Amyloid-carbon hybrid membranes for universal water purification

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Industrial development, energy production and mining have led to dramatically increased levels of environmental pollutants such as heavy metal ions, metal cyanides and nuclear waste. Current technologies for purifying contaminated waters are typically expensive and ion specific, and there is therefore a significant need for new approaches. Here, we report inexpensive hybrid membranes made from protein amyloid fibrils and activated porous carbon that can be used to remove heavy metal ions and radioactive waste from water. During filtration, the concentration of heavy metal ions drops by three to five orders of magnitude per passage and the process can be repeated numerous times. Notably, their efficiency remains unaltered when filtering several ions simultaneously. The performance of the membrane is enabled by the ability of the amyloids to selectively absorb heavy metal pollutants from solutions. We also show that our membranes can be used to recycle valuable heavy metal contaminants by thermally reducing ions trapped in saturated membranes, leading to the creation of elemental metal nanoparticles and films.

ater pollution affects millions of people worldwide^{1,2} and is a leading global risk factor for illness, disease and death³⁻⁵. It also affects plants and organisms living in lakes, rivers, oceans and ground water⁶⁻⁹. A major source of this pollution is unregulated industry¹⁰, from which increasing amounts of toxic pollutants¹¹ (heavy metal ions, organic waste and micro pollutants) are released directly into water bodies without adequate treatment. Mining of metals can also generate highly toxic cyanides in wastewater, which can have a long-term negative impact on nearby bodies of water¹².

Several methods exist to remove heavy metal ions from wastewater, and all come with their advantages and disadvantages¹³. Chemical precipitation is the most widely used method due to its efficiency, which under idealized conditions can be as high as ~99%14,15. However, the technique can produce secondary pollution in the form of high quantities of sludge and toxic fumes. Furthermore, it is expensive and is only suitable for treating water contaminated with high concentrations of heavy metals¹³. Sorbents or ion-exchange resins can also be used to remove heavy metal ions and other pollutants^{16,17}. However, the technology is costly, suited to small volumes of wastewater, and typically offers low efficiencies (60-90%)^{18,19} that also tend to decrease with prolonged use. Furthermore, fully saturated ionexchange resins must be converted by chemical reactions, which can also cause uncontrolled secondary pollution. Flotation or electrochemical treatment of wastewater can be very efficient in treating specific heavy metal ions^{20,21}, but they are demanding in terms of resources and thus they are cost-effective only in large-scale processing and are not suitable for decontamination at smaller scales.

Membrane purification is an alternative technology that can work in continuous operation²². It has several variants, including micellar-enhanced ultrafiltration²³, polymer-enhanced ultrafiltration²⁴, reverse osmosis²⁵ and nanofiltration²⁶, with efficiencies reaching 99% for idealized pH and concentration conditions. Yet, these technologies suffer from high intrinsic costs, membrane fouling and limiting feeding flows, which make their use impractical at industrially relevant scales¹³. Consequently, research is now focused on the development of new types of membrane by selfassembling adsorbents^{27,28} for fast and effective water purification. All of the above techniques also suffer from one main problem: specificity in heavy ion removal. In other words, although they can perform very well with a given heavy metal ion, they are highly inefficient in treating other metal contaminants, which makes their use very limited. More importantly, removal of several ions simultaneously is highly inefficient. For example, using commercial nanofiltration membranes, it has been shown that while the removal efficiency of nickel ions alone can reach $98\%^{26}$, the specific ion removal of nickel and cadmium together decreases to $\sim 83\%^{29}$. This hampers the use of this technology in treating environmentally polluted waters, in which several heavy metal ion pollutants typically coexist together. Finally, in all cases, the efficiency of the above technologies drops rapidly as soon as the feeding conditions depart from optimized pH and concentrations.

Indirect evidence is available that suggests a potential role of amyloid fibrils in the removal of toxic heavy metal ions, as metal binding has implications in the formation of the amyloid fibrils in neurodegenerative diseases. For example, Rufo and colleagues suggested that amyloid-forming peptides have a binding capability for zinc metal ions, which can catalyse fibril formation³⁰. It has also been proposed that the toxicity of protein aggregates is due to binding of heavy metal ions to the peptides³¹.

