

Electronic Supplementary Information (ESI)

**Development of a lateral flow test for rapid pyrethroid detection
using antibody-gated indicator-releasing hybrid materials**

Elena Costa,^a Estela Climent,^a Sandra Ast,^b Michael G. Weller,^a John Canning^c and Knut Rurack^{a,*}

^a*Bundesanstalt für Materialforschung und –prüfung (BAM), Richard-Willstätter-Str. 11, D-12489 Berlin, Germany*

^b*Australian Sensing and Identification Systems Pty Ltd, Sydney, NSW 2000 Australia*

^c*Interdisciplinary Photonics Laboratories, School of Electrical & Data Engineering, University of Technology Sydney, Sydney, NSW 2007 Australia*

Table of contents

S1. Reagents and general techniques	S2
S2. Synthetic procedures.....	S2
S3. Materials characterisation.....	S4
S4. Studies in suspension	S6
S5. Strip preparation and assembly.....	S6
S6. Analytical LFA studies	S8
S7. Uncertainty budget.....	S9
S8. References	S14

S1. Reagents and general techniques

Chemicals and solvents were purchased from Sigma-Aldrich, ACBR, Merck, and J.T. Baker in the highest quality available. The monoclonal anti-pyrethroid antibody was purchased from Eurofins Abraxis (clone number PY-I). Buffers were prepared with ultrapure reagent water, which was obtained by running demineralized water (by ion exchange) through a Milli-Q® ultrapure water purification system (Millipore Synthesis A10). Phosphate-buffered saline (PBS 80mM; 70 mmol L⁻³ Na₂HPO₄, 10 mmol L⁻³ NaH₂PO₄, 145 mmol L⁻³ NaCl, pH 7.5), was used for all procedures involving antibodies. Controlled release experiments were performed using a solution of PBS 80 mM containing 10% of iPrOH. Glass fibre membranes (GF/C grade)¹ were purchased from Whatman and the sample pad membrane employed (Ahlstrom Grade 142 microfibre glass) was provided by Kenosha. High-efficiency Sampling Swipes FS-03-E Nomex® were purchased from FLIR, whereas Teflon® PTFE-coated fiberglass Swipes were provided from High-tech-flon (Art. Nr. REF.55 455 25). UV/vis and fluorescence spectroscopy, elemental analysis, transmission and scanning transmission electron microscopy (TEM and STEM), N₂ adsorption-desorption, mass spectrometry, and NMR techniques were employed to characterize the synthesized compounds and materials and to test their behaviour towards phenoxybenzoic acid and permethrin. UV/vis spectra were measured with a Specord 210plus from Analytik Jena. Fluorescence measurements were carried out on a Fluoromax4 from HORIBA Scientific. Elemental analyses were performed on a Euro EA-Elementaranalysator. Thermogravimetric analyses were carried out on a STA7200 (Hitachi High-Tech Analytical Science) thermobalance, using in a first step a nitrogen atmosphere (80 mL min⁻¹) with a heating program consisting of a ramp of 10 °C min⁻¹ from 25°C to 600°C and in a second step an oxidant atmosphere (air, 80 mL min⁻¹) from 600°C until 1000°C with a heating program consisting of a ramp of 10 °C min⁻¹. TEM images were obtained with a. Tecnai G2 20 Twin transmission electron microscope. N₂ adsorption/desorption isotherms were recorded with a Micromeritics ASAP2010 automated sorption analyser. Mass spectra were measured with an Orbitrap Exactive ESI-HRMS and Agilent 1290 UHPLC with C18 column using Water/ACN (without FA) as solvent system connected to a Sciex 6500 TripleTOF (QTOF in positive ion mode). ¹H and ¹³C nuclear magnetic resonance (NMR) spectra were acquired with Bruker AV-500 and AV-600 spectrometers using residual protonated solvents as internal standards (¹H: δ[CDCl₃] = 7.24 ppm and ¹³C: δ[CDCl₃] = 77.23 ppm). A lateral flow reagent dispenser from ClaremontBio® was used to deposit the **APTES-MCM** material on zone B of the strips. 3D-box was printed with black PLA using an Ultimaker 3 printer. Lateral flow cassettes were purchased from Cangzhou Xingyuan Plastic Products Co. LEDs, and optical filters were purchased from Thorlabs. Photographs were taken with a Samsung Galaxy S7 and values retrieved from images via the integrated density, i.e., the product of mean grey value \bar{G} , $\bar{G} = (\text{red} + \text{green} + \text{blue})/3$, and the selected area a (in square pixels). The smartphone camera was set with a shutter speed of 1/100 s, a white balance at 4000 K, a macro focus, and an ISO value of 100. These settings yielded the best results as higher ISO values or longer exposure times resulted in distinctly more noise.

S2. Synthetic procedures

Synthesis of SBA-15 particles: SBA-15 of rod-like shape was synthesized with a poly (ethylene oxide)–poly (propylene oxide)–poly (ethylene oxide) triblock copolymer (P123, Aldrich, M_n=5800) as a structure-directing agent and TEOS as a silica source following reported procedures.² 4.0 g (0.69 mmol) P123 were dissolved in 120 mL water and 19.41 mL HCl conc. and then stirred at 50 °C for 1 h to dissolve the polymer. Then, 9.15 mL TEOS (41 mmol) were added dropwise into the homogeneous solution while stirring at 35 °C for 24 h. The obtained gel was aged at 115 °C in a polypropylene flask under static conditions for another 24 h. The

white solid obtained was centrifuged, washed 5-times with distilled water and air-dried at 115 °C in a vacuum for 48 h.

