Workshop of Research Laboratory for Quantum Beam Science on "Radiation Effects on DNA"

Lecture Hall, *The Institute of Scientific and Industrial Research* (SANKEN),

Osaka University,

Mihogaoka 8-1, Ibaraki, Osaka 565-0047, Japan

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Program

1830 Get-together party

Radiation Effects on DNA

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DNA is a genetic material with all information on the vital phenomenon. The radiation induces damage of DNA molecule, leading acute or evening damage such as carcinogenesis and shortened life. When DNAs in the reproductive cells are damaged, hereditary effect is possibly transferred to next generation. In other words, DNA damage generated by radiation can be inherited to the descendant through the reproductive cells. Thus, radiation effect on DNA is the key to elucidate the radiation effect on living thing. DNA is the target that should be defended most in living bodies against radiation.

Radiation induces DNA main strand scission and nuclear base damage. DNA main strand scission has two patterns of single strand and double strand scissions. The former can be exactly restored, while the latter induces genetic troubles such as the correction error and impossible correction error for DNA restoration, leading mutation and cell death. The nuclear base damage induces mutation directly and indirectly through DNA error restoration, relating to carcinogenesis and genetic effect.

Radiation effect on DNA depends on properties of the radiation. The low LET radiation such as X ray and gamma ray induce direct DNA strand scission and indirect nuclear base damage, while the high LET radiation such as neutron and alpha ray induce direct DNA damage. In addition, radiation effect on DNA depends on the environment of the target DNA such as temperature, oxygen concentration, regulation of chromatin, and others. Thus, the radiation damage on DNA is resulted from various reactions under the various conditions, and the reaction mechanisms are very complex. It is necessary to study the reactions and reaction mechanisms for radiation effect on DNA in detail under the various conditions.

Hole Transfer in DNA

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Double helical DNA carries information controlling our heredity by means of linear sequence of its nucleotide, G, A, C, and T. The oxidation potential of nucleotides increases in the order of G: 1.31 < A: 1.63 < C ~ T: 2.1 (V vs NHE) and thus G is the most subjective to one-electron oxidation. As a consequence, one-electron oxidation of DNA leads to the formation of G radical cation ($G^{\bullet+}$), a positive charge, in DNA. Based on the gel electrophoresis strand cleavage experiments, it has been demonstrated that a charge can migrate through DNA over long distance (> 100 Å). A model of a multi-step charge transfer mechanism, in which G acts as charge carriers, is the most widely adopted to describe the long-range charge-transfer process. The kinetics of charge transfer process in DNA was first reported by Lewis and Wasielewski *et al*., who determined the kinetics of single-step charge transfer between Gs across A-T base pair (*Nature*, **2000**, *406*, 51.). We have established the method for the observation of long-range charge transfer through DNA and determined the rate constants of charge transfer through various sequence patterns. Based on the determined rate constants, we can now estimate the charge transfer kinetics for a certain sequence and length of DNA. In other words, DNA can be consider to have another information, i.e., how fast a charge can migrate through it, and it would be possible to readout the sequence information of DNA by measuring the charge transfer kinetics. Herein, we will overview our strategy for the measurements of long-range charge transfer kinetics through DNA and our efforts in reading out the stored information of DNA, especially the sequence information about single nucleotide polymorphisms (SNPs), by measuring the charge transfer kinetics

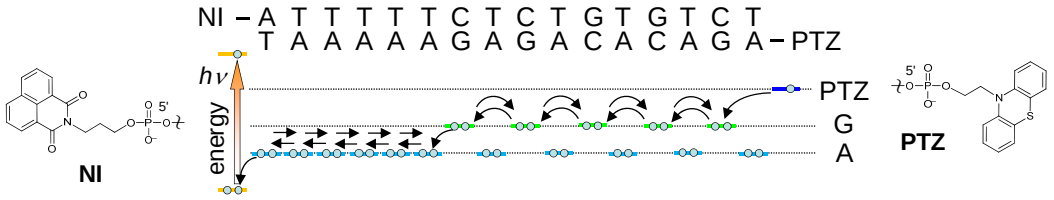

Figure 1. Strategy for the measurement of long-range charge transfer through DNA.

