

CLAIMS

1. A molecular design method for an ice growth inhibition material, comprising the following steps:

(1) constructing a library for structures of compound molecules, wherein the compound molecules comprise a hydrophilic group and an ice-philic group;

(2) simulating and evaluating the spreading performance of each of the compound molecules at an ice-water interface by adopting molecular dynamics (MD) simulation; and

(3) screening the compound molecules with desired affinities for ice and water; as the ice growth inhibition material.

wherein in the MD simulation of the step (2), simulation and calculation are performed on interactions between the compound molecules, interactions between the compound molecules and the water molecules, and interactions between the compound molecules and ice-water molecules.

wherein the interactions include the formation of a hydrogen bond, a Van der Waals interaction, an electrostatic interaction, a hydrophobic interaction, a π - π interaction.

2. The molecular design method according to claim 1, wherein the MD simulation of the step (2) is performed by GROMACS, AMBER, CHARMM, NAMD, or LAMMPS;

3. The molecular design method according to claim 1, wherein preferably, in the MD simulation of the step (2), a model of a water molecule is selected from models of TIP3P, TIP4P, TIP4P/2005, SPC, TIP3P, TIP5P and SPC/E.

4. The molecular design method according to claim 1, wherein preferably in the MD simulation of the step (2), a model of a water molecule is selected from TIP4P/2005 model of a water molecule;

5. The molecular design method according to claim 1, wherein preferably, in the MD simulation of the step (2), a force field parameter is provided by one of GROMOS, ESFF, MM force field, AMBER, CHARMM, COMPASS, UFF, CVFF and other force fields;

~~preferably, in the MD simulation of the step (2), simulation and calculation are performed on interactions between the compound molecules, interactions between the compound molecules and the water molecules, and interactions between the compound molecules and ice-water~~

~~molecules; for example, the interactions include the formation of a hydrogen bond, a Van der Waals interaction, an electrostatic interaction, a hydrophobic interaction, a π - π interaction and the like;~~

6. The molecular design method according to any one of claims 1 to 5, whereinpreferably, in the MD simulation of the step (2), a temperature and pressure are adjusted when the simulation and calculation are performed on the interactions between the molecules.;

7. The molecular design method according to claim 6, whereinpreferably, the temperature and the pressure are adjusted by using a V-rescale temperature regulator and a pressure regulator;

8. The molecular design method according to any one of claims 1 to 5, whereinpreferably, in the MD simulation of the step (2), a molecular configuration of the compound molecules is maintained by selecting a potential energy parameter.;

9. The molecular design method according to any one of claims 1 to 5, whereinpreferably, in the step (2), periodic boundary conditions are adopted for x-direction, y-direction and z-direction when an aqueous solution system is simulated; periodic boundary conditions are adopted for x-direction and y-direction when an ice-water mixed system is simulated.;

10. The molecular design method according to any one of claims 1 to 5, whereinpreferably, in the MD simulation of the step (2), a cubic or octahedral box of water is selected.;

11. The molecular design method according to claim 10, whereinand in the MD simulation of the step (2), a cubic box of water with dimensions of $3.9 \times 3.6 \times 1.0 \text{ nm}^3$ is preferredselected.;

12. The molecular design method according to any one of claims 1 to 5, whereinpreferably, a main chain of the compound molecules is a carbon chain or peptide chain structure.;

13. The molecular design method according to any one of claims 1 to 5, whereinpreferably, the hydrophilic group is a functional group capable of forming a non-covalent interaction with a water molecule, ~~for example, forming a hydrogen bond, a Van der Waals interaction, an electrostatic interaction, a hydrophobic interaction or a π - π interaction with water;~~ for example, the hydrophilic group may be selected from at least one of hydroxyl ($-\text{OH}$), amino ($-\text{NH}_2$), carboxyl ($-\text{COOH}$) and amido ($-\text{CONH}_2$), or, for example, from a compound molecule, such as a hydrophilic amino acid such as proline (L-Pro), arginine (L-Arg) and lysine (L-Lys), glucono

~~delta-lactone (GDL) and a saccharide, and a molecular fragment thereof;~~

14. The molecular design method according to claim 13, wherein the functional group is a functional group capable of forming a hydrogen bond, a Van der Waals interaction, an electrostatic interaction, a hydrophobic interaction or a π - π interaction with water.

