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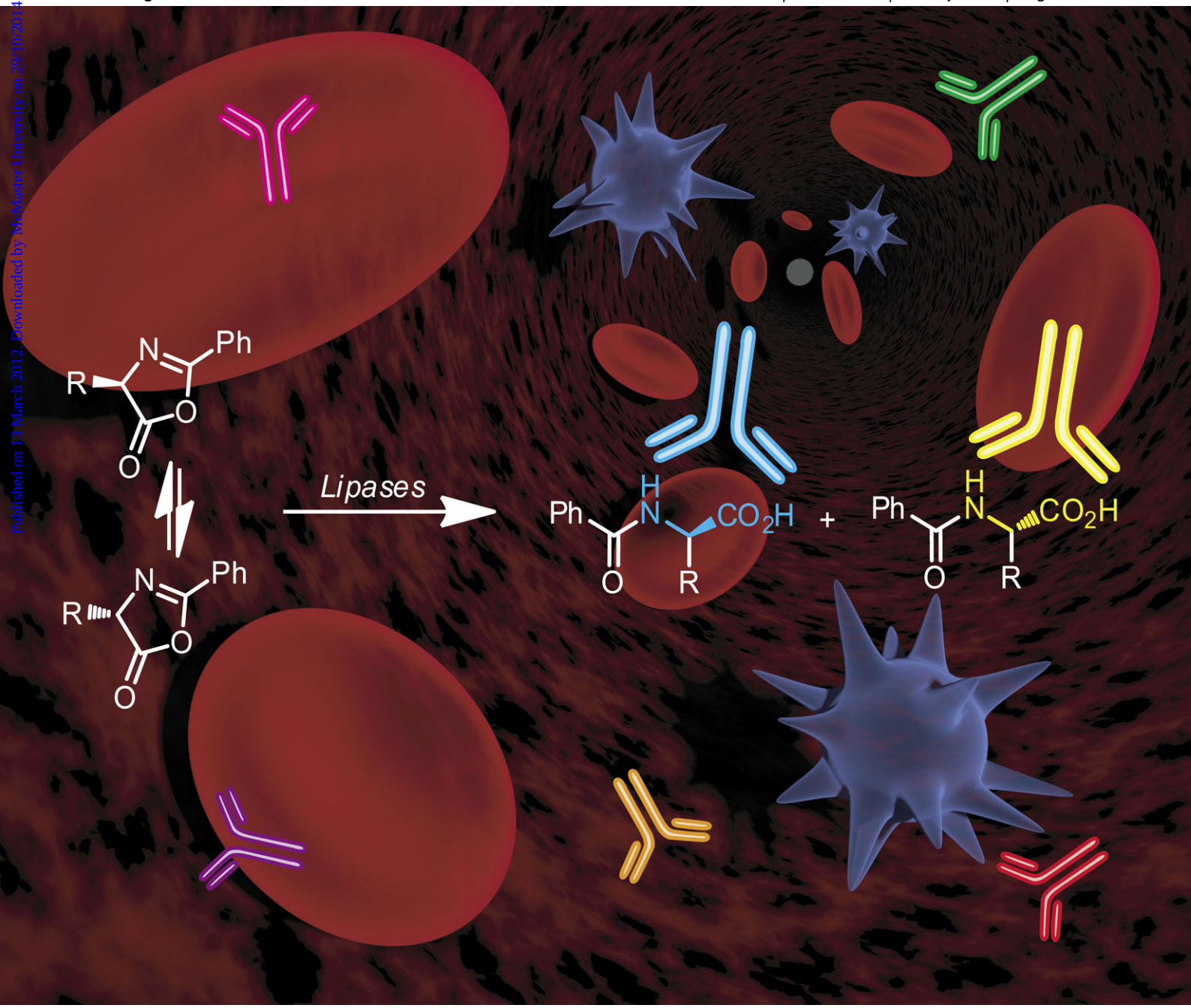

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Polyclonal antibodies: a cheap and efficient tool for screening of enantioselective catalysts†

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Enantioselective polyclonal antibodies have been produced and characterized to develop a high-throughput screening method for lipase activity fingerprinting, with a view to the enantioselective hydrolysis of azlactones.

