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(57) Abrégé/Abstract:
The present invention relates to emulsion-templated silica micro and nano-capsules- and methods for making them. In particular, the template emulsion is stabilized by a biosurfactant that also assists in nucleating the silica shell Mineralizing biosurfactants and stabilized micro- and nano-emulsions useful in forming the emulsion-templated micro- and nano-capsules, and methods for the use of the silica micro- and nano-capsules are also described.

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(54) Title: SILICA MICRO- AND NANO-CAPSULES AND METHODS FOR MAKING THEM

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SILICA MICRO- AND NANO-CAPSULES AND METHODS FOR MAKING THEM

Field of the Invention

The present invention relates to emulsion-templated silica micro- and nano-capsules and methods for making them. In particular, the template emulsion is stabilized by a biosurfactant that also assists in nucleating the silica shell. Mineralizing biosurfactants and stabilized micro- and nano-emulsions useful in forming the emulsion-templated micro- and nano-capsules, and methods for the use of the silica micro- and nano-capsules are also described.

Background of the Invention

The fabrication of silica nanocapsules has attracted much research and industrial interest owing to their unique morphology and diverse applications (Lou, 2008; Guerrero-Martínez, 2010; Schärtl, 2010). The core–shell structure allows encapsulation of fluorescent materials (Burns 2006), magnetic nanoparticles (Lu, 2007) and drugs (Barbé, 2004) in the core domain for imaging, sensing, and drug delivery, with a higher loading capacity than an equivalent solid nanoparticle. The silica shell is engineered around the core to provide: (i) a protective envelope with chemical and mechanical stability for storage and delivery; (ii) accessible pathways for adsorption, separation, and sustained release; and (iii) ease of surface modification with optical, magnetic, and/or biological functionalities enhancing the performance of nanocapsules for applications including biolabeling, controlled release, and targeted delivery.

Hard- and soft-templating approaches are commonly employed to construct silica nanocapsules. Hard templating of polystyrene latex spheres, for example, has been performed utilizing layer-by-layer assembly (Caruso, 1998), sodium silicate water-glass methodology (Cornelissen, 2003) and the Stöber synthesis (Kong, 2010). As an alternative to hard particle cores, soft emulsion droplets offer a number of advantages. Harsh processes (e.g., thermal decomposition and chemical dissolution) necessary for complete removal of the solid core are avoided and loading of a cargo is simplified as it can be solubilized in the core prior to shell formation (Hayashi, 2011; Chen, 2008; Li, 2010). Sol–

gel routes, including self-templating of an organosilica precursor (Hayashi, 2011) and interfacial polycondensation of hydrolyzed silicon alkoxide on ionic (Chen, 2008; Zhao, 2009; Li, 2010; Kuwahara, 2012) and non-ionic surfactants (Underhill, 2002; Jovanovic, 2005) have been developed to synthesize silica shells on oil-in-water (O/W) emulsions in the nanometer range. However, these soft-templating approaches variously incorporate steps generating adverse effects on the environment and biologically functional cargoes, for example, the use of extreme pHs and/or elevated temperatures or pressures, and involving chemical surfactants and oils that have limited pharmaceutical compatibility. An alternative pathway to silica microcapsules and nanocapsules using more benign reaction conditions and biocompatible components would, for some applications, remove restrictions inherent in current approaches.

Biomimetic templating offers mild processes (i.e., at near-neutral pH and ambient conditions) for the synthesis of silica-based materials as revealed by silica mineralization in organisms (Morse, 1999). However, there are no current biomimetic routes to emulsion-templated silica micro- and nano-capsules. A key limitation seems to be the identification of biocompatible agents (e.g., biomolecules) that can both stabilize an emulsion template and catalyze a silicification reaction.

There is a need for a simple, less harsh method of making silica micro- and nano-capsules that can be used in delivery of drugs, proteins, nanoparticles, pesticides, herbicides, and fluorescent or spin-responsive molecules for therapeutic, diagnostic, agricultural and environmental applications.

Summary of the Invention

The present invention is predicated in part on the discovery of biosurfactants that stabilize nanoemulsions can be used to nucleate silica on the stabilized nanoemulsion thereby forming silica nanocapsules.

In a first aspect of the invention there is provided a mineralizing biosurfactant comprising:

i) a surface-active polypeptide module at least 6 amino acid residues in length; and

ii) a charged peptide module 5 to 40 amino acid residues in length comprising at least one hydrogen bond donating amino acid residue and at least one positively charged amino acid residue;

wherein the surface-active polypeptide module and the charged peptide module are conjugated to one another.

In another aspect of the invention there is provided a stabilized microemulsion or nanoemulsion comprising an oil phase, an aqueous phase and a mineralizing biosurfactant of the invention, wherein the mineralizing biosurfactant is located at the interface between the oil and aqueous phases.

In a further aspect of the present invention, there is provided a silica micro- or nano-capsule comprising:

i) an oil core stabilized by a surface film of mineralizing biosurfactant of the invention; and

ii) a silica shell encapsulating the stabilized oil core.

In another aspect of the invention there is provided a method of making a silica micro- or nano-capsule comprising the steps of:

i) forming a stabilized microemulsion or nanoemulsion by mixing a composition comprising:

a) an oil phase;

b) an aqueous phase; and

c) a mineralizing biosurfactant of the invention; and

ii) mixing the microemulsion or nanoemulsion with silica or a silica precursor.

In a yet further aspect of the invention there is provided a composition comprising the micro- or nano-capsules of the invention and a carrier.

In another aspect of the invention there is provided a use of the micro- or nano-capsules of the invention to deliver a compound to a human, animal, pest or environment.

Brief Description of the Figures

Figure 1 is a schematic diagram showing the strategy for preparation of the emulsion-templated silica nanocapsules comprising a mineralizing biosurfactant. The mineralizing biosurfactant is comprised of a surface-active polypeptide module (Sur) and a charged peptide module (Si). Step (I) sonication of Miglyol® 812 oil in a SurSi solution followed by dialysis; Step (II) addition of tetraethoxysilane (TEOS) to nanoemulsion (bottom panel showing possible molecular interactions between peptide side chain groups and silica species); Step (III) interfacial polycondensation of silica species.

Figure 2 is a graphical representation of the effect of TEOS concentration (C_{TEOS}) and reaction time (t) on the diameter of the nanocapsules prepared from a nanoemulsion stabilized by SEQ ID NO:156 in 25 mM HEPES buffer, pH 7.5 (left panel). A photographic representation showing TEM images of individual silica nanocapsules (right panel) produced at pH 7.5 after 50 hours reaction of the nanoemulsion with 20 mM (top), 40 mM (middle) and 80 mM (bottom) TEOS in 25 mM HEPES buffer. Scale bars are 50 nm.

Figure 3 is a photographic representation showing TEM images of silica nanocapsules produced at pH 7.5 after 30 hours reaction of nanoemulsion stabilized by SEQ ID NO:156 with TEOS in 25 mM HEPES buffer at a TEOS concentration of a) 20 mM and b) 40 mM. Scale bars are 100 nm.

Figure 4 is a photographic representation of TEM images of silica nanocapsules produced at pH 7.5 after a) 20 hours, b) 30 hours, c) 40 hours and d) 50 hours reaction of nanoemulsion stabilized by SEQ ID NO:156 with 80 mM TEOS in 25 mM HEPES buffer. Scale bars are 100 nm.

Figure 5 is a photographic representation of TEM images of silica nanocapsules produced at pH a) 7.5 and b) pH 8 after 20 hours reaction of nanoemulsion stabilized by SEQ ID NO:156 with 80 mM TEOS in 25 mM HEPES buffer. Scale bars are 200 nm.

Figure 6 is a photographic representation of TEM images of silica nanocapsules loaded with 0.05 mg/mL fipronil in Miglyol® 812 oil produced at pH 7.5 after 30 hours reaction of fipronil-loaded nanoemulsions stabilized by SEQ ID NO:156 with a) 40 mM, b) 80 mM and c) 240 mM TEOS in 25 mM HEPES buffer, which resulted in silica nanocapsules having 8 ± 2 nm, 25 ± 3 nm and 44 ± 7 nm shell thickness respectively. Scale bars are 200 nm.

Figure 7 is a graphical representation of the efficacy of silica nanocapsules loaded with 0.05 mg/mL fipronil in Miglyol® 812 oil having 8 ± 2 nm (0.05F-NC8), 25 ± 3 nm (0.05F-NC25) and 44 ± 7 nm (0.05F-NC44) shell thicknesses compared to Milli-Q water (control), 0.05 mg/mL fipronil-loaded nanoemulsion stabilized by SEQ ID NO:156 (0.05F-NE) and commercially available Termidor® containing 0.05 mg/mL fipronil after direct treatment against worker termites of *Coptotermes acinaciformis*.

Figure 8 is a graphical representation of efficacy of silica nanocapsules loaded with 1 mg/mL fipronil in Miglyol® 812 oil having 44 ± 7 nm silica shell thickness (1F-NC44) as compared to Milli-Q water (control) and commercially available Termidor® containing 1 mg/mL fipronil after a feeding treatment against worker and soldier termites of *Coptotermes acinaciformis*.

Figure 9 provides block flow diagrams of the purification processes used to obtain high purity peptide of SEQ ID NO: 157. (a) Precipitation-based purification process and followed by (b) chromatography-based purification process.

Figure 10 is a photographic representation of SDS-PAGE analysis of SEQ ID NO: 157 after heating/cell lysis, contaminant precipitation and dilute precipitation steps, and IMAC purification step. (Lane 1) Total protein; (Lane 2) Resuspended precipitate and (Lane 3) supernatant after heating/cell lysis and contaminant precipitation step; (Lane 4) Solubilized precipitate after dilute precipitation step; (Lane 5) Pass-through fraction, (Lane 6) washing fraction and (Lane 7) collected elution fraction after IMAC purification step; (Lane 8) Novex® Sharp Pre-stain MW ladder.

Figure 11 is a graphical representation showing purification of SEQ ID No: 157 by (a) immobilized metal ion affinity chromatography (IMAC) and (b) desalting chromatography.

Figure 12 is a graphical representation showing analysis of high purity SEQ ID NO: 157 by using (a) reversed-phase high performance liquid chromatography (RP-HPLC) and (b) mass spectrometry (MS).

Figure 13 is a photographic representation showing TEM images of silica nanocapsules produced at pH 7.5 after 20 hours reaction of SEQ ID NO: 157-stabilized nanoemulsions (10 v/v% Miglyol® 812 oil) with 80 mM TEOS in 25 mM HEPES buffer. Scale bar is 200 nm.

Detailed description of the Invention

1. Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are described. For the purposes of the present invention, the following terms are defined below.

The articles "*a*" and "*an*" are used herein to refer to one or to more than one (i.e. to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

As used herein, the term "*about*" refers to a quantity, level, value, dimension, size, or amount that varies by as much as 30%, 25%, 20%, 15% or 10% to a reference quantity, level, value, dimension, size, or amount.

The term "*amphiphilic*" refers to molecules having both hydrophilic and hydrophobic regions. The term amphiphilic is synonymous with "amphipathic" and these terms may be used interchangeably.

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

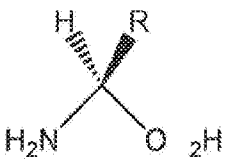
The term "*hydrophilic*" refers to a molecule or portion of a molecule that is attracted to water and other polar solvents. A hydrophilic molecule or portion of a molecule is polar and/or charged or has an ability to form interactions such as hydrogen bonds with water or polar solvents.

The term "*hydrophobic*" refers to a molecule or portion of a molecule that repels or is repelled by water and other polar solvents. A hydrophobic molecule or portion of a molecule is non-polar, does not bear a charge and is attracted to non-polar solvents.

As used herein, the term "*amino acid*" refers to an α-amino acid or a β-amino acid and may be a L- or D- isomer. The amino acid may have a naturally occurring side chain (see Table 1) or a non-naturally occurring side chain (see Table 2). The amino acid may also be further substituted in the α-position or the β-position with a group selected from -C_1-C_6alkyl, -$(CH_2)_nCOR_1$, -$(CH_2)_nR_2$, -PO_3H, -$(CH_2)_n$heterocyclyl or -$(CH_2)_n$aryl where R_1 is -OH, -NH_2, -NHC_1-C_3alkyl, -OC_1-C_3alkyl or -C_1-C_3alkyl and R_2 is -OH, -SH, -SC_1-C_3alkyl, -OC_1-C_3alkyl, -C_3-C_{12}cycloalkyl, -NH_2, -NHC_1-C_3alkyl or -NHC(C=NH)NH_2 and where each alkyl, cycloalkyl, aryl or heterocyclyl group may be substituted with one or more groups selected from -OH, -NH_2, -NHC_1-C_3alkyl, -OC_1-C_3alkyl, -SH, -SC_1-C_3alkyl, -CO_2H, -CO_2C_1-C_3alkyl, -$CONH_2$ or -$CONHC_1$-C_3alkyl.

Amino acid structure and single and three letter abbreviations used throughout the specification are defined in Table 1, which lists the twenty naturally occurring amino acids which occur in proteins as L-isomers.

TABLE 1



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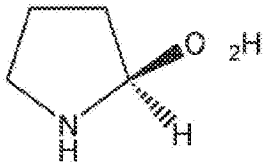
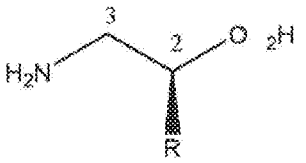


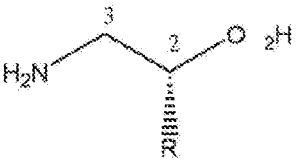
(2)

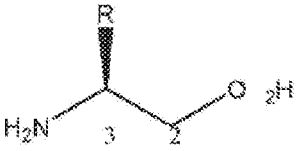
Amino Acid	Three-letter Abbreviation	One-letter symbol	Structure of side chain (R)
Alanine	Ala	A	$-CH_3$
Arginine	Arg	R	$-(CH_2)_3NHC(=N)NH_2$
Asparagine	Asn	N	$-CH_2CONH_2$
Aspartic acid	Asp	D	$-CH_2CO_2H$
Cysteine	Cys	C	$-CH_2SH$
Glutamine	Gln	Q	$-(CH_2)_2CONH_2$
Glutamic acid	Glu	E	$-(CH_2)_2CO_2H$
Glycine	Gly	G	-H
Histidine	His	H	$-CH_2$(4-imidazolyl)
Isoleucine	Ile	I	$-CH(CH_3)CH_2CH_3$
Leucine	Leu	L	$-CH_2CH(CH_3)_2$
Lysine	Lys	K	$-(CH_2)_4NH_2$
Methionine	Met	M	$-(CH_2)_2SCH_3$
Phenylalanine	Phe	F	$-CH_2Ph$
Proline	Pro	P	see formula (2) above for structure of amino acid
Serine	Ser	S	$-CH_2OH$
Threonine	Thr	T	$-CH(CH_3)OH$
Tryptophan	Trp	W	$-CH_2$(3-indolyl)
Tyrosine	Tyr	Y	$-CH_2$(4-hydroxyphenyl)
Valine	Val	V	$-CH(CH_3)_2$

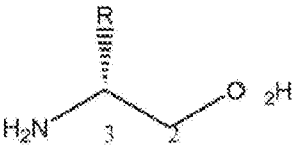
The term "*α-amino acid*" as used herein, refers to a compound having an amino group and a carboxyl group in which the amino group and the carboxyl group are separated by a single carbon atom, the α-carbon atom. An α-amino acid includes naturally occurring and non-naturally occurring L-amino acids and their D-isomers and derivatives thereof such as salts or derivatives where functional groups are protected by suitable protecting groups. The α-amino acid may also be further substituted in the α-position with a group selected from -C_1-C_6alkyl, -$(CH_2)_nCOR_1$, -$(CH_2)_nR_2$, -PO_3H, -$(CH_2)_n$heterocyclyl or -$(CH_2)_n$aryl where R_1 is -OH, -NH_2, -NHC_1-C_3alkyl, -OC_1-C_3alkyl or -C_1-C_3alkyl and R_2 is -OH, -SH, -SC_1-C_3alkyl, -OC_1-C_3alkyl, -C_3-C_{12}cycloalkyl, -NH_2, -NHC_1-C_3alkyl or -$NHC(C=NH)NH_2$ and where each alkyl, cycloalkyl, aryl or heterocyclyl group may be substituted with one or more groups selected from -OH, -NH_2, -NHC_1-C_3alkyl, -OC_1-C_3alkyl, -SH, -SC_1-C_3alkyl, -CO_2H, -CO_2C_1-C_3alkyl, -$CONH_2$ or -$CONHC_1$-C_3alkyl.

As used herein, the term "*β-amino acid*" refers to an amino acid that differs from an α-amino acid in that there are two (2) carbon atoms separating the carboxyl terminus and the amino terminus. As such, β-amino acids with a specific side chain can exist as the *R* or *S* enantiomers at either of the α (C2) carbon or the β (C3) carbon, resulting in a total of 4 possible isomers for any given side chain. The side chains may be the same as those of naturally occurring α-amino acids (see Table 1 above) or may be the side chains of non-naturally occurring amino acids (see Table 2 below).





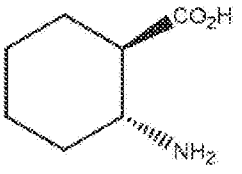


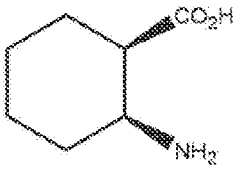


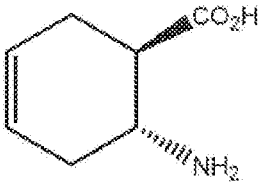
Furthermore, the β-amino acids may have mono-, di-, tri- or tetra-substitution at the C2 and C3 carbon atoms. Mono-substitution may be at the C2 or C3 carbon atom.

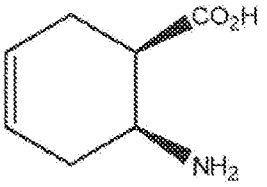
Di-substitution includes two substituents at the C2 carbon atom, two substituents at the C3 carbon atom or one substituent at each of the C2 and C3 carbon atoms. Tri-substitution includes two substituents at the C2 carbon atom and one substituent at the C3 carbon atom or two substituents at the C3 carbon atom and one substituent at the C2 carbon atom. Tetra-substitution provides for two substituents at the C2 carbon atom and two substituents at the C3 carbon atom. Suitable substituents include -C_1-C_6alkyl, -$(CH_2)_nCOR_1$, -$(CH_2)_nR_2$, -PO_3H, -$(CH_2)_n$heterocyclyl or -$(CH_2)_n$aryl where R_1 is -OH, -NH_2, -NHC_1-C_3alkyl, -OC_1-C_3alkyl or -C_1-C_3alkyl and R_2 is -OH, -SH, -SC_1-C_3alkyl, -OC_1-C_3alkyl, -C_3-C_{12}cycloalkyl, -NH_2, -NHC_1-C_3alkyl or -NHC(C=NH)NH_2 and where each alkyl, cycloalkyl, aryl or heterocyclyl group may be substituted with one or more groups selected from -OH, -NH_2, -NHC_1-C_3alkyl, -OC_1-C_3alkyl, -SH, -SC_1-C_3alkyl, -CO_2H, -CO_2C_1-C_3alkyl, -$CONH_2$ or -$CONHC_1$-C_3alkyl.

