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# Evidence of the use of silk by bronze age civilization for sacrificial purposes in the Yangtze River basin of China

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**Silk was the driving force behind the opening of the Silk Road, positioning this material as a global commodity that greatly influenced the progress of human civilization. Due to the natural protein properties of silk, the internal structure and external characteristics of silk cultural relics are irreversibly destroyed during the process of burial or when passed down through the generations until the production information and material components completely disappear, making it difficult to obtain direct archaeological evidence for pinpointing the origin of silk. The sacrificial pits at the Sanxingdui archaeologcal site, located in Guanghan, Sichuan, China, have been found with layers of ash above the layers of artifacts and some bronzes with fabric traces. Among the artifacts, one grid-like ware artifact first appeared in the Bronze Age in China. The two sides of the grid-like ware were grid-like ovals made of bronze material, and inside, there was an oval-shaped complete piece of jade with a bent back. Fabric traces were found on both the jade and bronze surfaces. In order to determine the specific function of fabric at this site, the developed silk fibroin immunoaffinity column (IAC) enrichment technique combined with enzyme-linked immunosorbent assay (ELISA), morphology observation and proteomics were used to identify mineralized fabric material and fabric residues in the ash layer. Silk residues were successfully detected, which confirmed the early use of silk as a material carrier to communicate between Heaven and Earth and provided archaeological evidence for the cultural origins of silk.**

Silk is an organic polymer material consisting of two silk fibroins wrapped in sericin[1]. As an object of archaeological interest, silk is not well preserved during long-term burial, and there are a large number of possible silk remnants, such as mineralized and charred forms, that cannot be identified even by basic morphology. In the early days of silk production, silk was not easy to obtain, and the silk that was produced was not considered an ordinary fabric, but rather, one whose important purposes included use in ritual sacrifice[2]. In the "Yue Ling" component of the *Li Ji*, the following is recorded: "When the work of sericulture was over, the cocoons were distributed to the women for their silk reeling, and then the weight of each was weighed to examine the achievements of each person, and the silk was used to make sacrificial clothes to worship Heaven and ancestors," which shows that the silk obtained from mulberry silkworm is mainly used for sacrificial temple clothing. Similarly, silk was also used as a sacrificial object, such as in the form of silk books or paintings on silk, with the silk serving as a carrier to convey the content of the calligraphy and painting upon it to Heaven. Silk manuscripts were also used to record the alliance between the two countries. There is a Chinese idiom "turning war into jade and silk," where the jade and silk are the materials for writing the nation's letter, either buried in the ground or burned with fire, which means that oaths could be made to Heaven[3]. However, due to the organic properties of silk and the ways in which it was used in ancient times, very few silk objects have been preserved, and most that were preserved are those that were attached to bronze and iron objects. This is because copper and iron ions are constantly released after burial, which can create a bacteriostatic microenvironment in the surrounding soil that is conducive to the preservation of the cultural relics such as silk that are made of organic materials that gradually become mineralized marks on the surfaces of bronze and iron ware[4–6].

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The Sanxingdui ruins in Southwest China's Sichuan Basin were the center of the Sanxingdui culture (1600–1100 BC). In 1986, two "sacrificial pits" ( No. 1 and No. 2) containing ivory, jade, bronze, gold and other materials were found in the soil collected by a local brick factory. From 2018 to 2019, six more similar "sacrificial pits" were discovered between the first two pits[15,13]. C tests confirmed that these pits all belonged to the same period, which was the late Shang Dynasty, 3148–2966 years ago[16]. A large number of bronze, jade, ivory and other inorganic cultural relics were unearthed in the pit. These relics were originally used for worship in temples and ancestral temples but were buried underground for unknown reasons[17]. Most of the artifacts showed signs of burning, and layers of ash were found above layers of artifacts in several pits[18]. After artifacts are burned, the probability of organic relics being preserved decreases. To study the protein in the discovered textile residue in the ash layer and the mechanism by which it was produced, an ash layer with a thickness of approximately 20 cm was extracted from sacrificial pit No. 4 (Fig. 1b), which is located in the northeast of the sacrificial pit group. When the No. 7 sacrificial pit was unearthed, it revealed the first discovery of Chinese Bronze Age grid-like ware (SI Appendix, Section S1). The outside of this artifact is an oval-shaped bronze grid-like object divided into upper and lower layers, with a complete oval-shaped jade object inside the grid-like ware. Fortuitously, mineralized fabric traces were found on both the jade and bronze grid surfaces when the soil was cleaned from them. The mineralized fabric sample was taken from the fragments shed during the cleaning of the grid-like ware (Fig. 1a). To study the relationships among the fabric, jade and grid-like ware, most of the fabric remnants were retained in their original positions during cleaning. The interpretation from the partially visible fabric residue traces of the cultural relics was that the jade had first been wrapped with fabric and placed in the grid-like ware, and then the grid-like ware was wrapped with another piece of fabric and placed in the pit (Fig. 2). Through three-dimensional video microscopy, it was found that both fabrics were plain weave fabric (SI Appendix, Section S2) but had different warp and weft densities and were produced with different weaving styles (Fig. 3). Moreover, the remnant fabric on the grid-like component was stained. The ash layers and mineralized fabric traces found in the Sanxingdui excavation were expected to provide archaeological evidence of the early use of silk. Therefore, researchers have been eager to find a reliable and accurate method to detect fabric residues in ash layers and mineralized fabric materials.

