Chemo-enzymatic cascades to produce cycloalkenes from bio-based resources

Shuke Wu1,3*, Yi Zhou1, Daniel Gerngross2, Markus Jeschek2 & Thomas R. Ward1*

Engineered enzyme cascades offer powerful tools to convert renewable resources into value-added products. Man-made catalysts give access to new-to-nature reactivities that may complement the enzyme's repertoire. Their mutual incompatibility, however, challenges their integration into concurrent chemo-enzymatic cascades. Herein we show that compartmentalization of complex enzyme cascades within E. coli whole cells enables the simultaneous use of a metathesis catalyst, thus allowing the sustainable one-pot production of cycloalkenes from oleic acid. Cycloheptene is produced from oleic acid via a concurrent enzymatic oxidative decarboxylation and ring-closing metathesis. Cyclohexene and cyclopentene are produced from oleic acid via either a six- or eight-step enzyme cascade involving hydration, oxidation, hydrolysis and decarboxylation, followed by ring-closing metathesis. Integration of an upstream hydrolase enables the usage of olive oil as the substrate for the production of cycloalkenes. This work highlights the potential of integrating organometallic catalysis with whole-cell enzyme cascades of high complexity to enable sustainable chemistry.
The exquisite performance and sophisticated orchestration of metabolic reaction networks have inspired the transition from single-step biocatalysis to cascade biocatalysis—interconnected enzymatic reactions in one pot. This strategy allows to minimize the isolation and workup of intermediates, thus reducing operation time, waste, and, sometimes, enhancing overall selectivity and yield. Furthermore, enzymes may be combined with organometallic catalysts to assemble chemo-enzymatic cascades to perform useful one-pot transformations unattainable with enzymes or small-molecule catalysts alone. In the past 6 years, significant progress have been made in combining enzymes with organometallic catalysts, including photocatalysts. For this purpose, different strategies were implemented to accommodate the incompatible reactivities in a single vessel. Despite these achievements, most of the chemo-enzymatic cascades reported to date are limited to one or two enzymatic steps. Quite on the contrary, the inherent sophistication of the metabolism in cells, which leverage the exquisite synthetic power of enzyme pathways, suggests the feasibility of much more complex reaction schemes. Synthetic biology and metabolic engineering hold great promise for sustainable chemical production from renewable resources via engineered microbial cell factories operating in single-vessel processes. Combining enzymes from different organisms substantially expands the scope of bioproduction; however, it remains limited to the existing enzymes’ repertoire. For example, the recent discovery of decarboxylases and decarboxylases has led to bioproduction of various hydrocarbons, including various linear alkanes, linear alkenes and aromatics (Fig. 1). However, to the best of our knowledge, cycloalkanes and cycloalkenes have not been reported via enzymatic pathways. Cycloalkanes are bulk petrochemicals widely employed in various industrial processes, including use as solvents, synthesis of cyclic compounds (e.g., cyclohexanol) and ring-opened chemicals (e.g., adipic acid). Currently, cycloalkanes are derived from fossil-fuels (e.g., steam cracking of naphtha and partial hydrogenation of benzene). The unavailability of enzymatic production is likely due to the limited number of enzyme-catalyzed C–C cyclization reactions. Indeed, most of these enzymes (e.g., terpene cyclases) are highly substrate-specific thus limiting their widespread implementation. Recent advances in heme-proteins that catalyze an abiotic carbene transfer offer a powerful means to generate cyclopropane or cyclopropene rings (C3).

Building on our previous work on ring-closing metathesis (RCM) in a biocompatible environment, we contemplated the possibility of capitalizing on RCM to construct cycloalkenes (C5–C7) from diolefins produced from renewable sources. Herein we report on our efforts to combine ring-closing metathesis with an engineered multi-enzyme cascade to produce cyclopentene, cyclohexene, and cycloheptene from olive oil-derivated intermediates (Fig. 1). This development, combining a concurrent RCM with up-to nine enzymatic steps hosted by whole cells of Escherichia coli in a single reaction vessel, showcases the potential of integrating biocompatible, new-to-nature reactions with synthetic biology at high sophistication.

