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(73) Patenthaver: **SmartDyeLivery GmbH, Botzstrasse 5, 07743 Jena, Tyskland**

(72) Opfinder: **Prof. Dr. Bauer, Michael, Botzstraße 10, 07743 Jena, Tyskland**
Prof. Dr. Schubert, Ulrich, Botzstraße 5, 07743 Jena, Tyskland
Dr. Gottschaldt, Michael, Friedrich-Engels-Straße 37, 07749 Jena, Tyskland
Dr. Schallon, Anja, Gartenstraße 22, 95367 Trebgast, Tyskland
Pietsch, Christian, Lutherstraße 64, 07743 Jena, Tyskland
Dr. Gonnert, Falk, Rathenaustraße 5, 07745 Jena, Tyskland
Dr. Recknagel, Peter, Zum Zimmergrund 7, 98529 Albrechts, Tyskland
Press, Adrian, Platanenstraße 16, 07747 Jena, Tyskland

(74) Fuldmægtig i Danmark: **Patrade A/S, Ceresbyen 75, 8000 Århus C, Danmark**

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Description

The present invention relates to nanostructured delivery systems according to the claims for use in the treatment of diseases of the liver and/or the kidneys, wherein the nanostructured delivery systems comprise one or more polymers and/or lipids as well as one or more polymethine dyes as targeting units, for targeted transport of the nanostructured delivery system into a target tissue. The invention also relates to target-specific transport of one or more active pharmaceutical ingredients into a specific target tissue (cell-specific targeting) and to the use of the nanostructured delivery system according to the invention for prevention and/or treatment of diseases of the liver and/or the kidneys.

In the prior art, the use of nanoparticles with coupled dyes is known in clinical diagnostics, for example regarding the detection of organ functions or protein expressions in the course of diagnosing pathogenic states, or regarding proteomic analyses. Here the coupled dyes, usually fluorescent dyes such as cyanines, are used as markers whose fluorescence and absorption properties are measured.

WO2012/013247A1 describes the use of polymethine fluorescent dyes for determining an organ function, in particular the function of liver or kidneys, wherein the dye is used as a marker in a tissue or a body fluid, such as blood or urine and is radioactively energized, and then the fluorescence emission of the dye is detected, the data is collected and analyzed to determine the organ function to be determined.

In WO2010/116209A1 describes how fluorescent dyes and fluorescence spectroscopy may be used to detect a clinical condition based on abnormal secretion of selectin.

In “Selective Imaging and Killing of Cancer Cells with Protein-Activated Near-Infrared Fluorescing Nanoparticles.” *Macromolecular bioscience* 11.7 (2011): 927-937, Rungta et al. describe nanoparticles comprising indocyanine green for the imaging and the treatment of liver cell cancer.

Furthermore, the use of nanoparticles is known in the prior art, wherein active ingredients are transported into a certain tissue in a targeted manner (Sheridan, C. “Proof of concept for next-generation nanoparticle drugs in humans.” Nature Biotechnol, 2012 30(6): pp. 471-3; Gratton, S.E.A., et al. “The effect of particle design on cellular internalization pathways.” *Proceedings of the National Academy of Sciences* 105.33 (2008): 11613-11618; Jiang, N., et al. “Targeted gene silencing of TLR4 using liposomal nanoparticles for preventing liver ischemia reperfusion injury.” *American Journal of Transplantation* 11.9 (2011): 1835-1844).

Targeted or cell-specific transport of an active ingredient, also known as “drug targeting” or “targeted drug delivery”, is understood to refer to the targeted and selective accumulation and release of an active ingredient is at a desired site of action, wherein the efficacy of action of the active ingredient is to be increased and the systemic side effects for the surrounding tissue are to be reduced. Active ingredients which are transported often include antibodies, peptides or small molecules, such as oligonucleotides or nucleic acids.

Active ingredient transporting nanoparticles known in the prior art are used in cancer therapy; they are working according to the following mechanisms: the nanoparticle is either provided with a shell layer or an antibody. If the nanoparticle is coated with an aqueous shell layer, it is rendered unrecognizable to the immune system. If this nanoparticle is injected and is not recognized by the

immune system, it diffuses through the fenestrated blood vessels, which are “leaky” in the tumor and exhibit significantly larger orifices (fenestrations) in comparison with normal blood vessels, and it is internalized via endocytosis by the surrounding cells, which also exhibit an increased permeability in comparison with healthy cells. Disadvantageously, not only desired cells endocytose the nanoparticle, but also other (healthy cells) to which the nanoparticle is transported nonspecifically via the blood vessels, which may cause serious adverse effects. Another disadvantage the limitation of this kind of transport to tumor tissue, i.e., there is no transport into any other tissue such as the liver or kidneys. This transport occurs passively and the uptake is nonspecific, i.e., nonselective. In the second method, the nanoparticle is provided with antibodies on its surface after synthesis. These constructs target cells carrying antigens to which these antibodies bind. This transport mechanism also is passive and nonselective.

The process of passive enrichment of nanoparticles, liposomes or macromolecules as described above is referred to as EPR effect („enhanced permeability and retention") and constitutes passive drug targeting. As already mentioned, it is disadvantageous that these transport processes do not occur actively and are non-selective.

Nowhere in the prior art there is a description of an active and selective transport- and/or delivery system, which is transported actively and selectively into a specific target tissue via specific targeting units and which allows at the same time the transport of (pharmaceutically) active ingredients into the target tissue (“drug targeting”; cell-specific targeting) and which not only enables accumulation of the delivery system and optionally the (pharmaceutically) active ingredient in the target tissue by way of the targeting unit, but also allows tracking and verifying of said accumulation.

Therefore, there is a need for providing an improved transport and/or delivery system that triggers an active and selective transport of delivery systems and active ingredients into a target tissue. There is also a need for using such a transport and/or delivery system for transporting active pharmaceutical ingredients for treatment of diseases.

Such a transport system is provided by the present invention. The present invention relates to a unique theragnostic system that may be combined in a variety of ways to transport actively and selectively various active pharmaceutical ingredients (e.g. hydrophilic, lipophilic, hydrophobic, amphiphilic, anionic and cationic substances) into a target tissue (targeted or cell-specific transport of an active ingredient or “drug targeting”).

In a first aspect, the present invention relates to a nanostructured delivery system according to the claims for use in the treatment of diseases of the liver and/or the kidneys, wherein the nanostructured delivery system comprises at least one polymer and/or at least one lipid and at least one polymethine dye, wherein the at least one polymethine dye acting as a targeting unit triggers the targeted transport of the nanostructured delivery system into a target tissue.

If the nanostructured delivery system according to the present invention comprises polymers, it is referred to herein as “nanoparticle”; if it comprises lipids it is referred to herein as a “liposome”. If the nanostructured delivery system according to the present invention comprises both, polymers and lipids, it is referred to herein as “nanoparticle” or as “liposome”. Accordingly, in the context of the present invention the terms “nanoparticle” and “liposome” are used synonymously and also relate to a nanostructured delivery system comprising both polymers and lipids.

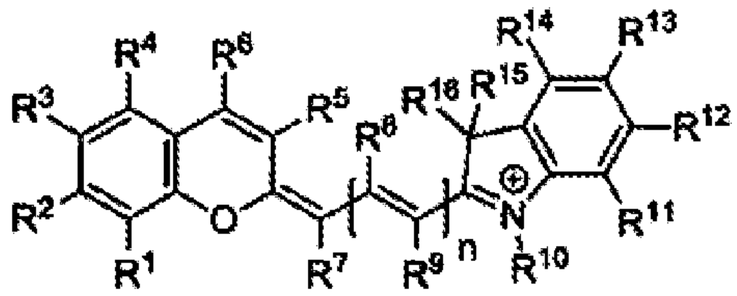
Nanoparticles are structures smaller than 1 µm in size, they may be composed of a plurality of molecules. Generally, they are characterized by a higher surface to volume ratio and thus offer a greater chemical reactivity. These nanoparticles may be composed of polymers wherein these polymers are characterized in that certain units (monomers) are repeating units. The polymers are covalently linked to each other by a chemical reaction involving the monomers (polymerization). If some of these polymers exhibited hydrophobic properties at least in part, they may form nanoscale structures (e.g., nanoparticles, micelles, vesicles) in an aqueous environment. Due to their hydrophobic properties, lipids may also be used to form nanoparticles (micelles, liposomes).

In a preferred embodiment, the present invention relates to a nanostructured delivery system, wherein the at least one polymer is selected from the group consisting of polyesters, poly(meth)acrylates, polystyrene derivatives, polyamides, polyurethanes, polyacrylonitriles, polytetrafluoroethylenes, silicones, polyethylene glycols, polyethylene oxides and polyoxazolines and their copolymers, preferably in a variety of compositions such as random, gradient, alternating, block, graft or star copolymers, or wherein the at least one lipid is selected from the group consisting of saturated and unsaturated fatty acids, preferably cholesterol, palmitic acid, phospholipids, sphingolipids and glycolipids. Preferably, the polymer and/or lipid according to the invention is/are a biocompatible polymer and/or lipid.

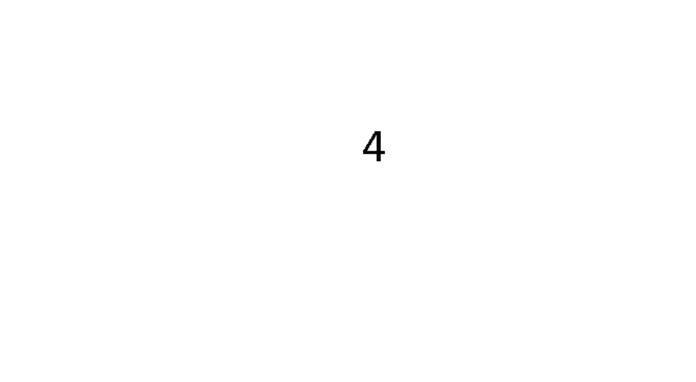
It is particularly preferred if the polymer according to the invention is a hydrophobic, hydrophilic, amphiphilic, anionic and/or cationic polymer. In particular, polymer is selected preferably from the group consisting of PLGA, PLA, PCL, PGA, PDMAEMA, PMMA, PMAA, PEI, PEtOx, PEG.

Substances which actively and selectively trigger the transport of the nanostructured delivery system according to the invention into a specific target tissue are referred to as a “targeting unit” in the context of this invention. Targeting units according to the invention are polymethine dyes. According to the invention, he terms “targeting unit” and “polymethine dye” are used synonymously.

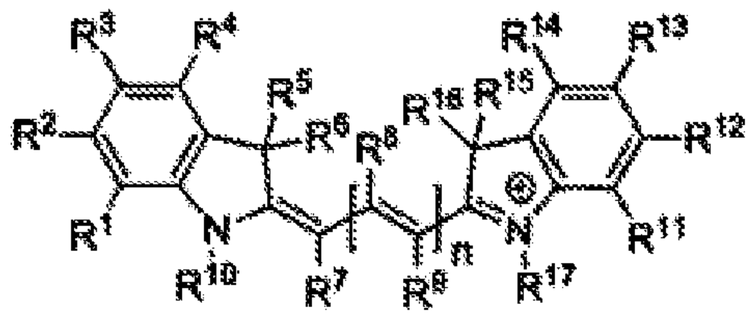
The polymethine dye according to the invention is a symmetrical or asymmetrical polymethine dye of the general structure I, II, III or IV:



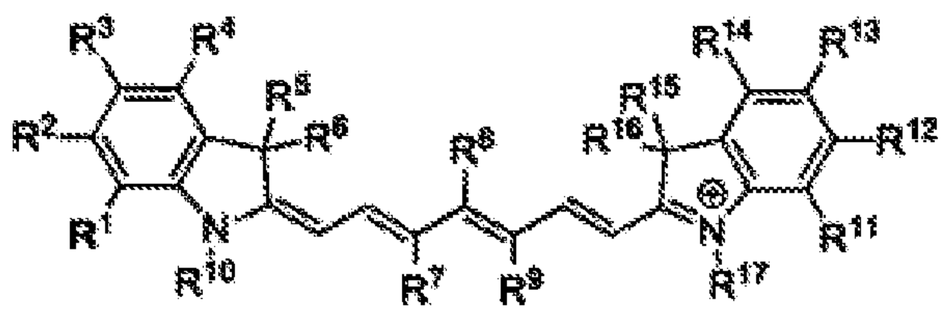
I



II



III



IV

wherein

a. n stands for the numerical values 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10,

b. R^1-R^{17} may be the same or different and maybe hydrogen, one or more alkyl, tert-alkyl, cycloalkyl or aryl-, carboxyaryl-, dicarboxyaryl-, heteroaryl- or heterocycloaliphatic radicals, alkyloxy-, alkylmercapto, heteroaryloxy-, heteroarylmercapto-, hydroxyl-, nitro- or cyano- group, an alkyl-substituted or cyclic amine function and/or two ortho-position radicals, e.g., R^3 and R^4 , R^{13} and R^{14} and/or R^1 and R^2 and R^{11} and R^{12} and/or R^7 and R^9 together may form an additional aromatic, heteroaromatic, aliphatic or heteroaliphatic ring,

c. at least one of the R^1 -R^{17} substituents has a solubilizing and/or ionizable or ionized substituent such as SO_3^- , (- SO_3H), PO_3^{2-}, COOH, OH or NR_3^+, cyclodextrins or sugar, which

determines the hydrophilic properties of these polymethine dyes, wherein this substituent may be bound to the polymethine dye also by a spacer group, and

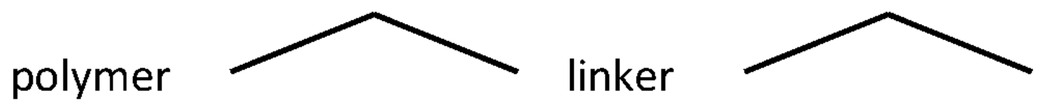
d. at least one of the R^1-R^{17} substituents has a reactive group (linker) such as isocyanates, isothiocyanates, hydrazines, amines, mono- and dichloro- or mono- and dibromotriazines, aziridines, epoxides, sulfonyl halides, acid halides, carboxylic anhydrides, N-hydroxysuccinimide esters, imido esters, carboxylic acids, glyoxal, aldehyde, maleimide or iodacetamide and phosphoramidite derivatives or azides, alkynes or olefins, wherein this substituent may be bound to the polymethine dye also by a spacer group,

e. the aromatic, heteroaromatic, aliphatic or heteroaliphatic spacer group consists of structural elements such as $[(CH_2)_a) - Y- (CH_2)_b]_c$ or $[(C_6H_4)_a) - Y- (C_6H_4)_b]$, where Y may be the same or different and comprises CR_2-, O-, S-, SO_2 , SO_2NH-, NR-, COO- or CONR functions, wherein it is bound to one of the R^1 -R^{17} substituents, and a and b may be the same or different and have numerical values of 0-18 and numerical values for c of 0-18,

f. the R^8 and R^9 substituents with corresponding n=2, 3, 4 or 5, may also be present 2x, 3x, 4x or 5x, and wherein these may be the same or different.

The targeting units (polymethine dyes) may be conjugated to the polymer via a linker.

general structure: polymer - linker - targeting unit:



The general structure of a linker according to the invention may be described as follows: at least one structural unit (polymer and/or targeting unit) carries a reactive group (linker), such as isocyanates, isothiocyanates, hydrazines, amines, mono- and dichloro- or mono- and dibromotriazines, aziridines, epoxies, sulfonyl halides, acid halides, carboxylic anhydrides, N-hydroxysuccinimide esters, imido esters, carboxylic acids, glyoxal, aldehyde, maleimide or iodacetamide and phosphoramidite derivatives or azides, alkynes or olefins, wherein these substituent may also be linked to the polymethine dye and/or to the polymer via a spacer group. By means of these reactive groups, the targeting unit is linked to the polymer (or vice versa) by a covalent bond.

The chemical bonds between polymer and/or the targeting unit and the linker may be selected to be biostable or biodegradable. One or more different targeting units may be bound to a polymer. Likewise, polymers provided with various targeting units may be combined in a nanoparticle. Thus, the polymer and the targeting unit or even both may be different. Instead of a polymer, a lipid may also be used under the same conditions as those described above, and a liposome may be used accordingly instead of a nanoparticle.

