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Optimizing Energy Transfer in Nanostructures Enables in vivo Cancer Lesion Tracking via Near-Infrared Excited Hypoxia Imaging

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Abstract: To explore highly-sensitive and low toxic techniques for tracking and evaluation of non-small cell lung cancer (NSCLC), one of the most mortal tumors in the world, is utterly imperative for doctors to select the appropriate treatment strategies. Herein, we suggest developing near-infrared (NIR) excited nanosensors, in which the donor and acceptor pairs within biological metal-organic frameworks (bio-MOFs) matrix are precisely controlled to rationalize upconversion Förster resonance energy transfer (FRET), for detecting O₂ concentration inside tumors with the reduced signal disturbance and health detriment. Under NIR excitation, as-fabricated core/satellite nanosensors exhibit much improved FRET efficiency and reversible hypoxic response with high sensitivity, which are effective both in vitro and in vivo (zebrafish) for cycling normoxia-hypoxia imaging. Significantly, combined with the reliable preclinical genetically engineered murine model, such nanosensors successfully realize tracking of in vivo NSCLC lesion upon the clear and gradient hypoxia signals without apparent long-term biotoxicity, illustrating their exciting potential for efficient NSCLC evaluation and prognosis.

Lung cancer is a severe threat to human being's health owing to its extremely high morbidity and mortality. Particularly, the non-small cell lung cancer (NSCLC) accounting for 85%~90% of all lung cancers has presented strong invasiveness and fast transfer.^[1,2] Therefore, non-intrusive and persistent observation of occurrence, development or prognosis of NSCLC (stage I to stage IV) is becoming pivotal.^[3,4] Unfortunately, current effective in vivo NSCLC lesion tracking still suffer great challenges. The classic non-intrusive imaging of NSCLC (*e.g.* magnetic response imaging (MRI), computed tomography (CT) and positron emission tomography (PET)) has different levels of harm to health due to radioactive risk, long usage intervals or low resolution.^[5-7] Furthermore, the preclinical murine model used for research is mostly the xenograft model that may not accurately simulate the cancer lesion progression owing to the big difference in the growth and development patterns versus human tumors, leading to disappointing results in clinical trials.^[8-13] Hence, novel imaging strategies combined with ideal preclinical models are highly needed to track the lesion progression of NSCLC towards the phased implement of personalized treatment.^[14]

Hypoxia is broadly recognized as one of the remarkable features in most cancers including NSCLC.^[15-17] Optical hypoxia imaging has received much attention due to their low biotoxicity and powerful visualizing capability with subcellular resolution, which might safely and conveniently meet various demands for tumor detections.^[18,19] The state-of-art non-intrusive optical hypoxia imaging is divided into two categories: redox degree and O₂ concentration.^[20] For the redox sensors, their photoluminescence (PL) intensity could be considerably changed under bioreductive microenvironment of hypoxia.^[21] However, the PL change is easily influenced by other redox substances irrelevant to hypoxia, such as glutathione or cysteine widely existing in cancer cells.^[20] With the development of tumors, the increase in inaccuracy of hypoxia signals may lead to misdiagnosis to patients. Alternatively, the PL intensity of some triplet metal-organic complexes can be largely quenched by O₂ molecules, offering the imaging opportunity directly based on O₂ concentration inside tumor

to reduce the unnecessary disturbances.^[22,23] But the requirement of excitation with high-energy ultraviolet (UV) or visible light decreases their stability and causes detrimental effect on healthy cells and organs,^[24,25] particularly in the late stage of cancer. Therefore, both categories have only achieved the limited success on *in situ* static observation of tumors in the xenograft murine model. The lesion progression detection of tumors by suitable non-intrusive optical hypoxia imaging in advanced preclinical models has never been touched, which will be very meaningful to predict clinical benefit against NSCLC lesion progression.

