

**UCC Library and UCC researchers have made this item openly available.
 Please [let us know](#) how this has helped you. Thanks!**

Title	Background-free fluorescence decay time sensing and imaging of pH with highly photostable diazaoxotriangulenium dyes
Author(s)	Dalfen, Irene; Dmitriev, Ruslan I.; Holst, Gerhard; Klimant, Ingo; Borisov, Sergey M.
Publication date	2018-12-06
Original citation	Dalfen, I., Dmitriev, R. I., Holst, G., Klimant, I. and Borisov, S. M. (2018) 'Background-free fluorescence decay time sensing and imaging of pH with highly photostable diazaoxotriangulenium dyes', <i>Analytical Chemistry</i> . doi:10.1021/acs.analchem.8b02534
Type of publication	Article (peer-reviewed)
Link to publisher's version	http://dx.doi.org/10.1021/acs.analchem.8b02534 Access to the full text of the published version may require a subscription.
Rights	© 2018, American Chemical Society. This document is the Accepted Manuscript version of a Published Work that appeared in final form in <i>Analytical Chemistry</i> after technical editing by the publisher. To access the final edited and published work see https://pubs.acs.org/doi/abs/10.1021/acs.analchem.8b02534
Embargo information	Access to this article is restricted until 12 months after publication by request of the publisher.
Embargo lift date	2019-12-06
Item downloaded from	http://hdl.handle.net/10468/7237

Downloaded on 2020-09-23T09:46:47Z

Article

Background-free fluorescence decay time sensing and imaging of pH with highly photostable diazaoxotriangulenium dyes

Irene Dalfen, Ruslan I Dmitriev, Gerhard Holst, Ingo Klimant, and Sergey M Borisov

Anal. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.analchem.8b02534 • Publication Date (Web): 06 Dec 2018Downloaded from <http://pubs.acs.org> on December 13, 2018

Just Accepted

“Just Accepted” manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides “Just Accepted” as a service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. “Just Accepted” manuscripts appear in full in PDF format accompanied by an HTML abstract. “Just Accepted” manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are citable by the Digital Object Identifier (DOI®). “Just Accepted” is an optional service offered to authors. Therefore, the “Just Accepted” Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the “Just Accepted” Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these “Just Accepted” manuscripts.



Background-free fluorescence decay time sensing and imaging of pH with highly photostable diazaoxotriangulenium dyes

Irene Dalfen,¹ Ruslan I. Dmitriev,^{2,3} Gerhard Holst,⁴ Ingo Klimant,¹ Sergey M. Borisov^{1*}

1. Institute of Analytical Chemistry and Food Chemistry, Graz University of Technology, Stremayrgasse 9, 8010 Graz, Austria

2. School of Biochemistry and Cell Biology, University College Cork, Cork, Ireland

3. Institute for Regenerative Medicine, I.M. Sechenov First Moscow State University, Moscow, Russian Federation

4. PCO AG, Donaupark 11, 93309 Kelheim, Germany

* sergey.borisov@tugraz.at

ABSTRACT

Novel fluorescent diazaoxatriangulenium (DAOTA) pH indicators for lifetime-based self-referenced pH sensing are reported. The DAOTA dyes were decorated with phenolic receptor groups inducing fluorescence quenching via photoinduced electron transfer mechanism. Electron-withdrawing chlorine substituents ensure response in the most relevant pH range (apparent pK'_a values ~ 5 and 7.5 for the *p,p*-dichlorophenol- and the *p*-chlorophenol-substituted dyes, respectively). The dyes feature long fluorescence lifetime (17-20 ns), high quantum yield ($\sim 60\%$) and high photostability. Planar optodes are prepared upon immobilization of the dyes into polyurethane hydrogel D4. Apart from the response in the fluorescence intensity, the optodes show pH-dependent lifetime behaviour which makes them suitable for studying 2D pH distribution with help of fluorescence lifetime imaging technique. The lifetime response is particularly pronounced for the sensors with high dye concentration (0.5-1% wt. in respect to the polymer) and is attributed to efficient homo-FRET mechanism.

KEYWORDS

Decay time; FRET; pH sensor; triangulenium; photoinduced electron transfer, Frequency Domain FLIM

INTRODUCTION

Many biological and ecological systems are based on a delicate equilibrium between a wide range of different parameters. One of the most important is the pH, which is of highest importance in biology, biotechnology, medicine, oceanography and environmental monitoring to mention only a few fields. For monitoring pH in these areas, fluorescent chemosensors proved to be promising analytical tools since they are free from electromagnetic interferences, enable non-invasive measurements and are available in a variety of formats ranging from planar optodes and fiber optic sensors to nanoparticles.¹ In contrast to other methods (e.g. electrochemical sensing) optical sensors enable imaging of pH distribution on

1
2
3 surfaces (with help of planar optodes) or in volume (with help of nanoparticles). Particularly, planar
4 optodes have been applied for imaging of pH gradients in marine sediments,^{2,3} rhizosphere,^{4,5} biofilms,⁶
5 wounds^{7,8} and microfluidic chips.^{9,10}
6
7

8 Optical pH sensors are generally manufactured via immobilization of a pH indicator into a
9 proton-permeable matrix, which ensures reusability of the sensor for multiple measurements. Among
10 numerous fluorescent pH probes reported¹¹ some most common representatives are xanthene dyes,^{12,13}
11 derivatives of 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS),^{14,15} naphthalimides^{16,17} and BODIPY
12 dyes.^{18–20} Unfortunately, the performance of pH sensors based on UV-Vis indicators can be
13 compromised by high levels of background fluorescence originating from biological species present in
14 the analysed sample or from optical components such as fibers and filters. An additional optical isolation
15 layer helps to minimize the autofluorescence in case of fiber-optic sensors and planar optodes but it can
16 also introduce problems associated with interaction of the dye and the components of optical isolation
17 (e.g. carbon black), and it is of course not possible at all in case of nanosensors. NIR excitable/emissive
18 probes such as seminaphthorhodafluors (SNARFs)²¹, aza-BODIPYs²² and dyes of other classes proved
19 to be advantageous due to low autofluorescence background in the NIR region. Unfortunately, most of
20 these dyes have significantly lower fluorescence quantum yields compared to UV-Vis dyes.
21
22

23 Another way to overcome the above limitation is to use an indicator with comparably long
24 luminescence decay time τ . Here, the background fluorescence can be eliminated in a time-resolved
25 measurement. Luminescent pH probes based on metal complexes (most prominently Ru(II)^{23–25} and
26 Eu(III)²⁶ complexes) proved to be particularly interesting candidates due to their decay times in
27 microsecond time domain. Apart from background-free measurements, some of these probes enable
28 self-referenced decay time read-out^{23,24} which is highly interesting for practical applications. Much more
29 common intensity-based indicators require addition of a luminescent reference to the sensor material to
30 ensure reliable read-out.^{1–10} This in turn may introduce numerous issues associated with chemical and
31 photochemical stability of the reference luminophore, leaching, oxygen cross-talk, inhomogeneous
32 distribution of the two components, inner-filter effects etc. In respect to the pH indicators based on
33 phosphorescence, long decay time typically results in a significant cross-sensitivity to molecular oxygen
34 which has to be compensated for. Although several fluorescent indicators with decay time read-out have
35 been reported, they either show comparably short wavelength of excitation and emission²⁷ or feature
36 fairly short decay times (< 7 ns in the “on” state),^{21,28,29} which makes background-free measurements
37 problematic. Some nanosensors based on quantum dots feature significantly longer decay time and
38 represent promising materials for pH sensing^{30–32} but toxicity and environmental impacts may be a
39 limitation here.
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54

55 Therefore, fluorescent dyes with decay times long enough to eliminate the autofluorescence³³
56 but short enough to avoid cross-sensitivity to oxygen, are of high interest as an alternative platform for
57 designing advanced pH optodes. Rich and co-workers showed that 90% of the background fluorescence
58 in tissues originates from emitters with the decay time of < 2.5 ns and about 10% from emitters with τ
59
60

1
2
3 ~ 7.5 ns.³³ Therefore, the overall autofluorescence is expected to decrease by about 10-fold after 7.5 ns
4 which appears to be a reasonable threshold for designing lifetime-based sensors for *in vivo* applications.
5 Among very few known representatives of the dyes with long fluorescence decay time,³⁴ triangulenium
6 dyes^{33,35} are particularly interesting due to combination of $\tau \sim 20$ ns and comparably long wavelengths
7 of absorption and emission which are similar to those of the established chromophores such as
8 rhodamines and BODIPYs. Although the pH-sensitive trihydroxytrioxatriangulene has been reported,³⁶
9 the fluorescence lifetimes of this dye do not exceed 4.3 ns in any protonation/deprotonation state, thus
10 eliminating the strongest advantage of this dye group. While this work was in progress, Sørensen and
11 co-workers reported a pH sensor based on a diazaoxatriangulenium (DAOTA⁺) dye decorated with a
12 phenolic receptor.³⁷ Since the sensing material has been characterized only for the fluorescence intensity,
13 it remained unclear if the lifetime properties of the chromophore were affected by modification and
14 immobilization.
15

