

Development of protein nanoparticles displaying IgG binding domain and luciferase

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Immunoassays play an important role in clinical, pharmaceutical and scientific researches. Especially the enzyme-linked immunosorbent assay (ELISA) is widely used due to its low background, wide dynamic range, simple operation and high specificity. However, the sensitivity of ELISA still has the potential to be improved.

In our previous research, we developed a fusion protein composed of elastin-like polypeptide (ELP) and polyaspartic acid (D). ELP is an amphipathic temperature-responsive recombinant protein. By incubating over the phase transition temperature, it can self-assemble into spherical nanoparticles with a hydrophobic core. In this study, we developed a new fusion protein ELP-D-C-N, ELP-D fused with IgG binding protein (C) and bright blue light-emitting luciferase NanoLuc (N). Based on the fusion protein ELP-D-C-N formed nanoparticles, a highly sensitive immunodetection system has been developed. The nanoparticle displays IgG binding domain and NanoLuc multivalently on its surface. Compared with traditional reporter enzyme-labeled monovalent constructs, multivalent antibody constructs can react with the antigen through an excess of labeled antibodies, and convert trace amounts of antigen into antigen-antibody complexes that can be detected by the reporter molecule. The apparent binding affinity is increased from several times to several hundred times, thereby more directly and significantly improving the sensitivity of the assay. In addition, the bioluminescence produced by NanoLuc through interaction with the substrate is a chemical process that does not require irradiation, which may increase the background signal, so a luminescence-based detection system may achieve a more sensitive ELISA.

The results of the application of the ELP-D-C-N protein nanoparticle in the immunoassay system showed that it increased the LOD from 341.19 pg/mL in the monomer by 10-fold to 34.8 pg/mL.

