Electronic Supporting Information for

# Towards Homogenous Multiwell Plate Based pH sensors using a Responsive Triangulenium Dye and an ATTO-647 reference dye

Magnus Christian Wied and Thomas Just Sørensen

## Contents

## Plate Reader Settings

All multiwell plate measurements were done using a PHERAstar FSX plate reader from BMG Labtech, utilising a standard Xenon flash lamp.

Measurement type was set to fluorescence with an optic module for excitation at 510 nm and dual emission collection at 590 nm and 665 nm (all with bandwidth 10 nm).

PMT gain value and focus height of the light beam was determined using the automated gain and focus calibration setting with the emission signal strength set to 85% of the detector maximum, as to avoid oversaturation. For each measurement calibration was done on well A1 (the top left).

Data collection was done using the top optic of the plate reader.

Measurements were done at ambient conditions without temperature control and no spoon type was used during measurements. Between measurements of each well a settling time of 0.5 s was used to alleviate possible crosstalk between wells.

Endpoint measurement was done with 20 flashes pr. well. Reading mode was repeating left to right of the multiwell plate.

# Sensor Fabrication

Table S1. Multiwell plate fabrication parameters

| Batch Loading Solution | I | II | III | IV | V | VI | VII | VIII |
|---|---|---|---|---|---|---|---|---|
| DAOTA@NP (mM) | 0.05 | 0.05 | 0.014 | 0.014 | 0.014 | 0.05 | 0.05 | 0.05 |
| NP Batch | I | I | II | II | II | III | III | IV |
| ATTO-647 (mM) | 0.05 | 0.025 | 0.025 | 0.025 | 0.025 | 0.025 | 0.1 | 0.1 |
| Triton X-100 (mg/ml) | None | None | None | None | None | 5.35 | 5.35 | 5.35 |
| Load volumes (µl) | 0-100 | 0-100 | 0-100 | 0-100 | 0-50 | 0-50 | 0-50 | 30+20 = 50 |
| Well vol. (µl) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Dye loading in sample (mM) | 0-0.05 | 0-0.025 | 0-0.025 | 0-0.025 | 0-0.013 | 0-0.025 | 0-0.05 | 0.015+0.02 = 0.035 |
| Plate type | **1** | **1** | **1** | **2** | **2** | **2** | **2** | **2** |
| Loading procedure | Automated mixing | Automated mixing | Automated mixing | Automated mixing | Premixed | Premixed | Premixed | Premixed |
| Purpose | Optimising | Optimising | Optimising | Optimising | Optimising | Optimising | Optimising | Sensing |

Table S2. DLS results of nanoparticle fabrications after several weeks.

| **NP Batch** | I | II | III | IV |
|---|---|---|---|---|
| **Mean Diameter (nm)** | 93.7 ± 5.5 | 48.1 ± 18.9 | 56.3 ± 40.0 | 32.0 ± 4.1 |

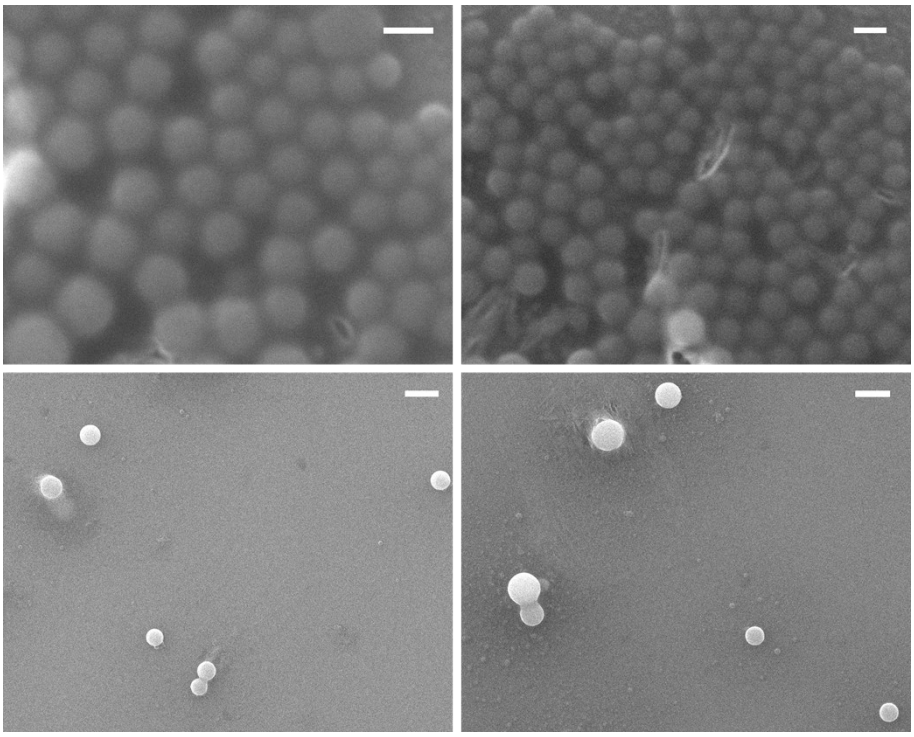

Figure S1. Scanning Electron Microscopy images taken of select nanoparticle batches. Top.) SEM images of Batch IV. The scale bar is 100 nm and accelerator voltage set to 5kV. Bottom.) SEM images of Batch V. Scale bar is 1 µm and accelerator voltage set to 15kV.

# Sensor Characterisation

## Batch I

Parameters:

- DAOTA:ATTO ratios: 100:0 - 80:20 - 60:40 - 40:60 – 20:80 – 0:100
- DAOTA NP conc: from dialysis (NP batch I)
- ATTO-647 conc: 0.05 mM -/- Triton X-100
- Plate: Greiner bio-one black F-bottom
- Vol pr. well: 100 ul
- Universal Buffer pH: 4.50 – 5.04 – 5.45 – 5.99 – 6.49 – 7.03 – 7.61 – 7.98
- D/A_001

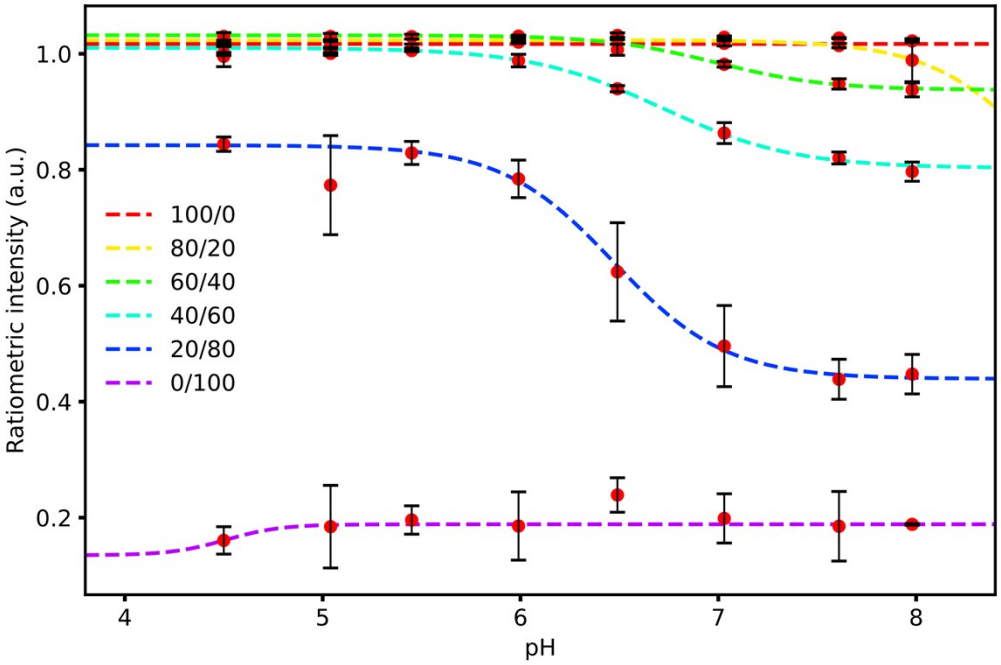


Figure S2. Response from batch I .

Table S3. Fit parameters for response functions for Batch I.

| DAOTA/ATTO-647 | a | $pK_a$ | k | $y_0$ | GoF ($\chi^2$) |
|---|---|---|---|---|---|
| **100/0** | 0.38 +/- 0.02 | 13.68 +/- 53.64 | 2.85 +/- 25.36 | 0.63 +/- 0.02 | 5.40*10^-27 |
| **80/20** | 0.41 +/- 0.03 | 8.66 +/- 1.07 | 3.54 +/- 3.72 | 0.62 +/- 0.03 | 0.152 |
| **60/40** | 0.09 +/- 0.02 | 6.99 +/- 0.19 | 3.58 +/- 1.94 | 0.94 +/- 0.02 | 0.963 |
| **40/60** | 0.21 +/- 0.04 | 6.72 +/- 0.18 | 3.0 +/- 1.6 | 0.8 +/- 0.03 | 0.976 |
| **20/80** | 0.4 +/- 0.07 | 6.47 +/- 0.37 | 3.53 +/- 2.91 | 0.44 +/- 0.06 | 0.995 |
| **0/100** | -0.05 +/- 3.59 | 4.51 +/- 19.41 | 7.17 +/- 88.41 | 0.19 +/- 0 | 0.943 |

Table S4. Sensor performance for Batch I given as confidence intervals on measured pH in three intervals.

| | DAOTA/ATTO-647 ratios (% vol.) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **100/0** | | **80/20** | | **60/40** | | **40/60** | | **20/80** | | **0/100** | |
| **Low/High pH** | - | + | - | + | - | + | - | + | - | + | - | + |
| **$pK_a$ +/- 0.25** | Inf | Inf | 1.11 | 1.18 | 0.35 | 0.26 | 0.29 | 0.24 | 0.45 | 0.49 | N/A | N/A |
| **$pK_a$ +/- 0.5** | Inf | Inf | 1.21 | 1.35 | 0.62 | 0.34 | 0.46 | 0.33 | 0.64 | 0.70 | N/A | N/A |
| **pH 7.2-7.4** | Inf | Inf | 4.62 | 2.68 | 0.25 | 0.31 | 0.32 | 0.42 | 0.99 | 1.47 | N/A | N/A |

