Light-driven catalytic regulation of enzymes at the interface with plasmonic nanomaterials

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ABSTRACT

Regulation of enzymes is highly relevant toward orchestrating cell-free and stepwise biotransformations, thereby maximizing their overall performance. Plasmonic nanomaterials offer a great opportunity to tune the functionality of enzymes, through their remarkable optical properties. Localized surface plasmon resonances (LSPR) can be used to modify chemical transformations at the nanomaterial's surface, upon light irradiation. Incident light can promote energetic processes, which may be related to an increase of local temperature (photothermal effects), but also to effects triggered by generated hot-spots or hot-electrons (photoelectronic effects). As a consequence, light irradiation of the protein-nanomaterial interface affects enzyme functionality. To harness these effects to finely and remotely regulate enzyme activity, the physicochemical features of the

nanomaterial, properties of the incident light and parameters governing molecular interactions must be optimized. In this perspective article, we discuss relevant examples that illustrate the use of plasmonic nanoparticles to control enzyme function through LSPR excitation. Finally, we also highlight the importance of expanding the use of plasmonic nanomaterials to the immobilization of multi-enzyme systems for light-driven regulation of cell-free biosynthetic pathways. Although this concept is living its infancy, we encourage the scientific community to advance in the development of novel light-controlled biocatalytic plasmonic nanoconjugates and explore their application in biosensing, applied biocatalysis, and biomedicine.

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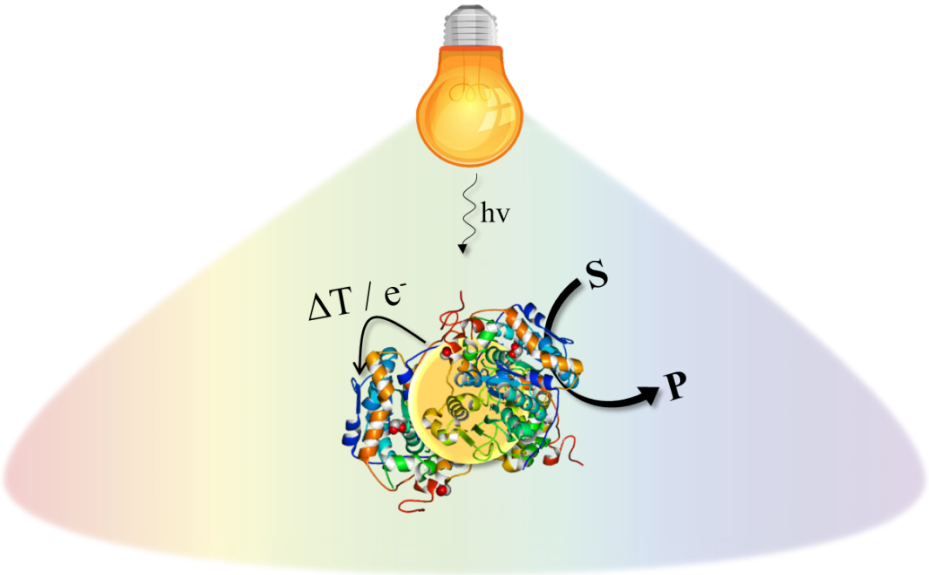

Remote control of extracellular enzyme functionality through external stimuli provides access to more complex and concurrent cell-free biosynthetic routes. Enzyme activity regulation additionally offers the possibility of orchestrating several enzymes to simultaneously work in tuned cascade reactions, which maximizes the efficiency of biosynthetic pathways. Inside the cell, enzymes are tightly regulated, both at genomic and proteomic levels,[1] however such a regulation is lost when they are isolated. Therefore, breakthroughs in this topic are expected to advance toward enzymatic designs with a finer control for fully artificial applications. Light is one of the most efficient stimuli to remotely control enzymatic activity. The bio-conjugation of enzymes to light-responsive molecules and polymers has been the most common approximation hereto.[2-4] Interfacing enzymes onto plasmonic nanomaterials has been recently introduced as a promising alternative tool to design bioconjugates with tailored properties for remote control of biocatalysis. As any immobilization protocol, the conjugation of enzymes onto nanoparticles must be designed to enhance both the activity and the stability of the resulting bioconjugates. Hence, nanoparticle size and shape, as well as the conjugation chemistry, are fundamental aspects to control the orientation, density and binding strength that ultimately influence the functionality of the immobilized enzymes.[5, 6] Not only the immobilization of enzymes on plasmonic nanomaterials may enhance their functional properties,[7, 8] but optimization of the interactions between enzymes and plasmonic nanoparticles additionally offers opportunities to develop nanoengineered materials for light-controlled biocatalysis.[9, 10] For example, combination of the remarkable biological functions of enzymes and the unique optical properties of plasmonic nanomaterials can contribute to applications including cancer therapy,[11-13] biosensing,[14-16] applied biocatalysis and biotransformations,[17, 18] and intra- or extra-cellular nanosurgery.[19-21]

