

COMPOSITIONS AND METHODS FOR TARGETED DELIVERY TO RETINAL CELLS

Technical field

5

The present invention relates, in general terms, to drug delivery, and more specifically to functionalised nanoparticles for targeted drug delivery to retinal cells.

Background

10

Retinal diseases are among the leading cause of vision impairment and blindness worldwide, and they impose a significant economic and societal burden due to their chronic and progressive nature. Degeneration or dysfunction of retinal ganglion cells (RGCs) is a hallmark of many retinal diseases, from glaucoma to optic neuropathies and diabetic
15 retinopathy. Because RGCs have limited capacity for self-repair, damage to these cells usually results in irreversible vision loss.

Delivering drugs to retinal cells presents challenges as the blood-retinal barrier effectively restricts the entry of most systemically administered drugs. Even when administered locally
20 to the eye, drug permeation to the retina can be limited by the densely packed cellular and extracellular matrix arrangement, as well as ocular clearance mechanisms. Targeting RGCs is particularly challenging as these cells are situated deep within the retina. Without selective delivery systems, drugs may not reach pharmacologically effective concentrations within the retina, and may exert unintended effects on other ocular cells, potentially worsening
25 vision impairment. There is thus a need for improved delivery systems that can specifically target retinal cells and improve cellular uptake.

It would be desirable to overcome or alleviate at least one of the above-described problems.

30 Summary

Disclosed herein is a nanoparticle comprising: a) a porous core at least partially coated by an ionic liquid; and b) a targeting moiety conjugated to the porous core.

Disclosed herein is a nanoparticle for targeted delivery to a retinal cell, the nanoparticle comprising: a) a porous core at least partially coated by an ionic liquid; and b) a targeting moiety conjugated to the porous core, wherein the targeting moiety binds to the retinal cell.

5 Disclosed herein is a method of preparing a nanoparticle as defined herein, the method comprising: a) synthesising a porous nanoparticle; b) contacting the porous nanoparticle with an ionic liquid to at least partially coat the nanoparticle with the ionic liquid; and c) conjugating a targeting moiety to the nanoparticle.

10 Disclosed herein is a pharmaceutical composition comprising a nanoparticle as defined herein, and a pharmaceutically acceptable carrier.

Disclosed herein is a nanoparticle as defined herein, for use as a medicament.

15 Disclosed herein is a method of delivering a therapeutic agent to a retinal cell, the method comprising contacting the cell with a nanoparticle or pharmaceutical composition as defined herein, wherein the nanoparticle comprises at least one therapeutic agent.

Disclosed herein is a method of delivering a therapeutic agent to a subject, the method
20 comprising administering a nanoparticle or pharmaceutical composition as defined herein to the subject, wherein the nanoparticle comprises at least one therapeutic agent.

Disclosed herein is a method of treating a retinal disease in a subject, the method comprising administering a nanoparticle or pharmaceutical composition as defined herein to the subject,
25 wherein the nanoparticle comprises at least one therapeutic agent.

Disclosed herein is a nanoparticle or pharmaceutical composition as defined herein, for use in treating a retinal disease in a subject, wherein the nanoparticle comprises at least one therapeutic agent.

30

Disclosed herein is the use of a nanoparticle or pharmaceutical composition as defined herein in the manufacture of a medicament for treating a retinal disease, wherein the nanoparticle comprises at least one therapeutic agent.

Disclosed herein is a method of labelling a retinal cell or tissue, the method comprising contacting the retinal cell or tissue with a nanoparticle as defined herein, wherein the nanoparticle comprises a detectable label.

5 Brief description of the drawings

Embodiments of the present invention will now be described, by way of non-limiting example, with reference to the drawings in which:

10 **Figure 1** shows Cell viability of ARPE-19 cells treated with ZIF-8 (Z), niosome (N), hierarchically porous ZIF-8 (HPZ) and niosome coated HPZ (HPZN). ARPE-19 cells were treated with increasing concentrations of nanoparticles (6.25–200 µg/mL) for 24 hours, and cell viability was assessed using the MTS assay. Significant cytotoxicity was observed at high concentrations, particularly at 100 µg/mL and 200 µg/mL for Z, HPZ, and HPZN. Data
15 are shown as mean ± SD of three independent experiments, with asterisks indicating statistically significant differences from the untreated control (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

Figure 2 shows cell viability of ARPE-19 cells treated with PEG-bridged metal-organic framework. ARPE-19 cells were treated with increasing concentrations of nanoparticles
20 (6.25–200 µg/mL) for 24 hours, and cell viability was assessed using the MTS assay. Significant cytotoxicity was observed at high concentration (200 µg/mL) for ZP3000 and ZP3000N. Data are shown as mean ± SD of three independent experiments, with asterisks indicating statistically significant differences from the untreated control (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

25 **Figure 3** shows cell viability of ARPE-19 cells treated with amino acid ionic liquid. ARPE-19 cells were treated with varying concentrations of nanoparticles (6.25–200 µg/mL) for 24 hours, followed by evaluation of cell viability using the MTS assay. No significant cytotoxicity was observed across the entire concentration range. Data are presented as mean ± SD of three independent experiments.

30 **Figure 4** shows cell viability of ARPE-19 cells treated with metal-organic framework-amino acid-based ionic liquid (ZIL). ARPE-19 cells were treated with increasing concentrations of nanoparticles (6.25–200 µg/mL) for 24 hours, and cell viability was assessed using the MTS assay. Significant cytotoxicity was observed at high concentration for these nanoparticles. Data are shown as mean ± SD of three independent experiments, with asterisks indicating

statistically significant differences from the untreated control (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

Figure 5 shows cell viability of ARPE-19 cells treated with hierarchical porous ZIF8 (HPZ) and hierarchical porous ZIF8-amino acid-based ionic liquid. ARPE-19 cells were treated with increasing concentrations of nanoparticles (6.25–200 $\mu\text{g/mL}$) for 24 hours, and cell viability was assessed using the MTS assay. Significant cytotoxicity was observed at high concentration for these nanoparticles. Data are shown as mean $\pm$ SD of three independent experiments, with asterisks indicating statistically significant differences from the untreated control (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

Figure 6 shows cell viability of ARPE-19 cells treated with PEG-bridged periodic mesoporous organosilica (PPMO₃₀₀₀ (P3), and PPMO₆₀₀₀ (P6)), and PPMO-amino acid IL including PPMO₃₀₀₀-Histidine Ionic Liquid (P3ILH or P3H), PPMO₃₀₀₀-Cysteine Ionic Liquid (P3ILC or P3C), PPMO₃₀₀₀-Glycine Ionic Liquid (P3ILG or P3G), PPMO₃₀₀₀-Lysine Ionic Liquid (P3ILL or P3L), PPMO₆₀₀₀-Histidine Ionic Liquid (P6ILH or P6H), PPMO₆₀₀₀-Cysteine Ionic Liquid (P6ILC or P6C), PPMO₆₀₀₀-Glycine Ionic Liquid (P6ILG or P6G), and PPMO₆₀₀₀-Lysine Ionic Liquid (P6ILL or P6L). ARPE-19 cells were treated with varying concentrations of Nanoparticles (6.25–200 $\mu\text{g/mL}$) for 24 hours, followed by evaluation of cell viability using the MTS assay. No significant cytotoxicity was observed across the entire concentration range. Data are presented as mean $\pm$ SD of three independent experiments.

Figure 7 shows images of ARPE19 and RGC5 cell lines stained using β -Tubulin antibody and DAPI (nuclear stain). The expression of β -Tubulin was observed in both RGC5 and ARPE19 cell lines.

Figure 8 shows images of ARPE19 and RGC5 cell lines stained using Thy1 antibody and DAPI (nuclear stain). The expression of Thy1 was observed only in RGC5.

Figure 9 shows comprehensive characterisation of the nanocarriers PPMO₃₀₀₀-GlyIL. The nanopores exhibit a narrow diameter range of approximately 7–10 nm, and from a vertical perspective, the uniform honeycomb-like structure of the pores is clearly visible (A). The pore size can be one of the controlling factors for targeted release, and changing the pore size may alter the characteristics of the nanocarrier. The scanning electron microscopy (SEM) image also reveals the nearly spherical shape and size of the particles (B). Additionally, the nanoporous structure of these nanocarriers was confirmed through X-ray diffraction (XRD) analysis (C). The XRD pattern shows a comparable profile, including a strong diffraction peak along with two weaker peaks, corresponding to Miller indices (211),

(220), and (332), which are characteristic of these nanomaterials.

Figure 10 shows fluorescence microscopic images of cellular uptake of sulforhodamine loaded on CD90 labelled PPMO-Glycine Ionic Liquid (Sulf@CD90@PPMO₃₀₀₀-GlyIL), DAPI-stained nuclei, and merged image in RCG5 and ARPE19 cells. The experiment was performed using three antibody/nanoparticle ratios: 1, 3, and 6 $\mu\text{g}/\mu\text{L}$ of antibody to 25, 75, and 150 $\text{ng}/\mu\text{L}$ of nanoparticles, respectively, as shown in Figures A, B, and C.

Figure 11 shows fluorescence microscopic images of cellular uptake of sulforhodamine loaded on CD90 labelled PPMO-Glycine Ionic Liquid (Sulf@CD90@PPMO₃₀₀₀-GlyIL), DAPI-stained nuclei, and merged image in RCG5 and ARPE19 cells. The experiment was performed using three antibody/nanoparticle ratios: 2.5, 7.5, and 15 $\text{ng}/\mu\text{L}$ of antibody to 25, 75, and 150 $\mu\text{g}/\mu\text{L}$ of nanoparticles, respectively, as shown in Figures A, B, and C.

Figure 12 shows fluorescence microscopic images of cellular uptake of sulforhodamine loaded on CD90 labeled PPMO-Glycine Ionic Liquid (Sulf@CD90@PPMO₃₀₀₀-GlyIL), DAPI-stained nuclei, and merged image in RCG5 and ARPE19 cells. The experiment was performed using three antibody/nanoparticle ratios: 10, 30, and 60 $\text{ng}/\mu\text{L}$ of antibody to 25, 75, and 150 $\mu\text{g}/\mu\text{L}$ of nanoparticles, respectively, as shown in Figures A, B, and C.

Figure 13 shows merged fluorescence microscopic images of cellular uptake of sulforhodamine loaded on CD90 labelled PPMO-Glycine Ionic Liquid (Sulf@CD90@PPMO₃₀₀₀-GlyIL)(P3GAS: NP with antibody) and DAPI-stained nuclei in RCG5 and ARPE19 cells. The experiment was performed using three antibody/nanoparticle ratios: 2.5, 1.25, and 0.625 $\text{ng}/\mu\text{L}$ of antibody to 25, 12.5, and 6.25 $\mu\text{g}/\mu\text{L}$ of nanoparticles, respectively, as shown in Figures A, B, and C. Nanoparticle without antibody (Sulforhodamine loaded PPMO-Glycine Ionic Liquid (Sulf@ PPMO₃₀₀₀-GlyIL)(P3GS)) was used as control. Scale bar: 200 μm .

Figure 14 shows merged fluorescence microscopic images of cellular uptake of EPO@CD90@PPMO₃₀₀₀-GlyIL (P3GAE) in RCG5 and ARPE19 cells. Erythropoietin (EPO) was stained with antibody (E2531 Sigma-Aldrich) and nuclei were stained with DAPI. The experiment was performed using three antibody/nanoparticle ratios: 2.5, 1.25, and 0.625 $\text{ng}/\mu\text{L}$ of antibody to 25, 12.5, and 6.25 $\mu\text{g}/\mu\text{L}$ of nanoparticles, respectively, as shown in Figures A, B, and C. Nanoparticle without antibody (EPO@PPMO₃₀₀₀-GlyIL (P3GE)) was used as control. Scale bar: 200 μm .

Figure 15 shows fluorescence microscopy images illustrating the delivery of erythropoietin (EPO)-loaded nanoparticles. Nanoparticles functionalised with the Thy1 antibody (P3GAE) are shown alongside control nanoparticles without antibody labels (P3GE). The nuclei are

stained with DAPI, while erythropoietin was visualised using an antibody. The merged image shows the overlap of DAPI and EPO staining, highlighting the localisation of EPO in relation to the cell nuclei in GCL, demonstrating the efficiency of targeted delivery using antibody-labelled nanoparticles compared to unlabelled controls. GCL: Ganglion Cell Layer; INL: Inner Nuclear Layer; ONL: Outer Nuclear Layer. (A) Scale bar: 200 μm . (B) Scale bar: 100 μm .

Figure 16 shows fluorescence microscopy images illustrating the delivery of erythropoietin (EPO)-loaded nanoparticles P3HE and P3HAE. Nanoparticles functionalised with the Thy1 antibody (P3HAE) is shown alongside control nanoparticles without antibody labels (P3HE). The nuclei are stained with DAPI, while erythropoietin was visualised using an antibody. The merged image shows the overlap of DAPI and EPO staining, highlighting the localisation of EPO in relation to the GCL nuclei. Scale bar: 50 μm .

Figure 17 shows fluorescence microscopy images illustrating the delivery of erythropoietin (EPO) loaded onto nanoparticles P3LAE and P3LE. Nanoparticles functionalised with the Thy1 antibody (P3LAE) are shown alongside control nanoparticles without antibody labels (P3LE). The nuclei are stained with DAPI, while erythropoietin was visualised using an antibody. The merged image shows the overlap of DAPI and EPO staining. Scale bar: 200 μm .

Figure 18 shows characterisation of the nanocarriers PPMO₃₀₀₀-HisIL. The nanopores display a narrow diameter range of approximately 7–10 nm, and the uniform honeycomb-like arrangement of the pores is distinctly visible from a vertical view (A). The pore size can be one of the controlling factors for targeted release, and changing the pore size may alter the characteristics of the nanocarrier. Scanning electron microscopy (SEM) images further reveal the particles' nearly spherical shape and consistent size (B). The nanoporous structure of these nanocarriers was also confirmed by X-ray diffraction (XRD) analysis (C), which shows a similar pattern, featuring a strong diffraction peak and two weaker peaks corresponding to Miller indices (211), (220), and (332), characteristic of these nanomaterials.

Figure 19 shows (A) Fluorescent imaging of nanoparticle delivery to retinal ganglion cells (RGCs). Nanoparticles labelled with FITC were administered to RGCs with (P3FGA) or without (P3FG) targeting Thy1 antibody. Images show DAPI-stained nuclei, FITC-labelled nanoparticles, and merged channels for co-localisation. In the antibody-targeted group, FITC signals localised primarily within GCL. (A) Scale bar: 200 μm . (B) Scale bar: 100 μm . (C) Scale bar: 50 μm .

Figure 20 shows fluorescent imaging of nanoparticle delivery to retinal ganglion cells (RGCs). Nanoparticles labelled with FITC were administered to RGCs with (P3GFA) or without (P3GF) targeting antibody Thy1. Images show DAPI-stained nuclei, FITC-labelled nanoparticles (green), and merged channels for co-localisation. Scale bar 200 μm . In the antibody-targeted group, FITC signals localised primarily within GCL. (A) Scale bar: 200 μm . (B) Scale bar: 100 μm . (C) Scale bar: 50 μm .

Figure 21 shows fluorescent imaging of FITC-labelled nanoparticles delivered to retinal ganglion cells (RGCs) using Thy1 antibody. Panels show DAPI-stained nuclei, FITC-labelled nanoparticles, Thy1-stained marker (red), and a merged view for co-localisation. The merged panel demonstrated nanoparticle entry into the cytoplasm of RGCs, where FITC signals overlap with Thy1-stained areas. Scale bar: 200 μm .

Figure 22 shows fluorescent imaging of FITC-labelled nanoparticles delivered to RGCs using Tuj1 antibody as an axonal marker. Panels show DAPI-stained nuclei, FITC-labelled nanoparticles, Tuj1-stained axonal marker, and a merged view for co-localisation. The merged image reveals FITC-labelled nanoparticles aligning with Tuj1-stained axonal regions. (A) Scale bar: 200 μm . (B) Scale bar: 100 μm .

Figure 23 shows fluorescent imaging of FITC-labelled nanoparticles delivered to RGCs using RBPMS antibody as a cytoplasmic marker. Panels display DAPI-stained nuclei, FITC-labelled nanoparticles, RBPMS-stained cytoplasmic marker, and a merged view. The merged image confirms nanoparticle localisation within cytoplasmic regions of RGCs, as shown by FITC signals overlapping with RBPMS staining. (A) Scale bar: 200 μm . (B) Scale bar: 100 μm .

Figure 24 shows fluorescent imaging of nanoparticle delivery to retinal ganglion cells (RGCs). Nanoparticles labelled with FITC were administered to RGCs with (P3GFA) or without (P3GF) targeting Thy1 antibody. Images show DAPI-stained nuclei, FITC-labelled nanoparticles, and merged channels for co-localisation. Scale bar 200 μm . In the antibody-targeted group, accumulation of nanoparticles in GCL was observed, demonstrating effective delivery. However, nanoparticles without conjugated antibody exhibit minimal FITC signal within GCL, highlighting the role of antibody targeting in nanoparticle localisation and uptake. Scale bar: 200 μm .

Figure 25 shows topical delivery of erythropoietin (EPO) via antibody-functionalized nanoparticles. Fluorescence microscopy images compare nanoparticles conjugated with anti-CD90 antibody (P3GAE) to control nanoparticles without antibody labeling (P3GE) following administration as eye drops. Cell nuclei are stained with DAPI (blue), and EPO is

detected using a green-fluorescent antibody. Merged images show colocalization of EPO with nuclei in the retinal ganglion cell layer (RGCL), indicating enhanced delivery by antibody-functionalized nanoparticles relative to controls. (A) Scale bar: 200 μm ; (B) Scale bar: 50 μm

- 5 **Figure 26** shows topical delivery of erythropoietin (EPO) via antibody-functionalized P3GACsE and P3GACsAE nanoparticles. Fluorescence microscopy images compare EPO@chitosan coated-CD90@PPMO3000-GlyIL (P3GACsE) to nanoparticles with dual-layer antibody coated antibody (P3GACsAE) following administration as eye drops. Cell nuclei are stained with DAPI (blue), and EPO is detected using a green-fluorescent antibody.
- 10 Merged images show colocalization of EPO with nuclei in the retinal ganglion cell (RGC) layer, indicating enhanced delivery by antibody-functionalized nanoparticles relative to controls. (A) Scale bar: 200 μm ; (B) Scale bar: 50 μm

- Figure 27** shows topical delivery of erythropoietin (EPO) via antibody-functionalized P3GACmE and P3GACmAE nanoparticles. Fluorescence microscopy images compare
- 15 EPO@CMC coated-CD90@PPMO3000-GlyIL (P3GACmE) to nanoparticles with dual-layer antibody coated antibody (P3GACmAE) following administration as eye drops. Cell nuclei are stained with DAPI (blue), and EPO is detected using a green-fluorescent antibody. Merged images show colocalization of EPO with nuclei in the retinal ganglion cell (RGC) layer, indicating enhanced delivery by antibody-functionalized nanoparticles relative to
- 20 controls. (A) Scale bar: 200 μm ; (B) Scale bar: 50 μm

Detailed description

- The inventors have developed a selective drug delivery system utilizing porous nanoparticles
- 25 that are coated with an ionic liquid and functionalized with cell-targeting moieties such as antibodies. The porous core provides a high surface area for drug loading, while the ionic liquid coating helps to reduce cytotoxicity and improve cellular uptake. Various porous materials including organic-inorganic hybrid materials such as periodic mesoporous organosilicas (PMOs) and metal-organic frameworks (MOFs) can be used.

