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COMPLEX SYSTEMS FOR DRUG TRANSPORT ACROSS CELL MEMBRANES

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Abstract. Targeted drug delivery to specific tissues or cell compartments in the human organism is an advantageous route to overcoming multidrug resistance or reducing undesired side effects of pharmaceuticals. Efficient specific targeting requires associating the drug with a carrier molecule. A multitude of compounds and assemblies have been tested as transporting moieties. This review summarizes the various classes of nanocarrier constructs, which have been proposed for transferring a class of potent chemotherapeutic agents, namely, anthracycline antibiotics through cell membranes. The building principles of the delivery systems are outlined and their pros and cons are discussed. Wherever available, the results from molecular simulations are presented. Special attention is paid to peptide-based systems in general and to a special type of peptides in particular – the cell-penetrating peptides, which may be used as building blocks of new systems for targeted drug delivery.

Keywords: drug delivery systems, anthracycline antibiotics, doxorubicin, cell-penetrating peptides, nanoparticles, biodegradable polymers

Introduction

Malignant neoplasm (cancer) is a disease related to uncontrolled growth and division of cells, which results in organs malfunction. The major part of neoplasms involves formation of carcinomas or tumors, which stem from the epithelial tissue. Another significant group are hematological cancers that affect blood or haematopoietic organs. These are classified as leukemia or lymphomas. Tumors formed by connective or muscle tissue also exist, the so-called sarcomas. Other types are nervous-endocrine tumors or neoplasms arising from embryonal tissue (malignant teratoma). Chemotherapy remains the most widely used method for cancer treatment where anthracycline antibiotics are among the most often applied therapeutic agents. These medicines, which can be obtained as natural extracts, have a broad toxicity spectrum towards neoplasms (Fujiwara et al., 1985). Their modifications can be synthesized in the lab as well. An example of a frequently applied semi-synthetic representative is doxorubicin (DOX) (Figure. 1).

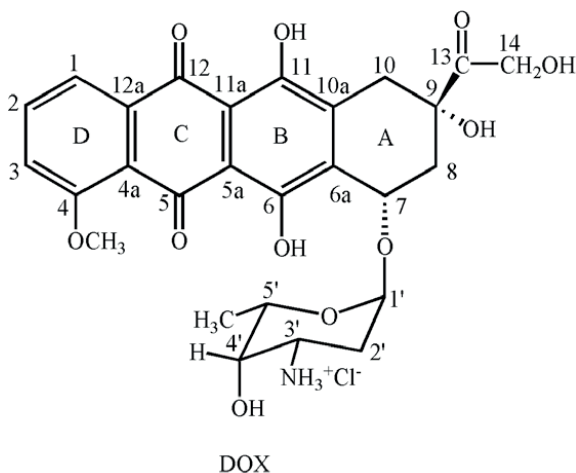


Figure 1. Chemical formula of doxorubicin

This chemotherapeutic is active against a wide range of tumors but its *administration in vivo* is accompanied by severe side effects: primarily cytotoxicity to undifferentiated cells, cardiotoxicity and myelosuppression, nausea and vomiting. Directed transport and delivery of the drugs to malignant tissues would drastically reduce the undesired side effects. This requires the development of drug delivery (DD) systems facilitating the transport of the active substance (Rabbani & Davoodi, 1994; Tewey et al., 1984). In most of the reported systems the drug is bound to an additional carrier biomolecule. The

complexes are modified in such a way as to discriminate malignant from healthy cells. Besides, efficient transport of the drug molecule necessitates its firm attachment to the carrier so that it does not dissociate prematurely. At the same, the drug should be able to leave the transporter after reaching the target cells. Consequently, the accent of research in this field is on systems, which are able to translocate directly across cell membranes. Then, the drug is released in the cell cytosol and enters the cell nucleus targeting the DNA molecules. Other advantages of such systems for drug delivery are their capacity to overcome multidrug resistance of cells and the possibility to avoid drug activation by external stimuli, e.g., X-ray irradiation, which is another important contributor to toxicity of chemotherapy.

Since anthracycline antibiotics are often applied as chemotherapeutics both in clinical practice and for development of new preparations for DD across cell membranes, one of their representatives – doxorubicin (Figure 1) – is selected as the delivery agent in this review. A brief summary of the systems offered so far for efficient and/or targeted drug delivery of chemotherapeutics to cells is presented. Several classes of complexes for transport of doxorubicin or its analogues are described. Their fundamental characteristics, composition, pros and cons are discussed.

The drug carriers reported in the literature can be divided in several categories depending on the transporting moiety. The most studies are devoted to complexes of the drugs with nanoparticles or with various peptides. The next few sections review also the published theoretical simulations addressing the translocation of drug-transporting complexes across cell membranes. Molecular dynamics (MD) simulations are a powerful tool for studying the mechanisms of cell membrane penetration at the molecular level. The focus of the paper is on the peptide-based transporters. Special emphasis is put on a class of peptides, which can translocate directly through cell membranes because they can be used as a constituent of novel promising systems for transport of doxorubicin.

Nanoparticles-based carriers

One of the approaches for reducing the drug toxicity and for ensuring its functionality is inclusion into nanoparticles tailored for directed transport, which are composed of biocompatible ingredients such as gelatin (Leo et al., 1997), chitosan (Janes et al., 2001), or lipid molecules. A basic stage of the construction of drug carrier nanoparticles is their modification with targeting ligands. Polyethylene glycol (PEG) grafted on the surface of the nanoparticles often serves as such (Lu et al., 2014). Antibodies are added in some cases, too (Lu et al., 2014). Other strategies for directed transport of the nanoparticles are the addition of peptide fragments, aptamers, polysaccharides, saccharides, folic acid,

etc. Apart from the type of the ligand, its density, size and surface nature are essential as well (Zhong et al., 2014). For example, aldehyde functionalization of doxorubicin-loaded particles significantly increases the drug concentration in cancer cells (Sagnella et al., 2014).

Transport with solid nanoparticles and nanotubes

Solid nanoparticles are often employed as transporters across the blood-brain barrier (Gil et al., 2009). There, the drug is adsorbed at the particle surface. However, additional encapsulation of the pharmaceutical by appropriate ligands is necessary. The particles enter the cell cytoplasm by two major mechanisms: endocytosis (Rajendran et al., 2010) or passive diffusion (Yang & Ma, 2010). When endocytosis is operative, the particle could be trapped inside the membrane, which could hinder its release in the cytosol (Gao et al., 2005; Verma & Stellacci, 2010). Hence, direct membrane penetration is the more efficient way but it is difficult to accomplish.

Ding et al. (2010) have carried out an MD simulation of a complex built of a nanoparticle (hydrophobic or hydrophilic) covered with ligands (lipids), which traverses a lipid bilayer (Figure 2). It has been found that the type of the ligands influences strongly the penetration capacity of the complex.

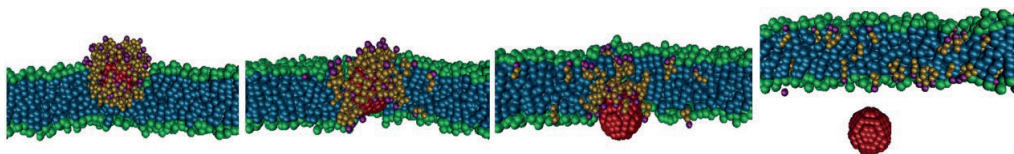


Figure 2. Model membrane translocation of a nanoparticle with diameter 3 nm wrapped with ligands with surface density of 3 nm⁻² (Ding et al., 2010)

Carbon nanotubes (SWCNTs) can also be employed for drug transport. The drug is located in the tube cavity and the surface of the nanotube is functionalized with various residues to enhance specific diffusion. An MD simulation has been performed to tackle the possibility for membrane translocation of a polar drug and paclitaxel (PTX, mitotic inhibitor widely used for chemotherapy) entrapped into a SWCNT. A dipalmitoylphosphatidylcholine (DPPC) bilayer models the cell membrane (Mousavi et al., 2013) (Figure 3).

