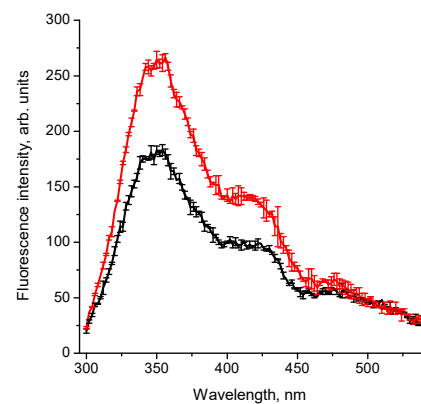


Supplementary Material

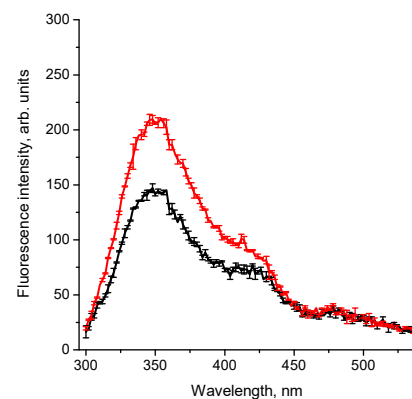
Retention of Activity by Antibodies Immobilized on Gold Nanoparticles of Different Sizes: Fluorometric Method of Determination and Comparative Evaluation

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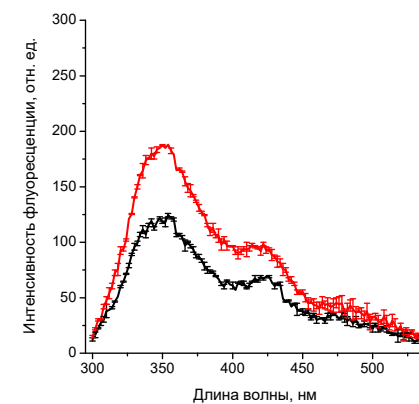
A. N. Bach Institute of Biochemistry, Research Center of Biotechnology, Russian Academy of Sciences, 119071 Moscow, Russia



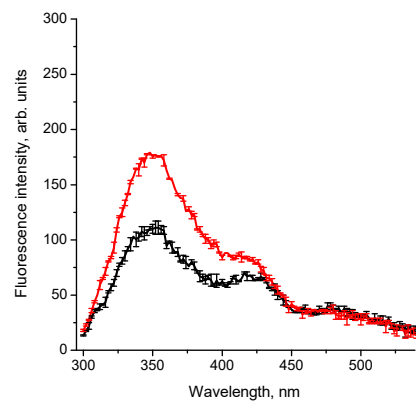
A



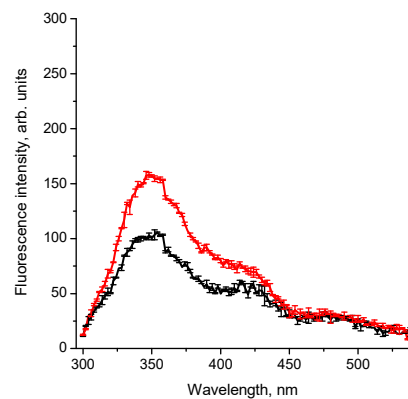
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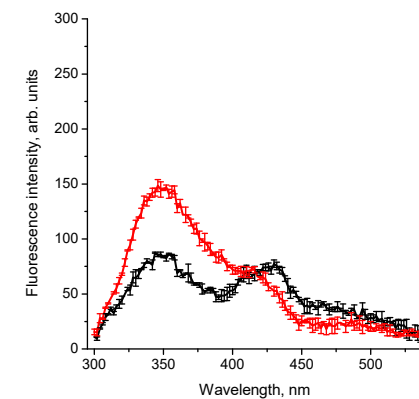
C



D



E



F

Figure S1. Fluorescence spectra of supernatants of conjugates GNP1-IgG (A), GNP2-IgG (B), GNP3-IgG (C), GNP4-IgG (D), GNP5-IgG (E), and GNP6-IgG (F). Red spectrum – after addition of 3.15 µg/mL IgG, black spectrum – without IgG.