In a preferred embodiment of the present invention, the at least one polymethine dye of the nanostructured delivery system is selected from the group consisting of DY-635, DY-680, DY-780, DY-880, DY-735, DY-835, DY-830, DY-730, DY-750, DY-850, DY-778, DY-878, DY-704, DY-804, DY-754, DY-854, DY-700, DY-800, ICG and DY-IRDYE 800CW. In addition, the polymethine dyes DY-630, DY-631, DY-632, DY-633, DY-634, DY-636, DY-647, DY-648, DY-649, DY-659, DY-651, DY-652, DY-590, DY-548, DY-495 and DY-405 are also preferred. Herein, polymethine dyes serve as targeting units, which

trigger a selective transport into hepatocytes or renal parenchyma cells. The general structures of a hepatocyte targeting unit according to the invention as well as a parenchyma cell targeting unit according to the invention and the corresponding examples are given in Table 2 in Figure 8. These targeting units exhibit selectivity for a cell type (hepatocyte or renal parenchyma cells) and may confer this cell selectivity to a nanoparticle or a liposome when they are bound to the latter by a chemical bond. The selectivity of the targeting unit originates from the interaction with influx transporters, which are expressed by the target cells. Moreover, the targeting units also exhibit fluorescent properties in the red to infrared range. These fluorescent properties may also be conferred to the nanostructured delivery system, more specifically, to the nanoparticle or the liposome, such that it is not only possible to detect the accumulation of dye but also (when coupled to a nanoparticle and/or to a liposome) the accumulation of the nanoparticle and/or of the liposome within blood and tissue.

In their function as targeting units, the polymethine dyes according to the invention transport the nanostructured delivery system selectively into the target tissue. This selectivity is crucial for successful transport into the “correct” tissue and only into said tissue and constitutes a very strong advantage in comparison with the prior art. The polymethine dyes serve as transporter ligands for tissue-specific transporters. The following properties are important in order for a polymethine dye to be suitable as transporter ligand: (1) hydrophobicity and (2) combination with the specific structure. These properties are essential for the recognition as a ligand by a tissue-specific transporter (selectivity of the dye). If the polymethine dye is coupled to a polymer or lipid, such that it is exposed to the outside after synthesis of the nanoparticle or the liposome, it confers its selectivity to the nanoparticle and/or the liposome. After systemic administration or topical application, the following processes occur which are essential for the selectivity of the nanoparticle and/or the liposome:

- The nanoparticle or liposome provided with the (at least one) exposed polymethine dye is passing various tissues.
- By means of its hydrophobicity and structure, the polymethine dye is recognized by the tissue-specific basolateral or apical influx transporters, followed by their interaction at the cell surface.
- The interaction of the polymethine dye with the influx transporter does not cause a direct transport of the entire nanostructured delivery system or nanoparticle or liposome across transporter because the polymethine dye in conjunction with the covalent and stably bond nanoparticle or liposome is characterized by high molecular weight and large size. The interaction of the polymethine dye with the influx transporter results instead in accumulation and immobilization of the nanoparticle or liposome on the surface of cell.
- The accumulation and immobilization of the nanoparticle or liposome on the cell surface enhances interaction between the cell membrane and nanoparticle or liposome such that cellular uptake (endocytosis) of the nanoparticle or liposome ensues.

Cell selectivity is caused by the specific interaction of the polymethine dye coupled to the nanoparticle or the liposome with the respective tissue specific influence transporter, by which it is recognized. Influx transporters for the polymethine dyes according to the invention were defined for hepatocytes and renal parenchyma cells.

Polymethine dyes according to the invention, which are taken up specifically by influx transporters localized at the basolateral membrane of hepatocytes, render the nanoparticle specific for hepatocytes. According to current scientific knowledge and according to the FDA, the following influx transporters are deemed hepatic influx transporters:

Name	Gene
OATP1B1, OATP-C, OATP2, LST-1	SLCO1B1
OATPB3,OATP8	SLCO1B3
OATP2B1	SLCO2B1
OATP1A2	SLCO1A2
NaDC3, SDCT2	SLC13A3
NTCP	SLC10A1
OCT1	SLC22A1
OCT3	SLC22A3
OAT2	SLC22A7
OAT1	SLC22A6
OAT3	SLC22A8
PGT	SLCO2A1

In particular, ligands of these transporters comprise all polymethine dyes having a structure such as that depicted in Table 2 (Figs. 8a-e), left column.

Polymethine dyes according to the invention taken up specifically by influx transporters of the basolateral membrane of renal parenchyma cells (mainly proximal tubular cells) render the respective nanoparticle specific for these types of cells. According to current scientific knowledge and according to the FDA, the following influx transporters are deemed renal parenchymal (mainly proximal tubular cells) influx transporters:

Name	Gene
OCT2	SLCO1B1
OAT1	SLCO1B3
OAT3	SLC22A8
OATP4A1	SLCO4A1
OATP4C1	SLCO4C1
OCT1	SLC22A1
OCT3	SLC22A3
PGT	SLCO2A1

In particular, ligands of these transporters comprise all polymethine dyes having a structure such as that depicted in Table 2 (Figs. 8a-e), right column.

A further preferred embodiment of the invention relates to a nanostructured delivery system, wherein the at least one polymethine dye triggers the uptake of the nanostructured delivery system

into the cells of the target tissue by means of at least one tissue-specific transporter. Particularly preferable, the tissue-specific transporter is selected from the group consisting of OATP1B1, OATP-C, OATP2, LST-1, OATP1B3, OATP8, OATP2B1, OATP1A2, NaDC3, SDCT2, NTCP, OCT1, OCT3, OAT2, OAT1, OAT3, PGT, OCT2, OAT1, OATP4A1, OATP4C1.

In the context of the invention, the terms “tissue-specific transporter”, “transporter” and “influx transporter” are used synonymously.

According to the invention, the terms “nanostructured delivery system”, “nanoparticle” and “liposome” are used in conjunction with the transport to and uptake into the target tissue by means of a tissue-specific transporter.

After uptake of the nanostructured delivery system and/or the nanoparticle or the liposome into the target tissue, both, the polymethine dye and an active pharmaceutical ingredient, also comprised by the present invention, are released.

Release of a nanoparticle as part of the nanostructured delivery system:

1. acidification of the endosome → destabilization of the nanoparticle, degradation of the polymer by spontaneous or enzymatic cleavage;
2. release of active substances (those which are capable of penetrating the endosome);
3. release of the active ingredient, desorption of the dye from the polymer;
4. polymer constituents are supplied to various metabolic pathways, dye is secreted.

Release of a liposome as part of the nanostructured delivery system:

1. uptake by endosomes → acidification → fusion of the liposome with the endosome membrane subsequent to endocytosis or direct fusion of the liposome with the cell membrane;
2. both pathways result in direct release of the active ingredient into the cytoplasm;
3. in the case of the targeting unit (polymethine dye) being coupled to the lipid by a biolabile bond, cleavage of the bond and cleaved and the dye is excreted. When using a biostable bond, the polymethine dye remains coupled to the lipid. If there is subsequently degradation of the lipid, the polymethine dye may be secreted with a small lipid radical. In all probability a portion of the lipid may be incorporated together with the polymethine dye into the cell membranes.

The nanostructured delivery system according to the invention further comprises at least one active pharmaceutical ingredient. In a preferred embodiment, at least one active pharmaceutical ingredient is selected from the group consisting of low-molecular weight substances, in particular inhibitors, inductors or contrast agents as well as higher molecular weight substances, in particular potentially therapeutically usable nucleic acids (e.g., short interferon RNA, short hairpin RNA, micro RNA, plasmid DNA) and proteins (e.g., antibodies, interferons, cytokines). The following table describes examples of active ingredients whose specific administration via the nanostructured delivery system of the present invention permits novel therapeutic options:

Active Ingredient	Examples of active ingredient	Treatment/ Disease	Organ/Tissue
glucocorticoids	decortin	organ transplants	liver, kidneys

cytostatics, alkylating agents	cyclophosphamide	organ transplants, tumors	liver, kidneys
antimetabolites	methotrexate	organ transplants, tumors	liver, kidneys
intercalating agents	mitoxantrone	organ transplants, tumors	liver, kidneys
antibodies	rituximab (anti-CD20), daclizumab (anti-CD25)	organ transplants, tumors	liver, kidneys
interferons	IFN-β, IFN-γ	organ transplants	liver, kidneys
Phospho-inositol-3-kinase inhibitors	D-116683, AS605240, IPI-145	tumors, sepsis	liver, kidneys
Coxibs	celecoxib, etoricoxib	acute renal failure	kidneys
JNK inhibitors	CC-401, celgene	Malaria	liver
X-ray contrast agents	peritrast	diagnosis e.g. of tumors	liver, kidneys
Paramagnetic X-ray contrast agents	gadopentetate dimeglumine (magnevist)	diagnosis e.g. of tumors	liver, kidneys

In a particularly preferred embodiment, the active pharmaceutical ingredient is a lipophilic, hydrophobic, hydrophilic, amphiphilic, anionic and/or cationic active pharmaceutical ingredient.

The term “active pharmaceutical ingredient” according to the invention is understood to refer to any organic or inorganic molecule, substance or compound having a pharmacological effect. The term “active pharmaceutical ingredient” is used synonymously herein with the terms “drug” and “medication”.

The nanostructured delivery system according to the present invention represents, as of to date, a unique theragnostic system, which may be combined a variety of ways in order to transport a wide variety of substances, in particular active pharmaceutical ingredients (e.g., hydrophilic or lipophilic small molecules, but also nucleic acids), actively and selectively into a specific target tissue. The transport of the active pharmaceutical ingredient is triggered by targeting units, polymethine dyes, as a component of the nanostructured delivery system which interacts with tissue-specific transporters on the target cell. By means of selecting the polymethine dyes (DY), the active pharmaceutical ingredients and the polymers and/or lipids as well as by varying their parameters, it is possible to generate nanoparticles and/or liposomes which are tailored individually to the respective application, in particular regarding the active pharmaceutical ingredient to be transported and/or the target tissue. In this way, it is possible to transport efficiently one or more active pharmaceutical ingredients as components of the nanostructured delivery system into a specific tissue or cell type (target tissue) and for release therein. The active pharmaceutical ingredients may comprise those exhibiting low or no bioavailability without being enclosed in a nanoparticle or a liposome or those exhibiting low or no *in vivo* stability or those being able to act only in specific organs and/or cells (target tissue). The specificity and accumulation of the nanostructured delivery system (nanoparticle or liposome) and/or components thereof such as polymers, lipids or active pharmaceutical ingredient(s) in the target tissue may be verified and tracked, i.e., detected by means of the fluorescence properties of the nontoxic polymethine dyes in the red to infrared range.

The term “target tissue” includes all tissues, organs or cells into which the transport of a nanostructured delivery system and/or components thereof, in particular an active pharmaceutical

ingredient, is possible and reasonable. In particular, target tissues include all tissues, organs or cells into which transport of one or more active pharmaceutical ingredient is possible and reasonable, for example, for treatment or diagnosis of a disease. Target tissues according to the invention are liver, kidneys and tumors originating from these tissues, e.g., hepatocellular carcinomas or hypernephromas. The terms “target tissue”, “target cell”, “cells of a target tissue” and “organ” are used synonymously in this context.

By conjugating the polymethine dyes according to the invention (hereinafter DY) to polymers or lipids, functionalized polymers (e.g., DY-PLGA, DY-PLA, DY-PCL) and/or functionalized lipids are produced. Subsequently, these are used for producing nanoparticles and/or liposomes, preferably by means of a single or double emulsion technique or precipitation technique. Here, the nanoparticles and/or liposomes may be tailored individually to the respective situation. The various possibilities are listed as examples in Table 1 (Figure 1). The polymethine dyes may be conjugated with a variety of different polymers and/or lipids, such that by means of the special combination of a polymethine dye with a lipid or polymer, highly selective nanostructured delivery systems may be provided. Synthesis of functionalized polymers is depicted schematically in Figure 2 and is illustrated in detail in Example 1. The production of functionalized polymers or lipids, nanoparticles and liposomes according to the invention as well as the inclusion of active pharmaceutical ingredients may be performed according to conventional methods known from the prior art. Preferred production processes are disclosed in the examples and figures of the present invention.

The disclosure further relates to a pharmaceutical composition containing a nanostructured delivery system according to the invention as well as suitable excipients and additives. The term “excipients and additives” is understood to refer to any pharmacologically acceptable and therapeutically expedient substance that is not an active pharmaceutical ingredient but may be formulated together with the active pharmaceutical ingredient in the pharmaceutical composition in order to influence qualitative properties of the pharmaceutical composition, particularly to improve them. Preferably, the additives and/or excipients do not exhibit any appreciably adverse pharmacological effects or they at least do not exert any adverse pharmacological effect with regard to the intended treatment. Suitable excipients and additives include, for example, pharmaceutically acceptable organic or inorganic acids, bases, salts and/or buffer substances. Examples of inorganic acids include hydrochloric acid, hydrobromic acid, nitric acid, sulfuric acid and phosphoric acid, wherein hydrochloric acid and sulfuric acid are preferred in particular. Examples of suitable organic acids include malic acid, tartaric acid, maleic acid, succinic acid, acetic acid, formic acid and propionic acid and, preferred in particular, ascorbic acid, fumaric acid and citric acid. Examples of pharmaceutically acceptable bases include alkali hydroxides, alkali carbonates and alkali ions, preferably sodium. Mixtures of these substances may be used in particular to adjust and buffer pH. Preferred buffer substances in the sense of the invention also include PBS, HEPES, TRIS, MOPS, as well as other physiologically acceptable buffer substances. Additional suitable excipients and additives include solubilizers or diluents, stabilizers, suspension mediators, preservers, fillers and/or binders as well as other conventional excipients and additives known in the prior art. The choice of excipients as well as the required quantities thereof will depend on the active pharmaceutical ingredient and its administration route. Preferably, pharmaceutical compositions according to the present invention are administered parenterally, particularly intravenously. Preparations in the form of suspensions and solutions as well as dry preparations which are easily reconstitutable are suitable for all parenteral applications.

A pharmaceutical composition may be produced by any method known in the state of the art.

Dosage of the components of a pharmaceutical composition depends on various factors, for example, the type of active pharmaceutical ingredient, the disease, the condition of the patient (mammal, preferably human) who the pharmaceutical composition is to be administered to, and on the administration route, e.g., parenterally, intravenously or by some other method. The skilled person is familiar with these parameters and thus, the determination of the dosage is subject to the general technical knowledge of those skilled in the art.

The disclosure further relates to the use of a nanostructured delivery system or a pharmaceutical composition according to the disclosure as a pharmaceutical drug.

The nanostructured delivery system according to the invention or a pharmaceutical composition according to the disclosure is for use in the treatment of diseases of the liver and/or kidneys, preferably infectious diseases involving damage to the liver and/or kidneys, for example, malaria and hepatitis C, liver failure, for example, drug-induced liver failure and fulminant liver failure, cirrhosis of the liver, for example, alcohol-induced cirrhosis of the liver, metabolic diseases of the liver, for example, Wilson's disease and Meulengracht's disease, excretory dysfunctions of the liver, liver tumors, primary liver tumors, for example, hepatocellular carcinomas, angiosarcomas and hepatoblastomas, renal tumors, primary renal tumors, for example, clear-cell carcinoma, papillary carcinoma and chromophobic carcinoma, various types of nephritis, chronic and acute renal failure and diseases that trigger subsequent damage to the liver and/or kidneys, for example, sepsis.

The nanostructured delivery systems and the targeting units according to the invention, in particular polymethine dyes, provide a unique possibility for combining diagnosis and treatment in one molecule. Thus, efficacy of treatment may be predicted on the one hand by evaluating the uptake of the unconjugated targeting structure, while treatment with the same targeting structure on the nanoparticle or liposome may also be monitored and controlled on the other. Due to the high flexibility of the targeting structure within the linker region, the targeting units may be chemically coupled to a wide variety of lipids and polymers. Due to the chemical structure of the targeting unit, it is very stable in contrast to biological targeting units (e.g., antibodies or peptides) and it may be subjected to chemical purification and analysis. Therefore, synthesis is high reproducible and controllable. Since the targeting unit functions as a ligand of tissue-specific transporters, it may be eliminated *in vivo* after desorption from the polymer thereby preventing intracellular accumulation and toxicity. Due to current developments in imaging, particularly in the field of multispectral optoacoustic tomography, the targeting unit may be detected directly. According to the invention, contrast media for use in computer- assisted X-ray tomography or magnetic resonance tomography may also be enclosed in the nanoparticles or liposomes such that they may be localized as well.

So far, the disclosed system is unique in its versatility and cell-specificity, since the system combines diagnosis and treatment via a dye serving as targeting unit, which in turn fluoresces in the red to infrared range and is eliminated in an extremely efficient manner, due to its selectivity for biotransporters, by the liver and kidneys.

The invention will now be illustrated in examples as illustrated in the figures:

In **Figure 1** an overview regarding possible variations of a nanoparticle according to the invention is shown, their influence concerning the physicochemical properties of the respective nanoparticles and the biological effects (Table 1).