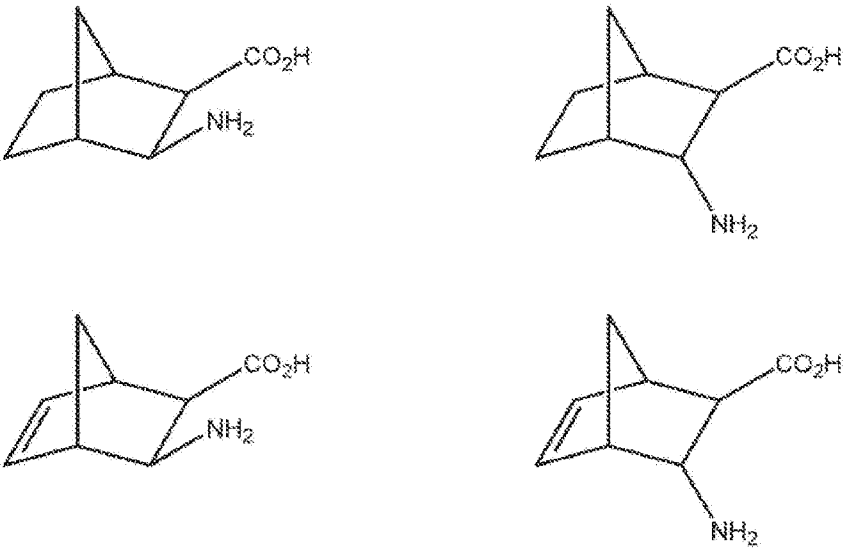
Other suitable β-amino acids include conformationally constrained β-amino acids. Cyclic β-amino acids are conformationally constrained and are generally not accessible to enzymatic degradation. Suitable cyclic β-amino acids include, but are not limited to, *cis*- and *trans*-2-aminocyclopropyl carboxylic acids, 2-aminocyclobutyl and cyclobutenyl carboxylic acids, 2-aminocyclopentyl and cyclopentenyl carboxylic acids, 2-aminocyclohexyl and cyclohexenyl carboxylic acids and 2-amino-norbornane carboxylic acids and their derivatives, some of which are shown below:











Suitable derivatives of β-amino acids include salts and may have functional groups protected by suitable protecting groups.

The term "*non-naturally occurring amino acid*" as used herein, refers to amino acids having a side chain that does not occur in the naturally occurring L-α-amino acids. Examples of non-natural amino acids and derivatives include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, *t*-butylglycine, norvaline, phenylglycine, ornithine, citrulline, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acids that may be useful herein is shown in Table 2.

TABLE 2

Non-conventional amino acid	Code	Non-conventional amino acid	Code
α-aminobutyric acid	Abu	L-N-methylalanine	Nmala
α-amino-α-methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
aminocyclopropane-carboxylate	Cpro	L-N-methylasparagine	Nmasn
		L-N-methylaspartic acid	Nmasp

	aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
	aminonorbornyl-carboxylate	Norb	L-N-methylglutamine	Nmgln
			L-N-methylglutamic acid	Nmglu
	cyclohexylalanine	Chexa	L-N-methylhistidine	Nmhis
5	cyclopentylalanine	Cpen	L-N-methylisoleucine	Nmile
	D-alanine	Dal	L-N-methylleucine	Nmleu
	D-arginine	Darg	L-N-methyllysine	Nmlys
	D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
	D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
10	D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
	D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
	D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
	D-isoleucine	Dile	L-N-methylproline	Nmpro
	D-leucine	Dleu	L-N-methylserine	Nmser
15	D-lysine	Dlys	L-N-methylthreonine	Nmthr
	D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
	D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
	D-phenylalanine	Dphe	L-N-methylvaline	Nmval
	D-proline	Dpro	L-N-methylethylglycine	Nmetg
20	D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
	D-threonine	Dthr	L-norleucine	Nle
	D-tryptophan	Dtrp	L-norvaline	Nva
	D-tyrosine	Dtyr	α-methyl-aminoisobutyrate	Maib
	D-valine	Dval	α-methyl-aminobutyrate	Mgabu
25	D-α-methylalanine	Dmala	α-methylcyclohexylalanine	Mchexa
	D-α-methylarginine	Dmarg	α-methylcylcopentylalanine	Mcpen
	D-α-methylasparagine	Dmasn	α-methyl-α-napthylalanine	Manap
	D-α-methylaspartate	Dmasp	α-methylpenicillamine	Mpen
	D-α-methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
30	D-α-methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
	D-α-methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
	D-α-methylisoleucine	Dmile	N-amino-α-methylbutyrate	Nmaabu

	D-α-methylleucine	Dmleu	α-napthylalanine	Anap
	D-α-methyllysine	Dmlys	N-benzylglycine	Nphe
	D-α-methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
	D-α-methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
5	D-α-methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
	D-α-methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
	D-α-methylserine	Dmser	N-cyclobutylglycine	Ncbut
	D-α-methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
	D-α-methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
10	D-α-methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
	D-α-methylvaline	Dmval	N-cylcododecylglycine	Ncdod
	D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
	D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
	D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
15	D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
	D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
	D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
	D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
	D-N-methylhistidine	Dnmhis	N-(hydroxyethyl))glycine	Nser
20	D-N-methylisoleucine	Dnmile	N-(imidazolylethyl))glycine	Nhis
	D-N-methylleucine	Dnmleu	N-(3-indolylyethyl)glycine	Nhtrp
	D-N-methyllysine	Dnmlys	N-methyl-γ-aminobutyrate	Nmgabu
	N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
	D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
25	N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
	N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
	N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
	N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
	D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
30	D-N-methyltyrosine	Dnmtyr	N-methylnapthylalanine	Nmanap
	D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
	γ-aminobutyric acid	Gabu	N-(*p*-hydroxyphenyl)glycine	Nhtyr

	L-*t*-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
	L-ethylglycine	Etg	penicillamine	Pen
	L-homophenylalanine	Hphe	L-α-methylalanine	Mala
	L-α-methylarginine	Marg	L-α-methylasparagine	Masn
5	L-α-methylaspartate	Masp	L-α-methyl-*t*-butylglycine	Mtbug
	L-α-methylcysteine	Mcys	L-methylethylglycine	Metg
	L-α-methylglutamine	Mgln	L-α-methylglutamate	Mglu
	L-α-methylhistidine	Mhis	L-α-methylhomophenylalanine	Mhphe
	L-α-methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
10	L-α-methylleucine	Mleu	L-α-methyllysine	Mlys
	L-α-methylmethionine	Mmet	L-α-methylnorleucine	Mnle
	L-α-methylnorvaline	Mnva	L-α-methylornithine	Morn
	L-α-methylphenylalanine	Mphe	L-α-methylproline	Mpro
	L-α-methylserine	Mser	L-α-methylthreonine	Mthr
15	L-α-methyltryptophan	Mtrp	L-α-methyltyrosine	Mtyr
	L-α-methylvaline	Mval	L-N-methylhomophenylalanine	Nmhphe
	N-(N-(2,2-diphenylethyl) carbamylmethyl)glycine	Nnbhm	N-(N-(3,3-diphenylpropyl) carbamylmethyl)glycine	Nnbhe
	1-carboxy-1-(2,2-diphenyl-	Nmbc		
20	ethylamino)cyclopropane			

The term "*alkyl*" as used herein refers to straight chain or branched hydrocarbon groups. Suitable alkyl groups include, but are not limited to methyl, ethyl, propyl, isopropyl, butyl, 25 isobutyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, dodecyl, tetradecyl, hexadecyl and octadecyl. The term alkyl may be prefixed by a specified number of carbon atoms to indicate the number of carbon atoms or a range of numbers of carbon atoms that may be present in the alkyl group. For example, C_1-C_3alkyl refers to methyl, ethyl, propyl and isopropyl.

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The term "*cycloalkyl*" as used herein, refers to cyclic hydrocarbon groups. Suitable cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl,

cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl, cycloundecyl and cyclododecyl.

The term "*heterocyclyl*" as used herein refers to 5 or 6 membered saturated, partially unsaturated or aromatic cyclic hydrocarbon groups in which at least one carbon atom has been replaced by N, O or S. Optionally, the heterocyclyl group may be fused to a phenyl ring. Suitable heterocyclyl groups include, but are not limited to pyrrolidinyl, piperidinyl, pyrrolyl, thiophenyl, furanyl, oxazolyl, imidazolyl, thiazolyl, isoxazolyl, pyrazolyl, isothiazolyl, pyridinyl, quinolinyl, isoquinolinyl, indolyl, benzofuranyl, benzothiophenyl, oxadiazolyl, tetrazolyl, triazolyl and pyrimidinyl.

The term "*aryl*" as used herein, refers to C_6-C_{10} aromatic hydrocarbon groups, for example phenyl and naphthyl.

The term "*α-helix breaking amino acid residue*" refers to an amino acid residue that has a low frequency of occurrence in known α-helical conformations and which promotes termination of an α-helix. α-Helix breaking amino acid residues may lack an amide hydrogen to participate in hydrogen bonding within the helix or may be too conformationally flexible or inflexible to form the constrained α-helical conformation in an energy efficient manner. Examples of α-helix breaking amino acid residues include, but are not limited to proline and glycine.

The term "*hydrophilic amino acid residue*" as used herein refers to an amino acid residue in which the side chain is polar or charged. Examples include glycine, L-serine, L-threonine, L-cysteine, L-tyrosine, L-asparagine, L-glutamine, L-aspartic acid, L-glutamic acid, L-lysine, L-arginine, L-histidine, L-ornithine, D-serine, D-threonine, D-cysteine, D-tyrosine, D-asparagine, D-glutamine, D-aspartic acid, D-glutamic acid, D-lysine, D-arginine, D-histidine and D-ornithine, especially L-serine, L-threonine, L-cysteine, L-tyrosine, L-asparagine, L-glutamine, L-aspartic acid, L-glutamic acid, L-lysine, L-arginine, L-histidine and L-ornithine.

As used herein, the term "*hydrophobic amino acid residue*" refers to an amino acid residue in which the side chain is non-polar. Examples include, but are not limited to L-alanine, L-valine, L-leucine, L-isoleucine, L-proline, L-methionine, L-phenylalanine, L-tryptophan, L-aminoisobutyric acid, D-alanine, D-valine, D-leucine, D-isoleucine, D-proline, D-methionine, D-phenylalanine, D-tryptophan, D-aminoisobutyric acid, L-cyclohexylalanine, D-cyclohexylalanine, L-cyclopentylalanine, D-cyclopentylalanine, L-norleucine, D-norleucine, L-norvaline, D-norvaline, L-*tert*-butylglycine, D-*tert*-butylglycine, L-ethylglycine and D-ethylglycine, especially L-alanine, L-valine, L-leucine, L-isoleucine, L-proline, L-methionine, L-phenylalanine, L-tryptophan and L-aminoisobutyric acid.

As used herein, the term "*positively charged amino acid residue*" refers to an amino acid residue having a side chain capable of bearing a positive charge. Examples include, but are not limited to L-lysine, L-arginine, L-histidine, L-ornithine, D-lysine, D-arginine, D-histidine and D-ornithine.

As used herein, the term "*negatively charged amino acid residue*" refers to an amino acid residue having a side chain capable of bearing a negative charge. Examples include, but are not limited to L-aspartic acid, L-glutamic acid, D-aspartic acid and D-glutamic acid.

As used herein, the term "*polar amino acid residue*" refers to an amino acid residue having a side chain that has a dipole moment. Examples of polar amino acid residues, include, but are not limited to glycine, L-serine, L-threonine, L-cysteine, L-tyrosine, L-asparagine, L-glutamine, D-serine, D-threonine, D-cysteine, D-tyrosine, D-asparagine and D-glutamine.

The term "*amino acid having a small side chain*" refers to amino acid residues having a side chain with 4 or less non-hydrogen atoms, especially 3 or less non-hydrogen atoms. Examples include, but are not limited to, glycine, L-alanine, L-valine, L-leucine, L-isoleucine, L-methionine, L-serine, L-threonine, L-cysteine, L-asparagine, L-aspartic acid, D-alanine, D-valine, D-leucine, D-isoleucine, D-methionine, D-serine, D-threonine, D-cysteine, D-asparagine and D-aspartic acid, especially glycine, L-alanine, L-valine, L-serine, L-threonine and L-cysteine.

The term "*conservative amino acid substitution*" refers to substituting one amino acid in a sequence with another amino acid that has similar properties of size, polarity and/or aromaticity and does not change the nature of activity of the peptide. For example, one polar amino acid residue may be substituted with another polar amino acid residue or an amino acid residue having a small side chain may be substituted with another amino acid residue having a small side chain.

The term "*liquid-liquid interface*" refers to the region forming the common boundary between the immiscible liquids, the oil phase and polar phase, in the nanoemulsion.

The terms "*self-assemble*", "*self-assembled*" and "*self-assembly*" refer to a population of peptide biosurfactant molecules with an affinity for the liquid-liquid interface and which relocate themselves from the bulk solution to the liquid-liquid interface.

The term "*surface-active polypeptide*" refers to a polypeptide that has both hydrophilic and hydrophobic residues and that has an affinity for the liquid-liquid interface and therefore is capable of self-assembly at the liquid-liquid interface either with or without structural modification in such a way that the hydrophobic and hydrophilic residues are able to preferentially partition into their miscible phases at the liquid-liquid interface.

As used herein, the term "*microemulsion*" refers to an oil-in-water emulsion having an average oil phase particle size of 900 nm to 100 μm especially between 900 nm and 50 μm, more especially between 900 nm and 5 μm.

As used herein, the term "*silica microcapsule*" refers to a structure comprising a core-shell structure having an oil core and a silica shell. The microcapsules have an average diameter of less than 150 μm, especially between 1 μm and 100 μm, more especially 1 μm and 30 μm, and most especially between 1 μm and 5 μm.

The term "*nanoemulsion*" refers to an oil-in-water emulsion having an average oil phase particle size of less than 900 nm, especially between 20 nm and 500 nm, more especially between 30 nm and 300 nm.

The term "*silica nanocapsule*" refers to a structure comprising core-shell structure having an oil core and a silica shell. The nanocapsules having an average diameter of less than 1 μm, especially between 50 nm and 750 nm, more especially between 70 nm and 500 nm, more especially between 80 nm and 400 nm.

2. Mineralizing Biosurfactants

In one aspect of the invention, there is provided a mineralizing biosurfactant comprising:

i) a surface-active polypeptide module at least 6 amino acid residues in length; and

ii) a charged peptide module 5 to 40 amino acid residues in length comprising at least one hydrogen bond donating amino acid residue and at least one positively charged amino acid residue;

wherein the surface-active polypeptide module and the charged peptide module are conjugated to one another.

The surface-active polypeptide module may be any peptide or protein that has a hydrophobic region and a hydrophilic region and is capable of self-assembly at a liquid-liquid interface, such as the interface between an oil phase and a polar or aqueous phase.

In some embodiments, the surface-active polypeptide module is a polypeptide or protein that has tertiary structure presenting defined hydrophobic and hydrophilic regions either before or after adsorption at the liquid-liquid interface. Typical proteins include food biosurfactants or portions thereof such as casein and lactoglobulin and the common protein lysozyme, all of which are known in the art to be surface active.

In embodiments where the surface-active polypeptide module is a peptide or polypeptide rather than a protein, the polypeptide module may have a limitation on the maximum

number of amino acid residues in the module. For example, the surface-active polypeptide module may be 6 to 130 amino acid residues in length.

In some embodiments, the surface-active polypeptide is a co-block polypeptide having a sequence comprising blocks of hydrophilic amino acid residues and blocks of hydrophobic amino acid residues, for example a sequence:

$$(O)_q(W)_r \qquad \text{or} \qquad (W)_q(O)_r$$

where O is a hydrophobic amino acid residue and W is a hydrophilic amino acid residue, q and r are at least each independently 1 and q + r is an integer of 6 to 130.

In some embodiments, the surface-active polypeptide module comprises a peptide able to structure in such a way as to form a hydrophobic face and a hydrophilic face thus imparting preferential absorption at a liquid-liquid interface. In some embodiments, the surface-active polypeptide module comprises an amphiphilic peptide.

In these embodiments, the surface-active polypeptide module comprises an amphiphilic peptide comprising an amino acid sequence:

$$[(a\ b\ c\ d\ d'\ e\ f\ g)_n(L)_m]_p$$

wherein n is an integer from 2 to 12;
amino acid residues a and d are hydrophobic amino acid resides;
amino acid d' is absent or is any amino acid residue;
at least one of residues b and c and at least one of residues e and f are hydrophilic amino acid residues and the other of amino acid resides b and c and e and f are any amino acid residue;
amino acid residue g is any amino acid residue;
L is a linking peptide sequence of 1 to 11 amino acid residues;
m is 0 or 1; and
p is an integer from 1 to 6.

In some embodiments, n is 2 to 8 or 2 to 6, especially 2 to 4.

Amino acid residues a and d are hydrophobic amino acid residues. In some embodiments, amino acid residues a and d are independently selected from L-alanine, L-valine, L-leucine, L-methionine, L-isoleucine, L-phenylalanine, L-tyrosine, D-alanine, D-valine, D-leucine, D-methionine, D-isoleucine, D-phenylalanine and D-tyrosine, especially L-alanine, L-methionine, L-valine and L-leucine.

Amino acid residue d' may be absent or may be any amino acid residue. In some embodiments, when present, d' is a hydrophobic amino acid residue. The residue d' may be included in longer sequences, for example, where n is 3, 6, 9 or 12, to counteract perturbations in helix turn when a helix is formed, that may result in misalignment of the hydrophobic residues on one face of the helix. In some embodiments, d' is present in the third, sixth, ninth and/or twelfth sequence of $(a\ b\ c\ d\ d'\ e\ f\ g)_n$ when n is 3, 6, 9 and 12, but is absent in the other (a b c d d' e f g) sequences in the amphiphilic peptide module. In some embodiments, when present, amino acid d' may be selected from L-alanine, L-valine, L-leucine, L-methionine, L-isoleucine, L-phenylalanine, L-tyrosine, D-alanine, D-valine, D-leucine, D-methionine, D-isoleucine, D-phenylalanine, D-tyrosine, especially L-alanine, L-methionine, L-valine and L-leucine.

At least one of b and c is a hydrophilic amino acid residue, such as L-serine, L-threonine, L-cysteine, L-tyrosine, L-asparagine, L-glutamine, L-aspartic acid, L-glutamic acid, L-lysine, L-histidine, L-ornithine, D-serine, D-threonine, D-cysteine, D-tyrosine, D-asparagine, D-glutamine, D-aspartic acid, D-glutamic acid, D-lysine, D-histidine and D-ornithine, especially L-serine, L-threonine, L-cysteine, L-tyrosine, L-asparagine, L-glutamine, L-aspartic acid, L-glutamic acid, L-lysine, L-histidine and L-ornithine. The other one of amino acid residues b and c is any amino acid residue, especially an amino acid residue that has a propensity to form α-helices, such as alanine, lysine, uncharged glutamic acid, methionine, leucine and aminoisobutyric acid or a small amino acid residue such as alanine, serine, valine, leucine or isoleucine, or a hydrophilic amino acid residue such as glutamine, asparagine, serine, glutamic acid and aspartic acid.

