Electronic Supplementary Material (ESI) for Analyst.

Supporting information for:

Influence of bovine and human serum albumin on the binding kinetics of biomolecular interactions

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Supplementary tables and figures:

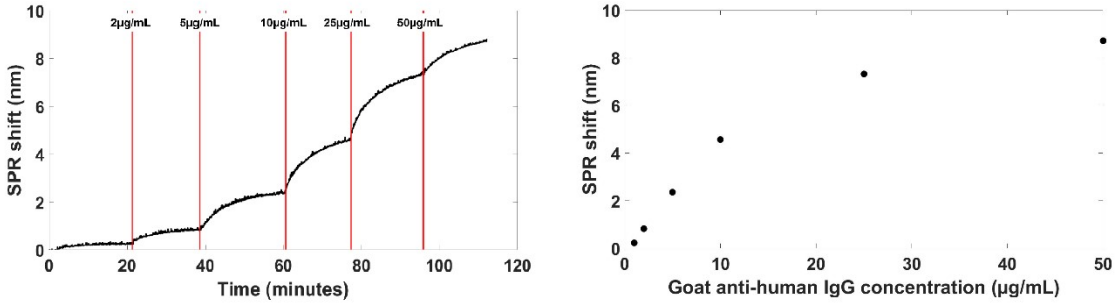

Figure S1. Typical sensorgram for goat anti-human IgGs in 0.1% BSA running buffer on a human IgG functionalized surface (left) and corresponding Langmuir isotherm calibration curve. (Right) Red lines represent the injection of the next concentrations. Concentrations are 1, 2, 5, 10, 25, and 50 µg/mL.

