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Bioorthogonal catalysis in complex media: Consequences of using polymeric scaffold materials on catalyst stability and activity

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ABSTRACT

Bioorthogonal catalysis using transition-metal-based complexes (TMCs) is a promising approach for converting substrates to desired products in complex cellular media. Notably, the *in situ* activation of prodrugs or synthesis of active drugs with the aim to complement existing treatments in diseases such as cancer has received significant attention. Whereas the focus has initially been on optimizing ligands to enhance the activity and stability of the metal complexes, more recently the benign effects of compartmentalization of the catalyst into homogeneous or heterogeneous scaffolds have been unveiled. Such tailor-made carrier materials not only afford active catalysts but also permit to guide the catalyst to the site of interest in *in vivo* applications. This review will emphasize the potential of synthetic amphiphilic polymers that form compartmentalized nanostructures for TMCs. The use of amphiphilic polymers is well established in the field of nanomedicine for *i.e.* drug delivery purposes, but their application as homogeneous carrier materials for TMCs has been less well explored. Since synthetic polymers are readily functionalized with ligands and targeting moieties, they can act as versatile catalysts carriers. After a short overview of the state-of-the-art in bioorthogonal catalysis using ligand-based TMCs, we summarize the advances in using homogeneous natural polymers as scaffolds and synthetic heterogeneous carrier materials for bioorthogonal catalysis. We end this review by highlighting the recent advances of catalysis in complex media using TMCs embedded in nanostructures formed by amphiphilic synthetic polymers. The combination of polymer science and homogeneous catalysis with the field of nanomedicine may open up new opportunities for advancing the exciting field of bioorthogonal catalysis for therapeutic applications.

1. Introduction

Progress in the field of catalysis has resulted in high selectivity, efficiency and versatility when converting substrates to desired products in different reaction media. Still, few synthetic catalysts can compete with the activity and selectivity of enzymes in water at low concentrations and mild temperatures. In fact, enzymes are unmatched in terms of rate and selectivity at mild conditions, which is a consequence of their folded, tertiary structure. Their perfectly defined three-dimensional structure results from a strictly defined length and amino acid sequence of the polypeptide chain. Directional hydrogen-bonding motifs that induce secondary structure elements *via* α-helix and β-sheet formation, additional hydrophobic effects and disulfide bonds complete nature's toolset to control the enzyme's three-dimensional structure [1–3]. The three-dimensionally controlled conformation of enzymes ensures a precise spatial positioning of crucial, catalytic amino acid residues, which is optimized for selective substrate binding [4]. These features result not only in unprecedented reactivity and selectivity, but also in compatibility with highly competitive and crowded environments, and complementarity to other types of enzymes. It is therefore no surprise that scientists have long aspired to mimic the catalytic features of these sophisticated systems. Great progress has been achieved to evolve enzymes to initiate nonnatural conversions and accept non-natural substrates [5]. Nevertheless, there remains a need to develop synthetic catalysts that perform well in aqueous media, not only for the further development of green chemical conversions in water, but also to augment the catalytic repertoire of enzymes with the versatility in bond breaking and forming reactions that transition metals offer. The latter is especially important in view of the recent developments in bioorthogonal catalysis, a relatively new field where transition metal

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complexes (TMCs) are applied in complex cellular media with the ultimate aim to activate prodrugs or synthesize drugs *in situ* and *in vivo*.

The importance of performing non-natural chemical reactions in *in vivo* conditions, coined "bioorthogonal chemistry" by Bertozzi and coworkers in 2003, was recently highlighted by awarding the Nobel prize in 2022. Bioorthogonal chemical reactions allow chemical modifications to take place in a selective manner in complex environments without interfering with the biological system [6]. The search for new bioorthogonal reactivities also turned to catalytic conversions and more specifically the use of TMCs [7–13]. This allowed to access new-to-nature reactions such as Suzuki-Miyaura couplings [14–16], Heck and Sonogashira reactions [17,18], carbene insertions [19], isomerizations [20], cyclizations [21], metathesis reactions [22–24], cycloaddition reactions [25], and a variety of metal-catalyzed bond cleavage reactions [12,26–33] in complex cellular media. Applying TMCs in biological environments comes with its own challenges. First, the catalysts have to be highly active and reach high turnover numbers to cope with the typical sub-micromolar concentrations in biological systems. Additionally, high catalyst stability is required due to the wide variety of competitive species present in complex biological media, which can interact with the metal. Furthermore, water-solubility, non-toxicity, and activity at physiological pH and temperature are necessary for the TMC to work in biological contexts [7,34]. Finally, when the *in situ* preparation or activation of therapeutical drugs are considered, the TMC has to be present at the site of interest when used *in vivo*.

Progress in developing TMCs for bioorthogonal reactions has been summarized in excellent recent reviews [8,9,11,13,35–39]. The importance of the ligand is well-recognized to boost the activity of transition-metal complexes, as well as for enhancing their stability. In addition, compartmentalization of the TMC has been found to enhance catalyst stability as well as provide opportunities to localize the TMC at the site of interest. A number of approaches have been evaluated to compartmentalize the TMC using both synthetic as well as natural carrier materials as shown in Fig. 1. Nevertheless, accurately localizing the TMCs *in vivo* to specific diseased organs or tumors while maintaining activity in a highly competitive environment remains a challenging endeavor. In this review, we will therefore focus on the advantages that well-designed polymeric scaffolds, both natural as well as synthetic, can offer, as they potentially protect the TMC from competitive species and carry the TMC to the desired location. In addition, all progress achieved in polymer-based nanocarriers that have been developed for drug

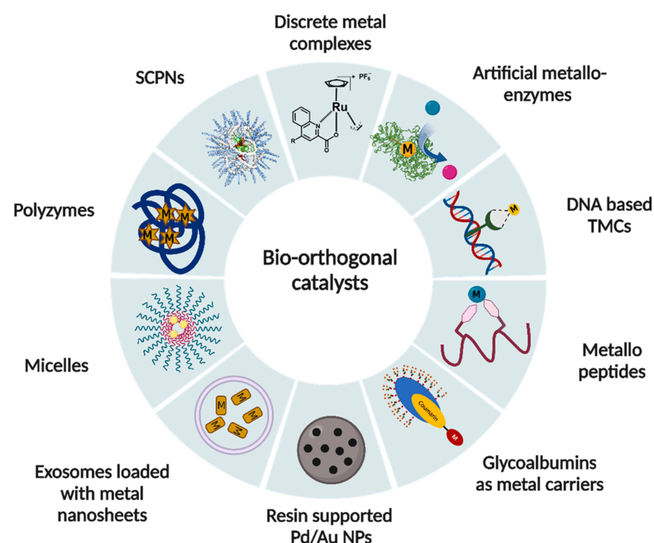

Fig. 1. An overview of synthetic or natural ligands and / or carrier materials that have been combined with TMCs to afford bioorthogonal TMC-based catalysts for *in vitro* or *in vivo* applications. Figure created with biorender.com.

delivery [40,41], can be included in the polymer scaffold design to target, actively or passively, the TMC to the desired location *in vivo*. Throughout this review, we use the nomenclature *in vitro* when the reactions are performed in the presence of cells (either bacterial or mammalian) and *in vivo* when the reactions are performed in animals such as zebrafish or rodents.

We structure this review as follows. First, we discuss briefly selected metal catalysts that have shown great promise in a range of bioorthogonal reactions and the effect of ligand tuning on the catalyst's stability and activity. We focus on the water solubility and stability of TMCs in complex media and in the presence of cells, and on reactions that either release an active drug or where a drug is formed from two separate substrates. Then, we discuss the use of natural polymers such as proteins and DNA as scaffolds for TMCs. Finally, synthetic polymers either present as heterogeneous or homogeneous scaffolds will be discussed in light of their applicability in bioorthogonal reactions. We end this review with summarizing the challenges ahead in this field, and how we believe that homogeneous water-compatible synthetic polymers could play a decisive role in addressing these challenges.

2. TMCs in bioorthogonal reactions: effect of ligand on catalyst stability and activity

While organometallic complexes such as *cis*-platinum have been used as metallodrugs for a long time [42], the application of TMCs to catalyze non-natural reactions in living cells is of more recent interest. Designing TMCs that are both stable and reactive in living cells is a challenging task, given that the complex sea of functionalities in cells, particularly thiols, can quickly deactivate them. In 2006, Streu and Meggers were the first to demonstrate the use of Ru-based TMCs as a bioorthogonal catalyst for the cleavage of allyl carbamates to their respective amines *in vitro* [26]. It was quickly realized that the nature of the ligands had a profound impact on the efficiency of the reaction as it can help to tune the reactivity and stability of these catalysts *in vitro*. Later studies focused on the ligand's effect on the catalyst's reactivity and stability by varying the ligands for dealkylation reactions (Fig. 2A) [27]. It was found that the 2-quinoline carboxylate-based Ru complex **2** showed a dramatic increase in the activity reaching up to 90 turnovers under biologically relevant conditions as compared to 4 turnovers by their previously reported [Cp*Ru(COD)Cl] complex **1** (Fig. 2A). Further, a significant boost in the activity was achieved reaching up to 270 turnovers with the introduction of π-donating methoxy and dimethylamino group onto the quinoline moiety **3** and **4** (Fig. 2A). The most active complex **4** enabled the uncaging of protected rhodamine **6** by cleaving allylcarbamate protected amines inside HeLa cells (Fig. 2B) [8]. In addition, the same Ru complex **4** successfully induced cell apoptosis in HeLa cells by uncaging protected doxorubicin **8**. In subsequent work, Ru complexes were fine-tuned using the 8-hydroxyquinolinate ligand leading to Ru complex **5**, which showed higher turnover (>300) in uncaging alloc-protected amines in blood serum (Fig. 2A).[43] Therefore, it became possible to activate protected doxorubicin **8** using very low concentrations of catalyst in Hela cell cultures (Fig. 2C). These findings highlight the fact that the reactivity of the Ru-complexes can be tuned by selecting appropriate ligands. However, the effect of ligands on catalyst's activity can differ in complex environments *versus* organic media as the rate determining step of the reactions can be shifted due to competing nucleophiles present in complex media. In earlier work, reducing the π-backbonding of bidendate ligand using methoxy and dimethylamino group boosted the catalytic activity of the Ru complexes. [27] An opposite trend in reactivity was observed by Kitamura and co-workers in organic solvents, where reduced π-backbonding of bidendate ligands diminished the catalytic activity of analogous Ru complexes [44].

After the pioneering studies of Meggers and coworkers on introducing Ru-based TMCs as a bioorthogonal tool [27], the field flourished with the application of new TMCs for a range of organic reactions in cells