We suggest constructing the O₂ concentration sensors for NSCLC monitoring excited by near-infrared (NIR) light so as to eliminate the negative effect of UV or visible light, based on the fact that NIR light is advantageous of deep tissue penetration, minimal biological damage and high spatial resolution.^[20,26-28] The typical structure of NIR light-excited sensors is encapsulation of rare-earth-doped upconversion nanoparticles (UCNPs) by polymer or silica matrix, in which O₂ indicators are immobilized. Taking advantage of Förster resonance energy transfer (FRET) process, the NIR light is up-converted into the desirable wavelength by UCNP donors to excite the O₂ indicators in the host matrix.^[29,30] It deserves pointing out that current NIR light-excited sensors, regardless of UCNPs imbedded in polymer film or UCNP core/silica shell structure, generally exhibit low FRET efficiency, insufficient O₂ sensitivity or large size, thus being unable for *in vivo* tumor hypoxia imaging.^[31,32]

In this work, biological metal-organic frameworks (bio-MOFs) that are formed via coordination between non-toxic metal ions and biomolecules are adopted as the matrix, because they not only inherit the features of conventional MOFs like abundant porosity for high drug loading and good stability in biological buffers but also possess additional advantages such as excellent biocompatibility^[33,34] and effective sensitization to fluorescent dyes in their well-defined anionic cavities.^[35,36] Then, we design and fabricate a unique core/satellite nanostructure,^[37,38] where many UCNPs as NIR antennas are attached onto the surfaces of bio-MOFs containing rich O₂ indicators, expecting to maximize the FRET

efficiency under NIR excitation. The genetically engineered murine model (GEMM)^[8] that may accurately mimic tumor microenvironment and progression of human lung cancer is introduced to provide reliable preclinical evaluation on the core/satellite nanosensors for in vivo tracking of NSCLC lesion via hypoxia imaging.

Synthesis process of the core/satellite nanostructures is schemed in **Figure 1a**. First, the NaYF₄:Yb,Tm@NaYF₄ core-shell UCNP (core = NaYF₄ : 20 mol% Yb³⁺, 0.5 mol% Tm³⁺; shell = NaYF₄) stabilized by oleic acid (OA) were prepared using a seed-mediated method,^[39] in which the pre-prepared NaYF₄:Yb,Tm UCNP with the average diameter of ~29.4 nm were introduced as the seeds (Figure S1a,b, Supporting Information). The transmission electron microscopy (TEM) images show that the monodispersed core-shell UCNP in the shape of cuboids have the average width of 31.3 nm and the average length of 38.7 nm (Figure 1b; Figure S1c-e, Supporting Information). Analysis by powder X-ray diffraction (XRD) pattern confirms the highly crystalline nature of core-shell UCNP with the hexagonal structure (Figure S2a, Supporting Information). Noteworthily, compared with core-only UCNP, the core-shell ones display much enhanced upconversion luminescence (UCL) intensity in the wavelength range of 300-500 nm excited by 980 nm NIR laser (Figure S2b, Supporting Information). Polyvinylpyrrolidone (PVP) was known as a good “glue” molecule between inorganic nanoparticles (NPs) and MOFs, so OA on the UCNP surface was then replaced by PVP via a ligand exchange process (Figure 1c; Figure S3, Supporting Information). In the next step, the core-shell UCNP stabilized with PVP were added into the mixed solution of Zn(Ac)₂, adenine (Ad) and 4,4'-biphenyldicarboxylic acid (BPDC), which was a typical recipe to synthesize bio-MOF-100.^[36] Upon heating at 85°C under stirring, a thin shell of bio-MOF-100 was spontaneously formed on the surface of UCNP within 2 min (Figure 1f). Soon afterwards, pure bio-MOF-100 nanocrystals began to appear, and simultaneously, the initially-formed bio-MOF-100 shell coated UCNP tended to spontaneously aggregate and attach onto the surface of bio-MOF-100 nanocrystals as the satellites (Figure 1g). Finally, the self-

limiting growth was completed when the dense layer of UCNPs was formed on the surface of bio-MOF-100 nanocrystals (Figure 1h-j), named as BMU core/satellite nanostructures. Both scanning electron microscopy (SEM) and TEM images demonstrate that as-synthesized BMU core/satellite nanostructures are monodispersed with an average size of 420 nm,^[40-42] where UCNPs are uniformly immobilized on the surface of bio-MOF-100 nanocrystals (Figure 1d,e; Figure S4, Supporting Information).