Combinatorial and parallel methods have become an important focus of research in catalysis. In particular, techniques allowing the simultaneous screening of numerous catalyst candidates have gained much attention over the past two decades as they accelerate the identification and optimization of interesting catalysts.[1] To fully realize the potential of combinatorial approaches to catalyst discovery, general and powerful high-throughput screening (HTS) methods are essential.[2]

A promising but still under-exploited HTS method relies on the use of antibody-based techniques (*i.e.* immunoassays) routinely used in the field of diagnosis but rarely exploited to solve chemical problems. Pioneering work in this field was done by D. S. Tawfik and D. Hilvert's groups who developed a so-called Cat-ELISA technique to identify biocatalysts.[3] Variants of this technique were then developed to reveal protease or esterase activities.[4] An interesting property of antibodies is their capacity to selectively bind chiral molecules. Stereoselective binding of antibodies was first demonstrated in 1928 when Karl Landsteiner developed the classic understanding of immunochemical molecular recognition.[5] Since this pioneering work, the capacity of antibodies to discriminate enantiomers was surprisingly largely ignored by the chemical community and antibody-based approaches for the detection of chiral compounds were developed only recently. Nowadays, the use of monoclonal antibodies (mAbs) as chiral selectors for the development of immunosensors allowing the precise and sensitive detection of enantiomeric impurities is largely demonstrated.[6]

During the course of our work dealing with the development of antibody-based screening techniques for the monitoring of chemical reactions,[7] our laboratory showed that enantioselective catalyst libraries can be easily screened by competitive immunoassays (ELISA).[8] Although very powerful (throughput >1000 yields and ee determination per day), this method requires preparation of mAbs which involves time-consuming and expensive techniques. Furthermore, since mAbs generally display very specific binding properties, few analytes can be detected. As a consequence, ELISA using mAbs allows catalyst screening of only a few, structurally close substrates and therefore cannot evaluate the substrate scope of catalysts.

On the other hand, polyclonal antibodies (pAbs) raised against small molecules (haptens) are easily obtained from serum after immunization of animals using hapten–protein conjugates. Although constituted of thousands of specific and non-specific antibodies, examples in the literature prove that pAbs displaying enantioselective binding properties can be obtained.[9] It should therefore be possible to develop immunoassays based on the use of enantioselective pAbs allowing the high-throughput screening of enantioselective catalysts. In this contribution we show that such a method can be elaborated at minimum cost and in only a few weeks.

The critical point for the development of such immunoassays is the preparation of pAbs with appropriate binding specificity. These pAbs should exhibit high chiral recognition while binding a panel of analytes. In other words, the binding selectivity of pAbs needs to be focused on the chiral center, while low binding specificity to other parts of the analyte structure is required. It is well known that the site of hapten–protein linkage orients antibody specificity.[10] Based on these data, we decided to produce stereoselective pAbs raised against *N*-Bz amino acids by immunizing rabbits with BSA-coupled optically pure *N*-Bz (*R*)- or (*S*)-lysine. The linking of the lysine haptens to BSA was carried out through the amine function in order to maximize exposure of the chiral center. After a few weeks, rabbit sera were collected and assayed for their ability to bind *N*-Bz (*R*)- and (*S*)-lysine. Standard ELISA experiments highlighted the excellent enantioselectivity of these pAbs (Fig. 1). Relative affinities (B/Bo 50% = $K_{d,\ app} \approx 0.1$ μM) of pAbs were in favor of the injected enantiomer by almost three orders of magnitude.

Control experiments proved that this high degree of stereoselectivity allowed ee determination of (*R*)/(*S*) mixtures of *N*-Bz-lysine with a precision of ±2% (Fig. 2).

