J Fluoresc (2014) 24:251–256
DOI 10.1007/s10895-013-1292-9

ORIGINAL PAPER

A Zinc-dipicolylethylenediamine Modified Near Infrared Fluorophore for Sensing of ATP

Michael Schäferling · Thomas Lang · Annette Schnettelker

Received: 15 April 2013 / Accepted: 21 August 2013 / Published online: 10 September 2013

Abstract The development of fluorescent probes for sensing of anions in biological environments is still a demanding task. Due to the structural versatility of biological active anions there are many challenges to cope with compared to fluoroionophores for the determination of metal cations. This concerns particularly the design of the recognition element, which has to provide a selective response, preferably unaffected by alterations of pH and ionic strength. Polyphosphate anions such as ATP are interesting targets in bioanalysis because they are involved in many enzymatic reactions and bear versatile biological functions. Zinc dipicolylamine complexes attached to fluorophores have been turned out to be promising candidates for ATP sensing with sufficient sensitivity and selectivity. We now report the first NIR probe that responds to ATP based on a zinc dipicolylethylenediamine receptor. It shows a "turn-on" fluorescence behavior which is selective to other polyphosphate species even at high ionic strength of the sample solution.

Keywords Fluorescent probe · Anions · ATP · Dipicolylethylenediamine · NIR dye

T. Lang · A. Schnettelker
Institute of Analytical Chemistry, Chemo- and Biosensors, University of Regensburg, 93040 Regensburg, Germany

M. Schäferling (✉)
Department of Biochemistry / Biotechnology, University of Turku, Tykistökatu 6A, BioCity 6th floor, Turku, Finland
e-mail: michael.schaeferling@utu.fi

Introduction

Adenosine-5′- triphosphate (ATP) is one of the most important nucleotide compounds occurring in living organisms and has different biochemical functions. As a source of chemical energy it is consumed in many enzymatic reactions, e.g. as substrate for ATPases that transport alkaline and alkaline earth metals or protons through the cell membrane. Protein kinases are responsible for the phosphorylation of proteins using ATP as substrate which regulates their biological function. The cellular signalling molecule cyclic adenosine monophosphate (cAMP) is built from ATP by catalysis of adenylyl cyclases. It is also assembled as one of the four nucleobases in the DNA backbone. Therefore, a tremendous versatility of luminescent probes for the determination of ATP was synthesized in the past decade [1, 2].

Foremost, this regards fluorophores functionalized with zinc dipicolylamine (DPA) complexes as recognition element for polyphosphates [3–8]. In many cases, binuclear complexes have been designed that bear two DPA-Zn^{2+} receptor units attached to xanthene or rhodamine chromophores. Besides, fluorophores bearing macrocyclic [9] or open chain polyamine receptors [10] have been applied as probes for ATP determination, also functionalized polythiophenes [11, 12] pyrenes [13] or sensitized lanthanides [14, 15]. Other attempts are based on the indicator displacement strategy [16], consisting of charge-transfer complexes between HPTS and a cyclic calixpyridinium tetracation [17] or a macrocyclic viologen–anthracene conjugate for the recognition of GTP [18].

The advantages of fluorescent molecular probes are that they can respond reversibly to ATP and no additional enzymes are required for its detection as in the case of the bioluminescent luciferase reaction. The addition of radioactively labeled

ATP to monitor the activity of the aforementioned enzymes can also be circumvented. However, most of these probes show a limited applicability in biological matrices, because they are prone to interferences and often lose their selectivity for ATP recognition at higher salt concentrations. Though, some reagents could be applied to monitor enzymatic reactions, e.g. a DPA-Zn^{2+} complex in a tyrosine kinase assay [7], a stacked pyrene excimer based system in an apyrase reaction [13], and a terbium norfloxacin complex for the determination of the activity of a bacterial adenylyl cyclase [19] or the GTPase activity of RAS proteins [20].

