Cell Reports
Physical Science

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Perspective

Goals, challenges, and perspectives in chemical transformations within living systems

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SUMMARY

Chemical transformations have been a central focus of chemistry for centuries, and the development of elegant catalytic systems working in live systems has recently distinguished them from traditional catalyst designs, enabling multifaceted benefits and opportunities in numerous areas. This perspective summarizes common strategies for enabling abiotic catalysis in live cells and animals and highlights the goals, challenges, and potential solutions associated with those modern catalytic systems.

PRELUDE

Chemical transformations have been a central focus of chemistry for centuries. Spanning from the ancient Taoist "Dan-Yao" (pills from those far-east alchemists) furnaces in the East to alchemists' feverish pursuit of the philosopher's stone in the West, the quest to discover efficient synthetic transformations to high-value materials remains a critical challenge. Enormous advances have been made over time not just in bond making and breaking but also in instrumentation. Where early chemists in the pre-atomic theory period prized Antoine Lavoisier's distinctive gooseneck flask, modern robot-controlled automated synthesis machines are becoming commonplace today. Throughout this time, there has been a constant pursuit of synthetic processes with higher efficiency and lower cost. The advent of modern chemistry and chemical engineering has accelerated this progress significantly with the exploration of chemical transformations in arenas previously thought to be intractable. Developing catalysts for synthetic reactions at the interface of chemistry and biology, for example, has presented numerous novel challenges and opportunities.

HARNESSING THE POWER OF NATURE

In the modern era, synthetic biology and bioengineering have emerged as powerful contributors to the field of chemical transformations. The exploitation of microorganisms for chemical synthesis has a rich historical lineage extending from the use of microorganisms for wine and vinegar making during the Neolithic era to contemporary examples wherein cells act as streamlined synthetic factories powered by enzymes expressed within. An exemplary case for the utilization of a natural system is the production of paclitaxel wherein fermentation eliminates the necessity for tree cutting or lengthy chemical syntheses using hazardous chemicals, thereby leading to energy conservation and the availability of this anticancer drug to a wider population (Figure 1, top). Furthermore, by genetically altering enzymes and cells, it is possible to further enhance the efficiency of existing biosynthetic processes and create novel ones. This approach is illustrated well in the case of artemisinic acid, the direct microbial precursor of artemisinin used for multidrug-resistant malaria treatment. Through the utilization of an optimized mevalonate pathway, along

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https://doi.org/10.1016/j.xcrp.2024.101896

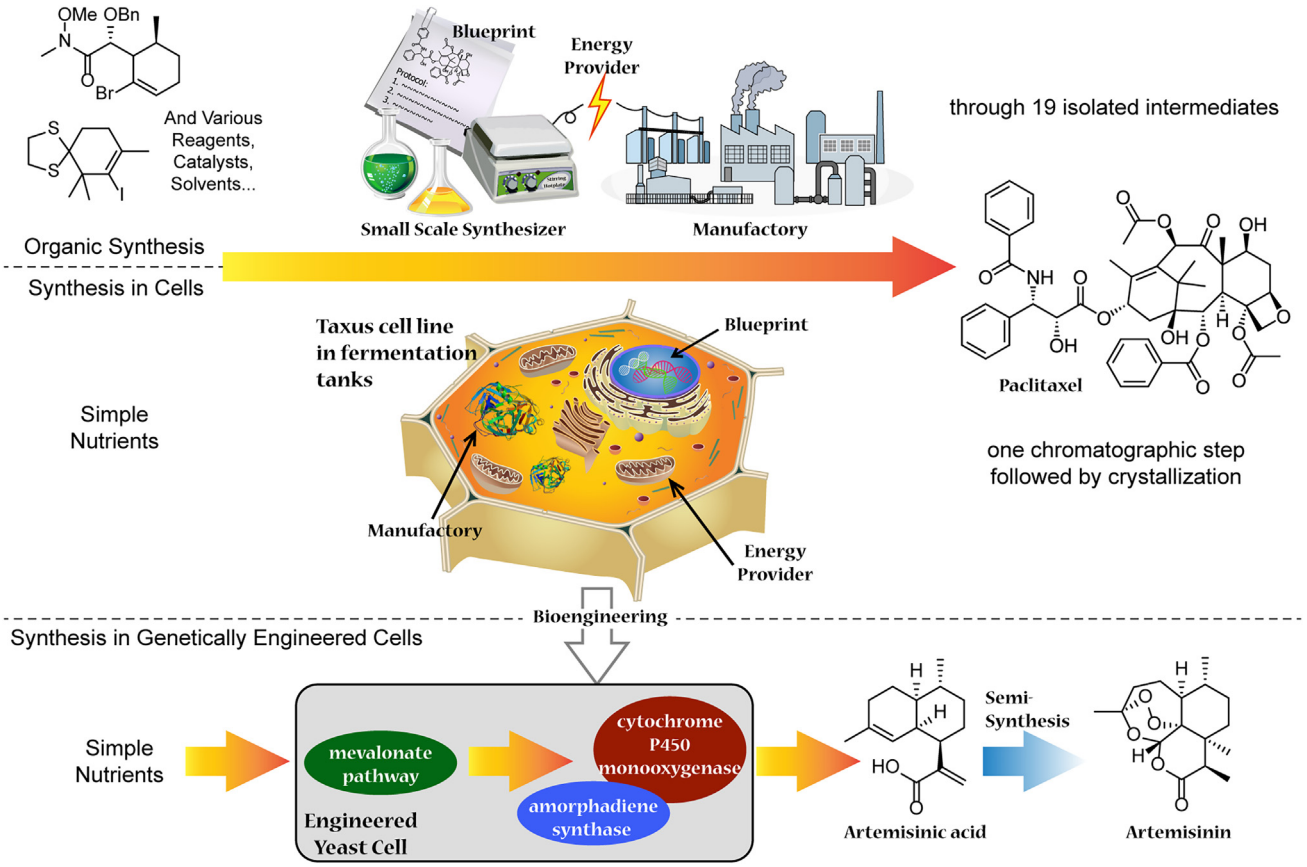

Figure 1. Two representative drug examples showing the use of organic synthesis and cell-based synthesis for improved efficiency and cost

Top: paclitaxel can be synthesized either by conventional, complicated, and high-cost organic synthesis process or by simple fermentation using natural biosynthesis. Bottom: artemisinin can be prepared by exploiting the mevalonate pathway.

with amorphadiene synthase and a novel cytochrome P450 monooxygenase from *Artemisia annua*, the genetically engineered yeast strain (*Saccharomyces cerevisiae*) cultivated by the Keasling group[1] exhibited proficiency in the generation of amorpha-4,11-diene, allowing artemisinic acid generation up to 100 mg/L through subsequent oxidation (Figure 1, bottom).

