

## Development of Fluorescent Protein Nanoparticles (FPNP) for Aptamer-Based Assay

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We synthesized fluorescent protein nanoparticles (FPNP) through the bacterial expression of hybrid gene consisting of self-assembled protein nanoparticle (PNP), peptide spacer, and fluorescent protein [GFP or DsRed] genes. The self-assembly activity of PNP that leads to the formation of nanoparticles (12 nm in diameter) and the conformational flexibility of C-terminus of PNP enabled GFPs (or DsReds) to be well displayed on the surface of each protein nanoparticle, resulting in the construction of green (or red) FPNP [gFPNP (or rFPNP)]. Compared to the spacer-free FPNP, the peptide spacer between PNP and GFP (or red) significantly increased the fluorescence intensity of FPNP due presumably to the decreased quenching effect among GFPs (or red). The developed gFPNP was used for an aptamer-based assay of platelet-derived growth factor B-chain homodimer (PDGF-BB). Moreover, the DNA aptamers that were chemically conjugated to the mutated GFPs further enhanced the fluorescence emission of gFPNP, resulting from the reduced fluorescence quenching due to electrostatic repulsion between DNA aptamers. This is a simple two-step assay without involving any signal amplification steps: the detection limit is 100 fM of PDGF-BB.