Periodic structures comprising lipids, polyelectrolytes, and structure-inducing soluble oligovalent linkers, and biological use thereof

The invention relates to a method for preparing pharmaceutically usable compositions which comprise periodic structures consisting of polyelectrolytes sandwiched between lipid aggregates having at least one charged component, and the use thereof; it further relates to a kit comprising, in a bottled or otherwise packaged form, at least one dose of said pharmaceutically usable composition.

Assemblies of lipid membranes and DNA have attracted large interest as artificial carriers of genetic material, being suitable for the use in gene therapy and DNA vaccination. For the purpose, various complexes of DNA and cationic lipids (CL) were tested without that the details of CL-DNA were completely understood or perfectly controlled to date.

The advantages of CL-DNA complexes over natural, viral gene vectors include the absence of a viral DNA and a greatly reduced immunogenicity. The main disadvantages are the poor reproducibility of CL-DNA complex formation and relatively low transfection efficiency in comparison with the viral vectors. In order to overcome said deficiencies, it is essential to master better the factors governing complex formation and also to maximise the payload of carriers.

When DNA is interspersed with a suspension of multilamellar lipid vesicles, multilamellar DNA-CL complexes also arise spontaneously. They comprise stacks of lipid bilayers alternating with monolayers of densely packed parallel DNA helices; often, more than 10 lamellae are counted in one complex.

CL-DNA complexes for practical applications are usually prepared by mixing DNA solution with an aqueous suspension of liposomes containing CLs. As a result, globular lipid-DNA particles often form, sometimes with a structure similar to that described in preceding paragraph. However, the size and morphology of the CL-DNA complexes, that stem from unilamellar vesicles, varies widely between the preparations; variability is a problem even within the same preparation. As any variability is prone to influence the transfection efficiency substantially, greater control over the key parameters of transfection/vaccination vehicle is desirable. This can only be based on a clear understanding of the process of (multilamellar) DNA-CL complex formation and on a good rationale for its modulation.

Based on a close examination of cryo electron-micrographs of multilamellar complexes formed from a suspension of unilamellar vesicles we previously suggested the following model for multilamellae formation:
Starting with a single template membrane, multilamellar structures emerge. This involves repeated, alternating adsorption of DNA and lipid layers: first, a DNA monolayer adsorbs to the template CL bilayer; then, a vesicle from the bulk adsorbs to the bulk side of the DNA monolayer, ruptures, and rolls its bilayer over the complex; the bulk side of this bilayer is now available for further DNA adsorption, and so on. In the systems containing lipid multilamellae, DNA can intercalate between the multilayers under the influence of mixing or osmotic stress, e.g. at the sites of maximum membrane curvature, which can easily lead to local bilayer rupture and DNA translocation. It was believed to date that salts play a role in this, but ruled by simple laws of electrostatics. These laws suggest that electrostatic interactions between the CL and DNA, as well as inter-DNA repulsion, will be screened progressively (in a square-root like fashion). A decreased DNA solubility and a lower affinity of oppositely charged (counter-ionic) surfaces to bind DNA polymer from the solution result from this.

Scientific publications reveal no consistent picture of the effect of EDTA, or other chelators, on lipid-DNA interaction or its biological effects. Some authors report positive while others observe negative or no effect of said additive.

An example for the former effect are asialofetuin-liposomes (AF-lps), developed as a vector for gene transfer to hepatocytes (Hara, T., Aramaki, Y., Takada, S., Koike, K., Tsuchiya, S., 1995, "Receptor-mediated transfer of pSV2CAT DNA to a human hepatoblastoma cell line HepG2 using asialofetuin-labelled cationic liposomes" in Gene 159: 167-174). Plasmid pSV2CAT DNA was associated with such liposomes (AF-lps-pSV2CAT). AF-lps was found to bind to HepG2 cells through specific interaction with asialoglycoprotein receptors (AGPR); internalisation follows by the receptor-mediated endocytotic pathway. Transfection of HepG2 cells with AF-lps-pSV2CAT was much stronger than the effects of either pSV2CAT associated with non-derivatised control lps (N-lps-pSV2CAT) or else a mixture of pSV2CAT and empty AF-lps. Pretreatment with EDTA- encapsulated AF-lps increased the transfection efficiency of AF-lps-pSV2CAT.

A negative example is given in the article of Watanabe, Y., Nomoto, H., Takezawa, R., Miyoshi, N., Akaike, T. entitled "Highly efficient transfection into primary cultured mouse hepatocytes by use of cation-liposomes: an application for immunization" J. Biochem. 116: 1220-1226, 1994. Specifically, four conventional artificial transfection vectors were examined. Amongst the four - DEAE-dextran, calcium phosphate, cation-multilamellar liposomes, and cation-liposomes (lipofection)- only the latter were highly efficient in primary cultured mouse hepatocytes, but less so in three other commonly used cell types (CHO-K1, COS-1, 3T3-L1). The transfection efficiency was strongly decreased (inhibited)

by EDTA, free low density lipoprotein (LDL), and an endocytosis inhibitor, cytochalasin B.

In a different study, cellular uptake and gene expression of plasmid DNA and its cationic liposome complexes were studied using primary cultures of bovine brain microvessel endothelial cells (BMEC). An avid association of naked plasmid DNA with the BMEC monolayer was observed at 37 °C, which is comparable to that of the DNA/liposome complex. The binding at 4 °C was saturable and significantly inhibited by polyanions involving polyinosinic acid and dextran sulfate; EDTA or polycytidylic acid had no effect (Nakamura et al., 1998).

We found unexpectedly that EDTA, a common constituent of many buffers with the charges of similar sign as DNA (a co-ion), drastically and reproducibly affects the morphology of CL-DNA complexes. We also observed that EDTA can promote efficiently the unilamellar-to-multilamellar structures transition. We consequently infer that oligovalent co-ions, including but not limited to EDTA, can beneficially affect the morphology of CL-DNA complexes for practical applications and should influence the efficacy of transfection in vitro and in vivo.

Why does the presence of EDTA in suspension so drastically alter the morphology of CL-DNA complexes? One possible, but as yet speculative, answer, by which the applicants wish not to be bound, is the mechanism by which assemblies form. It seems that the DNA chains, in the process of complex formation, function as 'molecular glue'. The application-relevant polyelectrolyte therefore can keep the substrate and adsorbed vesicles close together. Catalysed by such `glue´ action, the ruptured vesicles roll their bilayers over the substrate and form an adsorbed bilayer, which then improves/grows over time. Further layers develop similarly. EDTA can be involved in the process by either affecting the strength / range of

electrostatic interactions or else by more intimate (e.g. H-bond mediated) interaction with the polymer.

