様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　３ 年　 ６ 月 ２４ 日現在

機関番号：８２５０２
研究種目：基盤研究(C)（一般）
研究期間：2018～2020
課題番号：１８Ｋ０４９０９

研究課題名（和文）New quantum biosensors for quantitative molecular imaging assays of oxidative stress

研究課題名（英文）New quantum biosensors for quantitative molecular imaging assays of oxidative stress

研究代表者

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研究者番号：３０４６９９３６

交付決定額（研究期間全体）：（直接経費）　　3,400,000円

研究成果の概要（和文）：我々は、EPR、MRI、および光イメージングを用いて、生体内の酸化還元能力や酸化ストレスを追跡するための2つの量子センサーを開発した。いずれもTPP基を結合させた常磁性CD-TEMPOまたは反磁性CD-TEMPOHを被覆した量子ドットで構成され、TPP基は細胞内導入とミトコンドリアへの局在化を実現する。このセンサーは異なる増殖能を持つ単離細胞を用いた生体外での細胞内酸化還元状態のEPR/opticalイメージングや，腎機能障害を持つマウスを用いた生体内での酸化ストレスを検出するMRI実験に応用された。詳細はAnalytical Chemistry 2021年に掲載され報道発表された。

研究成果の学術的意義や社会的意義
Chemical structures are original. They allow analysis of intracellular superoxide in absolute units, which is impossible using conventional fluorescent/luminescent probes. Sensors are appropriate for diagnostics and control of therapy of free-radical diseases.

研究成果の概要（英文）：We developed two-set Qdot sensors for tracking total redox capacity and/or oxidative stress in living biological objects using EPR, MRI, and optical imaging: (i) QD@CD-TEMPO, and (ii) QD@CD-TEMPOH. Both sensors are composed of small-size QDs, coated with paramagnetic CD-TEMPO or diamagnetic CD-TEMPOH conjugated with triphenylphosphonium (TPP) groups. The TPP groups achieve intracellular delivery and mitochondrial localization. Nitroxide residues interact simultaneously with various oxidizers and reducers, and the sensors are transformed from paramagnetic form into diamagnetic form and vice-versa due to nitroxide redox cycling. Sensors were applied for EPR/optical imaging of intracellular redox-status in vitro on isolated cells with different proliferative indexes, as well as for noninvasive MRI of severe oxidative stress in vivo on mice with renal dysfunction. Details are described in Anal Chem 2021 and press-released.

研究分野：生物化学

キーワード：redox　Nitroxide　MRI　EPR　蛍光イメージング　センサー　量子ドット

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

1. Background at the beginning of research

(1) Oxidative stress (OxiStress) and total redox capacity (TRC) of cells, tissues and body fluids are crucial factors in pathogenesis of variety of diseases, as well as in chemo- and radiotherapy. They are recognized as new diagnostic markers in clinic. In this context, development of new low/zero toxic synthetic or genetically encoded redox-sensitive contrast probes and kits for TRC/OxiStress detection has a high clinical and social significance.

(2) All analytical methods, using already developed contrast probes (fluorescent, chemiluminescent, magnetic resonance, nuclear and ultrasound), allow partial evaluation of OxiStress and TRC due to information, obtained only for one or several redox-active compounds. These probes can not sense the total balance between oxidizers and reducers in biological object – major reason for controversial conclusions.

(3) We had a substantial background in development of biocompatible quantum dot-based probes, nitroxide-based contrast probes, and redox imaging.

2. Purpose of research

To develop new redox-sensitive contrast probe(s), which overcome the limitations of already existed on a market and/or reported in the literature for: (i) Quantitative Oxidative stress (OxiStress) In Vitro Assay & OxiStress In Vivo Mapping; (ii) and Quantitative Total Reducing Capacity (TRC) In Vitro Assay & TRC In Vivo Mapping; using fluorescence spectroscopy/imaging, EPR spectroscopy/imaging, and MRI – on living cells, body fluids, and experimental animals.

