

Designed cell penetrating peptide dendrimers efficiently internalize cargo into cells†

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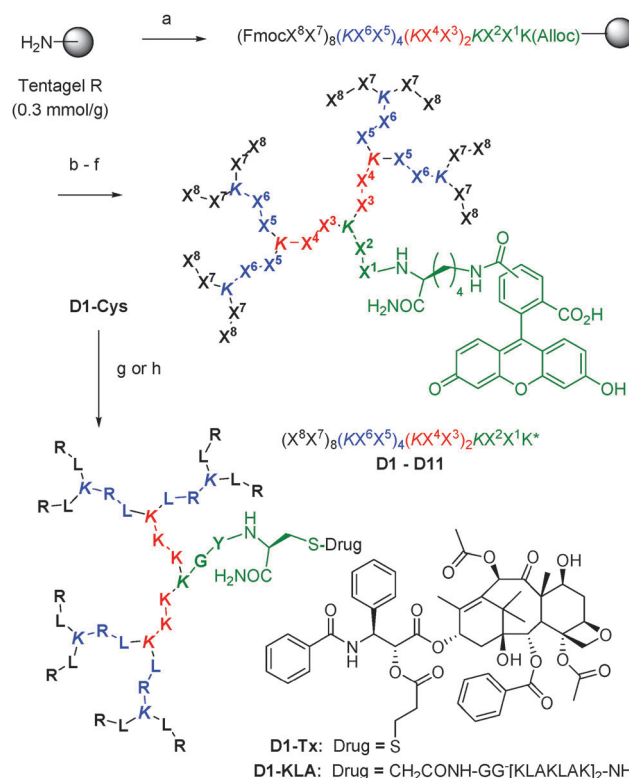
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Redesigning linear cell penetrating peptides (CPPs) into a multi-branched topology with short dipeptide branches gave cell penetrating peptide dendrimers (CPPDs) with higher cell penetration, lower toxicity and hemolysis and higher serum stability than linear CPPs. Their use is demonstrated by delivering a cytotoxic peptide and paclitaxel into cells.

The cell membrane poses an impermeable barrier for a large number of compounds, in particular peptides, limiting their use as drugs. Nevertheless the discovery of the cell penetrating properties of the HIV-1 Tat protein¹ led to the discovery of a variety of cell penetrating peptides (CPPs),² an effect which was later also reported for synthetic oligomer analogs of linear peptides,³ cyclic peptides,⁴ and various organic dendrimers.⁵ CPPs can serve as carriers for drug delivery, however they suffer from the typical metabolic instability of linear peptides and tend to be hemolytic and cytotoxic. Interestingly the cell internalization of CPPs can be enhanced by grafting them onto multivalent scaffolds, but their toxicity is usually also increased in such constructs despite the fact that their folding propensity is retained.⁶ We showed recently that peptide dendrimers containing very short mono- or dipeptide branches,⁷ while not able to form stable secondary structures,^{7b} are generally more resistant to proteolytic degradation compared to linear peptides,^{7c} and show almost no cytotoxicity when used for DNA transfection^{7f} and only very weak hemolysis when designed as antimicrobials,^{7e} which is probably a benefit of their particular molten globule conformation enforced by the dendritic topology.^{7a} Although no increase in cellular uptake was reported with branched octaarginines compared to linear (Arg)₈,⁸ we asked the question whether a broader survey of CPPs redesigned in a multi-branched topology might lead to cell penetrating peptide dendrimers (CPPDs) with higher cellular uptake, metabolic stability and lower toxicity compared to linear CPPs, and potential use as drug delivery agents.

