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Dyad beads and the combinatorial discovery of catalysts†

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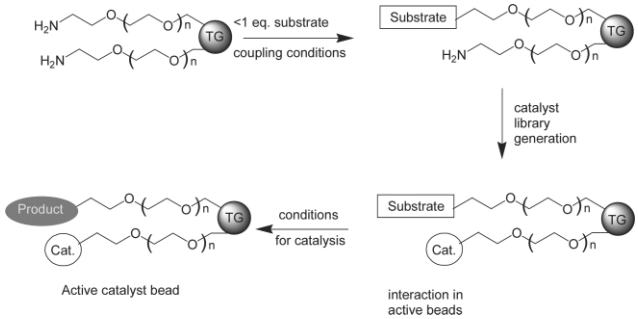
Dyad beads, bearing both a substrate and a catalyst, were prepared to enable direct split and mix bead based screening for catalysis.

Since its introduction combinatorial chemistry, in a variety of formats, has been extensively applied to the discovery of new pharmaceutical agents but it is only relatively recently that a range of combinatorial techniques, ranging from parallel synthesis to more sophisticated single bead processes, have been used for the discovery and development of new catalysts.[1–4] One of the pioneers in the field was Ellman who reported the synthesis of a small library of substituted 2-pyrrolidinemethanols[5] as ligands for the enantioselective addition of diethylzinc to aldehydes but this area has dramatically expanded since this time. This has included, for example, the generation of 'randomly' substituted polyallylamines with known mixtures of carboxylic acids and parallel screening for reduction, dehydration and phosphatase activities;[6] the extensive work of Hoveyda,[7] who has used libraries of Schiff base modified dipeptides as ligands to catalyse the addition of TMSCN to cyclohexene oxide in the presence of Ti(O*i*Pr)$_4$; the work of Jacobsen,[8] who used parallel synthesis and iterative positional scanning to investigate ligands for alkene epoxidation catalysts, to name but a few. Others have used a variety of parallel combinatorial techniques which have included capillary array electrophoresis,[9] electrospray mass spectrometry,[10] and CD-HPLC[11] to develop combinatorial processes for catalyst synthesis and screening.[12]

Recent advances in infra-red imaging have allowed the development of a method for the detection of active catalysts by thermographic imaging. Originally reported by Moates for the investigation of ignition temperatures of a small library of metal doped aluminas,[13] the technique has since been used by Morken and Reetz,[14] while Sutherland developed a 96 position array of thermistors as a flexible method of reaction temperature monitoring, without the need to use IR transparent materials.[15] A fluorescent assay for high throughput catalyst analysis was first described by Hartwig,[16] while Miller used a pH sensitive fluorophore to detect acid formed during a catalytic acyl transfer kinetic resolution on resin.[17]

Here we describe the development of a technique to allow the generation and screening of on-bead catalyst libraries prepared using split and mix techniques. The key component of this strategy was a method that would allow the evaluation of catalytic efficiency of compounds attached to single beads in the presence of the rest of the library and relied on the ability of compounds attached to the ends of the PEG chains on TentaGel resin to interact with each other within the same bead.[18] Thus by partially substituting TentaGel with a substrate for a catalytic reaction, and using the remaining free sites on this resin to generate a library of catalysts, catalytically active library members would be able to promote the reaction of substrate molecules mounted on the same bead by site-to-site interactions (Scheme 1). If the reaction being investigated was chosen such that the modification of the substrate under catalysed conditions led to a colour change, then beads containing active catalysts would display this colour change, allowing them to be selected from the library.

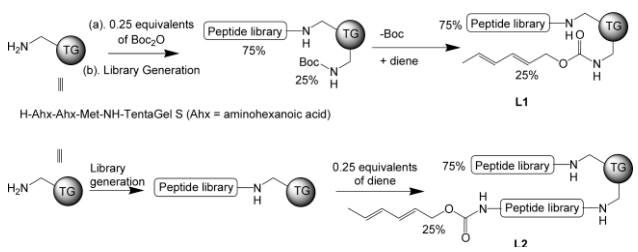


Scheme 1 Proposed method for the screening of catalytic activity within a split and mix library.

The Diels–Alder reaction was chosen for proof of principle studies. Thus (*E*,*E*)-2,4-hexadien-1-ol was attached to TentaGel resin *via* a urethane bond while *N*-fluorescein-5-yl maleimide was used as the dienophile.

Two, random, tripeptide libraries were prepared. In the first case the resin was partially substituted with a diene (25%), and the remaining free resin sites were used to generate the peptide library, while in the second case a portion of the *N*-termini of the peptides were capped with the diene (Scheme 2). The libraries were examined prior to screening and were found not to fluoresce (excitation between 450 and 490 nm with a suppression filter to block emissions below 515 nm).

Libraries were screened for catalytic activity against a range of controls consisting of unsubstituted TentaGel resin, an identical peptide library on TentaGel but with no diene attached and just the diene attached onto TG . Both libraries (L1) and (L2) were screened for Diels–Alder catalytic activity by treatment of a sample from each library with 0.01 equiv. of labelled maleimide in DMF and DCM and the uptake of the dye by the library beads was monitored under a microscope. Almost immediately several beads from each library were observed to be significantly fluorescent (Fig. 1) and these beads were photographed and picked for amino acid analysis. None of the control samples of resin showed any uptake apart from the diene labelled resin that took several days to react. Importantly samples from both libraries took up the dye to similar extents, suggesting that both were equally suited for the detection of



Scheme 2 Synthesis strategy for the two, random, 1000 member dyad bead libraries.[19] The diene was incorporated by the use of (*E*,*E*)-2,4-hexadienyl(4-nitrophenyl) carbonate.

† Iain Linguard, PhD Thesis, University of Southampton, September 2002.

