in vitro luciferase gene expression in A549 lung adenocarcinoma cells and selection of the most optimal mRNA:liposome ratios are the first steps towards this goal. The efficiency of mRNA/GL67 complexes will be compared to its 4th generation plasmid counterparts–pCpG-CMV-Luc/GL67 lipoplexes. This non-viral mRNA delivery system is potentially a more efficient way for delivering therapeutic genes specifically and directly to the respiratory tract. The respiratory tract is a very interesting and important target organ for gene therapy as it is affected by many acute and chronic diseases, such as cancer, cystic fibrosis, asthma, alpha-1-antytrypsin deficiency or respiratory infections. It is quite a special organ with the possibility of non-invasive, topical administration of a drug through the airways.

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A4
siRNA containing nanoparticles: stability of encapsulation and particle size

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A large effort is currently put into the development of nano-scaled carrier systems that can guide siRNA molecules to their target cells after intravenous injection. One of the main issues in this research is the integrity of the siRNA containing nanoparticles in the blood stream. The integrity of the nanoparticles comprises both the particle size and the stable encapsulation of siRNA. Techniques currently available for studying the disassembly and size distribution of siRNA containing nanoparticles are time-consuming and incompatible with biological fluids. We initially developed a fluorescence fluctuation spectroscopy (FFS) based method which allows us to monitor the integrity of siRNA-carrier complexes in less than one minute in complex biological media and at very low siRNA concentrations. Second, while the size distribution of the complexes can be easily measured in a clear dispersion by dynamic light scattering or electron microscopy, it cannot be measured in more complex biological media such as plasma or whole blood, which contain several different interfering components. To address this issue, we have developed a novel technique, based on single particle tracking (SPT) microscopy, for studying the size distribution (and aggregation) of nanoscopic drug complexes in biological fluids. For stabilization of the particle size of cationic lipid based nanoparticles, inclusion of lipids conjugated with PEG is widely used to sterically hinder aggregate formation. We have demonstrated that in order to obtain remaining siRNA complexation to the cationic liposomes, effective encapsulation inside the liposome, or in between lipid multilayers is required, since siRNA electrostatically bound to the outer side of the liposomes is quickly pushed away by the ubiquitous albumin molecules in blood which leads to siRNA degradation and loss of effectiveness. Formation of siRNA protecting multilayers is hindered by inclusion of PEG-lipids, a hurdle that needs to be overcome either by post-insertion of the PEG-lipid into multilayer containing siRNA-liposome complexes, or by efficient encapsulation of the siRNA inside the aqueous core of the PEGylated liposome. Size stabilization in buffer can be easily achieved by inclusion of minor percentages (~1%) of PEG-lipids. In whole blood however, we demonstrate that much higher percentages of PEG–lipids (5–10%) are required to achieve size stabilization. This requirement has not been previously considered because of the lack of a suitable technique to study the aggregation phenomena in whole blood. In our work we demonstrate that assaying the physicochemical properties of siRNA encapsulating nanoparticles should always be carried out in the biological media they are designed to be employed in. Two novel microscopy based methods were developed that enable such characterization in biological fluids such as serum, plasma or even whole blood.

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A5
Investigating the effects of cationic lipid-mediated toxicity and how to optimize liposomal systems for transfection purposes

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For magnetic resonance imaging (MRI) of therapeutic cells, these cells are often prelabelled in culture with iron oxide nanoparticles, enabling them to be non-invasively monitored by MRI following transplantation *in vivo*. Magnetoliposomes (MLs) are nanosized Fe3O4-cores (14 nm diameter) each surrounded by a lipid bilayer [1]. Different types of MLs have been utilised for biomedical research applications [2,3], where cationic MLs are more optimally suited for *in vitro* cell labelling [1]. Unfortunately, cationic lipids display several inherent properties which, to date, have not been clearly defined [4]. In the present work, cationic MLs as well as their non-iron oxide-containing vesicular counterparts were used to label NIH 3T3 fibroblasts. Using distearoyltrimethyl ammoniumpropane (DSTAP) as the cationic lipid the effects on cell physiology of the different particles was compared. Different amounts of DSTAP were used, indicating that when the cationic lipids exceed a certain safe threshold (3.33%), this affects cell viability by different mechanisms that are dependent and independent of actual nanoparticle internalization. Internalization-dependent mechanisms are closely linked to the induction of reactive oxygen species and altered Ca^{2+} homeostasis; the indirect mechanisms appear to indicate plasma membrane destabilization by means of transfer of the cationic lipid from the nanoparticles to the plasma membrane. The extent of cationic effects could be modified by: (1) the size of the liposome, (2) the presence of a stabilising iron oxide core, (3) the use of reactive oxygen species or Ca^{2+} channel inhibitors, (4) the nature of the cationic lipid and (5) the nature of the neutral matrix lipids. Based on these results, a novel cationic peptide-lipid conjugate (dipalmitoylphosphatidylethanolamine-

succinyl-tetralysine [DPPE-succ-(Lys)4]) was synthesized, which efficiently reduced cytotoxic effects and further augmented the internalization efficiency of the MLs [5]. In conclusion, the results indicate that the use of cationic lipids for transfection purposes should be carefully considered as they can induce severe cytotoxic effects. By carefully controlling the physicochemical properties of the liposomal systems used, many of the cytotoxic effects can immediately be reduced. These data highlight the need for careful optimization of cationic liposome formulations and that great advances can still be made with respect to diminished toxicity and enhanced internalization.

