

## Comprehensive screening of octopus amphiphiles as DNA activators in lipid bilayers: implications on transport, sensing and cellular uptake†

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Received 28th October 2010, Accepted 12th January 2011

DOI: 10.1039/c0ob00948b

Dynamic octopus amphiphiles contain one charged “head,” here a guanidinium cation, together several hydrophobic “tails” (or “tentacles”) that can be attached and exchanged *in situ* by reversible hydrazone formation. Quite surprisingly, their ability to activate DNA as transporters in lipid bilayer membranes was found to increase with the number of tails (up to four) as well as with their length (up to eight carbons). Both encouraged and puzzled by these results, we decided that a comprehensive screening of octopus amphiphiles with regard to number (from one to six) and length (from three to eighteen carbons) of their tails would be appropriate at this point. For this purpose, we here report the synthesis of cationic hexahydrazide peptide dendrons together with that of aldehydes with long, saturated, unsaturated and branched hydrophobic tails. Comprehensive screening of the completed collection of tails and heads reveals that the ability of octopus amphiphiles to activate DNA transporters shifts with increasing number of tails to decreasing length of the tails. Moreover, *cis*-alkenyl and branched alkyl tails are more active than their linear analogs, branched aromatic tails are best. These overall very meaningful trends for octopus amphiphiles will be of importance for sensing applications and fragrant cellular uptake.

## Introduction

The activity of dynamic polyion–counterion complexes in lipid bilayer membranes is of scientific interest with regard to topics as diverse as transmembrane transport,<sup>1–5</sup> voltage gating,<sup>5</sup> sensing<sup>1–3,6</sup> and cellular uptake.<sup>7–9</sup> In the presence of amphiphilic counterions, polyions such as anionic DNA and RNA<sup>7,8</sup> or the cationic cell-penetrating peptides (CPPs)<sup>9</sup> have been shown to become soluble in organic solvents (or bulk membranes) such as chloroform.<sup>5</sup> Moreover, counterion-activated CPPs or DNA/RNA have been shown to actually prefer hydrophobic over hydrophilic environments, that is partition from water into bulk membranes and lipid bilayer membrane. Moreover, they can carry hydrophilic counterions as large as fluorescent probes across bulk and intact lipid bilayer membranes.<sup>5</sup>

The activity of polyion–counterion complexes originates from the desire to minimize charge repulsion in weakly acidic or basic polyions where de-/protonation is impossible and only counterion scavenging can help.<sup>5</sup> By now, it is understood that counterion-mediated function accounts for the cellular uptake of CPPs and RNA/DNA,<sup>5,7–9</sup> the voltage gating of biological potassium

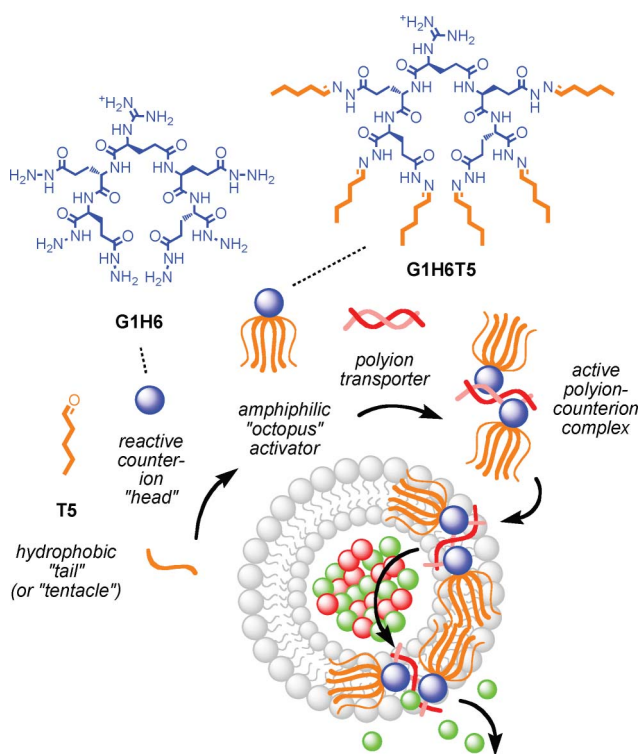
channels,<sup>5,9x</sup> the operation of membrane-based synthetic sensing systems<sup>10</sup> in many variations,<sup>1–3,6</sup> and the activity of many catalysts in organic synthesis.<sup>11</sup>

Recently, polyion–counterion complexes have been used to create the first differential sensing system<sup>10</sup> that works, like biological olfactory systems, in lipid bilayer membranes.<sup>1</sup> For this purpose, hydrophobic analytes such as octanal **T8** are reacted *in situ* with cationic hydrazides such as **G1H3** to yield amphiphilic cations such as **G1H3T8** (Fig. 1 and 2). These cationic amphiphiles then can function as activators of polyanionic cation transporters such as calf-thymus (ct) DNA in fluorogenic vesicles (Fig. 1). For pattern generation, analytes such as octanal **T8** are incubated with counterions (one to three guanidiniums, ammoniums, carboxylates or phosphonates) carrying one to four reactive hydrazides (e.g., **G1H1–G1H3**, Fig. 2).<sup>1</sup> The fluorescent response obtained from polyion activation with the different amphiphiles is then used to generate *n*-dimensional patterns that can be recognized by PCA or HCA.<sup>1–3</sup> This differential sensing approach was shown to discriminate enantiomers, *cis–trans* isomers and single atom homologs. Error-free recognition of all tested perfumes confirmed compatibility of the new sensing system with complex matrices from the supermarket.<sup>1</sup>

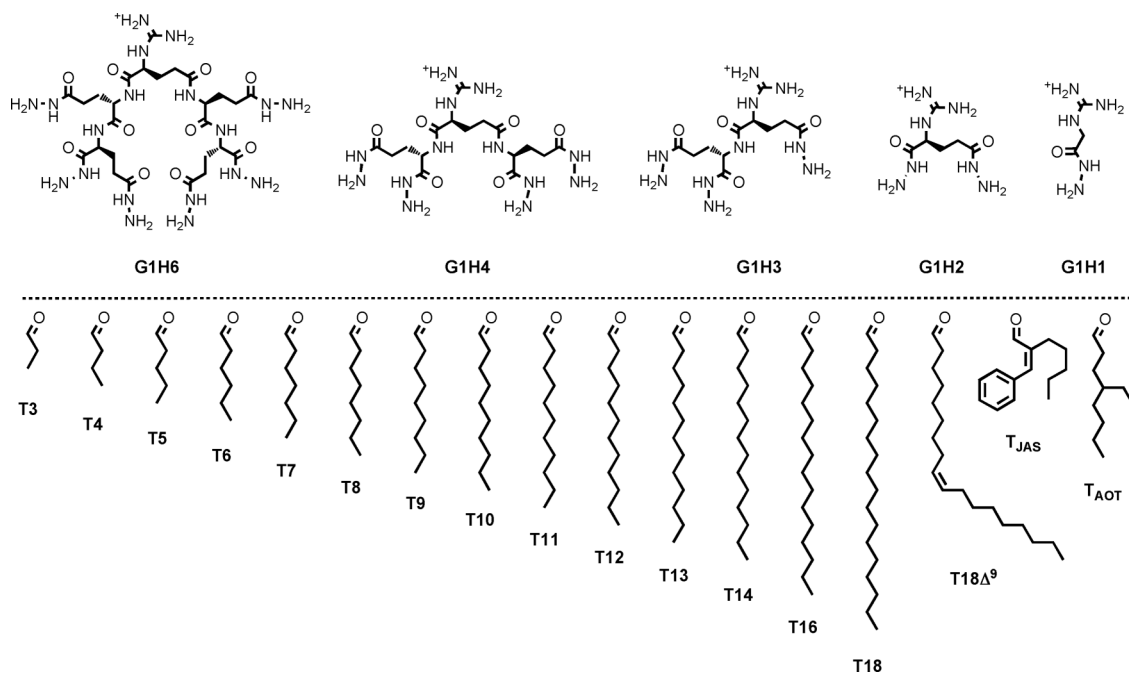
Covalent capture of hydrophobic “tails” by reactive counterion “heads” is of interest not only for differential sensing<sup>1–3,6,10</sup> but also for new approaches toward cellular uptake<sup>7–9</sup> and catalysis.<sup>11</sup> On the one hand, a high number of delivery agents can be prepared with essentially no effort for comprehensive screening.

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c0ob00948b



**Fig. 1** Ion transport by dynamic polyion-counterion complexes in fluorogenic vesicles. Hydrophobic “tails” (or “tentacles”, e.g., **T5**) are covalently captured by hydrophilic cations (e.g., hexahydrazides **G1H6**) to give six-tail amphiphiles (e.g., **G1H6T5**) that can activate polyanions (e.g., ctDNA) as transporters in lipid bilayers (here the example for fluorescence recovery in response to the export of trapped cationic quenchers (green) but not anionic fluorophores (red) is shown).



**Fig. 2** Reactive counterions **G1H1**–**G1H6** composed of one guanidinium cation (**G1**) and one to six hydrazides (**H**) for *in situ* reaction with hydrophobic tails (**T**) to yield amphiphilic hydrazones that can activate DNA as transporter in fluorogenic vesicles (see Fig. 1). **T<sub>AOT</sub>** for 4-ethyloctanal refers to the presence of a tail that is similarly branched as in the popular detergent AOT (Aerosol OT, di-2-ethyl-1-hexylsulfosuccinate), **T<sub>JAS</sub>** refers to jasminaldehyde as tail.

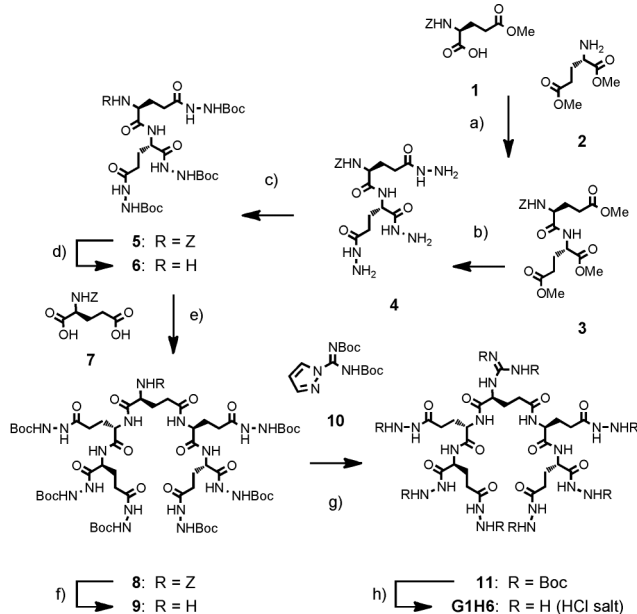
The dynamic nature of the non-viral vectors, on the other hand, can be used to add multifunctionality with regard to endosomal escape, targeting, fluorescence labeling, and so on.

Toward this end, we found that carboxylates are better than phosphonates to activate CPPs, and that gemini structures<sup>8</sup> are better than excessive “heads” or “tails”.<sup>3</sup> For the activation of DNA and RNA, guanidinium cations are better than ammonium cations, and activity increases in the guanidinium series with increasing number of tails from nearly inactive single-chain amphiphiles **G1H1T** to the most active “octopus” amphiphiles **G1H4T** with four hydrophobic tails or “tentacles”.<sup>2</sup> With one exception, these findings were all as expected from the literature.<sup>5–9</sup> They confirmed, *inter alia*, the importance of preorganized hydrogen-bonding as well as weak basicity/acidity to assist operational ion pairing with powerful proximity effects.<sup>5</sup> However, the increasing activity of cationic activators with increasing number of tails came as a surprise, culminating with best performances for octopus amphiphiles of significant structural complexity.<sup>2</sup> To clarify this puzzling octopus effect, we here report a comprehensive screening of dynamic amphiphiles with regard to number (from one to six) and length (from three to eighteen carbons) of their tails. The bottom line is that maximal activity of octopus amphiphiles shifts with increasing number of tails to shorter tail length, and that *cis*-unsaturated and branched alkyl tails are more active. These findings are of interest for applications toward sensing,<sup>6,10</sup> catalysis<sup>11</sup> as well as cellular uptake.<sup>7–9</sup>

## Results and discussion

The hexahydrazide peptide dendron<sup>12</sup> **G1H6** was synthesized in eight steps in overall 16% yield from the differently protected

glutamic acids **1** and **2** (Scheme 1). Their coupling to give triester **3** was followed by the conversion into trihydrazide **4**.<sup>1</sup> Hydrazide protection with Boc prepared for the hydrogenolytic Z removal in **5**.<sup>1</sup> The two mini-dendrons **6** were then attached to the glutamic acid core **7** to yield the desired scaffold. Chemoselective deprotection of **8** yielded amine **9**, which was guanidinylated with the standard reagent **10**. Acid-catalyzed removal of the eight Boc protecting groups in **11** gave the target molecule **G1H6**.



**Scheme 1** Reagents and conditions: (a) HBTU, DIEA,  $\text{CH}_2\text{Cl}_2$ , rt, 85%;<sup>1</sup> (b)  $\text{N}_2\text{H}_4$  monohydrate, MeOH, reflux, 90%;<sup>1</sup> (c)  $\text{Boc}_2\text{O}$ , DIEA, MeCN,  $\text{H}_2\text{O}$ , reflux, 90%;<sup>1</sup> (d)  $\text{H}_2$ , Pd/C, MeOH, rt, quant; (e) HBTU, DIEA,  $\text{CH}_2\text{Cl}_2$ , rt, 62%; (f)  $\text{H}_2$ , Pd/C, MeOH, rt, quant; (g) DIEA,  $\text{CH}_3\text{CN}$ , 50 °C, 61%; (h) 1 M HCl in  $\text{Et}_2\text{O}$ , reflux, 63% (8 steps, 16%).

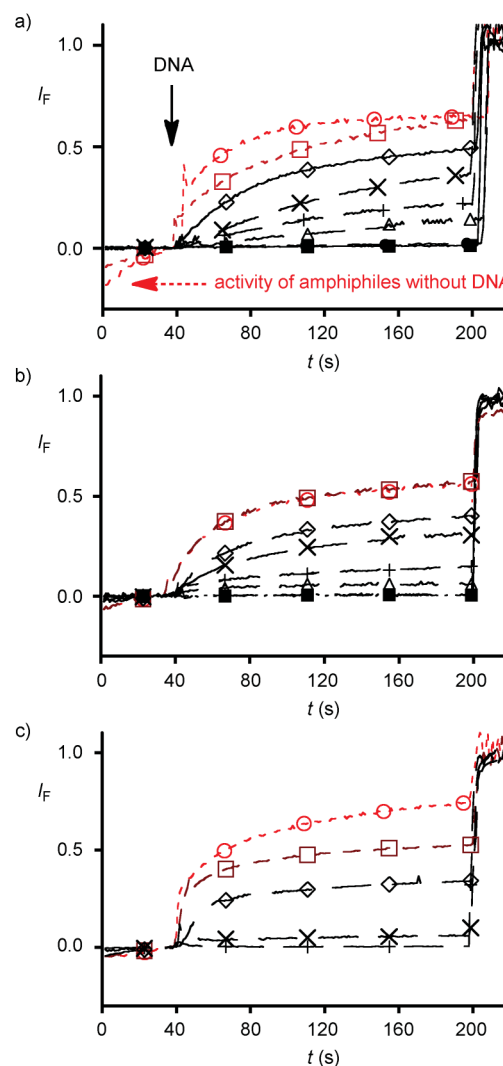
Tetrahydrazide mini-dendron **G1H4**, trihydrazide **G1H3**, dihydrazide **G1H2** and monohydrazide **G1H1** were prepared following previously reported procedures (Fig. 2).<sup>1,2</sup> Almost all tails **T** were commercially available. The 9-*cis*-octadecene-1-aldehyde **T18A**<sup>9</sup> and **T<sub>AOT</sub>**, the aldehyde with a 4-ethyl-1-octyl motif reminiscent of the branched tails in the reversed-micellar surfactant AOT, were accessible by PCC oxidation in one step following literature procedures.<sup>13</sup>

Hydrazides **G1H6**, **G1H4**, **G1H3**, **G1H2** and **G1H1** were converted into hydrazones under routine conditions,<sup>1-3,14</sup> that is by incubation for one hour with aldehyde tails **T** in DMSO at 60 °C. *In situ* hydrazone formation was confirmed by electrospray mass spectrometry (ESI-MS). Not further activated hydrazones are hydrolyzed under acidic conditions but are stable in neutral water, at least for the few minutes of a transport experiment.<sup>14</sup> Quantitative kinetics of hydrolysis as a function of pH are currently under investigation in the context of cellular uptake experiments.

The activity of new hexahydrazide **G1H6** with hydrophobic tails to activate polyanion transporters was explored under routine conditions in comparison with the previously reported **G1H1**–**G1H4**.<sup>1,2</sup> At the same time, the screening of tails was expanded from octanal **T8** or shorter to long, saturated, unsaturated and branched tails up to **T18**, **T18A**<sup>9</sup> and **T<sub>AOT</sub>**. Calf-thymus (ct) DNA was used as polyanion transporter, EYPC-LUVs  $\supset$  HPTS/DPX

as fluorogenic vesicles (*i.e.*, egg yolk phosphatidylcholine large unilamellar vesicles loaded with the anionic fluorophore 8-hydroxy-1,3,6-pyrenetrisulfonate and the cationic quencher *p*-xylene-bis-pyridinium bromide).<sup>1,2,5</sup> In this assay, counterion-activated DNA is thought to export the cationic quencher DPX from intact vesicles.<sup>5b</sup> This DPX export is detected as an increase in emission of the anionic fluorophore HPTS left behind. Although plausible and consistent with control experiments, we add that other interpretations for fluorescence recovery in the HPTS/DPX are always possible.<sup>5b</sup>

In a typical experiment, the counterion activator is added to EYPC-LUVs  $\supset$  HPTS/DPX first (Fig. 3). Fluorescence recovery before the addition of DNA transporters indicates that the



**Fig. 3** Changes in fractional fluorescence intensity  $I_F$  of HPTS ( $\lambda_{\text{ex}} = 413$  nm,  $\lambda_{\text{em}} = 510$  nm) during addition of (a) **G1H6T7** (1.25 (■), 3.75 (●), 6.25 (△), 10 (+), 12.5 (×), 25 (◇), 30 (□), 40 μM (○), final concentrations,  $t \sim 0$  s), (b) **G1H1T12** (0.375 (■), 1.25 (●), 6.25 (△), 12.5 (+), 20 (×), 30 (◇), 50 (□), 70 μM (○)) or (c) **G1H3T<sub>AOT</sub>** (1.25 (+), 3.25 (×), 6.25 (◇), 12.5 (□), 25 μM (○)), ctDNA (1.25 μg  $\text{mL}^{-1}$  final concentration,  $t \sim 40$  s) and Triton X-100 (excess,  $t \sim 200$  s) to EYPC-LUVs  $\supset$  HPTS/DPX. Data for the highest amphiphile concentrations are in colored in brown and red in each series to facilitate the comparison of activity before and after DNA addition (a, red arrow).

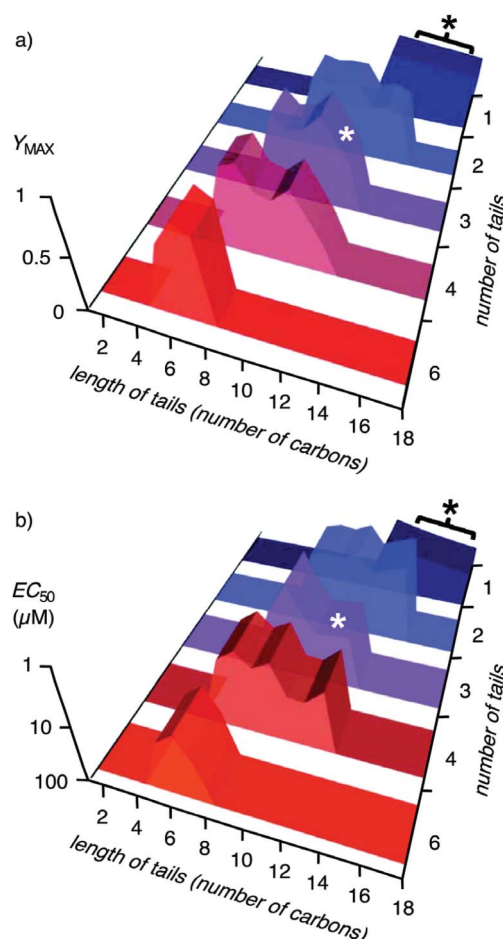
cationic amphiphile is a membrane-active surfactant (Fig. 3a, red arrow). With the current collection of amphiphiles, strong detergent effects were rare, whereas the onset of detergent activity at higher concentrations was observed occasionally. With octopus amphiphile **G1H6T7**, for example, the onset of detergent activity was observed around a critical concentration of 30  $\mu\text{M}$  (Fig. 3a,  $t = 0\text{--}40$  s). Interestingly, single-tail amphiphile with **G1H1T12**, featuring a classical surfactant motif with a long dodecyl tail, was inactive without DNA up to 70  $\mu\text{M}$  (Fig. 3b,  $t = 0\text{--}40$  s). The same was true for **G1H3T<sub>AOT</sub>**, containing three branched alkyl tails reminiscent of the classical detergent AOT (Fig. 3c,  $t = 0\text{--}40$  s).

Fluorescence recovery in response to the addition of ctDNA reports on active polyion-counterion complexes (Fig. 3,  $t = 40\text{--}200$  s, ctDNA alone is inactive). At the end of each experiment, the fluorescence response is calibrated to a fractional activity  $Y = 1.0$  by complete vesicle lysis with an excess of Triton X-100 (Fig. 3,  $t > 200$  s).

For quantitative characterization of polyion-counterion transporters, these experiments were repeated for different activator concentrations at constant polyion concentration first (Fig. 3). The fluorescence intensity just before lysis was taken as fractional activity  $Y$  and plotted as a function of activator concentration. The resulting dose-response curves were subjected to Hill analysis to yield the  $Y_{\text{MAX}}$ , the maximal accessible activity under these conditions, and the  $EC_{50}$ , the effective activator concentration needed to reach 50% of  $Y_{\text{MAX}}$  (Fig. S2, Table S1†).<sup>15</sup> For a comprehensive overview of the results for linear alkyl tails,  $Y_{\text{MAX}}$  and  $EC_{50}$  were then plotted as a function of the number and the length of the tails (Fig. 4).

In general, the activity of octopus amphiphiles shifted with increasing number of tails toward decreasing tail length (Fig. 4). Counterions with too few and too short tails were inactive because they are too hydrophilic to partition into the bilayer membrane. Counterions with too many and too long tails were inactive because they are too hydrophobic to reach the vesicles and precipitate instead. The results from this comprehensive mapping thus demonstrated that intermediate hydrophobicity is essential for activity, at least within the n-alkyl series.

Counterion activity was most interesting at the edges. The shortest functional tail was pentanal **T5**, addressable with the new octopus amphiphile **G1H6** only (Fig. 4, red). Hexanal **T6** already was active in both six-tail amphiphile **G1H6T6** and four-tail amphiphile **G1H4T6**. Heptanal **T7** was active in six-tail **G1H6T7**, four-tail **G1H4T7** and three-tail **G1H3T7**. With octanal **T8**, four-tail **G1H4T8**, three-tail **G1H3T8** and two-tail **G1H2T8** were sufficiently hydrophobic to activate DNA transporters, whereas six-tail **G1H6T8** was already too hydrophobic at this point to reach the membrane. Amphiphiles with fewer tails exhibited weaker selectivity compared to the six-tail **G1H6**, losing activity only with tails such as **T12** for four-tail **G1H4** and three-tail **G1H3**, and **T16** for two-tail **G1H2**. Most remarkable was single-tail amphiphile **G1H1**, which was inactive in all previous studies with shorter tails. With **T12**, **G1H1** suddenly became capable of activating DNA transporters in a significant manner (Fig. 4, dark blue; Fig. 3b). Together with **T13–T16**, **G1H1** exhibited a not further surprising detergent activity (not shown in Fig. 4\*), whereas DNA activation reappeared with **T18** (Fig. 4, dark blue).

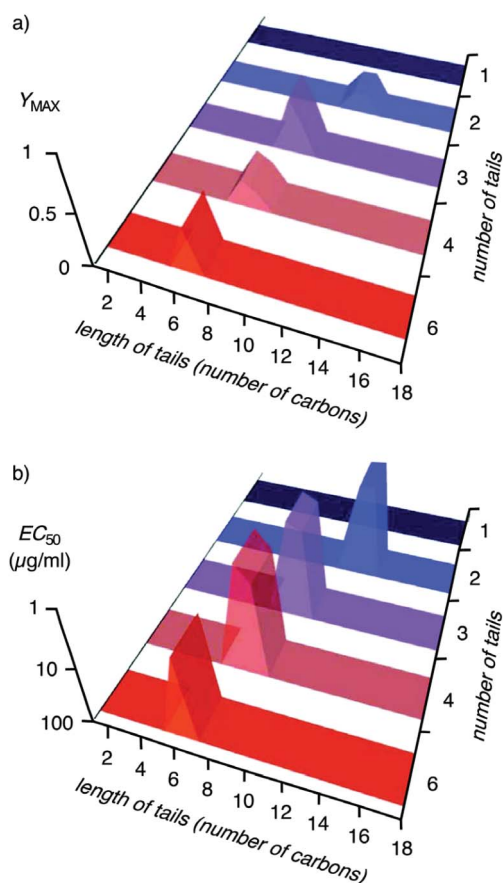


**Fig. 4** Dependence of (a)  $Y_{\text{MAX}}$  and (b)  $EC_{50}$  of counterion-activated DNA transporters on tail number and tail length, measured for varied counterion concentrations at constant ctDNA concentration (1.25  $\mu\text{g ml}^{-1}$ ). Results cover counterions with heads **G1H6** (with six tails, red), **G1H4** (four tails, garnet), **G1H3** (three tails, violet), **G1H2** (two tails, blue) and **G1H1** (one tail, dark blue) that are coupled with tails **T4** (4 carbons) to **T18** (18 carbons).  $Y_{\text{MAX}} < 0.2$  at 25  $\mu\text{M}$  was considered inactive and put to  $Y_{\text{MAX}} = 0.0$  and  $EC_{50} = 100$   $\mu\text{M}$  for contrast only. Some data points for **G1H1–G1H4** from ref. 1 and 2 are included. \* = Missing data points for membrane-active amphiphiles are bridged with straight lines to emphasize the trends for the polyion-counterion complexes of interest. All values are from Hill analysis of dose-response curves as in Fig. 3, compare Table S1†).<sup>15</sup>

To probe the validity and reproducibility of the results obtained for activator screening at constant DNA concentration and varied activator concentrations, the same activity mapping was made for varied DNA concentrations at constant counterion concentration (Fig. 5). The cut was placed at a quite challenging threshold of 6.15  $\mu\text{M}$  activator (compare Fig. 4b). The results from dose-response curves for DNA (Fig. 5) perfectly complemented the ones from dose-response curves for counterion activators (Fig. 4). Namely, with increasing number of tails, the ability to activate DNA shifted to shorter tails. The range covered reached from **T12** as best for two-tail **G1H2** to **T6** for six-tail **G1H6** (Fig. 5).

To outline the full potential of the concept of dynamic<sup>14</sup> counterion amphiphiles<sup>1–3</sup> for applications toward sensing,<sup>6,10</sup> catalysis<sup>11</sup> and cellular uptake,<sup>7–9</sup> the comprehensive analysis of octopus amphiphiles for linear and saturated alkyl tails **T4–T18**



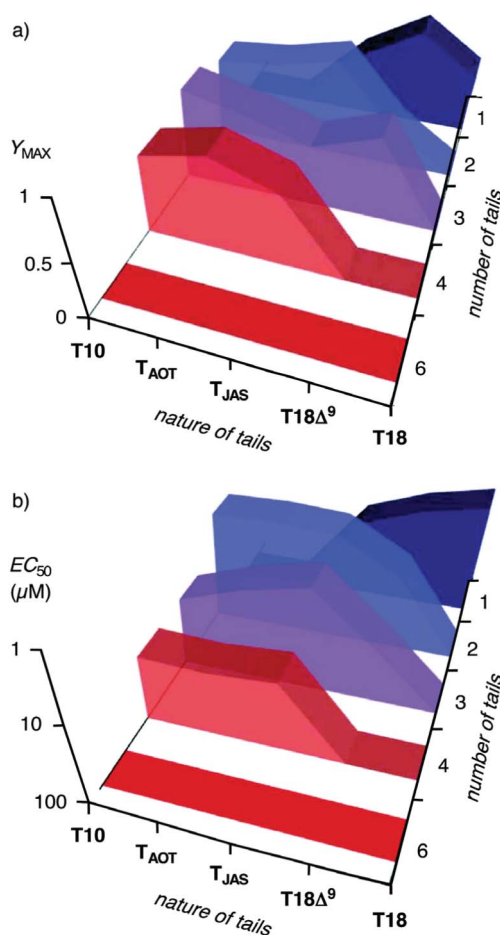


**Fig. 5** Dependence of (a)  $Y_{\text{MAX}}$  and (b)  $EC_{50}$  of counterion-activated DNA transporters on tail number and tail length, measured for varied ctDNA concentrations at constant counterion concentration (6.15  $\mu\text{M}$ ). Results cover counterions with heads **G1H6** (with six tails, red), **G1H4** (four tails, garnet), **G1H3** (three tails, violet), **G1H2** (two tails, blue) and **G1H1** (one tail, dark blue) that are coupled with **T4–T18** ( $Y_{\text{MAX}} < 0.2$  at 1.25  $\mu\text{g ml}^{-1}$  DNA was considered inactive and put to  $Y_{\text{MAX}} = 0.0$  and  $EC_{50} = 100 \mu\text{g ml}^{-1}$  for contrast only, compare Table S2†).<sup>15</sup>

was complemented with a full data set for unsaturated tails with central *cis* double bonds (**T18A**<sup>9</sup>), branched alkyl tails (**T<sub>AOT</sub>**), and branched alkyl-aryl tails (**T<sub>JAS</sub>**, Fig. 6).

Six-tail amphiphiles obtained with **G1H6** were inactive with all examples beyond linear saturated tails (Fig. 6). Confirming the results from the *n*-alkyl screening (Fig. 4), this general inactivity confirmed that the selected tails are all too hydrophobic for a six-tail dendron **G1H6**. Four-tail amphiphiles produced with **G1H4** functioned as DNA activators when coupled with the branched **T<sub>AOT</sub>** and jasminaldehyde **T<sub>JAS</sub>**, whereas the longer **T18A**<sup>9</sup> failed to generate activity despite the presence of a solubilizing *cis* double bond in the middle (Fig. 6).

Best results were secured with three-tail amphiphiles obtained from **G1H3** and two-tail amphiphiles obtained from **G1H2**, which were active with **T<sub>AOT</sub>**, **T<sub>JAS</sub>** and **T18A**<sup>9</sup> (Fig. 6). Unique again was the behavior of single-tail amphiphiles obtained from **G1H1** (Fig. 6, dark blue). The DNA activation with the unsaturated long-tail **T18A**<sup>9</sup> was as good as with the saturated analog **T18**. Here, **G1H1** differed from **G1H2** and **G1H3**, which were both active with the unsaturated **T18A**<sup>9</sup> but inactive with the saturated **T18**. However,



**Fig. 6** Dependence of (a)  $Y_{\text{MAX}}$  and (b)  $EC_{50}$  of counterion-ctDNA transporters on the nature of the tails, measured for varied counterion concentrations at constant DNA concentration (1.25  $\mu\text{g ml}^{-1}$ ). Results cover counterions with heads **G1H6** (with six tails, red), **G1H4** (four tails, garnet), **G1H3** (three tails, violet), **G1H2** (two tails, blue) and **G1H1** (one tail, dark blue) that are coupled with **T10** (longish, saturated), **T<sub>AOT</sub>** (branched), **T<sub>JAS</sub>** (branched, aromatic), **T18A**<sup>9</sup> (very long, unsaturated) and **T18** (very long, saturated.  $Y_{\text{MAX}} < 0.2$  at 25  $\mu\text{M}$  was considered inactive and put to  $Y_{\text{MAX}} = 0.0$  and  $EC_{50} = 100 \mu\text{M}$  to enhance contrast, compare Table S1†).<sup>15</sup>

**G1H1** was inactive with the detergent-like **T<sub>AOT</sub>**, where **G1H2** and **G1H3** both worked very well.

The only tail that generated activity independent of the number of tails attached to the head group (excluding the “hypervalent” **G1H6**) was the one derived from jasminaldehyde **T<sub>JAS</sub>** (Fig. 6). The  $EC_{50}$  values with **T<sub>JAS</sub>** were consistently in the lower micromolar range, namely  $EC_{50} = 10.6 \pm 0.7 \mu\text{M}$  with **G1H1**,  $EC_{50} = 4.1 \pm 0.7 \mu\text{M}$  with **G1H2**,  $EC_{50} = 4.4 \pm 0.6 \mu\text{M}$  with **G1H3** and  $EC_{50} = 11.8 \pm 1.6 \mu\text{M}$  with **G1H4** (Fig. 6b, Table S1†). The same trend was observed with the  $Y_{\text{MAX}}$  for **T<sub>JAS</sub>**, moving from  $Y_{\text{MAX}} = 0.50 \pm 0.02$  with **G1H1** over maximal  $Y_{\text{MAX}} = 0.81 \pm 0.11$  with **G1H2** to  $Y_{\text{MAX}} = 0.51 \pm 0.05$  with **G1H3** and  $Y_{\text{MAX}} = 0.62 \pm 0.06$  with **G1H4** (Fig. 6a, Table S1†). For comparison, the otherwise excellent **T18A**<sup>9</sup> was inactive with **G1H4**, or **T<sub>AOT</sub>**, otherwise outstanding, failed to work with **G1H1** ( $Y < 0.2$  at 25  $\mu\text{M}$ , Fig. 6, Table S1†).

## Conclusions

New concepts and methods for the identification of counterion activators of DNA (and the charge-inverted CPP) transporters in bilayer membranes are of interest because of their importance for general applications toward sensing,<sup>6,10</sup> catalysis<sup>11</sup> and cellular uptake.<sup>7–9</sup> The objective of this study was to elaborate a comprehensive map connecting the number, the length and the nature of the hydrophobic tails of single-head guanidinium activators of DNA transporters.

The three key findings are as follows. Firstly, the concept of dynamic<sup>14</sup> counterion activators,<sup>1–3</sup> where different tails can be characterized with essentially no synthetic effort, is confirmed as very important and productive from a methodological point of view. With this approach, it is no problem to rapidly produce and screen counterion libraries.

The second key finding is that with increasing number of tails, the maximal activity of counterion activators shifts to decreasing tail length. This finding highlights the fundamental importance of a balanced, intermediate amphiphilicity for activity. Hindered ion pairing of DNA phosphodiester with guanidinium cations hidden within multi-tail octopus amphiphiles was of interest as alternative explanation. However, altered DNA binding explained sharp drops in activity with single carbons added to or removed from the tails less convincingly than the need for balanced, intermediate hydrophobicity of the final active polyion-counterion complex, where small differences in amphiphile structure are amplified by multivalency.

The newly introduced octopus amphiphiles with a new record of six tails added *via* six hydrazones and a peptide dendron converging to one cationic guanidinium head turns out to be less interesting in this context because activity is limited to very short tails with 5–7 carbons only and often includes some non-specific leakage. This result suggests that the originally planned synthesis of “true” octopus amphiphiles with eight tentacles is redundant because activity is likely to further decrease. The newly introduced long tails with a new record of up to 18 carbons turn out to be very interesting because they bring activity to single-tail amphiphiles that have so far been useless.

Concerning the nature of the tails, we found that long *cis*-alkenyl and branched alkyl tails are more active than their linear analogs. The totally unpredictable identification of the branched and aromatic tail from jasminaldehyde **T<sub>JAS</sub>** as the one that works well ( $Y_{\text{MAX}} \geq 0.5$ ) with the highest number of different head groups underscores the power of “high-throughput” counterion screening with dynamic amphiphiles (Table S1,† Fig. 6). Dodecanal **T<sub>12</sub>** as closest competitor is poorly active with **G1H4** ( $Y_{\text{MAX}} = 0.24 \pm 0.07$ , Table S1,† Fig. 4a), all other tails fail with at least two head groups. Attractive applications of these overall very consistent lessons learned in this study toward fragrant cellular uptake are currently under investigation.

## Acknowledgements

We thank D. Jeannerat, A. Pinto and S. Grass for NMR measurements, the Sciences Mass Spectrometry (SMS) platform at the Faculty of Sciences, University of Geneva, for mass spectrometry services, and the University of Geneva, the NCCR Chemical

Biology, and the Swiss NSF for financial support. S.M. is an ERC Advanced Investigator.

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