Supporting Information for

Amplified Detection of Chemical Warfare Agents Using Two-Dimensional Chemical Potential Gradients

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1 Materials and supplies

3- (trimethoxysilyl)propyl methacrylate (≥98%), trichloro(1H,1H,2H,2H-perfluorooctyl)silane (97%), acrylamide/N,N’-methylenbisacrylamide mixture (AAm/BisAAm mixture, 37:1 weight ratio), N,N,N’,N’-tetramethylethylenediamine (99%), sodium hydroxide, sodium chloride, sodium nitrate, N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride (EDC), sulfanilic acid sodium salt hydrate, (2-aminoethyl)trimethylammonium chloride hydrochloride (AETMA), bromothymol blue solution (dye content 95 %), N,N’-dimethyl-9,9’-biacridinium dinitrate, and tetraethyl orthosilicate (TEOS), diethyl chlorophosphite (DCP), ethyl acetate, diisopropyl fluorophosphates (DFP), diisopropyl-fluorophosphatase (DSPase) were purchased from Sigma Aldrich. 2,2-diethoxyacetophenone (DEAP, 98%) and dimethyl sulfoxide (DMSO) and Sylgard® 184 were purchased from Alfa Aesar. Fluoride ion selective electrode, Ag/AgCl counter electrode and filling solutions were purchased from Microelectrodes, Inc. Fluoride ion standard solution were purchased from Fisher Scientific.

2 Synthesis and chemical modification of PAAm hydrogel films

2.1 Preparation of PAAm hydrogel precursor solution

0.30 g of a 37:1 ratio mixture of AAm and BisAAm, was dissolved in 5.0 g Millipore water and the resulting monomer solution was shaken for 5 min using a Vortex mixer. After that, 0.10 g of DEAP dissolved in 1.0 g of DMSO was poured into the monomer solution and mixed for 15 min on the Vortex mixture. To remove dissolved oxygen/air from the polymerization solution, it was purged with nitrogen gas for 10 min.

2.2 Preparation of hydrogel for optical measurements

The PAAm hydrogel precursor solution was filled into a cell and exposed to mercury UV lamp (Blak-
Ray® longwave lamp B-100AP, 365 nm, 100 W, intensity: 21700 μW/cm²) for 2 h to polymerize it. The cell consisted of a trichloro(1H,1H,2H,2H-perfluorooctyl)silane monolayer coated top glass slide and a 3-(trimethoxysilyl)propyl methacrylate monolayer coated bottom glass slide. A spacer was placed between the slides to control the thickness of the hydrogel. The resulting polyacrylamide (PAAm) hydrogel film was covalently attached to the bottom slide which had been coated with the 3-(trimethoxysilyl)propyl methacrylate monolayer. All samples were washed with Millipore water to remove byproducts and unreacted species and dried in air. The hydrogel film directly attached to the methacrylate functionalized glass substrate was used for the Cl⁻ and H⁺ transport studies.

2.3 Preparation of hydrogel for electrochemical measurements

Since fluoride ions react with SiO₂, an additional SU-8 layer was deposited at the hydrogel/glass slide interface (Figure S1). To remove any organic contaminants and form hydroxyl groups on the glass surface, a 25 × 25 mm² glass substrate was dipped in a 50 °C Nano-Strip® solution for 2 h. The substrates were then rinsed with DI water and dried with blowing N₂ gas. 1 mL of SU-8 2015 solution was spun-coat onto the glass substrate (500 rpm for 10 s followed by 2500 rpm for 30 s). The resulting coated substrate was baked on a hotplate at 60 °C for 15 min and 95 °C for 5 min, forming a cured SU-8 film with a thickness of 17±2 μm. The PAAm hydrogel precursor solution was then filled into the cell comprising the SU-8 coated glass slide and a trichloro(1H,1H,2H,2H-perfluoroctyl)silane monolayer coated top glass slide and exposed to UV light (Blak-Ray® longwave lamp B-100AP, 365 nm, 100 W, intensity: 21700 μW/cm²) for 2 h. After UV exposure, the substrate was placed onto a hot plate at a temperature of 95 °C for 5 min. to complete the cross-linking. The resulting PAAm hydrogel film was attached to the bottom slide which had been coated with the SU-8. All samples were washed with Millipore water to remove byproducts and unreacted species and dried in air.
2.4 Chemical modification of PAAm hydrogel films