The suitability of plasmonic nanomaterials to tune enzyme activity arises from their tunable localized surface plasmon resonances (LSPR), in the UV-Vis and near-infrared wavelength ranges.[22-24] Under incident light, conduction electrons in metal nanoparticles display coherent collective oscillations, thereby generating intense absorption and scattering at specific wavelengths, resulting in LSPR signals.[25, 26] It is well-known that the LSPR response is largely dictated by the dimensions and morphology of the plasmonic nanoparticles, but it is also highly sensitive to the interaction between biomolecules and the nanoparticles' surface.[27, 28] Therefore, LSPR excitation in resonance with light irradiation can be used as a potential tool to regulate protein functionality at the interface with the nanomaterial. For example, enzymes immobilized on the surface of

nanoparticles can be readily triggered by light irradiation, to enhance their catalytic activity.[9, 10, 17, 29] Harnessing LSPR, biocatalysis can be remotely regulated upon light irradiation to fine tuning artificial reaction biocascades. [9, 10, 17, 29-31] Hence, the use of light is a precise, sustainable, noninvasive and remote method to foster innovative solutions in the biocatalysis field through merging enzymes and plasmonic nanoparticles (Figure 1).[32, 33] This perspective provides an overview of this emerging research field, in an attempt to present and discuss the most exciting studies where light controls the enzyme activity at the interface with plasmonic nanomaterials, and ultimately offering our own outlook.

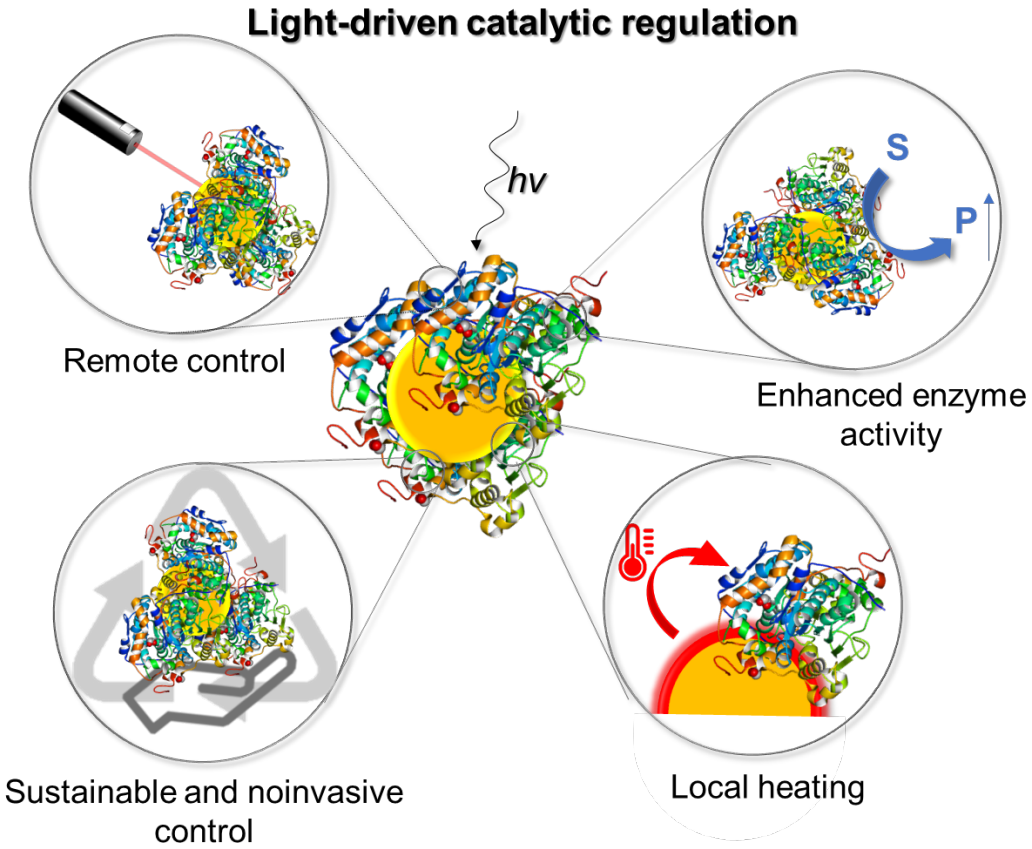

Figure 1. Schematic representation of some foreseen prospects for light-driven enzymatic activity regulation onto plasmonic nanoparticles. For clarity purposes, enzymes and nanoparticles are presented in similar sizes, but this is just a graphic representation, not necessarily the real scale.

