Selective detection of Hg^{2+} in the microenvironment of double-stranded DNA with an intercalator crown-ether conjugate†‡

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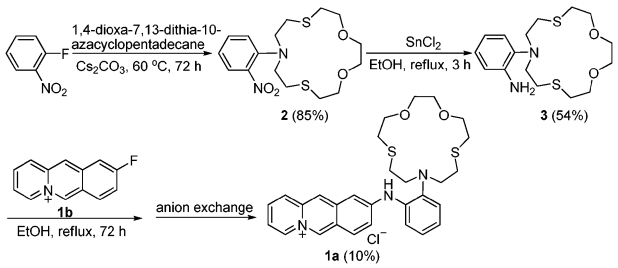
9-[2-(1,4-Dioxa-7,13-dithia-10-azacyclopentadecyl)phenyl]amino-benzo[*b*]quinolizinium enables the unambiguous fluorimetric and polarimetric detection of Hg^{2+} in the close proximity of double-stranded nucleic acids without interfering background signals from the complexes of this compound with Hg^{2+} or DNA alone.

Metal–DNA interactions have attracted much interest in nucleic acids chemistry due to their impact on the structure and function of DNA.[1] In particular, relatively soft metal cations, such as Hg^{2+}, associate reversibly with the endocyclic nitrogen atoms of purines and pyrimidines.[1c,2] Besides the acute toxic effects of Hg^{2+} ions, which are usually associated with protein–mercury interactions, the genotoxic effects due to the interactions of DNA with Hg^{2+} may lead to serious long-term health problems.[2a,3] For example, an increase of the concentration of heavy metals in the vicinity of DNA coincides with the formation of malignant tumors.[4] Considering the application of fluorescence spectroscopy as analytical tool in biochemistry and medicinal chemistry,[5] fluorescent probes may be employed to study the interactions between Hg^{2+} and DNA and the influence on physiological processes.[6] Nevertheless, such fluorescent probes need to be able to detect the proximate DNA or Hg^{2+} components at the same time, whereas the interaction of the probe with the separate components alone should not result in a fluorimetric response to avoid background signals. A resembling approach has been reported, in which the Hg^{2+}-mediated formation of T–Hg^{2+}–T base pairs induces the aggregation of DNA–nanoparticle conjugates, thus enabling the selective *colorimetric* detection of Hg^{2+}.[7] However, considering the higher sensitivity of fluorimetric detection as compared with photometric analysis, the development of complementary fluorimetric probes is still an attractive goal. Herein, we demonstrate that indeed the fluorescent probe **1a**, which exhibits a DNA-binding benzo[*b*]quinolizinium unit as well as a Hg^{2+}-sensitive receptor unit, may be employed as selective light-up probe for the simultaneous detection of Hg^{2+} and DNA.

Compound **1a** was synthesized by a 3-step reaction sequence (Scheme 1, *cf.* ESI†). Thus, nucleophilic substitution of *o*-fluoronitrobenzene with 1,4-dioxa-7,13-dithia-10-azacyclopentadecane and subsequent reduction of the nitro group by $SnCl_2$ yielded the aniline derivative **3**. Nucleophilic substitution of 9-fluorobenzo[*b*]quinolizinium **1b** with the aniline derivative **3** gave the benzo[*b*]quinolizinium–crown ether conjugate **1a**. After anion exchange (Cl^-), the product **1a** was isolated in 10% yield by column chromatography and subsequent recrystallization.



Scheme 1 Synthesis of the *N*-aryl-9-aminobenzo[*b*]quinolizinium **1a**.

The benzo[*b*]quinolizinium **1a** exhibits the characteristic absorption properties of *N*-aryl-9-aminobenzo[*b*]quinolizinium derivatives[8] with a broad long-wavelength absorption band at 400 nm in water, along with an unstructured CT band between 430 nm and 500 nm (*cf.* ESI†). This compound shows very low fluorescence quantum yields (<0.002) with the band maximum between 514 nm (water) and 533 nm (chloroform). The complexation of Hg^{2+} ions by **1a** with Hg^{2+} or ct DNA was determined by spectrophotometric titration in aqueous buffer solution (HEPES, 25 mM, pH 7.0; Fig. 1). Thus, the addition of Hg^{2+} induced a significant decrease of the absorption and a blue shift (7 nm) of the long-wavelength absorption maxima, most likely due to the decreased donor properties of the diaminophenyl substituent upon complexation of Hg^{2+}. At the same time, a new broad band developed between 480 nm and 580 nm, which indicates a pronounced charge-transfer process. Presumably, the complex formation induces a conformation in which the phenyl ring and the benzo[*b*]-quinolizinium chromophore are arranged towards each other such that a photoinduced charge transfer takes place efficiently. The plot of the absorption at 398 nm *versus* concentration of Hg^{2+} was fitted to a 1 : 1 binding stoichiometry with a

