

TiO₂-Based Photocatalysis at the Interface with Biology and Biomedicine

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TiO₂-based Photocatalysis at the Interface with Biology and Biomedicine

María Tomás-Gamasa* and José Luis Mascareñas*[a]

Abstract: The conversion of sunlight into chemical energy using the photosynthetic machinery is at the hearth of Nature and Life. Scientists have also learned to use the energy of light to promote a great variety of chemical reactions, most of them based on redox processes involving electron transfer steps. Indeed, the area of photoredox catalysis has recently emerged as one of the hottest fields in synthetic chemistry. Many of the photoredox reactions so far discovered take place in homogeneous phases, and rely on the use of soluble photoresponsive catalysts. However, along recent years, there have been also many advances in the area of heterogeneous photocatalysis, most of which are based on the use of semiconductor materials like TiO₂ as key photocatalytic system. These technologies have found different applications, especially in the field of sustainable chemistry and therapy. In this manuscript we review some of these applications, and the potential of TiO₂-based photocatalysts in biology and biomedicine.

1. Introduction

In recent years, photocatalysis has come to the forefront of synthetic chemistry, owing to its potential to promote transformations that cannot be accomplished using conventional methodologies. Photocatalysis refers to chemical reactions that take place in the presence of light and a small amount of a photoresponsive compound which after excitation induces energy or electron transfer-mediated chemical transformations. The latest case is the most common, and is known as photoredox catalysis.^[1]

Of the different light sources, sunlight is clearly the preferred choice, owing to its “green” nature: abundance, low cost, and environmentally benignity. Stimulated by the principles of sustainable chemical synthesis, researchers in last decades have made considerable efforts to use sunlight as a driving force for photocatalysis.^[2] The catalysts, which function as light-absorbing and electron-transfer species, must combine several features: redox-inactivity in the electronic ground states, optimal absorption properties, if possible in the visible region, and appropriate redox activity in the excited states.

In recent decades, three major groups of light-absorbing molecules/materials capable of promoting a wide range of redox activations from their excited states have been investigated: transition metal complexes (e.g. Ru(bpy)₃ and other Ru or Ir complexes) with strong MLCT (metal to ligand charge transfer)

absorptions, organic dyes such as xanthene, porphyrin or phthalocyanines (e.g., eosin Y), and colloidal semiconductor (SC) particles (e.g., TiO₂), which generate heterogeneous reaction mixtures.

This last type of photocatalysts are highly attractive owing to their ready availability, long-term photochemical stability and recyclability possibilities. In particular, TiO₂ possesses good mechanical resistance and stability in acidic and oxidative atmospheres, and presents high biocompatibility and safety toward both humans and the environment. These properties make TiO₂ a prime candidate for heterogeneous catalysis even in water or biological settings. Indeed, it has found applications that range from the degradation of chemical and biological contaminants, and the generation of bioactive reactive oxygen species (ROS), to the creation of photobiocatalysts.

2. Heterogeneous photocatalysis using semiconductors

The first report on the use of TiO₂ semiconductors as photocatalysts for the reduction of AuCl₃ to Au and AgNO₃ to Ag appeared in 1932.^[3] Six years later, TiO₂ was investigated as a photosensitizer to bleach dyes in the presence of O₂.^[4] but it was not until 1972 when Fujishima and Honda discovered the splitting of water over TiO₂ using UV light irradiation.^[5] This event marked the beginning of a new era in heterogeneous photocatalysis. Semiconductors absorb light photons to create active electrons and holes, which can promote the reduction and/or oxidation of different substrates, including water. Since then, investigations have been concentrated on understanding mechanisms, enhancing photocatalytic efficiencies or expanding the scope of the reactions.^[6] TiO₂ photocatalysis is now covering a variety of applications, from environmental and energy issues to biomedical problems.^[7]

2.1. Photophysical features

A semiconductor (SC) is characterized by an electronic structure in which the highest occupied energy band, called valence band (vb), and the lowest empty band, called conduction band (cb), are separated by a bandgap (E_g). The band gap of a semiconductor determines the wavelength range of the absorbed light. Under illumination with photons of energy equal or larger than the band gap, electrons in the valence band are excited to the conduction band, generating highly reactive electron-hole pairs (h⁺) (Figure 1, I). This electron-hole pair can evolve to form independent holes and electrons (Figure 1, II) or collapse by recombination (Figure 1, III). The photoinduced electrons and positively charged holes can migrate (Figure 1, IV) to the particle surface and participate in charge transfer

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reactions to reduce or oxidize species absorbed on the surface of the semiconductor (Figure 1, V). Electron transfer can occur between molecules at the surface or between a surface site and an adsorbate molecule. The semiconductors with either too wide or too narrow band gaps are unsuitable for practical use.

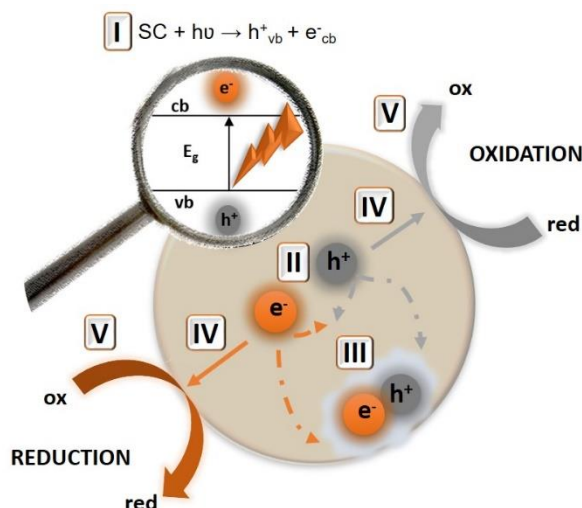


Figure 1. Schematic representation of heterogeneous photocatalytic processes occurring on an irradiated semiconductor (SC).

2.2. Types of semiconductors

Many semiconductor solid materials have been tested as photocatalysts. Basic prerequisites to fulfill this task are stability and durability under irradiation, as well as a high crystallinity and large surface area. Although metal chalcogenides exhibit a high initial photocatalytic activity, many of them cannot be used as general photocatalysts because they tend to undergo photocorrosion, and in aqueous phases they might dissolve under irradiation. Nevertheless, a number of metal chalcogenides can be safely used as photocatalytic semiconductors. They present adequate bandgap energies to be excited by UV, and eventually, by visible light, and the redox potentials of the edges of the vb and cb can promote a series of oxidative or reductive reactions. For instance, TiO_2 , ZnO , ZnS , CdS , Cu_2O and iron oxides have demonstrated their utility as photocatalytic materials (Figure 2).

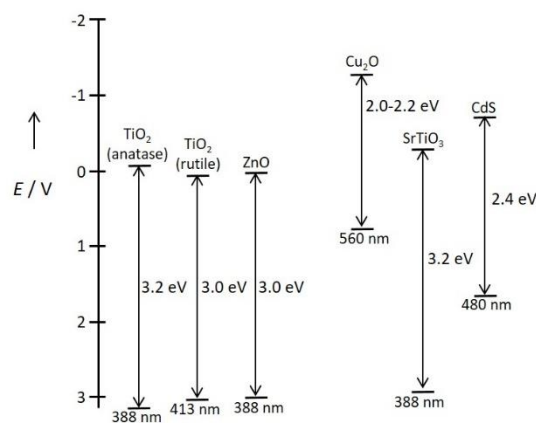


Figure 2. Valence and conduction bands, and wavelength thresholds for various semiconductors.

2.3. TiO_2 and modifications

Among the plentiful semiconductor materials, TiO_2 has been regarded as one of the most relevant for photocatalytic purposes, owing to its exceptional optical and electronic properties, ready availability and low cost, chemical and thermal stability, resistance to photocorrosion, high photocatalytic activity, and biological compatibility.^[7] However, the effectiveness of titania as photocatalyst depends on its crystal phase, impurities, particle size, surface area, crystallinity and other physicochemical parameters that strongly influence charge recombination and electron/hole trapping.

The practical application of TiO_2 is limited by two main factors. First, it suffers from inefficient utilization of solar energy, given that its wide band gap (over 3 eV) only allows a small portion of the solar spectrum to be absorbed (3–5%). Second, it presents a low photocatalytic quantum efficiency because the excited electrons and holes generated after irradiation can be readily deactivated by recombination, before they migrate to the surface to initiate redox reactions.

In view of these drawbacks, there has been a lot of work trying to improve the efficiency of photocatalytic processes promoted by TiO_2 , either by narrowing the semiconductor band gap (E_g) or by avoiding the recombination of the photogenerated electrons/holes.^[8] Most of the strategies have consisted of modifying the materials, but changing the operational conditions (solvent effect, additives, aeration, temperature, light source, pH value or reaction time) can also have a significant impact in the effectivity of the photocatalysis (Figure 3).

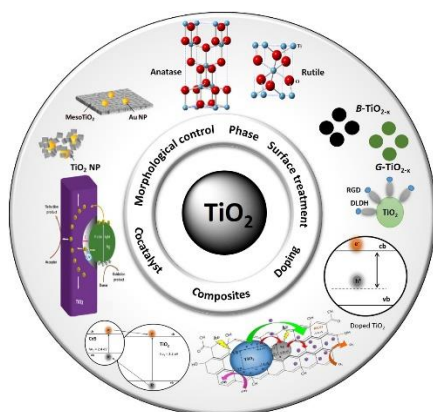


Figure 3. Examples of TiO_2 modifications. Images reproduced with permission from ref. 8b, 12b, 12e, 14.