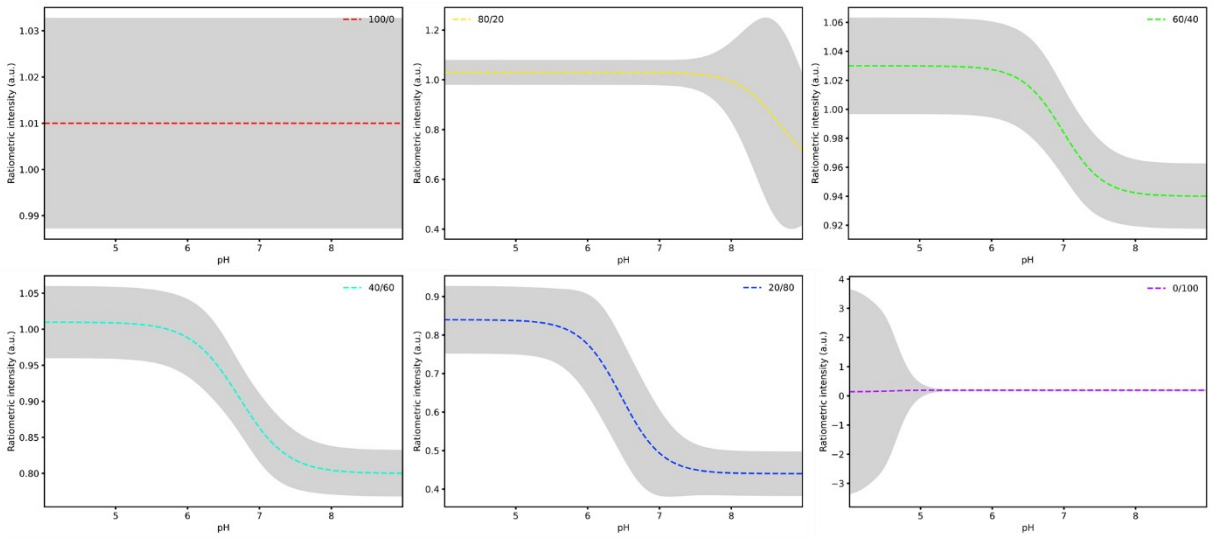


Figure S3. Response functions for Batch I shown with propagated error as shaded area.

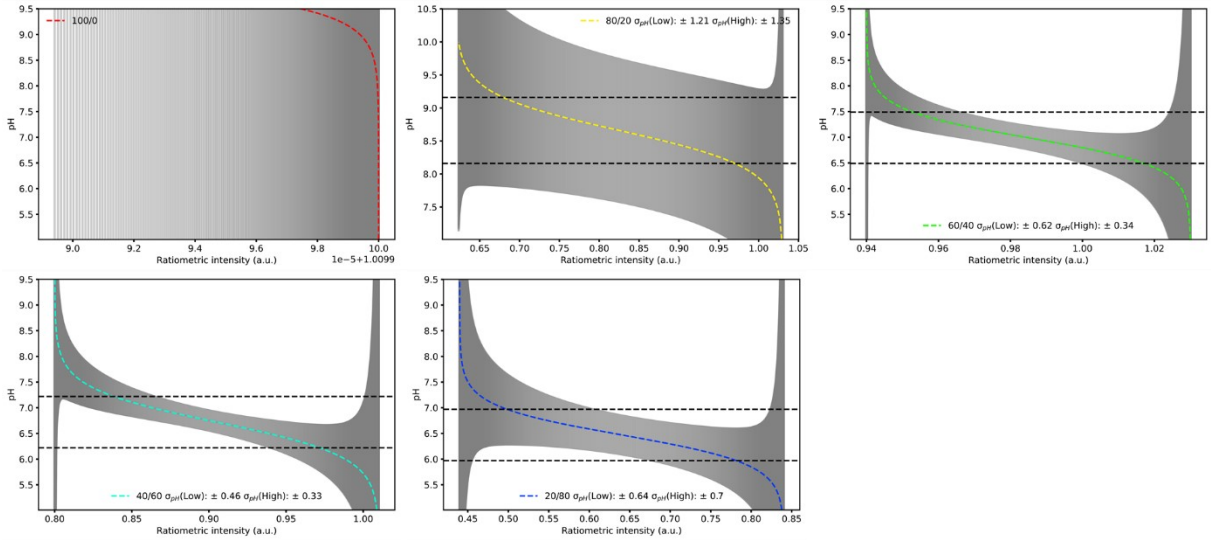


Figure S4. Evaluation functions for Batch I shown with propagated error as shaded area.

## Batch II

Parameters:

- DAOTA:ATTO ratios: 100:0 - 80:20 - 60:40 - 40:60 – 20:80 – 0:100
- DAOTA NP conc: from dialysis (NP batch I)
- ATTO-647 conc: 0.025 mM -/- Triton X-100
- Plate: Greiner bio-one black F-bottom
- Vol pr. well: 100 ul
- Universal Buffer pH: 4.50 – 5.03 – 5.47 – 6.05 – 6.57 – 7.14 – 7.65 – 7.93
- D/A_002

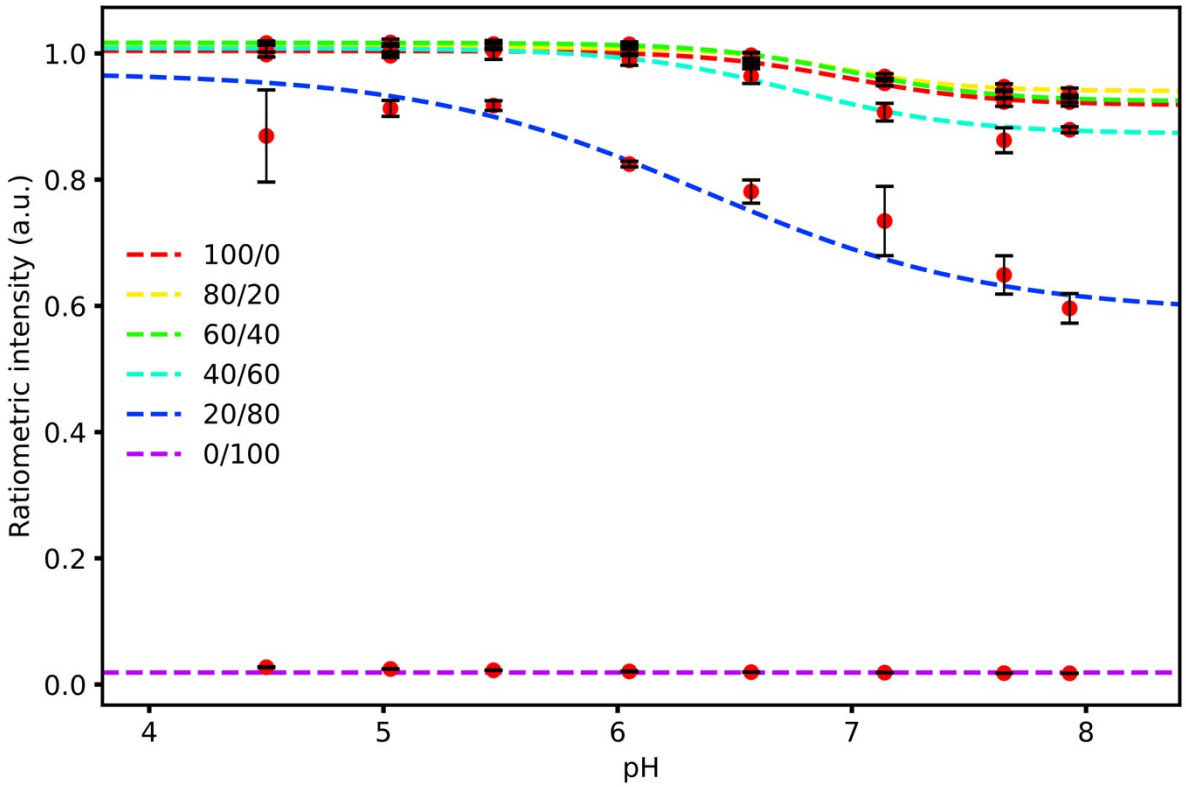


Figure S5. Response from Batch II.

Table S5. Fit parameters for response functions for Batch II.

| DAOTA/ATTO-647 | a | $pK_a$ | k | $y_0$ | GoF ($\chi^2$) |
|---|---|---|---|---|---|
| **100/0** | 0.09 +/- 0.02 | 6.98 +/- 0.18 | 3.19 +/- 1.01 | 0.92 +/- 0.02 | 0.329 |
| **80/20** | 0.07 +/- 0.02 | 6.96 +/- 0.2 | 3.85 +/- 2.07 | 0.94 +/- 0.01 | 0.976 |
| **60/40** | 0.09 +/- 0.02 | 7.0 +/- 0.1 | 3.21 +/- 1.19 | 0.92 +/- 0.01 | 0.999 |
| **40/60** | 0.14 +/- 0.02 | 6.77 +/- 0.27 | 2.74 +/- 1.7 | 0.87 +/- 0.02 | 0.982 |
| **20/80** | 0.38 +/- 0.15 | 6.38 +/- 0.28 | 1.62 +/- 1.08 | 0.59 +/- 0.01 | 0.430 |
| **0/100** | 0.16 +/- 0 | 3.61 +/- 91.52 | -76.33 +/- 179186.17 | -0.14 +/- 0 | 6.54*10^-38 |

Table S6. Sensor performance for Batch II given as confidence intervals on measured pH in three intervals.

| | DAOTA/ATTO-647 ratios (% vol.) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **100/0** | | **80/20** | | **60/40** | | **40/60** | | **20/80** | | **0/100** | |
| **Low/High pH** | - | + | - | + | - | + | - | + | - | + | - | + |
| **$pK_a$ +/- 0.25** | 0.26 | 0.21 | 0.31 | 0.25 | 0.22 | 0.16 | 0.36 | 0.32 | 0.71 | 0.55 | N/A | N/A |
| **$pK_a$ +/- 0.5** | 0.4 | 0.25 | 0.55 | 0.34 | 0.38 | 0.22 | 0.51 | 0.42 | 0.92 | 0.61 | N/A | N/A |
| **pH 7.2-7.4** | 0.21 | 0.23 | 0.25 | 0.32 | 0.15 | 0.19 | 0.38 | 0.48 | 0.77 | 0.91 | N/A | N/A |

