Supporting Information

Universal Biotin-PEG-Linked Gold Nanoparticle Probes for the Simultaneous Detection of Nucleic Acids and Proteins

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Table S1: Oligonucleotide Sequence Information

Name	Oligonucleotide Sequence
miRNA-1	5'-GAUGGAUAUGUCUUCAAGGAC-Biotin-3'
miRNA-2	5'-CCUUGGAGAAAUAUGCGUCAA-Biotin-3'
miRNA-3	5'-UGAGCCUCUGUGGUAGCCCUCA-Biotin-3'
miRNA-4	5'-UUAGAUGACCAUCAACAAACU-Biotin-3'
Capture Oligonucleotide (Complement to miRNA-1)	5'-GTCCTTGAAGACATATCCATC-Spacer18-NH_2-3'
Non-Complementary DNA Capture Oligonucleotide (negative control)	5'-GTGAGCACCATGGAG-Spacer18-Spacer18-NH_2-3'

*Spacer18: Hexaethylene glycol (Glen Research)

Figure S1: Biotin-PEG AuNP Probe Stability

Monothiol, cyclic disulfide, and trithiol biotin-PEG linked AuNP probes were synthesized for detecting biotinylated target molecules. The colloidal stability of these probes toward free thiols was assayed using dithiothreitol (DTT) in solution. DTT can replace thiols that are bound on the gold surface and eventually cause the gold colloid solution to aggregate, turning a purple color from the original pink dispersed colloidal state[1]. This experiment enabled us to investigate the stability of the biotin-PEG-AuNP probes toward free thiols.

Experimental Procedure: To 200 μL of each biotin-PEG linked AuNP probe (8 nM concentration of monothiol, dithiol, and trithiol linked AuNP probes), 40 μL of 100 mM DTT solution was added to the AuNP probes, mixed thoroughly, and kept at 25°C. After 24 hours of incubation there was a color change from pink to purple in the monothiol linked probe solution, but the dithiol and trithiol AuNP probes remained pink (Figure S1 a-c). From this study we concluded that dithiol and trithiol linked probes have greater stability against thiol exchange compared to monothiol probes due to ligand displacement between DTT and monothiol-PEG-biotin linkers on the AuNP surface. This behavior is important in potential cases where free thiols may be present in solution during use of the bioassay.

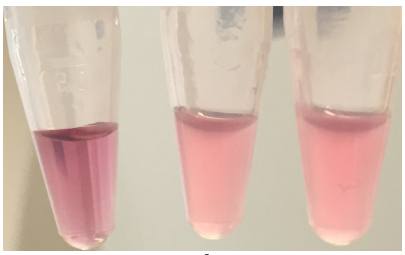

Figure S1. DTT AuNP probe colloidal stability study. (a) Monothiol-PEG-biotin AuNP (b) Dithiol-PEG-biotin AuNP (c) Trithiol-PEG-biotin AuNP

Figure S2: MicroRNA Detection Using 15 nm - 80 nm Biotin-PEG AuNP Probes

Detection of miRNA-1 using different-sized biotin-PEG-gold nanoparticle conjugates enables identification of biomolecule targets. Gold nanoparticles (100 pM) of varying diameters were functionalized with biotin-PEG-monothiol linkers and used to detect biotinylated miRNA-1. All sized AuNP probes detected the miRNA target with no detectable non-specific binding (Figure S2 a-g). Two experimental assay wells for each gold nanoparticle size are shown in Figure S2. Gold colloid (30 nm - 80 nm) was purchased from Ted Pella.

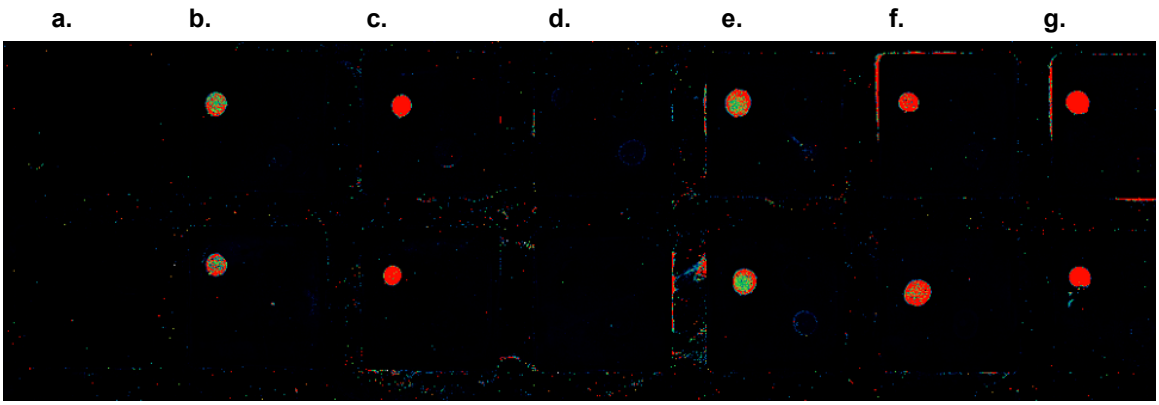

Figure S2. Detection of miRNA-1 using different sized biotin-PEG-AuNP probes. (a) Blank (no biotin-PEG-AuNP probe added) control well (b) d = 80 nm AuNP probe (c) d = 60 nm AuNP probe (d) Control (no miRNA target added) well (e) d = 50 nm AuNP probe (f) d = 30 nm AuNP probe (g) d = 15 nm AuNP probe

References

(1) Rosi, N. L., Giljohann, D. A., Thaxton, C. S., Lytton-Jean, A. K. R., Han, M. S., and Mirkin, C. A. (2006) Oligonucleotide-modified gold nanoparticles for intracellular gene regulation. *Science 312*, 1027-1030.