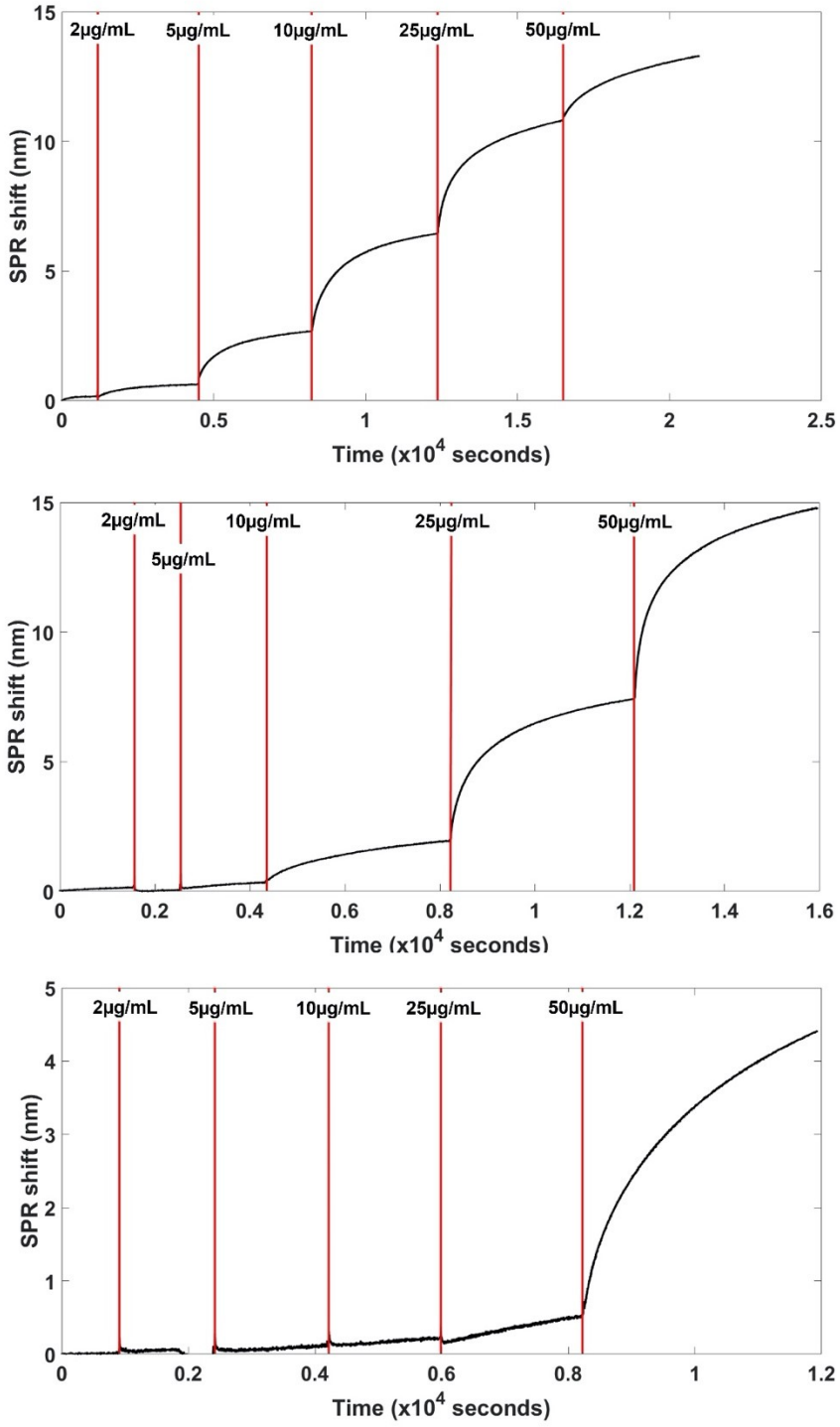

Figure S2. Sensorgrams of goat anti-human IgG solutions on a human IgG surface in 0.1% HSA buffer (top), 0.5% HSA buffer (middle), and 2.5% HSA buffer (bottom). Red lines represent time points at which an increased concentration of goat anti-human IgG was injected. The concentrations were 1, 2, 5, 10, 25, and 50 µg/mL. The varying time between injections is due to waiting for stabilization, defined as an increase of less than 10% of the total SPR shift in the last 10 minutes, before injecting the next solution.