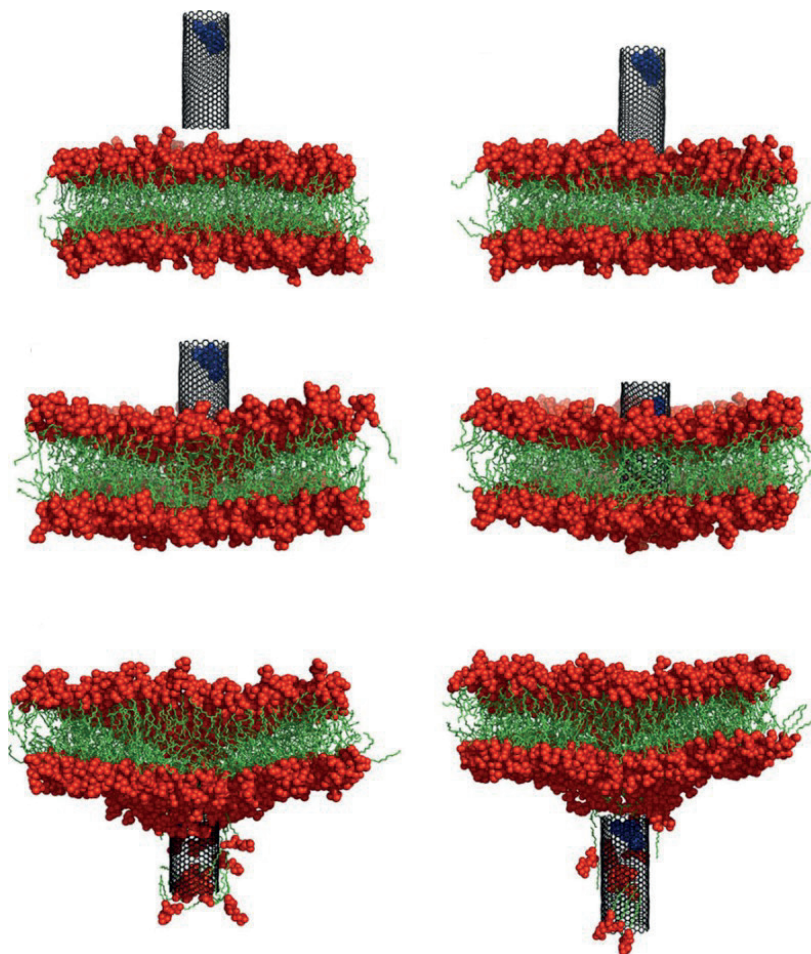


Fig. 3. Snapshots from the translocation of CNT–PTX through a lipid bilayer (Mousavi et al., 2013)

Similar simulations have been carried out for a PEG-modified SWCNT (Skandari & Al-Haik, 2013) entering the membrane lipid bilayer. The results signify that the presence of molecules PTX considerably increases the pulling force and the PTX-functionalized nanotube penetrates the membrane faster than the non-modified one. It has been shown that the presence of a small amount of water-soluble PEG chains bound covalently to the SWCNT inhibits the penetration.

One of the main disadvantages of transport systems based on spherical nanoparticles is their small diameter and the lack of suitable functional groups for binding hydrophobic drugs. Unfortunately, nanotubes are also difficult to implement directly in the medical practice due to their high toxicity.

Dendrimer-based transport

Dendrimers are particularly appealing for DD composites. They contain cavities of controlled size where therapeutics and other bioactive substances, as well as targeting ligands, can be encapsulated. Some of the most frequently used dendrimers are polyamidoamines (PAMAM), poly(L-lysine) dendrimers (PLL), polyester (PGLSA-OH), polypropylene (PPI) dendrimers or such based on poly(2,2-bishydroxymethyl) propanoic acid (bis-MPA).

A computer simulation has been made demonstrating that the lipid bilayer completely engulfs the dendrimer (Guo et al., 2013) (Figure 4).

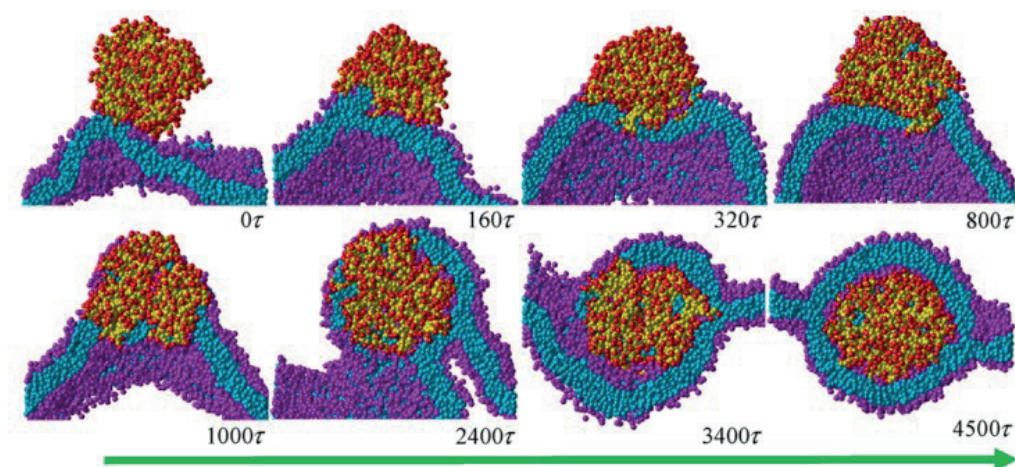


Figure 4. Local perturbation of the membrane lipid bilayer resulting from its interaction with a dendrimer DD system (Guo et al., 2013)

It has been shown that depending on the surface tension of the bilayer the interaction can take place in three different ways: penetration ($\sigma = 2.09 \text{ k}_B T/r_c$), penetration with partial wrapping ($\sigma = 0.10 \text{ k}_B T/r_c$), or complete wrapping ($\sigma = -0.72 \text{ k}_B T/r_c$) of the nanoparticle ($r_c \approx 0.7 \text{ nm}$).

Dendrimers can induce changes in the cell membrane (Ginzburg & Ballijepalli, 2007) and directly enter it thus leading to cell apoptosis (Nel et al., 2009; Leroueil et al., 2007). The main limitations for dendrimer-based drug transport are the variability of the drug release mechanisms (Lu et al., 2014) and of the release kinetics. Moreover, the pharmaceuticals encapsulated into dendrimers have a tendency to leave the matrix too fast – before reaching the target cells (Wolinsky & Grinstaff, 2008).

Transport with polymer nanoparticles

Among the most abundant drug transfer matrices are polymer nanoparticles. They can be divided into several groups: macromolecular, stealth, gelatin-based (nanospheres and nanocapsules), and nanovesicles (micelles and liposomes). They are based on biodegradable polymers and are much less toxic than metal nanoparticles. The constituent polymers have diverse structure. Molecular weight, composition, and functions are selected depending on the requirements of the cargo. Polymer nanoparticles are highly stable *in vivo* and are able to circulate in the bloodstream and to accumulate in tumors.

Liposomes covered with biocompatible polymers (pegylated; stealth) (Ruoslahti et al., 2010; Alberts & Garcia, 1997) are robust and suitable for long-circulating carriers delivering DOX to solid tumors (Gabizon & Martin, 1997). The registered low plasma concentrations of free doxorubicin after application of stealth liposomes indicate diminished tendency of the drug to accumulate into the myocard. Detoxication of the organism from liposomal DOX involves two mechanisms (Gabizon et al., 1994): (i) aided by an active substance, which extracts and destroys liposomes after metabolization; (ii) after decomposition of the liposomes the free DOX is bound to another carrier. On the basis of the efficient hydrophobic interaction with membranes, a complex of DOX with a hydrophobic cell-penetrating peptide PFVYLI loaded into a stealth liposome is suggested (Cai et al., 2014). It has been proven that the *in vivo* toxicity of DOX is reduced upon increasing the drug:lipid ratio. Hence, it is desirable to achieve highly efficient encapsulation of doxorubicin into the liposomes (Fritze et al., 2006). One of the main problems with the practical implementation of liposomes is the stability of the bilayer shell. Upon its premature degradation, which sometimes takes place, the drug is released in an unwanted spot in the organism.