DPA-Zn^{2+} based molecular sensors were even applied to image intracellular ATP stores of Jurkat cells [21] or to monitor ATP levels in HeLa cells [22]. Nevertheless, the applicability of these complexes for quantitative determination of ATP in complex biological samples is only limited because they are based on xanthene or rhodamine fluorophores with rather shortwave absorption and emission. The same is the case for the other indicator systems listed above. Near infrared absorbing and emitting dyes are usually preferred in bioanalysis, because only low background absorption and virtually no autofluorescence is present in the region between 650 and 1,200 nm [23, 24].

We now synthesized a fluorimetric probe for ATP which is based on a NIR cyanine dye attached to a dipicolylethylenediamine (DPED) chelator and can be excited in the optical biological window. We studied the fluorescence response to ATP and its selectivity to related species such has ADP, cAMP, pyrophosphate and phosphate ions. We also examined the influence of ionic strength on the sensor response by adding millimolar concentrations of Mg^{2+}, Na^{+} and K^{+}, which are required for the enzymatic reactions discussed above.

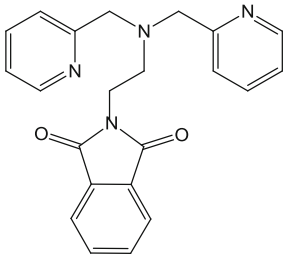
Materials and Methods

Chemicals and Reagents

All chemicals were obtained in analytical purity. Magnesium dichloride hexahydrate, tetrasodium pyrophosphate decahydrate (PPi), tris(hydroxyethyl)aminomethane (TRIS), and zinc chloride ($ZnCl_2$) were purchased from Merck KgaA (Darmstadt, Germany). Adenosine-5′-triphosphate disodium salt (ATP), Adenosine 5′- monopohosphate sodium salt (AMP), Adenosine-3′,5′-cyclic monophosphate sodium salt (cAMP), Di(2-picolyl)amine, and *N*-(2-bromoethyl)phthalimide were from Sigma-Aldrich (Steinheim, Germany). The NIR cyanaine dye S0378 was obtained from FEW Chemicals GmbH (Bitterfeld, Germany)

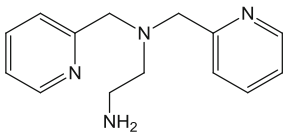
Synthesis

Synthesis of N-(2-[di(2-picolyl)amino]ethyl)phthalimide



Di(2-picolyl)amine (2.70 ml, 15.00 mmol), *N*-(2-bromoethyl)phthalimide (4.17 g, 16.42 mmol) and potassium carbonate (3.30 g, 23.88 mmol) were heated at 90 °C in DMF over-night. After cooling to room temperature the reaction mixture was poured onto ice-water for two hours. The crude product was filtered off and washed with cold water and was dried over calcium chloride in vacuum. An off white solid was obtained in a yield of 2.45 mg (6.58 mmol, yield: 43.87 %). ^{1}H- NMR ($CDCl_3$, 300 MHz) δ 2.79 (t, J=6.86, 2H), δ 3.79 (m, 6H), δ 6.99 (t, J=7.69 Hz, 2H), δ 7.31 (m, 4H), δ 7.71 (m, 4H), δ 8.34 (d, J=7.69 Hz, 2H); ^{13}C-NMR ($CDCl_3$) δ 36.04, δ 51.60, δ 60.20, δ 121.92, δ 122.98, δ 123.06, δ 132.20, δ 133.79, δ 136.18, δ 148.85, δ159.6, δ 168.06.

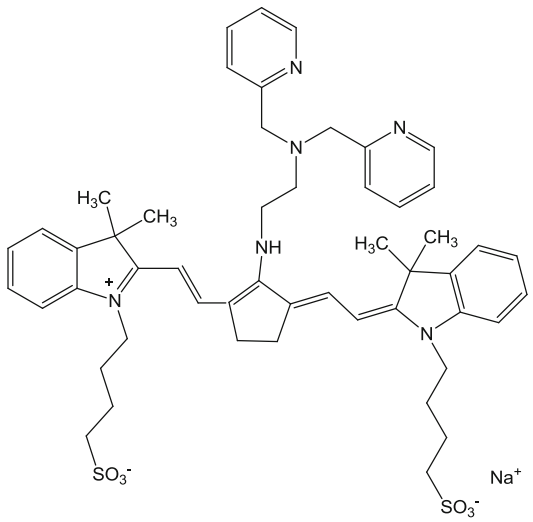
Synthesis of [Di(2-picolyl)amine]ethane-1,2-diamine



