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(54) **NANOGRAPHITE STRUCTURE/METAL NANOPARTICLE COMPOSITE**

NANOGRAPHITSTRUKTUR/METALLNANOPARTIKEL-VERBUND

COMPOSITE A NANOSTRUCTURE DE GRAPHITE/NANOPARTICULES METALLIQUES

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Description**Technical Field**

5 **[0001]** The present invention relates to a protein wherein a nanographite structure recognition peptide is fused or chemically bound to the surface of a cage protein such as ferritin, and a nanographite structure/metal nanoparticle composite constructed with the use of the protein, wherein a plurality of nanoparticles of an inorganic metal atom or an inorganic metal compound are supported on the nanographite structure, etc. For example, a nanographite structure/metal nanoparticle composite, wherein a plurality of nanoparticles are supported through a compound of graphite structure
10 having a nanometer-scale fine structure and a cage protein such as fusion ferritin which recognizes the compound specifically, can be advantageously used for semiconductors, nanobiotechnology, etc.

Background Art

15 **[0002]** As a crystal structure of carbon, diamond and graphite have been known from long time ago, and (C60) was found by R. E. Smalley, R. F. Curl and H. W. Kroto et al., in 1985 (for example, Non-Patent Document 1). C60 has a soccer ball-like structure comprising 12 pentagons and 20 hexagons, and other than C60, there are large basket-like molecules such as C70 and C76, and this series of molecules is called "fullerene". Further, carbon compounds with new structures previously unknown, such as "carbon nanotube" (Non-Patent Document 2; Patent Document 1) and "carbon nanohorn" (Non-Patent Document 3; Patent Document 1) were successively discovered by Sumio Iijima, in 1991 and 1999, respectively. All of these fullerenes, carbon nanotubes and carbon nanohorns comprise six- and five-membered rings of carbon atoms, and form nanometer-scale fine structures, and therefore, they have attracted a lot of attention as "nanographite structure" recently.

25 **[0003]** The reasons why nanographite structures attract a lot of attention include: "carbon nanotubes can have both properties of metal and semiconductor due to the difference in their chirality" (Non-Patent Document 4), "metal-doped fullerene exhibits superconductivity" (Non-Patent Document 5), "selective gas storage capability shown by carbon nanohorns" (Non-Patent Document 6), "ability of carbon nanohorn for the support and sustained release of pharmaceutical compounds" (Patent Document 2; Non-Patent Document 7), and the like. With the use of these characteristic properties,
30 nanographite structures are expected to be applied to new electrical materials, catalysts, optical materials, and other fields, more specifically, to wiring of semiconductors, fluorescent indicator tubes, fuel cells, gas storage, vectors for gene therapy, cosmetics, drug delivery systems, biosensors, etc.

[0004] One of the present inventors, Kiyotaka Shiba, and others have isolated a peptide motif which binds to a carbon nanohorn, one of nanographite structures, by the phage display technique (Patent Document 3; Non-Patent Document 8).

35 **[0005]** On the other hand, ferritin proteins have been known for long years as a protein which stores 'molecules of "iron", which is an essential metal and is toxic at the same time' in living bodies. Ferritin exists universally, from animals and plants to bacteria, and is deeply involved in the homeostasis of iron element in living bodies or in cells. Ferritin from higher eucaryotes such as human and horse forms a spherical shell structure consisting of a 24-mer approximately 12 nm in diameter, formed from peptide chains whose molecular weight is about 20 kDa, and has an interior space of 7 to 8 nm. Ferritin stores iron molecules in this interior space as a mass of nanoparticulate iron oxide. With regard to 24 subunits which constitute a protein spherical shell (cage), there are two types (type H and type L), and the ratio of these types varies depending on organism species and tissues.

40 **[0006]** Ferritin stores iron nanoparticles inside it under natural circumstances. However, under artificial circumstances, it has been revealed that ferritin can store the following substances in addition to iron: oxides of beryllium, gallium, manganese, phosphorus, uranium, lead, cobalt, nickel, chromium, etc.; and nanoparticles of semiconductors, magnets such as cadmium selenide, zinc sulfide, iron sulfide and cadmium sulfide. Consequently, applied researches of ferritin
45 in the fields of material engineering of semiconductors and health care have been actively conducted.

[0007]

Patent Document 1: Japanese Laid-Open Patent Application No. 2001-64004

50 Patent Document 2: Japanese Patent Application No. 2004-139247

Patent Document 3: Japanese Laid-Open Patent Application No. 2004-121154

Non-Patent Document 1: Nature, 318: 162-163, 1985

Non-Patent Document 2: Nature, 354: 56-58, 1991

Non-Patent Document 3: Chem. Phys. Lett., 309: 165-170, 1999

55 Non-Patent Document 4: Nature, 391: 59-62

Non-Patent Document 5: Nature, 350: 600-601

Non-Patent Document 6: Nikkei Science, 42, August issue, 2002

Non-Patent Document 7: Mol Pharmaceutics 1: 399

Disclosure of the Invention

5 Object to be Solved by the Invention

[0008] If it is possible to combine nanographite structures having excellent properties with metal-filled ferritin molecules, the development of composite materials having an unprecedented new function can be expected. In this case, a technique for making ferritin molecules efficiently recognize and bind to nanographite structures such as carbon nanotubes and carbon nanohorns, is required. An object of the present invention is to make it possible to efficiently recognize carbon nanotubes, carbon nanohorns or modifiers thereof and to support functional compounds thereon by fusing the ability of ferritin molecules capable of forming nanoparticles of inorganic metal atoms or inorganic metal compounds with nanographite structure recognition peptides. In addition, because ferritin molecules are capable of forming two-dimensional crystals at the interface and have an ability for molecular arrangement, an object of the present invention is to make it possible to align carbon nanotubes, carbon nanohorns with the use of the molecular arrangement ability of ferritin fused with nanographite structure recognition peptides.

Means to Solve the Object

20 **[0009]** The present inventors have made a keen study for solving the above-mentioned object, and have confirmed that a plurality of nanoparticles can be supported on a nanographite structure by the process comprising the steps of: fusing cDNA encoding the amino-terminal of horse spleen-derived type L ferritin molecule with DNA encoding a peptide consisting of the amino acid sequence shown by SEQ ID NO: 1; expressing a protein having the amino acid sequence shown by SEQ ID NO: 26 with the use of E. coli; purifying the protein and retaining nanoparticles of metal oxide in the interior space of the fusion protein thus obtained. This led to the completion of the present invention.

25 **[0010]** In other words, the present invention relates to: (1) a nanographite structure/metal nanoparticle composite, wherein a nanoparticle of an inorganic metal atom or an inorganic metal compound is retained in an interior space of a protein in which a nanographite structure recognition peptide is fused or chemically bound to a surface of a cage protein, and wherein a plurality of nanoparticles of an inorganic metal atom or an inorganic metal compound are supported on
30 a nanographite structure with the use of affinity of the nanographite structure recognition peptide to the nanographite structure; (2) the nanographite structure/metal nanoparticle composite according to (1) mentioned above, wherein the cage protein belongs to a ferritin protein family; (3) the nanographite structure/metal nanoparticle composite according to (2) mentioned above, wherein the ferritin protein family is ferritin; (4) the nanographite structure/metal nanoparticle composite according to (3) mentioned above, wherein the ferritin is higher eucaryote-derived ferritin; (5) the nanographite structure/metal nanoparticle composite according to (4) mentioned above, wherein the higher eucaryote-derived ferritin is horse spleen-derived type L ferritin; (6) the nanographite structure/metal nanoparticle composite according to (1) mentioned above, wherein the cage protein is derived from a bacterium; (7) the nanographite structure/metal nanoparticle composite according to (1) mentioned above, wherein the cage protein is a viral particle; (8) the nanographite structure/metal nanoparticle composite according to any one of (1) to (7) mentioned above, wherein the nanographite structure recognition peptide is a peptide consisting of an amino acid sequence shown by any one of SEQ ID NOs: 1 to 20; (9) the nanographite structure/metal nanoparticle composite according to any one of (1) to (7) mentioned above, wherein the nanographite structure recognition peptide is a peptide containing whole or part of an amino acid sequence shown by any one of SEQ ID NOs: 1 to 20 and capable of binding to a nanographite structure; (10) the nanographite structure/metal nanoparticle composite according to (8) or (9) mentioned above, wherein the amino acid sequence shown by any one of SEQ ID NOs: 1 to 20 is DYFSSPYEQLF (SEQ ID NO: 1); (11) the nanographite structure/metal nanoparticle composite according to (8) or (9) mentioned above, wherein the amino acid sequence shown by any one of SEQ ID NOs: 1 to 20 is YDPFHII (SEQ ID NO: 2); (12) the nanographite structure/metal nanoparticle composite according to any one of (1) to (11) mentioned above, wherein the nanoparticle of an inorganic metal atom or an inorganic metal compound is a metal nanoparticle; (13) the nanographite structure/metal nanoparticle composite according to any one of (1) to (11) mentioned above, wherein the nanoparticle of an inorganic metal atom or an inorganic metal compound is a metal compound nanoparticle; (14) the nanographite structure/metal nanoparticle composite according to (13) mentioned above, wherein the metal compound nanoparticle is a metal oxide nanoparticle; (15) the nanographite structure/metal nanoparticle composite according to (13) mentioned above, wherein the metal compound nanoparticle is a magnetic material nanoparticle; (16) the nanographite structure/metal nanoparticle composite according to any one of (1) to (15) mentioned above, wherein the metal is iron, beryllium, gallium, manganese, phosphorus, uranium, lead, cobalt, nickel, zinc, cadmium or chromium; (17) the nanographite structure/metal nanoparticle composite according to any one of (1) to (11) mentioned above, wherein the nanoparticle of an inorganic metal atom or an inorganic metal compound is a nanoparticle of iron oxide, a nanoparticle of cadmium selenide, a nanoparticle of zinc selenide, a nanoparticle of zinc

