NANOPARTICLES FOR PENETRATION OF BLOOD-BRAIN BARRIER

Fig. 3a

Abstract: The present invention relates to (a) a nanoparticle for penetration of a blood-brain barrier (BBB) comprising an organic nanoparticle core and a water-soluble multi-functional organic ligand coated on the nanoparticle core, and (b) a composition for brain imaging and (c) a pharmaceutical composition for brain-targeting thereof. The basic strategy adopted in the present invention is based on the finding that the water-soluble multi-functional organic ligand coated on the nanoparticle core permits the nanoparticle to penetrate the blood-brain barrier. The nanoparticle of the present invention may be efficiently used as a brain-imaging agent and in a drug delivery system into brain since the inorganic nanoparticle core coated with the water-soluble multi-functional organic ligand ensures an effective penetration of the blood-brain barrier even with no use of conventional adjuvants.
NANOPARTICLES FOR PENETRATION OF BLOOD-BRAIN BARRIER

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention relates to a nanoparticle for penetration of a blood-brain barrier, and a brain imaging agent and a brain-targeting pharmaceutical composition having the same.

DESCRIPTION OF THE RELATED ART

Nanotechnology is an emerging science that refers to the study of controlling the matter on an atomic and molecular scale, and generally it may be fused with various technologies in other scientific fields. Since nanotechnology is useful in development of advanced materials and devices, it has been extensively applied to electronics, material engineering, communication, machine, medicine, agriculture, energy, environment, and so on. In particular, nanoparticles are considered to be a crucial material because they have unique characteristics not found in micron-sized particles in various applications and exhibits a marked improvement of nano-characteristics compared to micron-sized materials.

In recent nanotechnology, the following fields have been being extensively researched: (a) synthesis of new nanoparticles and their nano-phenomena elucidation, (b) design and preparation of next generation nanodevices by nanoparticle self-assembly and integration processes, and (c) nano-medicine technologies in which nanotechnology is grafted into biology/medicine/biotechnology.

One of the most addressed researches in the nano-medicine is to provide both drug delivery systems for permitting selective and effective treatment of diseases and diagnostic agents for early detection of diseases. Thus, several types of nanoparticles such as an inorganic nanoparticle, a dendrimer, a hydrogel, a liposome, a micelle, a nanotube, a polymer nanoparticle, and a lipid nanoparticle have been
proposed during the last ten years, and some of them have been tested to have effects of interest in a clinical test and are now used in clinics (Kingsley J. D. etal. J. Neuroimmune Pharmacol. 2006, 1, 340).

Regardless of persistent researches, remarkable research products for diagnosis and treatment of diseases for brain have not been provided yet due to the presence of 'blood-brain barrier (BBB)'. This structure in the central nervous system where vessels are very closely surrounded by glia cells plays a role in transport of essential substances and restriction of toxic substances penetration from the brain for survival of neuronal cells. This blood-brain barrier blocks the penetration of not only various imaging agents for diagnosis but also drugs for brain disorders such as tumors, Alzheimer's disease and Parkinson's disease, which is a huge barrier to the development of diagnosis and treatment techniques for the brain.

Therefore, it is important to develop novel technologies by which imaging agents and drugs are allowed to penetrate into the blood-brain barrier. The current useful methods includes: a) temporal disruption of the blood-brain barrier by a chemical shock using adjuvants such as OX26 (antibody), OX26-polyethylene glycol, mannitol, and transferrin, and b) co-administration method in which the above-described medicines are chemically attached to drugs or imaging agents (M. Gumbleton etal. Journal of Drug Targeting 2006, 14, 191).

Likewise, previously suggested approaches for penetrating the blood-brain barrier by use of supplementary adjuvants or drug-conjugated particles are as follows:

US Pat. No. 6,117,454 discloses a novel method across the blood-brain barrier using nanoparticles coated with a pharmacological active polymer such as polyoxyethylene which functions to induce opening of the blood-brain barrier. US Pat. No. 6,821,594 discloses a method for diagnosing Alzheimer's disease using a conjugated material of mannitol and Gd, Mn, iron oxide as a drug for opening the blood-brain barrier and imaging agent, respectively.
US Pat. Appln. No. 2004-0131692 discloses nanoparticles for penetration of the blood-brain barrier, comprising a protein attached to a micelle in which apolipoprotein E as a drug for opening of the blood-brain barrier is composed. EP Pat. No. 1,071,408 discloses that the use of microparticles coated with polyethylene glycol complex is very useful in the penetration of drugs through the blood-brain barrier.

However, the utilization of supplementary adjuvants for crossing the blood-brain barrier has been not recommended because of: a) difficulty in continuous delivery due to recovery of the disrupted blood-brain barrier with the lapse of time, and b) likelihood of shock induction caused from disruption of the blood-brain barrier by drugs.

In this context, a few results on the nanoparticle which penetrates the blood-brain barrier with no help of BBB-crossing drugs have been recently reported.

*Angew Chem Int Ed, 2007, 46, 5397* and *Toxicological Science, 2006, 89, 338* disclose nanoparticles for crossing the blood-brain barrier without help of supplementary adjuvant. However, the nanoparticle is restricted to a manganese oxide nanoparticle encapsulated into a polyethylene glycol-phospholipid and a cobalt ferrite nanoparticle coated with silica, respectively.

Accordingly, it becomes significantly highlighted in the art to develop novel nanoparticles for penetration of the blood-brain barrier in much more efficient manner for treatment and diagnosis of brain diseases.

Throughout this application, various publications and patents are referred and citations are provided in parentheses. The disclosures of these publications and patents in their entities are hereby incorporated by references into this application in order to fully describe this invention and the state of the art to which this invention pertains.
SUMMARY OF THE INVENTION

The present inventors have made intensive studies to develop a novel nanoparticle to penetrate the blood-brain barrier and target the brain. As results, we have discovered that nanoparticles having an inorganic nanoparticle core of which the surface is coated with a water-soluble multi-functional organic ligand show excellent performance in penetration of the blood-brain barrier for brain targeting and the water-soluble multi-functional organic ligand moiety is mainly responsible for their BBB penetration abilities.

Accordingly, it is an object of this invention to provide a nanoparticle for penetration of a blood-brain barrier.

It is another object of this invention to provide a composition for brain imaging, comprising the nanoparticle for penetration of the blood-brain barrier.

It is still another object of this invention to provide a pharmaceutical composition for brain-targeting, comprising the nanoparticle for penetration of the blood-brain barrier.

Other objects and advantages of the present invention will become apparent from the following detailed description together with the appended claims and drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 represents a transmission electron microscopy (TEM) of several kinds of a blood-brain barrier-penetrating nanoparticle. The inorganic nanoparticle core is (A) Fe₃O₄, (B) MnFe₂O₄, (C) (Zn₀₄Fe₀₆)Fe₂O₄, (D) FePt-Au, and (E and F) Gd₂O₃.

Fig. 2 represents photographs of the blood-brain barrier-penetrating nanoparticle coated with several kinds of a water-soluble multi-functional organic ligand. The inorganic nanoparticle core is (A) Fe₃O₄, (B) MnFe₂O₄, (C) (Zn₀₄Fe₀₆)Fe₂O₄, (D) FePt-Au, and (E) Gd₂O₃. Each nanoparticle was easily dispersed...
in aqueous solution and coated with TMAOH, BSA, carbodextran and PAA-PEG (polyacrylic acid-polyethylene glycol) ligand.

Fig. 3a is a graph of T2 magnetic resonance imaging (MRI) signal in brain and liver of a rat with the changes of time at post-injection of the blood-brain barrier-penetrating nanoparticle coated with BSA, of which the inorganic nanoparticle core is MnFe$_2$O$_4$.

Fig. 3b represents MR image at the predetermined time points injected into a rat with the blood-brain barrier-penetrating nanoparticle coated with BSA, of which the inorganic nanoparticle core is MnFe$_2$O$_4$. The brain portion of rat is shown in color scale.

Fig. 4 is fluorescence microscope images representing long-term distribution of the blood-brain barrier-penetrating nanoparticle coated with BSA, of which the inorganic nanoparticle core is MnFe$_2$O$_4$, depending on the time.

Fig. 5 is fluorescence microscope images representing long-term distribution of the blood-brain barrier-penetrating nanoparticle coated with BSA, of which the inorganic nanoparticle core is Fe$_3$O$_4$, depending on the time.

Fig. 6a is a graph of T2 MRI signal in brain and liver of a rat with the changes of time at post-injection with the blood-brain barrier-penetrating nanoparticle coated with BSA, of which the inorganic nanoparticle core is (Zn$_{0.4}$Fe$_{0.6}$)Fe$_2$O$_4$.

Fig. 6b represents MR images at the predetermined time points injected into a rat with the blood-brain barrier-penetrating nanoparticle coated with BSA, of which the inorganic nanoparticle core is (Zn$_{0.4}$Fe$_{0.6}$)Fe$_2$O$_4$. The brain portion of rat is shown in color scale.

Fig. 7a is a graph of T2 MRI signal in brain of a rat with the changes of time at post-injection with the blood-brain barrier-penetrating nanoparticle coated with the water-soluble multi-functional organic ligand, carbodextran, of which the inorganic nanoparticle core is (Zn$_{0.4}$Fe$_{0.6}$)Fe$_2$O$_4$.

Fig. 7b represents MR images at the predetermined time points injected into a
rat with the blood-brain barrier-penetrating nanoparticle coated with the watersoluble multi-functional organic ligand, carbodextran, of which the inorganic nanoparticle core is \( (\text{Zn}_{0.4}\text{Fe}_{0.6})\text{Fe}_2\text{O}_4 \).

Fig. 8a is a graph of T2 MRI signal in the brain of a rat with the changes of time at post-injection with the blood-brain barrier-penetrating nanoparticle coated with SiO\(_2\) instead of the water-soluble multi-functional organic ligand, of which the inorganic nanoparticle core is \( (\text{Zn}_{0.4}\text{Fe}_{0.6})\text{Fe}_2\text{O}_4 \).

Fig. 8b represents MR images at the predetermined time points injected into a rat with the blood-brain barrier-penetrating nanoparticle coated with SiO\(_2\) instead of the water-soluble multi-functional organic ligand, of which the inorganic nanoparticle core is \( (\text{Zn}_{0.4}\text{Fe}_{0.6})\text{Fe}_2\text{O}_4 \). The brain portion of rat is shown in color scale.

**DETAILED DESCRIPTION OF THIS INVENTION**

In one aspect of this invention, there is provided a nanoparticle for penetration of a blood-brain barrier, comprising: (a) an inorganic nanoparticle core; and (b) a water-soluble multi-functional organic ligand coated on the nanoparticle core.

The present inventors have made intensive studies to develop a novel nanoparticle to penetrate the blood-brain barrier and target the brain without help of an additional adjuvant. As results, we have discovered that nanoparticles having an inorganic nanoparticle core of which the surface is coated with a water-soluble multi-functional organic ligand show excellent performance in penetration of the blood-brain barrier for brain targeting and the water-soluble multi-functional organic ligand moiety is mainly responsible for their BBB penetration abilities.

The nanoparticle for penetration the blood-brain barrier of the present invention may be very useful in brain imaging and drug delivery into brain.

The nanoparticle for penetration the blood-brain barrier of the present
invention is classified into two parts: (a) the inorganic nanoparticle core; and (b) the

The water-soluble multi-functional organic ligand coated on the nanoparticle core in the nanoparticle for penetration the blood-brain barrier of the present invention permits the nanoparticle to effectively penetrate the blood-brain barrier.

The inorganic nanoparticle core located at the most interior position in the nanoparticle of the present invention may include a variety of inorganic substances.

According to a preferable embodiment, the inorganic nanoparticle core includes: (i) a magnetic nanoparticle; (ii) an optical nanoparticle; (iii) a metal, an alloy, a metal chalcogen (Group 16 elements) or a metal pnicogen (Group 15 elements) nanoparticle; or a multi-component hybrid structure thereof.

The composition of substances described below refers to a stoichiometric ratio.

Preferably, the magnetic nanoparticle includes (i) an metal nanoparticle containing one or more elements selected from transition metal elements, metal and metalloid elements of Groups 13-16 elements, Lanthanide metal elements and Actinide metal elements; (ii) an alloy nanoparticle containing one or more elements selected from transition metal elements, metal and metalloid elements of Groups 13-16 elements, Lanthanide metal elements and Actinide metal elements; (iii) a oxide or metal ferrite nanoparticle containing one or more elements selected from transition metal elements, metal and metalloid elements of Groups 13-16 elements, Lanthanide metal elements and Actinide metal elements; or (iv) the multi-component hybrid structure thereof.