The presence of EDTA is not a prerequisite for DNA adsorption to the lipid layer, however. Rather, EDTA seems to influence, in a subtle fashion, the electrostatic interplay in interactions between the complex constituents. This may rely on the formation of inter-molecular hydrogen bonds, on very efficient electrostatic screening, which increases rapidly with ion valency or simply on modified Van der Waals interactions ('correlation forces'). It therefore stands to reason that molecules with force field similar to that of EDTA, most notably di- and oligovalent ions, can play a pivotal role in the creation of vectors suitable for of gene-therapy and / or DNA vaccination.

WO92/0666 discloses multilayer liposomes for thermal water encapsulation. The systems described therein generally form multilayer (periodical) structures spontaneously. This prior art comprises no teaching, how perdiodical structures can be built from monolayer (non-periodical) vesicles. Systems as disclosed in WO92/06666 are e.g. discussed in
Koltover, I., Salditt, T., Rädler, J.O. Safinya C.R. (1998). An Inverted Hexagonal Phase of Cationic liposomes/DNA Complexes Related to DNA Release and Delivery. Science 281:78-81 and
Rädler, J.O., Koltover I, Salditt, T., Safinya, C.R. (1997), Structure of DNA cationic liposome complexes: DNA intercallation in multilamellar membranes in distinct interhelical packing regimes Science 275:810-814.

Definitions:

An adsorbent, or less generally a carrier, means an aggregate, independent of its composition and/or the nature and /or the source of its generation, capable of associating with one or more charged macromolecules (adsorbates) suitable for a

practical purpose, such as application on or the delivery into mammalian body. The substrate for the positively charged adsorbates is typically negatively charged, while for the positively charged adsorbates negative adsorbents are preferable. The terms "adsorbate", "adsorbing (macro)molecule", "binding (macro)molecule", "associating (charged) (macro)molecule", etc., are therefore used interchangeably in this application to describe one of the participants in association between molecules which do not form an extended surface under the conditions chosen and the "adsorbent" or a "binding surface", etc., in above mentioned sense.

An associate, by the definition used in this application, is any complex between an adsorbate and a carrier (adsorbent). Such an associate typically comprises two or more different kind of molecules, at least one of which forms aggregates with one or several well defined surface(s), independent of the reason for the complex formation but excluding covalent binding. Adsorption of polyelectrolyte molecules onto an aggregate surface driven by electrostatic interactions between the differently charged system components is the most frequent type of association, and is also the main topic of this application. Binding of anionic DNA onto an aggregate surface containing cationic molecular `anchors´ is most prominent and practically relevant example for this.

An oligovalent linker is any molecule with two or more groups capable of binding, non-covalently, to other groups on different molecules. Very often, the binding is of electrostatic nature, such as between chelators and oppositely charged groups on various molecules / ions, but other types of binding, e.g. via hydrogen bonds or directed dispersion forces, are also possible.

A chelator, within the framework of this disclosure, denotes any molecule capable of bringing and/or keeping together two charged entities whether dissolved or (partially) aggregated, independent of whether or not the underlying force is of

electrostatic or some other origin. (Further possible interactions include H-bonds or other non-covalent forces.)

An extended stable lipid aggregate typically contains more than a few hundred molecules. Most often, and advantageously, it takes the form of a macroscopic monolayer or a bilayer, depending on the purpose of a formulation.

A lipid, in the sense of this invention, is any substance with characteristics similar to those of fats or fatty materials. In a rule, molecules of this type possess an extended apolar region (chain, X); often they also have a water-soluble, polar, hydrophilic group, so-called head-group (Y). Basic structural formula 1 for lipids therefore reads

$$X_m - Y_n \qquad (1)$$

n being greater than or equal to zero and m typically exceeding the value of 3 (and more often of 6 and in most cases of 10). Lipids with $n = 0$ are called apolar lipids; substances with $n \geq 1$ are polar. In this context, all amphiphiles are called simply lipids. For an explicit list and definitions previous patents and patent applications by one of the authors, such as German Patents DE 41 07 152.2 and DE 44 47 287.0, and International Patent Applications PCT/EP91/01596, PCT/EP96/04526, PCT/EP98/05539, PCT/EP98/06750, PCT/EP98/08421 and PCT/EP99/04659 should be considered, which are incorporated herein by reference.

In this description, all implicitly and explicitly mentioned lipids are well known in the art. Many lipids and phospholipids which form stable aggregates, most often in the form of bilayer vesicles are described in above mentioned patents and patent applications and are surveyed in 'Phospholipids Handbook' (Cevc, G., ed., Marcel

Dekker, New York, 1993); 'An Introduction to the Chemistry and Biochemistry of Fatty acids and Their Glycerides' (Gunstone, F.D., ed.) and 'Lipids' (D. M. Small, ed., Plenum Press, London). A survey of commercial surfactants, some of which may be suitable for the purposes of this application, is given in Handbook of Industrial Surfactants, M. Ash & I. Ash, eds., Gower).

A cationic amphiphile, with the basic formula similar to (1) except in that $Y \rightarrow Y^+$, which can act as an anchor for polyelectrolytes in the associate, is a substance that remains an integral part of the adsorbent during polyelectrolyte-substrate (carrier) association process. Monoamines, that advantageously can take the role of Y^+, include ethanolamine, methylamine, dimethylamine and trimethylamine, ethylamine, diethylamine and triethylamine, n-propylamine, n-butylamine, etc., furthermore methoxyamine, 2-methoxyethylamine, and 2-ethoxyethylamine; diamines, such as ethylenediamine, 1,3-diaminopropane, 1,3-diaminobutane, etc., hydrazine, putrescine, and cadaverine; polyamines, spermine and spermidine; amides, such as acetamide, propionamide, and isonicotinic acid hydrazide, or semicarbazide, etc.. For a comprehensive list, Handbook of Cationic Surfactants should be considered; specific examples used for the purpose of transgene delivery and an extensive list of cationic (amphiphilic) anchors is given in International Patent Application WO96-18372, US-Patent No. 5,910,487 and US-Patent No. 5,650,096, as well as "Cationic amphiphiles and plasmids for intracellular delivery of therapeutic molecules", Genzyme Corp. (USA), which are all incorporated herein by reference.