sulfide, or a nanoparticle of cadmium sulfide; (18) the nanographite structure/metal nanoparticle composite according to any one of (1) to (17) mentioned above, wherein the nanographite structure is a carbon nanotube or a carbon nanohorn; (19) the nanographite structure/metal nanoparticle composite according to (18) mentioned above, wherein the carbon nanotube or the carbon nanohorn is constituted of a carbon structure to which a functional group is added; (20) the nanographite structure/metal nanoparticle composite according to any one of (1) to (19) mentioned above, wherein the nanographite structure is two-dimensionally aligned on a substrate; (21) the nanographite structure/metal nanoparticle composite according to any one of (1) to (19) mentioned above, wherein the metal nanoparticle is two-dimensionally aligned on a substrate; and (22) the nanographite structure/metal nanoparticle composite according to (20) mentioned above, wherein the cage protein is removed.

[0011] The present invention also relates to: (23) a protein wherein a nanographite structure recognition peptide is fused or chemically bound to a surface of a cage protein; (24) the protein according to (23) mentioned above, wherein the cage protein belongs to a ferritin protein family; (25) the protein according to (24) mentioned above, wherein the ferritin protein family is ferritin; (26) the protein according to (25) mentioned above, wherein the ferritin is higher eucaryote-derived ferritin; (27) the protein according to (26) mentioned above, wherein the higher eucaryote-derived ferritin is horse spleen-derived type L ferritin; (28) the protein according to (24) mentioned above, wherein the ferritin protein family is derived from a bacterium; (29) the protein according to (23) mentioned above, wherein the cage protein is a viral particle; (30) the protein according to any one of (23) to (29) mentioned above, wherein the nanographite structure recognition peptide is a peptide consisting of an amino acid sequence shown by any one of SEQ ID NOs: 1 to 20; (31) the protein according to any one of (23) to (29) mentioned above, wherein the nanographite structure recognition peptide is a peptide containing whole or part of an amino acid sequence shown by any one of SEQ ID NOs: 1 to 20 and capable of binding to a nanographite structure; (32) the protein according to (30) or (31) mentioned above, wherein the amino acid sequence shown by any one of SEQ ID NOs: 1 to 20 is DYFSSPYEQLF (SEQ ID NO: 1); (33) the protein according to (30) or (31) mentioned above, wherein the amino acid sequence shown by any one of SEQ ID NOs: 1 to 20 is YDPFHII (SEQ ID NO: 2); (34) the protein according to any one of (23) to (33) mentioned above, wherein the nanoparticle of an inorganic metal atom or an inorganic metal compound is a metal nanoparticle; (35) the protein according to any one of (23) to (33) mentioned above, wherein the nanoparticle of an inorganic metal atom or an inorganic metal compound is a metal compound nanoparticle; (36) the protein according to (35) mentioned above, wherein the metal compound nanoparticle is a metal oxide nanoparticle; (37) the protein according to (35) mentioned above, wherein the metal compound nanoparticle is a magnetic material nanoparticle; (38) the protein according to any one of (22) to (36) mentioned above, wherein the metal is iron, beryllium, gallium, manganese, phosphorus, uranium, lead, cobalt, nickel, zinc, cadmium or chromium; (39) the protein according to (23) mentioned above, wherein the nanoparticle of an inorganic metal atom or an inorganic metal compound is a nanoparticle of iron oxide, a nanoparticle of cadmium selenide, a nanoparticle of zinc selenide, a nanoparticle of zinc sulfide, or a nanoparticle of cadmium sulfide; (40) the protein according to any one of (23) to (39) mentioned above, wherein the nanographite structure is a carbon nanotube or a carbon nanohorn; and (41) the protein according to (40) mentioned above, wherein the carbon nanotube or the carbon nanohorn is constituted of a carbon structure to which a functional group is added.

[0012] The present invention further relates to: (42) a method for retaining a nanoparticle of an inorganic metal atom or an inorganic metal compound in an interior space of the protein according to any one of (23) to (41) mentioned above, and supporting a plurality of nanoparticles of an inorganic metal atom or an inorganic metal compound on a nanographite structure with the use of affinity of the nanographite structure recognition peptide to the nanographite structure; (43) a method for producing a composite of a nanographite structure and nanoparticles of an inorganic metal compound, comprising the steps of: retaining a nanoparticle of an inorganic metal atom or an inorganic metal compound in an interior space of the protein according to any one of (23) to (41) mentioned above; supporting a plurality of nanoparticles of an inorganic metal atom or an inorganic metal compound on a nanographite structure with the use of affinity of the nanographite structure recognition peptide to the nanographite structure; and removing a protein moiety by a heat treatment; (44) a method for producing a composite of a nanographite structure and nanoparticles of an inorganic metal compound, comprising the steps of: retaining a nanoparticle of an inorganic metal atom or an inorganic metal compound in an interior space of the protein according to any one of (23) to (41) mentioned above; supporting a plurality of nanoparticles of an inorganic metal atom or an inorganic metal compound on a nanographite structure with the use of affinity of the nanographite structure recognition peptide to the nanographite structure; and removing a protein moiety by an electron beam treatment; (45) a method for aligning a nanographite structure by binding the nanographite structure to the protein according to any one of (23) to (41) mentioned above which has formed a two-dimensional crystal; and (46) a method for aligning a nanographite structure by binding the nanographite structure to the protein according to any one of (23) to (41) mentioned above which has formed a two-dimensional array.

Brief Description of Drawings

[0013]

[Fig. 1] This is a view showing the crystal structure of horse spleen-derived type L ferritin (LF) and the presentation site of DYFSSPYEQLF (SEQ ID NO: 1; N1 sequence). N-terminal site of the crystal structure of horse spleen-derived type L ferritin (LF0) is indicated in red. As shown in Fig. 1, because the N-terminal of LF0 is located outside of the molecule, a multiple number of N1 sequence can be presented by fusing the N-terminal with N1 sequence.

[Fig. 2] This is a frame format of the construction of N1-LF expression vector pKIS2. N1-LF recombinant ferritin expression vector pKIS2 was constructed by the process comprising the steps of: cutting pKITO, a horse spleen-derived type L ferritin expression vector, with restriction enzymes BamHI and SacI; inserting synthetic DNAs of SEQ ID NOs : 22 and 23 which had been annealed, and then cutting the resultant product with BamHI; to that site, inserting a short DNA fragment produced when pKITO had been cut with BamHI.

[Fig. 3] This is a view showing the result of polyacrylamide gel electrophoresis of the final purified preparation of N1-LF. By polyacrylamide gel electrophoresis of 3 pg of the final purified preparation of N1-LF, the uniformity was evaluated. When the preparation was separated by using a concentration gradient gel (15 to 25%) and stained with Coomassie brilliant blue, a protein band was observed only at the position corresponding to the molecular weight of the desired N1-LF. Based on the observation, it was possible to confirm the preparation was highly pure. The left lane indicates molecular weight markers corresponding to 97.4, 66.3, 42.4, 30.0, 20.1, 14.4 kDa in descending order. The right lane indicates the final purified preparation of N1-LF.

