Inorganic Nanomaterials with Intrinsic Singlet Oxygen Generation for Photodynamic Therapy

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Inorganic nanomaterials with intrinsic singlet oxygen ($^{1}\text{O}_2$) generation capacity, are emerged yet dynamically developing materials as nano-photosensitizers (NPSs) for photodynamic therapy (PDT). Compared to previously reported nanomaterials that have been used as either carriers to load organic PSs or energy donors to excite the attached organic PSs through a Foster resonance energy transfer process, these NPSs possess intrinsic $^{1}\text{O}_2$ generation capacity with extremely high $^{1}\text{O}_2$ quantum yield (e.g., 1.56, 1.3, 1.26, and 1.09) than any classical organic PS reported to date, and thus are facilitating to make a revolution in PDT. In this review, the recent advances in the development of various inorganic nanomaterials as NPSs, including metal-based (gold, silver, and tungsten), metal oxide-based (titanium dioxide, tungsten oxide, and bismuth oxyhalide), metal sulfide-based (copper and molybdenum sulfide), carbon-based (graphene, fullerene, and graphitic carbon nitride), phosphorus-based, and others (hybrids and MXenes-based NPSs) are summarized, with an emphasis on the design principle and $^{1}\text{O}_2$ generation mechanism, and the photodynamic therapeutic performance against different types of cancers. Finally, the current challenges and an outlook of future research are also discussed. This review may provide a comprehensive account capable of explaining recent progress as well as future research of this emerging paradigm.

1. Introduction

Photodynamic therapy (PDT) has emerged as an alternative tumor ablation approach with high spatio-temporal precision and reduced long-term morbidity.[1,2] In principle, PDT involves the utilization of three basic components: light, molecular oxygen ($\text{O}_2$), and a photosensitizer (PS).[3,4] to generate highly cytotoxic reactive oxygen species (ROS) to kill cancer cells through either type-I or type-II photochemical reaction mechanism.[5,6] In type-I mechanism, the excited triplet state PS directly interacts with adjacent biomolecules in cancer cells and generates radical cations or anions, which can subsequently interact with oxygen ($\text{O}_2$) to produce ROSs like superoxide anion ($\text{O}_2^-$), hydroxyl radical (·OH), hydrogen peroxide ($\text{H}_2\text{O}_2$), etc.[7,8] In the type-II mechanism, the excited triplet state PS directly sensitizes $\text{O}_2$ (the ground state is a triplet) and generates highly cytotoxic $^{1}\text{O}_2$ inside cancer cells.[9] Generally, cytotoxic $^{1}\text{O}_2$ has been recognized as one of the ROS responsive for PDT,[10] which can kill cancer cells through multifarious pathways, such as induction of cell apoptosis or necrosis, stimulation of robust inflammatory immune response, and disruption of tumor microvasculature.[3,5] Though PDT has shown quite appreciable performance in treating certain cancers (e.g., skin cancers, oral cancers),[11] it is still very challenging to serve as a first-line therapeutic modality for cancer in clinics due to the existing certain limitations, such as lack of an ideal PS with high tumor selectivity and ROS generation efficacy, lack of efficient methods to select right light dosimetry for PDT, and the lack of approaches for effective monitoring...
of treatment response. Among them, the lack of an ideal PS with desired properties is currently the key hurdle in the advancement and clinical applications of PDT. Though a variety of small organic PSs including porphyrin structures, synthetic dyes, and natural products have been used in clinics, most of them can only show limited PDT efficacy against cancers due to their inherent features, including poor water solubility, insufficient photostability, low extinction coefficient, low absorption in the near-infrared (NIR) region, insufficient 1O2 quantum yield, and poor cancer selectivity. Therefore, it is highly desirable to develop new PSs capable of overcoming the limitations of currently used organic PSs, thus augmenting therapeutic outcomes against cancers.

Over the past few decades, the advancement in nanotechnology has offered an alternative approach to refine the performance of current PSs and overcome some of the challenges for cancer PDT. A variety of nanomaterials, including biocompatible polymer-, metal-, carbon material-, silica-, and semiconductor-based nanomaterials have been actively developed as PSs for cancer PDT. Compared to classic organic PSs, the utility of these nanomaterials as inorganic nano-photosensitizers (NPSs) for cancer PDT has many advantages: 1) large extinction coefficients capable of instructing an efficient energy transfer process for photosensitization; 2) facile surface modification capable of conjugating target ligands and functional groups to improve tumor selectivity; 3) large surface-to-volume ratios due to their nanoscale size at least 1D within 100 nm; 4) high ability to take the enhanced permeability and retention (EPR) effect, capable of increasing accumulation in solid tumor tissues; 5) high ability to integrate PSs with chemodrugs and imaging modalities capable of achieving image-guided treatment against cancers. Generally, most of these nanomaterials utilized in PDT can serve as either carrier to load organic PSs or energy transducers to excite the attached PSs through an energy transfer process. Alternatively, recent studies have also shown that certain nanomaterials with unique optical properties can be designed as direct PSs with intrinsic 1O2 generation capacity, thereby they can produce 1O2 by themselves upon light irradiation. The direct generation of 1O2 from these inorganic nanomaterials without incorporating traditional organic PSs can effectively achieve high 1O2 quantum yield as they are highly resistant to photobleaching and possess a large extinction coefficient. Moreover, some of these nanomaterials can offer a different sensitizing mechanism to produce 1O2, as compared to that of carriers or energy transducers. As such, the development of inorganic nanomaterials as NPSs has attracted substantial research scrutiny among the scientific community and become a research hotspot, which can open promising avenues for the refinement of ideal NPSs for efficient PDT against cancers.

In this review, we summarize the recent advances in the development of various inorganic nanomaterials as direct 1O2 generative NPSs for cancer PDT. We exclusively focused on inorganic nanomaterial-based NPSs reported to date, including metal-based NPSs (gold, silver, and tungsten-based NPSs), metal oxide-based NPSs (titanium dioxide, tungsten oxide, and bismuth oxyhalide-based NPSs), metal sulfide-based NPSs (copper and molybdenum sulfide-based NPSs), carbon-based NPSs (graphene and graphitic carbon nitride-based NPSs), phosphorus-based NPSs, and others (hybrid-based NPSs and MXenes-based NPSs) (Scheme 1).

A summary of these NPSs is provided in Table 1, and the reported 1O2 quantum yield for each NPS (where available) is also listed. We also critically elaborate the 1O2 generation mechanism and the design principle of these newly emerged inorganic NPSs that endowed them to achieve a high 1O2 quantum yield. In the end, the existing challenges as well as future prospects are also discussed, which will assist and guide the material chemists to develop new nanomaterials as efficient NPSs for improving cancer PDT. Though many excellent and state-of-the-art reviews covering a range of topics related to PDT have been previously published even from our group,[4,16] the most of them focused on the discussion of the role of nanomaterials as 1) carriers to load organic PSs, 2) donors to excite the attached PSs (e.g., upconversion nanoparticle-based PSs), or 3) photothermal agents.[1,17,18] Until now, there is still no review paper to comprehensively discuss the role of inorganic nanomaterials as direct 1O2 generative materials for PDT. We hope that this first in-depth rigorous review regarding 1O2 generative NPSs can fully and strictly address the topic of advanced materials, providing recent progress as well as an outlook of future research of this emerging paradigm. We believed that this comprehensive account will provide sufficient guidance to biomaterial scientists and biological researchers to develop highly efficient next-generation inorganic PDT agents, as well as explore their therapeutic potential and toxicity in detail to accelerate their translation into clinics.

2. Design Principle of Inorganic NPSs and 1O2 Generation Mechanism

Under photo-excitation, the most of inorganic NPSs shifted from ground energy state (S0) to the singlet excited energy state (S1). In nanoseconds, they moved from S1 to a triplet energy state (T1) via intersystem crossing, followed by decay back to S0 by
Table 1. Summary of inorganic nanomaterial-based NPSs reported till date for ROS generation mediated cancer PDT.

| Class                      | Material          | Irradiation Source                     | \( \lambda_{\text{max}} \) | Quantum Yield | Tumor model | Injection mode/dose | Application                                                                 | Ref.       |
|----------------------------|-------------------|----------------------------------------|-----------------------------|---------------|-------------|---------------------|------------------------------------------------------------------------------|------------|
| **Metal-based NPs**        |                   |                                        |                             |               |             |                     |                                                                              |            |
| Au NPs                    | 100 W Hg lamp, pulsed laser | 514, 532 | 0.037, 0.07 | – | – | Mechanism exploration of \( ^1\text{O}_2 \) generation | [32,33]  |
| Ag NPs                    | 100 W Hg lamp | 530 | 0.155 | – | – | Mechanism exploration of \( ^1\text{O}_2 \) generation | [32]   |
| Pt NPs                    | 100 W Hg lamp | 508 | 0.085 | – | – | Mechanism exploration of \( ^1\text{O}_2 \) generation | [32]   |
| Au-Ag shell satellite     | Circular polarized light (6 mW cm\(^{-2}\)) | 532 | 1.09 | HeLa tumor | i.v. injection (100 nm) | Bimodal CT/PAI-guided PDT | [34] |
| Au NRs                    | Two-photon laser (300 W cm\(^{-2}\)), LED light (130 mW cm\(^{-2}\)) | 765, 808, 835, 940 | – | HeLa cells B16F0 melanoma tumor | i.v. injection (100 \( \mu \)g mL\(^{-1}\)) | Two-photon imaging and in vivo PDT, In vivo PDT/PTT | [35,37] |
| Au NEs                    | LED light, 130 mW cm\(^{-2}\) | 915, 1064 | 0.19, 0.22 | B16F0 tumor | Intratumoral injection (10 mg mL\(^{-1}\)) | In vivo PDT/PTT | [38] |
| Au NCS                    | 980 nm laser (0.5 W cm\(^{-2}\)) | 380 | 0.048 | HeLa cells, zebrafish | Microinjection (100 \( \mu \)g mL\(^{-1}\)) | In vivo FL imaging, gene delivery, in vitro PDT | [45] |
| Ag NCS                    | White light (150 mW cm\(^{-2}\)) | 480 | 1.26 | MCF-7 | 50-500 \( \mu \)m | FL imaging, in vitro PDT | [48] |
| **Metal oxide-based NPSs** |                   |                                        |                             |               |             |                     |                                                                              |            |
| TiO\(_2\) NPs             | CW NIR (200 mW cm\(^{-2}\)), (1.2 W cm\(^{-2}\)), 6 Gy for RT | 980 | 0.29 | B16F0 tumor, 4T1 tumor | Intratumoral injection (15 mg kg\(^{-1}\)), Intratumoral injection (1.4 mg mL\(^{-1}\)) | In vivo PDT/PTT, CT imaging-guided multimodal (PDT/PTT/RT) therapy | [65,66] |
| W\(_{18}\)O\(_{49}\) NWs | CW laser (0.5 W cm\(^{-2}\)) | 300 | – | U14 murine cervical carcinoma | i.v. injection (1 mg mL\(^{-1}\)) | Upconversion luminescence (UCL) and in vivo PDT | [69] |
| WO NPs                    | CW NIR II laser (2 W cm\(^{-2}\)) | – | – | HeLa tumor | Intratumoral injection (5 mg kg\(^{-1}\)) | In vivo PAI-guided PDT/PTT | [125] |
| BiOCl NSs                 | Mercury lamp (300 W cm\(^{-2}\)) | 350 | – | MCF-7 | (0-200 \( \mu \)g mL\(^{-1}\)) | In vitro PDT | [68] |
| UCNP/BOCl                | CW laser (0.5 W cm\(^{-2}\)) | 300 | – | U14 murine cervical carcinoma | i.v. injection (1 mg mL\(^{-1}\)) | UCL/CT imaging-guided in vivo PDT | [70] |
| BiOBi: Yb/Tm             | CW laser (0.9 W cm\(^{-2}\)) | 350 | – | U14 murine cervical carcinoma | Intratumoral injection (1 mg mL\(^{-1}\)) | UCL/CT imaging-guided in vivo PDT | [70] |
| **Carbon-based NPSs**      |                   |                                        |                             |               |             |                     |                                                                              |            |
| C60–PDA–rGO              | Xe lamp (50 W cm\(^{-2}\)) | – | – | HeLa cells | Intratumoral injection (10 mg kg\(^{-1}\)) | In vitro PDT/PTT | [85] |
| GQDs                      | CW laser (1 W cm\(^{-2}\)), two photon laser (2.64 mW cm\(^{-2}\)) | 300 | – | U251 gloma cells, Bacterial pathogens | 200 \( \mu \)g mL\(^{-1}\), 0–1 \( \mu \)g/mL\(^{-1}\) | In vitro PDT, TP imaging and antibacterial PDT | [87,89] |
| N-GQDs, Amino N-GQDs | CW (0.1 W cm\(^{-2}\)), Two photon laser (2.3936 mW cm\(^{-2}\)) | 0.60, 0.53 | – | Bacterial pathogens | 0–10 \( \mu \)g mL\(^{-1}\) | In vitro antibacterial PDT | [90] |
| GQDs                      | White light (80 W cm\(^{-2}\)) | 532 | 1.3 | MDA MB-231 tumor | Intratumoral injection (4 mg kg\(^{-1}\)) | In vivo bimodal FL/PAI-guided PDT/PTT | [14] |
| Au NRs@SiO\(_2\)-CDs     | CW laser (0.1 W cm\(^{-2}\) for PDT and 0.5 W cm\(^{-2}\) for PTT) | 808 | 1.3 | B16F0 tumor | i.v. injection (2 mg mL\(^{-1}\)) | In vivo bimodal FL/PAI-guided PDT/PTT | [92] |

(Continued)
Table 1. Continued.

| Class           | Material                     | Irradiation Source            | $\lambda_{\text{max}}$ | Quantum Yield | Tumor model | Injection mode/dose | Application                        | Ref. |
|-----------------|------------------------------|-----------------------------|-------------------------|---------------|-------------|---------------------|-----------------------------------|------|
|                 | UCNP-GQDs                    | CW laser, 980 nm laser       | 300                     | 1.5           | 4T1 tumor   | i.v. injection       | In vivo FL imaging-guided PDT     | [93] |
|                 | Gd@GCNs                     | LED light (100 mW cm$^{-2}$) | 550                     | 0.51          | SCC-7 tumor  | i.v. injection, (0.1 mmol Gd kg$^{-1}$) | In vivo bimodal FL/MRI-guided PDT | [94] |
|                 | C$_3$N$_4$ NSs               | LED light (20 mW cm$^{-2}$)  | 300                     | –             | HeLa cells   | (50 μg mL$^{-1}$)    | In vitro PDT                       | [98] |
|                 | g-C$_3$N$_4$ nanospheres     | Halogen lamp (300 W cm$^{-2}$) | 350                     | –             | MDA-MB-231 cells | (5–25 μg mL$^{-1}$) | In vitro PDT/PDT/chemotherapy     | [100]|
|                 | UCNP/C$_3$N$_4$ NSs          | CW laser (2.3 W cm$^{-2}$)   | 350                     | –             | H22 tumor    | i.v. injection (1 mg mL$^{-1}$) | In vivo PDT                        | [103]|
|                 | UCNP/C$_3$N$_4$ QDs          | CW laser (2.5 W cm$^{-2}$), (1.5 W cm$^{-2}$) | 350, 370 | –             | OEC-M1 cells, CAL-27 tumor | (250 μg mL$^{-1}$) | In vitro PDT, In vivo PDT | [104,105]|
|                 | Metal Sulfide-based NPSs      | MoS$_2$ QDs                 | 630                     | –             | –           | –                   | (100 μg mL$^{-1}$) $^1$O$_2$ detection in solution | [73] |
|                 | CuS NCs                      | CW NIR laser (0.6 W cm$^{-2}$) | 808                     | –             | B16 tumor   | Intratumoral injection (15 mg kg$^{-1}$) | In vivo PDT/PTT                    | [74] |
|                 | Phosphorus-based NPSs         | BP NSs                      | 450                     | 0.91          | MDA-MB-231 tumor | Intratumoral injection (500 μg mL$^{-1}$) | In vivo PDT                        | [107]|
|                 | UCNP/BP NSs                  | CW laser (1.44 W cm$^{-2}$)  | –                       | –             | U14 tumor   | i.v. injection (1 mg mL$^{-1}$) | In vivo PDT                        | [108]|
|                 | BP QDs                       | CW laser (80 W cm$^{-2}$ for PDT and 2 W cm$^{-2}$ for PTT) | 0.74                   | –             | 4T1 tumor    | Intratumoral injection (500 μg mL$^{-1}$) | In vivo PDT/PTT                   | [111]|
|                 | Hybrid-based NPSs            | Au NRs/TiO$_2$              | CW NIR I laser          | 808           | –           | –                   | Mechanism exploration of $^1$O$_2$ generation | [24] |
|                 | Au NPs/TiO$_2$               | Simulated sunlight, 532 nm laser | 532         | $2 \times 10^{-6}$ | –           | –                   | Mechanism exploration of $^1$O$_2$ generation | [25] |
|                 | Au NCs/TiO$_2$               | 650 nm laser (0.5W cm$^{-2}$) | 650                     | –             | U14 tumor   | i.v. injection (500 μg mL$^{-1}$) | In vivo PDT                        | [27] |
|                 | Au NPs/ZnO                  | Simulated sunlight          | 400                     | –             | –           | –                   | In vitro antibacterial PDT         | [116]|
|                 | Cs$_x$WO$_3$ NRs             | CW NIR (I, II) laser (2 W cm$^{-2}$) | 808         | –             | HeLa tumor  | Intratumoral injection (1 mg mL$^{-1}$) | In vivo bimodal CT/PAT-guided PDT/PDT | [121]|
|                 | W$_x$C NPs                  | CW NIR II laser (0.8 W cm$^{-2}$) | 1064       | –             | S180 tumor  | i.v. injection (10 mg kg$^{-1}$) | In vivo bimodal CT/PAT-guided PDT/PDT | [122]|
|                 | SnWO$_4$ NPs                | LED light (0.2 W cm$^{-2}$)  | 465                     | –             | 4T1 tumor   | Intraperitoneal injection (23 mg kg$^{-1}$) | In vivo PDT                        | [123]|
|                 | UCNPs@g-C$_3$N$_4$-Au$_2$      | CW laser (2.5 W cm$^{-2}$)   | –                       | 0.74          | H22 tumor   | i.v. injection (400 μg mL$^{-1}$) | In vivo trimodal CT/FL/MRI-guided PDT/PTT | [119]|

