Supporting Information

Less is More: A Comparison of Antibody–Gold Nanoparticle Conjugates of Different Ratios

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Table S1. The optimal operating parameters of ICP-MS measurements carried out with a quadrupole ICP-MS instrument Aurora M90 (Bruker Corp., USA)

Operating parameter	Value
Nebulizer gas flow	1.00 L/min
Auxiliary gas flow	2.00 L/min
Plasma gas flow	16.5 L/min
ICP RF power	1.40 kW
Scans/Replicates	30
Replicates/Sample	5
Replicate time	9.79 sec
Dwell time	50 ms
Stabilization delay	60 sec
Ion optics	Auto-optimized
Scanning mode	Peek hoping
Detector	Digital ETP AF250

Table S2. Primary data of ICP-MS measurements carried out with a quadrupole ICP-MS instrument Aurora M90 (Bruker Corp., USA)

	Parameters		
	OD_{520}	Au concentration, mg mL^{-1}	GNP concentration, nM
GNP	0.99	0.05	0.52
GNP with BSA	14.14	0.66	6.75

MAb-GNP conjugate without BSA	Parameters		
	OD_{520}	Au concentration, mg mL^{-1}	GNP concentration, nM
MAb (0.1)-GNP	1.02	0.06	0.58
MAb (0.3)-GNP	1.04	0.05	0.53
MAb (0.5)-GNP	1.06	0.05	0.55
MAb (1.5)-GNP	1.10	0.05	0.52
MAb (3)-GNP	1.13	0.06	0.58
MAb (5)-GNP	1.14	0.06	0.57
MAb (10)-GNP	1.15	0.05	0.49
MAb (19)-GNP	1.15	0.05	0.48

MAb-GNP conjugate with BSA	Parameters		
	OD_{520}	Au concentration, mg mL^{-1}	GNP concentration, nM
MAb (0.1)-GNP	12.88	0.68	6.93
MAb (0.3)-GNP	14.36	0.72	7.35
MAb (0.5)-GNP	14.53	0.72	7.32
MAb (1.5)-GNP	14.55	0.75	7.65
MAb (3)-GNP	13.94	0.67	6.82
MAb (5)-GNP	14.27	0.75	7.61
MAb (10)-GNP	14.28	0.72	7.29
MAb (19)-GNP	13.79	0.67	6.78

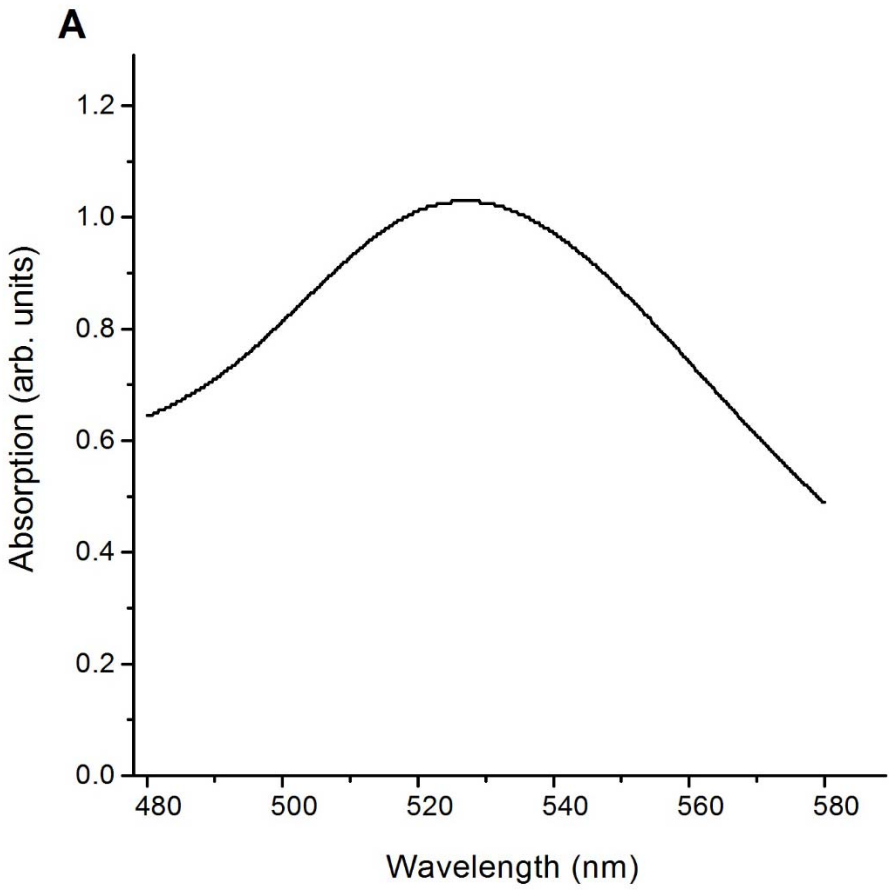
PAb-GNP conjugate without BSA	Parameters		
	OD_{520}	Au concentration, mg mL^{-1}	GNP concentration, nM
PAb (0.1)-GNP	1.02	0.06	0.56
PAb (0.3)-GNP	1.01	0.05	0.56
PAb (0.5)-GNP	1.04	0.05	0.50
PAb (1)-GNP	1.07	0.05	0.55
PAb (2)-GNP	1.09	0.05	0.50
PAb (3)-GNP	1.10	0.05	0.49
PAb (4)-GNP	1.10	0.05	0.47
PAb (6)-GNP	1.13	0.04	0.39