Crystal structure analysis from powder XRD pattern reveals that the BMU core/satellite nanostructures have two sets of characteristic peaks: cubic phase bio-MOF-100 and hexagonal phase UCNPs (Figure 1k,l), and no peak assigned to other impurities is detected. Furthermore, the pore structure of BMU core/satellite nanostructures was characterized by Brunauer-Emmett-Teller (BET) analysis (Figure 1m,n). The BET surface area of BMU core/satellite nanostructures is 365 m² g⁻¹ and the pore volume is 0.38 cm³ g⁻¹. Evidently, beside the intrinsic micropores at ~1 nm originated from bio-MOF-100 itself, a variety of mesopores and even large pores exist in BMU core/satellite nanostructures, which is beneficial for further loading of organic dyes or large drug molecules. The presence of both mesopores and large pores is mainly caused by etching of bio-MOF-100 matrix with MeOH in the mixed solvent during synthesis (Figure S5, Supporting Information).^[43]

It deserves mentioning that the assembly strategy of core/satellite nanostructures is universal and easily extended to other inorganic NPs of different size and shape combined with bio-MOF-100 matrix. For example, the promising plasmonic core/satellite nanostructures^[38] containing Au nanospheres or nanorods are successfully obtained (Figure S6, Supporting Information).

In view of the characteristic emission bands of NaYF₄:Yb,Tm@NaYF₄ core-shell UCNPs at 450 nm and 477 nm, the molecule tris(4,7-diphenyl-1,10-phenanthroline) ruthenium(II) dichloride ([Ru(dpp)₃]²⁺Cl₂) with strong and well-matched absorption in the wavelength region of 350-550 nm is chosen as the quenchable indicator for O₂ thanks to occurrence of

effective FRET between them (Figure S7a, Supporting Information). More importantly, the red emission of $[\text{Ru}(\text{dpp})_3]^{2+}\text{Cl}_2$ centered at 618 nm not only has negligible overlap with the emission bands of UCNP (Figure S7a, Supporting Information) but also can be dramatically quenched by O_2 with a linear Stern-Volmer relationship (Figure S8, Supporting Information).^[44] Zeta potential analysis indicates that $[\text{Ru}(\text{dpp})_3]^{2+}\text{Cl}_2$ (+43.1 mV) may be spontaneously loaded into the anionic framework of BMU core/satellite nanostructures (−8.05 mV) via electrostatic attraction (Figure S7b, Supporting Information). The successful loading of $[\text{Ru}(\text{dpp})_3]^{2+}\text{Cl}_2$ into BMU core/satellite nanostructures (named as BMU-Ru nanosensors, and their maximum loading content is 18.4 wt% estimated by Figure S9a in the Supporting Information) is confirmed by the obviously-reduced BET surface area ($82 \text{ m}^2 \text{ g}^{-1}$) and pore volume ($0.12 \text{ cm}^3 \text{ g}^{-1}$) (Figure S9b, Supporting Information) as well as the zeta potential change (Figure S7b, Supporting Information). Correspondingly, along with $[\text{Ru}(\text{dpp})_3]^{2+}\text{Cl}_2$ loading, the gradually-formed BMU-Ru nanosensors with good dispersion in water give rise to the color change of the mixed solution from white to orange, whereas no damage on BMU core/satellite nanostructures is discerned (Figure S9c,d, Supporting Information). In addition, BMU-Ru nanosensors also exhibit great stability in different physiological solutions and no obvious leaking in serum is observed, demonstrating their promising potential for biological applications (Figure S10 and Figure S11, Supporting Information).

