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A wavelength-ratiometric pH sensitive probe based on the boronic acid moiety and suppressed sugar response

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Abstract

We characterize a new water soluble fluorescent probe sensitive to changes in pH. The new probe shows spectral shifts and intensity changes in different pH media, in a wavelength ratiometric and colorimetric manner. Subsequently, changes in pH can readily be determined around the physiological level. The new probe's response is based on the ability of the boronic acid group to interact with strong bases like OH^-, changing from the neutral form of the boronic acid group [R-$B(OH)_2$] to the anionic R-$B^-(OH)_3$ form, which is an electron donating group. The presence of an electron deficient quaternary heterocyclic nitrogen center and a strong electron donating amino group in the 6-position, on the quinolinium backbone, provides for the spectral changes observed upon OH^- complexation. In addition, the presence of the amino group in the 6-position of the quinolinium backbone, suppresses the response of the boronic acid containing probe towards mono saccharides such as glucose and fructose, which are present in many biological fluids, allowing for the predominant pH sensitivity. Finally we compare our findings to those of a control compound that does not contain the boronic acid group.

Keywords: Ratiometric pH sensor; Suppressed sugar response; Boronic acid

1. Introduction

It is widely accepted that ratiometric or lifetime based methods offer intrinsic advantages for both chemical and biomedical fluorescence sensing [1,2]. Fluorescence intensity measurements are typically unreliable outside the laboratory and can require frequent calibration/s due to a variety of chemical, optical or other instrumental related factors. Unfortunately, while fluorescent probes are known to be useful for many applications such as in fluorescence microscopy, fluorescing sensing and DNA technology, most sensing fluorophores only display changes in intensity in response to analytes and hence relatively few wavelength ratiometric probes are available today [1,2]. Some useful wavelength ratiometric probes are available for pH, Ca^{2+} and Mg^{2+} [3,4], but the probes for

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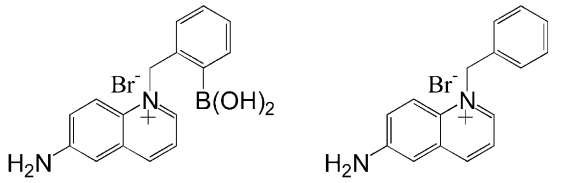
Nomenclature

BA	boronic acid
BAF and BAFs	boronic acid containing fluorophore/s
BAQ	*N*-benzyl-6-aminoquinolinium
BAQBA	*N*-(2-boronobenzyl)-6-aminoquinolinium bromide
HPTS	1-hydroxypyrene-3,6,8-trisulfonate
LED	light emitting diode
SNAFL	seminaphthofluoresceins
SNARF	seminaphthorhodafluors
TCSPC	time-correlated single photon counting

Na^+ and K^+ generally display small spectral shifts and negligible lifetime changes and are subsequently inadequate for quantitive sensing measurements [2].

The requirements of pH sensing have driven the development of several notable dyes in the past such as fluorescein [5–8], HPTS (1-hydroxypyrene-3,6,8-trisulfonate) [9–12], SNAFL (seminaphthofluoresceins) [1,13] and the SNARF (seminaphthorhodafluors) [1,13] pH probes. While these four probes are widely used and amongst the main pH probes today, they were historically developed due to the requirement for visible wavelength excitation, noting the expense and complexity of past UV excitation sources [1]. However, blue and UV, laser diode and light emitting diode sources are now readily available, allowing the possibility of using ratiometric probes at shorter wavelengths, which was previously not considered practical [1].

In this paper we characterize a new boronic acid containing quinolinium pH probe, which shows spectral shifts and intensity changes as a function of pH, in both a ratiometric and colorimetric manner, enabling pH to be sensed at near-physiological levels. The new probe BAQBA (Fig. 1), is readily water soluble, has a high quantum yield, is simple to synthesize, and works in both an excitation and fluorescence ratiometric manner. It is worth noting that both fluorescein and HPTS can only be used in an excitation wavelength ratiometric manner, with only one emission band



BAQBA **BAQ**

Fig. 1. Molecular structure of BAQBA (*N*-(2-boronobenzyl)-6-aminoquinolinium bromide) (left) and the control compound without the boronic acid moiety *N*-(benzyl)-6-aminoquinolinium bromide (right).

observed at ≈510 nm [1]. With an isobestic point at 358 nm this new probe can be readily used in a fluorescence ratiometric manner using a simple UV LED for excitation.