To extract enough silk residue for analysis, a large amount of sample from the ash layer was collected, and a large volume of extraction solution was required for pretreatment. However, the concentration of silk fibroin in the solution was too low, and many other organic degradation products were mixed in the solution. The existing chemical methods of purification and enrichment could not meet the requirements of this kind of sample pretreatment[6,19,20]. The immunoaffinity column (IAC) enrichment technique is a separation method

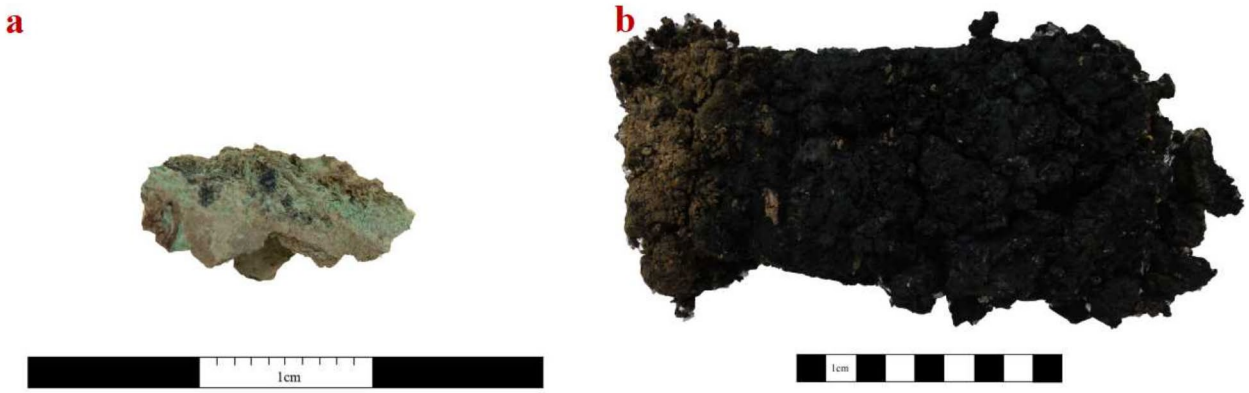


**Fig. 1**. Mineralized fabric sample (**a**) and ash layer sample from No. 4 sacrificial pit (**b**) unearthed at Sanxingdui Site.

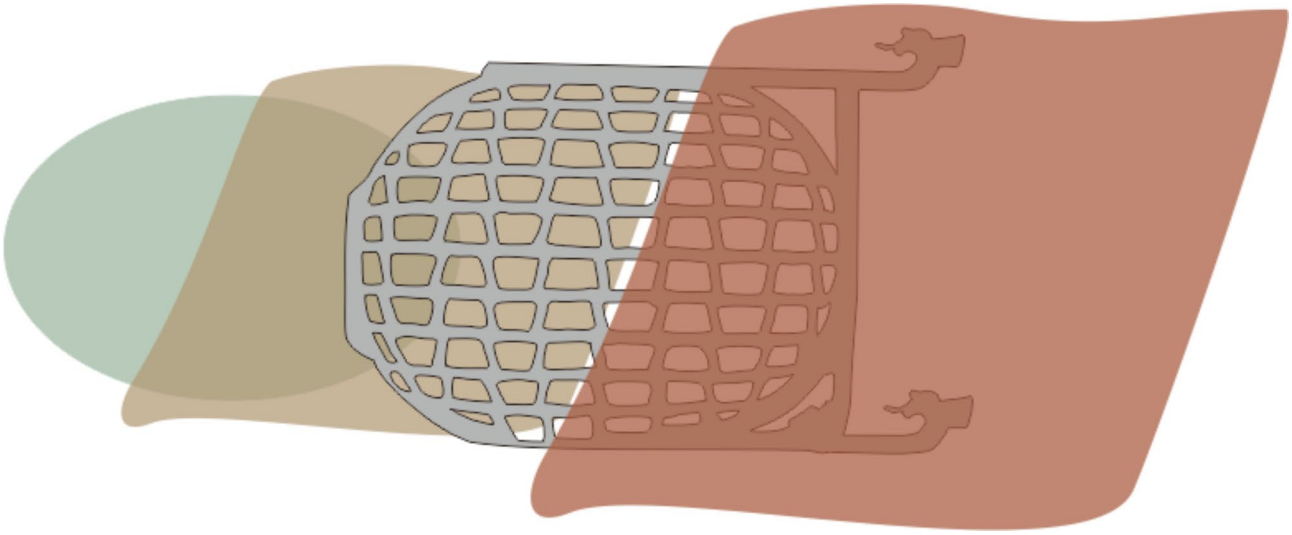

**Fig. 2**. Schematic diagram of fabric wrapped grid shape ware. From left to right are jade, silk fabric (multilayer), grid shape ware and silk fabric (multilayer).

