Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Detecting free hemoglobin in blood plasma and serum with luminescent lanthanide complexes

Frank Morgner, Alexandre Lecointre, Loïc J. Charbonnière and Hans-Gerd Löhmannsröben*

Supplemental Data

Figure S1:

Absorption spectrum of Hb and emission spectrum of Lumi4Tb, calculation of the overlap integral and the Förster radius.

Figure S2:

Normalized emission spectra of Lumi4Tb-Pep as a function of Hb-concentration.

Figure S3:

Hemoglobin-dependent luminescence decays of other LTC used.

Figure S4:

energy transfer sensitized luminescence decay curves of porphyrin in the presence of LTC in buffer, LTC concentration c = 2 nM, increasing porphyrin concentrations (0, 0.07, 0.4, 3.9, 19 µg mL^{-1}), λ_{ex} = 337 nm, λ_{em} = 655 nm.

Figure S5:

Change in LTC luminescence lifetimes τ_D in presence of Hb in dependence of the viscosity of the medium.

Figure S6

Change in LTC luminescence lifetimes τ_D dependent on hemoglobin concentration in blood plasma and buffer.

Figure S7

Chemical structure of the Terbium-complex Tb-Al086-NHS.

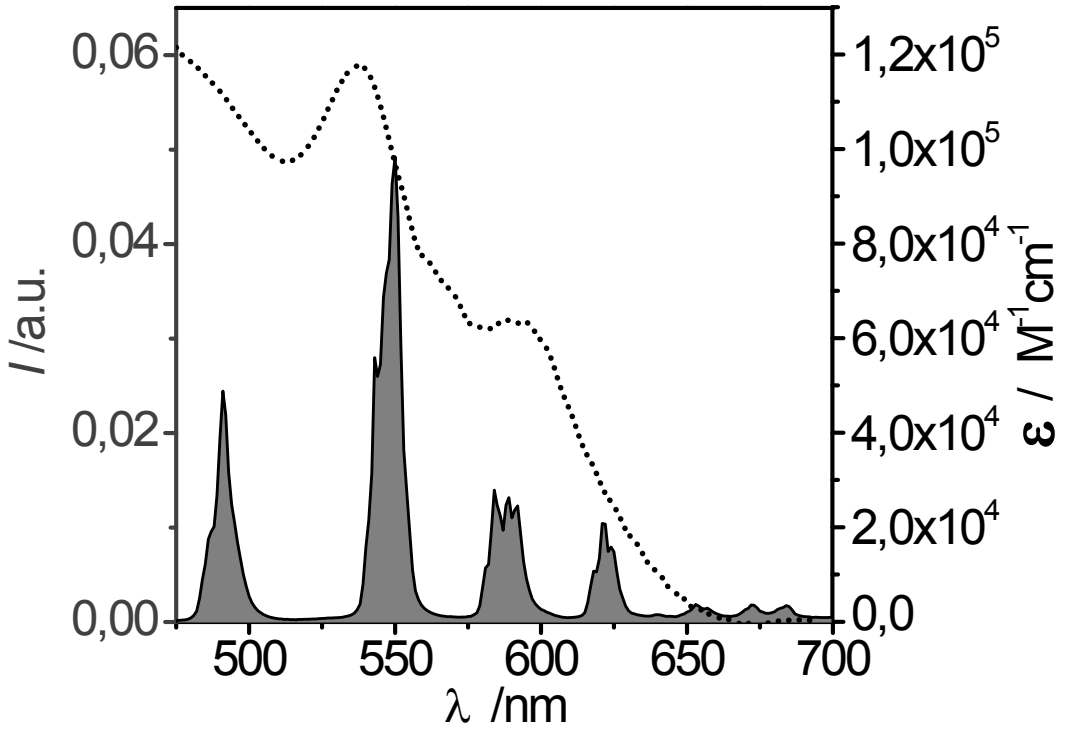


Figure S1

Spectral overlap of absorption spectrum of hemoglobin (dotted black) with the Lumi4Tb emission spectrum (solid grey). The spectral overlap is defined by the overlap integral *J*, with the normalized Lumi4Tb luminescence f_D and the Hb extinction coefficient ε_A. In the equation for calculating the Förster radius R_0 (donor-acceptor distance where FRET is 50 % efficient) *J* is one of the main contributors (besides the donor luminescence quantum yield Φ_D). κ^2 is an orientation factor (which is assumed to be 2/3 for our randomly oriented system), *n* is the refractive index of the surrounding medium and N_{Av} is Avogadro's number.