Synthesis of phenoxybenzoic acid (PBA) silane derivative I. The reaction was performed in inert atmosphere flowing Ar during the overall two steps synthetic procedure. Two anhydrous THF solutions (1 mL each) of *N*-hydroxysuccinimide (NHS, 138.1 mg; 1.2 mmol) and *N,N'*-dicyclohexylcarbodiimide (DCC, 247.2 mg; 1.2 mmol) were prepared. Both dissolved reagents were added to a PBA (214.3 mg, 1 mmol) solution in 1.5 mL anhydrous THF. The mixture was stirred, cooling the external environment with an ice bath for 2 h. In a second step, 3-(ethoxydimethylsilyl) propylamine (170 µL, 0.8 mmol) was dissolved in 1 mL of dry THF and added to the reaction mixture keeping it under stirring for 24 h at 5°C. The white solid formed (dicyclohexylurea, DCU) was removed, filtering the crude solution with cotton and resuspending the product in the residual supernatant in 4.5 mL of THF (0.7 mmol, 250 mg, considering 70% yield). The formation of the hapten derivative was confirmed by UPLC-MS and NMR. Exact mass $C_{20}H_{28}NO_3Si$ $[M+H]^+$ 358.1838; found 358.1866. 1H -NMR ($CDCl_3$, 400 MHz) δ 0.11 (s 6H $-(CH_3)_2$ Si), 0.65 (t, 2H, $-CH_2Si$), 1.17 (t, 3H, $-OCH_2CH_3$), 1.65 (tt, 2H, CH_2CH_2Si), 2.82 (q, 2H, $-OCH_2CH_3$), 3.43 (t, 2H, $-NHCH_2CH_2Si$), 7-7.9 (9H). ^{13}C -NMR ($CDCl_3$, 400 MHz) δ 0.1 (1C, $-OCH_2CH_3$), 14.2 (1C, $-CH_2Si$), 18.3 (1C, $-OCH_2CH_3$), 25.8 (1C, $-NHCH_2CH_2Si$), 43.1 (1C, $-NHCH_2CH_2$), 58.1 (1C, $-OCH_2CH_3$), 117 (1C, *Car*), 119 (3C, *Car*), 121 (1C, *Car*), 123 (1C, *Car*), 129 (3C, *Car*), 136 (1C, *Car*), 156 (1C, *Car*), 157 (1C, *Car*), 167 (1C, $NHCO$).

Synthesis of S1. 0.4 mmol PEG silane (3-[methoxy(polyethyleneoxy)propyl]) trimethoxysilane) were added to 200 mg SBA-15 suspended in 6 mL EtOH and left stirring overnight at 40 °C. Afterward, the material was washed with EtOH, centrifuged 3-times, and dried in a vacuum for 5 h. The template of the carriers was removed by extraction after the PEG coating. For this purpose, a suspension of 200 g of the modified material was suspended in 25 mL of EtOH abs. containing 0.2 mL of HCl 37% and stirred at 100 °C for 17 h. Finally, the material was washed with water and EtOH and dried at 50 °C overnight in a vacuum oven. In a second step, to load the dye into the pores, 100 mg of the carriers were suspended in 10 mL of acetonitrile (MeCN) solution containing sulforhodamine G (800 µmol dye /g solid). The solution was kept under stirring overnight at 45 °C. In the last step, to introduce the hapten moieties to the material, 100 µL (80 µmol) of PBA silane hapten derivative in THF solution were added, and the solution kept stirring for 5.30 h. The suspension was centrifuged (5 min, 9000 rpm) and washed 3-times with MeCN. The obtained material **S1** was dried 1 h in a vacuum and stored in the refrigerator.

Synthesis of S1-AB. For the final material, 5 µL of monoclonal anti-pyrethroid antibody (2.85 mg mL⁻¹) were added to 1 mg of **S1** suspended in 450 µL PBS solution (80 mM, pH 7.4). After 30 min, 50 µL of bovine serum albumin (BSA) solution (20 mg/400µL PBS 80 mM) were added, and the suspension was kept under rotation for an additional 1.5 h. Finally, the capped material **S1-AB** was centrifuged and washed 3-times with PBS 80 mM. The material was suspended in 1 mg mL⁻¹ PBS solution and stored in a refrigerator.

Synthesis of mesoporous silica MCM-41 nanoparticles. MCM-41 was prepared according to the literature.³ *N*-Cetyltrimethylammonium bromide (CTAB, 1.00 g, 2.74 mmol) was first dissolved (slow stirring at 30–35°C) in deionized water (480 mL). Then, 3.5 mL of NaOH 2M

were added to the CTAB solution, followed by adjusting the temperature to 80 °C. The solution was vigorously stirred, and tetraethylorthosilicate (TEOS, 5.00 mL; 25.7 mmol) was added dropwise to the surfactant solution. During the addition of TEOS, the formation of a white precipitate was observed. The mixture was stirred for 2 h. Finally, the solid product was centrifuged (20 min at 8500 rpm), washed with deionized water and ethanol (mixture ca. 2:1 H₂O:EtOH each time) until neutral pH, and dried at 60 °C overnight. In a second step, the as-synthesized solid was calcinated at a heating rate of 2 °C/min until 550 °C and left at 550 °C for 5 h using an oxidizing atmosphere to remove the template phase.

Synthesis of APTES-MCM. 100 mg of MCM-41 were dissolved in MeCN and stirred after the addition of 117 µL APTES (5 mmol g⁻¹) for 5.30 h. The obtained **APTES-MCM** was washed 2-times with 10 mL MeCN, centrifuged at 9000 rpm for 5 min and finally dried for 24 h in a vacuum.

S3. Materials characterisation

The porous structure of the prepared materials was confirmed by nitrogen adsorption-desorption studies, transmission electron microscopy (TEM), and scanning transmission electron microscopy (STEM).

Nitrogen adsorption-desorption studies of the SBA-15 material are shown in Figure S1. As can be seen, the isotherms showed a type-IV isotherm behaviour, typical for these types of mesoporous materials and containing a hysteresis loop in the region of 0.4–0.9 P/P₀, indicative of gas retention inside the pores and slower desorption through the more complicated morphology of the cavities full of vesicles and worm-like pores. Values of specific surface areas (in m² g⁻¹) were estimated using the Brunauer-Emmett-Teller (BET) theory as an evaluation method in a 0–1 range of (P/P₀). Pore size distribution curves were calculated using the desorption branch of the isotherms and the Barrett-Joyner-Halenda (BJH) method. The average pore diameter was extracted from the maximum of the peak in the distribution curves. SBA-15 material showed a specific surface area of 696 m² g⁻¹ with a narrow pore size distribution, a pore volume of 1.01 cm³ g⁻¹, and a pore diameter of 9.0 nm. For the materials **S1** and **S1-AB**, it was not possible to carry out any N₂ adsorption-desorption studies due to the small amount of material prepared. However, STEM and TEM images depicted in Figure S2 showed the typical porosity of the SBA-15 phase in all materials, indicating that the loading and functionalization of the material do not affect the structure of the inorganic scaffold.

