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(71) Applicants and
(72) Inventors: **MOROZOVA-ROCHE, Ludmilla** [RU/SE]; Kvartsvägen 52, S-907 41 Umeå (SE). **MALISAUSKAS, Mantas** [LT/NL]; Roeklaan 1, NL-5613 EG Eindhoven (NL).

(74) Agents: **ALBIHNS AB** et al.; P.O Box 5581, Linnégatan 2, S-114 85 Stockholm (SE).

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(54) **Title:** THIN METAL NANOWIRES PRODUCED BY BIOTEMPLATING

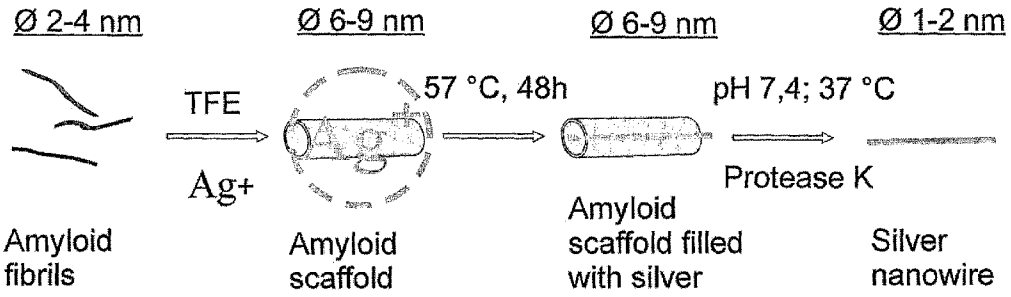

Figure 1

(57) **Abstract:** The present invention provides methods for the manufacturing of metal nanowires using protein fibrils as biotemplates.The methods comprise use of a solvent providing a dualeffect bypromoting the formation of protein fibrils of suitable size as well as acting as a reducing agent. The invention further provides metal nanowires.

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Thin metal nanowires produced by biotemplating.

Field of the invention

The present invention provides methods for the manufacturing of metal nanowires using protein fibrils as biotemplates. The methods comprise use of a solvent providing a dual effect by promoting the formation of protein fibrils of suitable size as well as acting as a reducing agent. The invention further provides metal nanowires produced by the methods according to the invention.

Background of the invention

The main challenge in the growing field of nanotechnology is the fabrication of nano-sized structures with controlled parameters. Nature provides us with an inspiration how to achieve this goal. The self-assembly of biological macromolecules into larger polymers and complexes with diverse structures is a very common event in live organisms. This phenomenon can be successfully explored for developing templates to cast inorganic nanostructures.

The ultra-thin nanowires are in great need for constructions of the conductive circuits in nano-scale electronics. The self-assembling biotemplates may enable us to grow the nano-structures directly in the required places in the nano-devices avoiding the stage of their positioning and micro-manipulations. The biotemplating involves two major steps; firstly, the regular and hierarchical assembly of biological molecules into the structural scaffold and then incorporation of the inorganic component with required properties. There are a number of previous attempts to use biotemplates for this purpose, among them the DNA molecules are most widely explored for manufacturing of conductive metal nanowires with diameters in the range of 20 to 100 nm and the lengths of 0.5 to 12 μm[1-3]. Utilization of viral capsids led to the synthesis of conductive and magnetic nanowires with 3 to 25 nm diameters, but the length was limited to a few hundred nanometers by the dimensions of capsids[4-6]. Recently, protein complexes have also been subjected to biotemplating. The polymerization of G-actin labelled with gold

nanoparticles, followed by the catalytic enlargement of the nanoparticles, yielded gold wires of 80-200 nm diameter and 1-4 μm in length[7]. The principle of metal coating of the biotemplates with silver and gold was used with genetically modified prion amyloid fibrils as templates, resulting in conducting wires of 100 nm diameter[8]. Another approach of casting silver nanowires inside the hollow part of chemically synthesized dipeptide nanotubes produced the wire of ca. 20 nm in diameter[9].

Over the last two decades the extensive studies of amyloids led to the concept that any polypeptide can assemble into this type of polymers as an alternative to its native functionally active conformation[10,11]. The amyloids possess a distinctive structural feature such as cross-β-sheet core packed by polypeptide chain and supported by a dense network of hydrogen bonding as well as by hydrophobic stacking of aromatic side-chains[12]. Consequently, amyloids are remarkably stable towards mechanical damage and chemical denaturation over a wide range of conditions, far exceeding similar properties of other macromolecules such as DNA[8]. Such properties make them particularly attractive for biotechnological applications, particularly as there is an unlimited natural source of these species. Lysozyme is a ubiquitous protein, which, can readily polymerize under tightly controlled conditions into regular and unbranched fibrous structures growing up to 10 μm in length[13,14].

Brief description of the invention

The present invention provides methods for the manufacturing of metal nanowires using protein fibrils as templates. The methods comprise the use of a solvent providing a dual effect by promoting the formation of protein fibrils of suitable size as well as acting as a reducing agent.

We applied naturally-occurring cross-β-sheet containing proteinaceous polymers known as amyloids to produce scaffolds for moulding silver nanowires. The diameter of the nanowires was within 1.0-2.5 nm and reached 1-1.5 μm length. Up to date this is the thinnest silver nanowires produced by biotemplating. These results demonstrate that the biotemplating expands boundaries for the nanoparticles production from tens to

sub-nanometer scale, opening new horizons in quantum electronics. They provide also an insight into the packing of amyloid core, demonstrating the absence of the cavities within individual fibrils.

