Supporting Information
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Mix-&-Read Determination of Mercury(II) at Trace Levels with Hybrid Mesoporous Silica Materials Incorporating Fluorescent Probes by a Simple Mix-&-Load Technique
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S.1 Synthesis and chemical characterization of materials

S1.1 Synthesis of non-porous silica microparticle support (N)
SiO$_2$ microparticles were prepared following a modified Stöber method.[1] A mixture containing ammonia solution (32%, 9 mL), pure ethanol (99 %, 16.25 mL) and milliQ water (24.75 mL) was deposited in a round-bottomed flask and stirred at 1000 rpm. Thereafter, a mixture of TEOS (4.5 mL) and ethanol (45.5 mL) was added and further stirred at 500 rpm for 2 h. The final silica particle product was washed with distilled water and ethanol and dried in a vacuum overnight.

S1.2 Synthesis of MCM-41 mesoporous silica nanoparticle support (MCM)
Mesoporous MCM-41 nanoparticles were synthesized as reported previously.[2] n-Cetyltrimethylammonium bromide (CTABr, 1.00 g, 2.74 mmol) was first dissolved in 480 mL of deionized water. Then, 3.5 mL of 2 M NaOH in deionized water were added to the CTABr solution, followed by heating the solution to 80 °C. Tetraethylorthosilicate (TEOS) (5.00 mL, 2.57·10$^{-2}$ mol) was then added dropwise to the surfactant solution. The mixture was stirred for 2 h to give a white precipitate. Finally, the solid product was centrifuged, washed with deionized water and ethanol, and dried at 60°C (MCM-41 as-synthesized).

Removal of the template was achieved suspending 1 g of the as-synthesized solids in 125 mL 1 M HCl in Ethanol (EtOH), keeping the suspension with constant agitation at 80 °C for 17 h. Subsequently, the particles were isolated by centrifugation (5 min at 10,000 rpm), washed with water until neutral pH and dried in a vacuum, yielding the extracted porous material MCM.

S1.3 Synthesis of APT-N, APT-MCM and APT-SBA materials
The amino-functionalized solid materials APT-N, APT-MCM and APT-SBA were prepared following literature procedures.[3] Modification of the surface with 3-aminopropyltriethoxysilane (APTES) groups was achieved by suspending 100 mg of each material (N, extracted MCM-41, MCM and extracted SBA-15, SBA) in 1.5 mL acetonitrile (MeCN), adding an excess of APTES (119.0 µL, 5 mmol g$^{-1}$) to each suspension and stirring the suspensions at room temperature for 5.5 h. Finally, the materials were centrifuged for 5 min at 10,000 rpm, washed two times with 1.5 mL MeCN and dried in a vacuum.

S1.4 Synthesis of PEG-N, PEG-MCM and PEG-SBA materials
The PEGylated solid materials PEG-N, PEG-MCM and PEG-SBA were prepared following a similar procedure as described before for APT-N, APT-MCM and APT-SBA, but using 2-\{methoxy-\[poly(ethyleneoxy)\]propyl\} trimethoxysilane instead of APTES. Briefly, 100 mg of each material (N, MCM, SBA) were suspended in 3 mL toluene, followed by addition of an excess of 2-\{methoxy[poly(ethyleneoxy)]propyl\} trimethoxysilane (227.0 µL, 5 mmol g$^{-1}$) and stirring the suspensions at room temperature for 5.5 h. Finally, the materials were centrifuged for 5 min at 10,000 rpm and washed twice with 1.5 mL of toluene before drying in a vacuum.
S1.5 Synthesis of EPO-N, EPO-MCM and EPO-SBA materials
The epoxy-functionalized solid materials EPO-N, EPO-MCM and EPO-SBA were also prepared as described before, employing 3-glycidoxypropyltrimethoxysilane. 100 mg of each material (N, MCM, SBA) were suspended in 1.5 mL MeCN, an excess of 3-glycidoxypropyltrimethoxysilane (167.4 µL, 5 mmol g⁻¹) was added and the suspensions were stirred at room temperature for 5.5 h. Finally, the materials were centrifuged for 5 min at 10,000 rpm, washed two times with 2 mL of MeCN and dried in a vacuum.

S1.6 Synthesis of PRO-N, PRO-MCM and PRO-SBA materials
The propyl-functionalized solid materials PRO-N, PRO-MCM and PRO-SBA were analogously prepared with propyltrimethoxysilane as functional silane. 100 mg of each material (N, MCM, SBA) were suspended in 3 mL MeCN, an excess of propyltrimethoxysilane (90.5 µL, 5 mmol g⁻¹) was added and the suspensions were stirred at room temperature for 5.5 h. Finally, the materials were centrifuged for 5 min at 10,000 rpm, washed twice with 2 mL MeCN and dried in a vacuum.

S1.7 Synthesis of xPRO-MCM material
To decorate the material with propyl groups only on the outer surface, 250 mg of as-synthesized MCM-41 were suspended in 7.5 mL MeCN before 220.2 µL propyltrimethoxysilane (5 mmol g⁻¹ solid) were added to the suspension. After stirring the suspension for 5.5 h at room temperature, the solid was centrifuged for 5 min at 10,000 rpm, washed twice with 5 mL MeCN and dried in a vacuum. In a second step, the surfactant of the propyl-modified silica material was removed by extraction with HCl in EtOH. Thus, 250 mg of the solid prepared previously were suspended in 25 mL of 1 M HCl in EtOH, stirred at 100 ºC for 15 h, centrifuged for 5 min at 10,000 rpm, washed with water until neutral pH and dried in a vacuum.

S1.8 General procedure for loading of BODIPY probe I into the materials
26 µmol L⁻¹ Solutions of I in MeCN were prepared. 20 mg of each material prepared previously (N, MCM, SBA, APT-N, APT-MCM, APT-SBA, PEG-N, PEG-MCM, PEG-SBA, EPO-N, EPO-MCM, EPO-SBA, PRO-N, PRO-MCM, PRO-SBA, xPRO-MCM and xPRO-SBA) were suspended in 4 mL of the solution of I, yielding suspensions with a final concentration of 5 µmol I (g solid)⁻¹. The suspensions were stirred for 24 h at room temperature, centrifuged (10 min at 6000 rpm) and washed with water until no fluorescence was observed in the supernatants under the UV lamp. Finally, the solids were dried in a vacuum for 12 h, yielding N-I, MCM-I, SBA-I, APT-N-I, APT-MCM-I, APT-SBA-I, PEG-N-I, PEG-MCM-I, PEG-SBA-I, EPO-N-I, EPO-MCM-I, EPO-SBA-I, PRO-N-I, PRO-MCM-I, PRO-SBA-I, xPRO-MCM-I and xPRO-SBA-I. The last largely removes residual solvent from the pores while the strongly adsorbed BODIPY probe molecules are retained.