Micelles do not need chemical modification of the bioactive substance. The drug is well solubilized by avoiding additional functionalization aimed at improving solubility. In addition, micelles reach cell membranes intact (Hubbell, 2003). The drug enters the cytoplasm primarily via diffusion after being released from the micelle and translocated

through the membrane. Adding surface groups increases the permeability of the endosomal membranes, which is witnessed by detection of micelles in the cytoplasm (Luo et al., 2002). Part of the methods applied for targeting DOX include biodegradable micelles (Park et al., 2009; Xiao et al., 2011). pH-sensitive micelles have been synthesized consisting of PEG shells and cores of poly(L-histidine) chains loaded with DOX (Gao et al., 2005). The micelles discriminate the low pH of tumors by ionization of the histidine groups. Other formulations include into the micelles amphiphilic co-polymers such as (β -amino ester)-*g*-octadecyl acrylate (Chen et al., 2011) or poly(D,L-lactic-co-glycolic acid) (Yoo & Park, 2001).

Nanospheres and *nanocapsules* pack drugs better in biological fluids compared to liposomes and micelles (Yoo et al. 2005). They also offer protection against enzymes during metabolism. They successfully pass the blood-brain barrier when loaded with DOX or other pharmaceuticals as loperamide or tubocurarine (Tosi et al., 2007). Nanoparticles of this kind allow overcoming the multidrug resistance *in vitro* (Evjen et al., 2011). Their metabolites (glycolic and lactic acid) enter the Krebs cycle where they are biodegraded to carbon dioxide and water and finally eliminated (Hyon, 2000).

Polymer nanoparticles are relatively stable drug transporters with high capacity. They enable entrapment of both hydrophilic and hydrophobic drugs and enhance the bioavailability of the active component. They also provide opportunities for directed transport to the cell nucleus. It is possible to release the drug spot-on in the organism. Some polymer nanoparticles are markedly less toxic and less hazardous.

Peptide-based drug carriers

Despite the multitude of reports on nanoparticle transporters, the efficient delivery of pharmaceuticals to the malignant cells remains a challenge. No complete biocompatibility combined with spatially addressable surfaces for efficient targeting of the desired tissues has been achieved. In addition, the nanoparticles stability is largely affected by the environment of the carrier. A plausible alternative is the development of single-macromolecule-based transporters. The macromolecule is usually chosen from naturally existing compounds *in vivo*, e.g., DNA (Jiang et al., 2012) or antibody (Dubowchik & Firestone, 1998; Trail et al., 1993; Willner et al., 1993). Currently, various peptide sequences are employed as the foundation of the transporter.

Delivery with natural proteins

The unique structure of proteins allows specific binding and targeting by utilizing different protein-binding ligands. Apart from that, special protein nanoparticles are

developed, which can form 3D-networks offering a variety of opportunities for reversible binding of bioactive components (Elzoghby et al., 2011; Elzoghby et al., 2012).

Animal proteins have the advantages of the synthetic polymers but they are also easily resorbed *in vivo* and their metabolites are of low toxicity (Leo et al., 1997). Gelatin is relatively non-specific to antigens and offers accessible functional groups for chemical modifications. It is suitable as medium for DD of anti-HIV (Jain et al., 2008), antimalarial (Bajpai & Choubey, 2006), antimicrobial (Nahar et al., 2008); Lee et al., 2011), antidiabetic (Devi et al., 2010; Zhao et al., 2012), or anticancer (Veis, 1964; Zworek et al., 2005) preparations. Collagen is biocompatible and with low antigen specificity. Biodegradable nanospheres have been obtained thereof (Marty et al., 1978), which have large surface area, high resorption capacity and possibility for delayed release of antimicrobial drugs and steroids (El-Samaligy & Rohdewald, 1983). *Albumin* is another frequently used carrier. It is proper since it metabolizes *in vivo* to harmless end products (Kratz, 2008; Elsadek & Kratz, 2012). It plays an important role in improving the pharmacokinetic profile and the targeting characteristics of a series of novel drug transporters (Park, 2012), which can overcome the blood-brain barrier (Dadparvar et al., 2011). *Caseins* self-organize into spherical micelles (Horne, 2006). Nanomicelles based on β -casein have been loaded successfully with model chemotherapeutics (Shapira et al., 2010).

Plant proteins are hydrophobic and offer opportunities for extended drug release (Lai & Guo, 2011). Nanoparticles built thereof do not require additional physical or chemical solidification treatment. Thus, the use of toxic chemical crosslinking agents is avoided. Plant proteins have functional groups, which can be utilized for adsorption or covalent binding of targeting molecules. *Lectins* are plant proteins, which are part of carbohydrate-binding non-immunogenic proteins/glycoproteins (Lehr, 2000). Wheat germ agglutinin, for example, is resistant to degradation in addition to its specific recognition and binding to glucosamine or acidic components of intestinal cell membranes (Pellegrina et al., 2005; Wen et al., 2011). Lectins are investigated as directed carriers for poorly available drugs and for glycotargeting of anticancer pharmaceuticals. Their well-expressed antitumor activity relies on inducing apoptosis or autophagy of tumor cells.

Among the most important pros of natural proteins is their biocompatibility because they are part of the normal metabolism of living organisms. The hydrolysis of the proteins by digestive enzymes generates bioactive peptides, which can express a number of physiological properties *in vivo* (Chen et al., 2006). The fact that proteins are involved in the metabolism poses one of the greatest dangers in their use as DD nanocarriers – they can be degraded before reaching the target tissues.

Synthetic peptides

Compared to proteins, peptides feature several benefits, e.g., pronounced stability, capability for easy targeting, and low immune response. They are implemented both as drug carriers and as directed nanoparticle transporters in general.

The utilization of synthetic peptides sensitive to cathepsin B as DD systems has been studied (Dubowchik & Radia, 1997; Dubowchik et al., 1997). Peptides like Gly-Phe-Leu-Gly (Kopeček & Duncan, 1987) or Ala-Leu-Ala-Leu (Troet et al., 1982) have been tested but out ruled as inappropriate. The reason is that they are too hydrophobic, which results in very slow release of the pharmaceutic and can lead to sedimentation or aggregation of the antibodies. The basic investigated structural unit is Z-Phe-Arg-X, where X is a fluoro-containing residue and Z – a capping group (Barrett, 1980). A systematic study describes the carrier properties of a series of peptides where arginine (Arg) is replaced by lysine (Lys), two protected arginine residues, or of a set of citrulline-containing compounds. In general, peptide-DOX substrates, in which the peptide is capable of self-degradation after binding to cathepsin B to release the drug, are well sustainable in human blood plasma. Other peptides like Tyr-D-Ala-Gly-Phe-D-Leu (Petri et al., 2007) and thyrotropin (releasing hormone) (Bodor et al., 1992) are employed as molecular ‘wrapping’ and delivery across the blood-brain barrier. These polypeptide materials allow control of the release of the therapeutic (Song et al., 2012). Another report focuses on successful DD based on a cyclized sequence Arg-Gly-Asp (RGD) conjugated to PEG-functionalized micelles loaded with DOX (Nasongkla et al., 2004). The same peptide has been used as a ligand for polyethylene oxide-modified micelles (PEO-PCL) apt for bladder cancer therapy (Zhou et al., 2013).

Antimicrobial peptides (AMPs) bind specifically to IL-4 receptors of atherosclerotic and cancer cells. The peptide CRKRLDRN has been conjugated to charged pH-sensitive micelles loaded with DOX (Wu et al., 2010). *In vivo* studies show much more efficient inhibition of cancer cells proliferation compared to free DOX. The peptide EGFR binds to some of the cell growth receptors and is suited for efficient directing of nanoparticles (Crawford, 2002).

Peptides are characterized by vast chemical functionality and are especially perspective constituents of preparations for specific DD. They have low cytotoxicity and high drug-binding capacity. Some of them permit controlled release of the therapeutic and express extra inhibiting activity of tumor growth. Moreover, they can be incorporated into the structure of micellar or vesicular formulations or other nanoparticles as targeting ligands. The versatility of studied peptide-based DD systems reveals the enormous potential for tuning of the peptide transporters depending on the specific goals or the type of the

drug. In order to gain full control on targeted DD, however, much more research (also at molecular level) is necessary.