More preferably, the magnetic nanoparticle includes: Ba, Cr, Mn, Fe, Co, Zn, Nb, Mo, Zr, Te, W, Pd, Gd, Tb, Dy, Ho, Er, Sm, Nd, $M^a_xM^b_y$ or $M^a_xM^b_yM^c_z$ ($M^a$ = one or more elements selected from the group consisting of Co, Fe, Mn, Ni, Mo, Si, Al, Cu, Pt, Sm, B, Bi, Cu, Sn, Sb, Ga, Ge, Pd, In, Au, Ag or Y, or Lu; $M^b$ or $M^c$ = one or more elements selected transition metal elements, metal and metalloid elements of Groups 13-16 elements, Lanthanide metal elements and Actinide metal elements;
0<x<20, 0<y<20, 0<z<20), M_d x O_y or M_d x M_e y O_z (M_d = one or more elements selected from transition metal elements selected from the group consisting of Ba, Cr, Mn, Fe, Co, Ni, Cu, Zn, Nb, Mo, Zr, W, Pd, Ag, Pt and Au, and Lanthanide metal elements and Actinide metal elements selected from the group consisting of Gd, Tb, Dy, Ho, Er, Sm and Nd; M_e = one or more elements selected from transition metal elements, metal and metalloid elements of Groups 13-16 elements, Lanthanide metal elements and Actinide metal elements; 0<x<16, 0<y<8, 0<z<8) or the metal ferrite nanoparticle; or the multi-component hybrid structure thereof.

Preferably, the optical nanoparticle includes: (i) a fluorescence emission nanoparticle; (ii) a nanoparticle representing surface plasmon resonance (SPR); (iii) a nanoparticle emitting Raman signal; or (iv) the multi-component hybrid structure thereof.

More preferably, the optical nanoparticle includes (i) a Group II/VI semiconductor nanoparticle; (ii) a Group III/V semiconductor nanoparticle; (iii) Au, Ag, Cu, Pt, Pd or Ni nanoparticle; (iv) the nanoparticle that a Raman dye is attached to the nanoparticle of the step (iii); (v) the nanoparticle containing a fluorescent dye in an inorganic matrix; or (vi) the multi-component hybrid structure thereof.

Preferably, the metal nanoparticle includes one or more elements selected from Group 1 metal elements, Group 2 metal elements, transition metal elements, metal and metalloid elements of Groups 13-16 elements, Lanthanide metal elements and Actinide metal elements, or the multi-component hybrid structure thereof, and more preferably Ba, Cr, Co, Mn, Fe, Ni, Cu, Zn, Nb, Pd, Ag, Pt, Au, Tb, Gd, Dy, Ho, Er, Sm, Nd or the multi-component hybrid structure thereof, and much more preferably transition metal elements selected from the group consisting of Co, Mn, Fe and Ni; Lanthanide metal elements and Actinide metal elements selected from the group consisting of Nd, Gd, Tb, Dy, Ho, Er and Sm; or the multi-component hybrid structure thereof.

Preferably, the alloy nanoparticle includes a M_f x M_9 y or M_f x M_9 y M_h z nanoparticle...
(M₁, M² and Mʰ each represents one or more elements selected from the group consisting of Group 1 metal elements, Group 2 metal elements, transition metal elements, metal and metalloid elements of Groups 13-16 elements, Lanthanide metal elements and Actinide metal elements; 0<x<20, 0<y<20, 0<z<20) or the multi-component hybrid structure thereof.

More preferably, the alloy nanoparticle includes the MᵢMᵢᵢ or MᵢMᵢᵢMᵢʰ nanoparticles (Mᵢ, Mᵢᵢ and Mᵢʰ each represents one or more elements selected from the group consisting of: Group 1 metal elements; Group 2 metal elements; transition metal elements selected from the group consisting of Ba, Cr, Mn, Fe, Co, Ni, Cu, Zn, Nb, Mo, Zr, Te, W, Pd, Ag, Pt and Au; metal and metalloid elements of Groups 13-16 elements; and Lanthanide metal elements and Actinide metal elements selected from the group consisting of Gd, Tb, Dy, Ho, Er, Sm and Nd; 0<x<20, 0<y<20, 0<z≤20) or the multi-component hybrid structure thereof.

Most preferably, the alloy nanoparticle includes MᵢᵢMᵢᵢ or MᵢᵢMᵢᵢMᵢʰ nanoparticles (Mᵢᵢ = one or more elements selected from the group consisting of transition metal elements selected from the group consisting of Ba, Cr, Mn, Fe, Co, Ni, Cu, Zn, Nb, Mo, Zr, W, Pd, Ag, Pt and Au, and Lanthanide metal elements and Actinide metal elements selected from the group consisting of Gd, Tb, Dy, Ho, Er, Sm and Nd; Mᵢᵢ or Mᵢʰ each represents one or more elements selected from the group consisting of Group 1 metal elements (Li or Na), Group 2 metal elements (Be, Ca, Mg, Sr, Ba or Ra), Group 13 elements (B, Al, Ga or In), Group 14 elements (Si or Ge), Group 15 elements (As, Sb or Bi), Group 16 elements (S, Se or Te), transition metal elements (Sr, Ti, V, Cu, Y, Zr, Nb, Mo, Tc, Ru, Rh, Pd, Ag, Cd, Hf, Ta, W, Re, Os, Ir, Pt, Au or Hg), Lanthanide metal elements and Actinide metal elements (La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm or Yb); 0<x<20, 0<y<20, 0<z<20] or the multi-component hybrid structure thereof.

Preferably, the metal chalcogen nanoparticle includes a MᵢᵢAy nanoparticle (Mᵢᵢ represents the element selected from Group 1 metal elements, Group 2 metal
elements, transition metal elements, metal and metalloid elements of Groups 13-15 elements, Lanthanide metal elements and Actinide metal elements; A is selected from O, S, Se, Te and Po; 0<x<32, 0<y<8), a $M_x^1 M_y A_z^1$ nanoparticle ($M^1$ and $M^0$ each represents one or more elements selected from Group 1 metal elements, Group 2 metal elements, transition metal elements, metal and metalloid elements of Groups 13-15 elements, Lanthanide metal elements and Actinide metal elements; A is selected from O, S, Se, Te and Po; 0≤x<32, 0<y≤32, 0<z<8), or the multi-component hybrid structure thereof.

More preferably, the metal chalcogen nanoparticle includes $M_x^1 A_y^1$ nanoparticle (with $M^1$ one or more elements selected from transition metal elements selected from the group consisting of Ba, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg, Nb, Mo, Zr, W, Pd, Ag, Pt and Au, or Groups 13-15 elements selected from the group consisting of Ga, In, Sn, Pb and Bi, or Lanthanide metal elements and Actinide metal elements selected from the group consisting of Gd, Tb, Dy, Ho, Er, Sm and Nd; A is selected from O, S, Se, Te and Po; 0<x<32, 0<y<8), or $M_x^1 M_y A_z^1$ nanoparticle ($M^1$ one or more elements selected from the group consisting of Group 1 metal elements, Group 2 metal elements, transition metal elements, metal and metalloid elements of Groups 13-15 elements, Lanthanide metal elements and Actinide metal elements; $M^0$ = one or more elements selected from transition metal elements selected from the group consisting of Ba, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg, Nb, Mo, Zr, W, Pd, Ag, Pt and Au, or Groups 13-15 elements selected from the group consisting of Ga, In, Sn, Pb and Bi, or Lanthanide metal elements or Actinide metal elements selected from the group consisting of Gd, Tb, Dy, Ho, Er, Sm and Nd; A is selected from O, S, Se, Te and Po; 0<x<16, 0<y<16, 0<z<8), or the multi-component hybrid structure thereof.

Most preferably, the metal chalcogen nanoparticle includes $M_x^1 M_y O_z$ nanoparticle ($M^1$ = one or more elements selected from the group consisting of Group 1 metal elements (Li or Na), Group 2 metal elements (Be, Ca, Mg, Sr, Ba or Ra), Group 13 elements (Ga or In), Group 14 elements (Si or Ge), Group 15
elements (As, Sb or Bi), Group 16 elements (S, Se or Te), transition metal elements (Sr, Ti, V, Cu, Y, Zr, Nb, Mo, Tc, Ru, Rh, Pd, Ag, Cd, Hf, Ta, W, Re, Os, Ir, Pt, Au or Hg), Lanthanide metal elements and Actinide metal elements (La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm or Yb); 0<x<20, 0<y<20, 0<z<20; M = one or more elements selected from transition metal elements selected from the group consisting of Ba, Cr, Mn, Fe, Co, Ni, Cu, Zn, Nb, Pd, Ag, Pt and Au, and Lanthanide metal elements and Actinide metal elements selected from the group consisting of Gd, Tb, Dy, Ho, Er, Sm and Nd; A is selected from O, S, Se, Te and Po; 0<z<8, 0<y<16, 0<z<8], or the multi-component hybrid structure thereof.

Preferably, the metal pnicogen nanoparticle includes a $M^k_A^{a+y}$ nanoparticle ($M^k$ represents the element selected from Group 1 metal elements, Group 2 metal elements, transition metal elements, metal and metalloid elements of Groups 13-14 elements, Lanthanide metal elements and Actinide metal elements; $A^a$ is selected from N, P, As, Sb or Bi; 0<x<32, 0<y<8), a $M^k_A^{a+y}$ nanoparticle ($M^k$ and $M^i$ each represents one or more elements selected from Group 1 metal elements, Group 2 metal elements, transition metal elements, metal and metalloid elements of Groups 13-14 elements, Lanthanide metal elements and Actinide metal elements; $A^a$ is selected from N, P, As, Sb and Bi; 0<x<32, 0<y<32, 0<z<8), or the multi-component hybrid structure thereof.

More preferably, the metal pnicogen nanoparticle includes $M^k_A^{a+y}$ nanoparticle ($M^k$ represents one or more elements selected from transition metal elements selected from the group consisting of Ba, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg, Nb, Mo, Zr, Te, W, Pd, Ag, Pt and Au, Groups 13-14 elements selected from the group consisting of Ga, In, Sn and Pb, or Lanthanide metal elements and Actinide metal elements selected from the group consisting of Gd, Tb, Dy, Ho, Er, Sm and Nd; $A^a$ is selected from N, P, As, Sb or Bi; 0<x<32, 0<y<8), or $M^k_A^{a+y}$ nanoparticle ($M^k$ = one or more elements selected from Group 1 metal elements, Group 2 metal elements, transition metal elements, metal and metalloid elements of Groups 13-14
elements, Lanthanide metal elements and Actinide metal elements; \( M^1 \) = one or more elements selected from transition metal elements selected from the group consisting of Ba, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg, Nb, Mo, Zr, Te, W, Pd, Ag, Pt and Au, Groups 13-14 elements selected from the group consisting of Ga, In, Sn and Pb, or Lanthanide metal elements and Actinide metal elements selected from the group consisting of Gd, Tb, Dy, Ho, Er, Sm and Nd; \( 0<x<20, \ 0<y<20 \); or (v) the metal group consisting of: (i) the magnetic nanoparticle \([M \ (M = Mn, Fe, Co, Ni, Gd, Tb, Dy, Ho, Er, Sm or Nd); M^a M^b \ (M^a and M^b each represents Mn, Fe, Co, Ni, Pt, Gd, Tb, Dy, Ho, Er, Sm or Nd); M^x Fe_y O_z \ (M^m = Ba, Mn, Fe, Co, Ni or Zn; 0<x<16, \ 0<y<16, \ 0<z<8); Zn_w M^b Fe_y O_z \ (M^p represents one or more elements selected from the group consisting of Group 1 metal elements, Group 2 metal elements, Group 13 elements, transition metal elements, Lanthanide metal elements and Actinide metal elements; 0<w<16, \ 0<x<16, \ 0<y<16, \ 0<z<8)] or the metal ferrite nanoparticle; (ii) the optical nanoparticle (the Group II/VI semiconductor nanoparticle selected from the group consisting of ZnS, ZnSe, ZnTe, CdS, CdSe, CdTe, HgS, HgSe and HgTe; the Group III/V semiconductor nanoparticle selected from the group consisting of GaN, GaP, GaAs, InN, InP and InAs; or the metal nanoparticle with optical property selected from the group consisting of Au, Ag, Cu, Pt and Ni); or (iii) the metal nanoparticle, \( M \ (M = Ba, Cr, Mn, Fe, Co, Zn, Nb, Mo, Zr, Te, W, Pd, Gd, Tb, Dy, Ho, Er, Sm or Nd); (iv) the alloy nanoparticle, \( M^x M^y \ (M^x and M^y each represents one or more elements selected from the group consisting of: Group 1 metal elements; Group 2 metal elements; transition metal elements selected from the group consisting of Ba, Cr, Mn, Fe, Co, Ni, Cu, Zn, Nb, Mo, Zr, Te, W, Pd, Ag, Pt and Au; Group 13 elements; Group 14 elements; Group 15 elements; Group 16 elements; Lanthanide metal elements and Actinide metal elements selected from the group consisting of Gd, Tb, Dy, Ho, Er, Sm and Nd; 0<x<20, \ 0<y<20); or (v) the metal
oxide nanoparticle, $M_{x}O_{y}$, in the metal chalcogen nanoparticle ($M^1 = $ one or more elements selected from Ba, Cr, Co, Fe, Mn, Ni, Cu, Zn, Nb, Pd, Ag, Au, Mo, Si, Al, Pt, Sm, B, Bi, Sn, Sb, Ga, Ge, Pd, In, Gd, Tb, Dy, Ho, Er, Sm and Nd; $0 < x < 16, 0 < y \leq 8$); or (vi) the multi-component hybrid structure thereof.

Most preferably, the inorganic nanoparticle core includes $M_{x}Fe_{y}O_{z}$ ($M^m = $ Ba, Mn, Fe, Co, Ni or Zn; $0 < x < 16, 0 < y < 16, 0 < z < 8$) or $Zn_{w}M_{x}Fe_{y}O_{z}$ ($0 < w \leq 16, 0 < x < 16, 0 < y < 16, 0 < z < 8$; $M^p$ represents one or more elements selected from the group consisting of Group 1 metal elements, Group 2 metal elements, Group 13 elements, transition metal elements, Lanthanide metal elements and Actinide metal elements).