To study the influence of the amount of probe I loaded into the particles containing mesopores or being non-porous and possible aggregate formation, N, APT-N, PEG-N, EPO-N, PRO-N, SBA, APT-SBA, PRO-SBA and xPRO-SBA (20 mg) were suspended in 4 mL of solutions containing I at 1.05 mmol L⁻¹, following the same procedure as above and leading to
suspensions with a final concentration of 200 µmol I (g solid)\(^{-1}\) and solids N-I', APT-N-I', PEG-N-I', EPO-N-I', PRO-N-I', SBA-I', APT-SBA-I', PRO-SBA-I' and xPRO-SBA-I'.

S1.9 General procedure for loading of BODIPY dye II into certain materials
To investigate the mechanism of Hg(II) detection at the molecular scale, BODIPY dye II was loaded into MCM, SBA and xPRO-SBA. For this purpose, fractions of 20 mg of these solids were suspended in 4 mL of solution II (26 µmol L\(^{-1}\)) in MeCN, yielding suspensions with a final concentration of 5 µmol II (g solid)\(^{-1}\). Work up as described above then led to MCM-II, SBA-II and xPRO-SBA-II.

S1.10 Quantification of organic groups grafted onto the materials.
Absorption measurements were carried out to determine the amount of BODIPYs I and II loaded into the materials. Additionally, elemental analyses were employed to evaluate the contents of organic moieties grafted to the materials plus the amount of I or II adsorbed.

In the indirect UV/vis approach, the concentration of the respective BODIPYs I and II in the aqueous solutions was determined from the absorbance at 520 nm (\(\lambda_{\text{max}}\)) before and after the loading process, using the Lambert-Beer law and the corresponding molar absorption coefficient of each dye. On the other hand, from elemental analysis of C, H, N and S it was possible to determine directly the total amount of organic material \(\alpha\) contained in the final materials, calculated as mmol (g solid)\(^{-1}\) using equation (S1):

\[
\alpha = \frac{\Delta W_i \%	imes 1000}{nM_i} \quad \text{(S1)}
\]

where \(\Delta W_i\%\) (i = C, N, H, S) are the weight percentages of carbon, nitrogen, hydrogen and sulphur. \(M_i\) is the corresponding atomic weight and \(n\) is the number of the corresponding atoms in one molecule. Thus, by considering C, N and S it is possible to estimate the amount of propyl, APTES, PEG and epoxy groups as well as BODIPYs I or II in the respective materials, using equation (S2)

\[
\alpha_i = A\alpha_{\text{APT-PEG-EPO-PRO}} + B\alpha_{\text{BODIPYI-II}} \quad \text{(S2)}
\]

where A and B are the number of the corresponding atom types in one molecule. All the results are collected in Table S1.

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Table S1. Moles of organic groups and probes/dyes per gram of solid (g⁻¹ solid), average coverage and distance between anchored groups for all materials.

|         | αEPO | αPEG | αAPTES | αProp | αBO-I | αBO-II |
|---------|------|------|--------|-------|-------|--------|
|         | µmol (g⁻¹ s.) | mmol (g⁻¹ s.) | mmol (g⁻¹ s.) | mmol (g⁻¹ s.) | µmol (g⁻¹ s.) | µmol (g⁻¹ s.) |
| MCM-I   | -    | -    | -      | -     | 0.17  | -      |
| SBA-I   | -    | -    | -      | -     | 0.45  | -      |
| N-I     | -    | -    | -      | -     | 0.07  | -      |
| APT-MCM-I | -  | 1.24 | -      | -     | 0.41  | -      |
| APT-SBA-I | -  | 1.64 | -      | -     | 0.35  | -      |
| APT-N-I | -    | -    | 0.45   | -     | 0.13  | -      |
| PEG-MCM-I | 0.44 | -    | -      | -     | 0.22  | -      |
| PEG-SBA-I | 0.62 | -    | -      | -     | 0.26  | -      |
| PEG-N-I | 0.12 | -    | -      | -     | 0.07  | -      |
| PRO-MCM-I | -  | -    | 1.13   | -     | 0.17  | -      |
| PRO-SBA-I | -  | -    | 1.22   | -     | 0.22  | -      |
| PRO-N-I | -    | -    | -      | 0.45  | 0.03  | -      |
| xPRO-MCM-I | -  | -    | 0.39   | -     | 0.12  | -      |
| xPRO-SBA-I | -  | -    | 1.07   | -     | 0.20  | -      |
| EPO-MCM-I | 0.68 | -    | -      | -     | 0.40  | -      |
| EPO-SBA-I | 0.71 | -    | -      | -     | 0.14  | -      |
| EPO-N-I | 0.33 | -    | -      | -     | 0.08  | -      |
| SBA-I'   | -    | -    | -      | -     | 2.59  | -      |
| N-I'     | -    | -    | -      | -     | 1.94  | -      |
| APT-SBA-I' | -  | 1.64 | -      | -     | 4.12  | -      |
| APT-N-I' | -    | -    | 0.45   | -     | 6.19  | -      |
| xPRO-SBA-I' | -  | -    | 1.07   | -     | 9.21  | -      |
| PRO-N-I' | -    | -    | -      | 0.45  | 2.03  | -      |
| PEG-N-I' | -    | -    | -      | -     | 2.56  | -      |
| EPO-N-I' | 0.33 | -    | -      | -     | 2.22  | -      |
| xPRO-SBA-II | -  | -    | 1.07   | -     | 0.14  | -      |
S.2 Spectroscopic measurements and LOD assessment

S2.1 Spectroscopic properties

Molar absorption coefficients and relative fluorescence quantum yields were determined as described in ref. [4]. The corresponding uncertainties of measurement can also be derived from the considerations detailed therein. Three different independent measurements were performed, employing Rhodamine 6G in ethanol as fluorescence standard ($\Phi = 0.95 \pm 0.015$).[5] To correct for scattered light in the absorption spectra of measurements involving particles, suspensions of the corresponding blank particles (containing no dye) at the respective concentration were performed and subtracted, see e.g. caption of Figure 4.

Figure S1. Enlarged, high-quality version of the photograph shown in the inset of Figure 3b, showing the color change from pink to yellow.

S2.2 Estimation of limit of detection.

