Cell-penetrating peptides as delivery vehicles for biology and medicine

Kelly M. Stewart,[b] Kristin L. Horton[b] and Shana O. Kelley*[a,b]

Received 2nd January 2008
First published as an Advance Article on the web 15th April 2008
DOI: 10.1039/b719950c

Cell-penetrating peptides (CPPs) have found numerous applications in biology and medicine since the first synthetic cell-permeable sequence was identified two decades ago. Numerous types of drugs have been transported into cells using CPPs, including small-molecule pharmaceuticals, therapeutic proteins, and antisense oligonucleotides. Improved agents for medical imaging have been generated by conjugation with CPPs, with the appended peptides promoting cellular uptake and in some cases, cell-type specificity. Organelle-specific CPPs have also been generated, providing a means to target specific subcellular sites. This review highlights achievements in this area and illustrates the numerous examples where peptide chemistry was exploited as a means to provide new tools for biology and medicine.

1. Introduction

The plasma membrane of eukaryotic cells is a tightly controlled barrier engineered to protect the cell from unregulated influx of bioactive molecules. For small-molecule and protein-based drugs that are not endogenous to the cell, traversing this membrane can involve hijacking a natural cellular process or achieving direct diffusion through the lipid bilayer. However, in many cases both modes of entry are inefficient for exogenous molecules. Thus, molecular transporters have long been sought that would facilitate the passage of pharmaceutically-active agents into living cells.

A major breakthrough in the identification of such transporters was realized in the late 1980s and early 1990s when a series of short peptide sequences were identified that efficiently crossed the plasma membrane.[1–3] One that received a great deal of attention was the Tat peptide, derived from the HIV Tat transactivator protein.[4] Originally, it was discovered that the full-length protein crossed the plasma membrane,[2] and subsequently, small fragments were identified that could efficiently enter cells.[4] These discoveries, along with those identifying other peptides with membrane-crossing activities,[5–9] served as the cornerstone for a new subfield focused on the use of cell-penetrating peptides (CPPs) as molecular transporters.

Today, thousands of studies have been performed characterizing and optimizing CPPs as cellular delivery agents.[7–11] Chemists and biochemists have developed many variations of peptide structures that have elucidated important molecular features of CPPs that modulate activity;[12–22] they have also used biophysical methods to characterize mechanisms underlying cellular uptake.[6,23–28] Researchers in both the biological and medical sciences have developed numerous applications of these constructs, illustrating the utility of CPPs in these fields.[7–11] A variety of intracellular cargoes have been transported by Tat or other CPPs, including DNA,[29] polymers,[30] nanoparticles,[31,32] and liposomes (Fig. 1).[33] Clearly, CPPs are a powerful tool for transporting diverse materials across the cell membrane and have attracted the interest of an interdisciplinary scientific community.

[a]*Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, Toronto, Canada*
[b]*Department of Biochemistry, Faculty of Medicine University of Toronto, Toronto, Canada. E-mail: shana.kelley@utoronto.ca*

Kristin L. Horton, Kelly M. Stewart and Shana O. Kelley

Kelly Stewart (center) is a PhD student with Dr Shana Kelley in the Department of Biochemistry at the U. Toronto. She earned a B.S. in Chemistry (Honors) from Penn State University in 2004.

Kristin Horton (left) is a PhD student in the Department of Biochemistry at U. Toronto. Horton completed a B.S. in Biochemistry (Tufts University) and a M.Sc. in Chemical Engineering (University of Rovirai Virgili, Spain).

Shana Kelley (right) is a Professor in the Departments of Biochemistry and Pharmaceutical Science at the University of Toronto. Kelley has co-authored over 50 scientific publications, and since 2000, Kelley has received a Research Corporation Innovation Award, a Dreyfus New Faculty Award, a NSF CAREER Award, Sloan Fellowship, a Dreyfus Teacher-Scholar Award, the Pittsburgh Conference Achievement Award and was named in Technology Review's *list of Top 100 innovators.*

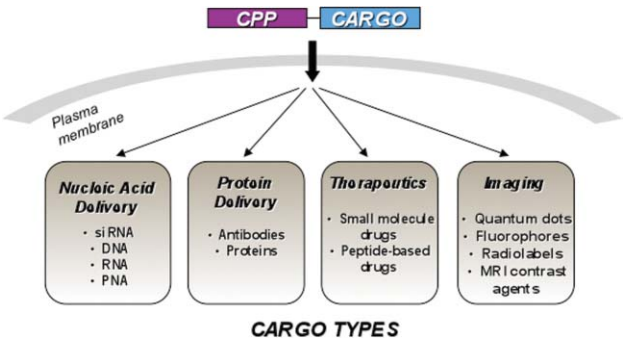

Fig. 1 Applications of cell-penetrating peptides as molecular delivery vehicles.

Harnessing the cell-penetrating properties of these peptides will have profound implications in both basic and medical research. The following review will focus on the applications of CPPs in medicine and as biological tools.

2. Cell penetrating peptides: background and mechanism of uptake

Over the last two decades, many different short peptide sequences have been identified that are able to transport diverse types of cargo molecules.[6,34] Examples of commonly used CPPs are included in Table 1. Some CPPs, like Tat and penetratin, are derived from natural sequences, while others are artificial constructs designed to capture the important features of natural systems. Some of the first examples of designed sequences included the polyarginines[35] and transportan.[36] This class of engineered molecules is rapidly expanding with the recent discoveries of new peptide sequences that are permeable to mammalian cells such as protamine,[37] maurocalcine,[38] and M918.[39] The continued expansion of this field indicates that chemical space is rich with peptide sequences that exhibit high levels of cellular uptake.

Obtaining information about general trends governing CPP uptake, such as the effects of peptide length, chemical properties, and size, has important implications for the rational engineering of CPPs. With knowledge concerning how physicochemical properties favor one mechanism of uptake over another, engineering new peptides with a desired uptake mechanism relevant to their application will be possible. Towards this end, numerous investigations have been conducted to elucidate how CPPs gain access to the interior of cells.[6,40] A variety of uptake mechanisms appear to be operative in different systems, and in some cases, the mechanism is cell-type or cargo-specific. It appears that CPPs can access the cell by two distinct routes: energy-dependent vesicular mechanisms, collectively referred to as endocytosis, or *via* a direct process involving translocation of the lipid bilayer (Fig. 2).

Table 1 Cell-penetrating peptides commonly used for delivery applications

Cell-penetrating peptide	Amino acid sequence
Polyarginines	**RRRRRRRRR** (R_9)
Tat_{49-57}	**RKKRRQRRR**
Penetratin (Antennapedia)	**R**Q**I**K**I**WFQN**RR**M**K**W**KK**
Pep-1	**K**ETWWETWWTEWSQP**KKKRK**V
Transportan	GWTLNSAGYLLG**K**INL**K**ALAALA**KK**IL
Nuclear localization sequences	VQ**RKRQK**LMP
	S**KKKKI**KV
	G**RKRKKR**T

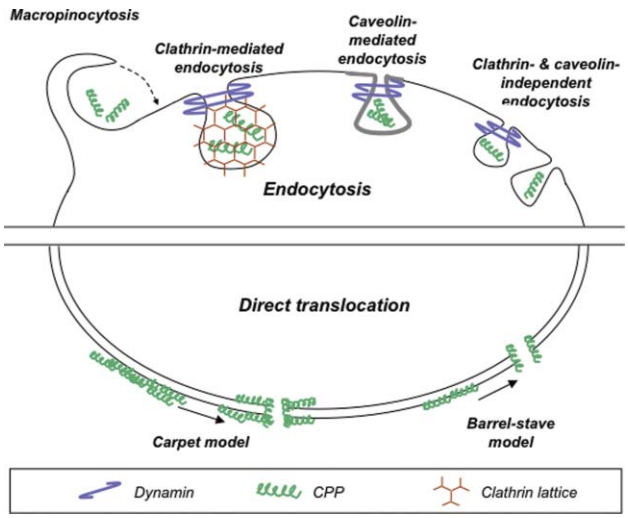

Fig. 2 Mechanisms of uptake across the plasma membrane.

Endocytosis, which includes phagocytosis and pinocytosis, is a regulated process used by the cell to internalize solutes and fluids in the extracellular matrix.[41] Reserved for specialized cells, such as macrophages and neutrophiles, phagocytosis is a complex process used to engulf large particles.[42,43] Pinocytosis occurs in all cell types and can be further classified into four mechanistically diverse pathways: macropinocytosis, clathrin-mediated, caveloae/lipid raft-mediated, and clathrin, caveolae-independent endocytosis.[41] While the exact mechanisms of each of these pinocytic processes differ with regard to vesicle structure and the machinery utilized, they all share a common outcome: extracellular molecules are encapsulated into lipid vesicles, which are internalized after resealing of the plasma membrane.[41] The fate of the solute molecules depends on their ability to escape lipid-encapsulated vesicles before they are trafficked back to the plasma membrane for recycling, or fused with lysosomes. It is important to recognize that this type of vesicular escape can limit the effectiveness of CPPs in delivery applications, as it may keep a cargo from reaching the desired intracellular site.

Results from a large number of studies suggest that the mechanism of endocytic uptake for a CPP is strongly dependent on the attached cargo.[44] For example, Tat has been shown to use lipid raft-mediated endocytosis when conjugated to a protein[45] and clathrin-dependent endocytosis[46] when conjugated to a fluorophore. Macropinocytosis has been implicated in the uptake of a variety of CPP–cargo conjugates,[39,47–49] suggesting that membrane ruffling aids the internalization of CPPs. Additionally, the electrostatic interaction of CPPs with surface proteoglycans has been shown to be responsible for the uptake of many CPPs.[48,50–54]

CPPs can also cross the membrane bilayer directly in an energy-independent process (Fig. 2).[36,55] Non-endocytic membrane permeation is thought to occur when the peptide has characteristics that are compatible with the bilayer or that sufficiently perturb the structural integrity of the membrane.[56,57] Peptides using a direct mechanism of uptake would be expected to display transport that

is sensitive to changes in membrane properties, such as fluidity and membrane potential. Biophysical models have taken concepts relevant to antimicrobial peptides to propose membrane structure perturbations that would facilitate transport, with the barrel-stave, inverted micelle, and carpet models all describing different types of transient structures that could be formed at the membrane surface and allow peptide entry.[58–61] Studies aimed at looking at the role of extracellular counterions have also lent support to proposed mechanisms involving direct uptake.[23–27]

Cellular uptake of polyarginines is sensitive to changes in membrane potential and independent of temperature, suggesting non-endocytic uptake.[35] In addition, translocation of Pep-1, penetratin, and polyarginines is driven by membrane potentials.[62,63] For these peptides, electrostatic interactions with cell surface molecules and negatively charged lipids[57,58,64,65] may initiate the penetration of the bilayer. This was observed for penetratin even in the absence of a membrane potential in model lipid vesicles where the fluorescently labeled peptide was able to translocate across a pure lipid bilayer *via* a non-pore-forming mechanism.[56] Furthermore, it is anticipated that even when endocytic pathways are relevant to uptake of CPPs, escape from the endosome by a physical mechanism is one way the peptide could reach cytoplasmic or other organelle targets.[49,62]

While debate about specific uptake mechanisms for CPPs is ongoing (for a detailed review readers are referred to ref. 6), it is now clear that the route of entry can be dependent on the identity of an attached cargo[44,66] (*e.g.* drug, fluorophore, nanoparticle, protein) and on cell type.[67] Moreover, uptake mechanism can be misassigned as a consequence of experimental artifacts, such as those resulting from cell fixation or omission of a proteolytic digestion.[68] In addition, some pharmacological agents used to probe specific mechanisms of endocytosis can interfere with multiple endocytic pathways as well as other cellular properties.[49,69] An added difficulty in the assignment of uptake mechanisms comes from the fact that a single CPP can simultaneously exploit multiple modes of uptake to enter cells.[40,44,70] Recently, this issue was explored in depth for cationic CPPs; in these detailed studies it was found that penetratin equally exploits macropinocytosis, clathrin-mediated endocytosis, and caveolae/lipid-raft-mediated endocytosis to enter HeLa cells.[40] Clearly, claims concerning dominant uptake mechanisms for CPPs must be made with great care.

