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Bioengineered Chimeric Spider Silk-Uranium Binding Proteins

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Abstract

Heavy metals constitute a source of environmental pollution. Here, novel functional hybrid biomaterials for specific interactions with heavy metals are designed by bioengineering consensus sequence repeats from spider silk of *Nephila clavipes* with repeats of a uranium peptide recognition motif from a mutated 33-residue of calmodulin protein from *Paramecium tetraurelia*. The self-assembly features of the silk to control nanoscale organic/inorganic material interfaces provides new biomaterials for uranium recovery. With subsequent enzymatic digestion of the silk to concentrate the sequestered metals, options can be envisaged to use these new chimeric protein systems in environmental engineering, including to remediate environments contaminated by uranium.

Keywords

bioremediation; heavy metals; proteins; spider silk; uranium

1. Introduction

The potential risks from heavy-metal pollution in the environment have prompted studies into remediation options. In particular, after the Fukushima nuclear incident,^[1] steps have been initiated for the treatment of sea water to determine the hazardous side-effects on ecological systems. The main source of pollutants from this catastrophe include nuclear fuel (uranium, plutonium) and fission products (strontium, selenium, technetium, cesium, iodine), which are toxic to living organisms. In order to identify options to recover and remove such contaminants, or to monitor the presence of such toxic metals, there are needs to develop new modes of recovery, with a goal for monitoring and remediation. Molecular biomimetics^[2] is an emerging approach in which hybrid technologies are developed by means of molecular biology tools and nanotechnology. Currently, plants or microorganisms are used to remove heavy metals like mercury,^[3] lead,^[4,5] and cadmium.^[4] Magnetotactic bacteria biomineralize magnetite (Fe_3O_4) or greigite (Fe_3S_4) within their magnetosomes.^[6] U(VI) reduction can be catalyzed by many microbes, the majority being metal- or sulfate-reducing bacteria. Successful uranium bioremediation requires strict anaerobic conditions, with the biomass-associated nanoparticles tending to aggregate, making it unlikely that they will be transported in groundwater.^[7] Understanding the fundamentals of biological mechanisms and chemical parameters to bioremediate natural systems to safely biomineralize heavy metals or convert them into insoluble forms will help in realizing options to generate environmentally benign aqueous-based biopolymer solutions for the removal of heavy metals, especially radionuclides.

Many metal sensors have been reported for the detection and treatment of uranium poisoning. A catalytic beacon sensor for uranium based on a DNAzyme was established with a detection limit of 11 parts per trillion.^[8] However, the practical application of these sensors in detecting uranium in contaminated soils was less optimal in terms of selectivity. Recombinant luminescent bacterial sensors have also been constructed for the determination of bioavailable fraction of specific metals like cadmium, zinc, mercury, and chromium in soil.^[9] Here, two bacterial recombinant heavy metal sensors were constructed based on two different receptor-reporter systems: one was inducible by Zn^{2+} , Cd^{2+} , and Hg^{2+} and the other by Cr(VI) and Cr(III) . These bacterial sensors were not specific to one heavy metal. These above examples illustrate the challenges in generating metal-specific sensors, which is further complicated when considering heavy metal recovery systems. In this case, avoidance of further insult to the environment, while optimizing metal-specific recovery needs, remains a major challenge.

Taking lessons from biology, proteins can be genetically engineered to selectively bind to inorganic compounds for applications in nano- and biotechnology. In this context, a chimeric spider silk protein was fused with uranium recognition motifs, to generate new protein designs that exploit the benefits of each component, but in a versatile materials-related format. These new proteins may find utility in chelation therapies to treat human exposures,^[10] environmental recovery operations including monitoring,^[8,11] nuclear waste management,^[12] developmental biology,^[13] and clinical toxicology.^[14] The use of metal-accumulating systems for the production of nanoparticles, and their assembly from silk, may allow control over the size, morphology, composition, and crystallographic orientation of

the particles. The potential of such biomimetic materials will also be relevant to the production of new advanced materials, with applications in metal and radionuclide detection used as molecular biomagnets, solar energy and electrical battery applications, and microelectronics.

