Electronic Supplementary Material (ESI)

A Supramolecular Sensing Array for Qualitative and Quantitative Analysis of Organophosphates in Water

Yuanli Liu and Marco Bonizzoni*

Department of Chemistry, The University of Alabama, Tuscaloosa, Alabama 35487, USA.

Table of Contents

Materials .................................................................................................................. S1
Structure of the PAMAM dendritic hosts............................................................... S2
Instrumentation and experimental conditions...................................................... S2
  Instrumentation .................................................................................................... S2
  Experimental conditions ..................................................................................... S3
Fluorescence titrations of (G5•MUP_m) ............................................................... S4
  Spectral properties of MUP .................................................................................. S4
  MUP binding to G5 dendrimer ........................................................................... S5
  MUP displacement experiments with organophosphates .................................. S5
Multivariate analyses ............................................................................................ S8
  Array plot of raw data ........................................................................................ S8
  Score plot for PCA analysis of organophosphates at 2 mM .............................. S10
  PCA loading plot for PCA analysis at 800 µM ................................................... S11
  LDA jackknife classification matrix ................................................................... S12
  LDA quantitation of hydrogen phosphate .......................................................... S14
  Calculation of confidence ellipsoids ................................................................. S15
Determination of the limits of detection ............................................................... S16
  Single analytes .................................................................................................... S16
  With interferents ................................................................................................ S17
References ............................................................................................................. S18

Materials

Amine-terminated PAMAM dendrimers with ethylenediamine core of generation 5 were purchased directly from the manufacturer, Dendritech Inc. and received as MeOH solution. 4-Methylumbelliferyl phosphate (MUP) and HEPES buffer (free acid) were purchased from VWR. Fluorescein, methylphosphonate, glyphosate, ethyl methylphosphonate, pinacolyl methylphosphonate and disodium phosphate were purchased from Sigma-Aldrich. Materials were used as received without further purification.
Structure of the PAMAM dendritic host

In this study we used an amine-terminated poly(amidoamine) (PAMAM) dendrimer of generation 5 (G5) with an ethylenediamine core. The structure of a small dendrimer (G1) belonging to this family is shown below, with each component generation highlighted in color for clarity. A dendrimer of generation 5 would be too cumbersome to draw in its entirety, but its structure can be inferred from the one shown. A G5 PAMAM dendrimer has a total of 128 amine groups on its surface. The terminal amines in these structures have been shown to be ca. 50% protonated in water at neutral pH, thereby providing a number of cationic binding sites with which the indicators and the organophosphates can interact.

Scheme 1. Representative structure of an amine terminated PAMAM dendrimer with 1,2-diaminoethane (ethylenediamine) core, in its approximate protonation state in water at neutral pH.

Instrumentation and Experimental Conditions

Instrumentation

Displacement titrations to confirm the ability of methylphosphonate (MPA) to displace fluorescein from its complex with the G5 PAMAM dendrimer were carried out on a benchtop instrument: an ISS PC1 spectrofluorimeter equipped with monochromators for wavelength selection and calibrated manual slits for resolution control. The excitation and emission channels on this instrument are also equipped with removable computer-controlled
high-aperture Glan-Thompson calcite polarizers. Excitation is provided by a 300 W high-pressure xenon arc lamp. Excitation correction is carried out through a rhodamine B quantum counter with a dedicated detector.

Spectroscopic data for multivariate analysis and displacement of 4-methylumbelliferyl phosphate presented in this paper were acquired on a BioTek Synergy II multimode microwell plate reader, capable of measuring absorbance spectra (through a monochromator), and steady-state fluorescence intensity and polarization (through bandpass filter sets and plastic sheet polarizers). The sample compartment in this instrument is electrically thermostatted and it was maintained at 25°C.

Absorbance spectra of MUP were measured on a Hewlett-Packard 8452a diode array UV-Vis spectrophotometer, with a cell holder connected to an external circulating water bath maintained at a constant 25°C.