3. Cell penetrating peptides: applications in drug delivery

Revolutionary advances in genomics and proteomics technology have led to the identification of molecular targets for treatment of different disease states.[71] The development of a successful drug requires access to a target of interest; therefore, for intracellular targets to be addressed, drug molecules must be cell-permeable. Traversing the plasma membrane is a challenge to the delivery of some therapeutics, with many drugs exhibiting desirable activities *in vitro* not displaying the requisite amount of lipophilicity allowing for membrane partitioning but simultaneously supporting aqueous solubility.[72] These incongruous requirements have limited the use of certain compounds and therefore, optimizing cellular delivery of therapeutics is an important priority. CPPs have been proven effective at increasing the efficacy of several therapeutics by improving cellular uptake, and the use of CPPs as molecular vehicles offers several advantages over other delivery vectors, including lower toxicity and more controlled administration.[73–75] Many studies have demonstrated successful CPP-facilitated intracellular transport of therapeutics, ranging from small molecules to large proteins or nucleic acids, demonstrating that CPP-mediated delivery of therapeutics is a promising approach.

4. Cellular delivery of proteins by CPPs

Administering exogenous proteins presents a valuable treatment for many disease states, but delivering these large macromolecules into cells is a challenging objective. Since the discovery was made that Tat was able to transport various proteins across cell membranes,[76] CPPs have been shown to be effective for delivering proteins ranging in size from 30 kDa (*e.g.* GFP) to 120–150 kDa (*e.g.* IgG) (Fig. 3). Administration of the 120 kDa β-galactosidase protein fused to Tat in mice resulted in the efficient penetration of all tissues, even crossing the blood–brain barrier; importantly, biological activity was maintained.[77] *In vivo* delivery of Fab fragments crosslinked with Tat, penetratin and other CPPs was shown to yield varied organ distributions and an overall increase in organ retention, suggesting that the peptide can play a role in tissue localization.[78]

Noncovalently-associated complexes of CPPs and proteins have proven effective for delivery. For example, the short amphipathic peptide, Pep-1, was shown to facilitate rapid and highly efficient cellular uptake of various peptides, proteins and even full-length antibodies.[79] This approach has the significant advantage of not requiring any chemical modification of the transporter or protein cargo, greatly simplifying the formulation of reagents.

CPP-mediated protein delivery has been used successfully to administer a variety of therapeutically relevant proteins, and has significant potential for cancer and stroke treatment.[64–70] A disulfide conjugate of Tat and an anti-tetanus Fab fragment was used to reverse nerve cell damage caused by tetanus toxin.[80] To treat ischemic brain damage, Tat has been fused to proteins that bind the postsynaptic density protein, PSD-95; the conjugate was effective in preventing excitotoxicity.[81] Tat conjugates with FNK, a stabilized variant of Bcl-x_L lacking the phosphorylation site, have been prepared to prevent cell death in the brains of mice.[82] The successful delivery of these proteins in an active form is a remarkable achievement, and has shown that CPP-mediated protein delivery is a generally-applicable tool.

CPP-mediated delivery of antibodies—large proteins that are notoriously difficult to deliver into cells—has also been achieved.[9,83-86] Tat has been used to deliver antibodies for radiotherapeutic applications (*e.g.*^{111}In-labeled anti-mouse IgG)[84] and for sensitizing cancer cells to cytotoxic therapies (anti-p21).[85] As well, penetratin has facilitated antibody delivery *in vivo* where uptake and retention in solid tumors of mice was improved.[86] The fact that CPPs have enabled antibody delivery highlights how broadly applicable these transporters are.

5. Cellular delivery of nucleic acids by CPPs

Cellular delivery of nucleic acids is another exceptionally challenging, but important, objective for the development of new

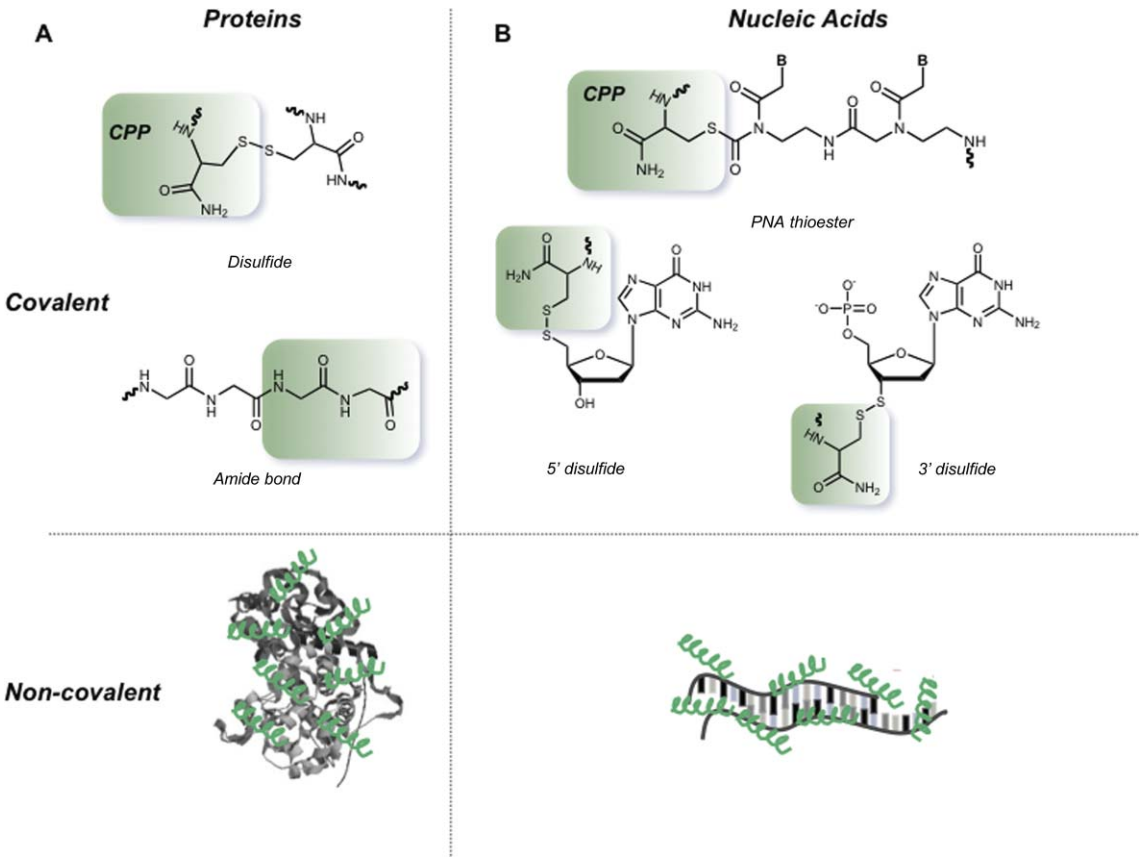

Fig. 3 Linkages of CPPs to nucleic acid and protein cargos.

therapeutics.[87] Currently, the delivery of these highly negatively charged biomolecules is typically achieved with methods that can be plagued by cellular toxicity or poor efficiency in certain cell types (*e.g.* lipofectamine or microinjection). CPPs offer qualities that could be beneficial for a non-viral gene delivery system, including the ability to easily functionalize the peptide structure (Fig. 3). While CPP–nucleic acid complexes can be trapped in endosomes, CPPs can be modified to promote endosomal escape, preventing degradation and allowing the nucleic acid to reach nuclear targets.[88–90] Additionally, peptide nucleic acids, which are highly stable, uncharged, protease- and nuclease-resistant oligonucleotide mimics, have been efficiently delivered by conjugation to various CPPs.[90–92]

RNAi technology has also been improved with CPP-aided delivery of siRNA.[93,94] The crosslinking of Tat to siRNA increased cellular uptake of the oligonucleotide without interfering with the perinuclear localization required for RNAi activity.[95] The Tat–siRNA complex silenced gene expression to a similar extent as one made with lipofectamine. Penetratin and transportan featuring terminal cysteines have been covalently attached to a 5′-thiol modified siRNA and produced expression knockdown for up to seven days.[96] As an alternative to covalent attachments, both a polyarginine CPP and a derivative of MPG (a fusion peptide between a hydrophobic segment of HIV-1 gp41 and nuclear localization sequence SV-40) were used for cellular delivery of exogenous siRNA as a non-covalent complex.[73,97] Even though many examples of successful CPP-mediated internalization of oligonucleotides exist, several studies with CPP–siRNAs revealed that Tat and penetratin alone affected gene expression and that penetratin–siRNA elicited an immunological response, challenging the use of these CPPs in delivery systems.[98,99] While these studies emphasize the need for caution and careful controls, the body of work in this area suggests that CPP-based delivery systems for oligonucleotides have great promise.

6. Cellular delivery of small-molecule drugs by CPPs

The efficacy of several small-molecule drugs has also been improved using CPPs as delivery vehicles (Fig. 4).[11,100,101] Polyarginine conjugates of the hydrophobic drug Paclitaxel were shown to greatly improve the water solubility and cellular uptake of this potent therapeutic.[11,102] CPP conjugates of cyclosporine A were shown to exhibit qualities beneficial for treatment of psoriasis and other skin conditions.[100] When topically administered, the polyarginine aids penetration to cells in the underlying tissue, delivering a therapeutically useful amount of the anti-inflammatory molecule by facilitating passage through the stratum corneum. CPPs have delivered photoactive drugs, *e.g.* the pro-drug 5-aminolevulinic acid, which is converted in the heme biosynthetic pathway to the photosensitizer, protoporphyrin IX; the delivery of this agent was made possible by coupling to penetratin.[103] CPPs can therefore be used to ameliorate properties of drugs—like extreme lipophilicity or poor trafficking—that limit their usefulness.

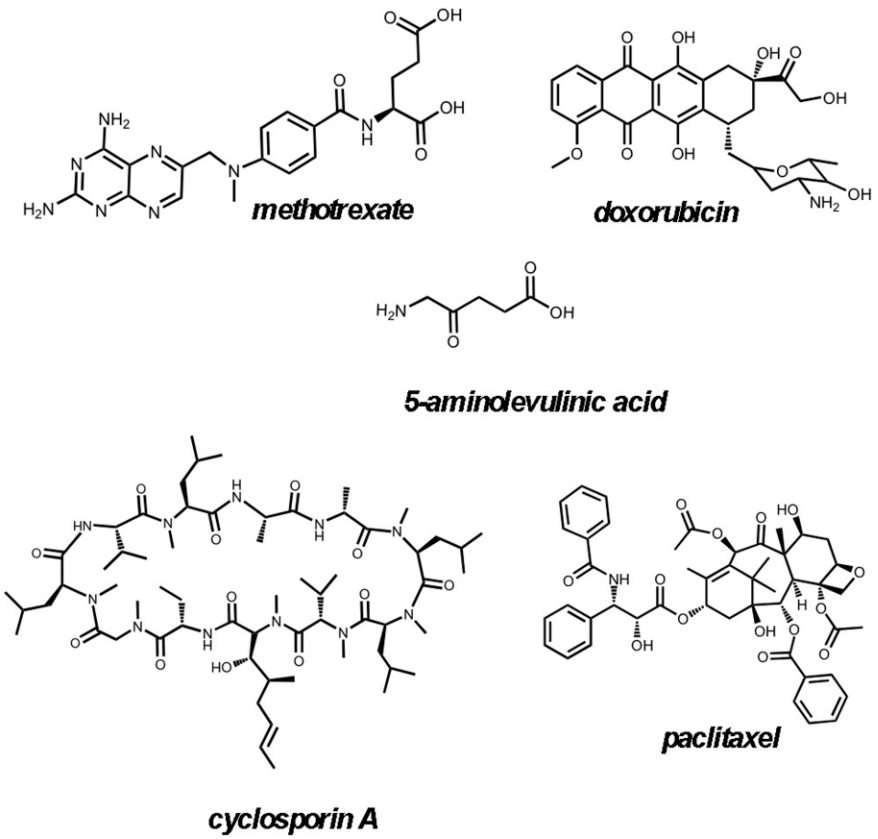

Fig. 4 Small molecule therapeutics successfully delivered by CPPs.