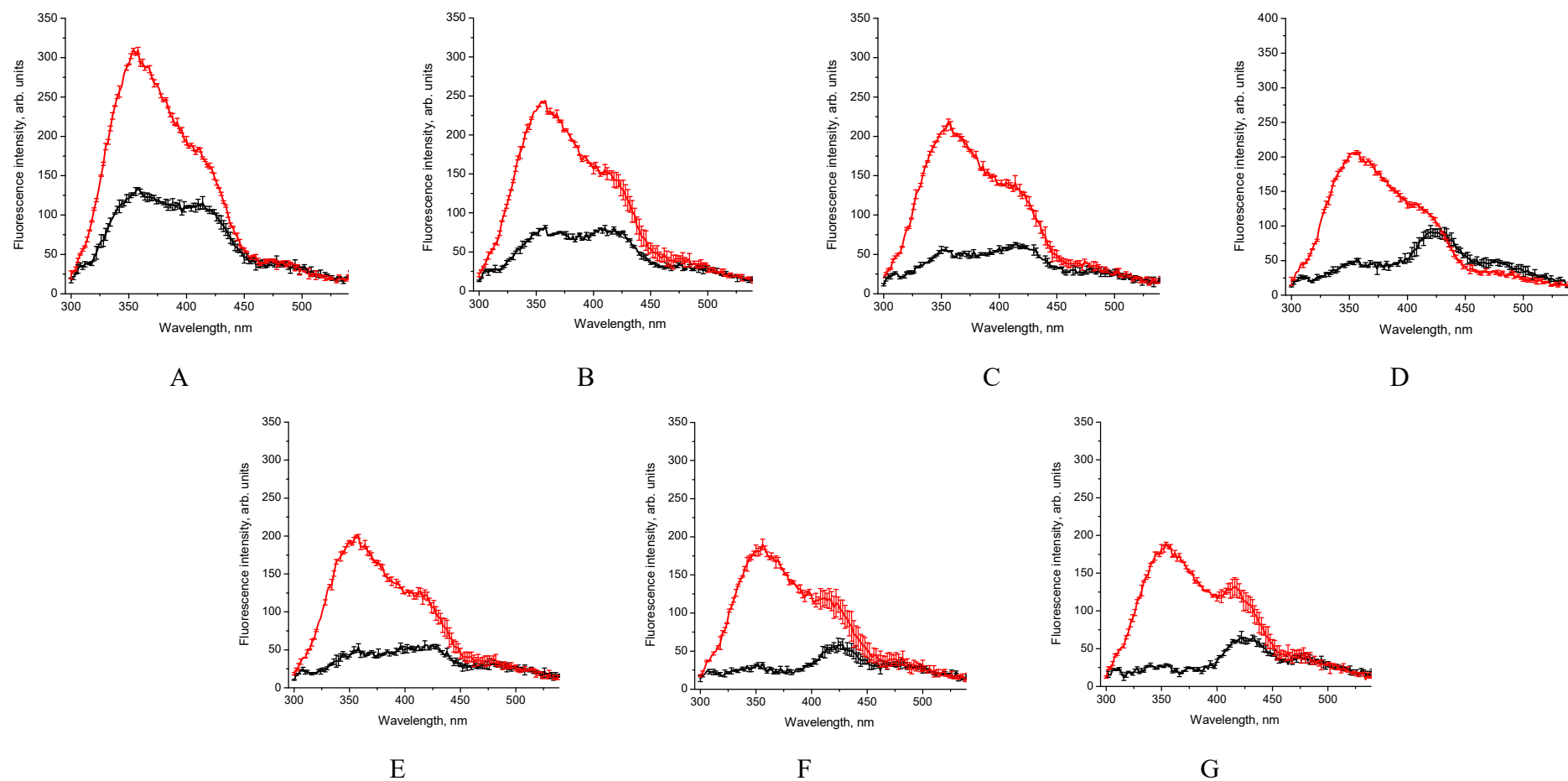


Figure S2. Fluorescence spectra of supernatants of the GNP1-IgG conjugate titrated with CRP at concentrations of 10 (A), 8 (B), 6 (C), 4 (D), 2 (E), 1 (F), and 0 (G) $\mu\text{g/mL}$. Red spectrum – after addition of 3.15 $\mu\text{g/mL}$ CRP, black spectrum – without CRP.

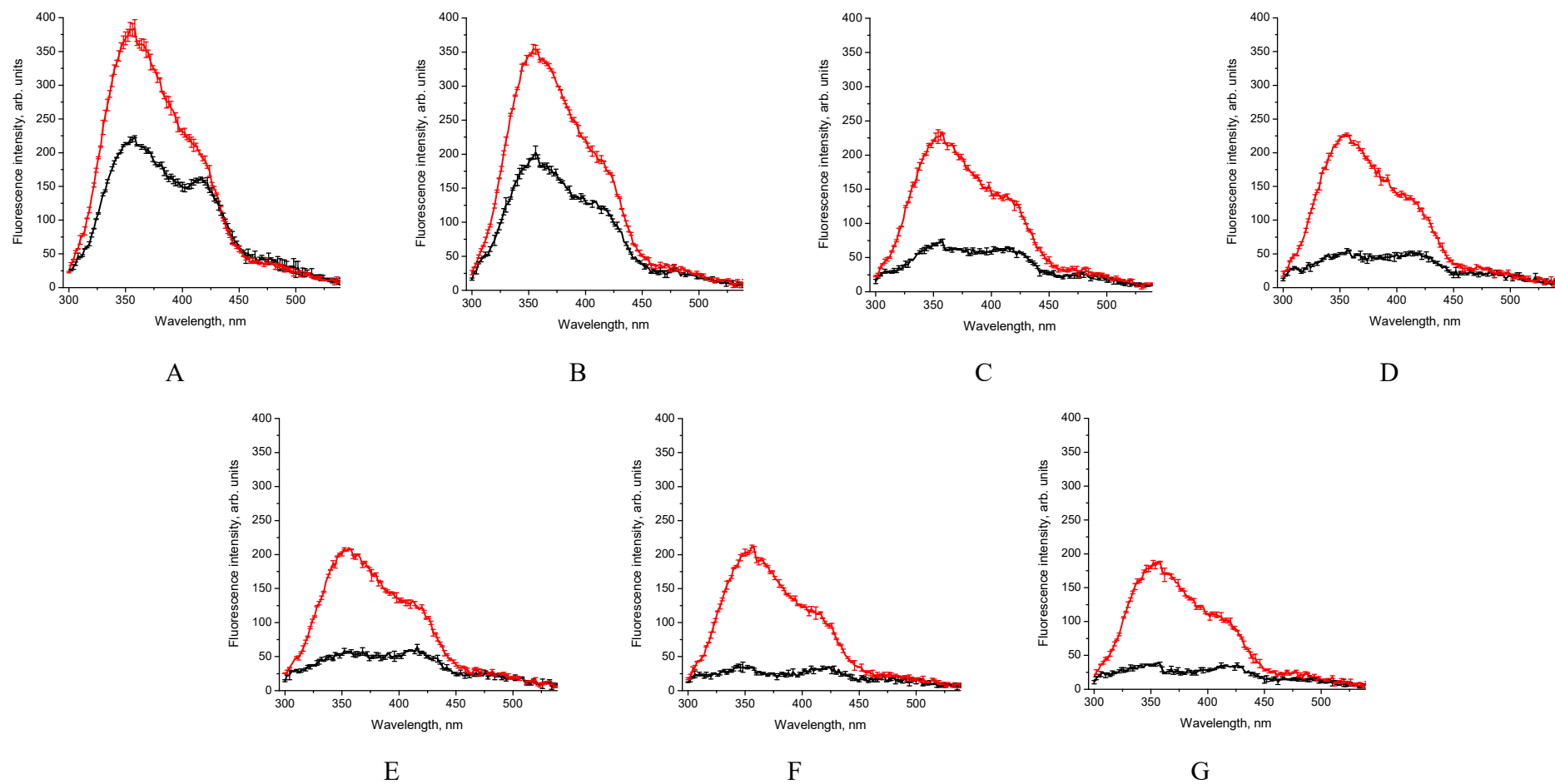


Figure S3. Fluorescence spectra of supernatants of the GNP2-IgG conjugate titrated with CRP at concentrations of 10 (A), 8 (B), 6 (C), 4 (D), 2 (E), 1 (F), and 0 (G) $\mu\text{g/mL}$. Red spectrum – after addition of 3.15 $\mu\text{g/mL}$ CRP, black spectrum – without CRP.

