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Doctoral Thesis

Development of fluorescent molecules and nanobioconjugates for cell imaging and singlet oxygen sensing

(細胞イメージングと一重項酸素センシングに向けた蛍光
分子とナノバイオコンジュゲートの開発)

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Recently, theranostics, a combination of diagnosis and therapy, has become a key modality in cancer management. Certain intrinsic limitations in conventional cancer diagnosis/therapy strategies lead to the development of nanomaterial-based therapeutics. Different nanoparticles have been developed into theranostics by combining multimodal contrast/imaging agents, drugs, and targeting moieties for cancer diagnosis, monitoring, therapy, and treatment follow-up. Theranostics for fluorescence imaging or fluorescence molecular tomography (FMT) using organic dyes and semiconductor nanocrystals with ligands/antibodies against cancer markers receive much attention in basic research and clinical applications. Also, nanomaterials combining FMT with chemotherapy, hyperthermia, or phototherapy enter the clinical stage. This thesis focuses on nanobioconjugates combining fluorescence probes, photosensitizers, fluorogenic sensors, and cancer-targeting biomolecules.

I use fluorescence probes such as semiconductor quantum dots (QDs) and nucleus staining Syto dyes to detect or image cancer cells. Also, QDs and porphyrins generate singlet oxygen ($^1\text{O}_2$), an essential reactive oxygen species (ROS) in photodynamic therapy (PDT), which is detected using a high sensitivity electron donor-acceptor (D-A) fluorogenic molecule. The cancer-targeting biomolecules include anticancer antibodies and a peptide. Rationally designed nanobioconjugates using the above components help me enrich and efficiently detect cancer cells in blood samples, and produce, detect, store and release $^1\text{O}_2$ in a solution or cells.

This thesis has five chapters, including general conclusions and perspectives. Chapter 1 of the thesis provides a general introduction to fundamental aspects of cancer management. Next, I discuss the significance of circulating tumor cells (CTCs)-based liquid biopsy and the current detection technologies for CTC isolation and enrichment. The importance of nanomaterials-based immunocapturing and optical detection based on the fundamental properties of nanomaterials are also discussed. Next, I discuss the role of $^1\text{O}_2$ in cancer therapeutics due to its cytotoxic effect on various biological substrates. I also discuss a few biological and chemical processes involved in the generation of $^1\text{O}_2$ followed by its detection using fluorescent molecular probes.

Chapter 2 provides the experimental procedures and techniques in this study. I discuss the procedure for the functionalization of silica, attachment of antibodies on functionalized silica

microparticles, QD labeling with cancer-specific antibodies, and the attachment of QD-antibody conjugates on the functionalized silica particles. I also discuss the synthesis of a $^1\text{O}_2$ sensor molecule, preparation of silica- $^1\text{O}_2$ sensor nanoassemblies, and the conjugation of cell-penetrating peptides on the nanoassembly. Next, I discuss the procedure for cell culture and cell labeling. I also discuss time-resolved fluorescence spectroscopy used in the characterization of CTCs. Finally, I discuss UV-vis absorption spectroscopy, steady-state and time-resolved fluorescence spectroscopy, single-particle microspectroscopy, laser scanning confocal microscopy, nuclear magnetic resonance spectroscopy, and scanning electron microscopy used in this thesis.

In chapter 3, I discuss a multimodal fluorescence microspectroscopic and mesenchymal-antigen specific detection, collection, and characterization of cancer cells. I use self-segregating immunosilica microparticles to capture the pre-labeled cells and the cells are identified from modalities such as multicolor images, multimodal fluorescence spectra, and fluorescence decay profile of nucleus staining dyes or QDs. The large size of silica microparticles prevents their endocytosis and help avoid an external force for cell separation, and the CD44 antigen-selective cell capturing help in an error-free cancer cell detection. The CD44-targeted cell collection combined with the above modalities shows a 9-fold detection accuracy for CTCs among blood cells.

