

Supplementary materials

Network of gold conjugates for enhanced sensitive immunochromatographic assays of troponins

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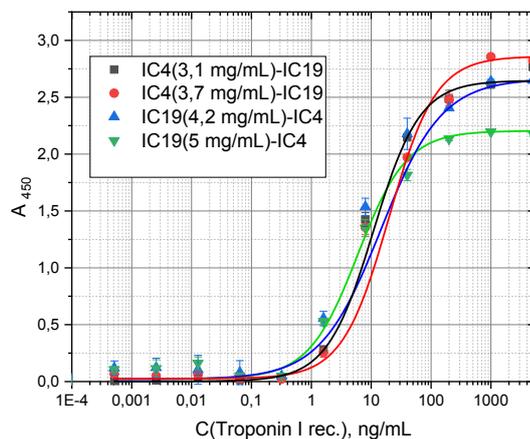
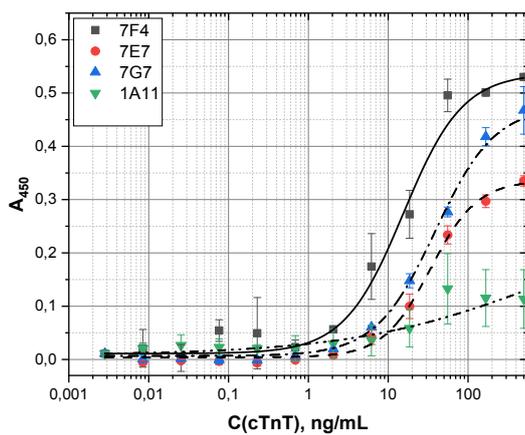


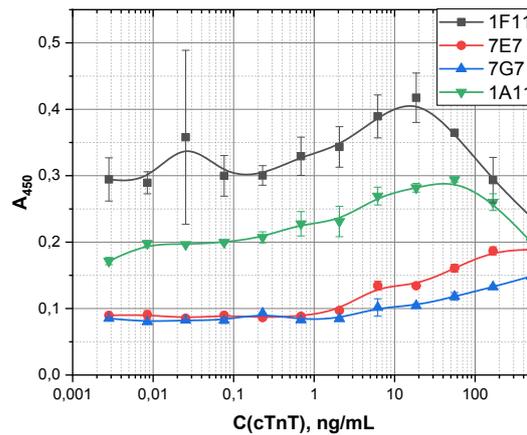
Fig. S1. ELISA for cTnI

Table S1. ELISA data for cTnI

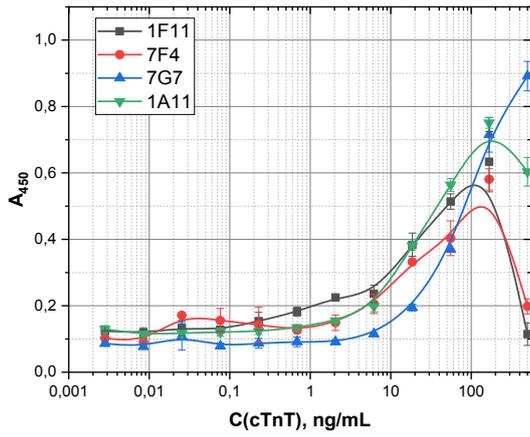
Combinations	IC10, ng/mL	IC20, ng/mL
IC4 (3.1)–IC19	1.52	3.15
IC4 (3.7)–IC19	2.50	5.43
IC19 (4.2)–IC4	1.01	2.73
IC19 (5.0)–IC4	0.66	1.50



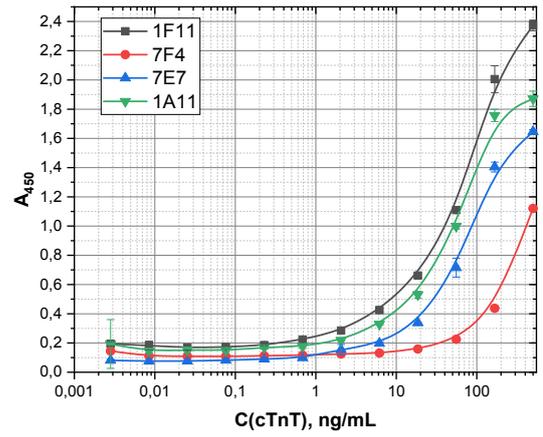
a)