15. The molecular design method according to claim 13, wherein the hydrophilic group is selected from at least one of hydroxyl (-OH), amino (-NH₂), carboxyl (-COOH) and amido (-CONH₂).

16. The molecular design method according to claim 13, wherein the hydrophilic group is selected from a compound molecule of a hydrophilic amino acid, glucono delta-lactone (GDL) and a saccharide, and a molecular fragment thereof.

17. The molecular design method according to claim 16, wherein the hydrophilic amino acid is selected from proline (L-Pro), arginine (L-Arg) and lysine (L-Lys).

18. The molecular design method according to any one of claims 1 to 5, wherein the ice-philic group is a functional group capable of forming a non-covalent interaction with ice, for example, forming a hydrogen bond, a Van der Waals interaction, an electrostatic interaction, a hydrophobic interaction or a π - π interaction with ice; illustratively, the ice-philic group may be selected from hydroxyl (-OH), amino (-NH₂), phenyl (-C₆H₅), pyrrolidinyl (-C₄H₈N) and the like, or, for example, from a compound molecule, such as an ice-philic amino acid such as glutamine (L-Gln), threonine (L-Thr) and aspartic acid (L-Asn), a benzene ring (C₆H₆) and pyrrolidine (C₄H₉N), and a molecular fragment thereof;

19. The molecular design method according to claim 18, wherein the ice-philic group is a functional group capable of forming a hydrogen bond, a Van der Waals interaction, an electrostatic interaction, a hydrophobic interaction or a π - π interaction with ice.

20. The molecular design method according to claim 18, wherein the ice-philic group is selected from hydroxyl (-OH), amino (-NH₂), phenyl (-C₆H₅), or pyrrolidinyl (-C₄H₈N).

21. The molecular design method according to claim 18, wherein the ice-philic group is selected

a compound molecule of an ice-philic amino acid, a benzene ring (C₆H₆) and pyrrolidine (C₄H₉N), and a molecular fragment thereof.

22. The molecular design method according to claim 21, wherein the ice-philic amino acid is selected a compound molecule of glutamine (L-Gln), threonine (L-Thr) and aspartic acid (L-Asn).

23. The molecular design method according to any one of claims 1 to 5, wherein preferably, the ice growth inhibition material is formed by covalently bonding a block comprising a hydrophilic group to a block comprising an ice-philic group, or is formed by ionically bonding a molecule comprising a hydrophilic group to a molecule comprising an ice-philic group.

24. The molecular design method according to any one of claims 1–35, further comprising a step of synthesizing the compound molecules,–

25. The molecular design method according to claim 24, for example the step of synthesizing the compound molecules are selected from polymerization, dehydration condensation, or biological fermentation of genetically engineered bacteria.

26. An ice growth inhibition material obtained by the molecular design method according to any one of claims 1–325; Preferably wherein, the ice growth inhibition material is an atactic polyvinyl alcohol (PVA) with a diad syndiotacticity r of 45%–60%–65% and a molecular weight of 10–500 kDa; preferably, the PVA has a diad syndiotacticity r of 50%–55% and a molecular weight of 10–30 kDa, or a combination of the atactic PVA with an amino acid, a polyamino acid, and/or a peptidic compound.

27. The ice growth inhibition material according to claim 26, wherein the PVA has a diad syndiotacticity r of 45-60%.

28. The ice growth inhibition material according to claim 26, wherein the PVA has a diad syndiotacticity r of 50%–55% and a molecular weight of 10–30 kDa.