3. Research methods

(1) Synthesis of nanoparticles and their characterization – DLS, fluorescence spectroscopy, EPR spectroscopy, MRI on phantoms;

(2) Biochemical analyses on model (cell-free) systems and cultured cells – fluorescence spectroscopy and microscopy, EPR spectroscopy, MRI on phantoms;

(3) Chemical (cell-free) model systems for detection of reduction and oxidation of both sensors;

(4) Cellular models of redox-imbalance and oxidative stress on isolated cells – with different proliferative activity (non-proliferating, slow-proliferating, rapidly proliferating);

(5) Animal model pf redox-imbalance and oxidative stress – kidney dysfunction on wild type mice, induced by hypercholesterolemia and accompanied by severe inflammation.

4. Research results

Details are described in our recently published paper in Analytical Chemistry 2021. The data were released in the press (in Japanese and English).

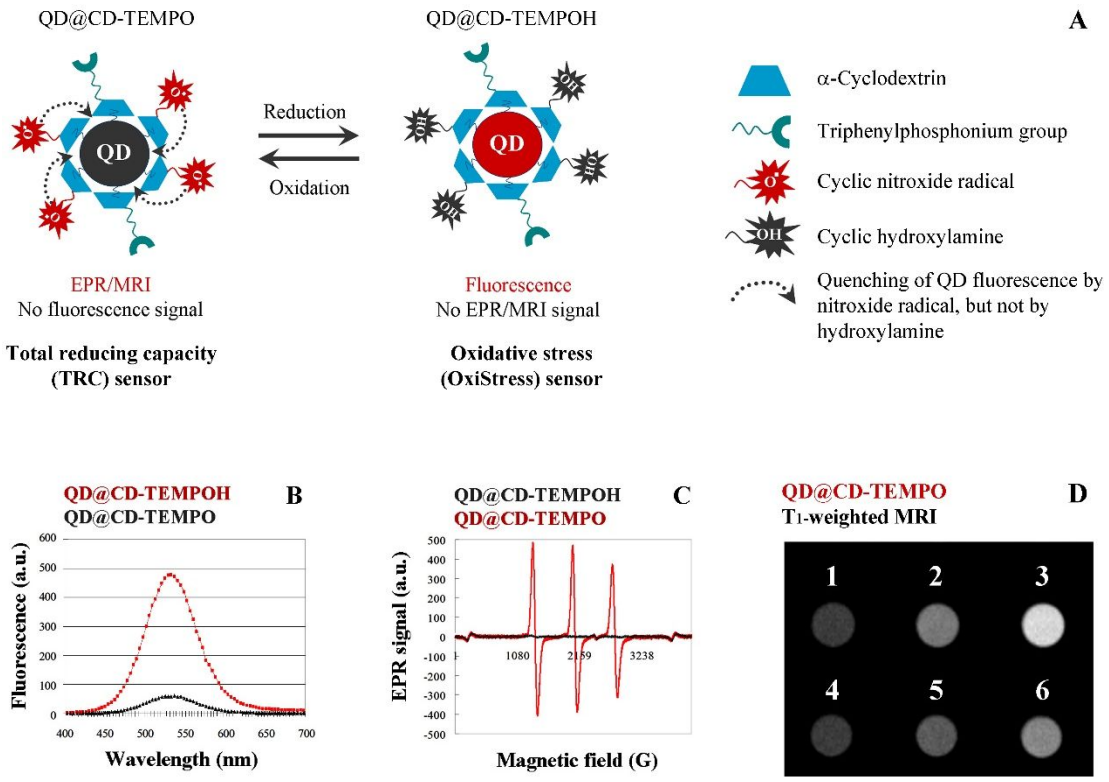

Figure 1. (A) Schematic presentation of quantum sensors: TRC sensor (QD@CD-TEMPO) and OxiStress sensor (QD@CD-TEMPOH). (B) Fluorescence spectra and (C) EPR spectra of QD@CD-TEMPOH (0.1 mM) and QD@CD-TEMPO (0.1 mM). Fluorescence spectra were recorded at λex=365 nm. EPR spectra were recorded at the conditions, described in "Materials and Methods". (D) T1W MRI on phantoms, containing: (1) deionized water; (2 and 3) QD@CD-TEMPO in 1 mM and 2 mM, respectively (dissolved in PBS); (4) phosphate-buffered saline (PBS); (5 and 6) mito-TEMPO in 1 mM and 2 mM, respectively (dissolved in PBS).