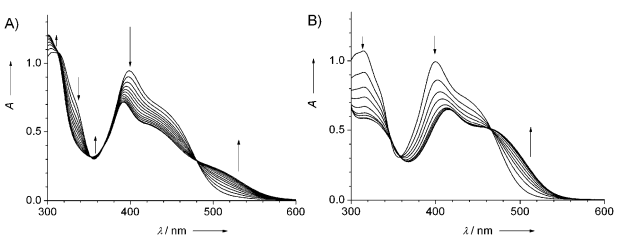


Fig. 1 Spectrophotometric titration of Hg^{2+} (A) or ct DNA (B) to **1a**. The arrows indicate the changes of the bands during titration. Conditions: c_{1a} = 50 μM; HEPES, 25 mM, pH 7.0; T = 20 °C.

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‡ This paper is dedicated to Professor Harald Günther, University of Siegen, on the occasion of his 75th birthday.

binding constant $K_b = 1.0 \times 10^5\ M^{-1}$ (*cf.* ESI†, Fig. S1).[9] The benzo[*b*]quinolizinium **1a** exhibits low quantum yields in water and in organic solvents such as MeOH, DMSO, CH_3CN, CH_2Cl_2, and $CHCl_3$ ($\phi_{fl} < 0.002$) which do not change significantly upon addition of Hg^{2+} (*cf.* ESI† Table S2). Moreover, the emission quantum yield of **1a** is also very low in media which resemble the physiological conditions in the cytosol with respect to the high concentration of biopolymers. Thus, the emission quantum yield of **1a** under such crowding conditions, as realized by a concentration of 40% w/v polyethylene glycol (PEG 400), is 0.001 and does not change upon addition of Hg^{2+} (*cf.* ESI† Table S2).

The addition of ct DNA to **1a** in HEPES buffer led to a significant decrease of the absorbance and to a red shift of 15 nm of the long-wavelength absorption maximum, which is characteristic of a DNA-binding process of cationic aromatic ligands.[10] The data from the photometric titration were represented as Scatchard plot and fitted to the neighbor-exclusion model[11] to give the binding constant $K_b = 1.6 \times 10^5\ M^{-1}$ and a binding site size $n = 1.3$ (*cf.* ESI†, Fig. S2). The association of **1a** with DNA was confirmed by CD spectroscopy.[12] Thus, at lower ligand-to-DNA ratio ($c_{ligand}/c_{DNA} = 0.1$) a negative ICD signal was observed in the long-wavelength absorption range of **1a** (Fig. 2A, line c) which usually indicates an intercalative binding mode of benzo[*b*]-quinolizinium derivatives with the long molecule axis parallel to the long axis of the intercalation pocket.[12,13] In contrast, at higher ligand-to-DNA ratios ($c_{ligand}/c_{DNA} = 0.2$), a bisignate signal pattern was observed with a maximum at 496 nm and a minimum at 440 nm (Fig. 2A, line b) which usually originates from additional exciton coupling of ligand aggregates that associate along the DNA backbone.[12,13] This change of binding mode with varying dye–DNA ratio is in accordance with the slight fading of isosbestic points at 467 nm during the photometric titration (Fig. 1B).

