(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 June 2020 (11.06.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/118136 A1

(51) International Patent Classification:
A61K 47/22 (2006.01) *A61K 47/26* (2006.01)
A61K 47/24 (2006.01)

(21) International Application Number:
PCT/US2019/064840

(22) International Filing Date:
06 December 2019 (06.12.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/776,005 06 December 2018 (06.12.2018) US

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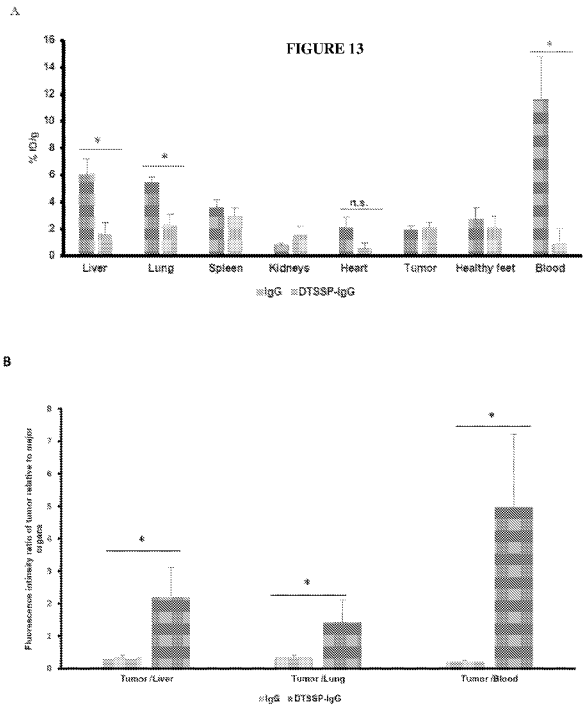
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

(54) Title: SELECTIVELY CLEAVABLE THERAPEUTIC NANOPARTICLES



(57) Abstract: Disclosed herein are nanoparticles for therapeutic and diagnostic use. The nanoparticles are designed to selectively accumulate in a tissue or organ of interest, and then release one or more therapeutically active or diagnostic agents. The nanoparticles include self-crosslinked therapeutic agents and therapeutic agents dispersed in a matrix.

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Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

SELECTIVELY CLEAVABLE THERAPEUTIC NANOPARTICLES

STATEMENT OF GOVERNMENT SUPPORT

This invention was made with government support under Grant no. CA135274 awarded by the National Institutes of Health. The government has certain rights in the invention.

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application 62/776,005, filed December 6, 2018, the contents of which are hereby incorporated in its entirety.

FIELD OF THE INVENTION

The invention is directed to nanoparticles containing one or more therapeutic agents. The nanoparticles selectively accumulate in a specified target tissue, at which point they release the active agent.

BACKGROUND

Monoclonal antibodies (mAbs) are an important class of therapeutic proteins which are used to treat a wide number of diseases, including cancers, autoimmune disorders, and inflammatory conditions. However, mAb-based medicines also have limitations that impact their clinical use; the most prominent challenges are their unfavorable pharmacokinetic properties and stability issues during manufacturing, transport and storage. Moreover, selective delivery of a mAb to a specific tissue remains an elusive goal. mAbs are typically administered parenterally (intramuscularly, subcutaneously or intravenously) and therefore the majority of the mAb is distributed in the plasma, rather than at the target tissue. Complicating matters, many mAbs suffer from relatively short *in vivo* half-lives, which necessitate frequent dosing in order to achieve meaningful concentrations of the mAb at the target tissue.

There remains a need for improved formulations for mAbs and other therapeutic agents. There remains a need for improved methods of selectively delivering mAbs and other therapeutic agents to specific tissue sites.

SUMMARY

Disclosed herein are improved pharmaceutical nanoparticle formulations for selectively delivering mAbs and other therapeutic agents to target tissues. The nanoparticles are selectively accumulated at inflammation sites and tumor tissues, where they are disintegrated thereby releasing the therapeutic agents.

The details of one or more embodiments are set forth in the descriptions below. Other features, objects, and advantages will be apparent from the description and from the claims.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. In vivo longitudinal bioluminescence imaging of acute and chronic inflammation in the right rear foot pad, but not the left rear foot pad, of mice. We induced local tissue inflammation by s.c. injection of 50 μg LPS. (A) Mice were given an i.p. injection of luminol (100 mg/kg) and imaged on day 3. (B) Mice were given an i.p. injection of lucigenin (25 mg/kg) and imaged on day 8.

Figure 2. Physical characterization of the PEGylated gold nanoparticles. (A-B) Representative TEM images of the selected 10 and 100 nm PEGylated gold nanoparticles. (C) Confirmation of PEG on the surface of 2 nm, 10 nm, 20 nm, 50 nm, 80 nm and 100 nm gold nanoparticles. Shown are OD490 values after samples were reacted with Lugol's solution. Data are mean ± S.E.M. (n = 3).

Figure 3: Pharmacokinetics of 2 nm, 10 nm, 20 nm, 50 nm, 80 nm, 100 nm and 200 nm fluorescently labeled gold nanoparticles in the inflamed foot in mice. Shown are in vivo fluorescence intensity-time profiles and selected pharmacokinetic parameters. Data are mean ± S.E. (n = 3-5). (a-c, $p < 0.05$).

Figure 4: Specificity towards the inflamed foot relative to the healthy foot and biodistribution of 2 nm, 10 nm, 20 nm, 50 nm, 80 nm, 100 nm and 200 nm fluorescent nanoparticles in other major organs. (A) In vivo fluorescence images of inflamed mouse feet vs. healthy feet at 24 h after i.v. injection of 2 nm, 10 nm, 100 nm and 200 nm nanoparticles. IF= Inflamed Foot. HF= Healthy Foot. (B) Specificity towards the inflamed foot for the 2 nm, 10 nm, 20 nm, 50 nm, 80 nm, 100 nm and 200 nm at 24 h post i.v. injection. The percent of specificity

was determined by subtracting the fluorescence intensity value of the healthy foot from the value of the inflamed foot, dividing by the value of the healthy foot, and then multiplying by 100.

Figure 5: Normalized fluorescence intensity values in major organs of mice 16 days after i.v. injection of gold nanoparticles. The gold nanoparticles were PEGylated and labeled with Cy 7.5. Data are mean ± S.E. (n = 4). (* $p < 0.05$) or (a-c, $p < 0.05$).

Figure 6: IgG and IgM specificity towards the inflamed foot relative to the healthy foot within the same mouse with chronic inflammation. (A) IgG fluorescence intensity profile in the rear feet of the mice. (B) IgM fluorescence intensity profile in the rear feet of the mice. (C) Profiles of IgG and IgM fluorescence intensity ratios of inflamed/healthy foot. Data are mean ± S.E. (n = 4). (* $p < 0.05$).

Figure 7: IgG and IgM specificity towards inflamed foot relative to healthy foot within the same mouse with acute inflammation. (A) IgG fluorescence intensity profile in the rear feet of the mice. (B) IgM fluorescence intensity profile in the rear feet of the mice. (C) Selected pharmacokinetic parameters of IgG and IgM, *Percentage of increase (+) or decrease (-) relatively to the healthy foot. (D) The percent of specificity was determined by subtracting the fluorescence intensity value of healthy foot from the value of the inflamed foot, dividing by the value of the healthy foot, and then multiplying by 100. Data are mean ± S.E. (n = 6). (* $p < 0.05$).

Figure 8. In vitro characterization and redox-sensitivity of the DTSSP-albumin nanoparticles. (A) TEM image of stable-albumin nanoparticles. (B) TEM image of DTSSP-albumin nanoparticles. (C) In vitro release of albumin from the stable-albumin nanoparticles and the DTSSP-albumin nanoparticles 2 h after pre-incubation in PBS or 1% 2-mercaptoethanol. Data are mean ± S.E. (n = 3).

Figure 9: Specificity and retention of free albumin, stable-albumin nanoparticles and DTSSP-albumin nanoparticles in the inflamed mouse foot. (A) In vivo specificity profile towards the inflamed foot of free albumin, stable-albumin nanoparticles, and DTSSP-albumin nanoparticles within 24 h post i.v. injection. The percent of specificity was determined by subtracting the fluorescence intensity values of the healthy foot from the values of the inflamed foot, then subtracting 1 and multiplying by 100. (B) In vivo fluorescence intensity values measured in the inflamed foot on days 6 and 7 after i.v. injection. (C) A representative in vivo fluorescence images of the inflamed mouse feet on day 6 after i.v. injection. Albumin from

bovine serum (BSA) is conjugated to Alexa Fluor™ 680. (D) Uptake and/or binding of fluorescein-labeled albumin by J774A.1 macrophages. J774A.1 cells (2 x 10^5) were seeded. Twenty hours later, the medium was replaced with serum-free DMEM containing fluorescein-labeled free albumin or albumin nanoparticles. The cells were washed after 50 min of incubation and lysed, and the fluorescence intensity was measured. Data are mean ± S.E. (n = 3 -5). (A-C, $p < 0.05$) or (*, $p < 0.05$) .

Figure 10: In vitro characterization of the DTSSP-IgG nanoparticles. (A) TEM image of the free IgG. (B) TEM image of the DTSSP-IgG nanoparticles.

Figure 11: Selected IgG and DTSSP-IgG-NPs PK parameters and the specificity of them towards the inflamed foot relative to the healthy foot within the same mouse with chronic inflammation. (A) IgG fluorescence intensity profile with selected pharmacokinetic parameters in the rear feet of the mice. (B) DTSSP-IgG-NPs fluorescence intensity profile with selected pharmacokinetic parameters in the rear feet of the mice. (C) Profiles of IgG and DTSSP-IgG-NPs fluorescence intensity ratios of inflamed/healthy foot. Data are mean ± S.E. (n = 4). (* $p \leq 0.05$).

Figure 12: IgG and IgM distribution in mice with M-Wnt tumors. (A) Percent of IgG or IgM detected in tumors, blood, and other key organs 24 h after i.v. injection in M-Wnt tumor-bearing mice. Shown are percent of dosed fluorescence intensity normalized to the weight of organs and tumors, or the volume of the blood. Values were after subtracting the mean values from the PBS group. (B) Ratios of IgG and IgM in tumor/organs. Data are mean ± S.D. (n = 3).

Fig. 13. Distribution IgG, free or in DTSSP-IgG-NPs, in mice with M-Wnt tumors.

(A) Percent of IgG detected in tumors, blood, and other key organs in M-Wnt tumor-bearing mice 24 h after i.v. injection with IgG, free or in DTSSP-IgG-NPs. Shown are percent of dosed fluorescence intensity normalized to the weight of organs and tumors, or the volume of the blood. Values were after subtracting the mean values from the PBS group. (B) Ratios of IgG in tumor/organs. Data are mean ± S.D. (n = 3).

Fig 14: TNF-α mAb released from DTSSP-TNF-α mAb nanoparticles is still functional and effective in binding to mouse TNF-α. The functionality of the DTSSP-TNF-α mAb nanoparticles is dependent on the concentration of a reducing agent such as GSH, which is known to be higher in synovial fluids and in some tumors, such as breast, ovarian, head and neck and lung cancer, than in blood or other healthy tissues. Data are mean ± S.D. (n = 3). Data

presented for three different mouse TNF-α concentrations; for each concentration leftmost bar: free anti-TNF-α; middle bar: GT003-anti-TNF-α- at high GTH level; rightmost bar: GT003-anti-TNF-α- at low GTH level

DETAILED DESCRIPTION

Before the present methods and systems are disclosed and described, it is to be understood that the methods and systems are not limited to specific synthetic methods, specific components, or to particular compositions. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Ranges may be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another embodiment includes¬ from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint.

"Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not.

Throughout the description and claims of this specification, the word "comprise" and variations of the word, such as "comprising" and "comprises," means "including but not limited to," and is not intended to exclude, for example, other additives, components, integers or steps. "Exemplary" means "an example of" and is not intended to convey an indication of a preferred or ideal embodiment. "Such as" is not used in a restrictive sense, but for explanatory purposes.

Disclosed are components that can be used to perform the disclosed methods and systems. These and other components are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these components are disclosed that while specific reference of each various individual and collective combinations and permutation of these may not be explicitly disclosed, each is specifically contemplated and described herein, for

all methods and systems. This applies to all aspects of this application including, but not limited to, steps in disclosed methods. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific embodiment or combination of embodiments of the disclosed methods.

Disclosed herein are therapeutic selectively cleavable nanoparticles that include at least one physiologically active agent. After administration to a patient in need thereof, the nanoparticles accumulate in a specific target tissue, e.g., tumor or inflamed tissues. Once the nanoparticles have reached the target tissue they are selectively disintegrated thereby releasing the active agent at the desired site.

The nanoparticle disclosed herein can have a variety of different particle sizes, depending on the exact target tissue. In some embodiments, the nanoparticles can have an average particle size (d50) from about 50-1,000 nm, from about 100-1,000 nm, from about 100-900 nm, from about 100-800 nm, from about 100-700 nm, from about 100-600 nm, from about 100-500 nm, from about 100-400 nm, from about 100-300 nm, from about 100-200 nm, from about 200-900 nm, from about 200-800 nm, from about 200-700 nm, from about 200-600 nm, from about 200-500 nm, from about 200-400 nm, from about 200-300 nm, from about 300-900 nm, from about 300-800 nm, from about 300-700 nm, from about 300-600 nm, from about 300-500 nm, or from about 300-400 nm.

A variety of different agents can be included in the nanoparticles. In some instances, the agent is a therapeutic agent (e.g., a therapeutic protein, peptide, small molecule, aptamer, or nucleic acid), which in other instances the agent has a diagnostic purpose, for instance a tracer element (e.g., a dye, a radionuclide, contrast agent, and the like). A preferred agent is a therapeutic protein, which includes PEGylated proteins, antibodies, and monoclonal antibodies ("mAbs"). The therapeutic protein can have a variety of different molecular weights. For instance, the therapeutic protein can have a molecular weight between about 10,000 Da and 100,000 kDa, between about 100,000 Da and 100,000 kDa, between about 500,000 Da and 100,000 kDa, between about 1-100,000 kDa, between about 1-75,000 kDa, between about 1-50,000 kDa, between about 1-25,000 kDa, between about 1-10,000 kDa, between about 1-5,000 kDa, between about 1-2,500 kDa, between about 1-1,000 kDa, between about 1-500 kDa, between about 10-500 kDa, between about 20-500 kDa, between about 20-400 kDa, between about 20-300 kDa, between about 20-200 kDa, between about 20-150 kDa, between about 20-

100 kDa, between about 20-75 kDa, between about 20-50 kDa, between about 50-500 kDa, between about 50-400 kDa, between about 50-300 kDa, between about 50-200 kDa, between about 50-150 kDa, between about 50-100 kDa, between about 50-75 kDa, between about 100-400 kDa, between about 100-300 kDa, or between about 100-200 kDa.