5. A method for screening an ice growth inhibition material, comprising: (1) determining the affinity of the ice growth inhibition material for water; and (2) determining the spreading performance of the ice growth inhibition material at an ice-water interface;

preferably, the step (1) is achieved by determining the solubility, the hydration constant, the dispersion size of the ice growth inhibition material in water, and/or the number of intermolecular hydrogen bonds formed between a molecule of the ice growth inhibition material and a water molecule.

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6. The method for screening an ice growth inhibition material according to claim 5, wherein the step (2) is achieved by determining the amount of the ice growth inhibition material absorbed on an ice surface by an ice adsorption experiment,

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the amount of the ice growth inhibition material absorbed on the ice surface = (the mass m_1 of the ice growth inhibition material adsorbed on the ice surface/the total mass m_2 of the ice growth inhibition material in an original solution comprising the ice growth inhibition material) $\times 100\%$.

7. The method for screening an ice growth inhibition material according to claim 5 or 6, wherein the ice adsorption experiment comprises:

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S1, preparing an aqueous solution of the ice growth inhibition material, and cooling to a supercooling temperature;

S2, placing a pre-cooled temperature regulating rod in the aqueous solution to induce an ice layer to grow on the surface of the temperature regulating rod, continuously stirring the aqueous solution to enable the ice growth inhibition material to be gradually adsorbed onto the surface of the ice layer, and keeping the temperature of the temperature regulating rod and the temperature of the aqueous solution at a supercooling temperature; and

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S3, determining the amount of the ice growth inhibition material absorbed on the ice surface; preferably, the temperature regulating rod is pre-cooled in one of modes of freezing by liquid nitrogen, dry ice or an ultra-low temperature refrigerator;

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preferably, the supercooling degree and the adsorption time are maintained unchanged during the ice adsorption experiment to ensure that the surface area of the resulting ice is maintained unchanged within an allowable error range;

preferably, wherein the ice growth inhibition material in the step S1 is fluorescently pre-labeled, for example, with fluorescein;

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preferably, if the ice growth inhibition material itself has absorption characteristics in an ultraviolet or fluorescence spectrum, no fluorescent label is required;

preferably, wherein the step S3 comprises:

S3a, taking out an ice block after adsorption, rinsing the ice block with purified water, and melting the ice block to give an adsorption solution of the ice growth inhibition material; and

S3b, determining the volume V of the melted adsorption solution of the ice growth inhibition material, determining the mass/volume concentration c of the ice growth inhibition material in the adsorption solution, and calculating the mass m_1 of the ice growth inhibition material adsorbed on the ice surface through the formula $m_1 = cV$;

5 preferably, wherein in the S3b, the concentration c is determined by ultraviolet-visible spectroscopy;

preferably, wherein the method is used for screening the material for inhibiting the growth of ice crystals.

10 8. The method for screening an ice growth inhibition material according to any one of claims 5–7, further comprising a step (3): evaluating the affinity of the material for water and the affinity of the material for ice, wherein the material with strong affinities for water and ice has good ice growth inhibition performance.

15 9. An ice adsorption experimental device for use in the method according to claim 6 or 7, comprising a multilayer liquid storage cavity, a temperature regulating rod and a temperature regulator, wherein the multilayer liquid storage cavity sequentially comprises an ice adsorption cavity, a bath cavity and a cooling liquid storage cavity from inside to outside, the temperature-regulating rod being arranged in the ice adsorption cavity, and the temperatures of the
20 temperature regulating rod and the liquid storage cavity being regulated by the temperature regulator;

