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(71) Applicant: **XYLOME CORPORATION** [US/US]; 510 Charmany Drive, Suite 62, Madison, WI 53719, Madison, Wisconsin 53719 (US).

(72) Inventors: **JEFFRIES, Thomas W.**; 5517 Greening Lane, Madison, Wisconsin 53705 (US). **KELLEHER, Thomas J.**; 1982 Calle Yucca, Thousand Oaks, California 91360 (US). **MOKRY, David Z.**; 5141 Prairie Rose Rd., Madison, Wisconsin 53704 (US). **TAYLOR, Richard**; 1938 Shelley Lane, Madison, Wisconsin 53704 (US). **GLUTH, Austin**; 2117 Hoel Cir., Stoughton, Wisconsin 53589 (US). **GOLD-**

ENBERG, Merrill S.; 3616 Radcliffe Rd., Thousand Oaks, California 91360 (US).

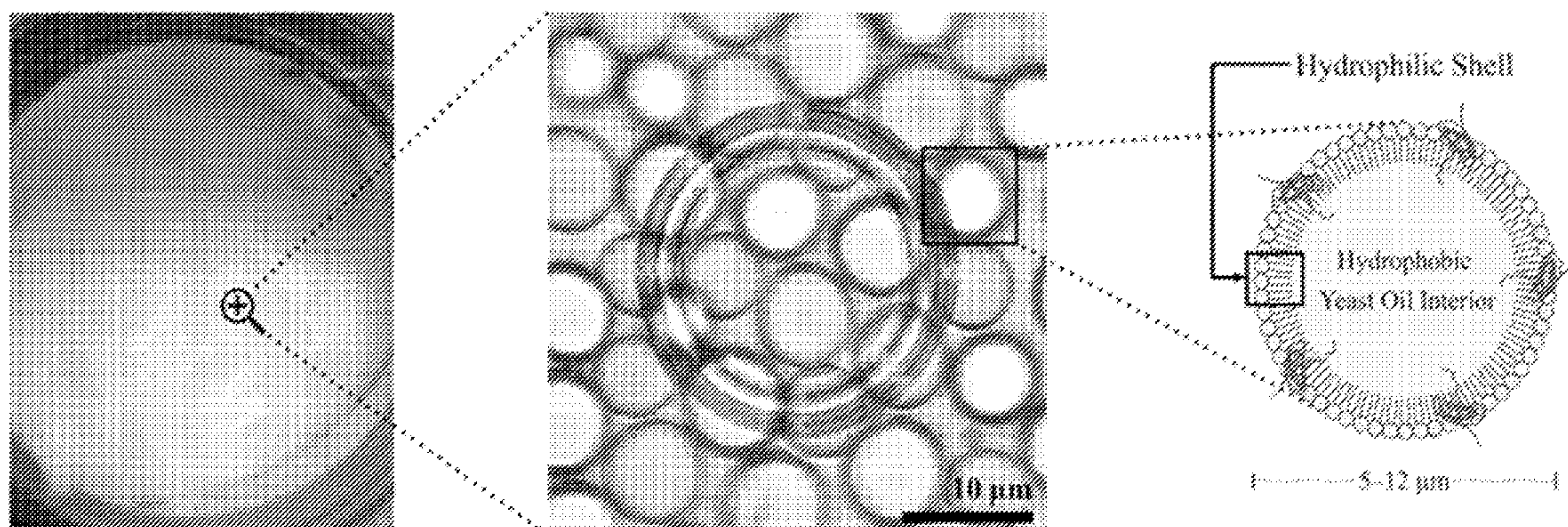
(74) Agent: **BLASIOLE, Daniel A.** et al.; 2 East Mifflin Street, Suite 600, Madison, Wisconsin 53703 (US).

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(54) Title: LIPID BODY COMPOSITIONS, PRODUCTS MADE THEREFROM, METHODS OF MAKING SAME, AND METHODS OF USE

FIG. 1



(57) Abstract: The invention provides lipid bodies isolated from yeast, compositions comprising the lipid bodies, products made from the lipid bodies, methods of making the lipid bodies, and methods of using the lipid bodies. The lipid bodies of the invention have an exceptionally large size and high internal neutral lipid content, providing a number of advantages for a variety of practical applications.

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LIPID BODY COMPOSITIONS, PRODUCTS MADE THEREFROM, METHODS OF
MAKING SAME, AND METHODS OF USE

CROSS-REFERENCE TO RELATED APPLICATIONS

5 Priority is hereby claimed to US Provisional Application 62/935,878, filed
November 15, 2019, and US Provisional Application 63/073,679, filed September 2, 2020,
each of which is incorporated herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

10 This invention was made with Government support under NSF SBIR grant No.
1632255 awarded by the National Science Foundation. The Government has certain rights
in this invention.

SEQUENCE LISTING

15 The instant application contains a Sequence Listing which has been submitted in
ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. The
ASCII copy, created on November 13, 2020, is named PCT--201113--
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bytes in size.

20

FIELD OF THE INVENTION

The invention is directed to lipid bodies and other useful biomaterials derived from
engineered hyper-lipogenic yeasts, methods for producing same, and methods of use.

25

BACKGROUND

There is a clear consumer interest in products that are free of petrochemicals,
detergent emulsifiers, and complex chemical compositions, particularly for cosmetics and
pharmaceutical compositions. Many cosmetic and skin care products are composed of a
number of chemical ingredients needed to make stable emulsions, and many of these
30 ingredients are contraindicated for the purpose of those products. There is also an interest
in sustainable products, especially consumer goods made without adverse environmental
impacts. Renewable sources of oils for consumer goods can be derived from plant seeds,
such as soybean, canola, rapeseed, and oil palm. These triglyceride-based oils are used in
pharmaceutical compositions, industrial applications, and biofuels. However, the cost and

complexity associated with processing these raw materials is significant, and involves seasonal harvesting, grinding, autoclaving, degumming, solvent isolation, and refining steps to obtain the pure vegetable oils. As a result, many industries need an alternative source of seed oils (Anon 2016). Palm oil is particularly recognized as being environmentally unsustainable due to the extensive tropical deforestation to accommodate more plantations to meet its growing demand, as it is used in half of all consumer goods in the U.S. One solution involves the substitution of single cell oils from organisms such as algae and yeast into the compositions of these products.

Oleaginous yeast and some algae are natural oil producers. On a carbon conversion basis, 2 to 3 times more lipid/g dry weight is generated by microbial sources when compared to seed oils. Algae cultivation for oil has low environmental impact, but cells grow slowly, to relatively low densities, and are difficult to harvest and process. Certain algae can accumulate lipids when cultivated on sunlight and CO₂, but carbon fixation by photosynthesis requires a great deal of metabolic energy, so cell growth and lipid accumulation is relatively slow. Some algae can grow heterotrophically on simple organic compounds dissolved in water, which greatly increases their rates of lipid accumulation. Algae, however, do not generally assimilate more complex organic materials such as starch, cellulosic, or hemicellulosic oligomers. On the other hand, heterotrophic Ascomyceteous and Basidiomyceteous oleaginous yeasts and filamentous fungi will readily assimilate these compounds. The growth rates of these organisms on simple or complex dissolved organic materials are much faster than algae under these conditions. Oil-producing yeasts have been isolated and studied over the past 60 years, yet an industrially effective way of recovering yeast oil from oleaginous yeast has eluded individuals skilled in the art with many methods requiring the use of various solvents in processes that have not been proven to be economical (Wang et al. 2016, Wenning et al. 2017, Severa et al. 2014, Nisha et al. 2012, Yu et al. 2015, Ochsenreither et al. 2016).

In addition to the oil itself, many current commercial products contain processed plant and animal oils and lipid enclosed structures in stable emulsions or droplets, the latter of which are canonically referred to as liposomes. The term "liposome" commonly refers to a spherical vesicle having at least one lipid bilayer, which usually encloses an aqueous core, but which can be modified to encompass hydrophobic inclusions (Schwendener et al. 2010). Artificial liposomes prepared from egg phosphatidyl choline are typically 85 +/- 32 nm in diameter prior to fractionation (Ingebrigsten et al. 2002). The outer or hydrophilic sides of liposomes are most often composed of phospholipids,

especially phosphatidylcholine derived from egg yolk or soy beans, but may also include other lipids, such as phosphatidylethanolamine or phosphatidylserine. These artificial structures impart stability to emulsions that can be used to maintain a suspension of hydrophobic compounds, which are otherwise not soluble in aqueous solutions.

5 Significant literature and expired patent publications exist related to liposomes dating back to the 1970s. The fatty acids and triglycerides for these lipid-based synthetic structures are generally created by combining chemicals, detergents, emulsifiers, and other agents.

Oleaginous yeasts accumulate their oil in lipid bodies. Lipid bodies are significantly larger than liposomes (generally $<2\ \mu\text{m}$ in diameter) and are irregular, lipid-
10 containing intracellular organelles. Lipid bodies have never previously been recognized as a useful free-standing product for commercialization, as alternatives to liposomes for commercial products, or as a useful intermediate composition of matter for solvent-free oil processing. Moreover, methods of generating isolated lipid bodies from either algae or oleaginous yeasts have not existed. While triglyceride oils from yeasts represent an
15 alternative to plant derived oils, if they were extracted from the cells using conventional means such as organic solvent mixtures of chloroform and methanol, these oils would still need to be combined with other emulsifiers and chemical agents to make liposomes. Therefore, yeast derived lipid bodies may be an alternative to plant-based oils and liposomes for certain applications, specifically to meet the growing consumer demand for
20 fewer chemical ingredients and sustainable products from encapsulated oil.

There is a need for the generation and isolation of lipid bodies suitable for industrial and consumer use.

SUMMARY OF THE INVENTION

25 The invention addresses the aforementioned needs by providing recombinant yeasts and methods for converting a wide range of carbon feedstocks and stillage organics into engineered lipid bodies that can be further processed to isolate lipid bodies, lipids, and other biomaterials.

The recombinant yeasts are specifically bioengineered for rapid lipid body
30 formation and can be used to make enlarged, engineered lipid bodies that can then be used as products themselves or further processed into other products and biological materials.

The present invention describes the genetic modification of oleaginous yeast to increase the intracellular concentration of oil production, and a series of processing steps to obtain single-cell oils, large lipid bodies, and other novel products. The high oil

concentrations attained by our novel engineered cells enables efficient recovery of the buoyant cells and lipid bodies through flotation, which consumes much less energy than centrifugation. Hydrolysis of the cell walls by chemical, thermophysical or enzymatic means can recover stable lipid bodies that can be used in compositions that will replace the use of oils in personal care and other products. Novel yeast oil extracts can be obtained through physical, thermophysical, or chemical treatments directly from lipogenic cells or the recovered lipid bodies for use in a wide range of products.

In an exemplary method of making the isolated lipid bodies, yeast cell walls of the recombinant yeasts can be hydrolyzed in heat $> 50^{\circ}\text{C}$ and acid (*e.g.*, sulfuric or phosphoric acid) resulting in spontaneous formation of two phases. The upper tan phase contains the crude lipid bodies, whereas the bottom aqueous phase contains the cell hydrolysate. The lipid bodies can then be washed in pure water. This can be followed by an alkali wash, which removes residual odor and shifts the appearance from tan to white. The lipid bodies can then be washed again and resuspended in a suitable compositions buffer.

The isolated lipid bodies of the invention are drastically larger than lipid bodies isolated from natural, un-modified yeasts. This makes them suitable for a number of useful applications.

The lipid bodies can be used as a platform or intermediate for cosmetic, pharmaceutical, industrial and biofuel products. The engineered lipid bodies are a particularly useful component of skin care products, pharmaceutical compositions involving hydrophobic medicines, consumer goods, food and feed products including aquaculture, and other industrial goods. The isolated lipid bodies of the invention can be applied directly to human skin as a moisturizer or used as a base platform for a wide range of skin and personal care products.

Aspects of the invention provide the ability to produce engineered lipid bodies without influencing the food supply, since the engineered lipid bodies from the recombinant yeast can be cultured in part on inexpensive industrial waste streams derived from ethanol plants or other industrial settings, such as cellulosic hydrolysates of agricultural residues or municipal waste treatment facilities (Leiva-Candia et al. 2014, Gong et al. 2012, Angerbauer et al. 2008, McNeil et al. 2018).

An extension of engineered lipid body technology is the facilitated processing of the isolated engineered lipid bodies to obtain yeast oil using inexpensive oil extraction methods. The identification and processing of biologically derived engineered lipid bodies from genetically modified yeast can facilitate the recovery of a yeast oil that is suitable for

biodiesel, other biofuels, and oleochemicals. The yeast oil could meet a significant fraction of the national transportation energy demand and decrease dependency on foreign oil. Alternatively, it could provide a palm oil substitute than can be used in the cosmetic, pharmaceutical and/or food and beverage industries.

5 Genetic modification of oleaginous yeast described herein provides the ability to produce biologically assembled lipid bodies that are easily isolated and processed into yeast oil in an engineered proportion without the use of solvents. Exemplary aspects of the present invention pertain to the creation of these engineered yeast, the subsequent aqueous processing to isolate lipid bodies, along with solvent or solvent-free oil extraction
10 techniques to isolate the oil contained within these lipid bodies. The development of these technologies has opened broad commercial applications not previously possible with native oleaginous yeasts. Lipid bodies and their applications can also replace the use of synthetic liposomes. Furthermore, the engineered isolated lipid bodies can be used as a product platform for sustainable consumer goods, pharmaceutical compositions, industrial
15 applications, and biofuels. This technology answers the demand of consumers for clean, sustainable, and simple products.

The objects and advantages of the invention will appear more fully from the following detailed description of the preferred embodiment of the invention made in conjunction with the accompanying drawings.

20

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. An exemplary cream compositions of the invention that includes lipid bodies in a water carrier (left); a photograph of lipid bodies relevant to the invention, exemplifying their uniquely large size (middle); and a schema showing the structure and
25 major constituents of the lipid bodies (right).

FIG. 2. Schematic diagram of a base vector used for creating genomic integrating cassettes. Restriction digest of a circular vector yields 2-4 fragments, depending on the construct. The integrating fragment contains native *L. starkeyi* promoters and terminators driving expression of a resistance and lipogenic gene. LoxP sequences flank the resistance
30 marker cassette to enable CRE mediated excision. The origin of replication (purple) and kanamycin resistance gene permit propagation and maintenance in *E. coli*, but are not maintained during the selection of transformants. Digest sites are marked with a scissors icon.

FIG. 3. Schema showing the strategy for creating first-, second-, third-, and fourth-generation engineered strains using several rounds of transformations, mating, and resistance marker removal. Marker removal, deemed “curing”, is denoted with an asterisk.

FIG. 4. Effect of combinatorial overexpression of genes on lipid accumulation as deemed by cumulative BODIPY fluorescence compared to the wild-type strain over 4 days of cultivation in sTS. Proteins overexpressed in each strain are as follows: 201 (*GUT1*, *GUT2*), 202 (*DGA1*, *DGA2*), 412 (*GUT1*, *GUT2*, *ME*, *SCT1*), 409 (*ACL1*, *ACL2*, *DGA1*, *DGA2*, *SCT1*), 414 (*ACL1*, *ACL2*, *DGA1*, *DGA2*, *ME*), 405 (*DGA1*, *DGA2*, *ME*, *SCT1*), 408 (*GUT1*, *GUT2*, *DGA1*, *SCT1*), and 403 (*DGA1*, *DGA2*, *ME*, *SCT1*). Strains 403 and 405 differ in the genomic site and/or number of copies of the Malic Enzyme (ME) cassette. The 403 strain was sequenced and has one copy of the Malic Enzyme overexpression construct, plus the native Malic Enzyme locus.

FIG. 5. Manipulation of cellular morphologies and intracellular lipid bodies resulting from overexpression of different proteins. Overexpression of *DGA1* by transforming cells once (*DGA1*) or twice (*DGA1...DGA1*) with the *DGA1* expression cassette results in multiple small intracellular lipid bodies, whereas overexpression of *GUT1* alone results in lipid bodies of various sizes and slow growth in media containing glycerol. The 403 strain overexpresses *DGA1*, *DGA2*, *ME*, and *SCT1*, and results in large uniform lipid bodies suitable for isolation and use in a number of products.

FIG. 6A. Growth rates (top panel) and glucose consumption (bottom panel) of strains 202 (*DGA1*, *DGA2*, shown as “DGA1+2”) and 403 (*DGA1*, *DGA2*, *ME*, and *SCT1*, shown as “XYL 403”). FIG. 6B. Cell and lipid yields of strains 202 (*DGA1*, *DGA2*, shown as “DGA1+2”) and 403 (*DGA1*, *DGA2*, *ME*, and *SCT1*, shown as “XYL 403.”) at T4 (65 h, left panel) and T18 (142 h, right panel) of growth.

FIG. 7. Comparison of lipid body diameters between those obtained from wild-type *L. starkeyi* and those obtained from the 403 strain (shown as “engineered lipogenic yeast”).

FIG. 8. Size distribution of lipid bodies obtained from the 403 strain (left panel) and those obtained from wild-type *L. starkeyi* (right panel). The Y axis on the left side of each bar graph indicates the percentage of total number of lipid bodies as shown with each individual blue bars. The Y axis on the right side of each bar graph indicates the cumulative percentage of lipid bodies by adding blue bar going from left to right as shown by the orange line. The numbers in brackets under each blue bar on the X axis of each bar

graph indicates the diameter range in microns represented by each blue bar. For example, [6.3,7.6], indicates a range of 6.3 μm to 7.6 μm .

FIG. 9. Cumulative volumes of wild-type and 403 lipid bodies as a function of the number of lipid bodies from each type.

5 FIG. 10. Lipid compositions of palm oil (“Palm Oil”), oil obtained directly from whole 403 cells (“XYL403 Strain Oil”), oil obtained directly from whole wild-type cells (“Wild-type Oil”), and oil obtained from 403 lipid bodies (“Lipid Body Oil”).

FIG. 11. UV-Vis scan of lipid obtained from a 403-strain lipid body preparation.

10 FIGS. 12A-12B. Merged lipid bodies up to 50 microns in diameter in the absence (FIG. 12A) or presence (FIG. 12B) of Sudan black, which stains lipids black.

FIGS. 13A-13B. Uptake of hydrophobic dyes by lipid bodies. Lipid bodies were mixed with Nile red (FIG. 13A) or Sudan black (FIG. 13B) and subsequently subjected to flotation.

15 FIGS. 14A-14C. Formation of skin barrier after administration of lipid bodies to skin. Shown are a micrograph of a control epithelial skin peel (FIG. 14A), a micrograph of a skin peel 10 hours after administering lipid bodies of the invention to the skin (FIG. 14B), and a micrograph of yeast lipid bodies overlaying epithelial cells.

FIG. 15. Micrograph of isolated, opened lipid-body envelopes.

20 FIG. 16. Micrograph (400x) of lipid bodies subjected to Sudan black in a strong ionic aqueous environment.

FIG. 17. Raman spectrograph of lipid-body envelope (shell) material and reference beta-glucan material, with the polysaccharide region indicated.

FIG. 18. Analysis of lipid constituents of lipid bodies.

25 FIG. 19. Results from exposure of patients to an exemplary lipid body-based skin cream of the invention.

FIGS. 20A and 20B. Properties of lipid bodies chelated to copper. FIG. 20A: Top view of lipid bodies migrating to a positive pole after chelating copper. FIG. 20B: Side view of lipid bodies that have become neutrally buoyant after chelating copper.

FIG. 21. Intrinsic fluorescence of lipid bodies of the invention.

30 FIG. 22. Titration of lipid bodies of the invention with 1M NaOH or 1M HCl.

DETAILED DESCRIPTION OF THE INVENTION

One aspect of the invention is directed to compositions comprising lipid bodies. As used herein, “lipid body” refers to structures comprising a hydrophobic core comprising

lipids such as triacylglycerols, steryl esters, and/or other lipids surrounded by at least one outer layer. The hydrophobic core is referred to herein as the “inner core.” The lipid present in the inner core is referred to herein as “core lipid” or “oil.” The outer layer is referred to herein as an “envelope.” The envelope comprise phospholipid, protein, and/or
5 other materials such as polysaccharide. The phospholipid may predominantly comprise phosphatidylethanolamine. When isolated, lipid bodies can also be designated “lipid droplets” (Uzuka et al. 1975). Lipid bodies or lipid droplets are distinct from synthetic liposomes, which may be most commonly comprised of phospholipid and emulsifiers.

The lipid bodies of the invention are preferably isolated. “Isolated” means that the
10 lipid bodies are not encompassed within an intact cell, such as a yeast cell. Isolated lipid bodies encompass lipid bodies that are isolated from intact cells and exist as-is in their originally isolated state or are modified in some way. Exemplary modifications are described in the following examples and include, without limitation, merging, impregnating with active agents, modifying surface charge, etc. In preferred versions the
15 isolated lipid bodies are yeast lipid bodies, meaning that they are originally produced in, and isolated from, yeast. An indication a lipid body is a yeast lipid body is that the lipid body comprises yeast protein. In some versions, the lipid bodies of the invention comprise isolated lipid bodies that are unmerged. That is, they are not merged after isolation to increase their size. In some versions, the lipid bodies of the invention comprise isolated
20 lipid bodies that are merged. That is, they are merged after isolation to increase their size.

The lipid bodies of the invention are preferably non-synthetic. As used herein, “non-synthetic” refers to lipid bodies produced from constitutive cellular components *in vivo* (i.e., within a cell, such as a yeast cell), as opposed to entities produced from individual isolated components (e.g., oil, phospholipids, etc.) *in vitro*. The term “non-
25 synthetic” refers to lipid bodies originally produced from constitutive cellular components *in vivo* but later modified in some way. Exemplary modifications are described in the following examples and include, without limitation, merging, impregnating with active agents, modifying surface charge, etc. In some versions, the lipid bodies of the invention comprise non-synthetic, isolated lipid bodies that are unmerged. That is, they are not
30 merged after isolation to increase their size. In some versions, the lipid bodies of the invention comprise non-synthetic, isolated lipid bodies that are merged. That is, they are merged after isolation to increase their size.

The lipid bodies of the invention comprise lipid bodies having a larger diameter than lipid bodies isolated from natural yeasts. The lipid bodies of the invention can have a

diameter of at least about 5 μm and up to about 60 μm microns or more. For example, the lipid bodies in various versions of the invention can have a diameter of at least about 5 μm , at least about 6 μm , at least about 7 μm , at least about 8 μm , at least about 9 μm , at least about 10 μm , at least about 15 μm , at least about 20 μm , at least about 25 μm , at least about 30 μm , at least about 35 μm , at least about 40 μm , or at least about 45 μm . The lipid bodies in various versions of the invention can have a diameter up to about 10 μm , up to about 15 μm , up to about 20 μm , up to about 25 μm , up to about 30 μm , up to about 35 μm , up to about 40 μm , up to about 45 μm , up to about 50 μm , up to about 55 μm , up to about 60 μm or more. The lipid bodies having these sizes can constitute at least about 1%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% of the total number lipid bodies in a given composition. The lipid bodies having these sizes can constitute up to about 5%, up to about 10%, up to about 15%, up to about 20%, up to about 25%, up to about 30%, up to about 35%, up to about 40%, up to about 45%, up to about 50%, up to about 55%, up to about 60%, up to about 65%, up to about 70%, up to about 75%, up to about 80%, up to about 85%, up to about 90%, up to about 95%, or up to about 100% of the total number lipid bodies in a given composition.

In some versions, from about 30% to about 80% of the number of lipid bodies in a given composition have a diameter of at least about 5 μm (such as from about 5 μm to about 10 μm or about 15 μm). In some versions, from about 20% to about 50% of the number of lipid bodies in a given composition have a diameter of at least about 6 μm , (such as about 6 μm to about 10 μm or about 15 μm). The lipid bodies in various versions of the invention have a diameter from about 5 to about 12 μm , from about 5 to about 50 μm , or from about 12 to about 50 μm .

In some versions of the invention, the lipid bodies in the composition have an average diameter of at least about 5 μm , at least about 5.5 μm , at least about 6 μm , or at least about 6.5 μm . In some versions, the lipid bodies in the composition have an average diameter up to about 7 μm , up to about 7.5 μm , up to about 8 μm , up to about 8.5 μm , up to about 9 μm , up to about 9.5 μm , up to about 10 μm , or more.

The lipid bodies in some versions have a larger average volume than lipid bodies derived from natural yeasts. In some versions, for example, the lipid bodies in the composition have an average volume of at least about 65 μm^3 , at least about 90 μm^3 , at

least about $120 \mu\text{m}^3$, or at least about $150,000 \mu\text{m}^3$. In some versions, the lipid bodies in the composition have an average volume up to about $180,000 \mu\text{m}^3$, up to about $220,000 \mu\text{m}^3$, up to about $270,000 \mu\text{m}^3$, up to about $320,000 \mu\text{m}^3$, up to about $380,000 \mu\text{m}^3$, up to about $450,000 \mu\text{m}^3$, up to about $525,000 \mu\text{m}^3$, or more.

5 The core lipid of the lipid bodies is preferably present in the lipid bodies in an amount of at least about 30% and up to about 90% or more by weight as determined by gravimetric analysis. In various versions, the core lipid is present in the lipid bodies in an amount of at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least
10 about 70%, at least about 75%, at least about 80%, or at least about 85% by weight as determined by gravimetric analysis. Alternatively or additionally, the core lipid is present in the lipid bodies in various versions in an amount up to about 60%, up to about 65%, up to about 70%, up to about 75%, up to about 80%, up to about 85%, up to about 90%, up to about 95%, or up to about 99% by weight as determined by gravimetric analysis.

15 The core lipid in the lipid bodies of the invention is preferably solid at a temperature within a range from about 10°C to about 30°C , such as a temperature within a range from about 15°C to about 25°C . Alternatively or additionally, the core lipid in the compositions is preferably liquid at a temperature within a range from about 30°C to about 60°C , such as a temperature preferably solid at a temperature within a range from about
20 35°C to about 55°C or a range from about 40°C to about 50°C . The core lipid in the lipid bodies of the invention can have a melting point at a temperature between about 25°C and about 50°C .

 In some versions, the compositions of the invention have a white or off-white color. The white or off-white color is different from the color of crude lipid bodies isolated
25 from yeast, which are typically tan or brown in color. The white or off-white color can be obtained by performing an alkaline wash of the crude lipid bodies, as described in detail below. An objective indicator of the white or off-white color is a spectral absorbance at about 400 nm of less than about 0.32 optical density (OD) units per gram of lipid body wet weight, such as less than about 0.3 OD units per gram of lipid body wet weight, less than
30 about 0.25 OD units per gram of lipid body wet weight, less than about 0.2 OD units per gram of lipid body wet weight, less than about 0.15 OD units per gram of lipid body wet weight, less than about 0.1 OD units per gram of lipid body wet weight, less than about 0.05 OD units per gram of lipid body wet weight, less than about 0.01 OD units per gram of lipid body wet weight, or about 0 OD units per gram of lipid body wet weight. Methods

for determining OD units per gram of lipid body wet weight are described in the following examples.

In some versions, the lipid bodies or the composition comprising the lipid bodies exhibit an absorbance at about 230 nm of at least about 0.01 OD units per gram of lipid
5 body wet weight, at least about 0.05 OD units per gram of lipid body wet weight, at least about 0.1 OD units per gram of lipid body wet weight, at least about 0.5 OD units per gram of lipid body wet weight, at least about 1 OD units per gram of lipid body wet weight, at least about 1.5 OD units per gram of lipid body wet weight, at least about 2 OD units per gram of lipid body wet weight, or at least about 2.5 OD units per gram of lipid
10 body wet weight. In some versions, the composition exhibits an absorbance at about 230 nm up to about 1.5 OD units per gram of lipid body wet weight, up to about 2 OD units per gram of lipid body wet weight, up to about 2.5 OD units per gram of lipid body wet weight, up to about 3 OD units per gram of lipid body wet weight, up to about 3.5 OD units per gram of lipid body wet weight, or more.

15 The compositions of the invention preferably comprise the lipid bodies at a concentration from about 1% or less to about 99% or more by dry weight. In various versions, the compositions of the invention comprise the lipid bodies at a concentration of at least about 1%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at
20 least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% by dry weight. Alternatively or additionally, the compositions in various versions comprise the lipid bodies at a concentration up to about 5%, up to about 10%, up to about 15%, up to about 20%, up to about 25%, up to about
25 30%, up to about 35%, up to about 40%, up to about 45%, up to about 50%, up to about 55%, up to about 60%, up to about 65%, up to about 70%, up to about 75%, up to about 80%, up to about 85%, up to about 90%, up to about 95%, or up to about 99% by dry weight. An exemplary range is a concentration from about 30% to about 55% by dry weight.

30 The compositions of the invention preferably comprise the lipid bodies at a concentration from about 1% or less to about 99% or more by dry weight. In various versions, the compositions of the invention comprise the lipid bodies at a concentration of at least about 1%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at

least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% by dry weight. Alternatively or additionally, the compositions in various versions comprise the lipid bodies at a concentration up to about 5%, up to about 10%, up to about 15%, up to about 20%, up to about 25%, up to about 30%, up to about 35%, up to about 40%, up to about 45%, up to about 50%, up to about 55%, up to about 60%, up to about 65%, up to about 70%, up to about 75%, up to about 80%, up to about 85%, up to about 90%, up to about 95%, or up to about 99% by dry weight. An exemplary range is a concentration from about 30% to about 55% by dry weight.

The composition can have a wide range of solids concentrations. The particular solids concentrations will depend on the desired form of the composition. In some versions, the composition has a solids concentration of at least about 5% w/w, at least about 10% w/w, at least about 15% w/w, at least about 20% w/w, at least about 25% w/w, at least about 30% w/w, at least about 35% w/w, at least about 40% w/w, at least about 45% w/w, at least about 50% w/w, at least about 55% w/w, at least about 60% w/w, at least about 65% w/w, at least about 70% w/w, at least about 75% w/w, or at least about 80% w/w. In some versions, the composition has a solids concentration up to about 15% w/w, up to about 20% w/w, up to about 25% w/w, up to about 30% w/w, up to about 35% w/w, up to about 40% w/w, up to about 45% w/w, up to about 50% w/w, up to about 55% w/w, up to about 60% w/w, up to about 65% w/w, up to about 70% w/w, up to about 75% w/w, up to about 80% w/w, up to about 85% w/w, up to about 90% w/w, or up to about 95% w/w.

In some versions, the composition of the invention comprises lipid in an amount of at least about 100 g/L, at least about 150 g/L, at least about 200 g/L, at least about 250 g/L, at least about 300 g/L, at least about 350 g/L, at least about 400 g/L, at least about 450 g/L, at least about 500 g/L, at least about 550 g/L, at least about 600 g/L, at least about 650 g/L, at least about 700 g/L, at least about 750 g/L, or at least about 800 g/L. In some versions, the composition of the invention comprises lipid in an amount up to about 150 g/L, up to about 200 g/L, up to about 250 g/L, up to about 300 g/L, up to about 350 g/L, up to about 400 g/L, up to about 450 g/L, up to about 500 g/L, up to about 550 g/L, up to about 600 g/L, up to about 650 g/L, up to about 700 g/L, up to about 750 g/L, up to about 800 g/L, up to about 850 g/L, or up to about 900 g/L. In some versions, at least about 50% by weight, at least about 55% by weight, at least about 60% by weight, at least about 65% by

weight, at least about 70% by weight, at least about 75% by weight, at least about 80% by weight, at least about 85% by weight, at least about 90% by weight, at least about 95% by weight, or at least about 99% by weight of the total lipid comprised by the composition is included as part of the lipid droplets.

5 In some versions, the composition of the invention comprises protein in an amount of at least about 0.01 g/L, at least about 0.05 g/L, at least about 0.1 g/L, at least about 0.5 g/L, at least about 1 g/L, at least about 2 g/L, at least about 3 g/L, at least about 4 g/L, at least about 5 g/L, at least about 6 g/L, at least about 7 g/L, at least about 8 g/L, at least about 9 g/L, at least about 10 g/L, at least about 11 g/L, at least about 12 g/L, at least about
10 13 g/L, or at least about 14 g/L. In some versions, the composition of the invention comprises protein in an amount up to about 0.5 g/L, up to about 1 g/L, up to about 2 g/L, up to about 3 g/L, up to about 4 g/L, up to about 5 g/L, up to about 6 g/L, up to about 7 g/L, up to about 8 g/L, up to about 9 g/L, up to about 10 g/L, up to about 11 g/L, up to about 12 g/L, up to about 13 g/L, up to about 14 g/L, or up to about 15 g/L. In some
15 versions, at least about 10% by weight, at least about 20% by weight, at least about 30% by weight, at least about 40% by weight, at least about 50% by weight, at least about 55% by weight, at least about 60% by weight, at least about 65% by weight, at least about 70% by weight, at least about 75% by weight, at least about 80% by weight, at least about 85% by weight, at least about 90% by weight, at least about 95% by weight, or at least about
20 99% by weight of the protein comprised by the composition is included as part of the lipid droplets.

In some versions, the lipid bodies in the composition comprise a lipid:protein ratio by mass from about 25:1 to about 1,000:1, such as from about 50:1 to about 500:1, from about 75:1 to about 300:1, or from about 100:1 to about 250:1.

25 In preferred versions, the protein in the lipid bodies comprises yeast protein. As used herein, “yeast protein” refers to any protein comprising an amino acid sequence specific to yeast. Determining whether the protein in lipid bodies comprises yeast protein can be performed by isolating the protein from the lipid bodies and determining the sequences and/or identities of the isolated protein by, for example, liquid chromatography
30 tandem mass spectrometry (LC-MS/MS). In some versions, the yeast protein in the lipid bodies comprises protein 212544 from *L. starkeyi*, a yeast homolog or ortholog thereof, or a protein comprising an amino acid sequence at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% identical to the amino acid sequence of protein 212544 from *L. starkeyi*. The amino acid sequence of protein 212544

is represented by SEQ ID NO:41. In some versions, the yeast protein in the lipid bodies comprises Oil1 (Bhutada et al. 2018), Sps4 (Garber et al. 1986), or any yeast homolog or ortholog of either of these proteins. In some versions, the yeast protein in the lipid bodies comprises any one or more of squalene epoxidase, sterol- Δ 24-methyltransferase, 5 oxidosqualene cyclase, long-chain fatty acid ligases, acylglycerol-3-phosphate dehydrogenase, alcohol acetyltransferase, TAG lipase. See, *e.g.*, Wältermann et al. 2005.

The compositions of the invention can further comprise a carrier for the lipid bodies. The carrier is preferably a liquid, semi-liquid, or a semi-solid. The carrier is preferably a polar or ionic substance. The carrier preferably comprises water. The carrier 10 may further include one or more excipients.

In some versions, the compositions comprise at least 50% w/w water, such as at least about 55% w/w water, at least about 60% w/w water, at least about 65% w/w water, at least about 70% w/w water, at least about 75% w/w water, at least about 80% w/w water, at least about 85% w/w water, at least about 90% w/w water, at least about 95% 15 w/w water, or at least about 99% w/w water.

The carrier may comprise one or more excipients. Exemplary excipients include humectants preservatives, antioxidants, emulsifiers, emollients, stabilizers, and thickening agents.

Exemplary humectants include propylene glycol, hexylene glycol, and butylene 20 glycol, aloe vera gel, alpha hydroxy acids such as lactic acid, egg yolk, egg white, glyceryl triacetate, honey, lithium chloride, molasses, polymeric polyols such as polydextrose, quillaia, sodium hexametaphosphate E452i, sugar alcohols (sugar polyols, *e.g.*, glycerol, sorbitol, xylitol, and maltitol), urea, castor oil, and hyaluronic acid, among others.

Exemplary preservatives include a citrates (*e.g.*, sodium citrate, anhydrous citric 25 acid, or tri-sodium citrate dihydrate), D-gluconolactone, benzoates (*e.g.*, sodium benzoate), sorbates (*e.g.*, potassium sorbate, calcium sorbate and sodium sorbate), propionates, nitrites, antioxidants that inhibit oxidation, sulfites (*e.g.*, sodium sulfite, sodium bisulfite, sodium metabisulfite, potassium bisulfite and potassium metabisulfite), vitamin E (tocopherol), vitamin C (ascorbic acid), butylated hydroxyanisole (BHA), 30 butylated hydroxytoluene (BHT), chelating agents that bind metal ions to prevent oxidation, disodium ethylenediaminetetraacetic acid (EDTA), polyphosphates, and D-gluconolactone, among others.

Exemplary antioxidants include vitamins such as vitamin A, vitamin C, and vitamin E; vitamin cofactors and minerals, such as coenzyme Q10, manganese, and iodide;

carotenoid terpenoids, such as alpha-carotene, astaxanthin, beta-carotene, canthaxanthin, cryptoxanthin, lutein, lycopene, and zeaxanthin; phenols or polyphenols. Exemplary phenols or polyphenols include flavonoids, phenolic acids and their esters, and non-flavonoid phenolics. Exemplary flavonoids include flavones, such as apigenin, luteolin, and tangeritin; flavonols, such as isorhamnetin, kaempferol, myricetin, proanthocyanidins, and quercetin; flavanones, such as eriodictyol, hesperetin, and naringenin; flavanols and their polymers, such as catechin, gallocatechin, epicatechin, epigallocatechin, theaflavin, thearubigins, isoflavone phytoestrogens, daidzein, genistein, and glycitein; stilbenoids, such as resveratrol and pterostilbene; and anthocyanins, such as cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin. Exemplary phenolic acids include chicoric acid, chlorogenic acid, cinnamic acid, ellagic acid, ellagitannins, gallic acid, gallotannins, rosmarinic acid, and salicylic acid. Exemplary non-flavonoid phenolics include curcumin, flavonolignans, xanthonenes, and eugenol.

Exemplary emulsifiers include polysaccharides (*e.g.*, starch), egg yolk, mustard, lecithin, sodium phosphates, mono- and diglycerides, sodium stearyl lactylate, diacetyl tartaric acid esters of mono- and diglycerides, cellulose, detergents (such as emulsifying wax, polysorbates, and ceteareths).

Exemplary emollients include shea butter, cocoa butter, mineral oil, lanolin, petrolatum, paraffin, beeswax, squalene, plant oils (*e.g.*, coconut, jojoba, sesame, almond, and other plant oils), cetyl alcohol, olive oil (oleic acid), and triethylhexanoin, among others.

Exemplary thickening agents include various polysaccharides and proteins. Examples of polysaccharide thickening agents include starches, microbial and vegetable gums, and sugar polymers. Examples of starches include arrowroot, cornstarch, katakuri starch, potato starch, sago, wheat flour, almond flour, tapioca, and their starch derivatives. Examples of microbial and vegetable gums include alginin, guar gum, locust bean gum, and xanthan gum. Examples of sugar polymers include agar, carboxymethyl cellulose, pectin, and carrageenan. Examples of proteins thickening agents include collagen, egg whites, and gelatin.

The compositions are preferably formulated as stabilized suspensions wherein the lipid bodies remain in suspension for a given period of time at room temperature (*e.g.*, about 20°C). The stabilized suspension can be achieved, for example, with various thickening agents or emulsifiers. In various versions, the compositions of the invention are formulated to maintain the lipid bodies in suspension for a period of time of at least about

10 days, at least about 20 days, at least about 30 days, at least about 40 days, at least about 50 days, at least about 75 days, at least about 100 days, at least about 200 days, at least about 300 days, at least about a year, at least about two years, or at least about three years at about 20°C.

5 The composition can be generated by heating the lipid bodies and/or the isolated lipid-body component with or without the carrier at a temperature greater than about 50°C, greater than about 60°C, greater than about 70°C, greater than about 80°C, greater than about 90°C, greater than about 100°C, greater than about 110°C, greater than about 120°C, greater than about 130°C, or greater than about 140°C and/or up to about 80°C, up to
10 about 90°C, up to about 100°C, up to about 110°C, up to about 120°C, up to about 130°C, up to about 140°C, or up to about 150°C.

 The carrier of the compositions can have a pH from about 0.5 or lower to about 12 or more. In some versions, the carrier has a pH from about 0.5 to about 4. In some versions the carrier has a pH from about 4 to about 9. In some versions the carrier has a
15 pH from about 9 to about 12.

 The composition can take any number of forms. Depending on the level of dilution or concentration of lipid bodies, the composition can be in a liquid form, a semi-solid form, or a solid form. The liquid and semi-solid forms can be suspensions. An exemplary liquid form includes the hair conditioner described in the following examples. Exemplary
20 semi-solid forms include gels, creams, paste, or ointments, such as the creams described in the following examples. An exemplary solid form includes the solid wax form described in the following examples. Any particular form of a substance for the purposes herein is understood to be the form of the substance at atmospheric pressure (101,325 Pa, 760 mm Hg, 29.9212 inches Hg, or 14.696 psi) and room temperature (23°C).

25 Certain embodiments of the present invention include active ingredients. The active ingredients are suitable for use in pharmaceutical compositions and methods of use. The active ingredient can be hydrophilic, lipophilic, amphiphilic, or hydrophobic. Such active ingredients can be any compound or mixture of compounds having therapeutic or other value when administered to an animal, particularly to a mammal, such as drugs,
30 nutrients, cosmeceuticals, diagnostic agents, nutritional agents, and the like. It should be appreciated that the categorization of an active ingredient as hydrophilic or hydrophobic may change, depending upon the particular salts, isomers, analogs, and derivatives used.

 In some embodiments, the active ingredient comprise a hydrophobic active ingredient. Hydrophobic active ingredients are compounds with little or no water

solubility. Intrinsic water solubilities (i.e., water solubility of the unionized form) for hydrophobic active ingredients are less than about 1% by weight, and typically less than about 0.1% or 0.01% by weight. In a particular aspect of this embodiment, the active ingredient is a hydrophobic drug. In other particular aspects, the active ingredient is a
 5 nutrient, a cosmeceutical, a diagnostic agent, or a nutritional agent.

Suitable hydrophobic active ingredients are not limited by therapeutic category, and can be, for example, analgesics, anti-inflammatory agents, anti-helminthics, anti-arrhythmic agents, anti-bacterial agents, anti-viral agents, anti-coagulants, anti-depressants, anti-diabetics, anti-epileptics, anti-fungal agents, anti-gout agents, anti-
 10 hypertensive agents, anti-malarials, anti-migraine agents, anti-muscarinic agents, anti-neoplastic agents, erectile dysfunction improvement agents, immunosuppressants, anti-protozoal agents, anti-thyroid agents, anxiolytic agents, sedatives, hypnotics, neuroleptics, β -Blockers, cardiac inotropic agents, corticosteroids, diuretics, anti-parkinsonian agents, gastro-intestinal agents, histamine receptor antagonists, keratolytics, lipid regulating
 15 agents, anti-anginal agents, cox-2 inhibitors, leukotriene inhibitors, macrolides, muscle relaxants, nutritional agents, opioid analgesics, protease inhibitors, sex hormones, stimulants, muscle relaxants, anti-osteoporosis agents, anti-obesity agents, cognition enhancers, anti-urinary incontinence agents, nutritional oils, anti-benign prostate hypertrophy agents, essential fatty acids, non-essential fatty acids, and mixtures thereof.

Specific, non-limiting examples of suitable hydrophobic active ingredients are:
 20 acetretin, albendazole, albuterol, aminoglutethimide, amiodarone, amlodipine, amphetamine, amphotericin B, atorvastatin, atovaquone, azithromycin, baclofen, beclomethasone, benezepril, benzonatate, betamethasone, bicalutanide, budesonide, bupropion, busulfan, butenafine, calcifediol, calcipotriene, calcitriol, camptothecin,
 25 candesartan, capsaicin, carbamezepine, carotenes, celecoxib, cerivastatin, cetirizine, chlorpheniramine, cholecalciferol, cilostazol, cimetidine, cinnarizine, ciprofloxacin, cisapride, clarithromycin, clemastine, clomiphene, clomipramine, clopidogrel, codeine, coenzyme Q10, cyclobenzaprine, cyclosporin, danazol, dantrolene, dexchlorpheniramine, diclofenac, dicoumarol, digoxin, dehydroepiandrosterone, dihydroergotamine,
 30 dihydrotachysterol, dirithromycin, donezepil, efavirenz, eposartan, ergocalciferol, ergotamine, essential fatty acid sources, etodolac, etoposide, famotidine, fenofibrate, fentanyl, fexofenadine, finasteride, fluconazole, flurbiprofen, fluvastatin, fosphenytoin, frovatriptan, furazolidone, gabapentin, gemfibrozil, glibenclamide, glipizide, glyburide, glimepiride, griseofulvin, halofantrine, ibuprofen, irbesartan, irinotecan, isosorbide

dinitrate, isotretinoin, itraconazole, ivermectin, ketoconazole, ketorolac, lamotrigine, lansoprazole, leflunomide, lisinopril, loperamide, loratadine, lovastatin, L-thyroxine, lutein, lycopene, medroxyprogesterone, mifepristone, mefloquine, megestrol acetate, methadone, methoxsalen, metronidazole, miconazole, midazolam, miglitol, minoxidil, mitoxantrone, montelukast, nabumetone, nalbuphine, naratriptan, nelfinavir, nifedipine, nilsolidipine, nilutamide, nitrofurantoin, nizatidine, omeprazole, oprevelkin, oestradiol, oxaprozin, paclitaxel, paracalcitol, paroxetine, pentazocine, pioglitazone, pizofetin, pravastatin, prednisolone, probucol, progesterone, pseudoephedrine, pyridostigmine, rabeprazole, raloxifene, rofecoxib, repaglinide, rifabutine, rifapentine, rimexolone, ritanovir, rizatriptan, rosiglitazone, saquinavir, sertraline, sibutramine, sildenafil citrate, simvastatin, sirolimus, spironolactone, sumatriptan, tacrine, tacrolimus, tamoxifen, tamsulosin, targretin, tazarotene, telmisartan, teniposide, terbinafine, terazosin, tetrahydrocannabinol, tiagabine, ticlopidine, tirofibrin, tizanidine, topiramate, topotecan, toremifene, tramadol, tretinoin, troglitazone, trovafloxacin, ubidecarenone, valsartan, venlafaxine, verteporfin, vigabatrin, vitamin A, vitamin D, vitamin E, vitamin K, zafirlukast, zileuton, zolmitriptan, zolpidem, and zopiclone. Of course, salts, isomers and derivatives of the above-listed hydrophobic active ingredients may also be used, as well as mixtures.

In some embodiments, the active ingredient comprises a hydrophilic active ingredient. Amphiphilic compounds are also included within the class of hydrophilic active ingredients. Apparent water solubilities for hydrophilic active ingredients are greater than about 0.1% by weight, and typically greater than about 1% by weight. In a particular aspect of this embodiment, the hydrophilic active ingredient is a hydrophilic drug. In other particular aspects, the hydrophilic active ingredient is a cosmeceutical, a diagnostic agent, or a nutritional agent.