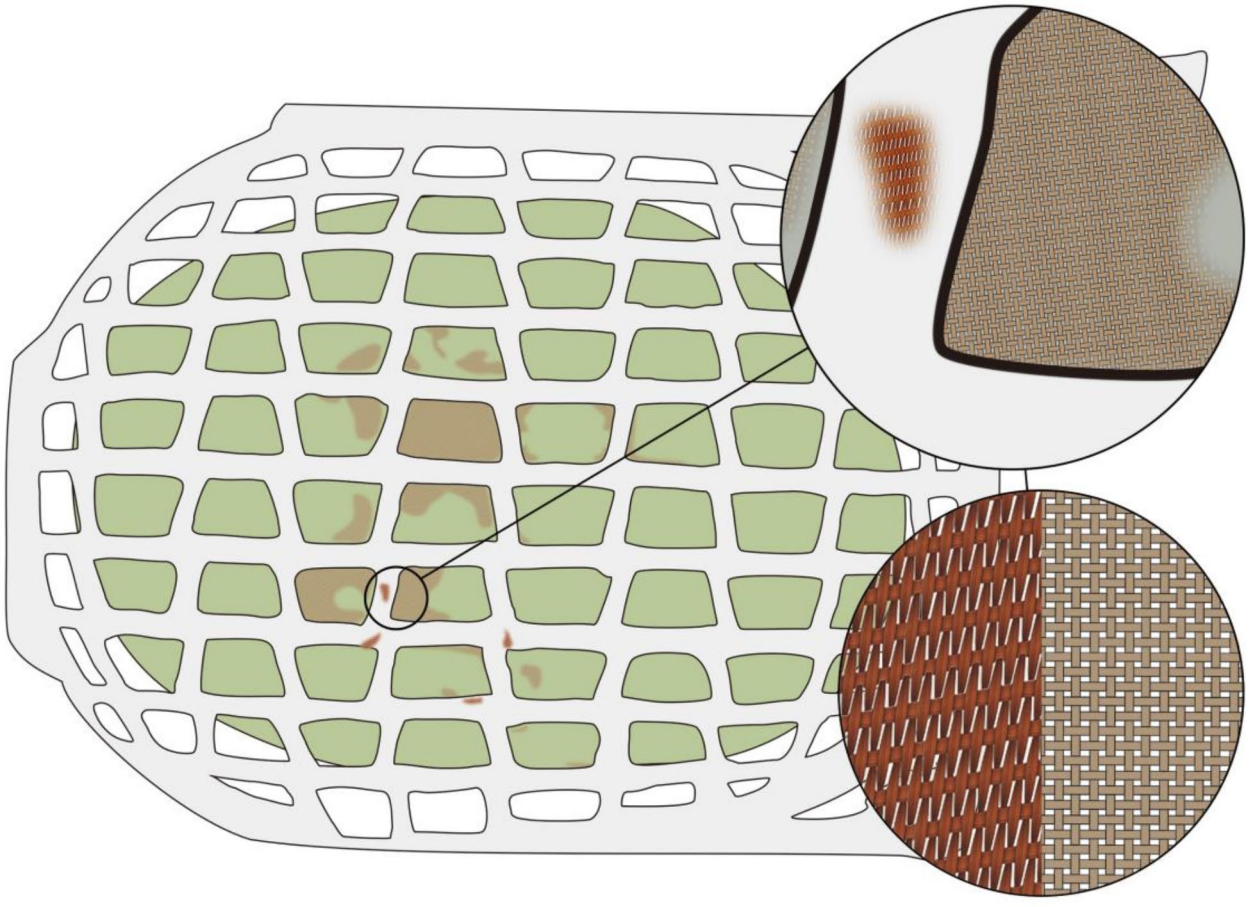

**Fig. 3**. Schematic diagram of fabric residue and weave structure.

that takes advantage of the specificity and reversibility of the reaction between antibodies and antigens. The antibodies are fixed on a chromatographic column, and the target components are obtained and enriched through sampling, washing and elution procedures[21–23]. This method can augment the available sample according to the requirements of different column capacities to achieve the necessary number of samples in one-time processing.

IAC has been used to extract and concentrate agricultural residues[24,25], mycotoxins[26], biomolecular residues[27,28], and other trace substances[29,30]. After enrichment and elution, the target substance can be directly detected by ELISA and high-performance liquid chromatography–mass spectrometry (HPLC-MS).

In this study, the physical and chemical properties of mineralized fabrics at the Sanxingdui site were analyzed by scanning electron microscopy (SEM), indirect ELISA (iELISA), X-ray 3D microscopy analysis and isotope technology. At the same time, a silk fibroin IAC was developed by coupling silk fibroin antibody and agarose microspheres, enabling high-efficiency enrichment and purification of silk fibroin in a large-volume extraction solution to be achieved. After enrichment, the trace fibroin in the ash layer was quickly and accurately identified via proteomics. This study provides archaeological evidence for the use of silk cultural relics as a material carriers of communication between Heaven and Earth, and also provides a new technology for expoloring the origin of silk.