[Fig. 4] This is a view showing the formation of nanoparticles of iron oxide in the interior space of N1-LF. It is an appearance of the solution at the time when nanoparticles of iron oxide were formed in the interior space of N1-LF. In control, no ferritin protein solution was contained. It can be seen from the color of the solution that nanoparticles of iron oxide were formed in the interior space of ferritin.

[Fig. 5] This is a photomicrograph taken by a transmission electron microscope showing the formation of nanoparticles of iron oxide in the interior space of N1-LF. The image of N1-LF stained with 1% aurothioglucose was observed with a JEOL1010, manufactured by JEOL Ltd., at 100 kV.

[Fig. 6] This is a photomicrograph taken by a transmission electron microscope showing the formation of nanoparticles of iron oxide in the interior space of LF0. The image of N1-LF stained with 1% aurothioglucose was observed with a JEOL1010, manufactured by JEOL Ltd., at 100 kV.

[Fig. 7] This is a view showing nanoparticles of metal oxide supported on a carbon nanohorn. By presenting a peptide capable of binding to carbon nanohorns to ferritin protein, N1-LF could specifically support a plurality of nanoparticles of metal oxide on the carbon nanohorn (left). With horse spleen-derived type L ferritin (LF0), it was impossible to support nanoparticles of metal oxide on the carbon nanohorn (right).

[Fig. 8] This is a view showing nanoparticles of metal oxide supported on a single-wall carbon nanotube. By presenting a peptide capable of binding to carbon nanohorns to ferritin protein, N1-LF could support a plurality of nanoparticles of metal oxide on the single-wall carbon nanotube.

Best Mode of Carrying Out the Invention

[0014] The nanographite structure/metal nanoparticle composite of the present invention is not particularly limited as long as it is a composite wherein a nanoparticle of an inorganic metal atom or an inorganic metal compound is retained in an interior space of a protein in which a nanographite structure recognition peptide is fused or chemically bound to a surface of a cage protein, and wherein a plurality of nanoparticles of an inorganic metal atom or an inorganic metal compound are supported on a nanographite structure with the use of affinity of the nanographite structure recognition peptide to the nanographite structure. In addition, the protein is not particularly limited as long as it is a protein wherein a nanographite structure recognition peptide is fused or chemically bound to a surface of a cage protein. Here, the cage protein of the present invention means a protein having a space inside it and capable of containing a substance.

[0015] Examples of the above-mentioned cage protein include ferritin protein family, those derived from bacteria, and viral particles. As the ferritin protein family, ferritin and apoferritin are exemplified, and, for example, type L or type H ferritin derived from higher eucaryotes such as horse spleen-derived type L ferritin is preferably exemplified. Examples of the cage proteins derived from bacteria include DpsA protein and MrgA protein, and examples of the viral particles include viral particles of retrovirus, adenovirus, rotavirus, poliovirus, cytomegalovirus, cauliflower mosaic virus, etc.

[0016] Examples of the above-mentioned nanographite structure include carbon nanotubes and carbon nanohorns, and in addition, a modified nanographite structure wherein a carbon nanotube or a carbon nanohorn is constituted of a carbon structure to which a functional group such as an amino group, a hydroxyl group, and a carboxyl group is added.

[0017] As the above-mentioned nanographite structure recognition peptide, a peptide consisting of an amino acid sequence shown by any one of SEQ ID NOs: 1 to 20 (see Patent Document 3; Non-Patent Document 8), and a peptide containing whole or part of an amino acid sequence shown by any one of SEQ ID NOs : 1 to 20 and capable of binding to a nanographite structure, are exemplified. Among them, a peptide of DYFSSPYEQLF (SEQ ID NO: 1) and a peptide of YDPFHI I (SEQ ID NO: 2) are preferably exemplified.

[0018] The site on the cage protein surface to which a nanographite structure recognition peptide is fused or chemically

bound is not particularly limited as long as it is a site where the nanographite structure recognition peptide can be bound to a nanographite structure. For example, in the case of ferritin, a loop structure site exposed on the ferritin surface, are exemplified in addition to amino terminals.

[0019] With regard to the method for fusing a nanographite structure recognition peptide to the surface of a cage protein such as ferritin, as described in Examples, the method can be conducted in accordance with the methods described in "Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY., 1989", "Current Protocols in Molecular Biology, Supplement 1-38, John Wiley & Sons (1987-1997)", etc. Further, as the method for chemically binding a nanographite structure recognition peptide to the surface of a cage protein such as ferritin, the methods described in literatures (Proteins second edition, T. E. Creighton, W. H. Freeman and Company, New York, 1993; and G. T. Hermanson, in Bioconjugate Techniques, ed. G. T. Hermanson, Academic Press, San Diego CA, 1996, pp. 169-186.) are exemplified.

[0020] Examples of the above-mentioned nanoparticles of an inorganic metal atom or an inorganic metal compound include: nanoparticles of metals such as iron, beryllium, gallium, manganese, phosphorus, uranium, lead, cobalt, nickel, zinc, cadmium or chromium; and nanoparticles of metal compounds such as nanoparticles of oxides, hydroxides, carbonate, etc. of these metals and nanoparticles of magnetic materials. Preferable examples include nanoparticles of iron oxide, nanoparticles of cadmium selenide, nanoparticles of zinc selenide, nanoparticles of zinc sulfide, and nanoparticles of cadmium sulfide.

[0021] By further fusing or chemically binding a functional peptide, for example, a peptide capable of binding to titanium, to the above-mentioned protein wherein a nanographite structure recognition peptide is fused or chemically bound to a surface of a cage protein, it is also possible to align nanographite structures on a titanium substrate, and moreover, to support a plurality of nanoparticles on the nanographite structures.

[0022] In the case of cage proteins such as ferritin capable of forming two-dimensional crystals, nanometer-sized pattern making are made possible by two-dimensional crystallization of the cage proteins. For instance, a nanographite structure/metal nanoparticle composite wherein nanographite structures are two-dimensionally aligned on a substrate, and a nanographite structure/metal nanoparticle composite wherein metal nanoparticles are two-dimensionally aligned on a substrate can be obtained by the process comprising the steps of: retaining a nanoparticle of an inorganic metal atom or an inorganic metal compound in an interior space of a protein wherein a nanographite structure recognition peptide is fused or chemically bound to a surface of a cage protein; supporting a plurality of nanoparticles of an inorganic metal atom or an inorganic metal compound on a nanographite structure with the use of affinity of the nanographite structure recognition peptide to the nanographite structure; and removing a protein moiety by a heat treatment or an electron beam treatment. The composite thus obtained can be the basic technique for high integration of memory devices, etc., in the field of semiconductors including memory devices.

[0023] In addition, a nanographite structure/metal nanoparticle composite wherein nanographite structures are two-dimensionally aligned on a substrate, and a nanographite structure/metal nanoparticle composite wherein metal nanoparticles are two-dimensionally aligned on a substrate can be obtained also by constituting cage proteins into a two-dimensional array, for example, by the method for immobilizing proteins on a substrate by forming a cross-linking between proteins or proteins and the substrate with the use of reactivity between a compound having a plurality of functional groups such as glutaraldehyde and a side chain of an amino acid which constitutes the protein: or by the method for immobilizing proteins by placing SAM (molecules capable of self-assembling into membranes) having a functional group on a substrate and forming a linkage between the functional group and a side chain of an amino acid which constitutes the protein. The composite thus obtained can be the basic technique for high integration of memory devices, etc., in the field of semiconductors including memory devices.

[0024] Hereinafter, the present invention is described more specifically with reference to Examples, however, the technical scope of the present invention is not limited to these exemplifications.

Example 1

[0025] The preparation of DNA (pKIS2) for expressing the fusion ferritinprotein (N1-LF, Fig. 1) wherein a nanographite structure recognition peptide consisting of the amino acid sequence shown by SEQ ID NO: 1 (N1) is fused with horse spleen-derived type L ferritin (LF) was conducted in accordance with the following procedure. In brief, an annealing reaction was conducted by: mixing 100 pmole/pl each of synthetic DNAs of SEQ ID NOs: 22 and 23 which are complementary to each other and encode Met, an initiation codon, and subsequently the amino acid sequence shown by SEQ ID NO: 21, and have a restriction enzyme BamHI linker sequence on the initiation codon side, and a restriction enzyme Sall linker sequence on the opposite side, in 50 mM NaCl, 10 mM Tris-HCl, 10 mM MgCl₂; heating the resultant mixture at 70° C for 10 minutes; and then slowly cooling the mixture to room temperature. Then, cDNA of horse spleen-derived type L ferritin digested a plasmid pKITO which had been cloned into downstream of tac promoter (Okuda et al. 2003, Biotechnology and Bioengineering, Vol 84, No. 2, p187-194) with restriction enzymes BamHI and Sall; a large DNA fragment, about 6 kb, separated by 1% agarose gel electrophoresis was purified with Gene Clean II kit (BIO101); the

purified substance was mixed with the aforementioned annealed DNA, and bound by using T4 DNA ligase.