Suitable hydrophilic active ingredients are not limited by therapeutic category, and can be, for example, analgesics, anti-inflammatory agents, antihelmintics, anti-arrhythmic agents, anti-bacterial agents, anti-viral agents, anti-coagulants, anti-depressants, anti-diabetics, anti-epileptics, anti-fungal agents, anti-gout agents, anti-hypertensive agents, anti-malarials, anti-migraine agents, anti-muscarinic agents, anti-neoplastic agents, erectile dysfunction improvement agents, immunosuppressants, anti-protozoal agents, anti-thyroid agents, anxiolytic agents, sedatives, hypnotics, neuroleptics, β -blockers, cardiac inotropic agents, corticosteroids, diuretics, anti-parkinsonian agents, gastro-intestinal agents, histamine receptor antagonists, keratolytics, lipid regulating

agents, anti-anginal agents, cox-2 inhibitors, leukotriene inhibitors, macrolides, muscle relaxants, nutritional agents, opioid analgesics, protease inhibitors, sex hormones, stimulants, muscle relaxants, anti-osteoporosis agents, anti-obesity agents, cognition enhancers, anti-urinary incontinence agents, nutritional oils, anti-benign prostate hypertrophy agents, essential fatty acids, non-essential fatty acids, and mixtures thereof

Likewise, the hydrophilic active ingredient can be a cytokine, a peptidomimetic, a peptide, a protein, a toxoid, a serum, an antibody, a vaccine, a nucleoside, a nucleotide, a portion of genetic material, a nucleic acid (*e.g.*, DNA, RNA, mRNA, including nucleic acids that would be included in a DNA or RNA vaccine), or a mixture thereof.

Specific, non-limiting examples of suitable hydrophilic active ingredients include: acarbose; acyclovir; acetyl cysteine; acetylcholine chloride; alatrofloxacin; alendronate; aglucerase; amantadine hydrochloride; ambenomium; amifostine; amiloride hydrochloride; aminocaproic acid; amphotericin B; antihemophilic factor (human), antihemophilic factor (porcine); antihemophilic factor (recombinant), aprotinin; asparaginase; atenolol; atracurium besylate; atropine; azithromycin; aztreonam; BCG vaccine; bacitracin; becalerin; belladonna; bepridil hydrochloride; bleomycin sulfate; calcitonin human; calcitonin salmon; carboplatin; capecitabine; capreomycin sulfate; cefamandole nafate; cefazolin sodium; cefepime hydrochloride; cefixime; cefonicid sodium; cefoperazone; cefotetan disodium; cefotaxime; cefoxitin sodium; ceftizoxime; ceftriaxone; cefuroxime axetil; cephalixin; cephalirin sodium; cholera vaccine; chorionic gonadotropin; cidofovir; cisplatin; cladribine; clidinium bromide; clindamycin and clindamycin derivatives; ciprofloxacin; clodronate; colistimethate sodium; colistin sulfate; corticotropin; cosyntropin; cromolyn sodium; cytarabine; dalteparin sodium; danaparoid; desferrioxamine; denileukin diflitox; desmopressin; diatrizoate meglumine and diatrizoate sodium; dicyclomine; didanosine; dirithromycin; dopamine hydrochloride; dornase alpha; doxacurium chloride; doxorubicin; etidronate disodium; enalaprilat; enkephalin; enoxaparin; enoxaprin sodium; ephedrine; epinephrine; epoetin alpha; erythromycin; esmolol hydrochloride; factor IX; famciclovir; fludarabine; fluoxetine; foscarnet sodium; ganciclovir; granulocyte colony stimulating factor, granulocyte-macrophage stimulating factor; growth hormones—recombinant human; growth hormone—bovine; gentamycin; glucagon; glycopyrolate; gonadotropin releasing hormone and synthetic analogs thereof; GnRH; gonadorelin; grepafloxacin; haemophilus B conjugate vaccine; Hepatitis A virus vaccine inactivated; Hepatitis B virus vaccine inactivated; heparin sodium; indinavir sulfate; influenza virus vaccine; interleukin-2; interleukin-3; insulin-human, insulin lispro;

insulin procine; insulin NPH; insulin aspart; insulin glargine; insulin detemir; interferon
 alpha; interferon beta; ipratropium bromide; ifosfamide; Japanese encephalitis virus
 vaccine; lamivudine; leucovorin calcium; leuprolide acetate, levofloxacin; lincomycin and
 lincomycin derivatives; lobucavir; lomefloxacin; loracarbef; mannitol; is measles virus
 5 vaccine; meningococcal vaccine; menotropins; mepenzolate bromide; mesalamine;
 methenamine; methotrexate; methscopolamine; metformin hydrochloride; metoprolol;
 mezocillin sodium; mivacurium chloride; mumps viral vaccine; nedocromil sodium;
 neostigmine bromide; neostigmine methyl sulfate; neurontin; norfloxacin; octreotide
 acetate; ofloxacin; olpadronate; oxytocin; pamidronate disodium; pancuronium bromide;
 10 paroxetine; perfloxacin; pentamidine isethionate; pentostatin; pentoxifylline; periciclovir;
 pentagastrin; pentholamine mesylate; phenylalanine; physostigmine salicylate; plague
 vaccine; piperacillin sodium; platelet derived growth factor-human; pneumococcal vaccine
 polyvalent; poliovirus vaccine inactivated; poliovirus vaccine live (OPV); polymyxin B
 sulfate; pralidoxime chloride; pramlintide, pregabalin; propafenone; propenthaline
 15 bromide; pyridostigmine bromide; rabies vaccine; residronate; ribavarin; rimantadine
 hydrochloride; rotavirus vaccine; salmeterol xinafoate; sinealide; small pox vaccine;
 solatol; somatostatin; sparfloxacin; spectinomycin; stavudine; streptokinase; streptozocin;
 suxamethonium chloride; tacrine hydrochloride; terbutaline sulfate; thiopeta; ticarcillin;
 tiludronate; timolol; tissue type plasminogen activator; TNFR:Fc; TNK-tPA;trandolapril;
 20 trimetrexate gluconate; trospectinomycin; trovafloxacin; tubocurarine chloride; tumor
 necrosis factor; typhoid vaccine live; urea; urokinase; vancomycin; valacyclovir;
 valsartan; varicella virus vaccine live; vasopressin and vasopressin derivatives;
 vecuronium bromide; vinblastine; vincristine; vinorelbine; vitamin B12 ; warfarin sodium;
 yellow fever vaccine; zalcitabine; zanamivir; zolendronate; zidovudine; pharmaceutically
 25 acceptable salts, isomers and derivatives thereof; and mixtures thereof.

In some versions, the active ingredient comprises a UV-absorbing agent. Non-
 limiting examples of UV-absorbing agents include oxybenzone, avobenzone, octisalate,
 octocrylene, homosalate, and octinoxate.

Regardless of whether or not the composition includes a UV-absorbing agent, the
 30 compositions preferably absorb light at a wavelength within a range from about 100 nm to
 about 400 nm, such as from about 150 nm to about 350 nm, or from about 190 nm to
 about 290 nm.

The compositions of the invention can comprise a cationic active ingredient. The
 cationic active agent can be solubilized in the carrier, bound to the envelope of the lipid

bodies, and/or bound to the isolated envelopes. A non-limiting cationic active ingredient includes copper.

The invention is also directed to compositions comprising components isolated from the lipid bodies of the invention. Exemplary components include isolated lipid-body envelopes and isolated lipid-body oil. Such compositions can further include a carrier. Such compositions can further include a cation bound to an isolated lipid-body envelope. Such compositions can comprise an active ingredient bound to the isolated lipid-body envelope. Such compositions can be formulated with any carriers or excipients described herein for lipid bodies.

Another aspect of the invention is directed methods of generating the compositions of the invention.

Such methods can involve disrupting yeast cells containing intracellular lipid bodies to release the lipid bodies therefrom. Disrupting the yeast cells is preferably performed in a manner that maintains the structural integrity of at least a large proportion of the lipid bodies. Maintaining the structural integrity of the lipid bodies can be determined by the lack of detectable significant amounts of – or even any – free oil being released. Exemplary methods of releasing the lipid bodies include cell-wall hydrolysis, enzymatic digestion, bead beating (*e.g.*, using a “bead beater” apparatus (Sitepu et al. 2014) with titanium beads), heating, sonicating, or any combination thereof. Exemplary enzymatic digestion methods are provided herein. Exemplary enzymes that can be used in the enzymatic digestion include yeast lytic enzymes. Exemplary yeast lytic enzymes include β -(1,3)-glucanases, such as those from EC 3.2.1.6 and 3.2.1.39. β -(1,3)-glucanases comprising a Ricin B Lectin binding domain are preferred, such as β -(1,3)-glucanases found in *Cellulosimicrobium cellulans* and *Trichoderma reesei*.

An exemplary method of cell-wall hydrolysis comprises treating the cells with acid. This acid treatment can be performed with heating for various lengths of time. The heating can include heating to temperatures from about 50°C to about 140°C or more. The lengths of time can range from about 30 minutes to about 18 hours. In some versions, the heating can comprise autoclaving. The autoclaving can be performed at about 121°C for about 45 minutes. The acid used to hydrolyze the cells can include sulfuric acid, phosphoric acid, oxalic acid, or citric acid, among others, or any combination thereof. The acid treatment can involve mixing cell suspensions with volumes of the acid. The mixing can include mixing the cell suspensions in 1- to 10-fold volumes of an acid solution. The acid solution in some versions includes the acid in an amount from about 0.1 N to about 3

N. In preliminary experiments, the lipid bodies were released by heat and bead beating or by the combination of heat, bead beating, and 0.2 to 0.5 N sulfuric acid. Surprisingly, control samples that experienced only heat and acid also released lipid bodies. Even more surprisingly, the lipid bodies could be recovered by mixing cell suspensions in 1- to 10-
5 fold volumes of 0.25 to 0.5 N sulfuric acid followed by autoclaving at 121°C for 45 minutes. Exemplary hydrolysis methods include mixing yeast cell suspensions with about 0.25 N sulfuric acid, about 0.5 N phosphoric acid, about 0.4 N oxalic acid, or about 2 N citric acid at about 50° to about 121°C for various lengths of time ranging from about 30 min up to about 18 h. The time, temperature, and acidity can be varied to attain hydrolysis
10 of the cell walls and release lipid bodies. All appropriate combinations of these acid hydrolysis steps preserve the lipid bodies.

Due to their large size and low density, the lipid bodies of the invention are easily separated by flotation, centrifugation, or any other means based on their density relative to the surrounding solution. The flotation or isolation by any other means allows for the
15 washing of the lipid bodies to remove the cell wall hydrolysate, nucleic acids, proteins, and other residual yeast and fermentation components. Accordingly, the lipid bodies released from the cells as outlined above can be recovered by centrifugation, with optional crude washing with water. The lipid bodies can be used directly as a product of commerce when a crude washed lipid body is sufficient.

20 The lipid bodies as isolated above have a tan to brown color, even after washing with water. These crude washed lipid bodies can be made particularly clean and nearly bright white by washing the released lipid bodies with an alkaline buffer. The buffer preferably has a pH from about 9.5 to about 13.5, such as from about 10.5 to about 12.5 or about 11.7. It is suspected that the alkaline washing removes entrained and bound proteins
25 and media. It is preferred to conduct the alkaline wash under reduced temperatures for relatively short, fixed durations, so that saponification reactions of the entrained lipids are minimized. The alkaline wash can include sodium hydroxide, (NaOH), sodium carbonate (washing soda, Na₂CO₃), and/or sodium percarbonate (Na₂CO₃•1.5 H₂O₂), as outlined in the following examples.

30 The lipid bodies are preferably alkaline washed to an extent that results in a white or off-white color, such that the resulting lipid bodies have a lower absorbance at 400 nm than the crude washed lipid bodies.

The alkaline-washed lipid bodies can be recovered as a useful intact commercial product by centrifugation, filtration, evaporation, or any other means of lipid body

isolation.

The isolated lipid bodies (whether merely water-washed, alkaline-washed, or non-washed) can be further processed to generate a number of useful biomaterials. The lipid bodies, for example, can be dried to generate a wax-like solid, as described in further
5 detail in the examples. The lipid bodies can also be processed to extract the oil therefrom. Any method can be used to extract the oil from the lipid bodies. In some versions, the oil is extracted using homogenization (2,500 to >35,000 psi) and/or solvents (hexane, chloroform/methanol, or any other similar solvent system) to produce a relatively pure yeast oil. If residual solvents are to be avoided, the lipid bodies can be dried or dried and
10 homogenized or pressed. The drying releases an amount of oil that can be collected. The released oil is comparable to palm-oil and is suitable for skin care and other person care or consumer products. The solvent-free oil extraction obtained by drying is a valuable commercial platforms for a wide range of products.

The yeast cells that generate the intracellular lipid bodies from which the isolated
15 lipid bodies are released are preferably recombinant yeasts. The recombinant yeasts are preferably derived from lipogenic yeasts. A preferred lipogenic yeast is *Lipomyces starkeyi*.

In preferred versions of the invention, the recombinant yeast used to generate the intracellular lipid bodies is a lipogenic yeast, such as *Lipomyces starkeyi* or any other
20 lipogenic yeast, that is genetically modified to express or overexpress a diacylglycerol acyltransferase, a malic enzyme, and a glycerol-3-phosphate acyltransferase.

In some versions, the diacylglycerol acyltransferase comprises the DGA1-1233 variant represented by SEQ ID NO:2, encoded by SEQ ID NO:1, or a protein comprising a sequence at least about 80%, at least about 85%, at least about 90%, or at least about 95%
25 identical to SEQ ID NO:2; the DGA2 represented by SEQ ID NO:4, encoded by SEQ ID NO:3, or a protein comprising a sequence at least about 80%, at least about 85%, at least about 90%, or at least about 95% identical to SEQ ID NO:4; or a combination thereof. The malic enzyme is preferably the ME represented by SEQ ID NO:6, encoded by SEQ ID NO:5, or a protein comprising a sequence at least about 80%, at least about 85%, at least
30 about 90%, or at least about 95% identical to SEQ ID NO:6. The glycerol-3-phosphate acyltransferase is preferably the SCT1 represented by SEQ ID NO:8, encoded by SEQ ID NO:7, or a protein comprising a sequence at least about 80%, at least about 85%, at least about 90%, or at least about 95% identical to SEQ ID NO:8.

In addition to the diacylglycerol acyltransferase, the malic enzyme, and the glycerol-3-phosphate acyltransferase, the recombinant yeast of the invention can also comprise additional modifications. An exemplary additional modification is a modification to express or overexpress an acetyl-CoA carboxylase, such as the Acc1 represented by SEQ ID NO:10, encoded by SEQ ID NO:9, or a protein comprising a sequence at least about 80%, at least about 85%, at least about 90%, or at least about 95% identical to SEQ ID NO:10, particularly an Acc1 variant comprising a serine or threonine at a position corresponding to position 639 of SEQ ID NO:10. Another exemplary additional modification is a modification to express or overexpress a glycerol-3-phosphate dehydrogenase, such as the GPD1 represented by SEQ ID NO:12, encoded by SEQ ID NO:11, or a protein comprising a sequence at least about 80%, at least about 85%, at least about 90%, or at least about 95% identical to SEQ ID NO:12. Another exemplary additional modification is a modification to express or overexpress a glucose-6-phosphate dehydrogenase, such as the ZWF1 represented by SEQ ID NO:14, encoded by SEQ ID NO:13, or a protein comprising a sequence at least about 80%, at least about 85%, at least about 90%, or at least about 95% identical to SEQ ID NO:14. Another exemplary additional modification is a modification to express or overexpress a 6-phosphogluconate dehydrogenase, such as the GND1 represented by SEQ ID NO:16, encoded by SEQ ID NO:15, or a protein comprising a sequence at least about 80%, at least about 85%, at least about 90%, or at least about 95% identical to SEQ ID NO:16. Another exemplary additional modification is a modification to express or overexpress a fatty acid synthase, such as the FAS2.2 represented by SEQ ID NO:18, encoded by SEQ ID NO:17, or a protein comprising a sequence at least about 80%, at least about 85%, at least about 90%, or at least about 95% identical to SEQ ID NO:18, and/or the FAS1.2 represented by SEQ ID NO:20, encoded by SEQ ID NO:19, or a protein comprising a sequence at least about 80%, at least about 85%, at least about 90%, or at least about 95% identical to SEQ ID NO:20. Another exemplary additional modification is a modification to express or overexpress a ATP citrate lyase, such as the ATP citrate lyase alpha subunit represented by SEQ ID NO:22, encoded by SEQ ID NO:21, or a protein comprising a sequence at least about 80%, at least about 85%, at least about 90%, or at least about 95% identical to SEQ ID NO:22, and/or the ATP citrate lyase beta subunit represented by SEQ ID NO:24, encoded by SEQ ID NO:23, or a protein comprising a sequence at least about 80%, at least about 85%, at least about 90%, or at least about 95% identical to SEQ ID NO:24. Another exemplary additional modification is a modification to express or overexpress a glycerol

kinase, such as the 1602 variant of GUT1 represented by SEQ ID NO:26, encoded by SEQ ID NO:25, or a protein comprising a sequence at least about 80%, at least about 85%, at least about 90%, or at least about 95% identical to SEQ ID NO:26, or the 1617 variant of GUT1 represented by SEQ ID NO:28, encoded by SEQ ID NO:27, or a protein comprising a sequence at least about 80%, at least about 85%, at least about 90%, or at least about 95% identical to SEQ ID NO:28. Another exemplary additional modification is a modification to express or overexpress a glycerol-3-phosphate dehydrogenase, such as the GUT2 represented by SEQ ID NO:30, encoded by SEQ ID NO:29, or a protein comprising a sequence at least about 80%, at least about 85%, at least about 90%, or at least about 95% identical to SEQ ID NO:30.

These exemplary additional modifications can be included in any combination. An exemplary combination includes expressing or overexpressing a glucose-6-phosphate dehydrogenase with a 6-phosphogluconate dehydrogenase and/or expressing or overexpressing a glycerol kinase with a glycerol-3-phosphate dehydrogenase.

In addition to expressing or overexpressing any one or more of the proteins listed above, the recombinant yeasts of the invention may be modified to reduce or ablate the activity of one or more native or non-native proteins. The recombinant yeasts, for example, may comprise a modification that reduces or ablates the activity of one or more of the following native proteins: acyl-CoA oxidase *POX1*, the multifunctional enzyme *FOX1*, UDP-glucose 6 dehydrogenase (UDPGH), Uridine 5'-diphospho-glucuronosyltransferase (UGT), and glycogen synthase. Acyl-CoA oxidases include enzymes falling under EC number 1.3.3.6. An exemplary acyl-CoA oxidase whose activity may be reduced or ablated includes POX1 (SEQ ID NO:32) encoded by *POX1* (SEQ ID NO:31) from *L. starkeyi*. Acyl-CoA oxidases catalyze the first step of beta oxidation. An exemplary multifunctional FOX 1 enzyme is the FOX1 from *L. starkeyi* (SEQ ID NO:34), which is encoded by *FOX1* (SEQ ID NO:33). This enzyme has both 3-hydroxyacyl-CoA dehydrogenase and enoyl-CoA hydratase activities. An exemplary UDP-glucose 6 dehydrogenase (UDPGH) is the UDPGH from *L. starkeyi* (SEQ ID NO:36), which is encoded by SEQ ID NO:35. An exemplary uridine 5'-diphospho-glucuronosyltransferase (UGT) is the UGT from *L. starkeyi* (SEQ ID NO:38), which is encoded by SEQ ID NO:37. An exemplary glycogen synthase is the glycogen synthase from *L. starkeyi* (SEQ ID NO:40), which is encoded by SEQ ID NO:39. Other suitable proteins whose activity may be reduced or ablated include those comprising amino acid sequences at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at

least about 99% identical to the sequences listed above. Other suitable proteins whose activity may be reduced or ablated include orthologs and paralogs of the proteins listed above. Other suitable proteins whose activity may be reduced or ablated include those comprising amino acid sequences at least about 80%, at least about 85%, at least about 5 90%, at least about 95%, at least about 97%, or at least about 99% identical to orthologs and paralogs of the proteins listed above.

Lipogenic (oleaginous yeasts), the biochemistry of lipid synthesis by yeasts, enzymes involved in substrate assimilation, genetic engineering, and organic substrate conversion are discussed in US 2018/0245109 A1 and US Patent 10,662,448 The entire 10 content of US 2018/0245109 A1 and US Patent 10,662,448 are incorporated herein by reference in their entirety. In addition to the modifications described herein, the recombinant yeast of the invention can also comprise any other modification described in US 2018/0245109 A1 and US Patent 10,662,448.

The compositions of the invention can be administered to an animal, such as a 15 human, for any of the many purposes described herein. Accordingly, Methods of the invention can include administering a composition of the invention to an animal. The administration can include topical administration, oral administration, and/or parenteral administration.

Some versions include applying a composition of the invention to a site on a 20 surface of an animal. The surface can include the skin, a mucous membrane, a buccal surface, the gums, a surface of the eye, etc. Applying the composition on a site on a surface can deposit envelopes of the lipid bodies and/or isolated envelopes on the site. The envelopes and/or the isolated envelopes can remain deposited on the site for a period of time after the application. The period of time can be at least about 30 minutes, at least 25 about 1 hour, at least about 2 hours, at least about 5 hours, at least about 7 hours, or at least about 10 hours and/or up to about 1 hour, up to about 2 hours, up to about 5 hours, up to about 7 hours, up to about 10 hours, up to about 15 hours, up to about 20 hours, or more.

Consumers increasingly desire pharmaceutical agents, drug delivery platforms, 30 cosmetic, and cosmeceutical compositions from sustainable sources with as few additional chemicals as possible. Topical compositions that minimize chemical exposures and are sustainable are needed as the planet's population grows.

Many diseases can be treated with medicinal compounds, but there are physical limitations in the ability to formulate, particularly hydrophobic drugs, at high doses. Most

current compositions for hydrophobic active ingredients require encapsulation, liposomes, or irritating emulsification agents. Personal care products also can contain undesirable chemicals and detergents. Most compositions are complex and have a dozen or more ingredients needed for a useful composition. The present invention addresses the challenge
5 of making compositions that carry high concentrations of hydrophobic substances while maintaining a pleasant aqueous feel.

There is significant medical need for improved oral drugs and vaccines due to consumer dislike of injections. The tolerance of an oral drug carrier system to stomach acid is an important property of oral drugs, which are absorbed in the intestines.
10 Additionally, the present invention addresses important challenges in the preparation of oral and respiratory compositions, which specifically require lipid protection of active ingredients and uniform particles in specific ranges to be effective.

For topical conditions such as burns and open sores covering the damaged tissue is important to facilitate the healing process. Sealing damaged tissue is problematic because
15 the covering and, in some cases, bandages need to be removed, thereby reopening the wound. Finally, the invention addresses compositions that impart a surface sealant to a treated area when used topically that naturally shed over an extended time.

As humans age, natural biological processes, physical, medical, and environmental conditions affect skin quality and appearance (Kvorning et al. 1949). In addition to age,
20 skin conditions can arise due to atmospheric dryness, liver spots, exposure to, salt, sun, water, alterations in diet, changes in weight, and other factors that can affect skin appearance, moisture, pliability, and elasticity (Jiang et al. 2011). The present invention provides compositions to address these conditions.

Similarly, age-related changes in other body parts are also observed, including
25 changes in hair, lips, and other body orifices such as the rectum. In all cases, these changes in epithelial tissues of the body can potentially progress to medical conditions including acne, acne scarring, rosacea, eczema, herpes, sun damage and psoriasis, all of which are hard to treat or cure. In some cases, exposure to sun damage may even progress to melanoma (Gershenwald et al. 2016). Many of the useful medicines that are applied
30 topically require compositions with a degree of hydrophobicity and compatibility with skin and other epithelial body parts. The ability to load active ingredients for addressing anti-aging strategies is an important element in skin care (Ganceviciene et al. 2012). Active ingredients such as vitamin A or retinol are associated with improvements to aging skin (Kafi et al. 2007). However, the loading of drugs into liposomes and emulsions are

frequently limited to concentrations of a few percent of active ingredient. This is because the loading capacity equals the mass of the active ingredient divided by the overall mass of a delivery particle, emulsion or micelle, which is limited by the volumetric loading capacity of small entities (generally less than 200 nm), due to the relatively small volume to surface ratio of those delivery approaches (McClements 2015). The invention comprises the uses of engineered biological organelles in compositions because of the engineered lipid bodies' large size (generally in the range of 4,000 nm to 12,000 nm) and associated high loading capacity due to their high volume to surface area ratio. Several additional properties, which are important for compositions of the invention are also described, including a range of robust features such as steam sterilizability and tolerance to gastric pH and more that are important for the compositions of the invention.

Many existing cosmetic and topical pharmaceutical preparations designed to treat skin conditions contain palm oil (US Patent 8,524,292). Compositions disclosed herein use engineered yeast lipid bodies and yeast lipid body oil, which is a palm-oil-biosimilar that is found inside of lipid bodies, as a key property imparting feature of the materials used in the invention.

Compositions of the invention comprise lipid bodies of the invention and/or components thereof, such as lipid body shells, envelopes, and associated biochemicals, including internal or externally added yeast oil along with other exogenous constituents or modifications. These components together or separately impart unique properties and important characteristics that enhance the utility of the compositions containing lipid bodies. Unless otherwise noted, the terms "lipid bodies", "yeast lipid bodies," or "large yeast lipid bodies" as used herein refer to "lipid bodies of the invention." Similarly, unless otherwise noted, the term "formulation(s)" as used herein refers to compositions of the invention that comprise lipid bodies and/or lipid body components.

Lipid bodies and their components provide multiple properties not readily available from other types of composition components, such as liposomes, nanoparticles, or emulsions. Lipid bodies provide a large internal hydrophobic zone with a high volumetric capacity to hold hydrophobic drugs and other ingredients. The lipid bodies also provide a smooth moisturizing matrix that feels aqueous and non-greasy and that can accommodate hydrophilic constituents in the aqueous interstitial layer between the lipid bodies. Lipid bodies, when applied and dried on the skin, provide a sealant property due in part to their encompassing envelope, which remains on the surface of the skin after the lipid body oil and aqueous components have been absorbed. The sealant property can

facilitate wound healing, burn repair, enhance the healing of damaged or blistered skin, reduce scar tissue formation and retain both hydrophobic and hydrophilic drugs and other key ingredients.

The lipid bodies can be isolated, refined, bleached, washed with water, saline, acid, or alkali, deodorized, purified, blended, coated with biopolymers, otherwise derivatized, or modified to achieve or take advantage of various properties that are relevant to compositions of the invention. Lipid bodies and lipid-body components, such as the lipid body surface shells and empty shell envelopes, can be chemically or enzymatically modified to impart additional unique properties to the compositions. Lipid bodies of the invention can be used directly or in combination with other substances to obtain novel features and preferred compositions that possess improved functionality. Compositions comprising the lipid bodies and/or lipid-body components can be blended as homogenous materials. The lipid bodies impart desirable features such as moisture retention, longer shelf life, better appearance, improved smell, high-levels of active-ingredient loading and suitable active-ingredient release, and other properties that render enhanced functionality or improved consumer acceptance. These characteristics are uniquely imparted to compositions comprising lipid bodies of the invention.

The compositions of the invention can be used in or as the following: Topical compositions and products, such as beauty agents, personal care products, skin care products, skin creams, lotions, salves, moisturizers, cosmetics, soaps, detergents, shampoos, and deodorants; Drug-delivery vehicles and systems, such as topical drugs, oral drugs and vaccines, cosmeceuticals, therapeutic solutions, parenteral solutions, biologic or biopharmaceutical delivery systems, and wound healing aids; Medicinal compositions and compositions such as anti-inflammatory solutions, pastes, and compositions, oral care products, ophthalmic compositions, vaginal delivery, otitis compositions and pharmaceutical products, and pharmaceutical research reagents for new drug development programs; Films and sprays such as water repellents, insect repellents, sunscreens, sanitizers and foods of various types, including cooking oil, shortening, vegetable ghee margarine, chocolate fats, ice cream fats, confectionary fats, non-dairy creamer, nutritional supplements, synthetic “meat free” compositions and meat substitutes, or as a carrier for food-related flavoring agents; Pet care products, including product compositions and pet and animal feeds and foods; and Other industrial compositions, including lubricants, slip agents, and biodiesel components and generally all those applications and compositions previously available only from palm oil or fractionated or derivatized palm oils.

The invention is directed to compositions comprising the lipid bodies and/or lipid-body components, either alone or in combination with exogenously added constituents, modifications, and/or derivatives.

5 The lipid bodies of the invention are large lipid bodies isolated from various genetically modified lipogenic yeasts and, optionally, lipid bodies that have been modified after isolation. They can be 5 to 12 microns or more in diameter resulting in volumes of 65 μm^3 to 525 μm^3 . The lipid-body components isolated from the lipid bodies of the invention include envelopes, oils, and other endogenous biochemical constituents. The lipid bodies, their components, and endogenous constituents alone or in combination with
10 exogenous constituents, coatings, derivatizations, and modifications comprise aspects of the invention.

The lipid bodies' hydrophobic interior contains oil into which hydrophobic drugs or compounds can be loaded. The hydrophilic exterior can carry hydrophilic drugs or compounds in a hydrophilic layer, on the hydrophilic surface or aqueous carrier of the
15 compositions of the invention. The compositions of the invention can accordingly incorporate both hydrophobic and hydrophilic substances, drugs and other beneficial oils, fatty acids, stabilizers, antimicrobial agents, and other compounds. The characteristics of the lipid bodies and lipid-body components impart the ability to carry large quantities of hydrophobic drugs, which normally have an oily feeling, in their interior as active
20 ingredients while maintaining an aqueous, non-greasy, and pleasant feeling when applied to the skin. Advantages for oral applications include acid stability and relative tastelessness.

The compositions of the invention may contain lipid body components, envelopes, and associated biochemicals, plus externally added reagents, ingredients, vitamins,
25 nutraceuticals, antioxidants, commercial additives, industrial additives, peptides, drugs or other exogenous constituents, including enzymatic and non-enzymatic protection from oxidation that is known to impact photodamaged skin. Additionally, enzymatic and non-enzymatic antioxidants may play a role in mitigating sun damaged skin and skin aging (Rhie et al. 2001, Shindo et al. 1994).

30 When used in topical, wound or burn applications, the compositions of the invention can impart key oil-based and/or water-based ingredients to the skin or application area and leave collapsed lipid body envelopes from the lipid bodies on the surface forming a protective seal, which is durable over a number of hours. Skin peel tests demonstrate that the sealing envelopes of the collapsed lipid bodies last for approximately

12 hours as a result of shedding that is analogous to the shedding of skin cells from healthy skin. The shedding of the lipid body envelopes is important to avoid accumulation and undesirable build-up on the skin upon repeated applications.

5 The lipid-body envelopes provide a stable lubricating property at temperatures up to 200°C. This lubricant property imparts useful features to industrial lubrication and in the operation of consumer devices that require a natural lubrication from a non-animal and non-petrochemical source. An example of mechanical lubrication is the lubrication of syringe plungers and auto-injector cartridge pistons, which are used for delivery of parenteral drugs by injection or for oral dosing in pediatric drug applications.

10 The internal yeast oil carried inside the lipid bodies or isolated therefrom and added as an additive to the compositions of the invention can provide enhanced functionality to compositions that require additional oiliness for specific purposes in a wide range of product compositions.

15 In some versions, the compositions of the invention are particularly suited for topical drug compositions and skincare products. Lipid bodies or their components can be blended with other constituents to produce customized compositions with desired properties such as melting point, consistency, stability, constituent release, miscibility, emulsion formation or other desired attributes, qualities, or features. The compositions can be used to treat, for example, a variety of skin and bodily conditions, including adverse and age-related skin or scalp conditions. The compositions can also be used as an effective moisturizer for prolonged maintenance of smooth healthy skin, particularly in cold, dry climates where skin is frequently subjected to chapping and other conditions.

20 The lipid bodies and lipid-body components address unmet needs and shortcomings of liposomes. First, the relatively low internal volume of conventional liposomes, due to their smaller diameters, results in a much smaller internal volume for carrying hydrophobic ingredients on a per unit basis. Second, their hydrophilic interiors require the use of emulsifiers and detergents to achieve solubility of hydrophobic ingredients. Such components are potential irritants.

30 The lipid bodies of the invention have an outer durable hydrophilic envelope, that collapses into a barrier once the oil and moisture have been imparted to the skin. The ability this lipid body component to form a seal over the area of composition application is an important property for topical drugs, once the drug, oil or active ingredients are imparted to the skin. The sealing property may play a role in enhanced wound healing

compositions. Liposomes and emulsions, in contrast, do not have robust envelopes that can form the protective covering over an area of application.

Important, unique properties of the lipid bodies of the invention for use in compositions of the invention include: (1) their relatively large diameter which gives them
5 a high internal hydrophobic volume; (2) their hydrophilic surface properties resulting in a smooth, non-greasy feel; and (3) their post-application sealing properties attributable to their external envelopes.

Other important properties of the lipid bodies of the invention include: the ability to carry large quantities of hydrophobic drugs, lipids, and hydrophobic active ingredients
10 onto the skin without feeling oily; the ability to be used as a relatively tasteless drug carrier for oral applications and provide the potential of delivering off-flavored drugs in a format that is acceptable to the palate; having envelopes that can be isolated to make additives and lubricants that are heat and friction tolerant in compositions of the invention (these components are also applicable as additives in compositions of various types); the
15 ability to provide compositions of the invention with lipid bodies that collapse after imparting their contents to the skin, yielding an overlapping layer of envelopes that form a clear imperceptibly thin protecting seal over the areas into which hydrophobic and hydrophilic ingredients have been applied; the ability to be modified, homogenized, or dehydrated to create envelopes that can be used to enhance compositions of large
20 molecules, biologics, and peptides for transport into the body; the ability for their surface charge state to be altered across a wide range of pH conditions (Some of these charges are useful in unaltered lipid-body compositions, while other charges are formed by chemical or enzymatic treatment of the lipid body surfaces; various charge states of the lipid bodies are associated with rapid repair of skin irritation or dryness, scar prevention, and healing
25 of blisters.); tolerance to heat and sterilization by autoclaving (*e.g.*, at 121°C), making them particularly useful for critical drug applications where subcutaneous injection may be applicable, for example, in vaccine development; stability to acid at pH levels down to 0.5 or lower (such as 0.4), enabling compositions that resist microbial contamination and resistance at gastric pH levels; low density, which is useful in concentrating hydrophobic
30 chemicals and other industrial applications; bright white color and oxidation resistance, even when heated or treated with acid at high temperatures; odor free, benign interaction with human skin enabling lipid bodies in cosmetic compositions that are non-irritating and well tolerated; smooth, aqueous, and pleasant feel under a wide range of conditions, which enables compositions having a significant interstitial aqueous zone surrounding the

exterior of the lipid bodies, which is can carry high concentrations of charged ingredients; melting temperature of internal oil that is a solid at room temperature and below and that melts at body temperature and temperatures above body temperature up to 200 °C. or more; high short-wavelength UV absorbance for utility with other sunblock ingredients; utility as a non-Newtonian viscosity modifier (*e.g.*, high viscosity at low shear rates and low viscosity at high shear rates), which can be useful for making the creams and compositions that spread easily but are resistant to dripping or running; ability to form stable suspensions when mixed with, *e.g.*, low concentrations of hydrophilic polymers and starches, despite their low density; the ability to maintain a net charge in compositions of the invention that are applicable to bioelectronics and biosensors; the ability to chelate divalent cations in the compositions of the invention that are useful, for example, in chelating copper in Wilson's disease and other metal-based pathologies; other properties arising from derivatization of lipid bodies for specific purposes in the compositions of the invention, such as the transport of nucleic acids in biological systems and humans in a manner that protect the nucleic acids, genes or other drugs from degradation or hepatic clearance; the lipid bodies are anionic at neutral pH and the charge can be manipulated by the environment around the lipid bodies. Coating, derivatizing and loading lipid bodies with cationic materials and metal ions can achieve variations in their charge state, which can be useful for broadening the range of active ingredients used in the compositions of the invention.

These distinguishing features offer advantages over the prior art. Liposomal delivery systems for topical pharmaceutical, cosmeceutical, and cosmetic ingredients have existed in the patent literature for many years, for example, in US 20050079210A1 and in many scientific publications. The well-established size of liposomes are between 10 and 200 nanometers (0.01 to 0.2 microns) in diameter, typically about 100 nanometers in diameter. No large and stable hydrophobic-interior liposomes with diameters in excess of 2.5 microns have been reported. The lipid bodies, by contrast, can have diameters between 5,000 and 10,000 nanometers (5-10 microns) or larger. The large size of the lipid bodies impart a number of advantages described herein.

According to the general nomenclature, all types of lipid bilayers surrounding an aqueous interior are generally known as liposomes. The methodology for liposome preparation has been known since being first described starting in the late 1960's (Demel et al. 1968, Alving et al. 1971, Bangham et al. 1974, Fishman et al. 1975, Braidman et al. 1976, Patel et al. 1976, Tentsova et al. 1976, Barsoum et al. 1980, Fitfield 1980, Szoka et

al. 1980, Mayer et al. 1986, Lopezberestein 1987, Egbaria et al. 1990). Large liposomes, sometimes called unilamellar vesicles are described in US Patent 5,004,611.

The lipid bodies of the invention can be included in compositions comprising or consisting of the lipid bodies and water. In such compositions, the lipid bodies form, in
5 order of increasing concentration from 1% to 80% of lipid bodies by dry weight, a white solution, a spray, a lotion, a cream or paste. A few stabilizing excipients may optionally be further included for long-term shelf-life.

The lipid bodies of the invention, for example, can be formulated as a single-component cream. The lipid bodies form a cream when formulated at a concentration
10 between 33% and 55% by dry weight. An antimicrobial agent, a humectant to prevent crusting, an antioxidant, and pH control can optionally be included to achieve long-term room temperature stability.

Lipid bodies of the invention have a high UV absorbance in the wavelength ranges between 190 nm and 290 nm, with an absorbance maximum at about 230 nm. The skin
15 and eyes are most sensitive to damage by UV light at 265–275 nm wavelength, which is in the lower UVC band that is absorbed by lipid bodies. However, most sunburn is caused by longer wavelengths, because those are more prevalent in sunlight at ground level. Accordingly, compositions containing the lipid bodies have sun blocking absorbance alone that can be enhanced when UV-absorbing agents are added. Examples of UV-
20 absorbing agents include oxybenzone, avobenzone, octisalate, octocrylene, homosalate, and octinoxate.

Many potential additives and active ingredients can be mixed with the lipid bodies or lipid-body components having the potential for skin discoloration. Successful
formulation of carotenoids requires an appropriate level of hydrophobicity and
25 compatibility with the components naturally found in skin and other epithelial body parts. The lipid bodies offer properties that can facilitate incorporation of fatty or hydrophobic ingredients.

Vitamin A is associated with carotenoid pigments as found in carrots, red bell peppers, and other pigmented foods. However, carotenoids have a limited application in
30 skin care because of the potential for skin discoloration. Successful formulation of carotenoids requires an appropriate level of hydrophobicity and compatibility with the components naturally found in skin and other epithelial body parts. The lipid bodies offer properties that can facilitate incorporation of fatty or hydrophobic ingredients.

In another example, vitamin E (including such forms as vitamin-E acetate) is an abundant, fat-soluble, lipophilic antioxidant that is important in maintaining healthy skin by preventing damage induced by free radicals and reactive oxygen species (Rhie et al. 2001, Shindo et al. 1994). Topical application of vitamin E effectively increases photoprotection. Vitamin E has very low solubility in water, but lipid bodies can facilitate incorporation of vitamin E as an ingredient in product compositions. Vitamin E can also act as a long-term antioxidant for room temperature shelf life.

Many other potential fatty or hydrophobic additives and active ingredients can be mixed with the lipid bodies, such as the hydrophobic compounds found in vegetable oils, nuts, and green leafy vegetables. Tocotrienols, for example, are similar to tocopherols except these have an unsaturated isoprenoid side chain. As antioxidants, these compounds are suitable additives to cosmeceuticals and personal care products. Specifically, erythema and skin aging effects are treated with topical lotions and emollients containing tocotrienols to address the underlying causes of skin and hair damage, such as free radicals generated by environmental factors. Tocotrienols can be effective in scavenging free radicals from lipid-rich layers of skin and scalp. Keratin in hair is also subject to oxidation by sunlight and harsh chemicals, and traditional remedies use added oil in conditioners or shampoos to ameliorate dry and brittle hair in addition to an antioxidant(s). The use of tocotrienols for treating skin and hair damage is disclosed in US Patent 5,545,398.

The lipid bodies offer properties that can facilitate incorporation of many plant and animal-derived substances. There are many additives and active ingredients that can be mixed with the lipid bodies or lipid-body components either singly or in combination with other additives.

Examples of skin care compositions can be found in US Patent 8,524,292. This patent discloses the use of various palm oil types for the formulation of cosmeceuticals and the utilization of red palm oil and other forms of palm oil, specifically palm stearin, palm olein, fractionated palm oils and blended, homogenized or votated (a type of blending) palm oil as a base for the formulation of skincare products, particularly those containing low but meaningful natural substances including oil isoforms, tocopherols, tocotrienols and carotenoids arising from crude red palm oil. The mixture of miscellaneous hydrophobic material including tocopherols, tocotrienols and various forms of palm oil described in US Patent 8,524,292 illustrates the heterogeneity of palm oil. The lipid bodies can facilitate incorporation of many ingredients in product compositions that currently rely on palm oil.

Goon et al. 2019 provides a review of the importance of palm oils in a broad range of applications for lipid-based compositions and drug delivery systems. Goon et al. also categorizes drug development compositions into the following: Type I, which is defined as any hydrophobic mixture of oil and active ingredients. Type II, which contain surfactants and is defined as self-emulsifying drug delivery systems or SEDDS. Type IIIa, which contains one or more co-surfactants or hydrophilic co-solvents defined as self-micro-emulsifying drug delivery systems or SMEDDS. Type IIIb containing a higher ratio of co-solvent than Type IIIa compositions and defined as self-nano-emulsifying drug delivery systems or SNEDDS.

10 The common feature of these preparations is the use of palm oils. The oil contained within the lipid bodies of the invention can be used as substitutes for the palm oil in any of these delivery systems and related compositions.

15 The simplicity of the compositions of the invention improves the ability to easily track drug loading and drug delivery to the patient and facilitates the development of analytical quality controls. The properties associated with the compositions of the invention can greatly facilitate topical drug development, manufacturability, and regulatory approvals because the exemplary source of the lipid bodies is a published GRAS yeast, *Lipomyces starkeyi*, which is manufactured in an engineered strain that has a completed EPA review and Regulatory exemption for the intended use in personal care products. *Lipomyces starkeyi* was identified in soil and on human skin as a non-pigmented oleaginous yeast Connell et al. 1953, Connell et al. 1954.

20 Any engineered hyper-lipogenic yeast harboring lipid bodies with an occupancy in the yeast of greater than 66% oil, to even more than 85% and as much as 95%-98% of the interior of the engineered yeast can be used for producing lipid bodies used in the compositions of the invention.

25 The lipid bodies can be used in compositions that include the addition of various, surfactants, alcohols, oils, dyes, and hydrophobic substances.

30 The lipid bodies, when recovered from fermentation, can be easily isolated with little residual odor. This can result in products that are nearly odor free, with only a slight wax scent remaining.

 One embodiment of the invention is directed to a biocompatible, cosmeceutical, topical compositions comprising of lipid bodies and/or lipid-body components, such as envelopes, and associated biochemicals, wherein the composition is substantially homogeneous. The composition can replace essential oils in skin, particularly dry skin.

Once the composition dries on the skin, lipid-body envelopes form a protective film that helps maintain a durable retention of oil and moisture on the skin. These films shed away from the skin over about 10 hours, as demonstrated by microscopic examination of face peels. This surprising and unique property of such compositions is particularly useful for skin care products and topical drug delivery.

Another embodiment of the present invention is a biocompatible, cosmeceutical, topical composition comprising the lipid bodies blended with yeast oil to alter the aqueous feel and acceptability of the composition when a slightly oily property is desired for the rapid replacement of oil levels in the skin. The composition can contain added yeast oil at levels across a range of mixture ratios, such as from about 10 to 90, w/w% oil or higher to modify the rate and oiliness of a moisturizer or skin softener.

In another embodiment, the lipid bodies are bound to, mixed, blended, emulsified with, or used to dissolve or suspend externally supplied hydrophobic or amphipathic substances. The resulting hydrophobic compositions have utility for research purposes, potential drug development systems and for the creation of a wide range of product compositions in which hydrophobic or semi-hydrophobic active ingredients are desired.

In another embodiment, the lipid bodies are used to bind externally applied hydrophobic or hydrophilic substances, such as hyaluronic acid, in combination with alcohols and surfactants to facilitate hydrophobic compositions, self-assembling microencapsulation compositions, and modified nanoencapsulation compositions useful in drug development and research, including, for example, hydrophobic dyes and/or other compounds. The resulting emulsions, and liposome-like structures are uniquely useful for creating stable hydrophobic compositions that have utility for research purposes, potential drug development systems and for the creation of a wide range of product compositions in which hydrophobic, semi-hydrophobic or amphipathic active ingredients are desired.

The lipid bodies and/or lipid body components can be useful in one or more drug delivery system categories in the following examples in various configurations:

- Tablets: Bi-layer, multi-layer, mini, lipid and oil encapsulated tablets.
- Capsules: Gel caps and blow-fill-finish configurations, lipid and oil contained within capsules, and time-release compositions.
- Powder blends: Microparticle-filled, microsphere-filled; Powders used to make parenteral and intravenous formulations.
- Tableting processes containing lipids and oils: Direct compression, dry granulation, wet granulation; Enteric coating.

- Ocular Controlled Release containing lipids and oils: Instant release; Sustained release; Enteric or delayed release.

- Oral Controlled Release containing lipids and oils: Instant release; Sustained release; Enteric or delayed release based on resistance to gastric acid at pH 0.5.

5 • Respiratory drug formulations, used nasally or inhaled, comprising lipid bodies for the delivery of drug substance.

- Rectal Formulations containing lipids and oils: Surface applications; Suppositories; Hemorrhoid creams; Aspirin-based systemic medicines; Anti-epileptics; Anti-nausea drugs and others.

10 • Topical, Liquids, Sprays, Lotions, Creams, containing lipids and oils; Transdermal cream; Topical sprays.

- Parenteral-solutions, Injectables, Auto-injector Cartridges, Pre-filled Syringe serum bags, drip formulations, parenteral nutrition.

Other drug delivery systems, containing lipids and oils as appropriate.

15 The lipid bodies and lipid-body components can be modified by emulsification, saponification, votation, compression, bleaching, alkaline cleaning, acidic cleaning, distillation, cold or cooling fractionation, and other physical or chemical treatments. The modifications can create stable compositions for skin care products, personal care products, soaps, shampoos, drug delivery systems for hydrophobic compositions, drug
20 delivery systems for self-assembling emulsifications and micrometer and sub-micrometer liposomal-like structures, drug delivery systems for medicinal products, pet care products, and other product compositions.