INVOLVEMENT OF CHEMISTS

Today, the development of intracellular catalysts is by no means the exclusive realm of synthetic biologists (Figure 2). Chemists are fully engaged in designing systems that perform catalytic functions previously attainable only by native enzymes, aiming both to enhance and extend the arsenal of naturally available tools. The most prominent instrument in chemists' toolbox is the method known as directed evolution, an iterative process that changes the primary coordination sphere and beyond[2] to create enzymes with desired traits through cycles of genetic diversification and subsequent selection. This iterative procedure can engender an enzyme with entirely novel catalytic abilities. The manifold potential of this technique was vividly demonstrated through the pioneering work of Arnold and co-workers who, among many other accomplishments, converted cytochrome P450 from a monooxidase to a carbene transferase capable of cyclopropanation reactions.[3] Another strategy, which can be traced back to Whitesides's visionary work[4] in the 1970s, involves the incorporation of an unnatural metal cofactor to generate novel artificial metalloenzymes

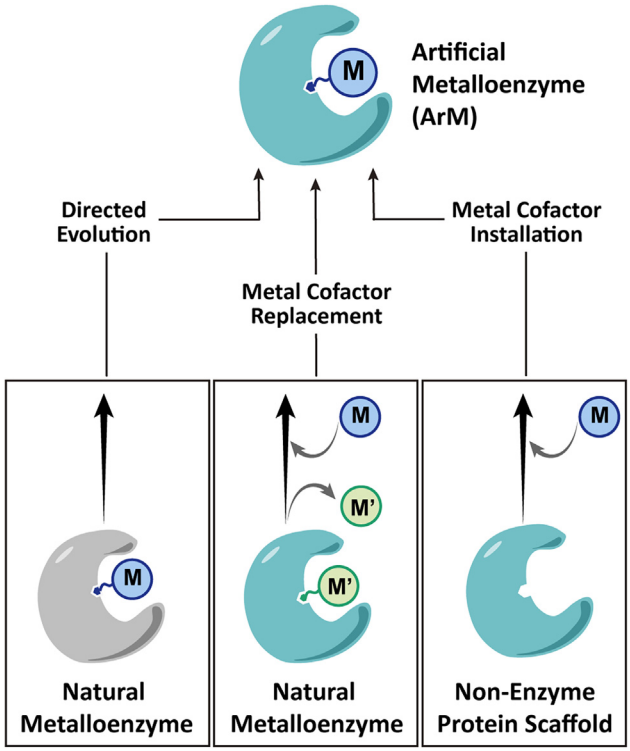

Figure 2. The three development pathways for artificial metalloenzymes

Artificial metalloenzymes can be prepared through iterative evolution of natural metalloenzymes, by replacing the natural cofactor with a synthetic alternative, or by installing a metal cofactor into otherwise catalytically inert protein scaffolds.

(ArMs). For example, Ward and co-workers presented the insertion of the Hoveyda-Grubbs catalyst directly into streptavidin to generate an engineered artificial metathase,[5] manifesting the strategy's simplicity and efficacy. An analogous approach with metal-incorporated albumin featured a surface coated with α(2,3)-linked sialic acid terminated *N*-glycans, and the artificial metathase was docked on SW620 colon adenocarcinoma cells to demonstrate pro-drug activation proximal to the cell surface via ring-closing metathesis.[6] Alternatively, the metal species in a naturally occurring cofactor can be changed to generate new catalytic abilities, as showcased by Hartwig et al. that the replacement of iron with other noble metals within myoglobin could grant the proteins the capability to catalyze C-H insertion reactions.[7] Importantly, the potential of cofactor manipulation can be further amplified through the integration of directed evolution. The synergistic cooperation of these approaches bestows the cofactor-modified enzymes with enhanced stability, enantioselectivity, and chemoselectivity, thereby expanding the spectrum of achievable chemical reactions and transformations. Yet, one should note that in many scenarios, it remains a challenge to introduce artificial metalloenzymes with synthetic metal cofactors into cells. Ward's work presented above has been a clever solution that has utilized bacterial periplasm to solve the issue.

INTRACELLULAR CATALYSIS: WHY AND HOW?

The advantages of intracellular catalysis

The inspiration to carry out abiotic reactions intracellularly originates, to a large extent, from the seminal discovery of bioorthogonal chemistry and its application to biomolecules reported by Bertozzi and co-workers.[8] Performing reactions

intracellularly offers myriad advantages. One emerging paradigm in bioorthogonal chemistry involves bypassing the long-standing medicinal chemistry problem of low cellular uptake of large molecules. Assembling drugs intracellularly means that many or all of the so-called Lipinski rules[9] can be entirely ignored. Furthermore, if the catalyst can be placed in specific tissues or intracellular locations, then the intracellular abiotic reaction strategy enables the production of desired molecules with unparalleled temporal and spatial precision. Assembling active agents exclusively within the places of interest overcomes the challenges associated with delivery to organelles or other cell-specific locations. The biological activity and uptake efficiency of small molecules are highly sensitive to structural modification, so integrating targeting moieties onto the catalysts presents a more versatile strategy to precisely "guide" the molecules of interest to the destination. Although the molecular building blocks must encounter the catalyst contemporaneously, this presents an additional advantage if both of these drug precursors exhibit selective uptake. As shown by Rideout over 30 years ago,[10] the cell selectivity could be amplified by the intracellular assembly of precursor compounds that showed even only small, individual selective uptakes.

Approaches to achieving intracellular catalysis

Given the challenge of expressing or delivering the enzymes or protein-based artificial enzymes to specific target locations within the cell, efforts have been focused on exogenous catalytic systems ranging from small molecular metal complexes to nanoparticles and polymers embedded with metal-organic contents.[11,12] Two of us reported in 2016 the first example of a polymeric enzyme mimic (PEM) performing *in cellulo* drug synthesis from two cell-permeable building blocks.[8] Another pioneering effort by Bradley and co-workers involved the loading of palladium-loaded microspheres[13] to target and prepare therapeutics within brain cancer cells,[14] and the system was later demonstrated in live animals.[15] These palladium nanoparticles could perform Suzuki coupling and depropargylation reactions, generating active anticancer agents in places of interest. Over time, a broad range of exogenous catalysts have been developed including polymer-based organic nanoparticles, inorganic-nanoparticle-based catalysts, catalytic spherical nucleic acids (SNAs), and fusogenic-liposome-based systems (Figure 3). Also, despite their complexities, artificial metalloenzymes may be used with existing cellular delivery systems[16,17] or simply as parts of whole-cell catalysts[18] to facilitate their usage as exogenous systems. Each of these systems has demonstrated its distinctive characteristics and capabilities to facilitate various chemical reactions as well as their limitations. Although they are currently at the proof-of-concept stage, they demonstrate tremendous potential as a new portal for drug and tool delivery in medicinal chemistry and chemical biology.