Accordingly, the present invention provides a method for the manufacture of metal nanowires, the method comprising incubating protein fibrils in a solution of a metal salt in a solvent, said solvent having a hydrophobic constant, logP, between 0 and 3.0 and further having a standard reducing potential, E_0, between 0 volts and 1.5 volts, allowing the formation of metal nanowires on the protein fibril templates.

The method can further comprise proteinase digestion of the protein fibril template following the formation of the metal nanowire. Proteinase digestion can be made by the use of proteinase K, other serine proteases like trypsin, α-chymotrypsin, β-chymotrypsin and subtilisin; cysteine proteases like bromelain, papain, chymopapain, ficin and sortase A, or aspartic proteases like rennin and pepsin.

Formation of protein fibril template is promoted by the use of a hydrophobic solvent. The solvent can have a hydrophobic constant, log P, defined as the log of the partition coefficient between octanol and water, which is between 0 and 3.0, preferably between 0.2 and 1.0, and even more preferably between 0.3 and 0.5.

$$\text{Log } P_{\text{octanol/water}} = \log \left(\frac{\text{concentration of solvent in octanol}}{\text{concentration of solvent in water}} \right)$$

The solvent can have a standard reducing potential, E_0, between -0.2 volts and 1.5 volts, preferably between 0 volts and 0.8 volts, such as between 0.1 volts and 0.7 volts, or even more preferably 0.2 volts and 0.6 volts.

A solvent with a lower reducing potential within the above ranges can be selected for the reduction of a metal with a lower reducing potential, e.g. silver having a reducing potential of 0.8 volts, a solvent with a higher reducing potential within the above ranges can be selected for the reduction of a metal with a higher reducing potential, e.g. gold having a reducing potential of 1.7 volts.

The solvent can be a halogenated alcohol, preferably a fluorinated alcohol. The fluorinated alcohol can be selected from a fluorinated ethanol, propanol, butanol, pentanol, hexanol, heptanol, octanol. The alcohol can be a mono, di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, or nona-fluorinated alcohol.

The solvent can be selected from

2-Fluoroethanol,
2,2-Difluoroethanol,
2,2,2-Trifluoroethanol,
1,1,1-Trifluoro-2-propanol,
3,3,3-Trifluoro-1-propanol,
Hexafluoro-2-propanol,
2,2,3,3-Tetrafluoro-1-propanol,
2,2,3,3,3-Pentafluoro-1-propanol,
4,4,4-Trifluoro-1-butanol,
2,2,3,4,4,4-Hexafluoro-1-butanol,
2,2,3,3,4,4,4-Heptafluoro-1-butanol,
3,3,4,4,4-Pentafluoro-2-butanol,
1,1,1,3,3-Pentafluoro-2-butanol,
5,5,5-Trifluoro-1-pentanol,
4,4,5,5,5-Pentafluoro-1-pentanol,
2,2,3,3,4,4,5,5-Octafluoro-1-pentanol,
6,6-Difluoro-1-hexanol,
7,7,7-Trifluoro-1-heptanol,
1,1,1-Trifluoro-2-octanol,
2,2,3,3-Tetrafluoro-1-octanol.

The protein fibrils are preferably amyloid fibrils.

The protein fibrils can be selected from lysozyme fibrils, Alzheimer β-amyloid fibrils, prion fibrils, insulin fibrils, bovine α-lactalbumin fibrils, S100A8 fibrils, S100A9 fibrils, α-synuclein fibrils.

The nanowire can be manufactured from a noble metal, i.e. silver, gold, platinum or palladium, or from another suitable metal such as copper.

The metal salt can be provided as any suitable salt, such as a nitrate, nitrite, carbonate, chloride, bromide, fluoride, iodide, acetate, sulphate.

The invention further provides metal nanowires produced by the methods of the invention.

The metal nanowires according to the invention have a diameter of less than 3 nm, preferably less than 2 nm.

The metal nanowires according to the invention have a length of more than 0.5 μm, preferably more than 1 μm, or even more preferably more than 2 μm.

Brief description of the figures.

Figure 1. Scheme of casting silver nanowires within amyloid scaffolds.

Figure 2. Silver nanoparticles formation by TFE reduction. Time course of silver reduction by TFE monitored by absorbance spectroscopy (a). The atomic force microscopy (AFM) images of the silver nanoparticles formed in TFE and the distributions of their sizes measured by AFM after 2 hours (b, c) and 72 hours (d, e) of incubation, respectively.

Figure 3. Silver nanowire formation in amyloid scaffolds. Thioflavin T binding by different amyloids measured by fluorescence (a). AFM images of original lysozyme amyloid fibrils (b); amyloid scaffolds incubated in TFE with silver (c) and without silver (e); silver nanowires released from the scaffolds after proteinase K digestion (d); the digestion of empty amyloid scaffolds by proteinase K (f).

Figure 4. AFM images of ultra thin silver nanowires, a network of silver nanowires (a), a single silver nanowire (b), the distribution of diameters of silver nanowires measured by AFM (c).

Figure 5. AFM images of amyloids with reducing agents. Amyloids in N,N-dimethylformamide (DMF) (a) and in citric acid (b).

Detailed description of the invention

The method used for nanowire production is outlined in Figure 1. According to the method of the invention two critical events, i.e. metal reduction and assembly of the nanowire within the biotemplate mould occur concomitantly. A key factor in this procedure is the use of a solvent exhibiting a dual effect, both promoting the formation of protein fibrils of suitable size, and acting a reducing agent, the solvent being exemplified by 2,2,2-trifluoroethanol (TFE).

