Abstract:

Novel nanolattices comprising cross-linked or chelated polymers having a desired polydispersity index suitable for delivery of pharmaceutical agents and complexes comprising same are described. Methods of making the nanolattices and use of the nanolattices to deliver pharmaceutical agents for use in a range of diagnostic, prophylactic, therapeutic, nutritional and/or research applications are also described.
NANOSTRUCTURES SUITABLE FOR DELIVERY OF AGENTS

This application claims priority from US provisional patent application No. 60/800,898 filed 16 May 2006, the disclosure of which is incorporated herein in its entirety.

Field of the Invention

The present invention relates to novel nanolattices suitable for delivery of pharmaceutical agents and complexes comprising the same. The present invention further extends to methods of making the nanolattices and use of such nanolattices to deliver pharmaceutical agents for use in a range of diagnostic, prophylactic, therapeutic, nutritional and/or research applications.

Background of the Invention

Many pharmaceutical agents, particularly protein drugs and vaccines are administered by parenteral delivery such as injection. The disadvantages associated with this form of administration include a potential lack of patient compliance, discomfort, expense, the need for training for administration and potential adverse reactions at the site of injection. A more desirable administration route for pharmaceuticals is an oral delivery system, however there are major challenges associated with oral delivery of pharmaceuticals. Successful oral delivery via the gastrointestinal tract of a host is hindered by a number of physical and chemical barriers, including degradation of the pharmaceutical agent by enzymes present in the gastrointestinal tract and poor permeability of pharmaceutical agents across the intestine to reach the systemic circulation and sites of action. Thus, an effective oral delivery system must be able to withstand the attack of endogenous enzymes, gastric acidity and intestinal alkalinity without losing activity, as well as being
able to penetrate and cross the gastrointestinal mucosa and enter into the blood stream, thereby delivering the pharmaceutical to the site where activity is required. Moreover, the delivery must take place at an appropriate rate to ensure the correct therapeutic dosage is delivered.

Targeted oral delivery of pharmaceutical agents such as peptides and proteins has in the past utilized various solid particulate systems in which the pharmaceutical is entrapped during particle preparation. For many proteins entrapment within such systems often leads to degradation of the pharmaceutical due to the use of organic solvents, cross-linking of the pharmaceutical to the particle matrix, or desolvation of the pharmaceutical.

In addition, the solid particles only passively and non-specifically entrap the pharmaceutical agent. These particles are also highly polydisperse meaning that some of the particles may be too big for uptake via intestinal epithelial cells, or alternatively some particles may be too small to effectively encapsulate the drug to be delivered. As a result, an accurate uptake of the pharmaceutical agent by a subject may not be readily determined, rendering the particles unsuitable for clinical application. The reason for this is that many drugs exhibit a narrow pharmaceutical index, meaning that an under supply of drug results in no pharmaceutical effect, while an over delivery of the drug results in a toxic dose. For example, an overdose of insulin will result in hypoglycemic shock, whereas an under supply results in ineffective control of blood sugar levels.

There is therefore a need for particles capable of carrying pharmaceutical agents that overcome or substantially ameliorate at least some of the deficiencies associated with known particles. Recently, nanotechnology has provided tools of constructing biostructures in a nano scale and such nanostructures can be applied in a variety of areas. Surprisingly, the inventors have produced nanostructures via the chelation of polymers which can entrap and deliver pharmaceutical agents without the use of solvents or
covalent cross-linking agents during the process of entrapment. The nanostructures of the invention involve the use of water-in-oil microemulsions and chelating agents to link the polymers during their formation. Diagnostic agents and pharmaceutically active agents, in particular proteins and peptides, are able to be entrapped at room temperatures allowing for the incorporation of heat-sensitive proteins. The size of the structures of the invention is particularly suitable for oral delivery and also for intravenous injection as their size is small enough for uptake into the endosomes of the intestinal epithelial cells following enteral administration, and sufficiently small to prevent non-specific trapping in the lung following parenteral injection.
Summary of the Invention

Surprisingly the present invention provides nanolattices capable of carrying a pharmaceutical agent. The nanolattices comprise at least one chelated or cross-linked polymer, wherein the nanolattices have a desired PDI. The nanolattices of the invention also have a desired size. Surprisingly, the present invention also provides methods for the preparation of the nanolattices of the invention involving micro-emulsion technology for the formation of the nanolattices and association of the pharmaceutical agent.

According to an aspect of the present invention there is provided nanolattices capable of carrying one or more pharmaceutical agents, the nanolattices comprising at least one cross-linked or chelated polymer, wherein the nanolattices have a polydispersity index (PDI) of between about 0.1 and about 0.4.

In an aspect of the invention the polymer is a polysaccharide.

In an aspect of the invention the nanolattice comprises a cross-linked polymer.

In another aspect of the invention the nanolattice comprises a chelated polymer.

In an embodiment, the nanolattices may comprise one or more pendant side-chains capable of interacting with one or more pharmaceutical agents. The interaction may be selected from one or more of the following: ionic interactions, hydrogen bonding, hydrophobic interactions, chelation, co-ordination bonding or pi-pi interactions.

According to another aspect, the present invention provides a process for preparing nanolattices having a PDI of between about 0.1 and 0.4, said method comprising:

(a) preparing separate mixtures comprising:
(i) a surfactant and a polymer in an aqueous phase; and
(ii) a surfactant and a non-polar phase; and
(b) combining mixtures (i) and (ii) from step (a) to form mixture (iii);
(c) subjecting mixture (iii) to mixing for a period sufficient to allow formation of a uniform water-in-oil emulsion;
(d) adding a cross-linking or metal chelating agent to the emulsion to form the nanolattices; and
(e) separating the nanolattices from the emulsion.

In one embodiment, co-surfactants are also added to (i) or (ii) from step (a) to form mixture (iii).

In one embodiment the polymer is cross-linked to form the nanolattices.

In another embodiment the polymer is chelated to form the nanolattices.

In another embodiment, the process for preparing nanolattices may further comprise surface modification of the nanolattices by one or more reagents to provide one or more pendant side chains.

In an additional embodiment, the polymer may be a polysaccharide, such as dextran. The amount of chelating agent added in step (d) may be between about 0.25 and 0.0005 mol equivalents of the polymer.

According to another aspect, the present invention provides a complex wherein said complex comprises nanolattices having a PDI of between about 0.1 and 0.4 prepared by a method comprising:
(a) preparing separate mixtures comprising:
   (i) a surfactant and a polymer in an aqueous phase; and
   (ii) a surfactant and a non-polar phase; and
(b) combining mixtures (i) and (ii) from step (a) to form mixture (iii);
(c) subjecting mixture (iii) to mixing for a period sufficient to allow formation of a uniform water-in-oil emulsion;
(d) adding a pharmaceutical agent to one or more of mixtures (i), and (iii) and the emulsion of step (c);
(e) adding a cross-linking or metal chelating agent to the emulsion formed in step (d) to form the nanolattices in the emulsion; and
(f) separating the nanolattices from the emulsion.

In one embodiment, the complex is for oral delivery.

In another embodiment, the complex is for subcutaneous delivery.

In another embodiment, the complex is for intravenous delivery.

In yet another embodiment, the complex is for pulmonary delivery.

In one embodiment the polymer is cross-linked to form the nanolattices.

In another embodiment the polymer is chelated to form the nanolattices.

In one embodiment, the pharmaceutical substance is added to the water-in-oil emulsion from step (c) and is further mixed to form the emulsion of step (d). The pharmaceutical substance interacts with the polymeric nanolattice by non-covalent means.
In another embodiment, one or more targeting moieties capable of being bound by, or binding to an intestinal epithelium of the subject, thereby allowing uptake and internalization of the complex, are associated with the polymer or the nanolatices.

The targeting moiety may be covalently bound to the polymer from step (a)(i) either directly or through a spacer or linker compound.

Alternatively, the targeting moiety may interact with the nanolattice by non-covalent means with or without linkers, spacers or other derivatisation.

In one embodiment, the targeting moiety present in the emulsion of step (d) before the chelation step.

In a preferred embodiment, the targeting moiety is added to the emulsion containing the chelated nanolatices of step (e).

In another embodiment, the targeting moiety is attached to the nanolattice via a pendant side chain of the nanolattice.

According to another aspect, the present invention provides a pharmaceutical composition comprising a complex of the invention and a pharmaceutically acceptable carrier and/or diluent.

According to still another aspect, the present invention provides a method of orally delivering a pharmaceutical agent to a subject in need of such pharmaceutical agent, said method comprising administering of a therapeutically effective amount of a complex of the invention, or a pharmaceutical composition of the invention comprising such pharmaceutical agent.
According to another aspect, the present invention is directed to a method for treating diabetes in a subject in need of said treatment, said method comprising administration of a therapeutically effective amount of a complex of the invention, or a pharmaceutical composition of the invention, wherein the pharmaceutical agent is insulin.

According to still another aspect, the present invention provides a method for treating cancer in a subject in need of said treatment, said method comprising administration of a therapeutically effective amount of a complex of the invention, or a pharmaceutical composition of the invention, wherein the pharmaceutical agent is a chemotherapeutic agent.

According to further aspects, the invention provides a method for treating inflammation and/or cardiovascular disease.

According to another aspect, the present invention provides a pendant functional group on the nanolattice, which is suitable for chelation of metals.

According to another aspect, the present invention provides a pendant functional group on the nanolattice to which is bound a metal.

According to another aspect the invention provides a nanolattice which has a pendant chelated metal group, which itself acts as a ligand for binding to a pharmaceutical agent.

According to another embodiment, there is provided a nanolattice, which comprises a pendant-chelated metal group, which acts as a ligand to which is bound a targeting agent.
According to yet another embodiment, there is provided a nanolattice, which comprises one or more targeting moieties capable of being bound by, or binding to a cancer cell of the subject, thereby allowing uptake and internalization of the complex, which are associated with the polymer or the nanolatices.

According to another embodiment, there is provided a nanolattice, which comprises one or more targeting moieties capable of being bound by, or binding to the vascular endothelium of the subject, thereby allowing uptake and internalization of the complex, which are associated with the polymer or the nanolatices.

Further aspects relate to the use of the complex of the invention for the manufacture of a medicament, and in particular medicaments for the treatmens of cancer and proliferative disorders, inflammation, cardiovascular diseases, diabeties and as an imaging or diagnostic agent or the delivery of monoclonal antibodies and Fc-fusion proteins.

These and other aspects, embodiments and preferments of the invention are further detailed in the description below and the claims that follow.
BRIEF DESCRIPTION OF THE FIGURES

Figure 1 graphically represents a comparison of sub cutaneous (SC) and oral administration (PO) of Anti-TNF molecules ALSTII administered in nanolattices.

Figure 2 graphically represents a comparison of sub cutaneous (SC) and oral (PO) administration of Anti-TNF molecules ALSTII administered in nanolattices.

Figure 3 graphically represents a comparison of glucose levels following administration of human insulin alone to insulin entrapped within nanolattices.

Figure 4 graphically represents a comparison of serum insulin levels in diabetic rats following SC administration of insulin within various nanolattice structures.

Figure 5 graphically represents a comparison of mean blood glucose levels in diabetic rats following SC administration of insulin within various nanolattice structures.

Figure 6 graphically represents the ratio of blood insulin to blood glucose levels in diabetic rats following administration of insulin within various nanolattice structures.

Figure 7 graphically represents a comparison of serum insulin levels in normal rats following SC administration of insulin within various nanolattice structures.

Figure 8 graphically represents a comparison of mean blood glucose levels in normal rats following SC administration of insulin within various nanolattice structures.

Figure 9 graphically represents a comparison of mean blood glucose levels in diabetic rats following PO administration of insulin within various nanolattice structures.
Figure 10 graphically represents a representative data for individual rats of mean blood glucose levels in diabetic rats following PO administration of insulin within nanolattice structures at pH 4.

Figure 11 graphically represents a comparison of mean blood glucose levels for SC administration of 5 month old nanolattice structures containing insulin to administration of insulin alone.

Figure 12 graphically represents the reduction in inflammation in C57/B1 mice administered ALST002 either alone (SC) or orally in Lc-biotin-targeted NL (PO)

Figure 13 graphically represents the reduction in inflammation in C57/B1 mice administered ALST002 either alone (SC) or orally in Lysyl-Riboflavin-targeted NL (PO)

Figure 14 graphically represents the reduction in inflammation in C57/B1 mice administered ALST002 either alone (SC) or orally in N-Asp-Cbl-targeted NL (PO)

Figure 15 graphically represents the reduction in inflammation in C57/B1 mice administered ALST002 either alone (SC) or orally in N-e-Lys-Folate-targeted NL (PO)

Figure 16 graphically represents the reduction in inflammation in C57/B1 mice administered ALST002 either alone (SC) or orally in DAP-Cbl-targeted NL (PO)

Figure 17 graphically represents the reduction in serum glucose levels in diabetic rats administered insulin within Cbl or biotin-targeted dextran nanolattices administered orally.
DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

It is to be understood that unless otherwise indicated, the subject invention is not limited to specific formulations, manufacturing methods, diagnostic methods, assay protocols, nutritional protocols, or research protocols or the like as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

The reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in the field of endeavour.

All cited references and publications referred to herein are incorporated by reference.

The term "nanolattice" as used herein refers to a scaffold nanostructure which is able to absorb between about 10% and about 99% of its weight in liquid. As such, such structure is elastic and spongy. The polymer of the lattice is normally added at around 100 mg/ml, which leaves around 90% water in the aqueous/nanolattice stage. The structures or lattices are on the nano scale having a diameter of from about 20 to 500 nm.

As used herein, the term "non-polar phase" refers to a phase comprising a solvent with a dipole moment below 1.35 Debye and a dielectric constant below 6.0. In certain embodiments, such solvents make up at least 10%, preferably at least 50%, more preferably at least 80% or even at least 97%, of the non-polar phase.
As used herein, the term "polydispersity index" (PDI) refers to an index of the ratio of the standard deviation of the size of the nanolattices to the mean of the size of the nanolattices. Preferably the polydispersity index is in the range of 0.1 to 0.4, and more preferably the PDI is between 0.15 and 0.3.

The physical characteristics of the nanolattices of the present invention can be evaluated using one or more of the following systems.

Mean particle size of the nanolattices can be determined by dynamic light scattering (DLS). DLS is particularly suited to determining small changes in mean diameter such as those due to adsorbed layers on particle surfaces or slight variations in manufacturing processes. Cumulative measurements can then be used to determine polydispersity index.

Surface morphology of nanoparticles structures such as nanolattices can be visualized by scanning electron microscopy. Typically, freeze-dried samples of nanolattice are coated by platinum prior to observation using an SEM. Various measurements can then be performed via image analysis using standard techniques.

DLS and SEM measurements can be used to infer parameters such as average cross-sectional length, the sum average of two or more linear measurements taken through the nanostructure.

Differential scanning calorimetry (DSC) can be used to study the effects of heat on the polymeric structure of the nanolattices. Thermal transitions, including glass transitions of a polymer can be determined. DSC can be used to understand the different characteristics of nanolattices formed by various chelating metal ions such as Zn$^{2+}$, Ca$^{2+}$ and Ni$^{2+}$. 
Surface charge of the nanolattices can be determined using zeta potential measurements. The zeta potential may be useful to study the effects of different charged pendant functional groups of the nanolattice polymers, the interaction between the nanolattice polymers and targeting agents, and the effect of pH on the retention characteristics of pharmaceutical agent release from the nanolattice structure.

Infrared spectroscopy can be used to further characterise particles such as nanolattices by the comparison of different particle spectra. Variables that alter infrared spectra include the presence of pendant side chains such as carboxylate groups, the interaction between the pendant side chains of the nanolattice with one or more pharmaceutical agents and the interaction between the pendant side chains of the nanolattice and a metal chelating agent.

UV-visible spectroscopy (UVS) can be used to further characterise nanoparticles such as nanolattices by the comparison of different particle spectra. In particular, UVS can help determine aggregation states of pharmaceutical agent loaded within the nanolattices.

The term "co-ordination bonding" as used herein is a type of bond in which one atom supplies both electrons. This term excludes a covalent bond in which each atom supplies one electron.

The terms "chelation" and "chelated" as used herein are understood to mean the formation of a heterocyclic complex between a ligand and a metal ion, wherein the bonding between the ligand and the metal ion is co-ordination bonding.

The term "cross-linked" as used herein means that covalent chemical linkages are introduced within and/or between constituent polymers of the nanolattices.
As used herein, the term "average cross-sectional length" refers to the sum average of two or more linear measurements taken through the nanostructure.

As used herein, the terms "active agent", "chemical agent", "pharmaceutical agent", "pharmacologically active agent", "medicament", "active" and "drug" are used interchangeably to refer to a chemical compound and in particular a protein or a peptide. In addition, the pharmaceutical agent may be an enzyme, a hormone, a toxin or an immunogen. The terms also encompass pharmaceutically acceptable and pharmacologically active ingredients of those active agents specifically mentioned herein including but not limited to salts, esters, amides, prodrugs, active metabolites, analogs and the like. When the terms "active agent", "chemical agent", "pharmaceutical agent", "pharmacologically active agent", "medicament", "active" and "drug" are used, then it is to be understood that this includes the active agent *per se* as well as pharmaceutically acceptable, pharmacologically active salts, esters, amides, prodrugs, metabolites, analogs, etc.

As used herein, the terms "pharmaceutical agent", "pharmacologically active agent", "active agent", "chemical agent", "medicament", "active" and "drug" include combinations of two or more active agents such as two or more proteins. A "combination" also includes multi-part such as a two-part composition where the agents are provided separately and given or dispensed separately or admixed together prior to dispensation.

The term "subject" as used herein refers to a vertebrate host.

The term "therapeutically effective amount" of a complex or a pharmaceutical composition as used herein is intended to include within its meaning a non-toxic but sufficient amount of the complex or the pharmaceutical composition of the invention
which comprises an amount of the pharmaceutical agent which produces the desired therapeutic effect. The exact therapeutically effective amount will vary depending on factors such as the type of disease, the age, sex, weight of the subject and mode of administration. Dosage regimen can be adjusted to provide the optimum therapeutic response. For example, several divided doses can be administered daily, or the dose can be proportionally reduced as indicated by the exigencies of the therapeutic situation.

The term "treating" or "treatment" as used herein refers to any and all uses which remedy a disease state or symptoms, prevent the establishment of disease, or otherwise prevent, hinder, ameliorate, retard or reverse the progression of disease or other undesirable symptoms in any way whatsoever.

As used herein, the term "targeting molecules" are molecules that are capable of being bound by, or binding to the intestinal epithelium of a subject, thereby allowing the uptake of itself and any conjugated moieties by the circulation or lymphatic drainage system of the subject. Targeting molecules also include those molecules for which there are natural receptors in the body, epithelium or cancer.

The present invention provides nanolattices capable of carrying a pharmaceutical agent, the nanolattices comprising at least one chelated or cross-linked polymer, wherein the nanolattices have a desired PDI.

Polymers suitable for use in the nanolattices may be any biodegradable polymer. The polymer may be natural, synthetic, semi-synthetic, native or modified.

The polymer may be selected from the group consisting of, but not limited to: proteins, poly-amino acids such as polyglutamic acid, polyaspartic acid, and polylysine; polyacrylamide; poly N-acylhydroxypropine esters; polyebac acid; polyfumaric acid;
polylactic acid; polyglycolic acid; polylactic-co-glycolic acid; polymers formed from hydroxyethylmethacrylate; polymers formed from ethylene bismethacrylate; carboxymethylcellulose; gum arabic; agarose; alginate; polyphosphate; heparin; gelatin; guaran; copolymers of sebacic acid and fumaric acid; copolymers of bis(carboxyphenoxy)propane and sebacic acid; poly(carboxyphenoxyacetic) acid; poly(carboxyphenoxyvaleric) acid; poly-ε-caprolactone and related polyesters; poly-ε-caprolactone-co-δ-valerolactone; poly-ε-caprolactone-co-DL-lactic acid; hyaluronic acid; chitin; chitosan; dextran; dextrin, carboxy-dextran, diethylaminoethyl dextran, aminoethyl dextran and dextran sulfate; collagen; albumin; fibrinogen; and other naturally occurring polymers, such as collagen, starch, amyllose, carboxymethyl amyllose, hydroxyethyl amyllose, hydroxyethyl amyllose, and cellulose, carboxymethyl cellulose, hydroxyethyl cellulose; agarose, pectic acid, alginic acid, gum Arabic, galactomannan, levan, hyaluronic acid; derivatives or mixtures thereof.

In one embodiment, the polymer may be a polysaccharide. In a preferred embodiment, the polymer is dextran or a derivative thereof. In another embodiment dextran is cross-linked to form the nanoparticle. In another embodiment carboxy alkly dextrans are chelated with metals to form the nanoparticle.

PDI is a measure of the variation of the sizes of the nanolattices in a preparation. A high PDI value, such as 0.7 to 1, suggests that there is a large variation in the size of nanolattices in a preparation and hence the nanolattices exhibit "polydispersity". A highly polydisperse preparation results in some structures being too big for uptake via intestinal epithelial cells, or alternatively some structures being too small to effectively encapsulate the drug to be delivered, such that an accurate uptake of the pharmaceutical agent by the subject may not be readily determined, leading to variable bioavailability.
A PDI of less than 0.3 indicates a state of near monodispersity, i.e. the state of uniform particle size in a preparation. The nanolattices of the present invention have a PDI of between about 0.1 to about 0.4, and preferably between about 0.12 to about 0.35. A more uniform sized dose provides better uptake.

The nanolattices preferably have a diameter of about 20 - 500 nm, more preferably in the range of between about 55 nm and 400 nm, and still more preferably between 75 and 300 nm. Larger diameter particles have been found to be unsuitable for intravenous injection due to hepatic and pulmonary clearance. Larger particles are also unsuitable for oral administration as these are either trapped in the intestinal mucous and as such do not reach the intestinal wall, or they are too big to enter the enterocyte, or the endosome that is used for uptake. Particles greater than 1 um have very low bioavailabilities whilst particles that are very small such as less than 20 nm have very low pay-loads and most of the pharmaceutical is on, or near the surface.

In an embodiment, the nanolattices may comprise one or more pendant side-chains capable of interacting with one or more pharmaceutical agent. The interaction may be selected from one or more of the following: ionic interactions, hydrogen bonding, hydrophobic interactions, chelation, co-ordination bonding or pi-pi interactions.

Pendant side chains of the nanolattice capable of having ionic interaction with the pharmaceutical agents may be moieties comprising functional groups of the following, including but not limited to: amino acids, including non-conventional amino acids, primary amines, secondary amines, tertiary amines, quaternary amines, amidines, aziridines, azetidines, carboxylates, dithioacids, primary imines, furans, guanidines, hydrazines, hydrazones, isocyanates, isothiocyanates, isothiazoles, imidazoles, nitro groups, oxalones, oxazoles, oxetanes, oximes, phosphodiesters, phosphoranes, pyrazoles, pyroles, pyridyls, pyrrolidines, pyridines, pyridazines, pyrimidines, pyrazines, thioacids,
thiazoles, thiocyanates, thiophosphoranes, thiranes, triazoles, 1,3,5-triazines, 1,2,4-triazines at a selected pH. Table 1A displays the amino acids that may provide functional groups for having ionic interaction with the pharmaceutical agents. Table 2A displays non-conventional amino acids that may provide functional groups for having ionic interaction with the pharmaceutical agents. However, it will be apparent to one skilled in the art that other moieties not listed herein but capable of having ionic interaction with the pharmaceutical agents may be included in the nanolattices.

For example, pendant side groups capable of having ionic interaction with the pharmaceutical agents may be provided by the α-carboxyl group of any amino acids or the α-amino group of any amino acids. Additionally, aspartic acid and glutamic acid may provide additional negatively charged moieties capable of having ionic interaction with the pharmaceutical agents. Alternatively, lysine, arginine and histidine may provide positively charged moieties capable of having ionic interaction with the pharmaceutical agents.

**TABLE IA - Amino acids**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>3-letter code</th>
<th>Single letter code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>Gly</td>
<td>G</td>
</tr>
<tr>
<td>Alanine</td>
<td>Ala</td>
<td>A</td>
</tr>
<tr>
<td>Valine</td>
<td>Val</td>
<td>V</td>
</tr>
<tr>
<td>Leucine</td>
<td>Leu</td>
<td>L</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Ile</td>
<td>I</td>
</tr>
<tr>
<td>Methionine</td>
<td>Met</td>
<td>M</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Phe</td>
<td>F</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>Trp</td>
<td>W</td>
</tr>
<tr>
<td>Proline</td>
<td>Pro</td>
<td>P</td>
</tr>
<tr>
<td>Amino acid</td>
<td>3-letter code</td>
<td>Single letter code</td>
</tr>
<tr>
<td>-----------------</td>
<td>--------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Serine</td>
<td>Ser</td>
<td>S</td>
</tr>
<tr>
<td>Threonine</td>
<td>Thr</td>
<td>T</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Cys</td>
<td>C</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Tyr</td>
<td>Y</td>
</tr>
<tr>
<td>Asparagine</td>
<td>Asp</td>
<td>N</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Gln</td>
<td>Q</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>Asp</td>
<td>D</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>Glu</td>
<td>E</td>
</tr>
<tr>
<td>Lysine</td>
<td>Lys</td>
<td>K</td>
</tr>
<tr>
<td>Arginine</td>
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</tr>
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<td>Histidine</td>
<td>His</td>
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**TABLE 2A** - Codes for non-conventional amino acids

<table>
<thead>
<tr>
<th>Non-conventional amino acid</th>
<th>Code</th>
<th>Non-conventional amino acid</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-aminobutyric acid</td>
<td>Abu</td>
<td>L-N-methylalanine</td>
<td>Nmala</td>
</tr>
<tr>
<td>α-amino-α-methylbutyrate</td>
<td>Mgbu</td>
<td>L-N-methylarginine</td>
<td>Nmarg</td>
</tr>
<tr>
<td>aminocyclopropane-carboxylate</td>
<td>Cpro</td>
<td>L-N-methyasparagine</td>
<td>Nmasn</td>
</tr>
<tr>
<td>aminoisobutyric acid</td>
<td>Aib</td>
<td>L-N-methylcysteine</td>
<td>Nmcys</td>
</tr>
<tr>
<td>aminonorbornyl-carboxylate</td>
<td>Norb</td>
<td>L-N-methylglutamine</td>
<td>Nmgln</td>
</tr>
<tr>
<td>cyclohexylalanine</td>
<td>Chexa</td>
<td>L-N-methylhistidine</td>
<td>Nmhis</td>
</tr>
<tr>
<td>cyclopentylalanine</td>
<td>Cpen</td>
<td>L-N-methylisoleucine</td>
<td>Nmile</td>
</tr>
<tr>
<td>Amino Acid</td>
<td>Simple Form</td>
<td>Full Form</td>
<td>Abbreviation</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-------------</td>
<td>-----------------------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>D-alanine</td>
<td>Dal</td>
<td>L-N-methylleucine</td>
<td>Nmleu</td>
</tr>
<tr>
<td>D-arginine</td>
<td>Darg</td>
<td>L-N-methyllysine</td>
<td>Nmlys</td>
</tr>
<tr>
<td>D-aspartic acid</td>
<td>Dasp</td>
<td>L-N-methylmethionine</td>
<td>Nmmet</td>
</tr>
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<td>D-cysteine</td>
<td>Dcys</td>
<td>L-N-methyleneurucine</td>
<td>Nmene</td>
</tr>
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<td>D-glutamine</td>
<td>Dgln</td>
<td>L-N-methylnorvaline</td>
<td>Nmnva</td>
</tr>
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Pendant side chains of the nanolattice capable of hydrogen bonding with the pharmaceutical agents may be selected from functional groups of the following, including but not limited to: amino acids, alcohols, aldehydes, primary amides, secondary amides, tertiary amides, primary amines, secondary amines, tertiary amines, amidines, anhydrides, azos, aziridines, azetidines, carboxylates, carbonates, carbamates, cyanates, dithioacids, di-sulfides, hydroxylamines, ketones, imides, primary imines, secondary imines, ethers, esters, epoxides, furans, guanidines, hydrazines, hydrazones, isocyantes, isothiocyanates, isothiazoles, isoxazoles, imidazoles, nitro groups, nitroso groups, oxalones, oxazoles, oxetanes, oximes, oxiranes, peroxys, phosphodiesters, phosphites, phosphates, phosphananes, phosphonates, pyrazoles, pyridyls, pyridines, pyridazines, pyrimidines, pyrazines, sulfones, sulfonamides, sulfites, sulfonates, thioacids, thiazoles, thiocyanates, thiocarbamates, thiocarbonates, thioethers, thioethanes, thiols, thioketones, thiophosphites, thiophosphates, thiophosphonates, thiophosphoranes, tetrahydro-thiophenes, thiophenes, thiranes, triazoles, 1,3,5-triazines, 1,2,4-triazines. However, it will be apparent for a skilled person in the art that other moieties not listed herein but capable of having hydrogen bonds with the pharmaceutical agents may be included in the nanolattices of the present invention.