Overlap integral

$$J(\lambda) = \int_0^\infty f_D(\lambda)\ \varepsilon_A(\lambda)\ \lambda^4\ d\lambda$$

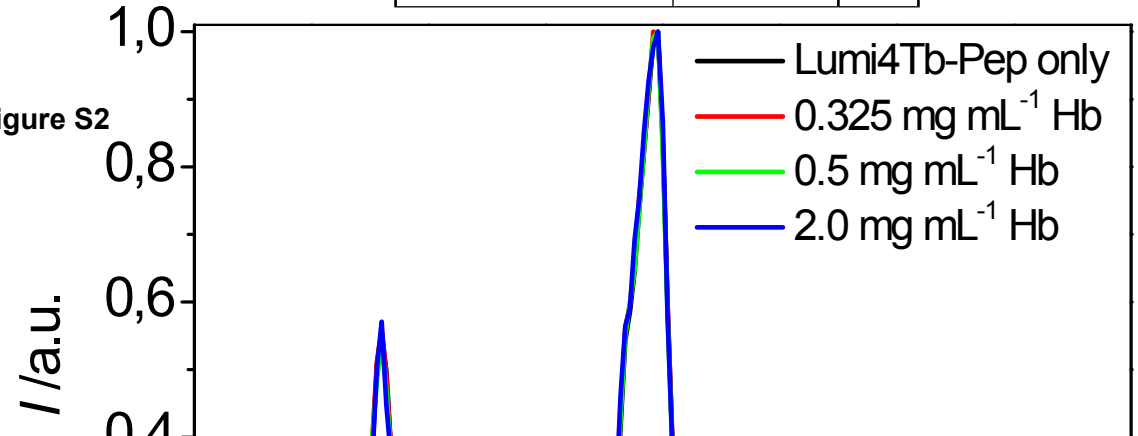
Förster radius

$$R_0^6 = \frac{9000(\ln 10)\Phi_D \kappa^2}{128\pi^5 n_r^4 N_{Av}} J(\lambda)$$

Förster radius R_0 for the FRET system Hb-Lumi4Tb.

	Hb	Unit
Lumi4Tb-NHS	**6.7**	**nm**

Figure S2



Emission spectra of Lumi4Tb-Pep in TRIS-buffer as a function of hemoglobin concentration (λ_{ex} = 340 nm), normalized to maximum of emission. There is no change in spectral shape clearly indicating the absence of reabsorption.

