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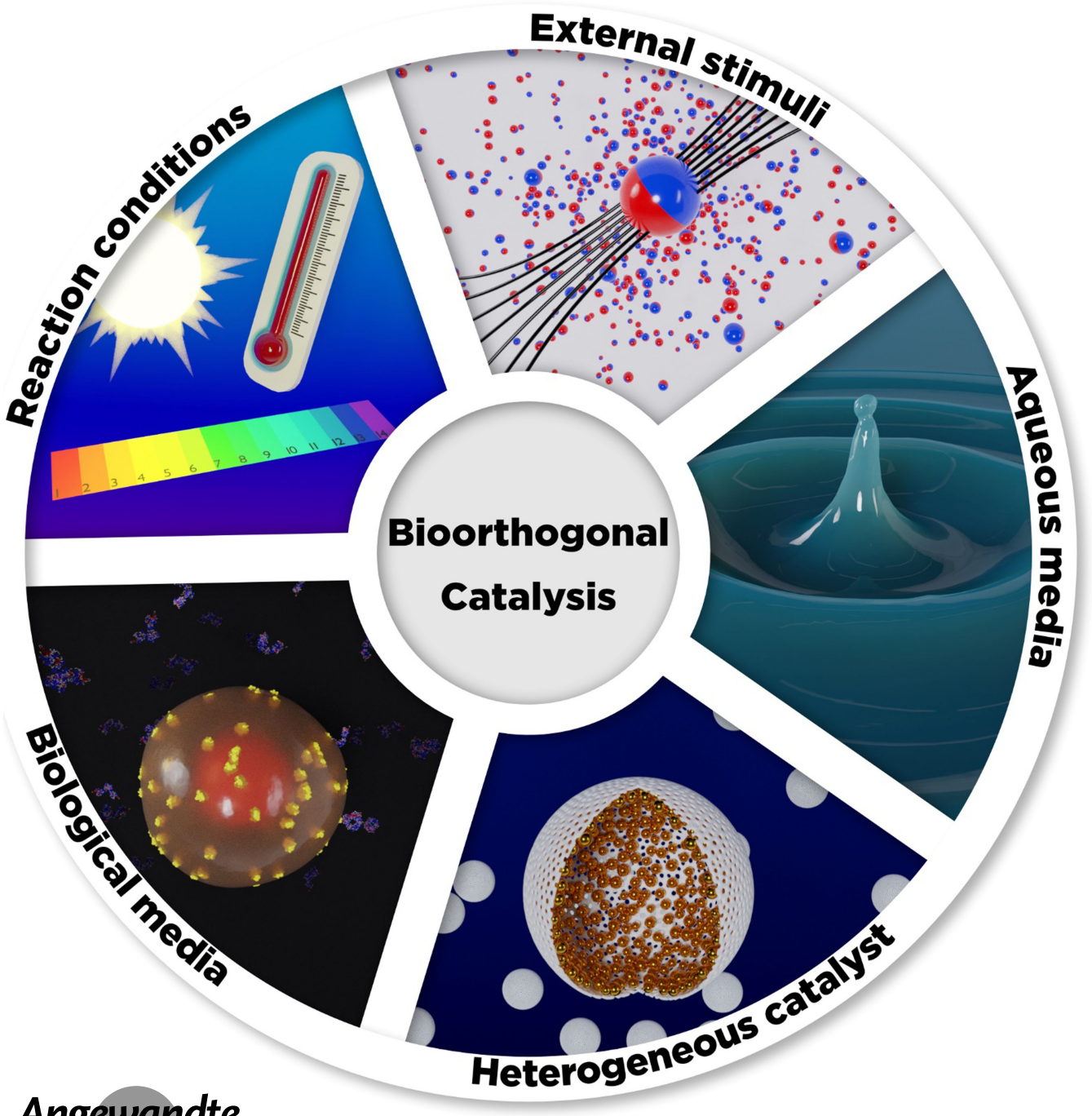
Bioorthogonal Chemistry

Nanostructured Heterogeneous Catalysts for Bioorthogonal Reactions

Ana Sousa-Castillo[+], *Andrea Mariño-López*[+], *Begoña Puértolas,* and Miguel A. Correa-Duarte**



Abstract: Bioorthogonal chemistry has inspired a new subarea of chemistry providing a powerful tool to perform novel biocompatible chemospecific reactions in living systems. Following the premise that they do not interfere with biological functions, bioorthogonal reactions are increasingly applied in biomedical research, particularly with respect to genetic encoding systems, fluorogenic reactions for bioimaging, and cancer therapy. This Minireview compiles recent advances in the use of heterogeneous catalysts for bioorthogonal reactions. The synthetic strategies of Pd-, Au-, and Cu-based materials, their applicability in the activation of caged fluorophores and prodrugs, and the possibilities of using external stimuli to release therapeutic substances at a specific location in a diseased tissue are discussed. Finally, we highlight frontiers in the field, identifying challenges, and propose directions for future development in this emerging field.

1. Introduction

In recent years, tremendous efforts have been focused to combine bio- and heterogeneous catalysis through the development of improved tools that enable rational catalyst design in complex biological media. The term bioorthogonality was first defined in the early 2000s, by Bertozzi and coworkers, and began with a precise definition in a very limited field.[1,2] Bioorthogonal chemistry refers to any chemical transformation that occurs in a selective and efficient manner within the complex biological environment of a cell, without interfering with native biochemical processes.[3,4] It has emerged as a prominent tool in chemical biology and has facilitated the study and rational modification of different biological processes. Such reactions are designed to monitor or modulate physiological and pathological processes and can be mediated by two or more mutually reactive chemical groups or in combination with non-biotic transition metals (e.g., Cu, Au, or Pd).[5,6] Since the coining of the term, the applicability of bioorthogonal reactions has largely broadened[7] and this concept has been used in many different fields including nanosensors, imaging, detection, diagnosis, drug delivery, and catalysis, among others.[8–12] Ideally, such bioorthogonal reactions must fulfill a number of requirements: 1) They must proceed in aqueous media; 2) they must happen at near physiological pH; 3) the reactions should be rapid at room temperature (or up to 37 °C); 4) they should involve low reactant concentrations, and 5) they should proceed in high yields.[13] With a plethora of modern catalysis and aqueous chemoselective reactions, this term has led to the synthesis of biomolecules (e.g., peptides),[14] synthetic polymers (e.g., polyethylene glycol),[15] and small molecules such as ligands (e.g., biotin),[16] drugs, and prodrugs,[17,18] and fluorescent dyes,[11] among a multitude of other possibilities. However, there are currently very few bioorthogonal reactions that are able to meet all requirements simultaneously.

