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Enzyme-controlled stereoselective radical cyclization to arenes enabled by metalloredox biocatalysis

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The effective induction of high levels of stereocontrol for free-radical-mediated transformations represents a notorious challenge in asymmetric catalysis. Herein, we describe a metalloredox biocatalysis strategy to repurpose natural cytochromes P450 to catalyse asymmetric radical cyclization to arenes through an unnatural electron transfer mechanism. Directed evolution afforded a series of engineered P450 aromatic radical cyclases with complementary selectivities: $P450_{arc1}$ and $P450_{arc2}$ facilitated enantioconvergent transformations of racemic substrates, giving rise to either enantiomer of the product with excellent total turnover numbers (up to 12,000). In addition to these enantioconvergent variants, another engineered radical cyclase, $P450_{arc3}$, permitted efficient kinetic resolution of racemic chloride substrates (*S* factor = 18). Furthermore, computational studies revealed a proton-coupled electron transfer mechanism for the radical–polar crossover step, suggesting the potential role of the haem carboxylate as a base catalyst. Collectively, the excellent tunability of this metalloenzyme family provides an exciting platform for harnessing free radical intermediates for asymmetric catalysis.

Due to their ability to exert excellent stereocontrol over challenging asymmetric transformations, enzymes are widely recognized as powerful tools to streamline the synthesis of chiral molecular scaffolds[1–4]. Until fairly recently, only a small set of biochemistries from nature's catalytic repertoire has been exploited to facilitate the synthesis and manufacturing of a relatively narrow range of value-added compounds. Unfortunately, the vast majority of privileged synthetic transformations, particularly those allowing for stereoselective C–C bond formation, are not present in the state-of-the-art biocatalytic toolbox[5]. Thus, to further advance the field of biocatalysis to the next level of sophistication and applicability, it is imperative to devise and optimize synthetically useful enzyme functions that are not presently known in the biological world[6–10].

Advances from mechanistic enzymology and structural biology have furnished invaluable insights into the molecular mechanism and structural basis of enzymatic machineries. Over the past decade, by cross-fertilizing the fields of synthetic chemistry and enzymology, biocatalysis researchers initiated a campaign to repurpose and evolve natural enzymes to catalyse unnatural reactions by leveraging the synthetic versatility of common cofactors illuminated by organic and organometallic chemists[8–10]. In nature, radical enzymes[11,12] such as radical *S*-Adenosyl methionine enzymes[12] facilitate challenging free radical transformations. However, despite their excellent selectivity and intriguing mechanism, these natural radical enzymes are not yet widely applied in synthetic chemistry and biotechnology. Very recently, new concepts and strategies in the emerging area of unnatural radical biocatalysis have led to several distinct activation modes to enable stereoselective transformations of open-shell intermediates[10,13,14]. Utilizing the strongly reducing excited-state flavin and nicotinamide

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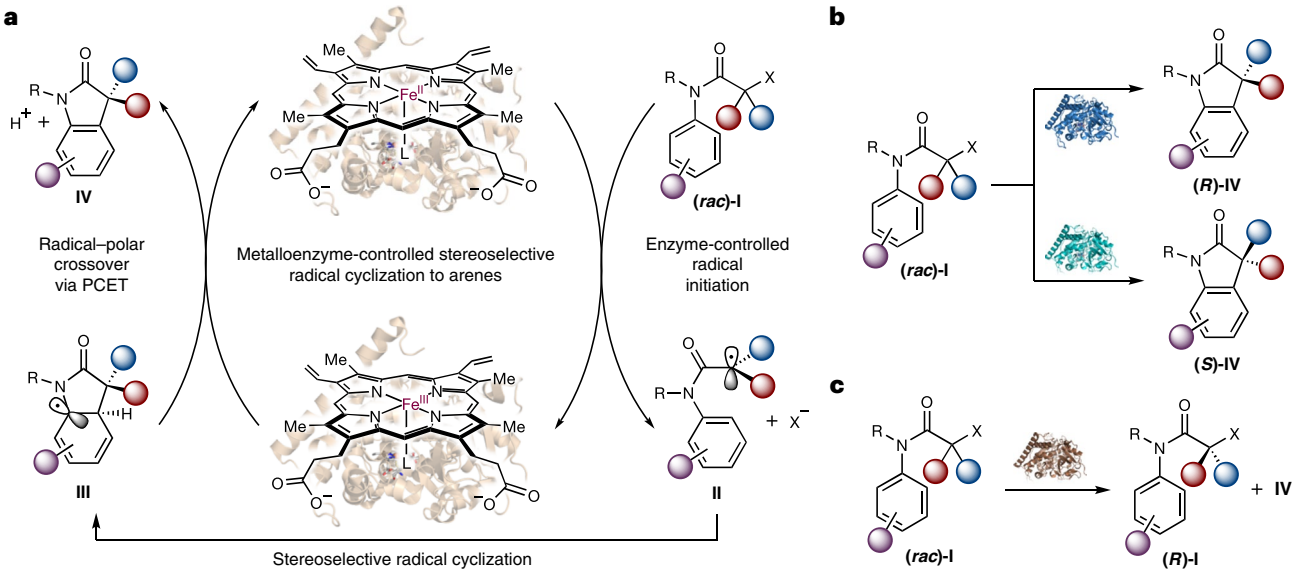

Fig. 1 | A metalloenzyme platform for stereoselective radical cyclization. **a**, Proposed catalytic cycle with a haemoprotein catalyst. **b**, Biocatalytic enantioconvergent radical cyclization leading to either enantiomer of the products. **c**, Biocatalytic kinetic resolution to prepare enantioenriched tertiary alkyl halides. L is an Fe-binding amino acid residue, which is serine in this work. X is either Br or Cl. PCET, proton-coupled electron transfer. Coloured spheres are generic substituents of the molecule.

cofactors[13–22], an elegant photoenzymatic strategy for asymmetric radical reactions was developed. In 2021 and 2022, by capitalizing on the innate redox activity of first-row transition-metal cofactors in common metalloproteins, haem[23,24] and non-haem[25] Fe enzymes were used via a metalloredox strategy to catalyse stereoselective atom-transfer radical reactions. Collectively, these emerging new-to-nature activation modes provide an exciting opportunity to evolve enzymes capable of imposing excellent stereocontrol over fleeting free radical intermediates, an objective that has long eluded small-molecule catalysis because of the inherent difficulties to induce asymmetry with free radical chemistry[26–28].