N-(2-[Di(2-picolyl)amino]ethyl)phtalimide (2.35 g, 6.31 mmol) was dissolved in boiling ethanol (40 ml) and hydrazine monohydrate (0.43 ml, 8.25 mmol) was added. After refluxing for four hours a white solid had precipitated. The reaction mixture was cooled to ambient temperature and conc. hydrochloric acid (10.00 ml) was added. The precipitate was filtered off after one hour and the filtrate adjusted to pH 10 with aqueous sodium hydroxide (1 mol L^{-1}). The solution was extracted 5 times with diethyl ether, and the combined organic phases were dried over magnesium sulfate. The solvent was removed in vacuum and the oily product was kept in the fridge over night to crystallize. A yellow solid was obtained in a yield of 650.00 mg (2.68 mmol, 42.47 %), ESI–MS: [M^{+}] (calculated): 242.3, [MH^{+}] (found): 243.0; ^{1}H- NMR ($CDCl_3$, 300 MHz) δ 2.69 (t, J=6.86, 2H), δ 2.81

(t, J=6.86, 2H), δ 3.84 (s, 4H), δ 7.14 (m, 2H), δ 7.46 (d, J= 7.69 Hz, 2H), δ 7.64 (m, 2H), δ 8.52 (d, J=7.69 Hz, 2H); ^{13}C-NMR ($CDCl_3$) δ 39.47, δ 57.06, δ 60.66, δ 122.10, δ 123.03, δ 136.48, δ 149.09, δ 159.55.

Synthesis of 2-[2-[2-[di(2-Picolyl)amine]ethane-1, 2-diamine]-3-[2-[1,3-dihydro-3,3-dimethyl-1-(4-sulfobutyl)-2H-indol-2-ylidene]-ethylidene]-1-cyclopenten-1-yl] -ethenyl]-3,3-dimethyl-1-(4-sulfobutyl)-3H-indolium sodium salt **2**



[Di(2-Picolyl)amine]ethane-1,2-diamine (650.00 mg, 2.68 mmol), FEW S 0378 **1** (1.58 g, 2.15 mmol), and triethylamine (0.36 ml, 2.60 mmol) were stirred in DMF at room temperature overnight while a change of colour from green to blue could be observed. The reaction mixture was poured into methyl-*tert*-butyl ether. The product precipitated and was collected by filtration. The crude product was washed with methyl-*tert*-butyl ether and dried in vacuum. A blue solid was obtained in a yield of 2.00 g (2.12 mmol, 79.10 %). HR-ESI–MS: [M-2H^{2+}] (calculated): 460.2159, [M-2H^{2+}] (found): 460.2164; ^{1}H-NMR (DMSO-d6, 600 MHz) δ 1.52 (s, 12H), δ 1.69 (m, 4H), δ 1.75 (m, 4H), δ 2.52 (t, J=7.14 Hz, 4H), δ 2.62 (m, 4H), δ 2.68 (m, 4H), δ 3.01 (t, J=6.00 Hz, 2H), δ 3.87 (t, J=6.00 Hz, 2H), δ 3.91 (m, 4H), δ 4.16 (t, J= 7.14 Hz, 1H), δ 5.61 (d, J=12.00 Hz, 2H), δ 7.04 (t, J= 8.00 Hz, 2H), δ 7.13 (d, J=8.00 Hz, 2H), δ 7.27 (m, 2H), δ 7.46 (d, J=8.00 Hz, 2H), δ 7.49 (d, J=8.00 Hz, 2H), δ 7.73 (d, J=8.00 Hz, 2H), δ 7.73 (d, J=12.00 Hz, 2H),δ 7.74 (d, J= 8.00 Hz, 2H), δ 8.46 (d, J=4.00 Hz, 2H); ^{13}C-NMR (DMSO-d6) δ 22.57, δ 22.70, δ 25.55, δ 25.96, δ 26.13, δ 26.21, δ 27.55, δ 36.00, δ 42.44, δ 43.85, δ 44.18, δ 45.83, δ 47.15, δ 49.00, δ 50.80, δ 51.08, δ 52.35, δ 58.89, δ 59.44, δ 96.45, δ 102.72, δ 109.29, δ 111.63, δ 122.16, δ 122.54, δ 122.69, δ123.28, δ 126.40, δ 128.35, δ 135.72, δ 136.90, δ 137.12, δ 139.81, δ 141.44, δ 142.33, δ 143.10, δ 148.78, δ 149.37, δ 158.44, δ 162.63, δ 164.52, δ 166.33, δ 170.85.