Biosensors based on bioengineered proteins have also been designed previously. Here, conformational changes caused by the binding of the metal ion to the engineered protein were tracked. A uranyl-responsive DNA-binding protein was genetically engineered by mutating three positions in a NikR template, a nickel-dependent transcriptional repressor from *Escherichia coli*.^[15] The basic coordination principles unique to actinyl ions, such as uranyl, can be applied in a protein framework to achieve selective uranyl binding.

Silks are biologically derived proteins that form fibers with exceptional mechanical properties, displaying high strength and toughness.^[16,17] These lustrous materials self-assemble in alternating crystalline and amorphous domains that impart unique mechanical properties. The application of silks in the field of biomaterials has been investigated actively due to the biocompatibility and biodegradability, and the ease with which silk proteins can be fabricated into particles, fibers, foams, and scaffolds from all-aqueous processing methods.^[18,19] The remarkable properties of these proteins prompt interest in their functionalization for enhancement in properties, such as for heavy metal sequestration and recovery. Recombinant spider silks considered in this study are comprised of the consensus repeat of spidroin 1 from the major ampullate (MaSp1) of *Nephila clavipes*. The repetitive stretch of hydrophobic glycine-alanine and alanine domains are responsible for formation of stable β -sheets that contribute to the mechanical strength, whereas the hydrophilic spacers comprised of glycine-glycine-x domains result in toughness.^[20,21] Spider silk inspired block copolymers designed by varying ratios of hydrophobic and hydrophilic domains have resulted in some understanding of the interplay between composition, assembly behavior and properties^[22–26] in order to bridge research and applications.

The successful chemical decoration chimeric silk protein materials generated by the fusion of spider silk with functional domains to provide multifunctional polymers and materials has been reported, including organic/ inorganic features for specific biological functions. For example, the incorporation of silica binding R5 peptide^[27] to recombinant silk resulted in the formation of new mineralized silk hybrid materials. In addition, hydroxyapatite was formed on recombinant spider silk fused with dentin matrix protein^[28] and chimeric silk-silver binding proteins displayed antimicrobial properties.^[29]

In order to develop new proteins that bind to uranium, a 33-amino acid sequence corresponding to helix-turn-helix motif of the calcium binding site I of protein calmodulin from *Paramecium Tetraurelia* was considered.^[30] The mutation of two residues of the metal coordinating loop provided access for peptide selective for uranyl ions. Designing proteins for sequestration or sensors capable of detecting a particular ion is challenging. In particular, many metal ions are similar, making them difficult to detect either at low concentrations or to avoid interference with other metals. Uranium can be present in number of oxidation states, of which +6 oxidation state is the uranylion (UO_2^{2+}) and the most stable in aerobic

and aqueous conditions. As a Lewis acid, UO_2^{2+} interacts with oxygen in the equatorial plane. UO_2^{2+} deposits in bone with metabolism similar to that of calcium, making ionic substitution a plausible hypothesis for some biochemical features of uranium. A number of serum proteins including transferrin,^[31] serum albumin,^[32] designed peptides^[15,30] and DNA^[33] interact with uranyl mainly through the carboxylic groups like aspartate and glutamate. In nature, certain bacteria, fungi, and plants reduce uranium in salts from its soluble +6 oxidation state to the less soluble +4 oxidation state. This change remains a promising approach for bioremediation by decreasing the bioavailability of uranium.^[34]

The present contribution describes the cloning and purification of recombinant silk-uranyl binding proteins and their ability to selectively respond to uranium ions. The goal of the study was to combine a calmodulin sequence mutated at two residues for increased binding of uranium with silk, to generate new uranium binding biomaterials. These fusion proteins enable new material designs with robust mechanical strength, biocompatibility and versatile processing in water, with specificity for binding to uranium. As a substrate for environmental use towards uranium collection and removal, this system is unique in the combination of material properties, selectivity and utility from a biodegradation and remediation perspective.

