Gold Nanobeads with Enhanced Absorbance for Improved Sensitivity in Competitive Lateral Flow Immunoassays

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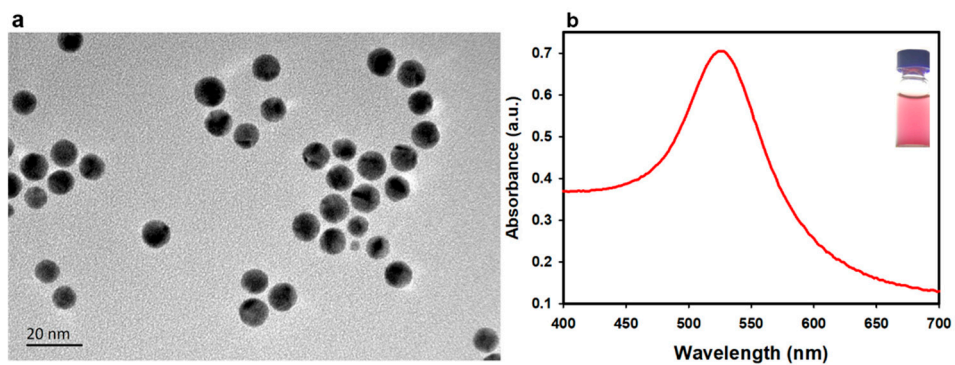
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Figure S1. Characterization of oleylamine-coated AuNPs. (a) TEM image. (b) UV-vis absorption spectrum.

Table S1. Synthesis conditions of different GNBs.

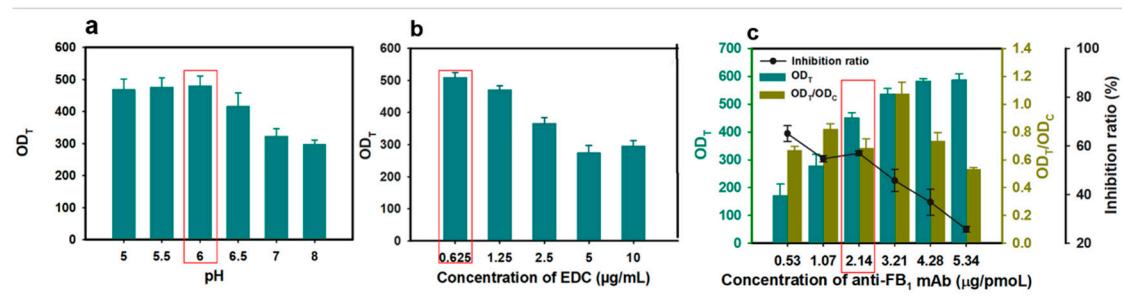| Size (nm)	Oleylamine-coated AuNPs (mg)	PMAO (mg)	SDS (mg)	Oil/water (µL/µL)	Ultrasonic power (960 W)
94 nm	10	2	6	50/500	8% (76.8 w)
129 nm	10	2	4	50/500	8% (76.8 w)
237 nm	10	2	2.5	20/500	8% (76.8 w)

Figure S2. Parameter optimization of GNB$_{94}$-LFIA for FB$_1$ detection. (a) Effect of pH for the conjugation of anti-FB$_1$ mAb to GNBs. (b) The EDC concentration for the anti-FB$_1$ mAb conjugation. (c) The optimal labeling amount of anti-FB$_1$ mAb on the GNB$_{94}$ surface.