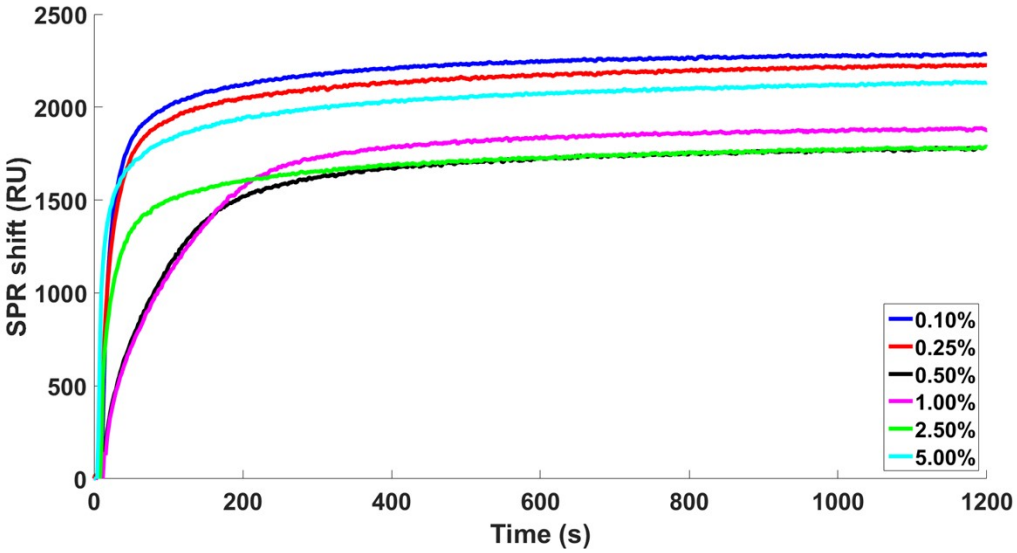

Figure S3. Binding curve for the immobilization of human gamma globulin on the gold prism surface for all experiments. The immobilization is performed in sodium acetate buffer in all cases (without HSA). These SPR chips served for the experiments with an HSA containing buffer for the analytical detection step and are therefore identified using the later HSA concentration to associate the curve with the binding obtained on this prism.

