Tailored fluorescent solvatochromic test strips for quantitative on-site detection of gasoline fuel adulteration

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I. Materials, methods and instrumentation

Kerosene and absolute ethanol (max 5 ppm carbonyl compounds) were commercially available at Aldrich and gasoline E5 and E10 grade were obtained from HEM, Shell and Esso gas stations in Berlin, Germany. The pre-treatment to remove fluorescent interferences followed either a laboratory-based protocol in which 10 wt% of active charcoal was suspended in fresh mixtures, stirred for 1 h, centrifuged and filtered to remove the charcoal, or an embedded protocol using a stainless steel in-line filter holder (47 mm, PALL) with active carbon paper filters (typically 4).[1]

Reagents for synthetic procedures were obtained from commercial suppliers and used without further purification, unless indicated. Air- and moisture-sensitive reactions were carried out with previously dried materials under N_2 atmosphere. TLC experiments were performed over Merck Silica Gel 60 F_{254} TLC. Reactions were monitored using a 254 nm hand-held lamp. Column chromatography was carried out with Merck Silica gel 60 (0.040–0.063 mm). NMR spectra were recorded on a 400 MHz (101 MHz for ^{13}C) Bruker AV 400 spectrometer at 300 K using residual protonated solvent signals as internal standard (^1H: δ ($CDCl_3$) = 7.26 ppm and ^{13}C: δ ($CDCl_3$) = 77.16 ppm). Assignments are based on chemical shifts. Mass spectra were measured on a Waters LCT Premier XE.

UV-vis absorption spectra were recorded on a Specord 210-Plus spectrophotometer from Analytik Jena. Steady-state fluorescence measurements were carried out on a FluoroMax-4 spectrofluorometer from Horiba Jobin-Yvon, using standard 10 mm path length quartz cells. All the solvents employed for the spectroscopic measurements were of UV spectroscopic grade (Aldrich).

The smartphone application ("App") was developed using Android Studio 2.0 and Android SDK tools API 22 (Android 5.1). The phone camera was set with a shutter speed of 1/1000 s, a white balance at 5000 K, a Macro Focus and ISO value of 1600 (kerosene) or 64 (ethanol).

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[1] Gotor, R.; Tiebe, C.; Schlischka, J.; Bell, J.; Rurack, K. *Energy & Fuels* **2017,** *31* (11), 11594-11600.

II. Procedures

4-((4-(Diphenylamino)phenyl)ethynyl)benzonitrile 1

Compound **1** was synthesized according a reported protocol with a 63 % yield.[2]

4-Bromo-*N*-(4-ethynylphenyl)-*N*-phenylaniline 3.

1 g (2.5 mmol) of 4,4'-Dibromotriphenylamine and 346 µL (2.5 mmol) of ethynyltrimethylsilane were dissolved in a 20 mL mixture of dry THF and Et_3N 1:1 v/v. The solution was deoxygenated by sparkling Ar(g) for 10 min. After that time, 87 mg of bis(triphenylphosphine)palladium(II) dichloride (0.13 mmol) and 47 mg copper(I) iodide (0.25 mmol) were added to the mixture and the reaction was heated for 3 h at 50 °C, before increasing to 80°C and keeping it overnight. Afterwards, the mixture was allowed to cool down, saturated NH_4Cl solution was added, and the products were extracted three times using CH_2Cl_2. The organic layer was washed with a saturated NaCl solution and dried with Na_2SO_4. 456 mg of an oily mixture were obtained. Consequently, 400 mg (0.98 mmol) of this product and 817 mg (5.9 mmol) of potassium carbonate were mixed in a 20 mL mixture of CH_2Cl_2 and MeOH 1:1 v/v. The reaction was kept for 1 h. Thereafter, saturated NH_4Cl solution was added, and the products were extracted three times using CH_2Cl_2. The organic layer was washed with saturated NaCl solution and dried with Na_2SO_4. A column chromatography (PET:CH_2Cl_2, 95:5 v/v) yielded 232 mg (30 %) of the desired product **3** after two steps. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.37 – 7.31 (m, 4H, Ar-H), 7.30 – 7.23 (m, 3H, Ar-H), 7.10 – 7.04 (m, 3H, Ar-H), 6.95 (d, *J* = 8.7 Hz, 4H, Ar-H), 3.01 (s, 1H, ≡CH). ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.8, 146.7, 146.3, 133.2, 132.4, 129.6, 126.0, 125.1, 122.5, 115.9, 115.5, 83.7, 83.7. HR-MS (ESI+): *m/z* calculated for $C_{20}H_{15}BrN$ $[M+H]^+$: 348.0388, found: 348.0397.

4-((4-((4-Bromophenyl)(phenyl)amino)phenyl)ethynyl)benzonitrile 4.