Cell-penetrating peptides

Certain short peptide sequences can penetrate cytoplasmic membranes. These are the so-called cell-penetrating peptides (CPPs). They can transport “cargos”, which are either covalently or non-covalently bound to them (Nakase et al., 2012) (Figure 5). The transporters condense tightly the active substance and direct towards the cell membrane receptors. After entering the cell they carry the cargo to a DNA section within the nucleus, which is responsible for gene expression and after releasing it induce endosomal excretion. Some CPPs are used in chemotherapy. The peptides belonging to this class have varying amino acid content and can be classified into two groups: they are either built of lysine or arginine, or are amphiphilic with alternating polar or charged amino acids and apolar or hydrophobic ones. The large positive charge of the polycationic peptides rules out passive diffusion through lipid bilayers at physiological pH. These CPPs usually contain the sequence Arg-Gly-Asp because it recognizes specific receptors on the cell membranes surface. Although CPPs are an excellent component of DD systems for the stage of cell penetration, they are relatively non-selective to particular cell types. Therefore, different strategies are devised for targeting CPP-based constructs to cancer cells and for increasing the intracellular supply of medicines. This is normally accomplished by binding the CPP to other peptides.

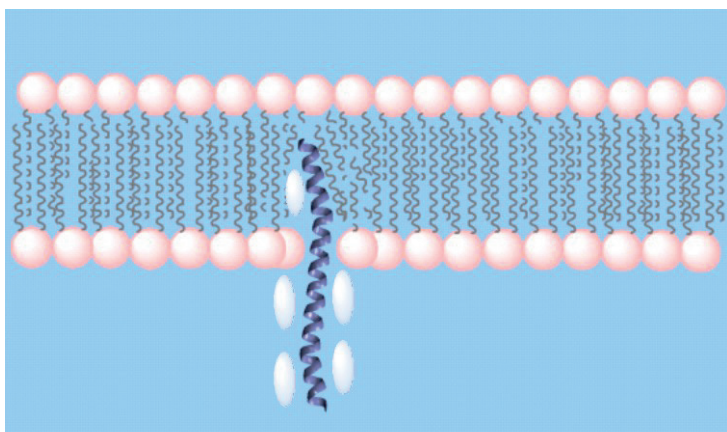


Figure 5. Schematic representation of cell membrane penetration of a drug-CPP complex

In order to understand and design in a rational way DD systems based on CPPs, it is important to reveal the way, in which they enter cells. Three major theories exist about the translocation mechanism of CPPs: direct membrane penetration, endocytosis-mediated transfer, and translocation via the formation of transient structures. At first the direct mechanism was considered as the only plausible route due to strong arginine-phosphate electrostatic interaction (Prochiantz, 2000).

Later, it was discovered that endocytosis has a vital role for translocation of basic and amphiphilic peptides and for arginine-rich sequences (Drin et al., 2003; Richard et al., 2003; Vendeville et al., 2004). Illustrative examples are the CPPs Tat and antennapedia (Antp). The accumulated experimental data about the endocytotic pathways are controversial. Several mechanisms are discussed: macropinocytosis, clathrin-mediated endocytosis (CME) (Arpino et al., 2008) and caveolae/raft-mediated endocytosis (Ferrari et al., 2003; Fittipaldi et al., 2003). Macropinocytosis (Wadia et al., 2004) is acknowledged as the main transfection mechanism operative arginine-rich CPPs due to the high density of positive charges. This has been confirmed by solid-state NMR (Hong & Su, 2011), by which translocation of Tat across cell membranes is monitored. Tünnemann et al. report that Tat conjugated to a small peptide cargo quickly penetrates cells by an endocytosis-independent mechanism whereas larger assemblies enter via endocytosis (Tünnemann et al., 2006). It is concluded that endocytosis is limited to high-molecular-weight complexes. Nevertheless, this conclusion contradicts several publications addressing penetration of the same CPP by a vesicular mode (Fischer et al., 2004; Potocky et al., 2003; Richard et al., 2005). In an attempt to explain these contradictions, Duchardt et al. (2007) have provided evidence that some peptides use the three endocytotic pathways simultaneously depending on the specific conditions.

Tat contains six arginine and two lysine residues. Consequently, it is highly positively charged and this guarantees its efficient cell membrane translocation. It interacts with various membrane components, which indicates that multiple penetration mechanisms exist. They can be divided into heparane sulphate-dependent and heparane sulphate-independent. It has been shown for Tat that it induces negative Gaussian curvature into the membrane – pores, micropinocytosis bumps, and endocytosis invaginations are formed (Mishra et al., 2008) and this has been outlined as the driving force for translocation.

12 hydrated models of Tat/lipid bilayer have been simulated. They differ in the peptide:lipid:water ratio (Herce & Garcia, 2007). The MD simulations last between 80 and 200 ns. A transfection mechanism has been proposed involving four stages: first, the peptide binds electrostatically to the lipid phosphate groups; second, highly charged peptide-lipid fractions group together; third, charged arginines are attracted to the

phosphate groups and start penetrating the lipid bilayer dragging along water molecules and bound lipids; fourth, a pore is formed in the membrane, which closes after letting several peptides in. A critical surface concentration of peptides exists above which the peptides act collectively facilitating the penetration.

Arginine homopolymers of average length penetrate cells much more efficiently than peptides with the same length but composed of lysine, ornithine, or histidine (Ziegler et al., 2005). Arginine-rich peptides traverse the membrane of various cell types without inducing high cytotoxicity (Futaki, 2008). Chains of 8 and 9 arginines have the best efficiency and the lowest toxicity. The potential of Arg-8 for transporting anticancer agents has been evaluated using DOX as a model compound. Upon comparison of the transfection efficiency of oligoarginines with various number of arginine or acyl residues, stearyl-octaarginine demonstrates the highest one (Nakase et al., 2012). This functionality can form up to five (two on average) hydrogen bonds with phosphates, sulphates, or carboxylates of cell membrane molecules. Hydrogen bonding also with amphiphilic counterions such as phosphatidylglycerol or lauryl further assists the peptide translocation across the cell membranes (Rothbard et al., 2004). Unlike oligoarginines, polyarginines need assisting factors to traverse cell membranes. This suggests that the hydrophobic anionic complex around guanidine-rich regions “captures” the cationic lipid structures with lipophilic anions acting as activators. Aromatic activators have been shown to be superior to aliphatic ones (Sakai & Matile, 2003; Sakai et al., 2006; Sakai et al., 2005; Hennig et al., 2008; Pantos et al., 2008; Nishihara et al., 2005; Perret et al., 2005). In spite of their deficiencies, it has been established that polyarginines are translocated more efficiently than other peptide homopolymers. Peptides containing lysine and arginine residues also facilitate resorption of daunomycin (DOX-related natural anthracycline) by cells (Shibagaki et al., 2011).

The behaviour of the isolated amino acid arginine inside the cell membrane has been studied both theoretically and experimentally (Szabó et al., 2010). The results from the simulations suggest that arginine and other positively charged amino acid residues can penetrate membranes and even reach their core as long as there are enough hydrophobic fragments assisting them.

Aiming at investigation of the arginine distribution within the membrane in direction normal to its surface, Dorairaj & Allen (2007) carry out atomistic MD simulation of the penetration process (Hristova & Wimley, 2011). They construct 80-residue-long leucine α -helix with one arginine in the middle and pull it across a lipid bilayer of 48 DPPC molecules in aqueous solution (8000 water molecules). Thereof, the free energy change is estimated by umbrella sampling. A very high energy barrier of 17

kcal/mol is obtained for the central Arg. It is seen that the amino acid drags along water molecules (forming water defects in the membrane hydrophobic core), ions, and lipid headgroups. It turns out that water molecules have the most essential stabilizing effect on the free energy change. The main stabilizing contribution is the electrostatic one, which is implicitly (by the Born model) taken into account in the simulations. The structure of the membrane is perturbed significantly. The conformations of Arg in various regions of the membrane are analyzed. While it appears to be very flexible in the aqueous phase, its conformational freedom is restricted inside the membrane. The virtual experiment shows that Arg can be found in a hydrocarbon medium but it prefers the membrane periphery.

