Electronic Supplementary Material (ESI) for RSC Advances.


**Supplementary information**

# Investigating Conjugated Polymer Nanoparticle Formulations for Lateral Flow Immunoassays

Moritz Schüller,a Annette Meister a, Mark Greenb and Lea Ann Dailey *c

## Brightness calculation

The amount of excitation light absorbed is given by Beer-Lambert law. (**Equation S1**) The Lambert-Beer law is an empiric finding and holds true for dilute solutions in which scattering of the light does not occur. However, in case of small NP with $d<<\lambda/\pi$ the main scattering mechanism is by Rayleigh scattering and the fraction of such is negligible in comparison to absorbed light.[1] Thus, the Lambert-Beer law can be well accepted for comparison of particles of similar geometry.

After absorption of light by the dye molecules, the amount of emitted fluorescent light is reduced by non-radiative relaxation, expressed as the quantum yield $\Phi$ (**Equation S2**). In fluorescence spectroscopy, the fluorescence intensity is further reduced by the primary and secondary inner filter effect as described in **Equation S3**.[2] The exponent contributes to the decrease in intensity by absorption at excitation and emission wavelength that has to pass through each half the path length of the cuvette. However, in an LFI, the emitted light is detected from the whole intersection of the NC membrane and the primary inner filter effect does not apply. Still, the secondary inner filter effect may reduce the intensity of emitted light. Thus, the brightness of the NP depending on concentration of dye is given by **Equation S4**.

The resulting function has a local maximum which describes the optimal dye loading concentration with maximal brightness. (**Figure S1**, solid line) However, if the extinction coefficient at emission wavelength is negligible, as expected for dyes with large Stokes-shift, the function has no maximum, but is steadily increasing. (**Figure S1**, segmented line) Furthermore, as the path length of light through NP typically does not exceed some hundred nm, the optical density in a particle is small. For example, in case of a NP that is composed of 100% PDOF with an extinction coefficient of $\varepsilon_{Ex}$=97.9 L/g/cm, an approximated density of $\delta$=1.05 g/cm$^3$ and a hypothetical path length of d=100 nm, the optical density is only $OD_{Ex}$=0.01. Even if the optical density at emission wavelength would be as high as it is at excitation wavelength, 25 NP would have to be stacked to reach the brightness maximum. For small optical densities the

brightness appears to be more or less linearly depending on dye concentration. To find a linear equation, that approximates the relation between brightness and dye concentration for small optical densities, **Equation S4** has to be derived for the concentration at c=0. The general derivation of **Equation S4** for concentration gives **Equation S5**. Inserting c=0 gives **Equation S6** and integration leads to the line function given by **Equation S7**. (**Figure S1**, dotted line) Likewise, it is published, that the brightness of a fluorophore is proportional to $\varepsilon_{Ex}*\Phi$.[3] As shown in the above considerations, this holds true for small optical densities, e.g. small particles or single dye molecules and is used likewise in this study.

$$\frac{I_{Abs}}{I_O} = 1 - 10^{-\varepsilon_{Ex}*c*d} = 1 - 10^{-OD_{Ex}} \quad (\mathbf{S1})$$

$$I_{Fl} = I_{Abs} * \phi \quad (\mathbf{S2})$$

$$I_{Obs} = I_{Fl} * 10^{-\frac{\varepsilon_{Ex}*c*d+\varepsilon_{Em}*c*d}{2}} \quad (\mathbf{S3})$$

$$\frac{I_{Obs}}{I_0} = (1 - 10^{-\varepsilon_{Ex}*c*d}) * \phi * 10^{-\varepsilon_{Em}*c*d} \quad (\mathbf{S4})$$

$$\frac{d\left(\frac{I_{Obs}}{I_0}\right)}{dc} = \ln(10) * \varepsilon_{Ex} * d * \phi * 10^{-\varepsilon_{Em}*c*d-\varepsilon_{Ex}*c*d} - \ln(10) * \varepsilon_{Em} * d * \phi * (1 - 10^{-\varepsilon_{Ex}*c*d}) * 10^{-\varepsilon_{Em}*c*d} \quad (\mathbf{S5})$$