Fluorescence emission and excitation spectra of suspensions of the materials were registered in the presence of various amounts of Hg(II), and the values of the fluorescence enhancement observed during the titrations were normalized to the initial intensities. Results were fitted to a four-parameter logistic fitting function (equation S3):

$$\frac{I}{I_0} = \frac{F_1-F_2}{(1+x/x_0)^p} + F_2$$  \hspace{1cm} (S3)

Here, $F_1$ and $F_2$ correspond to the minimum and maximum fluorescence observed, and $p$ corresponds to the slope of the sigmoidal curve. Limits of detection (LOD) were derived calculating the corresponding concentration of the signal of 3 times the standard deviation of the instrumental signal in the absence of sample ($3\sigma$ definition).
S.3 Materials design and morphological characterization

S3.1 General considerations

The functionalization of high-surface mesoporous silica materials with organic moieties is commonly achieved along two different routes, post-grafting or co-condensation. The first approach involves (at least) two steps, i.e., first the preparation of the inorganic scaffold and then its functionalization on the inner and/or outer surface with the desired organosilane, e.g., after removal of the templating surfactant. This approach guarantees that the mesostructured of the scaffold is retained. The second approach relies on the simultaneous (one-step) condensation of the structural silica precursor (e.g., TEOS) and one or more organosilanes, resulting in a distribution of the organic moieties within the silica walls as well as on the inner and outer surface. Such materials are usually much more disordered. For our present purposes, the co-condensation approach with either of the silanes used would not harbor any benefits, because functional groups in the silica walls would not be accessible for the dye and/or metal ion yet would only lead to different material morphologies and perhaps also stabilities, potentially making comparisons of the different materials problematic.

S3.2 Specific considerations

To be able to better compare the incorporation of organic moieties without an alteration of the mesostructure of the material, the post-grafting method was selected in this work. In doing so, the external surface is more accessible and is functionalized predominantly during the initial stages of the reaction. However, diffusion of reactants into the inner pore voids is also possible, allowing to control the degree of functionalization through reactant concentration and reaction time. The materials APT-MCM, APT-SBA, PEG-MCM, PEG-SBA, EPO-MCM, EPO-SBA, PRO-MCM and PRO-SBA were prepared in this way. When as-synthesized mesoporous materials which are still filled with the templating surfactant are subjected to post-grafting, it is possible to largely achieve selective functionalization of the external surface only. Diffusion of organosilane reactants into the pores is sterically hindered significantly by the surfactants. After grafting of an organosilane onto the external surface, the surfactant can be removed by extraction and the inner pores’ surface can be either left non-functionalized or can be reacted with a second organosilane.

The aim of this work was the development of a simple yet efficient material for the sensitive and selective recognition of mercury in aqueous environments. Thus, taking into account our previous studies with dyes and probes of similar architecture, we functionalized the surface of the silica materials with various functional silanes such as amino, poly(ethylene glycol), epoxy and propyl groups, to create different environments. Additionally, the effect of the pore size and diffusion times was investigated by using three families of materials, a first one based on non-porous silica microparticles with a specific surface area of ca. 50 m² g⁻¹ and two families more based on mesoporous materials, one on MCM-41 nanoparticles, with a specific surface area of ca. 1000 m² g⁻¹ and a pore diameter of ca. 2.5 nm, and one on SBA-15 microparticles, with a specific surface area of ca. 800 m² g⁻¹ and a pore diameter of ca. 8 nm. As described in Section S1 and the Experimental Section of the main paper, a total of 26 materials...
that either contain probe I or dye II have been prepared, N-I, MCM-I, SBA-I, APT-N-I, APT-MCM-I, APT-SBA-I, PEG-N-I, PEG-MCM-I, PEG-SBA-I, EPO-N-I, EPO-MCM-I, EPO-SBA-I, PRO-N-I, PRO-MCM-I, PRO-SBA-I, xPRO-MCM-I, xPRO-SBA-I, N-I’, SBA-I’, APT-N-I’, APT-SBA-I’, xPRO-SBA-I’, PEG-N-I’, EPO-N-I’, PRO-N-I’, and xPRO-SBA-II. The small library is illustrated in Scheme S1.

Scheme S1. Library of materials prepared.

Besides the synthesis and chemical characterization as detailed in S1, the morphology of the non-porous N and mesoporous silica particles MCM and SBA have been confirmed by transmission electron microscopy (TEM). As can be seen in Figure S2a, silica particles N show spherical particles with a diameter of ca. 450 nm, MCM show spherical particles with a diameter of ca. 100 nm (Figure S2c) and SBA are more hexagonal with a length of ca. 1 µm (Figure S2b). N2 adsorption-desorption measurements were also performed for the mesoporous silica particles MCM and SBA, yet not for bulk, non-porous N. The adsorption–desorption isotherms of extracted MCM-41 (MCM) and SBA-15 (SBA) particles show the typical type-IV isotherms of these materials. Two well distinguished adsorption steps in the isotherms are observed, ascribed to (i) nitrogen condensation inside the mesopores by capillarity, and (ii) nitrogen adsorption on the outer particle surfaces (Figure S2d). A clear type-H1 hysteresis loop with sharp adsorption and desorption branches is only observed in SBA, in which the nitrogen condensation takes place at a higher relative pressure (P/P0) with respect to MCM (0.7 vs. 0.3) due to its bigger pore size. The absence of a hysteresis loop in this interval for MCM suggests the existence of uniform cylindrical mesopores. TEM analysis also confirms the typical
hexagonal porosity and channels of MCM and SBA materials as alternating black and white stripes. BET specific surface values, pore volumes, and pore sizes calculated from N$_2$ adsorption–desorption isotherms for the materials MCM and SBA are detailed in Table S2.

**Figure S2.** a-c) TEM images of a) non-porous silica microparticles N, b) SBA and c) MCM mesoporous materials. d) N$_2$ adsorption–desorption isotherms for mesoporous materials MCM and SBA. The inset shows the pore size distribution.

**Table S2.** BET specific surface values, pore volumes, pore sizes and particle diameters calculated from N$_2$ adsorption–desorption isotherms and TEM measurements for selected materials.

|       | $S_{\text{BET}}$ (m$^2$ g$^{-1}$) | Pore volume$^a$ (cm$^3$ g$^{-1}$) | Pore diameter$^a$ (nm) | Particle diameter (nm) |
|-------|----------------------------------|----------------------------------|-------------------------|------------------------|
| MCM   | 970                              | 1.45                             | 2.55                    | 100                    |
| SBA   | 685                              | 1.01                             | 8.70                    | 1000                   |

$^a$ Volume ($V$) and diameter ($D$) of mesopore.

