Supplementary Material (ESI) for Chemical Science
This journal is (c) The Royal Society of Chemistry 2010

Pattern Generation with Synthetic Sensing Systems in Lipid Bilayer Membranes

Toshihide Takeuchi, Javier Montenegro, Andreas Hennig and Stefan Matile*

Department of Organic Chemistry, University of Geneva, Geneva, Switzerland, stefan.matile@unige.ch

Supporting Information

Table of Content

1. Supporting methods

1. Supporting methods

1.1. Materials and methods

As in refs. S1-S4, Supporting Information. Briefly, reagents for synthesis were purchased from Fluka and Aldrich, amino acid derivatives from Novabiochem and Bachem, buffers and salts of the best grade available from Fluka or Sigma-Aldrich and used as received. 8-Hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS) was from Sigma, and *p*-xylene-bis-pyridinium bromide (DPX) was from Invitrogen. Most of the odorants were purchased from Sigma, Fluka or Acros, and the others (enantiomeric citronellals, muscones and carvones) were gifts from Firmenich, Geneva. Egg yolk phosphatidylcholine (EYPC) was from Avanti Polar Lipids.

All reactions were performed under N_2 atmosphere. Unless stated otherwise, column chromatography was carried out on silica gel 60 (Fluka, 40-63 μm). Analytical thin layer chromatography (TLC) was performed in silica gel 60 (Fluka, 0.2 mm) and silica gel GF (Analtech, 1000 μm), respectively. $[\alpha]_D^{20}$ values were recorded on a Jasco P-1030 Polarimeter (reported for concentrations c in g / 100 ml solvent), melting points (Mp) on a heating table from Reichert (Austria). IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer (ATR, Golden Gate, unless stated otherwise) and are reported as wavenumbers ν in cm^{-1} with band intensities indicated as s (strong), m (medium), w (weak), b (broad). ^1H and ^{13}C NMR spectra were recorded (as indicated) either on a Bruker 300 MHz, 400 MHz or 500 MHz spectrometer and are reported as chemical shifts (δ) in ppm relative to TMS (δ = 0). Spin multiplicities are reported as a singlet (s), doublet (d), triplet (t), quartet (q) and quintet (quint) with coupling constants (*J*) given in Hz, or multiplet (m). ^1H

and ^{13}C resonances were assigned with the aid of additional information from 1D & 2D NMR spectra (H,H-COSY, DEPT 135, HSQC and HMBC). ESI-MS for the characterization of new compounds was performed on a Finnigan MAT SSQ 7000 instrument or a ESI API 150EX and are reported as mass-per-charge ratio *m/z* (intensity in %, [assignment]). Accurate mass determinations using ESI (HR ESI-MS) were performed on a Sciex QSTAR Pulsar mass spectrometer.

Vesicles were prepared with a Mini-Extruder from Avanti Polar Lipids (pore size 100 nm). Sample incubation with multiwell plates was done on a Rotamax 120 (Heidolph). Fluorescence measurements were performed with a FluoroMax-2 spectrofluorometer (Jobin-Yvon Spex) equipped with a stirrer and a temperature controller, or a FluoroMax-3 spectrofluorometer equipped with a multiwell plate reader (MicroMax 384). Fluorescence images were obtained with a Gene Genius (Syngene).

1.2. Abbreviations

Boc: *tert*-Butoxycarbonyl; Calculated: Calcd; Cbz: (Benzyloxy)carbonyl; CH_3CN: Acetonitrile; DCM: Dichloromethane; DIEA: N-Ethyl-N,N-diisopropyl amine; DMSO: Dimethylsulfoxide; DPX: *p*-Xylene-bispyridinium bromide; EYPC: Egg yolk phosphatidylcholine; Et_2O: Diethyl Ether; Glu: L-Glutamic acid; Gly: Glycine; HBTU: O-(Benzotriazol-1-yl)-N,N,N’,N’-tetramethyluronium hexafluorophosphate; HCl: Hydrochloric acid; HPTS: 8-Hydroxypyrene-1,3,6-trisulfonic acid trisodium salt; HR-MS (ESI): High resolution mass spectrometry (electrospray ionization); LUVs: Large unilamellar vesicles; Tris: Tris(hydroxymethyl)aminomethane; MeOH: Methanol; rt: Room temperature;

^{t}Bu: *tert*-Butyl.

1.3. Synthesis

1.3.1. Synthesis of reactive counterion A1H1

Compounds **A1H1.2** and **A1H1**. This compounds were prepared from the commercially available starting material **A1H1.1** as previously reported.[S4]

1.3.2. Synthesis of reactive counterion G1H1

G1H1.2. *General procedure A for hydrazidylation (amide synthesis) from carboxylic acids.* HBTU (906 mg, 2.39 mmol) and DIEA (2.04 ml, 11.95 mmol) were sequentially added to a solution of **G1H1.1** (500 mg, 2.39 mmol) in DCM (25 ml) under N_2. The reaction mixture was stirred for 5 min and a solution of NH_2-NHBoc (316 mg, 2.39 mmol) in DCM (5 ml) was added dropwise. The reaction mixture was stirred at rt for 1 hour and poured into a separation funnel containing DCM (15 ml) and $KHSO_4$ 1 M (35 ml); the aqueous phase was extracted with DCM (2 x 15 ml); the organic layer was washed with HCl 1 M (35 ml), $NaHCO_3$ saturated aqueous solution (35 ml), water (35 ml) and brine (35 ml) and dried over Na_2SO_4. After filtration and removal of solvent under vacuum, the residue was purified by silica gel column chromatography (DCM/MeOH 95:5, R_f 0.61) gave **G1H1.2** (679 mg, 88%) as a colorless oil. IR (neat): 3292 (m), 2979 (w), 1685 (s), 1236 (s), 1154 (s); ^{1}H NMR (400 MHz, $CDCl_3$): 8.63 (s, 1H), 7.38-7.29 (m, 5H), 7.10-6.84 (m, 1H), 5.98-5.62 (m, 1H), 5.12 (s, 2H), 4.92-3.75 (m, 2H), 1.45 (s, 9H); ^{13}C NMR (100 MHz,

$CDCl_3$): 169.3 (s), 156.8 (s), 155.5 (s), 136.0 (s), 128.47 (2x, d), 128.16 (2x, d), 128.06 (d), 81.9 (s), 67.2 (t), 42.9 (t), 28.1 (3x, q); MS (ESI, MeOH/H_2O 2:1): 346 (22, $[M+Na]^+$), 341 (93, $[M+NH_4]^+$), 324 (27, $[M+H]^+$), 268 (100, $[M-O^tBu+NH_4]^+$); HR-MS (ESI): Calcd for $C_{15}H_{21}N_3O_5Na$: 346.1373; found: 346.1357.

G1H1.3. *General procedure B for Cbz deprotection with H_2.* Compound **G1H1.2** (450 mg, 1.39 mmol) was dissolved in MeOH (25 ml) under N_2, and $Pd(OH)_2/C$ (20%) (49 mg, 0.07 mmol) was added in one portion. The reaction mixture was flushed with H_2 and stirred under H_2 atmosphere for 3 hours at rt. The resulting suspension was filtered trough celite, and the solvent was removed under vacuum to afford **G1H1.3** (261 mg, quantitative) as a colorless solid. Mp: 162-163 ^oC; IR (neat): 3374 (m), 3235 (m), 2653 (m, b), 1700 (s), 1252 (s); ^1H NMR (400 MHz, D_2O): 3.49 (s, 2H), 1.48 (s, 9H); ^{13}C NMR (100 MHz, D_2O): 173.9 (s), 157.1 (s), 83.2 (s), 41.8 (t), 27.5 (3x, q); MS (ESI, MeOH): 401 (44, $[2M+Na]^+$), 379 (100, $[2M+H]^+$), 212 (68, $[M+Na]^+$), 190 (87, $[M+H]^+$); HR-MS (ESI): Calcd for $C_7H_{17}N_3O_3$: 190.1191; found: 190.1199.

G1H1.4. *General procedure C for guanidinylation.* DIEA (678 μl, 3.96 mmol) and N,N'-di-Boc-*1H*-pyrazole-1-carboxamidine (369 mg, 1.18 mmol) were sequentially added to a solution of **G1H1.3** (150 mg, 0.79 mmol) in CH_3CN (20 ml) and water (1.5 ml) under N_2. The reaction mixture was stirred at 55 ^oC for 8 hours. The solvent was removed under vacuum and the residue was poured into a separation funnel containing AcOEt (25 ml) and HCl 1 M (25 ml). The aqueous phase was extracted with AcOEt (2x15 ml), and the combined organic layer was washed with water (25 ml) and brine (25 ml); dried over Na_2SO_4, filtered and removed solvent under vacuum. The crude product was purified by column chromatography (DCM/MeOH 9:1, R_f 0.43) to afford **G1H1.4** (253 mg, 74%) as a colorless solid. Mp: 96-97 °C; IR (neat): 3313 (m, b), 2977 (m), 2653 (m, b), 1615 (w), 1134 (s); ^{1}H NMR (400 MHz, $CDCl_3$): 11.41 (s, 1H), 9.08-8.82 (m, 2H), 6.85 (s, 1H), 4.28-4.17 (m, 2H), 1.51 (s, 9H), 1.49 (s, 9H), 1.47 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): 168.4 (s), 162.7 (s), 156.2 (s), 155.2 (s), 152.6 (s), 83.4 (s), 81.5 (s), 79.6 (s), 42.8 (t), 28.09 (3x, q), 28.06 (3x, q), 27.9 (3x, q); MS (ESI, MeOH): 454 (71, $[M+Na]^+$), 432 (70, $[M+H]^+$), 354 (42, $[M-Boc+Na]^+$), 254 (41, $[M-2Boc+Na]^+$), 101 (100, $[Boc]^+$); HR-MS (ESI): Calcd for $C_{18}H_{34}N_5O_7$: 432.2452; found: 432.2458.

G1H1. *General procedure D for Boc deprotection with HCl.* HCl (1 M in Et_2O) (10 ml) was added to a solution of **G1H1.4** (170 mg, 0.39 mmol) in DCM (5 ml) under N_2. The reaction mixture was stirred under reflux for 12 hours. The resulting suspension was then sonicated for 10 min and stirred again under reflux for 8 hours. The solvents were removed under vacuum and the colorless solid formed was dissolved in MeOH (0.5 ml), and Et_2O (20 ml) was added to obtain a colorless precipitate. The resulting suspension was centrifuged for 10 minutes and the solvent was discharged. The precipitate was washed with Et_2O (2x15 ml) and dried under high vacuum to afford **G1H1** (76 mg HCl salt, 97%) as a colorless solid. Mp: 124-125 °C; IR (neat): 3352 (m), 3183 (m), 2477 (m, b), 1646 (s), 1253 (s); ^{1}H NMR (400 MHz, D_2O/CD_3OD 2:1): 4.13 (s, 2H); ^{13}C NMR (100 MHz, D_2O): 168.9 (s), 157.9 (s), 42.7 (t); MS (ESI, MeOH): 132 (100, $[M+H]^+$); HR-MS (ESI): Calcd for $C_3H_{10}N_5O$: 132.0879; found: 132.0880.

1.3.3. Synthesis of reactive counterion A1H2

A1H2.2.[S5] *General procedure E for Boc protection*. DIEA (2.02 ml, 11.80 mmol) and Boc_2O (904 mg, 4.14 mmol) were sequentially added to a solution of **A1H2.1** (500 mg, 2.37 mmol) in DCM (50 ml) under N_2 and stirred at rt for 8 hours. The reaction mixture was then poured into a separation funnel containing HCl 1 M (45 ml); the aqueous phase was extracted with DCM (2x35 ml); the combined organic layer was washed water (25 ml) and brine (25 ml); dried over Na_2SO_4, filtered and removed solvent under vacuum. The crude product was purified by column chromatography (DCM/AcOEt 95:5) to afford **A1H2.2** (647 mg, quantitative) as a colorless oil. Spectroscopic data match those reported in the literature.[S5]

A1H2.3. *General procedure F for hydrazidylation from esters.* Hydrazine monohydrate (0.5 ml, 10.31 mmol) was added to a solution of **A1H2.2** (500 mg, 1.81 mmol) in MeOH (15 ml) under N_2. The reaction mixture was stirred at reflux for 12 hours. The solvent was removed under vacuum and the solid formed was washed with DCM (2x10 ml) and dried under high vacuum to afford **A1H2.3** (395 mg, 79%) as a colorless solid. Mp: 179-180 ^{o}C; $[\alpha]_D^{20}$: +1.8 (c = 0.1 in MeOH); IR (neat): 3313 (s), 2967 (w), 2941 (w), 1524 (s), 1251 (m); ^{1}H NMR (300 MHz, CD_3OD): 4.06-3.91 (m, 1H), 2.22 (t, ^3J(H,H) = 7.5 Hz, 2H), 2.09-1.79 (m, 2H), 1.44 (s, 9H); ^{13}C NMR (75 MHz, CD_3OD): 174.3 (s), 173.7 (s), 157.8 (s), 80.9 (s), 54.3 (d), 31.3 (t), 29.5 (t), 28.8 (3x, q); MS (ESI, MeOH): 573 (88, $[2M+Na]^+$), 551 (82, $[2M+H]^+$), 298 (94, $[M+Na]^+$), 276 (100, $[M+H]^+$), 176 (58, $[M\text{-}Boc+H]^+$); HR-MS (ESI): Calcd for $C_{10}H_{22}N_5O_4$: 276.1666; found: 276.1670.

A1H2. *General procedure G for Boc deprotection with TFA.* Trifluoroacetic acid (1 ml, 13.46 mmol) was added to a suspension of **A1H2.3** (250 mg, 0.90 mmol) in DCM (10 ml) under N_2. The reaction mixture was stirred at rt for 5 hours. The excess of TFA was removed by N_2 flow and the rest of the volatile materials were removed under vacuum. The solid formed was washed with DCM (2x10 ml) and dried under high vacuum to afford **A1H2** (367 mg TFA salt, 79%) of a colorless solid. Mp: 174-175 ^{o}C; $[\alpha]_D^{20}$: +15.1 (c = 0.1 in MeOH); IR (neat): 2928 (m, b), 2689 (m, b), 1662 (s), 1128 (s); ^{1}H NMR (400 MHz, D_2O): 4.12 (t, ^3J(H,H) = 6.5 Hz, 1H), 2.53 (t, ^3J(H,H) = 6.5 Hz, 2H), 2.28-2.16 (m, 2H); ^{13}C NMR (100 MHz, D_2O): 171.4 (s), 167.6 (s), 51.1 (d), 27.6 (t), 25.3 (t); MS (ESI, MeOH): 198 (19, $[M+Na]^+$), 176 (39, $[M+H]^+$), 158 (81, $[M-NH_2+H]^+$), 144 (100, $[M-N_2H_4+H]^+$); HR-MS (ESI): Calcd for $C_5H_{34}N_5O_2$: 176.1142; found: 176.1146.