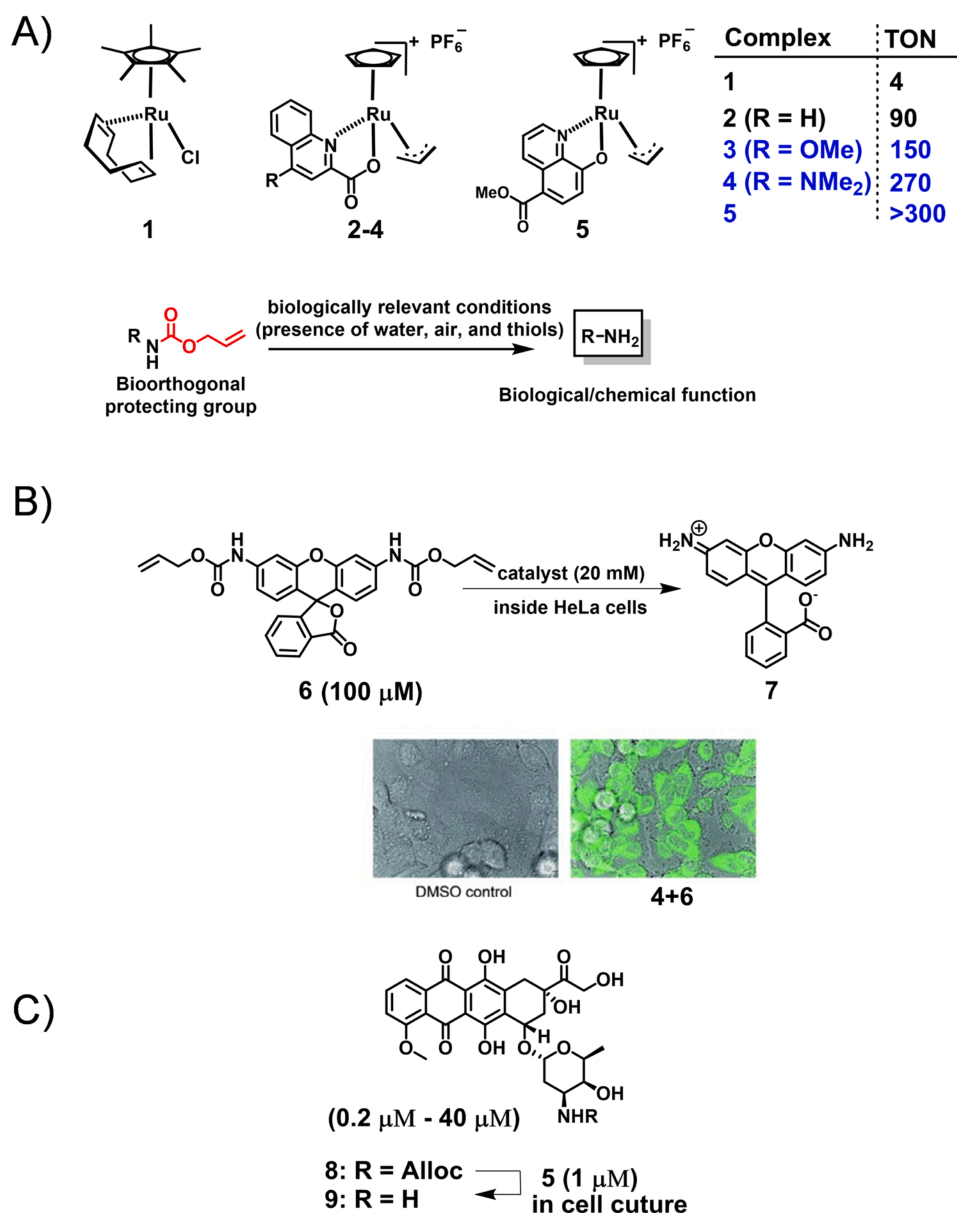

Fig. 2. A) Structure of Ru-complexes for catalytic cleavage of O-allyl carbamates with their corresponding turnover number (TON). B) Uncaging of protected rhodamine inside Hela cells using Ru catalyst **4**. C) Structure of protected doxorubicin uncaged in HeLa cell culture using Ru catalyst **5** [27].
Cell image adapted from Ref. [27] with permission.

and even living systems such as Pd(II)/(0) [29–31], Au [45,46], Cu [47, 48], Ir [49], Pt [50], and Fe [51], which opened up new avenues for *in situ* drug synthesis or prodrug activation. TMCs based on Pd(II)/Pd(0) were studied to catalyze deprotections and Suzuki-Miyaura cross--coupling reactions in biological settings owing to their low toxicity [52]. The oxidation state of Pd was found to have a large impact on the depropargylation reaction rate, which was studied in detail by Chen and co-workers (Fig. 3A) [53]. They screened heterogenous Pd(0) nanoparticles (Pd-NPs), Pd(0)/Pd(II) and Pd(IV) complexes for their catalytic efficiency in water. The conclusion was that Pd-NPs **12** and triphenylphosphine-based Pd(0) complex **14** (Fig. 3A) showed the highest reactivity in the deprotection of *N*-proc-caged coumarin **10** in water. They further performed metabolic incorporation of Neu5Proc **20** onto cell surface glycans and their *in situ* conversion to neuramic acid **21** using Pd-NPs (Fig. 3B).

More research on ligand tuning on Pd(II) complexes for intracellular catalysis was carried out by Mascareñas and co-workers (Fig. 4) [31]. They modified the solubility, stability and reactivity of the Pd(II) complex and observed that triphenylphosphine based complexes outperformed others in uncaging of protected dye **22** in cell lysates and even in mammalian cells. Moreover, both depropargylation and deallylation reactions were catalyzed, albeit with moderate activity. Interestingly, they could target the complex to the mitochondria of HeLa cells by using a phosphine ligand tethered to phosphonium and hydrophobic pyrene groups **32** (Fig. 4B) [31]. They also used the same targeting group to modify Meggers' Ru-catalyst **4** to selectively target the catalyst to mitochondria of HeLa cells and locally generate 2,4 dinitrophenol for mitochondrial depolarization. Thereby, they showed that ligand modifications can also allow targeting and preferential accumulation of catalysts to achieve functional alterations in living cells [54].

Cu-catalyzed click chemistry is widely used for a variety of transformations in biological settings such as modification of nucleic acids, proteins or virus particles [55]. Even after the introduction of strain-promoted azide-alkyne cycloaddition reactions, the catalytic nature of Cu click reactions lures chemists to devise complexes that are highly biocompatible and active [55]. Also here, the ligands played a

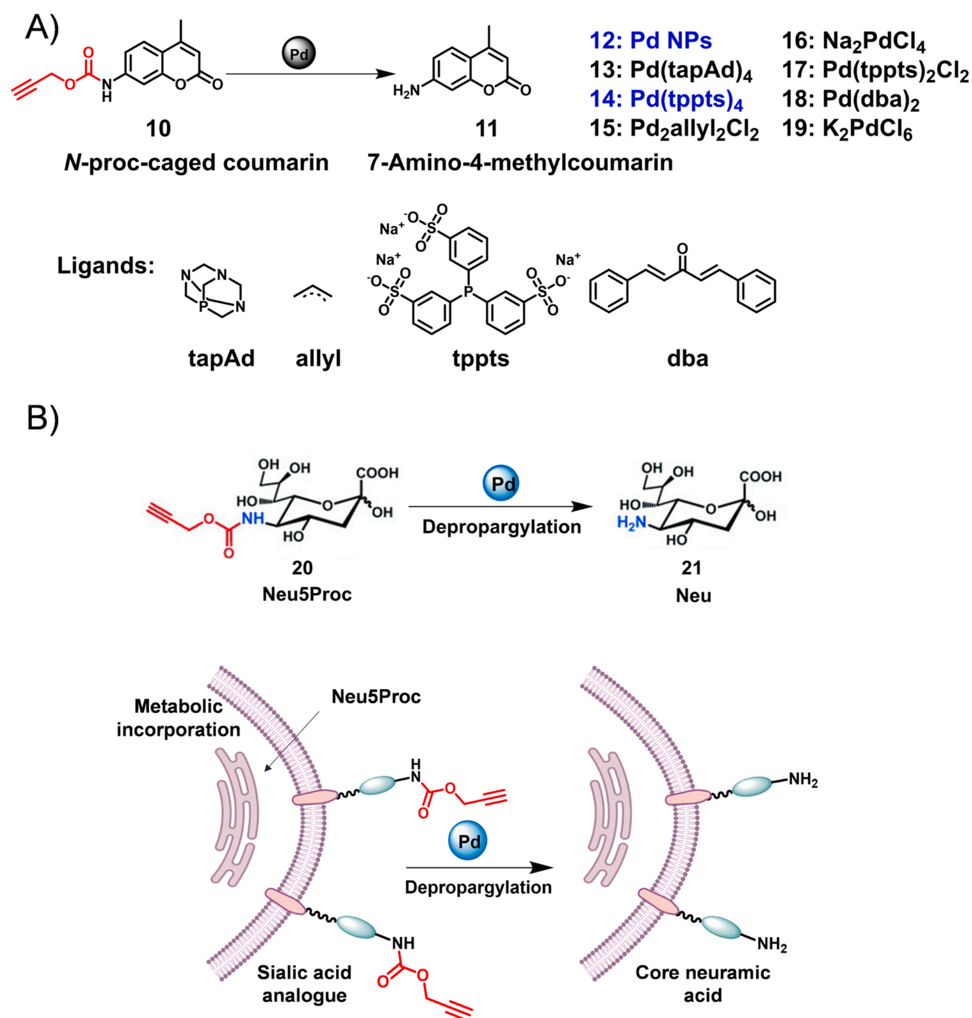

Fig. 3. A) Pd-based catalysts with different ligands screened for depropargylation of *N*-proc-caged coumarin (stochiometric amount of catalysts were used in water). B) Representation for metabolic incorporation of Neu5Proc and its *in situ* conversion to neuramic acid using Pd-mediated depropargylation [53]. Best-performing catalysts are marked in blue. Figure created with biorender.com.

significant role in balancing reactivity, stability, and toxicity as they aid in preventing the Cu(I) ion from oxidation and disproportionation. However, that balance is delicate: when the ligand-binding is too labile, the redox stability of the metal can be compromised but when the ligand-binding is strong, the catalytic activity of the metal can be suppressed [56]. Pezacki and co-workers studied the cellular consequences of Cu-complexes commonly used for bioconjugation reactions and found that the ligand environment affected the toxicity, cellular uptake, and lipid metabolism of mammalian cells [57]. The Cu(I)–L–histidine complex was tolerated in cells at millimolar concentrations for 3 days while other Cu-complexes that employ TBTA (tris(benzyltriazolylmethyl) amine) or BPS (bathophenanthroline disulfonate disodium salt) were toxic on prolonged exposure. The results confirmed that toxicity of the metal species is dependent on ligand environment and variation of the ligand could alter this toxicity by at least 3 orders of magnitude. Ligand specific ligation of Cu can not only prevent reactive oxygen species generation, but also helps to give antioxidant properties. Many biocompatible and amino acid based chelating ligands for Cu were developed later for bioconjugation reactions that exhibited efficient catalytic activity with low cytotoxicity [58].

So far, Cu has been mainly utilized for click reactions and depropargylation reactions [33,59]. Recently, Mascareñas and co-workers introduced Cu(II)-catalyzed carbene insertion reactions to catalyze the *in vitro* synthesis of quinoxaline derivatives [19]. This work highlighted the importance of developing new catalytic reactions that can be performed in biological settings. Here, fluorescent benzoquinoxalines were formed *in-situ*, of which one compound was a bio-active agent that altered the mitochondrial function of cells. Moreover, by conjugating the Cu-based catalyst to an integrin targeting moiety, it was possible to achieve cell-selective biological effects.

3. Natural polymers as scaffolds for TMCs

Scaffold-based catalysts have appeared in numerous recent examples of successful metal catalysis in living systems due to the improved stability and activities. An eligible scaffold for transition metal catalysts that works in a biological environment with high stability and catalytic efficiency should not only protect the catalytic center from deactivation, but should also keep the metal center accessible for substrates at low concentrations. In biology, catalytic metal centers are often shielded from the outer environment by scaffolding within a protein. Borrowing from this idea, transition metal catalysts have been anchored into a natural scaffold to create artificial metalloenzymes (ArMs). An appealing property of ArMs is that the transition metal catalysts and the natural scaffold can be adjusted separately by chemical and genetic methods. ArMs may combine beneficial aspects of organometallic and enzymatic catalysts, giving a way to create new-to-nature reactions.