16 In this contribution, the modification of diazaoxatriangulenium dyes with phenolate-based pH
17 receptors was chosen as a pathway to obtain pH indicators with long-lived fluorescence and attractive
18 spectral properties. These groups modulate the fluorescence of the dye via photoinduced electron
19 transfer (PET) but do not affect the advanced photophysical properties of the chromophore. Incorporated
20 in hydrogel matrices and nanoparticles the new indicators possess self-reference character due to
21 lifetime sensing capabilities, which favourably distinguishes them from most systems presented so far.
22 As will be demonstrated, planar optodes are particularly attractive for recording of pH distribution with
23 fluorescence lifetime imaging (FLIM) technique.
24
25

26 EXPERIMENTAL

27 *Materials*

28 Chemicals were purchased from various commercial providers (TCI (www.tcichemicals.com), Fluka
29 (www.analytix-shop.com/de/hersteller/fluka.html), Sigma Aldrich (www.sigmaaldrich.com), Acros
30 Organics (www.acros.com), Roth (www.carlroth.com/at/de), ABCR (www.abcr.de)) and used as
31 received. Macrolex Fluorescent Red G (3-(1,3-benzothiazol-2-yl)-7-(diethylamino)-2-oxo-2H-
32 chromene-4-carbonitrile) from TER chemicals (www.terchemicals.com) and the perylene dye Lumogen
33 Red from Kremer Pigmente (www.kremer-pigmente.com/de). Solvents were acquired from VWR
34 (<https://at.vwr.com/store>) and used without further purification.
35

36 Eudragit RL-100 copolymer (poly-(ethylacrylate-co-methylmethacrylate-co-trimethyl-aminoethyl
37 methacrylate), (average MW \approx 150000 Da, 8.85 – 11.96 wt. % trimethyl-aminoethyl methacrylate units
38 on dry substance) was from Degussa (www.evonik.com). Polyurethane hydrogels (Hydromed™ D1 and
39 D4) were purchased from CardioTech (www.cardiotech-inc.com). Poly(methyl methacrylate-co-
40 methacrylic acid (PMMA-MA, MW \approx 100000 Da, 10% methacrylic acid) and
41 poly(hydroxyethylmethacrylate) (pHEMA) (MW \approx 150000 Da) were obtained from Polysciences Inc.
42 (www.polysciences.com). Poly(styrenesulfonic acid), (PSSA, MW \approx 300000 Da), was from ABCR
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

(www.abcr.de) and Nafion® 117 (5% wt. solution in alcohols) from Aldrich. Poly(ethylene terephthalate) support Melinex 505 was purchased from Pütz (www.puetz-folien.com).

Synthesis

Synthesis of fluorescent DAOTA dyes was conducted similar to procedures reported previously by Laursen et al.³⁸⁻⁴⁰ Synthesis of tris(2,6-dimethoxyphenol)methanol (**a**) was performed according to the literature procedures.^{41,42}

Synthesis of *N*-(3,5-dichloro-4-hydroxy)phenyl-*N*-dodecyl DAOTA⁺ PF₆⁻ (**PhOHCl₂-DAOTA**)

1.25 g (2.84 mmol) of **a** were dissolved in ethanol and acidified with 1 mL aqueous HBF₄ (50 wt%, 16.1 mmol). After precipitation with cyclohexane and filtration the solid was dissolved in acetonitrile. 508 mg (2.86 mmol) of 3,5-dichloro-4-hydroxyaniline and a few drops of pyridine were added under stirring at room temperature. The resulting orange intermediate was precipitated in 250 mL 0.2 M aqueous KPF₆ and collected via filtration. The precipitate was dissolved in *N*-methylpyrrolidone and 2.73 g (14.7 mmol) dodecylamine were added. After 1 h of stirring at 95 °C under argon atmosphere, the intermediate was precipitated with aqueous KPF₆ solution and isolated via filtration. It was dissolved in molten PyHCl and the melt stirred at 180 °C for 45 min. **PhOHCl₂-DAOTA** was isolated via precipitation from aqueous KPF₆ and filtration. The product was purified via flash column chromatography on silica with DCM/MeOH (99/1 v/v). Yield: 97 mg (5%).

¹H NMR (300 MHz, CD₃OD): δ = 8.08 (td, J =8.4, 5.5 Hz, 2 H, aromat. CH), 7.91 – 7.79 (m, 1 H, aromat. CH), 7.59 (d, J =8.9 Hz, 1 H, aromat. CH), 7.46 (d, J =8.6 Hz, 1 H, aromat. CH), 7.27 (d, J =8.2 Hz, 1 H, aromat. CH), 7.20 (d, J =13.6 Hz, 3 H, 1 H: triangulenium aromat. CH, 2 H: substituent aromat. CH), 6.84 (d, J =8.6 Hz, 2 H, aromat. CH), 4.58 – 4.41 (m, 2 H, N-CH₂), 1.92 (p, J =8.1, 7.6 Hz, 2 H, aliphatic. CH₂), 1.62 (p, J =7.2 Hz, 2 H, aliphatic. CH₂), 1.46 (dq, J =11.6, 6.1, 5.3 Hz, 2 H, aliphatic. CH₂), 1.31 (d, J =16.4 Hz, 16 H, aliphatic. CH₂), 0.95 – 0.81 (m, 3 H, CH₃).

¹³C APT-NMR (76 MHz, CD₃OD): δ = 145.10, 143.64, 142.04, 140.82, 140.18, 139.53, 128.27, 127.89, 112.17, 110.63, 109.95, 109.05, 107.30, 64.72, 49.00, 33.34, 31.03, 30.72, 27.91, 27.20, 24.00, 14.71
MS MALDI-TOF: m/z [M⁺] calc. 611.22, found 611.12.

Synthesis of *N*-(3-chloro-4-hydroxy)phenyl-*N*-dodecyl DAOTA⁺ PF₆⁻ (**PhOHCl-DAOTA**)

The dye was prepared analogously to **PhOHCl₂-DAOTA** using 3-chloro-4-hydroxyaniline instead of 3,5-dichloro-4-hydroxyaniline. Yield: 32 mg (4%).

¹H NMR (300 MHz, CD₂Cl₂): δ = 8.13 (t, J =8.5 Hz, 1H, aromat. CH), 8.04 (t, J =8.6 Hz, 1 H, aromat. CH), 7.97 – 7.81 (m, 2H, 1 H: triangulenium aromat. CH, 1 H: substituent *p*-OH aromat. CH), 7.48 (d, J =8.8 Hz, 1 H, aromat. CH), 7.43 – 7.27 (m, 4 H, 3 H: Triangulenium aromat. CH, 1 H: substituent *p*-Cl aromat. CH), 7.19 – 7.07 (m, 1 H, substituent aromat. CH), 6.87 – 6.73 (m, 2 H, aromat. CH), 4.51 (t, J =8.3 Hz, 2 H, N-CH₂), 2.00 (p, J =7.5 Hz, 2 H, aliphatic. CH₂), 1.71 – 1.54 (m, 2 H, aliphatic. CH₂), 1.45 (s, 2 H, aliphatic. CH₂), 1.40 – 1.11 (m, 14 H, aliphatic. CH₂), 0.88 (t, J =6.5 Hz, 3 H, CH₃).

¹³C APT-NMR (76 MHz, CD₂Cl₂): δ = 145.10, 143.64, 142.04, 140.82, 140.18, 139.53, 128.27, 127.89, 112.17, 110.63, 109.95, 109.05, 107.30, 64.72, 49.00, 33.34, 31.03, 30.72, 27.91, 27.20, 24.00, 14.71
MS MALDI-TOF: m/z [M⁺] calc. 577.26, found 577.25.

Synthesis of N-(3-Morpholinopropyl)-N-dodecyl DAOTA⁺ PF₆⁻ (Morph-DAOTA)

Synthesis of Morph-DAOTA was conducted similarly to that of **PhOHCl₂-DAOTA** and **PhOHCl-DAOTA**. The order of substitution was reversed with the dodecyl chain introduced before the morpholino group. Yield: 34 mg, 10%.

¹H NMR (300 MHz, CD₂Cl₂): δ = 8.28 (t, 1H, aromat. C-H), 8.09 (t, 2H, aromat. C-H), 7.84 (d, J=4.7 Hz, 1H, aromat. C-H), 7.78 – 7.67 (m, 1H, aromat. C-H), 7.49 (d, J=8.3 Hz, 1H, aromat. C-H), 7.40 (d, J=7.6 Hz, 1H, aromat. C-H), 7.27 (d, J=4.3 Hz, 2H, aromat. C-H), 4.73 (s, 2H, N-CH₂), 4.59 – 4.39 (m, 2H, N-CH₂), 3.77 (s, 3H, morpholino N-CH₂), 3.70 – 3.48 (m, 3H, propyl N-CH₂), 2.65 (d, J=35.1 Hz, 4H, morpholino N-CH₂), 2.13 (d, J=10.5 Hz, 2H, propyl CH₂), 1.93 (s, 2H, aliphatic. CH₂), 1.61 (s, 2H, aliphatic. CH₂), 1.42 (s, 3H, aliphatic. CH₂), 1.26 (s, 10H, aliphatic. CH₂), 0.86 (d, J=6.7 Hz, 3H, CH₃)

¹³C APT-NMR (76 MHz, CD₂Cl₂): δ = 153.16, 141.63, 141.30, 140.54, 140.39, 140.14, 139.28, 112.01, 110.23, 109.59, 109.29, 107.99, 107.02, 106.35, 100.54, 67.25, 54.00, 48.94, 46.93, 32.45, 30.10, 29.87, 27.30, 26.26, 23.22, 14.42, 8.53

MS MALDI-TOF: m/z [M⁺] calc. 578.37, found 578.25.