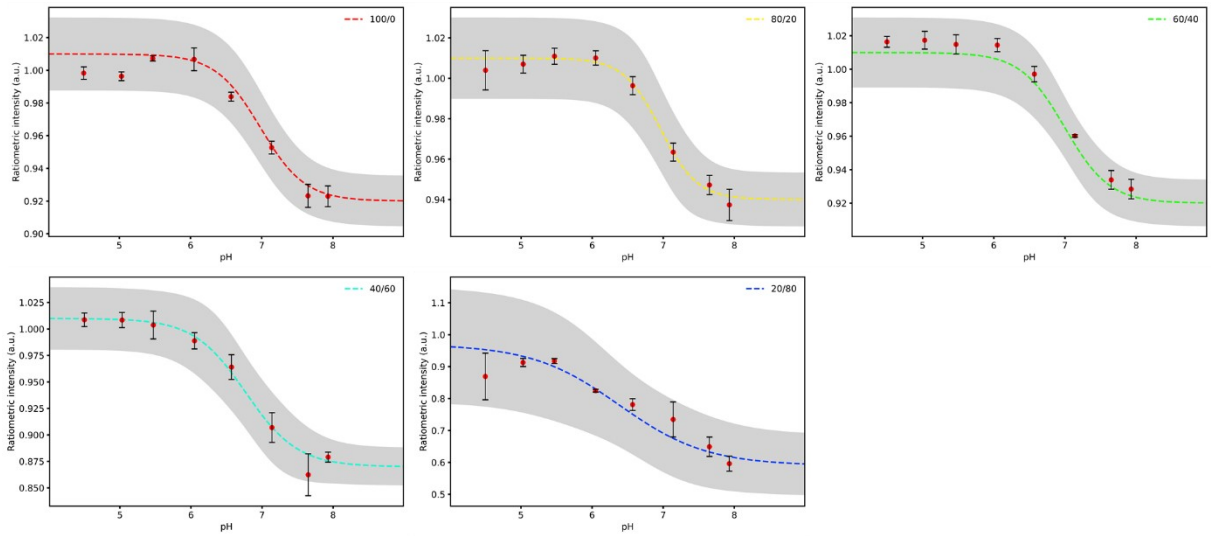


Figure S6. Response data and functions for Batch II shown with propagated error as shaded area.

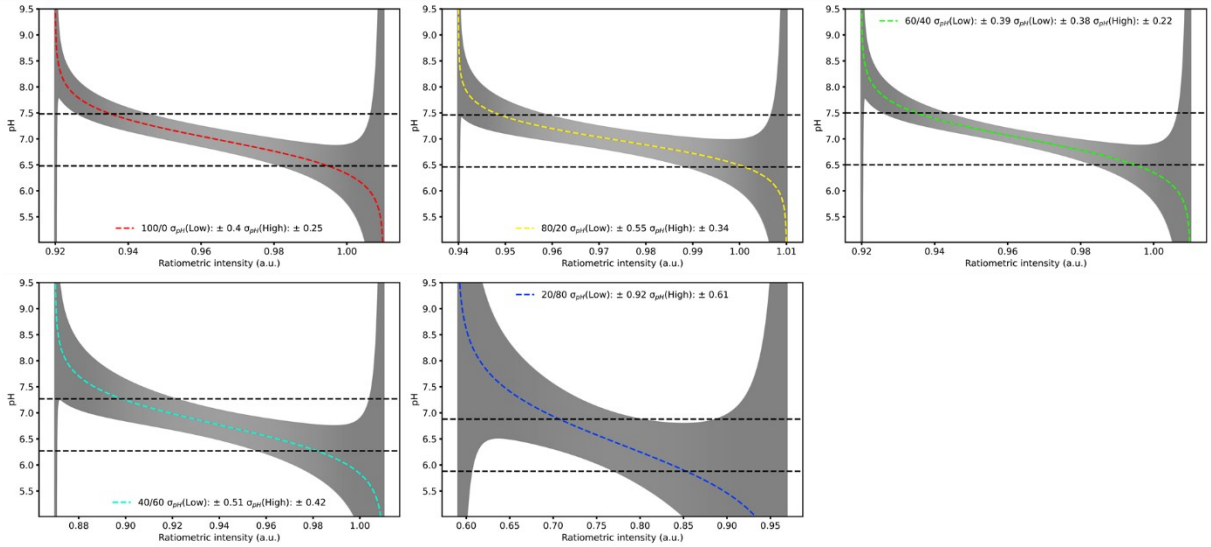


Figure S7. Evaluation functions for Batch II shown with propagated error as shaded area.

## Batch III

Parameters:

- Plate: Black Greiner Bio One 96 well – F-bottom
- DAOTA conc: 0.05 mM in Triton X-100 (5.35 mg/ml) (NP batch II)
  - Diluted 1:3 → 0.0125 mM
- ATTO-647 conc: 0.025 mM in water (no Triton X-100)
- Vol pr well: 100 µl
- pH series: 4.50 – 5.03 – 5.47 – 6.05 – 6.57 – 7.14 – 7.65 – 7.93
- D/A_003

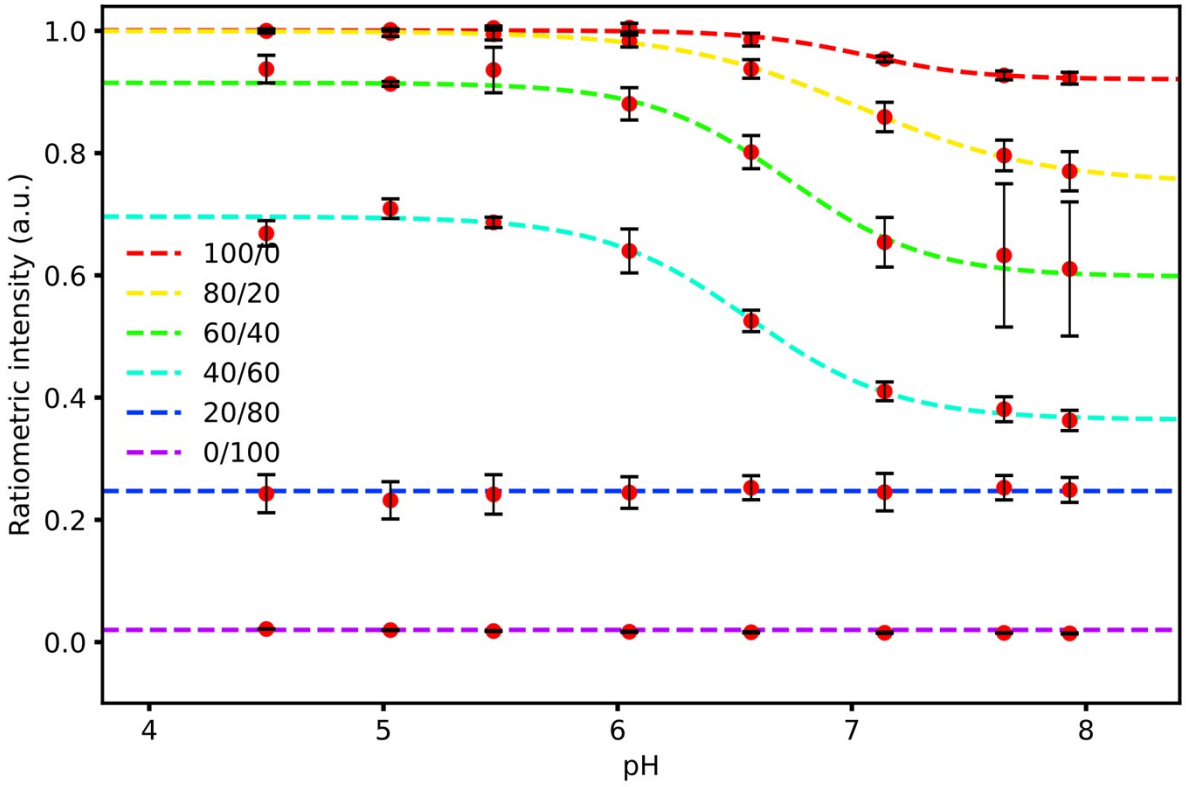


Figure S8. Response from Batch III.

Table S7. Fit parameters for response functions for Batch III.

| DAOTA/ATTO-647 | a | $pK_a$ | k | $y_0$ | GoF ($\chi^2$) |
|---|---|---|---|---|---|
| **100/0** | 0.08 +/- 0.02 | 7.05 +/- 0.18 | 4.1 +/- 3.1 | 0.92 +/- 0.02 | 0.947 |
| **80/20** | 0.25 +/- 0.11 | 7.03 +/- 0.52 | 2.54 +/- 1.59 | 0.75 +/- 0.11 | 0.999 |
| **60/40** | 0.32 +/- 0.18 | 6.73 +/- 0.46 | 3.38 +/- 3.4 | 0.6 +/- 0.17 | 0.982 |
| **40/60** | 0.33 +/- 0.05 | 6.56 +/- 0.15 | 3.2 +/- 1.6 | 0.36 +/- 0.03 | 0.949 |
| **20/80** | 49891.12 +/- 0 | -20.08 +/- 0 | 9.96 +/- 0 | 0.25 +/- 0 | 0.998 |
| **0/100** | 5875258.71 +/- 0 | 65.51 +/- 0 | -8.7 +/- 0 | 0.02 +/- 0 | 1.143*10^-14 |

Table S8. Sensor performance for Batch III given as confidence intervals on measured pH in three intervals.

| | DAOTA/ATTO-647 ratios (% vol.) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **100/0** | | **80/20** | | **60/40** | | **40/60** | | **20/80** | | **0/100** | |
| **Low/High pH** | - | + | - | + | - | + | - | + | - | + | - | + |
| **$pK_a$ +/- 0.25** | 0.34 | 0.27 | 0.74 | 0.61 | 0.75 | 0.60 | 0.24 | 0.22 | N/A | N/A | N/A | N/A |
| **$pK_a$ +/- 0.5** | 0.64 | 0.42 | 0.99 | 0.66 | 1.25 | 0.80 | 0.39 | 0.35 | N/A | N/A | N/A | N/A |
| **pH 7.2-7.4** | 0.23 | 0.33 | 0.61 | 0.63 | 0.77 | 1.07 | 0.44 | 0.65 | N/A | N/A | N/A | N/A |