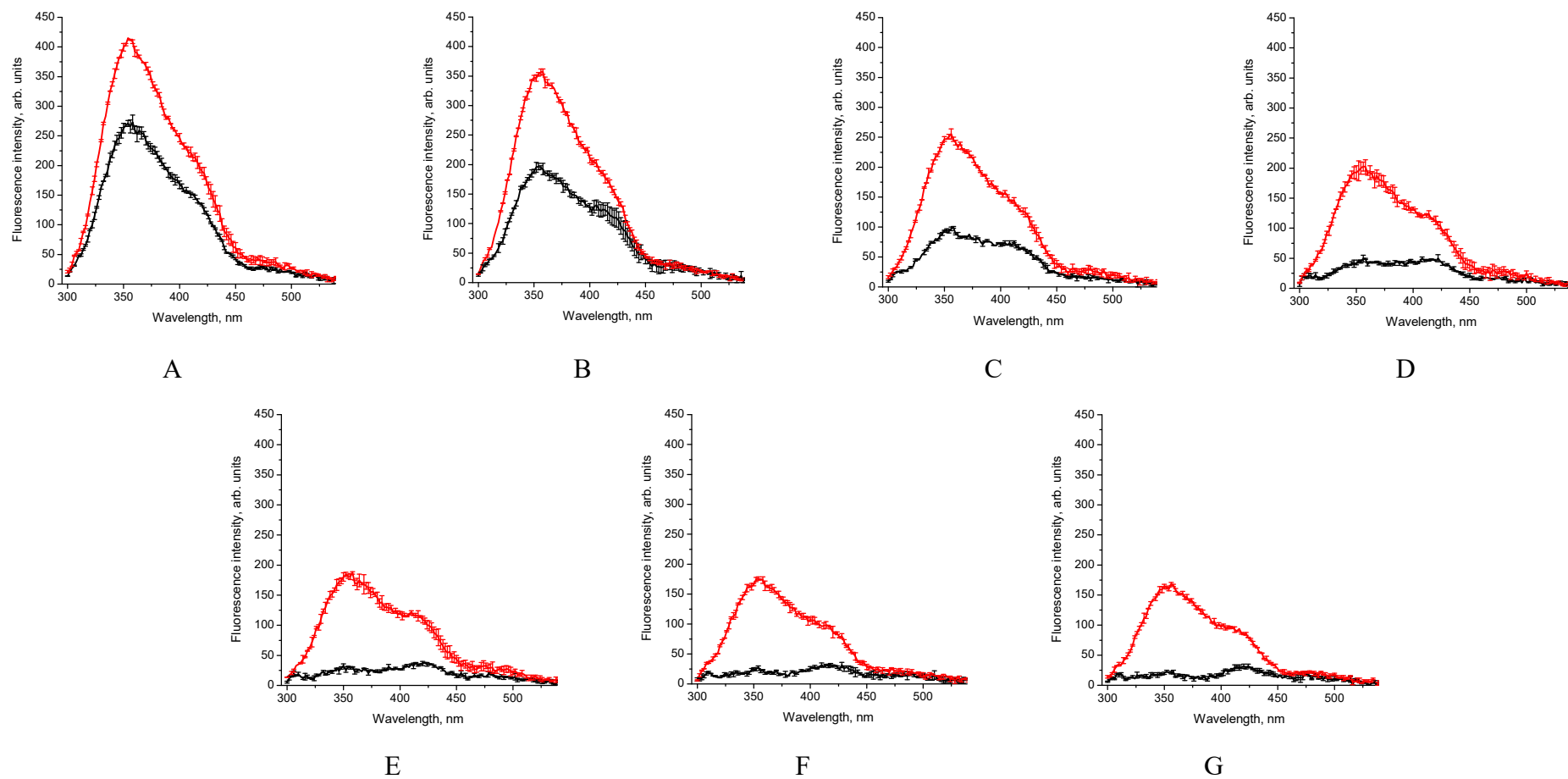


Figure S4. Fluorescence spectra of supernatants of the GNP3-IgG conjugate titrated with CRP at concentrations of 10 (A), 8 (B), 6 (C), 4 (D), 2 (E), 1 (F), and 0 (G) $\mu\text{g/mL}$. Red spectrum – after addition of 3.15 $\mu\text{g/mL}$ CRP, black spectrum – without CRP.