The optical property of BMU-Ru nanosensors in aqueous solution was investigated by UV-Vis and PL spectroscopy. Compared with BMU core/satellite nanostructures, the intensity of emission bands centered at 450 nm and 477 nm of BMU-Ru nanosensors is significantly reduced owing to the strong absorption by $[\text{Ru}(\text{dpp})_3]^{2+}\text{Cl}_2$ (Figure S12a, Supporting Information). Meanwhile, as shown in **Figure 2a**, BMU-Ru nanosensors exhibit new red emission maximized at 613 nm derived from $[\text{Ru}(\text{dpp})_3]^{2+}\text{Cl}_2$ under 980 nm NIR laser excitation (5 nm hypsochromic shift compared with free $[\text{Ru}(\text{dpp})_3]^{2+}\text{Cl}_2$ caused by FRET process).^[45] Except for the peak overlapping, the efficiency of FRET process is well known to

be determined by energy transfer distance between donors and acceptors as well as charge transfer pathway through the whole structures.^[46,47] The core/satellite structural merits of BMU-Ru nanosensors, *e.g.* high surface coverage of UCNP donors and strong intermolecular interaction between organic linkers of bio-MOF-100 and inside $[\text{Ru}(\text{dpp})_3]^{2+}\text{Cl}_2$ acceptors,^[45,48,49] greatly benefit occurrence of effective FRET (Figure S13, Supporting Information). Accordingly, the total FRET efficiency of BMU-Ru nanosensors is as high as 91.5% deduced from the UCL spectra (Figure S12a, Supporting Information);^[29,30] while the FRET efficiency from $^1\text{G}_4 \rightarrow ^3\text{H}_6$ UCL to $[\text{Ru}(\text{dpp})_3]^{2+}\text{Cl}_2$ at 477 nm reaches ~56% obtained by comparing the lifetimes of BMU core/satellite nanostructures ($\tau = 1230 \mu\text{s}$) and BMU-Ru nanosensors ($\tau = 541 \mu\text{s}$) (Figure S12b, Supporting Information). Remarkably, the FRET efficiency of BMU-Ru nanosensors is superior to that of the state-of-the-art reported FRET sensors (Table S1, Supporting Information).

To verify the potential of BMU-Ru nanosensors as NIR-excitable O_2 probes, the UCL spectra under various concentration of dissolved O_2 were recorded in aqueous solution under 980 nm NIR excitation. As displayed in Figure 2b, the red emission maximized at 613 nm of BMU-Ru nanosensors is gradually quenched with increase of the dissolved O_2 concentration. The change of the intensity ratio I_0/I of nanosensors before (I_0) and after (I) dissolving O_2 is polyexponential with the O_2 concentration ranging from 0 to 40 mg L^{-1} (inset in Figure 2b). It is noticed that the intensity ratio I_0/I is up to 3.6, indicating the high sensitivity of BMU-Ru nanosensors to O_2 under NIR excitation (Table S1, Supporting Information).^[30] Significantly, compared with free $[\text{Ru}(\text{dpp})_3]^{2+}\text{Cl}_2$ under 460 nm excitation, BMU-Ru nanosensors manifest the enhanced sensitivity at low O_2 concentration (0-5 mg L^{-1}) under 980 nm excitation, which is crucial for sensing or imaging of hypoxic regions in organisms. In addition to efficient FRET process, such sensitivity improvement is attributed to the steric restriction of $[\text{Ru}(\text{dpp})_3]^{2+}\text{Cl}_2$ inside bio-MOF-100 cavities, which prevents aggregation-induced quenching and suppresses Ru-N bond elongation in $[\text{Ru}(\text{dpp})_3]^{2+}\text{Cl}_2$, leading to largely increase of

emission intensity and lifetime (Figure S14, Supporting Information).^[50,51] Moreover, the local enrichment of O₂ in porous bio-MOF-100 matrix also benefits O₂ detection (Figure S15, Supporting Information).^[30,52]

The O₂ sensing performance of BMU-Ru nanosensors is further quantitatively determined via a ratiometric method.^[53] As demonstrated in Figure 2c, under 980 nm excitation, the R_I^0/R_I of BMU-Ru nanosensors is well linear with dissolved O₂ concentration from 0 to 8.2 mg L⁻¹ (normoxic condition), where $R_I^0 = I_{613}^0/I_{477}^0$ and $R_I = I_{613}/I_{477}$ represent the intensity ratio of O₂-sensitive luminescence at 613 nm against O₂-insensitive UCL at 477 nm in the absence and presence of O₂, respectively. From linear fit, the Stern-Volmer quenching constant K_{sv} is deduced to be 0.175 L mg⁻¹, allowing for accurately monitoring the low concentration of O₂ in the hypoxic tissues.