Optode Preparation

A solution containing 10% wt. polymer (Hydromed D4 polyurethane hydrogel) and 0.1% wt. dye (1 wt. % in respect to polymer) in EtOH:H₂O (9:1 v/v) was knife coated onto a poly(ethylene terephthalate) support with a bar film applicator (75 μ m wet film thickness) and the solvent was allowed to evaporate under ambient conditions. For the optodes containing 0.5, 0.3 and 0.15 % wt. of PhOHCl₂-DAOTA in hydrogel D4 the thickness of the wet film was 75, 200 and 200 μ m, respectively. This way, inner filter effect was avoided at higher dye concentrations and sufficient brightness of the optodes was ensured at lower dye concentrations. For imaging experiments diamond micropowder (1:1 w/w in respect to the polymer) was added to the sensor foil (1% dye w/w with respect to polymer).

Preparation of Nanoparticles and investigation of their properties in solutions and in cells is reported in supporting information.

Measurements

¹H and ¹³C NMR spectra were recorded on a Bruker AVANCE III instrument (300.36 MHz ¹H NMR, 75.53 MHz ¹³C NMR). Mass spectra were acquired in a positive reflector mode on a Micromass TofSpec 2E Time-of-Flight Mass Spectrometer (Bruker Ultraflex Extreme, www.bruker.com).

Absorption spectra were measured on a Varian Cary 50 UV-VIS spectrometer (www.agilent.com) in 10 mm precision cuvettes by Hellma Analytics (www.hellma-analytics.com). Emission spectra were recorded on a Hitachi-F-7000 fluorescence spectrometer (www.hitachi-hightech.com). Fluorescence lifetimes were acquired using time correlated single photon counting technique (TCSPC) on a FluoroLog[®] 3 spectrofluorometer (Horiba Scientific, www.horiba.com). Excitation was performed with a NanoLED ($\lambda = 453$ nm) controlled by a Delta Hub module and a pulse length of 1.1 ns. The average decay times were obtained from the fit of the decay profiles with biexponential decay model. Absolute quantum yields were determined on the FluoroLog[®] 3 spectrofluorometer equipped with a Quanta- Φ integrating sphere. Relative quantum yields were determined against Lumogen Red as reference dye ($\Phi = 0.96^{43}$). Nanoparticle size and surface charge were determined on a particle size analyzer Zetasizer Nano ZS (www.malvernpanalytical.com).

Photostability measurement

Photostability of PhOHCl-DAOTA was investigated in comparison with Macrolex[®] Fluorescent Red G and Rhodamine 101. The absorption of solutions of PhOHCl-DAOTA and the reference dyes in EtOH was measured every five minutes while illuminating the solutions with a high power LED ($\lambda = 528$ nm) (www.led-tech.de). The photon flux of the LED was measured using a LI-250A Light Meter from LICOR Biosciences (www.licor.com) and was $11700 \mu\text{mol s}^{-1} \text{m}^{-2}$, $9100 \mu\text{mol s}^{-1} \text{m}^{-2}$ and $8400 \mu\text{mol s}^{-1} \text{m}^{-2}$ for PhOHCl-DAOTA, Macrolex[®] Red and Rhodamine 101, respectively. The decrease in absorption was corrected to normalize the values for the identical light intensity.

pH Calibrations

Unless otherwise stated, all measurements were conducted at 25 °C with buffer concentration of 10 mM and ionic strength adjusted with NaCl. Absorption and emission spectra for the optode based on PhOHCl₂-DAOTA in D4 (1% wt.) were acquired at pH values between 3 and 9 in buffers with 10 mM buffer concentration and additional salt concentrations of 10 mM NaCl, 290 mM NaCl, 290 mM NaNO₃ and 100 mM NaClO₄ plus 190 mM NaCl. Boltzmann sigmoid function was used to fit the response curves: $y = A_2 + \frac{A_1 - A_2}{1 + e^{-\frac{pH - pK_a'}{dx}}}$, where A_2 and A_1 are the lower and upper limits of the calibration function, respectively, pK_a' is the point of inflection of the sigmoid and dx describes the slope in the point of inflection.

FD-FLIM Imaging

Imaging experiments were performed with a pco.flim camera from PCO (www.pco.de/de) operating in the frequency domain.^{44,45} The excitation source, a 488 nm laser diode (PhoxXplus 488-200 for pco.flim) from Omicron (www.omicron-laser.de), was modulated at 15 MHz modulation frequency (square wave

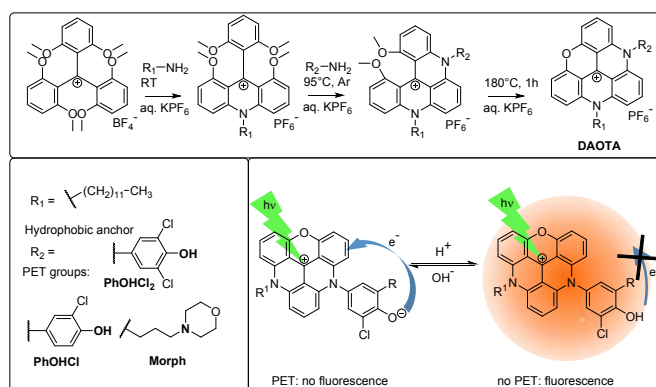
1
2
3 modulation). Average laser power was 100 mW. The NIS Elements AR microscopy software from
4 Nikon was used for data acquisition and processing. For microscopic measurements the sensor spots of
5 5 mm in diameter were cut out of the sensor foil and glued into a black 96 microwell plate from Greiner
6 bio-one (www.gbo.com). Images were acquired with a Nikon Eclipse Ti-S microscope equipped with a
7 Nikon Plan Apo 20x/0.75 objective and 0.7x camera adaptor mounted on the camera. The filter cube
8 included an excitation bandpass filter FF02-482/18, a dichroic mirror Di02-R488 and an emission
9 longpass filter BLP01-488R, all from Semrock (www.semrock.com). A dye-doped plate UMM/SFG
10 from Starna Scientific (www.starna.com) was used as a lifetime standard ($\tau = 3.75$ ns).
11
12
13
14
15

16 For macroscopic measurements, the sensor foil was fixed in the channel of the open microfluidic
17 chip (COP polymer, ChipShop, www.microfluidic-chipshop.com) with an epoxy glue. The chip was
18 bonded using a double side adhesive tape from ChipShop. The laser light was coupled into a 3 mm
19 liquid waveguide in combination with beam expansion optics to homogeneously illuminate the
20 microfluidic chamber under observation. A longpass filter OG 530 from Schott (www.schott.de) was
21 used in front of the macro lens Zeiss Makro-Planar 2/100 mm ZF (www.zeiss.com). Polystyrene-
22 immobilized Lumogen red was used as a lifetime standard ($\tau = 6.3$ ns as determined in TCSPC
23 experiments).
24
25
26
27
28
29
30
31

32 RESULTS AND DISCUSSION

33 *Synthesis*

34 Although triangulenium dyes have been primarily chosen for their extraordinary long fluorescence
35 lifetimes (~ 20 ns), these dyes also feature comparably long wavelengths of absorption and emission
36 (particularly for di-aza substituted representatives), which is favourable for biological applications. The
37 triangulenium structure offers a straightforward synthetic strategy to introduce functional groups
38 (Scheme 1). The dyes were substituted with a dodecyl chain on one nitrogen bridge as a hydrophobic
39 anchor to achieve good solubility in organic solvents and to prevent leaching from polymer matrices. A
40 pH-sensitive functionality was introduced via the second nitrogen bridge. Two different moieties were
41 investigated. The first group includes phenolic receptors which are known to induce efficient quenching
42 via photoinduced electron transfer when deprotonated.⁴⁶ Importantly, the pK_a of the receptor can be
43 tuned via the number of electron-withdrawing chlorine atoms attached to the phenyl ring. Mono- and
44 dichloro-substituted phenols (PhOHCl-DAOTA and PhOHCl₂-DAOTA, respectively) were selected to
45 cover the most relevant applications. A tertiary amine (morpholino group, Morph-DAOTA) was chosen
46 as the second receptor group. This group is potentially useful to achieve incomplete PET-quenching
47 rendering both forms of the indicator emissive.
48
49
50
51
52
53
54
55
56
57
58
59
60



Scheme 1: Synthesis of new pH sensitive DAOTA dyes and sensing mechanism of the indicators.

Photophysical properties

Absorption and emission spectra are similar for the new DAOTA dyes substituted with different PET groups. The absorption and the emission maxima are located at ~ 560 and 590 , respectively, resulting in a 30 nm Stokes shift (Figure 1, Table 1). The fluorescence quantum yields are comparable to those determined by Laursen et al. for the dipropyl-DAOTA dye (58% in acetonitrile)⁴⁷ with values between 48 and 63% in EtOH acidified with $\sim 0.1\%$ trifluoroacetic acid (TFA). Good quantum yields compensate for moderate molar absorption coefficients of $10 - 14 \cdot 10^3$ L mol⁻¹ cm⁻¹ ensuring acceptable brightness of the dyes. The fluorescence lifetimes are in the range of 17 to 19 ns in EtOH solution (Table 1), as has been expected for DAOTA compounds. The fluorescence of PhOHCl-DAOTA and PhOHCl₂-DAOTA is strongly quenched upon deprotonation of the receptor (Figure 1). In contrast, we did not observe any pH sensitivity for the Morph-DAOTA indicating that the tertiary amine is not an efficient PET quencher for the triangulenium chromophore.