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Excess Electron Transfer in DNA

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Hole and excess electron transfer processes in DNA have received great attention of many researchers in the various fields such as biomedical science and nanotechnology. From the viewpoint of radiation chemistry, the dynamics of hole and excess electron are important subject because radiation induced charged species have close relation to DNA damage and repair. Recently, the time-resolved spectroscopic measurements have revealed detailed mechanisms and kinetic parameters of the DNA-mediated hole transfer. The rate constants of the single hole hopping process in DNA have been determined to be on the order of 10^4-10^{10} s^{-1}. On the other hand, the rate constant of single electron hopping process in DNA has not been determined until now. Recently we have investigated excess electron transfer (EET) process in DNA using photosensitinzing donor-DNA-acceptor by means of femtosecond laser flash photolysis.[1] In the present study, we directly measured the EET rate in nicked dumbbell DNA.

In the present study, tetramer of thiophene (4T) and diphenyl acetylene (DPA) have been employed as the photosensitizing electron donor and acceptor of the donor-DNA-acceptor system, respectively (Fig. 1a, b). That is, photoexcited 4T can donate excess electron to thymine (T) in DNA, from which DPA can accept excess electron as indicated in Fig. 1c.

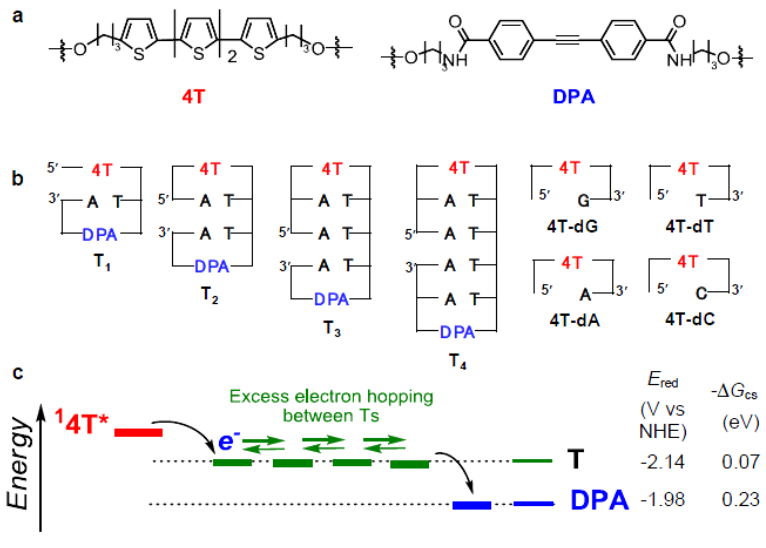

Fig. 1. (a, b) Structures of nicked dumbbell DNAs. (c) Expected charge transfer in DNA.

The sample was excited with the femtosecond laser pulse at 400 nm, which excites 4T selectively. The broad absorption band with a peak at >700 nm appeared immediately after the laser excitation is identified to ^{1}4T* (Fig. 2a, b). With the ^{1}4T* decay, an absorption band of 4T radical cation (4T$^{\bullet+}$) appeared at 675 nm, indicating the electron injection into adjacent thymine (T) within ~10 ps. On the other hand, a transient absorption band of DPA$^{\bullet-}$ appeared at 500 nm with a rising profile over 200 ps. Generation of DPA$^{\bullet-}$ slower than 4T$^{\bullet+}$ rise indicates to reduction of DPA by EET through the consecutive T sequence by the injected excess electron hopping. This is also supported by the fact that the generation of DPA$^{\bullet-}$ became slower with the number of intervening T, while the generation rate of 4T$^{\bullet+}$ is almost independent of the number of T. From the rise rate, the rate constant of single step negative charge hopping along T consecutive sequence of DNA was estimated to be $(4.2 \pm 0.5) \times 10^{10}$ s^{-1}. The estimated the rate is faster than that of the hole transfer rate.