The morphology has a strong influence on the photocatalytic performance of heterogenous materials like TiO_2 , due to the distinct active sites, facets, and associated adsorption-desorption ability of the reactants. For instance, using well-defined mesoporous or crystalline nanostructures can significantly affect not only the activity of the catalysts but also the selectivity, owing to confinement effects.^[9] Research attention is being increasingly directed towards engineering the surface structure of TiO_2 on the atomic level (namely morphological control of {001} facets at the range of micro and nanoscale).^[10] It is important to note that TiO_2 exists in two main crystallographic forms, *anatase* and *rutile*. While anatase presents an energy bandgap of 3.23 eV (388 nm), rutile exhibits a value of 3.02 eV (413 nm). Anatase has been found, in most of the cases, to be photocatalytically more active than rutile, likely because in the rutile phase the conduction band edge is at a relatively less negative position, which lowers oxygen adsorbing capacity and increases the recombination of the short-lived photocharges.

Besides, a large variation in the photocatalytic activity of the material depending on the origin of the titanium oxide has been observed. To avoid reproducibility issues, commercial TiO_2 from Degussa under the name P-25 (about 80% anatase, 20% rutile) prepared by spray pyrolysis, has become standard in the field. Photocatalytic reactions take place on the surface of the catalyst.^[11] Therefore, the selective adsorption ability of the photocatalytic material toward reactants and products is quite crucial for a selective photoreaction. It is possible to enhance the selectivity by surface adjustment,^[11a] e.g., modifying the surface charge/functional groups, or constructing specific structures. Actually, a weaker interaction between the reaction product and the photocatalyst might benefit the reaction, because the desired products can easily desorb from the photocatalyst surface once the transformation is achieved.

A conceptually different approach to enhance the solar absorption is based on the introduction of disorder in the surface layers of nanophase TiO_2 through hydrogenation. In this way, disorder-engineered TiO_2 nanocrystals, namely black titania nanoparticles, exhibit better solar-driven photocatalytic

activities.^[11b]

In order to photoreduce a chemical species, the cb of the semiconductor must be more negative than the reduction potential of the chemical species. In contrast, to photo-oxidize a chemical reactant, the potential of the vb of the semiconductor must be more positive than the oxidation potential of the chemical species. Doping TiO_2 with metals (Fe, Cu, Mo, Ru, Co, Rh, etc.), or nonmetal elements (B, C, N, F, S, etc.), usually leads to more efficient and selective redox processes. For instance, as it is displayed in Figure 4, after doping, the vb of TiO_2 presents a lower redox potential, suppressing the oxidation of OH^- by a photogenerated hole.

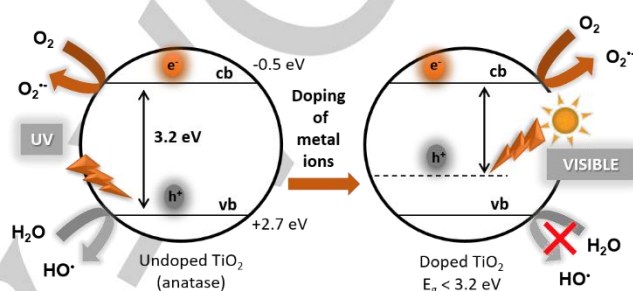


Figure 4. Schematic representation of the TiO_2 doping of metal ions: narrowing the bandgap and shifting light absorption into visible light range.

The use of cocatalysts can improve the charge separation and charge transfer processes, and also enhance both the reactivity and stability of the semiconductors. Metals, especially noble metals (i.e. Au, Ag, Pt or Pd), are the most often used cocatalysts due to their unique electronic, optical, and magnetic properties. Upon UV irradiation, small nanoparticles of these metals can act as electron traps to capture the conduction band electrons generated in the light absorbing TiO_2 , thereby decreasing the electron-hole recombination rates and enhancing lifetimes (Figure 5A).

Importantly, some noble metal nanoparticles (NPs) present the ability to strongly absorb visible or even NIR light due to surface plasmon resonance (SPR) effects.^[12] Indeed, metal NPs effectively act as miniature antennae to absorb light and inject electrons into the conduction band of the semiconductor (Figure 5B).^[13] The visible light absorbing characteristics of these systems can be particularly useful for biological applications.

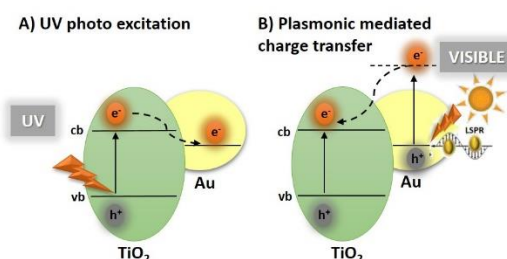


Figure 5. Schematic representation of charge photogeneration and transfer under A) UV and B) visible light irradiation, using a Au/ TiO_2 photocatalyst.

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The TiO_2 photoactivity can also be enhanced when used in the form of composites. For instance, in TiO_2 /carbon composites, the electron-hole pairs are generated upon TiO_2 excitation under UV light irradiation. These photogenerated electrons are then injected into the carbon material which acts as an electron sink (TiO_2 -C60, Figure 6A). Different carbon-materials, such as graphene (GR), carbon nanotubes (CNT) and C60 have been studied.^[14] In fact, TiO_2 /graphene composites have been established as viable photocatalysts due to the excellent electron transport properties of graphene, which decreases the recombination of electron and hole pairs. The presence of many oxygen-bearing functional groups in graphite oxide [GO] and reduced graphene oxide [rGO] helps TiO_2 to anchor on graphene sheets.^[15]

The combination of a CdS core with a TiO_2 semiconductor has been also extensively studied (Figure 6B).^[16] In this case, the irradiation is absorbed by CdS which transfers the electron to the conduction band of TiO_2 .

Z-scheme photocatalytic systems are an alternative and promising strategy for the improvement of photocatalysts, by

reducing the band gap of the semiconductors, while making the CB potential more negative and the VB potential more positive (Figure 6C).^[17]

3. Photocatalytic use of TiO_2 nanomaterials in aqueous and biological media

Many applications of heterogeneous photocatalysis are in the topic of environmental decontamination, degradation of pollutants and water purification,^[6e-g] but there is an increasing number of reports on their use for chemical reactions, and for biological or medicinal purposes, for instance, for the inactivation of microorganisms. Photodynamic therapy (PDT), which takes advantage of the generation of cytotoxic reactive oxygen species (ROS) under irradiation of TiO_2 surfaces, and photobiocatalysis, which combines both enzymatic and photocatalytic strategies, are promising biomedical applications.

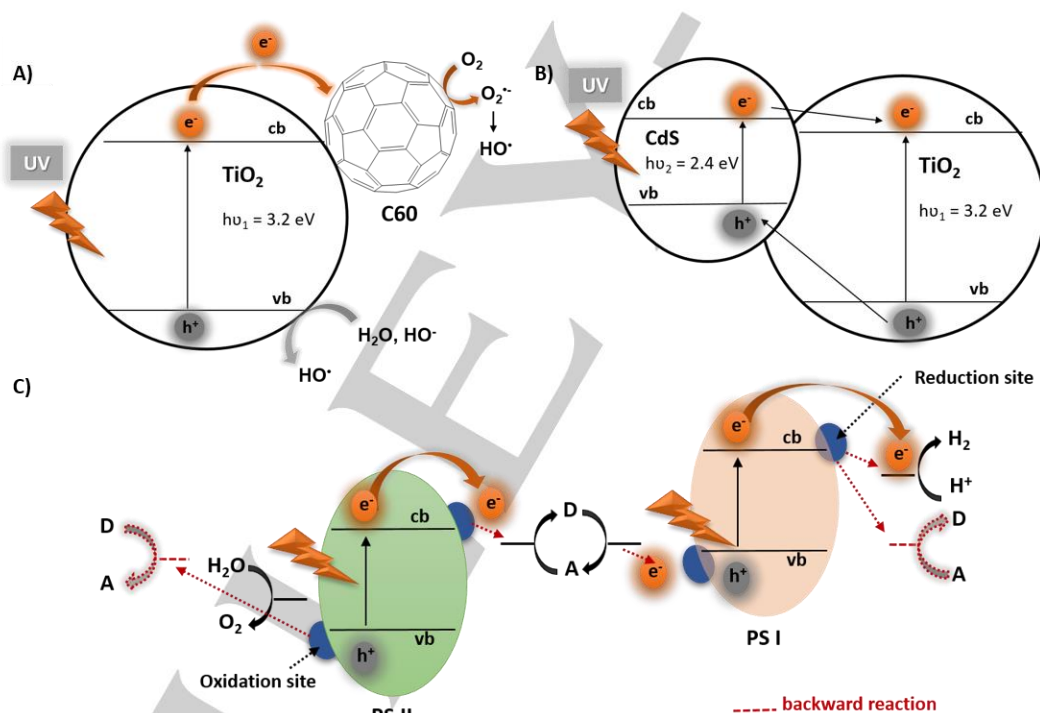


Figure 6. Schematic representation of the mechanism of excitation and charge transfer process between: A) C60 and TiO_2 in TiO_2 -C60 composites; B) CdS and TiO_2 in CdS- TiO_2 nanocomposites; C) First generation liquid-phase Z-scheme photocatalytic system.