The lipid bodies of the invention can form a moisturizing white cream (FIG. 1), either as a sole ingredient in a carrier (as shown) or in a stabilized composition for a
25 durable shelf-life at room temperature. The lipid bodies can be used directly in product compositions, which are composed of blended reagents or with other components, biochemicals, drugs, emulsification agents, alcohols, co-solvents, and any other appropriate additives to achieve desired compositions and moisturizing base cream for a wide range of products.

30 The lipid body components refer to any component derived or isolated from the lipid bodies of the invention. These can be used in the compositions of the invention. Exemplary lipid body components include lipid-body envelopes, broken envelope constituents, fragments, oil, and other associated biochemicals, such as protein, phospholipids, lipids, carbohydrates.

The oil contained in the exemplary lipid bodies is a yeast oil that can serve as a palm oil substitute or biosimilar. The term “palm oil” refers to oil recovered from the mesocarp of the fruit of oil palms, primarily the African oil palm *Elaeis guineensis*, and to a lesser extent from the American oil palm *Elaeis oleifera* and the maripa palm *Attalea*
 5 *maripa*.

The compositions of the invention include all compositions made with or comprising the lipid bodies and/or lipid-body components of the invention. The of the invention compositions of the invention may include, in addition to the lipid bodies and/or lipid body components, other ingredients, drugs, and or substances.

10 In nature, *Lipomyces starkeyi* and other oleaginous yeasts contain less than 45% lipid under the best conditions. The typical yeast oil synthesis rates are less than 0.26 g/l/hour and overall typical yields are less than 20 grams per liter when grown in fermentations. In most cases these yeasts have multiple, very small lipid bodies which can be seen under a microscope at 400x power. These small lipid bodies are not practically
 15 isolated or useful in the compositions of the invention.

The yeasts of the invention have product yields that are commercially viable and possess internal single lipid bodies that can occupy around 70%, and up to 95% or greater, of the internal area of the genetically engineered hyper-lipogenic yeast as large lipid bodies. The lipid bodies can be isolated from these strains by homogenization, mechanical
 20 disruption, or hydrolysis of yeasts and preparative washings. The isolation and washings of the lipid bodies can include acid hydrolysis, homogenization, and or multiple mild alkaline washes and neutralization steps.

The lipid bodies contain significant amounts of yeast oil that can be isolated by homogenization, hydrolysis, and/or dehydration followed by gravity decanting or, in a preferred manufacturing process, from extraction with suitable solvent, for example iso-
 25 hexane, n-hexane, n-pentane, chloroform/methanol or any other suitable solvent. Upon evaporative removal of the solvent, the resulting yeast oil is an unexpectedly pure triglyceride composition that contains the fatty acid content as shown in Table 1. Also shown in Table 1 is a comparison of the yeast oil isolated from yeast containing lipid
 30 bodies compared to tropical palm oil.

Table 1. The composition of triglyceride fatty acids isolated from lipid bodies of the invention compared to the fatty acid profile in palm oil .

Fatty Acid Profile of Oil from Exemplary Lipid Bodies of the Invention			Fatty Acid Profile of Palm Oil*
Fatty acid chain length	Fatty acid name	Percent of total fatty acids in exemplary lipid bodies of the invention#	Percent of total fatty acids and range in palm oil
C12:0	Lauric Acid	<u>0</u>	0.1-1.0
C14:0	Myristic Acid	<u>0.03</u>	0.9-1.5
C16:0	Palmitic Acid	40.08 - 43.0	41.8-46.8
C16:1	Palmitoleic Acid	5.08 - 7.0	0.1-0.3
C18:0	Steric Acid	4.81 - 7.0	4.2-5.1
C18:1	Oleic Acid	35.0 - 41.42	37.3-40.8
C18:2	Linoleic Acid	<u>2 - 8.6</u>	9.1-11.0
C18:3	Linolenic Acid	0.01 - 2	<0.05-0.6
C20:0	Arachidic Acid	<u>0.03</u>	0.2-0.7

*Kopas et.al. US 8,525292 B2

#: *Increased*, comparable, decreased

5 The fatty acid profile of oil from lipid bodies of the invention in comparison to palm oil is shown in FIG. 10.

10 The yeast oil isolated from yeast containing lipid bodies is comparable to palm oil with the exception that the content of palmitoleic acid (C16:1) that occurs in lipid body containing yeast at a level comparable to human skin and is nearly missing (<0.3%) in palm oil. US Patent 8,524,292 highlights the essential fatty acids, particularly palmitoleic acid, at 2-8% of the fatty acids found in skin. The fatty acid profile of the exemplary lipid bodies is similar to human skin. In particular, both contain about 5% palmitoleic acid, an essential fatty acid of skin. This essential fatty acid is almost absent in palm oil. The fatty acid profile of skin is shown in Table 2.

15 **Table 2.** The profiles of fatty acids of oil isolated from exemplary lipid bodies of the invention compared to lipids found in human skin.

Fatty acid name	Essential fatty acid profile (%)	
	Oil of the invention	Human skin lipids*
Palmitic Acid	40.08 - 43.0	30

Palmitoleic Acid	5.1 -7.0	8.0
Stearic Acid	4.8 - 7.0	13.0
Oleic Acid	35.0 - 41.4	17.0
Linoleic Acid	0.01 - 2%	14.0
Other Lipids, Waxes, Sebum and Cholesterol	~0-2%	45%

* US8,524,292 and Downing et al. 1969

The lipid bodies can impart oil to human skin when applied using the compositions of the invention. Because human skin can frequently be low in essential lipids, skin can be prone to dehydration, dryness, cracking, and other conditions associated with water loss and exposure to the environment. The comparability of the lipids in the lipid bodies to those in human skin makes the lipid bodies useful for treating conditions associated with dry skin. Applying the lipid-body containing creams of the invention to chapped hands makes the skin feel exceptionally smooth and moist and heals chapped skin in a manner not equaled with conventional hand creams. This observation is consistent with the profile of essential skin lipids and supports the suitability of the lipid bodies of the invention for providing the majority of human skin lipids in a proportion approaching the levels found in normal human skin. The lipid bodies can accordingly be used, with or without other ingredients, to restore oil and maintain moisture in skin.

The exemplary oil in lipid bodies have a relatively tight range and dominance of C16:0 and C18:1 lipids and an absence of lower chain saturated fatty acids. The oil from the exemplary lipid bodies are generated in controlled aerobic fermentation, so there are no plant contaminants such as polyphenols, phytophenols, phytoene, beta-carotene, phytofluene, neuroporene, zeacarotene, ubiquinone 10 (CoQ10), lycopene, tocopherol, tocotrienols, squalene, and potential pesticide residues, such as can be found in native tropical palm oils. When any desirable constituent is required in a composition, the composition can be made by adding the individual desired chemical or biochemical to the composition as needed to achieve a particular composition of the invention without the need for excessive co-contaminants, impacts to purify or difficult-to-manage quality controls.

The lipid bodies used in the compositions of the invention can carry hydrophobic chemicals, drugs, and other substances that bind to the hydrophobic interior of the lipid bodies that can be useful in treating skin, for example, lanolin. The use of lipid binding

dyes with visible spectrophotometric, microscopic, and/or fluorometric analyses is an excellent and easily quantifiable way to show how hydrophobic substances can bind to the lipid bodies. Examples of dyes that bind to the hydrophobic interior of the lipid bodies include BODIPY, Nile red, and Sudan black. Other actual drug loading and release
5 examples have been demonstrated using HPLC methods and or ultraviolet spectrophotometry to track drug incorporation into the compositions. The uptake and subsequent release of drug compounds provides a strong indication that the lipid bodies can be used as a carriers or vehicles for a range of compositions that incorporate hydrophobic and hydrophilic ingredients, including medicines, fragrances, cosmetic
10 ingredients, coloring agents and many other functional constituents.

The compositions of the invention need not exclude other more conventional compositional materials and systems. The lipid bodies can be modified chemically or enzymatically as type I (hydrophobic blends) or as type II (micro emulsions) containing emulsification agents such as lecithin. In some cases, when variable delivery rates are
15 desired, the lipid bodies can be blended with liposomes in a manner that creates a composition that may not be possible with either platform separately.

The lipid-body components, including envelopes and associated biochemicals, can be mixed with emulsification agents and other detergent-like agents to form other compositions of type III (micro and nano-emulsions) self-assembling emulsions similar to
20 type II but with or without co-solvents (Goon et al. 2019). Examples of drugs that can be formulated with lipid bodies include, Lidocaine, Benzocaine, Colchicine, Betamethasone, Phenytoin, Levadopa, Genistein, Paclitaxel, Aripiprazole, Chloramphenicol, Diclofenac acid, Sodium diclofenac, Ibuprofen, Vitamin E (tocopherols), Fish oils, Glucans, Carotene, Ketoprofen, Docetaxel, Tocopherol acetate, Tocotrienol, and any other suitable
25 hydrophobic or hydrophilic chemicals, drug, drug-delivery systems, food products, cosmeceuticals, cosmetics, skin care agents, personal care products, and other ingredients and composition components either singularly or in combinations with the lipid bodies and or associated components.

Compositions made with lipid bodies may include emulsification agents or
30 commercial reagents, for example, Capmul MCM (mono and di-glycerides of caprylic acid), Capmul PG-8, Captex 200 (mixed diesters of caprylic or capric acids in propylene glycol), Miglyol 812N, PEG 300, Tween 80, Polysorbate 80, Cremophor RH4 and other surfactants and emulsification agents alone or in combination with the compositions of the invention.

Other optional components that may be added to the compositions of the invention may include many types of other oils, for example, aloe vera, apricot oil, avocado oil, apricot kernel oil, fruit oils, borage oil, camellia oil, flower and seed oils of any type, coconut oil, passion fruit oil, safflower oil, rose hip oil, rice bran oil, soya oil, canola oil, 5 olive oil, shea butter oil, sunflower oil, wheat germ oil, tea tree oils, loquat oil, citronene, citrus oils, cannabidiol or CBD oil, and other oils alone or in combination within compositions based on lipid bodies. Additionally, the compositions of the invention may include other charged lipid bodies and or lipid body components, envelopes and associated biochemicals, fatty acids or synthetic chemicals such as organic and inorganic acids.

10 When important for particular compositions, esterification of fatty acids and alcohols can be made from yeast oils and other oils as straight or branched chain esters composed of either mono, di, tri, and tetra-esters. Depending on the chain length and structural arrangement of the fatty acids and alcohols, esters can be tailored to provide different physical properties and types of emollience that when mixed with lipid bodies 15 can impart the unique properties of lipid bodies to such compositions. For example, straight chain esters from cetylpalmitate and cetylstearyl stearate, which are solid at room temperature, may be used to increase the viscosity of emulsions and impart a dry emollience to the skin in compositions containing lipid bodies providing additional ingredients, yeast oil and or sealant properties that are provided from the lipid bodies.

20 Liquid branched chain esters, such as isopropyl myristate and cetostearyl ethylhexanoate, provide non-occlusive emollience with good spreading properties suitable for the compositions of the invention. Pentaerythrityl tetra-iso-stearate, a high molecular weight tetra-ester, confers a long-lasting emollience to skin creams and lotions, when in a composition with lipid bodies providing additional ingredients, yeast oil and or sealant 25 properties that are provided from the lipid bodies.

For sun protection compositions that are based on lipid bodies, the choice of emollient influences both the solubility of the protectant and ability of sunscreens to spread evenly. Preferred esters for sunscreen compositions are caprylic or capric triglycerides octyl methoxycinnamate, benzophenone-3, ethylhexyl hydroxystearate, other 30 related benzophenone structures, other aromatic ring structures and esters of C12 through C15 alkyl benzoate when in compositions based on lipid bodies providing additional ingredients, yeast oil and or sealant properties that are provided from the lipid bodies.

Other sunscreen agents such as zinc or titanium metals or other metal salts can be blended into lipid bodies to produce spreadable compositions, which can promote even

distribution on the skin and enhance the sun protection performance of a sunblock composition that is based on lipid bodies. Additionally, sunscreens with divalent or trivalent metals such as Cu^{2+} , Fe^{3+} , Co^{2+} , Mn^{2+} , Ti^{3+} , Zn^{2+} , and other metals and organic sun blocks can be formulated into lipid bodies either alone or in mixtures. The lipid bodies typically have net negative charge at typical pH's due to phospholipids and hydrophilic surface molecules, which facilitate inorganic sunblock compositions. These compositions can be enhanced by containing lipid bodies providing additional ingredients, yeast oil and or sealant properties are provided from the lipid bodies.

Natural and synthetic waxes can be formulated with lipid bodies and supplemented yeast oil to produce softened waxes, which are normally harder and more brittle with higher melting points than fats. Common waxes used in cosmetics include carnauba wax and beeswax, both of which can be made more pliable by the addition or blending with lipid bodies. Alternatively, synthetic waxes such as ethylene glycol diesters or triesters of long-chain C18 and C16 fatty acids from lipid bodies and associated yeast oil offer functional alternatives to confer rigidity and pliability to stick deodorants and other stick systems by modification of the synthetic wax crystallinity, while maintaining a high melting point. These compositions can be enhanced when containing lipid bodies providing additional ingredients, yeast oil and or sealant properties are provided from the lipid bodies.

Another common wax used in cosmetics is lanolin, which has chemical and physical similarities to the stratum corneum and lipids of human and animal skin. As a result, compositions disclosed herein can be used to help prevent water-loss from skin and maintain moisture with the combination of endogenous and or in combination with exogenous lipids plus the additional emollient and moisturizing properties of lanolin. Additionally, ethoxylated lanolin oils have been found to be effective in reducing the irritating effects of surfactants in detergents and soaps. Thus ethoxy-lanolin may be useful in improving the quality of lanolin-based soaps and detergents. These compositions can be enhanced by containing lipid bodies providing additional ingredients, yeast oil and or sealant properties are provided from the lipid bodies.

The compositions can be improved with respect oxidative properties by the addition of citric acid as a chelating agent and other agents such as vitamin E acetate. Furthermore, citric acid and D-gluconolactone plus sodium benzoate can provide a significant antimicrobial protection to the compositions.

Commercially available antimicrobial systems of a wide range of types and components are suitably effective when used in combination with the compositions of the invention. Significant shelf-lives have been demonstrated for compositions of the invention that are essentially aqueous mixtures and compositions containing lipid bodies.

5 It is best to uniformly add anti-oxidants such as vitamin E acetate and citric acid, a chelating agent, to the compositions when the compositions require significant shelf life at room temperature or at least temperatures less than 120° C. to avoid any unwanted localized effects that may arise from non-homogeneous mixing. Additionally, the addition of various starches and modified starches assist in preventing any separation for the
10 aqueous and interstitial components of compositions of the invention.

The compositions of the invention will enhance the utility of many products used in a wide range of applications because of the various properties of lipid bodies. The properties impart features and benefits to standard product compositions comprising a semi-solid, semi-solid, lotions and solution admixtures comprising lipid bodies with or
15 without other lipid body components and associated yeast oil. Key properties are the large size of the lipid bodies of the invention and their resultant high hydrophobic volume, stability under a wide range of pH and temperature conditions, the ability of lipid bodies to form a sealing layer after discharge of ingredients, and their ability to feel aqueous despite being full of oil. Other significant properties and example compositions containing the
20 lipid bodies and components of lipid bodies are described here.

The lipid bodies of the invention have utility where the compositions benefit from an essentially homogeneous state with differences in texture resulting from the effect of temperature on the melting of the lipids within the lipid bodies. In one configuration of the invention compositions can transform from a semi-solid to a liquid due to the melting
25 point of the yeast oil in the lipid bodies. In this case, the compositions can transform from a solid at room temperature to a less solid composition at temperatures above 80°F (27°C) and preferably above 85°F (29°C) and in some compositions more preferably above 90°F (32°C) and most preferably above 95°F (35°C). This melting property of lipids within the bodies is associated with a change in viscosity and texture yet the compositions can
30 continue to feel aqueous and smooth, despite the high content of yeast oil inside the lipid bodies or added to the compositions containing lipid bodies, for products, as described in the invention.

The lipid bodies used in the composition may be the direct product isolated by homogenization or by hydrolysis of hyper-lipogenic yeast cells as described herein,

followed by washing at various pH's (neutral, acidic, or alkaline) in order to isolate the lipid bodies. Other hydrophobic and hydrophilic partitioning methods are useful in preparing lipid bodies for the compositions in the invention. These processing steps are followed by removal of the solvent by evaporation or washing under a wide range of conditions as long as the lipid bodies do not become dry. Subsequently the lipid bodies can be further processed to make envelopes. Alternatively, lipid bodies can be chemically or enzymatically modified, derivatized or coupled to ligands that are useful for compositions, separations, and other consumer or industrial purposes.

Upon complete drying either in an oven or on the skin, the lipid bodies expel a significant fraction of their oil and the lipid bodies collapse resulting in envelopes or shells along with oil. Following drying the lipid body envelopes are not readily dispersed.

If the lipid bodies are washed and homogenized while wet, the envelopes release oil into aqueous or organic solvent solutions. One dense layer of separated components has a high concentration of lipid body envelopes, which are essentially opened shells. In general, both drying and homogenization disrupt the lipid bodies, which become permanently damaged as they release yeast oil from inside of the lipid bodies. Under these conditions the envelopes are disrupted, and envelopes can be formed.

In another embodiment, the compositions of the invention are obtained by hydrolysis of yeast in which an acid is used to hydrolyze away the cell walls of the yeast and the resulting lipid bodies are isolated by differential density and then washed with an alkaline solution followed by homogenization and solvent extraction followed by solvent evaporation to produce yeast oil that is separated from the lipid bodies envelopes, which can be used in oil-free versions of the compositions of the invention.

In another embodiment, yeast oil can be isolated from yeast containing lipid bodies and the isolated yeast can be added back to the compositions of the invention to increase oiliness or achieve other desirable behaviors in the composition of the invention.

The compositions of the invention may be topical, parenteral, injectable, oral, inhaled and administered as a cleaning composition for any type of object and composition used for medicine and drug delivery systems, pet care, pet medicines, and other acceptable applications of lipid body-based products containing 1% to 100% with or without yeast oil and lipid body components and or including any agent, emulsifier, co-solvent, natural or synthetic additive, flavoring, scent, or colorant as desired for the specific application of the composition.

The compositions of the invention can serve a wide range of drug delivery products, personal care, health care, skin care, hair care, pet care and other products for specific purposes. Of particular interest is the immediate impact on skin moisture and skin soothing, in which lipid bodies and/or lipid bodies are used as they impart significant
5 amounts of hydrophobic ingredients and drug to the user.

The compositions of the invention are surprisingly useful for skin conditions and this material is both pleasant and potentially useful for accelerated wound healing. The moisturizing properties and ability to impart hydrophobic and aqueous compositions to human skin can be beneficial for a number of human conditions that could benefit from
10 the compositions of the invention including dry skin, rashes, sunburn, wind burn, poison ivy, winter-season chapping, skin cracks, inflammatory diseases such as sebaceous gland disorders, e.g. acneiform disorders, such as acne vulgaris, rosacea, seborrhea, dermatoses, lupus erythematosus, systemic lupus, eczema, dermatitis and other skin disorders. Additionally, the compositions may be enhanced by the incorporation of drugs or
15 medicines into the human skin and other medicinal applications to further reduce inflammation through the addition of retinols, lidocaine, benzocaine, Benadryl, aspirin, ibuprofen, Tylenol, or any one or more anti-inflammatory agents, which are suitable for specific conditions for which the lipid bodies based products are being used. Additional ingredients may include nutraceuticals, cosmeceuticals, antioxidants, and any other
20 ingredient that improves the appeal, texture, odor, melting point, or any other property of the compositions. Finally, because of the mild soothing nature of the compositions of the invention, they are particularly suitable as a base for cosmetics and makeup in compositions that can be used day or night on or under makeup.

In practical terms, the compositions of lipid bodies and/or associated components
25 include compositions that can be used to decrease the recovery time associated with skin conditions or reduce the discomforts associated with scar tissue, such as tightness, by maintaining moisture in the healing tissue. When used as an admixture with other antioxidants, antibiotics, or analgesic (for pain), the compositions of the invention can be useful in patients who are either hospitalized or in-home care during recovery from
30 surgery or in compositions useful for soothing bed sores.

In related applications, the compositions of the invention can be the essential component of compositions used to reduce discoloration of scar tissue, discoloration of skin, alleviate parched skin associated with chemotherapy and radiation treatments, to

alleviate redness and scaling associated with psoriasis and extremely dry skin as is frequently found at the elbows or soles of the feet.

Because the compositions have a high hydrophobic carrying capacity, they can be effective vehicles for the delivery of carotenoids, vitamin E, retinol, tocotrienols, and other
5 tocopherols and medications to the skin of an individual. They can also retain antimicrobials, such as phenoxyethanol, sodium benzoate, benzalkonium chloride, and others. Similarly, compositions of the invention can be used for antibiotics such as griseofulvin, penicillin, daptomycin, bacitracin, neosporin, streptomycin, vancomycin, and other antibiotics.

10 The compositions may be applied to an individual's skin within a region in which skin moisturization, softening or soothing is desired. The compositions of the invention may be applied to the skin on the face, including the lips, or a region of inflammation, such as an area around an insect bite. The composition may be applied on a needed basis or as part of an on-going mitigation of an irritation over an extended period of time, such
15 as once a day, multiple times per day, once a week, or other periodic application scheduled as appropriated for the treatment of a skin condition.

Similarly, lipid bodies compositions can be used to impart medicines to the skin, for example, the application of pain reliving agents, lidocaine, benzocaine, gabapentin (neurontin) or other pain control substances as well as localized application of other
20 penetrating drugs formulated with lipid bodies, such as colchicine.

For application of the compositions of the present invention, the compositions of the invention can be used for facial skin and the following regimen is preferred, which involves cleansing the face thoroughly with a mild cleanser, applying compositions of the in a moderate to thin layer, and working the compositions into the skin by applying light
25 pressure and a spreading motion. Makeup may be applied on top of the cream.

The lipid bodies can be used in parenteral treatments, parenteral nutrition, oral applications, inhalation applications, and applications involving other fields of utility including, drug delivery. systems, medicinal applications, pet care, animal health, soaps, detergents, surfactants, industrial lubrication, machinery lubrication, leather conditioners,
30 rubber conditioners, coatings, surface protection, imparting water resistance, and compositions involving the addition of active ingredients, emulsification agents, coloring agents, agents that impart odor, texture or alter the physical properties or melting point of the compositions and other acceptable compositions depending on the desired impact.

The elements and method steps described herein can be used in any combination whether explicitly described or not.

All combinations of method steps as used herein can be performed in any order, unless otherwise specified or clearly implied to the contrary by the context in which the
5 referenced combination is made.

As used herein, the singular forms “a,” “an,” and “the” include plural referents unless the content clearly dictates otherwise.

Numerical ranges as used herein are intended to include every number and subset of numbers contained within that range, whether specifically disclosed or not. Further,
10 these numerical ranges should be construed as providing support for a claim directed to any number or subset of numbers in that range. For example, a disclosure of from 1 to 10 should be construed as supporting a range of from 2 to 8, from 3 to 7, from 5 to 6, from 1 to 9, from 3.6 to 4.6, from 3.5 to 9.9, and so forth.

All patents, patent publications, and peer-reviewed publications (*i.e.*, “references”) cited herein are expressly incorporated by reference to the same extent as if each
15 individual reference were specifically and individually indicated as being incorporated by reference. In case of conflict between the present disclosure and the incorporated references, the present disclosure controls.

All protein identification (PID) numbers provided herein refer to proteins in the
20 database of the Joint Genome Institute (JGI) of the United States Department of Energy. See, *e.g.*, Jeffries 2013.

It is understood that the invention is not confined to the particular construction and arrangement of parts herein illustrated and described, but embraces such modified forms thereof as come within the scope of the claims. Additionally, engineered lipid bodies exist
25 under a wide range of conditions including as intracellularly, extracellularly, crudely isolated, cleaned, purified or processed to other products both as intact lipid bodies or disassembled lipid bodies, which contain lipids, phospholipids, organellar proteins and dehydrated or packed forms that may be useful.

30 EXAMPLES

EXAMPLE 1

Background and Overview

Lipogenic yeasts such as wild-type *L. starkeyi* strains can be used as platform strains for metabolic engineering to produce lipids and lipid bodies. Given that the genome

of *L. starkeyi* NRRL Y-11557 has been sequenced (Grigoriev et al. 2012 and DOE JGI 2011), that it can grow on glycerol as a sole carbon source, and that it has a lack of a mucoid morphology in the tested experimental conditions, this public domain strain was selected as the exemplary platform yeast for metabolic engineering and for the development of both engineered yeast and associated engineered lipid bodies. Other wild-type *L. starkeyi* strains listed here and elsewhere, or other lipogenic yeasts, could also be engineered towards this purpose. The existence of an annotated genome for *Lipomyces starkeyi* greatly facilitates cloning and homologous expression. All wild-type strains evaluated have smaller quantities of lipids and small lipid body organelles that have not previously been isolated, purified and recognized as commercially valuable.

Fluorescent dyes such as Nile red or BODIPY (boron-dipyrromethene) can be used in rapid assays for cells or strains of cells showing greater relative lipid body size or accumulation. A series of washes to remove the media from the cells, followed by suspension of the cells in H₂O, can eliminate interference due to media components and reduce background fluorescence.

When examining cultures for increased lipid body size or accumulation it is important to distinguish higher levels of lipid due to higher cell density from higher levels of lipid due to higher lipid content per cell. The fluorescence response is therefore normalized to the cell density and confirmation of lipid body size is conducted microscopically.

Gene Cassette Selections and Primer Design

Gene cassette integration vectors were designed using a base vector as shown in FIG. 2 and having a sequence identical or similar to the exemplary base vector described in US 2018/0245109. Restriction digest or PCR amplification of a circular vector yielded 2-4 fragments, depending on the construct. The integrating fragment contained native *L. starkeyi* promoters and terminators driving expression of a resistance and lipogenic gene. LoxP sequences flanked the resistance marker cassette to enable CRE mediated excision. The origin of replication and kanamycin resistance gene permitted propagation and maintenance in *E. coli*, but were not maintained during the selection of transformants. Digest sites in FIG. 2 are marked with a scissors icon.

Measures were taken to ensure no antibiotic resistance gene was incorporated in the final yeast strain. The bacterial kanamycin resistance gene was not integrated into the yeast genome if it was interrupted by restriction digestion prior to transformation. The

presence of the loxP sites enabled excision of the resistance gene(s) by transient CRE recombinase expression. The LoxP sites themselves were modified (*i.e.*, dead) such that the product after one recombination event resisted further recombination, thereby protecting the genomic integrity of the organism. Genetic tools and selection markers
5 allowed for the removal of antibiotic selection markers, usually by excision or some other selective method. The gene overexpression cassettes therefore included a constitutive promoter, the gene(s) of interest, and one or more transcription terminator regions inserted into one of the multiple cloning sites (MCSs).

Genes were overexpressed to increase lipid body production through enhanced
10 lipogenesis under nutrient-rich conditions. These included the genes described in US 2018/0245109 A1, which include but are not limited to: acetyl-coenzyme-A carboxylase (LsACC1), delta-9 acyl-CoA desaturase (LsOLE1), ATP-citrate lyase alpha and beta subunits (LsACL1/LsACL2), two variants of glycerol kinase (LsGUT1-1602) and (LsGUT1-1617), two variants of diacylglycerol acyltransferase (LsDGA1-1233) and
15 (LsDGA1-1389) having different start sites, and malic enzyme cloned from genomic DNA (LsgME) and cDNA (LscME). Precise promoter, gene, and terminator sequences were selected based on Illumina RNAseq data and knowledge of promoter strength and expression patterns in this organism. All contained a single promoter, gene, and terminator region, with exception to cases in which a bidirectional promoter, natural or synthetic,
20 expresses two genes with different transcription terminator regions. Examples of this included constructs containing GUT1/GUT2 (GPD), ZWF1/GND1, LsAcl1/LsAcl2 and FAS2.2/FAS1.2. In some cases, these sequences were analyzed for the presence of specific restriction digest sites to determine into which one of two MCSs contained in the base vector they would be cloned. This step facilitated future subcloning into other
25 vectors. For example, the diacylglycerol acyltransferase (DGA1) construct was capable of being inserted upstream of the loxP site using the SbfI restriction enzyme to linearize the vector. Other constructs were capable of being inserted downstream of the opposing loxP site, using RsrII and AvrII to linearize the vector. In yet a third case, constructs were assembled solely from PCR fragments containing overlapping ends. The desired sequence
30 combinations and restriction enzyme sites were entered into the NEBuilder® assembly tool (nebuilder.neb.com/) with a minimum overlap setting of 20 base pairs to construct the primer sequences for performing the Gibson assembly reaction. Alternatively, this could be done manually without the use of special software.

gDNA Extraction and cDNA Library Creation

A Masterpure™ Yeast DNA Purification Kit (Epicentre, Madison, WI) was used to extract *L. starkeyi* genomic DNA (gDNA). Alternatively, DNA can be isolated using phenol/chloroform extraction followed by ethanol precipitation and resuspension in an aqueous solution. Nitrogen rich (YPD) or nitrogen limited (YPD 70:1 (C:N)) cultures were used for RNA extraction. The YPD 70:1 media contained only 3.64% and 1.82% percent of the yeast extract and peptone as YPD, respectively. A freshly saturated 5 mL culture of *L. starkeyi* grown under constant agitation at 30°C was pelleted by centrifugation, washed, suspended in 5 mL of sterile H₂O, then used to inoculate 50 mL of either YPD or YPD 70:1 media in a 125 mL shaker flask ≈ 0.8 OD₆₀₀ and allowed to incubate overnight at 30°C under 225 rpm. Cells were observed microscopically to determine lipid production in the YPD 70:1 media as compared to the YPD media, and the OD₆₀₀ was measured to calculate the quantity of cells to use in the RNA extraction protocol. RNA was extracted using an Rneasy Mini Kit (Qiagen), following enzymatic disruption. cDNA was synthesized from these RNA preparations using a QuantiTect® Reverse Transcription Kit (Qiagen), following the instructions of the manufacturer.

PCR of Fragments and Gibson Enzymatic Assembly

A detailed example of how some constructs were created using the Gibson *in vitro* enzymatic assembly method is provided. A representative base vector (FIG. 2) was linearized with the enzymes listed. Table 3 lists the gene targets, promoters, and terminators employed. All PCR amplifications were performed using Phusion High Fidelity Taq polymerase (NEB) and the manufacturer protocol in either 5X Phusion GC buffer (ACC1) or 5X Phusion buffer (all others). Annealing steps were carried out using the lowest T_m of the primer pair, or the experimentally optimized annealing temperature, where appropriate. The reaction products were analyzed by agarose gel electrophoresis containing ethidium bromide and subsequently visualized and photographed. Successful reactions of identical fragments were pooled and then all samples (including the digested base vector) were subjected to a PCR cleanup column (Qiagen), and then quantified on a Nanodrop 2000 instrument (Thermo Scientific) or a Qubit fluorimeter (Life Technologies). The vector and inserts were then added in equimolar quantities (0.1 pmoles/fragment) in a separate tube, and the final volume adjusted to 20 mL by the addition of 15 mL premade Gibson assembly reaction mix and water. All reactions were

allowed to incubate at 50°C for one hour, and then 5 mL of the assembly reaction was used to transform 20-30 mL of Endura™ DUO competent cells (Lucigen) using standard techniques. Transformants were selected on LB plates containing kanamycin, and positive candidates were identified by colony PCR and sent for sequencing confirmation.

- 5 All vectors were linearized so the entire target gene cassette, including the loxP flanked region, randomly integrates into the *L. starkeyi* genome as one fragment. Linearized DNA was ethanol precipitated and suspended in TE to increase its concentration (>160 Nm) and purity. To transform the cells, a procedure based on the lithium acetate method was used (Calvey et al. 2014, Gietz et al. 2002). A near stationary
- 10 phase culture of *L. starkeyi* was inoculated into 50 mL YPD to between 0.6 and 0.8 OD₆₀₀ and grown overnight to reach between 2.80 and 3.20 OD₆₀₀. The culture was harvested and washed 1-2 times with 25 mL sterile water, and resuspended to 1.5 mL in sterile water or 0.1M LiAc. Aliquots of 150 mL were dispensed into 1.5 mL tubes and centrifuged. The remaining cell pellet was then suspended in 360 mL transformation mix containing 240
- 15 mL 50% w/v PEG 3350, 50 mL boiled ssDNA, 36 mL 1.0 M LiAc, and 36 mL of the desired plasmid DNA (added last). Samples were incubated at 30°C for 3 hours, heat shocked at 40°C for 5 minutes, and then centrifuged to remove the transformation suspension. Cells were allowed to recover in 3 mL YPD for 4 hours before being plated onto selective media. After 6 days of growth, transformants were selected, catalogued by
- 20 size, and streaked onto YPD plates containing the appropriate antibiotic for creating glycerol stocks until characterization.

Table 3. Summary of gene targets, promoters, and terminators.

Target Gene	Full Name	Promoter/Terminator Pairing
ACC1	Acetyl-Coenzyme-A-carboxylase	Fructose 1,6-bisphosphate aldolase (promoter and terminator)
ACC1(S639A)	Acetyl-Coenzyme-A-carboxylase (S639A)	Fructose 1,6-bisphosphate aldolase (promoter and terminator)
ACC1(S1146A)	Acetyl-Coenzyme-A-carboxylase (S1146A)	Fructose 1,6-bisphosphate aldolase (promoter and terminator)
ACC1(S639A, S1146A)	Acetyl-Coenzyme-A-carboxylase (S639A, S1146A)	Fructose 1,6-bisphosphate aldolase (promoter and terminator)
ACL1/ACL2	ATP-citrate lyase, alpha and beta subunits	Enolase and promoter, Triose Phosphate Isomerase and ATPase

		(3900) terminator
DGA1(1233)	Diacylglycerol acyltransferase 1	Translation elongation factor EF-2 alpha promoter, Glyceraldehyde-3- phosphate dehydrogenase terminator
DGA1(1389)	Diacylglycerol acyltransferase 1	Translation elongation factor EF-2 alpha promoter, Glyceraldehyde-3- phosphate dehydrogenase terminator
DGA2	Diacylglycerol acyltransferase 2	Triose phosphate isomerase promoter, 3-phosphoglycerate kinase terminator
FAS1.2/2.2	Fatty Acid Synthase Complex Subunits 1.2 and 2.2	Various promoters, Triose phosphate isomerase and ATPase 3900 terminator
GDP1	NAD-dependent Glycerol-3- phosphate dehydrogenase	Pyruvate Kinase promoter and terminator
GUT1(1602)	Glycerol Kinase	ATPase 3900 promoter and terminator
GUT1(1617)	Glycerol Kinase	ATPase 3900 promoter and terminator
GUT2	FAD-dependent Glycerol-3- phosphate dehydrogenase	Pyruvate Kinase promoter and Triose phosphate isomerase terminator
cME	Malic Enzyme (cDNA)	Triose phosphate isomerase promoter and terminator
OLE1	Delta-9 acyl Coenzyme A desaturase	Glutamine synthetase promoter and terminator
SCT1	Glycerol 3- phosphate/dihydroxyacetone phosphate sn-1 acyltransferase	Citrate synthase promoter and terminator
SLC1	1-acyl-sn-glycerol-3-phosphate acyltransferase	Fructose 1,6-bisphosphate aldolase (promoter and terminator)
FOX2	3-hydroxyacyl-Coenzyme A	N/A, gene knocked out

	dehydrogenase, enoyl-CoA hydratase	
POX1	Fatty-acyl Coenzyme A oxidase	N/A, gene knocked out

Yeast Lipid Screening using Nile Red

Both YPD and Mts were used to screen for lipid accumulation in *L. starkeyi*. Starter cultures were inoculated with the desired transformants in 5 mL tubes containing YPD-NAT or YPD liquid media at 30°C under constant agitation. This lipid body screening protocol was based on Nile Red fluorescence adapted from a previous study (Sitepu et al. 2012). Samples and appropriate dilutions were then prepared in a 96-well black clear-bottomed plate to contain 100 mL in each well. Nile Red was prepared as a 2 mg/mL stock solution and then diluted to a 2X working concentration (8 µg/mL). Fluorescent signals and OD₆₃₀ readings were read independently from two plate readers (BioTek™ FLx800 and BioWhittaker™ Elx808, respectively). Data was analyzed by normalizing the fluorescent signal to the OD₆₃₀ of the culture. To enable comparisons between different runs, normalized fluorescence was standardized relative to the wild-type culture of each group.

Yeast Lipid Screening using BODIPY

Starter cultures were inoculated with the desired transformants in 5 mL tubes containing YPD liquid media and grown for 3 days at 30°C under constant agitation. After outgrowth, 200µL of each culture was added to a tube containing 5 mL of synthetic Thin Stillage (Sts) at a concentration of 0.6µg/mL of fresh BODIPY (boron-dipyrromethene) dye and monitored by OD₆₃₀ and fluorescence for 72 hours. Data was analyzed by normalizing the fluorescent signal to the OD₆₃₀ of the culture, or by simply summing the raw fluorescence values of each culture throughout the course of the experiment.

Lipid Extraction Analysis

An extraction protocol was used for crude gravimetric assessments of total lipid content based on the classic Bligh and Dyer method (Bligh et al. 1959) (20). First, 2 mL of cell culture was centrifuged at 3,000 rpm for 5 minutes in 15 mL falcon tubes, and the cell pellets frozen at -20 °C until lipid extraction analysis. Thawed cell pellets were suspended in 1 mL H₂O containing 200 mL of concentrated HCl, and the suspension was heated to 90 °C for 1 hour to lyse cells. Lipid was then extracted by addition of 6 mL of a 2:1 (v:v)

methanol:chloroform solution and 3 mL of 1M NaCl, followed by vortexing for 5 minutes. Tubes were then centrifuged at 3,000 rpm for 10 minutes to induce phase separation. The lipid-containing lower chloroform layer (≈ 2 mL) was then carefully removed using a glass Pasteur pipette, and transferred into a clean, pre-weighed, 5 mL glass vial. Finally, the extracted chloroform layer was completely evaporated by incubation in a 40 °C heat block under a constant stream of air for 1 hour. Vials were then re-weighed to determine the mass of extracted total lipids.

10 **Engineering 403 as an NHEJ-Deficient (*lig4Δ*) Strain for Further Metabolic Engineering**

The 403 strain was further engineered to facilitate homologous recombination by disrupting the non-homologous end joining (NHEJ) pathway, specifically by deleting the *LIG4* locus as described in US 2018/0245109. This greatly facilitates the knockout of other genes that are predicted to increase lipid yields, interfere with lipid catabolism, or even encode genes involved in potentially allergenic antigens. Examples of such genes include the acyl-CoA oxidase *POXI*, the multifunctional enzyme *FOX1*, UDP-glucose 6 dehydrogenase (UDPGH), Uridine 5'-diphospho-glucuronosyltransferase (UGT), and glycogen synthase, among others.

20 **Consolidation of Transformed Traits**

Not all genes evaluated on an individual basis in US 2018/0245109 A1 increased lipid production. However, various combinations of genes may enhance lipid production or growth synergistically, such as where bottlenecks might be relieved by gene overexpression. Various combinations of genes were therefore tested to determine which combinations resulted in improved lipid production, accumulation, and lipid body size.

Combinations of gene cassettes from top first-generation lipid producing strains (*i.e.*, strains with a single gene modification) were tested for enhanced lipid accumulation through yeast mating. Progeny were selected by spore isolation or selection on permissive media, granted the parental strains harbored resistances to different antibiotics. Strains derived by mating first-generation strains have two cassettes integrated in the genome, and were considered second-generation strains. Strains that were transformed twice with different cassettes were also considered second-generation strains. The limited number of resistance markers to use in selecting strains required resistance marker removal (curing) of strains to enable further metabolic engineering with additional gene cassettes.

Therefore, select second-generation strains were cured and transformed with additional cassettes to create third-generation strains. Third-generation strains were mated to create fourth-generation strains. Fourth-generation strains with prodigious lipid producing properties and capable of generating large intracellular lipid bodies were cured. FIG. 3 provides a diagram describing the creation of a generic fourth-generation strain from the starting wild-type strain. Creation of fifth- and sixth-generation strains followed the same strategy (i.e. transforming a “cured” fourth generation strain with cassettes containing resistance to different antibiotics to create fifth-generation strains, and then mating the fifth-generation strains together to create sixth-generation strains). The progressive improvement in lipid production as deemed by BODIPY fluorescence from select second- and fourth-generation strains are illustrated in FIG. 4.

Higher lipid production does not necessarily indicate larger intracellular lipid bodies, as an engineered strain could simply produce more, smaller lipid bodies per cell. Moreover, growth rates of highly lipogenic strains are typically impeded, presumably because lipid bodies restrict the available cytosol and other structures needed for robust protein synthesis. FIG. 5 demonstrates the difference in lipid body morphologies of various strains evaluated, along with isolated lipid bodies. Strain 403 produces lipid bodies that are the desired size and do not significantly decrease cell growth (approximately one large lipid body per cell).

20

Creation of the 202 and 403 Strains

Strain 202 was genetically modified from wild-type *L. starkeyi* strain NRRLY11557. It overexpressed two endogenous genes related to lipid production driven by native constitutive promoters. Specifically, this strain overexpressed two native genes encoding proteins with acyl transferase activity. These included diacylglycerol transferase 1 (*DGA1*, Protein ID # 6201) driven by the native transcription elongation factor EF-1 alpha promoter (*TEF1*, Protein ID # 63951) and the native glyceraldehyde-3-phosphate terminator (*TDH3*, Protein ID # 68472), and diacylglycerol transferase 2 (*DGA2*, Protein ID # 6231) driven by the native triose phosphate isomerase promoter (*TPI*, Protein ID # 196787) and the native phosphoglycerate kinase terminator (*PGK*, Protein ID # 75322). Vector backbones were obtained by amplification in *Escherichia coli* followed by restriction digests. Amplification of the DNA sequences of interest were created by polymerase chain reaction (PCR) from either cDNA (*DGA1*) or genomic DNA (*DGA2*), and PCR fragments and digested vectors were combined using Gibson isothermal

assembly. Cassettes were amplified in *Escherichia coli*, linearized by restriction digest, and transformed into *Lipomyces starkeyi* by random genomic integration. Transformations of each cassette were carried out independently in the wild-type strain, and screening revealed top performers of each. These were mated together, and the progeny were
 5 selected on plates containing the relevant antibiotics. Resistance genes to these antibiotics were removed using a CRE loxP based strategy, which results in a 34-base pair loxP scar sequence in place of the previously loxP flanked resistance marker cassette. This loxP sequence was not able to undergo further recombination, even in the presence of CRE. Nonetheless, the *CRE* recombinase gene was also removed during the excision process.
 10 Marker and *CRE* removal was confirmed by loss of resistance to antibiotics used in the original selection and PCR genotyping.

The 202 strain was further genetically modified to create the 403 strain. This strain overexpressed the same genes with the same promoters and terminators as described for the 202 strain in addition to native glycerol-3-phosphate o-acyltransferase 1 (*SCT1*,
 15 Protein ID# 235628) driven by the native *CIT1* (Protein ID# 47067) promoter and terminator and native malic enzyme (Protein ID# 72728) driven by the native triose phosphate isomerase (*TPI*, Protein ID # 196787) promoter and terminator. Construction of vector backbones used the same techniques as to make 202 with *SCT1* cloned from genomic DNA and malic enzyme cloned from cDNA. Transformations of each cassette
 20 were carried out independently in the 202 strain, and screening revealed top performers of each. These were mated and the progeny selected on plates containing the relevant antibiotics. Resistance genes were removed in the same manner as described above and confirmed by loss of resistance to antibiotics used in the original selection and PCR genotyping.

25

Strain 202 (DGA1+DGA2) Grows Faster Than Strain 403, But Strain 403 Accumulates More Lipid

Strains 202 (*DGA1*, *DGA2*) and 403 (*DGA1*, *DGA2*, *ME*, and *SCT1*) were inoculated successively to 5, 25 and 100 mL volumes of yeast extract (10 g/l) and peptone
 30 (20 g/l) plus glucose (40 g/l) and xylose (15 g/l) and cultivated at 28°C in 13-mL tubes, 125-mL baffled Erlenmeyer flasks, and 500-mL baffled Erlenmeyer flasks, respectively, while agitated at 250 rpm with periodic subculture. The densities of the 100-mL cultures were normalized and used as inocula for the bioreactors. Two bioreactors were set up with an initial target volume of 1.0 L with yeast extract (22 g) peptone (44 g) glucose (YPG)

medium at an initial concentration of 112.5 g glucose in a final volume of 1 liter. The yeast extract (Fisher) contained 11% w/w N and the peptone (Fisher) contained 15% w/w N. Initial total N in the bioreactor was calculated as 9 g; total carbon (glucose only) as 45 g. Initial C:N ratio was therefore $\approx 3.7:1$. The experiment was designed to pump into each
 5 bioreactor a glucose feed of 225 g glucose in 500 mL of H₂O after 90% of the initial glucose had been consumed. Initial bioreactor operating conditions were pH 5.2 to 5.3, Temp 28°C, DO, 100% saturation, agitation at 600 rpm with 1 v/v•min air sparged. Glucose was pumped into the reactors at 68, 94 and 99 h. At T4 (65 h) and T18 (142 h) samples were withdrawn for dry weight and lipid analysis.

10 As illustrated in FIG. 6A, the growth rates of the two yeast strains differed by ≈ 1.7 fold with the 202 and 403 strains attaining a final Ods of 182 and 403, respectively. The optical densities did not correlate directly with dry weight determinations or the lipid contents of the cells (FIG. 6B). At T4, the ratio of OD's for DGA1+2 to Xyl-403 was 1.7:1 while the ratios of their dry weights were 1.14:1. More interestingly, the ratios of
 15 their lipid contents were 0.83:1 at T4 and 0.7:1 at T18. The lipid content of the 403 strain was 55% greater than the 202 strain at the end of the experiment.

Fed-Batch Cultivation of the 403 Strain Enables Higher Growth and Lipid Accumulation

20 The 403 strain (*DGA1*, *DGA2*, *ME*, and *SCT1*) was cultivated in a 13-liter bubble bottle with an initial medium volume of 1.8 liters and an inoculum volume of 200 mL. The medium contained 333 g/l of cerelose (glucose monohydrate) plus 16.1 g/l Lallemand FNI yeast extract. One-third of the cerelose was present at the time of inoculation and two thirds were fed into the reactor after 72 h. This gave a final C:N ratio of 75:1. Cell growth
 25 was tracked by measuring packed wet weight, dry weight, and lipid content of cells. At the end of the fermentation, 2980 mL of medium contained 889 g of packed wet cells (≈ 300 g/L) with a dry weight of 109 g/l and a lipid yield of 68 g/l. The percent lipid concentration in the cells attained as measured by percent dry weight (w/w) was 62%.

30 Recovery of Lipid Bodies from Whole Broth by Acid Hydrolysis and Flotation

The 403 and 202 strains were cultivated as described above. After cultivation was terminated, the whole broth was transferred to 2-liter glass bottles. Concentrated sulfuric acid was added with mixing to a concentration of 0.5 N, and the acidified broths were autoclaved for 45 min at 212°C. In side experiments, phosphoric acid at 0.5 N was used in

place of sulfuric acid. After cooling, two phases emerged. An upper tan layer contained crude lipid bodies, and a lower layer contained cell hydrolysate.