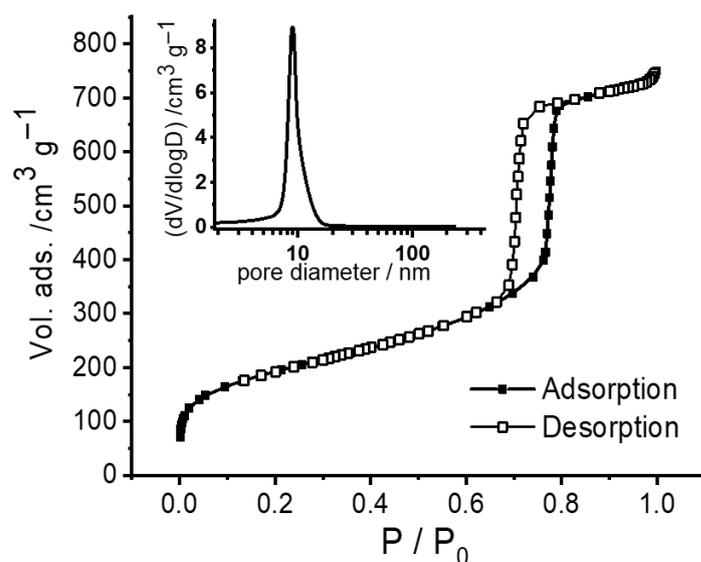


Fig. S1 Adsorption-desorption isotherms for the SBA-15 material prepared. Inset: Pore-size distribution of SBA-15.

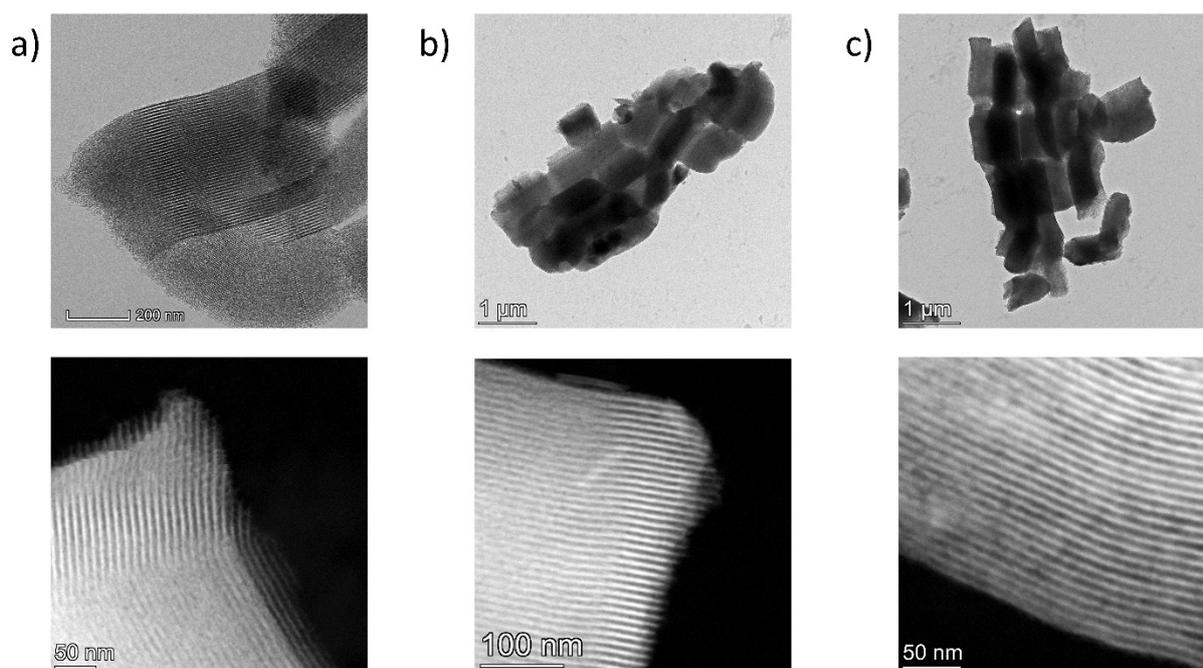


Fig. S2. TEM (Top) and STEM (bottom) images under the microscope for the materials a) SBA-15 b) S1 and c) S1-AB

To assess the amount of organic material loaded into the cavities and grafted onto the surface of the particles and their pores, elemental and thermogravimetric analyses were performed. Once the amount of C, H, S, and N in percentage was known, it was possible to calculate the amount of PEG, SRG dye a hapten derivative present on or inside the functionalized carriers. The obtained result was compared with TGA analysis, whose isothermal plots showed a steep weight loss between 200–600 °C, which was attributed to the decomposition of organic matter.

Tab. S1 Amounts of PEG, dye and hapten molecules on **S1** material (in mmol g⁻¹ and in % weight loss) estimated from elemental analysis (EA) and thermogravimetric analysis (TGA).

	<i>Sulphorhodamine G</i>	<i>Hapten</i>	<i>PEG silane</i>
S1 (mmol i / g solid)	0.27	0.03	0.35
S1-AB (mmol i / g solid)	0.14	0.03	0.35

S4. Studies in suspension

Release kinetics. PBA was used as a model analyte for type-I pyrethroids. 400 μL of 1 mg mL⁻¹ suspensions of **S1-AB** in PBS buffer (80 mM) were divided into two fractions. 200 μL of the suspension were diluted with 900 μL PBS and 120 μL iso-propanol (iPrOH), while another 200 μL were diluted with 900 μL PBS and 120 μL of a solution of 5 ppm PBA in iPrOH, resulting in a final sample composition containing 500 ppb PBA and 10% of iPrOH. Fractions of 150 μL were centrifuged in both cases directly at the beginning and after certain time intervals. 120 μL of the corresponding supernatants were collected, and their fluorescence registered at 550 nm ($\lambda_{\text{exc}} = 531$ nm). The increase in fluorescence signal with respect to the initial fluorescence was registered over time (Figure 1a).