Figure S3 Parameter optimization of GNB$_{129}$-LFIA for FB$_1$ detection. (a) Effect of pH for the conjugation of anti-FB$_1$ mAb to GNBs. (b) The EDC concentration for the anti-FB$_1$ mAb conjugation. (c) The optimal labeling amount of anti-FB$_1$ mAb on the GNB$_{129}$ surface.
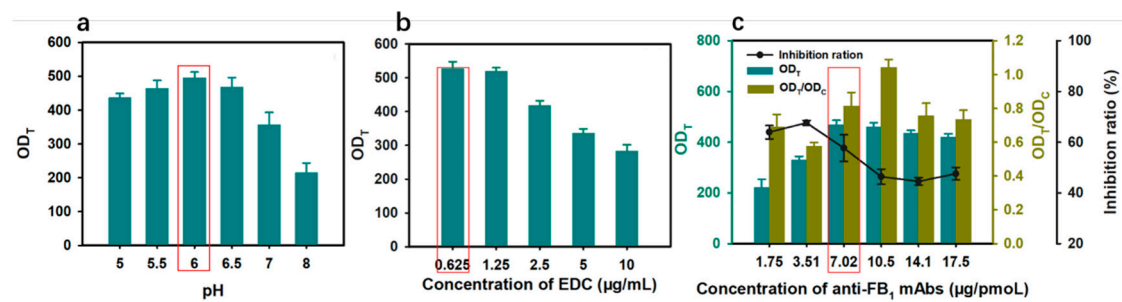
Figure S4 Parameter optimization of GNB_{237}-LFIA for FB_1 detection. (a) Effect of pH for the conjugation of anti-FB_1 mAb to GNBs. (b) The EDC concentration for the anti-FB_1 mAb conjugation. (c) The optimal labeling amount of anti-FB_1 mAb on the GNB_{237} surface.
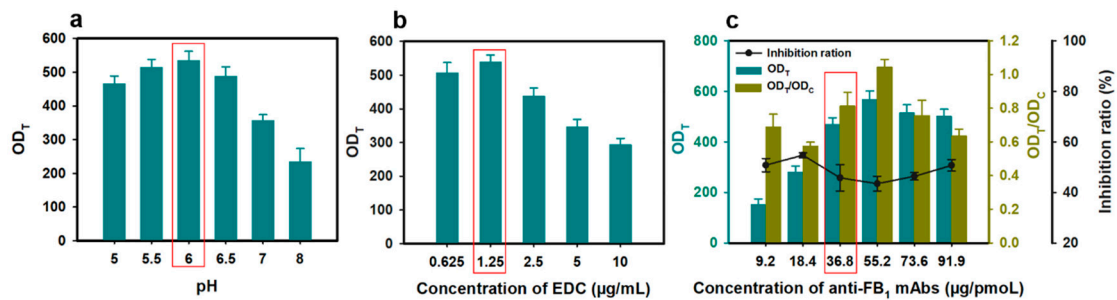
Table S2. Optimization of the parameters of the GNB$_{94}$-LFIA

No.	Concentration of FB$_1$-BSA (mg/mL)	Amount of GNB$_{94}$-probe (fmol)	Optical density of T-line (a.u)	Value of T/C	Inhibition rate (%)
1	1.33	1.32	843 ± 30	1.8 ± 0.3	19.4 ± 4.3
2	0.66	1.32	679 ± 23	1.5 ± 0.2	24.5± 3.3
3	0.33	1.32	554 ± 19	1.1 ± 0.2	36.8 ± 4.2
4	1.33	0.66	495 ± 24	1.6 ± 0.2	56.3 ± 5.3
5	0.66	0.66	356 ± 28	1.1 ± 0.3	49.3 ± 5.3
6	0.33	0.66	303 ± 10	0.9 ± 0.1	59.3± 5.8
7	1.33	0.33	298 ± 12	1.3 ± 0.1	56.4 ± 6.5
8	0.66	0.33	240 ± 14	1.0 ± 0.1	65.3 ± 10.3
9	0.33	0.33	197 ± 12	1.0 ± 0.1	46.3 ± 7.5

Note: The red mark indicates the optimal combination of conditions.