Here, we report the development of a hybrid composite membrane incorporating inexpensive and environmentally friendly β-lactoglobulin amyloid fibrils and activated carbon, and show that it can be used as a tool for the efficient removal of heavy metal ion pollutants from water. We also show how the same technology can be used to remove radioactive waste from water. We finally demonstrate that, in the case of expensive metal pollutants, the recovered ions can even be converted into valuable materials, turning a global risk challenge into a unique opportunity. Activated carbon is a well-known extraction medium as it readily adsorbs molecular and ionic species of heavy metals due to its large surface area $^{13}\!\!\!\!$ β -Lactoglobulin is a cheap edible milk protein, which can be easily converted into amyloid fibrils (Supplementary Fig. 1) with robust physical properties³². The stickiness and stiffness of the amyloid fibrils enable their assembly in combination with carbon-based materials³³, resulting in mechanically strong composite membranes. The amyloid fibrils

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Figure 1 | Schematic representation of the composite activated carbon-amyloid fibril adsorber membrane and the heavy metal ion purification process for polluted water. **a**, Structure of the β-lactoglobulin protein with the strongest heavy metal-binding motif highlighted, 121-cys, with a lead ion attached. **b**, Amyloid-forming 121-cys-containing fragment (LACQCL) from β-lactoglobulin with docked Pb metal ions. **c**, Schematic representation of heavy metal ion purification by amyloid-carbon adsorbers, and photographs of Na₂PdCl₄ solution changing colour from yellow to colourless after filtration due to the adsorption of palladium heavy metal ion pollutants onto the composite membrane. **d**, SEM image showing the surface of the composite membrane, with the visual aspect of the membrane shown in the inset. **e**, Higher-magnification SEM image of the membrane, demonstrating the assembly of the amyloid fibrils onto the activated carbon surface.

in the composite membrane play the main role of sequestering heavy metal pollutants from the liquid. Importantly, these amyloid fibrils also allow the reduction of membrane-immobilized metal ions into valuable metal nanoparticles at elevated temperatures³⁴ or via chemical routes.

β-Lactoglobulin protein and amyloids bind heavy metal ions

We first investigated the binding capacity of heavy metal ions to β-lactoglobulin fibrils and evaluated the binding performance using atomic absorption spectroscopy (AAS). The β-lactoglobulin membrane was prepared by a vacuum filtration method³³, and various heavy metal ions (potassium dicyanoaurate(1) (KAu(CN)₂), mercury chloride (HgCl₂), lead acetate (Pb(C₂H₃O₂)₄) and disodium tetrachloropalladate (Na₂PdCl₄)) were individually passed through it by means of vacuum-assisted or pressurized flow. The analysis showed that 154 µg of KAu(CN)₂, 10 µg of HgCl₂ and 3.32 mg of $Pb(C_2H_3O_2)_4$ were adsorbed by only 40 mg of amyloid fibrils. The strongest binding residue interactions of heavy metal ligands to bovine β-lactoglobulin protein and amyloid fibrils were studied in more detail using molecular docking methods³⁵. Figure 1a shows the lead ion's strongest binding motif for the native β-lactoglobulin protein at the cys-121 residue (other binding events of lower energy at different residues are not shown). Similarly, docking studies with HgCl₂ show that mercury ions bind at the same residue of β -lactoglobulin (Supplementary Fig. 2). This is in agreement with crystallographic studies on β -lactoglobulin protein crystals immersed in HgCl₂ salt solutions, which show that mercury ions bind at cys-121 (ref. 36). When β-lactoglobulin unfolds and hydrolyses to form amyloid fibrils, several peptides participate in fibril formation, and cys-121 is known to be involved³⁷. To generate, *in silico*, a model amyloid fibril based on cys-121-containing fragments from β -lactoglobulin, we navigated the energy landscape using zipperDB³⁸. The metal ions–ligand docking for the amyloid formed from the resulting segment (LACQCL) is shown in Fig. 1b for lead and in Supplementary Fig. 2c for mercury. This demonstrates that β -lactoglobulin amyloid fibrils possess effective binding sites capable of adsorbing multiple types of heavy metal ion, suggesting the generality of the ion-binding process. This was further confirmed experimentally by running systematic adsorption isotherms with the various metal ions considered in this work. The results indicated a general complexation process where more residues than cysteine may actually contribute to the observed overall binding (see Supplementary Fig. 3 and Supplementary Table 1 for details).