Releasing the absorbed photon energy to the surrounding or surface adsorbed molecular oxygen ($^1$O$_2$) to generate $^1$O$_2$. This mechanism is based on the type-II pathway of $^1$O$_2$ generation. Figure 1 demonstrates $^1$O$_2$ generation by Type-II PDT pathway along with the generation of other ROSs by Type-I pathway. Notably, the structural design of inorganic NPSs played a decisive role in $^1$O$_2$ generation. It has been demonstrated that molecular O$_2$ could selectively binds on a specific crystalline facet of nanomaterial, leading to morphology-dependent production of $^1$O$_2$. For example, oxygen exists as a molecular form when bind on the (111) crystalline facet of Ag NPs, but presents as an atomic form on both (100) and (110), thereby, $^1$O$_2$ can only be
formed on Ag NPs that possess Ag (111) surface. In addition, the size of inorganic NPSs also had a profound effect on $^1\text{O}_2$ generation. In general, the smaller size inorganic NPSs produced a high $^1\text{O}_2$ quantum yield as more amounts of $\text{O}_2$ were adsorbed on the surface of smaller NPs due to the high surface area to volume ratio. Whereas, for plasmonic NPs, the effect of size could be more complex, as plasmonic NPs with a bigger size generated more hot electrons compared to that of a smaller size, thus producing more $^1\text{O}_2$.[20] Surface modification of inorganic NPSs also played an important role in improving their ROS generation capacity. The chemical reduction of graphene oxide quantum dots (rGO-QDs) significantly lower the bandgap and valence band (VB), which led to generating more electron–hole pairs, resulting in enhanced ROS generation by rGOQDs than GOQDs under white light irradiation.[21] In addition, the coupling of two different inorganic NPs to develop hybrid-based inorganic NPSs is also an effective strategy to improve ROS yield due to slower electron–hole pairs recombination.[22] The fabrication process is also an important factor that endows inorganic NPSs with a high $^1\text{O}_2$ quantum yield. For example, the hydrothermal treatment of polythiophene engineers the energy levels of the resultant graphene QDs (GQDs), which lead to a huge energy gap of 49.3 kcal mol$^{-1}$ between the ground state (S0) and excited singlet state (S1) of GQDs. Given the fact that the formation of $^1\text{O}_2$ from $\text{O}_2$ requires an amount of 22.5 kcal mol$^{-1}$ energy, GQDs offered high $^1\text{O}_2$ quantum yield of 1.3 due to multistate sensitization $^1\text{O}_2$ generation mechanism, as the $^1\text{O}_2$ received an equal amount of energy on each transition either from S1 to T1 or from T1 to S0 to be converted into $^1\text{O}_2$, resulting in high $^1\text{O}_2$ generation.[24] In addition, strong acid oxidation of precursor material to fabricate GQDs results in GQDs with an ample amount of surface oxygenated functional groups, promoting an efficient $\text{O}_2$ quenching of triplet states, which leads to enhance $^1\text{O}_2$ quantum yield (1.56).[23]

In contrast to the type-II $^1\text{O}_2$ generation pathway, inorganic semiconductor NPSs and hybrid-based NPSs follow the type-I pathway of $^1\text{O}_2$ generation. Briefly, $\text{O}_2$ absorbed on the surface of inorganic NPSs especially TiO$_2$ is reduced to superoxide anion ($\text{O}_2^{-}$) by an electron in the conduction band (CB), which was then oxidized by the hole in the valence band (VB) to form $^1\text{O}_2$ (Figure 2).[24,25] It is noteworthy to mention that the hydrodynamic size and the band edge structures of inorganic metal-oxide NPSs are very crucial in the generation of ROS. Compared to their bulk counterparts, the smaller size NPSs generated more ROS under UV irradiation, presumably because the NPs had larger surface areas capable of providing more reaction sites for the absorption of UV light. Second, as ROS generation is dictated by the photoexcitation-driven interfacial electron transfer process, only the metal-oxide NPSs with band gap energy ($E_g$) smaller than the incident photon energy (i.e., $≈ 3.4$ eV for the 365 nm UV) can be photoexcited to generate electrons in the CB and holes in the VB, which subsequently react with an aqueous electron acceptor (e.g., molecular oxygen) and donor (e.g., $\text{H}_2\text{O}$ and $\text{OH}^{-}$), respectively, ultimately producing different types of ROS.[26]

Though hybrid-based inorganic NPSs followed the same $^1\text{O}_2$ generation mechanism as metal-oxide NPSs, they demonstrated an enhanced $^1\text{O}_2$ generation capacity due to reduced electron–hole pairs recombination than metal-oxide NPs alone, as electron could be transferred from semiconductor to the metal NPs or vice versa depending upon the excitation wavelength and Schottky barrier. Notably, the deposited metal NPs onto semiconductor NPs alter the electrical distribution of the hybrid, which hampered the recombination process. However, the size of semiconductor NPs, as well as the amount of loaded metal NPs,
should be considered because larger size semiconductor NPs significantly slow down the recombination process than smaller size NPs, resulting in improved ROS generation. Whereas, the higher amount of metal NPs would impede the adsorption of O$_2$ molecules on the surface of semiconductor NPs leading to low 1$O_2$ generation. We hope that this thorough and balanced discussion would greatly assist and guide the development of next-generation inorganic NPs with a high 1$O_2$ quantum yield.$^{[27]}$

3. Metal-Based NPSs

In recent years, noble metal nanomaterials (NMs) have gained significant attention due to their unique physiochemical and optical properties, which have found wide applications in industry, environment, and health.$^{[28]}$ Among these noble metal NMs, gold (Au) and silver (Ag) NMs have been of considerable research interest.$^{[29]}$ For example, Au NMs with diverse morphologies such as nanoparticles (Au NPs), nanorods (Au NRs), nanoclusters (Au NCs), nanoshells, nanoechinus (Au NE), and nanostars (Au NSs) have been actively developed. These Au NMs possess many versatile properties, such as high chemical inertness, easily tunable optical properties, high biocompatibility, large extinction coefficients and facile surface modifications.$^{[30]}$ More importantly, some well-designed AuNMs also possess strong localized surface plasmon resonance (LSPR) properties, which allowed them to efficiently absorb light and convert it into heat to trigger photothermal effect.$^{[31]}$ In addition, recent studies have also revealed that AuNMs could be directly sensitized to generate highly cytotoxic 1$O_2$, which make them potential candidate as an additional weapon in PDT capable of providing a synergistic effect of photothermal therapy (PTT) with PDT to improve therapeutic efficacy. Thus, AuNMs have found tremendous biomedical applications from diagnosis to therapy.

3.1. Gold-Based NPSs

In 2011, Raviraj et al. first reported an unprecedented observation that noble metal NPs could be directly sensitized to produce 1$O_2$ without using organic photosensitizers.$^{[32]}$ In this study, three different noble metal NPs, including Au NPs ($d = 22$ nm), Ag NPs ($d = 42$ and 55 nm) and Pt NPs ($d = 10$ nm) were synthesized. All these NPs exhibited one major strong LSPR band around 398–530 nm. Upon irradiation at the LSPR band of each NPs, the production of 1$O_2$ was monitored by the direct observation of 1$O_2$ phosphorescence at 1264 and 1268 nm (Figure 3A). The subsequent measurement of 1$O_2$ quantum yield using singlet oxygen sensor green (SOSG) as a fluorescent 1$O_2$ indicator demonstrated that Ag NPs possessed a higher value (0.155) as compared to Pt NPs (0.085) or Au NPs (0.037) (Figure 3B). It was plausible that
the energy transfer from LSPR of these metal NPs to molecular oxygen might be a possible reason to produce $^1$O$_2$ upon light irradiation. Based on these results, they subsequently revealed that the generation of $^1$O$_2$ was highly dependent on the morphologies of Au and Ag NPs.[13] They demonstrated that Ag decahedrons and Au triangular plates could produce strong $^1$O$_2$ phosphorescence emission at $\approx$1263 nm upon irradiation at 885 nm, while Ag nanocubes and Au decahedrons only produced negligible amount of $^1$O$_2$ (Figure 3C). Moreover, strong $^1$O$_2$ phosphorescence emission was also observed from penta-twinned Au NRs upon irradiation at a longitudinal LSPR band ($\approx$885 nm), while little $^1$O$_2$ was formed when exciting at the transverse LSPR band. Mechanism studies showed that the morphology-dependent production of $^1$O$_2$ from these nanomaterials was due to the selective binding of molecular O$_2$ on a specific crystalline facet (Figure 3D). For example, oxygen exists as a molecular form when binding on the Ag (111) surface but presents as an atomic form on both Ag (100) and Ag (110) surface, thereby, $^1$O$_2$ can only be formed on Ag NPs that possess Ag (111) surface like Ag decahedrons and Ag triangular plates.

In 2013, Pasparakis et al. studied the effect of different laser sources on the generation of $^1$O$_2$ from Au NPs by using continuous wave (CW) laser and pulsed laser sources.[33] Citrate stabilized Au NPs with a mean diameter of 40 nm and an LSPR band at 524 nm were prepared. The generation of $^1$O$_2$ was confirmed by direct observation of $^1$O$_2$ phosphorescence at 1270 nm and a gradual reduction in the absorption of 1,3-diphenylisobenzofuran (DPBF), a $^1$O$_2$ indicator. They found that the $^1$O$_2$ generation efficiency upon irradiation with pulsed laser (7 ns, 532 nm) was about 0.07, which was more than twofold higher than that with CW laser (0.03) at 532 nm. The difference in $^1$O$_2$ generation efficacy was presumably due to two different pathways involved in sensitizing O$_2$ during the irradiation of Au NPs. When irradiated under a CW laser, the $^1$O$_2$ was generated mainly through a plasmon and hot-electron emission mechanism. However, when irradiated under a pulsed laser, a combination of the plasmon-activated pathway and indirect photothermal pathway that potentially induces particle fragmentation and increases thermionic electron emission contributed largely to $^1$O$_2$ production. This study demonstrated that irradiation of Au NPs with a pulse laser was more efficient to trigger $^1$O$_2$ production compared to CW laser, which subsequently led to much higher cancer cell death. Afterward, Chadwick et al. confirmed that irradiation of Au NPs with a pulsed laser can proceed through the equilibrated hot electrons with temperature reached to several thousand degrees. In addition, they also found that the size of Au NPs had a profound effect on $^1$O$_2$ generation.[20] Larger Au NPs (46 nm) produced more $^1$O$_2$ as compared to smaller Au NPs (15 nm). This is because that Au NPs with a bigger size generated more hot electrons as compared to that of a smaller size.

In 2017, Gao et al. reported an intriguing study of fabricating DNA-driven shell-satellite (SS) Au-Ag plasmonic nanoparticles assemblies as chiral NPSs for cancer PDT.[34] The chiral SS nanoassemblies were prepared by hybridization of complementary DNA sequences modified Au NPs and Ag NPs, followed by galvanic replacement reaction and surface coupling with cysteine enantiomers (Figure 4A).

The as-prepared chiral nanoassemblies displayed strong chiroplasmonic activities in the visible region. Importantly, upon right circular polarized (RCP) light illumination of d-cysteine modified SS nanoassemblies that contained Au NPs with a mean size of $\approx$15 nm (SS15-d-Cys), very high ROS generation efficiency was observed. The $^1$O$_2$ quantum yield was estimated to be 1.09, which was more than four times higher than that
of protoporphyrin IX (PpIX) (0.23). Interestingly, irradiation of d-cysteine modified SS nanoassemblies with Au NPs at a size of ≈5 nm (SS5-d-Cys) only produced negligible ROS, indicating that the structure of the nanoassemblies also played an important role in the production of ROS (Figure 4B). Ascribed to the high 1O2 quantum yield of SS15-d-Cys, significant destruction of various tumor cells, including HeLa, MCF-7, HepG2, and Caco-2 tumor cells, was achieved upon irradiation of SS15-loaded cells with an RCP light (6 mW cm−2, 20 min) (Figure 4C). Meanwhile, SS15-d-Cys also showed dual-imaging potential as the strong signal was observed at 24 h post-injection for both computed tomography (CT) and photoacoustic imaging (PAI), respectively. In vivo studies on subcutaneous tumor-bearing mice were subsequently carried out, which showed remarkably stronger (CT) and (PAI) signal at 24 h post-injection, respectively, and the tumor was completely ablated under RCP light irradiation (Figure 4D–F). Thus, RCP light excitable SS nanostructures are exciting NPSs, which demonstrated the promising potential of dual-modal imaging-guided in vivo cancer therapy.

In addition to 0D Au NPs, 1D Au NRs have also shown the ability to directly generate 1O2 by taking advantage of their tunable LSPR bands. In 2012, Zhao et al. demonstrated that polyvinylpyrrolidone (PVP) coated Au NRs could be directly sensitized to generate 1O2 under two-photon excitation (TPE).[35] In their study, Au NRs with three different aspect ratios (≈3.5, ≈4.0, and ≈4.3) were prepared, with the maximum absorbance band at 765, 808, and 835 nm, respectively. Importantly, they demonstrated that all these three Au NRs exhibited large two-photon absorption cross-sections, greatly facilitating the absorption of two-photon energy for sensitizing O2. Upon TPE with an 808 nm femtosecond pulse laser, the photooxidation rates of 9, 10-anthracenediyl-bis (methylene) dimalonic acid (ABDA) in the presence of the Au NRs were much faster than that of either Rose Bengal (RB) or Indocyanine Green (ICG), revealing that the two-photon induced 1O2 generation capacity of these Au NRs was much larger than that of RB or ICG. Cell studies showed that the PVP-coated Au NRs could efficiently enter HeLa tumor cells and cause significant cell death (upto 85%) following irradiation with the 808 nm femtosecond laser for 15 min, suggesting that Au NRs could serve as efficient NPSs for two-photon induced PDT. Latterly, the same group further demonstrated that two-photon induced 1O2 generation abilities of Au nanospheres and short Au NRs (low aspect ratio) could be elevated when they were present in an aggregated form.[36] As Au NPs normally existed in an aggregated form when entering into tumor cells, it would be beneficial to use the aggregated Au NPs for PDT under TPE.