One important additional feature of this probe is that the affinity of the boronic acid group for monosachharides, such as for glucose and fructose, is similar to that of common boronic acid containing fluorophores, BAFs [14–16], but substantially less than for the BAFs produced by the addition of weaker electron donating groups, or indeed electron withdrawing groups in the 6-position on the quinolinium backbone [17]. Hence, the use of an amino group in the 6-position here is unique and suppresses the sugar response of this probe, enabling it to be of practical use for pH sensing in physiological fluids. Further details of the sugar affinity of BAQBA can be found elsewhere [17].

2. Experimental

2.1. Materials

All chemicals were purchased from Sigma at the highest purity available. The preparation of BAQBA and BAQ (Fig. 1) has recently been reported by us [17].

2.2. Methods

All solution absorption measurements were performed in a 4∗1∗1 cm quartz cuvette (Starna), using a Cary 50 Spectrophotometer from Varian. Fluorescence spectra were similarly collected on a Varian Eclipse spectrofluorometer with solution optical densities less than 0.2 and $\lambda_{ex} = 358$ nm.

Stability (K_S/units mM^{-1}) and Dissociation constants (K_D) were obtained by fitting the titration curves with sugar to the relation:

$$I = \frac{I_{min} + I_{max} K_{S[sugar]}}{1 + K_{S[sugar]}} \quad (1)$$

where I_{min} and I_{max} are the initial (no sugar) and final (plateau) fluorescence intensities of the titration curves, where $K_D = (1/K_S)$.

Time-resolved intensity decays were measured using reverse start-stop time-correlated single-photon counting (TCSPC) with a Becker and Hickl gmbh 630 SPC PC card and a un-amplifed MCP-PMT. Vertically polarized excitation at ≈372 nm was obtained using a pulsed LED source (1 MHz repetition rate) and a dichroic sheet polarizer. The instrumental response function was ≈1.1 ns fwhm. The emission was collected at the magic angle (54.7°), using a long pass filter (Edmund Scientific), which cut off wavelengths below 380 nm. The use of a pulsed 372 nm LED provided for excitation near-to the isobestic point at 358 nm (Fig. 2).

The intensity decays were analyzed in terms of the multi-exponential model:

$$I(t) = \sum_i \alpha_i \exp(-t/\tau_i) \quad (2)$$

where α_i are the amplitudes and τ_i the decay times, $\Sigma\alpha_i = 1.0$. The fractional contribution of each component to the steady-state intensity is given by:

$$f_i = \frac{\alpha_i \tau_i}{\sum_i \alpha_i \tau_i} \quad (3)$$

The mean lifetime of the excited state is given by:

$$\overline{\tau} = \sum_i f_i \tau_i \quad (4)$$

and the amplitude-weighted lifetime is given by:

$$\langle \tau \rangle = \sum_i \alpha_i \tau_i \quad (5)$$

The values of α_i and τ_i were determined by non-linear least squares impulse reconvolution with a goodness-of-fit χ^2_R criterion.

3. Results and discussion

Fig. 2 shows the change in absorbance for both BAQBA (top) and BAQ (bottom) as a function of pH. As the pH increases the absorption band at 388 nm decreases (for BAQBA), while the band at 340 increases. We can see significant changes in both the bands as the pH is altered. In contrast, BAQ shows a slight decrease in absorption as the pH is increased, which is attributed to the lack of a boronic acid group (Fig. 1) which is well known to complex strong bases such as the hydroxyl ion [18]. Subsequently, Fig. 3 shows the absorbance wavelength ratiometric plots for both BAQBA and BAQ based on the A_{340}/A_{388} nm bands. We were able to determine the pK_a for BAQBA to be ≈6.3, which is appropriate for near-physiological pH measurements. In contrast, BAQ, shows no or little response to changes in pH, where the small changes observed are attributed to the known quenching of the quinolinium nucleus by the hydroxyl ion at high pH [19].