$$\left.\frac{d\left(\frac{I_{Obs}}{I_0}\right)}{dc}\right|_{c=0} = \ln(10) * \varepsilon_{Ex} * d * \phi \quad (\mathbf{S6})$$

$$\frac{I_{Obs}}{I_0} = \ln(10) * \varepsilon_{Ex} * d * \phi * c \quad (\mathbf{S7})$$

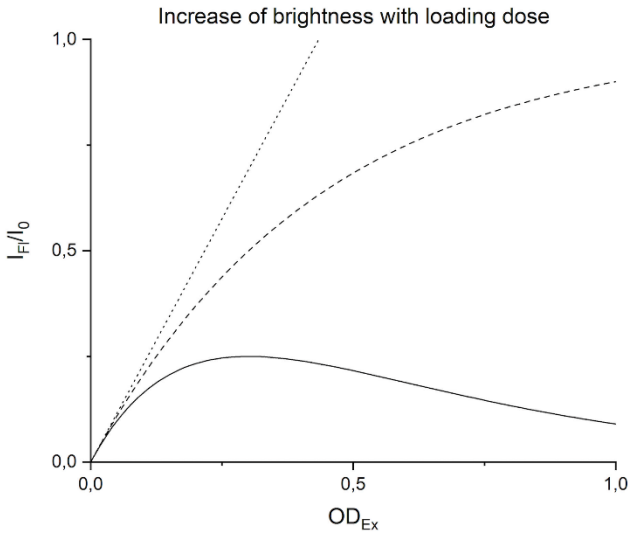


**Figure S1:** *Brightness as function of loading dose, represented by the optical density at excitation wavelength, for $\Phi$=1 and $OD_{Em}$=$OD_{Ex}$ (solid line) or $OD_{Em}$=0 (segmented line), calculated with **Equation 4** and tangent at $OD_{Ex}$=0 (dotted line), calculated with **Equation 7**.*