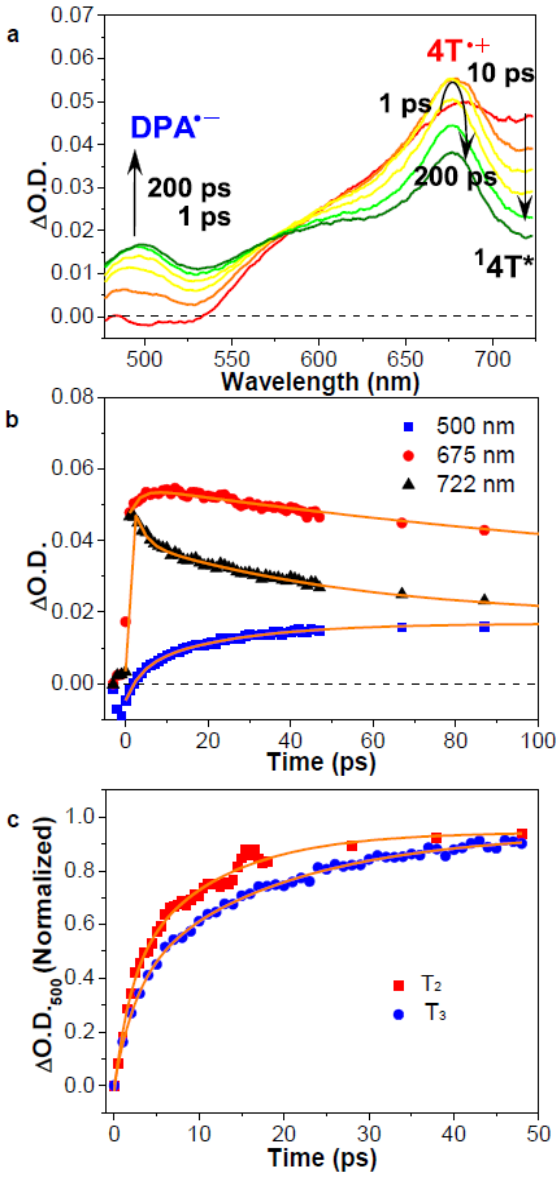

Fig. 2. (a) Transient absorption spectra of T_3 during laser flash photolysis. (b) Kinetic trances of ΔO.D. at 500, 675, and 722nm. (c) Kinetic trances of T_2 and T_3.

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Introduction of the Research Laboratory for Quantum Beam Science at SANKEN

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The Research Laboratory for Quantum Beam Science attached to the Institute of Scientific and Industrial Research (ISIR or Sanken), Osaka University is a joint-use laboratory in Osaka University. It was established in 2009 as a successor of the Radiation Laboratory in the Nanoscience and Nanotechnology Center of ISIR. All the facilities of the Radiation Laboratory were inherited, including a 40-MeV L-band electron linear accelerator (linac), a 150 MeV S-band electron linac, a 40 MeV RF-gun electron linac, and ^{60}Co γ-ray irradiation facility. At the laboratory, we conduct various researches in the fields of beam sciences and radiation sciences, including frontier beam science related to environmental materials science, new energy sources, and advanced medical technology as well as fundamental beam sciences. The research is also promoted on chemical reactions in ultra-short periods. Details of the man facilities are as follows;