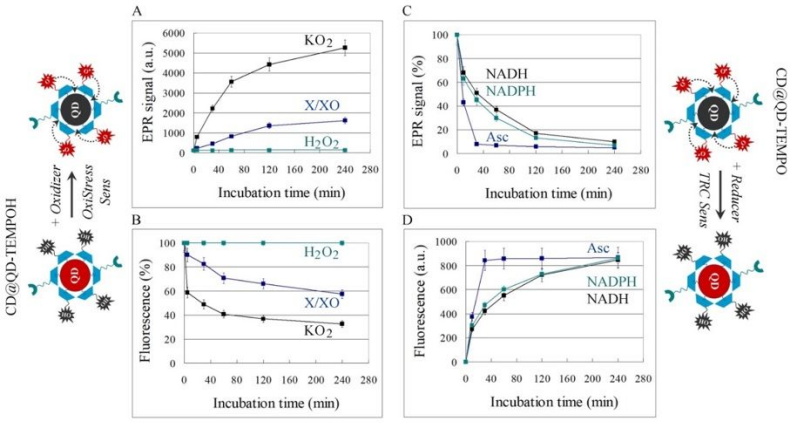

Figure 2. (A) Dynamics of EPR signal of QD@CD-TEMPOH (0.1 mM) in the presence of different oxidizers: 1 mM potassium superoxide; 1 mM hydrogen peroxide; 0.5 mM xanthine plus 0.1 U/mL xanthine oxidase (X/XO). (B) Dynamics of fluorescent signal of QD@CD-TEMPOH (0.1 mM) in the presence of different oxidizers: 1 mM potassium superoxide; 1 mM hydrogen peroxide; 0.5 mM xanthine plus 0.1 U/mL xanthine oxidase (X/XO). In (B), the data are presented as a percentage from the initial fluorescence signal of QD@CD-TEMPOH in deionized water (in the absence of oxidizer). (C) Dynamics of EPR signal of QD@CD-TEMPO (0.1 mM) in the presence of different reducers: 0.1 mM ascorbate (ASC); 0.1 mM NADPH; 0.1 mM NADH. (D) Dynamics of fluorescent signal of QD@CD-TEMPO (0.1 mM) in the presence of different reducers: 0.1 mM ascorbate (ASC); 0.1 mM NADPH; 0.1 mM NADH. In (C), the data are presented as a percentage from the initial EPR signal of QD@CD-TEMPO in deionized water (in the absence of reducer). The experiments were performed in deionized water, except for X/XO reaction, which was performed in 10 mM phosphate-buffered saline (pH 7.4), containing 25 μM DTPA to prevent Fenton's reactions. All data are Mean±SD from three independent experiments.