## F127-COOH characterization

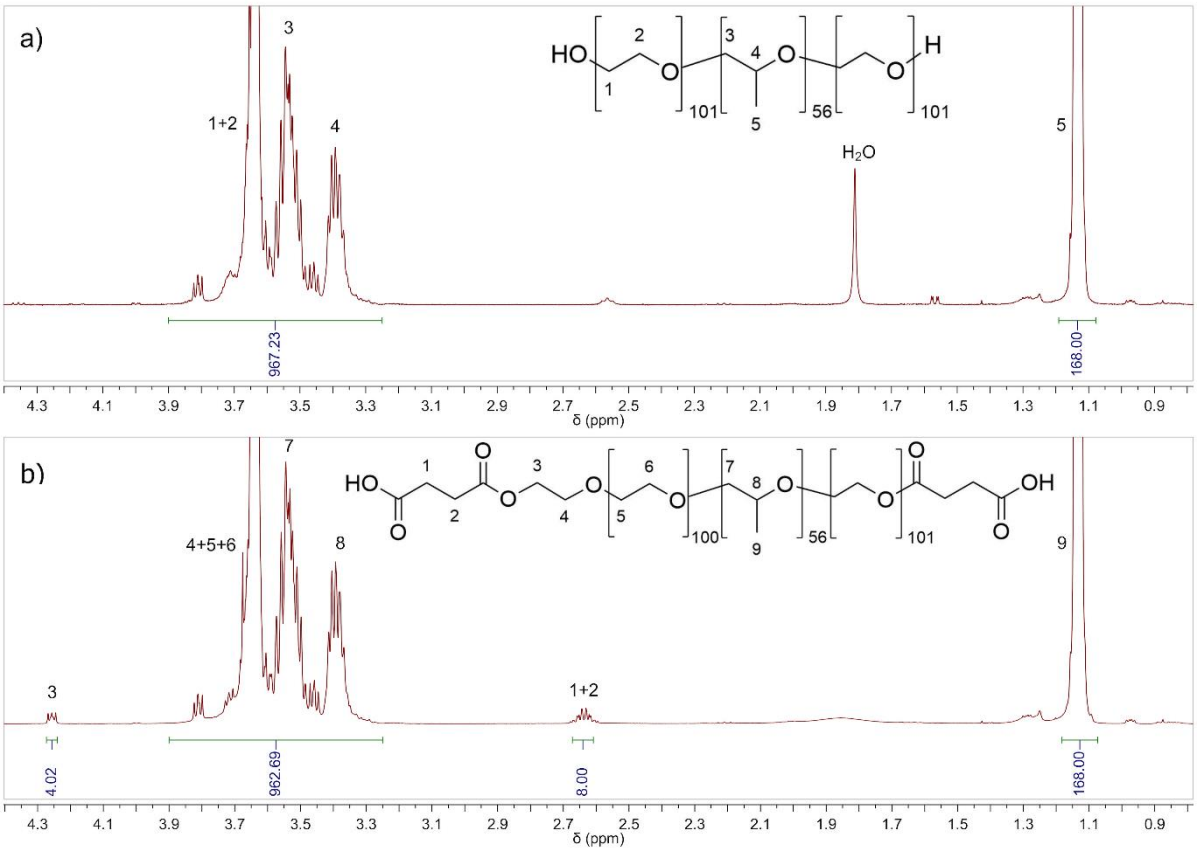


**Figure S2:** *$^1$H-NMR spectrum of Pluronic© F127 (a) and F127-COOH (b), recorded at 400 MHz in $CDCl_3$.*

## Si-NP composition

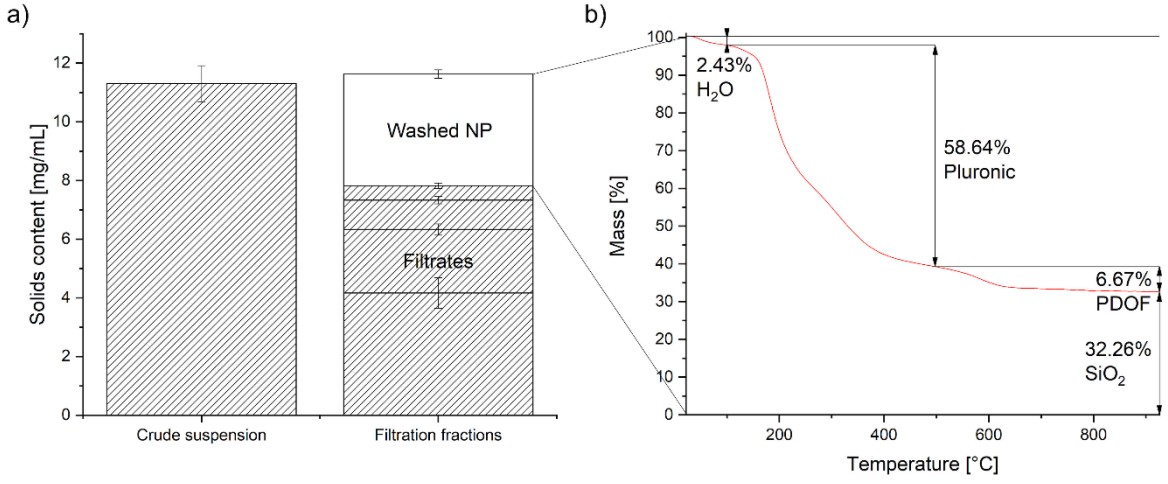


**Figure S3:** *Composition of PDOF-Si-NP suspension with LD=4% after production. Solid content of crude Si-NP compared to that of washed Si-NP plus the dry mass in filtrate fractions (a). Values represent mean ± standard deviation of n=3 Si-NP batches. TGA analysis of the washed NP fraction of a single Si-NP batch following lyophilisation (b).*