An anionic anchor differs from the corresponding cationic anchor functionally in the sign of its charges ($Y \rightarrow Y^-$). In principle, any group with a pK below the pH value of formulation containing periodic structures can take the role of Y^-. A representative list of such groups can be found in CRC Handbook of Chemistry and Physics, for example; a comprehensive list of corresponding anchors is given

in Handbook of Cationic Surfactants should be considered; both documents are incorporated herein by reference.

A polyelectrolyte (a charged macromolecule) is any straight or branched chain molecule with charges, which are more often than not concentrated in/along molecular segments, along the chain. This includes poly-cations as well as poly-anions, mixed forms being also possible.

The group of poly-anions encompasses, amongst others, oligo or polynucleotides, such as homo- or hetero-chains of desoxyribonucleic- (DNA) or ribonucleic acid (RNA), especially the genomic DNA, cDNA and mRNA that encode for therapeuticall their chemical, biological, or molecular biological (genetic) modifications or derivatives, etc., with at least 4 charges per chain. The group of nucleotides includes adenine, adenosine, adenosine-3',5'-cyclic monophosphate, n6,o2'dibutyryl, adenosine-3',5'-cyclic monophosphate, n6,o2'-dioctanoyl, adenosine, n6-cyclohexyl, salts of adenosine-5'-diphosphate, adenosine-5'-monophosphoric acid, adenosine-5'-o-(3-thiotriphosphate), salts of adenosine-5'-triphosphate, 9-beta-D-arabinoturanosyladenine, 1-beta-D-arabinoturanosylcytosine, 9-beta-D-arabinoturanosylguanine, 9-beta-D-arabinoturanosylguanin 5'-triphosphate, 1-beta-Darabinoturanosylthymine, 5-azacytidine, 8-azaguanine, 3'-azido-3'-deoxythymidine, 6-beniylaminopurine, cytidine phosphoramidite, beta-cyanoethyl diisopropyl, cytidine-5'-triphosphate, 2'-deoxyadenosine, 2'-deoxyadenosine 5'-triphosphate, 2'-deoxycytidine, 2'-deoxycytidine 5'-triphosphate, 2'-deoxyguanosin, 2'-deoxyguanosine 5'-triphosphate, 2',3'-dideoxyadenosine, 2',3'-dideoxyadenosine 5'-triphosphate, 2',3'-dideoxycytidine, 2',3'-dideoxycytidine 5'-triphosphate, 2',3'-dideoxyguanosine, 2',3'-dideoxyguanosine 5'triphosphate, 2',3'-dideoxyinosine, 2',3'-dideoxythymidine, 2',3'-dideoxythymidine 5'-triphosphate, 2',3'dideoxyuridin, n6-dimethylallyladenine, 5-fluoro-2'deoxyuridin, 5-fluorouracil, 5-fluorouridin, 5-

fluorouridin 5'-monophosphate, formycin a 5'-triphosphate, formycin b, guanosin-3'-5'-cyclic monophosphate, guanosin-5'-diphosphate-3'-diphosphate, guanosin-5'-o-(2thiotriphosphate), guanosin-5'-o-(3'-thiotriphosphate), guanosine 5'-triphosphate, 5'-guanylyl-imidodiphosphate, inosine, 5-iodo-2'-deoxyuridine, nicotinamide-adenine dinucleotides, nicotinamide-adenine dinucleotides, nicotinamide-adenine dinucleotide phosphate, oligodeoxythymidylic acid, (p(DT)10), oligodeoiythymidylic acid (p(DT)12-18), polyadenyl acid (poly A), polyadenyl acid-oligodeoxythymidynic acid, polycytidyl acid, poly(deoxyadenyl-deoxiythymidylic acid, polydeoxyadenylic-acid-oligodeoxythymidynic acid, polydeoxythymidylin acid, polyinosin acid-polycytidyl acid, polyuridynic acid, ribonuclein acid, tetrahydrouridin, thymidine, thymidin-3',5'-diphosphate, thymidine phosphoramidite, beta-cyanoethyl diisopropyl, thymidine 5'-triphosphate, thymin, thymine riboside, uracil, uridine, uridine-5'-diphosphoglucose, uridine 5'triphosphate, xanthine, zeatine, transeatine riboside, etc. Further suitable polymers are: poly(DA) ss, poly(A) ss, poly(C) ss, poly(G) ss, poly(U) ss, poly(DA)-(DT) ds, complementary homopolymers, poly (D(A-T)) ds, copolymers, poly(DG)•(DC) ds, complementary homopolymers, poly (d(GC)) ds copolymers, poly (d(L-C)) ds copolymers, poly(I)-poly(C) ds, etc.. Especially advantageous is the genomic DNA, cDNA and mRNA that encodes for therapeutically useful proteins as are known in the art, ribosomal RNA; further antisense polynucleotides, whether RNA or DNA, that are useful to inactivate transcription products of genes, and which are useful e.g. as therapies to regulate the growth of cells in diseased mammals; or ribozymes.

The group of poly-cations includes certain poly-amino acids, such as poly-lysine. More complete list can be found in pertinent scientific literature, primary sequence databanks, etc.. The chain molecules quoted in such lists, which can be formulated most advantageously following the prescriptions of this application, excel in biological activity and are typically either agonists or antagonists of

biological action, such as protein synthesis. Words poly-nucleic acids with sense or antisense have their common meaning, as used in term anti-sense DNA, for example.

The group of poly-cations includes certain poly-amino acids, such as poly-lysine. More complete list can be found in pertinent scientific literature, primary sequence databanks, etc.

The word biocide describes any ingredient added with the purpose of improving biological stability of the formulation. An exemplary list is given in International Patent Application by the same first applicant (cf. PCT/EP98/08421), which is incorporated herein by reference.

In order to solve the above-mentioned problems the inventions describes a method for preparing pharmaceutically usable compositions comprising periodic structures consisting of polyelectrolytes sandwiched between lipid aggregates having at least one charged component is described which is characterised in that

- a suspension of non-periodic, preferably mono- or bilayer like, lipid aggregates,
- a solution of polyelectrolyte molecules,
- a solution of oligovalent linkers

are separately made and then mixed to form said periodic structures, the simultaneous presence of said components catalysing the formation of said periodic structures comprising at least one layer of lipid component associated with a layer of polyelectrolyte molecules.

It is a preferred feature of said method that the formation of periodic structures does not take place or proceeds at least 10 time less rapidly if any of said components is left out.

Said lipid aggregates preferably have the original form of multilamellar, more preferably of unilamellar lipid vesicles or of freely suspended or supported lipid monolayers.

Accordingly, the polyelectrolytes are selected from the classes of poly-deoxyribonucleic acids, poly-ribonucleic acids, or derivatives thereof.