## Results

### SEM and X-ray 3D microscopy analysis

As seen from the morphology of the remnant mineralized fabric (Fig. 4), the longitudinal fiber morphology of the fabric was smooth and had an approximately triangular cross section, similar to silk[6,31]. Therefore, through SEM observation, the mineralized fabric was preliminarily identified as silk. The fiber impression was surrounded by minerals, and these minerals served as a carrier for the fabric. During the mineralization process, the organic components in the fibers were gradually lost[32], and a cavity was finally formed (Fig. 4a and b). An obvious color intensity distribution can be observed from two-dimensional X-ray three-dimensional microscope images, where the strength of the color is proportional to the density of the compound, that is, the brighter the color, the higher the substance density[33,34]. In general, the density of inorganic minerals is higher than that of natural fibers[35]. It can be seen from Fig. 4c that the brightness of different fibers presents an uneven distribution. Based on this, it is inferred that the density of the sample changes in space. The uneven density means that the mineralization rate of fiber mineralization in different regions is not exactly the same. In addition, the longitudinal morphology of the fiber bundle can be clearly observed in Fig. 4c, and the inner diameter of the mineralized fibers is close to the diameter of the silk fibers[1,36], which is about 15 ~ 20 μm. The difference of inner diameter between the fibers after mineralization is mainly due to the formation of mineralized layers with different thicknesses. The mineralized layer has a certain protective effect on the fiber, which can preserve part of the mineralized silk residue.

### iELISA analysis results

The positive result of the iELISA test was determined on the basis of the absorbance (OD) at 450 nm of the test sample being greater than that of the negative control plus 3 times the standard deviation[37]. The dashed line in Fig. 5 represents the critical value of this test. As shown in the figure, the test results for the mineralized samples and ash layer samples were both higher than the critical value, indicating positive results, i.e., that there were silk fibroin residues in both samples. These findings, together with the observed morphology of mineralized samples, indicate that the mineralized fiber can be defined as silk. In addition, the $OD_{450nm}$ value of the mineralized sample was significantly greater than that of the ash layer sample, and the OD value was positively correlated with the concentration of the sample, indicating that the silk residue content in the mineralized sample was greater than that in the ash layer, which suggests that mineralization effectively slowed the loss of the organic relic material. The silk residue was still detected in the ash layer, indicating that not all of the silk fabric was incinerated. During incineration, silk fibroin protein molecules rapidly remove alkyl, heteroatoms and hydrogen atoms; moreover, the molecular weight of silk decreases continuously, the carbon content increases continuously, and carbon is generated[38]. There were many interference effects in the ash layer samples, and the detected silk concentration was close to the critical value. The silk fibroin IAC was developed to enrich and purify the ash layer extract, and the ash layer sample was further analyzed by proteomics.

### Application of silk fibroin IAC

The silk fibroin monoclonal IAC was prepared (SI Appendix, Section S3) and applied to archaeological simulated soil samples. Proteomics was used to analyze the extraction solution of the archaeological simulated soil samples before and after enrichment, and the analysis results are shown in Table 1. A total of 437 proteins were detected in the unenriched sample extract solution, while only 13 proteins were detected in the enriched extract solution. Moreover, the heavy chain mulberry silk P05790 protein, the signature protein of mulberry silk, was detected in two samples[39,40]. A comparison of the proteomic analysis results before and after enrichment revealed that the protein types in the extracted solution after enrichment were significantly lower than those in the samples without enrichment, and the score for the P05790 protein was greater than that before enrichment (Table 2). The increase in score indicates that the reliability of the results increased[41–43]. These results show that the silk fibroin monoclonal IAC can also play a role in the enrichment of fibroin in complex media.

The Sanxingdui ash sample extraction solution before and after the silk fibroin monoclonal IAC enrichment was analyzed via proteomics. The results of the analysis with MaxQuant 1.6.1.0 software are shown in Fig. S4 and Fig. S5. Forty-four kinds of proteins were detected in the extraction solution before enrichment, but none of the proteins was silk fibroin. Although only 4 kinds of proteins were detected in the enriched extraction solution, one of the signature proteins of mulberry silk, P05790, was detected (Table 3)[39,40]. Comparing the results before and after enrichment reveals that the silk fibroin monoclonal IAC is effective for enrichment and purification of silk fibroin in archaeological soil samples. After enrichment, the types of identified proteins decreased significantly, but the silk fibroin content increased, fulfilling the needs of subsequent proteomic analysis.