[0026] Next, this DNA and pK1TO were digested by BamHI respectively and DNA fragments separated by 1% agarose gel electrophoresis, the former was a fragment of about 6 kb, and the latter was a fragment of about 300 bp, were purified with Gene Clean II kit (BIO101), the purified substances were bound by using T4 DNA ligase. The bound DNA was cloned into *E. coli* XLI-blue strain (hsdR17, supE44, recA1, endA1, gyrA46, thi, relA1, lac/F' [proAB+, lacI^qΔ (lacZ) M15;; Tn10 (tetR)]) in accordance with an ordinary method (Molecular Cloning Third Edition, Cold Spring Harbor Laboratory Press), and a clone into which a BamHI fragment of about 300 bp was inserted in the desired direction was determined by a dideoxy termination method (CEQ DTCS Quick start kit, Beckman, California), through DNA sequencing with the use of a primer (SEQ ID NO: 24) in a BamHI fragment of about 300 bp from pK1TO. For the migration and data analysis of the reactant, an automated capillary sequencer (CEQ2000, Beckman) was used (Fig. 2).

[0027] The fusion ferritin protein wherein a nanographite structure recognition peptide is fused with horse spleen-derived type L ferritin was expressed and purified as follows.

[0028] The *E. coli* XLI-blue strain was transformed with pK1S2 in accordance with an ordinary method, and a colony was picked up with a sterilized pick and shaking-cultured in 5 ml of LB medium at 37° C for 16 to 18 hours. Then this culture solution was transplanted to 1 liter of LB medium and shaking-culture was conducted at 37° C for another 16 to 18 hours. The *E. coli* was collected by centrifugation (Beckman J2-21M, JA-14 rotor, 5000 rpm, 5 minutes). The *E. coli* thus collected was washed with 80 ml of 50 mM Tris-HCl, pH 8.0, and collected by centrifugation (Kubota, 5922, RA410M2 rotor, 4000 rpm, 10 minutes) again. The collected *E. coli* was suspended in 30 ml of 50 mM Tris-HCl, pH 8.0, and an ultrasonic disruptor (BRANSON, SONIFIER 250, micro tip, output level maximum, duty cycle 50%, 2 minutes; this procedure was repeated 3 to 4 times) was used to obtain a solution of disrupted *E. coli* cells. The solution of disrupted *E. coli* cells was subjected to centrifugation (Kubota, 5922, RA410M2 rotor, 8000 rpm, 30 minutes) to collect soluble fractions. By putting the fractions into a warm bath at 65° C for 20 minutes, coexisting proteins were denatured. The denatured coexisting proteins which formed precipitates were removed by centrifugation (Kubota, 5922, RA410M2 rotor, 8000 rpm, 30 minutes), and the supernatant was collected.

[0029] To the collected supernatant, 5 M NaCl was added such that the final concentration was adjusted to 0.5 M, and the resultant mixture was stirred and allowed to stand still at room temperature for 5 to 10 minutes, and then a precipitate was collected by centrifugation (Kubota, 5922, RA410M2 rotor, 5000 rpm, 10 minutes). The precipitate was dissolved in 20 ml of 50 mM Tris-HCl (pH 8.0), and to this mixture, 5 M NaCl was added again such that the final concentration was adjusted to 0.5 M, and the resultant mixture was stirred and allowed to stand still at room temperature for 5 to 10 minutes, and then a precipitate was collected by centrifugation (Kubota, 5922, RA410M2 rotor, 5000 rpm, 10 minutes). The precipitate was further dissolved in 20 ml of 50 mM Tris-HCl (pH 8.0), and to this mixture, 5 M NaCl was added again such that the final concentration was adjusted to 0.375 M this time, and the resultant mixture was stirred and allowed to stand still at room temperature for 5 to 10 minutes, and then a precipitate was collected by centrifugation (Kubota, 5922, RA410M2 rotor, 5000 rpm, 10 minutes). The collected precipitate was dissolved in 10 ml of 50 mM Tris-HCl (pH 8.0).

[0030] In addition, the purification by gel filtration chromatography was conducted as needed. In other words, 200 to 500 μl of the purified preparation mentioned above was poured into an SW4000XL column (TOSOH) equilibrated with 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 1 mM NaN₃, purification and separation were conducted by chromatography at a flow rate of 1 ml/min, and a fraction corresponding to a ferritin 24-mer was collected (Fig. 3).

Example 2

[0031] It was confirmed by the following procedure that as in the case of recombinant apoferritin, the N1-LF obtained in Example 1 has an ability to form nanoparticles of iron oxide in its interior space.

To a solution comprising 50 mM HEPES-NaOH (pH 7.0) and 0.5 mg/ml N1-LF, 50 mM ammonium iron (II) sulfate hexahydrate was added at an amount, 1/10 of the volume of the solution (final concentration 5 mM), and the resultant mixture was allowed to stand still at room temperature overnight (Fig. 4). Subsequently, a procedure to precipitate excessive iron oxides by centrifugation (Kubota, 5922, RA410M2 rotor, 3000 rpm, 10 minutes) and remove them was repeated twice. Next, by a centrifugal operation with the use of an ultracentrifuge (Beckman, TLA 100.4 rotor, 50,000 rpm, 1 hour), N1-LF was precipitated. This precipitate was dissolved in 50 mM Tris-HCl (pH 8.0), overlaid on an equal amount of 15% sucrose solution, and N1-LF present in sucrose fractions was collected by conducting a centrifugal operation again with the use of the ultracentrifuge (Beckman, TLA 100.4 rotor, 50,000 rpm, 1 hour). With regard to the collected N1-LF, the formation of nanoparticles of iron oxide was confirmed by a transmission electron microscope (JEOL1010, 100 kV, stained with 1% aurothioglucose, Fig. 5). The collected N1-LF was dialyzed against 50 mM Tris-HCl (pH 8.0), then quantitated by BioRad Protein Assay (BioRad), and used for other experiments.

(Comparative example 1)

5 **[0032]** With regard to the horse spleen-derived type L ferritin (LFO), a recombinant was used as in the case of Example 1. The recombinant was prepared as follows. The E. coli XLI-blue strain was transformed with pKlTO in accordance with an ordinary method, and a colony was picked up with a sterilized pick and shaking-cultured in 5 ml of LB medium at 37° C for 16 to 18 hours. Then this culture solution was transplanted to 1 liter of LB medium and shaking-culture was conducted at 37°C for another 16 to 18 hours. The E. coli was collected by centrifugation (Beckman J2-21M, JA-14 rotor, 5000 rpm, 5 minutes). The E. coli thus collected was washed with 80 ml of 50 mM Tris-HCl (pH 8.0), and collected by centrifugation (Kubota, 5922, RA410M2 rotor, 4000 rpm, 10 minutes) again. The collected E. coli was suspended in 10 30 ml of 50 mM Tris-HCl (pH 8.0), and then an ultrasonic disrupter (BRANSON, SONIFIER 250, micro tip, output level maximum, duty cycle 50%, 2 minutes ; this procedure was repeated 3 to 4 times) was used to obtain a solution of disrupted E. coli cells. The solution of disrupted E. coli cells was subjected to centrifugation (Kubota, 5922, RA410M2 rotor, 8000 rpm, 30 minutes) to collect soluble fractions. By putting the fractions into a warm bath at 65°C for 20 minutes, coexisting proteins were denatured. The denatured coexisting proteins which formed precipitates were removed by 15 centrifugation (Kubota, 5922, RA410M2 rotor, 8000 rpm, 30 minutes), and the supernatant was collected.