Introducing transition metal catalysts to natural scaffolds can be

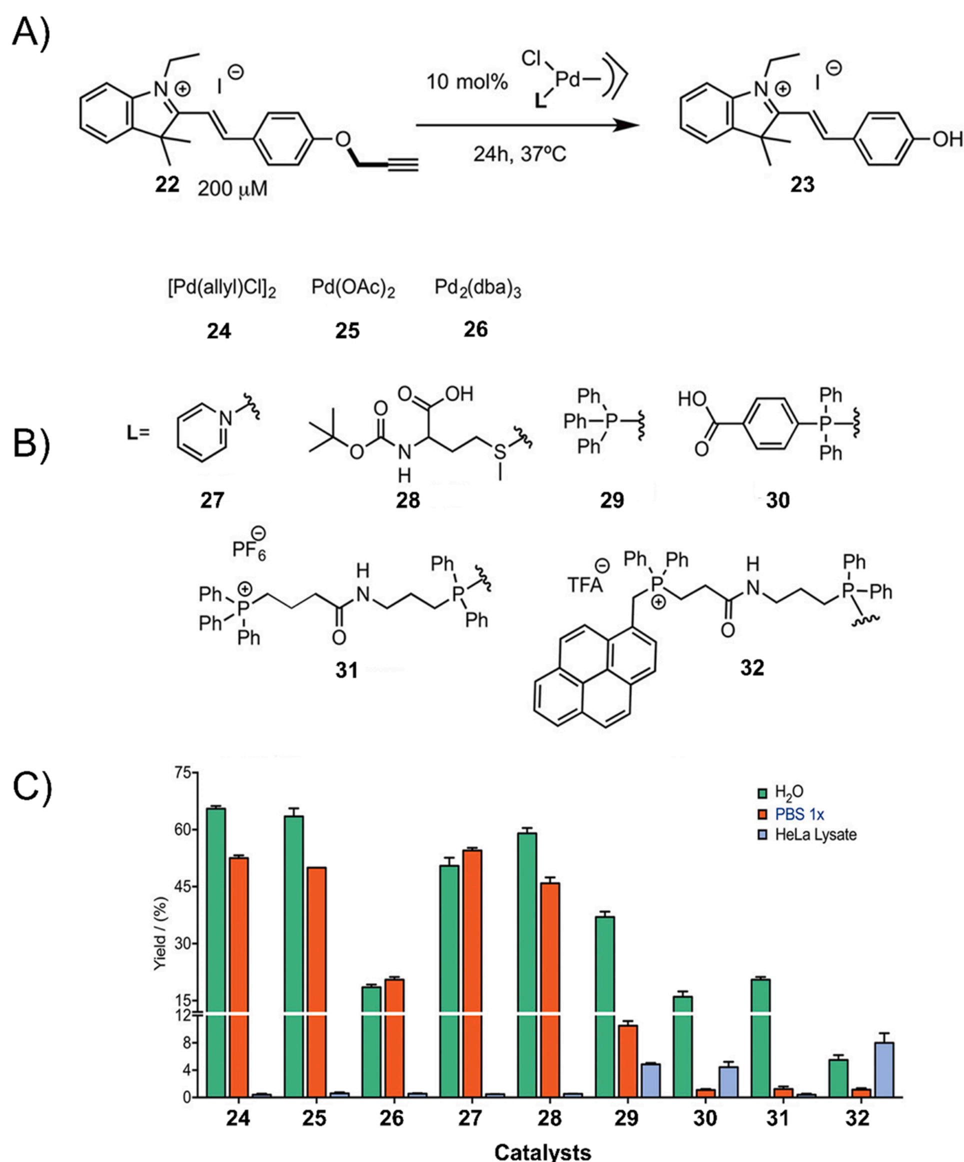

Fig. 4. A) Depropargylation reaction catalyzed by Pd-based catalysts. B) Pd-based catalysts with ligand modifications. C) Bar diagram representing the yield obtained for each catalyst in water, PBS and Hela cell lysate determined using HPLC-MS.
Image adapted from Ref. [31] with permission from the American Chemical Society.

achieved either by covalent or supramolecular anchoring [60,61]. Covalent anchoring requires a chemical reaction that links the TMC to the natural scaffold. This reaction enables precise control of the metal complex's location inside the natural scaffold. However, the number of handling steps in the covalent anchoring approach is limited and thus severely hinders options for optimization. Supramolecular anchoring, achieved by noncovalent interactions between the scaffold and TMCs, has the advantage of rapid optimization, due to the ease of spontaneous formation of the artificial metalloenzyme and we will therefore focus on the supramolecular approach in this review. The powerful and often exploited supramolecular anchoring approach relies on conjugating transition metal complexes to specific protein binders that drive the metal complexes to the natural scaffold. The most well-known application of this strategy is the biotin-streptavidin combination, first proposed by Whitesides [62], and later successfully implemented by the Ward laboratory [63].

Ward and co-workers investigated in detail the biotin-streptavidin technology for constructing artificial metalloenzymes [64,65]. Streptavidin is a tetrameric protein with four identical subunits which have an extraordinarily high affinity towards biotin, making their binding one of the most potent non-covalent interactions in nature. By combining biotinylated TMCs with a library of streptavidin mutants, significant diversity can be generated, allowing optimal artificial metalloenzymes' catalytic performance to be achieved. For example, the assembly of streptavidin-biotinylated-Hoveyda–Grubbs second-generation catalyst was reported for olefin metathesis in the periplasm of *E. coli* [22]. The periplasm of *E. coli* was selected as reaction compartment because it contains significantly less glutathione, which is known to deactivate metal catalysts [66]. The reactivity of a ring-closing metathesis of a diolefin substrate was monitored by fluorescence from the product, showing that ring-closing metathesis was only catalyzed effectively when the streptavidin and biotinylated-Ru-complex were assembled in the periplasm. To enhance the performance of the artificial metalloenzymes, different streptavidin mutants were screened, revealing that increased loop flexibility in streptavidin may help to improve activity of the artificial metalloenzymes.

Although the biotin-streptavidin technology proved successful in creating artificial metalloenzymes in the periplasm of *E. coli*, it required

effective penetration of biotinylated-Ru-complex across the *E. coli* outer membranes, which limited the accessibility of the metal complex towards streptavidin for proper assembly. To overcome this, a strategy for the assembly of streptavidin and the metal complex on the surface of *E. coli* was developed [67]. Hereby, Streptavidin (Sav) was fused to the anchor protein Lpp-OmpA (Fig. 5 A) which directs streptavidin from the cytoplasm towards the outer membrane of *E. coli* to enable convenient and prompt assembly with the biotinylated-Ru-complex (**BTL-Ru**) (Fig. 5B). The *in situ* assembly of artificial metalloenzymes enabled the deallylation reaction of an allylcarbamate-protected coumarin derivative **33** to obtain fluorescent aminocoumarin **34** (Fig. 5 C). Directed evolution allows the streptavidin mutant assembled with biotinylated-Ru-complex to display the highest activity *in vitro*, with the turnover number increased 5.7-fold compared to the wild type protein.

Apart from using streptavidin as the protein scaffold for shielding transition metal catalysts, other proteins also show great promise for *in vitro* catalysis. For example, Roelfes and co-workers reported the creation of artificial metalloenzymes based on a dimeric transcription factor LmrR [68]. LmrR has a sufficiently large hydrophobic binding pocket at the dimer interface, which can accommodate both substrates and metal catalysts, making it an interesting protein scaffold. The size of the pocket is readily adaptable depending on the bound guest molecule. One way of introducing TMCs to LmrR is to modify the protein scaffold with amino acids that can bind metal complex. For example, the incorporation of the unnatural amino acid (2,2′-bipyridin-5yl)alanine (BpyAla) in LmrR was reported for binding Cu [69]. The performance of generated metalloenzymes was subsequently evaluated in an asymmetric Friedel-Crafts alkylation reaction. The reaction results demonstrate that the protein scaffold was essential for obtaining decent enantioselectivities (up to 83%).

Next to introducing unnatural amino acids to the hydrophobic pocket of LmrR for binding transition metals, the two local tryptophan moieties in LmrR could also be applied for binding planar aromatic ligands complexed with transition metals. Roelfes and coworkers reported that the two tryptophan residues W96 and W96′ in LmrR bind and sandwich Cu(II)−phenanthroline *via* π-stacking interactions (Fig. 6), which was verified by tryptophan fluorescence and lifetime measurements [70]. By titrating Cu(II)-**L1** into a buffer solution containing LmrR, a dissociation constant (2.6 ± 2 μM) was found for binding of Cu(II) -**L1** to LmrR. The assembled artificial metalloenzymes exhibited excellent selectivity in catalyzing Friedel−Crafts alkylation of

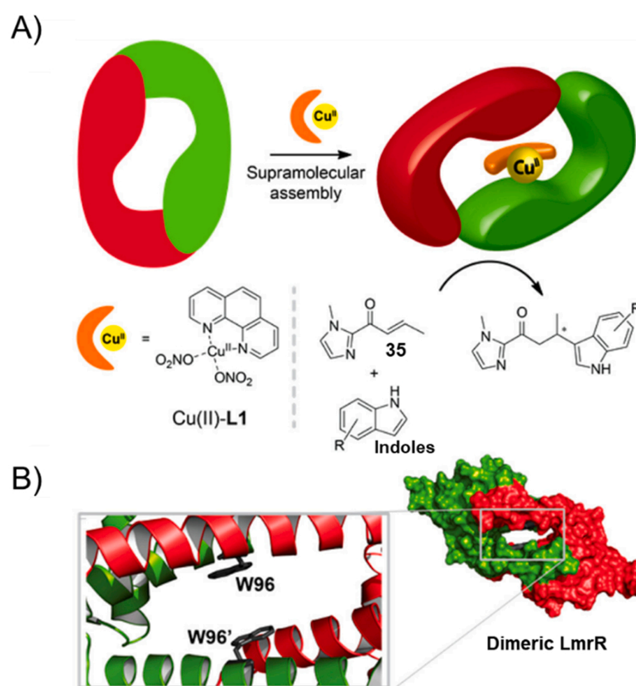

Fig. 6. A) Schematic representation of LmrR assembly with Cu(II)−phenanthroline at the dimer interface, followed by catalyzing Friedel–Crafts alkylation. B) Illustration of tryptophan residues W96 and W96′ displaying on the dimeric LmrR.
Image reproduced from Ref. [70] with permission from the American Chemical Society.

indoles and α,β-unsaturated 2-acylimidazoles **35** (enantiomeric excess, *ee*, up to 94%). The replacement of tryptophan residues with alanine results in significantly lowered *ee* (<5%), which further supports that the binding between Cu(II)−phenanthroline and tryptophan residues plays a key role in enantioselectivity.

This system was also introduced into a challenging environment by assembling Cu(II)−phenanthroline and LmrR in the cytoplasm of *E. coli* [71]. Dynamic nuclear polarization (DNP)-supported solid-state NMR spectroscopy provided direct evidence for the assembly of exogenous Cu

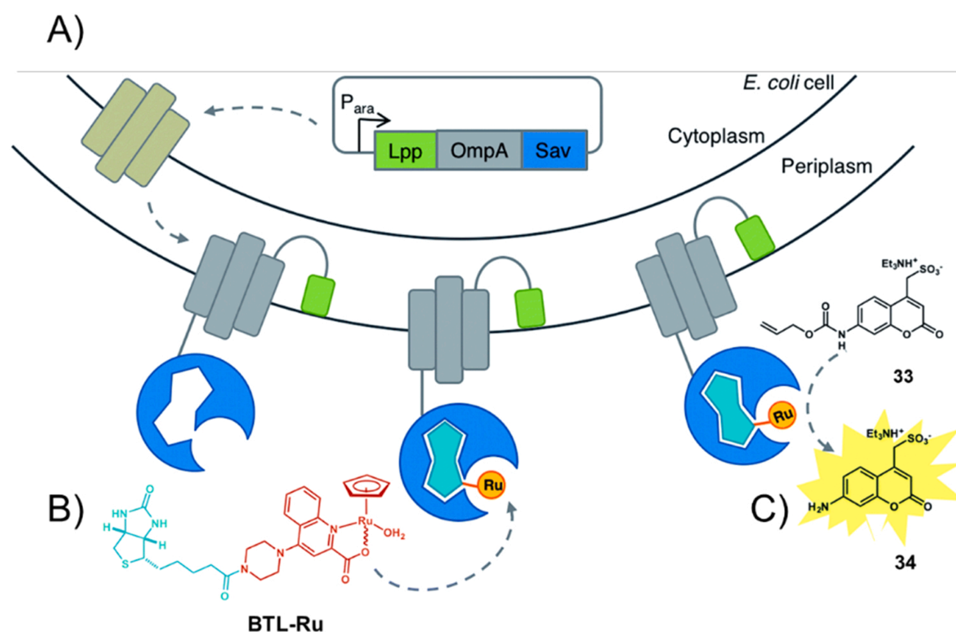

Fig. 5. The assembly of artificial metalloenzyme on the outer membrane of *E. coli* for deallylation reaction. A) Streptavidin (Sav) is fused to the C-terminus of an Lpp-OmpA fragment. Lpp-OmpA-Sav expression is first inducted in the cytoplasm and eventually anchored to the *E. coli* outer membrane. B) Biotinylated Ru-complex **BTL-Ru** assembled with streptavidin to afford an artificial deallylase. C) Allylcarbamate-protected coumarin **33** is converted into a fluorescent aminocoumarin **34** in the presence of the artificial deallylase on the *E. coli* surface.
Reproduced from Ref. [67] with permission from the Royal Society of Chemistry.