LIGHT AS THE MAJOR PLAYER IN THE FIELD: MECHANISMS BEHIND LIGHT-DRIVEN BIOCATALYSIS INTERFACING PLASMONIC NANOPARTICLES

Light irradiation can introduce enhanced energetic processes through different mechanisms into plasmonic nanoparticles, depending on the nanoparticle features, such as their size and morphology. For example, anisotropic nanoparticles can concentrate the generated energy at sharp corners or tips, with a significant enhancement of several orders of magnitude.[34] Plasmonic effects are thus expected to influence the functional properties of enzymes (activity and stability) in their neighborhood. Unfortunately, we are still lacking fundamental knowledge and understanding to properly explain the mechanisms underlying this phenomenon. It is well-established that chemical reactions triggered by plasmonic effects in nanoparticles are mainly driven by one or more of the following physical effects: (i) temperature increase promoted by incident light, also termed thermoplasmonics (Figure 2A); (ii) enhancement of the electromagnetic near-field at the nanoparticles surface, creating "hotspots" of concentrated energy per unit volume (Figure 2B); and (iii) light-induced hot electrons, where light promotes the transfer of an electron from the plasmonic nanoparticle to an adjacent molecule on their surface (Figure 2C).[34] On the other hand, in photo-biocatalysis, enzyme activity is enhanced by directly shining light onto the enzymes, usually oxidoreductases, which harbor a photoactive cofactor.[35, 36] The mechanisms driving such photobiocatalytic reactions are mainly related to the photoexcitation of such photoactive cofactors, which shuttle electrons between the substrates and the products (Figure 2D).[37-40] Hence, on the basis of this fundamental knowledge, some attempts have been made to address the study of the mechanisms governing light-driven biocatalysis of enzymes interfacing with plasmonic nanoparticles, which we briefly review in this section.

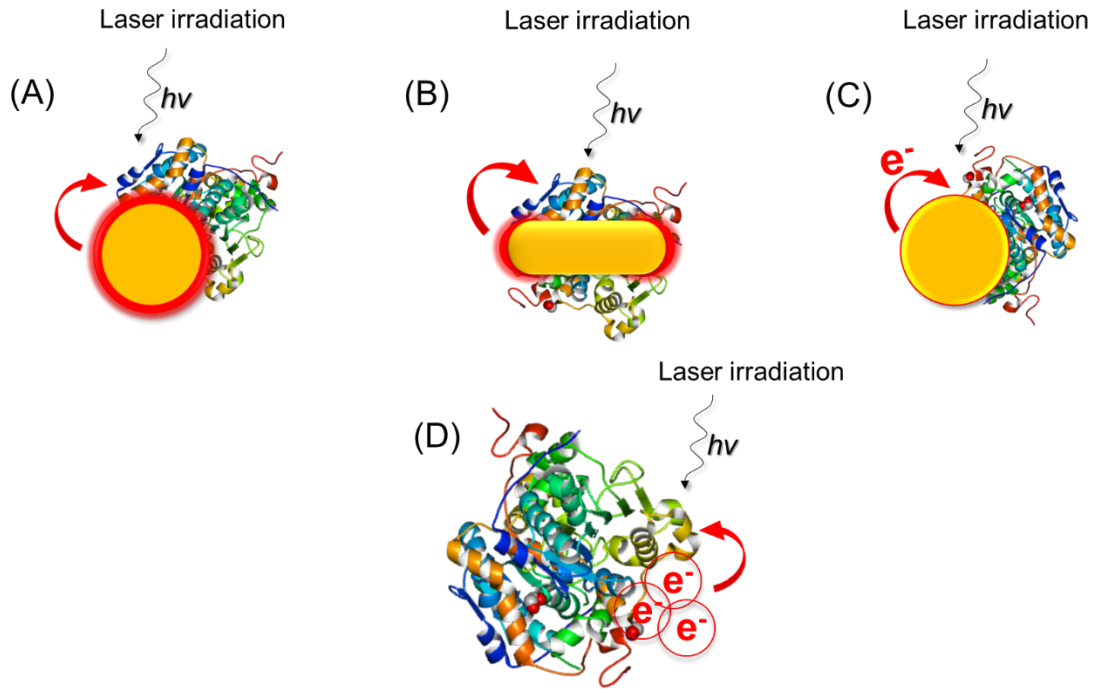

Figure 2. Schematic representation of potential mechanisms of enzymes interfacing with plasmonic nanomaterials, upon laser irradiation. (A-C) Concepts from nanoplasmonics through temperature increase (A), "hot-spots" generated from the enhancement of the optical near-field (B), transfer of hot electrons to the enzyme structure (C). (D) Concepts from photo-biocatalysis with photoinduction of electron transfer to the enzyme. For clarity purposes, enzymes and nanoparticles are presented in similar sizes, but this is just a graphic representation, not necessarily the real scale.