It is another preferred feature of the invention that said oligovalent linkers belong to the class of chelators. Another preference is to use polar lipids for forming lipid aggregates.

It is preferred that a suspension of lipid aggregates and a polyelectrolyte solution are mixed, to form a relatively stable suspension in a solution, and oligovalent linkers, preferably in a solution, are then added to start or to control otherwise the formation of said periodic structures. It then is advantageous that said periodic structures are suspended or remain suspended in the supporting solution after their formation.

According to an advantageous embodiment of the invention, the average size of plain lipid aggregates is between 30 nm and 5000 nm, preferably is between 20 nm and 1000 nm, more preferably is between 30 nm and 500 nm and most preferably is between 450 nm and 100 nm.

It is preferred that the concentration of at least one of the above-listed system components and / or the respective relative concentrations are used to control the speed of formation and / or the final size and / or the degree of periodicity for the structures generated in the system.

Accordingly, it may be advantageous to select the final size, which for spherical structures corresponds to diameter, of suspended periodic structures is between 10 nm and 10 μm, preferably is between 20 nm and 2.5 μm, even more advantageously is between 30 nm and 600 nm or even better between is 40 nm and 350 nm, and most preferably is between 50 nm and 200 nm.

Another important feature is the choice of final periodicity of said structures, which preferably should be between 2 and 100, more advantageously is between 4 and 50 and even more advantageously is between 8 and 25.

According to a preferred feature of the invention the chelator is selected amongst EDTA, EGTA, EDDA, EDDS (ethylenediamine-N,N'-disuccinic acid), iminodiacetic acid, or their salts, DMPS (2,3-dimercaptopropane-1-sulfonic-acid), 8-hydroxyquinoline, lipoic acid (thioctic acid), deferoxamine mesilate, polycarboxylate, 2-furildioxime, N-2-hydroxypropyl sulphonic acid aspartic acid, N-carboxymethyl N-2 hydroxypropyl 3 sulphonic acid, β-alanine N,N diacetic acid aspartic acid, N,N diacetic acid aspartic acid N-monoacetic acid, iminodisuccinic acid, is an amino acid based chelating agent, such as isoserine diacetic acid (ISDA), 2-phosphonobutane-1,2-4-tricarboxylic acid, GADS, alkyl iminodiacetic acid; dipicolinic acid; hydroxy-1,1-ethylidene diphosphonic acid (HEDP) or a derivative thereof, or is some other oligo- or poly-anions and cations, or any other molecules with several polar, polarisable, or otherwise associable groups, which often have hydrogen bond donors and/or acceptors on them.

It is also preferred to use lipids (or lipoids) from biological sources or made synthetically, directly or by modifying the former lipids, advantageously comprising a glyceride, glycerophospholipid, isoprenoidlipid, sphingolipid, steroid, sterine or sterol, a sulphur- or carbohydrate-containing lipid, or else, any other lipid which forms bilayers, in particular a half-protonated fluid fatty acid,

and very frequently a phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, a phosphatidic acid, a phosphatidylserine, a sphingomyelin or sphingophospholipid, glycosphingolipid (e.g. cerebroside, ceramidpolyhexoside, sulphatide, sphingoplasmalogene), a ganglioside or any other glycolipid or a synthetic lipid, in particular with oleoyl-, linoleyl-, linolenyl-, linolenoyl-, arachidoyl-, lauroyl-, myristoyl-, palmitoyl-, stearoyl chains, which can also be attached to the corresponding sphingosine base, is a glycolipid or any other diacyl-, dialkenoyl, dialkyl-lipid or branched aliphatic chain-lipid with two identical or mixed chains.

Cationic anchors which can be used particularly advantageously belong to the class of lipids with one or several aliphatic chains or other siutable apolar residues, if appropriate branched or derivatised, and a headgroup with one or several positive charges; the latter most often reside on a quaternary or ternary amine, which in case of monoamines includes ethanolamine, methylamine, dimethylamine and trimethylamine, ethylamine, diethylamine and triethylamine, n-propylamine, n-butylamine, etc., furthermore methoxyamine, 2-methoxyethylamine, and 2-ethoxyethylamine; diamines, such as ethylenediamine, 1,3-diaminopropane, 1,3-diaminobutane, etc., hydrazine, putrescine, and cadaverine; polyamines, spermine and spermidine; when an amide can be e.g. acetamide, propionamide, and isonicotinic acid hydrazide, or semicarbazide, etc.; an alkylamine or a polyalkylamine is also particularly useful. Preferable choices include N-[1-(2,3-diacyl)-, N-[1-(2,3-dialkyl)- or N-[1-(2,3-dialkenoyl)propyl]-N,N,N-trialkylammonium, -N,N-dialkylammonium or -N-alkylammonium salt, such as N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium bromide (DOTMA), 1,2-diacyloxypropyl-N,N-dialkyl-hydroxyalkyl ammonium salt, 1,2-dialkenoyloxypropyl-N,N,N-dialkyl-hydroxyalkyl ammonium salt, or -N,N-alkyl-hydroxyalkyl, or N,N,N-alkyl-dihydroxyalkyl, such as 1,2-dimyristyloxypropyl-N,N-dimethyl-hydroxyethyl ammonium bromide (DMRIE), [N-(N′, N′-

dialkylaminoethane) carbamyol] cholesterol, such as [N-(N′, N′-dimethylaminoethane) carbamyol] cholesterol (DC-Chol), or [N-(N′-alkylaminoethane) carbamyol] cholesterol, dialkylamidoglycyl spermine or spermidine, such as dioctadecylamidoglycyl spermidine (DOGS), diacyl, dialkenoyl or dialkyl diacylammonium or acylammonium salt, such as dimethyl dioctadecylammonium bromide (DDAB), 2,3-diacyl-, 2,3-dialkenoyl- or 2,3-dialkyl-N-[2(sperminecarbozamide-0-ethyl]-N,N-dialkyl- or N-alkyl-1-propanaminium trifluoroacetate, such as 2,3-dioleoyloxyl-N-[2(sperminecarbozamide-0-ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate (DOSPA), a 1-[2-(alkenoyloxy)-ethyl]-2-alkenoyl-3-(2-hydroxyalkyl) imidazolinium salt, such as 1--[2-(oleoyloxy)-ethyl]-2-oleyl-3-(2-hydroxyethyl) imidazolinium chloride (DOTIM), 1,2-dialkenoyloxy-3-(trialkylammonio)- or (dialkylammonio)- or alkylammonio-propane, such as 1,2-dioleoyloxy-3-(trimethylammonio) propane (DOTAP), 1,2-diacyl-3-trimethylammonium propane (TAP), 1,2-diacyl-3-dimethylammonium propane (DAP) or 1,2-diacyl-3-methylammonium propane (MAP), and fatty acid salts of quaternary amines.