The addition of Hg^{2+} to a solution of the ligand **1a** and ct DNA (ligand-to-DNA ratio: 0.15) led to a blue-shift (14 nm) of the absorption maxima of **1a** and to a strong decrease of the broad long-wavelength absorption band (Fig. 2B). The latter indicates that—other than in the **1a**–Hg^{2+} complex—the charge transfer between the aminophenyl substituent and the benzo[*b*]quinolizinium unit is no longer effective in the ternary complex between **1a**, DNA and Hg^{2+}. The plot of the absorption at 475 nm *versus* the concentration of Hg^{2+} was fitted to a 1 : 1 binding stoichiometry (**1a** : Hg^{2+}) with a binding constant of $K_b = 1.54 \times 10^6$ M (*cf.* ESI†, Fig. S3). The addition of Hg^{2+} to an aqueous solution of **1a** in the presence of ct DNA also led to a significant change of the ICD bands of the ligand. Specifically at a ligand-to-DNA ratio of 0.2, the bisignate ICD signal develops into a negative band, whose maximum is blue shifted by 8 nm (Fig. 2A, line d). These observations indicate that the intercalated ligand **1a** is still able to bind Hg^{2+}. Specifically, the CD-spectroscopic analysis of the ternary complex revealed that the ligand remains in the binding site of DNA after complexation of Hg^{2+}, because the ICD band of the intercalated ligand is maintained during titration (*cf.* ESI†, Fig. S4). To be stressed is the observation that even at a ligand-to-DNA ratio of 0.2 no bisignate ICD signal is observed, either because the ligand does not form aggregates at the DNA backbone in the presence of Hg^{2+}, or the aggregates do not induce a CD signal due to the lack of the CT absorption.[12] Most notably, the addition of Hg^{2+} to an aqueous solution containing both **1a** and ct DNA resulted in a significant enhancement of the fluorescence intensity by a factor of *ca.* 8 and a blue shift (17 nm) of the fluorescence maximum (Fig. 3A). In contrast, the emission intensity of the benzo[*b*]quinolizinium **1a** increases only slightly upon addition of Hg^{2+} (0.1 mM). And upon addition of DNA (0.15 mM, without Hg^{2+}) the maximum of the weak fluorescence band of **1a** is red shifted by 34 nm with essentially no enhancement of the intensity (Fig. 3B). Moreover, it was demonstrated that RNA does not interfere with the fluorimetric detection of Hg^{2+}. Thus, in the presence of both Hg^{2+} and RNA, the emission properties of **1a** change only to a small extent, whereas the addition of ct DNA to this mixture resulted in an increase of the fluorescence intensity (*cf.* ESI†, Fig. S5). Notably, the light-up effect of **1a** in the presence of DNA and Hg^{2+} was also observed under crowding conditions (*cf.* ESI†, Table S2). To assess the selectivity of the fluorescence response of the DNA-bound ligand **1a** towards selected cations, the fluorescence of **1a** was determined in the presence of ct DNA and excess of Cu^{2+}, Co^{2+}, Cd^{2+}, Ag^{+}, Zn^{2+}, Ni^{2+}, Ca^{2+}, Pb^{2+} (0.1 mM for each case), K^{+} (100 mM), and Mg^{2+} (10 mM). Notably, only Ag^{+} induced a fluorescence enhancement and interferes with the fluorimetric analysis of Hg^{2+}, whereas the other tested cations led to insignificant changes of the fluorescence intensity (*cf.* ESI†, Fig. S6, Table S3).

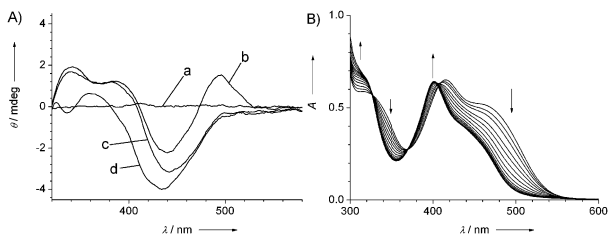

Fig. 2 A: CD spectra of **1a** ($c = 5 \times 10^{-5}$ M) with ct DNA; a: control; b: [ligand]/[DNA] = 0.2; c: [ligand]/[DNA] = 0.1; d: [ligand]/[DNA] = 0.2 and subsequent addition of Hg^{2+} ($c_{Hg} = 5 \times 10^{-5}$ M). B: Spectrophotometric titration of Hg^{2+} to **1a** in the presence of ct DNA in aqueous buffer (HEPES, 25 mM, pH 7.0; $c_{1a} = 50$ μM, $c_{DNA} = 0.3$ mM).