The multi-component hybrid structure includes two or more nanoparticles selected from the group consisting of metal, alloy, metal chalcogen or metal pnictogen nanoparticles described above, or one or more nanoparticles including both (i) the nanoparticle selected from the group consisting of metal, alloy, metal chalcogen or metal pnictogen nanoparticles described above and (ii) the nanoparticle selected from the group consisting of other metals (e.g., Au, Pt, Pd, Ag, Rh, Ru, Os or Ir), metal chalcogen and metal pnictogen. The multi-component hybrid structure has a core-shell, a multi-core shell, a heterodimer, a trimer, a multimer, a barcode or a co-axial rod structure.

The inorganic nanoparticle core indicated by metal, metal oxide, alloy, semiconductor and the multi-component hybrid structure thereof, can penetrate the blood-brain barrier by coating with the water-soluble multi-functional organic ligand to be explained below.

In addition, the nanoparticle of the present invention can be applied to administration of human body since the water-soluble multi-functional organic ligand improves water-solubility of the nanoparticle.

The water-soluble multi-functional organic ligand may be any one used ordinarily in the art.

According to a preferable embodiment, the water-soluble multi-functional
organic ligand includes (i) an attachment region (Li) to be linked to the inorganic nanoparticle core, and more preferably, includes \( L_1 \) as well as (ii) an active ingredient-binding region (Ln) for bonding of active ingredients, or (iii) a cross-linking region (Lm) for cross-linking between the water-soluble multi-functional organic ligands, or (iv) a region containing both the active ingredient-binding region (Ln) and the cross-linking region (Lm).

The term "attachment region (L)" refers to a portion of the water-soluble multi-functional organic ligand including a functional group capable of binding to the nanoparticle core, and preferably a terminal functional group. Accordingly, it is preferable that the attachment region includes the functional group having high affinity with the nanoparticle core. According to a preferable embodiment, the nanoparticle core can be attached to the attachment region by an ionic bond, a covalent bond, a hydrogen bond or a metal-ligand coordination bond.

The term "coating" refers to a surface treatment of the inorganic nanoparticle whereby the water-soluble multi-functional organic ligand is bound to the inorganic nanoparticle core through the chemical bonds, and preferably ionic bond, covalent bond, hydrogen bond, or metal-ligand coordination bond. The meaning of coating is different to that of the terms "encapsulating" or "entrapment" used ordinarily in the art. For example, the term "encapsulating" means that a particle is incorporated into a structure foremed by a substance (e.g., amphiphilic substance) which forms a micelle and contains any particle within its interior part, suggesting that it is different from "coating of the nanoparticle core" adopted in the present invention.

The attachment region may be varied depending on the substances constituting the nanoparticle. For example, the attachment region (Li) using ionic bond, covalent bond, hydrogen bond, or metal-ligand coordination bond may include 

\[-\text{CHO, -COOH, -NH}_2, -\text{SH, -CONH}_2, -\text{PO}_3\text{H}, -\text{OPO}_3\text{H}_2, -\text{SO}_3\text{H}, -\text{OSO}_3\text{H}, -\text{N}_3, -\text{NR}_3\text{OH} \quad (\text{R}=\text{C}_n\text{H}_{2n+1}, 0<n<16), -\text{OH, -SS-, -NO}_2, -\text{COX} (\text{X} = \text{F, Cl, Br or I}), -\text{COOCO-}, -\text{CONH-}, \text{or -CN.} \]

For nanoparticles to which the substance having the hydrophobic group as a
stabilizer is already bound, the coating with water-soluble multi-functional ligand may be accomplished through the attachment region (L₁) including a hydrocarbon chain having two or more carbon atoms.

The term "active ingredient-binding region (Ln)" means a portion of water-soluble multi-functional organic ligand containing the functional group capable of binding to chemical or biological substances, and preferably the other terminal group located at the opposite side from the attachment region. The functional group of the active ingredient-binding region may be varied depending on kinds of active ingredient and their formulae (Table 1). The active ingredient-binding region in this invention includes -SH, -COOH, -CHO, -NH₂, -OH, -PO₃H, -OPO₃H₂, -SO₃H, -OSO₃H, -NR₃⁺X⁺ (R = CₙH₂ₙ₊½, 0≤n≤18, 0<m<34, X = OH, Cl or Br), NR₄⁺X⁺ (R = CₙH₂ₙ₊½, 0<n<16, 0<m≤34, X = OH, Cl or Br), -N₃, -SCOCH₃, -SCN, -NCS, -NCO, -CN, -F, -Cl, -Br, -I, an epoxy group, -ONO₂, -PO(OH)₂, -C=NNH₂, -HC≡CH- and -C≡C-, but not limited to.

| TABLE 1. Examples of binding between the active ingredient-binding region of multi-functional organic ligand and the active ingredient |
|-------|-------|-------|
| R-NH₂ | R'-COOH | R-NHCO-R' |
| R-SH | R'-SH | R-SS-R' |
| R-OH | R'-{(Epoxyde group)} | R-OCH₂CH(OH)-R' |
| R-NH₂ | R'-{(Epoxyde group)} | R-NHCH₂CH(OH)-R' |
| R-SH | R'-{(Epoxyde group)} | R-SCH₂CH(OH)-R' |
| R-NH₂ | R'-COH | R-N=CH-R' |
| R-NH₂ | R'-NCO | R-NHCONH-R' |
| R-NH₂ | R'-NCS | R-NHCSNH-R' |
| R-SH | R'-COCH₃ | R-COCH₃S-R' |
| R-SH | R'-O(C=O)X | R-S(C=O)O-R' |
| R-(Aziridine group) | R'-SH | R-CH₂CH(NH₂)CH₂S-R' |
| R-CH=CH₂ | R'-SH | R-CH₂CH₂S-R' |
The term "cross-linking region (Lw)" refers to a portion of the multi-functional organic ligand including the functional group capable of cross-linking to an adjacent water-soluble multi-functional organic ligand, and preferably a side chain. The term "cross-linking" means that the multi-functional organic ligand is bound to another multi-functional organic ligand by intermolecular interaction. The intermolecular interaction includes, but not limited to, hydrogen bond, covalent bond (e.g., disulfide bond) or ionic bond. Therefore, the cross-linkable functional group may be variously selected according to the kind of the intermolecular interactions. For example, the cross-linking region may include SH, -CHO, -COOH, -NH₂, -OH, -PO₃H, -PO₄H₂, -SO₃H, -OSO₃H, -NR₃⁺X⁺ (R= CₙHₙ₀≤n≤16, 0<m<34, X = OH, Cl, Br), NR₄⁺X⁺ (R= CₙHₙ₀≤n≤16, 0<m<34, X = OH, Cl, Br), -N₃, -SCOCH₃, -SCN, -NCS, -NCO, -CN, -F, -Cl, -I, -Br, an epoxy group, -ONO₂, -PO(OH)₂, -C=NNH₂, -C≡C-, -C≡C- as the functional group, but not limited to.

According to a preferable embodiment, the water-soluble multi-functional organic ligand includes a biocompatible polymer, a peptide, a protein, an amphiphilic ligand, a nucleic acid, or a carbohydrate.
According to a preferable embodiment, the water-soluble multi-functional organic ligand coated on the inorganic nanoparticle core is a polymer. Preferable polymer includes, but not limited to, one or more polymer selected from the group consisting of polyphosphagen, poly(lactide), poly(lactide-co-glycolide), polycaprolactone, polyanhydride, polymaleic acid, a derivative of polymaleic acid, polyalkylcyanoacrylate, polyhydroxybutylate, polycarbonate, polyorthoester, polyethylene glycol, poly-L-lysine, polyglycolide, polymethyl methacrylate and polyvinylpyrrolidone.

Another example of the preferable water-soluble multi-functional organic ligand of the nanoparticle for penetration of the blood-brain barrier of the present invention is a peptide. Peptide is oligomer/polymer of several amino acids. Since the amino acids have -COOH and -NH₂ functional groups in both ends thereof, peptides naturally have the attachment region and the active ingredient-binding region. In addition, particular peptides that contain one or more amino acids having at least one of -SH, -COOH, -NH₂ and -OH as the side chain may be utilized as the preferable water-soluble multi-functional organic ligand.

In the nanoparticle for penetration of the blood-brain barrier of the present invention, still another example of the preferable water-soluble multi-functional organic ligand is a protein. Protein is a polymer composed of more amino acids than peptides, that is, composed of several hundreds or several hundred thousands of amino acids. Proteins contain -COOH and -NH₂ functional group at both ends, and also contain a lot of functional groups such as -COOH, -NH₂, -SH, -OH, -CONH₂, and so on. Proteins may be used as the water-soluble multi-functional organic ligand because they naturally contain the attachment region, the cross-linking region and the active ingredient-binding region as described in peptide. The preferable protein as the water-soluble multi-functional organic ligand includes simple protein, complex protein, inducible protein or an analog thereof. Much more preferable example of the water-soluble multi-functional organic ligand includes, but not limited to, a hormone,
a hormone analog, an enzyme, an enzyme inhibitor, a signal-transducing protein or its part, an antibody or its part, a light chain antibody, a binding protein or its binding domain, an antigen, an attachment protein, a structural protein, a regulatory protein, a toxic protein, a cytokine, a transcription factor, a blood coagulation factor and a plant defense-inducible protein. Most preferably, the water-soluble multi-functional organic ligand in the present invention includes, but not limited to, albumin, histone, protamine, prolamine, glutenin, antibody, antigen, avidin, cytochrome, casein, myosin, glycinin, carotene, hemoglobin, myoglobin, flavin, collagen, globular protein, light protein, streptavidin, protein A, protein G, protein S, immunoglobulin, lectin, selectin, angiopoietin, anti-cancer protein, antibiotic protein, hormone antagonist protein, interleukin, interferon, growth factor protein, tumor necrosis factor protein, endotoxin protein, lymphotoxin protein, tissue plasminogen activator, urokinase, streptokinase, protease inhibitor, alkyl phosphocholine, surfactant, cardiovascular pharmaceutical protein, neuro pharmaceutical protein and gastrointestinal pharmaceuticals. In particular, transferrin, albumin, hormone and cytokine may effectively used as the preferable water-soluble multi-functional organic ligand in the nanoparticle for penetration of the blood-brain barrier of the present invention.

Still another example of the preferable water-soluble multi-functional organic ligand in the present invention is a nucleic acid. The nucleic acid is oligomer consisting of many nucleotides. Since the nucleic acids have PO_4^- and -OH functional groups in both ends thereof, they naturally have the attachment region and the active ingredient-binding region (L_1-L_n) or the attachment region and the cross-linking region (L_L_n_i). Therefore, the nucleic acids may be useful in a phase-transfer ligand of this invention. In some cases, the nucleic acid is preferably modified to have the functional group such as -SH, -NH_2, -COOH or -OH at 3'- or 5'-terminal ends.

Still another example of the preferable water-soluble multi-functional organic
ligand in the nanoparticle for penetration of the blood-brain barrier of the present invention is an amphiphilic ligand including both a hydrophobic and a hydrophilic region.

In the nanoparticles synthesized in an organic solvent, hydrophobic ligands having long carbon chains coat the surface. The hydrophobic region of the amphiphilic ligand, which was added at that time, and the hydrophobic ligand on the nanoparticles are bound to each other through intermolecular interaction to stabilize the nanoparticles. Further, the outermost part of the amphiphilic ligand coated nanoparticles shows the hydrophilic functional group, and consequently water-soluble nanoparticles can be prepared. The intermolecular interaction includes a hydrophobic interaction, a hydrogen bond, a Van der Waals force, and so on. The portion which binds to the nanoparticles by the hydrophobic interaction is an attachment region (L₁), and further the amphiphilic cross-linking region (L_u) and the active ingredient-binding region (L_m) can be introduced therewith by an organochemical method. In addition, in order to increase the stability in an aqueous solution, amphiphilic polymer ligands with multiple hydrophobic and hydrophilic regions can be used. Cross-linking between the amphiphilic ligands can be also performed by a linker for enhancement of stability in an aqueous solution. Hydrophobic region of the amphiphilic ligand can be a linear or branched structure composed of chains containing 2 or more carbon atoms, more preferably an alkyl functional group such as ethyl, n-propyl, isopropyl, n-butyl, isobutyl, t-butyl, octyl, decyl, tetradecyl, hexadecyl, icosyl, tetracosyl, dodecyl, cyclopentyl, and cyclohexyl; a functional group having an unsaturated carbon chain containing a carbon-carbon double bond, such as ethynyl, propenyl, isopropenyl, butenyl, isobutenyl, octenyl, decenyl, and oleyl; and a functional group having an unsaturated carbon chain containing a carbon-carbon triple bond, such as propynyl, isopropynyl, butynyl, isobutynyl, octynyl and decenyl. In addition, examples of the hydrophilic region include the functional group being neutral at a specific pH, but being positively or
negatively charged at a higher or lower pH such as -SH, -COOH, -NH₂, -OH, -PO₃H, -PO₄H₂, -SO₃H, -SO₄H and -NR₄⁺X. Furthermore, preferable examples thereof include a polymer and a block copolymer, wherein monomers used therefore include ethylglycol, acrylic acid, alkylacrylic acid, ataconic acid, maleic acid, fumaric acid, acrylamidomethylpropane sulfonic acid, vinylsulfonic acid, vinylphosphoric acid, vinyl lactic acid, styrenesulfonic acid, allylammonium, acrylonitrile, N-vinylpyrrolidone and N-vinylformamide, but not limited thereto.