The chemical modification of PAAm films involved two steps, hydrolysis and EDC assisted coupling of functional groups (Figure S2). The hydrolysis process was performed by dipping a dry PAAm film for selected period of time in the hydrolysis solution containing NaOH (6 mmol), N,N,N′,N′-tetramethylethylenediamine (TMEDA) (18 mmol) and NaNO3 (3 mmol) in 20.0 g Millipore water. The partially hydrolyzed PAAm film, labeled as PAAm film with carboxylate is shown in Figure S2. The carboxylates were then transformed to the specific functionalities by conjugation with amine-appended molecules. For example, the quaternary ammonium functionalities was formed by immersing PAAm hydrogels containing carboxylate overnight in a solution of N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) (0.78 mmol), (2-aminoethyl)trimethylammonium chloride hydrochloride (AETMA) (0.78 mmol), NaNO3 (3 mmol) in 20 g of Millipore water. The gels were washed with Millipore water and dipped into a pH 7.0 phosphate buffer solution (SB 107-500) for 2 h and again washed with Millipore water several times.

Figure S2. Chemical modification of PAAm hydrogel with functionality R.
2.5 Directional and radially symmetric gradients

To create a directional gradient, a dry PAAm film was dipped for 24 h in a chamber where the hydrolysis solution was filled up to ca. 2 mm from the bottom edge of the film (see Figure S3a). As time passed, the hydrolysis solution diffused upward. Because the rate of the hydrolysis is slow at room temperature, this formed a gradient of carboxylate density across the PAAm film. The carboxylates were then transformed to the functionalities of interest by conjugation with amine-appended molecules.\(^1\) The radially symmetric gradient was fabricated following the procedure in Figure S3b. The localized hydrolysis which lead to the radially symmetric geometry was conducted using the setup shown in Figure S3b. The PDMS cap made from Sylgard® 184 was pressed against the dry PAAm film to prevent leakage of the hydrolysis solution. The hydrolysis time was fixed at 24 h. As time passed, the hydrolysis solution diffused radially. Because the rate of the hydrolysis is slow at room temperature, this formed a radially symmetric gradient of carboxylate density in the PAAm film. The center of the gradient was about 2.5 mm in diameter and the periphery of the gradient extended another ~2 mm outwards (the gradient diameter was controlled by the diameter of hydrolyzing solution injection needle, Figure S3b) and the gradient periphery was controlled by diffusion of the hydrolyzing solution into the gel). The carboxylates were then transformed to the functionalities of interest by conjugation with amine-appended molecules.

Figure S3. Schematics of setups for localized hydrolysis used for creating directional (a) and radially symmetric (b) chemical gradients in the PAAm films.
3 Molecular probes used in this study

![Chemical structures of the molecular probes used in this study.](image)

**Figure S4.** Chemical structures of the molecular probes used in this study.

### 3.1 Lucigenin

Lucigenin (N,N′-dimethyl-9,9′-biacridinium dinitrate) was selected as the fluorescent indicator for Cl− to demonstrate Cl− transport by directional and radially symmetric cationic gradients because of its fluorescence is quantitatively quenched by Cl− and it is insensitive to nitrate, phosphate and sulfate. The Stern-Volmer quenching constant of the quenching event was reported as $K_{SV} = 390$ M$^{-1}$. The quenching process is collisional; therefore, a chloride ion concentration-dependent fluorescence quenching was observed. Lucigenin absorbs light at both 368 nm and 455 nm, with the emission maximum at 505 nm. Its fluorescence emission has a quantum yield of ~0.6. Lucigenin was successfully used as Cl− indicator in liposomes and reconstituted membrane vesicles. The relationship between fluorescence intensity of the lucigenin and chloride concentration can be expressed by the Stern-Volmer equation:

$$\frac{F_0}{F} - 1 = K_{SV}[Cl] \quad \text{(eq S1)}$$

Where $F_0$ is the fluorescence intensity of the lucigenin in the absence of chloride ion, and $F$ is the fluorescence intensity in the presence of chloride ion. $[Cl]$ is the concentration of chloride ion.
3.2 Bromothymol blue