Polymer-based catalytic nanoparticles, sometimes referred to as PEMs, are systems that often involve a polymer-wrapped metal catalyst. Single-chain polymer nanoparticle (SCNP) catalysts are most closely related to enzymes both in structure and function.[19–22] SCNPs and enzymes have similar conformational features with compact, folded scaffolds forming binding and catalytic sites, although the former is less well defined. Other polymeric catalysts may utilize star polymers as scaffolds for more easily controlled core-shell structures, as exemplified by dense-shell nanoparticle (DSNP) catalysts.[23,24] Like the protein scaffolds in enzymes, the polymeric scaffolds grant protection and sometimes substrate selectivity for the catalytic centers while also offering tunable properties through structural alterations. For example, a phosphonium-oligo(ethylene glycol)-functionalized DSNP features membrane affinity and can function well on eukaryotic cell membranes. However, the challenges for PEMs are many. The most prominent one is their cytotoxicity, a price paid for

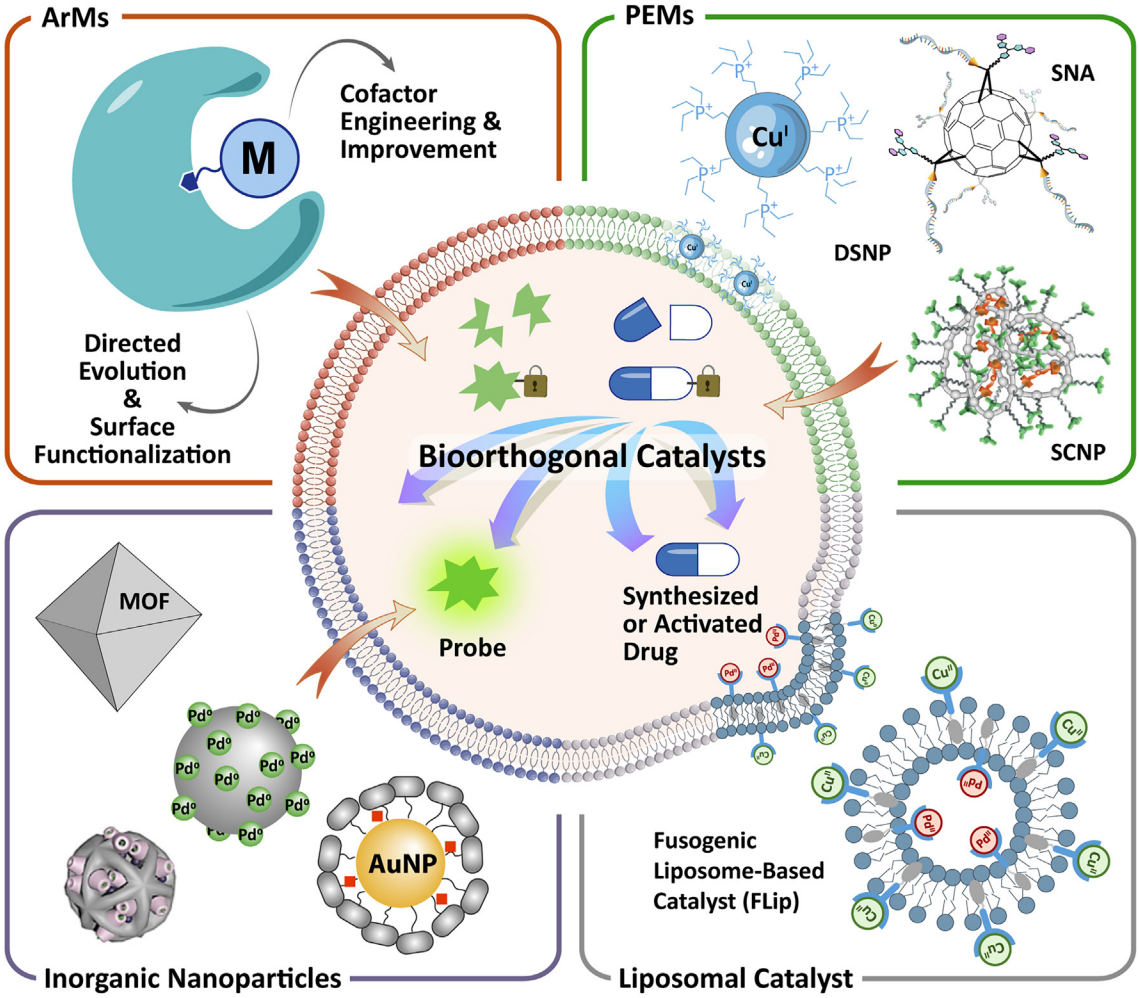

Figure 3. Illustration on the reported types of tools for performing metal catalysis inside living cells and animals

Various methods for achieving exogenous catalysis in living systems have been reported, from relatively simple ArMs to elaborate liposome-based designs.

enhancing their cell membrane penetration with membrane-disrupting lipo-cationic moieties usually introduced for this purpose. Other issues, such as non-specific absorption and instability *in vivo*, also call for improvements. One attractive alternative approach used SNAs as a catalyst development platform.[25] Although DNA has been explored as a metal catalytic scaffold[26] and SNAs can be viewed simply as a star polymer with nucleic acid arms, the design philosophy and properties of SNA scaffolds are distinct compared to traditional DNA or synthetic star polymer platforms like DSNPs. In addition to the already documented potential in nanomedicine,[27] SNAs may find extra usefulness in the intracellular catalysis area, as its anionic nature, excellent toxicity profile, cell penetration capability, synergy with enzymes,[28,29] and unmatched programmability[30] endowed its great potential in solving the issues currently faced by those PEMs and ArMs.