The fluorescence emission of BAQBA shows similar wavelength ratiometric behavior (Fig. 4 top), where $\lambda_{ex} = 358$ nm, i.e. at the isobestic point and the pK_a≈5.85. As the pH increases we typically see a decrease in fluorescence intensity of the 546 nm band, which is the uncomplexed form, while the band at 450 nm increases (complexed

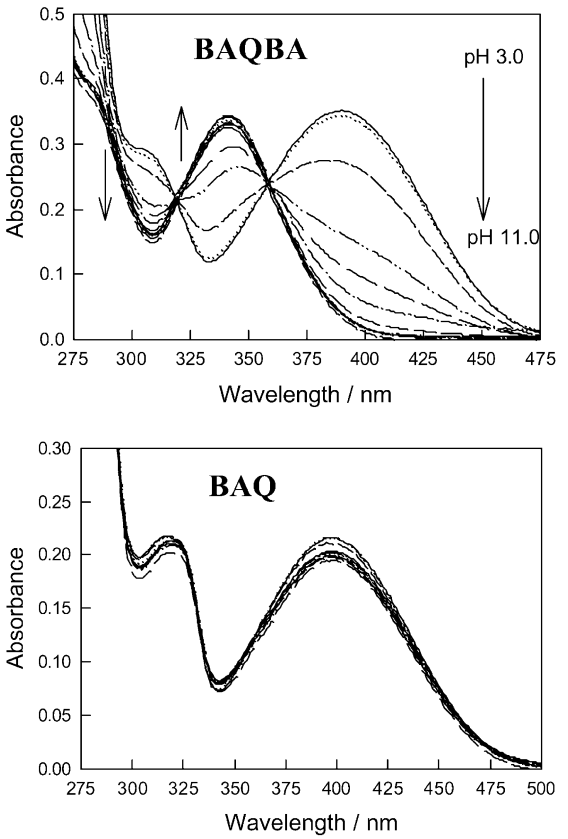


Fig. 2. Absorption spectrum of both BAQBA and BAQ with increasing pH, top and bottom respectively.

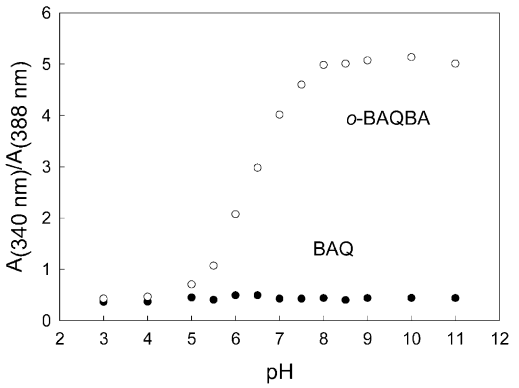


Fig. 3. Wavelength ratiometric plots for both BAQBA and BAQ based on the A_{340}/A_{388} nm bands in Fig. 2.

form). In contrast, BAQ shows a simple decrease in fluorescence intensity as the pH is increased (Fig. 4 bottom).

For the data shown in Fig. 4 we constructed the fluorescence emission ratiometric response (Fig. 5). It is interesting to compare the dynamic sensing range towards pH shown in both Figs. 3 and 5. Clearly a greater change is observed for the ratiometric absorption measurements, reflecting the differences in extinction coefficients and quantum yields of the OH^- unbound and bound forms respectively.

We additionally measured the lifetime/s for both BAQBA and BAQ as a function of pH (Fig. 6 and Table 1), using the time-correlated single-photon timing technique and the multiexponential model [1] [Eqs (2)–(5)]. Our analysis shows that both the mean and amplitude weighted lifetime of BAQ are typically reduced as the pH increases (Fig. 6 top