30

In an exemplary embodiment, nanoparticles targeting retinal ganglion cells (RGCs) were engineered by functionalizing ionic liquid-coated PMO or MOF nanoparticles with an antibody that binds to an RGC marker. *In vitro* and *in vivo* studies showed that the nanoparticles penetrated the retina, localised to the RGC layer, and were internalized by

RGCs. Furthermore, the nanoparticles successfully delivered erythropoietin (a glycoprotein with protective effects on retinal cells) into RGCs, thus showing the potential of the nanoparticles for targeted drug delivery to the retina.

- 5 Gene therapy is a promising strategy for preserving retinal neurons in conditions such as glaucoma, hereditary optic neuropathies and other degenerative retinal disorders. For example, the approved gene therapy product Luxturna (voretigene neparvovec) uses a viral vector to deliver a working copy of the RPE65 gene to treat an inherited retinal disease caused by RPE65 mutations. The nanoparticle system described herein has several
- 10 advantages for delivering gene therapy agents (including vectors, RNA and gene editing systems) compared to viral systems. For example, the nanoparticles may have a better safety profile as they do not elicit a strong immune response, allowing for repeated or long-term dosing without vector neutralisation or inflammatory complications that may occur with viral delivery. The nanoparticles are also readily engineered to accommodate a range of
- 15 nucleic acid sizes and formats, unlike the restrictive packaging capacities of viral vectors. Additionally, the nanoparticle platform incorporates targeting moieties that can provide more selective delivery to desired target cells.

- Accordingly, this disclosure provides nanoparticles for targeted delivery to retinal cells, and
- 20 methods for preparing the nanoparticles. Also provided are methods of treating retinal diseases and methods of labelling retinal tissues using the nanoparticles.

- Disclosed herein is a nanoparticle comprising: a) a porous core at least partially coated by an ionic liquid; and b) a targeting moiety conjugated to the porous core.

25

In one embodiment, the nanoparticle comprises: a) a porous core at least partially coated by an ionic liquid; and b) an antigen-binding molecule conjugated to the porous core, wherein the antigen-binding molecule binds to a retinal cell.

- 30 In one embodiment, the nanoparticle comprises: a) a periodic mesoporous organosilica (PMO) or metal-organic framework (MOF) core at least partially coated by an ionic liquid; and b) an antibody or antigen-binding fragment thereof conjugated to the porous core, wherein the antibody binds to a retinal ganglion cell (RGC).

In one embodiment, the nanoparticle comprises: a) a nanoparticle core comprising a polymeric-bridged periodic mesoporous organosilica (PMO) or a polymeric-bridged metal organic framework (MOF), coated with an amino acid-based ionic liquid; and b) an antibody specific to an RGC marker conjugated to the nanoparticle core for targeting RGCs.

5

Also disclosed herein is a method of preparing a nanoparticle as defined herein, the method comprising: a) synthesising a porous nanoparticle; b) contacting the porous nanoparticle with an ionic liquid to at least partially coat the nanoparticle with the ionic liquid; and c) conjugating a targeting moiety to the porous nanoparticle.

10

Disclosed herein is a method of delivering a therapeutic agent to a retinal cell, the method comprising contacting the cell with a nanoparticle as defined herein or a pharmaceutical composition containing such, wherein the nanoparticle comprises at least one therapeutic agent.

15

Disclosed herein is the use of a nanoparticle as defined herein as a medicament.

Also disclosed herein is a method of treating a retinal disease in a subject, the method comprising administering a nanoparticle as defined herein or a pharmaceutical composition containing such to the subject, wherein the nanoparticle comprises at least one therapeutic agent.

Also disclosed herein is a method of labelling a retinal cell or tissue, the method comprising contacting the retinal cell or tissue with a nanoparticle as defined herein, wherein the nanoparticle comprises a detectable label.

The nanoparticles of this disclosure provide improved delivery characteristics, which may comprise one or more of: (a) improved colloidal stability and/or dispersibility in aqueous and physiological media; (b) increased drug loading capacity, particularly for macromolecular drugs such as proteins, nucleic acids and gene editing systems; (c) loading of multiple drugs with different molecular weights; (d) reduced cytotoxicity, particularly in retinal cells and tissues; (e) improved tissue penetration, particularly through ocular tissues; (f) selective binding to retinal cells, e.g., to retinal ganglion cells; (g) increased uptake by retinal cells; (h) improved intracellular drug release; (i) controllable drug release, including

stimuli-responsive drug release; (j) increased circulatory half-life and/or tissue residence; and/or (k) reduced immunogenicity and immune clearance.

General definitions

- 5 For the purposes of this disclosure, porous materials are classified according to the International Union of Pure and Applied Chemistry (IUPAC) nomenclature for pore size distributions. A “micropore” refers to a pore with a pore size of less than 2 nm; a “mesopore” refers to a pore with a pore size in the range of 2 nm to 50 nm; and a “macropore” refers to a pore with a pore size of larger than 50 nm. A “hierarchically porous” material contains
10 pores that span two or more of the above size regimes.

- A “microporous material”, “mesoporous material”, and “macroporous material” refer respectively to a material with largest pore sizes in the microporous, mesoporous or microporous range. Microporous, mesoporous and macroporous materials may be
15 hierarchically porous. For example, a hierarchically porous mesoporous material may contain both micropores and mesopores, and a hierarchically porous macroporous material may contain micropores and/or mesopores in addition to macropores.

- As used herein, an “organic-inorganic network material” refers to a material in which
20 inorganic and organic components are chemically linked or structurally interconnected to form a unified three-dimensional network structure throughout the material. The inorganic component may include metal ions, metal clusters, silicon atoms, silicates, or other mineral-like structures, while the organic component may be, for example, organic ligands, polymers, organosilanes, or other carbon-based moieties. The organic and inorganic
25 components may be covalently bonded or connected through coordination, hydrogen bonding, or supramolecular interactions. The network may be ordered or disordered.

- As used herein, an “ionic liquid” refers to a salt, typically an organic salt, that exists in a liquid state at temperatures between about 20°C to about 50°C. Ionic liquids contain at least
30 one cationic and at least one anionic component, one or both of which may be an organic molecule.

A “retinal cell” herein includes any of the cell types that comprise the retina, such as photoreceptor cells (rods and cones), neurons (e.g., retinal ganglion cells, amacrine cells,

horizontal cells, bipolar cells), support cells (e.g., glial cells, retinal astrocytes), retinal pigment epithelial cells, and retinal vascular cells (e.g., vascular mural cells, vascular endothelial cells).

- 5 The terms “polypeptide”, “peptide” and “protein” are used interchangeably herein to refer to a polymer of amino acid residues and to variants and synthetic analogues of the same. Thus, these terms apply to amino acid polymers in which one or more amino acid residues is a synthetic non-naturally-occurring amino acid, such as a chemical analogue of a corresponding naturally-occurring amino acid, as well as to naturally-occurring amino acid
- 10 polymers. These terms do not exclude modifications, for example, glycosylations, acetylations, phosphorylations and the like. Soluble forms of the subject proteinaceous molecules are particularly useful. Included within the definition are, for example, polypeptides containing one or more analogues of an amino acid including, for example, unnatural amino acids or polypeptides with substituted linkages.

15

- The term “polynucleotide” or “nucleic acid” are used interchangeably herein to refer to a polymer of nucleotides, which can be mRNA, RNA, cRNA, cDNA or DNA. The term typically refers to polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The
- 20 term includes single and double stranded forms of DNA and RNA.

- The term “vector”, as used herein, refers to a nucleic acid construct designed for delivery to a host cell or for transfer between different host cells. As used herein, a vector can be viral or non-viral. The term “vector” encompasses any genetic element that is capable of
- 25 replication when associated with the proper control elements and that can transfer gene sequences to cells. A vector can include, but is not limited to, a cloning vector, expression vector, recombinant vector, plasmid, phage, transposon, cosmid, chromosome, virus, virion, etc.

- 30 As used herein, the term “viral vector” refers to a nucleic acid vector which contains at least one element of viral origin and which has the capacity to be packaged into a viral particle. The viral vector may contain a nucleic acid sequence encoding a therapeutic polypeptide or transcript as described herein in place of non-essential viral genes.

The term “pharmaceutical composition” or “pharmaceutical formulation” refers to a preparation which is in such form as to permit the biological activity of an active ingredient in the composition to be effective, and which contains no additional components which are unacceptably toxic to a cell or subject to which the composition or formulation would be administered. Such formulations are sterile. “Pharmaceutically acceptable” carriers and excipients (vehicles, additives) are those which can reasonably be administered to a subject to provide an effective dose of the active ingredient employed.

The terms “treating”, “treatment” and the like include relieving, reducing, alleviating, ameliorating or otherwise inhibiting the effects of a retinal disease or condition for at least a period of time. It is also to be understood that terms “treating”, “treatment” and the like do not imply that the disease, or a symptom thereof, is permanently relieved, reduced, alleviated, ameliorated or otherwise inhibited and therefore also encompasses the temporary relief, reduction, alleviation, amelioration or otherwise inhibition of the disease, or of a symptom thereof. Treatment may result in, for example, an improvement in visual acuity, or a return or approach to normal visual acuity.

As used herein, “contacting” refers to any suitable means for delivering, or exposing, a substance (e.g., a nanoparticle or therapeutic agent) to at least one cell. Exemplary delivery methods include, but are not limited to, direct delivery to cell culture medium, perfusion, injection, or other delivery method well known to one skilled in the art. In some embodiments, contacting comprises a step of mixing and/or dispensing, and/or a step of manipulating a delivery or mixing device.

As used herein, the term “administering” refers to the placement of a substance (e.g., a nanoparticle or therapeutic agent) into a subject by a method or route which results in at least partial delivery of the substance at a desired site. Pharmaceutical compositions comprising the nanoparticles or therapeutic agents disclosed herein can be administered by any appropriate route which results in an effective treatment in the subject.

As used herein a “therapeutically effective amount” or “effective amount” is an amount that is non-toxic to a cell or subject and sufficient to effect desired outcomes in the cell or subject (i.e., achieve therapeutic efficacy). For purposes of this disclosure, a therapeutically effective amount of a therapeutic agent is an amount that is sufficient to palliate, ameliorate, stabilise,

reverse, prevent, slow or delay the progression of a disease state. A therapeutically effective amount can be administered in one or more administrations.

It is recognised that the effective amount of therapeutic agent or composition will vary depending on the route of administration, the selected therapeutic agent or composition, and the species to which the therapeutic agent is administered. It is also recognised that one of skill in the art will determine appropriate effective amounts by taking into account such factors as metabolism, bioavailability, and other factors that affect levels of a therapeutic agent or composition following administration for different routes of administration.

10

The terms “patient”, “subject”, “host” or “individual” used interchangeably herein, refer to any subject, particularly a vertebrate subject, and even more particularly a mammalian subject, for whom therapy or prophylaxis is desired. Suitable vertebrate animals that fall within the scope of the invention include, but are not restricted to, any member of the subphylum Chordata including primates (e.g., humans, monkeys and apes, and includes species of monkeys such from the genus *Macaca* (e.g., cynomolgus monkeys such as *Macaca fascicularis*, and/or rhesus monkeys (*Macaca mulatta*)) and baboon (*Papio ursinus*), as well as marmosets (species from the genus *Callithrix*), squirrel monkeys (species from the genus *Saimiri*) and tamarins (species from the genus *Saguinus*), as well as species of apes such as chimpanzees (*Pan troglodytes*)), rodents (e.g., mice rats, guinea pigs), lagomorphs (e.g., rabbits, hares), bovines (e.g., cattle), ovines (e.g., sheep), caprines (e.g., goats), porcines (e.g., pigs), equines (e.g., horses), canines (e.g., dogs), felines (e.g., cats), avians (e.g., chickens, turkeys, ducks, geese, companion birds such as canaries, budgerigars etc.), marine mammals (e.g., dolphins, whales), reptiles (snakes, frogs, lizards etc.), and fish.

25 A preferred subject is a human in need of treatment for a disease or condition. However, it will be understood that the aforementioned terms do not imply that symptoms are present.

As used herein, “and/or” refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (or).

As used in this application, the singular form “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “an agent” includes a plurality of agents, including mixtures thereof.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising”, will be understood to imply the inclusion of a stated integer or step or group of integers or steps
 5 but not the exclusion of any other integer or step or group of integers or steps.

Throughout this specification and the claims which follow, unless the context requires otherwise, the phrase “consisting essentially of”, and variations such as “consists essentially of” will be understood to indicate that the recited element(s) is/are essential i.e. necessary
 10 elements of the invention. The phrase allows for the presence of other non-recited elements which do not materially affect the characteristics of the invention but excludes additional unspecified elements which would affect the basic and novel characteristics of the method defined.

15 Porous nanoparticles

The porous nanoparticles can be prepared from various materials, including organic (e.g., porous carbon, polymeric hydrogel, covalent organic framework, hydrogen-bonded organic framework, etc.), inorganic (e.g., mesoporous silica, metal-doped silica, alumina, aluminosilicates, metal or mixed oxide/sulphide/phosphate, etc.), and hybrid (containing
 20 both organic and inorganic moieties, e.g., periodic mesoporous organosilica, metal-organic framework, etc.).

In one embodiment, the porous core of the nanoparticle is hierarchically porous. Hierarchically porous materials and in particular hierarchically porous mesoporous materials
 25 can accommodate drug cargoes of various sizes, such as small molecules, proteins and/or nucleic acid vectors. They can provide higher drug loading and more controlled drug release.

In one embodiment, the porous core of the nanoparticle comprises mesopores. In one embodiment, the porous core of the nanoparticle comprises micropores and mesopores (i.e.,
 30 the nanoparticle is a hierarchically porous mesoporous material).

In one embodiment, the nanoparticle is characterised by an average pore size of about 7 nm to about 25 nm. For example, the pores may have an average size of about 7 nm, about 8 nm, about 9 nm, about 10 nm, about 11 nm, about 12 nm, about 13 nm, about 14 nm, about

15 nm, about 16 nm, about 17 nm, about 18 nm, about 19 nm, about 20 nm, about 21 nm, about 22 nm, about 23 nm, about 24 nm, or about 25 nm. Pore sizes in this range provide both high drug loading capacity and controlled release, especially for large macromolecules like antibodies, proteins, and nucleic acids.

5

In some embodiments, the total surface area of the nanoparticle is at least about 500 m²/g. In one embodiment, the total surface area of the nanoparticle is between about 500 m²/g to about 1000 m²/g.

- 10 In some embodiments, the porous core of the nanoparticle is an organic-inorganic network material, i.e., a material with a three-dimensional network structure made up of interconnected organic and inorganic components. The network structure of such a material typically contains inorganic centres that are connected (or bridged) by organic groups through a covalent linkage, metal-ligand coordination, or other supramolecular interactions.

15

- Organic-inorganic hybrid network materials combine the advantages of both the organic and inorganic domains, with properties not achievable with a purely organic or purely inorganic structure. The inorganic domain generally contributes mechanical strength, thermal and chemical stability, and defined pore architectures providing a high surface area for drug
20 loading. The organic component may provide for additional reactivity (e.g., for chemical or biological functionalisation), modulation of surface properties and particle degradation characteristics, and control of drug-particle interactions.

- In preferred embodiments, the organic-inorganic network material has an ordered network
25 structure with molecular-scale periodicity and/or a defined pore architecture. Such materials, which include periodic mesoporous organosilicas (PMOs) and a metal-organic frameworks (MOFs), can provide more consistent and predictable mass transport characteristics. In some embodiments, the organic-inorganic network material is a periodic mesoporous organosilica (PMO) or a metal-organic framework (MOF).

30

The pores of the organic-inorganic network material can be of various geometries and symmetries (e.g., cylinders, slits, cones, irregular, etc.), and may be separate or interconnected. In one embodiment, the nanoparticle comprises interconnected pores. An interconnected pore structure is preferred as it provides more efficient mass transport

pathways that can favour higher drug loading efficiency and a more controlled release profile.

In some embodiments, the nanoparticle is characterised by a diameter of about 50 nm to about 200 nm, such as a diameter of about 50 nm, about 60 nm, about 70 nm, about 80 nm, about 90 nm, about 100 nm, about 110 nm, about 120 nm, about 130 nm, about 140 nm, about 150 nm, about 160 nm, about 170 nm, about 180 nm, about 190 nm, or about 200 nm.

Periodic mesoporous organosilicas (PMOs)

In one embodiment, nanoparticles herein are periodic mesoporous organosilica (PMO) nanoparticles.

PMOs are composite materials containing a highly ordered silica structure with organic groups connecting (or bridging) silicon centres in the structure. Each individual organic group or linker is covalently bonded to two or more silicon atoms in the framework. This arrangement is repeated throughout the network with molecular-scale periodicity.

PMO nanoparticles may be synthesised by polycondensation of a bridged organosilane precursor. For example, bis-silylated precursors of Formula (I) may be used for synthesis:



wherein each OR_1 and OR_2 is, independently, a hydrolysable alkoxy group (typically methoxy or ethoxy), and X_1 is an organic bridging group or linker. Multiple bridged organosilane precursors may be used in a co-condensation reaction to create multifunctional organosilica materials. A silane precursor may also be added to adjust porosity and/or structural rigidity (e.g., tetraethyl orthosilicate (TEOS) or tetramethyl orthosilicate (TMOS)), or to introduce terminal functional groups (e.g., 3-aminopropyl triethoxysilane (APTES) for amine, 3-mercaptopropyl trimethoxysilane (MPTMS) for thiol, 3-glycidoxypropyl trimethoxysilane (GPTMS) for epoxy). The precursors are dissolved in a solution (usually a mixture of water and an organic solvent like ethanol or butanol), under acidic or basic conditions depending on the precursors.

A structure-directing agent such as a surfactant (e.g., cetyltrimethylammonium bromide (CTAB)), a block copolymer (e.g., a Pluronic P123, Pluronic F127), or an amphiphilic molecule may be introduced to the precursor mix to create the mesoporous architecture

through template-directed synthesis. The structure-directing agent self-assembles into micelles or other ordered supramolecular templates around which the organosilica framework condenses, creating a periodic arrangement of pores upon removal of the template (e.g., by solvent extraction, or other suitable methods). The mixture is typically
 5 aged or cured under static or stirring conditions often at an elevated temperature (i.e., hydrothermal treatment) to allow for further polycondensation and to improve the structural ordering and mechanical properties of the organosilica network. 1,3,5-trimethylbenzene (TMB) or 1,3,5-triisopropylbenzene can be added during synthesis to enlarge the mesopores.

- 10 By varying the precursors, templating agents and reaction conditions, PMO nanoparticles with various sizes, morphologies (e.g., spheres, rods, films, hollow, core-shell) and mesoporous structures may be synthesised. PMOs with defined pore geometry, symmetry, and connectivity patterns are known, such as KIT-6, SBA-15, SBA-16, FDU-12, MCM-41, and MCM-48. In one embodiment, the PMO nanoparticle is hierarchically porous.

15

In one embodiment, the PMO is characterised by a defined pore symmetry, including but not limited to a cubic or hexagonal pore symmetry. In one embodiment, the PMO is characterised by a defined pore symmetry selected from KIT-6 (cubic), SBA-16 (cubic), FDU-12 (cubic), MCM-48 (cubic), SBA-15 (hexagonal), and MCM-41 (hexagonal). In one
 20 embodiment, the PMO is characterised by a KIT-6 pore symmetry.

- A KIT-6 structure (Korea Advanced Institute of Science and Technology No. 6) is characterised by a three-dimensional highly interconnected bicontinuous pore system with pores ranging from 5–12 nm in diameter (but not limited to such). The KIT-6 architecture
 25 combines the benefits of large pore dimensions suitable for accommodating large therapeutic agents (such as proteins or nucleic acid therapeutics) with improved drug loading capacity and mass transport properties conferred by three-dimensional pore interconnectivity. KIT-6-type PMO nanoparticles are typically synthesised using triblock copolymer templates such as Pluronic P123 under acidic conditions, often with the addition of co-solvents such as n-
 30 butanol to control pore dimensions.