Firstly, it is demonstrated that the solvent reduces ionic silver into colloidal form following the reaction (1):

$$2\ Ag^{+}\ CF_3\text{-}CH_2\text{-}OH \rightarrow 2\ Ag^{\circ} + CF_3\text{-}CHO + 2H^{+} \qquad (1)$$

The reaction was monitored by changes of the absorbance spectra of the solution (Figure 2a), which were also corroborated by the colour developing from fully uncoloured to yellow/brown[15]. The samples were analysed by AFM, demonstrating the formation of uniform silver nanoparticles. Their size depended on the reduction time; 1.2 nm particles were formed after 2 hours and 35 nm after 72 hours, respectively (Figure 2b - 2e).

Secondly, the solvent induces the lateral assembly of individual protein fibrils into larger and thicker scaffolds, which trap silver in inter-fibrillar space and serve a role of the moulds. Before treatment with TFE the amyloid fibrils were characterised by 2-4 nm height in AFM images (Figure 3b), exhibiting typical amyloid tinctorial features

such as thioflavin T dye binding[16,17] (Figure 3a). After incubation with TFE in the presence and absence of silver ions fibrils become thicker with ca 6-9 nm height (Figure 3c, 3e), which corroborated with a slight decrease in thioflavin T binding due to a decrease in number of exposed thioflavin T binding sites (Figure 3a). In the fibrillar sample containing silver the yellow-brown colour was developed, indicating silver reduction.

In order to verify formation of silver nanowires, the proteinaceous moulding scaffolds were digested by proteinase K. In the sample without silver, the amyloid was fully removed after 24 hours of proteinase treatment. This was confirmed by the drop of the thioflavin T signal to the level of free dye in solution (Figure 3a) and by AFM (Figure 3e). In the sample where the silver nanowires were cast in the amyloid scaffold, the nanowires were released and observed by AFM (Figure 3d), while the thioflavin T signal also disappeared, indicating the degradation of amyloid structures (Figure 3a). The nanowires were characterised by having a diameter of 1-2.5 nm and a variable length from 0.5 to ca. 2 μm (Figure 3). The nanowires were straight or banded and sometimes a few nanowires were twisted around each other (Figure 3d, figure 4).

In control experiments the amyloids in the presence of $AgNO_3$ were treated with the reducing agents N,N-dimethylformamide (DMF) or citric acid. While both agents initiated the formation of silver nanoparticies[9,15], they did not induce fibril thickening, but rather caused their fragmentation to shorter species of 0.5-1 μm length and 2-4 nm in height (Figure 5). Consequently, after the treatment with proteinase K all amyloid material was removed but the silver nanowires were not detected. This indicates that fibrillar lateral assembly and formation of the structured, hollow fibrillar interface is essential for silver moulding, whiles the individual fibrils with a common diameter of 2 nm and less do not possess such a cavity.

Thus, naturally occurring proteinaceous amyloid polymers assembled into larger scaffolds can be used for casting metal nanostructures. This has been utilised for this purpose an abundant and cheep material such as hen lysozyme. The limit in biotemplating producing the ultra thin silver nanowire of ca. 1 nm diameter has been

reached, which is significantly below the dimensions attainable by standard electronic manufacturing processes and also one order of magnitude thinner than nanowires fabricated using other proteinaceous scaffolds[8,9]. The method according to the present invention allows removing the moulding scaffold by proteinase digestion compared to other approaches, in which the protein polymers remain within the formed metal wires[8].

For the first time it is surprisingly demonstrated that a fluorinated alcohol, TFE, can be used as a reducing agent and has potential applications in manufacturing metal nanoparticles. The major advantage of the methods according to the invention is the use of a solvent such as TFE, which compared to other combinations of solvents and reducing agents induces formation and stabilization of protein fibril scaffold simultaneously with metal ion reduction. Less suited reducing agents, such as citric acid and DMF causes fragmentation of the protein fibrils without the formation of metal nanowires.

The process of biotemplating can be used as a tool to get an insight on the properties of the scaffold itself, according to the present invention on amyloid. By analysing the X-ray diffraction pattern of fibrillar amyloids, Perutz and co-authors suggested that the amyloid fibrils are probably built of a few concentric cylindrical β-sheets containing the hollow part filled with water[18]. This hypothesis was supported by production of synthetic dipeptide amyloid-like nanotubes and filling them with silver of ca. 20 nm diameter[9]. However, the NMR-based analysis of amyloid fibrils of Aβ peptide and prions indicate the dense packing within fibrillar interior, which rules out the cavity in the protofilament core[19,20]. In this light the data directly demonstrate the absence of the hollow part within the individual amyloid fibrils, unlike synthetic peptide nanotubes, prior their assembly into the larger scaffold.

The methods according to the invention can be used to form nanowires from a noble metal, such as silver, gold, platinum, and palladium, or other suitable metal such as copper.

The methods according to the invention can be used to produce nanoparticles with controlled dimensions.

Materials and methods.

Materials

Hen egg white lysozyme was purchased from Sigma (USA). Amyloid was produced by incubation of protein in 20 mM glycine buffer at pH 2.5, 57°C. Proteinase K was purchased from Fermentas (Lithuania). DMF, TFE and citric acid were purchased from Fluka (Germany).

Thioflavin T binding assay.

Thioflavin T binding amyloid assay was carried out as described previously[13]. Fluorescence measurements were performed on a Jasco spectrofluorometer FP 6500, using excitation at 440 nm and measuring emission between 460 and 520 nm.

Atomic Force Microscopy.

AFM measurements were performed on a PICO PLUS microscope (Molecular Imaging, USA) in a tapping mode using a 100 μm scanner with acoustically driven cantilevers operating at a resonance frequency in 320-370 kHz range. Scanning resolution was 512x512 pixels. The scanning was performed in trace and retrace to avoid the scan artefacts. Specimens were diluted in MiliQ water, incubated on freshly cleaved mica for 10 min, subsequently washed 2 X 200 μ1 with MiliQ water and dried over night at room temperature. The parameters of nanostructures were measured by using a Scanning Probe Image Processor software (Image Metrology, Denmark).