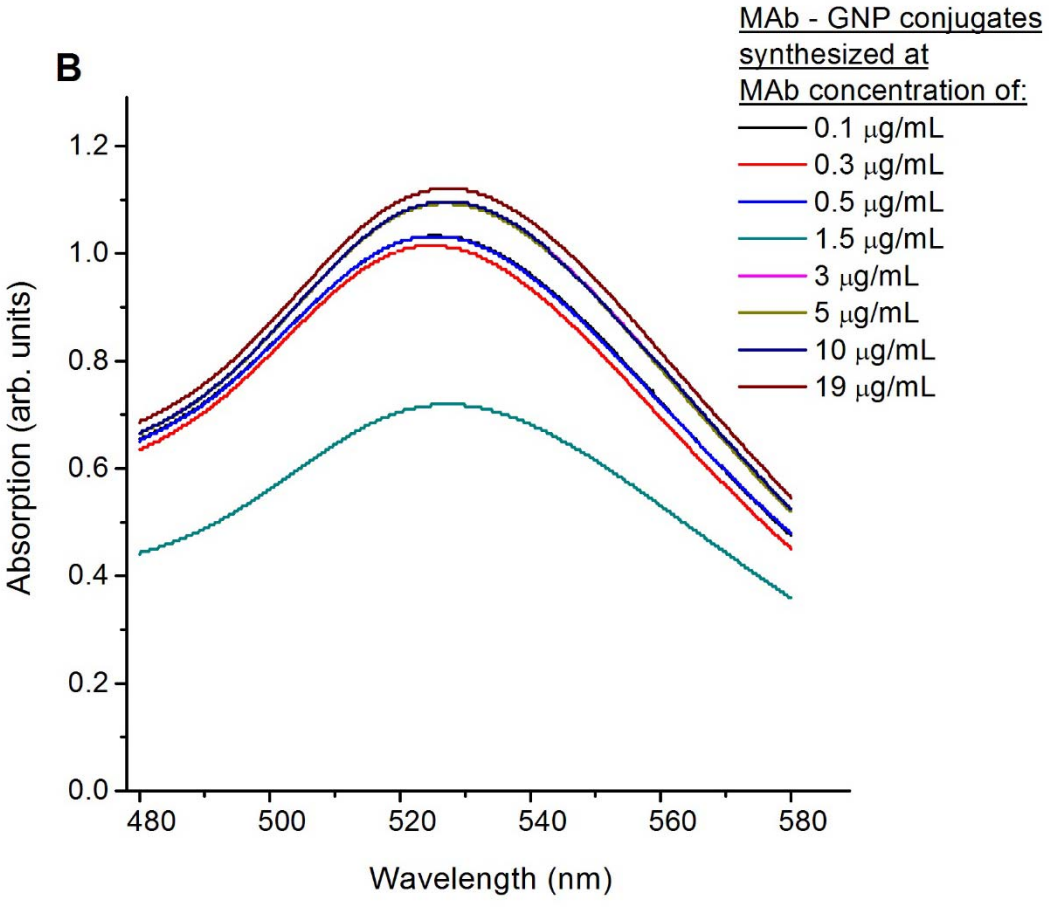
PAb-GNP conjugate with BSA	Parameters		
	OD_{520}	Au concentration, mg mL^{-1}	GNP concentration, nM
PAb (0.1)-GNP	14.75	0.77	7.81
PAb (0.3)-GNP	15.09	0.82	8.31
PAb (0.5)-GNP	14.52	0.79	8.07
PAb (1)-GNP	14.75	0.82	8.38
PAb (2)-GNP	14.05	0.81	8.20
PAb (3)-GNP	13.57	0.74	7.51
PAb (4)-GNP	15.00	0.80	8.17
PAb (6)-GNP	14.06	0.75	7.62

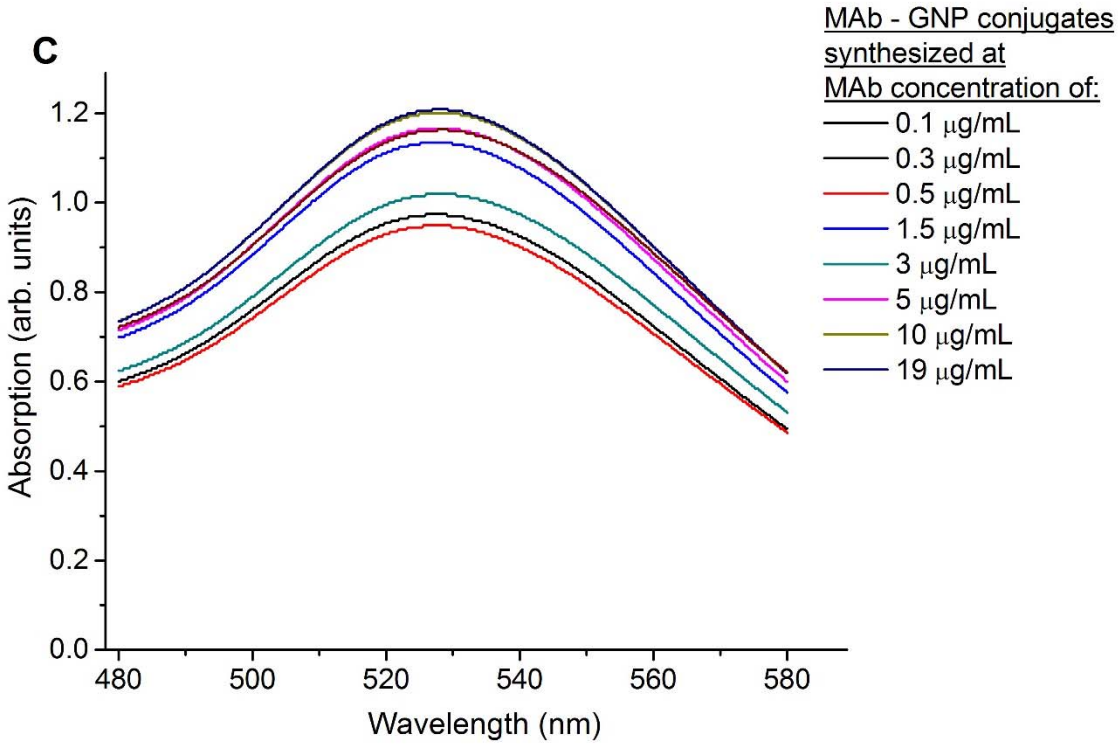
Table S3. Effect of concentration on limit of detection (LOD) of lateral flow immunoassay (LFIA) for different protein markers