Table S3. Optimization of the parameters of the GNB$_{129}$-LFIA

No.	Concentration of FB-BSA (mg/mL)	Amount of GNB$_{129}$-probe (fmol)	Optical density of T-line (a.u)	Value of T/C	Inhibition rate (%)
1	0.33	0.17	369 ± 33	2.4 ± 0.2	79.8 ± 2.2
2	0.66	0.17	254 ± 11	1.7 ± 0.0	58.6 ± 7.9
3	1.33	0.17	210 ± 4	1.3 ± 0.2	56.5 ± 11.2
4	0.33	0.34	384 ± 42	2.1 ± 0.3	72.4 ± 3.1
5	0.66	0.34	444 ± 72	1.8 ± 0.2	68.3 ± 3.4
6	1.33	0.34	535 ± 21	1.1 ± 0.4	67.4 ± 6.3
7	0.33	0.68	522 ± 27	1.8 ± 0.1	54.5 ± 1.2
8	0.66	0.68	658 ± 28	1.4 ± 0.0	55.1 ± 0.5
9	1.33	0.68	801 ± 23	0.8 ± 0.0	59.3 ± 8.1

Note: The red mark indicates the optimal combination of conditions.

Table S4. Optimization of the parameters of the GNB_{237}-LFIA

No.	Concentration of FB_1-BSA (mg/mL)	Amount of GNB_{237}-probe (fmol)	Optical density of T-line (a.u)	Value of T/C	Inhibition rate (%)
1	1.33	0.36	743 ± 50	1.5 ± 0.1	25.7 ± 2.3
2	0.66	0.36	579 ± 54	1.6 ±0.1	32.2 ± 2.3
3	0.33	0.36	553 ± 13	1.3 ± 0.0	44.8 ± 3.3
4	1.33	0.18	518 ± 26	1.5 ± 0.1	62.6 ± 6.2
5	0.66	0.18	484 ± 37	1.5 ± 0.1	56.6 ± 5.2
6	0.33	0.18	422 ± 3	1.3 ± 0.1	65.7± 4.3
7	1.33	0.09	341 ± 11	1.9 ± 0.0	45.6 ± 5.3
8	0.66	0.09	333 ± 19	1.6 ± 0.1	37.6 ± 3.2
9	0.33	0.09	308 ± 12	1.3 ± 0.1	42.8 ± 5.3

Note: The red mark indicates the optimal combination of conditions.
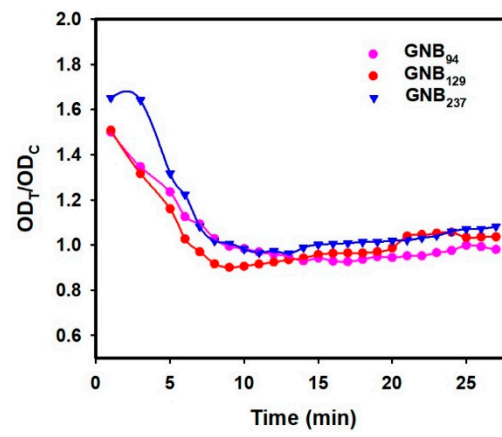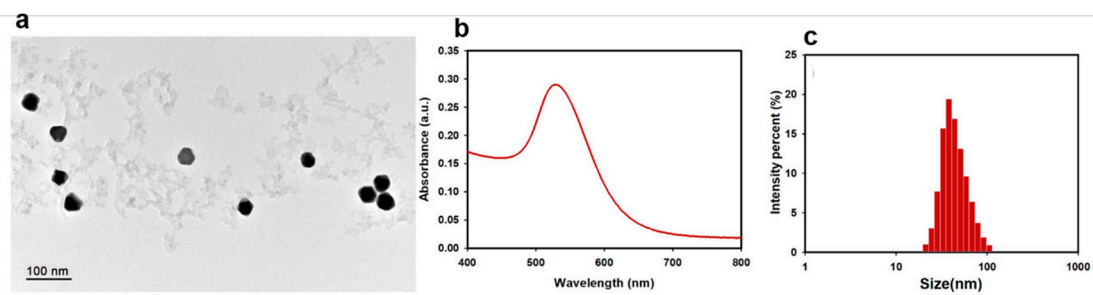
Figure S5 Immunoreaction dynamics of OD_T/OD_C at a FB_1 negative sample.