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A6
Intracellular iron oxide nanoparticle coating stability determines nanoparticle usability and cell functionality

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Iron oxide nanoparticles are routinely exploited as T2/T2* contrast agents [1]. One of the most active topics in this biomedical research area is the non-invasive imaging of pre-labelled stem or therapeutic cells upon transplantation *in vivo* in [2]. To this end, commercial particles such as Endorem® are frequently employed, however, the particles display several characteristics which makes them less suitable for *in vivo* labelling [3]. In the present work, the effects on cell physiology of in-house produced cationic magnetoliposomes (MLs), that is, 14-nm diameter iron oxide cores each individually enwrapped by a lipid bilayer containing 3.33% of distearoyltrimethyl ammoniumpropane (DSTAP)[4] – a cationic lipid – are compared with the effects of Resovist (carboxydextran), Endorem (dextran) and VSOP (citrate) iron oxide particles. When the particles are incubated at high dosages, reaching high intracellular iron levels, this results in a transient decrease in cell cycle progression, actin cytoskeleton remodelling and focal adhesion formation and maturation [5]. The extent of these effects is in line with the intracellular iron concentration and appears to be common for all particles. When reaching similar intracellular iron concentrations and when verifying that the different particles are routed along the same way and are therefore exposed to similar intracellular microenvironment at fixed time points, it is shown that intracellular stability of the coating molecules is of high importance. The results *in vitro* show that citrate-coated particles are rapidly degraded, whereas those coated with dextran are more stable, but still less than the MLs. The degradation of the particles can be shown by the increase in free ferric ions, and the distorted *r*1/*r*2 ratio of the particles, hampering their use for long-term imaging. Labelled cells further show increases in reactive oxygen species and transferrin receptor expression in C17.2 neural progenitor cells and impeded functionality of PC12 rat pheochromocytoma cells. The extent of these effects is in line with the degradability of the particles *in vitro*. The MLs appear to be the most stable particles and further show a high persistence of the label in continuously proliferating C17.2 cells. In conclusion, the results indicate that the type of coating material used is highly important with regard to maintaining cell functionality and stability of the label. Further characterization of cell-nanoparticle interactions is both warranted and needed [1].

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A7
Nuclear inclusion of inert and chromatin-targeted polystyrene beads and plasmid DNA containing nanoparticles

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Introduction and aim: The nuclear membrane is currently one of the major cellular barriers to the effective delivery of plasmid DNA (pDNA). Cell division has a positive influence on the transfection efficiency from naked pDNA and nanoparticles containing pDNA. At the end of mitosis, the pDNA near the chromatin is probably randomly included in the nuclei of daughter cells during reassemby of the nuclear envelope around chromatin. However, very little is known on the nuclear inclusion of nanoparticles during cell division. We were interested if inert nanospheres get randomly enclosed in artificial Xenopus nuclei and in nuclei of dividing cells. We investigated nanospheres with a different size and charge, and whether the enclosure could be enhanced by the use of chromatin binding peptides such as AT-hooks. *Material and methods*: Non-targeted positively charged, poly-ethyleneglycol (PEG)-ylated and negatively charged green fluorescent polystyrene nanospheres (Molecular Probes) of 100, 200 or 500 nm were used. The 100 nm nanospheres were also modified with Mel-28 (GPSKPRGRPPKHKAKT), mutated Mel-28 (GPSKPGGGPPGHKAKT) or HMGA2 (SPKRPRGRPKGSKNKS), containing an AT-hook or a mutated AT-hook (targeted nanospheres). Artificial nuclei were obtained with the 'Xenopus egg extract (XEE) nuclear assembly reaction'. The enclosure of the nanospheres in the artificial nuclei and upon microinjection was visualised by confocal fluorescence microscopy. *Results and conclusions*: Periodically the non-targeted nanospheres were able to get enclosed in the artificial nuclei but enclosure was rather limited. The enclosure of the positively charged spheres is higher than that of the negatively charged and the PEG-ylated variants, likely as a result of aspecific interactions with the nett negatively charged chromatin. Size is also important: spheres with a diameter of