

**Total protein concentration quantification using nanobeads**  
**with a new highly luminescent terbium (III) complex**

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SUPPORTING INFORMATION

1. NMR and mass spectroscopic characterization of [(tpip)<sub>3</sub>Tb]-1,4,8,9-tetraazatriphenylene complex - (Tpip)<sub>3</sub>Tb-(TAP)
2. REM images of nanoparticles
3. Results of DLS experiments
4. Results of the robustness test
5. The calculation of Förster radius for ((Tpip)<sub>3</sub>Tb-(TAP))-Cy5 donor-acceptor pair

# 1. NMR and mass spectroscopic characterisation of [(tpip)3Tb]-1,4,8,9-tetraazatriphenylene complex

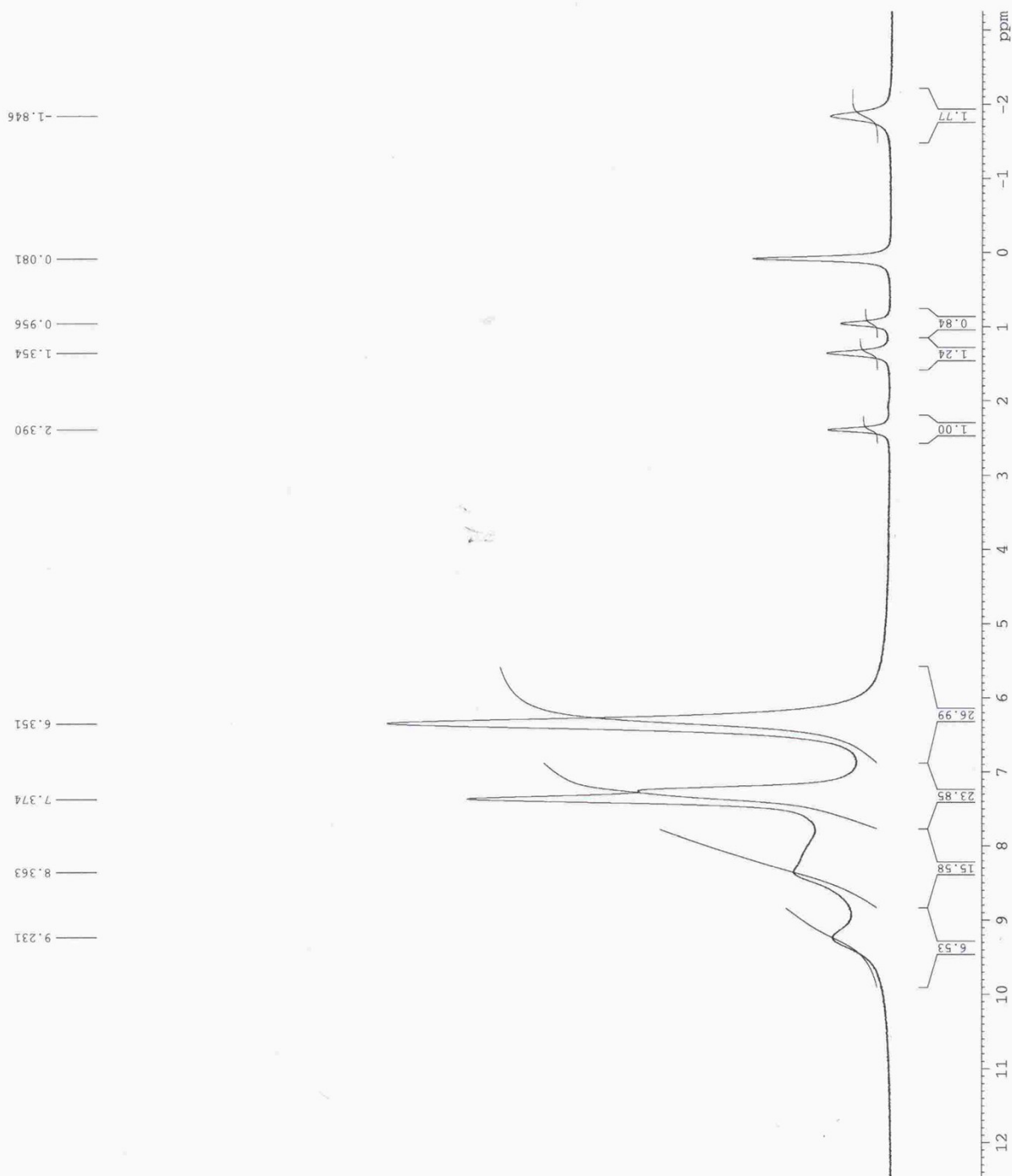


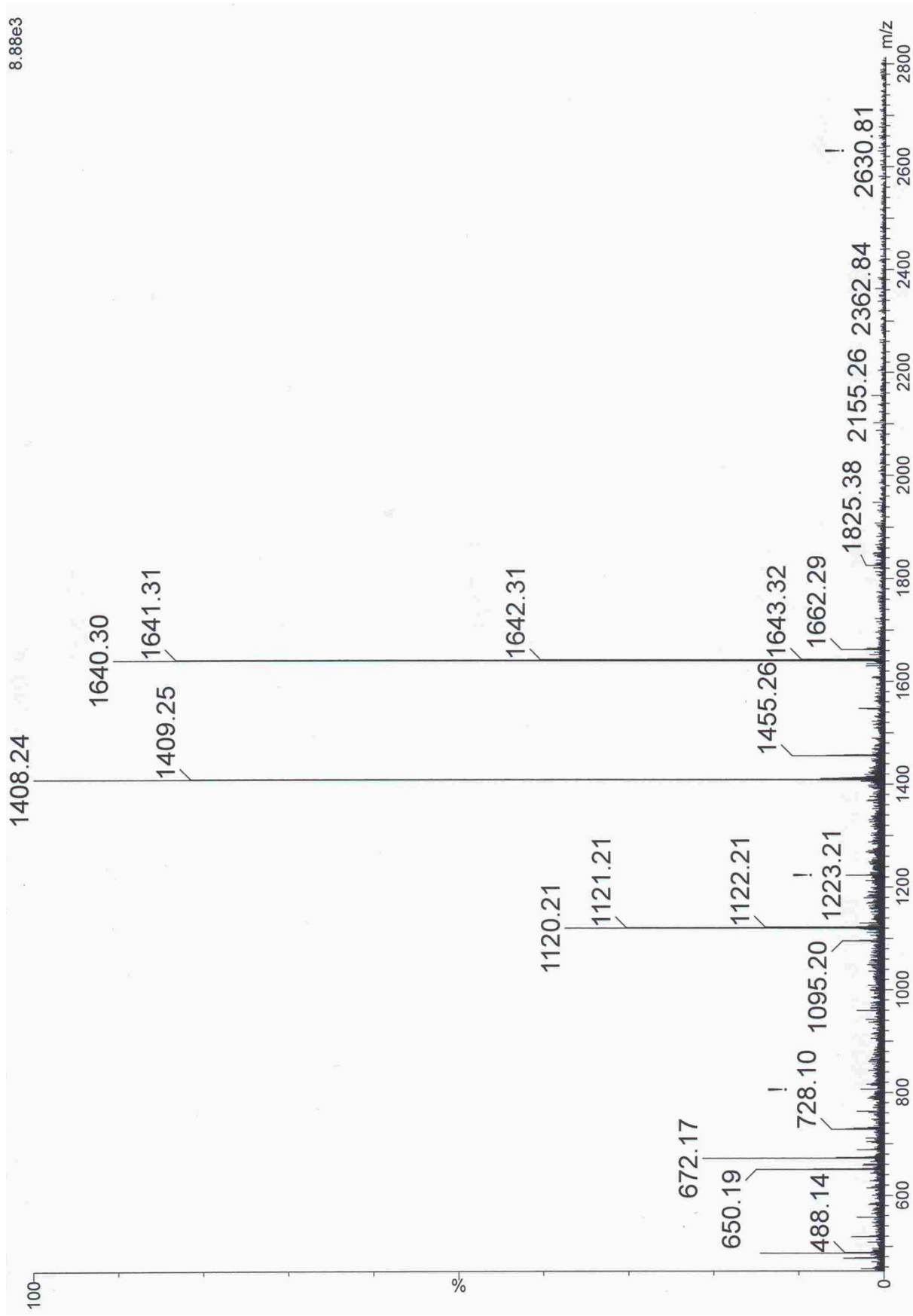
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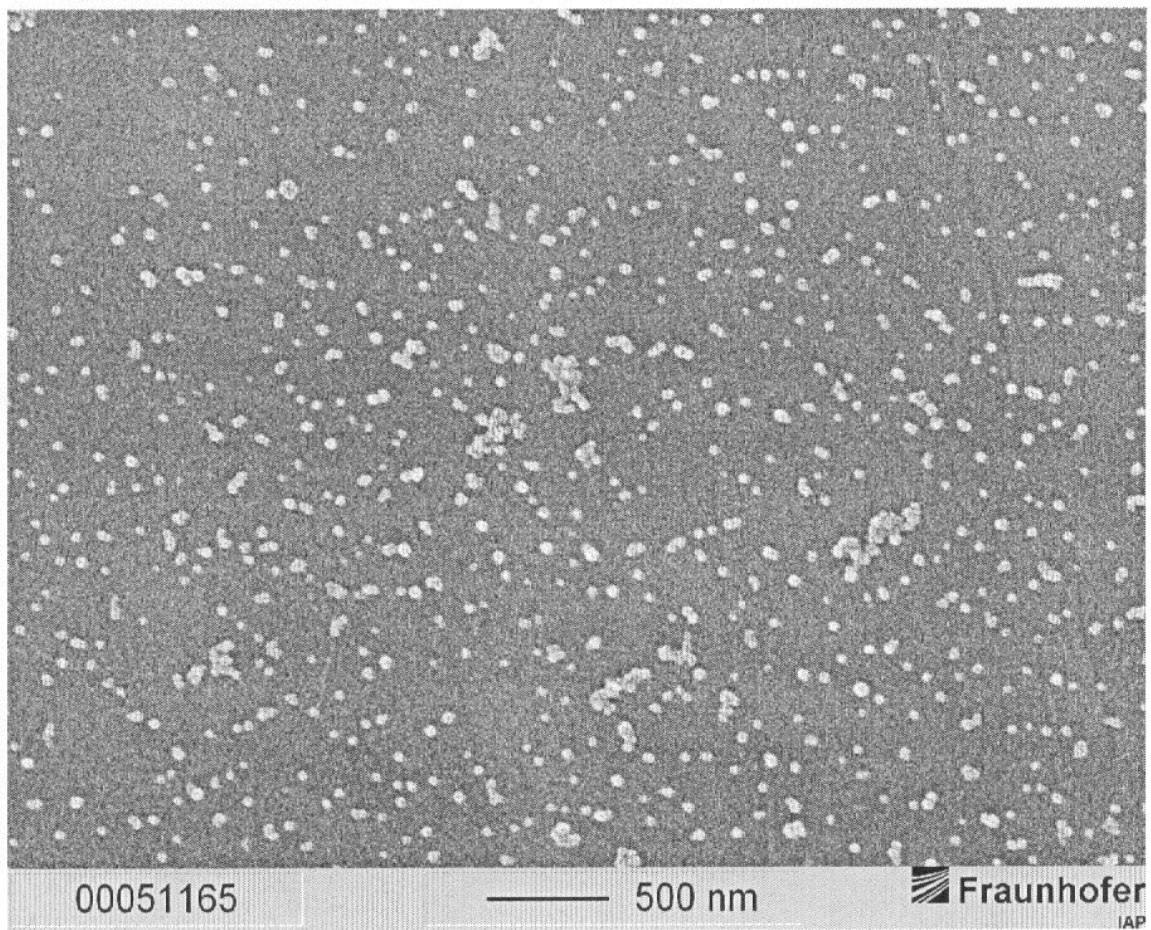
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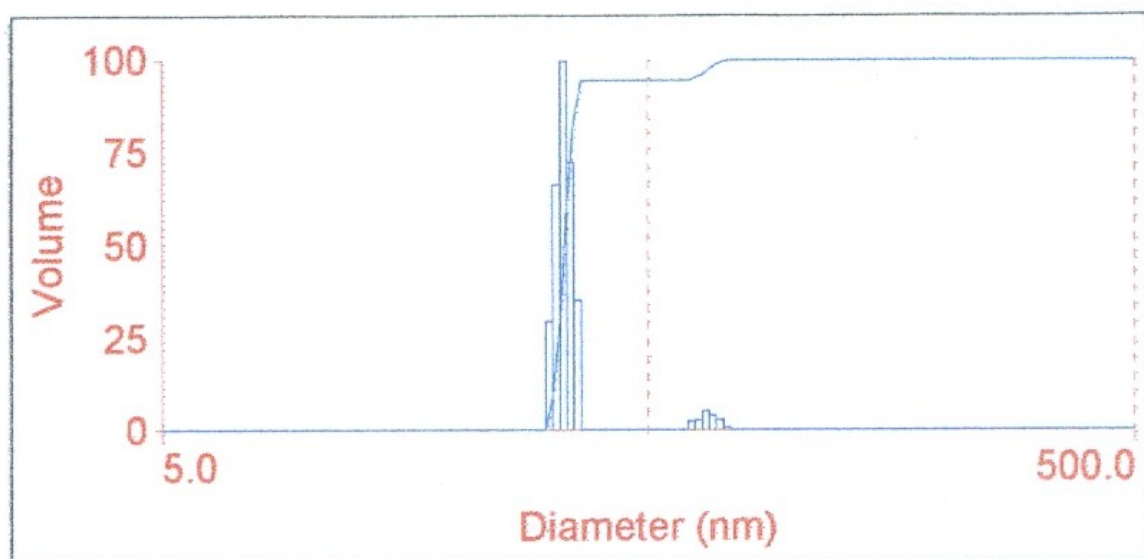