Figure 2 schematically depicts the functionalization of the polymers according to the invention. **A**: Synthesis of the functionalized PLGA polymer by EDC coupling of the polymethine dye DY635 to the carboxylic acid terminal group of the PLGA to form DY635-PLGA-NP (also referred to as DY635-PLGA; the two terms are used interchangeably in the present invention). **B**: SEC elugram of functionalized PLGA polymers using UV and IR detectors. Synthesis and functionalization are described in detail in Example 1.

Figure 3 demonstrates design and production of nanoparticles according to the invention. Individual ultrasonic steps are indicated by gray pins (arrows). A detailed description may be found in Example 2. **A**: Structure of the nanoparticles and as well as method of production using a single emulsion technique. The hydrophobic polymer is depicted in dark gray with the hydrophobic active ingredient shown in medium gray and the surfactant (tenside) in light gray. **B**: Structure of the nanoparticles and method of production using a double emulsion technique. The hydrophobic polymer is depicted in dark gray, the hydrophobic active ingredient in white. Again, the upper light gray layer represents water with surfactant. **C**: Overview (cross sections) of possible variations of a nanoparticle according to the invention and their influence concerning the physicochemical properties of the respective nanoparticles and the biological effects (Table 1). The hydrophobic polymer or lipid is depicted in black, a possible active pharmaceutical ingredient is shown in gray, galactose, and DY635 as the transporter mediating cell- specific uptake into hepatocytes, wherein only nanoparticles according to the invention retain their cell specificity.

In **Figure 4,** the results of a characterization regarding a selection of nanoparticles according to the invention are shown. Box plots comprise the 0.25 to 0.75 quantile. The median value is plotted as a horizontal bar, the mean as a cube. The whiskers each represent a highest and lowest value, respectively. **A**: The size of PLGA nanoparticles does not differ from that of siRNA/PEI-loaded PLGA nanoparticles (approximately 180 nm). DY635- PLGA nanoparticles, however, are significantly larger (approximately 260 nm). **B**: The zeta potential of PLGA nanoparticles is slightly negative. Due to the use of DY635-PLGA nanoparticles, the potential fluctuates into the weakly positive zeta potential (not significant). Due to the loading with the siRNA/PEI polyplexes (siRNA/PEI+PLGA nanoparticle), the zeta potential changes significantly and becomes strongly positive (+76 mV). **C**: For determining the amount of endotoxin, nanoparticle (NP) solutions in a concentration of 25 mg NP/mL, such as those also used in vivo, were investigated. The endotoxin load fluctuated between 0.4 mg/mL and 0.6 mg/mL in the samples. However, the value was always below the FDA limit value (2.5 ng/mL). **D**: NP solutions of 25 mg/mL are also used for the hemolysis and aggregation assays. Nanoparticles were used for the DY635-PLGA assay, such as those also used in the in vivo experiments. A further description of this can be found in Example 3.

In **Figure 5** uptake kinetics and characterization of RNAi into Hepa 1-6 cells *in vitro* are displayed. **A**: Diagram (heat map) is describing the time- and concentration-dependence of RNAi. The time is plotted in hours (h) on one axis, siRNA concentration (ng/100,000 cells) on the other axis. The change in HMGCR expression in comparison with untreated controls is shown as percentage in scaled greys (scale shown above the graph). For generation of points in the heat map, siRNA concentrations of 1, 5, 10, 25, 50, 100, 200 and 400 ng/100,000 cells were used and tested after 12, 16, 24, 32, 40 or 48 hours. Three independent replicates were generated for each point in time. The result was then corrected with respect to the gene expression level of HMGCR in untreated Hepa 1-6 cells and normalized using HPRT gene expression. **B**: Uptake of the nanoparticles into Hepa 1-6 cells after 0 and 30 minutes (min).

DY635 is visualized on LSM using the Cy5 channel. Cell nuclei were stained with DAPI after washing and fixing the cells. A further description of this process may be found in Example 4.

Figure 6 depicts organ specificity and kinetics of a nanoparticle according to the invention in liver, kidneys, spleen and heart. **A**: Comparison of the decay kinetics of DY635 vs. DY635-PLGA-NP. Averages are from 3 ROIs in the liver. Error bars represent SEM. **B + C**: Superimposing the images of Cy5 (DY635) channel (**B** light gray to white, **C** light gray) and DAPI (background) channel on the IVM at different points in time. **D - G**: organ sections at 5 µm, 10 minutes after injection of DY635-PLGA-NP. DY635-PLGA-NP and/or DY635 (Cy5 channel, imaged in green) and cell nuclei (DAPI-stained, imaged in red) are superimposed in the images. **F, G**: Here, stainings are superimposed onto the liver structure (shown in blue), visualized in phase contrast. A further description may be found in Example 5.

In **Figure 7**, the route of secretion of a nanoparticle according to the invention is depicted. The route of secretion of DY635- PLGA-NP: **A**: For calculating the plasma decay rate, a standard curve is generated using untreated plasma with DY635- PLGA nanoparticles. A standard series with DY635 in bile was used for the determination of bile secretion. **B**: In this figure, the percentage of DY635 "recovery" in the bile is shown. The measurement points were calculated based on data from A. The curve was approximated by using OriginPro 8.5, QuickFit: exponential decay with offset approximated.

Figure 8 shows targeting units according to the invention, namely polymethine dyes. In figure 8, the general structure for a hepatocyte targeting unit and the general structure for a renal parenchyma cell targeting unit is represented, specifying the linker bound to the polymer and/or lipid as well as examples of such hepatocyte targeting units and parenchyma cell targeting units (Table 2). The targeting units exhibit a selectivity for one cell type (hepatocytes or renal parenchyma cells) and may transfer this cell-selectivity to a nanoparticle or a liposome when bound to it by a chemical bond. The selectivity of the targeting unit occurs due to the interaction with influx transporters expressed by the target cells. The targeting units also have fluorescence properties ranging from red to infrared. Fluorescence properties may be transferred to the nanostructured delivery system, more specifically to the nanoparticle or the liposome, allowing not only for the detection of the accumulation of the dye in blood and in tissue, but also (if it is coupled to the nanoparticle and/or the liposome) of nanoparticles and/or liposomes.

Figure 9 relates to the efficacy of the nanostructured delivery systems according to the invention regarding the transport of an active pharmaceutical ingredient. **A**: Plasma cholesterol levels after two injections of the nanostructured delivery system transporting an HMGCR-siRNA and/or after two injections of a control substance. This figure shows a median bar plot, error bars describe the mean error of the standard deviation, numbers in the bars describe the number of animals in each group, significances were determined by a two-tailed U-test, ** significance level 0.01.

Figure 9 A: These results demonstrate that the utilization of the approach as described allows for a significantly lower the plasma cholesterol concentration. The organ-specific nanostructured delivery system exhibits the largest effect. It is clear from **Figure 9 B** that by means of the organ-specific nanostructured delivery system, an organ-specific and strong effect is achieved in hepatocytes. However, the nonspecific nanostructured delivery system does not exhibit any specific or lesser down-regulation of HMGCR.

In **Figure 10,** DY-635 utilized as an inventive polymethine dye targeting is shown to interact, according to the invention, with a human basolateral hepatocyte transporter. Figure 10 **A, B** show bar plots of the mean value, with error bars describing the mean error of the standard deviation. All experiments

here were performed six times each. The significances were determined by a bilateral U-test, **significance level 0.01, *significance level 0.01.

The invention is described below in more detail by way of examples, although it is not limited to them.

Examples

Example 1: Synthesis of functionalized polymers

The synthesized nanoparticles are based on the hydrophobic polymer poly(lactic-co-glycolic acid) (PLGA), which is biocompatible and biodegradable. This polymer can be bound covalently to an amine-functionalized dye on the basis of its active carboxylic acid group ("acid terminated") by means of coupling reagents such as EDC (1-ethy1-3-(3- dimethylaminopropyl) carbodiimide). Here, the polymethine dye DY- 635 was used (see Figure 2). Every 100th polymer chain was functionalized. The polymers were then separated from the free dye DY-635 by dialysis and purified by precipitation. The characterization was based on size exclusion chromatography (SEC), in which a UV/Vis detector and an RI (refractive index) detector were combined. Figure 2 shows the graphic plot of the synthesis and an SEC elugram.

Example 2: Production of nanoparticles

After functionalization of the polymers (Example 1), nanoparticles were produced by a single emulsion (**A**) and by a double emulsion (**B**) using high-frequency ultrasound, which promotes the formation of nanoscale particles with help of surface-active substances (surfactants), i.e., polyvinyl alcohol (PVA) in the present case. Therefore, the hydrophobic polymers were dissolved in ethyl acetate, a solvent that is immiscible with water (25 mg/mL). The surfactant used was 0.3% PVA (polyvinyl alcohol) in ultrapure water, where the total polymer concentration was 2.5 mg/mL. The ethyl acetate suspended polymer was added to water with surfactant and nanoparticles were formed by using ultrasound (A). If hydrophilic substances were to be enclosed, the hydrophilic substance was first dissolved in water and then added to the ethyl acetate suspended polymer and subjected to ultrasound. Next, water with surfactant was added and, again, nanoparticles were formed by using ultrasound. Figure 3 shows the results obtained by this emulsion technique. The nanoparticles with a diameter of approximately 200 nm produced in this way were then stirred in a stream of air until the entire organic solvent (ethyl acetate) had evaporated and the particles were thus stable in water. To remove the excess surfactant, the nanoparticles were washed thoroughly at least twice in ultrapure water. This may be supported by vortexing and incubation in an ultrasonic bath. In conclusion, the particles were lyophilized and their mass was determined.

Example 3: Characterization of the nanoparticles

Nanoparticles of DY-635-conjugated PLGA (DY635-PLGA-NP) were produced with constant parameters and reproduced. The assays used are explained below:

- Size: size determination of the various nanostructured delivery systems dissolved in deionized water by dynamic light scatter (for example, Zetasizer (Malvern Instruments GmbH)) or by electron micrographs.
- Shape: shape determination by electron micrographs.
- Charge: determining the electrophoretic signal (zeta potential, surface charge) of the various nanostructured delivery systems dissolved in deionized water using a Zetasizer (Malvern Instruments GmbH) for measurement.

- Endotoxins: endotoxin measurement by LAL chromogenic assay according to D. E. Guilfoyle, et al., Evaluation of a chromogenic procedure for use with the Limulus lysate assay of bacterial endotoxins drug products. *J Parenter Sci Technol,* 1985, 39(6): pp. 233-6.
- Hemolysis: measurement of the hemoglobin concentration of erythrocytes which were incubated with the particles in physiological buffer for one hour. The hemoglobin concentration measurable in the supernatant increases when there is damage to the erythrocyte membrane.
- Aggregation: Measurement of the absorption of erythrocytes incubated with the polymers in physiological buffer. Samples with cell aggregates show a lower absorption than homogeneously distributed, non-aggregated cells.

The results are shown in Figure 4. **A**: size, **B:** charge, **C**: endotoxin load. For the size measurement, the dynamic light scatter was used and the charge was determined by means of the zeta potential. **D**: In addition, it was shown that DY635- PLGA-NP dispersed in blood do not exhibit any lysing properties and do not cause erythrocyte aggregation. **E** and **F**: The particles are round or spherically shaped in electron micrographs (SEM), both unloaded and without targeting units (**E**) as well as loaded and with targeting units (**F**).

Example 4: Triggering drug-associated effects by RNAi and uptake *in vitro* ("proof of concept")

Procedure for Figure 6 A: Hepa 1-6 cells were cultured under standard culture conditions (37 C, 5% CO2, DMEM 4.5 g/L glucose, 10% heat-inactivated fetal bovine serum, 1% penicillin/streptomycin) in 6-well plates (100,000 cells per 9.6 cm^2). After 24 hours, various concentrations of the nanostructured delivery system, prepared as described in Example 7B, were added to the wells and incubated for various periods (concentrations and incubation times are disclosed in Figure 5 A). After incubation, the cells were washed with Hank's Balanced Salt Solution (HBSS) and lysed in RLT buffer (Qiagen GmbH), to which 1% p-mercaptoethanol was added. The mRNA was isolated from the lysate and analyzed using RT-qPCR. Subsequently, values were normalized to hypoxanthin-guanine- phosphoribosyl transferase expression and HMGCR expression (HMGCR: 3-hydroxy-3-methylglutaryl- coenzyme-A-reductase coenzyme or HMG-CoA) was compared with non-transfected Hepa 1-6 cells.

Procedure for Figure 6 B: Hepa 1-6 cells were cultured under standard culture conditions on chamber slides (Nunc, Thermo Scientific GmbH) (5,000 cells/1.5 cm2). After 24 hours, the cells were mixed with 100 μg/mL (final concentration) DY-635-modified nanostructured delivery system (produced as described in Example 7B). After incubating for 30 minutes under standard culture conditions with the nanostructured delivery system, the cells were washed with HBSS and fixed with 5% formalin (pH 7) for 15 minutes. Then, the microscope slides and the cells cultured thereon were washed and cell nuclei were stained with DAPI. For analysis by laser scanning microscopy, cells were moistened with VectaShield (Vector Labs, Inc.) and sealed with a cover glass. The nanostructured delivery system was detected at 633 nm (excitation) due to modification with DY-635, whereas cell nuclei were visualized at 460 nm (excitation).

The results are depicted in Figure 5 **A**: HMGCR gene expression in Hepa 1-6 cells could be downregulated up to 70% by siRNA transfection. HMGCR (3-hydroxy-3- methylglutaryl-coenzyme-A-reductase or HMG-CoA) represents the key enzyme of a central metabolic process – the biosynthesis of cholesterol. The downregulation of this metabolic gene as shown in this experiment demonstrates the efficient transport of the active ingredient according to the invention by the nanostructured delivery system. Furthermore, elevated plasma cholesterol levels are of central importance in the

development of arteriosclerosis. The treatment method presented here utilizing the inventive nanostructured delivery systems provides an interesting alternative to traditional treatment with statins, additionally, this constitutes a first step in the direction of gene-transport in humans exhibiting congenitally elevated cholesterol levels (familial hypercholesterolemia). **B**: This figure shows that DY635-PLGA-NP is taken up by Hepa 1-6 cells (murine hepatocyte cell line) within 30 minutes. Such a rapid and intense uptake of a nanoparticle has not been described before in the prior art.

Example 5: *In vivo* targeting: Organ specificity and description of the route of secretion

Production of the nanostructured delivery system for this experiment was carried out as described in Example 2 (B). For injection, the freeze-dried nanostructured delivery system was dissolved in a sterile 5% glucose solution (Glucosteril G5, Fresenius SE&Co KGaG) with the assistance of an orbital mixer and an ultrasonic bath.

Procedure (Figure 6 A): Mice or rats were catheterized intravenously (jugular vein). Next, the liver was prepared *ex situ* using an intravital microscope. Next, DY-635 (13 pmol/g body weight (BW)) or the nanostructured delivery system carrying a DY-635 modification (6.5 µg/g BW) was injected intravenously and the specific fluorescence of DY-635 was measured at 633 nm in the liver over time and various Regions of Interest (ROIs) were quantified in the images recorded over time. Figure 6 shows representative images of the measurement of DY-635 alone (Figure 6 B) or DY-635-modified nanostructured delivery systems (Figure 6 C). DY-635 or the DY-635-modified nanostructured delivery systems were detected by fluorescence of DY-635 at 633 nm. The structure of liver tissue was visualized by autofluorescence of NADH/NADH+ at 450 nm. For demonstration of organ specificity, male mice were injected with 6.5 µg of the DY-635-modified delivery system per g BW administered via a central intravenous catheter. Each animal was euthanized painlessly 10 minutes after injection and organs were removed and cryoprepared for histological workup. Next, 5 µm-thick sections of the organs were prepared using a cryotome; sections were then counterstained with DAPI. Subsequently, all organs were examined at the same settings with regard to DAPI-stained cell nuclei (at 430 nm) and with regard to nanoparticles (at 633 nm). Results are shown in Figure 6. **C**: DY635-PLGA-NP nanoparticles were taken up into hepatocytes after just 1 minute (cobblestone-type signal-rich areas; imaging after 1 and 10 minutes (1 min, 10 min)). After about 50 minutes (50 min), almost all DY635 dye has been eliminated from the liver. Similar results were found with DY635. **B**: On the whole, the results are similar to those obtained in earlier studies with pure DY635. **A**: The decay rate of DY635 in the liver is shown. The altered decay rate of DY635 intensity in the liver from DY635 and DY635-PLGA-NP indicates that DY635 is also still bound intracellularly to the PLGA polymer and is released and eliminated only after hydrolysis of PLGA. **D-G**: organ specificities which were verified by means of various organ sections are shown. After injection of DY635-PLGA-NP (green), marked accumulation is exhibited by liver tissue. Hardly any nanoparticles (spleen, heart) or no nanoparticles at all (kidneys), however, are visible in spleen (**E**), heart (**F**) and kidneys (**G**).