preferably, the temperature regulating rod is of a hollow structure made of a thermally conductive material, and the hollow structure of the temperature regulating rod is provided with a liquid inlet and a liquid outlet; the temperature regulator is a fluid temperature regulator and
25 is provided with a cooling liquid outflow end and a reflux end; two ends of the cooling liquid storage cavity is provided with a liquid inlet and a liquid outlet; the cooling liquid outflow end of the temperature regulator, the liquid inlet of the temperature regulating rod, the liquid outlet of the temperature regulating rod, the liquid inlet of a cooling liquid storage tank, the liquid outlet of the cooling liquid storage tank and the reflux end of the temperature regulator are
30 sequentially linked via pipelines through which a cooling liquid flows;

preferably, the multilayer liquid storage cavity is provided with a cover;

preferably, when the ice adsorption experimental device is used, the ice adsorption cavity is arranged to contain the aqueous solution of the ice growth inhibition material, and the bath cavity in the middle layer is arranged to contain a bath medium that is at a preset temperature,

for example, a water bath, an ice bath or an oil bath; after the preset temperature of the cooling liquid is reached, the cooling liquid flows out through the temperature regulator and flows into the hollow structure of the temperature-regulating rod to regulate the temperature of the temperature-regulating rod, then flows out from the liquid outlet of the temperature-regulating rod and flows into the cooling liquid storage cavity in the outer layer to maintain the temperature of the bath medium at the preset level, and then flows through the liquid outlet of the cooling liquid storage tank and the reflux end of the temperature regulator and enters the temperature regulator to circulate.

4029. A cryopreservation solution, comprising the biomimetic ice growth inhibition material according to any one of claims 426 to 28; preferably, the biomimetic ice growth inhibition material is one of or a combination of a polyvinyl alcohol (PVA), an amino acid or a polyamino acid, and/or a peptidic compound;—

30. The cryopreservation solution according to claim 29, wherein the cryopreservation solution further comprises a polyol, a water-soluble saccharide (or a derivative thereof such as water-soluble cellulose) and a buffer;—

31. The cryopreservation solution according to claim 29, wherein more preferably, the cryopreservation solution comprises the peptidic compound, and specifically comprising, per 100 mL, 0.1–50 g of the peptidic compound, 0–6.0 g of the PVA, 0–9.0 g of the polyamino acid or the amino acid, 0–15 mL of DMSO, 5–45 mL of the polyol, the water-soluble saccharide at 0.1–1.0 mol·L⁻¹, 0–30 mL of serum and the balance of the buffer;—

32. The cryopreservation solution according to claim 29, wherein the cryopreservation solution comprises the PVA, and specifically comprising, per 100 mL, 0.01–6.0 g of the PVA, 0–50 g of the peptidic compound, 0–9.0 g of the polyamino acid or the amino acid, 0–15 mL of DMSO, 5–45 mL of the polyol, the water-soluble saccharide at 0.1–1.0 mol·L⁻¹, 0–30 mL of serum and the balance of the buffer;—

33. The cryopreservation solution according to claim 29, wherein the cryopreservation solution comprises the amino acid or the polyamino acid, and specifically comprising, per 100 mL, 0.1–50 g of the amino acid or the polyamino acid, 0–6.0 g of the PVA, 0–15 mL of DMSO, 5–45 mL of the polyol, the water-soluble saccharide at 0.1–1.0 mol·L⁻¹, 0–30 mL of serum and the

balance of the buffer;.

34. The cryopreservation solution according to claim 29, wherein ~~most preferably~~, the content of the amino acid and/or the polyamino acid in the cryopreservation solution is 0.5–50 g, preferably 1.0–35 g, per 100 mL; for example, the content of the amino acid may be 5.0–35 g, preferably 15–25 g, in the presence of the amino acid; the content of the polyamino acid may be 0.5–9.0 g, preferably 1.0–5.0 g, in the presence of the polyamino acid;.

35. The cryopreservation solution according to claim 34, wherein the content of the amino acid and/or the polyamino acid in the cryopreservation solution is 1.0–35 g per 100 mL.

36. The cryopreservation solution according to claim 34, wherein the content of the amino acid in the cryopreservation solution is 5.0–35 g per 100 mL.

