Analyst

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/analyst

Journal Name

COMMUNICATION

NIR-emitting aza-BODIPY Dyes – New Building Blocks for Broad-range Optical pH Sensors

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Martin Strobl[a], Tanja Rappitsch[a], Sergey M. Borisov[a], Torsten Mayr[a] and Ingo Klimant[a]*

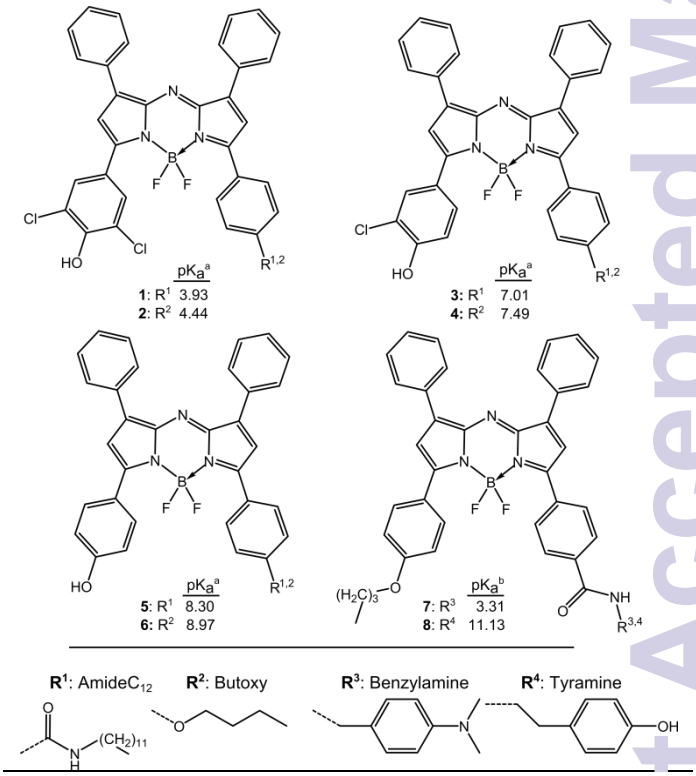
New aza-BODIPY indicators which cover the pH scale from 1.5 to 13 are presented. The new indicators feature absorption/emission bands in the red/near-infrared (NIR) spectral region, exhibit high molar absorption coefficients (~ 80 000 $M^{-1}cm^{-1}$) and show good quantum yields (~ 20 %). All dyes represent promising building blocks for the development of a broad-range sensor for various pH ranges. Combination of four of these pH indicators yields a pH sensor with an extended dynamic range from pH 2 to 9.

Determination of pH is probably the most frequently performed analytical measurement in a wide range of sciences and technology, including chemistry, biochemistry, biotechnology[1,2], medical diagnostics, biomedical research[3,4] and many industrial applications. Traditionally, electrochemical methods have been used for pH analysis providing accurate results within a relatively short time. In the last decade, optical pH sensors (pH optodes) were established. They offer important advantages compared to (potentiometric) glass pH electrodes and ion-sensitive field-effect transistors (ISFET). They show higher sensitivity within their dynamic range, enable contactless measurement, and are not prone to electromagnetic interferences. Additionally, the use of fiber-optic sensors allows a high degree of mechanical flexibility combined with ease of miniaturization, low production cost and the possibility of mass production[5–7]. A pH optode is composed of a pH sensitive indicator dye which is entrapped into a hydrophilic host polymer. The indicator dye possesses distinct optical properties associated with its protonated (acidic) and deprotonated (basic) form[8]. Depending on the concentration of hydrogen ions (pH), the absorption (color) or fluorescence emission of the indicator dye is altered, which is used as a source of analytical information.

Especially fluorescent pH sensors have gained considerable attention over absorption-based techniques due to high sensitivity and straightforward read-out in fiber-optic sensors[9–12].

However, the operating range of a pH indicator dye, which is controlled by its pK_a value, is fixed to a certain pH range and is limited to 3 pH units. Some attempts have been made to extend the dynamic range of optodes, for example by using a mixture of two or more pH indicators with different pK_a values or by using a single indicator with multiple dissociation constants. Several classes of fluorescent pH indicators, including coumarin-based dyes, naphthalimide derivatives or perylene bisimide probes were employed for designing a pH sensor covering a broad-range[13–17]. However, most broad-range pH sensors presented in literature suffer from several drawbacks such as complex calibration models, poor photostability

Table **1** Chemical structures of new aza-BODIPYs



[a] pK_a value from absorption and [b] apparent pK_a' value determined from emission spectra in EtOH/buffer mixture [1:1 (v/v)], ionic strength 150 mM. For reaction conditions, see Supporting Information.