Example 6: route of secretion of DY635-PLGA nanoparticles

By way of this experiment, plasma decay rate and bile secretion of DY635-PLGA nanoparticle and/or of polymethine dye DY635 were investigated. Male rats (strain: RccHan:WIST) are catheterized (placing catheters into the jugular vein, the carotid artery, and the common bile duct). Next, the substance to be tested is injected through the intravenous catheter. Subsequently, blood is drawn from the arterial catheter at short intervals, and bile is drawn from the catheter in the common bile duct. The blood is then processed further to obtain blood plasma. The, the amount of DY635 is measured by fluorimetry

based on a calibration curve. DY635-PLGA-NP could be detected in arterial blood at most after 4 minutes and was taken up almost completely into organs up to 20 minutes after injection of DY635-PLGA-NP, i.e., within 15 minutes (min). There was a slight delay because, as already described, DY635 must be released at first from the nanoparticles, followed by DY635 secretion into the bile (Figure 6, illustration A). The (calculated) 95% recovery of DY635 in bile also demstrates the high specificity of DY635-PLGA-NP for hepatocytes (Figure 6, illustration B).

Example 7: Inclusion of active pharmaceutical ingredients in nanoparticles

After functionalization of the polymers or lipids with the targeting unit (Example 1), nanoparticles were produced by single emulsion (A) and double emulsion (B).

(A) Nanoparticles from a single emulsion

If hydrophilic substances were to be included, the single emulsion technique was used. In this case, the active ingredient is enclosed in a hydrophobic polymer core by hydrophobic interactions. To this end, the active ingredient was dissolved together with the polymer in a suitable organic solvent. An organic solvent is suitable when it is neutral with respect to both the polymer and the active ingredient, i.e., it does not trigger any chemical changes therein and has no influence on their stability. Ethyl acetate was used in the present case. The mixture was overlayered with the hydrophilic solution. For stabilization of the nanoparticles and for increasing yield, a surfactant may be added to the hydrophilic solution in the case of double emulsion nanoparticles (cf. double emulsion nanoparticles). The two phases were combined by high-energy ultrasound emitted coaxially with an electrode immersed perpendicularly into the sample. This resulted in nanoparticles.

(B) Double emulsion nanoparticles

For production, hydrophobic polymers were dissolved at high concentration in a suitable solvent. An organic solvent is suitable when it is neutral with respect to both the polymer and the active ingredient, i.e., it does not not trigger any chemical changes therein and has no influence on their stability. Ethyl acetate was used in the present case. The concentration of the polymer depends on the size, hydrophobicity, solubility and stability of the polymer. Here, suitable concentrations are between 2 and 50 mg/mL. The active ingredient was dissolved in ultrapure water at a suitable concentration. A suitable concentration of active ingredient depends on the chemical properties of the active ingredient and the capacitance of the nanoparticles. Thereafter, the shell polymer dissolved in the organic solvent was overlayered with the active ingredient dissolved in aqueous solution. The polymer and the organic solvent had to be present in the sample in an excess of at least tenfold. Particles that were hydrophobic on the outside were formed by bombardment using high-energy ultrasound coaxially with an electrode immerse in the sample. The active ingredient was thereby enclosed in a hydrophobic core due to interaction with hydrophilic groups of the nanoparticle in the interior. In the second step, a suitable surfactant was dissolved in ultrapure water at a suitable concentration. A surfactant concentration is adequate when it produces enough nanoparticles. The concentration depends on the ambient conditions and must be determined experimentally. It is usually between 0.01 and 5% (w/v). Then, enough surfactant was added to the sample so that the concentration of polymer amounted to only at least 1/10 of the starting amount. Again, two phases were formed and were mixed by high-frequency ultrasound emitted coaxially to an electrode immersed perpendicularly into the sample. By mixing the surface-active substances (surfactants), i.e., polyvinyl alcohol in the present case, the formation of water-soluble nanoscale particles was ensured.

For the sake of illustration, a batch is described, in which hydrophilic small interferin RNA (siRNA) complexed with polyethyleneimine (PEI) was enclosed in PLGA nanoparticles. The PLGA was first modified with DY-635, so that one out of every 200 chains would carry a dye molecule:

1. 2.4 µL PEI (1 mg/ml) was mixed with 2µL siRNA (1 µg/µL) and mixed with 45.6 µL ultrapure water. The mixture is referred to below as a polyplex because the anionic siRNA and the cationic PEI interact with one another and a PEI binds and stabilizes the siRNA in a tight mesh network.
2. 325 mg DY-635-conjugated PLGA was dissolved in a total of 12.35 µL ethyl acetate.
3. 90 µL polymer solution from (2) was mixed with 50 µL polyplexes from (1) with high-frequency ultrasound (emitted as described above).
4. 1 ml PVA 0.3 wt% in ultrapure water was added to the mixture, which was then exposed to ultrasound.
5. The resulting nanoparticles were purified and freeze-dried.

Purification (for (A) and (B))

The nanoparticles produced in this way had a diameter which was a function of the size and the material of the vessels, the intensity of the ultrasound and the substance concentration; they were sized ranging from 120 nm to 220 nm. Under stable conditions, after producing the nanoparticles, the solvent was removed. To remove excess surfactant, the nanoparticles were washed several times (at least twice) by centrifuging, removing the supernatant and resuspending the nanoparticles in sterile ultrapure water. Then, the particles were lyophilized and their mass was determined.

Example 8: Inclusion of active pharmaceutical ingredients in liposomes

After functionalization of the polymers or lipids with the targeting unit (Example 1), liposomes were produced as follows:

1. Production of a 50 mM lipid solution from for example, 1:1 DOPC : DSPC (1,2-dioleolyl- sn-glycero-3- phosphocholine : 1,2-distearol-sn-glycero-3-phospholine) 30% cholesterol 5% N-dod-DOPE in chloroform/methanol (2:1 vol/vol). Before being used, the DOPC may be modified with a polymethine dye.
2. Evaporation of the chloroform/methanol solvent (approximately 30 min, 90 rpm) in a rotary evaporator.
3. Lipids were then dissolved in 1 ml 7:3 vol/vol mixed DMSO: EtOH.
4. Next, the hydrophilic dextran as the active ingredient was dissolved in a suitable buffer, namely PBS (phosphate buffered solution) to yield a concentration of 1 mg/ml.
5. 0.3 ml of the lipid solution was then added by drops to the dextran solution, was then kept in motion at 750 rpm on a magnetic agitator while the dropwise addition was underway.
6. The liposomes were subsequently separated in a mini-extruder.
7. Next, the liposome solution was aliquoted in 1 ml containers and then 10 times alternately frozen in liquid nitrogen and thawed in hot water.
8. Next, the liposomes were separated 10 times in the extruder.
9. Then, the liposomes were dialyzed in a prepared dialysis cassette (MWCO = 20 kDa) against PBS for 16 hours.
10. Next, the liposomes were freeze-dried, stored or used.

Example 9: Influencing cholesterol biosynthesis by the organ-specific transport of an siRNA against HMG-CoA reductase (HMGCR) in DY-635-modified nanostructured delivery systems

Male FVB/NRj mice (10 weeks old) were treated twice at intervals of 24 hours with the DY-635-modified nanostructured delivery system by i.v. injection by administering 6.5 µg of the nanostructured delivery system per kg body weight. The delivery system was produced as described in Example 7 (B), wherein PLGA-modified with 108 pg PEI was enclosed in 3 mg DY-635 for production of 3 pg siRNA against HMGCR or 3 pg scrambled siRNA (siRNA without effect). The animals were euthanized painlessly 16 hours after the second injection and both, blood and organs were removed for analysis. The blood was removed in lithium heparin monovettes and processed to obtain plasma. To determine efficacy of the treatment, the total plasma cholesterol was determined, and for determining specificity, the change in gene expression in various organs was determined in qPCR. These values were compared with cholesterol and HMGCR expression levels in healthy FVB/NRj mice (10 weeks old) and control groups. The control groups were composed of as follows:

- treatment with a DY-635-modified and, therefore, hepatocyte-specific nanostructured delivery system and an ineffective, scrambled siRNA;
- treatment with a nanostructured delivery system that did not contain any DY-635 modification but otherwise did not differ from the therapeutic construct;
- animals receiving only 5% glucose solution.

Example 10: Detecting interaction of DY-635 with hepatocytic transporters

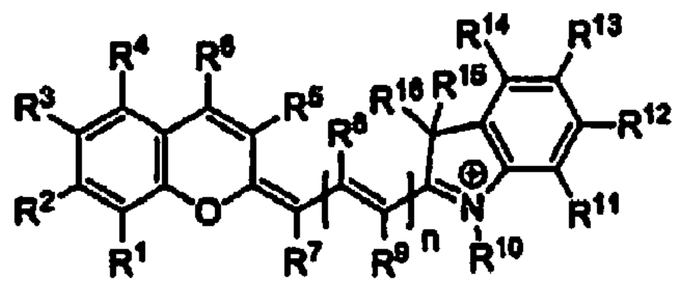
HEK-293T cells were transfected with human tissue-specific hepatocytic transporters. Then, the uptake of the polymethine dye DY-635 as a targeting unit for these tissue-specific transporters was investigated as shown in Figure 10 A. Cells were plated onto 96 well plates, incubated for 24 hours under standard conditions, then incubated 5 minutes with DY-635 (final concentration in the well: 10 µmol/l) after changing the medium. Next, cells were lysed and lysates were measured by fluorimetry. The quantity of DY-635 taken up was quantified by a DY-635 standard curve using the respective transporters which had been specifically inhibited for controls (inhibitors and the final concentrations used are shown in Table 3 below). In this experiment, it was found that DY-635 is a substrate for NTCP. The uptake by OCT1 may be considered negligible. As shown in Figure 10 B, the question of whether DY-635 binds as an inhibitor to basolateral hepatocytic transporters was investigated. For this purpose, HEK-293T cells transfected with tissue specific transporters were plated and incubated as described above. After 24 hours the cells were incubated for 5 minutes with either a radioactively labeled transporter- specific substrate or with radioactive-specific substrate together with a specific inhibitor or with DY-635 (10 µmol/l final concentration) (the substrates and their concentrations are shown in Table 3 below). The cells were then washed and lysed in the well. To quantify uptake, radioactive radiation of the substrates was used. It was found that DY-635 is a strong indicator for the presence of OATP1B1 and OATP1B3. OAT2 and OCT1 are also inhibited by DY-635. This illustrates the strong interaction of DY-635 with tissue-specific hepatocytic transporters. It may be concluded that by exposure of DY-635 to the surface of a nanostructured transporter system, immobilization on the cell surface of the hepatocyte occurs, resulting in subsequent endocytosis of the nanoparticle.

Table 3

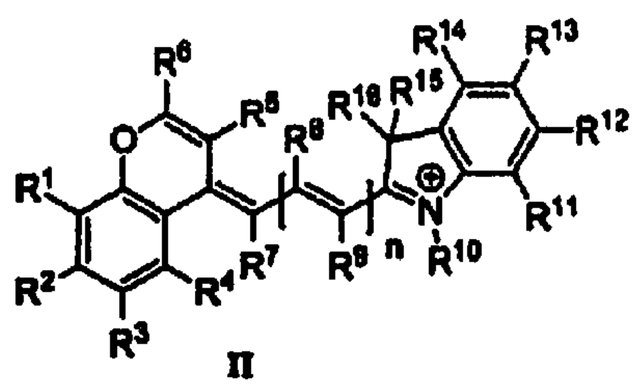
Human hepatocytic transporter	Radioactively labeled transporter-specific substrate/ concentration	transporter-specific inhibitor/ concentration
OATP1B1	[^{3}H]Estradiol/30nM	Rifampicin/5 μM
OATP1B3	[^{3}H]Sulfobromophthalein 50nM	Rifampicin/5 μM
OAT2	[^{3}H] cGMP/10 nM	indomethacine/100 μM
NTCP	[^{3}H] Estradio1/30 nM	Cyclosporin A/50 μM
NaDC3	[^{14}C] Succinate/10 μm	Succinate/100 μM
OCT1	[^{3}H] 1-Methyl-4- phenylpyridinium	Decynium22/50 μM

Patentkrav

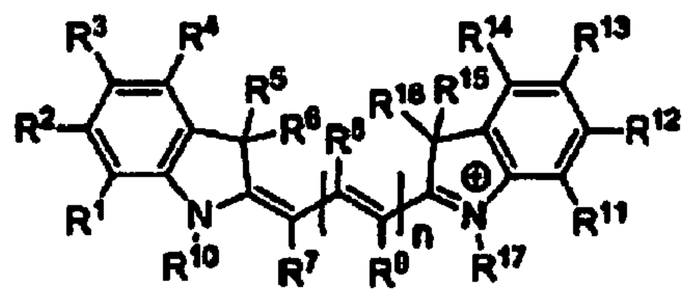
1. Nanostruktureret bærersystem omfattende i det mindste en polymer og/eller i det mindste et lipid og i det mindste et polymethinfarvestof til anvendelse til behandlingen af sygdomme i lever og/eller nyre, hvor det i det mindste ene polymethinfarvestof som målretningsenhed bevirker den målrettede transport af det nanostrukturerede bærersystem ind i et målvæv, og hvor det i det mindste ene polymethinfarvestof er et symmetrisk eller usymmetrisk polymethin med den generelle struktur I, II, III eller IV:



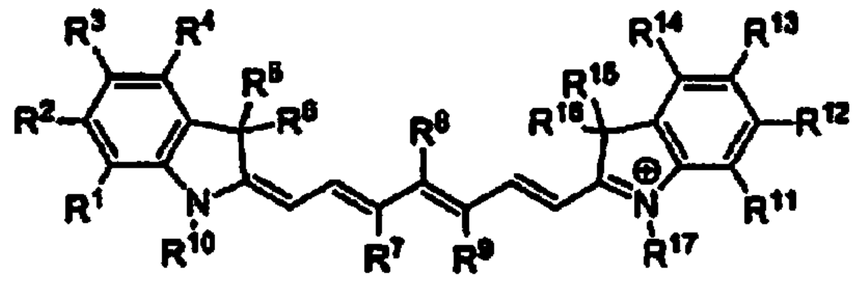
I



II



III



IV

hvor

a. n står for talværdierne 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, eller 10,

b. R^1-R^{17} er ens eller forskellige og kan være hydrogen, en eller flere alkyl-, tert-alkyl, cycloalkyl eller aryl-, carboxyaryl-, dicarboxyaryl-,

heteroaryl- eller heterocycloaliphatiske rester, alkyloxy-, alkylmercapto, aryloxy-, arylmercapto-, heteroaryloxy-, heteroarylmercapto-, en hydroxy-, nitro- eller cyanogruppe, en alkylsubsitueret eller cyclisk aminfunktion, og/eller to rester i orto-position, f.eks. R^3 og R^4, R^{13} og R^{14} og/eller R^1 og R^2 og R^{11} og R^{12} og/eller R^7 og R^9 tilsammen kan danne en yderligere aromatisk, hetero-aromatisk, aliphatisk eller heteroaliphatisk ring,

c. i det mindste en af substituenterne R^1- R^{17} bærer en solubiliserende henholdsvis ioniserbar eller ioniseret substituent såsom SO_3^-, (-SOsH), PO_3^{2-}, COOH, OH eller NR_3^+, cyclodextriner eller sukker, som bestemmer de hydrophile egenskaber af disse polymethinfarvestoffer, hvor denne substituent også via en spacergruppe kan være bundet til polymethinfarvestoffet, og

d. i det mindste en af substituenterne R^1-R^{17} bærer en reaktiv gruppe (linker) såsom isocyanater, isothiocyanater, hydraziner, aminer, mono- og dichlor- eller mono- og dibromtriaziner, aziridiner, epoxider, sulfonylhalogenider, syrehalogenider, carbonsyreanhydrider, N-hydroxysuccinimidestere, imido-estere, carbonsyrer, glyoxal, aldehyd, maleimid eller iodacetamid og phosphoramidit derivater eller azider, alkiner eller olefiner, hvor denne substituent også via en spacergruppe kan være bundet til polymethinfarvestoffet,

e. den aromatiske, heteroaromatiske, aliphatiske eller heteroaliphatiske spacergruppe består af strukturelementer såsom $-[(CH_2)_a-Y-(CH_2)_b]_c-$ eller $-[(C_6H_4)_a-Y-(C_6H_4)_b]-$, hvor Y er ens eller forskellig og indeholder CR_2-, O-, S-, SO_2, SO_2NH-, NR-, 000- eller CONR-funktioner, hvor den er på en af substituenterne R^1-R^{17}, og a og b er ens eller forskellige med talværdier på 0-18 og med talværdier for c på 0-18,

f. substituenterne R^8 og R^9 ved tilsvarende n= 2, 3, 4 eller 5 ligeledes kan være til stede 2, 3, 4 eller 5 gange, og disse kan være ens eller forskellige, og hvor

det nanostrukturerede bærersystem yderligere omfatter i det mindste et farmaceutisk aktivt stof.