Though the TPE Au NRs and Au nanospheres have shown promising applications in PDT, the requirement of expensive femtosecond lasers and high laser doses (usually 1–48 W cm−2 at 808 nm) has limited their clinical applications. In 2014, Vankayalaa et al. demonstrated the first example of Au NPs capable of initiating efficient in vivo PDT/PTT against B16F0 melanoma tumor xenografts under single-photon NIR laser irradiation at a very low power density (<130 mW cm−2).[17] In their study, cationic lipid (Lipofectamine 2000) coated Au NRs with an average length and diameter of 37.3 ± 2.4 nm and 11 ± 1.2 nm, respectively, were synthesized, which had a strong NIR absorption band at ≈808 nm. They demonstrated that the 1O2 could be produced only when the Au NRs were irradiated by a long NIR light (875–1100 nm), but not by a visible or short NIR light (Figure 5A). The measurement of 1O2 generation capacity in HeLa cells using the SOSG revealed a large amount of 1O2 generation upon irradiation with a 940 nm LED light, whereas little 1O2 generation was observed under 550 nm light irradiation (Figure 5B). More than ≈62% cells were dead when irradiating the Au NRs-loaded HeLa cells (25 µg mL−1) by 940 nm light, which was approximately tenfold higher as compared to that irradiation at 550 nm. They demonstrated that the 940 nm irradiation-induced ROS generation contributed mainly to cell death, which was more effective compared to the PTT effect at 550 nm. The subsequent in vivo studies in B16F0 melanoma tumor-bearing mice showed complete destruction of tumors upon irradiation with a 915 nm NIR laser at a power density of only 130 mW cm−2, which was more effective compared to the Au NRs-induced PTT effect upon irradiation by 780 nm light or chemotherapy with doxorubicin. Figure 5C illustrated the cellular events involved in the PDT/PTT mediated cellular destruction by photo-excited Au NRs. As the Au NRs possessed excellent photostability, high resistance to enzymatic degradations, and four to six orders higher extinction coefficients than organic PSs at NIR regions, the Au NRs reported in this work could serve as promising NPSs for effective cancer PDT.

To directly sensitize the formation of 1O2 for the treatment of deep-seated tumors, the same group lately reported a unique gold nanochinus structure (Au NE) as combinational PDT and PTT nanomaterials for ablation of tumors using NIR light in both the first and second biological windows.[38] A seed-growth procedure using double-chain cetyltrimethylammonium bromide (DC14TAB) surfactant was employed to synthesize the Au NEs, with a mean hydrodynamic size of 350 ± 50 nm. Both SEM and TEM images showed that the Au NEs contained many nanorods or multiple tips on the surface of each nanoparticle (Figure 6A). The Au NEs displayed a strong NIR absorption which covers both the NIR-I (650–960 nm) and NIR-II (1000–1300 nm) regions, presumably due to the enhanced LSPR property of the echinus-like structure (Figure 6B). Moreover, the Au NEs also showed large molar extinction coefficients (≈0.69 × 1012 m−1 cm−1 at 915 nm and ≈0.74 × 1012 m−1 cm−1 at 1064 nm), which were 7–9 orders higher than traditional organic PSs and 3–4 orders higher than other reported gold nanoparticles. It was notable that the Au NEs could be excited by NIR light at either the first (915 nm) or second (1064 nm) biological windows to sensitize the formation of an obvious 1O2 phosphorescence at ≈1267 nm, consistent with the two characteristic peaks (948 and 1074 nm) found in the excitation spectrum (Figure 6C). The 1O2 quantum yields were found to be 0.19 and 0.22 at 915 nm and 1064 nm light excitation, respectively. In addition to the generation of 1O2, they also demonstrated that the Au NEs could also produce heat under irradiation with 915, 940, or 1064 nm light, thus enabling bimodal PDT and PTT to kill tumor cells. After direct injection of the Au NEs into the subcutaneously implanted B16F0 tumors in living mice, a remarkable inhibition of tumor growth was achieved when the tumors were irradiated with 915 or 1064 nm light at a power density of merely 130 mW cm−2, which was approximately two to three times lower than the standards set by American National Standard Institute for skin burning.[19] The average tumor volume in mice at day 14 post-treatment with 1064 nm light irradiation was.
found to be 0.009 of initial tumor size, which was significantly lower than that with 808 nm light (53.33) or doxorubicin (57.52) (Figure 6D).

Moreover, tumor-bearing mice with 1064 nm light irradiation could survive for over 60 days, which was also significantly longer than that with 808 nm light (12 days) or doxorubicin (20 days). These results suggested that irradiation of Au NEs with light at NIR-II windows to trigger both PDT and PTT effects against tumors in vivo was superior to the mere PTT effect induced by the 808 nm light (Figure 6E). More importantly, the extremely large extinction coefficients of the Au NEs along with the deeper tissue penetration depth of the NIR-II light at 1064 nm could allow Au NEs to act as an effective agent for the treatment of deep-located tumors in vivo.

Considering that Au nanoclusters (Au NCs) with sizes comparable to the Fermi wavelength generally possess intriguing size-dependent optical, electronic, and chemical properties, they have recently attracted much attention for the applications in optoelectronic devices,\textsuperscript{[40]} catalysis,\textsuperscript{[41]} and fluorescent imaging.\textsuperscript{[42]} In 2009, Sakamoto et al. first employed single-molecule fluorescence spectroscopy (SMS) to elucidate the photoactivity of Au NCs.\textsuperscript{[43]} Au NCs with different sizes Au\textsubscript{n} (n, <12 or 17) and Au\textsubscript{m} (m, 19 or 21) were prepared via UV light irradiation of a poly(vinyl acetate) film containing HAuCl\textsubscript{4} and a radical precursor. SMS images showed that the fluorescence of Au\textsubscript{n} (n, <12 or 17) was significantly quenched by O\textsubscript{2} through an electron transfer, while the fluorescence of Au\textsubscript{m} (m, 19 or 21) increased greatly with increasing O\textsubscript{2} concentration from 0.3% to 95%, suggesting that an increase in atom number caused a remarkable change in the photoactivity. The increase in fluorescence intensity of Au\textsubscript{m} with the O\textsubscript{2} concentration could be due to the depopulation of the triplet state through energy transfer to O\textsubscript{2} molecules, which was confirmed by the appearance of 1O\textsubscript{2} phosphorescence signal at 1270 nm. These observations suggested that the cluster size as well as spin multiplicity have profound effects on the photochemical reactivity of Au NCs, which was helpful to guide the design of noble metal clusters with tunable photochemical properties for PDT. Encouraged by this, Das et al. elucidated that the orientation of O\textsubscript{2} molecules adsorbed on the surface of Au NCs with a different size was the key factor to influence the fluorescence intensity.\textsuperscript{[44]} Bovine serum albumin (BSA) stabilized Au NCs with a small size (Au\textsubscript{8}) had a superoxo type of O\textsubscript{2} orientation that could enhance the fluorescence emission and induce the formation of 1O\textsubscript{2} in the presence of O\textsubscript{2}. In contrast, the BSA stabilized Au NCs with a larger size (Au\textsubscript{25}) had a peroxo type of O\textsubscript{2} orientation that showed quenched fluorescence.

In 2015, Hwang’s group reported nucleus-targeting Au NCs for simultaneous fluorescence imaging, gene delivery, and...
PDT of tumors upon NIR light irradiation.\textsuperscript{[45]} In their study, 11-mercaptoundecanoic acid stabilized Au NCs (RS-Au NCs) were first synthesized, which were then coupled with the TAT peptide (N-GRKKRRQRRR-C) to afford the nucleus-targeting Au NCs (TAT-Au NCs) with a mean particle size of 3–5 nm and molecular weight of 2100 (Figure 7A). Both RS-Au NCs and TAT-Au NCs showed absorption in the entire spectral range (up to 900 nm) and bright red fluorescence (\( \approx 600 \text{ nm} \)) under UV exposure (Figure 7B, C), which could allow fluorescence monitoring of the uptake of Au NCs into live tumor cells. Confocal fluorescence imaging revealed that TAT-Au NCs exerted a higher accumulation inside the nucleus relative to that of RS-Au NCs, indicating that the presence of TAT on the surface of Au NCs could trigger efficient delivery into the nucleus (Figure 7D). Furthermore, the TAT-Au NCs also showed an ultrahigh gene transfection efficiency of \( \approx 81\% \) in HeLa cells, which was \( \approx 3.2\)-fold higher than that using LP2000, a commonly used gene carrier. The efficient transfection of the GFP gene in zebrafish using the pDNA-TAT AuNCs complexes was also realized (Figure 7E), suggesting that the TAT Au NCs could act as very effective gene carriers. They further revealed that both RS-Au NCs and TAT-Au NCs could directly sensitize the formation of \( ^1\text{O}_2 \) under irradiation at 980 nm light (Figure 7F), with \( ^1\text{O}_2 \) quantum yields of 0.048 and 0.046 for RS-Au NCs and TAT-Au NCs, respectively. The NIR light-activated formation of \( ^1\text{O}_2 \) could obviously elevate the intracellular ROS levels, thus causing significant DNA damage and inducing irreversible cell death. It was also found that the TAT-Au NCs could induce more cell death relative to the nucleus-nontargeting RS-Au NCs under 980 nm light irradiation, revealing that the delivery of PSSs inside the nucleus could trigger improved PDT effects to kill tumor cells. Two similar studies, which used thiolated Au NCs (Au\(_{25}\)(SR)\(_{18}\))\textsuperscript{[46]} and captopril-protected Au NCs (Au\(_{25}\)(Cap)\(_{18}\))\textsuperscript{[47]} capable of emitting red fluorescence and directly sensitizing the formation of \( ^1\text{O}_2 \) to kill cancer cells and microbial cells, respectively, have also been reported, supporting the high capacity of Au NCs for both fluorescence imaging and PDT.

3.2. Silver-Based NPSs

Though the Au NCs have been well studied as efficient NPSs for cancer PDT, the exploration of silver nanoclusters (Ag NCs) for controlling \( ^1\text{O}_2 \) generation is very rare due to the more difficult reduction of Ag\(^+\) into Ag as well as less stability of Ag NCs to the environment. In 2016, Yu et al. reported an elegant approach for the reliable synthesis of BSA-templated ultrasmall Ag NCs (BSA-Ag\(_{13}\) NCs), which showed improved stability and extremely high \( ^1\text{O}_2 \) generation capacity for cancer PDT.\textsuperscript{[48]} The key strategy utilized in the synthesis of BSA-Ag\(_{13}\) NCs was to dissolve the strong reducing agent NaBH\(_4\) in an alkaline NaOH solution, which could effectively prevent the decomposition of NaBH\(_4\) and remove less stable Ag NCs in the final product, thus facilitating to regulate the size and improve the stability of the BSA-templated Ag NCs (Figure 8A). They demonstrated that the as-formed BSA-Ag\(_{13}\) NCs exhibited a UV–vis absorption with a shoulder peak at 425 nm and a red emission at 625 nm (Figure 8B), with a
fluorescent quantum yield of only 0.4%. Notably, they found that the $^1\text{O}_2$ quantum yield of BSA-Ag$_{13}$ NCs was much higher (1.26) as compared to its analog BSA-Au$_{25}$ NCs (0.07) or RB (0.75). The mechanism studies using ultrafast laser spectroscopy like time-correlated single-photon counting and transient absorption techniques demonstrated that the majority of BSA-Ag$_{13}$ NCs could transit to triplet states via intersystem crossing upon photoexcitation (Figure 8C). The subsequent triplet-triplet transitions could prolong the excited electrons residing at the triplet states, which greatly increased the change to sensitize O$_2$ molecules.
to form $^1\text{O}_2$. Moreover, cellular studies on MCF-7 breast cancer cells revealed that the BSA-Au$_{25}$ NCs could easily enter the cells and effectively kill MCF-7 cells under white light irradiation (Figure 8D). These results suggested that the BSA-Au$_{25}$ NCs can act as promising NPSs to trigger effective PDT for cancer treatment.

4. Metal Oxide-Based NPSs

Metal oxide NMs have found promising biomedical applications for fluorescent labeling due to their well-known size dependent physiochemical and optical properties, such as high photostability, large extinction coefficients, high emission quantum yield, and easy surface modifications.\(^{[49]}\) Previously, metal oxides-based quantum dots (QDs) have been reported to sensitize the formation of $^1\text{O}_2$ under UV light excitation. However, the inherent cytotoxicity of these QDs due to the presence of heavy metal ions (e.g., cadmium) and low $^1\text{O}_2$ quantum yield restricts their use as a photosensitizer in clinical PDT.\(^{[50]}\) Alternatively, semiconductor NMs such as titanium dioxide (TiO$_2$), zinc oxide (ZnO), copper sulfide (CuS), molybdenum disulfide (MoS$_2$), and tungsten-based nanostructures have also been demonstrated to directly sensitize formation of $^1\text{O}_2$ upon light irradiation. Thus, in the forthcoming section, we will discuss the development of different types of metal oxide NMs as either UV/vis or NIR light triggered NPSs for cancer PDT.

4.1. Titanium Oxide

In the past few decades, TiO$_2$ NPs with a 0D structure have emerged as one of the most extensively studied photocatalysts that have been widely used for organic synthesis, bleaching processes, and the degradation of organic pollutants due to their high ability to generate ROS.\(^{[51]}\) In addition, TiO$_2$ NPs possess excellent photostability and good biocompatibility, which could allow them to be used as NPSs in PDT.\(^{[52]}\) It is found that irradiation of TiO$_2$ NPs under UV light can produce ROS such as $^1\text{O}_2$, OH, H$_2$O$_2$, and $^1\text{O}_2$, which are highly toxic capable of oxidizing proteins and lipids in cells, finally killing cancer cells. In 2004, Nosaka et al. first reported the direct observation of $^1\text{O}_2$ generation from TiO$_2$ NPs under 355 nm pulsed laser irradiation using a gated photon counting method.\(^{[53]}\) They found that the $^1\text{O}_2$ quantum yield of TiO$_2$ NPs was roughly estimated to be $\approx\,0.2$ using RB (0.8) as a reference. The plausible mechanism to produce $^1\text{O}_2$ could be due to the photocatalytic oxidation of $^3\text{O}_2$ at the surface of TiO$_2$ NPs. It was interesting that the lifetime of $^1\text{O}_2$ generated from TiO$_2$ NPs was found to be much higher (5 µs) in ethanol as compared to that in either water (2 µs) or water–ethanol mixture (1:1). They later investigated the formation and behavior of $^1\text{O}_2$ using 10 different commercially available TiO$_2$ NPs under different circumstances such as air, H$_2$O, D$_2$O, and ethanol.\(^{[54]}\) The lifetimes of $^1\text{O}_2$ from all the 10 TiO$_2$ NPs in various environments were found to be very short (2–3 µs), while the $^1\text{O}_2$ quantum yields were in the range from 0.12 to 0.38. They found that the lifetime of $^1\text{O}_2$ in the air was shorter compared to that in H$_2$O, but it was longer in ethanol. This difference could be due to the different electronic-to-vibrational deactivation processes among them. They also demonstrated that the amount of $^1\text{O}_2$ was the same when the TiO$_2$ NPs were present in either H$_2$O or D$_2$O due to the similar chemical properties. However, in ethanol suspension, most of the photogenerated holes were consumed during the oxidation of ethanol, resulting in the substantial reduction of the $^1\text{O}_2$ generation. Moreover, they found that the $^1\text{O}_2$ quantum yield decreased with increasing size of TiO$_2$ NPs when the size is greater than 20 nm (except P25 TiO$_2$), which was presumably due to that the amount of O$_2$ adsorbed on the surface of TiO$_2$ NPs decreased when the particle size increased (Figure 9A). Based on these observations, they proposed a plausible mechanism to illustrate the $^1\text{O}_2$ generation by TiO$_2$ NPs upon light irradiation: O$_2$ adsorbed on the surface of TiO$_2$ NPs is reduced to $^1\text{O}_2$ by an electron in the conduction band (CB), which was followed by oxidation by the hole in the valence band (VB) to form $^3\text{O}_2$. The subsequent study with surface-modified nanocrystalline TiO$_2$ NPs demonstrated a higher $^1\text{O}_2$ quantum yield (0.012) as compared to that of bare TiO$_2$ NPs (0.003).\(^{[55]}\)

