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What is claimed is:

CLAIMS

1. A method for preparing a phospholipid suspension, comprising
providing DPPA, DPPC and MPEG5000-DPPE stocks,
5 measuring calcium concentration of one or more of the DPPC, DPPA and MPEG5000-
DPPE stocks,
combining the DPPA, DPPC and/or MPEG5000-DPPE stocks with a non-aqueous
solvent to form a phospholipid solution, and
combining the phospholipid solution with an aqueous solvent to form a phospholipid
10 suspension.
2. A method for preparing a phospholipid suspension, comprising
providing DPPA, DPPC and MPEG5000-DPPE stocks,
measuring calcium concentration of one or more of the DPPC, DPPA and MPEG5000-
15 DPPE stocks,
combining DPPA, DPPC and/or MPEG5000-DPPE stocks having a combined measured
low calcium concentration with a non-aqueous solvent to form a phospholipid solution, and
combining the phospholipid solution with an aqueous solvent to form a phospholipid
suspension.
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3. A method for preparing a phospholipid suspension, comprising
combining a MPEG5000-DPPE stock, a DPPA stock, a DPPC stock and a non-aqueous
solvent, each with a characterized calcium concentration to form a phospholipid solution,
wherein the combined characterized calcium concentration of the MPEG5000-DPPE stock, the
25 DPPA stock, the DPPC stock and the non-aqueous solvent is a low calcium concentration, and
combining the phospholipid solution with an aqueous solvent to form a phospholipid
suspension.
4. A method for preparing a phospholipid suspension, comprising
30 selecting a MPEG5000-DPPE stock, a DPPA stock and a DPPC stock, one, two or all
three of which have a characterized calcium concentration, wherein the combined
characterized calcium concentration is a low calcium concentration,

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combining said MPEG5000-DPPE stock, DPPA stock, DPPC stock and a non-aqueous solvent to form a phospholipid solution, and

combining the phospholipid solution with an aqueous solvent to form a phospholipid suspension.

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5. A method for preparing a phospholipid suspension, comprising selecting a MPEG5000-DPPE stock, a DPPA stock and a DPPC stock, each with characterized calcium concentration, wherein the combined characterized calcium concentration is a low calcium concentration,

10 combining said MPEG5000-DPPE stock, DPPA stock, DPPC stock and a non-aqueous solvent to form a phospholipid solution, and

combining the phospholipid solution with an aqueous solvent to form a phospholipid suspension.

15 6. A method for preparing a phospholipid suspension, comprising measuring calcium concentration of a MPEG5000-DPPE stock, combining a MPEG5000-DPPE stock having a measured low calcium concentration with a DPPA stock, a DPPC stock, and a non-aqueous solvent to form a phospholipid solution, and

20 combining the phospholipid solution with an aqueous solvent to form a phospholipid suspension.

7. A method for preparing a phospholipid suspension, comprising measuring calcium concentration of a DPPC stock, combining a DPPC stock having a measured low calcium concentration with a DPPA stock, a MPEG5000-DPPE stock, and a non-aqueous solvent to form a phospholipid solution, and

combining the phospholipid solution with an aqueous solvent to form a phospholipid suspension.

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8. A method for preparing a phospholipid suspension, comprising measuring calcium concentration of a DPPA stock,

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combining a DPPA stock having a measured low calcium concentration with a DPPC stock, a MPEG5000-DPPE stock, and a non-aqueous solvent to form a phospholipid solution, and

combining the phospholipid solution with an aqueous solvent to form a phospholipid suspension.

9. A method for preparing a phospholipid suspension, comprising measuring calcium concentration of a non-aqueous solvent, combining a non-aqueous solvent having a measured low calcium concentration with a DPPA stock, a DPPC stock, and a MPEG5000-DPPE stock, to form a phospholipid solution, and combining the phospholipid solution with an aqueous solvent to form a phospholipid suspension.