We then looked at the scope of the method by determining the binding properties of pAbs using a panel of *N*-Bz amino

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† Electronic supplementary information (ESI) available: Experimental procedure for screening, complete screening results and analytical data of products. See DOI: 10.1039/c2cc31312j

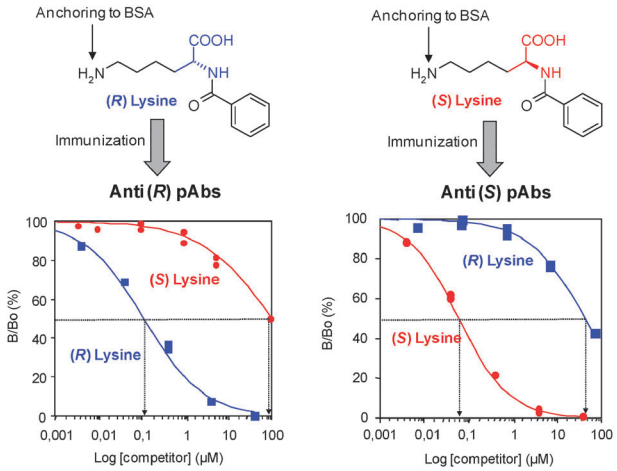


Fig. 1 Competitive ELISA of (*R*)- and (*S*)-*N*-Bz-lysine with pAbs (see ESI† for details).

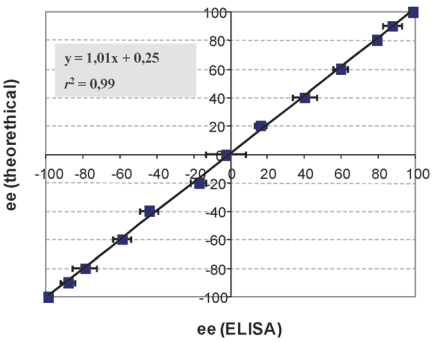


Fig. 2 ee measurements of (*R*) and (*S*)-*N*-Bz-lysine mixtures by ELISA using stereoselective pAbs.

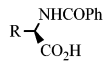
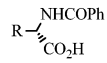
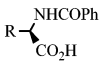
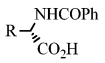
acids. As depicted in Table 1, the pAbs did not bind only lysine derivatives, but exhibited good affinities and high enantiomeric specificities for a series of aliphatic or aromatic *N*-Bz amino acids.

As the side chains of *N*-Bz amino acids are structurally different from the lysine hapten, lower binding affinities were observed as expected. However, in all cases the binding properties of the pAbs toward all tested *N*-Bz amino acids were good enough to allow the development of a screening procedure for the fast identification of enantioselective catalysts.

To this end, we were interested in the enantioselective ring opening of azlactones by water using lipases. This particular biocatalyzed dynamic kinetic resolution reaction leading to *N*-Bz amino acids has been previously reported under buffered conditions.[11] Because spontaneous hydrolysis of azlactones might cause low ee values,[12] we were interested in exploiting lipase activity in organic solvents. 11 azlactones were thus reacted with 9 lipases in 5 anhydrous solvents in the presence of 3 equivalents of H_2O. A series of experiments were also carried out in the presence of TEA to facilitate racemization. To look at possible spontaneous hydrolysis, control experiments performed without lipase were also carried out.

A total of 660 experiments were thus conducted in parallel and screened in one day with two jointly run ELISAs (one using anti-(*R*) pAbs and the other using anti-(*S*) pAbs).

Table 1 Enantioselective binding properties of pAbs

R	Anti (*R*) pAbs, $K_{d,app}$[a]/μM		Anti (*S*) pAbs, $K_{d,app}$[a]/μM	
				
	0.25	96.61	58.02	0.16
	0.11	40.80	25.60	0.04
	0.06	167.45	103.91	0.30
	0.43	150.23	150.62	1.42
	0.06	10.05	175.68	0.09
	0.03	2.74	13.35	0.19
	0.67	125.36	117.75	0.16
	0.53	141.25	148.69	0.81
	0.21	33.62	39.30	0.10
	0.23	111.82	185.24	0.19
	0.08	4.47	108.62	0.14
	0.14	92.42	43.52	0.07

[a] $K_{d,app}$ determined by competitive ELISA (B/Bo = 50%; see ESI).