37. The cryopreservation solution according to claim 34, wherein the content of the amino acid in the cryopreservation solution is 15–25 g per 100 mL.

38. The cryopreservation solution according to claim 34, wherein the content of the polyamino acid is 0.5–9.0 g.

39. The cryopreservation solution according to claim 34, wherein the content of the polyamino acid is 1.0–5.0 g.

40. The cryopreservation solution according to claim 30, wherein ~~preferably~~, the polyol ~~may be~~ is a polyol having 2–5 carbon atoms, preferably a diol having 2–3 carbon atoms, and/or a triol; such as any one of ethylene glycol, propylene glycol and glycerol, preferably ethylene glycol; preferably, the content of the polyol in the cryopreservation solution is 5.0–40 mL, for example, 6.0–20 mL, 9–15 mL, per 100 mL;.

41. The cryopreservation solution according to claim 40, wherein the polyol is a diol having 2–3 carbon atoms.

42. The cryopreservation solution according to claim 40, wherein the polyol is selected from any one of ethylene glycol, propylene glycol and glycerol.

43. The cryopreservation solution according to claim 40, wherein the polyol is ethylene glycol.

44. The cryopreservation solution according to claim 40, wherein the content of the polyol in the cryopreservation solution is 5.0–40 mL per 100 mL;

45. The cryopreservation solution according to claim 44, wherein the content of the polyol in the cryopreservation solution is 6.0–20 mL per 100 mL;

46. The cryopreservation solution according to claim 44, wherein the content of the polyol in the cryopreservation solution is 9–15 mL per 100 mL;

47. The cryopreservation solution according to claim 30, wherein preferably, the water-soluble saccharide is at least one of a non-reducing disaccharide, a water-soluble polysaccharide, a water-soluble cellulose and a saccharide anhydride, and, for example, is selected from sucrose, trehalose, hydroxypropyl methylcellulose and polysucrose;

48. The cryopreservation solution according to claim 47, wherein the water-soluble saccharide is a water-soluble cellulose.

49. The cryopreservation solution according to claim 47, wherein the water-soluble saccharide is selected from sucrose, trehalose, hydroxypropyl methylcellulose and polysucrose.

50. The cryopreservation solution according to claim 30, wherein preferably, the buffers may be selected from at least one of DPBS, hepes-buffered HTF and other cell culture buffers;

51. The cryopreservation solution according to claim 29, wherein the cryopreservation solution comprises preferably, the content of the DMSO in the cryopreservation solution is in a content of 0–10 mL, for example, 1.0–7.5 mL, per 100 mL;

52. The cryopreservation solution according to claim 51, wherein the cryopreservation solution comprises DMSO in a content of 1.0–7.5 mL per 100 mL.

53. The cryopreservation solution according to claim 29, wherein the cryopreservation solution

~~comprises the content of the serum in the cryopreservation solution is in a content of 0.1–30 mL, for example, 5.0–20 mL, per 100 mL;~~

54. The cryopreservation solution according to claim 53, wherein the cryopreservation solution comprises serum in a content of 5.0–20 mL per 100 mL.

55. The cryopreservation solution according to claim 30, wherein the cryopreservation solution comprises ~~the content of the water-soluble saccharide in the cryopreservation solution is in a content of~~ 0.1–1.0 mol·L⁻¹, for example, 0.1–0.8 mol·L⁻¹, per 100 mL;

56. The cryopreservation solution according to claim 55, wherein the cryopreservation solution comprises the water-soluble saccharide in a content of 0.1–0.8 mol·L⁻¹.

57. The cryopreservation solution according to claim 30, wherein the cryopreservation solution comprises ~~the content of the polyol in the cryopreservation solution is in a content of~~ 5.0–40 mL, for example, 6.0–20 mL, per 100 mL.

58. The cryopreservation solution according to claim 57, wherein the cryopreservation solution comprises the polyol in a content of, 6.0–20 mL per 100 mL.