127 mg (0.55 mmol) of 4-Iodobenzonitrile was dissolved in a 5 mL mixture of dry THF and Et_3N 1:1 v/v. The mixture was flushed with Ar(g) for 10 min and afterwards 20 mg of bis(triphenylphosphine)-palladium(II) dichloride (28 µmol) and 10 mg copper(I) iodide (56 µmol) were added to the mixture, followed by 200 mg (0.57 mmol) of **3.** The reaction was kept at room temperature overnight. Saturated NH_4Cl solution was then added, and the products were extracted three times using CH_2Cl_2. The organic layer was washed with saturated NaCl solution and dried with Na_2SO_4. A column chromatography (PET:CH_2Cl_2, 8:2 v/v) yielded 167 mg (65 %) of the desired product **4**. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.62 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.57 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.41 – 7.35 (m, 4H, Ar-H), 7.30 (dd, *J* = 8.7, 7.3 Hz, 2H, Ar-H), 7.14 – 7.08 (m, 3H, Ar-H), 7.03 – 6.97 (m, 4H, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.2, 146.5, 146.1, 132.9, 132.5, 132.0, 131.8, 129.6, 128.5, 126.3, 125.3, 124.3, 122.2, 118.6, 116.3, 115.3, 111.1, 94.2, 87.4. HR-MS (ESI+): *m/z* calculated for $C_{27}H_{18}BrN_2$ $[M+H]^+$: 449.0653, found: 449.0643.

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[2] Piao, M. J.; Chajara, K.; Yoon, S. J.; Kim, H. M.; Jeon, S.-J.; Kim, T.-H.; Song, K.; Asselberghs, I.; Persoons, A.; Clays, K.; Cho, B. R. *Journal of Materials Chemistry* **2006,** *16* (23), 2273-2281.

4-((4-((4-(5-Hydroxypent-1-yn-1-yl)phenyl)(phenyl)amino)phenyl)ethynyl)benzonitrile 5.

100 mg (0.22 mmol) of **4** and was dissolved in a 5 mL mixture of dry THF and Et_3N 1:1 v/v. The solution was deoxygenated by sparkling Ar(g) for 10 min. Thereafter, 15 mg of bis(triphenylphosphine)-palladium(II) dichloride (0.02 mmol) and 8 mg copper(I) iodide (0.04 mmol) were added to the mixture and allowed to react. Then, 30 µL (0.33 mmol) of 4-pentyn-1-ol was added and the mixture was heated overnight at 70 °C. Afterwards, the mixture was cooled down and the mixture was filtered through Celite and then saturated NH_4Cl solution was added. The products were extracted three times using CH_2Cl_2. The organic layer was washed with saturated NaCl solution and dried with Na_2SO_4. An isocratic column chromatography (cyclohexane:EtOAc, 8:2 to 7:3 v/v) yielded 78 mg (78 %) of the desired product **5**. ^1H NMR (400 MHz, Chloroform-*d*) δ 7.55 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.50 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.31 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.26 – 7.19 (m, 4H, Ar-H), 7.04 (dd, *J* = 6.2, 2.7 Hz, 3H, Ar-H), 6.94 (dd, *J* = 8.7, 2.8 Hz, 4H, Ar-H), 3.76 (t, *J* = 6.1 Hz, 2H, $\equiv C\text{-}CH_2\text{-}CH_2\text{-}\underline{CH_2}\text{-}OH$), 2.47 (t, *J* = 7.0 Hz, 2H, $\equiv C\text{-}\underline{CH_2}\text{-}CH_2\text{-}CH_2\text{-}OH$), 1.79 (p, *J* = 6.5 Hz, 2H, $\equiv C\text{-}CH_2\text{-}\underline{CH_2}\text{-}CH_2\text{-}OH$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.2, 146.6, 146.4, 132.9, 132.7, 132.0, 131.9, 129.6, 128.6, 125.5, 124.3, 124.1, 122.5, 118.6, 118.4, 115.3, 111.1, 94.3, 88.3, 87.4, 80.9, 61.9, 31.5, 16.1. HR-MS (ESI+): *m/z* calculated for $C_{32}H_{25}N_2O$ $[M+H]^+$: 453.1967, found: 453.1975.

4-((5-(4-((4-((4-Cyanophenyl)ethynyl)phenyl)(phenyl)amino)phenyl)pent-4-yn-1-yl)oxy)-4-oxobutanoic acid 2.