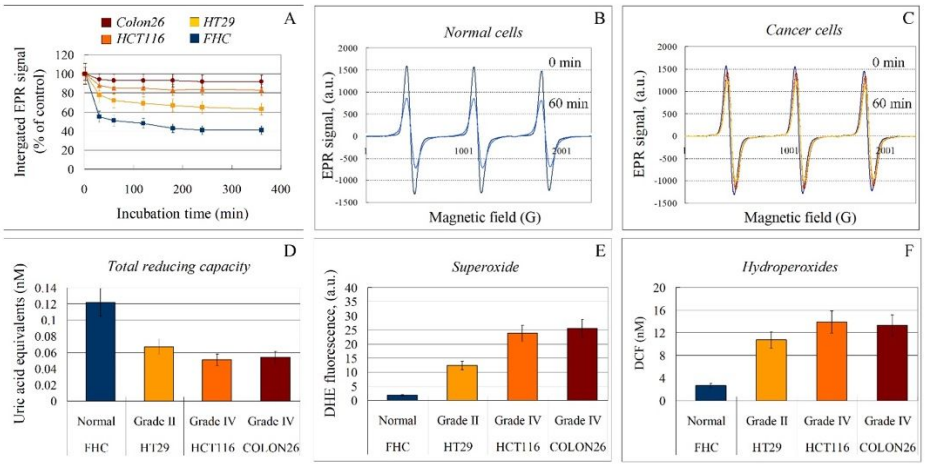

Figure 3. (A) Dynamics of EPR signal of QD@CD-TEMPO (0.1 mM) in cells with different proliferative index (1×10^6 cells/mL), within 6-hours of incubation. Data are presented as a percentage from the initial EPR signal of QD@CD-TEMPO in cell-free cultured medium, which was considered 100%. Cell viability did not change at 6-hours incubation and was ~91-94% (for normal cells) or 97-99% (for cancer cells). (B, C) Representative EPR spectra of QD@CD-TEMPO, recorded in cultured medium (0 min) and 60 min after addition to normal cells and cancer cells, respectively. (D, E, F). Total reducing capacity (in uric acid equivalents) and basic intracellular levels of superoxide (analyzed by DHE assay) and hydroperoxides (analyzed by DCF assay) in cells (1×10^6 cells/mL) with different proliferative index. In (A), (D), (E), and (F), the data are means±SD from three independent experiments.

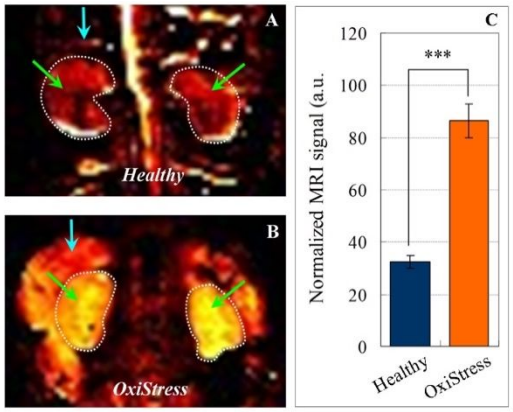

Figure 4. Representative images of extracted nitroxide-enhanced MRI signals in the kidneys of healthy mice (A) and mice with redox imbalance and severe oxidative stress (B), obtained by processing the original T_1W images using ImageJ. The images were obtained 3 min after intravenous injection of QD@CD-TEMPO in the tail vain. The green arrows indicate the kidney area. The blue arrows indicate the area outside the kidney. Redox imbalance and oxidative stress were induced by development of hypercholesterolemia and subsequent kidney dysfunction. (C) Normalized MRI signal intensity in the kidney area of healthy mice and mice with renal dysfunction, accompanied by redox imbalance. Data are means+/-SD from three mice in each group. ***$p<0.01$ (two-tiled p-value calculated by Student's t-test).

５．主な発表論文等

〔雑誌論文〕　計8件（うち査読付論文　3件／うち国際共著　3件／うちオープンアクセス　2件）

１．著者名 IVANOVA DONIKA、ZHELEV ZHIVKO、SEMKOVA SEVERINA、AOKI ICHIO、BAKALOVA RUMIANA	４．巻 39
２．論文標題 Resveratrol Modulates the Redox-status and Cytotoxicity of Anticancer Drugs by Sensitizing Leukemic Lymphocytes and Protecting Normal Lymphocytes	５．発行年 2019年
３．雑誌名 Anticancer Research	６．最初と最後の頁 3745～3755
掲載論文のDOI（デジタルオブジェクト識別子） 10.21873/anticanres.13523	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Bakalova Rumiana、Zhelev Zhivko、Miller Thomas、Aoki Ichio、Higashi Tatsuya	４．巻 2020
２．論文標題 Vitamin C versus Cancer: Ascorbic Acid Radical and Impairment of Mitochondrial Respiration?	５．発行年 2020年
３．雑誌名 Oxidative Medicine and Cellular Longevity	６．最初と最後の頁 1～12
掲載論文のDOI（デジタルオブジェクト識別子） 10.1155/2020/1504048	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 SEMKOVA SEVERINA、ZHELEV ZHIVKO、MILLER THOMAS、SUGAYA KIMIHIKO、AOKI ICHIO、HIGASHI TATSUYA、BAKALOVA RUMIANA	４．巻 40
２．論文標題 Menadione/Ascorbate Induces Overproduction of Mitochondrial Superoxide and Impairs Mitochondrial Function in Cancer: Comparative Study on Cancer and Normal Cells of the Same Origin	５．発行年 2020年
３．雑誌名 Anticancer Research	６．最初と最後の頁 1963～1972
掲載論文のDOI（デジタルオブジェクト識別子） 10.21873/anticanres.14151	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Bakalova Rumiana、Zhelev Zhivko、Miller Thomas、Aoki Ichio、Higashi Tatsuya	４．巻 28
２．論文標題 New potential biomarker for stratification of patients for pharmacological vitamin C in adjuvant settings of cancer therapy	５．発行年 2020年
３．雑誌名 Redox Biology	６．最初と最後の頁 101357～101357
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.redox.2019.101357	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