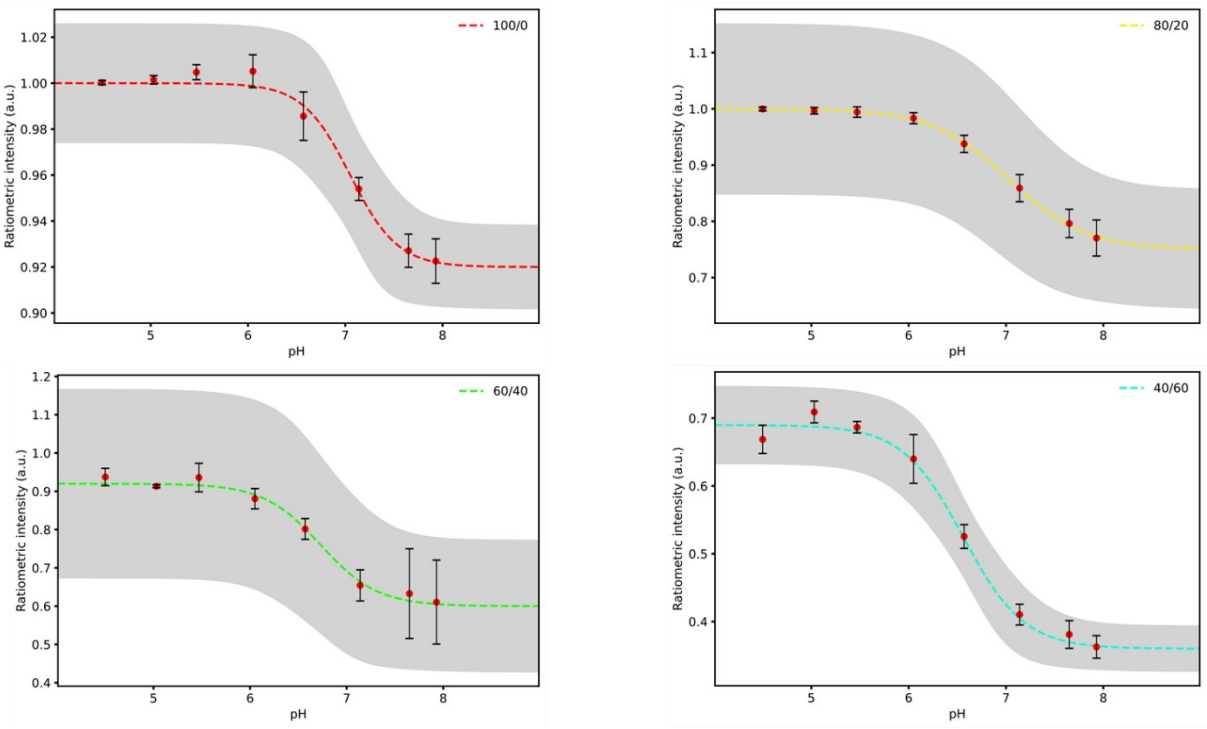


Figure S9. Response data and functions for Batch III shown with propagated error as shaded area.

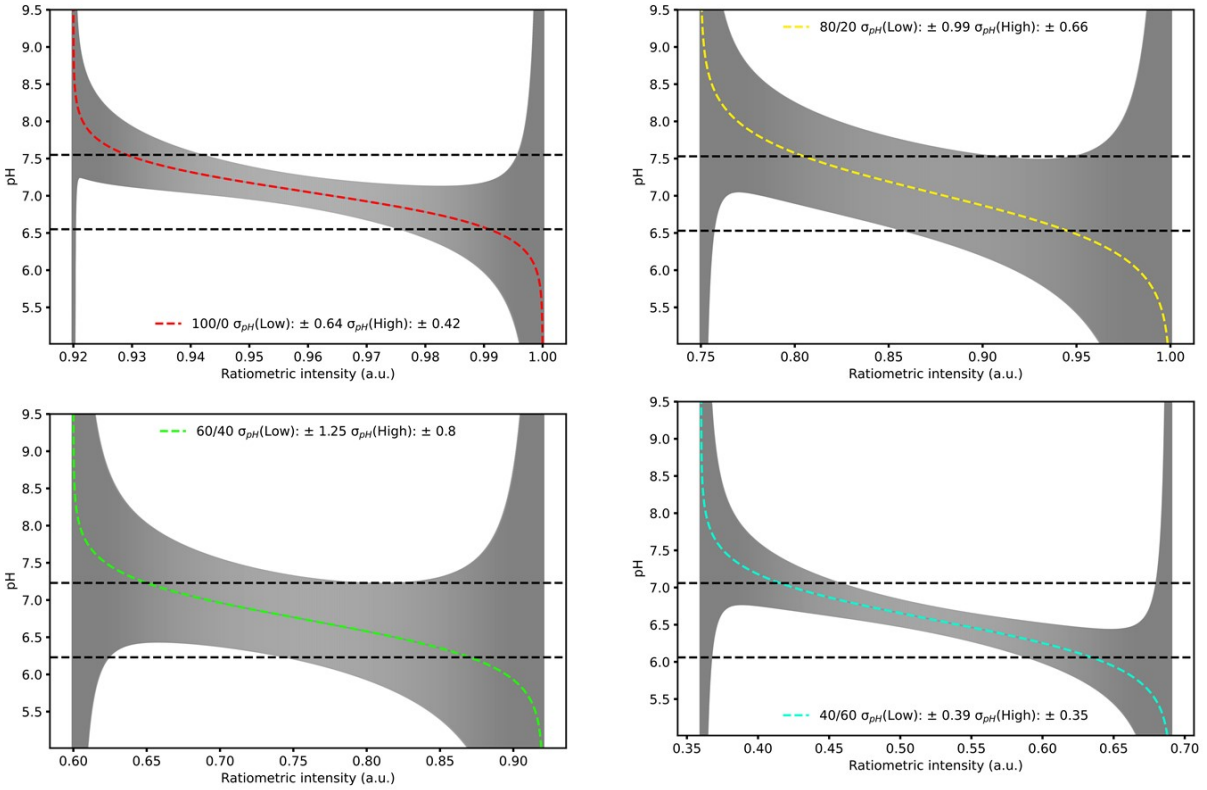


Figure S10. Evaluation functions for Batch III shown with propagated error as shaded area.

## Batch IV

Parameters:

- DAOTA:ATTO ratios: 100:0 - 80:20 - 60:40 - 40:60 – 20:80 – 0:100
- DAOTA NP conc: diluted 1:3 (NP batch II)
- ATTO-647 conc: 0.025 mM -/- Triton X-100
- Plate: Kartell - clear
- Vol pr. well: 100 ul
- Universal Buffer pH: 4.03 – 4.99 – 5.53 – 6.08 – 6.51 – 7.01 – 7.52 – 7.96
- D/A_004

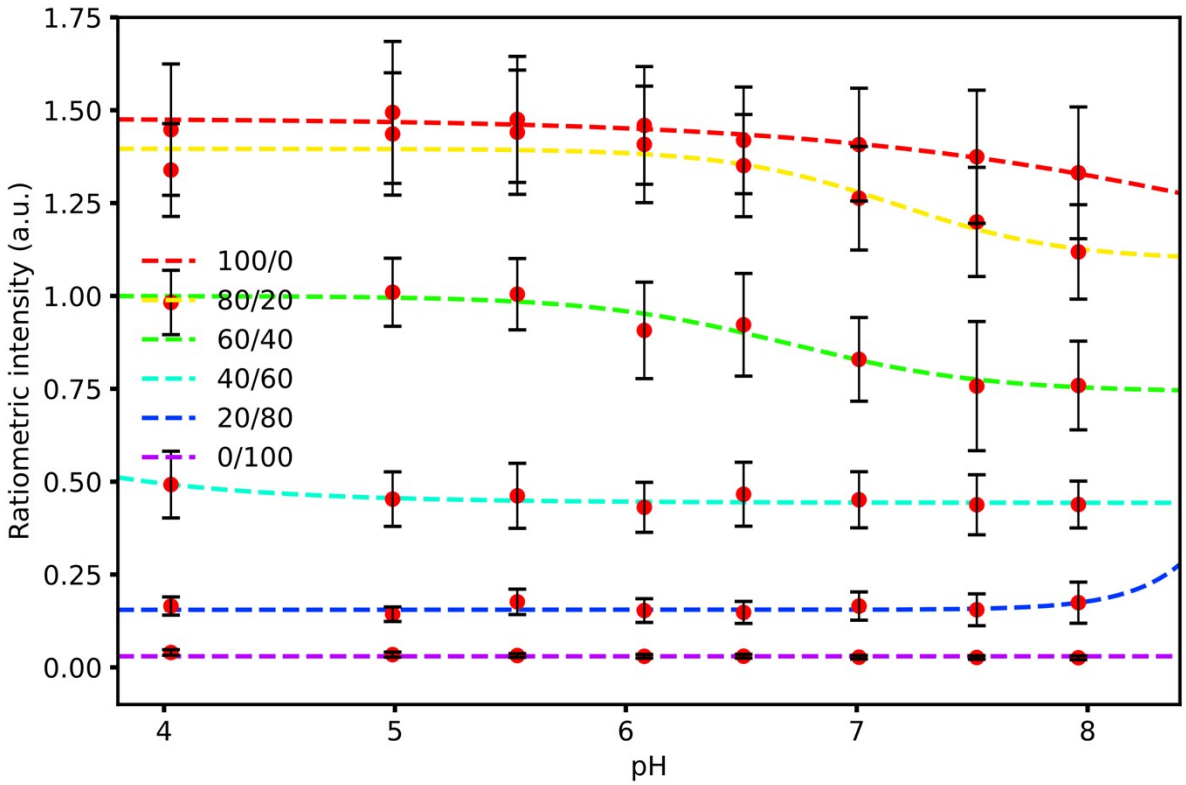


Figure S11. Response from Batch IV.