In chapter 4, I synthesize a $^1\text{O}_2$ sensor composed of an aminomethyl anthracene and a coumarin moieties to increase the efficiency of intracellular $^1\text{O}_2$ generation, detection, and release. I construct a nanoassembly of a sensitizer and the sensor and investigate the ability of the assembly to generate, store, sense, and release $^1\text{O}_2$ at the ensemble, single-particle, and cell levels. In all cases, the sensor shows an enormous fluorescence enhancement due to the reaction of $^1\text{O}_2$ generated by the photosensitizer. The mechanisms behind $^1\text{O}_2$ sensing and releasing are explained in detail in this chapter. The intracellular uptake ability of the nanoassembly and $^1\text{O}_2$ generation are studied after conjugating an RGD peptide to the assembly. The single-particle and cell imaging reveal continuous $^1\text{O}_2$ release and efficient cell death. In addition, the fluorescence from the photosensitizer and the sensor help colocalized cell imaging. Thus, this work highlights the utilization of programmed nanocarriers for multimodal cancer therapeutic strategies.

Chapter 5 is the general summary of the thesis and future prospects of nanobioconjugates and $^1\text{O}_2$ sensing-releasing probes for cancer therapy. Also, I explain the toxicity aspects and the significance to analyze the pharmacokinetics of nanobioconjugates.

The GLOBOCAN 2020 estimates by the International Agency for Research on Cancer report 10 million cancer deaths and 19.3 million new cancer cases.¹ Cancer remains a threat to human survival as the second leading cause of death in developing countries. Thus, improved diagnostic and targeted therapies are crucial for successful treatment and increased survival rates.² Chapter 1 outlines the causes and genetic alterations contributing to cancer, followed by different diagnostic and treatment modalities, including laboratory tests, tumor markers, biopsy, and imaging techniques used by clinicians. Next, I introduced liquid biopsy as an innovative tool for the early detection of cancer, where I explained the pros and cons between liquid biopsy and conventional tissue biopsy, among which circulating tumor cells (CTCs) serve as an excellent diagnostic and prognostic marker to enhance the treatment efficacy.³ Later, I discuss the current detection technologies for CTCs isolation based on their characteristic physical or biological properties different from hematologic cells. The immunoaffinity-based technique increases the sensitivity and reliability of CTC capture.^{4,5} Further, I discuss the advantages of nanomaterials for CTC enrichment, particularly silica- and quantum dots-based immunocapture and fluorescence detection of the CTCs. The next section explains the cytotoxic effects of singlet oxygen ($^1\text{O}_2$) on the oxidation of cellular macromolecules, cell signaling, and cell ablation by emphasizing its key role in cancer therapeutic application.⁶ Various methods for the endogenous and chemical generation of $^1\text{O}_2$ are explained, followed by luminescent molecular probes for its detection.⁷ The merit of anthracene-based chemical tools for $^1\text{O}_2$ detection is highlighted, and the photophysical factors that govern the $^1\text{O}_2$ quantum yield are also discussed. I finally conclude the chapter with the motivation to conduct my research.

In chapter 2, I have compiled the preparation and characterization of sensor molecules, quantum dots, and nanobioconjugates. Also, I outline the cell culture, cell labeling, and cell imaging techniques in addition to the various instruments used. The chemicals and reagents used for my research are listed under the Materials section. In the methods section, I discuss the preparation of different antibodies-functionalized silica microparticles. The size and morphology of the silica particles were examined by scanning electron microscopy. The general procedure for