To further develop and generalize the concept of metalloredox radical biocatalysis, we sought to develop a metalloenzyme-catalysed stereoselective addition of carbon-centred radicals to aromatic systems using easily available racemic α-halocarbonyls as substrates (Fig. 1)[29]. In this proposed catalytic cycle (Fig. 1a), the ferrous haem protein catalyst first reacts with the alkyl halide substrate **I** to furnish a highly reactive radical species **II** via single-electron transfer (SET). This incipient radical **II** subsequently adds to the aromatic ring, leading to a dearomatized radical intermediate **III**. Finally, the radical–polar crossover of **III** with the ferric haem protein furnishes the final product, regenerates the ferrous protein catalyst and completes the catalytic cycle. In this process, if the haem protein readily accommodates and transforms both enantiomeric forms of the organic halide substrate to the same radical intermediate, it would allow us to develop an enantioconvergent protocol[30] to convert racemic building blocks into enantioenriched products bearing a challenging quaternary stereocentre (Fig. 1b). Alternatively, if the haem protein catalyst effectively distinguishes the two enantiomers of the substrate and selectively converts one enantiomer, we would be able to develop a biocatalytic kinetic resolution[31,32] to prepare enantioenriched acyclic tertiary alkyl halides, whose enantioselective synthesis remain non-trivial (Fig. 1c)[33]. In asymmetric catalysis, engineering a set of structurally related yet functionally orthogonal catalysts to enable highly selective enantioconvergent transformation and kinetic resolution of the same racemic substrates remains a formidable task for both biocatalysts and small-molecule catalysts[30]. In light of the promiscuous nature of haem enzymes[34,35] as well as their ability to facilitate unnatural reactions as elegantly demonstrated previously[36,37], we postulated that haem-dependent radical cyclases could be evolved as a unifying platform to realize all these stereoselective processes as outlined in Fig. 1b,c.

Results and discussion

Discovery and directed evolution of radical cyclases P450$_{arc1-2}$

Using α-bromo-β-amidoester **1** as the model substrate, we commenced our investigation by evaluating a panel of haem proteins and their variants, including cytochromes P450, globins and cytochromes *c* as well as our recently evolved radical cyclase mutants[23], using intact *Escherichia coli* cells as biocatalysts (Fig. 2a). We focused our initial efforts on the asymmetric synthesis of 3,3-disubstituted oxindoles, in part due to the prevalence of these structural elements in bioactive natural products and medicinal agents[38]. Among all the haem proteins we tested, although many displayed encouraging initial activities, only a handful of variants from the cytochrome P450 superfamily showed moderate levels of enantioselectivity (Supplementary Table 1). In particular, P411$_{Diane2}$ and P411$_{Diane3}$, a set of closely related variants of serine-ligated CYP102A1 (ref. 39) (P450 from *Bacillus megaterium*) lacking the flavin adenine dinucleotide domain, which we previously engineered for enantioselective C–H amination[40,41], exhibited good activities with opposite enantiopreferences (P411$_{Diane2}$: 58% yield, **(*S*)-2a**:**(*R*)-2a** = 66:34; P411$_{Diane3}$: 62% yield, **(*S*)-2a**:**(*R*)-2a** = 36:64).

With P411$_{Diane2}$ and P411$_{Diane3}$ as initial hits for this novel enzyme function, we set out to engineer a set of enantiocomplementary radical cyclases for the catalytic asymmetric synthesis of 3,3-disubstituted oxindoles (Fig. 2b–d). To further improve the enantioselectivity of P411$_{Diane2}$ in this unnatural radical cyclization, by targeting amino acid residues in proximity to the haem cofactor, iterative rounds of site-saturation mutagenesis (SSM)[42] and screening were carried out. In each round of engineering, four active-site residues were randomized in parallel to provide a total of four single-site-saturation libraries. The selection of target residues for SSM was guided by our molecular docking studies (Supplementary Information). For each SSM library, 90 clones were screened in a 96-well plate. After four rounds of directed evolution of P411$_{Diane2}$, beneficial mutations W263Q, L181M, T438G and H266L were identified, furnishing P450$_{arc1}$ (Fig. 2c; arc, aromatic radical cyclase). Based on our quantum mechanics/

