Copper(II)quenched oligonucleotide probes for fluorescent DNA sensing

Jens Brunner and Roland Kraemer*

Supplementary material

Figure Captions

Scheme 1: Solid-phase syntheses of oligo **1**, **2**, **3**

Scheme 2: Synthesis of oligo **4**

Figures 1, 2, 3, 4: MALDI-TOF spectra of DNA **1**, **2**, **3** and **4** are given

General remarks: The solution of 50% saturated 6-aza-2-thiothymine in acetonitrile and 50% 0.1 M diammoniumcitrate in water was used as a matrix for MALDI-TOF analysis of the DNA conjugates. MALDI-TOF mass spectra were recorded on a Bruker BIFLEX III spectrometer.

Figure 5: Fluorescence recovery of Cu(II) quenched **1** by competing ligand 1,2-diaminoethane

General remarks: The fluorescence experiments were done on a Varian Eclipse Fluorescence Spectrophotometer with a Hellma 50 µl fluorescence cuvette. The exitation wavelength was λ = 494 nm, spectra were obtained from λ = 500 to 600 nm.

Figure 6: Fluorescence of Cu(II) quenched oligo **2** on addition of complementary **DNA 1**

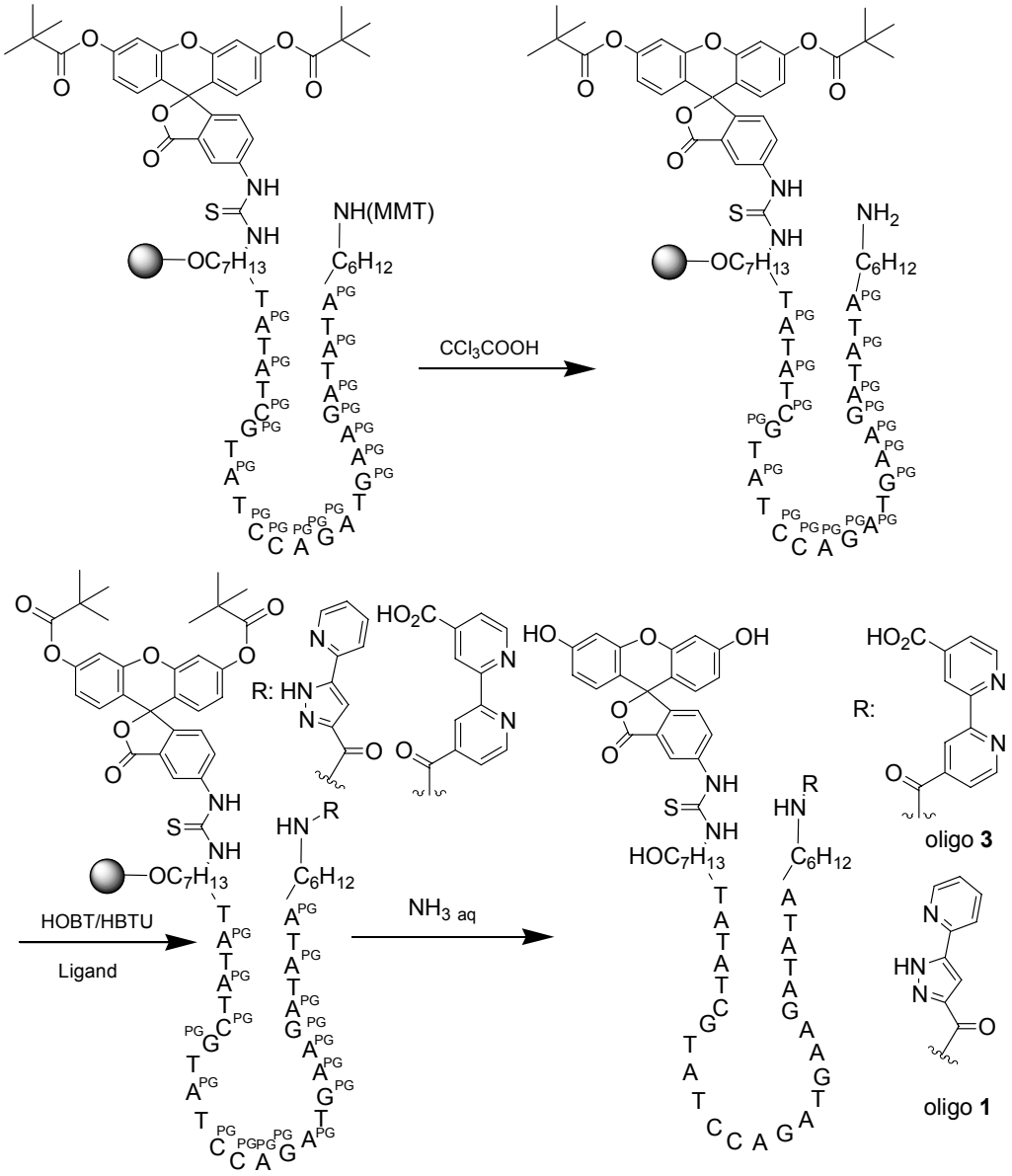
Figures 7, 8, 9, 10: Fluorimetric titrations of oligo **1**, oligo **2**, oligo **3** and oligo **4** with Cu(II)