Results
Design of chemo-enzymatic cascades. We applied a biocatalytic retrosynthetic analysis to design chemo-enzymatic cascades to produce cyclopentene (1a), cyclohexene (1b), and cycloheptene (1c) from oleic acid (6) (Fig. 2a, b). We hypothesized that cycloalkenes (1a–1c) may be accessed via ring-closing metathesis of the corresponding α,ω-dienes (2a–2c), which, in turn, can be produced from an oxidative bi-decarboxylation of ω,ω-dicarboxylic acids (4a–4c) using a decarboxylase (5a) (Fig. 2a). Exploratory studies using oleic acid (6) suggested that decarboxylation of carboxylate 6 affords 1,8-heptadecadiene (5), which may undergo ring-closing metathesis to afford cycloheptene 1c and 1-decene (Fig. 2b). To access the synthetically more useful cyclopentene (1a) and cyclohexene (1b) from oleic acid 6, we surmised that one could complement previously reported enzyme cascades— for the production of diacids from oleic acid—with a decarboxylase and an RCM catalyst. The diacids 4a and 4b are produced via a six- and four-enzyme-cascade, respectively (Fig. 2b). This is achieved via hydration of oleic acid (6) by OhyA2, followed by oxidation with MLADH to the corresponding keto-acid. Subjecting the keto-acid to a Baeyer–Villiger monoxygenase, using either PpBVMO or PfBVMO, followed by hydrolysis by TLL affords either sebacic acid (4b) or PfBVMO— or a hydroxy-carboxylic acid—with PpBVMO—. The latter may be oxidized by ChnD and ChnE to afford azelaic acid (4a) (Fig. 2b, Supplementary Fig. 1 for details). As olive oil is readily hydroyzed by TLL to afford oleic acid 6, one may thus be able to produce cycloalkenes 1a–1c, typically derived from petroleum-based feedstocks, from renewable resources via a concurrent chemo-enzymatic cascade.
Selection of the decarboxylase. To identify a suitable enzyme for the bis-decarboxylation of 4a-4c, we evaluated three oxidative decarboxylases, including: (i) a P450 monooxygenase OleT, combined with the putidaredoxin CamAB, (ii) a non-heme mononuclear iron oxidase UndA from *Pseudomonas*, and (iii) a membrane-bound desaturase-like enzyme UndB from *Pseudomonas*. These enzymes expressed well in recombinant *E. coli* strains as confirmed by SDS–PAGE analysis of the whole-cell protein extracts (Supplementary Fig. 2). The *E. coli* whole cells were used for the bis-decarboxylation of 4a-4c and decarboxylation of the intermediate ω-alkenoic acids (3a-3c) to afford the corresponding a,ω-dienes (2a-2c) in aqueous potassium phosphate buffer (KP). As summarized in Fig. 3a–c, both UndA and UndB displayed good activity towards medium-chain carboxytes 3a-3c, while OleT was inactive. Remarkably, only UndB catalyzed the bis-decarboxylation of 4a-4c to afford 2a-2c. These results are consistent with previous reports: OleT prefers long-chain saturated fatty acids, while UndA and UndB favor medium-chain fatty acids. Under optimized conditions, the bis-decarboxylation of 4a-4c in the presence of *E. coli* over-expressing UndB was improved to afford increased amounts of 2a (1600 μM, 32% conversion), 2b (1900 μM, 38% conversion) and 2c (800 μM, 16% conversion), respectively (Fig. 3a–c). In view of the hydrophobicity and low aqueous solubility of dienes 2a-2c, we examined a range of water-miscible and immiscible solvents as well as several surfactants added to the phosphate buffer. The results (Supplementary Fig. 3) suggest that n-dodecane (10%), abbreviated KP-Dod hereafter, and the non-ionic surfactant TPGS-750-M (1%) are compatible with UndB, maintaining >50% productivity for the conversion of 4b to 2b. Although isooctane is often used in conjunction of purified enzymes, it dramatically reduced the activity of UndB, probably due to the destabilization of the cell membrane. Thus, n-dodecane and TPGS-750-M were selected for further studies. Up to ≥80% conversion was obtained starting from 2 mM of 4a and 4b or 1 mM of 4c by *E. coli* (UndB) in KP buffer with or without either n-dodecane or TPGS-750-M (Supplementary Fig. 4).