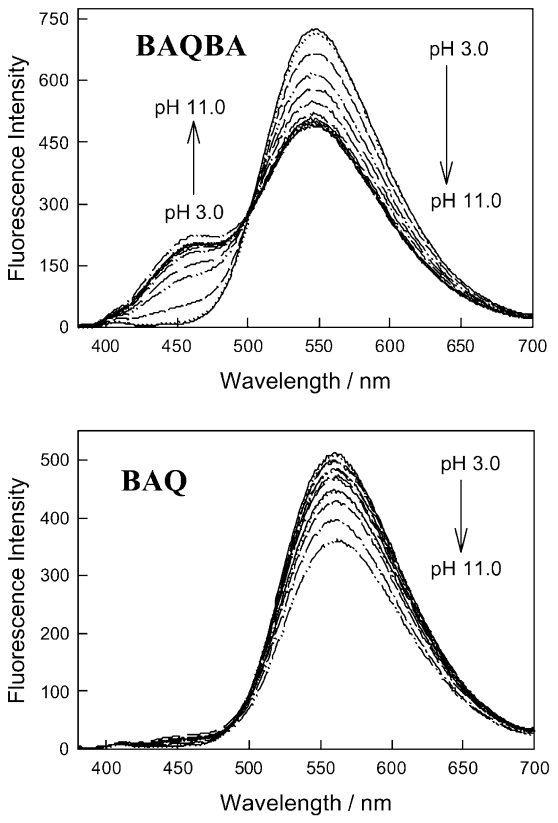


Fig. 4. Fluorescence emission spectra of BAQBA and BAQ with increasing pH, top and bottom respectively.

and bottom respectively), while BAQBA shows an increase in the mean lifetime as the pH increases. Table 1 also shows that the BAQBA data is well described by a triple exponential decay function above pH 5.5. Intuitively, this suggests that the additional ≈0.3 ns component is the lifetime of the complexed OH^- form, evident by the emission band at 450 nm, above pH 5.5, in Fig. 4. A comparison of both the BAQ and BAQBA data in Table 1, reveals that this short lived component is not present for BAQ as the pH increases, which is

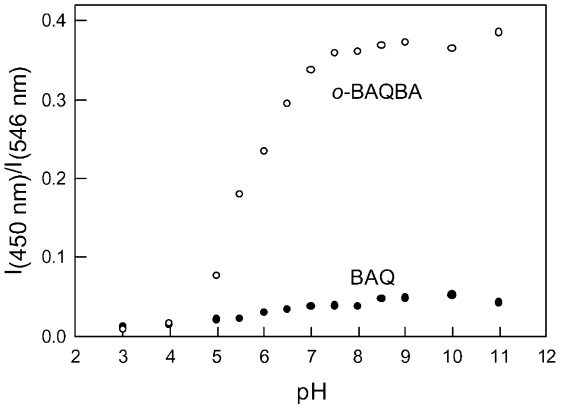


Fig. 5. Fluorescence emission wavelength ratiometric plots for both BAQBA and BAQ based on the I_{450}/I_{546} nm bands in Fig. 4.

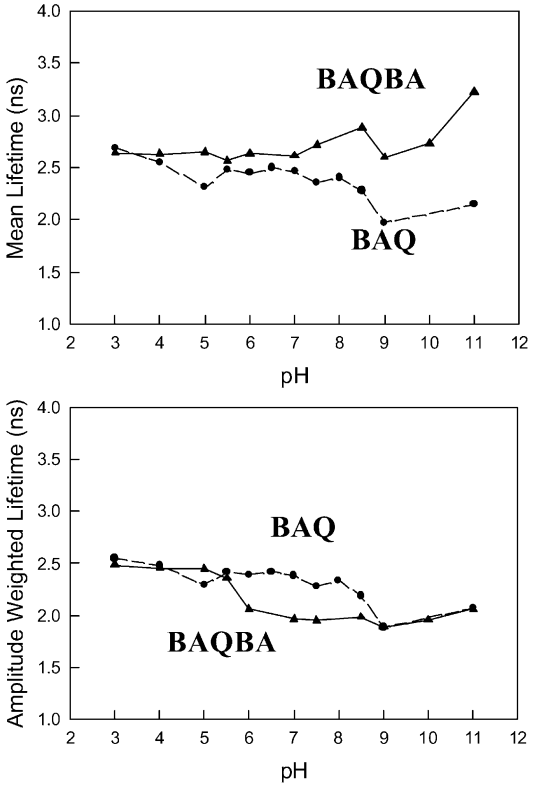


Fig. 6. Mean fluorescence lifetime for BAQBA (▲) and BAQ (●) with increasing pH (top) and the amplitude weighted lifetime/s (bottom).