Experimental conditions

Experiments were carried out in aqueous solutions buffered to pH 7.4 with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, 50 mM). Solutions were manually dispensed into each well of a microwell plate in 30 minutes by using Eppendorf Research multichannel pipettors and disposable plastic tips. The plates used were typically 96-well with clear bottom for absorbance, fluorescence and polarization measurement. The plates were made of non-treated (medium binding) polystyrene with black walls (to minimize scattered light) and clear flat bottoms. Each well contained 300 µL of solution. A new microwell plate was used for each experiment. Plates were read in a multimode plate reader right after preparation. The plate reader was thermostatted internally to 24°C. Reading time typically required 30-45 minutes per plate. No significant evaporation occurs in this time range, so we did not need to seal the plates.

For displacement experiments on the (G5•Fₙ) sensing ensemble, the concentration of fluorescein was kept constant at 2.0 x 10⁻⁶ M; the G5 dendrimer concentration was 1.0 x 10⁻⁶ M; the concentration of each organophosphate analyte was varied across the plate up to a maximum concentration ensuring complete displacement. Absorbance was collected at the following wavelengths: 460, 470, 500, 510, 520 and 600 nm; fluorescence intensity was collected in the following channels (λₑₓ/λₑₘ): 380/560 nm, 485/580 nm, 485/560 nm, 516/560 nm, and 516/580 nm; fluorescence anisotropy was collected in the following channels (λₑₓ/λₑₘ): 485/560 nm, 485/580 nm.

For displacement experiments on the (G5•MUPₘ) sensing ensemble, the concentration of 4-methylumbelliferyl phosphate was kept constant at 2.0 x 10⁻⁶ M; the G5 dendrimer concentration was 5.76 x 10⁻⁶ M. The concentration of organophosphates was varied over a range ensuring extensive displacement. Absorbance was collected at 320 nm; fluorescence intensity was collected with excitation at 380 nm and emission at 450 nm.

For a typical displacement experiment, a 96-well plate was laid out to contain the following: 6 replicates of free indicator; 6 replicates of buffer (used for blanking); 12 replicates of (G5•Fₙ) or (G5•MUPₘ) sensing ensemble solution; the rest of plate was used for the five analytes (Na₂HPO₄, GlyP, MPA, PMP and EMP), 12 replicates for each analyte.
Scheme 2. Typical layout of a 96-well plate used for displacement experiments.

Fluorescence titrations of (G5•MUP$_m$)

Spectral properties of 4-methylumbelliferyl phosphate (MUP)

Figure 1. Absorbance (blue) and fluorescence (green) spectra of 4-methylumbelliferyl phosphate (MUP). The fluorescence spectrum was acquired by exciting at 340 nm.
**MUP binding to G5 PAMAM dendrimers**

Binding of 4-methylumbelliferyl phosphate (MUP) to the G5 PAMAM dendrimer was first confirmed by a titration experiment on a benchtop spectrofluorimeter. The experiment was carried out in H2O buffered to pH 7.4 with 50 mM HEPES. The concentration of 4-methylumbelliferyl phosphate (MUP) was kept constant at 2.0 x 10^{-6} M; the G5 dendrimer concentration was varied over a range ensuring complete complexation of the dye. Fluorescence intensity spectra and single-wavelength (450 nm) anisotropy measurements were collected with excitation at 380 nm.

![Figure 2](image)

**Figure 2.** Binding titration of the 4-methylumberlliferyl phosphate dye (MUP) to G5 PAMAM dendrimer. The MUP concentration was held constant at 2.0x10^{-6} M; the concentration of dendrimer was varied over a range ensuring complete binding of the dye present in solution. In buffered water at pH 7.4 (HEPES 50 mM), 25°C. The fluorophore was excited at 380 nm; anisotropy was collected at 450 nm.