Bromothymol blue (4,4’-(1,1-dioxido-3H-2,1-benzoxathiole-3,3-diyl)bis(2-bromo-6-isopropyl-3-methylphenol)) was selected as the colorimetric indicator for hydrogen ion to demonstrate transportation of hydrogel ion by directional neutral-to-anionic gradient. It is a commercially available well-known pH indicator used in the applications, which require monitoring the change in pH from a relatively neutral pH (near 7). It is active at a pH range of 6.0 to 7.6. The protonated form of bromothymol blue has its peak absorption at 692 nm, and the deprotonated form has its peak absorption at 602 nm. Bromothymol blue is highly soluble in water. All these features make the bromothymol blue attractive for mapping the concentration of hydrogen ions.

4 Nerve agent simulants used in this study

![Chemical structures of common nerve agents, and safer simulants often used in studies.](image)

**Figure S5.** Chemical structures of common nerve agents, and safer simulants often used in studies.

Organophosphate-based nerve agents include the fluoride-containing “G series” and sulfur-containing “V series”. Of the G-series nerve agents, sarin (3) is the most common. It is volatile and extremely toxic (LD$_{50}$ = 70 µg/kg). Cyclosarin (5) is even more toxic (LD$_{50}$ = 17 µg/kg). Due to their extreme toxicity, the use of surrogates such as DCP (6, LD$_{50}$ = 11 mg/kg) and DFP (7, LD$_{50}$ = 2
mg/kg) is a common practice. They are structurally similar (contain P-F bond) to fluoride-containing G-series nerve agents and are safer to use (Figure S5). Note, many of the simulants are still rather toxic and must be handled with great care.

5 Enzyme used in this study and enzyme immobilization process

Enzymes possess a supreme degree of selectivity and high substrate turnover rates for catalytic reactions. A common hydrolase enzyme, DFPase (EC 3.1.8.2) was selected to catalyze the hydrolysis reaction of DFP. This enzyme specifically acts on ester bonds via phosphoric-triester hydrolases. Figure S6 shows how DFPase catalyzes the hydrolysis of DFP yielding HF.

![Enzymatic reaction of DFPase](Image)

**Figure S6.** Enzymatic reaction of DFPase.

In this study, DFPase was implanted in PAAm hydrogel matrix by photoactivated crosslinking reaction of a solution comprised of 2 mg/ml of enzyme in the PAAm hydrogel precursor solution. The process was done by spreading the solution on the pre-synthesized gels and covering this solution with a trichloro(1H,1H,2H,2H-perfluoroctyl)silane monolayer coated top glass slide followed by exposure to UV light (Blak-Ray® longwave lamp B-100AP, 365 nm, 100 W, intensity: 21700 μW/cm²) for 2 h. This process formed a ca. 5 μm-thick enzyme imbedded hydrogel layer on top of a gradient containing gel (Figure S7).
Figure S7. A thin layer of enzyme imbedded PAAm on neutral-to-cationic tertiary ammonium gradient containing hydrogel.

Literature reports indicate DFPase embedded within a polymeric matrix exhibited significantly increased shelf-life and pot-life stabilities at elevated temperatures. Catalytic activity of a native DFPase on DFP was reported as 81 µmol l⁻¹ s⁻¹ of F⁻, while an immobilized enzyme in poly(propylene sulfide) matrix was reported as 78 µmol l⁻¹ s⁻¹ of F⁻. The mesh size of our PAAm gel was calculated as 12 nm from the water content (93%) in the swollen state following the Ref, indicating there is ample spaces for the enzyme to retain its natural conformational structure.

6 Dye-silica gels preparation

To immobilize the bromothymol blue dye, it is enclosed in a silica gel matrix. The silica gel was prepared by adding 3–4 drops of concentrated hydrochloric acid to 38 mL of water. This solution was poured into a solution of TEOS (30 mL) and ethanol (31 mL) with constant stirring using a magnetic bar. The solution was heated at 60 °C for 2 h and dried in a vacuum oven at 60 °C for 24 h. The molar ratio was calculated as TEOS:ethanol:water to be 1:4:16. Once the silica gel is ready, 20 wt.% of the silica gel was dissolved in a 0.1% aqueous solution of bromothymol blue with stirring using a magnetic bar. The final dye-silica gels were ready to use (Figure S8).
Figure S8. Illustration of dye molecules immobilized within a silica gel network.