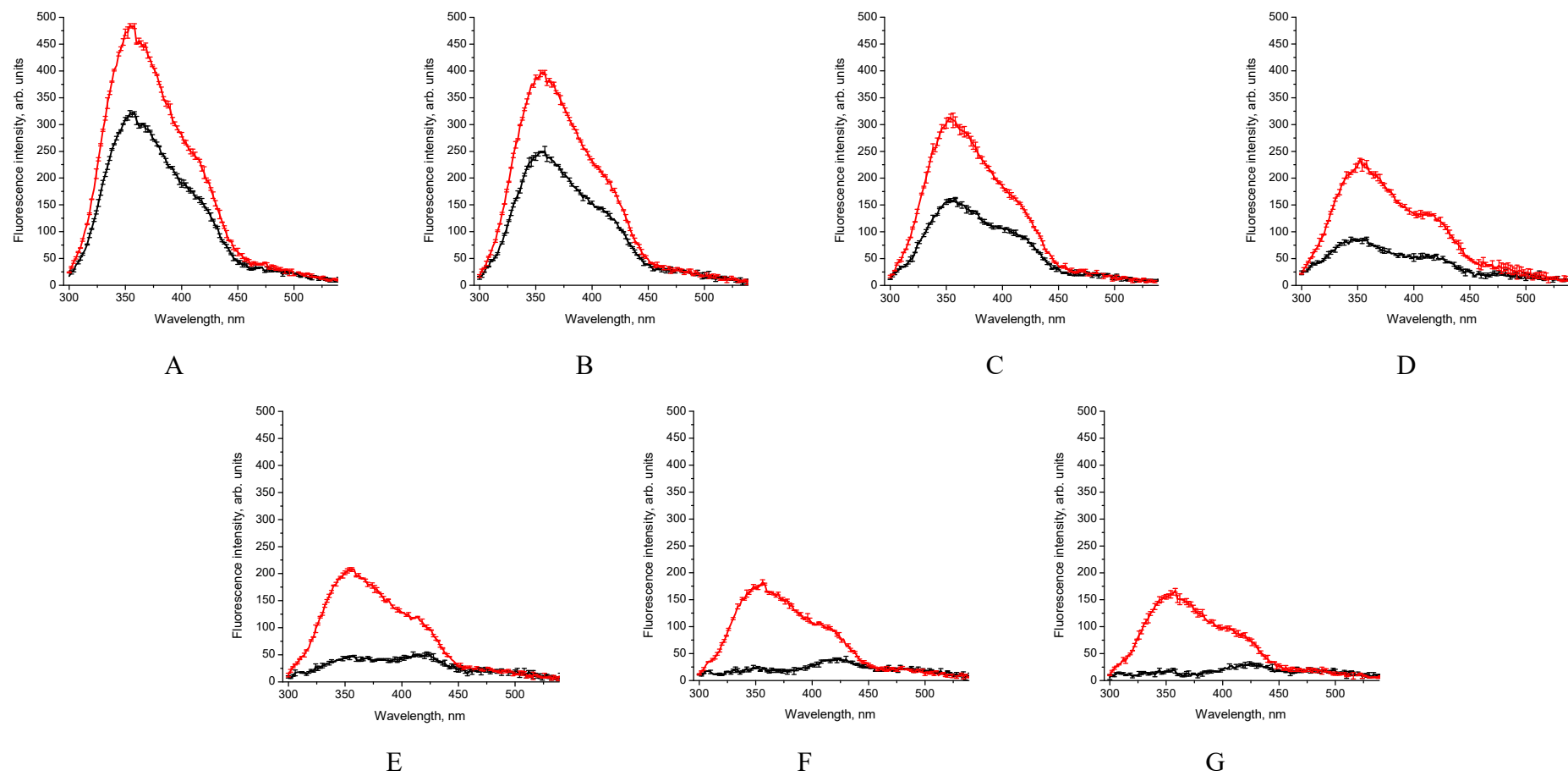


Figure S5. Fluorescence spectra of supernatants of the GNP4-IgG conjugate titrated with CRP at concentrations of 10 (A), 8 (B), 6 (C), 4 (D), 2 (E), 1 (F), and 0 (G) µg/mL. Red spectrum – after addition of 3.15 µg/mL CRP, black spectrum – without CRP.