20 mg of **5** (0.04 mmol), 0.53 mg of 4-dimethylaminopyridine (4.4 µmol) and 5 mg of succinic anhydride (0.05 mmol) were dissolved in 1.6 mL of CH_2Cl_2. To this mixture, 3.2 µL of Et_3N (0.05 mmol) were added, and the reaction was kept overnight at room temperature. Then, 1 mL of water was added, and the pH was adjusted to 2 with AcOH. The mixture was washed two times with water and a saturated NaCl solution. Next, it was purified using column chromatography (PET:EtOAc 3:7 v/v) yielding 21 mg (86 %) of the desired dye **2**. ^1H NMR (400 MHz, Chloroform-*d*) δ 7.54 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.49 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.31 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.24 – 7.19 (m, 4H, Ar-H), 7.06 – 7.00 (m, 3H, Ar-H), 6.93 (dd, *J* = 8.7, 4.2 Hz, 4H, Ar-H), 4.19 (t, *J* = 6.3 Hz, 2H, $\equiv C\text{-}CH_2\text{-}CH_2\text{-}\underline{CH_2}\text{-}O\text{-}$), 2.64 – 2.55 (m, 4H, $\text{-}CO\text{-}\underline{CH_2}\text{-}\underline{CH_2}\text{-}COOH$), 2.43 (t, *J* = 7.0 Hz, 2H, $\equiv C\text{-}\underline{CH_2}\text{-}CH_2\text{-}CH_2\text{-}O\text{-}$), 1.86 (p, *J* = 6.7 Hz, 2H, $\equiv C\text{-}CH_2\text{-}\underline{CH_2}\text{-}CH_2\text{-}O\text{-}$).$^{13}C$ NMR (101 MHz, $CDCl_3$) δ 176.9, 172.1, 148.2, 146.5, 146.5, 132.8, 132.7, 132.0, 131.8, 129.6, 128.6, 125.5, 124.3, 124.0, 122.4, 118.6, 118.3, 115.2, 111.0, 94.3, 88.2, 87.4, 81.0, 63.6, 28.9, 28.7, 27.8, 16.2. HR-MS (ESI+): *m/z* calculated for $C_{36}H_{29}N_2O_4$ $[M+H]^+$: 553.2127, found: 553.2120.

III. Photophysical properties

Tab. S1 Photophysical properties of dyes **1** and **2** in various solvents and gasoline.

Dyes	**1**				**2**			
Solvent	λ_{abs} / nm	λ_{em} / nm	Stokes shift / cm^{-1}	Φ_f	λ_{abs} / nm	λ_{em} / nm	Stokes shift / cm^{-1}	Φ_f
n-Hexane	380	401	1378	0.66	382	406	1547	0.45
Toluene	381	432	3099	0.95	385	436	3038	0.790
$CHCl_3$	384	472	4855	0.99	386	475	4854	0.99
EtOH	375	515	7249	0.78	379	521	7191	0.57
MeCN	372	543	8466	0.58	375	549	8452	0.37
Gasoline	379	430	3129	0.77	387	435	2851	0.69
Kerosene	384	406	1411	0.706	388	411	1442	0.714

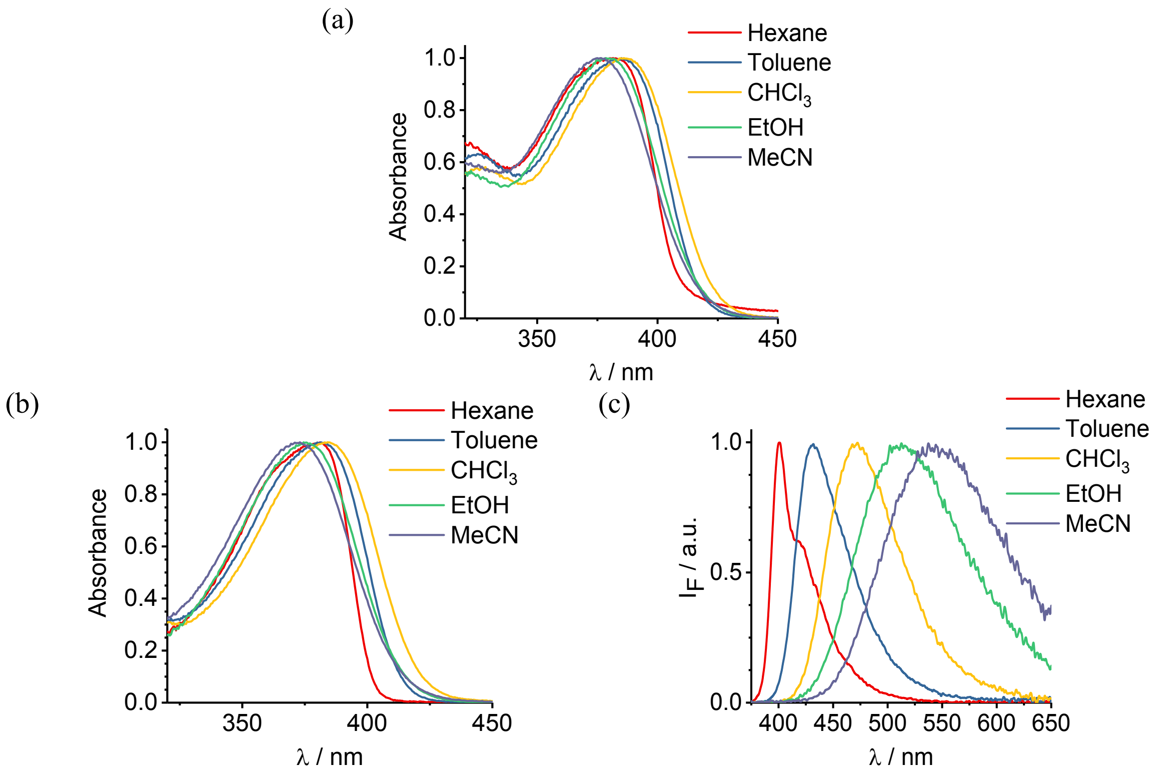

Fig. S2 Normalized absorption of (a) **2** and (b) **1** and normalized emission of (c) **1** in various solvents ([**1**] = [**2**] = 4 µM, λ_{exc} = 340 nm)