Beside the sensitivity, the reversibility, selectivity and photostability are another three important parameters to evaluate the sensor performance. The response of the BMU-Ru nanosensors toward O₂ under multiple hypoxia-hyperoxia cycles was monitored (Figure 2d). Clearly, the signal change of I_0/I vs. cycle time is reversible, and only slight emission decrease (5.3%) is found after 5 times hypoxia-hyperoxia cycles. Furthermore, the specificity of BMU-Ru nanosensors toward O₂ was examined in the acidic and reducing microenvironment with different reducing ions or agents (Figure 2e). Notably, without dissolved O₂, the limited emission change of BMU-Ru nanosensors is observed, implying their high selectivity toward O₂. The photostability of BMU-Ru nanosensors was also tested under exposure to 980 nm laser for 8 h (Figure 2f). Only ~10% decrease of the emission intensity demonstrates the robust stability of BMU-Ru nanosensors under NIR excitation. Altogether, the excellent reversibility, high selectivity and good photostability highlight that BMU-Ru nanosensors are perfect candidates for cycling hypoxia imaging under NIR excitation.

HeLa cell lines were selected as a bioassay model to inspect the performance of BMU-Ru nanosensors toward O₂ detection in vitro under physiological condition.^[54,55] Before

investigation, the cytotoxicity of BMU-Ru nanosensors was analyzed by CCK-8 assay on both the hypoxic and normoxia HeLa cell lines with the added amount of BMU-Ru nanosensors from 15-400 $\mu\text{g mL}^{-1}$ (Figure S16, Supporting Information). The cell viability of HeLa cell lines under the hypoxic environment remains above 80% even after incubation with 400 $\mu\text{g mL}^{-1}$ BMU-Ru nanosensors for 24 h, indicating the very low cytotoxicity of BMU-Ru nanosensors. Moreover, the BMU core/satellite nanostructures have also exhibited good endocytosis effect through the caveolae-mediated route and the internalization via an energy-dependent process (Figure S17, Supporting Information). Subsequently, BMU-Ru nanosensors were applied to monitor the intracellular O_2 level in living HeLa cell lines under decreased O_2 concentration (*e.g.* 20%, 10%, 5% and 0.1% O_2) excited by NIR laser (**Figure 3a,b**; Figure S18a, Supporting Information). Confocal laser scanning microscopy (CLSM) imaging displays that strong emission intensity ($\lambda_{\text{em}} = 600 - 625 \text{ nm}$) is detected from the cells incubated with BMU-Ru nanosensors at low O_2 concentration (*e.g.* 0.1% O_2). As comparison, with increase of O_2 concentration, the emission intensity is reduced gradually and shows negligible intracellular fluorescence under normoxic condition (20% O_2). The similar results are obtained in breast cancer MDA-MB-231 cell lines and squamous cell lung cancer (SQCLC), most common subtype of NSCLC, CH27 cell lines, illustrating the excellent cell type-independent hypoxia imaging ability of BMU-Ru nanosensors (Figure S19 and Figure S20, Supporting Information). Furthermore, the reversibility of BMU-Ru nanosensors was also assessed under alternatively incubating HeLa cell lines at 20% and 0.1% O_2 environment. As expected, very weak emission is discerned under the normoxia condition (Figure 3c1) whereas the remarkable increase of red emission in cells is distinguished after another 0.5 h incubation under the hypoxia condition (Figure 3c2). Importantly, such emission change in HeLa cell lines can be observed repeatedly for another three normoxia-hypoxia cycles (Figure 3d-f; Figure S18b and Figure S21, Supporting Information).