Another example of the preferable water-soluble multi-functional organic ligand in the nanoparticle for penetration of the blood-brain barrier of the present invention is a carbohydrate. More preferably, the carbohydrate includes, but not limited to, glucose, mannose, fucose, N-acetyl glucamine, N-acetyl galactosamine, N-acetylenuraminic acid, fructose, xylose, sorbitol, sucrose, maltose, glycoaldehyde, dihydroxyacetone, erythrose, erythrulose, arabinose, xylulose, lactose, trehalose, mellibose, cellobiose, raffmose, melezitose, maltorose, starchose, estrolose, xylan, araban, hexosan, fructan, galactan, mannan, agaropretin, alginic acid, carrageenan, hemicelluloses, hypromellose, amylose, deoxyacetone, glyceraldehyde, chitin, agarose, dextrin, ribose, ribulose, galactose, carboxy methylcellulose, glycogen dextran, carbodextran, polysaccharide, cyclodextran, pullulan, cellulose, starch, and glycogen.

According to the present invention, the compounds having the above-described functional group in nature may be used as the water-soluble multi-functional organic ligand. The compounds modified or prepared so as to have the above-described functional group according to a chemical reaction known in the art may be also used as the water-soluble multi-functional organic ligand.

According to a preferable embodiment, the water-soluble multi-functional organic ligand is cross-linked through cross-linking regions (Lm) or further molecular linker. The cross-linking permits the water-soluble multi-functional organic ligand to be firmly coated on the nanoparticle core. In particular, it is advantageous in the
senses that the nanoparticle for penetration of the blood-brain barrier of the present invention is administrated into the body. For example, in the case using proteins as the water-soluble multi-functional organic ligand, protein coating may be significantly stabilized by crosslinking the carboxyl and amine group of proteins using N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide (sulfo-NHS). Furthermore, protein coating may be remarkably stabilized by cross-linking between molecular linker (2,2-ethylenedioxy bis ethylamine) and the carboxyl group on the surface of protein using EDC and sulfo-NHS.

The nanoparticle for penetration of the blood-brain barrier of the present invention, to which a biomolecule (example: an antibody, a protein, an antigen, a peptide, a nucleic acid, an enzyme, a cell, etc.) or a chemical active substance (example: a monomer, a polymer, an inorganic support, a fluorescent substance, a drug, etc.) are bound may be used.

The biomolecule includes, but not limited to, an antibody, a protein, an antigen, a peptide, a nucleic acid, an enzyme and a cell, and preferably a protein, a peptide, DNA, RNA, an antigen, hapten, avidin, streptavidin, neutravidin, protein A, protein G, lectin, selectin, hormone, interleukin, interferon, growth factor, tumor necrosis factor, endotoxin, lymphotoxin, urokinase, streptokinase, tissue plasminogen activator, hydrolase, oxido-reductase, lyase, biological active enzymes such as isomerase and synthetase, enzyme cofactor and enzyme inhibitor.

The chemical active substance includes several functional monomers, polymers, inorganic substances, fluorescent organic substances, or drugs.

The example of the above-described monomer includes, but not limited to, a drug containing anti-cancer drug, antibiotics, Vitamin and folic acid, a fatty acid, a steroid, a hormone, a purine, a pyrimidine, a monosaccharide, and a disaccharide. The side chain of the above-described monomer includes one or more functional groups selected from -COOH, -NH₂, -SH, -SS-, -CONH₂, -PO₃H, -OPO₄H₂, -
PO_2(O_1R^1XOR^2) (R^1, R^2 = C_2H_Nu_wSx_Py_xz, X = -F, -Cl, -Br or -I, 0<s<20, 
0<t ≤ 2(s+u)+l, 0<u ≤ 2s, 0<w ≤ 2s, 0<x ≤ 2s, 0<y ≤ 2s, 0<z ≤ 2s), -SO_3H, -OSO_3H, -
NO_2, -CHO, -COSH, -COX, -COOCO-, -CORCO- (R = QH_m, 0<l ≤ 3, 0<m ≤ 2(l+1),)
- COOR, -CN, -N_3, -N_2, -NROH (R = C_2H_Nu_wSx_Py_xz, X = -F, -Cl, -Br or -I, 0<s<20, 
0<t ≤ 2(s+u)+l, 0<u ≤ 2s, 0<w ≤ 2s, 0<x ≤ 2s, 0<y ≤ 2s, 0<z<2s), -NR^1NR^2RR^3 (R^1,R^2,R^3
= C_2H_Nu_wSx_Py_xz, X = -F, -Cl, -Br or -I, 0<s<20, 0<t ≤ 2(s+u)+l, 0<u ≤ 2s, 0<w ≤ 2s,
0<x ≤ 2s, 0<y ≤ 2s, 0<z ≤ 2s), -CONHNR^1R^2 (R^1, R^2 = C_2H_Nu_wSx_Py_xz, X = -F, -Cl, -Br
or -I, 0<s≤20, 0<t<2(s+u)+l, 0<u ≤ 2s, 0<w ≤ 2s, 0<x ≤ 2s, 0<y ≤ 2s, 0<z ≤ 2s), -
NR^1NR^2RR^3 \ (R^1, R^2, R^3 = C_2H_Nu_wSx_Py_xz, X = -F, -Cl, -Br or -I, X' = F, Cl', Br'
or I', 0<s<20, 0<t ≤ 2(s+u)+l, 0<u ≤ 2s, 0<w ≤ 2s, 0<x ≤ 2s, 0<y ≤ 2s, 0<z ≤ 2s), -OH, -
SCOCH, -F, -Cl, -Br, -I, -SCN, -NCO, -OCN, -epoxide, -hydrazone, -alkene, and alkyn
group, but not limited to.

The example of the above-described bioactive chemical polymer includes
dextran, carbodextran, polysaccharide, cyclodextran, pullulan, cellulose, starch,
glycogen, monosaccharides, disaccharides and oligosaccharides, polyphosphagen,
polylactide, polylactide-co-glycolide, polycaprolactone, polyanhydride, polymaleic
acid and a derivative of polymaleic acid, polyalkylcyanoacrylate, polyhydroxybutylate,
polycarbonate, polyorthoester, polyethylene glycol, poly-L-lysine, polyglycolide,
polymethyl methacrylate, polymethylether methacrylate and polyvinylpyrrolidone,
but not limited to.

The example of the above-described bioactive inorganic substance includes a
metal oxide, a metal chalcogen compound, an inorganic ceramic material, a carbon
material, a semiconductor substrate consisting of Group II/VI elements, Group III/VI
elements and Group IV elements, a metal substrate, or complex thereof, and
preferably, SiO_2, TiO_2, ITO, nanotube, graphite, fullerene, CdS, CdSe, CdTe, ZnO,
ZnS, ZnSe, ZnTe, Si, GaAs, AlAs, Au, Pt, Ag or Cu.

The example of the above-described bioactive fluorescent substance includes
a fluorescent organic substance such as fluorescein and its derivatives, rhodamine
and its derivatives, lucifer yellow, B-phytoerythrin, 9-acrydine isothiocyanate, lucifer yellow VS, 4-acetamido-4'-isothio-cyanatostilbene-2,2'-disulfonate, 7-diethylamino-3-(4'-isothiocyanatophenyl)-4-methylcoumarin, succinimidyl-pyrenebutyrate, 4-acetoamido-4'-isothio-cyanatostilbene-2,2'-disulfonate derivatives, LC™-Red 640, LC™-Red 705, Cy3, Cy5, Cy5.5, Alexa dye series, resamine, isothiocyanate, diethyltriamine pentaacetate, 1-dimethylaminonaphthyl-5-sulfonate, l-anilino-8-naphthalene, 2-p-toluidinyl-6-naphthalene, 3-phenyl-7-isocyanatocoumarin, 9-isothiocyanatoacridine, acridine orange, N-(p-(2-benzoxazolyl)phenyl)meleimide, benzoxadiazol, stilbene and pyrene, and a fluorescent inorganic semiconductor nanoparticle (quantum dot), but not limited to.

The nanoparticle for penetration of the blood-brain barrier of the present invention refers to a particle of which the inorganic nanoparticle-core diameter is in a range of 1-1000 nm and preferably 2-500 nm. In addition, the nanoparticle for penetration of the blood-brain barrier of the present invention is dispersed in water to concentration of 1-500 mg/ml and preferably 1-100 mg/ml. The nanoparticle dispersed in water has hydrodynamic diameters in a range of preferably 1 nm-500 μm and more preferably 1 nm-200 μm.

According to a preferable embodiment, the nanoparticle for penetration of the blood-brain barrier of the present invention represents much higher distribution in brain than in heart, kidney, lung, muscle, spleen, lymph node, testes and thymus. The term "distribution" mentioned in a penetration ability of the blood-brain barrier of the nanoparticle refers to a localization status of the nanoparticle in various organs when intravenously injected.

As described above, brain localization of the nanoparticle for penetration of the blood-brain barrier is an interesting feature compared to the conventional particles for penetration of the blood-brain barrier. In more detail, the conventional particles for penetration of the blood-brain barrier induce brain localization through binding to a targeting ligand which can specifically bind to the particular biomolecule.
However, the nanoparticle for penetration of the blood-brain barrier of the present invention itself has very high distribution in brain only through the injection to the body.

This feature represents that the nanoparticle for BBB penetration may be used as a tool delivering the target substance into brain.

In addition, the nanoparticle for BBB penetration has an advantage of effectively penetrating the blood-brain barrier with no help of conventional adjuvants (e.g., mannitol).

In another aspect of this invention, there is provided a composition for brain imaging, comprising the nanoparticle for penetration of the blood-brain barrier as described above.

Since the present composition comprises the nanoparticle of this invention as active ingredients described above, the common descriptions between them are omitted in order to avoid undue redundancy leading to the complexity of this specification.

The nanoparticle for BBB penetration is very useful for brain imaging due to its localization in brain penetrating the blood-brain barrier at an excellent efficiency.

The brain-imaging agent composition may be applied to various imaging techniques. In the nanoparticles for BBB penetration prepared according to the present invention, the inorganic core nanoparticle having magnetic core may be used in a magnetic resonance imaging agent. In addition, the active ingredient-binding region of the multi-functional organic ligand in the present nanoparticle may be combined with a radioisotope, and may be used in SPECT (Single Photon Emission Computed Tomography) or PET (Positron Emission Tomography).

The active ingredient-binding region of the multi-functional organic ligand in the present nanoparticle may also be combined with the fluorescent substance, and may be used in an optical imaging and spectroscopy, or BL (bioluminescence).
imaging.

As a diagnostic agent, the active ingredient-binding region of the water-soluble multi-functional organic ligand in the present nanoparticle may be bound to barium sulfate and iodine for X-ray diagnosis (CT, computed tomography) or to a microbubble for ultrasonography diagnosis.

In MR imaging by the present brain-imaging composition, the present nanoparticle core for BBB penetration is preferably composed of magnetic or metal oxide nanoparticle and more preferably metal oxide nanoparticle represented by $M_{x}O_{y}$ or $M_{x}M^{n}O_{z}$. MR imaging method and device are disclosed in U.S. Pat. Nos. 6,119,032, 6,128,522, No. 6,127,825, No. 6,121,775, No. 6,119,032, No. 6,115,446, No. 6,111,410 and No. 602,891, which are incorporated herein by reference. The brain-imaging agent composition of this invention may be used as T1 and T2 brain-imaging agent.

