Supplementary information

The Supplementary information contains the following figures:

Fig. S1. Absorption spectrum of 2-nitrophenol in NaOH modified - agarose gel (1%). Gel volume (○) 150 µL, (■) 200 µL

Fig. S2. Effect of NaOH concentration on absorbance of 2-nitrophenol

Fig. S3. Absorption spectra in NQS modified - agarose gel (1%): (○) aniline coupled with NQS in gel against reagent blank, (■) NQS against agarose gel reagent blank

Fig. S4. Effect of NQS volume on absorbance of aniline coupled with NQS

Fig. S5. $^1$H-NMR for 3-amino-N-phenylpropanamide, TFA

Fig. S6. $^{13}$C-NMR for 3-amino-N-phenylpropanamide, TFA

Fig. S7. $^1$H-NMR for 3-amino-N-phenylpropanamide

Fig. S8. $^{13}$C-NMR for 3-amino-N-phenylpropanamide
The Supplementary information contains the following Tables:

Table S1. Effect of percentage agarose on exogenous VOC detection

Table S2. Effect of NaOH concentration on absorbance of aniline coupled with NQS

Table S3. Analytical data for 2-nitrophenol and aniline trapped in modified agarose gel
S1. Optimisation of percentage agarose

Percentage agarose had little effect on the absorbance readings when tested with 20 µg mL\(^{-1}\) aniline in BHI (Table S1). However, 2 % agarose gave slightly higher and 0.5 % agarose slightly lower absorbance readings with 50 µg mL\(^{-1}\) of 2-nitrophenol in BHI. In addition, gels containing 2 % agarose set more quickly than gels with a lower percentage of agarose. On that basis it was concluded that a 1% agarose gel was appropriate; this was applied to further work.

S2. Optimisation of 2-nitrophenol detection method

A yellow colour developed when agarose gel with added sodium hydroxide was exposed to 2-nitrophenol. Aliquots (100 µL, 150 µL and 200 µL) of 1% agarose with 2 mmol L\(^{-1}\) NaOH were tested with 50 µg mL\(^{-1}\) of 2-nitrophenol in BHI media. The smallest volume of agarose gel i.e. 100 µL was discarded due to downward curving of the surface of the gel. Absorption spectra of 2-nitrophenol trapped in agarose gel with both 150 µL and 200 µL of gel were generated (Figure S1). It was noted that an agarose gel volume of 150 µL produced the highest absorbance reading; on that basis 150 µL of agarose gel was used in all further work. In addition, the maximum absorption wavelength for 2-nitrophenol in 1 % agarose gel was 415 nm; all future 2-nitrophenol measurements were carried out at 415 nm.

The concentration range of sodium hydroxide added to agarose gel was assessed to determine the effect of increasing alkalinity on the yellow colour obtained after overnight incubation. Final NaOH concentrations of 0.1, 0.5, 1.0, 2.0 and 5.0 mmol L\(^{-1}\) in agarose
gel were tested with 50 µg mL\(^{-1}\) of 2-nitrophenol in BHI media. The Absorbance signal, and visual yellow colour development, increased with increasing NaOH concentration. However, there was a limited increase in absorbance and colour development at a NaOH concentration greater than 2 mmol L\(^{-1}\) (Figure S2); it was therefore concluded that the optimum NaOH in 1% agarose gel was 2 mmol L\(^{-1}\).

**S3. Optimisation of aniline detection method**

An orange colour developed when NQS modified agarose gel was exposed to aniline. An absorption spectrum of aniline with 150 µL of 1% agarose gel modified with NQS was produced (Figure S3). The maximum absorption wavelength was 470 nm. All future measurements with aniline were carried out at 470 nm. An absorption spectrum of NQS against blank agarose gel indicated a maximum absorption wavelength of 355 nm and no overlap with the spectrum obtained for aniline coupled with NQS.