The photo-induced increase of local temperature on plasmonic nanoparticles is arguably the most intensively explored effect to explain the mechanisms involved in enhanced enzymatic activity.[9, 10, 12, 13, 17, 29, 30, 41] Gold, the most common plasmonic nanomaterial, features inherent properties such as low specific heat capacity associated to high thermal conductivity, which render it an ideal candidate for thermally enhanced processes.[8] In principle, the activity of enzymes immobilized on the surface of plasmonic nanoparticles should rise along with the local temperature increase induced by light,[12] according to Arrhenius equation. However, a local high temperature can also promote a partial or even complete deactivation of the enzymes, due to conformational changes. This dichotomy makes it extremely important to precisely tune the heating ability of the involved plasmonic nanoparticles,[42] so that the enzyme surroundings can reach an optimal temperature ensuring maximum activity while avoiding deactivation.

On the other hand, hardly any studies are available that explore electron transfer between excited plasmonic nanoparticles and enzyme structures. One of the few, if not

the only example that addresses these light-driven electron transfer mechanisms uses a laccase immobilized onto gold nanoparticles (AuNPs) under green light.[30] Laccase is a multicopper oxidase that transfers electrons from the substrate to the molecular oxygen towards two copper clusters found at the enzyme's active site. More specifically, laccases work through a cooper atom (T1 site) that takes electrons from a reducing substrate, to subsequently transfer those electrons to a trinuclear cooper center (T2/T3 sites) that ultimately shuttles the electrons to the molecular oxygen as the final electron acceptor to produce water. It has been observed that green light irradiation increases the local temperature at the surface of AuNP biohybrids, promoting conformational changes in the immobilized laccase that likely alter electron transfer within its active site. Therefore, the local temperature increase at AuNPs surfaces seems to unpair the electron transfer between T1 and T2/T3 sites, which consequently diminishes the laccase activity (Figure 3A and B).

Mechanistic studies concerning electron transfer upon LSPR excitation are usually carried out using redox enzymes, because their mechanisms intrinsically involve the transfer of electrons. In an illustrative example, Wang *et al* identified hot charge carriers generated on AuNPs under visible light excitation (450, 532, 652, and 808 nm) in a plasmon-accelerated electrochemical reaction (PAER).[43] The experiments confirmed the presence of hot charge carriers, responsible for the enhanced electrocatalytic oxidation of glucose, as a model system. Although enzymes were not explored in this study, it highlights opportunities to exploit PAER with enzymes to regulate biocatalysis and elucidate the mechanisms involving electron transfer. For example, in the oxidation of D-glucose catalyzed by glucose oxidase (GOx), the mechanism involves molecular oxygen as an electron acceptor and a flavin cofactor as an electron shuttle.[44] The same concept could be expanded to other flavin oxidases that oxidize a diversity of substrates, such as alcohols, amino acids, and cholesterol, also using oxygen as the final electron acceptor.

In this context, it is clear that different events can concurrently occur upon light-driven biocatalysis through LSPR excitation, but some of them (i.e. temperature increase) are easier to identify than others (electronic effects). An interplay of different mechanisms can also result in one mechanism triggering another. Despite the few mechanistic studies involving plasmonic regulation of enzyme properties, we consider that the interactions between enzymes and nanomaterials are essential to harness the plasmonic effects upon light irradiation, as it happens in electrobiocatalysis, where electrons go back and forth

between the enzyme active sites and the electrodes.[8] In these cases, the distance, the number of attachments, the orientation and the density of the immobilized enzyme have been proven to play a central role in the interplay between biocatalysts and materials, for a variety of applications ranging from biosensing to biotransformations.[45] As an example, Kang *et al.* observed that the photothermal inactivation of α-chymotrypsin immobilized on AuNPs (irradiated at 532 nm) was strongly dependent on its distance to the AuNP surface.[13] Because of the rapid local temperature gradients generated in the region termed impact zone, enzyme deactivation occurred immediately under nanosecond laser pulses (Figure 3C). The authors additionally observed that the size of AuNPs (5-70 nm) was also a determining factor behind the temperature gradient responsible for protein inactivation: the larger particles induce larger impact zones at low laser power (Figure 3D). In this scenario, small AuNPs (5 nm) did not cause enzyme inactivation, while larger AuNPs (>50 nm) readily inactivated the enzymes upon laser irradiation. The AuNPs surface-to-volume ratio and the laser energy, related to the efficiency of heat dissipation to the surroundings, were argued to explain enzyme inactivation under irradiation conditions. Since the local temperature in the enzyme surroundings strongly depends on the distance between enzyme and nanoparticle surface, the heating and dissipation capacity of the nanoparticles, together with the intrinsic enzyme thermal stability, should determine the optimal immobilization distance in light-controlled biocatalysis. We suggest that further studies are needed to address the impact of protein orientation and the separation between enzymes and nanomaterials on photothermal effects. We foresee that protein engineering and chemical biology will facilitate these studies, *e.g.* by creating new enzyme variants with engineered surfaces, so as to precisely control their interaction with plasmonic nanomaterials. We therefore stress that, using plasmonic nanoparticles to manipulate enzyme activity is still highly challenging and demands a clear understanding of the mechanisms responsible for plasmon-enhanced biocatalysis. We envision plenty of opportunities to elucidate and dissect the mechanisms underlying LSPR effects on enzyme functionality. For instance, light-driven plasmonic effects may control the intermolecular interactions or bond breaking to timely and remotely activate or deactivate enzymes of interest.