General remarks: The fluorescence experiments were done on a Varian Eclipse Fluorescence Spectrophotometer with a Helma 50 µl fluorescence cuvette. The exitation wavelength was λ = 494 nm, spectra were obtained from λ=500 to 600 nm. The pyrene modified oligo **4** was excited at λ = 340 nm and spectra were obtained from λ = 350 to 550 nm.

Figures 11, 12: Comparison of optical and fluorescence spectra of oligo **1** and of calcein on titration with Cu(II)

General remarks: The UV-Vis absorption measurements were done on a Varian Cary 100 Bio Spectrophotometer. The fluorescence experiments were done on a Varian Eclipse Fluorescence Spectrophotometer. The exitation wavelength was λ = 494 nm, spectra were obtained from λ = 500 to 600 nm. Titration experiments were performed with a 50 µl fluorescence cuvette for oligo **1** and a 1000 µl fluorescence cuvette for Calcein.

Solid-phase syntheses of oligo **1,** oligo **2** and oligo **3**

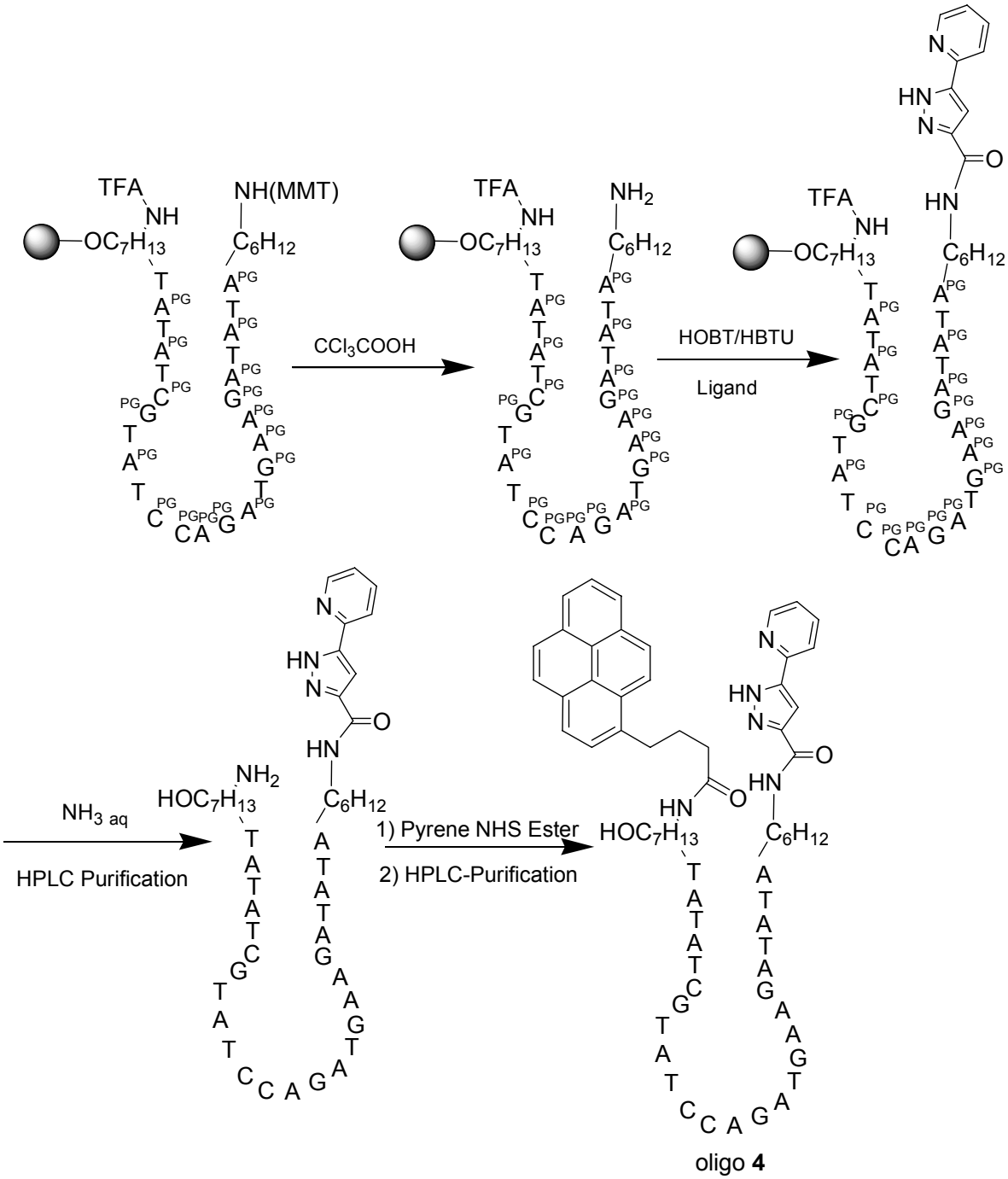


PG: Cytidine - benzoyl protected
Adenosine - benzoyl protected
Guanosine - isobutyryl protected

Scheme 1: Synthesis of oligo **1**, **2**, **3**; the 3´-fluorescein-5´- MMT protected aminolink hairpin-DNA on a CPG solid support was purchased from Metabion, Planegg-Martinsried (Germany). 1-Hydroxybenzotriazole (HOBT) was purchased from Acros Organics and O-(Benzotriazol-1-yl)-N,N,N´,N´-tetramethyluronium hexafluorphosphate (HBTU) was purchased from Fluka. The Ligand 2,2´-Bipyridine-4,4´-dicarboxylic acid was purchased from Sigma-Aldrich and Pyridyl-pyrazolyl carboxylic acid preparation was described earlier[11]. HPLC was performed at room temperature on a Shimadzu liquid chromatograph