Water molecules interact intensively with CPPs, which has been witnessed by NMR measurements (Dorairaj & Allen, 2007). Insertion of four Arg residues into a lipid bilayer leads to substantial deformation of the bilayer (Krepkiy et al., 2009). As a results of the large number of water molecules, which penetrate the membrane locally, the lipids interact directly with the Arg residues via hydrogen bonds and electrostatic attraction. Later, it became evident that a hydrogen bonding network existed. The capability of Arg to form hydrogen bonds and of the bilayer to deform are outlined as the main factors governing the translocation of arginine. Its transfection mechanism is based on the ability of this amino acid residue to take place in multiple electrostatic interactions and to form hydrogen bonds with lipid headgroups and water molecules. The deformation of the lipid bilayer results as a consequence of these interactions. Thus, arginine-containing peptides can bring into the hydrophobic membrane core polar groups and water molecules necessary for their stabilization in this unfriendly environment.

The four-stage translocation mechanism mentioned above for Tat has been witnessed in another theoretical study also for a peptide built of 9 positively charged arginine residues (Arg-9) (Herce et al., 2009). An MD simulation of length 500 ns has been performed. To validate the proposed translocation mechanism, model experiments have been carried out to check whether the ionic conductivity of the membrane increases after the addition of Arg-9. The results are affirmative. However, the outcome from *in vitro* tests with real cells is inconclusive – the effect is seen only in several cases.

Another important factor for the translocation of arginine-rich peptides is their secondary structure. The peptide penetratin is in the random coil conformation in aqueous solution and adopts α -helical shape at high lipid:peptide ratio (Freites et al., 2005; Caesar et al., 2006). Upon decrease of this ratio the share of β -sheet form increases (Magzoub et al., 2002). The amphiphilicity of penetratin is enhanced and its hydrophobic fragments interact directly with the non-polar part of the membrane. Branched arginine-containing

polymers are also efficient in this respect (Wender et al., 2000). The peptides with 6 or more amino acids are better, showing increase in the degree of penetration up to length of 15 amino acids. Oligomers of more than 15 can penetrate, too, but much less efficiently (Mitchell et al., 2000; Futaki et al., 2001).

The main advantage of CPPs as drug carriers is the lack of toxicity compared to other transporters such as liposomes, chemical polymers, etc. A serious obstacle for the implementation of CPPs is the fact that they provoke immune response. Hence, more knowledge about their translocation mechanisms and interactions *in vivo* is necessary. Molecular dynamics can be applied successfully to study problems related to structure and transfection mechanisms of CPPs.

Summary

The above brief review of various systems for delivery of antitumor drugs reveals that a wide variety of drug-carrier complexes exist up to date. Some of them, like liposomes, therapeutic-loaded micelles or drug-DNA and drug-peptide complexes show better characteristics than the unbound pharmaceuticals. However, the specificity of the tumors in terms of the type of tissues they affect and the individuality of the patients requires constant improvement of the chemotherapeutic drugs and of the DD and activation systems. Understanding and controlling the transporters is of utmost importance. Future research in this direction should be focused on microscopic description of different drug-biomolecule conjugates in terms of their capacity to traverse cell membranes, the ultimate goal being the development and implementation of the best components for an efficient targeted complex chemotherapeutic delivery construct. Therein, the drug should be bound tightly to the carrier, be able to reach the malignant cells, enter them and be released to act as a cytotoxic agent. The efficient transporter should be specific enough to allow targeting to neoplastic cells and to be biodegradable yielding non-toxic metabolites. These prerequisites imply several molecular building blocks of the delivery system, which should be mutually compatible and remain intact along the entire drug transduction path. Although optimization of the composition of such a transporter entails multistage experimental studies, this is worth the efforts since an improved carrier will offer solution for a significant part of the current problems related to the efficient targeted transport of anticancer drugs in general and of anthracycline antibiotics in particular.

The review is dedicated to the 90th anniversary of the Department of Physical Chemistry at Sofia University “St. Kliment Ohridski”.

REFERENCES

- Alberts, D.S. & Garcia, D.J. (1997). Safety aspects of pegylated liposomal doxorubicin in patients with cancer. *Drugs*, 54(4), 30-35.
- Arpino, G., Wiechmann, L., Osborne, C.K. & Schiff, R. (2008). Crosstalk between the estrogen receptor and the HER tyrosine kinase receptor family: molecular mechanism and clinical implications for endocrine therapy resistance. *Endocrinol. Rev.*, 29, 217-233.
- Bajpai, A.K. & Choubey, J. (2006). Design of gelatin nanoparticles as swelling controlled delivery system for chloroquine phosphate. *J. Mater. Sci. Mater. Med.*, 17, 345-358.
- Barrett, A.J. (1980). Fluorimetric assays for cathepsin B and cathepsin H with methylcoumarylamide substrates. *Biochem. J.*, 187, 909-912.
- Bodor, N., Prokai, L., Wu, W.M., Farag, H., Jonalagadda, S., Kawamura, M & Simpkins, J. (1992). A strategy for delivering peptides into the central nervous system by sequential metabolism. *Science*, 257, 1698-1700.
- Caesar, C., Esbjorner, E., Lincoln, P. & Norden, B. (2006). Membrane interactions of cell-penetrating peptides probed by tryptophan fluorescence and dichroism techniques: correlations of structure to cellular uptake. *Biochemistry*, 45, 7682-7692.
- Cai, D., Gao, W., He, B., Dai, W., Zhang, H., Wang, X., Zhang, X. & Zhang, Q. (2014). Hydrophobic penetrating peptide PFVYLI-modified stealth liposomes for doxorubicin delivery in breast cancer therapy. *Biomaterials*, 35, 2283-2294.
- Chen, J., Xing, M.M.Q. & Zhong, W. (2011). Degradable micelles based on hydrolytically degradable amphiphilic graft copolymers for doxorubicin delivery. *Polymer*, 52, 933-941.
- Chen, L., Remondetto, G.E. & Subirade, M. (2006). Food protein-based materials as nutraceutical delivery systems. *Trends Food Sci. Technol.*, 17, 272-283.
- Crawford, J. (2002). Clinical uses of pegylated pharmaceuticals in oncology. *Cancer Treat. Rev.*, 28, 7-11.
- Dadparvar, M., Wagner, S., Wien, S., Kufleitner, J., Worek, F., von Briesen, H. & Kreuter, J. (2011). HI 6 human serum albumin nanoparticles—development and transport over an in vitro blood-brain barrier model. *Toxicol. Lett.*, 206, 60-66.
- Devi, R., Singh, V. & Chaudhary, A.K. (2010). Antidiabetic activity of pilocarpus micophyllus extract on streptozotocin-induced diabetic mice. *Int. J. Pharm. Sci. Rev. Res.*, 5, 87-92.
- Ding, H.-m., Tian, W.-de. & Ma, Y.-q. (2010). Designing nanoparticle translocation through membranes by computer simulations. *ACS Nano*, 6, 1230-1238.
- Dorairaj, S. & Allen, T.W. (2007). On the thermodynamic stability of a charged arginine