2. Nanostruktureret bærersystem til anvendelse ifølge krav 1, hvor det i det mindste ene polymethinfarvestof via i det mindste en vævsspecifik transporter bevirker optagelsen af det nanostrukturerede bærersystem i målvævets celler.

3. Nanostruktureret bærersystem til anvendelse ifølge et af de foregående krav, hvor det i det mindste ene polymethinfarvestof er udvalgt fra gruppen bestående af DY635, DY-680, DY-780, DY-880, DY-735, DY-835, DY-830, DY-730, DY-750, DY-850, DY-778, DY-878, DY-704, DY-804, DY-754, DY-854, DY-700, DY-800, ICG og DY-IRDYE 800CW.

4. Nanostruktureret bærersystem til anvendelse ifølge et af de foregående krav, hvor den i det mindste ene polymer er udvalgt fra gruppen bestående af polyestere, poly(meth)acrylater, polystyren derivater, polyamider, polyurethaner, polyacrylnitriler, polytetrafluorethylener, siliconer, polyethylenglycoler, polyethylenoxider og polyoxazoliner og deres copolymerer, fortrinsvis i mest forskelligartet sammensætning, såsom statistisk, gradient, alternerende, blok, prop eller Stern copolymerer, eller det i det mindste ene lipid er udvalgt fra gruppen bestående af mættede og umættede fedtsyrer, fortrinsvis cholesterol, palmethylsyre, phospholipider, sphingolipider og glycolipider.

5. Nanostruktureret bærersystem til anvendelse ifølge et af de foregående krav, hvor den i det mindste ene vævsspecfikke transporter er udvalgt fra gruppen bestående af OATP1B1, OATP-C, OATP2, LST-1, OATP1B3, OATP8, OATP2B1, OATP1A2, NaDC3, SDCT2, NTCP, OCT1, OCT3, OAT2, OAT1, OAT3, PGT, OCT2, OAT1, OATP4A1, OATP4C1.

6. Nanostruktureret bærersystem til anvendelse ifølge et af de foregående krav, hvor det i det mindste ene farmaceutisk aktive stof er udvalgt fra gruppen bestående af lavmolekylære substanser, især induktorer eller kontrastmidler, og højeremolekylære substanser, især nukleinsyrer og

proteiner, fortrinsvis udvalgt fra gruppen bestående af glucokortikoider, cytostatika, antimetabolitter, interkalanter, antistoffer, interferoner, phospho-inositol-3-kinase inhibitorer, coxibe, JNK-inhibitorer.

7. Nanostruktureret bærersystem til anvendelse ifølge et af de foregående krav, hvor akkumulationen af det nanostrukturerede bærersystem og/eller dets bestanddele i et målvæv kan detekteres ved hjælp af fluorescensegenskaber af det i det mindste ene polymethinfarvestof.

8. Nanostruktureret bærersystem til anvendelse ifølge et af kravene 1 til 7, hvor sygdommen er udvalgt fra gruppen bestående af infektionssygdomme med beskadigelse af leveren og/eller nyren, leversvigt, levercirrhose, stofskiftesygdomme i leveren, ekskretoriske dysfunktioner i leveren, levertumorer, primære levertumorer, nyretumorer, primære nyretumorer, nephritider, kronisk og akut nyresvigt samt sygdomme, der forårsager en konsekutiv beskadigelse af leveren og/eller nyren.

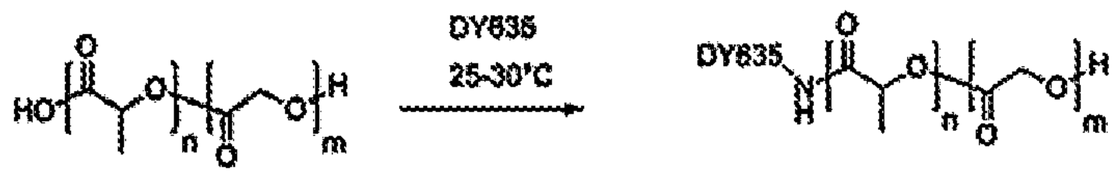
Tabelle 1

Variation	Effect on NP or on parts thereof	consequence
Change of the DY	altered charge of NP and altered functionalization	different target tissue, different target cell, altered transport efficiency
Change in the degree of subsitution in the DY	change in hydrophilic or lipophilic characteristics, respectively	renal/hepatic specificity of the dye/ the NP
Change in the number of conjugated double bonds in the DY	shift of emission and absorption spectra	detectability adjustment; simultaneous and concurrent detection of multiple dyes
Change of the shell polymer	altered endosomal or intracellular stability, respectively, of the NP; altered encapsulation efficiency	Control of the release rate and of the amount of the active ingredient transported in the NP and
Change in the active substance transported	potentially altered encapsulation efficiency; potential alteration of the NP's hydrophobic/hydrophilic equilibrium	Altered effect in target tissue
Change in the NP preparation method	size control, surface groups may be varied	passive uptake and accumulation in organs or cells, respectively, supporting active targeting
Change in NP stabilizers	altered stability during preparation, altered charge and size of NPs	alteration of the velocity of uptake and release

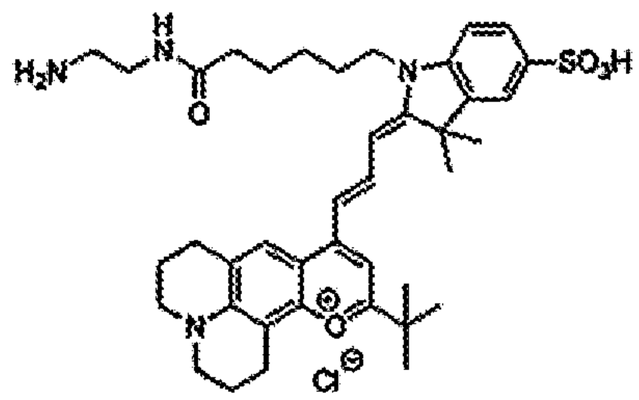
DY = polymethine dye
NP = nanoparticle

Figure 1

A



DY635 =



B

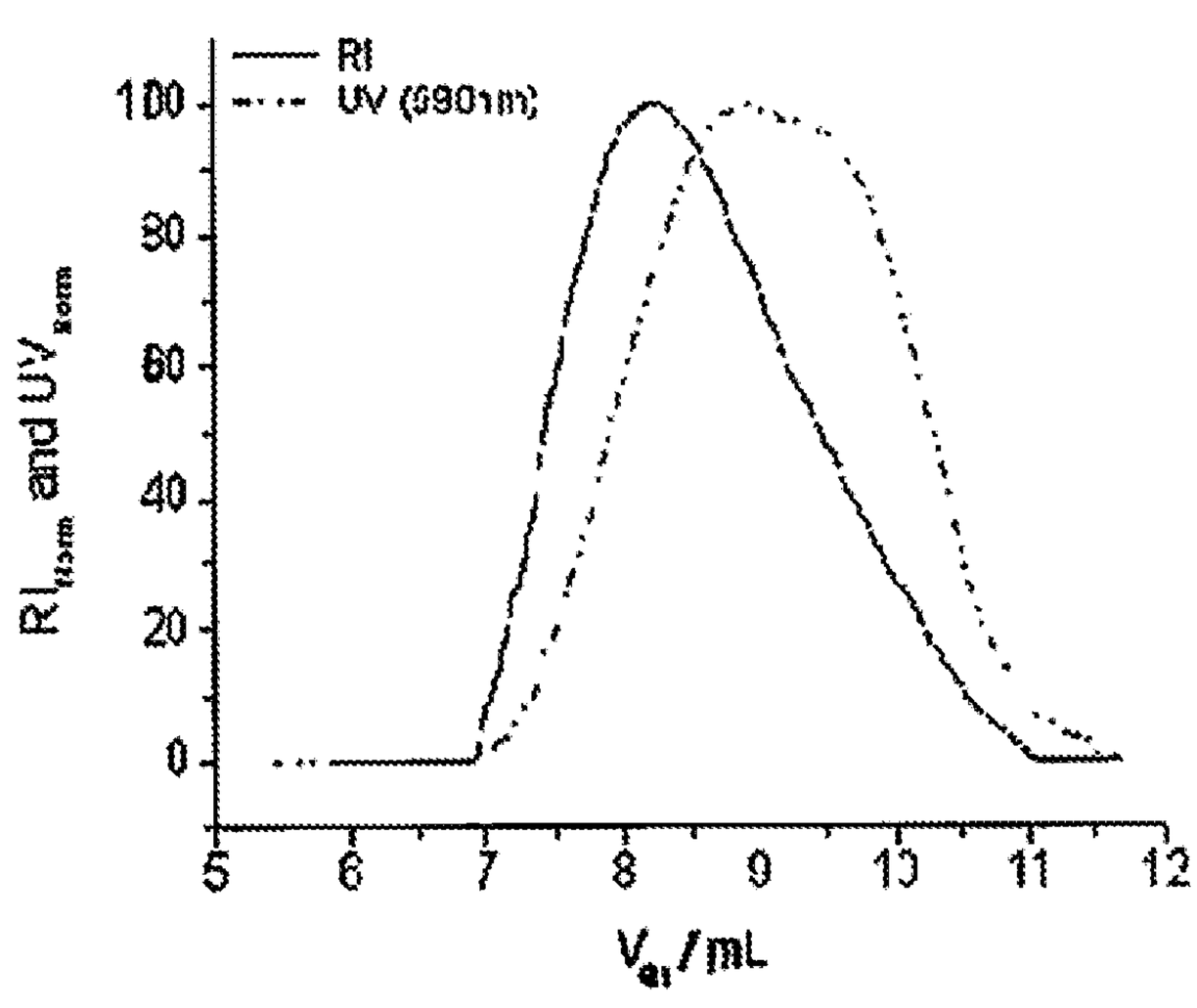


Figure 2

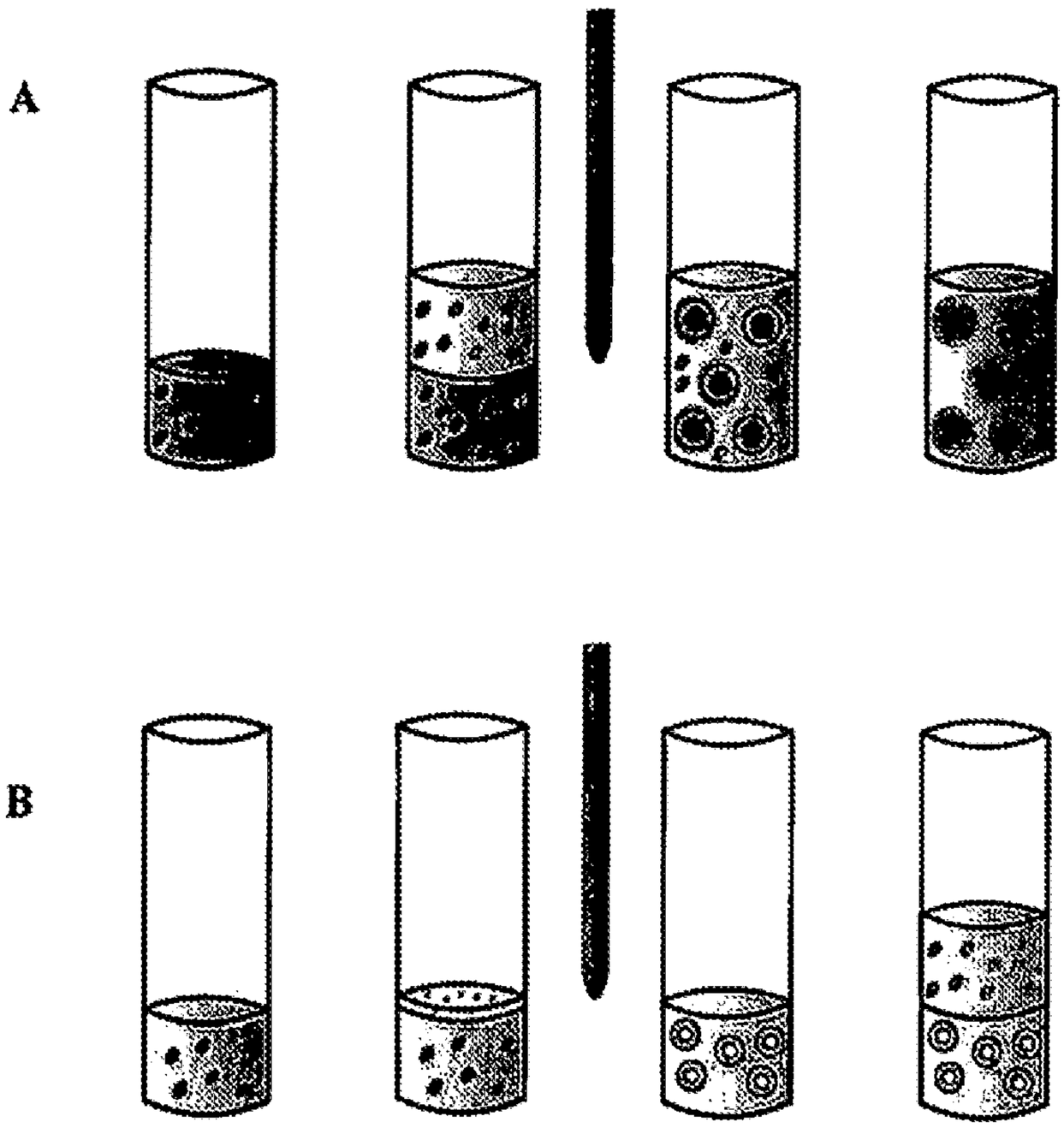
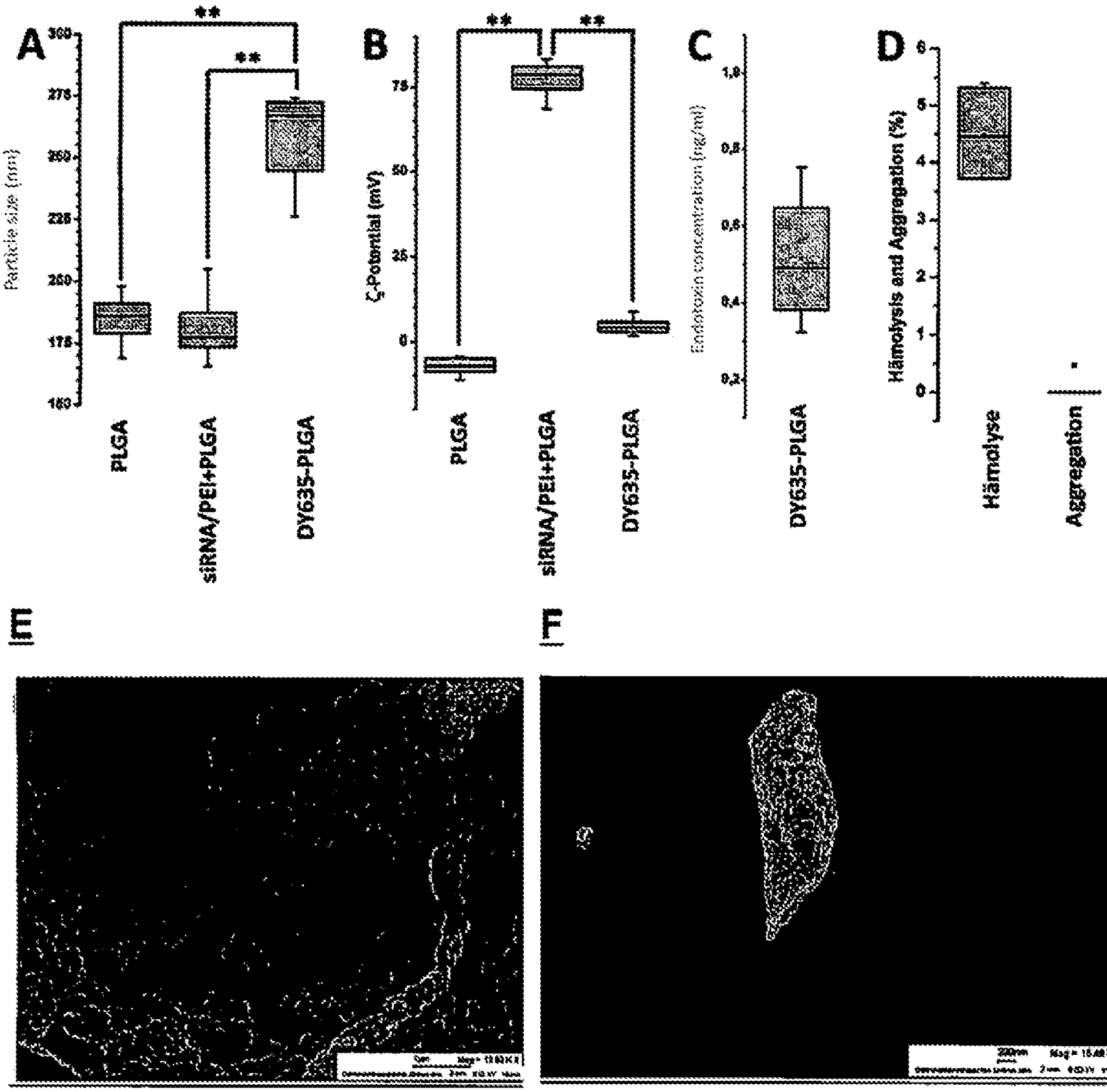