Pendant side chains of the nanolattice capable of having hydrophobic interaction with the pharmaceutical agents may be selected from the following, including but not limited to: alkanes, alkenes, alkynes, oxiranes, phenyls, pyridyls, pyrolidines, pyridines, pyridazines, pyrimidines, pyrazines, 1,3,5-triazines, 1,2,4-triazines, hydrophobic amino acids (such as, but not limited to alanine, valine, leucine, isoleucine, methionine, phenylalanine, tryptophan, tyrosine), lipids. However, it will be apparent for a skilled person in the art that other moieties not listed herein but capable of having hydrophobic interaction with the pharmaceutical agents may be included in the nanolattices of the present invention.
Pendant side chains of the nanolattice capable of co-ordination bonding to form chelates may be moieties comprising functional groups capable of donating a pair of electrons (Lewis bases). Such moieties are capable of chelating with metal ions, which in turn may act as ligands for binding to the pharmaceutical agents.

Pendant side chains of the nanolattice capable of chelating with metal ions, which in turn act as a ligand for binding to the pharmaceutical agents may be moieties including but not limited to: alcohols, aldehydes, primary amides, secondary amides, tertiary amides, primary amines, secondary amines, tertiary amines, amidines, anhydrides, azos, aziridines, azetidines, carboxylates, carbonates, carbamates, cyanates, dithioacids, disulfides, hydroxylamines, ketones, imides, primary imines, secondary imines, ethers, esters, epoxides, furans, guanidines, hydrazines, hydrazones, isocyanates, isothiocyanates, isothiazoles, isoazoles, imidazoles, nitriles, nitro groups, nitroso groups, oxalones, oxazoles, oxetanes, oximes, oxiranes, peroxys, phosphodiesters, phosphites, phosphates, phosphoranes, phosphonates, phosphoranes, pyrazoles, pyroles, pyridyls, pyrrolidines, pyridines, pyridazines, pyrimidines, pyrazines, sulfones, sulfonamides, sulfites, sulfonates, thioacids, thiazoles, thiocyanates, thiocarbamates, thiocarbonates, thioethers, thioethanes, thiol, thioketones, thiophosphites, thiophosphates, thiophosphonates, thiophosphoranes, tetrahydro-thiophenes, thiophenes, thiranes, triazoles, 1,3,5-triazines, 1,2,4-triazines. However, it will be apparent for a skilled person in the art that other moieties not listed herein but capable of chelation with the pharmaceutical agents via金属 ions may be included in the nanolattices of the present invention.

Additional pendant side chains of the nanolattice capable of chelating with metal ions, which in turn act as a ligand for binding to the pharmaceutical agents may include but are not limited to molecules such as: EDTA, iminodiacetic acid, o-hydroxy-benzyliminodiacetic acid, 3-aminobenyliminodiacetic acid, 6-Amino-1,4,8,11-tetraazacyclotetradecane, hydrazinonicotinamide (HYNIC), DTPA (N-
diethylenetriaminopentaacetic acid), DOTA (1,4,7,10-tetraazacyclododecane-
N,N',N",N"'-tetraacetic acid), TETA (1,4,8,11-tetraazacyclotetradecane-
1,4,8,-11-tetraacetic acid), NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid), histidine, 2-
nitroimidazole, picolinic acid, imidazole carboxylic acid, 2,4 dipicolinic acid, (2-
picolylamine-IV,N-diacetic acid), Dichloro-[4-(methyleneimino-diacetic acid)phenyl
(2,3' -diaminopropionamide), TRODAT, N-[[[(2-mercaptoethyl)amino]carbonyl]methyl]-
N-(2-mercaptoethyl)-6-aminohexanoic acid, deferoxamine, N-bis(2-pyridylmethyl)-L-
lysine, aspartic acid and glutamic acid, aminomalonic acid, 1,4, 8, 11-
tetraazacyclotetradecane (CYCLAM), bisaminothiols, dithiocarbamates, N-His, for
chelation of metals such as $^{99m}$Tc, 2,3-diaminopropionamide, or 2,3-diaminopropionic
acid for chelation of metals such as platinum.

Metal ions for use in chelation include but are not limited to: divalent or trivalent cations
of iron, manganese, chromium, cobalt, lead, silver, mercury, bismuth, plutonium,
cadmium, germanium, ruthidium, gold, indium, technetium, copper, zinc, gallium,
rhodium, palladium, platinum, nickel, gadolinium, erbium, europium, dysprosium,
yttrium, promethrium, lutetium and holmium and radioactive isotopes thereof, wherein
the metal ions may be complexed with one or more pharmaceutical agents. Examples of
pharmaceutical agents suitable for loading onto a metal chelated nanolattice include
insulin, which may be complexed with zinc.

In one embodiment, the pendant side chains may comprise chelating moieties to which
are bound metal ions, wherein the metal ions act as ligands for binding to one or more
pharmaceutical agents. Metal ions within the nanolattices include, but are not limited to
divalent or trivalent cations of iron, manganese, chromium, cobalt, lead, silver, mercury,
bismuth, plutonium, cadmium, germanium, ruthidium, gold, indium, technetium, copper,
zinc, gallium, rhodium, palladium, platinum, nickel, gadolinium, erbium, europium,
dysprosium, yttrium, promethrium, lutetium and holmium and radioactive isotopes
thereof, wherein the metal ions may be complexed with one or more pharmaceutical agents. Particularly preferred metals suitable for polymer chelation are magnesium, manganese, selenium, calcium and zinc, more preferably is calcium and zinc, and still more preferably is zinc.

Alternatively, pendant side chains of the nanolattice comprising chelating moieties may chelate directly to the metallic component of metallodrugs, such as cisplatin, carboxplatin, oxaliplatin, DACH-platinum, anthracyclines, daunomycin, doxorubicin and epirubicin.

Chelation of metals within the pendant side chains of the nanomatrix may be tailored to introduce specific charges to the chelated pendant side chain. For instance, chelation of metals via two, four or six carboxyl groups leads to a negatively charged pendant side chain. In contrast, chelation of metals via two, four or six amino groups leads to a positively charged pendant side chain. Whilst chelation via amino and carboxyl groups can lead to a neutral chelate.

Pendant side chains of the nanolattice capable of pi-pi interaction with the pharmaceutical agents may be selected from the following, including but not limited to alkenes, alkynes, furans, isothiazoles, isoxazoles, imidazole, oxalone, oxazoles, phenyl, pyrazoles, pyrole, pyridyl, pyridine, pyridazines, pyrimidines, pyrazines, thiophenes, triazines, 1,3,5-triazine, 1,2,4-triazine. However, it will be apparent for a skilled person in the art that other moieties not listed herein but capable of pi-pi interaction with the pharmaceutical agents may be included in the nanolattices of the present invention.

Suitable pharmaceutical agents that may be carried by the nanolattices include any water soluble solute, including, but not limited to peptides, proteins, polysaccharides, oligonucleotides, salts, sugars, nutrients, vitamins, minerals, acids, antioxidants, or any
biological active compounds for administration to a subject, such as a human, animal or other mammal. The pharmaceutical agent is selected based upon the intended application or therapy, wherein the effect of the pharmaceutical agent is suitable to treat a particular condition.

The pharmaceutical agent may be a chimeric molecule comprising an isolated protein or a fragment thereof, such as an extra-cellular domain of a membrane bound protein, linked directly to the constant (Fc) or framework region of a human immunoglobulin via one or more protein linkers. Such a chimeric molecule is also referred to herein as protein-Fc. Other chimeric molecules contemplated include the protein or protein-Fc or a fragment thereof, linked to a lipid moiety such as a polyunsaturated fatty acid molecule. Such lipid moieties may be linked to an amino acid residue in the backbone of the molecule or to a side chain of such an amino acid residue. The human immunoglobulin may be selected from IgGl, IgG2, IgG3, IgG4, IgAl, IgA2, IgM, IgE, IgD.

The pharmaceutical agent may further include a chimeric molecule comprising an isolated protein or a fragment thereof, such as an extra-cellular domain of a membrane bound protein, linked directly to the constant (Fc) or framework region of a mammalian immunoglobulin via one or more protein linker. In another embodiment, the mammal Fc or framework region of the immunoglobulin is derived from a mammal selected from the group consisting of primates, including humans, marmosets, orangutans and gorillas, livestock animals (e.g. cows, sheep, pigs, horses, donkeys), laboratory test animals (e.g. mice, rats, guinea pigs, hamsters, rabbits, companion animals (e.g. cats, dogs) and captured wild animals (e.g. rodents, foxes, deer, kangaroos). In another embodiment the Fc or framework region is a human immunoglobulin. In a particular embodiment the mammal is a human. Such a chimeric molecule is also referred to herein as protein-Fc. Other chimeric molecules contemplated by the present invention include the protein or protein-Fc or a fragment thereof linked to a lipid moiety such as a polyunsaturated fatty
acid molecule. Such lipid moieties may be linked to an amino acid residue in the background of the molecule or to a side chain of such an amino acid residue.

Suitable peptides and proteins that can be delivered via the nanolattices of the present invention include protein molecules, protein chimeric molecules, other chimeric molecules or fragments selected from the TNF superfamily (including but not limited to TNF-a, TNFRI, TNFR2, BAFF, OX-40, Lymphotoxin-a, Fas-ligand); chemokines (including but not limited to MCP-I, MIP-Ia, MIP-Ib, RANTES, IL-8 and viral like chemokine antagonist MC148); interleukins, interleukin receptors and antagonist (including but not limited to IL-1a, IL-1b, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-11, IL-12, IL-13, IL-15, IL-16, IL-18, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, their respective receptors including but not limited to IL-1Ra, IL-2Ra, IL-2Rb, IL-2Rg, IL-3Ra, IL-4Ra, IL-5Ra, IL-6Ra, IL-7Ra, IL-10Ra, IL-11Ra, IL-13Ra, IL-15Ra as well as IL-1R Antagonist); the interferon family (including but not limited to IFN-a2B, IFN-bl, IFN-g, IFN-y, IFN-aR2, IFN-aRa, IFN-gRb, IFN-gRb); lectins (including but not limited to CD209 type I and II, E-Selectin, L-Selectin, P-Selectin, Langerin); growth factors and their receptors (including but not limited to Amphiregulin, Angiopoietin, BDNF, beta-cellulin, BMPs (including but not limited to BMP-2, BMP-4, BMP-7), CNTF, cripto, ECGF-I, EGF, EGFR, EPO, FGFs and their receptors (including but not limited to FGF-I, FGF-2, FGF-5, FGF-7, FGF-9, FGF-II, FGF-12, FGF-13, FGF-14, FGF-14 FLAG, FGF-18, FGF-19, FGF-21, FGFR1, FGFR2, FGFR4, FGFR5), Flt3-Ligand and its receptor (including but not limited to Flt3), G-CSF, GDNF, GM-CSF, GM-CSF-R, hGH and its receptor (including but not limited to hGHR), IGF-I, IGFBP-3, M-CSF, Neuregulin, NGFs and its receptor (including but not limited to NGF-b, NGFR, NGFR), NT-3, PDGFs, TGFs and their receptors (including but not limited to TGF-a, TGF-b, TGFbR2), Trk-A, Trk-B, TPO, VEGFs and their receptors (including but not limited to VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-165, VEGFR); embryonic growth factors (including but not limited to Noggin, Nodal, SCF, Wnt5, Wnt-2, Wnt-3,
Wnt-3A, Wnt-4, Wnt-5A, Wnt-5A-FLAG-C, Wnt-5B, Wnt-6, Wnt-7A, Wnt-7B, Wnt-10A, Wnt-IOB, Wnt-1OB-FLAG-C, Wnt-11); adhesion molecules (including but not limited to adiponectin, ICAM), other cytokines and proteins, such as LIF, OSM, transferring and its receptor, hormones (including but not limited to insulin, calcitonin, adrenocorticotropic (ACTH), glucagon, somatostatin, somatotropin, thyrotrpin, parathyroid hormone, pigmentedary hormones, somatotropin, lutenizing hormone, chorionic gonadotropin, hypothalmic releasing factors, antidiuretic hormones, thymosin, parathyroid stimulating hormone, endorphins, enkephalins, biphelin and prolactin); antibiotics (including but not limited to gentamycin, amikacin), enzymes (including but not limited to activin A asparaginase, adenosine deaminase, BACE-I, caspase-1, fucosyltransferease, furin, mTACE, sialyltransferase ) Factor VIII, LH-RH analogues, and other biopharmaceuticals such as heparin and vaccines (for instance, vaccines for Hepatitis 'B' surface antigen, typhid and cholera vaccines), plasminogen activator inhibitors and fusion proteins including BAFFR-Fc.

In particular, suitable chimeric proteins that can be delivered via the nanolattices of the present invention include but is not limited to TNFRI-Fc, TNFR2-Fc, OX-40-Fc, MC148-Fc, IL-1Ra-Fc, IL-2Ra-Fc, IL-2Rb-Fc, IL-2Rg-Fc, IL-3Ra-Fc, IL-4Ra-Fc, EL-5Ra-Fc, IL-6Ra-Fc, IL-7Ra-Fc, IL-10Ra-Fc, IL-11Ra-Fc, IL-13Ra-Fc, IL-15Ra-Fc, IFN-aRa-Fc, IFN-aRb-Fc, IFN-gRa-Fc, IFN-gRb-Fc, CD209L-Fc, E-Selectin-Fc, L-Selectin-Fc, P-Selectin-Fc, Langerin-FLAG, EGFR-Fc, FGFR1-Fc, FGFR4-Fc, FGFR5-Fc, Flt3-Fc, hGHR-Fc, NGFR-Fc, TGFβRI-Fc, Trk-A-Fc, Trk-B-Fc, VEGFR-Fc, Wnt-5A-FLAG-C, Wnt-10B-FLAG-C and BAFFR-Fc.

Additional suitable proteins that can be delivered via the nanolattices of the present invention include monoclonal and polyclonal antibodies, single-chain antibodies, other antibody fragments, analogs and derivatives thereof. Polynucleotides, including antisense
oligonucleotides, aptamers and therapeutic genes can also be delivered using the methods and compositions of the present invention.

Anticoagulants, such as heparin, also can be delivered using the nanolattices of the invention. Still other suitable therapeutic agents for use in the present invention include bioactive molecules, such as anticancer drugs, e.g., doxorubicin, epirubicin and daunorubicin, vincristine, cisplatin, carboplatin, oxaliplatin, methotrexate, paclitaxol, taxol, camptothecin and camptothecin analogs, antibiotics, antipsychotics, antidepressants, and drugs for diabetes and cardiovascular disease.

Examples of pharmaceutical agents suitable for delivery by metal chelated nanolattices include insulin, which may be complexed with zinc, or arginine; and other metallodrugs, such as cisplatin, carboxplatin, oxaliplatin, DACH-platinum. Additional suitable pharmaceutical agents include the anthracyclines, daunomycin, doxorubicin and epirubicin.

**Preparation of the Nanolattices of the Present Invention**

According to a second aspect, the present invention provides a process for preparing nanolattices having a PDI of between about 0.1 and 0.4. Whilst there are a number of methods for the preparation of nanolattices of the present invention, preferred methods include the use of thermodynamically stabilized water-in-oil microemulsions. Alternatively, kinetically stabilized water-in-oil emulsions may be used to provide a suitable milieu for cross-linking polymers to form the nanolattices.
In general, the method of preparing the nanolattices of the present invention involves emulsification of an aqueous phase and an oil phase. Such method may be summarized as follows:

(a) preparing separate mixtures comprising:
   (i) a surfactant and a polymer in an aqueous phase; and
   (ii) a surfactant and a non-polar phase; and
(b) combining mixtures (i) and (ii) from step (a) to form mixture (iii);
(c) subjecting mixture (iii) to mixing for a period sufficient to allow formation of a uniform water-in-oil emulsion;
(d) adding a cross-linking or chelating agent to the emulsion to form the nanolattices; and
(e) separating the nanolattices from the emulsion.

In one embodiment, co-surfactants are also added to (i) or (ii) from step (a) to form mixture (iii).

In another embodiment the nanolattices are cross-linked.

In another embodiment the nanolattices are chelated.

Preferred aqueous phase ingredients may comprise water and any water-soluble components in water, including one or more pharmaceutical agent.

Preferred non-polar phase ingredients may comprise natural oils derived from plants or animals, such as vegetable oils, sunflower oils, coconut oils, almond oils; purified synthetic or natural di or triglycerides (such as Crodamol GTCC and Capmul MCM); phospholipids and their derivatives (such as lecithin or lysolecithin); fatty acid esters (such as isopropyl myristate, isopropyl palmitate, ethyl oleate, oleic acid ethyl ester);
hydrocarbons (such as hexane, the n-decane through n-octadecane series); glycerolysed fats and oils (such as glyceryl monooleate, glyceryl monicaprylate, glycerol monocaprate, propylene glycol moncaprylate, propyleme glycol monolaurate).

Other non-polar phase ingredients include, but are not limited to, Labrafil M 1944 CS, benzene, tetrahydrofuran, and n-methyl pyrrolidone, or halogenated hydrocarbons, such as methylene chloride, or chloroform. In a particular embodiment, the oil phase comprises Crodamol GTCC and Capmul MCM, at 3:1 ratio.

The non-polar component is either used alone or in combination with other non-polar components. For example, an oil or unique mixture of oils may require a different surfactant or mixture of surfactants or surfactants and co-surfactants to form an emulsion or a microemulsion with the aqueous phase, as can routinely be determined by those of skill in the art.

Surfactants used according to the invention are known surfactants in the art that reduce the interfacial tension between the non-polar and aqueous phases sufficiently to allow the formation of emulsions or microemulsions. Typically, surfactants are organic compounds that are amphiphatic, containing both hydrophobic groups and hydrophilic groups. Preferred surfactants include, but are not limited to, anionic surfactants such as fatty acid soaps (including sodium oleate, sodium palmitate, sodium myristate, sodium stearate); alkyl sulfates (including sodium dodecyl sulfate); alkyl benzenesulfonates; alkyl sulfonates; alkyl phosphates; acyl sulfates; or acyl sulfo succinates; cationic surfactants, such as alkyl primary, secondary, tertiary, or quaternary amines; alkyl pyridinium and quaternary ammonium salts; zwitterionic surfactants, for example, betaines, such as dodecyl dimethylammonium acetate, tetradecyldimethylammonium acetate, hexadecyldimethylammonium acetate, alkyl dimethylammonium acetate wherein the alkyl group averages about 14.8 carbon atoms in length, dodecyl dimethylammonium butanoate,
tetradecyldimethylammonium butanoate, hexadecyldimethylammonium butanoate,
dodecyldimethylammonium hexanoate, hexadecyldimethylammonium hexanoate,
tetradecyldiethylammonium butanoate, tetradecyldipropyl ammonium pentanoate,
cocobetaine, sulfobetaines (or sultaines), fatty acid ethanolamides such as cocamide
monoethanolamide and other zwitterionic surfactants such as 3-(N,N-dimethyl-N-
hexadecylammonio)-propane-1-sulfonate; 3-(N,N-dimethyl-N-hexadecylammonio)-2-
hydroxypropane-1-sulfonate; N,N-dimethyl-N-dodecylammonio acetate; 3-(N,N-
dimethyl-N-dodecylammonio)propionate; 2-(N,N-dimethyl-N-octadecylammonio)ethyl
sulfate; 3-(P,P-dimethyl-P-dodecylphosphonio)propane-1-sulfonate; 2-(S-methyl-S-tert-
hexadecylsulfo)ethane-1-sulfonate; 3-(S-methyl-S-dodecylsulfonio)propionate; N,N-
bis(oleylamidopropyl-N-methyl-N-carboxymethylammonium)betaine; N,N-
bis(stearamidopropyl)-N-methyl-N-carboxymethylammonium betaine; N-
(stearamidopropyl)N-dimethyl-N-carboxymethylammonium betaine; 3-(N-4-n-
dodecylbenzyl-N,N-dimethylammonio)propane-1-sulfonate; 3-(N-dodecylphenyl-N,N-
dimethylammonio)-propane-1-sulfonate; nonionic surfactants, for example, alcohol
ethoxylate, alkylphenol ethoxylate, alkyl polyglycosides, mono-, di- or glyceride esters,
polyglycerols, polyglycerol esters, phospholipids (such as lecithin), mono- or diglyceride
esters of citric acid, tartaric acid and lactic acid, sorbitan fatty acid esters (such as sorbitan
monostearate NF, sorbitan monooleate NF, sorbitan isosterate, sorbitan monolaurate),
polyoxylethylene sorbitan fatty acid esters (polysorbates) (such as polyoxylethylene
sorbitan monooleate, polysorbate 20 NF, polysorbate 20 NF3 EP, polysorbate 60 NF,
polysorbate 80 NF, polysorbate 80 NF, EP, JP, PEG-20 Sorbitan Isostearate),
polyethoxylated esters of acyl acids, copolymers of polyethylene oxide and polypropylene
oxide, polyoxylethylene fatty ethers (such as polyoxylethylene fatty ethers derived from
lauryl, cetyl, stearyl and oleyl alcohols, polyoxylethylene (4) lauryl ether, polyoxylethylene
(23) lauryl ether, polyoxylethylene (2) cetyl ether, polyoxylethylene (10) cetyl ether,
polyoxylethylene (20) cetyl ether, polyoxylethylene (2) stearyl ether, polyoxylethylene (10)
stearyl ether, polyoxylethylene (20) stearyl ether, polyoxylethylene (2) oleyl ether,
polyoxyethylene (10) oleyl ether, polyoxyethylene (20) oleyl ether, polyoxyethylene (21) stearyl ether), Cremophor. Surfactants listed herein may be used alone or in combination of each other.

Co-surfactants used according to the invention are known surface-active agents in the art that act, in addition to surfactants, to further lower the interfacial energy of an emulsion or a microemulsion. Preferred co-surfactants include, but are not limited to non-toxic amphophilic molecules; alcohols (including aliphatic alcohols, shorter chain alcohols, such as ethanol); fatty acid alcohols (such as n-alkane-1,2-diols); acids (such as acetic acid); esters (such as butyl lactate); any surfactants as herein listed or mixtures thereof.

In a particular embodiment, an aqueous phase may be prepared by mixing a suitable polymer and a non-ionic surfactant (for example Tween 80) in water. The ratio of the amount of surfactant added per gram of polymer may be between about 0.01 and 2 grams, or between about 0.05 and 1.5 grams, or between about 0.075 and 1.25 grams or between about 0.1 and about 1.0 grams or between about 0.1 and 0.75 grams or between about 0.1 and 0.5 grams. In one embodiment, the amount of surfactant added may be about 0.15 grams per gram of polymer.

An non-polar phase may be prepared by mixing a mineral oil and a non-ionic surfactant (for example Span 85). The ratio of the amount of surfactant added per gram of polymer may be between about 0.01 and 2 grams, or between about 0.05 and 1.5 grams, or between about 0.075 and 1.25 grams or between about 0.1 and about 1.0 grams or between about 0.1 and 0.75 grams or between about 0.1 and 0.5 grams. In one embodiment, the amount of surfactant added may be about 0.16 grams per gram of polymer.
The aqueous phase and the non-polar phase are combined and homogenized until a stable
emulsion is formed. Formation of the nanolattices is achieved by the addition of a cross-
linking agent or a chelating agent to the emulsion whilst the emulsion is maintained with
stirring. The resultant nanolattices are allowed to settle, washed in an organic solvent,
followed by distilled water and then lyophilized.

Cross-linking agents, which can be used in the formation of the nanolattice include, but
are not limited to bromoacetylbromide, bromoacetylchloride, 4,4'-difluoro-3,3'-
dinitrodiphenylsulfone, toluenediisocyanate, epichlorohydrin, phosphorus trichloride,
hexamethylene diisocyanate, bisoxiranes such as 1,4-butadiol diglycidoxy ether (1,4-
bis(2,3-epoxypropyl)butane), heterocyclic halides, s-triazines, such as cyanuric chloride,
2-amino-4,6-dichloro-s-triazine, 2-carboxymethylamino-4,6-dichloro-s-triazine, and 2-
carboxymethyloxy-4,6-dichloro-s-triazine, fluorotriazines, and fluoropyrimidines, 2,4,6-
trifluoro-5-chloropyrimidine, vinyl sulfone, p-benzoquinone, hydroxy-S-triazole and
those commercially available, for example, in Pierce Biotechnology, Inc. Preferred cross-
linking agents for use in the present invention include bisoxiranes, epichlorohydrin,
phosphorus oxychloride, divmethyl sulfone and p-benzoquinone.