Identification of the most suitable metathesis catalyst. To identify a biocompatible metathesis catalyst, we selected three commercial (Hoveyda)-Grubbs ruthenium(II) catalysts (Ru1, Ru2, Ru3, Supplementary Fig. 5), which have been reported to tolerate air and water. The RCM activity of Ru1, Ru2, Ru3 (100 μM, 2 mol%) was evaluated for diolein (7b, 5 mM) either in: (i) KP buffer, (ii) KP buffer and n-dodecane (10%), or (iii) in KP buffer containing the non-ionic surfactant TPGS-750-M (1%) (Fig. 3d). In all cases, the formation of cyclohexene 1b was detected. The biphasic system with 10% n-dodecane clearly outperformed both other systems. To our delight, Ru3 (Fig. 3d) afforded cyclohexene in >90% conversion for the biphasic system. Both other diolein substrates 2a and 2c afforded cyclopentene 1a (87% conversion) and cycloheptene 1c (72% conversion), respectively (Fig. 3e, f). RCM of dienes 2a and 2b (5 mM) in the presence of a lower catalyst Ru3 loading (50 μM, 1%) using the KP-Dod system afforded cycloalkanes 1a and 1b in 75% and 88% conversions respectively (corresponding to TTNs of 75 and 88). The commercially available catalyst Ru3 was thus selected for the implementation of the chemo-enzymatic cascades.
reaction in the presence of E. coli (UndB). The results are similar to those obtained in the concurrent cascade; high conversion (83%) was only achieved with Ru3 in KP-Dod (Fig. 4a, Supplementary Fig. 6). The sequential cascade however required 48 h instead of 24 h for the concurrent cascade to achieve comparable conversions. The reaction progress of the concurrent cascade (Supplementary Fig. 7) reveals minimal accumulation of intermediates 2b and 3b, thus highlighting that the cascade proceeds in a concurrent manner. Next, the E. coli (UndB) and Ru3 were used in the KP-Dod mixture to convert the diolefins 4a and 4c to...
cyclopentene 1a and cyclohexene 1c in high conversion (83% and 77% conversion, respectively, Fig. 4b, c).

A concurrent cascade to convert oleic acid to cyclohexene. Upon enzymatic decarboxylation, oleic acid (6) is converted to 1,8-heptadecadiene (5), a bio-based precursor of cyclohexene (1c, Fig. 2b). Accordingly, a two-step decarboxylation-metathesis cascade was engineered to convert oleic acid (6) to cyclohexene (1c). The E. coli strains over-expressing OleT, UndA, and UndB were examined for decarboxylation of oleic acid (6) (5 mM) in KP buffer, to afford 1,8-heptadecadiene (5). While OleT is known to display very modest activity toward Δ⁸ unsaturated fatty acids, E. coli (UndB) performed the best, giving 33% conversion to diene 5 (Fig. 5a). Upon combining E. coli (UndB) with Ru3, oleic acid (6) (2 mM) was converted to cyclohexene (1c) in the KP-Dod system in 44% conversion (Fig. 5b). The chemo-enzymatic cascade also performed well in a sequential mode: adding the catalyst Ru3 after 12 h allowed to maintain the same overall reaction time at the cost of a lower conversion however (24 h, 33% conversion, Fig. 5b). In a similar fashion, the two-step chemo-enzymatic cascade could be used to produce cyclopentene (1a) and cyclohexene (1b), but the corresponding fatty acids with a C=C bond at position 7 and 8 are not readily available. To access these cycloalkenes, we thus engineered the corresponding enzyme cascades in E. coli to produce the diolefins from oleic acid (6).

Engineering E. coli cells to convert oleic acid to diacids. To produce cyclopentene (1a) and cyclohexene (1b) from oleic acid (6), it is necessary to engineer enzyme cascades to produce the corresponding sebacic acid (4b) and azelaic acid (4a) from oleic acid (6). Two previous enzyme cascades have reported the conversion of oleic acid (6) to diacids 4a and 4b via hydration, oxidation, Baeyer–Villiger oxidation (with different regioselectivity) and hydrolysis by TLL (for 4b), as well as further double oxidation (for 4a) (Fig. 2b, Supplementary Fig. 1 for details). The reported cascades were accomplished by combining multiple E. coli strains as well as purified enzymes. We set out to implement this complex cascade in a single E. coli strain.

According to previous reports and our experience, the bottleneck in the above cascades is likely the Baeyer–Villiger oxidation by PbBVMO (for 4b) and PbBVMO (for 4a). Some N-terminal tags on BVMOs have been shown to improve the stability of the enzymes. Thus, fusion of BVMOs with different N-terminal tags was tested: the 6x Glu-tagged PbBVMO and 6x His-tagged PbBVMO outperformed other constructs, including BVMOs with no tag (Supplementary Figs. 8 and 9). A native fatty-acid transporter, FadL, was overexpressed and found to boost the hydration of oleic acid (6) in E. coli (Supplementary Fig. 10). The other enzymes (OhyA2, MIADH, TLL) expressed well in individual E. coli strains (Supplementary Fig. 11).