FigureS6 Characterization of traditional 40 nm AuNPs. (a) TEM images. (b) UV-Vis spectrum . (c) DLS analysis.
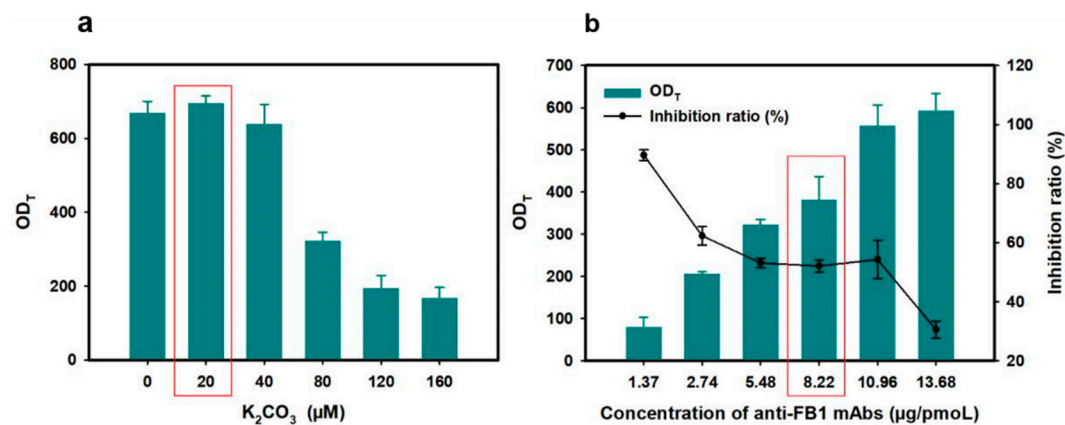
Figure S6. Parameter optimization of traditional AuNP$_{40}$-LFIA. (a) The K$_2$CO$_3$ concentration for the anti-FB$_1$ mAb conjugation. (b) The optimal labeling amount of anti-FB$_1$ mAb on the AuNP$_{40}$ surface.

Table S4. Optimization of the parameters of the AuNP$_{40}$-LFIA

No.	Concentration of FB$_1$-BSA (mg/mL)	Amount of AuNP$_{40}$-probe (fmol)	Optical density of T-line (a.u)	Value of OD$_T$/OD$_C$	Inhibition rate (%)
1	2.50	1.18	443 ± 23	2.6 ± 0.9	0.7 ± 0.0
2	2	1.18	353 ± 11	3.9 ± 1.0	0.7 ± 0.0
3	1.5	1.18	263 ± 8	3.8 ± 0.3	0.8 ± 0.0
4	2.50	2.36	754 ± 42	2.0 ± 0.3	0.5 ± 0.0
5	2	2.36	622 ± 72	1.9 ± 0.2	0.7 ± 0.0
6	1.5	2.36	541 ± 21	2.0 ± 0.34	0.7 ± 0.1
7	2.50	4.72	1091 ± 27	1.5 ± 0.0	0.6 ± 0.0
8	2	4.72	1006 ± 56	1.6 ± 0.0	0.6 ± 0.0
9	1.5	4.72	903 ± 92	1.6 ± 0.1	0.6 ± 0.0

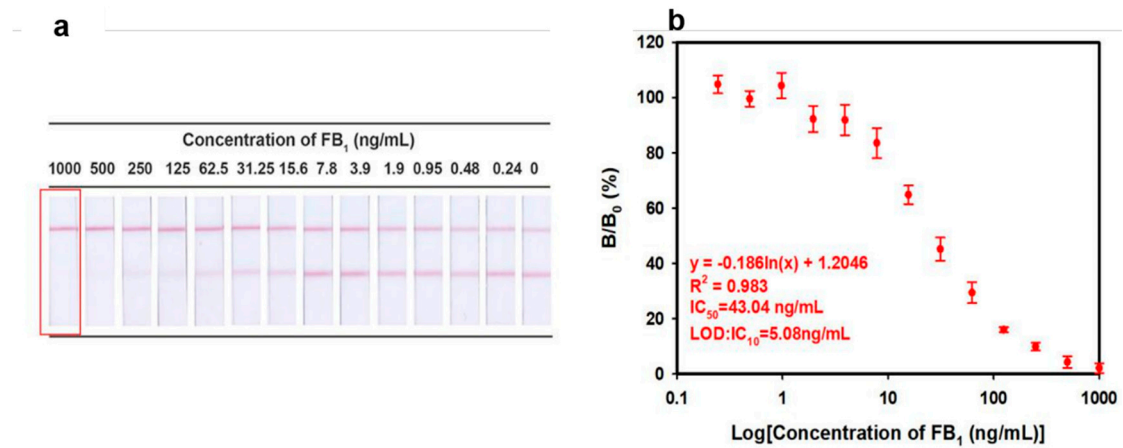**Note:** The red mark indicates the optimal combination of conditions.