[a] Institute of Analytical Chemistry and Food Chemistry
8010 Graz, Stremayrgasse 9/II AUSTRIA
E-mail: klimant@tugraz.at

Electronic Supplementary Information (ESI) available: See DOI: 10.1039/x0xx00000x

Table **2**: Photophysical properties of the aza-BODIPY dyes: absorbance maxima for the acidic ($\lambda_{abs\text{-}acid}$) and the basic forms ($\lambda_{abs\text{-}base}$), emission maxima for the acidic form ($\lambda_{em\text{-}acid}$), molar absorption coefficients (ε) and luminescence quantum yield (φ_F), n.m. not measureable.

dye	$\lambda_{abs\text{-}acid}/\lambda_{abs\text{-}base}$ [a] (nm)	$\lambda_{em\text{-}acid}$ [a] (nm)	ε (THF)[b] ($M^{-1}cm^{-1}$)	φ_F (THF) % acidic/basic	pK_{abs} (D4)	pK'_{em} (D4)	pK_{abs} [a]	pK'_{em} [a]
1	670/744	697	80500	17 / n.m.	4.66	4.25	3.93	3.87
2	683/755	715	88200	19 / n.m.	5.61	5.03	4.44	4.72
3	677/738	703	91600	20 / n.m.	6.66	6.32	7.01	6.47
4	687/750	724	93500	20 / n.m.	7.57	6.54	7.49	6.54
5	678/730	708	86300	22 / n.m.	8.21	7.59	8.30	8.02
6	690/743	729	95500	23 / n.m.	9.05	8.47	8.97	8.78
7[c]	675/675	704	85300	22 / n.m.	-	2.6	-	3.31
8[c]	675/675	703	84600	19 / n.m.	-	11.9	-	11.13

[a] (EtOH/H_2O-1:1), [b] for the protonated form, [c]No pH dependence in absorption.

and low brightness of pH dyes or excitation with UV or blue light [15], which limits their use for many applications.

The choice of the fluorophore class plays a key role in the design of fluorescent sensors with an extended dynamic range and require to fulfill the following criteria: (i) easy accessibility of the dye structure to modification including the introduction of electron-donating or -withdrawing groups for tuning the pK_a value. (ii) virtually identical spectral properties of the used indicator dyes. This means that the functionalities determining the pK_a value should have only minimal influence on absorption or emission spectra in order to allow excitation at the same wavelength and should not significantly affect the quantum yield or the absorption coefficient. (iii) outstanding photostability of all fluorescent probes or at least equal photo-degradation rates to ensure a consistent calibration function over time. (iv) suitability for immobilization of the indicator dye by covalent linkage or by adding a lipophilic moiety to structure. This prevents the dye from leaching out of the sensor material which ensures signal stability and keeps signal drift at a minimum

In many applications, e.g. for measuring in complex biological samples, it is preferable to use fluorophores with absorption/emission profiles in the long-wavelength spectral region (650 – 750 nm) which provides many advantages: lower auto-fluorescence of biomolecules, less scattering background and deep light penetration into (biological) probes.

Among all those fluorophores presented in literature, BF_2-chelated tetraarylazadipyrromethene dyes (aza-BODIPYs) proved to be excellent candidates capable of fulfilling the requirements mentioned above. These indicator dyes are exceptionally photo-stable, show sharp absorption/emission bands in the near-infrared region (NIR) and are highly accessible to structural modifications.[18] The pH sensitivity is obtained by a photoinduced electron transfer (PET) from an amino group or a phenolate to the aza-BODIPY's backbone causing effective fluorescence quenching in the deprotonated form.

Several pH sensitive aza-BODIPY dyes functionalized with amino- or hydroxyl subsituents were reported by O'Shea and co-workers[19–21]. Further work was done by Jokic *et al.*[22]. They showed that pK_a values can be tuned easily from 6 to 8, but spectral properties remain virtually identical. However, these probes cover only the near-neutral and basic range whereas aza-BODIPY dyes exhibiting pK_a values in the acidic and also very basic regions of the pH scale are not available, yet.