In order to combine all required activities for the conversion of oleic acid (6) to the dicarboxylate 4a or 4b in a single E. coli strain, we sought to optimize the absolute and relative expression levels of the corresponding enzymes relying on engineering of the respective ribosome binding sites (RBSs). Therefore, we performed combinatorial pathway optimization by applying the recently developed algorithm RedLib to design tailor-made degenerate RBS sequences. Briefly, degenerate RBSs were designed to achieve four different expression levels for each of the four involved genes with the goal to uniformly span the accessible range (Supplementary Fig. 20). For the production of sebacic acid (4b), primers with degenerate RBS (4x) were used to amplify PbBVMO, MIADH, OhyA2, and TLL (with the plasmid backbone). The resulting combinatorial library (combinatorial size: 4 × 4 × 4 × 4 = 256 variants) was assembled in vitro and used to transform E. coli (Fig. 6a). In total 576 of the resulting colonies were randomly picked (~89.5% statistical coverage), cultured in six 96-deep well plates and assayed for the conversion of oleic acid (6). The concentration of sebacic acid (4b) was quantified by UPLC analysis (Fig. 6b). The best four strains for each plate were cultured in flasks for further validation and quantification of the produced sebacic acid (4b) (Fig. 6c). The most promising strain, coined E. coli (C10) hereafter (P1-A4 in Fig. 6c), was selected for converting oleic acid (6) to sebacic acid (4b) in further...
Fig. 6 Engineering and screening a strain library for the production of sebacic acid (4b) from oleic acid (6). a) Construction of a plasmid library for the co-expression of PpBVMO, MIADH, OhyA2, and TLL with different expression levels, and screening of the resulting E. coli library to identify the most effective strain for the production of sebacic acid (4b). All E. coli strains included an additional plasmid harboring the fatty acid transporter FadL. b) Initial screening of 576 strains in six 96-well plates for production of sebacic acid (4b). c) Further validation and comparison of 24 strains (best four from each plate) for the production of sebacic acid (4b). The green column indicates the best strain E. coli (C10). The reactions were performed using oleic acid (6) (5 mM) and E. coli (10 g l⁻¹) in KP buffer (200 mM, pH 8.0, 1% glucose) at 30 °C for 24 h. Source data are provided as a Source Data file. Data in c) are mean values of triplicate experiments with error bars indicating standard deviations (n = 3).

Cascades for oleic acid to cyclopentene and cyclohexene. Cyclopentene (1a) may be produced from oleic acid (6) via a chemo-enzymatic cascade by combining E. coli (C9), E. coli (UndB) and Ru3 either in a concurrent- or a sequential cascade. Since E. coli (UndB) also catalyzes the decarbonylation of oleic acid (6) to form the diolein 1,8-heptadecadiene (5), E. coli (UndB) and Ru3 can only be added after the conversion of oleic acid to the corresponding diacids, sebacic acid (4b) and azelaic acid (4a), respectively. As highlighted in Fig. 7a, adding all three catalytic components simultaneously (i.e. E. coli (C9), E. coli (UndB) and Ru3), cyclopentene (1a) was produced in only 14% conversion from oleic acid 6 (2 mM). In contrast, the delayed addition of E. coli (UndB) and Ru3 led to cyclopentene (1a) in 65% conversion under the same reaction conditions. We surmise that the cross-reactivity of E. coli (UndB) is the major ground for the erosion of the conversion in the concurrent cascade. For the production of cyclohexene (1b) from oleic acid (6), the E. coli (C10), and E. coli (UndB) strains as well as the RCM catalyst Ru3 were combined in one pot either simultaneously or sequentially, (Fig. 7b). Again here, the delayed addition of E. coli (UndB) and Ru3 led to significantly higher conversions of cyclohexene (1b) (compare 6 % for the simultaneous vs. 22 % conversion for the sequential cascade starting with 2 mM oleic acid (6)). The modest conversion of cyclohexene (1b) is tentatively assigned to the low productivity of PpBVMO (Supplementary Fig. 14). The modest productivity of the former may be improved by engineering a more active BVMO. In summary, by combining two engineered E. coli strains and a ruthenium complex Ru3, cyclopentene (1a) and cyclohexene (1b) were produced from oleic acid (6) in one pot in titers 1.30 mM and 0.44 mM after 24 h, respectively.