In PET or SPECT images by the present composition for brain imaging, preferably the positron emitting radioisotope is bound to the water-soluble multi-functional organic ligand of the nanoparticle for BBB penetration. The examples of the positron emitting radioisotope includes, but not limited to, $^{10}$C, $^{11}$C, $^{13}$O, $^{14}$O, $^{15}$O, $^{12}$N, $^{13}$N, $^{15}$F, $^{17}$F, $^{18}$F, $^{32}$Cl, $^{33}$Cl, $^{34}$Cl, $^{43}$Sc, $^{44}$Sc, $^{45}$Ti, $^{51}$Mn, $^{52}$Mn, $^{52}$Fe, $^{53}$Fe, $^{55}$Co, $^{56}$Co, $^{58}$Co, $^{61}$Cu, $^{62}$Cu, $^{62}$Zn, $^{63}$Zn, $^{64}$Cu, $^{65}$Zn, $^{66}$Ga, $^{66}$Ge, $^{67}$Ge, $^{68}$Ga, $^{69}$Ge, $^{69}$As, $^{70}$As, $^{70}$Se, $^{71}$Se, $^{71}$As, $^{72}$As, $^{73}$Se, $^{74}$Kr, $^{74}$Br, $^{75}$Br, $^{76}$Br, $^{77}$Kr, $^{78}$Br, $^{78}$Rb, $^{79}$Rb, $^{79}$Kr, $^{81}$Rb, $^{82}$Rb, $^{84}$Rb, $^{84}$Zr, $^{86}$Y, $^{86}$Y, $^{87}$Y, $^{87}$Zr, $^{88}$Y, $^{89}$Zr, $^{92}$Tc, $^{93}$Tc, $^{94}$Tc, $^{95}$Tc, $^{95}$Ru, $^{95}$Rh, $^{96}$Rh, $^{97}$Rh, $^{98}$Rh, $^{99}$Rh, $^{100}$Rh, $^{101}$Ag, $^{102}$Ag, $^{102}$Rh, $^{103}$Ag, $^{104}$Ag, $^{105}$Ag, $^{106}$Ag, $^{108}$In, $^{109}$In, $^{110}$In, $^{115}$Sb, $^{116}$Sb, $^{117}$Sb, $^{116}$Te, $^{116}$Te, $^{117}$Te, $^{117}$I, $^{118}$I, $^{118}$Xe, $^{119}$Xe, $^{119}$I, $^{119}$Te, $^{120}$I, $^{120}$Xe, $^{121}$Xe, $^{121}$I, $^{122}$I, $^{123}$Xe, $^{124}$I, $^{126}$I, $^{128}$I, $^{129}$La, $^{130}$La, $^{131}$La, $^{132}$La, $^{133}$La, $^{135}$La, $^{136}$La, $^{140}$Sm, $^{141}$Sm, $^{142}$Sm, $^{144}$Gd, $^{145}$Gd, $^{145}$Eu, $^{146}$Gd, $^{146}$Eu, $^{147}$Eu, $^{147}$Gd, $^{148}$Eu, $^{150}$Eu, $^{190}$Au, $^{191}$Au, $^{192}$Au, $^{193}$Au, $^{193}$Tl, $^{194}$Tl, $^{194}$Au, $^{195}$Tl, $^{196}$Tl, $^{197}$Tl, $^{198}$Tl, $^{200}$Tl, $^{200}$Bi, $^{202}$Bi, $^{203}$Bi, $^{205}$Bi, $^{206}$Bi or derivatives thereof. PET imaging method and device are disclosed in U.S. Pat. No.
6,151,377, No. 6,072,177, No. 5,900,636, No. 5,608,221, No. 5,532,489, No. 5,272,343 and No. 5,103,098, which are incorporated herein by reference. SPECT imaging method and device are disclosed in US Pat. No. 6,115,446, No. 6,072,177, No. 5,608,221, No. 5,600,145, No. 5,210,421 and No. 5,103,098, which are incorporated herein by reference.

For obtaining BL (bioluminescence) image, a luminescent, fluorescent or chemiluminescent substance is bound to the water-soluble multi-functional organic ligand of the present nanoparticle for BBB penetration. General descriptions of BL imaging are disclosed in US Pat. No. 5,650,135. The example of the luminescent substance in BL imaging includes luciferase and aequorin.

To obtain CT images using the present composition for brain imaging, CT imaging may be carried out according to the methods disclosed in US Pat. No. 6,151,377, No. 5,946,371, No. 5,446,799, No. 5,406,479, No. 5,208,581 and No. 5,109,397.

The brain-imaging composition of the present invention is very useful for brain imaging of human body, particularly vessel of brain. The imaging process is as follows: 1) the diagnostically effective amount of imaging agent is administrated into human, and 2) the human body is scanned by various imaging method to obtain an optical image of brain.

The imaging agent of the present invention may be administrated together with a pharmaceutically acceptable carrier, which is commonly used in pharmaceutical formulations, but is not limited to, includes lactose, dextrose, sucrose, sorbitol, mannitol, starch, rubber arable, potassium phosphate, arginate, gelatin, potassium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrups, methylcellulose, methylhydroxy benzoate, propylhydroxy benzoate, talc, magnesium stearate, and mineral oils. Details of suitable pharmaceutically acceptable carriers and formulations can be found in Remington's Pharmaceutical Sciences (19th ed., 1995), which is incorporated herein by reference.
The imaging agent according to the present invention may be parenterally administered, and preferably, administered parenterally, e.g., by intravenous, intramuscular, intra-articular or intrathecal injection.

A suitable dosage amount of the imaging agent of the present invention may vary depending on pharmaceutical formulation methods, administration methods, the patient's age, body weight, sex, pathogenic state, diet, administration time, administration route, an excretion rate and sensitivity for a used contrast agent. The term "diagnostically effective amount" refers to an amount enough to show and accomplish images of human body and is generally administered with a daily dosage of 0.0001-100 mg/kg.

In still another of this aspect, there is provided a brain-targeting pharmaceutical composition, comprising: (a) a pharmaceutically effective amount of the nanoparticle for penetration of the blood-brain barrier in the present invention; and (b) a pharmaceutically acceptable carrier.

Since the present composition comprises the brain-targeting pharmaceutical composition of this invention as active ingredients described above, the common descriptions between them are omitted in order to avoid undue redundancy leading to the complexity of this specification.

In the brain-targeting pharmaceutical composition of this invention, the drug to be delivered into the brain is preferably bound to the water-soluble multi-functional organic ligand and more preferably the active ingredient-binding region of the water-soluble multi-functional organic ligand.

The drug linked to the particle for penetration of the blood-brain barrier is particularly limited and includes all drugs for treating a central nervous system-related disorder or condition. For example, the drug linked to the particle for penetration of the blood-brain barrier includes, but not limited to, drugs acting at synaptic sites and neuroeffector junctional sites; general and local analgesics and
anesthetics such as opioid analgesic and antagonist; hypnotics and sedatives; drugs for the treatment of psychiatric disorders such as depression and schizophrenia; anticonvulsants and anti-epileptics; drugs for treatment of Huntington's disease, Parkinson's disease and Alzheimer's disease; agents for protection and regeneration of nerves; trophic factors such as brain-derived neurotrophic factor, fibrotic neurotrophic factor and nerve growth factor; agents for treatment of CNS trauma or stroke; drugs for the treatment of addiction and drug abuse; antacoids and anti-inflammatory drugs; chemotherapeutic agents for parasitic infections and diseases caused by microbes; immunosuppressive agents and anti-cancer drugs; hormones and hormone antagonists; heavy metals and heavy metal antagonists; antagonists for non-metallic toxic agents; transmitters and their respective receptor agonists and receptor antagonists.

The pharmaceutical composition of the present invention may be administrated together with a pharmaceutically acceptable carrier, which is commonly used in pharmaceutical formulations, but is not limited to, includes lactose, dextrose, sucrose, sorbitol, mannitol, starch, rubber arable, potassium phosphate, arginate, gelatin, potassium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrups, methylcellulose, methylhydroxy benzoate, propylhydroxy benzoate, talc, magnesium stearate, and mineral oils. Details of suitable pharmaceutically acceptable carriers and formulations can be found in Remington's Pharmaceutical Sciences (19th ed., 1995), which is incorporated herein by reference.

The pharmaceutical composition according to the present invention may be parenterally administered, and preferably, administered parenterally, e.g., by intravenous, intramuscular, intra-articular or intrathecal injection. A suitable dosage amount of the imaging agent of the present invention may vary depending on pharmaceutical formulation methods, administration methods, the patient's age, body weight, sex, pathogenic state, diet, administration time, administration route, an excretion rate and sensitivity for a used contrast agent. The term “diagnostically
effective amount" refers to an amount enough to show and accomplish images of human body and is generally administered with a daily dosage of 0.0001-100 mg/kg. According to the conventional techniques known to those skilled in the art, the pharmaceutical composition may be formulated with pharmaceutically acceptable carrier and/or vehicle as described above, finally providing several forms including a unit dose form and a multi-dose form. Non-limiting examples of the formulations include, but not limited to, a solution, a suspension or an emulsion in oil or aqueous medium, an elixir, a powder, a granule, a tablet and a capsule, and may further comprise a dispersion agent or a stabilizer.

The features and advantages of the present invention will be summarized as follows:

(i) the present nanoparticle for BBB penetration includes the inorganic nanoparticle core and the water-soluble multi-functional organic ligand coated on the nanoparticle core.

(ii) the water-soluble multi-functional organic ligand coated on the nanoparticle core in the present nanoparticle for BBB penetration permits the nanoparticle to effectively penetrate the blood-brain barrier.

(iii) the nanoparticle for penetration of the blood-brain barrier of the present invention ensures an effective penetration of the blood-brain barrier even with no use of conventional adjuvants (e.g., mannitol).

(iv) the nanoparticle for penetration of the blood-brain barrier of the present invention may be used as a brain-imaging agent and as a drug delivery system into brain.

The present invention will now be described in further detail by examples. It would be obvious to those skilled in the art that these examples are intended to be more concretely illustrative and the scope of the present invention as set forth in the appended claims is not limited to or by the examples.
EXEMPLARY

EXAMPLE 1: Preparation of magnetic nanoparticles having Fe₃O₄ core
solubilized in water for BBB penetration

Water-insoluble nanoparticle having Fe₃O₄ core was synthesized according to
the method described in Korean Pat. No. 0604975. In detail, iron nitrate (Aldrich)
was thermally decomposed for 1 hr in 20 ml octylether (Aldrich) of 290°C including
0.1 M lauric acid (Aldrich) and 0.1 M lauryl amine (Aldrich), yielding iron oxide
nanoparticles with the size of 15 nm. The iron oxide nanoparticle was mixed with 5
ml of 1 M TMAOH (tetramethylammoniumhydroxide pentahydrate, Sigma) solution and
then dispersed in an aqueous solution (Hg. IA).

EXAMPLE 2: Preparation of magnetic nanoparticles having MnFe₂O₄ core
solubilized in water for BBB penetration

Water-insoluble nanoparticle having MnFe₂O₄ core was synthesized according
to the method described in Korean Pat. No. 0604975. To prepare MnFe₂O₄
nanoparticle with the size of 15 nm, iron nitrate acetylacetonate (Aldrich) and
manganese chloride (Aldrich) precursors were mixed at an equivalent ratio of 2:1
and was thermally decomposed for 2 hrs in 20 ml octylether (Aldrich) of 290°C
including 0.1 M lauril oleic acid (Aldrich) and 0.1 M lauryl oleamine (Aldrich), giving
MnFe₂O₄ nanoparticles. The synthesized MnFe₂O₄ nanoparticle with the size of 15 nm
was mixed with 5 ml of 1 M TMAOH solution and then dispersed in aqueous solution
(Fig. IB).

EXAMPLE 3: Preparation of magnetic nanoparticles having
(Zn₀.₄Fe₀.₆)Fe₂O₄ core solubilized in water for BBB penetration

Water-insoluble nanoparticle having (Zn₀.₄Fe₀.₆)Fe₂O₄ core was synthesized
according to the method described in Korean Pat. No. 0604975. In detail, ZnCl$_2$, FeCl$_2$ or MnCl$_2$, Fe(acac)$_3$ were thermally decomposed for 2 hrs at 200\textdegree C or 300\textdegree C in trioctylamine solution including 20 mmol of oleic acid and oleylamine, yielding (Zn$_{0.4}$Fe$_{0.6}$)Fe$_2$O$_4$ nanoparticles with the size of 15 nm. Since the synthesized nanoparticles with the size of 15 nm were dissolved in an organic solvent, they were solubilized using the following method (Fig. 1C). The (Zn$_{0.4}$Fe$_{0.6}$)Fe$_2$O$_4$ nanoparticles dispersed in 1 mL toluene (50 mg/mL) were precipitated by excessive ethanol and mixed with 5 mL of 1 M TMAOH solution, dispersing in aqueous solution (Fig. 1C).

EXAMPLE 4: Preparation of multi-functional hybrid structure nanoparticles having FePt-Au core solubilized in water for BBB penetration

The multi-functional hybrid structure nanoparticle of a dumbbell shape having FePt-Au core was synthesized according to the following method. First, FePt magnetic nanoparticle with the size of 6 nm was synthesized. The mixture of 0.5 mmol Pt(acac)$_2$ and 1 mmol Fe(CO)$_5$ was incubated for 2hrs at 100\textdegree C or 240\textdegree C under argon gas in the solution which 0.1 M oleic acid (Aldrich) and 0.1 M oleylamine (Aldrich) were added as a capping molecule. After the reaction is finished, the reaction solution was cooled to room temperature and the as-prepared FePt nanoparticle with the size of 6 nm was precipitated by ethanol and isolated. AuCl(PPh$_3$)$_2$, hexadecylamine and 1,2-dichlorobenzene were added to the prepared FePt nanoparticle with the size of 6 nm and incubated at 70\textdegree C for 30 min in a hydrogen gas atmosphere. When the color of solution turn to red, it was cooled to room temperature and was precipitated by ethanol for obtaining FePt (6 nm)-Au (10 nm) nanoparticles. The synthesized FePt-Au nanoparticles was mixed with 5 mL of 1 M TMAOH solution due to their insolubility in water and then dispersed in aqueous solution (Fig. 1D).

EXAMPLE 5: Preparation of magnetic nanoparticles having Gd$_2$O$_3$ core
solubilized in water for BBB penetration 1

The nanoparticle having Gd₂O₃ core was synthesized according to the following method. GdCl₃ • 6H₂O was dissolved in diethylene glycol solution to a concentration of 0.2 M and H₂O and NaOH with final concentration of 2.0 M and 0.2 M was added. The mixture solution was dissolved by heating to 140°C and further incubated for 1 day under heating condition to 180°C. The resulting synthetic nanoparticle was stable in aqueous solution (Fig. IE).