Inorganic nanoparticles are another major category of catalysts shown to work in living systems. Bradley's early example, as described above, falls in this category. Indeed, the catalytic properties of a diverse array of inorganic nanoparticles, including but not limited to metal nanoparticles,[12–15] metal-organic frameworks,[31,32] mesoporous silica

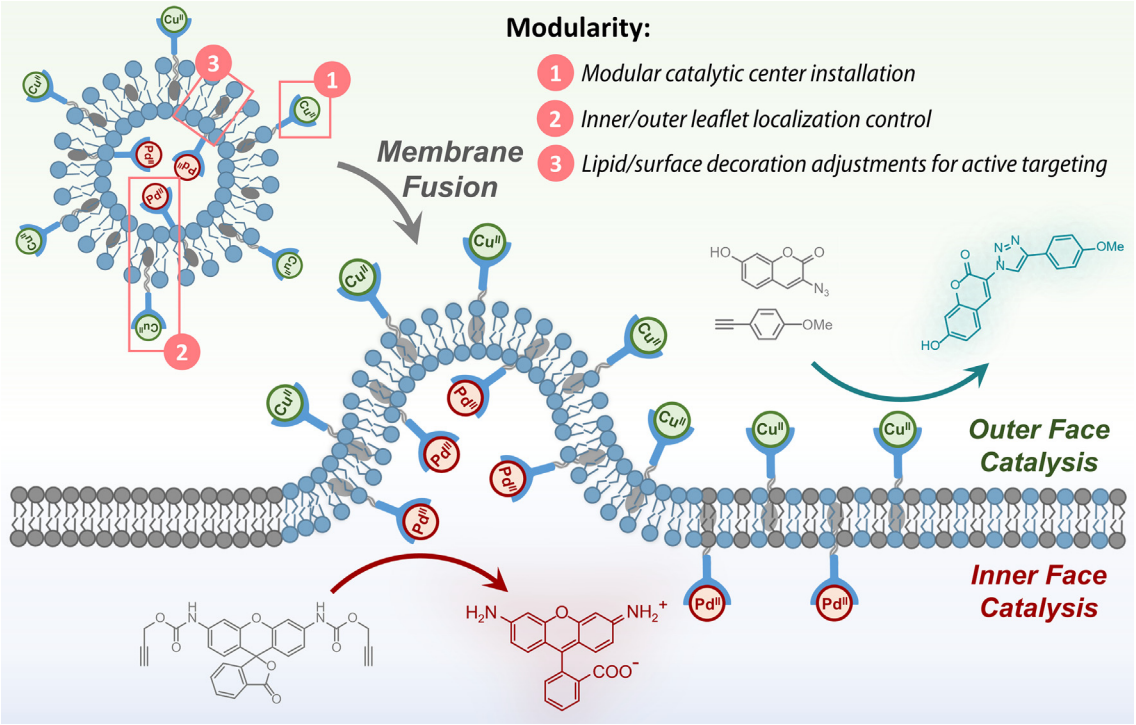

Figure 4. Cartoon illustration on the modular design and working mechanics of the MAC-LiFT system

Membrane-anchored catalyst via liposome-fusion-based transport (MAC-LiFT) embeds abiotic catalysts within a membrane, allowing precise targeting of subcellular sites.

nanoparticles,[33,34] nanosized metal oxides, and salts,[35] have been explored. Some of these inorganic catalysts, especially those related to reactions involving reactive oxygen species (ROS), are referred to as "nanozymes,"[36,37] and this concept was ranked among the "top ten emerging technologies in chemistry" by the IUPAC. Although the name "nanozyme" remains controversial to some extent,[38–40] partially due to insufficient mechanistic studies and limited reaction scope, its advantages are both clear and unique: stability in the bioenvironment, high efficiency, and low-cost preparation. These features make them ideal in certain application scenarios.[41] Also, as custom ligands can always be designed, optimized, and applied to the metal species within the nanomaterial, there is good reason to believe that further applications of these inorganic catalysts in living systems will be possible.

A novel strategy arising: MAC-LiFT

Among the notable advancements within the realm of artificial biocatalytic systems, a recent standout is the membrane-anchored catalyst via liposome-fusion-based transport (MAC-LiFT) approach[42] (Figure 4). The impetus for developing this approach arose from the advantages of its foundation: an interface engineering strategy. As the membrane serves as a barrier between the cytosol and the external environment, abiotic catalysts embedded in the membrane can conveniently accommodate substrates from one side and transport the products to the other side. The LiFT method provides a convenient and modular strategy to precisely modify the inner leaflet of cell membranes for catalytic purposes. Indeed, various strategies have been developed for integrating catalysts into distinct subcellular sites encompassing the outer membrane, endosomes, mitochondria, and cytosol, but the strategy to integrate metal complexes onto the inner leaflet of cell membranes remains a major challenge. Current methods for engineering the inner leaflet

of cell membranes encompass several approaches. These include using chemical inducers of dimerization to enable protein-protein interactions,[43] utilizing cell-penetrating peptides,[44] and genetic engineering of the inner leaflet domain of transmembrane proteins.[45] However, these techniques have been primarily utilized for incorporating proteins onto the inner leaflet, and adapting them for abiotic transition metal catalysts proves highly difficult.

The MAC-LiFT strategy was proposed by drawing inspiration from the LiFT process that was based on extracellular vesicle (EV) fusion, which integrates EV lipids and transmembrane receptors into the target cell's plasma membrane while maintaining precise orientation control. This strategy involves embedding metal catalysts on both the internal and external sides of the lipid bilayer of fusogenic liposomes, thereby enabling the precise placement of catalysts on either one or both sides of the membrane leaflets, with an emphasis on the interior leaflet via the liposomal fusion process.[42,46] Consequently, the cytoplasmic membrane emerges as a reliable shelter for housing metal centers, affording protection of metal complexes against deactivation in the cellular microenvironment, and significantly reducing the amount of catalyst required for effective intracellular catalysis, as well as ensuring intracellular transformations. It is notable that by anchoring metal catalysts on the cytoplasmic membrane's internal surface, the concentrations for Pd and Cu catalysts in cell lysate were about 1–3 ppb, marginally higher than the cell's background metal levels. However, the local concentration of Cu/Pd anchored on the membrane was apparently high enough to facilitate the catalysis, thanks to the excellent catalyst localization control. Such control, combined with the inner leaflet's protective role against intracellular glutathione (GSH), enabled efficient abiotic catalysis at unprecedented low metal concentrations that minimize cytotoxicity in tested cell lines.