Bioorthogonal reactions can be conducted using homogeneous, heterogeneous, or artificial enzymatic catalysis.[19] Homogeneous catalysts are generally soluble molecular or ionic compounds that coexist with the reactants in the same phase. The accessibility to the active sites and the easy tuning of the functional groups to provide chemo-, regio-, and enantioselectivity[20–23] result in good catalytic activities[24–26] that have been exploited in a number of industrial applications. Despite all the advantages, homogeneous catalysts face limitations in terms of stability and degradation under certain biological media. To overcome these limitations, the use of heterogeneous catalysts involving the immobilization of the active phase within (encapsulated catalyst) or over the surface (supported metal catalyst) of suitable materials offer the ease of handling, efficient recovery, enhanced stability and lifetime, and reusability. Nevertheless, as the reactants and the catalyst are in two different phases, typically in liquid and solid phases, respectively, selectivity issues and/or the leaching of the active phase into the solution[27] might compromise the overall catalytic performance. The thorough design of the catalyst can minimize these drawbacks.[19]

The advances in nanostructured catalytic materials in the past years provide a new vision for nanoscience-inspired design, synthesis, and formulation of efficient catalytic materials. Due to their small size, catalytically active nanoparticles have higher surface areas and therefore, increased exposed active sites which favor the interaction between the reactants and the surface of the catalysts.[28,29] Nanoparticles and nanostructured materials have traditionally played a critical role in the effectiveness of catalytic materials, but the precise control of the composition, surface structure, facet exposition, and chemical reactivity at the active sites is a grand challenge for the development of more efficient catalytic materials. Exciting advances have recently occurred in the synthesis of small metal and metal oxide nanoparticles with controlled size, shape, and specific surface orientations supported on appropriate host matrices or encapsulated that have demonstrated superior performance in targeted reactions.[30–34] These advances set the foundations for the rational design of catalysts with the specific surface and interface structures that are required for structure-sensitive catalytic reactions. Additionally, to fulfill the principles of bioorthogonal chemistry, the catalysts have to demonstrate functional compatibility with the physiological conditions, absence of toxicity, and biostability in complex environs,

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enabling their exploitation as extracellular or intracellular factories of bioactive agents.

Van de L'Isle et al. previously reported both homogeneous (e.g., organometallic complexes) and heterogeneous catalysts (e.g., nanodevices, such as metal–organic frameworks (MOFs) and nanozymes) for different bioorthogonal reactions.[35] In this Minireview, we focus on the applicability of heterogeneous catalysts consisting of supported or encapsulated metallic nanoparticles in three types of bioorthogonal reactions. We compile the progress of strategies to bioorthogonal reactions with particular emphasis on the heterogeneous catalysts reported in the literature in recent years. We highlight the emerging concepts and the possibilities of using nanostructured materials for the sustainable synthesis of organic compounds in living cells. We also outline hurdles that must be cleared if these transformations are to undergo practical implementation.

2. Bioorthogonal Reactions using Heterogeneous Catalysts

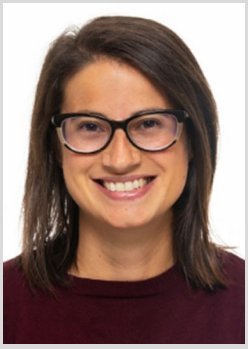
During the last years, different heterogeneous catalysts, mostly based on Pd, Au, and Cu were developed in order to catalyze a series of bioorthogonal reactions, such as fluorophore activation, anticancer drug synthesis via Suzuki–Miyaura cross-coupling, and prodrug activation via depropargylation reactions[6,36] (Table 1). In Section 2.1, we will first compile the studies related to Pd-based catalysts; Au- and Cu-based materials will be covered in Section 2.2; and finally, Section 3 is devoted to discussing recent developments in which Pd- and Au-containing catalysts are activated towards the desired transformation using external agents such as light or an alternating magnetic field.

2.1. Pd-Based Heterogeneous Catalysts

Palladium chemistry has been intensively studied, demonstrating its high catalytic efficiency and vast possibilities and applications in bioorthogonal reactions. Different approaches have been applied to stabilize the active phase, including the use of polymer- and silica-based supports, and the core–shell encapsulation into both organic and inorganic shells. All these strategies are described in this section.

2.1.1. Supported Pd Catalysts

The applicability of Pd-based heterogeneous catalysts in bioorthogonal catalysis was reported for the first time more than a decade ago by Yusop et al.[37] The catalyst consisted of Pd nanoparticles (PdNP) entrapped within polystyrene microspheres that are able to enter and synthesize exogenous materials in cells.[37-39] The catalytic activity of these structures was tested in the deprotection of the allyloxycarbonyl group of the bis-*N,N′*-allyloxycarbonyl Rh110, resulting in the liberation of the strongly fluorescent Rh110 in biological conditions. Additionally, they demonstrated the



Ana Sousa-Castillo completed her PhD in 2017 at the University of Vigo (Spain). In 2019 she moved to Munich (Germany) and joined Prof. Cortés laboratories at the LMU with a postdoctoral fellowship from Xunta de Galicia, Spain. She worked there for two years on colloidal synthesis and the characterization of plasmonic metal nanohybrids for photocatalysis. In April 2022 she returned to the group of Prof. Correa-Duarte. Her research interest focuses on the design of novel plasmonic hybrid nanoreactors for photocatalysis applications.

Andrea Mariño López obtained her BSc degree in Chemistry from Universidade de Vigo (Spain) in 2016, and her MSc degree in Molecular Nanoscience and Nanotechnology from Instituto de Ciencia de Materiales de Madrid-CSIC (Spain) in 2017. She is currently a PhD candidate at TeamNanoTech under the supervision of Prof. Miguel Á. Correa-Duarte and Prof. Moisés Pérez-Lorenzo. Her research interests focus on the fabrication and characterization of plasmonic platforms for applications in sensing and catalysis.

Begoña Puértolas is a distinguished researcher at the University of Vigo (Spain). She completed her PhD at ICB-CSIC (Spain) and then conducted postdoctoral research at ETH Zurich under the direction of Prof. Pérez-Ramírez from 2014 to 2020. Her current research interests focus on the development of chemocatalytic technologies for more sustainable chemical processes. This primarily comprises the design of heterogeneous catalysts combining state-of-the-art characterization techniques with catalytic tests and molecular modeling.

Miguel A. Correa-Duarte is Director of the Biomedical Research Center (CINBIO) and Associate Professor at the University of Vigo (Spain). He completed his PhD degree at the University of Vigo (Spain), then he worked as a postdoctoral researcher at the Center of European Advanced Studies and Research (Germany), and as a Visiting Assistant Research Professor at the Arizona State University (USA). His research interests include the synthesis and functionalization of nanomaterials, including core–shell nanohybrids, carbon nanotube nanocomposites, nanoreactors, and multifunctional nanomaterials for applications in sensing and catalysis.