PMO nanoparticles can be characterised by standard materials techniques (e.g., TEM, nitrogen sorption/BET, small-angle X-ray diffraction, FTIR, solid-state NMR, dynamic light

scattering, zeta potential, TGA/DSC, etc.) to confirm morphology, mesostructure, pore sizes, and functionalisation relevant to drug delivery performance.

Metal-organic frameworks (MOFs)

- 5 In one embodiment, nanoparticles herein are metal-organic framework (MOF) nanoparticles.

Metal-organic frameworks (MOFs) comprise metal nodes or centres (which may contain metal ions or metal clusters) connected (or bridged) by organic ligands to form an ordered
10 crystalline network with molecular-scale periodicity.

MOFs herein can include any useful metal. The composition can include one metal or a combination of two or more different metals. In addition, the composition can include the same metal having different coordination geometries. Exemplary metals include zinc (e.g.,
15 Zn^{2+}), iron (e.g., Fe^{3+}), zirconium (e.g., Zr^{4+}), copper (e.g., Cu^{2+}), cobalt (e.g., Co^{2+}), strontium (Sr^{2+}), aluminium (e.g., Al^{3+}), europium (Eu^{3+}), gadolinium (Gd^{3+}), nickel (e.g., Ni^{2+}), magnesium (e.g., Mg^{2+}), chromium (e.g., Cr^{3+}), and titanium (Ti^{4+}) and hafnium (Hf^{4+}).

- 20 The MOF may contain one or more metal clusters. Each cluster, in turn, may include a metal ion coordinated to one or more organic ligands. Common metal cluster configurations include dinuclear paddlewheel structures, trinuclear oxo-clusters, tetranuclear clusters, hexanuclear octahedral clusters, and higher-order polynuclear assemblies. More than one type of metal ion (from same or different metals) may be present within a cluster. Metal
25 clusters in the MOF may be the same or different (e.g., having different coordination geometries, different combinations of metals and ligands, etc.).

The MOF may contain monodentate organic ligands, multidentate organic ligands, or a mixture of both. Multidentate organic ligands may have a structure of Formula (II):



wherein each R_3 and R_4 is, independently, a metal-coordinating moiety; X_2 is an organic group or linker; and m and n are independently 1, 2, 3, 4, 5, or 6. For instance, if m and n are both one, then the ligand is a bidentate ligand. In another instance, if m is 1 and n is 2, then the ligand is a tridentate ligand (i.e., $\text{R}_3\text{-X}_2\text{-(R}_4)_2$, where each R_4 may be the same or

different moieties). R_3 and R_4 can be any useful coordinating moiety. Exemplary coordinating moieties include carboxyl, heterocyclyl, amino, phosphoryl, sulphonyl, as well as anionic forms thereof, e.g., carboxylate, azolate (such as imidazolate, pyrazolate, triazolate, tetrazolate), phosphate, phosphonate, sulphate, sulphonate, etc.

5

In some embodiments, the MOF comprises a ligand selected from a carboxylate, a dicarboxylate (e.g., oxalate, malonate, fumarate, succinate, glutarate, phthalate, isophthalate, terephthalate, etc.), an azolate (e.g., imidazolate, triazolate, pyrroldiazolate, etc.), and a bis-azolate (e.g., bis-imidazolate).

10

MOF nanoparticles may be synthesised by combining the metal ions (or metal clusters) with the organic ligands under conditions that promote controlled coordination-driven self-assembly into a crystalline framework. A skilled person may use various synthesis routes known in the art, including solvothermal, hydrothermal, microwave-assisted, sonochemical, and room-temperature approaches. Room-temperature methods are particularly advantageous as they allow encapsulation of drugs that may degrade when exposed to high temperatures.

15

Coordination modulators such as monocarboxylates (e.g., acetate, formate, benzoate or trifluoroacetate) may be added during synthesis to control nucleation kinetics and crystal growth rates. A higher concentration of modulators generally favours formation of smaller nanoparticles by promoting rapid nucleation while slowing crystal growth.

20

A structure-directing agent such as a surfactant may be added during synthesis to generate hierarchical porosity within the resulting MOF. The structure-directing agent self-assembles into micelles or other ordered supramolecular templates which direct the organisation of the metal-organic network during crystallisation. Following framework formation, removal of the structure-directing agent creates an array of secondary interconnected mesopores in addition to the intrinsic micropores of the MOF structure. Having hierarchical porosity in the MOF structure can enhance loading and diffusion of larger biomolecules or drug cargos.

25

30

By varying the metal ions / metal clusters, ligands (e.g., ligand length or functionality), and synthesis conditions, MOF nanoparticles with various sizes, crystalline symmetries and pore architectures may be synthesised. MOFs with defined network architectures are known, such

as ZIF-8, ZIF-11, ZIF-67, MOF-5, M-MOF-74, MOF-199, MOF-545, MIL-53, MIL-101, CAU-1, and UiO-66. In one embodiment, the MOF nanoparticle is hierarchically porous.

In one embodiment, the MOF is characterised by a network structure selected from ZIF-8, ZIF-11, ZIF-67, MOF-5, M-MOF-74, MOF-199, MOF-545, MIL-53, MIL-101, CAU-1, and UiO-66 MOF. In one embodiment, the MOF is characterised by a ZIF-8 network structure.

The ZIF-8 structure (zeolitic imidazolate framework 8) is made up of Zn^{2+} ions coordinated to 2-methylimidazolate-containing ligands. In certain embodiments, the ZIF-8 framework may contain a mixture of imidazolate and bis-imidazolate ligands. ZIF-8 exhibits chemical stability under physiological conditions, but undergoes pH-responsive degradation in acidic environments due to protonation of the imidazolate ligands. This pH sensitivity makes ZIF-8 particularly advantageous for intracellular drug delivery, as it enables drug release within the acidic compartments of endosomes and lysosomes following cellular uptake. ZIF-8 nanoparticles may be synthesised through room temperature aqueous precipitation, for example, by mixing a zinc salt (e.g., zinc nitrate, chloride or acetate) with one or more 2-methylimidazolate-containing ligands in an aqueous or alcoholic solvent system. A surfactant (e.g., sodium dodecylsulphate or cetyltrimethylammonium bromide) may be added to introduce mesoporosity and to produce hierarchically porous ZIF-8 nanoparticles.

MOF nanoparticles can be characterised by standard materials techniques (e.g., TEM, nitrogen sorption/BET, small-angle X-ray diffraction, FTIR, solid-state NMR, dynamic light scattering, zeta potential, TGA/DSC) to confirm morphology, pore size and structure, and functionalisation relevant to drug delivery performance.

25

Organic and polymer bridges

Organic-inorganic hybrid nanoparticles (such as PMOs and MOFs) comprise a network structure in which two or more inorganic centres are connected (or bridged) by an organic linker. The organic linker is incorporated into the network during nanoparticle synthesis and is distributed throughout the nanoparticle, including on the surface of the pores. The organic linker may be selected to confer desirable properties to the nanoparticles, such as biodegradability, biocompatibility, or sites for further chemical modification. The organic linker may be a monomeric or polymeric linker.

The organic linker connecting the inorganic centres may be a hydrophilic or hydrophobic linker. In one embodiment, the organic linker is a hydrophilic linker. Exemplary hydrophilic linkers include but are not limited to diethylene glycol (DEG), triethylene glycol (TEG), and polyethylene glycol (PEG). Such linkers may improve the colloidal stability and biocompatibility of the nanoparticles, and the interaction of the nanoparticles with coating materials.

The hydrophilicity or hydrophobicity of a molecule may be assessed by its logP value, i.e., the logarithm of its partition coefficient (P). P is defined as the ratio of the molecule concentration in a water-immiscible solvent (e.g., n-octanol) to its concentration in water at equilibrium. A higher logP value indicates greater hydrophobicity, whereas a lower or negative logP value indicates higher hydrophilicity. An increase in a logP value of 1 indicates a ten-fold increase in the concentration of the molecule in the organic phase as compared to the aqueous phase.

In some embodiments, the organic linker connecting the inorganic centres is a hydrolytically stable linker, i.e., the linker is substantially stable in water and does not react with water at useful pH values, including but not limited to, under physiological conditions for an extended period of time. The linker may be non-cleavable under physiological conditions and in an intracellular environment.

In other embodiments, the organic linker connecting the inorganic centres may be a hydrolytically unstable linker, i.e., the linker is degradable in water or in aqueous solutions, including for example, in a biological fluid or in an intracellular environment. Such linkers may be susceptible to cleavage (i.e., a cleavable linker), such as acid-induced cleavage, photo-induced cleavage, enzyme-induced cleavage, peptidase-induced cleavage, esterase-induced cleavage, and disulphide bond cleavage. Cleavable linkages include but are not limited to ester linkages; amide linkages; carbonate linkages; imine linkages resulting from reaction of an amine and an aldehyde; phosphate ester linkages formed by reacting an alcohol with a phosphate group; hydrazone linkages which are a reaction product of a hydrazide and an aldehyde; acetal linkages that are the reaction product of an aldehyde and an alcohol; and orthoester linkages that are the reaction product of a formate and an alcohol. Cleavable linkers allow modulation of nanoparticle degradation and drug release.

In some embodiments, the organic linker connecting the inorganic centres is a polymer. The polymer may be a linear or branched chain polymer. Branched polymers may be multiarm polymers or may contain one or more side chains attached to (or grafted onto) a main polymer chain.

5

Organic-inorganic network materials with inorganic centres that are bridged by polymers are also termed “polymer-bridged” or “polymeric-bridged” materials herein. For example, a PMO with a network structure that contains a polymer linker connecting silicon centres is also referred to as a “polymer-bridged PMO”. A MOF with a network structure that contains
 10 a polymer linker connecting metal centres is also referred to as a “polymer-bridged MOF”. Integration of the polymer into the network structure provides several benefits compared to conjugating the polymers to the nanoparticle surface. Internal polymer bridging provides uniform distribution of the polymer throughout the nanoparticle, while surface conjugation may be uneven. Furthermore, embedded polymer linkers can modulate pore size and particle
 15 degradation in ways not achievable through external conjugation, and are less likely to interfere sterically with targeting ligands or other surface modifications.

In one embodiment, the linking polymer is a hydrophilic polymer, such as (but not limited to) polyethylene glycol (PEG). In other embodiments, the linking polymer may be a
 20 hydrophobic polymer.

In one embodiment, the linking polymer is a charged polymer, such as a polycationic, polyanionic or mixed-charge polymer. Charged polymeric linkers can improve the solubility and biocompatibility of the MOF nanoparticles, and facilitate loading of charged therapeutic
 25 agents in the nanoparticles. In one embodiment, the linking polymer is a polycationic polymer, such as (but not limited to) polyethyleneimine (PEI). Polycationic polymers can improve loading of nucleic acid-based drugs (such as DNA vectors, mRNA, antisense compounds, and RNA interference agents) in the nanoparticles.

30 In some embodiments, the linking polymer is selected from polyethylene glycol (PEG), polyethylene imine (PEI), polyethylene glycol amine (PEGN), diethylene glycol (DEG)-grafted PEI, triethylene glycol (TEG)-grafted PEI, and PEG-grafted PEI.

The polymer linker may contain a polymer the comprises between 10 to 250 monomeric units. In one embodiment, the polymer comprises between 10 to 25 monomeric units. In one embodiment, the polymer comprises between 25 to 150 monomeric units. In one embodiment, the polymer comprises between 150 to 250 monomeric units.

5

The polymer may be characterised by a molecular weight of about 100 Da to about 10,000 Da, such as a molecular weight of about 100 Da, about 200 Da, about 300 Da, about 400 Da, about 500 Da, about 600 Da, about 700 Da, about 800 Da, about 900 Da, about 1000 Da, about 1500 Da, about 2000 Da, about 2500 Da, about 3000 Da, about 3500 Da, about 4000
 10 Da, about 4500 Da, about 5000 Da, about 5500 Da, about 6000 Da about 6500 Da, about 7000 Da, about 7500 Da, about 8000 Da, about 8500 Da, about 9000 Da, about 9500 Da, or about 10,000 Da. In one embodiment, the polymer has a molecular weight of about 100 Da to about 1000 Da. In another embodiment, the polymer has a molecular weight of about 1000 Da to about 10,000 Da. In one embodiment, the polymer has a molecular weight selected
 15 from about 300 Da, about 400 Da, about 600 Da, about 800 Da, about 3000 Da, about 4000 Da, about 6000 Da, or about 8000 Da. In one embodiment, the polymer has a molecular weight of about 3000 Da.

Selection of an appropriate polymer length may depend on factors such as surface
 20 accessibility of the polymer, particle stability, and drug cargo. For example, shorter polymers may be selected to increase packing density of the polymers on the nanoparticle surface, which can improve interaction with the drug cargo. Longer polymers can improve colloidal stability, reduce protein adsorption, enhance circulation time, and provide a more flexible spacer for conjugation.

25

Nanoparticle coating

The nanoparticle may be coated with one or more biocompatible materials (such as an ionic liquid, liposomes or niosomes) to reduce cytotoxicity, improve tissue penetration, increase drug loading, or to allow attachment of targeting moieties. The coating may form a
 30 continuous shell or partial layer surrounding the surface of the nanoparticle. The coating may be applied through adsorption, *in situ* assembly, solvent evaporation, covalent attachment, or other methods depending on the material to be coated.

In some embodiments, two or more coatings of different materials may be sequentially or simultaneously applied to generate multilayered shell structures. Each coating layer may serve a different functional role, such as controlling drug loading or release, enabling stimuli-responsive behaviour, or improving biocompatibility.

5

Ionic liquids

In preferred embodiments, the nanoparticle is coated at least partially by an ionic liquid (IL). The ionic liquid coating can serve various functions, including but not limited to: a) improving the dispersibility and colloidal stability of the nanoparticles in physiological media; b) providing sites for bioconjugation, including the attachment of targeting moieties; c) modulating surface charge to optimise particle targeting and/or uptake; d) as a solvent or carrier for hydrophobic drug molecules with poor aqueous solubility; e) as a stabiliser for macromolecular drugs such as proteins; f) improving transport across biological barriers such as the skin and conjunctiva; and g) reducing protein corona formation on the particle surface to increase circulatory half-life.

15

Preferably, the ionic liquid is biocompatible to minimise cytotoxicity and adverse immunological reactions. Biocompatible ionic liquids can be synthesised by selecting cations and anions derived from cell-endogenous or naturally occurring biomolecules or metabolites.

20

In one embodiment, the ionic liquid comprises a cation selected from choline, acetylcholine, glycine betaine and carnitine. In a preferred embodiment, the ionic liquid comprises a choline cation.

25

Exemplary anions for the ionic liquid include but are not limited to amino acids, fatty acids (saturated or unsaturated), and organic acids. In some embodiments, the ionic liquid comprises an anion selected from an amino acid, lactic acid; glycolic acid; malonic acid; maleic acid; glutaric acid; citric acid; geranic acid, gluconic acid; succinic acid, adipic acid; propionic acid, butyric acid, caproic acid, caprylic acid, capric acid, oleic acid, linoleic acid, octenoic acid, decenoic acid and citronellic acid.

30

In one embodiment, the ionic liquid comprises an amino acid anion. The amino acid is preferably a natural amino acid (including proteinogenic and non-proteinogenic amino

acids). The amino acid anion provides reactive sites (e.g., carboxyl, amine, thiol or hydroxyl groups) for conjugation of targeting ligands, therapeutic agents, or other labels. In one embodiment, the amino acid is selected from glycine, histidine, cysteine, alanine, valine, serine, and threonine, preferably from glycine, histidine and cysteine, more preferably from glycine and histidine.

In one embodiment, the ionic liquid is a choline-amino acid ionic liquid comprising a choline cation and an amino acid anion. In one embodiment, the ionic liquid is selected from choline-glycine, choline-histidine, choline-cysteine, choline-alanine, choline-valine, choline-serine, and choline-threonine ionic liquid, preferably from choline-glycine, choline-histidine, and choline-cysteine, more preferably from choline-glycine and choline-histidine.

The ionic liquid coating may comprise two or more ionic liquids, such as two or more choline-amino acid ionic liquids.

The molar ratio of cation to anion in the ionic liquid may be about 1:1 to about 1:1.2 to produce a coating that is close to electrically neutral. Alternatively, the ionic liquid may comprise an excess of cation or anion to improve interaction with a charged drug molecule or a biological target.

Synthesis of the ionic liquid may be carried out through conventional ion-exchange or neutralisation approaches. An exemplary synthesis of a choline-amino acid ionic liquid may comprise, for example, neutralising choline hydroxide or choline bicarbonate with a desired amino acid to yield the ionic liquid and water. Alternatively, a choline halide may be reacted with an amino acid in a basic solution (e.g., potassium hydroxide solution) to yield the ionic liquid by ion exchange.

The ionic liquid may be coated onto the nanoparticles by dispersing the nanoparticles in a suitable solvent, such as water or ethanol, and subjecting the mixture to stirring and/or elevated temperatures (e.g., up to 100°C) to adsorb the ionic liquid onto the nanoparticle surface.

Exterior coatings

The nanoparticle may be further coated with, e.g., niosomes, liposomes and/or biopolymers on an exterior surface of the nanoparticle. A niosomal or liposomal coating can impart a lipid-like interface to the nanoparticle surface that can increase tissue penetration and cellular uptake of the nanoparticle. Similarly, biopolymers such as chitosan or carboxymethyl cellulose can enhance mucoadhesion and improve cellular uptake. The exterior coating may improve the stability or dispersibility of the nanoparticles in formulations.

- 10 In one embodiment, the nanoparticle comprises a coating of a biopolymer on an exterior surface of the nanoparticle. The biopolymer may be, for example, chitosan, alginate, dextran, hyaluronic acid, or carboxymethyl cellulose.

- 15 In one embodiment, the nanoparticle comprises a coating of niosomes or liposomes on an exterior surface of the nanoparticle. Liposomes are synthetic vesicles comprising one or more phospholipid bilayers surrounding an aqueous core. The bilayers may comprise cholesterol and natural or synthetic phospholipids such as phosphatidylcholine, phosphatidylethanolamine, or mixtures thereof. A niosome is structurally similar to liposomes but formed from non-ionic surfactants and cholesterol. Liposomes and niosomes
20 herein may be unilamellar or multilamellar.

- Exemplary non-ionic surfactants used in niosome formulation include alkyl ethers and esters such as Span surfactants (e.g., Span 20, 40, 60, and 80), Tween surfactants (e.g., Tween 20, 40, 60, and 80), Brij surfactants (e.g., 35, 58, S10, S20, O10, O20, L4, and L23); glycol and glyceryl esters of fatty acids and their derivatives; polyoxyethylene ethers and esters of fatty acids and their derivatives; polyvinyl alcohols; and Triton X-100. Cholesterol may be incorporated at a molar ratio of 1:1 to 1:2 (surfactant:cholesterol). The niosomes may additionally contain other lipid or amphiphilic additives that can be used for further bioconjugation.

30

Niosomes and liposomes may be prepared by various methods known in the art, including thin-film hydration, solvent injection, reverse-phase evaporation, and microfluidic mixing. In a typical thin-film hydration process, a mixture of amphiphilic lipids/surfactants and cholesterol is dissolved in a volatile organic solvent, followed by solvent evaporation under

reduced pressure to form a thin film. Hydration of the film with an aqueous buffer induces spontaneous vesicle formation. The vesicles may be subjected to sonication or membrane extrusion to yield more uniform particles. Solvent injection involves dissolving the lipids/surfactants and cholesterol in a volatile organic solvent such as diethyl ether, which is then slowly injected into an aqueous phase maintained at an elevated temperature (typically 55–65°C) under continuous stirring. The ether rapidly evaporates as it contacts the warm aqueous solvent, leading to the formation of vesicles. This method typically produces small unilamellar vesicles with more uniform size distributions.