- side chain in a transmembrane helix. *Proc. Natl. Acad. Sci. USA*, 104, 4943–4948.
- Drin, G., Cottin, S., Blanc, E., Rees, A.R. & Temsamani, J. (2003). Studies on the internalization mechanism of cationic cell-penetrating peptides. *J. Biol. Chem.*, 278, 31192–31201.
- Dubowchik, G.M. & Radia, S. (1997). Monomethoxytrityl (MMT) as a versatile amino protecting group for complex prodrugs of anticancer compounds sensitive to strong acids, bases and nucleophiles. *Tetrahedron Lett.*, 38, 5257–5260.
- Dubowchik, G.M., King, H.D. & Pham-Kapлита, K. (1997). Efficient mitomycin C coupling with stable p-nitrophenyl-benzyl carbonates using n-hydroxybenzotriazole as a catalytic additive. *Tetrahedron Lett.*, 38, 5261–5264.
- Dubowchik, G.M. & Firestone, R.A. (1998). Cathepsin B-sensitive dipeptide prodrugs 1: a model study of structural requirements for efficient release of doxorubicin. *Bioorg. Medic. Chem. Lett.*, 8, 3341–3346.
- Duchardt, F., Fotin-Mleczek, M., Schwarz, H., Fischer, R. & Brock, R. (2007). A comprehensive model for the cellular uptake of cationic cell-penetrating peptides. *Traffic*, 8, 848–866.
- Elsadek, B. & Kratz, F. (2012). Impact of albumin on drug delivery -new applications on the horizon. *J. Control. Release*, 157, 4–28.
- El-Samaligy, M.S. & Rohdewald, P. (1983). Reconstituted collagen nanoparticles, a novel drug carrier delivery system. *J. Pharm. Pharmacol.*, 35, 537–539.
- Elzoghby, A.O., El-Fotoh, W.S. & Elgindy, N.A. (2011). Casein-based formulations as promising controlled release drug delivery systems. *J. Control. Release*, 153, 206–216.
- Elzoghby, A.O., Samy, W.M. & Elgindy, N.A. (2012). Albumin-based nanoparticles as potential controlled release drug delivery systems. *J. Control. Release*, 167, 168–182.
- Evjen, T.J., Hagtvet, E., Nilssen, E.A., Brandl, M. & Fossheim, S.L. (2011). Sonosensitive dioleoylphosphatidylethanolamine-containing liposomes with prolonged blood circulation time of doxorubicin. *Eur. J. Pharmac. Sci.*, 43, 318–324.
- Ferrari, A., Pellegrini, V., Arcangeli, C., Fittipaldi, A., Giacca, M. & Beltram, F. (2003). Caveolae-mediated internalization of extracellular HIV-1 tat fusion proteins visualized in real time. *Mol. Ther.*, 8, 284–294.
- Fischer, R., Köhler, K., Fotin-Mleczek, M. & Brock, R. (2004). A stepwise dissection of the intracellular fate of cationic cell-penetrating peptides. *J. Biol. Chem.*, 279, 12625–12635.
- Fittipaldi, A., Ferrari, A., Zoppé, M., Arcangeli, C., Pellegrini, V., Beltram, F. & Giacca, M. (2003). Cell membrane lipid rafts mediate caveolar endocytosis of HIV-1 tat fusion proteins. *J. Biol. Chem.*, 278, 34141–34149.

- Freites, J.A., Tobias, D.J., Heijne, G. & White, S.H. (2005). Interface connections of a transmembrane voltage sensor. *Proc. Natl. Acad. Sci. USA*, 102, 15059–15064.
- Fritze, A., Hens, F., Kimpfler, A., Schubert, R. & Peschka-Süss, R. (2006). Remote loading of doxorubicin into liposomes driven by a transmembrane phosphate gradient. *Biochim. Biophys. Acta*, 1758, 1633–1640.
- Fujiwara, A., Hoshino, T. & Westley, J.W. (1985). Anthracycline antibiotics. *Critical Rev. Biotechnology*, 3, 133–157.
- Futaki, S. (2008). Membrane permeable peptide vectors: chemistry and functional design for the therapeutic applications. *Adv. Drug Deliv. Rev.*, 60, 447–614.
- Futaki, S., Suzuki, T., Ohashi, W., Yagami, T., Tanaka, S., Ueda, K. & Sugiura, Y. (2001). Arginine-rich peptides An abundant source of membrane-permeable peptides having potential as carriers for intracellular protein delivery. *J. Biol. Chem.*, 276, 5836–5840.
- Gabizon, S. & Martin, F. (1997). Polyethylene glycol-coated (pegylated) liposomal doxorubicin: rationale for use in solid tumours. *Drugs*, 54(4), 15–21.
- Gabizon, A., Catane, R., Uziely, B., Kaufman, B., Safra, T., Cohen, R., Martin, F., Huang, A. & Barenholz, Y. (1994). Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes. *Cancer Res.*, 54, 987–992.
- Gao, H.J., Shi, W.D. & Freund, L.B. (2005). Optimal substrate shape for vesicle adhesion on a curved substrate. *Proc. Natl. Acad. Sci. U.S.A.*, 102, 3213–3218.
- Gao, Z.G., Lee, D.H., Kim, D.I. & Bae, Y.H. (2005). Doxorubicin loaded pH-sensitive micelle targeting acidic extracellular pH of human ovarian A2780 tumor in mice. *J. Drug Target.*, 13, 391–397.
- Gil, E., Li, J., Xiao, H. & Lowe, T. (2009). Dextran-based doxorubicin nanocarriers with improved tumor penetration. *Biomacromolecules*, 10, 505–516.
- Ginzburg, V.V. & Balijepalli, S. (2007). Modeling the thermodynamics of the interaction of nanoparticles with cell membranes. *Nano Lett.*, 7, 3716–3722.
- Guo, R., Mao, J. & Yan, L. (2013). Unique dynamical approach of fully wrapping dendrimer-like soft nanoparticles by lipid bilayer membrane. *ACS Nano*, 7, 10646–10653.
- Hennig, A., Gabriel, G.J., Tew, G.N. & Matile, S. (2008). Stimuli-responsive polyguanidino-oxanorbornene membrane transporters as multicomponent sensors in complex matrices. *J. Am. Chem. Soc.*, 130, 10338–10344.
- Herce, H.D. & Garcia, A.E. (2007). Molecular dynamics simulations suggest a mechanism for translocation of the HIV-1 TAT peptide across lipid membranes. *Proc. Natl. Acad. Sci. USA*, 104, 20805–20810.

- Herce, H.D., Garcia, A.E., Litt, J., Kane, R.S., Martin, P., Enrique, N., Rebolledo, A. & Milesi, V. (2009). Arginine-rich peptides destabilize the plasma membrane, consistent with a pore formation translocation mechanism of cell-penetrating peptides. *Biophys. J.*, 97, 1917–1925.
- Hong, M. & Su, Y. (2011). Structure and dynamics of cationic membrane peptides and proteins: insights from solid-state NMR. *Protein Sci.*, 20, 641–655.
- Horne, D.S. (2006). Casein micelle structure: models and muddles. *Curr. Opin. Colloid & Interface Sci.*, 11, 148–153.
- Hristova, K. & Wimley, W.C. (2011). A look at arginine in membranes. *J. Membrane Biol.*, 239, 49–56.
- Hubbell, J.A. (2003). Enhancing drug function. *Science*, 300, 595–596.
- Hyon, S.H. (2000). Biodegradable poly (lactic acid) microspheres for drug delivery systems. *Yonsei Med. J.*, 41, 720–734.
- Jain, S.K., Gupta, Y., Jain, A., Saxena, A.R., Khare, P. & Jain, A. (2008). Mannosylated gelatin nanoparticles bearing an anti-HIV drug didanosine for site-specific delivery. *Nanomedicine*, 4, 41–48.
- Janes, K.A., Fresneau, M.P., Marazuela, A., Fabra, A. & Alonso, M.J. (2001). Chitosan nanoparticles as delivery systems for doxorubicin. *J. Control. Release*, 73, 255–267.
- Jiang, Q., Song, C., Nangreave, J., Liu, X., Lin, L., Qiu, D., Wang, Z.-G., Zou, G., Liang, X., Yan, H. & Ding, B. (2012). DNA origami as a carrier for circumvention of drug resistance. *J. Am. Chem. Soc.*, 134, 13396–13403.
- Kopeček, J. & Duncan, R. (1987). Targetable polymeric prodrugs. *J. Control. Release*, 6, 315–327.
- Kratz, F. (2008). Albumin as a drug carrier: design of prodrugs, drug conjugates and nanoparticles. *J. Control. Release*, 132, 171–183.
- Krepkiy, D., Mihailescu, M., Freitas, J.A., Schow, E.V., Worcester, D.L., Gawrisch, K., Tobias, D.J., White, S.H. & Swartz, K.J. (2009). Structure and hydration of membranes embedded with voltage-sensing domains. *Nature*, 462, 473–479.
- Lai, L.F. & Guo, H.X. (2011). Preparation of new 5-fluorouracil-loaded zein nanoparticles for liver targeting. *Int. J. Pharm.*, 404, 317–323.
- Lee, E.J., Khan, S.A., Park, J.K. & Lim, K.H. (2011). Studies on the characteristics of drug-loaded gelatin nanoparticles prepared by nanoprecipitation. *Bioprocess Biosyst. Eng.*, 35, 297–307.
- Lehr, C.-M. (2000). Lectin-mediated drug delivery:: the second generation of bioadhesives. *J. Control. Release*, 65, 19–29.
- Leo, E., Vandelli, M.A., Cameroni, R. & Forni, F. (1997). Doxorubicin-loaded gelatin

