Supporting Information

Title of the primary article: Miniaturization of sensitive homogeneous time-resolved fluoroimmunoassays (TR-FIA) for point-of-care testing (POCT)

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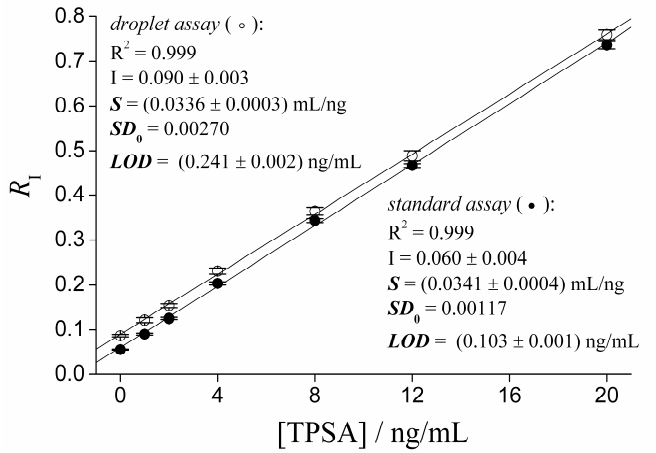

Figure S-1: TPSA calibration curve for standard immunoassay and one of the droplet immunoassays.

In order to analyze droplet (white dots) and standard immunoassay (black dots) a linear regression was performed and the correlation coefficient (R^2), the intercept (I), the sensitivity (***S***), the standard deviation of the background signal (***SD*$_0$**) and the limit of detection (***LOD***) were calculated.

Compared to the standard assay, a higher intercept (I) was found for the droplet assay. This was mainly caused by the "dark signal" arising from the microtiter plate, which gained influence on the detected intensities. When focusing the excitation laser beam in only a few µL sample volume, the laser light attenuation by the supernatant solution is decreased and an increased "dark signal" from the microtiter plate material is found in the measurement. This became perceivable at very low luminescence intensities resulting in a slightly increased signal in PMT A (whereas the signal in PMT D was uninfluenced) and consequently in an increased intensity ratio R_I.

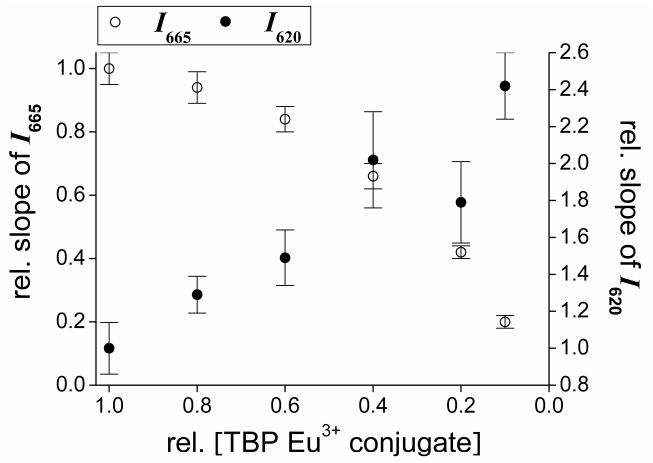

Figure S-2: Relative slope of the FRET signal (I_{655}) and the reference signal (I_{620}) for different TBP Eu^{3+} conjugate concentrations.

The signals of PMT A (I_{655}) and PMT D (I_{620}) were analyzed separately. The slope of the regression line of the FRET intensity I_{655} and the TBP Eu^{3+} luminescence intensity I_{620}, respectively, versus the TPSA concentration was normalized to the respective value at standard [TBP Eu^{3+} conjugate].