CPPs may also be useful for overcoming cancer-resistance in cells by diverting drugs away from efflux pathways. For example, doxorubicin conjugated to Tat[104] and transferrin[105,106] have been successful in exerting cytotoxic effects on doxorubicin-resistant cell lines. CPPs have enhanced uptake of methotrexate in cell lines resistant to this chemotherapeutic, further illustrating the therapeutic benefit of CPP-conjugated drugs.[101] While these drugs are inherently cell permeable, CPP-mediated delivery circumvents the effects of the efflux pumps and provides an effective means to override resistance.

7. Enhancement of activity for peptide-based drugs using appended CPPs

Peptide-based drugs represent another class of therapeutics that exhibit improved activity when CPPs are used for delivery (Fig. 5 and Table 2). For this class of agents, the shared structure of the drug and carrier simplifies synthesis, and thus this application of CPPs is particularly straightforward.

Anti-microbial peptides (AMPs), engineered by microbes to penetrate the cellular boundaries of other organisms, have been manipulated to exert their activity on human cancer cells.[107–113] Specificity for cancer cells, however, can be difficult to achieve with these systems.[109,110] The challenge in using these peptides as cancer-killing agents lies in directing their membrane-disrupting properties to diseased cells, leaving normal cells unperturbed. One very promising engineered sequence, $(KLAKLAK)_2$, has received a great deal of attention as a potential AMP-derived therapeutic.[114,115] Although the $(KLAKLAK)_2$ peptide does not perturb the plasma membrane, it disrupts mitochondria, inducing apoptosis when delivered into cells.[116] However, the low activity of the unmodified $(KLAKLAK)_2$ peptide towards cancer cells illustrates that the peptide cannot achieve therapeutically relevant intracellular concentrations alone.[116] Strategies employing CPPs to improve uptake of the AMP have achieved increased potency.[117,118] A $(KLAKLAK)_2$-polyarginine construct demonstrated improved IC_{50} values in comparison with doxorubicin, paclitaxel, methotrexate, cisplatin, and cyclophosphamide.[117] The activity of another mitochondria-disrupting peptide, Vpr, was also improved by conjugation to the CPP Tat.[119] Indeed, fusing domains that exert biochemical activity with CPPs leads to improved drugs, and the fact that these drugs can be generated completely *via* peptide synthesis makes their preparation facile.

Peptide fragments retaining the activity of full-length pro-apoptotic proteins are under development as anti-cancer agents.[120–125] These constructs have also been improved *via* conjugation to CPPs. Two examples of these are the BH3-helix from the Bcl-2 family and the N-terminal domain of the pro-apoptotic factor SMAC.[122–125] The BH3-only peptides have been investigated as therapeutics for cancer treatment, but are unable to cross the plasma membrane at levels that are therapeutically useful, prompting the development of strategies to improve intracellular accumulation.[120–122] Linkage of BH3-only therapeutic peptides

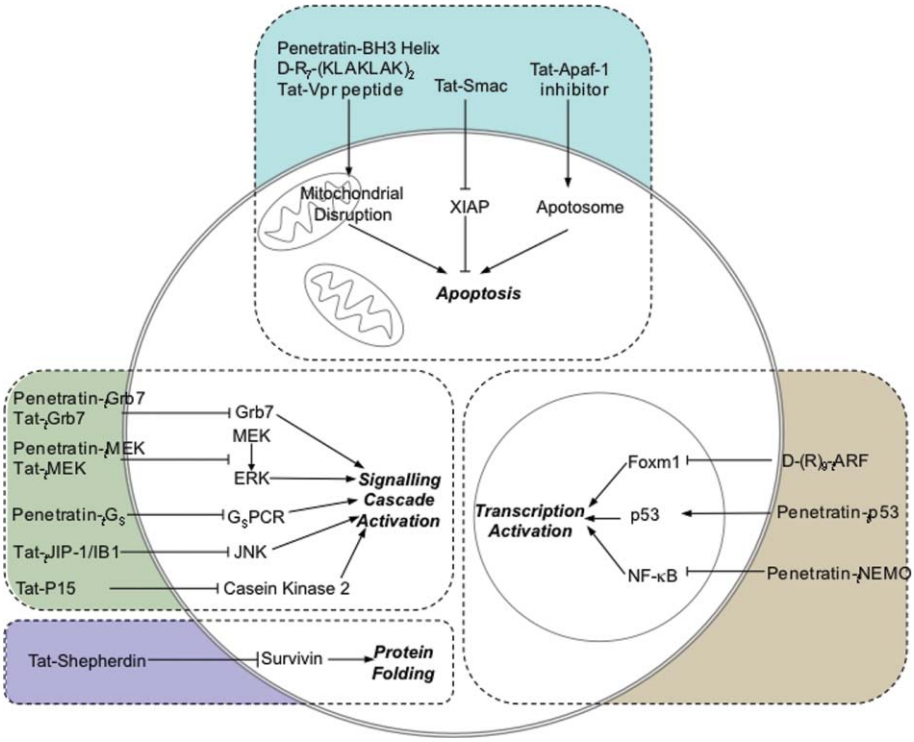

Fig. 5 Cellular targets of CPP-anticancer peptides. The subscript *t* preceding the peptide name denotes a truncated peptide of the full length protein: *ex.,* tNEMO.

to CPPs has been shown to improve their activity.[123,124] The antennapedia transduction domain fused to BH3 domains from Bak, Bax, and Bcl-2 was used with success, even in the presence of over-expressed anti-apoptotic Bcl-2 proteins Bcl-2 and Bcl-XL.[123,124] Thus, CPPs can be used to increase the activities of apoptotic peptides by facilitating cellular uptake.

Another mitochondrially-targeted apoptotic factor that is of interest as an anticancer agent, Smac/DIABLO, has been studied as a CPP fusion.[125] A seven amino acid sequence from the Smac N-terminus was linked to the Tat peptide, and potentiated apoptosis induced by various stimuli; in concert with TRAIL, this agent caused complete regression of an intracranial malignant glioma xenograft model in mice, in comparison to only partial growth inhibition by TRAIL or the Smac peptide alone.[125]

Peptide-based inhibitors are useful for blocking protein–protein interactions that are too large for small molecule interference, but often, sequences that produce significant disruption of a biomolecular interface *in vitro* do not exhibit efficient intracellular accumulation.[126] Delivery by CPPs has also been useful for this type of therapeutic. For example, penetratin and Tat improved uptake and activity of a peptoid inhibitor of the apotosome.[127] Another useful peptide inhibitor, shepherdin, blocks the interaction of a molecular chaperone, Hsp90, with the anti-apoptotic regulator survivin.[128] Shepherdin linked to Tat or antennapedia inhibited tumor cell growth in *in vitro* and *in vivo* models.[128] CPPs were also used to stimulate the uptake of a polyglutamine-binding peptide that inhibits aggregation of misfolded proteins responsible for neurodegenerative disorders.[129] Here again, by improving cellular delivery of these agents, CPPs are able to improve the inhibitory properties of agents with poor intrinsic cellular permeability.

Transcription factors are popular targets for anticancer agents, since these control downstream response to growth factors, apoptotic stimuli, and other cellular signals.[130] A peptide fragment of the tumor suppressor transcription factor p53 is known to stimulate the activity of wild type p53 and some mutant p53 cell lines.[131] Fused to the antennapedia transduction domain, the peptide markedly inhibited growth and colony formation in a mutant p53 cancer cell line,[131] and the peptide construct was used successfully *in vivo* to induce apoptosis through the Fas-FADD pathway.[132,133] Another transcription factor-targeting that has shown promising activity once conjugated to a CPP is one based on the natural inhibitor ARF (alternative reading frame protein); ARF targets Foxm1 (forkhead box m1), a transcription factor important to the development of hepatocellular carcinoma in humans.[134] The cell-permeable ARF peptide attached to nona-arginine reduced tumor size and number in a mouse model.[134] Another example of this type of approach involved inhibition of NEMO, a key regulator of NF-kB activation; a fusion of a peptide inhibitor with antennapedia was generated and inhibition was observed in cultured cells.[135] Inhibition of Stat3, a transcription factor found to be constitutively activated in some cancer cell lines, was achieved with a polyarginine transduction domain linked to a peptide aptamer inhibitor.[136] Modulating the levels of transcription allows control of cancer-related processes, and facilitating the delivery of agents interacting with transcription factors appears to be a powerful means of achieving such control.

Peptide-based inhibitors of signaling proteins have also been improved when prepared as CPP fusions.[81,137–144] Inhibition of casein kinase 2 by the peptide inhibitor P15 attached to Tat was found to induce apoptosis in a number of cancer cell lines and slowed the growth of TC-1 tumors *in vivo.*[142] In addition, a peptide

Table 2 Peptide drugs delivered by CPPs

Peptide drug	Target	CPP carrier	Model	IC50/μM	Ref
Apoptosis targeting					
$(klaklak)_2$	Mitochondrial disruption	D-(R)7, PTD-5	HT-1080, HCT-116, HT-29, MDA-MB-468, HeLa, PC-3, MIA PaCa-2, LL/2, MCA205, clinical head and neck tumor isolates	3–40	[99]
			HT-1080 & MCA205 xenografts		[100]
Smac-derived peptide	XIAP inhibition	Tat	SHEP, SH-SY5Y, U87MG, LN-18, LN-229, Panc-1, MCF-7, SV- FHAS, SMA560, human fibroblasts, human mammary epithelial cells, PBL, Schwann cells, primary neuroblastoma tumor cells, Human glioma xenograft	—	[102]
BH3 helix	Inhibition of pro-apoptotic Bcl-2 family members	Penetratin	HeLa, PBL HL-60 overexpressing Bcl-2	15–50	[103]
			BJAB, & Jurkat transfected with Bcl-2		[104]
HIV Vpr peptide	Mitochondrial disruption	Tat	HUVEC	15	[101]
Apaf-1 peptoid inhibitor	Activation of apotosome	Penetratin, Tat	U937, Saos-2	~ 1	[109]
Transcription targeting					
p53-derived peptide	Activation of p53-mediated transcription	Penetratin	Saos-2 with wt & mutant p53, HL-60, HeLa, SW480, EW36 & BL41	—	[112–114]
ARF-derived peptide	Inhibition of Foxm1-activated transcription	D-(R)9	Mouse model with induced liver tumors, U2OS clone C3, HepG2, PLC/PRF/5, Hep3B, HMEC-1	—	[77]
NEMO-derived peptides	NF-κB activation	Penetratin	Y79, transfected 70Z/3 & Jurkat	5–20	[115]
Signal cascade targeting					
P15 peptide	Casein kinase 2 activation	Tat	TC-1 HPV16-transformed cells, H-125 H-82, SiHa, CaSki, HeLa, & Jurkat	20–186	[117]
G7-18NATE (Grb7-derived peptide)	Grb7 activity	Penetratin, Tat	SK-BR-3, MDA-MB-231, MDA-MB-361, ZR-75–30, MCF-7, MC-10A, 3T3	~ 10	[120]
$G\alpha_s$-derived peptide	GPCR receptor	Penetratin	PC12, HMEC-1	5	[119]
JIP-1/IB1-derived peptide	JNK activity	Tat	Insulin-producing βTC-3 cells	—	[121]
MEK1-derived peptide	Inhibition of ERK activation	Penetratin, Tat	NIH 3T3, PC12, HeLa	0.2–1.5	[118]
Protein folding targeting					
Shepherdin	Survivin activity	Penetratin, Tat	HFF, HGF, WS-1, PC3, DU145, MCF-7, Raji, H-460, HeLa, MDA-MB-435LCC6, HCT116, JC, mouse embryonic fibroblasts, PMC, PC3, & MCF-7 mouse xenograft tumor models	~ 100	[110]

