colocalization degree over time. Additionally the internalization kinetics of integrin targeted micelles was compared to EGF targeted polyplexes that are well-known for their fast uptake kinetics [2]. The internalization pathway was then studied with inhibitor experiments and by colocalization with specific marker proteins. Our results reveal a strong competition between unspecific electrostatic interactions and specific receptor–ligand interactions that determines successful targeting of the micelles. Enhanced PEG shielding of the micelles leads to the reduction of electrostatic interactions resulting in a specific and faster internalization of the targeted micelles. Additionally we observed a considerable effect of the applied micelle concentration as well as the micelle size on their internalization properties. Our data lead to a more detailed understanding of the targeting effect than can be observed by conventional bulk instruments. The gained knowledge enables to maximize the therapeutic benefit of future gene vectors for clinical application.

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A46
A designer biomimetic vector for breast cancer gene therapy

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Introduction: Gene therapy holds the potential to cure many diseases, provided that the genetic or molecular basis is understood. In cancer, the delivery of therapeutic genes via viral vectors has proven more effective than the current alternative non-viral methods. However tissue specificity, high costs of production and safety remain major concerns with viral delivery. This study examines the use of a designer biomimetic vector (DBV), that is essentially a recombinant fusion protein, to deliver the therapeutic inducible nitric oxide synthase (iNOS) gene to breast cancer. The DBV is composed of several discrete motifs each designed with single function architecture including: (a) a DNA condensing motif (DCM) obtained from the adenovirus mu peptide, (b) a ZR-75-1 breast cancer cyclic targeting peptide (TP) for specific delivery of the nanoparticles, (c) an endosomal disruption motif (EDM) that mimics the influenza virus fusogenic peptide and (d) a nuclear localization signal (NLS), rev, obtained from the human immune-deficiency virus type-1. We now use this DBV to deliver the cytotoxic iNOS gene *in vitro* and the GFP reporter gene *in vivo* to ZR-75-1 tumours. *Methods*: The DBV was expressed in *Escherichia coli*, extracted with affinity chromatography and purified using size exclusion chromatography. The DBV was complexed to piNOS to form nanoparticles which were used either for characterisation via electrophoretic mobility shift assays, serum stability assays or dynamic light scattering analysis. ZR-75-1 breast cancer cells were transfected with DBV/piNOS nanoparticles and toxicity was quantified using the WST-1 cell toxicity and clonogenic assays. Over expression of iNOS was also confirmed via western blotting and greiss test. Finally ZR-75-1 intradermal tumours were grown using SCID models and the DBV/pEGFP-N1 nanoparticles were delivered both intratumourally and intravenously. Tumours and organs were excised and the GFP distribution was determined. *Results*: The DBV was effectively expressed in *E. coli* at approximately 3 mg/l yield. The DBV condenses piNOS into spherical nanoparticles between N:P ratios of 4–10. At a N:P ratio of 9, piNOS was fully condensed with an average size of 75.1 nm. Transfection with the DBV/piNOS nanoparticles resulted in a maximum of 62% cell kill. INOS overexpression was confirmed and total nitrite levels were in the range of 18 μM and comparable with lipofectamine/piNOS. Finally 48 h after i.v. injection of the DBV/pEGFP-N1 nanoparticles GFP protein was detected in all the organs. The addition of chloroquine (30 mg/kg I.P.) did not enhance the expression of GFP indicating functionality of the EDM. Furthermore the addition of nocodazole (3 mg/kg I.P.) resulted in a reduction in GFP expression again indicating NLS functionality *in vivo*. *Conclusions*: The DBV/piNOS nanoparticles gave significant cytotoxicity in ZR-75-1 breast cancer cells *in vitro* and with less than 20% transfection this indicates a bystander effect. Despite a lack of tumour targeting by the DBV vector *in vivo*, the data indicates that the DBV/pEGFP-N1 nanoparticles do not aggregate and can travel through the bloodstream with confirmation of gene expression in all the organs. Future studies will concentrate on using the human osteocalcin promoter (hOC) to transcriptionally target the iNOS plasmid to ZR-75-1 breast tumours.

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A47
Cellular delivery and biological activity of metall complex-peptide conjugates

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Bioorganometallic chemistry has become more and more important in several fields, especially in the development of new drugs for cancer treatment. A number of metal-based building blocks have promising features for applications in therapy and diagnosis. Introduction of a metal centre could add new features that may help to overcome some problems in cancer treatment. However the low water solubility and bioavailability of these organometallic compounds inhibits their therapeutic use in medicine. Therefore intracellular delivery of therapeutics is the challenging task in medicinal chemistry research. Recently, so-called cell-penetrating peptides (CPP) have emerged as potent tools to introduce substances into cells. CPP are an inhomogenic group of peptides that share the ability to translocate in a large number of different cell-lines without the need of any receptor or transporter molecule. Thereby they are capable to transport various cargos inside cells, like proteins, oligonucleotides, nanoparticles or small organic drugs. This work describes the coupling of metal-based building blocks to cell-penetrating peptides based on an antimicrobial peptide cathelicidin CAP18 or on the human peptide hormone calcitonin (hCT). Synthesis was achieved by solid phase peptide synthesis using standard Fmoc chemistry and activation by HOBt/DIC. Several different metal complexes have been investigated, for example, half-sandwich-complexes of different metals as iridium, manganese, rhodium or iron. To introduce the potential metal-specific activity to the bioconjugate, up to two organometal moieties were coupled either N-terminally, to a