IV. Test strip preparation and analyses

Preparation of M1 test strips

Whatman 1 paper filter (1×4 cm, 20 pieces) were added to a 100 mL toluene solution containing 2.5 mL of APTES and the mixture was agitated at 90 °C for 24 h. After that time the strips were washed thoroughly with EtOH and dried in air. Then, 10 mL of a $4\cdot10^{-4}$ M solution of **2** in CH_2Cl_2 and 0.8 mg (4 µmol) of DCC were mixed for 30 min. After that time, 280 mg of aminated Whatman paper 1 and 0.5 µL of Et_3N (4 µmol) were added to the mixture, and the reaction was kept for additional 2 h. The paper was washed several times with CH_2Cl_2 and then with EtOH and left to dry in air. Differential absorption analysis of the initial solution and final solutions, together with the washing fractions, revealed that the dye was grafted to the paper at a concentration of $3.3\ \mu mol\cdot g^{-1}{}_{paper}$.

Preparation of M2′ and M2 test strips

A mixture of 15 mL toluene and 1 mL APTES was added to 15 glass fiber test strips (Whatman glass fiber paper, 0.5×2.5 cm, 0.3 g). The reaction proceeded over 24 h at room temperature with constant stirring. The strips were then removed and washed 3 times with ethanol (3×15 mL) and dried in air for 15 min. Then, 10 mL of a 4.10^{-4} M solution of **2** in CH_2Cl_2 and 0.8 mg (4 µmol) of DCC were mixed for 30 min at room temperature. The test strips and 0.25 µL of triethylamine were added and stirred for 2 h at room temperature. The strips were then removed and washed 2 times with dichloromethane (2×10 mL) and 2 times with ethanol (2×10 mL) and dried in air for 15 min, yielding **M2′**. For the passivated strips **M2**, a mixture of 10 mL hexane and 1.5 mL HMDS was added to strips **M2′**. The reaction proceeded over 24 h at room temperature with constant stirring. Strips **M2** were then removed and washed 2 times with acetone (2×10 mL) and 2 times with ethanol (2×10 mL) and dried in air for 15 min.

Preparation of silica coated cellulose strips[3,4]

A mixture (7 mL) with pre-determined proportion of TEOS, distilled water, alcohol and catalyst (see table S2) was added to 3 cellulose test strips (Whatman 1 paper filter, 0.5×5 cm, 0.09 g). The reaction proceeded for 24 h at room temperature with constant stirring. The final hybrid material was removed and washed 3 times with ethanol (3×5 mL) and dried under reduced pressure. The percentage of silica incorporation was determined by weighing the hybrid material.

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[3] Sequeira, S.; Evtuguin, D. V.; Portugal, I.; Esculcas, A. P. *Materials Science and Engineering: C* **2007**, *27*, 172.

[4] Cai, J.; Liu, S.; Feng, J.; Kimura, S.; Wada, M.; Kuga, S.; Zhang, L. *Angewandte Chemie International Edition* **2012**, *51*, 2076.

Preparation of M3′ and M3 test strips

A mixture of 15 mL toluene and 1 mL APTES was added to 7 silica coated cellulose test strips (0.5×2.5 cm, 0.09 g) prepared following the entries #6 and #10 from Table S2. The reaction proceeded over 24 h at room temperature with constant stirring. The strips were then removed and washed 3 times with ethanol (3×15 mL) and dried in air for 15 min. Then, 10 mL of a 4.10^{-4} M solution of **2** in CH_2Cl_2 and 0.82 mg (4 μmol) of DCC were mixed for 30 min at room temperature. The test strips and 0.3 μL of triethylamine were added and stirred for 2 h at room temperature. The strips were then removed and washed 2 times with dichloromethane (2×10 mL) and 2 times with ethanol (2×10 mL) and dried in air for 15 min, yielding **M3′**. For the passivated strips **M3**, a mixture of 10 mL hexane and 1.5 mL HMDS was added to **M3′** strips. The reaction proceeded over 24 h at room temperature with constant stirring. Strips **M3** were then removed and washed 2 times with acetone (2×10 mL) and 2 times with ethanol (2×10 mL) and dried in air for 15 min. Differential absorption analysis of the initial solutions and final solutions after grafting of **2** revealed that the dye was grafted to **M3′** at concentrations of 9 $\mu mol \cdot g^{-1}_{paper}$ for strips prepared following entry #6 and 21 $\mu mol \cdot g^{-1}_{paper}$ for strips prepared following entry #10.