At least one of e and f is a hydrophilic amino acid residue, such as L-serine, L-threonine, L-cysteine, L-tyrosine, L-asparagine, L-glutamine, L-aspartic acid, L-glutamic acid, L-lysine, L-histidine, L-ornithine, D-serine, D-threonine, D-cysteine, D-tyrosine, D-asparagine, D-glutamine, D-aspartic acid, D-glutamic acid, D-lysine, D-histidine and D-ornithine, especially L-serine, L-threonine, L-cysteine, L-tyrosine, L-asparagine, L-glutamine, L-aspartic acid, L-glutamic acid, L-lysine, L-histidine and L-ornithine. The other one of amino acid residues e and f is any amino acid residue, especially an amino acid residue that has a propensity to form α-helices, such as alanine, lysine, uncharged glutamic acid, methionine, leucine and aminoisobutyric acid or a small amino acid residue such as alanine, serine, valine, leucine or isoleucine, or a hydrophilic amino acid residue such as glutamine, asparagine, serine, glutamic acid and aspartic acid.

Amino acid residue g may be any amino acid residue. In particular embodiments, amino acid residue g is a residue that has a propensity to form α-helices, such as alanine, lysine, uncharged glutamic acid, methionine, leucine and aminoisobutyric acid, especially alanine, lysine and uncharged glutamic acid.

In some embodiments, each amino acid residue b is independently selected from a small hydrophobic amino acid residue, such as alanine, leucine, valine, methionine and isoleucine, or a hydrophilic amino acid residue, especially a polar or charged amino acid residue such as L-serine, L-threonine, L-cysteine, L-tyrosine, L-asparagine, L-glutamine, L-lysine, L-arginine, L-histidine, aspartic acid and glutamic acid. In some embodiments, each b is independently selected from L-lysine, L-histidine, L-serine, L-alanine, L-asparagine and L-glutamine.

In some embodiments, each amino acid residue c is independently selected from a polar, positively charged or negatively charged amino acid residue, such as L-serine, L-threonine, L-cysteine, L-tyrosine, L-asparagine, L-glutamine, L-lysine, L-arginine, L-histidine, L-aspartic acid and L-glutamic acid. In some embodiments, each c is independently selected from L-glutamine, L-arginine, L-serine, L-glutamic acid and L-asparagine.

Each amino acid residue e is independently any amino acid residue and may be hydrophobic or hydrophilic. In some embodiments, each e is independently selected from L-alanine, L-valine, L-leucine, L-isoleucine, L-serine, L-threonine, L-aspartic acid and L-glutamic acid, especially L-alanine, L-serine and L-glutamic acid.

In some embodiments, each amino acid residue f is a polar, positively charged or negatively charged amino acid residue, such as L-serine, L-threonine, L-cysteine, L-tyrosine, L-asparagine, L-glutamine, L-lysine, L-arginine, L-histidine, L-aspartic acid and L-glutamic acid. In some embodiments, each f is independently selected from L-aspartic acid, L-glutamic acid, L-arginine, L-glutamine, L-histidine, L-lysine and L-asparagine.

Amino acid residue g is independently any amino acid residue and may be hydrophobic or hydrophilic. In some embodiments, the residue g is independently selected from a small hydrophobic residue or a charged or polar uncharged residue. In some embodiments, each g is independently selected from L-alanine, L-valine, L-leucine, L-isoleucine, L-serine, L-threonine, L-asparagine L-lysine, L-glutamic acid and L-glutamine, especially L-alanine, L-serine and L-glutamine.

The linking peptide sequence L may be absent (m=0) or may be a sequence of any amino acid residues. In some embodiments, the linking sequence L has 1 or 2 amino acid residues. In other embodiments, the linking sequences has at least three amino acid residues. In some embodiments, the linking peptide sequence is present between peptide sequences $(a\ b\ c\ d\ d'\ e\ f\ g)_n$ and enables folding of the peptide sequences $(a\ b\ c\ d\ d'\ e\ f\ g)_n$ allowing the peptide sequences $(a\ b\ c\ d\ d'\ e\ f\ g)_n$ to interact with one another and form folded tertiary structures such as 2, 3, 4 or 5 α-helix bundles.

In some embodiments, the linking peptide sequence L has 3 to 9, 3 to 7, 3 to 5 amino acid residues. In a particular embodiment, the linking peptide sequence L has 3 amino acid residues.

In some embodiments, the peptide sequence $(a\ b\ c\ d\ d'\ e\ f\ g)_n$ is α-helical or has α-helical propensity. In these embodiments, the linking peptide sequence L may comprise an amino acid residue that is an α-helix breaking amino acid residue.

This residue assists in terminating any α-helical structure formed by the preceding peptide $(a\ b\ c\ d\ d'\ e\ f\ g)_n$ and allowing the linking amino acid residues flexibility for folding. α-Helix breaking amino acid residues include amino acid residues that are unable to contribute to α-helical structure, such as proline, or have high flexibility, for example serine. The charged group on aspartic acid is also known to have low helix propensity. Common α-helix breaking amino acid residues include proline and glycine.

The linking peptide sequence also may include one or more residues that allow flexibility so that two adjacent peptides can fold so that they interact with one another. In particular embodiments, the linking peptide sequence allows the peptides $(a\ b\ c\ d\ d'\ e\ f\ g)_n$ to fold in a manner to form a 2, 3, 4 or 5 helix bundle, especially a 4-helix bundle, in bulk solution. In some embodiments, the flexibility is imparted by one or more amino acid residues having a small side chain, for example, glycine, serine, alanine, valine, cysteine and threonine. In some embodiments, these same amino acids play a dual role of conferring flexibility to the overall sequence of linking amino acid residues as well as helix termination.

When more than one linking peptide sequence is present in the polypeptide, for example, where m is 1 and p is 2 to 6, each linking sequence may be the same or different.

In some embodiments, the linking sequence comprises D-P-X where X is a small amino acid residue such as serine, glycine, cysteine or threonine. In some embodiments, the linking sequence comprises D-P-S. In some embodiments, the linking sequence is D-P-S.

In some embodiments, m is 1 and p is 1 to 6, especially 2 to 6, 3 to 6 or 3 to 5.

In other embodiments, m is 0 and p is 1. In these embodiments, the surface peptide module comprises an amino acid sequence:

$(a\ b\ c\ d\ d'\ e\ f\ g)_n$

wherein n is an integer from 2 to 12;

amino acid residues a and d are hydrophobic amino acid residues;

amino acid residue d' is absent or is any amino acid residue;

at least one of residues b and c and at least one of residues e and f are hydrophilic amino acid residues and the other of amino acid residues b and c and e and f are any amino acid residue;

amino acid residue g is any amino acid residue.

In some embodiments, the sequence $(a\ b\ c\ d\ d'\ e\ f\ g)_n$ may be shifted in sequence by one amino acid residue to form the sequence $(g\ a\ b\ c\ d\ d'\ e\ f)_n$. Sequence $(a\ b\ c\ d\ d'\ e\ f\ g)_n$ and sequence $(g\ a\ b\ c\ d\ d'\ e\ f)_n$ are interchangeable.

In some embodiments, the surface-active peptide module is selected from:

SEQ ID NO:1	MKQLADSVSRLEHA
SEQ ID NO:2	MKQLADSVSRLESA
SEQ ID NO:3	LMQLARQVSRLESA
SEQ ID NO:4	MKELADSVDRLESA
SEQ ID NO:5	MKQLADSVSHLEHA
SEQ ID NO:6	MEELADSVEELESA
SEQ ID NO:7	MKKLADSVKKLESA
SEQ ID NO:8	EISALEKEISALEK
SEQ ID NO:9	KISALKEKISALKE
SEQ ID NO:10	MKELADSVSRLEHA
SEQ ID NO:11	AKSLAESVSRLEHA
SEQ ID NO:12	MKQLADSLHQLARQ
SEQ ID NO:13	MKQLADSLMQLARQ
SEQ ID NO:14	LMQLARQMKQLADS
SEQ ID NO:15	LMQLARQLMQLARQ
SEQ ID NO:16	MKELADSLMQLARQ
SEQ ID NO:17	MKQLADSLHQLAHQ
SEQ ID NO:18	MEELADSLEELARQ

SEQ ID NO:19	MKKLADSLKKLARQ
SEQ ID NO:20	MKQLADSLHQLAHK
SEQ ID NO:21	MKELADSLHELARE
SEQ ID NO:22	MKELADSLHQLARQ
SEQ ID NO:23	MKQLADSLHELARQ
SEQ ID NO:24	MKELADSLHELARQ
SEQ ID NO:25	MKELADSLHQLARE
SEQ ID NO:26	MKQLADSLHELARE
SEQ ID NO:27	AKSLAESLHSLARS
SEQ ID NO:28	LHQLARQVSRLEHA
SEQ ID NO:29	LHQLARQVSRLEHA
SEQ ID NO:30	LMQLARQVSRLESA
SEQ ID NO:31	LMQLARQVDRLESA
SEQ ID NO:32	LHQLAHQVSHLEHA
SEQ ID NO:33	LEELARQVEELESA
SEQ ID NO:34	LKKLARQVKKLESA
SEQ ID NO:35	LHQLAHKVSHLEHA
SEQ ID NO:36	LHELAREVSRLEHA
SEQ ID NO:37	LHQLARQVSRLEHA
SEQ ID NO:38	LHELARQVSRLEHA
SEQ ID NO:39	LHELARQVSRLEHA
SEQ ID NO:40	LHQLAREVSRLEHA
SEQ ID NO:41	LHSLARSVSRLEHA
SEQ ID NO:42	AKSVAESLHSLARS
SEQ ID NO:43	AHSVAESLHSLARS
SEQ ID NO:44	AHSVAKSLHSLARS
SEQ ID NO:45	AHSVAESLHSLAES
SEQ ID NO:46	AQSVAQSLAQLAQS
SEQ ID NO:47	AESVAESLAELAES
SEQ ID NO:48	ANSVANSLANLANS
SEQ ID NO:49	ADSVADSLADLADS
SEQ ID NO:50	AQSVAESLAQLAES

SEQ ID NO:51	AESVAESLAELAES
SEQ ID NO:52	ANSVAESLANLAES
SEQ ID NO:53	ADSVAESLADLAES
SEQ ID NO:54	MKQLADSLHQLARQVSRLEHA
SEQ ID NO:55	MKQLADSLHQLARQVSRLEHA
SEQ ID NO:57	MKQLADSLMQLARQVSRLESA
SEQ ID NO:58	LMQLARQMKQLADSLMQLARQVSRLESA
SEQ ID NO:59	MKELADSLMQLARQVDRLESA
SEQ ID NO:60	MKQLADSLHQLAHQVSHLEHA
SEQ ID NO:61	MEELADSLEELARQVEELESA
SEQ ID NO:62	MKKLADSLKKLARQVKKLESA
SEQ ID NO:63	MKQLADSLHQLAHKVSHLEHA
SEQ ID NO:64	EISALEKEISALEKEISALEK
SEQ ID NO:65	KISALKEKISALKEKISALKE
SEQ ID NO:66	MKELADSLHELAREVSRLEHA
SEQ ID NO:67	MKELADSLHQLARQVSRLEHA
SEQ ID NO:68	MKQLADSLHELARQVSRLEHA
SEQ ID NO:69	MKQLADSLHQLARQVSRLEHA
SEQ ID NO:70	MKELADSLHELARQVSRLEHA
SEQ ID NO:71	MKELADSLHQLAREVSRLEHA
SEQ ID NO:72	MKQLADSLHELAREVSRLEHA
SEQ ID NO:73	AKSLAESLHSLARSVSRLEHA
SEQ ID NO:74	AKSVAESLHSLARSVSRLVEHA
SEQ ID NO:75	AHSVAESLHSLARSVSRLVEHA
SEQ ID NO:76	AHSVAKSLHSLARSVSRLVSHA
SEQ ID NO:77	AHSVAESLHSLAESVSELVSHA
SEQ ID NO:78	AQSVAQSLAQLAQSVSQLVSQA
SEQ ID NO:79	AESVAESLAELAESVSELVSEA
SEQ ID NO:80	ANSVANSLANLANSVSNLVSNA
SEQ ID NO:81	ADSVADSLADLADSVSPLVSDA
SEQ ID NO:82	AQSVAESLAQLAESVSELVSQA
SEQ ID NO:83	AESVAESLAELAESVSELVSEA

SEQ ID NO:84	ANSVAESLANLAESVSELVSNA
SEQ ID NO:85	ADSVAESLADLAESVSELVSDA
SEQ ID NO:86	MD(PS-MKQLADS-LHQLARQ-VSRLEHA-D)$_4$
SEQ ID NO:87	MD(PS-MKQLADS-LHQLARQ-VSRLEHA-D)$_2$
SEQ ID NO:88	MD(PS-AKSLAES-LHSLARS-VSRLEHA-D)$_4$
SEQ ID NO:89	MD(PS-AKSVAES-LHSLARS-VSRLVEHA-D)$_4$
SEQ ID NO:90	MD(PS-AHSVAES-LHSLARS-VSRLVEHA-D)$_4$
SEQ ID NO:91	MD(PS-AHSVAKS-LHSLARS-VSRLVSHA-D)$_4$
SEQ ID NO:92	MD(PS-AHSVAES-LHSLAES-VSELVSHA-D)$_4$
SEQ ID NO:93	MD(PS-AQSVAQS-LAQLAQS-VSQLVSQA-D)$_4$
SEQ ID NO:94	MD(PS-ANSVANS-LANLANS-VSNLVSNA-D)$_4$
SEQ ID NO:95	MD(PS-AQSVAES-LAQLAES-VSELVSQA-D)$_4$
SEQ ID NO:96	MD(PS-ANSVAES-LANLAES-VSELVSNA-D)$_4$.
SEQ ID NO:97	MD(PS-MKQLADS-LMQLARQ-VSRLESA-D)$_4$.
SEQ ID NO:98	MD(PS-LMQLARQ-MKQLADS-LMQLARQ-VSRLESA)$_4$.
SEQ ID NO:99	MD(PS-MKELADS-LMQLARQ-VDRLESA-D)$_4$.
SEQ ID NO:100	MD(PS-MKQLADS-LHQLAHQ-VSHLEHA-D)$_4$.
SEQ ID NO:101	MD(PS-MEELADS-LEELARQ-VEELESA-D)$_4$.
SEQ ID NO:102	MD(PS-MKKLADS-LKKLARQ-VKKLESA-D)$_4$.
SEQ ID NO:103	MD(PS-MKQLADS-LHQLAHK-VSHLEHA-D)$_4$.
SEQ ID NO:104	MD(PS-EISALEK-EISALEK-EISALEK-D)$_4$.
SEQ ID NO:105	MD(PS-KISALKE-KISALKE-KISALKE-D)$_4$.
SEQ ID NO:106	MD(PS-MKELADS-LHELARE-VSRLEHA-D)$_4$.
SEQ ID NO:107	MD(PS-MKELADS-LHQLARQ-VSRLEHA-D)$_4$.
SEQ ID NO:108	MD(PS-MKQLADS-LHELARQ-VSRLEHA-D)$_4$.
SEQ ID NO:109	MD(PS-MKELADS-LHELARQ-VSRLEHA-D)$_4$.
SEQ ID NO:110	MD(PS-MKELADS-LHQLARE-VSRLEHA-D)$_4$.
SEQ ID NO:111	MD(PS-MKQLADS-LHELARE-VSRLEHA-D)$_4$.
SEQ ID NO:112	MD(PS-AESVAES-LAELAES-VSELVSEA-D)$_4$.
SEQ ID NO:113	MD(PS-ADSVADS-LADLADS-VSPLVSDA-D)$_4$.
SEQ ID NO:114	MD(PS-AESVAES-LAELAES-VSELVSEA-D)$_4$.
SEQ ID NO:115	MD(PS-ADSVAES-LADLAES-VSELVSDA-D)$_4$.

SEQ ID NO:116 MDPS(MKQLADSLHQLARQVSRLEHA-DPS)$_3$MKQLADS-LHQLARQVSRLEHA-EPS
SEQ ID NO:117 Ac-AAAAAAD
SEQ ID NO:118 Ac-VVVVVVD
SEQ ID NO:119 Ac-VVVVVVDD
SEQ ID NO:120 Ac-LLLLLLDD
SEQ ID NO:121 Ac-GGGGDD
SEQ ID NO:122 Ac-GGGGGGDD
SEQ ID NO:123 Ac-GGGGGGGGDD
SEQ ID NO:124 Ac-GGGGGGGGGGDD
SEQ ID NO:125 Ac-VVVVVVKK
SEQ ID NO:126 Ac-LLLLLLKK
SEQ ID NO:127 Ac-AAAAAAK
SEQ ID NO:128 Ac-VVVVVVH
SEQ ID NO:129 Ac-LLLLLLK
SEQ ID NO:130 HHVVVVVV
SEQ ID NO:131 KVVVVVV

The charged peptide module may be any peptide that is capable of driving formation of an inorganic silica layer near a liquid-liquid interface. In some embodiments, the charged peptide module is a positively charged peptide module.

In some embodiments, the charged peptide module comprises 1 to 10 hydrogen bond donating amino acid residues, especially 1 to 8 hydrogen bond donating amino acid residues. In some embodiments, the hydrogen bond donating amino acid residues are independently selected from serine and tyrosine.

In some embodiments, the charged peptide module comprises 1 to 15 positively charged amino acid residues, especially 4 to 12 positively charged amino acid residues. In some embodiments, the positively charged amino acid residues are independently selected from lysine, arginine, histidine and ornithine, especially lysine, histidine and arginine.

In some embodiments, the charged peptide module comprises 1 to 10 uncharged or non-hydrogen bond donating amino acid residues, especially 1 to 5 uncharged or non-hydrogen bond donating amino acid residues. In particular embodiments, the uncharged or non-hydrogen bond donating are selected from polar amino acid residues and hydrophobic amino acid residues. In some embodiments, the uncharged or non-hydrogen bond donating amino acid residues are independently selected from glycine, alanine, valine, leucine, isoleucine, methionine, asparagine, glutamine, phenylalanine, tryptophan and aminoisobutyric acid, especially glycine, alanine, valine, leucine and isoleucine.

In some embodiments, the charged peptide module is selected from:

SEQ ID NO: 132 SSKKSGSYSGSKGSKRRIL
SEQ ID NO: 133 RKKRKKRKKRKKGGGY
SEQ ID NO: 134 SGSKGSKRRIL
SEQ ID NO: 135 KSGSYSGSKGSKRRIL
SEQ ID NO: 136 SGSKGSKRR
SEQ ID NO: 137 SSKKSGSYSGSKGSK
SEQ ID NO: 138 LIRRSSKKSGSY
SEQ ID NO: 139 SSKKSGSYRRIL
SEQ ID NO: 140 APPGHHHWHHH
SEQ ID NO: 141 KPSHHHHHTGAN
SEQ ID NO: 142 MSPHPHPRHHHT
SEQ ID NO: 143 MSPHHMHHSHGH
SEQ ID NO: 144 LPHHHHLHTKLP
SEQ ID NO: 145 APHHHHPHHLSR
SEQ ID NO: 146 RGRRRRLSCRLL
SEQ ID NO: 147 VKVKVKVKV^{D}P^{L}PTKVKVKVKV
SEQ ID NO: 148 VKVKVKVKV^{D}P^{L}PTKVEVKVKV
SEQ ID NO: 149 KIAALKQKIASLKQEIDALEYENDALEQ^{a}
SEQ ID NO: 150 KIRRLKQKNARLKQEIAALEYEIAALEQ^{a}
SEQ ID NO: 151 $CH_3(CH_2)_{14}CO$-AAAAKKKK
SEQ ID NO: 152 $CH_3(CH_2)_{14}CO$-AAAAHHHH
SEQ ID NO: 153 IIIK

SEQ ID NO: 154 CKKCKK[b]

a Self-assembling peptide fibres that are formed by block A (KIAALKQKIASLKQ) which complements D (EIAALEYEIAALEQ) and B (EIDALEYENDALEQ) complements C (KIRRLKQKNARLKQ). This leads to sticky end dimers that assemble further into fibres. The register of the assembly is partly maintained by the asparagine residues.

b The two tripeptides (CKK) are connected through a disulfide bridge (-S-S-).