The nanoparticles disclosed herein can include any number of different monoclonal antibodies. For instance, abagovomab, abciximab, abituzumab, abrezekimab, abrilumab, actoxumab, adalimumab, adecatumumab, aducanumab, afasevikumab, afelimomab, alacizumab pegol, alemtuzumab, alirocumab, altumomab pentetate, amatuximab, anatumomab mafenatox, andecaliximab, anetumab ravtansine, anifrolumab, anrukinzumab, apolizumab, aprutumab ixadotin, arcitumomab, ascrinvacumab, aselizumab, atezolizumab, atidortoxumab, atinumab, atorolimumab, avelumab, azintuxizumab vedotin, bapineuzumab, basiliximab, bavituximab, BCD-100, bectumomab, begelomab, belantamab mafodotin, belimumab, bemarituzumab, benralizuma, fasenramab, berlimatoxumab, bermekimab, bersanlimab, bertilimumab, besilesomab, bevacizumab, bezlotoxumab, biciromab, bimagrumab, bimekizumab, birtamimab, bivatuzumab mertansine, bleselumab, blinatumomab, blontuvetmab, blosozumab, bococizumab, brazikumab, brentuximab vedotin, briakinumab, brodalumab, brolucizumab, brontictuzumab, burosumab, crysvitamab, cabiralizumab, camidanlumab tesirine, camrelizumab, canakinumab, cantuzumab mertansine, cantuzumab ravtansine, caplacizumab, capromab pendetide, carlumab, carotuximab, catumaxomab, CBR96-doxorubicin immunoconjugate, cedelizumab, cemiplimab, cergutuzumab amunaleukin, certolizumab pegol, cetrelimab, cetuximab, cibisatamab, cirmtuzumab, citatuzumab bogatox, cixutumumab, clazakizumab, clenoliximab, clivatuzumab tetraxetan, codrituzumab, cofetuzumab pelidotin, coltuximab ravtansine, conatumumab, concizumab, cosfroviximab, CR6261, crenezumab, crizanlizumab, crotedumab, cusatuzumab, dacetuzumab, daclizumab, dalotuzumab, dapirolizumab pegol, daratumumab, dectrekumab, demcizumab, denintuzumab mafodotin, denosumab, depatuxizumab mafodotin, derlotuximab biotin, detumomab, dezamizumab, dinutuximab, diridavumab, domagrozumab, dorlimomab aritox, dostarlimab, drozitumab, ds-8201, duligotuzumab, dupilumab, durvalumab, dusigitumab, duvortuxizumab, ecromeximab, eculizumab, edobacomab, edrecolomab, efalizumab, efungumab, eldelumab, elezanumab, elgemtumab, elotuzumab, elsilimomab, emactuzumab, emapalumab, emibetuzumab, emicizumab, hemlibra, enapotamab vedotin, enavatuzumab, enfortumab vedotin, enlimomab pegol, enoblituzumab, enokizumab, enoticumab, ensituximab,

epitumomab cituxetan, epratuzumab, eptinezumab, erenumab, erlizumab, ertumaxomab, etaracizumab, etigilimab, etrolizumab, evinacumab, evolocumab, exbivirumab, fanolesomab, faralimomab, faricimab, farletuzumab, fasinumab, fbta05, felvizumab, fezakinumab, fibatuzumab, ficlatuzumab, figitumumab, firivumab, flanvotumab, fletikumab, flotetuzumab, fontolizumab, foralumab, foravirumab, fremanezumab, fresolimumab, frovocimab, frunevetmab, fulranumab, futuximab, galcanezumab, galiximab, gancotamab, ganitumab, gantenerumab, gatipotuzumab, gavilimomab, gedivumab, gemtuzumab ozogamicin, gevokizumab, gilvetmab, gimsilumab, girentuximab, glembatumumab vedotin , golimumab, gomiliximab, gosuranemab, guselkumab, ianalumab, ibalizumab, IBI308, ibritumomab tiuxetan, icrucumab, idarucizumab, ifabotuzumab, igovomab, iladatuzumab vedotin, IMAB362, imalumab, imaprelimab, imciromab, imgatuzumab, inclacumab, indatuximab ravtansine, indusatumab vedotin, inebilizumab, infliximab, inolimomab, inotuzumab ozogamicin, intetumumab , IOMAB-B, ipilimumab, iratumumab, isatuximab, iscalimab, istiratumab, itolizumab, ixekizumab, keliximab, labetuzumab, lacnotuzumab, ladiratuzumab vedotin, lampalizumab, lanadelumab, landogrozumab, laprituximab emtansine, larcaviximab, lebrikizumab, lemalesomab, lendalizumab, lenvervimab, lenzilumab, lerdelimumab, leronlimab, lesofavumab, letolizumab, lexatumumab, libivirumab, lifastuzumab vedotin, ligelizumab, lilotomab satetraxetan, lintuzumab, lirilumab, lodelcizumab, lokivetmab, loncastuximab tesirine, lorvotuzumab mertansine, losatuxizumab vedotin, lucatumumab, lulizumab pegol, lumiliximab, lumretuzumab, lupartumab amadotin, lutikizumab, mapatumumab, margetuximab, marstacimab, maslimomab, matuzumab, mavrilimumab, mepolizumab, metelimumab, milatuzumab, minretumomab, mirikizumab, mirvetuximab soravtansine, mitumomab, modotuximab, mogamulizumab, monalizumab, morolimumab, mosunetuzumab, motavizumab, moxetumomab pasudotox, muromonab-CD3, nacolomab tafenatox, namilumab, naptumomab estafenatox, naratuximab emtansine, narnatumab, natalizumab, navicixizumab, navivumab, naxitamab, nebacumab, necitumumab, nemolizumab, NEOD001, nerelimomab, nesvacumab, netakimab, nimotuzumab, nirsevimab, nivolumab, nofetumomab merpentan, obiltoxaximab, obinutuzumab, ocaratuzumab, ocrelizumab, odulimomab, ofatumumab, olaratumab, oleclumab, olendalizumab, olokizumab, omalizumab, omburtamab, OMS721, onartuzumab, ontuxizumab, onvatilimab, opicinumab, oportuzumab monatox, oregovomab, orticumab, otelixizumab, otilimab, otlertuzumab, oxelumab, ozanezumab, ozoralizumab, pagibaximab, palivizumab, pamrevlumab, panitumumab,

pankomab, panobacumab, parsatuzumab, pascolizumab, pasotuxizumab, pateclizumab, patritumab, PDR001, pembrolizumab, pemtumomab, perakizumab, pertuzumab, pexelizumab, pidilizumab, pinatuzumab vedotin, pintumomab, placulumab, plozalizumab, pogalizumab, polatuzumab vedotin, ponezumab, porgaviximab, prasinezumab, prezalizumab, priliximab, pritoxaximab, pritumumab, PRO 140, quilizumab, racotumomab, radretumab, rafivirumab, ralpancizumab, ramucirumab, ranevetmab, ranibizumab lucentis, ravagalimab, ravulizumab, raxibacumab, refanezumab, regavirumab, relatlimab, remtolumab, reslizumab, rilotumumab, rinucumab, risankizumab, rituximab, rivabazumab pegol, rmabrabishield, robatumumab, roledumab, romilkimab, romosozumab, rontalizumab, rosmantuzumab, rovalpituzumab tesirine, rovelizumab, rozanolixizumab, ruplizumab, SA237, sacituzumab govitecan, samalizumab, samrotamab vedotin, sarilumab, satralizumab, satumomab pendetide, secukinumab, selicrelumab, seribantumab, setoxaximab, setrusumab, sevirumab, SGN-CD19a, SHP647, sibrotuzumab, sifalimumab, siltuximab, simtuzumab, siplizumab sirtratumab vedotin, sirukumab, sofituzumab vedotin, solanezumab, solitomab, sonepcizumab, sontuzumab, spartalizumab, stamulumab , sulesomab, suptavumab, sutimlimab, suvizumab, suvratoxumab, tabalumab, tacatuzumab tetraxetan, tadocizumab, talacotuzumab, talizumab, tamtuvetmab, tanezumab, taplitumomab paptox, tarextumab, tavolimab, tefibazumab, telimomab aritox, telisotuzumab vedotin, tenatumomab, teneliximab, teplizumab, tepoditamab, teprotumumab, tesidolumab, tetulomab, tezepelumab, TGN1412, tibulizumab, tigatuzumab, tildrakizumab, timigutuzumab, timolumab, tiragotumab, tislelizumab, tisotumab vedotin, TNX-650, tocilizumab, tomuzotuximab, toralizumab, tosatoxumab, tositumomab, tovetumab, tralokinumab, trastuzumab, trastuzumab emtansine, TRBS07, tregalizumab, tremelimumab, trevogrumab, tucotuzumab celmoleukin, tuvirumab, ublituximab, ulocuplumab, urelumab, urtoxazumab, ustekinumab, utomilumab, vadastuximab talirine, vanalimabmab, vandortuzumab vedotin, vantictumab, vanucizumab, vapaliximab, varisacumab, varlilumab, vatelizumab, vedolizumab, entyvio, veltuzumab, vepalimomab, vesencumab, visilizumab, vobarilizumab, volociximab, vonlerolizumab, vopratelimab, vorsetuzumab mafodotin, votumumab, vunakizumab, xentuzumab, XMAB-5574, zalutumumab, zanolimumab, zatuximab, zenocutuzumab, ziralimumab, zolbetuximab, zolimomab aritox, and ponezumab can all be advantageously formulated into the inventive nanoparticles.

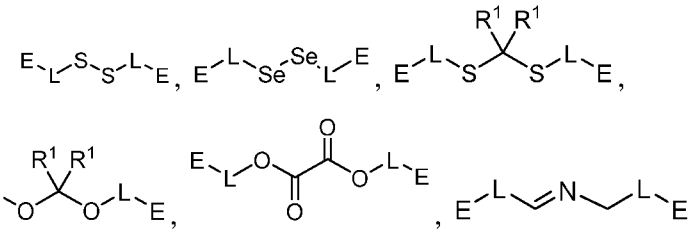
In some instances, the nanoparticle can include a therapeutic protein, for instance Lepirudin, Dornase alfa, Denileukin diftitox, Bivalirudin, Leuprolide, Peginterferon alfa-2a, Alteplase, Interferon alfa-n1, Darbepoetin alfa, Reteplase, Epoetin alfa, Salmon Calcitonin, Interferon alfa-n3, Pegfilgrastim, Sargramostim, Secretin, Peginterferon alfa-2b, Asparaginase, Thyrotropin Alfa, Antihemophilic Factor, Anakinra, Gramicidin D, Intravenous Immunoglobulin, Anistreplase, Insulin Regular, Tenecteplase, Menotropins, Interferon gamma-1b, Interferon Alfa-2a, Recombinant, Coagulation factor VIIa, Oprelvekin, Palifermin, Glucagon recombinant, Aldesleukin, Botulinum Toxin Type B, Lutropin alfa, Insulin Lispro, Insulin Glargine, Collagenase, Rasburicase, Imiglucerase, Alpha-1-proteinase inhibitor, Pegaspargase, Interferon beta-1a, Pegademase bovine, Human Serum Albumin, Eptifibatide, Serum albumin iodonated, Follitropin beta, Vasopressin, Interferon beta-1b, Hyaluronidase, Insulin, porcine, Digoxin Immune Fab (Ovine), Daptomycin, Pegvisomant, Botulinum Toxin Type A, Pancrelipase, Streptokinase, Alglucerase, Laronidase, Urofollitropin, Serum albumin, Choriogonadotropin alfa, Antithymocyte globulin, Filgrastim, Coagulation factor ix, Becaplermin, Agalsidase beta, Interferon alfa-2b, Oxytocin, Enfuvirtide, Idursulfase, Alglucosidase alfa, Exenatide, Mecasermin, Pramlintide, Galsulfase, Abatacept, Cosyntropin, Corticotropin, Insulin aspart, Insulin detemir, Insulin glulisine, Pegaptanib, Nesiritide, Thymalfasin, Defibrotide, Natural alpha interferon OR multiferon, Glatiramer acetate, Preotact, Teicoplanin, Sulodexide, Teriparatide, Liraglutide, Belatacept, Buserelin, Velaglucerase alfa, Tesamorelin, Taliglucerase alfa, Aflibercept, Asparaginase erwinia chrysanthemi, Ocriplasmin, Glucarpidase, Teduglutide, Insulin,isophane, Epoetin zeta, Fibrinolysin aka plasmin, Follitropin alpha, Romiplostim, Lucinactant, Aliskiren, Ragweed Pollen Extract, Somatotropin Recombinant, Drotrecogin alfa, Alefacept, OspA lipoprotein, Urokinase, Abarelix, Sermorelin, Aprotinin, Albiglutide, Ancestim, Antithrombin Alfa, Antithrombin III human, Asfotase Alfa, Autologous cultured chondrocytes, Beractant, C1 Esterase Inhibitor (Human), Coagulation Factor XIII A-Subunit (Recombinant), Conestat alfa, Daratumumab, Desirudin, Dulaglutide, Elosulfase alfa, Elotuzumab, Fibrinogen Concentrate (Human), Filgrastim-sndz, Gastric intrinsic factor, Hepatitis B immune globulin, Human calcitonin, Human clostridium tetani toxoid immune globulin, Human rabies virus immune globulin, Human Rho(D) immune globulin, Hyaluronidase (Human Recombinant), Immune Globulin Human, Turoctocog alfa, Tuberculin Purified Protein Derivative, Simoctocog Alfa, Sebelipase alfa, Sacrosidase, Prothrombin

complex concentrate, Poractant alfa, Pembrolizumab, Peginterferon beta-1a, Metreleptin, Methoxy polyethylene glycol-epoetin beta, Insulin Pork, Insulin Degludec, Insulin Beef, Thyroglobulin, Anthrax immune globulin human, Anti-inhibitor coagulant complex, Anti-thymocyte Globulin (Equine), C1 Esterase Inhibitor (Recombinant) , Chorionic Gonadotropin (Recombinant), Coagulation factor X human, Efmoroctocog alfa, Factor IX Complex (Human), Hepatitis A Vaccine, Human Varicella-Zoster Immune Globulin, Lenograstim, Pegloticase, Protamine sulfate, Protein S human, Sipuleucel-T, Somatropin recombinant, Susoctocog alfa, Thrombomodulin Alfa, and combinations thereof.

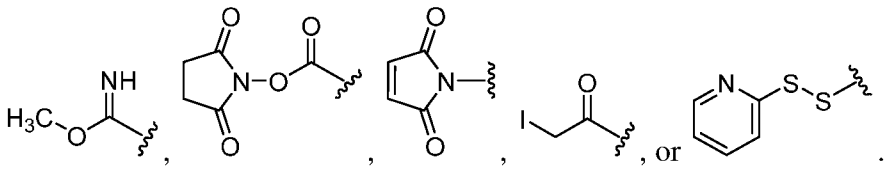
In some embodiments, the active agent itself can be crosslinked into nanoparticle form (i.e., self-crosslinked), while in other embodiments, the active agent can be dispersed in a matrix. The matrix can include crosslinked polymers. In other embodiments, the matrix can include a dispersion of non-covalently bound compounds, either polymers or small molecules. Non-covalent bonds include electrostatic, hydrophobic and van der Waals interactions. Exemplary systems of non-covalently bound nanoparticles include micelles, liposomes, dispersions and conglomerates. Lipids and other self-assembling compounds may be used in non-covalently bound dispersions.

In some embodiments, the crosslinks will include stimuli-cleavable crosslinks. Stimuli-cleavable crosslinks are those which are degraded by exposure to an appropriate trigger, for instance, a catalyst, oxidant, reductant, base, acid, radiation (e.g., UV, infrared, or microwave), ultrasound, heat, or magnetic field. Preferred triggers include oxidants such as reactive oxygen, which are produced in excess in some tumor and inflamed tissues. Exemplary functional groups which can serve as stimuli-cleavable crosslinks include disulfide bonds, trisulfide bonds, diselenide bonds, thioacetals, acetals, oxalates, imines, and short peptide sequences.

Because mAbs and other therapeutic proteins contain a variety of nucleophilic groups, they are especially suitable for self-cross linking into nanoparticles. In some embodiments, the mAb/protein can be dissolved in a suitable solvent and reacted with a crosslinking agent in a stoichiometry suitable to crosslink the mAb/protein into a nanoparticle. The crosslinking agent will contain at least two electrophilic groups capable of reacting with any of the thiol, amine, carboxylate, hydroxyl, or guanidine groups present in the amino acid side chain. In some instances, the crosslinking agent can have the formula:



wherein L represent a linking group, R^1 is in each case independently hydrogen or C_{1-6}alkyl, or where two R^1 groups form a ring; and E is an electrophilic group. Suitable electrophilic groups include imidoester, an N-hydroxysuccinimide ester, a maleimide, a vinyl sulfone, an epoxide, a haloacetyl, or a pyridyl disulfide. In certain embodiments, the crosslinker can include one or more moieties having the formula:



Suitable L groups include C_{1-10}alkyl and aryl groups, which may be substituted, or polyethylene glycol chains. The crosslinking agent may be provided in an amount from 10^{-5} wt% to 1 wt%, relative to the therapeutic agent. Other suitable ranges include 10^{-5} wt% to 0.1 wt%, 10^{-5} wt% to 10^{-2} wt%; 10^{-5} wt% to 10^{-3} wt%; 10^{-5} wt% to 10^{-4} wt%; 10^{-4} wt% to 1 wt%; 10^{-4} wt% to 0.1 wt%, 10^{-4} wt% to 10^{-2} wt%; 10^{-4} wt% to 10^{-3} wt%; 10^{-3} wt% to 1 wt%; 10^{-3} wt% to 0.1 wt%, 10^{-3} wt% to 10^{-2} wt%; 10^{-2} wt% to 1 wt%; and 10^{-2} wt% to 0.1 wt%.

In some embodiments, the physiologically active agent is dispersed in a crosslinked polymer matrix, wherein at least a portion of the crosslinks are stimuli-degradable crosslinks, as defined above. Exemplary polymers for crosslinking include polyphosphazenes, polycyanoacrylates, polyesters, polyhydroxyalkanoates, polyanhydrides, polydixanones, polyorthoesters, polyesteramides, polyamido amides, polythioesters, collagen, fibrin, fibrinogen, gelatin, polysaccharides, and combinations thereof. Suitable polyesters include poly(lactic acid), poly(glycolic acid), poly(caprolactone), poly(propylene fumarate), copolymers thereof, and combinations thereof. Suitable polysaccharides include chitosans, celluloses, modified celluloses, alginates, pectins, pullulans, hyaluronic acids, starches, amyloses, and dextrans. These polymers may be crosslinked using the same agents and techniques described about for crosslinking proteins. The crosslinking agent may be provided in an amount from 10^{-5} wt% to 1 wt%, relative to polymer to be crosslinked. Other suitable ranges include 1 wt% to 5 wt%, 2.5

wt% to 7.5 wt%, 5 wt% to 10 wt%, 7.5 wt% to 12.5 wt%, 10 wt% to 15 wt%, 12.5 wt% to 17.5 wt%, 15 wt% to 20 wt%, 1 wt% to 30 wt%, 5 wt% to 50 wt%, 10^{-5} wt% to 0.1 wt%, 10^{-5} wt% to 10^{-2} wt%; 10^{-5} wt% to 10^{-3} wt%; 10^{-5} wt% to 10^{-4} wt%; 10^{-4} wt% to 1 wt%; 10^{-4} wt% to 0.1 wt%, 10^{-4} wt% to 10^{-2} wt%; 10^{-4} wt% to 10^{-3} wt%; 10^{-3} wt% to 1 wt%; 10^{-3} wt% to 0.1 wt%, 10^{-3} wt% to 10^{-2} wt%; 10^{-2} wt% to 1 wt%; and 10^{-2} wt% to 0.1 wt%.