(II)−phenanthroline with protein LmrR in the cytoplasm of *E. coli*. Interestingly, NMR revealed that the Cu(II) state dominates the Cu-complex within the assembled artificial metalloenzymes, which confirms that cellular reductants such as glutathione do not play a significant role in reducing Cu(II) to Cu(I). This system proved to be versatile to obtain LmrR mutants with good catalytic efficiencies, which can be customized to catalyze mechanistically different reactions, such as Friedel–Crafts alkylation and Diels–Alder reaction [71].

Besides using proteins as scaffold, Roelfes and co-workers also reported DNA as a natural scaffold for TMCs. DNA, with its unique and programmable chiral structure, is an attractive natural scaffold for encapsulating transition metals. An aminomethylpyridine ligand complexed Cu(II) was reported to conjugate to a DNA intercalator *via* a short spacer for introducing Cu-complex to DNA (Fig. 7) [72]. The DNA intercalator 9-aminoacridine can insert itself between two DNA base pairs, driving the Cu-complex to reside near the DNA. The catalyzed reaction thus occurs in close proximity to the DNA helix, allowing the reaction to inherit the chirality of the DNA. To optimize the design, the spacer length n and the substituent R were tuned to obtain ligands **AMPA** (Fig. 7A). This system was evaluated in catalyzing Diels–Alder reaction of cyclopentadiene **36** with aza-chalcones **37** (Fig. 7B). The results show that the best enantioselectivities were obtained, up to 49% *ee* of the (-) enantiomer, when using a short spacer length of n = 2 or 3 and an arylmethyl group as the substituent.

The observation that a shorter spacer length between Cu-complex and DNA intercalator resulted in higher *ee*, suggested that the integration of metal binding moiety and DNA binding moiety without a spacer might increase the enantioselectivity further. For this purpose, Roelfes and coworkers used bidentate ligands that are known to bind DNA and at the same time complex with Cu(II). They developed polypyridine Cu (II) complexes such as Cu-4,4′-dimethyl-2,2′-bipyridine (Cu-dmbipy), Cu(II)− 1,10-phenanthroline (Cu-phen) and Cu-2,2′-bipyridine (Cu-bipy), which bind to DNA with moderate affinity[73]. It was found that this system resulted in rate accelerations and much higher *ee*, up to 90% *ee* for Cu(bipy) in catalyzing the Diels–Alder reaction. Significant rate accelerations were also observed in other Lewis acid-catalyzed reactions using this system, such as fluorinations [74], Michael additions [75] and hydrolytic epoxide ring opening [76].

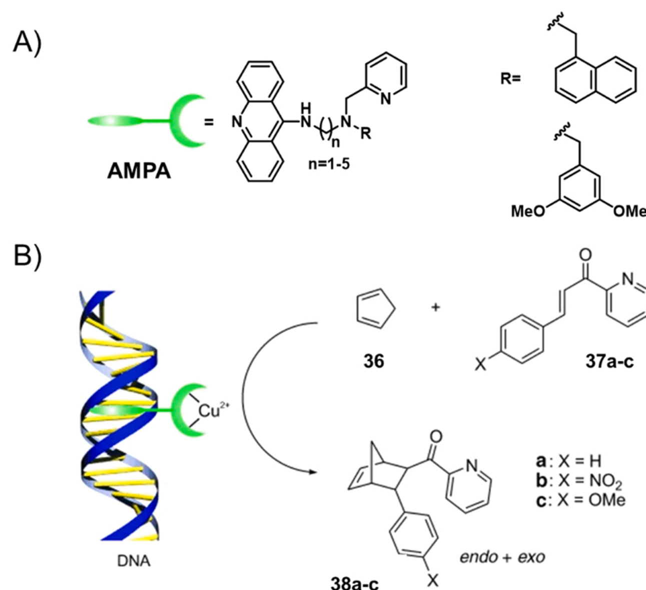

Fig. 7. A) Schematic representation of aminomethylpyridine conjugates to the DNA intercalator 9-aminoacridine *via* varied spacer length n and substituent R to form ligands **AMPA**. B) Cu-complexes in close proximity to the DNA helix for catalyzing Diels–Alder reaction of cyclopentadiene **36** with aza-chalcones **37**. Image reproduced from Ref. [72] with permission.

Apart from applying proteins and DNA as scaffolds to protect transition metal catalysts, Mascareñas and co-workers reported a synthetic metallopeptide using a peptide as the scaffold to assemble with Pd(II) for catalyzing depropargylation inside mammalian cells [77]. The peptide scaffold was mutated to introduce two histidine residues, which can bind Pd(II) to generate a stable Pd(II)-peptide complex (Fig. 8A). The Pd (II)-peptide complex can catalyze depropargylation reaction of substrate **39** in HeLa cells resulting in intensive magenta fluorescence, while only $PdCl_2$(COD) (COD = 1,8-cyclooctadiene) or substrate **39** displayed no fluorescence (Fig. 8B). The study revealed that the depropargylation efficiency of the substrate in living HeLa cells relied not only on an appropriate Pd source, but also on the histidine decorated peptide scaffold with high cell penetration ability.

Many of the elegant studies using artificial metalloenzymes in a range of catalytic reactions in living cells have focused on the use of bacterial cells. As a result, the translation to *in vivo* bioorthogonal catalysis for prodrug activation received less attention. A notable exception herein is the work of Tanaka and coworkers, who reported a glycolalbumin−Au(III) complex with organ targeting ability to localize catalytic activity inside live mice [78]. As coumarin is known for high binding affinity towards albumin pockets, in this work, they modified coumarin to link the Au(III) complex (Fig. 9 A) for bringing the TMC to glycolalbumin scaffold. Asparagine-linked glycan was immobilized on albumins to construct glycolalbumin with organ selectivity (Fig. 9B). The authors observed that (2−6)-disialoglycoalbumins (Sia) are retained within the liver for eventual urinary excretion while galactose-terminated glycoalbumins (Gal) are rapidly trafficked to the intestines. As a consequence, assembly of glycolalbumin and coumarin-Au(III) conjugate resulted in accumulation of the Au(III) complex in the desired organ. The Au(III) complexes were rapidly immobilized at glycan-targeted organs (within 30 min to the liver and intestine) without leaching or deactivation of the metal catalysts. In addition, glycolalbumin−Au(III) complexes successfully catalyzed the formation of amide bonds between propargyl ester modified imaging reagent and amines on the surface of the organ (Fig. 9C). These results are noteworthy as they show that biologically active TMCs can indeed be utilized in higher-level organisms.

4. Synthetic polymers as scaffolds for TMCs

4.1. Heterogeneous microspheres based on polystyrene-based beads

The inherent disadvantages of using "naked" transition metal complexes can be overcome by using heterogenous catalysts that possess improved stability in the presence of deactivating nucleophiles and proteins in complex media. In fact, the very first example of using Pd chemistry in mammalian cells was shown by Bradley and co-workers using heterogenous catalysts in 2011 [30]. Pd nanoparticles were trapped on 500 nm amino functionalized polystyrene based beads synthesized by dispersion polymerization from styrene-based monomers [79], followed by extensive crosslinking of the available amino groups, which allowed permanent entanglement of Pd(0) nanoparticles. This approach increased the biocompatibility of the NPs while retaining the catalytic activity amidst the complex environment in living cells. The heterogenous microspheres acted as 'Trojan horses' to deliver Pd nanoparticles into living cells, stayed in the cytoplasm for several days and performed allyl carbamate cleavage and Suzuki-Miyaura coupling reactions [14, 52]. It was proposed that these heterogenous catalysts can act as an artificial organelle with longer retention in cytoplasm of cells and with minimal leakage of catalysts thereby decreasing their toxicity [14,52, 80]. Even though the aforementioned reactions were catalytic in nature in a non-biological environment, it was found that this was not the case in living cells. The possibility of the Pd catalyst deactivating partially over time in the presence of intracellular components was therefore not completely ruled out [14]. Later, Bradley and co-workers developed fluorescent microspheres loaded with Pd nanoparticles and a cyclic

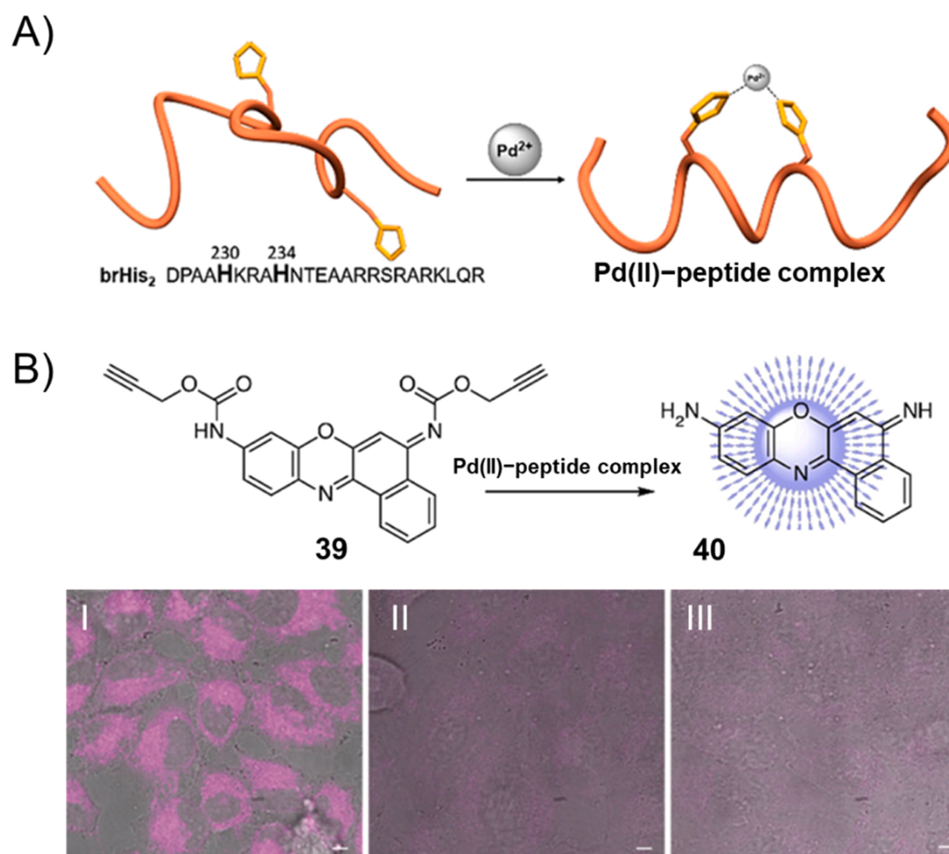

Fig. 8. A) A) Illustration on the bis-histidine modified peptide upon Pd(II) addition resulting in Pd(II)-peptide complex. B) Confocal images of Pd(II)-peptide complex catalyzing depropargylation reaction of substrate 39 in HeLa cells. (I) HeLa cells incubation with Pd(II)-peptide complex (Pd source: $PdCl_2$(COD)); (II) incubation with $PdCl_2$(COD) alone; (III) incubation with substrate 39 alone. Scale bar: 10 μm.
Image reproduced from Ref. [77] with permission.

peptide cRGD was conjugated to their surface as a targeting ligand (Fig. 10A). This allowed rapid and selective uptake in glioblastoma cells as well as dual drug synthesis. They performed simultaneous activation of a pro-drug of 5-fluorouracil (5-FU) **44** to 5-FU **45** and Suzuki-Miyaura coupling of two benign precursors **41** and **42** to form an anticancer agent PP-121 **43** (Fig. 10B). [81].