Reduction of silver.

The stock of 1M $AgNO_3$ in MiliQ water was diluted to a final concentration of 20 mM with the excess of appropriate reducing agents such as TFE, DMF or citric acid and subsequently incubated at 57°C for 72 hours. Reduction of silver was estimated by measuring absorbance spectra at 300-700 nm on a Lambda 40 spectrophotometer (Perkin Elmer, USA) as described previously[15].

Casting silver nanowires in amyloid fibrils.

Amyloid fibrils were sedimented for 30 min at 12000 g on a minicentrifuge (Eppendorf, Germany). The pellet was resuspended in 100 µl MiliQ water and sedimented again. $AgNO_3$ was added to the fibrillar pellet to 20 mM final concentration and the sample was incubated at room temperature for 24 h. The fibrils were centrifuged and an access of reducing agent was added to the pellet, following incubation for 48 h at 57°C. The complexes were centrifuged, resuspended at 100 mM Tris-HC1 pH 7.4 and 0.2 mg/ml protease K was added for 24 h at 37°C in order to achieve the full digestion of the amyloid scaffold. Consequently, the samples were analysed by AFM and ThT binding assay.

References

1. Braun, E., Eichen. Y., Sivan, U. & Ben-Yoseph, G. DNA-templated assembly and electrode attachment of a conducting silver wire. *Nature* **391,** 775 – 778 (1998).
2. Stoltenberg, R. M. & Woolley, A. T. DNA-templated nanowire fabrication *Biomed. Microdevices* **6**, 105 – 111 (2004).
3. Berti, L., Alessandrini, A. & Facci, P. DNA-templated photoinduced silver deposition. *J. Am. Chem. Soc.* **127**, 11216 – 11217, (2005).
4. Knez, M, *et al.* Biotemplate synthesis of 3-nm nickel and cobalt nanowires. *Nano Lett.* **3**, 1079 – 1082 (2003)
5. Baici, *S.*, *et al.* Copper nanowires within the central channel of tobacco mosaic virus particles. *Electrochim. Acta* **51,** 6251 – 6257 (2006).
6. Mao, C. *et al.* Virus-based toolkit for the directed synthesis of magnetic and semiconducting nanowires. *Science* **303**, 213 – 217 (2004).
7. Patolsky. F., Weizmann, Y. & Willner, I. Actin-based metallic nanowires as bionanotransporters. *Nat. Mater.* **3,** 692 – 695 (2004).
8. Scheibel, T *et al.* Conducting nanowires built by controlled self-assembly of amyloid fibers and selective metal deposition. *Proc. Natl. Acad. Sci. USA* 100, 4527 – 4532 (2003).
9. Reches, M. & Gazit, E. Casting metal nanowires within discrete self-assembled peptide nanotubes. *Science* **300,** 625 – 627 (2003).
10. Dobson, C. M. Protein folding and its links with human disease. *Biochem. Soc. Symp.* **68**, 1 – 26 (2001).
11. Chiti, F. & Dobson, C. M. Protein misfolding, functional amyloid, and human disease. *Annu. Rev. Biochem.* **75**, 333 – 366 (2006).
12. Jimenez, J. L. *et al.* Cryo-electron microscopy structure of an SH3 amyloid fibril and model of the molecular packing. *EMBO J.* **18**, 815 – 821 (1999).
13. Morozova-Roche, L. A. *et al.* Amyloid fibril formation and seeding by wild-type human lysozyme and its disease-related mutational variants. *J. Struct. Biol.* 1**30**, 339 – 351 (2000).

14. Krebs, M. R. *et al.* Formation and seeding of amyloid fibrils from wild-type hen lysozyme and a peptide fragment from the β–domain. *J Mol. Biol.* **300,** 541 – 549 (2000).

15. Pastoriza-Santos, L & Liz-Marzán, L. M. Reduction of silver nanoparticles in DMF. Formation of monolayers and stable colloids. *Pure Appl. Chem.* **72**, 83 – 90 (2000).

16. LeVine, H. 3rd. Thioflavine T interaction with synthetic Alzheimer's disease beta-amyloid peptides: detection of amyloid aggregation in solution. *Protein Sci.* **2**, 404 – 410 (1993).

17. Krebs, M. R., Bromley, E. H. & Donald, A. M. The Binding of thioflavin-T to amyloid fibrils: localisation and implications. *J. Struct. Biol.,* **149,** 30 – 37 (2005).

18. Perutz, M. F., Finch, J. T., Berriman, J. & Lesk, A. Amyloid fibers are water-filled nanotubes. *Proc. Natl. Acad. Sci. USA* **99**, 5591 – 5595 (2002).

19. Olofsson, A., Lindhagen-Persson, M., Sauer-Eriksson, A. E. & Ohman A. Amide solvent protection analysis demonstrate that Amyloid-β (1-40) and Amyloid-β (1-42) form different fibrillar structures under identical conditions. *Biochem J.* 404, 63 – 70 (2007).

20. Petkova, A. T., Yau, W. M. & Tycko, R. Experimental constraints on quaternary structure in Alzheimer's β-amy1oid fibrils. *Biochemistry* **45**, 498 – 512 (2006).

Claims

1. A method for the manufacture of metal nanowires, said method comprising incubating protein fibrils in a solution of a metal salt in a solvent, said solvent having a hydrophobic constant, logP, between 0 and 3.0 and further having a standard reducing potential, E_0, between -0.2 volts and 1.5 volts, allowing the formation of metal nanowires on the protein fibril templates.

