論文 / 著書情報
Article / Book Information

題目(和文)	
Title(English)	Construction of Multifunctional Temperature-responsive Protein Nanoparticles for Biomarker Detection
著者(和文)	WangGaoyang
Author(English)	Gaoyang Wang
出典(和文)	学位:博士(工学), 学位授与機関:東京工業大学, 報告番号:甲第12214号, 授与年月日:2022年9月22日, 学位の種別:課程博士, 審査員:小畠 英理,小倉 俊一郎,堤 浩,松田 知子,三重 正和
Citation(English)	Degree:Doctor (Engineering), Conferring organization: Tokyo Institute of Technology, Report number:甲第12214号, Conferred date:2022/9/22, Degree Type:Course doctor, Examiner:,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	要約
Type(English)	Outline

Doctoral Dissertation (Outline)

Construction of Multifunctional Temperature-responsive Protein Nanoparticles for Biomarker Detection

Gaoyang Wang

Supervised by Prof. Eiry Kobatake
Co-supervised by Prof. Masayasu Mie
Department of Life Science and Technology
Tokyo Institute of Technology

Qualitative and quantitative analysis of biomarkers is critical for early diagnosis and therapeutic prognosis of diseases. To selectively detect and quantify biomolecules of interest in complex biological samples, various nanoparticle-based biomolecular detection techniques have been developed. The main event in most nanoparticle-based assays is that the nanoparticles act as probes to bind to the specific marker of interest, thus providing specificity. Then, the multivalent effect of nanoparticles is used to act as a generator of the signal to enhance sensitivity. Elastin-like polypeptides (ELPs) based nanoparticles are protein-based biopolymers that not only have good biodegradability and biocompatibility, but also allow functional groups such as targeting ligands to be displayed homogeneously on their surface in a genetic and/or chemical adhesion manner, thus offering great potential for both *in vivo* and *in vitro* assays. The aim of this study is to develop ELP-based protein nanoparticles with recognition and bioluminescence by multifunctionalizing ELP to improve the sensitivity of detection and provide a possible approach for early diagnosis and prognostic monitoring of diseases.

The thesis consists of five chapters. The contents of each chapter are as follows.

The first chapter describes the background of the study. First, an overview of nanoparticles in assays is presented, followed by an introduction of the temperature-responsive properties of ELPs and the advantages of ELPs-based protein nanoparticles. Next, the ELPs protein nanoparticle-based detections are enumerated, as well as the advantages of ELPs protein nanoparticle-based detections. Finally, the purpose and significance of this study are presented.

In Chapter 2, to improve the sensitivity of detections based on the antigen-antibody recognition principle, new fusion proteins EDCN and EDNC were constructed using genetic engineering to fuse a Nanoluc luciferase (Nluc) derived from deep-sea shrimp and antibody-binding domain (C) derived from protein G into a fusion protein ED consisting of a hydrophobic block (PAVGV) of ELP and a polyaspartic acid (D) previously developed in our laboratory. The temperature responsiveness of ELP and the charge repulsion of aspartic acid were used to form core-shell nanoparticles with a hydrophobic core displaying antibody-binding domain and Nluc on the surface. The selective binding of IgG antibodies by antibody-binding domain was used to bind IgG antibodies to nanoparticle, and the selectivity and sensitivity of detection were evaluated. The results showed that the sensitivity of the EDCN and EDNC nanoparticle-based

detections were 10- and 13-fold higher than that of the conventional detections, respectively, indicating that this system has the potential to detect biomarkers with high sensitivity.

In Chapter 3, to achieve detection of biomarker cancer cells under more complex conditions, new fusion proteins were constructed by fusing Nluc and porcine circovirus type 2 replication initiation protein (Rep) into ELP-D, respectively. The nanoparticles were co-formed by heating above the inverse transition temperature. Rep was used to covalently bind DNA aptamer to confer recognition ability to the nanoparticles, and Nluc was used to confer signal amplification to the nanoparticles. Nanoparticles with different target binding abilities and luminescence were prepared by adjusting the ratio of Nluc and Rep, and their ability to detect tumour cells under complex conditions was evaluated. The results showed that these nanoparticle probes can recognition target tumor cells with high selectivity and detect them with high sensitivity and have the potential to detect biomarkers under complex conditions.

The capture, detection, recovery and release of tumor cells to downstream analysis is also an important step in the detection. It can provide useful information for studying tumorigenesis or developing personalized treatment regimens. Therefore, in Chapter 4, a new fusion protein EDNR was constructed by simultaneously fusing Nluc and Rep into ELP-D. Nanoparticles with bioluminescence, aptamer binding ability and biotinylation were co-formed by incubating EDNR with protein previously constructed in our laboratory which have the ability to be biotinylated because of its biotin acceptor peptides. The ability of protein nanoparticles to capture, detect, recover and release target tumor cells was verified by combining them with magnetic beads modified with streptavidin on the surface. The results show that selective recognition based on aptamer, signal amplification by Nluc, and biotin-streptavidin interactions resulted in nanoparticle-based detection with not only high selectivity and sensitivity, but also successful capture and release of cells with little or no damage.

Finally, Chapter 5 summarizes the results of this study and provides an outlook for future study.