Table S9. Fit parameters for response functions for Batch IV.

| DAOTA/ATTO-647 | a | $pK_a$ | k | $y_0$ | GoF ($\chi^2$) |
|---|---|---|---|---|---|
| **100/0** | 0.53 +/- 1.45 | 8.89 +/- 6.82 | 1.01 +/- 5.25 | 0.95 +/- 1.44 | 0.999 |
| **80/20** | 0.3 +/- 0.64 | 7.17 +/- 2.5 | 2.74 +/- 9.74 | 1.1 +/- 0.61 | 0.999 |
| **60/40** | 0.26 +/- 0.38 | 6.71 +/- 1.92 | 2.35 +/- 7.25 | 0.74 +/- 0.33 | 0.999 |
| **40/60** | 723.95 +/- 33647.23 | -2.71 +/- 28.48 | 1.42 +/- 3.7 | 0.44 +/- 0.06 | 0.999 |
| **20/80** | 2596.04 +/- 202249.46 | 10.75 +/- 8.49 | -4.23 +/- 24.81 | 0.15 +/- 0.02 | 0.992 |
| **0/100** | 45063.61 +/- 0 | 28.29 +/- 0 | -6.3 +/- 0 | 0.03 +/- 0 | 0.922 |

Table S10. Sensor performance for Batch IV given as confidence intervals on measured pH in three intervals.

| | DAOTA/ATTO-647 ratios (% vol.) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **100/0** | | **80/20** | | **60/40** | | **40/60** | | **20/80** | | **0/100** | |
| **Low/High pH** | - | + | - | + | - | + | - | + | - | + | - | + |
| **$pK_a$ +/- 0.25** | 9.45 | 8.81 | 3.54 | 2.92 | 2.7 | 2.31 | N/A | N/A | N/A | N/A | N/A | N/A |
| **$pK_a$ +/- 0.5** | 10.35 | 9.03 | 4.93 | 3.27 | 3.59 | 2.64 | N/A | N/A | N/A | N/A | N/A | N/A |
| **pH 7.2-7.4** | 20.89 | 18.13 | 2.93 | 2.91 | 2.62 | 3.06 | 120.97 | 155.75 | 30.73 | 28.65 | N/A | N/A |

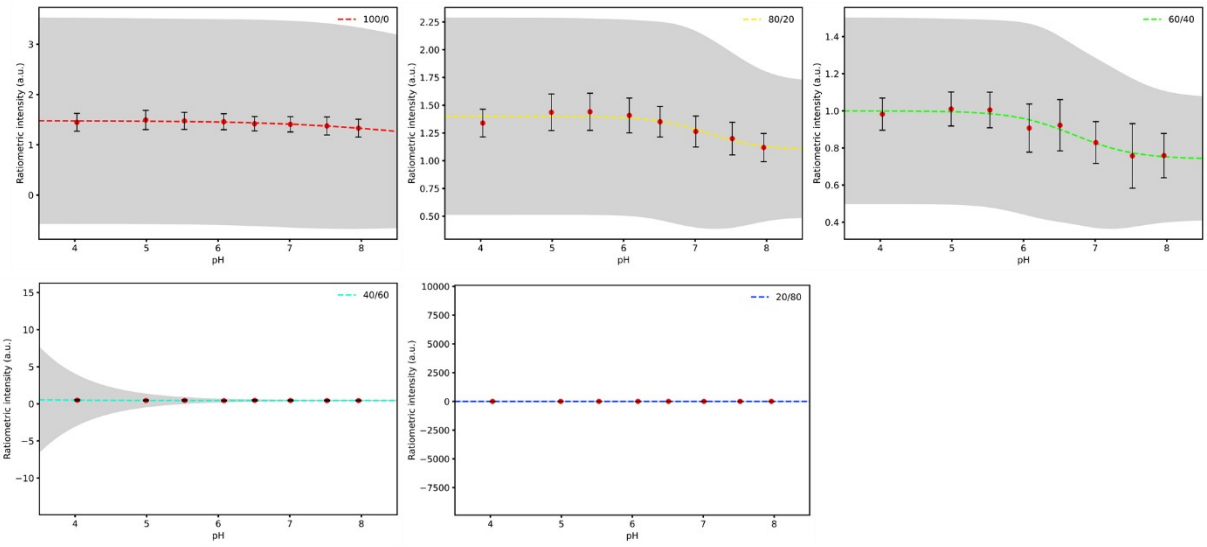


Figure S12. Response data and functions for Batch IV shown with propagated error as shaded area.

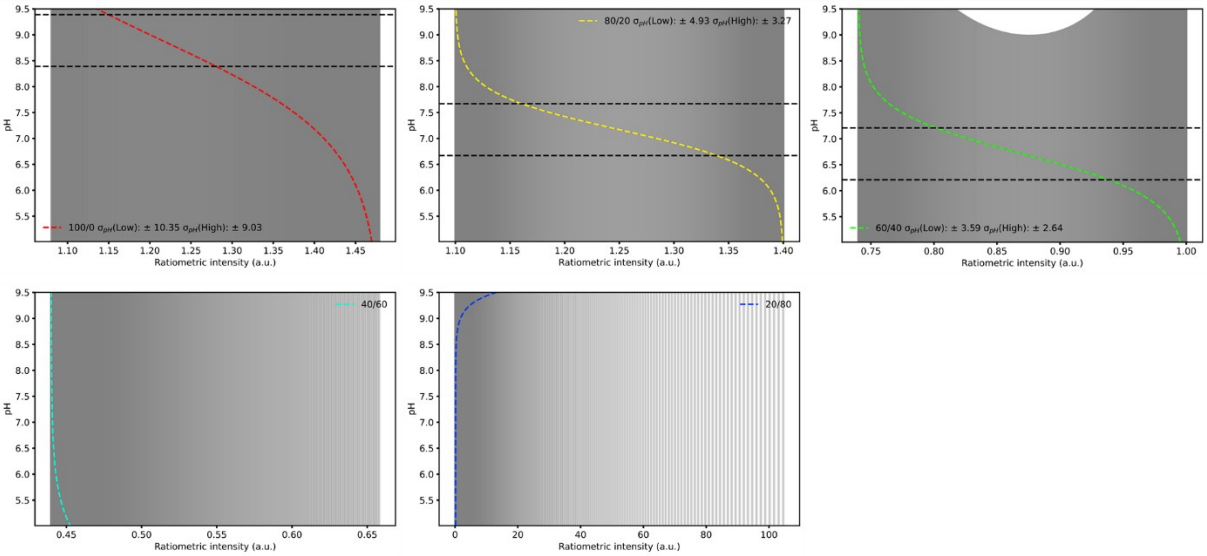


Figure S13. Evaluation functions for Batch IV shown with propagated error as shaded area.

## Batch V

Parameters:

- Premixed ratios
- DAOTA:ATTO ratios: 100:0 - 80:20 - 60:40 - 40:60 – 20:80 – 0:100
- DAOTA NP conc: diluted 1:3 (NP batch II)
- ATTO-647 conc: 0.025 mM -/- Triton X-100
- Plate: Kartell - clear
- Vol pr. well: 50 ul
- Universal Buffer pH: 4.01 – 5.05 – 5.66 – 6.18 – 6.53 – 6.99 – 7.47 – 7.79
- D/A_005

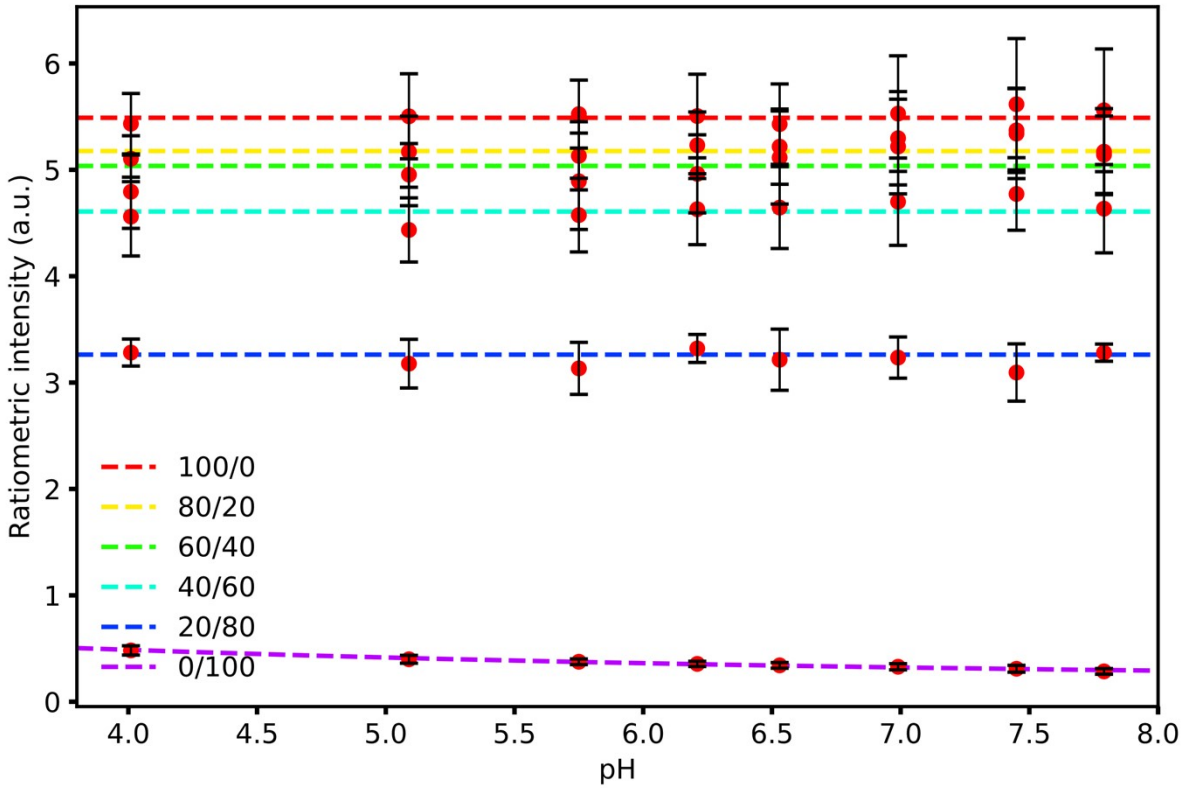


Figure S14. Response from Batch V.

Table S11. Fit parameters for response functions for Batch V.

| DAOTA/ATTO-647 | a | $pK_a$ | k | $y_0$ | GoF ($\chi^2$) |
|---|---|---|---|---|---|
| **100/0** | N/A | N/A | N/A | N/A | N/A |
| **80/20** | N/A | N/A | N/A | N/A | N/A |
| **60/40** | N/A | N/A | N/A | N/A | N/A |
| **40/60** | N/A | N/A | N/A | N/A | N/A |
| **20/80** | N/A | N/A | N/A | N/A | N/A |
| **0/100** | N/A | N/A | N/A | N/A | N/A |

Table S12. Sensor performance for Batch V given as confidence intervals on measured pH in three intervals.

| | DAOTA/ATTO-647 ratios (% vol.) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **100/0** | | **80/20** | | **60/40** | | **40/60** | | **20/80** | | **0/100** | |
| **Low/High pH** | - | + | - | + | - | + | - | + | - | + | - | + |
| **$pK_a$ +/- 0.25** | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| **$pK_a$ +/- 0.5** | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| **pH 7.2-7.4** | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |

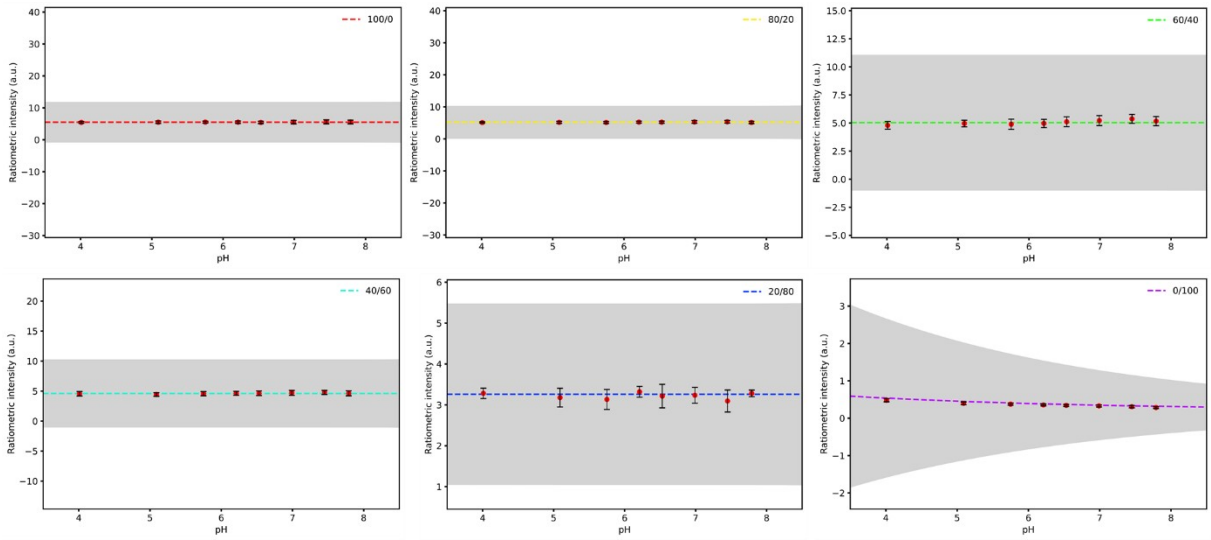


Figure S15. Response data and functions for Batch V shown with propagated error as shaded area.

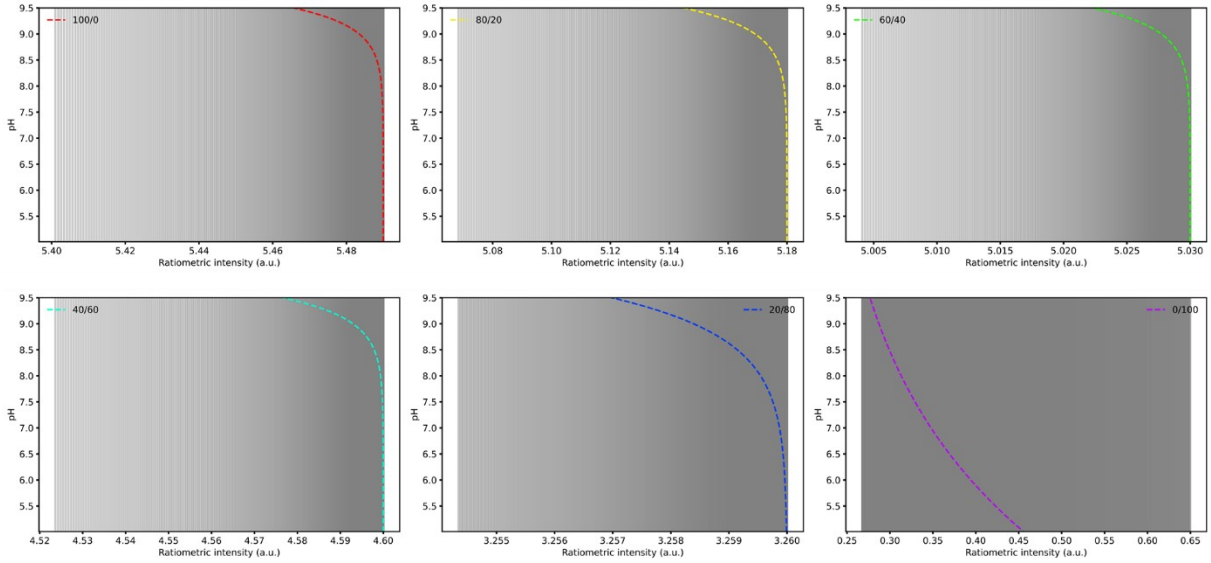


Figure S16. Evaluation functions for Batch V shown with propagated error as shaded area.

## Batch VI

Parameters:

- Premixed ratios
- DAOTA:ATTO ratios: 100:0 - 80:20 - 60:40 - 40:60 – 20:80 – 0:100
- DAOTA NP conc: from dialysis (NP batch III)
- ATTO-647 conc: 0.025 mM + Triton X-100 5.35 mg/ml
- Plate: Kartell - clear
- Vol pr. well: 50 ul
- Universal Buffer pH: 4.01 – 5.09 – 5.75 – 6.21 – 6.53 – 6.99 – 7.45 – 7.79
- D/A_006

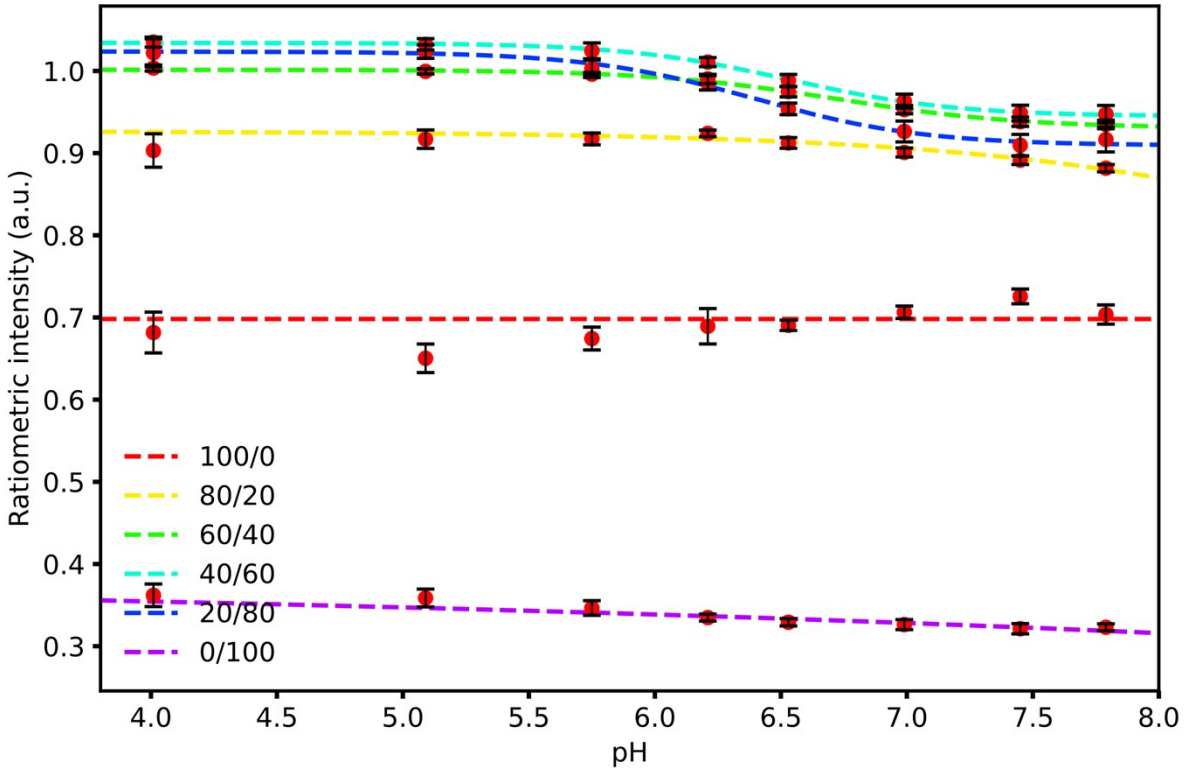


Figure S17. Response from Batch VI.

Table S13. Fit parameters for response functions for Batch VI.

| DAOTA/ATTO-647 | a | $pK_a$ | k | $y_0$ | GoF ($\chi^2$) |
|---|---|---|---|---|---|
| **100/0** | 0.19 +/- 0.12 | 10.75 +/- 46.17 | 3.71 +/- 53.21 | 0.51 +/- 0.12 | 0.210 |
| **80/20** | 0.34 +/- 0.05 | 9.42 +/- 0.86 | 1.12 +/- 0.67 | 0.59 +/- 0.05 | 0.843 |
| **60/40** | 0.07 +/- 0.02 | 6.72 +/- 0.27 | 2.69 +/- 1.51 | 0.93 +/- 0.02 | 0.994 |
| **40/60** | 0.09 +/- 0.02 | 6.53 +/- 0.26 | 3.05 +/- 2.22 | 0.94 +/- 0.02 | 0.999 |
| **20/80** | 0.11 +/- 0.04 | 6.39 +/- 0.22 | 2.97 +/- 2.39 | 0.91 +/- 0.03 | 0.994 |
| **0/100** | -7.05 +/- 4.7 | 32.78 +/- 13.57 | -0.18 +/- 0.09 | 0.39 +/- 0.04 | 0.899 |

Table S14. Sensor performance for Batch VI given as confidence intervals on measured pH in three intervals.

| | DAOTA/ATTO-647 ratios (% vol.) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **100/0** | | **80/20** | | **60/40** | | **40/60** | | **20/80** | | **0/100** | |
| **Low/High pH** | - | + | - | + | - | + | - | + | - | + | - | + |
| **$pK_a$ +/- 0.25** | N/A | N/A | 0.93 | 0.93 | 0.42 | 0.34 | 0.43 | 0.35 | 0.45 | 0.34 | N/A | N/A |
| **$pK_a$ +/- 0.5** | N/A | N/A | 0.98 | 0.98 | 0.61 | 0.41 | 0.68 | 0.46 | 0.75 | 0.48 | N/A | N/A |
| **pH 7.2-7.4** | 86336.4 | 40639.8 | 2.33 | 2.03 | 0.4 | 0.48 | 0.56 | 0.7 | 0.7 | 0.86 | 19.14 | 86336.4 |