Another key advantage inherent to the MAC-LiFT strategy lies in its pronounced modularity. By employing simple organic chemistry, metal complexes of interest can be tethered to hydrophobic lipid anchors, enabling the facile preparation of the corresponding catalytic liposomes. Subsequent immobilization of these metal complexes onto cell membranes can be easily achieved through established fusion protocols. More importantly, by simply quenching the initial metal catalyst located on the exterior side after the formation of the liposome and reintroducing a second metal catalytic moiety, it is possible to prepare asymmetric liposomal catalysts to functionalize a cell membrane with two types of bioorthogonal, spatially separated metal complexes, with one on the outer leaflet and one on the inner leaflet. The ability to perform two separate chemical transformations opens the door to numerous applications, including the potential for cascade reactions. Moreover, by adjusting the lipid formulations and components within the liposomes, targeted delivery and cell-specific fusion can be achieved, leveraging well-established advances in liposome technology. For instance, one could load cargo catalysts on the internal side of fusogenic liposomes and attach targeting motifs like aptamers or antibodies to their external surface. This receptor-mediated targeting can achieve cell-specific delivery of metal catalysts to target cells such as tumor cells, paving the way for the development of innovative targeted cancer therapies by directly synthesizing drug molecules inside target cells. Moreover, beyond metal catalysts, it is feasible to incorporate protein enzymes as biological catalysts into the cell membrane's inner leaflet. This can be easily achieved by employing proteases to degrade exogenous enzymes on the outer surface. These remarkable capabilities of the MAC-LiFT approach allow for the establishment of catalyst-protective systems using exclusively exogenous agents across a wide range of mammalian cells, thereby facilitating the convenient utilization of diverse bioorthogonal reactions in live cellular systems.

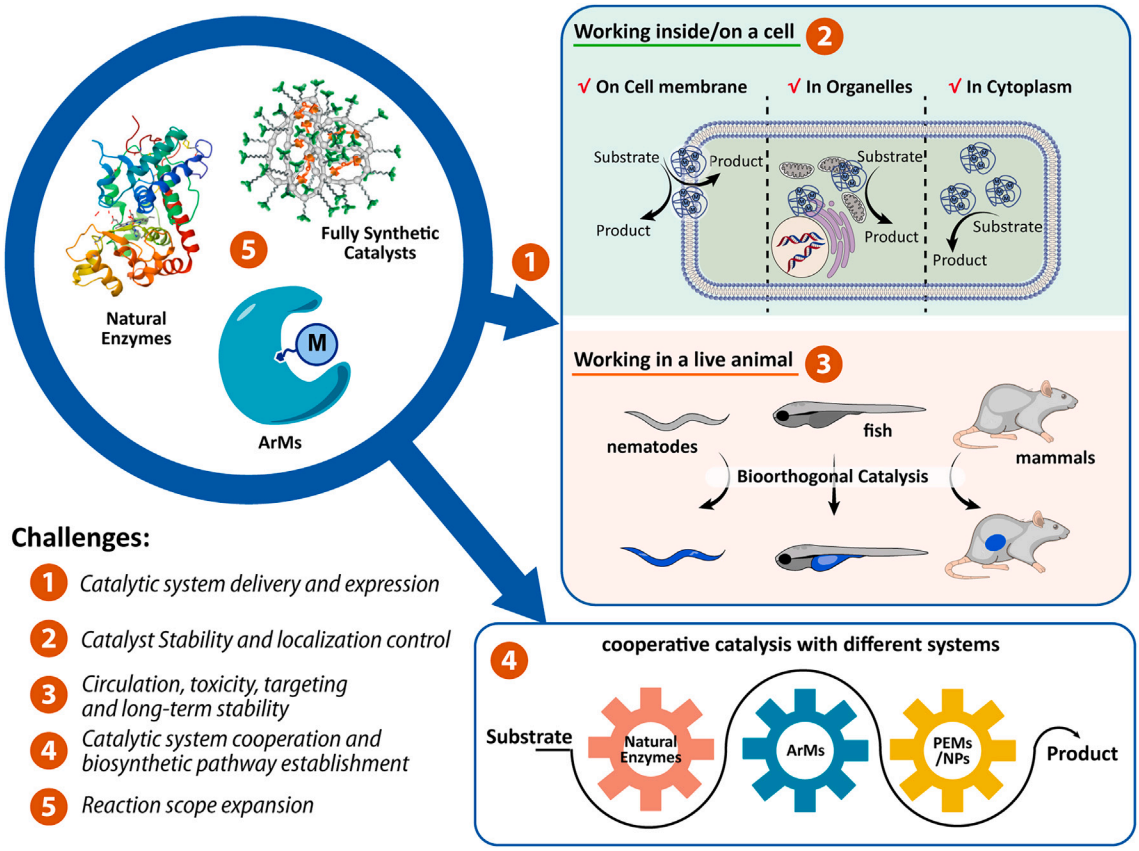

Figure 5. Future challenges and perspectives of the intracellular abiotic catalysis field

Key challenges include catalyst delivery and localization, stability, control of toxicity and pharmacokinetics, and developing more sophisticated catalytic networks.

CHALLENGES, POTENTIAL SOLUTIONS, AND VISTAS

Despite the enormous progress made to date in developing abiotic intracellular catalyst systems, their utilization is still at a relatively primitive stage facing a considerable number of hurdles (Figure 5). The primary challenge is the expansion of the reaction scope. Although catalysts mediating new abiotic transformations are continuously being reported, many factors are still limiting the expansion of the reaction scope as well as the efficiency of existing systems. In the case of directed evolution, the process can be time consuming and effort extensive, with the outcome not always fully predictable. For successful cases, the evolved enzymes may further face stability or low turnover issues in living systems and difficulties in intracellular delivery for certain application scenarios. The scope issue is even greater for those bringing exogeneous catalytic systems into the biological realm, as their functions rely on the successful "grafting" of metal catalytic moieties, which are frequently sensitive to various coordinating molecules in biologically relevant aqueous environments. In fact, most reports of polymeric catalysts (e.g., PEMs) have focused on simple transformations mediated by copper, ruthenium, palladium, gold, and iron, as well as ROS-involving catalysis for nanozymes. Novel catalytic structures with stability in biologically relevant conditions are in dire need for this field, but in developing new metal catalysts, organic chemists usually place the highest premium on the turnover number, rate, and selectivity of metal catalysts. This suggests that if one were to prioritize aqueous compatibility and bioorthogonality, one might find treasures

among the "discarded" structures. As illustrated in the early reports by Meggers, metal complexes with low but reliable catalytic activity in cells could be improved through structure tuning,[47,48] and this may be further enhanced by modern strategies such as SCNP wrapping and directed evolution.