In a preferred embodiment, thermodynamically stabilized water-in-oil microemulsions
are used in the preparation of the nanolattices of the present invention. Microemulsions
are quaternary systems composing of an non-polar phase, a water phase, surfactant/s and
a co-surfactant. These systems are thermodynamically stable and form spontaneously
upon mixing. They also possess specific physicochemical properties such as
transparency, optical isotropy, low viscosity and thermodynamic stability. Thus, in
contrast to standard emulsions which are opaque, microemulsions have an observable
transparency, which is due to the fact that the maximum size of the droplets of the
dispersed phase is not larger than one-fourth of the wavelength of visible light—approximately 150 nm. Thus the droplet diameter in stable microemulsions is usually within the range of 10-250 nm. The water droplet size in the microemulsions can be controlled by altering the amount of co-surfactant in the microemulsions. Alternatively, the percentage of the water phase can be varied.

The microemulsion used to form the nanolattices of the present invention may comprise an non-polar phase of between 2 and 98% by weight, most preferably between 10 and 90% by weight; a water phase of between 2 and 98% by weight, most preferably between 10 and 90 by weight; 0.1 to 90% by weight surfactant, preferably 1 to 90% by weight surfactant. The microemulsion may further comprise 0.1 to 90% by weight cosurfactant or cosolvent; preferably 1 to 90% by weight cosurfactant or cosolvent.

The microemulsion used to form the nanolattices of the present invention may further comprise solvents or other agents to enhance emulsion formation or stability. Other agents may be introduced to provide functions such as pH, ionic content, polymerisation, viscosity.

The microemulsions may also be generated using any suitable synthetic plastic or polymeric, monomeric or hybrid colloidal material.

In one instance, microemulsions were formed by mixing an oil phase composed of mixtures of oil (Crodamol GTCC and Capmul MCM, at 3:1 ratio) with the cosurfactants (Crillet 4 and Crill 4, 3:2 ratio) with one ninth volume of water containing the polymer to be cross-linked to form the nanolattice in solution. Polymer concentrations varied from 2 to 100 mg/ml.
Alternatively, a microemulsion can be formed by mixing 3.5 ml of hexane with 1.5 g m o f cosurfactant (Crillet 4 and Crill 4:1) and adding 260 ul of polymer solution at 2-100 mg/ml.

Additionally, a base stable microemulsion can be formed by mixing 16 gm of oil (Crodamol GTCC and Capmul, at 3:1 ratio) with 4 gm of the cosurfactant (Brij 72 and Brij 97, 3:1) and stirring until clear. The polymer solution is then added (0.5 ml 2-100 mg/ml).

Modification of nanolattices to provide pendant side-chains

In an embodiment of the second aspect, the process for preparing nanolattices may further comprise surface modification of the nanolattices by one or more reagents to provide one or more pendant side chains. The pendant groups may be introduced during the preparation of the nanolattices, or after preparation of the nanolattices.

Pendant side chains are introduced to the nanolattices by reaction of free reactive groups remaining after formation of the nanolattice, such as but not limited to hydroxyl groups, carboxyl groups, hydrazidyl groups, amino groups, imidazole or thiol groups with one or more reagents, wherein the reagents comprise suitable leaving groups.

In one embodiment, the free OH group of a nanolattice may react with a halo substituted carboxylic acid, such as chloro-, bromo-, iodo- acetic acids, chloro-, bromo-, iodo-hexanoic acids, producing nanolattices with pendant side chains comprising carboxylic moieties and hydrophobic moieties, which may be used to interact with pharmaceuticals.

For example:

\[
\text{Nanolattice-OH} + X-(\text{CH}_2)_n\text{-COOH} \rightarrow \text{Nanolattice-O-(CH}_2)_n\text{-COOH}
\]

wherein X is any halogen atom;
n is any integer $\geq 1$.

In another embodiment, the nanolattices may react with carbonyl electrophiles such as carbonyldiimidazole, phosgene, triphosgene, N,N'-disuccinimidyl carbonate, carbonyldipiperidine, 1,r-carbonyldi(1,2,4-triazole), di(2-pyridyl)ketone, or di(l-benzotriazolyl)carbonate, and carbonyldiimidazole. Following such reaction, the carbonyl groups are reacted with pendant side groups such as amino acids, diamines, thiols, diradical spacers.

In yet another embodiment, acid or acyl halides, such as thionyl chloride, phosphorus trichloride, phosphorus pentachloride can be reacted with the free OH groups of the nanolattice, after which suitable nucleophiles are added to provide pendant side chains according to the invention. For example,

$$\text{Nanolattice-OH} + R-COX \rightarrow \text{Nanolattice-O-C(O)-R}$$

wherein X is any halogen atom or other suitable leaving group.

Suitable acid pendant side chains for conjugation to the nanolattices of the present invention include, but are not limited to N-protected amino acids, carboxyl acids such as formic, acetic, propionic, butyric, valeric, caproic, caprylic, capric, lauric, myristic, palmitic, stearic, oleic, linoleic, linolenic, cyclohexanecarboxylic, penylacetic, benzoic, toulie, chlorbenzoic, bromohexanoic, nitrobenzoic, phthalic, isophthalic, terephthalic, salicylic, $\beta$-hydroxybenzoic, anthranilic, aminobenzoic, methoxybenzoic acids, and derivatives thereof.

Examples of acid halides include thionyl chloride, phosphorus trichloride, phosphorus pentachloride, 4-nitrophenylchloroformate, allylbromine, p-hexylbenzoyl chloride, 2-chloro-N,N-diethylaminoethane.
In another embodiment, alkyly halide derivatives of the nanolattices of the present invention can be prepared by reacting the hydroxyl groups on the nanolattices with concentrated HBr, or NaBr + H₂SO₄ plus heat, or PBr₃, or P + I₂. Alternatively, the alkyl halide derivatives of the nanolattices can be prepared by replacement of the -OH by X, using reagents such as phosphorus halides. Additionally, alkyl iodide derivatives of the nanolattices can be prepared from the bromide or chloride by treatment with a solution of sodium iodide in acetone.

The alkyl halide derivatives of the nanolattices may be represented by the following formula:

\[
\text{Nanolattice-} (\text{CH}_2)_n \text{X}
\]

wherein

- \( X \) is any halogen atom;
- \( n \) is any integer \( \geq 0 \).

Such alkyl halide derivatives of the nanolattices may be further reacted with by simple nucleophilic substitution in the following reactions:

- Nanolattice-(CH₂)ₙX + :OH' to form an alcohol derivative, Nanolattice-(CH₂)ₙOH;
- Nanolattice-(CH₂)ₙX + H₂O to form an alcohol derivative, Nanolattice-(CH₂)ₙOH;
- Nanolattice-(CH₂)ₙX + OR' to form an ether derivative, Nanolattice-(CH₂)ₙO R';
- Nanolattice-(CH₂)ₙX + ^\text{:C≡CR}' to form an alkyne derivative, Nanolattice-(CH₂)ₙ C≡CR';
- Nanolattice-(CH₂)ₙX + R'-M to form an alkane derivative, Nanolattice-(CH₂)ₙR';
- Nanolattice-(CH₂)ₙX + I to form an alkyl iodide derivative, Nanolattice-(CH₂)ₙI;
- Nanolattice-(CH₂)ₙX + :CN' to form a nitrile derivative, Nanolattice-(CH₂)ₙCN;
- Nanolattice-(CH₂)ₙX + R'COO' to form an ester derivative, Nanolattice-(CH₂)ₙOOC-R';
Nanolattice-(CH\textsubscript{2})\textsubscript{n}X + :NH\textsubscript{3}\textsuperscript{−} to form a primary amine derivative, Nanolattice-(CH\textsubscript{2})\textsubscript{n}NH\textsubscript{3};
Nanolattice-(CH\textsubscript{2})\textsubscript{n}X + :NH\textsubscript{2}R' to form a secondary amine derivative, Nanolattice-(CH\textsubscript{2})\textsubscript{n}NHR';
Nanolattice-(CH\textsubscript{2})\textsubscript{n}X + :NHR'R" to form a tertiary amine derivative, Nanolattice-(CH\textsubscript{2})\textsubscript{n}NHR'R";
Nanolattice-(CH\textsubscript{2})\textsubscript{n}X + :SH to form a thio derivative, Nanolattice-(CH\textsubscript{2})\textsubscript{n}SH;
Nanolattice-(CH\textsubscript{2})\textsubscript{n}X + :SR' to form a thioether derivative, Nanolattice-(CH\textsubscript{2})\textsubscript{n}SR';
Nanolattice-(CH\textsubscript{2})\textsubscript{n}X + ArH + AlCl\textsubscript{3} to form an alkylbenzene derivative, Nanolattice-(CH\textsubscript{2})\textsubscript{n}Ar.

In another embodiment, the free hydroxyl group in Nanolattice-OH can be oxidized to form a carboxylic acid nanolattice derivative by heating with reagents such as aqueous KMnO\textsubscript{4}. The potassium salt is then removed by addition of a mineral acid such as KOH.

In another embodiment, the free hydroxyl group in Nanolattice-OH can be oxidized to form the aldehyde Nanolattice-CH=O by the use OfK\textsubscript{2}C\textsubscript{2}O\textsubscript{7}:

$$\text{Nanolattice-OH} + \text{Cr}_2\text{O}_7 \rightarrow \text{Nanolattice-CH}=\text{O} + \text{Cr}^{+++},$$

which is further reacted to form a carboxylic acid nanolattice derivative, wherein the carboxylic acid pendant side groups may be used to interact with pharmaceuticals.

In another embodiment, chelators and/or metals are attached as pendant side chains, which may use to interact with pharmaceutical agents. For instance, the alpha amino and carboxylic acid group of an amino acid which has been attached as a pendant side chain, may be used to chelate metals as described herein. In this instance, an N,0 chelate is
formed between the chelate and the metal, which may be used to interact with one or more pharmaceutical agents.

Alternatively, the above chelate may be further reacted with the same or an alternative alpha amino and carboxylic acid to yield a second \( \text{N}_2\text{O} \) chelate with the same central metal atom. In this instance, the metal atom creates a "bridge" between the two chelating molecules.

In another embodiment, amino acids such as threonine, serine, lysine, glutamic acid, aspartic acid, histidine, cysteine, hydroxyproline, may be mixed 2:1 on a molar basis with metal ions including but are not limited to divalent or trivalent cations of iron, manganese, chromium, cobalt, lead, silver, mercury, bismuth, plutonium, cadmium, germanium, ruthidium, gold, indium, technetium, copper, zinc, gallium, rhodium, palladium, platinum, nickel, gadolinium, erbium, europium, dysprosium, yttrium, promethrium, lutetium and holmium and radioactive isotopes thereof. The resultant chelated moieties, such as but not limited to \( \text{Cu-LyS}_2, \text{Mg-LyS}_2, \text{Ca-LyS}_2 \), are neutrally charged, and may be conjugated to the nanolattices as pendant side chains.

In another embodiment, amino malonate may be mixed 2:1 on a molar basis with metal ions including but are not limited to divalent or trivalent cations of iron, manganese, chromium, cobalt, lead, silver, mercury, bismuth, plutonium, cadmium, germanium, ruthidium, gold, indium, technetium, copper, zinc, gallium, rhodium, palladium, platinum, nickel, gadolinium, erbium, europium, dysprosium, yttrium, promethrium, lutetium and holmium and radioactive isotopes thereof. Positively charged moieties formed between the amino groups and the metal ions and may be conjugated to the nanolattices as pendant side chains.
Pendant Side chains providing negative charge following chelation include those formed using linkage between carboxylic acids and metals. Thus amino malonate can be linked 2:1 with metals such as platinum, calcium, copper, magnesium, nickel, etc. Positively charged pendant side chains are those that are formed between amino groups and metals. Thus diamino-hexane, or diaminocyclohexane or derivatives thereof, can be linked 2:1 with metals such as platinum, calcium, copper, magnesium, nickel and the like.

According to another aspect, the present invention provides a complex for oral delivery wherein said complex comprises targeted nanolattices having a PDI of between about 0.1 and 0.4 prepared by a method comprising:

(a) preparing separate mixtures comprising:
   (i) a surfactant and a polymer in an aqueous phase; and
   (ii) a surfactant and a non-polar phase; and
(b) combining mixtures (i) and (ii) from step (a) to form mixture (iii);
(c) subjecting mixture (iii) to mixing for a period sufficient to allow formation of a uniform water-in-oil emulsion;
(d) adding a pharmaceutical agent to one or more of mixtures (i), (ii) and (iii) and the emulsion of step (c);
(e) adding a cross-linking agent or a metal chelating agent to the emulsion formed in step (d) to form the nanolattices in the emulsion; and
(f) separating the nanolattices from the emulsion,

wherein a targeting moiety is covalently bound to the polymer from step (a)(i) either directly or through a spacer or linker compound and the targeting moiety is capable of being bound by, or binding to an intestinal epithelium of the subject, thereby allowing uptake and internalization of the complex.
According to another aspect, the present invention provides a complex for oral delivery wherein said complex comprises targeted nanolattices having a PDI of between about 0.1 and 0.4 prepared by a method comprising:

(a) preparing separate mixtures comprising:
   (i) a surfactant and a polymer in an aqueous phase; and
   (ii) a surfactant and a non-polar phase; and
(b) combining mixtures (i) and (ii) from step (a) to form mixture (iii);
(c) subjecting mixture (iii) to mixing for a period sufficient to allow formation of a uniform water-in-oil emulsion;
(d) adding a pharmaceutical agent to one or more of mixtures (i), (ii) and (iii) and the emulsion of step (c);
(e) adding a cross-linking agent or a metal chelating agent to the emulsion formed in step (d) to form the nanolattices in the emulsion; and
(f) separating the nanolattices from the emulsion,

wherein a targeting moiety interacts with the nanolattice by non-covalent means with or without linkers, spacers or other devivatisation and the targeting moiety is capable of being bound by, or binding to an intestinal epithelium of the subject, thereby allowing uptake and internalization of the complex.

In one embodiment, the nanolattice is formed with a cross-linking agent.

In another embodiment, the nanolattice is formed with a chelating agent.

In one embodiment, one or more targeting moiety is attached to the nanolattice via a pendant side chain of the nanolattice.
Targeting agents according to the invention are selected from molecules which are known to be internalized by cells, or which have been shown to be transcytosed by cells, such as those of the gastro-intestinal epithelium.

Suitable targeting agents include but are not limited to water soluble vitamins such as vitamin C, analogues or derivatives thereof; folic acid and analogues or derivatives thereof (including but not limited to methotrexate, aminopterin, 10-deazaminopterin, 10-ethyl-10-deazaaminopterin, 5,10-dideazatetrahydrofolate, folinic acid, 7-hydOxyaminopterin); niacin (nicotinic acid, vitamin B3) and analogues or derivatives thereof (including but not limited to beta-hydroxybutyrate, acipimox, niceritrol, nicotinamide (niacin)); thiamine (vitamin B-1), analogues and derivatives thereof; riboflavin (vitamin B2)= and its analogues or derivatives thereof (including but not limited to 7-nor-7-chlororiboflavin, 8-nor-8-chlororiboflavin, 7-nor-7-bromoriboflavin, 8-nor-8-bromoriboflavin, 7α-methylriboflavin, 8α-methylriboflavin, 7α,8α-dimethylriboflavin, 7-nor-7-bromo-8α-methylriboflavin, 7α-methyl-8-nor-8-bromoriboflavin, 7-nor-7-chloro-8α-methylriboflavin, 7α-methyl-8-nor-8-chlororiboflavin, 8-nor-8-fluororiboflavin, 7-nor-7-chloro-8-nor-8-chlororiboflavin, 8-nor-8-aminoriboflavin, 7V(3)-methylriboflavin and 5-deaza-5-carbariboflavin; pyridoxine (vitamin B6), analogues or derivatives thereof; cyanocobalamin (vitamin B12), analogues or derivatives thereof, pantothentic acid (vitamin B5), analogues or derivatives thereof, and biotin, analogues or derivatives thereof.

Other targeting agents include viral haemmaglutinins, bacterial adhesins, transferrin, immunoglobulins, and immunoglobulin Fc fragments either derived therefrom or synthesized separately, bacterial invasins, toxins, and binding sub-units thereof, lectins, and the sugar binding moieties thereof, membrane transduction sequences thereof. Additionally fusion proteins of the aforementioned targeting agents can also be used.
Riboflavin (Rf) derivatives may be prepared by reaction of the primary OH group of Rf with diacids, di- or tri-carboxylic acids, or diamine acids.

Diacids acids suitable for linkage to Rf derivatives of the invention include, but are not limited to:- glutamic acid, aspartic acid, hydroxyglutamic acid, hydroxyisophthalic acid, 2, 4-diamino-pentanedioic acid, 2-amino-pentanedioic acid, 2-aminohexanedioic acid, mesoxalic acid, oxomalonic acid, ketomalonic acid, and alanosin.

Di-carboxy and tricarboxylic acids suitable for linkage to Rf derivatives of the invention include, but are not limited to:- agaricic acid, 2-hydroxy-1,2,3-nonadecanetricarboxylic acid, Cis, cis-1,3,5,Trimethylcyclohexane-1,3,5-tricarboxylic acid, Cis-Aconitic acid cis-Propene-1,2,3-tricarboxylic acid, Trimellitic acid, Benzene-1,2,4-tricarboxylic acid, Trimesic acid, Benzene-1,3,5-tricarboxylic acid, Cyclopropane-1,2,3-tricarboxylic acid, Carboxyaspartic acid, Carboxyglutamic acid (gamma-carboxy-D-glutamic acid, Malic acid (hydroxysuccinic acid).

Diamine acids suitable for linkage to Rf derivatives of the invention include, but are not limited to:- lysine, ornithine 2,5-diaminopentanoic acid NH₂(CH₂)₂CH(NH₂)COOH, diaminobenzoic acid, 2,4-diaminophenol, 2,3-diaminopropionic acidRf derivatives may be prepared by reaction of the primary OH group of Rf with diacids, di- or tri-carboxylic acids, or diamine acids. Diacids acids suitable for linkage to Rf derivatives of the invention include, but are not limited to:- 2,3-diaminopropionic acid, malonic acid, maleic acid, succinic acid, glutaric acid, methyl malonic acid, glutamic acid, aspartic acid, hydroxyglutamic acid, hydroxyisophthalic acid, hydroxyglutaric acid, 2,4-diamino-pentanedioic acid, 2-amino-pentanedioic acid-dimethyl ester, 2-aminohexanedioic acid, mesoxalic acid, oxomalonic acid, ketomalonic acid, HOOCOCOOH and alanosin. Di-carboxy and tricarboxylic acids suitable for linkage to Rf.
Biotin Analogues suitable for chelating include 3-(N-Maleimido-propionyl)biocytin: a thiol-specific biotinylating reagent, alpha-dehydrobiotin, Z- and E-4,5-dehydrodethiobiotin, norbiotinamine, dl-4 xi-(4-carboxybutyl)-5-carboxy-cis-hexahydropyrrolo (3,4-d)imidazol-2-one (N-carboxyazabiotin), dl-4xi-(2-carboxyethyl)-cis-hexahydropyrrolo-[3,4-d]imidazol-2-one (bisnorazabiotin), bis-allyloxy carbonyl biotin aldehyde, carboxybiotin and methyl biotin.

For the purpose of the invention vitamin B12 includes vitamin B12 or analogues thereof, such as described below.

In one embodiment, the targeting molecule is Vitamin B12 or an analogue thereof or a derivative of vitamin B12 or analogues thereof. Analogues of Vitamin B12 that may derivatized and thereby be used as targeting molecules in the complexes of the present invention include, but are not limited to: cyanocobalamin, aquacobalamin, adenosylcobalamin, methylcobalamin, hydroxycobalamin, cyanocobalamin carbanalide, 5-O-methylbenzylcobalamin, and the desdimethyl, monoethylamide and methylamide analogues of all of the preceding analogues, as well as coenzyme B12, 5'-deoxyadenosylcobalamin, chlorocobalamin, sulfocobalamin, nitrocoobalamin, thiocyanatocobalamin, 5,6-dichlorobenzimidazole, 5-hydroxybenzimidazole, trimethylbenzimidazole, adenosylcyanocobalamin, cobalamin lactone, cobalamin lactam, and analogues in which the cobalt is replaced by zinc or nickel or the corrin ring is replaced by a substituent which does not affect the binding capacity of the analogue to Castle’s intrinsic Factor.

Derivatives of Vitamin B12 or analogues thereof, for use as targeting molecules include but are not limited to: anilides, ethylamides, monocarboxylic and dicarboxylic acid derivatives of Vitamin B12 and its analogues, and also tricarboxylic acids or
propionamide derivatives of Vitamin B₁₂ or its analogues thereof, molecules in which alterations or substitutions had been performed to the Corrin ring, or where cobalt has been replaced by another metal ion, or various anionic or alkyl substituents have been added to the corrin ring such that the binding capacity of the molecule to Castle's intrinsic factor is unaffected.

Other derivatives of VB₁₂ or analogues thereof that may be used in the complexes of the present invention include 5′O-substituted VB₁₂ derivatives such as: hexyl-5′O-VB₁₂, dodecyl-5′O-VB₁₂, tetradecyl-5′O-VBi₂, hexadecyl-5′O-VB₁₂, octadecyl-5′O-VB₁₂, aminoethyl-5′O-VBi₂, aminobutyl-5′O-VBi₂, 1-ethyl-Phe-5′O-VBi₂, aminohexyl-5′O-VBi₂, aminododecanyl-5′O-VBi₂, succinylhydrazidyl-5′O-VBi₂, adipylhydrazidyl-5′O-VBi₂, phenylalanyl-5′O-VBi₂, glycyl-5′O-VBi₂, HO-Gly-5′O-VBi₂, VB₁₂-5′O-phenylalanine, VB₁₂-5′O-lysine, VBi₂-glycine. In one embodiment, the group attached to the 5′O position may constitute the pendant group of a nanolattice.

Additional VB₁₂ derivatives of the invention include, but are not limited to: agaric acid, 2-hydroxy-1,2,3-nonadecanetricarboxylic acid, cis, cis-1,3,5,Trimethylcyclohexane-1,3,5-tricarboxylic acid, cis-Aconitic acid cis-Propene-1,2,3-tricarboxylic acid, Trimellitic acid, Benzene-1,2,4-tricarboxylic acid, Trimesic acid, Benzene-1,3,5-tricarboxylic acid, Cyclopropane-1,2,3-tricarboxylic acid, Carboxyglutamic acid, Carboxyglutamic acid (gamma-carboxy-D-glutamic acid, Malic acid (hydroxysuccinic acid).

Upon binding of the complex of the invention to Castle's intrinsic factor, mucosal epithelial cells take up the intrinsic factor-complex and trans-epithelially transport the complex into the circulation or lymphatic drainage system where the pharmaceutical agent can be released. The nanolattices may degrade in vivo to release the pharmaceutical agent.
Chelators

Chelators that may be used in the complexes of the invention include 1,3,5-triaminocyclohexane, 1,3,5-triaminocyclohexane N-pyridine, 1,1-cyclobutanedicarboxylic acid, 1,2-Dimethyl-3-hydroxypyridin-4-one, 1,2-dimethyl-3-hydroxypyridin-4-one (Deferiprone), 1,4,7,10-tetraaza-4,7,10-tris(carboxymethyl)-1-cyclododecylacetyl-i?-(+)-R-methylbenzylamine (DOTA-MBA), 1,4,7,10-tetraazacyclododecane-Ν,Β,Ν,Β-tetraacetic acid (DOTA), 1,6-dimethyl-2-(1β-hydroxyethyl)-3-hydroxypyridin-4-one, 1-ethyl-2-(1β-hydroxyethyl)-3-hydroxypyridin-4-one, 1-hydroxypridin-2-one, 1-hydroxypridin-2-one, 2-Deoxy-2-(N-carbamoylmethyl-[N9-29-methyl-39-hydroxypyrid-49-one])-D-glucopyranose, 2-furoylcarboxaldehyde isonicotinoyl hydrazone (FIH), 2-hydroxy-1-naphthaldehyde benzoyl hydrazone, 2-hydroxy-1-naphthaldehyde isonicotinoyl hydrazone, 2-hydroxy-1-naphthaldehyde, isonicotinoyl hydrazone, 2-methyl-3-hydroxy-4H-benzopyran-4-one (MCOH), 2-pyridylcarboxaldehyde 2-thiophenecarboxyl hydrazone (PCTH), 2-pyridylcarboxaldehyde benzoyl hydrazone (PCBH), 2-pyridylcarboxaldehyde isonicotinoyl hydrazone (PCIH), 2-pyridylcarboxaldehyde isonicotinoyl hydrazone (PCIH), 2-pyridylcarboxaldehyde m-bromobenzoyl hydrazone (PCBBH), 2-pyridylcarboxaldehyde m-bromobenzoyl hydrazone (PCBBH), 2-pyridylcarboxaldehyde p-aminobenzoyl hydrazone (PCAH), 2-pyridylcarboxaldehyde p-hydroxybenzoyl hydrazone (PCHH), 2-pyridylcarboxaldehyde thiophenecarboxyl hydrazone (PCTH), 311 2-hydroxy-1-naphthaldehyde isonicotinoyl hydrazone, 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (Triapine®), 3-aminopyridine-2-carboxaldehyde thiosemicarbazone, 3-hydroxypyridin-2-one, 3-hydroxypyridin-4-one, 4-[3,5-bis-(hydroxyphenyl)-1,2,4-triazol-1-yl]-benzoic acid (ICL670A), aminocarboxylates, BAPTA/AM (1,2-bis(2-aminophenoxy)ethane- N,N,N9,N9-tetraacetic acid acetoxymethyl ester), catechols, CDTA cyclohexanediaminetetraacetic acid, cis-1,3,5-
triaminocyclohexane, clioquinol, DDC diethyldithiocarbamate, Defarasirox, Deferiprone (1,2-dimethyl-3-hydroxypyridin-4-one), Deferoxamine, Demercaptol, DFO desferrioxamine, DFOA, Diaminocyclohexane, diethylenetriaminepentaacetic acid; DMB, DMPA dimercaptopropionic acid, DMPS, DMSA dimercaptosuccinic acid, DPA (D-penicillamine), DTPA (diethylene triamine pentaacetic acid, EDTA (ethylenediaminetetraacetic acid), Ferroportin-1, hydroxamates, hydroxycarboxylates, hydroxypyridinones, IDA iminodiacetic acid, MECAM, N,N-bis-(2-hydroxybenzyl) ethylenediamine-N,N-diacetic acid (HBED), N,N'-bis(2-hydroxybenzyl)ethylenediamine- N,N'-diacetic acid (HBED), N,N'-Bis(3,4,5-trimethoxybenzyl)ethylenediamine-N,N'- diacetic acid, N,N-dimethyl-2,3-dihydroxybenzoic acid, N,N,N',N,N,N'-pentakis[(N- hydroxy-N-methyl)carbonyl]methyl]-2,6-diamino-4-azaheaxanoic hydrazide, NAPA N- acetyl-D-penicillamine, N-ethyl N, N, N-tris(pyrindylmethyl)-cis,cis, 1,3,5,- triaminocyclohexane, NOTP (1,4,7-triazacyclononane-1,4,7-trisfmethylene phosphonate), NOTPME (1,4,7-triazacyclononane-1,4,7-tris' methylene phosphonatomonoesterylery), N-pyridine, NTA nitrilotriacetic acid, oxalic acid, pyridoxal hydrochloride, pyridoxal isonicotinoyl hydrazone, pyridoxal isonicotinoyl hydrazone (PIH), pyridoxal metachlorobenzoyl hydrazone, pyridoxal metafluorobenzoyl hydrazone, pyridoxal paramethoxybenzoyl hydrazone, rhizoferrin, salicylaldehyde benzoyl hydrazone, staphloferrin, staphloferrin, succinic acid, tachpyridine, TETA (triethylenetetra-amine), tetraaza-4,7,10-tris(carboxymethyl)-1- cyclododecyldactetylbenzylamine (DOTA-BA), TREN-(Me-3,2-hydroxipyridonate) (HOPO), TRENCAM, Triapine, 3-aminopyridine-2-carboxyaldehyde thiosemicarbazone, TTD tetratethythiumsalisulfide, TTHA triethylenetetraminehexaacetic acid, Deferiprone (1,2-dimethyl-3-hydroxypyridin-4-one), HYNIC (6-hydrazinonicotinamide), HYNIC-Kp-DPPB and HYNIC-Ko-DPPB where (HYNIC ) 6-hydrazinonicotinamide; K) lysine; and DPPB ) diphenylphosphate-benzoic acid), HPO 3-hydroxypyridin-4-one, 1-(2'- carboxyethyl)-2-methyl-3-hydroxypyridin-4-one, 1-(3′-hydroxypropyl)-2-methyl-3- hydroxypyridin-4-one, 1-(2′-hydroxyethyl)-2-ethyl-3-hydroxypyridin-4-one,
tricoordinate ligand (5)-2-(2,4-dihydroxyphenyl)-4,5-dihydro-4-thiazolecarboxylic acid [4'-(HO)-DADMDFT], hexacoordinate derivative ([S,S]-U 1-bis[5-(4-carboxy-4,5-dihydrothiazol-2-yl)-2,4-dihydroxyphenyl]-4,8-dioxaundecane.