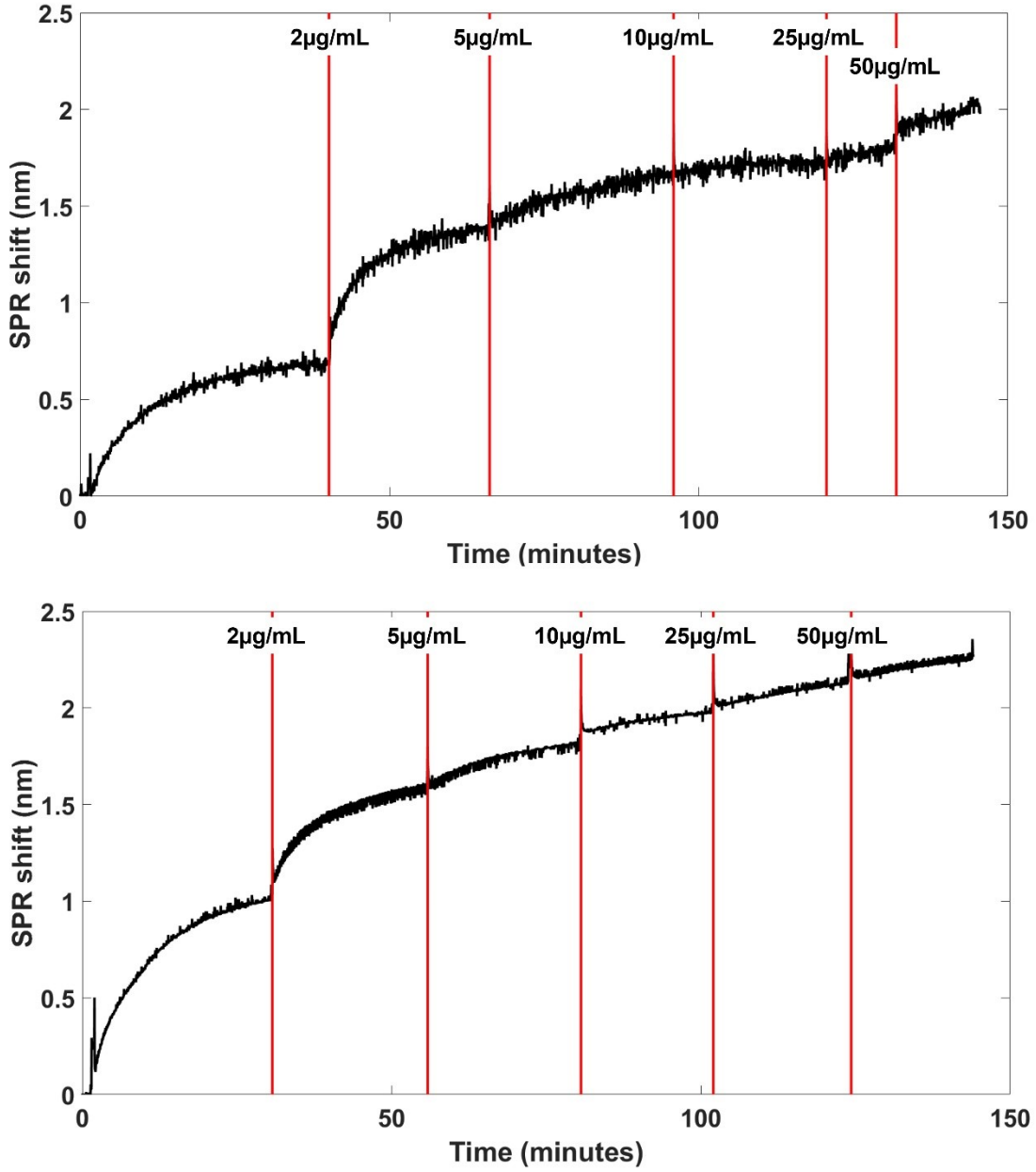

Figure S4. Sensorgrams obtained with analyte solutions of human IgGs on a surface of goat anti-human IgGs in buffer containing 0.1% BSA (top) or 0.1% HSA (bottom). Red lines represent time points at which an increased concentration of human IgG was injected. The concentrations were 1, 2, 5, 10, 25, and 50 μg/mL. The varying time between injections is due to waiting for stabilization, defined as an increase of less than 10% of the total SPR shift in the last 10 minutes, before injecting the next solution.