1.3.4. Synthesis of reactive counterion G1H2

G1H2.1.[S6] CbzCl (367 ml, 2.60 mmol) and DIEA (1.01 ml, 5.92 mmol) were sequentially added to a solution of **A1H2.1** (500 mg, 2.37 mmol) in CH_3CN (20 ml) and H_2O (3 ml) under N_2. The reaction mixture was stirred at rt for 1 hour. The solvent was removed under vacuum and the crude was purified by column chromatography (DCM/AcOEt 95:5, R_f 0.45) to afford **G1H2.1** (729 mg, quantitative) as a colorless oil. Spectroscopic data match those reported in the literature.[S6]

G1H2.2. Following the general procedure F (see A1H2.3), reaction of **G1H2.1** (500 mg, 1.62 mmol) with hydrazine monohydrate (0.5 ml, 10.31 mmol) in MeOH (25 ml) at reflux for 6 hours followed by removal of the solvent and washings with Et_2O

(2x10 ml) afforded **G1H2.2** (451 mg, 90%) as a colorless solid. Mp: 194-195 ^{o}C; $[\alpha]_D^{20}$: -18.8 (c = 0.1 in MeOH); IR (neat): 3290 (s), 3051 (w), 1655 (s), 1533 (s), 1254 (s); ^{1}H NMR (400 MHz, DMSO-d_6): 9.13 (s, 1H), 8.95 (s, 1H), 7.46 (d, ^{3}J(H,H) = 8.2 Hz, 1H), 7.40-7.29 (m, 5H), 5.01 (s, 2H), 4.22 (s, 2H), 4.16 (s, 2H), 3.92 (dt, ^{3}J(H,H) = 8.2 Hz, ^{3}J(H,H) = 6.1 Hz, 1H), 2.12-1.95 (m, 2H), 1.95-1.58 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): 170.92 (s), 170.77 (s), 155.8 (s), 137.0 (s), 128.4 (2x, d), 127.81 (2x, d), 127.77 (d), 65.4 (t), 53.1 (d), 29.9 (t), 28.2 (t); MS (ESI, MeOH): 332 (100, $[M+Na]^+$), 310 (59, $[M+H]^+$), 278 (63, $[M-N_2H_4+H]^+$), 248 (35, $[M-2(N_2H_4)+H]^+$), 234 (26, $[M-C_6H_5+H]^+$); HR-MS (ESI): Calcd for $C_{13}H_{19}N_5O_4Na$: 332.1329; found: 332.1320.

G1H2.3. Following the general procedure E (see A1H2.2), reaction of **G1H2.2** (450 mg, 1.45 mmol) with DIEA (1.23 ml, 7.25 mmol) and Boc_2O (870 mg, 3.98 mmol) in CH_3CN (35 ml) and H_2O (5 ml) and purification by column chromatography (DCM/AcOEt 9:1, R_f 0.49) afforded **G1H2.3** (583 mg, 79%) as a colorless solid. Mp: 97-98 ^{o}C; $[\alpha]_D^{20}$: -24.8 (c = 0.1 in MeOH); IR (neat): 3272 (m), 2980 (w), 1674 (s), 1243 (s), 1162 (s); ^{1}H NMR (400 MHz, $CDCl_3$): 9.93 (s, 1H), 9.64 (s, 1H), 7.43-7.25 (m, 5H), 6.54 (s, 2H), 5.63-5.52 (m, 1H), 5.12 (d, ^{3}J(H,H) = 12.5 Hz, 1H), 5.03 (d, ^{3}J(H,H) = 12.5 Hz, 1H), 4.38-4.26 (m, 1H), 2.45-2.26 (m, 2H), 2.07-1.96 (m, 1H), 1.85-1.74 (m, 1H), 1.47 (s, 9H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): 173.3 (s), 171.6 (s), 157.11 (s), 157.01 (s), 155.7 (s), 136.1 (s), 128.44 (2x, d), 128.11 (3x, d), 82.7 (2x, s), 67.0 (t), 52.3 (d), 29.4 (2x, t), 28.07 (3x, q), 28.02 (3x, q); MS (ESI, MeOH/H_2O 2:1): 532 (12, $[M+Na]^+$), 527 (65, $[M+NH_4]^+$), 510 (70, $[M+H]^+$), 398 (100, $[M-Cbz+Na]^+$); HR-MS (ESI): Calcd for $C_{23}H_{35}N_5O_8Na$: 532.2376; found: 532.2367.

G1H2.4. Following the general procedure B (see G1H1.3), reaction of **G1H2.3** (430 mg, 0.84 mmol) with $Pd(OH)_2/C$ (20%) (30 mg, 0.04 mmol) in MeOH (30 ml) under H_2 for 4 h followed by filtration through celite afforded **G1H2.4** (311 mg, quantitative) as a colorless solid. Mp: 131-132 ^{o}C; $[\alpha]_D^{20}$: -3.1 (c = 0.1 in MeOH); IR (neat): 3254 (w), 2977 (w), 1671 (s), 1154 (s); ^{1}H NMR (400 MHz, CD_3OD): 3.33 (t, ^3J(H,H) = 6.8 Hz, 1H), 2.31 (t, ^3J(H,H) = 6.8 Hz, 2H), 2.06-1.86 (m, 2H), 1.46 (s, 18H); ^{13}C NMR (100 MHz, CD_3OD): 176.8 (s), 175.0 (s), 158.36 (s), 158.27 (s), 82.2 (s), 53.6 (d), 32.4 (t), 30.9 (t), 28.7 (6x, q); MS (ESI, MeOH): 751 (80, $[2M+H]^+$), 398 (7, $[M+Na]^+$), 376 (100, $[M+H]^+$); HR-MS (ESI): Calcd for $C_{15}H_{30}N_5O_6$: 376.2190; found: 376.2190.

G1H2.5. Following the general procedure C (see G1H1.4), reaction of **G1H2.4** (150 mg, 0.40 mmol), DIEA (342 μl, 2.00 mmol) and N,N'-di-Boc-*1H*-pyrazole-1-carboxamidine (186 mg, 0.60 mmol) in CH_3CN (20 ml) and water (1.5 ml) at 55 ^{o}C for 8 hours and purification by column chromatography (DCM/MeOH 9:1, R_f 0.37) afforded **G1H2.5** (170 mg, 69%) as a colorless solid. Mp: 146-147 ^{o}C; $[\alpha]_D^{20}$: -10.7 (c = 0.1 in MeOH); IR (neat): 3274 (m, b), 2980 (w), 1610 (s), 1152 (s), 1130 (s); ^{1}H NMR (400 MHz, $CDCl_3$): 11.11 (s, 1H), 10.05 (s, 1H), 9.22 (s, 1H), 8.95 (s, 1H), 6.59-6.31 (m, 2H), 5.06-4.89 (m, 1H), 2.50-2.14 (m, 2H), 2.12-1.96 (m, 2H), 1.38 (s, 9H), 1.37 (s, 9H), 1.35 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$): 173.5 (s), 170.7 (s), 162.5 (s), 156.44 (s), 156.13 (s), 155.3 (s), 152.4 (s), 83.6 (s), 82.1 (s), 81.8 (s), 79.4 (s), 50.8 (d), 32.2 (t) 30.8 (t), 28.19 (3x, q), 28.14 (3x, q), 28.06 (3x, q), 27.97 (3x, q); MS (ESI, MeOH): 640 (28, $[M+Na]^+$), 618 (100, $[M+H]^+$), 418 (28, $[M-Boc+H]^+$), 318 (24, $[M-2Boc+H]^+$); HR-MS (ESI): Calcd for

$C_{26}H_{48}N_7O_{10}$: 618.3457; found: 618.3475.

G1H2. Following the general procedure D (see G1H1), reaction of **G1H2.5** (100 mg, 0.16 mmol) with 10 ml of HCl (1 M in Et_2O) in DCM (5 ml) for 12 hours under reflux followed by precipitation (Et_2O/MeOH 20:1, 10.5 ml), centrifugation and washings with Et_2O (2x15 ml) afforded **G1H2** (40 mg HCl salt, 76%) as a colorless solid. Mp: 182-183 ^{o}C; $[\alpha]_D^{20}$: -12.2 (c = 0.1 in MeOH); IR (neat): 3146 (s, b), 2902 (s, b), 2638 (s, b), 1666 (s), 1495 (s); ^{1}H NMR (400 MHz, D_2O/CD_3OD 2:1): 4.30-4.23 (m, 1H), 2.50-2.41 (m, 2H), 2.30-2.17 (m, 1H), 2.12-1.99 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD): 172.5 (s), 170.6 (s), 158.7 (s), 54.5 (d), 29.9 (t), 28.8 (t); MS (ESI, MeOH/H_2O 2:1): 218 (82, $[M+H]^+$), 186 (100, $[M-N_2H_4+H]^+$); HR-MS (ESI): Calcd for $C_6H_{16}N_7O_2$: 218.1359; found: 218.1364.

1.3.5. Synthesis of reactive counterion A1H3

A1H3.2.[S7] Following the general procedure A (see G1H1.2), reaction of **A1H3.1** (200 mg, 0.77 mmol) with HBTU (292 mg, 0.77 mmol), DIEA (657 μl, 3.85 mmol) and **A1H2.1** (162 mg, 0.77 mmol) in DCM (30 ml) and purification by column chromatography (DCM/MeOH 95:5, R_f 0.54) afforded **A1H3.2** (238 mg, 74%) as a colorless oil. $[\alpha]_D^{20}$: -11.8 (c = 0.1 in MeOH); IR (neat): 2939 (w, b), 2863 (w), 1607 (m), 835 (s); ^{1}H NMR (400 MHz, $CDCl_3$): 6.96 (d, ^{3}J(H,H) = 8.1 Hz, 1H), 5.25 (d, ^{3}J(H,H) = 8.1 Hz, 1H), 4.59 (td, ^{3}J(H,H) = 8.1 Hz, ^{3}J(H,H) = 5.1 Hz, 1H), 4.23-4.10 (m, 1H), 3.74 (s, 3H), 3.69 (s, 3H), 3.67 (s, 3H), 2.57-2.31 (m, 4H), 2.28-2.07 (m, 2H), 2.06-1.85 (m, 2H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): 173.84 (s), 173.15 (s), 171.86 (s), 171.47 (s), 155.5 (s) 80.1 (s), 53.6 (d), 52.5 (q), 51.89 (q), 51.86 (d), 51.62

(q), 30.1 (t), 29.9 (t), 28.2 (3x, q), 27.91 (t), 27.08 (t); MS (ESI, MeOH): 441 (80, $[M+Na]^+$), 419 (48, $[M+H]^+$), 341 (73, $[M-Boc+Na]^+$), 319 (100, $[M-Boc+H]^+$); HR-MS (ESI): Calcd for $C_{18}H_{30}N_2O_9Na$: 441.1843; found: 441.1843.

A1H3.3. Following the general procedure F (see A1H2.3), reaction of **A1H3.2** (500 mg, 1.19 mmol) and hydrazine monohydrate (0.5 ml, 10.31 mmol) in MeOH (25 ml) under reflux for 12 hours, removal of solvents and washings with DCM (2x10 ml) and Et_2O (2x10 ml) afforded **A1H3.3** (449 mg, 90%) as a colorless solid. Mp: 153-154 °C; $[\alpha]_D^{20}$: -26.6 (c = 0.1 in MeOH); IR (neat): 3290 (s), 3235 (m), 1630 (s), 1526 (s); ^1H NMR (400 MHz, D_2O): 4.29-4.21 (m, 1H), 4.03-3.96 (s, 1H), 2.35-2.21 (m, 4H), 2.13-1.76 (m, 4H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, D_2O): 174.4 (s), 173.86 (s), 173.68 (s), 171.8 (s), 157.4 (s), 81.6 (s), 54.1 (d), 51.7 (d), 29.77 (t), 29.64 (t), 27.5 (3x, q), 26.83 (t), 26.70 (t); MS (ESI, MeOH): 441 (67, $[M+Na]^+$), 419 (25, $[M+H]^+$), 341 (22, $[M-Boc+Na]^+$), 319 (40, $[M-Boc+H]^+$), 301 (44, $[M-Boc-NH_2+H]^+$), 243 (100, $[M-Glu(N_2H_8)_2+H]^+$); HR-MS (ESI): Calcd for $C_{15}H_{31}N_8O_6$: 419.2361; found: 419.2379.

A1H3. Following the general procedure G (see A1H2), reaction of **A1H3.3** (400 mg, 0.96 mmol) and TFA (1.0 ml, 13.46 mmol) in DCM (10 ml) under N_2 for 4 hours and solvent removal afforded **A1H3** (646 mg, 87%, TFA salt) as a colorless solid. Mp: >250 °C; $[\alpha]_D^{20}$: -6.9 (c = 0.1 in MeOH); IR (neat): 2887 (s, b), 2644 (s, b), 1674 (s), 1486 (s); ^1H NMR (400 MHz, D_2O): 4.47 (t, ^3J(H,H) = 6.3 Hz, 1H), 4.14 (t, ^3J(H,H) = 6.3 Hz, 1H), 2.61-2.41 (m, 4H), 2.34-1.97 (m, 4H); ^{13}C NMR (100 MHz, D_2O/TFA-*d* 8:2): 172.2 (s), 171.7 (s), 170.6 (s), 169.3 (s), 52.0 (d), 51.4 (d), 28.6 (t), 27.9 (t), 25.76 (t), 25.60 (t); MS (ESI, MeOH/H_2O 2:1): 359 (56, [M+H_2O+Na]$^+$), 341 (37, [M+Na]$^+$), 319 (100, [M+H]$^+$); HR-MS (ESI): Calcd for $C_{10}H_{23}N_8O_4$: 319.1836; found: 319.1842.