- nanoparticles stabilized by glutaraldehyde: involvement of the drug in the cross-linking process. *Int. J. Pharmac.*, 155, 75–82.
- Leroueil, P.R., Hong, S., Mecke, A., Baker, J.R., Orr, B.G. & Holl, M.M.B. (2007). Nanoparticle interaction with biological membranes: does nanotechnology present a Janus face? *Chem. Res.*, 40, 335–342.
- Lu, C.-T., Zhao, Y.-Z., Wong, H.L., Cai, J., Peng, L. & Tian, H.-Q. (2014). Current approaches to enhance CNS delivery of drugs across the brain barriers. *Int. J. Nanomed.*, 9, 2241–2257.
- Luo, L., Tam, J., Maysinger, D. & Eisenberg, A. (2002). Cellular internalization of poly(ethylene oxide)-*b*-poly(ϵ -caprolactone) diblock copolymer micelles. *Bioconjug. Chem.*, 13, 1259–1265.
- Magzoub, M., Eriksson, L.E. & Gräslund, A. (2002). Conformational states of the cell-penetrating peptide penetratin when interacting with phospholipid vesicles: effects of surface charge and peptide concentration. *Biochim. Biophys. Acta*, 1563, 53–63.
- Marty, J.J., Openheim, R.C. & Speiser, P. (1978). Nanoparticles - a new colloidal drug delivery system. *Pharm. Acta Helv.*, 53, 17–23.
- Mishra, A., Gordon, V.D., Yang, L., Coridan, R. & Wong, G.C. (2008). HIV TAT forms pores in membranes by inducing saddle-splay curvature: potential role of bidentate hydrogen bonding. *Angew. Chem., Int. Ed.*, 47, 2986–298.
- Mitchell, D.J., Kim, D.T., Steinman, L., Fathman, C.G. & Rothbard, J.B. (2000). Polyarginine enters cells more efficiently than other polycationic homopolymers. *J. Pept. Res.*, 56, 318–325.
- Mousavi, S.Z., Amjad-Iranagh, S., Nademi, Y. & Modarressi, H. (2013). Carbon nanotube encapsulated drug penetration through the cell membrane: an investigation based on steered molecular dynamics simulation. *Membrane Biol.*, 246, 697–704.
- Nahar, M., Mishra, D., Dubey, V. & Jain, N.K. Development, characterization, and toxicity evaluation of amphotericin B-loaded gelatin nanoparticles. (2008). *Nanomedicine*, 4, 252–261.
- Nakase, I., Akita, H., Kogure, K., Gräslund, A., Langel, U., Harashima, H. & Futaki, S. (2012). Efficient intracellular delivery of nucleic acid pharmaceuticals using cell-penetrating peptides. *Acc. Chem. Res.*, 45, 1132–1139.
- Nasongkla, N., Shuai, X., Ai, H., Weinberg, B.D., Pink, J., Boothman, D.A. & Gao, J. (2004). cRGD-functionalized polymer micelles for targeted doxorubicin delivery. *Angew. Chem., Int. Ed.*, 43, 6323–6327.
- Nel, A.E., Mädler, L., Velegol, D., Xia, T., Hoek, E.M.V., Somasundaran, P., Klaessig, F., Castranova, V. & Thompson, M. (2009). Understanding biophysicochemical interactions at the nano–bio interface. *Nat. Mater.*, 8, 543–557.

- Nishihara, M., Perret, F., Takeuchi, T., Futaki, S., Lazar, A.N., Coleman, A.W., Sakai, N. & Matile, S. (2005). Arginine magic with new counterions up the sleeve. *Org. Biomol. Chem.*, 3, 1659–1669.
- Pantos, A., Tsogas, I. & Paleos, C.A. (2008). Guanidinium group: a versatile moiety inducing transport and multicompartimentalization in complementary membranes. *Biochim. Biophys. Acta Biomembr.*, 1778, 811–823.
- Park, K. (2012). The optimal formulation variables for tumor targeting. *J. Control. Release*, 157, 315–316.
- Park, J., Fong, P.M., Lu, J., Russell, K.S., Booth, C.J., Saltzman, W.M. & Fahmy, T.M. (2009). PEGylated PLGA nanoparticles for the improved delivery of doxorubicin. *Nanomedicine: Nanotechnology, Biology & Medicine*, 5, 410–418.
- Pellegrina, C.D., Rizzi C., Mosconi, S., Zoccatelli G., Peruffo, A. & Chignola, R. (2005). Plant lectins as carriers for oral drugs: Is wheat germ agglutinin a suitable candidate? *Toxicol. Appl. Pharmacol.*, 207, 170–178.
- Perret, F., Nishihara, M., Takeuchi, T., Futaki, S., Lazar, A.N., Coleman, A.W., Sakai, N. & Matile, S. (2005). Anionic fullerenes, calixarenes, coronenes, and pyrenes as activators of oligo/polyarginines in model membranes and live cells. *J. Am. Chem. Soc.*, 127, 1114–1115.
- Petri, B., Bootz, A., Khalansky, A., Hekmatara, T., Müller, R., Uhl, R., Kreuter, J. & Gelperina, S. (2007). Chemotherapy of brain tumour using doxorubicin bound to surfactant-coated poly (butyl cyanoacrylate) nanoparticles: revisiting the role of surfactants. *J. Control. Release.*, 117, 51–58.
- Potocky, T.B., Menon, A.K. & Gellman, S.H. (2003). Cytoplasmic and nuclear delivery of a TAT-derived peptide and a β -peptide after endocytic uptake into HeLa cells. *J. Biol. Chem.*, 278, 50188–50194.
- Prochiantz, A. (2000). Messenger proteins: homeoproteins, TAT and others. *Curr. Opin. Cell Biol.*, 12, 400–406.
- Rabbani, A. & Davoodi, J. (1994). Effects of anthracycline antibiotic, daunomycin on thymus chromatin: the role of chromosomal proteins. *Gen. Pharmacol.*, 25, 787–793.
- Rajendran, L., Knölker, H.J. & Simons, K. (2010). Subcellular targeting strategies for drug design and delivery. *Nat. Rev. Drug Discovery*, 9, 29–42.
- Richard, J.P., Melikov, K., Vives, E., Ramos, C., Verbeure, B., Gait, M.J., Chernomordik, L.V. & Lebleu, B. (2003). Cell-penetrating peptides A reevaluation of the mechanism of cellular uptake. *J. Biol. Chem.*, 278, 585–590.
- Richard, J.P., Melikov, K., Brooks, H., Prevot, P., Lebleu, B. & Chernomordik, L.V. (2005). Cellular uptake of unconjugated TAT peptide involves clathrin-dependent endocytosis and heparan sulfate receptors. *J. Biol. Chem.*, 280, 15300–15306.