- 10 Niosomes or liposomes having a diameter of about 20 nm to about 200 nm may be used for coating the nanoparticles. To form the coating, the nanoparticle may be incubated with the niosomes at room temperature of at least 1 hr. Alternatively, the nanoparticles may be incubated with liposome/niosome-forming components (e.g., lipids, surfactants, cholesterol) under conditions that promote vesicle formation around the nanoparticle surface. This can be achieved using methods described above (such as thin film hydration or solvent injection).

The niosome coating on the nanoparticles can be characterised through various analytical techniques including electron microscopy, DLS (to measure changes in hydrodynamic diameter upon coating formation), zeta potential (to determine changes in surface charge), and spectroscopy (e.g., FTIR).

In one embodiment, the targeting moiety is conjugated to the niosomes, liposomes or biopolymer of the exterior coating.

25 Targeting moieties

The nanoparticles can incorporate targeting moieties that recognise and bind to molecular markers expressed on target cells or tissues, thereby enabling selective delivery of therapeutic agents. The molecular marker may be one that is uniquely expressed on a target cell (e.g., a cell type-specific marker or an altered protein), or one that is differentially expressed on the target cell (e.g., a protein that is overexpressed or aberrantly expressed on the target cell relative to a non-diseased cell).

The choice of targeting moiety depends on the intended cellular or biological target. Targeting moieties contemplated herein include ligands for cellular receptors (including but

not limited to hormone, metabolite, peptide, neurotransmitter, vitamin, fatty acid, lipid, growth factor, sugar, glycan, polysaccharide, glycolipid, glycoprotein, and lipoprotein ligands), and antigen-binding molecules that recognise specific antigens on the target cells. The targeting moiety may be a naturally-occurring or engineered molecule.

5

In one embodiment, the targeting moiety is an antigen-binding molecule that specifically binds an antigen on the target cell.

As used herein, the term “antigen-binding molecule” refers to any molecule capable of specifically binding to an antigen. Antigen-binding molecules include, but are not limited to, full-length antibodies (e.g., IgG, IgM, IgA, IgD, IgE), antibody fragments such as Fab and F(ab')₂, single-chain Fv (scFv), diabodies, triabodies, tetrabodies, minibodies, immunoglobulin new antigen receptors (IgNARs), variable new antigen receptors (VNARs), nanobodies (VHH domains) and aptamers. The term encompasses chimeric antibodies, humanised antibodies, and fully human antibodies. Additionally, antigen-binding molecules include non-immunoglobulin scaffold proteins such as affibodies, ankyrins, designed ankyrin repeat proteins (DARPs), fibronectin type III domain (FN3)-based scaffolds, anticalins, knottins, bicyclic peptides, and other engineered protein scaffolds that have been modified to confer specific antigen-binding properties.

20

In one embodiment, the antigen-binding molecule is an antibody or an antigen-binding fragment thereof.

The antigen-binding molecule may be monovalent (having one antigen-binding site), bivalent (having two antigen-binding sites for the same antigen), or multivalent (having multiple antigen-binding sites for the same antigen). The antigen-binding molecule may be monospecific (binding to a single antigen or epitope), bispecific (binding to two different antigens or epitopes), or multispecific (binding to multiple antigens or epitopes).

The term “epitope” refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antigen-binding molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antigen-binding molecules may bind to different areas on an antigen and may have different biological effects. Epitopes may be conformational or linear. A conformational epitope is produced, for example, by

spatially juxtaposed amino acids from different segments of a linear polypeptide chain. A linear epitope is one produced, for example, by adjacent amino acid residues in a polypeptide chain. In certain embodiments, an epitope may include moieties of a sugar, glycan, phosphoryl group, or other chemical substituent groups on the antigen.

5

For purposes of this disclosure, an antigen-binding molecule or antibody is considered to “specifically bind” to an antigen when it binds to the antigen with higher affinity than to unrelated antigens, typically with a dissociation constant (K_D) of 10^{-6} M or lower, more preferably 10^{-7} M or lower, as measured by standard binding assays such as surface plasmon
10 resonance, biolayer interferometry, or competitive binding.

The nanoparticle may contain more than one targeting moiety. In some embodiments, the nanoparticle comprises a plurality of targeting moieties that bind to one or more molecular markers on a single target cell. In other embodiments, the nanoparticle may comprise a
15 plurality of targeting moieties that bind to different molecular markers on two or more target cells.

In various embodiments, the targeting moiety is selected to bind to cells in the retina, cornea, lens or ocular vasculature. In one embodiment, the targeting moiety is selected to bind to a
20 retinal cell, such as to a surface marker on the retinal cell. The retinal cell may be, for example a neuron, neuronal support cell, epithelial cell, or vascular cell in the retina.

In one embodiment, the targeting moiety is selected to bind to a retinal ganglion cell (RGC). By way of non-limiting example, Thy1 (CD90), TuJ1 (β -tubulin III), and RBPMS (RNA-binding protein with multiple splicing) may be used as markers for targeting RGCs. Thus,
25 for example, antigen-binding molecules that bind to Thy1, TuJ1, or RBPMS may be conjugated to the nanoparticle to target drug delivery to RGCs.

Non-limiting examples of markers that may be used for targeting of other retinal and ocular
30 cell types are provided in Table 1.

Table 1. Exemplary surface markers for retinal cells.

Cell type	Surface marker
-----------	----------------

Retinal ganglion cells (RGCs)	CD90 (Thy1), CD171 (L1CAM), CD24, CD56 (NCAM1)
Photoreceptors (rods and cones)	CD73 (NT5E), CD24, CD133 (PROM1), CD26 (cones)
Retinal pigment epithelial cells (RPEs)	EPCAM (CD326), CD140b (PDGFR β), MERTK, Integrin α V β 5 (CD51/CD29)
Müller glia	GLAST (EAAT1), CD29, CD44
Retinal microglia	P2RY12, CD11b, CX3CR1, TREM2
Retinal endothelial cells	CD31, CD34, VEGFR2 (KDR), CD144 (VE-cadherin), ICAM-1 (CD54)
Retinal pericytes	CD146, PDGFR β (CD140b), NG2 (CSPG4)
Amacrine cells	CD56 (NCAM1), CHL1, CD24
Horizontal cells	Cadherins (CDH8, CDH9), CD171 (L1CAM)
Bipolar cells	CD24, ICAM family receptors
Corneal epithelial cells / Limbal stem cells	CD49f, CD44, EPCAM, ABCG2, CD200
Corneal endothelial cells	CD166 (ALCAM), CD31, CD54
Lens epithelial cells	CD324 (E-cadherin), CD44
Ocular vascular cells (choroid or retinal vasculature)	CD31, CD34, CD144, VEGFR2, ICAM-1, VCAM-1

The targeting moiety can be covalently or non-covalently attached to the nanoparticle core and/or to one or more of the nanoparticle coatings, for example, to functional groups in the nanoparticle material, or to functional groups provided by the ionic liquid or exterior coating.

5

A skilled person may employ bioconjugation chemistries well known in art, such as (but not limited to) amide bond formation, ester linkage, click chemistry, or thioether bonding, to conjugate the targeting moiety to the nanoparticle. In a non-limiting example, targeting moieties such as antibodies may be attached to amino acids in an amino acid-based ionic liquid coating through an amide or thioether bond, or to amine or carboxyl groups in niosomes or biopolymers on the exterior coating via an amide bond.

10

The density of targeting moieties on the nanoparticle may be selected to balance targeting efficiency (which favours a high density) with colloidal stability and immunogenicity

concerns. This density may be adjusted by varying the ratio of the targeting moiety to the nanoparticle during the conjugation step.

In some embodiments, the ratio of the targeting moiety to nanoparticle used for conjugation is between about 1:2500 to about 1:25,000 by weight. For example, a ratio of about 1:2500, about 1:5000, about 1:7500, about 1:10,000, about 1:12,500, about 1:15,000, about 1:17,500, about 1:20,000, about 1:22,500, or about 1:25,000 by weight of targeting moiety to nanoparticle may be used during the conjugation reaction.

In one embodiment, the ratio of the targeting moiety to the nanoparticle used for conjugation is selected from 1:2500, 1:10,000 and 1:25,000 by weight. In one embodiment, the ratio of the targeting moiety to the nanoparticle used for conjugation is about 1:10,000 by weight.

Therapeutic agents

Nanoparticles herein may contain one or more therapeutic agents, which may be incorporated into different compartments of the nanoparticles, such as within the pores, embedded in the ionic liquid, or adsorbed onto a nanoparticle surface.

As used herein, a “therapeutic agent” is any pharmacologically active agent which can exert a therapeutic or prophylactic effect on a target cell or subject. The therapeutic agent may be a natural or synthetic small molecule, polypeptide, nucleic acid, or gene editing system, including but not limited to: small organic or inorganic molecules; metabolites; hormones; signalling molecules; cytotoxins; radioisotopes; radioisotope-drug conjugates, anti-cancer compounds; anti-inflammatory compounds; antibiotics; antifungals; antivirals; immunosuppressants; immunomodulatory compounds; immune checkpoint inhibitors; analgesics; molecular glues; proteins or functional domains or fragments thereof; peptides; enzymes; growth factors; cytokines; chemokines; peptidomimetics; peptide analogues; antigen-binding molecules; antibodies or antigen-binding fragments thereof; bispecific antibodies; multispecific antibodies; nanobodies; immunoconjugates; antibody-drug conjugates; peptide-drug conjugates; degrader-antibody conjugates; antibody-oligonucleotide conjugates; bispecific T-cell engagers; proteolysis targeting chimeras; aptamers; nucleic acid vectors; gene therapy vectors; viral vectors; plasmids; minicircles; mRNA; tRNA; RNA interference compounds; siRNA; shRNA; antisense oligonucleotides; miRNA; antagomirs; nucleases for gene editing; CRISPR-associated nucleic acid constructs

or guide nucleic acids for gene editing; CRISPR-Cas systems; extracellular vesicles; exosomes; extracts made from biological materials such as bacteria, plants, fungi, or animal cells or tissues; and prodrugs of the above.

- 5 Particularly contemplated are therapeutic agents that may be administered to retinal cells to prevent or treat a retinal disease or condition. Non-limiting examples of such agents include erythropoietin (EPO), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), platelet-derived growth factor (PDGF), neurotrophin-3 (NT-3), ciliary neurotrophic factor (CNTF), retinoic acid, vitamin C (ascorbic acid), vitamin E (tocopherol),
 10 glycerol phosphate, a lithium salt, a corticosteroid, an omega-3 fatty acid, a nucleic acid inhibitor of a gene or transcript associated with a retinal disease, an antibody that binds to a molecular target associated with a retinal disease, a gene therapy vector, and a gene editing system. The therapeutic agent may improve RGC survival and reduce progression of a retinal disease.

15

As used herein, the term “small molecule” refers to a chemical agent (e.g., organic, inorganic or hybrid molecule) having a molecular weight less than about 1 kDa, including salts, solvates, and other pharmaceutically acceptable forms of such compounds.

- 20 In one embodiment, the therapeutic agent is a hydrophobic small molecule. In another embodiment, the therapeutic agent is a hydrophilic small molecule.

- A nucleic acid therapeutic, as described herein, can be a vector, an expression vector, an inhibitory nucleic acid, an aptamer, a template molecule or cassette (e.g., for gene editing),
 25 or a targeting molecule (e.g., for CRISPR-Cas technologies). The nucleic acid molecule can be RNA, DNA, peptide nucleic acids, or synthetic or modified versions thereof. In some embodiments, the nucleic acid is an inhibitory nucleic acid, e.g., a siRNA, shRNA, antisense oligonucleotide, miRNA, or guide nucleic acid.

- 30 Polypeptide therapeutics can be peptides (including cyclic, stapled and other non-linear or conformationally restricted peptides), proteins, functional protein domains or fragments, peptidomimetics, self-assembling peptide systems, fusion proteins that combine two or more proteins or functional proteins domains, and protein conjugates such as antibody-drug

conjugates, antibody-oligonucleotide conjugates, degrader-antibody conjugates and proteolysis targeting chimeras.

Gene therapy vectors herein are nucleic acid vectors used to deliver genetic material to a target cell or organism. The gene therapy vector may contain a transgene (a heterologous nucleic acid sequence) that is capable of expression in the cell. The transgene may, for example, replace a defective gene (such as a mutant gene associated with a retinal disease), augment expression of a gene (e.g., increase expression of an endogenous gene), or introduce a new gene or gene cluster into the cell. Suitable gene therapy vectors include non-viral vectors such as plasmids, transposons, minicircles and doggybone DNA, and viral vectors including adenoviral, adeno-associated viral (AAV), lentiviral, and retroviral vectors. Such vectors are widely known to a skilled person. The gene therapy vector may be episomal or integrative.

In certain embodiments, the therapeutic agent is a gene editing system. Gene editing systems typically comprise a site-specific nuclease that recognises a target nucleic acid sequence, and may further comprise a donor template nucleic acid for introducing specific sequence modifications at or adjacent to the target nucleic acid sequence. The target sequence may, for example, be in a gene, a gene regulatory region, a non-coding region, or a transcript.

The term “donor template nucleic acid”, as used herein, refers to a nucleic acid molecule that can be used by one or more cellular proteins to modify the sequence of a target nucleic acid after a site-specific nuclease described herein has altered the target nucleic acid. The donor template typically has some homology to the target sequence. In some embodiments, the donor template nucleic acid is a double-stranded nucleic acid molecule. In some embodiments, the donor template nucleic acid is a single-stranded nucleic acid molecule. In some embodiments, the donor template nucleic acid is linear molecule. In some embodiments, the donor template nucleic acid is circular (e.g., a plasmid). In some embodiments, the donor template nucleic acid is an exogenous nucleic acid molecule. In some embodiments, the donor template nucleic acid is an endogenous nucleic acid molecule (e.g., a chromosome). In some embodiments the donor template is a DNA molecule. In some embodiments, the donor template is an RNA molecule.

The site-specific nuclease may be a wild-type, engineered or chimeric nuclease. The nuclease induces a nick or double-strand break in a target site, which may then be repaired by the cell via non-homologous end joining (NHEJ) or homology-directed repair (HDR). In NHEJ, the double-strand breaks are repaired by direct ligation of the break ends to one another. As such, nucleotides are not inserted into the site, although some nucleic acid material may be lost, resulting in a deletion. In HDR, the donor template serves as a template for repair of the cleaved target DNA sequence, resulting in the transfer of genetic information from the donor nucleic acid to the target nucleic acid.

- 10 Gene editing systems may insert, delete or substitute one or more nucleic acid residues in a target nucleic acid sequence. Such modifications may be used for gene correction, gene replacement, gene insertion, gene disruption, gene mutation, gene tagging, etc.

In some embodiments, gene editing systems herein are selected from zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced palindromic repeats/CRISPR-associated nuclease (CRISPR/Cas) systems.

In one embodiment, the therapeutic agent is a CRISPR/Cas gene editing system. The CRISPR/Cas system may include a Cas nuclease and a guide RNA (gRNA), and may additionally comprise a donor template nucleic acid. The CRISPR/Cas system may comprise a plurality of gRNAs and/or donor templates for editing one or more nucleic acid sequences.

As used herein, the terms “guide nucleic acid”, “guide RNA”, “single guide RNA”, “gRNA” and “crRNA” refer to a nucleic acid comprising a guide sequence that determines the specificity of an enzyme (e.g., a Cas nuclease of a CRISPR/Cas system) to a target nucleic acid. The guide sequence may have at least partial complementarity with a target nucleic acid sequence, sufficient to hybridise with the target sequence and to direct sequence-specific binding of the enzyme to the target sequence.

30 In one embodiment, the CRISPR/Cas gene editing system is a CRISPR/Cas9 system. The CRISPR/Cas9 system may comprise a gRNA, which may comprise a CRISPR RNA (crRNA) containing the guide sequence. The gRNA may also comprise a trans-activating crRNA (tracrRNA) for processing the crRNA to its mature form. Alternatively, the gRNA

may be an engineered construct comprising both the crRNA and tracrRNA in a single nucleic acid molecule (i.e., a single guide RNA or sgRNA).

5 The CRISPR/Cas9 system may be a base editing system. In base editing systems, a catalytically impaired Cas9 (Cas9 nickase) is fused to a deaminase enzyme to direct conversion of specific nucleotides without creating double-strand breaks, thus minimising undesired mutations. For example, cytosine base editors (CBEs) can convert C≡G base pairs to T=A, while adenine base editors (ABEs) can convert A=T base pairs to G≡C.

10 In other embodiments, the CRISPR/Cas9 system may be a prime editing system. Prime editing systems include a Cas9 nickase that is fused to a reverse transcriptase, and a prime editing guide RNA (pegRNA) that contains both the guide sequence and the desired edit. Prime editing allows targeted edits without requiring donor templates or creating double-strand breaks.

15 In one embodiment, the CRISPR/Cas gene editing system is a CRISPR/Cas12 system, such as a CRISPR/Cas12a (also known as CRISPR/Cpf1) or CRISPR/Cas12b (also known as CRISPR/C2c1) system. In such an embodiment, the CRISPR/Cas system may comprise a single gRNA molecule containing the guide sequence.

20 Gene editing systems can be delivered as vectors (e.g., plasmid or viral vectors) encoding the components, as mRNA for transient expression, or as ribonucleoprotein (RNP) complexes comprising, e.g., pre-assembled Cas proteins and guide nucleic acids.

25 Therapeutic agents herein can be incorporated into the nanoparticles through various methods, including impregnation from solution, surface adsorption, or covalent conjugation. The therapeutic agent can be encapsulated in the nanoparticle pores or attached to a surface or coating of the nanoparticle. A skilled person may select a suitable loading approach that maintains the biological activity of the agent while providing desired release kinetics.

30

Detectable labels

In some embodiments, nanoparticles herein may contain a detectable label. The detectable label may be used to label a target cell or tissue upon nanoparticle binding and/or uptake, or to track biodistribution or cell/tissue uptake of the nanoparticle *in vitro* or *in vivo*.

- Non-limiting examples of detectable labels include fluorescent dyes (e.g., derivatives of fluorescein, coumarin, rhodamine, cyanine, BODIPY or squaraine), quantum dots, contrast agents (such as gadolinium complexes and iodinated compounds), radiolabelled compounds, and other imaging moieties for positron emission tomography (PET), single-photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), or optical imaging. Near-infrared dyes such as Cy5, Cy7, and indocyanine green (ICG) are particularly useful for *in vivo* imaging of deeper tissues.
- 10 In some embodiments, the detectable label is selected from FITC, rhodamine B, Cy3, Cy5, a quantum dot, and an AlexaFluor dye.

Nanoparticle preparation

- This disclosure also provides methods for preparing nanoparticles as described herein.
- 15 Disclosed herein is a method of preparing a nanoparticle as defined herein, the method comprising: a) synthesising a porous nanoparticle; b) contacting the porous nanoparticle with an ionic liquid to at least partially coat the nanoparticle with the ionic liquid; and c) conjugating a targeting moiety to the nanoparticle.
- 20 In some embodiments, step a) comprises synthesising a nanoparticle with an organic-inorganic network. In one embodiment, step a) comprises synthesising a PMO or MOF nanoparticle. In one embodiment, step a) comprises synthesising a polymer-bridged PMO or polymer-bridged MOF nanoparticle.
- 25 Synthesis of PMO and MOF nanoparticles may be by methods known in the art, such as methods described above. In one embodiment, hydrothermal synthesis is used for PMO synthesis. For example, to synthesise a PMO with KIT-6 pore architecture and a polymer bridge, a bis-silylated polymer (e.g., PEG-bis-Si(OEt)₃) may be mixed with a silane (e.g., 3-aminopropyltriethoxysilane (APTES)) and a Pluronic templating agent (e.g., P123) in a
- 30 1:1:7.35 ratio in an acidic mixture of water and n-butanol. The mixture may be cured in an autoclave (100°C –150°C) for polycondensation and network formation. The Pluronic may be removed by solvent extraction to give polymer-bridged PMO nanoparticles.