[0033] The supernatant was poured into Q-sepharose HP (Amersham), which is a carrier for anion exchange chromatography, equilibrated with 50 mM Tris-HCl (pH 8.0), and the elution was conducted with 100 ml of 100 to 500 mM sodium chloride concentration gradient (3 ml/min). About 40 ml of fractions containing LFO was concentrated by Centriprep 10 (Amicon) to 2.5 to 3 ml, and the resultant was poured into a 60 cm-long gel filtration chromatograph Sephacryl S-400 equilibrated with 50 mM Tris-HCl (pH 8.0), 150 mM NaCl (herein after referred to as TBS), and chromatography was conducted at a flow rate of 1.5 ml/min. Up to 100 µl of each fraction containing LFO was poured into an SW4000XL column equilibrated with 50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM NaN₃, and analyzed by chromatography at a flow rate of 1 ml/min, and a fraction corresponding to a ferritin 24-mer was confirmed and used for the experiment described below.

25 (Comparative example 2)

[0034] The formation of nanoparticles of iron oxide in the interior space of LFO obtained in Comparative example 1 was conducted in a same procedure as described in Example 2. The formation of nanoparticles was confirmed in a 30 same manner as described in Example 2, as well (Fig. 6).

Example 3

35 **[0035]** The following experiment was conducted in order to show that: though the N1-LF (SEQ ID NO: 26) having nanoparticles of iron oxide in its interior space obtained in Example 2 specifically binds to a nanographite structure, the LFO (SEQ ID NO: 25) having nanoparticles of iron oxide in its interior space obtained in Comparative example 2 cannot bind to a nanographite structure.

[0036] High-power CO₂ gas laser beam (output power 100 W, pulse width 20 ms, continuous wave) was emitted over the surface of carbon in a form of a sintered round bar in ambient pressure of 6×10^4 Pa of Ar gas, and the resultant soot-like substance was suspended in ethanol, then ultrasonic agitation (frequency 40 kHz, 60 minutes) and decantation were repeated 4 times to obtain single-wall carbon nanohorns. About 200 mg of the single-wall carbon nanohorns was put into 40 ml of nitric acid at a concentration of about 70%, and reflux was conducted for 1 hour at 130°C. After the reflux, the resultant was neutralized and washed by repeating dilution with ion-exchange water, centrifugation, and disposal of the supernatant, and water-soluble single-wall carbon nanohorns having a functional group (including a 40 carboxyl group) were prepared.

[0037] The carbon nanohorns were dissolved in 0.1% fetal bovine serum albumin, 0.05% polyoxyethylenesorbitan monolaurate [hereinafter referred to as Tween 20 (Sigma, St. Louis)] contained in TBS (hereinafter referred to as TBS-BT) such that the concentration was adjusted to 1 mg/ml. The carbon nanohorns were precipitated by a centrifugal operation (Kubota, 5922, AT-2018M rotor, 15000 rpm, 15 minutes), and the precipitate was suspended in TBS-BT such that the concentration was adjusted to 1 mg/ml. This operation was repeated 3 times, and subsequently the precipitated carbon nanohorns were suspended in TBS-BT containing N1-LF or LFO having 0.1 mg/ml of nanoparticles of iron oxide in its core, such that the concentration was adjusted to 1 mg/ml. The suspension was rotated and stirred for 12 hours at room temperature with a rotator RT-50 manufactured by Taitec. In order to remove ferritin molecules which had not bound, the carbon nanohorns were precipitated by a centrifugal operation (Kubota, 5922, AT-2018M rotor, 15000 rpm, 15 minutes), and the precipitate was washed 5 times with 400 µl of TBS containing 0.05% Tween 20, and then the solution was substituted with sterilized water for the demineralization of the precipitate. Thus treated precipitate was observed under a transmission electron microscope (TOPCON EM-002B, accelerating voltage 120 kV), and it was 55 observed that a plurality of nanoparticles of iron oxide were supported on a carbon nanohorn when N1-LF was mixed

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with carbon nanohorns, on the other hand in case of LF0, nanoparticles of iron oxide were not observed on carbon nanohorns. Based on the observation, it was confirmed that N1-LF has an ability to specifically bind to carbon nanohorns, and that the method for supporting nanoparticles on nanographite structures utilizing this ability is effective (Fig. 7).

5 Example 4

[0038] Hipco (Carbon Nanotechnologies Inc., Texas), a single-wall carbon nanotube synthesized by chemical vapor deposition, was treated with 1×10^{-5} Torr for 5 hours at 1750° C, and then reflux was conducted for 30 minutes at about 130° C in nitric acid at a concentration of about 70%. After that, neutralization with sodium hydroxide and washing with distilled water were conducted, and single-wall carbon nanotubes having a functional group (including a carboxyl group) were prepared.

[0039] The single-wall carbon nanotubes were dissolved in TBS-BT in a same manner as described in Example 3. The single-wall carbon nanotubes were precipitated by a centrifugal operation (Kubota, 5922, AT-2018M rotor, 15000 rpm, 15 minutes), and the resultant precipitate was suspended in TBS-BT again. This operation was repeated 3 times, and subsequently the precipitated single-wall carbon nanotubes were suspended, in a same manner as described in Example 3, in TBS-BT containing N1-LF having nanoparticles of iron oxide in its core. The suspension was rotated and stirred for 12 hours at room temperature with a rotator RT-50 manufactured by Taitec. In order to remove ferritin molecules which had not bound, the single-wall carbon nanotubes were precipitated by a centrifugal operation (Kubota, 5922, AT-2018M rotor, 15000 rpm, 15 minutes), and the precipitate was washed 5 times with TBS containing 0.05% Tween 20, and then the solution was substituted with sterilized water for the demineralization of the precipitate. Thus treated precipitate was observed under a transmission electron microscope (TOPCON EM-002B, 120 kV), and it was confirmed that a plurality of nanoparticles of iron oxide was supported on a carbon nanohorn when N1-LF was mixed with single-wall carbon nanotubes (Fig. 8).

25 SEQUENCE LISTING

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Claims

1. A nanographite structure/metal nanoparticle composite, wherein a nanoparticle of an inorganic metal atom or an inorganic metal compound is added and retained in an interior space of a ferritin in which a nanographite structure recognition peptide is fused to an N-terminal or a loop-structure site of the ferritin, and wherein a plurality of nanoparticles of an inorganic metal atom or an inorganic metal compound are supported on a nanographite structure with the use of affinity of the nanographite structure recognition peptide to the nanographite structure.
2. The nanographite structure/metal nanoparticle composite according to claim 1, wherein the ferritin is higher eucaryote-derived ferritin.
3. The nanographite structure/metal nanoparticle composite according to claim 2, wherein the higher eucaryote-derived ferritin is horse spleen-derived type L ferritin.
4. The nanographite structure/metal nanoparticle composite according to any one of the preceding claims, wherein

the nanographite structure recognition peptide is a peptide consisting of an amino acid sequence shown by any one of SEQ ID NOs: 1 to 20.

- 5 5. The nanographite structure/metal nanoparticle composite according to any one of the preceding claims, wherein the nanographite structure recognition peptide is a peptide containing whole or part of an amino acid sequence shown by any one of SEQ ID NOs: 1 to 20 and capable of binding to a nanographite structure.
- 10 6. The nanographite structure/metal nanoparticle composite according to claim 4 or 5, wherein the amino acid sequence shown by any one of SEQ ID NOs: 1 to 20 is DYFSSPYEQLF (SEQ ID NO: 1).
- 15 7. The nanographite structure/metal nanoparticle composite according to claim 4 or 5, wherein the amino acid sequence shown by any one of SEQ ID NOs: 1 to 20 is YDPFHII (SEQ ID NO: 2).
- 18 8. The nanographite structure/metal nanoparticle composite according to any one of the preceding claims, wherein the nanoparticle of an inorganic metal atom or an inorganic metal compound is a metal nanoparticle.
- 20 9. The nanographite structure/metal nanoparticle composite according to any one of claims 1 to 7, wherein the nanoparticle of an inorganic metal atom or an inorganic metal compound is a metal compound nanoparticle.
- 25 10. The nanographite structure/metal nanoparticle composite according to claim 9, wherein the metal compound nanoparticle is a metal oxide nanoparticle.
- 28 11. The nanographite structure/metal nanoparticle composite according to claim 9, wherein the metal compound nanoparticle is a magnetic material nanoparticle.
- 30 12. The nanographite structure/metal nanoparticle composite according to any one of the preceding claims, wherein the metal is iron, beryllium, gallium, manganese, phosphorus, uranium, lead, cobalt, nickel, zinc, cadmium or chromium.
- 35 13. The nanographite structure/metal nanoparticle composite according to any one of claims 1 to 7, wherein the nanoparticle of an inorganic metal atom or an inorganic metal compound is a nanoparticle of iron oxide, a nanoparticle of cadmium selenide, a nanoparticle of zinc selenide, a nanoparticle of zinc sulfide, or a nanoparticle of cadmium sulfide.
- 40 14. The nanographite structure/metal nanoparticle composite according to any one of the preceding claims, wherein the nanographite structure is a carbon nanotube or a carbon nanohorn.
- 45 15. The nanographite structure/metal nanoparticle composite according to claim 14, wherein the carbon nanotube or the carbon nanohorn is constituted of a carbon structure to which a functional group is added.
- 50 16. A method for adding and retaining a nanoparticle of an inorganic metal atom or an inorganic metal compound in an interior space of a ferritin in which a nanographite structure recognition peptide is fused to an N-terminal or a loop-structure site of the ferritin; and supporting a plurality of nanoparticles of an inorganic metal atom or an inorganic metal compound on a nanographite structure with the use of affinity of the nanographite structure recognition peptide to the nanographite structure.
- 55 17. A method for producing a composite of a nanographite structure and nanoparticles of an inorganic metal compound, comprising the steps of: adding and retaining a nanoparticle of an inorganic metal atom or an inorganic metal compound in an interior space of a ferritin in which a nanographite structure recognition peptide is fused to an N-terminal or a loop-structure site of the ferritin; supporting a plurality of nanoparticles of an inorganic metal atom or an inorganic metal compound on a nanographite structure with the use of affinity of the nanographite structure recognition peptide to the nanographite structure; and removing a protein moiety by a heat treatment.
18. A method for producing a composite of a nanographite structure and nanoparticles of an inorganic metal compound, comprising the steps of: adding and retaining a nanoparticle of an inorganic metal atom or an inorganic metal compound in an interior space of a ferritin in which a nanographite structure recognition peptide is fused to an N-terminal or a loop-structure site of the ferritin; supporting a plurality of nanoparticles of an inorganic metal atom or an inorganic metal compound on a nanographite structure with the use of affinity of the nanographite structure