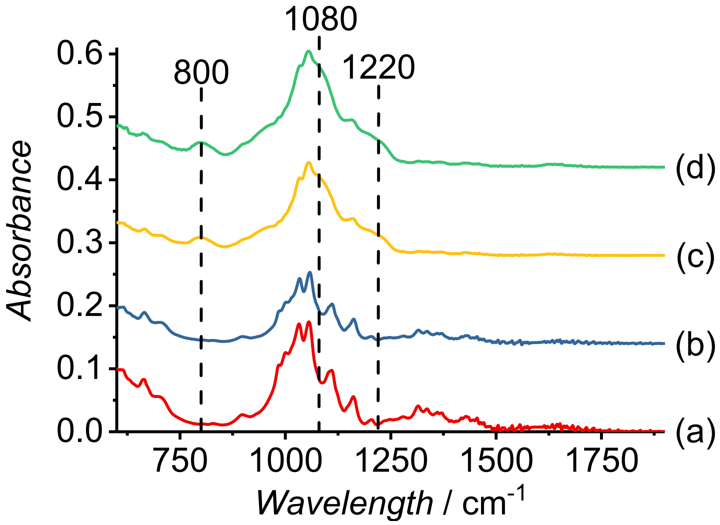

Fig. S3. Infrared absorption spectra acquired on a BRUKER-Equinox 55, IR-Scope with ATR-Objective of the cellulose without coating (a) and silica-coated cellulose strip obtained from entries #4 (b), #6 (c) and #10 (d) of Table S2. The bands at 800 and 1080 cm^{-1} are associated with rocking, bending (or symmetric stretching) and asymmetric stretching of SiO_2 inter-tetrahedral oxygen atoms. The band at 1080 cm^{-1} overlaps with a broad band between 1000 and 1150 cm^{-1} attributed to O–H bending of primary and secondary alcohol groups of cellulose. The presence of cyclic silica $(SiO)_6$ structures was confirmed as revealed from the characteristic band at 1220 cm^{-1}.

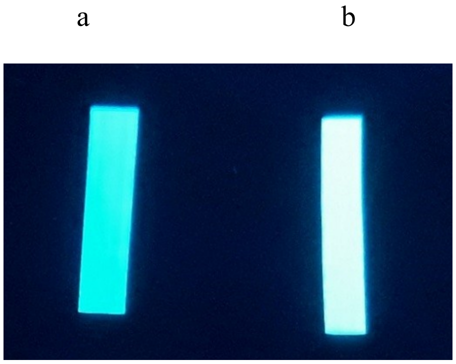

Fig. S4 Pictures upon UV excitation of the dried **M3** test strips obtained from silica-coated cellulose strips prepared following entries #6 (a) and #10 (b) of table S2 (λ_{exc} = 365 nm).

Tab. S2 Preparation and analyses of the silica coated cellulose strips and of the sensory test strips **M1**, **M2′**, **M2**, **M3′** and **M3**.

#	Water	Ethanol	TEOS	HNO_3 0.1M	NH_3 32%	Weight increase	Strain	λ_{em}(max) for		
								Kerosene	Gasoline	Ethanol
	mL	mL	mL	µL	µL	%	%	nm	nm	nm
1	1.8	4.3	0.7	210	-	-10.0	4.5	-	-	-
2	2.0	4.3	0.7	21	-	9.8	3.6	-	-	-
3	2.0	4.3	0.7	2	-	-1.5	3.1	-	-	-
4	2.0	4.5	0.3	210	-	-12.4	3.6	-	-	-
5	1.6	4.1	1.3	210	-	-1.6	4.1	-	-	-
6	1.8	3.9	1.1	-	226	23.8	3.0	-	-	-
7	1.9	3.9	1.1	-	90	13.4	3.6	-	-	-
8	2.0	3.9	1.1	-	9	-0.7	3.2	-	-	-
9	2.1	4.2	0.5	-	90	5.3	3.7	-	-	-
10	1.7	3.7	1.5	-	90	26.6	2.7	-	-	-
11	1,4	3,9	1,1	-	630	13.9	4.0	-	-	-
12	1,6	3,9	1,1	-	420	15.8	3.4	-	-	-
13	2,0	2,7	2,5	-	90	8.8	3.9	-	-	-
14	2,0	3,2	2,0	-	90	19.7	3.3	-	-	-
Cellulose	-	-	-	-	-	-	3.9	-	-	-
Glass fiber	-	-	-	-	-	-	3.1	-	-	-
M1	-	-	-	-	-	-	7.1	472	475	515
M2′	-	-	-	-	-	-	1.6	455	468	506
M2	-	-	-	-	-	-	2.6	431	468	513
M3′	-	-	-	-	-	-	4.6	470	470	515
M3	-	-	-	-	-	-	7.5	431	470	515