To get insight into the ROS generation mechanism,\(^{[26]}\) Li et al. investigated the ROS generation kinetics of seven different metal-oxide NPs (CeO$_2$, Fe$_2$O$_3$, SiO$_2$, Al$_2$O$_3$, ZnO, CuO, and TiO$_2$), and compared them to their bulk counterparts. They demonstrated that TiO$_2$ NPs and ZnO NPs produced three types of ROS ($^1\text{O}_2$, -OH, and $^1\text{O}_2$), whereas other metal-oxide NPs (e.g., CeO$_2$, SiO$_2$, Al$_2$O$_3$, and Fe$_2$O$_3$) produced only one or two types of ROS. CuO NPs did not generate any type of ROS. These results suggested that different metal-oxide NPs displayed distinct capacity to generate ROS upon light irradiation, with the order of TiO$_2$ NPs > ZnO NPs > Al$_2$O$_3$ NPs > SiO$_2$ NPs > Fe$_2$O$_3$ NPs > CeO$_2$ NPs > CuO NPs. Compared to their bulk counterparts, both TiO$_2$ and ZnO NPs generated more ROS under UV irradiation, presumably due to that NPs had larger surface areas capable of providing more reaction sites for the absorption of UV light. By comparing the electronic structures of metal-oxide NPs with the redox potentials of various ROS, they proposed that the band edge structures of metal-oxide NPs were crucial in the generation of ROS (Figure 9B). As ROS generation is dictated by the photoexcitation-driven interfacial electron transfer process, only the metal-oxide NPs with a band gap energy (Eg) smaller than the incident photon energy (i.e., \(\approx\,3.4\) eV for the 365 nm UV) can be photoexcited to generate excited electrons in CB and holes in the VB, which subsequently react with an aqueous electron acceptor (e.g., molecular oxygen) and donor (e.g., H$_2$O and OH$^-$), respectively, ultimately producing different types of ROS. Though TiO$_2$ NPs have exerted good ROS generation capacity, the requirement of UV light excitation has hampered their applications for in vivo PDT due to the low tissue penetration depth and UV light-induced damages to normal tissue. Considering that NIR light (650–1300 nm) can penetrate much deeper into tissues as compared to UV light, the incorporation of a light transducer in the TiO$_2$ NPs capable of converting NIR to UV light would be beneficial to engineer TiO$_2$ NPs as NIR-driven PFSs for in vivo PDT. Hence, upconversion nanoparticles (UCNPs) integration has been adopted to engineer TiO$_2$ NPs for the treatment of deep-seated tumors. Over the past decades, UCNPs made of lanthanide metals have exhibited unique optical properties of absorbing NIR light and emitting UV–vis light, which could serve as an efficient donor capable of transferring UV light to excite the attached TiO$_2$ NPs upon NIR light irradiation.\(^{[56]}\) Accordingly, a number of well-designed UCNPs with upconversion emission
spectra overlapped well with the absorption of the TiO2 NPs have been developed, allowing to build UCNP-TiO2 nanocomposites as NIR-driven ROS producers through an efficient energy transfer process.[57] In 2014, Zhang’s group reported a uniform core-shell UCNP-TiO2 nanocomposite consisting of a continuous layer of TiO2 coating on individual UCNP core. Under excitation with a 980 nm laser, the UCNP core could efficiently up-convert the 980 nm light to visible and UV light, which can subsequently activate the TiO2 layer to produce ROS like O2−, ·OH, and H2O2. They demonstrated that the as-prepared nanocomposite could enter into oral squamous carcinoma cells (OSCC) and kill more than 50% cells under 980 nm irradiation, indicating a potential NPSs for cancer PDT.[58] Based on this work, they later investigated the in vivo therapeutic applications of PEGylated UCNP-TiO2 composite against the OSCC tumor model.[59] After intratumoral injection of the PEG-UCNP-TiO2 into the tumor-bearing living mice, remarkable inhibition of tumor growth was achieved upon 980 nm light irradiation, which was approximately two to three times lower than the untreated groups. Importantly, no mice were died even after 60 days posttreatment, implying the excellent biocompatibility of the designed nanoconstruct. Meanwhile, Qu’s group reported another type of nanocomposite made from a mesoporous TiO2 (mTiO2) shell coated UCNPs for NIR-triggered synergistic chemo-photodynamic cancer therapy.[60] In their study, the mTiO2 shell in the nanocomposite could be activated by 980 nm light excitation to produce cytotoxic ROS through an efficient energy transfer from UCNP core to mTiO2. Moreover, the porous structures of mTiO2 shell could also load anticancer drugs (e.g., doxorubicin) for chemotherapy due to the large surface area. They found that the combined PDT and chemotherapy against cultured MDA-MB-231 breast cancer cells under 980 nm light excitation was more efficient compared to that of single PDT or chemotherapy.

To realize multimodality imaging-guided in vivo cancer PDT, Wu’s group recently developed folic acid-decorated NaGdF4:Yb/Tm@SiO2@TiO2 nanocomposites (FA-Gd-Si-Ti NPs) for both magnetic resonance imaging (MRI) and NIR-triggered PDT of cancer in living mice (Figure 10A).[61] In the structure of FA-Gd-Si-Ti NPs, the NaGdF4:Yb/Tm core could serve as both energy donors and T1-weighted MR contrast, and the TiO2 shell could serve as inorganic NPSs. Moreover, the presence of FA on the surface could selectively bind to the folate receptors overexpressed on some cancer cells, facilitating to enter into cancer cells. They demonstrated that the FA-Gd-Si-Ti NPs had a longitudinal T1 relaxivity of 4.53 mm−1 s−1, enabling to produce bright MR contrast in MCF-7 tumors (Figure 10B). After intratumoral injection into MCF-tumor-bearing mice, the tumor growth was significantly inhibited after irradiation of the MCF-7 tumors with a 980 nm laser. The tumor growth inhibition ratio was found to be ≈88.6% after 2 weeks, while the average body weights of mice were little changed during the course of treatment. Alternatively, Lin’s group reported similar TiO2-coated UCNP core/shell nanocomposites ((NaYF4:Yb4+, Tm3+@NaGdF4:Yb4+)@TiO2) as NIR light-activated NPSs for imaging-guided in vivo cancer PDT.[62] In their study, the polycrystalline anatase TiO2 NPs were successfully coated on the surface of the Yb/Tm-co-doped UCNP cores through hydrophilic polymer PVP-assisted one-step protocol. They demonstrated that the nanocomposite could be taken up by cancer cells via endocytosis and produce intracellular ROS upon 980 nm NIR laser irradiation, thus causing mitochondrial dysfunction and inducing cell apoptosis. The successful suppression of tumor growth in HeLa tumor-bearing mice that received intratumoral injection of the nanocomposites and 980 nm laser irradiation was also achieved, suggesting that the strategy of coupling TiO2 NPs with UCNPs was effective to sensitize formation of ROS for in vivo cancer PDT under NIR light irradiation. Different from the aforementioned UCNP-TiO2 nanocomplexes that used TiO2 as shells, Lin’s group latterly reported another folic acid-decorated core-shell-nanocomplexes (TiO2@Y2Ti2O7@YOF:Yb,Tm) (TYY), in which the TiO2 NPs were engineered as the core and the UCNPs of YOF:Yb,Tm with strong blue emissions were employed as the shell.[63] Moreover, an intermediate layer of Y2Ti2O7 NPs containing electron acceptors of Y3+ was also introduced to improve the photocatalytic activity. Besides ROS generation under 980 nm light excitation, the strong photothermal effect
could be simultaneously achieved owing to the nonradiative transition along with the recombination of electron-hole pairs. Figure 10C illustrates the plausible energy transfer process for the generation of ROS and heat from the TYY UCNPs under NIR light irradiation. Importantly, due to the presence of Yb\(^{3+}\) and Y\(^{3+}\), the designed TYY UCNPs also showed markedly enhanced CT contrast signal in vivo after injection (959.5 HU) compared to the control group (39.8 HU) (Figure 10D). Under 980 nm laser irradiation, the nanocomplexes showed appreciable combinational PDT and PTT effects by efficiently suppressing tumor growth in living mice (Figure 10E). Thus, TYY UCNPs could offer single NIR-light triggered CT-guided combinational in vivo cancer therapy.

Though the coupling of TiO\(_2\) NPs with UCNPs has shown promising results for in vivo PDT, the requirement of 980 nm NIR light as the irradiation source has brought an inherent drawback for in vivo applications. It is known that human tissues show high absorbance of light at 980 nm, which can shorten the penetration depth and induce overheating effect to cause normal tissue damage. Therefore, engineering of UCNPs-TiO\(_2\) nanocomposites capable of exciting by NIR light apart from the 980 nm wavelength could be beneficial for safe in vivo PDT, which was successfully demonstrated by Lin’s group.\(^{[64]}\) In their study, a well-defined core-shell UCNPs-TiO\(_2\) nanocomposite (UCNPs@mSiO\(_2@\)TiO\(_2\)) consisting of Nd\(^{3+}\)-sensitized UCNPs and TiO\(_2\) NPs were developed. The doping of Nd\(^{3+}\) into the outer layer of the UCNPs core could act as an efficient sensitizer for converting 808 nm NIR light to UV emission. To further improve the upconversion luminescence emission from UCNPs under 808 nm light irradiation, the UCNPs cores were smartly fabricated by introducing a quenching-shield layer to block the back energy transfer from the inner activator Tm\(^{3+}\) to Nd\(^{3+}\) in the outer layer. Meanwhile, UCNPs@mSiO\(_2@\)TiO\(_2\) also possessed dual-modal (CT/MRI) imaging features, offering high CT value (766.7 HU) after injection than control (23.53 HU) as well as T\(_1\) weighted longitudinal relaxivity \(r_1\) of 1.588 mm\(^{-1}\)s\(^{-1}\). In vivo studies showed that the 808 nm light-triggered PDT efficacy of UCNPs@mSiO\(_2@\)TiO\(_2\) in living mice was much higher compared to that with 980 nm or UV light. These results suggested that the 808 nm NIR light excitable UCNP-TiO\(_2\) nanocomposites could serve as promising NPSs for dual-modal imaging-guided safe and efficient cancer PDT.

### 4.2. Tungsten Oxide

Tungsten-based NMs belong to the family of transition metal oxide, are another kind of widely explored biomaterials as their LSPR properties can be easily tuned. Specifically, tungsten oxide-based 1D nanowires have been of particular interest due to their strong absorption in the NIR regions, high photothermal conversion efficiency, good O\(_2\) quantum yield, and large atomic number \((Z = 73)\). The first example of using tungsten oxide nanowires as NPS for cancer PDT was demonstrated by Hwang’s group in 2013.\(^{[65]}\) In their study, ultrathin PEGylated W\(_{18}\)O\(_{49}\) nanowires (PEG- W\(_{18}\)O\(_{49}\) NWs) with a length of 50 nm, a width of 4 nm,
and thickness of 1 nm were prepared, which showed extended absorption in the NIR region up to 1200 nm (Figure 11A). Under 980 nm excitation, the phosphorescence $^{1}\text{O}_2$ emission signal appeared at 1270 nm, indicating a $^{1}\text{O}_2$ quantum yield of 0.29. This result suggested that the PEG-W$_{18}$O$_{49}$ NWs hold a good ability to generate $^{1}\text{O}_2$ upon 980 nm NIR light irradiation. However, negligible $^{1}\text{O}_2$ was generated upon irradiation with an 808 nm laser, matching well to the corresponding excitation spectrum (Figure 11B). Cellular studies showed an obvious elevation of ROS levels inside HeLa cells following treatment with PEG-W$_{18}$O$_{49}$ NWs and 980 nm light irradiation, thus inducing significant cell death. A subsequent in vivo study on B16F0 melanoma tumor-bearing mice revealed successful destruction of tumors upon PDT with PEG-W$_{18}$O$_{49}$ NWs and 980 nm light irradiation. On day 10, the average tumor size was only 0.009% of the initial tumor size, which was much smaller compared to that following PTT with 808 nm irradiation (∼30.2%) or chemotherapy with doxorubicin (∼36%) (Figure 11C). Encouraged by this, Qiu et al. latterly reported PVP-decorated W$_{18}$O$_{49}$ NWs (PVP-W$_{18}$O$_{49}$ NWs) for combination PDT, PTT, and radiation therapy (RT) of cancers (Figure 11D). They demonstrated that the PVP-W$_{18}$O$_{49}$ NWs could produce remarkable heat and $^{1}\text{O}_2$ under 980 nm NIR laser irradiation, allowing to elicit PTT and PDT effects to kill tumor cells. Meanwhile, they could also act as radiation dose intensifying agents to enable additional RT due to the presence of heavy element W. The in vivo studies showed that mice administrated with the PVP-W$_{18}$O$_{49}$ NWs following by irradiation with a 980 nm NIR laser and gamma rays could completely eliminate the subcutaneous 4T1 murine breast tumors (Figure 11E), without tumor recurrence for at least 9 months. These results indicated that the PVP-W$_{18}$O$_{49}$ NWs with synergistic effects of PDT, PTT, and RT were very promising for cancer therapy.

### 4.3. Bismuth Oxyhalide

In addition to the previously described titanium and tungsten-based nanomaterials capable of direct generation of ROS to kill tumor cells upon light excitation, bismuth oxyhalide (BiOCl) is a new emerging metal oxide layered nanomaterial that possesses a tunable bandgap and fascinating physicochemical properties. Because of the unique layered structure, BiOCl showed great potential as a photocatalyst, which can be exploited for light-mediated therapeutic applications. For instance, Xiu et al. first investigated the ability of layered BiOCl for cancer PDT. In their work, two kinds of BiOCl nanostructures (nanoplates and nanosheets) were prepared by the hydrothermal method and further modified with polyetherimide (PEI) to ensure high aqueous solubility. Under low power UV light irradiation (2.2 mW cm$^{-2}$) for 10 min, BiOCl nanoplates and BiOCl nanosheets showed a dramatic decrease in the MCF cell viability (35% and 70%), respectively. Notably, TiO$_2$ did not show any therapeutic effect under this much low power UV irradiation. It has been observed that different electronic band structure and morphology of BiOCl nanoplates and BiOCl nanosheets influenced their cell uptake efficiency, and hence their PDT performance. Second, they
Figure 12. A) Schematic illustration for the formation of UCNPs@BiOCl composite. B) Time course absorbance spectrum of DPBF mixed with UCNPs@BiOCl at a wavelength of 410 nm under a 980 nm laser. C) Representative photographs of tumor-bearing mice and tumor tissue excised from (i) saline; (ii) pure UCNPs@BiOCl; (iii) 980 nm; and (iv) UCNPs@BiOCl with 980 nm on the 14 day. Reproduced with permission. Copyright 2017, Royal Society of Chemistry. D) CT images of tumor-bearing mice before (i) and after (ii) intratumor injection of BiOBr:Yb,Tm NSs. Reproduced with permission. Copyright 2017, Royal Society of Chemistry.

possessed different crystal facets (110, BiOCl nanoplates) and (001, BiOCl nanosheets), which strongly affected their in vitro PDT efficacies. Though both the nanostructures showed improved cell killing ability, they did not provide any evidence about the actual ROS specie responsible for inducing cell killing. On the other hand, low penetration depth and potential cytotoxicity associated with UV light remain a challenge in their clinical translation. Therefore, the UCNP@BiOCl nanohybrid was designed by Yang et al. to achieve NIR light-triggered PDT (Figure 12A).[69] Upon 980 nm light irradiation, UCNP@BiOCl showed higher \( \text{O}_2 \) production efficacy compared to UV light, which induced significant tumor cell destruction. This suggested that NIR light could replace UV/vis light for ROS generation (Figure 12B). Moreover, NIR light-induced in vivo PDT was successfully demonstrated in tumor-bearing mice (cervical carcinoma cell lines), showing the strongest tumor inhibition effect for mice treated with both UCNP@BiOCl and 980 nm light irradiation in comparison to control groups (Figure 12C). As UCNP@BiOCl increase the complexity of the system and demand careful consideration of excitation and emission wavelengths, they later developed self-activated Yb\(^{3+}/\text{Tm}^{3+}\) co-doped bismuth oxybromide (BiOBr) NSs under 980 nm light irradiation via simple co-precipitation approach and modified with PEG to endow excellent biocompatibility.[70] Due to self-activation, BiOBr:Yb\(^{3+}/\text{Tm}^{3+}\) NSs revealed high ROS generation capacity, offering exciting in vitro and in vivo therapeutic effects. Besides in vivo cancer therapy, BiOBr:Yb\(^{3+}/\text{Tm}^{3+}\) NSs also possess intrinsic fluorescence as well as due to Yb\(^{3+}/\text{Tm}^{3+}\) co-doping demonstrated far higher CT value after injection (976 HU) compared to without injection (63 HU) (Figure 12D), respectively, indicating their potential to serve as a dual-modal imaging agent capable of guiding precise cancer therapy in vivo. As the first demonstration of this kind, these findings will surely pave the way to develop a more self-activated nanoplatform for non-invasive deep cancer theranostics.

5. Metal Sulfides-Based NPSs

Apart from TiO\(_2\) NPs, MoS\(_2\), and CuS NPs with 0D nanostructures have also been reported to directly generate ROS upon light irradiation,[71,72] albeit most of them were widely used as photothermal agents. For example, small-size fluorescent MoS\(_2\) QDs with a mean diameter of \( \approx \) 14.7 nm have been synthesized using a tetrabutylammonium-assisted sonication approach.[73] Upon irradiation with a 630 nm light, the MoS\(_2\) QDs demonstrated a higher \( \text{O}_2 \) generation ability compared to that of an organic PS, PpIX, as indicated by both the reduction in DPBF absorption and increase in SOSG fluorescence intensity. Though the generation of \( \text{O}_2 \) upon irradiation of MoS\(_2\) QDs solution was demonstrated, whether the MoS\(_2\) QDs could serve as PSs to initiate PDT effect either in culture cells or in vivo was still unknown, which will require more biological studies in the future. In another study, Pellegri and coauthors reported small plasmonic CuS nanocrystals (CuS NCS) that showed high photothermal and photodynamic properties upon 808 nm NIR light irradiation, which could efficiently suppress B16 subcutaneous tumor growth in living mice.[74] Interestingly, they demonstrated that -OH but not \( \text{O}_2 \) was observed upon irradiation of the CuS NCS, which was mainly due to the heat-triggered release of Cu(I) ions capable of acting as a source of ROS generation.[75] Since the generation of
-OH and heat was independent of O₂, the CuS NCs might be feasible to treat hypoxic tumors in vivo.