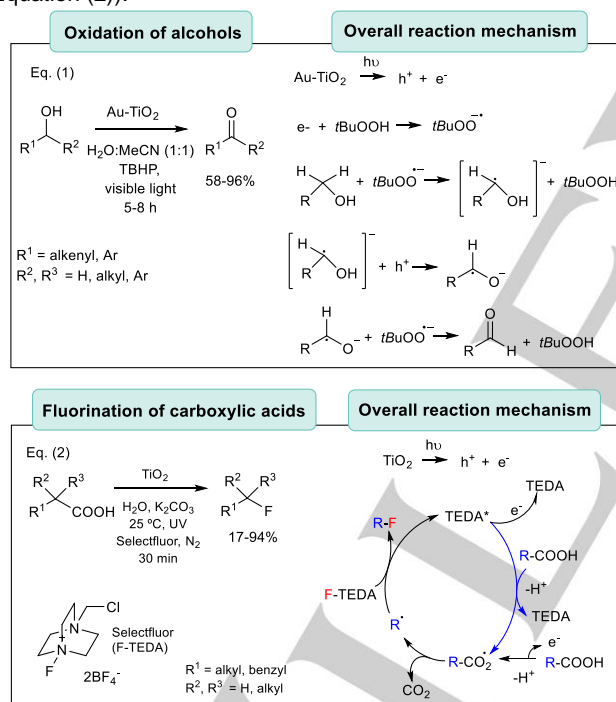
Since no external energy other than light is required, particles of titanium dioxide can be incorporated into biological systems and promote efficient redox processes, which take place with good reaction rates owing to the large surface area of TiO_2 .

3.1. Chemical transformations

In contrast to homogeneous photocatalysis, which has been extensively applied for synthetic purposes, applications of heterogeneous photocatalysis to promote chemical reactions are much less common.^[18] This is likely due to the notion that photocatalytic reactions promoted by semiconductors are difficult to control in terms of reactivity and selectivity. However, there are already several examples that demonstrate the viability

of using semiconductor photocatalysts to induce organic transformations.^[19] Some specific reactions promoted by TiO₂ are the oxidation of aliphatic and aromatic systems,^[19c,d] or alcohols,^[19e] the conversion of lactic acid into acetaldehyde,^[19f] the synthesis of α -chloro aryl ketones from aryldiazonium salts and terminal alkynes,^[19g] Ullmann reactions,^[19h] the conversion of amines to imines,^[19i,j] the N-alkylation of amines with alcohols,^[19k] the addition of amines to deficient alkenes,^[19l] heterocyclic functionalizations,^[19m,n] the Huisgen [3 + 2] cycloaddition of azides and alkynes,^[19o,p] or the addition of thiols to alkenes.^[19q]

An additional challenge in the area is associated to the possibility of performing heterogeneous photocatalytic transformations in aqueous media,^[20] or in biological related environments. Reports in this topic include the dechlorination of chlorobenzene to benzene (with core-shell Au@Pd/TiO₂),^[21] the reduction of dinitrophenyls,^[22] the degradation of nitrophenols,^[23] the oxidation of aromatic compounds,^[24] the oxidation of alcohols to aldehydes and ketones,^[25] (with Au/TiO₂ anatase, Scheme 1, Equation (1))^[25a] or the decarboxylative fluorination of carboxylic acids at room temperature with electrophilic fluorine reagents (Selectfluor, with P25, Scheme 1, Equation (2)).^[26]



Scheme 1. Examples of photocatalytic transformations with TiO₂ carried out in aqueous media, and mechanisms proposed by the authors.^[25a,26] The mechanism in Equation (1) is that proposed by the authors, but might need to be revised.

3.2. Oxidative degradations

The use of TiO₂ as semiconductor for degradation purposes is based on its ability to generate ROS under UV irradiation (Figure

7).^[27] Following UV excitation, the photo-generated electrons in the conduction band can be trapped by molecular oxygen (O₂) adsorbed on the titania particle, which is reduced to give a highly oxidizing superoxide radical anion (O₂^{•-}) (Equations (3)) that may further react with H⁺ to generate hydroperoxyl radical ([•]OOH) and hydrogen peroxide (H₂O₂) (Equations (4-5)).

These reactive oxygen species can contribute to the oxidative degradation of pollutants (Equation (6-7)). Meanwhile, the photo-generated holes can oxidize a water molecule or OH⁻ to yield the hydroxyl radical ([•]OH) which is a powerful oxidant (Equation (8)). The hydroxyl radicals can even produce mineralizations to CO₂ and H₂O (Equation (9)).

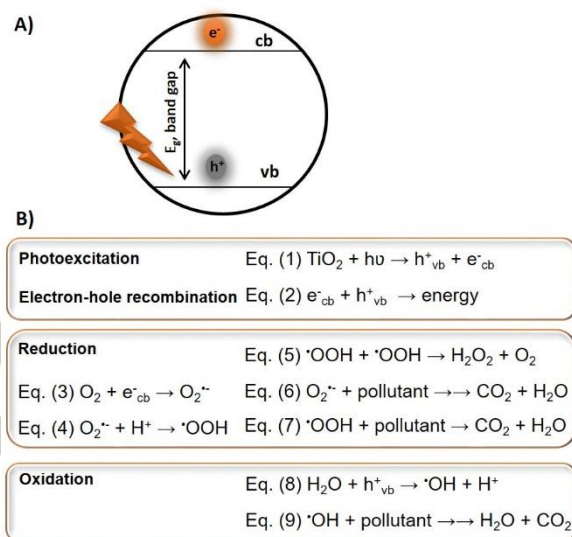


Figure 7. Schematic representation of the generation of reactive oxygen species (ROS) under UV irradiation.

TiO₂-based semiconductors are therefore being used for the photochemical degradation of a large variety of organic and inorganic pollutants such as dyes, pesticides, hydrocarbons, drugs, etc.^[28] There have been numerous reports on the photodegradation of dyes like methylene blue, methyl orange, rhodamine B, brilliant green phenol or acid red, using TiO₂-based photocatalysts under UV/visible light.^[29] Also, carbon-sensitized and nitrogen doped TiO₂ (C/N-TiO₂) have been employed for decomposing sulfanilamide (SNM) in which nitrogen doping is responsible of the absorption visible light shift of C/N-TiO₂.^[30] Zhang et al. reported humic acid removal from water through photocatalytic decomposition using hybrid Fe₂O₃/TiO₂ nanowires.^[31] Fe₂O₃ plays an important role in the biphasic catalyst by improving humic acid adsorption, increasing electron-hole separation by interfacial charge transfer, and absorbing visible light. Under UV-light irradiation, electron-hole pairs are generated on both TiO₂ and Fe₂O₃ (Figure 8A). Water oxidation by holes produces OH radicals, which in turn decompose humic acid molecules. Under visible-light irradiation, Fe₂O₃ absorbs visible light and transfer conduction band electrons to electron trapping sites of anatase TiO₂ (Figure 8B).

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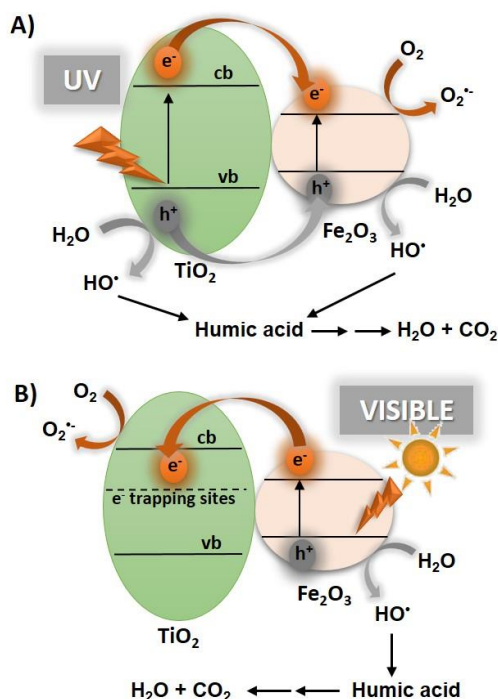


Figure 8. Proposed mechanism for the photocatalytic degradation of humic acid on $\text{Fe}_2\text{O}_3/\text{TiO}_2$ hybrid nanowires under A) UV and B) visible light irradiation.