Aim of this work was to develop a full set of aza-BODIPY indicators to enable pH-sensing over the entire pH range. We synthesized 8 new aza-BODIPY dyes with pK_a values evenly covering the pH scale from 2.06 to 11.90. The pK_a values are lowered by substitution of one or two chlorine atoms at the pH sensitive phenol group of the indicators (probe **1 - 4**). Additional fine tuning of the pK_a is achieved by introduction of either an electron-withdrawing carboxamide group (probe **1,3,5**) or an electron-donating butoxy moiety (probe **2,4,6**) as remote substituents. In order to cover extremely acidic or basic regions of the pH scale a new design concept for probe **7** and **8** was investigated. The pH-sensitive PET-group is covalently linked to the aza-BODIPY via a non-π-electron-conjugating spacer group [amide moiety with methylene group(s)]. Table **2** provides an overview of the spectral and photophysical properties of the new aza-BODIPY compounds. The molar absorption coefficients ε are ranging from 80 000 – 95 000 $M^{-1}cm^{-1}$ and fluorescence quantum yield are found to be within 17 -23 % in THF. The absorption of the protonated form of all dyes is similar (670-690 nm) and the emission maxima are located between 697-715 nm. pK_a values of the new probes were determined in mixtures of ethanol and aqueous buffer [1:1 (v/v)] and were derived from both absorption and emission spectra. Since in probe **1-6** the pH-sensitive phenol group is fully integrated into the dye's molecule, the absorption shifts bathochromically upon deprotonation whereas the absorption of probe **7** and **8** is pH independent. The fluorescence emission of the aza-BODIPY probes is quenched upon deprotonation which is characteristic for efficient photoinduced electron transfer from the protonated to the deprotonated form. The pH sensors were prepared via non-covalent entrapment of the indicator dye into commercially available polyurethane-based uncharged hydrogel (Hydromed D4) It exhibits high proton-permeability with a water uptake capacity of about 50 %. The pK_a values of the aza-BODIPY derivatives can be tuned over a wide