1．著者名 Lazarova D.、Shibata S.、Ishii I.、Zlateva G.、Zhelev Z.、Aoki I.、Higashi T.、Bakalova Rumiana	4．巻 38
2．論文標題 Nitroxide-enhanced magnetic resonance imaging of kidney dysfunction in vivo based on redox-imbalance and oxidative stress	5．発行年 2019年
3．雑誌名 General physiology and biophysics	6．最初と最後の頁 191～204
掲載論文のDOI（デジタルオブジェクト識別子） 10.4149/gpb_2019001	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

1．著者名 Zhelev Z., Georgieva E., Lazarova D., Semkova S., Aoki I., Higashi T., Bakalova R.	4．巻 なし
2．論文標題 “Redox imaging” to distinguish cells with different proliferative indexes: Superoxide, hydroperoxides and their ration as potential biomarkers.	5．発行年 2019年
3．雑誌名 Oxid. Med. Cell. Long.	6．最初と最後の頁 6373685
掲載論文のDOI（デジタルオブジェクト識別子） 10.1155/2019/6373685	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

1．著者名 Lazarova D., Shibata S., Ishii I., Zlateva G., Zhelev Z., Aoki I., Bakalova R.	4．巻 33:1
2．論文標題 Imaging of redox-balance and oxidative stress in kidney in vivo, induced by dietary cholesterol.	5．発行年 2019年
3．雑誌名 Biotechnol. Biotehnol. Equip.	6．最初と最後の頁 294-301
掲載論文のDOI（デジタルオブジェクト識別子） なし	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

1．著者名 Lazarova D., Shibata S., Ishii I., Zlateva G., Zhelev Z., Aoki I., Higashi T., Bakalova R.	4．巻 38:3
2．論文標題 Nitroxide-enhanced magnetic resonance imaging of kidney dysfunction in vivo based on redox-imbalance and oxidative stress.	5．発行年 2019年
3．雑誌名 Gen. Physiol. Biophys.	6．最初と最後の頁 191-204
掲載論文のDOI（デジタルオブジェクト識別子） なし	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計2件（うち招待講演　0件／うち国際学会　1件）

1．発表者名 Severina Semkova, Desislava Lazarova, Zhivko Zhelev, Biliana Pancheva Nikolova-Lefterova, Rumiana Bakalova, Ichio Aoki
2．発表標題 New redoxomic-based strategy in cancer; predictive medicine
3．学会等名 International Congress of Medical Sciences 2019（国際学会）
4．発表年 2019年

1．発表者名 Rumiana Bakalova, Severina Semkova, Zhivko Zhelev, Ichio Aoki
2．発表標題 Quantum sensors for mapping of oxidative stress in cells and tissues using EPR, MRI and optical imaging
3．学会等名 第14回日本分子イメージング学会総会・学術集会
4．発表年 2019年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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6．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考
研究協力者	青木　伊知男 (Aoki Ichio) (10319519)	国立研究開発法人量子科学技術研究開発機構・放射線医学総合研究所・分子イメージング診断治療研究部・グループリーダー (82502)	

7．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

8．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関			
ブルガリア	Trakia University	Sofia University	Bulgarian academy of science	
ブルガリア	Sofia University	Trakia University	Bulgarian Academy of Science	