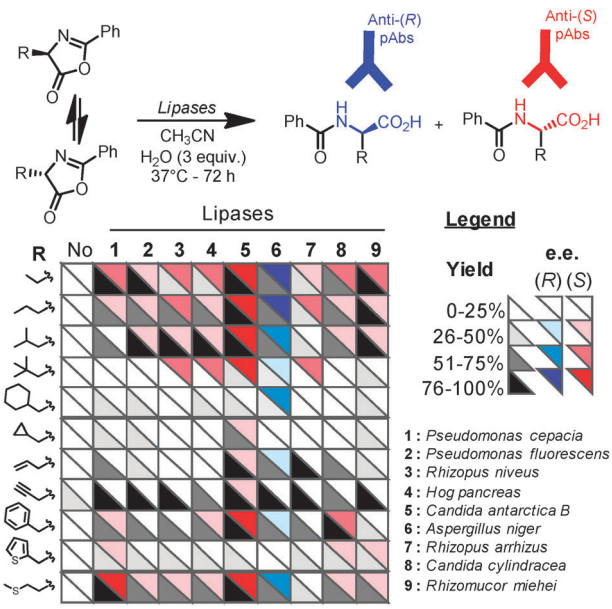


Fig. 3 Activity fingerprinting of 9 lipases for the water-promoted ring opening of azlactones.

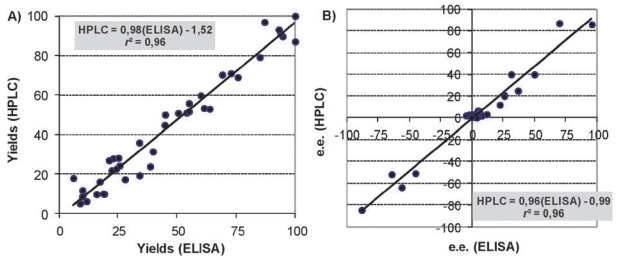
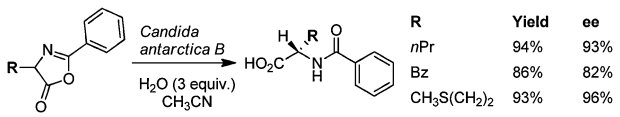


Fig. 4 HPLC/ELISA correlations for yields (A) and ee determination (B).



R	Yield	ee
*n*Pr	94%	93%
Bz	86%	82%
$CH_3S(CH_2)_2$	93%	96%

Scheme 1 Dynamic kinetic resolution of azlactones by *Candida antarctica B* (isolated yields, ees determined by chiral HPLC).

The screening revealed a strong influence of the solvent nature on the lipase activities and a dramatic effect of TEA which decreased both yields and ee (see ESI† for complete results). The best results, obtained in CH_3CN, are summarized in Fig. 3.

To confirm the validity of the method, 40 samples were randomly chosen across the range of ee and yield values and reanalyzed by chiral HPLC. The ELISA and HPLC measurements varied on average by ~10% and afforded excellent linear correlations (Fig. 4).

A key advantage of the method is the small quantity of substrate and enzyme needed for the screen. The sensitivity of the ELISA detection is such that 20 μL-scale reactions were run with only 10 μmoles of substrate and less than 0.1 mg of lipases. This method is therefore highly adapted to biocatalysts screening and should advantageously complete the panel of already described techniques.[13]

Candida antarctica B was the lipase that displayed broader substrate tolerance and generated the best yields and ee results. The activity of this enzyme was reproduced on a mmol scale reaction using three different azlactones. As expected, the desired *N*-Bz-amino acids were isolated in high yields and ees (Scheme 1).

In summary, we report a screening method that has at least two major advantages: it is cheap and fast to develop (stereoselective pAbs can be obtained in only 2 weeks, see ESI†) and it allows exploration of the scope of enantioselective catalysts using a panel of substrates. Since antibodies can be raised against virtually any kind of compound, we think that the technique can be extended to a panel of interesting chemical reactions.

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