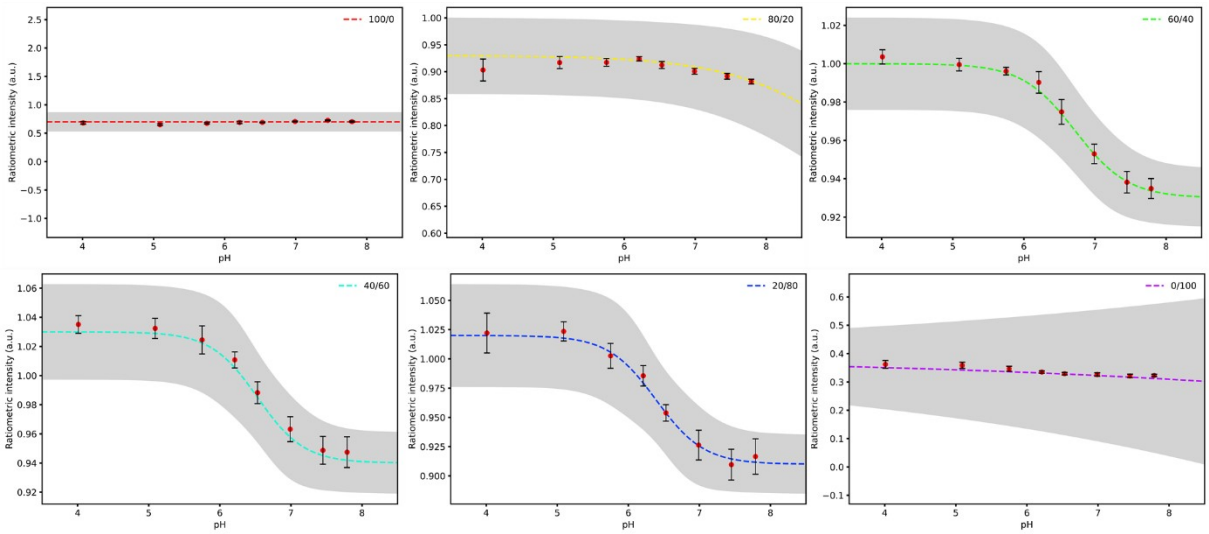


Figure S18. Response data and functions for Batch VI shown with propagated error as shaded area.

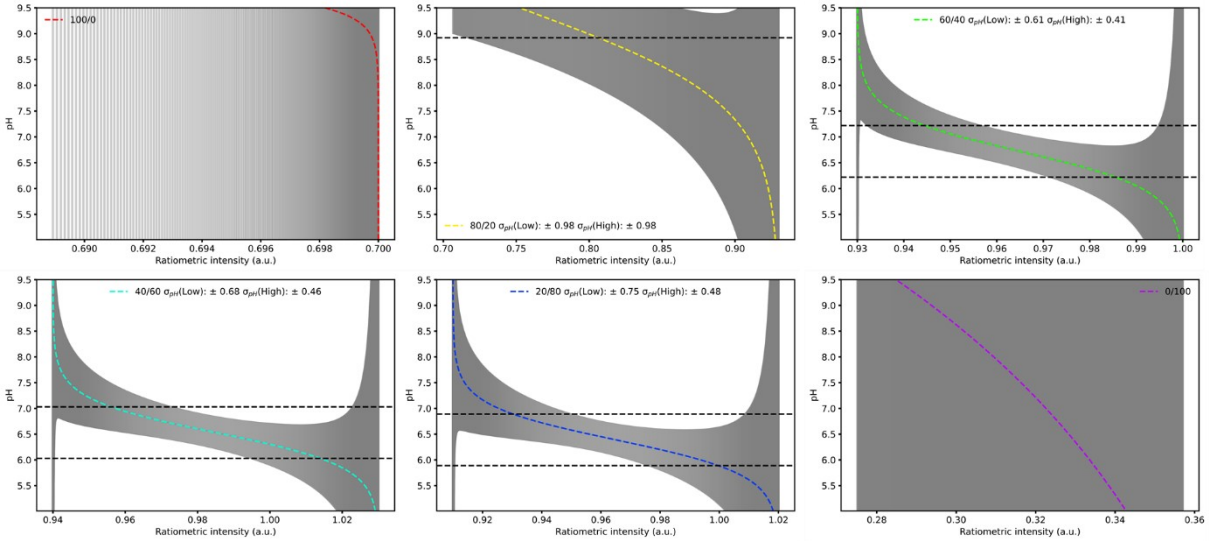


Figure S19. Evaluation functions for Batch VI shown with propagated error as shaded area.

## Batch VII

Parameters:

- Premixed ratios
- DAOTA:ATTO ratios: 100:0 - 80:20 - 60:40 - 40:60 – 20:80 – 0:100
- DAOTA NP conc: from dialysis (NP batch III) added Triton X-100 5.35 mg/ml
- ATTO-647 conc: 0.1 mM + Triton X-100 5.35 mg/ml
- Plate: Kartell - clear
- Vol pr. well: 50 ul
- Universal Buffer pH: 4.11 – 4.59 – 5.12 – 5.69 – 6.19 – 6.69 – 7.19 – 7.89
- D/A_007

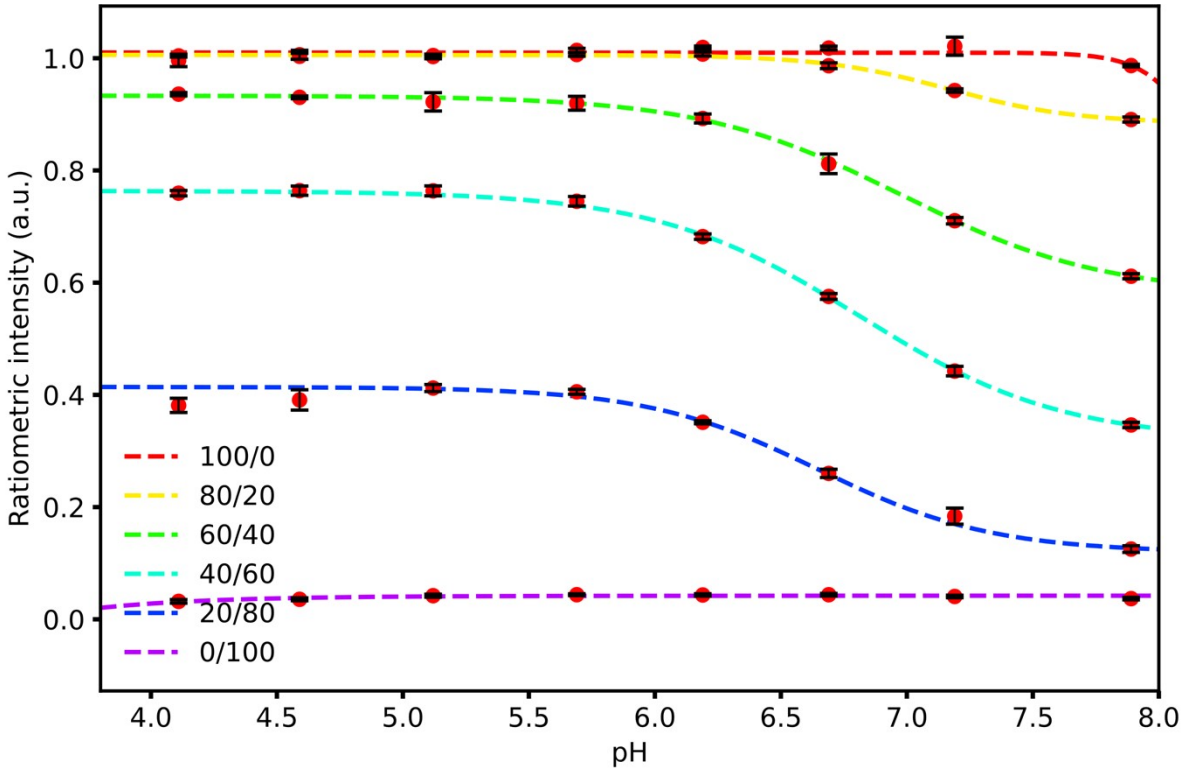


Figure S20. Response from Batch VII.

Table S15. Fit parameters for response functions for Batch VII.

| DAOTA/ATTO-647 | a | $pK_a$ | k | $y_0$ | GoF ($\chi^2$) |
|---|---|---|---|---|---|
| **100/0** | 0.52 +/- 0.03 | 8.26 +/- 0.27 | 8.36 +/- 6.22 | 0.49 +/- 0.03 | 0.0872 |
| **80/20** | 0.12 +/- 0.02 | 7.16 +/- 0.08 | 3.97 +/- 1.47 | 0.88 +/- 0.01 | 0.936 |
| **60/40** | 0.35 +/- 0.03 | 6.98 +/- 0.09 | 2.5 +/- 0.52 | 0.58 +/- 0.02 | 0.988 |
| **40/60** | 0.45 +/- 0.02 | 6.81 +/- 0.06 | 2.48 +/- 0.31 | 0.32 +/- 0.02 | 0.964 |
| **20/80** | 0.30 +/- 0.02 | 6.65 +/- 0.08 | 2.91 +/- 0.5 | 0.12 +/- 0.02 | 0.505 |
| **0/100** | -1.08 +/- 7.46 | 2.03 +/- 3.02 | 2.2 +/- 1.48 | 0.04 +/- 0 | 0.673 |

Table S16. Sensor performance for Batch VII given as confidence intervals on measured pH in three intervals.

| | DAOTA/ATTO-647 ratios (% vol.) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 80/20 | | 60/40 | | 40/60 | | 20/80 | |
| **Low/High pH** | - | + | - | + | - | + | - | + |
| **$pK_a$ +/- 0.25** | 0.2 | 0.14 | 0.15 | 0.12 | 0.1 | 0.13 | 0.17 | 0.96 |
| **$pK_a$ +/- 0.5** | 0.34 | 0.21 | 0.19 | 0.15 | 0.13 | 0.16 | 0.17 | 11.9 |
| **pH 7.2-7.4** | 0.11 | 0.13 | 0.12 | 0.15 | 0.18 | 0.28 | Inf | Inf |

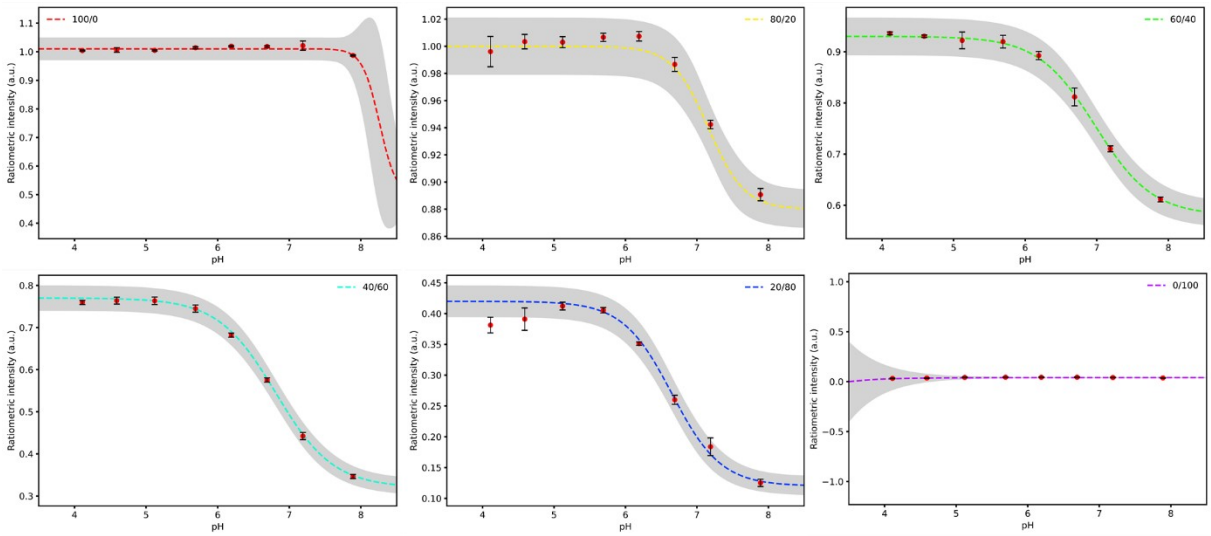


Figure S21. Response data and functions for Batch VII shown with propagated error as shaded area.