Concentration dependence studies. 333 μL of 1mg mL⁻¹ suspension of **S1-AB** in PBS buffer (80 mM) were diluted with 2200 μL PBS. The suspension was divided into fractions of 140 μL , and 16 μL of permethrin solutions in iPrOH containing different concentrations were added to the corresponding fractions. After 5 min, the fractions were centrifuged. 120 μL of the corresponding supernatants were collected, and their fluorescence registered at 550 nm ($\lambda_{\text{exc}} = 531$ nm). The increase in fluorescence signal with respect to the initial fluorescence was registered over time (Figure 1b).

System sensitivity via titration. Following a similar procedure, dye delivery from **S1-AB** was studied as a function of permethrin concentration. 333 μg **S1-AB** were suspended in 2.2 mL PBS, and the suspensions were divided into fractions of 140 μL . Afterwards, 16 μL of iPrOH solutions containing various amounts of permethrin were added to the corresponding fractions. After incubating for 5 min at room temperature, the suspensions were centrifuged, and the supernatants were collected for fluorescence measurements.

S5. Strip preparation and assembly

Conjugate pad synthesis. The conjugate pad was chemically modified with PEG moieties in a way that was similar to a procedure published by us recently.⁴ 1.7 mL H₂O (Milli-Q®), 3.7 mL EtOH abs., 900 μL TEOS, 600 μL PEG (3-[methoxy(polyethyleneoxy)propyl]) trimethoxysilane) and 90 μL NH₃ (32 % sol.) were added (respecting the order reported) to ca. 30 glass fibre strips of 5 × 0.5 cm, located in an 8 mL vial, and left under rotation overnight. On the next day, the

strips were washed 3-times with EtOH abs. and dried in a vacuum oven for 3 h. Finally, 10 μL of a solution of **S1-AB** in PBS (1 mg mL^{-1}) were deposited on the conjugate pad.

Test strip assembly. The lateral flow membranes were prepared by the assembly of a glass fibre membrane ($4 \times 0.5 \text{ cm}$), a conjugate pad consisting of a glass fibre membrane modified with PEG moieties and containing the sensing material ($0.5 \times 0.5 \text{ cm}$) and a sample pad out of glass fibre for the introduction of the liquid sample ($1 \times 0.5 \text{ cm}$), using Scotch tape for stabilisation. The hierarchical architecture is depicted in Figure S3. The conjugate pad half-covers the base membrane, protruding on end by ca. 0.25 cm. The sample pad fully covers the conjugate pad and protrudes by another 0.5 cm, see Figure S3. 2.5 μL of a suspension of **S1-AB** in PBS (1 mg mL^{-1}) were deposited on the conjugate pad by pipetting. **APTES-MCM** was deposited in a thin line at a distance of 2 cm from the conjugate pad with a lateral flow reagent dispenser to create a focussing line for the dye to facilitate optical detection. For that purpose, a suspension of 20 mg mL^{-1} **APTES-MCM** in phosphate buffer (80 mM) was dispensed onto a $30 \times 4 \text{ cm}$ glass fibre paper, using a syringe pump (with a flow of 0.3 mL min^{-1}) and a speed of 5 cm s^{-1} , remarking the line 10-times. Afterwards, the paper was dried for 1 h at room temperature in a vacuum and cut into the $4 \times 0.5 \text{ cm}$ pieces used as supporting strips.