mammalian cell culture is also discussed. The procedures for cell labeling using the nucleus staining dye, different QD-antibody conjugates, and the subsequent attachment of labeled cells on the surface of antibody functionalized silica microparticles are explained. This approach selectively attaches cancer cells to the surface of silica microparticles. With the help of time-resolved fluorescence spectroscopy, I performed the immunohistochemistry to distinguish the cancer cells on silica microparticles from the peripheral blood mononuclear cells (PBMCs). The spectrally and temporally resolved photocount maps help determine the levels of different antigens in the cells and distinguish the cancer cells from PBMCs. Next, I report the procedures for the synthesis of a singlet oxygen ($^1\text{O}_2$) sensor, incorporation of the sensor, and sensitizers such as tetrakis(4-carboxyphenyl)porphyrin (TCPP) and quantum dot 655 (QD655) into the silica microparticles covalently, and the conjugation of a cell-penetrating peptide RGD to the sensor-sensitizer-silica nanoassembly. The sensor is characterized by proton and carbon nuclear magnetic resonance (NMR) spectroscopy, which also helps determine the structure of the reaction intermediate. The $^1\text{O}_2$ sensing and releasing potentials of the sensor in the ensemble solution phase is realized from the steady-state absorption and fluorescence spectroscopic results. The $^1\text{O}_2$ generating-caging-releasing-sensing properties of the nanoassembly in the presence of a photosensitizer is studied through single-particle microspectroscopy measurements. The ability of the sensor embedded in the nanoassembly to release $^1\text{O}_2$ intracellularly is confirmed by confocal laser scanning microscopic imaging.

Liquid biopsy involving circulating tumor cells (CTCs) for the early detection and characterization of various carcinomas is a powerful noninvasive technique.⁸ To date, most of the detection technologies are based on single modality enrichment methods except for a few reports on dual modality-based methods.⁹ The collection efficiency and detection accuracy of CTCs are continuously sought after to improve the clinical prospects of CTC-based cancer detection.¹⁰ This chapter reports high-efficiency CTC collection and detection by combining the self-segregating multifunctional mesoporous silica microparticles conjugated to cancer cell-specific antibodies and spectrally- and temporally resolved fluorescence photocount maps of dye or quantum dot labels in the nucleus or the cell surface. The large size of the microparticles is favorable for preventing endocytosis common to nanoscale silica or magnetic particles in CTC detection and enables the gravity separation of CTCs without applying an external force. The significance of mesenchymal biomarker targeted CTC collection and detection is demonstrated to overcome the limitations of

the generally used EpCAM- or EGFR-based CTC enrichment methods. Also, the time-stamped and energy dispersed photocount histograms increase the modality of detection of triply labeled cancer cells into nine with the color, fluorescence wavelength, and lifetime resolutions. Here, the multicolor images of the collected cancer cells are associated with multiple fluorescence spectral maxima and lifetime values, increasing the CTC detection accuracy. Therefore, this multimodal detection method is expected to enable a more comprehensive collection of CTCs for advanced cancer screening and downstream assays.

Thus, I demonstrated an effective microspectroscopic detection technique for the collection and error-free detection of CTCs with an accuracy of 1-10 among 100 cancer cells in 1 mL blood samples. By pre-labeling cells with the desired antibodies without saturating the surface antigens, cancer cells were collected by the multifunctional and self-segregating immunosilica particles. The large-size, highly dense, and antibody-functionalization of silica particles were utilized to prevent the endocytosis and enable capturing of cancer cells without applying an external force. Besides, as an alternative to EpCAM antigen targeted CTC capturing, I focused on CD44 transmembrane stem cell marker, abundant in metastasized cells, resulting in high collection efficiency of cancer cells among the blood cells. Further, with the help of bright and narrowband emissive QDs labels, the isolated cancer cells were characterized by triple-fluorescence imaging and a six- to nine-fold fluorescence detection technique based on the spectral and lifetime resolutions, which are the fingerprints of the labeled cells. Hence, the multi-modality of this method is exemplified based on multicolor imaging and spectro-temporally resolved fluorescence detection technique, which can be developed for advanced cancer screening as well as post-surgical/ therapeutic clinical follow-up.