- L-band Electron Linac

The L-band electron linac was constructed in 1978 for generating an intense single-bunch electron beam in the picosecond time range. It is composed of a thermionic electron gun, three sub-harmonic bunchers (SHBs), a pre-buncher(PB), a buncher (B) and a 3 m long accelerating tube. The linac can be operated in four different modes; transient, steady, single-bunch and multi bunch modes. Electrons injected from the gun and passing through the SHB system are bunched to 20-30 ps with PB and B and accelerated up to 40 MeV through the acceleration tube driven with a klystron with the peak power of 30 MW at the RF frequency of 1.3 GHz. The maximum charge so far obtained in the single bunch mode is 91 nC/bunch, which is one of the highest beam intensities in the single bunch in the world. The high-brightness electron beam has been mainly used for researches on the transient phenomena in the range from nanoseconds to sub-picoseconds by means of pulse radiolysis, and developing a far-infrared free electron laser (FEL) as well as basic research on Self-Amplified Spontaneous Emission (SASE) in the far-infrared region. The linac was renewed in 2003 for the purpose of realizing high stability and reproducibility as well as easy operation.

- S-band Laser Photocathode RF Electron Linac

The laser-photocathode RF electron linear accelerator is a new electron accelerator generating a low-emittance and ultra short-bunch beam. It consists of a 1.6-cell S-band laser photocathode RF electron gun, a 3 m long S-band acceleration tube, and a magnetic pulse compression system. The picosecond electron beam is generated in the RF gun by irradiating a copper cathode using an all-solid-state Nd: YLF picosecond laser. A low-emittance electron beam generated with the RF gun is accelerated in the acceleration tube downstream of the RF gun, and the energy chirped electron beam is compressed into the

femtosecond range by rotating the longitudinal bunch phase space in the magnetic field.

- 150 MeV S-band Electron Linac

The 150 MeV S-band linac, which was developed in 1990, consists of tree acceleration tubes and a thermionic gun. It can accelerate an electron beam up to 100 MeV with the current of 0.25 A in the 2 μs long macropulse at a repetition rate of 30 Hz or less. This linac is exclusively used for production of the slow-positron beam.

- 60Co γ-ray irradiation facility

The 60Co γ-ray irradiation facility is equipped with three 370 TBq 60Co γ-ray sources. Two irradiation caves are available for the experiments. The facility is used for studies in the fields such as irradiation effects on materials and tissues, radiation induced polymerization, radiation damage on materials, radiation hazard on biological system and so on.

Radiation-induced damage to cellular DNA: formation and mechanistic insights.

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Emphasis has been placed in the last decade on the elucidation of the main degradation pathways of DNA model compounds mediated by •OH radical and one-electron oxidation reactions as the result of indirect and direct effects of ionizing radiation respectively. This has led to the isolation and characterization of almost 100 oxidized purine and pyrimidine nucleosides if hydroperoxide precursors and diastereomers are considered. However, far less information is currently available on the mechanisms of radiation-induced degradation of bases in cellular DNA mostly due partly to analytical difficulties. It may be reminded that the measurement of oxidized nucleosides and bases in nuclear DNA is still a challenging issue which until recently has been hampered by the use of inappropriate methods such as the GC-MS that have led to overestimated values of the lesions by factors varying between two and three orders of magnitude. At the present, using the accurate and sensitive HPLC/MS/MS assay, 11 single modified nucleosides and bases were found to be generated in cellular DNA upon exposure to gamma rays and heavy ions. This validates several of the •OH-mediated oxidation pathways of thymine, guanine and adenine that were previously inferred from model studies. The concomitant decrease in the yields of oxidized bases with the increase in the LET of heavy ions is accounted for by the preponderance of indirect effects in the damaging action of ionizing radiation on DNA. Further evidence for the major role played by •OH radical was provided by the results of irradiation of cells with high intensity 266 nm laser pulses. Under these conditions 8-oxo-7,8-dihydroguanine is mostly produced as the result of biphotonic ionization of DNA nucleobases and subsequent hole migration to guanine bases. It is likely that some of the oxidized bases that have been isolated as single lesions are in fact involved in clustered damage. Interestingly it was recently shown that a single oxidation hit is capable of generating complex lesions in DNA. Thus •OH-mediated abstraction at C4 of the 2-deoxyribose moiety gives rise to DNA strand cleavage together with the formation of a highly reactive aldehyde that undergoes an addition reaction to the amino group of a proximate cytosine, leading to 4 diastereomeric cycloadducts as components of likely interstrand cross-links. It was also shown that the (5'*R*)-5',8-cyclo-2'deoxyadenosine, a tandem lesion, that arises from intramolecular addition of the •OH-mediated C5' radical to the C8 position of the adenine moiety is generated in DNA, however, in a low yield upon exposure of cells to gamma radiation.