2. The method according to claim 1 further comprising proteinase digestion of the protein fibril template following the formation of the metal nanowires.

3. The method according to claim 2 wherein the proteinase digestion is made by the use of a proteinase selected from proteinase K, a serine protease such as trypsin, α-chymotrypsin, β-chymotrypsin and subtilisin; a cysteine protease such as bromelain, papain, chymopapain, ficin and sortase A, and an aspartic protease such as rennin and pepsin.

4. The method according to claim 1 wherein the solvent has a hydrophobic constant, log P, which is between 0 and 3.0, preferably between 0.2 and 1.0, and even more preferably between 0.3 and 0.5.

5. The method according to claim 1 wherein the solvent has a standard reducing potential, E_0, between -0.2 volts and 1.5 volts, preferably between 0 volts and 0.8 volts, such as between 0.1 volts and 0.7 volts, or even more preferably between 0.2 volts and 0.6 volts.

6. The method according to claim 1 wherein the solvent is a fluorinated alcohol.

7. The method according to claim 6 wherein the solvent is selected from a fluorinated ethanol, propanol, butanol, pentanol, hexanol, heptanol, octanol.

8. The method according to claim 7 wherein the fluorinated alcohol is selected from a mono, di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, and nona-fluorinated alcohol.

9. The method according to claim 6 wherein the solvent is selected from
2-Fluoroethanol,
2,2-Difluoroethanol,
2,2,2-Trifluoroethanol,
1,1,1-Trifluoro-2-propanol,
3,3,3-Trifluoro-1-propanol,
Hexafluoro-2-propanol,
2,2,3,3-Tetrafluoro-1-propanol,
2,2,3,3,3-Pentafluoro-1-propanol,
4,4,4-Trifluoro-1-butanol,
2,2,3,4,4,4-Hexafluoro-1-butanol,
2,2,3,3,4,4,4-Heptafluoro-1-butanol,
3,3,4,4,4-Pentafluoro-2-butanol,
1,1,1,3,3-Pentafluoro-2-butanol,
5,5,5-Trifluoro-1-pentanol,
4,4,5,5,5-Pentafluoro-1-pentanol,
2,2,3,3,4,4,5,5-Octafluoro-1-pentanol,
6,6-Difluoro-1-hexanol,
7,7,7-Trifluoro-1-heptanol,
1,1,1-Trifluoro-2-octanol,
2,2,3,3-Tetrafluoro-1-octanol.

10. The method according to claim 6 wherein the solvent is 2,2,2-Trifluoroethanol.

11. The method according to claim 1 wherein the protein fibrils are selected from lysozyme fibrils, Alzheimer β-amyloid fibrils, prion fibrils, insulin fibrils, bovine alpha-lactalbumin fibrils, S100A8 fibrils, S100A9 fibrils, and alpha-synuclein fibrils.

12. The method according to claim 1 wherein the metal is selected from a noble metal, such as silver, gold, platinum or palladium, and copper.

13. The method according to claim 1 wherein the metal salt is selected from a nitrate, nitrite, carbonate, chloride, bromide, fluoride, iodide, acetate, sulphate.

14. Metal nanowires produced according the method of any of claims 1 to 12.

15. Metal nanowires according to claim 13 having a diameter of less than 3 nm, preferably less than 2 nm.

16. Metal nanowires according to claim 13 having a length of more than 0.5 μm, preferably more than 1 μm, or even more preferably more than 2 μm..