**S.4 Spectroscopic studies with library of materials**
To assess the influence of the microenvironment on the response behavior of the system, materials MCM-I, SBA-I, APT-MCM-I, APT-SBA-I, PEG-MCM-I, PEG-SBA-I, EPO-
MCM-I, EPO-SBA-I, PRO-MCM-I, PRO-SBA-I, xPRO-MCM-I and xPRO-SBA-I were suspended in Milli-Q water (pH 7) in a concentration of 1 mg mL$^{-1}$. Then, 480 µL of each one of these suspensions were added to a fluorescence cuvette containing 2.0 mL of Milli-Q water (final solid concentration 0.19 mg mL$^{-1}$). In a first experiment, only the fluorescence emission ($\lambda_{ex}$ 490 nm) of all the suspensions was registered, and the different spectra were normalized to the content of I of each material. As can be seen in Figure S3, in all cases, the materials showed the typical BODIPY band centered at 538 nm. However, the fluorescence intensities were strongly dependent on the environment, which in turn is primarily determined by the organic moieties anchored to the inner and/or outer surface of the materials and their pore size. For both mesoporous scaffolds, MCM-41 and SBA-15, the materials modified with PEG and epoxy groups showed considerably high initial fluorescence. PEG and epoxy groups are both moderately polar and only differ in size, the pores of PEG-SBA-I accommodating the dye better than EPO-SBA-I. This sequence is reversed for MCM with distinctly smaller pores for which steric crowding of PEG moieties presumably will hamper diffusion and residence of the dye molecules. Moreover, whereas the degree of EPO functionalization is comparable for SBA and MCM, distinctly less (only ca. 70%) PEG is attached to MCM than SBA. In addition, the average fluorescence of a loaded dye molecule is very similar for PEG-SBA-I and EPO-SBA-I, and 1.5–2-times higher than for PEG-MCM-I and EPO-MCM-I. Obviously, dye I is less prone to aggregation or dimerization in the bigger pores of SBA, and PEG-SBA-I can accommodate more I in the pores than EPO-SBA-I. PEG functionalization seems to offer a larger volume of “organic space” in the pores than EPO does.

The presence of propyl groups on the entire surface of the materials allows a higher loading of dyes into the pores of SBA compared with MCM, yet the average fluorescence obtained from a dye molecule is now distinctly higher (ca. 2.5-times) for the material with the smaller pore diameter. Apparently, the short but hydrophobic coating of the larger pores leads to aggregation of the dyes taken up whereas in the MCM case, the dyes remain largely isolated, perhaps lining up in the channels as is well known from dye-loaded zeolites.[8] The difference in the degree of PRO coating of MCM and SBA is again small, stressing the absence of a size effect. For the materials only functionalized with propyl groups on the outer surface, the difference then is extreme, i.e., I being almost 10-times more fluorescent in xPRO-MCM-I than in xPRO-SBA-I. Both trends are reinforced, i.e., the fluorescence per dye molecule is twice as high in xPRO-MCM-I compared with PRO-MCM-I and even 3-times lower for xPRO-SBA-I compared with PRO-SBA-I, despite showing rather similar loadings for the two SBA materials. Whereas the behavior of xPRO-SBA-I is consistently similar to that of SBA-I, the outer surface functionalization having only a negligible effect for the micron-sized particles and the behavior of I being dominated by the native silica walls of the pores, xPRO-MCM-I differs also strongly from MCM-I. APT functionalization shows features of a small but highly polar coating, yet a considerable amount of free amino groups seems to have a detrimental effect on the fluorescence of I, most likely by electron transfer type quenching especially in the case of the marrow pores of APT-MCM-I, showing the lowest fluorescence.

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a The lengths between the Si atom and the terminal H atom of the elongated molecules have been determined to ca. 2.5 nm for PEG, ca. 0.8 nm for EPO and ca. 0.5 nm for both APT and PRO using ground-state geometry optimizations at semi-empirical AM1 level.
of all porous materials per dye unit. In general, moderately polar organic groups seem to accommodate I preferably in its monomer state.

To corroborate the interaction between the different organic groups as such and I, we compared the influence of the single species on the fluorescence of I (1.5 µM) in H2O, using 3 mM of aminopropyltrimethoxysilane (APTES), 2-[methoxy(polyethyleneoxy) propyl]trimethoxysilane (PEG), 3-glycidoxypropyltrimethoxysilane (EPO) and propyltrimethoxysilane (PRO) moieties. Figure S4a shows that in a neat aqueous environment, solely the polarity of the organic groups seems to govern the fluorescence yield of I, in line with the polarity-dependent fluorescence quantum yield of meso-(p-aminophenyl-)substituted BODIPYs.[7] The difference between this sequence PRO > PEG > EPO > APT (> H2O) and the sequences seen in the MCM-41 and SBA-15 materials suggests that confinement effects play a significant role for the hybrids. We thus also recorded the fluorescence of the different N-I materials in water, to get further insight into confinement effects vs. grafting state of the silanes. Figure S4b shows that qualitatively, grafting does not change decisively the influence of the organic groups. i.e., the fluorescence increases along the series N-I ~ EPO-N-I ~ APT-N-I < PEG-N-I < PRO-N-I. Confinement effects are thus essential in directing the behaviour of the mesoporous materials.

Figure S3. Fluorescence emission spectra (λex 490 nm) normalized to dye content of initial suspensions of materials MCM-I, SBA-I, APT-MCM-I, APT-SBA-I, PEG-MCM-I, PEG-SBA-I, EPO-MCM-I, EPO-SBA-I, PRO-MCM-I, PRO-SBA-I, xPRO-MCM-I and xPRO-SBA-I in Milli-Q water (0.19 mg mL⁻¹; pH 7); the content of BODIPY I is given in Table S1.
Figure S4. Fluorescence emission spectra (λ_{ex} 490 nm) normalized to dye content of a) I in Milli-Q water (1.5 µM; pH 7) in absence and in presence of 3 mM of APTES, PEG silane, EPO silane and PRO silane and b) suspensions of materials N-I, APT-N-I, PEG-N-I, EPO-N-I and PRO-N-I in Milli-Q water (0.19 mg mL\(^{-1}\); pH 7)

Keeping in mind that these differences in initial intensities could have an effect on the sensitivity, in a second series of experiments we registered the changes of the fluorescence of all suspensions of the materials prepared as a function of the concentration of Hg(II). Figure S5 depicts the respective fluorescence enhancement ratios (ΔF/F₀) upon titration with Hg(II). For the MCM-41 and SBA-15 materials families, such a fluorescence enhancement was observed in most cases and only xPRO-MCM-I and EPO-MCM-I offered virtually no response. For the non-porous control materials of type N, practically no response was observed, ΔF/F₀ remaining at ca. 0.2 even at high concentrations of Hg(II). We thus prepared a new series of materials N', using a much higher concentration of I during the synthesis (N-I', APT-N-I', PEG-N-I', EPO-N-I' and PRO-N-I'). The titrations carried out with those hybrids are shown in Figure S5c and reveal that indeed now a \(c_{\text{Hg(II)}}\)-dependent fluorescence enhancement was observed, but only at significantly elevated concentrations (LODs > 100 ppb). As explained in the manuscript, this enhanced fluorescence is due to the complex I–Hg(II) that has desorbed from the silica particles’ surface; after addition of Hg(II), shaking the suspension and centrifugation, the fluorescence was only observed in the supernatant.