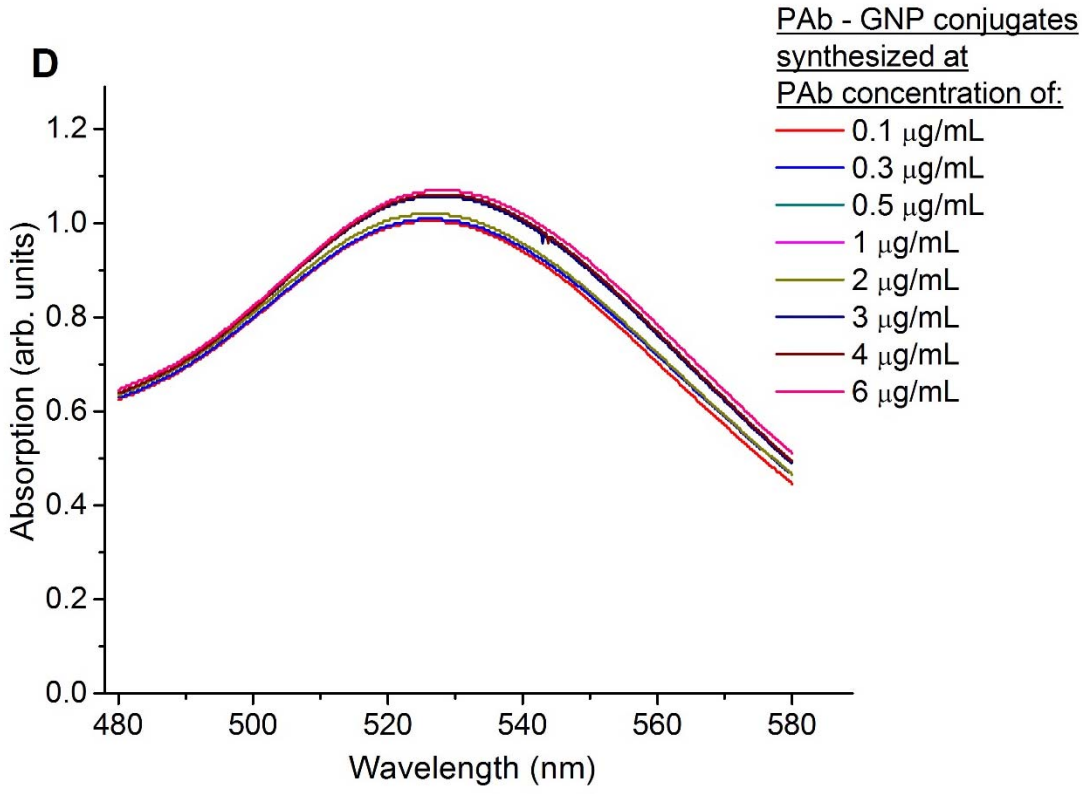
#	Antibody* specific to	Antibody concentration for conjugates' syntheses (μg mL^{-1})		LOD of LFIA using conjugate synthesized at different antibody's concentration (ng mL^{-1})	
		at the plateau of the flocculation curve (equal to the stabilizing concentration)	at the peak of the flocculation curve (less than the stabilizing concentration)	at the plateau of the flocculation curve	at the peak of the flocculation curve
1	Fatty acid binding protein	6	2	4.0	4.0
2	Troponin I	16	4	1.0	1.0
3	Myoglobin	8	3	3.0	3.0
4	C-reactive protein	14	4	600	600
5	D-dimer	8	3	100	100

*All antibodies are mouse monoclonal (Bialexa; Moscow, Russia, www.bialexa.ru)


A
Absorption (arb. units)
Wavelength (nm)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
480
500
520
540
560
580


B
Absorption (arb. units)
Wavelength (nm)
MAb - GNP conjugates synthesized at MAb concentration of:
0.1 μg/mL
0.3 μg/mL
0.5 μg/mL
1.5 μg/mL
3 μg/mL
5 μg/mL
10 μg/mL
19 μg/mL


C
MAb - GNP conjugates synthesized at MAb concentration of:
0.1 μg/mL
0.3 μg/mL
0.5 μg/mL
1.5 μg/mL
3 μg/mL
5 μg/mL
10 μg/mL
19 μg/mL
Absorption (arb. units)
Wavelength (nm)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
480
500
520
540
560
580


