(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 December 2011 (22.12.2011)

PCT

(10) International Publication Number
WO 2011/159529 A1

(51) International Patent Classification:
G01N 33/53 (2006.01)

(21) International Application Number:
PCT/US2011/039573

(22) International Filing Date:
8 June 2011 (08.06.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/354,821 15 June 2010 (15.06.2010) US

(71) Applicant *(for all designated States except US)*: **TRUSTEES OF DARTMOUTH COLLEGE** [US/US]; Technology Transfer Office, 11 Rope Ferry Road, Hanover, NH 03755-1404 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **SUPATTAPONE, Surachai** [US/US]; 11 Pine Drive, Hanover, NH 03755 (US). **MILLER, Michael, B.** [US/US]; 7 South Street, Apartment F, Hanover, NH 03755 (US).

(74) Agents: **LICATA, Jane Massey** et al.; Licata & Tyrrell P.C., 66 E. Main Street, Marlton, NJ 08053 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

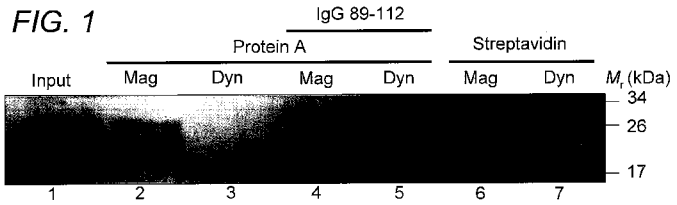
Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(54) Title: METHOD FOR NON-COVALENT IMMOBILIZATION OF INFECTIOUS PRION PROTEIN

FIG. 1



(57) Abstract: The present invention is method for non-covalently immobilizing an infectious prion protein using a magnetic substrate.

WO 2011/159529 A1

METHOD FOR NON-COVALENT IMMOBILIZATION OF INFECTIOUS PRION PROTEIN

Introduction

[0001] This application claims the benefit of priority of U.S. Provisional Application No. 61/354,821, filed June 15, 2010, the content of which is incorporated herein by reference in its entirety.

[0002] This invention was made with government support under grant number R01 NS046478 awarded by National Institutes of Health. The government has certain rights in the invention.

Background of the Invention

[0003] Prion diseases are fatal neurodegenerative illnesses that occur in genetic, sporadic and infectious forms (Glatzel, et al. (2005) *Arch. Neurol.* 62:545-552). From a public health perspective, prion diseases are challenging to control because infectious prions are highly resistant to environmental degradation (Brown & Gajdusek (1991) *Lancet* 337:269-270) and can potentially be transmitted by several different routes (Holada, et al. (2000) *Lancet* 356:1772; Ligios, et al. (2005) *Nat. Med.* 11:1137-1138; Mathiason, et al. (2006) *Science* 314:133-136; Seeger, et al. (2005) *Science* 310:324-326). The critical molecular event in the pathogenesis of prion diseases is the misfolding of the host-encoded prion protein (PrP^{C}) into an infectious isoform (PrP^{Sc}), but the mechanism of this conformational change remains unknown (Prusiner (1982) *Science* 216:136-144). Mature PrP molecules contain 208 amino acid residues, two N-linked glycosylation sites, an intramolecular disulfide bond and a C-terminal glycophosphatidylinositol anchor (Endo, et al. (1989) *Biochemistry* 28:8380-8388; Locht, et al. (1986) *Proc. Natl. Acad. Sci. USA* 83:6372-6376; Stahl, et al.

(1987) *Cell* 51:229-240; Turk, et al. (1988) *Eur. J. Biochem.* 176:21-30). Purified native PrP^{C} molecules containing only prion protein and co-purified lipids have been converted into infectious PrP^{Sc} molecules *de novo*, through an *in vitro* reaction requiring accessory polyanions (Deleault, et al. (2007) *Proc. Natl. Acad. Sci. USA* 104:9741-9746).

[0004] Mammalian prions occur in a variety of different "strains". Strains are defined as natural isolates of infectious prions characterized by distinctive clinical and neuropathological features, which are faithfully recapitulated upon serial passage within the same animal species (Bruce (1993) *Br. Med. Bull.* 49:822-838; Carlson (1996) *Curr. Top. Microbiol. Immunol.* 207:35-47). Strain diversity is associated with variations in PrP^{Sc} conformation (Bessen & Marsh (1992) *J. Virol.* 66:2096-2101; Collinge, et al. (1996) *Nature* 383:685-690; Peretz, et al. (2001) *Protein Sci.* 10:854-863; Safar, et al. (1998) *Nat. Med.* 4:1157-1165; Telling, et al. (1996) *Science* 274:2079-2082), but it remains unknown precisely which PrP^{Sc} conformers or domains are required to encode mammalian prion strain phenotypes.

[0005] Various methodologies have been developed to analyze and detect the various forms of PrP. For example, conformation-dependent immunoassays (CDI) have shown that prion-infected brains contain both protease-sensitive and protease-resistant PrP^{Sc} molecules (Safar, et al. (1998) *supra*). In addition, chemical cross-linking of recombinant PrP to nanoparticles has been suggested for use in *in vivo* and *in vitro* manipulation of prion proteins to facilitate structural analysis (Kouassi & Irudayaraj (2006) *J. Nanobiotech.* 4:8).

Summary of the Invention

[0006] The present invention is a method for immobilizing an infectious prion protein by contacting the infectious prion protein, *e.g.*, in a biological sample, with a magnetic substrate. In particular embodiments, immobilization is non-covalent and is carried out in the absence of a cross-linking agent. In some embodiments, the magnetic substrate is silanized and/or composed of an iron oxide. In other embodiments, the magnetic substrate is a microparticle, nanoparticle, or nanopowder.