The score for P05790 was low. To verify the results, secondary mass spectrometry was performed on the two specific peptides, whose sequences were NCGIPR and NCGIPRR, that were detected in the P05790 protein. The secondary mass spectrometry fragment ion peaks of these two peptides, from which the amino acid break sites

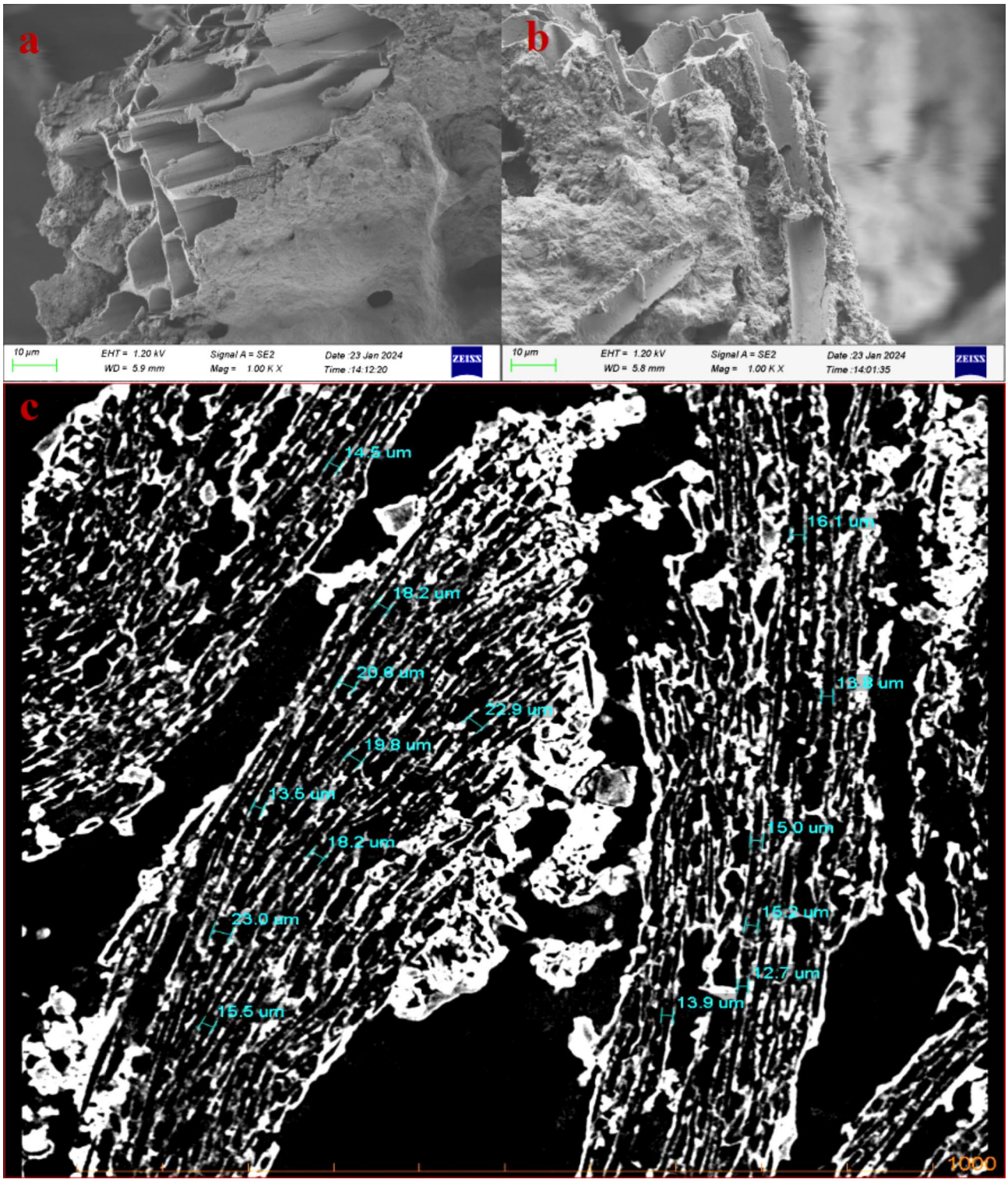


**Fig. 4**. The cross-sectional morphology (**a,b**) and two-dimensional longitudinal cross-section (**c**) of the mineralized sample.

can be seen, are shown in Figs. 6 and 7. The greater the amount of b and y ion information in the figure, the greater the number of peptide fragments obtained, and the greater the reliability of the matched proteins[44,45]. The b and y ions in the figure below matched this peptide regardless of whether they started at different fracture sites. This indicates that the peptide segment information was reliable; therefore, the silk fibroin protein identified by the peptide segment was also reliable, clearly indicating that there was silk fibroin in the ash layer. The presence of silk residues detected in the Sanxingdui sacrificial pits not only reflects that silk was used as a material carrier