6. Carbon-Based NPSs

In the past few decades, carbon NMs, including 0D fullerenes and carbon dots (C-dots), 1D carbon nanotubes, and 2D graphene, and graphitic carbon nitride have attracted tremendous attention in biomedical research due to their unique and prominent physicochemical and optical properties. For example, carbon NMs have the advantages of chemical inertness, good biocompatibility, high photostability, and tunable fluorescence emission ranging from visible to NIR-II windows, which make them highly attractive for biosensing and molecular imaging in vivo. Recently, well-designed carbon NMs like fullerenes (C₆₀) and GQDs have also been used as NPSs to trigger efficient PDT against tumors due to their high ability to sensitize the formation of ROS. In the following section, we will discuss the recent progress of using carbon NMs as NPSs for cancer PDT.

6.1. 2D Graphene

Fullerenes are soccer-ball-shaped NMs composed of sixty or seventy carbon atoms, which were discovered by Kroto et al in 1985. The photophysical properties of fullerenes such as photoabsorption, fluorescence, and phosphorescence have attracted people to explore its potential as NPSs for PDT. However, low solubility under physiological conditions has substantially hindered their applications in biological systems. To overcome this limitation, different functional groups (e.g., OH, COOH, and NH₂) have been introduced into the fullerenes to improve their aqueous solubility. These water-soluble fullerenes derivatives have shown a high ability to produce ROS like 1O₂, O₂⁻, and -OH under photoexcitation, which have been successfully applied to elicit PDT activity against viruses, bacteria, and cancer cells. For example, Tegos et al. prepared six fullerenes (C₆₀) derivatives functionalized with one, two, or three polar diserinol groups or cationic quarternary pyrroolidinium groups, and compared their PDT activities against gram-positive bacteria, gram-negative bacteria, and fungi upon white light irradiation. They found that the cationic fullerenes derivatives exerted a broad-spectrum antimicrobial activity, which can rapidly kill more than 99.99% of bacteria and fungi following illumination with white light. The antimicrobial PDT activities were better than that of neutral diserinol group-modified fullerenes. This was due to the increasing positive charge in the cationic group-modified fullerenes that could help to bind bacteria and overcome the microbial permeability barriers. These results suggested that cationic fullerenes could be used as effective NPSs for the treatment of localized infections. Besides the antimicrobial PDT activities, they also showed that the cationic group-functionalized fullerenes could trigger efficient PDT to kill cancer cells via both type-I and type-II photochemical processes under illumination.

In 2014, Hu et al. developed C₆₀-PDA–rGO nanohybrids consisting of C₆₀ fullerene, polydopamine (PDA)-coated rGO, and folic acid for targeting PDT/PTT synergistic activities to kill HeLa cells. In their study, the graphene oxide was reduced and coated by PDA to form PDA-rGO, which then subsequently reacted with folic acid-decorated C₆₀ fullerenes (FFA) via a Schiff base reaction or Michael addition. The as-prepared C₆₀-PDA-rGO showed a broad absorption extending to NIR regions. Upon irradiation with a Xe lamp equipped with a bandpass filter (400–1100 nm), an obvious production of ¹O₂ as indicated by p-nitroso- N,N’-dimethylaniline was observed in the C₆₀-PDA-rGO aqueous solution. Meanwhile, a remarkable increase in temperature (ΔT = 17.2 °C) was also achieved. Cellular studies showed that the C₆₀-PDA-rGO could efficiently enter into HeLa cells via folate receptor-mediated uptake, and exerted obvious cytotoxicity toward HeLa cells under irradiation by the Xe lamp (Figure 13). It was also found that the phototoxicity induced by C₆₀-PDA-rGO was higher than that induced by either PDA-rGO or FFA, suggesting that C₆₀-PDA-rGO with combined PDT and PTT effects was very promising for synergistic therapy of cancers. Based on this work, they latterly employed host–guest chemistry to develop GO-FA/PY-γ-CD/C₆₀ nanohybrids, which also showed synergistic PDT/PTT effects to efficiently kill cancer cells under Xe light irradiation.

In addition to fullerenes, another carbon allotrope, GQDs have also been developed as efficient NPSs for PDT. For example, Markovic et al. reported that GQDs could kill cancer cells by inducing both apoptosis and autophagy (programmed cell death). Briefly, GQDs with an average diameter of 56.6 nm and a height of 1.9 nm, respectively, were prepared by an electrochemical approach, showing strong absorption in the UV/vis region and the maximum fluorescence emission at 460 nm. Upon continuous laser irradiation (488 nm, 1 W cm⁻², 30 min), the prepared GQDs demonstrated a significant amount of ¹O₂ as determined by ¹O₂ sensitive substrate dihydrodorhamidine 123 and EPR spectroscopy. Due to the high ¹O₂ generation, GQDs induce oxidative stress which leads to the effective killing of human glioma cells. Notably, increased cell granularity and the downregulation of p62 protein were also observed upon exposure to photo-irradiated GQDs, suggesting that GQDs could induce both apoptotic and autophagic cell death pathways, which significantly contributed to higher cellular destruction. Importantly, photoexcited GQDs did not show any temperature increase, which clearly rules out the possibility of photothermal killing, suggesting that cellular killing is solely attributed to ¹O₂ generation and the activation of dual cell death pathways. Later, the same group reported the antibacterial activity of GQDs against gram-positive (S. aureus) and gram-negative (E. coli) bacteria, which also showed a significant reduction in bacterial colonies as observed by the standard plate count method, demonstrating excellent antibacterial activity of GQDs for both bacterial species. Though these findings indicated that GQDs could act as NPs to initiate efficient anti-tumor and anti-bacterial PDT, UV light excitation may damage the cells. To address this issue, Kuo et al. developed TPE GQDs by the ultrasonic shearing reaction, showing an absolute TPE cross-section of 47903 GM (Goeppert–Mayer). They demonstrated that under TPE excitation (808 nm, 2.64 mW) for only 15 s, GQDs could efficiently produce ¹O₂ and O₂⁻, which almost completely eliminate both gram +ve and gram –ve bacteria, respectively. Moreover, due to the strong TP luminescence (TPL), GQDs could also serve as a potential contrast agent, enabling imaging-guided TPE.
antibacterial PDT. To enhance the ROS generation capacity, the same group further designed one-photon excited nitrogen-doped GQDs (N-GQDs) and TPE amino-functionalized N-GQDs with a TPE cross-section of 54854 Gm, which also exhibited intrinsic imaging as well as enhanced TPL character.\textsuperscript{[90]} It has been notable that N doping of GQDs as well as amino functionalization of N-GQDs could readily increase the charge transfer efficiency, which in turn improved the ROS generation capacity, resulting in complete bacterial elimination under one photon (670 nm, 0.1 W cm\textsuperscript{−2}) excitation for 3 min and TPE (808 nm, 2.3936 mW) for only 12 s, respectively. Importantly, high antibacterial PDT efficacy could be attributed to high \( {^1}O_2 \) quantum yield, which was found to be 0.60 and 0.53 for N-GQDs and amino-N-GQDs, respectively, compared to 0.41 for unmodified GQDs. Alternatively, another work from Zhang et al. elucidated that the chemical reduction of GOQDs (rGOQDs) could also enhance the ROS generation capacity as a higher yield of ROS including \( {^1}O_2, \, O_2^- \), and \( H_2O_2 \) was achieved under white light irradiation compared to GOQDs.\textsuperscript{[21]} They suggested that the rGOQDs generated more electron–hole pairs due to the lower bandgap and VB than GOQDs, resulting in improved ROS generation and higher in vitro cancer PDT efficacy. In a subsequent study, Liu et al. provided another strategy to improve ROS yield by coupling GQDs with ZnO. Instead of \( {^1}O_2 \), the resultant hybrid ZnO/GQD showed increased \( \cdot OH \) formation, leading to improved antibacterial effects.\textsuperscript{[22]} However, due to the UV light absorption of the hybrid, they could be applied for wastewater treatment but are not suitable for in vivo anticancer therapy. Meanwhile, instead of chemical doping and functionalization, the latter approaches provided an effective way to improve the ROS yield of GQDs via surface modification and coupling. GQDs were modified with adenine (A-GQDs) by Wang’s group, showing efficient TPE cellular imaging and \( {^1}O_2 \) mediated bacterial killing under white light irradiation.\textsuperscript{[91]} Interestingly, A-GQDs could not get entry into the gram +ve bacteria due to the electrostatic repulsion, and thus A-GQDs are only effective against gram –ve bacteria. Though exciting antibacterial results have been achieved previously, certain limitations hindered the practical application of GQDs. For example, 1) the requirement of expensive femtosecond laser for TPE imaging-guided antibacterial PDT; 2) doping of GQDs to enhance ROS generation capacity is a laborious and time-consuming process, which demand careful consideration of sophisticated reactions; 3) though GQDs could act as a NPSs to initiate efficient anti-tumor and anti-bacterial PDT, their in vivo anticancer PDT applications are remain unverified.

Bearing this in mind, Ge et al. investigated the in vivo therapeutic applications of GQDs. They developed GQDs by hydrothermal treatment of polythiophene, exhibiting broad absorption ranges from 400–700 nm, deep red emission at 680 nm, and superior photostability than organic dye (PpIX) and CdTe QDs.\textsuperscript{[14]} It has been observed that GQDs generate \( {^1}O_2 \) through energy transfer (type-II) mechanism as evidenced by characteristic \( {^1}O_2 \) ESR signal (1:1:1) and phosphorescent \( {^1}O_2 \) emission signal at 1264 nm. Moreover, the \( {^1}O_2 \) quantum yield was determined by disodium 9,10 anthracendipropionic acid (Na\textsubscript{2}-ADPA), a chemical trapping agent, revealing that the GQDs possessed an extremely high \( {^1}O_2 \) quantum yield of 1.3, which was possibly due to the proposed multistate sensitization mechanism (Figure 14A). Briefly, according to the absorption and fluorescence spectra, the energy difference between ground state (S0) and excited singlet state (S1) of GQDs was estimated to be 49.3 kcal mol\textsuperscript{−1}, and the energy of T1 was calculated to be 22.5 and 26.5 kcal mol\textsuperscript{−1}. Whereas, the energy difference between S1 and T1 was estimated to be 22.8 to 26.5 kcal mol\textsuperscript{−1}. As the formation of \( {^1}O_2 \) from \( {^3}O_2 \) requires an amount of 22.5 kcal mol\textsuperscript{−1} energy, the \( {^1}O_2 \) received an equal amount of energy on each transition either from S1 to T1 or from T1 to S0 to be converted into \( {^1}O_2 \), resulting in high \( {^1}O_2 \) generation. Due to the high \( {^1}O_2 \) quantum yield, GQDs showed...
concentration-dependent toxicity as upon light irradiation using Xe lamp for 10 min, the cell viability decreased from 60% to 20% when the concentration of GQDs increased from 0.036 to 1.8 μm. The in vivo therapeutic efficacy of GQDs against subcutaneous breast cancer xenografts showed that Balb/c nu mice with intratumoral injection of GQDs (4 mg kg⁻¹) followed by white light irradiation (80 mW cm⁻²) for 10 min could efficiently destroy the tumors after 17 days compared to control groups, which showed significant tumor growth. Interestingly, high in vivo fluorescence intensity with a high signal-to-noise ratio was achieved, indicating that GQDs could also serve as an in vivo imaging agent (Figure 14B,C). Consequently, this work suggested that GQDs with excellent biocompatibility and higher ¹⁰⁰₂ quantum yield are a promising candidate for in vivo cancer diagnosis and therapy with enhanced therapeutic efficacy and lesser side effects.

To get multimodal imaging-guided synergistic PDT/PTT therapy, the same group developed Au NRs@silica-carbon dots (GNR@SiO₂-CD).[92] In the structure, GNRs with a strong longitudinal plasmon band centered at 810 nm could serve as a PA and PTT agent, while CDs act as FL imaging and PDT agent. Meanwhile, the coating of the SiO₂ layer onto GNRs avoids the complete fluorescence quenching of CDs. They demonstrated that the GNR@SiO₂-CD could produce remarkable heat and ¹⁰⁰₂ under 808 nm (0.5 W cm⁻²) and 635 nm (0.1 W cm⁻²) laser irradiation, allowing to elicit combined PTT and PDT effects to kill B16-F0 tumor cells (Figure 14D). In vivo studies on HeLa tumor-bearing mice following intratumoral injection of GNR@SiO₂-CDs showed remarkably increased FL and PAI signals, and the tumors were completely eliminated under irradiation with 808 nm and 635 nm light irradiation for 10 min, with no apparent side toxicity (Figure 14E,F). Though they demonstrated that synergistic PDT/PTT therapy far better than either PDT or PTT alone, the sequential irradiation by two different lasers to induce combined PTT/PDT effects prolongs the treatment time as well as complicates the treatment process. In addition, it is very hard to precisely align the two laser beams at the same position. Therefore, the development of different strategies or new NPs capable of inducing combined PDT/PTT effects under single laser irradiation is highly demanded.

Recently, Xing’s group covalently attached rhodamine derivative (TRITC) with UCNP-GQDs hybrid and illustrated mitochondrial-targeted NIR triggered in vivo PDT.[93] Due to the active mitochondrial targeting, UCNP-GQD/TRITC demonstrated in situ ¹⁰⁰₂ generation within mitochondria under NIR excitation (980 nm), which significantly lower the mitochondrial membrane potential, resulting in the initiation of irreversible tumor apoptotic pathway as verified by the 3.6-fold enhanced caspase activity for UCNP-GQD/TRITC compared to 2.5-fold for non-targeted UCNP-GQDs. Moreover, in vivo investigation further revealed effective tumor growth inhibition, suggesting the improved therapeutic performance of UCNP-GQD/TRITC than UCNP-GQDs, respectively. Subsequently, synergistic targeting was demonstrated by Liu et al. by attaching folic acid and mitochondrial targeting moiety (cabosubutyl triphenylphosphorium) with UCNPs-GQDs nanohybrid to establish synergistic targeting effects (Figure 15A).[23] Notably, dual targeting ligands improved the cellular uptake of UCN-GQDs composite in vitro, showing a 90% increase (3.95 μg per 10⁴ cells) compared to nanohybrid alone (2.08 μg per 10⁴ cells) as further characterized by TEM (Figure 15B). Moreover, under 980 nm laser irradiation...
(1 W cm\(^{-2}\)) for 10 min, the designed nanohybrid demonstrates efficient \(^1\)O\(_2\) generation efficiency as revealed by the 80% attenuation of ABDA absorption at 400 nm (Figure 15C). Interestingly, the current study employed the strong acid oxidation approach to develop GQDs which possess an ample amount of oxygenated functional groups, enabling efficient \(O_2\) quenching of triplet states, resulting in increased \(^1\)O\(_2\) quantum yield (1.56). Importantly, this is the highest \(^1\)O\(_2\) quantum yield ever reported by any organic PS or even inorganic NPS. Due to the highest \(^1\)O\(_2\) quantum yield and higher cellular uptake ability, dual-targeted UCNP-GQDs nanohybrid exerted significant cell killing and a higher degree of mitochondrial damage compared to either no or single-targeted UCNP-GQDs. Meanwhile, in vivo PDT evaluation using female Balb/C nude mice under 980 nm irradiation (0.5 W cm\(^{-2}\), 30 min) suggested higher uptake of intraperitoneally injected dual-targeted nanohybrid (0.21% ID per g) compared to FA (0.15%) or TPP (0.12%) targeted nanohybrid, respectively, resulting in remarkable tumor eradication (Figure 15D). Owing to the dual-targeting approach, this work will pave the way to improve the accumulation of nanoformulations for better therapeutic performance. In addition, due to the highest \(^1\)O\(_2\) quantum yield, the current nanotherapeutic agent could serve as high-efficiency NPSs for NIR excited deep-seated tumor PDT. Meanwhile, in another study Chen’s group developed ultrasmall (5 nm) gadolinium encapsulated graphene carbon NPs (Gd@GCNs), which could rapidly be cleared from the body through renal clearance, ensuring safe in vivo cancer PDT\(^{[94]}\). Under LED irradiation, these Gd@GCNs NPs demonstrated effective \(^1\)O\(_2\) generation with a quantum yield of about 0.51, which could effectively destruct cell/tumor both in vitro and in vivo, respectively. In addition, Gd@GCNs showed fluorescence imaging as well as \(T_1\)-weighted MRI with high longitudinal relaxivity (16.0 \(\times\) 10\(^{-3}\) m\(^{-1}\) s\(^{-1}\)), enabling dual-modal imaging-guided in vivo PDT (Figure 15E). Collectively, these excellent physicochemical and toxicological characters illustrated that Gd@GCNs is a state-of-the-art nanotheranostic tool with substantial potential for clinical translation.