59. The cryopreservation solution according to claim 29, wherein preferably, the pH of the cryopreservation solution is 6.5–7.6;

preferably, the PVA is selected from one of or a combination of two or more of an isotactic PVA, a syndiotactic PVA and an atactic PVA, and for example, the PVA has a diad syndiotacticity of 15%–65%, preferably a diad syndiotacticity of 45%–65%; preferably, the PVA may be selected from a PVA having a molecular weight of 10–500 kDa or higher;

60. The cryopreservation solution according to any one of claims 29 to 59, wherein preferably, the peptidic compounds are obtained by reacting ice-philic amino acids, such as threonine (L-Thr), glutamine (L-Gln) and aspartic acid (L-Asn), with other hydrophilic amino acids that may be selected from arginine, proline and alanine, or glucono delta-lactone (GDL) or saccharides.

61. The cryopreservation solution according to claim 60, wherein the ice-philic amino acids is

selected from threonine (L-Thr), glutamine (L-Gln) and aspartic acid (L-Asn).

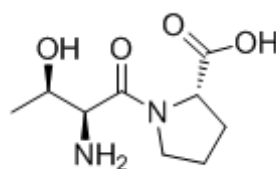
62. The cryopreservation solution according to claim 60, wherein ~~more preferably~~, the peptidic compound consists of no less than two amino acid units.

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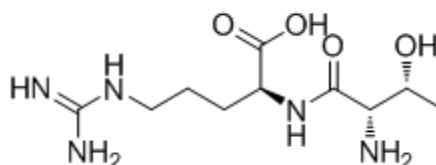
63. The cryopreservation solution according to claim 62, wherein the peptidic compound consists of ~~such as~~ 2–8 amino acid units.

64. The cryopreservation solution according to claim 60, wherein ~~more preferably~~, the peptidic compound has a structure of any one of formula (1) to formula (9):

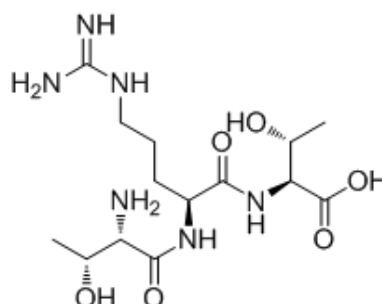
10



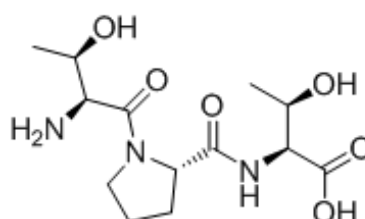
formula (1)



formula (2)

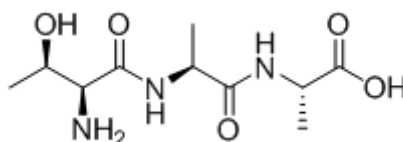


formula (3)

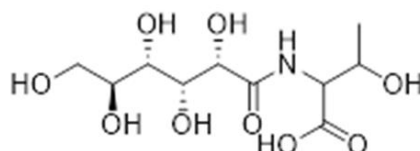


formula (4)

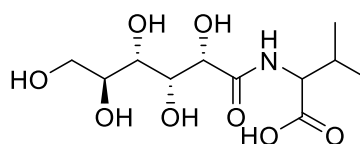
15



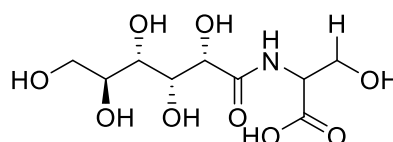
formula (5)