The lipid bodies in the upper layer were examined microscopically. Lipid bodies from the 202 strain were approximately half the diameter of those isolated from the 403 strain.

The pH was adjusted to 7.25 by the addition of 0.5 N NaOH in 32 g/l saline and the lipid bodies were separated from the hydrolysate by centrifugation at 4,000 rpm for 25 min. The dark brown hydrolysate was drawn off from the bottom of the centrifuge bottles. The lipid bodies were suspended in 800 mL of 32 g/l saline and centrifuged a second time at 4,000 rpm for 25 min. The 32 g/l saline provided osmotic support and additional buoyancy that enabled fractionation of larger lipid bodies from the 202 strain from smaller lipid bodies or non-lipogenic cells. No such separation was observed in the 403 cells. We infer from this observation that the higher lipid content of the 403 lipid bodies enables easier separation by flotation. In subsequent trials, washings were carried out with water or physiological saline rather than saline at 32 g/l.

Color and Odor Removal from Lipid Bodies using Alkaline Wash

Saline or water washes alone did not fully remove a tan color attributed to the hydrolysate in the procedures outlined above. The 403 lipid bodies recovered from the hydrolysate by washing were therefore suspended in 3 to 10 times their volume water or saline, and the pH was raised to 11.2-11.7 by the addition of NaOH (1.0 to 5.0 N). Dilute suspensions (e.g. 10-fold) resulted in more rapid separation of lipid bodies by flotation than in more concentrated suspensions (e.g. 2- to 3- fold), and the initial rise rate from the dilute suspension was significantly faster than the later rate as the lipid bodies became more concentrated at the surface. The alkaline-washed lipid bodies appeared bright and white and did not have any odd odors.

To compare various alkaline washes, 1137 mL of lipid bodies from the 403 strain in 1900 mL H₂O were divided into 3 aliquots of 379 mL each, transferred to 2-liter bottles; made up to 1800 mL with deionized water; adjusted to pH 11.7 with NaOH (5 N), 200 mL of Na₂CO₃ (1 M), or Na₂CO₃•1.5 H₂O₂ (0.5 N); and allowed to stand for 18 h. Table 4 outlines the various alkaline wash conditions.

Table 4. Comparison of alkaline wash conditions.

	Conc (M)	vol (mL)	Ph
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5 N NaOH	5	23.5	11.7
Na ₂ CO ₃	1	200	9.72
Na ₂ CO ₃ •1.5 H ₂ O ₂	0.5	200	9.88

The oxygen released by sodium percarbonate was excessive. It resulted in bright, clean lipid bodies and a decolorized solution, but it also disrupted the lipid bodies and left a significant fraction of them as an opalescent suspension. The wash with NaOH at pH 11.7 removed the most color. The lipid bodies resulting from the NaOH wash did not form as compact a layer as observed with the sodium carbonate wash. Also, microscopically, the lipid bodies with the sodium carbonate wash appeared to be larger and more spherical than the lipid bodies washed with either sodium hydroxide at pH 11.7 or with sodium percarbonate at pH 9.88.

In a follow-up experiment, lipid bodies were suspended in an aqueous solution of 1.5% Na₂CO₃ and 0.15% Na₂CO₃•1.5 H₂O₂. This yielded bright white lipid bodies with almost no odor and a more compact suspension. In another trial, lipid bodies in a greater than 5:1 lipid body:water suspension were separated from the hydrolysate by washing in 2 to 3 cycles of water, were then suspended in 1.5% Na₂CO₃, then adjusted to pH 11.7 briefly at room temperature as they rose to the surface of the container. Following removal of the alkaline wash, the lipid bodies were twice suspended in 4 to 9 volumes of water or physiological saline and the pH was adjusted to 11.2. Following the final wash, the lipid bodies were suspended in two volumes of physiological saline (8.8 g/l) plus sodium citrate, pH 4.5 and 0.5% w/v to 10% w/v glycerol. Higher concentrations of glycerol resulted in a more compact lipid body layer. The resulting lipid body suspension contained between 10% w/w and 60% w/w solids.

Properties of Isolated Lipid Bodies

The physical properties of lipid bodies isolated from the 403 strain and other strains (such as the wild type) were determined by a number of methods.

Packed cell volume: A suspension of lipid bodies were added to a graduated centrifuge tube and centrifuged in a swinging bucket rotor for 15 minutes at 3,000 to 5,000 rpm. The volume of the packed lipid bodies was measured.

Lipid body density (wet): The packed cell volume procedure was carried out in a tared, graduated centrifuge tube, and the tube plus the packed lipid bodies was weighed.

The weight of the tube was subtracted, and the resulting weight was divided by the volume.

Percent Solids: A volume of a lipid body composition was placed in a tared aluminum tray. The weight of the tray plus the lipid body composition was determined.

- 5 The aluminum tray was placed in a drying oven at 50°C for 3 to 12 h to allow evaporation of the liquid from the composition and weighed again. (Any method that permits evaporation of liquid can be used.) A ratio of the dry weight over the wet weight multiplied by 100 yields the percent solids (w/w) of the original lipid body composition. This is usually repeated in triplicate.

- 10 Grams Lipid Per Volume of Lipid Body Composition: An extraction protocol based on the classic Bligh and Dyer method (Bligh et al. 1959) was employed. 2 mL of lipid body composition was frozen at -20 °C for 30 minutes or held until lipid extraction. Thawed lipid bodies were supplemented with 0.5 mL H₂O and 300 μ L of concentrated HCl, and the suspension was heated to boiling for 1 hour to rupture the cell walls and
 - 15 membranes of the lipid bodies. Lipids were extracted by addition of 6 mL of a 2:1 (v:v) methanol:chloroform solution and 3 mL of 1M NaCl, followed by vortexing for 1 minute. Tubes were then centrifuged at 3,000 rpm for 15 minutes to induce phase separation. The lipid-containing lower chloroform layer was then carefully removed using a glass Pasteur pipette, and transferred into a tared aluminum weighing dish. This was greatly facilitated
 - 20 by first removing most of the upper phase. Finally, the extracted chloroform layer was completely evaporated by incubation in a hood under a constant stream of air or in a 50 °C incubator. The aluminum weighing dish was then re-weighed to determine the mass of the total extracted lipids per unit volume of lipid bodies from which lipid was extracted, and reported as grams of lipid per liter of lipid body composition (g/L).

- 25 Lipid body diameter and volume: Lipid bodies were suspended in water and placed under a weighted cover slip of a Petroff-Hauser counting chamber (Hausser Scientific, Horsham, PA). A photomicrograph of the lipid body suspension was taken. The dimension of the counting cells was 200 μ m on a side. The photomicrograph was calibrated using the dimensions of the counting chamber. Photomicrographs at different magnifications were
 - 30 calibrated by the relative apparent diameter of the oculus ring (ca. 50 μ m). The diameters of the lipid bodies in the photomicrograph were measured.

The diameters of lipid bodies from wild-type *L. starkeyi* and the 403 strain were determined using the methods outlined above. As shown in FIG. 7, the average diameter of the 403 lipid bodies was much larger (6.9 μ m $\pm$ 1.4 μ m) than the average diameter of the

wild-type lipid bodies ($3.417 \mu\text{m} \pm 0.708 \mu\text{m}$). The size distribution of lipid bodies from each source is shown in FIG. 8.

The volumes of the lipid bodies was calculated from the diameters ($4/3\pi r^3$). Because the diameter of the lipid body affects the volume as a function of the cube of the radius, the average volume of the 403 lipid bodies ($174.0 \mu\text{m}^3$) greatly exceeded the average volume of the wild-type lipid bodies ($20.9 \mu\text{m}^3$). As shown in FIG. 9, the cumulative volume of the 403 lipid bodies also greatly exceeded that of the wild-type cells.

Chloroform/methanol extractions of lipid from lipid bodies that have not been concentrated showed that the lipid body preparations have about 150 g/L lipid. Lipid body preparations having 58% w/w solids (typically condensed from the original lipid body preparations by half) have about 300 g/L lipid.

Protein concentration analyses on lipid body samples yielded between 0.588-1.58 g/L of protein. At a percent solids of about 58% w/w, the lipid body preparations have a protein concentration of about 1.18-3.16 g/L.

From the above data, the lipid body preparations have a lipid:protein ratio at a percent solids of about 58% w/w from about 250:1 to about 95:1.

The relative proportions of fatty acids in the 403 lipid bodies was determined and compared with those in the oils derived from palm oil, wild-type cells, and the 403 strain itself. See FIG. 10. The 403 oil and the lipid body oil derived from the 403 strain differed somewhat due to changes or losses in various fractions during purification of the lipid bodies from the whole cells. Both the characteristics of the 403 strain and the processing of lipid bodies makes the lipid profile different than the wild type, being observed as an increase in saturated fatty acids, and a decrease in most desaturated fatty acids. For example, in one experiment, the lipid profile from 403 lipid bodies was 42% palmitic acid (C16:0), 39% oleic acid (C18:1), 7% stearic acid (C18:0), 5% linoleic acid (C18:2), 4% palmitoleic acid (C16:1), and 3% other fatty acids. By contrast, the wild-type lipid profile (from whole cells) was 33% palmitic acid (C16:0), 42% oleic acid (C18:1), 3% stearic acid (C18:0), 3% linoleic acid (C18:2), 8% palmitoleic acid (C16:1), and 11% other fatty acids by weight.

UV-Vis scans (190 nm – 1100 nm) of 7 different lipid body preparations from the 403 strain were performed. The UV-Vis scans were essentially identical. Two peaks were present; a mild hump around 980 nm and a sharp peak (likely due to the protein present) around 230 nm. A representative scan is shown in FIG. 11.

Method for the Measurement of Residual Color in Isolated Lipid Bodies

Rationale: Lipid body preparations are made white to off white by a combination of aqueous washes at acidic and alkaline pH as described herein. Alkaline washes are particularly effective at removing residual fermentation components, proteins, and colored moieties to generate a white or colorless material.

Method: A quantitative measure of the amount of colored material in lipid bodies can be determined by extracting colored material from water-washed lipid bodies with 0.1 molar potassium hydroxide solution. The amount of extractable colored material is measured by absorbance at 400 nm in a 1.0 cm cuvette and normalized to the wet weight of packed, water-washed lipid bodies (nominally 1.0 gram).

1. Tare a 1.5 mL microfuge tube and add 1.0 to 1.4 mL of a suspension of water-washed lipid bodies. Larger tubes and larger volumes may be applied.
2. Centrifuge tubes at 13,000 to 1600 rpm for 3 to 4 min and pull off the supernatant water with a 1.0 mL syringe and needle (note: lipid bodies will form a layer on the surface); centrifuge a second time for 0.5 to 2 min and pull off the residual water on the bottom; weigh the tube plus wet pellet and subtract the tare weight to obtain the packed wet weight of lipid bodies.
3. Add 1.0 mL of 0.1 M KOH, mix well; centrifuge and remove the aqueous bottom layer. Filter through a 13 mm diameter, 0.22 μ m pore size syringe filter into a fresh tube.
4. Measure the optical density of the filtered solution in a 1.0 cm path length cuvette
5. Divide the optical density by the weight of the wet lipid body pellet to obtain the optical density “units” (OD units) per g lipid body wet weight.
- 6.

Example:

Lipid bodies from 403-strain washed once with water

Tube	1	2	3	4	OD/g wet
Tare	0.9977	0.985	0.9896	0.9821	
tare+ wet LB	2.2608	2.2902	2.2884	2.2526	
Tare+ pellet	1.3494	1.3316	1.3258	1.3667	
Net pellet	0.3517	0.3466	0.3362	0.3846	
OD @400 nm	1.108	1.075	1.07	1.173	Ave OD $\pm$ SD
OD/g wet	3.150	3.102	3.183	3.050	3.12 0.06

Lipid bodies from 403-strain washed with water and Na₂CO₃

Tube	1	2	3	4	OD/g wet	
Tare	0.9884	0.9882	0.9818	0.9886		
tare+ wet LB	1.5195	1.4835	1.3379	1.5053		
Net wet	0.5311	0.4953	0.3561	0.5167		
Net pellet	0.3666	0.3419	0.2458	0.3567		
OD @400 nm	0.324	0.324	0.324	0.324	Ave OD	± SD
	0.88	0.95	1.32	0.91	1.01	0.20

The extracted color was reduced from 3.12 OD units per g wet wt of lipid bodies to 1.01 OD units per g wet wt of lipid bodies.

Comments:

- 5 Extraction with 0.1 M NaOH to 1.0 NaOH can release similar amounts of color. In these experiments, the ratio of NaOH solution to lipid bodies was approximately 3:1. The ratio of solution : lipid bodies can range from 3:1 to 20:1. The lipid bodies have a nominal concentration of from 15% to 55% by dry weight.

10 The extraction is centrifuged and the color in the aqueous layer is measured spectrophotometrically at 400 nm. Filtration prior to OD reading is performed to avoid contamination of the sample with suspended lipid bodies. If filtration is applied forcefully, the lipid bodies may fragment and yield a slightly turbid suspension. A dilution of lipid bodies in 0.1M KOH range that provides an absorbance reading in the range of 0.05 to 0.8 can provide the most accurate measurement.

15 The total absorbance at 400 nm is determined accounting and adjusting for dilutions and lipid body weights to yield a maximum residual color released of 3.2 OD absorbance units per gram using packed lipid bodies at 15% to 55% (by dry weight) lipid bodies. The extraction mixture yields a brown to clear solution, which when centrifuged allows the aqueous layer to be measured spectrophotometrically at 400nm.

20 **Results:** An absorbance of 3.2 OD 400 nm per g lipid body wet weight was obtained for crude, water-washed, off-white lipid bodies. There is no minimum color specification or maximum whiteness of lipid body preparations of the invention. Very clean lipid bodies may not yield a measurable color in this quality control test.

25 Released residual color from 1 gram of packed crude lipid bodies was measured spectrophotometrically in a solution measured spectrophotometrically at 400 nm to

quantitate the worse-case residual color remaining in the lipid bodies. The absorbance was about 3.2 Optical Density units per gram wet weight of lipid bodies.

Properties and Uses of Lipid Body Preparations at Various Concentrations

5 Engineered lipid bodies produced and isolated using the methods described herein form stable emulsions or suspensions when suspended in water at concentrations greater than 25% w/w solids. In approximately 75% w/w suspensions of water and, optionally, other ingredients such as excipients and stabilizers, they form a thin suspension that can be useful as a hair conditioner. When suspended at concentrations greater than 33% w/w
10 solids, along with water and, optionally, other ingredients such as excipients and stabilizers, they form a cream suitable for application to hands. The cream is not greasy and does not leave an oily feeling upon drying – rather it leaves the hands smooth and soft. The lipid bodies form a thin flat layer on the surface of the skin which can constitute a protective coating. At concentrations from approximately 42% w/w to 66% w/w solids,
15 which can include other ingredients such as excipients and stabilizers, the lipid bodies form a cream that can be applied to the face to protect the skin and produce a barrier to preserve moisture. At the higher solids levels, the lipid bodies can be applied in a thick layer to abraded skin, lips, chapped, abraded, cut or fissured skin surfaces to form a protective coating that can help healing when combined with bandages or that can protect
20 against abrasion by medical devices. Application of lipid body cream can reduce itching and irritation.

The engineered lipid bodies are freely miscible with aqueous solutions and can be combined in appropriate ratios with various other agents or ingredients. The agents or ingredients can include excipients and stabilizers. Examples of suitable agents or
25 ingredients include glycerol, hyaluronic acid, vitamin E acetate, sodium benzoate, gluconolactone, retinol, benzoyl peroxide, hydrocortisone, clotrimazole, ketonazole, ecamsule, avobenzone, oxybenzone, titanium dioxide, sulisobenzene, zinc oxide, aloe vera, and others.

30 Exemplary Cream Preparation

An exemplary cream made from the 403 lipid bodies is made with the materials shown in Table 5. The cream is in the form of a lipid paste. The paste has a pH of 4.5. and is stabilized with an antioxidant agent and an anti-microbial agent in glycerol (10%) in

physiological saline. The final cream concentrate is adjusted to 40-60% w/w solids with 10% w/v glycerol and composition stabilizers by evaporation.

Table 5. Materials.

Description	Vendor	Units	Use
Commercial-grade or food-grade yeast extract	Yeast supplier (e.g. Lallemand FNI 100ag, agglomerated, dry, baker's yeast-sourced)	g/L (dry wt equivalence)	40 g/L x fermentation working volume
Cerelose, glucose-hydrate, GMO or non-GMO,	Tate and Lyle or CPC	g/L dry wt	Fed-batch starting at 2.5 g/l/hr increasing until a maximum OD 600nm. The second phase is a high cerelose feed that triggers lipogenesis
Sea Salt (pure food grade or better)	Kirkland, Fisher or equivalent	0.15 M salt solutions	Used in washes at alkaline and acidic Ph
Vitamin E-acetate	Fisher or equivalent, (USP or food grade)	1.5-2% (w/v) in the final product	0.45-0.9 grams per liter of fermentation depending on yield
Citric Acid	Fisher or equivalent, (USP or food grade)	3%	0.15 M concentration in final washes and product
Sodium Hydroxide	Fisher or equivalent, (USP or food grade)	As 1-5 N solutions	Used as a 5 N solution
GeoGuard-Ultra or D-Gluconolactone SB	Lonza	2%	Concentration in final product
Soy Peptone	Domo or equiv.	20% in YPD	Shake flask stage, only
Antifoam	Antifoam 204 or equivalent, non-	Sigma	Usage of ~0.1 mL/liter

	silicone		
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Fermentation Process Summary

5 The 403 strain can be used to produce a lipid body paste that is formed in a two-stage aerobic fermentation. An inoculation level of 10% v/v is desirable but not essential and a synchronized culture is not required for triggering secondary metabolism for lipid synthesis.

10 The first stage of the fermentation is a primary growth phase, which is carried out through the flask, seed, and early production stages of yeast proliferation. The optimum cerelese feed rates start at 2.5 g/L/hr at the outset of the fermenter with the feed rate building during rapid cell division. The secondary lipogenic phase is triggered by additional carbon in the form of cerelese, which is used to further promote lipogenesis of the cells under reduced aeration. The secondary stage is characterized by a lower oxygen demand, slower growth rate and intracellular lipid formation as large lipid bodies that can be seen microscopically at 400x.

15 *Inoculum Preparation Procedure*

20 An inoculum composed of the 403 strain is prepared from a working cell bank (WCB). The culture is thawed from a frozen WCB vial or an isolated colony on a YPD plate that was generated from a single passage from the WCB. Growth is in modified-YPD medium [20 g/L soy peptone, 20 g/L yeast extract and 40 g/L dextrose] in a 100 x 500 mL baffled shake flask at 28C at 300 rpm or faster.

25 After 48-56 hours, the culture is examined for pure culture status microscopically and then split into two 100 x 500 mL baffled shake flasks, which are grown at 28°C, at 300 rpm or faster.

30 At 48-56 hours at 28°C, the flasks are measured for Optical Density (OD600 nm). An OD₆₀₀ in the vicinity of at least 40 is expected. Both flasks are checked microscopically and, if pure, then both flask cultures can be used in the next scale, which can be a 12-Liter production bubble bottle containing 6-liters of medium containing only cerelese and yeast extract for product preparation.

 If the 12-liter bottle or intermediate fermenter is still part of the seed train, then expansion with modified-YPD medium is preferred. Other options and larger volume seed fermenters are expected to grow well under any closely related conditions.

 Additional scale-up steps use modified-YPD medium. The objective is to achieve

at least a 10-20% v/v inoculum for the production fermenter. The best seed stage process will be equipment and facility dependent.

Product stage fermentation uses a simple medium containing only cerelose and yeast extract.

5

Fermentation Overview

No extraordinary interventions, feeds or control cascades are required. Antifoam (0.5 mL per liter) is preferred during the proliferation stage. Having antifoam control available during first scale-ups will be helpful to determine how much, if any, additional antifoam is required.

With a 10-20% v/v inoculum, proliferative growth under aerobic conditions (1 vvm) is characterized by a declining Ph, dropping to about pH 4.0. but no less than pH 3.0. A generation-time of 6-8 hrs during the primary proliferative stage lasts for up to 3 days and there is consumption of both initial carbon and nitrogen by the yeast. An increasing glucose feed rate based on cell density is desired. Specific feed parameters will be equipment and facility dependent. If the glucose (cerelose) becomes exhausted, then the DO will rise but in most cases this effect is not seen, and it is easily reversed by increasing the cerelose feed rate.

The basic production medium is a yeast extract 40 g/L (Lallemand FNI 100ag agglomerated) and a cerelose feed starting at 2.5 g/L/hr which increases in the second stage of the fermentation until consumption of a total dextrose 350 g/L (Cerelose or dextrose) is complete or until growth slows to less than 1 generation per day. During the fermentation, the pH will decline from 6.5 to 4.5 and can be adjusted with NaOH (not NH₄OH) as appropriate.

Generally, a suitable final OD 600nm of above 100 is reached before glucose exhaustion. With proper adjustment of the growth phase and lipogenic phase, maximum OD 600 readings in excess of 170 can be achieved. If glucose exhaustion is approaching and the OD is sufficient, then it is acceptable to move to the lipogenesis phase earlier than 3 days.

Conversion to the lipogenesis phase can be achieved in several ways. First, the cells can naturally transition into lipogenesis as the cell density increases and oxygen becomes limiting. Second, the cells can be induced to transition by limiting the amount of nitrogen available for growth while providing excess of a readily assimilated carbon source. For example, the excess carbon can be achieved either through the addition a bolus

or surfeit of glucose monohydrate (cerelose) up to 350 g/L under reduced aeration (0.25 vvm) or by the gradual addition of cerelose at a rate in excess of its consumption. Following rapid cerelose addition the pH will typically drop so the pH can be adjusted back to 6.5 with a NaOH (other options for adjusting the pH may be acceptable). Adding a
5 carbon source results in a carbon-to-nitrogen ratio ~20:1 or greater. The carbon source may be in excess at the end of the lipogenic fermentation process at 48 hours, and growth may be slower with expected generation times of about 24 hours per cell division during this phase. Alternately, the addition of carbon sources may be tapered off near the end of the fermentation. As nitrogen is depleted or restricted, cell budding will cease, and the
10 cells will become larger. At the end of the fermentation, it is most desirable for > 80% of the cells to be homogeneous in size and for their diameters to approximate 10 μm or greater. As cell division stops and cell enlargement continues, the OD 600 will drop while cellular lipid content increases.

During lipogenesis, respiration does not cease, but the capacities of cells to take up
15 oxygen can be limited by the oxygen concentration and residual concentrations of oxygen in the medium can range from less than 1% to greater than 35% of air saturation. Higher cell densities and growth rates can be achieved by increasing the oxygen tension either by pressurizing the bioreactor or by adding supplemental O_2 .

Contamination checks are done microscopically and verify that the fermenter
20 content is a pure culture. Microscopically, the production of large lipid bodies can be seen in each cell, reaching more than 70% by volume per cell, but in some cases encompassing up to 95% of the intracellular volume.

Hydrolysis Process Steps

25 Following fermentation cell hydrolysis may be carried out in a separate reactor or in the bioreactor itself. The buoyancy of the lipogenic cells enables partial separation of the cells from the bulk of the medium simply by allowing the culture to stand without agitation. The cells will rise to the top of the reactor and the spent medium can be drawn off either for disposal or recycle. Aside from reducing water and waste disposal and
30 savings in medium constituents, it also reduces the amount of acid required for hydrolysis.

While cells and medium are still in the fermenter at the final volume the oxygen supply is shut off and the agitator is left on. Hydrolysis can be conducted by adding sulfuric acid to 0.25 M or phosphoric acid 0.5 M, followed by heating to a temperature of 75 to 121°C while mixing for 2 hours. At the lower temperature, heating is continued for

up to 18 h and at the higher temperature, the heating can be as short as 1 h. Mixing may be intermittent or absent. Other methods of cell hydrolysis are described elsewhere herein. The hydrolyzed cells are then allowed to stand at 90°C for an additional 4 to 22 hr with the impeller and air off. During this latter part of the acid hydrolysis, the lipid bodies are
5 allowed to float to the surface.

After the cream rises, the bottom culture medium and cell wall hydrolysate are removed by siphoning or decanting, measured, and recycled or discarded. An estimated volume of the lipid body suspension is made, which is useful information needed as an in-process control and for establishing the approximate wash volumes that will be needed in
10 the next washing steps.

In the low pH wash steps lipid bodies float to the top of a 10-liter bottle in 2 to 4 hours. During the subsequent alkali wash, steps may take up to 6 hours and the rise rate is approximately 1/3 the rate observed at lower pHs.

Following hydrolysis, the lipid bodies are still tan, and the solution is still acidic.
15 The lipid bodies are washed with water in a 10X total dilution after being cooled to 20°C. The goal is to remove cell wall debris and the brown/tan color from the cream.

In the water washes, agitation can be used to break up the lipid bodies to suspend and wash the cream layer. The lipid bodies or cream will rise at initial rates of about 0.25 to 0.5 inches per minute followed by slower rise rates as the lipid bodies accumulate at the
20 top of the vessel. The greater the initial dilution, the faster the initial rise rate. During the water wash, the cream may be taken through rapid short cycles (i.e. after the lipid bodies rise to the top 33% of the volume) or they may be left overnight. Water washing totaling 10X volumes is performed with one or more dilutions with either one 10X exchange or multiple exchanges at lesser volumes. It is acceptable for the flotation to be facilitated by
25 moving the cream layer into a horizontal separation tank (e.g. whey/curd tank) that can speed-up the subsequent flotation steps.

Alkali Washes

The water-washed cream is then washed with alkali in a 10X or greater fold
30 dilution of the cream layer in water adjusted to pH 11.7 with a sodium hydroxide stock solution that is between 1-5 N. FIG. 12D shows the release of additional brown color with the first alkali wash and the whitening of the lipid bodies.

The alkali washes are repeated at pH 11-11.2 as needed until the absorbance at 400 nm is equal to background absorbance of a buffer blank and visual appearance of the lipid

bodies is no longer tan. Multiple washes may be required to remove all of the brown color.

Care should be taken during the alkali washes. Alkali at pH 11.7 above 20°C can hydrolyze the triglyceride fatty acid ester linkages and result in “soap”, which forms bubbles. Slow addition of the sodium hydroxide stock solution while mixing is preferred.

- 5 Wash solutions should be introduced with minimal of air entrainment to avoid bubble formation. Foaming or bubble formation may denature the lipid bodies on the bubble surface, which results in lower yield and lesser quality.

In all alkali wash steps, the sodium hydroxide stock solution is added slowly with agitation at a cool temperature of ~20°C. To reach a 10X wash dilution, more cycles at
10 lower dilution may be required (e.g. two 5-X dilution washes or three 4x dilution washes) to reach the 10X. Flotation of the cream in a 5-X dilution should take 2.5 to 3 hours per cycle at a height of 3 feet during the alkali wash step.

The bottom solution after each cycle is measured and discarded and the floating dilute cream is retained. Removal of the first pH 11.7 wash (minimum of 10X wash)
15 followed by a second 5X or greater dilution will reduce the pH to 11 – 11.2. A pH of 11-11.2 using a sodium hydroxide solution (1-5 N) is maintained as needed in subsequent washes to achieve a clear bottom without brown color

Adding the alkali too fast will cause loss of lipids due to saponification. This will contribute to a white hazy or white turbidity in the discarded bottom once the brown color
20 is gone. There is no corrective action for this phenomenon.

pH Adjustment to pH 4.5 and Buffer Exchange

The lipid bodies achieve a white to off-white color due to the alkali washes. After the alkali washes, the pH is adjusted to 4.5 ± 0.1 with 2 N citric acid and diluted to achieve
25 a 5X dilution. Mixing is performed to suspend the cream, then the cream is allowed to stand to achieve lipid body flotation. The bottom layer is siphoned off, measured, and discarded as in earlier steps. The approximate cream volume is determined.

Once a clear bottom is achieved, it is important to reduce the pH to 4.5 as soon as possible. Lipid bodies should not be left at pH >11 for more than 12 hours at 20°C.

30

Composition Buffer Exchange, Safety Screening and Bulk Product Final Formulation

Next, the washed cream is diluted 6X with a stock bulk product composition buffer at pH 4.5 that is made with 0.15 M sodium chloride (physiologic saline 8.8 g/L), containing 3% w/v sodium citrate, 0.25% w/v sodium benzoate, and 10% w/v glycerol.

The suspension is then made acidic to exactly pH 4.5 +/- 0.1 using citric acid. Mixing is continued for at least 30 minutes and the final pH of 4.5 +/- 0.1 is verified and adjusted if needed with NaOH or citric acid (as needed) to achieve a pH of 4.5. Perform this correction carefully and only once.

5 Protective screening is performed. The watery dilute cream is passed through a screened funnel with a tight mesh 100-500 microns (0.5mm) to ensure removal of sand, glass shards, metal particles and other particulates or globs. The screened suspension is then centrifuged or dried in a scraping bowl vacuum drier, and the final pellicle is harvested in the next step.

10 Recovery of a concentrated paste as a pellicle involves centrifugation in a swinging bucket centrifuge or decreamers or a bowl scraping vacuum drier. If a vacuum drier is used, the composition buffer should be adjusted so that at the end of the evaporation the concentration of glycerol is up to 10% w/v and other components return to the correct concentration when the cream achieves a 40% w/w solids concentration. If using
15 centrifugation, then the centrifugation should be about 7000 rpm (approximately 8-10,000 g) for 20-30 minutes conditions for other or larger equipment are not known at this time and will be facility dependent. The harvested concentrated pellicle should be at or slightly exceed 40% w/w solids and 60% w/w water.

The concentrated pellicle is weighed and 2% GeoGuard-Ultra (Lonza) or
20 equivalent (D-Glucolactone SB) and 1% vitamin E-acetate are added, and the cream is checked and adjusted if necessary to pH 4.5 +/- 0.1 with NaOH (1N) or citric acid (1N).

The solids concentration of 40% w/w is measured and any downward adjustments in concentration can be made using a stock final composition buffer at pH 4.5 containing physiologic saline, 3% w/v sodium citrate, 10% w/v glycerol and 2% w/v GeoGard-Ultra
25 or equivalent. This adjusting buffer specifically excludes additional Vit-E-acetate.

The harvested pellicle is weighed and should have a consistency thicker than yogurt and thinner than whipped cream cheese. The color should be white to off-white and a microscopic purity (under 400x or 1000x power and photographed) is checked for particles and contaminants (e.g. gross cell wall fragments).

30 If the harvested concentrated pellicle is weighed and is too thick then the final product will be adjusted to 40% w/w/ solids and 60% w/w water, using a composition buffer at 4.5 containing 0.15N sodium chloride (physiologic saline 8.8 g/L), 3% w/v sodium citrate and 2% w/v GeoGuard-Ultra [D-gluconolactone SB (sodium benzoate)].

The paste can be uniformly mixed for 30 minutes followed by pasteurization in an

air-tight container at 75°C. +/- 10°C for 2 hours, prior to bottling. If a scraping bowl evaporator is used, then all of these formulation steps can be performed in that unit.

The material is then allowed to cool to room temperature in a closed container in a laminar flow hood or other suitable clean area prior to packaging. Using sterilized tubing
5 and a peristaltic pump, appropriately sized containers are filled with the finished cream using sterile bottles polystyrene bottles or sanitized pales. An air tight seal that is robust enough for shipping is preferred for the packaging. Conventional 5-gallon food-grade pales many be appropriate at approximately 19 Kg per pale.

10 *Notes*

The foregoing process is based on production runs from 3 to 45 liters (working volumes). The total quantity of reagents will be scale dependent. Where possible, materials are defined as units per liter. The product and process are robust across a range of fermentation conditions.

15 The recovery process takes advantage of the unique low-density properties of the cells and lipid bodies. The process was developed to minimize mechanical requirements and centrifugation steps. Alternative cream separation technologies may be applicable.

The product is resistant to heat and freezing when maintained in an air tight container.

20 The product is sensitive to complete drying, which can irreversibly change the product.

Recovery of Yeast Oil

The lipid bodies from the 403 strain can be used as a source of yeast oil. Recovery
25 of yeast oil from *Lipomyces* and other oleaginous yeast has posed a significant challenge to the production of yeast derived lipids and the ability to make a palm oil substitute. In one embodiment, the washed large crude engineered lipid bodies can be dried by any manner, and due to their large size and high oil content, a significant portion of yeast oil is unexpectedly released without the use of solvents or mechanical extraction. For example,
30 a clear oil can be collected by spray drying lipid bodies. In another example, a thin layer of lipid bodies is spread onto a surface and sloped surface at 50°C, from which the free oil flows as it is expressed from the drying lipid bodies. The elevated temperature is employed because yeast oil, like palm oil, is a solid at room temperature.

Solid and Semi-Solid Lipid Body Preparations

We unexpectedly generated a unique solid byproduct associated with the residual lipid composition that emerges after drying the 403 lipid bodies. This was obtained after a thin layer of lipid bodies were spread onto a sloped surface and incubated at 50°C after oil
5 expression. The structure exists with or without yeast oil removal, and it can be used as a thermally stable wax with a temperature tolerance of at least 200°C before deforming or oxidizing. This lipid material has an unexpectedly high resistance to thermal deformation and oxidation and is useful as a lubricant or specialty packing material, as well as for a range of other commercial and industrial applications, particularly in the area of
10 sustainable lubricants.

In another embodiment, when hydrolyzed or partially hydrolyzed and washed lipid bodies are incubated for 12 to 24 h in 50 to 65°C in alkali at pH 11.2 to 11.7, a temperature-dependent polymer is extracted from the material. When cooled to less than 25°C, the extracted polymer turns the extractive, alkaline aqueous solvent into a gel. When
15 the gel is heated to greater than 45°C and the pH is adjusted to less than 8.4, the gel disperses.

EXAMPLE 2

Exemplary Base Compositions of the Invention

20 An exemplary base composition of the invention comprises a stable, moisturizing composition, which is either used alone or used as a predominant carrier of other active ingredients or agents in compositions comprised of lipid bodies or lipid body derived components. In one embodiment (1A), the inventors developed a lipid body cream at a defined water content for further compositions using *Lipomyces* lipid bodies. The lipid
25 bodies were harvested from cells of genetically engineered *Lipomyces starkeyi* strain XYL-403 or XYL-12Lx13L or equivalent strains and were cultivated in bioreactors, as disclosed above. The processing can provide range of qualities from the crudest composition through subsequent processing steps up to the purest grade of lipid bodies. Several grades of lipid bodies are produced during processing and can be used to meet
30 various requirements of specific compositions of the invention. The lipid bodies can be crudely isolated and formulated or highly purified and formulated as required for the specific purpose. For example, an industrial lubrication composition containing lipid bodies as a lubricating ingredient will not require the same level of purity and control expected when lipid bodies are used for an oral or parenteral drug composition.

For simplicity, one embodiment of making purified lipid bodies in the compositions of the invention is presented. In this embodiment, processed lipid bodies are formulated in phosphate buffered saline at pH 6.5-7.5 and sterilized with heat to make a composition that is essentially a simple solution suitable for pharmaceutical purposes.

5 In another embodiment of the present example, the hyper-lipogenic engineered yeast were grown in fermentations from 2 to 45 liters and the post-harvest process leading to usable lipid bodies. Starting conditions at harvest and yeast cell wall hydrolysis resulted in an initial dry weight that was 26.54% solids in 372 grams of raw lipid body suspension that was stabilized with respect to microbial contamination with citrate and sodium
10 benzoate. The lipid bodies were washed to remove fermentation contaminants and yeast proteins. Final lipid bodies were measured by dry weight and the appropriate amount of excipients were combined as a slurry of 12 g glycerol, 7.2 g sodium citrate, 4.8 g gluconolactone, 2.16 g sea salt, and 1.2 g vitamin E acetate in 100 mL of water to provide the correct final concentration of each excipient once the mixture reached the final lipid
15 body concentration of 52%. During the excipient introduction into the composition or during excipient measurements, the slurry was mechanically mixed at 50 to 60°C to ensure homogeneity. The volume of the excipients for this single iteration was 88.1 mL. An HPLC check of the composition slurry showed 119 grams/liter of glycerol as a marker for the progress of the evaporation. The lipid body and excipient suspension was evaporated
20 at 50°C under gentle agitation on a rotary shaker set initially at a slow speed of about 100 rpm to avoid loss through splashing or sloshing. The net wet starting mass of 485 g took 6 hours to reduce to the target of 211 g. The final material was approximately 52% yeast lipid bodies containing about 5% glycerol, 3% sodium citrate, 0.9% sodium chloride from sea salt, 2% D-gluconolactone, 0.2% sodium benzoate, and 0.5% vitamin-E acetate at a
25 pH of about 4.5. Final checks on dry weight were done in duplicate using a weight to weight method, and a single HPLC analysis was performed to verify the excipient concentrations in which an arbitrary amount of cream near 0.100 grams was placed into a tared microfuge tube and subsequently combined with a calculated amount of water to achieve a 1:10 dilution. This was vortexed and dispensed into an HPLC vial through a
30 filter without centrifugation. The glycerol content was 5.3%, the nominal value being 5%. The composition of the invention comprises lipid bodies in combination with stabilizing ingredients to impart a durable shelf-life and commercial utility in a wide range of industries and markets. The compositions can be adjusted with additional additives and variable amounts of additives to create a stable useful product for any specific purpose.

Composition 2A is a composition that comprises a physiological level of saline and other excipients in a 52% lipid-body composition for use in personal-care and topical drug products.

5 **Composition 2A**

Ingredient	Final Amount ^a
Yeast Lipid Bodies	52%
Glycerol	5%
Sodium chloride	0.9%
Sodium citrate	3%
D-gluconolactone	2%
Sodium benzoate	0.2%
Vitamin E acetate	0.5%

^a by dry wt

Composition 2A is a stable composition of the invention that is suitable alone or in combination with other ingredients, drug mixtures, cosmetics, and other compositions of the invention. The composition described in this example has been tested as a hand cream, a face cream, and a carrier for various drugs.

Composition 2B is a composition of the invention that contains glycerol and other excipients in a 52% lipid-body composition for use in personal-care and topical drug products. This composition is identical to Composition 2A except sodium chloride was replaced with water or omitted. This composition is an example of how a modification of Composition 2A can be made, when physiological saline is not needed for incorporation of a drug or active ingredient, depending on the specific product or market of use.

Composition 2B

Ingredient	Final amount ^a
Yeast Lipid Bodies	52%
Glycerol	5%
Sodium citrate	3%
D-gluconolactone	2%

Sodium benzoate	0.2%
Vitamin E acetate	0.5%

^aby dry wt

Composition 2B describes a basic composition used for combinations of other ingredients and other compositions of the invention. Composition 2B was tested as a face cream, and
5 it proved to be tolerated and well received by the subjects.

Composition 2C

Ingredient	Final amount ^a
Yeast Lipid Bodies	52%
Glycerol	3%
Sodium citrate	3%
D-gluconolactone	2%
Sodium benzoate	0.2%
Vitamin E acetate	0.5%
Modified starch	2%

^a by dry wt.

10 Composition 2C is identical to 2B except that 2% (by dry weight) modified starch (Cargill Star Power Design) was used to inhibit long-term separation by flotation of the lipid bodies in the compositions of the invention. Additionally, glycerol levels were lowered to 3% to reduce transient stickiness as the compositions dried on the skin. This composition comprises a composition that is modified to help maintain a homogeneous
15 mixture and enhanced shelf-life when the composition is prepared for consumer goods, topical drug delivery or any other composition where long-term resistance to component separation or flotation is required. Modifications of this composition are possible depending on the specific consumer product or drug product or markets being addressed by the product. For example, Composition 2C was mixed with lidocaine, an analgesic,
20 which produced a smooth feeling cream that dried and sealed the area of skin that it was

applied. Composition 2C describes a basic composition of the invention that can be used for combinations of other ingredients and other compositions.

EXAMPLE 3

5 A 52% yeast lipid-body composition of the invention was shown to have direct utility when tested as a face cream on humans. Human test data was obtained using Compositions 2A, 2B, 2C and 3. The data demonstrate the utility of a 52% yeast lipid-body composition of the invention as described in Compositions 2A-2C and 3, when applied to human skin. Initial testing in humans is summarized as follows. The base skin
10 composition comprised of lipid bodies was self-administered by health subjects and the data set was obtained from 579 exposures in 21 individuals in the age range of 28 to 65 with an average use of 27.6 skin treatments per subject. In the various compositions comprising lipid bodies that were stabilized at pH 4-5, testing in human volunteers was conducted as a face cream composition.

15 This trial suggested that the compositions of the invention were well-tolerated by subjects, who had a general satisfaction with the moisturizing benefits of the composition as a cosmetic skin care product. Any number of variations of the present embodiments can be used directly or used as a base composition for the incorporation of topical or systemic drugs and other active ingredients depending on the specific purposes of the desired
20 compositions.

Composition 3

Ingredient	Final amount by dry wt
Yeast Lipid Bodies in Water	52%
Glycerol	5.0%
Sodium chloride (optional)	0.9%
Sodium citrate	3.0%
D-gluconolactone	2.0%
Sodium benzoate	0.2%

The compositions described in Compositions 2A-2C and 3 were the base compositions
25 that included additives for maintaining long-term cream moisture and microbial stability at room temperature.

EXAMPLE 4

Yeast XYL-403 was grown under standard fermentation conditions. The yeast will grow under glucose fed-batch conditions or other acceptable carbon sources until a cell concentration has reached 100 or greater optical density units at 600 nm, then the carbon feed is increased until the accumulation of oil in lipid bodies inside of the cell exceeds 50% of the cell volume and, preferably, as much as 85% or greater of the volume inside of the hyper-lipogenic cells. The high-lipid-containing cells can be hydrolyzed with phosphoric acid and the lipid bodies isolated as described herein or the cells can be homogenized to release yeast oil, which is useful as an additive to some compositions of the invention or as a stand-alone yeast oil product for applications that currently use tropical palm oil or similar oils. The composition can comprise lipid bodies along with additional yeast oil or any other modifying oil to cause the composition to be more hydrophobic and to impart a lasting oil sensation on the skin that some individuals find desirable.

As shown in Composition 4A below, compositions of the invention can contain yeast oil, which is a palm-oil-biosimilar. The completed fermentation, containing yeast with internal lipid bodies can be homogenized with a pressure drop homogenizer at an appropriate pressure to lyse the cells and rupture the internal lipid bodies to release the yeast oil into the culture broth. Yeast oil is separated from the homogenates by mixing the material with an equal volume of n-hexane or preferably, iso-hexane, pentane or other appropriate solvent. The yeast oil enters the solvent, which is isolated and then evaporated to produce a pure yeast oil that can be used directly on the skin or added to various compositions that may contain lipid bodies. The yeast oil is essentially water-free and solvent-free following a period in which the oil is held at a temperature between 60° C. and 90 ° C to meet residual solvent and moisture specifications. The yeast oil and or lipid bodies can be used directly as a moisturizing agent on the skin in the compositions of the invention.

In the present example the isolated yeast oil composition was used with or without additional stabilizers such as 0.25% vitamin-E acetate as an antioxidant. This yeast oil alone or in compositions of the invention can be used directly as a moisture retention agent and a lipid replenisher for the skin.

Composition 4A

Ingredient	Final amount by dry wt
Yeast Lipid Bodies in Water	52%
Glycerol	5.0%
Sodium chloride (optional)	0.9%
Sodium citrate	3.0%
D-gluconolactone	2.0%
Sodium benzoate	0.2%
Yeast oil from lipid body containing yeast	0-40%

When applied to the skin, the combined lipid bodies and yeast oil composition is associated with a supple appearance and significant water repellence for human volunteers who repeatedly used this product composition and who have sensed a smoother skin. The composition comprised of lipid bodies is useful for the protection of skin that is exposed to weather, frequent washing (dish pan hands) or any other consumer or drug product where a protective oil or increased hydrophobicity is required. Any increased level of desired oil-feel can be accomplished by adding various amounts of yeast oil to the compositions of the invention. Additional amounts of added yeast oil in the compositions of the invention can be desirable depending on the specific requirements of the consumer product, industry or market. Compositions of the invention containing added yeast oil were found to be desirable by human test subjects when used as a skin or face cream.

Composition 4B is a composition made only from the yeast oil component of lipid bodies where the composition is modified with increased oil relative to Composition 4A up to the point of containing essentially only the yeast oil component of lipid bodies. The example compositions shown below are with and without Vitamin-E acetate as an antioxidant. This composition comprises the yeast oil component of lipid bodies, where a product composition that is very water resistant is required.

Composition 4B

Ingredient	Final amount by dry wt
Yeast Oil	99.95%
Vitamin E acetate	0.05%

Alternatively, the composition can be prepared without an antioxidant, as shown in Composition 4C:

Composition 4C

Ingredient	Final amount by dry wt
Yeast oil	99.95%

5

The embodiment of Composition 4C was found to be desirable as a lipid which melts at body temperature and is a solid at room temperature. The palm oil biosimilar nature of the yeast oil is useful in many applications that currently use tropical palm-oil.

In an embodiment such as Composition 4D, lipid bodies will provide a sealant
10 film. In this embodiment a composition is processed the same as Composition 4A, except the resulting composition contains both yeast oil component of lipid bodies that are mixed 1:1 or 1:2 or any other ratio with lipid bodies in the composition of Composition 4A.

Composition 4D

Ingredient	Final amount by dry wt
Yeast Oil	50%
lipid bodies	26%
Glycerol	2.5%
Sodium chloride	0.45%
Sodium citrate	1.5%
D-gluconolactone	1.0%
Sodium benzoate	0.1%
Vitamin E acetate	0.25%

15

Yeast oil was added to the composition described in Composition 4A and mixed 1:1 with a 52% lipid-body composition to produce a composition that contains 50% yeast oil and 26% lipid bodies with the remainder of the product composition being composed of water and stabilizing excipient as described in Composition 2A. The composition is a
20 blended product that was about 50% yeast oil and 26% lipid bodies. This blended composition can be used directly as a moisturizing agent on the skin and is associated with the appearance of fewer fine wrinkles of the skin as observed by the feedback of 20 human

volunteers, who used the product over a period of several weeks. Additionally, this composition can be used as a carrier of hydrophobic drugs in topical and oral applications and in many other applications, products, and markets.

5 **EXAMPLE 5**

Properties and Utility of Creams

In a preferred embodiment the lipid bodies are made into creams containing yeast lipid bodies at sufficient concentrations that will feel smooth when applied to the face, hands or other parts of the skin. The compositions that are prepared as creams with a lipid-
10 body concentration at or above 52%, referred to herein as Composition 5, have been applied to skin and these compositions of the invention dry relatively quickly, typically in less than 5 minutes. Within that time, the yeast oil plus moisture components of compositions are absorbed into the skin. The exterior shell-like structure of the yeast lipid bodies are composed of a thin layer of proteins, phospholipids, glucan-like structures, and
15 other constituents that form a microscopically thin protective layer over the lipid core of the lipid bodies. The emptied lipid body shells form a durable barrier, which can be visualized and quantitated using skin peel methods and a microscope. The duration of retained protection is greater than 50% by area of treated skin at 10 hours as observed in skin peel testing.