In some embodiments, the nanoparticles will be held together using non-covalent interactions. A preferred system includes acid-sensitive lipids, which can be agglomerated into nanoparticles containing one or more active agents, and which selectively degrade at pH levels lower than found in healthy, non-gastric tissue. Suitable agglomerates include micelles, liposomes, and non-ordered clusters. Exemplary acid sensitive lipids include 1,2-dipalmitoyl-sn-glycero-3-succinate, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-succinate, N-palmitoyl homocysteine, cholesteryl hemisuccinate, N-(4-carboxybenzyl)-N,N-dimethyl-2,3-bis(oleoyloxy)propan-1-aminium, PEG-poly(monomethylitaconate)CholC6, and others. The agglomerates can further include a stabilizer, for instance a cholesterol or succinate derivative, e.g., cholesterol hemisuccinate, tocopherol hemisuccinate.

In some embodiments, especially involving small molecule therapeutics, the active agent can be loaded onto smaller nanoparticles, e.g., having a particle size less than 100 nm, less than 75 nm, less than 50 nm, less than 25 nm, less than 10 nm, or less than 5 nm, and then incorporated into the stimuli-cleavable nanoparticles as described above. The larger delivery nanoparticle ensures the agent is preferentially delivered to tumor or inflammation sites, and the smaller nanoparticle increases the persistence of the agent subsequent to the disintegration of the larger delivered nanoparticles.

In certain embodiments, the crosslinks are cleaved by irradiation, for instance x-ray irradiation. A composition can be administered to a patient, either systemically or locally to a desired tissue or tumor location. Once a therapeutic concentration of nanoparticles has accumulated in the tumor or tissue of interest, the tumor or tissue of interest can be exposed to irradiation, for instance, x-ray irradiation, to cleave the nanoparticle and release the active agent. The total amount of irradiation applied can be between 0.1-100 Gy, between 1-50 Gy, between 1-25 Gy, between 1-15 Gy, between 1-10 Gy, between 5-15 Gy, between 10-20 Gy, between 10-30 Gy, between 10-40 Gy, or between 15-50 Gy.

EXAMPLES

The following examples are for the purpose of illustration of the invention only and are not intended to limit the scope of the present invention in any manner whatsoever.

Lugol's solution, Tris-EDTA (TE), sodium dodecyl sulfate, Triton X-100, N,N-dimethyl-9,9-biacridinium dinitrate (Lucigenin), lipopolysaccharides (LPS) from *Salmonella enterica* serotype enteritidis, 3-aminophthalhydrazide, 5-amino-2,3-dihydro-1,4-phthalazinedione (Luminol sodium salt), bovine serum albumin (BSA) (lyophilized powder, ≥96%), DTSSP (3,3'-dithiobis(sulfosuccinimidyl propionate)), sulfo-SMCC (sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate) were from Sigma-Aldrich (St. Louis, MO). Albumin-Alexa Fluor™ 647 conjugate and albumin from bovine serum (BSA)-FITC conjugate were from ThermoFisher (Waltham, MA). Normal mouse IgG Alexa Fluor® 647 and normal mouse IgM Alexa Fluor® 647 were from Santa Cruz Biotechnology (Dallas, TX). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), and streptomycin/penicillin were from Invitrogen (Carlsbad, CA).

Example 1

PEGylated gold nanoparticles of the following sizes, 2, 10, 20, 50, 80, 100 and 200 nm, labeled with Cy7.5 were from NANOCS (New York, NY). The nanoparticles have a uniform size distribution measured by dynamic light scattering (DLS) and transmission electron microscopy (TEM) by the manufacturer. In addition, the size of the 10 and 100 nm were confirmed using DLS and TEM. Hydrodynamic size and zeta potential were measured using a Malvern ZetaSizer ZS (Westborough, MA). For TEM, nanoparticles were deposited onto copper grids, stained with phosphotungstic acid (PTA) (2% w/v) and dried overnight. Before administration, the fluorescence intensity of nanoparticles was measured using IVIS, and nanoparticles that showed higher fluorescent intensity were diluted with PBS so that all the nanoparticles in suspension had a similar fluorescence intensity. The content of polyethylene glycol (2000) (PEG) on the surface of the nanoparticles was measured using an iodide staining method with Lugol's solution. Briefly, 150 µl of nanoparticles (7×10^{12} nanoparticles/ml) were added to a solution that contained 950 µl of PBS (pH 7.4, 10 mM) and 68 µl of Lugol's solution.

After 5 min of incubation at room temperature, the absorbance (OD490 nm) was measured using a BioTek Synergy HT Multi-Mode Microplate Reader.

BSA was used to formulate the DTSSP-albumin nanoparticles via a desolvation technique. BSA was dissolved at a concentration of 25 mg/ml in 10 mM sodium chloride solution (pH 9.0). The resulting solution was filtered through a 0.22 μm filtration unit (Schleicher und Schüll, Dassel, Germany). An aliquot (1.0 ml) of the BSA solution was transformed into nanoparticles by dropwise addition of 4.0 ml of a desolvating agent (i.e. ethanol/methanol, 50/50%) under stirring (500 rpm) at room temperature. After the desolvation process, 100 μl of 1% DTSSP in water was added to induce protein crosslinking. The crosslinking process was performed over a time period of 24 h at room temperature under stirring. Similarly, stable-albumin nanoparticles were prepared using 100 μl of 1% Sulfo-SMCC as a crosslinker. TEM, nanoparticles were deposited onto copper grids, stained with phosphotungstic acid (PTA) (2% w/v) and dried overnight.

For animal studies, 5 mg of the albumin-Alexa Fluor™ 647 conjugate was added to 20 mg of BSA to prepare fluorescently labeled DTSSP-albumin nanoparticles or stable-albumin nanoparticles. For the uptake study, 1 mg of the albumin-FITC conjugate was added to 24 mg of BSA to prepare fluorescently labeled DTSSP-albumin nanoparticles. The particle size, polydispersity index (PDI), and zeta potential of the nanoparticles were determined using a Malvern Zeta Sizer Nano ZS.

Final formulation	**Particles size (nm)**	**PDI**	**Zeta potential (mV)**
Stable-Albumin-NPs	187.3 ± 21	0.26 ± 0.02	-26.0 ± 2.1
DTSSP-Albumin-NPs	186.3 ± 12	0.27 ± 0.03	-23.7 ± 2.0

Data are mean ± SD (n = 3). PDI, polydispersity index.

For in vitro release study, 1% of 2-mercaptoethanol (Hercules, CA) was prepared in PBS (10 mM, pH 7.4) to test the stability of the DTSSP-albumin nanoparticles in redox conditions. DTSSP-albumin nanoparticles or stable-albumin nanoparticles were collected by centrifugation (17,500 × g, 30 min, 4 °C), resuspended in 1 ml of 1% of 2-mercaptoethanol in PBS or PBS alone (10 mM, pH 7.4), and then placed in shaker incubator (MAQ 5000, MODEL 4350, Thermo Fisher Scientific, Waltham, MA) (100 rpm, 37 °C). After 2 h, the tubes were centrifuged (17,500 × g, 30 min), and the amount of albumin released (i.e. in the supernatant) was measured using

Bradford assay by measuring the absorbance at 595 nm with a BioTek Synergy HT Multi-Mode Microplate Reader.

Normal mouse IgG Alexa Fluor® 647 was used to formulate the DTSSP-IgG-NPs via the desolvation technique as previously described. IgG was diluted at a concentration of 500 μg/ml in 10 mM sodium chloride solution (pH 9.0). The resulting solution was filtered through a 0.22 μm filtration unit. An aliquot (1.0 ml) of the IgG solution was transformed into nanoparticles by dropwise addition of 4.0 ml of a desolvating agent (i.e. ethanol/methanol, 50/50%) under stirring (500 rpm) at room temperature. After the desolvation process, 100 μl of 0.04% DTSSP in water was added to induce particle crosslinking. The crosslinking process was performed under stirring over a time period of 24 h at room temperature. The particle size, polydispersity index (PDI), and zeta potential of the nanoparticles were determined using a Malvern Zeta Sizer Nano ZS. TEM, nanoparticles were deposited onto copper grids, stained with phosphotungstic acid (PTA) (2% w/v) and dried overnight.

Murine macrophage J774A.1 cells (American Type Culture Collection, Manassas, VA) were seeded in a 12-well plate (2×10^5 cells/well). To study the effect of the nanoparticle size on their uptake and/or binding by the cells, Free BSA-FITC conjugate or fluorescently labeled DTSSP-albumin nanoparticles were added into the cell culture medium. After 50 min of co-incubation, the cells were washed with PBS (10 mM, pH 7.4) and lysed with a lysis solution that contained 2% (v/v) sodium dodecyl sulfate and 1% Triton X-100. The fluorescence intensity in the cell lysate was measured using a plate reader (Ex = 485 nm, Em = 528 nm). Bradford protein assay did not show any significant difference in the total protein concentrations in the lysates among the groups.

All animal studies were conducted in accordance with the U.S. National Research Council Guidelines for the care and use of laboratory animals. The animal protocol was approved by the Institutional Animal Care and Use Committee at The University of Texas at Austin. Female C57BL/6 mice (6-8 weeks) were from Charles River Laboratories (Wilmington, MA). For imaging, mice were fed with alfalfa-free diet (Harlan, Indiana) to minimize unwanted background signals. An LPS-induced mouse model of chronic inflammation was established follows. Briefly, LPS was dissolved in sterile PBS (pH 7.4, 10 mM) at a concentration of 1 mg/ml. On day 0, 50 μl of the solution was injected into the right hind footpad of each mouse. For the acute inflammation study, acute inflammation was confirmed on day 3 using an IVIS®

Spectrum (Caliper, Hopkinton, MA) with a bioluminescence imaging system 20 min following intraperitoneal (i.p.) injection of luminol (100 mg/kg) (exposure time 60 s, large binning, field B). For the chronic inflammation studies, chronic inflammation was confirmed on day 8 using an IVIS® Spectrum with a bioluminescence imaging system 20 min following i.p. injection of lucigenin (15 mg/kg) (exposure time 60 s, large binning, field B). Only mice that showed significant acute or chronic inflammation in the right foot were used.

Upon the confirmation of chronic inflammation in the right rear foot, mice were randomly assigned to groups and injected i.v. with PBS or gold nanoparticles of different particle sizes (i.e. 2, 10, 20, 50, 80, 100 and 200 nm). These nanoparticles are non-degradable thus excluding resorption as a variable. Mice were imaged using the IVIS® Spectrum 3, 6, 12, and 24 h and 2, 4, 8, and 16 days after the injection. At the end of the study, mice were euthanized to collect the inflamed foot and major organs (i.e. heart, kidneys, liver, spleen, and lungs). All samples were weighed and imaged using an IVIS® Spectrum. All fluorescent units are in photons per second per centimeter square per steradian ($p/s/cm^2/sr$).

Similarly, groups of mice with chronic inflammation in the right foot were i.v. injected with PBS, free albumin, stable-albumin-NPs or DTSSP-albumin-NPs (albumin-Alexa Fluor™ 647, 0.32 mg/kg). Mice were imaged using an IVIS® Spectrum 3, 6, 12, 24 h and 2, 4, 6 and 7 days after the injection. Data were analyzed using PK Solver. A similar study was also carried out using fluorescently labeled DTSSP-IgG-nanoparticles (IgG, 2 μg/kg). Mice were imaged using IVIS® Spectrum 3, 6, 12, 24 h and 2 and 4 days after the injection.

Finally, the distribution of IgG and IgM, both fluorescently labeled, in acute or chronic inflammation sites after they were i.v. injected in mice with LPS-induced inflammation were studied similarly. Upon confirmation of acute or chronic inflammation in the right rear foot, mice were randomly assigned to groups and i.v. injected with PBS, IgG or IgM (IgM, 40 μg/kg; IgG, 20 μg/kg to account for difference in fluorescence intensities). Mice were imaged using the IVIS® Spectrum 3, 6, 12, and 24 h after the injection to determine specificity of IgG and IgM to the inflammation sites within the first 24 h.

Statistical analyses were completed by performing analysis of variance followed by Fisher's protected least significant difference procedure. A p value of ≤ 0.05 (two-tail) was considered significant.

Example 2

Redox-sensitive IgG nanoparticles were prepared as in Example 1. Briefly, normal mouse IgG Alexa Fluor® 647 from Santa Cruz Biotechnology (Dallas, TX) was diluted to a concentration of 100 µg/ml in a 10 mM sodium chloride solution, pH 9.0. Aliquots (1.0 ml) of the IgG solution were transformed into nanoparticles by dropwise addition of 4.0 ml of a desolvating agent (i.e. ethanol/methanol, 50%/50%) under stirring (500 rpm) at room temperature. After the desolvation process, 100 µl of a 3,3'-dithiobis(sulfosuccinimidyl propionate) in water solution (i.e. DTSSP, 0.004%) were added to induce particle crosslinking (i.e. 24 h at room temperature under stirring). Particle size was measured using a Malvern Nano ZS and morphology examined using transmission electron microscopy.

M-Wnt mammary tumor cells (basal-like, triple-negative, claudin-low) were cloned from spontaneous mammary tumors in MMTV-Wnt-1 transgenic mice in a congenic C57BL/6 background. M-Wnt cells were cultured in RPMI 1640 medium at 37°C and 5% CO_2. The medium was supplemented with 10% fetal bovine serum (FBS), 100 U/mL of penicillin, and 100 µg/mL of streptomycin. All cell culture medium and reagents were from Invitrogen (Carlsbad, CA). Animal study was conducted in accordance with the U.S. National Research Council Guidelines for the care and use of laboratory animals. The animal protocol was approved by the Institutional Animal Care and Use Committee at The University of Texas at Austin. Female C57BL/6 mice (6-8 weeks) were from Charles River Laboratories (Wilmington, MA). M-Wnt tumors were established by injecting M-Wnt tumor cells (5×10^5 cells/mouse) subcutaneously in the ninth mammary fat pad of the mice. When tumors reached 6-9 mm in diameter, mice were i.v. injected with PBS, IgG, IgM, or DTSSP-IgG. Both IgG and IgM (Sant Cruz Biotechnology) were fluorescently labeled with Alexa Fluor® 647. The dose of IgM was 40 µg/kg, 20 µg/kg for IgG so that the fluorescence intensities of the two antibodies injected in each mouse were similar. Mice were euthanized 24 h later to collect blood, tumor, and major organs (e.g. heart, kidneys, liver, spleen, and lung, gastrointestinal tract). All samples were then imaged using an IVIS Spectrum (Caliper, Hopkinton, MA) (Em/Ex of 465/600 nm).

IgG and IgM are natural, large biologic molecules with particle size in the nanometer scale (i.e. IgG, ~ 10 nm; IgM, ~150 nm). To preliminarily study the distribution of IgG and IgM

in tumor tissues, relative to other key organs, we injected (i.v.) M-Wnt tumor-bearing mice with fluorescently labeled IgG or IgM. Shown in Fig. 12A are the percentages of injected IgG and IgM that were detected in tumors, blood, and key organs, 24 h after the injection. IgM and IgG showed similar weight-normalized levels in tumor tissues, but the weight- or volume-normalized levels of IgM in the liver, lung, and blood are significantly lower than those of the IgG (Fig. 12A). Shown in Fig. 12B are the ratios of IgG and IgM levels in tumor issues, relative to in liver, lung, and blood, clearly indicating that the IgM has more specific distribution to tumors than IgG.

IgGs, such as anti-PD-1 monoclonal antibodies, are used extensively in clinics to treat various types of cancers, but are associated with severe adverse events, likely relative to their non-specific distribution upon injection. Currently, there is effort in developing anti-PD-1 IgM antibodies. However, as mentioned above, the affinity of IgMs is not as high as IgGs. We therefore tested whether crosslinking IgG into redox-sensitive nanoparticles will increase its specific distribution in tumors. Tumor cells are known to be in a state of redox imbalance, resulting in increased oxidants within the tumor microenvironment. We synthesized redox-sensitive IgG nanoparticles (i.e. DTSSP-IgG-NPs) with a hydrodynamic protein size of about 170 ± 21 nm and i.v. injected them, or free IgG, into mice with M-Wnt tumors.

As shown in Fig. 13A, DTSSP-IgG-NPs and IgG have similar levels of weight-normalized distributions in tumors, but the levels of IgG in liver, lung, and blood, when given as DTSSP-IgG-NPs, are significantly lower, compared to when given as free IgG. Shown in Fig. 13B are the ratios of IgG in tumor issues, relative to liver, lung, and blood, 24 h after mice were injected with free IgG or IgG in DTSSP-IgG-NPs, indicating that formulating IgG into DTSSP-IgG-NPs may increase its specific distribution to tumors. Currently, we are testing the affinity of IgG released from the DTSSP-IgG-NPs.