Brief Description of the Drawings

[0007] Figure 1 shows binding of PrP^{Sc} to MAGNABIND beads. RML scrapie-infected mouse brain homogenate was incubated with MAGNABIND (*Mag, lanes 2, 4, and 6*) or DYNAL (*Dyn, lanes 3, 5, and 7*) magnetic beads bearing Protein A or Streptavidin. One set of Protein A reactions (*lanes 3-4*) was co-incubated with IgG 89-112, which recognizes PrP^{Sc}. Following incubation, beads were washed, subjected to Proteinase K digestion, and bound molecules were analyzed by anti-PrP (6D11) immunoblot.

[0008] Figure 2 shows binding of PrP^{Sc} to MAGNABIND Protein A beads treated for protein disruption. MAGNABIND Protein A beads were pre-treated by Proteinase K digestion (25 μg/mL), boiled (95°C for 10 minutes), or centrifuged (14,000x*g* for 10 minutes at 22°C). Untreated control DYNAL Protein A and MAGNABIND Protein A beads were also tested. Following these treatments, beads were washed, and incubated with RML scrapie-infected mouse brain homogenate overnight. Bound PrP^{Sc} molecules were detected by Proteinase K digestion and anti-PrP (6D11) immunoblot.

[0009] Figure 3 shows binding specificity of MAGNABIND beads. Binding of PrP^{Sc} and PrP^{C} molecules to MAGNABIND

Protein A. RML scrapie-infected or uninfected mouse brain homogenates were incubated with MAGNABIND Protein A beads for two hours in tris-buffered saline with 3% NP-40 and 3% TWEEN 20. (Figure 3A) Input, supernatant (Sup) and bound fractions were analyzed for PrP molecules by anti-PrP (6D11) immunoblot. The scrapie brain bound fraction was also analyzed for PrP^{Sc} by Proteinase K digestion (+PK). (Figure 3B) Input and bound fractions were analyzed for total protein by silver staining. All samples were analyzed on the same gel, with white lines indicating excised intervening lanes.

[00010] Figure 4 shows protein misfolding cyclic amplification (PMCA) reactions seeded with MAGNABIND-bound PrP^{Sc}. RML mouse (Figure 4A) or Sc237 hamster (Figure 4B) brain homogenates before (input) or after binding by MAGNABIND Protein A beads (Mag) or by DYNAL Protein A (Dyn) were used to seed PMCA reactions. Normal mouse (Figure 4A) or hamster (Figure 4B) brain homogenates were used as substrate. Prior to PMCA, one set of beads was washed with Sarkosyl detergent (Sarkosyl, Figure 4B). Each reaction mixture was analyzed before (-) or after (+) PMCA. PrP^{Sc} molecules were detected by Proteinase K digestion and anti-PrP (6D11) immunoblot.

[00011] Figure 5 binding of PrP^{Sc} to MAGNABIND, silanized nano-magnetite, and unsilanized nano-magnetite. RML scrapie-infected mouse brain homogenate was incubated with various quantities of MAGNABIND Protein A beads (0.005 - 0.125 mg), silanized magnetite nanoparticles (10 nm size, 0.005 - 2 mg), silanized magnetite nanopowder (<50 nm size, 0.005 - 2 mg), unsilanized magnetic nanoparticles (0.0015 - 2 mg), or unsilanized magnetic nanopowder (0.0015 - 2 mg). PrP^{Sc} molecules were detected by Proteinase K digestion and anti-PrP (6D11) immunoblot.

[00012] Figure 6 shows binding of diverse strain PrP^{Sc} molecules to MAGNABIND or DYNAL beads. Prion-infected brain homogenates from various strains (mouse RML, mouse Me7, hamster 139H) were incubated overnight with MAGNABIND Protein A or DYNAL Protein A beads. Input and bound PrP^{Sc} molecules were detected by Proteinase K digestion and anti-PrP (6D11) immunoblot.

Detailed Description of the Invention

[00013] PrP^{C} is known in the art as the naturally expressed glycoprotein PrP^{C}, also known as PrP-sen, which is found in the neurons of mammals. Not to be held to any particular mechanism of action, it is believed that contact between PrP^{C} and an infectious prion or PrP^{Sc} brings about a conformational change in PrP^{C}, converting it from a protein primarily composed of alpha-helices to a protein primarily composed of beta-sheets. This conversion creates a protease resistant, prion protein (*i.e.*, PrP^{Sc}, PrP-res) associated with prion disease. Therefore, the term "infectious prion protein" is intended to mean a prion protein which is protease resistant and causes a prion-associated disease.

[00014] It has now been found that magnetic substrates, *e.g.*, magnetic iron oxide substrates, non-covalently bind the infectious conformer of the prion protein, Prp^{Sc}, selectively and with high affinity (see Figures 1 and 2). Furthermore, immobilized Prp^{Sc} serves as a competent seed for prion amplification techniques such as Protein Misfolding Cyclic Amplification (PMCA)(See Figure 4). Moreover, magnetic substrates bind to Prp^{Sc} molecules from a variety of prion isolates in different animal species (Figures 3 and 6), but do not bind the normal conformer of the prion protein, Prp^{c}, or the vast majority of other proteins. Immobilization of Prp^{Sc} appeared to be independent

of substrate size and coating (*i.e.*, silanization) as silanized and unsilanized magnetic beads, nanoparticles or nanopowders could immobilize prion protein (see Figure 5).

[00015] Accordingly, the present invention features methods for immobilizing infectious prion protein using a magnetic substrate, compositions containing substrate-bound Prp^{Sc}, and methods of using the same. The magnetic substrate of the invention can be ferromagnetic, paramagnetic or superparamagnetic. Paramagnetic materials are characterized by a weak, positive magnetic susceptibility and by their inability to remain magnetic in the absence of an applied magnetic field. Ferromagnetic materials have high, positive magnetic susceptibilities and maintain their magnetism in the absence of an applied field. Like paramagnetic materials, superparamagnetic materials are characterized by an inability to remain magnetic in the absence of an applied magnetic field. Superparamagnetic materials can have magnetic susceptibilities nearly as high as ferromagnetic materials and far higher than paramagnetic materials (Bean & Livingston (1959) *J. Appl. Phys.* 30(Suppl.):1205). In particular embodiments, the magnetic substrate of the invention is paramagnetic or superparamagnetic.