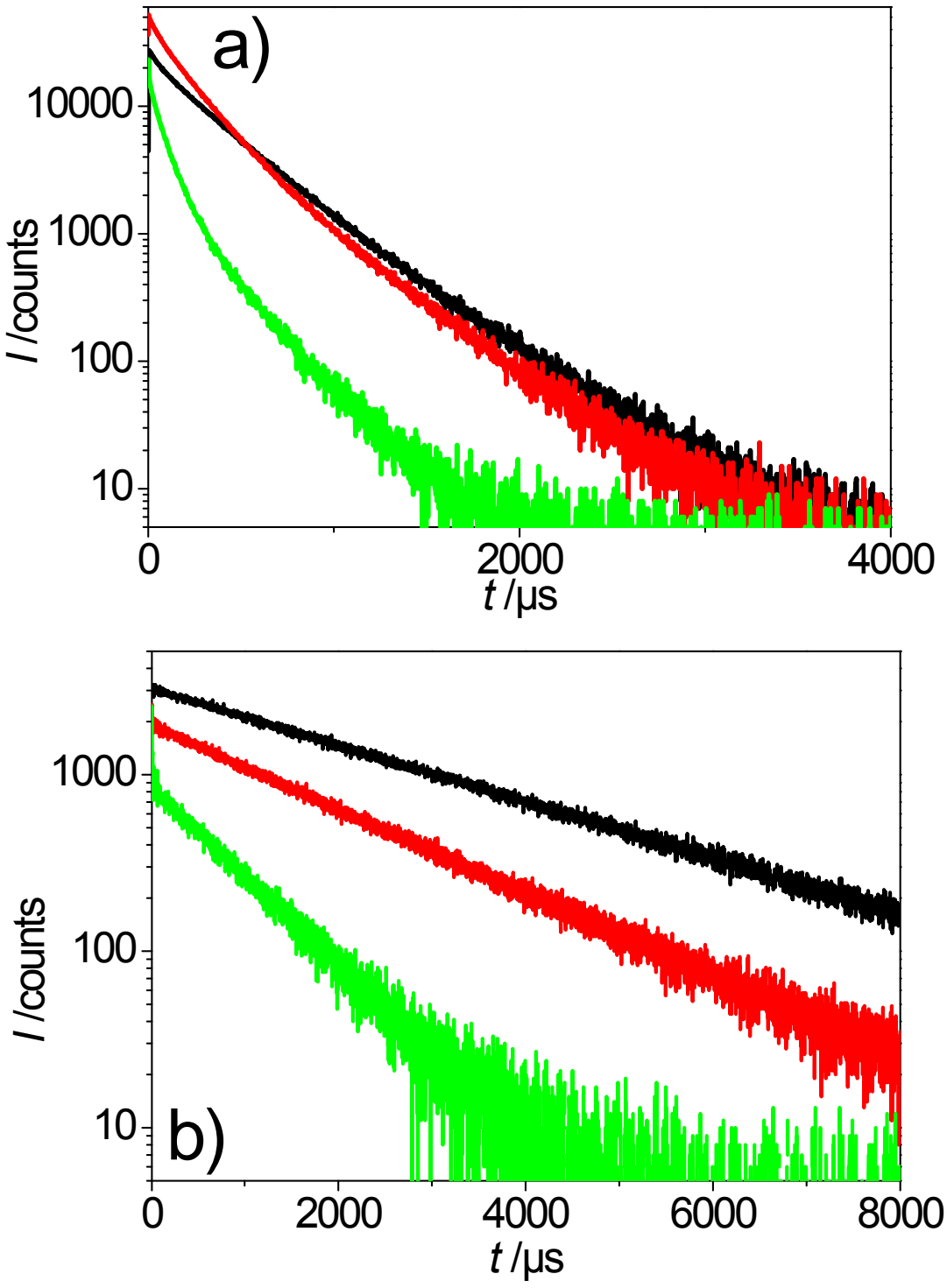
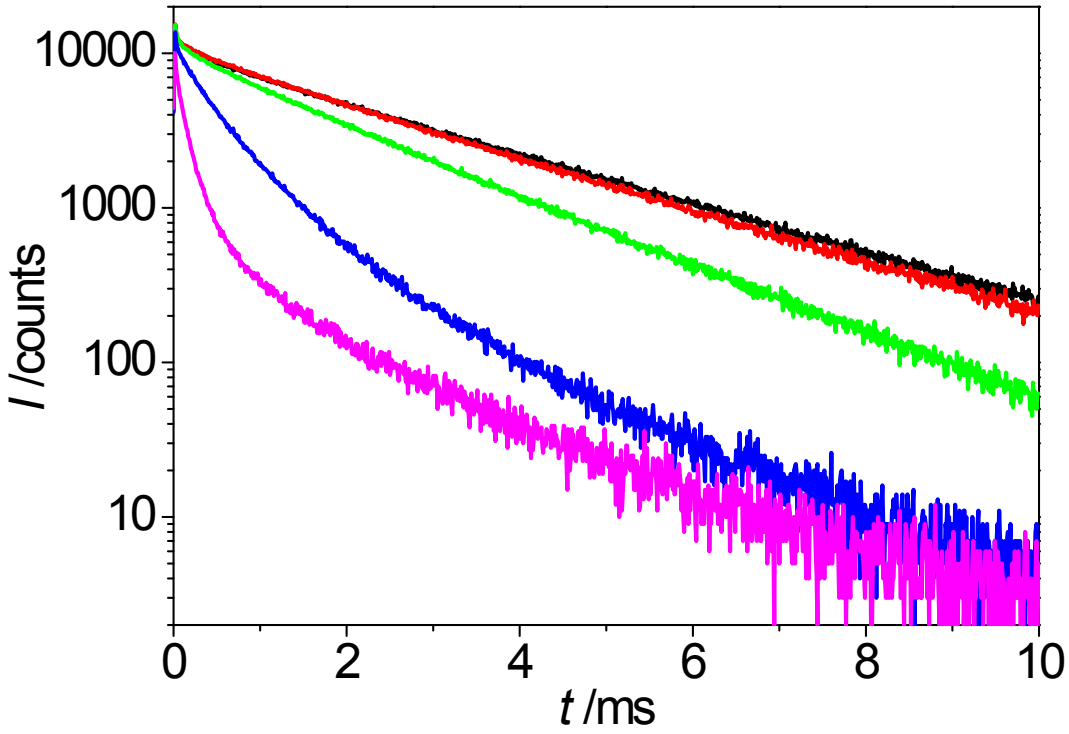