Intracellular singlet oxygen ($^1\text{O}_2$) generation and detection play a key role in PDT to treat various cancers.¹¹⁻¹³ Although photosensitizer nanoparticles and organic molecules promise photodynamic therapy, the PDT efficacy is limited due to certain existing limitations or their inherent features.¹⁴ Thus, programmed nanomaterials for controlled photosensitization and intracellular $^1\text{O}_2$ generation, trapping, and releasing have great potential to augment cancer therapy.¹⁵ This chapter introduces a novel sensor-sensitizer incorporated multifunctional mesoporous silica nanoassembly promising for efficient PDT. I demonstrate the potential of this nanoassembly to produce, store, release, and sense $^1\text{O}_2$ at the single-particle level and in living

cells. The small size of the nanoassembly, within the diffusion length of $^1\text{O}_2$ in cells and tissues, allows for extremely efficient $^1\text{O}_2$ storing and releasing. I illustrate the potential of the nanoassembly for $^1\text{O}_2$ -mediated therapy by synthesizing an anthracene-coumarin-dioctylamine conjugate. The $^1\text{O}_2$ sensing efficiency of this molecule is studied using steady-state absorption and fluorescence spectroscopy. The fluorescence of coumarin in the sensor or the nanoassembly is quantitatively quenched by the efficient intramolecular electron transfer from the aminomethyl anthracene moiety and under one- or two-photon excitation, a >230-fold fluorescence enhancement is observed, which is the highest compared to the previously reported molecules. High sensitivity and controlled release of $^1\text{O}_2$ are attained using a UV or Vis source. The sensor also shows good photostability in room light, unlike the commercial $^1\text{O}_2$ probes. Further, the conjugation of cell-penetrating ligands to the nanoassembly enables its utilization for biological applications. The cellular uptake of the nanoassembly is facilitated by macropinocytosis or passive delivery, which is confirmed by confocal imaging. In addition, the nanoassembly provides the red fluorescence of the photosensitizer and intense blue emission from the uncaged sensor for synergized fluorescence-guided cell imaging and $^1\text{O}_2$ induced cell death.

Thus, I constructed a nanomaterial scaffold for intracellular $^1\text{O}_2$ production, trapping, sensing, and controlled release by the co-assembly of a $^1\text{O}_2$ generator, a PS, and an anthracene-coumarin dyad-based sensor molecule. The sensitivity of this nanoassembly was studied using steady-state absorption and fluorescence measurements in solutions and living cells, which revealed a significant fluorescence intensity enhancement of the sensor, outraging the commercially employed $^1\text{O}_2$ sensors. The photostability and spatiotemporal controlled release ability of the nanoassembly were monitored at the single-particle level in various steps of light-induced photoactivation. The UV-light induced spatiotemporal controlled release of $^1\text{O}_2$ underscores the potential of this nano-scaffold as an intracellular $^1\text{O}_2$ probe. Further, the fluorescence cell images showed that the NP-RGD peptide conjugate is internalized and readily accumulated in the cytoplasm. Under high power laser irradiation, $^1\text{O}_2$ -induced cell death was observed. Therefore, the nanoassembly can be utilized for synergized fluorescence-guided tumor cells diagnosis and PDT. Additionally, with the incorporation of efficient photothermal agents, the design can be extended further for image-guided PDT and PTT.

Cancer has become the most devastating disease affecting the healthy longevity plans of developing and developed economies. The challenges facing humanity face are control measures against cancers, such as error-free diagnosis and efficient treatment. Along with various biomedical imaging techniques such as magnetic resonance imaging (MRI), X-ray computed tomography (CT), ultrasound (US), positron emission tomography (PET), and single-photon emission computed tomography (SPECT), solid and fluid sample biopsies help cancer diagnosis by tissue/cell imaging and gene profiling. Also, novel chemotherapeutics and emerging nanomaterials-based phototherapy play important roles in cancer management.¹⁶ In this thesis, I summarized a silica particles-based platform for collecting and detecting cancer cells in the blood and the generation, storing, releasing, and sensing of singlet oxygen, an important component in photodynamic therapy (PDT).