**MUP displacement experiments with organophosphates**

Dye displacement experiments on the (G5•MUP_m) sensing ensemble were carried out on a BioTek Synergy II multimode microwell plate reader. The concentration of 4-methylumbelliferyl phosphate (MUP) was kept constant at 2.0 x 10^{-6} M; the G5 dendrimer concentration was 5.76 x 10^{-6} M in H2O buffered to pH 7.4 with 50 mM HEPES. Fluorescence intensity was collected with excitation centered at 380 nm and emission centered at 450 nm.
Figure 3. Dye displacement from the \((G5\cdot MUP_m)\) sensing ensemble as a function of the molar concentration of methylphosphonate (MPA).

Figure 4. Dye displacement from the \((G5\cdot MUP_m)\) sensing ensemble as a function of the molar concentration of glyphosate (GlyP).
Figure 5. Dye displacement from the (G5•MUP$_m$) sensing ensemble as a function of the molar concentration of ethyl methylphosphonate (EMP).

Figure 6. Dye displacement from the (G5•MUP$_m$) sensing ensemble as a function of the molar concentration of pinacolyl methylphosphonate (PMP).
Multivariate analyses

Array plot ("heat map") of raw data

The first step towards multivariate analysis was the visualization of the complex response obtained from the multicomponent array. The response is best presented visually as an array plot (i.e. a “heat map”) as the one presented in the main manuscript (Figure 6) and reported below as well, for convenience.

Figure 7. A visual map of the array’s experimental response to target phosphates ([analyte] = 800 μM), highlighting its rich cross-reactive behavior. A key to the measurement channel codes used is provided below.

The following measurement channels are references in the array plot above:

<table>
<thead>
<tr>
<th>Code</th>
<th>Measurement channel</th>
</tr>
</thead>
<tbody>
<tr>
<td>A320</td>
<td>Absorbance at 320 nm</td>
</tr>
<tr>
<td>A460</td>
<td>Absorbance at 460 nm</td>
</tr>
<tr>
<td>A470</td>
<td>Absorbance at 470 nm</td>
</tr>
<tr>
<td>A500</td>
<td>Absorbance at 500 nm</td>
</tr>
<tr>
<td>A510</td>
<td>Absorbance at 510 nm</td>
</tr>
<tr>
<td>A520</td>
<td>Absorbance at 520 nm</td>
</tr>
<tr>
<td>F380-560</td>
<td>Fluor. em. through a 450/50 nm filter, with exc. through a 330/20 nm filter</td>
</tr>
<tr>
<td>F380-560</td>
<td>Fluor. em. through a 560/40 nm filter, with exc. through a 380/40 nm filter</td>
</tr>
<tr>
<td>F485-560</td>
<td>Fluor. em. through a 560/40 nm filter, with exc. through a 485/20 nm filter</td>
</tr>
<tr>
<td>F485-580</td>
<td>Fluor. em. through a 580/50 nm filter, with exc. through a 485/20 nm filter</td>
</tr>
<tr>
<td>F516-560</td>
<td>Fluor. em. through a 560/40 nm filter, with exc. through a 516/20 nm filter</td>
</tr>
<tr>
<td>F516-580</td>
<td>Fluor. em. through a 580/50 nm filter, with exc. through a 516/20 nm filter</td>
</tr>
</tbody>
</table>
The array plot was generated using Wolfram Inc.’s Mathematica v. 9.0. The data in each channel spans vastly different ranges: for instance, absorbance typically ranges between 0 and 0.1 units, raw fluorescence emission ranges up to $10^6$ units, and polarization between 0 and 350 milliunits. Each channel was therefore rescaled to range from 0 to 1, so that values from different measurement methods could be compared directly. The rescaled array was then used as input to Mathematica’s ReliefPlot[] function, using a color gradient to highlight the differences between data points. In this color scheme, black = zero, and bright yellow = 1. Color shades from black through green to bright yellow indicate intermediate values.