Figure 1

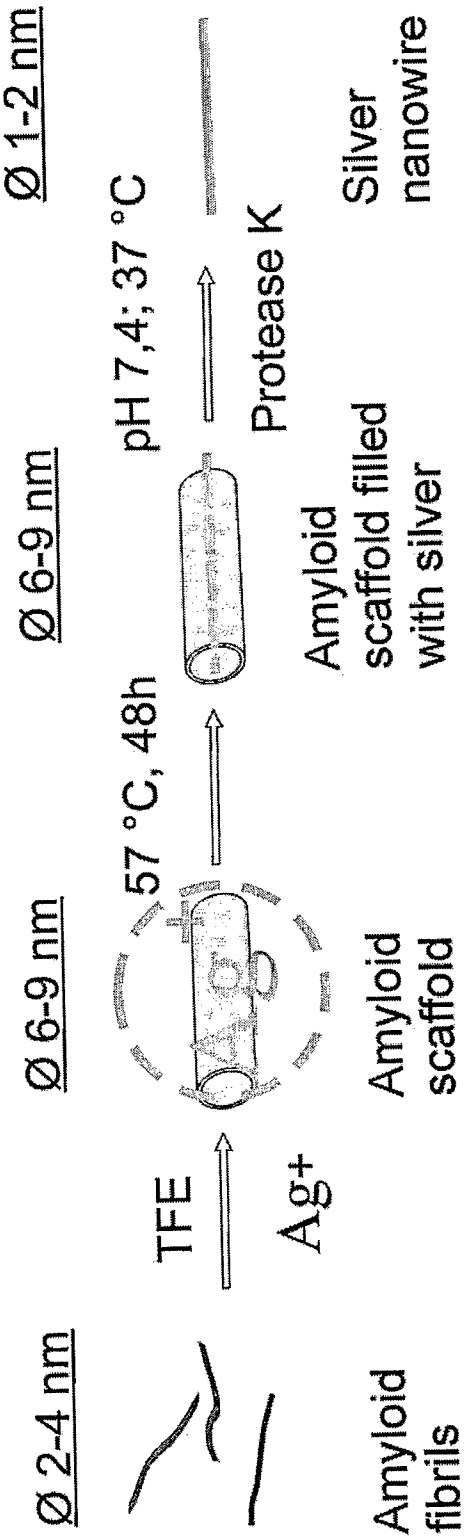
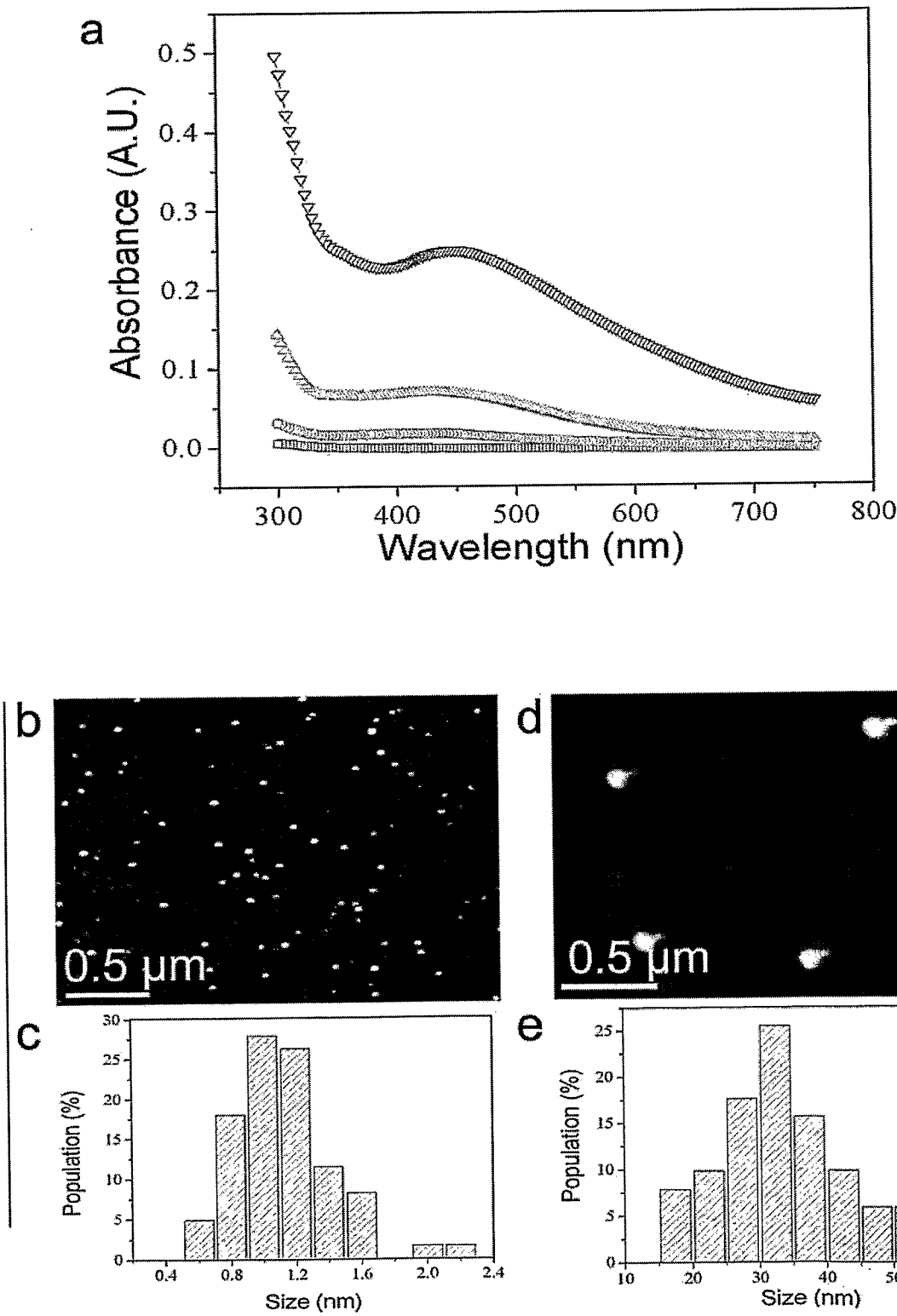

Ø 2-4 nm
Amyloid fibrils
TFE
Ag+
Ø 6-9 nm
Amyloid scaffold
57 °C, 48h
Ø 6-9 nm
Amyloid scaffold filled with silver
pH 7,4; 37 °C
Protease K
Ø 1-2 nm
Silver nanowire