V. Sensing of gasoline/kerosene and gasoline/ethanol mixtures

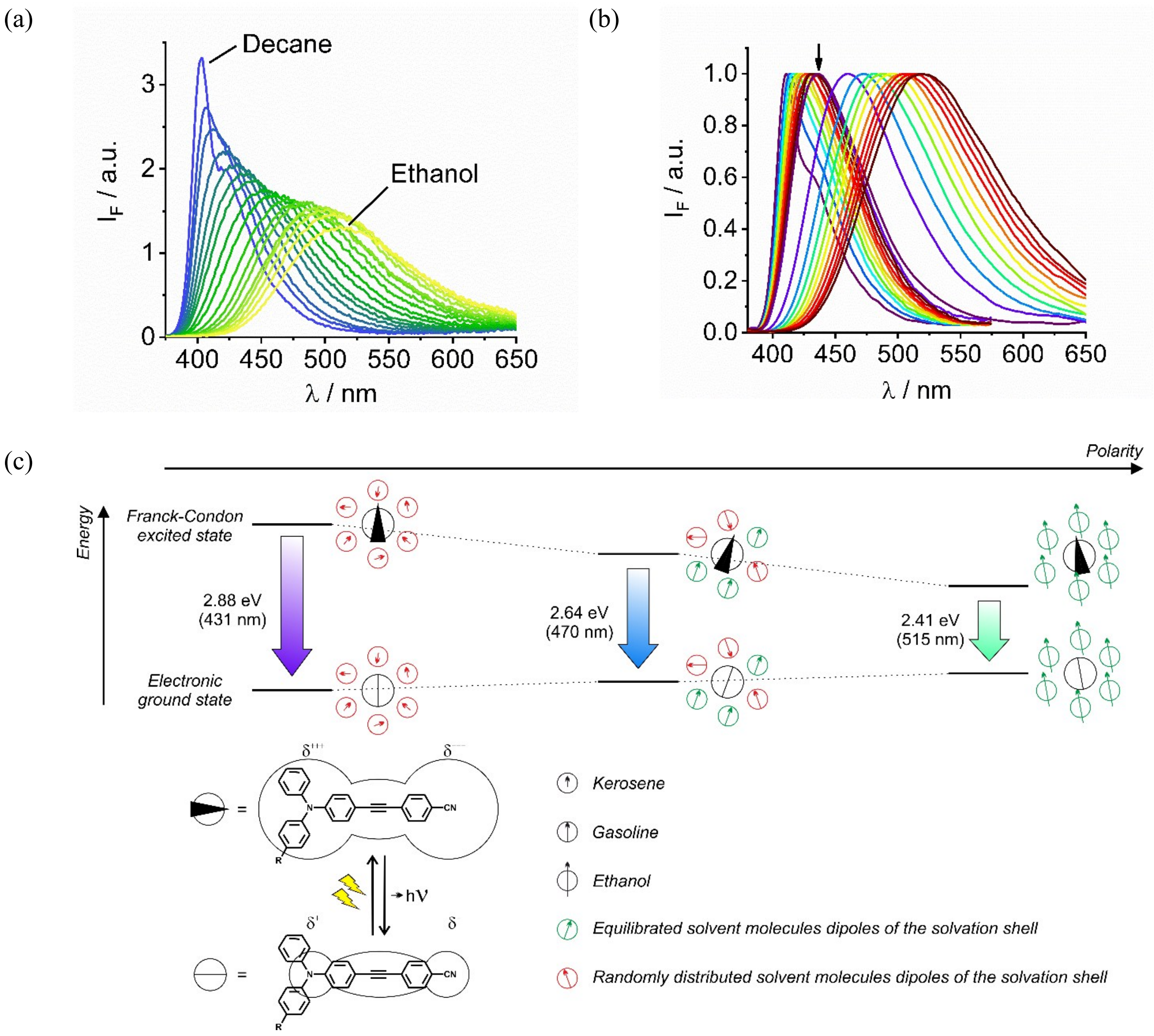

Fig. S5 Emission spectra of (a) **1** in decane/ethanol mixtures and (b) **2** in gasoline/kerosene and gasoline/ethanol mixtures with arrow signaling gasoline spectra and steps of 10 vol% ([**1**] = [**2**] = 4 μM, λ_{exc} = 340 nm). (c) Scheme representing the sensing mechanism that occurs inside the fluorometric test strips (i.e. **M3**) upon solvation in various type of fuels. The solvation of the charge transfer dyes **1** or **2**, which have only moderate dipole moments in the ground state but very pronounced dipole moments in the excited state, leads to solvent relaxation upon Franck-Condon excitation.[5] The more polar the solvent is the higher the stabilization of the excited and the higher the destabilization of the ground state of the two species involved in the vertical emission process, hence the larger the red-shift of the emission spectrum.