Cascades to convert olive oil to cycloalkenes. Currently, oleic acid is usually manufactured from plant oils (e.g. canola oil, olive oil) on a very large scale.62 To directly utilize these attractive bio-based feedstocks (or even waste oil), we further extend the chemo-enzymatic cascades from a readily available plant oil–olive oil (7), as an example. The enzymatic hydrolysis of olive oil (7) produces oleic acid (6) under mild conditions and is compatible with the implementation of enzyme-cascades. We thus engineered E. coli (TLL) cells expressing the lipase from Thermomyces lanuginosus for the hydrolysis of olive oil (7) to oleic acid (6). The E. coli (TLL) cells were lyophilized and stored. Using the lyophilized cells (1 g l⁻¹), oleic acid (6) was produced with titers ranging from 1.1-2.5 mM from olive oil 7 (1-2 g l⁻¹) in 1-3 h (Supplementary Fig. 15). To produce cycloheptene (1c) from olive oil 7, lyophilized E. coli (TLL), E. coli (UndB) and Ru3 were combined simultaneously or sequentially in one pot. As presented in Fig. 8a, 610–670 µM of cycloheptene (1c) was produced from olive oil (7, 1 g l⁻¹) via this chemo-enzymatic cascade in a concurrent mode as well as two complementary sequential modes. A third sequential cascade afforded cycloheptene (1c) in lower concentration (330 µM). We hypothesize that the lower conversion may be due to the limited RCM reaction time applied in the third sequential cascade. For the production of cyclopentene (1a) from olive oil (7), lyophilized E. coli (TLL), E. coli (C9), E. coli (UndB) and Ru3 were combined sequentially in one pot: to our delight, 760 µM of cyclopentene (1a) was produced from olive oil (7, 1 g l⁻¹) via this extended chemo-enzymatic cascade that includes nine enzymatic reactions preceding the final ring-closing metathesis step (Fig. 8b). Similarly, combining lyophilized E. coli (TLL), E. coli (C10), E. coli (UndB) and Ru3 sequentially, enabled the production of cyclohexene (1b) in 710 µM from olive oil (7, 1 g l⁻¹) via a chemo-enzymatic cascade that includes seven
enzymatic steps preceding the final RCM reaction (Fig. 8c). Assuming an oleic acid accounts for 70% of the fatty acids in olive oil, the production of cycloalkenes (1a-1c) in 670-760 μM corresponds to around 29–33% conversion from olive oil (7, 1 g l⁻¹). Although the overall conversion to cycloalkenes is rather modest, the chemo-enzymatic cascades showcase the feasibility to produce cycloalkenes from a renewable feedstock. Currently, the performance of the cascades is limited by the modest decarboxylase activity of UndB, which we surmise may be overcome by directed evolution.63–67 Considering that oleic acid and diacids are often encountered by microbes, discovering and evolving yet unknown decarboxylases specific for oleic acid or diacids may allow to overcome the current bottleneck of the cascades. In analogy to the discovery of an efficient decarboxylase,68 which led to the efficient production of styrene via a two-step deamination-decarboxylation cascade,68 the discovery and/or evolution of a more efficient decarboxylase may allow to significantly enhance the yields of cyclohexene 1c via the two-step decarboxylation-metathesis cascade. Similarly, the extended cascades towards cyclopentene 1a and cyclohexene 1b, may be further improved by relying on recently evolved69 or discovered70 BVMOs. Nevertheless, the extended one-pot chemo-enzymatic cascades that include (up to) nine enzymatic steps demonstrates the possibility to integrate a transition metal-catalyzed reaction with synthetic biology at high complexity.

Discussion

In summary, we have integrated a ruthenium-catalyzed ring-closing metathesis reaction as the last step of complex enzyme cascades for the one-pot production of cyclopentene (1a), cyclohexene (1b), and cycloheptene (1c) from olive oil (7) and its derivatives. The development of an aqueous-n-dodecane two-phase system enabled the combination of a ruthenium-based catalyst Ru3 with a delicate membrane-bound decarboxylase as part of extended enzyme cascades in E. coli whole cells, including up to nine enzymatic steps. The (chemo)-bioproduction of cycloalkenes from bio-based resources significantly expands the product realm accessible thanks to synthetic biology. The efficiency of the chemo-enzymatic cascades may be further engineered via the identification and/or the directed evolution of (more active) enzymes. The complementarity of homogeneous catalysts and enzymes allows the introduction of new-to-nature reactions with whole-cell enzyme cascades, thus synergizing the synthetic power of organometallic chemistry and synthetic biology.