1.3.6. Synthesis of reactive counterion G1H3

G1H3.2.[S8] Following the general procedure A (see G1H1.2), reaction of **G1H3.1** (200 mg, 0.67 mmol) with **A1H2.1** (141 mg, 0.67 mmol), DIEA (572 μl, 3.35 mmol) and HBTU (253 mg, 0.67 mmol) in DCM (25 ml) and purification by column chromatography (DCM/MeOH 95:5, R_f 0.61), afforded **G1H3.2** (257 mg, 85%) as a colorless solid. Mp: 120-121 °C; $[\alpha]_D^{20}$: -37.1 (c = 0.1 in MeOH); IR (neat): 3289 (m), 2955 (w), 1721 (s), 1214 (s); ^{1}H NMR (400 MHz, $CDCl_3$): 7.40-7.31 (m, 5H), 7.13-7.05 (m, 1H), 5.70 (d, ^3J(H,H) = 7.5 Hz, 1H), 5.10 (s, 2H), 4.59 (dt, ^3J(H,H) = 7.5 Hz, ^3J(H,H) = 6.9 Hz, 1H), 4.31 (dt, ^3J(H,H) = 7.5 Hz, ^3J(H,H) = 6.9 Hz, 1H), 3.74 (s, 3H), 3.68 (s, 3H), 3.66 (s, 3H), 2.55-2.32 (m, 4H), 2.27-2.09 (m, 2H), 2.07-1.89 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): 174.1 (s), 173.5 (s), 172.1 (s), 171.4 (s), 156.3 (s), 136.4 (s), 128.7 (2x, d), 128.4 (2x, d), 128.3 (d), 67.3 (t), 54.1 (d), 52.80 (q), 52.14 (q), 52.13 (q), 51.99 (d), 30.19 (t), 30.18 (t), 28.5 (t), 27.1 (t); MS (ESI, MeOH/H_2O 2:1): 475 (97, [M+Na]$^+$), 470 (39, [M+NH_4]$^+$), 453 (100, [M+H]$^+$), 391 (93, [M-2OMe+H]$^+$); HR-MS (ESI): Calcd for $C_{21}H_{29}N_2O_9$: 453.1867; found: 453.1856.

G1H3.3. Following the general procedure F (see A1H2.3), reaction of **G1H3.2** (500 mg, 1.10 mmol) with hydrazine monohydrate (0.5 ml, 10.31 mmol) in MeOH (25 ml) and water (5 ml) under reflux for 12 hours followed by removal of solvents and washings with Et_2O (2x15 ml) afforded **G1H3.3** (402 mg, 81%) as a colorless solid.

Mp: 210-211 °C; $[\alpha]_D^{20}$: -22.3 (c = 0.1 in MeOH); IR (neat): 3288 (s), 3056 (w), 1638 (s), 1610 (s), 1243 (s); ^{1}H NMR (400 MHz, D_2O): 7.17-6.80 (m, 5H), 4.69-4.63 (m, 2H), 3.99 (dd, ^3J(H,H) = 8.7 Hz, ^3J(H,H) = 4.8 Hz, 1H), 3.71 (dd, ^3J(H,H) = 8.7 Hz, ^3J(H,H) = 4.8 Hz, 1H), 2.13-1.86 (m, 4H), 1.82-1.66 (m, 2H), 1.60-1.45 (m, 2H); ^{13}C NMR (100 MHz, D_2O/ DMSO-d_6/TFA-d 1:1:1): 174.6 (s), 173.4 (s), 172.2 (s), 172.1 (s), 158.5 (s), 137.6 (s), 130.0 (2x, d), 129.69 (2x, d), 129.20 (d), 68.1 (t), 55.3 (d), 52.5 (d), 30.3 (t), 30.1 (t), 27.79 (t), 27.22 (t); MS (ESI, MeOH): 475 (100, $[M+Na]^+$); HR-MS (ESI): Calcd for $C_{18}H_{29}N_8O_6$: 453.2204; found: 453.2194.

G1H3.4. Following the general procedure E (see A1H2.2), reaction of **G1H3.3** (350 mg, 0.77 mmol) with DIEA (661 μl, 3.87 mmol) and Boc_2O (970 mg, 4.45 mmol) in CH_3CN (25 ml) and H_2O (5 ml) and purification by column chromatography (DCM/MeOH 9:1, R_f 0.48), afforded **G1H3.4** (459 mg, 79%) as a colorless solid. Mp: 128-129 °C; $[\alpha]_D^{20}$: -49.3 (c = 0.1 in MeOH); IR (neat): 3277 (m, b), 2981 (w), 1666 (s), 1152 (s); ^{1}H NMR (400 MHz, CD_3OD): 7.42-7.29 (m, 5H), 5.16-5.03 (m, 2H), 4.51-4.41 (m, 1H), 4.16-4.04 (m, 1H), 2.51-1.90 (m, 8H), 1.48 (s, 27H); ^{13}C NMR (100 MHz, $CDCl_3$/DMSO-d_6 5:1): 171.99 (s), 171.46 (2x, s), 171.04 (s), 156.0 (s), 155.80 (s), 155.08 (s), 154.9 (s), 135.0 (s), 127.33 (2x, d), 127.11 (2x, d), 127.00 (d), 80.66 (s), 80.12 (s), 80.48 (s), 65.7 (t), 52.2 (d), 49.4 (d), 28.3 (3x, t), 27.0 (9x, q), 25.4 (t); MS (ESI, MeOH/H_2O 2:1): 775 (17, $[M+Na]^+$), 770 (32, $[M+NH_4]^+$), 753 (100, $[M+H]^+$), 653 (42, $[M-Boc+H]^+$), 553 (25, $[M-2Boc+H]^+$), 453 (27, $[M-3Boc+H]^+$); HR-MS (ESI): Calcd for $C_{33}H_{52}N_8O_{12}Na$: 775.3596; found: 775.3592.

G1H3.5. Following the general procedure B (see G1H1.3), reaction of **G1H3.4**

(410 mg, 0.54 mmol) with $Pd(OH)_2/C$ (20%) (20 mg, 0.03 mmol) in MeOH (20 ml) under H_2 for 4h and filtration through celite gave **G1H3.5** (334 mg, quantitative) as a colorless solid. Mp: 141-142 °C; $[\alpha]_D^{20}$: -35.2 (c = 0.1 in MeOH); IR (neat): 3278 (m), 2980 (w), 1662 (s), 1156 (s); ^1H NMR (400 MHz, CD_3OD): 4.41 (t, ^3J(H,H) = 7.8 Hz, 1H), 3.39 (dd, ^3J(H,H) = 7.8 Hz, ^3J(H,H) = 5.6 Hz, 1H), 2.47-1.82 (m, 8H), 1.49 (s, 27H); ^{13}C NMR (100 MHz, CD_3OD): 176.7 (s), 175.1 (s), 174.46 (s), 174.32 (s), 158.60 (s), 158.15 (s), 157.8 (s), 82.41 (s), 82.23 (s), 82.16 (s), 54.8 (d), 52.5 (d), 32.3 (t), 30.97 (2x, t), 30.68 (t), 28.72 (3x, q), 28.69 (6x, q); MS (ESI, MeOH): 641 (17, $[M+Na]^+$), 619 (100, $[M+H]^+$), 519 (11, $[M\text{-}Boc+H]^+$); HR-MS (ESI): Calcd for $C_{25}H_{47}N_8O_{10}$: 619.3409; found: 619.3395.

G1H3.6. Following the general procedure C (see G1H1.4), reaction of **G1H3.5** (200 mg, 0.32 mmol), DIEA (276 μl, 1.61 mmol) and N,N'-di-Boc-*1H*-pyrazole-1-carboxamidine (149 mg, 0.48 mmol) in CH_3CN (20 ml) and water (1.5 ml) at 55 °C for 8 hours and purification by column chromatography (DCM/MeOH 9:1, R_f 0.39) afforded **G1H3.6** (168 mg, 61%) as a colorless solid. Mp: 231-232 °C; $[\alpha]_D^{20}$: -34.9 (c = 0.1 in MeOH); IR (neat): 3273 (m), 2980 (m), 2653 (m, b), 1667 (s), 1154 (s); ^1H NMR (400 MHz, $CD_3OD/CDCl_3$ 1:1): 4.71-4.60 (m, 1H), 4.48-4.39 (m, 1H), 2.47-2.01 (m, 8H), 1.55 (s, 9H), 1.48 (s, 36H); ^{13}C NMR (100 MHz, $CDCl_3$): 173.27 (s), 173.21 (s), 172.5 (s), 171.9 (s), 170.8 (s), 162.5 (s), 156.59 (s), 156.23 (s), 155.7 (s), 152.4 (s), 83.2 (s), 81.78 (s), 81.75 (s), 81.40 (s), 79.3 (s), 52.2 (d), 50.2 (d), 30.91 (t), 30.06 (t), 29.4 (t), 28.0 (3x, q), 27.96 (9x, q), 27.85 (3x, q), 27.23 (t); MS (ESI, MeOH): 883 (10, $[M+Na]^+$), 861 (100, $[M+H]^+$), 761 (19, $[M\text{-}Boc+H]^+$), 661 (14, $[M\text{-}2Boc+H]^+$); HR-MS (ESI): Calcd for $C_{36}H_{65}N_{10}O_{14}$: 861.4676; found: 861.4643.

G1H3. Following the general procedure D (see G1H1), reaction of **G1H3.6** (130 mg, 0.15 mmol) in HCl (10 ml, 1 M in Et_2O) and DCM (5 ml) under reflux for 18 hours followed by precipitation (Et_2O/MeOH 20:1, 10.5 ml) and washings with Et_2O (2x15 ml) afforded **G1H3** (55 mg HCl salt, 72%) as a colorless solid. Mp: 210-211 ^{o}C; $[\alpha]_D^{20}$: +3.2 (c = 0.1 in MeOH); IR (neat): 3152 (s, b), 2916 (s, b), 2649 (s, b), 1656 (s), 1500 (s); ^1H NMR (400 MHz, D_2O): 4.42 (dd, $^3J(H,H) = 8.4$ Hz, $^3J(H,H) =$ 6.0 Hz, 1H), 4.31 (dd, $^3J(H,H) = 8.4$ Hz, $^3J(H,H) = 6.0$ Hz, 1H), 2.53-2.42 (m, 4H), 2.31-2.03 (m, 4H); ^{13}C NMR (100 MHz, D_2O): 172.48 (s), 172.40 (s), 171.8 (s), 170.9 (s), 156.7 (s), 54.0 (d), 51.5 (d), 28.76 (t), 28.46 (t), 27.0 (t), 25.7 (t); MS (ESI, MeOH/H_2O 2:1): 361 (87, $[M+H]^+$), 329 (100, $[M-N_2H_4+H]^+$); HR-MS (ESI): Calcd for $C_{11}H_{25}N_{10}O_4$: 361.2054; found: 361.2056.

1.4. General procedures for vesicle experiments

1.4.1. Vesicle preparations

Following the general procedures in ref. S9, a thin lipid film was prepared by evaporating a solution of 25 mg EYPC in 1 ml MeOH/$CHCl_3$ (1:1) on a rotary evaporator (room temperature) and then *in vacuo* overnight. The resulting film was hydrated with 1.0 ml buffer (5 mM HPTS, 16.5 mM DPX, 10 mM Tris, 72 mM NaCl, pH 7.4) for more than 30 min, subjected to freeze-thaw cycles (5×) and extrusions (15×) through a polycarbonate membrane (pore size, 100 nm). Extravesicular components were removed by gel filtration (Sephadex G-50) with 10 mM Tris, 107 mM NaCl, pH 7.4. Final conditions: ~5 mM EYPC; inside: 5 mM HPTS, 16.5 mM

DPX, 10 mM Tris, 72 mM NaCl, pH 7.4; outside: 10 mM Tris, 107 mM NaCl, pH 7.4.

1.4.2. Preparation of odorant hydrazones

Adapting the general procedures in ref. S10, 10 μl of 100 mM reactive counterion (**A1H1-G1H3**) in DMSO was mixed with 2 equivalent of odorant for each hydrazide of the reactive counterion (e.g., 30 μl of 200 mM odorant in DMSO for reactive counterions with three hydrazides) and adjusted the total volume to 100 μl with DMSO. The mixture was stirred at 60˚C for 1 hr, and the resulting hydrazone was then used for DNA activation experiments.

1.4.3. DNA activation experiments

Adapting the general procedures in ref. S9, EYPC-LUV stock solutions (5 μl) were diluted with a buffer (10 mM Tris, 107 mM NaCl, pH 7.4) and placed in a thermostated fluorescence cuvette (25 ˚C) and gently stirred (total volume in the cuvette, ~2000 μl; final lipid concentration, ~13 μM). HPTS efflux was monitored at λ_{em} 511 nm (λ_{ex} 413 nm) as a function of time after addition of activator (odorant hydrazones, 10 μl in DMSO) at t = 0 s, transporter (calf thymus DNA, 20 μl stock solution in buffer, 1.25 μg/ml final concentration) at t = 40 s and 1.2% aqueous triton X-100 (40 μl, 0.024% final concentration) at t = 200 s. Fluorescence intensities were normalized to fractional emission intensity I(t) using equation (*S1*)

$$I(\mathrm{t}) = (I_\mathrm{t} - I_0) / (I_\infty - I_0) \qquad (S1)$$

where $I_0 = I_t$ at DNA addition, $I_\infty = I_t$ at saturation after lysis. Effective concentration for hydrazone EC_{50} and Hill coefficient n were determined by plotting the fractional activity Y (= I(t) at saturation just before lysis, t = ~190 s) as a function of hydrazone concentration $c_{\text{hydrazone}}$ and fitting them to the Hill equation (*S2*)

$$Y = Y_0 + (Y_{\text{MAX}} - Y_0) / \{1 + (EC_{50} / c_{\text{hydrazone}})^n\} \qquad (S2)$$

where Y_0 is Y without hydrazone, Y_{MAX} is Y with an excess hydrazone at saturation, EC_{50} is the concentration of hydrazone required to reach 50% activity and n is the Hill coefficient (e.g. Fig. S4). Results are summarized in Table S1.