7 Theory and modeling for ion transportation

Attempts have been made to model the diffusion of solutes in gels\(^6\) accounting the effects of specific binding\(^7\) and electrostatic potential in charged systems.\(^8\) The transportation of solute molecules through a gel is also hindered by the crosslinked polymer matrix. As diffusion progresses, the free volume of the gel reduces and the hydrodynamic drag subsequently increases. As a result, a physical obstruction against the motion of the solute molecules is being generated. In the system presented in here, one should also consider the direct attachment of solution molecules to binding sites on the polymer chain, and the electrostatic field induced by the charged binding sites. In addition to that lucigenin dye was dispersed to map the position of chloride ion. We could not exclude the binding interaction between lucigenin and chloride. A complete theoretical model of these systems is complex, and beyond the scope of this manuscript. However, to get an idea about the diffusion coefficient and the gradient imposed drift velocity, COMSOL multiphysics program (ver. 5.2) was used to model the system using a two-dimensional diffusion-convection equation,

\[
\frac{\partial c}{\partial t} = \nabla \cdot [D(x, y)\nabla c] + \nabla \cdot [\mathbf{v}(x, y)c]
\]  

(eq S2)
where $D(x, y)$ is the local diffusion coefficient, $\vec{v}(x, y)$ is the gradient-imposed drift velocity, and $c$ is the concentration of ions. For the simplicity, we assume that both diffusion coefficient and drift velocity (since the gradient slope is steep) are constant in the gradient region.

### 7.1 Diffusion of Cl$^-$ in hydrogel

It is important to ascertain the rate of Cl$^-$ diffusion in a gradient-free hydrogel. The experiments were performed with ca. 30 μm thick hydrogel films covalently attached to acrylate-functionalized glass substrates and carried out at 100% RH. The thickness of the film is significantly less than the gradient length (several millimeters), and therefore, ion transportation is effectively two-dimensional. To remove residual Cl$^-$ remaining from the gradient fabrication, the film was dipped in 3 mM NaNO$_3$ for 2 h and then 5 mM NaOH for 2 h. Finally, the films were washed with Millipore water several times.

To indicate the presence of Cl$^-$ in the hydrogel, 1 mM aqueous lucigenin, a fluorescent chloride indicator was sprayed (~75 μg/cm$^2$) on the surface of the films using an atomizer spray and Cl$^-$ (0.1 M HCl) was dosed using a sharp tip. The presence of Cl$^-$ quenched the fluorescence of the lucigenin and the quenched spot was monitored continuously under a fluorescence microscopy as shown in Figure S9b. It was seen that the quenched spot spread isotropically. A good fit was achieved from a COMSOL model (Figure S9c) using a diffusion coefficient of $1 \times 10^{-9}$ m$^2$/s. This value of the diffusion coefficient is comparable to the reported value for Cl$^-$ in water.$^9$
Figure S9. Diffusion of Cl⁻ in a gradient-free PAAm hydrogel. (a) Schematic, and (b) experimental fluorescence images, (c) COMSOL modelled concentration maps at increasing times. Line profiles of fluorescence intensity (d) experimental and (e) modelled intersecting the origin of dosing spot by the dashed lines in (b,c). The experimental images in (b) were taken using an inverted fluorescence light microscopy (Axiovert 200M, Carl Zeiss Inc., X-Cite® 120 excitation light source with mercury lamp, 470Ex./515Em. FITC chroma Filter Set 41025, 2.5×/0.075 object and Zeiss AxioCam HRC color CCD camera). (d) Line profiles of the intensity of gray color obtained from the images (b) using ImageJ. (e) Line profiles of the intensity of white color obtained from the images (c) using COMSOL.
Directional transport of Cl⁻ using ammonium gradient

Figure S10. Transportation of Cl⁻ in a PAAm hydrogel by a neutral-to-cationic directional gradient. (a) Schematic, and (b) experimental fluorescence images, (c) COMSOL modelled concentration maps at increasing times. (d,e) The intensities as a function of time at two spots indicating my arrows in (b,c). Symbols are the intensity of the grey color obtained from the images (b) using ImageJ. Solid lines are the fits using COMSOL. The experimental images in (b) were taken using an inverted fluorescence light microscopy (Axiovert 200M, Carl Zeiss Inc., X-Cite® 120 excition light source with mercury lamp, 470Ex./515Em. FITC chroma Filter Set 41025, 2.5×/0.075 object and Zeiss AxioCam HRC color CCD camera).