Pulmonary and liver adverse events are commonly associated with monoclonal antibodies that have been approved for clinical use, although the mechanisms underlying such adverse effects are generally not known. For pulmonary adverse effects there are four main categories: interstitial pneumonitis and fibrosis; acute respiratory distress syndrome (ARDS), bronchiolitis obliterans organizing pneumonia (BOOP), and hypersensitivity reactions. Liver is an Fc-receptor rich organ that helps to increase the circulation and retention time of monoclonal antibodies. A possible side effect of antibody therapy is the cytokine-release syndrome that may lead to auto-

immune complications via interactions with Fc receptors. Due to the longer exposure, life-threatening and fatal cytokine release syndrome has been reported with antibody therapies (e.g. Rituximab for treatment of chronic lymphocytic leukemia (CLL) and non-Hodgkin's lymphomas). The high concentrations of IgG in lung and liver are probably related to the high residual plasma concentrations of the IgG in those organs, which may also be related to the adverse events caused by monoclonal antibodies in the lung and liver.

Example 3: Confirmation of the functionality of TNF-α released from redox-sensitive TNF-α mAb nanoparticles (DTSSP-TNF-α mAb-nanoparticles)

Redox-sensitive TNF-α mAb nanoparticles were prepared as in Example 1 and 2. Briefly InVivoMAb anti-mouse TNFα (TNF-α mAb) from Bio-X-Cell (West Lebanon, NH) was diluted to a concentration of 1 mg/ml in a 10 mM sodium chloride solution, pH 9.0. Aliquots (1.0 ml) of the TNF-α mAb solution were transformed into nanoparticles by dropwise addition of 4.0 ml of a desolvating agent (i.e. ethanol/methanol, 50%/50%) under stirring (500 rpm) at room temperature. After the desolvation process, 100 µl of a 3,3'-dithiobis(sulfosuccinimidyl propionate) in water solution (i.e. DTSSP, 0.04%) were added to induce particle crosslinking (i.e. 24 h at room temperature under stirring).

The particles were centrifuged at 15,000 rpm for 30 min, then the pellet was suspended in 1 ml solution that contain about 1.6 or 0.8 nmoles of glutathione (Sigma, St. Louis, MO). The mixture was then placed in shaker incubator (MAQ 5000, MODEL 4350, Thermo Fisher Scientific, Waltham, MA) for about 1.5 h (150 rpm, 37 °C) to allow the TNF-α mAbs to release from the nanoparticles. To determine the ability of TNF-α mAb in binding mouse TNF-α, about 12.5 µg (0.5 ml of 25 µg/ml added to 0.5 ml of the samples) of free TNF-α mAb or redox-sensitive TNF-α mAb nanoparticles were incubated with different concentrations of mouse TNF-α (125, 62.5 and 31.25 pg/ml, final concentration) and then placed in shaker incubator for about 2 h (150 rpm, 37 °C). The concentrations of TNF-α in the samples were measured using a Mouse TNF-α ELISA MAX™ Standard from BioLegend (San Diego, CA). Results were expressed as the percent of TNF-α bound by the anti-TNF-α mAb using the following equation:

% TNF-α bound = 100 x 1 - (OD of mouse TNF-a bound to anti-TNF-α mAb)/(OD of mouse TNF-α alone).

Results are shown in Figure 14.

The compositions and methods of the appended claims are not limited in scope by the specific compositions and methods described herein, which are intended as illustrations of a few aspects of the claims and any compositions and methods that are functionally equivalent are intended to fall within the scope of the claims. Various modifications of the compositions and methods in addition to those shown and described herein are intended to fall within the scope of the appended claims. Further, while only certain representative compositions and method steps disclosed herein are specifically described, other combinations of the compositions and method steps also are intended to fall within the scope of the appended claims, even if not specifically recited. Thus, a combination of steps, elements, components, or constituents may be explicitly mentioned herein or less, however, other combinations of steps, elements, components, and constituents are included, even though not explicitly stated. The term "comprising" and variations thereof as used herein is used synonymously with the term "including" and variations thereof and are open, non-limiting terms. Although the terms "comprising" and "including" have been used herein to describe various embodiments, the terms "consisting essentially of" and "consisting of" can be used in place of "comprising" and "including" to provide for more specific embodiments of the invention and are also disclosed. Other than in the examples, or where otherwise noted, all numbers expressing quantities of ingredients, reaction conditions, and so forth used in the specification and claims are to be understood at the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, to be construed in light of the number of significant digits and ordinary rounding approaches.

CLAIMS

What is claimed is:

1. A therapeutic nanoparticle comprising stimuli-cleavable crosslinks, and at least one physiologically active agent, wherein the nanoparticle has a particle size of between about 100-1,000 nm, and wherein the nanoparticle undergoes stimulus-directed degradation.
2. The nanoparticle of claim 1, wherein the nanoparticle has a particle size from about 50-900 nm, from about 100-800 nm, from about 100-700 nm, from about 100-600 nm, from about 100-500 nm, from about 100-400 nm, from about 100-300 nm, from about 100-200 nm, from about 200-900 nm, from about 200-800 nm, from about 200-700 nm, from about 200-600 nm, from about 200-500 nm, from about 200-400 nm, from about 200-300 nm, from about 300-900 nm, from about 300-800 nm, from about 300-700 nm, from about 300-600 nm, from about 300-500 nm, or from about 300-400 nm.
3. The nanoparticle of claim 1, wherein the at least one physiologically active agent comprises one or more therapeutic agents or diagnostic agents.
4. The nanoparticle of claim 3, wherein the at least one physiologically active agent comprises at least one therapeutic protein, small molecule therapeutic agent, peptide, aptamer, or nucleic acid.
5. The nanoparticle of claim 4, wherein the at least one physiologically active agent comprises a protein having a molecular weight between about 10,000 Da and 100,000 kDa, between about 100,000 Da and 100,000 kDa, between about 500,000 Da and 100,000 kDa, between about 1-100,000 kDa, between about 1-75,000 kDa, between about 1-50,000 kDa, between about 1-25,000 kDa, between about 1-10,000 kDa, between about 1-5,000 kDa, between about 1-2,500 kDa, between about 1-1,000 kDa, between about 1-500 kDa, between about 10-500 kDa, between about 20-500 kDa, between about 20-400 kDa, between about 20-300 kDa, between about 20-200 kDa, between about 20-150 kDa, between about 20-100 kDa, between about 20-75 kDa, between about 20-50 kDa, between about 50-500 kDa, between about 50-400 kDa,

between about 50-300 kDa, between about 50-200 kDa, between about 50-150 kDa, between about 50-100 kDa, between about 50-75 kDa, between about 100-400 kDa, between about 100-300 kDa, or between about 100-200 kDa.

6. The nanoparticle of claim 1, wherein the at least one physiologically active agent comprises a PEGylated protein.
7. The nanoparticle of claim 1, wherein the at least one physiologically active agent comprises at least one antibody.
8. The nanoparticle of claim 1, wherein the at least one physiologically active agent comprises at least one monoclonal antibody.
9. The nanoparticle of claim 1, wherein the physiologically active agent is crosslinked.
10. The nanoparticle of claim 1, wherein the physiologically active agent is dispersed in a matrix.
11. The nanoparticle of claim 1, wherein the physiologically active agent is dispersed in a matrix comprising one or more polymers or lipids.
12. The nanoparticle of claim 1, wherein the physiologically active agent is dispersed in a crosslinked polymer matrix, wherein the crosslinked polymer is selected from the group consisting of polyphosphazenes, polycyanoacrylates, polyesters, polyhydroxyalkanoates, polyanhydrides, polydixanones, polyorthoesters, polyesteramides, polyamido amides, polythioesters, collagen, fibrin, fibrinogen, gelatin, polysaccharides, and combinations thereof.
13. The nanoparticle of claim 1, wherein the crosslinked polymer matrix comprises a polyester selected from the group consisting of poly(lactic acid), poly(glycolic acid), poly(caprolactone), poly(propylene fumarate), copolymers thereof, and combinations thereof.
14. The nanoparticle of claim 1, wherein the crosslinked polymer comprises a polysaccharide selected from the group consisting of chitosans, celluloses, modified celluloses, alginates, pectins, pullulans, hyaluronic acids, starches, amyloses, dextrans
15. The nanoparticle of claim 1, wherein the physiologically active agent is loaded into smaller nanoparticles having a particle size no greater than 50 nm, said smaller nanoparticles dispersed in a matrix.

16. The nanoparticle of claim 1, wherein the stimuli-cleavable crosslinks are degraded upon exposure to oxidant, reductant, acid, irradiation, ultrasound, heat or magnetic exposure.
17. The nanoparticle of claim 1, wherein the stimuli-cleavable crosslinks are degraded upon exposure to oxidant.
18. The nanoparticle of claim 1, wherein the stimuli-cleavable crosslinks are degraded upon exposure to reductant.
19. The nanoparticle of claim 1, wherein the stimuli-cleavable crosslinks are degraded upon exposure to acid.
20. The nanoparticle of claim 1, wherein the stimuli-cleavable crosslinks are degraded upon exposure to heat.
21. The nanoparticle of claim 1, wherein the stimuli-cleavable crosslinks are degraded by infrared radiation, microwave irradiation, or UV irradiations.
22. The nanoparticle of claim 1, wherein stimuli-cleavable crosslinks are degraded by reactive oxygen species.
23. The nanoparticle of claim 1, wherein the stimuli-cleavable crosslinks comprise functional groups selected from disulfide bonds, trisulfide bonds, diselenide bonds, thioacetals, acetals, oxalates, imines, and peptide bonds.
24. The nanoparticle of claim 1, wherein the stimuli-cleavable crosslinks comprise disulfide bonds.
25. The nanoparticle of claim 1, wherein the stimuli-cleavable crosslinks comprise trisulfide bonds.
26. The nanoparticle of claim 1, wherein the stimuli-cleavable crosslinks comprise diselenide bonds.
27. The nanoparticle of claim 1, wherein the stimuli-cleavable crosslinks comprise thioacetals.
28. The nanoparticle of claim 1, wherein the stimuli-cleavable crosslinks comprise acetals.
29. The nanoparticle of claim 1, wherein the stimuli-cleavable crosslinks comprise oxalates.

30. The nanoparticle of claim 1, wherein the stimuli-cleavable crosslinks comprise imines.
31. The nanoparticle of claim 1, wherein the stimuli-cleavable crosslinks comprise peptide bonds.
32. The nanoparticle of any preceding claim, wherein the nanoparticle has a particle size from about 50-900 nm, from about 100-800 nm, from about 100-700 nm, from about 100-600 nm, from about 100-500 nm, from about 100-400 nm, from about 100-300 nm, from about 100-200 nm, from about 200-900 nm, from about 200-800 nm, from about 200-700 nm, from about 200-600 nm, from about 200-500 nm, from about 200-400 nm, from about 200-300 nm, from about 300-900 nm, from about 300-800 nm, from about 300-700 nm, from about 300-600 nm, from about 300-500 nm, or from about 300-400 nm.
33. The nanoparticle of any preceding claim, wherein the at least one physiologically active agent comprises one or more therapeutic agents or diagnostic agents.
34. The nanoparticle of any preceding claim, wherein the at least one physiologically active agent comprises at least one therapeutic protein, small molecule therapeutic agent, peptide, aptamer, or nucleic acid.
35. The nanoparticle of any preceding claim, wherein the at least one physiologically active agent comprises a protein having a molecular weight between about 10,000 Da and 100,000 kDa, between about 100,000 Da and 100,000 kDa, between about 500,000 Da and 100,000 kDa, between about 1-100,000 kDa, between about 1-75,000 kDa, between about 1-50,000 kDa, between about 1-25,000 kDa, between about 1-10,000 kDa, between about 1-5,000 kDa, between about 1-2,500 kDa, between about 1-1,000 kDa, between about 1-500 kDa, between about 10-500 kDa, between about 20-500 kDa, between about 20-400 kDa, between about 20-300 kDa, between about 20-200 kDa, between about 20-150 kDa, between about 20-100 kDa, between about 20-75 kDa, between about 20-50 kDa, between about 50-500 kDa, between about 50-400 kDa, between about 50-300 kDa, between about 50-200 kDa, between about 50-150 kDa, between about 50-100 kDa, between about 50-75 kDa, between about 100-400 kDa, between about 100-300 kDa, or between about 100-200 kDa.

36. The nanoparticle of any preceding claim, wherein the at least one physiologically active agent comprises a PEGylated protein.
37. The nanoparticle of any preceding claim, wherein the at least one physiologically active agent comprises at least one antibody.
38. The nanoparticle of any preceding claim, wherein the at least one physiologically active agent comprises at least one monoclonal antibody.
39. The nanoparticle of any preceding claim, wherein the at least one physiologically active agent comprises a monoclonal antibody selected from abagovomab, abciximab, abituzumab, abrezekimab, abrilumab, actoxumab, adalimumab, adecatumumab, aducanumab, afasevikumab, afelimomab, alacizumab pegol, alemtuzumab, alirocumab, altumomab pentetate, amatuximab, anatumomab mafenatox, andecaliximab, anetumab ravtansine, anifrolumab, anrukinzumab, apolizumab, aprutumab ixadotin, arcitumomab, ascrinvacumab, aselizumab, atezolizumab, atidortoxumab, atinumab, atorolimumab, avelumab, azintuxizumab vedotin, bapineuzumab, basiliximab, bavituximab, BCD-100, bectumomab, begelomab, belantamab mafodotin, belimumab, bemarituzumab, benralizuma, fasenramab, berlimatoxumab, bermekimab, bersanlimab, bertilimumab, besilesomab, bevacizumab, bezlotoxumab, biciromab, bimagrumab, bimekizumab, birtamimab, bivatuzumab mertansine, bleselumab, blinatumomab, blontuvetmab, blosozumab, bococizumab, brazikumab, brentuximab vedotin, briakinumab, brodalumab, brolucizumab, brontictuzumab, burosumab, crysvitamab, cabiralizumab, camidanlumab tesirine, camrelizumab, canakinumab, cantuzumab mertansine, cantuzumab ravtansine, caplacizumab, capromab pendetide, carlumab, carotuximab, catumaxomab, CBR96-doxorubicin immunoconjugate, cedelizumab, cemiplimab, cergutuzumab amunaleukin, certolizumab pegol, cetrelimab, cetuximab, cibisatamab, cirmtuzumab, citatuzumab bogatox, cixutumumab, clazakizumab, clenoliximab, clivatuzumab tetraxetan, codrituzumab, cofetuzumab pelidotin, coltuximab ravtansine, conatumumab, concizumab, cosfroviximab, CR6261, crenezumab, crizanlizumab, crotedumab, cusatuzumab, dacetuzumab, daclizumab, dalotuzumab, dapirolizumab pegol, daratumumab, dectrekumab, demcizumab, denintuzumab mafodotin, denosumab, depatuxizumab mafodotin, derlotuximab biotin, detumomab,

dezamizumab, dinutuximab, diridavumab, domagrozumab, dorlimomab aritox, dostarlimab, drozitumab, ds-8201, duligotuzumab, dupilumab, durvalumab, dusigitumab, duvortuxizumab, ecromeximab, eculizumab, edobacomab, edrecolomab, efalizumab, efungumab, eldelumab, elezanumab, elgemtumab, elotuzumab, elsilimomab, emactuzumab, emapalumab, emibetuzumab, emicizumab, hemlibra, enapotamab vedotin, enavatuzumab, enfortumab vedotin, enlimomab pegol, enoblituzumab, enokizumab, enoticumab, ensituximab, epitumomab cituxetan, epratuzumab, eptinezumab, erenumab, erlizumab, ertumaxomab, etaracizumab, etigilimab, etrolizumab, evinacumab, evolocumab, exbivirumab, fanolesomab, faralimomab, faricimab, farletuzumab, fasinumab, fbta05, felvizumab, fezakinumab, fibatuzumab, ficlatuzumab, figitumumab, firivumab, flanvotumab, fletikumab, flotetuzumab, fontolizumab, foralumab, foravirumab, fremanezumab, fresolimumab, frovocimab, frunevetmab, fulranumab, futuximab, galcanezumab, galiximab, gancotamab, ganitumab, gantenerumab, gatipotuzumab, gavilimomab, gedivumab, gemtuzumab ozogamicin, gevokizumab, gilvetmab, gimsilumab, girentuximab, glembatumumab vedotin , golimumab, gomiliximab, gosuranemab, guselkumab, ianalumab, ibalizumab, IBI308, ibritumomab tiuxetan, icrucumab, idarucizumab, ifabotuzumab, igovomab, iladatuzumab vedotin, IMAB362, imalumab, imaprelimab, imciromab, imgatuzumab, inclacumab, indatuximab ravtansine, indusatumab vedotin, inebilizumab, infliximab, inolimomab, inotuzumab ozogamicin, intetumumab , IOMAB-B, ipilimumab, iratumumab, isatuximab, iscalimab, istiratumab, itolizumab, ixekizumab, keliximab, labetuzumab, lacnotuzumab, ladiratuzumab vedotin, lampalizumab, lanadelumab, landogrozumab, laprituximab emtansine, larcaviximab, lebrikizumab, lemalesomab, lendalizumab, lenvervimab, lenzilumab, lerdelimumab, leronlimab, lesofavumab, letolizumab, lexatumumab, libivirumab, lifastuzumab vedotin, ligelizumab, lilotomab satetraxetan, lintuzumab, lirilumab, lodelcizumab, lokivetmab, loncastuximab tesirine, lorvotuzumab mertansine, losatuxizumab vedotin, lucatumumab, lulizumab pegol, lumiliximab, lumretuzumab, lupartumab amadotin, lutikizumab, mapatumumab, margetuximab, marstacimab, maslimomab, matuzumab, mavrilimumab, mepolizumab, metelimumab, milatuzumab, minretumomab, mirikizumab, mirvetuximab soravtansine, mitumomab,