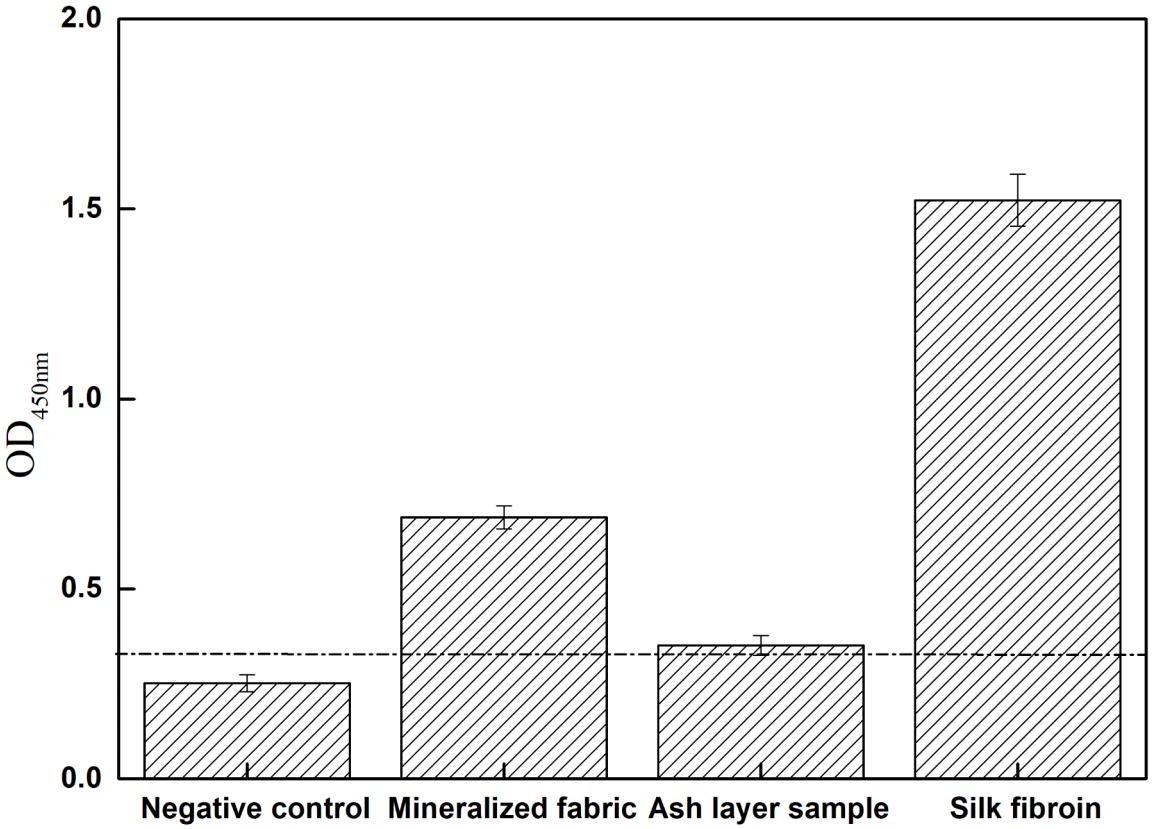


**Fig. 5**. ELISA results of mineralized sample and ash layer sample.

| Sample | Number of identified proteins | Number of identified peptides | Number of identified PSM |
|---|---|---|---|
| Pre-enrichment | 437 | 440 | 489 |
| Post-enrichment | 13 | 21 | 23 |

**Table 1**. Proteomic analysis results of the extraction solution from archaeological simulated soil sample.

| Sample | Accession number | Description | Peptides | Unique peptides | Score | Species |
|---|---|---|---|---|---|---|
| Pre-enrichment | P05790 | Fibroin heavy chain | 1 | 1 | 199.8 | BOMMO |
| Post-enrichment | P05790 | Fibroin heavy chain | 7 | 7 | 323.31 | BOMMO |

**Table 2**. Results of mulberry silk signature protein in archaeological simulated soil sample.

| Accession number | Description | Peptides | Unique peptides | Score | Species |
|---|---|---|---|---|---|
| P05790 | Fibroin heavy chain | 2 | 2 | 36.600 | BOMMO |
| A0A8R2MAX3 | Uncharacterized protein | 1 | 1 | 37.308 | \ |
| A0A8R2R2R6;A0A8R2CA75 | Uncharacterized protein | 1 | 1 | 9.4103 | \ |
| A0A8R2DL44 | Uncharacterized protein | 1 | 1 | 8.7166 | \ |