Table 1: Summary of the reported bioorthogonal reactions using heterogeneous catalysts. The asterisk (*) refers to a number of organic molecules without a common nomenclature.

	Reaction	Product	Catalyst	References
Fluorophore activation	Carbamate cleavage	Rhodamine 110 (Rh110)	Supported Pd catalyst	[6, 37, 38, 61]
			Encapsulated Pd catalyst	[55]
	Dealkylation		Supported Pd catalyst	[36, 43, 46]
			Pd–Ti catalyst	[50]
			Au catalyst	[59]
			Au–Ti catalyst	[63]
			Fe–Au/Pd/Pt catalyst	[62]
		Resorufin	Encapsulated Pd catalyst	[53]
		*	Pd-loaded exosomes	[52]
		Phenol derivatives	Encapsulated Pd catalyst	[54]
	Oxidation	Rhodamine 123 (Rh123)	Au–Ti catalyst	[63]
	Carbamate cleavage	Coumarin	Supported Pd catalyst	[61]
			Cu catalyst	[53]
	Azide–alkyne cycloaddition	*	Cu catalyst	[5]
		*	Cu catalyst	[58]
	Alkynyl carbocyclization	*	Encapsulated Pd catalyst	[65]
Drug synthesis	Suzuki–Miyaura cross-coupling	*	Supported Pd catalyst	[37]
		PP-121	Supported Pd catalyst	[43, 44]
		BODIPY dye	Supported Pd catalyst	[43]
		*	Supported Pd catalyst	[61]
		4-(thiophen-2-yl)benzaldehyde	Encapsulated Pd catalyst	[52]
Prodrug activation	Carbamate cleavage	Amsacrine	Supported Pd catalyst	[37]
		Gemcitabine	Supported Pd catalyst	[6, 41]
		Doxorubicin (DOX)	Supported Pd catalyst	[48]
	Dealkylation	Vorinostat	Supported Pd catalyst	[42]
			Pd–Ti catalyst	[50]
			Au catalyst	[59]
		5-fluorouracil (5-FU)	Supported Pd catalyst	[36, 41, 44, 45, 61]
			Fe–Pd catalyst	[64]
			Au catalyst	[59]
		SN-38	Supported Pd catalyst	[45]
		DOX	Supported Pd catalyst	[46]
			Encapsulated Pd catalyst	[51]
			Au catalyst	[59]
		Monomethyl auristatin E (MMAE)	Encapsulated Pd catalyst	[51]
		Panobinostat	Encapsulated Pd catalyst	[53]
		4-hydroxytamoxifen (4-OHT)	Encapsulated Pd catalyst	[55]
	Azide–alkyne cycloaddition	*	Cu catalyst	[53]
		Reveratrol analogue	Cu catalyst	[5]
		*	Cu catalyst	[57]
	Hydrogenation reaction	Ibuprofen	Supported Pd catalyst	[49]

activity of this catalyst in the Suzuki–Miyaura cross-coupling reaction within living cells, using HeLa cells as a model system. After this work, PdNP immobilized in polymeric resins, most of them based on a procedure previously

developed in the Bradley group,[40] were used as efficient heterogeneous catalysts for several bioorthogonal reactions. Using these materials, different prodrugs were developed in order to be activated by Pd-mediated bioorthogonal reactions. This spatially targeted masking/activation strategy would serve to expand the use of potent cytotoxic drugs, such as 5-FU, which has a long history in oncology practice but whose clinical activity is limited due to safety concerns. In 2014, Weiss et al., demonstrated that alkylation of the N1 position of 5-FU suppresses the cytotoxic properties of the drug.[36] The obtained propargyl derivative, 5-fluoro-1-propargyl-uracil (Pro-5FU), displays excellent stability in cell culture and high sensitivity to Pd^0-mediated catalysis, allowing the bioorthogonal generation of 5-FU under biocompatible conditions (Figure 1a). It was demonstrated that Pro-5FU, in the presence of Pd^0-resins, exhibits antiproliferative properties equivalent to unmodified 5-FU in colorectal and pancreatic cancer cells. They also reported the bioorthogonal generation of cytotoxic gemcitabine, which is an antimetabolite antineoplastic agent widely employed to treat several difficult-to-cure cancerous processes, from the combined treatment of extracellular Pd^0-resins and the propargyl (*N*-Poc)-protected precursor in pancreatic cancer cell culture (Figure 1d).[6] For the development of the prodrug, different carbamate derivatives were studied; it was found that *N*-Poc group exhibited higher sensitivity than allyl (*N*-alloc) or benzyl carbamate (*N*-Cbz) groups to Pd-mediated cleavage in biological conditions. This was also demonstrated for the activation of carbamate-protected Rh110 in zebrafish. Another example of Pd^0-resins catalyzing *N*-dealkylation processes for the activation of a prodrug was described one year later.[41] The propargylation of the N3 position of floxuridine (FUdR) gave rise to a biochemically stable derivative (Pro-FUdR) which had lower pharmacological activity than the drug. After incubation in cell media, the combination of Pro-FUdR with Pd^0-resins