A
B
C
Dyeass
Galactose
Galactose
Galactose
Dyeass
Dyeass
no cell specificity
cell specificity for hepatocytes
no cell specificity
cell specificity for hepatocytes

Figure 3



** level of significance: 0.99; p < 0.0025, respectively, U-test

Figure 4

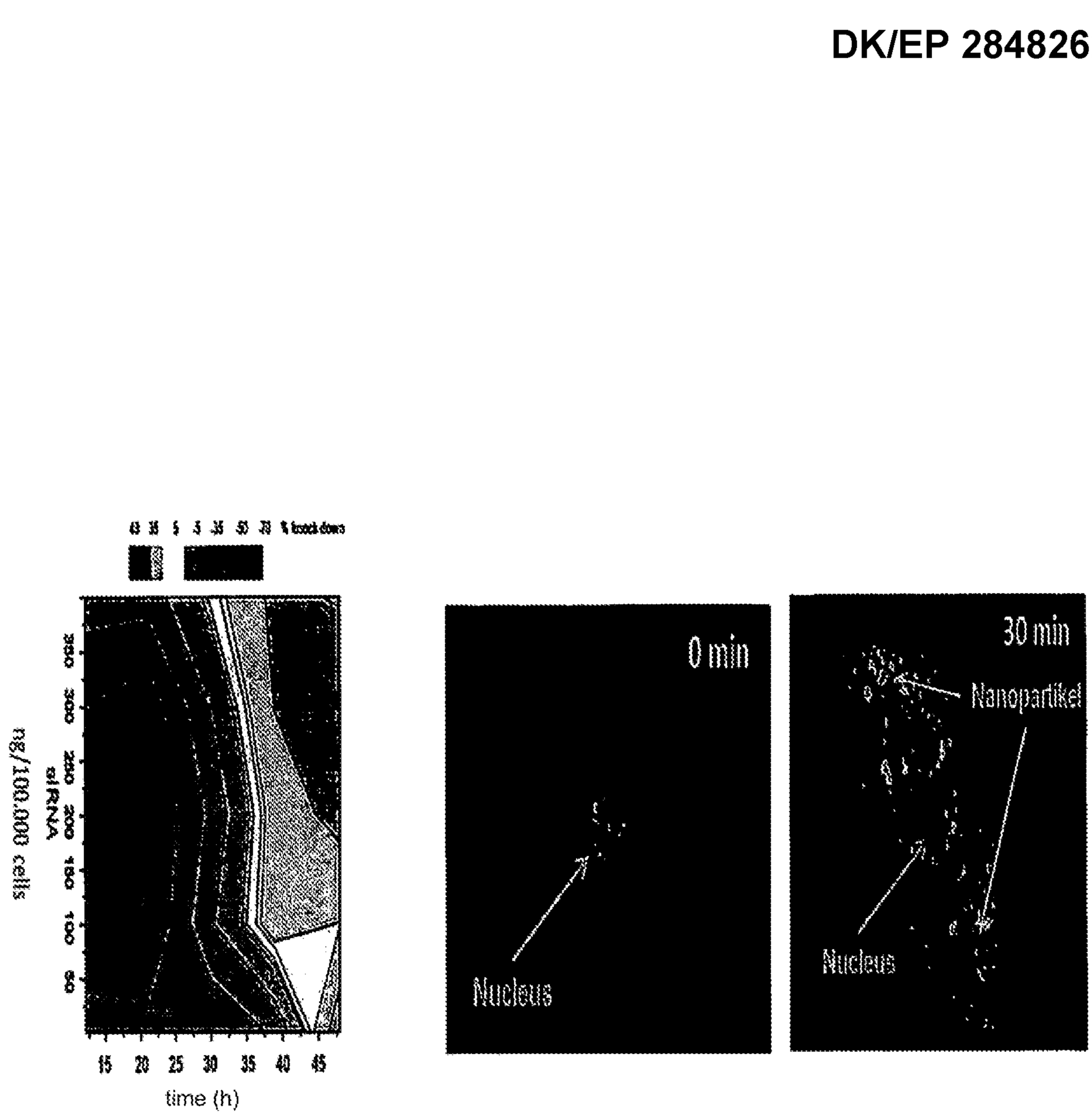

siRNA
ng/100,000 cells
time (h)
0 min
Nucleus
30 min
Nanopartikel
Nucleus

Figure 5

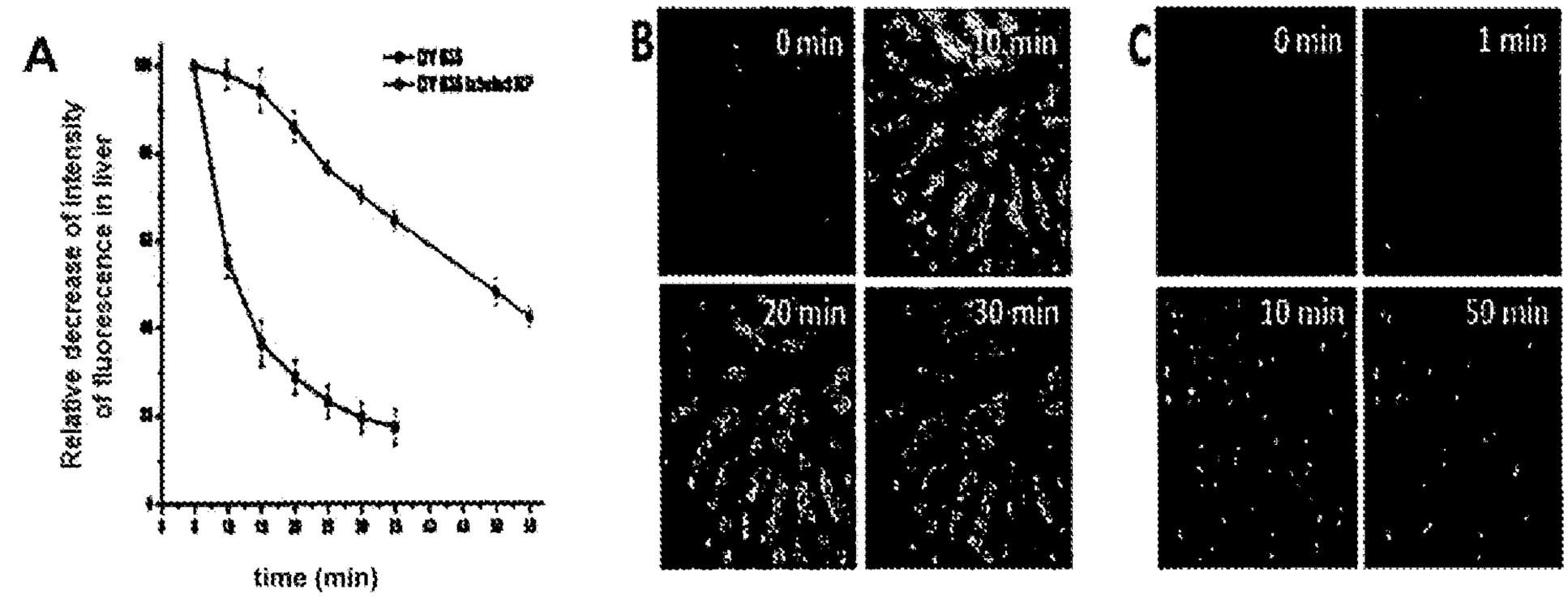

A
Relative decrease of intensity of fluorescence in liver
time (min)
B
0 min
10 min
20 min
30 min
C
0 min
1 min
10 min
50 min
D
liver
E
spleen
F
heart
G
kindey

Figure 6

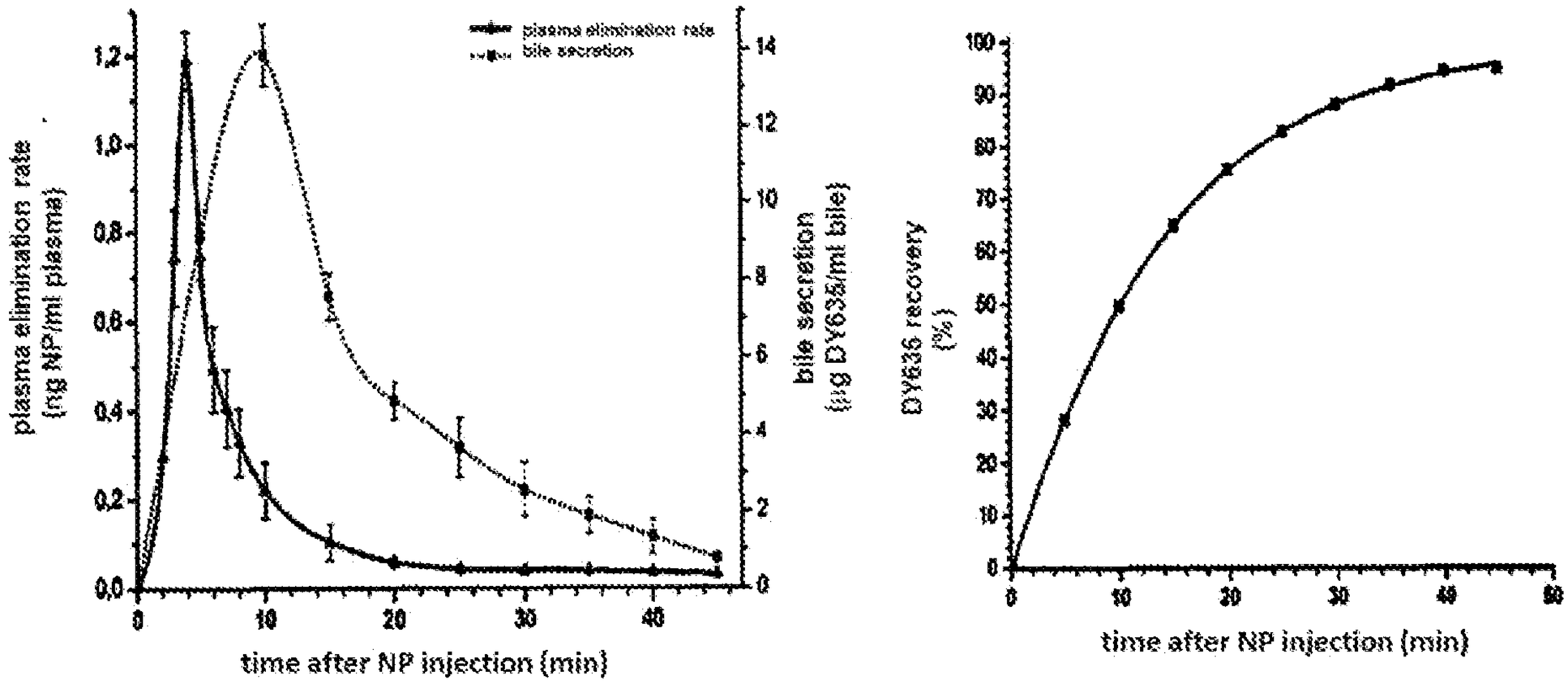

plasma elimination rate
bile secretion
plasma elimination rate (ng NP/ml plasma)
bile secretion (µg DY635/ml bile)
time after NP injection (min)
DY635 recovery (%)
time after NP injection (min)

Figure 7

Tabelle 2:

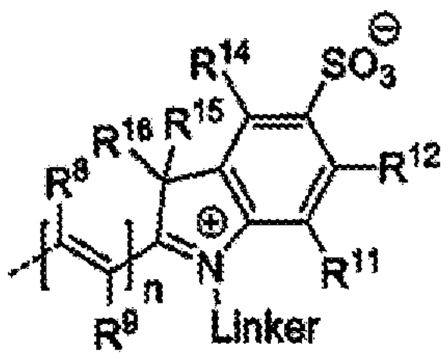
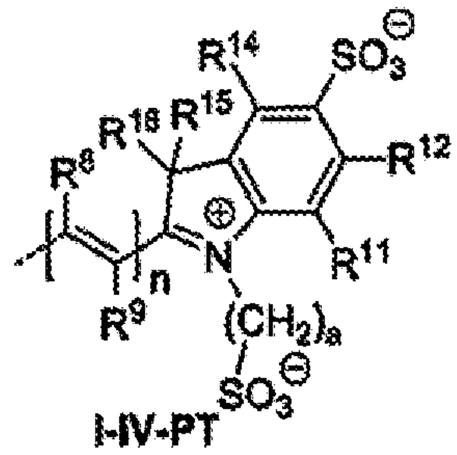
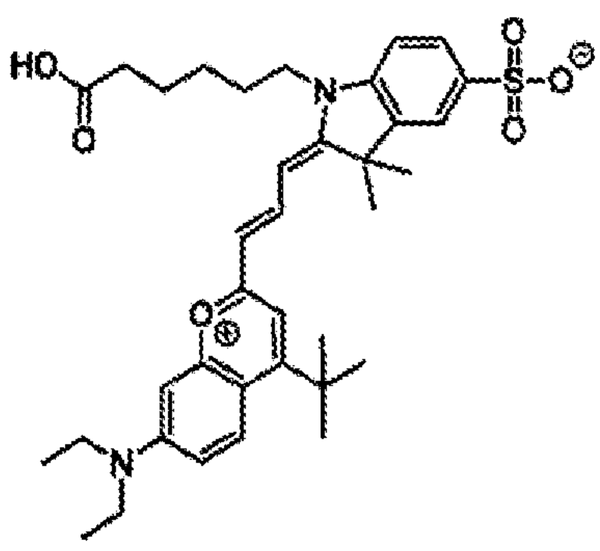
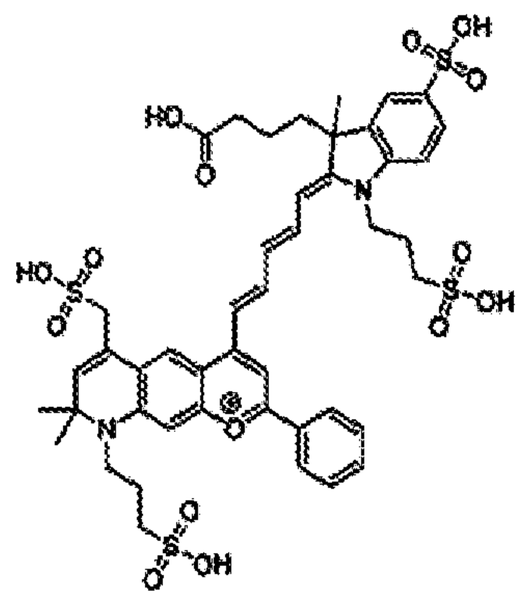
general structure hepatocyte targeting unit	**general structure renal targeting unit**
structures I-IV with $R^{13} = SO_3^-$ (or $-SO_3H$), and with R^{17} = linker to polymer  **I-IV-H**	structures I-IV with $R^{13} = SO_3^-$ (or $-SO_3H$), R^{15} oder R^{16} = linker to polymer and $R^{17} = -(CH_2)_a-SO_3^-$ with a ranging from 1 - 18  **I-IV-PT**
examplary structures for hepatic targeting	**examplary structures for targeting renal parenchyma cells (espec. proximal tubule cells)**
DY-680 	**DY-778** 