2. Experimental Section

2.1. Cloning of Uranium-Binding Sequence into pET30L Vector Containing Silk Modules

The vector pET30a(+) (Novagen, San Diego, CA) was used for the construction of the vector pET30L carrying the silk block copolymer and its assembly was described in our prior studies.^[28] The spider silk block copolymer was cloned together with six histidine residues to facilitate purification. The clone containing the bacterial plasmid pUC carrying the uranium binding cDNA sequence was designed from Genscript. The clone with pUC plasmid carrying the uranium binding cDNA sequence was inoculated in Luria Bertani (LB) medium and grown overnight at 37 °C with shaking (200 rpm). The plasmid was extracted using Qiagen miniprep kit for plasmid isolation and digested with *NheI* & *SpeI* enzymes (New England Biolabs) which cuts in the regions flanking the uranyl binding cDNA sequence. The digestion product was run in a 0.8% agarose gel and the band for uranyl cDNA sequence was purified using a QIAquick gel extraction kit (Qiagen). For insertion of the clone in the expression vector pET30L, the vector was digested with *SpeI*, dephosphorylated with alkaline phosphatase, calf intestinal (CIP) enzyme (New England Biolabs) and run on 0.8% agarose gel. The linearized vector was purified using the QIAquick gel extraction kit. The clone was then inserted in the linearized vector pET30L. The ligation reaction was carried out with T4 DNA ligase enzyme (New England Biolabs).

E. coli DH5α cells (Invitrogen) were transformed with the ligation product and transformants were identified by incubation on agar plates containing 25 μg mL⁻¹ kanamycin. The presence of uranyl insert sequence in the pET30L silk 6mer vector was confirmed by DNA sequencing using T7 and T7 terminator sequences. The new constructs were named

6merU1 and 6merU2 with one and two repeats of the uranyl binding sequences, respectively.

2.2. Protein Expression, Purification, and Identification

Both 6merU1 and 6merU2 proteins, along with control 6mer (silk alone) were expressed in *E. coli*, RY-3041 strain, a mutant strain of *E. coli* BLR(DE3) defective in the expression of SlyD protein.^[35,36] Cells were cultivated at 37 °C in Luria Bertani (LB) medium, with 25 µg mL⁻¹ kanamycin until an OD₆₀₀ between 0.6 and 0.8 was reached. At this point, expression was induced by adding isopropyl-β-D-thiogalactoside (IPTG, Invitrogen) to a final concentration of 0.5×10^{-3} M. After 5 h, cells were harvested by centrifugation at 6500 rpm. The cell pellet was resuspended in a denaturing buffer (0.100 M NaH₂PO₄, 0.010 M Tris/HCl, 8 M urea, pH = 8.0) and left overnight with stirring for complete cell lysis. Insoluble cell fragments and soluble proteins present in the cell lysate were separated by centrifugation at 11 000 rpm. The supernatant was mixed with Ni-NTA resin (Qiagen) and left for 2 h with stirring. The supernatant/Ni-NTA resin mixture was loaded onto a glass econo-column (Biorad) and washed several times with denaturing buffer at pH = 8.0 and with washing buffer at pH = 6.3 and pH = 5.9. The protein 6merU1 was eluted using denaturing buffer at pH = 4.5. The purified protein was dialyzed first against a 0.020 M sodium acetate buffer followed by extensive dialysis against water using cellulose ester snake skin membranes with 100–500 Da molecular-weight cut-off (SpectraPor Biotech). Finally, the dialyzed proteins were lyophilized.

