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

amino acid side chain or in between two amino acids. Cellular uptake of the new bioconjugates was investigated with different methods like fluorescence microscopy, atom absorption spectroscopy or flow cytometry. High accumulation could be observed in different tumour cells. Furthermore, cell viability assays showed that those organometallic peptide conjugates are very potent and possess promising cytotoxic properties.

See references below for additional reading
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A48
Polyelectrolite complex based microspheres for colon specific anticancer drug delivery

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Localized delivery of chemotherapeutic agents has long been the aim of clinical colon cancer therapy in order to limit the indiscriminate activity of many anti-cancer drugs on rapidly dividing cells, including normal tissues. The ideal drug delivery system (DDS) is envisioned to selectively and efficiently transport the anticancer drug to the target cells. It will not only minimize side effects associated with inappropriate drug distribution, but will also enhance therapeutic efficacy by increasing local drug concentration. The goal of our study was to develop wheat-germ aglutinin (WGA) functionalized chitosan-Ca-alginate microspheres (MS) loaded with acid-resistant nanoparticles (NP) of 5-FU, as colon targeting DDS and evaluate its *in vitro* efficacy and *in vivo* biodistribution. The rationale behind the design of the formulation is the presence of high level of polysaccharides of microbial origin in the human colon and the possibility of direct binding of MS to the mucosal surface by nonspecific or specific ligand–receptor interactions using biological molecules (WGA), thus enabling active uptake of 5-FU in the target cancer cells. A simple one-step spray drying procedure was used to produce polyanion/polycation MS loaded with acid-resistant NP of 5-FU with mean diameter of ~14.74 μm, high production yield (~50%) and encapsulation efficiency (~72%). Using 1,1′-Carbonyl-diimidazol as a surface group activation agent, successful conjugation of WGA to MS surface was achieved (~50%). Haemagglutination test confirmed that WGA, treated by covalent coupling procedure, still retained its specific carbohydrate binding activity on the surface of MS. *In vitro* efficiacy was evaluated by investigating 5-FU permeability and [methyl-3H]thimidine uptake in Caco-2 cells. The cumulative amount of transported 5-FU through Caco-2 cells was 15.1% and 6.5% for 5-FU solution and WGA conjugated MS, respectively. Cell culture studies also indicated a marked decrease in [methyl-3H]thimidine uptake for WGA decorated MS compared to 5-FU solution, suggesting that immobilization of WGA onto MS surface, due to the improved interaction and enhanced tissue accumulation of 5-FU could led to improved efficacy in targeted anticancer colon therapy. *In vivo* biodistribution studies were conducted with oral administration of ^{99m}Tc labeled MS on fasted male Wistar rats. The imaging was performed at different time intervals post administration. The results showed that MS traversed fairly quickly through upper part of GI tract and resided in the colon for relatively longer period of time, probably due to the particle size, pH dependent swelling and surface properties of the MS. Overall, the results of this work showed that cross-linked polycation/polyanion MS loaded with 5-FU and decorated with WGA, were able to effectively deliver 5-FU to colon region, thus affecting the transport of 5-FU into the cells and consequently improving the efficacy.

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A50
Engineering macrophages to synthesize recombinant adenoviruses in hypoxic areas of human prostate tumours

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Background: Like many other forms of human malignancy, prostate carcinomas contain multiple regions of transient and chronic hypoxia. New therapies targeting the hypoxic areas of tumours need to be designed as these sites are highly resistant to conventional cancer therapies. We have recently shown that macrophages accumulate in these hypoxic areas of prostate tumours, so we investigated the possibility of using these cells to deliver therapeutic genes to these otherwise inaccessible sites. *Materials and methods*: We designed a novel system in which macrophages are used to deliver hypoxia-regulated therapeutic adenovirus. In this approach, macrophages are co-transduced with a hypoxically activated E1A/B plasmid and an a hypoxia-regulated E1A/B construct and an E1A-dependent oncolytic adenovirus, whose proliferation is restricted to prostate tumor cells using prostate-specific promoter elements from the TARP, PSA and PMSA genes. *Results*: When co-cultured with prostate tumour spheroids, these 'armed' macrophages migrated into the hypoxic centres of the 3D tumour masses where E1A/B protein expression was upregulated, resulting in replication of the latent E1A/B-deficient adenovirus. Multiple copies of the virus (~5000/macrophage) were released and infected neighbouring prostate tumour cells, resulting in widespread gene expression. Systemic administration of co-transduced macrophages into mice bearing human prostate xenografts resulted in their subsequent trafficking into the hypoxic areas of tumours leading to viral replication and widespread infection of neighboring tumour cells, resulting in the marked inhibition of tumor growth and reduction of pulmonary metastases. *Conclusions*: We show for the first time that macrophages can be engineered to express high titres of a therapeutic adenovirus