modotuximab, mogamulizumab, monalizumab, morolimumab, mosunetuzumab, motavizumab, moxetumomab pasudotox, muromonab-CD3, nacolomab tafenatox, namilumab, naptumomab estafenatox, naratuximab emtansine, narnatumab, natalizumab, navicixizumab, navivumab, naxitamab, nebacumab, necitumumab, nemolizumab, NEOD001, nerelimomab, nesvacumab, netakimab, nimotuzumab, nirsevimab, nivolumab, nofetumomab merpentan, obiltoxaximab, obinutuzumab, ocaratuzumab, ocrelizumab, odulimomab, ofatumumab, olaratumab, oleclumab, olendalizumab, olokizumab, omalizumab, omburtamab, OMS721, onartuzumab, ontuxizumab, onvatilimab, opicinumab, oportuzumab monatox, oregovomab, orticumab, otelixizumab, otilimab, otlertuzumab, oxelumab, ozanezumab, ozoralizumab, pagibaximab, palivizumab, pamrevlumab, panitumumab, pankomab, panobacumab, parsatuzumab, pascolizumab, pasotuxizumab, pateclizumab, patritumab, PDR001, pembrolizumab, pemtumomab, perakizumab, pertuzumab, pexelizumab, pidilizumab, pinatuzumab vedotin, pintumomab, placulumab, plozalizumab, pogalizumab, polatuzumab vedotin, ponezumab, porgaviximab, prasinezumab, prezalizumab, priliximab, pritoxaximab, pritumumab, PRO 140, quilizumab, racotumomab, radretumab, rafivirumab, ralpancizumab, ramucirumab, ranevetmab, ranibizumab lucentis, ravagalimab, ravulizumab, raxibacumab, refanezumab, regavirumab, relatlimab, remtolumab, reslizumab, rilotumumab, rinucumab, risankizumab, rituximab, rivabazumab pegol, rmabrabishield, robatumumab, roledumab, romilkimab, romosozumab, rontalizumab, rosmantuzumab, rovalpituzumab tesirine, rovelizumab, rozanolixizumab, ruplizumab, SA237, sacituzumab govitecan, samalizumab, samrotamab vedotin, sarilumab, satralizumab, satumomab pendetide, secukinumab, selicrelumab, seribantumab, setoxaximab, setrusumab, sevirumab, SGN-CD19a, SHP647, sibrotuzumab, sifalimumab, siltuximab, simtuzumab, siplizumab sirtratumab vedotin, sirukumab, sofituzumab vedotin, solanezumab, solitomab, sonepcizumab, sontuzumab, spartalizumab, stamulumab , sulesomab, suptavumab, sutimlimab, suvizumab, suvratoxumab, tabalumab, tacatuzumab tetraxetan, tadocizumab, talacotuzumab, talizumab, tamtuvetmab, tanezumab, taplitumomab paptox, tarextumab, tavolimab, tefibazumab, telimomab aritox, telisotuzumab vedotin, tenatumomab, teneliximab,

teplizumab, tepoditamab, teprotumumab, tesidolumab, tetulomab, tezepelumab, TGN1412, tibulizumab, tigatuzumab, tildrakizumab, timigutuzumab, timolumab, tiragotumab, tislelizumab, tisotumab vedotin, TNX-650, tocilizumab, tomuzotuximab, toralizumab, tosatoxumab, tositumomab, tovetumab, tralokinumab, trastuzumab, trastuzumab emtansine, TRBS07, tregalizumab, tremelimumab, trevogrumab, tucotuzumab celmoleukin, tuvirumab, ublituximab, ulocuplumab, urelumab, urtoxazumab, ustekinumab, utomilumab, vadastuximab talirine, vanalimabmab, vandortuzumab vedotin, vantictumab, vanucizumab, vapaliximab, varisacumab, varlilumab, vatelizumab, vedolizumab, entyvio, veltuzumab, vepalimomab, vesencumab, visilizumab, vobarilizumab, volociximab, vonlerolizumab, vopratelimab, vorsetuzumab mafodotin, votumumab, vunakizumab, xentuzumab, XMAB-5574, zalutumumab, zanolimumab, zatuximab, zenocutuzumab, ziralimumab, zolbetuximab, zolimomab aritox, ponezumab, and combinations thereof.

40. The nanoparticle of any preceding claim, wherein the physiologically active agent is a therapeutic protein selected from Lepirudin, Dornase alfa, Denileukin diftitox, Bivalirudin, Leuprolide, Peginterferon alfa-2a, Alteplase, Interferon alfa-n1, Darbepoetin alfa, Reteplase, Epoetin alfa, Salmon Calcitonin, Interferon alfa-n3, Pegfilgrastim, Sargramostim, Secretin, Peginterferon alfa-2b, Asparaginase, Thyrotropin Alfa, Antihemophilic Factor, Anakinra, Gramicidin D, Intravenous Immunoglobulin, Anistreplase, Insulin Regular, Tenecteplase, Menotropins, Interferon gamma-1b, Interferon Alfa-2a, Recombinant, Coagulation factor VIIa, Oprelvekin, Palifermin, Glucagon recombinant, Aldesleukin, Botulinum Toxin Type B, Lutropin alfa, Insulin Lispro, Insulin Glargine, Collagenase, Rasburicase, Imiglucerase, Alpha-1-proteinase inhibitor, Pegaspargase, Interferon beta-1a, Pegademase bovine, Human Serum Albumin, Eptifibatide, Serum albumin iodonated, Follitropin beta, Vasopressin, Interferon beta-1b, Hyaluronidase, Insulin, porcine, Digoxin Immune Fab (Ovine), Daptomycin, Pegvisomant, Botulinum Toxin Type A, Pancrelipase, Streptokinase, Alglucerase, Laronidase, Urofollitropin, Serum albumin, Choriogonadotropin alfa, Antithymocyte globulin, Filgrastim, Coagulation factor ix, Becaplermin, Agalsidase beta, Interferon alfa-2b, Oxytocin, Enfuvirtide, Idursulfase,

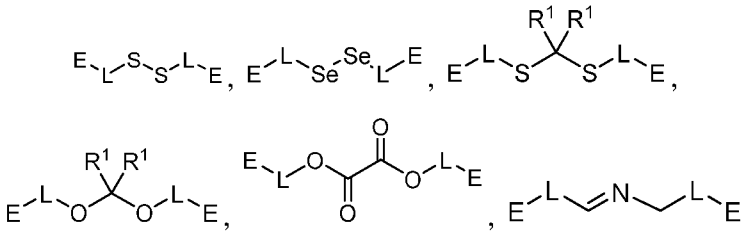
Alglucosidase alfa, Exenatide, Mecasermin, Pramlintide, Galsulfase, Abatacept, Cosyntropin, Corticotropin, Insulin aspart, Insulin detemir, Insulin glulisine, Pegaptanib, Nesiritide, Thymalfasin, Defibrotide, Natural alpha interferon OR multiferon, Glatiramer acetate, Preotact, Teicoplanin, Sulodexide, Teriparatide, Liraglutide, Belatacept, Buserelin, Velaglucerase alfa, Tesamorelin, Taliglucerase alfa, Aflibercept, Asparaginase erwinia chrysanthemi, Ocriplasmin, Glucarpidase, Teduglutide, Insulin,isophane, Epoetin zeta, Fibrinolysin aka plasmin, Follitropin alpha, Romiplostim, Lucinactant, Aliskiren, Ragweed Pollen Extract, Somatotropin Recombinant, Drotrecogin alfa, Alefacept, OspA lipoprotein, Urokinase, Abarelix, Sermorelin, Aprotinin, Albiglutide, Ancestim, Antithrombin Alfa, Antithrombin III human, Asfotase Alfa, Autologous cultured chondrocytes, Beractant, C1 Esterase Inhibitor (Human), Coagulation Factor XIII A-Subunit (Recombinant), Conestat alfa, Daratumumab, Desirudin, Dulaglutide, Elosulfase alfa, Elotuzumab, Fibrinogen Concentrate (Human), Filgrastim-sndz, Gastric intrinsic factor, Hepatitis B immune globulin, Human calcitonin, Human clostridium tetani toxoid immune globulin, Human rabies virus immune globulin, Human Rho(D) immune globulin, Hyaluronidase (Human Recombinant), Immune Globulin Human, Turoctocog alfa, Tuberculin Purified Protein Derivative, Simoctocog Alfa, Sebelipase alfa, Sacrosidase, Prothrombin complex concentrate, Poractant alfa, Pembrolizumab, Peginterferon beta-1a, Metreleptin, Methoxy polyethylene glycol-epoetin beta, Insulin Pork, Insulin Degludec, Insulin Beef, Thyroglobulin, Anthrax immune globulin human, Anti-inhibitor coagulant complex, Anti-thymocyte Globulin (Equine), C1 Esterase Inhibitor (Recombinant) , Chorionic Gonadotropin (Recombinant), Coagulation factor X human, Efmoroctocog alfa, Factor IX Complex (Human), Hepatitis A Vaccine, Human Varicella-Zoster Immune Globulin, Lenograstim, Pegloticase, Protamine sulfate, Protein S human, Sipuleucel-T, Somatropin recombinant, Susoctocog alfa, Thrombomodulin Alfa, and combinations thereof.

41. The nanoparticle of any preceding claim, wherein the physiologically active agent is crosslinked.

42. The nanoparticle of any preceding claim, wherein the physiologically active agent is dispersed in a matrix.
43. The nanoparticle of any preceding claim, wherein the physiologically active agent is dispersed in a matrix comprising one or more polymers or lipids.
44. The nanoparticle of any preceding claim, wherein the physiologically active agent is dispersed in a crosslinked polymer matrix, wherein the crosslinked polymer is selected from the group consisting of polyphosphazenes, polycyanoacrylates, polyesters, polyhydroxyalkanoates, polyanhydrides, polydixanones, polyorthoesters, polyesteramides, polyamido amides, polythioesters, collagen, fibrin, fibrinogen, gelatin, polysaccharides, and combinations thereof.
45. The nanoparticle of any preceding claim, wherein the crosslinked polymer matrix comprises a polyester selected from the group consisting of poly(lactic acid), poly(glycolic acid), poly(caprolactone), poly(propylene fumarate), copolymers thereof, and combinations thereof.
46. The nanoparticle of any preceding claim, wherein the crosslinked polymer comprises a polysaccharide selected from the group consisting of chitosans, celluloses, modified celluloses, alginates, pectins, pullulans, hyaluronic acids, starches, amyloses, dextrans
47. The nanoparticle of any preceding claim, wherein the physiologically active agent is loaded into smaller nanoparticles having a particle size no greater than 50 nm, said smaller nanoparticles dispersed in a matrix.
48. The nanoparticle of any preceding claim, wherein the stimuli-cleavable crosslinks are degraded upon exposure to oxidant, reductant, acid, irradiation, ultrasound, heat or magnetic exposure.
49. The nanoparticle of any preceding claim, wherein the stimuli-cleavable crosslinks are degraded by infrared radiation, microwave irradiation, or UV irradiations.
50. The nanoparticle of any preceding claim, wherein stimuli-cleavable crosslinks are degraded by reactive oxygen species.
51. The nanoparticle of any preceding claim, wherein the stimuli-cleavable crosslinks comprise functional groups selected from disulfide bonds, trisulfide bonds, diselenide bonds, thioacetals, acetals, oxalates, imines, and peptide bonds.

52. A method of preparing the nanoparticle of any preceding claim, comprising contacting the physiologically active agent with at least one crosslinking agent at a concentration and time sufficient to prepare a crosslinked nanoparticle.

53. The method of any preceding claim, wherein the crosslinking agent has the formula:

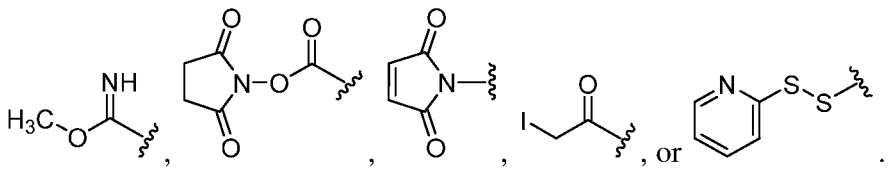


wherein L represent a linking group, R^1 is in each case independently hydrogen or C_{1-6}alkyl, or where two R^1 groups form a ring; and

E is an electrophilic group.

54. The method of any preceding claim, wherein E comprises an imidoester, an N-hydroxysuccinimide ester, a maleimide, a haloacetyl, or a pyridyl disulfide.

55. The method of any preceding claim, wherein E has the formula:



56. The method of any preceding claim, wherein L is selected from C_{1-10}alkyl, poly(ethylene glycol), or aryl.

57. The method of any preceding claim, wherein the crosslinking agent is provided in an amount from 0.00001-0.1 wt%, relative to the physiologically active agent.