Figure 2


a
Absorbance (A.U.)
0.0
0.1
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0.3
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300
400
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Population (%)
0
5
10
15
20
25
30
0.4
0.8
1.2
1.6
2.0
2.4
Size (nm)
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0
5
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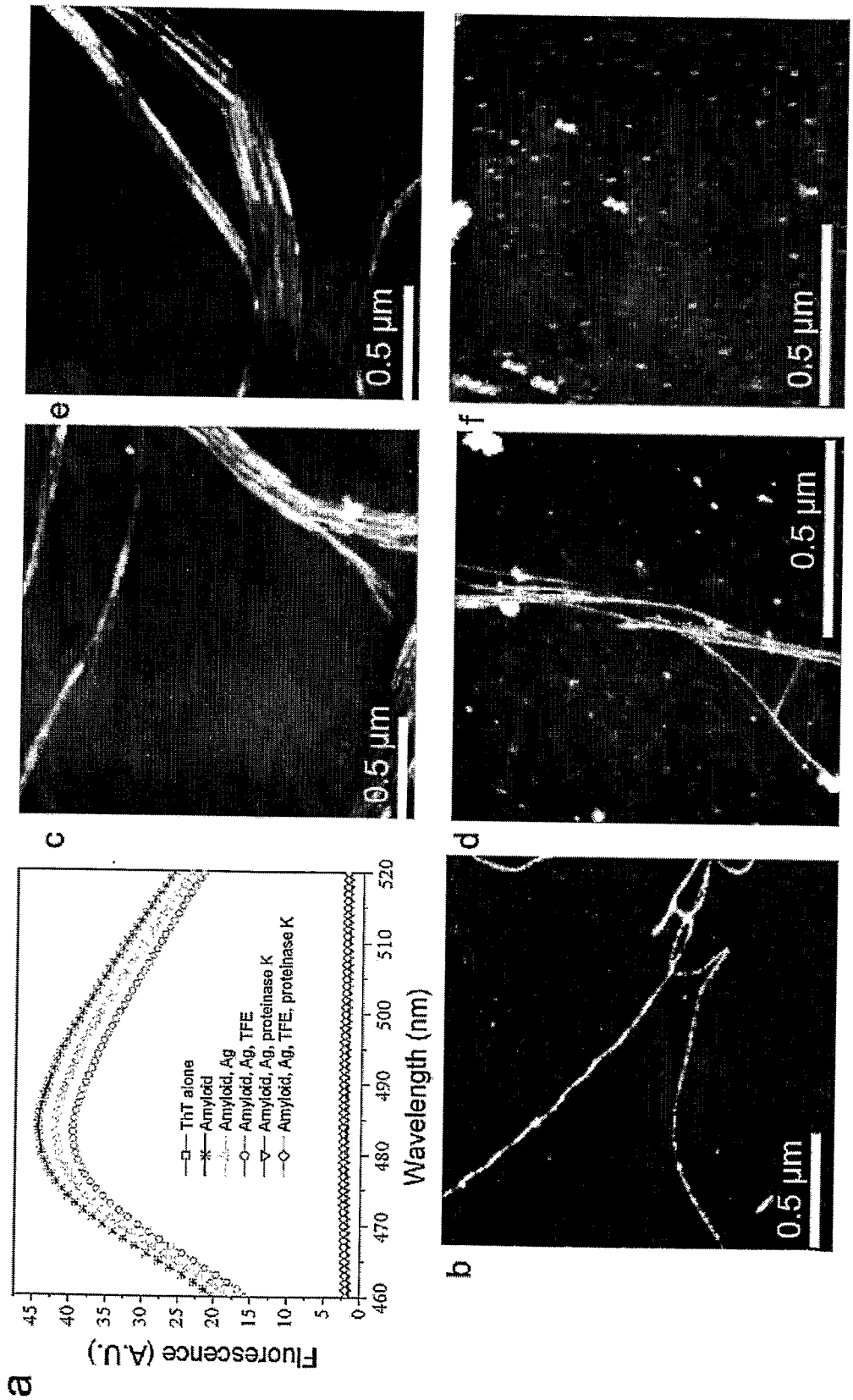
40
50
60
Size (nm)

Figure 3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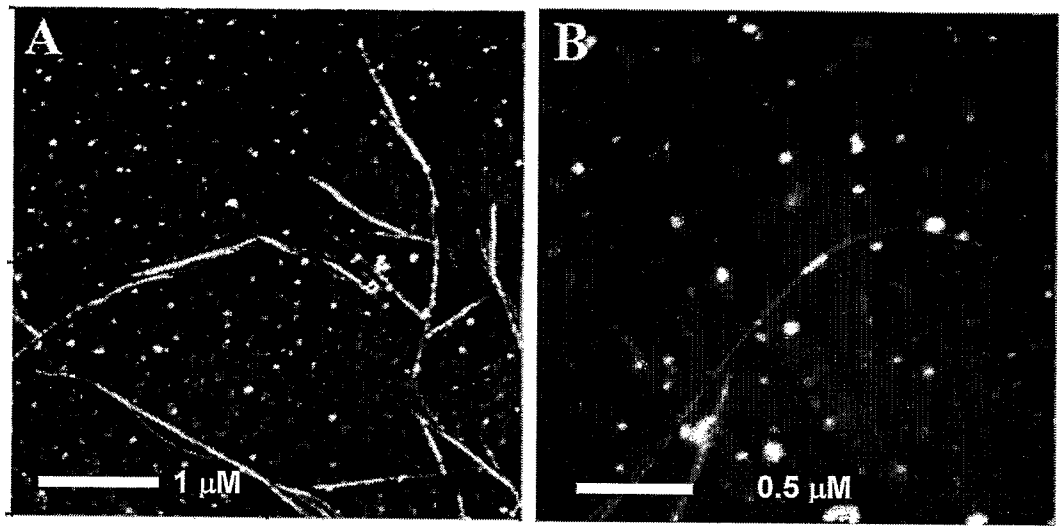
Amyloid, Ag, TFE, proteinase K
b
c
d
e
f
0.5 µm