HT-1080 fibrosarcoma; *HCT-116 & HT-29* colorectal carcinoma; *MDA-MB-468 & MCF-7* breast carcinoma; *PC-3* prostate carcinoma; *MIA PaCa-2 & Panc-1* pancreatic adenocarcinoma; *LL/2* Lewis lung carcinoma, *MCA205* murine fibrosarcoma; *SHEP & SH-SY5Y* neuroblastoma; *U87MG, LN-18, & LN-229* malignant glioma; *SV-FHAS* astrocytes; *SMA560* murine glioma; *PBL* peripheral blood lymphocytes; *HL-60* myeloid leukemia; *BJAB*; *Jurkat* T cell leukemia; *HUVEC* human umbilical vein endothelial cells; *U937* histocytic lymphoma; *Saos-2 & U2OS* clone C3 osteosarcoma; *SW480* colon carcinoma; *Ew36 & BL41* Burkitt lymphoma; *HepG2, PLC/PRF/5, & Hep3B* hepatoma; *HMEC-1*; *Y79* human retinoblastoma; *TC-1* mouse lung HPV 16-transformed cells; *H-125 & H-460* non-small cell lung cancer; *H-82* small-cell lung cancer; *SiHa, CaSki, & HeLa* cervical carcinomas; *SK-BR-3, MDAMB- 231, MDA-MB-361, & ZR-75–30* breast cancer cell lines; *MC-10A* non-malignant human mammary epithelial; *3T3* mouse fibroblast; *PC12*; *HMEC-1* human microvascular endothelial cells; *βTC-3*; *HFF* foreskin fibroblasts; *HGF* gingival fibroblasts; *WS-1* skin fibroblasts; *PC3 & DU145* prostate adenocarcinoma; *Raji* B lymphoma; *MDA-MB-435LCC6* melanoma; *JC* mammary carcinoma; *PMC* peripheral blood mononuclear cells.

inhibitor designed to block the interaction of ERK (extracellular signal-regulated kinase) and MEK (MAPK/ERK kinase), two members of a MAPK (mitogen-activated protein kinase) signaling pathway involved in growth and proliferation, exhibited increased potency when bound to Tat or antennapedia.[141] Chimeric peptides comprising a peptide transduction domain linked to an inhibitor domain were shown to disrupt signaling mediated by NMDAR (*N*-methyl-D-aspartate receptor),[81] GPCR (G-protein coupled receptor),[140] receptor tyrosine kinase,[139] JNK (c-Jun N-terminal kinase),[138] and protein kinase C.[137] Fusion peptides have also been developed that contain two additional functional domains in addition to a transduction domain.[145] Two examples of these combined Tat with a targeting ligand for the CXC chemokine receptor 4 as well as an anticancer peptide; a p53-activating peptide and a cyclin-dependent kinase 2 antagonist peptide were both successfully delivered to tumor cells in this way.[145] In addition, delivery of a δPKC-specific inhibitor with Tat is currently being investigated as a potent therapeutic agent for patients with acute myocardial infarction.[143,144]

While significant advances in CPP-mediated drug delivery have appreciably improved delivery of therapeutics, further optimization of peptide carrier systems is critical. In addition, CPP

pharmacology and toxicity needs to be extensively examined *in vivo*. The effort put forth on these fronts will be extremely beneficial, as finding a potentially universal peptide carrier for each class of therapeutic molecules (nucleic acids, small molecules, peptides, and proteins) will facilitate the development of potential clinically-relevant delivery systems.

8. CPP-mediated delivery of imaging agents

The ability to visualize internal features and physiological structures of living organisms and observe cellular functions *in vivo* is vital for understanding, diagnosing, and treating disease. For example, directly visualizing diseased tissue during surgical procedures and identifying pre-disease states in patients will have profound implications in medicine. In biomedical research, assessing stem cell differentiation or following the dynamics of the immune cells in living animals will provide a fundamental understanding of these biological processes. Non-invasive biomedical imaging techniques such as fluorescence imaging and magnetic resonance imaging (MRI) have been developed for these types of *in vivo* applications. As some useful imaging agents are not able to penetrate cells or tissues, CPPs have proven useful in this field as delivery vehicles (Fig. 6).

Fluorescence imaging relies on tracking molecular imaging agents and has been shown to be useful for visualizing tissues *in vivo*.[146,147] However, poor cellular uptake, inadequate targeting, and the lack of photostability of fluorophores can be problematic. CPPs have been combined with imaging labels in an effort to optimize aspects of *in vivo* fluorescence imaging related to cellular targeting.

Various fluorophore–CPP conjugates with improved stability and uptake have been developed as imaging agents.[148,149] For example, fluorescein-doped monodisperse silica particles of approximately 70 nm in diameter were modified with Tat peptides for cellular delivery and shown to efficiently cross the blood–brain barrier, labeling the neuronal tissue of rats *in vivo*.[148] Another very elegant example of the application of CPPs for imaging was recently reported by Tsien and coworkers (Fig. 7).[149] A fluorescein-peptide hairpin was designed that took advantage of the increased number of extracellular proteases surrounding tumor tissues. The construct consisted of a polyarginine peptide covalently attached to a polyanionic segment, which would only be internalized upon proteolytic cleavage of the anionic domain and—because the protease targeted is overexpressed on cancerous cells—selectively label tumors.

The development of semiconductor nanocrystals, or quantum dots (QDs), has presented a robust alternative to molecular fluorophores that may eventually dominate imaging applications. A QD's size and composition results in quantum confinement of electrons, and as a consequence, these materials exhibit many advantageous optical characteristics.[150] Along with a strong luminescence, QDs are characterized by a resistance to photobleaching, remarkably long luminescent lifetimes, broad adsorption and narrow emission profiles.[151] Even with these ideal properties, QDs are not always stable in a biological environment and have little cellular uptake, presenting a hurdle for use in *in vivo* imaging.

The functionalization of QDs with CPPs has been shown to improve biocompatibility. For example, Tat-modified CdS:Mn/ZnS quantum dots were delivered intra-arterially into rats and were able to efficiently label brain tissue within minutes.[152] The Tat-QDs not only crossed the blood–brain barrier, but were visualized with a hand-held lamp, further demonstrating the potential of this type of imaging agent for the visualization of diseased tissues in surgical procedures. Other CPPs such as Pep-1,[153] and polyarginines[154,155] have also been used for the cellular delivery of QDs. While non-covalent delivery of QDs has been achieved, this is specific to

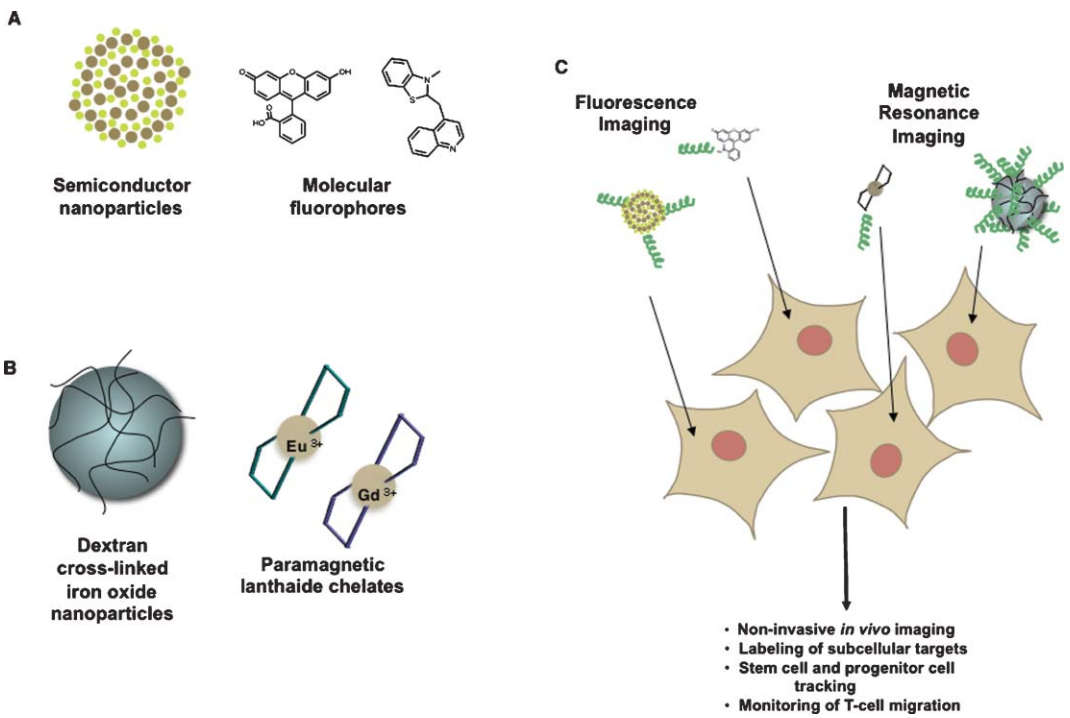

Fig. 6 CPP-mediated delivery of imaging agents.

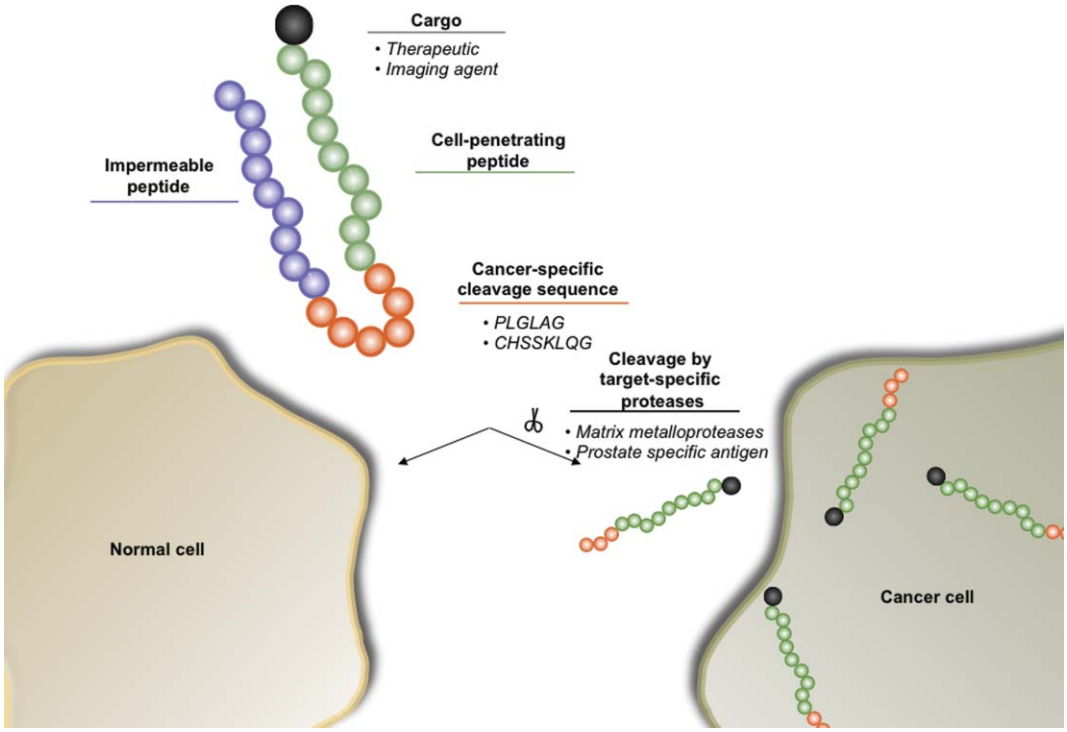

Fig. 7 Engineering cancer specificity into CPP-delivered therapeutics using protease-triggered cellular uptake.

Pep-1;[153] all other CPPs appear to require chemical conjugation to facilitate cellular uptake.

Attachment of CPPs to QDs has been carried out in multiple ways: *e.g.* with covalent bonding between a cysteine and lysine residues,[152] using biotin-conjugated peptides to streptavidin-coated nanoparticles,[154,155] or *via* a polyhistidine peptide linker exhibiting affinity for the metals contained within nanocrystals.[156] In addition, tioprion, a molecule with both a terminal thiol and carboxylic acid, has been used to functionalize QDs with Tat *via* a two-step process.[157]

CPPs have additionally been exploited for use in the delivery of MRI contrast agents.[32,158,159] MRI avoids artifacts caused by light scattering by relying on magnetic spin coupling of protons in water molecules, which are enhanced with the administration of magnetic contrast agents. However, a major barrier associated with this imaging technique is the poor cell permeability and non-specific localization of many agents. Consequently, extracellular labeling[160] or microinjection are commonly employed.[161] Using CPPs to enhance the cellular delivery of contrast agents in MRI represents an alternative solution with many advantages.