D
PAb - GNP conjugates synthesized at PAb concentration of:
0.1 μg/mL
0.3 μg/mL
0.5 μg/mL
1 μg/mL
2 μg/mL
3 μg/mL
4 μg/mL
6 μg/mL
Absorption (arb. units)
Wavelength (nm)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
480
500
520
540
560
580

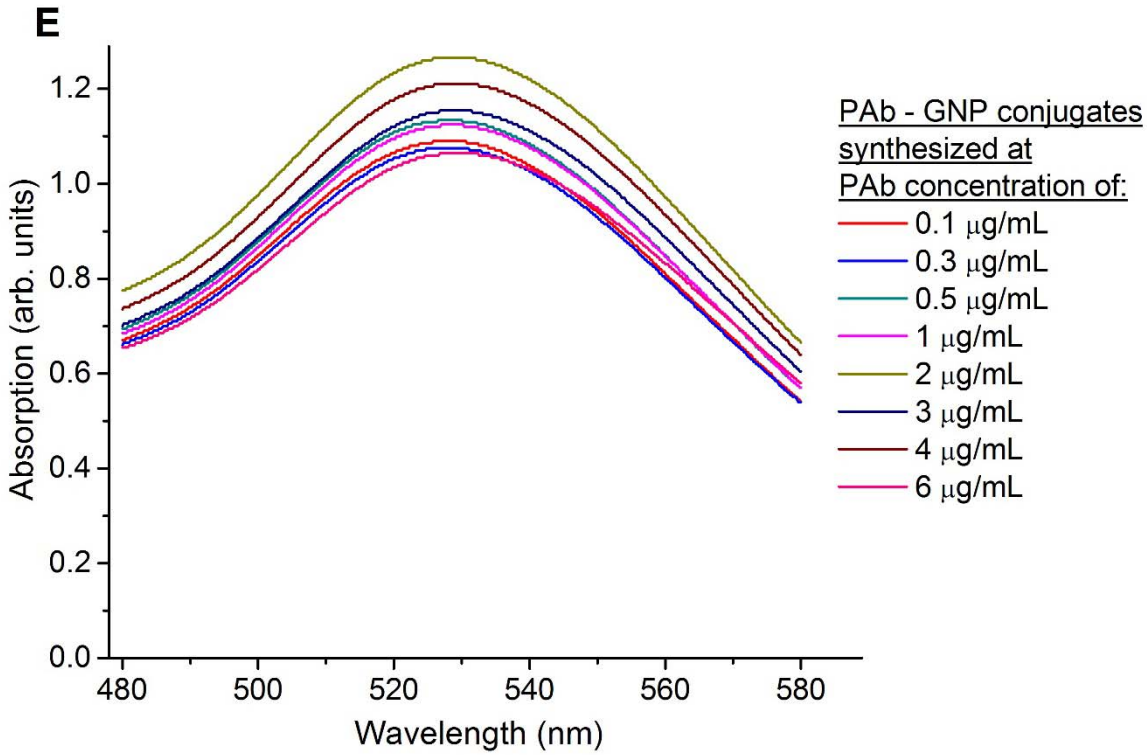


Figure S1. Absorption spectra of GNPs (A), MAb-GNP conjugates without BSA (B), MAb-GNP conjugates with 0.25% BSA (C), PAb-GNP conjugates without BSA (D), and PAb-GNP conjugates with 0.25% BSA (E)

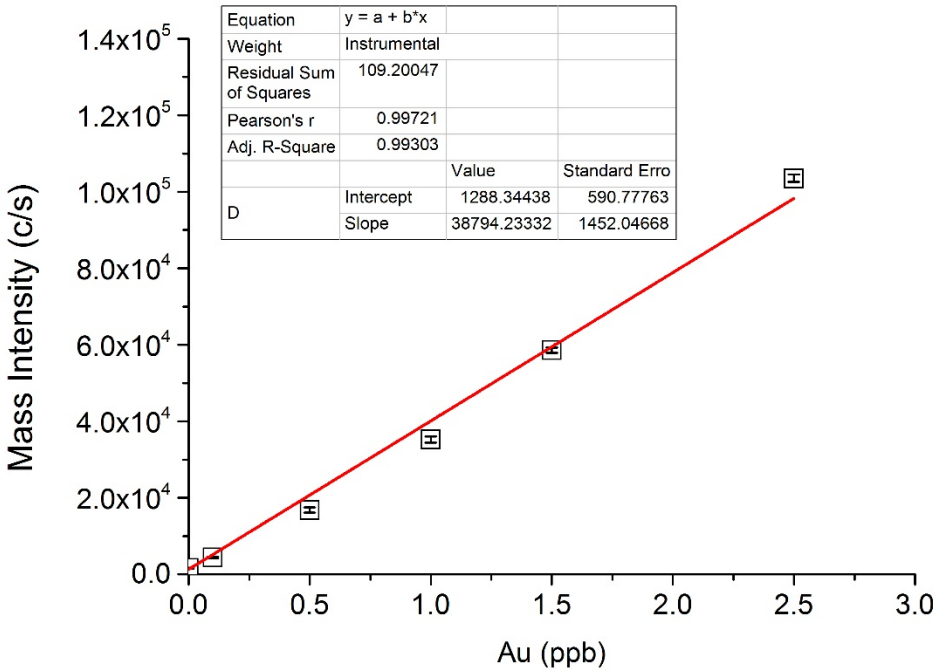


Figure S2. Calibration curve of Au concentration. ICP-MS measurements carried out with a quadrupole ICP-MS instrument Aurora M90 (Bruker Corp., USA)