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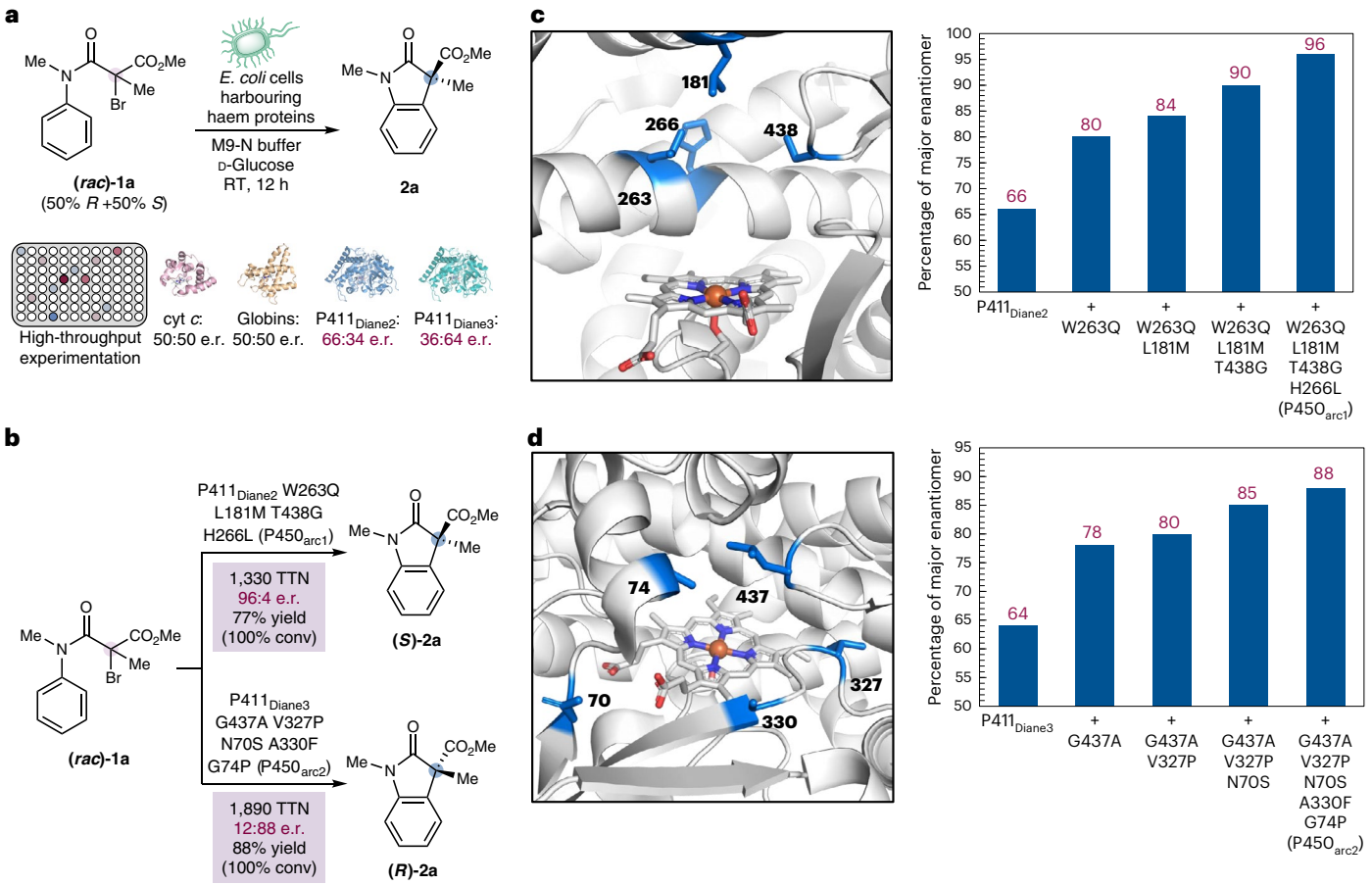

Fig. 2 | Discovery and engineering of enantioconvergent P450 radical cyclases. a, Evaluation of haem protein catalysts for enantioconvergent radical cyclization. **b**, Evolved final variants $P450_{arc1}$ and $P450_{arc2}$ as orthogonal biocatalysts for enantioconvergent radical cyclization. **c**, Directed evolution of $P450_{arc1}$. **d**, Directed evolution of $P450_{arc2}$. Both active site illustrations were made on the basis of the crystal structure of a closely related P450 variant (Protein Data Bank ID: 5UCW).

molecular mechanics investigation[24], the newly introduced glutamine at residue 263 likely engages the carbonyl group of the amide substrate through hydrogen bonding, thus facilitating substrate activation and enhancing enantiocontrol[24]. Under standard conditions, final variant $P450_{arc1}$ afforded the radical cyclization product (*S*)-2a in 77% ± 3% yield, 1,330 ± 50 total turnover number (TTN) and 96:4 e.r., as determined by chiral high-performance liquid chromatography analysis (Fig. 2b). Similarly, the enantioselectivity of $P411_{Diane3}$ could also be optimized through directed evolution. Accumulating five beneficial mutations G437A, V327P, N70S, A330F and G74P, $P450_{arc2}$ was developed to provide **(*R*)-2a** in 88% ± 1% yield, 1,890 ± 30 TTN and 12:88 e.r. (Fig. 2d). We note that when previously developed photoenzymatic conditions were applied, substrates bearing a small α-substituent such as **1a** provided modest enantioselectivities (78:22 e.r.) favouring the (*S*)-enantiomer[18]. Thus, the rapid engineering of enantiodivergent radical metalloenzymes $P450_{arc1}$ and $P450_{arc2}$ to access both the (*R*)-enantiomer and the (*S*)-enantiomer demonstrated the power of this adaptive metalloenzyme platform to solve difficult problems in asymmetric catalysis. Additionally, steady-state kinetic studies showed that our evolved enzyme $P450_{arc1}$ exhibited a k_{cat} of 0.54 ± 0.03 s^{-1}, which represented a 15-fold improvement relative to its parent $P411_{Diane2}$ (k_{cat} = 0.036 ± 0.004 s^{-1}). The K_M of $P450_{arc1}$ was found to be similar to that of the parent enzyme (Supplementary Table 10). The k_{cat} of $P450_{arc1}$ is similar to that of previously engineered new-to-nature radical C–H azidases[25]. Although these unnatural radical enzymes have not yet reached the catalytic efficiency of natural systems, further directed evolution may lead to enhanced enzyme kinetics for unnatural metalloredox radical biocatalysis.