recognition peptide to the nanographite structure; and removing a protein moiety by an electron beam treatment.

Patentansprüche

- 5 1. Nanographitstruktur/Metallnanopartikel-Verbund, wobei ein Nanopartikel eines anorganischen Metallatoms oder einer anorganischen Metallverbindung zu einem inneren Raum eines Ferritins zugegeben und darin zurückgehalten wird, bei dem ein Nanographitstruktur-Erkennungspeptid mit einem N-Terminus oder einer Stelle einer Loop-Struktur des Ferritins fusioniert ist, und wobei eine Vielzahl von Nanopartikeln eines anorganischen Metallatoms oder einer
10 anorganischen Metallverbindung durch eine Nanographitstruktur unter Verwendung der Affinität des Nanographitstruktur-Erkennungspeptids zu der Nanographitstruktur getragen werden.
- 15 2. Nanographitstruktur/Metallnanopartikel-Verbund gemäß Anspruch 1, wobei das Ferritin ein von einem höheren Eukaryonten abgeleitetes Ferritin ist.
- 20 3. Nanographitstruktur/Metallnanopartikel-Verbund gemäß Anspruch 2, wobei das von einem höheren Eukaryonten abgeleitete Ferritin ein aus Pferdemicz abgeleitetes Ferritin Typ L ist.
- 25 4. Nanographitstruktur/Metallnanopartikel-Verbund gemäß irgendeinem der vorangehenden Ansprüche, wobei das Nanographitstruktur-Erkennungspeptid ein Peptid ist, das aus einer Aminosäuresequenz besteht, die durch irgendeine von SEQ ID NOs: 1 bis 20 gezeigt ist.
- 30 5. Nanographitstruktur/Metallnanopartikel-Verbund gemäß irgendeinem der vorangehenden Ansprüche, wobei das Nanographitstruktur-Erkennungspeptid ein Peptid ist, das die gesamte oder einen Teil einer Aminosäuresequenz enthält, die durch irgendeine von SEQ ID NO: 1 bis 20 gezeigt sind, und in der Lage ist, an eine Nanographitstruktur zu binden.
- 35 6. Nanographitstruktur/Metallnanopartikel-Verbund gemäß Anspruch 4 oder 5, wobei die durch irgendeine von SEQ ID NO: 1 bis 20 gezeigte Aminosäuresequenz DYFSSPYEQLF (SEQ ID NO:1) ist.
- 40 7. Nanographitstruktur/Metallnanopartikel-Verbund gemäß Anspruch 4 oder 5, wobei die durch irgendeine von SEQ ID NO: 1 bis 20 gezeigte Aminosäuresequenz YDPFHII (SEQ ID NO: 2) ist.
- 45 8. Nanographitstruktur/Metallnanopartikel-Verbund gemäß irgendeinem der vorangehenden Ansprüche, wobei das Nanopartikel eines anorganischen Metallatoms oder einer anorganischen Metallverbindung ein Metallnanopartikel ist.
- 50 9. Nanographitstruktur/Metallnanopartikel-Verbund gemäß irgendeinem der Ansprüche 1 bis 7, wobei das Nanopartikel eines anorganischen Metallatoms oder einer anorganischen Metallverbindung ein Metallverbindung-Nanopartikel ist.
- 55 10. Nanographitstruktur/Metallnanopartikel-Verbund gemäß Anspruch 9, wobei das Metallverbindung-Nanopartikel ein Metalloxid-Nanopartikel ist.
11. Nanographitstruktur/Metallnanopartikel-Verbund gemäß Anspruch 9, wobei das Metallverbindung-Nanopartikel ein Nanopartikel aus magnetischem Material ist.
12. Nanographitstruktur/Metallnanopartikel-Verbund gemäß irgendeinem der vorangehenden Ansprüche, wobei das Metall Eisen, Beryllium, Gallium, Mangan, Phosphor, Uran, Blei, Cobalt, Nickel, Zink, Cadmium oder Chrom ist.
13. Nanographitstruktur/Metallnanopartikel-Verbund gemäß irgendeinem der Ansprüche 1 bis 7, wobei das Nanopartikel eines anorganischen Metallatoms oder einer anorganischen Metallverbindung ein Nanopartikel aus Eisenoxid, ein Nanopartikel aus Cadmiumselenid, ein Nanopartikel aus Zinkselenid, ein Nanopartikel aus Zinksulfid oder ein Nanopartikel aus Cadmiumsulfid ist.
14. Nanographitstruktur/Metallnanopartikel-Verbund gemäß irgendeinem der vorangehenden Ansprüche, wobei die Nanographitstruktur ein Kohlenstoff-Nanorohr oder ein Kohlenstoff-Nanotrichter (Kohlenstoff-nanohorn) ist.

15. Nanographitstruktur/Metallnanopartikel-Verbund gemäß Anspruch 14, wobei das Kohlenstoff-Nanorohr oder der Kohlenstoff-Nanotrichter aus einer Kohlenstoffstruktur besteht, welcher eine funktionelle Gruppe zugefügt ist.
- 5 16. Verfahren zum Zufügen und Halten eines Nanopartikels eines anorganischen Metallatoms oder einer anorganischen Metallverbindung in einem inneren Raum eines Ferritins, bei dem ein Nanographitstruktur-Erkennungspeptid mit einem N-Terminus oder einer Stelle einer Loop-Struktur des Ferritins fusioniert ist, und Tragen einer Vielzahl von Nanopartikeln eines anorganischen Metallatoms oder einer anorganischen Metallverbindung auf einer Nanographitstruktur, unter Verwendung der Affinität des Nanographitstruktur-Erkennungspeptids zu der Nanographitstruktur.
- 10 17. Verfahren zum Herstellen eines Verbunds einer Nanographitstruktur und eines Nanopartikels einer anorganischen Metallverbindung, die die folgenden Stufen umfasst: Zugeben und Halten eines Nanopartikels eines anorganischen Metallatoms oder einer anorganischen Metallverbindung in einem inneren Raum eines Ferritin, bei dem ein Nanographitstruktur-Erkennungspeptid an einen N-Terminus oder eine Stelle einer Loop-Struktur des Ferritins fusioniert ist, Tragen einer Vielzahl von Nanopartikeln eines anorganischen Metallatoms oder einer anorganischen Metallverbindung auf einer Nanographitstruktur unter Verwendung der Affinität des Nanographitstruktur-Erkennungspeptids zu der Nanographitstruktur und Entfernen eines Proteinrestes durch eine Hitzebehandlung.
- 15 18. Verfahren zur Herstellung eines Verbunds aus einer Nanographitstruktur und Nanopartikeln einer anorganischen Metallverbindung, das die folgenden Stufen umfasst: Zugeben und Halten eines Nanopartikels eines anorganischen Metallatoms oder einer anorganischen Metallverbindung in einem inneren Raum eines Ferritin, bei welchem ein Nanographitstruktur-Erkennungspeptid an einen N-Terminus oder eine Stelle einer Loop-Struktur des Ferritins fusioniert ist, Tragen einer Vielzahl von Nanopartikeln eines anorganischen Metallatoms oder einer anorganischen Metallverbindung auf einer Nanographitstruktur, unter Verwendung der Affinität des Nanographitstruktur-Erkennungspeptids zu der Nanographitstruktur, und Entfernen eines Proteinrestes durch eine Behandlung mit einem Elektronenstrahl.
- 20 25