Fourteen peptide dendrimers were prepared by SPPS with sequence $(X^8X^7)_8(KX^6X^5)_4(KX^4X^3)_2KX^2X^1K^*$ (K = branching lysine) and labeled with 5(6)-carboxyfluorescein (CF) attached to the ϵ -amino group of the core lysine residue (K^*) (Scheme 1). Variable residues (X) were assigned to amino acids found in the linear cationic CPP Tat¹ or the amphipathic CPPs TP10⁹ and pVEC¹⁰ to form dendrimers with Arg and Lys (**D1–D4** and **D11**) or only Lys (**D5–D10**) as cationic



Scheme 1 Synthesis of CPPD. Conditions: (a) Fmoc solid-phase peptide synthesis (SPPS), X^i = amino acid, K = branching lysine; (b) PhSiH_3 , $\text{Pd(PPh}_3)_4$; (c) 5(6)-carboxyfluorescein, HOBT, DIC; (d) piperidine/DMF; (e) optional: Ac_2O ; (f) $\text{CF}_3\text{CO}_2\text{H}/i\text{Pr}_3\text{SiH}/\text{H}_2\text{O}$, then preparative HPLC; (g) $\text{ClCH}_2\text{CO-GG-[KLAKLAK]}_2\text{-NH}_2$, DIEA; (h) PTX-PDP, DIEA. See ESI† for details.

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Table 1 Synthesis and cellular uptake of CF-labelled CPPs and CPPDs

Cpd.	Sequence ^a	Yield ^b (mg (%))	+ ^c	Arg	Lys	Hyd. ^d	MFI av. (1 μM)	(10 μM) ^e	Hemolysis ^f (μg mL ⁻¹ /μM)
							HeLa, CHO, Jurkat		
Tat	*-YGRKKRRQRRR	90.9 (26)	8	6	2	1	2, 2, 11	14, 23, 65	125/44
D1	(RL) ₈ (KRL) ₄ (KKK) ₂ KGYK*	14.7 (3)	24	12	4	13	11, 10, 49	214, 114, 1450	31/4
D2	(RL) ₈ (KLL) ₄ (KKK) ₂ KGYK*	9.6 (6)	20	8	4	17	20, 22, 108	158, 175, 631	31/4
D3	(RL) ₈ (KNN) ₄ (KKK) ₂ KGYK*	23.3 (5)	20	8	4	9	5, 4, 40	64, 48, 210	125/17
D4	(GY) ₈ (KKR) ₄ (KQQ) ₂ KRRK*	13.2 (2)	18	6	4	12	3, 4, 27	19, 51, 112	63/9
AcD4	(AcGY) ₈ (KKR) ₄ (KQQ) ₂ KRRK*	4.7 (1)	10	6	4	12	3, 2, 9	15, 25, 65	250/38
TP10	*-AGYLLGKINLKALAALAKKIL	51.3 (15)	4	—	4	15	7, 6, 23	152, 186, 385	31/10
D5	(AL) ₈ (KIK) ₄ (KLA) ₂ KKIK*	33.4 (5)	13	—	5	25	4, 9, 28	46, 122, 118	63/11
D6	(LA) ₈ (KKL) ₄ (KKL) ₂ KYAK*	55.1 (8)	14	—	6	24	2, 5, 7	17, 21, 31	1000/163
AcD6	(AcLA) ₈ (KKL) ₄ (KKL) ₂ KYAK*	9.0 (7)	6	—	6	24	2, 7, 14	35, 40, 64	n.d.
D7	(IK) ₈ (KLA) ₄ (KLA) ₂ KKIK*	6.4 (1)	17	—	9	21	5, 14, 29	33, 51, 355	125/19
AcD7	(AcIK) ₈ (KLA) ₄ (KLA) ₂ KKIK*	3.0 (1)	9	—	9	21	5, 14, 190	33, 259, 862	63/10
D8	(LA) ₈ (KIK) ₄ (KLA) ₂ KKIK*	11.1 (2)	13	—	5	25	2, 4, 11	20, 30, 71	63/11
D9	(LA) ₈ (KLA) ₄ (KIK) ₂ KKIK*	8.2 (2)	11	—	3	27	2, 2, 10	14, 14, 61	125/22
D10	(LA) ₈ (KLA) ₄ (KLA) ₂ KKIK*	12.3 (3)	9	—	1	29	3, 2, 5	9, 8, 36	250/48
pVEC	*-LLIILRRIRKQAHASHK	94.3 (26)	6	4	2	8	36, 71, 84	126, 210, 292	4/1
D11	(LI) ₈ (KRK) ₄ (KRA) ₂ KHSK*	15.0 (2)	18	6	4	18	3, 12, 58	60, 106, 968	500/70
AcD11	(AcLI) ₈ (KRK) ₄ (KRA) ₂ KHSK*	7.0 (5)	10	6	4	18	4, 8, 26	44, 105, 385	n.d.