To determine the limits of detection (LOD) the concentration dependencies were fitted using a four-parameter logistic function. The LODs were calculated adding three times the standard deviation (3σ definition) from the minimum signal observed for the corresponding curve. Values of LOD found are listed in Table S3. From the results obtained it is possible to conclude that xPRO-SBA-I offered the best performance, with a limit of detection of 12 ppt, which is below the toxicity level of Hg(II) in drinking water established by the EPA (2 ppb). However, it is important to remark that most of the other materials prepared offered also an excellent sensitivity below the toxicity level.
Figure S5.- Fluorescence enhancement ratio ($\Delta F/F_0$) registered at 538 nm ($\lambda_{ex}$ 490 nm) for a) SBA type materials, b) MCM-41 type and c) N type materials suspended in Milli-Q water (pH 7) in presence of varying amounts of Hg(II).

Table S3. Limits of detection (LOD) for Hg(II) determination for all materials in Milli-Q water (pH 7)

| Material       | LOD / ppb | Material       | LOD / ppb |
|----------------|-----------|----------------|-----------|
| xPRO-SBA-I     | 0.012     | xPRO-MCM-I     | -         |
| PRO-SBA-I      | 0.075     | PRO-MCM-I      | 2.68      |
| SBA-I          | 0.25      | MCM-I          | 0.086     |
| PEG-SBA-I      | 1.18      | PEG-MCM-I      | 0.151     |
| EPoSBA-I       | 1.47      | EPOMCM-I       | -         |
| APT-SBA-I      | 155       | APT-MCM-I      | 3.6       |

Analysing the behaviour of the materials prepared in more detail, we observed that for instance the presence of APT groups produced a strong fluorescence enhancement only in the presence
of higher amounts of Hg(II), whereas a less polar microenvironment increases sensitivity, at least for the SBA family. A reduced microenvironment induces (a) an intrinsically higher fluorescence of BODIPY I (less excited-state intramolecular charge transfer formation) and (b) favours complexation of Hg(II) in the crown ether (higher binding constants). However, and as we have seen before for MCM-based fatty acid-detection materials,[9] the surface functionalization of such porous materials does not change the microenvironmental polarity of the entire pore void, but mainly that of the interface close to the pore wall. Already for the narrow pores of MCM, the polarity of the inner pore void and of the interface close to the wall are significantly different.[9] Comparison of the present materials of the SBA and the MCM families supports these earlier findings, as the sequence of functional groups governing the LOD is largely different for MCM (Table S3). For instance, both propyl-functionalized MCM materials show only inferior responses, the hydrophobic groups together with the smaller pore size most likely hampering largely diffusion of the hydrated Hg(II) ions and counterions into the pores. On the basis of all the effects observed, we can conclude that various different factors determine the performance of the materials, such as polarity/hydrophobicity, pore size, solvent-dependent fluorescence quantum yields of the fluorescent probe and solvent-dependent complexation constants, all of them being connected in a non-trivial manner, finally leading to xPRO-SBA-I being the most potent material.

S.4.1 Cross-reactivity of PRO-SBA-I

Having found an excellent discrimination of xPRO-SBA-I for Hg(II) against Ni(II), Cu(II) and Ag(I), see Figure 7, we also evaluated the effect of other cations on the analogous material PRO-SBA-I. Titrations with perchlorate salts of the cations Hg(II), Cu(II), Ni(II) and Ag(I) (0.1–10⁶ nM) were performed, following the same procedure as outlined in the manuscript. As can be seen in Figure S6, the response pattern is qualitatively similar with Ag(I) showing a somewhat higher cross-reactivity for PRO-SBA-I than for xPRO-SBA-I (Figure 7), more comparable to free I in MeCN. These findings support the unique properties of xPRO-SBA-I for the selective and sensitive determination of Hg(II).

![Figure S6](image)

**Figure S6.** Fluorescence enhancement ratio ($\Delta F/F_0$) registered at 538 nm ($\lambda_{ex}$ 490 nm) of PRO-SBA-I suspended in Milli-Q water (pH 7) in presence of several amounts of Hg(II), Ni((II)), Cu(II) and Ag(I).
S.5 Demonstration of mechanism

S.5.1 Titrations with xPRO-SBA-II

Figure S7. A) Fluorescence emission spectra ($\lambda_{\text{ex}}$ 510 nm) of xPRO-SBA-II suspended in Milli-Q water (0.11 mg mL$^{-1}$; pH 7) in presence of several amounts of Hg(II) (from 0 to 10 ppm of Hg(II)). b) Corresponding fluorescence excitation spectra ($\lambda_{\text{em}}$ 540 nm).

S.5.2 Dye leaching studies

Figure S8. Representation of the leaching studies of xPRO-SBA-I' and APT-N-I' in absence and in presence of 2.5 ppm of Hg(II) a) before and b) after centrifugation. After centrifugation only xPRO-SBA-I' containing the complex I–Hg(II) remains fluorescent, whereas the supernatant is non-fluorescent under irradiation with a home-made light source ($\lambda_{\text{ex}}$ 470 nm). On the other hand, APT-N-I' in the presence of Hg(II) is only weakly fluorescent after centrifugation yet the supernatant is highly fluorescent, indicating that the complex desorbed from the surface and diffused into the solution.
S.6 Extraction Hg(II) from fish tissues

Extraction of the total mercury from fish muscle ERM-BB422 with a known content was performed following a reported procedure\[10\] using i) an ultrasound-assisted extraction procedure (UEP), ii) a microwave acid digestion method and iii) UV irradiation.