The in vivo hypoxia imaging of BMU-Ru nanosensors with negligible toxicity (Figure S22, Supporting Information) was checked with the zebrafish system. A cerebral anoxia model of zebrafish was built up through abolishment of cardiac contractility, which was induced by incubation of zebrafish embryos with 2,3-butanedione (BDM).^[56] Note that after washing with PBS solution to remove BDM, the effect of BDM on cardiac contractility of zebrafish can be reversed. As shown in Figure 3g1, the 5-day-old zebrafish is incubated with BMU-Ru nanosensors for 1 h, and no emission is observed under CLSM imaging. Once BDM is introduced into the system, the rapid increase of emission in the brain of zebrafish caused by cerebral anoxia is found, and the UCL intensity is nearly saturated after 300 s (Figure 3g4). In contrast, disappearance of emission is discerned when BDM is washed out with PBS, demonstrating that the concentration of O₂ in the brain of zebrafish is recovered (Figure 3h1). Similarly to cycling imaging on HeLa cell lines, the process of emission recovery and quenching combined with normoxic and hypoxic conditions is well reversible in zebrafish system (Figure 3h,i).

Encouraged by the above excellent hypoxia imaging ability both in vitro and in vivo, BMU-Ru nanosensors are finally employed to track the NSCLC lesion progression under NIR laser excitation. The ability of BMU-Ru nanosensors for hypoxia imaging was firstly assessed with tumor-bearing mice, which were prepared by implanting 1×10^6 MDA-MB-231 cell lines at the right hind legs.^[57,58] After the xenograft diameter of tumor reached 8 mm, BMU-Ru nanosensors were injected into the tumor site and muscle area (Figure S23, Supporting Information), respectively. Evidently, the emission signal in tumor site is very strong compared with that in the normal area, demonstrating that BMU-Ru nanosensors have an excellent hypoxic response on the low O₂ condition inside the tumor. Afterwards, *Kras*^{G12D};*Lkb1*^{lox/lox} (KL) GEMM of NSCLC was prepared by treating with 5×10^6 p.f.u. *adeno-Cre* intranasally for faithfully preclinical investigation of NSCLC lesion.^[59] At various time points (2, 8, 12 and 16 weeks), BMU-Ru nanosensors were injected via the tail vein for

hypoxia detection of tumor region through the enhanced permeability and retention (EPR) effect in the lung, and simultaneously the magnetic response imaging (MRI) was performed for comparison.^[60,61] As manifested in **Figure 4a** and Figure S24 in the Supporting Information, the UCL signal of BMU-Ru nanosensors begins to appear within 8 weeks, presenting an early stage in tumor development.^[6] As time prolongs to 12 weeks, the signal accounts for 40~50% area of lung and meanwhile the signal intensity becomes stronger, illustrating that the lesion of NSCLC becomes worsen and causes more hypoxia state. While at 16 weeks, observation of 70%~80% signal coverage accompanied by more and larger red color signal area in lung indicates that lung hypoxia further increases and NSCLC deteriorates into the late stage. Note that these signals are not affected by phagocytosis of spleen and liver (Figure S25, Supporting Information),^[62,63] and is well consistent with that of MRI in the same region of lung (Figure 4b, marked with red circles), namely, the larger and clearer white signal region after 12 weeks suggests growing up and deteriorating of tumor. The NSCLC lesion was also confirmed by the immunofluorescence staining of lung sections with the hypoxia inducible factor 1 α (HIF-1 α), carbonic anhydrase 9 (CAIX) and transforming growth factor- β (TGF- β) at different intervals. As displayed in Figure 4c-e, HIF-1 α , CAIX and TGF- β are all expressed more and more obviously during the tumor malignant progression, demonstrating the lower oxygenation, the higher intracellular acidity and more immunosuppression and angiogenesis in the tumor region, respectively.^[64-66] Therefore, based on the gradually deepening hypoxic state inside the NSCLC tumor, BMU-Ru nanosensors could not only detect NSCLC at the early stage but also monitor the NSCLC lesion easily with clear and gradient signals at different stages of tumors under NIR laser excitation, suggesting its great potential in detection of cancer progression for efficient evaluation and prognosis of cancer treatment strategies.

