Electronic Supplementary Information (ESI)

Associative Chemosensing by Fluorescent Macrocycle-Dye Complexes – A Versatile Enzyme Assay Platform Beyond Indicator Displacement

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Materials and Methods

All starting materials were purchased from Sigma Aldrich and used as received unless stated otherwise. Peptides were purchased from BIOSYNTAN GmbH. CB8 was synthesized according to literature procedures[1] but can also be purchased from Strem or Sigma. MDPP[2] was synthesized from 1,3,8,10-tetrahydro-2,9-dimethyl-2,9-diazadibenzo<cd,lm>perylene,[3] according to literature procedures.[2] Likewise, MDAP[4] was synthesized from 1,3,6,8-tetrahydro-2,7-dimethyl-2,7-diazapyrene,[3] following the DDQ-oxidation procedure for MDPP.[2] PDI was synthesized according to literature procedures.[5] UV/vis spectra were recorded on a Varian Cary 4000 UV-Vis spectrophotometer and fluorescence spectra were recorded on a Cary Eclipse spectrofluorimeter.

Determination of binding constants by fluorescence titrations

Spectroscopic titrations were carried out in H_2O unless stated otherwise. To a solution of the host-dye reporter-pair was added stepwise a solution of the analyte and the fluorescence or UV/Vis spectra were recorded. The normalized emission at 450 nm (CB8•MDAP), 510 nm (CB8•MDPP), or 550 nm (CB8•PDI) were fitted with an equation for a 1:1 binding by a least-squares fit.[13] The resulting K_a values are reported in Table S1. Note that the high host-dye binding affinities correspond to an almost quantitative degree of host-dye complexation, such that the subsequent binding of the analyte can be treated independently.

Experimental details for the investigated enzymatic reactions

Oxidation of phenols, anilines, and naphthols by horseradish peroxidase (HPR, from Armoracia rusticana): Temperature: 22°C. Medium: 10 mM sodium phosphate buffer, pH 4.0, with 120 µM NaI and 100 µM H_2O_2. Substrates: each at 46 µM. Enzyme: 0.6 µg ml^{-1}. Receptors: a) CB8•MDAP (5 µM). Wavelengths: λ_{exc} = 420 nm; λ_{em} = 450 nm. b) CB8•MDPP (5 µM). Wavelengths: λ_{exc} = 441 nm; λ_{em} = 510 nm and c) CB8•PDI (5 µM). Wavelengths: λ_{exc} = 500 nm; λ_{em} = 550 nm. See Fig. 1a in the main text and Fig. S3.

Oxidation of Trp by horseradish peroxidase (HPR, from Armoracia rusticana): Temperature: 22°C. Medium: 10 mM sodium phosphate buffer, pH 4.0, with 120 µM NaI and 100 µM H_2O_2. Substrates: Trp (0-24 µM). Enzyme: 12 µg ml^{-1}. Receptor: CB8•MDAP (5 µM). Wavelengths: λ_{exc} = 420 nm; λ_{em} = 450 nm. The pH-rate dependence was determined with 20 µM Trp, 120 µM NaI, 100 µM H_2O_2.and in 12 µg ml^{-1} HRP in 10 mM sodium phosphate buffer adjusted with HCl to pH 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0. See Fig. 1b in the main text.

Oxidation of catechol (1,2-DHB) by horseradish peroxidase (HPR, from Armoracia rusticana): Temperature: 22°C. Medium: 10 mM sodium phosphate buffer, pH 4.0, with 120 µM NaI and 100 µM H_2O_2. Substrates: 1,2-DHB (0-200 µM). Enzyme: 0.24 µg ml^{-1}, 0.6 µg ml^{-1} and 1.2 µg ml^{-1}. Receptor: CB8•MDAP (5 µM). Wavelengths: λ_{exc} = 420 nm; λ_{em} = 450 nm (Fig. S1). The enzyme concentration-rate dependence was determined with 46 µM 1,2-DHB, 120 µM NaI, and 100 µM H_2O_2 in 10 mM sodium phosphate buffer pH 4.0 and 0.24-12 µg ml^{-1} HRP (Fig. S2).

Oxidation of Trp by lactoperoxidase (LAP, from bovine milk): Temperature: 22°C. Medium: 10 mM sodium phosphate buffer, pH 5.0, with 120 µM NaI and 100 µM H_2O_2. Substrate: Trp 20 µM. Enzyme: 0.06-6.0 µg ml^{-1}. Receptor: CB8•MDPP (5 µM). Wavelengths: λ_{exc} = 441 nm; λ_{em} = 510 nm (Fig. S4).

Oxidation of thioanisole by chloroperoxidase (CPO, from C. fumago): Temperature: 22°C. Medium: 10 mM sodium phosphate buffer, pH 4.5, with 100 µM NaCl and 200 µM H_2O_2. Substrate: thioanisole (0-20 µM). Enzyme: 25 µg ml^{-1}. Receptor: CB8•MDAP (5 µM). Wavelengths: λ_{exc} = 420 nm; λ_{em} = 450 nm (Fig. 1c in the main text).

Oxidation of 4-chlorophenol by laccase (from T. versicolor) and mediators: Temperature: 22°C, Medium: 10 mM sodium phosphate buffer, pH 5.0. Substrate: 4-chlorophenol (20 µM). Enzyme: 80 µg ml^{-1}. Mediators: 2,2'-azino-bis(3-

ethylbenzothiazoline-6-sulphonic acid (ABTS, 0-120 μM) or N-Hydroxyl-phthalimide (NHA, 0-100 μM). Receptor: CB8•MDAP (5 μM). Wavelengths: λ_{exc} = 420 nm; λ_{em} = 450 nm (Fig. S5).

Oxidation of 4-methoxybenzalcohol by alcohol dehydrogenase (ADH, equine): Temperature: 22°C. Medium: 10 mM sodium phosphate buffer, pH 7.5. Substrate: 4-methoxybenzalcohol (100 μM). Enzyme: 3.75 μg ml^{-1}. Cofactor: nicotinamide adenine dinucleotide (NAD^+, 1 mM). Receptor: CB8•MDAP (5 μM). Wavelengths: λ_{exc} = 420 nm; λ_{em} = 450 nm. (Fig. 1d in the main text).