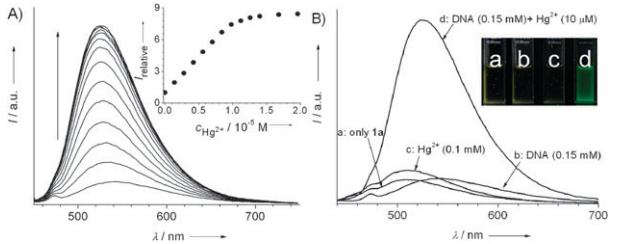

Fig. 3 A: Spectrofluorimetric titration of Hg^{2+} to **1a** in the presence of ct DNA in aqueous buffer (HEPES, 25 mM, pH 7.0; $\lambda_{ex} = 407$ nm, $c_{1a} = 10$ μM, $c_{DNA} = 0.15$ mM); inset: plot of the emission intensity at 525 nm *versus* Hg^{2+} concentration. B: Emission spectra of **1a** (10 μM) in the presence of ct DNA (0.15 mM), Hg^{2+} (0.1 mM), or ct DNA and Hg^{2+} (0.15 mM and 10 μM resp.).

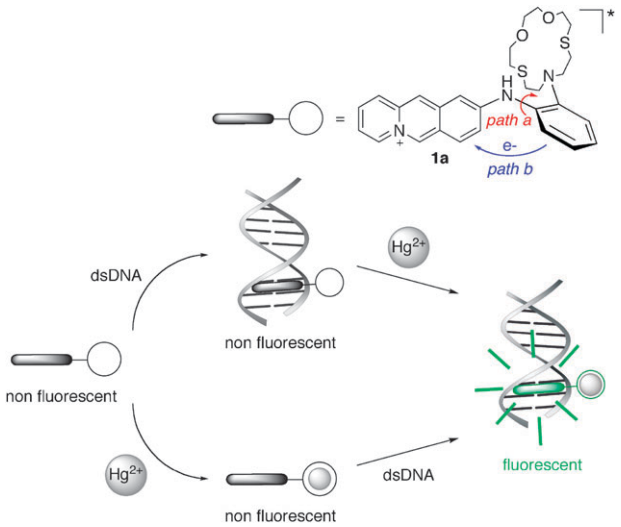

Fig. 4 Deactivation pathways of the excited state of **1a** (path a: rotation about N–C_{ar} bond; path b: photoinduced electron transfer), and schematic representation of fluorimetric detection of Hg^{2+} ions in the microenvironment of ds DNA by suppression of both deactivation pathways in the excited state.

Notably, neither the complexation of Hg^{2+} nor the intercalation into DNA has a significant influence on the very weak emission intensity of **1a**. This behavior may be explained by the two possible pathways through which the excited state may be deactivated, namely photoinduced electron transfer from the aminophenyl unit to the excited benzo[*b*]quinolizinium (Fig. 4, path b) and rotation about the C–N bond (Fig. 4, path a), as has been demonstrated in detail for the 9-[(4-dimethylamino)phenyl]-aminobenzo[*b*]quinolizinium.[8] Thus, if only one of these deactivation pathways is suppressed, namely by complexation of the azacrown ether donor functionality or by intercalation of the ligand, the emission is still efficiently quenched. However, if both pathways are suppressed by simultaneous complexation and intercalation, the emission intensity increases as observed for the ligand **1a** in the presence of DNA and Hg^{2+}. Thus the combination of **1a**, DNA, and Hg^{2+} constitutes a DNA-based molecular logic gate of the AND type,[7,14] *i.e.* the output (fluorescence enhancement) of the gate occurs only if both inputs, DNA and Hg^{2+}, are present. In addition, this system enables the sensitive and selective detection of Hg^{2+} ions under physiological conditions, even in the presence of potentially competing metal cations. Specifically, the sensitivity of fluorimetric detection of Hg^{2+} was determined to be 39 nM, *i.e.*, 7.8 ppb (*cf.* ESI†). Even more notable is the fact that the fluorescent probe **1a** enables the unambiguous detection of Hg^{2+} in the close proximity of double-stranded nucleic acids, *i.e.* at a position where mercury develops its genotoxicity; and there is no interfering background signal due to association with Hg^{2+} or DNA alone, respectively. Most remarkably, the strong influence of the Hg^{2+} concentration on the CD spectrum of the ternary complex is also not influenced by separate **1a**–Hg^{2+} or **1a**–DNA interactions, so that the combination of fluorimetric and polarimetric titrations even enables the 3D analysis of the Hg^{2+} concentration (*cf.* ESI†, Fig. S7).