Anionic anchors which are selected most often, or with an advantage, carries a carboxylate, succinate, sulfosuccinate, sulphate, sulphonate, ether sulphate, phosphate, phosphonate or amine oxide, or other anionic substances which also appear in anionic linkers, with some preference for long-chain fatty acid derivatives, alkylsulphate-, phosphate or phosphonate salts, cholate-, deoxycholate-, glycodeoxycholate-, taurodeoxycholate-salts, dodecyl- dimethyl-aminoxides, especially lauroyl- or oleoylsulphate-salts, sodium deoxycholate, sodium glycodeoxycholate, sodium oleate, sodium elaidate, sodium linoleate, sodium laurate or sodium myristate.

It is advantageous if the concentration of charged anchors used in the mixing process, relatively to the concentration of the lipids that form basic aggregates, is

in the range 1-80 mol-%, more preferably is 10-60 mol-%, and most preferably is 20-50 mol-%, the specific chosen value also depending on the selected polyelectrolyte concentration; higher concentrations of latter ingredient typically require a relatively high concentration of charged anchor molecules.

It is furthermore advantageous if the total lipid concentration, including charged anchors and basic lipids in the aggregates is 0.0005-30 w-%, more preferably is 0.001-20 w-%, even more preferably is 0.01-15 w-%, and most preferably is 0.05-10 w-%.

It may be advantageous as well that the bulk polyelectrolyte concentration is selected to be in the range 0.0005-30 w-%, more preferably is 0.001-20 w-%, even more preferably is 0.01-15 w-%, and most preferably is 0.05-10 w-%.

Preferably, the specific total lipid concentration and polyelectrolyte concentration values are chosen so as to ensure that the resulting periodic structures carry less than 50 % of the original charge density and more preferably less than 25 % of residual charge.

Furthermore, it is preferred that the concentration and the composition of background electrolyte is chosen so as to maximise the positive effect of charge-charge interactions on the association and normally is below I = 1, more preferably below 0.5 and even more preferably is between 0.01 and 0.3.

According to a preferred feature of the invention the formation of (mixed) lipid suspension is induced by substance addition into the fluid phase, evaporation from a reverse phase, by using an injection- or a dialysis procedure, with the aid of mechanical stress, such as shaking, stirring, vibrating, homogenisation, ultrasonication, shear, freezing and thawing, or filtration using convenient driving pressure.

It is another preferred feature of the invention that the lipid(s) and charged anchor molecules are separately mixed, if required in an organic solution (which in case is eliminated in due time), and the resulting suspension is combined with the solution of polyelectrolytes and the chosen linkers solution under the action of mechanical energy.

It may be advantageous to generate the starting suspension of lipid aggregates or to achieve the final mixing by filtration, suitably elevated pressure or velocity homogenisation, shaking, stirring, mixing, or by means of any other controlled mechanical fragmentation.

Preferably, the formation of aggregates with the desired size is ensured by filtration, the filtering material having pores sizes between 0.02 μm and 0.8 μm, very frequently between 0.05 μm and 0.4 μm, and most frequently between 0.08 μm and 0.2 μm, several filters being potentially used in a row or sequentially.

According to another preferred feature, the composition of periodic structures is prepared just before the application, if convenient, from a suitable concentrate or a lyophilisate.

It is another characteristic feature of the invention that the composition comprising periodic structures, prepared according to the above-described method, is used to manipulate cells, their metabolism, reproduction or survival. It then may be of an advantage if the composition is used in or on the mammalian body, preferably as drug, drug depot, or some other kind of device with a desirable medical or biological action.

It may then be advantageous if said structures contain oligo- or poly-nucleic acids that are either sense or antisense or else comprise an expressible form of a transgene, and are used to deliver said nucleic acids into cells. Preferably, said composition is used for gene delivery, gene therapy or any other kind of modulation of genetic action or in bioengineering; it may furthermore be advantageous for said transgene to encode a protein, which preferably is selected from the group consisting of a ligand, a receptor, an agonist of a ligand, an agonist of a receptor, an antigonist of a ligand, and an antigonist of a receptor. It then also is preferred that said protein is a soluble protein.

It is a preferred feature of said inventive to use transgene which expresses antisense RNA.

Another preferred use of said composition refers to selection of above-mentioned cells in a mammal with a disorder or a potential disorder, said use then being for treating the disorder or for preventing the potential disorder, as in the case of vaccination. Said disorder preferably is an inflammatory disease, dermatosis, kidney or liver failure, adrenal insufficiency, aspiration syndrome, Behcet syndrome, blood disorder, such as cold-haemagglutinin disease, haemolytic anemia, hypereosinophilia, hypoplastic anemia, macroglobulinaemia, trombocytopenic purpura, a bone disorder, cerebral oedema, Cogan´s syndrome, congenital adrenal hyperplasia, connective tissue disorder, such as lichen, lupus erythematosus, polymyalgia rheumatica, polymyositis and dermatomyositis, epilepsy, an eye disorder, such as cataracts, Graves´ ophthalmopathy, haemangioma, herpes infection, neuropathy, retinal vasculitis, scleritis, a gastro-intestinal disorder, such as inflammatory bowel disease, nausea and oesophageal damage, hypercalcaemia, an infection, e.g. of the eye (as in infections mononucleosis), Kawasaki disease, myasthenia gravis, one of pain syndromes, such as postherpetic neuralgia, polyneuropathy, pancreatitis, respiratory disorder,

such as asthma, rheumatoid disease or osteoarthritis, rhinitis, sarcoidosis, skin disease, such as alopecia, eczema, erythema multiforme, lichen, pemphigus and pemphigoid, psoriasis, pyoderma gangrenosum, urticaria, a thyroid or vascular disorder.

Another advantageous piece of the invention is a kit comprising, in a bottled or otherwise packaged form, at least one dose of the pharmaceutically usable composition prepared according to the above-described method designed to be used in or on a mammal for prophylactic purposes, e.g. in the course of vaccination, or for therapy.

The following two examples (cf. comparative panels 1 and panel 2) illustrate the determining influence of chelator (linking agent) EDTA on the formation of lipid multilayers from a suspension of originally unilamellar / monolayer systems. (Electron density profiles given in both examples, pertaining to the aggregates at the air-water interface, were determined with a laboratory built X-ray reflectometer and are shown in Figure 1.)