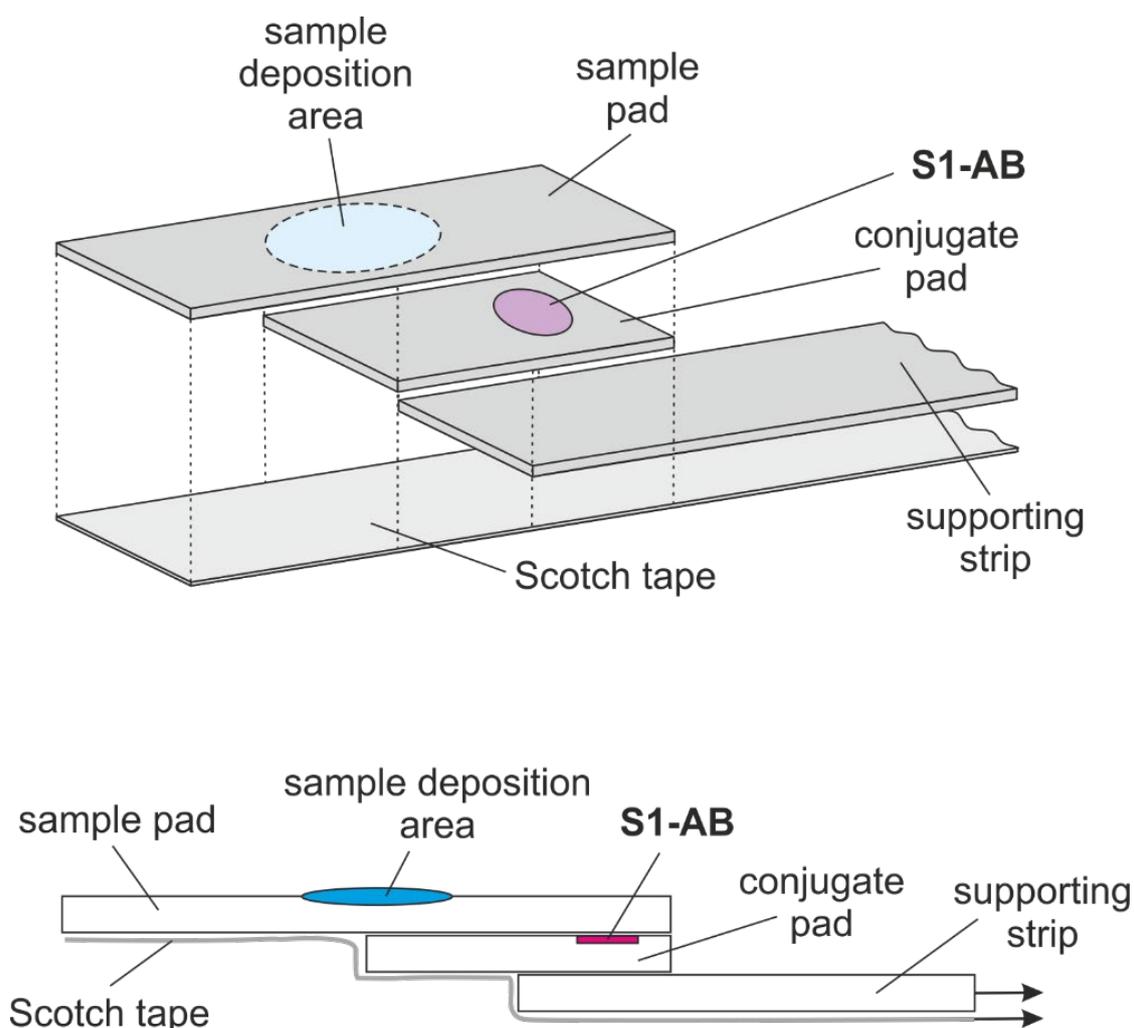


Fig. S3. Architecture of the test strip: assembly of layers at the sample application and interaction side of the strip in blow-up (top) and side (bottom) views.

S6. Analytical LFA studies

Analytical LFA experiments. When the LFA experiments were carried out with the same buffer as in the studies performed in vials/cuvettes, a high fluorescence background was observed, resulting in a high uncertainty of the measurements. This auto-fluorescence of the membrane was presumably caused by the organic co-solvent interacting with the Scotch tape. The amount of organic solvent was thus reduced to 1% of iPrOH, entailing the desired reduction in background fluorescence while not compromising SRG signal intensities and travel speed on the strip. Typically, blank and spiked samples (120 μ L, in 80 mM PBS and containing 1% of iPrOH) were thus analysed for the presence and absence of permethrin. Images of the smartphone setup and a strip are shown in Figure S4. Reference image analysis was done on a computer with ImageJ.⁵

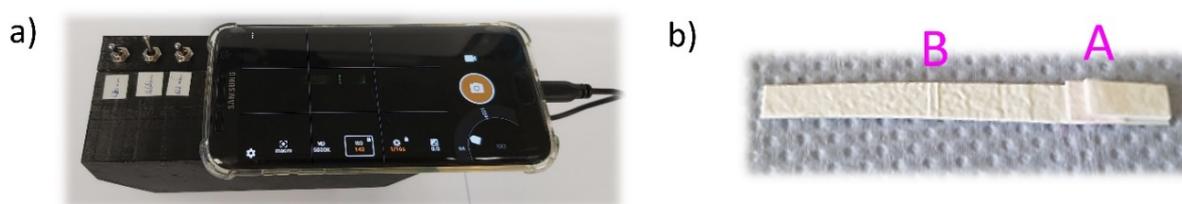


Fig. S4. (a) 3D-printed smartphone case containing a green LED as the excitation source (522 nm), a short-pass filter (532 nm) and a long-pass filter (550 nm). (b) Image of the sensing membrane prepared to contain the conjugate pad (A) and the **APTES-MCM**-containing collection line B.

Swipe sampling experiments. Two commercial materials, Nomex[®] and Teflon[™], often used for swipe sampling were assessed with respect to their efficiency to collect pyrethroid residues from conventional glass surfaces. For that purpose, 1 mL of an iPrOH solution containing a mixture of permethrin and phenothrin (0.1% w/v) was sprayed on a glass beaker surface. In parallel, 1 mL of iPrOH was sprayed on another beaker. A third beaker containing no solution sprayed on the surface was used as a reference. Swipe strips of 2.5 \times 2 cm of Nomex[®] and Teflon[™] materials were prepared, and the surfaces were wiped once with the materials. Remobilization was done with a commercial disposable eye drop capsule, rinsing the swipe strip with 300 μ L of a PBS solution containing 1% of iPrOH; the solutions were collected in a cap of a conventional glass vial. Aliquots of the solutions were transferred to the lateral flow cassettes containing the sensing membranes with the same capsule (Figure S5b). After 2 min of development, the case was opened, and the strip was introduced into the smartphone holder. The fluorescence in zone B was registered following the same procedure as described in the main manuscript. Figure S5a shows the integrated densities in the absence and presence of the mixture of the pyrethroids on the glass surface, referenced to the non-sprayed glass surface. As can be seen, the Teflon[™] swipe strips, which performed much better than the Nomex[®] strips, were able to extract the pyrethroids from the glass surface efficiently down to the ppb concentration range.