20 When the lipid bodies in various compositions of the invention are applied on the surface of the skin, the moisture and oil are absorbed by the skin and the external shell-like structures of the lipid bodies collapse into pancake-like structures that aggregate as a film to form a protective seal over the skin. This layer along with the oil and other composition constituents can be very soothing on chapped hands, sores, or abrasions. These creams are
25 suitable for use as a base composition for a wide number of topical medicines, cosmetics, personal care products, consumer goods and industrial goods of all type and many other applications, particularly when a durable sealant property is desirable. Here we focus on examples of how the lipid bodies can enhance skin care products, keeping in mind that these are best examples for base composition of many other products using ingredients
30 that can be mixed into the creams having the described sealant properties.

Composition 5 and the other compositions containing lipid bodies are useful when applied to chapped hands or sun-burnt skin or burnt skin. Dry, chapped hands resulting from frequent washing can become irritated, red and sore due to repeated washing or exposure to organic chemicals such as ethanol, acetone, soaps and other agents.

Application of yeast lipid body or lipid body plus yeast lipid oil-based compositions can greatly ease itching and soreness of skin irritation. In recent months, the long-term wearing of face masks, nasal accessories on CPAP machines and other areas of direct skin contact by abrasive materials are treatable with the compositions of the invention.

5 In Composition 5, and other compositions of the invention, a composition containing lipid bodies is useful as lip balm and for potential with or without anti-viral or analgesic drugs for cold for cold sores. Cold sores on lips can often appear in winter months when the skin is exposed to dry air and other irritants. Application of a high concentration of yeast lipid body or yeast lipid oil creams can greatly soothe itching and
10 can be formulated with antiviral compounds or analgesic agents for pain treatment, for example, gabapentin (Nerontin), lidocaine, benzocaine, and others. A preparation of lipid bodies

 Composition 5 and other compositions of the invention are useful for the treatment of fissures. Mucosal membranes can develop fissures or cracking due to dry air or other
15 irritations. Application of a layer of yeast lipid body or yeast oil-based compositions can soothe itching and promote healing of irritated or inflamed skin. Post shaving application of the compositions of the invention have also proved satisfactory to human subjects in reducing irritation.

 Composition 5 and other compositions containing lipid bodies are useful as a
20 treatment for burns and sunburns. First degree burns and sunburns causing redness and irritation can be soothed by the application of a yeast lipid body or yeast oil cream. They are particularly effective with first and second degree burns in which the skin blisters and peels. Additionally, the lipid bodies can be loaded with hydrophobic antifungals, such as fluconazole and other for the treatment or prevention of fungal infections, which are
25 common with severe burns.

EXAMPLE 6

Examples of Stabilized Compositions

 A range of embodiments are described in this example. The lipid bodies and
30 associated component compositions and applications listed above in the invention can be formulated into the stand-alone basic compositions listed below. The best examples of various practical base compositions use antimicrobial stabilizing ingredients in compositions based on lipid bodies.

Many variations to the basic compositions are possible. The following is not intended to limit the range of the compositions of the invention nor to limit the range of product areas addressed by the invention.

5 The options for various compositions are assembled as a suspension of yeast lipid bodies and information about the starting dry weight and the target concentration, then the final composition preparations are dehydrated to hit the desired final composition targets for all ingredients.

10 Lipid body concentrations around 20 to 25% solids are further concentrated by evaporation using shaker trays with bowls, or various mixing tanks, or vacuum mixer-driers that can be used to achieve the desired evaporation. The general method to achieve final compositions is evaporation, which will concentrate lipid bodies to a final concentration that is about 50% to 52% by dry weight not including other excipients.

15 The evaporation bowl or container is mixed at 50 to 350 rpm at 55 to 60°C either at atmospheric pressure or under partial vacuum in order to concentrate the solids to a final target concentration of 50% to 52% plus the weight (not including) of various excipients for a final dry weight that is 8-12% or higher, depending on the specific additives used in any of the following examples and steps: The yeast lipid body suspension is added to the bowl based on its initial dry solids content and the total wet weight is determined. The total solids is calculated as a product of the percent solids and the total wet weight;

20 Glycerol, anhydrous citric acid and tri-sodium citrate trihydrate along with Polysorbate 80 and other ingredients can be mixed into a concentrated solution and added together in a predetermined ratio; Glucuronolactone and vitamin E acetate are added separately as powders; The mixture is evaporated to a final weight that will yield the following mixtures.

25

Composition 6A

Component	Amount (Fraction)
Lipid body solids	0.487
Yeast oil supplement	0
Water	0.450
Excipients:	
Glycerol	0.017
Hyaluronic acid	0.000
Citric acid, anhydrous	0.005

Tri-sodium citrate dihydrate	0.008
Polysorbate 80	0.009
Gluconolactone	0.019
Vit. E acetate	0.005
Total excipients (fraction)	0.063

Composition 6B

Component	Fraction
Lipid body solids	0.433
Yeast oil supplement	0.103
Water	0.400
Excipients:	
Glycerol	0.015
Hyaluronic acid	0.000
Citric acid, anhydrous	0.005
Tri-sodium citrate dihydrate	0.007
Polysorbate 80	0.010
Gluconolactone	0.021
Vit. E acetate	0.005
Total excipients (fraction)	0.063

Composition 6B is a composition that replaces a portion of the oil in lipid body solids with yeast oil extracted from lipid bodies. This can yield a smoother cream.

Composition 6C

Component	Fraction
Lipid body solids	0.423
Yeast oil supplement	0.101
Water	0.390
Excipients:	
Glycerol	0.030
Hyaluronic acid	0.002
Citric acid, anhydrous	0.012

Tri-sodium citrate dihydrate	0.018
Polysorbate 80	0.004
Gluconolactone	0.016
Vit. E acetate	0.004
Total excipients (fraction)	0.086

In addition to a yeast oil supplement, the composition in Composition 6C includes hyaluronic acid and a higher concentration of glycerol to reduce separation of lipid bodies from the aqueous layer and to maintain a high humectant level.

5

Composition 6D

Component	Fraction
Lipid body solids	0.392
Yeast oil supplement	0.159
Water	0.362
Excipients:	
Glycerol	0.028
Hyaluronic acid	0.003
Citric acid, anhydrous	0.012
Tri-sodium citrate dihydrate	0.019
Polysorbate 80	0.006
Gluconolactone	0.015
Vit. E acetate	0.004
Total excipients (fraction)	0.083

Composition 6D contains a higher concentration of yeast oil supplement to increase the smoothness and hydrophobicity of the cream along with a higher concentration of emulsifier.

10

Composition 6E

Component	Fraction
Lipid body solids	0.496
Yeast oil supplement	0.0
Water	0.458

Excipients:	
Glycerol	0.0092
STARDESIGN powder	0.0000
Citric acid, anhydrous	0.0054
Tri-sodium citrate dihydrate	0.0083
Gluconolactone	0.0191
Vit. E acetate	0.0048
Polysorbate 80	0.0000
Total excipients (fraction)	0.0467

Composition 6E is a basic cream containing yeast lipid bodies similar to Composition 1A above. However, in this composition the citric acid concentrations are moderately higher to impart a greater buffering and ionic strength, which are potentially useful for improving antimicrobial stability to the compositions without damaging the feel of the lipid bodies.

Composition 6F

Component	Fraction
Lipid body solids	0.494
Yeast oil supplement	0.0
Water	0.456
Excipients:	
Glycerol	0.0091
STARDESIGN powder	0.0027
Citric acid, anhydrous	0.0054
Tri-sodium citrate dihydrate	0.0083
Gluconolactone	0.0190
Vit. E acetate	0.0048
Polysorbate 80	0.0000
Total excipients (fraction)	0.0493

Composition 6F is similar to Composition 2A but it also contains a small amount of STARDESIGN powder, a modified starch, to reduce water separation.

Composition 6G

Component	Fraction
Lipid body solids	0.305
Yeast oil supplement	0.0
Water	0.6193
Excipients:	
Glycerol	0.0124
STARDESIGN powder	0.0217
Citric acid, anhydrous	0.0073
Tri-sodium citrate dihydrate	0.0113
Gluconolactone	0.0185
Vit. E acetate	0.0046
Polysorbate 80	0.0000
Total excipients (fraction)	0.0757

Composition 6G (without yeast oil) is similar to Composition 6F but the amounts of STARDESIGN powder and citric acid are substantially higher.

5

Composition 6H

Component	Fraction
Lipid body solids	0.3096
Yeast oil supplement	0.0469
Water	0.6155
Excipients	
Glycerol	0.0114
STARDESIGN powder	0.0199
Citric acid, anhydrous	0.0081
Tri-sodium citrate dihydrate	0.0125
Gluconolactone	0.0185
Vit. E acetate	0.0046
Polysorbate 80	0.0009
Total excipients (fraction)	0.0750

Composition 6H (with yeast oil) contains a small amount of yeast oil supplement along with an emulsifier to increase smoothness and a lower amount of STARDESIGN powder than found in Composition 6G.

5 **Composition 6I**

Component	Fraction
Lipid body solids	0.0
Yeast oil supplement	0.2695
Water	0.6288
Excipients:	
Glycerol	0.0129
STARDESIGN powder	0.0377
Citric acid, anhydrous	0.0113
Tri-sodium citrate dihydrate	0.0174
Gluconolactone	0.0180
Vit. E acetate	0.0045
Polysorbate 80	0.0055
Total excipients (fraction)	0.1017

Composition 6I has been designed to use yeast oil supplement alone without the addition of lipid body solids. It is a semi-transparent cream that is quite smooth, but with more of an oily feel than other compositions. This composition is particularly oil with a lubricating quality on the skin and in applications involving moving parts.

Composition 6J

Component	Target	Final
Lipid body solids	33%	29.7%
Yeast oil supplement	0	0
Water	55%	60.4%
Excipients:		
Glycerol*	2.00%	1.80%
STARDESIGN powder*	3.50%	3.15%
Citric acid, anhydrous*	1.18%	1.06%
Tri-sodium citrate dihydrate*	1.82%	1.64%

Gluconolactone	2.00%	1.80%
Vit. E acetate	0.50%	0.45%
Polysorbate 80**	0.00%	0.00%
Total	11.0%	9.91%

*Based on net water; **Based on oil content

Composition 6J uses a moderate amount of STARDESIGN powder to stabilize the lipid body suspension at a lower concentration of lipid bodies and without the addition of supplemental yeast oil.

Composition 6K

Component	Target	Final
Lipid body solids	33%	31.3%
Yeast oil supplement	5%	4.4%
Water	51%	57.1%
Excipients:		
Glycerol*	2.00%	1.14%
STARDESIGN powder*	3.50%	2.00%
Citric acid, anhydrous*	1.18%	0.67%
Tri-sodium citrate dihydrate*	1.82%	1.04%
Gluconolactone	2.00%	1.86%
Vit. E acetate	0.50%	0.46%
Polysorbate 80**	0.10%	0.09%
Total	11.0%	7.3%

*Based on net water; **Based on oil content

In Composition 6K, a moderate amount of STARDESIGN powder along with Polysorbate 80 is used to stabilize a lower concentration of lipid body solids along with yeast oil to make a smoother preparation.

Composition 6L

Component	Target	Final
Lipid body solids	0%	0%
Yeast oil supplement	30%	20.79%

Water	51%	69.3%
Excipients:		
Glycerol*	2.05%	1.42%
STARDESIGN powder*	6.00%	4.16%
Citric acid, anhydrous*	1.18%	0.82%
Tri-sodium citrate dihydrate*	1.82%	1.26%
Gluconolactone	2.00%	1.80%
Vit. E acetate	0.50%	0.45%
Polysorbate 80**	0.62%	0.43%
Total	13.6%	10.3%

*Based on net water; **Based on oil content

Composition 6L uses a higher concentration of STARDESIGN powder to stabilize a preparation that contains no lipid body solids and only a moderate amount of yeast oil.

5 The resultant composition was starchy and prone to separation upon transient heating.

The series of compositions comprising lipid bodies, modified with excipients in a range of concentrations are useful for consumer products, drug delivery systems, and a wide range of industrial and markets applications.

10 **EXAMPLE 7**

Preparation of antiviral hydrophobic surfactant compositions

Compositions of the invention can be made to enhance the utility of sanitizers, which are typically very drying to the skin. In this example, lipid bodies are formulated to produce an anti-viral composition, although similar applications exist for anti-bacterial,
15 anti-fungal and anti-protozoan sanitizers.

Viruses are not generally susceptible to antibiotics such as antibacterial, antifungal or antiparasitic agents because they target physiological functions unique to the pathogen. Viruses that infect human cells generally bind to surface membrane proteins and then undergo transmembrane transport their DNA or RNA into the target cells where the viral
20 machinery takes over the cell metabolism and forces it to make many copies of itself. Virus infection can be blocked either by preventing contact between the viral surface proteins and the receptor proteins on the target cell or by disrupting the receptor proteins or viral membranes of the virus itself, depending on the specific viral pathogen. The latter is particularly useful as a viral disinfectant. Viral disinfectants are well known, for

example, ethanol or isopropyl alcohol. However, these common sanitizing agents are particularly drying to the skin. Utilizing any number of compositions of the invention can offset the drying impact of the sanitizers on human skin.

Animal viruses are generally susceptible to surfactants such as soap. Their outer
5 surface includes a lipid bilayer membrane derived from the cytosolic membrane of their host cell. In another embodiment an antiviral composition comprising lipid bodies is made by adding antiviral substances. Viruses can be destroyed by surfactants and chaotropic agents that break up lipid bilayer membranes of enveloped viruses and dissociate the viral nucleic acids (RNA or DNA) from the viral packaging proteins. In this example, lipid
10 bodies in a base composition are supplemented with a surfactant to achieve an anti-viral composition. Useful surfactants include sodium lauryl sulfate and other detergents at concentrations of less than 10% or alkylation agents such as benzalkonium chloride at concentrations of less than 5%.

The objective of these compositions is to produce a persistent surfactant hand
15 cream that will disrupt viral particles, leave a protective layer on the skin and allow for safe, effective removal of contaminants by washing with soap and water, while slowing the desiccation impact of the antiviral agents. Similar approaches are available for antibacterial and antifungal sanitizers that could benefit from compositions of the invention either as part of the composition or as a follow-up hand moisturizer.

20

EXAMPLE 7A

Formulation procedure: Weigh out a quantity of yeast oil; Add Polysorbate 80 equal to one-third of the mass of oil and mix to form an emulsion; Weigh out a quantity of Aloe vera juice equal to the yeast oil plus polysorbate; Add hyaluronic acid equal to 0.5%
25 of the mass of the Aloe vera juice and mix to dissolve; Blend the yeast oil plus polysorbate emulsion with the Aloe vera plus hyaluronic acid to form an even suspension.

EXAMPLE 7B

Variations to the formulation procedure of Example 7A: The Aloe vera juice may
30 be concentrated prior to mixing with hyaluronic acid; The concentration of hyaluronic acid may be decreased to 2% or increased up to 0.8% to alter thickness of the surfactant cream; Glucuronic acid, sodium benzoate, citric acid, vitamin E and/or STARDESIGN starch may be added; Other antiviral compounds may be added, e.g. ethanol, isopropyl alcohol, benzalkonium chloride and others.

EXAMPLE 8

In this example, one or more of base Compositions 2-6L, lipid bodies simply sterilized in phosphate buffered physiological saline (PBS), and any other composition
5 described herein are used in combination with other substances, preferably drugs and topical medicinal compositions. In this example, the compositions take up hydrophobic and hydrophilic drugs, which mix uniformly into base compositions. drugs loaded into lipid bodies by the inventors were Lidocaine, Griseofulvin, Benzocaine, Colchicine, Hydroxychloroquine, and many other representative drug substances. The embodiments
10 included drugs for a variety of conditions and administration points, for example, oral, ophthalmic drugs, internal medicines, parenteral and subcutaneous treatments, vaccines, antibiotics, biologics and many other medical applications and compositions, will benefit from the properties of the lipid bodies. Of particular utility is an embodiment that is simply processed, and purified lipid bodies suspended in PBS and which are then steam
15 sterilized at 121°C. This composition was prepared at 27% lipid bodies as measured by dry weight at pH 6.8. The sterile lipid bodies are particularly useful in drug applications, carrier components of vaccines, suitable for coating with cationic biopolymers and suitable for formulation with active drug ingredients for a range of administrations sites. In this embodiment, PBS lipid bodies prepared as described in this example are essentially
20 tasteless and suitable for the development of oral drug forms and oral vaccines.

EXAMPLE 9

In this example, one or more of base Compositions 2-6L and any other composition described herein, are used in any combination with other substances,
25 preferably cosmetics and cosmeceutical compositions. The lipid body based compositions take up and mix with compounds such as steroids, cholesterol, pigments, dyes, flavors, water insoluble vitamins, hydrophobic peptides, amino acids such as phenyl alanine, poly-aromatic compounds and many other generic and brand name substances and ingredients that are useful in preparing compositions for a wide range of pharmaceutical, cosmetics
30 and cosmeceutical applications. Lipid bodies of Compositions 2-6L were washed in a 1:10 mixture of compositions to 95% ethanol and the dehydrated lipid bodies were recovered by centrifugation. The pelleted lipid bodies were resuspended in deionized water and the reduction and effective removal of excipients and particularly the removal of sodium benzoate produced a lipid body suspension which could be filtered through a 0.2 micron

syringe filter and the resultant filtrate measured by UV scan from 220-299 nm and determined to have a minimal UV absorbance. Using the experimental composition of washed lipid bodies or suspensions of PBS sterilized lipid bodies, UV absorbing substance could be loaded into the lipid bodies and the filtration used to quantitate the amount of the

5 UV absorbing material that was bound to the lipid bodies. This method proved particularly effective in evaluating the bound vs unbound levels for hydrophobic substances and drug models such as aromatic amino acids, lidocaine, benzocaine, benzoate, Nile red and Sudan black. The resultant compositions of the invention indicated that aromatic and polyaromatic compound load to various degrees into the lipid bodies as a function of their

10 solubility into the internal neutral triglycerides of the compositions.

EXAMPLE 10

In this example, one or more of base Compositions 2-6L and any other composition described herein are used in any combination with other substances,

15 preferably skin care products and skin healing compositions. In this example, the lipid body based compositions take up and mix with compounds such as lanolin, glycerol, polyglycols, polymers, peptides, oils, vitamins, hyaluronic acid, collagen, peptides, hydrophilic amino acids, non-aromatic amino acids, and many other generic and brand name substances and ingredients that are useful in preparing skin care compositions for a

20 wide range of skin ailments. The lipid body base composition of the invention was capable of being formulated with a range of hydrophilic substances on the hydrophilic surface of lipid bodies and inter-lipid body spaces in example compositions. The method was applied to dehydrated and washed or pure lipid bodies in PBS when there was a significant interstitial space for hydrophilic substances. In some cases, such as lidocaine, the

25 ingredient partitioned into both the hydrophobic interior and the hydrophilic exterior of the compositions.

EXAMPLE 11

In this example, one or more of base Compositions 2-6L and any other

30 composition described herein are used in any combination with other substances, preferably pet care products and pet veterinary compositions. In this example, the lipid body based compositions take up and mix with compounds such as lice and tick medicines, shampoo, fragrances, odor blocking agents, oils, vitamins, hyaluronic acid, collagen, peptides, amino acids such as phenyl alanine, and many other generic and brand

name substances and active ingredients that are useful in preparing pet care compositions for a wide range of pet ailments and pests. The lipid body base compositions of the invention have been developed with Environmental Working Group (EWG) additives in mind so as to maximize the safety of the compositions for pets and humans.

5

EXAMPLE 12

In this example, one or more of base Compositions 2-6L, simpler, cruder compositions containing only lipid bodies and or lipid-body envelopes and biochemicals, and any other composition described herein are used in any combination with other substances, preferably with industrial applications such as but not limited to lubrication, sealants, coatings, oils, greases, biofuels, fuels, insulation, conductive compositions, and many other industrial applications. In this example, the lipid body based compositions take up and mix with compounds such as aviation, machinery operation, agriculture, pharmaceutical operations, manufacturing, transportation, and other industrial areas and many other generic and brand name substances and ingredients that are useful in preparing industrial compositions for a wide range of industrial purposes. In this example, yeast containing lipid bodies were dried and oil released by dehydration was removed and the residual lipid body shells were heat resistant up to 200°C. and provided a heat tolerant waxy lubricant, which could be used directly or mixed with other agents to provide an industrially useful lubricant composition of the invention.

EXAMPLE 13

In this example, one or more of base Compositions 2-6L, simpler compositions containing only lipid bodies and or lipid-body envelopes and biochemicals, and any other composition described herein are used in any combination with other substances, preferably with nanotechnology applications such micro-electronics, biometrics, castings, mold release agents, plastics, polymeric compositions, separation resins, battery elements, photovoltaics, optics, lasers, light filters, whitening agents, dyes, coloring agents, precipitation agents, chelators, organic chemistry reactions and many other physical and chemical or organic chemistry uses, including catalysis, photocatalysis and other compositions that have practical value for many emerging areas of technology. The lipid bodies of the invention are mobile in an electric field established in deionized water. The current flow is on and across the exterior of the lipid bodies with the neutral triglyceride interiors acting as an oil-base resistor to electrical flow. With low levels of divalent cations

present, the lipid bodies with their phospholipid exteriors are bridged and aggregate but remain mobile in an electric field, moving toward the positive cathode. The utility of spherical structures in bio-electrical applications are not fully understood at this time. As a proof of lipid body charge state, excess salt was used in the electrical field and the surface charges of lipid bodies are nullified and electrical mobility of the lipid bodies declines.

EXAMPLE 14

To evaluate drug uptake and delivery into the compositions, several standard analytical methods are used. One method utilized an instrument for UV-VIS spectroscopy. For this example, samples were run on a Perkin-Elmer Lambda 35 UV-VIS Spectrometer with a 4-sided clear quartz cuvette (Fisher) and a 1 mL sample of PBS (Dulbecco's Phosphate Buffered Saline, Sigma) used as the blank before every run.

Another preferred method used a High-Pressure Liquid Chromatography (HPLC) instrument for analysis. Samples were run on an Agilent Technologies 1260 Infinity HPLC in a reverse phase isocratic mode using UV detection at 210 nm and 10 μ L of sample injection. The column was a Hypersil Gold column (250x4.6 mm, Thermo Scientific) and the mobile phase was a 1:1 suspension of acetonitrile to aqueous buffer (HPLC grade water) mixed with 5 mM ammonium acetate and 0.02% (of total volume) formic acid. The flow rate was 1.2 mL/min and the column temperature was 22°C. Peak detection and quantitation were determined using ChemStation software and reference standards.

The following non-limiting examples are provided for illustrative purposes and represent the incorporation of drugs in the compositions and their release into aqueous media. The lipid bodies contained approximately 50% lipid and 50% aqueous material. Using these methods, it is shown that many drugs can form useful compositions with unique properties that depend on high loading and unloading volumes.

EXAMPLE 15

Colchicine, an alkaloid for gout, was determined to form a suitable topical drug composition using the compositions of the invention. Specifically, in this example, an aqueous drug plus lipid-body composition was created.

A 55% aqueous and 45% drug-containing lipid-body composition was prepared by the following procedure. A 2 mg/mL colchicine (TCI, Tokyo Chemical Industry) stock was prepared by vigorous mixing of 20mg of colchicine and 10 mL Phosphate Buffered

Saline (PBS). To 1 g lipid body was added 100 μ L of 2 mg/mL colchicine stock followed by vigorous mixing. A 300mg sample of the resulting topical composition was taken out and placed in the bottom of a 2 mL Eppendorf tube with 1mL PBS layered on top and incubated for 15 minutes at room temperature without agitation. This mixture was
5 centrifuged for 2 minutes at 14000 RPM in an Eppendorf Centrifuge 5415C to separate the lipid and aqueous layers and once complete, the aqueous layer was removed and stored in a new 2 mL Eppendorf tube at 4C.

Results: UV spectroscopy was run and the peak on the spectrum was at 350 nm with an absorbance of 0.82 and a baseline of 0.57 yielding a total absorbance of 0.25. A
10 total of 5.9 μ g/mL was released calculated using the published Extinction Coefficient of $\sim$ 17 mM units at 350.5 nm. This corresponds to 9.8 % drug release from the drug containing lipid body into 1 mL PBS at room temperature. Thus, colchicine can be loaded and released in an aqueous environment from a lipid-body composition. Since colchicine is a potent drug, the resultant level of released drug is believed to be useful for the
15 treatment of gout.

EXAMPLE 16

In another example, colchicine was loaded into and released from a lipid-body composition containing a larger aqueous fraction. The procedure uses a 62% liquid and
20 38% drug-containing lipid-body composition that was prepared in a similar manner to Example 18, except a 5mg/mL stock of colchicine was used (10 mg of colchicine and 2 mL PBS) with 200 μ L of PBS added and the homogeneous mixture was allowed to sit in 1mL PBS at room temperature.

Results: The absorbance of the peak at 350 nm was not readily visible due to
25 extension light scattering and thus a 1/10 dilution in PBS was needed to determine the 350 nm absorbance of 0.22. A total of 51.8 μ g/mL drug released corresponds to 34.5% of the total colchicine. By HPLC (350nm, 2.9 minutes), 61.5 μ g/mL released corresponds to 41% colchicine released, which is similar to the value calculated by UV. Thus, colchicine can be loaded and have an enhanced release from a lipid-body composition containing a higher
30 water content than Example 18. Under these conditions, the lipid-body composition retains a pleasant, smooth, and aqueous feeling.

EXAMPLE 17

Colchicine was loaded into and released from a lipid-body composition containing an even larger aqueous fraction than in Example 19. The procedure and composition is a 67% liquid and 33% drug-containing lipid-body composition prepared in a similar manner to Example 19 except 178 μ L of PBS was added.

Results: Due to the high absorbance of the peak at 350 nm, a 1 to 3 dilution followed a 1 to 2 dilution which led to a 350 nm absorbance of 0.16. A total of 22.6 μ g/mL of colchicine was released corresponding to 15.1 % of the total compound loaded. By HPLC, 38.3 μ g/mL or 25.5% of the compound was released, which is almost double that calculated by UV. Thus, colchicine can be loaded and have an enhanced release from higher water content lipid-body compositions than in Examples 18 and 19.

EXAMPLE 18

Colchicine was loaded as a dry powder into a lipid-body composition and then colchicine was released from a lipid-body composition using a dry starting drug powder. The procedure used 30 mg of colchicine powder, which was vigorously mixed into and with 1g of lipid bodies. A 300 mg sample was removed and placed in the bottom of a 2mL Eppendorf tube, 2 mL PBS layered on top and incubated for 15 minutes at room temperature. The sample was centrifuged for 2 minutes at 1400 RPM in an Eppendorf 5415C Centrifuge and the aqueous layer removed and placed in a new 2 mL Eppendorf tube to be run on the UV-VIS spectrophotometer described in Example 17.

Results: Due to large scattering a 200-fold dilution was needed to determine the peak at the 350nm absorbance of 0.72. A total of 3,390 μ g/mL was released, corresponding to 37.6% of the total colchicine present. Thus, colchicine can be loaded as a dry powder and released from a 50% lipid-body composition.

EXAMPLE 19

In another example, colchicine was loaded as a dry powder into a cream of lipid bodies, plus additional PBS and then the colchicine was released from a lipid body cream and PBS composition using a dry starting drug. The procedure used a sample that was prepared in a similar manner to Example 22, except 33 μ L of PBS was added to the PBS mixture and lipid-body composition.

Results: Due to large scattering a 100-fold dilution was needed to obtain a 350 nm absorbance of 1.7 corresponding to 4000 μ g/mL released or 44% of the total colchicine.

Increasing the volume of the release medium increases the amount of colchicine released. These results support the properties of loading and delivery of topical drugs, which may require dosing control provided by adjusting the lipid body cream and PBS levels in the final topical drug composition.

5

EXAMPLE 20

In this example, another drug, hydroxychloroquine, which is used as an antimalarial, amebicide, and also to treat lupus, rheumatoid arthritis, and putatively, Covid-19, can be formulated and benefit from incorporation into lipid bodies. The procedure used a 5 mg/mL stock of hydroxychloroquine (Cayman), which was prepared by vigorously mixing 10mg of hydroxychloroquine and 2 mL PBS. Then to 1g of lipid bodies was added 100uL of 5mg/mL hydroxychloroquine stock followed by vigorous mixing. A 300mg sample was removed and placed in the bottom of a 2 mL Eppendorf tube with 1mL PBS layered on top and incubated for 15 minutes at room temperature. As in earlier examples, the mixture was centrifuged, and the aqueous layer removed for analysis.

Results: Due to the light scattering, a 6-fold dilution was needed to determine the absorbance at 343 nm, which gave a value of 0.65. The concentration from the peak at 343 nm was determined using the stock solution as a reference standard. The released hydroxychloroquine was determined to be 5.66 µg/mL, corresponding to a total hydroxychloroquine release of 10.4%. Thus, hydroxychloroquine can be loaded and released from a hydroxychloroquine lipid-body composition in a therapeutic concentration.

25 **EXAMPLE 21**

Chloroquine, an antimalarial, an amebicide, and used to treat lupus, rheumatoid arthritis, and putatively, Covid-19, was prepared in a lipid-body composition. The procedure used a 5 mg/mL stock solution of chloroquine (Alfa Aesar), which was prepared by vigorously mixing 10 mg chloroquine and 2mL PBS. Then, to 1 g of lipid bodies, 100 µL of 5 mg/mL chloroquine stock was added and vigorously mixed. A 300 mg sample was removed and placed in the bottom of a 2 mL Eppendorf tube with 1 mL PBS layered on top and incubated and centrifuged as described in earlier examples.

Results: Due to the high light scattering, a 6-fold dilution was performed. This led to an absorbance of 0.40 at 343 nm and a calculated concentration of 12.1 µg/mL,

indicating a total chloroquine release of 8.1%. Of note, the absorbance of the peak at 343 nm at a 6-fold dilution was not consistent with the standard, therefore a 1 to 3 dilution was done, which gave an absorbance too high to calculate. Then a 1 to 2 dilution was prepared, and a useful absorbance peak was obtained at 343 nm, giving a value of 0.45 and the
5 baseline 0.05A, which is a total of 0.40A. The concentration from the peak at 343 nm was 12.12 mg/mL using the standard and a ratio, and a total release of 8.1%. Thus, chloroquine can be loaded and released from a large yeast lipid-body composition at therapeutic levels.

EXAMPLE 22

10 Losartan, an ACE Inhibitor, and a putative Covid-19 treatment was prepared in a lipid-body composition. The procedure used a 5 mg/mL stock of Losartan Potassium Salt (Cayman), which was vigorously mixed after combining 10 mg of Losartan with 2 mL PBS. Then, to 1 g of lipid bodies was added 100 μ L of 5 mg/mL Losartan stock and vigorously mixed. A 300 mg sample was removed and placed in the bottom of a 2 mL
15 Eppendorf tube with 1 mL PBS layered on top and incubated and centrifuged as described in previous examples.

Results: The Losartan sample were analyzed by HPLC and spectroscopically, which gave peak heights at 3.9 minutes at 210 nm. The measured value was compared to a 20 mg/mL Losartan reference standard. From the peak heights, the Losartan released into
20 1 mL PBS had a concentration of 54.4 μ g/mL corresponding to a total release of 36.3%. Thus, Losartan can be loaded and released from a Lipid body.

EXAMPLE 23

Travoprost, a prostaglandin analogue used for glaucoma, was prepared in a lipid-
25 body composition. The procedure (#1) used 1g of lipid bodies, which was added with 2 μ L of Travoprost (25 mg/mL, Cayman Chemical Company) and vigorously mixed. A 300 mg sample was removed and placed in the bottom of an Eppendorf tube with 1mL PBS layered on top and incubated and processed as before and stored in a 2 mL HPLC vial.

A second procedure used a 10 μ L sample of Travoprost stock (25 mg/mL), which
30 was evaporated under nitrogen flow to a final weight of 250 μ g. To this concentrate of Travoprost was added 1g of lipid bodies followed by vigorous mixing. A 100 mg sample was removed and place in an Eppendorf followed by the addition of 1 mL PBS. The remaining steps followed procedure 1, which utilized HPLC analysis.

Results, first procedure: The HPLC results of the Travoprost sample and the 25 $\mu\text{g/mL}$ Travoprost in ethanol sample gave the same peak height of 1.7 at around 4 minutes at 210 nm. With an initial concentration of 50 $\mu\text{g/mL}$, or a 50% of Travoprost released. Results, second procedure: The HPLC results of Travoprost indicated a 60.7 % release.

5 Thus, the drug Travoprost can be loaded and released from lipid bodies under different preparation conditions and concentrations. Compositions include utilizing lipid bodies, which have a diameter larger than 5 μm and are derived from any engineered hyper-lipogenic yeast.

10 **EXAMPLE 24**

In one example, the absorbance at 260 nm was used for determining Lidocaine binding and partitioning into lipid bodies using a UV spectrophotometry. Lipid bodies were cleared of excipients and antimicrobial preservative by undergoing a 1:10 wash in ethanol followed by rehydration in distilled water. To improve the UV signal and
15 minimize turbidity from the lipid bodies, the lipid bodies were filtered through an 0.2 μm filter before measurement. Elimination of the background noise and minimizing dilutions improves the absorbance of UV-absorbing drugs such as lidocaine. The methodology used a lidocaine stock of 50 mg in 950 μL (50 mg/mL), which was used to load various concentrations of lidocaine into the lipid-body compositions of the invention. In this
20 example, 2 μL of a 50 mg/mL stock or 100 μg of lidocaine was mixed with 100 μL of washed lipid bodies in 1M NaCl or in deionized water and incubated 10 minutes before removal of the lipid bodies by 0.2 μm filtration.

The resulting UV absorbances were:

Concentration of Lidocaine	UV Absorbance	Lidocaine in Aqueous Cream Fraction	Lidocaine Bound to Lipid Bodies
Control: 500 μg Lidocaine without Lipid Bodies and Filtered	1.06	100%	0%
500 μg Lidocaine in Water	0.884	83%	16%
500 μg Lidocaine in Salt	0.76	72%	28%

25

The results highlight the ability to influence the partitioning of drug into the lipid body sphere using a high ionic strength imparted by salt, when formulating a large yeast lipid body. Similar results were obtained with phenyl alanine, benzocaine and griseofulvin with some variation depending on the hydrophobicity of each ingredient.

5

EXAMPLE 25

In this example, fermentations of different engineered hyper lipogenic *Lipomyces starkeyi* were used to make different diameter lipid bodies. Using the engineered hyper-lipogenic strain XYL-403, compositions of lipid bodies were prepared with a diameter
10 from 6.9 +/- 1.4 microns, as shown in the figure below, although some size manipulation is possible using fermentation conditions, when measured under a microscope using a calibrated Petroff Hauser counter. Compositions of lipid bodies were prepared with a diameter up to 12 microns using other strains of engineered hyper-lipogenic strains, for example, XYL-610, as measured using a calibrated Petroff Hauser counter and a
15 microscope ocular ring with a 20 micron span under 1000x power. The following photo of lipid bodies was obtained and observed to be approximately 10-12 microns when compared to a 20 µm diameter ocular reference-ring at 1000x power with a phase contrast microscope. These large lipid bodies present a much larger internal hydrophobic volume than commercial liposomes and therefore a greater capacity to carry hydrophobic
20 ingredients.

EXAMPLE 26

In this example, fermentations of different engineered hyper lipogenic *Lipomyces starkeyi* were used to create lipid bodies. Using the engineered hyper-lipogenic strain
25 XYL-403, compositions of lipid bodies were prepared with a diameter from 6.9 +/- 1.4 microns when measured under a microscope using a calibrated Petroff Hauser counter. Following the initial preparation of lipid bodies, the lipid bodies were fused using high levels of salt at 1M NaCl, solvents such as ethanol at 20% (v/v) in water, and application of shear force using centrifugation (FIG. 12A). Homogenization, filtration, or chemical
30 treatments can be used as alternatives to or in additional centrifugation to induce shear force. Thus, compositions utilizing even larger lipid bodies (e.g., between 12 to 50 µm) can be developed using physically modified and merged lipid bodies. The advantages of larger lipid bodies can be extended using high total volumes of drug carrying capacity.

Once such example used Sudan black, which is nearly 100% taken up into the lipid bodies to demonstrate the larger hydrophobic capacity of very large lipid bodies (FIG. 12B). This example opens the possibility of even higher volumetric carrying capacity of lipid-body compositions of the invention when expanded to the upper limit of known
5 diameter.

EXAMPLE 27

In this example, a composition utilizing lipid body components, envelopes, associated yeast oils and other biochemicals derived from lipid bodies was shown to be
10 possible by the isolation of opened envelopes by passage of a 20% suspension of lipid bodies through a pressure-drop homogenizer. The homogenized solution was centrifuged at high speed in an Eppendorf centrifuge in the presence of a hexane solvent. The yeast oil, which was derived from the lipid bodies was separated into the solvent layer and recovered by evaporation. Following the same centrifugation, the interface was observed
15 to have a layer, which when isolated and examined under a microscope at 400x power was shown to have disrupted envelopes. Additionally, there is a light water interacting layer that is thought to be phospholipid, some protein(s) and other biochemicals, which remain in the bottom aqueous layer as a turbid haze. Finally, heavy solids pelleted to the bottom of the centrifuge tube. This simple process identified multiple components of lipid bodies,
20 which may prove useful when formulated back into the lipid body cream or compositions. To demonstrate this point, the isolated yeast oil was mixed back with a composition at a level of 10, 15 and 40% and mixed to form a homogeneous composition with a somewhat oilier feel.

This example shows that components of lipid bodies can be used independently or
25 in combination with lipid-body compositions to modify the feel and utility of final compositions.

EXAMPLE 28

In this example, lipid bodies at about 50% concentration in base composition of
30 example 1A were subjected to the addition of concentrated Nile red, which had been solubilized in DMSO and used in this example as a surrogate for a hydrophobic compound or hydrophobic drug. The lipid-body composition was mixed with Nile red for 10 minutes and centrifuged. After centrifugation, the lipid bodies separate by flotation and all of the Nile red entered into the lipid bodies. The lipid bodies remain hydrophilic on the exterior

of the lipid body spheres and continue to feel aqueous. The observed uptake of Nile red was quantitatively complete, moving the Nile red into the lipid bodies that float upon centrifugation (FIG. 13A).

In another version of this example, lipid bodies at approximately 50% by dry weight were mixed with a second hydrophobic model drug compound, Sudan black. After about 10 minutes, the mixture was centrifuged, and the lipid bodies floated with essentially all of the Sudan black loaded into the lipid bodies. This example was used to demonstrate the uptake of a second model compound representing the behavior of hydrophobic materials or drugs as they load into lipid bodies. Similar to the Nile red results, Sudan black is shown to be taken up by lipid bodies (FIG. 13B).

This example shows the ability of the lipid bodies to hold a hydrophobic ingredient.

EXAMPLE 29

Lipid bodies have a unique property of collapsing and sealing a surface once all other hydrophilic and hydrophobic components are absorbed into a treated surface. Lipid bodies formulated as an approximately 50% lipid body cream were applied uniformly to the face of a test subject. The lipid-body composition was allowed to dry and remain on the skin. After a few minutes, the water and oil components of the composition were absorbed into the skin and a water repellent layer remained as a durable sealant on the skin. After 10 hours, the remaining lipid bodies were removed with clear tape (3M Corp.) and this skin peel was examined at 1000x power under a microscope to observe the durability of the lipid body envelopes as they overlaid epithelial cells from the test subject's face. Lipid bodies, when applied and dried on the skin form pancaked envelopes, which have the property of collapsing to form a sealing surface (FIGS. 14A-C). When the lipid-body compositions discharge of hydrophobic and hydrophilic ingredients, pancaked envelopes form a flexible barrier that is essentially invisible to the naked eye. This example shows that lipid bodies can sealing epithelial cells and promote the protective retention of ingredients, which are absorbed into the skin. This unique property is especially useful in the field of topical drug administration, cosmetic applications and in other fields.

EXAMPLE 30

Lipid bodies were formulated to be approximately 50% by dry weight as a cream, which was modified, homogenized or dehydrated to create broken or modified envelopes (FIG. 15) that can be used to enhance the formulation of large molecules, biologics and peptides for transport into the human body at any point of entry. This example applies to compositions containing lipid bodies with or without other lipid body components, such as envelopes. The opened envelope is shown here.

An envelope is the result of homogenizing a yeast lipid body of the invention. The formation of envelopes can then be blended with proteins, peptides, nucleic acids, or adjuvants for vaccines and treated with a biopolymer such a chitosan to product a drug or vaccine delivery system. Once mixed with an active ingredient, the envelopes can be coated or blended with a biopolymer of any type to produce a protected closed entity, which can carry ingredients, including large molecules, nucleotides, and proteins, in a protected state.

EXAMPLE 31

When homogenized yeast lipid bodies are solvent extracted with hexane or pentane, the resulting opened envelope material described in Example 30 can be harvested by centrifugation and dried. The lipid body envelopes were thought to be a mixture of phospholipids, proteins, and glucan-like polysaccharides. Surprisingly, the envelope material of the invention was not consistent with chitin when evaluated by Raman spectrophotometry. The unique envelope material is a hard material that is heat tolerant. Additionally, in the polysaccharide region of a Raman spectra, the spectra are relatively close to a reference yeast beta-glucan (FIG. 17). The beta-glucan identification was further supported by the appearance of free glucan monomers following treatment with lyticase or equivalent beta-1,3 glucanase. These observations show that lipid body envelopes are unique with a robust shell.

EXAMPLE 32

In this example, lipid bodies were observed to have properties related to the interplay of hydrophobic and ionic environments. Lipid bodies are surprisingly stable under a wide range of ionic strengths, pH conditions and variable hydrophobicity. To demonstrate the aggressive binding of a hydrophobic substances when applying a strong ionic aqueous environment, lipid bodies were mixed into a 1 M sodium chloride

environment. A diluted sample was placed on a microscope slide with a cover slip and a drop of Sudan black was added to the edge of the cover slip and allowed to flow into the lipid bodies that were in the high salt environment. The charged environment was used to drive the model hydrophobic compound, Sudan Black, into the lipid bodies with a surprising effect as shown in FIG. 16.

The aqueous exterior and charge state of lipid bodies across a wide range of pH conditions can be used to enhance drug loading, permitting specialized compositions to exist. Some of these charges are useful in unaltered lipid-body compositions while other charges are formed by chemical or enzymatic treatment of lipid body surfaces with applications for soothing the skin, rapid repair of skin irritation, dryness, scar prevention and healing of blisters when applied by individuals with those conditions. This example shows that modification of the environment of lipid-body compositions can be used to improve drug uptake, particularly hydrophobic drugs such as those used for chemotherapy such as doxorubicin, and inflammatory conditions such as steroid-like compounds.

EXAMPLE 33

In this example, lipid bodies are released from the engineered yeast by mixing the *Lipomyces starkeyi* with 0.5 M to 1 M phosphoric acid, or 0.25 M to 0.5 M sulfuric acid, or any other acid with equivalent strengths and then placing the lipid bodies at 65°C., or preferably at 80°C, or even more preferably 121°C, for a period of time between 30 minutes and 18 hours, depending on the temperature of treatment. Using the high temperature acid hydrolysis used to isolate lipid bodies, this example illustrates the release of lipid bodies that can be observed because they float above the hydrolyzed yeast debris.

This example demonstrates the heat tolerance of lipid bodies under acid conditions at elevated temperatures, which are useful in the manufacturing of specific compositions of the invention. In fact, the lipid bodies have an ability to be treated at sterilizing temperatures as found in an autoclave e.g. 121°C. This property makes the compositions of the invention particularly useful for critical drug applications, where an aseptic product or subcutaneous injection may be applicable, for example in vaccine development. This example shows the tolerance of lipid bodies to very low pHs.

EXAMPLE 34

In this example, a deterioration of lipid bodies was observed when dehydrated. Lipid bodies, approximately 75 grams of a 50% lipid body suspension by dry weight were

spread on a metal pan and placed into a 65°C oven and allowed to completely dehydrate overnight. In the drying process, oil was expelled from the lipid bodies and the aqueous portion of the mixture was removed by heat. The expelled oil could be captured by tilting the tray and allowing the oil to pour into a 50 mL test tube. The yield of oil captured in this fashion was approximately half of the yeast oil that could have been isolated by chloroform: methanol extraction. The residual material after heating and oil recovery is also of value, as discussed below.

EXAMPLE 35

In this example, the residual material which remains after heating, drying, and removal of yeast oil from lipid bodies results in an interesting lubricant material. The dried material has a high lubricity and may be a useful component of industrial lubrication. The material has a slippery waxy feel at a wide range of temperatures. When heated on a hot plate the envelope material does not change in lubricity, color, or texture up to 200°C. Above 240°C, the material begins to turn brown. The lubricity of heat oxidized material was not considered, but this discolored material may still have lubricant properties. Material heated below 200°C was applied to both sides of a flat metal surface and the resultant lubricant appeared to have a durable and useful property of reducing frictional wear. This example shows that lipid bodies and envelopes, when dried, can be isolated to make a material with significant lubricity for lubricants that are heat and friction tolerant.

EXAMPLE 36

In this example, lipid-body compositions have extreme acid stability at pH levels at or below 0.9. Similarly, lipid bodies have alkaline tolerance, which facilitates transient removal of impurities, but more importantly this property broadens the useful range of pH conditions from very acidic to pH 9 and transiently to pH 11.7. This range can be used for loading various hydrophobic and hydrophilic ingredients into lipid-body compositions.

EXAMPLE 37

In this example, the lipid bodies were mixed with 2x-10x levels of water, water plus saline, or water plus citrate, at pH ranges from 1 to 11.7 at room or elevated temperatures. It was noted that some deterioration of yeast oil by saponification was observed at the highest alkaline pHs particularly when combined with elevated temperatures of 50-65°C, so the duration of lipid body exposure was minimized at high

pH levels, above 11, at all temperatures. Otherwise the lipid body suspensions exhibit good flotation, particularly when the pH was below 7.5, with initial upward boundary rates of 5 to 7 cm per hour or greater. The flotation rates are somewhat slower at a pH above 8.5. As the compression density of the floating lipid bodies increases during the flotation process, the upward rate of flotation slows. However, the property is particularly useful for washing and formulating by buffer exchange. Flotation in a water plus saline solution can be used to wash the lipid bodies.

In this example, bodies lipid have rapid flotation due to their very low buoyant density, which is useful in concentrating the lipid bodies that are loaded with hydrophobic chemicals and other industrial applications. The property is commercially useful for washing, processing, and buffer exchange steps leading up to and during the production of final compositions. This example shows the utility of the very low buoyant density of lipid bodies for washing and cleaning and isolating lipid bodies from residual reagents and chemicals.