Spectroscopy

Fluorescence spectroscopy was carried out with an Aminco Bowman Series 2 spectrometer. For the intensity measurements the excitation wavelength was set to λ_{exc}=670 nm and the emission was measured at λ_{em}=720 nm. The bandwidth of the filter was 8 nm. They were carried out room temperature.

Absorption measurements were recorded with a Varian Cary spectrometer at room temperature. The solution in each cuvette had a final volume of 1 mL, containing 20 μmol L^{-1} NIR ligand **2**, 60 μmol L^{-1} Zn^{2+} and 0.5 mmol L^{-1}ATP.

Phosphate Assays

All data points represent the mean value of four individual measurements. All experiments were performed at room temperature. The fluorescence responses were recorded in a 1 mL quartz cuvette (Hellma GmbH & Co. KG, Mühlheim).

The solution in each well had a final volume of 200 μ L. The different phosphates were added in varying concentrations from 50–500 μmol L^{-1} yielding the specified final concentrations.

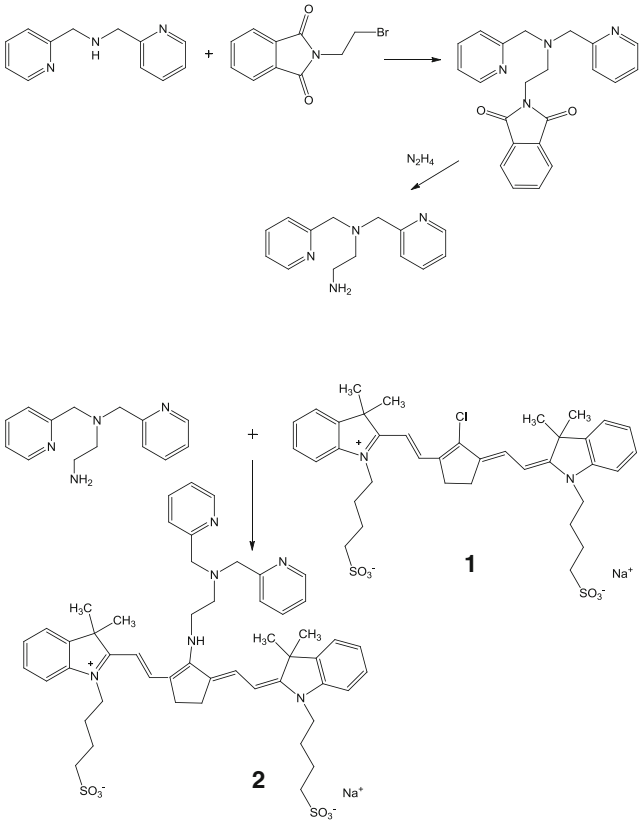
Different buffer compositions have been used to study the influence of ionic strength:

(a) TRIS buffer 24 mmol L^{-1}, pH 7.4
(b) TRIS buffer 24 mmol L^{-1}, containing 6 mmol L^{-1} Na^+, 3 mmmol L^{-1} K^+, and 0.5 mmmol L^{-1} Mg^{2+}
(c) TRIS buffer 24 mmol L^{-1} containing 80 mmol L^{-1} Na^+, 40 mmmol L^{-1} K^+, and 0.5 mmmol L^{-1} Mg^{2+}

Results and Discussion

Synthesis of the NIR Ligand

The synthesis of the NIR probe was accomplished in three steps (Scheme 1). First, dipicolylamine (DPA) was converted to a tertiary amine with *N*-(2-bromoethyl) phthalimide. Then the phthalimide was split off to form the DPED chelator with a primary amino linker. This was coupled to the cyanine based NIR fluorophore **1** to yield the ligand **2** for complexation of Zn^{2+}.