Methods

Materials. Ru1, Ru2, Ru3, and n-dodecane were purchased from Sigma-Aldrich and used without further purification. Except for OleT, the genes of all the other enzymes were synthesized from Gene Universal. The recombinant E. coli strains were engineered using standard molecular cloning techniques by restriction enzymes or Gibson assembly. All the other chemicals were purchased from commercial suppliers and used without further purification. See Supplementary Table 1 for all the primers used in this study.

Preparation of engineered E. coli whole cells. LB medium (1 ml) with appropriate antibiotics was inoculated with recombinant E. coli cells and cultured for 8–10 h at 37 °C 250 rpm. Then, the cells were transferred to modified M9 medium (50 ml, with 2% glucose and 0.6% yeast extract) in a baffled flask to grow at 37 °C until OD₆₀₀ of the culture reached 0.6–0.8. At this time, IPTG was added to a final concentration of 0.5 mM. The culture was carried on at 22 °C for 12–14 h. The E. coli cells were harvested by centrifugation and immediately used in the enzyme cascades. For E. coli (TLL), the cells were harvested, washed with water, lyophilized, stored at 4 °C, and added as powder when needed.

Typical procedure for performing chemo-enzymatic cascades. Substrate stock solutions were prepared by dissolving in EtOH: 4a-4c (250 mM), 6 (350 mM), and 7 (100 g l⁻¹, emulsion). Stock solutions of cells were prepared by resuspending freshly harvested E. coli (C9), E. coli (C10) and/or E. coli (UndB) cells in KP buffer (200 mM, pH 8.0). The density of cells (OD₆₀₀) was determined with a UV-spectrometer. Stock solutions of Ru3 (5 mM) were freshly prepared by dissolving Ru3 in DMF: EtOH 1: 1. To an air-tight reaction tube (25 ml) with screw-cap, stock solutions of cells, KP buffer (200 mM, pH 8.0) and stock solution of glucose (50%) were added to form a system (0.5 ml) with required density/concentrations.
(cells: 10 g\(^{-1}\), glucose: 1%). Then, \(\text{n-dodecane (50 \mu l)}\) and stock solutions of Ru3 (5–10 \mu l) were added to the reaction tube. The stock solutions of the substrates (2–10 \mu l) were added last. The reaction tubes were sealed and incubated at 250 rpm, 30 °C for 24 h. For the cascades in the sequential mode, the appropriate amounts of cells, \(\text{n-dodecane (50 \mu l)}\) and Ru3 were added at the specified time. Upon completion, the reaction tubes were incubated on ice for 15–20 min before opening these (to minimize the loss of volatile alkenes) and adding of EIOP (1 ml, containing 1 \mu M of acetonaphen as internal standard). The reaction products were extracted, dried over \(\text{Na}_2\text{SO}_4\), and analyzed by GC–MS. See Supplementary Methods for details. See Supplementary Figs. 16 and 21 for the calibration curves. See Supplementary Figs. 17–19 for the representative GC–MS chromatograms.

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

**Data availability**

The DNA sequences of the synthetic genes are available in the SI file and they have been deposited in GenBank with the accession codes (UndA: MN125553; UndB: MN125554; PfBVMO: MN125555; PpBVMO: MN125556; MlADH: MN125557; OhyA2: MN125558; TLL: MN125559; ChnD: MN125560; ChnE: MN125561). The source data used to generate Figs. 3–8 and Supplementary Figs. 2–4, 6–16 and 20–21 are provided as a Source Data file. The other data that support the findings of this study are available from the corresponding authors upon request.

**Code availability**

The source code for RedLibs algorithm is available at GitHub: https://github.com/dgerngross/RedLibs.

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Author contributions
T.R.W. and S.W. conceived and designed the project. S.W. and Y.Z. performed the experiments and analyzed the results. M.J. and D.G. designed RBS libraries in silico. T.R.W. supervised the whole project. S.W. and T.R.W. wrote the manuscript with inputs from all authors.

Competing interests
The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to S.W. or T.R.W.