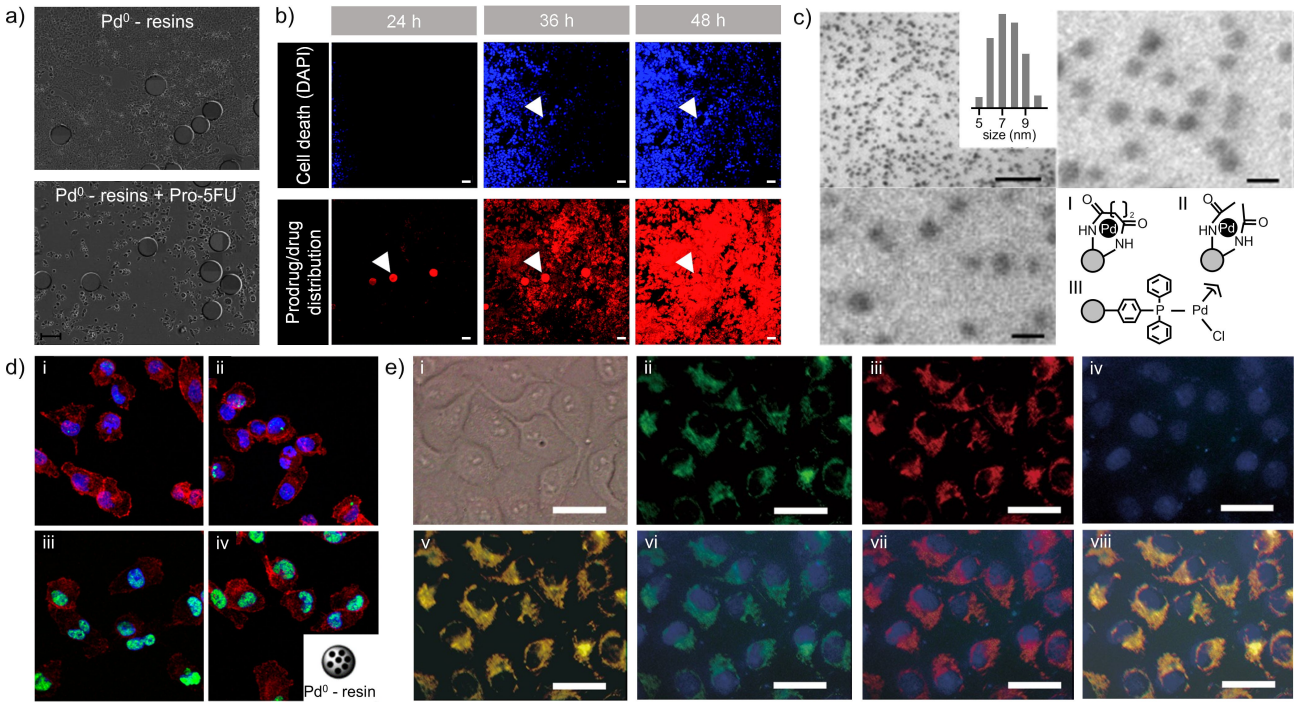


Figure 1. a) Phase-contrast images of cells after 4 days of treatment. Pd^0-resins are identified as spheres of 150 mm. Scale bar: 150 mm. b) Live-cell imaging study of the Pd-mediated conversion of inactive prodrug into cytotoxic drug in a human prostate tumor explant model bearing 21 day-in-tumor after treatment with the prodrug in the presence of 4′,6-diamidino-2-fenilindol (DAPI) for 24, 36, and 48 h. The presence of the Pd-based catalysts, indicated with white arrows, is identified by a bright fluorescence signal in the red channel. Scale bar: 30 μm. c) TEM images of cross-sectional resin beads of (first row) XL-RC Pd (cross-linked resin-captured Pd) magnified by approximately 5×10^4 (scale bar: 100 nm) and size distribution of PdNP, and original XL-RC Pd; (second row) XL-RC Pd after six reuses magnified by approximately 3×10^6 (scale bar: 10 nm) and the different Pd supported catalysts investigated. d) Merge fluorescent images of Mia PaCa-2 cells 24 h after treatment with (i) 0.67 mg cm^{-3} Pd^0-resin (negative control); (ii) prodrug (300 nM, negative control); (iii) gemcitabine (300 nM, positive control); and (iv) 0.67 mg cm^{-3} of Pd^0-resin + prodrug (300 nM, BOOM activation assay). The inset in (iv) corresponds to a scheme of the catalyst used in this study. Fluorescent labels: Hoechst 33342 for cell nuclei (blue), Alexa Fluor 594 phalloidin for F-actin (red), and anti-phospho-histone γ-H2AX + Alexa Fluor 488 secondary antibody for phosphorylated γ-H2AX (green). e) Collection of the fluorescence images of the Pd-based catalyst catalyzing the Suzuki–Miyaura coupling reaction with UV light activation: (i) bright-field image of HeLa cells; (ii) emission of the fluorescent probe (green channel); (iii) the mitochondrion labeled with MitoTracker®Red CMXRos (red channel); (iv) the cell nucleus labeled with Hoechst 33258 (blue channel); (v) merging with (ii) and (iii); (vi) merging with (ii) and (iv); (vii) merging with (iii) and (iv); (viii) merging with (ii), (iii), and (iv) (scale bar = 10 μm). Reprinted with permission from (a) Jason T. Weiss et al., [36] Copyright 2014, (b) Thomas L. Bray et al.,[46] Copyright 2018 Royal Society of Chemistry, c) Jin Ku Cho et al.,[40] Copyright 2006 American Chemical Society, (d) Jason T. Weiss et al.,[6] Copyright 2014 American Chemical Society, (e) Faming Wang et al.,[61] Copyright 2018 Springer Nature.

led to a cytotoxic effect similar to that seen in cells incubated with unmodified FUdR, demonstrating the efficacy of the deactivation strategy and the rapid reaction kinetics of the Pd-mediated *N*-depropargylation process. Moreover, a new bioorthogonal prodrug of suberoylanilide hydroxamic acid (SAHA) described as pharmacologically inactive and metabolically stable was designed.[42] This drug, aka vorinostat, was the first histone deacetylases inhibitor to be approved by the U.S. Food and Drug Administration (FDA) to treat cutaneous T-cell lymphoma. However, its dose-limiting side effect and poor pharmacokinetic properties stimulated the development of an inactive precursor of this drug which was obtained by alkylation of the OH group of the hydroxamic acid. This inactive precursor was efficiently uncaged by Pd-functionalized resins in cell culture models of glioma and lung cancer.

Using polymer-supported PdNP, Indrigo et al., reported the first example of in situ drug synthesis by a cross-coupling reaction.[43] The anticancer agent PP-121 was synthesized from two benign precursors, iodopyrazole and a boronic ester, by C–C bond formation via a Suzuki–Miyaura cross-coupling reaction. The catalyst consists of biologically compatible Pd^0 nanoparticles trapped within a physical polymer framework, formed by the generation of modular sintered aminomethyl polystyrene resin beads. The catalytic activity of this structure was first confirmed by the decaging of bis-propargyloxycarbonyl (Proc) Rh110 in a biological medium, which was subsequently applied in the synthesis of PP-121. In the latter case, the authors showed that the conversion of Pro-5FU to toxic 5-FU occurs simultaneously under biological conditions.[44] The concurrent generation of two anticancer drugs via two different Pd-catalyzed reactions, i.e., in situ synthesis and decaging, enhances the therapeutic effect of the drugs. For that purpose, they developed a catalyst that consisted of PdNP-functionalized fluorescent microspheres decorated with the cyclic-RGD cancer targeting functionality (cRGDfE-PdNP), which demonstrated rapid and selective uptake in glioblastoma cells. One year later, Adam et al., also showed the occurrence of two reactions taking place at the same time under biological conditions, but in this case, they were the first to report the concomitant uncaging of two clinically approved drugs by the same bioorthogonal method.[45] The anticancer properties of this compound were restored in the presence of a heterogeneous Pd-based catalyst, consisting of PdNP supported on an amino resin, to kill colorectal cancer and glioma cells, proving the efficacy of this novel masking strategy for aromatic hydroxyls. This prodrug was also tested in combination with the Pro-5FU, previously synthetized by the same group,[46] demonstrating that both drugs could be released simultaneously by the same bioorthogonal triggering mechanism.