EXAMPLE 6: Preparation of magnetic nanoparticles having Gd₂O₃ core solubilized in water for BBB penetration 2

The nanoparticle having Gd₂O₃ core was synthesized according to the method described in Korean Pat. No. 0604975. In detail, Gd(acac)₂ was thermally decomposed for 2 hrs at 200°C or 300°C in trioctylamine solution including 20 mmol of oleic acid and oleylamine, yielding Gd₂O₃ nanoparticles with the size of 15 nm. Since the synthesized nanoparticles with the size of 15 nm were dissolved in the organic solvent, they were solubilized using the following method (Fig. 1C). The Gd₂O₃ nanoparticles dispersed in 1 mL toluene (50 mg/mL) were precipitated by excessive ethanol and mixed with 5 mL of 1 M TMAOH solution, dispersing in aqueous solution (Fig. IF).

EXAMPLE 7: Preparation of BBB-penetrating nanoparticles having Fe₃O₄ core coated with BSA, carbodextran, or polyacrylate-polyethylene glycol (PAA-PEG)

The water-soluble nanoparticles coated with BSA, carbodextran or PAA-PEG were synthesized according to the methods described in Korean Pat. Nos. 0652251, 064976 and 0713745.

PAA-PEG polymer was prepared according to the following method. 0.72 g of PAA (M.W. 2,000) was dissolved in 10 mL of dichloromethane and mixed with 0.8 g
of N-hydroxysuccinimide (NHS). 1.1 g of dicyclohexylcarbodiimide (DCC) was added to the mixture and incubated for 24 hrs. The resulting NHS-modified PAA was separated using a silica column chromatography and the solvent was removed, obtaining white solid materials. 0.8 g of the white solid material was dissolved in DMF solution and mixed with 2 g of NH₂-PEG-OH, followed by incubating for 24 hrs. Eventually, 50% PEG substituted PAA-PEG was yielded.

200 mg of BSA (bovine serum albumin, Aldrich), carbodextran (Amersham Bioscience) or PAA-PEG were added to the water-soluble Fe₃O₄ nanoparticles (5 mL) synthesized in Example 1 and incubated for 24 hrs at room temperature. The reaction solution was separated using a Sephacryl S-300 column (GE healthcare, USA) and non-reactive excess BSA, carbodextran or PAA-PEG were removed, isolating the nanoparticles. The isolated nanoparticles were concentrated to 5 mL with a Centricon YM100 filter. This solution was mixed with 50 mm EDC (Pierce), 5 mM sulfo-NHS (Pierce) and 20 mg 2,2-(ethylenedioxy)bis(ethylenamine), and incubated for 2 hrs, purifying Fe₃O₄ nanoparticles coated with cross-linked-BSA, carbodextran or PAA-PEG using Sephadex G25 column (GE healthcare, USA) (Fig. 2A) (Table 2. Nos. 1-4).

**EXAMPLE 8: Preparation of BBB-penetrating nanoparticles having MnFe₂O₄ core coated with BSA, carbodextran, or polyacrylate-polyethylene glycol (PAA-PEG)**

The water-soluble nanoparticles coated with BSA, carbodextran or PAA-PEG were synthesized according to the methods described in Korean Pat. Nos. 0652251, 064976 and 0713745. In addition, PAA-PEG polymer was prepared according to the method described in Example 7.

200 mg of BSA, carbodextran, or PAA-PEG were added to the water-soluble MnFe₂O₄ nanoparticles (5 mL) synthesized in example 2 and incubated for 24 hrs at room temperature. The reaction solution was separated using a Sephacryl S-300...
column and non-reactive excess BSA, carbodextran or PAA-PEG were removed, isolating the nanoparticles. The isolated nanoparticles were concentrated to 5 ml with the Centricon YM100 filter. This solution was mixed with 50 mM EDC, 5 mM sulfo-NHS and 20 mg 2,2-(ethylenedioxy)bis(ethylenamine), and incubated for 2 hrs, obtaining MnFe₂O₄ nanoparticles coated with cross-linked-BSA, carbodextran or PAA-PEG. The MnFe₂O₄ nanoparticles coated with cross-linked-BSA, carbodextran or PAA-PEG were purified using the Sephadex G25 column (Fig. 2B) (Table 2. Nos. 5-8).

EXAMPLE 9: Preparation of BBB-penetrating nanoparticles having (Zn₀.₄Fe₀.₆)Fe₂O₄ core coated with BSA, carbodextran, or polyacrylate-polyethylene glycol (PAA-PEG)

The water-soluble nanoparticles coated with BSA, carbodextran or PAA-PEG were synthesized according to the methods described in Korean Pat. Nos. 0652251, 064976 and 0713745. In addition, PAA-PEG polymer was prepared according to the method described in Example 7.

200 mg of BSA, carbodextran or PAA-PEG were added to the water-soluble (Zn₀.₄Fe₀.₆)Fe₂O₄ nanoparticles (5 ml) synthesized in example 3 and incubated for 24 hrs at room temperature. The reaction solution was separated using the Sephacryl S-300 column and non-reactive excess BSA, carbodextran or PAA-PEG were removed, isolating the nanoparticles. The isolated nanoparticles were condensed to 5 ml with the Centricon YM100 filter. This solution was mixed with 50 mM EDC, 5 mM sulfo-NHS and 20 mg 2,2-(ethylenedioxy)bis(ethylenamine), and incubated for 2 hrs, obtaining (Zn₀.₄Fe₀.₆)Fe₂O₄ nanoparticles coated with cross-linked-BSA, carbodextran or PAA-PEG. The (Zn₀.₄Fe₀.₆)Fe₂O₄ nanoparticles coated with cross-linked-BSA, carbodextran or PAA-PEG were purified using the Sephadex G25 column (Fig. 2C) (Table 2. Nos. 9-12).

EXAMPLE 10: Preparation of BBB-penetrating nanoparticles having FePt-
Au core coated with BSA, carbodextran, or polyacrylate-polyethylene glycol (PAA-PEG)

The water-soluble nanoparticles coated with BSA, carbodextran or PAA-PEG were synthesized according to the methods described in Korean Pat. Nos. 0652251, 064976 and 0713745. In addition, PAA-PEG polymer was prepared according to the method described in Example 7.

200 mg of BSA, carbodextran or PAA-PEG were added to the water-soluble FePt-Au nanoparticles (5 mL) synthesized in example 4 and incubated for 24 hrs at room temperature. The reaction solution was separated using the Sephacryl S-300 column and non-reactive excess BSA, carbodextran, or PAA-PEG were removed, isolating the nanoparticles. The isolated nanoparticles were condensed to 5 mL with the Centricon YM100 filter. This solution was mixed with 50 mm EDC, 5 mM sulfo-NHS and 20 mg 2,2-(ethylenedioxy)bis(ethyleneamine), and incubated for 2 hrs, obtaining FePt-Au nanoparticles coated with cross-linked-BSA, carbodextran or PAA-PEG. The FePt-Au nanoparticles coated with cross-linked-BSA, carbodextran or PAA-PEG were purified using the Sephadex G25 column (Fig. 2D) (Table 2, Nos. 13-16).

EXAMPLE 11: Preparation of BBB-penetrating nanoparticles having Gd$_2$O$_3$ core coated with BSA, carbodextran, or polyacrylate-polyethylene glycol (PAA-PEG)

The water-soluble nanoparticles coated with BSA, carbodextran or PAA-PEG were synthesized according to the methods described in Korean Pat. Nos. 0652251, 064976 and 0713745. In addition, PAA-PEG polymer was prepared according to the method described in Example 7.

200 mg of BSA, carbodextran or PAA-PEG were added to the water-soluble Gd$_2$O$_3$ nanoparticles (5 mL) synthesized in examples 5-6 and incubated for 24 hrs at room temperature. The reaction solution was separated using the Sephacryl S-300 column and non-reactive excess BSA, carbodextran or PAA-PEG were removed,
isolating the nanoparticles. The isolated nanoparticles were condensed to 5 mL with the Centricon YM100 filter. This solution was mixed with 50 mM EDC, 5 mM sulfo-NHS and 20 mg 2,2-(ethylenedioxy)bis(ethylenamine), and incubated for 2 hrs, obtaining Gd₂O₃ nanoparticles coated with cross-linked-BSA, carbodextran or PAA-PEG. The Gd₂O₃ nanoparticles coated with cross-linked-BSA, carbodextran or PAA-PEG were purified using the Sephadex G25 column (Hg. 2E) (Table 2. Nos. 17-20).

The blood-brain barrier-penetrating nanoparticles prepared in the above-described examples 1-10 were summarized as Table 2:

**TABLE 2. List of Blood-brain barrier-penetrating nanoparticles**

<table>
<thead>
<tr>
<th>No.</th>
<th>Chemical formula of inorganic core nanoparticle</th>
<th>Core size (nm)</th>
<th>Water-soluble multi-functional ligand*</th>
<th>Hydrodynamic size (nm)</th>
<th>Salt (NaCl) concentration (mM)</th>
<th>pH stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fe₃O₄</td>
<td>15</td>
<td>TMAOH</td>
<td>~ 18</td>
<td>~ 200</td>
<td>6~9</td>
</tr>
<tr>
<td>2</td>
<td>Fe₃O₄</td>
<td>15</td>
<td>BSA</td>
<td>~ 24</td>
<td>~ 2000</td>
<td>1~13</td>
</tr>
<tr>
<td>3</td>
<td>Fe₃O₄</td>
<td>15</td>
<td>carbodextran</td>
<td>~ 26</td>
<td>~ 2000</td>
<td>1~13</td>
</tr>
<tr>
<td>4</td>
<td>Fe₃O₄</td>
<td>15</td>
<td>PAA-PEG</td>
<td>~ 25</td>
<td>~ 2000</td>
<td>1~13</td>
</tr>
<tr>
<td>5</td>
<td>MnFe₂O₄</td>
<td>15</td>
<td>TMAOH</td>
<td>~ 17</td>
<td>~ 200</td>
<td>6~9</td>
</tr>
<tr>
<td>6</td>
<td>MnFe₂O₄</td>
<td>15</td>
<td>BSA</td>
<td>~ 23</td>
<td>~ 2000</td>
<td>1~13</td>
</tr>
<tr>
<td>7</td>
<td>MnFe₂O₄</td>
<td>15</td>
<td>carbodextran</td>
<td>~ 29</td>
<td>~ 2000</td>
<td>1~13</td>
</tr>
<tr>
<td>8</td>
<td>MnFe₂O₄</td>
<td>15</td>
<td>PAA-PEG</td>
<td>~ 25</td>
<td>~ 2000</td>
<td>1~13</td>
</tr>
<tr>
<td>9</td>
<td>(Zn₉₋₄Fe₀₋₆)Fe₂O₄</td>
<td>15</td>
<td>TMAOH</td>
<td>~ 19</td>
<td>~ 200</td>
<td>6~9</td>
</tr>
<tr>
<td>10</td>
<td>(Zn₉₋₄Fe₀₋₆)Fe₂O₄</td>
<td>15</td>
<td>BSA</td>
<td>~ 26</td>
<td>~ 2000</td>
<td>1~13</td>
</tr>
<tr>
<td>11</td>
<td>(Zn₉₋₄Fe₀₋₆)Fe₂O₄</td>
<td>15</td>
<td>carbodextran</td>
<td>~ 28</td>
<td>~ 2000</td>
<td>1~13</td>
</tr>
<tr>
<td>12</td>
<td>(Zn₉₋₄Fe₀₋₆)Fe₂O₄</td>
<td>15</td>
<td>PAA-PEG</td>
<td>~ 24</td>
<td>~ 2000</td>
<td>1~13</td>
</tr>
<tr>
<td>13</td>
<td>FePt-Au</td>
<td>17</td>
<td>TMAOH</td>
<td>~ 20</td>
<td>~ 200</td>
<td>6~9</td>
</tr>
<tr>
<td>14</td>
<td>FePt-Au</td>
<td>17</td>
<td>BSA</td>
<td>~ 28</td>
<td>~ 2000</td>
<td>1~13</td>
</tr>
<tr>
<td>15</td>
<td>FePt-Au</td>
<td>17</td>
<td>carbodextran</td>
<td>~ 30</td>
<td>~ 2000</td>
<td>1~13</td>
</tr>
</tbody>
</table>
Further animal experiments were carried out using the BSA-coated nanoparticle among nanoparticles for crossing the blood-brain barrier prepared according to the examples above.

**EXAMPLE 12: In vivo MRI mouse experiment using BBB-penetrating nanoparticles having MnFe$_2$O$_4$ core**

To determine whether BSA-coated MnFe$_2$O$_4$ nanoparticles synthesized in the example 8 can cross the blood-brain barrier of mouse using MRI, the experiment was carried out as follows. 2 mg/ml of MnFe$_2$O$_4$ nanoparticles coated with BSA were intravenously injected through the tail of Balb/c mouse (n=4) in an amount of 25 mg/kg. MRI was measured at 10 min interval at pre- and post-injection with nanoparticle. T2 signal intensity in brain portion of MRI was measured to examine whether the nanoparticles penetrate into brain. Meanwhile, T2 signal intensity in liver as a control was measured. 3T system (Acheiva; Philips Medical Systems. Best, the Netherlands) having a sense-flex-M coil was used in measurement of MRI. The results of MRI were obtained using T2 FSE (fast spin echo sequence). The illustrative parameters were as follows: resolution = 256 x 256 μm, slice thickness = 1 mm, TE = 100 ms, TR = 4000 ms, FOV= 10 x 10 cm$^2$, number of excitation = 2.