Unciti-Broceta and co-workers used a similar approach and reported extracellular microdevices using TentaGel® resins (crosslinked polystyrene matrix with grafted PEG chains for additional biocompatibility) that are 150 μm in diameter to trap Pd(0) nanoparticles [29]. They showed for the first time the activation of an anticancer pro-drug, pro-5FU, near cancer cells using Pd catalysts. They also elegantly implanted a single Pd(0) resin into the yolk sac of zebra fish, which displayed excellent bio-compatibility and local catalytic activity by activating a non-fluorescent lipophilic pro-dye based on rhodamine 110 [29]. This work paved the way for further developments of bioorthogonally activated pro-drug approach in *in vivo* disease models. In subsequent work, they optimized the size of the resins to obtain the best Pd loading capacity and catalytic efficacy for ultrasound guided intra-tumoral implantation in mouse prostate tumors. The size of 30 μm Pd-devices was found to be optimal for performing this procedure and these NPs did not leak out from the tumors, were not toxic to the animal, and did not affect the tumor growth. By activating a pro-dye based on rhodamine 110, they demonstrated for the first time that a bioorthogonal catalyst can be hosted in a tumor *in vivo* without negatively impacting the turnover rate of the devices over time[80]. They also demonstrated the capacity of these devices to sequester circulating pro-doxorubicin and generate cytotoxic levels of doxorubicin drug in tumor explants. Moreover, they showed that these Pd(0) devices can uncage a variety of newly developed pro-drugs based on active metabolite of irinotecan [82], vorinostat [83], floxuridine [84], and gembicitabine [85]. Most recently, Unciti-Broceta and coworkers marked another milestone in the field by developing a novel Pd activable 5-FU prodrug that can be administered orally as it can evade anabolic and catabolic drug pathways. This pro-drug was activated by intratumorally implanted Pd resins inside a tumor xenograft in mice [86]. This study highlights the potential of using heterogeneous Pd(0) resins for site specific activation of orally administered pro-drugs for new side-effect-free cancer therapies. The work was recently extended to other bioactive molecules as reported by Kane and co-workers, where Pd-NPs were immobilized on TentaGel® resins to activate a prodrug of resatorvid, an anti-inflammatory agent in *in vitro* conditions [87]. In addition to this, Qu and co-workers reported studies on photo-sensitive Pd loaded silica nanoparticles functionalized with azobenzene units that allow light regulated catalyst activation [88]. Another novel approach was pursued by Gu and co-workers, where a bioorthogonal catalytic patch was developed with Pd-NPs coated on TiO_2 nanosheets that can activate pro-drugs in sub-cutaneous tumors in mice [89].

A formidable challenge in the field is to bring the catalytic system to the site of interest, which is especially challenging with heterogeneous systems. Santamaria and coworkers together with Unciti-Broceta developed new therapeutic vectors combining the targeting ability of cancer-derived exosomes and catalytic potential of Pd nanosheets for the release of anti-cancer drug Panobinostat [90]. These heterogenous vectors not only had the ability to enter cancer cells and remain catalytically active, they also preferentially targeted their progenitor cells from other cells. Further, they perfected the protocol to load Pd-nanosheets inside the exosomes using a mild reduction process using

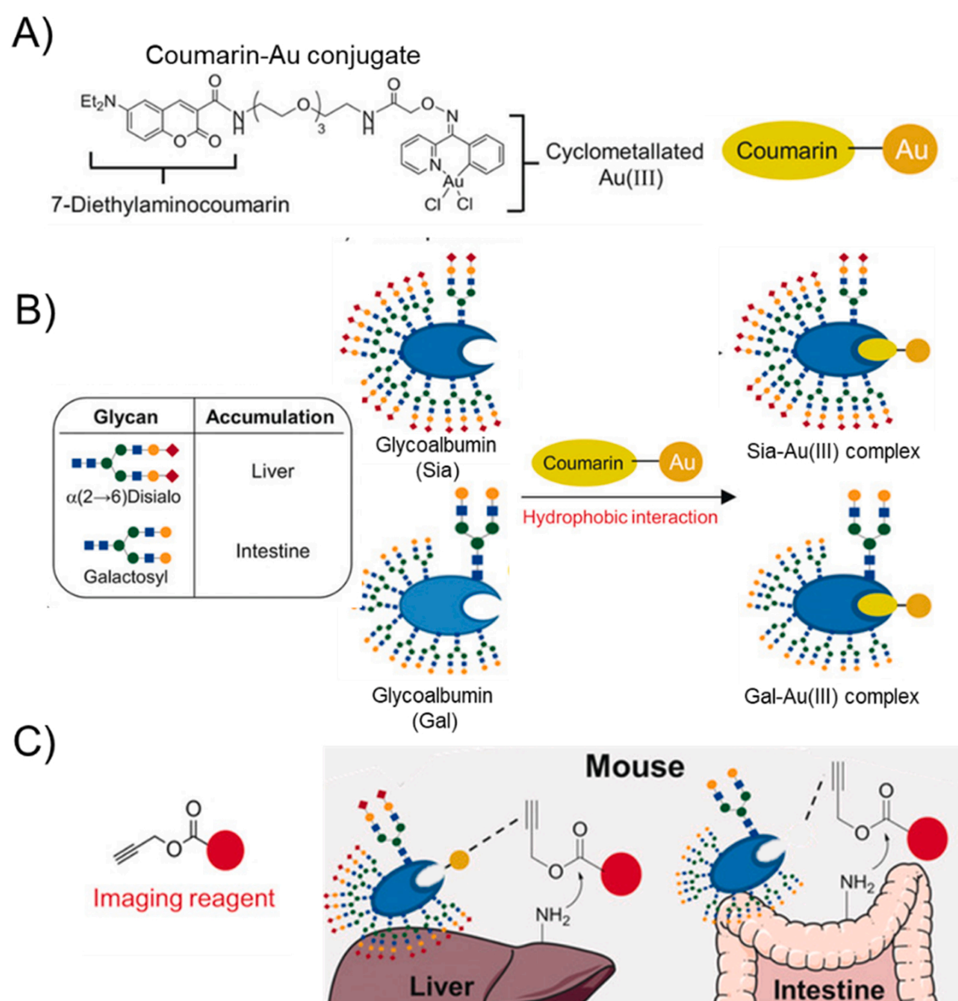

Fig. 9. A) Modification of 7-diethylaminocoumarin to conjugate Au(III) complex. B) Assembly of glycoalbumins and coumarin-Au conjugate to produce Sia-Au(III) and Gal-Au(III) complex for liver and intestine selective localization. C) Amide bond formation between the propargyl ester of imaging reagent and surface amino groups of targeted organs, catalyzed by Sia-Au(III) and Gal-Au(III) complex. Sia and Gal were prepared with α(2−6)-disialoglycoalbumin and galactosylglycoalbumin, respectively.
Image reproduced from Ref. [78] with permission.

CO without disrupting the membranes thereby protecting surface integrins responsible for targeting [91].

Gold and copper have also been studied extensively as bioorthogonal catalysts, and the polymer resin-based approach can significantly increase their biocompatibility while lowering toxicity. The first example of heterogenous Au-based catalysis in living systems was reported by Unciti-Broceta and co-workers where intracranial activation of a bioorthogonal probe in zebra fish using 75 µm Au-resins was performed [45]. Moreover, they reported an inspiring example of *in vivo* heterogenous Au-based catalysis, where localized uncaging of a neuroactive agent, the anxiolytic drug fluoxetine, was demonstrated in the head of zebrafish, which decreased their locomotor activity compared to their normal mode (Fig. 11) [46]. Also, a novel strategy was developed to increase the number of active metal centers on the outer layer of the polymer scaffold. This was achieved by using an electrostatic loading method to load negatively charged Au-NPs at the positively charged outer amphiphilic layer of NH_2-PEG-grafted polystyrene micro-implants with a diameter of 75 µm (Fig. 11A). The study highlights the numerous exciting applications of bioorthogonal chemistry where in this case modification of cognition activity was possible by localized release of a neuroactive agent in the brain of an animal (Fig. 11B-E). This is a promising strategy to treat localized neurological disorders such as neuropathic pain if it can be extended to damaged nerves in the future.

The use of Cu as a bioorthogonal catalyst was always perceived to be limited due to the inherent toxicity of Cu(I) salts [92]. However, Bradley and co-workers developed non-toxic, biocompatible and implantable heterogenous Cu-based catalysts for *in vitro* synthesis of a combretastatin analogue, an anticancer agent, using an azide-alkyne cycloaddition. In addition, the *in vivo* synthesis of a fluorophore based on coumarin was achieved in the yolk sac of zebra fish embryos, while no toxicity was observed [47].

4.2. Homogeneous water-compatible polymers as carriers materials for TMCs

While the number of successful examples to catalytically activate prodrugs or make drugs *in vivo* using heterogeneous TMCs is increasing rapidly [8,35,93], there is an intrinsic challenge to heterogeneous systems, namely that they either need to be implanted at the tumor site using microsurgery or require a delivery vehicle such as exosomes to reach the site of interest. An interesting alternative is the use of nanoparticles based on water-compatible synthetic copolymers. Borrowing from the advances in the field of nanomedicine [40,94,95], such synthetic polymeric nanoparticles could offer several advantages. For example, polymeric NPs form homogeneous, compartmentalized structures in water that possess hydrophobic interiors, in which apolar TMCs can be physically or covalently trapped and hydrophobic substrates can be sequestered affording high local concentrations. In addition, it is straightforward to attach peripheral targeting groups to help guide the NPs to the tumor sites. Finally, the size and shape of these polymeric NPs is easily controlled by the length and microstructure of the amphiphilic polymers. As a result, amphiphilic block copolymers that from nanoparticles [17], cationically charged polymers that form micelles, so called polyzymes [96], and random amphiphilic heterocopolymers that

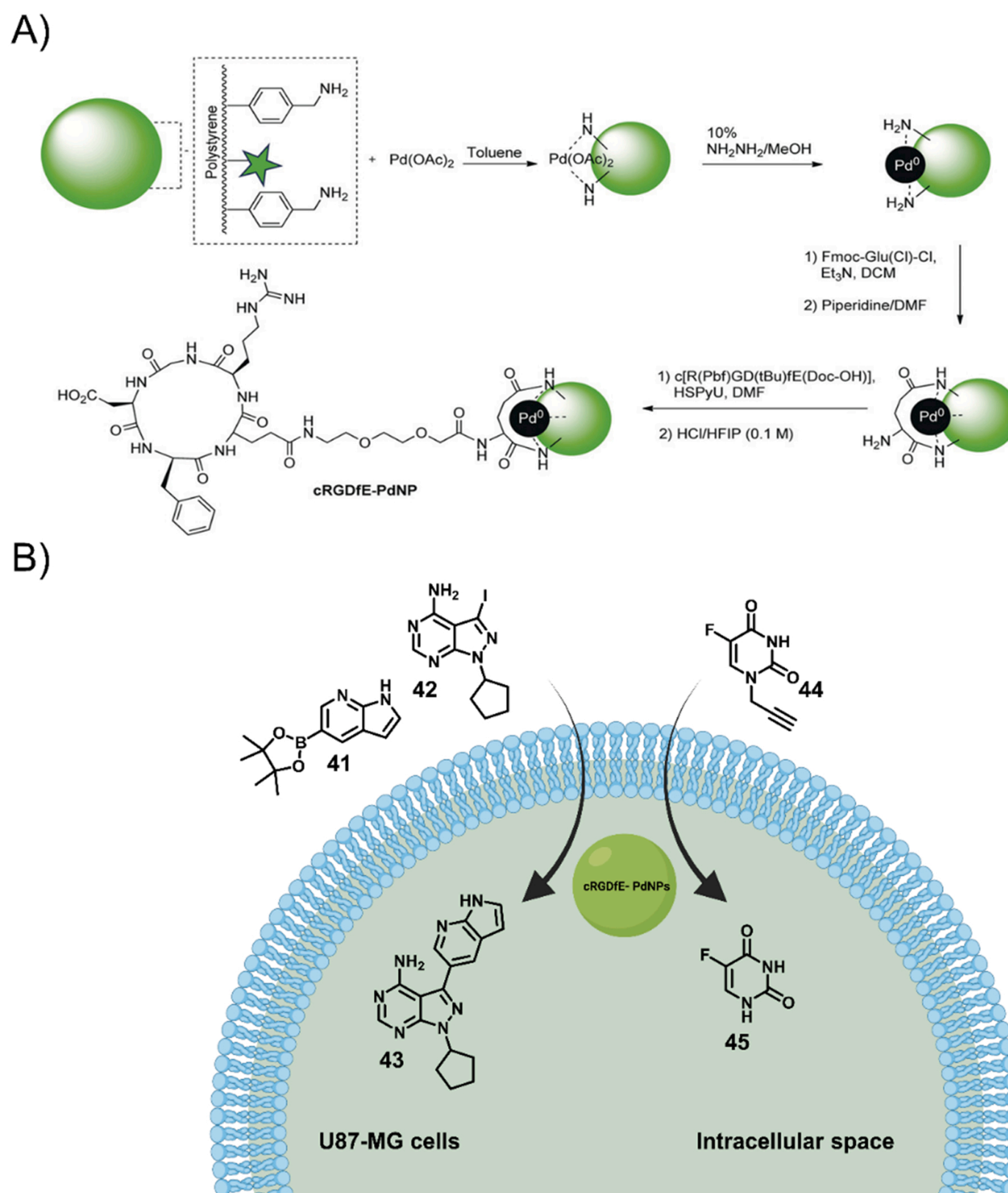

Fig. 10. A) Synthesis of RGD functionalized polystyrene Pd-NPs by Clavadetscher et al. B) Representation of in-cell dual drug synthesis of PP 121 **43** and 5-fluorouracil **45** by these Pd-NPs. [81] Figure created with biorender.com.
Image reproduced with permission from Ref. [81].

collapse/fold into single chain polymeric nanoparticles [48,97] have been actively investigated in the last decade.