For the ultrasound sonication, two portions of ca. 0.2 g were weighed in 25 mL flasks. After a mixture of 4 mL of HNO\(_3\) (65%) : H\(_2\)O\(_2\) (30%) 2:1 was added to the samples, the flasks were placed inside an ultrasonic bath with an ultrasonic energy of 37 kHz and were left for 11 min at 80°C. Thereafter, the samples were allowed to cool down to room temperature, followed by centrifugation at 4000 rpm for 5min. 1 mL of supernatant of each flask was diluted to 50 mL with Milli-Q water and solutions were stored in the freezer for further analysis.

For the acid digestion using microwave, two portions of ca. 0.25 g were weighed into PTFE tubes, and 4 mL of HNO\(_3\) (65%) were added to one portion and 6 mL of a mixture of HNO\(_3\) (65%) : H\(_2\)O\(_2\) (30%) 2:1 was added to the second portion. The samples were digested for 1–2 min until complete dissolution. The digestion tubes were cooled and the resulting solution was diluted up to 50 mL with Milli-Q water and left for further analysis.

Finally, UV extraction was performed using also two portions of 0.25 g. Both portions were incubated in 2 mL HNO\(_3\) (65%) for 1 h before addition of 0.5 mL HClO\(_4\) (70%) or 0.5 mL of H\(_2\)O\(_2\) (30%). Then, the samples were irradiated under a UV lamp for 3 h (LAR Analytik & Umweltmeßtechnik GmbH, NI. UO25E5; Tp: PLYser; 1991 V 230 KW 0.7). Finally, the acid extracts were transferred to 50 mL volumetric flasks, and the volume was adjusted to 50 mL with Milli-Q water. All conditions employed for the extraction are summarized in Table S4.

Extraction of Hg(II) from commercial fishes (tuna, pollock, madjes and mackerel) was done by crushing portions of these fishes and drying on petri dishes overnight at 85°C. Extraction was performed under UV irradiation following procedure as described above, employing ca. 0.25 g of each fish tissue and incubating with a mixture of 2 mL HNO\(_3\) (65%) and 0.5 mL HClO\(_4\) (70%) (tuna and pollock) or 2 mL HNO\(_3\) (65%) and 1.5 mL HClO\(_4\) (70%) (matjes and mackerel). Samples were also irradiated under a UV lamp for 3 h (LAR Analytik & Umweltmeßtechnik GmbH, NI. UO25E5; Tp: PLYser; 1991 V 230 KW 0.7) to convert all possibly contained organic mercury to inorganic mercury. Finally, the acid extracts were transferred to 50 mL volumetric flasks, and the volume was adjusted to 50 mL with Milli-Q water.

Determination of Hg(II) was carried out using our sensing material xPRO-SBA-I and CV-AES as control. For this purpose, extracts were diluted (1/5) and left at neutral pH. 0.5 mL of these neutralized suspensions were mixed with 2.07 mL of a suspension of xPRO-SBA-I (0.17 mg mL\(^{-1}\)) in phosphate buffer (10 mM, pH 7), and the initial mercury concentration in the tissues was then determined using linear regression by standard addition method.
Table S4. Conditions employed for the extraction of total mercury from certified reference material CRM-BB422

| Sample | Extraction method | Acids employed |
|--------|-------------------|----------------|
| UEP-1  | Ultrasound        | 4 mL of HNO₃ (65%) : H₂O₂ (30%) 2:1 / 50 mL Milli-Q water |
| UEP-2  | Ultrasound        | 4 mL of HNO₃ (65%) : H₂O₂ (30%) 2:1 / 50 mL Milli-Q water |
| MW-1   | Microwave         | 6 mL of HNO₃ (65%) : H₂O₂ (30%) 2:1 / 50 mL Milli-Q water |
| MW-2   | Microwave         | 4 mL of HNO₃ (65%) / 50 mL Milli-Q water |
| UV-1   | UV                | 2 mL HNO₃ (65%) + 0.5 mL HClO₄ (70%) / 50 mL Milli-Q |
| UV-2   | UV                | 2 mL HNO₃ (65%) + 0.5 mL H₂O₂ (30%) / 50 mL Milli-Q |

S.7 Time-resolved fluorescence spectroscopy

The fluorescence lifetimes τᵢ were determined with a unique customized laser impulse fluorometer with picosecond time resolution described elsewhere. In a typical experiment, the sample was excited at 488 nm with ca. 10⁵ photons per pulse. A solution of glycogen in water was used to measure the instrumental response function (IRF). The fluorescence decay profiles of the suspensions in water were registered at 540 nm employing a 500 nm cut-off filter to exclude scattered photons of the particles. The fluorescence lifetime profiles were analyzed to fit to a multi-exponential model, showing in all the cases non-exponential decay kinetics in absence and in presence of varying amounts of Hg(II). Non-exponentiality because of microstate heterogeneity is a well-known phenomenon for dyes in confined media such as zeolites or mesoporous silica. The results of the average fluorescence lifetimes τₐᵥ calculated according to eq. (S4) are listed in Table S5, representative decays are shown in Figure S9.

\[
τ_{av} = \frac{\sum_i a_i τ_i}{\sum_i a_i}
\]  

(S4)

In absence of Hg(II), average lifetimes of 1.89 ns and 1.29 ns are observed for xPRO-SBA-I and xPRO-SBA-I’, respectively. The lower value in case of xPRO-SBA-I’ also corroborates the presence of aggregates/dimers when more dye is loaded into the pores, leading to a higher number of species with reduced fluorescence. The presence of Hg(II) then produces an increase in the average fluorescence lifetimes, correlating in both cases with Hg(II) concentration and increasing from 1.8 ns to 2.2 ns for xPRO-SBA-I and from 1.3 ns to 4.0 ns for xPRO-SBA-I’ upon addition of up to 2.5 ppm of Hg(II). This increase in average lifetime is higher for xPRO-SBA-I’ compared with xPRO-SBA-I, which agrees well with the steady-state fluorescence enhancement observed for the materials in suspension in the conventional fluorometer measurements (Figure 6) or shown in Figure S8. Apparently, the larger pores accommodate the complexes in a rather well-solvated state, showing decay kinetics that resemble more its behavior in neat solution.
Table S5. Average fluorescence lifetimes obtained through non-exponential fitting of the corresponding fluorescence decays of suspensions of \textit{xPRO-SBA-I} and \textit{xPRO-SBA-I′} in the presence of several amounts of Hg(II).