equipped with UV-Vis detector and column oven. HPLC was done on a EC 250 x 4.6 mm Nucleosil 300-5 C4. Gradients of CH_3CN (solvent B) and 0.1M triethylammoniumacetate in water (solvent A) were used: 1min at 0% B; in 20 min from 0%-30% B; in 6min from 30%-90% B; 10min at 90% B.

Synthesis of oligo **4**



oligo **4**

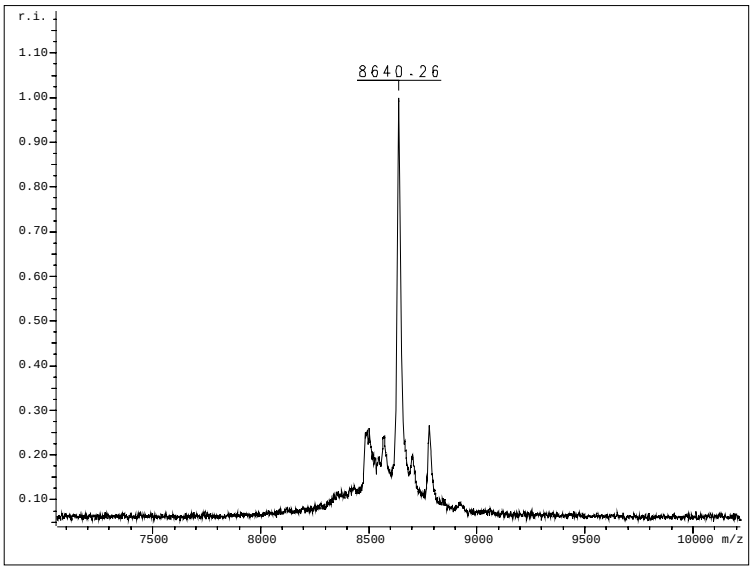
PG: Cytidine - benzoyl protected

Adenosine - benzoyl protected

Guanosine - isobutyryl protected

Scheme 2: Synthesis of oligo **4**; the 3´-TFA(Trifluoracetic acid) protected C7-aminolink and 5´- MMT protected C6-aminolink hairpin-DNA on a CPG solid support was purchased from Metabion, Planegg-Martinsried (Germany). 1-Pyrenebutyric acid N-hydroxysuccinimide ester was purchased from Sigma-Aldrich. 1-Hydroxybenzotriazole (HOBT) was purchased from Acros Organics and O-(Benzotriazol-1-yl)-N,N,N´,N´-tetramethyluronium hexafluorphosphate (HBTU) was purchased from Fluka. The preparation of the ligand Pyridyl-pyrazolyl carboxylic acid was described earlier[11]. HPLC was performed at room temperature on a Shimadzu liquid chromatograph equipped with UV-Vis detector and column oven. HPLC was done on a EC 250 x 4.6 mm Nucleosil 300-5 C4. Gradients of CH_3CN (solvent B) and 0.1M triethylammoniumacetate in water (solvent A) were used: 1min at 0% B; in 20 min from 0%-30% B; in 6min from 30%-90% B; 10min at 90% B.