Substrate scope of evolved P450 radical cyclases

Using whole *E. coli* cells harbouring newly evolved P450 radical cyclases, we next examined the substrate scope of this enantioconvergent radical C–C bond formation (Fig. 3a). Radical precursors with various α-substituents, including a methyl (**2a**), an ethyl (**2b**), a propyl (**2c**), an allyl (**2d**) and an isopropyl (**2e**), were all transformed with excellent enantioselectivities under these biocatalytic conditions, showcasing the versatility of engineered biocatalysts. Moreover, aromatic rings bearing a diverse range of *para*-substituents, including a fluorine (**2f**), a chlorine (**2g**), a bromine (**2h**), an iodine (**2i**), a methoxy (**2j**), a methyl (**2k**), an ethyl (**2l**) and an isopropyl (**2m**), all underwent radical cyclization with excellent TTNs and enantioselectivities. In addition to methyl esters (**2a**–**2m**), ethyl esters (**2n**) were also excellent substrates. Additionally, the *N*-ethyl substrate **1o** could also be successfully converted into the corresponding enantioenriched product **2o**. The absolute stereochemistry of **2i** was determined by single-crystal X-ray diffraction analysis. Notably, gram-scale biotransformations could be conveniently carried out with slightly improved yield and identical enantioselectivity (**2b** and **2i**), further demonstrating the synthetic utility of these newly evolved enzymes. Furthermore, by lowering the cell density of these whole-cell biotransformations (OD_{600} = 30, 20, 10 and 5), evolved enzymes were able to provide the C–C bond formation product **2b**

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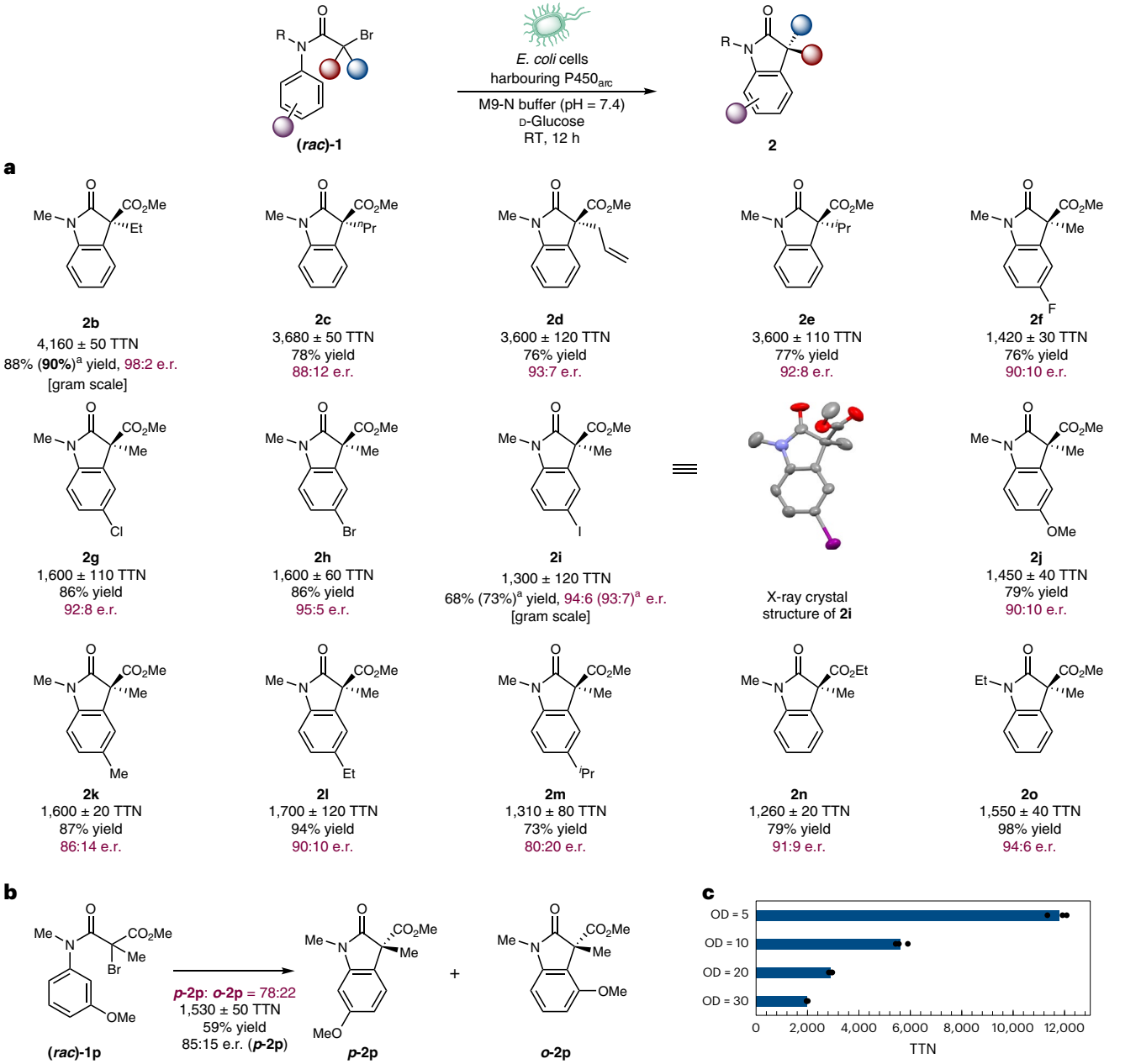

Fig. 3 | Substrate scope of P450$_{arc}$-catalysed enantioconvergent radical cyclization. a, Substrate scope of α-bromoesters (OD_{600} = 15–35 unless otherwise noted; Supplementary Methods). All the reactions were performed in triplicate and averaged results and standard deviations are provided. **b**, Biocatalytic site-selective radical cyclization of *meta*-substituted substrate **1p**. **c**, Whole-cell radical cyclization of **1b** with high TTNs. [a]Gram-scale reaction.

with up to 12,000 ± 300 TTN (Fig. 3c) without lowering the yield and enantioselectivity (88% yield, 98:2 e.r.).