Protein sequencing (Tufts Core Facility, Boston, MA) and SDS-PAGE were used to confirm protein identity. For SDS-PAGE, proteins were mixed with NuPAGE LDS sample buffer (Invitrogen) and heated at 80 °C for 10 min. The samples were separated using a bis-tris 4–12% gel (Invitrogen) and stained with colloidal blue staining kit (Invitrogen).

2.3. Preparation of Uranyl Solutions

All aqueous solutions were prepared with pure water (18.2 MΩ cm resistivity, MilliQ station, Millipore). A stock solution of uranyl nitrate (0.010 M) was prepared and acidified at pH = 2.0 using nitric acid to avoid formation of hydroxides and stored at 4 °C. Working solutions were prepared daily by diluting the stock solution in 1×10^{-3} M phosphate buffer pH = 6.5.

2.4. Circular Dichroism (CD)

CD spectra were obtained from Aviv, Model 410 instrument (Biomedical Inc., NJ, USA), equipped with a thermostatic cell holder. Peptides were dissolved at 10×10^{-6} M in phosphate buffer, pH = 6.5. Spectra were run at room temperature from 190 to 260 nm using 0.1 cm quartz cuvette. Each spectra represents average of three spectra, obtained with an integration time of 2 s every 0.5 nm. The secondary structures were determined by submitting CD spectra to the online server <http://www.ogic.ca/projects/k2d2> using the K2D2 program. K2D2^[37] server is a neural network online program that uses self-organized map (SOM) of spectra from proteins with known structure to deduce maps of protein structures whose secondary structure need to be estimated.

2.5. General Procedure for Fluorescence Quenching Experiments

Fluorescence quenching experiments were realized using a microplate reader (SpectraMax M2, Molecular devices) top read equipped with a Xenon flash lamp (1 J per flash). Data obtained from quenching experiments were collected with SoftMax Pro software (Molecular Devices). In a typical experiment, different concentrations of uranyl ions were added to the microplate wells and excited at a wavelength of 266 nm with a time delay of 800 μ s after which fluorescence was measured at the emission wavelength of 522 nm corresponding to the uranyl ion peak. Next, varying amounts of chimeric proteins were added and incubated for 15 min followed by measurements, and the decrease in the intensity was recorded. All of the measurements were performed in triplicate.

2.6. Data Analysis

Fractions of an aqueous solution of protein were added successively to 100 mM solution of uranyl nitrate in 1×10^{-3} M phosphate buffer at pH = 6.5. The fluorescence spectra of uranyl ion ($\lambda_{\text{ex}} = 266$ nm) was recorded after each addition. The graph intensity at 522 nm as a concentration of uranium added is then simulated by the binding isotherm corresponding to a 1:1 complex between uranium and protein, according to the relationship deduced from the expression of dissociation constant:

$$(I_0 - I) = (I_0 - I)_{\text{max}} \frac{\left\{ \left(4[U]_0 + \frac{K_d}{[P]} \right) - \sqrt{\left(4[U]_0 + \frac{K_d}{[P]} \right)^2 - 16[U]_0^2} \right\}}{4[U]_0} \quad (1)$$

where I_0 = intensity of the maximum emitted fluorescence without protein, I = intensity of the maximum emitted fluorescence with protein, $[U]_0 = 1 \times 10^{-4}$ M; K_d = dissociation constant of the complex, and $[P]$ denotes the amount of protein added to the solution. The experimental data and also the interpretation thereof by means of the above equation are represented in Figure 5.