Scheme 1 Synthesis of the DPED ligand **2**. The primary amine of the DPED chelator was obtained by cleavage of the *N*-alkylated phthalimid with hydrazine (Gabriel synthesis). This was coupled to the commercially available NIR dye **1** to yield the DPED functionalized dye **2** as chelator for Zn^{2+}

Binding of Zn^{2+} and ATP Sensitivity

The DPED-Zn^{2+} complex were formed in situ by mixing a TRIS buffered solution of **2** with $ZnCl_2$. DPA-based ligands show a good selectivity for Zn^{2+} over other biologically relevant ions such as Mg^{2+}, Ca^{2+}, K^+ and Na^+ [25]. We varied the ratio of ligand to Zn^{2+} at a fixed ligand concentration of 20 μmol L^1 to identify the mixture which shows the highest signal increase after addition of 500 μmol L^{-1} ATP. It emerged that an excess of Zn^{2+} to ligand **2** of 3:1 provides the best response with a 2.2-fold increase of the fluorescence intensity (Fig. 1).

Absorption Spectroscopy

The replacement of chlorine in the NIR dye **1** by the DPED ligand causes a hypsochromic shift of its absorption maximum from 800 nm to 650 nm. As a result of the addition of Zn^{2+} a bathochromic shift occurs (Fig. 2). The zinc complex shows an absorption maximum at λ_{abs}=670 nm. This is in accordance with the results obtained by Kiyose et al., [26], who designed a ratiometric probe for Zn^{2+} based on a related

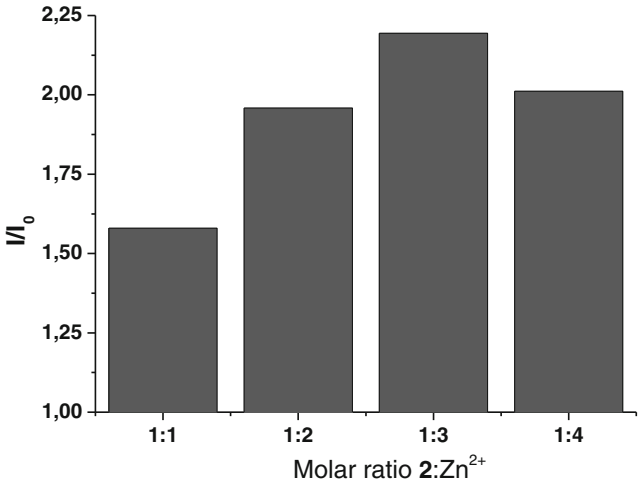


Fig. 1 Referenced fluorescence response I/I_0 after addition of 500 μmol L^{-1} ATP to mixtures with different ratios of ligand **2** (c=20 μmol L^1 to Zn^{2+} in TRIS buffer (pH 7.4). I_0 represents the fluorescence intensity prior to ATP addition. λ_{exc}=670 nm, λ_{em}=720 nm

DPED probe. This shift can be further increased by the addition of higher concentrations of Zn^{2+}. Addition of 500 μmol L^{-1} ATP leads to a slight increase of the absorbance. Overall, the effect of the studied phosphates on the absorption spectrum of the Zn^{2+}- complex with **2** is only weak.

Fluorescence Response to ATP

This picture changes if the fluorescence properties of the complex are examined. First, the fluorescence of the ligand is somewhat quenched in presence of Zn^{2+}. Secondly, a significant increase of the fluorescence intensity can be observed after addition of ATP. As outlined above, the maximum at λ_{em}=723 nm shows a 2.2-fold enhancement (Fig. 3).