Since luminescence of some cationic dyes is known to be efficiently quenched by chloride ions^{48,49}, this potential cross-talk was investigated. As can be seen from Figure S-20 (ESI), chloride ions do not notably affect the fluorescence properties of the triangulenium dyes even at very high concentration of 290 mM.

Table 1: Photophysical properties of DAOTA⁺ dyes in ethanol, acidified with $\sim 0.1\%$ TFA.

	$\lambda_{\max, \text{abs}}$ [nm]	$\lambda_{\max, \text{em}}$ [nm]	ϵ [L mol ⁻¹ cm ⁻¹] (n=3)	$\Phi^{(a)}$ [%] (n=6)	τ [ns] (n=3)
PhOHCl₂-DAOTA	562	589	13800 ± 1400	63 ± 18	17.3 ± 0.1
PhOHCl-DAOTA	562	590	10600 ± 700	59 ± 7	17.7 ± 0.1
Morph-DAOTA	559	589	9800 ± 100	48 ± 12	19.0 ± 0.9

(a) Fluorescence quantum yields are determined as an average value of the absolute and relative quantum yields

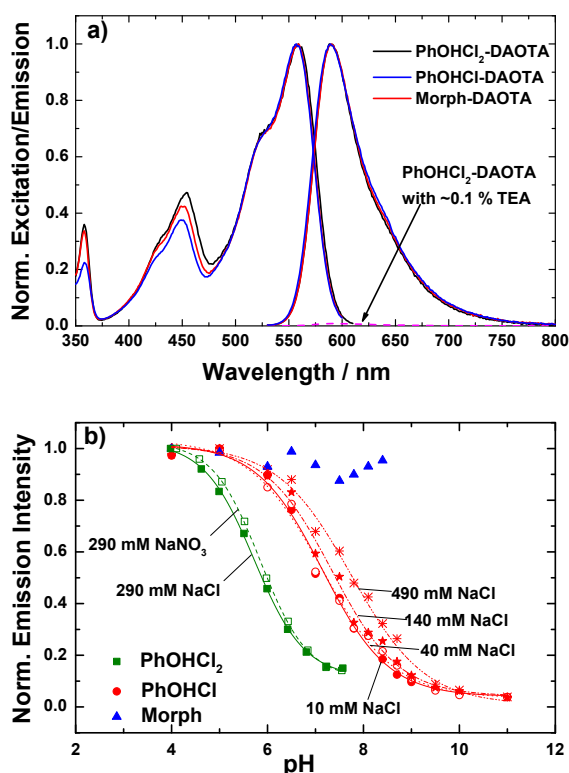


Figure 1: **a)** Absorption and emission spectra for the dyes in EtOH solution (except otherwise stated, all the solutions were acidified with $\sim 0.1\%$ TFA) **b)** calibration plots for the dyes dissolved in EtOH : aqueous buffer (1:1 v/v). The buffer concentration in the solution was 10 mM, the salt (NaCl or NaNO₃) concentration is indicated for each calibration; ($\lambda_{\text{exc}} = 560$ nm, $\lambda_{\text{em}} = 590$ nm).

Photostability

To access the photostability of the triangulenium system, the EtOH solution of PhOHCl-DAOTA was illuminated with a high power green LED. Two fluorescent dyes with similar spectral properties were used for comparison: the coumarin dye Macrolex[®] Red and Rhodamine 101. As can be seen (ESI Figure S-21), only minor degradation (3%) of PhOHCl-DAOTA was detected even after 1 h of continuous illumination. In the same conditions about 1% of Macrolex[®] Red and 15% of Rhodamine 101 degraded, confirming excellent photostability of the triangulenium chromophore.

pH Optodes

pH optodes were manufactured via physical entrapment of the indicator dyes into a polyurethane hydrogel D4 (water uptake 50%). Evidently, the spectral properties of the immobilized indicators are very similar to the dissolved ones (Figure 2). Even at high concentrations (1% wt.) the absorption spectra remain virtually unaltered (Figure 2a) which indicates absence of dye aggregation. Deprotonation of the

receptor results in a minor bathochromic shift of the absorption spectra and almost complete quenching of fluorescence (Figure 2b). This response is reversible, showing almost no hysteresis (ESI Figure S-22). Similar to the behaviour in solution, Morph-DAOTA immobilized into D4 hydrogel did not show any pH sensitivity in the pH range from 3 to 12. (ESI Figure S-23).

Despite the positive charge of the triangulenium dyes in the protonated form and zwitterionic character of the deprotonated species, the optodes showed no leaching of the indicators in aqueous media during exposure to various buffers for several hours/overnight (ESI Figure S-24). This confirms the efficiency of the strategy based on a lipophilic anchor group in the indicator molecule.

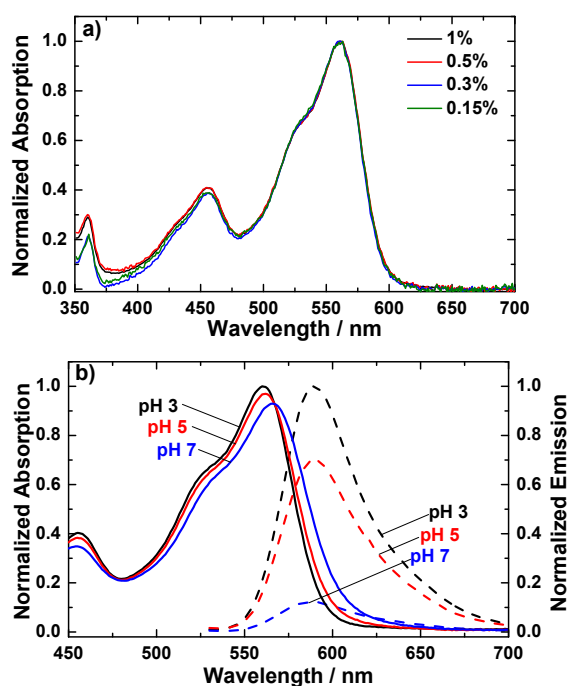


Figure 2: a) Absorption spectra of different concentrations of PhOHCl₂-DAOTA in D4. b) pH dependency of the absorption and emission ($\lambda_{\text{exc}} = 560 \text{ nm}$) spectra of PhOHCl₂-DAOTA dye 1% wt. in D4.

pH Dependence of Fluorescence Intensity

Similar to solutions (Figure 1b), the pH sensors show typical sigmoidal dependency of both fluorescence intensity and decay time. As expected, the sensors based on PhOHCl₂-DAOTA and PhOHCl-DAOTA indicators cover different dynamic ranges (Figure 3a) making them suitable for a variety of potential applications ranging from biotechnology to biology and medicine.

The cross-talk of optical pH sensors to ionic strength resulting from different charges of the protonated and deprotonated forms is well documented in literature. Not surprisingly, this cross-talk was also observed for the sensors based on triangulenium dyes (Figure 3b). In fact, the apparent pK'_a value of the PhOHCl₂-DAOTA embedded into D4 hydrogel (1% wt. of the dye) increased from 4.71 ± 0.01

1
2
3 to 5.00 ± 0.04 on going from 10 mM NaCl to 290 mM NaCl (overall ionic strength of ~ 20 and 300 mM,
4 respectively).

5
6 Subsequently, we investigated the effect of different cations and anions on the pH sensing
7 behaviour of D4-immobilized PhOHCl₂-DAOTA, keeping total ionic strength constant. Whereas
8 several common metal cations used in physiologically relevant concentrations did not affect the
9 fluorescence properties of the sensor (ESI Figure S-25), we found a strong influence of the anion nature
10 on the response curve (Figure 3b). In fact, the apparent pK'_a value increased from 5.00 ± 0.04 for 290
11 mM NaCl to 5.38 ± 0.04 for 290 mM NaNO₃. This shift was even more pronounced in presence of the
12 perchlorate ion (addition of 100 mM NaClO₄ to 190 mM NaCl); the material showing the apparent
13 pK'_a value of 5.54 ± 0.09 . Importantly, the nature of the anions similarly affects the apparent
14 pK'_a values obtained from the absorption spectra (Figure 3c). Interestingly, the observed trend correlates
15 well with the position of the anions in the Hofmeister series.⁵⁰

16
17 Polyurethane hydrogels are reported to be heterogeneous featuring microphase separation and
18 physical cross-linking typical for polyurethanes⁵¹ which is due to combination of hydrophobic
19 interactions (aggregation of hydrophobic domains in order to minimize the surface area contacting with
20 bulk water)⁵² and hydrogen bonding between NH groups and urethane carbonyl oxygen or ether oxygen
21 atoms.⁵³ Therefore, the above behaviour may be due to formation of stable ion pairs between the dye
22 and the anion and the associated changes in the dye localization in the heterogeneous hydrogel matrix.
23 This can affect both the fluorescence properties and dissociation equilibrium due to different activity of
24 the protons and changes in microsolvation in the not purely aqueous medium. In fact, only marginal
25 difference between the pK_a values obtained in 290 mM NaCl and 290 mM NaNO₃ were observed for
26 the dissolved indicator (Figure 1b). Moreover, whereas in solution the emission intensity of the dye
27 appears not to be affected by the nature of the anion (ESI Figure S-20), the situation is opposite for the
28 hydrogel-immobilized dye (Figure 3d). Here a strong enhancement of the fluorescence intensity in the
29 row Cl⁻ < NO₃⁻ < ClO₄⁻ was observed. Immobilization of the indicator into the blends of hydrogel D4 and
30 Nafion® 117 membrane (a perfluorinated polymer with charged sulfonate-groups) virtually eliminated
31 the anion interference albeit at the cost of strong dependency of the apparent pK_a of the indicator to ionic
32 strength explained by the highly charged character of the matrix (ESI Figure S-27). Based on above
33 considerations, immobilization of the triangulenium indicators into homogeneous polymeric matrixes
34 (e.g. acrylamides, acrylates etc.) or their covalent attachment to the polymer support (e.g. cross-linked
35 polymeric microparticles, silica-gel beads etc.) should be considered in future as a possible way to
36 minimize the anion influence.