Additional chelators include DFO, deferiprone, PCTH, PCBBH, PCBH, HBED, ICL670A, MCOH, TREN-(Me-3,2-HOPO), PIH, PCIH, PCHH, PCAH and FIH.

Stability of Metal Complexes. Representative examples of the stability constants of different chelating pairs may vary.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Stability Constant</th>
</tr>
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<tbody>
<tr>
<td>[Ag(NH₃)₂]⁺</td>
<td>1.4 x 10⁷</td>
</tr>
<tr>
<td>[Ag(CN)₂]⁻</td>
<td>1.0 x 10²¹</td>
</tr>
<tr>
<td>[Fe(CN)₆]⁴⁻</td>
<td>1.0 x 10⁴⁴</td>
</tr>
<tr>
<td>[Fe(CN)₆]³⁻</td>
<td>1.0 x 10³¹</td>
</tr>
<tr>
<td>[Co(NH₃)₆]³⁺</td>
<td>1.3 x 10⁵</td>
</tr>
<tr>
<td>[Co(NH₃)₅(N₂)]⁴⁻</td>
<td>2.3 x 10³⁴</td>
</tr>
<tr>
<td>[Co(CN)₆]³⁻</td>
<td>1.0 x 10⁶⁴</td>
</tr>
<tr>
<td>[Co(H₂NCH₂CH₂NHH₂)₃]⁴⁺</td>
<td>9.1 x 10¹³</td>
</tr>
<tr>
<td>[Co(H₂NCH₂CH₂NHH₂)₃]³⁺</td>
<td>4.8 x 10⁴⁸</td>
</tr>
<tr>
<td>[Ni(NH₃)₆]⁺⁺</td>
<td>4.1 x 10⁸</td>
</tr>
<tr>
<td>[Ni(H₂NCH₂CH₂NHH₂)]⁺⁺</td>
<td>2.5 x 10¹⁸</td>
</tr>
</tbody>
</table>

Stability of chelates is much higher than monodentate ligands.

Metals for chelation include aluminum, antimony, arsenic, beryllium, bismuth, cadmium, cobalt, copper, gadolinium, gallium, gold, iron, lanthanum, lead, manganese, mercury, nickel, platinum, organic tin, samarium, thallium, zirconium and zinc, and dietary
supplemental chelates. More preferably, the chelates are calcium, magnesium and zinc, most preferably zinc.

Amino acids suitable for substitution of the polymers of the lattice include amino-glycine, Diaminopropionic acid, Diaminobutyric acid, Dehydroalanine, Fluoro-alanine, Chloro-alanine, Azetidine-2-carboxylic acid, aminobutyric acid, cyanoalanine, amino-isobutyric acid, homocysteine, methylene-glutamic acid, hydroxy-glutamic acid, ornithine, difluoromethyl-ornithine, cyclopropyl-alanine, tert-butyl-alanine, propargyl-glycine, 2-allyl-glycine, tert-butyl-glycine, alla-threonine, Ureido-alanine, pyroglutamic acid, carboyglutamic acid, 2,6-Diamino-4-hexynoic acid, 4-amino-piperidine-4-carboxylic acid, B-(1-cyclopentenyl)-alanine, B-Cyclopentyl-alanine, thioproline, 3, 4-dehydroproline, methyl-valine, hydroxyproline, citrulline, thiocitrulline, aminoadipic acid, 4,5-dehydro-lysine, 6-hydroxy-lysine, B-cyclohexyl-alanine, B,B-dicyclohexyl-alanine, 4-fluoro-proline, methyl-proline, norvaline, 6-diao-5-oxo-norleucine, homocitrulline, 5-methyl-thiocitrulline, 2-aminoheptanedioic acid, Hydroxy-norarginine, homoarginine, penicillamine, 4,5-dehydro-leucine, allo-isoleucine, pipecolic acid, cyclohexyl-glycine, B-(2-thoazolyl)-alanine, B-(1,2,4-triazol-1-yl)-alanine, B-(2-thienyl)-serine, aminosuberic acid, amino-arginine, methyl-arginine, l-aminocyclopentane-carboxylic acid, methyl-leucine, Norleucine, octahydroindole-2-carboxylic acid, methyl-phenylalanine, 2-mercapto-histidine, 4-nitro-phenylalanine, B-(3,4-di-hydroxy-phenyl)-serine, 4-carboxy-phenylalanine, methyl-histidine, 2,5-diido-histidine, B-(2-thienyl)alanine, B-(3-benzothienyl)-alanine, phenylglycine, 4-bromo-phenylalanine, homophenylalanine, 4-azido-phenylalanine, 4-cyano-phenylalanine, 3,5-dinitro-tyrosine, 3,5-dibromo-tyrosine, 1-methyl-histidine, 3-methyl-histidine, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, B-(1-Naphthyl)-alanine, 4-iodo-phenylalanine, 3- Fluoro-phenylalanine, 4-tert-butyl-phenylalanine, a-methyl-tryptophan, 3-iodotyrosine, 3-nitro-tyrosine, 3,5-diiodo-tyrosine, B-(2-pyridyl)-alanine, B-(3-pyridyl)alanine, Spinacine, B-(2-naphthyl)-alanine, 3,4-dichloro-phenylalanine, 4-chloro-phenylalanine,
Additional reagents for modification of the hydroxyl groups of VB12 or riboflavin include chloroacetic acid, 3-Chloropropylamine.HCl, 4-Nitrobenzyl chloroformate, Sodium Chloroacetate, 4-chlorobenzoic acid, 2-chlorobenzoic acid, 3-chlorobenzoic acid, 3-chloropropyl amine, Epichlorohydrin, Ethylchloroacetate, Bromo-benzyl bromide, Bromoethylammonium bromide, 3-bromo-l,2-propandiol, Bromoisobutyric acid, Bromoisovaleric acid, Bromoacetylchloride, 4,4'-difluoro-3',5'-dinitrodiphenylsulfone, toluenediisocyanate, Hexamethylenediisocyanate, Bisoxiranes such as 1,4-butanediol diglycidyl ether (1,4-bis(2,3-epoxypoxypropoxy)butane), Heterocyclic Halides, s-Triazines, such as cyanuric chloride, 2-amino-4,5-dichloro-s-triazine, 2-carboxymethylamino-4,6-
dichloro-s-triazine, and 2-carboxymethyloxy-4,6-dichloro-s-triazine, fluoropyrimidine, Vinyl sulfone, p-benzoquinone, Hydroxy-S-triazole

Polymers
Polyglutamate, Polyaspartate, polyphosphazenes, polyanionic polysaccharides, poly(hydroxamic acid), poly(salicylate), poly(catecholate), poly(aminooxime), polyaminocarboxylates, dendrimers, Poly(L-glutamic acid)-cystamine-DOTA, polyaminophosphonic acid, polyphosphates

DNA Transfection
During the process of transfection chromosomal or plasmid DNA is complexed with calcium chloride in solution. The resultant calcium-DNA mix is then applied to the surface of cell monolayers and one to two days later the cells screened for the presence of marker DNA. One major problem with this method is that the calcium-DNA complex so formed is highly variable and also cannot be stored readily. In contrast Calcium:DNA nanolattices (as per example B13) are much more uniform and can be stored either as the final ethanol pellet or frozen as a solution.

Imaging
Nanolattices of the invention may include imaging agents, in combination with targeting agents. Suitable imaging agents include any metal capable of being detected in a diagnostic procedure can be employed as the Metal in a nanolattice chelate. Suitable ions include the following Antimony-124, Antimony-125, Arsenic-74, Barium-103, Barium-140, Beryllium-7, Bismuth-206, Bismuth-207, Cadmium-109, Cadmium-1 Ism, Calcium-45, Cerium-139, Cerium-141, Cerium-144, Cesium-137, Chromium-51, Cobalt-56, Cobalt-57, Cobalt-58, Cobalt-60, Cobalt-64, Erbium-169, Europium-152, Gadolinium-153, Gold-195, Gold-199, Hamium-175, HamiumO175-181, Indium-II, Iridium-192, Iron-55, Iron-59, Krypton-85, Lead-210, Manganese-54, Mercury-197, Mercury-203,

**Hydrazidyl Derivatives**

Hydrazidyl derivatives that may be linked to the targeting agent or the polymer include, but are not limited to adipic acid dihydrazide, carbohydrazide, ethylmalonic acid dihydrazide, glutaric acid dihydrazide, isophthalic acid dihydrazide, maleic acid dihydrazide, malonic acid dihydrazide, naphthalene dihydrazide, oxalic acid dihydrazide, pimelic acid dihydrazide, sebacic acid dihydrazide, suberic acid dihydrazide, succinic acid dihydrazide, 9,10-dihydro-9,10-ethanoanthracene-ll,12-dicarboxylic acid dihydrazide, terathalic acid dihydrazide, thiocarbazide, 2,3,4,5-tetrahydrophthalic acid dihydrazide, 4,4'-oxy-bis(benzenesulfonyl hydrazide).

In addition, hydrazidyl compounds may be made from derivatisation of the carboxyl group(s) on amino acids. Dihydrazides may formed from various diacids including aspartic acid, glutamic acid, or diacids of the general formula NH₂NH-CO-R-CO-NHNH₂, where R can equal any alkyl or aryl containing group, or where CO-R-CO could be replaced by CO or CS.

Additionally, targeting agents may be chelated to the nanolattice by reaction of a targeting agent with the alpha amine of an amino acid, followed by modification of the free carboxyl group with hydrazine to form a terminal hydrazide. Suitable amino acids
include, but are not limited to 6-aminonicotinic acid, 4-amino-3-phenylbutyric acid, p-aminophenylacetic acid, 3-amino-2-naphthoic acid, 3-amino-4-hydroxybutyric acid, p-amino-hippuric acid, aminobutyric acid, aninocapric acid, 1-aminoanthraquinone-2-carboxylic acid, aminoadipic acid, m-aminobenzoic acid, o-aminobenzoic acid, anthranilic acid, aminoheptanoic acid, 7-amino-4-methyl-3-coumarinylacetic acid, Aminoacidipic acid, glycine, proline, hydroxyproline, alanine, valine, glutamine, glutamic acid, asparagine, aspartic acid, phenylalanine, tyrosine, threonine, serine, tryptophan, histidine, leucine, isoleucine, cystine, methionine, ornithine, arginine, and lysine. Alternative amino acids include Amino-Glycine, Diaminopropionic acid, Diaminobutyric acid, Dehydroalanine, Fluoro-alanine, Chloro-alanine, Azetidin-2-carboxylic acid, aminobutyric acid, cyanoalanine, cyano-isobutyric acid, homocysteine, methylene-glutamic acid, hydroxy-glutamic acid, ornithine, difluoromethyl-ornithine, cyclopropylalanine, tert-butylylalanine, propargyl-glycine, 2-allyl-glycine, tert-butyl-glycine, allathreonine, Ureido-alanine, pyroglutamic acid, carboxyglutamic acid, 2,6-Diamino-4-hexynoic acid, 4-amino-piperidine-4-carboxylic acid, B-(1-cyclopentenyl)-alanine, B-Cyclopropyl-alanine, thiolproline, 3, 4-dehydro-proline, methyl-valine, hydroxyproline, citrulline, thiocitrulline, aminoacidipic acid, 4,5-dehydro-lysine, 6-hydroxy-lysine, B-cyclohexyl-alanine, B,B-dicyclohexyl-alanine, 4-fluoro-proline, methyl-proline, norvaline, 6-diazo-5-oxo-norleucine, homocitrulline, 5-methyl-thiocitrulline, 2-aminoheptanedioic acid, Hydroxy-norarginine, homoarginine, penicillamine, 4,5-dehydro-leucine, allo-isoleucine, piperolic acid, cyclohexyl-glycine, B-(2-thiazolyl)-alanine, B-(1,2,4-triazol-1-yl)-alanine, B-(2-thienyl)-serine, aminosuberic acid, aminoarginine, methyl-arginine, 1-amino-cyclopentanecarboxylic acid, methyl-leucine, Norleucine, octahydroindole-2-carboxylic acid, methyl-phenylalanine, 2-mercaptotimididine, 4-nitro-phenylalanine, B-(3,4-dihydroxy-phenyl)-serine, 4-carboxyphenylalanine, methyl-histidine, 2,5-diido-histidine, B-(2-thienyl)alanine, B-(3-benzothienyl)-alanine, phenylglycine, 4-bromo-phenylalanine, homophenylalanine, 4-
azido-phenylalanine, 4-cyano-phenylalanine, 3,5-dinitro-tyrosine, 3,5-dibromo-tyrosine, 1-methyl-histidine, 3-methyl-histidine, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, B-(1-Naphthyl)-alanine, 4-iodo-phenylalanine, 3-Fluoro-phenylalanine, 4-tert-butyl-phenylalanine, a-methyl-tryptophan, 3-iodotyrosine, 3-nitro-tyrosine, 3,5-diiodo-tyrosine, B-(2-pyridyl)-alanine, B-(3-pyridyl)alanine, Spinacine, B-(2-naphthyl)-alanine, 3,4-dichloro-phenylalanine, 4-chloro-phenylalanine, 4-chloro-phenylalanine, 4-fluoro-phenylalanine, 4-methyl-tryptophan, 5-methyl-tryptophan, 7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, Thyronine, B-(2-quinolyl)-alanine, 3-aminotyrosine, 4-amino-phenylalanine, B,B-diphenyl-alanine, 4-methyl-phenylalanine, 2,3,4,5,6-pentafluoro-phenylalanine, 4-benzoyl-phenylalanine, 1,2,3,4-tetrahydronorharman-3-carboxylic acid, 3-hydroxymethyl-4-isopropylidene-tyrosine.

Complexes of the invention may be synthesized by conjugating the nanolattices to the targeting molecule, via one or more pendant groups of the nanolattices. The pendant groups may be free reactive groups on the cross-linking agents that remain after the cross-linking procedure has been completed in the preparation of the nanolattices, for example a bifunctional molecule such as an amino carboxylic acid, a diamine, a dithiol or a dicarboxylic acid. Further examples of cross-linking agents that may be used for conjugation to the targeting molecules include: N-(4-azidophenylthio)-phthalimide, 4,4'-dithiobisphenylazide, dithio-bis-(succinimidyl-propionate), dimethyl-3,3'-dithio-bis-propionimidate.2HCl, 3,3'-dithio-bis-Csulfosuccinimidyl-propionate), ethyl-(4-azidophenyl)-l,3'-dithiopropionate, sulfo-succinimidyl-2-(m-azido-o-nitrobenzamido)-ethyl-1,3'-dithiobutyrimidate.HCl, N-succinimidyl-(4-azido-phenyl)-1,3'dithiopropionate, sulfo-succinimidyl-2-(m-azido-o-nitro-benzamido)-ethyl-1,3'-dithiopropionate, sulfo-succinimidyl-2-(p-azido-salicylamido)-ethyl-1,3'-dithiopropionate, N'-succinimidyl-3-(2-pyridylthio)propionate; sulfosuccinimidyl-(4-azidophenylthio)-propionate, 2-
iminothiolane, disuccinimidyl tartrate and bis-[2-(succinimidylloxycarbony-ethyl]-sulfone.

In one embodiment, where the targeting molecule is VB_{12}, the targeting molecule may be attached to the nanolattices via a pendant group as follows:

\[
\text{EDAC} \\
\text{Nanolattice-NH-C(})CH_{2}\text{COOH + VB}_{12}\text{N}-\text{NH}_2 \rightarrow \text{Nanolattice-NH-C(}O\text{)(CH}_{2}\text{n-C(}O\text{-NH-VB}_{12}
\]

In one embodiment, the targeting molecule is not linked to the nanolattices via an ester linkage.

In an alternative embodiment, the VB_{12} targeting molecule may be attached directly to the nanolattice via reaction of a compound having the formula VB_{12}-X, wherein X is a leaving group, for example a halide, a tosylate or a mesylate, with a nanolattice comprising a nucleophilic group. An example of this embodiment is given below:

\[
\text{Nanolattice-OH + VB}_{12}\text{-Br} \rightarrow \text{Nanolattice-O-VB}_{12}
\]

In a further alternative embodiment, the nucleophilic group may be located on the VB_{12} molecule, and the leaving group may be located on the nanolattice.

The present invention further provides a pharmaceutical composition comprising the complexes of the invention and a pharmaceutically acceptable carrier and/or diluent. Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, anti-bacterial and anti-fungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art and except insofar as any conventional media or agent is incompatible with the modulator; their use in the pharmaceutical compositions is
contemplated. Supplementary active compounds can also be incorporated into the compositions.

As such, the present invention provides a method of orally delivering a pharmaceutical agent, such as those described in the present invention, to a subject in need of such pharmaceutical agent, said method comprising administering of a therapeutically effective amount of a complex of the invention, or a pharmaceutical composition thereof comprising such pharmaceutical agent.

In one embodiment, the pharmaceutical agent in the complexes or the pharmaceutical composition of the invention is insulin. Accordingly, the present invention is directed to a method for treating diabetes (including diabetes mellitus) in a subject in need of said treatment, said method comprising administration of a therapeutically effective amount of the complex or the pharmaceutical composition of the invention, wherein the pharmaceutical agent is insulin. Such treatment may be used alone or in conjunction with another drug or therapy.

In another embodiment, the pharmaceutical agent in the complexes or the pharmaceutical composition of the invention is EPO. Accordingly, the present invention is directed to a method for treating a number of conditions in a subject in need of said treatment, said method comprising administration of a therapeutically effective amount of the complex or the pharmaceutical composition of the invention, wherein the pharmaceutical agent is EPO. In particular, the pharmaceutical composition of the present invention comprising EPO may be used in treatment alone or in conjunction with another drug or therapy for indications including anaemia, such as anemia of chronic disease; microangiographic hemolytic anemia; anaemia associated with chronic renal failure (including patients on dialysis or not); anaemia associated with end stage renal disease; anaemia resulting from or in association with dialysis; acute lymphocytic leukaemia; anaemia in patients with
non-myeloid malignancies where anaemia is due to effect of concomitantly administered chemotherapy; anaemia associated with HIV infection or treatment; anaemia associated with cancer chemotherapy; anaemia due to radiotherapy or associated with hormonal therapy or immunotherapy; for the reduction of allogeneic blood transfusion in anaemic patients undergoing non-cardiac, non-vascular surgery; anaemia due to antiviral therapies (e.g. in treatment of hepatitis C or ADDS), inflammatory bowel disease, blood loss (e.g. due to surgery, gastrointestinal ulcers or childbirth), rheumatoid arthritis, myelosuppressive therapy, chronic or congestive heart failure and malaria.

In another aspect, the pharmaceutical composition of the present invention comprising EPO may be used in treatment alone or in conjunction with another drug or therapy for non-hematopoietic indications, such as, for general maintenance, protection and repair of the nervous system (e.g. focal brain ischemia, concussive brain injury, experimental autoimmune encephalomyelitis (EAE), kainate-induced seizures, epilepsy, cerebral ischemia, spinal cord, injury, hypoxia, lower back pain, diabetic neuropathy, multiple sclerosis, schizophrenia, for neuroprotection purposes (e.g. in stroke patients and to prevent brain injury due to perinatal asphyxia); retinal degeneration; beta-thalassemia; neuropsychiatric porphyria; glaucoma; neonatal necrotizing enterocolitis; enhancing autologous stem cell transplantation; increasing platelet reactivity and counts e.g. in alcoholic liver cirrhosis.

In another embodiment, the pharmaceutical agent in the complexes or the pharmaceutical composition of the invention is GM-CSF. Accordingly, the present invention is directed to a method for treating a number of conditions in a subject in need of said treatment, said method comprising administration of a therapeutically effective amount of the complex or the pharmaceutical composition of the invention, wherein the pharmaceutical agent is GM-CSF. In particular, the pharmaceutical composition of the present invention comprising GM-CSF may be used alone or in conjunction with other biologies, drugs or
therapies (e.g. surgery, radiotherapy, bone marrow transplantation, peripheral stem cell transplantation; conventional chemotherapy, combined chemotherapy including cisplatin, streptozocin, doxorubicin, fluorouracil, mitomycin C, busulfan, oblimersen, etoposide, vincristine, cyclophosphamide, gemcitabine, pacitaxel, taxol, cytabarine; antivirals including didanosine, AZT, ribavirin, viramidine, zidovudine, dideoxycytidine; other therapies including antibiotics, cytokines, growth factor, small molecule pharmaceuticals) for several types of leukaemia, including acute myeloid leukaemia and acute lymphoblastic leukaemia, and various pathologies associated with anti-cancer chemotherapy, such as leukopenia, (a reduction in leukocytes); neutropenia, including febrile neutropenia in patients receiving chemotherapy for acute myeloid leukaemia or in other high risk patients, neutropenia after consolidation chemotherapy for acute myeloid leukaemia and in myelodysplastic syndrome, induced neutropenia in HIV patients with Kaposi sarcoma; facilitating engraftment in bone marrow or peripheral stem cell transplants; assisting failed or delayed bone marrow engraftment; accelerating myeloid recovery after autologous bone marrow or peripheral stem cell (peripheral blood progenitor cell, PBPC) transplantation (for example, in patients with non-Hodgkin's lymphoma); Hodgkin's disease; and increasing the mobilisation and yield of PBPC to be collected for transplantation.

In another aspect, the pharmaceutical composition of the present invention comprising GM-CSF may be used alone or in conjunction with other biologies, drugs or therapies as an adjunct to increase chemotherapy dose-intensity; to provide a priming effect in chemotherapy (i.e. to alter cell cycle kinetics to increase cytotoxicity); to act as a hematopoietic protectant in chemotherapy; to reverse neutropenia in combined radio- and chemotherapy; to prevent or treat mucosal damage (mucositis), which occurs as a side effect of chemotherapy; to reduce severity of fungal infections in leukemia patients; as vaccine adjuvant for the contribution of maturation of dendritic cells and antigen presenting cells; as adjuvant for several vaccines including hepatitis B and influenza; as
an adjunct to treat bacterial and fungal infections in preterm infants with sepsis; as an adjuvant to prevent infections in organ transplantation patients who are immunosuppressed; to inhibit HIV replication, to facilitate increased CD4 counts and decreased infections in AIDS patients; as a tumour vaccine in order to enhance immune response to tumour antigen epitope peptides in melanoma; to promote wound repair, such as non-healing leg ulcers; to stimulate arteriogenesis, for instance, in peripheral artery disease; to stimulate of axonal regeneration in nervous system injury; to protect the brain following stroke; to treat pulmonary alveolar proteinosis; and to reduce of graft versus host disease, for instance, in stem cell transplantation.