The lone electron pair of the secondary amine in the DPED ligand increases the electron density of the fluorophore. The binding of Zn^{2+} reverses this effect, lowering the electron-

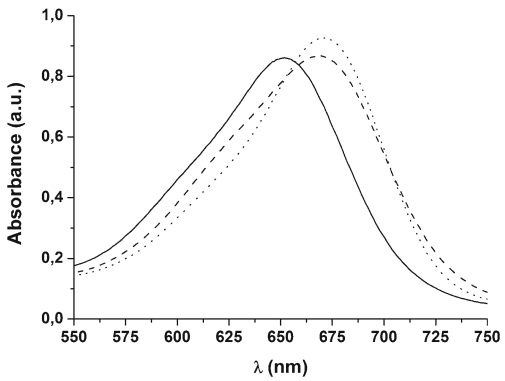


Fig. 2 Absorption spectra of **2** (*solid line*), **2**-Zn^{2+} (*dashed line*), and **2**-Zn^{2+} + ATP (*dotted line*) with 20 μmol L^{-1} ligand **2**, 60 μmol L^{-1} Zn^{2+} and 0.5 mmol L^{-1}ATP in TRIS buffer

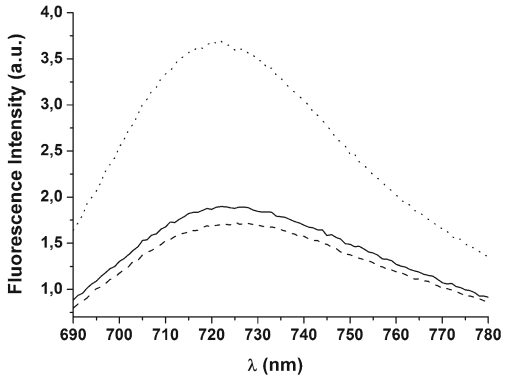


Fig. 3 Fluorescence spectra of **2** (*solid line*), **2**-Zn^{2+} (*dashed line*), and **2**-Zn^{2+} + ATP (*dotted line*) with 20 μmol L^{-1} ligand **2**, 60 μmol L^{-1} Zn^{2+} and 0.5 mmol L^{-1}ATP in TRIS buffer

donating ability of the amine to the fluorophore which leads to a fluorescence quenching [26]. From this behaviour it is apparent that no photoinduced electron transfer takes place from the lone electron pairs of the ethylenediamine bridge to the fluorophore [27]. Several mechanisms for the fluorescence increase after addition of ATP have been discussed in literature, which are most likely different in case of binuclear complexes than in the mononuclear system presented here. Obviously, the coordination of ATP is accompanied by structural changes or an increase of the electron density in the NIR fluorophore.

As already highlighted, many probes for ATP recognition suffer from a strong interference by ionic strength. Therefore, we tested the effect of increasing alkaline and alkaline earth metal concentrations on the ATP detection. A TRIS buffer containing 6 mmol L^{-1} Na^{+}, 3 mmmol L^{-1} K^{+} and 0.5 mmmol L^{-1} Mg^{2+} (in form of their chlorides) showed a dumping effect on the fluorescence increase in presence of increasing ATP concentrations, but the effect is comparatively low. Figure 4 shows the linear range of the response. The slope of the linear fit is diminished from 3.05 10^{-3} in TRIS to 1.69 10^{-3} in the saline buffer. The signal increase in TRIS buffer is saturated at ATP concentrations higher than 350 μmol L^{-1}. The dynamic range is extended up to 500 μmol L^{-1} in the saline TRIS. The dumping effect of ionic strength can be countermanded by applying higher probe concentrations.

Selectivity of the Response

In the following, we measured the fluorescence increase of the NIR probe in presence of ATP, ADP, cAMP, pyrophosphate and phosphate to show the selectivity of the response. Therefore, the ionic strength of the buffer was further increased by addition of 80 mmol L^{-1} Na^{+}, 40 mmmol L^{-1} K^{+} and 0.5 mmmol L^{-1} Mg^{2+}. This represents ion concentrations that are adequate for a high activity of the ATP-converting enzymes highlighted above.