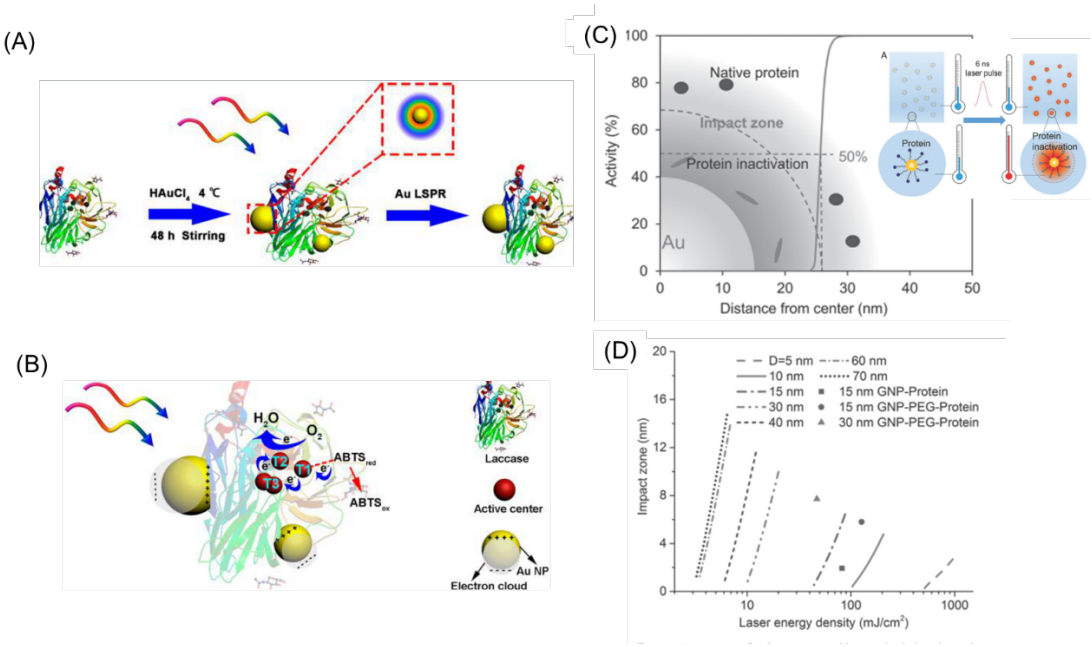

Figure 3. Light effect on enzymes interfacing with plasmonic nanomaterials. Temperature effect (A) and electron transfer effect (B) in laccase-AuNPs bioconjugates, under green light irradiation. Reproduced with permission from ref. 30. Copyright 2015 American Chemical Society. (C) Impact zone on the nanoparticle surface for α-chymotrypsin inactivation and (D) effect of AuNP size and laser energy on impact zone. Adapted with permission from ref. 13. Copyright 2017 John Wiley and Sons. For clarity purposes, enzymes and nanoparticles are presented in similar sizes, but this is not necessarily the real scale.

PLASMONIC NANOMATERIALS AS AN OPPORTUNITY TO INTRODUCE REMOTE CONTROL IN ENZYME ACTIVITY

The response of plasmonic materials under irradiation conditions, and thus their effect on enzyme functionality, can be regulated by tuning parameters of the incident light such as wavelength, power, and illumination time. Some examples illustrate how light decreases the activity of enzymes immobilized on plasmonic nanoparticles. Guo *et al.* observed[30] a decrease in the activity of laccase-AuNP bionanohybrids by 8.1% under visible light ($\lambda > 420$ nm) illumination for 3 minutes, with recovery upon turning the light off. This effect was wavelength-dependent, since green light ($\lambda = 500 – 560$ nm) induced the lowest enzyme activity, likely due to resonance with the LSPR of spherical AuNPs (λ ~520 nm). Interestingly, the intensity of green light - ranging 0 - 60 $mW \cdot cm^{-2}$ - followed a negative correlation with the activity of the immobilized laccase. Similar results were obtained for horseradish peroxidase (HRP) immobilized onto spherical AuNPs upon laser

irradiation at λ = 532 nm,[41] suggesting that enzyme inactivation is related to released heat under resonance conditions. Another interesting example of enzyme inactivation by heat generation was provided by Thompson *et al*.[12] By using gold nanorods (AuNRs) (λ at ~785 nm) in resonance with incident near-infrared (NIR) laser irradiation (λ = 785 nm), the authors showed selective control of a cascade reaction induced by glucose oxidase (GOx) immobilized on AuNRs and free horseradish peroxidase (HRP), through selective inactivation of GOx (Figure 4A). As the bi-enzyme system is controlled through the activity of one of the enzymes – GOx – this is a one-time (shot) control because once GOx is inactivated upon light irradiation, the bi-enzyme cascade cannot be subsequently recovered.