In one embodiment, room temperature synthesis is used for MOF synthesis. For example, to synthesise a hierarchically porous MOF with a ZIF-8 structure and a polymer bridge, a zinc salt (e.g., zinc acetate) may be slowly added to a bis-imidazolate polymer (e.g., PEG-bis-imidazole) in water or methanol at about 25°C in the presence of about 0.02% w/v of a
 5 surfactant (e.g., SDS). A 5:1 ratio of the polymer and zinc salt may be used. The MOF nanoparticles are formed as a precipitate.

The nanoparticle is contacted with an ionic liquid for a time and under conditions for forming at least a partial coating of the ionic liquid on the nanoparticles. The coating may rely on
 10 adsorption of the ionic liquid on the surface of the nanoparticle. By way of non-limiting example, to form a coating of a choline-amino acid ionic liquid around the nanoparticles, the nanoparticles may be mixed with the ionic liquid in water or ethanol at a temperature of about 100°C to adsorb the ionic liquid to the nanoparticles.

15 The ratio of the ionic liquid to the nanoparticle used for coating may be about 1:1 to about 1:5 by weight. For example, a ratio of about 1:1, about 1:2, about 1:3, about 1:4, or about 1:5 of ionic liquid to nanoparticle by weight may be used for coating.

In some embodiments, the nanoparticle is also contacted with niosomes, liposomes and/or a
 20 biopolymer for a time and under conditions for forming a coating of those materials on an exterior surface of the nanoparticles. For example, the nanoparticles may be mixed with the niosomes, liposomes or biopolymer at room temperature to allow adsorption of the particles or biopolymer to the exterior surface of the nanoparticle.

25 The targeting moiety may be conjugated to the nanoparticle core and/or to one or more nanoparticle coatings (e.g., to the ionic liquid coating, or to an exterior niosome coating) by bioconjugation methods known to the skilled person. Conjugation of an antibody to the nanoparticle may rely on amide bond formation between the antibody and the ionic liquid coating and/or the niosome/liposome/biopolymer coating. In preferred embodiments, the
 30 targeting moiety is conjugated to a nanoparticle coating (e.g. to the ionic liquid coating and/or to an exterior niosome or biopolymer coating) to ensure adequate surface display for targeting.

The targeting moiety and the nanoparticle may be mixed at a ratio of between about 1:2500

to about 1:25,000 by weight for conjugation. In one embodiment, the targeting moiety and the nanoparticle are mixed at a ratio of about 1:10,000 for conjugation.

5 Nanoparticle concentrations of up to about 100 $\mu\text{g}/\mu\text{l}$ (such as between about 25 $\mu\text{g}/\mu\text{l}$ to about 75 $\mu\text{g}/\mu\text{l}$) may be used for conjugating the targeting moiety to reduce nanoparticle aggregation during conjugation.

10 The preparation of the nanoparticle may further comprise contacting the nanoparticle with one or more therapeutic agents or detectable labels to incorporate the therapeutic agent(s) or detectable label(s) in the nanoparticle. The therapeutic agent or detectable label may be encapsulated within the nanoparticle, or may be attached to the surface of the nanoparticle material or coating(s) by covalent or non-covalent means.

15 The method of drug loading will depend on the physicochemical characteristics of the nanoparticle material, nanoparticle coating(s), and the therapeutic agent. Some embodiments of the method comprise selecting a nanoparticle with a pore size and surface properties that complements the therapeutic agent. For example, nanoparticles with larger mesopores (e.g., larger than 10 nm) and containing PEI polymer bridges may be used to carry macromolecular nucleic acid therapeutics such as RNA and plasmid vectors.

20 In one embodiment, the therapeutic agent is incorporated by passive diffusion into the nanoparticle's internal pores, with loading driven, e.g., by interactions of the therapeutic agent with the pore walls such as hydrophobic interactions or electrostatic interactions with charged polymer bridges in the pores. Many kinds of drugs, including small molecule, 25 nucleic acid, peptide or protein drugs, may be loaded in this way. The nanoparticles may be immersed in a solution of the drug to allow diffusion. The drug may be loaded before the ionic liquid coating is applied and before conjugation of the targeting moiety.

30 In one embodiment, the therapeutic agent is incorporated by diffusion into or adsorption to the ionic liquid coating. Small molecule drugs, proteins and nucleic acids may be loaded in this manner. The ionic liquid coating can improve the solubility of hydrophobic drugs, and stabilise proteins and nucleic acids against degradation.

Nucleic acid therapeutics, including DNA, RNA, oligonucleotides, and gene-editing

complexes, may bind through electrostatic interaction with the ionic liquid coating or with a biopolymer coating on the nanoparticle exterior. Peptide and protein drugs may similarly be loaded through electrostatic interaction, hydrogen bonding or coordination with functional groups present in the ionic liquid coating or an exterior biopolymer coating.

5

Alternatively, the therapeutic agent may be covalently bonded to the nanoparticle core or coating(s), for example, using a biodegradable linker that is cleaved intracellularly.

The temperature, pH, buffer ionic strength, and ratio of drug to nanoparticles may be suitably varied to achieve a desired loading efficiency while maintaining bioactivity of the drug.

10

For nanoparticles with a total surface area of 500–1000 m²/g, exemplary weight ratios (% w/w) of drug to nanoparticle that may be used for loading are provided below.

Small molecules (<1 kDa): ~10–30% w/w

15 Peptides (~0.5–5 kDa): ~1–10% w/w

Peptide nucleic acids (1–10 kDa): ~1–10% w/w

Small RNA, e.g., siRNA, miRNA (~13–15 kDa): ~1–10% w/w

Proteins, e.g., enzymes (~10–200 kDa): ~5–20% w/w

Ribonucleoprotein (RNP) complexes, e.g., CRISPR/Cas systems (~190 kDa): ~1–3% w/w

20 mRNA (0.5–5 MDa): ~1–5% w/w

Plasmid vectors (~1–7 MDa): ~1–5% w/w

Drug combinations: ~5–15% w/w

In one embodiment, the loading of the therapeutic agent or detectable label occurs before conjugation of the targeting moiety. Alternatively, the therapeutic agent or label may be loaded after the targeting moiety has been conjugated to the nanoparticle.

25

Pharmaceutical compositions

The nanoparticles of the present disclosure may be formulated as pharmaceutical compositions (such as infusible or ophthalmic compositions) for clinical use and may comprise a pharmaceutically acceptable carrier, diluent or adjuvant.

30

By “pharmaceutically acceptable carrier” is meant a pharmaceutical vehicle comprised of a material that is not biologically or otherwise undesirable, i.e., the material may be

administered to a subject along with the nanoparticle without causing any or a substantial adverse reaction. Carriers may include excipients and other additives such as diluents, detergents, colouring agents, wetting or emulsifying agents, pH buffering agents, preservatives, and the like.

5

Representative pharmaceutically acceptable carriers include any and all solvents, dispersion media, coatings, surfactants, antioxidants, preservatives (e.g., antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, drugs, drug stabilisers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavouring agents, dyes, polymers, polymeric matrices, and such like materials and combinations thereof, as would be known to one of ordinary skill in the art (see, for example, *Remington's Pharmaceutical Sciences*, 18th Ed. Mack Printing Company, 1990, pp. 1289-1329, incorporated herein by reference). Except insofar as any conventional carrier is incompatible with the nanoparticle, its use in the pharmaceutical compositions is contemplated.

The present disclosure provides methods for the production of pharmaceutically useful nanoparticle compositions. Such methods of production may comprise isolating and mixing a nanoparticle as defined herein with a pharmaceutically acceptable carrier, diluent or adjuvant.

The pharmaceutical compositions may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, eye drops, contact lenses, and implants. The preferred form depends on the intended mode of administration and therapeutic application. Suitable pharmaceutical compositions may be administered systemically (e.g., intravenously), or locally to the eye (e.g., intraocularly, topically, using a contact lens). A preferred mode of administration is parenteral (e.g., intravenous, intraocular, topical).

In some embodiments, the compositions or pharmaceutical compositions herein are in the form of injectable, infusible or instillable solutions.

The solutions may be formulated for systemic administration (e.g., intravenous infusion) or local administration to the eye (e.g., intraocular injection, topical instillation).

In some embodiments, compositions and pharmaceutical compositions herein are ophthalmic compositions suitable for administration to the eye, for example, via extraocular (topical), periocular or intraocular routes.

5

In one embodiment, the pharmaceutical composition is formulated for topical administration to the eye. In one embodiment, the topical formulation is in the form of an eye drop. Other formulations for topical application to the eye are also possible, such as gels, ointments, and sprays.

10

For eye drop formulations, the nanoparticles may be dispersed in sterile water or an aqueous buffer adjusted for isotonicity and refractive compatibility. A mucoadhesive polymer (e.g., hyaluronic acid, chitosan, carboxymethyl cellulose) may be included to prolong residence time on the ocular surface and enhance ocular penetration. Surfactants or wetting agents may be included to improve nanoparticle dispersion and enhance corneal wetting.

15

In one embodiment, the nanoparticle comprises a biopolymer coating. Exemplary biopolymer include but are not limited to chitosan and carboxymethyl cellulose. Chitosan, a natural cationic polysaccharide, can enhance formulation stability through its mucoadhesive properties and its ability to form polyelectrolyte complexes that protect active drug compounds. Carboxymethyl cellulose (CMC) may function as a viscosity-modifying and stabilising agent, preventing nanoparticle aggregation and sedimentation, thereby maintaining homogeneity and consistent flow characteristics required for injectable formulations. These polymers can contribute to an extended shelf-life and improved performance of liquid ready-to-use products.

20

25

In one embodiment, the pharmaceutical composition is formulated for periocular or intraocular administration. Periocular and intraocular formulations may take the form of an injectable solution. Such solutions may be injected to the sub-conjunctival, sub-Tenon's, or orbital floor regions for the periocular route, or through intracameral, intravitreal, suprachoroidal or supraciliary routes for the intraocular administration.

30

In one embodiment, the pharmaceutical composition is formulated for intravascular injection or infusion..

Compositions suitable for injectable use include sterile aqueous solutions (where water-soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In such cases, the composition must be sterile and should
5 be fluid to the extent that easy syringability exists. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof.

Injectable formulations also include *in situ* gelling formulations that exist as low-viscosity
10 solutions at room temperature for easy instillation, but undergo sol-to-gel transition upon contact with the warmer ocular surface (32–37°C). Such formulations allow the formation of nanoparticle depots within the eye that can prolong drug release within the eye. The injection volume is typically about 25–100 µl for human eyes.

15 In some embodiments, the pharmaceutical composition may be incorporated into an ophthalmic device such as a contact lens, ocular insert, or ocular implant. For example, the nanoparticles may be embedded within the polymeric matrix of a hydrogel lens or coated on its surface for sustained release of the nanoparticles during wear. Alternatively, the nanoparticles may be loaded into a biodegradable ocular film or implant designed for
20 placement in the conjunctival sac, scleral pocket, or intraocular space, providing sustained release over days to weeks.

Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus of the nanoparticle composition may be administered,
25 several divided doses may be administered over time, or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. A nanoparticle composition of the present disclosure can be administered on multiple occasions. Intervals between single dosages can be daily, weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood or ocular levels of the
30 nanoparticle or therapeutic agent in the subject. Alternatively, the nanoparticle composition can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the nanoparticle or drug in the subject.

It may be advantageous to formulate compositions in dosage unit form for ease of administration and uniformity of dosage. “Dosage unit form” as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutically acceptable carrier. The specification for the dosage unit forms is dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in subjects.

10

Dosage values may vary with the type and severity of the disease or condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens may be adjusted over time according to individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

15

Targeted drug delivery

For targeted drug delivery to retinal cells, the nanoparticles may be functionalised with targeting moieties that bind to retinal cell markers. Retinal cells encompass neurons (e.g., retinal ganglion cells), neuronal support cells (e.g., Müller glia cells), retinal pigment epithelial cells, and retinal vascular cells (e.g., vascular endothelial cells) in the retina.

20

In one embodiment, antigen-binding molecules that bind specifically to Thy1 (CD90), TuJ1 (β -tubulin III), or RBPMS (RNA-binding protein with multiple splicing) are conjugated to the nanoparticles to provide targeting to retinal ganglion cells (RGCs). Additional ocular cell markers are provided in Table 1.

25

Targeted delivery may comprise contacting the retinal cell with nanoparticles loaded with one or more therapeutic agents. In various embodiments, the contacting may be carried out *in vitro*, *ex vivo* or *in vivo*. In some embodiments, the retinal cell is a cell in a subject, and the contacting step comprises administering the nanoparticle to the subject.

30

The density of the targeting moiety on the nanoparticle may be adjusted to achieve a desired targeting specificity. In some embodiments, the nanoparticle provides a targeting specificity of at least 80%, at least 85%, at least 90%, or at least 95% for the retinal cell.

5 Treatment of retinal diseases

Nanoparticle compositions described herein may be used to deliver one or more therapeutic agents for treating or preventing a retinal disease or condition.

- 10 Disclosed herein is a method of treating a retinal disease in a subject, the method comprising administering a nanoparticle as defined herein to the subject, wherein the therapeutic agent is comprised in the nanoparticle.

- Disclosed herein is a nanoparticle as defined herein, for use in treating a retinal disease in a
15 subject, wherein the nanoparticle comprises a therapeutic agent.

- Disclosed herein is the use of a nanoparticle as defined herein in the manufacture of a medicament for treating a retinal disease in a subject, wherein the nanoparticle comprises a therapeutic agent.

20

- The retinal disease may be a retinal vascular disease or an inherited disease. Retinal diseases and conditions include, without limitation, glaucoma, optic neuropathy, diabetic retinopathy, sickle cell retinopathy, ischemic retinopathy, retinopathy of prematurity, infectious retinitis, retinitis pigmentosa, Stargardt's disease, optic nerve injury, age-related macular
25 degeneration (AMD), uveitis, macular edema, neovascular glaucoma, proliferative vitreoretinopathy, vitelliform maculopathy, and choroidal neovascularisation.

- The nanoparticles may be administered intravenously (e.g., by infusion or injection), intraocularly (e.g., by injecting into the vitreous body of the eye), or topically to the eye
30 (e.g., by eye drop).

In some embodiments, the nanoparticle is provided at a concentration of up to about 100 µg/mL.

Labelling of retinal tissues

In some embodiments, nanoparticle compositions described herein may incorporate a detectable label and may be used to label a retinal cell or tissue.

5

Disclosed herein is a method of labelling a retinal cell or tissue, the method comprising contacting the retinal cell or tissue with a nanoparticle as defined herein, wherein the nanoparticle comprises a detectable label. Such methods may be used to monitor biodistribution, tissue distribution, or particle uptake of the nanoparticles.

10

The method further comprise detecting a signal generated by the detectable label, such as a fluorescent, luminescent, colorimetric, radioactive, magnetic resonance, or thermal signal, to image the retinal cell or tissue.

15

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

20

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

25

30

Unless otherwise defined, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs.

Certain embodiments of the invention will now be described with reference to the following examples which are intended for the purpose of illustration only and are not intended to limit the scope of the generality hereinbefore described.

EXAMPLES

Example 1: Synthesis of core nanostructures

- 5 **Synthesis of niosomes:** 20 mg of non-ionic surfactant (Span 80, Tween 80, Tween 60, or Span 60) and 40 mg cholesterol was dissolved in 6 mL of diethyl ether. This mixture was combined with 2 mL of methanol, which had been pre-mixed with a weighed quantity of the drug. The resulting solution was then slowly injected into 10 mL of phosphate buffer (pH 7.4) at a rate of 1 mL/min using a microsyringe. The solution was continuously stirred with
10 a magnetic stirrer while maintaining the temperature at 60–65°C. The temperature difference between the phases caused vaporisation, leading to the formation of niosomes.

- Synthesis of the choline-amino acid ionic liquid (IL):** Choline chloride (ChCl) and amino acids (cysteine, lysine, glycine, and histidine) were added to a potassium hydroxide-ethanol
15 solution to obtain ionic liquids, referred to as CysIL, LysIL, GlyIL, and HisIL, respectively. The mole ratio of choline chloride, potassium hydroxide, and amino acid was maintained at 1:1:1.05. The reaction was conducted for 8 hours at 30°C under ambient pressure. After the reaction, the mixture was filtered to separate the insoluble by-product, potassium chloride. The solvent was then evaporated to yield the crude product. This crude product was washed
20 with acetonitrile to remove excess unreacted amino acids, which precipitated out.

- Synthesis of ZIF-8 metal-organic framework:** 0.3 g of zinc nitrate hexahydrate [$\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$] was dissolved in 11.3 g of methanol (Acros Organics, anhydrous, water content <50 ppm). A solution containing 0.68 g of 2-methylimidazole ($\text{C}_4\text{H}_6\text{N}_2$, Aldrich,
25 98%) and 11.3 g of methanol was then added to the zinc-based solution, and the mixture was stirred for 1 hour. The resulting particles were separated from the gel by centrifugation at 14,000 rpm and rinsed with methanol. This procedure was repeated twice. The final ZIF-8 powder was dried overnight at 75°C. Then, ZIF-8 were coated by niosomes to generate niosome-coated ZIF-8 (ZN).

30

Synthesis of hierarchically porous ZIF-8 (HPZ): Microporous ZIF-8 was synthesised in a water phase at room temperature. First, 6.25 g 2-methylimidazole was dissolved in 45 mL deionized (DI) water to form clear solution A. Then 0.01 g SDS was added to solution A. After that 1.25 g $\text{Zn}(\text{CH}_3\text{COO}^-)_2 \cdot 2\text{H}_2\text{O}$ was dissolved in 5 mL DI water to form clear

solution B. Then, solution B was slowly added to solution A and stirred for 24 h at room temperature, and the mixed solution gradually became white and turbid. After the reaction was complete, the mixture was centrifuged at 14,000 rpm, washed with deionised water and anhydrous ethanol, and dried under vacuum for 12 h at 60°C to obtain a white powder solid.

- 5 Then, HPZ were coated by niosomes to generate niosome-coated hierarchically porous ZIF-8 (HPZN).

- Synthesis of KIT-6 periodic mesoporous organosilica:** Pluronic P123 triblock copolymer and n-butanol (n-BuOH) were used as structure-directing agents. The process began by
- 10 dissolving 4.0 g of P123 in a solution of 144 g deionized (DI) water and 7.9 g of 35 wt% HCl while stirring at 35°C. Next, 4.0 g of n-BuOH was added to the mixture. After stirring for 1 hour, 8.6 g of TEOS was added to the clear solution. The mixture was then stirred vigorously at 35°C for 24 hours. Following this, the temperature was increased to 100°C, and the mixture was aged under static conditions for another 24 hours. The resulting white
 - 15 precipitate was filtered without additional washing and dried under vacuum at 100°C overnight. The template was removed by briefly washing with an ethanol/HCl solution after calcination at 550°C for 6 hours.

Example 2: Synthesis of novel nanostructures

- 20 **Synthesis of PEG-bridged ZIF-8:** A solution of PEGX-linked bis-imidazole in MeOH (150 mL) was added to a fresh prepared Zn-based solution of 3.6 g of zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) dissolved in MeOH (150 mL), and allowed to vigorously stir for 2 h. The gel-like product was centrifuged at 14000 rpm and washed thoroughly with (3×50) MeOH. The resultant PEGX-bridged Metal Organic Framework (or PEGX-bridged ZIF, X was the
- 25 MW of PEG in PEG-linked bis-imidazole) powder was dried overnight at 75°C to obtain PEG₂₀₀-bridged Metal Organic Framework (PEG₂₀₀ bridged ZIF (ZP200)) and PEG₃₀₀₀-bridged Metal Organic Framework (PEG₃₀₀₀ bridged ZIF (ZP3000)). Then, the PEGX-bridged Metal Organic Framework was coated by niosomes to generate Niosome Coated PEG₂₀₀-bridged Metal–Organic Framework (PEG₂₀₀ bridged ZIF (ZP200N)), and Niosome
- 30 Coated PEG₃₀₀₀-bridged Metal–Organic Framework (PEG₃₀₀₀ bridged ZIF (ZP3000N)).