MALDI-mass spectra of oligo **1**, oligo**2**, oligo **3** and oligo **4**



Figue 1: MALDI Spectrum of oligo **1** M_{calc}=8638 ($M\text{-}H^+$) ; M_{found}=8640

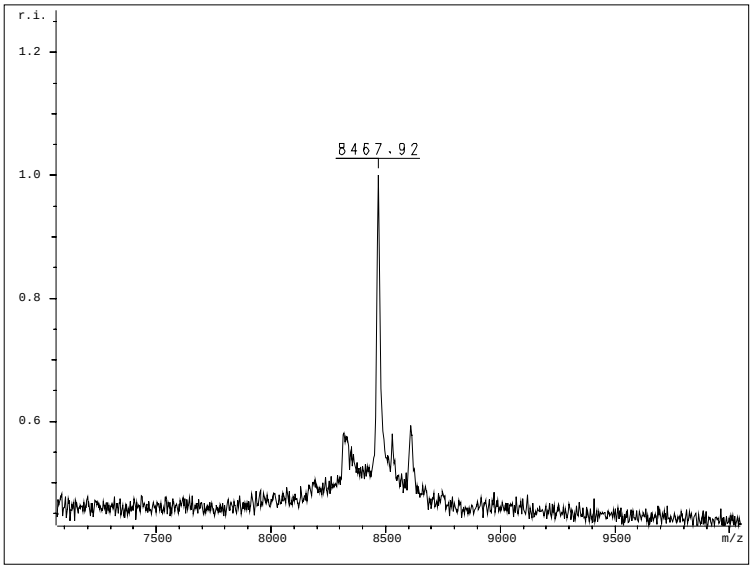


Figure 2: MALDI Spectrum of oligo **2** M_{calc}=8467 ($M\text{-}H^+$) ; M_{found}=8468

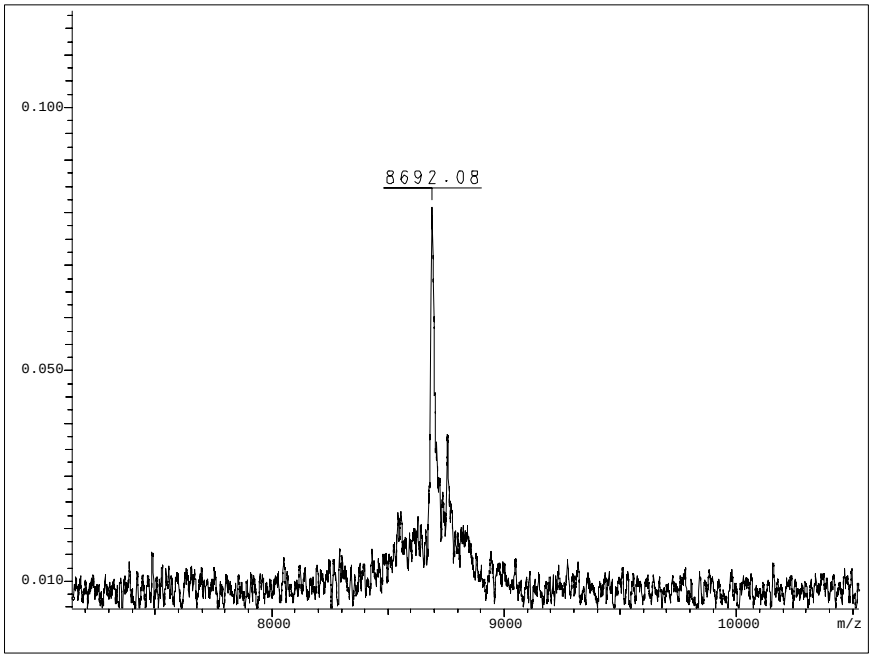


Figure 3: MALDI Spectrum of oligo **3** M_{calc}=8691 ($M\text{-}H^+$) ; M_{found}=8692