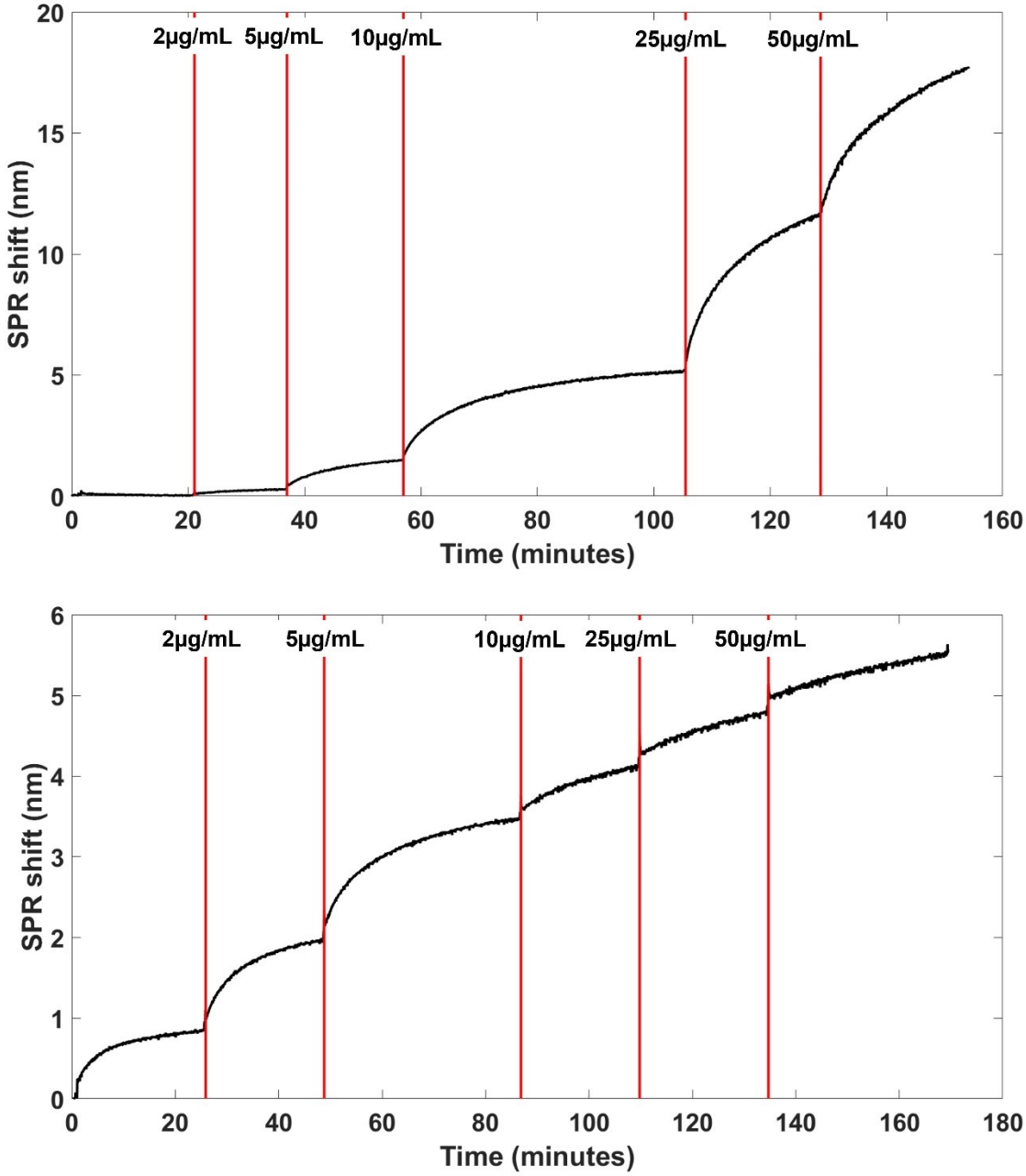

Figure S5. Sensorgrams obtained with analyte solutions of either goat anti-human IgGs on a human IgG surface (top) or human IgGs on a goat anti-human IgG surface (bottom) in 0.1% HSA buffer and the capture protein bound to Protein G. Red lines represent time points at which an increased concentration of analyte was injected. The concentrations are 1, 2, 5, 10, 25 and 50 µg/mL. The varying time between injections is due to waiting for stabilization, defined as an increase of less than 10% of the total SPR shift in the last 10 minutes, before injecting the next solution.

Table S1. Interaction parameters from calibrations of goat anti-human IgGs in a BSA containing buffer on a surface functionalized with human IgGs.

BSA fraction (m/v, %)	B_{max} (nm)	k_{on} (10^3 $M^{-1}s^{-1}$)	k_{off} (10^{-3} s^{-1})	K_D (µM)
0.1	9.6 ± 0.6	8 ± 6	0.2 ± 0.1	0.025 ± 0.009
0.25	14.4 ± 0.5	10 ± 3	0.3 ± 0.2	0.03 ± 0.02
0.5	8 ± 2	8 ± 3	0.3 ± 0.1	0.031 ± 0.007
1	14 ± 9	9.6 ± 0.5	0.31 ± 0.07	0.032 ± 0.006
2.5	13 ± 1	10 ± 1	0.2 ± 0.1	0.022 ± 0.008
5	11.5 ± 0.8	10 ± 1	0.2 ± 0.2	0.02 ± 0.02

Table S2. Interaction parameters from calibrations of goat anti-human IgGs in a HSA containing buffer on a surface functionalized with human IgGs.

HSA fraction (m/v, %)	B_{max} (nm)	k_{on} (10^3 $M^{-1}s^{-1}$)	k_{off} (10^{-3} s^{-1})	K_D (µM)
0.1	15 ± 7	10 ± 10	0.2 ± 0.4	0.039 ± 0.008
0.25	31 ± 3	0.2 ± 0.04	0.7 ± 0.2	0.37 ± 0.05
0.5	(2 ± 3) *10^4	0.004 ± 0.007	1.6 ± 0.4	500 ± 800
1	(7 ± 2) *10^3	0.004 ± 0.002	0.7 ± 0.5	190 ± 70
2.5	(0 ± 1) *10^5	0.001 ± 0.003	(2 ± 3)*10^{-8}	(1 ± 4)*10^{-4}
5	N/A	N/A	N/A	N/A

Table S3. Interaction parameters from calibrations of goat anti-human IgGs on a surface of human IgG or of human IgG on a surface of goat anti-human IgG in a 0.1% protein buffer.