The safety of nanostructures is pre-requirement for their application in nanomedicine,^[67] so the biodistribution of BMU-Ru nanosensors was analyzed in vivo. To quantify the content of

BMU-Ru nanosensors in major organs, BMU-Ru nanosensors were intravenously injected into the mice and the Y content was measured by inductively coupled plasma mass spectrometry (ICP-MS). As shown in **Figure 5a** and Figure S26 in the Supporting Information, BMU-Ru nanosensors in major organs reach maximum at 3 days post injection and then obviously decrease over time, leading to effective clearance or biodegradation from major organs after 28 days post injection. Particularly, little long-term accumulation of BMU-Ru nanosensors in lung would facilitate detection of lung cancer lesion. Moreover, to evaluate the relative long-term toxicity of BMU-Ru nanosensors, the major organs of mice after 28 days post injection were collected for histopathological analysis. The hematoxylin and eosin (H&E) staining images show that there is no obvious injury in major organs of mice after 28 days post injection (Figure 5b). This result discloses that BMU-Ru nanosensors have no apparent long-term biotoxicity after intravenous administration and might be safely used for in vivo application.

In conclusion, a preclinical case for tracking of NSCLC lesion progression with well-designed hypoxia nanosensors, in which UCNP as antennas convert NIR light into the visible light absorbed by the O₂ indicator of [Ru(dpp)₃]²⁺Cl₂ within bio-MOF-100 nanocrystals via FRET process, has been successfully developed. Notably, the BMU-Ru core/satellite nanostructures exhibit the unprecedented NIR induced FRET efficiency owing to their structural merits including integration of multiple UCNP donors and [Ru(dpp)₃]²⁺Cl₂ acceptors in close distance as well as the limited aggregation and steric restriction of [Ru(dpp)₃]²⁺Cl₂ inside the porous bio-MOF-100. When used as hypoxia sensors, BMU-Ru nanostructures are characteristic of great sensitivity, reversible response, high selectivity and robust stability that enable detecting low concentration O₂ under NIR excitation. Most importantly, in vivo NSCLC lesion tracking evaluated by the preclinical GEMM is for the first time achieved without apparent long-term biotoxicity based on optical hypoxia monitoring using the BMU-Ru nanosensors. The strategy of optimizing energy transfer in the

nanostructures for preclinical examination will open up the avenues toward reliable non-invasive detection of various tumor lesion progression by next generation optical sensors.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

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Figure 1. Preparation and Characterization of BMU core/satellite nanostructures. a) Strategy for fabrication of sensors based on UCNP, bio-MOF-100 and $[\text{Ru}(\text{dpp})_3]^{2+}\text{Cl}_2$ for cycling hypoxia response under NIR excitation. TEM images of b) OA-stabilized $\text{NaYF}_4:\text{Yb,Tm}@\text{NaYF}_4$ UCNP and c) PVP-stabilized $\text{NaYF}_4:\text{Yb,Tm}@\text{NaYF}_4$ UCNP. d) SEM image and e) TEM image of BMU core/satellite nanostructures. TEM images of BMU core/satellite nanostructures obtained with different reaction time: f) 2 min, g) 5 min, h) 10 min, i) 20 min and j) 4 h. k) XRD pattern of BMU core/satellite nanostructures. l) Magnified XRD pattern at small angles of 3° - 15° shown in k) and simulated pattern of bio-MOF-100. m) Nitrogen adsorption/desorption isotherm of BMU core/satellite nanostructures. n) Barrett-Joyner-Halenda pore size distribution of BMU core/satellite nanostructures.

Figure 2. O₂ sensing performances of BMU-Ru nanosensors. a) UCL spectrum of BMU-Ru nanosensors without dissolved O₂. b) O₂ dependent UCL spectra of BMU-Ru nanosensors with various concentration of dissolved O₂. Inset shows the calibration plots of free [Ru(dpp)₃]²⁺Cl₂ (blue curve, λ_{ex} = 460 nm) and BMU-Ru nanosensors (red curve, λ_{ex} = 980 nm) with various concentration of dissolved O₂ (0-40 mg L⁻¹). c) Stern-Volmer plot of UCL quenching of BMU-Ru nanosensors against dissolved O₂ concentration (0-8.2 mg L⁻¹). The O₂ quenching data are analyzed quantitatively by the Stern-Volmer equation: $R^0/R_I = 1 + K_{SV}[\text{O}_2]$. d) UCL response of BMU-Ru nanosensors upon alternation of hypoxia (0 mg L⁻¹) and hyperoxia (40 mg L⁻¹) cycles. e) Relative emission intensity of BMU-Ru nanosensors in acidic (pH = 5) and reducing microenvironment with presence of different reducing ions or agents (400 μM) in water without dissolved O₂. The abbreviation of Hcy, GSH, Cys, DTT, BME and AA represents homocysteine, reduced glutathione, cysteine, dithiothreitol, 2-mercaptoethanol and ascorbic acid, respectively. f) Comparison of photostability of BMU-Ru nanosensors under continuous excitation by 980 nm laser (0.8 W cm⁻²) and 465 nm lamp (0.1 W cm⁻²) for 8 h under ambient condition.