2.7. Fluorescence Quenching

The dynamic or static nature of the fluorescence quenching was investigated for the chimeric protein uranyl systems. Graphs were plotted according to the Stern-Volmer equation:^[38]

$$\frac{F_0}{F} = 1 + k_q \tau_0 [Q] = 1 + K_{SV} [Q] \quad (2)$$

where F_0 and F are the relative fluorescence intensities of uranyl nitrate and proteins (6mer, 6merU1, and 6merU2); $[Q]$ the quencher concentration (M), k_q the biomolecular quenching rate constant ($\text{L mol}^{-1} \text{s}^{-1}$); τ_0 the lifetime of the fluorophore in the absence of uranium (s); and K_{SV} the Stern-Volmer constant ($\text{L} \cdot \text{mol}^{-1}$). If the evolution of F_0/F plots, according to the concentration of quencher, is linear for the whole range of quencher concentrations, fluorescence quenching can be attributed either to being purely dynamic, or purely static. The latter mechanism being due to the formation of a ground-state nonfluorescent complex. In contrast, if the ratio F_0/F is not linear and shows an upward

curve at higher quencher concentrations, the fluorescence quenching mechanism can be attributed to the presence of simultaneous dynamic and static quenching. In the latter situation, from the bimolecular quenching rate constant value k_q , determined in the linear range of the F_0/F ratio, the initial fluorescence quenching mechanism can be determined. Typically, if k_q is much higher than $10^{10} \text{ L mol}^{-1} \cdot \text{s}^{-1}$, i.e., the upper value possible for diffusion limited quenching in most solutions at room temperature, the fluorescence quenching mechanism is initially a static one, whereas with the lower k_q values it is initially a dynamic quenching.^[38]

2.8. Statistics

All of the experiments were performed with a minimum of $N = 3$ for each data point. Statistical analysis was performed by a chi-squared test to evaluate the data using IgorPro5.3 to have a good fit.

3. Results and Discussion

3.1. Production of Recombinant Uranyl-Binding Fusion Pproteins

The design and expression of two new chimeric proteins was based on the fusion of consensus repeats of spider silk replicated six times along with incorporation of the nucleotides for a C-terminal 33-amino acid sequence inspired by calmodulin that recognizes uranyl ions, using techniques previously described (Figure 1).^[19,27] The presence of single and double repeats of uranyl binding sequences was confirmed by DNA sequencing (Tufts Core Facility, Boston, MA) and expression of chimeric proteins was carried out in *E. coli*. SDS-PAGE (Figure 2) indicated that both expression and purification of proteins were successful and protein sequencing confirmed the N-terminal sequence. The theoretical molecular weights of the silk 6mer, silk 6merU1, and silk 6merU2 were 20.9, 24.8, and 28.6 kDa, respectively. The appearance of the proteins around 28 kDa was mainly due to the hydrophobic amino acid composition of the chimeric proteins, inhibiting the proper migration of bands in aqueous buffers.^[28] After purification, dialysis and lyophilization, the yields of the 6mer, 6merU1 and 6merU2 were approximately 20 mg L^{-1} each.

3.2. Metal Binding Assessed by Circular Dichroism (CD)

CD spectra of the chimeric proteins 6merU1, 6merU2, and control 6mer were recorded in phosphate buffer (10^{-3} M phosphate buffer, pH = 6.5) in the absence of metal ions (Figure 3). Metal free spectra of the recombinant proteins indicated a dominant β -hairpin conformation characterized by a negative minimum at approximately 203 nm, corresponding to the spider silk. However, there were conformational changes for 6merU1 and 6merU2 upon the addition of uranyl ions. Initially, the addition of eight equivalents of uranyl ions led to peaks shifted towards 206 nm, and the effect was prominent for 6merU2 with 16 equivalents of uranyl nitrate, forming a distinct α -helical shoulders at 206 nm and 222 nm, apart from the β -hairpin conformation from the spider silk. These results are in agreement with the stabilization of the native α -helical conformation by uranyl binding.^[30] Table 1 indicates the fraction of secondary structures of these recombinant systems. Deconvolution of spectra indicated an increased α -helix content for both 6merU1 and 6merU2, from 4.7 to 8.2% and from 2.6 to 10.6% in the absence and presence of uranium nitrate, respectively,

from the uranyl binding motif. The significant b-hairpin conformation was from the spider silk. In contrast, the control 6mer showed only 4.7% α -helical content with uranyl nitrate.