- Rothbard, J.B., Jessop, T.C., Lewis, R.S., Murray, B.A. & Wender, P.A. (2004). Role of membrane potential and hydrogen bonding in the mechanism of translocation of guanidinium-rich peptides into cells. *J. Am. Chem. Soc.*, 126, 9506–9507.
- Ruoslahti, E., Bhatia, S.N. & Sailor, M.J.J. (2010). Targeting of drugs and nanoparticles to tumors. *J. Cell Biol.*, 188, 759–768.
- Sagnella, S.M., Duong, H., MacMillan, A., Boyer, C., Whan, R., McCarroll, J.A., Davis, T.P. & Kavallaris, M. (2014). Dextran-based doxorubicin nanocarriers with improved tumor penetration. *Biomacromolecules*, 15, 262–275.
- Sakai, N. & Matile, S. (2003). Anion-mediated transfer of polyarginine across liquid and bilayer membranes. *J. Am. Chem. Soc.*, 125, 14348–14356.
- Sakai, N., Takeuchi, T., Futaki, S. & Matile, S. (2005). Direct observation of anion-mediated translocation of fluorescent oligoarginine carriers into and across bulk liquid and anionic bilayer membranes. *Chem-BioChem*, 6, 114–122.
- Sakai, N., Futaki, S. & Matile, S. (2006). Anion hopping of (and on) functional oligoarginines: from chloroform to cells. *Soft Matter*, 2, 636–641.
- Shapira, A., Markman, G., Assaraf, Y.G. & Livney, Y.D. (2010). β -casein-based nanovehicles for oral delivery of chemotherapeutic drugs: drug-protein interactions and mitoxantrone loading capacity. *Nanomedicine*, 6, 547–555.
- Shibagaki, N., Okamoto, T., Mitsui, H., Inozume, T., Kanzaki, M. & Shimada, S. (2011). Novel immunotherapeutic approaches to skin cancer treatments using protein transduction technology. *J. Dermat. Sci.*, 61, 153–161.
- Skandani, A.A. & Al-Haik, M. (2013). Reciprocal effects of the chirality and the surface functionalization on the drug delivery permissibility of carbon nanotubes. *Soft Matter*, 9, 11645–11649.
- Song, W., Li, M., Tang, Z., Li, Q., Yang, Y., Liu, H., Duan, T., Hong, H. & Chen, X. (2012). Methoxypoly (ethylene glycol)-block-Poly (L-glutamic acid)-loaded cisplatin and a combination with iRGD for the treatment of non-small-cell lung cancers. *Macromol. Biosci.*, 12, 1514–1523.
- Szabó, R., Bánóczy, Z., Mező, G., Láng, O., Köhidai, L. & Hudecz, F. (2010). Daunomycin-polypeptide conjugates with antitumor activity. *Biochim. Biophys. Acta*, 1798, 2209–2216.
- Tewey, K.M., Rowe, T.C., Yang, L., Halligan, B.D. & Lin, L.F. (1984). Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. *Science*, 226, 466–468.
- Tosi, G., Costantino, L., Rivasi, F., Ruozzi, B., Leo, E., Vergoni, A.V., Tacchi, R., Bertolini, A., Vandelli, M.A. & Forni, F. (2007). Targeting the central nervous system: *in vivo*

- experiments with peptide-derivatized nanoparticles loaded with loperamide and rhodamine-123. *J. Control. Release.*, 122, 1–9.
- Trail, P.A., Willner, D., Lasch, S.J., Henderson, A.J., Hofstead, S., Casazza, A.M., Firestone, R.A., Hellström, I. & Hellström, K.E. (1993). Cure of xenografted human carcinomas by BR96-doxorubicin immunoconjugates. *Science*, 261, 212–215.
- Trouet, A., Masquelier, M., Baurain, R. & Deprez-De Campaneere, D. (1982). A covalent linkage between daunorubicin and proteins that is stable in serum and reversible by lysosomal hydrolases, as required for a lysosomotropic drug-carrier conjugate: in vitro and in vivo studies. *Proc. Natl. Acad. Sci. U.S.A.*, 79, 626–629.
- Tünnemann, G., Martin, R.M., Haupt, S., Patsch, C., Edenhofer, F. & Cardoso, M.C. (2006). Cargo-dependent mode of uptake and bioavailability of TAT-containing proteins and peptides in living cells. *FASEB J.*, 20, 1775–1784.
- Veis, A. (1964). *The macromolecular chemistry of gelatin*. New York: Academic Press.
- Vendeville, A., Rayne, F., Bonhoure, A., Bettache, N., Montcourrier, P. & Beaumelle, B. (2004). HIV-1 Tat enters T cells using coated pits before translocating from acidified endosomes and eliciting biological responses. *Mol. Biol. Cell*, 15, 2347–2360.
- Verma, A. & Stellacci, F. (2010). Effect of surface properties on nanoparticle–cell interactions. *Small*, 6, 12–21.
- Wadia, J.S., Stan, R.V. & Dowdy, S.F. (2004). Transducible TAT-HA fusogenic peptide enhances escape of TAT-fusion proteins after lipid raft macropinocytosis. *Nature Med.*, 10, 310–315.
- Wen, Z., Yan, Z., Hu, K., Pang, Z., Cheng, X., Guo, L.R., Zhang, Q., Jiang, X., Fang, L. & Lai, R. (2011). Odorranalectin-conjugated nanoparticles: preparation, brain delivery and pharmacodynamic study on Parkinson's disease following intranasal administration. *J. Control. Release*, 151, 131–138.
- Wender, P.A., Mitchell, D.J., Pattabiraman, K., Pelkey, E.T., Steinman, L. & Rothbard, J.B. (2000). The design, synthesis, and evaluation of molecules that enable or enhance cellular uptake: peptoid molecular transporters. *Proc. Natl. Acad. Sci.*, 97, 13003–13008.
- Willner, D., Trail, P.A., Hofstead, S.J., King, H.D., Lasch, S.J., Braslawsky, G.R., Greenfield, R.S., Kaneko, T. & Firestone, R.A. (1993). (6-Maleimidocaproyl) hydrazone of doxorubicin: a new derivative for the preparation of immunoconjugates of doxorubicin. *Bioconj. Chem.*, 4, 521–527.
- Wolinsky, J.B. & Grinstaff, M.W. (2008). Therapeutic and diagnostic applications of dendrimers for cancer treatment. *Adv. Drug Deliv. Rev.*, 60, 1037–1055.
- Wu, X.L., Kim, J.H., Koo, H., Bae, S.M., Shin, H., Kim, M.S., Lee, B.H., Park, R.W., Kim, I.S., Choi, K., Kwon, I.C., Kim, K. & Lee, D.S. (2010). Tumor-targeting peptide

- conjugated pH-responsive micelles as a potential drug carrier for cancer therapy. *Bioconj. Chem.*, 21, 208–213.
- Xiao, K., Luo, J., Li, Y., Lee, J.S., Fung, G. & Lam, K.S. (2011). PEG-oligocholeic acid telodendrimer micelles for the targeted delivery of doxorubicin to B-cell lymphoma. *J. Control. Release*, 155, 272–281.
- Yang, K. & Ma, Y.-Q. (2010). Computer simulation of the translocation of nanoparticles with different shapes across a lipid bilayer. *Nat. Nanotechnol.*, 5, 579–583.
- Yoo, H.S. & Park, T.G. (2001). Biodegradable polymeric micelles composed of doxorubicin conjugated PLGA–PEG block copolymer. *J. Control. Release*, 70, 63–70.
- Yoo, J.Y., Kim, J.M., Seo, K.S., Jeong, Y.K., Lee, H.B. & Khang, G. (2005). Characterization of degradation behavior for plga in various ph condition by simple liquid chromatography method. *Biomed. Mater Eng.*, 15, 279–288.
- Zhao, Y.Z., Li, X., Lu, C.T., Xu, Y.Y., Lv, H.F., Dai, D.D., Zhang, L., Sun, C.Z., Yang, W., Li, X.K., Zhao, Y.P., Fu, H.X., Cai, L., Lin, M., Chen, L.J. & Zhang, M. (2012). Experiment on the feasibility of using modified gelatin nanoparticles as insulin pulmonary administration system for diabetes therapy. *Acta Diabetol.*, 49, 315–325.
- Zhong, Y., Meng, F., Deng, C. & Zhong, Z. (2014). Ligand-directed active tumor-targeting polymeric nanoparticles for cancer chemotherapy. *Biomacromolecules*, 15, 1955–1969.
- Zhou, D., Zhang, G. & Gan, Z. (2013). c(RGDfK) decorated micellar drug delivery system for intravesical instilled chemotherapy of superficial bladder cancer. *J. Control. Release*, 169, 204–210.
- Ziegler, A., Nervi, P., Dürrenberger, M. & Seelig, J. (2005). The cationic cell-penetrating peptide CPP(TAT) derived from the HIV-1 protein TAT is rapidly transported into living fibroblasts: optical, biophysical, and metabolic evidence. *Biochemistry*, 44, 138–148.
- Zwizorek, K., Kloeckner, J., Wagner, E. & Coester, C. (2005). Gelatin nanoparticles as a new and simple gene delivery system. *J. Pharm. Pharm. Sci.*, 7, 22–28.

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