Reduction of benzaldehyde by alcohol dehydrogenase (ADH, equine): Temperature: 22°C. Medium: 10 mM sodium phosphate buffer, pH 7.5. Substrate: benzaldehyde (80 μM). Enzyme: 3.75 μg ml^{-1}. Cofactor: reduced nicotinamide adenine dinucleotide ($NADH,H^+$, 1 mM). Receptor: CB8•MDAP (5 μM). Wavelengths: λ_{exc} = 420 nm; λ_{em} = 450 nm. (Fig. S6)

Hydrolysis of bovine serum albumin (BSA) by pepsin (porcine): Temperature: 22°C. Medium: 10 mM sodium phosphate buffer, pH 2.0. Substrate: bovine serum albumin (BSA, 80 μg ml^{-1}). Enzyme: 0-7 μg ml^{-1}. Receptor: CB8•MDDP (5 μM). Wavelengths: λ_{exc} = 441 nm; λ_{em} = 510 nm (Fig. 1f in the main text).

Hydrolysis of AlaPhe, AspPhe, TrpGlyGly, and GlyTrpGly by L-leucine aminopeptidase Type IV (LAP, from porcine kidney): Temperature: 37°C. Medium: 10 mM sodium buffer, pH 8.0. Substrate: (20 μM). Enzyme: 50 μg ml^{-1}. The enzyme was incubated in 1 mM $MgCl_2$ solution at 37°C for 2 h. Receptor: CB8•MDDP (5 μM). Wavelengths: λ_{exc} = 441 nm; λ_{em} = 510 nm (Fig. 1e in the main text and Fig. S7).

Hydrolysis of Trp–Leu_6–NH_2 by L-leucine aminopeptidase Type IV (LAP) (from porcine kidney): Temperature: 22°C. Medium: 10 mM sodium buffer, pH 8.0. Substrate: Trp–Leu_6–NH_2 (40 μM). Enzyme: 50 μg ml^{-1}. The enzyme was incubated in 1 mM $ZnCl_2$ or $MgCl_2$ solution at 37°C for 2 h. Receptor: CB8•MDDP (5 μM). Wavelengths: λ_{exc} = 441 nm; λ_{em} = 510 nm (Fig. S8).

Hydrolysis of hippuryl-L-phenylalanine (Hip-Phe) by carboxypeptidase A, Type II (from bovine pancreas): Temperature: 22°C. Medium: 10 mM sodium buffer, pH 8.0. Substrate: Hip-Phe (50 μM). Enzyme: 10 μg ml^{-1}. The enzyme was incubated in 1 mM NaCl for activation. Receptor: CB8•MDDP (5 μM). Wavelengths: λ_{exc} = 441 nm; λ_{em} = 510 nm (Fig. S9).

Hydrolysis of phenyl acetate by porcine liver esterase (PLE, porcine): Temperature: 22°C. Medium: 10 mM sodium phosphate buffer, pH 7.0. Substrate: phenyl acetate (5-80 μM). Enzyme: 1.25 μg ml^{-1}. Receptor: CB8•MDAP (5 μM). Wavelengths: λ_{exc} = 420 nm; λ_{em} = 450 nm (Fig. S10 and Fig. 1g in the main text).

Hydrolysis of phenyl phosphate and naphthyl phosphate by alkaline phosphatase (ALKP, from bovine intestinal mucosa): Temperature: 37°C. Medium: 10 mM sodium phosphate buffer, pH 7.5. Substrate: phenyl- (5-50 μM) or naphthyl phosphate (5-30 μM). Enzyme: 16 μg ml^{-1}. Receptor: CB8•MDAP (5 μM). Wavelengths: λ_{exc} = 420 nm; λ_{em} = 450 nm (Fig. S11)

Hydrolysis of benzylpenicillin by penicillinase (from bacillus cereus): Temperature: 25°C. Medium: 10 mM sodium phosphate buffer, pH 7.8. Substrate: benzylpenicillin (500 μM). Enzyme: 0.2 μg ml^{-1}. Receptor: CB8•MDAP (5 μM). Wavelengths: λ_{exc} = 420 nm; λ_{em} = 450 nm (Fig. S12).

Hydrolysis of phenyl-β-D-galactopyranoside by β-galactosidase (β-gal) (from A. oryzae): Temperature: 22°C. Medium: 10 mM sodium phosphate buffer, pH 5.0. Substrate: phenyl-β-D-galactopyranoside (10-50 μM). Enzyme: 43 μg ml^{-1}. Receptor: CB8•MDAP (5 μM). Wavelengths: λ_{exc} = 420 nm; λ_{em} = 450 nm. The pH dependence of β-galactosidase activity was determined with 20 μM phenyl-β-D-galactopyranoside in 10 mM sodium phosphate buffer adjusted with HCl/NaOH to pH 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0 (Fig. S13).

Description of the enzyme assay with fluorescent chemosensors

The working principle of our assay is shown in Scheme 1 in the main text and Scheme S1 below. A fluorescent chemosensor (C) is in a dynamic equilibrium with (excess) of substrate (S), which is converted by an enzyme (E) into the product (P). The observed emission intensity (I_{obs}) of the reaction mixture is the sum of the individual emission of free and bound C, *i.e.*, $I_{obs} = I(C) + I(C•S) + I(C•P)$, where the individual contributions change as the enzymatic reaction proceeds. Introducing $I_0(C)$ and $c_0(C)$, the emission intensity and concentration of the unbound chemosensor in the absence of S and P, and defining $QE(S)$ and $QE(P)$ as the quenching efficiencies of S and P in their complexes with C, I_{obs}, can be expressed as:

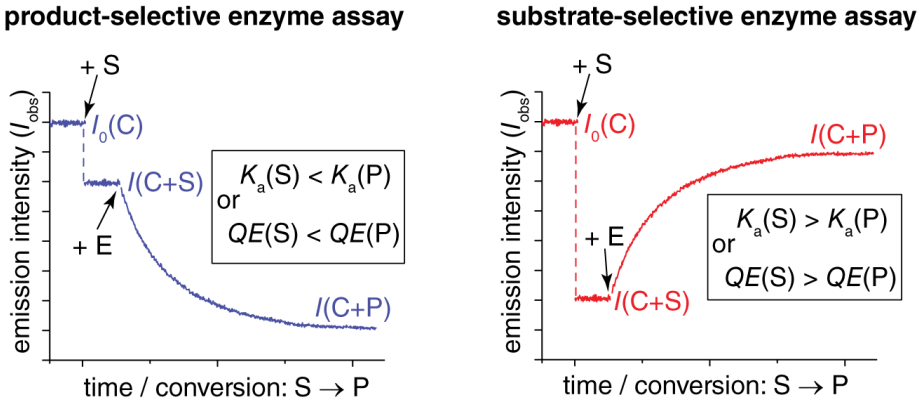
$$I_{obs} = I_0(C)•c_0(C)^{-1}•(c(C) + c(C•S)•[1 - QE(S)] + c(C•P)•[1 - QE(P)]) \quad (1)$$

$$\text{with } c_0(C) = c(C) + c(C•S) + c(C•P) \quad (2)$$

The equilibrium concentrations of $c(C)$, $c(C•S)$ and $c(C•P)$ are further subject to the binding equations:

$$K_a(S) = c(C•S)•c(C)^{-1}•c(S)^{-1} \quad (3)$$

$$K_a(P) = c(C•P)•c(C)^{-1}•c(P)^{-1} \quad (4)$$



Scheme S1: Fluorescence kinetic traces for enzymatic reactions conducted in the presence of the associative chemosensing ensembles. First, addition of S to R reduces the emission intensity due to partial complexation of R by S. Subsequent addition of E starts the enzymatic conversion of S to P, which result in a time-resolved change in emission intensity. In the product-selective assay variant (left), P has a higher affinity for R (K_a) than S, such that the fluorescence decreases. The same net effect (continuous fluorescence decrease) results if the fluorescence quenching efficiency (QE) of R•P is higher than that of R•S, then a decrease in the emission intensity will be observed during the course of the enzymatic reaction. For the substrate-selective case (right), where the conditions are opposite, a fluorescence increase results.

For most of the substrate/product/chemosensor combinations investigated herein, both $QE(S)$ and $QE(P)$ reach 100% (see also Table S1), such that a change in I_{obs} is only observed if $c(C)$, *i.e.*, the fraction of unbound, fluorescent chemosensor, changes in the course of the enzymatic reaction. This means that an enzymatic conversion of S to P can be monitored with the fluorescent chemosensor whenever $K_a(S) \neq K_a(P)$. Specifically, a decrease in I_{obs} is expected for $K_a(S) < K_a(P)$, which is termed product-selective enzyme assay (Scheme S1). Conversely, an increase of I_{obs} is seen for $K_a(S) > K_a(P)$, the substrate-selective mode. Please note that the ratio of chemosensor to substrate should be adjusted such that a fraction of chemosensor remains uncomplexed at any time. In principle, suitable conditions can be predicted by solving the aforementioned coupled equations when the affinities and QE values are known for the substrate and all potential (side)products of the enzymatic reaction. Practically, this will be rarely the case, but is also not necessary: Suitable starting conditions can be experimentally found by 1) adjusting the substrate to chemosensor ratio such that significant but not quantitative quenching is obtained in the desired reaction buffer, and 2) initiating the enzymatic reaction and recording the kinetics. If the chemosensor is product-selective, *i.e.*, $K_a(S) < K_a(P)$, then a saturation of C by P could occur during the enzymatic reaction, impeding the analysis of

the full kinetic trace. Nevertheless, initial rates would still be extractable. To test if the chemosensor becomes saturated, the reaction can be repeated with a higher substrate loading, or more substrate or product can be added to the reaction mixture *after* the reaction has been completed. In either case, if unbound chemosensor is still available, then the emission intensity should further decrease. If not, then either c_0(S) should be reduced or c_0(C) should be increased, or – if both is not possible – a chemosensor with a lower affinity for the product should be employed.

If the chemosensor is substrate-selective, *i.e.*, $K_a(S) > K_a(P)$, then saturation of the chemosensor will not occur during the enzymatic conversion. However, if the chemosensor strongly competes with the enzyme for the substrate, then the apparent enzymatic rate could be slightly reduced. An excess of substrate should therefore be used in order to avoid this problem, however, within the limit of not fully saturating the chemosensor. If this is not possible, then a chemosensor with a lower affinity for the substrate can be used to remedy. Notably, a small chemosensor library with different affinities for the substrate and product of an enzymatic reaction can be readily obtained with different dyes (see below), circumventing the cumbersome chemical modification of the macrocyclic host.

For some substrate-product pairs QE(S) or QE(P) do not reach 100%. If $QE(S) \neq QE(P)$, then I_{obs} reports on the concentrations of substrate and product in the course of the enzymatic reaction even if both the substrate and the product have a very similar affinity for the chemosensor. Specifically, $QE(S) < QE(P)$ leads to a decrease in I_{obs}, similar to the observation for a product-selective enzyme assay, and $QE(S) > QE(P)$ results in an increase, as for a substrate-selective assay (see also Scheme S1). In other words, enzymatic reactions involving substrate-product pairs with $K_a(S) \approx K_a(P)$ that are generally undetectable by competitive tandem assays can in principle be monitored by the associative variant if any photophysical differences between the substrate and product, such as their quenching efficiencies or spectral changes, can be exploited. For the rare combination of $K_a(S) > K_a(P)$ and $QE(S) < QE(P)$, or if $K_a(S) < K_a(P)$ and simultaneously $QE(S) > QE(P)$ the net changes in I_{obs} can be small. This is very unlikely, and, in fact, was never the case for any of the combinations we tested.

Specific description of the enzyme assays for CB8-based associative chemosensors

We investigated the photophysical response of analyte binding to selected CB8•dye chemosensor complexes. MDAP,[4,6] MDPP,[6-7] and a perylene-bisdiimide derivative (PDI)[5] were chosen as dicationic, fluorescent dyes (Scheme 1 in the main text) because of their high affinity ($> 10^5$ M^{-1}) [4-5,7] for CB8 and because their emission and absorbance lies in the reliably addressable visible region of the spectrum.