In another embodiment, the pharmaceutical agent in the complexes or the pharmaceutical composition of the invention is TNFRII or chimeric molecules thereof, such as TNFRII-Fc. Accordingly, the present invention is directed to a method for treating a number of conditions in a subject in need of said treatment, said method comprising administration of a therapeutically effective amount of the complex or the pharmaceutical composition of the invention, wherein the pharmaceutical agent is TNFRII or chimeric molecules thereof, such as TNFRII-Fc. In particular, the pharmaceutical composition of the present invention comprising TNFRII or chimeric molecules thereof may be used alone or in conjunction with other biologies, drugs or therapies in the treatment of infectious diseases such as HIV; hepatitis C; HIV-I-associated tuberculosis; SARS; coronavirus infection; severe sepsis; septic shock, gram negative and gram positive bacteremia; endotoxic shock; arthritis including rheumatoid arthritis, polyarticular juvenile rheumatoid arthritis (JRA), spondyloarthropathy, psoriatic arthritis, severe gouty arthritis, ankylosing spondylitis, juvenile idiopathic arthritis, chronic polyarthritis, systemic lupus; pain such as in rheumatoid arthritis, pain and swelling after oral surgery, temporomandibular disorders, chronic back and/or neck disc-related pain, acute, severe sciatica, pain due to bone metastasis, sciatica due to herniated nucleus pulposus, complex regional pain syndrome - Type 1 (CRPS 1); psoriasis; asthma; allergic and non-allergic inflammatory responses in
the airways; Wegener's granulomatosis; dermatomyositis; polymyositis; uveitis; non-infectious scleritis; myelodysplastic syndrome; Graves' ophthalmopathy; iritis in patients with ankylosing spondylitis; vasculitis; small vessel vasculitis; relapsing panniculitis; tumor necrosis factor receptor associated periodic syndrome (TRAPS); Weber-Christian disease (WCD); Behcet's disease; Churg-Strauss vasculitis; Churg-Strauss-Syndrome; polyarteritis nodosa; giant cell arteritis; sarcoidosis; polymyositis/dermatomyositis; Sjogren's syndrome; sleepiness in patients with sleep apnea e.g. due to obstructive sleep apnea in obesity; multicentric reticulohistiocytosis; pyoderma gangrenosum; Takayasu arteritis; cardiac mitochondrial dysfunction, oxidative stress, and apoptosis in heart failure; Adult-onset Stills disease (AOSD); Crohn's disease; alcoholic hepatitis; myositis; giant cell arteritis; spontaneous endometriosis; chronic infantile neurological cutaneous articular (CINCA) syndrome; Guillain-Barre syndrome; sarcoidosis; aphthous stomatitis; peri-prosthetic osteolysis e.g. following total hip replacement; primary amyloidosis; hyperimmunoglobulinemia and periodic fever syndrome; male and female infertility; inner ear inflammation; Langerhans-cell histiocytosis; immune thrombocytopenic purpura; chronic inflammatory demyelinating polyneuropathy; multicentric reticulohistiocytosis; autoimmune dacryoadenitis; peripheral neuropathy e.g. in celiac disease; polychondritis; pneumatosis cystoides intestinalis; neurosarcoidosis; pigmented villonodular synovitis; necrotizing vasculitis; acute childhood ulcerative colitis; inflammatory bowel disease; Kawasaki disease; myopathy e.g. in Duchenne muscular dystrophy (DMD); ocular inflammation in Adamantiades-Behcet disease; acrodermatitis continua of Hallopeau; hidradenitis suppurativa; renal amyloidosis; indeterminate colitis; post-transplant obliterative bronchiolitis; pyostomatitis vegetans; SAPHO syndrome; necrobiosis lipoidica; Red man syndrome; cancer e.g. breast cancer including in combinations with chemotherapy or other biological therapies; cancer-related cachexia; cutaneous T-cell lymphomas; graft rejection phenomena such as graft-versus host disease (GVHD) (e.g. acute non-infectious lung injury (idiopathic pneumonia syndrome, IPS) and subacute pulmonary dysfunction after allogeneic stem cell transplantation); lung graft ischemia-
reperfusion injury; severe steroid-refractory acute GVHD; in hematopoietic stem cell transplants; in organ transplants e.g. chronic graft injury e.g. in renal allografts. For the treatment of rheumatoid arthritis, the pharmaceutical composition comprising TNFRII or chimeric TNFRII molecule can also be administered in combination with methotrexate. In yet another embodiment, the present invention is administered in combination with other biologically active molecules, such as Leflunomide, Azathioprin, cyclosporine A or sulfasalazine or other monoclonal antibodies (e.g. anti-TNF antibodies, antibodies to Mac I or LFA I) or other receptor associated with TNF production including IL-1 or IL-2 receptors.

In another embodiment, the pharmaceutical agent in the complexes or the pharmaceutical composition of the invention is a chemotherapeutic agent. Accordingly, the present invention is directed to a method for treating cancer in a subject in need of said treatment, said method comprising administration of a therapeutically effective amount of the complex or the pharmaceutical composition of the invention, wherein the pharmaceutical agent is a chemotherapeutic agent. Such treatment may be used alone or in conjunction with another drug or therapy. The chemotherapeutic agent may be cisplatin, carboxplatin, oxaliplatin, DACH-platinum, anthracyclines, daunomycin, doxorubicin or epirubicin.

In another embodiment the invention provides a complex of DNA/Ca++ that has been formed in a microemulsion of the invention and precipitated by addition of ethanol. The pellet can be washed with ethanol, resuspend in water and freeze dried.

In an extension to the previous embodiment the DNA/Ca++ nanolattice can be used to increase the frequency of Cellular transfection.
In yet another embodiment of the invention, the nanolattice comprises DNA molecules that are chelated via calcium or magnesium ions and comprise a targeting agent of the invention that is suitable for Oral delivery of DNA.

In yet another embodiment of the invention, the nanolattice comprises siRNA molecules that are chelated via calcium or magnesium ions and comprise a targeting agent of the invention that is suitable for Oral delivery of siRNA.

In a further embodiment there is provided a nanolattice comprising entrapped Metal. Any metal capable of being detected in a diagnostic procedure in vivo or in vitro can be employed as the metal in the nanolattice. Particularly, any radioactive metal ion capable of producing a diagnostic result in a human or animal body or in an in vitro diagnostic assay may be used in the practice. Metals of the present invention that can be used such as in the detection of capillary blockage or tumour masses include Antimony-124, Antimony-125, Arsenic-74, Barium-103, Barium-140, Beryllium-7, Bismuth-206, Bismuth-207, Cadmium-109, Cadmium-115m, Calcium45, Cerium-139, Cerium-141, Cerium-144, Cesium-137, Chromium-51, Cobalt-56, Cobalt-57, Cobalt-58, Cobalt-60, Cobalt-64, Erbium-169, Europium-152, Gadolinium-153, Gold-195, Gold-199, Hafnium-175, Hafnium-175-181, Indium-111, Iridium-192, Iron-55, Iron-59, Krypton-85, Lead-210, Manganese-54, Mercury-197, Mercury-203, Molybdenum-109 99, Neodymium-147, Neptunium-237, Nickel-63, Niobium-95, Osmium-185+191, Palladium-103, Platinum-195m, Praseodymium-143, Promethium-147, Protactinium-233, Radium-226, Rhenium-186, Rubidium-86, Ruthenium-103, Ruthenium-106, Scandium44, Scandium-46, Selenium-75, Silver-II0m, Silver-III, Sodium-22, Strontium-85, Strontium-89, Strontium-90, Sulfur-35, Tantalum-182, Technetium-99m, Tellurium-125, Tellurium-132, Thallium-204, Thorium-228, Thorium-232, Thallium-170, Tin-I 13, Titaniu44, Tungsten-185, Vanadium-48, Vanadium49, 20 Ytterbium-169, Yttrium-88, Yttrium-90, Yttrium-91, Zinc-65, andZirconium-95.
In yet another embodiment of the invention nanolattices are produced that are sufficiently small for pulmonary delivery. Such NL must be of sufficiently small size to travel through the bronchi, bronchiols, and into the alveoli, without impacting upon the walls of the pulmonary system.

In another embodiment of the invention the nanolattices comprise vaccines, which are suitable for oral administration. Further, these nanolattices can also comprise adjuvants such as Inulin to help stimulate the immune response.

The present invention is further described by the following non-limiting examples.
EXAMPLES

Preparation of nanolattices.

A.1 Preparation of Carboxymethyl Dextran
Dextran T70 (100 gm) of average molecular weight of 70,000 Daltons was dissolved at 50 mg/ml in 1 M Na₂CO₃ and was reacted with 200 gm sodium chloroacetate. The reaction was heated to 80°C and allowed to proceed for 3 hours. The Carboxy-methyl dextran (CM-Dextran) was then purified by positive pressure dialysis against DW via tangential flow filtration and lyophilized. The carboxymethyl pendant side chains comprise carboxylic acid moieties that may be used to interact with pharmaceutical agents, or may be used to conjugate to targeting agents, or are available for chelation. The presence of the carboxy group on the resulting product was verified by a negative zeta potential, and the ability to chelate copper when copper chloride was added to a solution of 50 mg/ml CM-dextran. A spectral change was seen in the 600-900 nm range.

A.2 Preparation of acetylated dextran (AcDex) using bromoacetic acid.
Dextran (10g) was added to 100 g of bromoacetic acid dissolved in 30 ml of dioxane and stirred for 20 hours at room temperature. The resultant acetylated dextran was washed extensively with distilled water via tangential flow filtration using a 10-30 kDa membrane. The acetyl pendant side chains comprise carboxylic acid moieties that may be used to interact with pharmaceutical agents, metals and/or targeting agents.

A.3 Preparation of Carboxymethyl dextran using chloroacetic acid
Dextran (2 gm) was dissolved at 50 mg/ml in 1 M Na₂CO₃ and was reacted with 2 gm chloroacetic acid (Fluka #24520) which had been neutralized via an equal molarity of sodium hydroxide. The reaction was heated to 80°C and allowed to proceed for 3 hours. The carboxymethyl dextran was then purified by positive pressure dialysis via tangential flow filtration and lyophilized. The carboxymethyl pendant side chains
comprise carboxylic acid moieties that may be used to interact with pharmaceutical agents, metals and/or targeting agents.

A.4 Preparation of Carboxyhexyl dextran using bromohexanoic acid.
Dextran (2 gm) was dissolved at 50 mg/ml in 1 M Na₂CO₃ and was reacted with 2 gm bromohexanoic acid (Fluka #24520) which had been neutralized via an equal molarity of sodium hydroxide. The reaction was heated to 80°C and allowed to proceed for 3 hours. The Carboxyhexyl-dextran was then purified by positive pressure dialysis via tangential flow filtration and lyophilized. The hexanoic acid pendant side chains comprise ionic and hydrophobic moieties that may be used to interact with pharmaceutical agents, metals and/or targeting agents.

A.5 Preparation of Aminohexyl-dextran using epichlorohydrin
Dextran (2 gm) was dissolved at 50 mg/ml in 1 M Na₂CO₃ and was reacted with 2 gm epichlorohydrin in the presence of 50 mg/ml NaHBO₃. The reaction was heated to 80°C and allowed to proceed for 3 hours. 2 gm diaminohexane was added and the reaction was allowed to proceed overnight. The aminohexyl-dextran was then purified by positive pressure dialysis via tangential flow filtration and lyophilized. The hexylamine pendant side chains comprise ionic and hydrophobic moieties that may be used to interact with pharmaceutical agents, metals and/or targeting agents.

A.6 Preparation of Aminohexyl-dextran using POCl₃
Dextran (2 gm) was dissolved at 50 mg/ml in 1 M Na₂CO₃ and was reacted with 2 gm POCl₃ in the presence of 50 mg/ml NaHBO₃. The reaction was heated to 80°C and allowed to proceed for 3 hours. 2 gm diaminohexane was added and the reaction was allowed to proceed overnight. The Aminohexyl-dextran was then purified by positive pressure dialysis via tangential flow filtration and lyophilized. The hexylamine pendant
side chains comprise ionic and hydrophobic moieties that may be used to interact with pharmaceutical agents, metals and/or targeting agents.

A.7 Preparation of aminoethyl-dextran using $\text{POCl}_3$

Dextran (2 gm) was dissolved at 50 mg/ml in 1 M $\text{Na}_2\text{CO}_3$ and was reacted with 2 gm $\text{POCl}_3$ in the presence of 50 mg/ml $\text{NaHBO}_3$. The reaction was heated to $80^\circ\text{C}$ and allowed to proceed for 3 hours. 1 ml diaminoethane was added and the reaction was allowed to proceed overnight. The aminoethyl-dextran was then purified by positive pressure dialysis via tangential flow filtration and lyophilized. The ethylamine pendant side chains comprise amino moieties that may be used to interact with pharmaceutical agents, metals and/or targeting agents.

A.8 Preparation of 4-fluorobenzene sulfonate derivatized dextran

Dextran (1 gm) can be reacted with fosyl chloride (600 mg) (4-fluorobenzenesulfonyl chloride, Aldrich) in the presence of triethylamine (60 ul) and DMAP (160 mg) as a catalyst in ultra-dry DMSO. The reaction yields the 4-fluorobenzene sulfonate derivatized Dextran, which can be used immediately stored at $4^\circ\text{C}$ or in water. The unreacted fosyl chloride is removed by precipitation with acetone, or ethyl acetate. The pendant side chains comprise sulfonate moieties that may be used to interact with pharmaceutical agents, metals and/or targeting agents.

A.9 Preparation of p-toluenesulfonyl chloride derivatized Dextran

Dextran (1 gm) can be reacted with tresyl chloride (p-toluenesulfonyl chloride) and DMAP (160 mg) as a catalyst in ultra-dry DMSO. The reaction yields the p-toluenesulfonyl chloride derivatized Dextran, which can be used immediately stored at $4^\circ\text{C}$ or in water. The unreacted tresyl chloride is removed by precipitation with acetone,
or ethyl acetate. The pendant side chains comprise sulfonyl moieties that may be used to interact with pharmaceutical agents, metals and/or targeting agents.

A.10 Preparation of Lysyl-Folate

Folate (1 gm) was dissolved at 24.4 mg/ml in DW. NHS (0.5 gm) was dissolved at 92 mg/ml in DMF and added to the folate. Solid EDAC at ~ 4-fold excess (3.1 gm) was added as powder to the NHS/folate mix, while stirring rapidly. The reaction was allowed to proceed for 20 mins. At this stage complexes of Lysyl-Copper-Lysine at 100 mg/ml in 1% NaHCO3 (5.8 gm in 58 ml) was added to folate-NHS and reacted overnight. The reaction mix was precipitated with acetone to 70% and then the pellet was dissolved in 100 ml 50 mM EDTA. The soluble material was loaded onto Dowex 1 X 2 resin. AU of the Lys-Cu-Lys stuck to the column. The Lysyl-folate in the flow through was loaded onto XAD-16 resin and washed with DW. Residual Lys-Cu-Lys washed through. The Lysyl-folate was eluted with 4 x 100 ml 75% MeOH. After which the product was rotary evaporated, resuspended in distilled water and lyophilized.

A.11 Preparation of Lysyl-Biotin (Biocytin)

D-Biotin (1 gm) was dissolved at 48.8 mg/ml in DW. NHS (0.5 gm) was dissolved at 92 mg/ml in DMF and added to the biotin. Solid EDAC at ~ 4-fold excess (3.1 gm) was added as powder to the NHS/Biotin mix, while stirring rapidly. The reaction was allowed to proceed for 20 mins. At this stage complexes of Lysyl-Copper-Lysine at 100 mg/ml in 1% NaHCO3 (5.8 gm in 58 ml) was added to Biotin-NHS and reacted overnight. The reaction mix was precipitated with acetone to 70% and then the pellet was dissolved in 100 ml 50 mM EDTA. The soluble material was loaded onto Dowex 1 X 2 resin. All of the Lys-C-Lys stuck to the column. The biocytin in the flow through was loaded onto XAD-16 resin and washed with DW. Residual Lys-Cu-Lys washed through. The biocytin was eluted with 4 x 100 ml 75% MeOH. After which the product was rotary evaporated, resuspended in distilled water and lyophilized. Mass spectral analysis of biocytin showed a Molecular ion of 373.
A.12 Preparation of 5'Lysyl-VB12

Vitamin B12 (Sigma) was dissolved at 100 mg/ml in DMSO. Solid \text{1,2-Carbonyl-di(1,2,4-triazole}} was added to a final concentration of 25 mg/ml and reacted for 30 minutes. The activated derivative was precipitated with ethyl acetate and the supernatant decanted. The pellet was then dissolved in a solution of DMSO containing 50 mg/ml Lysyl-copper-lysine and 100 mg/ml TEA. The reaction was allowed to proceed overnight. The product was precipitated with acetone, and the resultant pellet dissolved in distilled water. The product was purified by chromatography on S-Sepharose. The eluted material was concentrated on XAD-16 resin, eluted with methanol, rotary evaporated to remove methanol and lyophilized. Mass spectral analysis of the lysyl-VB12 showed the presence of the product of Molecular Weight 1527.5 and a \text{A} mass of 764.

A.13 Preparation of Lysyl-e-VB12

The eVB12-carboxylic acid derivative (1 gm) (Wockhardt) was dissolved at 100 mg/ml in DW. NHS (0.5 gm) was dissolved at 92 mg/ml in DMF and added to the VB12. Solid EDAC at ~ 4-fold excess (3.1 gm) was added as powder to the NHS/eVB12 mix, while stirring rapidly. The reaction was allowed to proceed for 20 mins. At this stage complexes of Lysyl-Copper-Lysine at 100 mg/ml in 1\% NaHCO3 (5.8 gm in 58 ml) was added to eVB12-NHS and reacted overnight. The reaction mix was precipitated with acetone to 70\% and then the pellet was dissolved in 100 ml 50 mM EDTA. The soluble material was loaded onto Dowex 1 X 2 resin. All of the Lys-Cu-Lys stuck to the column. The Lysyl-eVB12, which appeared in the flow through was loaded onto XAD-16 resin and washed with DW. Residual Lys-Cu-Lys washed through. The product was eluted with 4 x 100 ml 75\% MeOH. After which the product was rotary evaporated, resuspended in distilled water and lyophilized.

A.14 Preparation of glutathionyl-VB12
Hydroxy-cobalamin (Aventis) was dissolved at 100 mg/ml in 2.5% acetic acid. Solid zinc was added as a powder to a final concentration of 10 mg/ml and allowed to reduce Colli to CoI for 5 minutes. An equal weight of glutathione as a powder was added and reacted overnight. The product, Glutathionyl-S-Co-Cbl was purified by Reverse Phase HPLC. Mass spectral analysis of glutathionyl-S-Co-Cbl showed the presence of the product of Molecular Weight 1636 and the \( \gamma_2 \) mass of 818.

### A.15 Preparation of cysteinyl-VBl2
Hydroxy-cobalamin was dissolved at 100 mg/ml in 2.5% acetic acid. Solid zinc was added as a powder to a final concentration of 10 mg/ml and allowed to reduce Colli to CoI for 5 minutes. An equal weight of cysteine as a powder was added and reacted overnight. The product was purified by Reverse Phase HPLC.

### A.16 Preparation of Lysyl-Riboflavin.
Riboflavin (Sigma-Aldrich) was dissolved at 37.6 mg/ml in DMSO and heated until in solution. A 2.5 molar excess of Solid 1,1'-carbonyl-di(1,2,4-triazole) (150 mg) was added and reacted for 20 minutes. A two-fold weight excess of Lys-Cu-Lys dissolved in DMSO was added plus 300 \( \mu \)l TEA. The reaction was allowed to proceed overnight, and then the reaction was precipitated at pH 4.5. The product was dissolved in EDTA and applied to a Dowex 1x2 resin and the flow-through applied to XAD resin and washed with 0.2M EDTA. The product was eluted with methanol, rotary evaporated and lyophilized.

**Preparation of charged pendant side chains suitable for ionic interaction.**

### A.17 Malonato-N-Acetyl-5'OVBl2
Carboxy-acetyl-5 O-VB\(_{12}\) (generous gift of Dr Tai Le) was reacted with TSTU. The product was further reacted with diethylaminomalonate hydrochloride. The resultant product was purified by RP-HPLC and then lyophilized. The side groups were removed by five minute treatment with 0.1 M NaOH. The resultant malonato-N-O-VB\(_{12}\) is suitable
for ionic interaction with diamino pendant groups, or for chelation of metals such as platinum, calcium, copper, magnesium, nickel or for ionic interaction with diamino pendant side groups on the nanolattice.

A.18 Diaminopropylaminohexyl-5O-VB12
Boc-diamoBoc-propionic acid was reacted with TSTU and the product was then reacted with aminohexyl-5O-VB12. The product was purified by RP-HPLC and lyophilized. The product was then deprotected with neat TFA.

A.19 Diaminopentaacetic acid.
DTPA-dianhydride was dissolved in DMF and added dropwise to diaminoethane. The product was purified by extraction into ethylacetate. The product was lyophilized.

A.20 Derivatization of charged dextran suitable for the preparation of nanolattices according to the invention
Dextran is suspended in dichloromethane at 50 mg/ml. CDT is added at 20% weight for weight and allowed to react for 20 minutes. Diethyaminomalonate was dissolved in DMF plus an equal weight of triethylamine and added at 20 % weight for weight to DN. The reaction was allowed to proceed overnight, afterwhich the modified dextran was treated with NaOH to 0.1 M for 30 minutes to remove the protecting groups. The product was purified by tangentional flow filtration against distilled water.

A.21 Conjugation of targeting agent to amino-malonate modified dextran
Amino-malonate-dextran (see example above) was mixed with 5% weight NHS, then activated by 10% EDAC for 20 minutes. The activated malonic acid groups were mixed with an amino-containing targeting agent dissolved in 10% NaHCO3. The amino-
malonate modified detrzn then comprised negatively charged pendant side chains to which is attached the TA, and to which can be added the pharmaceutical agent. Suitable amino containing targeting agents include but are by no means limited to lysyl-vitamin B12, glutathionyl vitamin B12, lysyl-riboflavin, lysyl-biotin.

A.22 Preparation of Calcium-ADP linkers
ADP was dissolved at 100 mg/ml in DW and a half-molar amount of CaCl2 dissolved at 100 mg/ml slowly dripped into the solution. The resultant ADP-Ca-ADP chelate was lyophilized.

A.23 Preparation of a cross-linked dextran nanolattice (DNL)
Dextran (Ave mol. Wt 70kDa; Fluka) was solubilized at 1 g/15 ml in 0.5M NaOH. Tween 80 was added at 0.15 g/g dextran and allowed to disperse by rapid mixing until uniform. An oil phase was prepared as a separate solution of 60 g of heavy mineral oil (per g dextran) with 0.16 g of Span 85 (per g dextran) and mixed vigorously.

The two phases were mixed and homogenized until a uniform micro-emulsion was formed. The micro-emulsion was then stirred at 4°C (Ice bath) using a mechanical stirrer at a suitable speed to maintain the micro-emulsion. Once the emulsion had cooled to 20°C POCl3 was added (at a specified ratio to the polymer according to Table 3A) and stirring was continued for 4hrs. The pH of the suspension was brought to pH 7.0 using Cone. HCl and an equal volume of n-hexane (Lab scan) was added to the cross-linked preparation and stirred well to allow for extraction of the water phase. The particles were then allowed to settle. The organic phase containing the oil was decanted and the particulate suspension was washed with 3 x 1 volume of n-hexane. The water phase was dialysed against a 100 kDa cut-off membrane to remove the salt and the particles were then lyophilised and stored in moisture free environment until further use. The
polydispersity index for various preparations of dextran nanolattice was found to range between 0.1 and 0.3 as determined by dynamic light scattering (see Table 3).

<table>
<thead>
<tr>
<th>Dextran Nanolattice (DNL)</th>
<th>Crosslinker: Polymer Ratio</th>
<th>PDI</th>
<th>Size average</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNL 1</td>
<td>1:4</td>
<td>0.11</td>
<td>97.6 nm</td>
</tr>
<tr>
<td>DNL 2</td>
<td>1:8</td>
<td>0.205</td>
<td>132.5 nm</td>
</tr>
<tr>
<td>DNL 3</td>
<td>1:12.5</td>
<td>0.205</td>
<td>111.3 nm</td>
</tr>
<tr>
<td>DNL 4</td>
<td>1:20</td>
<td>0.205</td>
<td>175 nm</td>
</tr>
</tbody>
</table>

**A.24 Preparation of a cross-linked dextran nanolattice**

27.5 g of sodium hydroxide and 100 g of dextran were added to 1375 g of distilled water. The mixture was stirred until all components were dissolved. Tween 80 (41.5g) was added to the mixture and stirred at approximately 1000 rpm for 10 minutes until it was fully dissolved. Mineral oil phase (6100g) was prepared by adding Span 85 (158.5g) to the oil and homogenising briefly. While homogenising at setting 6, the water phase was added into the oil phase. The mixture was homogenised until fully dispersed and until the micro-emulsion reached 60°C. The micro-emulsion was transferred into the fume hood on ice bath, stirred at 1000 rpm until the micro-emulsion was cooled to 30°C while stirring.

The dextran solution was cross-linked at 30°C by slowly adding POCl₃ (7.0ml) and stirring on ice for 4 hours. The micro-emulsion was neutralised with 8.8M HCl. Hexane (7500 ml) was added and stirred for approximately 10 min to wash the emulsion. Nanolattices were allowed to settle overnight. The hexane/oil phase was removed from the top. A further 750 ml hexane was added and quickly washed by swirling. The material was transferred to a separating funnel, the water phase collected (approximately
1500 ml). Another 750 ml hexane, and approximately 500 ml distilled water was added. The nanolattices were clarified by centrifugation at 5000 rpm for 20 minutes. The clarified water phase was make up to 4 L with distilled water, and washed by filtration using Tangential Flow Filtration on 100,000 MW cut-off Millipore Biomax cartridge. When the conductivity reached that of the distilled water, it was concentrated down to 2.5 L and lyophilised. The polydispersity index for various preparations of dextran nanolattice was found to range from 0.1 to 0.3.

A.25 Preparation of bisoxirane cross-linked nanolattices using kinetically stabilized emulsions

A solution of dextran 50 mg/ml in 1M NaOH containing 2 mg/ml NaBH4 plus 3% Tween 80 is emulsified in heavy mineral oil containing 12% Span 85 at 80°C. The micro-emulsion is rapidly cooled to room temperature. Then 5 ml of 1,4 butandioldiglycidoxyether is added and allowed to react for 5 to 10 hours. The dextran nanolattice is precipitated by the addition of hexane, and washed twice with additional hexane before being further washed extensively with distilled water via tangential flow filtration using a 100-300 kDa membrane.

A.26 Preparation of epichlorohydrin cross-linked nanolattices

A solution of dextran 50 mg/ml in 1M NaOH containing 2 mg/ml NaBH4 plus 3% Tween 80 is emulsified in heavy mineral oil containing 12% Span 85 at 80°C. The micro-emulsion temperature is lowered to 60°C then 5 ml of epichlorohydrin is added and allowed to react for 2 hours. The dextran nanolattice is precipitated by the addition of hexane, and washed twice with additional hexane before being further washed extensively with distilled water via tangential flow filtration using a 100-300 kDa membrane.
A.27 Preparation of divinylsulfone cross-linked nanolattices
A solution of dextran 50 mg/ml in 1 M Na\textsubscript{2}CO\textsubscript{3} plus 3% Tween 80 is emulsified in heavy mineral oil containing 12% Span 85 at 80°C. The micro-emulsion temperature is rapidly lowered to room temperature then 5 ml of divinylsulfone is added and allowed to react for 2 hours. The dextran nanolattice is precipitated by the addition of hexane, and washed twice with additional hexane before being further washed extensively with distilled water via tangential flow filtration using a 100-300 kDa membrane.