For the preparation of comparative example 1 (cationic vesicles / liposomes in absence of EDTA) and of example 2 (cationic vesicles / liposomes in presence of EDTA), the following general experimental conditions were used: a solution of linear DNA fragments with an average length of 6 000 bp was prepared by digesting calf thymus DNA (Sigma Chemical Co., USA) with the restriction endonuclease EcoRV (Stratagene, USA). The fragments were purified by repeated phenol/chloroform extraction according to standard procedures and exhaustive dialysis against 25 mM triethanolamine buffer (pH adjusted to 7.4 with HCl) through a membrane with a molecular weight cut-off of 3 kDa.
The lipid mixture used in this study consisted of the cationic lipid 3β[N-(N′,N′-dimethylaminoethane)-carbamoyl] cholesterol (DC-Chol, Bachem Biochemica,

Heidelberg, Germany) and the zwitterionic lipid 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC, Avanti Polar Lipids, Alabaster, AL, USA) at a molar ratio of 2:3. Part of the mixture was dissolved in chloroform to a total lipid concentration of 1 g/L. The other part of the mixture was used to prepare small unilamellar vesicles by repeated sequential extrusion through 400 nm to 50 nm polycarbonate filters in the buffer described above. Quasi-elastic light scattering revealed final vesicle diameters of 63 (±22) nm.

To measure X-ray reflectivity, as shown in Figure 1, a Langmuir trough was filled with DNA solution to which vesicle suspension was added. To this, 0.54 mM EDTA was added to induce multilayer formation. The final DNA concentration in the solution was 6 mg/L, total vesicles concentration was 100 mg/L. A Langmuir film at the air water interface was prepared by spreading said mixture from a chloroform solution onto the surface of the suspension of vesicles and DNA solution. To suppress capillary waves, a film of only 300 μm thickness was used. The temperature was kept constant at
25 (±0.1) °C .
The X-ray reflectometer was laboratory made. A sealed 3 kW Molybdenum anode serves as X-ray source. A Germanium solid state detector (Silena, Milan, Italy, E.U.) with an energy resolution of about 1 % was used to analyse the specularly reflected beam. The source as well as the detector were mounted on goniometers. The Langmuir trough was positioned on a lifting jack between the two goniometers and enclosed in a gas-tight box to avoid water evaporation. In this study, the reflectometer was operated in the energy dispersive mode with angles fixed at $\alpha = 8.61$ mrad. In the energy dispersive mode, the fact was exploited that the wave vector transfer q is a function of X-ray energy and incident angle α. The energy spectrum of the specularly reflected beam was multiplied with a previously recorded calibration function, to correct for the energy distribution of the source, detector sensitivity and secondary effects, such as

scattering at the water vapour in the sample chamber. From the calibrated energy spectrum the reflectivity R(q) was obtained. A more detailed description of the method is given in Vierl, U., Cevc, G., Metzger, H. 1995 "Energy-Dispersive X-ray Reflectivity Study of the Model Membranes at the Air/Water Interface" in Biochim. Biophys. Acta. 1234: 139-143.

The upper panel of Figure 1 gives the electron density profile of a lipid monolayer at the air-water interface in contact with the suspension of corresponding vesicles and DNA without chelators (linkers) added (example 1). Only a mixed (cationic) lipid monolayer is observed at all times. In contrast to this, the electron density profile illustrated in lower panel of Figure 1 (example 2) pertains to the air-water interface covered with a mixed (cationic) lipid monolayer in contact with a suspension of corresponding vesicles and a solution of DNA with EDTA added, after 100 hours of incubation. The underlying molecular structure is shown for better reflectogram understanding. The multilayers apparent in the lower panel consist of lipid bilayers alternating with monolayers of DNA. Low electron densities correspond to the hydrocarbon tail region of lipid bilayers. High electron densities correspond to the lipid headgroup regions and intercalated DNA layers. The vertical grid corresponds to the repeat distance of surface adsorbed CL-DNA multilayers. As the only difference between examples 1 and 2 is the presence of EDTA the importance of latter is evident.

The validity of general conclusions is not restricted to the narrowness of illustrated examples, described here in detail, nor is the use of resulting associates only useful in the field of human or veterinary medicine.



CLAIMS

1. Method for preparing pharmaceutically usable compositions comprising periodic structures consisting of polyelectrolytes sandwiched between the lipid aggregates having at least one charged component **characterised in that**

- a suspension of non-periodic, preferably mono- or bilayer like, lipid aggregates,
- a solution of polyelectrolyte molecules,
- a solution of oligovalent linkers

are separately made and then mixed to form said periodic structures, the simultaneous presence of said components catalysing the formation of said periodic structures comprising at least one layer of lipid component associated with a layer of polyelectrolyte molecules.

2. The method according to claim 1, **characterised in that** the formation of such structures does not take place or proceeds at least 10 time less rapidly if any of said components is left out.

3. The method according to claims 1 or 2, **characterised in that** lipid aggregates originally have the form of multilamellar, more preferably unilamellar lipid vesicles or freely suspended or supported lipid monolayers.



4. The method according to any one of the preceding claims, **characterised in that** said polyelectrolytes are selected from the classes of poly-deoxyribonucleic acids, poly-ribonucleic acids, or derivatives thereof.

5. The method according to any one of the preceding claims, **characterised in that** said oligovalent linkers belong to the class of chelators.

6. The method according to claim 5, **characterised in that** polar lipids are used to form lipid aggregates.

7. The method according to any one of the preceding claims, **characterised in that** a suspension of lipid aggregates and a polyelectrolyte solution are mixed, to form a relatively stable suspension in a solution, and oligovalent linkers, preferably in a solution, are then added to start or to control otherwise the formation of said periodic structures.

8. The method according to claim 7, **characterised in that** said periodic structures are suspended or remain suspended in the supporting solution after their formation.

9. The method according to any one of the preceding claims, **characterised in that** the average size of plain lipid aggregates is between 30 nm and 5000 nm, preferably is between 20 nm and 1000 nm, more preferably is between 30 nm and 500 nm and most preferably is between 450 nm and 100 nm.

10. The method according to claims 7 to 9, **characterised in that** the concentration of at least one of the system components listed in claim 1 and / or the respective relative concentrations are used to control

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the speed of formation and / or the final size and / or the degree of periodicity for the structures generated in the system.

