**3**).[6] The maximum temperature attained during the hydrolysis of **1** to **2** was determined in the presence of various amounts of **3** (Figure 3). Potassium clavulanate (**3**) was serially diluted from the back to the front of the plate in column 1 and from the front to the back in column 2, again to minimize the effects of any adventitious temperature gradients in the incubator. The effects of enzyme inhibition are clearly visible and the data resemble inhibitor titration data measured by more conventional techniques. This observation suggests that the method could be used not just for inhibitor discovery but also for a crude ranking of inhibitory efficiency, thus making it a promising technique for adaptation to high-throughput enzyme-inhibitor screening.

In their seminal work on IR-thermographic catalyst screening Reetz and co-workers investigated the enantioselectivity of the hydrolysis of epoxides catalyzed by transition metal ion complexes of salen (salen = *N*,*N*′-bis(salicylidene)ethylenedi-

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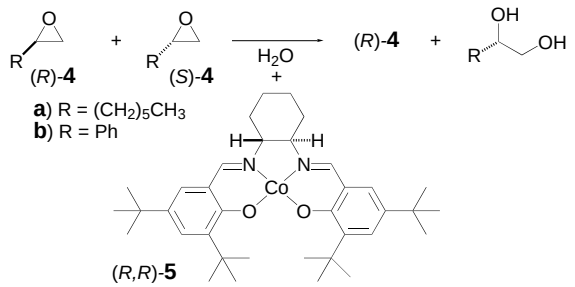
amine dianion).[3a] To compare our thermistor array methodology with IR thermography for chemical catalyst screening we therefore decided to investigate the same chemical system. Optically pure cobalt(II)–salen complexes are efficient and highly enantioselective catalysts for the hydrolysis of terminal epoxides (Scheme 2).[7] Initially we investigated the effect of



Scheme 2. Catalytic enantioselective hydrolysis of terminal epoxides using the cobalt(II)–salen system.[7]

catalyst concentration on the hydrolysis of *rac*-1,2-epoxyoctane (*rac*-**4a**) by the cobalt(II)–(*R*,*R*)-salen catalyst ((*R*,*R*)-**5**). Different amounts of (*R*,*R*)-**5** were added to the wells of a microtiter plate containing *rac*-**4a** at a fixed concentration (Figure 4). The reaction was initiated by dispensing a solution

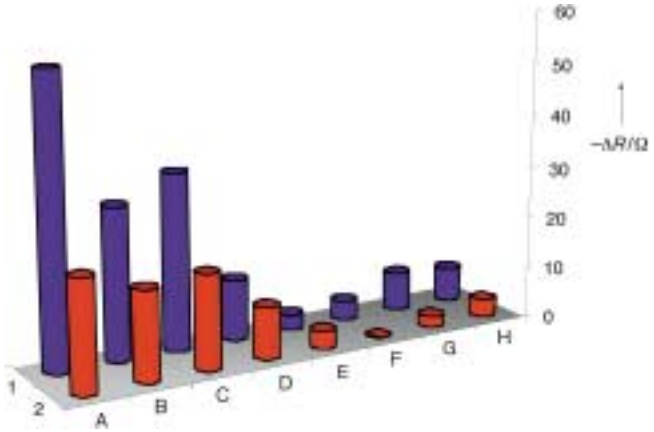


Figure 4. Effect of decreasing the concentration of catalyst (left to right, blue) and substrate (left to right, red) for the hydrolysis of *rac*-**4a** catalyzed by (*R*,*R*)-**5**.[9]

of water in *rac*-1,2-hexanediol (30% (v/v)) into each well. The temperature of each reaction was then monitored for 120 min. The maximum temperature in each well was observed after approximately 45 minutes and showed a reasonable correlation with the concentration of the catalyst (Figure 4). An experiment to investigate the effect of varying the concentration of the substrate with a fixed concentration of catalyst was also performed in the same microtiter plate. Again the apparatus was capable of distinguishing between the maximum temperatures attained in each reaction.