EXAMPLE 38

In this example, a comparative evaluation was made comparing an existing commercial large liposome to the lipid bodies of the invention. The analysis was conducted using published data from a commercial liposome provider and data gathered by microscopic evaluation of lipid bodies using a Petroff Hauser Counter ocular scaling on a phase contrast microscope at either 400x or 1000x magnification. Additional data was obtained by quantitation of yeast oil levels in lipid bodies, using a solvent extraction followed by gravimetric analysis, which provided confirmation that the lipid bodies have an interior that is predominantly yeast oil, existing as triglycerides when measured by thin layer chromatography. The comparative analysis is shown in Table 6.

Table 6.

Impact of Volume on Lipid Content and Drug Loading Potential	Diameter in microns (μm)	Volume (Cubic μm)	Lipid content in 5 mL	Estimated Hydrophobic Drug Loading Capacity
Lipid Bodies of the Invention	~7 microns (6-10 μm)	174 cubic μm	~2500 mg	10-20 mg/mL

FormuMax™ PEGylated liposomes*	0.1 microns	0.00052 cubic μm	220 mg	1-2 mg/mL
Comparative Difference	6.8 microns per particle	332,314x- fold delta	11.4x- fold	10 x per mL of applied formulation

*Data from FormuMax Scientific Inc.

A primary observation of this analysis resides in the fact that the lipid bodies have a surprisingly thin but robust envelope that allows them to be large and stable with a significantly larger internal hydrophobic interior due to the engineering of the yeast metabolism as described herein. Large commercial liposomes are relatively small, typically less than 0.2 μm in diameter and they contain a significant amount of phospholipids required to make liposomes or stable emulsions in conventional compositions of hydrophobic ingredients. The lipid bodies of the invention have a significant and unexpectedly large internal hydrophobic volume.

EXAMPLE 39

Lipid bodies were harvested from engineered *Lipomyces starkeyi* according to the methods described above. Following the initial isolation by hydrolysis, the resulting lipid bodies are tan and have an odor similar to the fermentation medium. When appropriately washed in mild to moderate alkaline conditions, the lipid bodies become bright white using a wide range of basic reagents. In this example, the lipid bodies were washed with 14 g/l sodium carbonate plus 1 g/l of sodium percarbonate at a pH of 8.5 to 9.5. The resulting wash results in the removal of the tan color and residual fermentation smell leaving a bright white and appealing cream.

In a similar example, the lipid bodies were treated with a sodium hydroxide solution which was added slowly with mixing to the lipid bodies until a pH of 11.7 was reached. The mixture was separated by flotation. The product of the sodium hydroxide treatment was a bright white cream with no residual smell from the fermentation medium.

In another version of this example, lipid bodies obtained from the fermentation as described above were mixed with either 14 or 15 g/l of sodium carbonate, 1 g/l of sodium percarbonate to obtain a pH of 8.5-9.5. The treated lipid bodies were made more alkaline by the addition of sodium hydroxide until a pH of 11.5 was obtained. Mixing was allowed to proceed for 10 minutes to 1 hour and then the pH was lowered with citric acid to pH 7.0 or below, until any excessive CO₂ was displaced from the mixture. The resulting lipid

bodies were bright white without smell and in all cases the result was the same for all cases within this example. The use of additional sodium hydroxide did not remove any more color than just the sodium carbonate and sodium percarbonate treatment. Additionally, raising the pH above 11 can trigger undesirable saponification, unless the
 5 intention is to form a soap in a composition. The lipid bodies are visualized as a bright white cream that dominates the appearance of lipid bodies compositions.

This example shows the bright white color of either lipid bodies alone or when formulated for microbial and oxidative stability. The example supports the stability of the lipid bodies under elevated temperatures and acid conditions used in their isolation and
 10 subsequent washing. The lipid bodies, produced in each of the cases of this example and in many more preparations, had a near-odorless state with a sooth pleasant aqueous feel, even though they are loaded with yeast oil.

EXAMPLE 40

In this example, lipid bodies were prepared with approximately a 50% concentration as measured by dry weight in stabilizing excipients that in total make the minimal base composition of lipid bodies. The ~50% lipid body cream was diluted in a stabilizing excipient buffer described in Example 2B to determine what level of interstitial aqueous fraction was possible and still maintain a white appearance and other unique
 20 properties of the compositions of the invention. The resulting creams and diluted creams (lotions) were evaluated for visual appearance and microscopic appearance. Additionally, the dilutions were applied to the back of the hands of three test subjects and a consensus opinion about texture was generated. Table 7 shows the dilutions and proportion of interstitial aqueous portions evaluated by feel and consistency.

25

Table 7.

Sample Description	Concentration of lipid bodies	Interstitial Aqueous Fraction	Appearance	Texture description and Feel
Starting Formulation (see Example 43)	~50%	~50%	White Cream, non-dripping on inversion	Like Soft Cream-Cheese with a smooth feel
Dilution 1	30-33%	67-70%	White Cream,	Like Yogurt

			non-dripping on inversion	with a smooth feel
Dilution 2	18-25%	75-82%	While Lotion, drips	Like whole milk with a smooth feel
Dilution 3	12 %	88%	White Lotion, viscous* and drips	Like 2% fat milk with a smooth feel
Dilution 4	6%	94%	White Lotion low viscosity drips	Like skim milk with a smooth feel
	3%	97%	White Thin Lotion, drips	Like skim milk that is diluted 1:2 with water

*The lipid body suspension is non-Newtonian when measured on a viscometer, values not reported.

5 The conditions listed in Table 7 have the property of a significant interstitial aqueous zone around the exterior of lipid bodies, while maintaining a solid white appearance, when displayed against a black background. Textures and viscosities vary with dilution, but the color remains a solid white for both creams and lotions down to a 3% concentration of lipid bodies.

10 Having a wide range of available aqueous fractions, while maintaining a solid white color, is important for the inclusion of hydrophilic ingredients containing lipid bodies. The retention of a solid white appearance important and masking benefits when blending in off-color ingredients. The density of light absorbance was pronounced even in a suspension containing 3% lipid bodies, which appears as a solid color.

EXAMPLE 41

15 In this example, lipid bodies were prepared as described in Example 43. The alkaline washed and pH adjusted materials were homogenized and extracted with chloroform/methanol extraction to establish the 100% value to total oil. When compared to the dry weight of the lipid bodies in the preparation, the oil content of the large lipid bodies approaches a total oil content of greater than 90% by gravimetric analysis and
20 some preparations greater than 90%, when considering both triglycerides, phospholipids

and lipid containing envelope proteins. The chloroform/methanol extraction is the gold standard for yeast oil measurement. When lipid bodies are extracted with hexane, then the resulting oil level in the lipid bodies appears to be 75-80% of the total gravimetric mass of lipid bodies. In this example, an analysis was conducted to evaluate the relative quantity of envelope phospholipids compared to neutral triglycerides (yeast oil). As a positive control, egg yolk was used to inform the expected locations of various lipid classes. The results suggest that the envelopes and exterior of the lipid bodies contain relatively small amounts of phospholipid when compared to amounts of internal neutral yeast oil as shown in FIG. 18. The presence of low levels of phospholipids was confirmed by lipidomic analysis with LC-MSMS (liquid chromatography-mass spec-mas spec).

The data in this example shows the high level of yeast oil triglycerides (neutral lipids) in the lipid bodies, *e.g.*, an internal lipid content of at least 66% and as much as 90% lipid by gravimetric analysis. The example contributes to the explanation of the relatively small occupancy of the outwardly facing envelopes.

EXAMPLE 42

Whole yeast containing lipid bodies were homogenized and the yeast oil extracted with pentane, plus an emulsion breaker such as ethanol. The pentane was slowly removed by evaporation at room temperature and the yeast oil formed a solid above the liquid in which it was suspended. The same result can be obtained by first isolating the lipid bodies and then homogenizing them. The yeast oil was then removed and placed on a tin tray and warmed to 40-50°C, after which the yeast oil became a liquid. This is a unique and important property of this component of lipid bodies because of the frequent need to have products melt when applied to the skin or consumed. The following picture demonstrates the melting point transition of the yeast oil component of lipid bodies.

The fact that the lipid bodies have the property of melting at body temperature and above is important for imparting hydrophobic ingredients into the skin as a mobile liquid. Alternatively, there may be instances when restricting mobility of a formulated drug or composition may be required and, in that case, the ingredient will remain in a solid state by maintaining the temperature at or below room temperature. Additionally, lipid bodies can be frozen and melted through multiple cycles providing that free oil is not present that could float to the top of a composition when warmed. The base composition with 50% lipid bodies tolerates multiple cycles of being frozen and thawed without injury to the lipid body spheres.

EXAMPLE 43

The UV absorbance of lipid bodies (FIG. 11) corresponds to the high light absorbance and reflectance described in Example 44. However, the absorbing wavelengths
5 of lipid bodies are in the UV regions between 290 nm and 190 nm that are important for blocking the harmful rays of the sun. However a valid sunblock composition could be enhanced by using lipid bodies to take-up additional sunblock ingredients, particularly if those ingredients can be facilitated by the hydrophobic or hydrophilic nature of the compositions of the invention that suggest that sunblock agents need only protect against
10 longer wavelength UVC rays when such agents are loaded into the compositions of the invention.

This example shows the utility of lipid bodies in sunblock preparations, such as when sunblock reagents are added. There is a need for safer alternative sunblock reagents that can be made to feel smooth and aqueous.

15

EXAMPLE 44

In this example, the viscometric properties were measured in a lipid body-based composition that contained 30% to 50% lipid bodies by dry weight. This observation demonstrates that lipid body cremes exhibit the property of non-Newtonian viscosity. The
20 utility of non-Newtonian viscosity means that compositions of the invention will have a high viscosity at low shear rates, and have a lower viscosity at higher shear rates. This is particularly useful for making the creams and compositions that spread easily but are resistant to dripping.

25 **EXAMPLE 45**

Compositions containing 3% to 50% lipid bodies were mixed with 2% to 5% modified starch (Star-Design Power, Cargill). The resulting compositions had the property of stable suspensions despite low density of lipid bodies, as low as 6% when left to stand for 4 hours. The inhibition of flotation at a fixed level of modified starch at 2% was
30 adequate to maintain the suspension of lipid-body compositions for longer periods of time with increasing lipid body concentrations and or increasing concentrations of modified starch. In this example, lipid-body compositions containing 2% modified starch in a composition with 30% lipid bodies remained suspended without separation for 90 days at room temperature. This example highlights the important shelf-life compositions that are

possible when lipid-body compositions include a low concentration of hydrophilic polymers and starches. In another version of this example, hyaluronic acid, glycerol, Polysorbate-80, and non-modified starch can be used to achieve stable suspensions of lipid body containing compositions.

5

EXAMPLE 46

As a cosmetic composition, lipid bodies have the property of being relatively benign on human skin and have a demonstrated low irritation level in human exposures. An interim test with a skin cream formulated with approximately 50% lipid body skin cream was performed (FIG. 19) and shows no adverse events or skin irritations. This example shows that the lipid bodies, when used in all types of compositions, are benign and non-irritating on the skin, as shown in this cosmetic composition data above.

Currently the largest commercially available liposomes for hydrophobic drug loading are from FormuMax™ and those are PEGylated liposomes that are 0.1 microns. Table 8 illustrates the relative sized and volumetric values for FormuMax™ hydrophobic PEGylated liposomes in comparison to lipid bodies of the invention.

Table 8. Comparison of lipid bodies compared to FormuMax™ Liposomes.

Impact of Volume on Lipid Content and Drug Loading Potential	Diameter in microns (μm)	Volume (Cubic μm)	Lipid content in 5 mL	Hydrophobic Drug Loading Capacity
Lipid bodies of the invention	~7 microns (6-10 μm)	174 cubic μm	~2500 mg	10-20 mg/mL
FormuMax™ PEGylated liposomes*	0.1 μm	0.00052 cubic μm	220 mg	1-2 mg/mL
Comparative Difference	6.8 microns per particle	332,314x-fold delta	11.4x-fold	10 x per mL

*Data and Trademark of FormuMax Scientific Inc.

20

EXAMPLE 47

Lipid bodies were exposed to an electric field using copper electrodes and they migrated toward the positive (cathode) pole (FIG. 20A). During the course of electrophoresis, they accumulated copper. When repeated with simple mixing, the binding

of copper was independent of the electrical field. Unexpectedly for a lipid composition, lipid bodies of the invention chelate copper and perhaps other divalent metals. The chelation of copper by lipid bodies of the invention has medical utility in Wilson's disease and the addition of copper to lipid bodies of the invention increases the density of lipid
5 bodies such that they become neutrally buoyant in water, property that is useful in maintaining stable suspensions of lipid bodies in some applications.

Wilson disease is found worldwide, with an estimated prevalence of 1 case per 30,000 live births in most populations, although data from population screening by molecular sequencing in the United Kingdom suggest a potentially higher prevalence,
10 perhaps as frequent as 1 case in 7021. Assuming a prevalence of 1 in 10,000 to 30,000, approximately one person in 90 carries an abnormal copy of the *ATP7B* gene. However, in some isolated populations, the prevalence is much higher. One of the highest reported prevalence was from a small mountain village on the island of Crete, where Wilson disease was diagnosed in 1 in 15 births. Excess copper in the diet leads to pathologic
15 accumulation of the metal in the liver, cornea, and brain in patients with insufficient copper transporter proteins.

Engineered lipid bodies of the invention effectively chelate copper and become blue from copper, which is displaced from the wire electrodes in an aqueous electrophoresis setting, using in a 12 Volt DC, 1.75-amp system. Upon chelation of
20 copper, the structure and charge properties of lipid bodies remain unchanged, but the density of lipid bodies increases (FIG. 20B).

EXAMPLE 48

The invention of lipid bodies of the invention that can be manipulated with respect
25 to density is a very useful property for shelf-life stabilization of lipid bodies of the invention suspension in dilute compositions comprising lipid bodies of the invention. Finally, the lipid bodies of the invention can also be loaded with high levels of divalent cations such as copper to achieve an excess of charge, which leads to a change in electrophoretic migration at neutral pH (FIG. 20A). The property of having a net positive
30 charge at neutral pH can facilitate the development of drug and nucleic acid therapeutics comprised of lipid bodies of the invention.

EXAMPLE 49

Sterile lipid bodies of the invention were found to be essentially tasteless. A 27% lipid bodies of the invention suspension in phosphate buffered saline at neutral pH was tasted and found to have no specific taste other than that of the slightly salty phosphate buffered saline. The property of being tasteless is an important property of lipid bodies of the invention for use in oral drug delivery systems and oral or respiratory vaccines comprising lipid bodies of the invention.

EXAMPLE 50

Lipid bodies of the invention can be coated with a cationic polymer such as chitosan or specific cationic peptides or cationic sections of larger proteins and ligands. Additionally, lipid bodies of the invention treated with or coupled to cationic peptides can enhance the ability of lipid bodies of the invention to delivery drugs and genes in a manner described by Hashida et al. 2015. Cationic peptides can play a role in transporter-based import across the intestinal mucosal layer or enhance endocytosis across the mucosal layer. Similarly, cardiotoxins can facilitate transport across phospholipid monolayers as described in Bougis et al. 1981. The property of transmucosal transport is a useful property in the development of therapeutic products, which incorporate specific drugs, nucleotides, genes, plasmids and other genetic and immune modulators comprising lipid bodies of the invention as a carrier in such therapeutics.

EXAMPLE 51

This example demonstrates intrinsic fluorescence of lipid bodies of the invention. Water (H₂O), phosphate buffered saline (PBS) buffer, or lipid bodies at the indicated percent dry weight in PBS (0.125%, 0.25%, and 0.5%) were subjected to six sets of the indicated excitation and emission wavelength pairings. In neither scenario did the lipid bodies display a higher intrinsic fluorescence than both the water or PBS samples, and in most cases were identical to the PBS sample (FIG. 21). In the case of the 360 nm/460 nm excitation and emission pairing, the lipid bodies had a slightly higher intrinsic fluorescence over the PBS sample, but were lower or comparable with the water sample (FIG. 21). These results demonstrate the feasibility of detecting fluorescent compounds or drugs loaded into lipid bodies by monitoring an increase in overall fluorescence at established excitation and emission wavelengths of the lipid body loaded fluorescent compounds or drugs relative to unloaded lipid bodies.

EXAMPLE 52

This example demonstrates the titration of lipid bodies with 1 M NaOH or 1 M HCl. Water-washed lipid bodies at 10-15% dry weight in water, as well as the water (free of lipid bodies) used to wash the lipid bodies (liquid suspension), were subjected to titration with either 1 M NaOH or 1 M HCl. Titration of the lipid bodies with 1 M NaOH displayed a slight buffering capacity above pH 7 compared to the aqueous wash liquid, and was especially pronounced above pH 9.0 (FIG. 22). However, the titration of lipid bodies with 1 M HCl achieved acidic pH more readily compared to the aqueous resuspension liquid (FIG. 22). A number of explanations could describe this behavior, such as saponification of lipid bodies under alkaline pH conditions, and the displacement of free water by lipid bodies allowing sharper changes in pH where no buffering capacity is present. These results demonstrate a unique titration profile of lipid bodies compared to the liquid in which they are suspended.

EXAMPLE 53**Enzymatic Treatments**

Cell lysis is among the most difficult process steps for recovering oil from yeast, especially for the recovery of highly purified lipid free from odor and color. The present examples show a scalable process for lipid body recovery that involves hydrolysis with 0.5 M phosphoric acid and heating at 90 to 121°C followed by washing. The oil itself can be recovered by direct high-pressure homogenization of the cells following alkali treatment and organic extraction. This process yields a high-quality oil, but it depends on the use of a very high-pressure and expensive homogenization system.

Beta-(1,3)-glucanases digest the cell walls of yeasts. These are often known as yeast cell wall degrading enzymes (YCWDE) or yeast lytic enzymes (YLE). Originally, yeast cell wall degrading enzymes were derived from snail gut (Giaja 1914, Uzuka et al. 1975). Many different microbial sources were later described (Kitamura et al.). Such enzymes have been used to make yeast protoplasts from viable cells to transform cells with DNA (Marchand et al. 2007), to release endogenous or heterologous enzymes (Andrews et al. 1987 *Trends in Biotechnology*) or to extract carotenoids (Michelon et al. 2012), among other uses.

The enzymes produced by *Oerskovia xanthineolytica* (Jeffries et al. 1981, Mann et al. 1978) (aka: *Arthrobacter luteus* (Kitamura et al. 1971, Kanedo et al. 2014),

Cellulosimicrobium cellulans) include at least three enzymes, two of which possess the Ricin B Lectin binding domain, which enables these enzymes to attach to the yeast cell walls and release pentameric oligosaccharides from the cell walls (Jeffries et al. 1981). A continuous cultivation process allows rapid and economical production of YLE from *O. xanthineolytica* (Andrews et al. 1987 *Biotechnol Bioeng*).

Yeast Lytic Enzymes

Bacterial endo-1,3- β -glucanases (EC 3.2.1.6 and 3.2.1.39) are integral for assimilating fungal cell walls as a food source in nature, because they catalyze the hydrolysis of cell wall β -1,3-glucosidic linkages (Tanabe et al. 2011). Bacteria from the species *Cellulosimicrobium cellulans*, previously known as *Oerskovia xanthineolytica* and *Arthrobacter luteus*, produces yeast cell wall degrading enzymes. These bacteria are various strains of high GC nocardoid actinomycetes.

Three genes encoding endo- β -1,3-glucanases from two *C. cellulans* have been cloned and characterized (Ferrer 2006). The β glII and β glIIA genes from strain DSM 10297 (also known as *O. xanthineolytica* LL G109) encode proteins of 40.8 and 28.6 kDa, respectively, whereas the β -1,3-glucanase gene from strain ATCC 21606 (also known as *A. luteus* 73-14) encode a 54.5 kDa protein (GenBank: BAA04892.1). β glII and β glIIA have catalytic domains assigned to GH family 16 of glycosyl hydrolases, whereas the catalytic domain from the 54.5kDa glucanase belongs to GH family 64. both β glII and the 54.5 kDa β -1,3-glucanase are multidomain proteins, having a lectin-like C-terminal domain that has been assigned to GH family 13 of carbohydrate binding modules, and that confers to β -1,3-glucanases the ability to lyse viable yeast cells.

In cloned, nuclease and protease-free formulations, yeast cell wall degrading enzymes are used to release protoplasts from *S. cerevisiae* and other yeasts (Ferrer 2006). One characteristic of the principal enzyme known to produce yeast protoplasts (β -1,3-glucanase I) is its capacity to rapidly solubilize insoluble laminarin and produce laminaripentaose. Because of the oligomeric nature of this product, few reducing groups are formed, but the ricin binding domain enables it to readily attack yeast cell walls. Enzymes belonging to this class are known from *C. cellulans* (Tanabe et al. 2011) (*Oerskovia xanthineolytica*) (Jeffries et al. 1981, Mann et al. 1978), *Arthrobacter sp.* YCWD3 (Doi et al. 1986), *Arthrobacter luteus* (Tanabe et al. 2011, Watanabe et al. 2014, Aono et al. 1995, Nakabayashi et al. 1998), and *Streptomyces matensis* (Shrestha et al. 2011). A second enzyme, β -1,3-glucanase II, attacks oligomeric β -1,3-glucans and reduces

them to glucose, and β -1,3-linked dimeric oligosaccharides. It can also release trimeric β -1,3- β -1,6-branched oligomers.

A number of methods for producing yeast lytic enzymes have been described. See DeWi et al. 2016 for the production of β -glucanase production by *Bacillus circulans*.
 5 None of the three GH-16 β -1,3-glucanases found in *Bacillus circulans* include the Ricin B Lectin domain that enables binding to yeast cell walls; hence they are predicted to be less effective than the enzyme from *C. cellulans*. See Ferracini-Santos et al. 2008 for the production of alkaline protease from *Cellulosimicrobium cellulans*. See Mann et al. 1978 for the production of yeast lytic activities from *O. xanthineolytica*. See Andrews et al.
 10 1987 (*Biotechnol Bioeng*) for the induction of yeast lytic enzymes in *O. xanthineolytica*. See Andrews et al. 1987 (*Trends in Biotechnology*).

Strain, Media, and Cultivation of *C. cellulans* to Produce Yeast Lytic Enzyme

There were two motives of this example: (1) to dispose of the acid hydrolysate
 15 and/or (2) eliminate the need for acid hydrolysis by replacing phosphoric acid with a yeast cell wall degrading enzyme (YCWDE).

The strain of *Cellulosimicrobium cellulans* used herein to demonstrate the utility of yeast lytic enzymes for recovery of lipid bodies and oil was NRRL B-4567, which corresponds to ATCC 21606, but other strains possessing the appropriate enzymes may be
 20 used. Strain NRRL B-4567 was used by Ferrer et al. 2006. A sequencing project for this strain has been announced by Peking University (*Cellulosimicrobium cellulans* strain: ATCC 21606 Genome sequencing and assembly; NCBI Accession PRJNA505736). An assembly is available (NCBI Accession ASM523930v1).

This strain has at least one glycosyl hydrolase GH64 “Ricin-type beta-trefoil lectin
 25 domain protein”, which releases laminaripentose ($[\beta$ -D-glucosyl-(1,3)]₄- β -D-glucosyl and which rapidly breaks down the β -D-(1,3/1,6)-glucan in the yeast cell walls (NCBI Accession WP_137279716.1, *Cellulosimicrobium cellulans*, family GH64 ricin-type beta-trefoil lectin domain protein; SEQ ID NO:42). It also has at least one glycosyl hydrolase (GH16) “Family 16 glycosylhydrolase”, which hydrolyses the laminaripentose to glucose.
 30 Both of these proteins have the Ricin B Lectin binding domain, which enables the enzymes to attach to the yeast cell walls (NCBI Accession WP_137281286.1, *Cellulosimicrobium cellulans*, family GH16 glycosylhydrolase; SEQ ID NO:43).

In these examples, *C. cellulans* NRRL B-4567 is used as the producer organism and autolyzed nutritional yeast (AY) yeast glucan (YG) and/or a phosphoric acid

hydrolysate of *L. starkeyi* (LS Hyd) as medium components. The AY, YG and LS Hyd media were all adjusted to between 6.8 and 7.2 prior to inoculation. AY medium contained the components shown in Table 9. The YG medium contained the components shown in Table 10.

5

Table 9. AY medium.

Component	Amount (g)
Peptone	2.5
Yeast extract	2.5
K ₂ HPO ₄	1.3
Glucose	0.5
Autolysed yeast	10.0
DH ₂ O QS	1.0 L
pH	6.93

Table 10. YG medium.

Component	Amount (g)
K ₂ HPO ₄	0.65
KH ₂ PO ₄	2.5
(NH ₄) ₂ SO ₄	0.5
NaCl	2.5
MgSO ₄ •7H ₂ O	0.12
yeast extract	1.5
β-glucan	10.0
Water	1.0 L

10 The LS Hyd was used directly after adjusting the pH of the phosphoric acid hydrolysate from 1.2 to between 6.9 and 7.1 by the addition of KOH. The starting H₃PO₄ concentration was relatively high (ca. 56 g/l or 0.5 M), so the medium prepared from the hydrolysate was strongly buffered and did not shift significantly during the fermentation.

15 **Assays**

 The presence of yeast lytic activity can be assayed by the methods of Dewi et al. 2016, Jeffries et al. 1981, or others. Substrates may comprise live yeasts suspended in a

buffered osmotic support such as 1- or 2-M KCl, sorbitol, or some other osmotic support with or without supplemental dithiothreitol. Yeast lytic activity may be quantified by optical density readings before and after osmotic shock, from which one can measure the rate or extent of yeast lysis. Activity may also be assayed using isolated yeast cell walls, beta-(1,3)-glucan isolated from yeast or other sources as the substrate. Enzyme activity may be quantified by measuring the release of reducing sugars using the DNS or Nelson-Somogyi methods or by separating the products of the enzymatic hydrolysis on an HPLC column. Other dye-release methods from appropriate substrates may also be used to assess activity.

10

Preparation of Lytic Enzyme

The LS Hyd was used directly after adjusting the pH of the phosphoric acid hydrolysate from 1.2 to between 6.9 and 7.1 by the addition of KOH. The starting H₃PO₄ concentration was relatively high (ca. 56 g/l or 0.5 M), so the medium prepared from the hydrolysate was strongly buffered and did not shift significantly during the fermentation. LS Hyd medium alone produces an active *Lipomyces* lytic enzyme after 96 h of incubation in a flat-bottom 125 mL Erlenmeyer at 28°C, shaken at 250 rpm. The LS Hyd substrate is high in phosphate and nutrients and does not reliably produce large titers of yeast lytic enzymes. Moreover, the large amount of residual hydrolyzed yeast proteins and wall material make the LSHyd *C. cellulans* lytic enzyme complex and when used to recover lipid bodies or oil, the product is contaminated with the hydrolysate degradation products and proteins.

The AY medium was made up as indicated, autoclaved and distributed to baffled 125 mL Erlenmeyer flasks, inoculated with a small amount of NRRL B-4567 from a fresh plate and incubated with shaking at 250 to 350 rpm, 28°C for 24 to 48 h. Samples are removed after 7, 12, 18, 24 and 48 h and tested for their abilities to hydrolyze 1% yeast glucan and to release lipid bodies from whole cell broth. In this example, the pH of the reaction mixture comprising 5, 10 or 20% AY enzyme preparation ranged from 5.5 to 7.5. Reaction mixtures with the pH at 7.0 had higher activity than reaction mixtures at pH 5.5. The temperature of incubation ranged from 30°C to 50°C. The reaction mixtures incubated at 50°C had higher activity than reaction mixtures at 30°C.

The YG medium was likewise made up as indicated, distributed to baffled Erlenmeyer flasks, autoclaved, inoculated with NRRL B-4567 and incubated with shaking at 250 to 350 rpm, 28°C for 24 to 48 h. Samples were removed after 7, 12, 18, 24 and 48 h

and tested for their abilities to hydrolyze 1% yeast glucan and to release lipid bodies from whole cell broth. The cell lytic activities were similar to those observed with AY medium. Samples from the AY and YG cultures were also examined microscopically. NRRL B-4567 initially grows with a thin filamentous morphology. With time, the filaments grow
5 thicker and break into rods, which then progress to the stage of small spherical cells. The optimal time for cell transfer to larger scale is when the cells are in the rod stage.

Release of Lipid Bodies

For the purposes of this example, release of lipid bodies was assessed by direct
10 inspection of cell suspensions, microscopically, or by HPLC of the cell-free assay medium following treatment of highly lipogenic *Lipomyces starkeyi* with enzyme preparations. In the first instance, enzyme preparations were added to whole broth containing highly lipogenic cells in which the pH ranged from 5.0 to 7.5. Alternately, the cells may be suspended in water, physiological saline or 1 M KCl along with a 0.1 to 0.025 M
15 phosphate buffer of the corresponding pH. The concentration of lipogenic cells used as a substrate can range from 10% to 50% on a volumetric basis. The cell suspension plus the enzyme with or without additional buffer or osmotic support is incubated at 37 to 50°C for 2 to 24 h and the cells are examined directly, microscopically or by separating the supernatant lipid bodies from the enzyme plus buffer reaction mixture and measuring the
20 amount of glucose and oligosaccharides released. The concentration of crude enzyme broth can range from 1% to 20% of the reaction volume depending on the cell concentration, the enzymatic activity and the length of the incubation.

In direct observation, active preparations caused the lipid bodies to clump and go to the surface of the vessel. In small-volume assays of 5 mL, the lipid bodies clumped at
25 the top of the tube. This can be discerned either by the length of time that it takes for the layer of cells at the top of the tube to be disrupted by mixing or by the rapidity with which the cells rise to the surface following mixing. In both cases, the enzyme-treated cells or lipid bodies are compared to cells in a control tube that receives either buffer without enzyme or a heat-killed enzyme preparation. This result can be observed with 5, 50, 250,
30 500 and 1000 mL reaction mixtures or larger. The reaction rate is accelerated if mixing is applied during the incubation. Following enzymatic treatment, the lipid bodies can be separated from the hydrolysate by dilution and washing with water or physiological saline. The lipid bodies rise to the surface and the hydrolysate can be drawn off from the lower level.

In microscopic observation, the enzymatically prepared lipid bodies appear smooth and unlike untreated cells they do not contain intracellular constituents. The lipid bodies tend to clump together and form flat surfaces with adjacent lipid bodies. Prior to washing fragments of intracellular constituents can be seen microscopically.

- 5 HPLC analysis of the cell-free, lipid body-free reaction medium reveals the accumulation of glucose, disaccharide and higher oligosaccharides in the reaction medium as compared to the cell suspensions incubated without enzyme or active enzyme.

Experimental Determination of pH and Temperature for Enzymatic Hydrolysis

- 10 Enzymatic cell wall lysis and release of lipid bodies can be done optimally in whole broth following cultivation of the lipogenic cells and adjustment of the broth to pH 7.0. The crude enzyme preparation is added to the cell suspension and the broth is again adjusted to the optimal pH as necessary. The broth plus enzyme is agitated without aeration and the temperature is raised to 50°C. Samples are periodically removed and
15 assessed microscopically or by centrifugation and HPLC analysis of the cell-free broth. Typically, a final fermentation may contain 30% cells by packed volume, which will require dilution prior to recovery of the lipid bodies.

Scale Up

- 20 The fermentation can be scaled up in a fed-batch mode. In this example, 25 to 50 mL of AY or YG medium in a 125 to 250 mL baffled or flat-bottom Erlenmeyer flask is inoculated with NRRL B-4567 for 15 to 20 h. The inoculum is then transferred to a 3-liter bioreactor pH 5.3 and agitated at 200 to 700 rpm with 1 L air per L medium per minute (1 v/v min). The AY or YG medium is fed into the reactor slowly so as to keep the culture
25 under glucose limitation. After 20 to 24 h, the reactor is harvested, the suspended cells are removed by centrifugation and the enzyme preparation is stabilized by refrigeration or the addition of 0.1% sodium benzoate.

Properties of the Lipid Bodies Following Isolation

- 30 Following washing and isolation, the enzymatically prepared lipid bodies are osmotically more fragile than lipid bodies prepared using phosphoric acid hydrolysis. They appear to have a thinner membrane and a more hydrophobic surface.

Breakage using low pressure homogenization: The enzymatically treated lipid bodies may be homogenized immediately following incubation at 50°C. One aspect of

their physical properties is that they can be disrupted more readily at a lower homogenization pressure than whole cells either with or without treatment with alkali. Their subsequent extraction with hexane or pentane and ethanol yields a clean oil without the formation of a rag layer or particulates.

- 5 *Release of lipid from enzymatic lipid bodies following osmotic shock:* If the enzymatically treated lipid bodies are suspended in a strong osmotic medium such as 15 g/L of sodium carbonate plus 1 g/L of sodium percarbonate plus 55 g/L of sodium citrate and then immediately diluted into a 10-fold volume of water, they will rupture and release their contents

10

Production of Lipid Body Creams from Enzymatically Recovered Lipid Bodies

- When the enzymatically treated lipid bodies are used to make a cream, the texture of the cream is such that it is smoother and does not readily separate. The resulting enzymatically treated lipid bodies may be suitable for formulation with more hydrophilic
- 15 constituents than the phosphoric acid treated lipid bodies.

EXAMPLE 54

A summary of the some conclusions from the foregoing examples is as follows.

- The lipid bodies of the invention can have a diameter larger than 5 μm , a diameter
- 20 from 5 μm to 12 μm , and/or can be merged to have a diameter of 12 to 50 μm or more.

The lipid bodies can be processed to generate isolated components thereof, such as envelopes, yeast oils and other lipid body components or biochemicals.

- The lipid bodies and/or any one or more components thereof can be formulated to generate compositions of the invention for drug delivery, consumer products, industrial
- 25 products, and other products.

The compositions of the invention can be formulated in the form of pastes, creams, lotions, and solutions.

The compositions of the invention can include from 1% to 99% lipid bodies, when measured as a percent dry weight of the formulation

- 30 The compositions of the invention can be formulated in an aqueous mixture that can carry hydrophobic drugs, lipids, and hydrophobic active ingredients on the interior of the lipid bodies, while simultaneously carrying large quantities of hydrophilic drugs in the exterior of the lipid bodies.

The compositions of the invention can be applied to the skin without feeling oily.

The compositions of the invention comprising lipid bodies of the invention, when applied to the skin, collapse to form a sealing and protectant surface once the lipid bodies in the composition discharge their content. The compositions quickly dry on human skin after the discharge of hydrophobic and hydrophilic ingredients, drugs, and additives, 5 impart a durable protecting layer from the exterior envelopes. The collapse of the lipid body envelopes can seal and retain any ingredients discharged by the composition. This can occur for a period of at least 10 hours. Such compositions can be used as topical drug compositions in the field of topical drug administration and other medical or veterinary fields. These compositions can be important replacements for liposomes and emulsions, 10 which fail to provide retention and protection of treated areas.

The lipid bodies can be modified, charge manipulated, homogenized, and/or dehydrated to create envelopes.

The envelopes can be included in compositions of the invention with or without intact lipid bodies to serve as modified surfaces that can change the types of active 15 ingredients loaded in the compositions. The envelopes can enhance the formulation of large molecules, biologics, and/or peptides for transport into the human body at any point of entry.

The lipid bodies and/or envelopes in the compositions of the invention can have an adjustable and modified surface charge state across a wide range of pH conditions, 20 permitting improved product compositions for specific applications. Some of these charge modifications are useful in unaltered lipid-body compositions. Other charges are formed by chemical or enzymatic treatment of lipid body surfaces. Compositions with lipid bodies or lipid-body envelopes with modified surface charge states can include anti-inflammatory drugs, steroid-like compounds, or other active ingredients. The compositions can contain 25 both hydrophobic and hydrophilic active ingredients. The compositions can be used as a topical composition for soothing the skin, rapid repair of skin irritation, burns, dryness, scar prevention and healing of blisters when applied by individuals with those conditions.

The lipid bodies have tolerance to heat and the ability to be sterilized by autoclaving at 121°C. This makes the lipid bodies particularly useful for critical drug 30 applications, such as subcutaneous injection or vaccines.

The compositions of the invention can include lipid bodies supplemented with yeast oils and/or exterior envelopes that can be isolated by dehydration. Such compositions of the invention have significant lubricity and can be used as industrial or medical lubricants that are heat and friction tolerant.

The lipid bodies of the invention have extreme acid stability to pH levels at or below 0.9, making acidic product compositions and industrial compositions possible.

The lipid bodies of the invention have mild alkaline tolerance, which facilitates transient stability to pH levels at or above 9 to about 12. This is useful in conducting
5 chemical modifications of lipid bodies containing product compositions for medical compositions, consumer care products, cleaners, and de-oiling purposes by the creation of saponified product compositions.

The lipid bodies of the invention have low buoyant density, which is useful in concentrating hydrophobic chemicals and other commercial applications for industrial
10 compositions, such as compositions for the concentration and cleanup of hydrophobic toxic substances and other environmental uses.

Lipid bodies having a diameter 5-12 microns or greater provide internal capacity much greater than conventional liposomes and emulsions and can be used in for medicinal or veterinary product compositions where emulsifiers are undesirable or potentially
15 irritating.

The lipid bodies and compositions thereof can be bright white and oxidation resistant, even when heated or treated with acid at high temperatures. This property is useful in compositions in which oxidation needs to be avoided, either for performance or consumer acceptance.

20 The lipid bodies and compositions thereof can have a solid white color and a smooth aqueous and pleasant feel under a wide range of conditions and dilutions. This can be a valuable characteristic in many consumer products, medical compositions, and industrial compositions.

The compositions of the invention can include a significant interstitial and surface
25 aqueous zone around the exterior of lipid bodies. This can be important for carrying high concentrations of charged ingredients, hydrophilic drugs, and moisture to areas of application.

The lipid bodies of the invention can have internal lipid contents of at least 66% and as much as 90% or higher, when considering both triglycerides and phospholipids,
30 that are useful as biochemical components and/or for loading hydrophobic drugs in medicinal compositions.

The encapsulated oil in the lipid bodies have a melting temperature at approximately human body temperature and above. The external addition of yeast oil isolated from the lipid bodies imparts a soft-to-liquid texture to elevated-temperature

compositions and a softer or even liquid state when warmed above body temperatures. Such compositions can be useful in consumer goods, medical compositions, personal-care compositions, and industrial compositions, where a temperature sensitive state and texture transition to a more liquid state is important for the performance of the composition.

5 The encapsulated oil in the lipid bodies is a solid at room temperature and below. This property can be exploited to impart a solid texture to room-temperature compositions and a softer or even liquid state when warmed to body temperatures. Such compositions can be useful in consumer goods, medical compositions, personal-care compositions, and industrial compositions, where a temperature-sensitive state and texture transition to a
10 more solid state, for example ice cream or deodorant sticks, is important for the performance of the composition.

 The lipid bodies have high UV absorbance. This property makes the lipid bodies useful for sunblock compositions or other compositions in which UV absorbance is important. The sunblock compositions can include the lipid bodies as the sole UV
15 absorbing agent or can include the lipid bodies in addition to other UV absorbing agents. Sunblock compositions that include additional UV absorbing agents have native UV absorbance and enhanced UV absorbance.

 The lipid bodies have a higher viscosity at low shear rates and a lower viscosity at high shear rates, a characteristic of non-Newtonian viscosity modifiers. This is a property
20 useful for making the creams and compositions that spread easily but are resistant to dripping. The compositions of the invention can include lipid bodies with or without other viscosity modifiers or ingredients.

 The lipid bodies can be formulated as stable suspensions, despite the low density of lipid bodies, when mixed with low concentrations of hydrophilic polymers, starches, or
25 other reagents. Such compositions can be useful for applications in which a lack of separation of the composition components is preferred.

 The lipid bodies and components thereof can be odor free as a result of purifying the lipid bodies using water, acids, bases, and microbial stabilizers, and can be combined with or without antimicrobial agents, to both remove yeast smell from the lipid bodies and
30 components thereof and prevent the development of unwanted smells in the final compositions.

 The lipid bodies are benign on human skin and have demonstrated low irritation level, when used in compositions of the invention that come into contact with human skin. This hypoallergenic quality of can be important in many medical compositions, veterinary

compositions, personal care product compositions, and industrial compositions that may come into contact with human skin.

The lipid bodies can serve as a source of palm-oil-substitute, which is a fundamental feedstock material and basic for many product compositions, for example, in
5 drug delivery systems, medicinal applications, nutritional uses, industrial uses, personal care, skin care, pet care, and many other formulated products.

Topical compositions of the invention have the property of sealing the area of skin where it has been applied. The durability of the sealing and protection of the area of application is durable with minimal shedding over an extended period of time exceeding
10 4-hours, and in some cases over 10-hours and in some cases over 12-24 hours to help maintain skin moisture and lipid retention in human skin, hair or other tissues without undesirable accumulation.

The lipid bodies and lipid-body components can be easily combined with a wide range of ingredients of all types to make acceptable compositions for a wide range of
15 products across industries. The added ingredients can be hydrophobic, amphiphilic and/or hydrophilic.

The lipid bodies can be composed of at least 50% yeast oil and as much as 98% yeast oil. The yeast oil is capable of being used in product compositions that currently utilize palm oil or palm oil derivatives or fractionations of palm oil, where consumer
20 preference is to use a palm-free product compositions for environmental or other reasons.

The compositions of the invention can enhance skin care and wound healing aids, particularly moisturizers for skin. The compositions of the invention can be used to restore lipids to and heal dry skin, burnt skin, and protected skin. These applications are complemented by the unique protective film from the lipid bodies, which is formed when
25 the lipid bodies impart their lipids to the skin.

The lipid bodies can be formulated in water-repellent compositions, which can be used in any industry where water resistance and lubrication is appropriate.

The lipid bodies can be formulated without chemical emulsifiers or other chemicals frequently associated with personal care and cosmetic products. Product
30 compositions comprised solely of lipid bodies have the property of easy mixing at elevated pasteurizing and or sterilizing temperatures. The pasteurized lipid bodies can be used in the sanitary production of medicines, nutrients, snacks, animal nutrition, parenteral nutrition, food emulsifications, nutritional water-resistant foods, aquaculture feeds, and

thermally stable oils for cooking, frying, baking, microwavable food compositions, and ready-to-use food compositions.

The easy combination and mixability of the lipid bodies lends them to generating compositions for oral care, ocular care, mouth washes, topical and oral anti-infectives, antibiotics, antifungals and other medical applications to other body parts including inhalation therapies, intestinal therapies and composition for the movement of drug through the stomach. Such compositions can include, in addition to lipid bodies, other drug emulsifications, drug compositions that require various levels of hydrophobicity and or hydrophilicity, and, in some cases, product compositions that protect against light, moisture, oxidation, radiation, and/or sun-exposure.

The compositions of the invention can include other oils, vegetable oils, synthetic oils, biochemicals, RNA, DNA, peptides, dyes, colorants, vaccine antigens and adjuvants, carbohydrates, sugars, fibers, cellulose or other substance or combination of substances.

The compositions of the invention can deliver amphipathic substance, steroids, hormones, carotenoids, vitamins, nutraceuticals, cosmeceuticals, essential fatty acids, essential lipids, essential amino acids, other amino acids, RNA, DNA, oligonucleotides, synthetic genes and other nutritional, medical or cosmetic applications and or research reagents.

The lipid bodies and/or lipid-body components can modify skin care products that are capable of impacting the skin or skin wrinkles, rashes, inflammations, irritations, blisters, dry skin remediations, sunburn, sun blocks, and any medical condition appearing or experienced on the skin. The resulting composition can include night creams, day creams, cosmetics, cosmetic bases, beauty agents, hair and scalp gels, conditioners, anti-fungal treatments, and deodorants..

The lipid bodies and/or lipid-body components are capable of impacting facial skin appearance, skin wrinkles, rashes, inflammations, irritations, blisters, dry skin remediations, sunburn, sun blocks, and any medical or cosmetic condition appearing or experienced on the facial skin.

The lipid bodies can be combined with or without other liposome products, nanoparticles, macro emulsification, hydrophobic carrier, micro emulsification reagents or nano-emulsification agents with or without co-solvents and other solvents. The compositions can be used as insecticides, herbicides, algacides, pesticides in general, rodenticide, sanitization of water systems, sanitization of water towers, cleaning agents, clearing heat exchangers and boiler tubes, sanitization of cooling towers, ship bottom

paints containing time-release anti-fouling agents, other marine compositions and other similar product applications .

The compositions of the invention can be used in product compositions for electronics, insulation, capacitance, resistance, optical properties, radio frequency modulation, corrosion resistance, lubricity at high temperatures and other industrial applications.

Compositions of the invention comprising the lipid bodies or components thereof can be used for research and scientific compositions. The lipid bodies and/or lipid-body components can be modified or derivatized with other chemicals or ligands for making separations media or chromatographic resins, membranes, films, coatings, reflectance modifiers, transmission modifiers, clear films, colored films, opacity on glass or crystals, and any other industrial or research applications or used in product compositions for marine, outer-space, on Mars, on the Moon and in all composition applications for micro-electronics, high-power electrical systems related insulation, capacitance, resistance, optical properties, radio frequency modulation, corrosion resistance, lubricity at high temperatures and other industrial applications.

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CLAIMS

We claim:

1. A composition comprising isolated yeast lipid bodies, wherein:
each lipid body comprises an envelope surrounding an inner core;
the envelope comprises phospholipid, protein, and polysaccharide;
the inner core comprises lipid; and
the lipid bodies comprise a set of lipid bodies having a diameter of at least 5 μm .
2. The composition of claim 1, wherein a number of the lipid bodies in set is at least 30% of a total number of the lipid bodies in the composition.
3. The composition of any one of claims 1-2, the lipid bodies comprise a subset of the lipid bodies within the set, wherein the lipid bodies in the subset have a diameter of at least 6 μm .
4. The composition of claim 3, wherein a number of the lipid bodies in subset is at least 20% of a total number of the lipid bodies in the composition.
5. The composition of any prior claim, wherein the lipid bodies in the composition have an average diameter from 5 μm to 10 μm .
6. The composition of claim 1, wherein the set of the lipid bodies comprises lipid bodies having a diameter of at least 12 μm .
7. The composition of any one of claims 1 and 6, wherein the lipid bodies in the set have a diameter up to 60 μm .
8. The composition of any prior claim, wherein the lipid bodies in the composition comprise a lipid:protein ratio by mass from 50:1 to 500:1.
9. The composition of any prior claim, wherein the lipid bodies in the composition comprise yeast protein.

10. The composition of any prior claim, wherein the lipid in the inner cores of the lipid bodies in the set is present in an amount of at least 60% w/w of the lipid bodies in the set as determined by gravimetric analysis.

11. The composition of any prior claim, wherein the lipid in the inner core of the lipid bodies has a melting point at a temperature between 25°C and 50°C.

12. The composition of any prior claim, wherein the lipid bodies absorb light at a wavelength within a range from 190 nm to 290 nm.

13. The composition of any prior claim, wherein the composition exhibits an absorbance at 230 nm of at least 0.5 OD units per gram of lipid body wet weight.

14. The composition of any prior claim, wherein the composition is white in color.

15. The composition of any prior claim, wherein the composition exhibits an absorbance at 400 nm of less than 0.32 OD units per gram of lipid body wet weight.