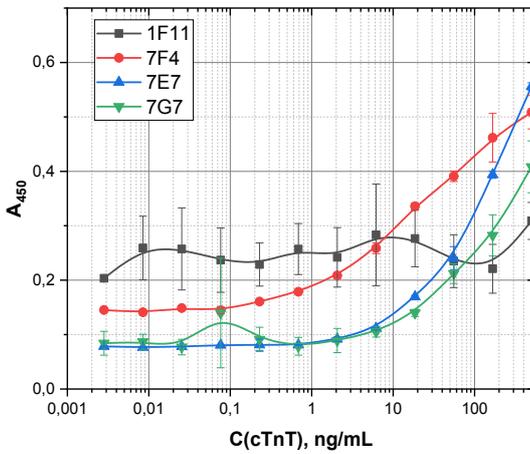
b)



c)



d)



f)

Fig. S2. ELISA for cTnT: a) 1F11cc immobilised; b) 7F4 immobilised; c) 7E7 immobilised; d) 7G7 immobilised; f) 1A11 immobilised

Table S2. ELISA data for cTnT

Immobilised	Abs-biotin				
	1F11	7F4	7E7	7G7	1A11
1F11		IC10 1.88 IC20 4.2 ΔA 0.53365	IC10 6.9 IC20 12.5 ΔA 0.34187	IC10 5.1 IC20 10.9 ΔA 0.43598	IC10 0.6 IC20 9.8 ΔA 0.15237
7F4	IC10 - IC20 - ΔA 0.04943		IC10 2.95 IC20 5.98 ΔA 0.08836	IC10 10.8 IC20 24.9 ΔA 0.06202	IC10 - IC20 - ΔA 0.0339
7E7	IC10 0.82 IC20 2.43 ΔA 0.57266	IC10 3.1 IC20 6.4 ΔA 0.50067		IC10 18 IC20 35.4 ΔA 0.77634	IC10 5.8 IC20 10 ΔA 0.49697
7G7	IC10 1.7 IC20 8.7 ΔA 2.19357	IC10 171 IC20 319 ΔA 0.99508	IC10 12.7 IC20 25.1 ΔA 1.55846		IC10 11.7 IC20 21.4 ΔA 1.83289
1A11	IC10 - IC20 - ΔA 0.13284	IC10 0.83 IC20 2.4 ΔA 0.38482	IC10 14 IC20 36.6 ΔA 0.48243	IC10 18 IC20 27.9 ΔA 0.30601	