In the context of the development of new prodrugs that are activated through a bioorthogonal Pd-mediated bond-cleavage reaction, different prodrugs of DOX were synthesized by masking the NH_2 group as a carbamate group.[46] Different cytotoxicity studies were performed and the compound with the highest therapeutic window was tested by vivo cardiotoxicity and assay on the heart of developing zebrafish, showing clinically relevant result for a DOX prodrug for the first time in this animal model. The authors also proposed the surgical implantation of a polymer-based system functionalized with PdNP as a catalyst for drug conversion and release at the disease site minimizing adverse effects in distant organs and tissues. After the optimization of the size and performance of this material, they tested its catalytic capacity to generate fluorescent Rh110 upon Pd-mediated O-depropargylation of non-fluorescent bis-*N*,*N*′-(propargyloxycarbonyl) Rh110 under cell culture conditions. The optimized catalyst showed adequate biocompatibility and stability in living tissue. In addition, it demonstrated high echogenicity, thus enabling precise injection into a tumor via ultrasound-guided intratumoral insertion. Ex vivo tests showed the catalyst to be non-perturbing to the mice, and resilient and biocompatible at the tumor location. More recently, the same group has reported the development of a palladium-activated 5-FU prodrug that is completely innocuous and increases the safety profile of 5-FU chemotherapy regimens. i.e., the anabolic activation and catabolic processing metabolization routes.[47] The prodrug was locked in a lactim-type structure by alkylating the O atoms of 5-FU and was subjected to in vivo pharmokinetic analysis, which showed the prodrug is rapidly and completely absorbed after oral administration and exhibits a longer half-life than 5-FU, thereby improving the drug metabolism and pharmacokinetics properties relative to 5-FU. They also showed for the first time that an inactive drug precursor can be administered orally and locally converted to active drugs inside a tumor xenograft by intratumorally inserted Pd catalysts, overall demonstrating that bioorthogonal chemistry can be used to improve both tolerability and pharmokinetic issues of approved therapeutics. On a different note, Chen et al., reported the preparation of ultrathin TiO_2 nanosheets that are employed as a support for depositing Pd catalysts. They demonstrated the catalytic efficacy of this device in the activation of a prodrug, alloc-DOX, at a tumor site in a spatiotemporally controlled manner.[48] Nowadays, this type of nanocatalyst has even been used for the in situ synthesis of chiral compounds. Du et al., presented a Pd catalyst, consisting of chirally modified PdNP immobilized on large-pore mesoporous silica particles, which catalyzed an asymmetric transfer hydrogenation reaction for chiral ibuprofen synthesis.[49]

Strategies to boost the activity of Pd in certain reactions have been devised by introducing additional transition metals such as Ti. In this direction, a drug-eluting system that can be implanted inside tumors to release cytotoxic agents only at the disease tissue was developed by Rubio-Ruiz.[50] It consisted of a Ti substrate coated with metallic Pd that was prepared by the magnetron sputtering method and demonstrated high catalytic efficiency for the uncaging of a prodye to generate fluorescent Rh110 upon Pd-mediated O depropargylation. The bioorthogonal uncaging of an anticancer drug, in this case *O*-(4-propagyloxybenzyl)suberoylanilidehydroxamate (POB-SAHA), which was previously designed by Rubio-Ruiz et al.,[42] was also studied. The results indicated no cytotoxicity induced by the Ti-[Pd] devices and the in situ uncaging of POB-SAHA by the

catalyst showed a drop of the cell viability similar to that obtained by direct treatment with the active drug SAHA.

2.1.2. Encapsulated Pd Catalysts

The developed bioorthogonal catalysts demonstrated high reactivity and efficient activation of caged compounds in living systems. However, they typically suffer from biocompatibility, stability, or toxicity issues. To alleviate these issues, encapsulated Pd catalysts have been synthesized and used for different in vitro experiments, as shown in Figure 2. Miller et al., designed a catalyst using PdNP based on the polymeric micellar encapsulation of bis[tri(2-furyl)phosphine]palladium(II) dichloride ($PdCl_2(TFP)_2$) following a bioorthogonal cleavage strategy that consisted of coupling Pd-catalyzed deallylation with a self-immolative linker.[51] This linker has been functionalized with 1) a nanoencapsulation anchor, 2) the caged drug payload, and 3) a bioorthogonally cleavable protective group (allyloxycarbonyl) that is removed upon exposure to a triggering agent. The aliphatic C16 chain that forms the anchor enables a nanoencapsulation efficiency of the prodrug higher than 90 % with acceptable size and polydispersity along with a high cytotoxicity upon prodrug activation. The bioorthogonal activating agent leads to drug uncaging and activation in a controlled manner. The researchers applied this strategy to two model anticancer drugs, i.e., MMAE and DOX, which contain primary amines that are critical for their drug activity. Upon encapsulation into nanoparticles, it was demonstrated their ability to kill cancer cells only after selective activation by PdNP (Figure 2a). Another encapsulation strategy was developed by Destito et al.,[52] who synthesized a biocompatible Pd-based nanoreactor consisting of hollow mesoporous silica nanoshells with an inner layer of PdNP. The thickness and porosity of the nanoshells was tuned in order to allow an effective flow of low-molecular-weight reactants and products, while avoiding the leakage of metal nanoparticles and the entrance of large biomolecules that could passivate the catalytic surface. The resulting catalyst exhibits high yields in O-depropargylation and Suzuki–Miyaura cross-coupling reactions in water and at physiologically compatible temperatures, thus avoiding the use of organic solvents and high temperatures typically applied in this type of reaction. Furthermore, these nanoreactors were tested in cell culture medium and also in the presence of Vero mammalian living cells, demonstrating a higher biocompatibility than state-of-the-art homogeneous