The chemosensing ensembles bind strongly ($K_a = 10^3$-10^6 M^{-1}) to a wide range of analytes with an aromatic ring as recognition motif, *e.g.*, phenols, phenylalanine-, and tryptophan derivatives (Table S1).[27] Noteworthy, the affinities of the CB8 complexes are largely dependent on the hydrophilicity of the analyte, because the desolvation of hydrophilic analytes from water is energetically unfavorable.[8] Thus, more polar analytes usually possess lower K_a values than less polar ones. For instance, Phe and Trp bind approximately 50 times stronger to CB8•MDPP than their hydroxylated analogues Tyr and 5-hydroxytryptophan (5HO-Trp), respectively (Table S1). Consequently, chemical reactions that increase the hydrophilicity of an aromatic species, as is often the case for enzymatic oxidations, lead to an increase in fluorescence intensity (substrate-selective enzyme assay). Negatively charged analytes display generally a much weaker binding affinity for CB*n* macrocycles than their positively or non-charged analogues. This means that enzymatic reactions resulting in a removal of a negative charge, *e.g.*, decarboxylations or dephosphorylations, are characterized by $K_a(S) < K_a(P)$, and, thus, by a reduction of I_{obs} (product-selective assay). Conversely, esterases, peptidases, and kinases can create a negative charge close to the aromatic recognition motif, or, in the case of tyrosine phosphorylation, at the anchor itself, resulting in $K_a(S) > K_a(P)$ and an associated emission increase (substrate-selective case).

It is worth noting that both, the absolute as well as the relative affinities between analytes are largely dependent on the chemical nature of the dye, *i.e.*, it functions both as a signaling unit and as a modulator of the concave molecular recognition

element. Modification of the dye is therefore a practical way to fine-tune the chemosensor-binding properties to the required or desired absolute and relative affinity of substrate and product.

Inspection of Table S1 illustrates that some analytes have much lower *QE* values than others do, in particular benzene and its alkyl-substituted derivatives. Furthermore, the extent of fluorescence quenching also depends on the dye component of the chemosensor. For instance, CB8•MDAP and CB8•MDPP respond to the presence of benzene with only partial emission quenching. Finally, the emission of the CB8•PDI chemosensor is quenched by nitrogen-containing analytes but not by phenols, naphthols, and many other aromatic hydrocarbons.[5]

Supplementary Tables

Table S1. Quenching efficiencies (*QE*) and binding constants (K_a) for the formation of chemosensor•analyte complexes in water, determined by fluorescence titrations.

Chemosensor	Analyte	QE	K_a / (10^3 M^{-1})
CB8•MDAP	benzene	0.3	4
CB8•MDAP	Phe	0.5	5
CB8•MDAP	phenylethylamine	0.4	8
CB8•MDAP	aniline	0.9	33
CB8•MDAP	phenol	>0.9	56
CB8•MDAP	4-ethoxy-phenol	>0.9	1
CB8•MDAP	1,2-dihydroxybenzene	>0.9	20
CB8•MDAP	1,4-dihydroxybenzene	>0.9	13
CB8•MDAP	*p*-benzoquinone	>0.9	0.5
CB8•MDAP	phenyl-β-D-galactopyranoside	>0.9	30
CB8•MDAP	Trp	>0.9	150
CB8•MDAP	5HO-Trp	>0.9	8
CB8•MDAP	tryptamine	>0.9	50
CB8•MDAP	serotonin	>0.9	5
CB8•MDDP	benzene	< 0.1	n.d.
CB8•MDDP	Phe	0.2	400
CB8•MDDP	aniline	0.7	20
CB8•MDDP	phenol	0.7	180
CB8•MDDP	Tyr	>0.9	8
CB8•MDDP	Trp	>0.9	1200
CB8•MDDP	5HO-Trp	>0.9	22
CB8•PDI	benzene	<0.1	n.d.
CB8•PDI	aniline	0.6	50
CB8•PDI	phenol	<0.1	n.d.
CB8•PDI	indole	0.6	33

Table S2. Emission enhancement factors (after full substrate conversion) and relative initial rates for the oxidation of phenols (each 46 μM) by HRP with H_2O_2 (100 μM) and NaI (120 μM) in sodium phosphate buffer (pH 4, 10 mM), recorded in the presence of the chemosensor CB8•MDAP (5 μM).

Substrate	I/I_0	Relative rate
4F-phenol	2.1	1.0
indole	2.4	1.1
Phenol	1.9	1.4
Trp	1.8	1.6
4Br-phenol	1.6	3.0
4Me-phenol	1.5	3.0
4Cl-phenol	1.1	3.5
4I-phenol	2.2	3.5
4MeO-phenol	1.2	5.3
1,4-dihydroxybenzene	1.7	10.8
2-naphthol	12.5	26.0
2,7-dihydroxynaphthalene	7.9	36.4

Supplementary Figures

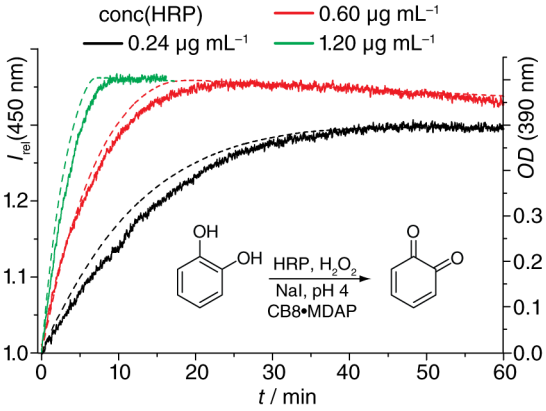


Fig. S1: Oxidation of catechol (46 µM) by H_2O_2 (100 µM) with horseradish peroxidase (HRP, 0.24-1.20 µg ml^{-1}) in 10 mM phosphate buffer (pH 4) with 120 µM iodide; detection *via* increase in *OD* at 390 nm in the absence of chemosensor (dashed line) and *via* increase in emission intensity at 450 nm of the chemosensor in the presence of CB8•MDAP (5 µM).