| Hg(II) / ppb | \(\tau_{\text{av}}\) \textit{xPRO-SBA-I} / ns | \(\tau_{\text{av}}\) \textit{xPRO-SBA-I′} / ns |
|--------------|----------------------------------------|----------------------------------|
| 0            | 1.89                                   | 1.29                             |
| 2.5          | 1.79                                   | 1.51                             |
| 25           | 1.96                                   | 2.82                             |
| 250          | 2.17                                   | 3.09                             |
| 2500         | 2.20                                   | 4.05                             |

The fluorescence decay behavior was also studied in the solid state, to verify the behavior of the materials spotted on the strips and measured in the dry state. For this purpose, suspensions were spotted on glass slides, to avoid scattering of the strips in the laser experiments, and exposed to various amounts of Hg(II). The fluorescence lifetime profiles were analyzed in this case with a PC using the software package FLA900 (Edinburgh Instruments) to allow for convolution with a distribution of decay components, the confinement plus drying potentially increasing the microheterogeneity of the probes’ environment.\[^{[12]}\] This model assumes that the molecular emitters show a quasi-continuous distribution of lifetimes (or reaction rates) which can be utilized to recover the decay kinetics.\[^{b}\] The envelope of such a lifetime distribution function usually shows several peaks, corresponding to the mean lifetimes \(\langle \tau \rangle\) (or centers of gravity) of certain preferred species or environmental situations, and each of the peaks is characterized by a specific width or spread \(\sigma\).

Analysis of the data revealed at bi-modal distributions of lifetimes in both materials in the absence of Hg(II) and multi-modal distributions in its presence. When focusing at that part of the species that correspond to the highly emissive complex \(\text{I–Hg(II)}\), the lifetime of the center of gravity increased from 3.7 ns via 4.0 ns to 5.2 ns when increasing the Hg(II) concentrations from 2.5 via 25 to 250 ppb and using \textit{xPRO-SBA-I} as the sensor material. These changes were much more pronounced than for \textit{xPRO-SBA-I′}, for which already at 2.5 ppb the limiting value of 5.7 ns was reached and this value remained unchanged upon further increasing Hg(II) concentration (Figure S9). For \textit{xPRO-SBA-I′}, Hg(II) seems to be bound only by rather mobile species of \textit{I} residing in the pores whereas in the case of \textit{xPRO-SBA-I} complexation seems to occur primarily in \textit{I} that is residing closer to the pore walls.

\[^{b}\] Quasi-continuous refers to the finite number of single lifetimes that the software employed allows to fit to a given set of data. Here, the best results were obtained with the maximum number of lifetimes (100) that the FLA900 program package allows used for the 4k data sets analyzed.
Figure S9. Representative decays of dyes and materials. a) Dye I and its Hg(II) complex in H$_2$O and MeCN; $\lambda_{ex}$ 488, $\lambda_{em}$ 540 nm, no filter. b) Aqueous suspensions of xPRO-SBA-I in the presence of different concentrations of Hg(II); $\lambda_{ex}$ 488, $\lambda_{em}$ 540 nm, with 500 nm cut-off filter. c) Aqueous suspensions of xPRO-SBA-I’ in the presence of different concentrations of Hg(II); $\lambda_{ex}$ 488, $\lambda_{em}$ 540 nm, with 500 nm cut-off filter. d) Slides with xPRO-SBA-I’ after exposure to various concentrations of Hg(II); $\lambda_{ex}$ 488, $\lambda_{em}$ 540 nm, with 500 nm cut-off filter.
S.8 Relative Measurement Uncertainties in a Conventional Assay

Because of the multiplicative and quotient forms of the respective equations and because correlations between the quantities are assumed to be negligible, summation of the squares of the relative uncertainties was performed.\cite{13}

S.8.1 Conventional assay in suspension

S.8.1.1 Preparation of suspensions and stock solutions

a) Weighing of ca. 4 mg of the corresponding material (balance Mettler Toledo MS204S ± 0.1 mg); $u_{rel}^w = 2.5\%$

b) Dissolving in 1 mL H$_2$O pH 7 (Eppendorf Reference pipette ± 0.006 mL); $u_{rel}^s = 0.6\%$

c) Diluting stock suspensions in H$_2$O pH 7, phosphate buffer (10 mM; pH 7), acetate buffer (10 mM; pH 4) or natural waters tested for kinetic or titration experiments (70 µL stock suspension in 2 mL H$_2$O pH 7 (Eppendorf Reference pipettes 100 ± 0.8 µL and 5000 ± 60 µL). $u_{rel}^d = (0.8+1.2)\%; 2\%$

d) Weighing of ca. 10 -20 mg of the corresponding salts AgClO$_4$, Ni(ClO$_4$)$_2$, Cu(ClO$_4$)$_2$ and Hg(ClO$_4$)$_2$ (balance Mettler Toledo MS204S ± 0.1 mg); $u_{rel}^{st} = 1\%$

e) Dissolving corresponding salts in 1 mL H$_2$O pH 7 (Eppendorf Reference pipette ± 0.006 mL); for obtaining Hg(II), Ag(I), Cu(II), and Ni(II) stock solutions of 10000 ppm; $u_{rel}^s = 0.8\%$

h) Diluting stock solution of Hg(II), Ag(I), Cu(II), and Ni(II) in H$_2$O pH 7 for obtaining standard Hg(II) 10×, 100×, 1000×, 1·10^4×, 1·10^5×, 1·10^6×, 1·10^7×, 1·10^9×, 1·10^{10}× in H$_2$O pH 7 (Eppendorf Reference pipette ± 0.02 µL). Successive dilution of the mother solution: 10µL in 100 µL MeCN; $n \times u_{rel}^d = n \times (1+0.8)\%; n = 1; 10×, n = 2; 100×, n = 3; 1000×, n = 4; 5·10^4×, n = 5; 5·10^5×, n = 6; 5·10^6×, n = 7; 5·10^7×, n = 8; 5·10^8×, n = 9; 5·10^9×, n = 10; 5·10^{10}×.$

S.8.1.2 Assay execution and preparation of measurement solutions

a) Taking 1-5 µL of corresponding standard metal solutions with 2.57 mL of suspensions of respective materials in H$_2$O pH 7, phosphate buffer (10 mM; pH 7), acetate buffer (10 mM; pH 4) or natural waters tested. (Eppendorf Reference pipette ± 0.01 mL); $u_{rel}^w = 0.8\%$

b) Contribution from cell length (± 0.01 mm); $u_{rel}^L = 0.1\%$

S.8.1.3 Fluorescence measurements

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

b) For the fluorescence intensities at $\lambda_i$, the maximum possible error amounts to: $u_{rel}^{em}/F_{\lambda_i} \leq 0.05\%$
S.8.1.4 Experimental standard deviation for replicate measurement

\[ u_{rel}^{\prime} \leq 2.6 \% \]