**Table 3**. Proteomic analysis results of Sanxingdui ash layer sample extraction solution enriched by IAC.

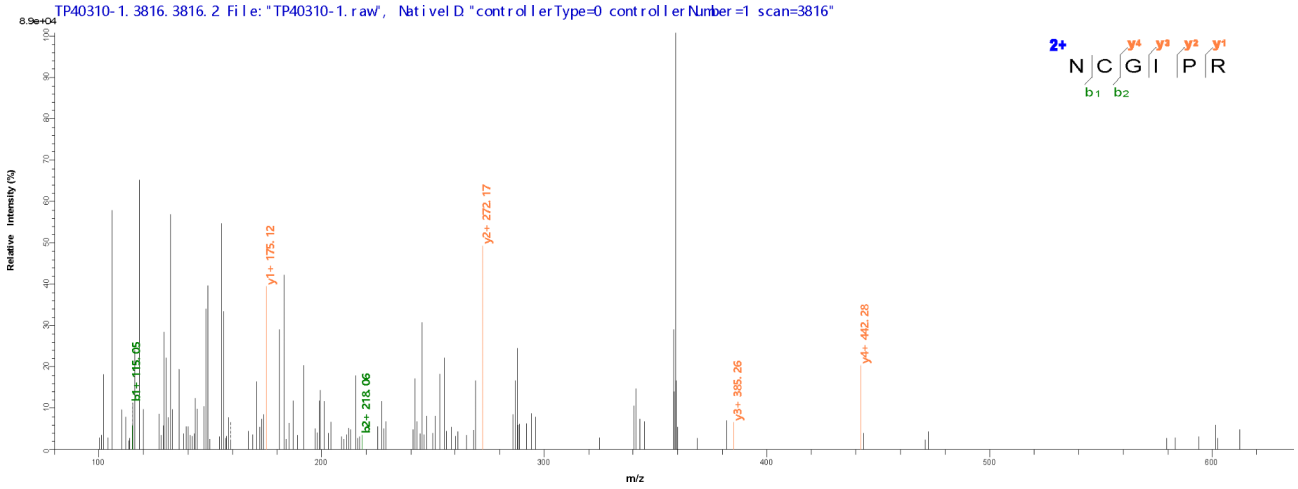


**Fig. 6**. Secondary fragment ion peak of NCGIPR polypeptide.

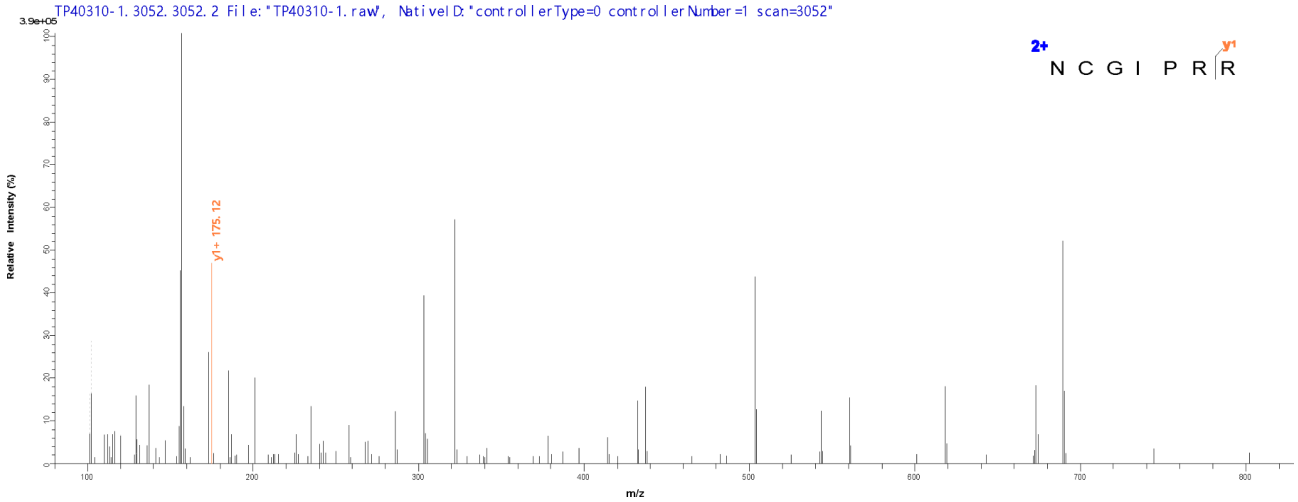


**Fig. 7**. Secondary fragment ion peak of NCGIPRR polypeptide.

for communication between Heaven and Earth but also provides archaeological evidence of the Southwest Silk Road, confirming that silk was already used in the ancient Shu State more than 3,000 years ago.

## Discussion

Silk is a protein that is easily degraded when buried. Moreover, burning and high temperatures undermine the preservation of silk, and most of the cultural relics unearthed in Sanxingdui have traces of burning. Moreover, silk residue is mixed with ash, soil and various artifacts in the pit, which presents a great challenge for identifying silk in the pit. Thus, it is not difficult to imagine how over the long course of history, the silk buried in the pit has lost its original appearance and now exists as a residue or trace that is difficult to identify with the naked eye. The silks from the Sanxingdui site show these extreme states of mineralization and conversion to ash and no longer belong to the category of textiles in the traditional sense. The developed silk fibroin IAC can enrich and extract silk residues from archaeological collections and effectively improve proteomics detection of silk proteins. The discovery of silk and its residues at the Sanxingdui sacrificial pits is reliant on the use of new technologies that greatly improve the extraction level of organic residues.

The essence of textile mineralization is that the organic components in the fabric are replaced by inorganic substances. From the existing studies on fiber mineralization, it can be seen that the essence of mineralization is the migration, enrichment and exchange of different ions in a certain liquid and substance, and finally the process of precipitation and mineralization due to changes in condition[46]. From the morphology of Sanxingdui mineralized silk the preservation state of fibers can be seen. The fibers were hollow, which indicated a high degree of mineralization, but there were differences in the degree of mineralization of silks in different regions. Mineralization involves an ion exchange process, so the area near the copper will show a higher degree of mineralization. In addition, copper ions penetrate the interior of the fiber as water molecules enter and

accumulate in specific areas of the fiber under the action of gravity. These areas are more prone to mineralization due to longer exposure to copper ions. The morphology of fiber is preserved during mineralization, which provided the morphological information for residue identification.