Figure 8

table 2 continued

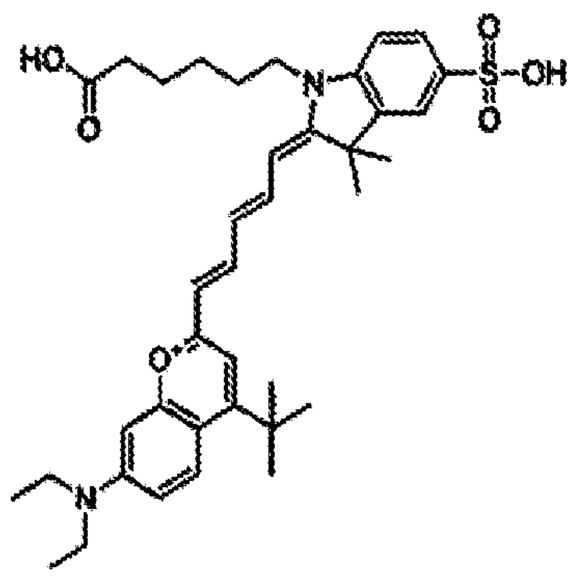
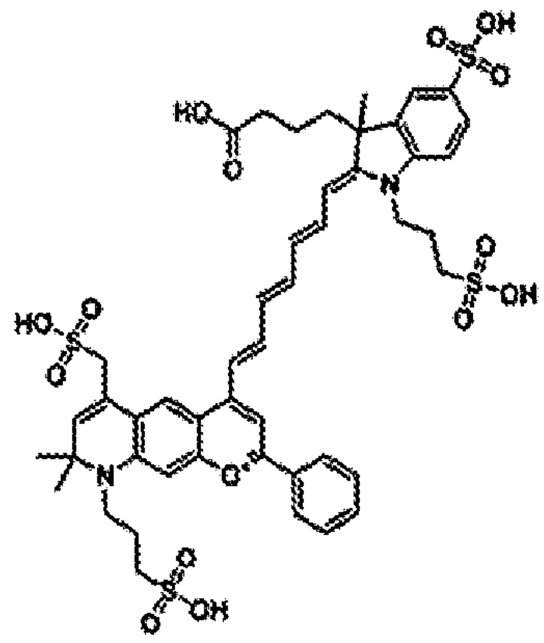
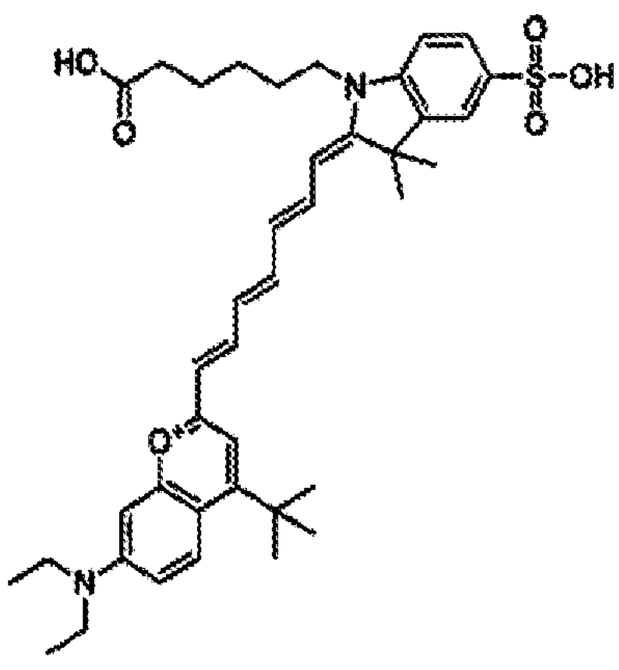
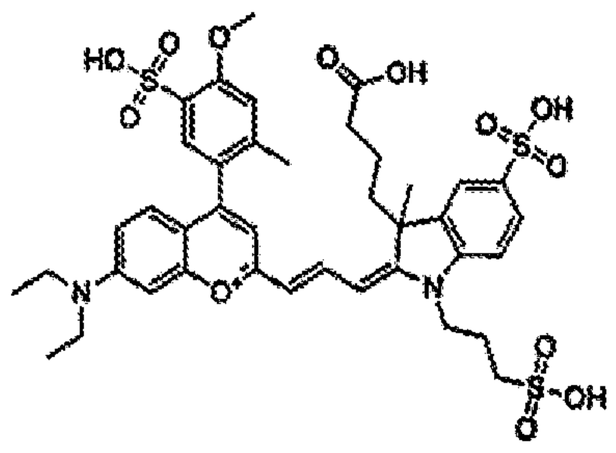
DY-780 	**Bsp. 1.2: DY-878** 
DY-880 	**DY-704** 

Figure 8 continued

table 2 continued

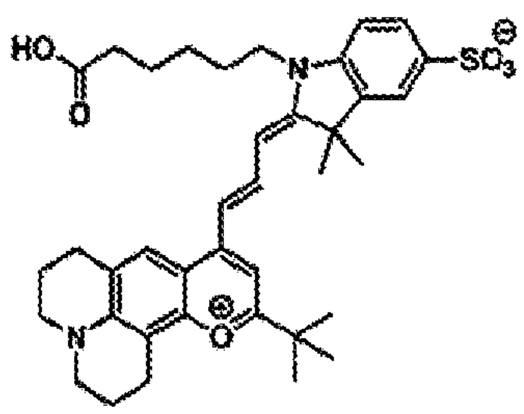
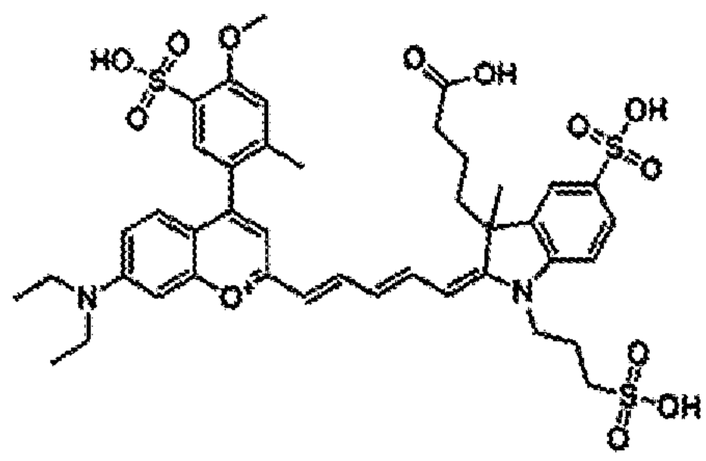
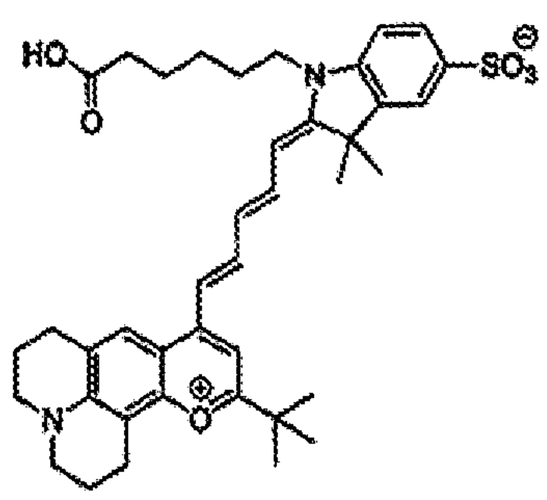
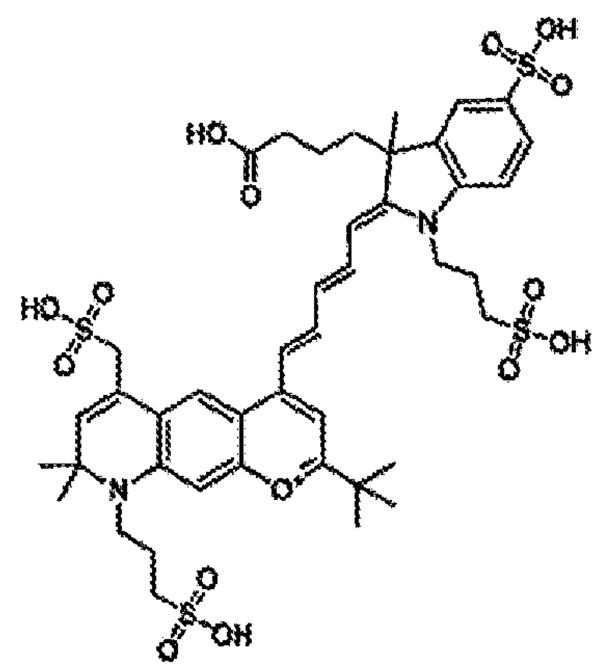
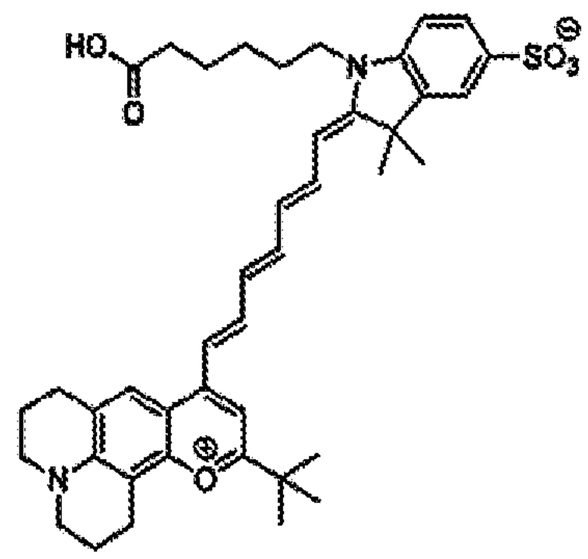
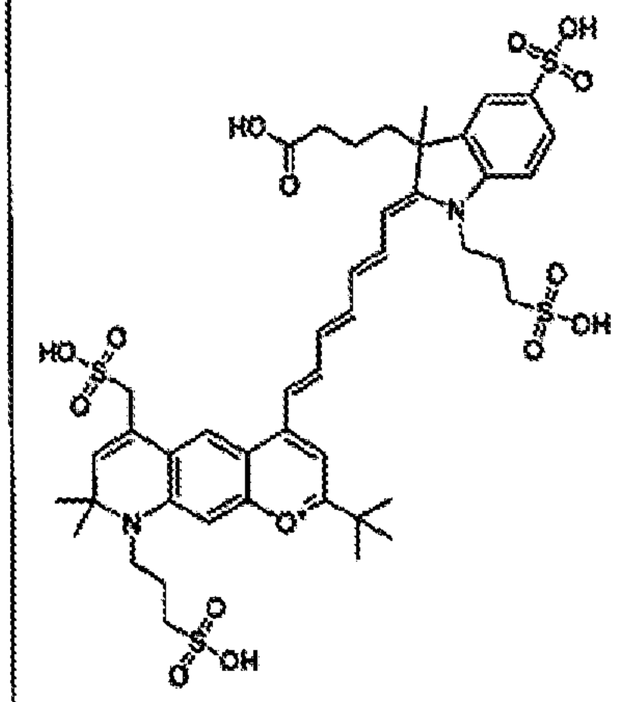
DY-635 	**DY-804** 
DY-735 	**DY-754** 
DY-835 	**DY-854** 

Figure 8 continued

table 2 continued

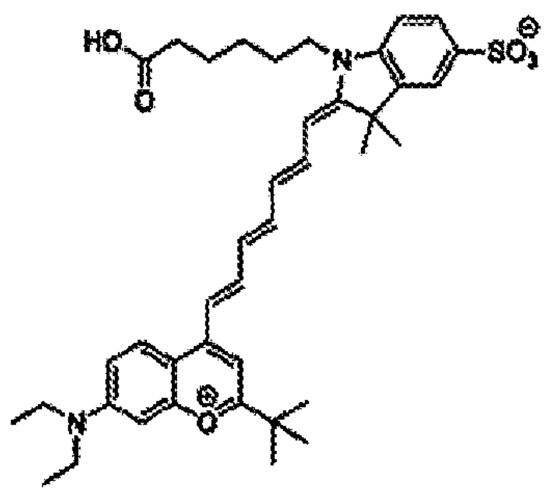
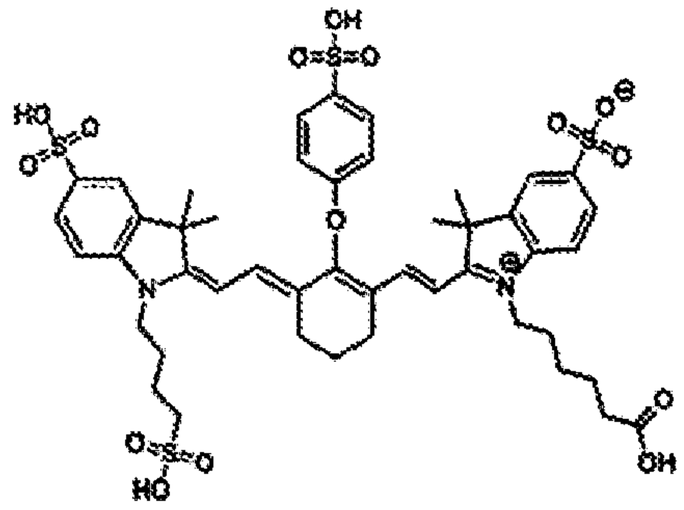
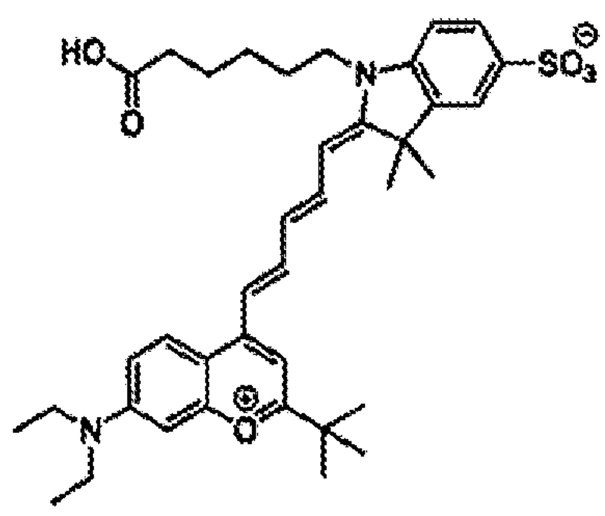
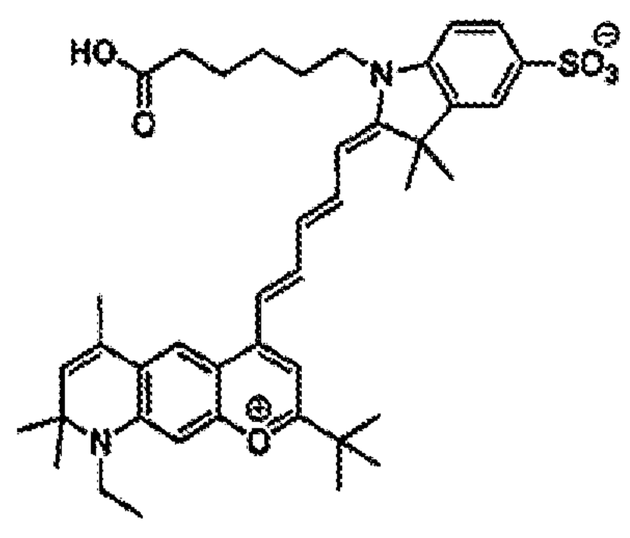
DY-830 	**IRDYE 800CW** 
DY-730 	
DY-750 	

Figure 8 continued

table 2 continued

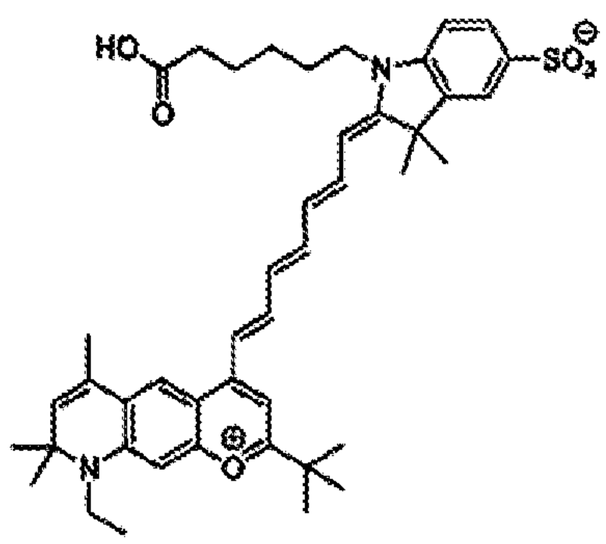
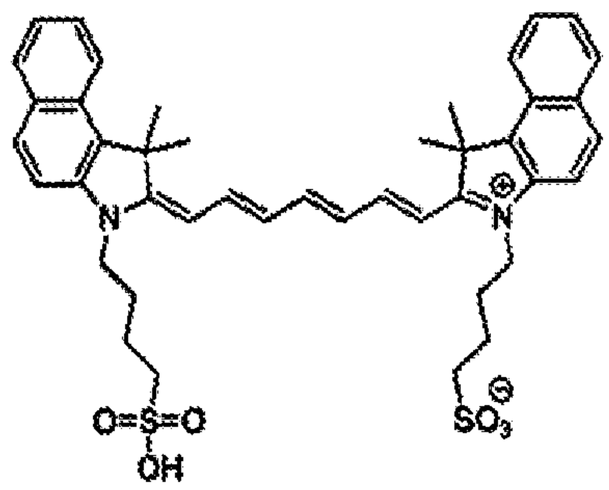
DY-850 	
ICG 	