In some embodiments, the surface-active polypeptide module and the charged peptide module are conjugated directly to one another via an amide bond. In other embodiments, the surface-active polypeptide module and the charged peptide module are conjugated to one another by a linker. In a particular embodiment, the surface--active polypeptide module and the charged peptide module are conjugated directly to one another via an amide bond.

In some embodiments, where the surface-active polypeptide and the charged peptide are conjugated to one another by a linker, the linker is a peptide linker of 1 to 10 amino acid residues in length, especially 1 to 5 amino acid residues in length. The linker sequence may be formed from any amino acid residue. In some embodiments, the amino acid sequence linking the surface-active polypeptide module and the charged peptide module comprises an α-helix breaking amino acid residue. This residue assists in terminating any α-helical structure in the surface-active polypeptide and may further provide flexibility in the amino acid linking sequence to orientate the charged peptide module away from the liquid-liquid interface into the aqueous or polar phase when the mineralizing biosurfactant is located at a liquid-liquid interface. α-Helix breaking amino acid residues include amino acid residues that are unable to contribute to α-helical structure, such as proline, have high flexibility, for example, serine. The charged group on aspartic acid is also known to have low helix propensity Common α-helix breaking amino acid residues include proline and glycine.

The amino acid sequence linking the surface-active polypeptide module and the charged polypeptide module may also include one or more residues that impart flexibility. In some embodiments, the flexibility is imparted by one or more amino acid residues having a small side chain, for example, glycine, serine, alanine, valine, cysteine and threonine. In some embodiments, these same amino acids play a dual role of conferring flexibility to the overall sequence of linking amino acid residues as well as helix termination.

In some embodiments, the surface-active polypeptide module is located at the N-terminus of the mineralizing biosurfactant and the charged peptide module is located at the C-terminus of the mineralizing biosurfactant. In other embodiments, the surface-active polypeptide module is located at the C-terminus of the mineralizing biosurfactant and the charged peptide module is located at the N-terminus of the mineralizing biosurfactant. In particular embodiments, the surface-active polypeptide module is located at the N-terminus of the mineralizing biosurfactant and the charged peptide module is located at the C-terminus of the mineralizing biosurfactant.

In some embodiments, the charged peptide module is attached to a side chain of an amino acid residue of the surface-active peptide module. For example, the C-terminal carboxylic acid of the charged peptide module may be linked to an amine group of a lysine or ornithine residue in the surface active polypeptide via an amide bond. Alternatively, the N-terminal amino group of the charged peptide module may be linked to a side chain carboxylic acid of an amino acid residue in the surface-active polypeptide module via an amide bond.

In some embodiments, the mineralizing biosurfactant has a free N-terminal amino group and a free C-terminal carboxy group. In other embodiments, the N-terminal amino group is capped with an N-terminal capping group and/or the C-terminal carboxy group is capped with a C-terminal capping group. In particular embodiments, the N-terminal amino group is capped with an N-terminal capping group and the C-terminal carboxy group is capped with a C-terminal capping group.

As used herein, the N-terminal capping group, when present, is any group that blocks the reactivity of the N-terminal amino group. Suitable examples include acyl groups such as acetyl (ethanoyl), propanoyl, butanoyl, pentanoyl and hexanoyl, especially acetyl.

As used herein, the C-terminal capping group, when present, is any suitable group that blocks the reactivity of the C-terminal carboxyl group. Suitable examples include amino groups thereby forming an amide. Examples include $-NH_2$, -NH(alkyl) and $-NH(alkyl)_2$.

Suitable mineralizing biosurfactants include any combination of a surface-active polypeptide of SEQ ID NO: 1 to 131 and a charged peptide of SEQ ID NO: 132 to 154; especially:

SEQ ID NO: 155 Ac-MKQLAHSVSRLEHA-SSKKSGSYSGSKGSKRRIL-NH_2

SEQ ID NO: 156 Ac-MKQLAHSVSRLEHA-RKKRKKRKKRKKGGGY-NH_2

SEQ ID NO: 157 MDPSMKQLADSLHQLARQVSRLEHADPSMKQLADSLHQ LARQVSRLEHADPSMKQLADSLHQLARQVSRLEHADPSMKQLADSLHQLARQVS RLEHAEPS-RKKRKKRKKRKKGGGY

Without wishing to be bound by theory, it is postulated that the surface-active polypeptide module (Sur peptide module) self-assembles at the liquid-liquid interface to stabilize the nanoemulsion droplet. In some instances, stabilization may be enhanced by intermolecular interactions between laterally oriented side chains of adjacent surface-active polypeptide modules at the interface. In cases where the charged peptide module has multiple charges, the high charge on this module may provide additional DLVO stabilization at the interface. While the surface-active polypeptide module is at the interface, the charged peptide module (Si peptide module) extends into the aqueous phase of the micro- or nano-emulsion. The amino acids of the charged peptide module, such as lysine, arginine, serine and tyrosine, interact with silica species in the aqueous phase by providing cationic charges and hydrogen bonding sites. These amino acids may act as the nucleation sites for reactive silanolate (≡Si-O^-) and silanol (≡Si-OH) species that participate in silicification and direct the silca growth through condensation of these species, forming siloxane (≡Si-O-Si≡) at the oil-water interface. This process is schematically outlined in Figure 1.

The mineralizing biosurfactant or the surface-active polypeptide module or charged peptide module may be prepared by methods known in the art, such as solid phase synthesis or solution phase synthesis using Fmoc or Boc protected amino acid residues. Alternatively, the mineralizing biosurfactant may be prepared by recombinant techniques as known in the art using standard microbial culture technology, genetically engineered microbes and recombinant DNA technology (Sambrook and Russell, Molecular Cloning: A Laboratory Manual (3rd Edition), 2001, CSHL Press).

In brief, the genetically engineered microbes contain a polynucleotide sequence that comprises a nucleotide sequence that encodes the polypeptide. The nucleotide sequence is operably linked to a promotor sequence. The microbes may be any microbes suitable for use in culturing processes such as fermentation. Examples of suitable microbes include *E. coli*, *Saccharomyces cerevisiae*, *Bacillis subtilis*, and *Piccia pastoris*, especially *E. coli*. Any culturing process may be used, for example, fermentation. During the culturing process the microbes express the polypeptide.

Once culturing is complete, the microbial cells may be further treated in the culture medium, for example, the fermentation broth, or may be isolated and stored or resuspended in the same or different media. Cells may be isolated by commonly used techniques such as centrifugation or filtration. Optionally cells may undergo a cell-conditioning step after cell recovery. For example, the cells may be collected and re-suspended in water or buffered solution prior to storage or use.

After culturing, the microbial cells are disrupted to provide a disruptate composition comprising soluble proteins and cell debris. Cell disruption may be achieved by means known in the art including mechanical means and non-mechanical means. Small scale disruption may be achieved by methods such as sonication or homogenization. Large scale disruption may be achieved by mechanical means such as bead milling, homogenization and microfluidization, or non-mechanical means including physical means such as decompression, osmotic shock and thermolysis; chemical means such as antibiotics, chelating agents, chaotropes, detergents, solvents, hydroxide and hyperchlorite; and enzymatic means such as lytic enzymes, autolysis and cloned phage lysis.

Optionally after the cell disruption step, solid cell debris is removed by techniques known in the art such as centrifugation or filtration. Removal of cell debris provides a solution of soluble cell proteins that includes the polypeptide mineralizing biosurfactant.

Purification of the polypeptide from other contaminating cell proteins and polypeptides may be achieved by treating the cell disruptate, either directly from cell disruption or

clarified by removal of insoluble cell debris, with a kosmotropic salt in an amount suitable to salt-out cell derived contaminants but salt-in the mineralizing surfactant.

The kosmotropic salt may be formed from a kosmotropic ion. Examples of kosmotropic ions include sulfate, carbonate, phosphate, lithium, fluoride, calcium and acetate. The counterion of the salt may be any suitable counterion of opposite charge.

The amount of kosmotropic salt is an amount suitable to precipitate contaminating proteins and polypeptides but not the polypeptide mineralizing biosurfactant of interest. This amount can be readily determined by those skilled in the art by exposing a sample of cell disruptate, with or without clarification, to a range of salt concentrations, separating the precipitate, and supernatant and analyzing the supernatant to determine the amount of contaminating proteins in the supernatant and pellet by SDS-PAGE or HPLC. In some embodiments, the amount of kosmotropic salt is in the range of 0.2 M and 2.0 M. In some embodiments, the amount of kosmotropic salt is in the range of 0.2 M and 0.5 M, for example, about 0.25 M. In other embodiments, the amount of kosmotropic salt is in the range of 0.5 M and 2.9 M, for example, 1.0 M and 2.0 M, especially about 1.5 M.

After treatment with the kosmotropic salt, the precipitate containing cell contaminants and the supernatant containing the polypeptide may be separated by methods known in the art, such as gravity sedimentation, centrifugation or filtration.

Alternatively, established chromatography methods may be used to separate the cell contaminants and mineralizing biosurfactant.

In some embodiments, where the surface-active polypeptide module has a folded tertiary structure such as a 4-helix bundle, the mineralizing biosurfactant or the surface-active polypeptide may be prepared recombinantly and purified and isolated using a thermal purification method in which the cell disruptate is exposed to a kosmotropic salt at an elevated temperature such as above 45°C or above 60°C or between 85°C and 100°C, such a purification method is described in detail in WO 2012/079125.

In particular embodiments, the mineralizing biosurfactant is prepared as one polypeptide sequence optionally including amino acid linking sequences.

In other embodiments, the surface-active polypeptide module and the charged peptide module are prepared separately and conjugated together by methods known in the art. For example, the C-terminus of one module may be conjugated to the N-terminus of the other module by peptide bond formation (amide bond formation), as is well known in the art. In brief, the C-terminal or side chain carboxylic acid is activated by formation of a reactive species such as an acyl chloride, anhydride, a carbodiimide, a phosphonium species and the like, and the activated carboxylic acid is reacted with the N-terminal or side chain amino group to form an amide bond.

3. Micro- and Nano-emulsions and Micro- and Nano-capsules

In another aspect of the present invention, there is provided a micro- or nano-emulsion comprising the mineralizing biosurfactant described above.

The micro- or nano-emulsion is typically an oil-in-water micro- or nano-emulsion where an oil droplet is dispersed in a polar or aqueous phase. The microemulsion has an average oil droplet size of 900 nm to 100 μm, especially between 900 nm and 50 μm, more especially between 900 nm and 5 μm. The nanoemulsion has an average oil droplet size of less than 900 nm, especially in the range of between 20 and 750 nm or 30 and 500 nm, more especially in the range of 30 to 300 nm.

The micro- or nano-emulsions include a polar or aqueous phase and an oil phase wherein the oil phase is immiscible with the polar or aqueous phase. Suitable polar or aqueous phases include water, buffer, methanol, ethanol, propanol and mixtures thereof. Suitable oil phases include neutral esters of saturated coconut and palm kernel oil-derived caprylic and capric fatty acids and glycerine or propylene glycol such as Miglyol® 810, 812, 818, 829 and 840, edible oils such as olive oil, sunflower oil, safflower oil, grapeseed oil, sesame oil, coconut oil, canola oil, corn oil, flaxseed oil, palm oil, palm kernel oil, peanut oil and soyabean oil, or triglycerides rich in unsaturated fatty acids or mixtures thereof.

In some embodiments, the oil phase is present in the initial emulsion in an amount between 0.5 to 10% v/v, especially 0.5 to 5% v/v, more especially 1 to 3% v/v, most especially about 2% v/v.

The micro- and nano-emulsions of the invention may be prepared by methods known in the art for preparing micro- and nano-emulsions, for example, high energy mixing, couette shear, homogenization, sonication, dropwise dispersion or the use of microfluidic platforms. For example, the micro- or nano-emulsion may be prepared by mixing the oil and aqueous phase where the aqueous phase comprises the at least one mineralizing biosurfactant, by ultrasonication or high shear mixing. Micro-emulsions may be prepared using lower concentrations of biosurfactant and lower energy mixing than used for nano-emulsions.

In some embodiments, stabilization of the mineralizing biosurfactant at the liquid-liquid interface results from DLVO interactions. In some embodiments, the aqueous phase further comprises a component that enhances interaction of the side chains of the amino acid residues of the surface-active polypeptide module in the mineralizing biosurfactant at the liquid-liquid interface. This enhances the stability of the micro- or nano-emulsion against coalescence. Suitable components include metal ions which may form bridges between two charged laterally oriented side chains of the surface-active peptide which are located on adjacent polypeptide biosurfactants. Suitable metal ions include calcium ions and magnesium ions, transition metal ions such as titanium ions, vanadium ions, chromium ions, manganese ions, iron ions, cobalt ions, nickel ions, copper ions, zinc ions and molybdenum ions, and lanthanide ions such as lanthanum ions, cerium ions, praseodynium ions, neodynium ions, promethium ions, samarium ions, europium ions, gadolinium ions, terbium ions, dysprosium ions, holmium ions, erbium ions, thulium ions, ytterbium ions, and lutetium ions. In particular embodiments, the metal ions are selected from one or more of calcium, magnesium and transition metal ions, especially calcium, magnesium, copper, nickel and zinc ions, more especially zinc ions.

The metal ions may be included in the micro- or nano-emulsion aqueous or polar phase in the form of a salt. The salt may be selected for suitability for the use of the micro- or

nano-emulsion or subsequent micro- or nano-capsule. Suitable salts include halides, such as fluorides, chlorides, bromides and iodides, phosphates, sulfates and the like, especially chlorides such as zinc chloride.

In some embodiments, the oil core of the nanoemulsion comprises a compound for delivery to a human or animal body, such as a pharmaceutical or veterinary product, or a compound for delivery to an environment, such as a household, industrial or agricultural environment, for example a pesticide, herbicide, microbicide and the like.

In some embodiments, the compound to be delivered to the human or animal body or environment is sparingly soluble, slightly soluble, very slightly soluble or practically insoluble in water but is very soluble in the oil phase of the micro- or nano-emulsion. In other embodiments, the pharmaceutically active agent is soluble, freely soluble or very soluble in water and is included in the micro- or nano-emulsion oil phase on a nanoparticle such as a dendrimer, mesoporous silica nanoparticle or a polymeric nanoparticle such as those made of polycaprolactone (PCL) or polylactic-co-glycolic acid (PGLA), or in an aqueous micro- or nano-droplet within the oil phase, or by oil-phase solubilisation using a surfactant or polymer to alter the surface properties of the pharmaceutically active agent from hydrophilic to hydrophobic.

Suitable sparingly soluble, slightly soluble, very slightly soluble and insoluble pharmaceutically active agents include cancer drugs such as taxol, paclitaxel, docetaxel, carbazitaxel, camptothecin, 10-hydroxycamptothecin, irinotecan, doxorubicin, etoposide, temolzolomide, teniposide, amsacrine, actinomycin D, ellipticine and bis-dioxopiperazines such as ICRF-1893; corticosteroids such as methylprednisilone, prednisilone, prednisone, betametasone and budesonid; metalloprotease inhibitors such as marimastat, and steroid hormones such as testosterone, progesterone and levonorgestrel.

Suitable pharmaceutically active agent soluble in aqueous solution include protein antigens or siRNA. In some embodiments, the protein antigens may elicit an immunogenic response against an invading pathogen, for example, in infectious disease. In other embodiments, the protein antigen may elicit a tolerogenic response in an autoimmune disease or in organ

replacement or repair. Suitable protein antigens include tumour-associated cell lysates, CD1, CD3, CD4, CD5, CD8, CD15, CD27, CD30, CD31, CD44, C47, LRRC16 and prostate specific antigen (PSA) or other protein antigens associated with autoimmunity or infectious disease. Tumour cell lysates are derived from a patient's tumour and contain antigens specific to the tumour from which they are derived optionally together with cytokines, allowing for the use of micro- and nano-emulsions directed to personal medicine approaches.

In some embodiments, where a tolerogenic response is required from the pharmaceutically active agent, the micro- or nano-emulsion further comprises at least one inhibitor of NF-κB in an amount sufficient to inhibit the NF-κB pathway of the antigen presenting cells to which the antigen is being delivered. Suitable NF-κB inhibitors include antioxidants, proteosome and protease inhibitors of REL/NF-κB, phosphorylation and/or degradation inhibitors and other NF-κB inhibitors. Suitable antioxidants include α-lipoic acid, α-tocopherol, aged garlic extract (allicin), 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), allopurinol, 5,6,3',5'-tetramethoxy-7,4'-hydroxyflavone, bis-eugenol, butylated hydroxyanisole (BHA), 3,4-dihydroxycinnamic acid, curcumin, diethyldithiocarbamate, ethyl pyruvate, folic acid, glutathione, hydroquinone, melatonin, N-acetyl-cysteine, quercetin, spironolactone and vitamin C. Suitable proteosome and protease inhibitors of REL/NF-kB include N-acetyl-leucinyl-leucinyl-norleucinyl (ALLnL), N-acetyl-leucinyl-leucinyl-methionyl (LLM), carbobenzoxyl-leucinyl-leucinyl-norvalinyl (Z-LLnV), carbobenzoxyl-leucinyl-leucinyl-leucinyl (Z-LLL), bortezomib, cyclosporine A, tacrolimus, disulfiram, N-acetyl-DL-phenylalanine-β-naphthyl ester, N-benzoyl L-tyrosine-ethyl ester, 3,4-dichloroisocoumarin, diisopropyl fluorophosphate, N-α-tosyl-L-phenylalanine chloromethyl ketone and N-α-tosyl-L-lysine chloromethyl ketone. Suitable phosphorylation or degradation inhibitors include desloratiadine, salmeterol, fluticasone propionate, LY29, LY30, evodiamine, geldanamycin, 4-(2'-aminoethyl)amino-1,8-dimethylimidazo(1,2-a)-quinoxaline, cobrotoxin, nitric oxide, thienopyridine, 2-amino-6-[2-(cyclopropylmethoxy)-6-hydroxyphenyl]-4-piperidin-4-yl nicotinonitrile, N-(4-hydroxyphenyl)retinamide, scytonemin, zerumbone, silibinin, sulfasalazine, quercetin, rosmarinic acid, staurosporine, gamma-tocotrienol, thalidomide, anethole, benzylisothiocyanate, digitoxin, interferon-α, methotrexate, capsaicin, genistein and

ursodeoxycholic acid. Other NF-kB inhibitors include α-pinene, indole-3-carbinol, 1,2,3,4,6-penta-O-galloyl-β-D-glucose, selenomethionine, neomycin, rapamycin, ethylpyruvate, 2-acetylaminofluorene, 7-amino-4-methylcoumarin, camptothecin, cinnamaldehyde, clarithromycin, erythromycin, glycyrrhizin, linoleic acid, 2-methoxyestradiol, prostaglandin E2, rapomycin, raloxifene, ribavirin, ritonavir, rosiglitazone and xylitol.