In 2017, Weissleder and coworkers used poly(lactic-*co*-glycolic acid)-*b*-polyethylene glycol based NPs to encapsulate the hydrophobic bis[tri(2-furyl)phosphine] Pd(II) dichloride (Pd-TFP) catalyst **48** for bioorthogonal prodrug activation (Fig. 12A) [17]. The NPs were around 60 nm in size and demonstrated good stability in biological solutions. Taking advantage of the enhanced permeability and retention (EPR) effect, the Pd-NPs accumulated at tumor sites in mouse models of cancer. Interestingly, at the tumor site the Pd-NPs activated different model prodrugs, which inhibited tumor growth, and increased the survival of tumor-bearing mice (Fig. 12B). Detailed studies showed that the NPs were taken up by cells *via* an endocytic/macropinocytic process that results predominantly in lysosomal uptake [98]. In addition, their efficacy was further improved by a dual action whereby both the Pd-catalysts as well as the prodrug embedded in NPs were colocalized in the tumor cells *via* the EPR effect. These detailed studies showed that the use of synthetic polymers, in this case an FDA approved amphiphilic block copolymer, is a viable strategy to simultaneously protect the catalyst from deactivation in complex media as well as deliver the catalytic system to the site of interest *in vivo*.

In 2020, Rotello and coworkers developed the concept of polyzymes, which are poly(oxanorborneneimides) with pendant cationic tetra-alkylamines that ensure water solubility (Fig. 13) [99]. In water, these

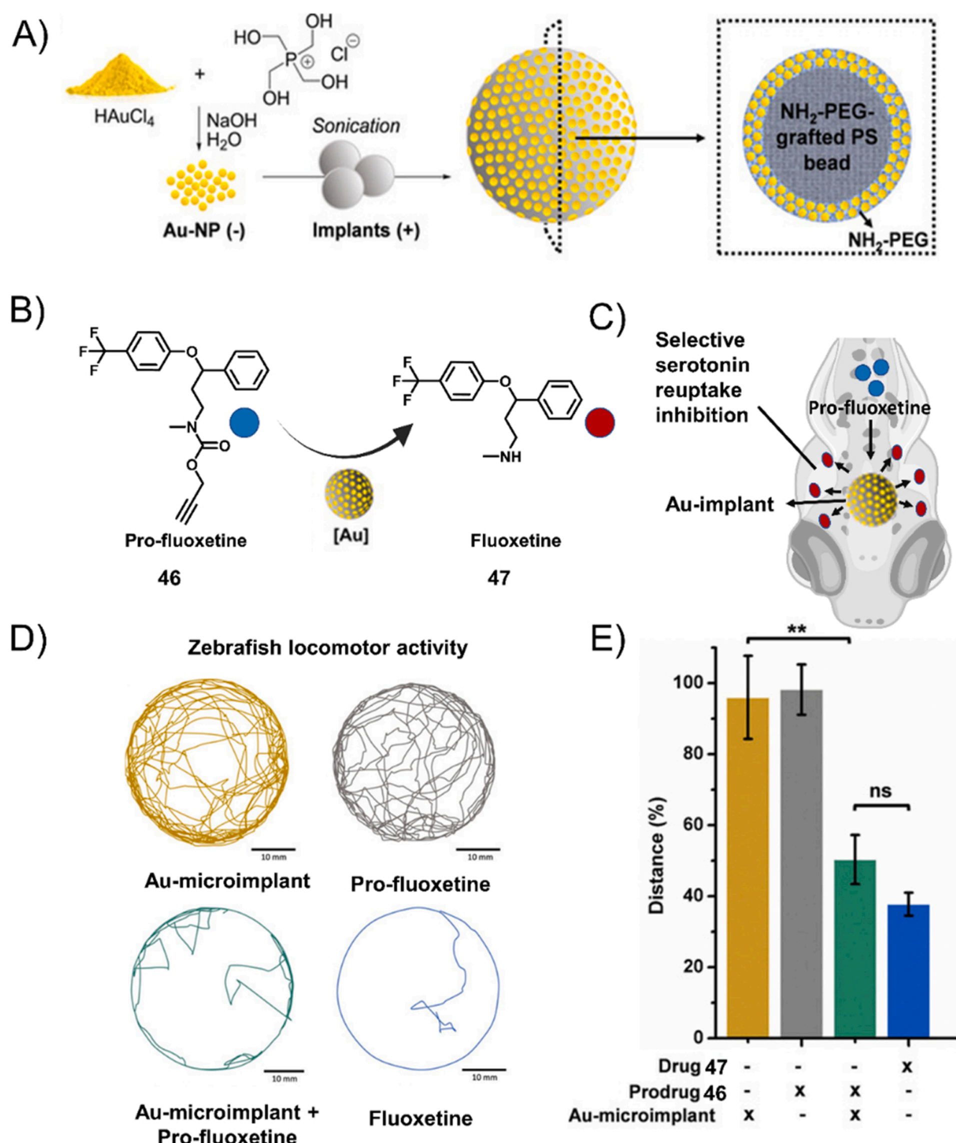

Fig. 11. A) Synthesis of polystyrene PEGylated Au-NPs by Ortega-Liebana et al. B) Generation of anxiolytic drug **47** from inactive precursor **46** by Au-NPs. C) Representation for generation of anxiolytic drug **47** in brain of zebrafish by localized Au-NPs. D) Representative images of the tracked distances of individual zebrafish in a cell culture dish under different treatments. E) Percentage distance swum by zebrafish embryos when treated relative to the DMSO-treated control [46].
Image adapted from Ref. [46]

polymers form nanoparticles of around 50 nm in size in which the TMC is non-covalently embedded in the hydrophobic interior (Fig. 13B). These systems rely on the hydrophobicity of the TMC to retain the catalyst in the hydrophobic parts of the nanoparticles. Their results showed that the cationic nature of the polyzymes helped to transport a porphyrin based iron catalyst [Fe(TPP)]Cl **49** across a bacterial biofilm. When adding appropriately protected resorufin, the formation of fluorescent resorufin indicated that the protective group was cleaved, indicating that the Fe-catalyst remained active in the biofilm. Next, appropriately protected prodrugs of the antibacterial agents moxifloxacin and ciprofloxacin, were activated by the polyzyme, and a decreased viability of the biofilms was observed (Fig. 13C). As the polyzymes showed no acute cytotoxicity for fibroblast cells, which are mammalian cells, the authors continued with bioorthogonally activating prodrugs in the presence of HeLa cells[96]. Here, the Meggers Ru-catalyst [26] was formulated in the hydrophobic compartments of the nanoparticles. Approximately four Ru centers were present per nanoparticle, which showed a size of around 80 nm. The size of the polyzymes remained similar in 10% fetal bovine serum for at least 3 days, highlighting the protective capability of the polymer towards the embedded Ru-TMC. Ru-catalyzed deallylation of protected mitoxantrone, a cytotoxic drug, in the presence of HeLa cells showed a reduced cell viability, indicative of the release of the activated drug.

Our group started to explore the use of amphiphilic random heterocopolymers that fold into defined conformations in water as catalyst carriers in 2011 (Fig. 14A) [100]. Engineering the microstructure of the amphiphilic copolymer by balancing the ratio of hydrophilic, hydrophobic and hydrogen-bonding grafts, permitted single polymer chains to spontaneously form particles of nanometer size (5–20 nm), which are referred to as single chain polymeric nanoparticles (SCPNs) [101]. These particles comprise an inner hydrophobic compartment, and a Jeffamine-based hydrophilic periphery. Compared to the previously applied block copolymer NPs[17], these particles are much smaller and do not have a critical micelle concentration because they are unimolecular. Upon incorporating TMCs into the hydrophobic interior of the SCPNs [102,103], reactions proceeded at good rates and high lipophiloselectivity for Ru-based transfer hydrogenations and oxidations [103]. Hydrophobic substrates were converted at a much higher rate than hydrophilic substrates in competition reactions, indicating that the logP of the substrate plays an important role in enhancing the local concentration for high logP substrates, and hereby the reaction rate.

As a consequence of these promising early findings, our group started to explore TMC-based SCPNs for bioorthogonal catalysis [97]. In biological environments, both high turnover frequency (TOF) of the TMC as

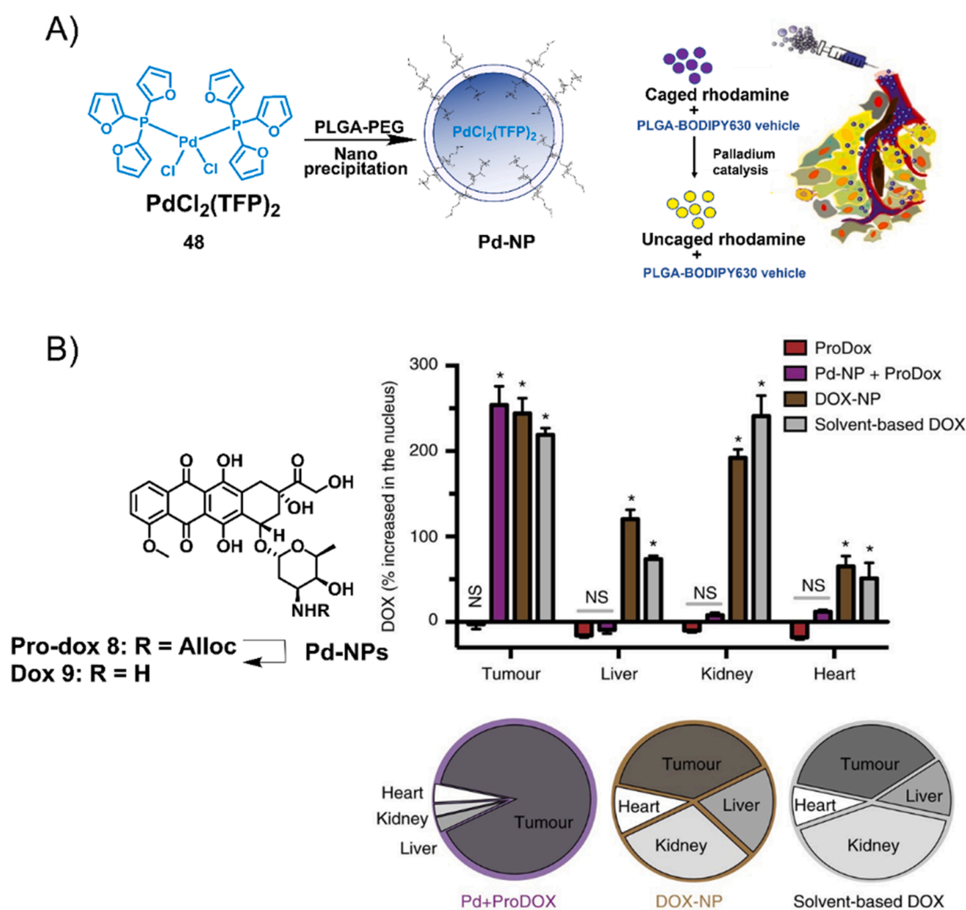

Fig. 12. A) Nanoencapsulation of Pd-NPs using PLGA-PEG surfactant, followed by intravenous administration into live xenograft cancer mice models for activation of caged rhodamine and pro-doxorubicin. B) Activation of pro-doxorubicin **8** using Pd-NPs leading to drug accumulation in tumor nuclei of ovarian tumors, but not in the nuclei of key organs related to toxicity. The tumors and organs were excised and imaged for colocalization between intrinsic doxorubicin fluorescence and DNA after 24 h, pie chart shows the accumulation of nuclear doxorubicin in ovarian tumors and key organs relating to toxicity.
Image reproduced with permission from Ref. [17].