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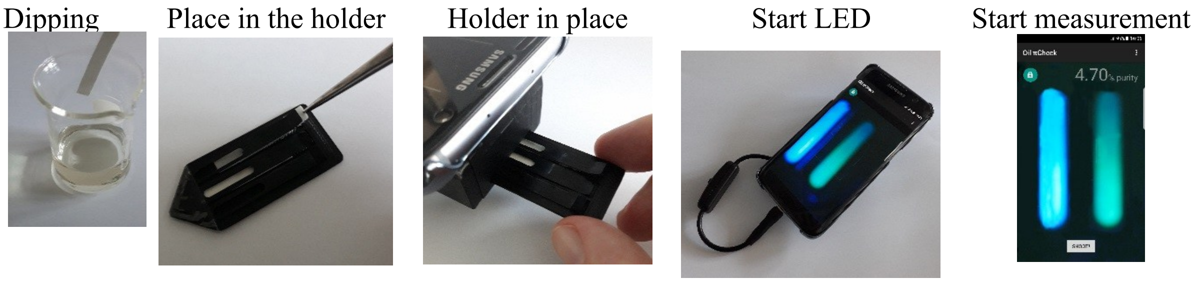
[5] B. Valeur, *Molecular Fluorescence: Principles and Applications*, **2001** Wiley-VCH Verlag GmbH.



Dipping Place in the holder Holder in place Start LED Start measurement

Fig. S6 Successive steps to analyze the purity of a gasoline fuel: dip in sample, place in the holder, start the LED, place the holder and press the shoot button to obtain directly the gasoline purity on screen.

Tab. S3 Hue values of **M3** after dipping in kerosene, gasoline E5 or ethanol upon various ISO parameters with phone camera settings of 1/1000 s for the shutter speed, 5000 K for white balance and a Macro Focus.

Fuel	**ISO 64**	**ISO 1600**
Kerosene	239.2 ± 1.0	224.2 ± 0.8
Gasoline E5	239.0 ± 1.0	220.8 ± 0.4
Ethanol	199.4 ± 0.8	186.0 ± 0.9

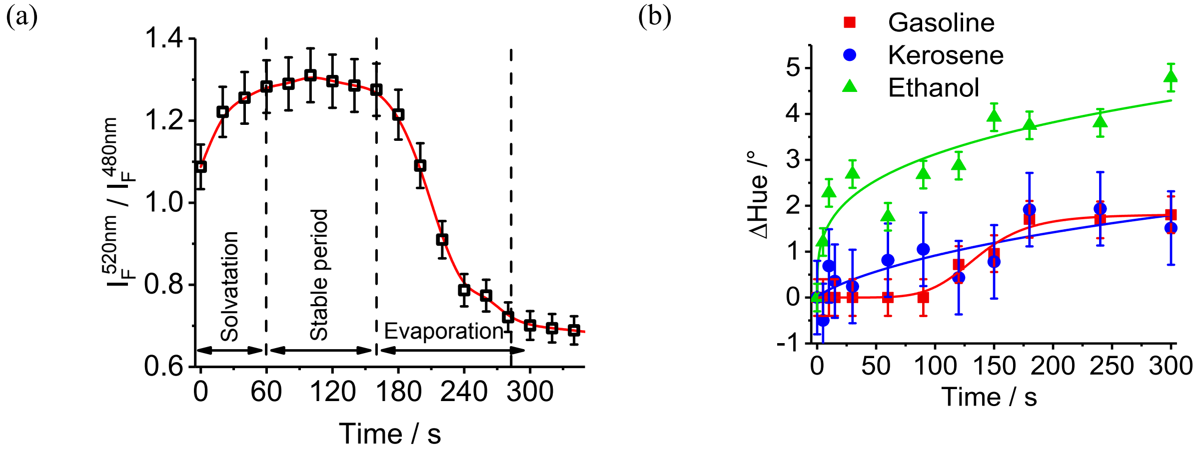
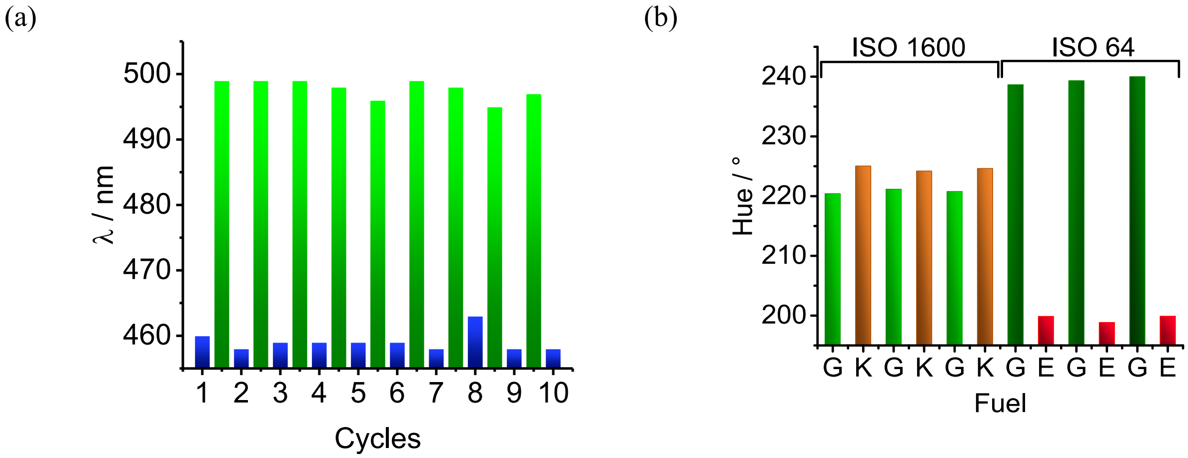


Fig. S7 (a) 520/480 nm fluorescence intensity ratio vs time of **M1** test-strip wetted with a drop (approx. 0.2 mL) of 2:8 gasoline/ethanol mixtures (λ_{exc} = 340 nm). Fluorescence signals of the test strips upon UV excitation (λ_{exc} = 365 nm) were monitored through an optical fiber placed below the strip coupled to an Ocean Optics USB2000+ spectrometer. (b) Hue variation over time after dipping of **M3** test-strip in gasoline, kerosene or ethanol (λ_{exc} = 380 nm).