We found the resulting array plot very useful. First, we used it extensively to quickly check the quality of acquired data. In fact, replicate measurements for the same analyte/concentration combination should afford identical values; in practice, however, this is of course not always the case. We found it quite easy to spot “bad” data points (e.g. obvious outliers due to simple mistakes in plate preparation in such a “heat map”.

Additionally, the array plot presents us with an immediate idea of how much variability/cross reactivity is present in the array under study, and is therefore very helpful during method development and optimization, because it takes advantage of the human brain’s exceptional natural prowess at identifying patterns without having to proceed through the full computer analysis.

Not all of the differential behavior implied by the array plot can be readily explained. To name one example, exposure of the array to EMP clearly results in low fluorescence of the MUP dye (in the F330-450 channel), whereas the very similar PMP analyte does not. This may be due to actual quenching of the fluorescence through an unidentified process involving EMP, but not PMP. One might also note, however, that the absorbance of MUP in the presence of EMP is much lower than that measured in the presence of PMP for the same dye/dendrimer combination (see the A320 channel). This may suggest the alternate explanation that the difference in fluorescence might be due to lower excitation efficiency in the case of EMP instead. Although very intriguing, instances of the behavior outlined above would be very hard to explain individually. The power of multivariate detection and pattern recognition methods comes from the very ability to capture all these minute hints and details, and turn them into discriminatory power.
**Score plot for PCA analysis of organophosphates at 2 mM**

To evaluate the discriminatory ability of the two member (G5•Fₙ) + (G5•MUPₘ) sensor array for organophosphates, we used principal component analysis (PCA) and linear discriminant analysis (LDA)² as data reduction and pattern recognition methods.

PCA analyses were performed as implemented in the commercial MINITAB® program (release 16 for Windows). LDA analyses were performed as implemented in the commercial SYSTAT® program (release 12 for Windows) program.

PCA is a non-supervised statistical tool that facilitates data reduction of a multidimensional data set into a lower dimensional space with minimal loss of information content.³ LDA is a supervised statistical approach for clusters analysis, LDA also allowed us to check for classification accuracy by using a leave-one-out routine.

Reported below are the score plots obtained from the PCA and LDA analysis.

![Score plot](image)

**Figure 8.** Two-dimensional score plot from PCA analysis of the qualitative discrimination experiments carried out at 2 mM concentration of organophosphates. No significant improvement in clustering or cluster dispersion were observed over the corresponding experiment carried out at 800 μM.
PCA loading plot obtained from the array response at 800 µM

A loading plot describes the relative contributions of the original (experimental) variables to each principal component in a PCA analysis. In particular, we show below the contributions to the first two principal components, PC1 and PC2, because these are the ones we have actually used for discrimination. For instance, the graph below shows that most information contained in the second principal component (PC2) derives from changes in the fluorescence emission of fluorescein at 560 nm when this fluorophore was excited at 516 nm. On the other hand, the information gained from observing changes in the emission of fluorescein at 560 nm when excited at 485 nm mostly contributes to the information captured by PC1.

A loading plot can be particularly useful in pinpointing redundant or useless instrumental variables in the design phases of an array such as the one presented here. In the case discussed, we did not pursue any further reduction of the instrumental response acquired because the use of a plate reader allowed us to acquire as much information as desired with essentially no extra effort on the part of the operator.

Figure 9. Two-dimensional loading plot for PC1 and PC2 in the PCA analysis of the qualitative discrimination experiments carried out at 800 µM concentration of organophosphates.
**LDA jackknife classification matrix**

Using LDA we were able to perform jackknife analysis to check the correctness of sample classification using our system. In this case, jackknife analysis consisted in systematically performing the LDA analysis leaving out one of the observations from the complete sample set at a time. The results of the analysis on this reduced set are then used to reclassify the corresponding omitted sample, and checking if it is indeed assigned to the known correct category. The results of this analysis are presented in the table below.

As is evident from the table, the system was able to reclassify all samples correctly.

**Table 1.** Results from jackknife analysis on the LDA classification of organophosphates at 800 μM analyte concentration.