Superparamagnetic iron oxide nanoparticles are sensitive and biologically compatible contrast agents for MRI.[162] Weissleder and coworkers have reported that 40 nm dextran cross-linked iron oxide nanoparticles could be functionalized with CPPs to increase levels of cellular internalization.[163] Superparamagnetic nanoparticles with >10 attached Tat sequences exhibited a 100-fold increase in cellular uptake[31] and were retained in HeLa cells for up to 7 days.[164] Tat-iron oxide nanoparticles have shown significant promise for *in vivo* imaging as these agents exhibit good tissue penetration.[165] In addition, they allowed for *in vivo* tracking of stem cells[32] and T-cell migration,[166] two applications that necessitate real-time monitoring. Importantly, CPP-modified contrast agents did not interfere with biological function; stem cells loaded with the nanoparticles exhibited normal differentiation[32] and T-cells demonstrated normal activation for immune response.[166]

Paramagnetic cations such as gandolinium (Gd^{3+}) can be used as MRI contrast agents. By conjugating CPPs to the ion chelators, lanthanide contrast agents can be delivered into cells.[167,168] The macrocyclic chelators 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA) and diethylenetriaminepentaacetic acid anhydride (DTPA) have been covalently linked with Tat and polyargine peptides to aid with plasma membrane permeability.[158,159,167–169] Alternatively, Polyakov *et al.* used a peptide-based metal chelator (ε-KGC) attached to a Tat peptide for the intracellular delivery of diagnostic metal complexes.[170]

CPP-derivatized contrast agents have been further engineered to gain cellular specificity. To obtain access to the nuclear compartment, a homologue of penetratin was linked to the nuclear localization sequences of SV40T-antigen, which rapidly entered cells after 10 minutes, achieved nuclear localization, and exhibited a stable imaging signal for as long as 48 hours.[171] To achieve tissue specificity, an imaging construct was designed that consisted of a Gd^{3+} complexed with terminal lysines of a PNA sequence which was linked to a penetratin analogue *via* a disulfide bond.[172] The PNA sequence was complementary to the oncogene *c-myc* and upon binding to the target mRNA, trapped the lanthanide ion inside the cell. While the CPP portion was responsible for cell permeability, the PNA allowed for tumor specificity both in cancer cell lines and in rat adenocarcinoma. This design was improved by using a more stable metal complex and developing a continuous

solid-phase synthesis scheme for each portion of the contrast agent (*i.e.* CPP, PNA, chelating agent).[173] In addition to tissue labeling, synthetic contrast agents featuring a PNA sequence have potential for use in cellular and whole-body gene expression imaging.

9. Organelle-specific delivery with CPPs

In addition to facilitating transport across the plasma membrane, applications of CPPs where other cellular barriers are crossed can be envisioned. The ability to target specific organelles creates opportunities to study biological processes at the subcellular level and to deliver therapeutics to targets within cellular compartments. Currently, the nucleus and the mitochondria have been successfully targeted with CPPs. As the storehouse of genomic DNA, the nucleus is a desirable target and the necessary destination for agents used in gene therapy.[174] The mitochondrion is an especially interesting organelle for drug therapy given its role in the pathology of cancer, neurodegenerative diseases, and others where reactive oxygen species are linked with disease progression.[175–177]

One effective strategy for organelle-specific targeting is the use of signal peptides, used by cellular machinery to identify newly translated peptides and traffic them to the correct destination in the cell.[178,179] Nuclear localization sequences (NLS), highly cationic peptides approximately 10 amino acids in length, exhibit high levels of cell-permeability.[174,180,181] NLS peptides, most notably the NLS from simian virus 40 (SV40) large T antigen, have been used in a number of studies to drive uptake of DNA for nonviral gene therapy.[174] Most studies have focused on the ability of the NLS sequences to drive localization of the DNA into the nucleus, aiding uptake efficiency with transfection agents or microinjection,[182–186] but there are several examples of unaided uptake of the DNA–peptide complexes into the cell, with the cell-penetrating properties of the NLS peptides driving translocation.[187–190] Although all of the studies showed an improvement in nuclear localization, a smaller number demonstrated improved transfection efficiency.[182–186] In some studies, the DNA was encapsulated in polymer nanospheres[191] or phage particles with NLS peptide displayed on the exterior.[192,193] Antisense oligonucleotides were also delivered successfully through NLS derivatization, blocking translation of Bcl-2 and PKC-α in two cancer cell lines.[194] The NLS peptides have been demonstrated to guide uptake and nuclear localization of other species, including gold nanoparticles,[195] carboplatin-based anti-cancer therapeutics,[196] and green fluorescent protein (GFP).[197]

Several examples of mitochondrial targeting using CPPs exist where artificial, rather than natural signal sequences, were used.[198–200] Naturally occurring mitochondrial signaling sequences can be quite long, and often require the presence of a full-length protein for mitochondrial import. As an alternative approach, Szeto and coworkers used tetrapeptide sequences to localize antioxidants to mitochondria.[198] The sequences included the unnatural amino acid dimethyltyrosine (dmt), which has radical scavenging properties. Upon induction of oxidative stress by *tert*-butylhydroperoxide, cells treated with the antioxidant peptides had decreased levels of mitochondrial reactive oxygen species and halted the progression of apoptosis.[198] No information was collected on these compounds, however, to document their cellular permeability or to identify critical functional groups that are responsible for mitochondria localization.

Recently, we developed a class of mitochondria-penetrating peptides (MPPs) with a number of unique and useful features.[199] These sequences, containing both cationic and lipophilic residues, were designed and engineered to display levels of cellular uptake that rival conventional CPPs, but with strong mitochondrial localization not attainable with other sequences. Moreover, the exact physiochemical properties leading to the organellar specificity of these mitochondria-penetrating peptides were identified, providing an important conceptual framework for understanding how mitochondrial localization can be achieved with synthetic compounds (Fig. 8). By studying a panel of MPPs with different charges and lipophilicities, we were able to observe that both of these molecular-level properties strongly impact mitochondrial specificity. Critical lipophilicity thresholds were identified that control organellar localization, and it was discovered that these thresholds are strongly dependent on molecular charge. The MPPs represent a promising new class of CPPs that will enable mitochondria-specific delivery of cargo.

Our laboratories have used organelle-specific CPPs to trigger oxidative stress selectively in different subcellular compartments, allowing the oxidative stress response induced by stress originating at different subcellular sites to be monitored.[200] The sequence F-r-F-K was shown to localize specifically to the mitochondria of HeLa and MRC-5 cells, while the sequence R-r-R-K was shown to localize to the nuclei. This difference in subcellular sequestration was used to study the cellular response to site-specific oxidative stress (Fig. 9).[200] To mediate the production of ROS, the peptides were linked to the singlet oxygen-sensitizer thiazole orange (to). The response of HeLa cells to the differential subcellular oxidative stress was characterized by monitoring cytotoxicity, apoptosis levels, gene expression, and survival signal transduction pathway activation. Interestingly, it was found that increased levels of apoptosis were observed when ROS were produced in the nucleus *versus* the mitochondria. Additionally, it was found that different survival pathways were activated by oxidative stress in these organelles, with the PKC pathway activated by to-R-r-R-K induced damage, the ERK signaling pathway by to-F-r-F-K, and the PI3K pathway by both agents. Gene expression profiling also revealed differences in the cellular response to mitochondrial and nuclear oxidative stress, with upregulation of a number of growth factors observed specifically when oxidative stress originated in mitochondria. These studies demonstrated that oxidative stress in the mitochondria elicits a different cellular response than when oxidative stress is localized in the nucleus.

Summary and outlook

The multiple studies described here highlight the numerous applications of CPPs as powerful delivery agents. Cellular delivery of many different types of cargos has been improved *via* conjugation to CPPs, improving the performance of agents useful for imaging and as therapeutics. A survey of the many examples where CPPs have been applied to systems where enhanced cellular uptake was desired indicates that CPPs represent a general solution and powerful tool for the development cell-permeable agents.

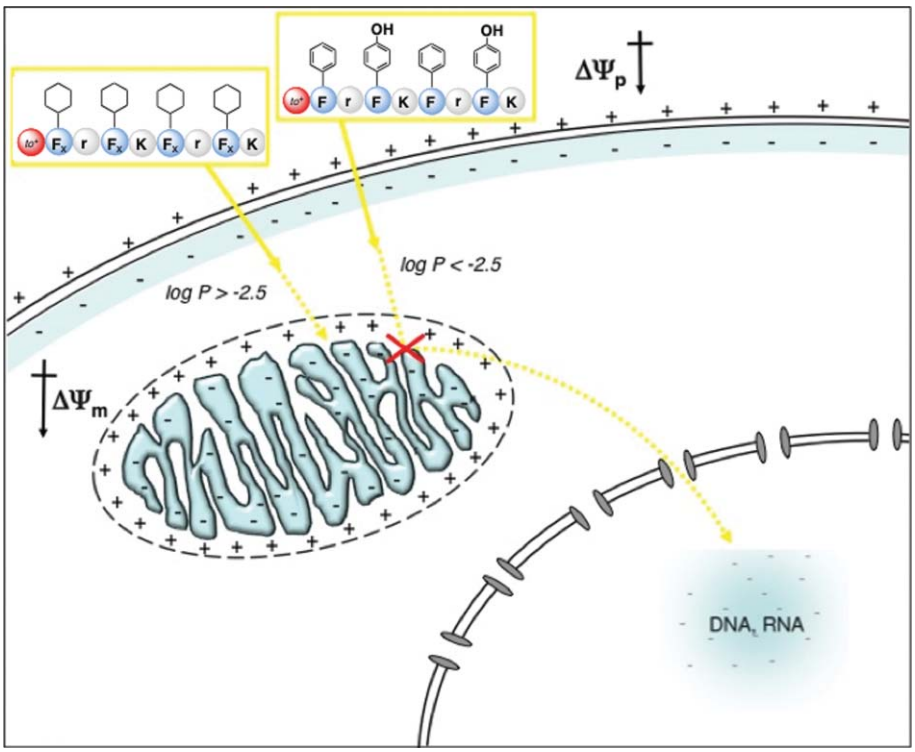

Fig. 8 Mitochondrial targeting of CPP-like sequences.[199] Modulation of the lipophilicity of synthetic cell-penetrating peptides controls intracellular localization. Synthetic octamers of the general sequence XrXKXrXK (X = phenylalanine, cyclohexylalanine (F_X), or tyrosine) localized to the mitochondria above a lipophilicity threshold (log*P* −2.5) or to the nucleus and cytosol below this lipophilicity threshold.

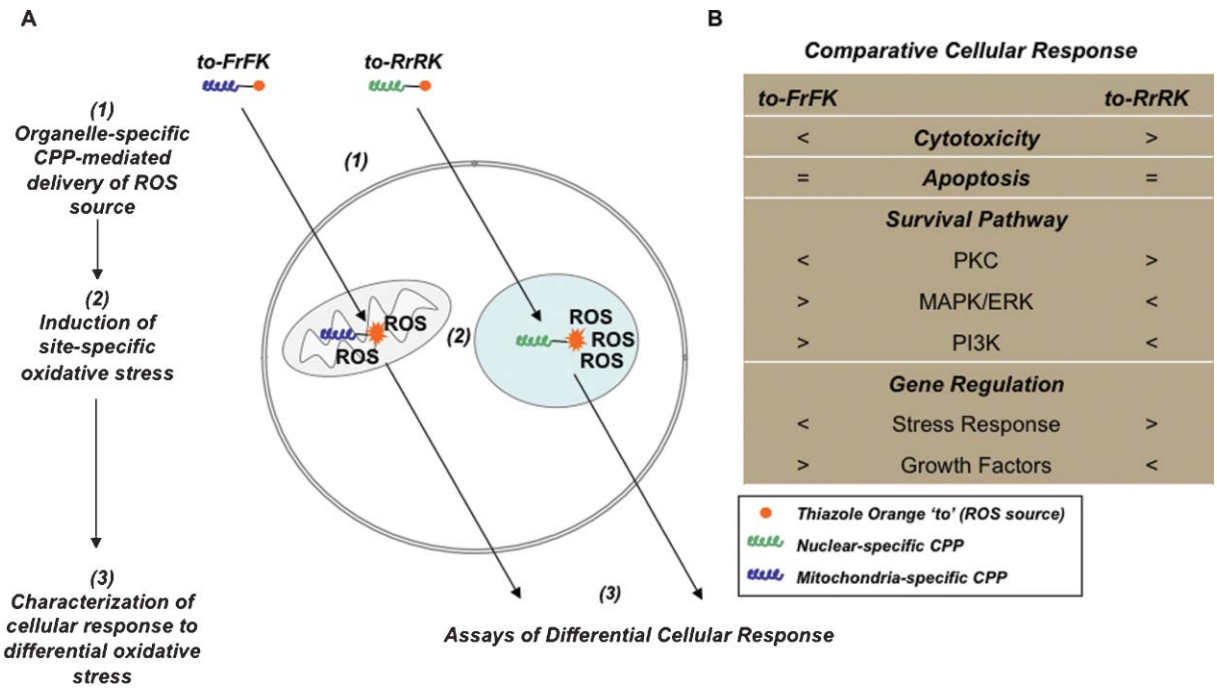

Fig. 9 Delivery of organelle-specific oxidants.[200] **A**. CPP-mediated mitochondria- or nucleus-specific delivery of the ROS source thiazole orange (1) enabled the induction of site-specific oxidative stress (2) and analysis of differential cellular response to nuclear *vs.* mitochondrially-localized oxidative stress (3). **B**. Analysis of the stress response by cellular assays and gene profiling exposed differences in the response to mitochondrially (to-FrFK) or nuclear (to-RrRK) localized oxidative stress.