1.4.4. Odorant sensing experiments (direct detection; Table S2)

2 μl of reactive counterions (**A1H2-G1H3**) in DMSO (**A1H2**, 100 mM; **G1H2**, 30 mM; **A1H3**, 60 mM; **G1H3**, 10 mM) and 2 μl of increasing concentrations of odorant in DMSO were mixed with 16 μl DMSO in a tube (total 20 μl), and the mixture was stirred at 60˚C for 30 min. 10 μl of the resulting reaction mixture was then used as an activator for DNA activation experiments following the above procedure 1.4.3. The formation of hydrazones was confirmed by ESI-MS. The final concentration of the reactive counterions in each cuvette is 50 μM (**A1H2**), 15 μM (**G1H2**), 30 μM (**A1H3**) and 5 μM (**G1H3**). Fluorescence intensities were normalized to fractional emission intensity I(t) using equation (*S1*). Effective concentration for odorant EC_{50} and Hill coefficient n were determined by plotting the fractional activity Y (= I(t) at saturation just before lysis, t = ~190 s) as a function of odorant concentration c_{odorant} and fitting them to the Hill equation (*S3*)

$$Y = Y_0 + (Y_{\mathrm{MAX}} - Y_0) / \{1 + (EC_{50} / c_{\mathrm{odorant}})^n\} \qquad (S3)$$

where Y_0 is Y without odorant, Y_{MAX} is Y with an excess odorant at saturation, EC_{50} is the concentration of odorant required to reach 50% activity and n is the Hill coefficient (e.g. Fig. S6).

1.4.5. Odorant sensing experiments (inverse detection; Table S3)

2 μl of reactive counterions (**A1H2-G1H3**) in DMSO (**A1H2**, 100 mM; **G1H2**, 30 mM; **A1H3**, 60 mM; **G1H3**, 10 mM), 2 μl of increasing concentrations of odorant in DMSO and 2 μl of **O5** (octanal) in DMSO (200 mM for **A1H2**; 100 mM for **G1H2** and **A1H3**; 40 mM for **G1H3**) were mixed with 14 μl DMSO in a tube (total 20 μl), and the mixture was stirred at 60˚C for 30 min. 10 μl of the resulting reaction mixture was then used as an activator for DNA activation experiments following the above procedure (1.4.3). The final concentrations of the reactive counterion and **O5** in each cuvette are 50 μM **A1H2** and 100 μM **O5**, 15 μM **G1H2** and 50 μM **O5**, 30 μM **A1H3** and 50 μM **O5**, and 5 μM **G1H3** and 20 μM **O5**. Fluorescence intensities were normalized to fractional emission intensity $I(\mathrm{t})$ using equation (*S1*). Inhibitory concentration for odorant IC_{50} and Hill coefficient n were determined by plotting the fractional activity Y_{i} (= $I(\mathrm{t})$ just before lysis, t = ~190 s) as a function of odorant concentration c_{odorant} and fitting them to the Hill equation (*S4*)

$$Y_{\mathrm{i}} = Y_{\mathrm{MIN,i}} + (Y_{\mathrm{MAX,i}} - Y_{\mathrm{MIN,i}}) / \{1 + (c_{\mathrm{odorant}} / IC_{50})^n\} \qquad (S4)$$

where $Y_{\mathrm{MAX,i}}$ is Y in the absence of odorant, $Y_{\mathrm{MIN,i}}$ is Y with an excess odorant, IC_{50} is the concentration of odorant required for 50% inactivation and n is the Hill coefficient (e.g. Fig. S7).

1.4.6. Perfume sensing experiments (Table S4)

2 μl of reactive counterions (**A1H2-G1H3**) in DMSO (**A1H2**, 100 mM; **G1H2**, 30 mM; **A1H3**, 60 mM; **G1H3**, 10 mM) was mixed with the increasing volumes of perfume (**P1-P6**) and adjusted the total volume to 20 μl with DMSO, and the mixture was stirred at 60˚C for 30 min. 10 μl of the resulting reaction mixture was then used as an activator for DNA activation experiments following the above procedure (1.4.3). The final concentration of the reactive counterion in each cuvette is 50 μM (**A1H2**), 15 μM (**G1H2**), 30 μM (**A1H3**) and 5 μM (**G1H3**). Fluorescence intensities were normalized to fractional emission intensity I(t) using equation (*S1*). Effective volume for odorant V_{50} and Hill coefficient n were determined by plotting the fractional activity Y (= I(t) at saturation just before lysis, t = ~190 s) as a function of perfume volume in a cuvette V and fitting them to the Hill equation (*S5*)

$$Y = Y_0 + (Y_{\mathrm{MAX}} - Y_0) / \{1 + (V_{50} / V)^n\} \qquad (S5)$$

where Y_0 is Y without perfume, Y_{MAX} is Y with an excess perfume at saturation, V_{50} is the perfume volume required to reach 50% activity and n is the Hill coefficient (e.g. Fig. S8).

1.4.7. Multiwell assays

Adapting the general procedures in ref. S11, 2 μl of reactive counterions (**G1H2** and **G1H3**) in DMSO (**G1H2**, 30 mM; **G1H3**, 10 mM) was mixed with the increasing volumes of perfume (**P1-P6**) and adjusted the total volume to 20 μl with DMSO, and the mixture was stirred at 60˚C for 30 min. 5 μl of the resulting reaction mixture and 5 μl of the transporter (calf thymus DNA, 125 μg/ml) were added to the diluted EYPC-LUV solution in a 96-well plate (total volume in a well, 250 μl; final lipid concentration, 50 μM; final DNA concentration, 2.5 μg/ml; final reactive-counterion concentration, 60 μM and 20 μM, respectively). After 1 hr incubation with Rotamax 120 (Heidolph), the fluorescence image of the plate was obtained with Gene Genius (Syngene) (λ_{ex} 365 nm, λ_{em} 500-600 nm). HPTS efflux could be monitored at λ_{em} 511 nm (λ_{ex} 413 nm) as a function of time using FluoroMax-3 spectrofluorometer equipped with a multiwell plate reader (MicroMax 384).

1.4.8. Data analysis

Statistical evaluation of above data was performed using principal component analysis (PCA) and hierarchical clustering analysis (HCA).[S12] PCA is a statistical unsupervised method to reduce a multidimensional data set to a lower dimensional one for easier interpretation, which is achieved by calculating orthogonal eigenvectors in the direction of the maximum variance within that data set. HCA is an unsupervised method to classify the analytes by measuring the interpoint distances (in this study, the Euclidean distance) between all analytes in the multidimensional space, providing graphic output in the form of a 2-D dendrogram. Ward’s method was used to define clusters, which takes into consideration the minimum variance between the analytes. All data analysis was performed with MYSTAT software (SYSTAT).

1.5. References

S1. (a) V. Gorteau, M. D. Julliard and S. Matile, *J. Membr. Sci.*, 2008, **321**, 37-42. (b) V. Gorteau, G. Bollot, J. Mareda and S. Matile, *Org. Biomol. Chem.*, 2007, **5**, 3000-3012. (c) V. Gorteau, G. Bollot, J. Mareda, A. Perez-Velasco and S. Matile, *J. Am. Chem. Soc.*, 2006, **128**, 14788-14789.

S2. A. Perez-Velasco, V. Gorteau and S. Matile, *Angew. Chem. Int. Ed.*, 2008, **47**, 921-923.

S3. R. S. K. Kishore, V. Ravikumar, G. Bernardinelli, N. Sakai and S. Matile, *J. Org. Chem.*, 2008, **73**, 738-740.

S4. R. E. Dawson, A. Hennig, D. P. Weimann, D. Emery, V. Ravikumar, J. Montenegro, T. Takeuchi, S. Gabutti, M. Mayor, J. Mareda, C. A. Schalley and S. Matile, *Nat. Chem.*, 2010, **2**, 533-538.

S5. Y.-C. Wu, M. Liron and J. Zhu, *J. Am. Chem. Soc.*, 2008, **130**, 7148-7152.

S6. A. Ardá, R. G. Soengas, M. I. Nieto, C. Jiménez and J. Rodríguez, *Org. Lett.*, 2008, **10**, 2175-2178.

S7. A. Pichette, N. Voyer, R. Larouche and J.-C. Meillon, *Tetrahedron Lett.*, 1997, **38**, 1279-1282.

S8. Y. Shunichi, T. Shiro and W. Mitsuyoshi, *Tetrahedron Lett.*, 1970, **18**, 1501-1504.

S9. T. Takeuchi and S. Matile, *J. Am. Chem. Soc.*, 2009, **131**, 18048-18049.

S10. S. Litvinchuk, H. Tanaka, T. Miyatake, D. Pasini, T. Tanaka, G. Bollot, J.

Mareda and S. Matile, *Nat. Mater.*, 2007, **6**, 576-580.

S11. G. Das, P. Talukdar and S. Matile, *Science*, 2002, **298**, 1600-1602.

S12. P. C. Jurs, G. A. Bakken and H. E. McClelland, *Chem. Rev.*, 2000, **100**, 2649-2678.

2. Supporting figures

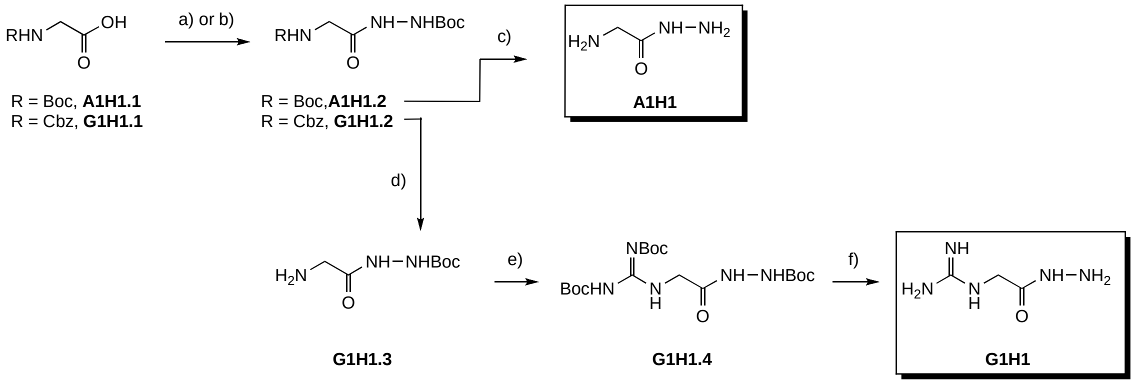

Fig. S1. Synthesis of reactive counterions **A1H1** and **G1H1**. a) For R = Boc: NH_2-NHBoc, HBTU, DIEA, DCM, rt, 90%; b) for R = Cbz: NH_2-NHBoc, HBTU, DIEA, DCM, rt, 88%; c) 1 M HCl in Et_2O, DCM, reflux, 86%; d) Pd/C, H_2, MeOH, rt, quantitative; e) N,N'-di-Boc-*1H*-pyrazole-1-carboxamidine, DIEA, CH_3CN, 55 ^oC, 74%; f) 1 M HCl in Et_2O, DCM, reflux, 97%.

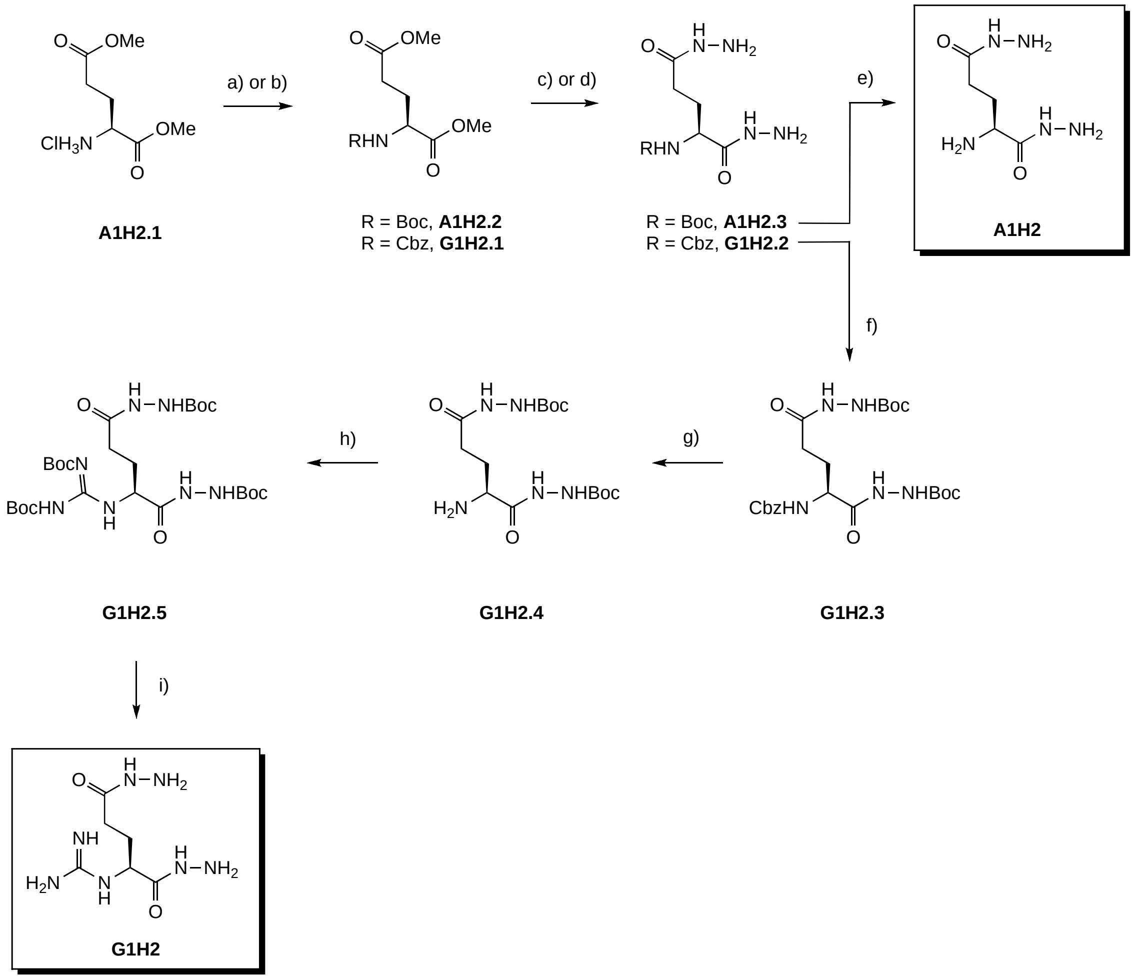

Fig. S2. Synthesis of reactive counterions **A1H2** and **G1H2**. a) For R = Boc: Boc_2O, DIEA, DCM, rt, quantitative; b) for R = Cbz: CbzCl, DIEA, CH_3CN, H_2O, quantitative; c) For R = Boc: Hydrazine monohydrate, MeOH, reflux, 79%; d) for R = Cbz: Hydrazine monohydrate, MeOH, reflux, 90%; e) TFA/DCM 1:1, rt, 79%; f) Boc_2O, DIEA, CH_3CN, H_2O, rt, 79%; g) Pd/C, H_2, MeOH, rt, quantitative; h) N,N'-di-Boc-*1H*-pyrazole-1-carboxamidine, DIEA, CH_3CN, H_2O, 55 ^oC, 69%; i) 1 M HCl in Et_2O, DCM, reflux, 76%.