^a One letter codes for amino acids. Branching diamino acids are shown in italics. * = 5(6)-carboxyfluorescein amidated to the Lys side chain (K*) or the N-terminus (*) added as the last coupling step, see ESI for details. Ac = acetyl. All peptides are carboxamide (CONH₂) at the C-terminus. ^b Yields given for RP-HPLC purified products as TFA salts. ^c + = number of cationic amino acid side chains (Lys and Arg) and free N-termini. ^d Hyd = hydrophobic residues: Ala, Ile, Leu and Tyr. ^e Flow cytometry data. MFI average = average of the mean fluorescence intensity of the tested peptides over background. Three independent experiments are used to get values. ^f Hemolysis assay detailed in ESI. n.d. = not determined.

residues, featuring various ratios and distribution of cationic and hydrophobic side chains in the branches and optional acetylation of N-termini to reduce the number of positive charges (Table 1).

CD spectra of CPPDs show random coil signals independent of amino acid composition consistent with the disordered conformation

of branched peptides and similar to Tat and pVEC (Fig. 1a and Fig. S1, ESI[†]). CPPD uptake by HeLa, CHO and Jurkat cells was evaluated by flow cytometry after 1 h incubation of the cells with 1 μ M or 10 μ M compounds (Table 1, Fig. 1b–d and Fig. S2, ESI[†]). A strong fluorescence increase was observed with arginine containing