In some embodiments, the compound to be delivered is a veterinary compound. In some embodiments, the veterinary compound is a protein associated with animal disease, such as VirB91, or other proteins associated with animal diseases, such as cattle diseases.

In some embodiments, the compound to be delivered is a compound suitable for use as a diagnostic agent, including but not limited to, fluorescent agents, magnetic particles and other imaging agents. Suitable fluorescent agents include fluorophores such as green fluorescent protein, fluorescein, rhodamine, eosin, indocarbocyanine, merocyanine, nile red, nile blue, cresyl violet, proflavin, acridine orange, acridine yellow, auramine, malachite green, crystal violet and porphin. Suitable contrast agents for imaging include gadolinium compounds such as gadoterate, gadodiamide, gadopentetate, gadoteridol, gadoversetamide, gadoxetate, gadobutrol, gadobenate and Gd-DOTA, techtinium compounds such as techtinium sestamibi, techtinium bicisate and techtinium tetrafosmin, iodine compounds such as metrizamide, ioxaglate, ioversol, iopamidol and iohexol, indium compounds such as indium petreotide, fluorine compounds such as fluorodeoxyglucose or fluorine 19; iron oxides such as feridex, resovist, sinenem, lumirem and clariscan; and others such as gold nanoparticles and iodine.

In some embodiments, the compound to be delivered is a pesticide such as an acaricide, an avicide, algicide, antifouling agent, antimicrobial, antifeedant, bactericide, biocide, chemisterilant, fungicide, herbicide, herbicide safener, insect attractant, insect repellent, mammal repellent, bird repellent, insecticide, fumigant, disinfectant, sanitiser, mating disrupter, miticide, molluscicide, nematicide, ovicide, pheromone, plant activator, plant growth regulator, rodenticide, synergist, termiticide or virucide. Suitable pesticides include, but are not limited to, fipronil, organochlorides such as aldrin, chlordane, chlordecone, DDT, dieldrin, endosulfan, endrin, heptachlor, hexachlorobenzene, lindane

(gamma-hexachlorocyclohexane), methoxychlor, mirex, pentachlorophenol and TDE, organophosphates such as acephate, azinphos-methyl, bensulide, chlorethoxyfos, chlorpyrifos, chlorpyriphos-methyl, diazinon, dichlorvos (DDVP), dicrotophos, dimethoate, disulfoton, ethoprop, fenamiphos, fenitrothion, fenthion, fosthiazate, malathion, methamidophos, methidathion, mevinphos, monocrotophos, naled, omethoate, oxydemeton-methyl, parathion, parathion-methyl, phorate, phosalone, phosmet, phostebupirim, phoxim, pirimiphos-methyl, profenofos, terbufos, tetrachlorvinphos, tribufos and trichlorfon, carbamates such as aldicarb, bendiocarb, carbofuran, carbaryl, dioxacarb, fenobucarb, fenoxycarb, isoprocarb and methomyl, 2-(1-Methylpropyl)phenyl methylcarbamate, pyrethroids such as allethrin, bifenthrin, cyhalothrin, lambda-cyhalothrin, cypermethrin, cyfluthrin, deltamethrin, etofenprox, fenvalerate, permethrin, phenothrin, prallethrin, resmethrin, tetramethrin, tralomethrin and transfluthrin, neonicotinoids such as acetamiprid, clothianidin, imidacloprid, nitenpyram, nithiazine, thiacloprid and thiamethoxam, ryanoids such as rynaxypyr, insect growth regulators including benzoylureas such as diflubenzuron and flufenoxuron, methoprene, hydroprene and tebufenozide. Suitable herbicides include synthetic auxins such as 2,4-D, dicamba, fluroxypyr and picloram, pyridine herbicides such as clopyralid and aminopyralid, triazine herbicides such as atrazine, and other herbicides such as glufosinate ammonium, fluazifop, glyphosate, imazapyr, imazapic, imazamox, metolachlor, paraquat pendimethalin, sodium chlorate and triclopyr.

In some embodiments, the micro- or nano-emulsion formed may be concentrated prior to use by methods known in the art including gravitational or centrifugal separation.

In yet another aspect of the present invention, there is provided a silica micro- or nano-capsule comprising an oil droplet stabilized by a mineralizing biosurfactant as described above and a silica shell encapsulating the stabilized oil core.

The oil droplet may be any oil suitable for solubilizing or carrying the compound to be delivered. Suitable oil phases include neutral esters of saturated coconut and palm kernel oil-derived caprylic and capric fatty acids and glycerine or propylene glycol such as Miglyol® 810, 812, 818, 829 and 840, edible oils such as olive oil, sunflower oil, safflower oil, grapeseed oil, sesame oil, coconut oil, canola oil, corn oil, flaxseed oil, palm oil, palm

kernel oil, peanut oil and soyabean oil, or triglycerides rich in unsaturated fatty acids or mixtures thereof.

In some embodiments, the oil droplet further comprises a compound for delivery to an environment, such as a household, industrial or agricultural environment, for example a pesticide, herbicide, microbicide and the like as described above.

The microcapsules have an average diameter of less than 150 μm, especially between 1 μm and 100 μm, more especially between 1 μm and 30 μm and most especially bewteen 1 μm and 5 μm

The nanocapsules having an average diameter of less than 1 μm, especially between 50 nm and 750 nm, more especially between 70 nm and 500 nm, more especially between 80 nm and 400 nm.

The thickness of the silica shell may be adapted to assist with the release of the compound to be delivered. In some embodiments, the silica shell has a thickness in the range of 5 to 100 nm, 10 to 60 nm or 10 to 50 nm. In embodiments where release is less inhibited, the silica shell thickness may be in the range of 10 to 40 nm, 10 to 30 nm, 10 to 20 nm, especially 10 to 20 nm. In embodiments where release is desired to be slower or inhibited, the thickness of the silica shell may be in the range of 30 to 80 nm, 40 to 70 nm or 40 to 60 nm, especially 40 to 50 nm.

In some embodiments, the silica shell is an amorphous silica shell.

In some embodiments, the nanocapsule further includes a pharmacokinetic modifying agent and/or a targeting agent located on the surface of the micro- or nano-capsule. The pharmacokinetic agent and/or targeting agent are attached to the surface of the micro- or nano-capsule using methods known in the art. For example, carboxylic acids on the pharmacokinetic agent or targeting agent may be covalently linked to the hydroxy group of silanol groups on the silica shell surface. Alternatively, the surface of the silica shell may be coated with amine functionality, for example, with (3-aminopropyl)triethoxysilane

(APTES) and their pharmacokinetic agents bearing a carboxylic acid may be coupled by methods known for amine formation, such as with a carbodiimide and base, for example, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinamide (NHS). In some embodiments, the pharmacokinetic modifying agent is a polyalkylene glycol, a polyalkyloxazoline such as polyethyloxazoline (PEOX), or polyvinylpyrolidone, especially a polyalkylene glycol such as polyethylene glycol or polypropylene glycol, more especially polyethylene glycol (PEG). The polyethylene glycol may have a molecular weight between 2500 and 25000 Da, especially 2500 and 20000 Da. In a particular embodiment, the PEG has a molecular weight of about 5000 Da. The targeting agent may be any molecule that binds to a cell surface receptor in the target tissue or organ to which the nanocapsule is to be delivered. For example, the targeting agent may be a small molecule such as folate or an oestrogen, peptides such as tumor-targeting peptide or may be an antibody or antibody fragment such as an scFv or diabody, directed to a particular cell surface receptor.

Suitable targeting peptides include Lyp-1 (CGNKRTRGC), Bombesin peptide (QQRLGNQWAVGHLM) and suitable tumor-targeting peptides include RGD peptides such as RGD, cyclic RGD dimer and CRGDKRGPDEC (iRGD).

Suitable antibodies or fragments thereof include anti-PSMA and anti-HER2, target receptors for IL-6, IFN8, VEGF, EGF, CA-125, Clec9A, Clec12A, TNF-α, CD4, CD8, CD19, CD64, CD3, CD28, CD40, CD326 and CD20 (rituximab), or monoclonal antibodies targeting cancer-associated proteoglycans such as melanoma-associated proteoglycans, or cancer-associated gangliosides, for example, GD2 and GD3.

The silica micro- and nano-capsules of the present invention may be prepared using the micro- or nano-emulsion of the invention as a template to nucleate silica onto the stabilized micro- or nano-emulsion.

In another aspect of the present invention, there is provided a method of making a silica micro- or nano-capsule comprising the steps of:

A) forming a stabilized micro- or nano-emulsion by mixing a composition comprising:
   a) an oil phase;
   b) an aqueous phase; and
   c) a mineralizing biosurfactant according to the invention; and
B) mixing the micro- or nano-emulsion with silica or a silica precursor.

The micro- or nano-emulsion formed is a micro- or nano-emulsion of the invention as described above.

The silica or silica precursor is any silica containing compound that will react with the positively charged peptide module of the mineralizing biosurfactant and form the silica shell. Suitable silica or silica precursors include alkoxylated silanes such as tetraethoxysilane, tetramethoxysilane, methyltriethoxysilane, phenyltriethoxysilane, trimethylethoxysilane, sodium silicate ($Na_2Si_3O_7$), dipotassium silicon triscatecholate ($K_2[Si(C_6H_4O_2)_3.2H_2O$), silica sol (silica nanoparticles with diameter of 10-12 nm, 40% SiO_2, 0.4% Na_2O), ethylene glycol modified silane ($SiC_2H_8O_2)_4$), and the like.

The concentration of silica or silica precursor used in the reaction will vary depending on the desired thickness of the silica shell. Increasing the silica or silica precursor concentration can increase the thickness of the silica shell. Suitable concentrations of silica or silica precursor are in the range of 10 to 100 mM, especially 20 to 80 mM.

The reaction time can also be used to vary the thickness of the silica shell of the nanocapsule. Increasing the reaction time may increase the thickness of the silica shell. Reaction times may range from 1 hour to 100 hours, for example 10 hours to 80 hours, 20 to 70 hours or 30 to 50 hours, including all numbers of hours and parts thereof inbetween.

The pH of the reaction conditions for mixing the composition comprising micro- or nano-emulsion and silica precursor may be determined by the mineralizing biosurfactant used for stabilizing the micro- or nano-emulsion. In some embodiments, the pH used is suitable to maximise the charges on the charged peptide module and the hydrogen bonding propensity of the charged peptide module of the mineralizing biosurfactant. In some

embodiments, the pH is between 7 and 9, especially 7 and 8.5 or 7 and 8, more especially about 7.5. Varying the pH of the reaction composition comprising micro- or nano-emulsion and silica precursor may also be used to vary the thickness of the silica shell.

The thickness of the silica shell may be tuned for a particular thickness by varying the concentration of the silica or silica precursor, varying the pH of the reaction composition and/or varying the reaction time.

In some embodiments, the silica shell has a thickness in the range of 5 to 100 nm, especially 5 to 20 nm. In some embodiments, the release of the compound to be delivered is by diffusion of the compound through the silica shell. In some embodiments, the release of the compound to be delivered is released at least in part, by the breakdown of the silica shell. In some embodiments, the thickness of the silica shell allows control of the rate of release of the active payload.

The silica or silica precursor is mixed with the stabilized nanoemulsion by gentle stirring. Suitable stirring methods will be known to those in the art, for example, mechanical stirring or magnetic stirring.

In yet another embodiment, there is provided a composition comprising a micro- or nano-capsule of the invention together with a suitable carrier.

Suitable carriers are determined by the use of the composition. For example, compositions for use in pharmaceutical or diagnostic applications will include a pharmaceutically acceptable carrier, compositions for use in veterinary applications will include a veterinary acceptable carrier, compositions for use in agricultural, household or environmental applications will include carriers acceptable for use in these environments. A person skilled in the art could determine suitable carriers for a specific use.

4. Applications

The micro- or nano-capsules of the present invention can be used to deliver one or more compounds such as pharmaceutical or veterinary products, agricultural products such as herbicides or insecticides or environmental or household products such as insecticides.

In some cases, the micro- or nano-capsule will be delivered to an environment that will cause rapid breakdown of the silica shell and immediate release of the active payload as a bolus dose. In other cases, the nanocapsule will be delivered to an environment which will cause gradual breakdown of the silica shell causing delayed release of the active payload. In yet other cases, the nanocapsule enables slowed release of the active payload over a period of time. In some embodiment, the active payload diffuses out of the micro- or nano-capsule over a period of time. In other embodiments, the active payload is released by diffusion at different times as the silica shell breaks down.

The micro- or nano-capsules of the invention may be useful in delivery of pharmaceutical compounds, particularly compounds that have limited solubility in aqueous or polar solvents. The micro- or nano-capsules may be tailored to have a particular silica shell thickness to allow controlled release such as immediate release, delayed release or slow release of the pharmaceutical compound. In some embodiments, a composition comprising multiple micro- or nano-capsules having different silica shell thicknesses to provide an extended release or slow release profile.

In some embodiments, the active payload is a diagnostic agent which is delivered to allow imaging such as magnetic resonance imaging or fluorescent imaging. In these embodiments, the micro- or nano-capsule may have a silica shell thickness that is suitable for delivery of the imaging agent as a bolus. Optionally the micro- or nano-capsule may be further modified to include a targeting agent such that the imaging agent is delivered to the target tissue or organ.

In some embodiments, the active payload is an immunogenic compound such as an antigen to provide a vaccine. In some embodiments, the antigen-loaded micro- or nano-capsule is stable until it is delivered to the individual in need of vaccination. Advantageously, these nanocapsule vaccine compositions may have an extended shelf life compared to solutions of antigen and/or may be stable without refrigeration allowing storage without cold chain requirements. The thickness of the silica shell on the nanocapsule shell may also be tailored to give a rate of delivery of antigen over a period of time and thereby providing a "single shot" vaccine. In some embodiments, the kinetics of release of the active

compound may be further controlled by incorporating the compound in nanoparticles that are released from the micro- or nano-capsules and then the compound is released from the nanoparticles.

In some embodiments, the active payload is a small molecule such as a metalloprotease inhibitor, optionally incorporated into a nanoparticle, that is released to treat a human or animal disease, for example, laminitis in horses.

In some embodiments, the active payload is a pesticide such as an insecticide or combination of insecticides. The pesticidal micro- or nano-capsule compositions may be tailored to provide storage stability yet allow rapid release of the pesticide to provide fast "knock-down" of the invading pest, or may include a thick silica shell allowing slow release of a pesticidal amount of compound over a period of time to give long term protection of an agricultural, industrial or household environment. In particular embodiments, the active payload is a termiticide and the composition is suitable for preventing or controlling termite infestations.

In some embodiments, the active payload is a pesticide such as a herbicide or combination of herbicides. The herbicidal nanocapsule compositions may be tailored to allow immediate release of the herbicide to provide rapid control of weeds or unwanted plants, or may include a thick silica shell allowing slow release of a herbicidal amount of compound over a period of time to give long term control or prevention of weed infestation.

EXAMPLES

SEQ ID NO:155 (M_w 3643.2) and SEQ ID NO:156 (M_w 3632.4) peptides with 95% purity were custom synthesized by Peptide 2.0 Inc. (Chantilly, VA, USA). The peptides were dissolved in water and lyophilized prior to use, and the concentrations were determined by reversed-phase high-performance liquid chromatography (RP-HPLC). Miglyol® 812 (Axo Industry SA, Wavre, Belgium) was purified by passing the oil through dry silica gel column prior to use. 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid buffer (HEPES, ≥99%), zinc chloride ($ZnCl_2$, ≥98%) and tetraethoxysilane (TEOS, ≥99%) were obtained from Sigma and used as received. Water was obtained from a Milli-Q (Millipore, New

South Wales, Australia) system with a 0.22 μm filter and had a resistivity of >18.2 MΩ cm. Piranha solution for acid washing of glassware was prepared from equal volumes of 30% hydrogen peroxide (Rowe Scientific Pty Ltd, Queensland, Australia) and 98% sulfuric acid (Chem-Supply Pty Ltd, South Australia, Australia). All glassware used to hold peptide solution was (i) soaked in 1% detergent solution (Decon 90, Decon Laboratories Ltd, East Sussex, U.K.), (ii) rinsed with 6 volumes of Milli-Q water, (iii) soaked for 15 mins in piranha solution, and (iv) rinsed with 10 volumes of Milli-Q water.

The size distribution profile of the nanoemulsions and nanocapsules was determined by dynamic light scattering (DLS, Malvern Zetasizer Nano ZS) and the samples were diluted by a factor of 100 prior to the measurement. The morphology and shell thickness of the nanocapsules were examined by transmission electron microscopy (TEM, JEOL 1010, 100 kV accelerating voltage). Samples (2 μL) were taken directly from the reaction mixtures, deposited onto Formvar-coated copper grids (200 mesh), and left to air-dry prior to examination. Samples were also analyzed for high-resolution (HR) TEM (JEOL 2100, 200 kV accelerating voltage) equipped with an energy dispersive X-ray spectroscopy (EDS) detector. The formation of amorphous silica was verified by selected area electron diffraction (SAED) in HRTEM.

Example 1: Precipitation of silica by biosurfactants

To test whether biosurfactants SEQ ID NO:155 and SEQ ID NO:156 were able to precipitate silica, separate aqueous solutions of SEQ ID NO:155 and SEQ ID NO:156 (400 μM) in HEPES buffer (25 mM) at pH 7.5 and 8.0 were prepared and TEOS (80 mM) was added. A solution of each biosurfactant was retained without TEOS as a control. The mixtures were gently stirred at room temperature. A transparent gel-like precipitate formed within a few minutes in those compositions which had TEOS added whereas the control remained a solution. The precipitate was isolated and extensively washed with water and vacuum dried. The precipitate was analyzed by elemental analysis using EDS in conjunction with scanning electron microscopy (SEM, JEOL 6610). The analysis showed the precipitate to be silica (SiO_2). Excess oxygen, carbon and sulfur also found in the analysis were consistent with entrapped peptide. This demonstrated that the peptides of SEQ ID NO:155 and SEQ ID NO:156 can nucleate silica.

Example 2: Formation of Nanoemulsions with SEQ ID NO:155 and SEQ ID NO:156

Separate solutions of SEQ ID NO:155 (400 μM) and SEQ ID NO:156 (400 μM) in HEPES buffer (25 mM, pH 7.0) in the presence of $ZnCl_2$ (800 μM) were mixed with Miglyol® 812 (2% v/v) by sonication (Branson Sonifier 450, 10 W, 4 x 30 s burst and interspersed with cooling in an ice bath for 60 s). After sonication, the compositions were macroscopically identical and homogeneous with cloudy appearance.

The nanoemulsions were analyzed by dynamic light scattering (DLS). The number-average diameter (d_n) of the oil droplets in the nanoemulsions were ~80 nm (polydispersity index (PDI) = 0.329) for SEQ ID NO:155 and ~40 nm (PDI = 0.293) for SEQ ID NO:156.

The nanoemulsion compositions (1 mL) were dialyzed against HEPES buffer (500 mL, 25 mM, pH 7.5 or 8.0) using a 10 kDa cellulose membrane to adjust pH of each nanoemulsion and to remove residual peptide. After dialysis for 20 hours there was only a slight increase, approximately 5%, in the number-average diameter of nanoemulsions stabilized by either SEQ ID NO:155 or SEQ ID NO:156 and the PDIs remained similar. The nanoemulsions were stable to dialysis.