Since the uranyl binding sequence was adopted from a mutated calmodulin motif, we wanted to evaluate the selectivity of the recombinant proteins with other metal ions, in particular calcium. Interestingly, the α -helical content of 6merU1 and 6merU2 were lower than in the presence of uranyl ions (6.2 and 7.6%, respectively). These results showed that the recombinant proteins were able to bind uranium in its uranyl form selectively. Thus, the genetically engineered proteins were selective towards uranyl binding and spider silk alone was not altered in terms of secondary structure.

The calcium binding to calmodulin peptides has been studied by circular dichroism where the addition of calcium ions stabilizes the α -helices of the helix-loop-helix motif.^[39] Interestingly no ordered structure was observed in the presence of calcium ions, even when a 50-fold excess of Ca^{2+} versus the mutated peptide was added.^[30] No evidence of the affinity of lanthanide binding to the mutated peptide was obtained from fluorescence measurements showing that the mutated peptide could bind to uranium in the uranyl form with high selectivity. Comparing these results with ours, the recognition motif plays an important role in uranium binding.

3.3. Fluorescence Quenching

Fluorescence titrations of 6merU1, 6merU2, and the control 6mer protein was achieved by titrating solutions of UO_2^{2+} in 10^{-3} M phosphate buffer at pH = 6.5. As an example, fluorescence spectra for 6merU2 is represented in Figure 4. The fluorescence intensities of the uranyl ions decreased concomitantly with increasing protein concentrations. Furthermore, the spectra showed there was no significant λ_{em} shift with the addition of proteins. These data indicate that the proteins interacted with UO_2^{2+} and quenched their fluorescence without affecting their microenvironmental properties, as the protein does not contain tryptophan residues (intrinsic fluorescent probe) and there was no shift in the peak – either blue or red λ_{em} with conformational changes of the protein.

3.4. Chelation of Heavy Metals with Chimeric Peptides

The mutation of the calmodulin peptide by replacing aspartic acid with threonine (at positions 20 and 24, PDB code 1EXR) was based on the finding that the hydrogen atoms of the hydroxyl groups could be positioned at a distance from the uranyl ion compatible with the formation of hydrogen bonds between the hydroxyl groups of the amino acid side chain and the oxygen atoms of the uranyl core, thereby selective to the uranyl ion instead of calcium ion despite similar charges.^[30] Furthermore, the reduction of the negative charge of the binding loop also reduced electrostatic interactions, enabling selective uranyl ion binding. The binding properties of the chimeric peptides for uranyl were studied using time resolved fluorescence spectroscopy.

A spectrum of 10^{-4} M solution of uranyl nitrate in 10^{-3} M phosphate buffer (pH = 6.5) presented several fluorescence maxima that reflect the symmetrical vibration of U—O bond of the uranyl core. Upon increasing concentrations of peptides, a decrease in intensity of free

uranyl fluorescence was observed. Since no modification in the uranyl pattern was observed in the titration, this decrease could be attributed to static quenching phenomenon and to the formation of uranyl/peptide complex in the solution. Fitting of the experimental fluorescence data (Figure 5) using an equilibrium corresponding to the formation of a monomeric complex in solution led to apparent equilibrium constants of 3.1 ± 1.1 , 1.8 ± 0.2 , and $1.9 \pm 0.2 \times 10^{-6}$ M for the 6mer alone, 6merU1, and 6merU2 proteins, respectively. Although, the dissociation constants were lower for the 6merU1 and 6merU2, indicating higher binding affinity in comparison to the control 6mer, there was no difference between the two chimeric proteins. Previous studies have shown that the calmodulin peptide coordinates with uranium with a dissociation constant of between 3.8 and 18×10^{-6} M.^[30] Moreover, since phosphates are known to be strong chelators of uranyl ions,^[40]

$$K_d \left(PO_4^{3-} \right) = 10^{-13.7} \text{ M}, K_d \left(HPO_4^{2-} \right) = 10^{-7.7} \text{ M}, K_d \left(H_2PO_4^- \right) = 10^{-2.7} \text{ M},$$

this result is indicative of high affinity of the peptide for UO_2^{2+} .