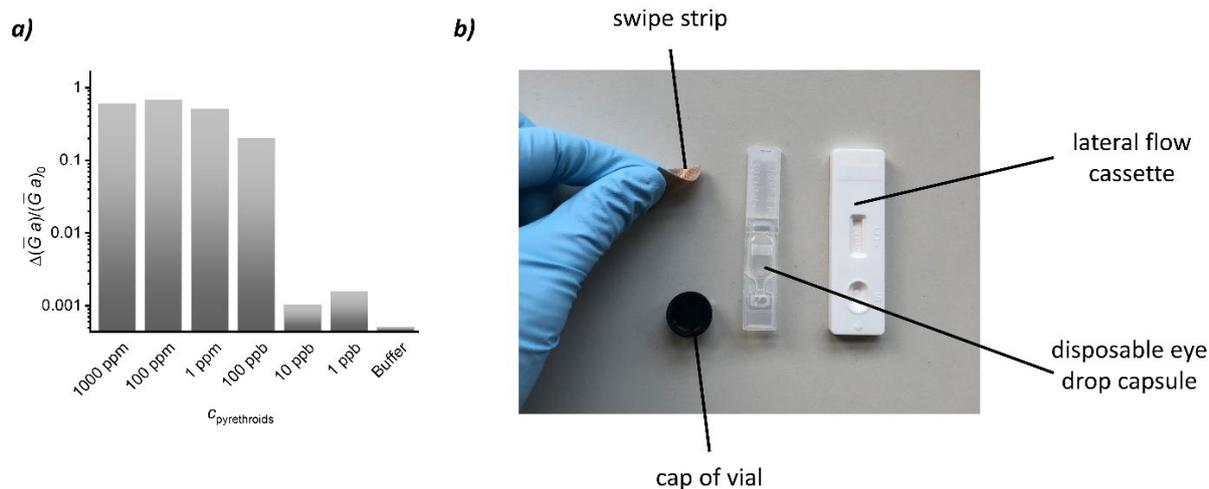


Fig. S5. (a) Integrated density registered for the images taken with the 3D-printed holder and the smartphone after wiping glass surfaces sprayed with different concentrations of pyrethroids, referenced against the non-sprayed glass. (b) Image of the parts used in this simple simulated swipe sampling scenario.

S7. Uncertainty budget

Relative measurement uncertainties and limits of detection (LOD). Because of the multiplicative and quotient forms of the respective equations and because correlations between the quantities are assumed to be negligible, the summation of the squares of the relative uncertainties was performed.^{6,7}

1.- Conventional assay:

1.1 Preparation of capped material and stock solutions:

- a) Weighing of ca. 1 mg of **S1** (balance Mettler Toledo 1 ± 0.01 mg); $u_{rel}^w = 1\%$
- b) Dissolving in 450 μ L PBS (Eppendorf Reference pipette ± 0.0006 mL); $u_{rel}^d = 0.6\%$
- c) Weighing ca. 10 mg BSA (balance Mettler Toledo 1 ± 0.01 mg); $u_{rel}^{w2} = 0.1\%$
- d) Dissolving in 200 μ L PBS (Eppendorf Reference pipette ± 0.0006 mL) and taking 50 μ L of the solution; $u_{rel}^{d2} = 1.2\%$
- e) Washing and dividing of the suspension to obtain suspensions of **S1-AB** of 1 mg mL⁻¹ (Eppendorf Reference pipette ± 0.0006 mL); $u_{rel}^{d3} = 0.6\%$.
- f) Weighing of ca. 1 mg of 3-PBA or permethrin (balance Mettler Toledo: ± 0.01 mg); $u_{rel}^{ws} = 1\%$
- g) Dissolving in 0.1 mL iPrOH (Eppendorf Reference pipette ± 0.0006 mL) for obtaining 3-PBA or permethrin of 10 g L⁻¹ in iPrOH; $u_{rel}^{ds} = 0.6\%$
- h) Diluting of stock solutions in iPrOH for obtaining standard permethrin, phenothrin or etofenprox solutions of 100 mg L⁻¹ and 1 mg L⁻¹ in iPrOH (Eppendorf Reference pipette ± 0.001 mL); $u_{rel}^{ds2} = 1\%$

i) Preparing permethrin, phenothrin or etofenprox standards in iPrOH (100 μL) from 1 mg L^{-1} solution of standards permethrin, phenothrin or etofenprox 1 mg L^{-1} , by dilution in iPrOH: Successive dilution of the mother solution: 100 μL in 100 μL iPrOH; $n \times u_{rel}^{ds3} = n \times (1) \%$; $n = 1$; 2 \times , $n = 2$; 4 \times , $n = 3$; 8 \times , $n = 4$; 16, $n = 5$; 32 \times , $n = 6$; 64 \times , $n = 7$; 128 \times , $n = 8$; 256 \times , $n = 9$; 512 \times , $n = 10$; 1024 \times , $n = 11$; 2048 \times , $n = 12$; 4096 \times .

1.2 Assay execution and preparation of measurement solutions:

a) Mixing capped material suspension **S1-AB** (0.33 mg mL^{-1}) with 2.2 mL PBS (Eppendorf Reference pipette ± 0.00625 mL); $u_{rel}^{d4} = 1.2 \%$

b) Fractionation of the suspension (0.14 mL) (Eppendorf Reference pipette ± 0.0006 mL); $u_{rel}^{d5} = 0.6 \%$

c) Addition of 16 μL analyte soln. at c_n in iPrOH (Eppendorf Reference pipette ± 0.0003 mL); $u_{rel}^{d6} = 2 \%$

d) Transfer of the fractions into a 10 mm optical path length quartz cell. Since no dilution step is involved, only contribution from cell length (± 0.01 mm); $u_{rel}^L = 0.1 \%$

1.3 Fluorescence measurements:

a) The relative uncertainty of the emission spectrum across the respective wavelength range: $u_{rel}^f \leq 5 \%$

b) For the fluorescence intensities at λ_i , the maximum possible error amounts to; $u_{rel}^{f2} \leq 0.05 \%$

1.4 Experimental standard deviation for replicate measurements: $u_{rel}^s \leq 5 \%$

1.5 Relative uncertainty: $u_{rel}^u \leq 6.1 \%$

$$\text{Relative uncertainty : } u_{rel}^2 = u_{rel}^w{}^2 + u_{rel}^d{}^2 + u_{rel}^{w2}{}^2 + u_{rel}^{d2}{}^2 + u_{rel}^{d3}{}^2 + u_{rel}^{ws}{}^2 + u_{rel}^{ds}{}^2 + u_{rel}^{ds2}{}^2 + n * u_{rel}^{ds3}{}^2 + u_{rel}^{d4}{}^2 + u_{rel}^{d5}{}^2 + u_{rel}^{d6}{}^2 + u_{rel}^L{}^2 + u_{rel}^f{}^2 + u_{rel}^{f2}{}^2 + u_{rel}^s{}^2 + u_{rel}^u{}^2$$