To demonstrate the capability of the method to monitor enantioselective catalysis (*R*,*R*)-**5** and (*S*,*S*)-**5** were individually mixed with each enantiomer of styrene oxide (**4b;** Figure 5). As expected, on addition of water, the temperatures increased most in the cases where the chirality of the catalyst matched that of the substrate. That reaction only took place in the matched cases was demonstrated by ^{1}H NMR spectroscopy. The small temperature increases observed in the nonmatched cases are possibly a result of mixing effects or thermal nonequivalence of the reaction components prior to mixing.

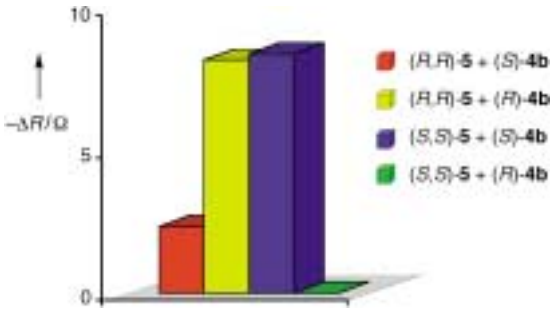


Figure 5. Demonstration of the enantioselectivity obtained using the thermistor array. Water was added to wells containing the catalyst (3.3 μM) and substrate (6.3 M) in toluene.

In conclusion we have demonstrated that thermistor arrays are an attractive alternative to IR thermography for the detection of temperature changes associated with chemical and biochemical reactions. Our method is more sensitive than IR thermography (100 μK changes can be reliably detected), can be carried out in a closed incubated system, and produces data that are easily processed and are amenable to operation at high-throughput. Furthermore it has the scope to be fully automated and the inexpensive thermistor array can easily be removed and replaced with similar arrays of probes to monitor, for example, pressure, pH, dissolved oxygen, or specific ions. Further advances in miniaturization should allow the construction of arrays of multiple probes to monitor several of these variables simultaneously.

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[9] The plate was prepared with a solution of *rac*-**4a** at 4.7 M in *rac*-1,2-hexanediol in wells A1-H1; 4.3 M in A2; 3.7 M in B2; 3.3 M in C2; 2.7 M in D2; 2.3 M in E2; 1.7 M in F2; 1.3 M in G2, and 0.7 M in H2 and with (*R*,*R*)-**5** at 5.8 μM in A2-H2 and A1; 4.9 μM in B1; 4.1 μM in C1; 3.3 μM in D1; 2.5 μM in E1; 1.7 μM in F1; 0.8 μM in G1; and 0 μM in B1. Reaction was initiated by the addition of water.

$[Cu(2\text{-pyrazinecarboxylato})_2HgI_2]\cdot HgI_2$: An Open Noninterpenetrating $Cu^{II}-Hg^{II}$ Mixed-Metal Cuboidal Framework Encapsulating Nearly Linear HgI_2 Guest Molecules**

Yu-Bin Dong, Mark D. Smith, and Hans-Conrad zur Loye*

Self-assembly of organic ligands and inorganic metal ions is one of the most efficient and widely utilized approaches for the construction of organic/inorganic coordination polymers. During the past decades unimetallic coordination polymers, which contain only one kind of metal center, have been the predominant synthetic target in this rapidly expanding area of research.[1] To date, numerous unimetallic coordination polymers with impressive structural motifs have been successfully synthesized. In contrast, the chemistry of bimetallic coordination polymers has received considerably less attention, although bimetallic extended structures based on inorganic counterions such as cyanide (Prussian blue phases) and thiocyanate have been reported.[2]

Bimetallic coordination polymers have the potential to exhibit interesting physical properties such as electrical conductivity or magnetic ordering that result from interactions between two distinct metal centers connected by a suitable linker. For example, complex magnetic behavior in $Cu^{II}-Mn^{II}$ mixed-metal coordination polymers was reported by Kahn et al.[3] Likewise, a series of mixed-metal molecular magnets $[Cp^*_2Z^{III}][M^{II}M^{III}(ox)_3]$ (Z^{III} = Co, Fe; M^{III} = Cr, Fe; M^{II} = Mn, Fe, Co, Cu, Zn; $Cp^* = \eta^5$-C_5Me_5) based on bridging oxalato (ox) ligands was reported by Coronado et al.[4] Of course, novel physical properties are not the only reason for pursuing the synthesis of bimetallic coordination polymers; propagation of the structural preferences (influenced by oxidation state and coordination predisposition) of two different metal centers in mixed-metal systems should result in a broader palette of polymer structural motifs than is achievable in unimetallic systems, and this may in turn lead to better understanding of composition – structure relationships.