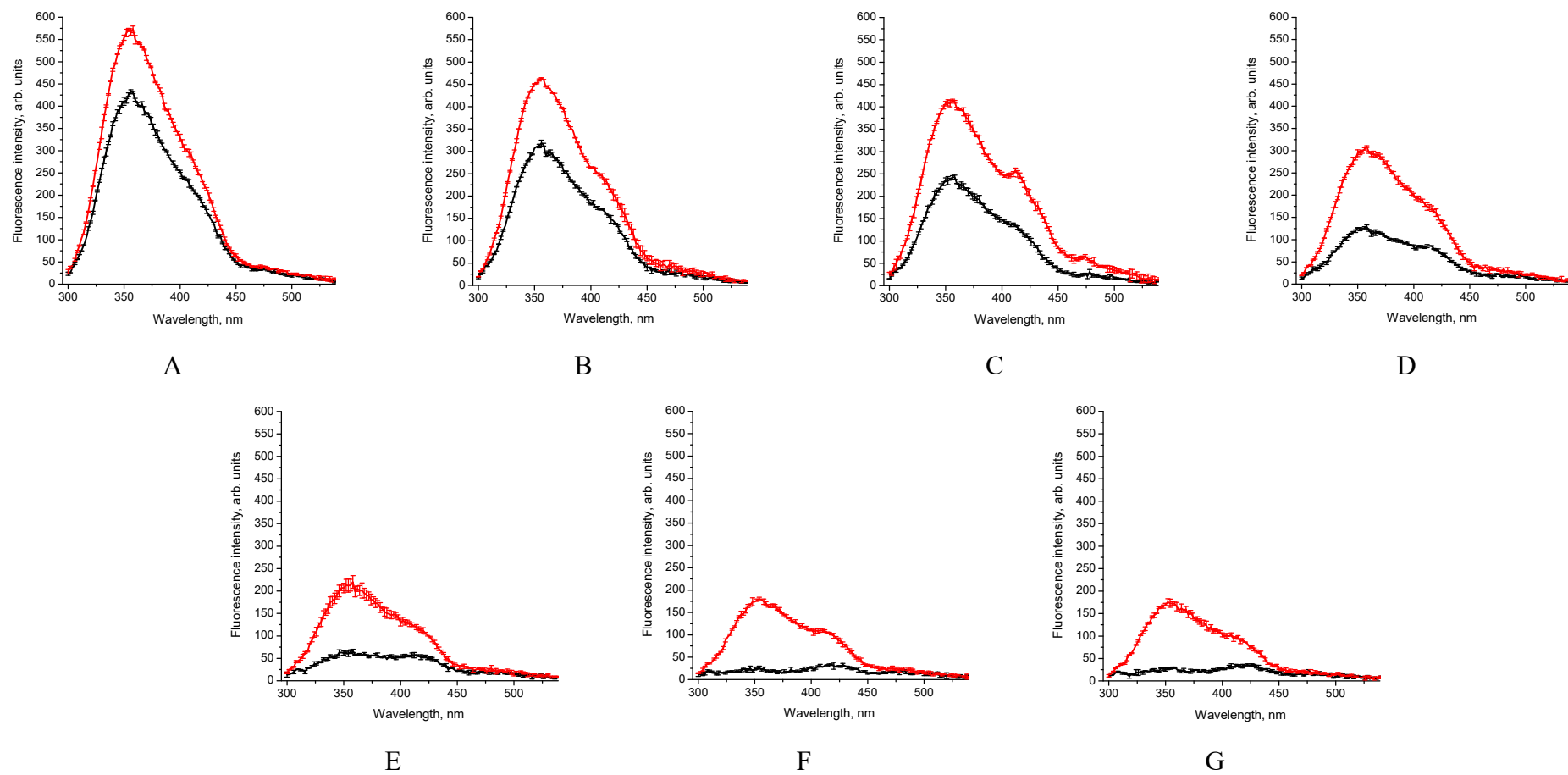


Figure S6. Fluorescence spectra of supernatants of the GNP5-IgG conjugate titrated with CRP at concentrations of 10 (A), 8 (B), 6 (C), 4 (D), 2 (E), 1 (F), and 0 (G) $\mu\text{g/mL}$. Red spectrum – after addition of 3.15 $\mu\text{g/mL}$ CRP, black spectrum – without CRP.

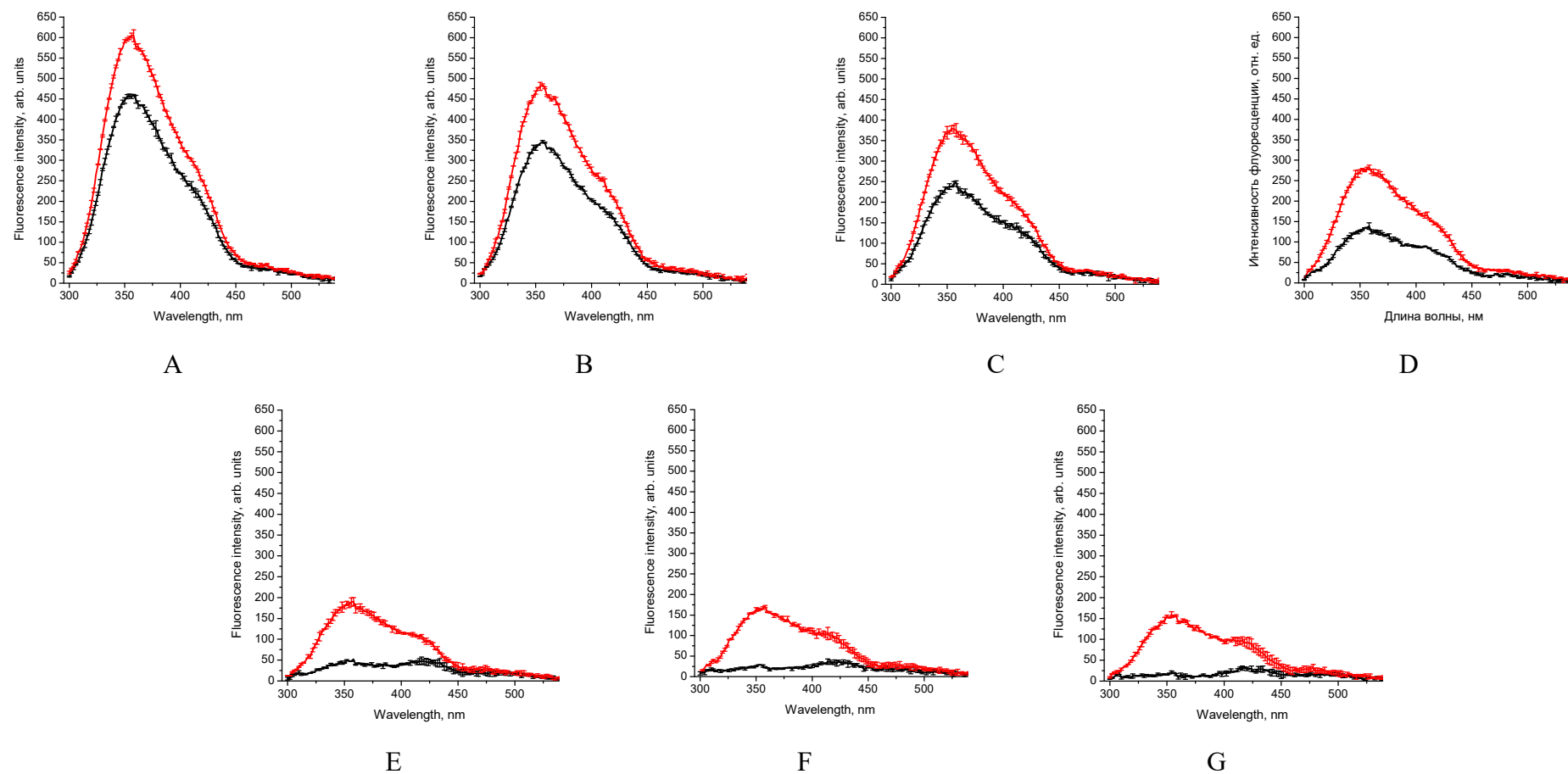


Figure S7. Fluorescence spectra of supernatants of the GNP6-IgG conjugate titrated with CRP at concentrations of 10 (A), 8 (B), 6 (C), 4 (D), 2 (E), 1 (F), and 0 (G) µg/mL. Red spectrum – after addition of 3.15 µg/mL CRP, black spectrum – without CRP.