Surface functionalization	Buffer protein	B_{max} (nm)	k_{on} (10^3 $M^{-1}s^{-1}$)	k_{off} (10^{-3} s^{-1})	K_D (μM)
Human IgG	BSA	9.6 ± 0.6	8 ± 6	0.2 ± 0.1	0.025 ± 0.009
Human IgG	HSA	15 ± 7	10 ± 10	0.2 ± 0.4	0.039 ± 0.008
Goat anti-human IgG	BSA	1.8 ± 0.5	200 ± 100	0.6 ± 0.9	0.004 ± 0.003
Goat anti-human IgG	HSA	0.9 ± 0.4	300 ± 200	0.02 ± 0.2	$(0.7 \pm 5)*10^{-4}$

Table S4. Interaction parameters from calibrations of antibodies with or without protein G as a surface binding in a 0.1% HSA buffer.

Surface functionalization	B_{max} (nm)	k_{on} (10^3 $M^{-1}s^{-1}$)	k_{off} (10^{-3} s^{-1})	K_D (μM)
Human IgG	15 ± 7	10 ± 10	0.2 ± 0.4	0.039 ± 0.008
Protein G + Human IgG	40 ± 30	4 ± 3	1.3 ± 0.9	0.4 ± 0.5
Goat anti-human IgG	0.9 ± 0.4	300 ± 200	0.02 ± 0.2	$(0.7 \pm 5)*10^{-4}$
Protein G + Goat anti-human IgG	3.6 ± 0.4	33 ± 6	0.62 ± 0.06	0.019 ± 0.004

Table S5. Interaction parameters from calibrations of goat anti-human IgGs in dilutions of bovine serum on a surface functionalized with human IgGs.

Bovine serum fraction		B_{max}	k_{on}	k_{off}	K_D
Dillution	**% (v/v)**	**(nm)**	**(10^3 $M^{-1}s^{-1}$)**	**(10^{-3} s^{-1})**	**(µM)**
1:1	**100**	9.6 ± 0.6	8 ± 6	0.2 ± 0.1	0.025 ± 0.009
1:2	**50**	10.2 ± 0.4	5 ± 2	0.2 ± 0.1	0.03 ± 0.03
1:4	**25**	10 ± 1	8 ± 1	0.31 ± 0.01	0.04 ± 0.007
1:8	**12.5**	10.9 ± 0.5	9 ± 1	0.29 ± 0.05	0.031 ± 0.009
1:16	**6.25**	10.2 ± 0.2	7.4 ± 0.7	0.28 ± 0.06	0.04 ± 0.02
1:32	**3.125**	11 ± 3	10 ± 10	0.4 ± 0.2	0.03 ± 0.01
1:64	**1.5625**	11 ± 2	9 ± 3	0.36 ± 0.07	0.04 ± 0.01
1:128	**0.78125**	11.6 ± 0.9	7 ± 7	0.3 ± 0.1	0.04 ± 0.02

Table S6. Interaction parameters from calibrations of goat anti-human IgGs in dilutions of IgG depleted human serum on a surface functionalized with human IgGs.

Human serum fraction		B_{max}	k_{on}	k_{off}	K_D
Dilution	**% (v/v)**	**(nm)**	**(10^3 $M^{-1}s^{-1}$)**	**(10^{-3} s^{-1})**	**(µM)**
1:1	**100**	N/A	N/A	N/A	N/A
1:2	**50**	2400 ± 400	$(9 \pm 2)*10^{-4}$	0.11 ± 0.03	116 ± 2
1:4	**25**	3000 ± 2000	$(9 \pm 5)*10^{-4}$	$(1 \pm 3)*10^{-7}$	$(1 \pm 3)*10^{-4}$
1:8	**12.5**	7000 ± 9000	0.002 ± 0.002	0.2 ± 0.1	100 ± 100
1:16	**6.25**	$(4 \pm 5) *10^4$	0.002 ± 0.002	2.0 ± 0.6	1000 ± 1000
1:32	**3.125**	5 ± 1	2.1 ± 0.6	1.3 ± 0.5	0.6 ± 0.4
1:64	**1.5625**	2 ± 2	10 ± 20	0.2 ± 0.4	0.1 ± 0.2
1:128	**0.78125**	2 ± 1	5 ± 8	0.2 ± 0.4	0.1 ± 0.2