## Optimisation of antibody conjugation

For optimisation of the conjugation conditions the reaction was performed as described in the methods section. Rabbit IgG solutions of 551, 367, 184, 50, 25 or 10 µL (55.1, 36.7, 18.4, 5.0, 2.5 or 1.0 µg, 0.1 g/L), corresponding to 75%, 50%, 25%, 75, 35 or 1% of theoretical antibody surface coverage of NP were used. Alternatively, for investigation of the effect of pH on the conjugation reaction, MES buffer (40 µL, 1 M, pH=6.5) was used instead of HEPES together with rabbit IgG solutions of 367, 1834, 73, 25, 13 or 5 µL (73.4, 36.7, 14.7, 5.0, 2.5 or 1.0 µg, 0.2 g/L), corresponding to 100%, 50%, 20%, 7%, 3% or 1% of theoretical antibody surface coverage of NP.

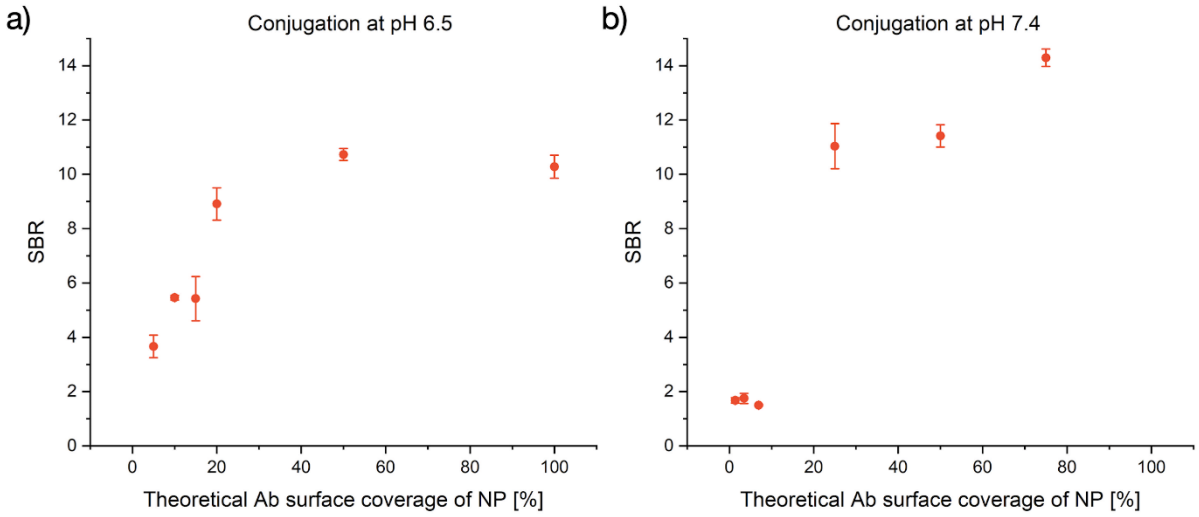


**Figure S4:** *Dipstick LFI signal performance of CN-PPV-Pdots conjugated at pH=6.5 (a) or pH=7.4 (b). SBR versus IgG amount used for conjugation expressed as percentage of NP surface, that would be covered by the Ab in case of complete reaction. Values are mean ± standard deviation of 3 individual test strips.*

## References


(1) Hlaing, M.; Gebear-Eigzabher, B.; Roa, A.; Marcano, A.; Radu, D.; Lai, C. Y. *Opt. Mater. (Amst).* **2016**, *58*, 439–444.

(2) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Lakowicz, J. R., Ed.; Springer US: Boston, MA, 2006.

(3) Lavis, L. D.; Raines, R. T. *ACS Chem. Biol.* **2008**, *3* (3), 142–155.