TiO_2 -based photocatalysts have also been used for the photodegradation of antibiotics in water media.^[32] For example, Poullos et al. found that commercial TiO_2 P25 is able to photocatalytically degrade chloramphenicol under UV light irradiation (in 90 min).^[33] Similarly, Zhou, Vilar and Mantzinos also reported the high efficiency of TiO_2 towards the photocatalytic degradation of tetracycline, oxytetracycline and sulfamethoxazole solution.^[34] Since then, different drugs, like atorvastatin calcium, diclofenac sodium, fluoxetine, ketoconazole, ibuprofen, dexamethasone, fluvoxamine, tioconazole, naphazoline hydrochloride, valsartan, guaifenesin or paracetamol, have been degraded under UV and visible radiation in the presence of supported titania-based catalysts.^[35] In all cases, it has been suggested that the degradation processes are initiated by ROS species formed by electron transfer between the excited photocatalyst surface and water.^[32]

3.3. Photoinactivation of microorganisms

The use of TiO_2 photocatalysts for the inactivation of microorganisms, also as part of decontamination tactics, is catching up an increasing attention of the scientific community.^[36] Heterogenous photocatalysis offers several advantages over other alternatives owing to the capability of achieving simultaneous oxidative and reductive reactions, the low cost of the materials, and the possibility of using solar light. Among all microbes, the presence of bacteria in water is considered an indicator of water pollution. Since the introduction

of the novel concept of photochemical sterilization by Matsunaga et al.,^[37] many photochemical bacterial inactivation studies using TiO_2 have been reported.^[36,38] Researchers have demonstrated that nano- TiO_2 particles can show a strong disinfecting activity against both Gram-negative (*E. Coli*, *Salmonella*, *Shigella*, *Pseudomonas*, *Cyanobacterias* or *Baumannii*) and Gram-positive pathogens (*Lactobacillus acidophilus*, *Streptococcus* or *P. Aureus*) using UV or visible light.^[39]

The mechanism proposed for the antibacterial activity is summarized in the Figure 9A (*E. Coli*).^[36a,40] The oxidation of components of the cell wall favors the entrance of ROS in the cell, where they negatively interfere with DNA replication and respiratory activity. Ultimately, ROS attack leads to the loss of cell viability and cell death. Gram-positive bacteria are more resistant to photocatalytic inactivation than Gram negative. The effectiveness of TiO_2 for the photocatalytic degradation of bacteria is highly influenced by the crystal phase and particle size of the material. For instance, smaller TiO_2 nanoparticles with an anatase phase have higher affinity to the cell surfaces and induce a heavier oxidative damage and toxicity to the bacterial cells. The bactericidal effect is also highly correlated with the dispersion state of the TiO_2 nanoparticles in the medium. Indeed, hybrid titania nanoparticles suspended in aqueous solution have a high specific surface, therefore providing a better accessibility to bacteria cells and a more efficient effect of the UV irradiation than TiO_2 nanopowder. TiO_2 thin films have also been studied.^[41]

Matsunaga et al. found an *E. Coli* inactivation of 99% in deionized water using a solar simulator and a TiO_2 -immobilized acetylcellulose membrane.^[42] Doping can also shift the bacteria inactivation to visible light. This effect has been studied with nonmetals,^[43] as well as with metal ions such as Ni^{2+} , Cu^{2+} , Cd^{2+} .^[44] The beneficial effect of the noble metal is also observed with titania loaded with either gold or silver, which exhibits enhanced photocatalytic killing of bacteria under visible light illumination due to the localized surface plasmon resonance (LSPR) properties.^[45] For instance, Ag/TiO_2 -N photocatalytic systems have allowed the inactivation of *A. Baumannii* bacteria.^[45b]

More recently, an organic-inorganic-based graphitic carbon nitride ($\text{g-C}_3\text{N}_4$)/ TiO_2 has been shown to inactivate bacteria under visible light irradiation (Figure 9B).^[46] The enhancement is the result of a synergistic effect between $\text{g-C}_3\text{N}_4$ and TiO_2 , which improves light absorption and the effective separation of photo-generated electron-hole pairs as previously commented. Other examples involve the use of graphene nanosheet- TiO_2 composites,^[47] TiO_2 - Bi_2WO_6 ,^[48] TiO_2 - Al_2O_3 films^[49] or CdSe quantum dots/graphene/ TiO_2 nanosheets composites.^[50]

Similarly to the inactivation of bacteria, photoexcited TiO_2 can cause damage to fungi.^[51] For instance, it has been shown that rutile- TiO_2 nanorod arrays present activity against *Candida albicans* when irradiated with UV light.^[52] Nevertheless, the rigid cell wall of filamentous and unicellular fungi, composed mainly of soluble and insoluble polysaccharide polymers, make them more resistant than bacterial cells to ROS attack.

A common surrogate virus, such as bacteriophage MS2^[53] can be inactivated by composite SiO_2 - TiO_2 nanomaterials. These

composites have markedly higher photocatalytic inactivation rates (up to 270%) compared to unmodified TiO_2 , due to an improved adsorptive density. Doping TiO_2 nanoparticles with noble metals like silver also leads to a higher photocatalytic

activity for virus inactivation,^[54] primarily by allowing a higher $\cdot\text{OH}$ production, necessary for the degradation.

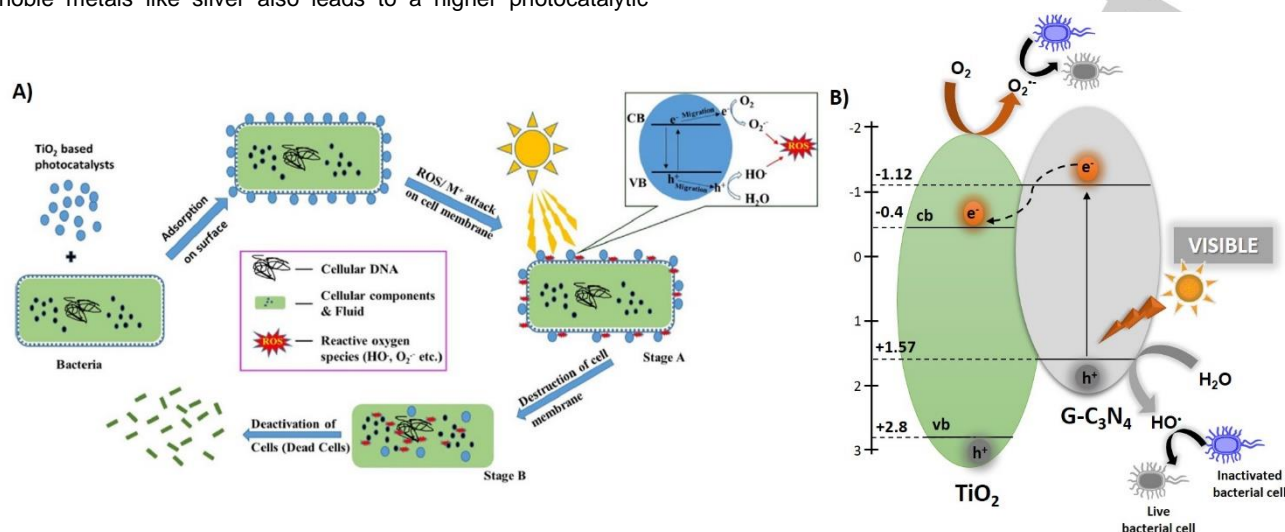


Figure 9. A) Possible mechanism of the photocatalytic inactivation of bacteria. Part A is reproduced with permission from ref. 36a; B) Schematic representation of a photocatalytic antibacterial process with graphitic carbon nitride TiO_2 composites.

Much more challenging is the application of TiO_2 photo disinfection to the destruction of more resistant protozoa. *Cryptosporidium parvum* and *Giardia lamblia* are two of the most chlorine resistant microorganisms with notable adverse effects on humans studied to date. García et al. demonstrated that waters containing these two protozoa at low concentrations can be efficiently disinfected in continuous flow by using a commercial fibrous ceramic TiO_2 photocatalysts.^[55] The efficiency of the photocatalytic disinfection is largely enhanced by adding a small concentration of chlorine. Under the same conditions and radiance power, UV light without any photocatalyst is significantly less efficient.

3.4. Photodynamic therapy

TiO_2 as photosensitizer for PDT. Photodynamic therapy (PDT) is used in biomedicine for different purposes. It is based on the use of photosensitizers molecules (PS), which are noncytotoxic in the dark, but can be activated with light of a specific wavelength. This activation triggers a local generation of cytotoxic species (ROS, which apart from the radicals mentioned along this review also includes singlet oxygen),^[27,56] which can lead to the damage and destruction of the target cells, including tumor cells. Conventional photodynamic therapy is restricted to the treatment of superficial and flat lesions as most of the available PSs are activated by near UV and visible light (400–700 nm), which has limited tissue penetration depth. For deeper penetration of light, wavelength of the irradiated beam should better be in the near-infrared (NIR) window (700–1100 nm). Recently, nanomaterials with photocatalytic behavior are attracting much attention as promising photosensitizers to develop new photodynamic therapeutic strategies. While titanium dioxide shows very weak or no toxicity in vitro and in

vivo, which corroborates its high biocompatibility, it can become cytotoxic when irradiated with UV light. This is because of its photocatalytic property to generate reactive oxygen species (ROS) under UV irradiation. Thus, TiO_2 -based biomaterials are promising candidates for their medical use in PDT.

Fujishima et al. were the first to report the photokilling of malignant HeLa cells grown on a polarized illuminated TiO_2 film electrode.^[57] Five years later, they replaced the electrodes by small TiO_2 nanoparticles and demonstrated that they could be incorporated into the cancerous cells by phagocytosis over a 24 h incubation period, showing no cytotoxicity.^[58] However, they could sensitize the photodamage and suppress the tumor growth of the cells after irradiation.^[59] Other work demonstrated that the photokilling action of TiO_2 particles could be enhanced through the addition of superoxide dismutase, which converts superoxide radicals into hydrogen peroxide.^[60]

Since these discoveries, TiO_2 has been subjected to intensive research as a photosensitizer for PDT. Titania has proved its phototoxicity upon UV light exposure against different type of cancer cells (e.g. cervical cancer cells (HeLa), glioma cells (C6, GMB, U87), bladder cancer cells (T24), leukemic cells (HL60, THP-1 U937) or colon carcinoma cells (LoVo Ls-174-t)).^[61]

Despite the above interesting PDT properties of TiO_2 nanoparticles, they still present important drawbacks for clinical use, such as insufficient selectivity and low efficiency because of the lack of cell-specific accumulation of TiO_2 on cancer cells. Hence, it is necessary to modify the nanoparticles with biomolecules that can specifically target cancer cells for improving efficiency and selectivity. Moreover, visible- and NIR responsive particles are highly desirable.