When *meta*-substituted arene **1p** was applied, without further engineering, P450$_{arc2}$ overrode inherent substrate selectivity to furnish ***para*-2p** as the major product in 78:22 regioisomeric ratio, 1,530 ± 50 TTN and 85:15 e.r. This result highlighted the potential of metalloenzymes to exert regiocontrol over free-radical-mediated transformations. By contrast, previously developed radical cyclization using photoredox and small-molecule Cu catalysts furnished racemic oxindole **2p** as a mixture of *para*- and *ortho*-product in an approximately 1:1 ratio, slightly favouring ***ortho*-2p** (with photoredox catalyst Ir(ppy)$_3$: ***p*-2p**:***o*-2p** = 44:56; with Cu(tris(2-pyridyl)methylamine)Br: ***p*-2p**:***o*-2p** = 39:61; Supplementary Methods). The non-selective nature of these reactions underscored the challenge of imposing regiocontrol over radical cyclization using conventional approaches.

Next, we sought to generalize this metalloenzymatic radical process to the conversion of α-chloro substrates. Gratifyingly, it was found that P450$_{arc1}$ and P450$_{arc2}$–P74G–L436R–F330V allowed the enantioconvergent conversion of chloride **3a** with orthogonal enantiopreferences (Fig. 4a). Under optimized reaction conditions (OD_{600} = 120), P450$_{arc1}$ gave rise to ***(S)*-2a** in 85% yield, 450 ± 60 TTN and 94:6 e.r. Under similar conditions with a lower cell density (OD_{600} = 30), the enantiocomplementary variant P450$_{arc2}$–P74G–L436R–F330V furnished ***(R)*-2a** in 64% yield, 1,200 ± 110 TTN and 12:88 e.r. (Fig. 4a). Furthermore, another enzyme variant from this evolutionary lineage, P450$_{arc3}$ (P450$_{arc1}$–G438T–L266H–L78C–V328E–S332A), was found to promote effective kinetic resolution of **3a** (Fig. 4b). With P450$_{arc3}$, at a conversion of 55%, ***(R)*-3a** was recovered in 94:6 e.r. This corresponds to an *S* factor of 18 ($S = \log[(1-c)(1-\text{e.e.})]/\log[(1-c)(1+\text{e.e.})]$, where *c* is conversion and e.e. is enantiomeric excess, e.e. = $([R]-[S])/([R]+[S])$), indicating

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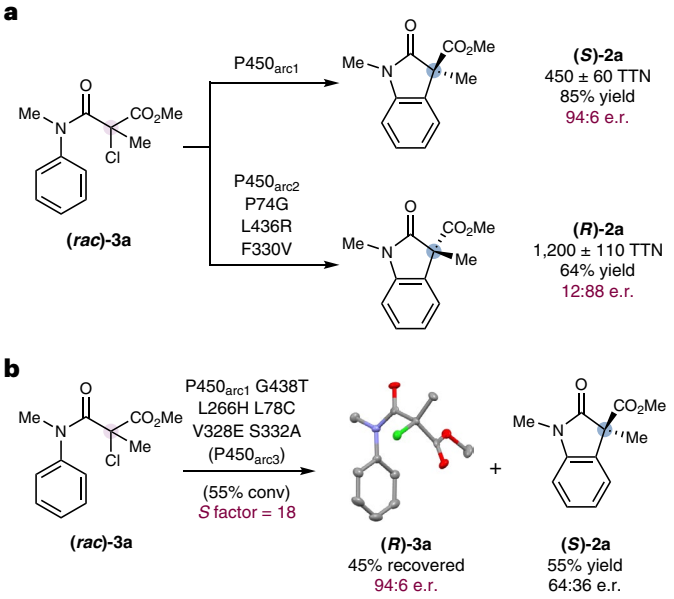

Fig. 4 | P450$_{arc}$-catalysed stereoselective transformations of α-chloro substrate 3a. a, P450$_{arc1}$ and P450$_{arc2}$–P74G–L436R–F330V-catalysed enantioconvergent transformation of **3a** with complementary enantiopreferences. **b**, P450$_{arc3}$-catalysed radical kinetic resolution of **3a**. All the reactions were performed in triplicate and averaged results and standard deviation are provided.

excellent enantiodiscrimination by this kinetic resolution enzyme. Additionally, P450$_{arc3}$ furnished cyclization product **2a** in 55% yield and 64:36 e.r. Additionally, it was found that **(*S*)-3a**, the enantiomer undergoing faster radical cyclization with P450$_{arc3}$, exhibited the same stereochemistry at the α-position as the major enantiomeric product **(*S*)-2a** (Fig. 4b). Together, these results demonstrated the highly malleable nature of this metalloenzyme platform to affect both enantioconvergent radical cyclization and kinetic resolution with evolutionarily related mutants.

Mechanistic and computational studies

Enzymes capable of accommodating and transforming both enantiomers of a racemic substrate that are configurationally stable into the same major enantiomeric product are rare in natural biosynthetic machineries, despite a handful of recently developed new-to-nature biocatalytic enantioconvergent transformations[15,16,18,40]. To date, detailed enantioconvergent mechanisms of these unnatural biocatalytic processes remain poorly understood. With a panel of highly efficient P450$_{arc}$ enzymes permitting the enantioconvergent transformation of both α-bromo and α-chloro substrates, we studied the evolution of the e.r. of formed products and recovered substrates as a function of substrate conversion (Fig. 5).

First, when the α-bromo substrate **1b** was used, with P450$_{arc2}$ as the biocatalyst (Fig. 5a), the e.r. of product **2b** remained constant (97:3) throughout the course of the reaction, with **(*R*)-2b** as the major enantiomeric product. During this enantioconvergent transformation, as the reaction proceeded, gradual enrichment of **(*S*)-1b** in recovered **1b** was observed, showcasing kinetic resolution. With P450$_{arc2}$, this kinetic resolution occurred with low levels of enantiodiscrimination, as evidenced by a small *S* factor of 1.4 ± 0.1. Thus, although **(*R*)-1b** underwent faster conversion relative to its enantiomer **(*S*)-1b**, product **2b** derived from **(*R*)-1b** and **(*S*)-1b** exhibited identical e.r.