Fluorescence detection of the repair of radiation-induced DNA damage using synthetic oligonucleotides

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Thymine glycol (Tg) is a major type of oxidative DNA damage that results from the reaction of a thymine base with reactive oxygen species generated by ionizing radiation. There are four diastereomers of this damaged base, i.e. (5*R*,6*S*), (5*R*,6*R*), (5*S*,6*R*) and (5*S*,6*S*), but Tg exists as either the (5*R*) *cis–trans* pair [(5*R*)-Tg] or the (5*S*) *cis–trans* pair [(5*S*)-Tg] in solution, due to epimerization at the C6 position. It has been reported that these two isomeric pairs are formed in equal amounts in γ-irradiated DNA. We previously succeeded in the synthesis of DNA building blocks of (5*R*,6*S*)- and (5*S*,6*R*)-Tg and their incorporation into oligonucleotides (1,2). Since the (5*R*,6*S*) isomer was preferentially formed by the oxidation of protected thymidine with OsO_4, we sought for conditions to improve the yield of the (5*S*,6*R*) isomer and found that the Sharpless asymmetric dihydroxylation reaction, using an ionic liquid as a co-solvent, produced a satisfactory result (3).

Tg is repaired by the base excision repair pathway, and the enzymes responsible for its initial step include *Escherichia coli* endonuclease III (Endo III) and human NTH1. We have developed a method for the detection of this repair step by fluorescence (4). The probes were 13-base-pair hairpin-shaped oligonucleotides containing each isomer of Tg or 5,6-dihydrothymine (DHT) as a damaged base at the center, and had a fluorophore and a quencher at the 5' and 3' ends, respectively. Fluorescence was detected when the phosphodiester linkage at the damage site in the probe was cleaved by the enzyme, because the short fragment bearing the fluorophore could not be hybridized to the quencher strand at the incubation temperature. The substrate specificity was shown using *E. coli* and human enzymes. There was no difference between (5*R*)- and (5*S*)-Tg when *E. coli* Endo III was used, whereas a large increase in the fluorescence intensity was observed only for (5*R*)-Tg in the case of human NTH1. Fluorescence measurement also showed that the probe containing DHT was cleaved by the human enzyme more efficiently than by *E. coli* Endo III under the conditions where the two enzymes exhibited a similar activity for (5*R*)-Tg. These results agreed with the previous reports in which the substrate specificity was determined by using ^{32}P-labeled substrates. In order to use these probes in living cells, nuclease-resistant phosphorothioate linkages were incorporated. *In vitro* and *ex vivo* experiments revealed that the phosphodiester linkages between the fluorophore/quencher and the terminal nucleoside were hydrolyzed unless they were changed into the phosphorothioate. Using a probe containing normal phosphodiester linkages only at the sites surrounding Tg, the initial step of the base excision repair in HeLa cells was clearly detected.

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Dynamics of Radical Cations of Guanine and Adenine in Duplex DNA Studied by Pulse Radiolysis