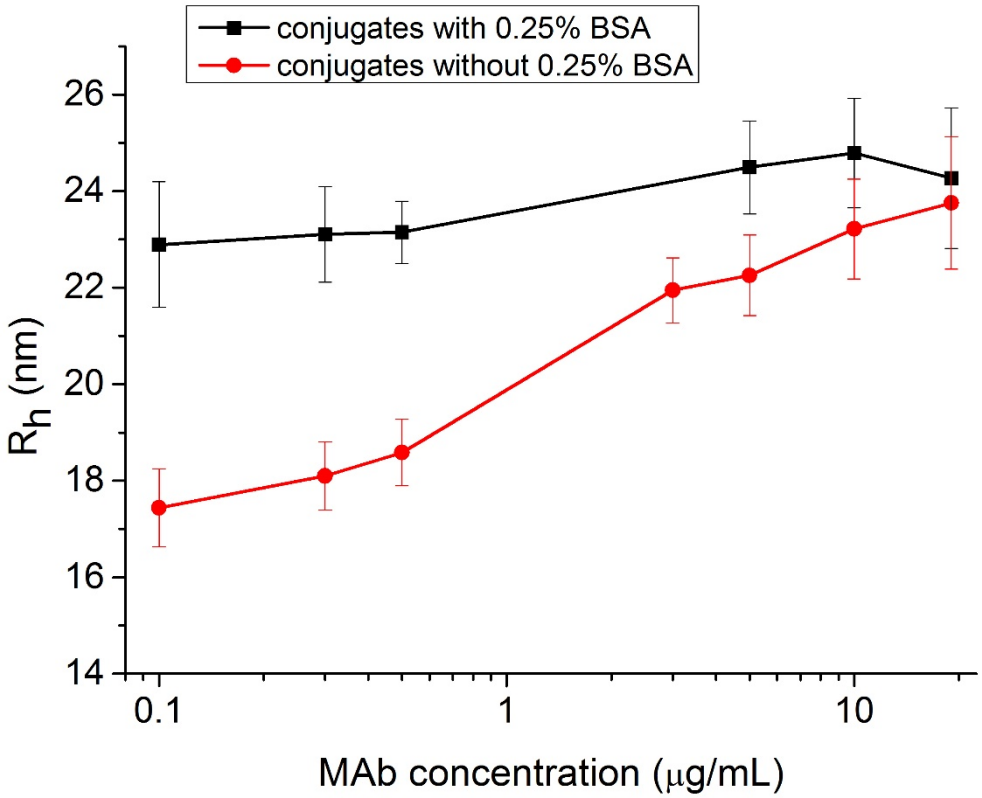
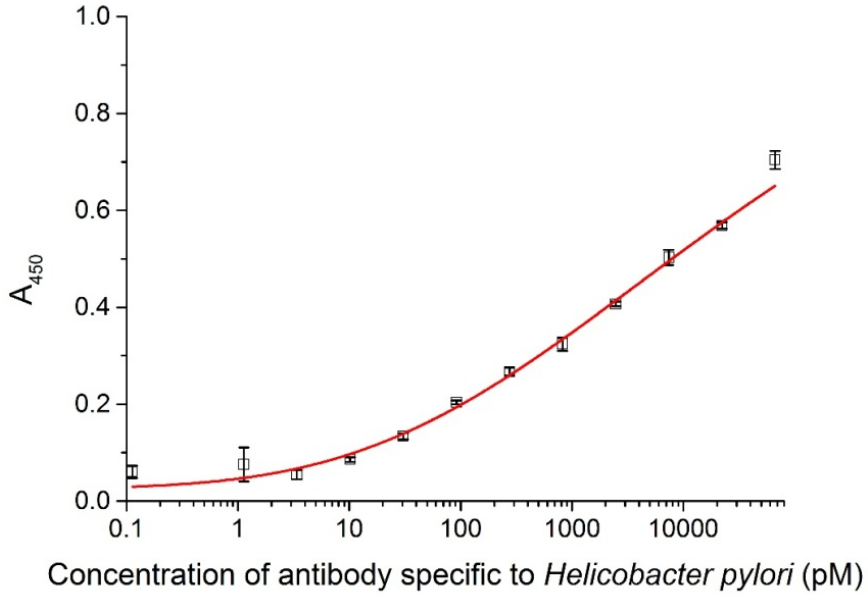


Figure S3. Plots of the hydrodynamic radius of the MAb-GNP vs. the concentration of the MAb used for the conjugation