TiO_2 phototoxicity depends not only on the photocatalyst concentration and light dose but also on its morphology. For that reason, the development of nanomaterials with different

structures has been addressed (e.g. nanoparticles vs. nanotubes or nanorods). As recently shown by Zhang et al., biocompatible 1D inorganic nanomaterials like TiO₂ nanofibers with mixed phases have been employed for inducing cytotoxic effects in HeLa cells.^[62] Unlike TiO₂ nanoparticles, which tend to aggregate and lack the ability to accumulate specifically in cancer cells, TiO₂ nanofibers can disperse quite well in aqueous solutions and attach on cell surfaces. More importantly, these nanofibers exhibit a high killing efficiency, which is attributed to the inhibition of recombination of photo generated electrons and holes, due to a stable interface between phases in a single nanofiber. This promotes efficient charge separation and electron transfer processes to produce more ROS.

One important aspect to be considered is that the effectiveness of PDT is quite limited in hypoxic environments, such as solid tumors. During PDT, molecular oxygen is rapidly consumed by many organic PSs to produce cytotoxic reactive oxygen species, creating transient hypoxia that further decreases the therapeutic effects. For that motive, Achilefu et al. have designed a ROS-producing hybrid nanoparticle-based photosensitizer capable of maintaining high levels of ROS under both normoxic and hypoxic conditions (Figure 10). Conjugation of a ruthenium complex (**N₃**) to a TiO₂ nanoparticle affords TiO₂-N₃, and upon exposure to light the ruthenium complex can inject electrons into TiO₂, which allows to increase the production of ROS.^[63]

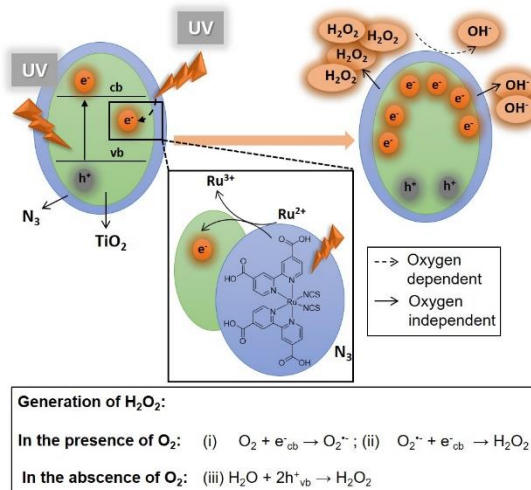


Figure 10. Schematic representation of electron injection to TiO₂ by N₃, enhancing H₂O₂ and ·OH production.

Selectivity. The absence of selectivity of bare TiO₂ lacking specific targeting ligands, can induce damage to surrounding healthy tissues, which restricts its applicability. Therefore, there have been many efforts to produce targeted-delivery nanocomplexes that produce controlled cytotoxicity. For instance, TiO₂ nanoparticles have been linked to integrin derivatives making PDT more selective. Since cancer cells often overexpress integrin receptors (e.g. αvβ3) on their cell surface, by introducing an Arg-Gly-Asp (RGD) recognition motif, Fabian and coworkers have developed an integrin-targeted nanomedicine for PDT. This nanobiocomplex is UVA switchable,

highly selective towards cancer cells and quite effective in producing cell death (Figure 11A).^[64]

Another example is based on the preparation of antibody–TiO₂ bioconjugates. Simply by changing the type of antibody, various kinds of cancer cells could be targeted. This method shows a high efficiency for photokilling LoVo cancer cells, even using very low concentration of the antibody–TiO₂ bioconjugate.^[65] Human brain cancer cells (GMB cells) have been also targeted following this strategy (Figure 11B and C).^[66] Folic acid (FA) has also been used with nitrogen-doped TiO₂ nanoconjugates as an effective targeting agent for killing KB cells under visible light irradiation.^[67]

Expanding the spectrum from UV to visible and IR wavelengths. The requirement of UV light for activating TiO₂ has been a major impediment to its practical clinical usage. Besides causing damage to cells and tissues, UV light is strongly absorbed and scattered by tissues, thereby limiting its penetration depth to only fractions of a millimeter. Clinical application of TiO₂ would thus be restricted only to superficial malignancies, or those at cavitary sites whose accessibility could only be reached by optical fibers delivering the UV light. Therefore, certain modifications of this material leading to the improvement of its photosensitizing properties in the extended spectrum from UV to visible light or even NIR have been explored.

The development of new TiO₂-based photosensitizers which can be excited with longer wavelengths is mainly based on doping approaches. Doped constructions with Fe, Pt or Zn,^[68] or N^[69] have demonstrated photokilling efficiency on a series of cell lines (murine melanoma cells (B16-F10), fibroblasts (NIH 3T3), human cervical carcinoma cells (HeLa) and leukemia cells (K562)).

Ag and Au nanoparticles can by themselves exhibit also photothermal toxicity due to their plasmonic absorption. Satisfactorily, the photocatalytic cytotoxicity of Ag@TiO₂ requires only one-fourth of the Vis light irradiation time than TiO₂ alone at the same concentration, presenting a three-times-higher killing efficiency.^[70]

Also, Ag and Fe have been combined in Ag@Fe-doped TiO₂ composite nanoclusters, and exhibit higher photocatalytic killing activity than Fe-doped TiO₂ when irradiated under visible light. The promoting effect of the Ag nanoparticles deposited on the Fe doped TiO₂ surface shows strong absorption in the visible region due to localized surface plasmon resonance of Ag, and inhibits the recombination of photoelectrons and holes better than Fe-doped TiO₂.^[71]

Dyes such phthalocyanines (Pc) have also been used to increment the absorption of TiO₂ nanoparticles in the visible region, and the photokilling activity of this combination has been evaluated in different cell lines.^[72] Also, other light-sensitive photosensitizers such Chlorin e6 have been linked to TiO₂ NPs for PDT in glioblastoma U87 cells.^[73]

A composite architecture with a Zn-metalloporphyrin self-assembly nanocrystal coated by an homogeneous TiO₂ layer has been described as an excellent photosensitizer.^[74]

Nanocomposites composed of TiO₂ and reduced graphene,^[75]

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CdS^[76] or PtCl₄^[77] are excellent PDT visible light photosensitizers and have demonstrated a relatively high antiproliferative efficiency in HepG2, leukemia HL60 or mouse melanoma cells (S-91), respectively.

The use of 'invisible' near-infrared (NIR) light affords greater tissue penetration depth because most tissues are 'transparent' to this wavelength. As previously commented, it is possible to introduce structural changes in TiO₂ in order to facilitate a NIR absorption of the semiconductor. For instance, by strong ultrasonication, black titania *B*-TiO_{2-x} is facily and irreversibly converted into stable green *G*-TiO_{2-x}, which presents enhanced NIR absorption (920 nm).

Chemical modifications of TiO₂ can also expand the response range to the near infrared (NIR) light region. For instance, the employment of noble metals is reflected in an interesting example with black anatase TiO_{2-x} nanotubes modified with Au clusters, which serve as photosensitizers.^[78] When excited by

NIR light, the nanoplatform shows a noticeable improved therapeutic efficacy *in vivo* and *in vitro*.

The targeting-PDT strategy has also been used in the case of NIR-responsive systems. Very recently, mitochondria-targeted PDT has emerged as new approach for increasing therapeutic efficiency and safety, minimizing side effects.

In this sense, black titania was synthesized from P25 (pristine titania) through an aluminum reduction method. Then it was transformed into *G*-TiO_{2-x} by strong ultrasonication and subsequently conjugated with triphenylphosphonium (TPP), a cation that selectively accumulates in the mitochondria. Both *in vitro* and *in vivo* results demonstrate the capability of *G*-TiO_{2-x} with TPP functionalization for simultaneously mitochondria-targeting and PDT, under NIR laser irradiation, and using a low dosage (Figure 11D-E).^[79]