[00016] Paramagnetic substrates of the invention preferably contain transition metal ions, such as iron, manganese, gadolinium, and/or copper ions. Additional materials suitable for preparation of paramagnetic substrates include transition metals such as titanium, vanadium, chromium, cobalt, and nickel, lanthanide metals such as europium, and/or actinide metals such as protactinium. These metals may be independently selected or excluded for use in different embodiments of the invention. Paramagnetic ions have unpaired electrons, resulting in a positive magnetic

susceptibility. Preferably, the paramagnetic substrates contain a relatively non-toxic metal such as iron.

[00017] Superparamagnetic substrates are composed of substances like ferrite which are ferromagnetic in bulk but which, because of the very small particle size, have lost their permanent magnetism. Generally, superparamagnetic particles have a particle size that ranges from about 30 to 50 nanometers (about 300 to 500 angstroms (Å)). Particles in this size range are impacted by both thermal effects, which quench the magnetic field, and magnetic ordering effects, with the result that the magnetic vector is unstable and fluctuates in the same way as for paramagnetic materials. Superparamagnetic materials possess high magnetic susceptibility and crystalline structures found in ferromagnetic materials, but rapidly lose their magnetic properties in the absence of an applied magnetic field. Superparamagnetic materials are preferably iron oxides such as iron hydroxide, iron oxides, iron oxide hydrates, or iron mixed oxides. Superparamagnetic particles exhibit stronger magnetic effects than paramagnetic particles of an equivalent size. For example, an iron oxide superparamagnetic particle may exhibit a magnetic field that is about 50,000 times stronger than the magnetic field exhibited by a similarly-sized gadolinium-based paramagnetic particle. Accordingly, in particular embodiments, the magnetic substrate of the invention contains an iron oxide.

[00018] Preferably, the magnetic substrate of the invention is a microparticle (*e.g.*, a bead having a diameter in the range of 1 to 500 μm), nanoparticle ((*e.g.*, a particle having a diameter in the range of 1 to 999 nm), or nanopowder. The selection of the magnetic substrate may be dependent on a number of factors including, *e.g.*, the

intended used of the substrate. For example, a microparticle may be desirable when the substrate is used for diagnostic detection of a prion disease or disinfection of a blood sample, whereas a nanoparticle or nanopowder may be desirable when the magnetic substrate is used in the treatment of prion diseases, *i.e.*, such substrates would reduce the chance of triggering an immune response or thrombosis. Moreover, a small size helps to enhance the half life of the particles in circulation. The size of the magnetic nanoparticles may be controlled, for example, by selection of reaction conditions such as temperature, presence and type of stabilizing agent, ratio of metallic salts to surfactants, and the like. See, *e.g.*, Murray, et al. (2001) *IBM. J. Res. Dev.* 45:47-56.

[00019] Substrates of the invention can be manufactured to be chemically and magnetically stable, and to have a high magnetic moment. Stability may optionally be enhanced, for example, by coating the magnetic substrate with a noble metal surface. Such a surface can improve both oxidative and magnetic stability. Methods of coating magnetic nanoparticles with a noble metal shell are known in the art. See, *e.g.*, Park, et al. (2001) *J. Am. Chem. Soc.* 123:5743-5746.

[00020] As demonstrated herein, silanized magnetic substrates also selectively and efficiently bind infectious prion protein. Accordingly, the present invention also embraces the use of a magnetic substrate that is silanized. Silanization can be carried out as described herein or by any suitable conventional method using organic or inorganic molecules or with organic-inorganic mixed structures (Durdureanu-Angheluta, et al. (2008) *Dig. J. Nanomater. Biostruct.* 3:33-40). For example, magnetic substrates coated with 3-(trimethoxy-silyl)propyl methacrylate, 3-

aminopropyltriethoxysilane, allyltriethoxysilane or methyltriethoxysilane can be used to confer a hydrophobic or hydrophilic nature to the magnetic substrates described herein and determine stability of the same in adequate solvents. Moreover, when the magnetic substrate of the invention is a nanoparticle or nanopowder, silanization promotes self-assembly of the nanoparticles or nanopowder thereby increasing their dimensions (Durdureanu-Angheluta, et al. (2008) *supra*).

[00021] The results herein demonstrate that contact of an infectious prion protein with a magnetic substrate (optionally silanized) non-covalently immobilizes the infectious prion protein. In this respect, the immobilization of an infectious prion protein in accordance with the present invention is carried out in the absence of a cross-linking agent or other functional or reactive group that covalently binds or has the potential to covalently bind (*i.e.*, in the presence of a crosslinking agent) the prion protein to the magnetic substrate. Functional or reactive groups conventionally used in the art for covalent binding include, *e.g.*, carbodiimides, ketones, imides, oximes, thioesters, thioamines, and the like. Examples of cross-linking agents include but are not limited to *N*-hydroxysuccinimide used in carbodiimide activation, dimethyl suberimidate, glyoxal, glutaraldehyde, epichlorohydrin, recombinant protein linkers or spacers and the like.

[00022] The analysis of biological samples (*e.g.*, for research, diagnostic or forensic purposes) begins with complex mixtures such a blood, serum or cell suspensions that contain not only the analytes of interest, but also a great variety of constituents which may interfere with the intended analysis. As with standard analytical chemical

separation, it is generally desirable if not necessary to separate the analyte fraction of sample from the remainder. The suitability of certain magnetic particles for this purpose has been widely documented in the prior art, said particles, when used in the microwell format, generally requiring a high magnetic susceptibility to permit their collection and immobilization within reasonable time in magnetic field gradients which may be generated in a laboratory setting by use of permanent magnets. In this regard, the immobilization of prion proteins with magnetic substrates is of particular use in methods of analyzing prion proteins, wherein prion proteins in a sample are immobilized with a magnetic substrate, the magnetic substrate is washed to remove mobile (non-immobilized) constituents of the original mixture and the bound prion protein is analyzed, *e.g.*, by PMCA or immunoassay. In certain embodiments, magnetic capture of the magnetic substrate during wash cycles employs permanent magnets known in the prior art which achieve temporary immobilization. Moreover, miniaturization of the assay environment ensures that particles always reside within a short distance of typically not more than 100 μm from the nearest bounding surface of the reaction vessel, thereby reducing the time required to collect particles of given magnetic susceptibility from suspension into a magnetic gradient, or, conversely, to minimize the requisite magnetic susceptibility to ensure trapping within a given collection time, typically not more than 5 minutes and preferably not more than 0.5 minutes, by a magnetic field and field gradient of given strength.