A.28 Preparation of p-benzoquinone cross-linked nanolattices
A solution of dextran 50 mg/ml in 1 M NaHCO\textsubscript{3} plus 3% Tween 80 is emulsified in heavy mineral oil containing 12% Span 85 at 80°C. The micro-emulsion temperature is rapidly lowered to room temperature then 5 ml of p-benzoquinone at 20% in ethanol added and allowed to react for 2 hours. The dextran nanolattice is precipitated by the addition of hexane, and washed twice with additional hexane before being further washed extensively with distilled water via tangential flow filtration using a 100-300 kDa membrane.

A.29 Preparation of acetylated dextran nanolattices (DNL) using bromoacetic acid.
DNL (10g) prepared as described in Example A.23 added to 100 g of bromoacetic acid dissolved in 30 ml of dioxane and stirred for 20 hours at room temperature. The resultant acetylated nanolattices are washed extensively with distilled water via tangential flow filtration using a 100-300 kDa membrane. The acetyl pendant side chains comprise carboxylic acid moieties that may be used to interact with pharmaceutical agents.

A.30 Preparation of DNL-O-acetic acid using chloroacetic acid
DNL (2 g) prepared as described in Example A.23 is dissolved at 50 mg/ml in 1 M Na\textsubscript{2}CO\textsubscript{3} and is reacted with 2 g chloroacetic acid (Fluka #24520) which had been
neutralized via an equal molarity of sodium hydroxide. The reaction is heated to 80°C and allowed to proceed for 3 hours. The DNL-acetate is then purified by positive pressure dialysis via tangential flow filtration and lyophilized. The acetic acid pendant side chains comprise carboxylic acid moieties that may be used to interact with pharmaceutical agents.

A.31 Preparation of DNL-hexanoic acid using bromohexanoic acid
DNL (2 g) prepared as described in Example A.23 is dissolved at 50 mg/ml in 1 M Na₂CO₃ and is reacted with 2 g bromohexanoic acid (Fluka #24520) which had been neutralized via an equal molarity of sodium hydroxide. The reaction is heated to 80°C and allowed to proceed for 3 hours. The DNL-hexanoate is then purified by positive pressure dialysis via tangential flow filtration and lyophilized. The hexanoic acid pendant side chains comprise ionic and hydrophobic moieties that may be used to interact with pharmaceutical agents. Other carboxylic acid derivatives of DNL may be prepared analogously, e.g., by using other halo substituted carboxylic acids or active derivatives thereof (anhydrides, acid halides, etc.).

A.32 Preparation of DNL-O-hexylamine using epichlorhydrin
DNL (2 g) prepared as described in Example A.23 is dissolved at 50 mg/ml in 1 M Na₂CO₃ and is reacted with 2 g epichlorhydrin in the presence of 50 mg/ml NaHBO₃. The reaction is heated to 80°C and allowed to proceed for 3 hours. 2 g diaminohexane is added and the reaction is allowed to proceed overnight. The DNL-hexylamine is then purified by positive pressure dialysis via tangential flow filtration and lyophilized. The hexylamine pendant side chains comprise ionic and hydrophobic moieties that may be used to interact with pharmaceutical agents.

A.33 Preparation of DNL-O-hexylamine using POCl₃
DNL (2 g) prepared as described in Example A.23 is dissolved at 50 mg/ml in 1 M Na$_2$CO$_3$ and is reacted with 2 g POCl$_3$ in the presence of 50 mg/ml NaHBO$_3$. The reaction is heated to 80 °C and allowed to proceed for 3 hours. 2 g diaminohexane is added and the reaction is allowed to proceed overnight. The DNL-hexylamine is then purified by positive pressure dialysis via tangential flow filtration and lyophilized. The hexylamine pendant side chains comprise ionic and hydrophobic moieties that may be used to interact with pharmaceutical agents.

A.34 Preparation of DNL-O-ethylamine using POCl$_3$
DNL (2 g) prepared as described in Example A.23 is dissolved at 50 mg/ml in 1 M Na$_2$CO$_3$ and is reacted with 2 g POCl$_3$ in the presence of 50 mg/ml NaHBO$_3$. The reaction is heated to 80 °C and allowed to proceed for 3 hours. 1 ml diaminoethane is added and the reaction is allowed to proceed overnight. The DNL-ethylamine is then purified by positive pressure dialysis via tangential flow filtration and lyophilized. The ethylamine pendant side chains comprise amino moieties that may be used to interact with pharmaceutical agents.

A.35 Preparation of 4-fluorobenzene sulfonate derivatized DNL
DNL (1 g) prepared as described in Example A.23 can be reacted with fosyl chloride (600 mg) (4-fluorobenzenesulfonyl chloride, Aldrich) in the presence of triethylamine (60 ul) and DMAP (160 mg) as a catalyst in ultra-dry DMSO. The reaction yields the 4-fluorobenzene sulfonate derivatized DNL, which can be used immediately stored at 4°C or in water. The unreacted fosyl chloride is removed by precipitation with acetone, or ethyl acetate. The pendant side chains comprise sulfonate moieties that may be used to interact with pharmaceutical agents.
A.36 Preparation of p-toluenesulfonyl chloride derivatized DNL

DNL (1 g) prepared as described in Example A.23 can be reacted with tresyl chloride (p-toluenesulfonyl chloride) and DMAP (160 mg) as a catalyst in ultra-dry DMSO. The reaction yields the p-toluenesulfonyl chloride derivatized DNL, which can be used immediately stored at 4 °C or in water. The unreacted tresyl chloride is removed by precipitation with acetone, or ethyl acetate. The pendant side chains comprise sulfonyl moieties that may be used to interact with pharmaceutical agents.

A.37 Preparation of Trimellitic-Copper chelates

Trimellitic acid was neutralized with a three molar equivalent of sodium hydroxide. The trimellitic acid. 3 NaOH was mixed with 0.5 molar equivalents of copper dichloride. There resultant complex was lyophilized. The complex provides a di-carboxy metal-chelate-linker suitable for linkage to the nanolattices as pendant side chains or targeting molecules.

A.38 Preparation of Trimellitic-Calciura chelates

Trimellitic acid is neutralized with a three molar equivalent of sodium hydroxide. The trimellitic acid. 3 NaOH is mixed with 0.5 molar equivalents of calcium dichloride. There resultant complex is lyophilized. The complex provides a di-carboxy metal-chluate-linker suitable for linkage to the nanolattices as pendant side chains or targeting molecules.

A.39 Preparation of charged pendant side chains suitable for ionic interaction.

(a) Malonato-N-Acetyl-5′OVBi2

Carboxy-acetyl-5′O-VBi2 is reacted with TSTU. The product is further reacted with diethylaminomalonate hydrochloride. The resultant product is purified by RP-HPLC and then lyophilized. The side groups are removed by five minute treatment with 0.1 M NaOH. The resultant malonato-N-0-VBi2 is suitable for ionic interaction with diamino
pendant groups, or for chelation of metals such as platinum, calcium, copper, magnesium, nickel or for ionic interaction with diamino pendant side groups on the nanolattice.

(b) Diaminopropylaminohexyl-5O-VB12
Boc-diaminoBoc-propionic acid was reacted with TSTU and the product was then reacted with aminohexyl-5O-VB12. The product was purified by RP-HPLC and lyophilized. The product was then deprotected with neat TFA.

(c) Diaminopenta-acetic acid.
DTPA-dianhydride was dissolved in DMF and added dropwise to diaminoethane. The product was purified by extraction into ethylacetate. The product was lyophilized.

A.40 Derivatization of charged dextran nanolattices
Dextran nanolattices are suspended in dichloromethane at 50 mg/ml. CDT is added at 20% weight for weight and allowed to react for 20 minutes. Diethylaminomalonate is dissolved in DMF plus an equal weight of triethylamine and added at 20% weight for weight to DNL. The reaction is allowed to proceed overnight, after which the lattices were treated with NaOH to 0.1 M for 30 minutes. The product is purified by tangential flow filtration against distilled water.

A.41 Conjugation of targeting agent to nanolattices
Amino-malonate-DLN are mixed with 5% weight NHS, then activated by 10% EDAC for 20 minutes. The activated DLN are mixed with an amino-containing targeting agent dissolved in 10% NaHCO3. The DLN then will comprise negatively charged pendant side chains to which is attached the TA, and to which can be added the pharmaceutical agent.
A.42 Complexation of insulin with negatively charged DLN
Recombinant human insulin (Wockhardt) was dissolved at 100 mg/ml in 0.1 N HCl. The solution was adjusted to pH 4.0 with sodium hydroxide. Negatively charged DNL-TA complexes were added as a powder (10x weight DNL: insulin) and the insulin allowed to bind via electrostatic interaction. The product was lyophilized.

B.1 Complexation of insulin with negatively charged dextran polymer
Insulin was dissolved at 100 mg/ml in 0.1 N HCl. The solution was adjusted to pH 4.0 with sodium hydroxide. Negatively charged Dextran-TA complexes were added as a powder (10x weight dextran polymer:insulin) and the insulin allowed to bind via electrostatic interaction. The product was lyophilized.

B.2 Preparation of Folate-targeted Carboxymethyl Dextran
CM-Dextran was dissolved at 50 mg/ml in distilled water. N-Hydroxysuccinimide was dissolved at 100 mg/ml in DMF, and added at 1:2 w/w to CM-Dextran. EDAC added as powder at an equal weight to CMD. Activation proceeded for 30 minutes after which Lysyl-Folate, dissolved at 25 mg/ml in 10% NaHCO₃ was added 1:3 w/w to CMD. The solution was reacted ON and then the Folate-CM-dextran was dialysed exhaustively against distilled water and lyophilized.

B.3 Preparation of Vitamin B12-targeted Carboxymethyl Dextran
CM-Dextran was dissolved at 50 mg/ml in distilled water. N-Hydroxysuccinimide was dissolved at 100 mg/ml in DMF, and added at 1:2 w/w to CM-Dextran. EDAC added as powder at an equal weight to CMD. Activation proceeded for 30 minutes after which Lysyl-vitamin B12, dissolved at 25 mg/ml in 10% NaHCO₃ was added 1:3 w/w to CMD. The solution was reacted ON and then the VB12-CM-dextran was dialysed exhaustively against distilled water and lyophilized.
B.4 Preparation of Riboflavin-targeted Carboxymethyl Dextran
CM-Dextran was dissolved at 50 mg/ml in distilled water. N-Hydroxysuccinimide was dissolved at 100 mg/ml in DMF, and added at 1:2 w/w to CM-Dextran. EDAC added as powder at an equal weight to CMD. Activation proceeded for 30 minutes after which Lysyl-riboflavin, dissolved at 25 mg/ml in 10% NaHCO₃ was added 1:3 w/w to CMD. The solution was reacted ON and then the riboflavin-CM-dextran was dialysed exhaustively against distilled water and lyophilized.

B.5 Preparation of Biotin-targeted Carboxymethyl Dextran
CM-Dextran was dissolved at 50 mg/ml in distilled water. N-Hydroxysuccinimide was dissolved at 100 mg/ml in DMF, and added at 1:2 w/w to CM-Dextran. EDAC added as powder at an equal weight to CMD. Activation proceeded for 30 minutes after which Lysyl-biotin, dissolved at 25 mg/ml in 10% NaHCO₃ was added 1:3 w/w to CMD. The solution was reacted ON and then the biotin-CM-dextran was dialysed exhaustively against distilled water and lyophilized.

B6. Preparation of anti-TNF-receptor II loaded Riboflavin-targeted Carboxymethyl Dextran nanolattices
Riboflavin-targeted Carboxymethyl Dextran was dissolved at 100 mg/ml in DW. An oil phase composed of mixtures of oil (Crodamol GTCC (Croda Surfactants) and Capmul MCM (Croda Surfactants), at 3:1 ratio) with surfactants and cosurfactants (Crillet 4 (Croda Surfactants) and Crill 4 (Croda Surfactants), 3:2 ratio). The Crodamol/Capmul mix was mixed with the Crillet 4/Crill4 mix at a ratio of 7:3. The microemulsion oils were then mixed with one ninth volume of water containing the riboflavin-CMD in solution. Microemulsion (ME) formation occurred following gentle shaking of the oil and water phases. The ME was allowed to form over 30 minutes. One tenth weight of TNF-RII-Fc (ALS 00T2), the production and purification of which is described in published
PCT application WO06/079176, as a 100 mg/ml solution was added to the Rf-CMD/ME mix and allowed to mix for 30 minutes. An equal weight of ZnCl₂ to polymer as a 1 M solution (volume:weight) was then added to the above ME and allowed to associate for 30 minutes. Two-fold volume excess of ethanol was then added to precipitate the formed nanolattice. The lattices were allowed to settle and the supernatant decanted. The pellet was then washed with ethanol. The settled nanolattices were resuspended in DW and lyophilized.

B.7 Preparation of anti-TNF-receptor II loaded Folate-targeted Carboxymethyl Dextran nanolattices

Folate-targeted Carboxymethyl Dextran was dissolved at 100 mg/ml in DW. An oil phase composed of mixtures of oil (Crodamol GTCC (Croda Surfactants) and Capmul MCM (Croda Surfactants), at 3:1 ratio) with surfactants and cosurfactants (Crillet 4 (Croda Surfactants) and Crill 4 (Croda Surfactants), 3:2 ratio) The Crodamol/Capmul mix was mixed with the Crillet 4/Crill4 mix at a ratio of 7:3. The ME oils were then mixed with one ninth volume of water containing the folate-CMD in solution. Microemulsion formation occurred following gentle shaking of the oil and water phases. The ME was allowed to form over 30 minutes. One tenth weight of TNF-RII (as described in B.6) as a 100 mg/ml solution was added to the folate-CMD/ME mix and allowed to mix for 30 minutes. An equal weight of ZnCl₂ to polymer as a 1 M solution (volume:weight) was then added to the above ME and allowed to associate for 30 minutes. Two-fold volume excess of ethanol was then added to precipitate the formed nanolattice. The lattices were allowed to settle and the supernatant decanted. The pellet was then washed with ethanol. The settled nanolattices were resuspended in DW and lyophilized.

B.8 Preparation of anti-TNF-receptor II loaded cobalamin-targeted Carboxymethyl Dextran nanolattices
Cobalamin-targeted Carboxymethyl Dextran (prepared according to the method of B.3) was dissolved at 100 mg/ml in DW. An oil phase composed of mixtures of oil (Crodamol GTCC (Croda Surfactants) and Capmul MCM (Croda Surfactants), at 3:1 ratio) with surfactants and cosurfactants (Cril 4 (Croda Surfactants) and Crill 4 (Croda Surfactants), 3:2 ratio) The Crodamol/Capmul mix was mixed with the Cril 4/Crill4 mix at a ratio of 7:3. The ME oils were then mixed with one ninth volume of water containing the cobalamin-CMD in solution. Microemulsion formation occurred following gentle shaking of the oil and water phases. The ME was allowed to form over 30 minutes. One tenth weight of TNF-RII (as described in B.6) as a 100 mg/ml solution was added to the cobalamin-CMD/ME mix and allowed to mix for 30 minutes. An equal weight of ZnCl₂ to polymer as a 1 M solution (volume:weight) was then added to the above ME and allowed to associate for 30 minutes. Two-fold volume excess of ethanol was then added to precipitate the formed nanolattice. The lattices were allowed to settle and the supernatant decanted. The pellet was then washed with ethanol. The settled nanolattices were resuspended in DW and lyophilized.

B.9  Preparation of Insulin loaded Riboflavin-targeted Carboxymethyl Dextran nanolattices

Riboflavin-targeted Carboxymethyl Dextran was dissolved at 100 mg/ml in DW. An oil phase composed of mixtures of oil (Crodamol GTCC (Croda Surfactants) and Capmul MCM (Croda Surfactants), at 3:1 ratio) with surfactants and cosurfactants (Cril 4 (Croda Surfactants) and Crill 4 (Croda Surfactants), 3:2 ratio). The Crodamol/Capmul mix was mixed with the Cril 4/Crill4 mix at a ratio of 7:3. The microemulsion oils were then mixed with one ninth volume of water containing the riboflavin-CMD in solution. Microemulsion (ME) formation occurred following gentle shaking of the oil and water phases. The ME was allowed to form over 30 minutes. One tenth weight of insulin as a 100 mg/ml solution in 0.1 M HCl was added to the Rf-CMD/ME mix and allowed to mix for 30 minutes. An equal weight of ZnCl₂ to polymer as a 1 M solution
(volume:weight) was then added to the above ME and allowed to associate for 30 minutes. Two-fold volume excess of ethanol was then added to precipitate the formed nanolattice. The lattices were allowed to settle and the supernatant decanted. The pellet was then washed with ethanol. The settled nanolattices were resuspended in DW and lyophilized.

**B.10 Preparation of insulin loaded Folate-targeted Carboxymethyl Dextran nanolattices**

Folate-targeted Carboxymethyl Dextran was dissolved at 100 mg/ml in DW. An oil phase composed of mixtures of oil (Crodamol GTCC (Croda Surfactants) and Capmul MCM (Croda Surfactants), at 3:1 ratio) with surfactants and cosurfactants (Crillet 4 (Croda Surfactants) and Crill 4 (Croda Surfactants), 3:2 ratio) The Crodamol/Capmul mix was mixed with the Crillet 4/Crill4 mix at a ratio of 7:3. The ME oils were then mixed with one ninth volume of water containing the folate-CMD in solution. Microemulsion formation occurred following gentle shaking of the oil and water phases. The ME was allowed to form over 30 minutes. One tenth weight of insulin as a 100 mg/ml solution in 0.1 M HCl was added to the folate-CMD/ME mix and allowed to mix for 30 minutes. An equal weight of ZnCl₂ to polymer as a 1 M solution (volume:weight) was then added to the above ME and allowed to associate for 30 minutes. Two-fold volume excess of ethanol was then added to precipitate the formed nanolattice. The lattices were allowed to settle and the supernatant decanted. The pellet was then washed with ethanol. The settled nanolattices were resuspended in DW and lyophilized.

**B.11 Preparation of insulin loaded cobalamin-targeted Carboxymethyl Dextran nanolattices**

Cobalamin-targeted Carboxymethyl Dextran (prepared according to the method of B.3) was dissolved at 100 mg/ml in DW. An oil phase composed of mixtures of oil (Crodamol GTCC (Croda Surfactants) and Capmul MCM (Croda Surfactants), at 3:1 ratio) with
surfactants and cosurfactants (Crillet 4 (Croda Surfactants) and Crill 4 (Croda Surfactants), 3:2 ratio) The Crodamol/Capmul mix was mixed with the Crillet 4/Crill4 mix at a ratio of 7:3. The ME oils were then mixed with one ninth volume of water containing the cobalamin-CMD in solution. Microemulsion formation occurred following gentle shaking of the oil and water phases. The ME was allowed to form over 30 minutes. One tenth weight of insulin as a 100 mg/ml solution in 0.1 M HCl was added to the cobalamin-CMD/ME mix and allowed to mix for 30 minutes. An equal weight of ZnCl₂ to polymer as a 1 M solution (volume:weight) was then added to the above ME and allowed to associate for 30 minutes. Two-fold volume excess of ethanol was then added to precipitate the formed nanolattice. The lattices were allowed to settle and the supernatant decanted. The pellet was then washed with ethanol. The settled nanolattices were resuspended in DW and lyophilized.

B.12 Preparation of insulin loaded cobalamin-targeted Carboxymethyl Dextran nanolattices

Cobalamin-targeted Carboxymethyl Dextran (100 gm) prepared according to the method of B.3) was dissolved at 100 mg/ml in DW (1 litre). An oil phase composed of mixtures of oil (Crodamol GTCC (Croda Surfactants) and Capmul MCM (Croda Surfactants), at 3:1 ratio) with surfactants and cosurfactants (Crillet 4 (Croda Surfactants) and Crill 4 (Croda Surfactants), 3:2 ratio) The Crodamol/Capmul mix was mixed with the Crillet 4/Crill4 mix at a ratio of 7:3. The ME oils (9 litres) were then mixed with one ninth volume of water containing the cobalamin-CMD in solution. Microemulsion formation occurred following gentle stirring of the oil and water phases. The ME was allowed to form over 30 minutes. One fifteenth weight of insulin (2 gm) as a 100 mg/ml solution in 0.1 M HCl was added to the cobalamin-CMD/ME mix and allowed to mix for 30 minutes. An equal weight of 1 M ZnCl₂ solution to polymer (vol:wt) was then added to the above ME and allowed to associate for 30 minutes. The ME mix containing insulin-Cbl-NL was added to 20 litres of rapidly stirring ethanol was then added to precipitate the formed nanolattice. The lattices were allowed to settle and the supernatant decanted. The
pellet was then washed with ethanol (5 litres). The settled nanolattices were resuspended once more in 5 litres ethanol, allowed to settle once more. The supernatant decanted and the pellet resuspended in DW and lyophilized. The resultant material was stored for 5 months before testing for stability.

C.1 Production of Lysyl-folate targeted nanolattices containing an anti-TNF molecule.

Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. The anti-TNF molecule, ALSO0T2 (as described in B.6) was dissolved at 1-100 mg/ml in distilled water. The anti-TNF molecules were added to ME composition to a final of 10% anti-TNF (w/w dextran) solution in the ME. The protein was then added to a final concentration of 10% protein:CM-dextran. The solution was stirred for 30 minutes, afterwhich ZnCl₂ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30 minutes. Lysyl-Foate targeting agent (10% w/w Lysyl-folate:CM dextran), also dissolved in ME was then added to the Zn:protein:CM-dextran ME. The solution was stirred for a further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL were left to settle, afterwhich the supernatant was decanted. The NL were then washed two times with ethanol, resuspended in distilled water, and lyophilized.

C.2 Production of Hydrazidyl-biotin targeted nanolattices containing an anti-TNF molecule.

Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. The anti-TNF
molecule, ALS00T2 (as described in B.6) was dissolved at 1-100 mg/ml in distilled water. The anti-TNF molecules were added to ME composition to a final of 10% anti-TNF (w/w dextran) solution in the ME. The protein was then added to a final concentration of 10% protein:CM-dextrans. The solution was stirred for 30 minutes, after which ZnCl₂ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30 minutes. Hydrazidyl-biotin as a targeting agent (10% w/w Hydrazidyl biotin:CM dextran), also dissolved in ME was then added to the Zn:protein:CM-dextran ME. The solution was stirred for a further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL were left to settle, after which the supernatant was decanted. The NL were then washed two times with ethanol, resuspended in distilled water, and lyophilized.

C.3 Production of Long-chain hydrazidyl-biotin targeted nanolattices containing an anti-TNF molecule.

Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. The anti-TNF molecule, ALS00T2 (as described in B.6) was dissolved at 1-100 mg/ml in distilled water. The anti-TNF molecules were added to ME composition to a final of 10% anti-TNF (w/w dextran) solution in the ME. The protein was then added to a final concentration of 10% protein:CM-dextran. The solution was stirred for 30 minutes, after which ZnCl₂ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30 minutes. Long-chain hydrazidyl biotin targeting agent (10% w/w LC-biotin:CM dextran), also dissolved in ME was then added to the Zn:protein:CM-dextran ME. The solution was stirred for a further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL
were left to settle, after which the supernatant was decanted. The NL were then washed two times with ethanol, resuspended in distilled water, and lyophilized.

**C.4 Production of Biocytin targeted nanolattices containing an anti-TNF molecule.**

Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. The anti-TNF molecule, ALS00T2 (as described in B.6) was dissolved at 1-100 mg/ml in distilled water. The anti-TNF molecules were added to ME composition to a final of 10% anti-TNF (w/w dextran) solution in the ME. The protein was then added to a final concentration of 10% protein:CM-dextran. The solution was stirred for 30 minutes, after which ZnCl$_2$ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30 minutes. Lysyl-Biotin (Biocytin) targeting agent (10% w/w Biocytin:CM dextran), also dissolved in ME was then added to the Zn:protein:CM-dextran ME. The solution was stirred for a further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL were left to settle, after which the supernatant was decanted. The NL were then washed two times with ethanol, resuspended in distilled water, and lyophilized.

**C.5 Production of Lysyl-riboflavin targeted nanolattices containing an anti-TNF molecule.**

Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. The anti-TNF molecule, ALS00T2 (as described in B.6) was dissolved at 1-100 mg/ml in distilled water. The anti-TNF molecules were added to ME composition to a final of 10% anti-
TNF (w/w dextran) solution in the ME. The protein was then added to a final concentration of 10% protein:CM-dextran. The solution was stirred for 30 minutes, after which ZnCl₂ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30 minutes. Lysyl-riboflavin targeting agent (10% w/w Lysyl-riboflavin:CM dextran), also dissolved in ME was then added to the Zn:protein:CM-dextran ME. The solution was stirred for a further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL were then washed two times with ethanol, resuspended in distilled water, and lyophilized.

C.6 Production of Diaminopropyl-vitamin B₁₂ targeted nanolattices containing an anti-TNF molecule.

Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. The anti-TNF molecule, ALS00T2 (as described in B.6) was dissolved at 1-100 mg/ml in distilled water. The anti-TNF molecules were added to ME composition to a final of 10% anti-TNF (w/w dextran) solution in the ME. The protein was then added to a final concentration of 10% protein:CM-dextran. The solution was stirred for 30 minutes, after which ZnCl₂ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30 minutes. Diaminopropyl-vitamin B₁₂ (DAP-VB₁₂) targeting agent (10% w/w DAP-VB₁₂:CM dextran), also dissolved in ME was then added to the Zn:protein:CM-dextran ME. The solution was stirred for a further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL were left to settle, after which the supernatant was decanted. The NL were then washed two times with ethanol, resuspended in distilled water, and lyophilized.
C.7 Production of Diaminopropyl-riboflavin targeted nanolattices containing an anti-TNF molecule.

Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. The anti-TNF molecule, ALS00T2 (as described in B.6) was dissolved at 1-100 mg/ml in distilled water. The anti-TNF molecules were added to ME composition to a final of 10% anti-TNF (w/w dextran) solution in the ME. The protein was then added to a final concentration of 10% protein:CM-dextran. The solution was stirred for 30 minutes, after which ZnCl₂ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30 minutes. Diaminopropyl-riboflavin (DAP-Rf) targeting agent (10% w/w DAP-Rf:CM dextran), also dissolved in ME was then added to the Zn:protein:CM-dextran ME. The solution was stirred for a further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL were left to settle, after which the supernatant was decanted. The NL were then washed two times with ethanol, resuspended in distilled water, and lyophilized.