To achieve sufficient signal changes, the concentration of ligand **2** and Zn^{2+} were increased to 40 μmol L^{-1} and 120 μmol L^{-1}, respectively. This is at the expense of the sensitivity and accuracy of ATP recognition, as the standard deviations become rather large applying these high concentrations. However, the selectivity of the fluorescence response compared to ADP and other phosphate species is sufficient, as it was observed also in case of other DPA-Zn^{2+} based probes for ATP. Under these conditions the fluorescence increases even by150% in presence of 500 μmol L^{-1} ATP, whereas the same concentration of ADP induces only a 40 % increase, and cAMP showed no effect in this concentration range (Fig. 5). The same is the case for inorganic phosphate or pyrophosphate anions (results not shown). From the standard deviation (SD) and the linear fit of the ATP response (not shown) a limit of detection for nucleotide determination can be calculated around 50 μmol L^{-1} (3 x SD/slope).

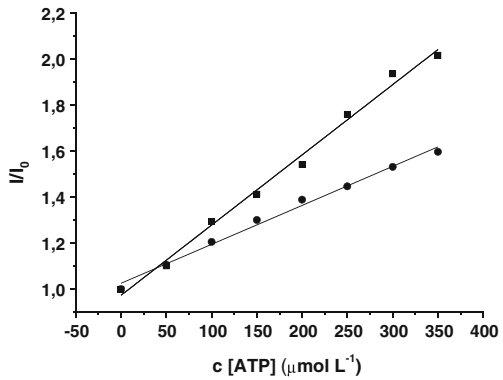


Fig. 4 Referenced fluorescence response of **2**-Zn^{2+} (20:60 μM) to increasing concentrations of ATP in TRIS buffer (■) and in TRIS buffer containing 6 mM NaCl, 3 mM KCl and 0.5 mM $MgCl_2$ (●). Linear fits: slope = 3.05 10^{-3}, intercept = 0.97, R^2 = 0.99 (■); slope = 1.69 10^{-3}, intercept = 1.03, R^2 = 0.99 (●).

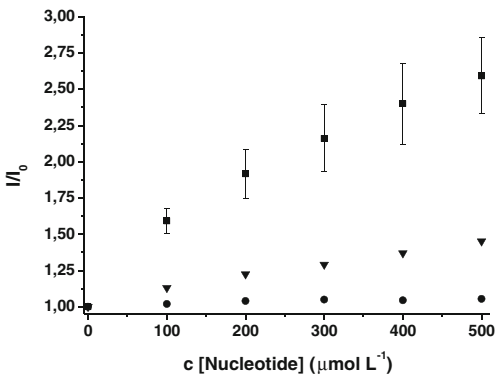


Fig. 5 Referenced fluorescence response of **2**-Zn^{2+} to increasing concentrations of ATP (■), ADP (▼) and cAMP (●) in TRIS buffer containing 80 mM NaCl, 40 mM KCl and 0.5 mM $MgCl_2$. Standard deviations are exemplarily shown for ATP and grow from 5 to 10 %.

It should be noted that much higher fluorescence responses were obtained in case of binuclear DPA-Zn^{2+} complexes coupled to shorter chromophores. Ojida et al. [21] reported a 30-fold increase of the fluorescence of a xanthene dye bearing two Zn^{2+} receptors after addition of a 10 –fold excess of ATP with a superior limit of detection. Apparently, in such cases a much higher impact on the aromatic chromophore system can be achieved by coordination of ATP. Nevertheless, the fluorescence response provided by this new NIR probe is clearly high enough to be quantitatively determined.

As there is no fluorescence increase observed in presence of phosphate and pyrophosphate, it is evident that electrostatic interactions between Zn^{2+} and phosphate groups are not decisive for the sensing mechanism. The nucleoside is required to induce the change of the structural or electronic properties of the cyanine dye. The phosphate seems only being responsible for the coordination to the zinc center. It is likely that π-stacks are formed between the aromatic nucleobase and the chromophore.