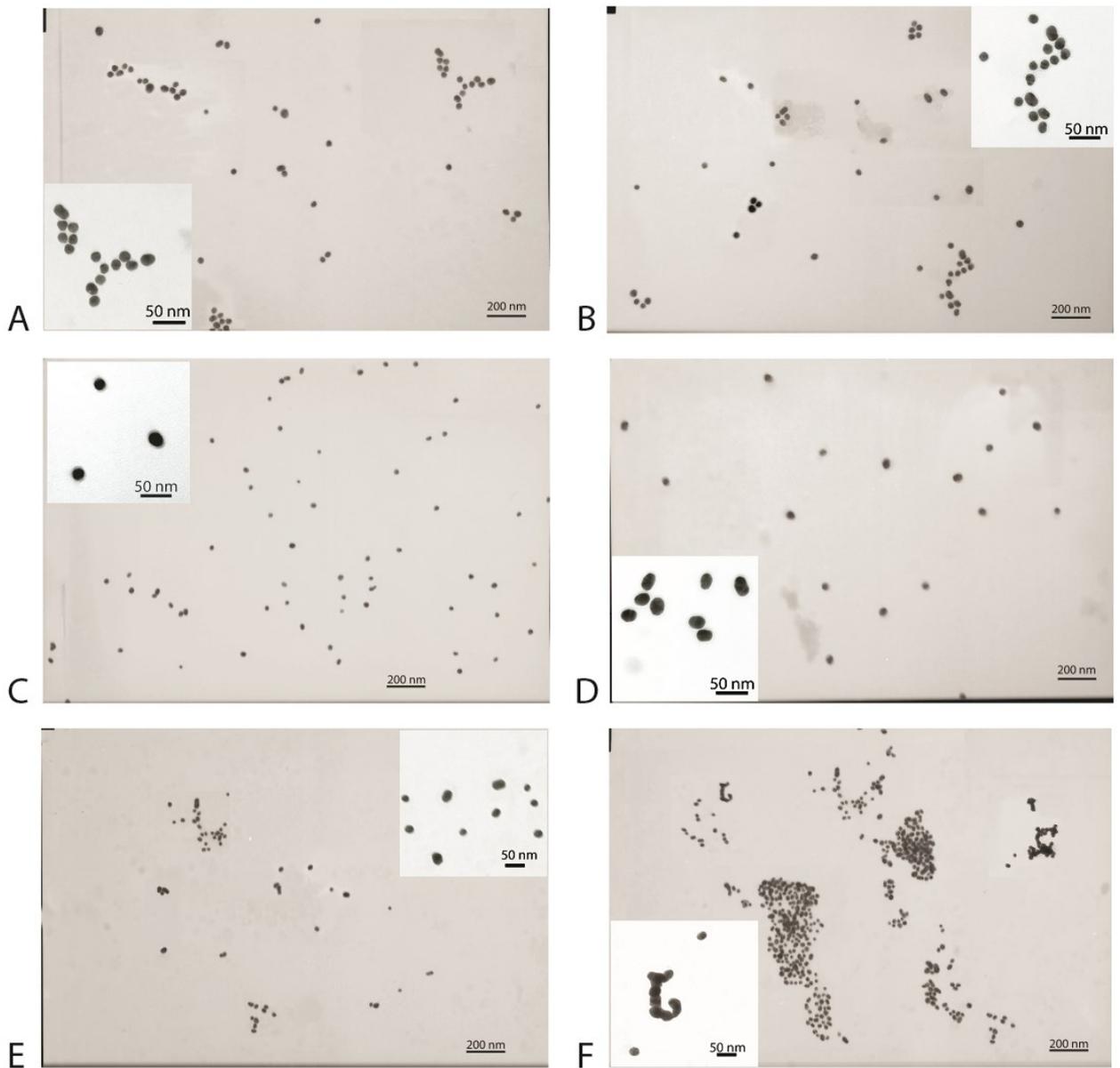


Figure S3. TEM data for native GNPs and its conjugates for cTnT detection (as example): (A) GNPs 29.3 ± 0.9 nm; (B) GNP-Abs/cTnT 31.5 ± 0.5 nm; (C) GNP-Stp 32.1 ± 0.8 nm; (D) GNP-BSA-biotin 29.8 ± 0.8 nm; (E) GNP-Abs/cTnT-biotin 32.7 ± 3.8 ; (F) GNP-Stp : GNP-BSA-biotin : GNP-Abs/cTnT-biotin 32.2 ± 10.7 nm

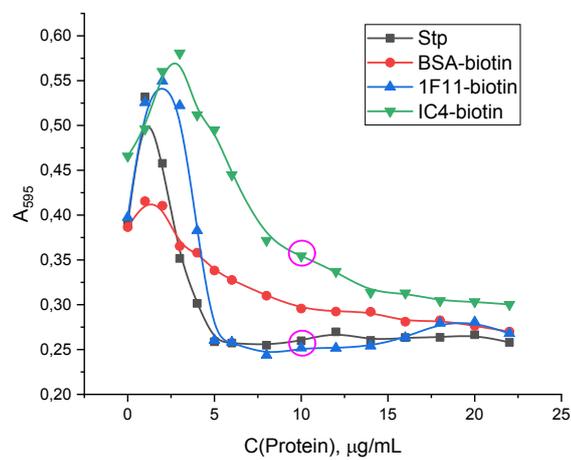


Fig. S4. Absorption curves for proteins

Table S3. Effect of optical density on detecting GNP-Abs conjugates on vLODs

Analyte	A(GNP–Abs)			
	LoD, ng/mL			
	1	2	4	5
cTnI	100	100	33.3	11.1
cTnT	33.3	11.1	11.1	11.1

Table S4. Influence of the concentration of antibodies immobilised in the analytical zone on vLoDs

Analyte	C(Abs immobilised), mg/mL		
	LoD, ng/mL		
	1	1.5	2
cTnI	100	33.3	11.1

Table S5. Influence of the concentration of detergent Tween-20 in the analytical zone on LoDs