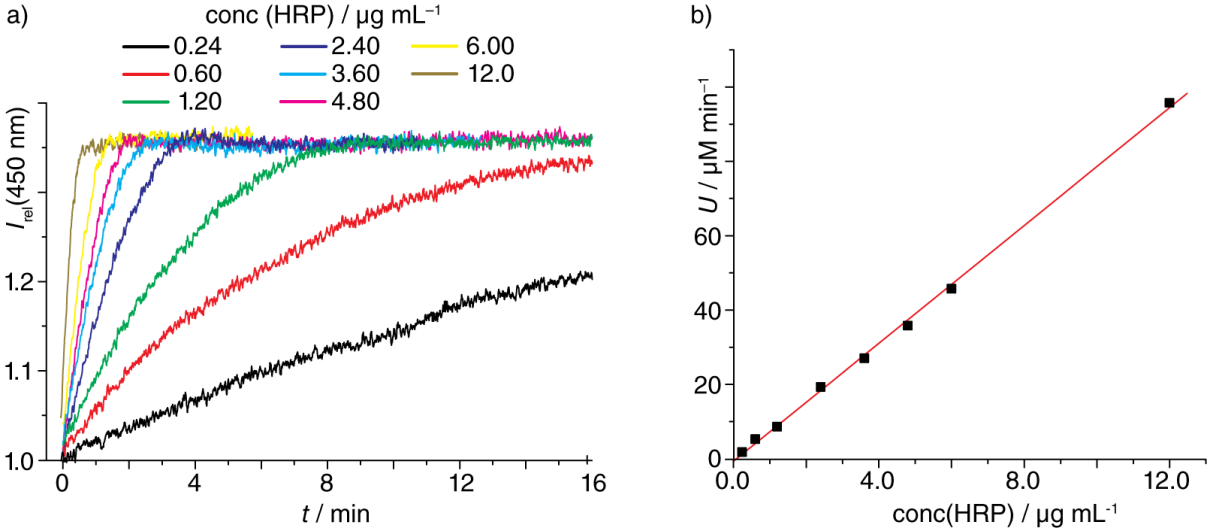


Fig. S2: Oxidation of catechol (46 µM) by H_2O_2 (100 µM) with horseradish peroxidise (HRP, 0.24-12.0 µg ml^{-1}) in 10 mM phosphate buffer (pH 4) with 120 µM iodide. a) Kinetic traces upon varying the enzyme concentration in the presence of CB8•MDAP (5 µM, λ_{em} = 450 nm). b) The initial rates are linearly proportionally to the enzyme concentration.

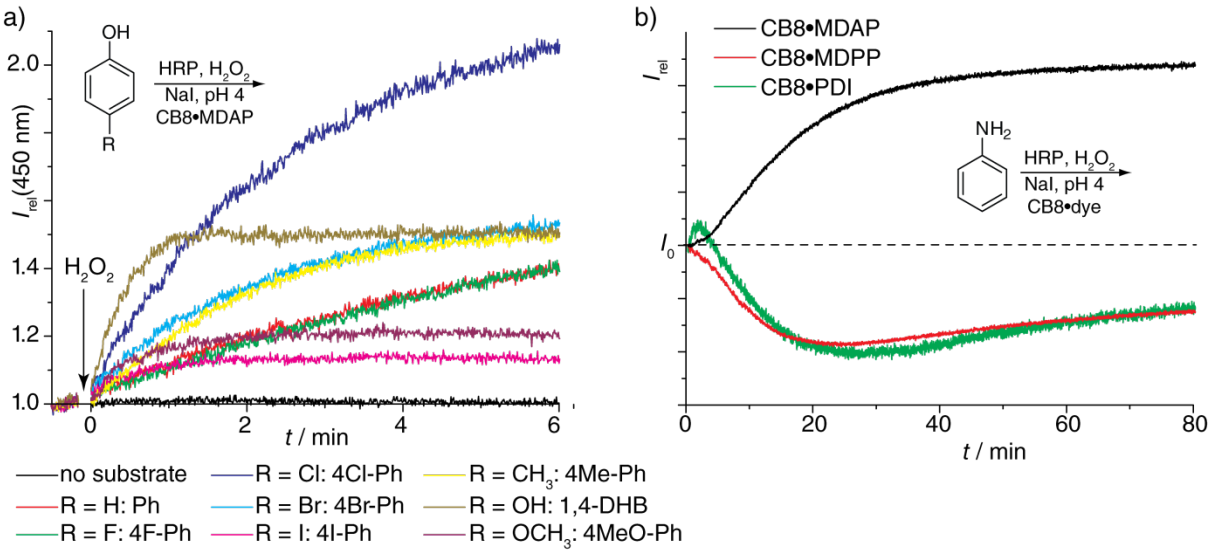


Fig. S3: a) Oxidation of various 4-substituted phenols (each 46 μM) by H_2O_2 (100 μM) with horseradish peroxidise (HRP, 0.6 μg mL^{-1}) in 10 mM phosphate buffer (pH 4) with 120 μM iodide and CB8•MDAP (5 μM) as the chemosensor. b) Oxidation of aniline (46 μM) to polyaniline by H_2O_2 (100 μM) with horseradish peroxidise (HRP, 0.6 μg mL^{-1}) in 10 mM phosphate buffer (pH 4) with 120 μM iodide; the fluorescence intensity was monitored in the presence of either CB8•MDAP, CB8•MDPP, or CB8•PDI (each 5 μM) at 450 nm, 510 nm, and 550 nm, respectively.

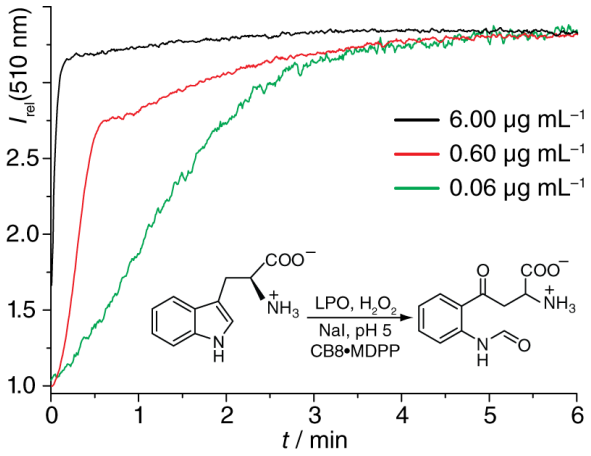


Fig. S4: Oxidation of Trp (20 μM) by H_2O_2 (100 μM) with lactoperoxidase (LPO, 0.06-6.0 μg ml^{-1}) in 10 mM phosphate buffer (pH 5) with 120 μM iodide; the fluorescence intensity was monitored at 510 nm in the presence of CB8•MDPP (5 μM).