Second, when P450$_{arc1}$ was applied to transform **1b** (Fig. 5b), although the product enantiopreference was reversed to favour **(*S*)-2b**, the same (*R*)-enantiomer of **1b** still underwent faster conversion, similar to that with P450$_{arc2}$. Furthermore, as the reaction proceeded to higher conversions, the e.r. of product **2b** increased, indicating that the slow-reacting enantiomer **(*S*)-1b** was converted to **2b** with a slightly higher e.r. Similar to P450$_{arc2}$, P450$_{arc1}$ also displayed a low kinetic-resolution selectivity toward the substrate (*S* factor = 1.8 ± 0.1).

Third, starting from the α-chloro substrate **3a**, P450$_{arc1}$ furnished the same major enantiomeric product **(*R*)-2a** as that from the bromo substrate **1a** (Fig. 5c). Kinetic resolution of the substrate was also observed (*S* factor = 3.4 ± 0.5), with **(*R*)-3a** being transformed faster than **(*S*)-3a**. Using the same biocatalyst P450$_{arc1}$, the (*R*)-enantiomer of both the chloro (**3a**) and the bromo (**1a**) substrate underwent faster transformation. Interestingly, as the reaction proceeded, a slight decrease in the e.r. of **2a** was observed, suggesting that the slow-reacting **(*S*)-3a** furnished **2a** with a lower enantioselectivity. This trend is contrary to that of the bromo substrate **1b** with the same enzyme variant (vide supra). Together, these experiments demonstrate that for all these newly evolved enantioconvergent biocatalysts, kinetic resolution of the substrate occurs with modest selectivities, despite the ability of these biocatalysts to fully transform the racemic substrate with excellent product enantioselectivities. Furthermore, all three possible product e.r. evolution patterns, including constant e.r. (Fig. 5a), slowly increasing e.r. (Fig. 5b) and slowly decreasing e.r. (Fig. 5c), were uncovered in this study, revealing subtle differences in enzymatic stereocontrol over enantioconvergent radical cyclization.

To provide further insights into the reaction mechanism, we performed density functional theory (DFT) calculations using a model Fe porphyrin system, which has been used in our previous computational studies (Fig. 6)[23,24,40,41]. DFT calculations showed that the Fe porphyrin catalyst remains at high spin throughout the catalytic cycle[23,24]. Due to the relatively weak C–Br bond in α-bromo-β-amidoester **1a** (bond-dissociation enthalpy = 47.6 kcal mol^{-1}), the Fe-catalysed radical initiation to afford an α-carbonyl radical **4a** is highly exergonic (ΔG = −35.5 kcal mol^{-1}). With this model system bearing an anionic methoxide axial ligand, the radical initiation step prefers an outer-sphere dissociative electron transfer mechanism ($\Delta G^{\ddagger}$ = 2.0 kcal mol^{-1}) over the inner-sphere electron transfer pathway (**TS1** in Fig. 6, $\Delta G^{\ddagger}$ = 15.5 kcal mol^{-1}). The subsequent radical cyclization to the pendant aromatic ring has an activation barrier ($\Delta G^{\ddagger}$) of 14.2 kcal mol^{-1}, indicating a sufficient lifetime for the enantioconvergent conversion of racemic starting materials via the conformational change and C–C bond rotation of radical **4a**. For the radical–polar crossover step, several proton/electron transfer pathways from the dearomatized radical intermediate **8a** to the oxindole product **2a** were considered. The most favourable mechanism features a concerted proton–electron transfer (CPET)[43], as previously studied in a related haem system[44], where electron transfer from **8a** to the haem Fe and proton transfer from **8a** to the haem propionate occur in a concerted manner. This CPET pathway displays a low free-energy barrier of 1.1 kcal mol^{-1}. Stepwise proton transfer–electron transfer and electron transfer–proton transfer processes are kinetically less favourable, because of the higher barriers to form the relatively unstable intermediates **11** and **12**, respectively. This finding indicates that the carboxylate group of the haem cofactor may serve as a base catalyst to facilitate the radical–polar crossover event, demonstrating the potential role of the haem cofactor as a bifunctional catalyst to greatly lower the activation barrier of radical–polar crossover. Although other basic residues in the enzyme active site may serve as the base catalyst, these DFT calculations suggest that this CPET process is more favourable, as it bypasses the formation of high-energy intermediates resulting from stepwise electron or proton transfer. Additionally, as this CPET enables a fast and irreversible process to trap the cyclized radical intermediate **8a**, radical cyclization (**TS2** in Fig. 6) is expected to be the enantioselectivity-determining step with the enzyme catalyst. This mechanistic scenario is consistent with kinetic isotope effect experiments that suggest irreversible radical cyclization because of the kinetically facile trapping of **8a** (see Supplementary Fig. 7).