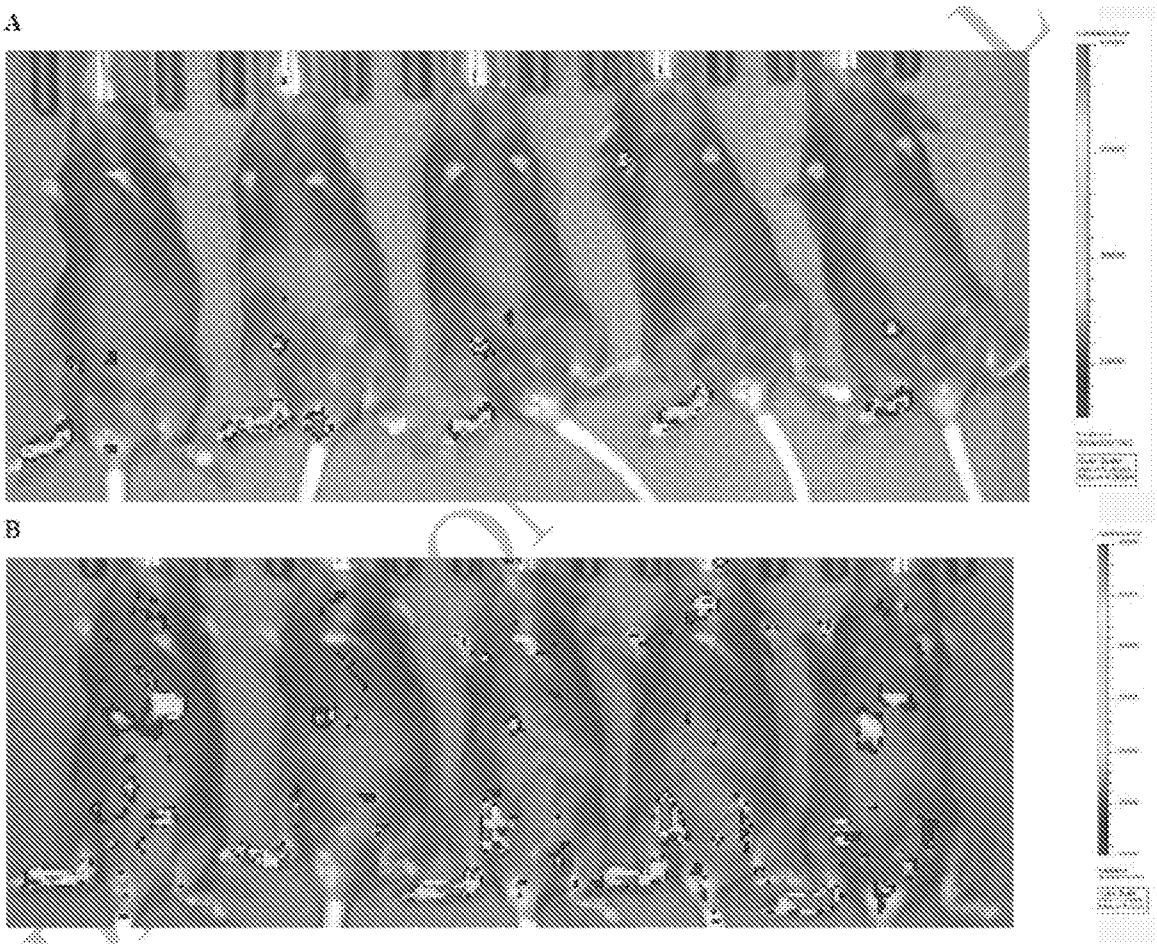


FIGURE 1

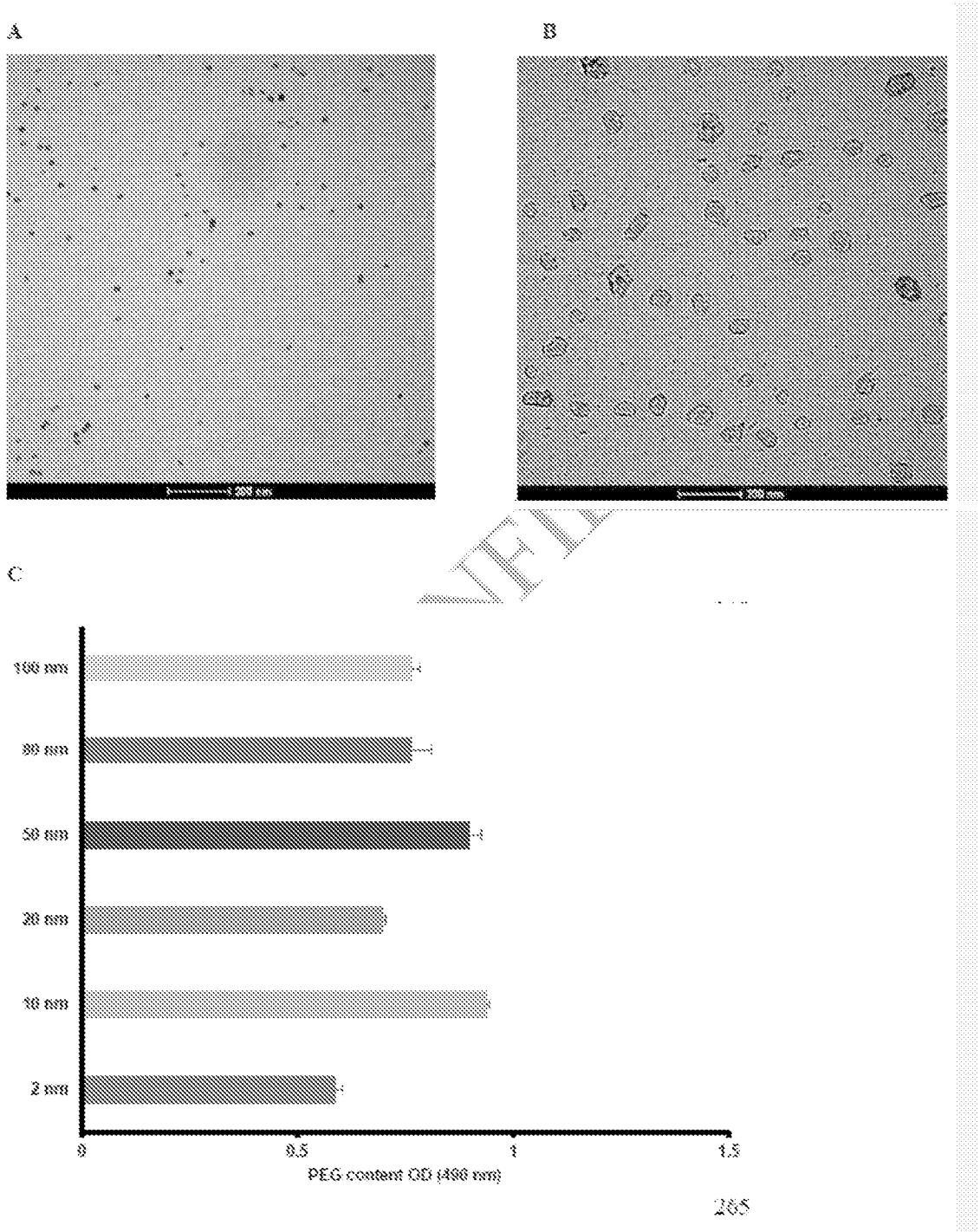

A
B
C
100 nm
80 nm
50 nm
20 nm
10 nm
2 nm
0
0.5
1
1.5
PEG content OD (490 nm)
265

FIGURE 2

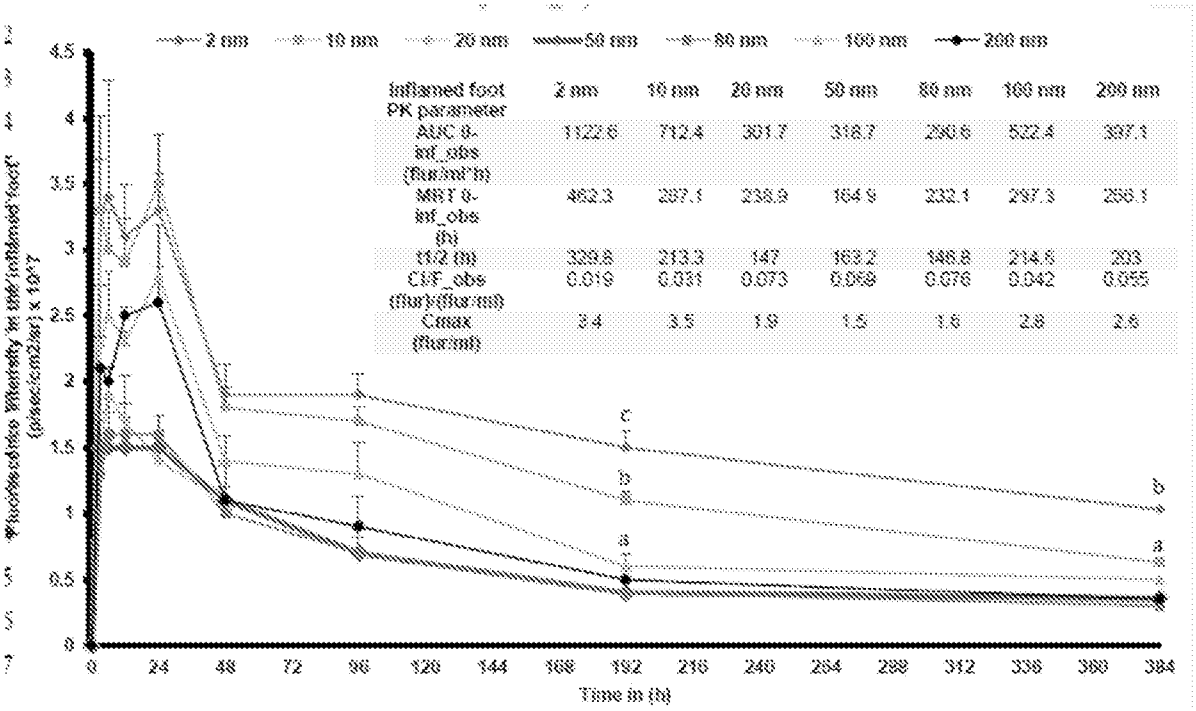

2 nm
10 nm
20 nm
50 nm
80 nm
100 nm
200 nm
Inflamed foot PK parameter
AUC 0-inf_obs (flur.ml*h)
MRT 0-inf_obs (h)
t1/2 (h)
CL/F_obs ((flur)/(flur.ml))
Cmax (flur.ml)
1122.6 712.4 301.7 318.7 250.6 522.4 397.1
462.3 287.1 238.9 164.9 232.1 297.3 256.1
329.8 213.3 147 163.2 146.8 214.6 203
0.019 0.031 0.073 0.059 0.076 0.042 0.055
3.4 3.5 1.9 1.5 1.6 2.8 2.6
Time in (h)

FIGURE 3

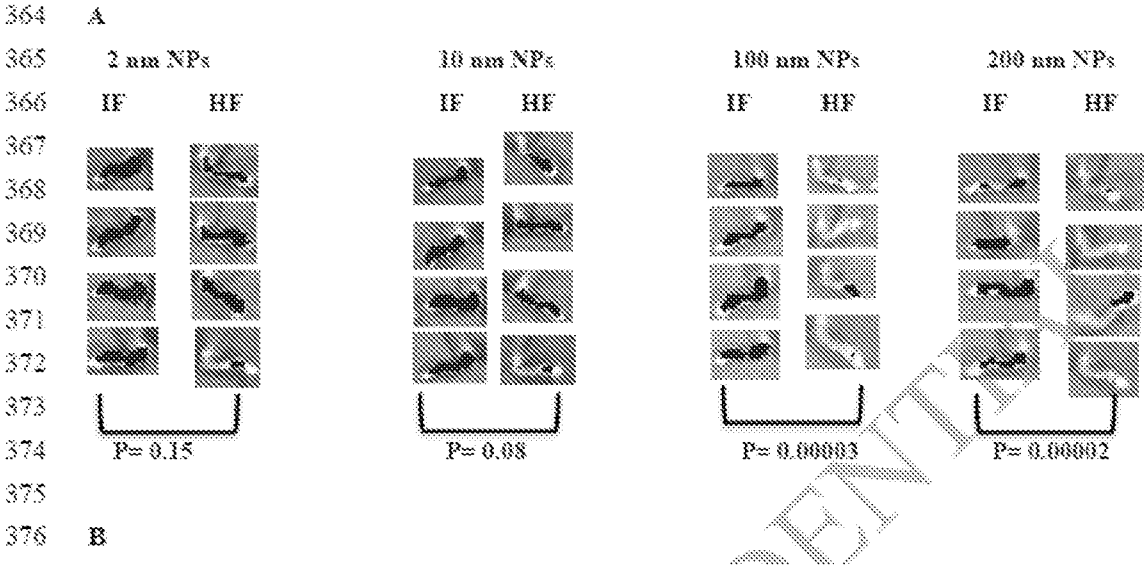

A
2 nm NPs
10 nm NPs
100 nm NPs
200 nm NPs
IF
HF
P= 0.15
P= 0.08
P= 0.00003
P= 0.00002
B
% of specificity toward inflamed host
0
5
10
15
20
25
2 nm
10 nm
20 nm
50 nm
80 nm
100 nm
200 nm

FIGURE 4

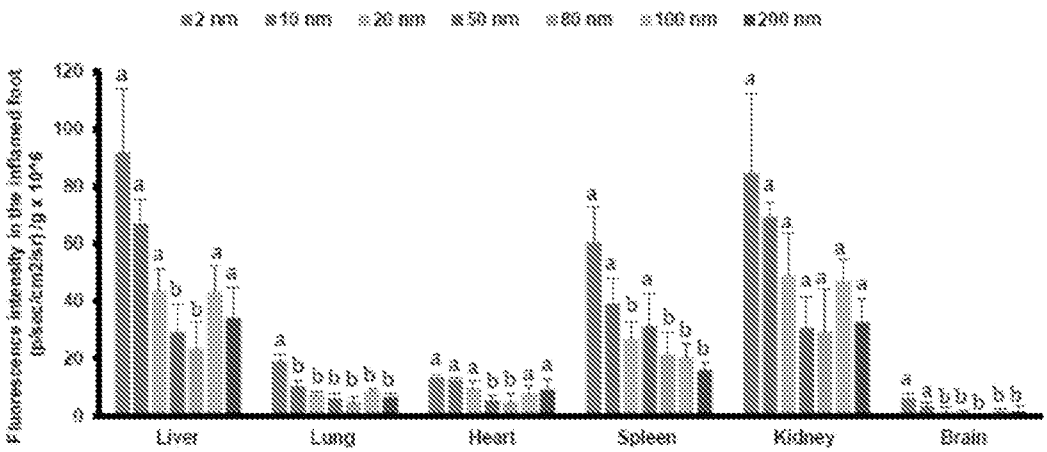

2 nm
10 nm
20 nm
50 nm
80 nm
100 nm
200 nm
Liver
Lung
Heart
Spleen
Kidney
Brain

FIGURE 5

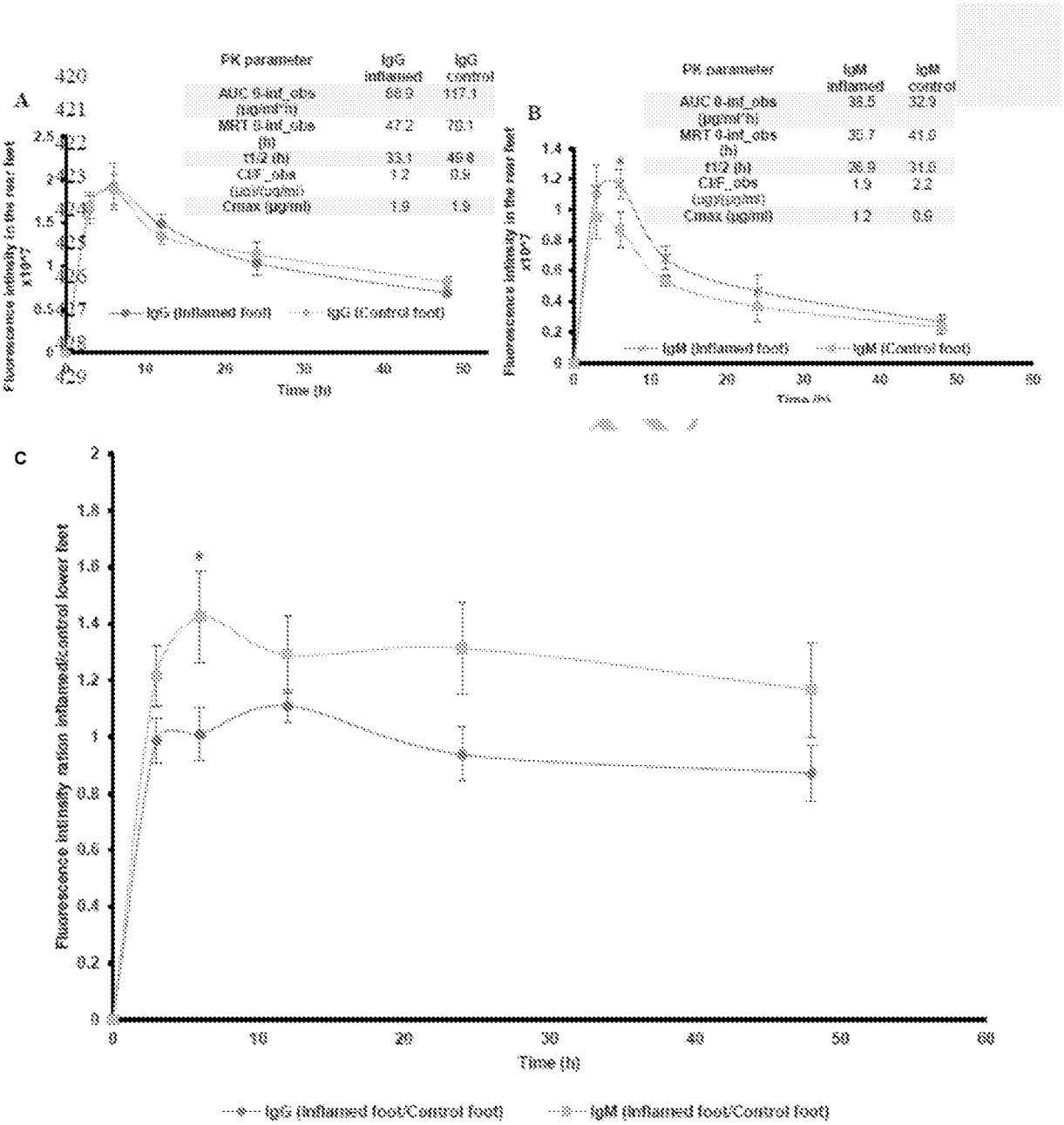
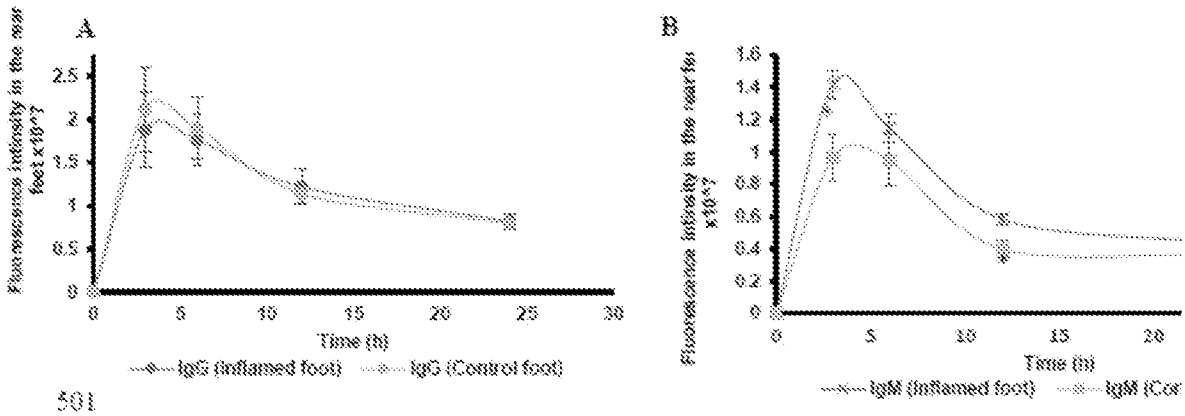

A
PK parameter
IgG inflamed
IgG control
AUC 0-inf_obs (pg/ml*h)
MRT 0-inf_obs (h)
t1/2 (h)
CL/F_obs (ml/h/kg/ml)
Cmax (pg/ml)
IgG (Inflamed foot)
IgG (Control foot)
Time (h)
B
PK parameter
IgM inflamed
IgM control
IgM (Inflamed foot)
IgM (Control foot)
C
Fluorescence intensity ratio inflamed/control lower foot
Time (h)
IgG (Inflamed foot/Control foot)
IgM (Inflamed foot/Control foot)