10. A method for preparing a phospholipid suspension, comprising selecting a MPEG5000-DPPE stock characterized as having no or low calcium concentration, combining said MPEG5000-DPPE stock, a DPPA stock, a DPPC stock and a non-aqueous solvent to form a phospholipid solution, and combining the phospholipid solution with an aqueous solvent to form a phospholipid suspension.

11. A method for preparing a phospholipid suspension, comprising combining a MPEG5000-DPPE stock, a DPPA stock, a DPPC stock and a non-aqueous solvent to form a phospholipid solution characterized as having no or low calcium concentration, and combining the phospholipid solution with an aqueous solvent to form a phospholipid suspension.

12. A method for imaging a subject comprising combining a phospholipid suspension with a perfluorocarbon gas to form an ultrasound contrast agent comprising phospholipid-encapsulated gas microspheres,

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administering the ultrasound contrast agent to a subject, and
obtaining one or more contrast-enhanced ultrasound contrast images of the subject,
wherein the phospholipid suspension is prepared by the method of any one of claims 1-37.

5 13. A method for imaging a subject comprising
 combining a phospholipid suspension with a perfluorocarbon gas to form an ultrasound
contrast agent comprising phospholipid-encapsulated gas microspheres,
 administering the ultrasound contrast agent to a subject, and
 obtaining one or more contrast-enhanced ultrasound contrast images of the subject,
10 wherein the phospholipid suspension is prepared by a method comprising
 measuring calcium concentration of MPEG5000-DPPE stock,
 combining a MPEG5000-DPPE stock having a measured low calcium concentration
with a DPPA stock, a DPPC stock, and a non-aqueous solvent to form a phospholipid solution,
 and
15 combining the phospholipid solution with an aqueous solvent to form the phospholipid
suspension.

 14. A method for imaging a subject comprising
 combining a phospholipid suspension with a perfluorocarbon gas to form an ultrasound
20 contrast agent comprising phospholipid-encapsulated gas microspheres,
 administering the ultrasound contrast agent to a subject, and
 obtaining one or more contrast-enhanced ultrasound contrast images of the subject,
wherein the phospholipid suspension is prepared by a method comprising
 selecting a MPEG5000-DPPE stock characterized as having no or low calcium
25 concentration,
 combining said MPEG5000-DPPE stock, a DPPA stock, a DPPC stock and a non-
aqueous solvent to form a phospholipid solution, and
 combining the phospholipid solution with an aqueous solvent to form a phospholipid
suspension.

30 15. A method for imaging a subject comprising

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combining a phospholipid suspension with a perfluorocarbon gas to form an ultrasound contrast agent comprising phospholipid-encapsulated gas microspheres,
administering the ultrasound contrast agent to a subject, and
obtaining one or more contrast-enhanced ultrasound contrast images of the subject,

5 wherein the phospholipid suspension is prepared by a method comprising
combining a MPEG5000-DPPE stock, a DPPA stock, a DPPC stock and a non-aqueous solvent to form a phospholipid solution characterized as having no or low calcium concentration, and
combining the phospholipid solution with an aqueous solvent to form a phospholipid
10 suspension.

16. A method for preparing a phospholipid suspension, comprising
individually combining DPPA, DPPC and MPEG5000-DPPE stocks with a propylene glycol (PG) -comprising non-aqueous solvent, in a low or no calcium condition, to form a
15 phospholipid solution, and
combining the phospholipid solution with an aqueous solvent to form a phospholipid suspension.

17. A method for preparing a phospholipid suspension, comprising
20 sequentially combining DPPA, DPPC and MPEG5000-DPPE stocks with a PG-comprising non-aqueous solvent, in a low or no calcium condition, in an order-independent manner, to form a phospholipid solution, and
combining the phospholipid solution with an aqueous solvent to form a phospholipid suspension.

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18. A method for preparing a phospholipid suspension, comprising
combining, in a methanol and toluene-free condition, DPPA, DPPC and MPEG5000-DPPE stocks to form a phospholipid blend,
combining the phospholipid blend with a PG-comprising non-aqueous solvent, in a low
30 or no calcium condition, to form a phospholipid solution, and
combining the phospholipid solution with an aqueous solvent to form a phospholipid suspension.