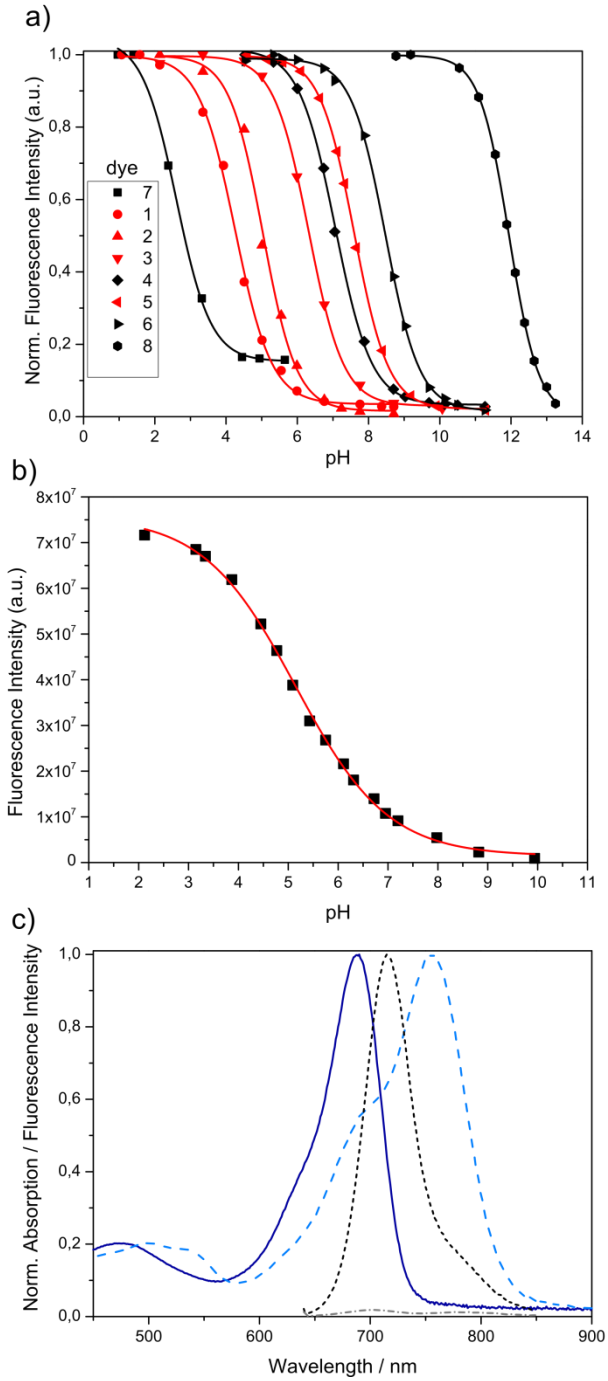


Figure **1 a)** Calibration curves of probe 1-8, determined in D4 from emission spectra. Combination of probe (1,2,3,5) (red) in D4 yields a broad-range sensor **(b)** with an extended dynamic range from pH 2 to 9. c) Absorption of protonated (blue) and deprotonated (dashed-blue) form and fluorescence (dotted black) of the broad-range sensor.

range by introducing electron-withdrawing/donating subsituents in adjacent or remote positions. When probe **5** (with pK_a of 8.21) is modified at the *m*-position with a chlorine atom, the pK_a drops to 6.66 (**3**). If two chlorine atoms are substituted in the *m*-positions the pK_a further decreases to 4.66 (**1**). On the other hand, when the amide-functionality in the *p*-position of the Ar^2 ring of dye **(1, 3, 5)** is replaced by the electron-donating butoxy group (**2**, **4**, **6**), the pK_a value increases by approximately 0.9 pH units respectively. The apparent pK_a values in D4 derived from fluorescence emission are 0.4 - 0.6 pH units lower than those determined from absorption data. This may be explained by the fact that the dye's concentration is much higher in D4 than in the solution. Consequently, the indicator molecules are close enough to enable intermolecular radiationless energy transfer (FRET), which results in a lowered apparent pK_a' value [22].

These novel pH sensing materials exhibit dynamic ranges which cover the pH scale from pH 2 to pH 13. Moreover, the new aza-BODIPY dyes show virtually identical spectral properties and thus provide the possibility of designing a pH sensor with an extended dynamic range. Probes **1**, **2**, **3** and **5** (ratio 1:1:1:1) were mixed together in the same host polymer (D4) for this purpose. Indeed, the new sensor provides a dynamic range from 2 to 9, thus an extended working range by ~ 4 pH units compared to sensors based on a single indicator. Simple Boltzmann-fitting yields a smooth sigmoidal calibration curve (R^2 0.998) which is not distorted due to evenly distributed pK_a values.

It should be emphasized that the aza-BODIPY dyes show outstanding photostability, even under extreme illumination with a 642-nm high-power 10 W LED array for 3 hours (6300 µmol $s^{-1}m^{-2}$, see supporting information [SI]) and outperform established prominent NIR-chromophores such as cyanine dyes or lipophilic SNARF derivatives[23,24].

Additionally, the photostability of the aza-BODIPY dyes is not affected by substitution of electron-donating or -withdrawing groups. This is in contrast to other classes of pH indicators (e.g. fluorescein) where pK_a determining functionalities have a significant impact on the photostability and therefore the development of a pH sensor with broadened operating range is compromised (see SI)[25]. Moreover, cross-sensitivity to ionic strength is minimized due to the facts that aza-BODIPY dyes carry only one charge in their deprotonated form and that they are embedded into a neutral polymeric matrix [26]. All dyes were modified with an alkyl chain. Consequently, due to pronounced hydrophobicity the indicator dyes do not leach out from hydrogel D4 into the aqueous solution but are not prone to aggregation within the hydrophilic host polymer, even at high concentrations. (see SI). The aza-BODIPY dyes show good solubility in organic solvents (e.g. THF, CH_2CL_2) and are virtually insoluble in water. Notably, probes 1,3,5 exhibit a carboxy moiety before they are functionalized with an alkyl chain. Ongoing work will focus on the covalent immobilization of the aza-BODIPY dyes to a polymeric matrix via amide bond formation.

We presented a set of novel NIR-emitting aza-BODIPY indicators which cover the pH scale from 1.5 to 13. All dyes exhibit virtually identical spectral and photophysical properties and, thus, represent building blocks for the development of a broad-range sensor for various pH-ranges. We have shown that combination of four of these remarkably photo-stable pH dyes yields a pH sensor with an extended dynamic range from pH 2 to 9. Especially for biotechnological applications there is a surprising scarcity of pH dyes which exhibit pK_a values at acidic pH and additionally have absorption and emission bands in the red/near-infrared region. Here, aza-BODIPY dyes fulfill these requirements which make them ideal fluorophores for pH determination in most fermentation processes and also complex biological samples (e.g. growth media).