11. The method according to claims 7 to 10,
characterised in that the final size, which for spherical structures corresponds to diameter, of the suspended periodic structures is between 10 nm and 10 µm, preferably is between 20 nm and 2.5 µm, even more advantageously is between 30 nm and 600 nm or even better between 40 nm and 350 nm, and most preferably is between 50 nm and 200 nm.

12. The method according to claims 7 to 11,
characterised in that the final number of periods in said structure is between 2 and 100, advantageously is between 4 and 50 and even more advantageously is between 8 and 25.

13. The method according to any one of the preceding claims,
characterised in that the chelator is selected amongst EDTA, EGTA, EDDA, EDDS (ethylenediamine-N,N'-disuccinic acid), iminodiacetic acid, or their salts, DMPS (2,3-dimercaptopropane-1-sulfonic-acid), 8-hydroxyquinoline, lipoic acid (thioctic acid), deferoxamine mesilate, polycarboxylate, 2-furildioxime, N-2-hydroxypropyl sulphonic acid aspartic acid, N-carboxymethyl N-2 hydroxypropyl 3 sulphonic acid, ß-alanine N,N diacetic acid aspartic acid, N,N diacetic acid aspartic acid N-monoacetic acid, iminodisuccinic acid, is an amino acid based chelating agent, such as isoserine diacetic acid (ISDA), 2-phosphonobutane-1,2-4-tricarboxylic acid, GADS, alkyl iminodiacetic acid; dipicolinic acid; hydroxy-1,1-ethylidene diphosphonic acid (HEDP) or aderivative thereof, or is some other oligo- or poly-anion and cation, or any other molecule with several polar, polarisable, or otherwise associable groups, which often have hydrogen bond donors and/or acceptors on them.



14. The method according to any one of the preceding claims, **characterised in that** the lipid (or lipoid) stems from a biological source or is made synthetically, directly or by modifying the former lipid, and advantageously comprises a glyceride, glycerophospholipid, isoprenoidlipid, sphingolipid, steroid, sterine or sterol, a sulphur- or carbohydrate-containing lipid, or else, any other lipid which forms bilayers, in particular a half-protonated fluid fatty acid, and very frequently a phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, a phosphatidic acid, a phosphatidylserine, a sphingomyelin or sphingophospholipid, glycosphingolipid (e.g. cerebroside, ceramidpolyhexoside, sulphatide, sphingoplasmalogene), a ganglioside or any other glycolipid or a synthetic lipid, in particular with oleoyl-, linoleyl-, linolenyl-, linolenoyl-, arachidoyl-, lauroyl-, myristoyl-, palmitoyl-, stearoyl chains, which can also be attached to the corresponding sphingosine base, is a glycolipid or any other diacyl-, dialkenoyl, dialkyl-lipid or branched aliphatic chain-lipid with two identical or mixed chains.

15. The method according to any one of the preceding claims, **characterised in that** the cationic anchor belongs to the class of lipids with one or several aliphatic chains or other, suitable apolar residues, the former being potentially branched or derivatised, and a headgroup with one or several positive charges, said headgroup most often being a monoamine, including ethanolamine, methylamine, dimethylamine and trimethylamine, ethylamine, diethylamine and triethylamine, n-propylamine, n-butylamine, etc., furthermore methoxyamine, 2-methoxyethylamine, and 2-ethoxyethylamine; a diamine, e.g. ethylenediamine, 1,3-diaminopropane, 1,3-diaminobutane, etc., hydrazine, putrescine, and cadaverine; a polyamine, e.g. spermine or spermidine; an amide, such as acetamide, propionamide, an isonicotinic acid hydrazide, a semicarbazide, etc..

16. The method according to claim 15,

characterised in that the cationic anchor is selected from N-[1-(2,3-diacyl)-, N-[1-(2,3-dialkyl)- or N-[1-(2,3-dialkenoyl)propyl]-N,N,N-trialkylammonium, -N,N-dialkylammonium or -N-alkylammonium salt, such as N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium bromide (DOTMA), 1,2-diacyloxypropyl-N,N-dialkyl-hydroxyalkyl ammonium salt, 1,2-dialkenoyloxypropyl-N,N,N-dialkyl-hydroxyalkyl ammonium salt, or -N,N-alkyl-hydroxyalkyl, or N,N,N-alkyl-dihydroxyalkyl, such as 1,2-dimyristyloxypropyl-N,N-dimethyl-hydroxyethyl ammonium bromide (DMRIE), [N-(N′, N′-dialkylaminoethane) carbamyol] cholesterol, such as [N-(N′, N′-dimethylaminoethane) carbamyol] cholesterol (DC-Chol), or [N-(N′-alkylaminoethane) carbamyol] cholesterol, dialkylamidoglycyl spermine or spermidine, such as dioctadecylamidoglycyl spermidine (DOGS), diacyl, dialkenoyl or dialkyl diacylammonium or acylammonium salt, such as dimethyl dioctadecylammonium bromide (DDAB), 2,3-diacyl-, 2,3-dialkenoyl- or 2,3-dialkyl-N-[2(sperminecarbozamide-0-ethyl]-N,N-dialkyl- or N-alkyl-1-propanaminium trifluoroacetate, such as 2,3-dioleoyloxyl-N-[2(sperminecarbozamide-0-ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate (DOSPA), a 1-[2-(alkenoyloxy)-ethyl]-2-alkenoyl-3-(2-hydroxyalkyl) imidazolinium salt, such as 1--[2-(oleoyloxy)-ethyl]-2-oleyl-3-(2-hydroxyethyl) imidazolinium chloride (DOTIM), 1,2-dialkenoyloxy-3-(trialkylammonio)- or (dialkylammonio)- or alkylammonio-propane, such as 1,2-dioleoyloxy-3-(trimethylammonio) propane (DOTAP), 1,2-diacyl-3-trimethylammonium propane (TAP), 1,2-diacyl-3-dimethylammonium propane (DAP) or 1,2-diacyl-3-methylammonium propane (MAP), and fatty acid salts of quaternary amines.



17. The method according to any one of the preceding claims, **characterised in that** said anionic anchor carries a carboxylate, succinate, sulfosuccinate, sulphate, sulphonate, ether sulphate, phosphate, phosphonate or amine oxide, or other anionic substances which also appear in anionic linkers, with some preference for long-chain fatty acid derivatives, alkylsulphate-, phosphate or phosphonate salts, cholate-, deoxycholate-, glycodeoxycholate-, taurodeoxycholate-salts, dodecyl- dimethyl-aminoxides, especially lauroyl- or oleoylsulphate-salts, sodium deoxycholate, sodium glycodeoxycholate, sodium oleate, sodium elaidate, sodium linoleate, sodium laurate or sodium myristate.