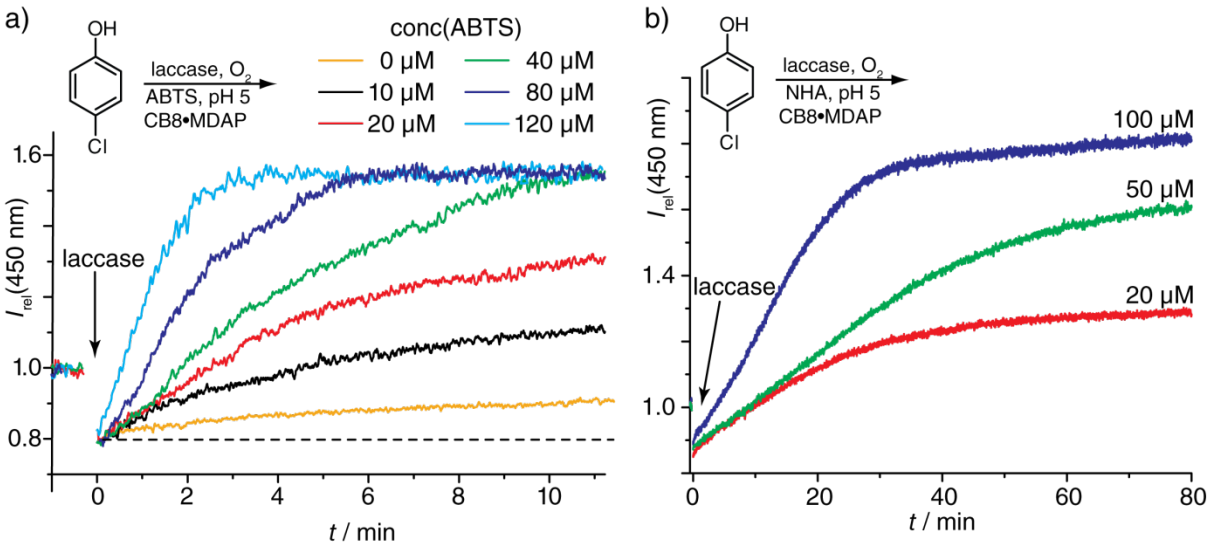


Fig. S5: a) Oxidation of 4-chlorophenol (46 μM) with laccase (80 μg mL^{-1}) in aerated 10 mM phosphate buffer (pH 5) in the presence of or 2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulphonic acid (ABTS, 0-120 μM) as the mediator and CB8•MDAP (5 μM) as the chemosensor; the fluorescence intensity was monitored at 450 nm; the initial drop in I_{rel} upon addition of laccase is due to impurities in the commercial laccase sample. b) Oxidation of 4-chlorophenol (46 μM) with laccase (80 μg mL^{-1}) in aerated 10 mM phosphate buffer (pH 5) in the presence of presence of or N-hydroxy-phthalimide (NHA, 0-100 μM) as the mediator and CB8•MDAP (5 μM) as the chemosensor; the fluorescence intensity was monitored at 450 nm.

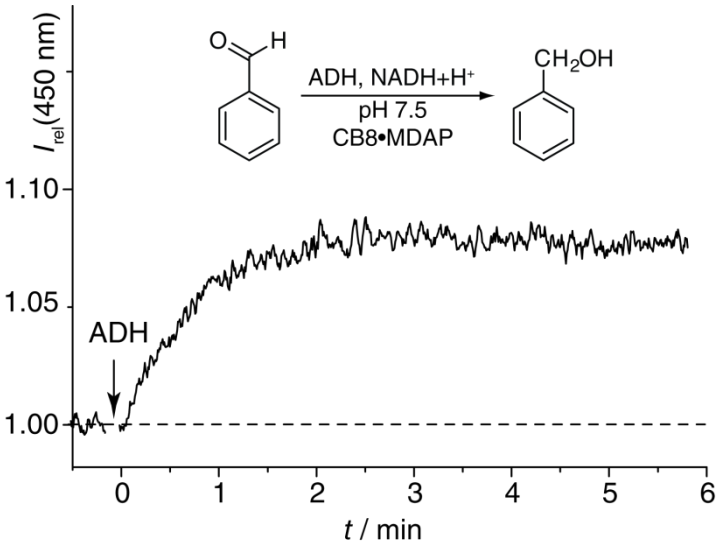


Fig. S6: Reduction of benzaldehyde (80 μM) by reduced nicotinamide adenine dinucleotide ($NADH,H^+$, 1 mM) and alcohol dehydrogenase (ADH, 3.75 μg ml^{-1}) in 10 mM sodium phosphate buffer (pH 7.5); the fluorescence intensity was monitored at 450 nm in the presence of CB8•MDAP (5 μM).

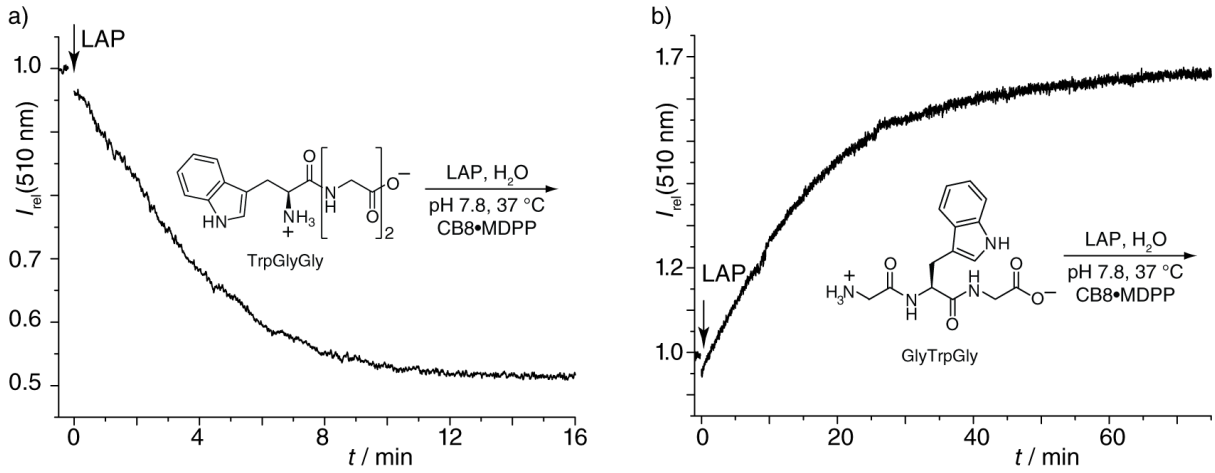


Fig. S7: Hydrolysis of a) TrpGlyGly and b) GlyTrpGly (each 20 µM) by leucine aminopeptidase (LAP, 50 µg mL^{-1}) in 10 mM phosphate buffer (pH 7.8) at 37 °C in the presence of CB8•MDPP (5 µM); the fluorescence intensity was monitored at 510 nm.