A



B

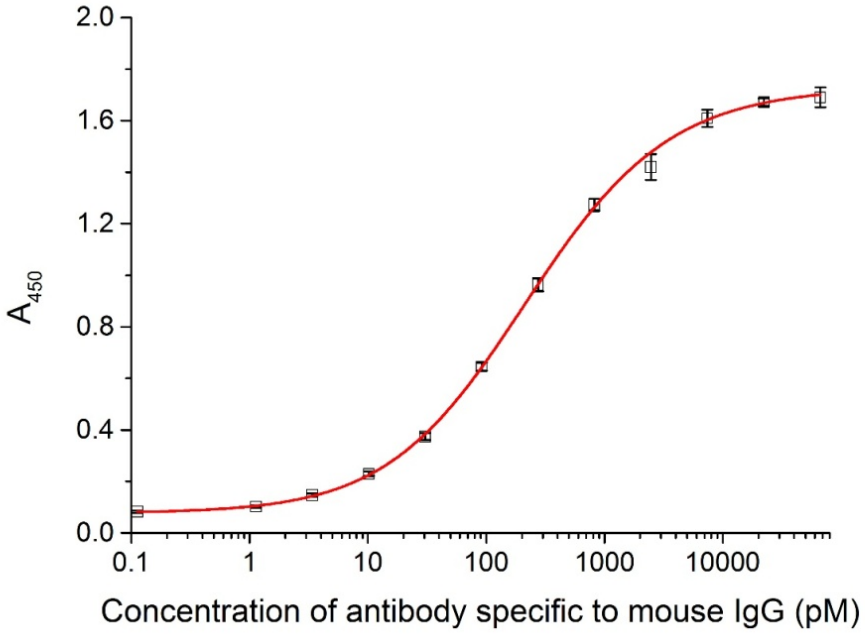
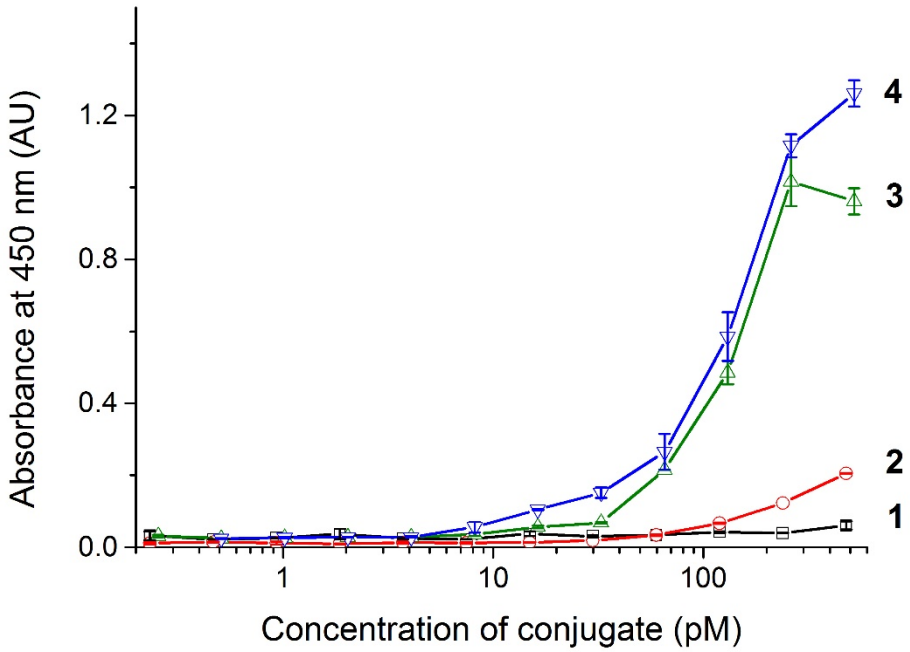
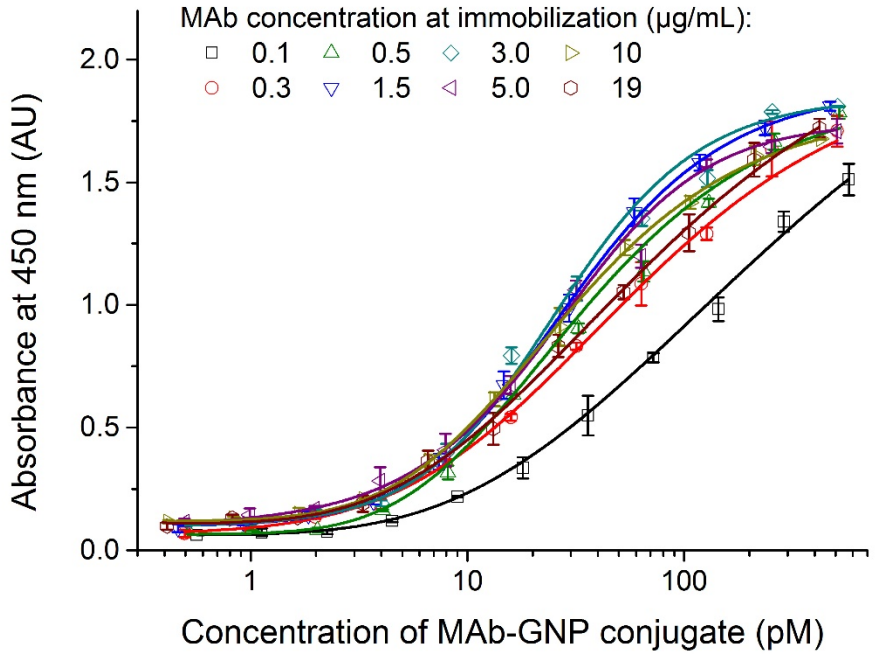


Figure S4. Concentration dependence for antigen-capturing activity of the MAb (A) and PAb (B)

A


Absorbance at 450 nm (AU)
0.0
0.4
0.8
1.2
1
10
100
Concentration of conjugate (pM)
4
3
2
1

B


MAb concentration at immobilization (µg/mL):
0.1
0.5
3.0
10
0.3
1.5
5.0
19
Absorbance at 450 nm (AU)
0.0
0.5
1.0
1.5
2.0
1
10
100
Concentration of MAb-GNP conjugate (pM)

