Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Synthetic Polyion-Counterion Transport Systems in Polymersomes and Gels

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Supporting Information

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1. Supporting methods

1.1. Materials and methods

As in refs. S1-S7, Supporting Information. Briefly, reagents were purchased from Fluka and Aldrich, 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. Egg yolk phosphatidylcholine (EYPC) was from Avanti Polar Lipids. Most of the odorants were purchased from Sigma, Fluka or Acros, and the others (enantiomeric citronellals) were gifts from Firmenich, Geneva. Oleyl aldehyde was synthesized from the commercial available alcohol as described previously.[S5]

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 of the gel plates were obtained with a Gene Genius (Syngene).

1.2. Abbreviations

$CHCl_3$: Chloroform; DMSO: Dimethylsulfoxide; DPX:

p-Xylene-bispyridinium bromide; EYPC: Egg yolk phosphatidylcholine; HPTS 8-Hydroxy-1,3,6-pyrenetrisulfonate; LUVs: Large unilamellar vesicles; MeOH: Methanol; PDMS-PMOXA: Poly(dimethylsiloxane)-b-poly(2-methyloxazoline); rt: Room temperature; Tris: Tris(hydroxymethyl)aminomethane.

1.3. Synthesis of reactive counterions and polymers

Reactive counterions **G1H2**, **G1H3** and **G1H4** were synthesized and characterized following reported procedures.[S6]

Diblock copolymer poly(dimethylsiloxane)-b-poly(2-methyloxazoline) (PDMS-PMOXA) was synthesized and characterized following reported procedures.[S7]

1.4. General procedures for vesicle experiments

1.4.1. Vesicle preparations

a) PDMS-PMOXA-LUVs⊃HPTS/DPX

Adapting the general procedures in ref. S5, a thin lipid film was prepared by evaporating a solution of 5 mg of PDMS-PMOXA block copolymer in 1 ml $CHCl_3$ on a rotary evaporator (room temperature) and then *in vacuo* overnight. The resulting film was hydrated with 1 ml buffer (5 mM HPTS, 16.5 mM DPX, 10 mM Tris, 72 mM NaCl, pH 7.4) for 12 hours and extruded (15×) through a polycarbonate membrane (pore size, 200 nm). Extravesicular components were removed by gel filtration (Sephadex G-50) with 10 mM Tris, 107 mM NaCl, pH 7.4. Final conditions: ~ 819 μM

PDMS-PMOXA; 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.

The polymersomes formed by PMOXA-PDMS block copolymer have been characterized using light dynamic and static light scattering, transmission electron microscopy and cryo-transmission microscopy as described in S7.

b) EYPC-LUVs⊃HPTS/DPX

Following the general procedures in ref. S5, 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 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. S5, 30 μl of 25 mM of the reactive counterion (**G1H2**, **G1H3**, **G1H4**) in DMSO were mixed with 2 equivalent of aldehyde for each hydrazide of the reactive counterion (e.g., 45 μl of 100 mM odorant in DMSO for the reactive counterion with three hydrazides) and the total volume was adjusted to 300 μl with DMSO. The mixture was stirred at 60˚C for 1 hour, and the resulting

hydrazone was then used for DNA activation experiments.

1.4.3. DNA solution activation experiments

a) PDMS-PMOXA-LUVs⊃HPTS/DPX

Adapting the general procedures in ref. S5, PDMS-PMOXA-LUVs stock solutions (25 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 PDMS-PMOXA concentration, ~ 10 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, 10-120 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.

b) EYPC-LUVs⊃HPTS/DPX

Adapting the general procedures in ref. S5, EYPC-LUVs 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.

For EYPC and PDMS-PMOXA solution experiments the fluorescence intensities were normalized to fractional emission intensity $I(t)$ using equation (*S1*)

$$I(t) = (I_t - I_0) / (I_\infty - I_0) \qquad (S1)$$

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, $t \sim 190$ s) as a function of hydrazone concentration $c_{hydrazone}$ and fitting them to the Hill equation (*S2*)

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

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. S3). Results are summarized in Table S1 and S2.

All of these transports experiments were carried out in triplicates for **G1H3** and the different tails **Tn**, with independent hydrazone formation reactions for each dose response curve. The results showed differences for each amphiphile (**G1H3Tn**) as well as good reproducibility as depicted by the low values of the standard deviation of the key parameters EC_{50}, Y_{MAX} and Hill coefficient (Tables S1 and S2) and by good resolution in the PCA score plots (Fig. 5, see 1.6).

1.5. General procedures for the gel supported assays

1.5.1. Preparation of the gel plate

200 mg of agar were suspended in 20 ml of aqueous buffer (10 mM Tris, 107 mM NaCl, pH =7.4), the suspension was boiled until the solution was clear and the mixture was allowed to cool down to rt. When the temperature was below 35 ^{o}C, 1 ml of the stock vesicles solution (see 1.4.1) was added under vigorous stirring. Immediately after vesicles mixing, the solution was poured into a disposable Petri plate (100 mm diameter) and let cool down for 15 min. When the gel formation was complete, small holes were made in the gel surface with the help of a 50 µl micropipette. 2 holes in the top and bottom of the plate for detergents and 3 horizontal rows of five holes for polyion-counterion complexes (Fig. 6). The plate was then ready for detergent and polyion-counterion addition.

1.5.2. Gel activation experiments

Once the plate was ready, 4 µl of detergents and 4 µl of polyion-counterion complexes were added with a micropipette in the corresponding holes:

- Detergent (top and bottom holes of the plate): 4 µl of Triton X-100 (1.2% w/w in H_2O) for EYPC gels and 4 µl of Tween 20 (50% w/w in H_2O) for PDMS-PMOXA gels.

- Polyion-counterion complexes (three horizontal lines of five holes): 4 µl of a

mixture of **G1H3Tn**/ctDNA (3:1 v/v). $[\mathbf{G1H3Tn}]_{variable}$ = 625 uM, 1.25 mM, 2.5 mM, 5 mM, 10 mM solutions in DMSO. $[ctDNA]_{cte}$ = 1mg/ml solution in aqueous buffer (10 mM Tris, 107 mM NaCl, pH 7.4).

The gel was incubated at rt and the fluorescence image of the plate was obtained (without removing the gel from the plate) with Gene Genius (Syngene) (λ_{ex} 365 nm, λ_{em} 500-600 nm).

Pictures of the plates after 5 min of incubation time were opened with ImageJ and the background was subtracted. Rectangles for the different lanes of holes were drawn and the spot fluorescence intensity for each plot lane was obtained. After normalization and statistical treatment of this plot lanes areas the dose response curves were plotted.

Pictures of the EYPC gels showed a permanent broadening of the spots which resulted in the collapse of the lanes after 3 hours incubation time. Pictures of the PDMS-PMOXA gels showed better contrast and no increase of the spot, however they faded after long incubation times (> 3 hours).

For EYPC and PDMS-PMOXA gel experiments the fluorescence intensities were normalized to fractional emission intensity *I* using equation (*S3*)

$$I = (I_t) / (I_\infty) \qquad (S3)$$

where $I_\infty = I_t$ at saturation after lysis (average of the two detergent maximum values). Effective concentration for hydrazone EC_{50} and Hill coefficient *n* were determined by plotting the fractional activity *Y* (= *I* of the spots from the gel picture after 5 min incubation time) as a function of hydrazone concentration $c_{hydrazone}$ and fitting them to

the Hill equation (*S2*)

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

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. Results are summarized in Tables S3 and S4.

1.6. Data analysis

Statistical evaluation of above data was performed using principal component analysis (PCA).[S8] 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. All data analysis was performed with MYSTAT software (SYSTAT).

1.7. References

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2. Supporting figures

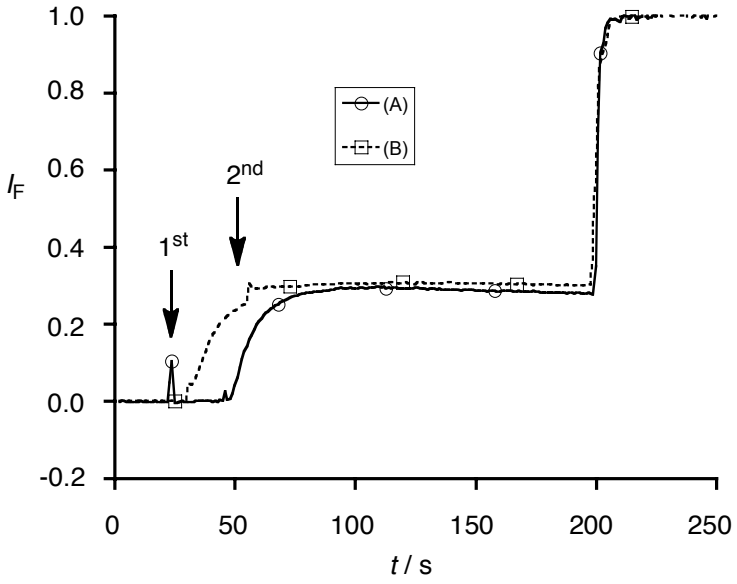

Fig. S1. Normalized kinetics showing the detergent effect for **G1H2T8** in PDMS-PMOXA-LUVs⊃HPTS/DPX. Increase in HPTS fluorescence after: (A) addition of (calf thymus DNA, 1.25 μg/ml final concentration, t ~ 30 s), amphiphile (**G1H2T8**, 25 μM final concentration, t ~ 45 s) and triton X-100 (0.024% final concentration, t ~ 200 s) ○; (B) addition of amphiphile (**G1H2T8**, 25 μM final concentration, t ~ 30 s), transporter (calf thymus DNA, 1.25 μg/ml final concentration, t ~ 45 s) and triton X-100 (0.024% final concentration, t ~ 200 s) □.

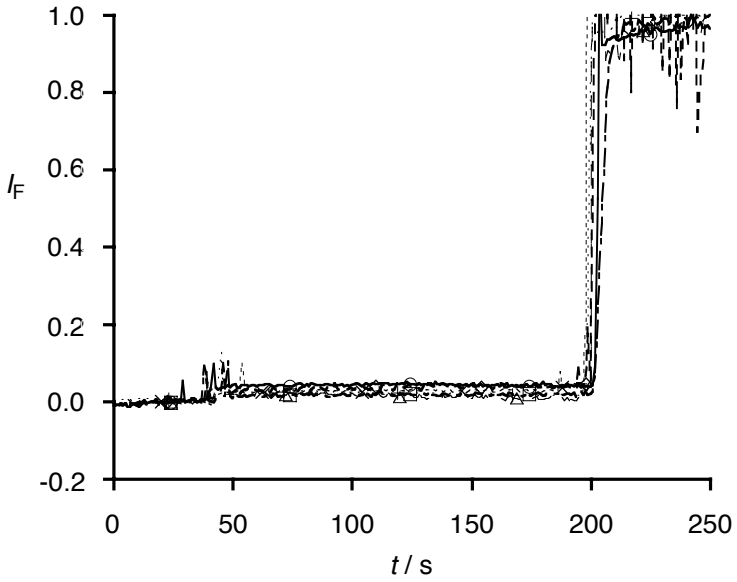

Fig. S2. Normalized kinetics showing the lack of activity for octopus amphiphiles (**G1H4Tn**) in PDMS-PMOXA-LUVs⊃HPTS/DPX. No increase in HPTS fluorescence is observed after sequential addition of amphiphile **G1H4T7** (1.25 (△), 10 (+), 50 (◇), 75 (□), 100 μM (○), final concentrations, $t \sim 0$ s), ctDNA (1.25 μg/ml final concentration, $t \sim 40$ s) and triton X-100 (excess, $t \sim 200$ s) to PDMS-PMOXA-LUVs⊃HPTS/DPX.

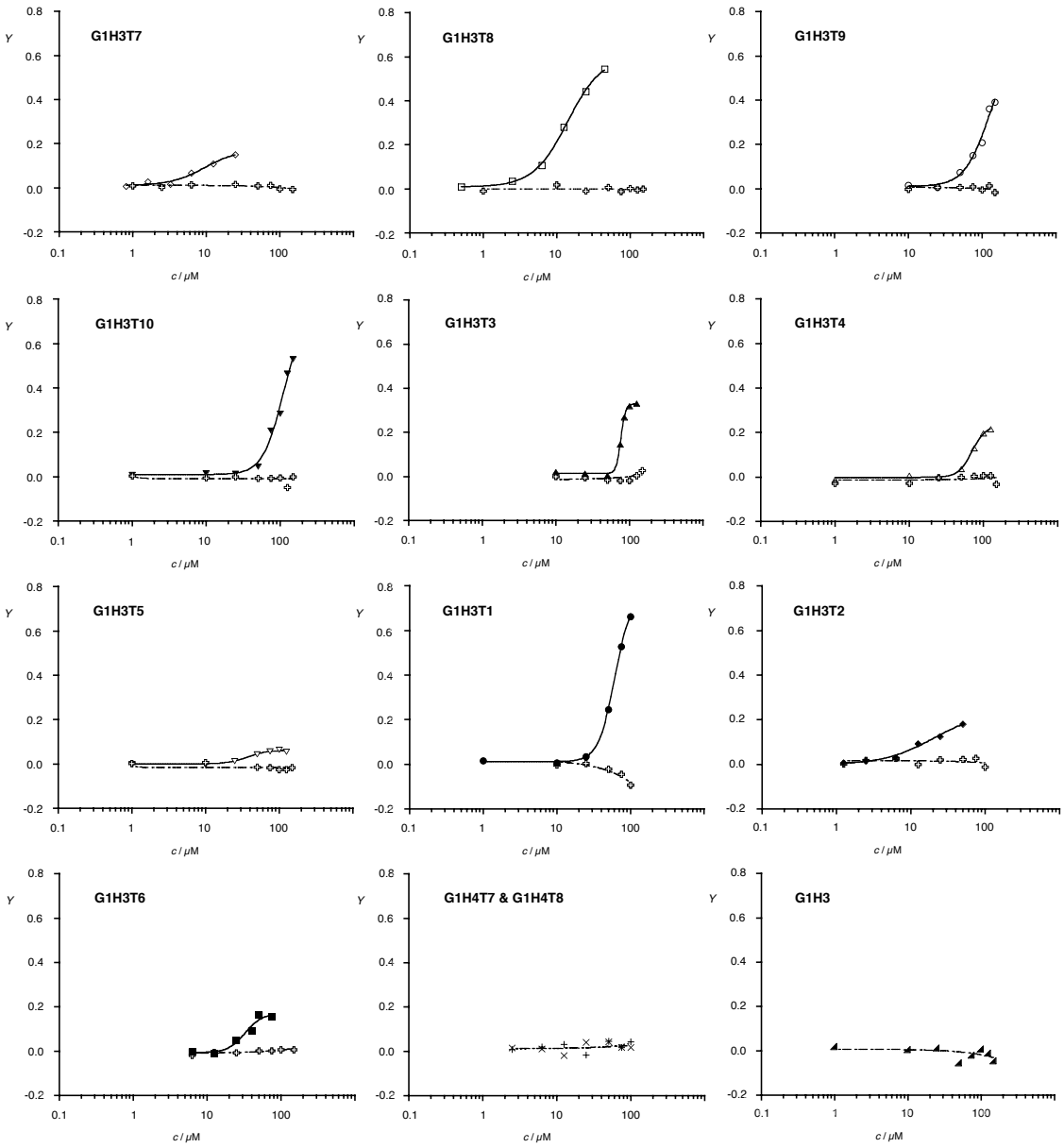

Fig. S3. Dose-response curves for DNA activation in polymersomes for the 10 tails **Tn** (**T7** (◇), **T8** (□), **T9** (○), **T10** (▼), **T3** (▲), **T4** (△), **T5** (▽), **T1** (●), **T2** (◆) and **T6** (■)) coupled with **G1H3**, obtained by plotting the fractional activity *Y* as a function of **G1H3Tn** concentration. Negative control experiment for each pure aldehyde tail is shown (**Tn**, ✣). Negligible activity for the four tails hydrazones **G1H4T7** (×), **G1H4T8** (+) and the pure hydrazide head **G1H3** (◢) are also shown.

3. Supporting Tables

Table S1. EC_{50}, Y_{MAX} and n for **G1H3Tn** in solution for three independent hydrazone formation experiments.[a]

Odorant	EC_{50} (μM)[b]	Y_{MAX} (%)[c]	n[d]	EC_{50} (μM)[b]	Y_{MAX} (%)[c]	n[d]	EC_{50} (μM)[b]	Y_{MAX} (%)[c]	n[d]
		1st			2nd			3rd	
T1	61.1	75.6	3.9	62.3	79.0	5.0	57.9	71.2	4.6
T2	14.1	27.8	2.2	9.8	16.8	2.3	20.4	23.3	1.3
T3	76.7	32.9	13.9	76.8	36.1	11.7	82.9	42.2	9.3
T4	78.9	19.1	2.0	70.6	23.0	4.8	63.9	19.9	3.8
T5	43.5	5.5	4.5	41.3	7.5	4.5	37.0	6.1	4.0
T6	32.9	17.1	3.7	37.7	19.8	2.5	40.0	22.2	2.4
T7	8.6	14.6	2.4	8.2	17.2	1.5	9.6	17.1	1.9
T8	14.7	60.3	1.3	14.2	60.8	1.8	14.3	53.4	1.9
T9	124.9	64.7	2.6	124.9	57.1	2.6	123.7	67.6	3.6
T10	99.2	69.2	6.1	109.4	72.8	3.2	96.7	64.6	4.8

[a]All data were obtained from Hill analysis of dose response curves for hydrazones **G1H3Tn** (n = **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, **9**, **10**, variable concentrations) and detected with calf thymus DNA in PDMS-PMOXA-LUVs⊃HPTS/DPX. [b]Effective concentration for hydrazones needed to reach $Y_{MAX}/2$. [c]Maximal activity. [d]Hill coefficient.

Table S2. Average values ± standard deviation of the EC_{50}, Y_{MAX} and n for characterized amphiphilic hydrazones.[a] and ESI-MS.[e]

Tail	*Reactive counterion*	EC_{50} (μM)[b]	Y_{MAX} (%)[c]	n[d]	ESI-MS[e] Found	ESI-MS[e] Expected for $[M+H]^+$
T1	**G1H3**	60.4 ± 1.9	75.3 ± 3.2	4.5 ± 0.6	877.7	877.6
T2	**G1H3**	14.7 ± 4.3	22.6 ± 4.5	1.9 ± 0.6	913.5	913.6
T3	**G1H3**	78.8 ± 2.9	37.1 ± 3.9	11.6 ± 2.3	770.0	769.6
T4	**G1H3**	71.1 ± 6.2	20.6 ± 1.7	3.5 ± 1.5	769.7	769.6
T5	**G1H3**	40.6 ± 2.7	6.4 ± 0.8	4.3 ± 0.3	769.7	769.6
T6	**G1H3**	36.9 ± 3.0	19.7 ± 2.1	2.9 ± 0.7	1106.1	1105.9
T7	**G1H3**	8.8 ± 0.6	16.3 ± 1.2	2.0 ± 0.4	649.8	649.5
	G1H4	--[f]	--[f]	--[f]	889.0	888.6
T8	**G1H3**	14.4 ± 0.2	58.2 ± 3.4	1.7 ± 0.3	692.0	692.0
	G1H2	--[g]	--[g]	--[g]	438.7	438.6
	G1H4	--[f]	--[f]	--[f]	944.5	944.7
T9	**G1H3**	124.5 ± 0.5	63.1 ± 4.4	2.9 ± 0.6	733.7	733.6
	G1H2	--[g]	--[g]	--[g]	466.5	466.4
T10	**G1H3**	101.8 ± 5.5	68.9 ± 3.4	4.7 ± 1.5	775.8	775.6

[a]All data were obtained from Hill analysis of dose response curves for tails **Tn** (n = **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, **9**, **10**, variable concentrations) coupled with the reactive counterions **G1Hm** (m = **2**, **3**, **4**) and detected with calf thymus DNA in PDMS-PMOXA-LUVs⊃HPTS/DPX. 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]Negligible activity at 100 μM final concentration in cuvette. [g]Only detergent effect at 25 μM final concentration in cuvette.

Table S3. Values of the EC_{50}, Y_{MAX} and (Y_{MAX}/EC_{50}) for comparison of hydrazones activities in solution and gel experiments (plate a).[a]

Tail	Vesicles	Sol or Gel[b]	Y_{MAX} (%)[c]	EC_{50}[d]	n[e]
T8	EYPC	Sol	59.0	2.0 µM	1.6
		Gel	40.7	1.9 mM	2.9
	PDMS-PMOXA	Sol	60.8	14.2 µM	1.8
		Gel	32.4	1.4 mM	3.8
T10	EYPC	Sol	77.3	12.6 µM	2.7
		Gel	37.3	2.3 mM	2.4
	PDMS-PMOXA	Sol	64.7	96.7 µM	4.7
		Gel	28.1	1.7 mM	3.4
T9	EYPC	Sol	62.9	5.6 µM	1.9
		Gel	79.8	4.5 mM	1.9
	PDMS-PMOXA	Sol	67.6	123.7 µM	3.6
		Gel	47.5	4.1 mM	2.6

[a]All sensing data were obtained from Hill analysis of dose response curves for tails **Tn** (n = **1**, **3**, **7**, **8**, **9**, **10**) coupled with the reactive counterion **G1H3** and detected with calf thymus DNA in EYPC or PDMS-PMOXA-LUVs⊃HPTS/DPX (Sol or Gel). [b]Sol: solution experiment in fluorescent cuvette; Gel: gel experiment in solid plate. [c]Maximal activity. [d]Effective concentration for odorants needed to reach $Y_{MAX}/2$ (µM for solution and mM for gel experiments). Concentration refers to the final concentration of hydrazone in cuvette for solution experiments and to the concentrations in the 4 µl stock solutions for the gel experiments. [e]Hill coefficient.

Table S4. Values of the EC_{50}, Y_{MAX} and (Y_{MAX}/EC_{50}) for comparison of hydrazones activities in solution and gel experiments (plate b).[a]

Tail	Vesicles	Sol or Gel[b]	Y_{MAX} (%)[c]	EC_{50}[d]	n[e]
T9	EYPC	Sol	62.9	5.6 µM	1.9
		Gel	38.5	3.2 mM	1.4
	PDMS-PMOXA	Sol	67.6	123.7 µM	3.6
		Gel	57.8	4.6 mM	2.7
T1	EYPC	Sol	73.4	3.3 µM	3.4
		Gel	65.4	3.7 mM	1.5
	PDMS-PMOXA	Sol	75.6	61.1 µM	3.9
		Gel	86.1	4.5 mM	2.7
T3	EYPC	Sol	74.7	4.2 µM	3.0
		Gel	29.5	2.6 mM	2.1
	PDMS-PMOXA	Sol	32.9	76.7 µM	13.9
		Gel	16.0	3.6 mM	3.1

[a]All sensing data were obtained from Hill analysis of dose response curves for tails **Tn** (n = **1**, **3**, **7**, **8**, **9**, **10**) coupled with the reactive counterion **G1H3** and detected with calf thymus DNA in EYPC or PDMS-PMOXA-LUVs⊃HPTS/DPX (Sol or Gel). [b]Sol: solution experiment in fluorescent cuvette; Gel: gel experiment in solid plate. [c]Maximal activity. [d]Effective concentration for odorants needed to reach $Y_{MAX}/2$ (µM for solution and mM for gel experiments). Concentration refers to the final concentration of hydrazone in cuvette for solution experiments and to the concentrations in the 4 µl stock solutions for the gel experiments. [e]Hill coefficient.