Analyte	Tween 20, %			
	LoD, ng/mL			
	0.1	0.25	1.0	2.0
cTnT	100	33.3	11.1	33.3

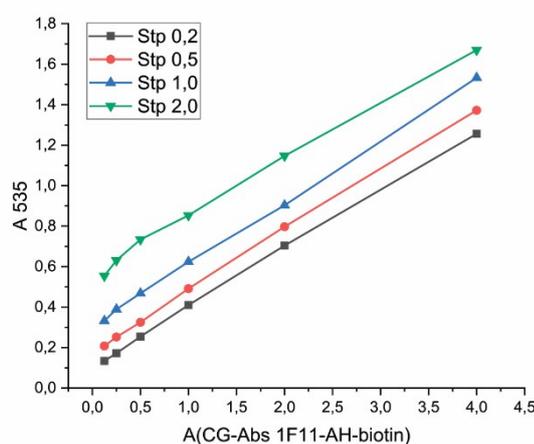


Fig. S5. Dependences of the optical density of a mixture of GNPs–Stp and GNPs–Abs/cTnT conjugates on the concentration of GNPs–Abs–biotin

Table S6. Theoretical calculations to determine the minimum and maximum amount of CG–Stp conjugate for complete binding of all available biotin groups

Parameter	Minimum amount of GNP–Stp conjugate		Maximum amount of CG–Stp conjugate	
	GNP–BSA–biotin	GNP–Abs–biotin	GNP–BSA–biotin	GNP–Abs–biotin
Number of protein molecules per 1 GNP, pcs	50	196	2110	202
Number of biotin molecules per 1 GNP, pcs	756	2943	31658	2038
Number of GNPs per test strip, pcs	8E+09	4E+11	8E+09	4E+11
Number of biotin molecules per test strip, pcs	6E+12	1E+13	2E+14	1E+13
Number of molecules Stp for all biotin, pcs	2E+12	4E+12	8.7E+13	4E+12
Number of GNP–Stp particles, pcs	1.6E+10	3E+10	1.5E+11	7.5E+09
A(GNP–Stp)	0.08	0.17	0.85	0.04
Total A(GNP–Stp)		0.25		0.89

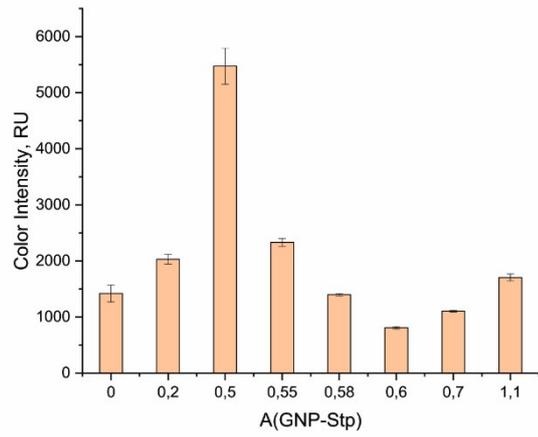


Fig. S6. Varying amounts of GNP–Stp conjugate in the three-component system for C (cTnT) = 3.7 ng mL⁻¹

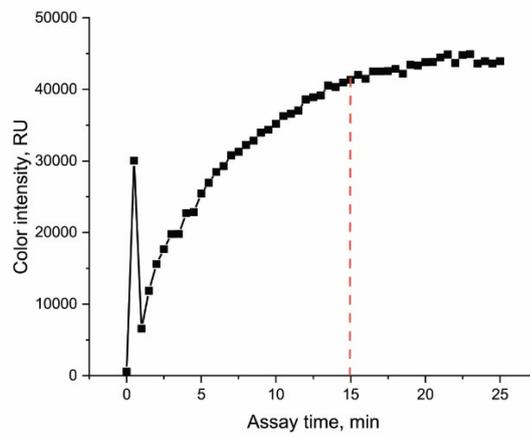


Fig. S7. Kinetics of staining development in the analytical zone (for cTnT)

Table S7. Specificity of the test system

Protein	Human serum albumin	Human immunoglobulins G		
Photo				
				Control zone
				Test zone
C(protein), µg mL ⁻¹	25	2500	25	5000