37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

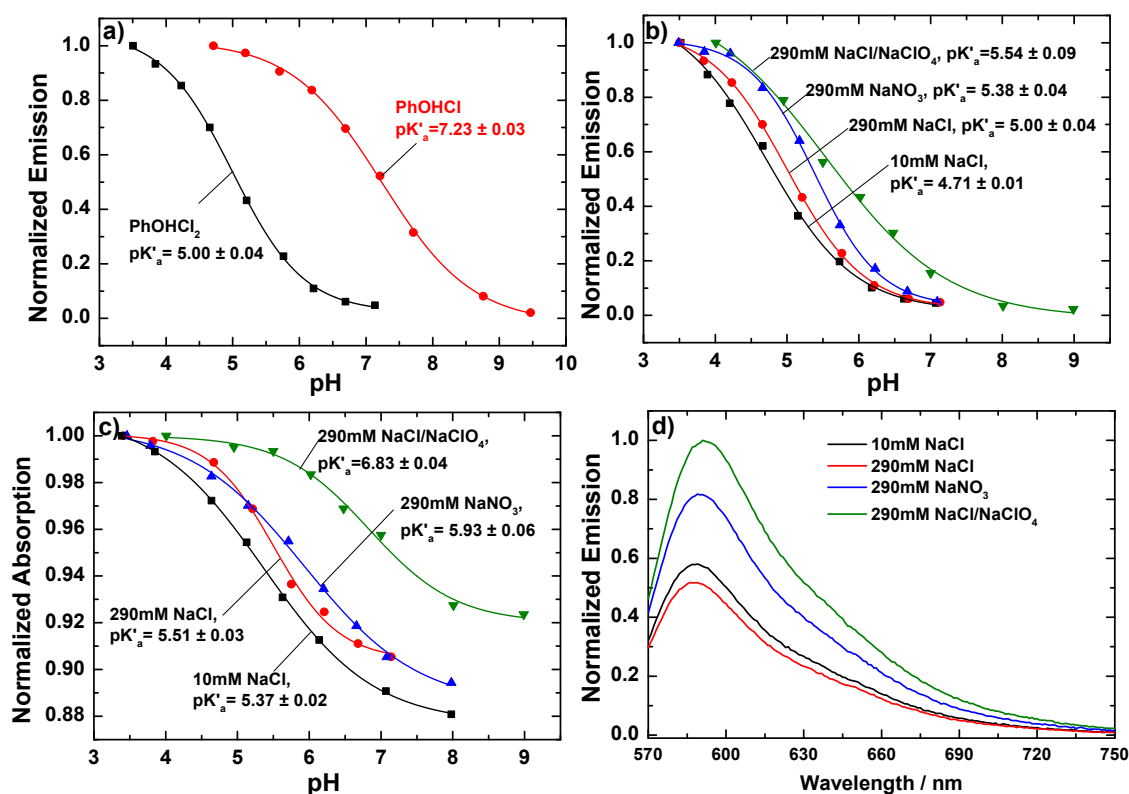


Figure 3: a) Fluorescence response of the PhOHCl₂-DAOTA and PhOHCl-DAOTA embedded into hydrogel D4 (1% wt. of the dye, 290 mM NaCl); b) and c) pH dependency of fluorescence ($\lambda = 590$ nm) and absorption ($\lambda = 560$ nm), respectively, of PhOHCl₂-DAOTA in D4 (1% wt. of the dye) in buffers with different ionic strength and type of anions; d) Influence of ionic strength and type of anion on the fluorescence intensity of the same material measured at pH 3; buffer concentrations in all cases 10 mM; ($\lambda_{\text{exc}} = 560$ nm).

pH Dependence of Fluorescence Decay Time

Fluorescence lifetime measurements in EtOH:buffer solutions revealed that the decay time was independent on pH (17.3 ns, ESI Figure S-28). Such behaviour is not unexpected since only the protonated form of the dye is emissive and “visible” for the decay time measurement. In contrast, a clear pH dependency of the average decay time was observed for the polymer-immobilized dyes (Figure 4). More detailed investigation revealed strong dependency of the calibration plots on the concentration of the immobilized dye (Figure 4).

This unexpected lifetime sensitivity is attributed to the homo-FRET process. In fact, the overlap between absorption and emission spectra is clearly visible (Figure 2). Moreover, despite rather modest bathochromic shift of the absorption spectrum, the overlap increases significantly upon deprotonation of the dye. The FRET efficiency and the observed lifetime change is the highest for 1% dye doping (~2-fold lifetime change from pH 3 to pH 8). On the contrary, it is the least pronounced (only 15% decrease in the same pH range) for the lowest concentration of the dye in the polymer (0.15% wt.). Nevertheless,

even the lowest concentration of the dye in the optode is much higher than for the solutions (~ 2.5 mM for the swollen hydrogel and $10 \mu\text{M}$ for the solution) where no lifetime change was recorded. Clearly, because of the self-referenced character of the decay time, the new materials are very promising for a lifetime-based micro- and macroscopic imaging using a FLIM technique. Imaging of pH with planar optodes gains importance in marine biology^{54,55}, soil and plant research^{4,5}, medicine⁷ and other areas and is currently performed either in ratiometric mode or using a so called dual lifetime referencing technique. These methods typically require addition of other luminescent reference emitters which makes the system more complex and does not eliminate some interferences (e.g. from light scattering or background fluorescence). The advantage of the new optode for lifetime imaging of pH is strong modulation of the decay time which however remains sufficiently long to discard the autofluorescence from the sample. Moreover, the decay only slightly deviates from the mono-exponential behaviour (ESI Figure S-29) which can also be useful for background elimination and simplified data processing.

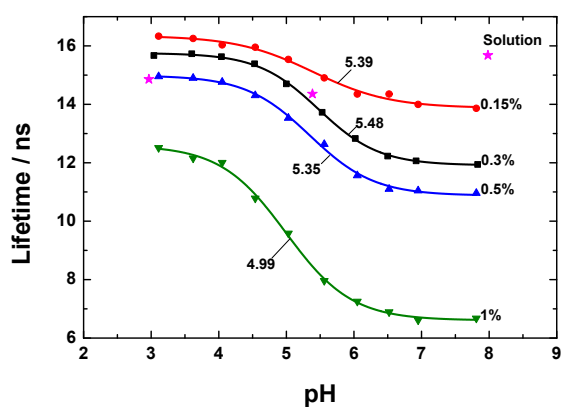


Figure 4: Response of the fluorescence lifetimes to pH for different concentrations of PhOHCl_2 -DAOTA in D4 (% wt.) and of $10 \mu\text{M}$ dye solution (MeOH:buffer 30:70) at $25 \text{ }^\circ\text{C}$, 10 mM buffer and 140 mM NaCl; the inflection points of the curves are marked in the graph.

FD-FLIM imaging with the planar optodes

In order to demonstrate the practical applicability of the sensors we interrogated them with a new frequency-domain lifetime imaging camera, pco.flim.^{44,45} The pH dependencies of the fluorescence decay time obtained in the imaging experiments (Figure 5a) and TCSPC (Figure 4) are similar. The planar optode placed inside the chamber of a microfluidic chip (Figure 5b) proved to be excellently suitable for monitoring of pH gradients (Figure 5c and 5d). This can be useful, for instance, for monitoring enzymatic reactions⁵⁶ and for isoelectric focusing.^{9,10} The experiment also shows that the planar optode in combination with the FD-FLIM camera can also be used on macroscale for other experiments, for example monitoring pH in biofilms⁶ or during wound healing.^{7,8}

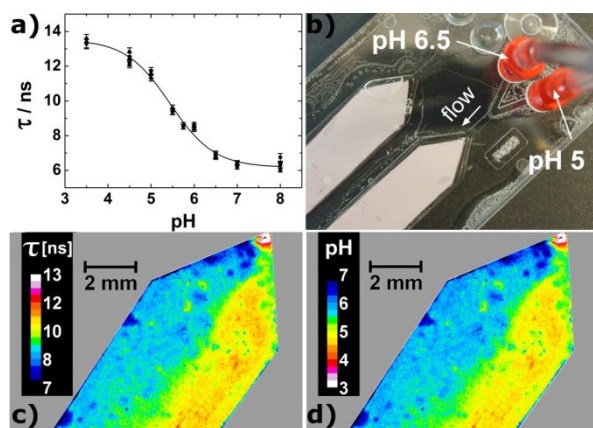


Figure 5: FLIM imaging of pH with the planar optode based on PhOHCl₂-DAOTA in D4 (1 % wt). (a): pH dependency of the fluorescence lifetime obtained in the microscopic imaging experiment (read-out area ~0.9x0.9 mm). The foil has been measured at ≥ 3 different spots for each pH value. (b): photographic image of the microfluidic chip with integrated sensor foil. (c) and (d): false color images of decay time and pH distribution, respectively, for the microfluidic chip simultaneously flushed with buffers of pH 6.5 and 5.0 (speed 21 $\mu\text{L min}^{-1}$).