It is important to note that the binding studies reported here were conducted at pH = 6.5. We anticipate that changes in pH would have some impact on the binding. In the present study we balanced metal solubility, the pI of the silk (≈ 4.2) and the anticipated environmental pH range where these new materials were most likely to be used (e.g., a range of pH 6–7.5 to cover groundwater, marine waters, and general drinking water).

3.5. Fluorescence Quenching Mechanisms

In order to determine the quenching mechanisms between the recombinant proteins and uranyl ions, fluorescence titration was tested using Stern-Volmer plots (Figure 6). Based on the experimental data, at lower concentrations of the protein, Stern-Volmer graphics were linear. The graph suggests that fluorescence quenching was not initiated with dynamic quenching but by static quenching, starting with the formation of a complex between the protein and uranyl ions. Thus, $\log K_{sv}$ values were determined to be 7.2, 5.1, and 7.3 for 6mer, 6merU1, and 6merU2, respectively. These values show that the binding of uranium is according to: 6merU2 > 6mer > 6merU1. However, using this Stern-Volmer approach, only the linear range of F_0/F ratio can be used, thus excluding higher concentrations. In addition, the number of binding sites and their densities could not be obtained.

4. Conclusion

Environmental contamination can lead to public health concerns and ecosystem damage. These factors drive the demand for biosensors and remediation options to deal with silent contaminants. In the present study, the synthesis of new spider silk uranyl binding proteins was demonstrated through bioengineering of varying repeats of uranyl binding sequence adopted from a mutated calmodulin sequence in combination with self-assembling spider silk sequences. The resulting proteins showed selective binding of the uranyl cation by circular dichroism and fluorescence measurements. This approach provides a new means of fabricating metal ion binding materials with potential utility in remediation, based on the extensive literature on materials fabrication from silks. Further, new biosensors based on spider silk can also be considered where selective metal binding can be exploited. The functionalization of chimeric spider silk proteins with cell binding domains, cytokines,

antimicrobial and silver binding peptides have resulted in enhanced properties related to cell adhesion, mineralization and nanostructure formation, respectively. These new uranyl binding systems add to this repertoire of functionalized silks wherein environmental remediation and related needs can be addressed. Future studies will involve understanding how the heavy metal-chelating biopolymers coat and interact with contaminated surfaces to develop novel and safe decontamination methods for removal of radio-nuclides, thereby allowing increased efficiency of decontamination without adding new toxicity or disposal problems in the process.

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6mer (Mol. Wt.: 20.9 kDa)
 MHHHHHHSSGLVPRGSGMKETAAKFERQHMDSPDLGTDDDDKAMAASGRGG
 LGGQQGAGAAAAAGGAGQGGYGGGLGSQGTSGRGGLGGQQGAGAAAAAGGAGQG
 GYGGGLGSQGTSGRGGLGGQQGAGAAAAAGGAGQGGYGGGLGSQGTSGRGGLGG
 QGAGAAAAAGGAGQGGYGGGLGSQGTSGRGGLGGQQGAGAAAAAGGAGQGGY
 GLGSQGTSGRGGLGGQQGAGAAAAAGGAGQGGYGGGLGSQGTSG

6merU1 (Mol. Wt.: 24.8 kDa)
 MHHHHHHSSGLVPRGSGMKETAAKFERQHMDSPDLGTDDDDKAMAASGRGG
 LGGQQGAGAAAAAGGAGQGGYGGGLGSQGTSGRGGLGGQQGAGAAAAAGGAGQG
 GYGGGLGSQGTSGRGGLGGQQGAGAAAAAGGAGQGGYGGGLGSQGTSGRGGLGG
 QGAGAAAAAGGAGQGGYGGGLGSQGTSGRGGLGGQQGAGAAAAAGGAGQGGY
 GLGSQGTSGRGGLGGQQGAGAAAAAGGAGQGGYGGGLGSQGTSGEQIAEFKEAFA
LCTKDGTGYITTKELGTCMRSLTS