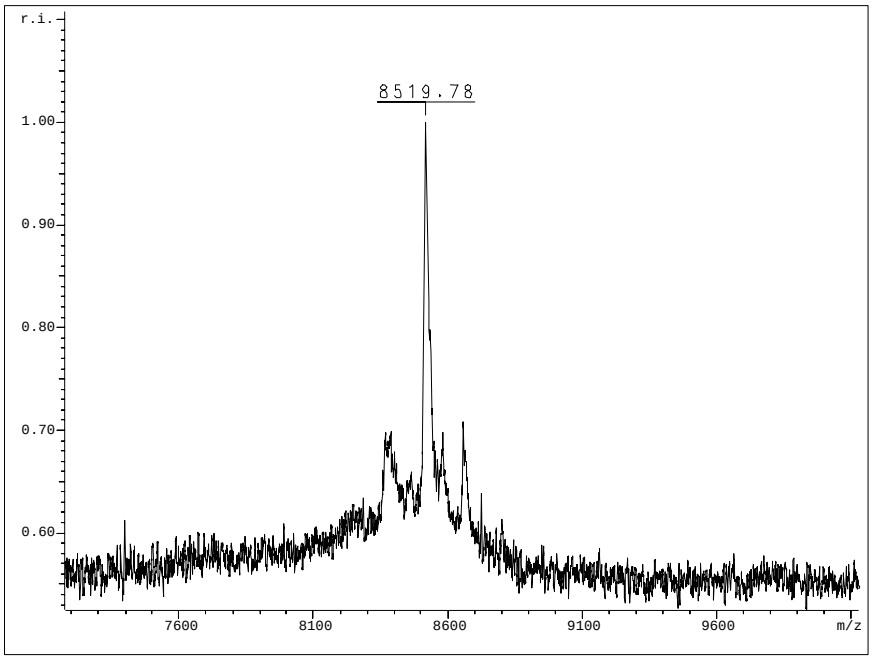


Figure 4: MALDI Spectrum of oligo **4** M_{calc}=8516 ($M\text{-}H^+$); M_{found}=8520

Fluorescence recovery of Cu(II) quenched **1** by competing ligand 1,2-diaminoethane

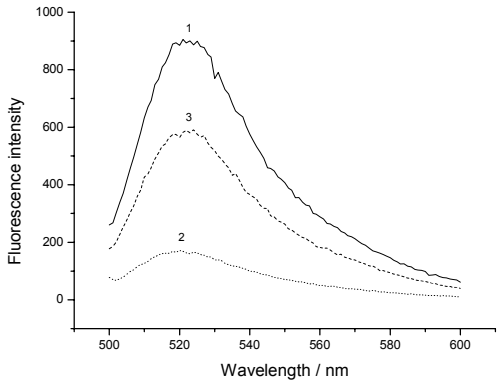
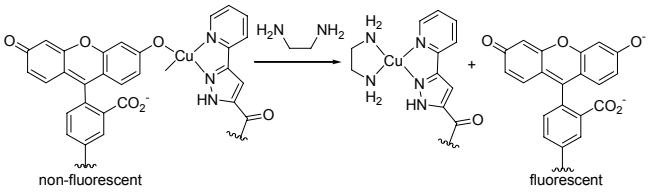


Figure 5: Fluorescence intensities: 0.1 µM oligo **1** (1), addition of 5 µM Cu(II)SO_4 (2), addition of 5 µM 1,2-diaminoethane (3), 10 mM MOPS pH 7, 1 M NaCl, T = 25°

We suggest that the quenching process is triggered by intramolecular coordination of a phenolate donor of fluorescein, which is more basic and therefore a better donor than carboxylate [1], to a free coordination site of the (pypz) Cu^{2+} moiety of **1**. This is indicated by 58% recovery of fluorescence when 1,2-diaminoethane (en, 5 µM) is added to the solution containing 5 µM Cu^{2+} and 0.1 µM **1**. Formation of a ternary complex [Cu^{2+}(**1**)(en)] is expected which compares well to the complex [Cu^{2+}(2,2´-bipyridine)(en)] for which crystallographic [2] data are available: the four in-plane sites of copper are blocked by the two bidentate chelators and not available for coligand coordination. 58 % quenching-inactive ternary complex [Cu^{2+}(**1**)(en)] and 42 % quenching active [Cu^{2+}(**1**)] fit a calculated species distribution using published stability data of the en/Cu^{2+} system [3], a Cu^{2+}(**1**) formation constant logK = 6.3 (see above), and assuming for the association of **1** and Cu^{2+}(en) a logK value of 5.9, somewhat smaller than the Cu^{2+} binding constant of **1** [4].



Fluorescence quenching by intramolecular fluorescein coordination at Cu^{2+}(**1**)

[1] At pH 7 the nonfluorescent carboxylate monoanion (pK_a=4.3) of fluorescein is in equilibrium with the strongly fluorescent carboxylate+phenolate dianion, pKa = 6.7 at 21°C and 70 mM NaCl, S. A. Smith, W. A. Pretorius, *Water SA*, **2002**, *28*, 395-402.

[2] C. C. Su, Y. L. Lin, S. J. Liu, T. H. Chang, S. L. Wang, L. F. Liao, *Polyhedron,* **1993**, *12*, 2687-2696.

[3] R. Griesser, H. Sigel, *Inorg. Chem.,* **1970**, *9*, 1238-1243. The program „Species Ver. 1.2" by L.D. Pettit, Academic Software **1999**, was used for calculation of species distribution.

[4] This is a reasonable assumption since the association constant for 2,2'-bipyridine and Cu^{2+}(en) is 1.2 logK values smaller than for 2,2'-bipyridine and Cu^{2+} (logK=8) [3].