pH nanosensors

Several types of nanoparticles with immobilized indicators were prepared in order to explore the self-referenced lifetime measuring capabilities. In order to ensure high efficiency of homo-FRET, comparably high concentration of the dye was used (0.75% wt.). Nanoparticles prepared upon immobilization of PhOHCl₂-DAOTA into anionic PMMA-MA showed aggregation in buffer solutions, which might be due to destabilization of the particle by the cationic dye. The cationic RL-100 nanoparticles (Z-potential of +41 mV), on the contrary, were stable in water and in buffers. Their size (16 nm, PDI=0.410) was significantly below the size of the RL-100 particles doped with a hydrophobic oxygen indicator (30-40 nm).^{57,58} The dyes showed no leaching from the nanoparticles in aqueous buffer solution evidenced by formation of colored precipitate and uncoloured solution upon coagulation of the beads at high concentration (~3 mg/mL) and high ionic strength (300 mM). For both dyes in RL-100 nanoparticles, pH calibrations in fluorescence intensity and decay time mode yielded sigmoidal calibration plots (ESI, **Error! Reference source not found.**).

PhOHCl₂-DAOTA was also immobilized in poly(styrene-sulfonic acid) (PSSA) which forms very small (6 nm) and highly negatively-charged (Z-potential of -49 mV) nanogel. The fluorescence intensity decreased by about 80% on going from pH 4.5 to 7.5, showing the characteristic sigmoidal response with an apparent pK'_a of 5.98. Change in fluorescence decay time, however, was not observed. This indicates that the PSSA dispersion cannot provide the spatial proximity necessary for efficient homo-FRET.

As expected, the inflection point of decay time measurements for PhOHCl₂-DAOTA embedded in the positively charged RL-100 nanoparticles was about one pH unit lower than in the uncharged

1
2
3 hydrogel D4 (4.02 and 5.00, respectively). On the contrary, the pK_a' value for the dye in negatively
4 charged PSSA (5.98) was about one pH unit higher than in D4.
5

6 Due to the possibility of the self-referenced read-out, the RI-100 based nanosensors were
7 expected to be highly promising for intracellular pH imaging. Although they showed no evident toxicity
8 after internalization by the human colon cancer HCT116 cells (ESI, Fig. S-32), the staining was very
9 fast (1 h or less) and highly similar to the free dye, which may indicate leaching of the dye from the
10 nanoparticles inside the cells, due to their small size (ESI, Fig. S-33, S-34). For comparison, the
11 nanoparticles prepared from the same polymer typically require three hours and more for cell
12 internalization.^{58,59} The assumption of leaching was also supported by the FLIM measurements in cells,
13 when no reliable pH response and loss of mitochondrial-like localization after dissipation of
14 mitochondrial pH gradient were observed (ESI, Fig. S-35, S-36). Therefore, for intracellular pH FLIM
15 sensing, it appears to be essential to completely eliminate 'in-cell' leaching of the dye out of the
16 nanosensors, which can be possible by using longer (or/and branched) anchor groups or via covalent
17 grafting of the indicators into the polymer.
18
19
20
21
22
23
24
25
26
27

28 CONCLUSIONS

29
30 In this work, we presented synthesis and characterization of new fluorescent pH indicators based on
31 diazoxatriangulenium dyes. The dyes were rationally designed to bear both a pH-sensing functionality
32 and a hydrophobic anchor group for non-covalent immobilization into hydrogel matrices and
33 nanoparticles. The dyes feature absorption and emission in the green-orange part of the spectrum and
34 possess excellent photostability. Importantly, the pH indicators retain extremely long fluorescence decay
35 times enabling efficient elimination of autofluorescence in time-resolved measurements.
36
37
38
39

40 Chlorinated phenols proved to be excellent receptors, which enable pH modulation via
41 photoinduced electron transfer. The dyes decorated with mono- and di-chlorinated moieties possess
42 largely different pK_a' values and therefore enable high versatility of potential applications.
43 Unexpectedly, we have found strong pH dependency of the fluorescence decay time for the indicators
44 immobilized into a polyurethane hydrogel and polymeric nanoparticles. Being concentration-dependent
45 and not observed for the dissolved indicators this behaviour is attributed to homo-FRET. Self-referenced
46 character of the lifetime measurement makes the new materials particularly attractive for pH sensing
47 and imaging. Whereas pH measurement in cells with help of nanosensors proved to be impossible due
48 to dye leaching in the intracellular environment, the planar optodes were shown to be promising for
49 imaging of pH distribution with help of a FD-FLIM camera. This paves the way to a variety of
50 applications eliminating the need for the reference luminophore which so far has to be added into the
51 sensing material for reliable measurement. Covalent immobilization of the dyes into the polymers
52 represent the next logical step in order to eliminate leaching of the indicators out of the nanoparticles
53 and possibly reduce the cross-sensitivity of the sensor response to the nature of anion used.
54
55
56
57
58
59
60

ACKNOWLEDGMENTS

This work was partly supported by the Science Foundation Ireland (SFI) grant 13/SIRG/2144 (RID).
The authors thank Prof. T. Mayr (TU Graz) for providing the microfluidic chips.

SUPPORTING INFORMATION

Additional Experimental Data. Analytical data for structure analysis (^1H , COSY, APT and HSQC NMR, Maldi-TOF MS) Figures S1-S19. Details on characterization of optodes (leaching, reversibility, hysteresis, calibration, interferences and cell staining / viability / FLIM studies, Figures S20-S36).

REFERENCES

- (1) McDonagh, C.; Burke, C. S.; MacCraith, B. D. Optical Chemical Sensors. *Chem. Rev.* **2008**, *108* (2), 400–422. <https://doi.org/10.1021/cr068102g>.
- (2) Stahl, H.; Glud, A.; Schröder, C. R.; Klimant, I.; Tengberg, A.; Glud, R. N. Time-Resolved PH Imaging in Marine Sediments with a Luminescent Planar Optode: PH Imaging in Marine Sediments. *Limnol. Oceanogr. Methods* **2006**, *4* (10), 336–345. <https://doi.org/10.4319/lom.2006.4.336>.
- (3) Larsen, M.; Borisov, S. M.; Grunwald, B.; Klimant, I.; Glud, R. N. A Simple and Inexpensive High Resolution Color Ratiometric Planar Optode Imaging Approach: Application to Oxygen and PH Sensing.: A Simple RGB Based Planar Optode Imaging Approach. *Limnol. Oceanogr. Methods* **2011**, *9* (9), 348–360. <https://doi.org/10.4319/lom.2011.9.348>.
- (4) Hofer, C.; Santner, J.; Borisov, S. M.; Wenzel, W. W.; Puschenreiter, M. Integrating Chemical Imaging of Cationic Trace Metal Solutes and PH into a Single Hydrogel Layer. *Anal. Chim. Acta* **2017**, *950*, 88–97. <https://doi.org/10.1016/j.aca.2016.11.004>.
- (5) Koop-Jakobsen, K.; Mueller, P.; Meier, R. J.; Liebsch, G.; Jensen, K. Plant-Sediment Interactions in Salt Marshes – An Optode Imaging Study of O₂, PH, and CO₂ Gradients in the Rhizosphere. *Front. Plant Sci.* **2018**, *9*. <https://doi.org/10.3389/fpls.2018.00541>.
- (6) Moßhammer, M.; Strobl, M.; Köhl, M.; Klimant, I.; Borisov, S. M.; Koren, K. Design and Application of an Optical Sensor for Simultaneous Imaging of PH and Dissolved O₂ with Low Cross-Talk. *ACS Sens.* **2016**, *1* (6), 681–687. <https://doi.org/10.1021/acssensors.6b00071>.
- (7) Schreml, S.; Meier, R. J.; Wolfbeis, O. S.; Landthaler, M.; Szeimies, R.-M.; Babilas, P. 2D Luminescence Imaging of PH in Vivo. *Proc. Natl. Acad. Sci.* **2011**, *108* (6), 2432–2437. <https://doi.org/10.1073/pnas.1006945108>.
- (8) Meier, R. J.; Schreml, S.; Wang, X.; Landthaler, M.; Babilas, P.; Wolfbeis, O. S. Simultaneous Photographing of Oxygen and PH In Vivo Using Sensor Films. *Angew. Chem.* **2011**, *123* (46), 11085–11088. <https://doi.org/10.1002/ange.201104530>.
- (9) Jezierski, S.; Belder, D.; Nagl, S. Microfluidic Free-Flow Electrophoresis Chips with an Integrated Fluorescent Sensor Layer for Real Time PH Imaging in Isoelectric Focusing. *Chem Commun* **2013**, *49* (9), 904–906. <https://doi.org/10.1039/C2CC38093E>.
- (10) Poehler, E.; Herzog, C.; Lotter, C.; Pfeiffer, S. A.; Aigner, D.; Mayr, T.; Nagl, S. Label-Free Microfluidic Free-Flow Isoelectric Focusing, PH Gradient Sensing and near Real-Time Isoelectric Point Determination of Biomolecules and Blood Plasma Fractions. *The Analyst* **2015**, *140* (22), 7496–7502. <https://doi.org/10.1039/C5AN01345C>.
- (11) Han, J.; Burgess, K. Fluorescent Indicators for Intracellular PH. *Chem. Rev.* **2010**, *110* (5), 2709–2728. <https://doi.org/10.1021/cr900249z>.
- (12) Offenbacher, H.; Wolfbeis, O. S.; Furlinger, E. Fluorescence Optical Sensors for Continuous Determination of Near-Neutral PH Values. *Sens. Actuators* **1986**, *9* (1), 73–84. [https://doi.org/10.1016/0250-6874\(86\)80008-7](https://doi.org/10.1016/0250-6874(86)80008-7).
- (13) Whitaker, J. E.; Haugland, R. P.; Prendergast, F. G. Spectral and Photophysical Studies of Benzo[c]Xanthene Dyes: Dual Emission PH Sensors. *Anal. Biochem.* **1991**, *194* (2), 330–344. [https://doi.org/10.1016/0003-2697\(91\)90237-N](https://doi.org/10.1016/0003-2697(91)90237-N).
- (14) Wolfbeis, O. S.; Furlinger, E.; Kroneis, H.; Marsoner, H. Fluorimetric Analysis: 1. Untersuchung von Fluoreszenzindikatoren Zur Messung von PH-Werten Im