Figure S3

a) Luminescence decay curves of Eu-TBP in the presence of hemoglobin in buffer, Eu-TBP concentration c = 0.2 nM, increasing hemoglobin concentrations (black – 0 mg mL^{-1}, red - 0.5 mg mL^{-1}, green - 2.0 mg mL^{-1}), λ_{ex} = 337 nm, λ_{em} = 620 nm.

b) Luminescence decay curves of Tb-AI086-NHS in the presence of hemoglobin in buffer, LTC concentration c = 0.2 nM, increasing hemoglobin concentrations (black – 0 mg mL^{-1}, red - 0.5 mg mL^{-1}, green - 2.0 mg mL^{-1}), λ_{ex} = 337 nm, λ_{em} = 495 nm.



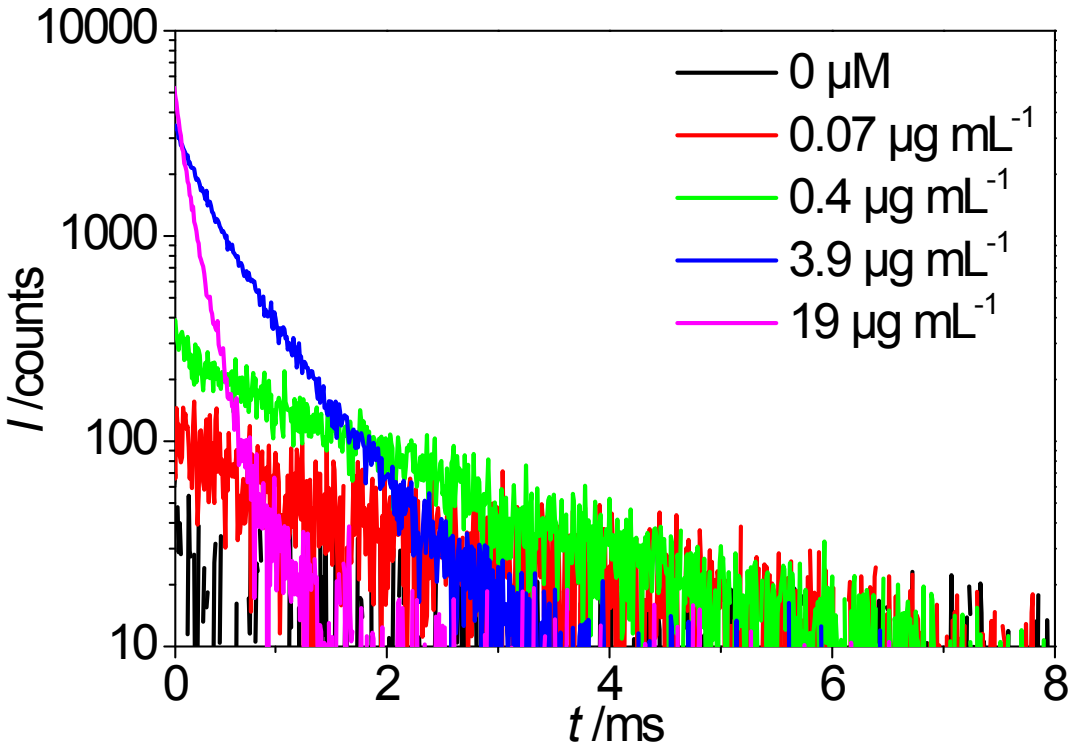


Figure S4

a) Representative luminescence decay curves of LTC (Lumi4Tb-Pep shown) in buffer, LTC concentration c = 2 nM, increasing porphyrin concentrations (black – 0 μg mL^{-1}, red - 0.067 μg mL^{-1}, green - 0.4 μg mL^{-1}, blue - 3.9 μg mL^{-1}, magenta - 19 μg mL^{-1}), λ_{ex} = 337 nm, λ_{em} = 495 nm.