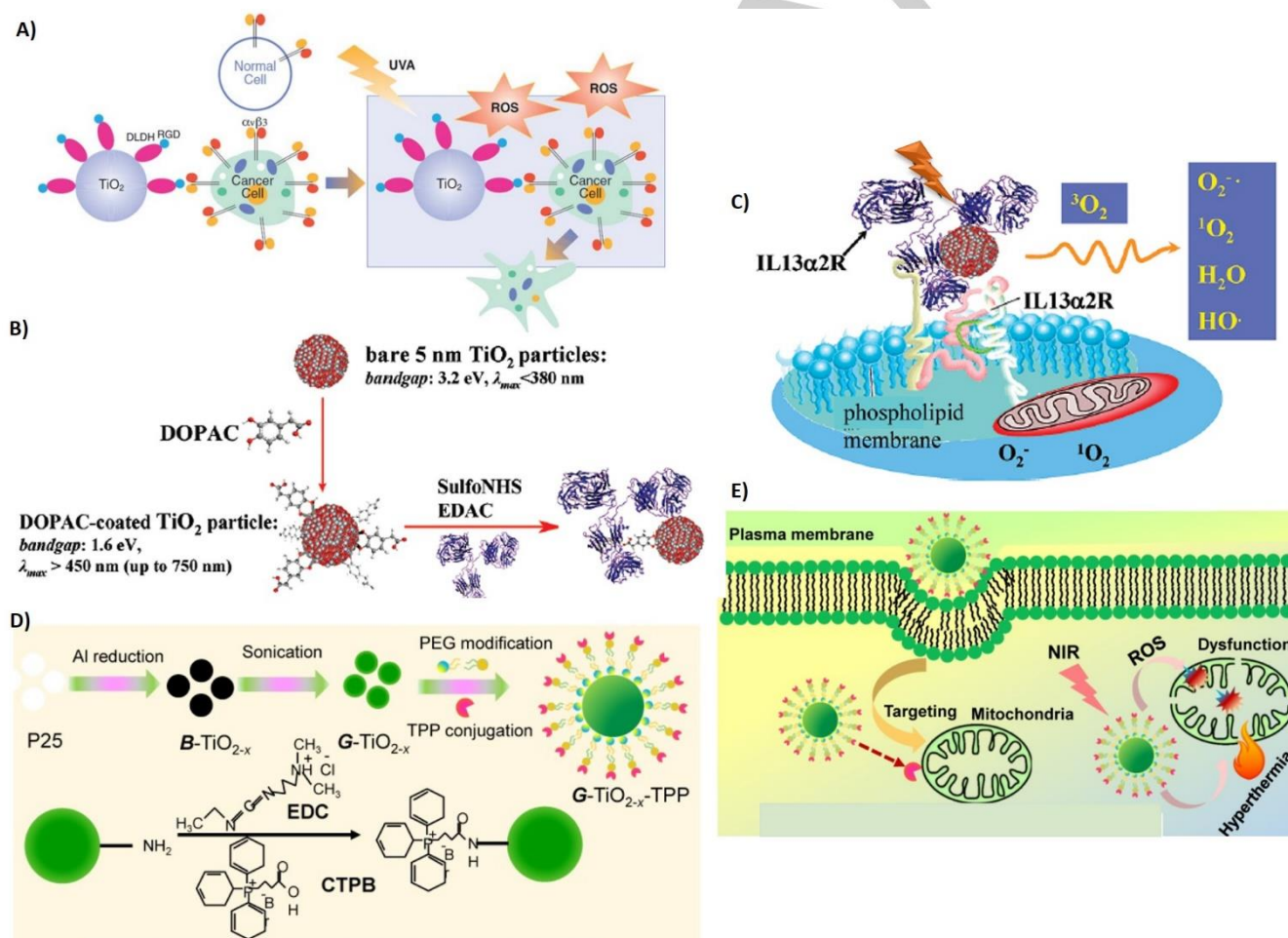


Figure 11. A) Integrin targeted PDT by the nanobiocomplex upon UV illumination. Part A is reproduced with permission from ref. 64; B) Nanobiocomposites composed of 5 nm TiO₂ and IL13R-recognizing antibody linked via DOPAC linker to recognize and bind exclusively to surface IL13R; C) Visible light photoexcitation of the nanobio hybrid in aqueous solution. Parts B,C are reproduced with permission from ref. 66; D) Schematic representation of the preparation and surface modification of green titania (*G*-TiO_{2-x}); E) Mitochondria-targeted cancer phototherapy. Parts D,E are reproduced with permission from ref. 79.

An alternative to match the deeply penetrating NIR light with the absorption spectrum of semiconductors at UV range, is the employment of a light transducer that can convert NIR to UV light (UCN upconversion nanoparticles).

Nano-materials made of rare earth metals have the unique ability to absorb NIR light and emit higher energy light in the UV/visible spectrum based on the process termed 'upconversion'.^[80] Besides utilizing safe and deeply penetrating NIR light, these upconversion nanoparticles are endowed with the advantage of highly unusual optical properties such as photostability, near-zero autofluorescence background and tunable emission colors to suit different activation wavelengths of diverse photoactivable compounds. This makes them a versatile yet powerful tool for diverse photoactivation and imaging applications.

Combination of upconversion (UC) materials with TiO_2 provides a good solution to increase the utilization of NIR light for TiO_2 photocatalysts. Recently, several attempts have been made to synthesize composite materials consisting of a TiO_2 shell and a UC core, being NaYF_4 the host material of choice due to its higher upconversion efficiency.

The upconversion of NIR to UV light requires spectra overlap between the emitted UV and the maximum absorption wavelength of the coated TiO_2 . The NIR responsive nanocomposites are effective in killing cancer cells in vitro, and even in vivo (Figure 12). However, some of their limitations are the lack of uniformity in size and shape, and the difficulty to control the amount of photosensitizer (PS) loaded in close proximity to the UCN constructs, which is very important for an efficient upconversion and generation of ROS.^[81] To overcome these limitations, researchers have used a continuous layer of PS (TiO_2) individually coated on each UCN core to give a well-defined core-shell structure with uniform size, shape and dispersion. This allows a controllable and uniform TiO_2 loading onto individual UCN cores, and eliminates the possibility of PS leakage, thus ensuring significant ROS generation for effective and repeatable PDT.^[82]

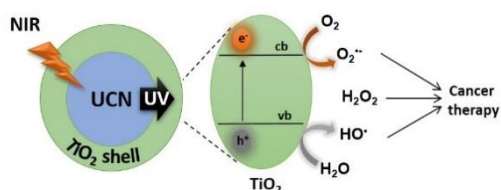


Figure 12. Schematic diagram of TiO_2 -UCN composites, and their use for promoting cancer cell death.

In order to overcome the overheating generated by a 980 nm irradiation, and to further improve the tissue penetration depth, Lin et al. have designed multifunctional TiO_2 nanoparticles replacing Y^{3+} by Nd^{3+} -sensitized UCNPs for 808 nm NIR light. The in vitro and in vivo biocompatibility and cytotoxicity against tumor cells of this multifunctional NIR-PDT nanoplateform has

been systematically investigated and compared with others that respond to light of other wavelengths. In vivo results indicate that a 808 nm NIR light-mediated PDT shows much higher antitumor efficacy than that observed with 980 nm and UV light, due to the better tissue penetration depth.^[83]

It is worthy to note that photodynamic therapy is not limited to cancer therapy, and can be also used for many other diseases, like rheumatoid arthritis (RA), or diabetes mellitus (DM), one of the more common human diseases resulting in hyperglycemia. Treatment with tetra sulphonatophenyl porphyrin (TSP) containing TiO_2 appears to produce an ameliorating effect in both cases, by significantly decreasing the IL-17 and TNF- α levels in blood serum in the RA^[84] or by lowering the blood glucose level in type II DM.^[85]

Combination of PDT with photothermal- and chemotherapy.

Photodynamic therapy (PDT) can be also combined with chemotherapy. Towards this end, the metallic NPs must combine a photoresponsive chromophore, a tumor-targeted biocompatible nanocarrier and an effective anticancer drug. Colocalization of all the components in the target cells usually requires encapsulation.^[86]

Singh et al. have designed a construction composed of three main ingredients, targeting folic acid decorated TiO_2 NPs, 8-dihydroxy coumarin as chromophore and the well-known chemotherapeutic drug chlorambucil. The system allows an efficient destruction of MDA-MB breast cancer cells with UV-vis light of ≥ 410 nm via the synergic effect of both PDT and chemotherapy (Figure 13A).^[86a]

Near infrared (NIR) light mediated upconversion nanoparticles have also been introduced in order to enhance the NPs anticancer efficiency.^[87] In this last case, a UV cleavable o-nitrobenzyl derivative linker (TC linker) has been prepared as "gate" to encapsulate anticancer agent doxorubicin (DOX) inside mSiO_2 . Under NIR irradiation, the UV emission can excite TiO_2 and also induce the photodegradation of the TC linker for drug release (Figure 13B).^[87c]

The gold-plasmonic photogenerated heat can also be used synergistically with the production of ROS in the cancer cell-killing activity of Au- TiO_2 nanocomposites.^[88] For instance, a novel plasmon enhanced PDT- and PTT (photothermal therapy)-controlled drug release system for cancer cell therapy has been recently presented.^[89] Indocyanine green (ICG) has been used as NIR photosensitizer conjugated site-specifically at the ends of gold nanorods (AuNRs). The surface plasmon resonance of the AuNRs is carefully tuned to overlap with the exciton absorption of indocyanine green (ICG). Because the AuNRs also cause local increases in temperature upon NIR excitation, DNA strands loaded with doxorubicin (DOX) were attached to the AuNRs sidewalls. It was found that the synergistic effect of generating $^1\text{O}_2$ and delivering DOX by photothermal activation leads to a significant improvement in antitumor effects (Figure 13C).