## 2. REM image of nanoparticles



### 3. Results of DLS experiments



**Multimodal Size Distribution**

d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)
27.4	0	0	39.6	0	95	57.3	0	95
28.3	0	0	41.0	0	95	59.3	0	95
29.3	0	0	42.4	0	95	61.3	2	95
30.3	0	0	43.8	0	95	63.4	3	96
31.3	29	9	45.3	0	95	65.6	5	98
32.4	66	30	46.9	0	95	67.8	4	99
33.5	100	61	48.5	0	95	70.1	3	100
34.6	72	84	50.1	0	95	72.5	1	100
35.8	35	95	51.8	0	95	75.0	0	100
37.1	0	95	53.6	0	95	77.6	0	100
38.3	0	95	55.4	0	95	80.2	0	100

#### 4. Results of the robustness test

Compound	Tolerable concentration [v/w %]
Sodium dodecyl sulphate	0.0002%
Cetyl trimethylammonium bromide	0.0003%
Sorbitan trioleate	2%
Polyethylene glycol sorbitan monolaurate	1%
Polyethylene glycol tert-octylphenyl ether	3%

The tolerable concentration is a concentration of a surfactant that resulted in maximum 20% change in the signal.

#### 5. The calculation of Förster radius for the (Tpip)3Tb-(TAP)-Cy5 donor-acceptor pair

The Förster radius  $R_0$  for the ((Tpip)3Tb-(TAP))-Cy5 donor-acceptor pair has been determined using following equation:

$$R_0 = \sqrt[6]{\frac{9\Phi_0(\ln 10)\kappa^2}{128\pi^5 n^4 N_A} J(\lambda)}$$

where  $\Phi_0$  is the donor quantum yield equal to 0.5 (determined using integrating sphere),  $\kappa^2$  is the dipole orientation factor taken as equal to 2/3 (random dipole orientation),  $N_A$  is the Avogadro's number ( $6.02 \times 10^{23} \text{ mol}^{-1}$ ) and  $n$  is the refractive index of the surrounding medium (water  $n = 1.33$ ). Based on the above equation and that the spectral overlap  $J(\lambda)$  is  $(3.2 \pm 0.10) \times 10^{15} \text{ L mol}^{-1} \text{ cm}^{-1} \text{ nm}^4$ , the Forster radius  $R_0$  for the (Tpip)3Tb-(TAP)-Cy5 donor-acceptor pair was found to be  $(5.6 \pm 0.1) \text{ nm}$ .