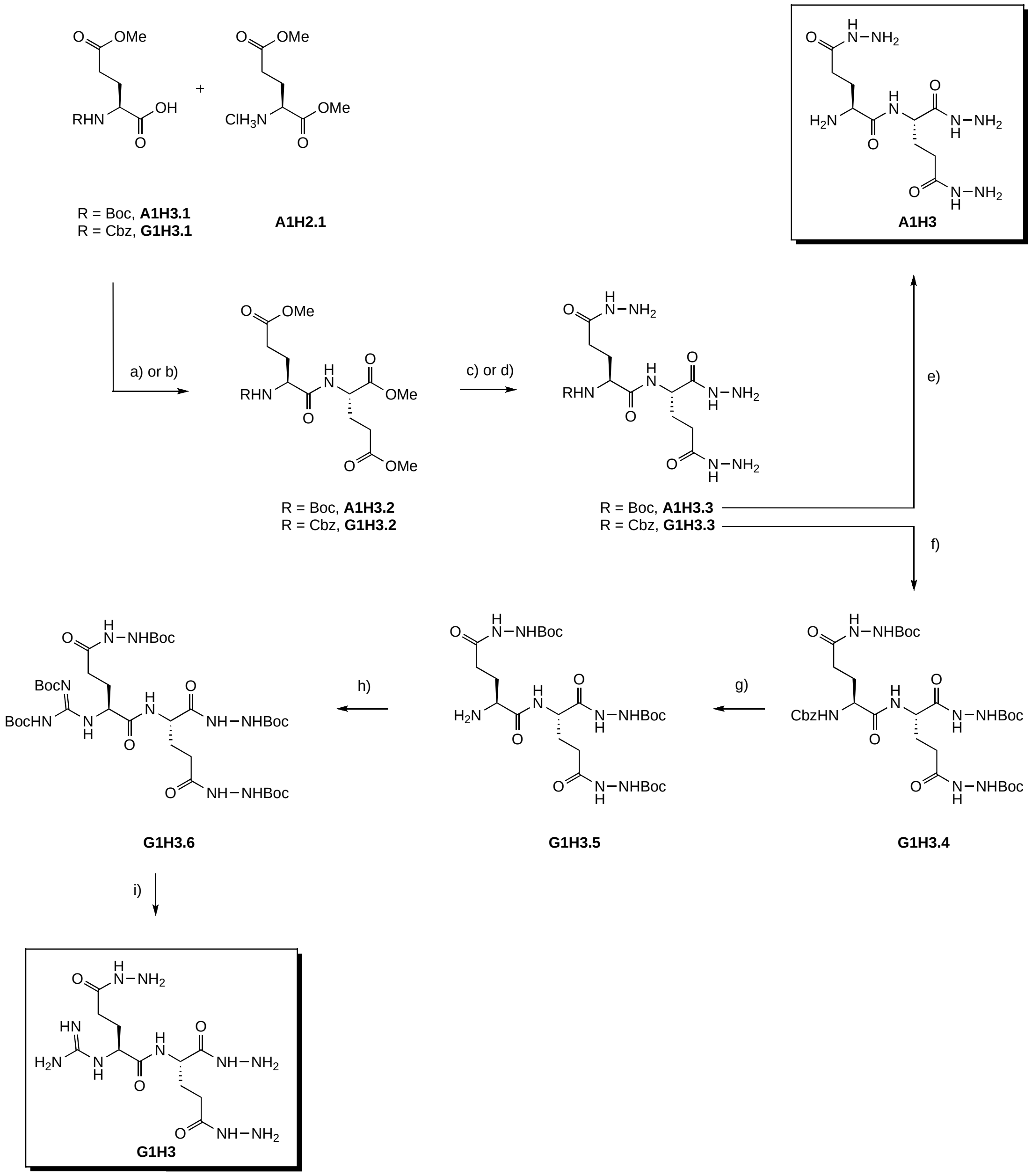

Fig. S3. Synthesis of reactive counterions **A1H3** and **G1H3**. a) For R = Boc: HBTU, DIEA, DCM, rt, 74%; b) for R = Cbz: HBTU, DIEA, DCM, rt, 85%; c) for R = Boc: Hydrazine monohydrate, MeOH, reflux, 90%; d) for R = Cbz: Hydrazine monohydrate, MeOH, H_2O, reflux, 81%; e) TFA/DCM 1:1, rt, 87%; f) Boc_2O, DIEA, CH_3CN, H_2O, rt, 79%; g) Pd/C, H_2, MeOH, rt, quantitative; h) N,N'-di-Boc-*1H*-pyrazole-1-carboxamidine, DIEA, CH_3CN, H_2O, 55 ^{o}C, 61%; i) 1 M HCl in Et_2O, DCM, reflux, 72%.

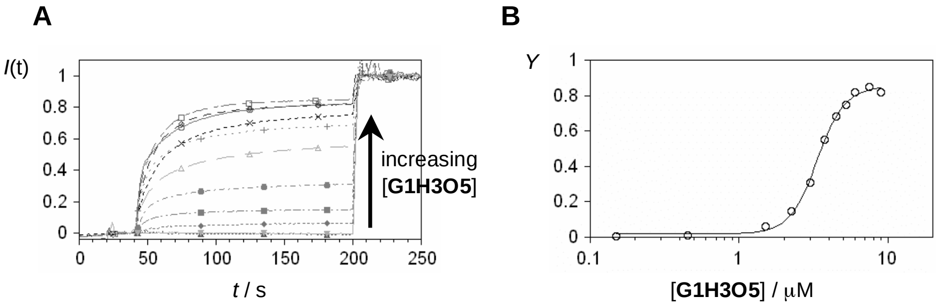

Fig. S4. (**A**) Representative normalized kinetics of transporter-mediated increase in HPTS fluorescence, following addition of transporter (calf thymus DNA, 1.25 μg/ml final concentration) at t = 40 s and triton X-100 (0.024% final concentration) at t = 200 s, demonstrating increasing transporter activity with increasing concentrations of **G1H3O5** hydrazone (0.15-9 μM). (**B**) Representative dose-response curve for DNA activation with **G1H3O5** hydrazone obtained by plotting the fractional activity Y as a function of **G1H3O5** hydrazone concentration, giving EC_{50} for **G1H3O5** hydrazone of 3.4 ± 0.1 μM, Y_{MAX} of 85.3 ± 1.7% and Hill coefficient n of 4.6 ± 0.4.

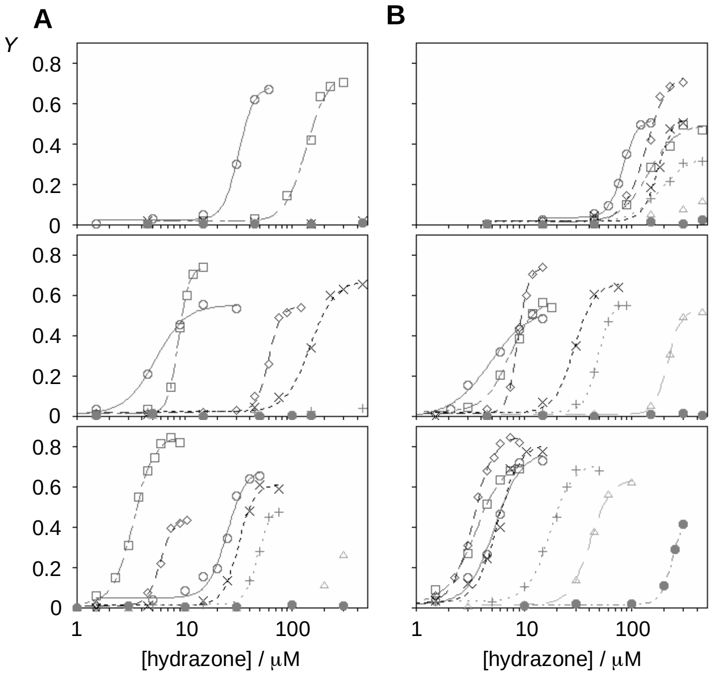

Fig. S5. Dose-response curves for 7 odorants (**A**; **O5**, □; **O8**, ○; **O16**, ●; **O17**, △; **O18**, ×; **O24**, ◊; **O29**, +) and for 7 linear alkyl aldehydes with different chain length (**B**; **O1**, ●; **O2**, △; **O3**, +; **O4**, ×; **O5**, ◊; **O6**, □; **O7**, ○) derivatized with three different reactive counterions (from upper to lower: **G1H1**, **G1H2** and **G1H3**).

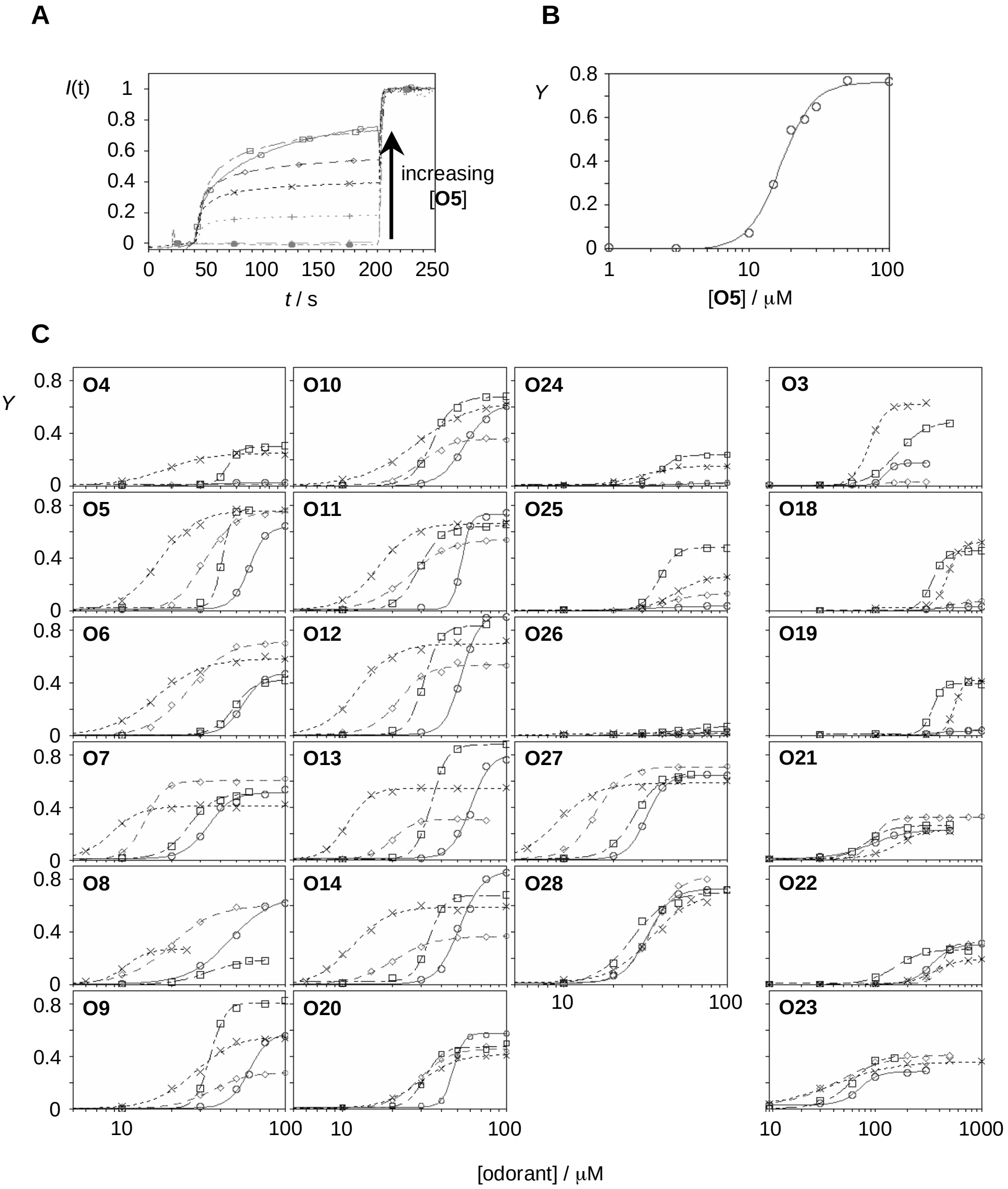

Fig. S6. Odorant sensing with the direct detection method. (**A**) Representative normalized kinetics of transporter-mediated increase in HPTS fluorescence, following addition of transporter (calf thymus DNA, 1.25 µg/ml final concentration) at *t* = 40 s and triton X-100 (0.024% final concentration) at *t* = 200 s, demonstrating increasing transporter activity with increasing concentrations of an odorant (**O5**, 1.5-100 µM final) coupled with a reactive counterion (**G1H3**, 5 µM final). (**B**) Representative dose-response curve for DNA activation with **O5** coupled with **G1H3**, obtained by plotting the fractional activity *Y* as a function of **O5** concentration, giving EC_{50} for **O5** of 20.5 ± 1.3 µM, Y_{MAX} of 75.1 ± 3.4% and Hill coefficient *n* of 3.2 ± 0.6. (**C**) Dose-response curves for odorants coupled with **A1H2** (50 µM, ○), **G1H2** (15 µM, ◊), **A1H3** (30 µM, □) and **G1H3** (5 µM, ×). A different set of reactive-counterion concentrations was used for derivatization of **O3**, **O18**, **O19** and **O21-O23** (**A1H2** and **A1H3**, 50 µM; **G1H2** and **G1H3**, 30 µM) because of weak responsiveness.