As shown in Fig. 3a, it was demonstrated that T2 signals in brain were decreased at post-injection and the maximal reduction of signal was reached at
about 20 min. At about 30 min, T2 signal in brain was recovered to 80% compared to the signal of pre-injection. The reason why the signal in brain is dropped until 20 min is due to accumulation of nanoparticles in vessel of brain and hereafter the T2 signal is raised since the nanoparticles remaining in vessel which are unable to cross the blood-brain barrier were washed. Therefore, it is supposed that the MRI signal after 30 min is practically caused from the blood-brain barrier-penetrated nanoparticles. On the other hand, T2 signal in liver as control was not changed at pre- and post-injection with nanoparticle, demonstrating that the nanoparticles of the present invention penetrates the blood-brain barrier selectively and have effective brain imaging effect. Fig. 3b represented the brain portion as MRI image of Hg. 3a in color scale. In Fig. 3b, the red color before nanoparticle injection represents that MRI signals are absent, and each blue and green color after 20 min and 50 min represents that MRI signals are constantly detected in brain.

EXAMPLE 13: Ex vivo biodistribution of BBB-penetrating nanoparticles having MnFe$_2$O$_4$ core

To investigate biodistribution of BSA-coated MnFe$_2$O$_4$ nanoparticles synthesized in the Example 8, tissue analysis of Balb/c mouse injected with nanoparticle according to the method of the Example 11 was carried out (Fig. 4). Tissues were analyzed by a fluorescence microscope using a green fluorescent substance (fluorescein) attached to the surface of nanoparticles. Tissue analysis was performed as follows: each tissue of mice (brain, liver, kidney, heart, lung, muscle, spleen, lymph node, testes, and thymus) was analyzed using a fluorescence microscope at 5 min, 30 min, 60 min, 4 hrs, 16 hrs, 48 hrs and 7 days. As represented in Fig. 4, green fluorescence in brain was constantly detected until 1 hr, demonstrating that the nanoparticles of the present invention penetrate the blood-brain barrier and exist in brain. However, it is evident that in other internal organs, the nanoparticles was hardly detected or rapidly disappeared within a short time.
although it is observed.

**EXAMPLE 14: Ex vivo biodistribution of BBB-penetrating nanoparticles having Fe₃O₄ core**

To investigate biodistribution of BSA-coated Fe₃O₄ nanoparticles synthesized in the Example 7, tissue analysis of Balb/c mouse injected with Fe₃O₄ nanoparticle according to the method of the Example 11 was carried out (Fig. 5). Tissues were analyzed by a fluorescence microscope using fluorescein attached to the surface of nanoparticles. Tissue analysis was performed as follows: each tissue of mice (brain, liver, kidney, heart, lung, muscle, spleen, lymph node, testes, and thymus) was analyzed using a fluorescence microscope at 5 min, 30 min, 60 min, 4 hrs, 16 hrs, 48 hrs and 7 days. As represented in Fig. 5, green fluorescence in brain was constantly detected until 30 min, demonstrating that the nanoparticles of the present invention penetrate the blood-brain barrier and exist in brain. In case of Fe₃O₄ nanoparticle, the nanoparticles were detected in the internal organ such as liver until 60 min after 30 min.

**EXAMPLE 15: In vivo MRI mouse experiment using BBB-penetrating nanoparticles having (Zn₀₄Fe₀₆)Fe₂O₄ core**

To examine whether BSA-coated (Zn₀₄Fe₀₆)Fe₂O₄ nanoparticles synthesized in the Example 9 can cross the blood-brain barrier of mouse using MRI, the experiment was carried out as follows. 2 mg/ml of (Zn₀₄Fe₀₆)Fe₂O₄ nanoparticles coated with BSA were intravenously injected through the tail of Balb/c mouse (n=4) in an amount of 25 mg/kg. MRI was measured at 10 min interval at pre- and post-injection with nanoparticle. T2 signal intensity in brain portion of MRI was measured to investigate whether the nanoparticles penetrate into brain. Meanwhile, T2 signal intensity in liver as a control was also measured.

3T system (Acheiva; Philips Medical Systems. Best, the Netherlands) having a
sense-flex-M coil was used in measurement of MRI. The results of MRI were obtained using T2 FSE (fast spin echo sequence). The illustrative parameters were as follows: resolution = 256 x 256 µm, slice thickness = 1 mm, TE = 100 ms, TR = 4000 ms, FOV= 10 x 10 cm^2, number of excitation = 2.

As shown in Fig. 6a, it was demonstrated that T2 signals in brain were decreased at post-injection with the nanoparticle of the present invention and the maximal reduction of signal was reached at about 20 min. At about 30 min, T2 signal in brain was recovered a little and hereafter maintained in a constant level. In addition, it is supposed that the MRI signal after 30 min is practically caused from the blood-brain barrier-penetrated nanoparticles. On the other hand, T2 signal in liver as control was not changed at pre- and post-injection with nanoparticle, demonstrating that the nanoparticles of the present invention penetrates the blood-brain barrier selectively and have effective brain imaging effect. Fig. 6b represented the brain portion as MRI image of Fig. 6a in color scale. In Fig. 6b, the red color before nanoparticle injection represents that MRI signals are absent, and the blue colors after 20 min and 50 min represents that MRI signals are constantly detected in brain.

EXAMPLE 16: Experiment for BBB penetration using the carbodextran-coated nanoparticle having (Zn_{0.4}Fe_{0.6})Fe_2O_4 core

The (Zn_{0.4}Fe_{0.6})Fe_2O_4 nanoparticles synthesized according to Korean Pat. No. 0604975 were coated with the water-soluble multi-functional ligand, carbodextran, according to the methods described in Korean Pat. Nos. 0652251, 064976 and 0713745. 2 mg/ml of (Zn_{0.4}Fe_{0.6})Fe_2O_4 nanoparticles were intravenously injected through the tail of Balb/c mouse (n=3) in an amount of 25 mg/kg. MRI was measured at 10 min interval at pre- and post-injection with nanoparticle. T2 signal intensity in brain portion of MRI was measured to investigate whether the
nanoparticles penetrate into brain. MRI was measured according to the same method represented in Example 12 and performed using coronal imaging.

Fig. 7a is a T2 MRI signal graph in brain of a rat at 10 min intervals. Fig. 7b represents color images in brain portion of coronal MRI image photographed. Red color indicates that T2 signal is weak and the migration to blue color is in accordance with increase in T2 signal. According to Fig. 7a, it was demonstrated that T2 signals in brain were decreased at post-injection with the nanoparticle until about 20 min (= signal increase). After that, T2 signal in brain was again increased and hereafter maintained in a constant value (2,000 msec). This is the value reduced up to 30% level compared to that at pre-injection with nanoparticle (2,800 msec), supposing that the amount of the nanoparticle is penetrated into brain through crossing the blood-brain barrier. As explained above, it is supposed that T2 signals reduced to about 1,700 msec until 20 min are derived from the nanoparticles present at vessel of brain immediately after injection. This is demonstrated in MRI images of Fig. 7b. Overall, the green and blue colors are detected at 20 min post-injection with the nanoparticle compared to those at pre-injection with the nanoparticle, and the green colors at 50 min post-injection with the nanoparticle.

As a result, it could be appreciated that the nanoparticles coated with the water-soluble multi-functional ligand penetrates the blood-brain barrier.

**COMPARATIVE EXAMPLE:** Experiment for BBB penetration using the \( (\text{Zn}_{0.4}\text{Fe}_{0.6})\text{Fe}_2\text{O}_4 \) nanoparticles with no use of the water-soluble multi-functional ligand.

The \((\text{Zn}_{0.4}\text{Fe}_{0.6})\text{Fe}_2\text{O}_4 \) nanoparticles synthesized according to Korean Pat. No. 0604975 were coated with other kind of surface modified substance, SiO\(_2\), instead of the water-soluble multi-functional ligand using the method described in J. Ying et al. *J. Am. Chem. Soc.*, 2005, 127, 4990. In detail, the nanoparticle and an emulsifying agent (Igepal CO-520, Sigma) were mixed in cyclodexan and then TEOS
(Tetraethylorthosilicate, Sigma) was added to the solution. Consequently, the surface of the nanoparticle was coated with the silica. It was evaluated using MRI whether the nanoparticle prepared penetrates the blood-brain barrier of mouse.

2 mg/ml of the nanoparticles were intravenously injected through the tail of Balb/c mouse (n=3) in an amount of 25 mg/kg. MRI was measured at 10 min interval at pre- and post-injection with nanoparticle. T2 signal intensity in brain portion of MRI was measured to examine whether the nanoparticles penetrate into brain. MRI was measured according to the same method represented in Example 12 and performed using axial imaging.

Fig. 8a is a T2 MRI signal graph in brain of a rat at 10 min intervals. Fig. 7b represents color images in brain portion of coronal MRI images Red color indicates that T2 signal is weak and the migration to blue color is in accordance with increase in T2 signal. As shown in Fig. 8a, T2 signal in brain was not changed at pre- and post-injection with the nanoparticle, suggesting that the nanoparticles do not reach at brain. This is demonstrated in MRI images of Fig. 8b. Therefore, it is demonstrated that image signal intensity at pre-/post-injection with the nanoparticle is not changed. As a result, it could be appreciated that in the nanoparticles without the water-soluble multi-functional ligand adopted in the present invention, the nanoparticles do not penetrate the blood-brain barrier regardless of the nanoparticle having the same core.

Having described a preferred embodiment of the present invention, it is to be understood that variants and modifications thereof falling within the spirit of the invention may become apparent to those skilled in this art, and the scope of this invention is to be determined by appended claims and their equivalents.
What is claimed is:

1. A nanoparticle for penetration of a blood-brain barrier, comprising: (a) an inorganic nanoparticle core; and (b) a water-soluble multi-functional organic ligand coated on the nanoparticle core.

2. The nanoparticle for penetration of the blood-brain barrier according to claim 1, wherein the nanoparticle passes the blood-brain barrier to be distributed in the brain with no use of an additional adjuvant.

3. The nanoparticle for penetration of the blood-brain barrier according to claim 1, wherein the inorganic nanoparticle core comprises (i) a magnetic nanoparticle; (ii) an optical nanoparticle; (iii) a metal, an alloy, a metal chalcogen or a metal pnictogen nanoparticle; or (iv) a multi-component hybrid structure nanoparticle thereof.

4. The nanoparticle for penetration of the blood-brain barrier according to claim 3, wherein the magnetic nanoparticle core comprises (i) the metal nanoparticle containing one or more elements selected from transition metal elements, metal and metalloid elements of Groups 13-16 elements, Lanthanide metal elements and Actinide metal elements; (ii) the alloy nanoparticle containing one or more elements selected from transition metal elements, metal and metalloid elements of Groups 13-16 elements, Lanthanide metal elements and Actinide metal elements; (iii) an oxide or metal ferrite nanoparticle containing one or more elements selected from transition metal elements, metal and metalloid elements of Groups 13-16 elements, Lanthanide metal elements and Actinide metal elements; or (iv) the multi-component hybrid structure nanoparticle thereof.

5. The nanoparticle for penetration of the blood-brain barrier according to claim 4, wherein the magnetic nanoparticle core comprises Mn, Fe, Co, Ni, Gd, M_{x}^{a}, M_{y}^{b},
M^a M^b M^c (M^a = one or more elements selected from the group consisting of Co, Fe, Mn, Ni, Mo, Si, Al, Cu, Pt, Sm, B, Bi, Cu, Sn, Sb, Ga, Ge, Pd, In, Au, Ag or Y, or Lu; M^b or M^c = one or more elements selected transition metal elements, metal and metalloid elements of Groups 13-16 elements, Lanthanide metal elements and Actinide metal elements; 0<x<20, 0<y<20, 0<z<20), M^d_x O_y M^e_x M^f_y O_z (M^d = one or more elements selected from transition metal elements selected from the group consisting of Ba, Cr, Mn, Fe, Co, Ni, Cu, Zn, Nb, Mo, Zr, W, Pd, Ag, Pt and Au, and Lanthanide metal elements and Actinide metal elements selected from the group consisting of Gd, Tb, Dy, Ho, Er, Sm and Nd; M^e = one or more elements selected from transition metal elements, metal and metalloid elements of Groups 13-16 elements, Lanthanide metal elements and Actinide metal elements; 0< x ≤ 16, 0< y< 8, 0< z< 8) or the metal ferrite nanoparticle; or the multi-component hybrid structure thereof.

6. The nanoparticle for penetration of the blood-brain barrier according to claim 3, wherein the optical nanoparticle core comprises (i) a fluorescence emission nanoparticle; (ii) a nanoparticle representing surface plasmon resonance (SPR); (iii) a nanoparticle emitting Raman signal; or (iv) the multi-component hybrid structure thereof.

7. The nanoparticle for penetration of the blood-brain barrier according to claim 6, wherein the optical nanoparticle core comprises (i) a Group II/VI semiconductor nanoparticle; (ii) a Group III/V semiconductor nanoparticle; (iii) Au, Ag, Cu, Pt, Pd or Ni nanoparticle; (iv) the nanoparticle that a Raman dye is attached to the nanoparticle of (iii); (v) the nanoparticle containing a fluorescent dye in an inorganic matrix; or (vi) the multi-component hybrid structure thereof.