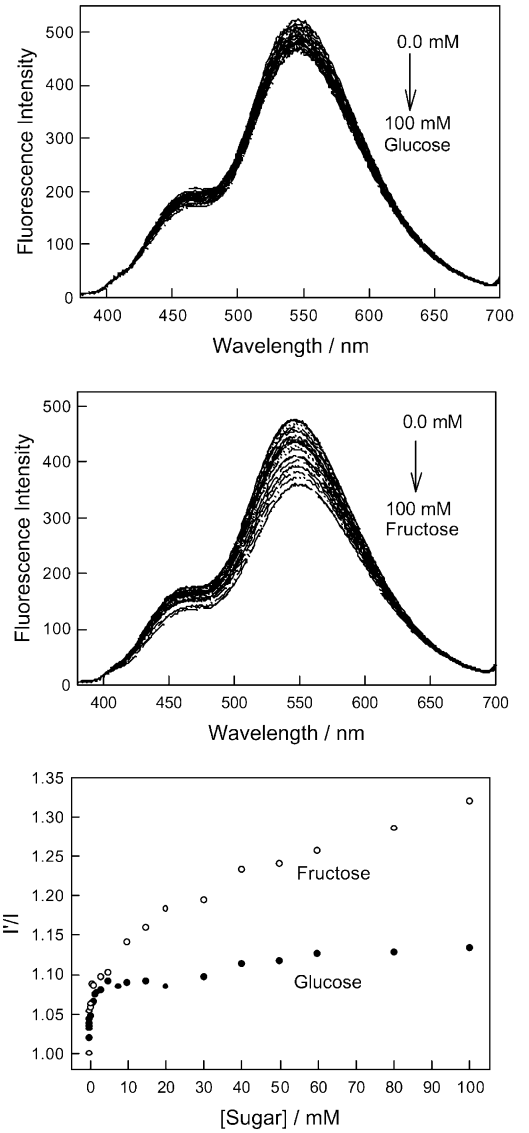


Fig. 7. Response of BAQBA to both glucose (top), fructose (middle) and the ratio plot in the absence of sugar, $\boldsymbol{I}'$, and in the presence of sugar, $\boldsymbol{I}$ (bottom).

Table 1
Multiexponential Intensity decay of BAQ and BAQBA

pH	τ_1 (ns)	α_1	τ_2 (ns)	α_2	τ_3 (ns)	α_3	χ^2
BAQ							
3[a]	2.47	0.98	6.99	0.02	–	–	1.15
4[a]	2.33	0.89	3.66	0.11	–	–	1.06
5[b]	2.16	0.63	2.53	0.37	–	–	1.03
5.5[b]	2.15	0.69	3.01	0.31	–	–	1.02
6[b]	2.22	0.81	3.13	0.19	–	–	1.10
6.5[b]	2.24	0.85	3.44	0.15	–	–	1.08
7[b]	2.17	0.81	3.30	0.19	–	–	1.03
7.5[b]	2.05	0.76	2.99	0.24	–	–	1.03
8[b]	2.13	0.79	3.12	0.21	–	–	1.07
8.5[b]	1.99	0.83	3.16	0.17	–	–	1.14
9[b]	1.71	0.84	2.83	0.16	–	–	1.03
11[c]	1.79	0.66	2.62	0.34	–	–	1.16
BAQBA							
3[a]	2.06	0.68	3.40	0.32	–	–	1.06
4[a]	2.03	0.69	3.44	0.31	–	–	1.02
5[b]	1.97	0.69	3.52	0.31	–	–	1.18
5.5[b]	1.24	0.28	2.79	0.72	–	–	1.52[d]
6[b]	1.79	0.51	0.18	0.16	3.38	0.33	0.99
7[b]	1.73	0.49	0.22	0.19	3.41	0.32	1.03
7.5[b]	1.93	0.61	0.29	0.22	4.25	0.17	1.10
8.5[b]	1.94	0.61	0.29	0.23	4.63	0.16	1.15
9[b]	1.76	0.52	0.27	0.25	3.59	0.23	1.22
10[c]	1.89	0.51	0.27	0.25	3.77	0.24	1.03
11[c]	1.97	0.59	0.27	0.25	5.22	0.16	1.13

[a] Acetate buffer.
[b] Phosphate buffer.
[c] Carbonate buffer.
[d] A triple exponential function could not describe this data set well.

expected as BAQ doesn't form a boronate complex with hydroxyl ions.