Tab. S2. Calculation of relative errors in a conventional assay in suspension

Solutions	Relative error of the measurement	%
Standard	$u_{rel}^2 = u_{rel}^w{}^2 + u_{rel}^d{}^2 + u_{rel}^{w2}{}^2 + u_{rel}^{d2}{}^2 + u_{rel}^{d3}{}^2 + u_{rel}^{ws}{}^2 + u_{rel}^{ds}{}^2 + u_{rel}^{ds2}{}^2$ $+ 0 * u_{rel}^{ds3}{}^2 + u_{rel}^{d4}{}^2 + u_{rel}^{d5}{}^2 + u_{rel}^{d6}{}^2 + u_{rel}^L{}^2 + u_{rel}^f{}^2$ $+ u_{rel}^{f2}{}^2 + u_{rel}^s{}^2 + u_{rel}^u{}^2$	5.1
8x	$u_{rel}^2 = u_{rel}^w{}^2 + u_{rel}^d{}^2 + u_{rel}^{w2}{}^2 + u_{rel}^{d2}{}^2 + u_{rel}^{d3}{}^2 + u_{rel}^{ws}{}^2 + u_{rel}^{ds}{}^2 + u_{rel}^{ds2}{}^2$ $+ 3 * u_{rel}^{ds3}{}^2 + u_{rel}^{d4}{}^2 + u_{rel}^{d5}{}^2 + u_{rel}^{d6}{}^2 + u_{rel}^L{}^2 + u_{rel}^f{}^2$ $+ u_{rel}^{f2}{}^2 + u_{rel}^s{}^2 + u_{rel}^u{}^2$	5.3
64x	$u_{rel}^2 = u_{rel}^w{}^2 + u_{rel}^d{}^2 + u_{rel}^{w2}{}^2 + u_{rel}^{d2}{}^2 + u_{rel}^{d3}{}^2 + u_{rel}^{ws}{}^2 + u_{rel}^{ds}{}^2 + u_{rel}^{ds2}{}^2$ $+ 6 * u_{rel}^{ds3}{}^2 + u_{rel}^{d4}{}^2 + u_{rel}^{d5}{}^2 + u_{rel}^{d6}{}^2 + u_{rel}^L{}^2 + u_{rel}^f{}^2$ $+ u_{rel}^{f2}{}^2 + u_{rel}^s{}^2 + u_{rel}^u{}^2$	5.6
512x	$u_{rel}^2 = u_{rel}^w{}^2 + u_{rel}^d{}^2 + u_{rel}^{w2}{}^2 + u_{rel}^{d2}{}^2 + u_{rel}^{d3}{}^2 + u_{rel}^{ws}{}^2 + u_{rel}^{ds}{}^2 + u_{rel}^{ds2}{}^2$ $+ 9 * u_{rel}^{ds3}{}^2 + u_{rel}^{d4}{}^2 + u_{rel}^{d5}{}^2 + u_{rel}^{d6}{}^2 + u_{rel}^L{}^2 + u_{rel}^f{}^2$ $+ u_{rel}^{f2}{}^2 + u_{rel}^s{}^2 + u_{rel}^u{}^2$	5.9
4096x	$u_{rel}^2 = u_{rel}^w{}^2 + u_{rel}^d{}^2 + u_{rel}^{w2}{}^2 + u_{rel}^{d2}{}^2 + u_{rel}^{d3}{}^2 + u_{rel}^{ws}{}^2 + u_{rel}^{ds}{}^2 + u_{rel}^{ds2}{}^2$ $+ 12 * u_{rel}^{ds3}{}^2 + u_{rel}^{d4}{}^2 + u_{rel}^{d5}{}^2 + u_{rel}^{d6}{}^2 + u_{rel}^L{}^2 + u_{rel}^f{}^2$ $+ u_{rel}^{f2}{}^2 + u_{rel}^s{}^2 + u_{rel}^u{}^2$	6.1

2 Lateral flow assay

2.1 Preparation of suspensions and stock solutions:

- a) Weighing of ca. 1 mg of **S1** (balance Mettler Toledo 1 ± 0.01 mg); $u_{rel}^w = 1\%$
- b) Dissolving in 450 μ L PBS (Eppendorf Reference pipette ± 0.0006 mL); $u_{rel}^d = 0.6\%$
- c) Weighing ca. 10 mg BSA (balance Mettler Toledo 1 ± 0.01 mg); $u_{rel}^{w2} = 0.1\%$
- d) Dissolving in 200 μ L PBS (Eppendorf Reference pipette ± 0.0006 mL) and taking 50 μ L of solution; $u_{rel}^{d2} = 1.2\%$
- e) Washing and divide the suspension to obtain to obtain suspensions of **S1-AB** of 1 mg mL⁻¹ (Eppendorf Reference pipette ± 0.0006 mL); $u_{rel}^{d3} = 0.6\%$.
- f) Weighing of ca. 1 mg of Permethrin (balance Mettler Toledo: ± 0.01 mg); $u_{rel}^{ws} = 1\%$
- g) Dissolving in 0.1 mL iPrOH (Eppendorf Reference pipette ± 0.0006 mL) for obtaining permethrin standard of 10 g L⁻¹ in iPrOH; $u_{rel}^{ds} = 0.6\%$
- h) Diluting of stock solutions in iPrOH for obtaining standard Permethrin solutions of 100 mg L⁻¹ in PBS : iPrOH (99:1, Eppendorf Reference pipette ± 0.001 mL); $u_{rel}^{ds2} = 3\%$

i) Preparing permethrin standard in PBS containing iPrOH (500 μL) from 100 mg L^{-1} solution of standard permethrin 1 mg L^{-1} , by dilution in PBS: Successive dilution of the mother solution: 100 μL in 100 μL in PBS : iPrOH (99:1); $n \times u_{rel}^{ds3} = n \times (1) \%$; $n = 1$; 2 \times , $n = 2$; 4 \times , $n = 3$; 8 \times , $n = 4$; 16, $n = 5$; 32 \times , $n = 6$; 64 \times , $n = 7$; 128 \times , $n = 8$; 256 \times , $n = 9$; 512 \times , $n = 10$; 1024 \times , $n = 11$; 2048 \times , $n = 12$; 4096 \times .