8. The nanoparticle for penetration of the blood-brain barrier according to claim 3,
wherein the metal nanoparticle core comprises one or more elements selected from Group 1 metal elements, Group 2 metal elements, transition metal elements, metal and metalloid elements of Groups 13-16 elements, Lanthanide metal elements and Actinide metal elements, or the multi-component hybrid structure thereof.

9. The nanoparticle for penetration of the blood-brain barrier according to claim 8, wherein the metal nanoparticle core comprises a transition metal element selected from the group consisting of Co, Mn, Fe and Ni; Lanthanide metal element and Actinide metal element selected from the group consisting of Nd, Gd, Tb, Dy, Ho, Er and Sm; or the multi-component hybrid structure thereof.

10. The nanoparticle for penetration of the blood-brain barrier according to claim 3, wherein the alloy nanoparticle comprises a \( M^x M^y \) or \( M^x M^y M^z \) nanoparticle \( (M^f, M^g \) and \( M^h \) each represents one or more elements selected from the group consisting of Group 1 metal elements, Group 2 metal elements, transition metal elements, metal and metalloid elements of Groups 13-16 elements, Lanthanide metal elements and Actinide metal elements; \( 0<x<20, 0<y<20, 0<z<20 \) or the multi-component hybrid structure thereof.

11. The nanoparticle for penetration of the blood-brain barrier according to claim 10, wherein the alloy nanoparticle comprises the \( M^x M^y \) or \( M^x M^y M^z \) nanoparticle \( (M^f, M^g \) and \( M^h \) each represents one or more elements selected from the group consisting of: Group 1 metal elements; Group 2 metal elements; transition metal elements selected from the group consisting of Ba, Cr, Mn, Fe, Co, Ni, Cu, Zn, Nb, Mo, Zr, Te, W, Pd, Ag, Pt and Au; metal and metalloid elements of Groups 13-16 elements; and Lanthanide metal elements and Actinide metal elements selected from the group consisting of Gd, Tb, Dy, Ho, Er, Sm and Nd; \( 0<x<20, 0<y<20, 0<z<20 \) or the multi-component hybrid structure thereof.
12. The nanoparticle for penetration of the blood-brain barrier according to claim 3, wherein the metal chalcogen nanoparticle core comprises:

- a $M^A_y$ nanoparticle ($M$ represents the element selected from Group 1 metal elements, Group 2 metal elements, transition metal elements, metal and metalloid elements of Groups 13-15 elements, Lanthanide metal elements and Actinide metal elements; $A$ is selected from O, S, Se, Te and Po; $0 < x < 32$, $0 < y < 8$);
- a $M^A_yM^A_z$ nanoparticle ($M$ and $M^l$ each represents one or more elements selected from Group 1 metal elements, Group 2 metal elements, transition metal elements, metal and metalloid elements of Groups 13-15 elements, Lanthanide metal elements and Actinide metal elements; $A$ is selected from O, S, Se, Te and Po; $0 < x < 32$, $0 < y < 32$, $0 < z < 8$); or
- the multi-component hybrid structure thereof.

13. The nanoparticle for penetration of the blood-brain barrier according to claim 3, wherein the metal pnicogen nanoparticle core comprises:

- a $M^A_y$ nanoparticle ($M$ represents the element selected from Group 1 metal elements, Group 2 metal elements, transition metal elements, metal and metalloid elements of Groups 13-14 elements, Lanthanide metal elements and Actinide metal elements; $A$ is selected from N, P, As, Sb or Bi; $0 < x < 32$, $0 < y < 8$);
- a $M^A_yM^A_z$ nanoparticle ($M$ and $M^l$ each represents one or more elements selected from Group 1 metal elements, Group 2 metal elements, transition metal elements, metal and metalloid elements of Groups 13-14 elements, Lanthanide metal elements and Actinide metal elements; $A$ is selected from N, P, As, Sb and Bi; $0 < x < 32$, $0 < y < 32$, $0 < z < 8$); or
- the multi-component hybrid structure thereof.
14. The nanoparticle for penetration of the blood-brain barrier according to claim 3, wherein the inorganic nanoparticle core comprises:

(i) the magnetic nanoparticle \( [M (M = \text{Mn, Fe, Co, Ni, Gd, Tb, Dy, Ho, Er, Sm or Nd}; M^a\text{M}^\beta (M^a\text{and } M^\beta \text{each represents Mn, Fe, Co, Ni, Pt, Gd, Tb, Dy, Ho, Er, Sm or Nd}); \text{M}^m_\text{xFe}_y\text{O}_z (\text{M}^m = \text{Ba, Mn, Fe, Co, Ni or Zn}; 0<x<16, 0<y<16, 0<z<8}); \text{Zn}_w\text{M}^m_\text{xFe}_y\text{O}_z (\text{M}^m \text{represents one or more elements selected from the group consisting of Group 1 metal elements, Group 2 metal elements, group 13 elements, transition metal elements, Lanthanide metal elements and Actinide metal elements}; 0<w<40, 0<x<40, 0<y<40, 0<z<8]) \text{or the metal ferrite nanoparticle;}

(ii) the optical nanoparticle (the Group II/VI semiconductor nanoparticle selected from the group consisting of ZnS, ZnSe, ZnTe, CdS, CdSe, CdTe, HgS, HgSe and HgTe; the Group III/V semiconductor nanoparticle selected from the group consisting of GaN, GaP, GaAs, InN, InP and InAs; or the metal nanoparticle with optical property selected from the group consisting of Au, Ag, Cu, Pt and Ni);

(iii) the metal nanoparticle, \( M (M = \text{Ba, Cr, Mn, Fe, Co, Zn, Nb, Mo, Zr, Te, W, Pd, Gd, Tb, Dy, Ho, Er, Sm or Nd}); \text{M}^m_\text{xM}^\beta_\text{y} (\text{M}^m \text{and } M^\beta \text{each represents one or more elements selected from the group consisting of: Group 1 metal elements; Group 2 metal elements; transition metal elements selected from the group consisting of Ba, Cr, Mn, Fe, Co, Ni, Cu, Zn, Nb, Mo, Zr, Te, W, Pd, Ag, Pt and Au; Group 13 elements; Group 14 elements; Group 15 elements; Group 16 elements; and Lanthanide metal elements and Actinide metal elements selected from the group consisting of Gd, Tb, Dy, Ho, Er, Sm and Nd}; 0<x<20, 0<y<20); \text{or the metal oxide nanoparticle, } M^1_\text{xO}_y, \text{ in the metal chalcogen nanoparticle } (M^1 = \text{one or more elements selected from Ba, Cr, Co, Fe, Mn, Ni, Cu, Zn, Nb, Pd, }
15. The nanoparticle for penetration of the blood-brain barrier according to any one of claims 3-15, wherein the multi-component hybrid structure has a core-shell, a multi-core shell, a heterodimer, a trimer, a multimer, a barcode or a co-axial rod structure.

16. The nanoparticle for penetration of the blood-brain barrier according to claim 1, wherein the water-soluble multi-functional organic ligand comprises an attachment region (U) to be linked to the surface of the inorganic nanoparticle core.

17. The nanoparticle for penetration of the blood-brain barrier according to claim 16, wherein the attachment region is bound to the surface of the inorganic nanoparticle core through one or more bonds selected from the group consisting of an ionic bond, a covalent bond, a hydrogen bond, a hydrophobic interaction and a coordination bond.

18. The nanoparticle for penetration of the blood-brain barrier according to claim 16, wherein the water-soluble multi-functional organic ligand comprises an active ingredient-binding region (Ln) for bonding of active ingredients, and/or a cross-linking region (Lm) for cross-linking between water-soluble multi-functional organic ligands.

19. The nanoparticle for penetration of the blood-brain barrier according to claim 16, wherein the attachment region (U) comprises a functional group selected from the group consisting of -CHO, -COOH, -NH₂, -SH, -CONH₂, -PO₃H, -OPO₄H, -SO₃H, -
OSO₃H, -N₃, -NR₃OH (R=CₙH₂₄₋₁₆, 0<n<16), -OH, -SS-, -NO₂, -COX (X = F, Cl, Br or I), -COOCO-, -CONH-, -CN and hydrocarbon having two or more carbon atoms.

20. The nanoparticle for penetration of the blood-brain barrier according to claim 18, wherein the active ingredient-binding region (Ln) comprises one or more functional groups selected from the group consisting of -CHO, -SH, -COOH, -NH₂, -OH, -PO₃H, -PO₄H₂, -SO₃H, -OSO₃H, -NR₃⁺X⁺ (R=CₙH₄₋₁₆, 0<n<16, 0<m≤34, X = OH, Cl or Br), NR₄⁺X⁺ (R=CₙH₄₋₁₆, 0<n<16, 0<m<34, X = OH, Cl, Br), -N₃, -SCOCH₃, -SCN, -NCS, -NCO, -CN, -F, -Cl, -Br, an epoxy group, -ONO₂, -PO(OH)₂, -C=NNH₂, -HC=CH- and -C≡C-.

21. The nanoparticle for penetration of the blood-brain barrier according to claim 18, wherein the cross-linking region (Lm) comprises one or more functional groups selected from the group consisting of -SH, -CHO, -COOH, -NH₂, -OH, -PO₃H, -PO₄H₂, -SO₃H, -OSO₃H, -NR₃⁺X⁺ (R=CₙH₄₋₁₆, 0<n≤16, 0<m<34, X = OH, Cl or Br), NR₄⁺X⁺ (R=CₙH₄₋₁₆, 0<n≤1₆, 0≤m≤34, X = OH, Cl, Br), -N₃, -SCOCH₃, -SCN, -NCS, -NCO, -CN, -F, -Cl, -Br, an epoxy group, -ONO₂, -PO(OH)₂, -C=NNH₂, -HC=CH- and -C≡C-.

22. The nanoparticle for penetration of the blood-brain barrier according to claim 1, wherein the water-soluble multi-functional organic ligand comprises a chemical monomer, a polymer, an amphiphilic ligand, a carbohydrate, a peptide or a protein.

23. The nanoparticle for crossing the blood-brain barrier according to claim 22, wherein the water-soluble multi-functional organic ligand comprises at least one polymer selected from the group consisting of polyphosphagen, polylactide, polylactide-co-glycolide, polycaprolactone, polyanhydride, polymaleic acid, a derivative of polymaleic acid, polyalkylcyanoacrylate, polyhydroxybutylate, polycarbonate, polyorthoester, polyethylene glycol, poly-L-lysine, polyglycolide,
polymethyl methacrylate and polyvinylpyrrolidone.

24. The nanoparticle for penetration of the blood-brain barrier according to claim 22, wherein the water-soluble multi-functional organic ligand comprises one or more carbohydrates selected from the group consisting of glucose, mannose, fucose, N-acetyl glucosamine, N-acetylneuraminic acid, fructose, xylose, sorbitol, sucrose, maltose, glycoaldehyde, dihydroxyacetone, erythrose, erythrulose, arabinose, xylulose, lactose, trehalose, mellibiose, cellobiose, raffinose, melezitose, maltoriose, stachyose, estrodose, xylan, araban, hexosan, fructan, galactan, mannan, agaropeptin, alginic acid, carrageenan, hemicellulose, hypromellose, amylose, deoxyacetone, glyceraldehyde, chitin, agarose, dextrin, ribose, ribulose, galactose, carboxy methylcellulose, glycogen dextran, carbodextran, polysaccharide, cyclodextran, pullulan, cellulose, starch and glycogen.

25. The nanoparticle for penetration of the blood-brain barrier according to claim 22, wherein the water-soluble multi-functional organic ligand comprises a peptide having an amino acid residue comprising the functional group selected from the group consisting of -SH, -COOH, -NH₂ and -OH as a side chain.

26. The nanoparticle for penetration of the blood-brain barrier according to claim 22, wherein the water-soluble multi-functional organic ligand is selected from the group consisting of albumins, avidins, antibodies, cytochromes, caseins, myosins, glycinins, transferrins, hormones, cytokines, carotenes, collagens, globular proteins and light proteins.

27. The nanoparticle for penetration of the blood-brain barrier according to claim 18, wherein the water-soluble multi-functional organic ligand is cross-linked through the cross-linking region (Lm) or an additional molecular linker.
28. A composition for brain imaging, comprising the nanoparticle for penetration of the blood-brain barrier according to any one of claims 1-27.

29. The composition according to claim 28, comprising a MRI (magnetic resonance imaging) brain-imaging agent.

30. A pharmaceutical composition for brain targeting, comprising the nanoparticle for penetration of the blood-brain barrier according to any one of claims 1-27.
Fig. 1
Fig. 2
Fig. 3a

![Graph showing T2 intensity (msec) over time for Brain and Liver.

Fig. 3b

![Images showing pre, 20 min, and 50 min T2 intensity images for a mouse model.]
Fig. 4

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Fig. 6a

![Graph showing T2 (msec) vs. time (min) for brain and liver]  

Fig. 6b

![Imaging sequences showing T2 values: pre, 20 min, 50 min]
Fig. 7a

![Graph showing T2 intensity (msec) over time.](image)

Fig. 7b

![Images of brain MRI scans showing T2 signal change over time.](image)
Fig. 8a

![Graph showing T2 intensity (msec) over time (pre, immed, 10, 20, 30, 40, 50 min post).]

Fig. 8b

![Images showing T2 scan at pre, post 10 min, and post 50 min.]