Synthesis of ionic liquid-coated ZIF-8 (ZIL): In order to synthesise histidine ionic liquid coated ZIF-8 (ZILH), cysteine ionic liquid coated ZIF-8 (ZILC), glycine ionic liquid coated ZIF-8 (ZILG), and lysine ionic liquid coated ZIF-8 (ZILL), 0.06 g of amino acid based-IL

(CysIL, LysIL, GlyIL, and HisIL) and 4.8 mL of MeOH were stirred for 1 h. Then 80 mg of ZIF-8 was added. It was stirred for 7 h at 35°C. It was placed at a temperature of 100°C for 2 h. Then the residue was washed 2 times with EtOH and separated by centrifuging at 14000 rpm for 5 min.

5

Synthesis of ionic liquid-coated hierarchically porous ZIF-8 (HPZIL): 0.06 g of amino acid IL (ZILH, ZILC, ZILG, ZILL) and 4.8 mL of methanol were stirred for 1 h. Then 80 mg of HPZ was added. It was stirred for 7 h at 35°C. It was placed at a temperature of 100°C for 2 h. Then the residue was washed 2 times with ethanol and separated by centrifuging at 14,000 rpm for 5 min to achieve HPZIL including hierarchically porous ZIF-8-histidine ionic liquid (HPZILH), hierarchically porous ZIF-8-cysteine ionic liquid (HPZILC), hierarchically porous ZIF-8-glycine ionic liquid (HPZILG), and hierarchically porous ZIF-8-lysine ionic liquid (HPZILL).

10

Synthesis of PEG-bridged KIT-6 PMO (PPMO): PPMO was prepared using bis-PEG-Si(OEt)₃ and aminopropyl triethoxysilane (APTES) as the Si resources. Briefly, n-BuOH (24.5 mmol, 1.8 g) was added to a mixture of P123 (MW = 5800, 1.9 g, 0.34 mmol), H₂O (72.0 g, 4 mol), and HCl (2 M), and then allowed to stir at 35°C for 60 min. Then bis-PEGX-Si(OEt)₃ (X = MW = 200, 3000, and 6000) and APTES were separately poured into the later mixture, such a way that the molar ratio in the final mixture was 0.25 APTES: 0.25 bis-PEG-Si(OEt)₃: 0.034 P123: 1.0 HCl: 2.45 BuOH: 400 H₂O. The mixture was continuously stirred for a day at 35°C, followed by keeping without stirring in a Teflon coated autoclave at 130°C for 3 days. The solid residue was washed thoroughly with water, separated, and then dried in the air atmosphere. Subsequently, the copolymer templating agent from the PMO materials was removed through two solvent extraction processes. First step was performed as follow: 200 mg of as-synthesised sample was stirred in a solution of 100 mL of EtOH and 3.0 g of HCl at 60°C for 12 h, and then the solid residual was separated and dried. Next, for further solvent extraction, H₂SO₄ treatment was done on the resultant solid by two successive circles with 100 mL of 48 wt% H₂SO₄ solution per 1.0 g of the as-synthesised sample at 90°C for 12 h. The resultant solid was filtered off, washed until the eluent became neutral, and then the residual solid was dried at room temperature in a vacuum oven to achieve PPMO₃₀₀₀ (P3), and PPMO₆₀₀₀ (P6) by using PEG MW = 3000, and PEG MW = 6000, respectively.

20

25

30

Synthesis of ionic liquid-coated PPMO: 0.06 g of amino acid IL (ZILH, ZILC, ZILG, ZILL) and 4.8 mL of methanol were stirred for 1 h. Then 80 mg of PPMO₃₀₀₀ (P3), and PPMO₆₀₀₀ (P6) were added. The mixture was stirred for 7 h at 35°C, then placed at a temperature of 100°C for 2 h. Then the residue was washed 2 times with ethanol and separated by centrifuging at 14,000 rpm for 5 min to obtain PPMO-amino acid IL including PPMO₃₀₀₀-Histidine Ionic Liquid (P3ILH or P3H), PPMO₃₀₀₀-Cysteine Ionic Liquid (P3ILC or P3C), PPMO₃₀₀₀-Glycine Ionic Liquid (P3ILG or P3G), PPMO₃₀₀₀-Lysine Ionic Liquid (P3ILL or P3L), PPMO₆₀₀₀-Histidine Ionic Liquid (P6ILH or P6H), PPMO₆₀₀₀-Cysteine Ionic Liquid (P6ILC or P6C), PPMO₆₀₀₀-Glycine Ionic Liquid (P6ILG or P6G), and PPMO₆₀₀₀-Lysine Ionic Liquid (P6ILL or P6L).

Example 3: Cytotoxicity of Nanoparticles via MTS Assay

To evaluate the cytotoxicity of various nanoparticle concentrations on ARPE-19 cells, an MTS assay was conducted based on a colorimetric protocol modified to reduce interference from culture media, as suggested by Huang et al. This method reliably measures cell viability without significant media-induced amplification of formazan production, ensuring that absorbance readings reflect actual cell numbers.

For the assay, ARPE-19 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) high glucose medium supplemented with 10% KnockOut™ Serum Replacement (KOSR), 1% penicillin/streptomycin, 1% non-essential amino acids, and 1% Glutamax. Cells were subcultured to reach a density of 1×10^4 cells/100 μ L and then seeded into the wells of a 96-well plate, avoiding edge wells to eliminate edge effects. The plate was incubated at 37°C for 24 hours in a 5% CO₂ humidified atmosphere to allow cell adherence. Following incubation, the culture medium was removed, and cells were treated with a twofold serial dilution of nanoparticle concentrations, ranging from 6.25 to 200 μ g/mL, to determine the concentration range that does not compromise cell viability. After a 24-hour exposure to nanoparticles, the culture medium was replaced with MTS reagent prepared in DPBS supplemented with 4.5 g/L glucose, at a volume of 100 μ L per well. The MTS reagent solution included 4 mL of MTS reagent in 16 mL DPBS with 0.09 g of D-(+)-Glucose for a total of 20 mL. Cells were incubated with MTS at 37°C for 1–4 hours in a 5% CO₂ humidified incubator. A parallel experiment was conducted in cell-free wells to determine the background absorbance, enabling accurate correction of readings for non-cellular components. Absorbance at 490 nm was recorded using a 96-well plate reader, and the

results provided a quantitative measure of viable cells, allowing identification of nanoparticle concentrations that are non-toxic to ARPE-19 cells. The MTS assay was performed in triplicate for each treatment condition to ensure statistical reliability and reproducibility of the results, with the mean absorbance values calculated and reported along
 5 with standard deviations (Figures 1–6).

ZIF-8 (Z), Niosome (N), hierarchically porous ZIF-8 (HPZ), and niosome-coated HPZ (HPZN): In the MTS assay, concentrations of nanoparticles below 100 µg/mL demonstrated no significant reduction in cell viability, indicating that these lower doses are well-tolerated
 10 by ARPE-19 cells. However, a noticeable decline in cell viability was observed at concentrations of 100 µg/mL and 200 µg/mL of Z, HPZ and HPZN, suggesting a dose-dependent cytotoxic effect for these nanoparticles. Niosomes may improve cellular uptake and decreasing the cellular toxicity.

PEG-bridged ZIF-8: The MTS analysis showed that there was no significant reduction in cell viability at any of the nanoparticle concentrations evaluated in this study except for 200 µg/mL of ZP3000 and ZP3000N.

Choline-amino acid ionic liquid: The MTS assays demonstrated that there was no
 20 significant reduction in cell viability at any of the nanoparticle concentrations evaluated in this study. This suggests that the nanoparticles at the tested dosages did not produce cytotoxic effects, highlighting their biocompatibility and potential appropriateness for future experimental applications.

Ionic liquid-coated ZIF-8 (ZIL): The viability of ARPE-19 cells significantly decreased at the highest concentrations of 100 µg/mL and 200 µg/mL. This further suggests that compound ZIF-8 exhibits a dose-dependent cytotoxic effect at these elevated concentrations.

Ionic liquid-coated hierarchically porous ZIF-8 (HPZIL): We also observed that the
 30 viability of ARPE-19 cells significantly decreased at the highest concentrations of 100 µg/mL and 200 µg/mL. These results also further highlights that compound ZIF-8 exhibits a cytotoxic at high concentrations. Hierarchical porous ZIF8 may improve the cargo release pattern.

Ionic liquid-coated PEG-bridged KIT-6 (PPMO): The MTS assays showed no significant decrease in cell viability at any of the nanoparticle concentrations tested in this cluster. This indicated that the nanoparticles, at the dosages used, did not exhibit cytotoxic effects, demonstrating their biocompatibility and potential suitability for future experiments.

5

Some of the nanoparticles tested showed no toxicity in the range of 6.25 to 200 ng/ μ L, while the others exhibited no toxicity at concentrations below 100 ng/ μ L. This indicates that these lower levels are safe for cellular use. Consequently, for subsequent *in vitro* analyses, nanoparticle concentrations were carefully chosen to remain below this threshold to ensure cell viability and minimize any potential cytotoxic effects. This selection approach not only preserves the integrity of the cellular model but also allows for a more accurate assessment of the nanoparticles' effects in future experiments.

10

Example 4: Thy1/CD90 is a specific marker for RGC5 cells

15

To achieve targeted delivery to RGCs, we first validated the specificity of Thy1 (aka CD90) as a specific cell surface marker for these cells. This validation is crucial because the effective targeting of nanoparticles relies heavily on the accurate recognition of unique surface markers that distinguish RGCs from surrounding cell types. By confirming that anti-Thy1 antibody binds selectively to RGCs, we can conjugate it to our nanoparticles, thereby enhancing the specificity of the nanoparticle targeting. This approach not only increases the likelihood of successful delivery of nanoparticles and their cargos but also minimizes off-target effects, which are common challenges in drug delivery systems. This strategic design leverages the natural affinity between the antibody and its target, paving the way for more effective treatments and improved outcomes in future applications.

25

Cell culture

The RGC5 cells (passages 5 to 9) and ARPE19 cells (passages 6 to 12) were provided from the Royan Stem Cell Bank grow in an DMEM-high glucose medium, which consists of 10% FBS, 1% penicillin-streptomycin, and 1% L-glutamine. Cell cultivation was performed to conduct absorption test experiments in 24-well plates, with 25,000 cells (RGC5) and 1,000,000 (ARPE19) cultured in each well. When cells reached approximately 70–80% confluence, they were detached from the culture vessel using a 10% trypsin-EDTA solution. The trypsin was neutralised with fresh complete medium, and the cell suspension was then

30

split into new sterile culture dishes or flasks. Cells were cultured and maintained under sterile conditions in a controlled environment with a temperature of 37°C and 5% CO₂.

Antibody selection for RGC cells

- 5 For selecting the specific antibody for RGC cells, we evaluated the expression profile of two known proteins including β III tubulin (Tuj1), and Thy1 on the RGC5 and ARPE19 cell lines with immunostaining technique. The cells from each group were fixed for 15 min in 4% paraformaldehyde. After washing with PBS, permeabilising with Triton X-100 (Sigma-Aldrich) for 10 min with gentle rocking and blocking process has been done for the
- 10 nonspecific background immunoreactivity. Non-specific sites were blocked with 3% BSA + 2.5% NGS (Normal Goat serum)/PBS for 1 h. Then the cells exposed with primary antibody, β III tubulin (ab18207) and Thy1 (Cell signaling 13801s) (1:200) in blocking buffer 3 h at 37°C, then, rinsed 1×10 min in PBS 0.1% Tx100 and 1×10 min in blocking buffer (RT). Secondary antibody: Goat anti-mouse & Rat Alexa-fluor 546 (1:500) in blocking buffer 1 h
- 15 at RT were used for 2 h. Images were captured using an IX71 fluorescent (Olympus, Japan).

Cellular particle absorption

- In this study, we conducted a particle uptake experiment with PPMO₃₀₀₀-Glycine Ionic Liquid nanoparticles (P3ILG or P3G), to assess the cellular absorption of particles. The
- 20 investigation was carried out precisely 48 hours after initiating the cell culture. The primary aim was to understand how cells interacted with and internalised particles under controlled conditions. The absorption test is carried out 48 hours after cell seeding. This time frame provides an optimal opportunity for the cells to reach their best morphological state. The procedure involved introducing particles into a predetermined volume of the culture medium
- 25 (2 ml). To ensure uniform distribution of particles within the medium, we employed a brief sonication process with intermittent paused (4-minute sonication process with 10-second breaks every 50 seconds).

- Subsequently, we added 150 μ l of particles to each well. The cells were then allowed to
- 30 incubate for three hours before fixation with a 4% paraformaldehyde solution. Finally, DAPI staining was used to visualize and stain the cell nuclei with DAPI (Sigma-Aldrich, D-8417), enabling the assessment of particle uptake by the cells.

Results

Immunostaining was employed to validate the expression of β -tubulin in both RGC5 and ARPE19 cell lines. The results demonstrated that β -tubulin was expressed in both cell lines, as shown in Figure 7. This indicates that β -tubulin is not specific to RGC5 cells and can also be expressed by ARPE19 cells.

5

To assess Thy1 expression, immunostaining was performed on RGC5 and ARPE19 cell lines. The data revealed that Thy1 was expressed exclusively in RGC5 cells, while no expression was detected in ARPE19 cells, as presented in Figure 8. These findings confirm that Thy1 serve as a specific marker for RGC5 cells.

10

Example 5: Conjugation of anti-Thy1 antibody to nanoparticles improves uptake in RGC5 cells

Synthesis of sulforhodamine-loaded anti-CD90 antibody-labelled glycine ionic liquid-coated PPMO (Sulf@CD90@PPMO₃₀₀₀-GlyIL)

15

2 mg of sulforhodamine dye and 1 mL of DMSO and PPMO₃₀₀₀-GlyIL (P3G) were shaken for 3 h in a shaker in the dark at room temperature. Then it was centrifuged at 14,000 rpm for 10 min. It was washed with 1 mL of ethanol and centrifuged again. The supernatant was separated and the precipitate (Sulf@PPMO₃₀₀₀-GlyIL) was stored in a freezer.

20

1.5 μ g of anti-CD90 antibody (22.5 μ L PBS, pH 7.4) was added to 5 mL of PBS (0.1 M, pH 5.8). The suspension was then added with 45 mg of DCC and 22.5 mg of NHS. After 30 min, the suspension was added dropwise with 15 mg of Sulf@PPMO₃₀₀₀-GlyIL dissolved in 4.5 mL of PBS (0.1 M, pH 5.8) at room temperature ($25 \pm 5^\circ\text{C}$) under sustained magnetic stirring for 12 h. The solution was at 14000 rpm and 4°C for 30 min to remove redundant DCC and unmodified CD90. The solution was centrifuged twice using the previously described conditions, and Sulf@CD90@PPMO₃₀₀₀-GlyIL (P3GAS) were obtained as precipitates. Figure 9 provides a comprehensive characterization of the PPMO₃₀₀₀-GlyIL, showcasing their pore structure, particle morphology, and crystalline properties through various analytical techniques.

30

In vitro uptake assay

To evaluate the impact of antibody conjugation on nanoparticle uptake, different ratios of antibody to nanoparticles were prepared (Table 1) in 600 μ L and were sonicated and applied

on RGCs and ARPE19 cells as control, in 3 replicates. Then the cells incubated 37°C for 3 h. After washing with PBS 2×5 min, the cells stained with DAPI and observed with fluorescent microscope (IX71; Olympus, Japan).

5 **Table 1.** Ratios of antibody to nanoparticles used for conjugation.

Name	Antibody concentration (ng/μl)	Nanoparticle concentration (μg/μl)
A0/N25	0	25
A0/N75	0	75
A0/N150	0	150
A1/N25	1	25
A3/N75	3	75
A6/N150	6	150
A2.5/N25	2.5	25
A5/N75	7.5	75
A15/N150	15	150
A10/N25	10	25
A20/N75	30	75
A60/N150	60	150

Figures 10–12 illustrate the nanoparticle uptake in ARPE19 and RGC5 cells treated with sulforhodamine-loaded nanoparticles prepared using different antibody/nanoparticle (Ab/NP) ratios and concentrations. In this experiment, the successful uptake of nanoparticles into the cytoplasm (visible as fluorescence within the cells) was used to assess the effectiveness of the Ab/NP ratios and concentrations. A higher presence of red fluorescence in the cytoplasm indicated more effective uptake. The results confirmed that RGC5 cells showed a significantly greater uptake of sulforhodamine-labelled nanoparticles compared to the ARPE19 control cells, which exhibited minimal internalisation. Specifically, analysis revealed that the Ab/NP concentrations of 1:25, 3:75, 2.5:25, 7.5:75, 10:25, and 30:75 (Table 1) effectively facilitated nanoparticle entry into the cytoplasm of RGC5 cells, with clear red fluorescence observed within these cells. In contrast, the control cells did not display significant cytoplasmic uptake, with the particles appearing randomly distributed and distant from the nuclei, likely trapped between cells.

Based on these observations, a 2.5 ng/ μ l : 25 μ g/ μ l ratio of antibody to nanoparticles was selected to further assess the impact of antibody conjugation on nanoparticle uptake. RGC5 cells were treated with nanoparticles both with and without antibody labelling. Three antibody/nanoparticle concentrations: 2.5, 1.25, and 0.625 ng/ μ L of antibody to 25, 12.5, and 6.25 μ g/ μ L of nanoparticles, respectively, were tested as shown in Figure 13. Nanoparticle without antibody (Sulforhodamine-loaded PPMO-Glycine Ionic Liquid (Sulf@PPMO₃₀₀₀-GlyIL)(P3GS)) was used as control. The results demonstrated a significant enhancement in nanoparticle uptake when nanoparticles were conjugated with antibodies. Specifically, in cells exposed to antibody-labelled nanoparticles, a markedly higher accumulation of nanoparticles was observed within the cytoplasm, with fluorescence intensity indicating strong intracellular presence. In contrast, cells treated with non-labelled nanoparticles displayed minimal uptake, with particles either absent from the cytoplasm or probably loosely bound to the cell surface. Based on this result, antibody/nanoparticle concentration ratio of 1.25 ng/ μ L of antibody to 12.5 μ g/ μ L of nanoparticles was selected for *in vivo* study.

Example 6: Uptake of erythropoietin-loaded anti-CD90-labelled glycine ionic liquid-coated PPMO (EPO@CD90@PPMO₃₀₀₀-GlyIL) nanoparticles *in vitro*

Erythropoietin is known for its neuroprotective and anti-apoptotic properties and has shown promise in protecting retinal ganglion cells (RGCs) from degeneration or death caused by various retinal diseases and injuries, including glaucoma, diabetic retinopathy, optic neuritis, ischemic optic neuropathy, and retinal ischemia and degeneration.

Preparation of EPO@PPMO₃₀₀₀-GlyIL and EPO@CD90@PPMO₃₀₀₀-GlyIL

0.06 g of glycine-based Ionic liquid (GlyIL) and 4.8 mL of methanol were mixed for 1 h. Then 80 mg of PPMO₃₀₀₀ was added. It was stirred for 7 h at 35°C. It was placed at a temperature of 100°C for 2 h. the solid residue washed 2 times with ethanol and collected by centrifuge at 14000 rpm for 5 min to have PPMO₃₀₀₀-GlyIL.