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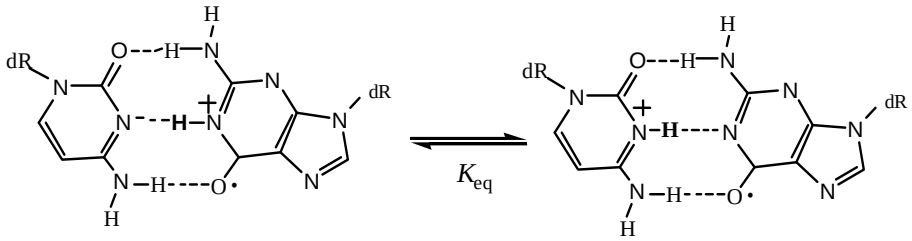
Oxidative damage of DNA via radical cation is a common event and causes the important biological processes of mutagenesis, cancer, and cellular radiation damage. The electron-loss center generated in duplex DNA ultimately reaches at guanine (G) sites via hole migration and the guanine radical cation ($\mathbf{G}^{+\cdot}$) thus formed migrates by a hopping mechanism until it is trapped in a chemical reaction with H_2O or O_2. The $\mathbf{G}^{+}\bullet$ of deoxyguanosine (dG) has a pK_a of 3.9, and it rapidly loses a proton of N1 to yield the guanine neutral radical, **G(-H)•**. In the guanine radical cation/cytosine base pair ($\mathbf{G}^{+}\bullet$/C**),** on the other hand, it is not clear whether the proton of N1 of G does or does not shift to C, in eq (1).



(1)

It has been demonstrated that adenine (A) plays a hole carrier in long A-T stretches. Once a hole resides on an A base, as an A cation radical ($\mathbf{A}^{+}\bullet$), hole transfer can occur over long A sequences rapidly and efficiently (A-hopping) if G does not interrupt the A sequence. This implies that the direct stacking between adjacent same-strand A bases is important for the A-hopping mechanism. However, the mechanistic detail of A hopping remains unclear. In this report, the spectroscopic measurement in dynamics of $\mathbf{G}^{+}\bullet$[1, 2] and $\mathbf{A}^{+}\bullet$[3] in oligonucleotides (ODNs) was investigated by nanoseconds pulse radiolysis.

Pulse radiolysis experiments in the presence of ammonium persulfate involve the almost instantaneously generation of $SO_4^{-}\bullet$, which in turn can oxidize bases unselectively. Previous report showed that $\mathbf{G}^{+}\bullet$ was formed within 50 ns after pulse radiolysis, followed by deprotonation to yield **G(-H)•**.[1] In experiments using 5-substituted cytosine-modified ODN, substitution of the cytosine C5 hydrogen by a methyl group increased the rate constant of deprotonation, whereas replacement by bromine decreased the rate constant. Kinetic solvent isotope effects on the kinetics of dG and ODN duplexes were examined in H_2O and D_2O. The rate constant of formation of **G(-H)•** in dG was 1.7-fold larger in H_2O than D_2O, whereas the rate constant in the ODN duplex was 3.8-fold larger in H_2O than D_2O. These results suggest that the formation of **G(-H)•** from $\mathbf{G}^{+}\bullet$ in the ODN corresponds to the

deprotonation of the oxidized hydrogen-bridged (**G**$^{+}$•-C) base pair by a water molecule.

Among the systematic series of G-containing ODNs, the characteristic absorption maxima of **G**$^{+}$• intermediate around 400 nm were shifted to the longer wavelength in the order of G < GG < GGG. In contrast, the spectra of **G(-H)•** were not affected by the sequence and was essentially similar to that of free dG. These results suggest that the positive charge in **G**$^{+}$• in ODN is delocalized over the extended π orbitals of DNA base, whereas **G(-H)•** is essentially localized on guanine base. The rate constant of the deprotonation was found to change by the sequence of ODNs, where adjacent bases of the guanine base are important factor of the deprotonation.