Fluorescence of Cu(II) quenched oligo **2** on addition of complementary **DNA 1**

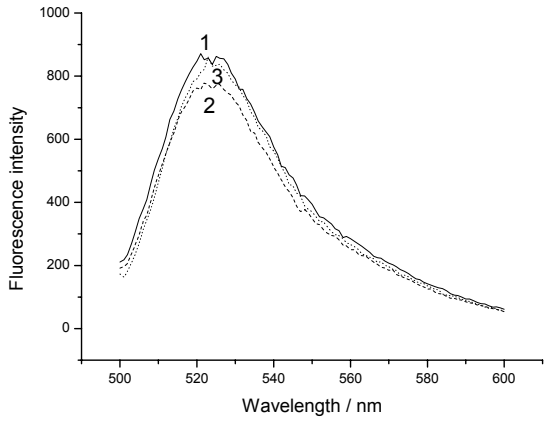


Figure 6: Quenching experiments with oligo **2**: 0.1 µM oligo **2** (1), addition of 5 µM Cu(II)SO_4 (2), addition of 0.5 µM **DNA 1** (3); 10 mM MOPS pH7, 1 M NaCl, T=25°

Only 5% quenching was observed for oligo **2** after addition of $CuSO_4$ due to unspecific coordination of Cu^{2+} Ions to nucleobases.

Fluorimetric titrations of oligo **1**, oligo **2**, oligo **3** and oligo **4** with Cu(II)

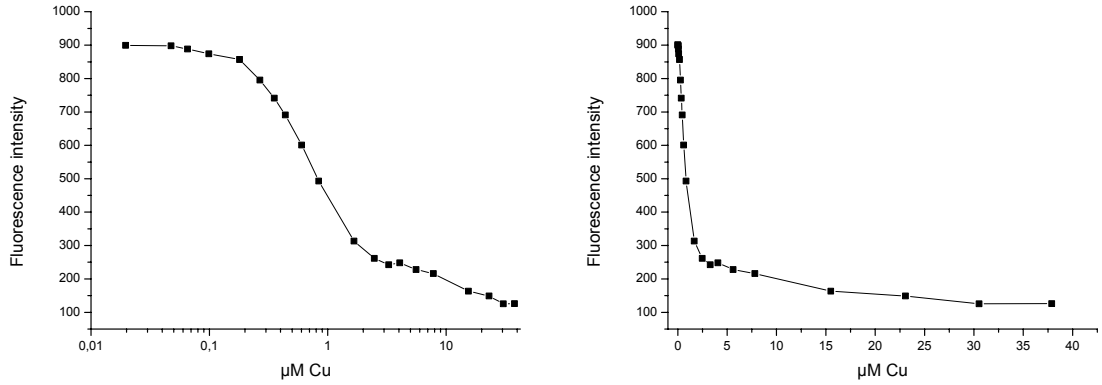


Figure 7: Fluorimetric titration (excitation at λ = 494 nm, emission at λ = 522 nm) of oligo **1** (0.1 μM) with $CuSO_4$, logarithmic scale (left), linear scale (right). 10 mM MOPS pH 7, 1 M NaCl, T = 25°

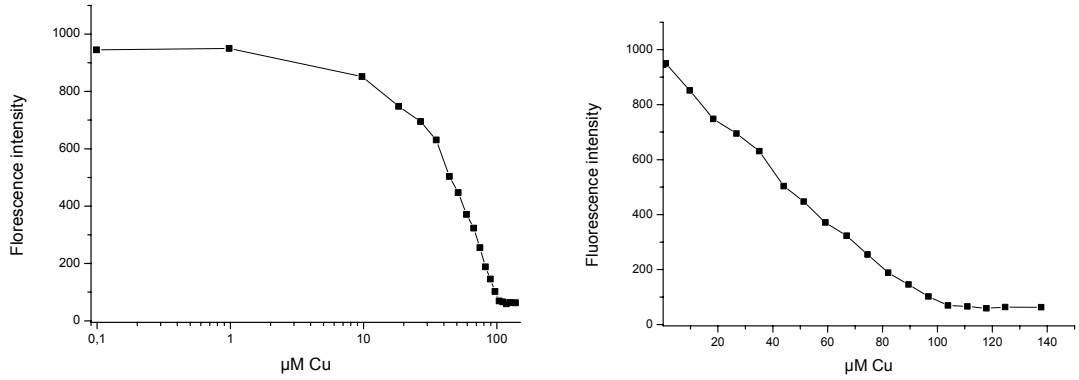


Figure 8: Fluorimetric titration (excitation at λ = 494 nm, emission at λ = 522 nm) of oligo **2** (0.1 μM) with $CuSO_4$, logarithmic scale (left), linear scale (right). 10 mM MOPS pH 7, 1 M NaCl, T = 25°