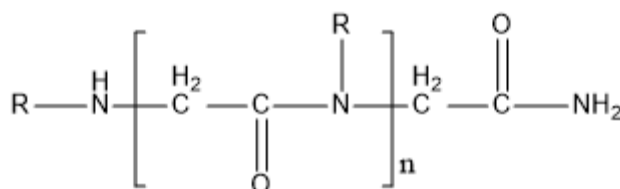
formula (6)



formula (7)



formula (8)



formula (9),

wherein, R in the formula (9) is selected from substituted alkyl and unsubstituted alkyl, and the substituent may be selected from -OH, -NH₂, -COOH, and -CONH₂ and the like; for example, R is substituted or unsubstituted C₁₋₆ alkyl, and preferably R is -CH₃, -CH₂CH₃, -CH₂CH₂COOH; n is an integer no less than 1 and no more than 1000;

65. The cryopreservation solution according to claim 64, wherein R is substituted or unsubstituted C₁₋₆ alkyl.

66. The cryopreservation solution according to claim 65, wherein R is -CH₃, -CH₂CH₃, or -CH₂CH₂COOH.

67. The cryopreservation solution according to claim 29, preferably, wherein the amino acid is an amino acid comprising an ice-philic group and a hydrophilic group, the polyamino acid is a polyamino acid consisting of an amino acid comprising an ice-philic group and an amino acid comprising a hydrophilic group;

68. The cryopreservation solution according to claim 67, wherein and the polyamino acid preferably has a degree of polymerization of 2–40.

69. The cryopreservation solution according to claim 68, wherein, for example the polyamino acid has a degree of polymerization of 6, 8, 15 and or 20 and the like, and for example;

70. The cryopreservation solution according to claim 67, wherein the polyamino acid is one of or a combination of two or more of poly-L-proline, poly-L-arginine;

71. The cryopreservation solution according to claim 67, wherein the amino acid is selected from one or two of arginine, threonine, proline, lysine, histidine, glutamic acid, aspartic acid, glycine and the like;

72. The cryopreservation solution according to claim 67, wherein the amino acid is such as a combination of arginine and threonine; or a polyamino acid consisting of the above amino acids.

11. A freezing equilibration solution, comprising, per 100 mL, 5.0–45 mL of a polyol and the balance of a buffer;

preferably, the freezing equilibration solution further optionally comprises 0–15 mL of DMSO, 0–30 mL of serum, and/or 0–5.0 g of a PVA.

73. A cryopreservation reagent, comprising the cryopreservation solution according to any one of claims 40–29 to 72 and the a freezing equilibration solution according to claim 11, wherein the freezing equilibration solution comprises, per 100 mL, 5.0–45 mL of a polyol and the balance of a buffer.

74. The cryopreservation reagent according to claim 73, wherein the freezing equilibration

solution further optionally comprises 0–15 mL of DMSO, 0–30 mL of serum, and/or 0–5.0 g of a PVA.

4375. Use of the cryopreservation solution according to any one of claims 10–29 to 72 or the freezing equilibration solution according to claim 11 or the cryopreservation reagent according to claim 12–73 or 74 for cryopreservation of cells, tissues and organs;

4476. The use according to claim 4375, wherein the cells are germ cells or stem cells; for example, the germ cells are selected from oocytes and sperms, and the stem cells are selected from embryonic stem cells, various mesenchymal stem cells (for example umbilical cord mesenchymal stem cells, adipose mesenchymal stem cells and bone marrow mesenchymal stem cells), and hematopoietic stem cells;
preferably, the tissue is an ovarian tissue or embryonic tissue;
preferably, the organ is an ovarian organ.

77. The use according to claim 76, wherein the germ cells are selected from oocytes and sperms, and the stem cells are selected from embryonic stem cells, various mesenchymal stem cells, and hematopoietic stem cells.

78. The use according to claim 77, wherein the mesenchymal stem cells are umbilical cord mesenchymal stem cells, adipose mesenchymal stem cells or bone marrow mesenchymal stem cells.

79. The use according to claim 75, wherein the tissue is an ovarian tissue or embryonic tissue.

80. The use according to claim 75, wherein the organ is an ovarian organ.