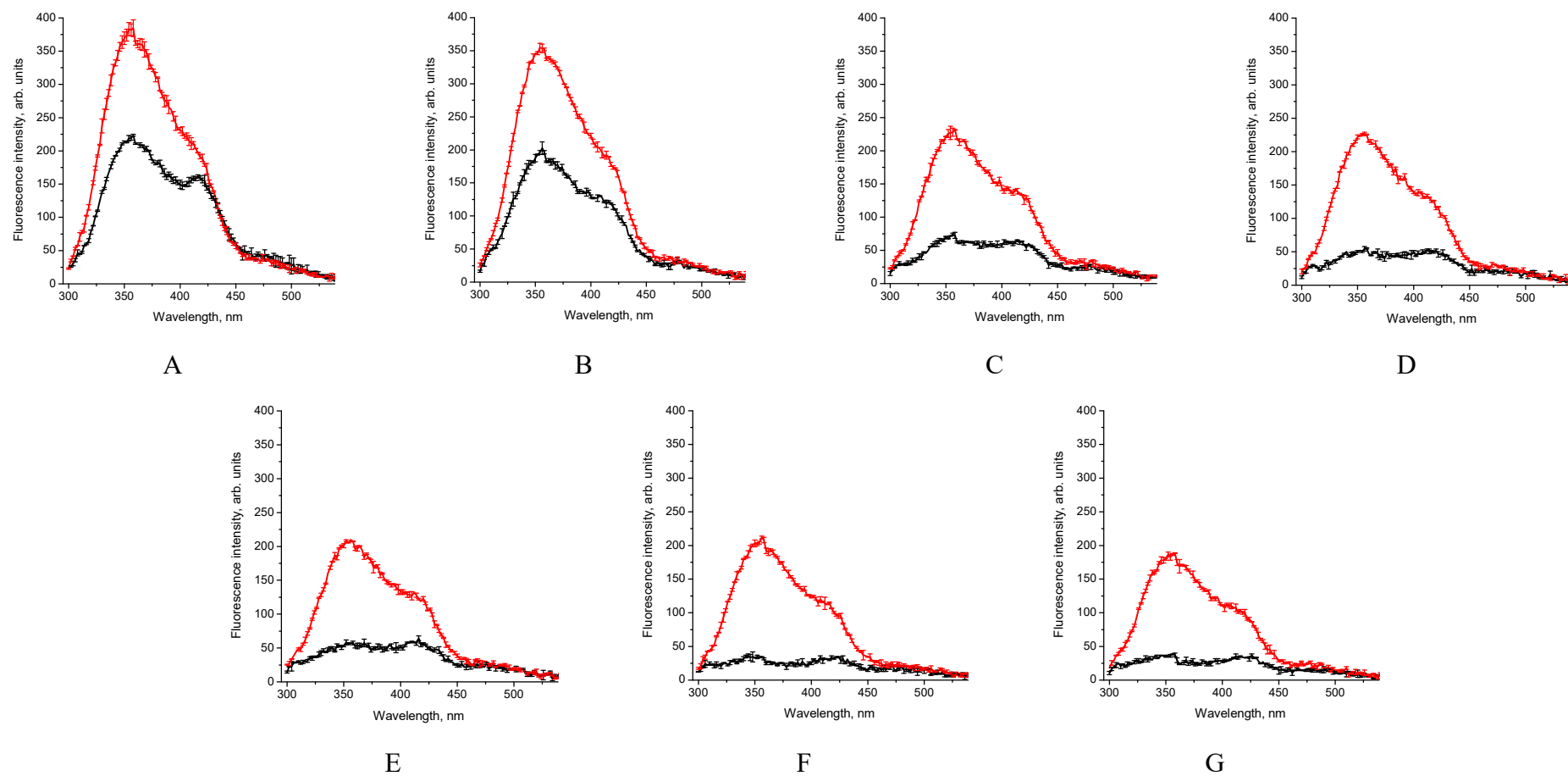


Figure S8. Fluorescence spectra of supernatants of the GNP2-IgG conjugate (monolayer) titrated with CRP at concentrations of 10 (A), 8 (B), 6 (C), 4 (D), 2 (E), 1 (F), and 0 (G) $\mu\text{g/mL}$. Red spectrum – after addition of 3.15 $\mu\text{g/mL}$ CRP, black spectrum – without CRP.