(c)

Time after test strip preparation (months):		0	1	10
Hue values / °	Ethanol (ISO 64)	182	181	181
	Gasoline (ISO 64)	239	240	242
	Kerosene (ISO 1600)	218	219	218

Fig. S8 (a) Emission maxima of **M1** test-strip after addition of 30 µL of gasoline (blue) and ethanol (green) for ten cycles (λ_{exc} = 340 nm). (b) Hue value upon successive dipping of **M3** test-strip in gasoline (G), kerosene (K) and ethanol (E) (λ_{exc} = 380 nm). (c) Stability of the test strips upon long-term storage at room-temperature in a dark and dry place.

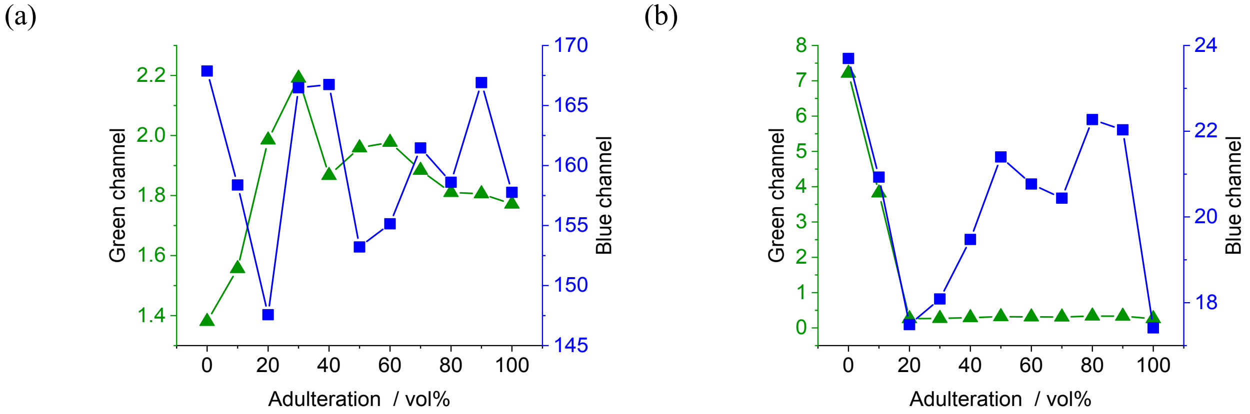


Fig. S9 Respective Green and Blue channels signals measured from **M3** test-strips when dipped in (a) gasoline E5/kerosene and (b) gasoline E5/ethanol mixtures (λ_{exc} = 380 nm).

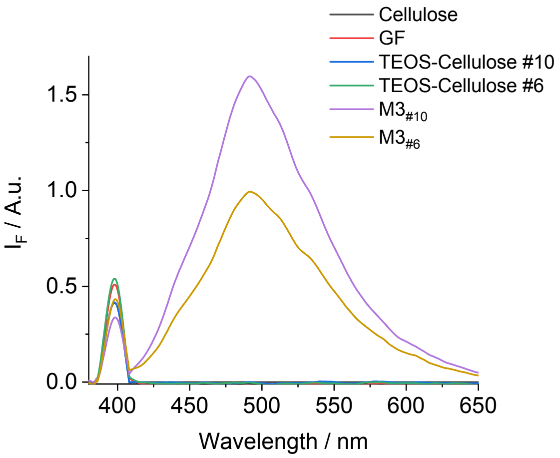


Fig. S10 Emission spectra of various dry strips (cellulose, glass fiber paper (GF), TEOS coated cellulose type #6 and #10, $\mathbf{M3}_{\#6}$ and $\mathbf{M3}_{\#10}$) obtained from replacing the smartphone detection by an optical fibre to a USB spectrometer (STS-UV Ocean Optics), ($\lambda_{exc} = 380$ nm).

Tab. S4 Hue values of **M3** after dipping in kerosene/gasoline E5/ethanol mixtures recorded with two different ISO parameters and phone camera settings of 1/1000 s for the shutter speed, 5000 K for white balance and a Macro Focus.

Fuels proportion / %			**Aspect**	**Hue / °**	
Kerosene	Gasoline	Ethanol		ISO 64	ISO 1600
33	33	33	Biphasic	*n.m.*	*n.m.*
10	50	40	Clear	233.6	194.5
40	50	10	Turbid	235.9	204.5

n.m.: not measured