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19. A method for preparing a phospholipid suspension, comprising
combining DPPA, DPPC and MPEG5000-DPPE stocks with a blend solvent to form a
phospholipid blend,

5 evaporating the blend solvent to form a dried phospholipid blend,
combining the dried phospholipid blend with a PG-comprising non-aqueous solvent, in
a low or no calcium condition, to form a phospholipid solution, and
combining the phospholipid solution with an aqueous solvent to form a phospholipid
suspension.

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20. A method for preparing a phospholipid suspension, comprising
combining DPPA, DPPC and MPEG5000-DPPE stocks with a blend solvent to form a
phospholipid blend,

precipitating, in a MTBE-free condition, the phospholipid blend using a second blend
15 solvent,
combining the precipitated phospholipid blend with a non-aqueous solvent, in a low or
no calcium condition, to form a phospholipid solution, and
combining the phospholipid solution with an aqueous solvent to form a phospholipid
suspension.

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21. A method for imaging a subject comprising
combining a phospholipid suspension with a perfluorocarbon gas to form an ultrasound
contrast agent comprising phospholipid-encapsulated gas microspheres,
administering the ultrasound contrast agent to a subject, and
25 obtaining one or more contrast-enhanced ultrasound contrast images of the subject,
wherein the phospholipid suspension is prepared by the method of any one of claims 42-47.

22. A composition comprising
a phospholipid solution comprising DPPA, DPPC and MPEG5000-DPPE in a non-
30 aqueous solvent and having a low calcium concentration.

23. A composition comprising

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a phospholipid solution comprising DPPA, DPPC and MPEG5000-DPPE in a non-aqueous solvent, wherein the DPPA, DPPC and MPEG5000-DPPE and the non-aqueous solvent have a combined characterized calcium ion content that is low.

- 5 24. A method of ultrasound contrast imaging a subject comprising
- (a) activating a phospholipid suspension with a perfluorocarbon gas to form lipid-encapsulated gas microspheres, wherein the phospholipid suspension comprises a phospholipid solution having one or more phospholipids and a non-aqueous solvent, one or more of which has a characterized low calcium concentration,
- 10 (b) administering the lipid-encapsulated gas microspheres to a subject, and
- (c) obtaining an ultrasound image of the subject.

25. A method of ultrasound contrast imaging a subject comprising
- (a) activating a phospholipid suspension with a perfluorocarbon gas to form lipid-
- 15 encapsulated gas microspheres, wherein the phospholipid suspension comprises a phospholipid solution having one or more phospholipids and a non-aqueous solvent and made under a methanol and toluene free condition and a methyl t-butyl ether free condition, wherein one or more of the phospholipids and non-aqueous solvent has a low calcium concentration,
- (b) administering the lipid-encapsulated gas microspheres to a subject, and
- 20 (c) obtaining an ultrasound image of the subject.

26. A method for preparing lipid-encapsulated gas microspheres comprising combining one or more phospholipids and a non-aqueous solvent to form a phospholipid solution, wherein one or more of the phospholipids and/or the non-aqueous
- 25 solvent has a characterized low calcium concentration,
- combining the phospholipid solution with an aqueous solution to form a phospholipid suspension, and
- activating the phospholipid suspension with a perfluorocarbon gas to form lipid-encapsulated gas microspheres.

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27. A method for preparing lipid-encapsulated gas microspheres comprising combining one or more phospholipids and a non-aqueous solvent, in a methanol and toluene free and methyl t-butyl ether free condition, to form a phospholipid solution, wherein one or more of the phospholipids and/or the non-aqueous solvent has a low calcium

5 concentration.

combining the phospholipid solution with an aqueous solution to form a phospholipid suspension, and

activating the phospholipid suspension with a perfluorocarbon gas, to form lipid-encapsulated gas microspheres.

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