References

1 M. Green and P. M. Loewenstein, *Cell*, 1988, **55**, 1179–1188.
2 A. D. Frankel and C. O. Pabo, *Cell*, 1988, **55**, 1189–1193.
3 D. Derossi, A. H. Joliot, G. Chassaing and A. Prochiantz, *J. Biol. Chem.*, 1994, **269**, 10444–10450.
4 K. Green, M. D. Brand and M. P. Murphy, *Diabetes*, 2004, **53**, S110–S118.
5 S. Deshayes, M. C. Morris, G. Divita and F. Heitz, *Cell. Mol. Life Sci.*, 2005, **62**, 1839–1849.
6 R. Fischer, M. Fotin-Mleczek, H. Hufnagel and R. Brock, *ChemBioChem*, 2005, **6**, 2126–2142.
7 B. Gupta, T. S. Levchenko and V. P. Torchilin, *Adv. Drug Delivery Rev.*, 2005, **57**, 637–651.
8 A. Joliot and A. Prochiantz, *Nat. Cell Biol.*, 2004, **6**, 189–196.
9 E. L. Snyder and S. F. Dowdy, *Pharm. Res.*, 2004, **21**, 389–393.
10 M. Zhao and R. Weissleder, *Med. Res. Rev.*, 2004, **24**, 1–12.
11 E. A. Goun, T. H. Pillow, L. R. Jones, J. B. Rothbard and P. A. Wender, *ChemBioChem*, 2006, **7**, 1497–1515.
12 B. Geueke, K. Namoto, I. Agarkova, J. C. Perriard, H. P. Kohler and D. Seebach, *ChemBioChem*, 2005, **6**, 982–985.
13 T. Hitz, R. Iten, J. Gardiner, K. Namoto and D. Seebach, *Biochemistry*, 2006, **45**, 5817–5829.
14 M. Rueping, Y. Mahajan, M. Sauer and D. Seebach, *ChemBioChem*, 2002, **3**, 257–259.
15 J. Farrera-Sinfreu, E. Giralt, S. Castel, F. Albericio and M. Royo, *J. Am. Chem. Soc.*, 2005, **127**, 9459–9468.
16 S. Pujals, E. Sabido, T. Tarrago and E. Giralt, *Biochem. Soc. Trans.*, 2007, **35**, 794–796.
17 H.-H. Chung, G. Harms, C. M. Seong, B. H. Choi, C. Min, J. P. Taulane and M. Goodman, *Pept. Sci.*, 2004, **76**, 83–96.
18 S. E. Eldred, M. R. Pancost, K. M. Otte, D. Rozema, S. S. Stahl and S. H. Gellman, *Bioconjugate Chem.*, 2005, **16**, 694–699.
19 T. B. Potocky, A. K. Menon and S. H. Gellman, *J. Biol. Chem.*, 2003, **278**, 50188–50194.
20 T. B. Potocky, A. K. Menon and S. H. Gellman, *J. Am. Chem. Soc.*, 2005, **127**, 3686–3687.
21 T. B. Potocky, J. Silvius, A. K. Menon and S. H. Gellman, *ChemBioChem*, 2007, **8**, 917–926.
22 J. Yang, K. Wang, J. Driver, J. Yang and A. R. Barron, *Org. Biomol. Chem.*, 2007, **5**, 260–266.
23 M. Nishihara, F. Perret, T. Takeuchi, S. Futaki, A. N. Lazar, A. W. Coleman, N. Sakai and S. Matile, *Org. Biomol. Chem.*, 2005, **3**, 1659–1669.
24 F. Perret, M. Nishihara, T. Takeuchi, S. Futaki, A. N. Lazar, A. W. Coleman, N. Sakai and S. Matile, *J. Am. Chem. Soc.*, 2005, **127**, 1114–1115.
25 N. Sakai and S. Matile, *J. Am. Chem. Soc.*, 2003, **125**, 14348–14356.
26 N. Sakai, T. Takeuchi, S. Futaki and S. Matile, *ChemBioChem*, 2005, **6**, 114–122.
27 T. Takeuchi, M. Kosuge, A. Tadokoro, Y. Sugiura, M. Nishi, M. Kawata, S. Matile and S. Futaki, *ACS Chem. Biol.*, 2006, **1**, 299–303.
28 M. Tang, A. J. Waring and M. Hong, *J. Am. Chem. Soc.*, 2007, **129**, 11438–11446.
29 A. Astriab-Fisher, D. Sergueev, M. Fisher, B. R. Shaw and R. L. Juliano, *Pharm. Res.*, 2002, **19**, 744–754.
30 A. Nori, K. D. Jensen, M. Tijerina, P. Kopeckova and J. Kopecek, *Bioconjugate Chem.*, 2003, **14**, 44–50.
31 M. Zhao, M. F. Kircher, L. Josephson and R. Weissleder, *Bioconjugate Chem.*, 2002, **13**, 840–844.
32 M. Lewin, N. Carlesso, C. H. Tung, X. W. Tang, D. Cory, D. T. Scadden and R. Weissleder, *Nat. Biotechnol.*, 2000, **18**, 410–414.
33 V. P. Torchilin, R. Rammohan, V. Weissig and T. S. Levchenko, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 8786–8791.
34 F. Hudecz, Z. Banoczi and G. Bsik, *Med. Res. Rev.*, 2005, **25**, 679–736.
35 D. J. Mitchell, D. T. Kim, L. Steinman, C. G. Fathman and J. B. Rothbard, *J. Pept. Res.*, 2000, **56**, 318–325.
36 M. Pooga, M. Hallbrink, M. Zorko and U. Langel, *FASEB J.*, 1998, **12**, 67–77.
37 F. Reynolds, R. Weissleder and L. Josephson, *Bioconjugate Chem.*, 2005, **16**, 1240–1245.
38 S. Boisseau, K. Mabrouk, N. Ram, N. Garmy, V. Collin, A. Tadmouri, M. Mikati, J.-M. Sabatier, M. Ronjat, J. Fantini and M. D. Waard, *Biochim. Biophys. Acta*, 2006, **1758**, 308–319.
39 S. El-Andaloussi, H. J. Johnsson, T. Holm and U. Langel, *Mol. Ther.*, 2007, **15**, 1820–1826.
40 F. Duchardt, M. Fotin-Mleczek, H. Schwarz, R. Fischer and R. Brock, *Traffic*, 2007, **8**, 848–866.
41 S. D. Conner and S. L. Schmid, *Nature*, 2003, **422**, 37–43.
42 A. Aderem and D. M. Underhill, *Annu. Rev. Immunol.*, 1999, **17**, 593–623.
43 J. A. Champion and S. Mitragotri, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 4930–4934.
44 J. R. Maiolo, M. Ferrer and E. A. Ottinger, *Biochim. Biophys. Acta*, 2005, **1712**, 161–172.
45 A. Fittipaldi, A. Ferrari, M. Zoppe, C. Arcangeli, V. Pellegrini, F. Beltram and M. Giacca, *J. Biol. Chem.*, 2003, **278**, 34141–34149.
46 J. P. Richard, K. Melikov, H. Brooks, P. Prevot, B. Lebleu and L. V. Chernomordik, *J. Biol. Chem.*, 2005, **280**, 15300–15306.
47 J. S. Wadia, R. V. Stan and S. F. Dowdy, *Nat. Med.*, 2004, **10**, 310–315.
48 I. Nakase, A. Tadokoro, N. Kawabata, T. Takeuchi, H. Katoh, K. Hiramoto, M. Negishi, M. Nomizu, Y. Sugiura and S. Futaki, *Biochemistry*, 2007, **46**, 492–501.
49 A. T. Jones, *J. Cell. Mol. Med.*, 2007, **11**, 670–684.
50 E. Barany-Wallje, S. Keller, S. Serowy, S. Geibel, P. Pohl, M. Bienert and M. Dathe, *Biophys. J.*, 2005, **89**, 2513–2521.
51 J. P. Richard, K. Melikov, H. Brooks, P. Prevot, B. Lebleu and L. V. Chernomordik, *J. Biol. Chem.*, 2005, **280**, 15300–15306.
52 S. Futaki, T. Suzuki, W. Ohashi, T. Yagami, S. Tanaka, K. Ueda and Y. Sugiura, *J. Biol. Chem.*, 2001, **276**, 5836–5840.
53 A. Ziegler, P. Nervi, M. Durrenberger and J. Seelig, *Biochemistry*, 2005, **44**, 138–148.
54 G. M. K. Poon and J. Gariepy, *Biochem. Soc. Trans.*, 2007, **35**, 788–793.
55 B. Christiaens, J. Grooten, M. Reusens, A. Joliot, M. Goethals, J. Vandekerckhove, A. Prochiantz and M. Rosseneu, *Eur. J. Biochem.*, 2004, **271**, 1187–1197.
56 P. E. G. Thoren, D. Persson, M. Karlsson and B. Norden, *FEBS Lett.*, 2000, **482**, 265–268.
57 S. T. Henriques, J. Costa and M. A. R. B. Castanho, *Biochemistry*, 2005, **2005**, 10189–10198.
58 M. E. Herbig, K. Weller, U. Krauss, A. G. Beck-Sickinger, H. P. Merkle and O. Zerbe, *Biophys. J.*, 2005, **89**, 4056–4066.
59 D. Derossi, S. Calvet, A. Trembleau, A. Brunissen, G. Chassaing and A. Prochiantz, *J. Biol. Chem.*, 1996, **271**, 18188–18193.
60 A. H. A. Clayton, B. W. Atcliffe, G. J. Howlett and W. H. Sawyer, *J. Pept. Sci.*, 2006, **12**, 233–238.
61 L. E. Yandek, A. Pokorny, A. Floren, K. Knoelke, U. Langel and P. F. F. Almeida, *Biophys. J.*, 2007, **92**, 2434–2444.
62 S. Henriques, M. Melo and M. Castanho, *Biochem. J.*, 2006, **399**, 1–7.
63 D. Terrone, S. L. W. Sang, L. Roudaia and J. R. Silvius, *Biochemistry*, 2003, **42**, 13787–13799.
64 J. B. Rothbard, T. C. Jessop and P. A. Wender, *Adv. Drug Delivery Rev.*, 2005, **57**, 495–504.
65 S. M. Fuchs and R. T. Raines, *Biochemistry*, 2004, **43**, 2438–2444.
66 G. Tunnemann, R. M. Martin, S. Haupt, C. Patsch, F. Edenhofer and M. C. Cardoso, *FASEB J.*, 2006, **20**, 1775–1784.
67 X. Zhang, L. Wan, S. Pooyan, Y. Su, C. R. Gardner, M. J. Leibowitz, S. Stein and P. J. Sinko, *Mol. Pharm.*, 2004, **1**, 145–155.
68 J. P. Richard, K. Melikov, E. Vives, C. Ramos, B. Verbeure, M. J. Gait, l. V. Chernomordik and B. Lebleu, *J. Biol. Chem.*, 2003, **278**, 585–590.
69 M. Fretz, J. Jin, R. Conibere, N. A. Penning, S. Al-Taei, G. Storm, S. Futaki, T. Takeuchi, I. Nakase and A. T. Jones, *J. Controlled Release*, 2006, **116**, 247–254.
70 I. Nakase, M. Niwa, T. Takeuchi, K. Sonomura, N. Kawabata, Y. Koike, M. Takehashi, S. Tanaka, K. Ueda, J. C. Simpson, A. T. Jones, Y. Sugiura and S. Futaki, *Mol. Ther.*, 2004, **10**, 1011–1022.
71 R. Frank and R. Hargreaves, *Nat. Rev. Drug Discovery*, 2003, **2**, 566–580.
72 C. Lipinski and A. Hopkins, *Nature*, 2004, **432**, 855–861.
73 S. Veldhoen, S. D. Laufer, A. Trampe and T. Restle, *Nucleic Acids Res.*, 2006, **34**, 6561–6573.
74 O. Zelphati, L. S. Uyechi, L. G. Barron and F. C. Szoka, Jr, *Biochim. Biophys. Acta*, 1998, **1390**, 119–133.
75 S. M. Moghimi, P. Symonds, J. C. Murray, A. C. Hunter, G. Debska and A. Szewczyk, *Mol. Ther.*, 2005, **11**, 990–995.
76 S. Fawell, J. Seery, Y. Daikh, C. Moore, L. L. Chen, B. Pepinsky and J. Barsoun, *Proc. Natl. Acad. Sci. U. S. A.*, 1994, **91**, 664–668.