16. The composition of any prior claim, further comprising a carrier for the lipid bodies.

17. The composition of claim 16, wherein the carrier comprises water.

18. The composition of any one of claims 16-17, wherein the carrier comprises an excipient.

19. The composition of claim 18, wherein the excipient comprises one or more of a humectant, a preservative, an antioxidant, an emulsifier, an emollient, and a thickening agent.

20. The composition of any one of claims 16-19, wherein the lipid bodies are suspended in the carrier and remain in suspension for at least 40 days at 20°C.

21. The composition of any one of claims 16-20 wherein the carrier has a pH of from 0.5 to 4.

22. The composition of any one of claims 16-20, wherein the carrier has a pH of from 4 to 9.

23. The composition of any one of claims 16-20, wherein the carrier has a pH of from 9 to 12.

24. The composition of any prior claim, wherein the carrier further comprises an isolated lipid-body component comprising at least one of isolated lipid-body envelopes and isolated lipid-body lipid.

25. The composition of any prior claim, wherein the composition is in the form of a cream or a paste.

26. The composition of any prior claim, wherein the composition further comprises an active ingredient.

27. The composition of claim 26, wherein the active ingredient comprises a hydrophobic active ingredient.

28. The composition of any one of claims 26-27, wherein hydrophobic active ingredient is disposed within the inner core of the lipid bodies.

29. The composition any one of claims 26-28, wherein the active ingredient comprises a hydrophilic active ingredient.

30. The composition of claim 29, wherein the hydrophilic active ingredient is disposed within a carrier or adhered to the envelopes of the lipid bodies.

31. The composition of any one of claims 26-30, wherein the active ingredient comprises a UV-absorbing agent.

32. The composition of any one of claims 1-25, wherein the composition comprises a hydrophobic active ingredient disposed within the inner core of the lipid bodies and a

hydrophilic active ingredient disposed within a carrier or adhered to the envelopes of the lipid bodies.

33. The composition of any prior claim, wherein the composition further comprises a cation bound to the envelope of the lipid bodies.

34. A composition comprising:
an isolated lipid-body envelope comprising phospholipid, protein, and polysaccharide;
and
a carrier.

35. The composition of claim 34, wherein the composition further comprises a cation bound to the isolated lipid-body envelope.

36. The composition of claim 34, wherein the composition further comprises an active ingredient bound to the isolated lipid-body envelope.

37. A method of preparing isolated yeast lipid bodies or a product derived therefrom, the method comprising disrupting yeast cells containing intracellular lipid bodies to release the lipid bodies therefrom.

38. The method of claim 37, further comprising culturing a recombinant lipogenic yeast to generate the intracellular lipid bodies, wherein the recombinant yeast is genetically modified to overexpress a DGA1, a DGA2, a malic enzyme, and an SCT1.

39. The method of any one of claims 37-38, wherein the disrupting the yeast cells comprises hydrolyzing the yeast cells with acid.

40. The method of any one of claims 37-39, wherein the disrupting the yeast cells comprises enzymatic digestion of the yeast cells with a yeast lytic enzyme.

41. The method of any one of claims 37-40, further comprising washing the released lipid bodies with an alkaline buffer having a pH from 10.5 to 12.5 to thereby generate alkaline-washed lipid bodies.

42. The method of claim 41, wherein the alkaline-washed lipid bodies have a lower absorbance at 400 nm than the released lipid bodies.

43. The method of any one of claims 37-42, further comprising drying the released lipid bodies or the alkaline-washed lipid bodies to generate dried lipid bodies.

44. The method of claim 43, further comprising collecting oil from the dried lipid bodies.

45. A method comprising administering the composition of any one of claims 1-36 to an animal.

46. The method of claim 45, wherein the administering comprises at least one of topical administration, oral administration, and parenteral administration.

47. The method of any one of claims 45-46, wherein the administering comprises applying the composition to a site on a surface of an animal.

48. The method of claim 47, wherein the applying deposits the envelopes of the lipid bodies on the site.

49. The method of claim 48, wherein the envelopes remain deposited on the site for a period of at least one hour after the applying.

50. The method of any one of claims 47-49, wherein the surface comprises skin.

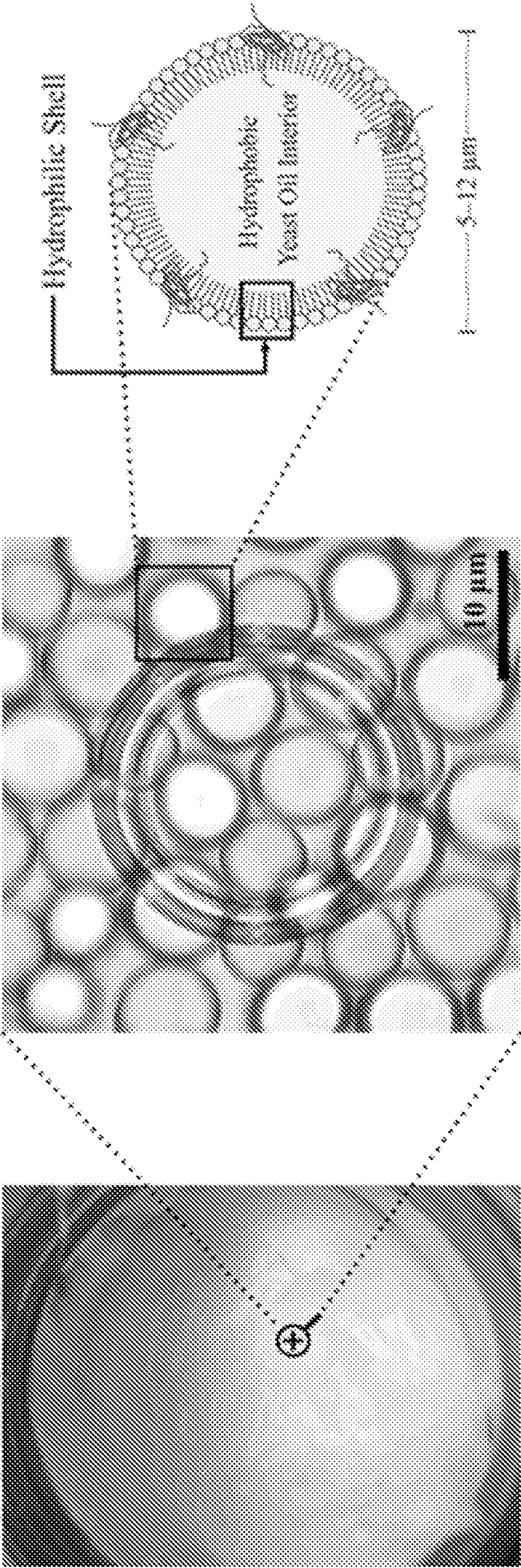
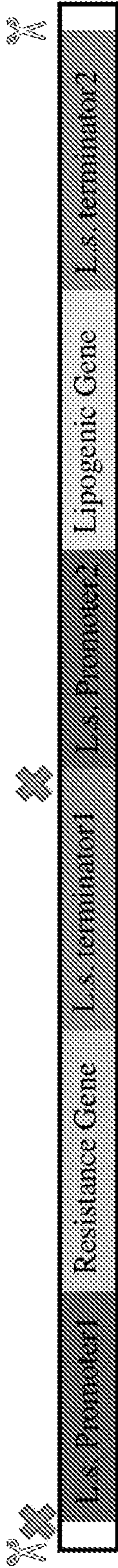


FIG. 1

Fragment 1:



Other Fragments:

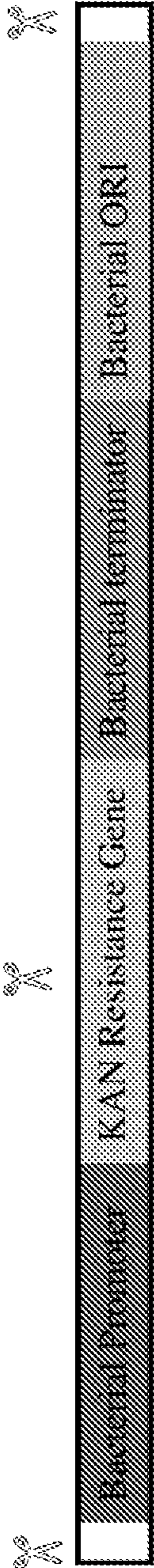


FIG. 2

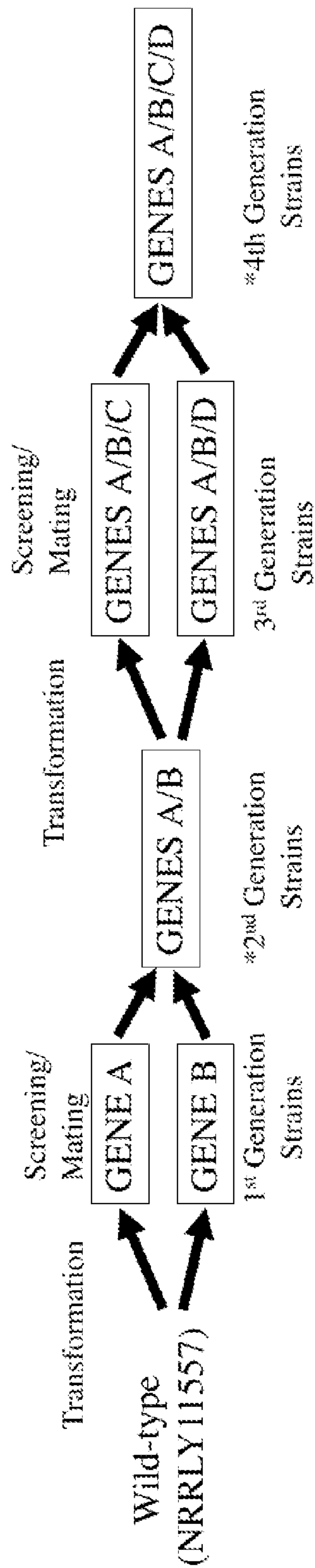


FIG. 3

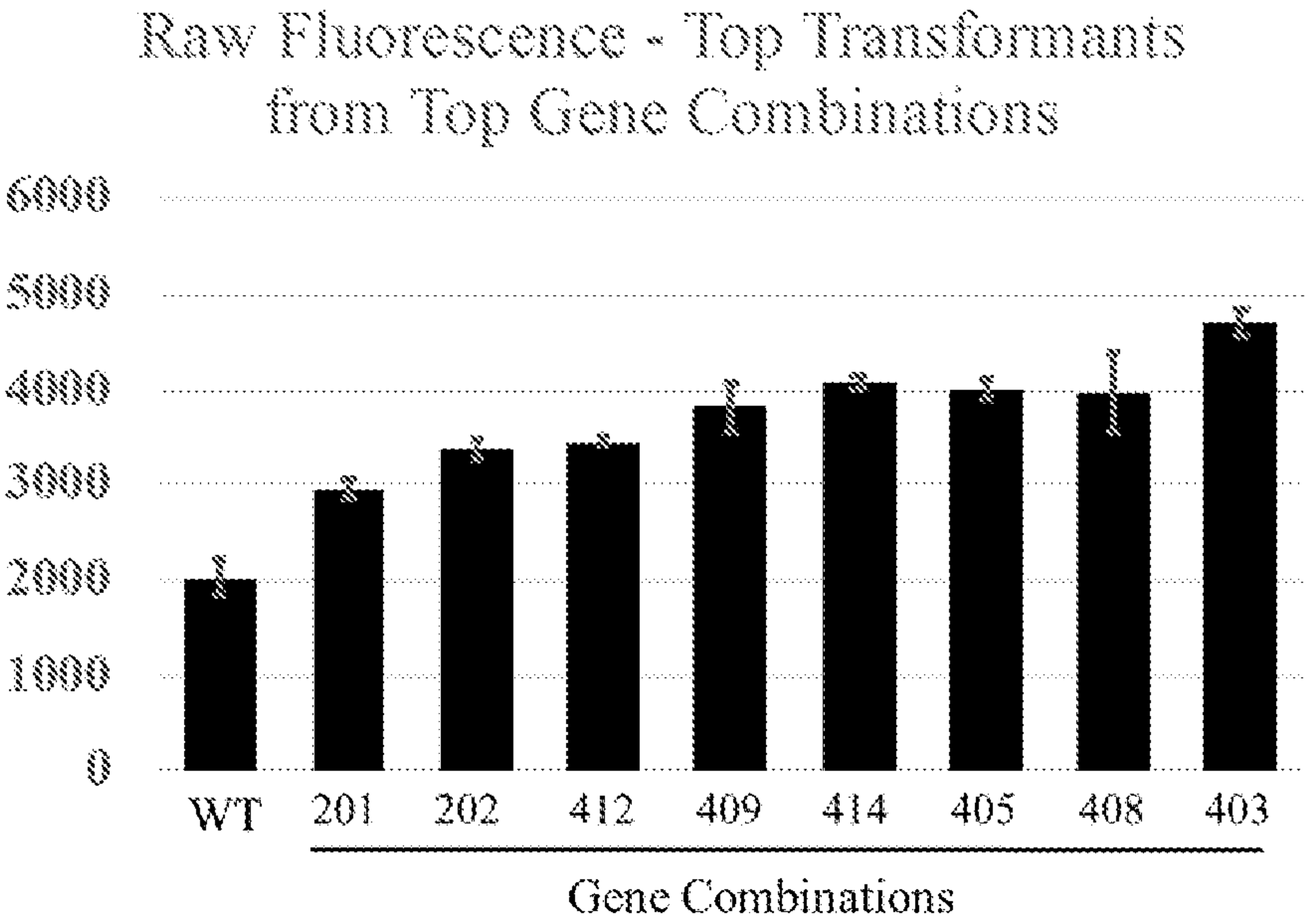


FIG. 4

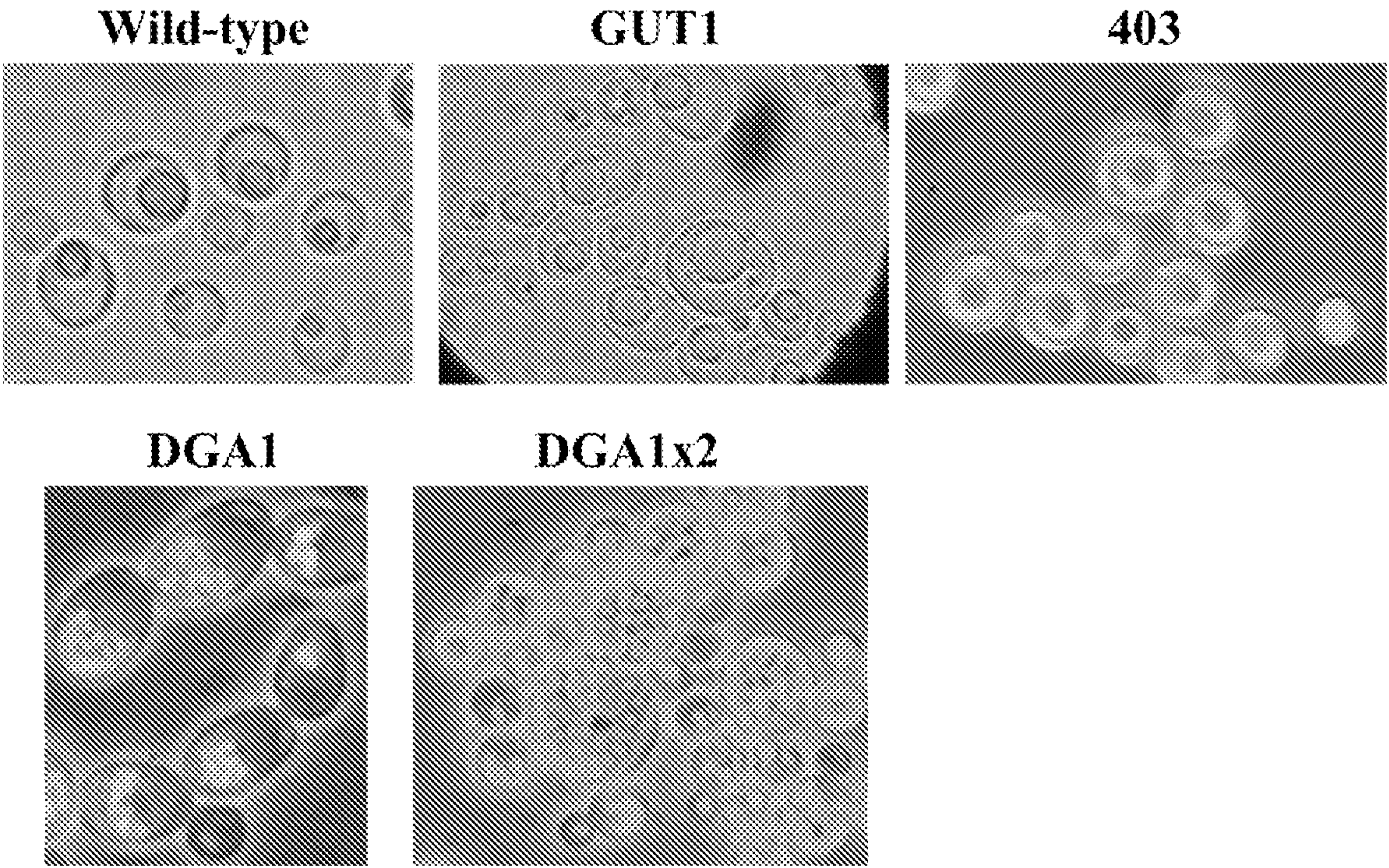


FIG. 5

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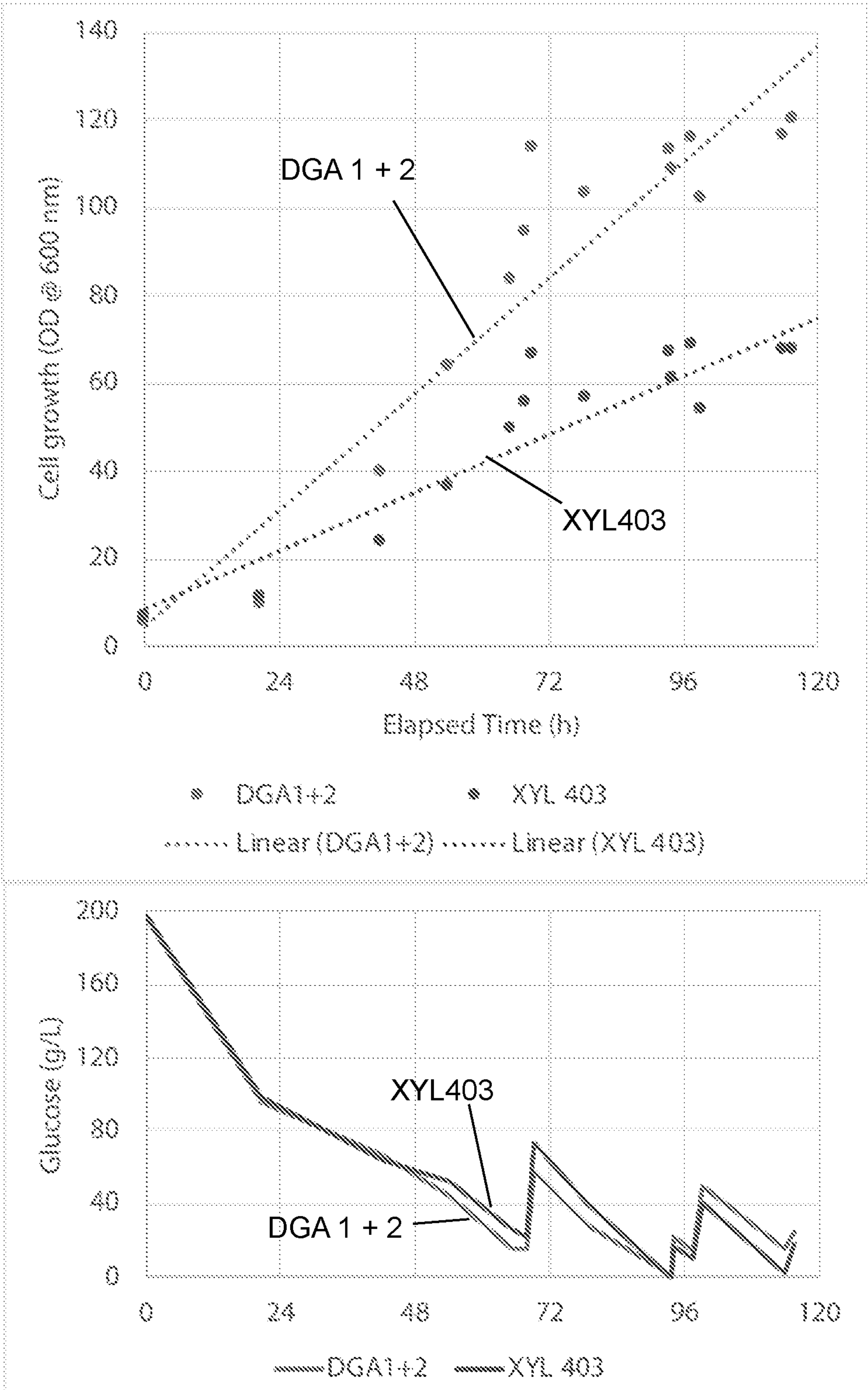


FIG. 6A

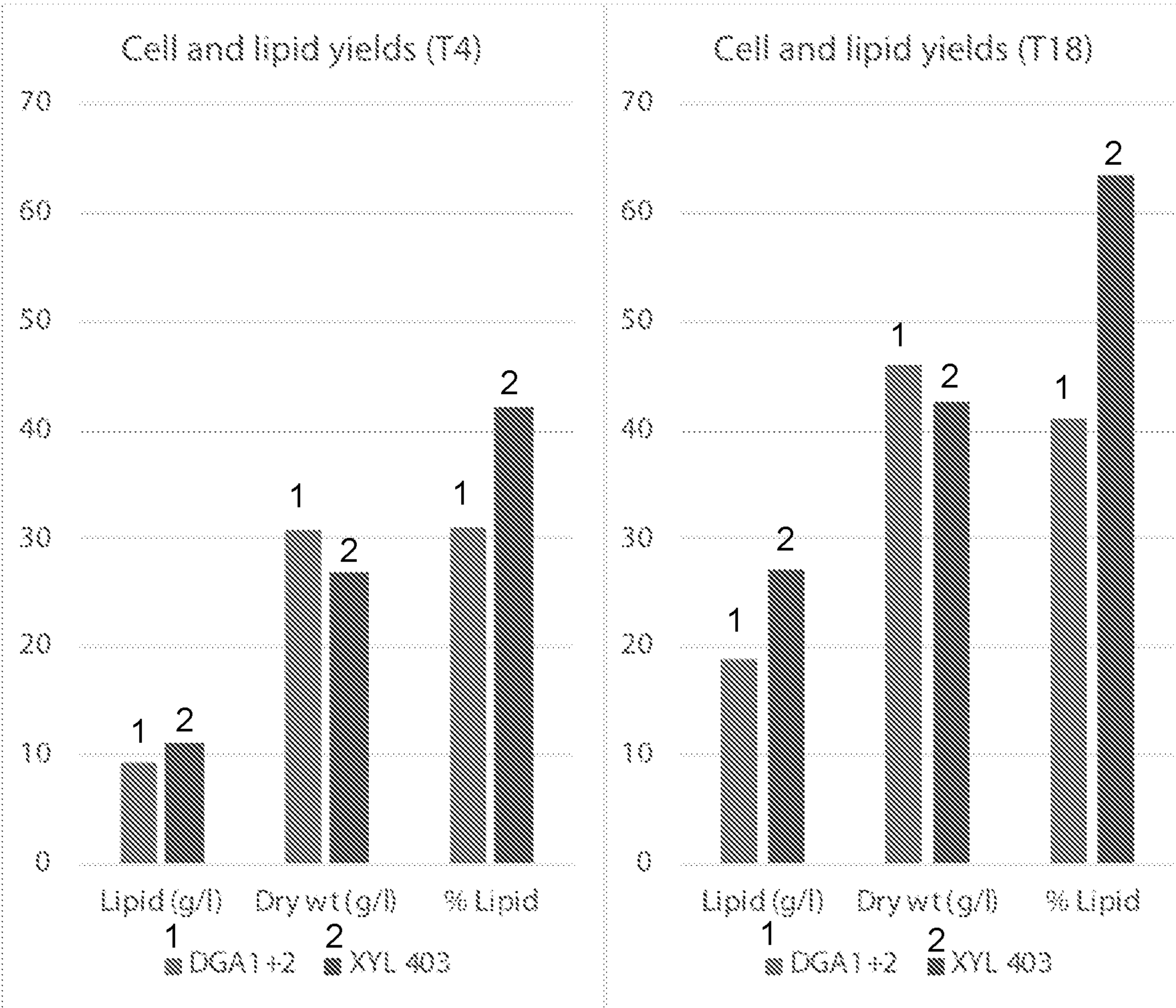


FIG. 6B

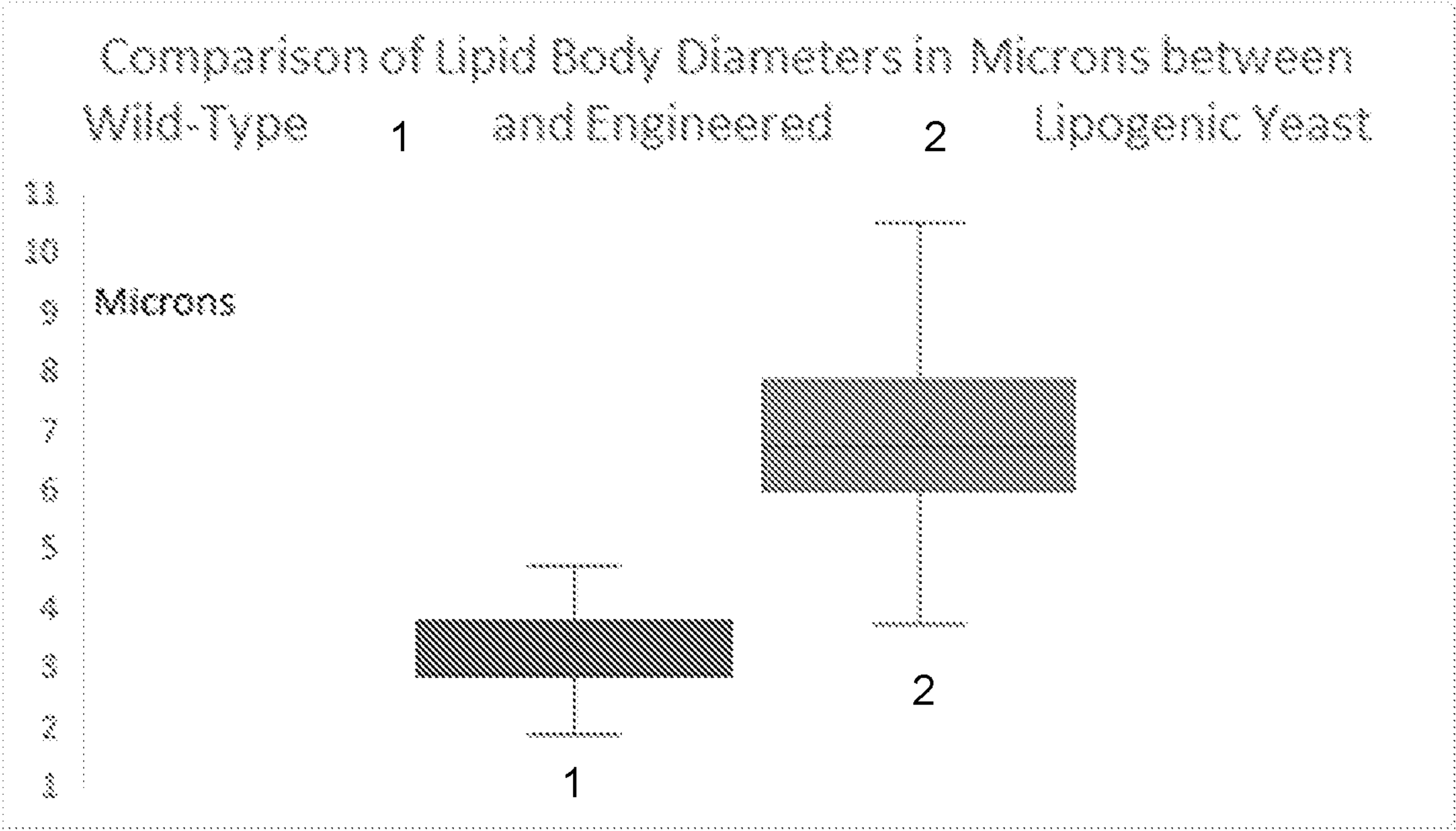
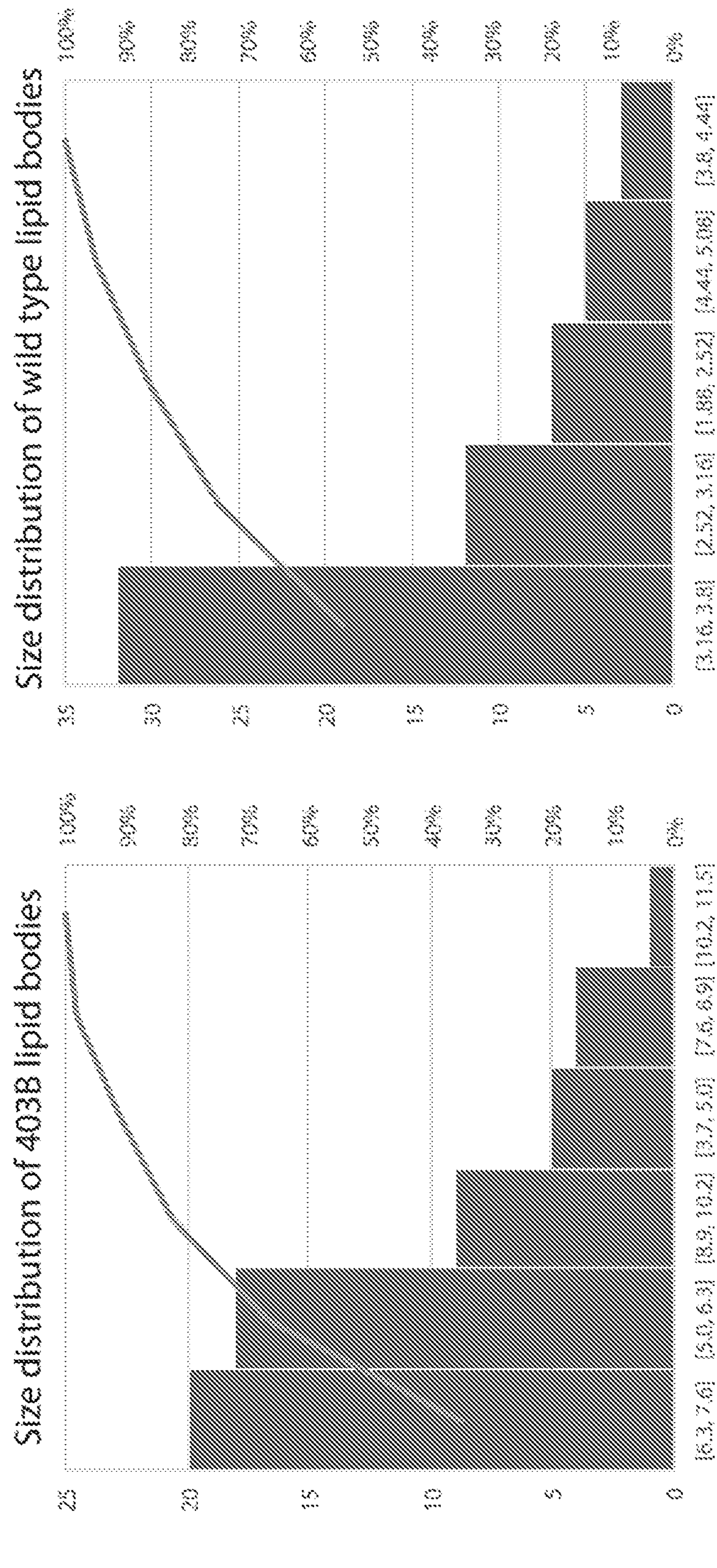