- Neutralbereich. *Fresenius Z. Für Anal. Chem.* **1983**, 314 (2), 119–124.
<https://doi.org/10.1007/BF00482235>.
- (15) Zhujun, Z.; Seitz, W. R. A Fluorescence Sensor for Quantifying PH in the Range from 6.5 to 8.5. *Anal. Chim. Acta* **1984**, 160, 47–55. [https://doi.org/10.1016/S0003-2670\(00\)84507-9](https://doi.org/10.1016/S0003-2670(00)84507-9).
- (16) Niu, C.-G.; Zeng, G.-M.; Chen, L.-X.; Shen, G.-L.; Yu, R.-Q. Proton “off-on” Behaviour of Methylpiperazinyl Derivative of Naphthalimide: A PH Sensor Based on Fluorescence Enhancement. *The Analyst* **2004**, 129 (1), 20–24.
<https://doi.org/10.1039/B309594K>.
- (17) Li, Z.-Z.; Niu, C.-G.; Zeng, G.-M.; Liu, Y.-G.; Gao, P.-F.; Huang, G.-H.; Mao, Y.-A. A Novel Fluorescence Ratiometric PH Sensor Based on Covalently Immobilized Piperazinyl-1,8-Naphthalimide and Benzothioxanthene. *Sens. Actuators B Chem.* **2006**, 114 (1), 308–315. <https://doi.org/10.1016/j.snb.2005.05.018>.
- (18) Gotor, R.; Ashokkumar, P.; Hecht, M.; Keil, K.; Rurack, K. Optical PH Sensor Covering the Range from PH 0–14 Compatible with Mobile-Device Readout and Based on a Set of Rationally Designed Indicator Dyes. *Anal. Chem.* **2017**, 89, 8437–8444.
<https://doi.org/10.1021/acs.analchem.7b01903>.
- (19) Strobl, M.; Walcher, A.; Mayr, T.; Klimant, I.; Borisov, S. M. Trace Ammonia Sensors Based on Fluorescent Near-Infrared-Emitting Aza-BODIPY Dyes. *Anal. Chem.* **2017**, 89 (5), 2859–2865. <https://doi.org/10.1021/acs.analchem.6b04045>.
- (20) Tahirbegi, I. B.; Ehgartner, J.; Sulzer, P.; Zieger, S.; Kasjanow, A.; Paradiso, M.; Strobl, M.; Bouwes, D.; Mayr, T. Fast Pesticide Detection inside Microfluidic Device with Integrated Optical PH, Oxygen Sensors and Algal Fluorescence. *Biosens. Bioelectron.* **2017**, 88, 188–195. <https://doi.org/10.1016/j.bios.2016.08.014>.
- (21) Szmecinski, H.; Lakowicz, J. R. Optical Measurements of PH Using Fluorescence Lifetimes and Phase-Modulation Fluorometry. *Anal. Chem.* **1993**, 65 (13), 1668–1674.
<https://doi.org/10.1021/ac00061a007>.
- (22) Staudinger, C.; Borisov, S. M. Long-Wavelength Analyte-Sensitive Luminescent Probes and Optical (Bio)Sensors. *Methods Appl. Fluoresc.* **2015**, 3 (4), 042005.
<https://doi.org/10.1088/2050-6120/3/4/042005>.
- (23) Clarke, Y.; Xu, W.; Demas, J. N.; DeGraff, B. A. Lifetime-Based PH Sensor System Based on a Polymer-Supported Ruthenium(II) Complex. *Anal. Chem.* **2000**, 72 (15), 3468–3475. <https://doi.org/10.1021/ac000111g>.
- (24) Gonçalves, H. M. R.; Maule, C. D.; Jorge, P. A. S.; Esteves da Silva, J. C. G. Fiber Optic Lifetime PH Sensing Based on Ruthenium(II) Complexes with Dicarboxybipyridine. *Anal. Chim. Acta* **2008**, 626 (1), 62–70. <https://doi.org/10.1016/j.aca.2008.07.044>.
- (25) Malins, C.; Glever, H. .; Keyes, T. .; Vos, J. .; Dressick, W. .; MacCraith, B. . Sol-Gel Immobilised Ruthenium(II) Polypyridyl Complexes as Chemical Transducers for Optical PH Sensing. *Sens. Actuators B Chem.* **2000**, 67 (1–2), 89–95.
[https://doi.org/10.1016/S0925-4005\(00\)00411-1](https://doi.org/10.1016/S0925-4005(00)00411-1).
- (26) Blair, S.; Lowe, M. P.; Mathieu, C. E.; Parker, D.; Senanayake, P. K.; Katakly, R. Narrow-Range Optical PH Sensors Based on Luminescent Europium and Terbium Complexes Immobilized in a Sol Gel Glass. *Inorg. Chem.* **2001**, 40 (23), 5860–5867.
<https://doi.org/10.1021/ic010371w>.
- (27) Ryder, A. G.; Power, S.; Glynn, T. J. Evaluation of Acridine in Nafion as a Fluorescence-Lifetime-Based PH Sensor. *Appl. Spectrosc.* **2003**, 57 (1), 73–79.
- (28) Lin, H.-J.; Szmecinski, H.; Lakowicz, J. R. Lifetime-Based PH Sensors: Indicators for Acidic Environments. *Anal. Biochem.* **1999**, 269 (1), 162–167.
<https://doi.org/10.1006/abio.1999.4011>.