We have initiated a synthetic program for the preparation of mixed-metal coordination polymers, in which the concepts of self-assembly and metal-containing building block ligands were combined to yield a series of novel bimetallic framework structures. These materials are based on [M(2-pyrazinecarboxylato)$_2$]$\cdot n\,H_2O$ and [M(methylpyrazine-5-carboxylato)$_2$]$\cdot n\,H_2O$ ($M = Cu^{II}$, Co^{II}, Ni^{II}, Zn^{II}; $n = 0$, 2, 3) and were synthesized by treating these metal-containing ligands with a variety of metal salts.[5] Here we report on the first hydrothermally synthesized $Cu^{II}-Hg^{II}$ mixed-metal framework material, namely, [Cu(2-pyrazinecarboxylato)$_2$HgI$_2$]$\cdot$ HgI_2 (**1**), which is constructed from [Cu(2-pyrazinecarboxylato)$_2$] building blocks and HgI_2 linkers. This neutral, cuboidal framework structure possesses regular square channels (dimensions 7.24 × 7.24 Å)[6] in which, surprisingly, uncoordinated, nearly linear HgI_2 molecules are encapsulated. While it is quite common to find solvated species in the void spaces of coordination polymers, we believe the presence of a free inorganic salt to be quite novel.

Reaction of [Cu(2-pyrazinecarboxylato)$_2$] (**2**) or [Cu(2-pyrazinecarboxylato)$_2$]$\cdot 2\,H_2O$ (**3**)[5b, 7] with HgI_2 (1:2 molar ratio) in water under hydrothermal conditions (130 °C, 24 h), afforded the title compound as blue-green orthorhombic plate crystals in quantitative yield. Single-crystal structure analysis[8] revealed a three-dimensional extended cuboidal framework based on [Cu(2-pyrazinecarboxylato)$_2$] and HgI_2 building blocks. Each Cu^{II} center has a slightly distorted $\{CuN_4O_2\}$ octahedral coordination sphere, with two nitrogen donors and two oxygen donors of two 2-pyrazinecarboxylato ligands in the equatorial plane. The coordination sphere is completed by the terminal nitrogen donors of two neighboring [Cu(2-pyrazinecarboxylato)$_2$] building blocks in the axial positions. The framework Hg^{II} centers reside in a distorted $\{HgI_2O_2\}$ tetrahedral coordination environment that is composed of two iodide ions (Hg–I 2.603(2), 2.633(2) Å; I-Hg-I 158.79(6)°) and two oxygen atoms of two adjacent [Cu(2-pyrazinecarboxylato)$_2$] building blocks (Hg–O 2.532(9) Å). The two copper-bound carboxylate oxygen atoms interact very weakly with the Hg^{II} centers (Hg···O 3.119(4) Å). The Cu^{II} and Hg^{II} centers are linked by the carboxylate moiety of 2-pyrazinecarboxylate in a bis-monodentate fashion to form linear [Cu(2-pyrazinecarboxylato)$_2$HgI$_2$] mixed-metal chains (Figure 1). Identical chains, rotated along the chain direction by 90°, are interconnected through Cu^{II} nodes into a three-dimensional cuboidal network. As shown in Figure 2, each cuboidal box consists of eight Cu^{II} atoms at the corners, which are connected by four long carboxylate-Hg-carboxylate (Cu···Cu 9.99(4) Å) and eight shorter pyrazine (Cu···Cu 7.24(4) Å) linkers that make up the 12 edges. This cuboidal $Cu^{II}-Hg^{II}$ structural motif is clearly different from those of

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