To exploit this unique property of amyloid fibrils more efficiently, hybrid adsorber membranes were created by mixing the amyloids with activated carbon. This allowed the exchange area of the membrane to be increased, while combining the mechanical properties of the carbon with the capacity of the fibrils to remove the toxic heavy metal ions.

Amyloid membranes remove metal ions and radioactive waste

Figure 1c presents a schematic representation of the adsorption of heavy metal ions onto the composite adsorber membrane during the filtration process. The assembly of the resulting hybrid films was simple and resulted in robust, homogenous films (inset of Fig. 1d and Supplementary Fig. 4). Figure 1d,e shows scanning electron microscopy (SEM) images of the surface of the composite membrane. At high magnification (Fig. 1e), amyloid fibrils on the

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Figure 2 | Concentrations of heavy metal and radioactive pollutants before and after filtration through the amyloid fibril-activated carbon hybrid adsorber membrane. a, Potassium dicyanoaurate (i). b, Mercury chloride. c, Lead acetate. d, Disodium tetrachloropalladate. e, Uranyl acetate. f, Phosphorus-32. Insets: plots on a logarithmic scale. Further insets in c and d: solution colour change before and after filtration. All error bars represent standard deviation from triplicate experiments.

activated carbon surface are resolved, demonstrating the formation of uniform composite membranes.

The composite membrane is able to adsorb several toxic heavy metal ions present as environmental water pollutants very well. To illustrate the generality of this approach, the ions considered in this study were typical pollutants of environmental and industrial relevance, including KAu(CN)₂, HgCl₂, Pb(C₂H₃O₂)₄ and Na₂PdCl₄. Before filtration, the concentration range used was adjusted to match values typical of severely polluted waters. At these concentrations, the pollutants are present in a molecular ionic form, as verified by several control experiments (Supplementary Fig. 5). Figure 2a–d presents the concentration values for the different heavy metal ions before and after filtration. In Fig. 2e,f, the same concepts are applied to the removal of radioactive waste from water.

The performance of the membrane was good for all four commonly found environmental metal pollutants and the two tested radioactive compounds. The first heavy metal pollutant investigated was $KAu(CN)_2$ (at a concentration similar to industrial pollutant wastewater: 50 ml, 30 ppm, pH 6.8), for which AAS measurements were performed before and after filtration. The measurements demonstrated a reduction in gold concentration from 30 ppm to 0.105 ppm after filtration (Fig. 2a). To investigate the full potential of these membranes, a more highly concentrated $KAu(CN)_2$ solution (561 ppm) was filtered. In this case, membrane efficiency



Figure 3 | Efficiency of the composite membrane in removing pollutants simultaneously and after several cycles of filtration. a, Simultaneous single filtration event of water contaminated by four different heavy metal ions. **b**, Concentration of mercury chloride filtered by the same composite membrane with 7.4 wt% of amyloid fibrils for ten consecutive cycles. **c**, Efficiency of the composite membrane with different ratios of amyloid fibrils and controls with alternative membranes based on cellulose. **d**, Comparison of the relative specific adsorption capacity per filtration cycle of β -lactoglobulin amyloid fibrils (β -LG) and activated carbon (AC) for the four Au, Pd, Hg and Pb ion contaminants, by processing 50 ml of 0.013 mg ml⁻¹ salt concentration. The relative adsorption capacity per filtration cycle of the amyloid fibrils (in μ g mg⁻¹) is in all cases superior to the capacity of the activated carbon, by one to three orders of magnitude. All error bars represent standard deviation from triplicate experiments.

was also very good, with the filtered solution having a residual concentration of pollutant of only 0.134 ppm. The composite membrane thus efficiently adsorbed KAu(CN)₂, reducing the concentration of pollutant gold ions by four orders of magnitude, with an adsorption efficiency of 99.98%.

The second pollutant studied was HgCl₂. An 84 ppm (pH 4) solution was filtered and the corresponding concentrations of mercury atoms before and after filtration are shown in Fig. 2b. The AAS measurements estimated that the mercury atom concentration reduced from 84 ppm to less than the AAS detection threshold (<0.4 ppm) after filtration, that is, a reduction of at least three order of magnitude, or >99.5%.