Figure S7. Qualitative and quantitative assay for FB$_1$ in PBS buffer using traditional AuNP$_{40}$-LFIA. (a) The prototypes of AuNP$_{40}$-LFIA strips responding to varying FB$_1$ concentrations. (b) Linear dependences against FB$_1$ concentrations of four AuNP$_{40}$-LFIA strips.
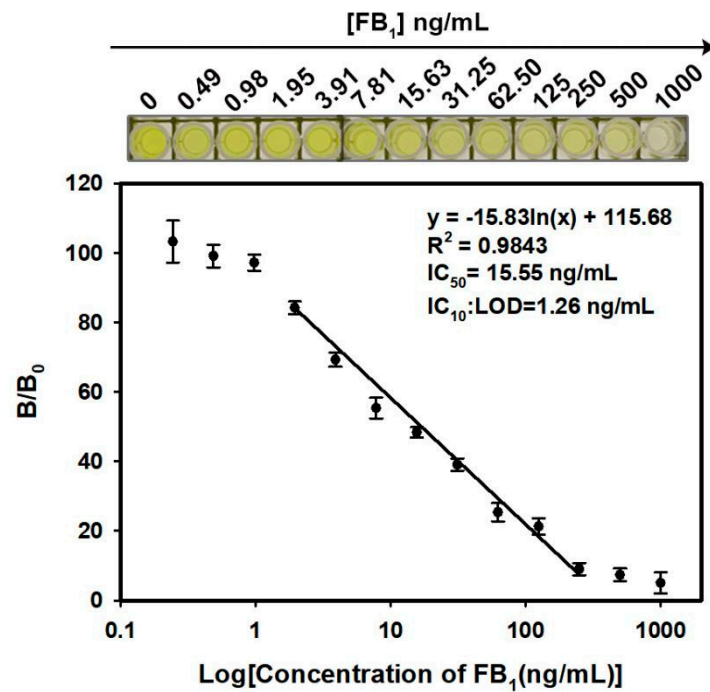
Figure S8. Quantitative assay of the conventional HRP-based ELISA for FB$_1$ detection.

Table S5. A comparison of our proposed GNB$_{129}$-LFIA with other reported LFIA methods for the determination of FB$_1$.

Label	Signal output	Linear range	Detection limit	Ref
Gold or silver nanoparticles and CdSe/ZnS quantum dots	Fluorescence	NA	1.56-6.25 ng/mL[a]	[1]
AuNPs	Colorimetry	NA	5 ng/mL[a]	[2]
AuNPs	Colorimetry	2-40 ng/mL	2 ng/mL[a]	[3]
AuNPs	Colorimetry	NA	3200 μg/kg [a]	[4]
AuNPs	Colorimetry	NA	50 ng/mL[b]	[5]
AuNPs	Colorimetry	NA	1000 μg/kg[b]	[6]
AuNPs	Colorimetry	4-80 ng/mL	3.72 ng/mL[a]	[7]
Urchin-like AuNPs	Colorimetry	NA	5 ng/mL[b]	[8]
GNBs	Colorimetry	3.9-125 ng/mL	1.79 ng/mL[a]	This work

Note: 'NA' indicates the "Not available". 'a' indicates "IC$_{10}$", and 'b' indicates "cutoff limit".

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