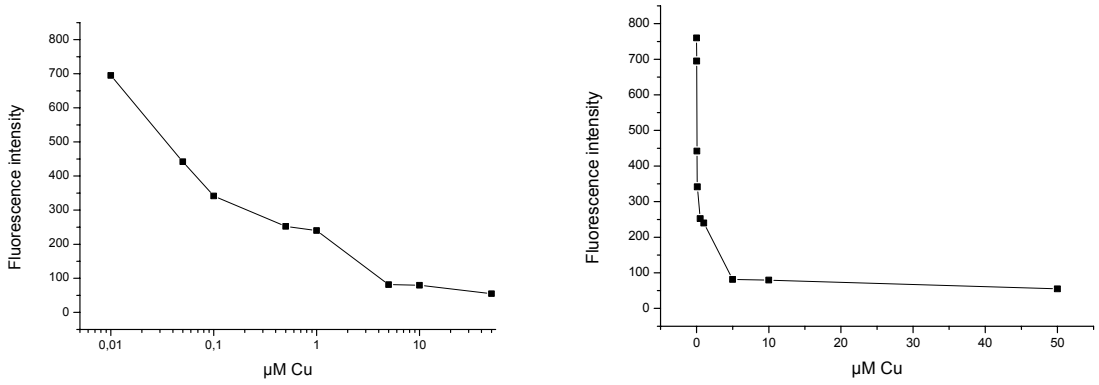


Figure 9: Fluorimetric titration (excitation at λ = 494 nm, emission at λ = 522 nm) of oligo **3** (0.1 µM) with $CuSO_4$, logarithmic scale (left), linear scale (right). 10 mM MOPS pH 7, 1 M NaCl, T = 25°

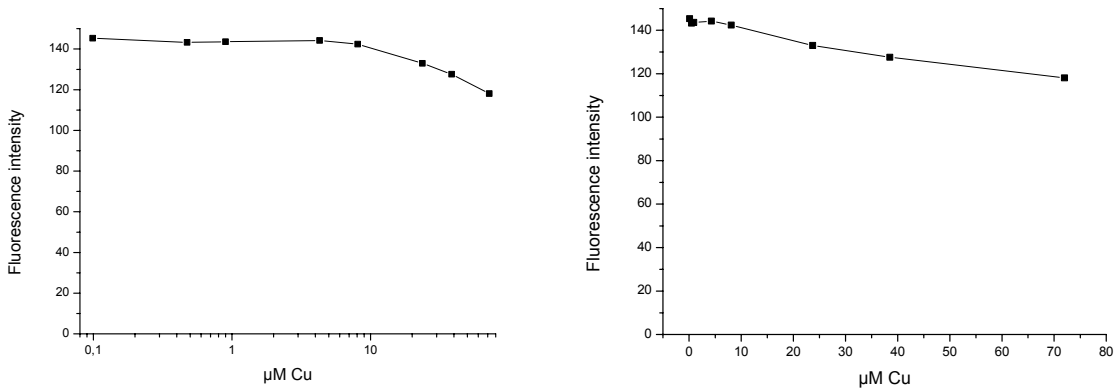


Figure 10: Fluorimetric titration (excitation at λ = 340 nm, emission at λ = 381 nm) of oligo **4** (0.1 µM) with $CuSO_4$, logarithmic scale (left), linear scale (right). 10 mM MOPS pH 7, 1 M NaCl, T = 25°

Fluorimetric and photometric titrations of calcein and oligo **1** with Cu(II)