A range of NQS volumes added to agarose gel was tested (Figure S4); a 0.5 % NQS solution was added to 50 mL gel and the effect of amount of NQS in the gel was determined. NQS volumes of 0.05, 0.1, 0.125, 0.25, 0.5, 1.0 mL in 50 mL agarose gel were tested with 20 µg mL\(^{-1}\) aniline in BHI media. The orange colour became more intense as NQS volume increased. However, at higher NQS volumes (1.0 and 0.5 mL), a red precipitate formed in the gel, therefore 0.25 mL NQS (0.5 %) was applied to further work.
The addition of NaOH to agarose gel and its effect on colour development was investigated. Final NaOH concentrations of 0, 0.01, 0.1, 0.5, 1.0 and 2.0 mmol L\(^{-1}\) NaOH in 50 mL agarose gel with 0.25 mL NQS (0.5\%) were tested with 20 \(\mu\)g mL\(^{-1}\) aniline in BHI media (Table S2). The addition of NaOH had no effect on absorbance readings; therefore NaOH was omitted from all further work with aniline.

**S4 Analytical calibration data for 2-nitrophenol and aniline**

The linear range for 2-nitrophenol in BHI media, measured at 415 nm, was 10 - 50 \(\mu\)g mL\(^{-1}\); whereas for aniline, measured at 470 nm, it was 5 - 20 \(\mu\)g mL\(^{-1}\) in BHI media. All measurements were taken following 18 h of incubation at 37 °C. Analytical data for the quantification of 2-nitrophenol and aniline trapped in modified agarose gels are given in Table S3.
S5. Synthesis of 3-amino-N-phenylpropanamide, TFA

![Chemical structure]

[a] DCCI, N-hydroxy succinimide [b] TFA

S6. Analytical data for 3-amino-N-phenylpropanamide, TFA

3-Amino-N-phenylpropanamide, TFA salt, obtained as an off white powder, m.p. 105-107 °C. LRMS (ESI) for C₉H₁₂N₂O.

Calculated mass of molecular ion 165.21 [M+H]+. Measured mass: 164.93.

¹H-NMR (400 MHz; d₆-DMSO) δH 10.13 (1H, s, NH), 7.75 (3H, s, NH₃⁺), 7.55 (2H, d, J = 7.6 Hz, Ar-H), 7.26 (2H, t, J = 8.0 Hz, Ar-H), 7.00 (1H, t, J = 7.4 Hz, Ar-H), 3.04 (2H, t, J = 7.0
Hz, $CH_2$), 2.65 (2H, $t$, $J = 6.6$ Hz, $CH_2$) (Figure S5); $^{13}$C-NMR (101 MHz; $d_6$-DMSO) $\delta_C$ 168.3 (C=O), 159.0 (q, $J = 38$ Hz, $CF_3CO_2^-$),
139.7 (Ar-C), 129.3 (Ar-C), 124.0 (Ar-C), 119.6 (Ar-C), 115.9 (q, $J = 288$ Hz, $CF_3CO_2^-$), 35.5 ($CH_2$), 33.9 ($CH_2$) (Figure S6).

S7. Analytical data for 3-amino-N-phenylpropanamide

3-Amino-N-phenylpropanamide was obtained as a white solid. $^1$H-NMR (400 MHz; CDCl$_3$) $\delta_H$ 9.98 (1H, broad s, NH), 7.51 (2H, d, $J = 8$ Hz, Ar-$H$), 7.26 (2H, $t$, $J = 8$ Hz, Ar-$H$), 7.02 (1H, t, $J = 8$ Hz, Ar-$H$), 3.05 (2H, broad m, $CH_2$), 2.43 (2H, $t$, $J = 6$ Hz, $CH_2$). (Figure S7). This data is consistent with that reported in the literature.$^{15}$

$^{13}$C-NMR (101 MHz; CDCl$_3$) $\delta_C$ 171.2 (C=O), 138.5, (Ar-C), 129.0 (Ar-C), 123.9 (Ar-C), 119.9 (Ar-C), 38.8 ($CH_2$), 38.0 ($CH_2$). (Figure S8).
Fig. S1. Absorption spectrum of 2-nitrophenol in NaOH modified - agarose gel (1%). Gel volume (○) 150 µL, (●) 200 µL
Fig. S2. Effect of NaOH concentration on absorbance of 2-nitrophenol

50 µg mL⁻¹ 2-nitrophenol in BHI; 150 µL gel; overnight incubation.
Fig. S3. Absorption spectra in NQS modified - agarose gel (1%): (○) aniline coupled with NQS in gel against reagent blank, 

(●) NQS against agarose gel reagent blank
Fig. S4. Effect of NQS volume on absorbance of aniline coupled with NQS

20 µg mL$^{-1}$ aniline in BHI, 150 µL gel, overnight incubation.
Fig. S5. $^1$H-NMR for 3-amino-N-phenylpropanamide, TFA