Collecting and analyzing circulating tumor cells (CTCs) by liquid biopsy is promising for cancer detection and treatment follow-up. This is a less invasive tool, helping frequent assays to study the formation of secondary tumors and determine tumor heterogeneity. Technologies for CTC enrichment relying on the physical or biological characteristics of CTCs can be classified into immunocapture and biophysical methods. In the immunocapture technique, cells are collected using positive or negative immunomagnetic enrichment or microfluidics or nanomaterials. In contrast, biophysical techniques such as microfluidics, membrane filtration, and dielectrophoresis involve the isolation of cells based on the size, density, or electrical charge. Following the isolation, CTCs are often identified by various optical techniques. These optical methods suffer from low throughput and decreased cell viability despite the advantages. Further, morphological and gene alterations such as epithelial-to-mesenchymal transition (EMT) downregulate the epithelial phenotypes, lowering the CTC collection efficiency. In chapter 3, I showed an effective method for collecting and characterizing cancer cells in the blood, which adds a new dimension to liquid biopsy for CTC detection. For the cancer cell collection/isolation, I used stem cell antigen-specific immunosilica particles. Also, I used anticancer antigen-specific immuno quantum dots (QDs) and nucleus staining dyes for error-free CTC detection. The large size of silica microparticles helped gravity separation and collection of the silica-cell complexes, thus eliminating an external centrifugal/magnetic force.

The narrow and bright orange-red emission of two types of QDs, the green emission of the nucleus stain, and the unique photoluminescence lifetimes of these labels helped multiplexed CTC imaging and detection. Also, by using the CD44 antibody, this work overcomes the limitation of EpCAM-based CTC enrichment techniques that cause false positives due to the presence of such antigen in other cells and false negatives due to the EMT transition of CTCs. Thus, by integrating triple fluorescence color, spectral maxima, and lifetime values, this work achieved a nine-fold CTC detection accuracy. This work highlights the clinical relevance of multimodal CTC collection and detection by a noninvasive approach, promising for cancer screening and post-surgical or therapeutic follow-up. This technique helps identify CTCs at the single-cell level. This CTC detection method can be translated into clinical technology by combining a spectro-temporal sensor and disposable silica/silane-coated membranes to enable on-site dip-detection of cancer cells in the blood. Also, the cells collected using large silica particles or membranes maintain their viability for subculturing and genetic profiling of CTCs.

I focus on singlet oxygen detection in the fourth chapter. Singlet oxygen ($^1\text{O}_2$) is a highly reactive species with a strong cytotoxic effect, and it induces the oxidation of various biomolecules. It is vital in regulating intracellular signaling pathways and treating cancers by PDT. The short $^1\text{O}_2$ lifetime demands accurate and sensitive probes for its detection. Different methods for detecting $^1\text{O}_2$ include electron-para-magnetic resonance (EPR), phosphorescence, UV-vis spectrophotometry, fluorescence, and chemiluminescence. EPR is used for quantifying and detecting $^1\text{O}_2$ using sterically hindered secondary amine probes such as 2,2,6,6-tetramethylpiperidine (TEMP). But the complexity of analysis and expensive instrumentation limits its utility. Phosphorescence of $^1\text{O}_2$ at 1270 nm has a low quantum efficiency resulting in a weak signal-to-noise ratio and demanding high sensitivity cooled detectors. Absorbance-based spectrophotometric techniques use probes, such as 9,10-diphenyl anthracene, forming endoperoxides on reaction with $^1\text{O}_2$. But poor photostability of the intermediate and the limited sensitivity of spectrophotometry often limit this technique. Chemiluminescence probes such as 2-methyl-6-phenyl-3,7-dihydroimidazo[1,2- α] pyrazine-3-one (CLA) also exhibit good $^1\text{O}_2$ sensitivity, but it also reacts with superoxide anion, lowering $^1\text{O}_2$ selectivity. Fluorescence probes based on organic molecules ensure high $^1\text{O}_2$ detection sensitivity. This technique provides high spatiotemporal resolutions to study the $^1\text{O}_2$ generation or decay kinetics. But some sensors show self-sensitization-induced degradation and reactivity to other ROS such as hydroxyl radical or