6merU2 (Mol. Wt.: 28.6 kDa)
 MHHHHHHSSGLVPRGSGMKETAAKFERQHMDSPDLGTDDDDKAMAASGRGG
 LGGQQGAGAAAAAGGAGQGGYGGGLGSQGTSGRGGLGGQQGAGAAAAAGGAGQG
 GYGGGLGSQGTSGRGGLGGQQGAGAAAAAGGAGQGGYGGGLGSQGTSGRGGLGG
 QGAGAAAAAGGAGQGGYGGGLGSQGTSGRGGLGGQQGAGAAAAAGGAGQGGY
 GLGSQGTSGRGGLGGQQGAGAAAAAGGAGQGGYGGGLGSQGTSGEQIAEFKEAFA
LCTKDGTGYITTKELGTCMRSLTSEQIAEFKEAFALCTKDGTGYITTKELGTCMR
SLTS

Figure 1.

Protein sequences of recombinant spider silk proteins with their molecular weights. Top: 6mer (silk control); middle: 6merU1; bottom: 6merU2. Underline denotes the uranyl binding sequence.

Figure 2.
SDS-PAGE of purified recombinant proteins visualized with colloidal blue stain: lane M – Marker, lane 1–6mer, lane 2– 6merU1, and lane 3–6merU2.

Figure 3.

Circular dichroism spectra of 2×10^{-6} M recombinant proteins (A) 6merU1, (B) 6merU2, and (C) 6mer in 10^{-3} M phosphate buffer, pH = 6.5, in the absence and presence of uranyl/calcium ions. Recombinant proteins in the absence of salt (shaded diamond); in the presence of 8 (shaded triangle) and 16 (shaded square) equivalents of uranyl ions; and in the presence of 16 equivalents of calcium ions (asterisks). CD values are molar ellipticity.

Figure 4.

As a representation, titration of 10^{-4} M solution of uranyl nitrate in 10^{-3} M phosphate buffer, pH = 6.5 with 6merU2 (from top to bottom: 0, 50, 100, 250, 750, 2000 10^{-9} M) is indicated. The intensity concomitantly decreased with addition of the protein indicating the complexation of uranyl ion with the protein. Some spectra have been omitted for clarity.

Figure 5.

Plot of differences in fluorescence intensity versus the concentration of added recombinant proteins. (A) 6merU1, (B) 6merU2, (C) 6mer and their corresponding binding isotherms. Chi-squared tests were performed to evaluate fit.

Figure 6. Stern-Volmer plot of 6merU1 (shaded triangle), 6merU2 (shaded square) and control 6mer (shaded circle) titrated with uranyl ions to determine the mechanism of fluorescence quenching.

Table 1

Estimated secondary structure of recombinant spider silk chimeras by circular dichroism after deconvolution by K2D2 software.

Structure motif	Conditions	Content of structural motif [%]		
		6mer	6merU1	6merU2
α -helix	(-) salt	7.9	4.7	2.6
	(+) 8 equiv. UO^{2+}_2	4.7	7.1	10.0
	(+) 16 equiv. UO^{2+}_2	4.7	8.2	10.6
	(+) 16 equiv. Ca^{2+}_2	4.7	6.2	7.6
b-turn	(-) salt	29.1	29.6	40.5
	(+) 8 equiv. UO^{2+}_2	29.6	30.0	29.7
	(+) 16 equiv. UO^{2+}_2	29.6	28.7	42.1
	(+) 16 equiv. Ca^{2+}_2	29.6	28.7	29.3