When $Pb(C_2H_3O_2)_4$ (pH 3.7) was investigated, the concentration of lead atoms was reduced from 65 ppm to less than the AAS detection threshold (<0.02 ppm) after filtration, corresponding to a reduction of at least four orders of magnitude, or >99.97%. The inset of Fig. 2c shows the change in solution colour before and after filtration, with the solution becoming completely colourless due to adsorption of the lead atoms onto the adsorber membrane surface.

The last heavy metal pollutant investigated to illustrate the generality of this filtration approach was Na_2PdCl_4 . In this case, the concentration of the solution was measured by UV–visible absorption spectroscopy, and was found to reduce from 12.2 ppm to <0.019 ppm after filtration, giving a colourless solution (Fig. 2d, inset), with an efficiency of at least three orders of magnitude, or >99.84%.

The performance of the membrane was found to be similar for decontamination of water from radioactive waste. The first radioactive compound studied was uranyl acetate. Concentrations before and after filtration were measured by UV–visible absorption spectroscopy, and starting solutions were adjusted to remain below the legal radioactivity level threshold (Supplementary Fig. 6). A reduction from an initial feeding concentration of 1,980 ppm to

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Figure 4 | Turning toxic gold ions into non-toxic gold nanoparticles and films. a, UV-visible spectra of the catalytic reduction of p-nitrophenol (PNP) to p-aminophenol (PAP) by recovered gold nanoparticles. Insets: dispersion of the gold nanoparticles and scheme for catalytic reduction. b, Plot of In A (absorption at 400 nm) versus time for the catalytic reduction of PNP, showing a first-order reaction. **c**,**d**, Visual appearance of a gold film recovered at 1,200 °C (**c**) and its electrically conductive behaviour (**d**). Inset in **d**: lithography etching on the surface of the recovered gold film by a focused ion beam (scale bar, 50 μm).

12.9 ppm was measured, a contaminant removal efficiency of three orders of magnitude, or 99.35%.

The second radioactive contaminant studied was phosphorus-32 and in this case concentrations were acquired by measuring ionizing radiation with a Geiger counter (see Methods and Supplementary Fig. 6). The initial concentration, adjusted to 12.5 nM to comply with radiation safety issues, decreased to 0.015 nM after filtration, resulting in a contaminant removal efficiency of three orders of magnitude, or 99.88%. These results illustrate the universal role of the present approach to water decontamination.

Environmentally polluted waters typically present multiple metal contaminants simultaneously, and the currently used technologies are highly inefficient in such scenarios. Figure 3a shows that our technology is well suited to adsorbing several heavy metal pollutants simultaneously. When filtering water presenting four heavy metal series at the same time, the efficiency per individual contaminant remained essentially unaffected when compared to filtration with single ions—between 99.54 and 99.92%, depending on the pollutant considered.

In Fig. 3b, the reusability of the membrane was investigated by filtering ten consecutive cycles of 50 ml of $HgCl_2$ (89 ppm) with the same adsorber membrane. The results show that the adsorber membrane works efficiently up to and beyond ten cycles of filtration. The increase in the concentration of residual $HgCl_2$ from

0.4 to 4.2 ppm over the ten cycles is possibly due to a slight saturation of the membrane after several cycles of filtration. It is worth highlighting, however, that the 10×50 ml = 0.5 l volume of water contaminated by mercury was processed using a 77 mg membrane containing only 7.4 wt% protein, that is, 5.7 mg, nearly 90,000 times less than the quantity of processed contaminated water. By extrapolating, 1 kg of protein could be used to treat 90,000 l of contaminated water.

The role of the amyloid fibrils in the performance of these hybrid membranes was further investigated by preparing membranes with various concentrations of amyloid fibrils mixed with activated carbon. Their performance was then compared over several filtration cycles of 50 ml of more concentrated HgCl₂ (1,904 ppm) to make saturation of the membranes experimentally accessible, using cellulose membranes as a control. The measured HgCl₂ concentration before and after filtration is shown in Fig. 3c.