The directional transport of Cl⁻ using a neutral-to-cationic quaternary ammonium gradient with a length of ca. 7 mm (confirmed by Raman measurement¹) was formed to transport Cl⁻, since the
quaternary ammonium ion functionality reversibly binds Cl-. Figure 10b(i) shows the result when a tip coated with Cl- (0.1 M HCl) was contacted with the dye loaded hydrogel surface, leaving a trace of chloride ions, which quenched the fluorescence. It was seen that the quenched spot spreads anisotropically, with the overall transport being towards the quaternary ammonium-rich end of the gradient. Figure 10c shows a model of the ion transport under directional field velocity (eq S2). The fluorescence intensities at two spots indicating by arrows in Figure 10b,c were plotted in Figure 10d,e. A good fit was achieved with a diffusion coefficient of $2.3 \times 10^{-09}$ m$^2$/s and a drift velocity of $1.1 \times 10^{-06}$ m/s.

8 Electrochemical measurement

8.1 Fluoride ion selective electrochemical sensor

The fluoride ion electrode contains an internal fluoride ion selective crystal composed of 3% EuF$_2$-doped LaF$_3$ with an internal NaF filling solution (Optimum Results A, Cat. No. 900061, Thermo Scientific) (Figure S11a,b). The fluoride ion selective crystal was pushed into a PVC barrel. To prevent any leakage, the crystal/PVC interfaces was sealed by epoxy glue. The active area of the crystal was measured as $\sim$2 mm in diameter. The reference electrode is composed of an internal Ag/AgCl electrode with an internal filling solution of 3M KCl saturated with AgCl. Electrodes have been stored dry. Filling solutions were refilled each day. The electrodes were immersed into the filling solution for at least 30 min before making any measurements. The electrode potentials were directly measured in millivolts using Mettler Toledo™ S220 SevenCompact™ pH/Ion benchtop meter.

To determine the electrode operation, a standard calibration curve was generated by measuring electrode potentials of standard solutions and plotted on the linear axis against their concentrations on the log axis shown in Figure S11c. The standard solution contains Total Ionic Strength Adjustment Buffer (TISAB) at a recommended dose to ensure a constant background ionic strength, decomplexes fluoride ions and adjusts the solution pH. When a stable reading was displayed, the electrode potential
was recorded in millivolts. Ideally, there should be a 54 to 60 mV difference between two concentrations with 10-fold difference in concentration at room temperature (between 20 to 25°C). Errors in the electrode potential were calculated from the several identical measurements done at different times before starting each electrochemical measurement. This thus indicates the stability of the fluoride electrode. The fluoride electrode used in this study showed a good stability as low as $1 \times 10^{-4}$ M concentration.

Figure S11. Fluoride ion selective miniature electrode. (a) Fluorescence images of the electrode tip: i) 3% EuF$_2$-doped LaF$_3$ crystal was forced into a PVC barrel. (ii) epoxy glue at the crystal/PVC interface provides resistant to damage by inorganic solutions. (b) Schematic diagram of a fluoride meter. Ag/AgCl was used a reference electrode, (c) Standard calibration curve to determine fluoride ion sensor performance.

8.2 Activity of fluoride ion in tertiary ammonium group functionalized hydrogel

The fluoride ion selective electrode converts the activity of F$^-$ dissolved in a solution into an electrical potential. The voltage is dependent on the logarithm of the ionic activity, according to the Nernst equation. However, in this manuscript the F$^-$ are being transported by a cationic quaternary ammonium-based gradient, with electrostatic interactions between the cationic quaternary
ammonium group and the fluoride ion being the primary driving force for the F⁻ transport. The activity of the F⁻ is being reduced by the ionic interactions, and thus the change in F⁻ activity with hydrogel functionalization needed to be ascertained. To ascertain the F⁻ activity in presence of quaternary ammonium groups, the hydrogel was functionalized with quaternary ammonium groups according to the following procedure and the F⁻ activity in this hydrogel was compared the activity of F⁻ in a pristine hydrogel.