77 S. R. Schwarze, A. Ho, A. Vocero-Akbani and S. F. Dowdy, *Science*, 1999, **285**, 1569–1572.
78 S. Kameyama, M. Horie, T. Kikuchi, T. Omura, T. Takeuchi, I. Nakase, Y. Sugiura and S. Futaki, *Bioconjugate Chem.*, 2006, **17**, 597–602.
79 M. C. Morris, J. Depollier, J. Mery, F. Heitz and G. Divita, *Nat. Biotechnol.*, 2001, **19**, 1173–1176.
80 S. K. Rodal, G. Skretting, O. Garred, F. Vihardt, B. van Deurs and K. Sandvig, *Mol. Biol. Cell*, 1999, **10**, 961–974.
81 M. Aarts, Y. Liu, L. Liu, S. Besshoh, M. Arundine, J. W. Gurd, Y.-T. Wang, M. W. Salter and M. Tymainski, *Science*, 2002, **298**, 846–849.
82 S. Asoh, I. Ohsawa, T. Mori, K.-i. Katsura, T. Hiraide, Y. Katayama, M. Kimura, D. Ozki, K. Yamagat and S. Ohta, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 17107–17112.
83 J. S. Wadia and S. F. Dowdy, *Adv. Drug Delivery Rev.*, 2005, **57**, 579–596.
84 B. Cornelissen, M. Hu, K. McLarty, D. Costantini and R. M. Reilly, *Nucl. Med. Biol.*, 2007, **34**, 37–46.
85 M. Hu, J. Wang, P. Chen and R. M. Reilly, *Bioconjugate Chem.*, 2006, **17**, 1280–1287.
86 R. K. Jain, *Nat. Med.*, 1998, **4**, 655–657.
87 P. Jarver, K. Langel, S. El-Andaloussi and U. Langel, *Biochem. Soc. Trans.*, 2007, **35**, 770–774.
88 S. Abes, H. M. Moulton, P. Clair, P. Prevot, D. S. Youngblood, R. P. Wu, P. L. Iversen and B. Lebleu, *J. Controlled Release*, 2006, **116**, 304–313.
89 P. Lundberg, S. El-Andaloussi, T. Sutlu, H. Johansson and U. Langel, *FASEB J.*, 2007, **21**, 2664–2671.
90 S. Abes, J. J. Turner, G. D. Ivanova, D. Owen, D. Williams, A. Arzumanov, P. Clair, M. J. Gait and B. Lebleu, *Nucleic Acids Res.*, 2007, **35**, 4495–4502.
91 L. Fisher, U. Soomets, V. C. Toro, L. Chilton, Y. Jiang, U. Langel and K. Verfeldt, *Gene Ther.*, 2004, **11**, 1264–1272.
92 Y. Wolf, S. Pritz, S. Abes, M. Bienert, B. Lebleu and J. Oehlke, *Biochemistry*, 2006, **45**, 14944–14954.
93 B. R. Meade and S. F. Dowdy, *Adv. Drug Delivery Rev.*, 2007, **59**, 134–140.
94 B. R. Meade and S. F. Dowdy, *Adv. Drug Delivery Rev.*, 2008, **60**, 530–536.
95 Y.-L. Chiu, A. Ali, C.-y. Chu, H. Cao and T. M. Rana, *Chem. Biol.*, 2004, **11**, 1165–1175.
96 A. Muratovska and M. R. Eccles, *FEBS Lett.*, 2004, **558**, 63–68.
97 P. Kumar, H. Wu, J. L. McBride, K.-E. Jung, M. Hee Kim, B. L. Davidson, S. Kyung Lee, P. Shankar and N. Manjunath, *Nature*, 2007, **448**, 39–43.
98 S. A. Moschos, S. W. Jones, M. M. Perry, A. E. Williams, J. S. Erjefalt, J. J. Turner, P. J. Barnes, B. S. Sproat, J. J. Gait and M. A. Lindsay, *Bioconjugate Chem.*, 2007, **18**, 1450–1459.
99 S. A. Moschos, A. E. Williams and M. A. Lindsay, *Biochem. Soc. Trans.*, 2007, **35**, 807–810.
100 J. B. Rothbard, S. Garlington, Q. Lin, T. Kirschberg, E. Kreider, P. L. McGrane, P. A. Wender and P. A. Khavari, *Nat. Med.*, 2000, **6**, 1253–1257.
101 M. Lindgren, K. Rosenthal-Aizman, K. Saar, E. Eiriksdottir, Y. Jiang, M. Sassian, P. Ostlund, M. Hallbrink and U. Langel, *Biochem. Pharmacol.*, 2006, **71**, 416–425.
102 T. A. Kirchberg, C. L. VanDeusen, J. B. Rothbard, M. Yan and P. A. Wender, *Org. Lett.*, 2003, **5**, 3459–3462.
103 M. J. Dixon, L. Bourre, A. J. MacRobert and I. M. Eggleston, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 4518–4522.
104 J. F. Liang and V. C. Yang, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 5071–5075.
105 M. Mazel, P. Clair, C. Rousselle, P. Vidal, J.-M. Scherrmann, D. Mathieu and J. Temsamani, *Anti-Cancer Drugs*, 2001, **12**, 107–116.
106 M. Fritzer, T. Szekeres, V. Szuts, H. N. Jarayam and H. Goldenberg, *Biochem. Pharmacol.*, 1996, **51**, 489–493.
107 S. Johnstone, K. Gelmon, L. Mayor, R. Hancock and M. Bally, *Anti-Cancer Drug Des.*, 2000, **15**, 151–160.
108 C. Leuschner and W. Hansel, *Curr. Pharm. Des.*, 2004, **10**, 2299–2310.
109 J. Mader and D. Hoskin, *Expert Opin. Invest. Drugs*, 2006, **15**, 933–946.
110 N. Papo and Y. Shai, *Biochemistry*, 2003, **42**, 9346–9354.
111 A. Risso, E. Braidot, M. Sordano, A. Vianello, F. Macri, B. Skerlavaj, M. Zanetti, R. Gennaro and P. Bernardi, *Mol. Cell. Biol.*, 2002, **22**, 1926–1935.
112 A. Risso, M. Zanetti and R. Gennaro, *Cell. Immunol.*, 1998, **189**, 107–115.
113 P. Warren, L. Li, W. Song, E. Holle, Y. Wei, T. Wagner and X. Yu, *Cancer Res.*, 2001, **61**, 6783–6787.
114 M. Javadpour and M. Barkley, *Biochemistry*, 1997, **36**, 9540–9549.
115 M. Javadpour, M. Juban, W.-C. J. Lo, S. Bishop, J. Alberty, S. Cowell, C. Becker and M. McLaughlin, *J. Med. Chem.*, 1996, **39**, 3107–3113.
116 H. Ellerby, W. Arap, L. Ellerby, R. Kain, R. Andrusiak, G. Del Rio, S. Krajewski, C. Lombardo, R. Rao, E. Ruoslahti, D. Bedesen and R. Pasqualini, *Nat. Med.*, 1999, **5**, 1032–1038.
117 B. Law, L. Quinti, Y. Choi, R. Weissleder and C. Tung, *Mol. Cancer Ther.*, 2006, **5**, 1944–1949.
118 J. Mai, Z. Mi, S. Kim, B. Ng and P. Robbins, *Cancer Res.*, 2001, **61**, 7709–7712.
119 A. Borgne-Sanchez, A. Langonne, L. Baux, H. Lecoeur, D. Chauvier, M. Lassalle, O. Deas, J. Biere, M. Brabant, P. Roux, C. Pechoux, J. Briand, J. Hoebeke, A. Deniaud, C. Brenner, P. Rustin, L. Edelman, D. Rebouillat and E. Jacotot, *Cell Death Differ.*, 2007, **14**, 422–435.
120 L. Walensky, A. Kung, I. Escher, T. Malia, S. Barbuto, R. Wright, G. Wagner, G. Verdine and S. Korsmeyer, *Science*, 2004, **305**, 1466–1470.
121 N. Brewis, A. Phelan, N. Normand, E. Choolun and P. O'Hare, *Mol. Ther.*, 2003, **7**, 262–270.
122 J. Wang, Z. Zhang, S. Choksi, S. Shan, Z. Lu, C. Croce, E. Alnemri, R. Korngold and Z. Huang, *Cancer Res.*, 2000, **60**, 1498–1502.
123 H. Vieira, P. Boya, I. Cohen, C. El Hamel, D. Haouzi, S. Druillenec, A. Belzacq, C. Brenner, B. Roques and G. Kroemer, *Oncogene*, 2002, **21**, 1963–1977.
124 E. Holinger, T. Chittenden and R. Lutz, *J. Biol. Chem.*, 1999, **274**, 13298–13304.
125 S. Fulda, W. Wick, M. Weller and K.-M. Debatin, *Nat. Med.*, 2002, **8**, 808–815.
126 G. Prive and A. Melnick, *Curr. Opin. Genet. Dev.*, 2006, **16**, 71–77.
127 M. Orzaez, L. Mondragon, I. Marzo, G. Sanclimens, A. Messeguer, E. Perez-Paya and M. Vicent, *Peptides*, 2007, **28**, 958–968.
128 J. Plescia, W. Salz, F. Xia, M. Pennati, N. Zaffaroni, M. Daidone, M. Meli, T. Dohi, P. Fortugno, Y. Nefedova, D. Gabrilovich, G. Colombo and D. Altieri, *Cancer Cell*, 2005, **7**, 457–468.
129 H. A. Popiel, Y. Nagai, N. Fujikake and T. Toda, *Mol. Ther.*, 2007, **15**, 303–309.
130 N. Finnberg and W. El-Deiry, *Cancer Biol. Ther.*, 2004, **3**, 614–616.
131 G. Selivanova, V. Iotsova, I. Okan, M. Fritsche, M. Strom, B. Groner, R. Grafstrom and K. Wiman, *Nat. Med.*, 1997, **3**, 632–638.
132 P. Senatus, Y. Li, C. Mandigo, G. Nichols, G. Moise, Y. Mao, M. Brown, R. Anderson, A. Parsa, P. Brandt-Rauf, J. Bruce and R. Fine, *Mol. Cancer Ther.*, 2006, **5**, 20–28.
133 Y. Li, Y. Mao, R. Rosal, R. Dinnen, A. Williams, P. Brandt-Rauf and R. Fine, *Int. J. Cancer*, 2005, **115**, 55–64.
134 G. Gusarova, I. Wang, M. Major, V. Kalinichenko, T. Ackerson, V. Petrovic and R. Costa, *J. Clin. Invest.*, 2007, **117**, 99–111.
135 F. Agou, G. Courtois, J. Chiaravalli, F. Baleux, Y. Coic, F. Traincard, A. Israel and M. Veron, *J. Biol. Chem.*, 2004, **279**, 54248–54257.
136 K. Nagel-Wolfrum, C. Buerger, I. Wittig, K. Butz, F. Hoppe-Seyler and B. Groner, *Mol. Cancer Res.*, 2004, **2**, 170–182.
137 C. Ioannides, R. Freedman, R. Liskamp, N. Ward and C. O'Brian, *Cell. Immunol.*, 1990, **131**, 242–252.
138 T. Borsello and G. Forloni, *Curr. Pharm. Des.*, 2007, **13**, 1875–1886.
139 S. Pero, G. Shukla, M. Cookson, J. S. Flemer and D. Krag, *Br. J. Cancer*, 2007, **96**, 1520–1525.
140 A. D'Ursi, L. Giusti, S. Albrizio, F. Porchia, C. Esposito, G. Caliendo, C. Gargini, E. Novellino, A. Lucacchini, P. Rovero and M. Mazzoni, *Mol. Pharmacol.*, 2006, **69**, 727–736.
141 B. Kelemen, K. Hsiao and S. Goueli, *J. Biol. Chem.*, 2002, **277**, 8741–8748.
142 S. Perea, O. Reyes, Y. Puchades, O. Mendoza, N. Vispo, I. Torrens, A. Santos, R. Silva, B. Acevedo, E. Lopez, V. Falcon and D. Alonso, *Cancer Res.*, 2004, **64**, 7127–7129.
143 F. Ikeno, K. Inagaki, M. Rezaee and D. Mochly-Rosen, *Cardiovasc. Res.*, 2007, **73**, 699–709.
144 L. Chen, H. Hahn, G. Wu, C.-H. Chen, T. Liron, D. Schechtman, G. Cavallaro, L. Banci, Y. Guo, R. Bolli, G. W. Dorn, II and D. Mochly-Rosen, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 11114–11119.
145 E. L. Snyder, C. C. Saenz, C. Denicourt, B. R. Meade, X. S. Cui, I. M. Kaplan and S. F. Dowdy, *Cancer Res.*, 2005, **65**, 10646–10650.