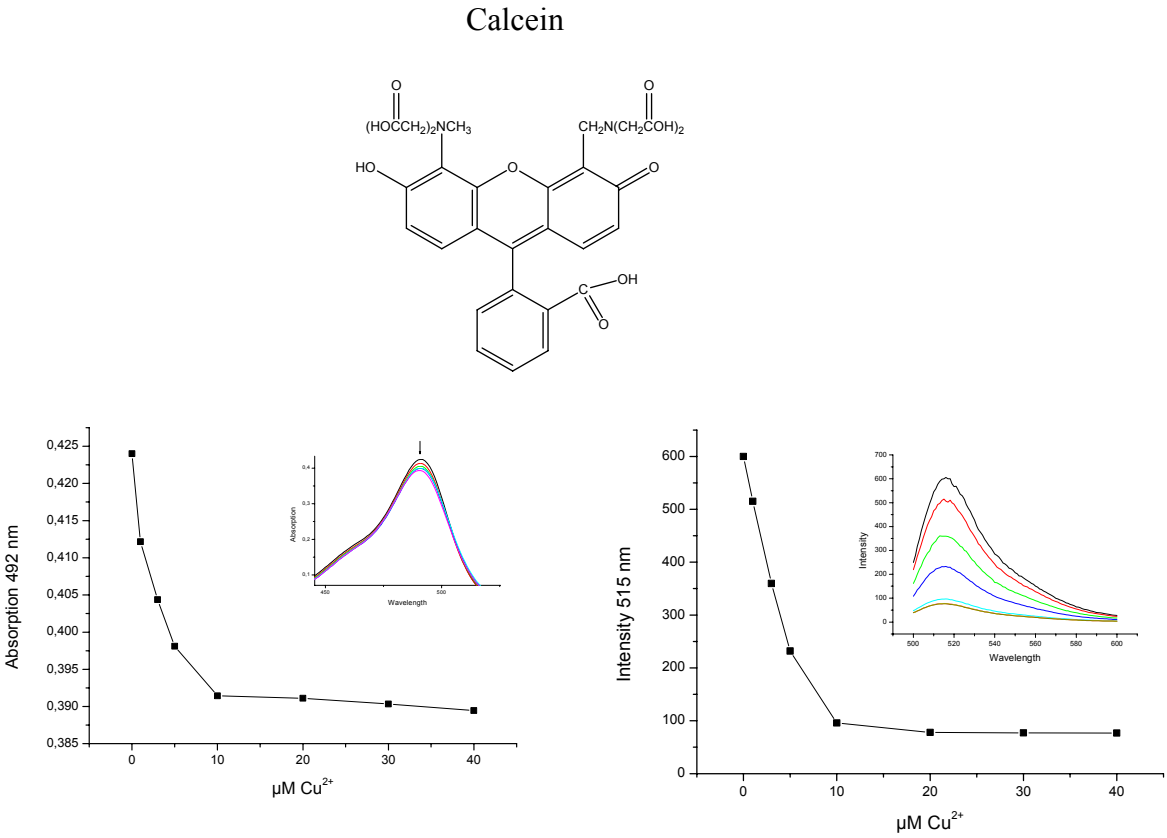


Figure 11: top: Structure of Calcein ; bottom left: Copper(II) titration absorption measurement of Calcein; bottom right: Copper(II) titration fluorescence measurement of Calcein; 10 µM Calcein, 10 mM MOPS pH7, 1 M NaCl, T = 25°C

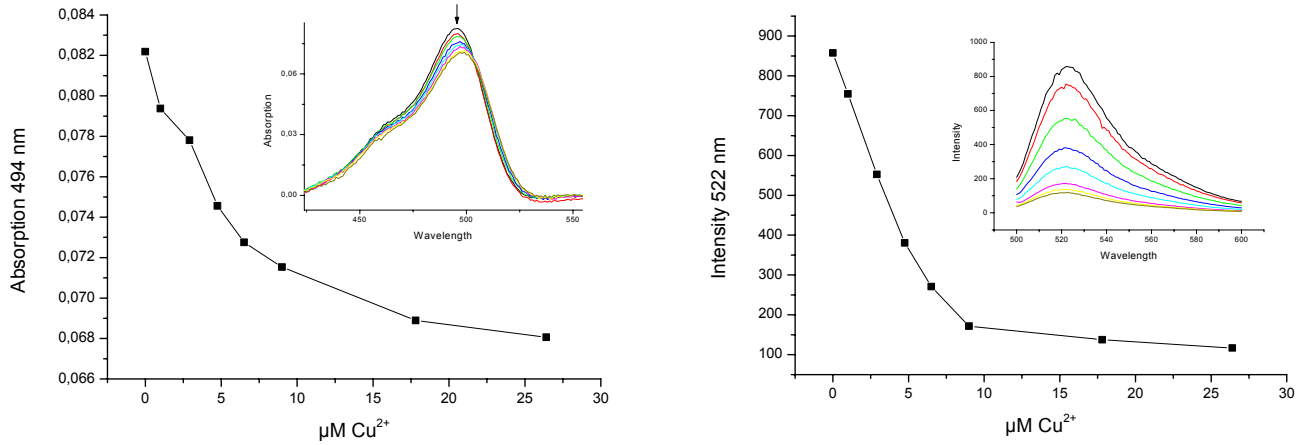


Figure 12: left : Copper(II) titration absorption measurement; right: Copper(II) titration fluorescence measurement; 5 µM oligo**1**, 10 mM MOPS pH7, 1 M NaCl, T = 25°C

Absorption and fluorescence spectra of calcein (Fig. 7) and oligo **1** (Fig. 8) show similar decrease of absorption and fluorescence after adition of $CuSO_4$. In case of strong Cu^{2+} binder Calcein, a 1:1 complex is apparently formed quantitatively on addition of 1 equivalent metal ion (= 10 μM). In case of oligo **1**, decrease of optical optical absorbance and fluorescence is more sluggish due to weaker Cu(II) binding by the pyridyl-pyrazole chelator.