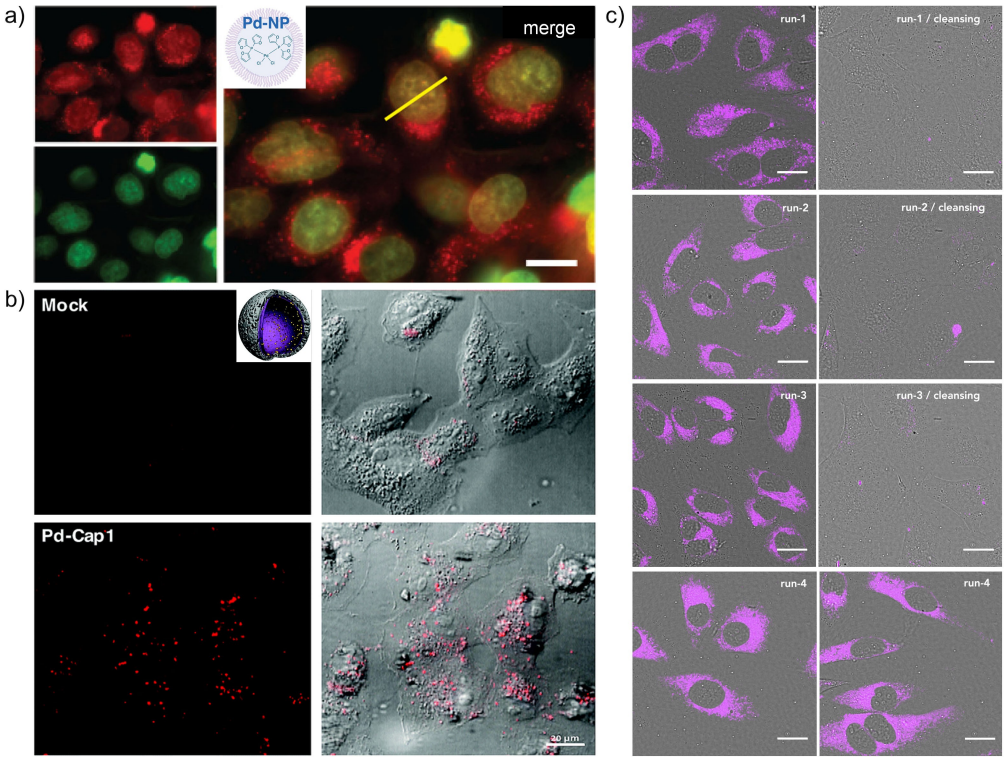


Figure 2. a) Fluorescence microscopy of HT1080 tumor cells treated with PdNP with C16proDOX for 24 h (scale bar = 20 µm). The inset corresponds to a scheme of the catalyst used in this study. b) Micrographs of Vero cells incubated for 30 min in DMEM containing Pd-Cap1 and 50 mM of the probe HBTPQ, washed, and analyzed. The inset corresponds to a scheme of the catalyst used in this study. c) First, second, and third reaction runs using the same nanoreactor-preloaded cells and recorded by confocal microscopy; left: after 3 h incubation with bis-propargyl carbamate-protected cresyl violet; right: after washing with phosphate-buffered saline and leaving the cells for 3 h for further cleansing. The two images in the fourth row correspond to the fourth deprotection run of bis-propargyl carbamate-protected cresyl violet with the same nanoreactor-preloaded cells (scale bar = 200 µm). Reprinted with permission from (a) Jason Miles A. Miller et al.,[51] Copyright 2018 American Chemical Society, (b) Paolo Destito et al.,[52] Copyright 2019 Royal Society of Chemistry, (c) Raquel Martínez et al.,[54] Copyright 2020 Elsevier.

$[Pd(allyl)Cl]_2$ catalysts and the ability to catalyze a bioorthogonal depropargylation reaction (Figure 2b). A different approach was developed by Sancho-Albero et al.,[53] who exploited the intrinsic capability of exosomes to target tumor cells in a spatio-temporally selective manner. These exosome-based artificial vesicles contain ultrathin Pd nanosheets capable of entering cancer cells and delivering their catalytic cargo. The authors demonstrated the activation of a prodrug of the clinically approved anticancer drug panobinostat in a specific cell type by Pd-mediated dealkylation (Figure 2c). Another alternative proposed by del Pino and co-workers was the synthesis of nanoreactors composed of a Pd nanocube core and a nanometric imidazolate framework that can catalyze the activation of pro-fluorophores both in biological media and inside living cells. The fabricated MOF shell plays a double role, preserving the integrity of the catalytic chamber and providing substrate selectivity and biocompatibility.[54] Another work involving the use of MOF structures with PdNP was described by Chen et al., for the bioorthogonal activation of fluorescent Rh110 in aqueous solution and living cells. They also demonstrate the activation of chemically caged 4-hydroxytamoxifen (4-OHT), which is a high-affinity binding ligand for a protein stabilizing domain, ER50. This strategy can contribute to the regulation of protein function in cancer cells.[55]

2.2. Cu- and Au-Based Heterogeneous Catalysts

Most of the studies of heterogeneously catalyzed bioorthogonal reactions are based on Pd-containing systems. However, other metals, such as Cu and Au, were also evaluated. In particular, inspired by the applicability of Cu complexes to catalyze bioorthogonal reactions under homogeneous conditions, Clavadetscher et al., developed biocompatible and implantable heterogeneous Cu catalysts for azide–alkyne cycloaddition processes avoiding the inherent toxicity of the Cu catalyst for in vivo applications.[5] In the field of bioorthogonal reactions using homogeneous catalysts, Cu complexes were extensively applied, but their use for the in situ generation of active compounds by coupling two inert partners, such as prodrugs, has not been reported. Cu nanoparticles (CuNP) were synthesized by reduction of $Cu(OAc)_2$ incubated in the presence of an amino-functionalized resin, resulting in CuNP entrapped within the polymeric support. This catalyst was used to activate a coumarin profluorophore and to catalyze the in situ synthesis of an anticancer agent by the cycloaddition reaction of two component halves. These reactions were conducted both in vitro and in vivo. After a single bead of the catalyst had been implanted into the yolk sac of zebrafish embryos, no toxicity was observed. Moreover, Wang et al., developed a mitochondria-targeted heterogeneous catalyst for azine–alkyne cycloaddition (AAC) reactions.[56] In this case, MOFs were used as scaffolds to stabilize and protect CuNP. Subsequently, these structures were functionalized with 3-carboxypropyltriphenylphosphonium bromide (TPP) which acts as a mitochondria-targeting group. This catalyst showed good stability and high catalytic activity for the production of a fluorescent molecule and the in situ synthesis of a resveratrol analogue in mitochondria. Along these lines, the use of CuNP for this purpose was also reported by Qu and co-workers. They prepared a catalyst through the in situ growth of CuNP on mesoporous carbon nanospheres, which can be externally activated employing near-infrared (NIR) in order to catalyze AAC reactions in vitro and in vivo.[57] Gao et al., also proposed another Cu-based catalyst for these cycloaddition reactions. They integrated a Cu^I-stabilizing ligand into mesoporous organosilica frameworks. This selective nanoreactor presents pores that provide molecular sieving properties, allowing the access of small molecules while avoiding the accessibility of macromolecules. The implementation of this bioorthogonal catalyst has been demonstrated both in vitro and in animals, such as zebrafish embryos.[58]