A 100 µm-thick PAAm hydrogel on SU-8 layer was synthesized following the procedure in SI Section 2.3. The quaternary ammonium functionalized PAAm hydrogel were fabricated by first forming a partial carboxylate group in the PAAm film through hydrolysis (hydrolysis time = 6h) of the amide groups on the PAAm following the procedure in SI Section 2.4. The final gels were then dipped into 7.0 buffer solution for 2 h and washed with Millipore water several times.

**Figure S12.** Activity of fluoride ion in presence of quaternary ammonium group. (a) Schematic diagram of pristine PAAm and quaternary ammonium functionalized PAAm hydrogels. (b) Plot of given dosing concentration of fluoride ion in gels versus measured concentration of the respective fluoride ion by electrochemical sensors. Dotted line shows dosing concentration of the fluoride ion for better comparison. The electrochemical sensor limit of detection is shown by the grayed-out region. Errors in the electrode potential were calculated from three identical measurements.

A control dosing of fluoride ion solution was applied on two different hydrogels (pristine PAAm and
tertiary ammonium functionalized PAAm hydrogel) using atomizer from a 10 mM NaF aqueous solution (shown in Figure S12a). Surfaces of the electrodes placed the top surface of the gel (Figure S12a). The experiment was carried out at 100% RH. Figure S12b shows a plot of dosing concentration versus measured concentration using fluoride meter.

The activity of F⁻ in presence of ammonium ion is defined as

\[ a = \gamma \times \frac{c_i}{c^0} \]

(eq S3),

Where \( \gamma \) is the relative activity coefficient, a dimensionless quantity which relates the activity to a measured F⁻ concentration \( c_i \) in a pristine gel and dosing concentration \( c^0 \). It was observed that due to the electrostatic interactions between fluoride ion and tertiary ammonium ion, the relative activity coefficient in the ammonium functionalized gel was found to be 0.16 ±0.02.

8.3 Concentration and detection of F⁻ using a radially symmetric gradient

To evaluate the fluoride ion concentration (an important property for many detection schemes) by a radially symmetric neutral-to-cationic quaternary ammonium PAAm hydrogels with a thickness of 100 µm were synthesized following the procedure in SI Section 2 and the excess Cl⁻ was removed following the procedure mentioned in the previous section. The gels were dosed with different concentrations of F⁻ using an aqueous NaF solution sprayed onto the film surface using an atomizer. The gels were left in a 100% RH chamber for 15 minutes to allow F⁻ to diffuse to the center of the gradient (use of a 100% RH chamber ensured the hydrogel did not dry out). After that the activities of F⁻ were measured at two different locations (center and outside of the gradient) of the gels (Figure S13a,b). It was seen that the electrode potential is almost 30 mV different between two locations shown in Figure S13c. After converting the electrode potential into concentration of F⁻ and correcting the offsets value (due to the electrostatic interaction between F⁻ and the ammonium group), we found a 30-fold increase in concentration of fluoride ion at the center of the gradient shown in Figure S13d with respect to the dosing concentration.
Figure S13. Fluoride ion concentration and detection using electrochemical sensors. (a,b) Schematic diagram of experimental setup to monitor fluoride ion activities. The gel contains a neutral-to-cationic radially symmetric gradient to concentrate fluoride ion. Fluoride ion activities were measured at two different regions of the gel – (a) at the center of the gradient and (b) outside of the gradient. (c) The gels were dosed with NaF at difference concentration and electrode potentials were measured by fluoride meter. (d) The electrode potentials were converted to concentration of fluoride ion. Activity coefficient was used to calibrate actual concentration of ion in gradient gel. The dotted line represents the dosing concentration. The electrochemical sensor limit of detection is shown by the grayed-out region. Errors in the electrode potential were calculated from three identical measurements.

8.4 Sarin-simulant hydrolyses and the activities of fluoride ion

A 100 µm-thick PAAm hydrogel on SU-8 layer was synthesized following the procedure in SI Section 2.3. The quaternary ammonium functionalized PAAm hydrogel was fabricated by first forming a partial carboxylate group in the PAAm film through hydrolysis (hydrolysis time = 6h) of the amide groups on the PAAm following the procedure in SI Section 2.4. The final gels were then dipped into 7.0 buffer solution for 2 h and washed with Millipore water several times. To remove free Cl⁻ from the gel, the film was washed with 3 mM AgNO₃ and then Millipore water. It is to be noted that AgCl is insoluble in water, therefore residual Cl⁻ in the gel (if any) is precipitated. However, AgF
is highly soluble in water, therefore if Ag⁺ is present in the gel after this procedure, F⁻ could still diffuse throughout the gel. After that a thin layer of enzyme containing gel was fabricated on pristine and functionalized hydrogel respectively following the procedure in SI Section 5.