Example 3: Formation of nanocapsules from nanoemulsions

Each of the dialyzed nanoemulsion compositions from Example 2 at pH 7.5 or 8.0 were divided into a number of separate glass vials. One composition of each peptide nanoemulsion at each pH was retained as a control. To the other compositions, three concentrations of TEOS were added to separate vials, 20 mM, 40 mM and 80 mM, and the compositions were gently stirred for 20 to 50 hours. The compositions were then analyzed by DLS and a size increase consistent with the formation of a thick layer surrounding the oil droplet core in TEOS-containing solutions was observed. The results for nanocapsules comprising SEQ ID NO:156 are shown in Figures 2 to 5.

Figure 2 demonstrates that the size of the nanocapsules formed with SEQ ID NO: 156 increases with concentration of TEOS added and the reaction time. These results were confirmed by TEM where the presence of the silica shell was shown and an increase in

thickness with increasing TEOS concentration (Figure 3) and increasing reaction time (Figure 4) was demonstrated. SAED indicated the silica shells exhibit amorphous form as indicated by a diffuse ring pattern for the silica shell.

After 20 hours silicification of peptide nanoemulsions with 80 mM TEOS in 25 mM HEPES buffer, DLS results revealed a decrease in the polydispersity indexes of the nanocapsules formed with SEQ ID NO:155 at pH 7.5 (PDI = 0.242) and SEQ ID NO:156 at pH 7.5 (PDI = 0.146) and pH 8.0 (PDI = 0.233). This suggests the mineralizing biosurfactants are covered by a condensed silica network after silicification yielding uniform structures. An increased PDI was observed for SEQ ID NO:155 at pH 8 (PDI = 0.794) suggesting flocculation of the nanocapsules.

The shell thickness of at least 100 individual nanocapsules were measured by TEM (a dark ring around the perimeter of the bright core). The silica layer formed on SEQ ID NO:155 and SEQ ID NO:156 nanoemulsions at pH 7.5 was 22 ± 5 nm and 12 ± 2 nm thick respectively and at pH 8.0, 35 ± 5 nm and 27 ± 6 nm respectively. This demonstrates that the thickness of the silica shell can be tuned by adjusting pH as shown on SEQ ID NO:156 nanoemulsions in Figure 5.

Varying reaction time and TEOS concentration also affected the thickness of the silica shell. As expected, the nanocapsule silica shell thickness increased regularly with TEOS concentration (Figure 3) and reaction time (Figure 4). For example, after 30 hours silicification of SEQ ID NO:156 nanoemulsions with 20 mM, 40 mM and 80 mM TEOS in 25 mM HEPES buffer, pH 7.5, the thickness of the silica shell was measured at 5 ± 1 nm, 10 ± 2 nm and 16 ± 3 nm respectively (Figure 3). Once the nanocapsules reached about 20 nm (C_{TEOS} = 80 mM, t = 40 hr) flocculation of the nanocapsules started to occur. This demonstrates that well dispersed nanocapsules having different silica shell thickness may be obtained by an interplay between TEOS concentration and reaction time.

Example 4: Synthesis of biocide-loaded silica nanocapsules

Nanoemulsion was prepared by homogenization of fipronil-loaded Miglyol® 812 at 2% v/v in HEPES buffer (25 mM, pH 7.5) containing the biosurfactant peptide Ac-

MKQLAHSVSRLEHA-RKKRKKRKKRKKGGGY-$CONH_2$ (SEQ ID NO: 156, 400 μM) and $ZnCl_2$ (800 μM) using an ultrasonicator (Branson Sonifier 450, 10 W, 4 x 30 s bursts and interspersed with cooling in an ice bath for 60 s). The fipronil-loaded nanoemulsion (1 mL) was dialyzed against HEPES buffer (500 mL, 25 mM, pH 7.5) using 10 kDa cellulose membrane to adjust pH of the nanoemulsion and to remove residual peptide.

Aliquots (400 μL) of the nanoemulsions were transferred into 4 mL glass vials and TEOS at concentrations of 40 mM, 80 mM and 240 mM was added. The compositions were gently stirred at room temperature for 30 hours to provide oil-core/silica-shell nanocapsules.

The loaded nanocapsules have dense core under transmission electron microscope as shown in Figure 6, in contrast to the light core shown by unloaded nanocapsules (Figures 2-5). Nanocapsules with three different silica shell thicknesses of 8 ± 2 nm, 25 ± 3 nm and 44 ± 7 nm were produced using 40 mM (Figure 6a), 80 mM (Figure 6b) and 240 mM (Figure 6c) TEOS, respectively.

The fipronil-loaded nanocapsules formed at each concentration of TEOS were analyzed directly by TEM. Samples of each thickness nanocapsules were also dialyzed against Milli-Q water to remove unreacted TEOS.

Example 5: *In vivo* efficacy of fipronil-containing silica nanocapsules on termites

Termites were collected from a *Coptotermes acinaciformis* colony and active worker termites were selected for the experiment. Prior to experiment, the termites were incubated at 28 ± 1°C in Petri dishes containing Whatman® cellulose filter paper to assess termite survival under incubation conditions. After 4 days, 26 ± 8% mortality was observed.

Six samples were prepared and analyzed for response of fipronil.

Control: Milli-Q water

Termidor®: commercially-available Termidor® containing 0.05 mg/mL fipronil

0.05F-NE: nanoemulsion containing 0.05 mg/mL fipronil (as prepared in Example 4 without biosilicification step)

0.05F-NC8: silica nanocapsules loaded with 0.05 mg/mL fipronil having a silica shell thickness of 8 ± 2 nm (as prepared in Example 4)

0.05F-NC25: silica nanocapsules loaded with 0.05 mg/mL fipronil having a silica shell thickness of 25 ± 3 nm (as prepared in Example 4)

0.05F-NC44: silica nanocapsules loaded with 0.05 mg/mL fipronil having a silica shell thickness of 44 ± 7 nm (as prepared in Example 4)

Treatment with each fipronil formulation or water against a group of termites was conducted in five replicates. Termite response (mortality) was examined during the treatment. Ten active worker termites were placed into a Petri dish (92 × 16 mm). Prior to treatment, termites in the Petri dishes were incubated at 2 °C for 2 mins to slow termite movement. Each of the fipronil formulations or water (5.5 µL) was topically applied on the dorsal thorax of termites. In cases where a droplet was misplaced or ran off, the treated termite was excluded from testing. Topically-dosed termites were left in Petri dishes until the droplet dried. Termites were then transferred to clean Petri dishes lined with a filter paper (42.5 mm, Whatman®). Each Petri dish was placed into a controlled environment chamber. The chamber (65 × 47 × 37.5 cm) containing a thermometer, a lid and a 11.5-L metal container with water and heater, maintained the temperature at 28 ± 1°C, high humidity and, once covered with insulating material, total darkness (except during observation). Preliminary study showed that termites could survive under these conditions for at least 4 days. Mortality was recorded at 1, 2, 3, 4, 5, 6, 10, 22, 24 and 27 h after treatment (Figure 7). The Petri dishes were removed from the chamber whenever needed and the lids were opened with the least possible disturbance. A termite was considered dead when it was on its back or side (not supported by legs) and not able to move even after prodding with a soft brush.

Rapid mortality of termites was observed when they were treated with commercially-available Termidor® containing fipronil or 0.05F-NE. Termidor® gave a burst release of fipronil during the first hour resulting in 50% mortality with 100% mortality at 4 h (Figure 7). 0.05F-NE exhibited a two-step release profile. The first stage of fipronil release showed a significant initial burst effect, reflected by the mortality of almost 50% within the first 4

h, and followed by a more sustained release over 22 h (Figure 7). 0.05F-NE gave a slightly reduced mortality within the time frame as compared to Termidor®. In contrast, silica nanocapsules showed more sustained release as a result of the silica shell. First mortality was found after 4 h (6%), 6 h (4%) and 10 h (8%) for 0.05F-NC8, 0.05F-NC25 and 0.05F-NC44, respectively (Figure 7). Mortality then gradually increased in a way dependent on shell thickness, suggesting that the silica shell provided an effective barrier for the controlled diffusion of encapsulated fipronil.

Example 6:

Fipronil-loaded silica nanocapsules with 44 ± 7 nm shell thickness was evaluated for the slow release performance by remote feeding treatment on termites *Coptotermes acinaciformis*. For feeding treatment, 1 mg/mL fipronil solubilized in Miglyol® 812 was used as oil phase and fipronil-loaded nanocapsule having a 44 nm shell (1F-NC44) was then prepared using 240 mM TEOS as previously described in Example 4. Three samples were compared, including Milli-Q water as a control, commercially-available Termidor® containing 1 mg/mL fipronil and 1F-NC44. Treatment of each fipronil formulation or water was conducted in four or two replicates, respectively. Termite response (mortality) was examined during the treatment.

Termites (7.5 g ≈ 1,500 workers and soldiers of *Coptotermes acinaciformis*) were placed into 750 mL containers with 150 g roasted *Nasutitermes magnus* mound material and 50 g boiled water. The mound material was roasted in an oven at 200 °C for 60 minutes to reduce the incidence of microbes and then ground with a mortar and pestle. The material is a slightly nutritious building substrate and was included to mimic the termite natural environment. A clear vinyl tubing (150 × 6 mm diameter) connected the container to a 70 mL feeding vial. Each vial was filled with moist α-cellulose bait (50 g boiled water and 20 g α-cellulose). Each assembly was placed into a controlled-environment chamber. The chamber (65 × 47 × 37.5 cm) containing a thermometer, a lid and a 11.5-L metal container with water and heater was maintained at 26 ± 1°C, high humidity and, once covered with insulating material, total darkness (except during observation). The termites in the ten assemblies were allowed to acclimatize to these conditions for 4 days and began feeding on the bait as indicated by the presence of enclosed tunnels and chambers built in the cellulose. On day 4, 0.5 mL Termidor®, 0.5 mL 1F-NC44 or 0.5 mL water was transferred

into each feeding vial. The assembled feeding devices were removed from the chamber and observed whenever needed with the least possible disturbance. Observation of each assembly was made daily using a Termatrac® T3i device (Termatrac® Pty Ltd, Beenleigh, Queensland, Australia) to detect movement of living termites. Lack of movement indicated 100% mortality of termites and destructive inspections were made to confirm. The termite colony in one Termidor® container (out of four) remained alive at 7 days because termites did not enter into the vial containing Termidor®-treated cellulose. Similarly, two IF-NC44 containers (out of four) were still alive at 7 days because the tube entrance to the vial containing IF-NC44-treated cellulose was blocked with mud built by termites. These containers were not included for further consideration. The two control colonies survived throughout the study.

It was observed that termites built mud and cellulose surrounding the connector tubes as well as galleries within the baits, indicating that termites were responding to the cellulose. As termites carried the cellulose from the feeding vial back to the container, they transferred it to other termites within the colony. Termidor®-treated cellulose caused 100% mortality to termite colonies after 3 days, whereas more delayed mortality (6 days) was observed after treatment using IF-NC44-treated cellulose (Figure 8). Delayed mortality as for the silica nanocapsule increases the likelihood of horizontal transfer of fipronil hence allowing improved area-wide control of termite populations.

Example 7: Design and production of a recombinant protein capable of making silica nanocapsules

To develop a scalable and sustainable technology to make silica nanocapsules, a mineralizing biosurfactant protein (MDPS MKQLADS LHQLARQ VSRLEHA DPS MKQLADS LHQLARQ VSRLEHA DPS MKQLADS LHQLARQ VSRLEHA DPS MKQLADS LHQLARQ VSRLEHA EPS-RKKRKKRKKRKKGGGY, SEQ ID NO: 157), which can be produced directly from recombinant DNA, was designed consisting of one surface active polypeptide module ((MD(PS-MKQLADS-LHQLARQ-VSRLEHA-D)$_4$, SEQ ID NO: 86) capable of stabilizing nanoemulsions and another positively charged peptide module (RKKRKKRKKRKKGGGY, SEQ ID NO: 133) inducing biosilicification at oil–water interfaces at near neutral pH. SEQ ID NO: 157 can be produced at high level of solubility in genetically-modified of the industrially relevant bacterium *Escherichia coli*.

With an aim of obtaining high purity of SEQ ID NO: 157, a precipitation-based process has been developed based on purification method of SEQ ID NO: 86 described in WO 2012/079125 (Figure 9a) coupled with chromatography technique (Figure 9b).

Expression construct and transformation: pET-48b(+) plasmid with nucleotide sequence encoding for SEQ ID NO: 157 (1 µL) (Protein Expression Facility, The University of Queensland) was added into *E. coli* BL21(DE3) competent cells (50 µL) and incubated on ice for 30 min, heat-shocked at 42 °C for 45 s, and incubated on ice for further 2 min. Then, 0.95 mL Luria Bertani (LB) medium (10 g/L NaCl, 10 g/L tryptone, 5 g/L yeast extract, water) was added into the cell suspension and incubated at 37 °C, 220 rpm for 1 h. The cell suspension (100 µL) was plated on LB agar (10 g/L NaCl, 10 g/L tryptone, 5 g/L yeast extract, 15 g/L agar, water) and incubated at 37 °C, 180 rpm for 16 h. Following transformation, a single colony was picked from the plate, placed in 5 mL 2× yeast extract and tryptone (2YT) medium (5 g/L NaCl, 16 g/L tryptone, 10 g/L yeast extract, water), and incubated at 37°C, 180 rpm for 16 h. All media for cultures were supplemented with 15 µg/mL antibiotic (i.e., kanamycin sulfate). To preserve the clones, the overnight culture (0.5 mL) and 60 v/v% glycerol (0.5 mL) were mixed well, frozen in liquid nitrogen, and stored at −80°C for later use.

Protein expression: A single colony from a freshly streaked plate was inoculated into 5 mL 2YT medium and incubated at 30 °C, 180 rpm for 16 h. 2YT medium (800 mL) was inoculated with 800 µL of the overnight culture (OD_{600} = 2) in a 2.5 L-baffled shake flask and incubated at 37 °C, 180 rpm until OD_{600} reached approximately 0.5. Protein expression was induced by adding isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 1 mM and then incubated at 37 °C, 180 rpm for further 4 h. A final OD_{600} of 2 was routinely obtained. Cell pellet was harvested by centrifugation at 4 °C, 6250 ×*g* for 10 min, washed with the supernatant (40 mL) and then centrifuged at 4 °C, 4700 ×*g* for 10 min. The cell pellet was stored at −80 °C until further processing. All media for cultures were supplemented with 15 µg/mL kanamycin sulfate.

Heating/cell lysis, contaminant precipitation and dilute precipitation: The cell pellet was resuspended in 80 mL lysis buffer (25 mM sodium phosphate, 1.2 M NaCl, 1 M Na_2SO_4, pH 7.5). The mixture was transferred into a 250 mL-conical flask and then incubated at 90 °C with stirring at 1000 rpm for 30 min in a thermostatic bath equipped with an electronic

contact thermometer (IKA®-Werke GmbH & Co. KG, Germany). During the heating, the opening of the flask and the bath were covered with aluminium foil to minimize evaporation. The cell lysate was then centrifuged at 20 °C, 51500 ×*g* for 5 min. The supernatant was collected and diluted 5-fold with water, and the pH was adjusted to pH 3.5 by adding 10 N HCl to allow precipitation of SEQ ID NO: 157. Then, the mixture was centrifuged at 4 °C, 33750 ×*g* for 30 min. The precipitated sample was resuspended in equilibrium buffer (25 mM sodium phosphate, 1.2 M NaCl, 20 mM imidazole, pH 7.5) and solubilized by adjusting the pH to pH > 10 using 5 N NaOH.

The protein solution produced after heating/cell lysis, contamination precipitation and dilute precipitation consisted of SEQ ID NO: 157 based on the SDS-PAGE result (Figure 10, lane 4). Although the protein could be used to facilitate formation of nanoemulsions, the nanoemulsions gave negative surface charge due to the presence of significant amount of DNA contamination at a concentration of 63.18 ± 6.14 ng DNA/mg SEQ ID NO: 157, and, as a result, silica shell could not be formed at the interfaces. Therefore, a further purification process using chromatography method was developed to remove DNAs from SEQ ID NO: 157 (Figure 9b).

Chromatography: Immobilized metal ion affinity chromatography (IMAC) was conducted by using an ÄKTA Explorer 10 system (GE Healthcare, Sweden) with Ni^{2+} charged IMAC Sepharose High Performance resin (GE Healthcare, UK) packed into a 15 mm diameter Omnifit glass column (Omnifit, NJ, USA) to a bed height of 75 mm which is equivalent to 5.5 mL column volume (CV). Prior to loading, the protein solution was filtered using a 0.45 µm syringe filter with MF-Millipore® mixed cellulose ester membrane (Millipore, Australia), and the column was equilibrated with 3 CV of equilibrium buffer (25 mM sodium phosphate, 1.2 M NaCl, 20 mM imidazole, pH 7.5). The protein solution was then loaded into the column at a flow rate of 1 mL/min. Unbound components including DNAs as indicated by higher UV absorbance at 260 nm than at 280 nm were washed out with 2 CV of equilibrium buffer. Bound protein was then eluted using 2.5 CV of elution buffer (25 mM sodium phosphate, 1.2 M NaCl, 500 mM imidazole, pH 7.5) (Figure 11a). The eluted fraction was mainly SEQ ID NO: 157 based on the SDS-PAGE analysis (Figure 10 lane 7) with significantly reduced DNA fraction of 1.90 ± 0.48 ng DNA/mg SEQ ID NO: 157. To chelate Ni^{2+} ions leaked after IMAC step, the pH of the collected fractions was

adjusted to pH 5.8 by adding 10 N HCl, and ethylenediaminetetraacetic acid disodium salt (EDTA) was added to a final concentration of 20 mM.

Following the IMAC process, desalting of the protein solution with water was conducted by using an ÄKTA Explorer 10 system with a Sephadex G-25 resin (GE Healthcare, UK) packed into a 20 mm diameter Omnifit glass column to a bed height of 129 mm (CV = 22.8 mL). The protein solution was loaded into the column pre-equilibrated with water at a flow rate of 1 mL/min. The protein fraction after desalting process was collected (Figure 11b) and lyophilized at –55°C, 0.08 mbar for 16 h.

After the chromatography purification steps, DNA fraction in SEQ ID NO: 157 solution is 1.82 ± 0.46 ng DNA/mg SEQ ID NO: 157 with final yield of SEQ ID NO: 157 of approximately 7.22 mg. As analyzed by reversed-phase high performance liquid chromatography (RP-HPLC) and mass spectrometry (MS), high purity SEQ ID NO: 157 can be obtained (Figure 12a), and the calculated molecular weight of SEQ ID NO: 157 based on the mass spectrum (Figure 12b) is 13308.16 Da which is very close to its theoretical molecular weight i.e., 13299.31 Da.