C.8 Production of Aspartate-vitamin B12 (Asp-VB12) targeted nanolattices containing an anti-TNF molecule.

Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. The anti-TNF molecule, ALS00T2 (as described in B.6) was dissolved at 1-100 mg/ml in distilled water. The anti-TNF molecules were added to ME composition to a final of 10% anti-TNF (w/w dextran) solution in the ME. The protein was then added to a final concentration of 10% protein:CM-dextran. The solution was stirred for 30 minutes,
aflerwhich ZnCl$_2$ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-
dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30
minutes. Asp-VB12 targeting agent (10% w/w Asp-VB12:CM dextran), also dissolved in
ME was then added to the Zn:protein:CM-dextran ME. The solution was stirred for a
further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume)
of ethanol. The NL precipitated immediately in the ethanol. The NL were left to settle,
afterwhich the supernatant was decanted. The NL were then washed two times with
ethanol, resuspended in distilled water, and lyophilized.

C.9 Production of Glutathionyl-vitamin B12 targeted nanolattices containing an
anti-TNF molecule.

Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the
CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion
according to the invention. The solution was stirred gently for 30 minutes. The anti-TNF
molecule, ALS00T2 (as described in B.6) was dissolved at 1-100 mg/ml in distilled
water. The anti-TNF molecules were added to ME composition to a final of 10% anti-
TNF (w/w dextran) solution in the ME. The protein was then added to a final
concentration of 10% protein:CM-dextran. The solution was stirred for 30 minutes,
afterwhich ZnCl$_2$ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-
dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30
minutes. Glutathionyl-VB12 targeting agent (10% w/w Glutathionyl-VB12:CM dextran),
also dissolved in ME was then added to the Zn:protein:CM-dextran ME. The solution
was stirred for a further 30 minutes and was then added to a rapidly stirring two-fold
excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL
were left to settle, afterwhich the supernatant was decanted. The NL were then washed
two times with ethanol, resuspended in distilled water, and lyophilized.
ClO Production of Cysteinyl-vitamin B12 targeted nanolattices containing an anti-TNF molecule.

Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. The anti-TNF molecule, ALS00T2 (as described in B.6) was dissolved at 1-100 mg/ml in distilled water. The anti-TNF molecules were added to ME composition to a final of 10% anti-TNF (w/w dextran) solution in the ME. The protein was then added to a final concentration of 10% protein:CM-dextran. The solution was stirred for 30 minutes, after which ZnCl₂ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30 minutes. Cysteinyl-VB12 targeting agent (10% w/w Cysteinyl-VB12:CM dextran), also dissolved in ME was then added to the Zn:protein:CM-dextran ME. The solution was stirred for a further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL were left to settle, after which the supernatant was decanted. The NL were then washed two times with ethanol, resuspended in distilled water, and lyophilized.

ClI Production of Asp-biotin targeted nanolattices containing an anti-TNF molecule.

Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. The anti-TNF molecule, ALS00T2 (as described in B.6) was dissolved at 1-100 mg/ml in distilled water. The anti-TNF molecules were added to ME composition to a final of 10% anti-TNF (w/w dextran) solution in the ME. The protein was then added to a final concentration of 10% protein:CM-dextran. The solution was stirred for 30 minutes, after which ZnCl₂ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30
minutes. Asp-biotin targeting agent (10% w/w Asp-biotin:CM dextran), also dissolved in ME was then added to the Zn:protein:CM-dextran ME. The solution was stirred for a further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL were left to settle, after which the supernatant was decanted. The NL were then washed two times with ethanol, resuspended in distilled water, and lyophilized.

C.12 Production of targeted nanolattices containing the anti-TNF molecule, Enbrel.
Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. The anti-TNF molecule, Enbrel™ was dissolved at 1-100 mg/ml in distilled water. The anti-TNF molecules were added to ME composition to a final of 10% anti-TNF (w/w dextran) solution in the ME. The protein was then added to a final concentration of 10% protein:CM-dextran. The solution was stirred for 30 minutes, after which ZnCl₂ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30 minutes. Lysyl-Vitamin B12 targeting agent (10% w/w Lysyl-Vitamin B12:CM dextran), also dissolved in ME was then added to the Zn:protein:CM-dextran ME. The solution was stirred for a further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL were left to settle, after which the supernatant was decanted. The NL were then washed two times with ethanol, resuspended in distilled water, and lyophilized.

C.13 Production of targeted nanolattices containing the anti-TNF molecule, Humira.
Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. The anti-TNF molecule, Humira™ was dissolved at 1-100 mg/ml in distilled water. The anti-TNF molecules were added to ME composition to a final of 10% anti-TNF (w/w dextran) solution in the ME. The protein was then added to a final concentration of 10% protein:CM-dextran. The solution was stirred for 30 minutes, after which ZnCl₂ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30 minutes. Lysyl-Vitamin B12 targeting agent (10% w/w Lysyl-Vitamin B12:CM dextran), also dissolved in ME was then added to the Zn:protein:CM-dextran ME. The solution was stirred for a further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL were left to settle, after which the supernatant was decanted. The NL were then washed two times with ethanol, resuspended in distilled water, and lyophilized.

C.14 Production of targeted nanolattices containing the anti-TNF molecule, Remicade.

Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. The anti-TNF molecule, Remicade™ was dissolved at 1-100 mg/ml in distilled water. The anti-TNF molecules were added to ME composition to a final of 10% anti-TNF (w/w dextran) solution in the ME. The protein was then added to a final concentration of 10% protein:CM-dextran. The solution was stirred for 30 minutes, after which ZnCl₂ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30 minutes. Lysyl-Vitamin B12 targeting agent (10% w/w Lysyl-Vitamin B12:CM dextran), also dissolved in ME was
then added to the Zn:protein:CM-dextran ME. The solution was stirred for a further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL were left to settle, after which the supernatant was decanted. The NL were then washed two times with ethanol, resuspended in distilled water, and lyophilized.

C.15 Production of Lysyl-Cbl-targeted nanolattices containing the anti-TNF molecule, ALST002.
Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. The anti-TNF molecule, ALST002 was dissolved at 1-100 mg/ml in distilled water. The anti-TNF molecules were added to ME composition to a final of 10% anti-TNF (w/w dextran) solution in the ME. The protein was then added to a final concentration of 10% protein:CM-dextran. The solution was stirred for 30 minutes, after which ZnCl₂ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30 minutes. Lysyl-Vitamin B₁₂ targeting agent (10% w/w Lysyl-Vitamin B₁₂:CM dextran), also dissolved in ME was then added to the Zn:protein:CM-dextran ME. The solution was stirred for a further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL were left to settle, after which the supernatant was decanted. The NL were then washed two times with ethanol, resuspended in distilled water, and lyophilized.

C.16 Production of Lysyl-folate targeted nanolattices containing insulin.
Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. Insulin was
dissolved at 1-100 mg/ml in 0.1 N HCl. The insulin was added to ME composition to a final of 10% insulin (w/w dextran) solution in the ME. The protein was then added to a final concentration of 10% protein:CM-dextran. The solution was stirred for 30 minutes, after which ZnCl₂ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30 minutes. Lysyl-Vitamin B12 targeting agent (10% w/w Lysyl-Vitamin B12:CM dextran), also dissolved in ME was then added to the Zn:protein:CM-dextran ME. The solution was stirred for a further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL were left to settle, after which the supernatant was decanted. The NL were then washed two times with ethanol, resuspended in distilled water, and lyophilized.

C.17 Production of Hydrazidyl-biotin targeted nanolattices containing Insulin.
Carboxy-nethyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. Insulin was dissolved at 1-100 mg/ml in 0.1 N HCl. The insulin was added to ME composition to a final of 10% insulin (w/w dextran) solution in the ME. The protein was then added to a final concentration of 10% protein:CM-dextran. The solution was stirred for 30 minutes, after which ZnCl₂ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30 minutes. Hydrazidyl-biotin as a targeting agent (10% w/w Hydrazidyl-biotin:CM dextran), also dissolved in ME was then added to the Zn:protein:CM-dextran ME. The solution was stirred for a further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL were left to settle, after which the supernatant was decanted. The NL were then washed two times with ethanol, resuspended in distilled water, and lyophilized.
C.18 Production of Long-chain hydrazidyl-biotin targeted nanolattices containing Insulin.

Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. Insulin was dissolved at 1-100 mg/ml in 0.1 N HCl. The insulin was added to ME composition to a final of 10% insulin (w/w dextran) solution in the ME. The protein was then added to a final concentration of 10% protein:CM-dextran. The solution was stirred for 30 minutes, after which ZnCl$_2$ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30 minutes. Long-chain-hydrazidyl biotin (10% w/w:CM dextran), also dissolved in ME was then added to the Zn:protein:CM-dextran ME. The solution was stirred for a further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL were left to settle, after which the supernatant was decanted. The NL were then washed two times with ethanol, resuspended in distilled water, and lyophilized.

C.19 Production of Biocytin targeted nanolattices containing insulin

Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. Insulin was dissolved at 1-100 mg/ml in 0.1 N HCl. The insulin was added to ME composition to a final of 10% insulin (w/w dextran) solution in the ME. The protein was then added to a final concentration of 10% protein:CM-dextran. The solution was stirred for 30 minutes, after which ZnCl$_2$ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30 minutes. Biocytin as a targeting agent (10% w/w Biocytin:CM dextran), also dissolved in ME was then added to the Zn:protein:CM-dextran ME. The solution was stirred for a
further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL were left to settle, after which the supernatant was decanted. The NL were then washed two times with ethanol, resuspended in distilled water, and lyophilized.

C.20 Production of Lysyl-riboflavin targeted nanolattices containing Insulin.
Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. Insulin was dissolved at 1-100 mg/ml in 0.1 N HCl. The insulin was added to ME composition to a final of 10% insulin (w/w dextran) solution in the ME. The protein was then added to a final concentration of 10% protein:CM-dextran. The solution was stirred for 30 minutes, after which ZnCl$_2$ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30 minutes. Lysyl-Riboflavin B12 targeting agent (10% w/w Lysyl-Riboflavin:CM dextran), also dissolved in ME was then added to the Zn:protein:CM-dextran ME. The solution was stirred for a further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL were left to settle, after which the supernatant was decanted. The NL were then washed two times with ethanol, resuspended in distilled water, and lyophilized.

C.21 Production of Diaminopropyl-vitamin B12 targeted nanolattices containing Insulin.
Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. Insulin was dissolved at 1-100 mg/ml in 0.1 N HCl. The insulin was added to ME composition to a final of 10% insulin (w/w dextran) solution in the ME. The protein was then added to a
final concentration of 10% protein: CM-dextran. The solution was stirred for 30 minutes, after which ZnCl₂ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30 minutes. Diamino-propyl-Vitamin B₁₂ targeting agent (10% w/w Diaminopropyl-Vitamin B₁₂:CM-dextran), also dissolved in ME was then added to the Zn:protein:CM-dextran ME. The solution was stirred for a further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL were left to settle, after which the supernatant was decanted. The NL were then washed two times with ethanol, resuspended in distilled water, and lyophilized.

C.22 Production of Diaminopropyl-riboflavin targeted nanolattices containing Insulin.

Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. Insulin was dissolved at 1-100 mg/ml in 0.1 N HCl. The insulin was added to ME composition to a final of 10% insulin (w/w dextran) solution in the ME. The protein was then added to a final concentration of 10% protein:CM-dextran. The solution was stirred for 30 minutes, after which ZnCl₂ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-dextran) was added to the proteur.CM-dextran ME. The solution was stirred for 30 minutes. Diaminopropyl-riboflavin as a targeting agent (10% w/w DAP-Rf: CM dextran), also dissolved in ME was then added to the Zn:protein:CM-dextran ME. The solution was stirred for a further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL were left to settle, after which the supernatant was decanted. The NL were then washed two times with ethanol, resuspended in distilled water, and lyophilized.
C.23 Production of Aspartate-vitamin B12 targeted nanolattices containing Insulin.

Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. Insulin was dissolved at 1-100 mg/ml in 0.1 N HCl. The insulin was added to ME composition to a final of 10% insulin (w/w dextran) solution in the ME. The protein was then added to a final concentration of 10% protein:CM-dextran. The solution was stirred for 30 minutes, after which ZnCl₂ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30 minutes. Aspartate-Vitamin B12 targeting agent (10% w/w Asp-Vitamin B12:CM dextran), also dissolved in ME was then added to the Zn:protein:CM-dextran ME. The solution was stirred for a further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL were then washed two times with ethanol, resuspended in distilled water, and lyophilized.

C.24 Production of Glutathionyl-vitamin B12 targeted nanolattices containing Insulin.

Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. Insulin was dissolved at 1-100 mg/ml in 0.1 N HCl. The insulin was added to ME composition to a final of 10% insulin (w/w dextran) solution in the ME. The protein was then added to a final concentration of 10% protein:CM-dextran. The solution was stirred for 30 minutes, after which ZnCl₂ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30 minutes. Glutathionyl-Vitamin B12 targeting agent (10% w/w Glutathionyl-Vitamin
B12:CM dextran), also dissolved in ME was then added to the Zn:protein:CM-dextran ME. The solution was stirred for a further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL were left to settle, afterwhich the supernatant was decanted. The NL were then washed two times with ethanol, resuspended in distilled water, and lyophilized.

C.25 Production of Cysteiny1-vitamin B12 targeted nanolattices containing Insulin.

Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. Insulin was dissolved at 1-100 mg/ml in 0.1 N HCl. The insulin was added to ME composition to a final of 10% insulin (w/w dextran) solution in the ME. The protein was then added to a final concentration of 10% protein:CM-dextran. The solution was stirred for 30 minutes, afterwhich ZnCl₂ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30 minutes. Cysteiny1-Vitamin B12 as a targeting agent (10% w/w Cysteiny1-Vitamin B12:CM dextran), also dissolved in ME was then added to the Zn:protein:CM-dextran ME. The solution was stirred for a further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL were left to settle, afterwhich the supernatant was decanted. The NL were then washed two times with ethanol, resuspended in distilled water, and lyophilized.

C.26 Production of Asp-biotin targeted nanolattices containing Insulin.

Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. Insulin was dissolved at 1-100 mg/ml in 0.1 N HCl. The insulin was added to ME composition to a final of 10% insulin (w/w dextran) solution in the ME. The protein was then added to a
final concentration of 10% protein: CM-dextran. The solution was stirred for 30 minutes, after which ZnCl₂ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30 minutes. Asp-biotin as a targeting agent (10% w/w Lysyl-Vitamin B12:CM dextran), also dissolved in ME was then added to the Zn:protein:CM-dextran ME. The solution was stirred for a further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL were left to settle, after which the supernatant was decanted. The NL were then washed two times with ethanol, resuspended in distilled water, and lyophilized.

C.27 Production of N-e-Lys-Cbl targeted nanolattices containing Insulin
Carboxy-methyl dextran (1 gm) was dissolved at 100 mg/ml in distilled water. Once the CM-dextran was dissolved it was added to a 9-fold volume excess of microemulsion according to the invention. The solution was stirred gently for 30 minutes. Insulin was dissolved at 1-100 mg/ml in 0.1 N HCl. The insulin was added to ME composition to a final of 10% insulin (w/w dextran) solution in the ME. The protein was then added to a final concentration of 10% protein:CM-dextran. The solution was stirred for 30 minutes, after which ZnCl₂ as 0.1 N solution in 10% water in ME (10 ml for each gram of CM-dextran) was added to the protein:CM-dextran ME. The solution was stirred for 30 minutes. N-e-Lysyl-Cbl as a targeting agent (10% w/w Lysyl-Vitamin B12:CM dextran), also dissolved in ME was then added to the Zn:protein:CM-dextran ME. The solution was stirred for a further 30 minutes and was then added to a rapidly stirring two-fold excess (by volume) of ethanol. The NL precipitated immediately in the ethanol. The NL were left to settle, after which the supernatant was decanted. The NL were then washed two times with ethanol, resuspended in distilled water, and lyophilized.

D.1 Induction of inflammation in the foot of BAlb/C mice.
Mice were injected with 50 ul of 1% (10 mg/ml) Carrageenan into the footpad. The level of swelling in the foot was monitored, initially for 2, 4, and 6 hours after injection and subsequently once or twice per day. Inflammation measurement was performed using a paper caliper.

D.2 Reduction in inflammation of mice with carrageenan induced inflammation in the foot of BAIb/C mice.

Mice (Balb/C) were injected with 50 ul of 1% (10 mg/ml) Carrageenan into the right foot pad. Immediately after injection the mice were also treated with the following:

i) Nil (n=5)

ii) TNF-Receptor 2 VB12-nanolattices (0.2 mg in 2 rhg nanolattices) SC (n=5)

iii) TNF-Receptor 2 VB12-nanolattices (1.0 mg in 10 mg nanolattices) PO (n=5)

Nanolattices were prepared as described in B8. Additionally, mice received daily dosing for 7 days, with footpads measured at T0 and T6 hrs. Treatment was ceased on Day 8 of the experiment. Examination of the footpads showed that there was good reduction in inflammation in all the treated mice, regardless of whether the ALSTII was injected subcutaneously (SC) or administered orally (PO). The data in Figure 1 shows that the oral nanolattice technology is active in this model, to a similar level regardless of oral or subcutaneous administration.

D.3 Comparison in reduction in inflammation of mice following oral administration of VB12-targeted nanolattices containing a TNF-RII-Fc fusion protein

VB 12-targeted nanolattices were prepared (according to the method of B8) containing the TNF-RII-Fc fusion protein, ALS00T2, as described above. Mice (Balb/C) were injected with 50 ul of 1% (10 mg/ml) Carrageenan into the right foot pad. Immediately after injection the mice were also treated with the following:

i) Nil (n=5)
ii) TNF-Receptor 2 VB 12-nanolattices (0.2 mg in 2 mg nanolattices) SC (n=5)

iii) TNF-Receptor 2 VB 12-nanolattices (1.0 mg in 10 mg nanolattices) PO (n=5)

Additionally, mice received daily dosing for 7 days, with footpads measured at T0 and T6 hrs. Treatment was ceased on Day 8 of the experiment. Examination of the footpads showed that there was good reduction in inflammation in all the treated mice, regardless of whether the ALSTII was injected SC or administered orally. The data in Figure 2 shows that the oral nanolattice technology is active in this model, to a similar level regardless of oral or subcutaneous administration.

D.4 Modification of release from nanolattices by modification of the concentration of ZnCl₂ used in the formation of the lattices.

Recombinant human insulin (Wockhardt) was radiolabeled with ¹²⁵I via the chloramine T method of Greenwood et al. Cobalamin-modified carboxy-dextran (Example B.3), was dissolved at 100 mg/ml in DW. The dextran was then added to a 9-fold excess of microemulsion (see standard method). Once the ME had formed insulin (dissolved at 100 mg/ml in 0.1N HCl, with pH adjusted to 3.5) was added to the Dextran ME at 10% w/w insulin:dextran. The solution was stirred gently for 30 minutes and then separated into 6 equal portions. ZnCl₂ (IM solution) was added to ME at 10% by volume and the following volumes were added to the insulin/dextran ME:- 0, 1.25, 2.5, 5.0, 7.5 and 10 ml. The solution was stirred gently for 30 minutes followed by isolation of the nanolattices via standard method. The NL were freeze-dried, resuspended in DW and centrifuged at 10k for 10 minutes. Particles were analysed by DLS. Representative sizes (DLS) and polydispersity of the particles prior to 0.45 um filtration, and after centrifugation are given below.
The starting material was also centrifuged for 10 min at 10K. Representative sizes are cited below, along with recovery yields.

<table>
<thead>
<tr>
<th>Particle ZnCl₂</th>
<th>Z Av</th>
<th>PDI</th>
<th>Filtered Z Av</th>
<th>Filtered PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>542</td>
<td>0.465</td>
<td>288</td>
<td>0.18</td>
</tr>
<tr>
<td>1.25</td>
<td>625</td>
<td>0.385</td>
<td>273</td>
<td>0.198</td>
</tr>
<tr>
<td>2.5</td>
<td>748</td>
<td>0.333</td>
<td>342</td>
<td>0.244</td>
</tr>
<tr>
<td>5.0</td>
<td>928</td>
<td>0.482</td>
<td>234</td>
<td>0.295</td>
</tr>
<tr>
<td>7.5</td>
<td>884</td>
<td>0.575</td>
<td>192</td>
<td>0.275</td>
</tr>
<tr>
<td>10</td>
<td>1034</td>
<td>0.725</td>
<td>163</td>
<td>0.249</td>
</tr>
</tbody>
</table>

D.5 Reduction in Serum Glucose following sub-cutaneously administered targeted Insulin-containing nanolattices

Female Wistar rats were made diabetic via the sub-cutaneous injection of 50 mg/kg streptozotocin. Blood glucose levels were monitored with time. When serum levels rose to greater than two time normal, the rats were regarded as diabetic. These animals were then used in subsequent oral studies. For SC studies rats received a dose of 400 µg/kg human insulin alone or entrapped within nanolattices (prepared according to the method of B11) (loaded at 10% by weight). Blood glucose was determined at TO, T1, T2, T3, T4, T5, T6 and 7 hours using Acucheck glucose monitor. Data in Figure 3 is presented as a comparison to glucose levels at the initiation of the experiment (TO).
Data presented demonstrate that insulin administered in nanolattices results in lower serum glucose levels for more than 6 hours after sub-cutaneous administration of insulin-loaded nanolattices than if the insulin is injected alone.

Table 1a: Average percentage of blood glucose from time of sub-cutaneous administration nanolattice insulin (NL) in diabetic rats

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T0</td>
</tr>
<tr>
<td>Control - No treatment</td>
<td>100</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin (sc)</td>
<td>100</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices(sc)</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 1b: Mean blood glucose (mmol/L) measured over time

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T0</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin (sc)</td>
<td>20.975</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices(sc)</td>
<td>19.525</td>
</tr>
</tbody>
</table>

D. 6 Alteration of insulin release from Nanolattices by chelation-linking: Blood glucose reduction of subcutaneously administered insulin-nanolattices in diabetic rats - Pharmacokinetic studies of SC insulin in Diabetic Rats

Pharmacokinetic studies have been performed in which radiolabeled ^{125}\text{I}-insulin has been incorporated within nanolattices chelated with increasing levels of zinc according to the method of D4. The resultant preparations were freeze-dried until used. Female in-bred
Wistar rats were injected with 50 mg/kg streptozotocin injected SC in order to induce diabetes. One week following streptozotocin treatment, diabetes was confirmed by blood glucose monitoring with glucose levels reaching greater than twice the normal level increasing from normal levels of 4-6 mmol/L to 15-30 mmol/L. Once the diabetic status of the animals was established nanolattices were injected sub-cutaneously into normal and diabetic rats (n=3 per group). Separate groups of animals received $^{125}$I-labelled insulin alone, and radiolabeled insulin in combination with non-cross-linked dextran. At 15, 30, 60, 120, 240 and 360 minutes post injection blood was obtained from the tail vein of rats and samples separated for calculation of insulin concentration (by radioactivity) and biological activity by measurement, using an AccuChek glucose meter. Data is represented as cpm (1,000 cpm = 1 ug insulin per ml), blood glucose, or the ratio of (serum insulin):(blood glucose reduction) in Figures 4, 5 and 6 respectively.

Comparison of serum levels of $^{125}$I-Insulin with reduction in serum glucose levels demonstrated that formation of the nanolattices resulted in a delay in the appearance of insulin in the serum, which was paralleled with the reduction in serum glucose level. Additionally calculation of the ratio of serum insulin to the reduction in serum glucose levels demonstrated that the response of diabetic animals to the lattice enclosed material was more uniform with time than that seen with insulin alone or insulin co-administered with dextran. Data suggests that insulin hyporesponsiveness observed in diabetic animals can be reduced by the administration of the insulin entrapped within zinc chelated nanolattices.
Table 2a: Average percentage of blood glucose from time of sub-cutaneous administration nanolatice insulin (NL) chelated with various amounts of ZnCl₂ in diabetic rats

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin (sc)</td>
<td>0</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices no chelation (sc)</td>
<td>66.0</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices chelated with 0.25 M ZnCl₂ (sc)</td>
<td>64.8</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices chelated with 0.5 M ZnCl₂ (sc)</td>
<td>65.7</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices chelated with 1.0 M ZnCl₂ (sc)</td>
<td>67.5</td>
</tr>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>240</td>
</tr>
<tr>
<td></td>
<td>360</td>
</tr>
<tr>
<td>100</td>
<td>66.0</td>
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<td>100</td>
<td>36.8</td>
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<td>100</td>
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<td>100</td>
<td>70.8</td>
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<td>64.8</td>
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<td>100</td>
<td>37.6</td>
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<td>100</td>
<td>70.7</td>
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<td>100</td>
<td>75.0</td>
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<td>100</td>
<td>54.5</td>
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<td>70.1</td>
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<td>100</td>
<td>79.0</td>
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<td>67.5</td>
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<tr>
<td>100</td>
<td>48.7</td>
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<tr>
<td>100</td>
<td>39.2</td>
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<tr>
<td>100</td>
<td>53.2</td>
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<tr>
<td>100</td>
<td>78.1</td>
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<tr>
<td>100</td>
<td>86.1</td>
</tr>
<tr>
<td>100</td>
<td>72.9</td>
</tr>
<tr>
<td>100</td>
<td>57.1</td>
</tr>
<tr>
<td>100</td>
<td>48.6</td>
</tr>
<tr>
<td>100</td>
<td>50.8</td>
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<tr>
<td>100</td>
<td>84.0</td>
</tr>
<tr>
<td>100</td>
<td>84.2</td>
</tr>
</tbody>
</table>
Table 2b: Mean blood glucose (ramol/L) measured over time for sub-cutaneous injection of nanolattice insulin (NL) chelated with various amounts of ZnCl₂ in diabetic rats

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin (sc)</td>
<td>26</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices no chelation (sc)</td>
<td>27</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices chelated with 0.25 M ZnCl₂ (sc)</td>
<td>29</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices chelated with 0.5 M ZnCl₂ (sc)</td>
<td>28</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices chelated with 1.0 M ZnCl₂ (sc)</td>
<td>26</td>
</tr>
</tbody>
</table>

Table 2c: Calculation of Cmax and Tmax for sub-cutaneous injection of nanolattice insulin (NL) chelated with various amounts of ZnCl₂ in diabetic rats

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Tmax (mins)</th>
<th>Cmax ug/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin (sc)</td>
<td>120</td>
<td>1.0</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices no chelation (sc)</td>
<td>60</td>
<td>1.0</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices chelated with 0.25 M ZnCl₂ (sc)</td>
<td>240</td>
<td>0.8</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices chelated with 0.5 M ZnCl₂ (sc)</td>
<td>240</td>
<td>0.8</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices chelated with 1.0 M ZnCl₂ (sc)</td>
<td>360</td>
<td>0.8</td>
</tr>
</tbody>
</table>
D.7 Release of insulin from Nanolattices can be altered by chelation: Blood glucose reduction of subcutaneously administered insulin-nanolattices in normal rats - Pharmacokinetic studies of SC insulin in Normal Rats

Pharmacokinetic studies have been performed in which radiolabeled $^{125}$I-insulin has been incorporated within nanolattices chelated with increasing levels of zinc according to the method of D4. The resultant preparations were freeze-dried until used. Female in-bred (Wistar) rats were injected sub-cutaneously with $^{125}$I-labelled insulin alone, and radiolabeled insulin in combination with non-cross-linked dextran and dextran NL chelated with increasing amounts of ZnCl$_2$. At 15, 30, 60, 120, 240 and 360 minutes post injection blood was obtained from the tail vein of rats and samples separated for calculation of insulin concentration (by radioactivity) and biological activity by measurement using an AccuChek glucose meter. Data is represented as cpm (1,000 cpm = 1 ug insulin per ml) or blood glucose in Figures 7 and 8 respectively.