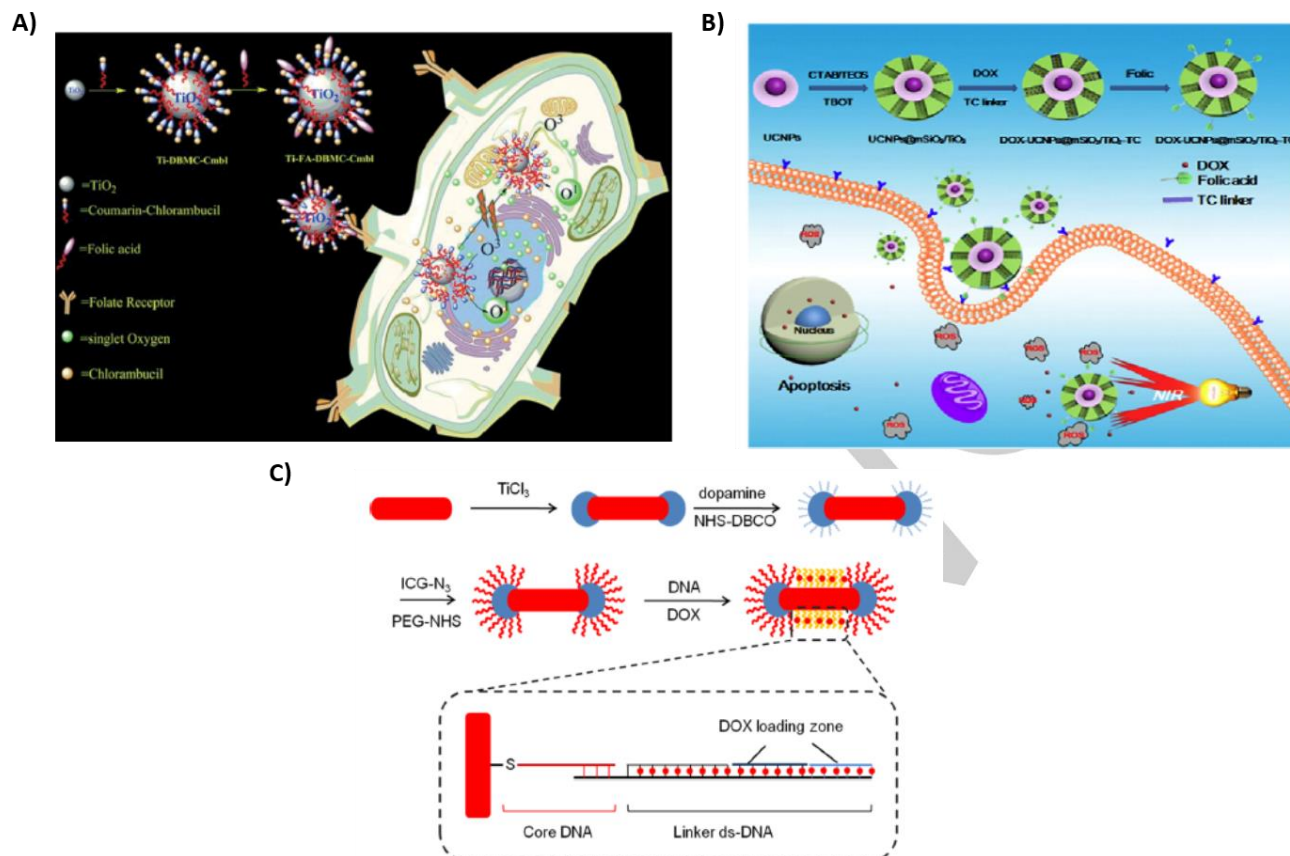


Figure 13. A) Schematic representation of the effect of combination therapy of folic acid decorated TiO_2 NPs loaded with a coumarin-chlorambucil conjugate on cancer cells. Part A is reproduced with permission from ref. 86a; B) Illustration of the synthesis and chemo/photothermal synergistic therapy under single 980 nm near-infrared light. Part B is reproduced with permission from ref. 87c; C) Schematic representation of the overall process to produce ICG and DOX-loaded TiO_2 -modified AuNRs. First, porous TiO_2 caps were synthesized at the tips of AuNRs (AuR@TiO_2). Next, ICG was site-specifically attached to TiO_2 by first reacting with dopamine followed by NHS-DBCO and azide functionalized ICG. The ICG-conjugated AuNR@TiO_2 was next reacted with thiolated CG-containing DNA strands that were then loaded with DOX. Part C is reproduced with permission from ref. 89.

4. Photobiocatalysis

Enzymes present in natural systems show extraordinary properties in terms of activity, specificity and efficiency, and therefore have found wide applications in the chemical, food and pharmaceutical industries. Most enzymes operate under benign aqueous conditions and at room temperature and generate products with exquisite selectivities. In recent years there has been an increasing interest in combining enzymes with photoresponsive materials in order to obtain smart catalysts with improved properties.^[90] In particular, the combination of high catalytic rates exhibited by enzymes with the outstanding photophysical properties of nanomaterials can provide catalytic methodologies involving electron transfer processes.^[91] The most widely used photobiocatalysts make use of conduction

band electrons of semiconductors, which are transferred to the redox center of enzymes. The photoactivation can be achieved through a direct (Figure 14, path II) or indirect transfer, using a mediator (Figure 14, path I).^[90b]

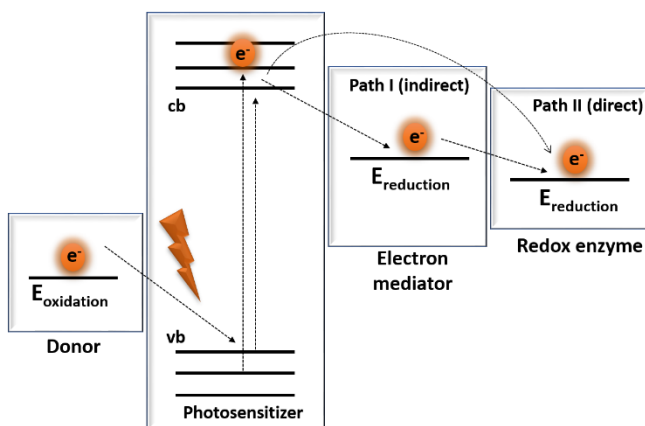


Figure 14. Schematic illustration of direct and indirect photoactivation of redox enzymes by photoinduced electron transfer.

4.1. Photobiocatalysis using mediators or cofactors

A number of cofactor-dependent enzymes such as oxidoreductases and transferases can catalyze complex reactions under mild conditions. Thus, numerous efforts have been made over the past decades to accomplish an *in situ* regeneration of the cofactor from their oxidized or reduced counterpart.

Nicotinamide adenine dinucleotide (NADH) is one of the most important cofactors used by oxidoreductases. Up to now, a number of strategies have been devised for the regeneration of NADH. Photochemistry is especially appealing because of the possibility of using clean and cheap solar energy. In the photochemical process for the regeneration of NADH, the photosensitizer is used as a dispersed semiconductor material, such as P-doped TiO_2 or CdS- TiO_2 .^[92] One of the first examples for *in situ* NAD(P)H regeneration is displayed in Figure 15.^[92b] It consists of TiO_2 -CdS nanotubes acting as photoelectrodes. CdS absorbs visible light and injects one electron into the conduction band of TiO_2 . This injection is thermodynamically favored due to more negative conduction band potential of CdS than TiO_2 . Due to its morphology, it allows a fast migration of the electron through the nanotube over long distances, favoring charge separation. These electrons are transferred to NAD^+ through a rhodium complex acting as a mediator. Finally, triethanolamine (TEOA), is used as a sacrificial electron donor.

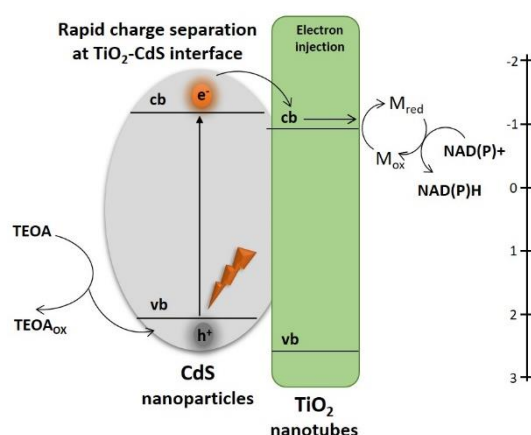


Figure 15. Schematic representation of the electron transfer pathway in the photoregeneration system of NADH by a TiO_2 -CdS nanotube.

4.2. Photobiocatalysis without mediators or cofactors

As an alternative to the above enzyme cofactor regeneration processes, it is also possible to design photoactive semiconductor constructs that directly exchange electrons with the active site of enzymes (Figure 14, path II).^[93]

Photobiocatalysts for hydrogen evolution. The generation of hydrogen from water is one of the hottest topics in science. In this field, platinum-containing semiconductors have been the main choice for many years;^[7] however, Pt suffers from several drawbacks such as the lack of selectivity (at low potentials it catalyzes the reduction of O_2) or its sensitivity towards poisoning inhibitors (e.g., CO).

Considering the well-documented efficiency of hydrogenases (H_2 ases) in electrochemical hydrogen generation,^[94] it is not surprising that these enzymes had been applied for the photobiological generation of hydrogen using TiO_2 as photocatalyst.

[FeFe]- and [NiFe]-hydrogenases are efficient natural catalysts that can catalyze the reduction of protons to H_2 at enzyme active sites composed of iron- or nickel/iron complexes.^[95] Some authors have identified a specially suited hydrogenase from *Desulfomicrobium baculatum*, *Ds* [NiFeSe]-hydrogenase, which not only binds strongly to TiO_2 , but also sustains a high electrocatalytic activity under mild conditions, an important requirement for the photocatalytic production of H_2 . It contains a terminal selenocysteine coordinated to nickel at the [NiFe]-active site and three [4Fe4S]-clusters for intraprotein electron transfer. These systems use either high-energy irradiation (UV) for band gap excitation or visible light sensitization with a dye complex, for injecting electrons into the conduction band.