- 1
2
3 (29) Aigner, D.; Dmitriev, R. I.; Borisov, S. M.; Papkovsky, D. B.; Klimant, I. PH-Sensitive
4 Perylene Bisimide Probes for Live Cell Fluorescence Lifetime Imaging. *J Mater Chem B*
5 **2014**, 2 (39), 6792–6801. <https://doi.org/10.1039/C4TB01006J>.
6
7 (30) Orte, A.; Alvarez-Pez, J. M.; Ruedas-Rama, M. J. Fluorescence Lifetime Imaging
8 Microscopy for the Detection of Intracellular PH with Quantum Dot Nanosensors.
9 *ACS Nano* **2013**, 7 (7), 6387–6395. <https://doi.org/10.1021/nn402581q>.
10
11 (31) Tang, R.; Lee, H.; Achilefu, S. Induction of PH Sensitivity on the Fluorescence Lifetime
12 of Quantum Dots by NIR Fluorescent Dyes. *J. Am. Chem. Soc.* **2012**, 134 (10), 4545–4548.
13 <https://doi.org/10.1021/ja300276s>.
14
15 (32) Chen, C.; Zhang, P.; Zhang, L.; Gao, D.; Gao, G.; Yang, Y.; Li, W.; Gong, P.; Cai, L.
16 Long-Decay near-Infrared-Emitting Doped Quantum Dots for Lifetime-Based in Vivo
17 PH Imaging. *Chem. Commun.* **2015**, 51 (56), 11162–11165.
18 <https://doi.org/10.1039/C5CC03046C>.
19
20 (33) Rich, R. M.; Stankowska, D. L.; Maliwal, B. P.; Sørensen, T. J.; Laursen, B. W.;
21 Krishnamoorthy, R. R.; Gryczynski, Z.; Borejdo, J.; Gryczynski, I.; Fudala, R.
22 Elimination of Autofluorescence Background from Fluorescence Tissue Images by Use
23 of Time-Gated Detection and the AzaDiOxaTriAngulenium (ADOTA) Fluorophore.
24 *Anal. Bioanal. Chem.* **2013**, 405 (6), 2065–2075. <https://doi.org/10.1007/s00216-012-6623-1>.
25
26 (34) Draxler, S.; Lippitsch, M. E. PH Sensors Using Fluorescence Decay Time. *Sens.*
27 *Actuators B Chem.* **1995**, 29 (1–3), 199–203. [https://doi.org/10.1016/0925-4005\(95\)01683-X](https://doi.org/10.1016/0925-4005(95)01683-X).
28
29 (35) Badri P., M.; Rafal, F.; Sangram, R.; Rutika, K.; Thomas J., S.; Bo W., L.; Zygmunt, G.;
30 Ignacy, G. Long-Lived Bright Red Emitting Azaoxa-Triangulenium Fluorophores.
31 *PLoS ONE* **2013**, 8 (5). <https://doi.org/10.1371/journal.pone.0063043>.
32
33 (36) Westerlund, F.; Hildebrandt, C. B.; Sørensen, T. J.; Laursen, B. W.
34 Trihydroxytrioxatriangulene-An Extended Fluorescein and a Ratiometric PH Sensor.
35 *Chem. - Eur. J.* **2010**, 16 (10), 2992–2996. <https://doi.org/10.1002/chem.200902965>.
36
37 (37) Frankær, C. G.; Hussain, K. J.; Rosenberg, M.; Jensen, A.; Laursen, B. W.; Sørensen, T. J.
38 Biocompatible Microporous Organically Modified Silicate Material with Rapid
39 Internal Diffusion of Protons. *ACS Sens.* **2018**, 3 (3), 692–699.
40 <https://doi.org/10.1021/acssensors.8b00024>.
41
42 (38) Laursen, B. W.; Sorensen, T. J.; (Vorname). Synthesis of Super Stable Triangulenium
43 Dye. *J. Org. Chem.* **2009**, 74 (8), 3183–3185. <https://doi.org/10.1021/jo9002486>.
44
45 (39) Laursen, B. W.; Krebs, F. C.; Nielsen, M. F.; Bechgaard, K.; Christensen, J. B.; Harrit, N.
46 2,6,10-Tris(Dialkylamino)Trioxatriangulenium Ions. Synthesis, Structure, and
47 Properties of Exceptionally Stable Carbenium Ions. *J. Am. Chem. Soc.* **1998**, 120 (47),
48 12255–12263. <https://doi.org/10.1021/ja982550r>.
49
50 (40) Laursen, B. W.; Krebs, F. C. Synthesis, Structure, and Properties of Azatriangulenium
51 Salts. *Chem. - Eur. J.* **2001**, 7 (8), 1773–1783. [https://doi.org/10.1002/1521-3765\(20010417\)7:8<1773::AID-CHEM17730>3.0.CO;2-F](https://doi.org/10.1002/1521-3765(20010417)7:8<1773::AID-CHEM17730>3.0.CO;2-F).
52
53 (41) Wittig, G.; Pockels, U. Über Den Austausch von Aromatisch Gebundenem Brom
54 Gegen Lithium Mittels Phenyl-Lithiums. *Berichte Dtsch. Chem. Ges.* **1939**, 72, 89–92.
55 <https://doi.org/10.1002/cber.19390720120>.
56
57 (42) Martin, J. C.; Smith, R. G. Factors Influencing the Basicities of Triarylcarbinols. The
58 Synthesis of Sesquixanthidrol. *J. Am. Chem. Soc.* **1964**, 86 (11), 2252–2256.
59 <https://doi.org/10.1021/ja01065a030>.
60
61 (43) Seybold, G. New Perylene and Violanthrone Dyestuffs for Fluorescent Collectors. *Dyes*
62 *Pigments* **1989**, 11 (4), 303–317. [https://doi.org/10.1016/0143-7208\(89\)85048-X](https://doi.org/10.1016/0143-7208(89)85048-X).

- 1
2
3 (44) Chen, H.; Holst, G.; Gratton, E. Modulated CMOS Camera for Fluorescence Lifetime
4 Microscopy: Modulated CMOS Camera for Fluorescence Lifetime Microscopy.
5 *Microsc. Res. Tech.* **2015**, 78 (12), 1075–1081. <https://doi.org/10.1002/jemt.22587>.
6
7 (45) Franke, R.; Holst, G. A. Frequency-Domain Fluorescence Lifetime Imaging System
8 (Pco.Flim) Based on a in-Pixel Dual Tap Control CMOS Image Sensor. *SPIE*
9 *Proceedings*; **2015**; Vol. 9328, pp 93281K-9328-19.
10 (46) Aigner, D.; Freunberger, S. A.; Wilkening, M.; Saf, R.; Borisov, S. M.; Klimant, I.
11 Enhancing Photoinduced Electron Transfer Efficiency of Fluorescent PH-Probes with
12 Halogenated Phenols. *Anal. Chem.* **2014**, 86 (18), 9293–9300.
13 <https://doi.org/10.1021/ac502513g>.
14 (47) Bogh, S. A.; Simmermacher, M.; Westberg, M.; Bregnhøj, M.; Rosenberg, M.; De Vico,
15 L.; Veiga, M.; Laursen, B. W.; Ogilby, P. R.; Sauer, S. P. A.; et al.
16 Azadioxatriangulenium and Diazaoxatriangulenium: Quantum Yields and
17 Fundamental Photophysical Properties. *ACS Omega* **2017**, 2 (1), 193–203.
18 <https://doi.org/10.1021/acsomega.6b00211>.
19 (48) Huber, C.; Klimant, I.; Krause, C.; Wolfbeis, O. S. Dual Lifetime Referencing as
20 Applied to a Chloride Optical Sensor. *Anal. Chem.* **2001**, 73 (9), 2097–2103.
21 <https://doi.org/10.1021/ac9914364>.
22 (49) Masih, D.; Aly, S. M.; Alarousu, E.; Mohammed, O. F. Photoinduced Triplet-State
23 Electron Transfer of Platinum Porphyrin: A One-Step Direct Method for Sensing
24 Iodide with an Unprecedented Detection Limit. *J. Mater. Chem. A* **2015**, 3 (13), 6733–
25 6738. <https://doi.org/10.1039/C4TA07033J>.
26 (50) Baldwin, R. L. How Hofmeister Ion Interactions Affect Protein Stability. *Biophys. J.*
27 **1996**, 71 (4), 2056–2063. [https://doi.org/10.1016/S0006-3495\(96\)79404-3](https://doi.org/10.1016/S0006-3495(96)79404-3).
28 (51) Fernández-d'Arlas, B.; Rueda, L.; Fernández, R.; Khan, U.; Coleman, J. N.; Mondragon,
29 I.; Eceiza, A. Inverting Polyurethanes Synthesis: Effects on Nano/Micro-Structure and
30 Mechanical Properties. *Soft Mater.* **2010**, 9 (1), 79–93.
31 <https://doi.org/10.1080/1539445X.2010.525173>.
32 (52) Hoare, T. R.; Kohane, D. S. Hydrogels in Drug Delivery: Progress and Challenges.
33 *Polymer* **2008**, 49 (8), 1993–2007. <https://doi.org/10.1016/j.polymer.2008.01.027>.
34 (53) Furukawa, M.; Kojio, K.; Kugumiya, S.; Uchiba, Y.; Mitsui, Y. Microphase Separation
35 of Bulk and Ultrathin Films of Polyurethane Elastomers. *Macromol. Symp.* **2008**, 267 (1),
36 9–15. <https://doi.org/10.1002/masy.200850702>.
37 (54) Köhl, M.; Polerecky, L. Functional and Structural Imaging of Phototrophic Microbial
38 Communities and Symbioses. *Aquat. Microb. Ecol.* **2008**, 53, 99–118.
39 <https://doi.org/10.3354/ame01224>.
40 (55) Han, C.; Yao, L.; Xu, D.; Xie, X.; Zhang, C. High-Resolution Imaging of PH in Alkaline
41 Sediments and Water Based on a New Rapid Response Fluorescent Planar Optode. *Sci.*
42 *Rep.* **2016**, 6 (1). <https://doi.org/10.1038/srep26417>.
43 (56) Gruber, P.; Marques, M. P. C.; Sulzer, P.; Wohlgemuth, R.; Mayr, T.; Baganz, F.; Szita,
44 N. Real-Time PH Monitoring of Industrially Relevant Enzymatic Reactions in a
45 Microfluidic Side-Entry Reactor (MSER) Shows Potential for PH Control. *Biotechnol. J.*
46 **2017**, 12 (6), 1600475. <https://doi.org/10.1002/biot.201600475>.
47 (57) Fercher, A.; Borisov, S. M.; Zhdanov, A. V.; Klimant, I.; Papkovsky, D. B. Intracellular
48 O₂ Sensing Probe Based on Cell-Penetrating Phosphorescent Nanoparticles. *ACS Nano*
49 **2011**, 5 (7), 5499–5508. <https://doi.org/10.1021/nn200807g>.
50 (58) Müller, B. J.; Zhdanov, A. V.; Borisov, S. M.; Foley, T.; Okkelman, I. A.; Tsytsarev, V.;
51 Tang, Q.; Erzurumlu, R. S.; Chen, Y.; Zhang, H.; et al. Nanoparticle-Based
52 Fluoroionophore for Analysis of Potassium Ion Dynamics in 3D Tissue Models and In
53 Vivo. *Adv. Funct. Mater.* **2018**, 28 (9), 1704598.
54 <https://doi.org/10.1002/adfm.201704598>.
55
56
57
58
59
60

- 1
2
3 (59) Dmitriev, R. I.; Borisov, S. M.; DÜssmann, H.; Sun, S.; Müller, B. J.; Prehn, J.;
4 Baklaushev, V. P.; Klimant, I.; Papkovsky, D. B. Versatile Conjugated Polymer
5 Nanoparticles for High-Resolution O₂ Imaging in Cells and 3D Tissue Models. *ACS*
6 *Nano* **2015**, 9 (5), 5275–5288. <https://doi.org/10.1021/acsnano.5b00771>.
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30

31 **For TOC only**