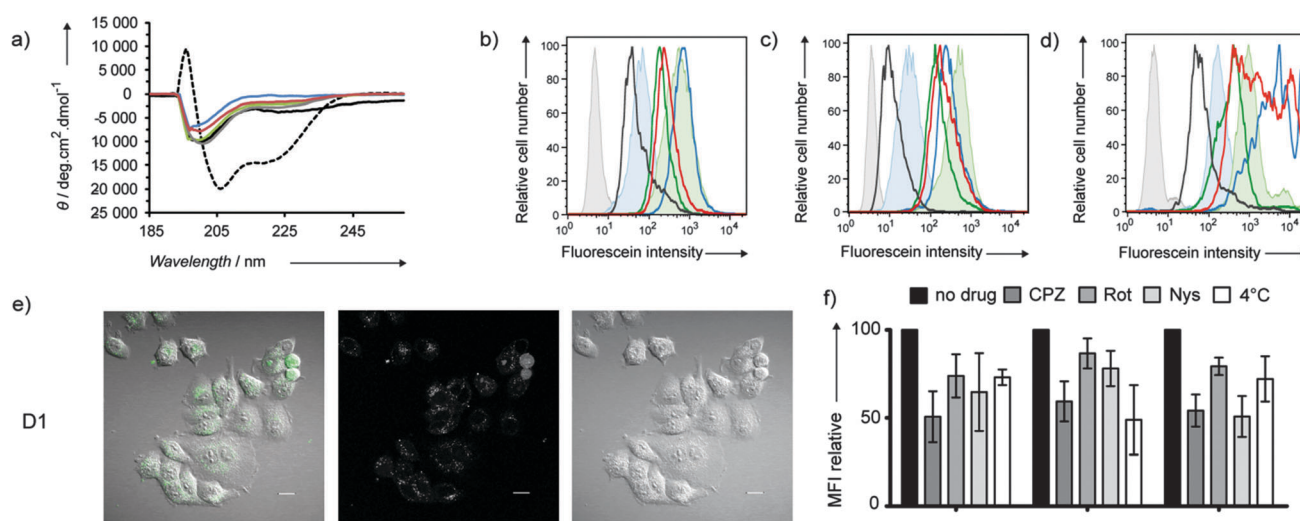


Fig. 1 (a) CD measurements of **D1** (blue), **D5** (green), **D10** (grey), and **D11** (red). **Tat** (black), **TP10** (dotted black) in PBS buffer (pH 7.4). (b–d) Flow cytometry histograms for HeLa (b), CHO (c) and Jurkat (d) cells after 1 h incubation with 10 μ M of peptide dendrimers at 37 °C. **D1** (blue), **D5** (green), **D10** (black), and **D11** (red). Untreated cells (grey), **Tat** (filled blue), **TP10** (filled green). (e) Observed uptake of **D1** (10 μ M) after 1 h incubation at 37 °C on HeLa cells in live confocal microscopy of the differential interference contrast (DIC, right panel) and the CF fluorescence at 525 nm (middle panel). The left panel shows the merged images of the DIC and fluorescence pictures. White bar = 20 μ m. See Fig. S5 (ESI[†]) for data with CPPD **D11**. (f) Uptake levels of **D1** and **D11** in HeLa cells in the presence of various inhibitors. Cells were pretreated 30 min with 50 μ M chlorpromazine (CPZ), 20 μ M rottlerin (Rot), 25 μ g mL⁻¹ nystatin (Nys) or cooled down at 4 °C prior to 1 h incubation with 10 μ M CPPD or linear octaarginine (R8) in the presence of an inhibitor. Error bars represent the SD of three independent experiments. See also Fig. S6 (ESI[†]) for CHO cells.

dendrimers **D1** and **D2** (staining 10–15 times higher than the parent linear Tat), **D11** and **AcD11** (staining comparable to the parent linear pVEC). Exchanging the eight leucines in the G2-branches of **D2** for polar asparagines to form **D3** decreased the MFI average in spite of the same total number of cationic residues (8 Arg and 4 Lys), highlighting the importance of hydrophobic residues for uptake. **D5** and **AcD7** with only lysine as a cationic residue also showed strong fluorescence comparable to the parent linear TP10, implying that arginine is not necessary for cellular uptake of CPPD. The remaining CPPDs showed MFI average comparable to linear Tat. Hydrophobic **D10** with only one cationic side chain was the least efficient CPPD in the library.

Fixed confocal microscopy images with HeLa and CHO cells confirmed the trends seen in flow cytometry and revealed the cellular localization of peptide dendrimers (Fig. S3, ESI†). For the Arg containing dendrimers **D1**, **D2**, **D3** and **D11**, and **D5** with only Lys as a cationic residue, images showed a strong staining of the cytoplasm similar to Tat in both cell lines. In contrast to PAMAM dendrimers,^{5b} acetylation of N-terminal amino groups did not block cellular uptake of CPPDs, although differences of subcellular localization were visible between **D11** (homogeneous staining) and **AcD11** (punctuated patterns). Live confocal microscopy experiments were performed with **D1** and **D11** to confirm cellular uptake and distribution (Fig. 1e, Fig. S5 and S6, ESI†). After 1 h, dendrimers were found inside cells localized in the cytoplasm in a punctuated pattern suggesting confinement in endosomes.

Both energy-dependent endocytosis and energy-independent or direct translocation across the cell membrane have been proposed as uptake mechanisms for linear CPPs.¹¹ The uptake of dendrimer **D1** by HeLa cells was reduced by chlorpromazine, indicating clathrin dependent uptake, and to a lesser extent by rottlerin (macropinocytosis inhibitor) and nystatin (caveola/lipid raft-dependent endocytosis inhibitor) (Fig. 1f). Uptake was only 30% lower at 4 °C compared to that at 37 °C, suggesting that direct membrane translocation also takes place for **D1**. Dendrimer **D11** showed a similar pattern however with reduced uptake at 4 °C, indicating lower self-translocating ability probably due to its smaller number of positive charges and different distribution of Arg residues in the branches compared to **D1**. Results with CHO cells (Fig. S6, ESI†) show primarily a clathrin dependent uptake for both **D1** and **D11** and a reduced uptake at 4 °C (energy-dependent uptake mostly) in line with previous observations that the cell type plays a critical role in the internalization mechanism of CPPs. In the case of CPPDs, clathrin mediated uptake appears to be the major process of internalization taking place in both cell lines.

CPPDs were not significantly toxic to HeLa or CHO cells (up to 10 µM, 24 h, Fig. S4, ESI†). However less than 50% viable Jurkat cells remained after 24 h exposure to 10 µM pVEC, **D1**, **D2**, **D7**, **D11** or **AcD11**, which belong to the most cell penetrating compounds. While hemolysis was very strong for the linear CPP pVEC (4 µg mL⁻¹), CPPDs were less hemolytic including those with strong cell penetrating properties (30–500 µg mL⁻¹, Table 1).