Light-driven biocatalysis on plasmonic nanoparticles becomes more attractive when using thermophilic enzymes, since they are highly active and stable at high temperature (>70 ºC). The local heating triggered by the LSPR effect may be thus transferred to thermophilic enzymes immobilized on plasmonic NPs, thereby enhancing their catalytic activity. This concept has been demonstrated for several classes of temperature-resistant enzymes. Li *et al*. compared the enzymatic performance of three enzymes with different thermal stability, conjugated to AuNRs and irradiated with a NIR laser (808 nm).[17] The results were compared for a mesophilic enzyme pig pancreatic lipase (PPL), a thermophilic protease from *Sulfolobus tokodaii* strain T7 (ST0779), and a hyperthermophilic protease from *Aeropyrum pernix* strain K1 (APE1547), with optimal temperatures of 37, 70 and 90 °C, respectively. Under laser irradiation, the activity of the enzymes immobilized onto AuNRs increased by 75.3%, 118.4% and 218.4%, respectively, compared to their free enzyme counterparts. It was interesting to confirm that more thermophilic enzymes underwent a higher activity enhancement by the photothermal effect as a consequence of LSPR illumination. To further concentrate the heating generated by the plasmonic effect in the enzyme surroundings, Blankschien *et al*. trapped AuNRs conjugated with a thermophilic glucokinase from *Aeropyrum pernix* into alginate macrobeads.[29] Under 800 nm laser irradiation, the enzymatic activity was found to increase by 60%, compared to alginate-trapped enzyme, whereas a negligible effect was observed by illuminating the same biohybrid in dispersion (outside the beads). This study shows how a surrounding matrix can preclude fast heat dissipation, thereby preserving the generated heat to enhance enzymatic activity. However, entrapment of the biohybrids in macroporous hydrogels brings along a 10-fold decrease in enzymatic

activity, likely due to mass transport restrictions for the substrate to reach the enzyme active sites. To overcome mass transport limitations, other solutions have been proposed to confine the heat generated by photothermal effects at the nanoscale. Tadepalli *et al*. explored two strategies to encapsulate bionanohybrids through either *in situ* polymerization or by means of metal-organic frameworks (MOF) (Figure 4B and C).[9, 10] The enzymatic activity of HRP immobilized on AuNRs and protected by either a polymer layer or MOF architecture, was stimulated by approximately 110% and 80% under laser irradiation (808 nm, 400 $mW \cdot cm^{-2}$), respectively, compared to the dark reaction. These strategies showed that local heating may be preserved at the enzymes surroundings, thereby stimulating their activity at the NP interface. Hence, nanoecpasultation seems to be a suitable strategy to promote photothermal effects in enzymes of different nature and origin (besides thermophilic) without jeopardizing their stability under the resulting high local temperatures induced by LSPR illumination.

From the studies reported during the past decade, we conclude that the activity of enzymes immobilized on plasmonic nanomaterials upon light irradiation depends on various parameters related to enzyme nature and type, nanomaterial properties (morphology, size, LSPR signal) and laser features (power, wavelength, illumination time). It is important to mention that nanoparticle concentration in the dispersion, regarding the density of the immobilized enzyme, is decisive to tune the heat output transfer in these systems, and therefore controlling bionanohybrid activity under irradiation.[46, 47] Finally, we identify scenarios where the enzyme activity either increases or decreases upon light irradiation. However, the vast majority of studies are focused on quantifying the effect of light once the enzyme is immobilized without accounting for the enzyme activity lost upon conjugation. To evaluate the effect of light on the enhancement of enzyme functionally, we encourage reporting the specific activity of the enzyme before and after immobilization, under irradiation and non-irradiation conditions.