120 μ L of EPO and 1 mL of PBS (0.1 M, pH 5.8) and PPMO₃₀₀₀-GlyIL were shaken for 3 h on a shaker in the dark and at room temperature. Then, it was centrifuged at 14,000 rpm for 10 min. It was washed with 1 mL of PBS and centrifuged again. The supernatant was separated and the precipitate (EPO@PPMO₃₀₀₀-GlyIL(P3GE)) was stored in a freezer.

1.5 µg of anti-CD90 antibody (22.5 µL PBS, pH 7.4) was added to 5 mL of PBS (0.1 M, pH 5.8). The suspension was then added with 45 mg of DCC and 22.5 mg of NHS. After 30 min, the suspension was added dropwise with 15 mg of EPO@PPMO₃₀₀₀-GlyIL dissolved in 4.5 mL of PBS (0.1 M, pH 5.8) at room temperature (25± 5 °C) under sustained magnetic stirring for 12 h. The solution was at 14000 rpm and 4 °C for 30 min to remove redundant DCC and unmodified CD90. The solution was centrifuged twice using the previously described conditions, and EPO@CD90@PPMO₃₀₀₀-GlyIL (P3GAE) was obtained as precipitates.

10

In vitro uptake assay

Three antibody/nanoparticle ratios: 2.5, 1.25, and 0.625 ng/µL of antibody to 25, 12.5, and 6.25 µg/µL of nanoparticles, respectively, were used to test the uptake of Ab/NP in RGCs and ARPE19 cells. Nanoparticle without antibody (EPO@PPMO₃₀₀₀-GlyIL) (P3GE)) was used as control.

15

The results revealed a significant increase in nanoparticle uptake in RGC5 cells when the particles were conjugated with antibodies (Figure 14). Specifically, cells exposed to antibody to NP ratio of 2.5:25 showed a much higher accumulation within uptake with strong fluorescence intensity indicating substantial intracellular presence. In contrast, cells treated with non-labelled nanoparticles exhibited minimal uptake, with particles largely absent from the cytoplasm or weakly associated with the cell surface. ARPE19 cells, used as a control, also showed no significant nanoparticle uptake, further emphasising the specificity of antibody-mediated targeting in RGC5 cells. These findings suggest that antibody labelling also enhances targeted EPO@CD90@PPMO₃₀₀₀-GlyIL (P3GAE) uptake, promoting more efficient delivery into RGC5 cells.

20

25

Example 7: Uptake of EPO@CD90@PPMO₃₀₀₀-GlyIL nanoparticles *in vivo*

Building on the findings in Example 6, we proceeded to test a 2.5 ng/µL: 25 µg/µL ratio of antibody to nanoparticles with and without erythropoietin for *in vivo* delivery.

30

C57BL/J6 male mice, approximately 8–10 weeks of age were kept on a 12 h day/night cycle. All procedures on the mice followed institutional guidelines and with the ARVO statement

for the use of animals in ophthalmic and vision research. The mice were anaesthetised with a 1:4 mixture of Xylazine/Ketamine.

In addition to the age-matched intact group mice that did not receive any surgery, other mice
5 received 2 µl intraocular (i.o.) infusions of Antibody/NPs. The i.o. injections were made via approaching the dorsal limbus and performed with a 30-gauge needle connected to a 10 µl Hamilton syringe.

One day following i.o. injection, the animals were sacrificed under anaesthesia and their
10 eyes harvested. The anterior segment that included the lens was removed. The posterior eyecup and optic nerves were fixed by immersion in 4% paraformaldehyde for 1 h at room temperature, cryoprotected in 30% sucrose/PBS for 24 h at 4°C, and then embedded in optimal cutting temperature compound (OCT/Leica Microsystems). The specimens were cryo-sectioned into 8-µm sections and mounted on Superfrost plus slides (Thermo Scientific,
15 4951, Germany). The immunohistofluorescent study was carried out by blocking the sections in PBS that contained 5% normal goat serum and permeabilised with 0.3% Triton X-100 for 1 h at room temperature. Then, sections were incubated with primary antibodies diluted in blocking solution, overnight at 4°C. After three successive washes with PBS/Tween, the slides were incubated with proper secondary antibodies for 1 h at room
20 temperature. Nuclei were counterstained with DAPI.

In the control group, where nanoparticles lacked antibody conjugation (EPO@PPMO₃₀₀₀-GlyIL (P3GE)), minimal erythropoietin staining was observed in the ganglion cell layer, indicating limited uptake (Figure 15). Notably, when nanoparticles were conjugated with
25 antibodies, antibody staining revealed robust erythropoietin uptake directly into the cytoplasm of retinal ganglion cells (Figure 15). These findings clearly demonstrate that antibody conjugation in EPO@CD90@PPMO₃₀₀₀-GlyIL (P3GAE) significantly enhanced nanoparticle uptake by cells in GCL, as the antibody-functionalised particles effectively delivered erythropoietin within target cells.

30

Example 8: Uptake of EPO@CD90@PPMO₃₀₀₀-HisIL and EPO@CD90@PPMO₃₀₀₀-LysIL nanoparticles *in vivo*

Following the successful *in vivo* uptake results observed with EPO@CD90@PPMO₃₀₀₀-GlyIL described in Example 7, the nanoparticles were modified by replacing amino acid

glycine with histidine and lysine. In this experiment, four types of nanoparticles were evaluated including antibody-conjugated (EPO@CD90@PPMO₃₀₀₀-HisIL (P3HAE) and EPO@CD90@PPMO₃₀₀₀-LysIL (P3LAE)) and non-antibody-conjugated (EPO@PPMO₃₀₀₀-HisIL (P3HE) and EPO@PPMO₃₀₀₀-LysIL (P3LE)) forms.

5

Preparation of nanoparticles

In order to produce PPMO₃₀₀₀-HisIL and PPMO₃₀₀₀-LysIL, 0.06 g of Histidine-based Ionic liquid (HisIL), or Lysine-based Ionic liquid (LysIL), respectively, and 4.8 mL of methanol were mixed for 1 h. Then 80 mg of PPMO₃₀₀₀ was added. It was stirred for 7 h at 35°C. It was placed at a temperature of 100°C for 2 h. the solid residue washed 2 times with ethanol and collected by centrifuge at 14,000 rpm for 5 min.

To obtain EPO@PPMO₃₀₀₀-HisIL (P3HE) and EPO@PPMO₃₀₀₀-LysIL (P3LE), 120 µL of EPO and 1 mL of PBS (0.1 M, pH 5.8) and PPMO₃₀₀₀-HisIL and PPMO₃₀₀₀-LysIL (P3GL), respectively, were shaken for 3 h on a shaker in the dark and at room temperature. Then, it was centrifuged at 14,000 rpm for 10 min. It was washed with 1 mL of PBS and centrifuged again. The supernatant was separated and the precipitate was stored in a freezer.

To obtain EPO@CD90@PPMO₃₀₀₀-HisIL (P3HAE) and EPO@CD90@PPMO₃₀₀₀-LysIL (P3LAE), 1.5 µg of anti-CD90 antibody (22.5 µL PBS, pH 7.4) was added to 5 mL of PBS (0.1 M, pH 5.8). The suspension was then added with 45 mg of DCC and 22.5 mg of NHS. After 30 min, the suspension was added dropwise with 15 mg of EPO@PPMO₃₀₀₀-HisIL (P3HE) and EPO@PPMO₃₀₀₀-LysIL (P3LE), respectively, dissolved in 4.5 mL of PBS (0.1 M, pH 5.8) at room temperature (25± 5 °C) under sustained magnetic stirring for 12 h. The solution was at 14000 rpm and 4°C for 30 min to remove redundant DCC and unmodified CD90. The solution was centrifuged twice using the previously described conditions, and P3HAE and P3LAE were obtained as precipitates.

In vivo uptake assay

In vivo delivery was performed as described in Example 7 using four types of nanoparticles including antibody conjugated (P3HAE and P3LAE) and non-antibody-conjugated (P3HE and P3LE) forms.

30

When antibody-conjugated nanoparticle P3HAE were introduced, they demonstrated successful accumulation of erythropoietin in GCL, as evidenced by robust staining within the cytoplasm. Conversely, the antibody-conjugated nanoparticles P3LAE showed minimal erythropoietin staining in the ganglion cell layer, indicating ineffective uptake. In the non-
 5 conjugated forms of both types, uptake was also limited, with minimal staining observed in GCL (Figures 16 and 17).

These findings not only demonstrate that antibody conjugation significantly enhances P3HAE uptake into RGCs, but also underscore the importance of nanoparticle structure in
 10 achieving efficient delivery.

Figure 18 provides a comprehensive characterisation of the PPMO₃₀₀₀-HisIL nanoparticles, showcasing their pore structure, particle morphology, and crystalline properties through various analytical techniques.

15

Example 9: Antibody-conjugated CD90@PPMO₃₀₀₀-GlyIL nanoparticles are internalised by RGCs *in vivo*

To confirm that the observed *in vivo* uptake of EPO seen in Example 7 was due to the direct internalisation of nanoparticles and not merely due to the release of EPO by the nanoparticle
 20 with subsequent uptake of EPO by the cells, the CD90@PPMO₃₀₀₀-GlyIL nanoparticles were labelled with FITC. FITC labelling allows visual tracking of the nanoparticle itself within target cells through fluorescence imaging. This approach distinguishes whether the nanoparticle was localised into RGCs rather than only delivering its cargo extracellularly. In this experiment, two relatively similar nanostructured nanoparticles were prepared and
 25 tested in mouse retinas.

Preparation of CD90/FITC labelled PPMO-Glycine Ionic Liquid (CD90/FITC@PPMO₃₀₀₀-GlyIL) (P3GFA)

0.06 g of glycine-based Ionic liquid (GlyIL) and 4.8 mL of methanol were mixed for 1 h.
 30 Then 80 mg of PPMO₃₀₀₀ was added. It was stirred for 7 h at 35°C. It was placed at a temperature of 100°C for 2 h. the solid residue washed 2 times with ethanol and collected by centrifuge at 14000 rpm for 5 min to have PPMO₃₀₀₀-GlyIL. 15 mg of PPMO₃₀₀₀-GlyIL (was added to a solution of Fluorescein thiocyanate (FITC) (3 mg) in 1 mL DMF and the mixture was stirred in the dark overnight. After that 2 mL deionized water was added to the

mixture and the orange solid was centrifuged. The obtained solid was thoroughly washed with deionized water and EtOH. Then, FITC@PPMO₃₀₀₀-GlyIL (P3GF) gave via vacuum drying as an orange powder. 1.5 µg of anti-CD90 antibody (22.5 µL PBS, pH 7.4) was added to 5 mL of PBS (0.1 M, pH 5.8). The suspension was then added with 45 mg of DCC and 22.5 mg of NHS. After 30 min, the suspension was added dropwise with 15 mg of FITC@PPMO₃₀₀₀-GlyIL dispersed in 4.5 mL of PBS (0.1 M, pH 5.8) at room temperature (25 ± 5 °C) under sustained magnetic stirring for 12 h. The solution was at 14000 rpm and 4 °C for 30 min to remove redundant DCC and unmodified CD90. The solution was centrifuged twice using the previously described conditions, and CD90/FITC@PPMO₃₀₀₀-GlyIL (P3GFA) were obtained as precipitates.

Preparation of anti-CD90 antibody-labelled FITC grafted glycine ionic liquid-coated PPMO (CD90@FITC-PPMO₃₀₀₀-GlyIL (P3FGA))

5 µL of 3-aminopropyltrimethoxysilane was poured to 10 mg FITC dissolved in 10 mL DMF, and allowed to stir at room temperature overnight under inert atmosphere. The solvent was evaporated under vacuum and dark condition. The residue was added to 100 mg of PPMO₃₀₀₀ dispersed in 10 mL EtOH and refluxed at 70 °C and dark condition. The solid product was centrifuged and washed 2 times with EtOH. FITC-PPMO₃₀₀₀ was obtained as a yellow-wish powder, after vacuum drying. 0.06 g of glycine-based Ionic liquid (GlyIL) and 4.8 mL of methanol were mixed for 1 h. Then 80 mg of FITC-PPMO₃₀₀₀ was added. It was stirred for 7 h at 35°C. It was placed at a temperature of 100 °C for 2 h. the solid residue washed 2 times with ethanol and collected by centrifuge at 14000 rpm for 5 min to have FITC-PPMO₃₀₀₀-GlyIL (P3FG). 1.5 µg of anti-CD90 antibody (22.5 µL PBS, pH 7.4) was added to 5 mL of PBS (0.1 M, pH 5.8). The suspension was then added with 45 mg of DCC and 22.5 mg of NHS. After 30 min, the suspension was added dropwise with 15 mg of FITC-PPMO₃₀₀₀-GlyIL dispersed in 4.5 mL of PBS (0.1 M, pH 5.8) at room temperature (25± 5 °C) under sustained magnetic stirring for 12 h. The solution was at 14000 rpm and 4 °C for 30 min to remove redundant DCC and unmodified CD90. The solution was centrifuged twice using the previously described conditions, and CD90@FITC-PPMO₃₀₀₀-GlyIL (P3FGA) were obtained as precipitates.

In vivo uptake assay

In vivo delivery was performed as described in Example 7. Four nanoparticle formulations were analysed including control (without antibody conjugation, P3GF and P3FG) and

antibody-conjugated (with Thy1 antibody, P3GFA and P3FGA). Initially, lyophilised nanoparticle powder was reconstituted in 300 ml of PBS, sonicated, and then applied at a concentration of 240 $\mu\text{l/ml}$ in a 12-well plate. In addition, 2 μl of the nanoparticle suspension was injected into the vitreous of mice. Absorption was assessed at 24 hours following
 5 fixation of the cells and mouse eyes with paraformaldehyde.

Images showed that antibody-conjugated green nanoparticles (P3GFA and P3FGA) were clearly detectable within the GCL (Figures 19 and 20). In both P3FG and P3GF groups, the presence of nanoparticles was significantly enhanced when conjugated with the Thy1
 10 antibody, indicating improved uptake and localisation within the target cells.

By confirming the direct uptake of FITC-labelled nanoparticles, this experiment provides stronger evidence of the ability of the nanoparticles to reach and enter the target cells, thereby validating its functionality as a precise delivery vehicle.
 15

Example 10: Antibody-conjugated CD90@PPMO₃₀₀₀-GlyIL nanoparticles localise to the retinal ganglion cell layer (GCL)

The results of Example 9 clearly showed that nanoparticles CD90/FITC@PPMO₃₀₀₀-GlyIL (P3GFA) and CD90@FITC-PPMO₃₀₀₀-GlyIL (P3FGA) labelled with FITC could target
 20 cells in retinal ganglion cell layer. This experiment aimed to verify that FITC-labelled nanoparticles could selectively localise to RGCs in the retinal ganglion cell layer, excluding other cell types and thus ensuring the precision necessary for therapeutic applications.

To address this, we utilised three unique biomarkers specific to RGCs (Thy1, Tuj1, and
 25 RBPMS). Co-localisation of the FITC signal with each of these target cell markers can strengthen the evidence that P3FGA and P3GFA directly enters mouse RGCs in retinal ganglion cell layer.

In vivo uptake assay

In vivo delivery was performed as described in Examples 7 and 9. Four nanoparticle formulations were analysed including control (without antibody conjugation, P3GF and P3FG) and antibody-conjugated (with Thy1 antibody, P3GFA and P3FGA). RGCs were stained with specific antibodies for cytoplasmic and axonal markers: Thy1 (Cell Signaling, 13801s), Tuj1 (Abcam, 18207), and RBPMS (Phosphosolutions, 1830).
 30

Microscopic analysis showed co-localisation of FITC-labelled nanoparticles with the RGC-specific markers (Figures 21–23), confirming that the nanoparticles were predominantly associated with RGCs and not with other cells in the layer. This successful targeting demonstrates the specificity of our approach, indicating that FITC-labelled nanoparticles can deliver agents directly to RGCs in a complex cellular environment, thereby minimising off-target effects and enhancing potential therapeutic precision.

Example 11: Antibody-conjugated CD90@PPMO₃₀₀₀-HisIL nanoparticles are internalised by RGCs *in vivo*

Following the successful results observed with EPO@CD90@PPMO₃₀₀₀-HisIL described in Example 8, we aimed to confirm that the observed *in vivo* uptake of CD90@PPMO₃₀₀₀-HisIL was due to the direct internalisation of nanoparticle by RGCs. We labelled CD90@PPMO₃₀₀₀-HisIL with FITC. FITC labelling allows us to visually track the presence of the nanoparticle itself within target cells through fluorescence imaging.

Preparation of CD90/FITC@PPMO₃₀₀₀-HisIL (P3HFA)

0.06 g of His-based Ionic liquid (HisIL) and 4.8 mL of methanol were mixed for 1 h. Then 80 mg of PPMO₃₀₀₀ was added. It was stirred for 7 h at 35°C. It was placed at a temperature of 100°C for 2 h. the solid residue washed 2 times with ethanol and collected by centrifuge at 14000 rpm for 5 min to have PPMO₃₀₀₀-HisL.

15 mg of PPMO₃₀₀₀-GlyIL was added to a solution of Fluorescein thiocyanate (FITC) (3 mg) in 1 mL DMF and the mixture was stirred in the dark overnight. After that 2 mL deionized water was added to the mixture and the orange solid was centrifuged. The resulted solid was thoroughly washed with deionized water and EtOH. Then, FITC@PPMO₃₀₀₀-HisIL (P3HF) gave via vacuum drying as an orange powder.

1.5 µg of anti-CD90 antibody (22.5 µL PBS, pH 7.4) was added to 5 mL of PBS (0.1 M, pH 5.8). The suspension was then added with 45 mg of DCC and 22.5 mg of NHS. After 30 min, the suspension was added dropwise with 15 mg of FITC@PPMO₃₀₀₀-HisIL dispersed in 4.5 mL of PBS (0.1 M, pH 5.8) at room temperature (25± 5 °C) under sustained magnetic stirring for 12 h. The solution was at 14000 rpm and 4 °C for 30 min to remove redundant DCC and unmodified CD90. The solution was centrifuged twice using the previously

described conditions, and CD90/FITC@PPMO₃₀₀₀-HisIL (P3HFA) were obtained as precipitates.

In vivo uptake assay

- 5 *In vivo* delivery was performed as described in Examples 7 and 9. Two nanoparticle formulations were analysed: control (without antibody conjugation, labelled as P3HF) and antibody-conjugated (with Thy1 antibody, labelled as P3HFA). Figure 24 shows that antibody-conjugated green nanoparticles were clearly detectable within the GCL. In P3HFA group, the presence of nanoparticles was significantly enhanced, indicating improved uptake and localization within the target cells. However, nanoparticles without conjugated antibody showed lower accumulation of nanoparticles in GCL.

Example 12: Topical administration of nanoparticles for erythropoietin (EPO) delivery

- While intravitreal injection is effective for delivering macromolecules, including CRISPR-Cas RNPs and other large cargoes, it is invasive, requires clinical expertise, and is not ideal for repeated or long-term dosing. For drug delivery, non-invasive routes are clinically preferable. To assess the translational potential of the nanoparticle platform for topical drug delivery to the eye, we evaluated the topical administration of nanoparticles carrying erythropoietin (EPO) using eye drops. Demonstrating nanoparticle-mediated delivery of EPO into relevant ocular tissues following topical application supports the feasibility of a patient-friendly, repeatable, and non-invasive therapeutic approach.

Preparation of nanoparticles

- PPMO₃₀₀₀-Glycine Ionic Liquid nanoparticles (P3G) were prepared as described in Example 2. P3GE and P3GAE were prepared as described in Example 6.