[00023] In addition to diagnostic applications, immmobilization of prion proteins with magnetic substrates can be used to effectively remove infectious prion proteins

from blood and/or plasma supplies. Moreover, it is contemplated that magnetic nanoparticles could be of use in capturing infectious prion protein *in vivo* thereby facilitating the prevention or treatment of a prion-associated disease. In each application, a sample (*e.g.*, a blood sample) or subject (a subject diagnosed with an infectious prion protein) is provided with a magnetic nanoparticles so that the infectious prion protein binds to the magnetic nanoparticle and the infectious prion protein-bound magnetic nanoparticle is removed using, *e.g.*, a magnet.

Example 1: Materials and Methods

[00024] *Preparation of Scrapie-Infected and Uninfected Brain Homogenate*. CD-1 Mouse (strains RML, Me7, and 301C) and Syrian hamster (Sc237, 139H, Drowsy) scrapie-infected brains were homogenized (Covidien tissue grinder, Mansfield, MA) to 10% in phosphate-buffered saline (PBS, pH 7.4; Cellgro, Manassas, VA). Uninfected CD-1 mouse and Syrian hamster brains (Biochemed, Winchester, VA) were homogenized in the same manner. Homogenates were initially clarified by centrifugation at 200x*g* for 30 seconds and stored at -70°C. Freshly clarified 5% homogenate for each experiment was prepared by adding an equal volume of tris-buffered saline (TBS: 50 mM Tris, 200 mM NaCl, pH 7.5), vortexing for 15 seconds, sonicating (Misonix 4000 with Microplate Horn; Qsonica, Newtown, CT) for 1 minute, and centrifuging at 500x*g* for 15 minutes.

[00025] *Preparation of Nanoparticles and Nanopowder*. Iron (II,III) oxide (Fe_3O_4, magnetite) 10 nm nanoparticles (Sigma, St. Louis, MO) in toluene were mixed with an equal volume of methanol, and separated by a Magnetic Particle Separator (PureBiotech, Middlesex, NJ). Iron (II,III) oxide

(Fe_3O_4, magnetite) <50 nm nanopowder was also obtained from Sigma. To silanize (Norén & Kempe (2009) *Int. J. Pept. Res. Ther.* 15:287-292), nanoparticles or nanopowder was resuspended in methanol to 0.11 mg/mL, to which was added 1/10 volume 3-(Trimethoxy-silyl)propyl methacrylate (Sigma). Each was sonicated for 1 minute at 70% power, then incubated for 4.5 hours at 25°C with 300 rpm shaking. Each was then rinsed in methanol, then ethanol.

[00026] *Binding Assays*. MAGNABIND (Pierce, Rockford, IL) or DYNAL (Invitrogen, Carlsbad, CA) magnetic beads, bearing either Protein A or Streptavidin, were magnetically separated from solution. Unless otherwise noted, 25 μL beads (5 μg/mL) were rinsed twice in 500 μL PBS, then incubated in 150 μL assay buffer (TBS, 1% TRITON X-100, 1% TWEEN 20) with 5 μL clarified 5% brain homogenate overnight at room temperature with 10 rpm end-over-end rotation. IgG 89-112 anti-PrP^{Sc} antibody (Moroncini, et al. (2004) *Proc. Natl. Acad. Sci.* 101:10404-9) was added to designated samples at 7.5 μg/mL. Beads were separated from solution and rinsed twice in 500 μL wash buffer (TBS, 0.05% TWEEN 20) before analysis of bound molecules. PrP^{Sc}-PrP^{C} comparison reactions were carried out in TBS with 3% NP-40 and 3% TWEEN 20 for 2 hours, followed by four 1 mL washes in TBS with 2% Sarkosyl.

[00027] *Protein Misfolding Cyclic Amplification (PMCA)*. Following binding, samples were resuspended in 10% CD-1 mouse or Syrian hamster brain homogenate, which was prepared in Soto conversion buffer (PBS, 1% TRITON X-100, Roche Complete mini protease inhibitor; Castilla, et al. (2006) *Methods Enzymol.* 412:3-21) with additional 4 mM EDTA. One round of PMCA included 30 second microplate horn

sonication pulses every 30 minutes for 24 hours at 90% power.

[00028] *Prion Protein Detection*. Bound PrP^{Sc} was detected by subjecting beads to limited proteolysis in 50 μL (25 μg/mL for mouse, 50 μg/mL for hamster) Proteinase K (Roche, Indianapolis, IN) in PBS, 1% TRITON X-100. Proteolysis proceeded for 30 minutes (mouse) or 60 minutes (hamster) at 37°C and 750 rpm shaking, and was terminated by addition of 17 μL 4X sample buffer (217 mM tris pH 6.8, 8.7% (w/v) sodium dodecyl sulfate, 21% (v/v) glycerol, 0.02% (w/v) bromophenol blue, 3M β-mercaptoethanol) and 10-minute incubation at 95°C. PrP was detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), semi-dry transfer to PVDF membrane, immunoblot with anti-PrP antibody 6D11, horseradish peroxidase (HRP)-conjugated anti-mouse sheep antibody, and enhanced chemiluminescence (SUPERSIGNAL West Femto Substrate; Pierce, Rockford, IL). Signals were visualized by a FUJI (Fujifilm) LAS-3000 chemiluminescence documentation system.