The affinity of boronic acid for diols is well-known [14–16]. Subsequently, we tested the response of BAQBA towards both glucose and fructose. No response was observed, as expected, for BAQ (data not shown). A comparison of Fig. 7 top and middle, shows a similar affinity of BAQBA for both fructose and glucose. While the emission spectra of BAQBA show similar bands, as in the presence of OH^-, i.e. at 450 and 546 nm, the bands do not show increasing and decreasing intensities in the presence of sugar, but instead simply show an overall decrease as sugar concentration increases. Subsequently, Fig. 7 bottom shows the response curves to sugars normalized by the response of BAQBA in the absence of sugar, I'. Using Eq. (1), we were able to determine the binding (stability) constants to be $\approx$1.04 and $\approx$0.06 mM^{-1} for glucose and fructose respectively. In contrast these binding constants can be tuned *much higher* by replacing the 6-amino group to a less efficient electron donating group, or indeed, electron withdrawing groups as mentioned earlier [17].

With a slight sugar response evident, (relevant to similar structures but with different substituents [17]), we tested the ability of BAQBA to sense pH in the presence of 50 mM glucose and 50 mM fructose (Fig. 8 top and middle respectively). As we can see the boronic acid containing quinolinium type fluorophore responds well towards pH in the presence of the sugar interferents (Fig. 8 bottom shows the respective ratiometric plot from the 546 and 450 nm bands, where we can see that 50 mM glucose has little effect on the probe's overall response to pH, by comparing with the

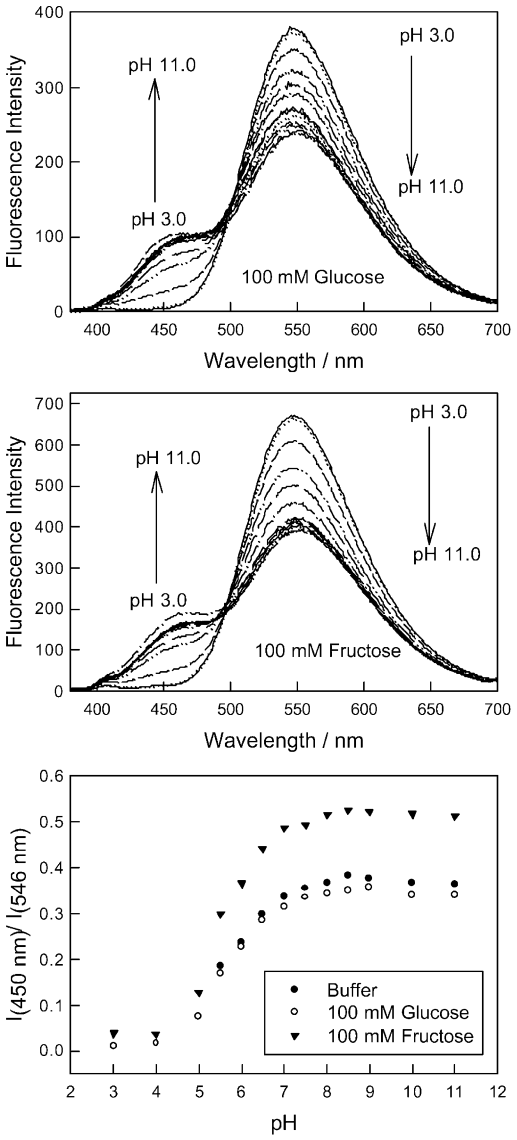


Fig. 8. Fluorescence emission spectra for BAQBA with increasing pH in the presence of 100 mM glucose (top), 100 mM fructose (middle) and the fluorescence emission wavelength ratiometric plots based on the I_{450}/I_{546} nm bands (bottom).

buffer (no sugar) titration curve. While a 50 mM fructose background has an effect, as shown in Fig. 8 bottom, it should be noted that fructose levels in blood are typically 10→100 fold lower for a healthy person than used here, hence BAQBA is likely to be suitable for physiological pH measurements.

4. Conclusions

We have characterized a new water-soluble probe, BAQBA, with regard to pH. This new probe responds well to pH changes around 7. In addition we have shown that the pH can readily be determined in a background of 50 mM sugar allowing its potential use in physiological measurements in both an excitation and emission ratiometric manner.

Acknowledgements

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