- Preparation of EPO@chitosan coated-CD90@PPMO₃₀₀₀-GlyIL (P3GACsE):** 1.5 µg of anti-CD90 antibody (22.5 µL PBS, pH 7.4) was added to 5 mL of PBS (0.1 M, pH 5.8). The suspension was mixed with 45 mg of DCC and 22.5 mg of NHS. After 30 min, the suspension was added dropwise to 15 mg of P3G in 4.5 mL of PBS (0.1 M, pH 5.8) at room temperature (25 ± 5 °C), and the mixture was stirred overnight. The solution was centrifuged at 14,000 rpm and 4°C for 30 min to remove excess DCC and unmodified CD90 antibody. The solution was centrifuged twice using the previously described conditions, and CD90@PPMO₃₀₀₀-GlyIL (P3GA) were obtained as precipitates. Then 120 µL of EPO and 1

mL of PBS (0.1 M, pH 5.8) and P3GA were shaken for 3 h on a shaker in the dark and at room temperature. Then, it was washed and centrifuged to give EPO@P3GA (P3GAE). Then it was added to a clear solution of 7.5 mg of chitosan (Cs) in 5.6 mL of 1% acetic acid. After 30 min, 8.4 mg of sodium tripolyphosphate (TPP), previously dissolved in 5.6 mL of distilled water, was added dropwise to the solution. The mixture was then stirred for 1 h at room temperature in the dark, followed by centrifugation, and the precipitated material was collected as P3GACsE.

- Preparation of EPO@CD90@chitosan coated-CD90@PPMO3000-GlyIL (P3GACsAE):** 1.5 μ g of anti-CD90 antibody (22.5 μ L PBS, pH 7.4) was added to 5 mL of PBS (0.1 M, pH 5.8). The suspension was then added with 45 mg of DCC and 22.5 mg of NHS. After 30 min, the suspension was added dropwise with 15 mg of P3G dispersed in 4.5 mL of PBS (0.1 M, pH 5.8) at room temperature (25 ± 5 °C) under sustained magnetic stirring overnight. The solution was at 14,000 rpm and 4 °C for 30 min to remove redundant DCC and unmodified CD90 antibody. The solution was centrifuged twice using the previously described conditions, and CD90@PPMO3000-GlyIL (P3GA) were obtained as precipitates. Then 120 μ L of EPO and 1 mL of PBS (0.1 M, pH 5.8) and P3GA were shaken for 3 h on a shaker in the dark and at room temperature. Then, it was washed and centrifuged to give EPO@P3GA (P3GAE). Then it was added to a clear solution of 7.5 mg of chitosan (Cs) in 5.6 mL acetic acid 1%. Following 30 min, 8.4 mg of sodium tripolyphosphate (TPP), previously dissolved in 5.6 mL of distilled water, was added dropwise to the solution. The mixture was then stirred for 1 h at room temperature in the dark, followed by centrifugation, and the precipitated material was collected as P3GACsE.
- 1.5 μ g of CD90 antibody (22.5 μ L PBS, pH 7.4) was added to 5 mL of PBS (0.1 M, pH 5.8). The suspension was then added with 45 mg of DCC and 22.5 mg of NHS. After 30 min, the suspension was added dropwise with 15 mg of P3GACsE dispersed in 4.5 mL of PBS (0.1 M, pH 5.8) at room temperature (25 ± 5 °C) under sustained magnetic stirring overnight. The solution was at 14,000 rpm and 4 °C for 30 min to remove redundant DCC and unmodified CD90 antibody. The solution was centrifuged twice using the previously described conditions, and EPO@CD90@P3GACs (P3GACsAE) were obtained as precipitates.

Preparation of EPO@CMC coated-CD90@PPMO3000-GlyIL (P3GACmE): 1.5 μ g of CD90 antibody (22.5 μ L PBS, pH 7.4) was added to 5 mL of PBS (0.1 M, pH 5.8). The

suspension was then added with 45 mg of DCC and 22.5 mg of NHS. After 30 min, the suspension was added dropwise with 15 mg of P3G dispersed in 4.5 mL of PBS (0.1 M, pH 5.8) at room temperature ($25 \pm 5^\circ\text{C}$) under sustained magnetic stirring overnight. The solution was centrifuged at 14,000 rpm and 4°C for 30 min to remove redundant DCC and unmodified CD90 antibody. The solution was centrifuged twice using the previously described conditions, and CD90@PPMO₃₀₀₀-GlyIL (P3GA) were obtained as precipitates. Then 120 μL of EPO and 1 mL of PBS (0.1 M, pH 5.8) and P3GA were shaken for 3 h on a shaker in the dark and at room temperature. Then, it was washed and centrifuged to give EPO@P3GA (P3GAE). Then it was added to a clear solution of 5% w/v carboxymethylcellulose (CMC) in PBS (pH 7.4). After stirring for 30 min, 4 mL glutaraldehyde 0.1% was added to the mixture and the mixture was allowed to stir for 2 h at room temperature in the dark. The solid residue was separated by centrifugation, and the precipitated material was collected as EPO@CMC coated-CD90@PPMO₃₀₀₀-GlyIL (P3GACmE).

15

Preparation of EPO@CD90@CMC coated-CD90@PPMO₃₀₀₀-GlyIL (P3GACmAE):
1.5 μg of CD90 antibody (22.5 μL PBS, pH 7.4) was added to 5 mL of PBS (0.1 M, pH 5.8). The suspension was then added with 45 mg of DCC and 22.5 mg of NHS. After 30 min, the suspension was added dropwise with 15 mg of P3G dispersed in 4.5 mL of PBS (0.1 M, pH 5.8) at room temperature ($25 \pm 5^\circ\text{C}$) under sustained magnetic stirring overnight. The solution was at 14,000 rpm and 4°C for 30 min to remove excess DCC and unmodified CD90 antibody. The solution was centrifuged twice using the previously described conditions, and CD90@PPMO₃₀₀₀-GlyIL (P3GA) were obtained as precipitates. Then 120 μL of EPO and 1 mL of PBS (0.1 M, pH 5.8) and P3GAmA were shaken for 3 h on a shaker in the dark and at room temperature. Then, it was washed and centrifuged to give EPO@P3GA (P3GAE). Then it was added to a clear solution of 5% w/v carboxymethylcellulose CMC in PBS (pH 7.4). After stirring for 30 min, 4 mL glutaraldehyde 0.1% was added to the mixture and allowed to stir for 2 h at room temperature in the dark. The solid residue was separated by centrifugation, and the precipitated material was collected as P3GACmE.

30

1.5 μg of CD90 antibody (22.5 μL PBS, pH 7.4) was added to 5 mL of PBS (0.1 M, pH 5.8). The suspension was then added with 45 mg of DCC and 22.5 mg of NHS. After 30 min, the suspension was added dropwise with 15 mg of P3GACmE dispersed in 4.5 mL of PBS (0.1 M, pH 5.8) at room temperature ($25 \pm 5^\circ\text{C}$) under sustained magnetic stirring overnight. The

solution was centrifuged at 14,000 rpm and 4°C for 30 min to remove excess DCC and unmodified CD90 antibody. The solution was centrifuged twice using the previously described conditions, and EPO@CD90@CMC coated-CD90@PPMO₃₀₀₀-GlyIL (P3GACmAE) was obtained.

5

Topical delivery

C57BL/6 mice were used for all *in vivo* studies. Animals were anesthetized using a ketamine-xylazine mixture prepared at a 1:4 ratio and administered subcutaneously. For topical delivery experiments, 15 µL of the nanoparticle formulation was applied to each eye. Applications were performed every 10 minutes for a total of four treatments. Mice were maintained for 24 hours post-application before tissue collection. Antibody-conjugated green nanoparticles were applied to the ocular surface. Four eyes per group were collected for histological examination. Eyes were fixed in 4% paraformaldehyde (PFA) for 1 hour at room temperature, transferred to a sucrose/PBS solution for cryoprotection, and stored at 4°C. Each eye was cryosectioned at a thickness of 8 µm, and six slides were prepared per sample, with each slide containing 12–14 tissue sections.

Tissue sections were washed in PBS for 5 minutes and permeabilized with 0.5% Triton X-100 for 30 minutes. Sections were blocked with 10% normal goat serum for 10 minutes and incubated overnight at 4°C with rabbit anti-erythropoietin (EPO) antibody at a dilution of 1:200. The following day, slides were washed three times in PBS for 5 minutes each and incubated for 3 hours at room temperature with goat anti-rabbit Alexa Fluor 485 (green) secondary antibody at a dilution of 1:300. Sections were washed again three times in PBS, counterstained with DAPI for 5 minutes, washed once more, and mounted using Fluor-mount. Imaging was performed using a BX51 microscope (Olympus, Japan).

Results

The delivery of erythropoietin (EPO) using six nanoparticle formulations were analysed including control (without antibody conjugation, P3GE), antibody-conjugated (P3GAE), EPO@chitosan coated-CD90@PPMO₃₀₀₀-GlyIL (P3GACsE), EPO@CD90@chitosan coated-CD90@PPMO₃₀₀₀-GlyIL (P3GACsAE), EPO@CMC coated-CD90@PPMO₃₀₀₀-GlyIL (P3GACmE), and EPO@CD90@CMC coated-CD90@PPMO₃₀₀₀-GlyIL (P3GACmAE).

In the control group, where nanoparticles lacked antibody conjugation (P3GE), only minimal erythropoietin staining was detected in the ganglion cell layer, indicating poor penetration and uptake following topical administration. In sharp contrast, antibody-conjugated nanoparticles (P3GAE) produced strong intracellular erythropoietin signals within retinal ganglion cells. This robust staining pattern confirms that the functionalized nanoparticles successfully traversed the ocular surface barriers and delivered their cargo directly into the target neuronal layer using only eye-drop administration. These results provide clear evidence that our antibody-conjugated nanoparticle platform enables efficient, non-invasive delivery to the ganglion cell layer (Figure 26).

We then coated the nanoparticles with chitosan (Cs) or carboxymethyl cellulose (Cm) to enhance stability in ready-to-use liquid formulations, improve formulation uniformity, and facilitate injectability. Both polymers are known to extend shelf-life and improve the performance of liquid ready-to-use products.

Chitosan, a natural cationic polysaccharide, enhances formulation stability through its mucoadhesive properties and its ability to form polyelectrolyte complexes that protect active components from degradation. Carboxymethyl cellulose (CMC) functions as a viscosity-modifying and stabilising agent, preventing nanoparticle aggregation and sedimentation, thereby maintaining homogeneity and consistent flow characteristics required for injectable formulations. Together, these polymers contribute to extended shelf-life and improved performance of liquid ready-to-use products.

Figures 27A and 28A show that antibody-conjugated nanoparticles coated with chitosan or carboxymethyl cellulose (P3GACsE and P3GACmE) were clearly detectable within the ganglion cell layer (GCL). To assess whether these polymer coatings might impede nanoparticle delivery to the GCL, we also evaluated dual-layer antibody-coated formulations (EPO@CD90@chitosan-coated-CD90@PPMO3000-GlyIL, P3GACsAE; and EPO@CD90@CMC-coated-CD90@PPMO3000-GlyIL, P3GACmAE). The results showed no significant difference between single-layer and dual-layer antibody coatings, indicating that chitosan and carboxymethyl cellulose do not hinder antibody function or delivery efficiency (Figure 27B and 28B).

It will be appreciated that many further modifications and permutations of various aspects of the described embodiments are possible. Accordingly, the described aspects are intended to embrace all such alterations, modifications, and variations that fall within the spirit and scope of the appended claims.

CLAIMS

1. A nanoparticle comprising:
 - a) a porous core at least partially coated by an ionic liquid; and
 - b) a targeting moiety conjugated to the porous core.
2. The nanoparticle of claim 1, wherein the porous core is hierarchically porous.
3. The nanoparticle of claim 1 or 2, wherein the porous core comprises mesopores.
4. The nanoparticle of any one of claims 1 to 3, wherein the porous core is an organic-inorganic network material.
5. The nanoparticle of claim 4, wherein the organic-inorganic network material is a periodic mesoporous organosilica (PMO) or a metal-organic framework (MOF).
6. The nanoparticle of claim 4 or 5, wherein the organic-inorganic network material comprises a network structure comprising two or more inorganic centres connected by an organic polymer.
7. The nanoparticle of claim 6, wherein the organic polymer is selected from polyethylene glycol (PEG), polyethylene imine (PEI), polyethylene glycol amine (PEGN), diethylene glycol (DEG)-grafted PEI, triethylene glycol (TEG)-grafted PEI, and PEG-grafted PEI.
8. The nanoparticle of claim 6 or 7, wherein the organic polymer is characterised by a molecular weight of about 100 Da to about 10,000 Da.
9. The nanoparticle of any one of claims 5 to 8, wherein the PMO is characterised by a defined pore symmetry selected from KIT-6 (cubic), SBA-16 (cubic), FDU-12 (cubic), MCM-48 (cubic), SBA-15 (hexagonal), and MCM-41 (hexagonal).

10. The nanoparticle of any one of claims 5 to 8, wherein the MOF is characterised by a network structure selected from ZIF-8, ZIF-11, ZIF-67, MOF-5, M-MOF-74, MOF-199, MOF-545, MIL-53, MIL-101, CAU-1, and UiO-66.
11. The nanoparticle of any one of claims 1 to 10, wherein the porous core is characterised by an average pore size of about 7 nm to about 25 nm.
12. The nanoparticle of any one of claims 1 to 11, wherein the ionic liquid comprises a choline cation.
13. The nanoparticle of any one of claims 1 to 12, wherein the ionic liquid comprises an amino acid anion.
14. The nanoparticle of claim 13, wherein the amino acid is selected from glycine, histidine, cysteine, alanine, valine, serine or threonine.
15. The nanoparticle of any one of claims 1 to 14, wherein the ionic liquid comprises a cation to anion ratio of about 1:1 to about 1:1.2.
16. The nanoparticle of any one of claims 1 to 15, wherein the nanoparticle comprises a coating of niosomes, liposomes and/or a biopolymer on an exterior surface of the nanoparticle.
17. The nanoparticle of any one of claims 1 to 16, wherein the nanoparticle is characterised by a diameter of about 50 nm to about 200 nm.
18. The nanoparticle of any one of claims 1 to 17, wherein the targeting moiety is an antigen-binding molecule.
19. The nanoparticle of claim 18, wherein the antigen-binding molecule is an antibody or an antigen-binding fragment thereof.

20. The nanoparticle of claim 18 or 19, wherein the antigen-binding molecule binds specifically to Thy1 (CD90), TuJ1 (β -tubulin III), or RBPMS (RNA-binding protein with multiple splicing).
21. The nanoparticle of any one of claims 1 to 20, wherein the targeting moiety binds to a retinal cell (RGC).
22. The nanoparticle of claim 21, wherein the retinal cell is a retinal ganglion cell (RGC).
23. The nanoparticle of any one of claims 1 to 22, wherein the nanoparticle comprises at least one therapeutic agent.
24. The nanoparticle of claim 23, wherein the at least one therapeutic agent is selected from a small molecule, polypeptide, nucleic acid, gene editing system, and a fusion thereof.
25. The nanoparticle of claim 23 or 24, wherein the at least one therapeutic agent is selected from erythropoietin (EPO), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), platelet-derived growth factor (PDGF), ciliary neurotrophic factor (CNTF), retinoic acid, a lithium salt, glyceryl phosphate, a corticosteroid, an omega-3 fatty acid, vitamin E (tocopherol), and vitamin C (ascorbic acid).
26. The nanoparticle of claim 23 or 24, wherein the at least one therapeutic agent is a nucleic acid vector.
27. The nanoparticle of claim 23 or 24, wherein the at least one therapeutic agent is a CRISPR-Cas gene editing system.
28. The nanoparticle of any one of claims 1 to 27, wherein the nanoparticle comprises a detectable label.
29. The nanoparticle of any one of claims 1 to 27, wherein the nanoparticle comprises:

- a) a nanoparticle core comprising a polymeric-bridged periodic mesoporous organosilica (PMO) or a polymeric-bridged metal organic framework (MOF) coated with an amino acid-based ionic liquid; and
 - b) an antibody specific to an RGC marker conjugated to the nanoparticle core for targeting RGCs.
30. A nanoparticle for targeted delivery to a retinal cell, the nanoparticle comprising:
- a) a porous core at least partially coated by an ionic liquid; and
 - b) a targeting moiety conjugated to the porous core, wherein the targeting moiety binds to the retinal cell.
31. A method of preparing a nanoparticle of any one of claims 1 to 30, the method comprising:
- a) synthesising a porous nanoparticle;
 - b) contacting the porous nanoparticle with an ionic liquid to at least partially coat the nanoparticle with the ionic liquid; and
 - c) conjugating a targeting moiety to the porous nanoparticle.
32. The method of claim 31, wherein the ratio of the ionic liquid to the nanoparticle in step b) is about 1:1 to about 1:5 by weight.
33. The method of claim 31 or 32, wherein the ratio of the targeting moiety to the nanoparticle in step c) is about 1:2500 to about 1:25,000 by weight.
34. The method of any one of claims 1 to 33, further comprising a step of coating an exterior surface of the nanoparticle with niosomes, liposomes and/or a biopolymer.
35. The method of any one of claims 1 to 34, further comprising a step of contacting the nanoparticle with at least one therapeutic agent or detectable label to incorporate the at least one therapeutic agent or detectable label in the nanoparticle.
36. A pharmaceutical composition comprising a nanoparticle of any one of claims 1 to 30, and a pharmaceutically acceptable carrier.

37. The pharmaceutical composition of claim 36, wherein the pharmaceutical composition is formulated for administration intravenously, intraocularly, or topically to the eye.
38. The pharmaceutical composition of claim 37, wherein the pharmaceutical composition is formulated as an eye drop.
39. A nanoparticle of any one of claims 1 to 30, for use as a medicament.
40. A method of delivering a therapeutic agent to a retinal cell, the method comprising contacting the cell with a nanoparticle of any one of claims 1 to 30 or a pharmaceutical composition of any one of claims 36 to 38, wherein the nanoparticle comprises at least one therapeutic agent.
41. The method of claim 40, wherein the nanoparticle is provided at a concentration of up to about 100 $\mu\text{g/mL}$.
42. The method of claim 40 or 41, wherein the method is an *in vitro* method.
43. A method of delivering a therapeutic agent to a subject, the method comprising administering a nanoparticle of any one of claims 1 to 30 or a pharmaceutical composition of any one of claims 36 to 38 to the subject, wherein the nanoparticle comprises at least one therapeutic agent.
44. The method of claim 43, wherein the subject is suffering from a retinal disease.
45. A method of treating a retinal disease in a subject, the method comprising administering a nanoparticle of any one of claims 1 to 30 or a pharmaceutical composition of any one of claims 36 to 38 to the subject, wherein the nanoparticle comprises at least one therapeutic agent.
46. The method of claim 45, wherein the retinal disease is selected from glaucoma, optic neuropathy, diabetic retinopathy, sickle cell retinopathy, ischemic retinopathy, retinopathy of prematurity, infectious retinitis, retinitis pigmentosa, Stargardt's disease, optic nerve injury, age-related macular degeneration (AMD), uveitis, macular

edema, neovascular glaucoma, proliferative vitreoretinopathy, vitelliform maculopathy, and choroidal neovascularisation.

47. The method of claim 45 or 46, wherein the nanoparticle or pharmaceutical composition is administered intravenously, intraocularly, or topically to the eye.
48. A nanoparticle of any one of claims 1 to 30 or a pharmaceutical composition of any one of claims 36 to 38, for use in treating a retinal disease in a subject, wherein the nanoparticle comprises at least one therapeutic agent.
49. Use of a nanoparticle of any one of claims 1 to 30 or a pharmaceutical composition of any one of claims 36 to 38 in the manufacture of a medicament for treating a retinal disease in a subject, wherein the nanoparticle comprises at least one therapeutic agent.
50. A method of labelling a retinal cell or tissue, the method comprising contacting the retinal cell or tissue with a nanoparticle of any one of claims 1 to 30, wherein the nanoparticle comprises a detectable label.