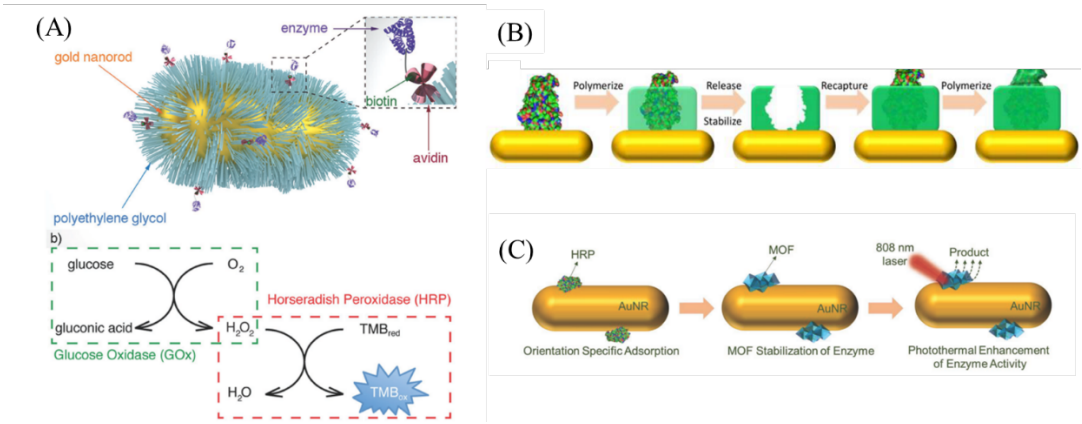

Figure 4. Examples of enzyme inactivation or activation onto plasmonic nanoparticles upon laser irradiation. (A) Strategy to selective targeting of GOx or HRP immobilized onto PEG-AuNRs, upon NIR laser irradiation. Reproduced with permission from ref. 12. Copyright 2017 John Wiley and Sons. (B,C) Schematic representation of strategies to confine the heat generated at AuNRs to enhance HRP enzymatic activity by *in situ* polymerization (B) and by using MOFs (C). Reproduced with permission from ref. 9. Copyright 2017 American Chemical Society. Reproduced with permission from ref. 10. Copyright 2018 John Wiley and Sons.

CELL-FREE PLASMONIC REGULATION OF BIOSYNTHETIC PATHWAYS

Metabolic networks within living cells have evolved for billions of years to maximize fitness and ensure optimal cell survival and reproducibility within specific environments. Hence, the cell metabolism relies on a multitude of chemical reactions catalyzed by highly chemo-, regio- and stereo-selective enzymes. These biological catalysts are perfectly orchestrated to work simultaneously within the cell milieu, under similar reaction conditions. Although some enzymatic reactions are compartmentalized, the vast majority of them occur inside the cytosol. For this reason, the regulation of enzyme activity is extremely important to efficiently route biosynthetic pathways. Within the cells, such metabolic pathways are mainly regulated at the genomic level by enhancing or suppressing the expression of the genes encoding the enzymes involved in the target pathway, and at the protein level through allosterism and post-translational modifications. In the former case, gene regulation pathways respond to both metabolite accumulation and environmental changes, by either increasing or decreasing the intracellular enzyme concentrations. In contrast, the latter case promotes conformational changes in enzymes, thereby modulating their activity through partial proteolysis or chemical modification.

Human beings exploit metabolic networks for their survival but also for technological purposes. Since the beginning of biotechnology in the 7th century B.C, ancient societies made use of enzymes for food processing (wine, beer, bread…). Nowadays, enzymes are routed to catalyze non-natural biosynthetic pathways demanded by modern biotechnological applications in chemical manufacturing, biosensing and biomedicine.[31] These applications often require the enzyme to work far from physiological conditions. Therefore, driving the enzymes out of their natural environment makes us face technological challenges related to their functionality toward non-natural substrates and their responsiveness to external stimuli. Protein engineering has successfully adapted the functionality of a myriad natural enzymes to artificial synthetic reactions,[48, 49] yet the regulation of such artificial cascades must be engineered.

In recent years, many approaches have been developed to artificially regulate the catalytic activity of isolated enzymes. With the revolution of cell-free synthetic biology, more complex biosynthetic pathways are assembled to work under non-physiological conditions. Defining synthetic biology as the discipline that integrates the engineering principles behind biological processes, materials chemistry and engineering can contribute to expand this concept beyond DNA modifications. Through the incorporation of biological machinery (i.e multi-enzyme systems) at the interfaces between stimuli-responsive materials (i.e. plasmonic nanoparticles), biological processes can gain novel functionalities. This revolution is still seeking radical solutions to control the activity of enzymes, so that their performance can be tuned in the context of cell-free biosynthetic pathway.

