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)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	生命理工学 系 コース	申請学位（専攻分野）： Academic Degree Requested	博士 （ 工 学 / Doctor of engineering ）
学生氏名： Student's Name	WANG Gaoyang	指導教員（主）： Academic Supervisor(main)	小畠英理
		指導教員（副）： Academic Supervisor(sub)	三重正和

要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

The detection of biomarkers is one of the best solutions to achieve a non-invasive approach to early diagnosis and management of patients. The unique properties of nanoparticles can enhance the selectivity and sensitivity of detection and quantification of biomolecules of interest in complex biological samples. Elastin-like polypeptides (ELPs) are synthetic biopolymers that possess not only the small size and large surface area-to-volume ratio of conventional nanoparticles, but also the unique properties of natural proteins, such as biodegradability and biocompatibility and high tolerance to functional molecules. The fusion protein ELP-D, previously developed in our laboratory based on the ELP hydrophobic block (42 repeats of the pentapeptide (PAVGV)) and polyaspartic acid (D) chain, can form controllable size, stable nanoparticles by responding to the inverse transition temperature (Tt) and shows a high tolerance to functional proteins on its surface. The high sensitivity of nanoparticle-based biomarker detection depends mainly on the binding efficiency between the probe and the target, and the ability to convert the target recognition event into a measurable signal. Therefore, this study aims to develop multifunctional nanoparticles as detection tools by improving these two aspects to provide a new possibility to break the limitations of bioassay and analysis.

In Chapter 2, an antibody-binding domain (C) derived from protein G and a small but stable and strongly luminescent NanoLuc luciferase (Nluc) were fused into ELP-D using genetic engineering to immobilize antibodies on nanoparticles to confer the nanoparticles antigen recognition ability and to amplify the detection signal, thus enabling highly sensitive detection of the target. After characterization of the constructed protein nanoparticles, their potential to be used as probes and signal converters in high-sensitivity detection systems was investigated by ELISA. The sensitivity of detecting human serum album (HSA) was evaluated using anti-HSA nanoparticle conjugates as probes. The results showed that the sensitivity of the detections based on the constructed nanoparticles could be improved by at least 10-fold over the sensitivity of the conventional single-molecule-based detection, suggesting that these multivalent Nluc and antibodies displayed nanoparticles have the potential to be applied in high-sensitivity detection systems.

In Chapter 3, "chemical antibody" aptamers, which can bind targets strongly but with no or little off-target reaction, are used to instead of conventional antibodies to confer recognition and binding ability to nanoparticles, thus enabling detection of biomarkers such as tumor cells under complex conditions. To bind the aptamers to the nanoparticles, a replication initiation protein (Rep) derived from porcine circovirus 2, which can covalently bind to the DNA aptamer, was genetically engineered into the C-terminus of ELP-D to construct a new fusion protein EDR. The nanoparticles were co-formed by heating above the inverse transition temperature with the fusion protein EDN, which was constructed by fusing Nluc into the C-terminus of ELP-D. The ratios of the EDR and EDN were adjusted to optimize the nanoparticles for optimal target recognition and signal amplification. When the aptamer-nanoparticle conjugates contact with targets, they are captured by Rep-mediated covalent conjugated aptamer on the nanoparticle, and the marker is then detected by bioluminescence derived from Nluc displayed on the same nanoparticle. Here, as a model, transmembrane glycoprotein mucin 1 (MUC1) aptamer displaying nanoparticles were constructed. The aptamer-nanoparticle conjugates could detect MUC1-positive cells, such as human breast cancer cells MCF-7, with high sensitivity in a complex environment (the mixture of MCF-7 cells and human embryonic kidney cells 293 (HEK293) with low expression of MUC1). Thus, the Nluc and aptamer displaying nanoparticles have the ability to detect targets in a complex environment.

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. New nanoparticles were co-formed by mixing EDNR with the

protein EDNB previously constructed in our laboratory that can be biotinylated due to the presence of biotin acceptor peptides. In this nanoparticle, aptamers are used to target and capture targets, Nluc is used for signal generation, and biotin is used to bind streptavidin magnetic beads so that the captured cells can be recovered by the beads, and finally, the cells were released by the disassembling of nanoparticles caused by cooling. The results of the detection using MCF-7 and HEK293 as models showed that MCF-7 cells could be recovered by magnetic beads mediated by nanoparticles, while HEK293 could not, and the capture and release efficiency were 70% and 81%, respectively. The above results indicate that the constructed multifunctional nanoparticles have the potential to be applied to capture, detect, recover, and release tumor cells in complex environments.

Therefore, multifunctional temperature-responsive protein nanoparticles with recognition and signal amplification were constructed by adding functional proteins to ELP-D. These nanoparticles achieved highly sensitive biomarker detection and tumor cell capture and release by adding functionality, which has the potential to be applied in clinical research.