<table>
<thead>
<tr>
<th>OPs</th>
<th>EMP</th>
<th>GlyP</th>
<th>HPO₄²⁻</th>
<th>MPA</th>
<th>PMP</th>
<th>%correct</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMP</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>GlyP</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>HPO₄²⁻</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>MPA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>PMP</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 2.** Results from jackknife analysis on the LDA classification of methylphosphonate (MPA) samples at concentrations ranging from 10 μM to 2 mM.

<table>
<thead>
<tr>
<th>MPA</th>
<th>10 μM</th>
<th>40 μM</th>
<th>100 μM</th>
<th>400 μM</th>
<th>1 mM</th>
<th>2 mM</th>
<th>%correct</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 μM</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>88</td>
</tr>
<tr>
<td>40 μM</td>
<td>1</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>88</td>
</tr>
<tr>
<td>100 μM</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>400 μM</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>1 mM</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>2 mM</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>96</td>
</tr>
</tbody>
</table>
Table 3. Results from jackknife analysis on the LDA classification of glyphosate (GlyP) samples at concentrations ranging from 10 µM to 2 mM.

<table>
<thead>
<tr>
<th>GlyP</th>
<th>10 µM</th>
<th>40 µM</th>
<th>100 µM</th>
<th>400 µM</th>
<th>1 mM</th>
<th>2 mM</th>
<th>%correct</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 µM</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>88</td>
</tr>
<tr>
<td>40 µM</td>
<td>1</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>88</td>
</tr>
<tr>
<td>100 µM</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>400 µM</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>1 mM</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>2 mM</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>96</td>
</tr>
</tbody>
</table>

Table 4 Results from jackknife analysis on the LDA classification of hydrogen phosphate (HPO₄²⁻) samples at concentrations ranging from 10 µM to 2 mM.

<table>
<thead>
<tr>
<th>Na₂HPO₄</th>
<th>10 µM</th>
<th>40 µM</th>
<th>100 µM</th>
<th>400 µM</th>
<th>1 mM</th>
<th>2 mM</th>
<th>%correct</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 µM</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>40 µM</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>100 µM</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>400 µM</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>1 mM</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>2 mM</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>100</td>
</tr>
</tbody>
</table>
Figure 10. Quantitative analysis of Na$_2$HPO$_4$ using the proposed two component array and LDA analysis for clustering.
**Calculation of the confidence ellipsoids**