superoxide. Therefore, stable $^1\text{O}_2$ fluorescence probes are continuously sought after, which, combined with $^1\text{O}_2$ storing and releasing capabilities add new dimensions to cancer PDT. I developed a silica nanosystem that generates, store, release and sense $^1\text{O}_2$ in a spatiotemporally controlled manner for synergized fluorescence-based cell imaging and photodynamic therapeutic applications. A $^1\text{O}_2$ sensor was synthesized, and its photophysical properties were investigated at the ensemble-solution and single-particle levels and in living cells. Further, a sensor-sensitizer nanoassembly was constructed by modifying the nanosilica with a porphyrin photosensitizer, $^1\text{O}_2$ sensor, and cell-penetrating RGD ligands for intracellular $^1\text{O}_2$ delivery and detection. The sensor exhibited ca. 230-fold $^1\text{O}_2$ sensitivity in two-step light irradiation, which is the highest sensitivity reported so far. Further, the nanoassembly showed efficient cell internalization on conjugation with RGD peptide, which was confirmed by the uniform distribution of the porphyrin and the sensor fluorescence in the cytoplasm. Cell death by apoptosis was observed under continuous laser irradiation of the labeled cells. Although the sensor shows blue fluorescence, Thus, this work expands the scope for developing new NIR-emissive and two-photon excitation-based $^1\text{O}_2$ probes with high fluorescence sensitivity. Also, by incorporating efficient photothermal agents, the design can be further extended to image-guided combined PDT-photothermal therapy.

While nanoparticles (NPs) are enormously utilized in biomedical and commercial products such as electronic gadgets, cosmetics, food additives, surface coatings, and paints, their impacts on human health and the environment need careful attention. NPs toxicity depends on various physiochemical parameters such as the size, shape, composition, morphology, dose, surface smoothness/roughness and modifications, durability, or solubility.¹⁷ These properties determine the extent of biological interactions of NPs with proteins, cells, or tissues.¹⁸ The administration of NPs can be intravenous, subcutaneous, inhalation, transdermal, intraperitoneal, and oral. Among these routes, the small-size NPs facilitate their entry mainly by inhalation (lungs), injection (gastrointestinal tract), dermal penetration, nervous system uptake, etc., which later translocate to the circulatory and lymphatic systems.^{19,20} Recent research results connect asthma, lung cancer, bronchitis, Parkinson's disease, and Alzheimer's disease with NP inhalation. Crohn's disease and colon cancers are caused by NP ingestion, whereas the presence of NPs in the blood can result in blood clots, heart diseases, and arrhythmia. NPs are also associated with autoimmune diseases such as rheumatoid arthritis and lupus erythematosus and can adversely affect the liver, spleen, etc., during their translocation. This thesis includes cell-based studies such as membrane labeling

and intracellular delivery of CdSe/ZnS QDs and silica NPs. Although these NPs do not show cytotoxic effects on cultured cells, detailed pharmacokinetic studies are needed before their *in vivo* applications.