Figure 3. CLSM images both in vitro and in vivo. a,b) CLSM images of HeLa cell lines incubated with BMU-Ru nanosensors under various O₂ concentrations (0.1%, 5%, 10% and 20% O₂): a1-a4) dark-field images and b1-b4) merged images. c-f) CLSM images of HeLa cell lines loaded with BMU-Ru nanosensors and exposed to cycles of normoxia (20% O₂) and hypoxia (0.1% O₂): c1) HeLa cell lines incubated with BMU-Ru nanosensors under normoxia for 1 h; c2) HeLa cell lines incubated under hypoxia after c1) for another 0.5 h. d-f) HeLa cell lines during the repeated process of normoxia (d1, e1, f1) and hypoxia (d2, e2, f2) for another three times. g) Zebrafish after incubation with BMU-Ru nanosensors for 1 h followed by addition of 15 mM BDM at g1) 0 s, g2) 60 s, g3) 180 s and g4) 300 s, respectively. h1) After BDM is washed out, red emission in the brain of zebrafish disappears. h2) After adding BDM again, red emission in the brain is recovered. i1,i2) The process was repeated three times at least. $\lambda_{\text{ex}} = 980 \text{ nm}$, $\lambda_{\text{em}} = 600 - 625 \text{ nm}$; Scale bar: a-f) 50 μm and g-i) 300 μm . The imaging experiments were repeated independently three times and similar results were received.

Figure 4. Lung cancer lesion tracking of BMU-Ru nanosensors. a) UCL images and b) MRI images of the mice lung after intravenous injection of BMU-Ru nanosensors at different intervals (2, 8, 12 and 16 weeks). c-e) Immunofluorescence staining of lung sections with c) HIF-1 α , d) CAIX and e) TGF- β antibodies at different intervals, respectively. The imaging experiments were repeated independently three times and similar results were received. $\lambda_{\text{ex}} = 980 \text{ nm}$; $\lambda_{\text{em}} = 600 - 625 \text{ nm}$. Scale bar: 200 μ m.

Figure 5. Biodistribution and histopathological analysis of BMU-Ru nanosensors. a) Biodistribution of BMU-Ru nanosensors in major organs after administration for 1, 3, 7, 14 and 28 days. The concentration of BMU-Ru nanosensors was normalized as the percentage of the injected dose (ID) per gram of each organ (%ID/g). N.S. represents no statistical significance, * represents $p < 0.05$, ** represents $p < 0.01$. b) H&E staining of the major organ slices of mice untreated and treated with BMU-Ru nanosensors after 28 days. The imaging experiments were repeated independently three times and similar results were received. Scale bar: 100 μm .

The table of contents entry:

A well-designed hypoxia nanosensor has been developed, in which the donors and acceptors are precisely controlled to optimize energy transfer. This near-infrared excited core/satellite nanosensor exhibits great sensitivity, reversible response, high selectivity and robust stability for cycling normoxia-hypoxia imaging. More importantly, combined with the reliable preclinical model, *in vivo* lung cancer lesion tracking is for the first time achieved.

Keywords: hypoxia imaging, metal-organic frameworks, near-infrared excitation, core/satellite nanostructures, cancer lesion tracking

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Title

Optimizing Energy Transfer in Nanostructures Enables *in vivo* Cancer Lesion Tracking via Near-Infrared Excited Hypoxia Imaging

TOC Figure: