Supporting Information

**GeT peptides: a single-domain approach to gene delivery.**

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**Abbreviations**

DIPEA – diisopropylethylamine; EDTA – ethylenediaminetetraacetic acid; Flu – carboxyfluorescein; Fmoc – 9-fluorenlymethoxycarbonyl; GeT – gene transporter; HBTU – O-benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluorophosphate; RP-HPLC – reversed phase high pressure liquid chromatography; MALDI-ToF – matrix-assisted laser desorption/ionization time of flight; TAMRA - tetramethyl-6-carboxyrhodamine; TIS – triisopropyl silane; TFA – trifluoroacetic acid.

**Experimental Notes**

**Peptide synthesis.** Peptides were assembled on a Liberty-1 microwave peptide synthesizer (CEM Inc.). Standard solid-phase Fmoc-based protocols using HBTU/DIPEA for amino-acid couplings and Rink amide MBHA resin were used. lam-GeT was synthesized according to the published protocols.29 non-GeT peptide was designed as described elsewhere.33 5-(and 6-) carboxyfluorescein succinimidyl ester was used to label the N-terminus of GeT and the lysine side chain of lam-GeT directly on resin. Synthesized peptides were purified by RP-HPLC after deprotection (95% TFA, 2.5% TIS, 2.5% water) and workup. The identities of the peptides were confirmed by analytical RP-HPLC and MALDI-ToF:

MS [M+H]+: GeT – m/z 2319.1 (calc), 2320.1 (observed); flu-GeT – m/z 2677.4 (calc), 2677.6 (observed); lam-GeT – m/z 3378.2 (calc.), 3379.1 (observed); lam-flu-GeT – m/z 3736.5 (calc.), 3736.6 (observed); non-GeT – m/z 2358.6 (calc), 2357.8 (observed).

Peptide sequences are shown in Table S1.

**High Performance Liquid Chromatography.** Analytical and semi-preparative gradient RP-HPLC was performed on a JASCO HPLC system using Vydac C5 analytical (5 μm, 4.6 mm i.d. x 250 mm) and C18 semi-preparative (5 μm, 10 mm i.d. x 250 mm) columns. Both analytical and semi-prep runs used a 10-60% B gradient over 60 min at 0.5 mL/min and 4.5 mL/min respectively with detection at 214 nm, 280 nm and 520 nm (fluorescence emission). Buffer A – 5% and buffer B – 95% aqueous CH3CN, 0.1% TFA.

**Circular Dichroism.** Circular dichroism spectroscopy was performed on a JASCO J-810 spectropolarimeter fitted with a Peltier temperature controller. All measurements were taken in ellipticities in mdeg and converted to molar ellipticities ([θ],...
deg cm\(^2\) dmol\(^{-1}\)) by normalizing for the concentration of peptide bonds. Aqueous peptide solutions (300 μL volume; 4-10 μM in peptide) were prepared in filtered (0.22 μm) 10 mM phosphate buffer, pH 7.4. CD spectra recorded in the presence of synthetic membranes are for lipid/peptide molar ratio of 100:1 (up to 1 mM total lipid, 4-10 μM peptide).

Linear Dichroism. Solution-phase flow linear dichroism spectroscopy was performed on a Jasco-810 spectropolarimeter using a photo elastic modulator 1/2 wave plate. A micro-volume quartz Couette flow cell with ~0.25 mm annular gap and quartz capillaries were purchased from Kromattec Ltd, UK. Molecular alignment is achieved through the constant flow of the sample solution between two coaxial cylinders – a stationary quartz rod and a rotating cylindrical capillary. LD spectra were acquired with laminar flow obtained by maintaining the rotation speed at 3000 rpm and processed by subtracting non-rotating baseline spectra. LD spectra recorded in the presence of synthetic membranes, DLPC and DLPC:DLPG (3:1), were prepared at a lipid:peptide molar ratio of 100:1 (3 mM total lipid, 30 μM peptide).

Synthetic membranes. Dilaurylphosphatidylcholine (DLPC) and its mixtures with dilaurylphosphatidylglycerol (DLPG) were used to prepare zwitterionic and anionic membranes respectively using published protocols. Typically, unilamellar vesicles of DLPC and DLPC-DLPG (3:1 molar ratio) were prepared by dissolving dry lipids in chloroform/methanol (2:1 v/v) followed by evaporating the solvents and hydrating the residue to 10 mg/mL total lipid concentration in 10 mM MOPS, 10 mM NaCl, pH 7.4. The suspension was then extensively vortexed, sonicated (30°C), and extruded using a hand-held extruder (Avanti polar lipids) (twenty times, polycarbonate filter, 0.1 μm) to give a clear solution, and analyzed (100 nm) by photon correlation spectroscopy.

Photon Correlation Spectroscopy. Vesicles were re-suspended to final concentration of 1 mg/mL and were analysed on a Zetasizer Nano (ZEN3600), Malvern Instruments (Worcestershire, UK). DLS batch measurements were carried out in a low volume disposable cuvette at 25°C. Hydrodynamic radii were obtained through the fitting of autocorrelation data using the manufactures software, Dispersion Technology Software (DTS version 5.10).

Cell culture

*Human dermal fibroblasts* (ATCC number PCS-201-012,) were maintained in Medium 106 with low serum growth supplement (Gibco) and antibiotics (gentamicin and amphotericin B) in 25 cm\(^2\) flasks. For growth the cells were incubated at 37°C, 5% CO\(_2\).

*Pichia pastoris* (X33) yeast cells (Invitrogen) were incubated overnight in Yeast Peptone Dextrose media at 37°C, 220 rpm (orbital 50 mm).

Minimum inhibitory concentrations (MIC) were determined by broth microdilution on *Escherichia coli* (K12) according to the Clinical and Laboratory Standards Institute. Typically, 90 μL of 0.5 - 1 x 106 CFU per ml of each bacterium in Mueller Hinton media (plus cations) broth (Oxoid) were incubated in 96 well micro-titre plates with serial two-fold dilutions of the peptides from 100 μM to 0.78 μM. Minimum inhibitory concentrations (MIC’s) were defined as the lowest peptide concentration with no visible growth of bacteria from the MIC micro-titer plates after 24 hours at 37 °C.
Transfection assays

**Peptide transfection:** Exponentially growing human fibroblasts were cultured in two-well chambers (Labtek) at a seeding density of 10^3 cells/cm^2 overnight at 37°C, 5% CO_2_. Following incubation the culture medium was aspirated, and the cells were twice washed with Medium 106, which was followed up by incubation with fluorescently labeled peptide at set concentrations (0.1-10 μM), first for 15 min in serum-reduced Opti-MEM and then after washing with Medium 106 for up to 24 hours. Control experiments performed using free dyes and free DNAs (TAMRA-ssDNA and pEGFP) gave no transfection.

**Liopfectamine-mediated transfection:** Human dermal fibroblasts were cultured as above in two-well chambers (Labtek), and were then transfected with pEGFP (0.2 μg) using Lipofectamine Ltx (Invitrogen) at about 40-60% confluency as recommended by to the proprietary protocols. After 3 hours of incubation cells were twice washed with Medium 106. EGFP expression was monitored at different time points (from 3 hours to over a week) using confocal fluorescent microscopy.

**Transfection of peptide/DNA complexes:** Peptide/TAMRA-ssDNA and peptide/pEGFP complexes (P/N ratios 0.2-1, with pEGFP at 0.2 μg) were incubated with the same cell population as above for 30 min and 3 hours respectively in serum-reduced Opti-MEM. Cells were then twice washed and incubated with Medium 106.

**Peptide transfection into Pichia pastoris:** Following an overnight incubation at 37 °C, 220 rpm, *P. pastoris* cells were centrifuged, the pellet was re-suspended in PBS to obtain OD600nm = 0.5 AU. The cells were incubated with the fluorescently labeled peptide at 10 μM for 30 min, upon which cells were centrifuged and washed with PBS.

For all assays, 10-μL cell suspensions were mounted onto glass microscope slides and observed using confocal fluorescence microscopy.

**Confocal fluorescence microscopy**

Fluorescence was monitored using an Olympus IX81 confocal microscope at 488 nm using an x60 objective with appropriate filter. Life cell imaging was performed under controlled environmental conditions (37 °C, 5% CO2). Images (2D and 3D stacks) were processed using Imaris v5.1 and Image J software.

### Table and Figures

**Table S1. Peptide and DNA constructs used in the study**

<table>
<thead>
<tr>
<th>Construct name</th>
<th>Sequence^a</th>
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<tr>
<td>GeT KIAKLKAKIQKLQKIAK</td>
<td>GeT KIAKLKAKIQKLQKIAK</td>
</tr>
<tr>
<td>flu-GeT flu-KIAKLKAKIQKLQKIAK</td>
<td>flu-GeT flu-KIAKLKAKIQKLQKIAK</td>
</tr>
<tr>
<td>lam-GeT cyclo(YisSRN)-GG-KGG-KIAKLKAKIQKLQKIAK</td>
<td>lam-GeT cyclo(YisSRN)-GG-KGG-KIAKLKAKIQKLQKIAK</td>
</tr>
<tr>
<td>lam-flu-GeT cyclo(YisSRN)-GG-K(FLU)-GG-KIAKLKAKIQKLQKIAK</td>
<td>lam-flu-GeT cyclo(YisSRN)-GG-K(FLU)-GG-KIAKLKAKIQKLQKIAK</td>
</tr>
<tr>
<td>non-GeT ac-IAALEYEIAALEKEIAALE</td>
<td>non-GeT ac-IAALEYEIAALEKEIAALE</td>
</tr>
<tr>
<td>CF508-G-TAMRA 5’-GGCACCATTAAAGAAAATATCATCTG-3’-TAMRA</td>
<td>CF508-G-TAMRA 5’-GGCACCATTAAAGAAAATATCATCTG-3’-TAMRA</td>
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^a^lower case denotes D-serine; ^b^cyclo peptides linked via the side chain of the asparagine residues
Figure S1. Confocal fluorescence micrographs of human dermal fibroblasts incubated with fluorescein-labeled GeT peptides (no DNA) for 15 min (left column) and 36 hrs (right column) at different concentrations.
Figure S2. Comparative transfection efficiency of pEGFP/GeT as a function of N/P charge ratios at two different time points: 3 and 24 hours, dark and light bars respectively. Lipofectamine Ltx was used as a benchmark. The efficiency of transfection was determined by counting fluorescent cells (EGFP expressing cells). The total number of cells was taken as 100%.

Figure S3. Confocal fluorescence micrographs of human dermal fibroblasts incubated with pEGFP/GeT for 18 hrs (left) and 48 hrs (right).
**Figure S4.** Differential interference contrast (a) and confocal fluorescence (b and c) micrographs of human dermal fibroblasts incubated with pEGFP/Lipofectamine for 36 (a and b) and 72 hrs (c).

**Figure S5.** Confocal fluorescence micrographs of *Pichia pastoris* incubated with flu-GeT: fluorescence (a), bright field (b) and 3D (c) representations. Incubations were for 30 min at 37°C, at 10 μM peptide.

**Figure S6.** Folding of GeT. (a) CD spectra upon mixing with anionic membranes (solid line) and after a 3-hour incubation (dotted line), 4 μM peptide. (b) LD spectra acquired upon mixing with anionic membranes (dotted line) and after a 2-hour incubation, 30 μM peptide.
Figure S7. CD spectra for GeT at pH 1.5 (solid line) and pH 5 (solid line and black squares), 4 μM peptide.

Figure S8. CD spectra for GeT at 60 μM (solid line and black discs) and 100 μM (dashed line and white discs) in phosphate buffer pH 7.4.
Figure S9. RP-HPLC traces for (a) GeT, (b) lam-flu-GeT (b) and flu-GeT (c): absorbance was recorded at 214 nm (black and red lines) and fluorescence emission at 520 nm (blue lines).

References