The **A**$^{+}$• of dA, produced by oxidation with SO_4^{-}•, rapidly deprotonated to form the neutral A radical (A(-H)•) with a rate constant of 2.0×10^7 s^{-1} and a pK_a value of 4.2, as determined by transient spectroscopy. A similar process was observed in experiments performed on a variety of double-stranded ODNs containing adenine•thymine (A•T) base pairs. The transient spectrum of **A**$^{+}$• in an ODN composed of alternating A•T pairs was essentially identical to that of free dA, and differed from the spectra of ODNs containing AA and AAA. In contrast, the spectra of A(-H)• were not affected by the sequence. These results suggest that the positive charge on **A**$^{+}$• in ODNs is delocalized as the dimer stabilized by π-orbital stacking between adjacent As. The rate constants for deprotonation of **A**$^{+}$• in ODNs containing AA and AAA ($0.9\sim1.1 \times 10^7$ s^{-1}) were a factor of two smaller than the rate constants for deprotonation of **A**$^{+}$• in ODNs containing alternating A•T and dA (2.0×10^7 s^{-1}). This suggests that the formation of a charge resonance stabilized dimer **AA**$^{+}$• in DNA produced a significant barrier to deprotonation.

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New Quantum-Beam Development for Radiation Therapy

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A new quantum-beam for the intensity modulated radiation therapy (IMRT) has been developed by using a photocathode rf gun electron LINAC in Sanken, Osaka University. IMRT is one of the most advanced radiation therapies, in which the body damage of the radiation can be avoided by hitting only cancer part. In the advanced IMRT, the high contrast of the radiation dose of the cancer part to the health part should be acquired. However, the human body is always moving by the breathing and the pulsation. Therefore, the accurate beam control techniques on the beam shaping, the beam intensity modulation, and the irradiation timing, are necessary.

The laser photocathode rf gun can generate high quality and low emittance electron beam [1]. The electron beam shape was changed by controlling the UV laser shape which excites the laser photocathode of the rf-gun. We demonstrated the production of various shapes of the electron beams, such as triangle, square, and very small dotted pattern [2].

For the more accurate beam shaping and beam intensity modulation, a digital micro mirror device (DMD) which can control the laser light with a high speed and a high spatial resolution was inserted the UV laser line. By transferring images to the DMD and drawing them continuously, the shape and intensity of the electron beam was modulated dynamically. The irradiation timing also could be controlled by using the DMD.

Although the highly-controlled modulated electron beam is enough effective to the IMRT, the X-ray is usually more convenient for the radiation therapy, because the penetration range is longer. The X-ray conversion from the electron beam with maintaining the shape and intensity modulation was succeeded by the parametric X-ray radiation (PXR) method using a silicon crystal wafer in air.

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Real-time and In vivo Monitoring of Reactive Oxygen Species (ROS) Based on Designed Functional Materials

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Determination of Reactive oxygen species (ROS) is of great physiological and pathological importance since ROS is considered to be involved in the etiology of aging, cancer, and progressive neurodegenerative diseases such as Parkinson's disease. The quantative monitoring of the local concentration of ROS has been a relatively challenging analytical problem because of the low concentration, high reactivity and short lifetime in biological systems. One of the research interests of our group is focusing on real-time and in vivo detection of ROS. Firstly, our group has systematically investigated the fabrication, characterization, especially the electrochemical properties of the functional nanostructures-biomolecule hybrid systems. The combination of facilitated electron transfer on the nanostructured functional materials, and the inherent electrocatalytic activities of biomolecules, provides a number of biocompatible methodologies for the determination of ROS. The second part is "plasmon-enhanced analytical performance in biosensors for ROS". Surface plasmon resonance is the collective oscillations of conductive electrons induced by the electric field of visible light, which is strongly dependent on the size, shape of nanoparticles and the inter-distance between nanoparticles. We have developed a plasmon-induced photoelectrochemical strategy for determination of ROS with enhanced analytical performance through direct electron transfer of protein at TiO_2 nanoneedles film loaded with gold nanoparticles. Both anodic and cathodic redox currents have been amplified under visible light irradiation. The sensitivity of the present biosensor is 4-fold larger than that obtained at Au/TiO_2 film without visible light illumination. The third part is "functional biomimetic enzyme for detection of ROS from living cells". We have reported a series of facile and efficient methods for real-time analysis of ROS from living cells with high selectivity and relatively long stability, through direct electron transfer of biomimetic enzymes.

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Discussion