well as high conformational stability of the SCPN are crucial to ensure good catalyst performance over time. Experiments using a covalently attached solvatochromic dye Nile Red indicated that the compartmentalized nature of the SCPNs remained intact in cellular growth media, in the cell's cytosol and, when an optimized microstructure was used, also inside lysosomal compartments of HeLa cells [105]. In addition, the Jeffamine-based SCPNs showed low toxicity and were taken up by endocytosis only slowly, requiring incubation times of 24 h. By attaching triphenylphosphine-, bipyridine-, or phenantroline-based ligands to the SCPNs, catalytic conversions such as Cu(I)-catalyzed click reactions and depropargylations [104,106], and Pd(II)-catalyzed depropargylations, proceeded fast in buffered aqueous media. Interestingly, the use of the dimethylpropargyl group rather than the commonly used propargyl group significantly accelerated the deprotection reactions rate when Cu(I) was used as the catalysts [104]. However, a reduction of activity was observed in the presence of the cell culture medium DMEM with 10% fetal bovine serum. Interestingly, adding HeLa cells did not further reduce activity, suggesting that the hydrophobic proteins in the serum likely represent the most deactivating factor for the Pd(II)- and Cu(I)-based SCPNs. This can be rationalized by two factors (or a combination of the two): (i) the sequestration of hydrophobic substrates by serum protein binding or (ii) the serum proteins inactivate the catalyst. The fact that the catalyst in DMEM reached a plateau before full conversion, indicated that the catalyst loses efficacy over time. Gratifyingly, the results also showed that a pro-rhodamine dye was still successfully converted into a fluorescent product by depropargylation when the SCPN-based catalysts were added to the cultured HeLa cells (Fig. 14B,C). Recent investigations into the details of Pd(II)-catalyzed depropargylations showed that the catalytic activity of nanoparticles in complex media can be enhanced by selecting the appropriate the ligand–metal complex and that the rate of the reaction increased for hydrophobic substrates [107]. Triphenylphosphine ligands showed higher activity than the bipyridine-based ligands. Deconstruction of the catalytic system revealed that covalent attachment of the ligand to the polymer backbone is necessary to retain catalytic activity in cell culture medium, as non-covalently embedded hydrophobic Pd-based catalysts were strongly deactivated. Promising results were obtained in the activation of anti-cancer pro-drugs based on 5-FU, paclitaxel, and doxorubicin, albeit that in the cell culture medium DMEM the success of the reaction depended on the type of protecting group and hydrophobicity of the prodrug.

The previously discussed SCPNs were based on amphiphilic polyacrylamide-based polymers with Jeffamine@ 1000 grafts to impart water solubility. In contrast, the group of Zimmerman focused on polynorbornene-based amphiphilic polymers made *via* Ru-catalyzed ring-opening metathesis polymerization (ROMP) with both water soluble imidazolium groups and aspartate-bearing grafts for Cu-binding (Fig. 15A) [48]. After the addition of Cu(II), particles with sizes < 10 nm were formed in water, which were referred to as Cu-crosslinked single-chain metal-organic nanoparticles (Cu-MONPs). By *in situ* reduction to Cu(I) using sodium ascorbate (NaAsc), the Cu-MONPs showed very high activities in azide-alkyne click reactions at ppm catalyst concentration, also in the presence of different cell types (Fig. 15 B,C) [48,108]. The rate of reaction was found to have a strong correlation with charge and hydrophobicity of the alkyne substrate, with more hydrophobic substrates and negatively charged substrates reacting faster. Interestingly, the alkyne substrate must enter the particles' interior in order for the reaction to occur, which corroborated that binding of the substrate to the particles interior was crucial for turnover. Recently, Bai and co-workers introduced a polymerization-crosslinking method to obtain polynorbornene-based dense shell nanoparticle scaffolds with the aim to embed the Cu-containing nanoparticles (NPs) in

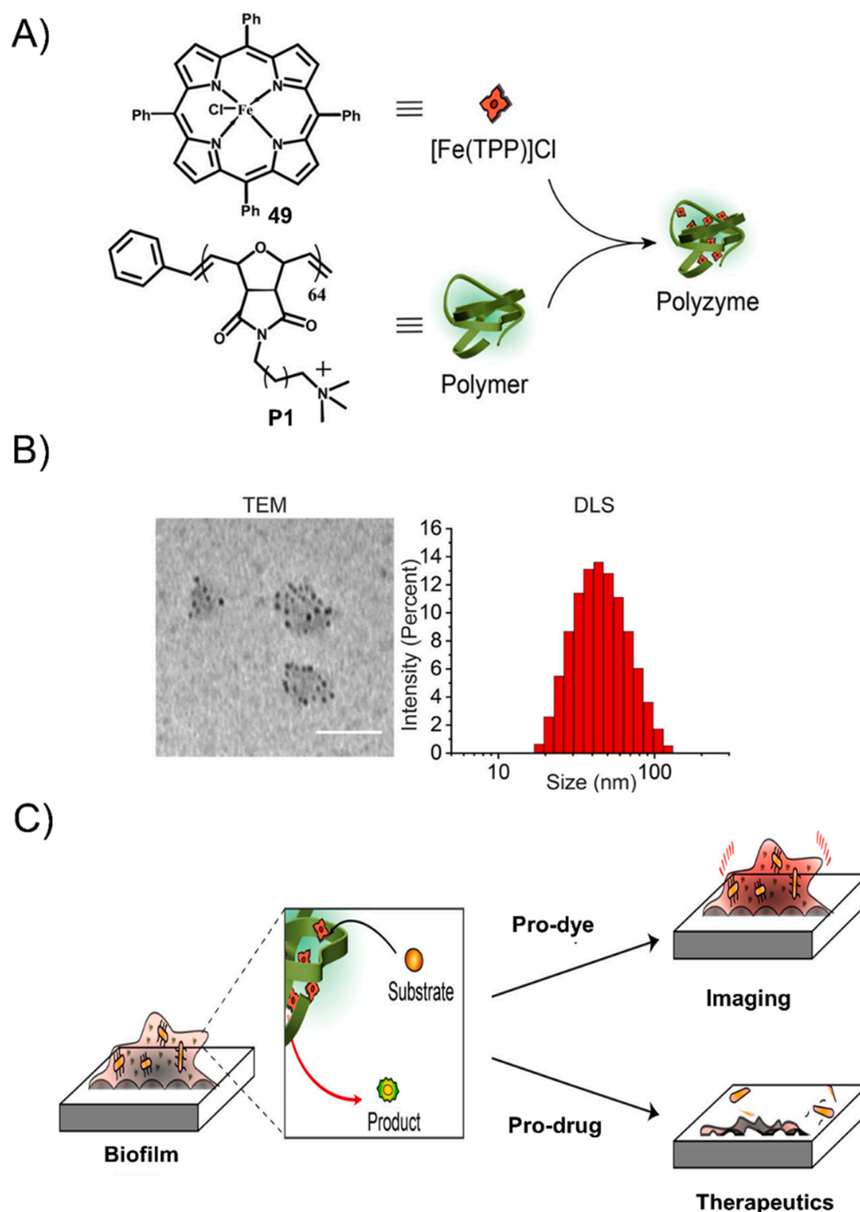

Fig. 13. A) Structure of Fe catalyst **49** and poly(oxanorborneneimides) polymer **P1** forming polyzyme. B) Size of polyzymes ~50 nm monitored by transmission electron microscopy and dynamic light scattering. C) Representation of the bioorthogonal activation by polyzymes for imaging and eradication of bacterial biofilms. Image reproduced with permission from Ref. [99].

the membrane of cells [109]. Such embedding was envisaged to reduce catalyst poisoning on the one hand but at the same time allow the synthesis of drug targets of low membrane permeability inside the membrane, hereby facilitating the uptake. A systematic investigation on the nature of the cationic group used for water-compatibility —phosphonium, imidazolium or ammonium— revealed a dramatic effect on the cytotoxicity of the NPs on mammalian NIH/3T3 cells, with the phosphonium-based NPs showing the best biocompatibility. In addition, a balance between water-soluble phosphonium and oligo-ethylene glycol pendants was necessary to retain the NPs in the membrane. Interestingly, different monomolecular and bimolecular reactions at low concentrations were possible as well the cell internalization of less-cell-permeable molecules by synthesizing them directly inside the membrane.

Zimmerman and co-workers also investigated intramolecularly crosslinked polyacrylamide-based Cu-SCNPs comprising a tris (triazolylmethyl)amine-based ligand for Cu-binding and cationically charged grafts for water solubility [110]. These particles showed very high activity for click reactions, and were also very efficient for click reactions on modified protein surfaces and cell surface glycans. The catalysis was proposed to occur through an "attach mode" where the SCNPs reversibly bind protein surfaces through multiple hydrophobic and electrostatic contacts. The polyacrylamide-based systems were further functionalized with light-activatable Ru-catalysts (Fig. 16A) [111]. The co-delivery of these Ru-SCPNs with β-galactosidase (β-gal) was possible *via* endosomal uptake into HeLa cells (Fig. 16B). An azide-containing doxorubicin derivative **57** and a galactose-masked combretastatin A4 **58** were chosen as the prodrugs, able to be activated *via* light-activated Ru-catalysis and β-gal induced galactose cleavage, respectively (Fig. 16C). After intracellular activation, both anticancer agents were released as evidenced by the significant reduction in cell viability. Further work [112] using neutral rather than cationically charged polymers and replacing Cu by Ru to afford Ru-SCNPs showed that also allylcarbamate cleavage reactions were

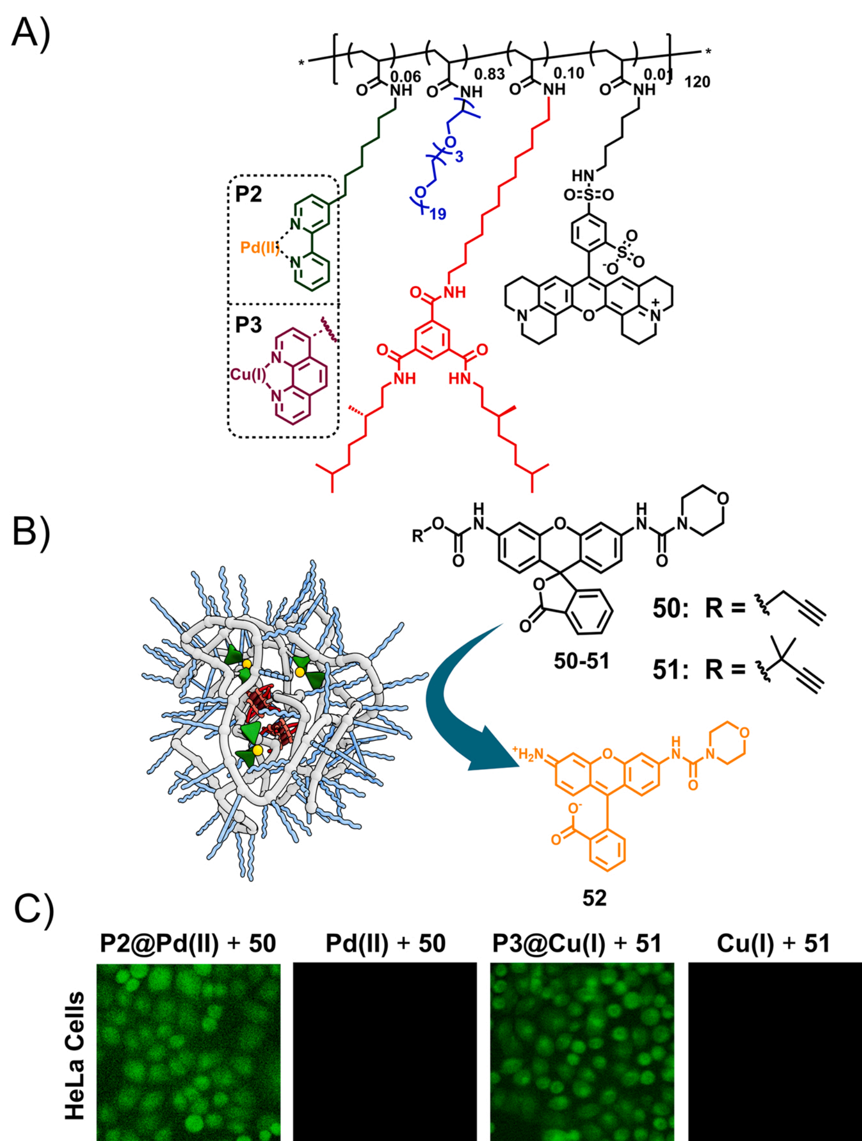

Fig. 14. A) Structure of polyacrylamide-based polymers developed by Palmans and co-workers for depropargylation reactions in biological settings [104]. B) Structure of protected rhodamines **50** and **51** for activation. C) Activation of pro-dye **50** and **51** in HeLa cells using Pd(II) and Cu(I) catalysts, respectively.

feasible in biologically relevant conditions using a modified Meggers-type Ru catalyst [43]. In the presence of DMEM and DMEM containing 5 mM glutathione, the Ru-SCNP showed decent conversions. In HeLa cell lysate, in contrast, lower initial rates and lower percent conversions were observed, a consequence of the complex environment of the cell lysate. The lower activity of Ru-SCNP in cell lysate was assigned to a reduced ability to bind substrates and due to unfolding of the polymer chain that results in enhanced interactions of the TMC with specific cellular biomacromolecules.