Alternatively, we may look for better ways to protect the metal catalytic centers in cells. Synthetic polymers are used to "wrap" and protect internal catalytic centers, and those polymer-based "click" catalysts[11,20,21,23,49] can be viewed as the "protected versions" of Ting's intracellular catalytic system[50] for much-improved stability, and the protein scaffolds for ArMs work similarly.[51–53] One can also attempt to locate the catalytic systems in the "cleaner" places inside cells, such as bacterial periplasm,[5,54] certain subcellular structures,[55] and membranes,[23,42] so that the catalysts have lower risks of being poisoned by external nucleophiles. Additionally, since external energy such as light irradiation may be introduced to promote reactions as part of the catalyst design,[56] one can think of "on-demand" activation of the catalyst, which is responsive to an external stimulus. An early example of this is a $Ru(bpy)_3$-containing SCNP that functions as an azide reductase with light as the trigger.[49] The on-demand activation of catalysts not only allows them to be tailored to specific applications but also potentially provides a way to protect the catalytic species through a "pro-catalyst" approach: a "hibernating" analog may be a solution for some highly sensitive catalytic species so that it may maintain a stable or even inert chemical structure for a longer period of time until it is activated for work in cells or live animals using, for example, light triggers. Such a strategy will also allow the catalyst to withstand long blood circulation times required for catalyst targeting, enlarging the benefits of therapeutics based on on-demand drug synthesis.

Certainly, localization control of catalysts, or targeted catalysis under many contexts, is in urgent demand not only for stability improvement. Successful chemical biology and medicinal applications of those in-cell catalysts require specific targeting capabilities to organs, tissues, cells, or even organelles, thereby maximizing the benefit from the on-site generation of molecules of interest. In this aspect, SNA catalysts and the MAC-LiFT strategy reveal great potential by having functional DNAs (aptamers) as modular targeting moieties[57,58] as part of the catalyst design. An alternative pathway is to utilize antibodies as part of the catalyst scaffold. Metal installation through direct chemical modification of an affibody has already been practiced,[59] and this affibody-catalyst conjugate (Ru-HER2) selectively binds to the HER2 receptor on cancer cells and catalyzes the *in situ* activation of the gemcitabine pro-drug, resulting in enhanced anticancer activity through a synergistic effect of HER2 signaling pathway blockade and cell-selective DNA damage from on-site-prepared gemcitabine. Despite not being demonstrated yet, various antibodies and affibodies may be utilized as ArM scaffolds so that ArMs with innate targeting capabilities may be made possible. Consequently, with these available strategies, one can expect the benefits of on-site generation of active molecules to be further revealed and boosted in possibly the next decade.

A critical analysis of artificial catalysts that work in cells indicates that none reported thus far can match natural enzymes in their diversity of substrates and reactions, or their specificity and yields, but the gap is becoming narrower over time. Importantly, many of the abiotic reaction catalysts developed thus far perform reactions that have no counterpoint in nature, and they may work together to allow multistep, abiotic tandem reactions in cells. The azide reductase described above is an example: it was able to complex β-galactosidase and deliver it to cells for tandem catalysis.[49] For efficient tandem reactions, localization control over individual catalytic systems

also becomes more important because the catalytic centers must be in proximity to each other to facilitate the cascading process. Indeed, from natural product synthesis to drug delivery, those in-cell synthetic systems are becoming increasingly complex and require multifaceted considerations and upgrades in design. To make them more prevalent and useful across multiple fields, scientists must become more familiar with running reactions in cells and knowing the locations and ways to place the catalysts in live systems so that a lot of challenging problems may become more manageable. Thereafter, this field of research is likely to attract increased interest from the general audience.

ACKNOWLEDGMENTS

The authors thank the National Science Foundation of China (22361142830 and 22374043), the Huxiang Youth Talent Support Program of Hunan Province (2022RC1107), the Hunan Provincial Science and Technology Department (2022SK2003, 2024JJ2010, and 2022JJ10007), the Open Research Fund of Songshan Lake Materials Laboratory (2023SLABFN22), and the US-Israel Binational Science Foundation (2020144) for financial support.

AUTHOR CONTRIBUTIONS

Conceptualization, all authors; visualization, T.W., H.X., and Y.B.; writing – original draft, T.W., Y.C., H.X., and Y.B.; writing – review & editing, S.C.Z., H.X., and Y.B.; resources, S.C.Z., H.X., and Y.B.; funding acquisition, S.C.Z., H.X., and Y.B.; supervision, H.X. and Y.B. T.W. and Y.C. are the co-first authors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Ro, D.-K., Paradise, E.M., Ouellet, M., Fisher, K.J., Newman, K.L., Ndungu, J.M., Ho, K.A., Eachus, R.A., Ham, T.S., Kirby, J., et al. (2006). Production of the antimalarial drug precursor artemisinic acid in engineered yeast. Nature *440*, 940–943.

2. Van Stappen, C., Deng, Y., Liu, Y., Heidari, H., Wang, J.-X., Zhou, Y., Ledray, A.P., and Lu, Y. (2022). Designing Artificial Metalloenzymes by Tuning of the Environment beyond the Primary Coordination Sphere. Chem. Rev. *122*, 11974–12045.

3. Coelho, P.S., Brustad, E.M., Kannan, A., and Arnold, F.H. (2013). Olefin Cyclopropanation via Carbene Transfer Catalyzed by Engineered Cytochrome P450 Enzymes. Science *339*, 307–310.

4. Wilson, M.E., and Whitesides, G.M. (1978). Conversion of a protein to a homogeneous asymmetric hydrogenation catalyst by site-specific modification with a diphosphinerhodium(I) moiety. J. Am. Chem. Soc. *100*, 306–307.

5. Jeschek, M., Reuter, R., Heinisch, T., Trindler, C., Klehr, J., Panke, S., and Ward, T.R. (2016). Directed evolution of artificial metalloenzymes for in vivo metathesis. Nature *537*, 661–665.

6. Eda, S., Nasibullin, I., Vong, K., Kudo, N., Yoshida, M., Kurbangalieva, A., and Tanaka, K. (2019). Biocompatibility and therapeutic potential of glycosylated albumin artificial metalloenzymes. Nat. Catal. *2*, 780–792.

7. Key, H.M., Dydio, P., Clark, D.S., and Hartwig, J.F. (2016). Abiological catalysis by artificial haem proteins containing noble metals in place of iron. Nature *534*, 534–537.

8. Prescher, J.A., and Bertozzi, C.R. (2005). Chemistry in living systems. Nat. Chem. Biol. *1*, 13–21.

9. Lipinski, C.A. (2004). Lead- and drug-like compounds: the rule-of-five revolution. Drug Discov. Today Technol. *1*, 337–341.