### 6.2. Graphitic Carbon Nitride

Graphitic carbon nitride nanosheets (g-C\(_3\)N\(_4\) NSs) are another newly reported 2D carbon-based NM, which are mainly composed of carbon and nitrogen atoms\(^{[95]}\). Intriguingly, g-C\(_3\)N\(_4\) NSs possess high biocompatibility, high photoluminescence (PL) quantum yields, and facile surface modification\(^{[96]}\), which could facilitate their biomedical applications. Recently, the use of g-C\(_3\)N\(_4\) NSs as NPSs capable of producing \(^1\)O\(_2\) for cancer PDT has also been reported\(^{[97]}\). For instance, ultrasonic exfoliation of bulk g-C\(_3\)N\(_4\) was performed by Lin et al. to develop g-C\(_3\)N\(_4\) NSs possessing a lateral dimension of 40 nm and a thickness of 1.1 nm, respectively\(^{[98]}\). The resultant g-C\(_3\)N\(_4\) NSs showed
efficient in vitro ROS generation under LED light irradiation (405 nm, 20 mW cm\(^{-2}\), 10 min) as monitored by the bright intracellular green fluorescence from DCFH-DA, which results in dose and irradiation time-dependent induction of HeLa cells death. In the meantime, HeLa cells incubated with g-C\(_3\)N\(_4\) NSs also demonstrate bright blue color emission under 405 nm excitation wavelength, suggesting the potential of g-C\(_3\)N\(_4\) NSs as an imaging agent. Moreover, to the high surface area to volume ratio of 2D NMs, g-C\(_3\)N\(_4\) NSs also offer pH-dependent drug loading and release of DOX, and thus could act as a pH-responsive drug nanocarrier (Figure 16A). Meanwhile, both g-C\(_3\)N\(_4\) NSs and DOX have been loaded into zeolitic-imidazolate framework-8 (ZIF-8) by Chen et al., which also showed pH-responsive drug release and \(^{1}\text{O}_2\) generation under visible light irradiation, leading to enhanced therapeutic efficacy.\(^{99}\) Similar to g-C\(_3\)N\(_4\) NSs, HA functionalized graphitic hollow C\(_3\)N\(_4\) nanospheres (GHCSNS) also showed imaging-guided dual-modal chemo/PDT.\(^{100}\) Overall, these findings illustrated that g-C\(_3\)N\(_4\) based nanostructures could serve as a multifunctional nanoagent, offering stimuli-responsive chemotherapy as well as imaging-guided PDT. Though g-C\(_3\)N\(_4\) NSs have shown promising PDT efficacy in vitro, the lower triplet exciton yield limited their ROSs generation efficiency. It has been noted that triplet exciton yield could be enhanced by incorporating carbonyl groups into the g-C\(_3\)N\(_4\) NSs via a simple oxidation approach.\(^{101}\) Interestingly, carbonyl incorporation remarkably reduced the energy gap of singlet to triplet states as well as promoting spin-orbit coupling, which in turn facilitate higher \(^{1}\text{O}_2\) generation through the type-II process as well as suppress other ROS species. In contrast, unoxidized pristine g-C\(_3\)N\(_4\) only produces \(^{\cdot}\text{OH}\) (Figure 16B,C). Conclusively, this work established a new understanding that how the excitonic process influences the formation of \(^{1}\text{O}_2\) in g-C\(_3\)N\(_4\), which would be helpful to design NPSs with highly efficient \(^{1}\text{O}_2\) generation. In a subsequent study, Ju et al. suggested that the integration of metal ions (Cu\(^{2+}\)) into the g-C\(_3\)N\(_4\) is also an effective way to enhance the ROS generation capacity.\(^{102}\) As Cu\(^{2+}\) integration lowered the energy gap between singlet and triplet states of g-C\(_3\)N\(_4\), the resultant Cu\(^{2+}\)-g-C\(_3\)N\(_4\) NSs could efficiently catalyze the formation of \(\text{O}_2^{\cdot -}\) and \(^{\cdot}\text{OH}\) from \(\text{O}_2\) and \(\text{H}_2\text{O}_2\), respectively, compared to unmodified g-C\(_3\)N\(_4\) NSs. In addition to improved ROS generation, Cu\(^{2+}\)-g-C\(_3\)N\(_4\) also showed the potential to effectively reduce glutathione (GSH) level by oxidizing GSH into GSSH (Figure 16D,E). As a high level of endogenous GSH significantly consumes the generated ROS, oxidized GSSH could not consume the ROS, which in turn augment the intracellular ROS. Moreover, in the presence of GSH, Cu\(^{2+}\)-g-C\(_3\)N\(_4\) exhibited enhanced intracellular DCFH fluorescence (74%) compared to g-C\(_3\)N\(_4\) NSs (20%), confirming the depletion of GSH level. Thus, Cu\(^{2+}\)-g-C\(_3\)N\(_4\) illustrated improved in vitro PDT outcomes due to the enhanced ROS generation and efficient depletion of GSH. Though g-C\(_3\)N\(_4\) NSs, CNO and Cu\(^{2+}\)-g-C\(_3\)N\(_4\) hybrid have been demonstrated as potential NPSs for cancer PDT, only in vitro investigation of these NMs has been conducted. Thus, there is still a lack of evidence showing their potential for in vivo cancer treatment. Furthermore, absorption in the UV/vis region significantly limits their in vivo therapeutic applications due to the poor tissue penetration depth of UV/vis light.

Figure 16. A) Schematic illustration of g-C\(_3\)N\(_4\) NSs as potential NPS and pH-responsive drug nanocarrier for cancer therapy. Reproduced with permission.\(^{98}\) Copyright 2014, Royal Society of Chemistry. B) The absorbance of TMB oxidation with pristine g-C\(_3\)N\(_4\) and CNO monitored at 380 nm in the presence of different scavengers. C) ESR spectra of different samples in the presence of TEMP. Reproduced with permission.\(^{101}\) Copyright 2016, Wiley-VCH. D) Illustration of g-C\(_3\)N\(_4\) NSs as NPS for PDT and Cu\(^{2+}\)-g-C\(_3\)N\(_4\) NSs with enhanced PDT through the synergistic effect of extra ROS generation and GSH depletion. E) Proposed mechanism of GSH reduction and enhanced cytotoxicity. Reproduced with permission.\(^{102}\) Copyright 2016, Wiley-VCH.
To develop NIR excitable g-C₃N₄ based NPSs, Feng et al. integrated UCNP with g-C₃N₄ NS, which can efficiently convert NIR light (980 nm) and emit UV/vis light that matches well with the absorption of g-C₃N₄ NSs. Hence, under 980 nm light excitation, the designed g-C₃N₄/UCNP NCs showed high intracellular \( ^1O_2 \) generation as verified by the bright green fluorescence of DCFH, which induces significant HeLa cell destruction in vitro. Subsequently, in vivo PDT investigation utilizing the intravenous injection of g-C₃N₄/UCNP NCs (100 mL, 1 mg mL\(^{-1}\)) into HeLa tumor-bearing mice was also conducted, showing a remarkable inhibition of tumor growth in mice receiving both g-C₃N₄/UCNP NCs and 980 nm light irradiation (2.5 W cm\(^{-2}\)). In contrast, control groups showed a negligible growth inhibition effect. Meanwhile, UCL of UCNP and intrinsic fluorescence character of g-C₃N₄ also offer imaging-guided in vivo PDT. Thus, these results indicate the suitability of g-C₃N₄/UCNP as a NIR-triggered theranostic agent.

7. Phosphorus-Based NPSs

Black phosphorus (BP) belongs to the class of attractive 2D inorganic NM that possess sheet-like morphology with a lateral size of 100 nm to a few μm, while the thickness is within a few atoms (typically <5 μm), tunable band gap, and unusual structural anisotropy. Importantly, structural anisotropic feature significantly contributes to its exceptional optical and electronic properties, which differentiate it from other 2D-NM, for example, graphene, tungsten diselenide (WSe₂), MoS₂, and boron nitride (BN). Though BP has been widely explored in electronic industry, unfortunately, little attention has been paid to explore its potential photodynamic therapeutic applications. Hence, studies reported the therapeutic applications of BPs are surprisingly rare. Recently, Wang et al. provided an unprecedented observation that ultrathin BP nanosheets (NSs) could sensitize \( ^1O_2 \) generation for efficient PDT. They performed the exfoliation of bulk BP sample to develop BP NSs with a diameter of about several 100 nm and a height of 2.0 nm, respectively (Figure 18A). The resultant BP NSs demonstrated effective \( ^1O_2 \) generation efficiency as confirmed by the gradual reduction in DPBF absorbance at 410 nm, an enhanced ESR signal, and the appearance of \( ^1O_2 \) phosphorescence signal at 1270 nm, respectively (Figure 18B,C). Further, by taking RB as the reference (0.86), the \( ^1O_2 \) quantum yield of BP NSs was calculated to be 0.91, which was much higher than the most other reported 2D-NM-based NPSs. The cellular studies showed that the designed BP NSs exhibited negligible dark toxicity, while a dramatic reduction in cellular viability was observed upon light irradiation at 660 nm (1 W cm\(^{-2}\)) for 10 min. Subsequently, in vivo PDT evaluation using Balb/c nude mouse after intratumoral injection of BP NSs and light ir-
radiation suggested a significant reduction in tumor growth as compared to control groups (Figure 18D), which is further confirmed by immunohistochemical analysis of tumor tissues.

Though BP NSs showed very high $^{1}O_2$ quantum yield and efficient in vivo antitumor effects, the absorption in the UV/vis region does not allow deep penetration, and second, there is a strong concern regarding potential cytotoxicity associated with UV light. To address these issues, Lin’s group designed NIR excitable UCNP@BP nanocomposite for deep cancer PDT.\cite{108} NaGdF4:Yb,Er@Yb@Nd@Yb UCNPs and BP NSs were first surface modified with polyacrylic acid and N-methyl-2-pyrrolidinone, respectively, followed by integrated them by electrostatic interaction to prepare UCNP@BP (Figure 18E). They demonstrated that 808 nm light irradiation was more appropriate to sensitize high $^{1}O_2$ generation by UCNP@BP than 650 or 980 nm as monitored by a reduction in DPBF absorbance at 410 nm (Figure 18F). Both in vitro and in vivo investigations demonstrated remarkable cell/tumor destruction under 808 nm laser irradiation for 5 min (Figure 18G). Collectively, this study indicated the high ability of UCNP@BP for single NIR laser triggered in vivo deep cancer PDT.

Though single treatment modality is offering exciting therapeutic results, multimodal therapy could completely eradicate the tumor and thus demonstrate far superior antitumor efficacy than a single treatment approach. Bearing this in mind, Chen et al. developed a multimodal therapeutic platform based on BP NSs, which could induce chemotherapy/PDT/PTT under single laser irradiation.\cite{109} They demonstrated that high surface area to volume ratio of BP NSs could allow high drug loading capacity (95% in weight) of electrostatically assembled doxorubicin (DOX, a chemotherapeutic drug). Further, due to the broad absorption from visible to NIR region, BP NSs demonstrated efficient photothermal conversion efficacy as temperature increased from 23 to 45 °C under 808 nm light irradiation (1 W cm$^{-2}$, 3 min). These results suggested that BP NSs could enable the photothermal release of DOX (90% release) to induce chemotherapy as well as...
could also act as a PTT agent to induce PTT. Besides previously reported BP NSs (PDT agent), the current BP-DOX nanoplatorm could induce synergistic chemotherapy/PDT/PTT under 660 nm and 808 nm, respectively, resulting in significant 4T1 cell/tumor destruction both in vitro and in vivo. Though previous studies efficiently demonstrated the in vivo therapeutic performance of BP NSs, the continuous consumption of tissue oxygen to generate $^1O_2$ could result in tumor hypoxia, which ultimately limits the PDT efficacy. Incorporation of catalase enzyme into therapeutic formulations is an effective strategy to overcome tumor hypoxia, but enzyme denaturation during both storage and handling is a major concern, which impedes their practical applicability. Alternatively, it is well documented that Pt NPs could act as an artificial catalase enzyme, offering facile synthesis, high stability, excellent catalytic activity, and biocompatibility. Keeping this in mind, Liu’s group decorated BP NSs with Pt NPs through an in situ growth approach to overcome tumor hypoxia (Figure 19A). Upon the addition of $H_2O_2$ and light irradiation (660 nm), Pt@BP hybrid showed more reduction in DPBF absorption (35%) than either BP NSs alone or Pt@BP hybrid (<10%) without the presence of $H_2O_2$, implying that Pt NPs could efficiently decompose $H_2O_2$ and alleviate tissue oxygen level, resulting in enhanced PDT efficacy (Figure 19B). This was further confirmed by immunohistochemical staining, which revealed the downregulation of hypoxia-inducible factor (HIF-1). Due to the effective decomposition of $H_2O_2$, Pt@BP hybrid demonstrated high in vitro and in vivo PDT performance against 4T1 cells/tumor-bearing mice (Figure 19C). Upon the addition of $H_2O_2$, the 4T1 cell/tumor-bearing mice showed significantly enhanced PDT efficacy.

Li et al. reported the design of BP quantum dots (BP QDs) as theranostic probes for bioimaging and synergistic PDT/PTT of cancers. They used a liquid exfoliation approach to prepare BP QDs with a mean diameter of 2.5 nm, which was further modified with hydrophilic PEG5000 polymers to improve their water solubility and biocompatibility. The synthesized BP QDs exhibited a broad absorption band across the UV and NIR regions, and good photoluminescence property (excitation $= 500$ nm, emission $= 577$ nm), indicating the potential for bioimaging. The $^1O_2$ generation was verified by DPBF and ESR spectroscopy and the $^1O_2$ quantum yield was found to be $≈ 0.74$ as monitored by PL spectra (Figure 19D). In addition to $^1O_2$ generation, BP QDs also showed significant temperature increase, which indicated that they could also be used as a PTT agent (Figure 19E). The combined in vitro PDT/PTT cellular studies showed that BP QDs could enter HepG2 liver tumor cells, causing more than 80% cell death upon irradiation with 625 nm (80 mW cm$^{-2}$, 10 min) and 808 nm (2 W cm$^{-2}$, 2 min) laser for combined PDT/PTT, demonstrating superior synergistic anticancer efficacies over single treatment modality. Moreover, the combinational PDT/PTT modalities could also allow BP QDs to efficiently eradicate tumors in 4T1 tumor-bearing mice (Figure 19F). Importantly, due to their small size, BP QDs could easily and rapidly remove from the body without exerting any potential cytotoxicity, thus offering safe in vivo cancer therapy.

For instance, Guo et al. verified the renal clearance of BP QDs by monitoring the P content in urine. They demonstrated that more than 65% of BP QDs were found in the urine within 8 h of intravenous injection, confirming their effective clearance through the excretory system. Further, TEM analysis of BP QDs collected from urine sample show the same morphology and hydrodynamic size, indicating their intact nature, which could allow
safe long-term mediated cancer PDT. Meanwhile, under 670 nm light irradiation, BP QDs effectively generate $^{1}$O$_2$ which could result in anti-cancer PDT effects both in vitro and in vivo. In addition to combined PDT/PTT, Zhang et al. developed MRI guided synergistic PDT/PTT platform by assembling Fe$_3$O$_4$-carbon dots (Fe$_3$O$_4$-CD) with BP QDs and revealed that the resultant Fe$_3$O$_4$-CD@BP possess high transverse relaxivity ($r_2$) of 50.867 × 10$^{-3}$ m$^{-1}$ s$^{-1}$ with a concentration-dependent darkening effect, enabling enhanced T$_2$ MRI in vivo.$^{[111]}$ Taken together, such observations evidently demonstrated that BP QDs could serve as an effective theranostic agent capable of diagnosis as well as initiate synergistic PDT/PTT against cancers under NIR light irradiation. Though combined PDT/PTT activity of BP QDs has been successfully demonstrated in vivo by different groups, the requirement of two different laser is a major concern in their practical clinical application. Therefore, the development of BP QDs which could trigger combine PDT/PTT under single NIR laser is highly demanded.