[00029] *Silver Stain Detection of Total Protein*. Following SDS-PAGE, the gel was fixed overnight in 50% ethanol/10% acetic acid, then treated with two 10 minute washes in 10% ethanol to remove SDS. Next, the gel was incubated for 2 minutes in Farmer's solution (0.3 g sodium thiosulfate, 0.15 g potassium ferricyanide, 0.05 g sodium carbonate in 100 mL water), followed by four 20-minute washes in water, then 12 minutes of silver staining (0.2 g silver nitrate in 100 mL water). Gel was then treated with developer (3 g sodium carbonate, 50 μL fresh 37% formaldehyde, 100 mL water) for a short rinse and subsequent approximate 8 min. incubation). Progression of staining was halted by addition of stop solution (5% acetic acid in water).

What is claimed is:

1. A method for immobilizing an infectious prion protein comprising contacting an infectious prion protein with a magnetic substrate so that the infectious prion protein is immobilized.

2. The method of claim 1, wherein contact of the infectious prion protein with the magnetic substrate is carried out in the absence of a cross-linking agent.

3. The method of claim 1, wherein the magnetic substrate comprises an iron oxide.

4. The method of claim 1, wherein the magnetic substrate is silanized.

5. The method of claim 1, wherein the magnetic substrate is a microparticle, nanoparticle, or nanopowder.

6. The method of claim 1, wherein the infectious prion protein is present in a biological sample.

7. An infectious prion protein non-covalently bound to a magnetic substrate immobilized by the method of claim 1.

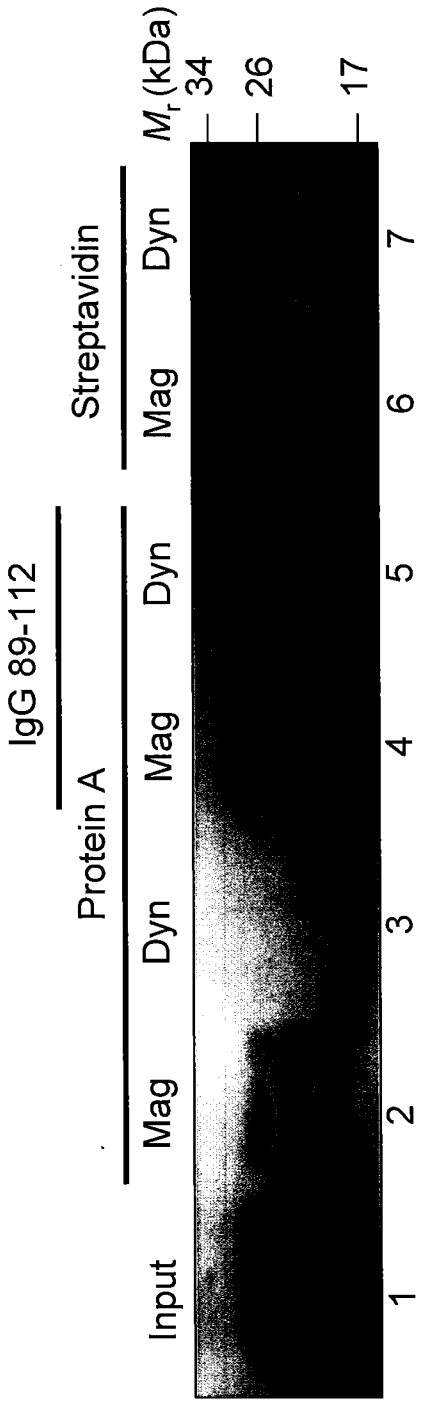

IgG 89-112
Protein A
Streptavidin
Input
Mag
Dyn
Mag
Dyn
Mag
Dyn
1
2
3
4
5
6
7
M_r (kDa)
34
26
17

FIG. 1

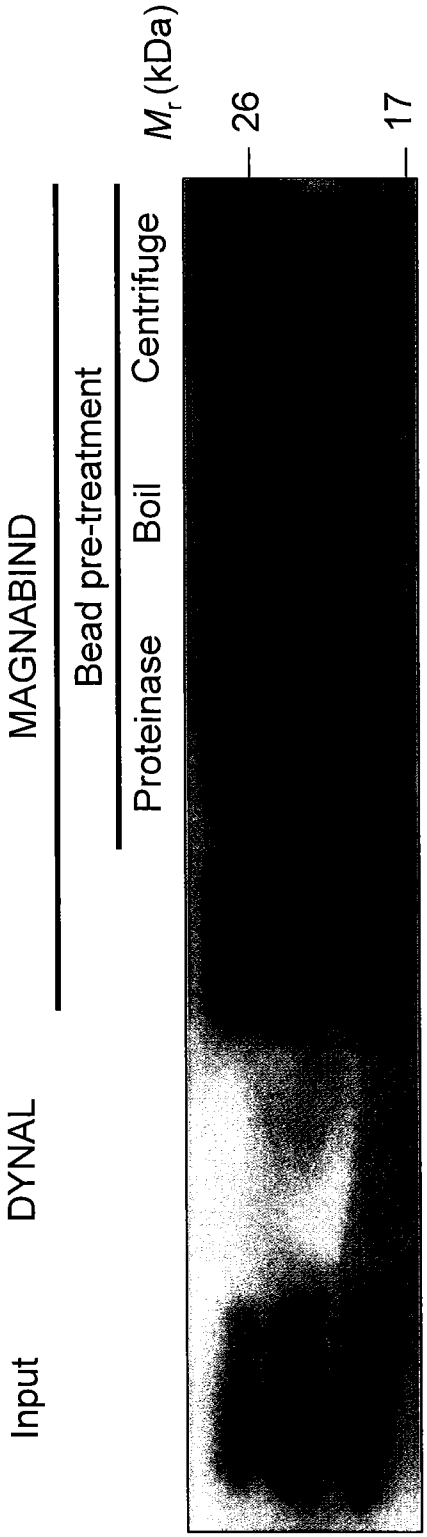

Input
DYNAL
MAGNABIND
Bead pre-treatment
Proteinase
Boil
Centrifuge
M_r (kDa)
26
17