Conclusion and Outlook

We have developed a fast and straightforward synthesis of a NIR probe for the detection of ATP. It is based on a DPED chelator that is coupled to a cyanine fluorophore. The zinc complex is able to coordinate polyphosphates, particularly ATP, which is accompanied by a significant fluorescence increase. The response after addition of ATP is stronger than in case of ADP or cAMP, whereas no reaction was observed with phosphate and pyrophosphate. This indicates that polyphosphate ions play an essential role for the coordination to Zn^{2+}, but the nucleobase is the crucial factor for the fluorescence response. Summarized, this NIR probe for ATP determination has the following quality characteristics:

- Good water solubility
- Excitation and emission in the optical biological window
- Selectivity of ATP recognition even at high ionic strength required in enzymatic assays
- Fast response
- Large Stokes shift (~ 55 nm)

Nevertheless, it should be pointed out that as in case of all long-wave cyanine dyes with expanded chromophores this probe is prone to photobleaching. We observed a loss of fluorescence intensity of 50 % after 60 min continuous excitation applying standard settings of the fluorescence spectrometer.

Acknowledgment The authors thank the Deutsche Forschungsgemeinscha (DFG) for financial support (project SCHA 1009/4-3)

References

1. Spangler C, Schäferling M, Wolfbeis OS (2008) Microchim Acta 161:1–39
2. Zhu Y, Xu Z, Yoon J (2011) Chem Soc Rev 40:2222–2235
3. Moro AJ, Schmidt J, Doussineau T, Lapresta-Fernandéz A, Wegener J, Mohr GJ (2011) Chem Comm 47:6066–6068
4. Moro AJ, Cywinski PJ, Körsten S, Mohr GJ (2010) Chem Comm 49: 4919–4923
5. Ojiida A, Park SK, Mito-oka Y, Hamachi I (2002) Tetradedron Lett 43:6193–6195
6. Ojida A, Miyahara Y, Wongkongkatep J, Tamaru S, Sada K, Hamachi I (2006) Chem As J 1:555–563
7. Jang YJ, Jun EJ, Lee YJ, Kim YS, Kim JS, Yoon JJ (2005) Org Chem 70:9603–9606
8. Lee DH, Kim SY, Hong JI (2004) Angew Chem Int Ed 43:777–4780
9. Fenniri H, Hosseini MW, Lehn JM (1997) Helv Chim Acta 80:786–803
10. Vance DH, Czarnik AWJ (1994) Am Chem Soc 116:9397–9398
11. Li C, Numata M, Takeuchi M, Shinkai S (2005) Angew Chem Int Ed 44:6371–6374
12. Spangler C, Lang T, Schäferling M (2012) Dyes Pigments 95:194–200
13. Xu Z, Singh NJ, Lim J, Pan J, Kim HN, Park S, Kim KS, Yoon JJ (2009) Am Chem Soc 131:15528–15533
14. Schäferling M, Wolfbeis OS (2007) Chem Eur J 13:4342–4349
15. Miao Y, Liu J, Hou F, Jiang C (2006) J Luminesc 116:67–72
16. Wiskur SL, Ait-Haddou H, Lavigne JJ, Anslyn EV (2001) Acc Chem Res 34:963–972
17. Atilgan S, Akkaya EU (2004) Tetrahedron Lett 45:9269–9271
18. Neelakandan PP, Hariharan M, Ramaiah D (2006) J Am Chem Soc 128:11334–11335
19. Spangler CM, Spangler C, Göttle M, Shen Y, Tang WJ, Seifert R, Schäferling M (2008) Anal Biochem 381:86–93
20. Spangler C, Spangler CM, Spörner M, Schäferling M (2009) Anal Bioanal Chem 394:989–996
21. Ojida A, Takashima I, Kohira T, Nonaka H, Hamachi I (2008) J Am Chem Soc 130:12095–12101
22. Kurishita Y, Kohira T, Ojida A, Hamachi I (2010) J Am Chem Soc 132:13290–13299
23. Kobayashi H, Ogawa M, Alford R, Choyke PL, Urano Y (2010) Chem Rev 110:2620–2640
24. Ueno T, Nagano T (2011) Nat Methods 8:642–645
25. Tien Ngo H, Liu X, Jolliffe KA (2012) Chem Soc Rev 41:4928–4965
26. Kiyose K, Kojima H, Urano Y, Nagano T (2006) J Am Chem Soc 128:6548–6549
27. de Silva AP, Gunaratne HQN, Gunnlaugsson T, Huxley AJM, McCoy CP, Rademacher JT, Rice TE (1997) Chem Rev 97:1515–1566