8. Hybrid-Based NPSs

Though metal oxides especially TiO$_2$ have shown remarkable biomedical applications both in vitro and in vivo as we have discussed in detail in the previous section. However, absorption in the UV region due to the high band gap is a major hurdle in the practical clinical applications of TiO$_2$ based NPSs. To address this issue, UCNPs have been integrated with TiO$_2$ based NPSs as we discuss several recent examples of this approach in the previous section. However, the integration of UCNPs increases the complexity of the system and requires careful consideration of excitation and emission wavelengths. Alternatively, plasmonic metal nanocrystals have high absorption/scattering cross-sections and thus, can enhance and extend the light absorption of TiO$_2$ NSs through scattering, absorption enhancement, and hot electron injection.$^{[114]}$ Encouraged by this, plasmonic metal NSs have been successfully incorporated into TiO$_2$ NSs to harvest visible or NIR light for the generation of $^{1}$O$_2$ and other ROS species. Therefore, hybrid NSs are another class of attractive NMs that have been exploited as NPSs to overcome the shortcomings of TiO$_2$ based NPSs.

In 2014, Fang et al. reported Au/TiO$_2$ core–shell NSs for the plasmon-enhanced generation of ROS.$^{[24]}$ In this work, the TiO$_2$ shell was coated onto the pregrown Au NRs to develop Au/TiO$_2$ core–shell hybrid NSs. The resultant hybrid NSs have an average diameter of 32 nm and a length of 91 nm, respectively. The $^{1}$O$_2$ generation efficiency of the Au/TiO$_2$ core–shell NS was determined by ADBA, showing a 43% reduction in ADBA absorbance after 2 h NIR laser illumination (809 nm, 8.5 W cm$^{-2}$) as compared to 10% for the uncoated Au NRs and hollow TiO$_2$, respectively. This suggested that a significant amount of $^{1}$O$_2$ was generated by Au/TiO$_2$ core–shell NS in a time-dependent manner. In addition to $^{1}$O$_2$–OH was also detected using terephthalic acid (TA), which can react with -OH to generate a fluorescent product. However, the fluorescence intensity of TA was five times stronger than uncoated Au NRs and hollow TiO$_2$ NSs after 2 h of illumination, indicating that the LSPR phenomenon of plasmonic metal (Au NRs) plays a key role in enhanced ROS generation from core/shell structure. In addition, the recombination efficiency of photogenerated charge carriers (electron–hole pairs) is significantly lower in the core/shell structure than individual NSs, leading to enhanced ROS generation. They proposed the ROS generation mechanism from the hybrid NSs: under plasmon resonant excitation of Au NRs, electrons having energies higher than that of the Schottky barrier could be directly transferred into the CB of TiO$_2$ and reduced the adsorbed O$_2$ molecules on the surface of TiO$_2$ to generate $^{1}$O$_2$. The resultant $^{1}$O$_2$ interacts with the remaining hole in the Au NRs or surrounding water to generate $^{1}$O$_2$ or -OH ($^{1}$O$_2$+H$_2$O). Encouraged by this finding, Saito et al. reported another example of Au NPs/TiO$_2$ hybrid NSs and studied the influence of Au loading onto TiO$_2$ and the effect of TiO$_2$ particle size on ROS generation. They used 13 commercially available TiO$_2$ powders to develop Au NPs/TiO$_2$ hybrid NSs. They revealed that under visible light irradiation, Au NPs/TiO$_2$ hybrid NSs could also produce both $^{1}$O$_2$ and $^{3}$O$_2$$^{[24]}$ with a quantum yield of 8 × 10$^{-6}$ and 2 × 10$^{-6}$, respectively. Additionally, the phosphorescent $^{1}$O$_2$ emission signal at 1260 nm further verified $^{1}$O$_2$ generation from the Au NPs/TiO$_2$ hybrid. Moreover, the mechanism of $^{1}$O$_2$ and $^{3}$O$_2$ generation from Au NPs/TiO$_2$ was similar as proposed by Feng et al. (Figure 20B,C). Importantly, Au NPs/TiO$_2$ hybrid containing larger TiO$_2$ particle size generates more $^{1}$O$_2$– and $^{1}$O$_2$ due to the slow recombination of electron–hole pairs in larger Au NPs/TiO$_2$ than smaller size TiO$_2$ as confirmed by laser spectroscopy. Alternatively, a higher amount of Au loading on TiO$_2$ seriously impeded the adsorption of oxygen molecules on the surface of TiO$_2$ leading to low $^{1}$O$_2$ generation. In another subsequent study, instead of electron transfer from Au NPs to TiO$_2$, Meng’s group suggested that Au NPs/TiO$_2$ hybrid behaves differently under different light excitation (sunlight and visible light)$^{[115]}$. They demonstrated that under sunlight irradiation, an electron could also be injected from TiO$_2$ to Au NPs, while under visible light irradiation (>420 nm), SPR induced near field enhancement effect as well as hot electron injection from Au NPs to TiO$_2$. Both the enhancement mechanisms lead to a dramatic increase in the generation of ROS species (·OH and $^{1}$O$_2$) and charge carriers (electron and hole) as monitored by ESR spectroscopy. Though metal/semiconductor hybrid NSs demonstrated an efficient ROS generation especially $^{1}$O$_2$ particularly in the context of PDT, their suitability for in vitro and in vivo PDT are still unexplored.

Recently, in vivo PDT evaluation of Au nanoclusters (Au$_{25}$ NC) stabilized black TiO$_2$ nanotubes (B-TiO$_2$ NTs) were first reported by Yang et al.$^{[27]}$ Using TiO$_2$ NPs as the substrate, TiO$_2$ NTs with a thickness of 2 nm and a width of 15 nm, respectively, were prepared by the hydrothermal approach and then undergo high pressure gaseous (H$_2$) reduction to develop B-TiO$_2$ NTs. They suggested that the reduction of anatase TiO$_2$ could extend the absorption of TiO$_2$ NTs from UV to the visible and even NIR region, allowing deeper tissue penetration depth. On the other hand, the reduction process increased the density of Ti$^{4+}$ on the surface of B-TiO$_2$ NTs, enabling effective suppression of electron–hole pairs recombination. Moreover, the reduced B-TiO$_2$ NTs were further modified with Au$_{25}$ NC to develop hybrid NSs (Figure 21A). Interestingly, the deposition of Au$_{25}$ NC further hampered the recombination of charge carriers by changing the electrical distribution in the hybrid, demonstrating enhanced ROS generation efficacy. Instead of $^{1}$O$_2$ generation, Au$_{25}$/B-TiO$_2$ NTs generate efficient O$_2$– and ·OH (Figure 21B), which could induce more
Figure 20. A) Proposed mechanism of $\text{^1O}_2$ by Au NRs core/TiO$_2$ shell nanocomposite under 809 nm light illumination. Reproduced with permission.[24] Copyright 2014, Royal Society of Chemistry. B) Plausible mechanism for the generation of $\text{O}_2^-$ and $\text{^1O}_2$ on AuNP/TiO$_2$ excited with visible light. C) Phosphorescent $\text{^1O}_2$ emission spectra for AuNP/TiO$_2$ (blue ■), bare TiO$_2$ (green ◆), and AuNP (purple ▲). Reproduced with permission.[25] Copyright 2014, American Chemical Society. D) Mechanistic illustration of the enhanced generation of charge carriers of ROS in Au@TiO$_2$ (i). However, the enhancement mechanism is different under full light (ii) and visible light (ii). $E_c$ and $E_v$ represent the CB and VB of TiO$_2$, respectively. $E_F$ represents the Fermi level of Au. Reproduced with permission.[115] Copyright 2018, Royal Society of Chemistry.

Figure 21. A) Schematic process for the synthesis of Au$_{25}$/B-TiO$_{2-x}$ NTs. B) The photocatalytic water splitting mechanism on Au$_{25}$/B-TiO$_{2-x}$ NTs. C) Representative photographs of tumor-bearing mice and tumor from those treated with different conditions upon 650 nm laser irradiation on the 14th day. Reproduced with permission.[27] Copyright 2017, Wiley-VCH.

than 80% cell death relative to the B-TiO$_2$ NTs or TiO$_2$ NTs under 650 nm light illumination (0.5 W cm$^{-2}$, 10 min). This suggested that the Au$_{25}$ NC and the increased density of Ti$^{4+}$ could trigger an improved PDT effect to kill tumor cells. The subsequent in vivo studies in murine cervical cancer mice were performed by intravenously injected Au$_{25}$/B-TiO$_2$ NTs following irradiation with a 650 nm laser, showing significant destruction of tumors, which was more effective compared to the B-TiO$_2$ NTs or TiO$_2$ NTs alone (Figure 21C). As the Au$_{25}$/B-TiO$_2$ NTs possessed excellent in vivo PDT performance at NIR regions, the hybrid NSs...
reported in this work could serve as outstanding NPSs for effective cancer PDT.

Instead of using TiO$_2$, He et al. developed zinc oxide (ZnO)/Au hybrid NSs (ZnO/Au) and investigated their photocatalytic and antibacterial activity.$^{[116]}$ They employed the photoreduction approach, in which AuCl$_4^-$ were reduced by photogenerated electrons from the ZnO, resulting in ZnO/Au hybrid. This method offers two advantages: 1) smaller size Au NPs (<3 nm) on the surface of ZnO, and 2) this approach is eco-friendly and cost-efficient as it does not require any additional reactants. They further revealed the injection of an electron from semiconductor (ZnO) to metal (Au) under photoexcitation (Figure 22A), leading to suppress electron–hole pairs recombination, resulting in enhanced ROS generation as confirmed by four times higher photocatalytic degradation of methylene blue (MB) and salicylic acid (SA) by hybrid NSs than ZnO NPs alone. To get a clear insight, ESR spectroscopy with spin trapping and spin labeling agents was employed to investigate the actual ROS species produced by ZnO/Au hybrid and photogenerated charge carriers (electron, hole). They demonstrated that three ROS species including O$_2^-$, ·OH, and 1O$_2$ were produced by hybrid NSs upon 5 min irradiation as verified by a characteristic four-line spectrum 1:2:2:1 and a three-line spectrum 1:1:1, respectively. It is notable that the increased amount of Au loading onto ZnO significantly enhanced the photocatalytic performance of ZnO. Due to the enhanced ROS generation capacity, the antibacterial activity of ZnO/Au hybrid was also evaluated against gram-positive S. aureus and gram-negative E. coli, showing that hybrid NSs were about three times more effective in killing bacterial cells as compared to pure ZnO NPs (Figure 22B). Due to high power white light excitation, the present hybrid NSs is not suitable for in vivo cancer PDT, but holds a promising potential for use in water purification and antibacterial products. Though in vitro and in vivo PDT applications of hybrid NSs are not widely explored, the current observations and detailed mechanistic investigations will truly pave the way to explore more hybrid NSs (e.g., Au/MoS$_2$) for ROS generation as well as the development of advanced strategies to tune the absorption of hybrid NSs in NIR region for in vivo cancer PDT.

In another study, Zhang et al. integrated plasmonic Au NPs with PEI functionalized 2D BP NSs through electrostatic interaction to enhance the 1O$_2$ generation capacity and photothermal performance of BP NSs (Figure 23A).$^{[117]}$ As metal NPs possess an exciting LSPR phenomenon which could enhance the light absorption, as well as 1O$_2$ generation ability of nearby PS via local field enhancement effect, BP-PEI/Au hybrid presented 3.9-fold enhanced 1O$_2$ generation and 1.7-fold high PTT efficiency of BP NSs compared to BP-PEI alone under 670 nm laser irradiation (Figure 23B,C). Additionally, in vitro and in vivo studies using HepG2 cell/tumor mice further verified the enhanced dual-modal PDT/PTT therapy as complete tumor eradication was
achieved without any side effects (Figure 23D). This study highlighted the potential of plasmonic materials to incorporate with either 2D NPSs or other NPSs to enhance therapeutic performance. Encouraged by this, Lin’s group combined Fe₃O₄ NPs with BPs@Au for MRI guided synergistic PDT/PTT. The BPs@Au@Fe₃O₄ composite was developed via simple electrostatic attraction among BP NSs, Au@glutathione (Au@GSH), and Fe₃O₄@polyetherimide (Fe₃O₄@PEI) NPs, showing higher ROS production as well as higher photothermal conversion efficiency under 650 nm light irradiation (0.5 W cm⁻²) compared to BP NSs alone, BPs@Au, and BPs@Fe₃O₄. This suggested that both Au and Fe₃O₄ NPs could participate synergistically to enhance the photothermal performance, resulting in high PTT efficacy of BPs@Au@Fe₃O₄ composite (Figure 23E). Owing to the tumor targeting and MRI-guiding ability of Fe₃O₄ NPs, a high transverse relaxivity (r₂) of 54.5 × 10⁻³ m⁻¹ s⁻¹ was achieved with a concentration-dependent darkening effect as monitored by T₂-weighted MRI studies (Figure 23F). Further, a significant visible signal attenuation was observed in vivo, indicating the potential of MR contrast enhancement effect of BPs@Au@Fe₃O₄ NPs.

Apart from single ¹⁰⁰⁵ NPSs, Feng et al. first time designed dual-inorganic NPSs (g-C₃N₄, Au₂₅ NCs) based strategy for multimodal imaging and cancer PDT. The UCNPs@g-C₃N₄-Au₂₅-PEG hybrid effectively promotes the electron–hole pair separation, and thus exhibited high ¹⁰⁰⁵ generation ability with a quantum yield of 0.74, allowing to induce remarkable cellular/tumor destruction both in vitro and in vivo (Figure 24B). It has been noted that UCNPs@g-C₃N₄-Au₂₅-PEG do not produce significant heat at 980 nm light irradiation both in vitro and in vivo, suggesting that the cell death is solely attributed to high ¹⁰⁰⁵ generation. In addition to UCL imaging, the currently developed UCNPs also exhibited a longitudinal (r₁) relaxivity of 0.9195 mm⁻¹ s⁻¹ with a concentration-dependent brightening effect as well as a far superior CT value of 424.3 HU (Hounsfield unit) after injection compared to without injection (39.8 HU) (Figure 24C), making them suitable candidates for simultaneous tri-modal imaging with UCNPs@g-C₃N₄-Au₂₅-PEG could offer 3D whole-body imaging.

Figure 23. A) Schematic view of the biofunction of BP-PEI/AuNPs. B) Normalized absorbance of ABDA at 380 nm during photodecomposition by ROS generation upon 670 nm laser irradiation under different materials. C) Temperature elevation curves of different solutions over four rounds of on/off cycling under 670 nm laser at a power density of 1 W cm⁻². D) Tumor photographs after indicated treatments at 14th day. Reproduced with permission.[117] Copyright 2018, Wiley-VCH. E) The corresponding temperature profiles of the HeLa cells incubated with indicated materials as a function of 650 nm laser irradiation time (0.5 W cm⁻²). F) In vivo MR images of a mouse before and after injection of BPs@Au@Fe₃O₄. Reproduced with permission.[118] Copyright 2017, Wiley-VCH.
and high-resolution imaging to guide precise cancer therapy in vivo. Inspired by this, they also reported enhanced $T_1/T_2$ weighted MRI from Fe$_3$O$_4$@UCNPs@g-$C_3$N$_4$ system with a calculated longitudinal ($r_1$) and transverse ($r_2$) relaxivity of 2.545 and 23.080 mm$^{-1}$ s$^{-1}$, respectively (Figure 24D).\cite{120} Due to an improved magnetic guided accumulation and type-I ROS species generation, Fe$_3$O$_4$@UCNPs@g-$C_3$N$_4$ completely inhibited the tumor growth without any perceived adverse effects. In conclusion, these findings depicted the theranostic applications of g-$C_3$N$_4$-based nanoconstructs, which will truly shine some light on the development of high-efficiency NIR excitable g-$C_3$N$_4$-based 2D-NMs as NPSs for multimodal imaging-guided cancer therapy.

Instead to gold-based hybrids, Guo et al. reported tungsten-based metal semiconductor hybrid consists of polyelectrolyte multilayers (PEM) coated cesium tungstate nanorods ($Cs_xWO_3$ NRs@PEM) as a multifunctional nanotheranostic agent for bi-modal (CT/PAI) imaging-guided PDT/PTT of tumors.\cite{121} In their study, $Cs_xWO_3$ NRs@PEM with a mean diameter of 13 nm and a length of 70 nm, respectively, were prepared, showing strong absorption in NIR regions that cover both biological windows I and II (Figure 25A). They demonstrated that the strong NIR absorbance could be used for PAI, and the high atomic number ($Z = 73$) of the W element could offer strong X-ray attenuation ability for X-ray CT imaging. Typically, a high CT/PAI contrast signal with remarkably enhanced CT value was achieved after injection (606.6 HU) than without injection (278.5 HU). The ability of simultaneous CT/PAI imaging with $Cs_xWO_3$ NRs@PEM could offer 3D whole-body and high-resolution images to guide precise cancer therapy in vivo (Figure 25D,E). Besides CT/PAI bimodal imaging, $Cs_xWO_3$ NRs@PEM could also generate both heat and $^{13}$O$_2$ upon irradiation at either 1064 or 880 nm light. It was notable that both the temperature increment and $^{13}$O$_2$ generation under 1064 nm irradiation were larger compared to that under 880 nm irradiation, which could help to penetrate deeper to treat deep-seated tumors (Figure 25B,C). In vivo studies on HeLa tumor-bearing mice following intratumoral injection of $Cs_xWO_3$ NRs@PEM showed remarkably increased CT and PAI signals, and the tumors shrank obviously after irradiation with 880 nm or 1064 nm light for 10 min (Figure 25F). Importantly, four and three out of five tumors were completely eliminated under irradiation with 1064 nm and 880 nm light, respectively, with no obvious side toxicity. These results suggested that $Cs_xWO_3$ NRs@PEM could act as an efficient theranostic agent for dual-modal imaging-guided combination PDT/PTT of tumors under irradiation with biological window NIR-II light, which might be useful for the treatment of deep-located tumors.