FIG. 2

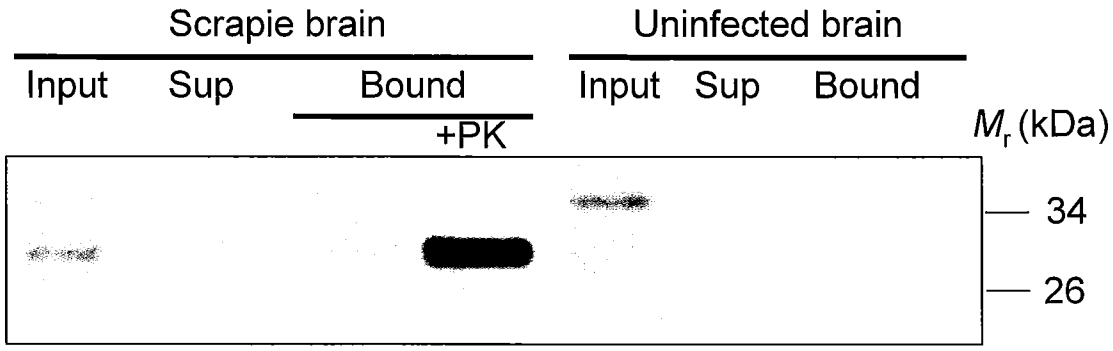

Scrapie brain
Uninfected brain
Input
Sup
Bound
+PK
Input
Sup
Bound
M_r (kDa)
34
26

FIG. 3A

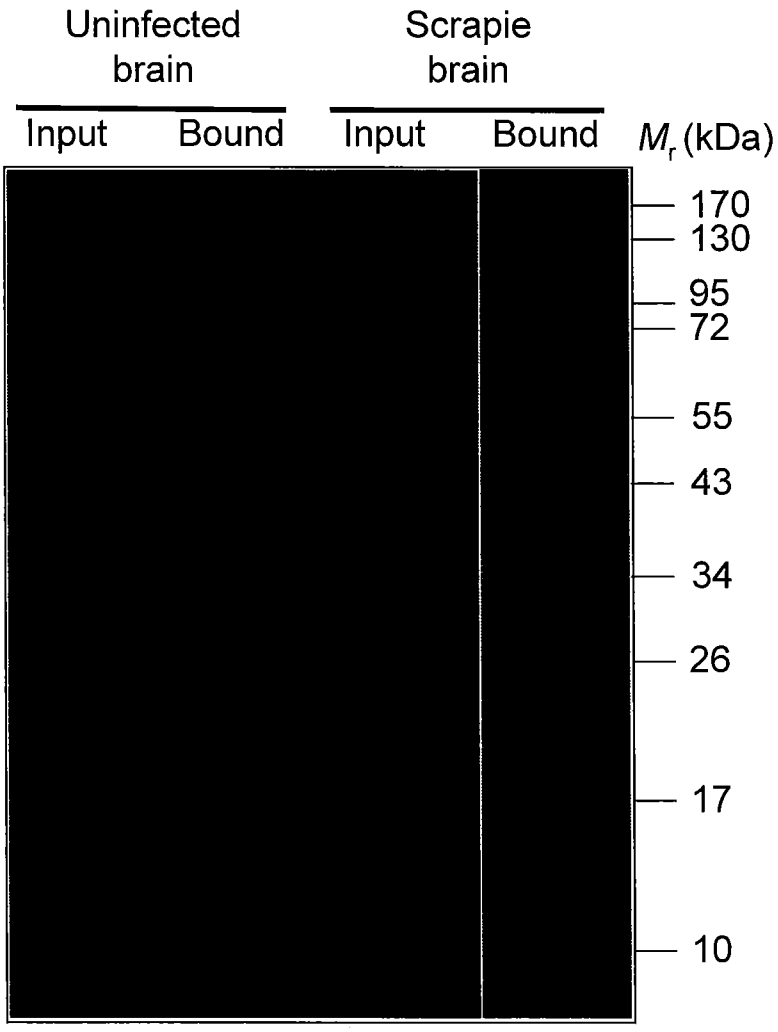

Uninfected brain
Scrapie brain
Input
Bound
Input
Bound
M_r (kDa)
170
130
95
72
55
43
34
26
17
10