Figure 4


A
1 μM
B
0.5 μM

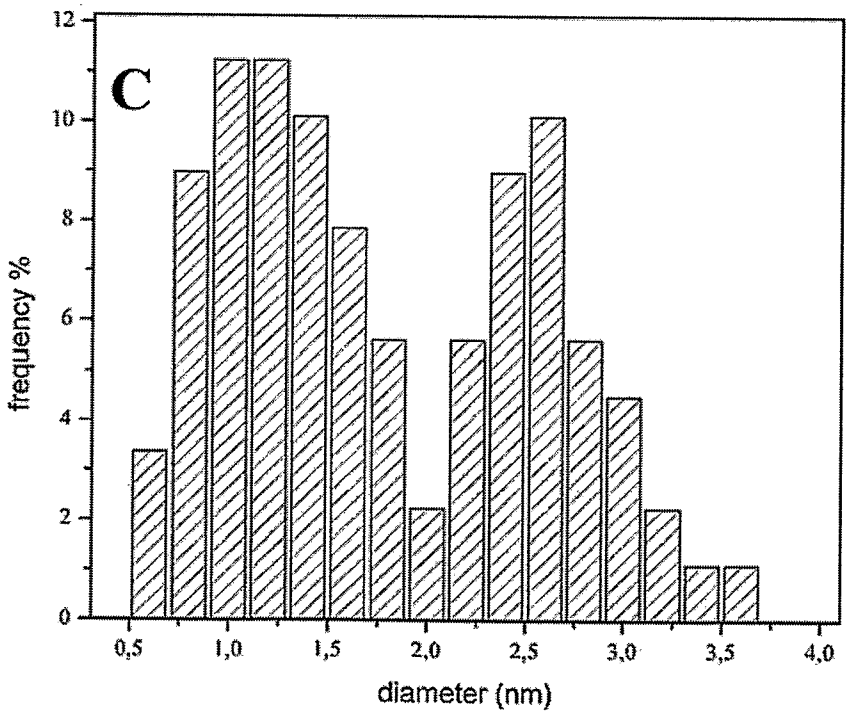
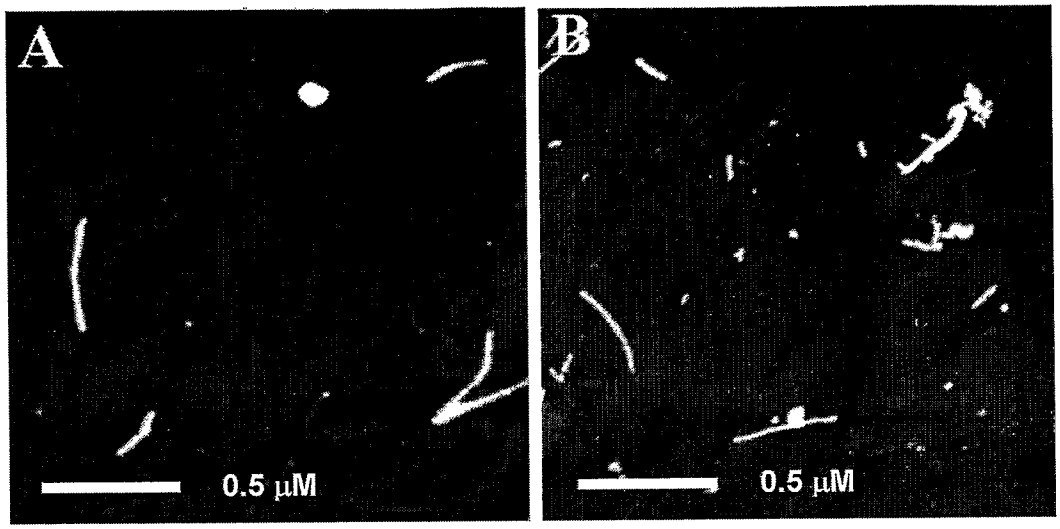

C
frequency %
0
2
4
6
8
10
12
0,5
1,0
1,5
2,0
2,5
3,0
3,5
4,0
diameter (nm)

Figure 5



INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2008/050348

A. CLASSIFICATION OF SUBJECT MATTER

IPC: see extra sheet
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)

IPC: B28B, C01G, C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-INTERNAL, WPI DATA, PAJ, EMBASE, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Reches, M et al, "Casting Metal Nanowires Within Discrete Self-Assembled Peptide Nanotubes", Science 25 April 2003, Vol. 300, p 625 - 627, entire document --	1-16
Y	EP 1264919 A2 (POSTECH FOUNDATION), 11 December 2002 (11.12.2002), claims 1-9, abstract, paragraphs [0001],[0003]-[0005], [0012]-[0018],[0024]-[0025]	1-13
X	--	14-16

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 July 2008	22 -07- 2008
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Nora Bucht/PR Telephone No. +46 8 782 25 00

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Hamada, D et al, "Engineering amyloidogenicity towards the development of nanofibrillar materials", Trends in Biotechnology February 2004, Vol. 22, No. 2, p. 93 - 97, entire document	1-16
Y	Scheibel, T et al, "Conducting nanowires built by controlled self-assembly of amyloid fibers and selective metal deposition", PNAS April 15 2003, Vol. 100, No. 8, p. 4527 - 4532, entire document	1-16
Y	Song, Y et al, "Synthesis of peptide-nanotube platinum-nanoparticle composites", Chem. Commun. 2004, p. 1044 - 1045, entire document	1-16
A	Padalkar, S et al, "Alpha-synuclein as a template for the synthesis of metallic nanowires", Nanotechnology 2007, Vol. 18, p. 1 - 9, entire document	1-16
Y	US 20070059727 A1 (DEYMIER, P ET AL), 15 March 2007 (15.03.2007), abstract, paragraphs [0010],[0013],[0024],[0028],[0069]-[0070], [0072]-[0073],[0111]-[0116]	1-16
A	Gazit, E, "Use of biomolecular templates for the fabrication of metal nanowires", FEBS Journal 2007, Vol. 274, p. 317 - 322, entire document	1-16

International patent classification (IPC)
B28B 3/00 (2006.01)
C07K 14/47 (2006.01)
C01G 5/00 (2006.01)

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Cited literature, if any, will be enclosed in paper form.

EP	1264919	A2	11/12/2002	KR	2002093209	A	16/12/2002
				KR	20020093209	A	16/12/2002
				US	6762331	B	13/07/2004
				US	20020185368	A	12/12/2002
US	20070059727	A1	15/03/2007	WO	2006119472	A	09/11/2006