Prior to the work on Cu catalysts, the first example of bond-cleavage chemistry mediated by a heterogeneous Au-based catalyst in living systems was developed by Pérez-López et al.[59] Au catalysis has been extensively studied over the last decades, due to its interesting properties such as greener and recyclable material, stability and safety in handling, and activity at or even below room temperature. Another interesting property is its preference to coordinate with alkynes in the presence of other functional groups. In their study, the authors developed a heterogeneous catalyst formed by Au nanoparticles (AuNP) embedded in a resin. The solid support protects AuNP from large thiol-rich biomolecules, which present high affinity for Au, while allowing alkyne-functionalized small molecules to enter and undergo Au-mediated chemistry under biological conditions. The catalytic activity of the Au-based resins was demonstrated by the strong fluorescence of Rh110 observed after the *O*-depropargylation reaction. This catalyst also triggered the bioorthogonal uncaging of cytotoxic precursors, providing a novel and safe method to activate prodrugs. In particular, Pro-FUdR, POB-SAHA, and N-Poc-DOX were evaluated and all of them displayed a potent anticancer activity in combination with the Au-based resins demonstrating the capability of Au to cleave both *O*- and *N*-propargyl groups from different molecules in human lung cancer A549 cells. Furthermore, this catalyst also enabled the locally controlled release of a fluorescent dye (Rh110) in the brain of a zebrafish, which represents the first Au-catalyzed bioorthogonal organometallic reaction to be locally performed in the brain of a living animal. Alternatively, Ortega-Liebana et al., developed a fully biocompatible Au-polymer composite that enabled the localized uncaging of a neuroactive agent, i.e., the anxiolytic agent fluoxetine, at the central nervous system of zebrafish resulting in the modification of cognitive activity, particularly, influencing their swim pattern.[60] These microreactors were prepared by loading positively charged NH_2-PEG-grafted polystyrene microimplants with negatively charged AuNP resulting in the accumulation of AuNP at the outer amphiphilic layer of the microimplant. This made it possible to simultaneously trap and shield the AuNP with a protein-

repelling material and to facilitate small molecules' access to the active sites of the composite.

3. Externally Activated Heterogeneous Catalysts

One of the main issues encountered in bioorthogonal chemistry is the ability of the catalysts to control reactions on demand, as happens in reactions in living systems. The activation of the catalytic processes in response to an extrinsic stimulus is an important factor for their practical implementation.[61] Some examples of these heterogeneous catalysts activated on demand using different stimuli are compiled in this section and summarized in Figure 3.

3.1. Light-Induced Bioorthogonal Reactions

One of these external stimuli is light, since it is a highly selective external trigger to activate certain chemical reactions. In this sense, Wang et al., developed the first heterogeneous Pd-based catalyst that could effectively mediate in situ bioorthogonal reactions through light (Figure 3a).[61] The catalyst consisted of macroporous silica nanoparticles that were used as a scaffold to stabilize Pd^0 nanoparticles. This structure was subsequently modified with an azobenzene group (ASP) and then reversibly functionalized with cyclodextrin (CD). When these compounds are attached to the structure, the activity is inhibited. Due to the isomerization of ASP under UV illumination, the CD blocker can be released from the catalyst, triggering the catalytic activity, or recombined again under visible light and returned to its original state. This light-induced catalyst was applied in the cleavage reaction of *N*-alloc coumarin in solution and different types of reactions in HeLa cells, such as the cleavage reaction of alloc-Rh110, the synthesis of a fluorescent dye via Suzuki–Miyaura cross-coupling reaction, and the conversion of prodrug Pro-5FU into 5-FU by *N*-dealkylation. The light-induced catalysis approach was also reported in reference [62]. The authors synthetized plasmonically integrated nanoreactors (PINER), which consist of different metal nanocrystals located in the inner part of a porous silica capsule, coated with a tannic acid-Fe^{III} (TA–Fe) nanofilm and, finally, a cover of Au-nanospheroids with close interparticle nanogap growth around the capsule (Figure 3b). The TA–Fe coordination polymer nanofilm directs the growth of Au-nanospheroids while protecting the internal catalytic nanocapsules from overgrowth. The surrounding plasmonic component acts as a NIR-photothermal transducer and it allows an external control and acceleration of the reaction upon irradiation with a NIR-laser. They tested the catalytic efficiency of this structure using three different metals, i.e., Au, Pd, and Pt, in the deprotection reaction of Poc-Rho to Rh110 using a NIR laser, and obtained the highest yield in the case of the Au-containing catalyst. Some of these nanoreactors were successfully applied in other chemical transformations, including depropargylation, deallylation, and C–C cross-coupling reactions on a variety of substrates in biological medium. Owing to the catalytic efficiency and high stability and biocompatibility of Au-PINERs, researchers applied this material over two different cell-lines (LNCaP and MDA-MB-231) demonstrating the high biocompatibility and cell internalization of these nanoreactors. In addition, by the activation of fluorescence probes inside living cells, they proved the