Comparison of serum levels of $^{125}$I-Insulin with reduction in serum glucose levels demonstrated that formation of nanolattices resulted in a delay in the appearance of insulin in the serum, which was paralleled with the reduction in serum glucose level. Additionally calculation of the ratio of serum insulin to the reduction in serum glucose levels demonstrated that the response of diabetic animals to the lattice enclosed material was more uniform with time than that seen with insulin alone or insulin co-administered with dextran. Data suggests that there is enhanced evidence of insulin hyporesponsiveness in normal animals that can be reduced somewhat by the administration of the insulin entrapped within zinc chelated nanolattices. The comparative data with diabetic animals suggests that there is considerable evidence of homeostasis in the response of normal rats to external glucose. This homeostasis would be further enhanced in animals that received a slower addition of insulin nanolattices such as following oral administration. It is thus highly likely that given the prolonged delivery
of low doses of insulin when the Vitamin B12 uptake system is used for oral delivery, the effective delivery of insulin will be masked in normal animals/humans.

**Table 3a:** Average percentage of blood glucose from time of sub-cutaneous administration nanolatice insulin (NL) chelated with various amounts of ZnCl₂ in normal rats

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin (sc)</td>
<td>100</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices no chelation (sc)</td>
<td>100</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices chelated with 0.25 M ZnCl₂ (sc)</td>
<td>100</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices chelated with 0.5 M ZnCl₂ (sc)</td>
<td>100</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices chelated linked with 1.0 M ZnCl₂ (sc)</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 3b: Mean blood glucose (mmol/L) measured over time for sub-cutaneous injection of nanolattice insulin (NL) chelated with various amounts of ZnCl$_2$ in normal rats

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin (sc)</td>
<td>7</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices no chelation (sc)</td>
<td>6</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices chelated with 0.25 M ZnCl$_2$ (sc)</td>
<td>11</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices chelated with 0.5 M ZnCl$_2$ (sc)</td>
<td>8</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices chelated with 1.0 M ZnCl$_2$ (sc)</td>
<td>11</td>
</tr>
</tbody>
</table>

Table 3c: Calculation of Cmax and Tmax for sub-cutaneous injection of nanolattice insulin (NL) chelated with various amounts of ZnCl$_2$ in normal rats

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Tmax (mins)</th>
<th>Cmax (ug/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin (sc)</td>
<td>120</td>
<td>0.95</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices no chelation (sc)</td>
<td>120</td>
<td>0.95</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices chelated with 0.25 M ZnCl$_2$ (sc)</td>
<td>120</td>
<td>1.1</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices chelated with 0.5 M ZnCl$_2$ (sc)</td>
<td>240</td>
<td>0.6</td>
</tr>
<tr>
<td>0.1 mg (0.4 mg/kg) human insulin in nanolattices chelated with 1.0 M ZnCl$_2$ (sc)</td>
<td>120</td>
<td>0.65</td>
</tr>
</tbody>
</table>
D.8 Blood glucose reduction of orally administered nanolattice insulin in diabetic rats

Comparative formulation optimisation studies utilising different formulations of nanolattice insulin were used to evaluate the pharmacodynamic effect of orally administered insulin. Diabetic rats (four female rats per group) received a 0.5 ml dose of test lattices dissolved in water. Nanolattices were administered by oral gavage. Test lattices included various formulations which included a vitamin B12 preparation that had previously shown significant blood glucose reduction, and a further three formulations that each received the same dose of test lattices made up at pH 2, 3 or 4 of the insulin solution according to the method of B11. Controls were administered 0.5ml of water. Rats were fasted for 3 hours prior to treatment administration and allowed food and water ad lib post-oral gavage. Blood samples were taken at T=0, 2, 4, 6 and 8 hours post oral dosing.

The results showed that rats administered nanolattices formulated using insulin solution at pH 4 had optimal glucose lowering effect relative to controls (see Figure 9).

Representative data from individual rats is shown for the optimal formulation (pH 4.0) group. It can be seen that each animal in the group responded in a similar fashion, showing a reduction in serum glucose to normal levels, with a prolonged reduction for greater than 4 hours (See Figure 10).

Oral administration of targeted insulin-nanolattices resulted in a steady decrease in the blood glucose levels of diabetic rats, to levels similar to normal rats. The glucose reduction action of nanolattice insulin was consistent and sustained to at least 8 hours post feeding. In addition, the glucose reduction profile was prolonged with an absence lack of a significant spike in anti-glycaemic effect. These data demonstrated the capacity
of the vitamin B12 transport system to promote the uptake of insulin entrapped within nanolattices.

**Table 4a**: Average percentage of blood glucose in diabetic rats from oral administration of nanolattice insulin (NL) incorporated at various pH's

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>TIME</th>
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</tr>
</thead>
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<td>T0</td>
<td>T2</td>
<td>T4</td>
<td>T6</td>
<td>T8</td>
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<tr>
<td>Control</td>
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<td>91.5</td>
<td>89.4</td>
<td>84.8</td>
<td>79.4</td>
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<tr>
<td>Insulin NL - Targeted</td>
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<td>73.3</td>
<td>67.4</td>
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<td>79.7</td>
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<td>55.7</td>
<td>52.4</td>
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<td>56.9</td>
<td>42.1</td>
<td>42.6</td>
<td>39.8</td>
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**Table 4b**: Mean blood glucose (mmol/L) measured over time in diabetic rats for oral administration of nanolattice insulin (NL) incorporated at various pH's

<table>
<thead>
<tr>
<th>Treatment Groups</th>
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</tr>
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<tbody>
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<td>18.0</td>
<td>18.3</td>
<td>17.2</td>
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<td>10.8</td>
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<td>10.4</td>
<td>10.1</td>
</tr>
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<td>Insulin NL - pH 4</td>
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<td>6.5</td>
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D.9 Stability of nanolattice insulin stored at room temperature and tested by SC injection in normal rats

Vitamin B12-targeted insulin nanolattices, prepared according to Example B12, were stored at room temperature for 5 months and the efficacy of the nanolattices in glucose reduction studied in diabetic animals. Normal rats (three female rats per group) received a 0.2 ml dose of test lattices dissolved in water. Nanolattices were administered by sub-
cutaneous injection. Controls were administered 0.2 ml of water. Rats were fasted for 3 hours prior to treatment administration and allowed food and water ad lib post-oral gavage. Blood samples were taken at T=0, 15, 30, 60, 120 and 240 minutes post SC dosing.

The results showed that rats administered nanolattices formulated and stored for 5 month still contained bioactive insulin (see Figure 11).

Representative data shows that the two preparations of insulin nanolattices stored for 5 months had equal activity in reduction in serum glucose. Furthermore the lattices showed prolonged release of insulin as judged by modification of serum glucose.

Table 5a : Average percentage of blood glucose in normal rats from SC administration of stored insulin nanolatices

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<tr>
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<td>45.1</td>
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Table 5b: Mean blood glucose (mmol/L) measured over time in normal rats from SC administration of stored insulin nanolattices (NL)

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D.10 Enhanced uptake of nucleic acid molecules, including antisense oligonucleotides

Experiments are performed to ascertain the efficiency of nanolattices of the invention to facilitate the uptake of antisense oligonucleotides.

HeLa cells are incubated with the following: 1. 2µg of antisense; 2. 2µg of antisense plus Fugene (3µl); 3. Fugene only; 4. No treatment; 5. 2µg of antisense plus nanolattice; and 6. Nanolattice only; or with 7. Antisense only (10µg); 8. Antisense only (10µg) plus Fugene; 9. Fugene only; 10. No treatment; 11. Antisense (10µg) plus nanolattice; and 12. Nanolattice only.

The antisense molecules are fluorescently labelled with FIC. Cells are incubated for 24 hours at 37°C at which time the cells are harvested using trypsin, washed and resuspended in 100 µl of paraformaldehyde. Each cell sample is then analysed for the level of mean fluorescence using flow cytometry. It is experimentally shown that the nanolattices enhance uptake of nucleic acid molecules, including antisense oligonucleotides.
E.1 Comparison in reduction in inflammation of mice following oral administration of VB12-targeted nanolattices containing a TNF-RII-Fc fusion protein

Targeted nanolattices containing ALST002 were prepared that had been coated with Long-chain Biotin (according to the method of C3), N-Asp-VB12 (method of C8), N-e-Lys-Riboflavin (method of C5), or N-e-Lys-Folate (method of Cl) containing the anti-TNF-RII-Fc fusion protein, ALS00T2, as described above. Mice (C57/BL) were injected with 50 ul of 1% (10 mg/ml) Carrageenan into the right footpad. Immediately after injection the mice (n=5 per group) were also treated with 0.2 mg of ALS00T2 either alone or in NL, injected SC, or were fed 1.0 mg ALS00T2 in NL. Additionally, mice received daily dosing for 7 days, with footpads measured at T0 and T6 hrs. Treatment was ceased on Day 8 of the experiment. Examination of the footpads showed that there was good reduction in inflammation in all the treated mice, regardless of whether the ALSTII was injected SC or administered orally. The data in Figure 12 demonstrates that NL targeted with 'LC-Biotin have similar activity to ALSTII injected SC. Similar effectiveness was seen with PO administration of NL targeted with Lysyl-riboflavin (see Fig. 13), Asp-Cbl (see Fig 14), Lysyl-folate (see Fig 15) and DAP-Cbl (see Fig 16).

E.2 Blood glucose reduction of orally administered nanolattice insulin in diabetic rats

Targeted nanolattices containing Insulin were prepared that had been coated with N-e-Lysyl-Cbl (according to the method of C27), or N-e-Lysyl-Biotin N-Asp-VB12 (method of C19). Diabetic rats (four female rats per group) received a 0.5 ml dose of test lattices dissolved in water. Nanolattices were administered by oral gavage. Controls were administered 0.5ml of water. Prior to feeding rats were fasted for 3 hours and allowed food and water ad lib post-oral gavage. Blood samples were taken at T=0, 2, 4, 6 and 8 hours post oral dosing. The results shown in Fig. 17 demonstrate that oral administration of insulin-loaded nanolattices targeted with either lysyl-Cbl or lysyl-biotin to diabetic
rats, could successfully lower the levels of serum glucose. The blood glucose levels were still reducing at 6 hours after administration.

**F.I  In Vivo Studies Using a Pharmaceutical Composition Comprising Insulin-Loaded Nanolattices of the Present Invention**

The individual subjects of the *in vivo* studies described herein are warm-blooded vertebrate animals, which includes humans. In particular, volunteers have a diagnosis of insulin-dependent type I diabetes. The clinical trial is subjected to rigorous controls to ensure that individuals are not unnecessarily put at risk and that they are fully informed about their role in the study.

Preferably to account for the psychological effects of receiving treatments, the trial is conducted in a double-blinded fashion. Volunteers are randomly assigned to placebo or a pharmaceutical composition comprising insulin-loaded nanoscaffold treatment groups. Furthermore, the relevant clinicians are blinded as to the treatment regime administered to a given subject to prevent from being biased in their post-treatment observations. Using this randomization approach, each volunteer has the same chance of being given either the pharmaceutical composition or the placebo.

Volunteers receive either the pharmaceutical composition or placebo for an appropriate period with biological parameters associated with insulin-dependent type I diabetes being measured at the beginning (baseline measurements before any treatment), end (after the final treatment), and at regular intervals during the study period. Such measurements include the levels of insulin in body fluids, tissues or organs compared to pre-treatment levels. Other measurements include, but are not limited to, indices of insulin-dependent type I diabetes such as the incidence of blood glucose daily glycemic excursions, serum titers of pharmacologic indicators of disease such as C-peptide levels in response to meal
challenges, hemoglobin AIc levels or toxicity as well as ADME (absorption, distribution, metabolism and excretion), body weight and blood pressure measurements.

Information recorded for each patient includes age (years), gender, height (cm), family history of disease state or condition (yes/no), motivation rating (some/moderate/great) and number and type of previous treatment regimens for insulin-dependent type I diabetes.

Volunteers taking part in this study are adults aged 18 to 65 years and roughly an equal number of males and females participate in the study. Volunteers with certain characteristics are equally distributed for placebo and the insulin-loaded nanoscaffold treatment. In general, at the conclusion of the study, the volunteers treated with placebo have little or no response to treatment, whereas the volunteers treated with the pharmaceutical composition comprising insulin-loaded nanoscaffold show positive trends in one or more of the following measures, including but not limited to: significant reductions in the average blood glucose daily glycemic excursions; significant increases in C-peptide levels in response to meal challenges; reduced hemoglobin AIc levels.

F.2  In Vivo Studies Using a Pharmaceutical Composition Comprising (anti-TNF molecule)-Loaded Nanolattices of the Present Invention

The individual subjects of the in vivo studies described herein are warm-blooded vertebrate animals, which includes humans, hi particular, volunteers have a diagnosis of a chronic inflammatory condition such as rheumatoid arthritis. The clinical trial is subjected to rigorous controls to ensure that individuals are not unnecessarily put at risk and that they are fully informed about their role in the study.

Preferably to account for the psychological effects of receiving treatments, the trial is conducted in a double-blinded fashion. Volunteers are randomly assigned to placebo or a
pharmaceutical composition comprising an (anti-TNF molecule)-loaded nanolattice treatment groups. Furthermore, the relevant clinicians are blinded as to the treatment regime administered to a given subject to prevent from being biased in their post-treatment observations. Using this randomization approach, each volunteer has the same chance of being given either the pharmaceutical composition or the placebo.

Volunteers receive either the pharmaceutical composition or placebo for an appropriate period with biological parameters associated with the inflammatory condition being measured at the beginning (baseline measurements before any treatment), end (after the final treatment), and at regular intervals during the study period. Such measurements include the levels of anti-TNF molecule in body fluids, tissues or organs compared to pre-treatment levels. Other measurements include, but are not limited to, indices of rheumatoid arthritis such as the extent of joint swelling and serum titers of pharmacologic indicators of disease such as TNF-alpha levels or toxicity as well as ADME (absorption, distribution, metabolism and excretion), body weight and blood pressure measurements.

Information recorded for each patient includes age (years), gender, height (cm), family history of disease state or condition (yes/no), motivation rating (some/moderate/great) and number and type of previous treatment regimens for insulin-dependent type I diabetes.

Evaluation of treatment is graded by four categories, namely, cured, obviously effective, effective and non-effective. "Cured" is where the joint or joints show no sign of swelling or associated pain/tenderness. "Obviously effective" is where the joint or joints show a substantial diminution of swelling (more than 60%) accompanied by a marked reduction of joint pain and tenderness. "Effective" is where the joint or joints show a diminution of swelling of between 20 to 60% accompanied by a mild diminution of associated joint pain and tenderness. "Non-effective" is where the swelling of the joint or joints is
diminished by less than 20% and there is no perceived improvement to associated joint pain.

Volunteers taking part in this study are adults aged 18 to 65 years and roughly an equal number of males and females participate in the study. Volunteers with certain characteristics are equally distributed for placebo and the (anti-TNF molecule)-loaded nanolattice treatment. In general, at the conclusion of the study, the volunteers treated with placebo have little or no response to treatment, whereas the volunteers treated with the pharmaceutical composition comprising insulin-loaded nanoscaffold show positive trends in one or more of the following measures, including but not limited to: significant reductions in the extent of joint swelling; significant reductions in the extent of associated joint pain/tenderness; significant reductions in TNF-alpha levels in body fluids. In particular, the (anti-TNF molecule)-loaded nanolattices of the present invention are obviously effective or effective on most patients in the (anti-TNF molecule)-loaded nanolattice treatment group.

Physical Characterisation

G.1 Mean particle size and polydispersity index (PDI)
Mean particle size and size distribution of nanolattice preparations loaded with various pharmaceutical agents are analysed using dynamic light scattering using standard methods on a Malvern Data Sizer NanoZS. Aliquots of lyophilized nanolattice preparations were re-suspended in DI water prior to measurement. Mean particle size and polydispersity index were obtained from the cumulative measurements.

G.2 Zeta potential
Zeta potential of various nanolattice preparations are determined by phase analysis light scattering using standard instruments, for example, a Zetasizer (NanoZS90, Malvern,
UK). Aliquots of lyophilized nanolattice preparations are re-suspended in DI water prior to measurement. Zeta potential is calculated from the electrophoretic mobility using standard methods, for example, the Smoluchowski approximation approach.

G.3 Scanning Electron Microscopy
Surface morphology of nanolattice preparations are visualized by scanning electron microscopy using standard methods. Freeze-dried samples of re-suspended nanolattice preparations are coated by platinum prior to scanning. Standard image analysis methods are employed to determine nanolattice shape and size.

G.4 Infrared spectroscopy
IR spectra of nanolattice preparations are characterised by infrared spectroscopy using standard methods. IR profiles of various nanolattice preparations are compared to determine presence and interactions of pendant side chains such as carboxylate groups, interactions between the pendant side chains of the nanolattice with one or more pharmaceutical agents and interactions between the pendant side chains of the nanolattice and a metal chelating agent.

G.5 UV-visible spectroscopy (UVS)
UV-vis spectra of nanolattice preparations are characterised by UV-vis spectroscopy using standard methods. UV-vis spectral profiles of various nanolattice preparations are compared to determine aggregation states of pharmaceutical agent loaded within the nanolattices.

G.6 Differential scanning calorimetry (DSC)
Polymeric structure of nanolattice preparations are characterised by DSC using standard methods. Thermal transition characteristics of various nanolattice preparations are
compared to determine the resulting glass transitions as a result of using different types and/or ratios of chelating metal ions.

Those skilled in the art will appreciate that the invention described herein the susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in the specification, individually or collectively, and any and all combinations of any two or more of said steps of features.
Claims

1. A composition comprising a plurality of nanolattices capable of carrying one or more pharmaceutical agents, wherein the nanolattices comprise at least one cross-linked or chelated polymer and the nanolattices have a polydispersity index (PDI) from about 0.1 to about 0.4.

2. A composition according to claim 1, wherein the nanolattices have an average cross-sectional length of about 20—500 nm.

3. A composition according to claim 1, wherein the polymer is a polysaccharide.

4. A composition according to claim 3, wherein the polysaccharide is dextran or derivative thereof, preferably carboxy methyl dextran.

5. A composition of claim 1, wherein the nanolattices comprise one or more pendant side-chains capable of interacting with one or more pharmaceutical agents.

6. A composition of claim 5, wherein the interaction is one or more of: ionic interactions, hydrogen bonding, hydrophobic interactions, chelation, co-ordination bonding and pi-pi interaction.

7. A composition according to claim 1, wherein the pharmaceutical agent is insulin or an anti-TNF protein.

8. A composition according to claim 1, wherein the nanolattice comprises a chelated polymer.
9. A composition according to claim 8, wherein the polymer is chelated by zinc, calcium, magnesium, manganese or selenium.

10. A composition according to claim 9, wherein the chelating agent is zinc (Zn$^{2+}$).

11. A composition according to claim 1, wherein the nanolattice comprises a cross-linked polymer.

12. A composition according to claim 11, wherein the polymer is cross-linked with a bisoxirane, epichlorohydrin, phosphorusoxychloride, divinylsulfone or p-benzoquinone.

13. A composition according to claim 1, wherein one or more targeting moieties capable of being bound by, or binding to an intestinal epithelium of the subject are associated with the polymer or the nanolattices.

14. A composition according to claim 13, wherein the targeting moiety is covalently bound to the polymer either directly or through a spacer or linker compound.

15. A composition according to claim 13, wherein the targeting moiety interacts with the nanolattice by non-covalent means with or without linkers, spacers or other derivatisation.

16. A composition according to claim 13, wherein the targeting moiety is attached to the nanolattice via a pendant side chain of the nanolattice.

17. A composition according to claim 13, wherein the targeting moiety is biotin, vitamin B12, riboflavin, folate or a derivative thereof.
18. A composition according to claim 1 which is for oral delivery.

19. A process for preparing nanolattices having a PDI of between about 0.1 and 0.4, said method comprising:
   (a) preparing separate mixtures comprising:
      (i) a surfactant and a polymer in an aqueous phase; and
      (ii) a surfactant and a non-polar phase; and
   (b) combining mixtures (i) and (ii) from step (a) to form mixture (iii);
   (c) subjecting mixture (iii) to mixing for a period sufficient to allow formation of a uniform water-in-oil emulsion;
   (d) adding a cross-linking or metal chelating agent to the emulsion to form the nanolattices; and
   (e) separating the nanolattices from the emulsion.

20. A process according to claim 19, wherein co-surfactants are also added to (i) or (ii) from step (a) to form mixture (iii).

21. A process according to claim 19, wherein the polymer is cross-linked to form the nanolattices.

22. A process according to claim 19, wherein the polymer is chelated to form the nanolattices.

23. A process of claim 19, wherein the nanolattices may further comprise surface modification by one or more reagents to provide one or more pendant side chains.

24. A process of claim 19, wherein the amount of chelating agent added in step (d) is from about 0.25 to 0.0005 mol equivalents of the polymer.
25. A process for preparing nanolattices having a PDI of between about 0.1 and 0.4 prepared by a method comprising:
   (a) preparing separate mixtures comprising:
   (i) a surfactant and a polymer in an aqueous phase; and
   (ii) a surfactant and a non-polar phase; and
   (b) combining mixtures (i) and (ii) from step (a) to form mixture (iii);
   (c) subjecting mixture (iii) to mixing for a period sufficient to allow formation of a uniform water-in-oil emulsion;
   (d) adding a pharmaceutical agent to one or more of mixtures (i), and (iii) and the emulsion of step (c);
   (e) adding a cross-linking or metal chelating agent to the emulsion formed in step (d) to form the nanolattices in the emulsion; and
   (f) separating the nanolattices from the emulsion.

26. A process according to claim 25, wherein the pharmaceutical substance is added to the water-in-oil emulsion from step (c) and is further mixed to form the emulsion of step (d).

27. A process according to claim 25, wherein the pharmaceutical substance interacts with the polymeric nanolattice by non-covalent means.

28. A process according to claim 25, wherein one or more targeting moieties capable of being bound by, or binding to an intestinal epithelium of the subject, thereby allowing uptake and internalization of the complex, are associated with the polymer or the nanolattices.
29. A process according to claim 25, wherein the targeting moiety may be covalently bound to the polymer from step (a)(i) either directly or through a spacer or linker compound.

30. A process according to claim 25, wherein the targeting moiety may interact with the nanolattice by non-covalent means with or without linkers, spacers or other derivatisation.

31. A process according to claim 25, wherein the targeting moiety is present in the emulsion of step (d) before the chelation step.

32. A process according to claim 25, wherein the targeting moiety is added to the emulsion containing the chelated nanolattices of step (e).

33. A process according to claim 25, wherein the targeting moiety is attached to the nanolattice via a pendant side chain of the nanolattice.

34. A pharmaceutical composition comprising a complex according to claim 1 in association with a pharmaceutically acceptable carrier and/or diluent.

35. A method of delivering a pharmaceutical agent to a subject in need of such pharmaceutical agent, said method comprising administering of a therapeutically effective amount of the complex of claim 1.

36. A method of claim 35, wherein the pharmaceutical agent is insulin, a chemotherapeutic agent, an anti-inflammatory agent, an imaging or diagnostic agent, a monoclonal antibody, an Fc-fusion protein or a nucleic acid.
37. A method of claim 36, wherein the pharmaceutical agent is insulin.

38. A method of claim 35, wherein the pharmaceutical agent is delivered orally.

39. A method for the treatment of diabetes comprising the step of administering a complex of claim 1 to a subject in need of such treatment.

40. A method of claim 36, wherein the pharmaceutical agent is an anti-inflammatory agent.

41. A method of claim 40, wherein the anti-inflammatory agent is an anti-TNF protein.

42. A method for the treatment of an inflammatory condition comprising the step of administering a complex of claim 1 to a subject in need of such treatment.

43. Use of a complex of claim 1 in the manufacture of a medicament.
Fig. 1
Fig. 3
Fig. 4
Fig. 5
Fig. 7
Fig. 8
Fig. 9
Fig. 11
Fig. 12
Fig. 13
Fig. 14
Fig. 15
Fig. 17
INTERNATIONAL SEARCH REPORT

A61K 9/00 (2006.01)  A61K 31/74 (2006.01)  A61K 9/107 (2006.01)  A61K 38/28 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPIDS, CA, Google Scholar, Google Patents, PubMed: Nanolattice, nanolatice, lattice, nanostructure, pharmaceutical, drug, active, medicament, medicine, insulin, anti-TNF, crosslinked polymer, chelated polymer, polysaccharide, dextran, polydispersity index, Russell-Jones

C DOCUMENTS CONSIDERED TO BE RELEVANT

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* Further documents are listed in the continuation of Box C

**X** Document member of the same patent family

**T** Later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

**Y** Document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

**R** Document member of the same patent family

Date of the actual completion of the international search 12 June 2007

Date of mailing of the international search report 18 JUL 2007

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Form PCT/ISA/210 (second sheet) (April 2007)
### INTERNATIONAL SEARCH REPORT

**INTERNATIONAL SEARCH REPORT**

**C (Continuation).** DOCUMENTS CONSIDERED TO BE RELEVANT

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This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report: The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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