FIG. 7



Diameter Distribution in microns Diameter Distribution in microns

FIG. 8

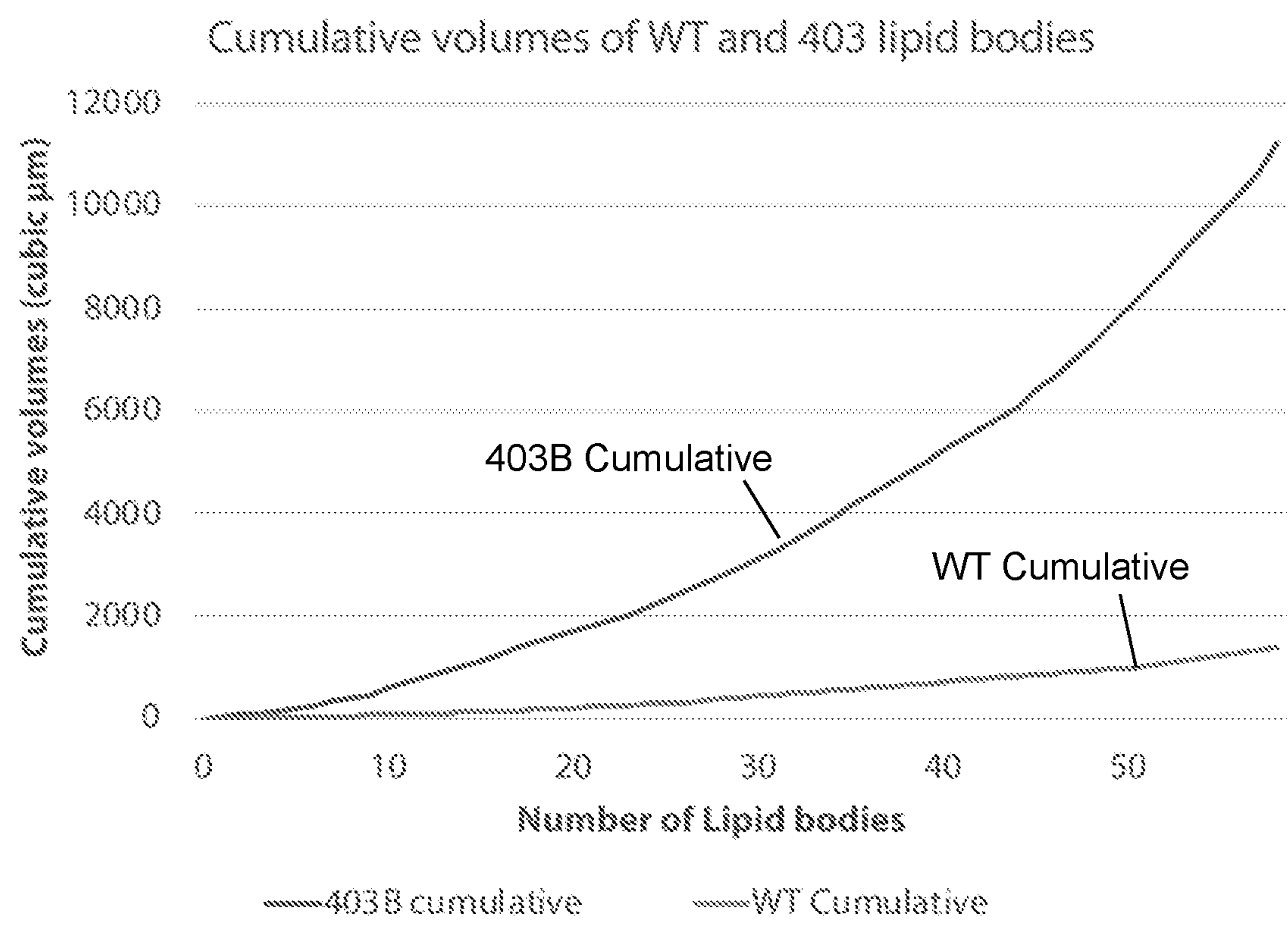


FIG. 9

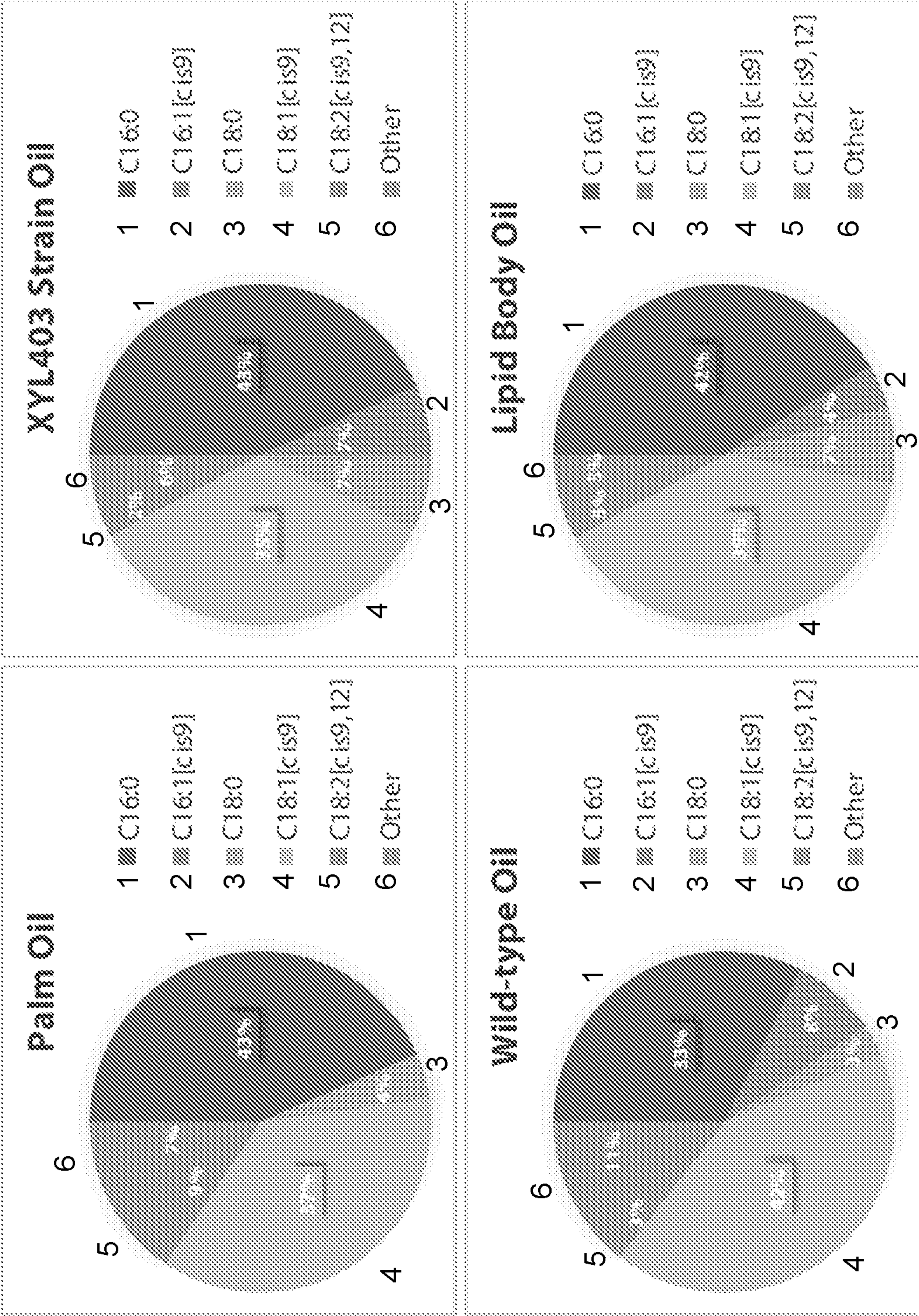


FIG. 10

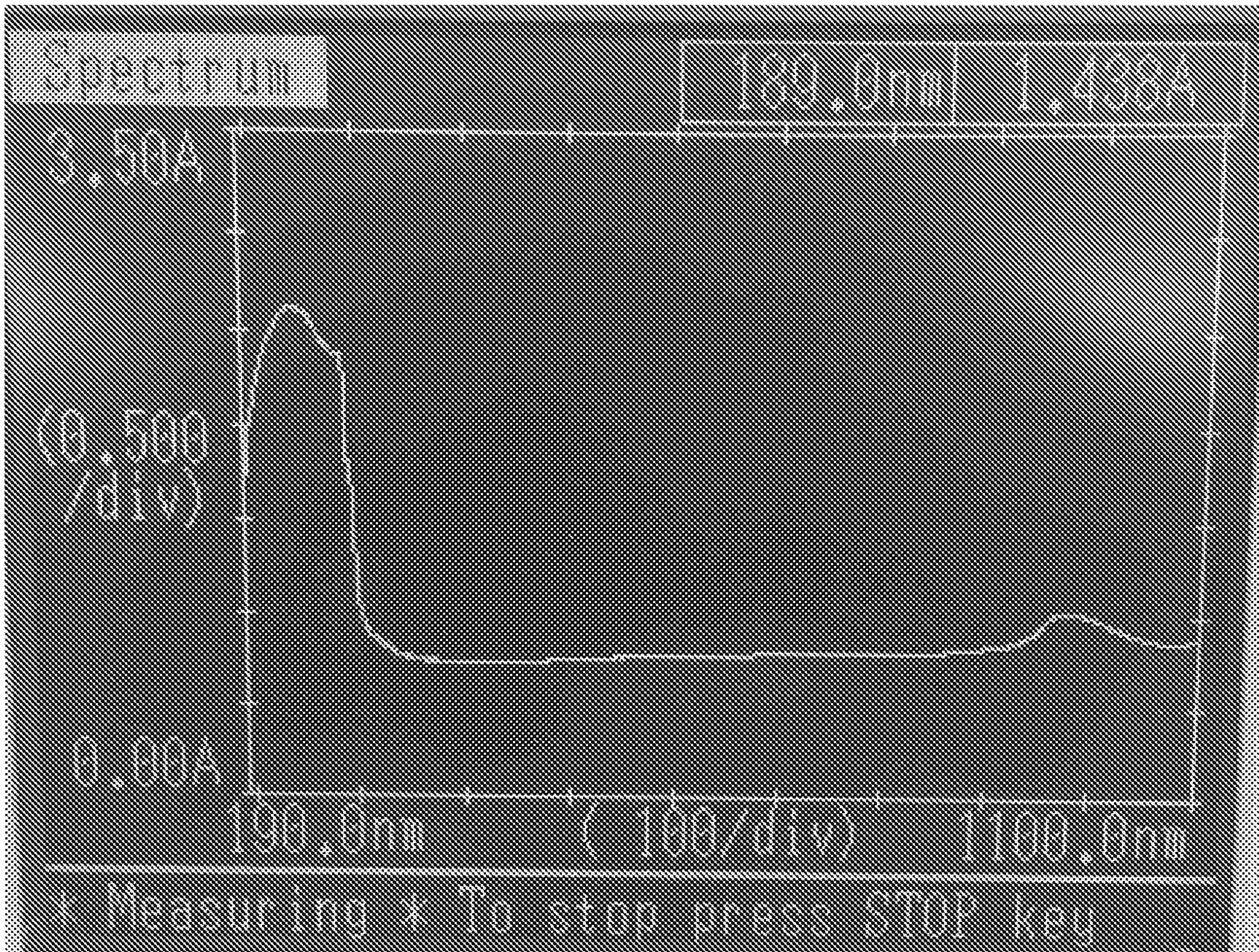


FIG. 11

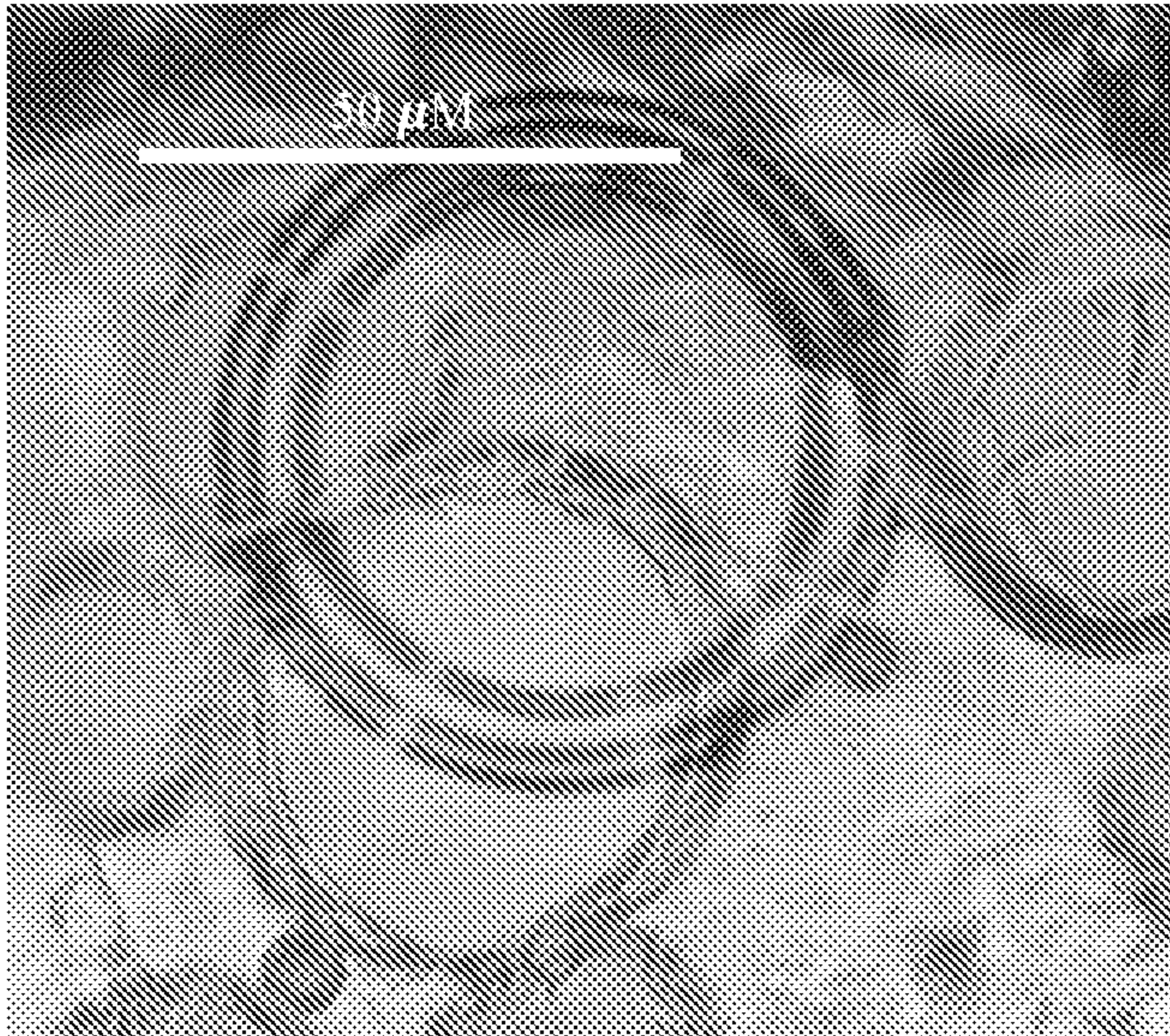


FIG. 12A

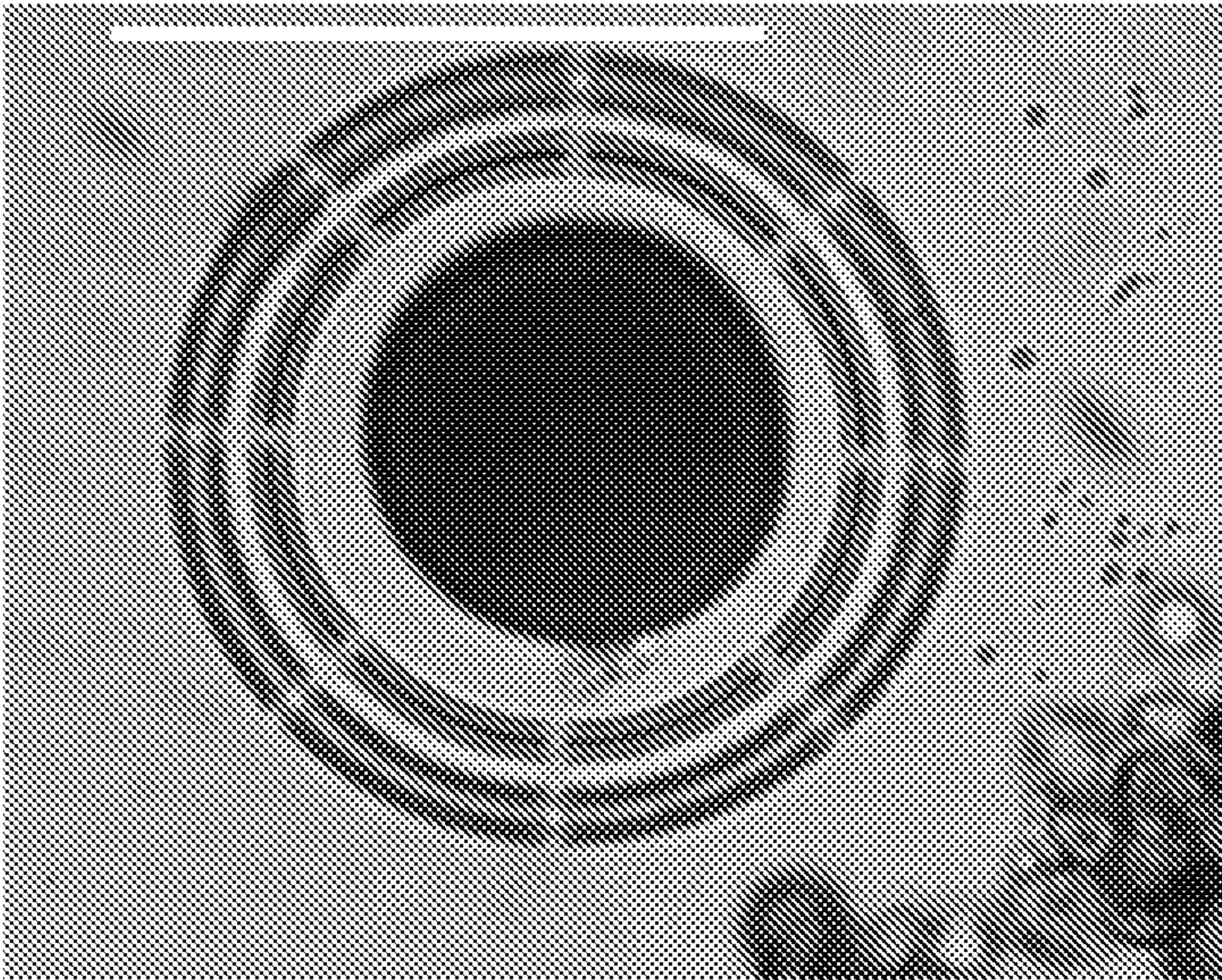


FIG. 12B

13/20



FIG. 13A



FIG. 13B

14/20

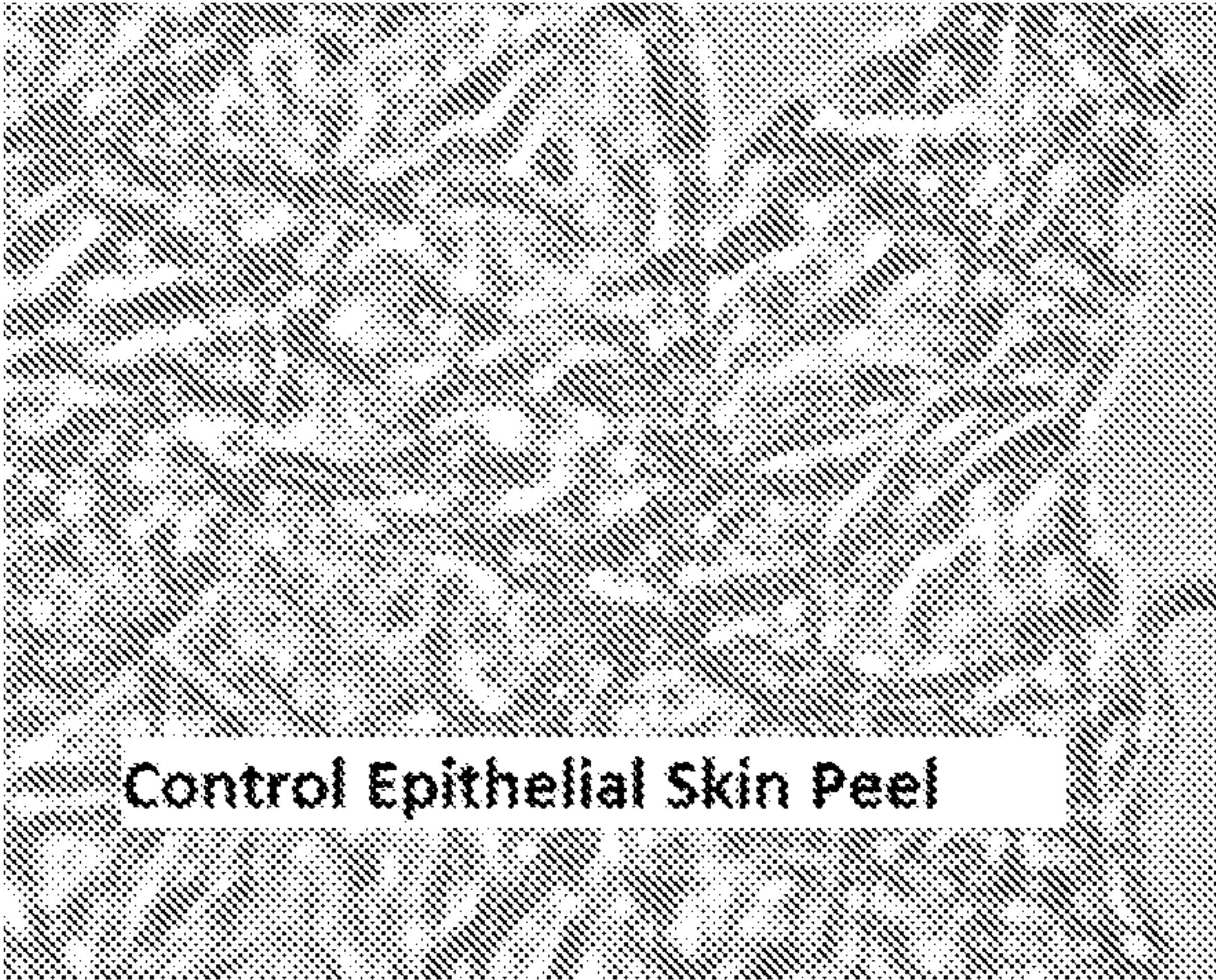


FIG. 14A

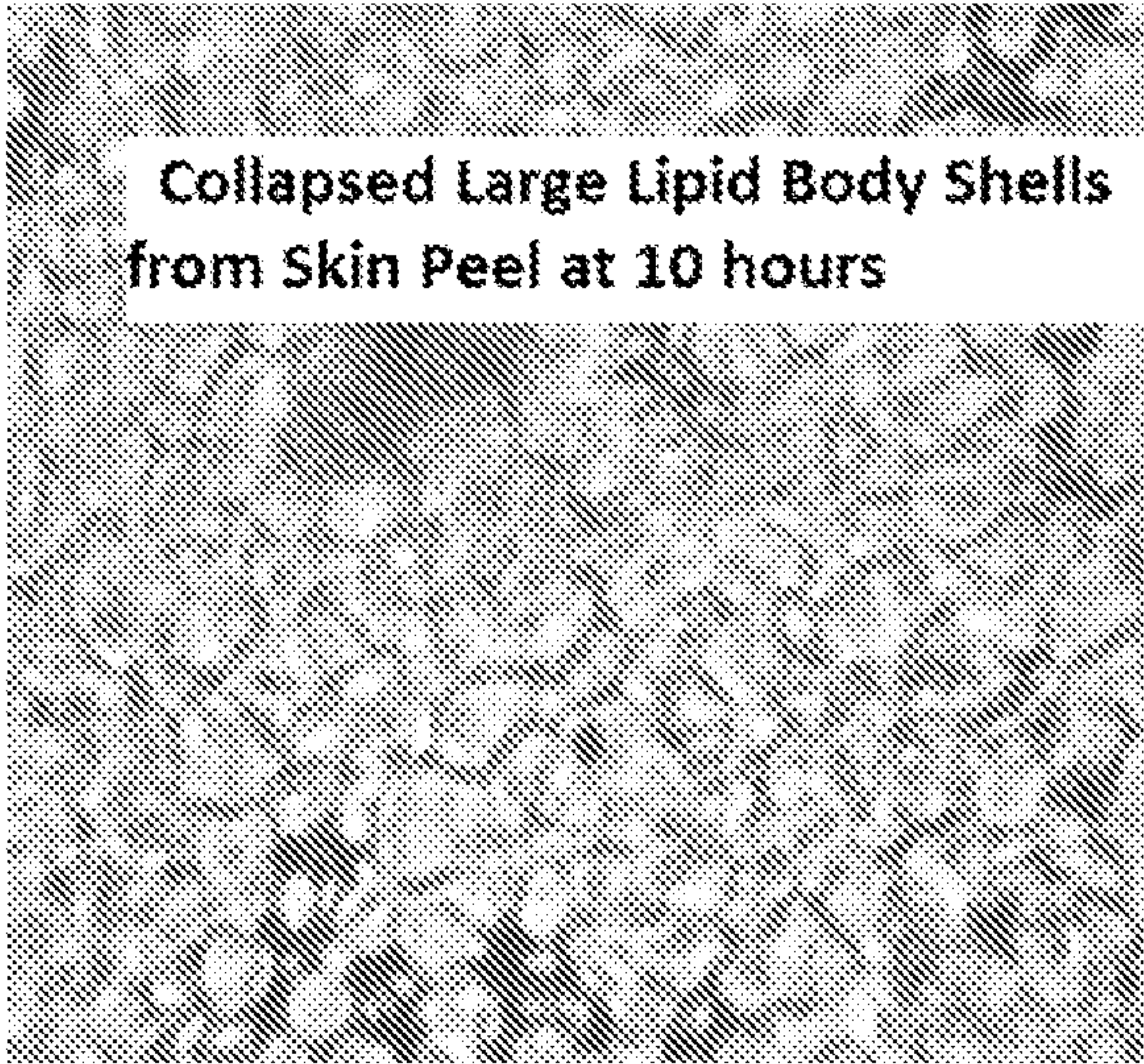


FIG. 14B

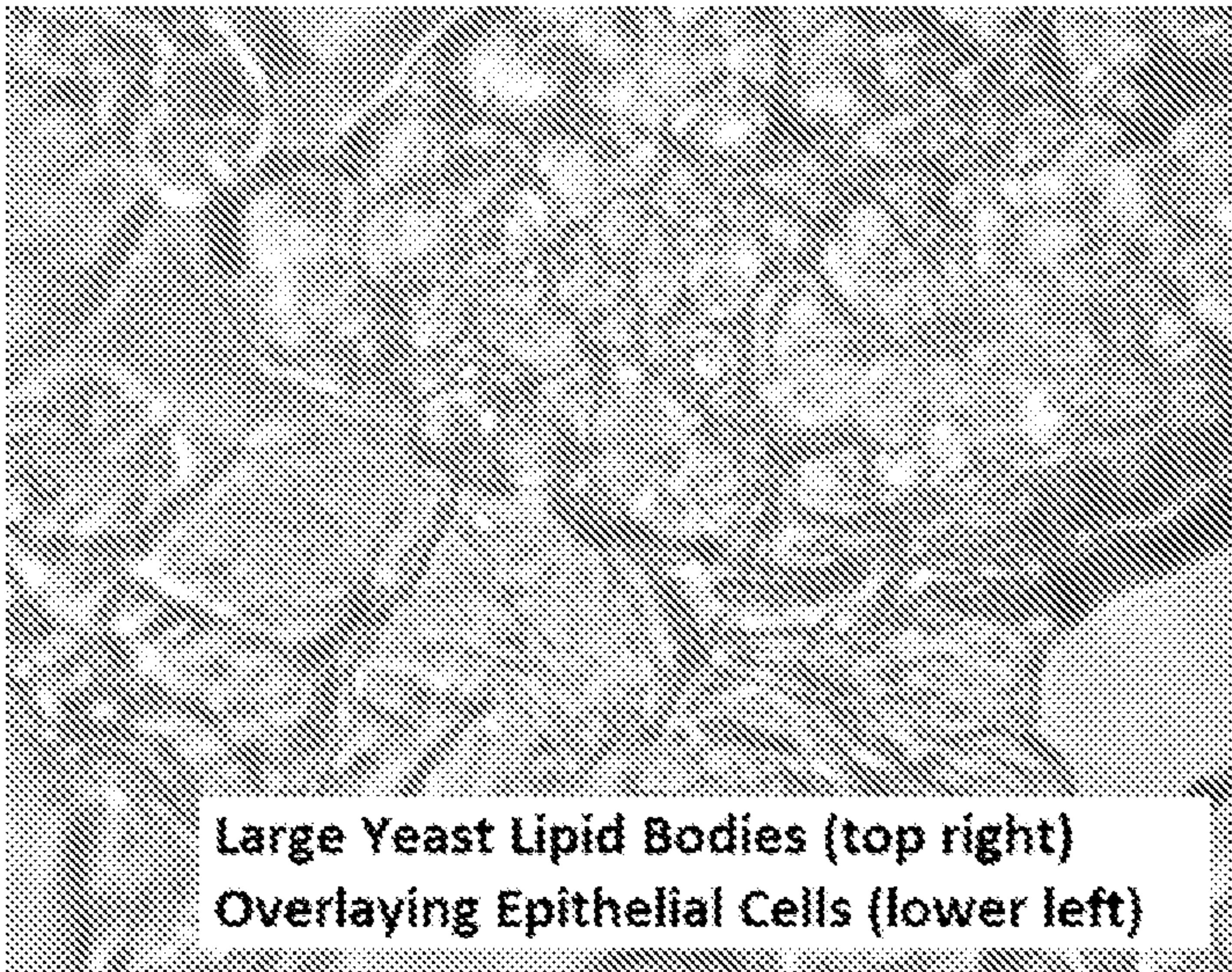


FIG. 14C

15/20

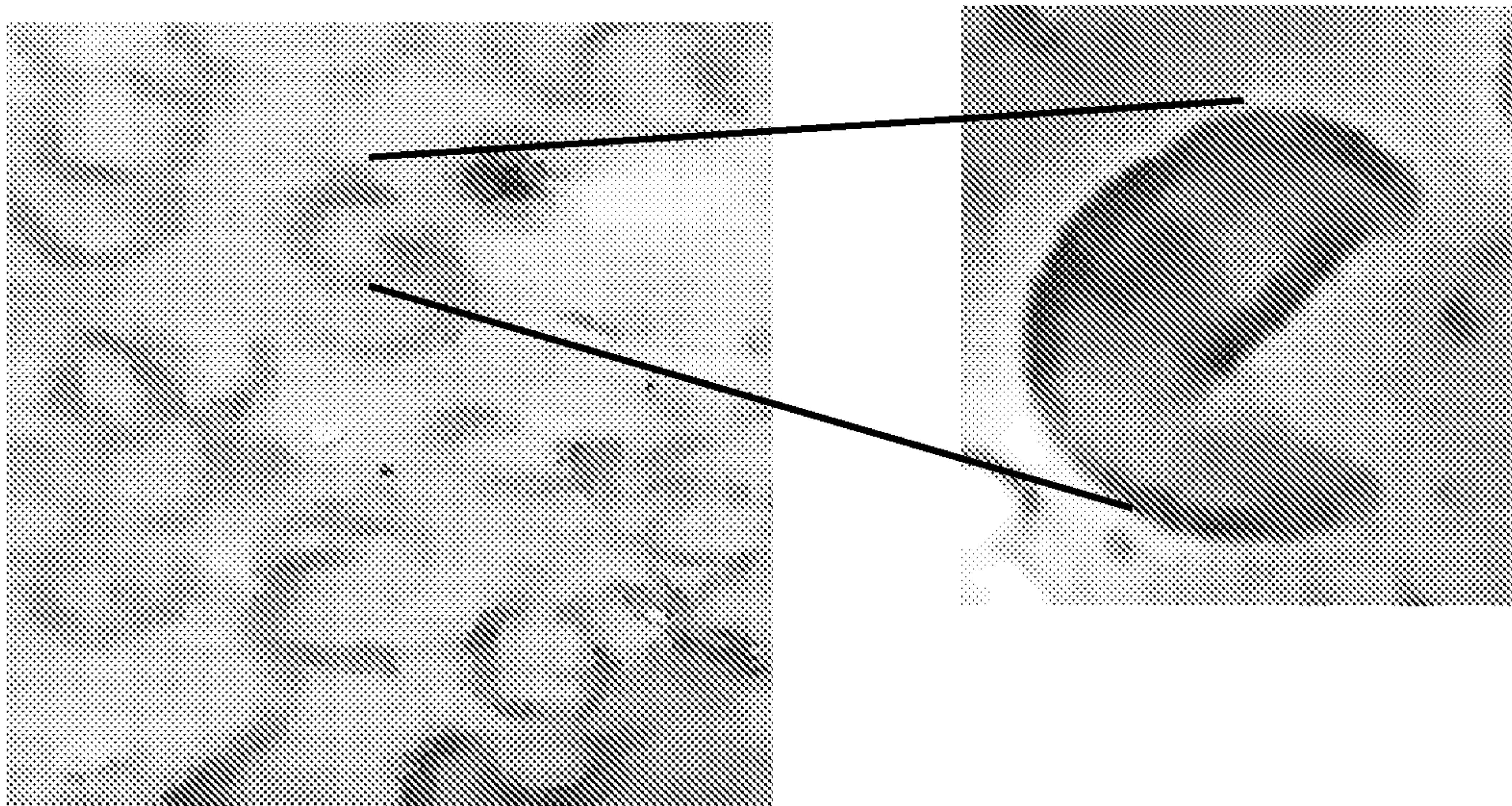


FIG. 15

400x

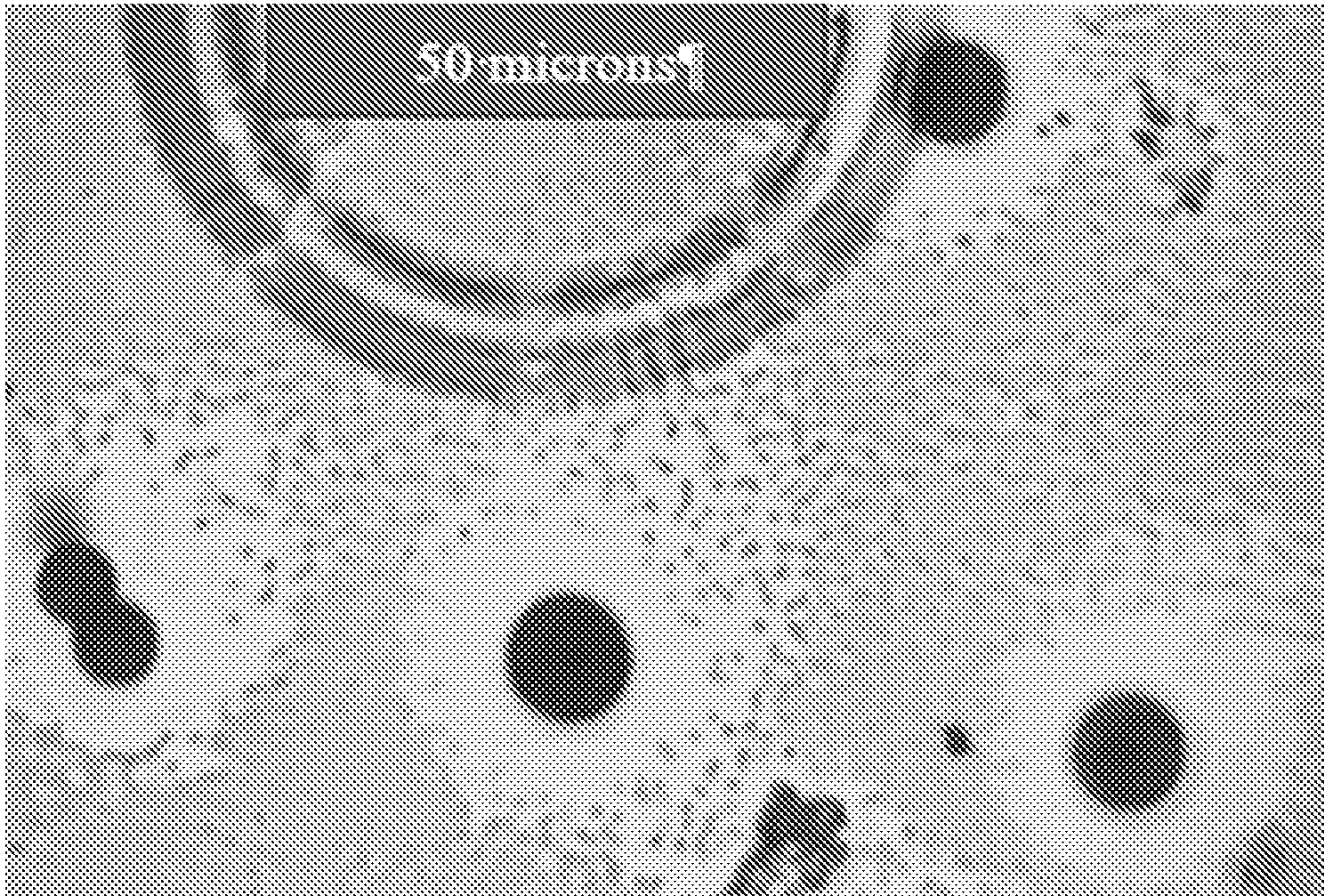


FIG. 16

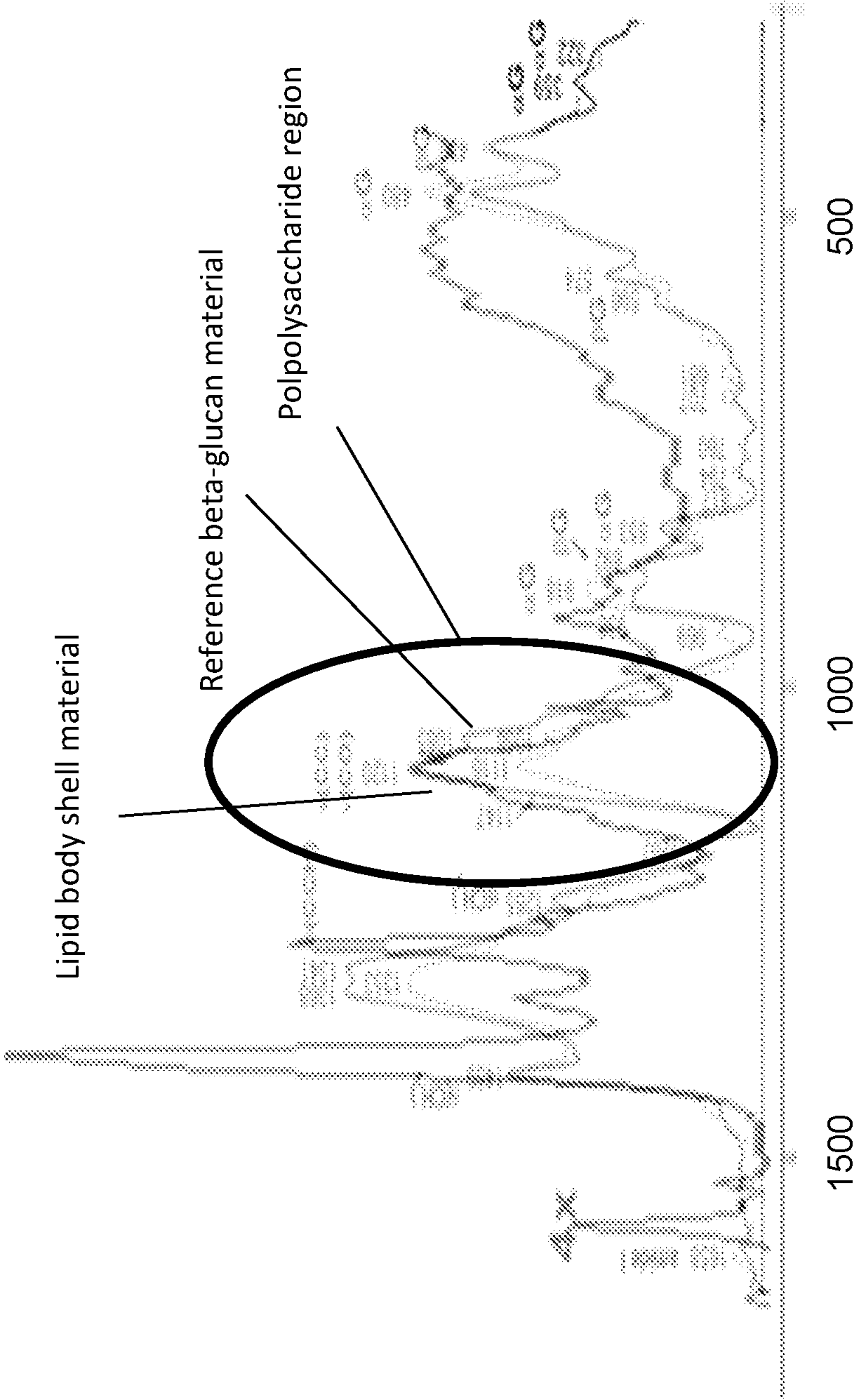


FIG. 17

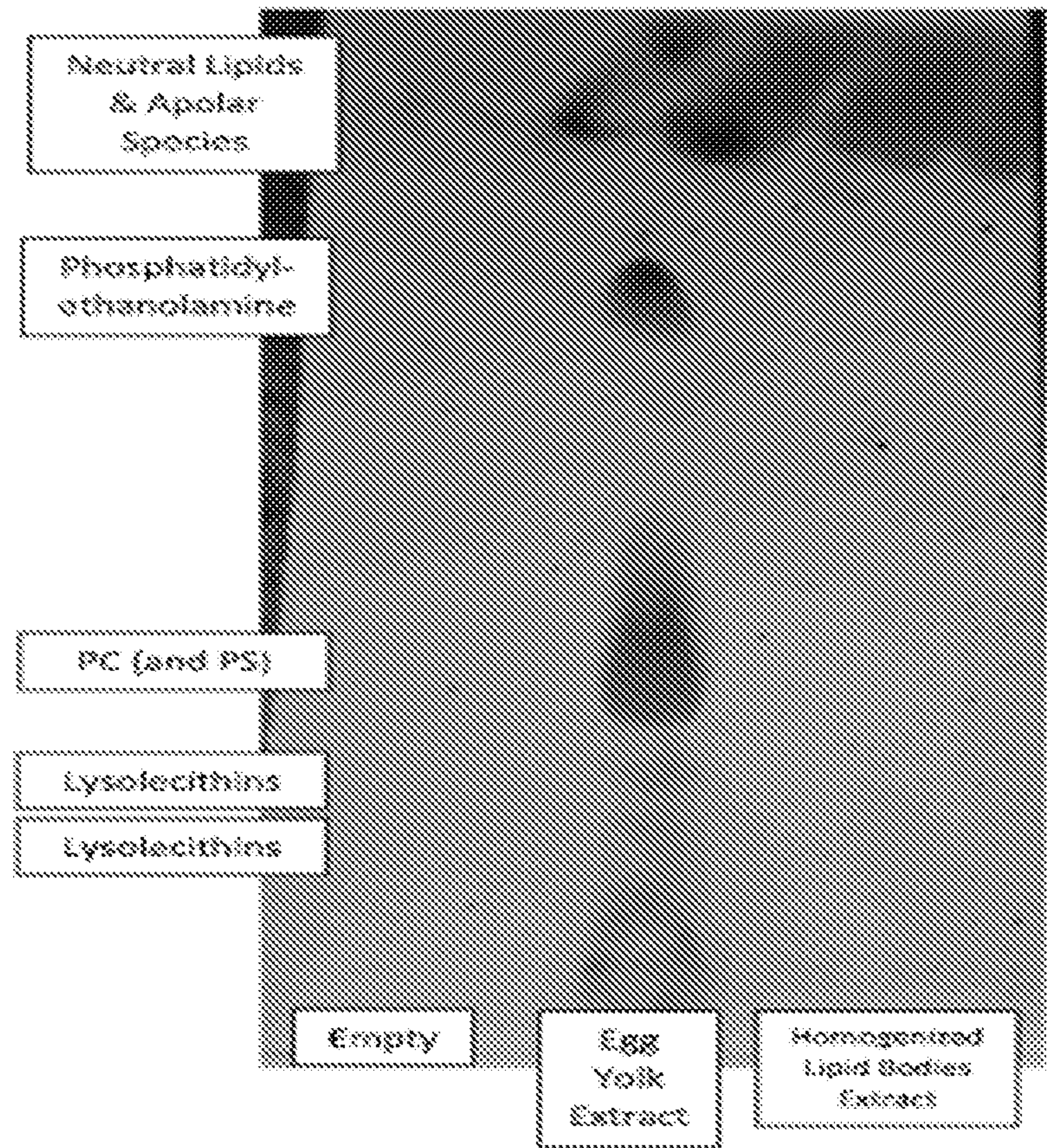


FIG. 18

45.5 Average Age
28-65 Age Range
27.6 Average uses per person
579 Exposures
0 Number of adverse events and skin irritations

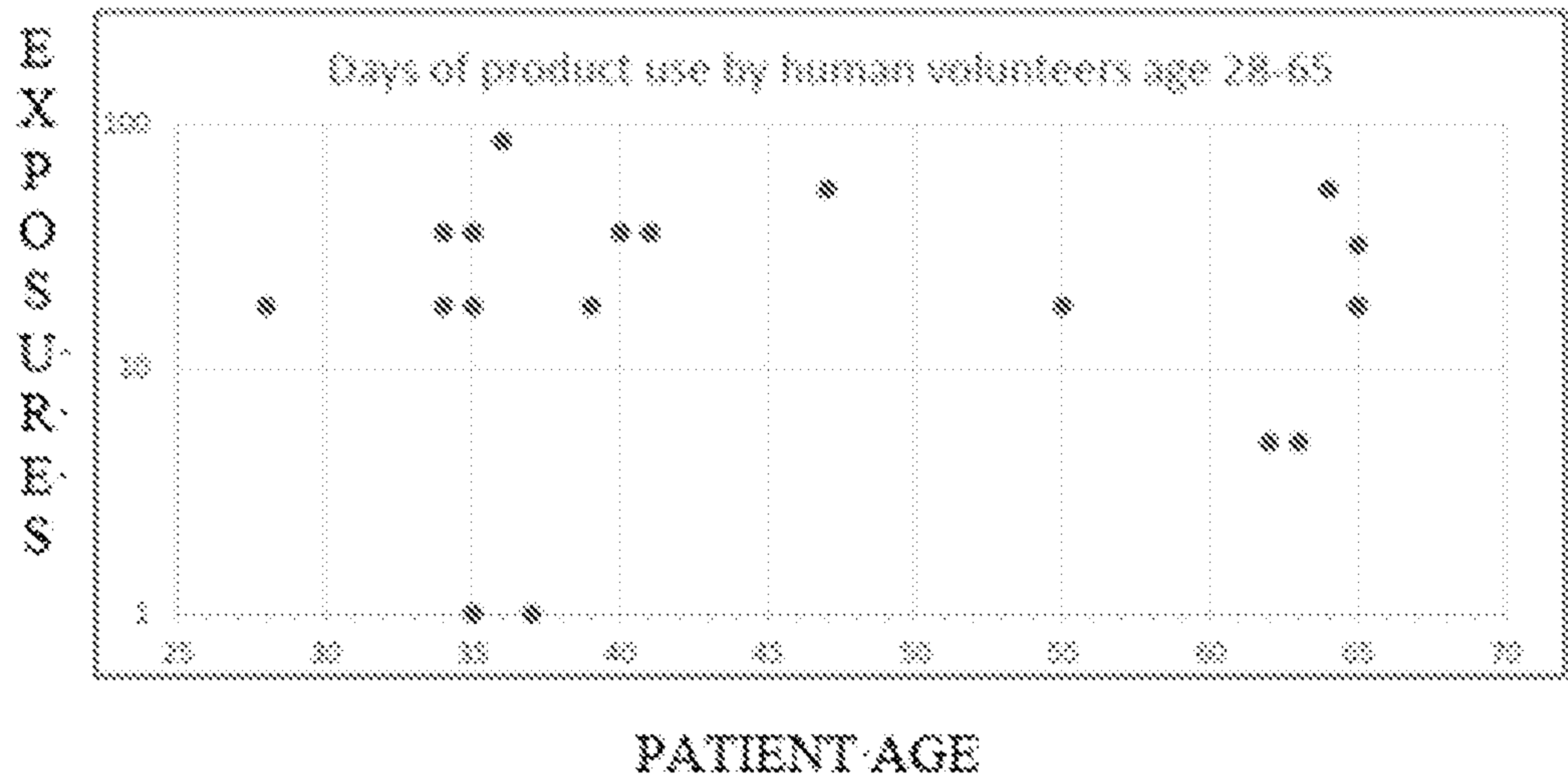


FIG. 19



FIG. 20A

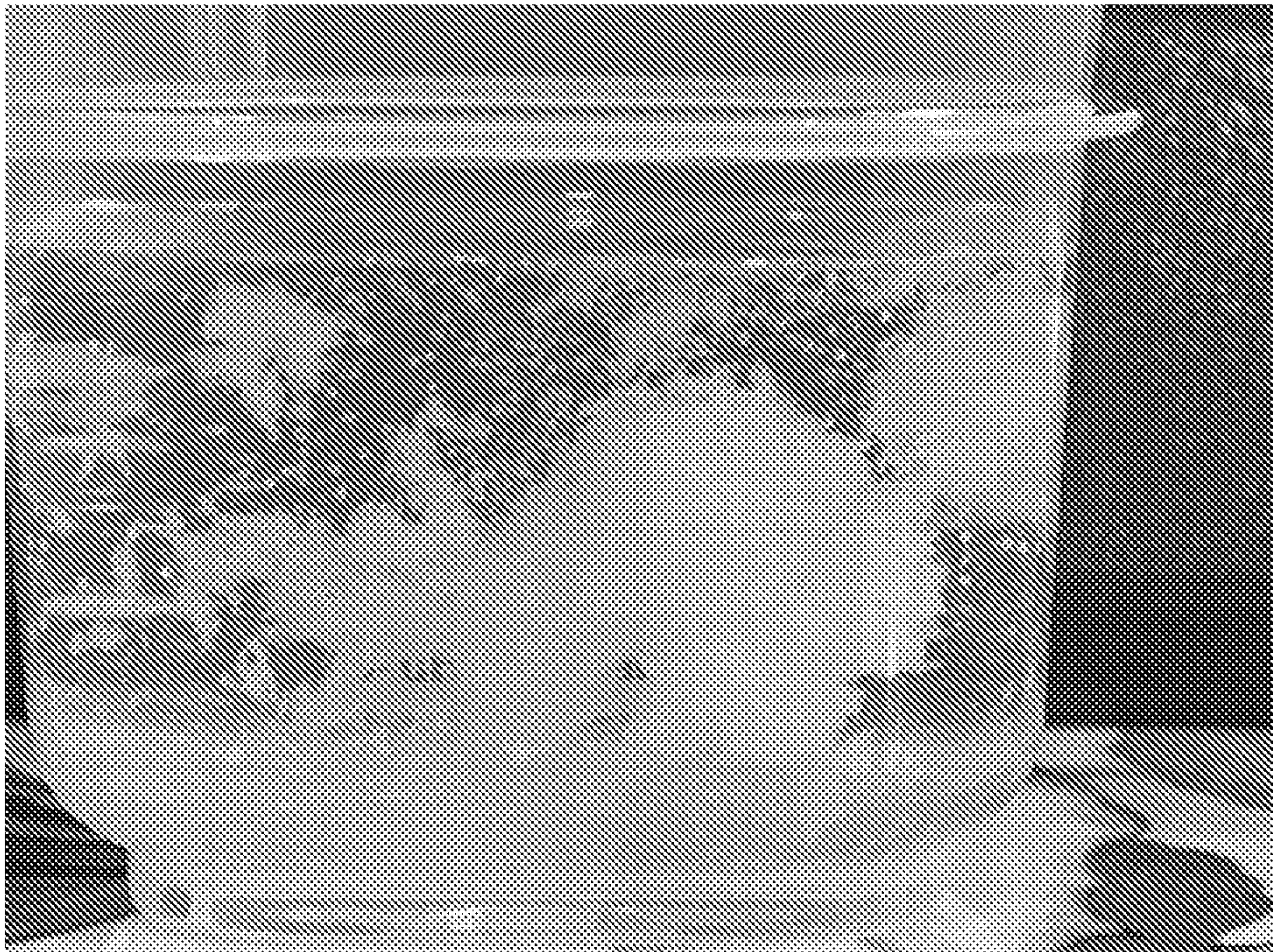


FIG. 20B

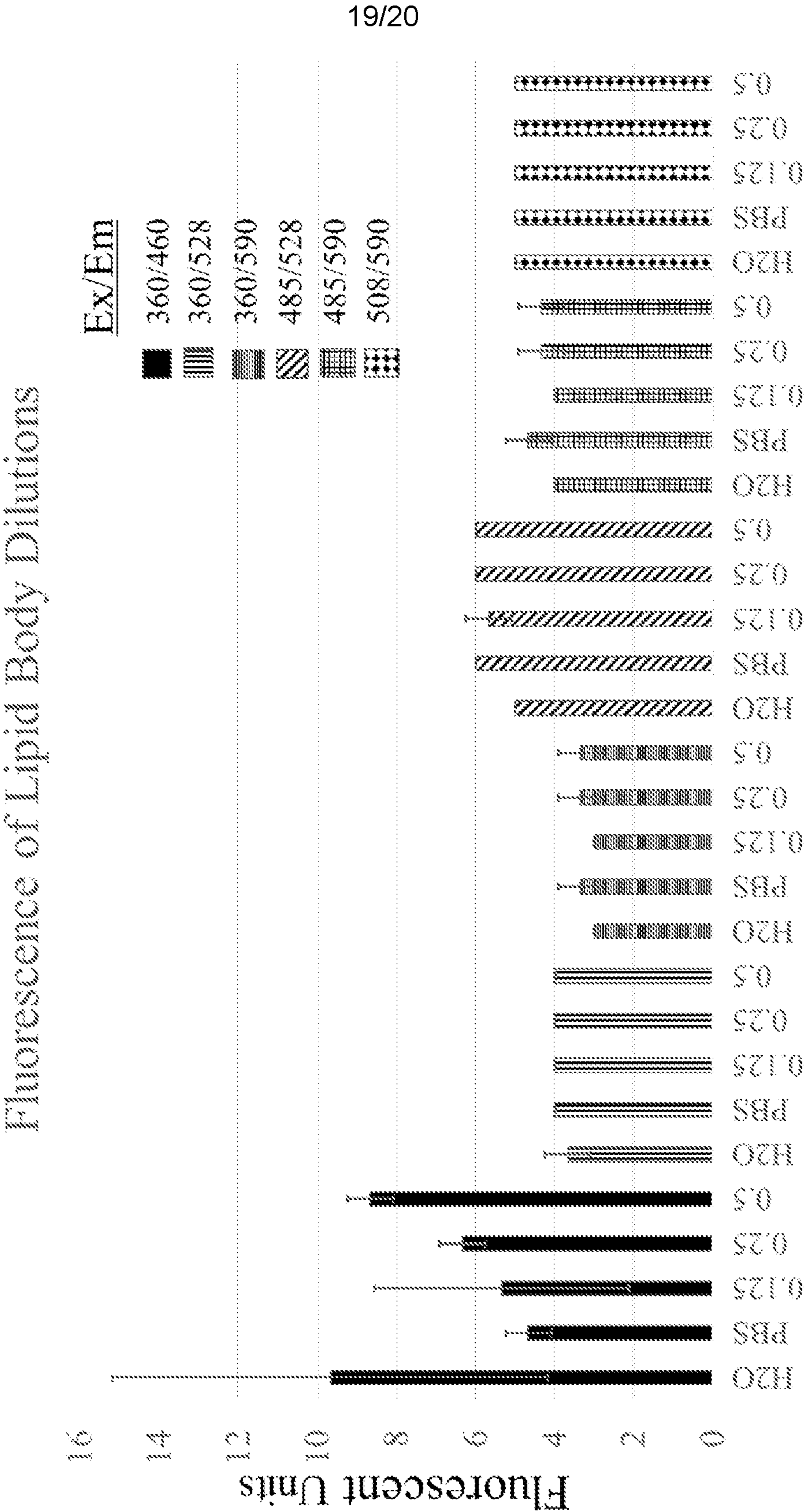


FIG. 21

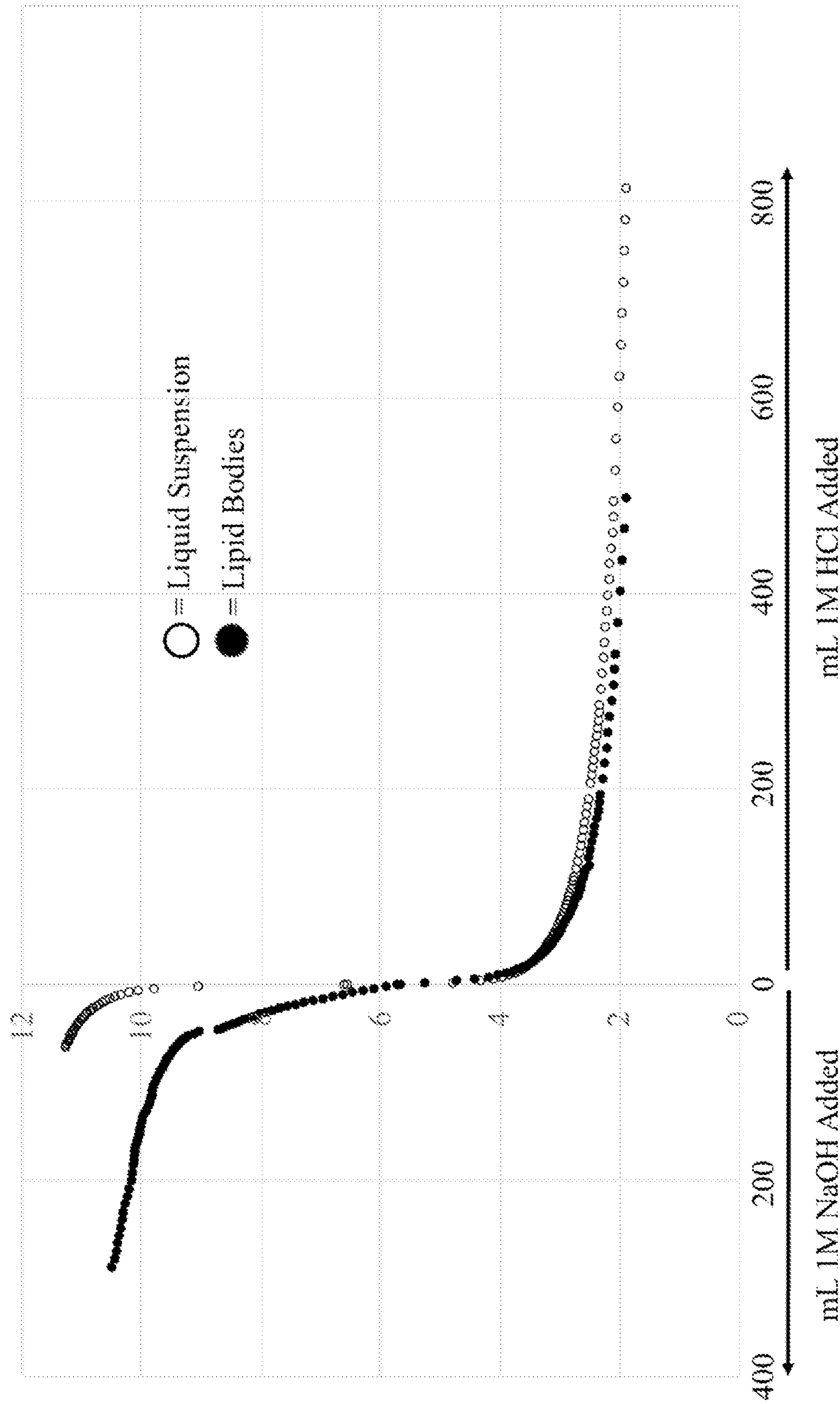


FIG. 22

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/060443

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.: 5, 8-33, 40-50
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:

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.:

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/US2020/060443

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01H 1/00; A01H 5/00; A01H 5/10; C07H 21/04; C07K 14/415; C12N 1/20; C12N 9/10 (2021.01)
CPC - C07K 14/195; C07K 14/415; C12N 1/20; C12N 9/1029; C12P 7/64; C12P 7/6409; C12P 7/6463 (2021.01)

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	US 2014/0024714 A1 (WIJESUNDERA et al) 23 January 2014 (23.01.2014) entire document	1-4, 6, 7, 34-36
X	US 2018/0245109 A1 (XYLOME CORPORATION) 30 August 2018 (30.08.2018) entire document	37-39
A	WO 2011/127118 A1 (ALGENETIX, INC et al) 13 October 2011 (13.10.2011) entire document	1-4, 6, 7, 34-39
A	US 2013/0289289 A1 (FRANZOSI et al) 31 October 2013 (31.10.2013) entire document	1-4, 6, 7, 34-39
A	US 2013/0217083 A1 (NORTHERN ARIZONA UNIVERSITY et al) 22 August 2013 (22.08.2013) entire document	1-4, 6, 7, 34-39

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

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Date of the actual completion of the international search

23 January 2021

Date of mailing of the international search report

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P.O. Box 1450, Alexandria, VA 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Blaine R. Copenheaver

Telephone No. PCT Helpdesk: 571-272-4300