10. Rideout, D. (1994). Self-Assembling Drugs: A New Approach to Biochemical Modulation in Cancer Chemotherapy. Cancer Invest. *12*, 189–269.

11. Bai, Y., Feng, X., Xing, H., Xu, Y., Kim, B.K., Baig, N., Zhou, T., Gewirth, A.A., Lu, Y., Oldfield, E., and Zimmerman, S.C. (2016). A Highly Efficient Single-Chain Metal–Organic Nanoparticle Catalyst for Alkyne–Azide "Click" Reactions in Water and in Cells. J. Am. Chem. Soc. *138*, 11077–11080.

12. Clavadetscher, J., Hoffmann, S., Lilienkampf, A., Mackay, L., Yusop, R.M., Rider, S.A., Mullins, J.J., and Bradley, M. (2016). Copper Catalysis in Living Systems and In Situ Drug Synthesis. Angew. Chem. Int. Ed. *55*, 15662–15666.

13. Yusop, R.M., Unciti-Broceta, A., Johansson, E.M.V., Sánchez-Martín, R.M., and Bradley, M. (2011). Palladium-mediated intracellular chemistry. Nat. Chem. *3*, 239–243.

14. Clavadetscher, J., Indrigo, E., Chankeshwara, S.V., Lilienkampf, A., and Bradley, M. (2017). In-Cell Dual Drug Synthesis by Cancer-Targeting Palladium Catalysts. Angew. Chem. Int. Ed. *56*, 6864–6868.

15. Bray, T.L., Salji, M., Brombin, A., Pérez-López, A.M., Rubio-Ruiz, B., Galbraith, L.C.A., Patton, E.E., Leung, H.Y., and Unciti-Broceta, A. (2018). Bright insights into palladium-triggered local chemotherapy. Chem. Sci. *9*, 7354–7361.

16. Brown, T.D., Whitehead, K.A., and Mitragotri, S. (2019). Materials for oral delivery of proteins and peptides. Nat. Rev. Mater. *5*, 127–148.

17. Fu, A., Tang, R., Hardie, J., Farkas, M.E., and Rotello, V.M. (2014). Promises and Pitfalls of Intracellular Delivery of Proteins. Bioconjugate Chem. *25*, 1602–1608.

18. Wittwer, M., Markel, U., Schiffels, J., Okuda, J., Sauer, D.F., and Schwaneberg, U. (2021). Engineering and emerging applications of artificial metalloenzymes with whole cells. Nat. Catal. *4*, 814–827.

19. Terashima, T., Mes, T., De Greef, T.F.A., Gillissen, M.A.J., Besenius, P., Palmans, A.R.A., and Meijer, E.W. (2011). Single-Chain Folding of Polymers for Catalytic Systems in Water. J. Am. Chem. Soc. *133*, 4742–4745.

20. Chen, J., Wang, J., Bai, Y., Li, K., Garcia, E.S., Ferguson, A.L., and Zimmerman, S.C. (2018). Enzyme-like Click Catalysis by a Copper-Containing Single-Chain Nanoparticle. J. Am. Chem. Soc. *140*, 13695–13702.

21. Liu, Y., Pujals, S., Stals, P.J.M., Paulöhrl, T., Presolski, S.I., Meijer, E.W., Albertazzi, L., and Palmans, A.R.A. (2018). Catalytically Active Single-Chain Polymeric Nanoparticles: Exploring Their Functions in Complex Biological Media. J. Am. Chem. Soc. *140*, 3423–3433.

22. Sathyan, A., Deng, L., Loman, T., and Palmans, A.R. (2023). Bioorthogonal catalysis in complex media: Consequences of using polymeric scaffold materials on catalyst stability and activity. Catal. Today *418*, 114116.

23. Deng, Y., Wu, T., Chen, X., Chen, Y., Fei, Y., Liu, Y., Chen, Z., Xing, H., and Bai, Y. (2023). A Membrane-Embedded Macromolecular Catalyst with Substrate Selectivity in Live Cells. J. Am. Chem. Soc. *145*, 1262–1272.

24. Lu, Q., Bai, S., Chen, Z., Zheng, N., Feng, X., and Bai, Y. (2020). A Dense-Shell Macromolecular Scaffold for Catalyst- or Substrate-Guided Catalysis in a Cellular Environment. ACS Mater. Lett. *2*, 89–94.

25. Zhang, B., Bai, S., Chao, X., Wu, T., Chen, Z., Cheng, Z., Xiao, Y., Zhang, K., and Bai, Y. (2021). Molecularly pure miktoarm spherical nucleic acids: preparation and usage as a scaffold for abiotic intracellular catalysis. Chem. Sci. *12*, 15843–15848.

26. Roelfes, G., and Feringa, B.L. (2005). DNA-Based Asymmetric Catalysis. Angew. Chem. Int. Ed. *44*, 3230–3232.

27. Cutler, J.I., Auyeung, E., and Mirkin, C.A. (2012). Spherical Nucleic Acids. J. Am. Chem. Soc. *134*, 1376–1391.

28. Yan, J., Tan, Y.-L., Lin, M.-j., Xing, H., and Jiang, J.-H. (2020). A DNA-mediated crosslinking strategy to enhance cellular delivery and sensor performance of protein spherical nucleic acids. Chem. Sci. *12*, 1803–1809.

29. Brodin, J.D., Sprangers, A.J., McMillan, J.R., and Mirkin, C.A. (2015). DNA-Mediated Cellular Delivery of Functional Enzymes. J. Am. Chem. Soc. *137*, 14838–14841.

30. Jones, M.R., Seeman, N.C., and Mirkin, C.A. (2015). Programmable materials and the nature of the DNA bond. Science *347*, 1260901.

31. Zhu, J., You, Y., Zhang, W., Pu, F., Ren, J., and Qu, X. (2023). Boosting Endogenous Copper(I) for Biologically Safe and Efficient Bioorthogonal Catalysis via Self-Adaptive Metal–Organic Frameworks. J. Am. Chem. Soc. *145*, 1955–1963.

32. Martínez, R., Carrillo-Carrión, C., Destito, P., Alvarez, A., Tomás-Gamasa, M., Pelaz, B., Lopez, F., Mascareñas, J.L., and del Pino, P. (2020). Core-Shell Palladium/MOF Platforms as Diffusion-Controlled Nanoreactors in Living Cells and Tissue Models. Cell Rep. Phys. Sci. *1*, 100076.