Revendications

- 30 1. Composite à nanostructure de graphite/nanoparticules métalliques, dans lequel une nanoparticule d'un atome métallique inorganique ou d'un composé métallique inorganique est ajoutée et retenue dans un espace intérieur d'une ferritine dans lequel un peptide de reconnaissance d'une nanostructure de graphite est fusionné à un site N-terminal ou ayant une structure en boucle de la ferritine, et dans lequel une pluralité de nanoparticules d'un atome métallique inorganique ou d'un composé métallique inorganique sont supportées sur une nanostructure de graphite en utilisant l'affinité du peptide de reconnaissance de la nanostructure de graphite pour la nanostructure de graphite.
- 35 2. Composite à nanostructure de graphite/nanoparticules métalliques selon la revendication 1, dans lequel la ferritine est une ferritine dérivée d'un eucaryote supérieur.
- 40 3. Composite à nanostructure de graphite/nanoparticules métalliques selon la revendication 2, dans lequel la ferritine dérivée d'un eucaryote supérieur est une ferritine de type L dérivée de rate de cheval.
- 45 4. Composite à nanostructure de graphite/nanoparticules métalliques selon l'une quelconque des revendications précédentes, dans lequel le peptide de reconnaissance de la nanostructure de graphite est un peptide consistant en une séquence d'acides aminés montrée par l'une quelconque des SEQ ID NO: 1 à 20.
- 50 5. Composite à nanostructure de graphite/nanoparticules métalliques selon l'une quelconque des revendications précédentes, dans lequel le peptide de reconnaissance de la nanostructure de graphite est un peptide contenant l'intégralité ou une partie de la séquence d'acides aminés montrée par l'une quelconque des SEQ ID NO: 1 à 20 et capable de se lier à une nanostructure de graphite.
- 55 6. Composite à nanostructure de graphite/nanoparticules métalliques selon la revendication 4 ou 5, dans lequel la séquence d'acides aminés montrée par l'une quelconque des SEQ ID NO: 1 à 20 est DYFSSPYEQLF (SEQ ID NO: 1).
7. Composite à nanostructure de graphite/nanoparticules métalliques selon la revendication 4 ou 5, dans lequel la séquence d'acides aminés montrée par l'une quelconque des SEQ ID NO: 1 à 20 est YDPFHII (SEQ ID NO: 2).

8. Composite à nanostructure de graphite/nanoparticules métalliques selon l'une quelconque des revendications précédentes, dans lequel la nanoparticule d'un atome métallique inorganique ou d'un composé métallique inorganique est une nanoparticule métallique.
- 5 9. Composite à nanostructure de graphite/nanoparticules métalliques selon l'une quelconque des revendications 1 à 7, dans lequel la nanoparticule d'un atome métallique inorganique ou d'un composé métallique inorganique est une nanoparticule d'un composé métallique.
- 10 10. Composite à nanostructure de graphite/nanoparticules métalliques selon la revendication 9, dans lequel la nanoparticule d'un composé métallique est une nanoparticule d'un oxyde métallique.
11. Composite à nanostructure de graphite/nanoparticules métalliques selon la revendication 9, dans lequel la nanoparticule d'un composé métallique est une nanoparticule d'un matériau magnétique.
- 15 12. Composite à nanostructure de graphite/nanoparticules métalliques selon l'une quelconque des revendications précédentes, dans lequel le métal est le fer, le béryllium, le gallium, le manganèse, le phosphore, l'uranium, le plomb, le cobalt, le nickel, le zinc, le cadmium ou le chrome.
- 20 13. Composite à nanostructure de graphite/nanoparticules métalliques selon l'une quelconque des revendications 1 à 7, dans lequel la nanoparticule d'un atome métallique inorganique ou d'un composé métallique inorganique est une nanoparticule d'oxyde de fer, une nanoparticule de sélényde de cadmium, une nanoparticule de sélényde de zinc, une nanoparticule de sulfure de zinc, ou une nanoparticule de sulfure de cadmium.
- 25 14. Composite à nanostructure de graphite/nanoparticules métalliques selon l'une quelconque des revendications précédentes, dans lequel la nanostructure de graphite est un nanotube de carbone ou un nanocornet de carbone.
- 30 15. Composite à nanostructure de graphite/nanoparticules métalliques selon la revendication 14, dans lequel le nanotube de carbone ou le nanocornet de carbone est constitué d'une structure de carbone à laquelle est ajouté un groupe fonctionnel.
- 35 16. Procédé pour ajouter et retenir une nanoparticule d'un atome métallique inorganique ou d'un composé métallique inorganique dans un espace intérieur d'une ferritine dans lequel un peptide de reconnaissance d'une nanostructure de graphite est fusionné à un site N-terminal ou ayant une structure en boucle de la ferritine ; et supporter une pluralité de nanoparticules d'un atome métallique inorganique ou d'un composé métallique inorganique sur une nanostructure de graphite en utilisant l'affinité du peptide de reconnaissance de la nanostructure de graphite pour la nanostructure de graphite.
- 40 17. Procédé pour produire un composite d'une nanostructure de graphite et de nanoparticules d'un composé métallique inorganique, comprenant les étapes consistant à : ajouter et retenir une nanoparticule d'un atome métallique inorganique ou d'un composé métallique inorganique dans un espace intérieur d'une ferritine dans lequel un peptide de reconnaissance d'une nanostructure de graphite est fusionné à un site N-terminal ou ayant une structure en boucle de la ferritine ; supporter une pluralité de nanoparticules d'un atome métallique inorganique ou d'un composé métallique inorganique sur une nanostructure de graphite en utilisant l'affinité du peptide de reconnaissance de la nanostructure de graphite pour la nanostructure de graphite ; et éliminer un fragment protéique au moyen d'un traitement par la chaleur.
- 45 18. Procédé pour produire un composite d'une nanostructure de graphite et de nanoparticules d'un composé métallique inorganique, comprenant les étapes consistant à : ajouter et retenir une nanoparticule d'un atome métallique inorganique ou d'un composé métallique inorganique dans un espace intérieur d'une ferritine dans lequel un peptide de reconnaissance d'une nanostructure de graphite est fusionné à un site N-terminal ou ayant une structure en boucle de la ferritine ; supporter une pluralité de nanoparticules d'un atome métallique inorganique ou d'un composé métallique inorganique sur une nanostructure de graphite en utilisant l'affinité du peptide de reconnaissance de la nanostructure de graphite pour la nanostructure de graphite ; et éliminer un fragment protéique au moyen d'un traitement par un faisceau d'électrons.
- 50 55