S.8.1.5 Relative uncertainty for experiments carried out in suspension

\[ u_{rel}^{2} = u_{rel}^{2} + u_{rel}^{2} + u_{rel}^{2} + u_{rel}^{2} + n \cdot u_{rel}^{2} + u_{rel}^{2} + u_{rel}^{2} + u_{rel}^{2} + \left( \frac{u_{ref}^{2}}{F \lambda} \right) ^{2} \]

\[ u_{rel}^{2} \leq 8.1 \% \]

Table S7. Calculation of relative errors during assays in suspension.

| Solutions       | Relative error on the measurement | \( u_{rel}^{\prime} \) % |
|-----------------|----------------------------------|--------------------------|
| Stock solution  |                                  |                          |
|                  | \( u_{rel}^{1} = u_{rel}^{2} + u_{rel}^{2} + u_{rel}^{2} + u_{rel}^{2} + 0 \cdot u_{rel}^{2} + u_{rel}^{2} + u_{rel}^{2} + u_{rel}^{2} + \left( \frac{u_{ref}^{2}}{F \lambda} \right) ^{2} + u_{rel}^{2} \) | 6.7                      |
| \( 10^{3} \times \) |                                  |                          |
|                  | \( u_{rel}^{2} = u_{rel}^{2} + u_{rel}^{2} + u_{rel}^{2} + u_{rel}^{2} + 3 \cdot u_{rel}^{2} + u_{rel}^{2} + u_{rel}^{2} + u_{rel}^{2} + \left( \frac{u_{ref}^{2}}{F \lambda} \right) ^{2} + u_{rel}^{2} \) | 7.1                      |
| \( 10^{5} \times \) |                                  |                          |
|                  | \( u_{rel}^{2} = u_{rel}^{2} + u_{rel}^{2} + u_{rel}^{2} + u_{rel}^{2} + 5 \cdot u_{rel}^{2} + u_{rel}^{2} + u_{rel}^{2} + u_{rel}^{2} + \left( \frac{u_{ref}^{2}}{F \lambda} \right) ^{2} + u_{rel}^{2} \) | 7.4                      |
| \( 10^{8} \times \) |                                  |                          |
|                  | \( u_{rel}^{2} = u_{rel}^{2} + u_{rel}^{2} + u_{rel}^{2} + u_{rel}^{2} + 8 \cdot u_{rel}^{2} + u_{rel}^{2} + u_{rel}^{2} + u_{rel}^{2} + \left( \frac{u_{ref}^{2}}{F \lambda} \right) ^{2} + u_{rel}^{2} \) | 7.8                      |
| \( 10^{10} \times \) |                                  |                          |
|                  | \( u_{rel}^{2} = u_{rel}^{2} + u_{rel}^{2} + u_{rel}^{2} + u_{rel}^{2} + 10 \cdot u_{rel}^{2} + u_{rel}^{2} + u_{rel}^{2} + u_{rel}^{2} + \left( \frac{u_{ref}^{2}}{F \lambda} \right) ^{2} + u_{rel}^{2} \) | 8.1                      |

S.8.2 Conventional Assay on strips

S.8.2.1 Strip preparation

a) Weighing of ca. 4 mg of the corresponding material (balance Mettler Toledo MS204S ± 0.1 mg); \( u_{rel}^{w} = 2.5 \% \)

b) Dissolving in 0.8 or 0.4 mL H2O pH 7 to have stock suspensions of 5 mg mL\(^{-1}\) and 10 mg mL\(^{-1}\) (Eppendorf Reference pipette ± 0.006 mL); \( u_{rel}^{d} = 0.6 \% \)

c) Deposition of 2 µL of suspension onto the strip (Eppendorf Reference pipette ± 0.02 µL); \( u_{rel}^{dep} = 2 \% \)

S.8.2.2 Preparation of stock solutions
(as described in 7.1.1 e) and h).

S.8.2.3 Assay execution

Deposition of 2 µL of Hg(II) solutions onto the strip (Eppendorf Reference pipette ± 0.02 µL);

\( u_{rel}^{a} = 2 \% \)
S.8.2.4 Fluorescence measurements
Relative uncertainty of the fluorescence intensities < 1 mV at maximum voltage (2000 mV):
\[ u_{\text{rel}}^{\text{em}} \leq 3.5\% \]

S.8.2.5 Strip positioning in reader
\[ u_{\text{rel}}^{\text{er}} \leq 3.3\% \]

S.8.2.6 Digital camera measurements
Relative uncertainty of the photograph acquisition (dark current noise) and RGB values acquisition:
\[ u_{\text{rel}}^{\text{ph}} \leq 4.5\% \]

S.8.2.7 Experimental standard deviation for replicate measurements
\[ u_{\text{rel}} \leq 5\% \]

S.8.2.8 Relative uncertainty
\[
\begin{align*}
    u_{\text{rel}}^{\text{reader}}^2 &= u_{\text{rel}}^w + u_{\text{rel}}^r + u_{\text{rel}}^{\text{dep}} + u_{\text{rel}}^{\text{wst}} + u_{\text{rel}}^d + n * u_{\text{rel}}^d + u_{\text{rel}}^e + u_{\text{rel}}^r + u_{\text{rel}}^r \leq 8.7 \\
    u_{\text{rel}}^{\text{camera}}^2 &= u_{\text{rel}}^w + u_{\text{rel}}^r + u_{\text{rel}}^{\text{dep}} + u_{\text{rel}}^{\text{wst}} + u_{\text{rel}}^d + n * u_{\text{rel}}^d + u_{\text{rel}}^e + u_{\text{rel}}^r + u_{\text{rel}}^r \leq 8.6
\end{align*}
\]

Table S8. Calculation of relative errors during assays using strips

| Instrument | Solutions | Relative error on the measurement | % |
|------------|-----------|-----------------------------------|----|
| Reader     | 10^2×     | \[ u_{\text{rel}}^{\text{reader}}^2 \] | 8.1 |
| Camera     |           | \[ u_{\text{rel}}^{\text{camera}}^2 \] | 8.3 |
| Reader     | 10^4×     | \[ u_{\text{rel}}^{\text{reader}}^2 \] | 8.3 |
| Camera     |           | \[ u_{\text{rel}}^{\text{camera}}^2 \] | 8.5 |
| Reader     | 10^6×     | \[ u_{\text{rel}}^{\text{reader}}^2 \] | 8.6 |
| Camera     |           | \[ u_{\text{rel}}^{\text{camera}}^2 \] | 8.7 |
S.9 References

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