References

1. H. Sung, J. Ferlay, R. L. Siegel, M. Laversanne, I. Soerjomataram et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, *CA Cancer J. Clin.* **2021**, *71*, 209.
2. S. H. Jafari, Z. Saadatpour, A. Salmaninejad, F. Momeni, M. Mokhtari et al. Breast cancer diagnosis: Imaging techniques and biochemical markers, *J. Cell Physiol.* **2018**, *233*, 5200.
3. M. Arechederra, M. A. Avila, C. Berasain, Liquid biopsy for cancer management: a revolutionary but still limited new tool for precision medicine, *Adv. Lab. Med.* **2020**, *1*.
4. C. H. Chu, R. Liu, T. O. Ahmadov, B. E. Swain, M. Boya et al. Negative enrichment of circulating tumor cells from unmanipulated whole blood with a 3D printed device, *Sci. Rep.* **2021**, *11*, 20583.
5. X. Li, Y. Li, W. Shao, Z. Li, R. Zhao, Strategies for enrichment of circulating tumor cells, *Trans. Cancer Res.* **2020**, *9*, 2012.
6. Z. Y. Jin, H. Fatima, Y. Zhang, Z. Shao, X. J. Chen, Recent advances in bio-compatible oxygen singlet generation and its tumor treatment, *Adv. Therap.* **2022**, *5*, 2100176.
7. Y. You, Chemical tools for the generation and detection of singlet oxygen, *Org. Biomol. Chem.* **2018**, *16*, 4044.
8. D. E. Campton, A. B. Ramirez, J. J. Nordberg, N. Drovetto, A. C. Klein et al. High-recovery visual identification and single-cell retrieval of circulating tumor cells for genomic analysis using a dual-technology platform integrated with automated immunofluorescence staining, *BMC Cancer* **2015**, *15*, 360.
9. J. M. Park, J. Y. Lee, J. G. Lee, H. Jeong, J. M. Oh et al. Highly efficient assay of circulating tumor cells by selective sedimentation with a density gradient medium and microfiltration from whole blood, *Anal. Chem.* **2012**, *84*, 7400.
10. T. H. Kim, M. Lim, J. Park, J. M. Oh, H. Kim et al. FAST: Size-selective, clog-free isolation of rare cancer cells from whole blood at a liquid-liquid interface, *Anal. Chem.* **2017**, *89*, 1155.
11. K. Liu, X. Liu, Q. Zeng, Y. Zhang, L. Tu et al. Covalently assembled NIR nanoplatfor for simultaneous fluorescence imaging and photodynamic therapy of cancer cells, *ACS Nano* **2012**, *6*, 4054.

12. N. Zhao, B. Ding, Y. Zhang, J. L. Klockow, K. Lau et al. Reactive oxygen species and enzyme dual-responsive biocompatible drug delivery system for targeted tumor therapy, *J. Control. Release* **2020**, *324*, 330.
13. Z. Cao, D. Li, J. Wang, X. Yang, Reactive oxygen species-sensitive polymeric nanocarriers for synergistic cancer therapy, *Acta Biomater.* **2021**, *130*, 17.
14. M. Das, A. Solanki, A. Ganesh, S. Thakore, Emerging hybrid biomaterials for oxidative stress induced photodynamic therapy, *Photodiagnosis Photodyn. Ther.* **2021**, *34*.
15. Q. Liu, J. Tian, Y. Tian, Q. Sun, D. Sun et al. Near-infrared-II nanoparticles for cancer imaging of immune checkpoint programmed death-ligand 1 and photodynamic/immune therapy, *ACS Nano* **2021**, *15*, 515.
16. D. Wang, H. Wu, S. Z. F. Phua, G. Yang, W. Qi Lim et al. Self-assembled single-atom nanozyme for enhanced photodynamic therapy treatment of tumor, *Nat. Commun.* **2020**, *11*, 1.
17. D. B. Zhu, D. Xing, Y. D. Wei, X. Li, B. Gao, Evaluation of the degree of medical radiation damage with a highly sensitive chemiluminescence method, *Luminescence* **2004**, *19*(5), 278.
18. T. A. Saleh, Nanomaterials: Classification, properties, and environmental toxicities, *Environ. Technol. Innov.* **2020**, *20*, 101067.
19. C. Buzea, I. I. Pacheco, K. Robbie, Nanomaterials and nanoparticles: Sources and toxicity, *Biointerphases* **2007**, *2*(4).
20. S. Sharifi, S. Behzadi, S. Laurent, M. L. Forrest, P. Stroeve et al. Toxicity of nanomaterials, *Chem. Soc. Rev.* **2012**, *41*, 2323.