These results illustrate that, without the amyloid fibrils, no retention of $HgCl_2$ occurs, the cellulose membrane being highly inefficient. Also, the membrane with 7.4 wt% amyloid has a much higher adsorption efficiency than the 2 wt% amyloid hybrid membrane. At 7.4 wt% amyloid, the composite membrane adsorbs mercury ions with an initial >99.99% efficiency, and the concentration of residual ions dropped by more than four orders of magnitude. In contrast, reducing the amount of amyloid fibrils

to 2 wt% rapidly depressed the initial adsorption efficiency to ~80% and the membrane saturated much more rapidly. Notably, only a 5.4 wt% increase in amyloid content led to an ~5,000 times increase in the adsorption efficiency of the membrane (Fig. 3c, first cycle), demonstrating that the amyloid fibrils in the hybrid membranes play a crucial role in the effective adsorption of heavy metal ions.

Indeed, by performing filtration experiments on fresh hybrid amyloid-carbon membranes and membranes made of 100% amyloid fibrils, we can resolve the specific adsorption efficiency per mg and per cycle of the amyloid and the activated carbon (see Fig. 3d and Supplementary Information for details). The results show that, under the filtration flow rate and concentration considered, the amyloid fibril adsorption capacity per individual filtration cycle is one (Hg) to three (Au, Pb, Pd) orders of magnitude larger than that of activated carbon (Fig. 3d). These values were obtained on fresh membranes far from saturation. The total adsorbed weight of metal ions by membranes that had reached saturation is several orders of magnitude larger and is comparable with the weight of the membrane itself (see adsorption isotherms in Supplementary Fig. 3 and Supplementary Table 1 for saturation of amyloids alone and the discussion of Fig. 4c,d for saturation of the hybrid membrane). Thus, when higher concentrations (or larger volumes of contaminated water) have to be treated, the process can be scaled up efficiently by simply increasing the concentration of amyloid in the membrane. For example, Fig. 3c shows that approximately one order of magnitude efficiency in heavy metal ions removal can be gained for several cycles by increasing the concentration of amyloid from 7.4 to 29 wt%. It should be noted, however, that on completely removing the activated carbon from the membrane, the porosity of the membrane decreases significantly and, with it, the operating water processing flow rate. Therefore, the results presented here are based on an optimal amyloid-carbon composition allowing both high efficiency and processing flow rates at the same time.

From metal pollutants to valuable materials

Although environmentally polluted water may contain several heavy metal pollutants, contamination by a single heavy metal is relevant in industrial wastewater and thus opens up metal recovery strategies. Beside their high affinity for metal ions, amyloid fibrils also have the capability to reduce, via a bio-mineralization process, metal ions into metal nanoparticles³⁴. Thus, the presence of protein fibrils inside an adsorber membrane also enables the recovery of expensive heavy metal ions, by thermally or chemically converting them from pollutants to valuable nanoparticles. This process is illustrated here with the most valuable of the studied toxic pollutants, $KAu(CN)_2$, which is a notable pollutant found in wastewater from the electroplating industry, but is also a possible precursor to gold nanoparticles. To this end, the membrane obtained after filtration of the KAu(CN)₂, the concentration of which was adjusted to mimic industrially relevant pollution streams (Fig. 2a), was placed in a furnace at 750 °C for 3 h. After thermal reduction and cooling, the residues were redispersed in water, and the sample colour appeared red (inset of Fig. 4a) due to the formation of gold nanoparticles (confirmed by AAS) and the degradation of the carbon (Supplementary Fig. 8). After removal of organic hashes and purification, AAS revealed that more than 70% of the original gold ions were recovered from the filtrate into nanoparticulate elemental gold. Alternatively, conversion of trapped gold ions into elemental gold could also be achieved via chemical reduction (Supplementary Fig. 9).

These particles are non-toxic compared to the initial cyanide complex solution, and are also a valued material, which can be further processed using a multitude of technologies. For example, the catalytic activity of the as-reduced nanoparticles was investigated by photometric monitoring of p-nitrophenol (PNP) reduction to p-aminophenol (PAP)³⁹. Figure 4a shows the time-dependent

UV–visible absorption analysis of the conversion of PNP to PAP with the gold nanoparticles acting as a catalyst. The UV–visible absorption spectrum of PNP shows a characteristic peak at 400 nm, then as the reduction process progresses, the peak at 400 nm vanishes, whereas a peak at 300 nm emerges. After reduction, the PNP solution changes colour from light yellow to transparent (Supplementary Fig. 10). We followed the intensity of the peak at 400 nm to rationalize the kinetics of the PNP reduction. The reaction was found to be of first order, and K_{cat} was calculated to be 2.96×10^{-2} min⁻¹ at a gold nanoparticle concentration of 3 µg ml⁻¹ (Fig. 4b). This indicates a catalytic performance comparable to or slightly better than that of equivalent commercial gold nanoparticles (Supplementary Fig. 11).