Fig. 1

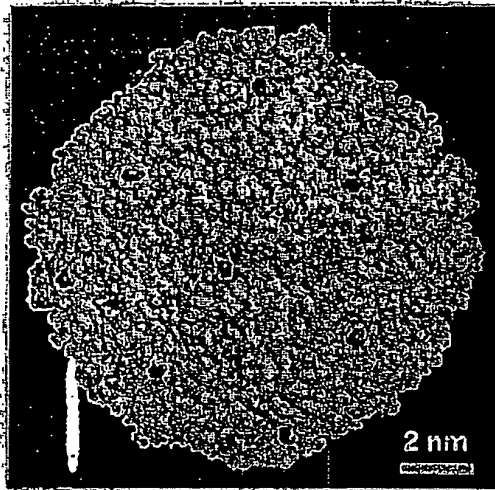


Fig. 2

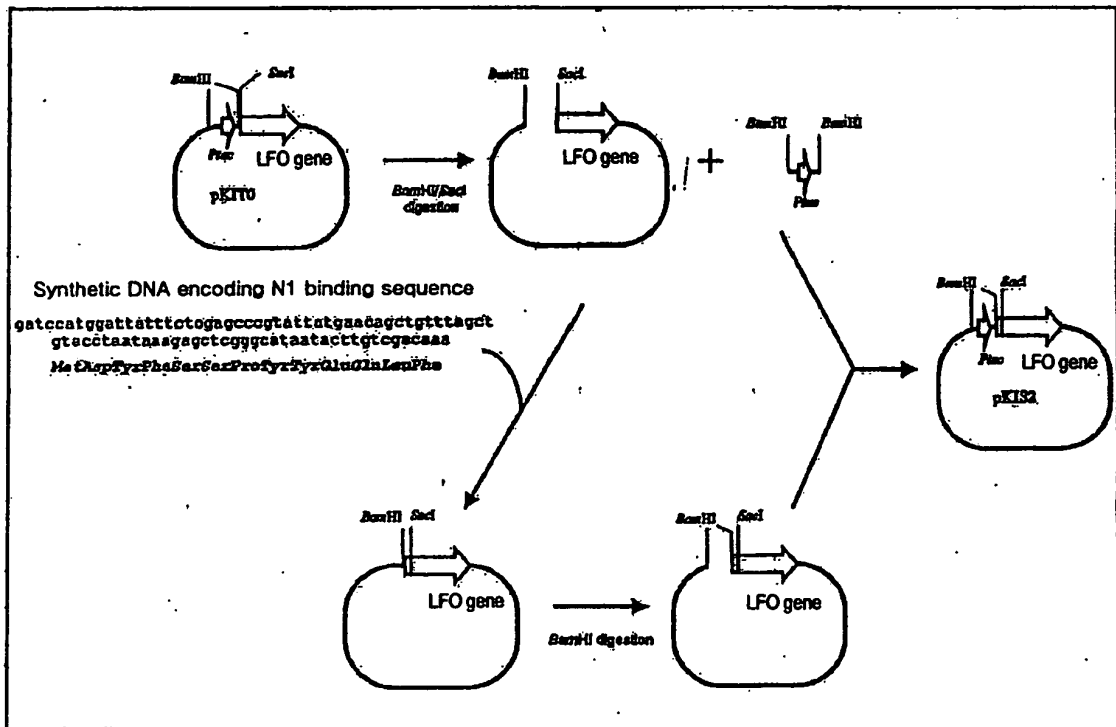


Fig. 3

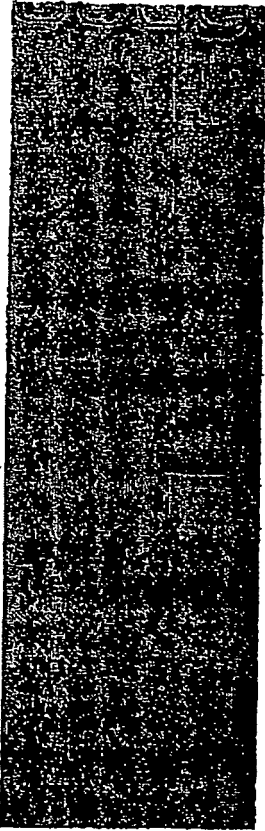
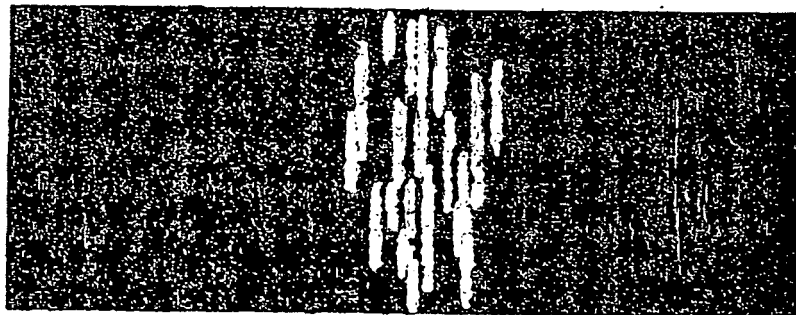


Fig. 4



control

LFO

N1-LF

Fig. 5

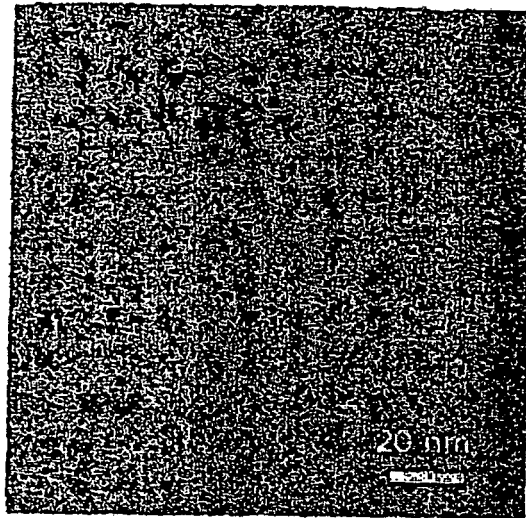


Fig. 6

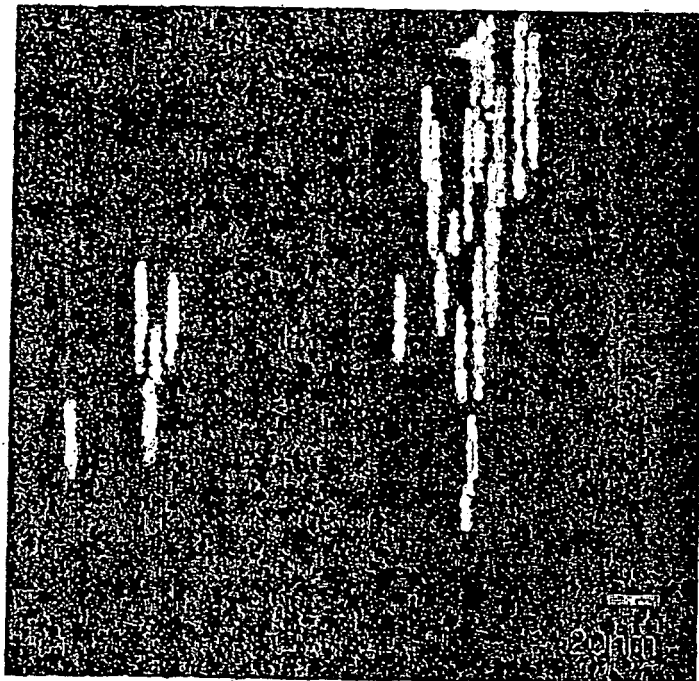


Fig. 7

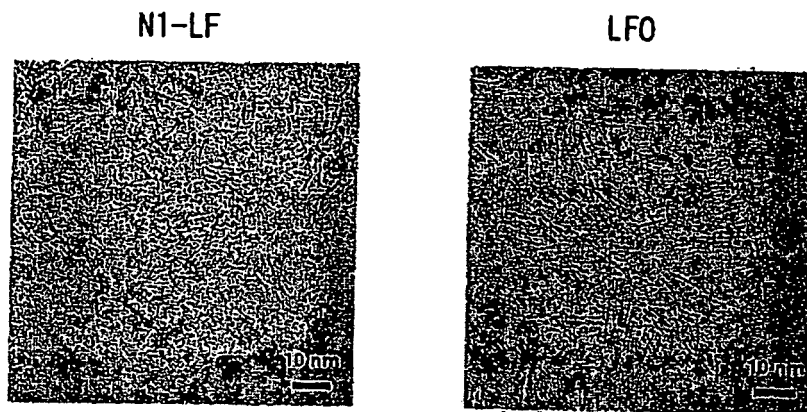
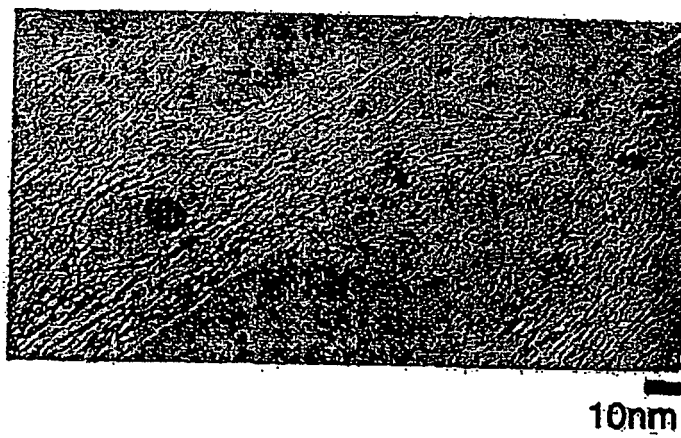


Fig. 8



REFERENCES CITED IN THE DESCRIPTION

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