Dendrimers **D1** and **D11** combined efficient uptake into cells with moderate toxicity and were further investigated. Degradation experiments in human serum showed that 40% of **D1** and **D11** were still unchanged after 12 h while linear Tat was completely

Table 2 Cytotoxicity of CPPD-drug conjugates^a

Cpd.	IC ₅₀ HeLa cells	IC ₅₀ CHO cells
KLA	>> 20 µM (100%) ^b	>> 20 µM (93%) ^b
D1-KLA	4.6 ± 0.2 µM	7.8 ± 0.6 µM
D11-KLA	6.0 ± 0.3 µM	8.9 ± 0.6 µM
Tat-KLA	10.1 ± 0.5 µM	~ 20 µM (48%) ^b
Paclitaxel (PTX)	8.4 ± 0.6 nM ^c	~ 4 µM (60%) ^b
D1-PTX	47 ± 15 nM	~ 4 µM (57%) ^b
D11-PTX	41 ± 9 nM	~ 4 µM (46%) ^b

^a Cell survival measured after 24 h (KLA series) or 72 h (paclitaxel series) with the WST-8 assay. ^b % of surviving cells at the indicated concentration, which was the highest measured. No IC₅₀ is given when the full inhibition curve could not be measured. ^c With 1.6% DMSO added to solubilise paclitaxel.

degraded, in line with previous reports that peptide dendrimers are more resistant to proteolysis than linear peptides (Fig. S7, ESI†).^{7d-f} To evaluate the ability of **D1** and **D11** to deliver cargos into cells, dendrimers were conjugated *via* a thioether bridge to the α-helical tetradecapeptide [KLAKLAK]₂ (**KLA**), an antimicrobial peptide that does not penetrate mammalian cells but induces cell death by disrupting the mitochondrial membrane if delivered into the cytosol.¹² The resulting conjugates **D1-KLA** and **D11-KLA** (Scheme 1) were found to be significantly more cytotoxic to HeLa and CHO cells compared to **Tat-KLA** at 10 µM (Fig. S8 and S9, ESI†), while **KLA** alone or an ungrafted dendrimer were not toxic (Table 2, Fig. S10, ESI†). Covalent conjugation to CPPD does not change the bioactivity of **KLA** since disulfide bridged conjugates to both **D1** and **D11** showed cytotoxicity similar to the thioether adducts (Fig. S11, Table S1, ESI†).

In a further example, **D1** and **D11** were conjugated to paclitaxel (PTX), a notoriously insoluble anticancer drug whose solubility and targeting can be significantly improved by incorporation into a variety of nanocarriers including dendrimers and peptides.¹³ CPPD conjugates **D1-PTX** and **D11-PTX** were prepared by disulfide bond formation between **D1-Cys** or **D11-Cys** and paclitaxel-2'-(3-(2-pyridyldithio)) propionate (PTX-PDP, Scheme 1). The dithiopropionyl linker has been reported for PTX-octaarginine¹⁴ and for dendrimer drug conjugates¹⁵ and is susceptible to intracellular reductive cleavage. Our CPPD-PTX conjugates were water soluble and stable in cell culture over the time of the experiment, yet retained most of the selective cytotoxicity of PTX to HeLa cells over CHO cells (Table 2, Fig. S12, ESI†).

In summary, redesigning CPPs into G3 peptide dendrimers with short dipeptide branches gave CPPDs with stronger cellular uptake, lower cytotoxicity and hemolysis, and higher stability towards serum degradation compared to their linear counterpart. Cellular uptake was observed in a diversity of sequences containing either Arg or Lys as a cationic residue and a balanced ratio of hydrophobic residues. Dendrimer **D1** inspired by the Tat peptide and **D11** inspired by pVEC efficiently localized in the cytoplasm and delivered cytotoxic cargo into cells. The low intrinsic toxicity and hemolysis of CPPDs might result from their inability to fold into amphipathic α-helical membrane lytic aggregates. The higher cellular uptake of CPPDs compared to linear CPPs probably reflects in part their larger size, which is remarkably obtained without increased synthetic complexity since the described CPPDs are



obtained in only 12 SPPS coupling steps warranting low production costs. Due to their ease of synthesis and favorable properties CPPDs represent a promising and versatile new class of cell penetrating devices.