The gold nanoparticles were further processed into bulk gold by melting them at 1,200 °C and pressing the gold solid into a film by applying hydraulic pressure (Fig. 4c). Figure 4d presents the voltage-current behaviour of the resulting high-purity film (Supplementary Fig. 12), which shows pure ohmic behaviour, with a conductivity of $\sim 2.2 \times 10^7$ S m⁻¹. The inset of Fig. 4d shows that the surface of the recovered gold film can be rendered lithography-grade by sculpturing any pattern by focused ion beam lithography. Palladium nanoparticles were also recovered following the same method, and these nanoparticles also exhibited catalytic behaviour (Supplementary Fig. 14). Notably, the recovery process is cost-effective, with 100 mg gold (Fig. 4c) recovered using a single saturated membrane of 77 mg carbon and 5.7 mg β -lactoglobulin; that is, the recovered gold had a value of about 200 times the original value of the saturates.

In summary, we have developed an efficient hybrid membrane consisting of amyloid fibrils and activated carbon, which can be used to purifying wastewater that contains toxic heavy metal and nuclear waste environmental pollutants, even in simultaneous ions removal mode. The membranes can be used for several cycles of wastewater purification with minimal reduction in performance. The technology is flexible, performs well over a broad pH range (Supplementary Fig. 15), is scalable, and can be implemented easily using inexpensive protein precursors. In Supplementary Fig. 16 we show how amyloid fibrils produced from the very inexpensive whey protein (the industrial precursor of β-lactoglobulin) also allow multiple cycles of ion removal with comparable efficiency. Individual performances of hybrid membranes made of other types of amyloid protein, or composites thereof, are also compared in Supplementary Fig. 17. The membranes can also be used to remove bacteria from water via size exclusion filtration (Supplementary Fig. 18), further broadening their scope in water purification applications. Finally, the membranes also allow the most valuable heavy metal contaminants to be recycled by thermally or chemically reducing the ions trapped within the saturated membranes, converting them into elemental metal nanoparticles and films.

By exploiting the high metal-binding feature of the amyloid fibrils or the high porosity of the activated carbon, the membrane composition can be adjusted to optimize its performance as a water purification membrane or a metal recycling membrane, respectively (for a discussion see Supplementary Table 1). The intermediate concentrations reported here are designed to ideally serve both purposes simultaneously. Due to the affordable nature of the membrane constituents and the inexpensive processes used for both adsorption and metal recovery, this technology could be of significant use in addressing the global problem of wastewater treatment and environmental pollution.

Methods

Methods and any associated references are available in the online version of the paper.

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Author contributions

S.B. performed the experiments, analysed the results and wrote the manuscript. R.M. designed the study, analysed the data and wrote the manuscript. Both authors discussed the results and commented on the manuscript.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to R.M.

Competing financial interests

The authors are the inventors of a patent filed by ETH Zurich related to the work presented here (EP2921216 and WO2015140074).

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Methods

BioPURE β -lactoglobulin was purchased from the Technische Universität Munich, Department of Food Process Engineering and Dairy Technology, Munich, Germany. For a discussion of the purification of the β -lactoglobulin and the detailed preparation of amyloid fibrils see ref. 40. Potassium dicyanoaurate(1), mercury chloride, lead acetate and disodium tetrachloropalladate were purchased from Sigma-Aldrich. Radioactive phosphorus [γ -³²P] (Perkin Elmer) labelled with double-stranded DNA was prepared by incubating primer (VBC Biotech, 23-mer, TAA TAC GAC TCA CTA TAG GGA GA) with the template (VBC Biotech), following the manufacturer's protocol.