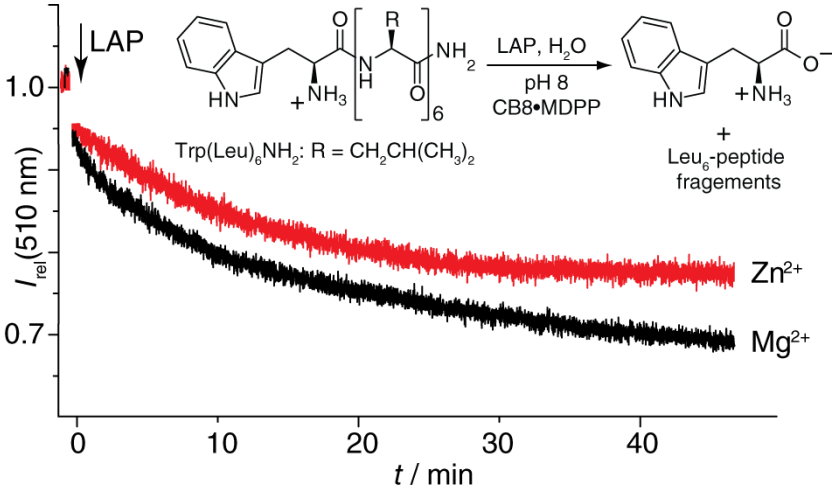


Fig. S8: Hydrolysis of Trp–Leu_6–NH_2 peptide (40 µM) by leucine aminopeptidase (LAP, 50 µg mL^{-1}) in 10 mM phosphate buffer (pH 8) in the presence of CB8•MDPP (5 µM). The enzyme was incubated in 1 mM $ZnCl_2$ or $MgCl_2$ solution at 37°C for 2 h in order to pre-activate it; the fluorescence intensity was monitored at 510 nm.

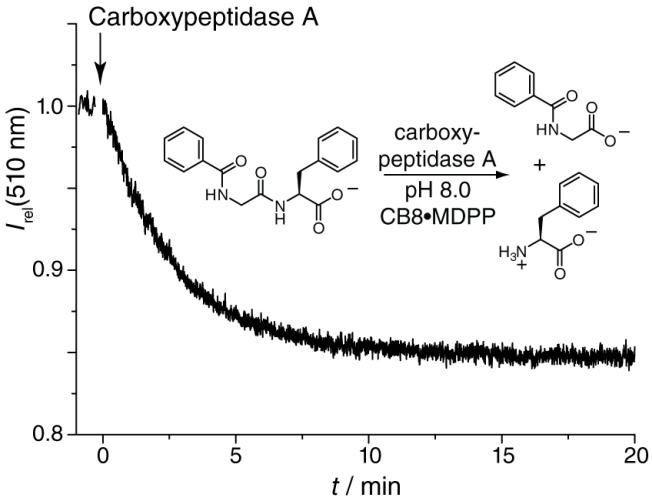


Fig. S9: Hydrolysis of hippuryl-L-phenylalanine (50 μM) by carboxypeptidase A (10 μg ml^{-1}) in 10 mM phosphate buffer (pH 8.0) in the presence of CB8•MDPP (5 μM); the fluorescence intensity was monitored at 510 nm.

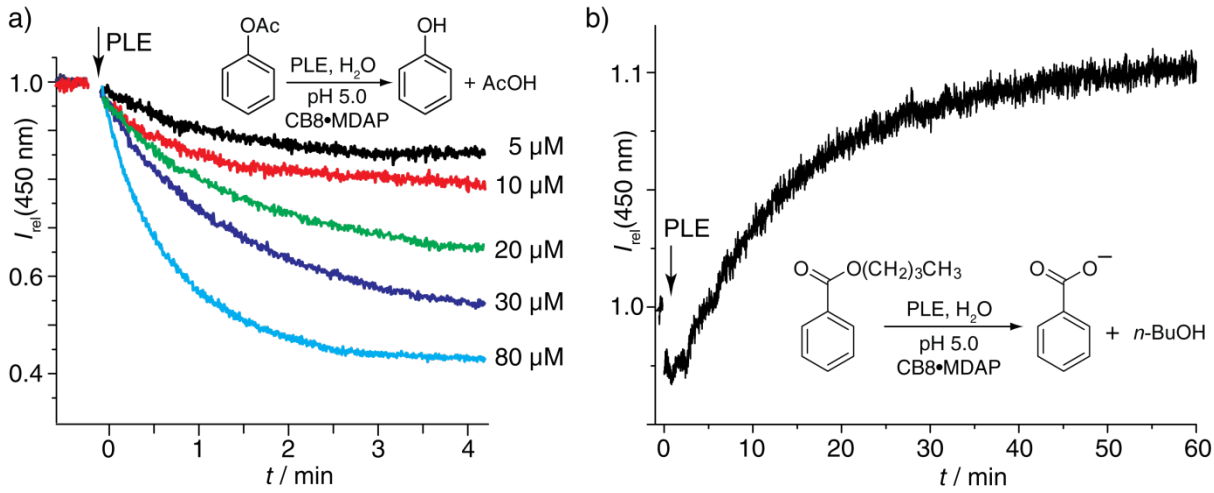


Fig. S10: a) Hydrolysis of phenyl acetate at different concentrations by porcine liver esterase (PLE, 80 μg mL^{-1}) in 10 mM phosphate buffer (pH 5) in the presence of CB8•MDAP (5 μM); the fluorescence intensity was monitored at 450 nm. b) Hydrolysis of *n*-butylbenzoate (200 μM) by porcine liver esterase (PLE, 80 μg mL^{-1}) in 10 mM phosphate buffer (pH 5) in the presence of CB8•MDAP (5 μM); the fluorescence intensity was monitored at 450 nm.