5. Conclusions and outlook

In 2006, the first example of bioorthogonal catalysis using a Ru-based homogeneous catalyst to activate a prodye in the presence of living cells was reported by Meggers and Streu [26]. This imaginative work sparked the interest of different scientific communities and functioned as a bridge between the traditional fields of homo-/heterogeneous catalysis and polymer science, with that of nanomedicine. Despite great progress and the Nobel prize in 2022 underlining the importance of the field of bioorthogonal chemistry, the number of examples of TMC-based catalysts that successfully function in *in vivo* models is still limited. This can be rationalized by the high degree of interdisciplinarity that is required between the fields of catalysis, carrier material development and medicinal chemistry. Progress in this area requires a number of issues to be addressed simultaneously rather than separately. First, the TMC, its carrier material, and the combination of the two should be non-toxic. While *in vitro* cell studies are a good start to establish toxicity effects, there are still only few studies that have been conducted in living systems and that have investigated long-term toxicity effects. Second, the TMC needs to show high activity, which is typically achieved by optimization of the ligand-metal complex. However, results obtained in organic media do not necessarily hold in aqueous media, and even less so in complex biological media. Third, long term stability of the catalyst is needed in the presence of competitive compounds that abound in biological media. As this is a consequence of the metal-ligand interaction, and since higher binding constants result in lower catalyst activity, the effects of ligand-metal binding strength on activity and stability typically require a compromise. Fourth, for *in vivo* prodrug activation or *in situ* drug formation for therapeutic applications, the TMC needs to accumulate or be present at a site of interest. While elegant approaches have been worked out for heterogeneous systems, such as localizing the catalysts *via* microsurgery

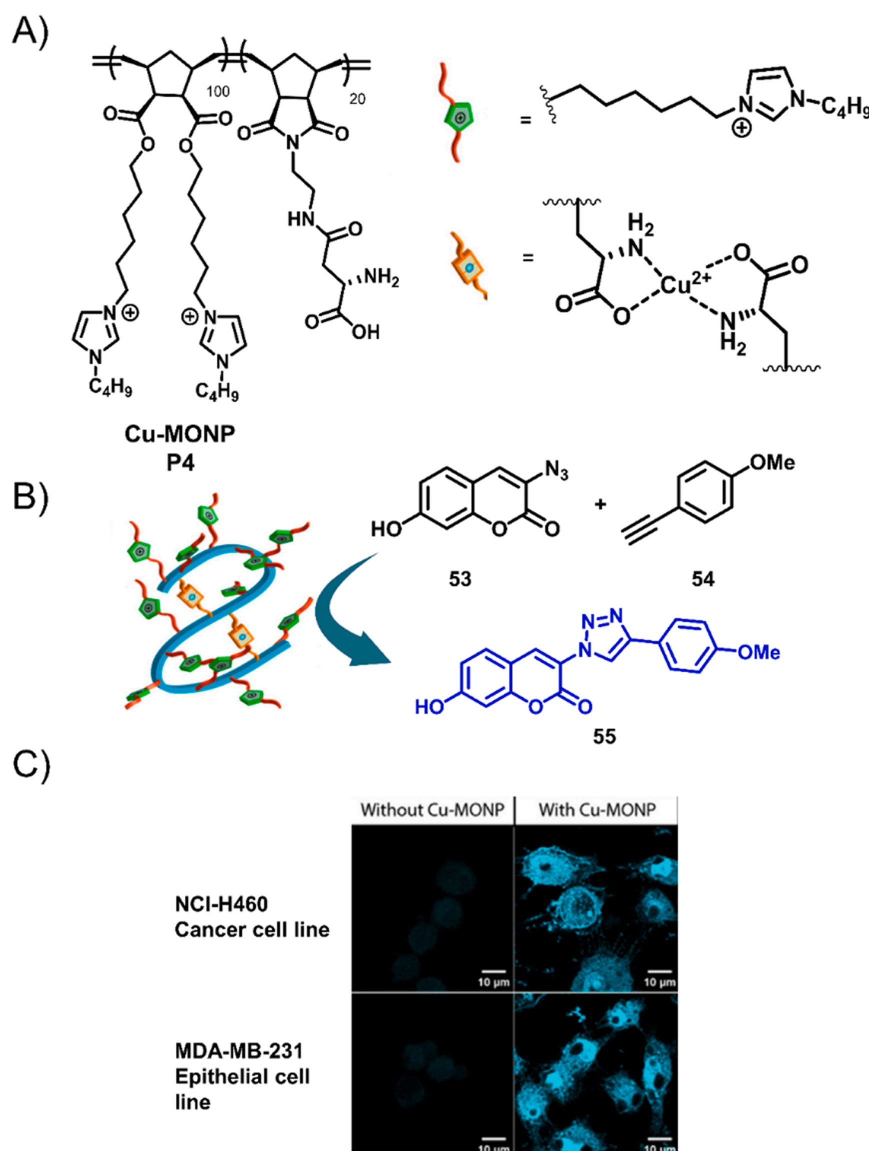

Fig. 15. A) Structure of polynorbornene-based polymer **P4** developed by Zimmerman and co-workers for intracellular click reactions. B) Fluorogenic click compounds used for reactions in living cells **53** and 54 C) Confocal microscopy study of intracellular catalysis of the click reaction between **53** and **54** in cancer and epithelial cell lines using Cu-MONP.
Image reproduced with permission from Ref. [48].

or the use of exosomes for Pd-nanoparticles, only very few non-invasive targeting approaches have been evaluated till now for homogeneous systems.

The use of amphiphilic polymeric carriers to which suitable ligands are covalently bound, can offer solutions and can help address some of the issues raised above. Many amphiphilic polymers are available that are non-toxic, and some are even FDA approved. In addition, amphiphilic polymers form compartmentalized nanostructures of tunable size, with hydrophobic regions, which comprise the TMC. The hydrophobic regions will help sequester hydrophobic substrates, and when these are in the vicinity of the TMC, the high local concentration of both substrates and catalyst will result in an increase of the reaction rates. Stably folded polymeric NPs that mimic the folding of polypeptides into enzymes, can likely also help protect the TMC by shielding it from interactions with competitive compounds, hereby enhancing stability. From the nanomedicine field, a vast knowledge is available on desired sizes and shapes of polymeric carrier materials. In addition, synthetic polymers can be easily functionalized with selective targeting groups that can assist in guiding the TMC-based NPs to the site of interest. Despite the current debate on the EPR effect [113] and the challenges associated with targeted nanoparticle delivery to tumor sites [114], combined with the vast differences that exist between different types of tumors, advances in the field of nanomedicine will be beneficial to help design suitable carriers for TMCs [115]. All in all, the versatility of synthetic polymers, and the ability to control their microstructure and hereby the superstructures they form, make homogeneous polymer-based TMCs highly complementary to heterogeneous TMC-NP systems and protein-based carrier systems. A further integration of the fields of polymer chemistry, homogeneous catalysis and nanomedicine will undoubtedly provide new opportunities for discoveries and advancements in the exciting field of bioorthogonal catalysis for therapeutic applications.

CRediT authorship contribution statement

All authors contributed to the writing of the manuscript, TL wrote the introduction and critically read the whole manuscript, LLD wrote the part on natural scaffolds for TMC based catalysis, AP wrote the abstract,

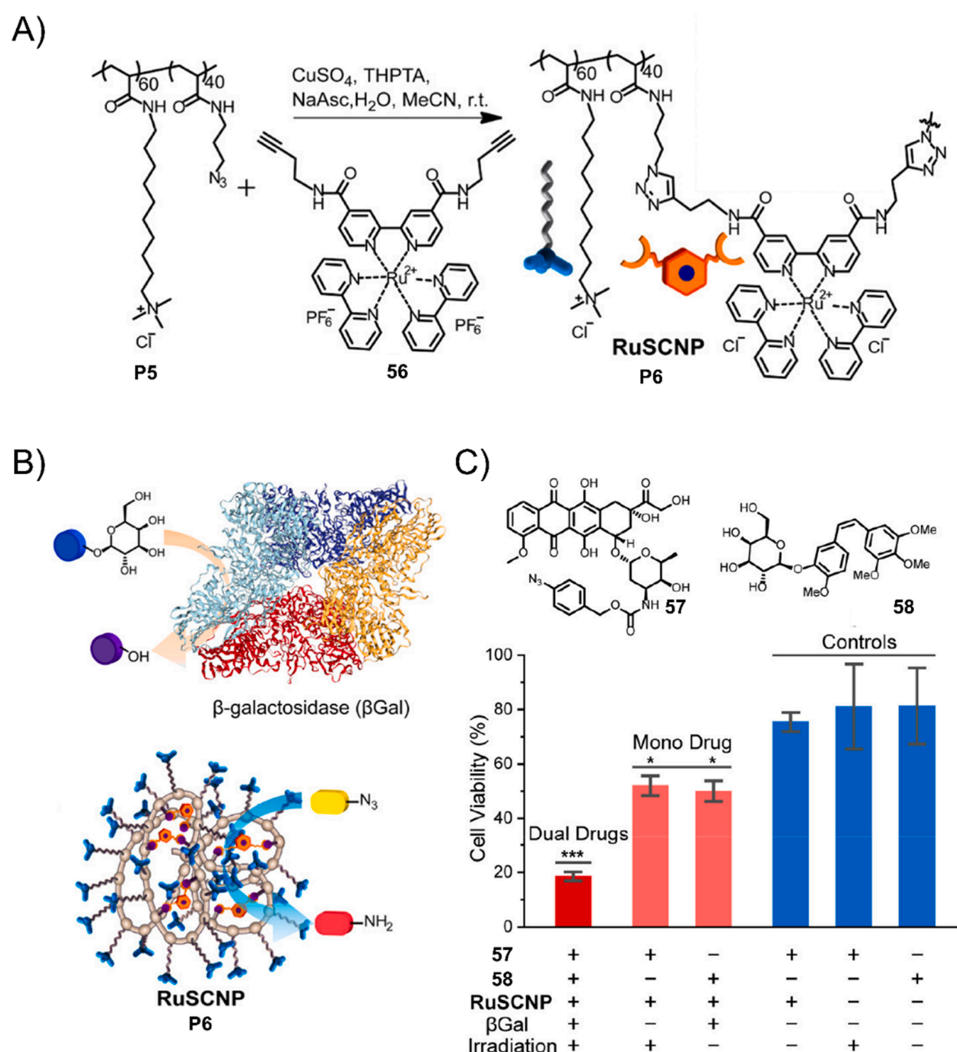

Fig. 16. A) Synthesis of RuSCNP using Ru catalyst **56**. B) Illustration of dual catalysis performed by β-galactosidase and RuSCNP. C) Intracellular dual drug activation of drugs **57** and **58** in HeLa cells monitored by cell viability assay by performed by β-galactosidase and RuSCNP.
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conclusions and homogeneous polymer scaffolds for TMC based catalysis, AS wrote the other parts. AS and LLD made the images for the review, and contributed equally.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests. Anjana Sathyan and Linlin Deng reports financial support was provided by Horizon Europe.

Data Availability

No data was used for the research described in the article.

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