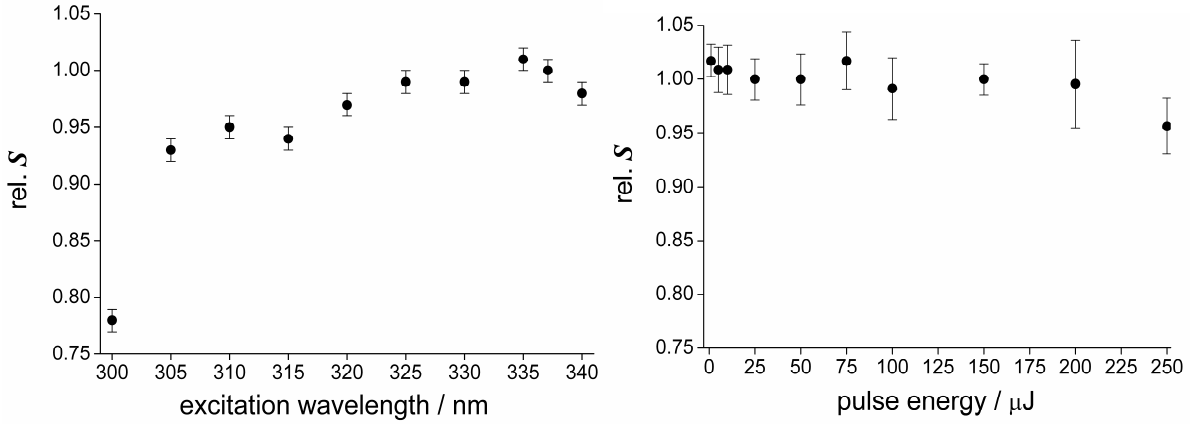

Figure S-3: Sensitivity S for the optimization of excitation wavelength and pulse energy. S was normalized to the respective value at λ_{ex} = 337.1 nm and E_{ex} = 25 μJ, respectively.

Table S-1: Luminescence intensity I_{620} (mean values) for the POCT assays relative to the respective values of the standard TPSA immunoassay.

reference assay: I_{620} (standard) = (55,600 ± 900) a.u.		
POCT assay	rel. I_{620} at E_{ex} = 1 µJ	rel. I_{620} at E_{ex} = 5 µJ
Standard concentration of all assay components	0.06 ± 0.01 (I_{620} = (3,200 ± 340) a.u.)	0.26 ± 0.02 (I_{620} = (14,400 ± 1,000) a.u.)
0.6-fold [TBP Eu^{3+} conjugate]	0.03 ± 0.004 (I_{620} = (1,700 ± 200) a.u.)	0.12 ± 0.02 (I_{620} = (6,800 ± 700) a.u.)
0.6-fold [TBP Eu^{3+} conjugate] and latex particles	0.11 ± 0.01 (I_{620} = (6,400 ± 600) a.u.)	0.44 ± 0.06 (I_{620} = (24,200 ± 2,600) a.u.)

Table S-2: Standard deviation of the background signal SD_0 (mean values) for the POCT assays relative to the respective values of the standard TPSA immunoassay.

reference assay: SD_0 (standard) = 0.00130 a.u.		
POCT assay	rel. SD_0 at E_{ex} = 1 µJ	rel. SD_0 at E_{ex} = 5 µJ
Standard concentration of all assay components	1.59 (SD_0 = 0.00207 a.u.)	0.84 (SD_0 = 0.00109 a.u.)
0.6-fold [TBP Eu^{3+} conjugate]	2.85 (SD_0 = 0.00370 a.u.)	1.36 (SD_0 = 0.00177 a.u.)
0.6-fold [TBP Eu^{3+} conjugate] and latex particles	1.19 (SD_0 = 0.00155 a.u.)	0.60 (SD_0 = 0.00078 a.u.)

Table S-3: Sensitivity *S* (mean values) for the POCT assays relative to the respective values of the standard TPSA immunoassay.

reference assay: ***S*** (standard) = (0.0313 ± 0.0012) mL/ng		
POCT assay	rel. ***S*** at E_{ex} = 1 µJ	rel. ***S*** at E_{ex} = 5 µJ
Standard concentration of all assay components	0.91 ± 0.08 (***S*** = (0.0284 ± 0.0014) mL/ng)	0.86 ± 0.09 (***S*** = (0.0269 ± 0.0017) mL/ng)
0.6-fold [TBP Eu^{3+} conjugate]	1.26 ± 0.18 (***S*** = (0.0394 ± 0.0040) mL/ng)	1.35 ± 0.11 (***S*** = (0.0423 ± 0.0017) mL/ng)
0.6-fold [TBP Eu^{3+} conjugate] and latex particles	1.29 ± 0.10 (***S*** = (0.0404 ± 0.0015) mL/ng)	1.33 ± 0.13 (***S*** = (0.0417 ± 0.0022) mL/ng)

Table S-4: Detection limit *LOD* (mean values) for the POCT assays relative to the respective values of the standard TPSA immunoassay.

POCT assay	rel. ***LOD*** at E_{ex} = 1 µJ	rel. ***LOD*** at E_{ex} = 5 µJ
Standard concentration of all assay components	1.74 ± 0.17	0.97 ± 0.11
0.6-fold [TBP Eu^{3+} conjugate]	2.26 ± 0.36	1.01 ± 0.08
0.6-fold [TBP Eu^{3+} conjugate] and latex particles	0.92 ± 0.08	0.45 ± 0.05