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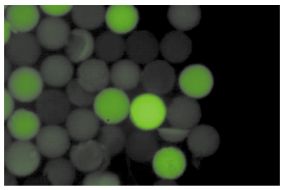

Fig. 1 Beads under catalyst screening conditions. It can be clearly seen that some beads have taken up the dye to a far greater level than their neighbours ($\times$ 100).

catalysts. Secondly, DCM was a better solvent for the reaction, leading to higher levels of dye uptake (measured by fluorescent imaging).

Twenty active beads were picked and submitted for Edman sequencing. From these beads fifteen sequences were obtained. Table 1 shows the results from each "sequenceable" bead.

The high degree of correlation between L1 and L2 for the first two positions in the library supports the screening technique as a detection method for catalysis and shows that the uptake of the dye was clearly dependent on the peptide sequence, and was not a random occurrence. Indeed the presence of arginine groups indicates Brønstead acid catalysis, known catalysts for Diels–Alder chemistry, while there might also be some proximity effects caused by interactions with the dye and the peptide on the bead. The third position was found to be much more random (less important?), indeed from seven beads isolated from the reactions carried out in DMF only 1 bead had arginine at the *N*-terminal position, with a predominance of hydrophobic residues. However when screening in DCM 50% of the residues identified at the *N*-terminus were arginine.

Having established a method for monitoring the reaction, experiments were carried out to investigate the catalytic activity of the compounds identified from the catalyst library screening. Thus the resin bound peptide[20] (Arg_3-Ahx-Ahx-Met) was prepared by solid phase synthesis and examined as a catalyst for the Diels–Alder reaction between hexadienyloxycarbonyl benzylamine and *N*-(5-fluoresceinyl) maleimide.

Equimolar solutions of *N*-(5-fluoresceinyl) maleimide and hexadienyloxycarbonyl benzylamine (3.5 mM in water/THF) were split into two portions and allowed to react at room temperature. The first portion was allowed to react without catalyst (control) and to the second was added 10 mol% of the solid phase catalyst. Aliquots were taken at regular intervals and analysed by RP-HPLC. The reaction was observed to be second order, *k* for each experiment were determined and showed a rate acceleration of 3.4 fold over the uncatalysed reaction (a similar rate enhancement was determined for the solution peptide Arg_3-εAhx-εAhx-Met-NH_2, Ahx = aminohexanoic acid).

In conclusion a new method for the discovery of catalysts from combinatorial libraries, which relies on site to site substrate–catalyst interaction within flexible PEG based TentaGel resin, has been developed and used to examine a split and mix library of small peptides for activity as Diels–Alder catalysts. Peptide sequences obtained from the "hits" were found to have a high degree of correlation. The hits were synthesised and analysis undertaken to determine the level of activity of these compounds as Diels–Alder catalysts. The compound was found to be a catalyst for the Diels–Alder reaction between *N*-(5-fluoresceinyl) maleimide and hexadienyloxycarbonyl benzylamine although the rate accelerations observed were poor, presumably due to the very high local concentration found within beads. These results show that the technique described for the discovery of new catalysts from large split and mix libraries could be a valuable new method in the combinatorial discovery and development of catalysts. The technique can readily be adapted to any catalytic bond forming reaction, as long as one substrate can be immobilised onto a solid support and the other labelled with a dye.[21]

Table 1 Sequencing results from 15 active catalyst beads.

(Library), Solvent	AA^3	AA^2	AA^1
(L1), DMF	Phe	Arg	Arg
(L1), DMF	Gln	Arg	Arg
(L1), DMF	Ser	Gly	Arg
(L1), DMF	Phe	Arg	Arg
(L2), DMF	Val	Leu	Arg
(L2), DMF	Arg	Val	Arg
(L2), DMF	Ala	Arg	Gln
(L2), DCM	Arg	Gln	Arg
(L1), DCM	Gly	Arg	Arg
(L1), DCM	Arg	Arg	Arg
(L1), DCM	Leu	Arg	Arg
(L2), DCM	Ala	Arg	Arg
(L2), DCM	Arg	Leu	Leu
(L2), DCM	Gln	Val	Arg
(L2), DCM	Arg	Ala	Ala
Consensus Sequence	Arg (5)	Arg (8)	Arg (12)

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19 Library synthesis was carried out using Fmoc chemistry and a random split and mix process on H-Ahx-Ahx-Met-NH-TentaGel S resin using ten Fmoc amino acids (Fmoc-Phe-OH, Fmoc-Ala-OH, Fmoc-Ser-(OtBu)-OH, Fmoc-Gly-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Trp-OH, Fmoc-Val-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Leu-OH, Fmoc-Gln-OH) in equal ratios. Removal of the terminal Fmoc group and the side chain protecting groups (TFA/H_2O/PhOH/TIPS) was followed by capping 25% of the sites with (*E*,*E*)-2,4-hexadienyl(4-nitrophenyl) carbonate to give library (L2). For library (L1) 25% of the sites on the resin were initially capped with a Boc group before split and mix synthesis. Following Boc deprotection, the 25% of the remaining amino groups on the resin were capped with (*E*,*E*)-2,4-hexadienyl(4-nitrophenyl) carbonate to give after removal of the *N*-terminal Fmoc group library (L1).
20 The catalyst was synthesised by solid phase peptide synthesis on aminomethyl TentaGel resin using the Fmoc strategy. The arginine groups were introduced with their side chains protected with the 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) group and peptides were deprotected with TFA/water/TIPS (95 : 2.5 : 2.5).
21 A recent paper was published describing a similar concept: P. Krattiger, C. McCarthy, A. Pfaltz and H. Wennemers, *Angew. Chem., Int. Ed.*, 2003, **42**, 1722–1724.