FIGURE 6



501

502

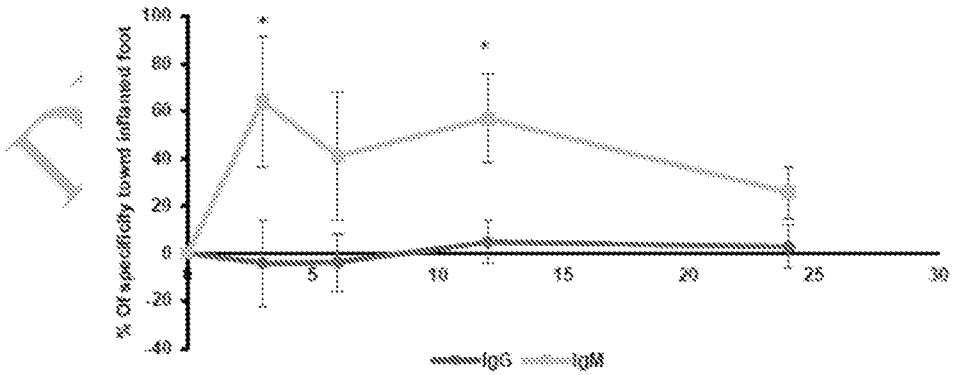
503 C

504

PK parameter	IgG Inflamed foot	IgG Control foot	IgM Inflamed foot	IgM Control foot
AUC 0-inf_obs (µg/ml*h)	52.5	50.33	27.5	21.8
MRT 0-inf_obs (h)	24.6	21.2	17.7	20.4
t1/2 (h)	17	14.6	12.2	13.9
CL/F_obs ((µg)/(µg/ml))	3	3.6	2.7	3.4
Cmax (µg/ml)	1.88	2.12	1.42	0.96

506 508 510 511

512 D



513

22

FIGURE 7

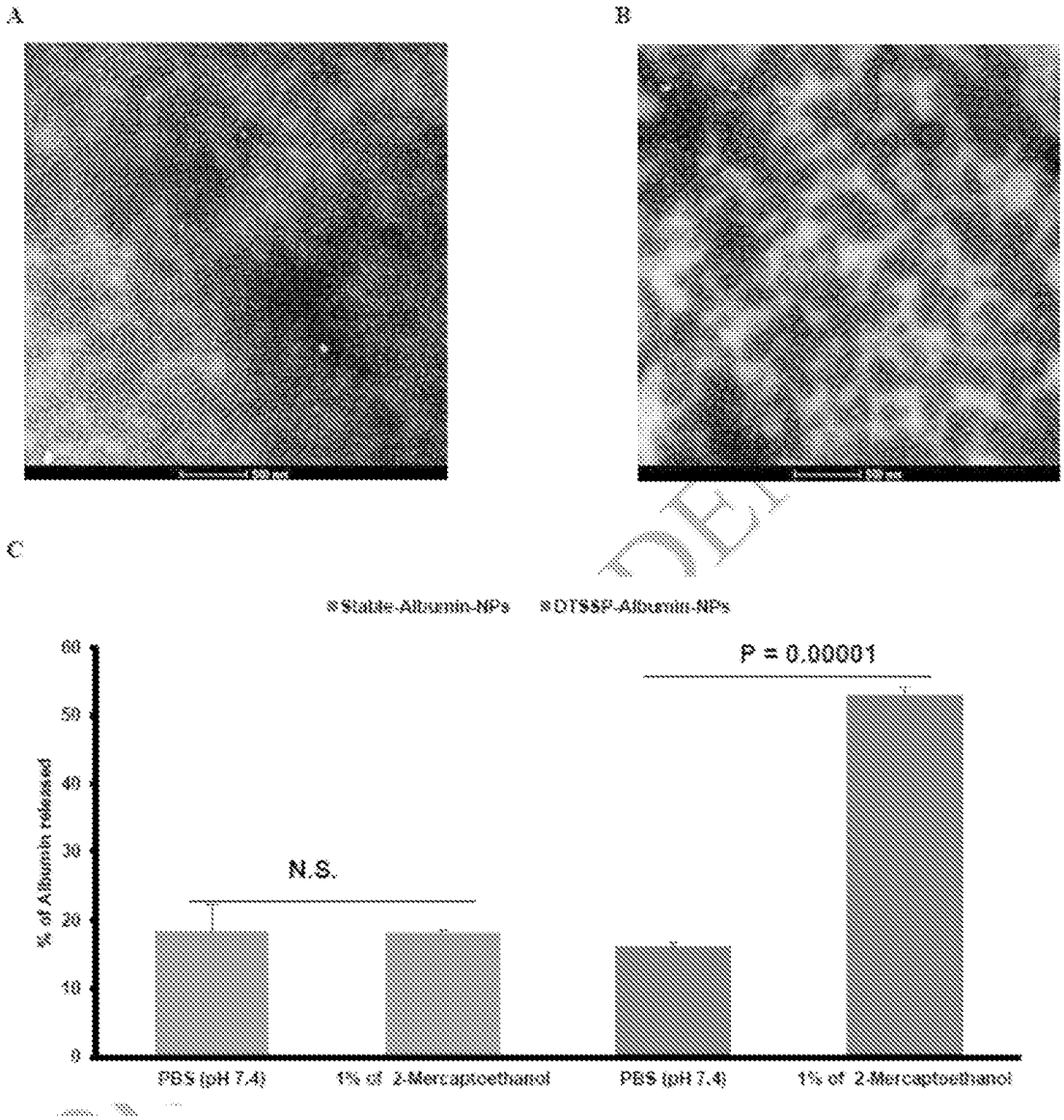

A
B
C
Stable-Albumin-NPs
DTSSP-Albumin-NPs
P = 0.00001
N.S.
% of Albumin released
60
50
40
30
20
10
0
PBS (pH 7.4)
1% of 2-Mercaptoethanol
PBS (pH 7.4)
1% of 2-Mercaptoethanol

FIGURE 8

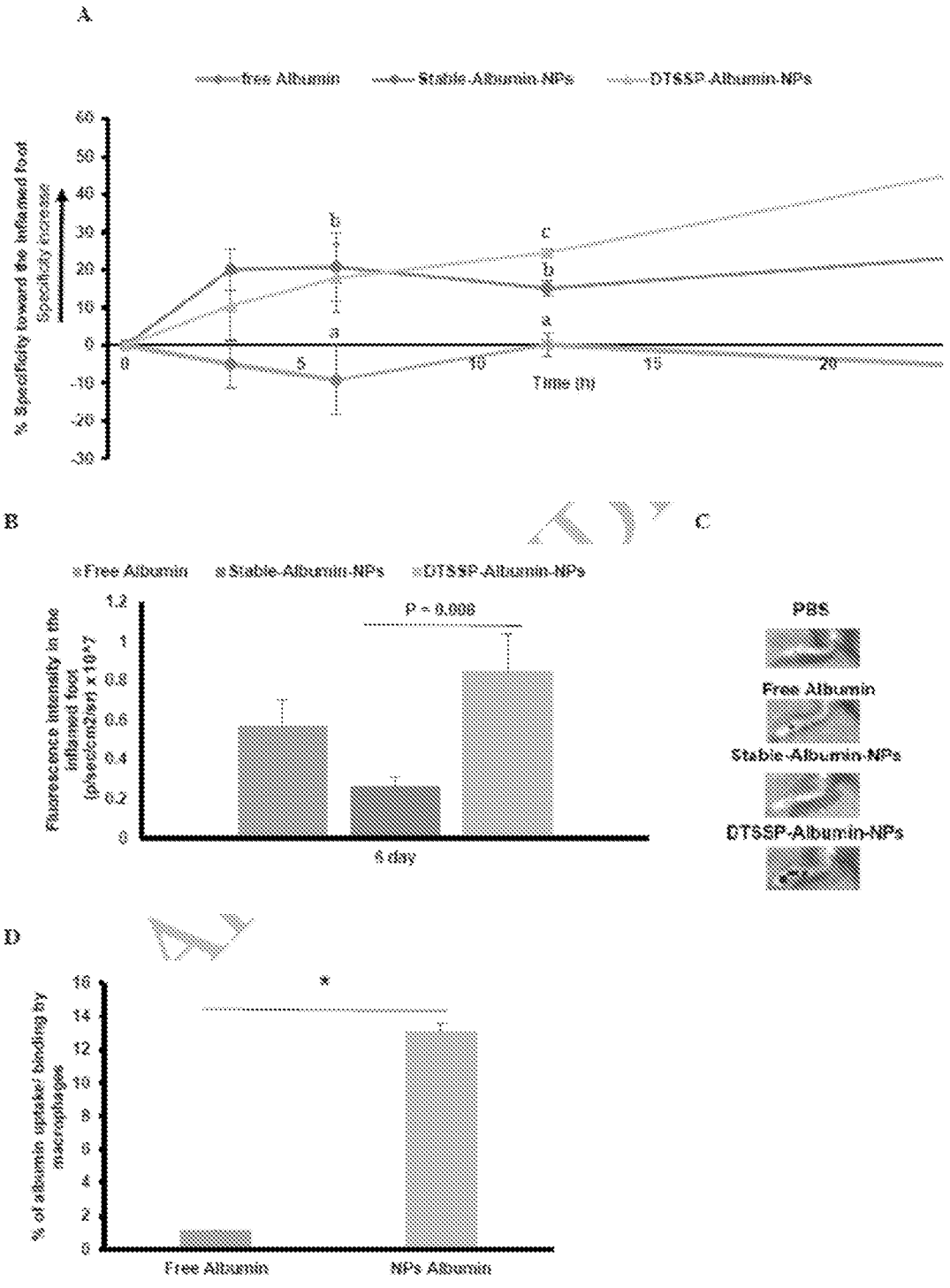

A
free Albumin
Stable-Albumin-NPs
DTSSP-Albumin-NPs
% Specificity toward the inflamed foot
Specificity increase
Time (h)
B
C
Free Albumin
Stable-Albumin-NPs
DTSSP-Albumin-NPs
P = 0.008
Fluorescence intensity in the inflamed foot (p/sec/cm2/sr) x 10^7
5 day
PBS
Free Albumin
Stable-Albumin-NPs
DTSSP-Albumin-NPs
D
% of albumin uptake binding by macrophages
Free Albumin
NPs Albumin

FIGURE 9

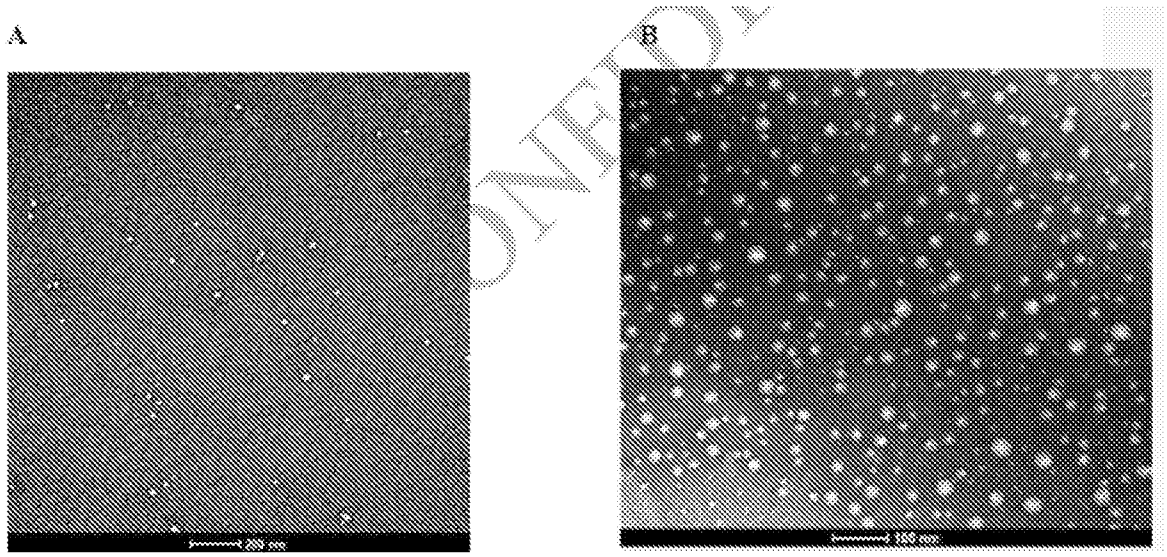


FIGURE 10

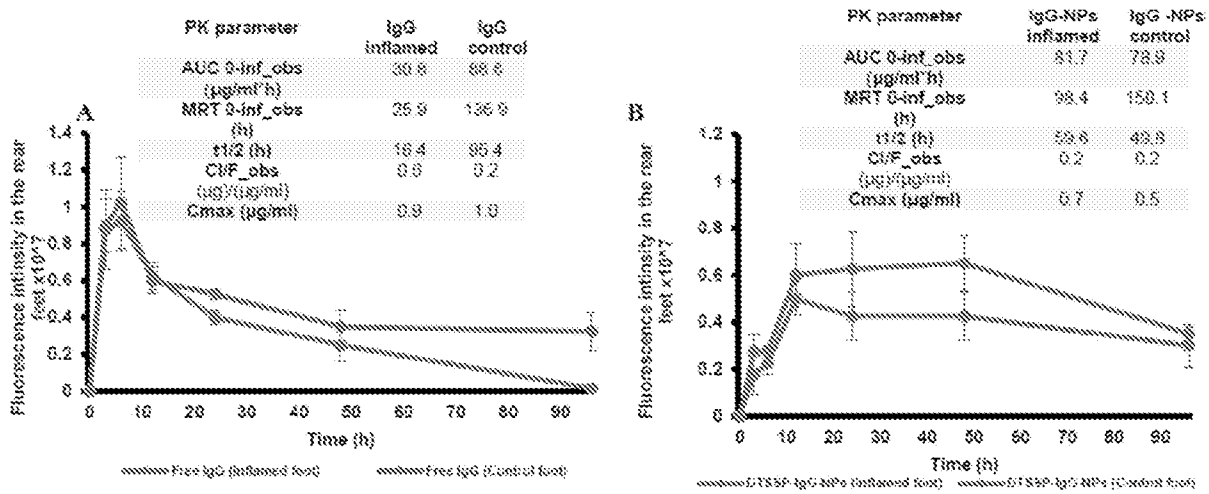


FIGURE 11A-B

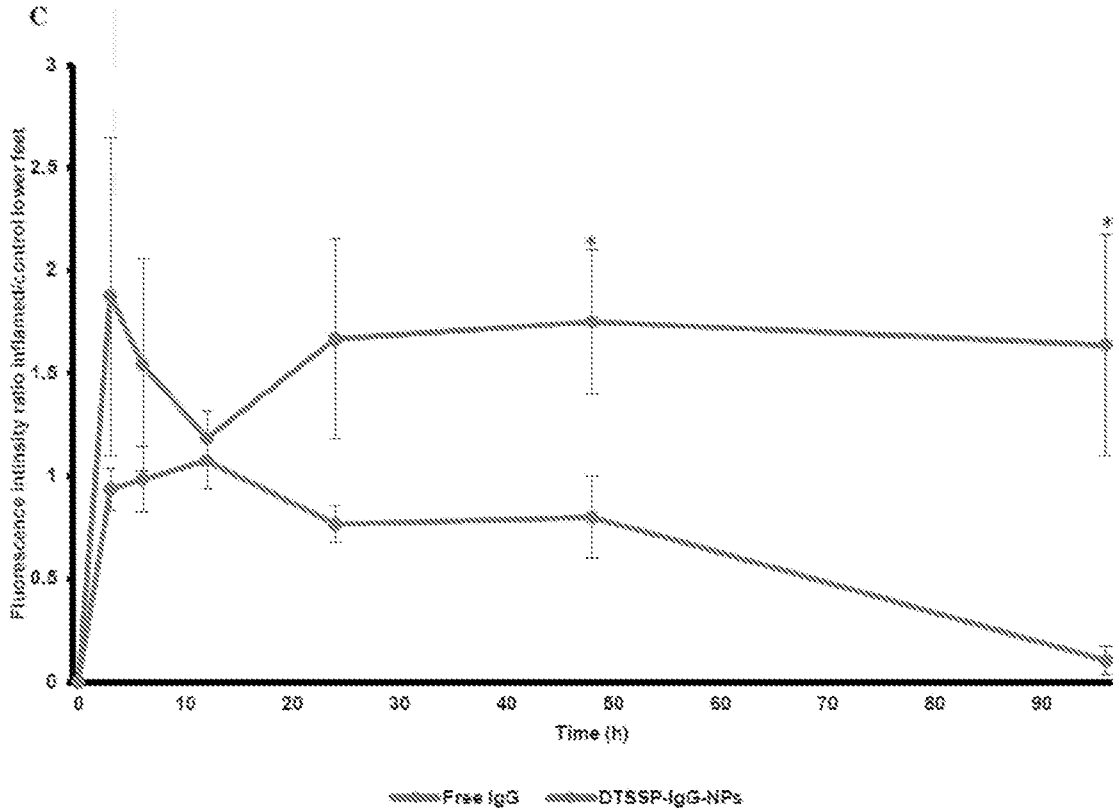

C
Fluorescence intinsity ratio inflamed:control lower feet
0
0.5
1
1.5
2
2.5
3
0
10
20
30
40
50
60
70
80
90
Time (h)
Free IgG
DTSSP-IgG-NPs