Instead of type-II ($^{1}$O$_2$) ROS species, hyaluronic acid (HA) functionalized tungsten carbide (W$_2$C) NPs developed by Yang’s group showed both type-I ($^{1}$OH) and type-II ($^{1}$O$_2$) ROS species, respectively, under normoxic conditions (Figure 26A).\cite{122} Meanwhile, under hypoxic conditions, W$_2$C NPs could also generate hydroxyl radicals as evidenced by EPR spectroscopy (Figure 26B) and in vitro DCFH assay. This illustrated that W$_2$C NPs are suitable NPSs to treat the deep-seated hypoxic tumor. Attributed to the high in vitro ROS generation capacity and photothermal conversion efficiency of W$_2$C NPs, significant destruction of cells/tumors were achieved under 1064 nm laser (0.8 W cm$^{-2}$) irradiation both in vitro and in vivo, respectively, without any obvious side effects. Apart from therapeutic performance, W$_2$C NPs also showed enhanced CT/PA contrast images, enabling them to track cancer therapy in vivo. These findings suggested...
Figure 25. A) Absorption spectra of Cs$_x$WO$_3$ NR@PEM aqueous suspension with different concentrations and corresponding linear fit between concentration and absorption (inset: the top curve represents absorption intensity at 1064 nm and the bottom curve represents absorption intensity at 880 nm). B) ESR spectra of Cs$_x$WO$_3$ NR@PEM with TEMP probes under 10 min irradiation. C) Temperature variation of tumors on different groups during laser irradiation. D) CT images of mice before (i) and after (ii) intratumoral injection of Cs$_x$WO$_3$ NR@PEM (4 mg mL$^{-1}$, 50 μL). E) In vivo PAT images of HeLa-tumor-bearing mice before and after intratumoral injection (2 mg mL$^{-1}$, 50 μL) for different times. F) Representative photos of HeLa-tumor-bearing mice and tumors after 14 d treatment with Cs$_x$WO$_3$ NR@PEM under 808 nm (i) 5 min, (ii) 10 min and 1064 nm (iii) 5 min, and (iv) 10 min, respectively. Reproduced with permission.$^{[121]}$ Copyright 2016, Wiley-VCH.

Figure 26. A) Schematic illustration of the application of HA-W$_2$C NPs for 1064 nm activated tumor dual-type PDT, PTT, and PA/X-ray CT dual-modal bioimaging. B) ESR spectra of HA-W$_2$C NPs solutions before and after irradiation for 8 min: (i) and (ii) under normoxic conditions, and (iii) under hypoxic conditions. Reproduced with permission.$^{[122]}$ Copyright 2018, Springer.
the promising potential of W₂C NPs as a multimodal theranostic NPSs for cancer PDT of normoxic as well as hypoxic tumors. Importantly, in contrast to previously reported semiconductor-based tungsten nanostructures, W₂C NPs exhibited metallic character as revealed by diffuse reflectance spectrum and X-ray photoelectron spectroscopy, respectively. Though both type-I and type-II ROS species were successfully detected, but their mechanism of generation remains elusive, which needs further exploration to advance the treatment of deep-seated hypoxic tumors.

Apart from 1D tungsten-based nanomaterials that have been widely used to treat deep-seated tumors, Seidl et al. reported 0D tin tungstate (β-SnWO₄) NPs as inorganic NPS for PDT of surface located tumors under blue light illumination. In their work, β-SnWO₄ NPs with an average size of ≈8 nm were prepared, showing maximum absorption in the UV region with an emission at 470 nm, and further functionalized electrostatically with protamine to improve membrane penetration and cell uptake ability (Figure 27A). The O₂ generation efficiency of the β-SnWO₄ NPs in HeLa cells upon blue light illumination was evaluated by 2,7-dichlorodihydrofluorescein diacetate (DHFA), showing a bright intracellular green fluorescence, which was not observed in HeLa cells treated with either β-SnWO₄ or blue light illumination alone (Figure 27B). Due to an efficient in vitro O₂ generation, β-SnWO₄ NPs exhibited effective killing of both HepG2 and HeLa cells under blue LED light illumination for 5 min. Subsequently, the application of β-SnWO₄ NPs to trigger PDT of xenograft 4T1 breast tumors in living mice was conducted, which demonstrated remarkable tumor growth inhibition as compared to that of doxorubicin-treated or untreated control mice (Figure 27C). Importantly, histological examination of lymph nodes revealed that the β-SnWO₄ NPs exhibited similar anti-metastatic effect as that of doxorubicin, but at much-reduced side effects as determined by blood counts. These results suggested that β-SnWO₄ NPs could be a new addition in already available inorganic NPSs, which will be much efficacious for the treatment of metastatic tumors and near-surface PDT.

Meanwhile, in a recent work reported by Bu’s group suggested the coupling of UCNPs with SnWO₄ NPs to induce NIR-triggered PDT and RT, offering dual-modal therapy of deep-seated tumors. Subsequently, to avoid the complexity of the system and extend the absorption of 0D tungsten NPs from UV to NIR region, Wang et al. prepared urchin-like tungsten suboxide (WO₅) NPs for photoacoustic (PA) imaging-guided PDT/PTT in the second biological window. The designed WO₅ NPs showed broad absorption in the entire absorption spectrum (500–2500 nm) (Figure 27D), demonstrating significant heat and O₂ generation under 1064 nm NIR laser irradiation, enabling effective HeLa tumor cell killing via PTT and PDT effects. The in vivo studies on HeLa tumor mice administered intratumorally with the WO₅ NPs showed remarkably enhanced PA signal (Figure 27E), and the tumors were completely eliminated after irradiation with a 1064 nm NIR laser (2 W cm⁻²) for 10 min.
ment of nanoparticles through trans-endothelial pathway could be demonstrated that nanoparticles extravasate into solid tumor through major organs. Warran Chan and coworkers recently demonstrated that nanoparticles of varying diameter, morphologies, and surface chemistry. [128] Third, the clearance of these NPSs刨路ways to elucidate how different tumor vessels and trans-endothelial pathways respond to nanoparticles of varying diameter, morphology, and surface chemistry. [128] Third, the clearance of these NPSs is a major hurdle in their bench to bedside translation, which is also a major hurdle to their application.

9. Current Challenges and Future Prospects

As aforementioned, $^1O_2$ generative nanomaterials are a rising platform that successfully demonstrated exciting results in PDT. In fact, nanomaterial-based NPSs overcome and resolved most of the issues associated previously with classical organic PSs and showed remarkable therapeutic outcomes. Although these NPSs showed promising results owing to their highest $^1O_2$ quantum yield, there are still many challenges that demand careful consideration for future clinical applications of these NPSs.

First, like organic PSs, the majority of currently reported NPSs, especially semiconductor NMs, are still activated under U/VIS light irradiation, which restricts their therapeutic applications to near-surface tumors as well as peripheral and endoscopically accessible regions due to limited tissue penetration depth of U/VIS light. Currently, several strategies have been adopted, most notably decorating UCNPs onto semiconductors to tune their absorption from U/VIS to NIR region (biologically transparent window), which promises deeper tissue penetration depth and less attenuation by biological tissues. However, UCNPs-based tunable absorption approach did not show satisfactory results due to certain limitations such as limited choices of excitation wavelengths (usually 980 nm), low extinction coefficient, and requirement of high laser intensity to generate upconversion visible light luminescence, which is far beyond the skin tolerance limit. [126]

Second, NPSs showed <10% accumulation in tumor region. To increase tumor enrichment, high dosage are usually administered intravenously, which can induce undesired side-toxicity to the major organs. Warran Chan and coworkers recently demonstrated that nanoparticles extravasate into solid tumor through trans-endothelial route rather than endothelial gaps. They suggested that by modulating the tumor endothelium, the enrichment of nanoparticles through trans-endothelial pathway could be improved. [127] Whereas, an in detail study should be conducted to elucidate how different tumor vessels and trans-endothelial pathways respond to nanoparticles of varying diameter, morphology, and surface chemistry. [128] Third, the clearance of these NPSs is an important question mark as they remained in the vital or-gans (e.g., liver, spleen, etc.) for longer periods after systemic administration and are nonbiodegradable, thus pose serious risk of long-term biotoxicity. Yu et al. reported that rather than larger size Au NRs, smaller size Au NRs rapidly cleared from the body. [129]

Although these results are quite encouraging, it remains difficult to generalize which hydrodynamic size of NPSs is more appropriate for photodynamic tumor treatment in vivo as the nature of interaction between these NPSs and the biological systems is still unknown. In addition, in vivo biodistribution, long-term toxicity, and biosafety profile of these rapidly developing NPSs are yet undefined, which is also a major hurdle in their bench to bedside applications. [130] Furthermore, several factors such as size, surface chemistry, and inherent chemical constituents of nanomaterials are well known to modulate potential cytotoxic effects. For instance, CTAB-functionalized Au NRs presented extensive cellular toxicity and low stability in physiological conditions. It has been found that CTAB as a surfactant disrupted the integrity of cell membrane by inducing multiple defects. [131] Therefore, biocompatible surfactants should be considered for the stabilization of NPSs to ensure nontoxicity, biocompatibility, and good physio-logical stability. Recently, several studies have reported that many inorganic nanomaterials especially gold and carbon, if surface-functionalized with a biocompatible agent (e.g., PEG) and an appropriate size, do not induce any noticeable toxic effect in cultured cells or mouse tumor models.

In addition to the above-mentioned challenges, some future directions should be explored further to achieve high antitumor effects and to serve the suffering humanity. Since NIR laser can achieve deeper penetration within body, new NPSs that could be irradiated to directly generate a large amount of $^1O_2$ at NIR-I or even NIR-II window should be developed to treat deep-seated tumors in vivo. In particular, NIR-II activatable NPSs would not only resolve an issue of low penetration depth but also reduce the overall complexity and cost of the system. [132] Recently, Huang et al. demonstrated an innovative strategy to modulate the plasmonic peak of gold-based theranostic systems in NIR-II region to achieve deep-seated tumor theranostics. [133] Meanwhile, biocompatible and biodegradable NPSs (e.g., black phosphorus NSs, hybrid-based NPSs) capable of reducing side toxicity in vivo are greatly needed. [134] A recent report from Wang’s group demonstrated a combination of nanomaterials both as exciting moiety and as NPS. Plasmonic Au NRs were used as a primary exciting moiety to activate the attached carbon dots-based NPSs due to the near field enhancement effect, showing high $^1O_2$ generation under NIR light as reported from QD’s alone. [92] In our opinion, this is the next generation of PDT, which used nanomaterial to excite the attached NPS. This new direction not only overcomes most of the issues of traditional PDT based on organic PS but also paves the way for the development of multifunctional nanothera-nostic agents exclusively based on nanomaterials. Moreover, this approach should be expanded to other nanomaterials by taking careful considerations of the spectral overlap of the maximum absorption wavelength of both the nanomaterials. Fourth, Zhang et al. first time reported the in vivo high PDT efficacy based on dual organic PSs coupled with UCNPs. [135] Very recently, Hou et al. use a combination of organic PS (Hypocrellin A) and inorganic PS (TiO$_2$ NPs) by integrating with UCNPs, while Feng et al. utilized nanomaterial-based dual inorganic NPSs (g-C$_3$N$_4$ and Au$_{25}$ nanoclusters) coupled with UCNPs and evaluated their in vivo anti-tumor performance. [136] Both observations demonstrated the high PDT response and better tumor inhibition compared to single PS. Hence, this approach should be explored further to understand how dual NPSs could be used to enhance the $^1O_2$ quantum yield, which is of course indirectly improve the treatment outcomes. Furthermore, the utilization of dual NPSs having maximum absorption at NIR and U/VIS region will provide an edge to the scientific community to get the ultimate therapeu-tic results by taking the advantage of an entire light spectrum (200–1000 nm) capable of treating the near surface and deep-seated tumors, simultaneously. As we all know that single treatment modality is not sufficient to completely eradicate the tumor and there is a consensus among scientific community regarding
this issue. Therefore, new NPs should be developed, which will enable simultaneous PDT and PTT. Recently, Vankayala et al. and Wang et al. reported Au NRs and CuS nanocrystals as both PDT and PTT agents. Therefore, it is highly demanding that multifunctional nanoagents with multimodal imaging and synergistic therapy should be developed to win this battle against cancer. Metal-organic frameworks (MOFs) are new and exciting material, showing remarkable contribution in PDT as a nanocarrier due to their certain inherent features such as adjustable pore sizes, crystalline nature, design flexibility, tunable chemical environment, biocompatibility, high porosity, and high surface area. Recently, MOFs as a nanocarrier, have been successfully integrated with organic PSs and exhibited satisfactory therapeutic performance. Therefore, it is highly demanding to multifunctional nanoagents with multimodal imaging and synergistic therapy should be developed to win this battle against cancer. Metal-organic frameworks (MOFs) are new and exciting material, showing remarkable contribution in PDT as a nanocarrier due to their certain inherent features such as adjustable pore sizes, crystalline nature, design flexibility, tunable chemical environment, biocompatibility, high porosity, and high surface area. Recently, MOFs as a nanocarrier, have been successfully integrated with organic PSs and exhibited satisfactory therapeutic performance. Therefore, it is highly demanding that multifunctional nanoagents with multimodal imaging and synergistic therapy should be developed to win this battle against cancer. Metal-organic frameworks (MOFs) are new and exciting material, showing remarkable contribution in PDT as a nanocarrier due to their certain inherent features such as adjustable pore sizes, crystalline nature, design flexibility, tunable chemical environment, biocompatibility, high porosity, and high surface area. Recently, MOFs as a nanocarrier, have been successfully integrated with organic PSs and exhibited satisfactory therapeutic performance. Therefore, it is highly demanding that multifunctional nanoagents with multimodal imaging and synergistic therapy should be developed to win this battle against cancer. Metal-organic frameworks (MOFs) are new and exciting material, showing remarkable contribution in PDT as a nanocarrier due to their certain inherent features such as adjustable pore sizes, crystalline nature, design flexibility, tunable chemical environment, biocompatibility, high porosity, and high surface area. Recently, MOFs as a nanocarrier, have been successfully integrated with organic PSs and exhibited satisfactory therapeutic performance. Therefore, it is highly demanding that multifunctional nanoagents with multimodal imaging and synergistic therapy should be developed to win this battle against cancer. Metal-organic frameworks (MOFs) are new and exciting material, showing remarkable contribution in PDT as a nanocarrier due to their certain inherent features such as adjustable pore sizes, crystalline nature, design flexibility, tunable chemical environment, biocompatibility, high porosity, and high surface area. Recently, MOFs as a nanocarrier, have been successfully integrated with organic PSs and exhibited satisfactory therapeutic performance. Therefore, it is highly demanding that multifunctional nanoagents with multimodal imaging and synergistic therapy should be developed to win this battle against cancer. Metal-organic frameworks (MOFs) are new and exciting material, showing remarkable contribution in PDT as a nanocarrier due to their certain inherent features such as adjustable pore sizes, crystalline nature, design flexibility, tunable chemical environment, biocompatibility, high porosity, and high surface area. Recently, MOFs as a nanocarrier, have been successfully integrated with organic PSs and exhibited satisfactory therapeutic performance. Therefore, it is highly demanding that multifunctional nanoagents with multimodal imaging and synergistic therapy should be developed to win this battle against cancer. Metal-organic frameworks (MOFs) are new and exciting material, showing remarkable contribution in PDT as a nanocarrier due to their certain inherent features such as adjustable pore sizes, crystalline nature, design flexibility, tunable chemical environment, biocompatibility, high porosity, and high surface area. Recently, MOFs as a nanocarrier, have been successfully integrated with organic PSs and exhibited satisfactory therapeutic performance. Therefore, it is highly demanding that multifunctional nanoagents with multimodal imaging and synergistic therapy should be developed to win this battle against cancer.
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