Example 8: Formation of nanocapsules from nanoemulsions using recombinant protein

SEQ ID NO: 157 was used to facilitate formation and stabilization of nanoemulsions, and subsequently direct nucleation and growth of silica shell encasing the nanoemulsion template. A solution of SEQ ID NO: 157 (115 μM) in HEPES buffer (25 mM, pH 7.5) was mixed with Miglyol® 812 oil (10% v/v) by sonication at 10 W using Branson Sonifier 450 for 4 × 30 s burst and interspersed with cooling in an ice bath for 60 s. An aliquot of SEQ ID NO: 157 nanoemulsions (400 μL) was transferred to a glass vial and added with TEOS (80 mM). The mixture was then stirred at room temperature for 20 h to form silica shell surrounding the nanoemulsions. The outer diameter and shell thickness of the nanocapsules as measured by TEM were 249 ± 29 nm and 26 ± 2 nm as shown in Figure 13.

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The Claims Defining the Invention are as Follows:

1. A mineralizing biosurfactant comprising:

   i) a surface-active polypeptide module at least 6 amino acid residues in length, wherein the surface-active polypeptide module comprises an amino acid sequence selected from:

      a) $(a\ b\ c\ d\ d'\ e\ f\ g)_n$;

         wherein n is an integer from 2 to 12;

         amino acid residues a and d are hydrophobic amino acid residues;

         amino acid residue d' is absent or is any amino acid residue;

         at least one of residues b and c and at least one of residues e and f are hydrophilic amino acid residues and the other of amino acid residues b and c and e and f are any amino acid residue;

         amino acid residue g is any amino acid residue; or

      b) $(O)_q(W)_r$ or $(W)_q(O)_r$

         wherein O is a hydrophobic amino acid residue;

         W is a hydrophilic amino acid residue;

         q and r are at least 1 and q + r is an integer between 6 and 130;

         wherein n is an integer from 2 to 12;

      and

   ii) a charged peptide module 5 to 40 amino acid residues in length comprising at least one hydrogen bond donating amino acid residue and 4 to 12 positively charged amino acid residues;

wherein the surface-active polypeptide and the charged peptide modules are conjugated to one another.

2. The mineralizing biosurfactant according to claim 1 wherein the surface-active polypeptide is an amphiphilic polypeptide.

3. The mineralizing biosurfactant according to claim 2 wherein the amphiphilic polypeptide has α-helical secondary structure with a hydrophobic face and a hydrophilic face.

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4. The mineralizing biosurfactant according to any one of claims 1 to 3 wherein n is 2 to 4.

5. The mineralizing biosurfactant according to any one of claims 1 to 4 wherein each amino acid residue a and d is independently selected from L-alanine, L-valine, L-leucine, L-methionine, L-isoleucine, L-phenylalanine, L-tyrosine, D-alanine, D-valine, D-leucine, D-methionine, D-isoleucine, D-phenylalanine and D-tyrosine.

6. The mineralizing biosurfactant according to any one of claims 1 to 5 wherein each amino acid residue b is independently selected from alanine, leucine, valine, methionine, isoleucine, L-serine, L-threonine, L-cysteine, L-tyrosine, L-asparagine, L-glutamine, L-lysine, L-arginine, L-histidine, aspartic acid and glutamic acid.

7. The mineralizing biosurfactant according to any one of claims 1 to 6 wherein each amino acid residue c is independently selected from L-serine, L-threonine, L-cysteine, L-tyrosine, L-asparagine, L-glutamine, L-lysine, L-arginine, L-histidine, L-aspartic acid and L-glutamic acid.

8. The mineralizing biosurfactant according to any one of claims 1 to 7 wherein each amino acid residue e is independently selected from L-alanine, L-valine, L-leucine, L-isoleucine, L-serine, L-threonine, L-aspartic acid and L-glutamic acid, especially L-alanine, L-serine and L-glutamic acid.

9. The mineralizing biosurfactant according to any one of claims 1 to 8 wherein each amino acid residue f is independently selected from L-serine, L-threonine, L-cysteine, L-tyrosine, L-asparagine, L-glutamine, L-lysine, L-arginine, L-histidine, L-aspartic acid and L-glutamic acid.

10. The mineralizing biosurfactant according to any one of claims 1 to 9 wherein each amino acid residue g is independently selected from L-alanine, L-valine, L-leucine, L-isoleucine, L-serine, L-threonine, L-asparagine L-lysine, L-glutamic acid and L-glutamine.

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11. The mineralizing biosurfactant according to any one of claims 1 to 10 wherein the positively charged peptide module comprises 1 to 8 hydrogen bond donating amino acid residues.

12. The mineralizing biosurfactant according to any one of claims 1 to 11 having one of the following sequences:

Ac-MKQLAHSVSRLEHA-SSKKSGSYSGSKGSKRRIL-NH_2 SEQ ID NO:155

Ac-MKQLAHSVSRLEHA-RKKRKKRKKRKKGGGY-NH_2 SEQ ID NO:156

MDPSMKQLADSLHQLARQVSRLEHADPSMKQLADSLHQLARQVSRLEHA DPSMKQLADSLHQLARQVSRLEHADPSMKQLADSLHQLARQVSRLEHAEP S-RKKRKKRKKRKKGGGY SEQ ID NO: 157.

13. A stabilized micro- or nano-emulsion comprising an oil phase, an aqueous phase and a mineralizing biosurfactant according to any one of claims 1 to 12, wherein the mineralizing biosurfactant is located in the region of the interface between the oil and aqueous phases.

14. The stabilized micro- or nano-emulsion according to claim 13 wherein the micro- or nano-emulsion is an oil-in-water emulsion.

15. The stabilized micro- or nano-emulsion according to claim 13 or claim 14 further comprising a metal ion selected from calcium, magnesium, copper, nickel and zinc ions.

16. A silica micro- or nano-capsule comprising an oil core stabilized by a surface film of mineralizing biosurfactant according to any one of claims 1 to 12 and a silica shell encapsulating the stabilized oil core.

17. The silica nanocapsule according to claim 16 having an average diameter of between 70 and 500 nm.

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18. The silica microcapsule according to claim 16 having an average diameter of between 1 μm and 5 μm.

19. The silica micro- or nano-capsule according to any one of claims 16 to 18 wherein the silica shell has a thickness in the range of 5 nm to 100 nm.

20. The silica micro- or nano-capsule according to any one of claims 16 to 19 wherein the oil core further comprises a compound for delivery to a human or animal or a household, industrial or agricultural environment.

21. The silica micro- or nano-capsule according to claim 20 wherein the compound is selected from a pharmaceutical agent, a veterinary agent, a diagnostic agent and a pesticide.

22. The silica micro- or nano-capsule according to any one of claims 16 to 21 further comprising a pharmacokinetic modifying agent and/or a targeting agent, wherein said pharmacokinetic modifying agent and/or a targeting agent are located on the surface of the micro- or nano-capsule.

23. A composition comprising the micro- or nano-capsule according to any one of claims 16 to 22 together with an acceptable carrier.

24. A method of making a silica micro- or nano-capsule comprising the steps of:

A) forming a stabilized micro- or nano-emulsion by mixing a composition comprising:
   a) an oil phase;
   b) an aqueous phase; and
   c) a mineralizing biosurfactant according to any one of claims 1 to 12; and

B) mixing the nanoemulsion with silica or a silica precursor.

25. The method according to claim 24 wherein the micro- or nano-emulsion is formed by mixing, sonification or homogenisation.

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26. The method according to claim 24 or 25 wherein the silica or silica precursor is selected from tetraethoxysilane, tetramethoxysilane, sodium silicate ($Na_2Si_3O_7$), dipotassium silicon triscatecholate ($K_2[Si(C_6H_4O_2)_3.2H_2O$), silica sol (silica nanoparticles with diameter of 10-12 nm, 40% SiO_2, 0.4% Na_2O), ethylene glycol modified silane ($SiC_2H_8O_2)_4$), methyltriethoxysilane, phenyltriethoxysilane and trimethylethoxysilane.

27. The method according to claim 26 wherein the silica or silica precursor is tetraethoxysilane.

28. The method according to any one of claims 24 to 27 wherein the reaction of step B) is mixed for 10 to 80 hours.

29. The method according to any one of claims 24 to 28 wherein the reaction of step B) is carried out at a pH between 7 and 8.5.

30. Use of the nanocapsules of claim 20 or 21 to deliver a compound to a human, animal, pest or environment.

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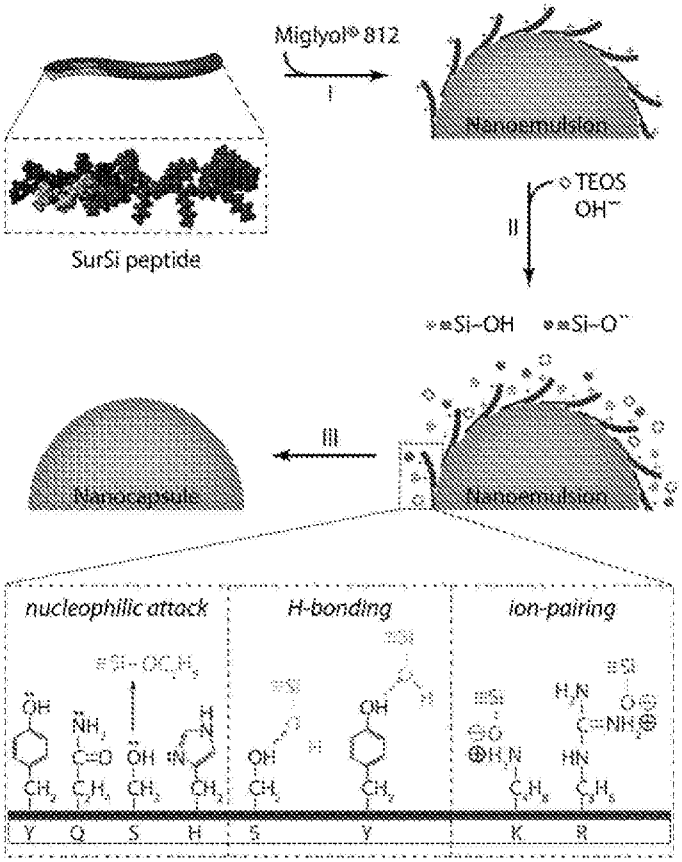
Miglyol® 812
I
Nanoemulsion
TEOS
OH⁻
II
SurSi peptide
≡Si-OH ≡Si-O⁻
III
Nanocapsule
Nanoemulsion
nucleophilic attack
H-bonding
ion-pairing
Y Q S H
S Y
K R

Figure 1

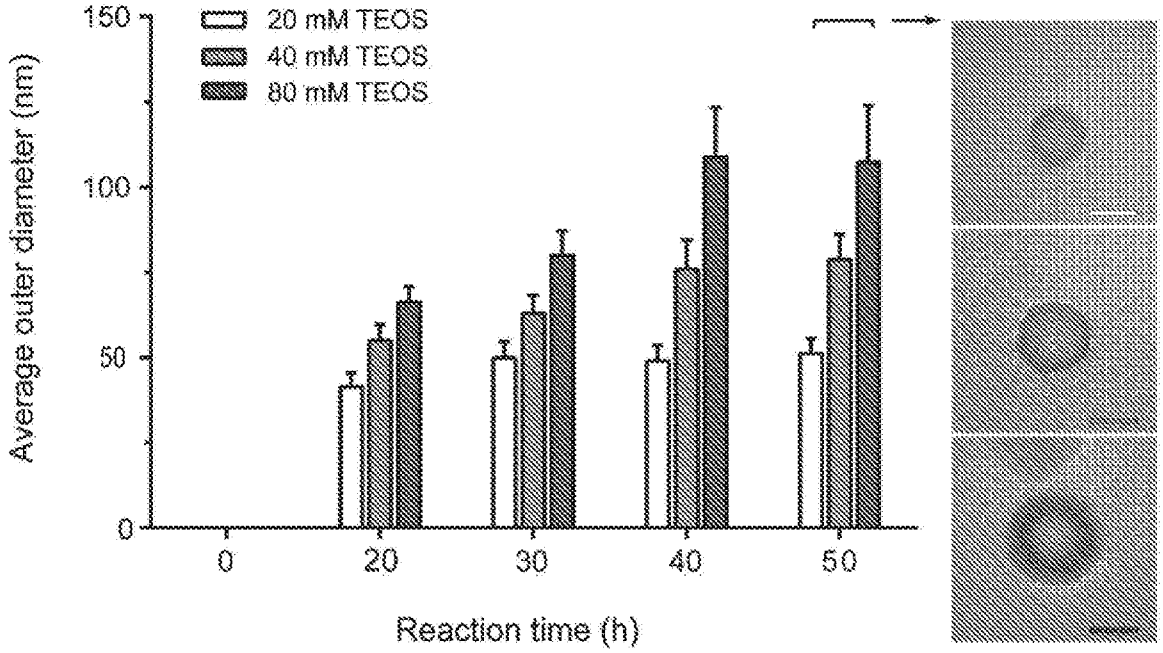
20 mM TEOS
40 mM TEOS
80 mM TEOS
Average outer diameter (nm)
150
100
50
0
0
20
30
40
50
Reaction time (h)