Two dimensional confidence ellipsoids were calculated for each cluster obtained from multivariate analysis, using the `EllipsoidQuantile` routine implemented in the `MultivariateStatistics` package included in Wolfram’s *Mathematica* v. 9.0. A confidence level of 95% was used for the calculation. Below we present a sample from the code used for the calculation of ellipsoids for the PCA score plot obtained from the quantitative analysis of five phosphates at 800 μM (shown in the main manuscript).

```mathematica
Needs["MultivariateStatistics"]

clusters = {
  (* HPO4 *) {{4.595, 1.3278}, {4.4469, 1.3409}, {3.8197, 0.9827}, {4.0072, 0.3554}, {4.132, -0.2919},
               {3.7958, -0.3026}, {3.8881, -0.5625}, {4.1658, -0.4699}, {4.0111, -1.2171}, {4.2003, 0.1917},
               {3.9648, -0.4244}, {4.1041, 0.7991}},
  (* glyphosate, GlyP *) {{2.2132, 0.8528}, {1.8003, -0.5182}, {1.7108, -0.5196}, {1.4471, -1.7224},
                          {2.0336, -1.1863}, {2.7774, -1.4338}, {2.9171, -1.6059}, {2.0374, -0.3667}, {1.5909, -1.2102},
                          {1.8385, -0.6981}, {2.1545, -0.9167}, {2.4665, -0.5139}},
  (* methylphosphonate, MPA *) {{0.5216, 0.4442}, {0.4957, 0.0118}, {0.3068, 0.3268},
                              {0.3502, 0.532}, {0.1376, 1.8099}, {0.7292, 0.0181}, {0.6441, -0.6905}, {0.7045, 1.446},
                              {0.7952, 0.4222}, {-0.1989, 2.7435}, {0.3252, 2.1756}, {0.0987, 0.8203}},
  (* ethyl methylphosphonate, EMP *) {{-3.3983, -1.9903}, {-3.2053, -2.7178}, {-3.4333, -1.2986},
                                    {-3.2386, -2.1931}, {-3.5412, -2.9791}, {-2.91, -2.8402}, {-3.0403, -0.8911}, {-2.9578, -2.2513},
  (* pinacolyl methylphosphonate, PMP *) {{-3.2401, 2.0537}, {-3.3055, 2.8588}, {-3.3052, 3.1924},
                                           {-3.0948, 1.5971}, {-3.3752, 1.6276}, {-3.002, 0.5658}, {-3.3387, 1.295}, {-3.5327, 1.5524},
                                           {-3.008, 1.9775}, {-3.1863, 2.643}, {-3.9753, 1.8969}, {-3.0819, 1.3473}}
};

Table[InputForm@Graphics@EllipsoidQuantile[clusters[[i]], 0.95], {i, 1, 5, 1}];

ellipsoids = %[[All, 1, 1, 1, 3, 2, 1]];
Determination of limits of detection (LOD)

*Single analytes*

The limit of detection was calculated according to the generally accepted method proposed by Kaiser, based on the average response (Ave.) of the sensing system to a blank, and the standard deviation associated with repeated measurements of that response (σ). In particular we used the fluorescence emission response for our determinations.

A calibration curve was also established for each analyte for which we determined a limit of detection. The limit of detection was calculated as the concentration that would generate a signal equal to the average blank response plus three times the standard deviation of that response. Examples are shown below for methylphosphonate and glyphosate.

![Graph showing calibration data and fitted first-order response curves for the determination of the limits of detection of the proposed method, for methylphosphonate (MPA, top) and glyphosate (GlyP, bottom).](image)

**Figure 11** Calibration data and fitted first-order response curves for the determination of the limits of detection of the proposed method, for methylphosphonate (MPA, top) and glyphosate (GlyP, bottom).
Limit of detection for MPA in the presence of interferents

One of the most interesting applications of the method we propose is not only to determine the concentration of organophosphates, but also to distinguish between herbicides and nerve gas related compounds. In view of this, we determined the limit of detection of our method for methylphosphonate (MPA), an indicator of nerve gas use or manufacture, in the presence of a large excess of glyphosate, a commonly used organophosphate herbicide.

We prepared a solution containing the \((G^5\cdot F_n)\) sensor and 40 \(\mu\)M glyphosate: this is a concentration of glyphosate ca. 10x the EPA-mandated limit (0.7 ppm \(\approx 4 \mu\)M). We then titrated this solution with aliquots of methylphosphonate (MPA) and monitored the fluorescence emission to determine the limit of detection for MPA in these competitive conditions. As shown by the titration profile (Figure 12, right), quantitative results can still be obtained for micromolar concentrations of MPA. The LOD increases in comparison to that measured for MPA in the absence of GlyP (0.3 mg/L \(\approx 2 \mu\)M), but it is still within the micromolar range. We have also included a spectrum of free fluorescein in the titration below (Figure 12 left, blue spectrum), to gauge the remaining dynamic range available for further displacement.

![Fluorescence Spectra](image1)

**Figure 12.** Competition experiment for the determination of the limit of detection for methylphosphonate (MPA). Aliquots of MPA were added to a solution of the \((G^5\cdot F_n)\) sensor that also contained 40 \(\mu\)M glyphosate. A regression line was fitted to the linear portion of the response curve, and the limit of detection was calculated considering 3x the standard deviation associate with repeated measurements of the initial solution in absence of MPA.
References