146 J. Rao, A. Dragulescu-Andrasi and H. Yao, *Curr. Opin. Biotechnol.*, 2007, **18**, 17–25.
147 S. Andersson-Engels, C. af Klinteberg, K. Svanberg and S. Svanberg, *Phys. Med. Biol.*, 1997, **42**, 815–824.
148 S. Santra, H. Yang, D. Dutta, J. T. Stanley, P. H. Holloway, W. Tan, B. M. Moudgil and R. A. Mericle, *Chem. Commun.*, 2004, **24**, 2810–2111.
149 T. Jiang, E. S. Olson, Q. T. Nguyen, M. Roy, P. A. Jennings and R. Y. Tsien, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 17867–17872.
150 A. P. Alivisatos, *Science*, 1996, **271**, 933–937.
151 I. L. Medintz, H. T. Uyeda, E. R. Goldman and H. Mattoussi, *Nat. Mat.*, 2005, **4**, 435–446.
152 S. Santra, H. Yang, J. T. Stanley, P. H. Holloway, B. M. Moudgil, G. Walter and R. A. Mericle, *Chem. Commun.*, 2005, 3144–3146.
153 S. M. Rozenzhak, M. P. Kadakia, T. M. Caserta, T. R. Westbrook, M. O. Stone and R. R. Naik, *Chem. Commun.*, 2005, 2217–2219.
154 B. C. Lagerholm, M. Wang, L. A. Ernst, D. H. Ly, H. Liu, M. P. Bruchez and A. S. Waggoner, *Nano Lett.*, 2004, **4**, 2019–2022.
155 J. Silver and W. Ou, *Nano Lett.*, 2005, **5**, 1445–1449.
156 J. B. Delehanty, I. L. Medintz, T. Pons, F. M. Brunel, P. E. Dawson and H. Mattoussi, *Bioconjugate Chem.*, 2006, **17**, 920–927.
157 J. M. de la Fuente, M. Fandel, C. C. Berry, M. Riehle, L. Cronin, G. Aitchison and A. S. G. Curtis, *ChemBioChem*, 2005, **6**, 989–991.
158 M. Liu, Y.-M. Guo, J.-L. Yang, P. Wang, L.-Y. Zhao, N. Shen, S.-C. Wang, X.-J. Guo and Q.-F. Wu, *Acta Biochim. Biophys. Sin.*, 2006, **38**, 865–873.
159 M. J. Allen, K. W. MacRenaris, P. N. Venkatasubramanian and T. J. Meade, *Chem. Biol.*, 2004, **11**, 301–307.
160 E. H. Ohlstein, A. M. Romanic, L. V. Clark, R. D. Kapadia, S. K. Sarkar, R. Gagnon and S. Chandra, *Cardiovasc. Res.*, 2000, **47**, 759–768.
161 A. Y. Louie, M. M. Huber, E. T. Ahrens, U. Rothbacher, R. Moats, R. E. Jacobs, S. E. Fraser and T. J. Meade, *Nat. Biotechnol.*, 2000, **18**, 321–325.
162 S. Saini, D. D. Stark, P. F. Hahn, J. Wittenberg, T. J. Brady and J. T. Ferrucci, Jr., *Radiology*, 1987, **162**, 211–216.
163 L. Josephson, C.-H. Tung, A. Moore and R. Weissleder, *Bioconjugate Chem.*, 1999, **10**, 186–191.
164 A. M. Koch, R. Reynolds, M. F. Kircher, H. P. Merkle, R. Weissleder and L. Josephson, *Bioconjugate Chem.*, 2003, **14**, 1115–1121.
165 P. Wunderbaldinger, L. Josephson and R. Weissleder, *Bioconjugate Chem.*, 2002, **13**, 264–268.
166 C. H. Dodd, H.-C. Hsu, W.-J. Chu, P. Yang, H.-G. Zhang, J. D. Mountz, Jr, K. Zinn, J. Forder, L. Josephson, R. Weissleder, J. M. Mountz and J. D. Mountz, *J. Immunol. Methods*, 2001, **256**, 89–105.
167 M. J. Allen and T. H. Meade, *JBIC, J. Biol. Inorg. Chem.*, 2003, **8**, 746–750.
168 R. Bhorade, R. Weissleder, T. Nakakoshi, A. Moore and C.-H. Tung, *Bioconjugate Chem.*, 2000, **11**, 301–304.
169 M. Liu, Y.-M. Guo, Q.-F. Wu, J.-l. Yang, P. Wang, S.-c. Wang, X.-j. Guo, Y.-Q. Qiang and X.-Y. Duan, *Biochem. Biophys. Res. Commun.*, 2006, **341**, 133–140.
170 V. Polyakov, V. Sharma, J. L. Dahlheimer, C. M. Pica, G. D. Luker and D. Piwnica-Worms, *Bioconjugate Chem.*, 2000, **11**, 762–771.
171 S. Heckl, J. Debus, J. Jurgen, R. Pipkorn, W. Waldeck, H. Spring, R. Rastert, C. W. von der Lieth and K. Braun, *Cancer Res.*, 2002, **62**, 7018–7024.
172 S. Heckl, R. Pipkorn, W. Waldeck, H. Spring, J. Jenne, C.-W. v. d. Lieth, H. Corban-Wilhelm, J. Debus and K. Braun, *Cancer Res.*, 2003, **63**, 4766–4772.
173 W. Su, R. Mishra, J. Pfeuffer, K.-H. Wiesmuller, K. Ugurbil and J. Engelmann, *Contrast Media Mol. Imaging*, 2007, **2**, 42–49.
174 R. Cartier and R. Reszka, *Gene Ther.*, 2002, **9**, 157–167.
175 L. Galluzzi, N. Larochette, N. Zamzami and G. Kroemer, *Oncogene*, 2006, **25**, 4812–4830.
176 M. P. Murphy and R. A. Smith, *Adv. Drug Delivery Rev.*, 2000, **41**, 235–250.
177 V. Weissig, S. V. Boddapati, G. G. M. D'Souza and S. M. Cheng, in *Drug Design Reviews*, 2004, vol. 1, pp. 15–28.
178 J. Davis, M. Kakar and C. Lim, *Pharm. Res.*, 2007, **24**, 17–27.
179 D. Christophe, C. Christophe-Hobertus and B. Pichon, *Cell. Signalling*, 2000, **12**, 337–341.
180 D. Goldfarb, J. Gariepy, G. Schoolnik and R. Kornberg, *Nature*, 1986, **322**, 641–644.
181 A. Ragin and J. Chmielewski, *J. Pept. Res.*, 2004, **63**, 155–160.
182 J. Ludtke, G. Zhang, M. Sebestyen and J. Wolff, *J. Cell Sci.*, 1999, **112**, 2033–2041.
183 M. Zanta, P. Belguise-Valladier and J. Behr, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 91–96.
184 P. Collas and P. Alestrom, *Biochem. Cell Biol.*, 1997, **75**, 633–640.
185 P. Collas, H. Husebye and P. Alestrom, *Transgenic Res.*, 1996, **5**, 451–458.
186 A. Subramanian, P. Ranganathan and S. Diamond, *Nat. Biotechnol.*, 2000, **17**, 873–877.
187 A. Kichler, C. Leborgne, E. Coeytaux and O. Danos, *J. Gene Med.*, 2001, **2**, 135–144.
188 M. Morris, L. Chaloin, J. Mery, F. Heitz and G. Divita, *Nucleic Acids Res.*, 1999, **27**, 3510–3517.
189 D. Singh, R. Kiarash, K. Kawamura, E. LaCasse and J. Gariepy, *Biochemistry*, 1998, **37**, 5798–5809.
190 F. Zhang, P. Andreassen, P. Fender, E. Geissler, J. Hernandez and J. Chroboczek, *Gene Ther.*, 1999, **6**, 171–181.
191 O. Jeon, H. Lim, M. Lee, S. Song and B. Kim, *J. Drug Target.*, 2007, **15**, 190–198.
192 T. Akuta, A. Eguchi, H. Okuyama, T. Senda, H. Inokuchi, Y. Suzuki, E. Nagoshi, H. Mizuguchi, T. Hayakawa, K. Takeda, M. Hasegawa and M. Nakanishi, *Biochem. Biophys. Res. Commun.*, 2002, **297**, 779–786.
193 A. Eguchi, H. Furusawa, A. Yamamoto, T. Akuta, M. Hasegawa, Y. Okahata and M. Nakanishi, *J. Controlled Release*, 2005, **104**, 507–519.
194 L. Benimetskaya, N. Guzzo-Pernell, S. Liu, J. Lai, P. Miller and C. Stein, *Bioconjugate Chem.*, 2002, **13**, 177–187.
195 A. Tkachenko, H. Xie, D. Coleman, W. Glomm, J. Ryan, M. Anderson, S. Franzen and D. Felheim, *J. Am. Chem. Soc.*, 2003, **125**, 4700–4701.
196 O. Aronov, A. Horowitz, A. Gabizon, M. Fuertes, J. Perez and D. Gibson, *Bioconjugate Chem.*, 2004, **15**, 814–823.
197 K. Wagstaff, D. Glover, D. Tremethick and D. Jans, *Mol. Ther.*, 2007, **15**, 721–731.
198 K. Zhao, G.-M. Zhao, D. Wu, Y. Soong, A. V. Birk, P. W. Schiller and H. H. Szeto, *J. Biol. Chem.*, 2004, **279**, 34682–34690.
199 K. L. Horton, K. S. Stewart, S. B. Fonseca, Q. Guo and S. O. Kelley, *Chem. Biol.*, 2008, **15**, DOI: 10.1016/j.chembiol.2008.03.015.
200 K. Mahon, T. Potocky, D. Blair, M. Roy, K. Stewart, T. Chiles and S. Kelley, *Chem. Biol.*, 2007, **14**, 923–930.