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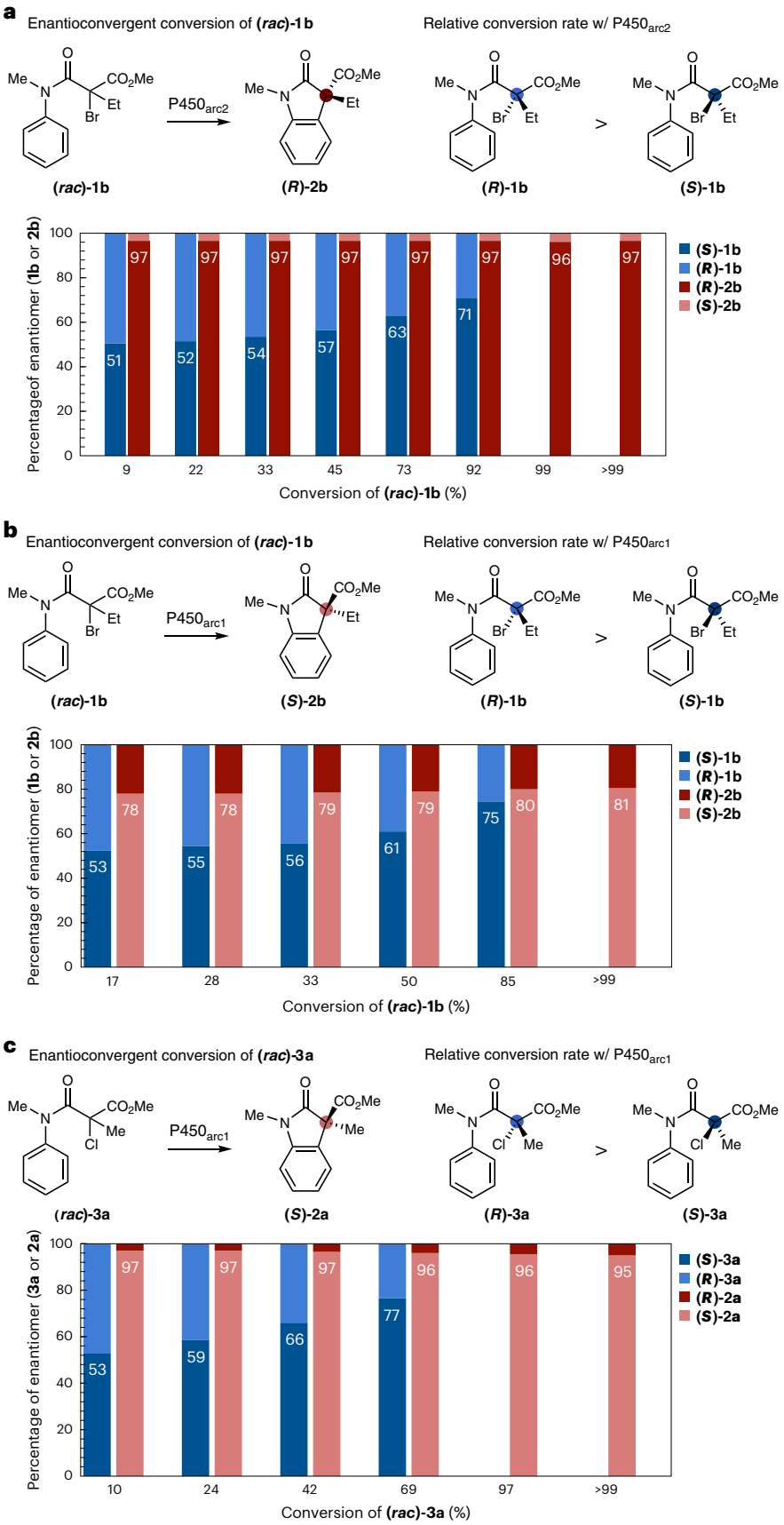

Fig. 5 | Time course of P450$_{arc}$-catalysed enantioconvergent radical cyclization processes. a, P450$_{arc2}$-catalysed enantioconvergent radical cyclization of (***rac***)-**1b**. **b**, P450$_{arc1}$-catalysed enantioconvergent radical cyclization of (***rac***)-**1b**. **c**, P450$_{arc1}$-catalysed enantioconvergent radical cyclization of (***rac***)-**3a**.

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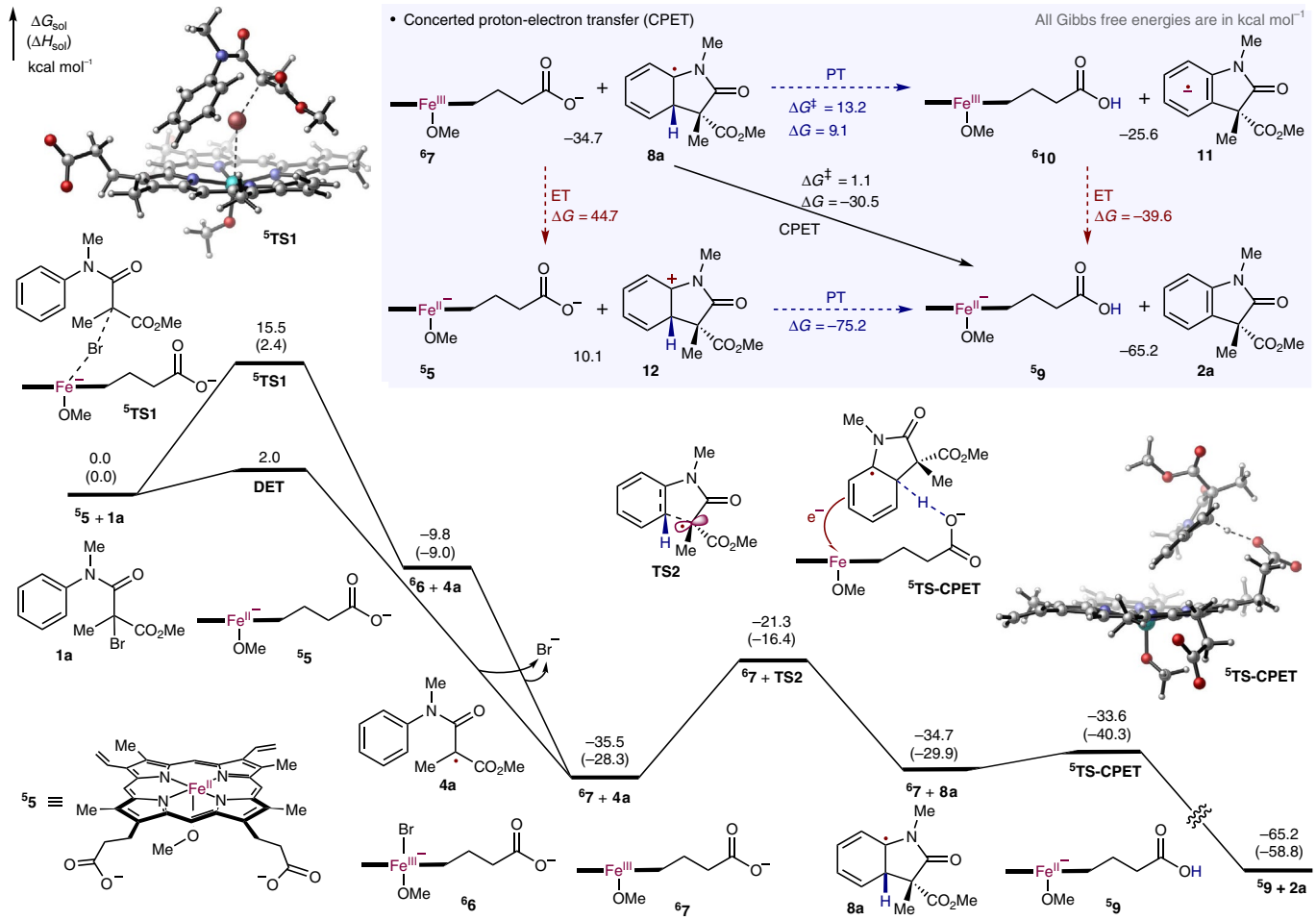