C

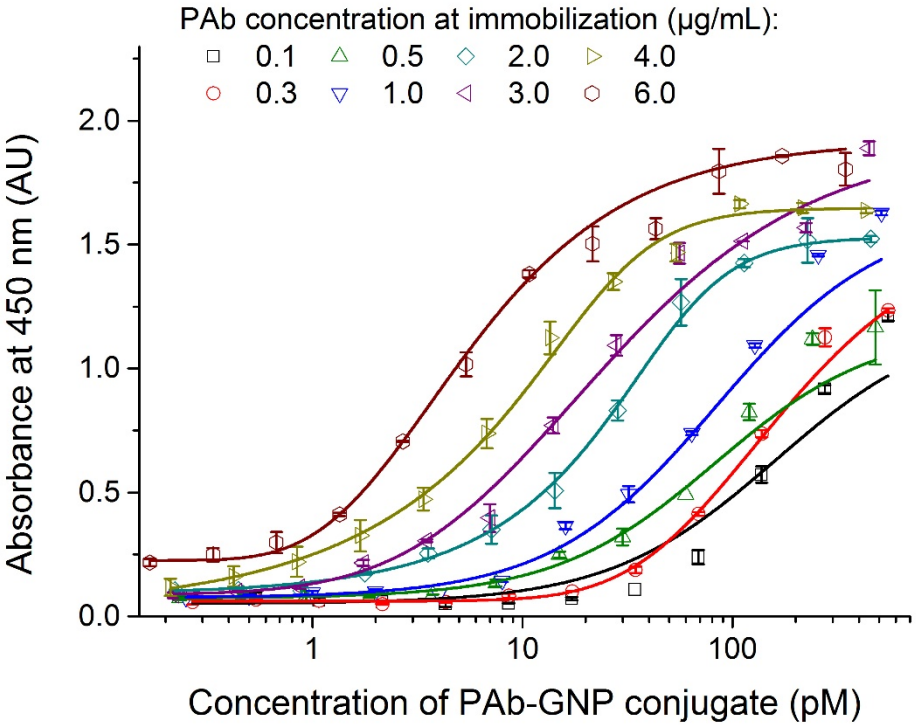
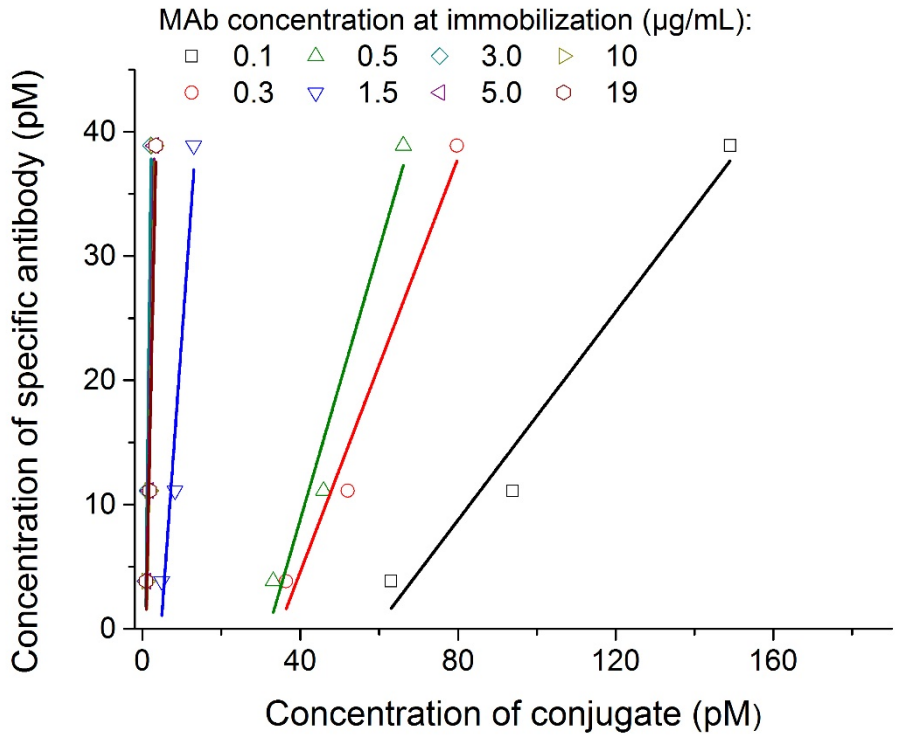


Figure S5. Dependencies of non-specific bindings of conjugates with 0.25% of BSA (1-2)) and without 0.25% of BSA (3-4) (A); dependencies of bindings of MAb-GNP (B); and PAb-GNP (C)