33. Du, Z., Liu, C., Song, H., Scott, P., Liu, Z., Ren, J., and Qu, X. (2020). Neutrophil-Membrane-Directed Bioorthogonal Synthesis of Inflammation-Targeting Chiral Drugs. Chem *6*, 2060–2072.

34. Du, Z., Yu, D., Du, X., Scott, P., Ren, J., and Qu, X. (2019). Self-triggered click reaction in an Alzheimer's disease model: in situ bifunctional drug synthesis catalyzed by neurotoxic copper accumulated in amyloid-β plaques. Chem. Sci. *10*, 10343–10350.

35. Zhao, H., Zhao, C., Liu, Z., Yi, J., Liu, X., Ren, J., and Qu, X. (2023). A Polyoxometalate-Based Pathologically Activated Assay for Efficient Bioorthogonal Catalytic Selective Therapy. Angew. Chem. Int. Ed. *62*, e202303989.

36. Wei, H., and Wang, E. (2013). Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes. Chem. Soc. Rev. *42*, 6060–6093.

37. Wu, J., Wang, X., Wang, Q., Lou, Z., Li, S., Zhu, Y., Qin, L., and Wei, H. (2019). Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes (II). Chem. Soc. Rev. *48*, 1004–1076.

38. Scott, S., Zhao, H., Dey, A., and Gunnoe, T.B. (2020). Nano-Apples and Orange-Zymes. ACS Catal. *10*, 14315–14317.

39. Wei, H., Gao, L., Fan, K., Liu, J., He, J., Qu, X., Dong, S., Wang, E., and Yan, X. (2021). Nanozymes: A clear definition with fuzzy edges. Nano Today *40*, 101269.

40. Robert, A., and Meunier, B. (2022). How to Define a Nanozyme. ACS Nano *16*, 6956–6959.

41. Liang, M., and Yan, X. (2019). Nanozymes: From New Concepts, Mechanisms, and Standards to Applications. Acc. Chem. Res. *52*, 2190–2200.

42. Chen, Y., Wu, T., Xie, S., Bai, Y., and Xing, H. (2023). Orientation-controlled membrane anchoring of bioorthogonal catalysts on live cells via liposome fusion–based transport. Sci. Adv. *9*, eadg2583.

43. Ishida, M., Watanabe, H., Takigawa, K., Kurishita, Y., Oki, C., Nakamura, A., Hamachi, I., and Tsukiji, S. (2013). Synthetic Self-Localizing Ligands That Control the Spatial Location of Proteins in Living Cells. J. Am. Chem. Soc. *135*, 12684–12689.

44. Scott, H.L., Nguyen, V.P., Alves, D.S., Davis, F.L., Booth, K.R., Bryner, J., and Barrera, F.N. (2015). The Negative Charge of the Membrane Has Opposite Effects on the Membrane Entry and Exit of pH-Low Insertion Peptide. Biochemistry *54*, 1709–1712.

45. Peruzzi, J.A., Galvez, N.R., and Kamat, N.P. (2023). Engineering transmembrane signal transduction in synthetic membranes using two-component systems. Proc. Natl. Acad. Sci. USA *120*, e2218610120.

46. Lin, M., Chen, Y., Zhao, S., Tang, R., Nie, Z., and Xing, H. (2022). A Biomimetic Approach for Spatially Controlled Cell Membrane Engineering Using Fusogenic Spherical Nucleic Acid. Angew. Chem. Int. Ed. *61*, e202111647.

47. Streu, C., and Meggers, E. (2006). Ruthenium-Induced Allylcarbamate Cleavage in Living Cells. Angew. Chem. Int. Ed. *45*, 5645–5648.

48. Völker, T., Dempwolff, F., Graumann, P.L., and Meggers, E. (2014). Progress towards Bioorthogonal Catalysis with Organometallic Compounds. Angew. Chem. Int. Ed. *53*, 10536–10540.

49. Chen, J., Li, K., Shon, J.S.L., and Zimmerman, S.C. (2020). Single-Chain Nanoparticle Delivers a Partner Enzyme for Concurrent and Tandem Catalysis in Cells. J. Am. Chem. Soc. *142*, 4565–4569.

50. Uttamapinant, C., Tangpeerachaikul, A., Grecian, S., Clarke, S., Singh, U., Slade, P., Gee, K.R., and Ting, A.Y. (2012). Fast, Cell-Compatible Click Chemistry with Copper-Chelating Azides for Biomolecular Labeling. Angew. Chem. Int. Ed. *51*, 5852–5856.

51. Roelfes, G. (2019). LmrR: A Privileged Scaffold for Artificial Metalloenzymes. Acc. Chem. Res. *52*, 545–556.

52. Heinisch, T., and Ward, T.R. (2016). Artificial Metalloenzymes Based on the Biotin–Streptavidin Technology: Challenges and Opportunities. Acc. Chem. Res. *49*, 1711–1721.

53. Fischer, S., Ward, T.R., and Liang, A.D. (2021). Engineering a Metathesis-Catalyzing Artificial Metalloenzyme Based on HaloTag. ACS Catal. *11*, 6343–6347.

54. Liang, A.D., Serrano-Plana, J., Peterson, R.L., and Ward, T.R. (2019). Artificial Metalloenzymes Based on the Biotin–Streptavidin Technology: Enzymatic Cascades and Directed Evolution. Acc. Chem. Res. *52*, 585–595.

55. Schunck, N.S., and Mecking, S. (2022). In Vivo Olefin Metathesis in Microalgae Upgrades Lipids to Building Blocks for Polymers and Chemicals. Angew. Chem. Int. Ed. *61*, e202211285.

56. Deng, Y., Dwaraknath, S., Ouyang, W.O., Matsumoto, C.J., Ouchida, S., and Lu, Y. (2023). Engineering an Oxygen-Binding Protein for Photocatalytic CO2 Reductions in Water. Angew. Chem. Int. Ed. *62*, e202215719.

57. Keefe, A.D., Pai, S., and Ellington, A. (2010). Aptamers as therapeutics. Nat. Rev. Drug Discov. *9*, 537–550.

58. Zhou, J., and Rossi, J. (2017). Aptamers as targeted therapeutics: current potential and challenges. Nat. Rev. Drug Discov. *16*, 181–202.

59. Zhao, Z., Tao, X., Xie, Y., Lai, Q., Lin, W., Lu, K., Wang, J., Xia, W., and Mao, Z.-W. (2022). In Situ Prodrug Activation by an Affibody-Ruthenium Catalyst Hybrid for HER2-Targeted Chemotherapy. Angew. Chem. Int. Ed. *61*, e202202855.