Fig. 6 | Reaction energy profile of biocatalytic radical cyclization to arenes using a model system for serine-ligated P450$_{arc}$. Density functional theory calculations were carried out at the (U)B3LYP-D3/6-311+G(d,p)–LANL2TZ(f)/SMD(chlorobenzene)//(U)B3LYP-D3/6-31G(d)–LANL2DZ level of theory. PT, proton transfer; ET, electron transfer.

Conclusion

We have developed a unifying metalloenzyme platform for the asymmetric radical cyclization to arenes, allowing challenging quaternary stereogenic centres to be formed with excellent enantioselectivities. Directed evolution enabled the rapid engineering of an orthogonal set of P450 radical cyclases P450$_{arc1–2}$, allowing either enantiomeric product to be accessed via enantioconvergent radical cyclization. Furthermore, kinetic resolution biocatalyst P450$_{arc3}$ was also developed, giving rise to enantioenriched tertiary alkyl chlorides. Thus, closely related biocatalysts were engineered to catalyse both enantioconvergent transformation and kinetic resolution of the same racemic substrates via a common radical mechanism. DFT calculations suggest that the radical–polar crossover event with ferric haem is facilitated by proton-coupled electron transfer and the C–C bond-forming radical addition determines the stereoselectivity of enantioconvergent processes. Collectively, the promiscuous nature and the excellent tunability of this metalloenzyme platform highlight its potential to tackle challenging problems in asymmetric radical transformations via unnatural biocatalysis.

Methods

Expression of P450$_{arc}$ variants

E. coli (*E. cloni* BL21(DE3)) cells carrying plasmid encoding the appropriate P450$_{arc}$ variant were grown overnight in 3 ml of Luria-Bertani medium with 0.1 mg/mL ampicillin (LB$_{amp}$). Preculture (1.5 ml) was used to inoculate 28.5 ml of Hyperbroth (AthenaES) with 0.1 mg/mL ampicillin (HB$_{amp}$) in an Erlenmeyer flask (125 mL). This culture was incubated at 37 °C and 230 r.p.m. for 2 h. It was then cooled on ice for 20 min and induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside and 1.0 mM 5-aminolevulinic acid (final concentrations). Expression was conducted at 22 °C and 150 r.p.m. for 20 h. *E. coli* cells were then transferred to a conical tube (50 ml) and pelleted by centrifugation (3,000*g*, 5 min, 4 °C). Supernatant was removed and the resulting cell pellet was resuspended in M9-N buffer to OD$_{600}$ = 5–60 (usually 15–30). An aliquot of this cell suspension (2 ml) was taken to determine protein concentration using the pridine hemochromagen assay after lysis by sonication.

Stereoselective radical cyclization using whole *E. coli* cells harbouring P450$_{arc}$

Suspensions of *E. coli* (*E. cloni* BL21(DE3)) cells expressing the appropriate P450$_{arc}$ variant in M9-N buffer (typically OD$_{600}$ = 30) were kept on ice. In another conical tube, a solution of D-glucose (500 mM in M9-N) was prepared. The suspension of *E. coli* cells expressing P450$_{arc}$ (typically OD$_{600}$ = 30, 345 µl) and the solution of D-glucose (40 µl of 500 mM stock solution in M9-N buffer) were added to a vial (2 ml). This vial was then transferred into an anaerobic chamber, where the organic substrate (15 µl of stock solution (267 mM in EtOH)) was added. The final reaction volume was 400 µl; the final concentrations were 10 mM substrate and 50 mM D-glucose. (Note: the reaction performed with *E. coli* cells resuspended to OD$_{600}$ = 30 indicates that 345 µl of OD$_{600}$ = 30 cells were added, and likewise for other reaction OD$_{600}$ descriptions.) The vials

were sealed and agitated in a Corning digital microplate shaker at room temperature and 680 r.p.m. for 12 h.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All data are available in the main text and the Supplementary Information or available from the authors upon reasonable request. X-ray crystal structures of **2i** and **(*R*)-3a** are available free of charge from the Cambridge Crystallographic Data Centre under reference numbers CCDC 2184585 and 2184586. Plasmids encoding $P450_{arcs}$ reported in this study are available for research purposes from Y.Y. under a material transfer agreement with the University of California Santa Barbara.

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Author contributions

Y.Y. conceived and directed the project. W.F., N.M.N., Y.Z. and B.K.-H. designed and performed the experiments. Y.F. carried out the computational studies with P.L. providing guidance. Y.Y., Y.F. and P.L. wrote the manuscript with the input of all other authors.

Competing interests

Y.Y., W.F., N.M.N. and Y.Z. are inventors on a patent application (US provisional patent no. 63/477,081) submitted by the University of California Santa Barbara that covers stereoselective biocatalytic radical addition to arenes. The remaining authors declare no competing interests.

Additional information

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Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

Correction

Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

Graph analysis

Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).

Multivariate modeling and predictive analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.