Preparation of adsorber membranes. Hybrid composite adsorber membranes were prepared to adsorb the toxic heavy metal and radioactive pollutants. Typically, to yield a 2 wt% protein hybrid membrane, 5 ml of a 10 wt% dispersion of activated carbon was mixed with 0.5 ml of a 2 wt% β-lactoglobulin (pH 2) protein fibril solution. A 1 ml volume of this solution was vacuum-filtered using 0.22 µm cellulose filters (diameter, 25 mm). 7.4 wt% protein hybrid membranes were prepared by filtering 1 ml solution containing 5.7 mg β-lactoglobulin fibrils and 71.5 mg activated carbon dispersion. 29 wt% protein hybrid membranes were prepared in the same way by vacuum filtering 1 ml solution containing 20.4 mg amyloid fibrils and 50 mg activated carbon dispersion. The extreme adhesiveness and stiffness of the protein fibrils enabled the homogeneous assembly of the hybrid amyloid fibrils–activated carbon composite adsorption membranes. These adsorption membranes were then used as is to adsorb heavy metal ion pollutants from environmentally polluted water and used for high-temperature reduction in the recovery of the heavy metals.

Atomic absorption spectroscopy. The metal content was measured by atomic absorption spectroscopy using an AA240Z Zeeman graphite-furnace (GTA 120) equipped with a PSD 120 programmable sample dispenser. The sample was digested with aqua regia and the metal content was measured by atomic absorption spectroscopy in triplicate. A separate calibration was performed by measuring solutions with various concentration regimes.

Radioactivity measurements. Radioactivity measurements were performed by establishing calibration curves with a Thermo Scientific series 900 mini monitor Geiger counter. The total level of radiation was maintained systematically below the acceptable threshold set by federal laws.

Scanning electron microscopy. SEM imaging was carried out using a Zeiss SEM (LEO 1530). The catalytic adsorber membrane was initially glued to the metal substrate and the surface of the amyloid hybrid membrane was scanned at an accelerating voltage of 3 keV.

Molecular modelling and docking. The molecular docking program ArgusLab 4.0.1 was used to generate an ensemble of docked conformations. β-Lactoglobulin apo-protein (unbound protein) was used for docking studies. The known crystal structure of β -lactoglobulin (PDB ID: 3NPO) was obtained from the RCSB Protein Data Bank. The protein sequence was submitted to the zipperdb server to generate amyloid-forming segments. The submitted sequences were broken up into six-residue fragments to yield model amyloid fibrils from short hydrolysed fragments. The amyloid-forming segment with the sequence of cys 121 and with the best energy and shape complementarity was selected. The ArgusDock exhaustive search docking engine, with a grid resolution of 0.40 Å, was used. The docking precision was set to regular precision, and the flexible ligand docking mode was used for the docking run. To identify the binding sites in β lactoglobulin, blind docking was carried out and the grid size was set to 146, 156 and 132 Å along the x, y and z axes, respectively. During docking, a maximum of ten conformers were considered. The lowest energy conformation was used for further analysis

Atomic force microscopy. The atomic force microscope tapping mode was used on a Nanoscope VIII Multimode Scanning Force Microscope (Veeco). The solutions were deposited onto freshly cleaved mica sheets, incubated for 2 min, rinsed with Milli-Q water and dried by nitrogen. For all experiments, MPP-11100-10 tips for the tapping mode in soft tapping conditions were used (Veeco) at a vibrating frequency of 300 kHz. Images were simply flattened using the Nanoscope 8.1 software, and no further image processing was carried out.

Transmission electron microscopy. Transmission electron microscopy (TEM) imaging was performed on carbon-coated copper grids, which were previously glow discharged for 45 s (Emitech K100X, GB). A 3.5 µl volume of the dispersion was placed on the grids and, after waiting 1 min for settlement, excess liquid was drained off with a piece of filter paper. Dried grids were examined by bright-field TEM (FEI, model Morgagni, NL) operated at 100 kV.

UV-visible spectrophotometry. Ultraviolet measurements were used to follow the reduction of metal ions from salts to nanoparticles using a CARY-100 Bio UV-visible spectrophotometer.

References

 Jung, J.-M., Savin, G., Pouzot, M., Schmitt, C. & Mezzenga, R. Structure of heatinduced β-lactoglobulin aggregates and their complexes with sodium-dodecyl sulfate. *Biomacromolecules* 9, 2477–2486 (2008).