b) Energy transfer sensitized luminescence decay curves of porphyrin in the presence of LTC in buffer, LTC concentration c = 2 nM, increasing porphyrin concentrations (0, 0.07, 0.4, 3.9, 19 μg mL^{-1}), λ_{ex} = 337 nm, λ_{em} = 655 nm.

With the increase in porphyrin concentration LTC luminescence intensity decreases and the luminescence decays become multiexponential. At the same time sensitized emission intensity is increasing for the porphyrin acceptors.

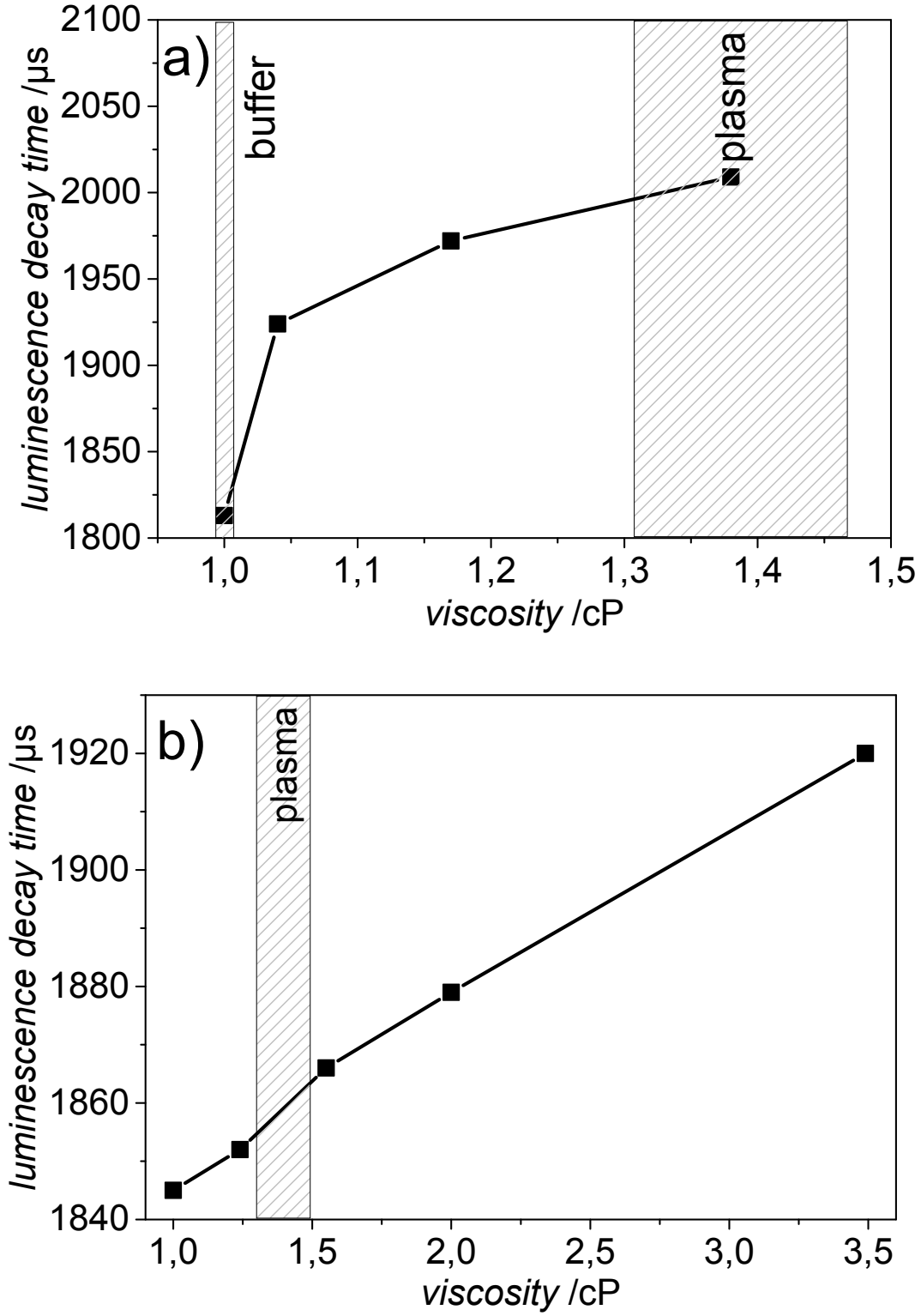


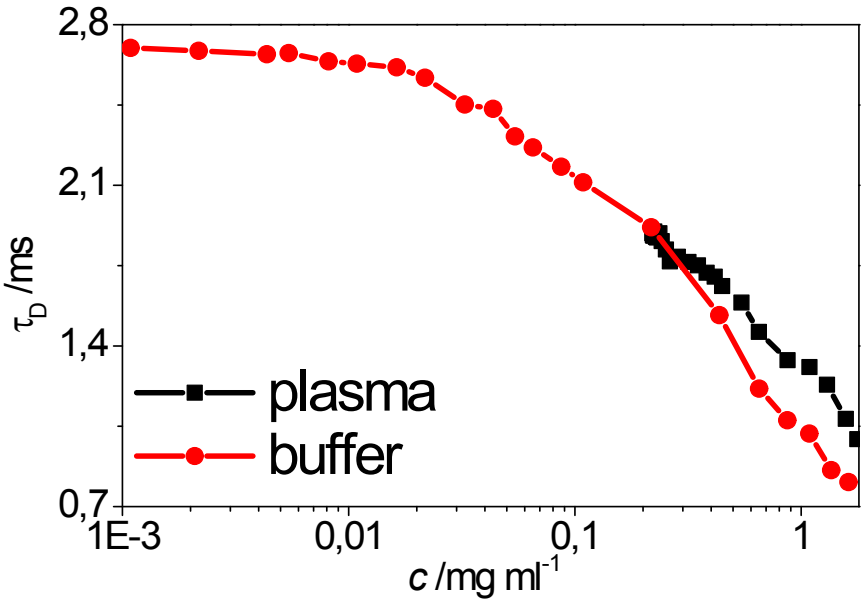
Figure S5

Change in LTC luminescence decay times τ_D in presence of Hb (c = 0.33 mg mL^{-1}) in dependence of viscosity of the medium.

- **a)** in a mixture of water/glycerol
- **b)** in a mixture of water/glycerol containing 8% bovine serum albumin

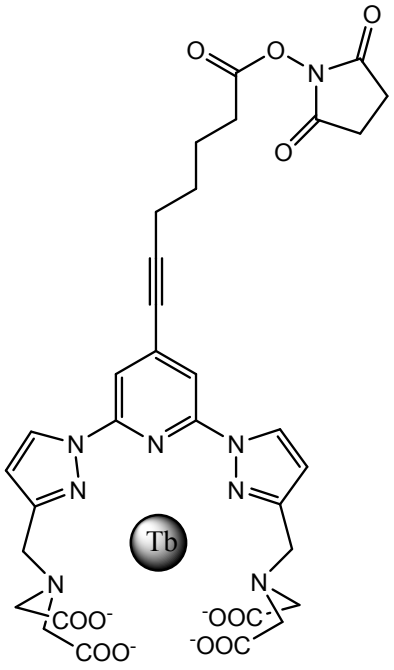
It is obvious that viscosity influences the luminescence decay time of LTC in presence of Hb. The more viscous the medium the longer is the luminescence decay time due to a reduction of the diffusion of the molecules and therefore their interaction.

The effect is more pronounced in pure buffer. Proteins, such as BSA, seem to reduce the influence of viscosity.



Supplemental Data Figure 6

Change in LTC luminescence lifetimes τ_D dependent on hemoglobin concentration in blood plasma and buffer. Due to extraction process plasma always contains some free hemoglobin. For comparison the initial concentration of free hemoglobin was estimated via TMB-method to be 0.24 mg/ml hemoglobin for plasma used. Estimation of initial concentration of free Hb via luminescence lifetimes of LTC yields 0.22 mg/ml which is in very good agreement.



Supplemental Data Figure 7

Chemical structure of the Terbium-complex Tb-Al086-NHS.