For instance, an assembly consisting of TiO_2 -P25 as semiconductor and a ruthenium polypyridyl dye containing peripheral phosphonic groups to bind the TiO_2 surface as a light harvester, has been combined with a [NiFeS]-hydrogenase to produce H_2 . The system exhibits a turnover frequency of 50 (mol

H_2)^{s-1} (mol enzyme)⁻¹ at pH 7 and 25 °C, upon visible light irradiation. The enzyme is very stable (even after prolonged exposure to air), and does not require cofactors or mediators. However, a sacrificial electron donor such as TEOA or water is required (Figure 16A).^[96]

Another semi-biological system consisting of TiO₂ modified with polymeric carbon nitride and an immobilized H₂ase has been demonstrated to work as a photobiocatalyst for solar light driven H₂ production. By improving the surface interaction of the enzyme with the light harvesting CN_x material, specifically by adsorption of the enzyme onto the TiO₂ surface, H₂ generation is drastically improved in the absence of an external soluble redox mediator.^[97]

Photobiocatalysis for the reduction of CO₂. In biology, the interconversion of CO and CO₂ is catalyzed by a class of enzymes called carbon monoxide dehydrogenases (CODHs). Therefore, these enzymes can be combined with photocatalytic systems to promote the reduction of CO₂ to CO.

Since the first report of CO₂ photoreduction by a semiconductor particle suspension by Inoue *et al.*^[100,98] many efforts have focused on the use of TiO₂ as photocatalytic material for this type of reactions. An extraordinary catalyst for CO₂ photoreduction features a photocatalyst like TiO₂ linked electronically to a light capturing moiety, and a CO₂-reducing enzyme CODH I.^[99] The photocatalytic system operates by photoinduced electron injection from a ruthenium polypyridyl complex to the active center of CODH I through the intermediacy of TiO₂ NPs, using mercaptoethylsulphonate (MES) as a sacrificial electron donor, with no need of a cofactor or mediator (Figure 16B). It has been found that the photocatalytic activity of the system increases with the loading of enzyme, which indicates that the rate-determining steps in this photobiocatalytic CO₂ reduction to CO are the processes taking place at the enzyme. However, the photobiocatalytic system decreases in activity in a few hours. This lack of stability under the operation conditions contrasts with that of the analogous photobiocatalyst for H₂ evolution based on dehydrogenases.

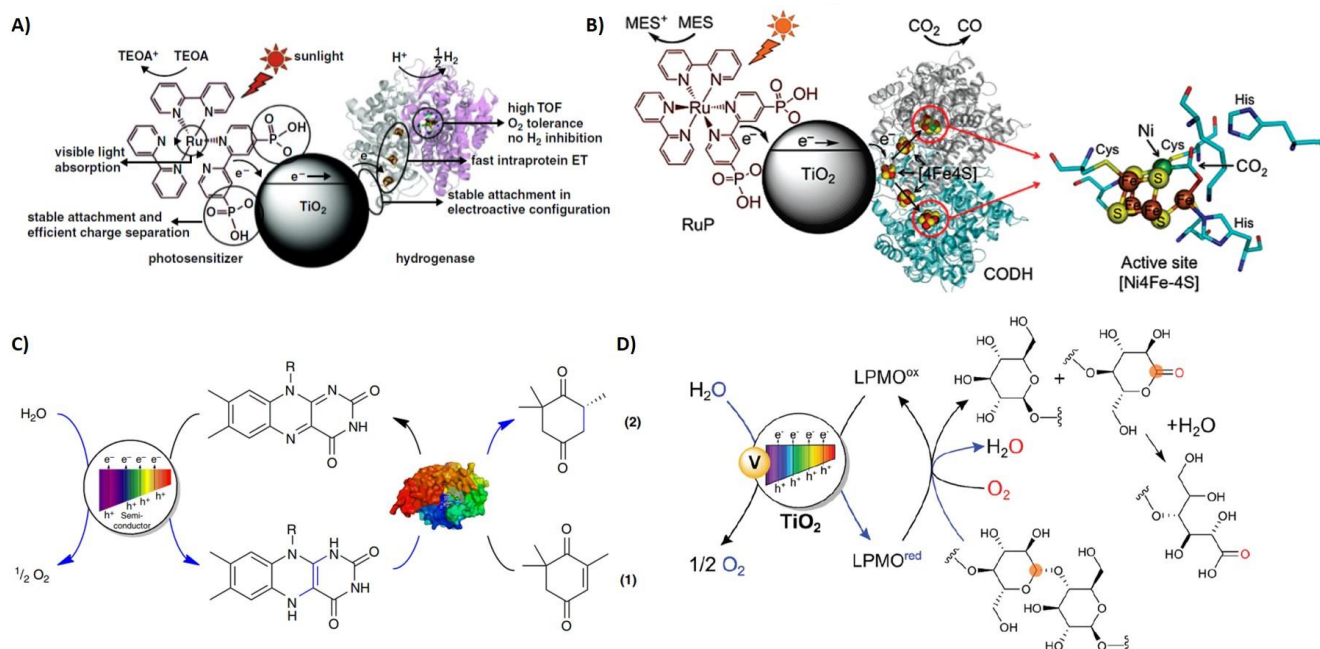


Figure 16. A) Schematic representation of visible-light driven H₂ evolution with RuP-TiO₂-H₂ase. Part A is reproduced with permission from ref. 96b; B) Schematic representation of the CO₂ photoreduction with RuP-TiO₂-CODH I. A catalytic intermediate of the active site of the closely related enzyme CODH II with bound substrate (CO₂, indicated with an arrow) is also shown. Part B is reproduced with permission from ref. 99a; C) Photobiocatalytic enantioselective reduction of ketosiphonone to (R)-levodione by an oxidoreductase enzyme mediated by FADH₂ using Au/TiO₂ as a semiconductor. Part C is reproduced with permission from ref. 100; D) Photobiocatalytic oxidation of glycosidic bonds using LPMO. The oxygenation leading to cellulose chain cleavage occurs at the C-1 position (orange circle) and the resulting lactone then spontaneously hydrolysed to the corresponding aldonic acid. Part D is reproduced with permission from ref. 101.

H₂O as sacrificial donor. The combination of photocatalysis and biocatalysis usually requires sacrificial electron donors (such as TEOA and MES, reported in the previous examples). Several studies have shown that redox-active buffers or even water alone can serve as electron sources. In a pioneering work,

Hollmann and Corma^[100] employed light-driven, TiO₂-promoted water oxidation to achieve biocatalytic redox reactions. With this proof-of-concept study, these authors demonstrated that catalytic water oxidation mediated by robust TiO₂ semiconductors could be productively coupled to

oxidoreductases achieving redox reactions. They combined the photocatalytic activity of gold nanoparticles (NPs) supported on TiO₂ with the enzymatic activity of oxidoreductases, and FAD⁺ as a mediator, to achieve the stereospecific hydrogenation of the conjugated C=C bond of ketoisophorone. In the photocatalytic system, water becomes the only sacrificial electron donor present in the medium, generating oxygen in the process. Electrons in the conduction band of TiO₂ or on the gold NPs reduce FAD⁺ to the corresponding reduced form FADH₂, that is the cofactor necessary to activate the oxidoreductase. After activation, the enzyme exhibits activity to convert ketoisophorone to the corresponding (R)-levodione (Figure 16C).

Bissaro and coworkers have also made use of this type of strategy with lytic polysaccharide monooxygenases (LPMOs), enzymes that need an external electron donor to promote the cleavage of a glycosidic bond, one of the most stable bonds in Nature. They have demonstrated that vanadium-doped TiO₂ (V-TiO₂), can provide the electrons needed through water oxidation for the photocatalytic LPMO activation in the absence of redox mediators (Figure 16D).^[101]

It has also been recently shown that visible light photocatalysis can use water for the in situ generation of H₂O₂, needed for the peroxidases to generate the catalytic active species. These are a class of antioxidant enzymes that catalyze a plethora of interesting transformations, like C-H oxidations and halogenations.^[102]

A water/Au-TiO₂/visible light approach was developed by Hollmann and coworkers, who established an efficient and stereoselective photobiocatalytic oxyfunctionalisation of hydrocarbons using peroxidases.^[103] König and Gulder also described the use of vanadium-dependent haloperoxidases (VHPO) for bromination reactions.^[104]

5. Conclusion and outlook

This review highlights the enormous potential of titanium dioxide, a readily accessible photoactive material, for different biological-related applications, that range from microorganism removal or photodynamic therapy to the development of photobiocatalysts. Despite the advances produced in recent years, fundamental research is still needed in order to improve the photocatalyst performance and efficiencies, and the expansion of the catalyst absorption to the visible or infrared zone. The heterogeneous nature of the semiconductor, its ready availability and combination versatility, together with its biocompatibility, promise to yield many new developments in the fields of chemical and cell biology and biomedicine. It is even expected that some of the heterogeneous constructs can be translated to the clinic. Especially appealing is the possibility of achieving bioorthogonal and intracellular photocatalytic processes with abiotic substrates, and therefore complement current methods based on metal catalysis.^[105] Indeed, we predict that the area of bioorthogonal photocatalysis, and especially using heterogeneous photocatalysts, will be a hot research area in the years to come.

Future efforts will also focus on further expanding the combination of photocatalysts with other enzymes, in order to achieve new type of transformations.

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Keywords: heterogeneous photocatalysis • TiO₂ • bioorthogonal • photodynamic therapy • photobiocatalysis

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