FIG. 3B

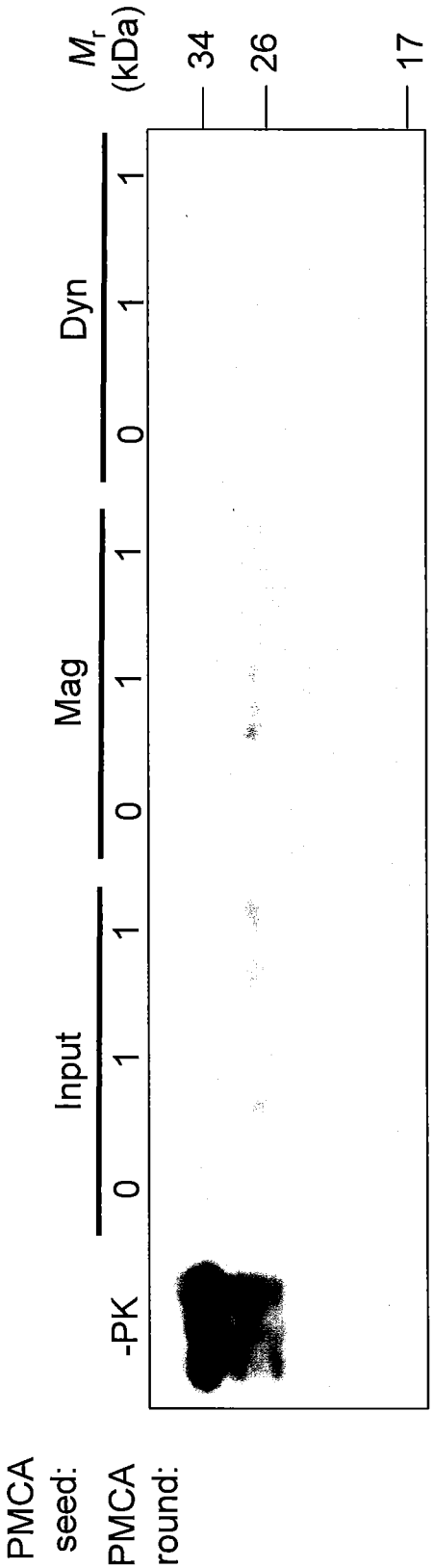

PMCA seed:
PMCA round:
-PK
0
Input
1
1
0
Mag
1
1
0
Dyn
1
1
M_r (kDa)
34
26
17

FIG. 4A

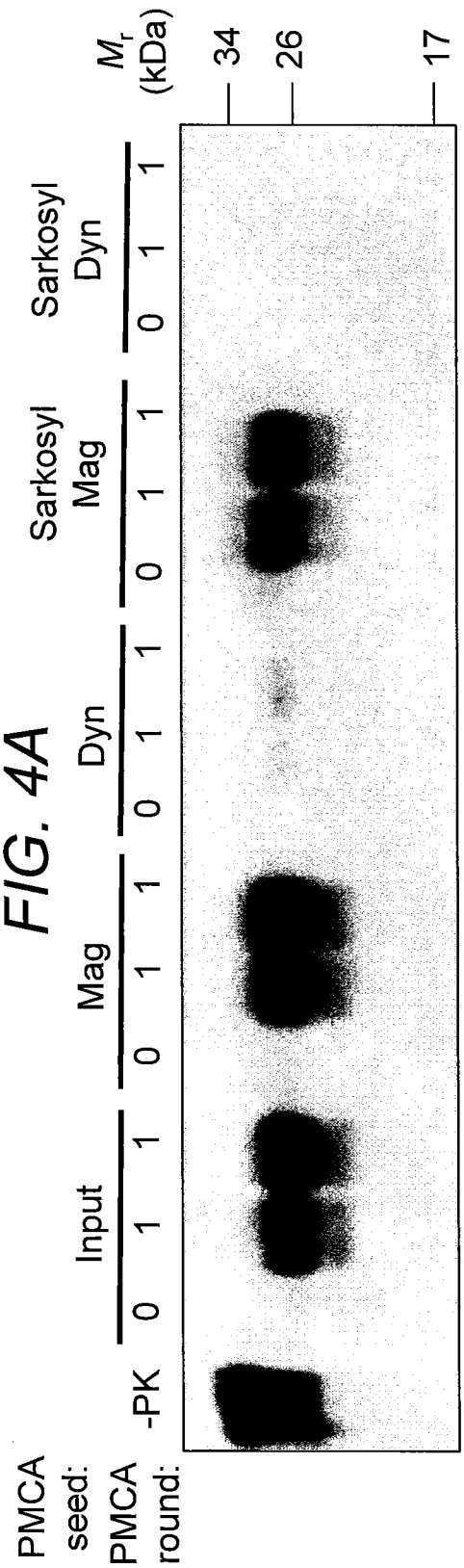

PMCA seed:
PMCA round:
-PK
0
Input
1
1
0
Mag
1
1
0
Dyn
1
1
0
Sarkosyl Mag
1
1
0
Sarkosyl Dyn
1
1
M_r (kDa)
34
26
17