- **Label**: SRR ER1_Proton-2-1.png
  - **Type**: single_pulse

---

Filename = SRR ER1_Proton-2-1.png
Author = delta
Experiment = proton
Sample_ID = SRR ER1
SdsetID = DSCP-06
Creation_Time = 10-SEP-2014 09:48:19
Revision_Time = 10-SEP-2014 09:49:23
Current_Time = 10-SEP-2014 09:49:24

- **Field_strength**: 9.40974e+07 (900 MHz)
- **X_Avg_Duration**: 1.183851950 (s)
- **X_Domain**: 1.0
- **X_Freq**: 500.72019636 (MHz)
- **X_Offset**: 5.0 (ppm)
- **X_Fs**: 16394
- **Resolution**: 0.48794505 (Hz)
- **X_ref**: 7.0030042 (ppm)
- **X_res**: 0.002400948 (ppm)
- **FDomain**: Proton
- **Fp**: 565.72019636 (MHz)
- **Fp_Offset**: 5.0 (ppm)
- **Tri_Freq**: 565.72019636 (MHz)
- **Tri_Offset**: 5.0 (ppm)
- **Clipped**: FALSE
- **Zoom**: 1
- **Total_Images**: 1
- **Relaxation_Delay**: 5 (s)
  - **Recvr_Gain**: 40
  - **Temp_Gain**: 10.4145
  - **X_W_Weight**: 10.455 (u)
  - **X_Avg_Time**: 1.183851950 (s)
  - **X_Angle**: 413 (deg)
  - **X_Ato**: 0.99 (dB)
  - **X_Pulse**: 5.329 (ms)
  - **Tri_Mode**: OFF
  - **Tri_Mode**: OFF
  - **Emute_Freq**: FALSE
Fig. S6. $^{13}$C-NMR for 3-amino-N-phenylpropanamide, TFA
Fig. S7. $^1$H-NMR for 3-amino-N-phenylpropanamide
Fig. S8. $^{13}$C-NMR for 3-amino-N-phenylpropanamide
Table S1. Effect of percentage agarose on exogenous VOC detection

<table>
<thead>
<tr>
<th>% Agarose*</th>
<th>2-Nitrophenol (absorbance at 415 nm)†</th>
<th>Aniline (absorbance at 470 nm)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.138</td>
<td>0.132</td>
</tr>
<tr>
<td>1</td>
<td>0.147</td>
<td>0.134</td>
</tr>
<tr>
<td>2</td>
<td>0.165</td>
<td>0.124</td>
</tr>
</tbody>
</table>

*150 µL volume; †50 µg mL⁻¹ 2-nitrophenol in BHI, overnight incubation; ‡20 µg mL⁻¹ aniline in BHI, overnight incubation.
Table S2. Effect of NaOH concentration on absorbance of aniline coupled with NQS

<table>
<thead>
<tr>
<th>NaOH concentration (mmol L(^{-1}))(^\dagger)</th>
<th>Absorbance at 470 nm(^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.123</td>
</tr>
<tr>
<td>0.01</td>
<td>0.124</td>
</tr>
<tr>
<td>0.1</td>
<td>0.129</td>
</tr>
<tr>
<td>0.5</td>
<td>0.125</td>
</tr>
<tr>
<td>1</td>
<td>0.125</td>
</tr>
<tr>
<td>2</td>
<td>0.09</td>
</tr>
</tbody>
</table>

\(^*\)20 µg mL\(^{-1}\) aniline in BHI; \(^\dagger\)0.25 mL NQS (0.5 %) in 150 µL gel.
Table S3. Analytical data for 2-nitrophenol and aniline trapped in modified agarose gel

<table>
<thead>
<tr>
<th>VOC</th>
<th>Linear range (µg mL⁻¹)</th>
<th>y = mx + c</th>
<th>Correlation coefficient</th>
<th>n*</th>
<th>λ_max (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Nitrophenol</td>
<td>10 - 50</td>
<td>y = 0.0024x + 0.0055</td>
<td>0.9998</td>
<td>4</td>
<td>415</td>
</tr>
<tr>
<td>Aniline</td>
<td>5 - 20</td>
<td>y = 0.0095x + 0.0165</td>
<td>0.9901</td>
<td>4</td>
<td>470</td>
</tr>
</tbody>
</table>

*n, number of points on calibration curve.