Acknowledgments

Financial support by the European Union FP7 Project BIOINTENSE – Mastering Bioprocess integration and intensification across scales (Grant Agreement Number 312148) is gratefully acknowledged.

References

1 A. S. Jeevarajan, S. Vani, T. D. Taylor and M. M. Anderson, *Biotechnol. Bioeng.*, 2002, **78**, 467–472.
2 G. T. John, D. Goelling, I. Klimant, H. Schneider and E. Heinzle, *J. Dairy Res.*, 2003, **70**, 327–333.
3 W. Zhang, B. Tang, X. Liu, Y. Liu, K. Xu, J. Ma, L. Tong and G. Yang, *Analyst*, 2009, **134**, 367–371.
4 B. Tang, F. Yu, P. Li, L. Tong, X. Duan, T. Xie and X. Wang, *J. Am. Chem. Soc.*, 2009, **131**, 3016–3023.
5 X.-D. Wang and O. S. Wolfbeis, *Anal. Chem.*, 2013, **85**, 487–508.
6 F. J. Arregui, M. Otano, C. Fernandez-Valdivielso and I. R. Matias, *Sens. Actuators B Chem.*, 2002, **87**, 289–295.
7 D. Wencel, T. Abel and C. McDonagh, *Anal. Chem.*, 2014, **86**, 15–29.
8 O. S. Wolfbeis, *Fiber optic chemical sensors and biosensors*, CRC Press, 1991.
9 D. Aigner, S. M. Borisov, F. J. Orriach Fernández, J. F. Fernández Sánchez, R. Saf and I. Klimant, *Talanta*, 2012, **99**, 194–201.
10 D. Aigner, B. Ungerböck, T. Mayr, R. Saf, I. Klimant and S. M. Borisov, *J. Mater. Chem. C*, 2013, **1**, 5685–5693.
11 D. Aigner, S. M. Borisov and I. Klimant, *Anal. Bioanal. Chem.*, 2011, **400**, 2475–2485.
12 A. Lobnik, I. Oehme, I. Murkovic and O. S. Wolfbeis, *Anal. Chim. Acta*, 1998, **367**, 159–165.
13 L. Shen, X. Lu, H. Tian and W. Zhu, *Macromolecules*, 2011, **44**, 5612–5618.
14 C.-G. Niu, X.-Q. Gui, G.-M. Zeng and X.-Z. Yuan, *Analyst*, 2005, **130**, 1551–1556.
15 G. Nishimura, Y. Shiraishi and T. Hirai, *Chem. Commun.*, 2005, 5313–5315.
16 A. S. Vasylevska, A. A. Karasyov, S. M. Borisov and C. Krause, *Anal. Bioanal. Chem.*, 2007, **387**, 2131–2141.
17 J. Qi, D. Liu, X. Liu, S. Guan, F. Shi, H. Chang, H. He and G. Yang, *Anal. Chem.*, 2015, **87**, 5897–5904.
18 J. Killoran, L. Allen, J. F. Gallagher, W. M. Gallagher and D. F. O'Shea, *Chem. Commun. Camb. Engl.*, 2002, 1862–1863.
19 J. Killoran, S. O. McDonnell, J. F. Gallagher and D. F. O'Shea, *New J. Chem.*, 2008, **32**, 483–489.
20 J. Murtagh, D. O. Frimannsson and D. F. O'Shea, *Org. Lett.*, 2009, **11**, 5386–5389.
21 S. O. McDonnell and D. F. O'Shea, *Org. Lett.*, 2006, **8**, 3493–3496.
22 T. Jokic, S. M. Borisov, R. Saf, D. A. Nielsen, M. Kühl and I. Klimant, *Anal. Chem.*, 2012, **84**, 6723–6730.
23 S. M. Borisov, K. Gatterer and I. Klimant, *Analyst*, 2010, **135**, 1711–1717.
24 M. E. Cooper, S. Gregory, E. Adie and S. Kalinka, *J. Fluoresc.*, 2002, **12**, 425–429.
25 C. R. Schröder, B. M. Weidgans and I. Klimant, *Analyst*, 2005, **130**, 907–916.
26 B. M. Weidgans, C. Krause, I. Klimant and O. S. Wolfbeis, *The Analyst*, 2004, **129**, 645–650.