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Notes and references

- (a) S. Futaki, J. Seery, Y. Daikh, C. Moore, L. L. Chen, B. Pepinsky and J. Barsoum, *Proc. Natl. Acad. Sci. U. S. A.*, 1994, **91**, 664–668; (b) E. Vivès, P. Brodin and B. Lebleu, *J. Biol. Chem.*, 1997, **272**, 16010–16017.
- (a) S. Futaki, T. Suzuki, W. Ohashi, T. Yagami, S. Tanaka, K. Ueda and Y. Sugiura, *J. Biol. Chem.*, 2001, **276**, 5836–5840; (b) S. B. Fonseca, M. P. Pereira and S. O. Kelley, *Adv. Drug Delivery Rev.*, 2009, **61**, 953–964; (c) E. Koren and V. P. Torchilin, *Trends Mol. Med.*, 2012, **18**, 385–393.
- (a) I. Peretto, R. M. Sanchez-Martin, X. H. Wang, J. Ellard, S. Mittoo and M. Bradley, *Chem. Commun.*, 2003, 2312–2313; (b) J. H. Moon, W. McDaniel, P. Maclean and L. F. Hancock, *Angew. Chem., Int. Ed.*, 2007, **46**, 8223–8225; (c) E. R. Gillies, F. Deiss, C. Staedel, J. M. Schmitter and I. Huc, *Angew. Chem., Int. Ed.*, 2007, **46**, 4081–4084; (d) C. B. Cooley, B. M. Trantow, F. Nederberg, M. K. Kiesewetter, J. L. Hedrick, R. M. Waymouth and P. A. Wender, *J. Am. Chem. Soc.*, 2009, **131**, 16401–16403; (e) E. I. Geihe, C. B. Cooley, J. R. Simon, M. K. Kiesewetter, J. A. Edward, R. P. Hickerson, R. L. Kaspar, J. L. Hedrick, R. M. Waymouth and P. A. Wender, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 13171–13176; (f) U. Sternberg, E. Birtalan, I. Jakovkin, B. Luy, U. Schepers, S. Brase and C. Muhle-Goll, *Org. Biomol. Chem.*, 2013, **11**, 640–647.
- (a) Y. U. Kwon and T. Kodadek, *Chem. Biol.*, 2007, **14**, 671–677; (b) G. Lattig-Tunnemann, M. Prinz, D. Hoffmann, J. Behlke, C. Palm-Apergi, I. Morano, H. D. Herce and M. C. Cardoso, *Nat. Commun.*, 2011, **2**, 453; (c) D. Mandal, A. Nasrolahi Shirazi and K. Parang, *Angew. Chem., Int. Ed.*, 2011, **50**, 9633–9637; (d) Z. Qian, T. Liu, Y. Y. Liu, R. Briesewitz, A. M. Barrios, S. M. Jhiang and D. Pei, *ACS Chem. Biol.*, 2013, **8**, 423–431.
- (a) B. Aussedat, E. Dupont, S. Sagan, A. Joliot, S. Lavielle, G. Chassaing and F. Burlina, *Chem. Commun.*, 2008, 1398–1400; (b) L. Albertazzi, M. Serresi, A. Albanese and F. Beltram, *Mol. Pharmaceutics*, 2010, **7**, 680–688; (c) R. J. Amir, L. Albertazzi, J. Willis, A. Khan, T. Kang and C. J. Hawker, *Angew. Chem., Int. Ed.*, 2011, **50**, 3425–3429.
- (a) K. Sheldon, D. Liu, J. Ferguson and J. Gariepy, *Proc. Natl. Acad. Sci. U. S. A.*, 1995, **92**, 2056–2060; (b) K. S. Kawamura, M. Sung, E. Bolewska-Pedyczak and J. Gariepy, *Biochemistry*, 2006, **45**, 1116–1127; (c) A. M. Angeles-Boza, A. Erazo-Oliveras, Y.-J. Lee and J.-P. Pellois, *Bioconjugate Chem.*, 2010, **21**, 2164–2167; (d) G. A. Eggimann, S. Buschor, T. Darbre and J.-L. Reymond, *Org. Biomol. Chem.*, 2013, **11**, 6717–6733.