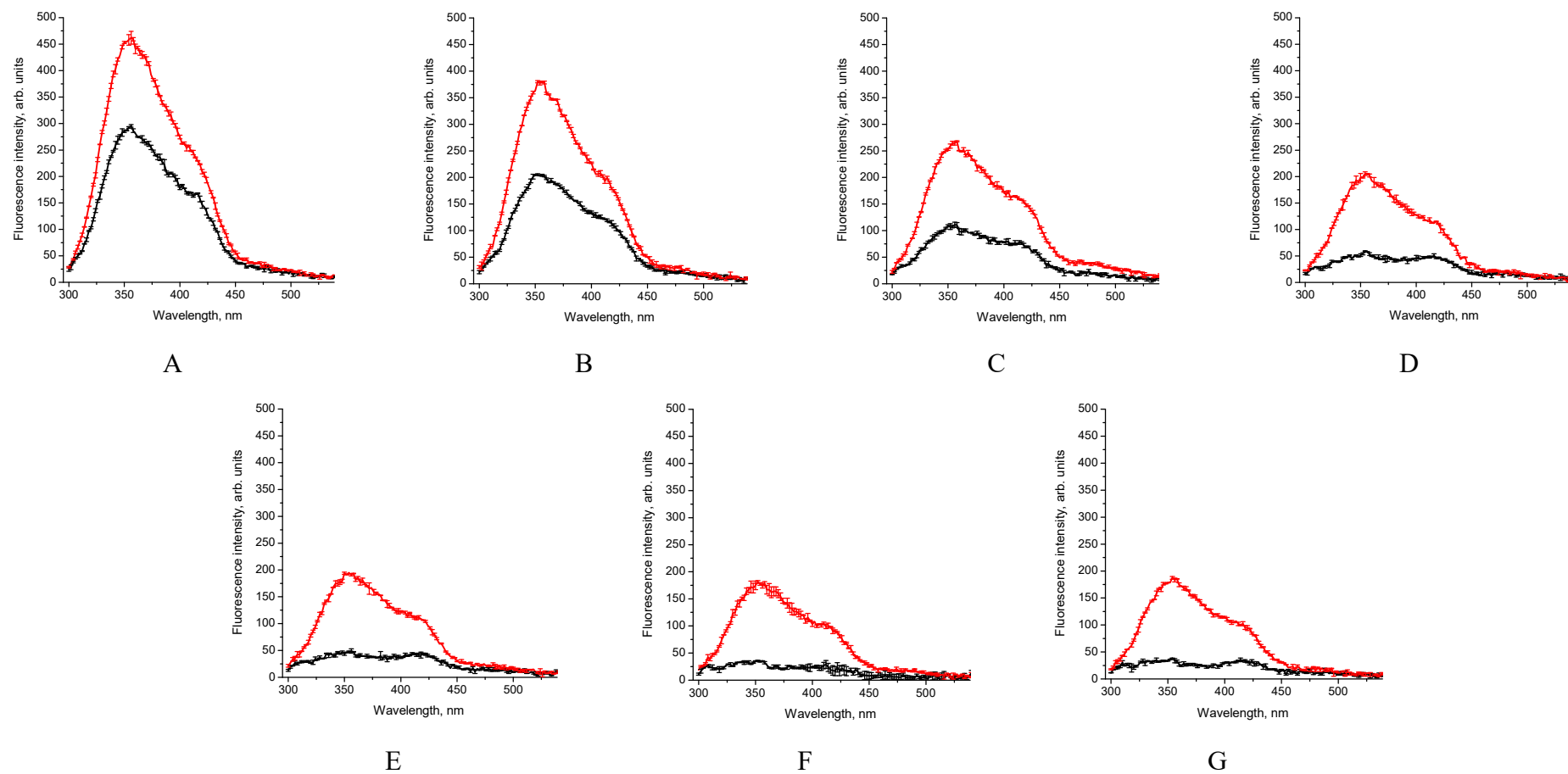


Figure S9. Fluorescence spectra of supernatants of the GNP2-IgG conjugate (1/2 monolayer) titrated with CRP at concentrations of 10 (A), 8 (B), 6 (C), 4 (D), 2 (E), 1 (F), and 0 (G) $\mu\text{g/mL}$. Red spectrum – after addition of 3.15 $\mu\text{g/mL}$ CRP, black spectrum – without CRP.

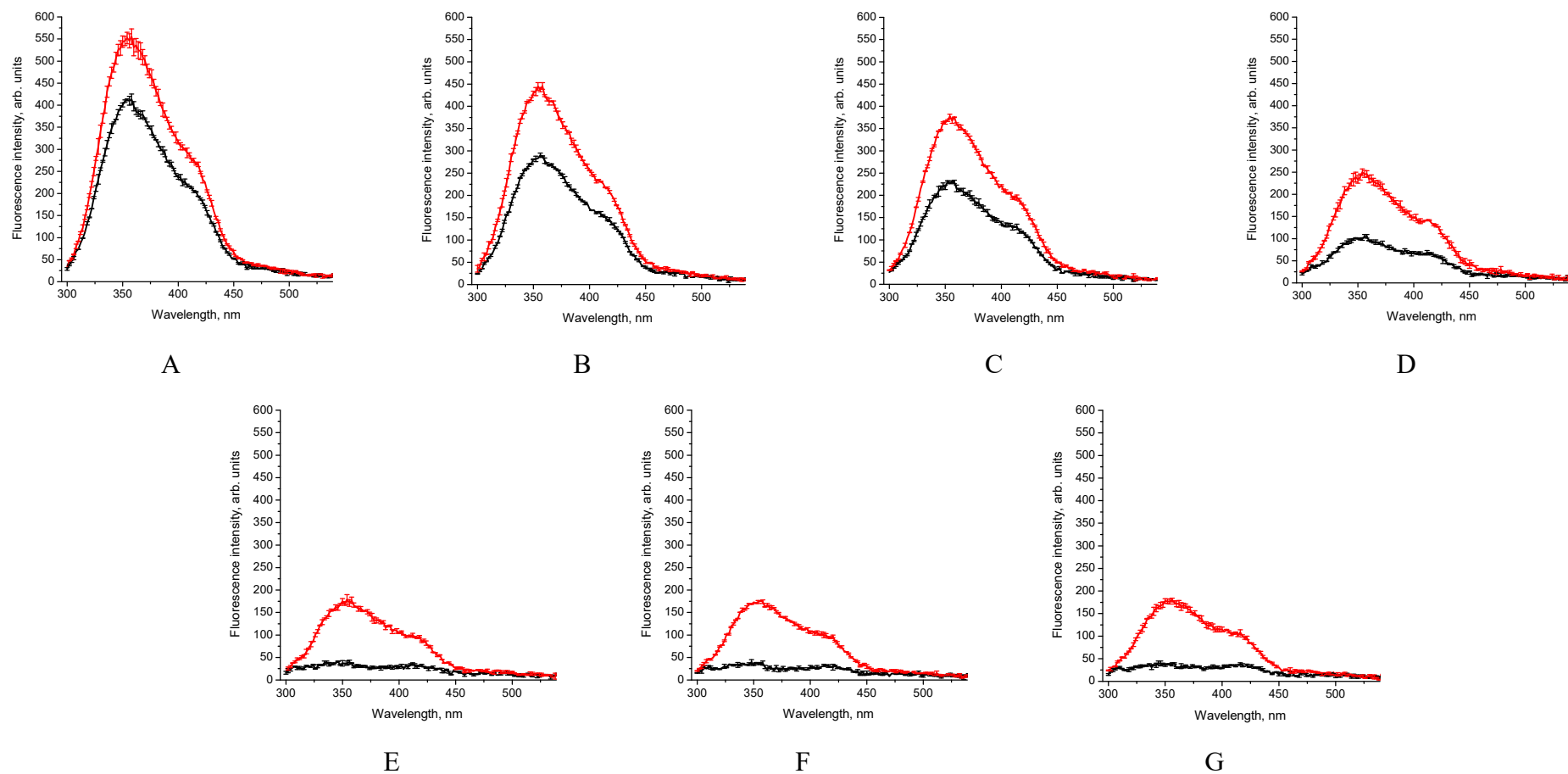


Figure S10. Fluorescence spectra of supernatants of the GNP2-IgG conjugate (1/4 monolayer) titrated with CRP at concentrations of 10 (A), 8 (B), 6 (C), 4 (D), 2 (E), 1 (F), and 0 (G) µg/mL. Red spectrum – after addition of 3.15 µg/mL CRP, black spectrum – without CRP.