18. The method according to any one of the preceding claims, **characterised in that** the concentration of charged anchors used in the mixing process relatively to the concentration of the lipids that form basic aggregates is in the range 1-80 mol-%, more preferably is 10-60 mol-%, and most preferably is 20-50 mol-%, the specific chosen value also depending on the selected polyelectrolyte concentration, higher concentrations of latter ingredient typically requiring higher relative concentration of charged anchor molecules.

19. The method according to any one of the preceding claims, **characterised in that** the total lipid concentration, including charged anchors and basic lipids in the aggregates, is 0.0005-30 w-%, more preferably is 0.001-20 w-%, even more preferably is 0.01-15 w-%, and most preferably is 0.05-10 w-%.

20. The method according to any one of the preceding claims, **characterised in that** the bulk polyelectrolyte concentration is in the range 0.0005-30 w-%, more preferably is 0.001-20 w-%, even more preferably is 0.01-15 w-%, and most preferably is 0.05-10 w-%.



21. The method according to any one of the preceding claims, **characterised in that** the specific total lipid concentration and polyelectrolyte concentration values are chosen so as to ensure that the resulting periodic structures carry less than 50 % of the original charge density and more preferably less than 25 % of residual charge.

22. The method according to any one of the preceding claims, **characterised in that** the concentration and the composition of background electrolyte is chosen so as to maximise the positive effect of charge-charge interactions on the association and normally is below I = 1, more preferably below 0.5 and even more preferably is between 0.01 and 0.3.

23. The method according to any one of the preceding claims, **characterised in that** the formation of (mixed) lipid suspension is induced by substance addition into the fluid phase, evaporation from a reverse phase, by using an injection- or a dialysis procedure, with the aid of mechanical stress, such as shaking, stirring, vibrating, homogenisation, ultrasonication, shear, freezing and thawing, or filtration using convenient driving pressure.

24. The method according to any one of the preceding claims, **characterised in that** the lipid(s) and charged anchor molecules are separately mixed, if required in an organic solution, which in case is eliminated in due time, and the resulting suspension is combined with the solution of polyelectrolytes and the chosen linkers solution under the action of mechanical energy.

25. The method according to any one of the preceding claims, **characterised in that** the starting suspension of lipid aggregates is generated or the final mixing is achieved by filtration, suitably elevated pressure or velocity



homogenisation, shaking, stirring, mixing, or by means of any other controlled mechanical fragmentation.

26. The method according to claim 25,
characterised in that the formation of aggregate with desired size is ensured by filtration, the filtering material having pores sizes between 0.02 µm and 0.8 µm, very frequently between 0.05 µm and 0.4 µm, and most frequently between 0.08 µm and 0.2 µm, several filters being potentially used in a row or sequentially.

27. The method according to any one the preceding claims,
characterised in that the formulation of periodic structures is prepared just before the application, if convenient from a suitable concentrate or a lyophilisate.

28. Use of a pharmaceutically usable composition comprising periodic structures prepared according to any one of the preceding claims to manipulate cells, their metabolism, reproduction or survival.

29. The use of a pharmaceutically usable composition comprising periodic structures according to claim 28, in or on the mammalian body, preferably as drug, drug depot, or some other kind of device with a desirable medical or biological action.

30. The use of a pharmaceutically usable composition comprising periodic structures according to claims 28 or 29, said structures contain oligo- or poly-nucleic acids that are either sense or antisense or else comprise an expressible form of a transgene, and are used to deliver said nucleic acids into cells.

31. The use of a pharmaceutically usable composition comprising periodic structures according to claim 30 for gene delivery, gene therapy or any other kind of modulation of genetic action or in bioengineering.

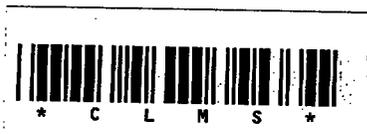
32. The use of a pharmaceutically usable composition comprising periodic structures according to claim 31,
characterised in that said transgene encodes a protein.

33. The use of a pharmaceutically usable composition comprising periodic structures according to claim 32,
characterised in that said protein is selected from the group consisting of a ligand, a receptor, an agonist of a ligand, an agonist of a receptor, an antigonist of a ligand, and an antigonist of a receptor.

34. The use of a pharmaceutically usable composition comprising periodic structures according to claim 33,
characterised in that said protein is a soluble protein.

35. The use of a pharmaceutically usable composition comprising periodic structures according to claim 30,
characterised in that said transgene expresses antisense RNA.

36. The use of a pharmaceutically usable composition comprising periodic structures according to claim 28,
characterised in that said cells are in a mammal with a disorder or a potential disorder and the method is for treating the disorder or for preventing the potential disorder, as in the case of vaccination.



37. The use of a pharmaceutically usable composition comprising periodic structures according to claim 36,
characterised in that said disorder comprises an inflammatory disease, dermatosis, kidney or liver failure, adrenal insufficiency, aspiration syndrome, Behcet syndrome, blood disorder, such as cold-haemagglutinin disease, haemolytic anemia, hypereosinophilia, hypoplastic anemia, macroglobulinaemia, trombocytopenic purpura, a bone disorder, cerebral oedema, Cogan's syndrome, congenital adrenal hyperplasia, connective tissue disorder, such as lichen, lupus erythematosus, polymyalgia rheumatica, polymyositis and dermatomyositis, epilepsy, an eye disorder, such as cataracts, Graves' ophthalmopathy, haemangioma, herpes infection, neuropathy, retinal vasculitis, scleritis, a gastro-intestinal disorder, such as inflammatory bowel disease, nausea and oesophageal damage, hypercalcaemia, an infection, e.g. of the eye (as in infections mononucleosis), Kawasaki disease, myasthenia gravis, one of pain syndromes, such as postherpetic neuralgia, polyneuropathy, pancreatitis, respiratory disorder, such as asthma, rheumatoid disease or osteoarthritis, rhinitis, sarcoidosis, skin disease, such as alopecia, eczema, erythema multiforme, lichen, pemphigus and pemphigoid, psoriasis, pyoderma gangrenosum, urticaria, a thyroid or vascular disorder.

38. A kit comprising, in a bottled or otherwise packaged form, at least one dose of the pharmaceutically usable composition prepared according to any one of the preceding claims 1 to 27 designed to be used in or on a mammal for prophylactic purposes, e.g. in the course of vaccination, or for therapy.