Figure 8 continued

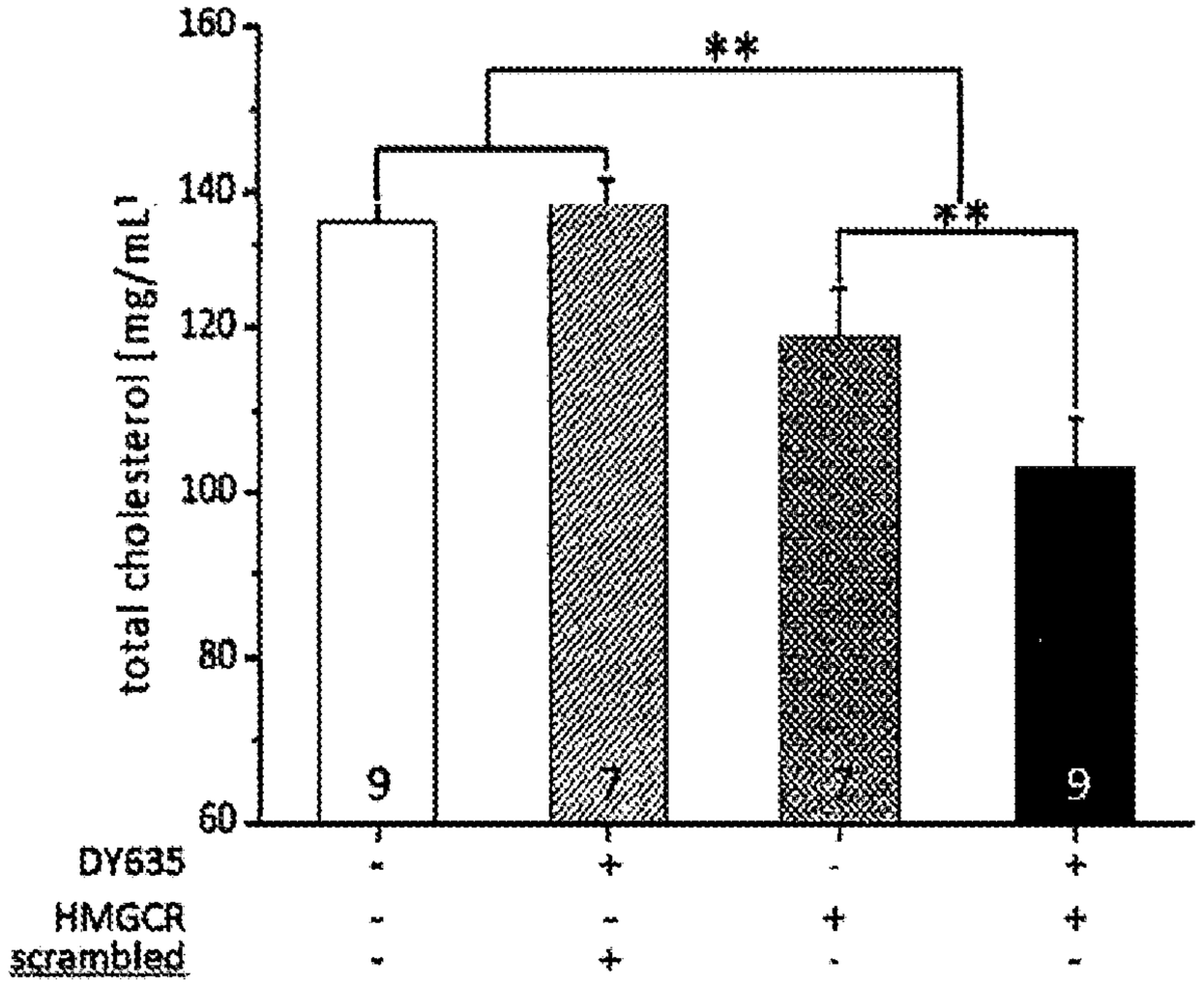


Figure 9A

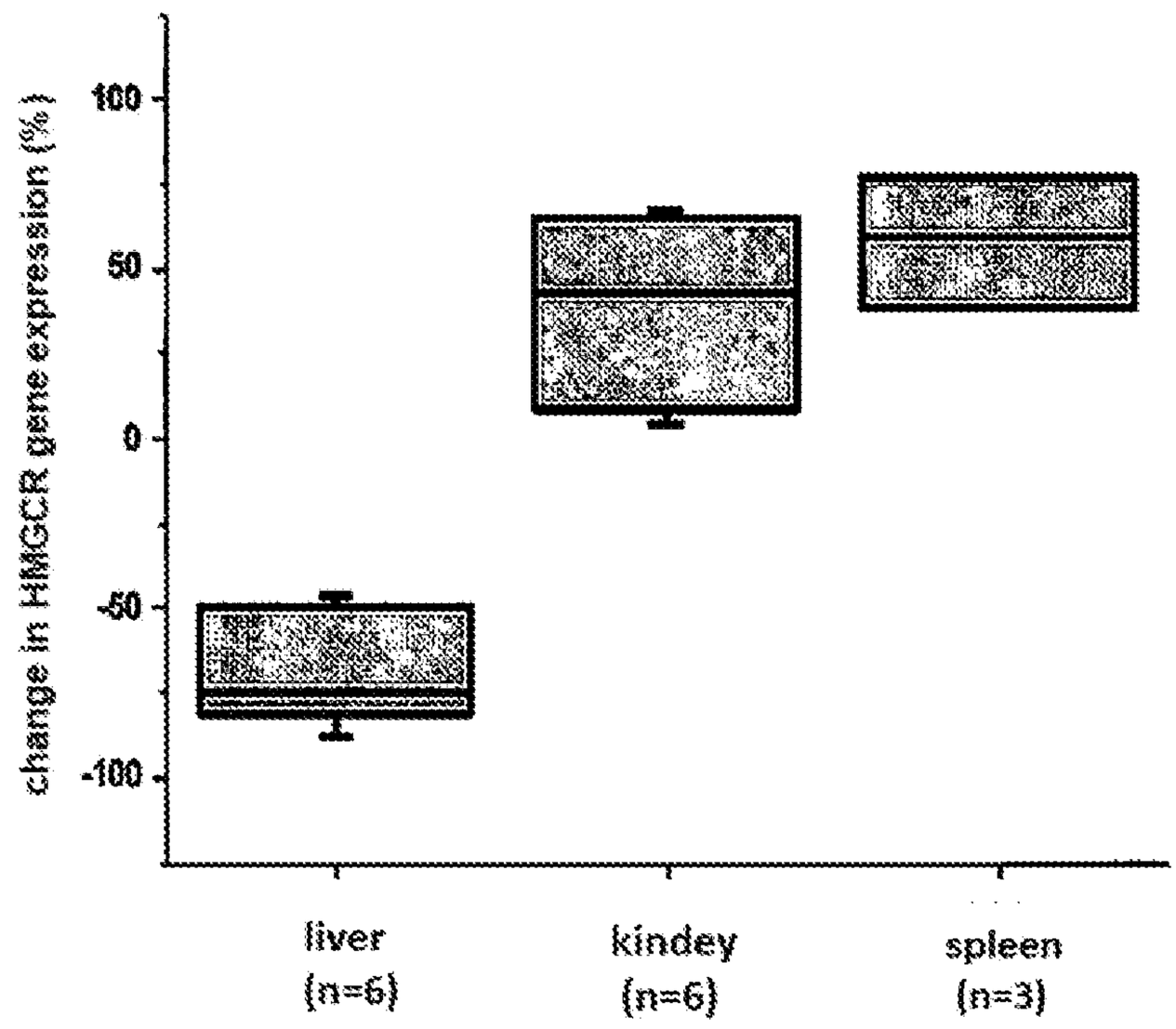
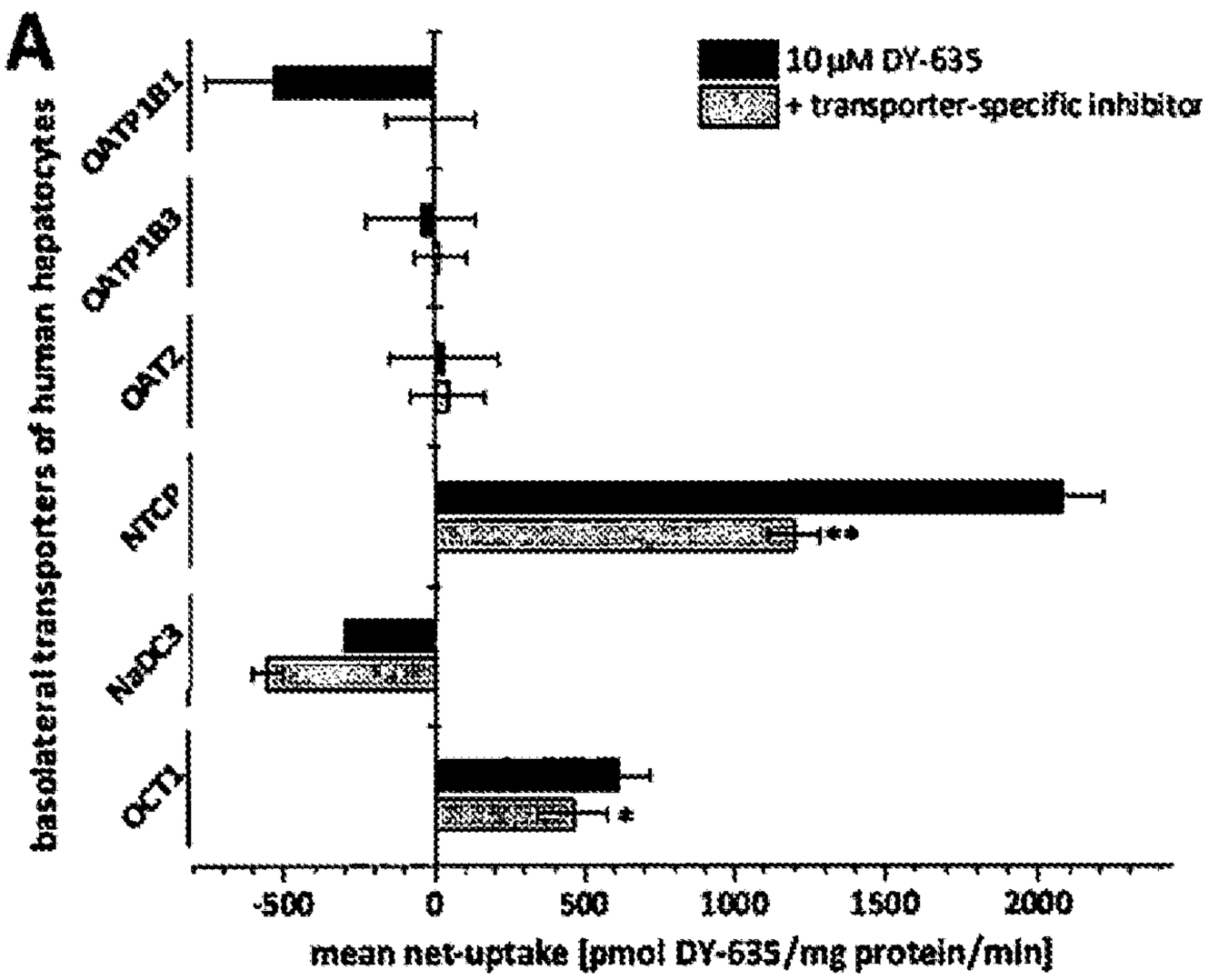


Figure 9B


A
basolateral transporters of human hepatocytes
OATP1B1
OATP1B3
OAT2
NTCP
NaDC3
OCT1
10 µM DY-635
+ transporter-specific inhibitor
-500
0
500
1000
1500
2000
mean net-uptake [pmol DY-635/mg protein/min]

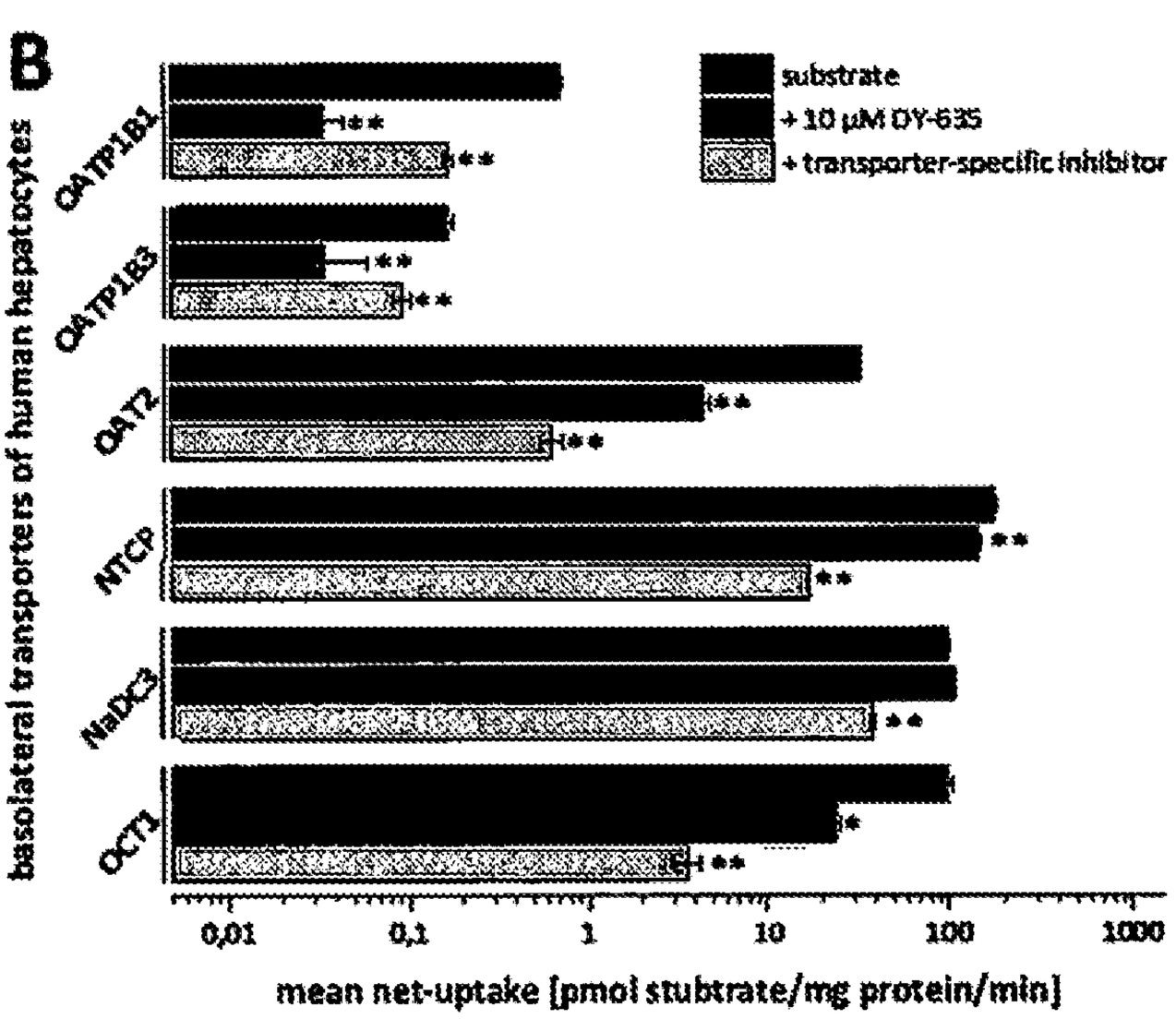

B
basolateral transporters of human hepatocytes
OATP1B1
OATP1B3
OAT2
NTCP
NaDC3
OCT1
substrate
+ 10 µM DY-635
+ transporter-specific inhibitor
0,01
0,1
1
10
100
1000
mean net-uptake [pmol stubtrate/mg protein/min]

Figure 10