Figure 2

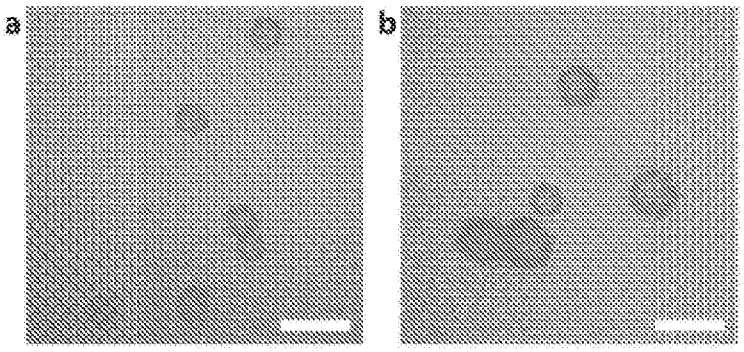

Figure 3

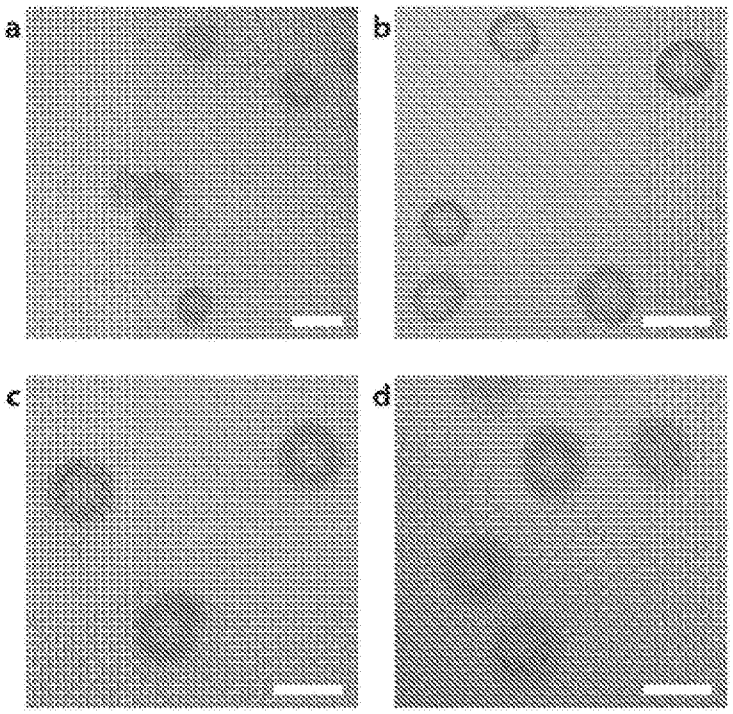

Figure 4

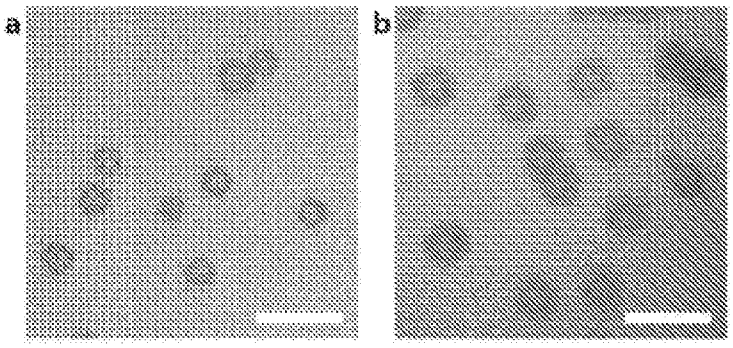

Figure 5

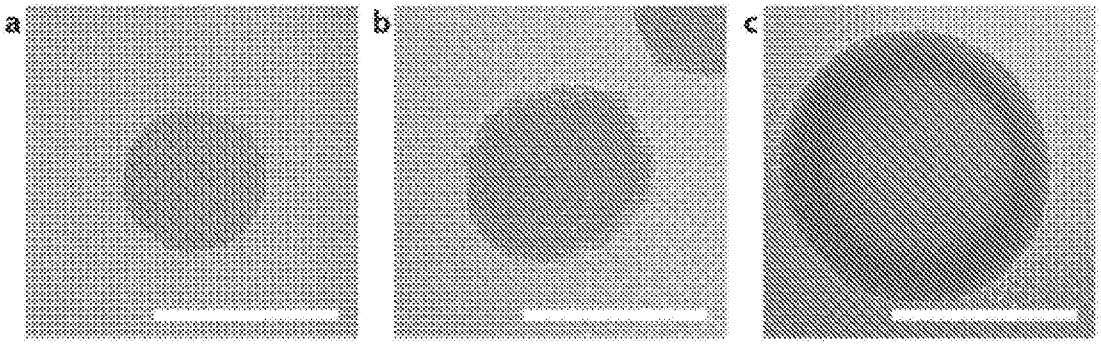

Figure 6

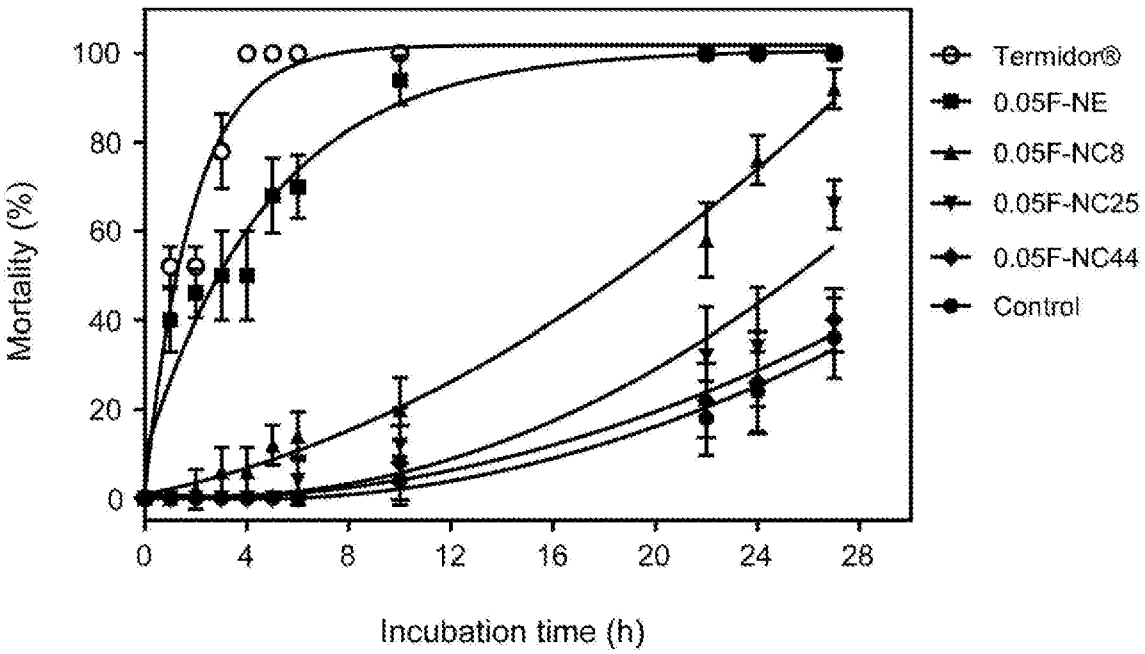

Figure 7

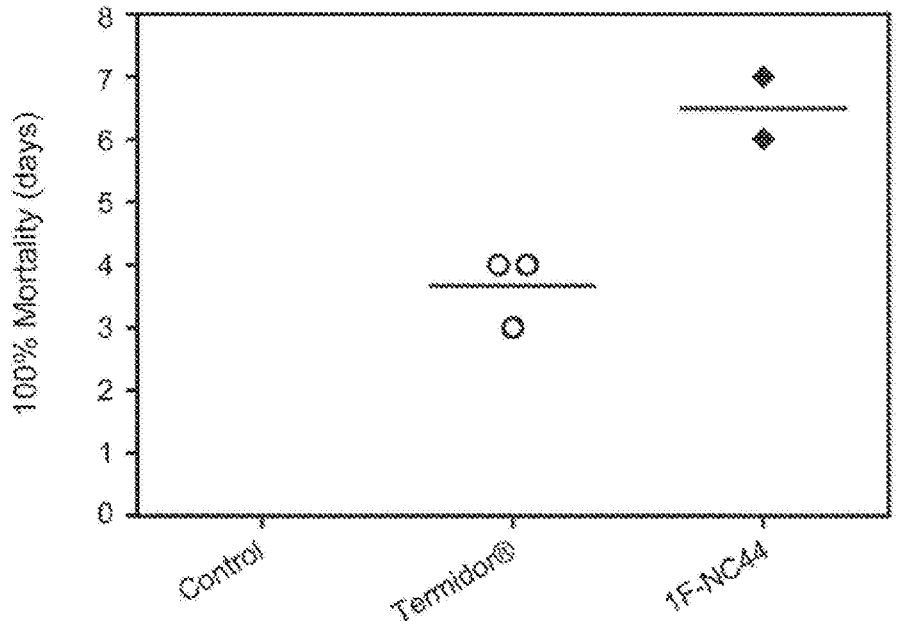

Figure 8

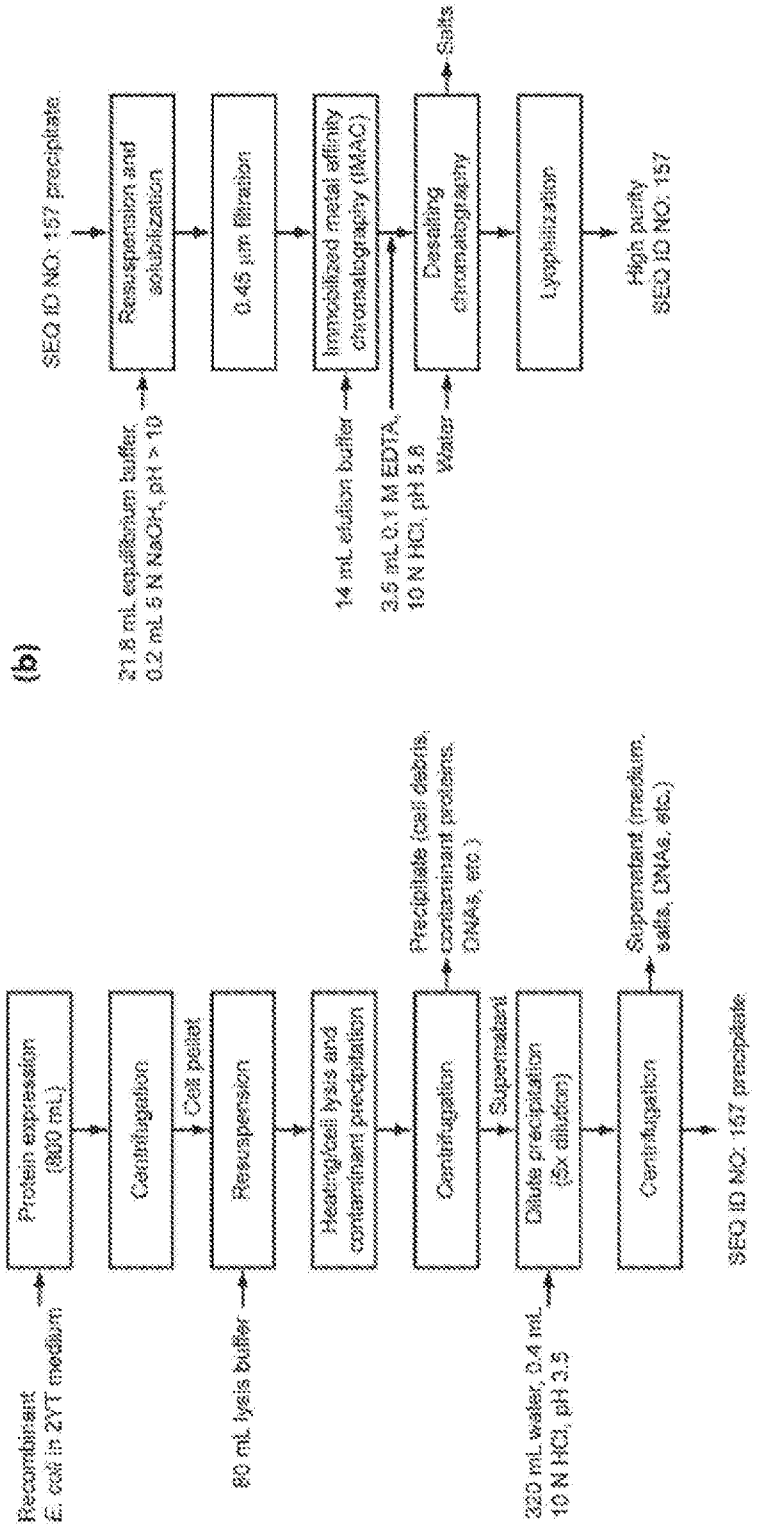
(a)
Recombinant E. coli in 2YT medium
Protein expression (800 mL)
Centrifugation
Cell pellet
80 mL lysis buffer
Resuspension
Heating/cell lysis and contaminant precipitation
Centrifugation
Precipitate (cell debris, contaminant proteins, DNAs, etc.)
Supernatant
220 mL water, 0.4 mL 10 N HCl, pH 3.5
Dilute precipitation (5x dilution)
Centrifugation
Supernatant (medium, salts, DNAs, etc.)
SEQ ID NO: 157 precipitate
(b)
SEQ ID NO: 157 precipitate
21.8 mL equilibrium buffer, 0.2 mL 6 N NaOH, pH > 10
Resuspension and solubilization
0.45 µm filtration
14 mL elution buffer
3.5 mL 0.1 M EDTA, 10 N HCl, pH 5.8
Water
Immobilized metal affinity chromatography (IMAC)
Desalting chromatography
Salts
Lyophilization
High purity SEQ ID NO: 157

Figure 9

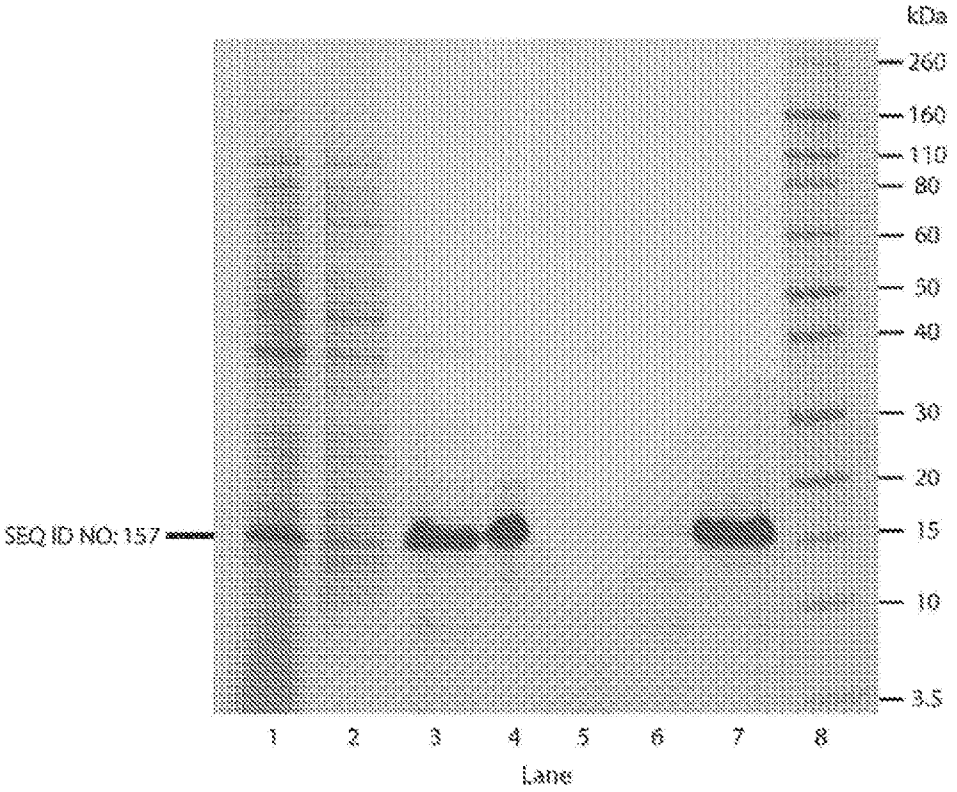
kDa
260
160
110
80
60
50
40
30
20
15
10
3.5
SEQ ID NO: 157
1
2
3
4
5
6
7
8
Lane

Figure 10

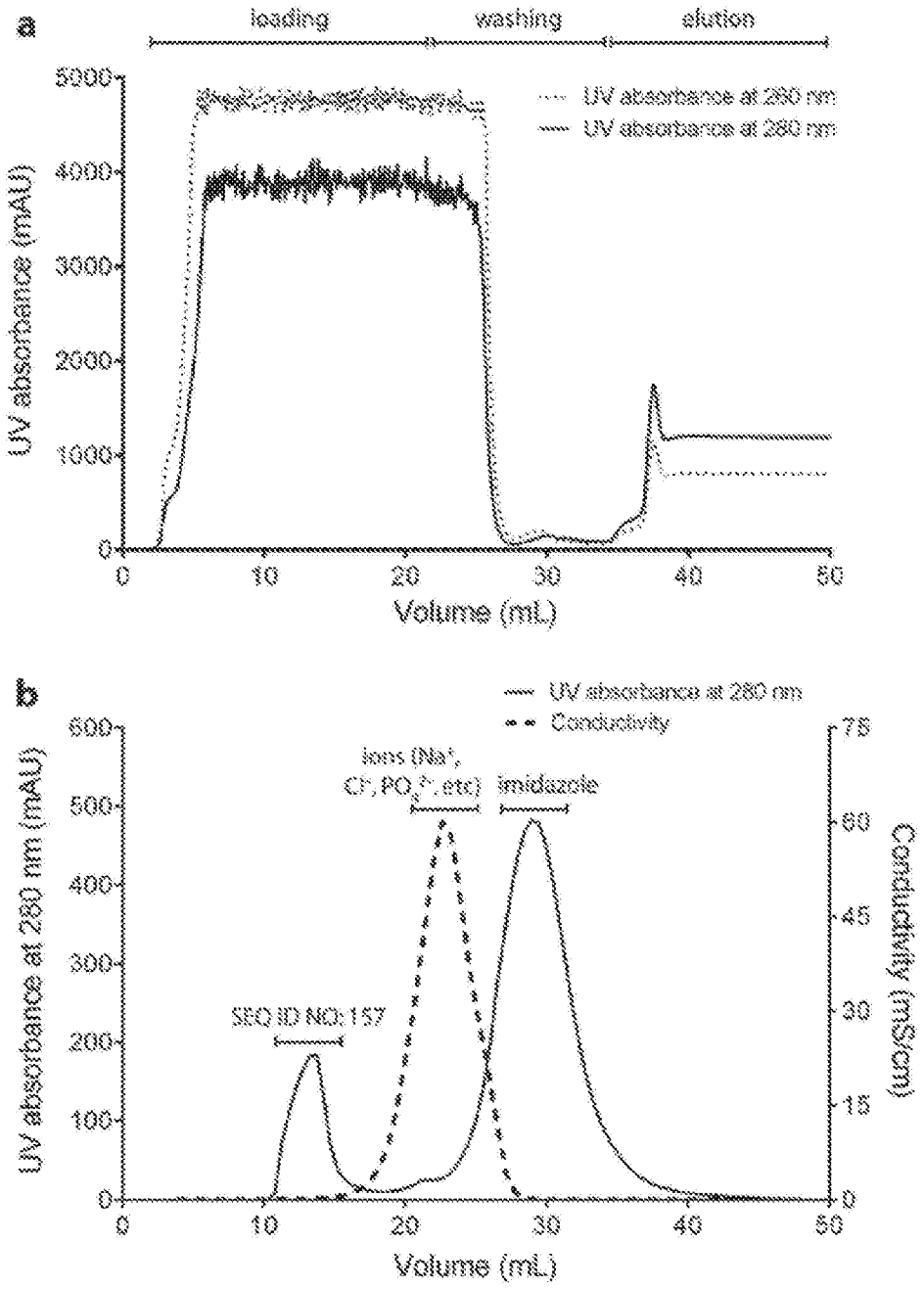
a
loading
washing
elution
UV absorbance at 280 nm
UV absorbance at 280 nm
UV absorbance (mAU)
Volume (mL)
b
UV absorbance at 280 nm
Conductivity
ions (Na+, Cl-, PO4 3-, etc)
imidazole
SEQ ID NO: 157
UV absorbance at 280 nm (mAU)
Conductivity (mS/cm)
Volume (mL)

Figure 11

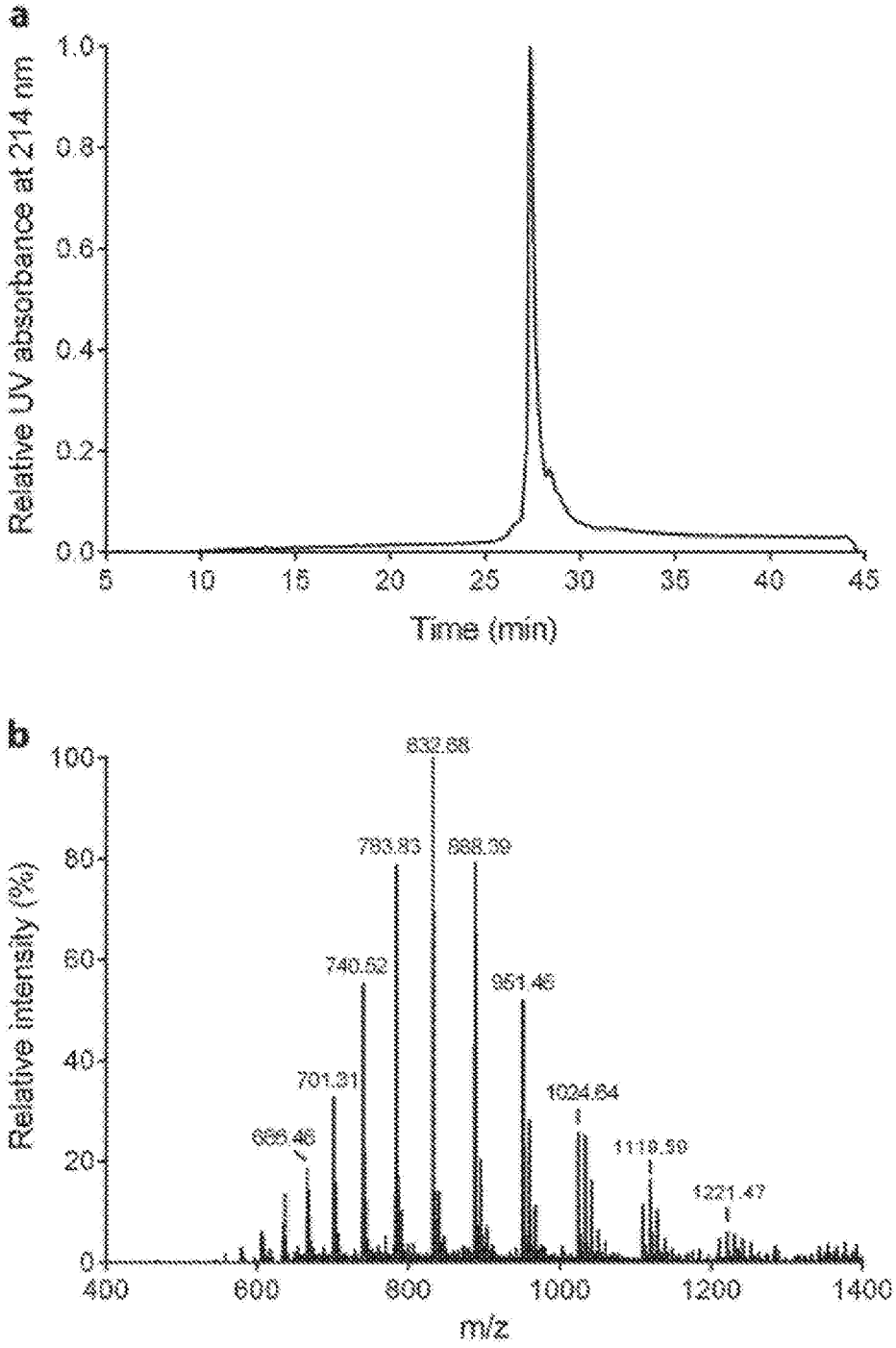
a
Relative UV absorbance at 214 nm
1.0
0.8
0.6
0.4
0.2
0.0
5
10
15
20
25
30
35
40
45
Time (min)
b
Relative intensity (%)
100
80
60
40
20
0
832.68
793.83
888.39
740.62
951.46
701.31
1024.84
666.46
1118.59
1221.47
400
600
800
1000
1200
1400
m/z

Figure 12

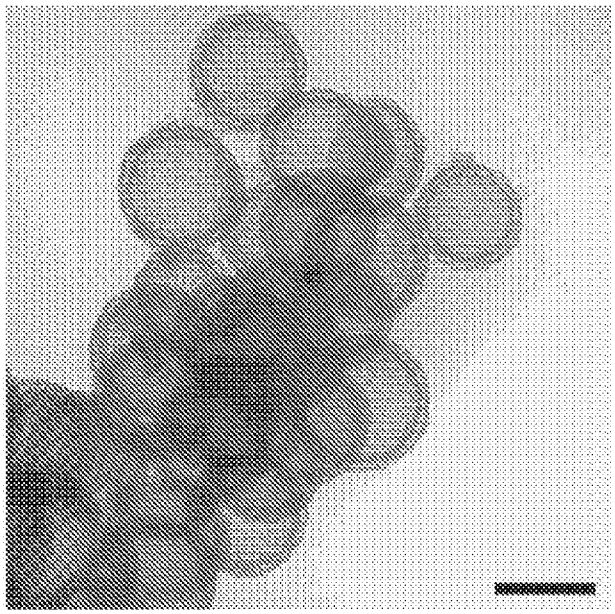

Figure 13