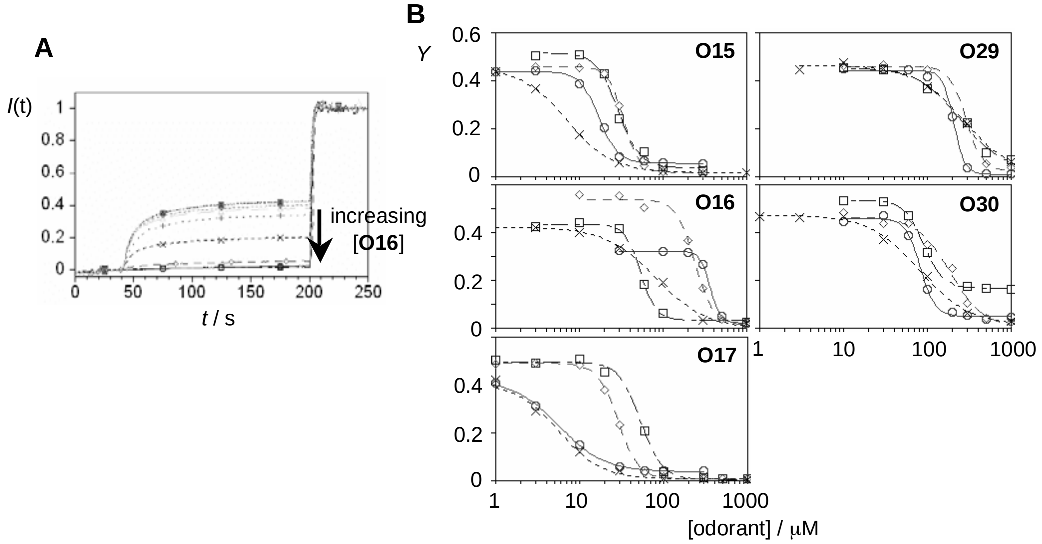

Fig. S7. Odorant sensing with the inverse detection method. (**A**) Representative normalized kinetics of transporter-mediated increase in HPTS fluorescence, following addition of transporter (calf thymus DNA, 1.25 μg/ml final concentration) at *t* = 40 s and triton X-100 (0.024% final concentration) at *t* = 200 s, demonstrating increasing transporter activity with increasing concentrations of an odorant (**O16**, 0.1-1000 μM final) coupled with a reactive counterion (**G1H3**, 5 μM final) in the presence of a competitor (**O5**, 20 μM). (**B**) Dose-response curves for odorants coupled with **A1H2** (50 μM, ○), **G1H2** (15 μM, ◊), **A1H3** (30 μM, □) and **G1H3** (5 μM, ×) in the presence of a competitor (**O5**, 100 μM for **A1H2**; 50 μM for **G1H2** and **A1H3**; 20 μM for **G1H3**).

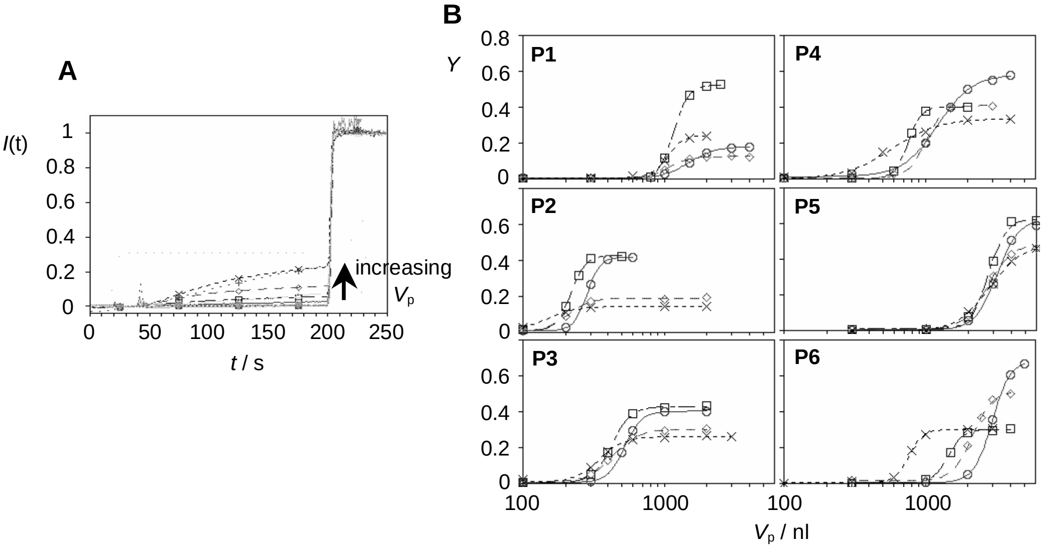

Fig. S8. Perfume sensing with the direct detection method. (**A**) Representative normalized kinetics of transporter-mediated increase in HPTS fluorescence, following addition of transporter (calf thymus DNA, 1.25 μg/ml final concentration) at t = 40 s and triton X-100 (0.024% final concentration) at t = 200 s, demonstrating increasing transporter activity with increasing volumes (V_p) of a perfume (**P1**, 100-4000 nl final) coupled with a reactive counterion (**G1H3**, 5 μM final). (**B**) Dose-response curves for perfumes coupled with **A1H2** (50 μM, ○), **G1H2** (15 μM, ◊), **A1H3** (30 μM, □) and **G1H3** (5 μM, ×).

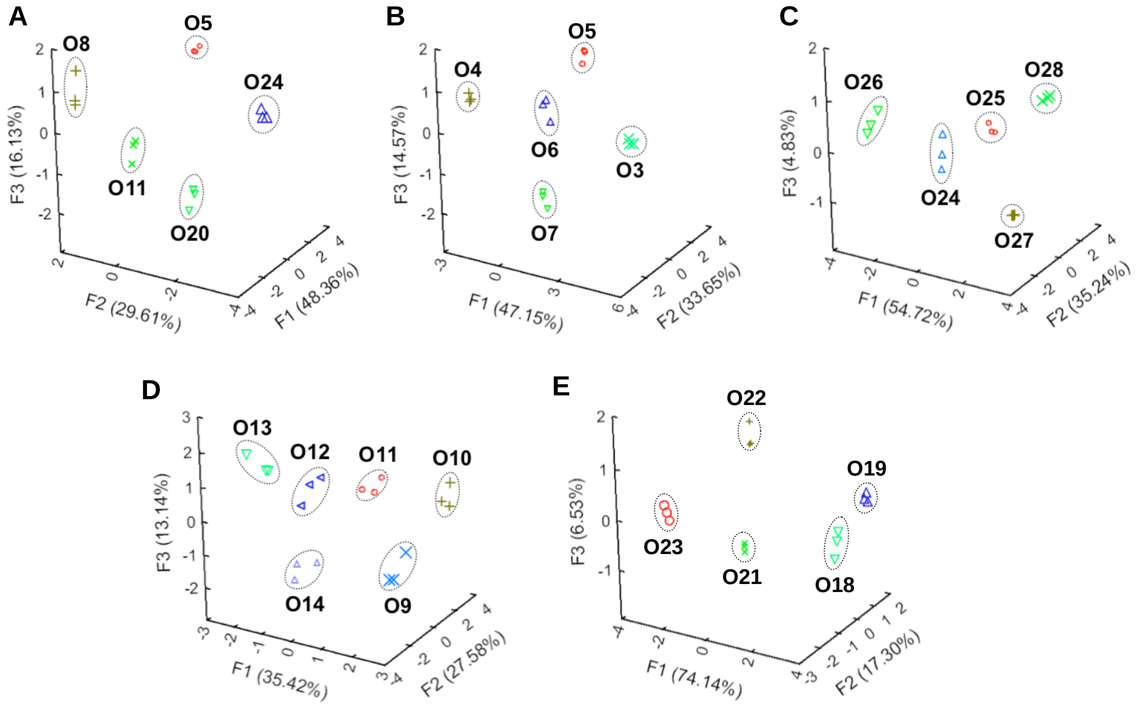

Fig. S9. PCA score plots for 26 odorants detected with the direct sensing method. (**A**) Discrimination of representative odorants. Octanal (**O5**, ○), methylundecanal (**O8**, +), citral (**O11**, ×), perillaaldehyde (**O20**, ▿) and cinnamaldehyde (**O24**, ▵). (**B**) Discrimination of linear alkyl aldehydes with different chain length. Hexanal (**O3**, ×), heptanal (**O4**, +), octanal (**O5**, ○), nonanal (**O6**, ▵) and decanal (**O24**, ▿). (**C**) Discrimination of cinnamaldehyde and close derivatives. Cinnamaldehyde (**O24**, ▵), methylcinnamaldehyde (**O25**, ○), hydrocinnamaldehyde (**O26**, ▿), cyclosal (**O27**, +) and jasminaldehyde (**O28**, ×). (**D**) Discrimination of cis/trans isomers and enantiomers. *trans*,*cis*-nonadienal (**O9**, ×), *trans*,*trans*-nonadienal (**O10**, +), citral (**O11**, ○), (±)-citronellal (**O12**, ◃), (+)-citronellal (**O13**, ▿) and (-)-citronellal (**O14**, ▵). (**E**) Discrimination of enantiomers. (-)-carvone (**O18**, ▿), (+)-carvone (**O19**, ▵), (-)-muscone (**O21**, ×), (±)-muscone (**O22**, +) and civetone (**O23**, ○).

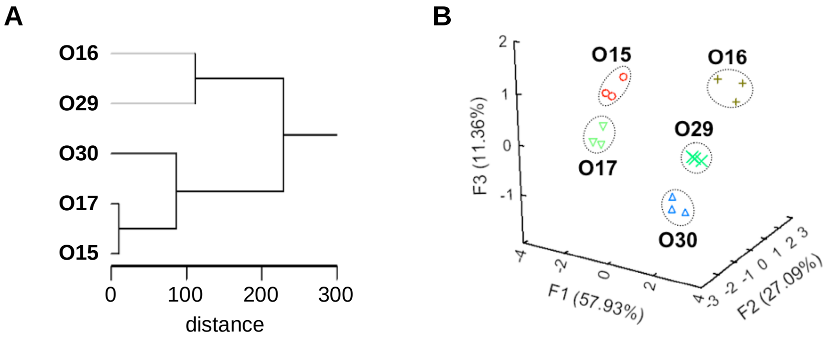

Fig. S10. (**A**) HCA dendrogram for 5 odorants detected with the inverse sensing method, showing the Euclidean distances between average values from three independent trials. (B) PCA score plot. Hydroxycitronellal (**O15**, ○), acetone (**O16**, +), isovaleraldehyde (**O17**, ▿), *p*-anisaldehyde (**O29**, ×) and vanillin (**O30**, ▵).

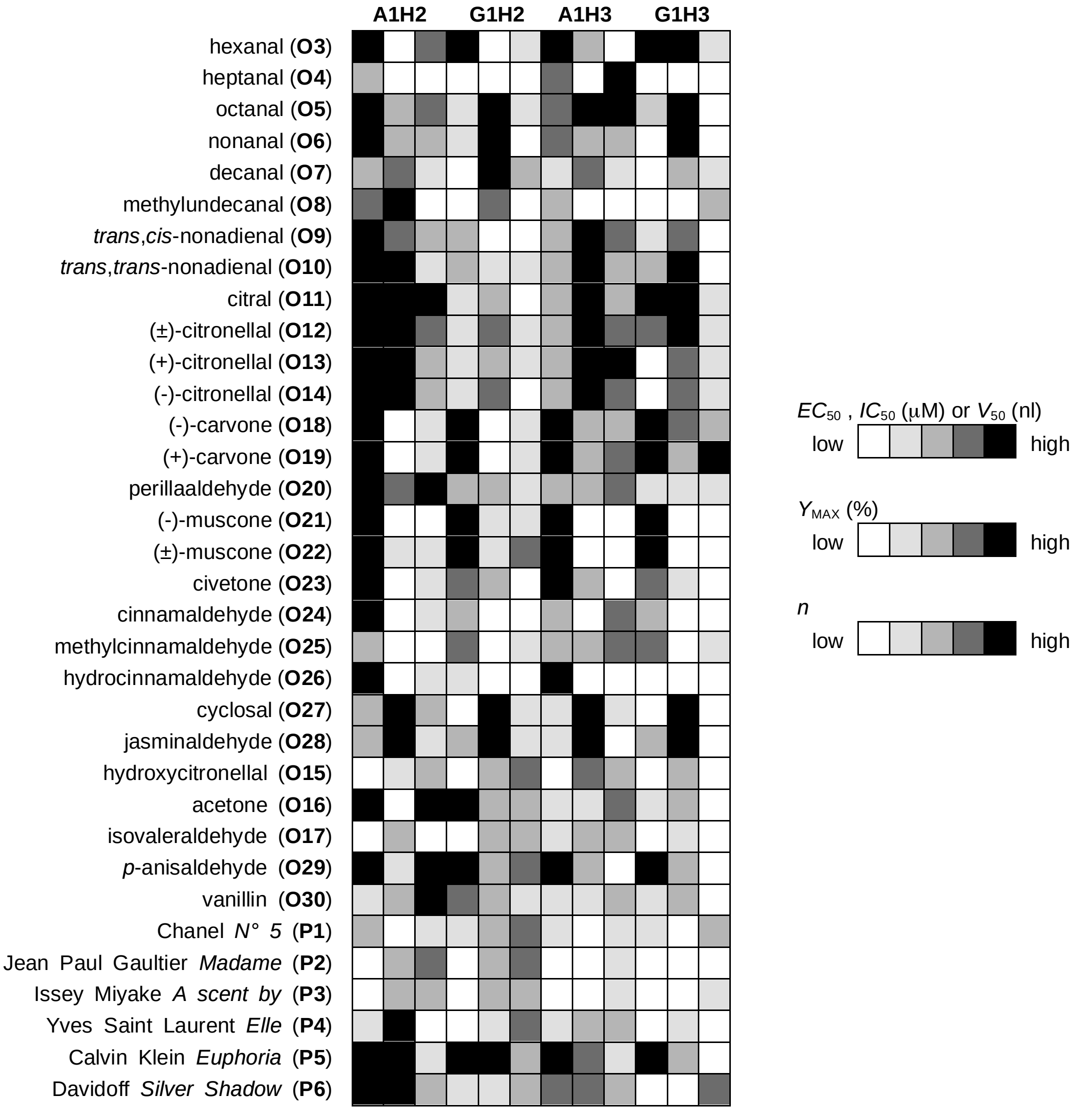

Fig. S11. 12-D pattern generation for 28 odorants and 6 perfumes with reactive counterions (**A1H2-G1H3**) and three readouts each (from left to right: EC_{50} (10-60 μM), Y_{MAX} (20-70%) and n (2-12) for **O3-O14** and **O18-O28** (direct detection, HCA/PCA: Figs. 2, 3C, S9); IC_{50} (0-250 μM), response (20-70%) and n (1-6) for **O15-O17**, **O29** and **O30** (inverse detection, HCA/PCA: Fig. S10); V_{50} (500-3000 nl) Y_{MAX} (20-70%) and n (2-12) for **P1-P6**; direct detection, HCA/PCA: Figs. 3B, 3C), expressed as 5 different tones of black and white.

3. Supporting tables

Table S1. EC_{50}, Y_{MAX} and n for hydrazones.[a]

odorant		reactive counterion	EC_{50} (μM)[b]	Y_{MAX} (%)[c]	n[d]
O1	Butanal	**G1H1**	NA[e]	NA[e]	NA[e]
		G1H2	NA[e]	NA[e]	NA[e]
		G1H3	240.1 ± 4.0	48.9 ± 2.2	7.6 ± 0.6
O2	pentanal	**G1H1**	>200	>10	ND[f]
		G1H2	214.7 ± 2.8	52.8 ± 1.0	7.3 ± 0.7
		G1H3	41.0 ± 1.1	64.0 ± 1.8	4.6 ± 0.5
O3	hexanal	**G1H1**	185.1 ± 19.0	34.3 ± 3.7	3.2 ± 1.0
		G1H2	48.4 ± 0.9	56.7 ± 1.5	6.9 ± 0.9
		G1H3	17.3 ± 0.6	71.2 ± 2.6	4.3 ± 0.6
O4	heptanal	**G1H1**	172.8 ± 7.6	53.2 ± 4.2	6.3 ± 1.8
		G1H2	29.2 ± 1.3	66.1 ± 3.2	4.7 ± 1.2
		G1H3	5.7 ± 0.3	81.4 ± 4.9	4.3 ± 1.0
O5	octanal	**A1H1**	198.8 ± 8.5	68.2 ± 3.3	4.1 ± 0.6
		G1H1	134.8 ± 7.7	74.5 ± 4.6	4.5 ± 1.0
		A1H2	35.7 ± 0.8	78.9 ± 1.6	5.4 ± 0.7
		G1H2	8.8 ± 0.1	74.2 ± 1.8	9.0 ± 0.9
		A1H3	12.1 ± 0.4	66.9 ± 0.9	3.4 ± 0.3
		G1H3	3.4 ± 0.1	85.3 ± 1.7	4.6 ± 0.4
O6	nonanal	**G1H1**	141.5 ± 10.7	49.7 ± 3.0	3.4 ± 0.8
		G1H2	7.4 ± 0.3	58.3 ± 2.5	3.6 ± 0.6
		G1H3	3.5 ± 0.2	73.3 ± 2.9	3.3 ± 0.4
O7	decanal	**G1H1**	82.3 ± 1.5	52.3 ± 1.4	6.5 ± 0.8
		G1H2	4.8 ± 0.6	53.7 ± 4.4	2.3 ± 0.5
		G1H3	5.3 ± 0.3	77.6 ± 4.9	3.5 ± 0.7
O8	methylundecanal	**G1H1**	31.8 ± 1.0	68.9 ± 3.1	6.1 ± 1.4
		G1H2	5.4 ± 0.3	55.3 ± 1.8	3.1 ± 0.5
		G1H3	24.7 ± 1.4	69.0 ± 5.1	4.9 ± 1.3
O9	*trans,cis*-nonadienal	**G1H3**	3.5 ± 0.2	61.3 ± 4.1	3.7 ± 0.9
O10	*trans,trans*-nonadienal	**G1H3**	3.4 ± 0.2	58.8 ± 2.7	3.9 ± 0.7
O11	citral	**A1H2**	20.5 ± 0.5	41.5 ± 1.3	5.5 ± 0.7
		G1H2	13.4 ± 0.2	67.8 ± 1.3	5.7 ± 0.6
		A1H3	26.3 ± 0.8	62.0 ± 1.8	3.3 ± 0.3
		G1H3	3.7 ± 0.1	74.3 ± 1.5	4.6 ± 0.3
O12	(±)-citronellal	**G1H2**	13.9 ± 0.5	66.9 ± 2.4	4.9 ± 0.8
		G1H3	4.2 ± 0.2	75.9 ± 3.1	3.0 ± 0.4
O13	(+)-citronellal	**G1H3**	3.7 ± 0.1	68.0 ± 2.1	2.6 ± 0.2
O14	(-)-citronellal	**G1H3**	4.1 ± 0.1	76.6 ± 2.0	3.9 ± 0.3
O15	hydroxycitronellal	**G1H2**	NA[e]	NA[e]	NA[e]
		G1H3	NA[e]	NA[e]	NA[e]
O16	acetone	**G1H1**	NA[e]	NA[e]	NA[e]
		G1H2	NA[e]	NA[e]	NA[e]
		G1H3	NA[e]	NA[e]	NA[e]
O17	isovaleraldehyde	**G1H1**	NA[e]	NA[e]	NA[e]
		G1H2	NA[e]	NA[e]	NA[e]
		G1H3	>200	>30	ND[f]
O18	(-)-carvone	**G1H1**	NA[e]	NA[e]	NA[e]
		G1H2	146.4 ± 7.2	67.4 ± 2.8	3.8 ± 0.7
		G1H3	31.5 ± 1.4	61.3 ± 2.6	5.9 ± 1.0
O19	(+)-carvone	**G1H3**	33.0 ± 0.4	63.6 ± 0.8	4.1 ± 0.1
O20	perillaaldehyde	**A1H2**	43.9 ± 0.5	74.6 ± 1.4	10.4 ± 0.9
		G1H2	12.9 ± 0.6	61.4 ± 2.9	4.9 ± 1.0
		A1H3	32.4 ± 0.3	37.3 ± 0.6	6.0 ± 0.4
		G1H3	5.1 ± 0.2	66.5 ± 3.9	5.5 ± 1.3
O21	(-)-muscone	**G1H3**	23.0 ± 2.1	33.5 ± 2.1	2.6 ± 0.6
O22	(±)-muscone	**G1H3**	33.0 ± 3.9	33.8 ± 1.9	2.4 ± 0.5
O23	civetone	**G1H3**	13.6 ± 0.9	34.7 ± 1.3	3.0 ± 0.5
O24	cinnamaldehyde	**A1H2**	224.7 ± 8.3	52.7 ± 3.7	8.7 ± 4.3
		G1H2	60.2 ± 1.4	54.8 ± 1.9	7.6 ± 1.2
		A1H3	29.0 ± 1.8	24.6 ± 1.2	4.1 ± 0.9
		G1H3	6.0 ± 0.1	44.8 ± 1.5	7.1 ± 1.0
O25	methylcinnamaldehyde	**G1H2**	21.3 ± 0.7	57.9 ± 2.0	4.6 ± 0.6
		G1H3	4.3 ± 0.1	44.0 ± 0.9	5.1 ± 0.7
O26	hydrocinnamaldehyde	**G1H2**	75.8 ± 2.9	75.3 ± 3.9	4.4 ± 0.6
		G1H3	15.2 ± 0.9	73.1 ± 6.1	7.3 ± 4.1
O27	cyclosal	**G1H3**	3.3 ± 0.2	73.4 ± 2.7	3.4 ± 0.7
O28	jasminaldehyde	**G1H3**	5.0 ± 0.1	61.3 ± 1.7	6.1 ± 0.9
O29	*p*-anisaldehyde	**G1H1**	NA[e]	NA[e]	NA[e]
		G1H2	NA[e]	NA[e]	NA[e]
		G1H3	47.8 ± 1.7	50.7 ± 3.5	7.1 ± 1.6
O30	vanillin	**G1H1**	NA[e]	NA[e]	NA[e]
		G1H2	NA[e]	NA[e]	NA[e]
		G1H3	NA[e]	NA[e]	NA[e]

[a] All data were obtained from Hill analysis of dose response curves for hydrazones **A1H*n*O*m*** and **G1H*n*O*m*** (n=**1**-**3**, m=**1**-**30**; variable concentrations) and detected with calf thymus DNA in HPTS/DPX-LUVs. [b] Effective concentration for hydrazones needed to reach $Y_{\mathrm{MAX}}/2$. [c] Maximal activity. [d] Hill coefficient. [e] NA, no activity. [f] ND, not determined.

Table S2. EC_{50}, Y_{MAX} and n for odorants detected with the direct sensing method,[a] and ESI-MS.

odorant	reactive counterion	EC_{50} (μM)[b]	Y_{MAX} (%)[c]	n[d]	ESI-MS[e]	
					found	expected for $[M+H]^+$
O3[f]	**A1H2**	120.6 ± 2.7	17.2 ± 0.9	8.5 ± 0.3	340.5	340.3
	G1H2	104.4 ± 4.3	3.4 ± 0.3	5.1 ± 0.1	382.3	382.3
	A1H3	162.9 ± 4.5	49.8 ± 0.8	3.2 ± 0.2	565.4	565.4
	G1H3	90.3 ± 3.3	63.1 ± 1.1	5.4 ± 0.2	607.3	607.4
O4	**A1H2**	33.0 ± 1.6	2.6 ± 0.1	3.2 ± 0.1	369.3	368.3
	G1H2	18.1 ± 0.9	1.5 ± 0.2	2.9 ± 0.2	410.3	410.3
	A1H3	44.5 ± 0.8	29.6 ± 0.7	11.4 ± 0.5	607.5	607.5
	G1H3	17.9 ± 0.6	24.3 ± 0.8	3.1 ± 0.2	649.8	649.5
O5	**A1H2**	73.0 ± 0.5	49.5 ± 1.7	9.4 ± 0.5	396.5	396.6
	G1H2	33.1 ± 1.0	74.7 ± 0.9	4.8 ± 0.1	438.7	438.6
	A1H3	41.4 ± 0.8	74.3 ± 2.9	14.7 ± 0.6	649.8	649.9
	G1H3	20.1 ± 0.5	75.4 ± 0.7	3.2 ± 0.1	692.0	692.0
O6	**A1H2**	57.9 ± 2.5	48.0 ± 0.4	6.9 ± 0.1	424.5	424.4
	G1H2	28.1 ± 2.2	70.4 ± 0.9	3.9 ± 0.3	466.5	466.4
	A1H3	49.3 ± 1.2	43.8 ± 1.3	6.9 ± 0.2	691.8	691.6
	G1H3	18.2 ± 2.0	57.9 ± 1.1	3.1 ± 0.5	733.7	733.6
O7	**A1H2**	34.3 ± 0.9	52.4 ± 1.0	5.4 ± 0.3	452.4	452.4
	G1H2	13.8 ± 0.8	60.1 ± 0.4	6.8 ± 0.4	494.5	494.4
	A1H3	26.4 ± 1.2	50.8 ± 1.0	5.8 ± 0.2	733.7	733.6
	G1H3	8.8 ± 0.2	42.1 ± 1.0	4.5 ± 0.2	775.8	775.6
O8	**A1H2**	46.6 ± 1.1	66.2 ± 2.4	3.3 ± 0.2	508.5	508.5
	G1H2	19.6 ± 0.9	59.9 ± 2.4	3.2 ± 0.3	550.3	550.5
	A1H3	35.7 ± 2.4	18.9 ± 1.4	3.9 ± 0.2	817.7	817.7
	G1H3	10.4 ± 0.7	26.8 ± 0.3	6.2 ± 0.2	859.7	859.7
O9	**A1H2**	59.9 ± 0.7	57.5 ± 2.2	6.7 ± 0.2	416.4	416.3
	G1H2	34.2 ± 2.2	28.4 ± 1.6	3.7 ± 0.2	458.5	458.3
	A1H3	33.8 ± 0.8	81.3 ± 0.6	9.7 ± 0.2	679.5	679.5
	G1H3	27.2 ± 1.4	55.5 ± 1.4	3.4 ± 0.1	721.5	721.5
O10	**A1H2**	55.6 ± 0.8	61.6 ± 0.8	5.4 ± 0.1	416.0	416.3
	G1H2	31.2 ± 1.3	36.2 ± 0.9	5.1 ± 0.3	458.4	458.3
	A1H3	35.3 ± 0.3	69.9 ± 2.4	6.7 ± 0.3	679.5	679.5
	G1H3	30.5 ± 2.3	64.1 ± 0.8	2.7 ± 0.2	721.5	721.5
O11	**A1H2**	53.2 ± 0.4	73.9 ± 0.5	15.5 ± 1.4	444.5	444.6
	G1H2	28.0 ± 0.2	49.2 ± 1.2	3.9 ± 0.2	486.7	486.7
	A1H3	35.4 ± 1.2	62.7 ± 1.6	6.9 ± 0.1	721.8	722.0
	G1H3	16.0 ± 0.8	63.1 ± 2.8	4.0 ± 0.1	763.8	764.0
O12	**A1H2**	58.4 ± 0.7	88.0 ± 1.7	9.2 ± 0.1	448.4	448.4
	G1H2	22.3 ± 0.3	52.0 ± 1.5	4.8 ± 0.1	490.7	490.4
	A1H3	35.3 ± 1.1	84.9 ± 2.0	8.9 ± 0.2	727.7	727.6
	G1H3	11.8 ± 0.5	65.1 ± 0.7	4.1 ± 0.1	770.0	769.6
O13	**A1H2**	59.0 ± 1.9	79.6 ± 2.0	7.9 ± 0.9	448.4	448.4
	G1H2	21.5 ± 1.1	40.8 ± 3.2	4.8 ± 0.0	490.4	490.4
	A1H3	33.9 ± 0.5	67.6 ± 1.9	10.4 ± 0.6	727.5	727.6
	G1H3	10.6 ± 0.3	52.5 ± 1.9	5.9 ± 0.3	769.7	769.6
O14	**A1H2**	52.7 ± 0.7	88.0 ± 1.7	6.4 ± 0.3	448.4	448.4
	G1H2	22.8 ± 0.5	52.8 ± 1.9	3.5 ± 0.2	490.6	490.4
	A1H3	33.3 ± 1.8	80.7 ± 1.3	8.2 ± 0.2	727.5	727.6
	G1H3	11.2 ± 0.5	58.5 ± 1.2	4.0 ± 0.2	769.7	769.6
O18[f]	**A1H2**	348.4 ± 9.1	2.7 ± 0.3	4.5 ± 0.3	440.5	440.3
	G1H2	487.0 ± 7.6	6.7 ± 0.7	4.4 ± 0.1	482.5	482.3
	A1H3	339.9 ± 6.4	44.3 ± 1.4	6.8 ± 0.4	715.5	715.5
	G1H3	486.7 ± 9.9	53.1 ± 1.2	7.6 ± 0.2	757.7	757.5
O19[f]	**A1H2**	351.2 ± 6.3	4.0 ± 0.5	5.1 ± 0.1	440.5	440.3
	G1H2	576.5 ± 10.2	4.7 ± 0.2	4.6 ± 0.1	482.4	482.3
	A1H3	350.9 ± 10.7	40.2 ± 1.1	9.4 ± 0.4	715.5	715.5
	G1H3	530.9 ± 15.5	42.0 ± 0.8	10.2 ± 0.4	757.7	757.5

O20	**A1H2**	51.9 ± 4.1	56.2 ± 1.3	13.6 ± 0.2	440.5	440.6
	G1H2	33.1 ± 1.1	42.1 ± 1.9	4.7 ± 0.2	482.5	482.6
	A1H3	32.6 ± 0.6	45.1 ± 2.3	8.4 ± 0.2	715.8	716.0
	G1H3	29.7 ± 1.9	33.7 ± 2.7	4.1 ± 0.2	757.8	758.0
O21[f]	**A1H2**	95.1 ± 3.7	23.3 ± 0.6	2.7 ± 0.2	616.8	616.6
	G1H2	95.9 ± 4.1	31.0 ± 1.2	5.8 ± 0.3	658.8	658.6
	A1H3	82.6 ± 2.3	26.5 ± 1.1	3.4 ± 0.2	979.8	979.8
	G1H3	161.4 ± 4.2	23.0 ± 0.8	3.7 ± 0.2	1022.0	1021.9
O22[f]	**A1H2**	111.7 ± 1.8	31.7 ± 1.6	4.5 ± 0.2	616.7	616.6
	G1H2	138.5 ± 2.0	32.6 ± 0.9	8.8 ± 0.3	658.7	658.6
	A1H3	76.2 ± 3.7	27.3 ± 0.4	3.3 ± 0.2	979.8	979.8
	G1H3	181.3 ± 4.9	19.6 ± 1.6	3.7 ± 0.2	1021.8	1021.9
O23[f]	**A1H2**	69.7 ± 1.6	28.9 ± 0.7	4.8 ± 0.2	640.8	640.6
	G1H2	46.2 ± 1.3	40.9 ± 0.6	2.0 ± 0.1	682.5	682.6
	A1H3	62.8 ± 2.5	43.7 ± 1.3	3.1 ± 0.1	1015.8	1015.8
	G1H3	42.7 ± 1.3	36.3 ± 0.7	1.3 ± 0.2	1057.6	1057.9
O24	**A1H2**	55.0 ± 1.7	2.9 ± 0.7	4.7 ± 0.2	404.5	404.5
	G1H2	33.3 ± 1.7	2.1 ± 0.2	3.8 ± 0.1	446.3	446.5
	A1H3	39.0 ± 0.2	23.6 ± 1.2	8.0 ± 1.1	661.5	661.8
	G1H3	30.5 ± 1.3	15.2 ± 0.2	3.9 ± 0.1	703.8	703.8
O25	**A1H2**	33.1 ± 3.4	3.3 ± 0.6	2.9 ± 0.1	432.5	432.2
	G1H2	42.1 ± 1.6	12.2 ± 1.4	4.5 ± 0.3	474.3	474.3
	A1H3	37.6 ± 0.7	47.4 ± 1.1	9.0 ± 0.6	703.5	703.4
	G1H3	46.4 ± 2.2	27.6 ± 2.7	4.7 ± 0.1	745.5	745.4
O26	**A1H2**	65.7 ± 2.6	3.5 ± 0.2	5.8 ± 0.6	408.1	408.2
	G1H2	21.5 ± 2.9	1.3 ± 0.2	2.1 ± 0.2	450.5	450.3
	A1H3	53.9 ± 2.8	7.8 ± 0.1	3.4 ± 0.4	667.5	667.4
	G1H3	7.8 ± 0.4	1.9 ± 0.2	3.9 ± 0.4	709.5	709.4
O27	**A1H2**	31.0 ± 0.8	63.6 ± 0.9	6.9 ± 0.3	520.3	520.4
	G1H2	15.3 ± 0.5	68.4 ± 2.6	5.3 ± 0.2	562.5	562.4
	A1H3	27.7 ± 1.1	67.0 ± 1.2	5.3 ± 0.1	835.5	835.6
	G1H3	9.2 ± 0.2	59.4 ± 1.8	3.7 ± 0.2	877.7	877.6
O28	**A1H2**	31.4 ± 0.8	71.9 ± 2.5	5.7 ± 0.1	544.5	544.4
	G1H2	34.8 ± 0.2	82.9 ± 1.2	5.3 ± 0.1	586.5	586.4
	A1H3	26.3 ± 0.4	74.1 ± 3.6	3.7 ± 0.2	871.5	871.6
	G1H3	33.9 ± 0.6	71.4 ± 2.6	3.8 ± 0.3	913.5	913.6

[a] All sensing data were obtained from Hill analysis of dose response curves for odorants **O***n* (*n*=**3-14**, **18-28**; variable concentrations) coupled with reactive counterions **A1H***m* or **G1H***m* (*m*=**2-3**; **A1H2**, 50 μM; **G1H2**, 15 μM; **A1H3**, 30 μM; **G1H3**, 5 μM) and detected with calf thymus DNA in HPTS/DPX-LUVs. EC_{50}, Y_{MAX} and *n* are average values (± standard deviation) from three independent experiments. [b] Effective concentration for odorants needed to reach $Y_{MAX}/2$. [c] Maximal activity. [d] Hill coefficient. [e] ESI-MS of the reaction mixtures with excess odorants. [f] Different reactive-counterion concentrations were used (**A1H2**, 50 μM; **G1H2**, 30 μM; **A1H3**, 50 μM; **G1H3**, 30 μM).

Table S3. IC_{50} and n for odorants detected with the inverse sensing method,[a] and ESI-MS.

odorant	reactive counterion	competitor	IC_{50} (μM)[b]	response (%)[c]	n[d]	ESI-MS[e]	
						found	expected for [M+H]+
O15	**A1H2**	**O5** (100 μM)	17.6 ± 0.9	37.5 ± 1.8	3.8 ± 0.1	484.4	484.4
	G1H2	**O5** (50 μM)	32.0 ± 1.8	45.6 ± 4.6	4.6 ± 0.2	526.5	526.4
	A1H3	**O5** (50 μM)	27.6 ± 1.4	52.0 ± 3.7	3.3 ± 0.3	781.8	781.6
	G1H3	**O5** (20 μM)	7.4 ± 0.6	42.4 ± 2.1	1.7 ± 0.1	823.8	823.6
O16	**A1H2**	**O5** (100 μM)	375.3 ± 12.2	29.7 ± 2.3	7.6 ± 0.3	256.1	256.2
	G1H2	**O5** (50 μM)	238.2 ± 1.3	49.4 ± 5.5	3.7 ± 0.3	298.3	298.2
	A1H3	**O5** (50 μM)	58.8 ± 5.7	39.4 ± 1.0	4.9 ± 0.3	439.5	439.3
	G1H3	**O5** (20 μM)	83.8 ± 4.7	42.2 ± 0.8	1.5 ± 0.1	441.5[f]	481.3
O17	**A1H2**	**O5** (100 μM)	6.5 ± 0.8	40.7 ± 2.1	1.6 ± 0.1	312.5	312.2
	G1H2	**O5** (50 μM)	30.0 ± 3.2	48.1 ± 0.3	3.8 ± 0.3	354.3	354.3
	A1H3	**O5** (50 μM)	53.2 ± 2.7	48.7 ± 2.3	3.1 ± 0.3	523.4	523.4
	G1H3	**O5** (20 μM)	6.6 ± 1.2	39.8 ± 1.3	1.8 ± 0.1	565.5	565.4
O29	**A1H2**	**O5** (100 μM)	205.4 ± 7.0	39.9 ± 3.0	6.7 ± 0.1	412.4	412.2
	G1H2	**O5** (50 μM)	291.1 ± 8.5	47.5 ± 3.6	4.4 ± 0.1	454.4	454.2
	A1H3	**O5** (50 μM)	243.5 ± 6.1	46.9 ± 3.6	1.6 ± 0.1	673.3	673.3
	G1H3	**O5** (20 μM)	267.1 ± 3.7	47.3 ± 3.2	1.5 ± 0.1	715.5	715.3
O30	**A1H2**	**O5** (100 μM)	86.3 ± 3.3	41.4 ± 0.6	5.2 ± 0.3	444.0	444.2
	G1H2	**O5** (50 μM)	182.2 ± 6.5	45.4 ± 3.0	2.2 ± 0.2	486.5	486.2
	A1H3	**O5** (50 μM)	92.3 ± 3.8	37.9 ± 1.3	3.6 ± 0.1	721.2	721.3
	G1H3	**O5** (20 μM)	77.9 ± 1.7	43.4 ± 2.3	1.6 ± 0.1	763.5	763.3

[a] All sensing data were obtained from Hill analysis of inhibitory dose response curves for odorants **O***n* (n=**15**-**17**, **29**, **30**; variable concentrations) coupled with reactive counterions **A1H***m* or **G1H***m* (m=**2**-**3**; **A1H2**, 50 μM; **G1H2**, 15 μM; **A1H3**, 30 μM; **G1H3**, 5 μM) in the presence of a competitor odorant (**O5**, final concentrations are 100 μM for **A1H2**, 50 μM for **G1H2** and **A1H3**, and 20 μM for **G1H3**) and detected with calf thymus DNA in HPTS/DPX-LUVs. IC_{50}, response and n are average values (± standard deviation) from three independent experiments. [b] Inhibitory concentration for odorants needed to reach $Y_{MAX}/2$. [c] Response (%) = $Y_{MAX,i}$-$Y_{MIN,i}$. [d] Hill coefficient. [e] ESI-MS of the reaction mixtures with excess odorants. [f] ESI-MS corresponding to **G1H3**(**O16**)$_2$ (expected for [M+H]$^+$, 441.3), indicating the incomplete hydrazone formation due to poor reactivity of **O16**.

Table S4. V_{50}, Y_{MAX} and n for perfumes detected with the direct sensing method.[a]

perfume		reactive counterion	V_{50} (nl)[b]	Y_{MAX} (%)[c]	n[d]
P1	Chanel	**A1H2**	1530 ± 30	17.2 ± 0.9	4.9 ± 0.2
	N° 5	**G1H2**	1120 ± 60	12.8 ± 0.4	5.7 ± 0.1
		A1H3	1110 ± 50	49.7 ± 3.3	8.2 ± 0.3
		G1H3	1090 ± 40	23.8 ± 0.7	7.7 ± 0.2
P2	Jean Paul Gaultier	**A1H2**	283 ± 9	43.4 ± 1.8	9.5 ± 0.2
	Madame	**G1H2**	211 ± 9	19.0 ± 0.3	5.5 ± 0.1
		A1H3	216 ± 6	40.1 ± 2.5	8.3 ± 0.1
		G1H3	141 ± 11	14.3 ± 0.4	3.8 ± 0.1
P3	Issay Miyake	**A1H2**	528 ± 21	41.2 ± 2.4	7.1 ± 0.1
	A scent by	**G1H2**	417 ± 17	29.9 ± 1.1	4.6 ± 0.2
		A1H3	414 ± 11	43.6 ± 0.6	6.5 ± 0.2
		G1H3	346 ± 2	25.6 ± 0.6	4.9 ± 0.2
P4	Yves Saint Laurent	**A1H2**	1240 ± 21	59.1 ± 2.2	3.7 ± 0.2
	Elle	**G1H2**	1020 ± 21	40.3 ± 0.6	7.2 ± 0.4
		A1H3	768 ± 29	39.0 ± 1.0	9.6 ± 0.1
		G1H3	604 ± 21	33.3 ± 0.4	2.7 ± 0.1
P5	Calvin Klein	**A1H2**	3250 ± 100	62.7 ± 0.9	5.6 ± 0.2
	Euphoria	**G1H2**	2780 ± 70	50.3 ± 2.7	5.0 ± 0.3
		A1H3	2820 ± 60	61.3 ± 1.2	7.2 ± 0.2
		G1H3	2760 ± 60	49.2 ± 1.7	3.7 ± 0.1
P6	Davidoff	**A1H2**	3020 ± 50	69.6 ± 0.6	6.6 ± 0.2
	Silver Shadow	**G1H2**	2190 ± 50	51.5 ± 0.6	6.2 ± 0.2
		A1H3	1420 ± 30	30.4 ± 1.1	7.9 ± 0.2
		G1H3	756 ± 14	29.6 ± 1.5	8.9 ± 0.3

[a] All sensing data were obtained from Hill analysis of dose response curves for perfumes **P***n* (n=**1**-**6**; variable volumes) coupled with reactive counterions **A1H***m* or **G1H***m* (m=**2**-**3**; **A1H2**, 50 μM; **G1H2**, 15 μM; **A1H3**, 30 μM; **G1H3**, 5 μM) and detected with calf thymus DNA in HPTS/DPX-LUVs. V_{50}, Y_{MAX} and n are average values (± standard deviation) from three independent trials. [b] Perfume volumes needed to reach $Y_{MAX}/2$. [c] Maximal activity. [d] Hill coefficient.