- (a) S. Javor, E. Delort, T. Darbre and J. L. Reymond, *J. Am. Chem. Soc.*, 2007, **129**, 13238–13246; (b) S. Javor and J. L. Reymond, *J. Org. Chem.*, 2009, **74**, 3665–3674; (c) P. Sommer, V. S. Fluxa, T. Darbre and J. L. Reymond, *ChemBioChem*, 2009, **10**, 1527–1536; (d) J. L. Reymond and T. Darbre, *Org. Biomol. Chem.*, 2012, **10**, 1483–1492; (e) M. Stach, N. Maillard, R. U. Kadam, D. Kalbermatter, M. Meury, M. G. P. Page, D. Fotiadis, T. Darbre and J.-L. Reymond, *MedChemComm*, 2012, **3**, 86–89; (f) A. Kwok, G. A. Eggimann, J. L. Reymond, T. Darbre and F. Hollfelder, *ACS Nano*, 2013, **7**, 4668–4682.
- S. Futaki, I. Nakase, T. Suzuki, Z. Youjun and Y. Sugiura, *Biochemistry*, 2002, **41**, 7925–7930.
- (a) M. Pooga, M. Hallbrink, M. Zorko and U. Langel, *FASEB J.*, 1998, **12**, 67–77; (b) U. Soomets, M. Lindgren, X. Gallet, M. Hallbrink, A. Elmquist, L. Balaspiri, M. Zorko, M. Pooga, R. Brasseur and Ü. Langel, *Biochim. Biophys. Acta*, 2000, **1467**, 165–176.
- (a) A. Elmquist, M. Lindgren, T. Bartfai and Ü. Langel, *Exp. Cell Res.*, 2001, **269**, 237–244; (b) A. Elmquist, M. Hansen and Ü. Langel, *Biochim. Biophys. Acta*, 2006, **1758**, 721–729.
- (a) H. Brooks, B. Lebleu and E. Vives, *Adv. Drug Delivery Rev.*, 2005, **57**, 559–577; (b) G. M. Poon and J. Gariepy, *Biochem. Soc. Trans.*, 2007, **35**, 788–793; (c) A. Ziegler, *Adv. Drug Delivery Rev.*, 2008, **60**, 580–597.
- S. Futaki, M. Niwa, I. Nakase, A. Tadokoro, Y. Zhang, M. Nagaoka, N. Wakako and Y. Sugiura, *Bioconjugate Chem.*, 2004, **15**, 475–481.
- (a) J. J. Khandare, S. Jayant, A. Singh, P. Chandna, Y. Wang, N. Vorsa and T. Minko, *Bioconjugate Chem.*, 2006, **17**, 1464–1472; (b) P. Zhao and D. Astruc, *ChemMedChem*, 2012, **7**, 952–972; (c) R. Colombo, M. Mingozzi, L. Belvisi, D. Arosio, U. Piarulli, N. Carenini, P. Perego, N. Zaffaroni, M. De Cesare, V. Castiglioni, E. Scanziani and C. Gennari, *J. Med. Chem.*, 2012, **55**, 10460–10474.
- (a) E. A. Dubikovskaya, S. H. Thorne, T. H. Pillow, C. H. Contag and P. A. Wender, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 12128–12133; (b) P. A. Wender, W. C. Galliher, N. M. Bhat, T. H. Pillow, M. M. Bieber and N. N. Teng, *Gynecol. Oncol.*, 2012, **126**, 118–123.
- (a) R. S. Navath, Y. E. Kurtoglu, B. Wang, S. Kannan, R. Romero and R. M. Kannan, *Bioconjugate Chem.*, 2008, **19**, 2446–2455; (b) Y. E. Kurtoglu, R. S. Navath, B. Wang, S. Kannan, R. Romero and R. M. Kannan, *Biomaterials*, 2009, **30**, 2112–2121.