Table S1. Results of measuring the fluorescence of supernatant solutions after centrifugation of conjugates with GNPs(1-6) and calculated concentrations of IgG in solutions and in the conjugates. Fluorescence signals are average fluorescence values at 350 nm for 2 replicates with subtracted background values for supernatants after centrifugation of pure GNPs(1-6) preparations stabilized with PEG-SH.

N	Particle diameter, nm	Added antibodies, $\mu\text{g/mL}$	Fluorescence in supernatants, arb. units	Error, %	Fluorescence in supernatants with added 3.15 $\mu\text{g/mL}$ IgG, arb. units	Error, %	Calibration signal from 3, 15 $\mu\text{g/mL}$ IgG, arb. units	IgG in supernatants, $\mu\text{g/mL}$	IgG in conjugates, $\mu\text{g/mL}$
1	14.8	12	144.7	3.5	224.4	3.2	73.5	6.2	5.8
2	23.5	7.4	108	1.1	172.2	1.3	79.2	4.3	3.1
3	28.6	6.2	85.1	1.2	150.8	0.3	75.6	3.5	2.68
4	34	5.1	76.1	3.6	143.2	0.3	71.3	3.4	1.76
5	43.3	4.0	71.4	1.8	125.3	1.2	73.8	3.0	0.99
6	54.5	3.2	55	1.5	115.6	2.7	71.6	2.4	0.74

Table S2. Calculated concentrations of CRP bound to conjugates of GNPs(1-6) and CRP unbound (remaining in supernatants).

	GNP1				GNP 2			
Added CRP, $\mu\text{g/mL}$	Bound CRP, $\mu\text{g/mL}$	Error, $\mu\text{g/mL}$	Unbound CRP, $\mu\text{g/mL}$	Error, $\mu\text{g/mL}$	Bound CRP, $\mu\text{g/mL}$	Error, $\mu\text{g/mL}$	Unbound CRP, $\mu\text{g/mL}$	Error, $\mu\text{g/mL}$
0	0	—	0	—	0	—	0	—
1	0.94	0.004	0.06	0.004	0.96	0.005	0.04	0.005
2	1.62	0.098	0.38	0.098	1.82	0.008	0.18	0.008
4	3.61	0.017	0.39	0.017	3.54	0.060	0.46	0.060
6	5.41	0.023	0.60	0.023	4.61	0.021	1.39	0.021
8	7.04	0.027	0.96	0.027	4.8	0.024	3.20	0.024
10	8.27	0.049	1.73	0.049	—	—	—	—

continues

	GNP3				GNP4			
Added CRP, $\mu\text{g/mL}$	Bound CRP, $\mu\text{g/mL}$	Error, $\mu\text{g/mL}$	Unbound CRP, $\mu\text{g/mL}$	Error, $\mu\text{g/mL}$	Bound CRP, $\mu\text{g/mL}$	Error, $\mu\text{g/mL}$	Unbound CRP, $\mu\text{g/mL}$	Error, $\mu\text{g/mL}$
0	0	—	0	—	0	—	0	—
1	0.92	0.008	0.08	0.008	0.88	0.007	0.12	0.007
2	1.83	0.017	0.17	0.017	1.45	0.019	0.55	0.019
4	3.53	0.031	0.47	0.031	2.44	0.040	1.55	0.040
6	4.5	0.045	1.46	0.045	3.07	0.046	2.93	0.046
8	4.56	0.102	3.44	0.102	3.43	0.089	4.57	0.089

continued

	GNP5				GNP6			
Added CRP, $\mu\text{g/mL}$	Bound CRP, $\mu\text{g/mL}$	Error, $\mu\text{g/mL}$	Unbound CRP, $\mu\text{g/mL}$	Error, $\mu\text{g/mL}$	Bound CRP, $\mu\text{g/mL}$	Error, $\mu\text{g/mL}$	Unbound CRP, $\mu\text{g/mL}$	Error, $\mu\text{g/mL}$
0	0	—	0	—	0	—	0	—
1	1	—	0	—	0.78	0.007	0.21	0.007
2	1.24	0.019	0.75	0.019	1.32	0.043	0.68	0.043
4	2.2	0.022	1.80	0.022	1.59	0.032	2.41	0.032
6	2.22	0.081	3.78	0.081	0.88	0.222	5.12	0.222
8	2.02	0.047	5.98	0.047	—	—	—	—

Table S3. Calculated concentrations of CRP bound to conjugates of GNP2 with different contents of IgG.

	GNP2 conjugate, 1/4 of IgG monolayer		GNP2 conjugate, 1/2 of IgG monolayer		GNP2 conjugate, IgG monolayer	
Added CRP, $\mu\text{g/mL}$	Bound CRP, $\mu\text{g/mL}$	Error, $\mu\text{g/mL}$	Bound CRP, $\mu\text{g/mL}$	Error, $\mu\text{g/mL}$	Bound CRP, $\mu\text{g/mL}$	Error, $\mu\text{g/mL}$
0	0	0	0	0	0	0
1	0.85	0.069	0.96	0.112	0.77	0.146
2	1.85	0.093	1.70	0.114	1.54	0.126
4	2.37	0.005	3.52	0.081	3.42	0.186
6	1.55	0.027	4.36	0.003	5.22	0.054
8	2.23	0.176	4.18	0.217	—	—
10	1.57	0.003	4.42	0.168	6.29	0.017