Notes and references

1 (*a*) G. L. Eichhorn and J. J. Butzow, *Proc. Int. Symp. Biomol. Struct. Interactions, Suppl. J. Biosci.*, 1985, **8**, 527; (*b*) M. T. Rodgers and P. B. Armentrout, *J. Am. Chem. Soc.*, 2000, **122**, 8548; (*c*) I. Onyido, A. R. Norris and E. Buncel, *Chem. Rev.*, 2004, **104**, 5911; (*d*) J. Müller, *Eur. J. Inorg. Chem.*, 2008, 3749; (*e*) C. Ruan and M. T. Rodgers, *J. Am. Chem. Soc.*, 2009, **131**, 10918.

2 (*a*) E. Sletten and W. Nerdal, in *Metal Ions in Biological Systems*, ed. A. Sigel and H. Sigel, Marcel Dekker, 1997, p. 479; (*b*) H. Sigel, *Chem. Soc. Rev.*, 1993, **22**, 255; (*c*) J. V. Burda, J. Sponer, J. Leszczynski and P. Hobza, *J. Phys. Chem. B*, 1997, **101**, 9670; (*d*) Y. Miyake, H. Togashi, M. Tashiro, H. Yamaguchi, S. Oda, M. Kudo, Y. Tanaka, Y. Kondo, R. Sawa, T. Fujimoto, T. Machinami and A. Ono, *J. Am. Chem. Soc.*, 2006, **128**, 2172; (*e*) Y. Tanaka, S. Oda, H. Yamaguchi, Y. Kondo, C. Kojima and A. Ono, *J. Am. Chem. Soc.*, 2007, **129**, 244.

3 M. E. Crespo-Lopez, G. L. Macedo, S. I. D. Pereira, G. P. F. Arrifano, D. L. W. Picanco-Diniz, J. L. M. do Nascimento and A. M. Herculano, *Pharmacol. Res.*, 2009, **60**, 212.

4 (*a*) M. Tabata, A. K. Sarker and E. Nyarko, *J. Inorg. Biochem.*, 2003, **94**, 50; (*b*) S. S. Babkina and N. A. Ulakhovich, *Bioelectrochemistry*, 2004, **63**, 261; (*c*) Z. Hossain and F. Huq, *J. Inorg. Biochem.*, 2002, **91**, 398.

5 (*a*) A. P. H. de Silva, Q. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.*, 1997, **97**, 1515; (*b*) B. Valeur, *Molecular Fluorescence: Principles and Applications*, Wiley-VCH, Weinheim, 2002.

6 E. M. Nolan and S. J. Lippard, *Chem. Rev.*, 2008, **108**, 3443.

7 X. Xue, F. Wang and X. Liu, *J. Am. Chem. Soc.*, 2008, **130**, 3244.

8 A. Granzhan, H. Ihmels and G. Viola, *J. Am. Chem. Soc.*, 2007, **129**, 1254.

9 J. Polster and H. Lachmann, *Spectrometric Titrations: Analysis of Chemical Equilibria*, Wiley, VCH, Weinheim, 1989.

10 (*a*) H. Ihmels and D. Otto, *Top. Curr. Chem.*, 2005, **258**, 161; (*b*) U. Pindur, M. Jansen and T. Lemster, *Curr. Med. Chem.*, 2005, **12**, 2805; (*c*) M. Sinan, M. Panda, A. Ghosh, K. Dhara, P. E. Fanwick, D. J. Chattopadhyay and S. Goswami, *J. Am. Chem. Soc.*, 2008, **130**, 5185.

11 J. D. McGhee and P. H. von Hippel, *J. Mol. Biol.*, 1974, **86**, 469.

12 B. Norden and T. Kurucsev, *J. Mol. Recognit.*, 1994, **7**, 141.

13 H. Ihmels, K. Faulhaber, C. Sturm, G. Bringmann, K. Messer, N. Gabellini, D. Vedaldi and G. Viola, *Photochem. Photobiol.*, 2001, **74**, 505.

14 K. Szacilowski, *Chem. Rev.*, 2008, **108**, 3481. Most recent examples: T. Li, E. Wang and S. Dong, *J. Am. Chem. Soc.*, 2009, **131**, 15082; T. Konry and D. R. Walt, *J. Am. Chem. Soc.*, 2009, **131**, 13232.