A



B

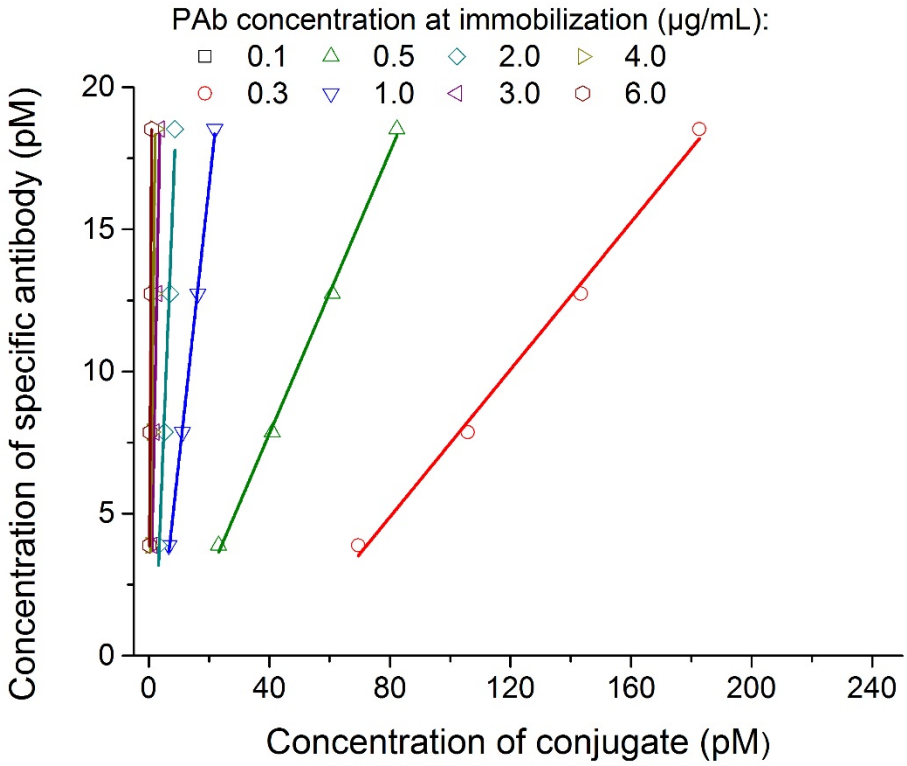
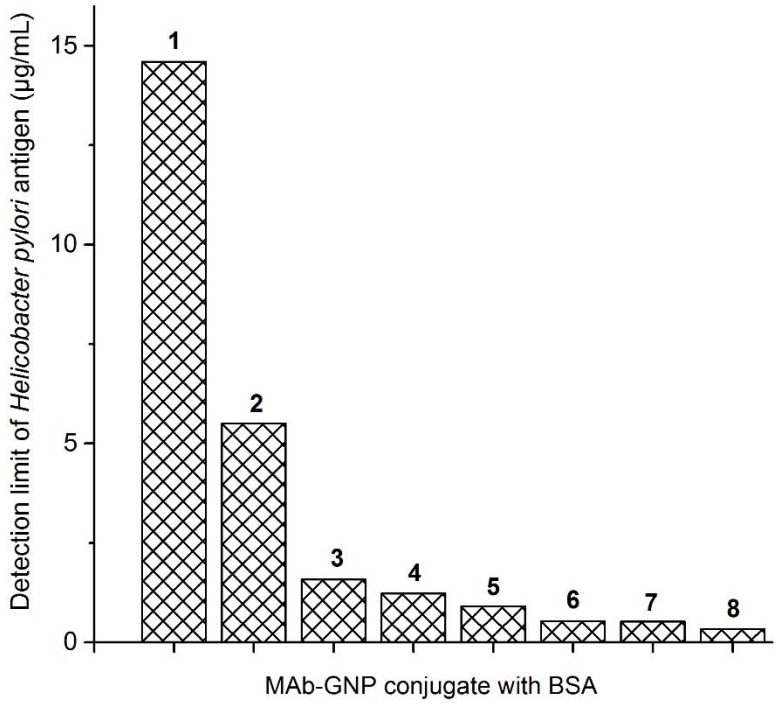


Figure S6. Dependencies of the antibody concentration on the MAb-GNP (A) and PAb-GNP (B) concentrations

A



B

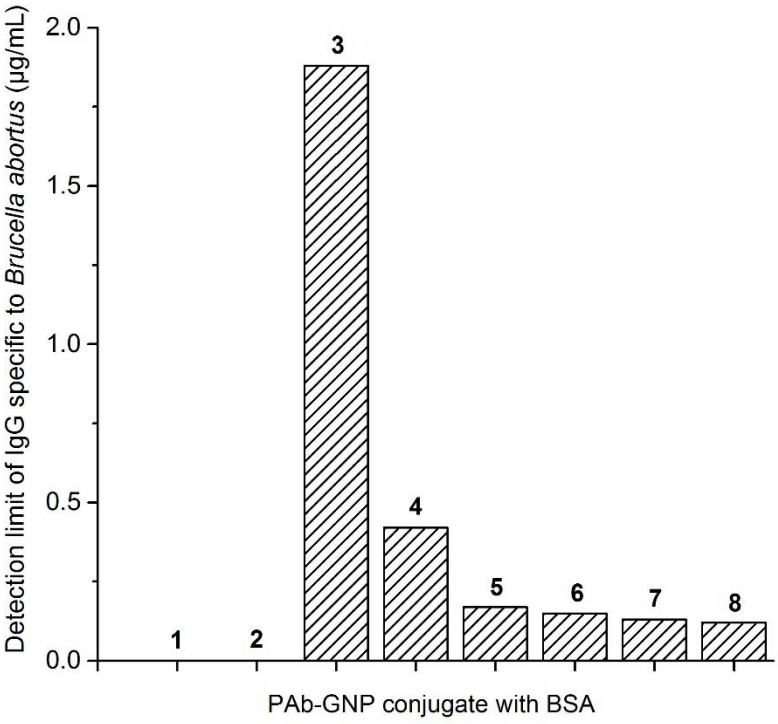


Figure S7. Dependence of the detection limit on the composition of the conjugate:

(A) For *H. pylori* and MAb-GNP conjugates; 1-8 corresponds to the MAb concentrations used for the conjugate synthesis: 0.1, 0.3, 0.5, 1.5, 3, 5, 10, and 19 µg mL^{-1};

(B) For IgG specific to *B. abortus* and PAb-GNP conjugates; 1-8 corresponds to the PAb concentrations used for the conjugate synthesis: 0.1, 0.3, 0.5, 1, 2, 3, 4, and 6 µg mL^{-1}