Figure S14. (a) Hydrolysis of sarin simulant, DFP, in the presence of immobilized DFPase in the PAAm hydrogel. (b) Schematic diagram of a 5±2 µm-thick layer of enzyme imbedded hydrogel on a pristine PAAm and a quaternary ammonium functionalized PAAm respectively. Enzyme catalyzes DFP to produce fluoride ion, which then interact with the quaternary ammonium ion (c) Plot of dosing concentration of fluoride ion in gels versus measured concentration of the fluoride ion by electrochemical sensor. The dashed line shows the dosing concentration. The electrochemical sensor limit of detection is shown by the grayed-out region. Errors in the electrode potential were calculated from three identical measurements.

9 Sensor evaluation relative to AEGL level 1 for airborne sarin

To categorize the risk of chemical agents to the general population, the United States Environmental Protection Agency (US EPA) published Acute Exposure Level Guidelines (AEGLs) as guidance in dealing with releases of chemicals into the air. Of the AEGLs, the AEGL Level 1 (AEGL-1) is the mildest effect category. The individual exposed to AEGL-1 could experience noticeable eye discomfort, irritation, or non-sensory effects. These symptoms are reversible upon termination of
exposure. AEGL-1 for sarin is $6.9 \times 10^{-9}$ mg/cm$^3$. To evaluate our sensor, we assume the sensor is exposed to a flowing stream of AEGL-1 sarin for 10 min. Table S1 shows the effect and our assumptions if a 100 µm-thick gel with 4 cm$^2$ surface area is exposed to AEGL-1 sarin. We assume the sarin molecules adsorb into the gel and are hydrolyzed by DFPase yielding F$^-$, which is then concentrated by a neutral-to-cationic radially symmetric gradient. If the concentration enhancement factor is 30-fold, the F$^-$ generated by 55.2 ng of sarin will be concentrated in a volume of $7.0 \times 10^{-4}$ cm$^3$, resulting in a F$^-$ concentration of $2.9 \times 10^{-4}$ M (Table S2). Note, that for a 4 cm$^2$ substrate and a 0.07 cm$^2$ area of the center of the gradient, the theoretical maximum concentration enhancement is 57-fold. The F$^-$ detection limit of the electrochemical sensor demonstrated here in an unfunctionalized hydrogel is $1.0 \times 10^{-4}$ M. Due to electrostatic interactions between F$^-$ and the tertiary ammonium groups appended on the hydrogel, with the current system, the F$^-$ detection limit of the electrochemical sensor is $6.0 \times 10^{-4}$ M, just slightly above AEGL-1 for sarin.

**Table S1.** Parameters and assumptions for AEGL-1 exposure of sarin to a gel.

| Parameters and Assumptions                  | Value                         |
|--------------------------------------------|-------------------------------|
| Concentration of the exposed sarin         | $6.9 \times 10^{-9}$ mg/cm$^3$|
| Exposure time                              | 10 min                        |
| Exposure flow rate                         | 200 cm$^3$/min                |
| Sticking probability on gel                | 100%                          |
| Thickness of the gel                        | 100 µm                        |
| Area of the gel surface                    | $2 \times 2$ cm$^2$          |
| Area of the gradient center                | 0.07 cm$^2$                   |
| Concentration enhancement                  | 30-fold                       |
Table S2. Calculations of gradient directed concentration of sarin after exposure of AEGL-1.

| Calculated Parameters                                      | Value       |
|------------------------------------------------------------|-------------|
| Amount of sarin adsorbed in 4 cm² gel                      | 55.2 ng     |
| Concentration of sarin molecules in gel                    | 9.8 × 10⁻⁶ M|
| Concentration of F⁻ at the center of the gradient          | 2.9 × 10⁻⁴ M|
| Equivalent amount of sarin at the center of the gradient   | 29.2 ng     |

10 References

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