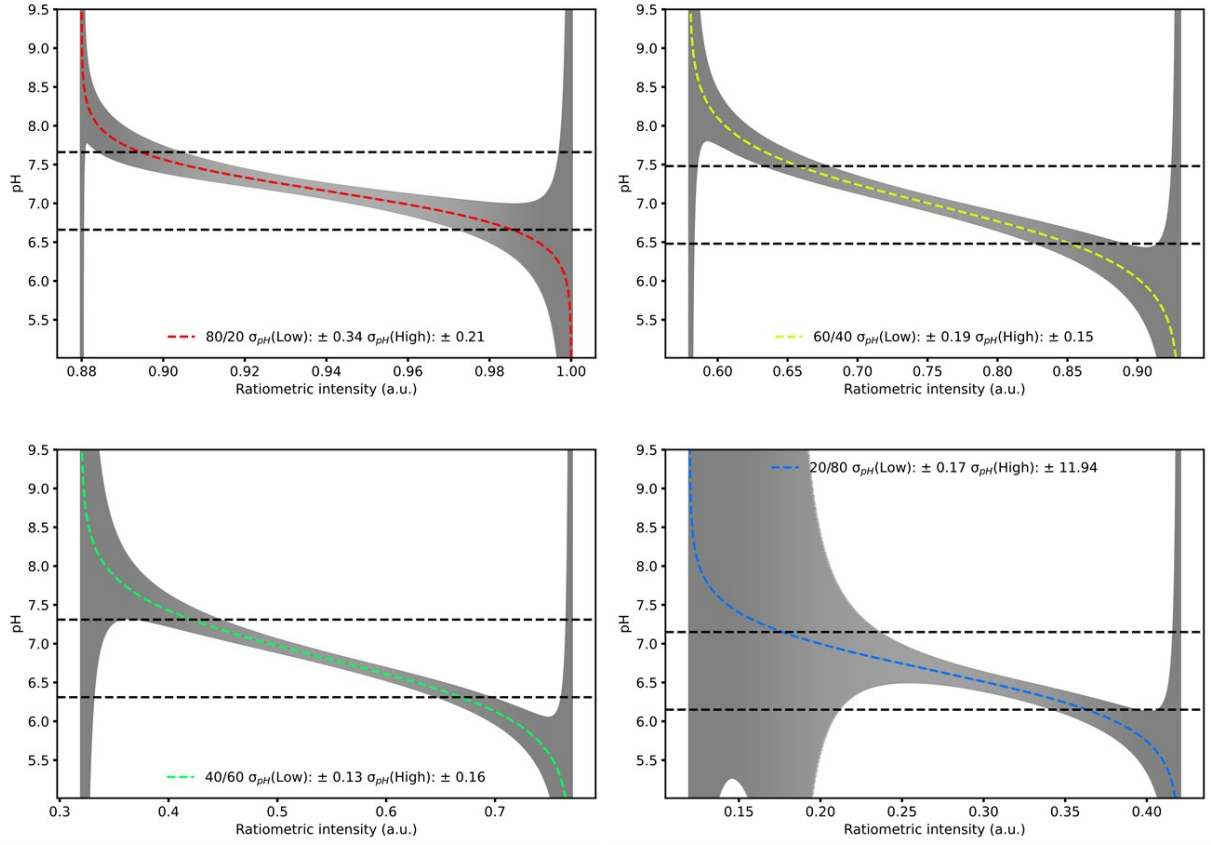


Figure S22. Evaluation functions for Batch VII shown with propagated error as shaded area.

# Sensor Testing

## Batch VIII

Parameters:

- Premixed ratios
- DAOTA:ATTO ratio: 60:40
- DAOTA NP conc: from dialysis (NP batch IV) added Triton X-100 5.35 mg/ml
- ATTO-647 conc: 0.1 mM + Triton X-100 5.35 mg/ml
- Plate: Kartell - clear
- Vol pr. well: 50 ul
- Universal Buffer pH: 4.50 – 4.99 – 5.49 – 6.05 – 6.51 – 6.79 – 7.24 – 7.48 – 7.77 – 7.98
- D/A_007

Table S17. Results of triplicate pH measurements using the multiwell plate based pH sensor compared to pH determined using a pH meter, day 1.

| | **MOPS** | | **MOPS known** | | **HEPES** | | **MOPSO** | |
|---|---|---|---|---|---|---|---|---|
| | pH sensor (pH-meter) | ΔpH | pH sensor (pH-meter) | ΔpH | pH sensor (pH-meter) | ΔpH | pH sensor (pH-meter) | ΔpH |
| **A** | 6.47 +/- 0.02 (6.53) | -0.06 | 6.75 +/- 0.02 (6.75) | -0.00 | 6.89 +/- 0.02 (6.87) | +0.02 | 6.92 +/- 0.07 (7.09) | -0.17 |
| **B** | N/A (7.74) | N/A | 7.10 +/- 0.02 (7.11) | -0.01 | N/A (7.58) | N/A | 7.78 +/- 0.30 (7.56) | +0.22 |
| **C** | 7.02 +/- 0.06 (7.13) | -0.11 | 7.30 +/- 0.03 (7.21) | +0.09 | N/A (7.88) | N/A | 7.33 +/- 0.13 (7.28) | +0.05 |
| **D** | 6.77 +/- 0.02 (6.73) | +0.04 | 7.51 +/- 0.02 (7.30) | +0.21 | 7.18 +/- 0.02 (7.05) | +0.13 | 6.79 +/- 0.07 (6.79) | +0.00 |
| **E** | 7.52 +/- 0.12 (7.39) | +0.13 | 8.08 +/- 0.23 (7.41) | +0.67 | N/A (8.13) | N/A | 6.54 +/- 0.06 (6.56) | -0.02 |
| **F** | 6.78 +/- 0.02 (6.91) | -0.13 | 8.14 +/- 0.15 (7.62) | +0.52 | 7.38 +/- 0.02 (7.28) | +0.10 | 6.12 +/- 0.04 (6.21) | -0.09 |

Table S18. Results of triplicate pH measurements using the multiwell plate based pH sensor compared to pH determined using a pH meter, day 5.

| | **MOPS** | | **MOPS known** | | **HEPES** | | **MOPSO** | |
|---|---|---|---|---|---|---|---|---|
| | pH sensor (pH-meter) | ΔpH | pH sensor (pH-meter) | ΔpH | pH sensor (pH-meter) | ΔpH | pH sensor (pH-meter) | ΔpH |
| **A** | 6.47 +/- 0.02 (6.56) | -0.09 | 6.75 +/- 0.02 (6.78) | -0.03 | 6.89 +/- 0.02 (6.89) | +0.00 | 6.92 +/- 0.07 (7.09) | -0.17 |
| **B** | N/A (7.76) | N/A | 7.10 +/- 0.02 (7.15) | -0.05 | N/A (7.62) | N/A | 7.78 +/- 0.30 (7.58) | +0.20 |
| **C** | 7.02 +/- 0.06 (7.14) | -0.12 | 7.30 +/- 0.03 (7.25) | +0.05 | N/A (7.95) | N/A | 7.33 +/- 0.13 (7.32) | +0.01 |
| **D** | 6.77 +/- 0.02 (6.71) | +0.06 | 7.51 +/- 0.02 (7.35) | +0.16 | 7.18 +/- 0.02 (7.06) | +0.12 | 6.79 +/- 0.07 (6.79) | +0.00 |
| **E** | 7.52 +/- 0.12 (7.44) | +0.08 | 8.08 +/- 0.23 (7.46) | +0.62 | N/A (8.13) | N/A | 6.54 +/- 0.06 (6.46) | -0.08 |
| **F** | 6.78 +/- 0.02 (6.91) | -0.13 | 8.14 +/- 0.15 (7.64) | +0.50 | 7.38 +/- 0.02 (7.33) | +0.05 | 6.12 +/- 0.04 (6.06) | +0.06 |

Table S19. Comparison of results of pH measurements using a pH meter, day 1 and day 5.

| | **MOPS** | | **MOPS known** | | **HEPES** | | **MOPSO** | |
|---|---|---|---|---|---|---|---|---|
| | pH sensor (pH-meter) | ΔpH | pH sensor (pH-meter) | ΔpH | pH sensor (pH-meter) | ΔpH | pH sensor (pH-meter) | ΔpH |
| **A** | 6.56 (6.53) | -0.03 | 6.78 (6.75) | -0.03 | 6.89 (6.87) | -0.02 | 7.09 (7.09) | -0.00 |
| **B** | 7.76 (7.74) | -0.02 | 7.15 (7.11) | -0.04 | 7.62 (7.58) | -0.04 | 7.58 (7.56) | -0.02 |
| **C** | 7.14 (7.13) | -0.01 | 7.25 (7.21) | -0.04 | 7.95 (7.88) | -0.07 | 7.32 (7.28) | -0.05 |
| **D** | 6.71 (6.73) | +0.02 | 7.35 (7.30) | -0.05 | 7.06 (7.05) | -0.02 | 6.79 (6.79) | -0.00 |
| **E** | 7.44 (7.39) | -0.05 | 7.46 (7.41) | -0.05 | 8.13 (8.13) | -0.00 | 6.46 (6.56) | +0.10 |
| **F** | 6.91 (6.91) | -0.00 | 7.64 (7.62) | -0.02 | 7.33 (7.28) | -0.05 | 6.06 (6.21) | +0.15 |