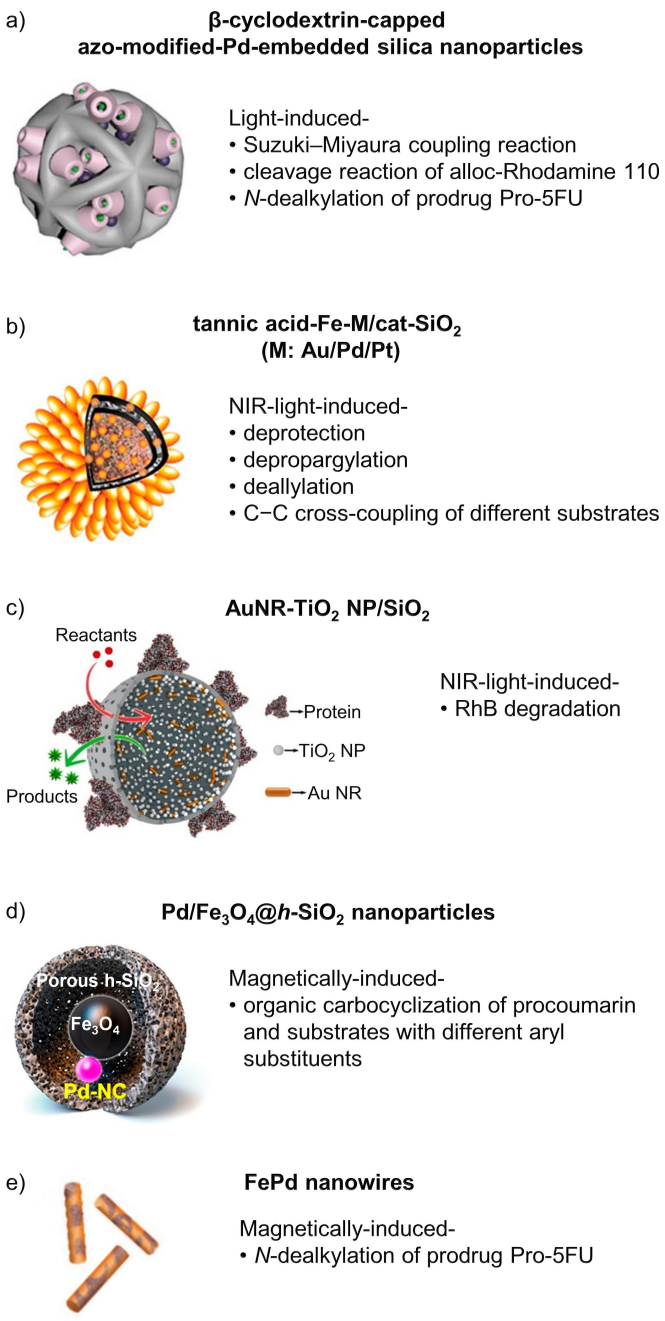


Figure 3. Summary of the different strategies utilized to induce a series of chemical transformations. NC stands for nanocrystals. Reprinted with permission from (a) Faming Wang et al.,[61] Copyright 2018 Springer Nature, (b) Amit Kumar et al.,[62] Copyright 2019 American Chemical Society, (c) Ana Sousa-Castillo et al.,[63] Copyright 2020 American Chemical Society, (d) Jihwan Lee et al., [65] Copyright 2020 American Chemical Society, (e) Salvador Pané et al.,[64] Copyright 2018 Wiley-VCH.

possibility of catalyzing intracellular transformations. One year later, Sousa-Castillo et al., developed a nanoreactor that can be remotely activated by NIR light to catalyze chemical transformations in biological media.[63] It consists of mesoporous hollow silica capsules with Au nanorods and TiO_2 nanoparticles adsorbed onto the inner wall of the silica shell (Figure 3c). TiO_2 is combined with Au to extend its photocatalytic activity from the ultraviolet part of the solar radiation spectrum to the first biological transparency window of the NIR range (650–950 nm) as a result of the hot-electron injection from the metal to the semiconductor. The photocatalytic capabilities of these hybrid capsules were demonstrated with different degradation reactions of a fluorescent substrate, both in aqueous solution and in complex biological media. Preliminary experiments in the presence of living mammalian cells were also performed without any apparent modification of the cellular viability, indicating the confinement of the produced reactive oxygen species inside the capsules. Furthermore, the degradation of bulky dyes, such as methylene blue, does not takes place, demonstrating the size-selective molecular diffusion through the mesoporous silica of these nanoreactors.

3.2. Magnetically Induced Bioorthogonal Reactions

Another strategy for the external activation of bioorthogonal reactions is the development of magnetically driven structures. Hoop et al., combined the bioorthogonal activation of prodrugs with the site-specific targeting of the compounds.[64] They reported a nanorobot consisting of FePd nanowires prepared by template-assisted electrodeposition that was capable of performing the bioorthogonal conversion of the latent Pro-5FU to the active chemotherapeutic 5-FU in an in vitro breast cancer model without significant cytotoxic effect (Figure 3e); this turned-on catalytic activity was ascribed to the ferromagnetic properties of the Fe present in the catalyst. Furthermore, these magnetic and bioorthogonal active nanostructures were applied to an in vivo mouse model of human breast cancer xenografts in nude mice. The catalyst was injected in the tumor and its therapeutic potential for Pd-mediated activation of Pro-5FU was demonstrated by significantly reducing the tumor growth. A similar approach was reported by Lee et al.[65] The catalyst is composed by a hollow porous silica capsule that encapsulates the active Pd phase (Figure 3d). This metal is grown over a magnetic Fe_3O_4 core under magnetic induction. Upon exposure to a magnetic field, the Fe_3O_4 core acts as a nanoheater, transferring energy to Pd that is then able to catalyze several intramolecular carbocyclization reactions, generating fluorescent products in aqueous and physiological conditions. The magnetic-field-induced heating of the nanoreactors is highly localized inside the capsules, resulting in the local temperature increase at the catalytic sites, thus avoiding stabilization and reactivity problems.

4. Summary and Outlook

This Minireview highlights the enormous potential of combining two different fields that have been intensely studied in recent years: bioorthogonal chemistry and heterogeneous catalysis. The catalysts are typically composed of non-biotic transition metals, such as Pd, Cu, or Au, that have the potential for both acute toxicity and genotoxicity and are capable of catalyzing reactions of huge interest, such as the synthesis of different drugs or the activation of fluorophores or prodrugs. These unique capabilities could be complemented by targeting and stimuli-responsive properties to provide enhanced spatiotemporal control of drug activation, rendering localized therapy to reduce the off-target effects. Pd-based catalysts are the most widely used materials in bioorthogonal reactions, both those in the form of nanoparticles supported on polymer and silica structures, and the encapsulated counterparts, which avoid biocompatibility, stability, and toxicity issues. The efficiency of these catalysts carrying out bioorthogonal reactions in biological medium with minimal impact on cell viability has entailed a great advance in the biological field. Their versatility provides multiple opportunities for developing heterogeneous catalysts with improved performance as they move towards the clinic. Still, the catalytic efficiency and rate, the stability and durability of the transition metal catalysts, and the deactivation due to biological nucleophiles needs serious consideration. Their inherent toxicity also entails major concern. Improving the catalyst activity or the application of other low-toxicity metal catalysts could eventually address these issues. Moreover, the metabolism and long-term effects of the heterogeneous catalysts are additional barriers to be tackled before translating to clinic. Apart from the materials choice, prodrug design can also greatly affect the performance of bioorthogonal catalysts as they might alter pharmacokinetics and physicochemical properties, thus expanding or constraining the final application of the drug. The in situ synthesis of compounds and the delivery of drugs at the site of their action might imply an important milestone in disease treatment in the not too distant future. In vivo studies generally using zebrafishes have demonstrated promising catalytic efficiencies. However, it will be necessary to extend these studies to another animal models to verify the feasibility of the targeted bioorthogonal catalysis. Another important point to consider will be the ability to control these reactions by external agents. As discussed, some studies tackling the catalyst activation by means of light or an external magnetic field have been already performed. This is expected to go a long way toward localized therapies and preventing healthy tissue damage. In addition to the application of these techniques for cancer therapies, studies should be focused on other biomedical purposes, such as the treatment of antibacterial diseases. Despite the numerous advances in recent years, a there is still a long way to go in the field of bioorthogonal chemistry.

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Conflict of Interest

The authors declare no conflict of interest.

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