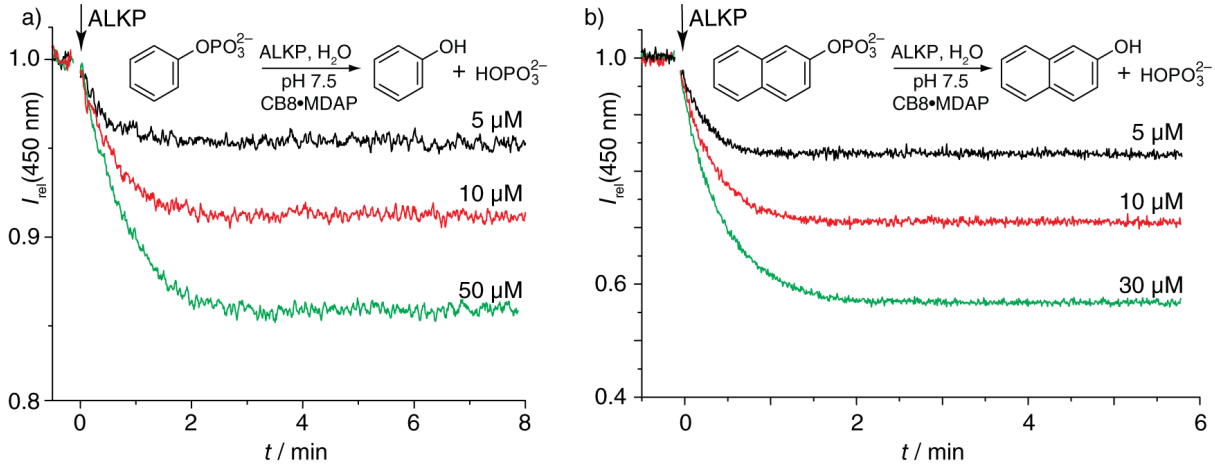


Fig. S11: a) Hydrolysis of 2-napthyl phosphate at different concentrations by alkaline phosphatase (ALKP, 16 μg mL^{-1}) in 10 mM phosphate buffer (pH 7.5) in the presence of CB8•MDAP (5 μM); the fluorescence intensity was monitored at 450 nm. No enzymatic reaction was observed for diphenyl phosphate as the substrate.

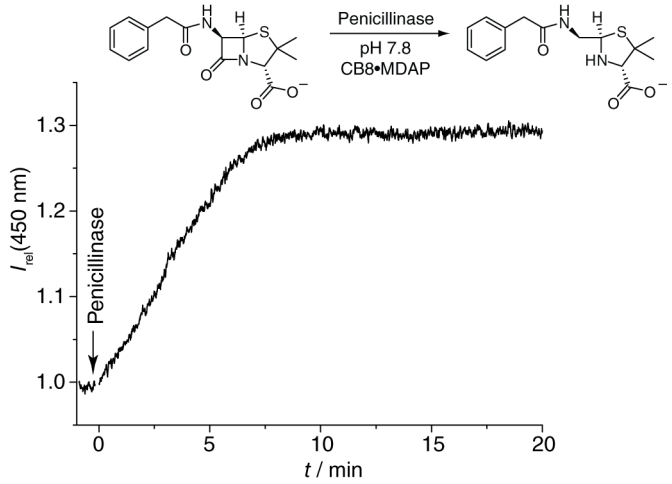


Fig. S12: Hydrolysis of benzylpenicillin (500 μM) by penicillinase (0.2 μg mL^{-1}) in 10 mM sodium phosphate buffer (pH 7.8) in the presence of CB8•MDAP (5 μM); the fluorescence intensity was monitored at 450 nm.

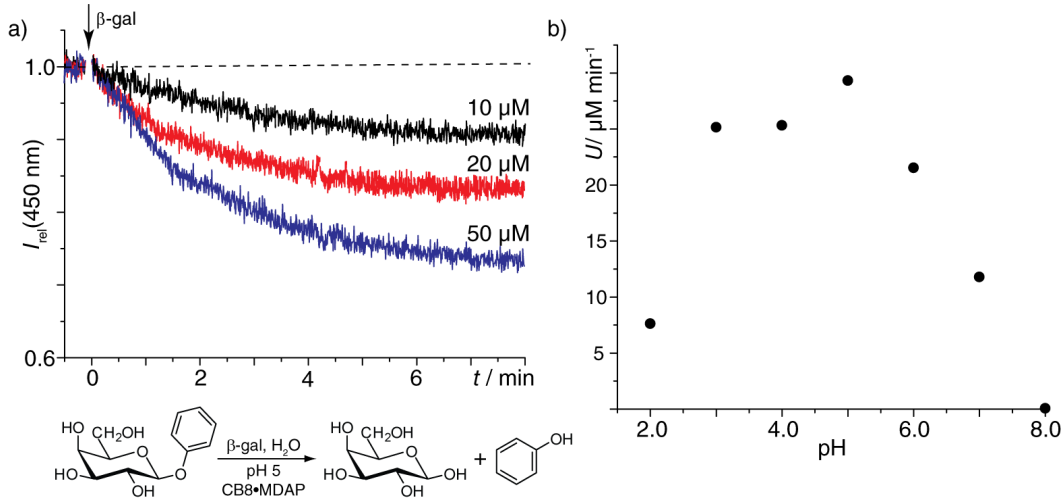


Fig. S13: a) Hydrolysis of phenyl-β-D-galactopyranoside at different concentrations by β-galactosidase (β-gal, 43 μg mL^{-1}) in 10 mM phosphate buffer (pH 7.5) in the presence of CB8•MDAP (5 μM); the fluorescence intensity was monitored at 450 nm. b) Initial rates for the hydrolysis of 20 μM phenyl-β-D-galactopyranoside at different pH.

Supplementary References

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