2.2 Strip preparation:

a) Deposition of 2.5 μL of **S1-AB** suspension (1 mg mL^{-1}) onto the strip (Eppendorf Reference pipette \pm 0.00003 mL); $u_{rel}^{d2} = 1.4 \%$

2.3 Integration of the signal using ImageJ:

a) Relative uncertainty of the fluorescence intensities. $u_{rel}^c \leq 5 \%$

2.4 Experimental standard deviation for replicate measurements: $u_{rel}^{sc} \leq 5 \%$

2.5 Relative uncertainty: $u_{rel}^u \leq 5.3 \%$

$$\text{Relative uncertainty: } u_{rel}^2 = u_{rel}^w{}^2 + u_{rel}^d{}^2 + u_{rel}^{w2}{}^2 + u_{rel}^{d2}{}^2 + u_{rel}^{d3}{}^2 + u_{rel}^{ws}{}^2 + u_{rel}^{ds}{}^2 + u_{rel}^{ds2}{}^2 + n * u_{rel}^{ds3}{}^2 + u_{rel}^{d2}{}^2 + u_{rel}^c{}^2 + u_{rel}^{sc}{}^2$$

Tab. S3. Calculation of relative errors in a lateral flow assay using a camera

Solutions	Relative error of the measurement	%
Standard	$u_{rel}^2 = u_{rel}^w{}^2 + u_{rel}^d{}^2 + u_{rel}^{w2}{}^2 + u_{rel}^{d2}{}^2 + u_{rel}^{d3}{}^2 + u_{rel}^{ws}{}^2 + u_{rel}^{ds}{}^2 + u_{rel}^{ds2}{}^2 + 0 * u_{rel}^{ds3}{}^2 + u_{rel}^{d2}{}^2 + u_{rel}^c{}^2 + u_{rel}^{sc}{}^2$	4.1
8x	$u_{rel}^2 = u_{rel}^w{}^2 + u_{rel}^d{}^2 + u_{rel}^{w2}{}^2 + u_{rel}^{d2}{}^2 + u_{rel}^{d3}{}^2 + u_{rel}^{ws}{}^2 + u_{rel}^{ds}{}^2 + u_{rel}^{ds2}{}^2 + 3 * u_{rel}^{ds3}{}^2 + u_{rel}^{d2}{}^2 + u_{rel}^c{}^2 + u_{rel}^{sc}{}^2$	4.4
64x	$u_{rel}^2 = u_{rel}^w{}^2 + u_{rel}^d{}^2 + u_{rel}^{w2}{}^2 + u_{rel}^{d2}{}^2 + u_{rel}^{d3}{}^2 + u_{rel}^{ws}{}^2 + u_{rel}^{ds}{}^2 + u_{rel}^{ds2}{}^2 + 6 * u_{rel}^{ds3}{}^2 + u_{rel}^{d2}{}^2 + u_{rel}^c{}^2 + u_{rel}^{sc}{}^2$	4.7
512x	$u_{rel}^2 = u_{rel}^w{}^2 + u_{rel}^d{}^2 + u_{rel}^{w2}{}^2 + u_{rel}^{d2}{}^2 + u_{rel}^{d3}{}^2 + u_{rel}^{ws}{}^2 + u_{rel}^{ds}{}^2 + u_{rel}^{ds2}{}^2 + 9 * u_{rel}^{ds3}{}^2 + u_{rel}^{d2}{}^2 + u_{rel}^c{}^2 + u_{rel}^{sc}{}^2$	5.0
4096x	$u_{rel}^2 = u_{rel}^w{}^2 + u_{rel}^d{}^2 + u_{rel}^{w2}{}^2 + u_{rel}^{d2}{}^2 + u_{rel}^{d3}{}^2 + u_{rel}^{ws}{}^2 + u_{rel}^{ds}{}^2 + u_{rel}^{ds2}{}^2 + 12 * u_{rel}^{ds3}{}^2 + u_{rel}^{d2}{}^2 + u_{rel}^c{}^2 + u_{rel}^{sc}{}^2$	5.3

3 General considerations on reproducibility, specificity, and stability of the assay

During the course of this work, the same assay has been carried out with strips prepared and LFA test assembled from different batches of materials (Whatman and Kenosha membranes, see Section S1) over a period of more than three months by different operators, which allowed us to determine the reproducibility across such more general parameter variations to a relative error of 34%. It has to be kept in mind, however, that system preparation in our research lab is and was essentially manual so that, once manufactured in a more automatized fashion when aiming at commercialization, the batch-to-batch and day-to-day reproducibility would be improved.

Class specificity was found to comply with that reported by the manufacturer.⁸

Regarding the stability of the components of the system, stock solutions of **S1-AB** (1 mg mL⁻¹) suspended in 1 mL PBS are stable for one month when kept in the refrigerator. The un-capped pre-prepared scaffold material **S1** (SBA functionalized with PEG and hapten and loaded with dye) is stable for more than 1 year, as assessed by regularly capping small portions of stored **S1** and running assays for comparison alongside.

In addition, after spotting onto a glass fibre membrane functionalized with 40% PEG, the capped material **S1-AB** is stable for at least three months when stored in the refrigerator.

S8. References

1. W. Hickel, *Mar. Ecol. Prog. Ser.*, 1984, **16**, 185-191.
2. K. Zhuang, G. Yin, X. Pu, X. Chen, X. Liao, Z. Huang and Y. Yao, *MRS Commun.*, 2016, **6**, 449-454.
3. Q. Cai, Z.-S. Luo, W.-Q. Pang, Y.-W. Fan, X.-H. Chen and F.-Z. Cui, *Chem. Mater.*, 2001, **13**, 258-263.
4. R. Gotor, J. Bell and K. Rurack, *J. Mater. Chem. C*, 2019, **7**, 2250-2256.
5. C. A. Schneider, W. S. Rasband and K. W. Eliceiri, *Nat. Methods*, 2012, **9**, 671-675.
6. Evaluation of Measurement Data—Guide to the Expression of Uncertainty in Measurement; Joint Committee for Guides in Metrology JCGM, Paris, 1 Edn, 2008, corrected version 2010.
7. B. Walter, G. C. Maurice and M. H. Peter, *Metrologia*, 2006, **43**, S161.
8. Pyrethroids ELISA Kits PN 500201, PN 500204, Eurofins Abraxis, Inc, Warminster, PA.