FIGURE 11C

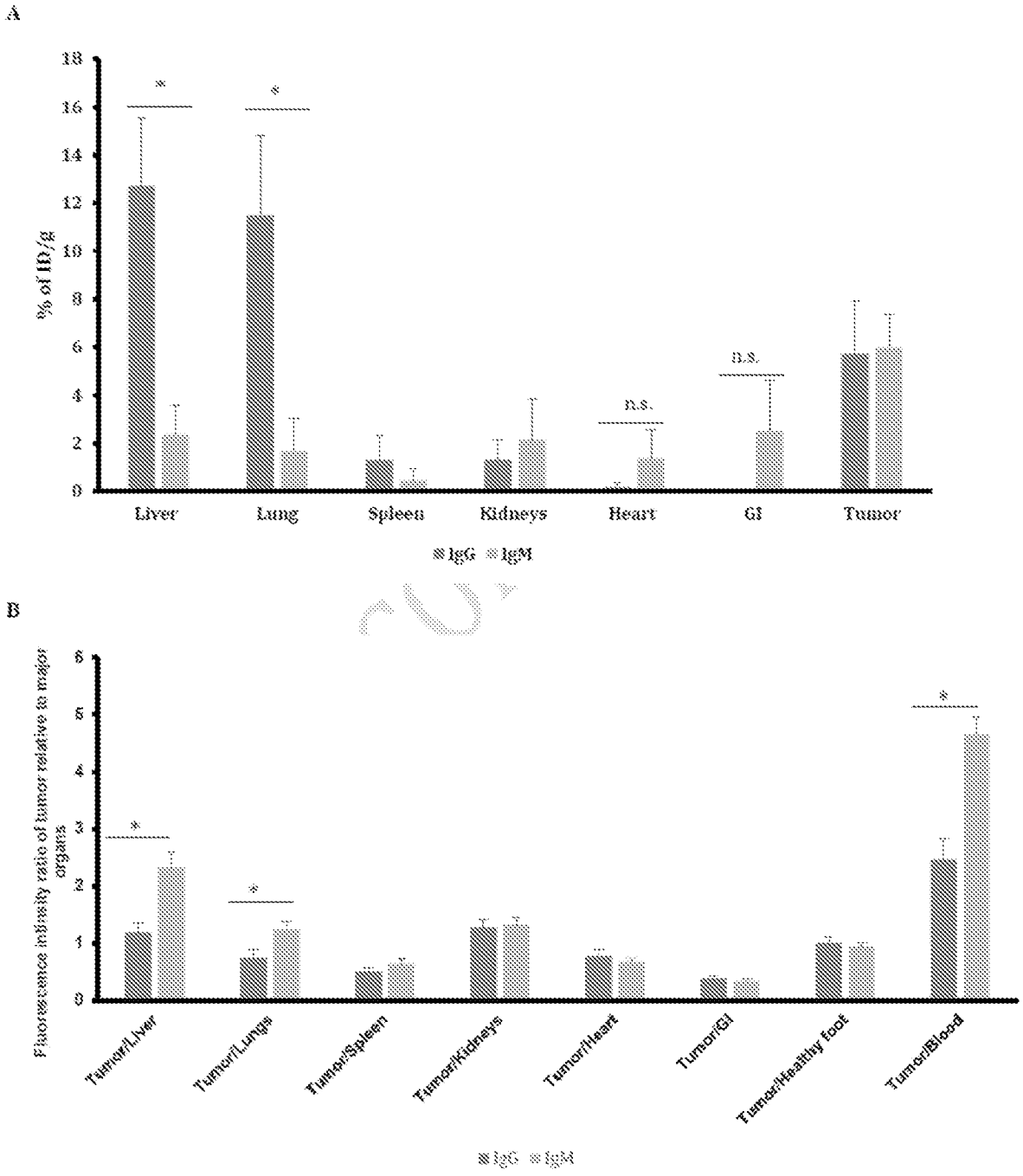

A
% of ID/g
Liver
Lung
Spleen
Kidneys
Heart
GI
Tumor
n.s.
IgG IgM
B
Fluorescence intensity ratio of tumor relative to major organs
Tumor/Liver
Tumor/Lung
Tumor/Spleen
Tumor/Kidneys
Tumor/Heart
Tumor/GI
Tumor/Healthy foot
Tumor/Blood
IgG IgM

FIGURE 12

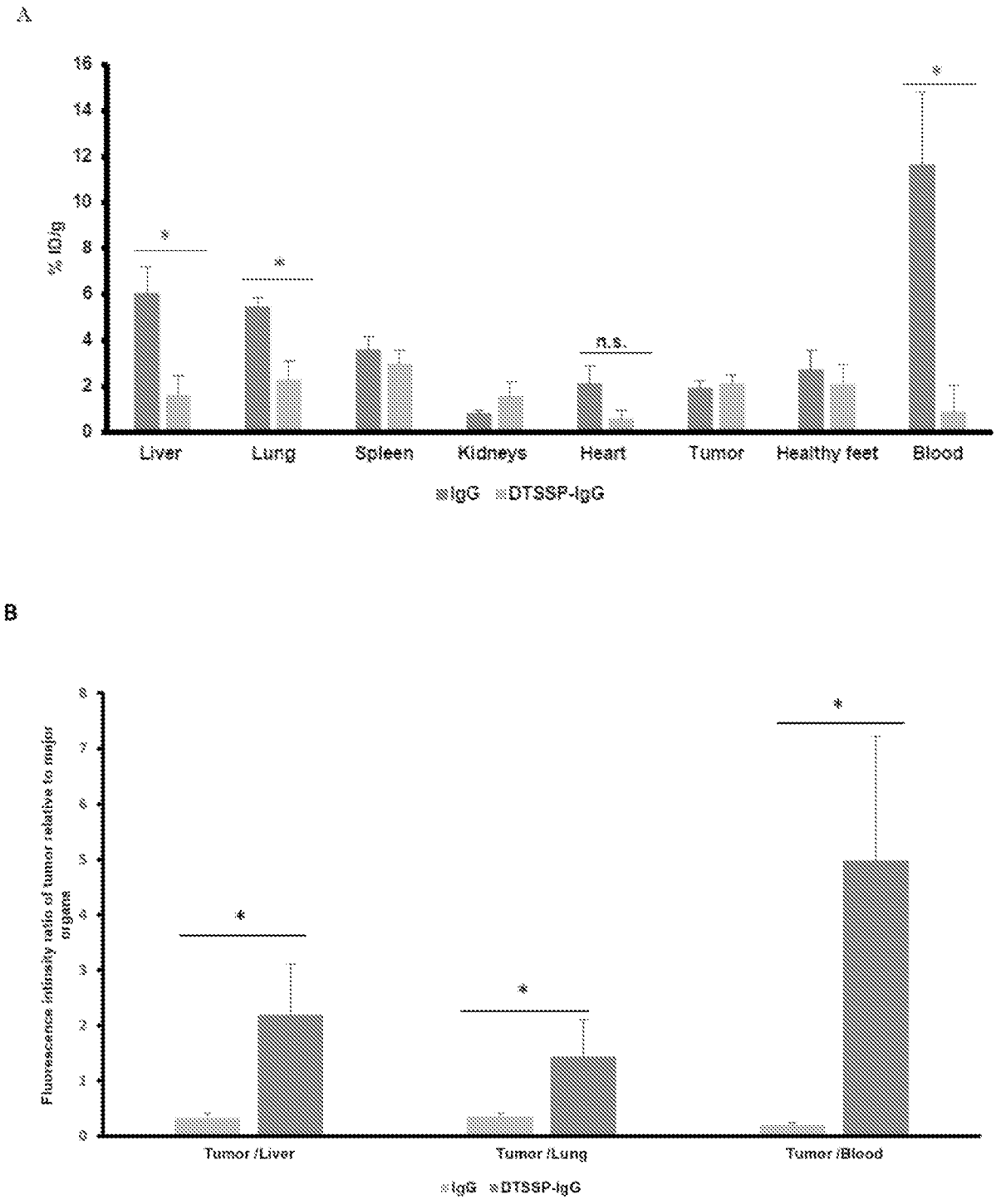

A
% ID/g
16
14
12
10
8
6
4
2
0
*
*
n.s.
*
Liver
Lung
Spleen
Kidneys
Heart
Tumor
Healthy feet
Blood
IgG
DTSSP-IgG
B
Fluorescence intensity ratio of tumor relative to major organs
*
*
*
Tumor /Liver
Tumor /Lung
Tumor /Blood
IgG
DTSSP-IgG

FIGURE 13

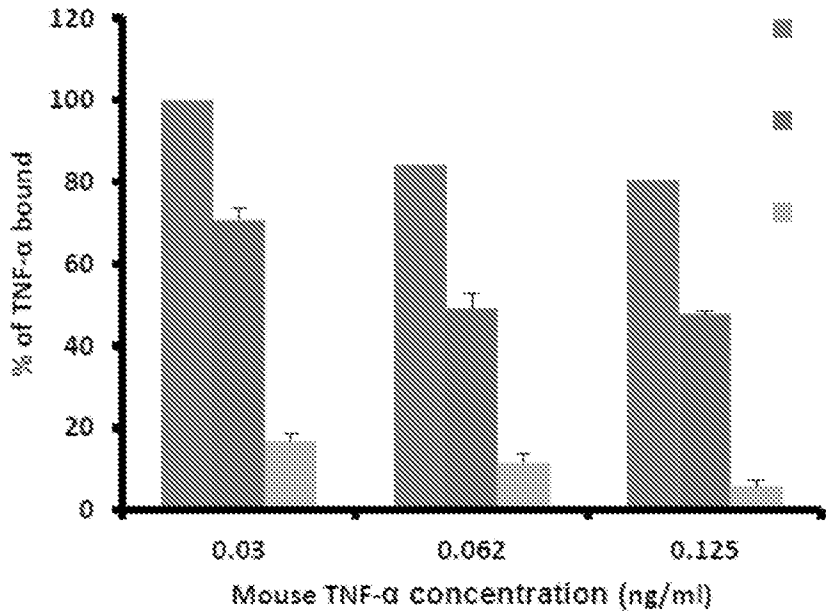

120
100
80
60
40
20
0
% of TNF-α bound
0.03
0.062
0.125
Mouse TNF-α concentration (ng/ml)

FIGURE 14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/64840

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 47/22; A61K 47/24; A61K 47/26 (2020.01)

CPC - A61K 47/20; A61K 47/22; A61K 47/24; A61K 47/26; A61K 9/107; A61K 9/1075

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	Aldayel et al. "Acid-Sensitive Sheddable PEGylated PLGA Nanoparticles Increase the Delivery of TNF-a SiRNA in Chronic Inflammation Sites." Molecular Therapy - Nucleic Acids, vol. 5, 2016, doi:10.1038/mtna.2016.39.	1-5, 11, 32/(1-5, 11) --- 6-7, 12-14, 16-17, 23-24, 32/(6-7, 12-14, 16-17, 23-24)
Y	US 2012/0230913 (Johnston et al.) 13 September 2012 Entire document, especially Abstract, para [0003]; [0130]; [0154]	6-7, 32/(6-7)
Y	US 5,464,932 A (Allcock et al.) 7 November 1995 Entire document, especially Abstract, col 2 ln 47-50; col 3 ln 25-31; col 30 ln 28, 56-58	12, 32/12
Y	US 2009/0117070 A1 (Daniloff et al.) 7 May 2009 Entire document, especially Abstract, para [0077]; [0185]; [0190]; [0203]-[0205]; [0242]; [0322]	13-14, 16-17, 23-24, 32/(13-14, 16-17, 23-24)
A	Joseph et al. "Chapter 7 - Peptide and Protein-Based Therapeutic Agents.? Emerging Nanotechnologies for Diagnostics, Drug Delivery and Medical Device, Elsevier, 2017, p. Abstract.	1
A	US 7,381,805 B2 (Germansen et al.) 3 June 2008 Entire document, especially col 1 ln 25-30	6
A	WO 2015/143010 A1 (The Trustees of the University of Pennsylvania) 24 September 2015 Entire document, especially Abstract, page 4 ln 6-7; page 22 ln 16-19; page 27 ln 16-18	12

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 February 2020	16 APR 2020
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No 571-273-8300	Authorized officer Lee Young Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/64840

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	"Hydrogen Peroxide." Wikipedia, Wikimedia Foundation, 29 Nov. 2018, https://en.wikipedia.org/w/index.php?title=Hydrogen_peroxide&oldid=871266610	16-17
A	Uchiyama et al. "Degradation of Phospholipid Polymer Hydrogel by Hydrogen Peroxide Aiming at Insulin Release Device." Biomaterials, vol. 24, no. 28, 2003, Abstract. doi:10.1016/s0142-9612(03)00441-1.	16-17
A	Guo et al. "Advances in Redox-Responsive Drug Delivery Systems of Tumor Microenvironment." Journal of Nanobiotechnology, vol. 16, no. 1, 2018, doi:10.1186/s12951-018-0398-2.	23-24

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/64840

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 33-57
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-- See Extra sheet --

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-7, 11-14, 16-17, 23-24, and 32/(1-7, 11-14, 16-17, 23-24)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/64840

Box III Observations where unity of invention is lacking:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-32 directed to a therapeutic nanoparticle comprising: stimuli-cleavable crosslinks, and at least one physiologically active agent, wherein the nanoparticle has a particle size of between about 100-1,000 nm, and wherein the nanoparticle undergoes stimulus-directed degradation. The therapeutic nanoparticle of claim 1 will be searched to the extent that the therapeutic nanoparticle encompasses a therapeutic nanoparticle comprising: stimuli-cleavable crosslinks selected from the first choice of claim 23 (i.e., disulfide bonds), and at least one physiologically active agent selected from first choice of claim 4 (i.e., therapeutic protein) dispersed in a matrix selected from the first choice of claim 11 (i.e., a polymer matrix) that may comprise a polyester of claim 13 or a polysaccharide of claim 14, wherein the nanoparticle has a particle size of between about 100-1,000 nm, and wherein the nanoparticle undergoes stimulus-directed degradation, via the selected first choice from claim 16 (i.e., exposure to an oxidant). It is believed that claims 1-7, 11-14, 16-17, 23-24, and 32/(1-7, 11-14, 16-17, 23-24) read on this first named invention and thus these claims will be searched without fee to the extent that they encompass the first species of claim 1. Applicant is invited to elect additional composition(s), wherein each additional composition elected will require one additional invention fee. Applicants must specify the claims that encompass any additionally elected composition. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the '+' group(s) will result in only the first claimed invention to be searched. An exemplary selection would be a therapeutic nanoparticle comprising: stimuli-cleavable crosslinks selected from the second choice of claim 23 (i.e., trisulfide bonds), and at least one physiologically active agent selected from fourth choice of claim 4 (i.e., aptamer) and dispersed in a matrix selected from the first choice of claim 11 (i.e., a polymer matrix)) that may comprise a polyester of claim 13 or a polysaccharide of claim 14, wherein the nanoparticle has a particle size of between about 100-1,000 nm, and wherein the nanoparticle undergoes stimulus-directed degradation, via the selected second choice from claim 16 (i.e., exposure to a reductant) (claims 1-5, 11-14, 16, 18, 23, 25, 32/(1-5, 11-14, 16, 18, 23, 25)).

The group of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I+ includes the technical feature of a unique nanoparticle composition, which is not required by any other invention of Group I+.

Common technical features:

The inventions of Group I+ share the technical features of a therapeutic nanoparticle comprising: stimuli-cleavable crosslinks, and at least one physiologically active agent, wherein the nanoparticle has a particle size of between about 100-1,000 nm, and wherein the nanoparticle undergoes stimulus-directed degradation.

These shared technical features, however, do not provide a contribution over the prior art, as being anticipated by the document titled, "Acid-Sensitive Sheddable PEGylated PLGA Nanoparticles Increase the Delivery of TNF-a siRNA in Chronic Inflammation Sites" to Aldayel et al. (hereinafter 'Aldayel') which discloses a therapeutic nanoparticle (page 2 col 1 para 1 - '...we showed that the acid-sensitive sheddable PEGylated PLGA nanoparticles significantly increase the accumulation/distribution of the TNF-siRNA encapsulated...') comprising: stimuli-cleavable crosslinks (page 3 col 1 para 1 - '...Importantly, the acid-sensitive sheddable nature of the PEGylation is expected to facilitate the shedding of the PEG chains from the nanoparticles...'; see the document entitled, "Chapter 7 - Peptide and Protein-Based Therapeutic Agents" to Joseph et al., see Abstract; see instant claim 31); at least one physiologically active agent (Abstract - '...Herein we report that innovative functionalization of the surface of siRNA-incorporated poly (lactic-co-glycolic) acid (PLGA) nanoparticles significantly increases the delivery of the siRNA in the chronic inflammation sites in a mouse model...'; page 2 col 1 para 1 '...we showed that the acid-sensitive sheddable PEGylated PLGA nanoparticles significantly increase the accumulation/distribution of the TNF-siRNA encapsulated in them in the inflamed mouse foot...'; see instant claim 4) wherein the nanoparticle has a particle size of between about 100-1,000 nm (see page 2, Table 1 - '...Particle sizes (nm)... AI-siRNA-NPs... 193 +/- 9... AS-siRNA-NPs... 192 +/- 12...') and wherein the particle under goes stimulus-directed degradation (page 2 col 2 para 2-page 3 col 1 para 1 - '...Importantly, the acid-sensitive sheddable nature of the PEGylation is expected to facilitate the shedding of the PEG chains from the nanoparticles in the relatively lower pH environment in chronic inflammation sites after extravasation...'; page 6 col 2 para 3 - '...we prepared PLGA nanoparticles incorporated with siRNA using the standard double emulsion method with a new acid-sensitive stearoyl PEG molecule...'; see instant claim 19).

As said composition and method were known in the art at the time of the invention, these cannot be considered special technical features, that would otherwise unify the inventions of Groups I+.

The inventions of Group I+ thus lack unity under PCT Rule 13.