FIG. 4B

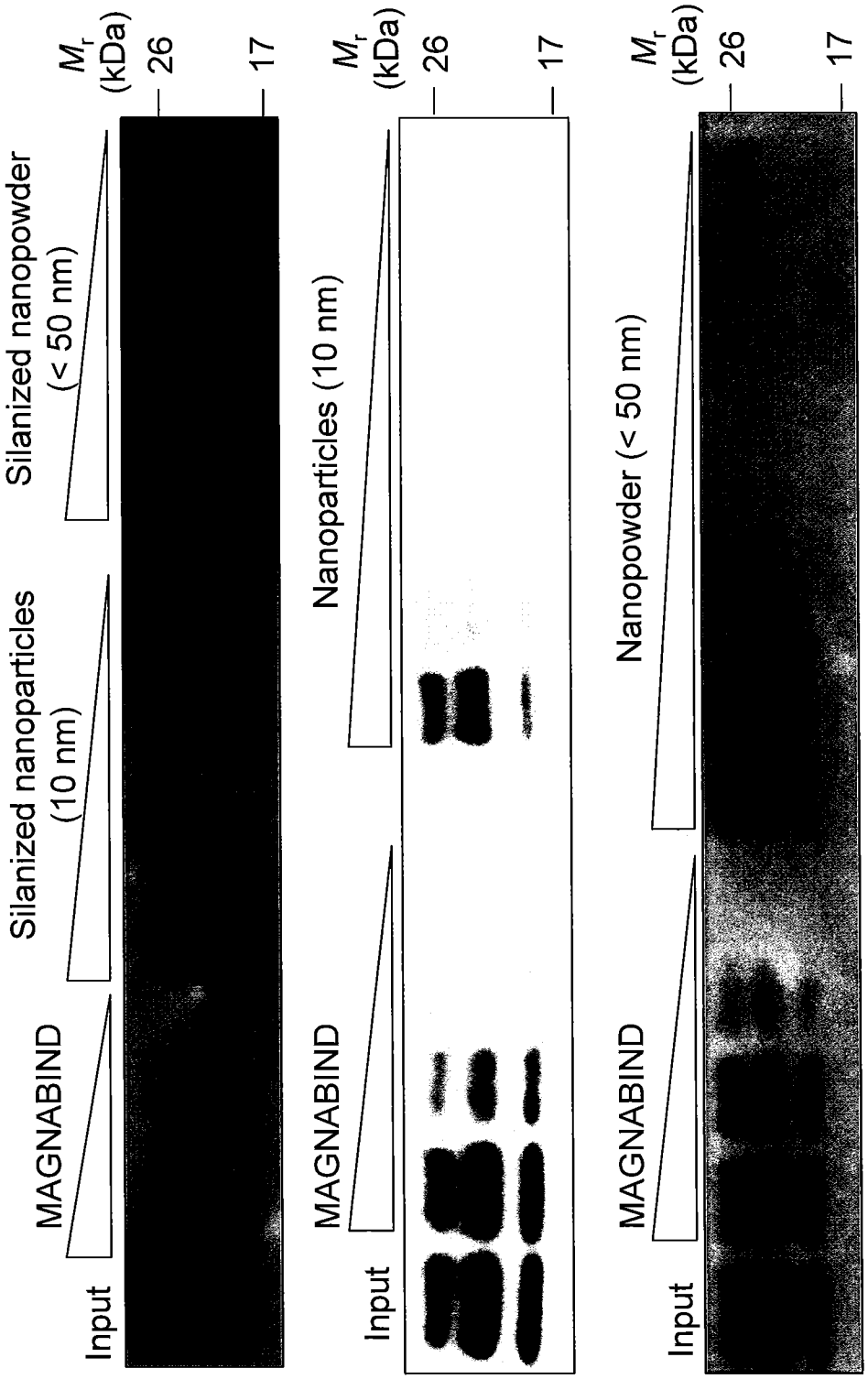

Input
MAGNABIND
Silanized nanoparticles (10 nm)
Silanized nanopowder (< 50 nm)
M_r (kDa)
26
17
Input
MAGNABIND
Nanoparticles (10 nm)
M_r (kDa)
26
17
Input
MAGNABIND
Nanopowder (< 50 nm)
M_r (kDa)
26
17

FIG. 5

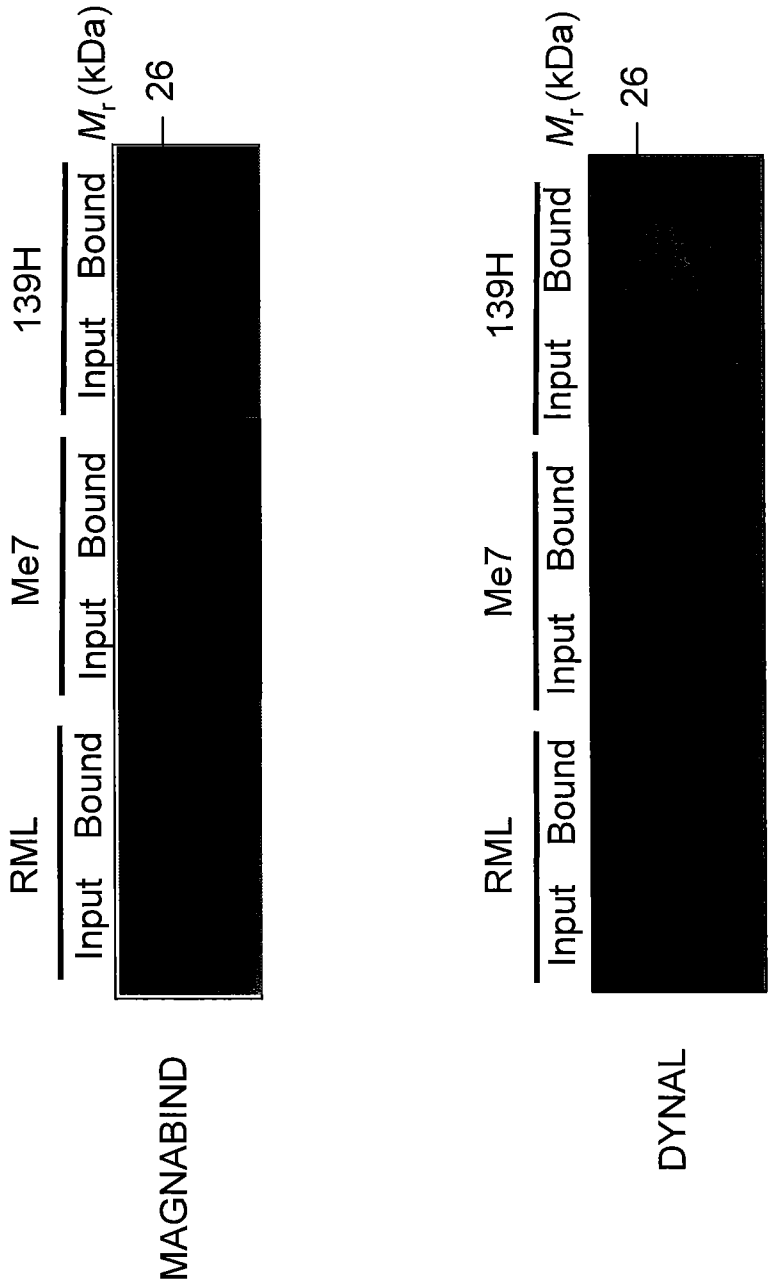

MAGNABIND
RML
Me7
139H
Input
Bound
Input
Bound
Input
Bound
M_r (kDa)
26
DYNAL
RML
Me7
139H
Input
Bound
Input
Bound
Input
Bound
M_r (kDa)
26

FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/39573

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/53 (2011.01)
USPC - 435/7.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC - 435/7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 435/173.1, 174; 530/350 (text search, see terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(PGPB,USPT,EPAB,JPAB); Google/Scholar (text search, see terms below)
Search Terms: Prion, iron, magnet$, bind$, immobiliz$, iron oxide, magnetic bead, microparticle, nanoparticle, nanopowder, non-covalent$, silaniz$

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6,916,419 B2 (PRUSINER et al.) 12 July 2005 (12.07.2005); (col 8, ln 45-48), (col 9, ln 6-15), (col 16, ln 5-10,23-26), (col 17, ln 8-13), (col 18, ln 59-67), (col 19, ln 1-3)	1-3, 5-7
---		---
Y		4
Y	US 2003/0219801 A1 (LIPSHUTZ) 27 November 2003 (27.11.2003); para [0057]	4
A	US 2004/0052833 A1 (PRUSINER et al.) 18 March 2004 (18.03.2004); paras [0170]-[0185], [0208]-[0216]	

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 October 2011 (20.10.2011)	04 NOV 2011
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774