Enzyme cascades based on NADH-dependent alcohol dehydrogenases have been controlled using light as an external stimulus, through different approaches. The combination of enzymes and materials with photochromic functionality is usually exploited for this aim. The most common approach comprises mixing soluble enzymes and photoactive materials in one-pot, which couples a photocatalytic step with a biocatalytic one.[50] Whereas the enzymes consume the redox cofactor to perform the reaction of interest, the light-irradiated material replenishes the pool of the cofactor demanded by the enzyme. A similar strategy has been recently adopted for the photocatalytic *in situ* production of H_2O_2 employed by perooxygenases.[51]

Events where light remotely controls the activity of more than one enzyme attached to the surface of light-responsive or plasmonic nanomaterials are however scarce. The assembly of two NADH-dehydrogenases in DNA origami scaffolds is one of the few

examples where light can directly control the activity of two enzymes.[52] This system relies on DNA strands functionalized with azo groups that control the relative position of each enzyme, bridging them together and activating the cascade under visible light, but separating and inhibiting them under UV irradiation. More complex systems where more than two enzymes are simultaneously controlled by light have not been reported so far. We can definitely see here an opportunity for the use of plasmonic effects (photothermal or electron transfer) interfacing with multi-enzyme systems. In fact, one can envision in the long term a cohort of bionanoplasmonic hybrids whose catalytic activities are activated under different irradiation conditions (lasers with different wavelengths). In this scenario, different enzymes could be remotely activated through either simultaneous (two lasers at a time) or sequential (one laser after another) light stimuli to orchestrate a given enzymatic cascade reaction on demand. An enzyme cascade composed of thermophilic enzymes and thermolabile cofactors transforming temperature-sensitive substrates would be an excellent proof of this concept. While the enzymes interfacing the nanoplasmonic materials operate at their optimal temperature under irradiation conditions due to local heating, the bulk remains at room temperature avoiding the deterioration of both substrates and cofactors.

CHALLENGES AND PERSPECTIVES

Light-regulation at the interface between enzymes and nanomaterials is an outstanding concept to control enzymatic activity through remote stimuli. Regulation of enzymes is particularly relevant for sequential enzymatic reactions, since pairing the activities of all involved enzymes is paramount to optimally drive the chemical flux toward the target product. When enzyme cascades simultaneously work in one pot, their activities must be orchestrated to avoid accumulation of inhibiting intermediates and toxic by-products. Within living cells, such biosynthetic orchestration is met through sophisticated genomic and proteomic regulation pathways that control the expression level and post-translational modifications of the enzyme working in the cell milieu. When using enzymes for *ex vivo* applications, enzyme regulation becomes an arduous task that requires artificial actuators to remotely exert control over the enzymatic activities. These challenges open up the opportunity of using plasmonic nanomaterials, which harness the incident light to tune enzyme functionalities at the protein-nanomaterial interface.

Heat generated at the surface of plasmonic nanoparticles is attractive to manipulate the catalytic properties of enzymes, but effective strategies to protect enzyme

activity are still challenging. Even though progress has been made for thermophilic enzymes, little has been developed for mesophilic enzymes in terms of enzymatic activity enhancement. Strategies for remote-control of mesophilic enzymes are highly relevant because these enzymes represent the majority of applications in biocatalysis and biotransformations, overall when compared to thermophilic ones. Hence, plasmonic nanoparticles can introduce numerous advantages for remote-control of biocatalysis using mesophilic enzymes if we can find the optimal trade-off between activity and stability under light-induced local heating. Besides increasing the local temperature, LPSR effects can also generate hot-electrons at the surface of plasmonic NPS, which may induce electronic effects on enzymatic mechanisms. Unfortunately such light-driven plasmonic effects have been rarely exploited to control enzyme activity. Here, we identify a scientific niche to remotely regulate the activity of redox enzymes with catalytic mechanisms relying on electron transfer. New discoveries in this direction are yet to come.

Controlling more than one enzyme simultaneously is an unmet challenge using plasmonic nanomaterials. Even using phenomena other than plasmonics, few examples have been reported where light itself can control a multi-enzyme process. Therefore, the creativity window stays open toward the design of novel biohybrids, where the interface between materials and enzymes comes to play in the presence of an external stimulus such as light. Hence, enzymatic material interfaces are needed to allow efficient light control over enzyme functionality. Protein engineering is a promising route toward gaining control over orientation, number of attachments and distance between the enzyme and the plasmonic nanomaterial.

The revolution of synthetic biology has arrived to cell-free systems, where many isolated enzymes work simultaneously in the same pot and under the same conditions. It is thus the right time to introduce regulators that exert control over cell-free enzymatic systems to up- or down-regulate enzymes without altering the reaction medium. To that aim, such remote regulators must respond to external stimuli as plamonic nanomaterials do to light. Hence, we envision enzymes immobilized on plasmonic nanomaterials as new tools to endow cell-free synthetic biology with spatio-temporal control. Additionally, the immobilization of enzymes on light-responsive materials, such as plasmonic nanoparticles, is likely to pave the way to more futuristic applications like the chemical communication between compartmentalized cell-free systems, or even living cells. More

rational interfaces between enzymes and nanomaterials must therefore be created and new theoretical knowledge must be acquired to better understand the mechanisms governing the enzyme functionality at the interface with plasmonic nanomaterials.

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