Sanxingdui is the capital city of the ancient Shu State and was founded by the ancient Shu ancestors. The sericulture and silk weaving industry in Sanxingdui has a long history, and the historical accounts about sericulture in ancient China are mostly related to the land of the Shu State[47]. Sima Qian wrote in the "Annals of the Five Emperors" in the *Records of the Grand Historian* that "the Yellow Emperor lived on the hill of Xuanyuan and married the daughter of Xiling family." Huang Di Yuan Concubine Leizu Xiling taught others to raise silkworms, and the legend Leizu is from the people of Chengdu in Sichuan. "Shiwen" says that "Shu" refers to "mulberry worms," that is, "silkworm larvae." The character "Shu" is related to the initial sericulture," referring to the beginning of the Shu State and the people practicing sericulture, for whom sericulture was central to their economic activities[48]. However, no silk or its residues have been found in Sanxingdui's previous archaeology. Mineralized fabrics and silk residues are of great significance to the study of ancient silk production technology, economy and trade, cultural exchanges and the origin of silk[13,49]. At the Sanxingdui sacrificial pits, post-incineration silk residues and silk traces attached to the surfaces of bronzes and jades were found, providing archaeological evidence for exploring the uses of silk, especially the silk traces on the surfaces of the grid-like ware and jade. These discoveries suffice to verify that silk was used as a material carrier for communication between Heaven and Earth at that time. There is a record called "Jiatu Zhijia" about emperor Yao passing the throne to emperor Shun that states emperor Yao was ordered by Heaven to pass the throne to Yu Shun, and the divine turtle conveyed the order of emperor Yao passing the throne to Yu Shun on behalf of the emperor of Heaven. The shape of the "Jiatu" described in this text is very similar to that of the grid-like ware unearthed at the Sanxingdui site, so the silk remnants on the surface of the jade and grid-like ware can be surmised to have been the material carriers of communication between Heaven and Earth. Silk was also used in burial clothing, as wearing silk after death would help people communicate with Heaven. Evidence for this can be found in the literature. In the *Book of Rites*, it is stated that "linen is used for clothing of the living, and silk is mainly used for clothing of corpses."

In summary, we have developed a silk fibroin IAC that can effectively enrich the residues of silk in archaeological samples. Combining it with ELISA and proteomics techniques can significantly improve the detection level of silk residues, thereby preventing the loss of valuable information from a large number of precious silk cultural relics. The first discovery of silk traces and residues at the Sanxingdui archaeological site, providing archaeological evidence for the use of silk in ritual contexts and providing more information on how the ancient Shu people of 3,000 years ago expressed their religion. Given that the target detection substance has a protein property, this technology is expected to be applied to the trace exploration of early human covert materials such as fur, which have been used in history but have now become invisible archaeological remains, and further improve the detection level of organic textile materials.

## Methods

### Extraction of silk fibroin from mineralized fabric or cultural relics samples

Three micrograms of mineralized sample and 5 g of ash layer sample were placed in silk protein extraction solution and dissolved in a water bath at $95 \pm 2$ ℃ for 45 min. After cooling, the supernatant was centrifuged, and the supernatant was extracted to conduct the iELISA.

### iELISA analysis

First, 100 μL/well of the test liquid, a blank control and a negative control were added to two 96-well iELISA plates, and the plates were incubated overnight at 4 °C. The negative control was that PBS was added to the test liquid instead of silk fibroin antibody during the experiment. and silk fibroin solution was used as a positive control. Then, the liquid was removed, and 200 μL of washing buffer was added to each well to wash the unbound proteins 3 times for 2 min each. Two hundred microliters of blocking solution was then added to the wells and incubated for 2 h at 37 °C, after which 200 μL of washing buffer was added to wash the plate 3 times for 2 min each. The silk fibroin polyclonal antibody was diluted 2000 times with blocking solution, 100 μL/well of diluted silk fibroin antibody was added to test liquid and positive control wells, and the plates were incubated at 37 °C for 1 h. Then, 100 μL of washing buffer was added to wash the plate 3 times for 2 min each. The goat anti-rabbit IgG-HRP antibody was diluted 5000 times with blocking solution, 100 μL/well of goat anti-rabbit IgG-HRP antibody was then added, and the plate was incubated for 1 h at 37 ℃. Then, 200 μL of washing buffer was added to wash the plate 3 times. Finally, 100 μL/well of TMB solution was added, and the plate was incubated in the dark for 10 min. Then, 50 μL/well of 2 mol/L $H_2SO_4$ solution was added to terminate the reaction, and the absorbance of the sample was measured by a microplate reader at 450 nm.

### Preparation and enrichment of simulated archeological soil sample

First, 5 mg of silk fibroin powder was weighed, dissolved in deionized water, mixed with 30 g of soil sample and placed in an oven at 100 ℃ until there was no moisture. The dried sample was removed, cooled to room temperature, and ground to ensure that the silk fibroin was evenly mixed with the soil sample. Twenty milliliters of silk fibroin extraction solution was mixed with the soil sample, and the silk fibroin was extracted at 60 °C. After 60 min, the supernatant was collected by centrifugation. To fully extract silk fibroin from the sample, the extracted soil sample was washed, filtered and centrifuged with 10 mL of silk fibroin extraction solution, and the supernatant was collected. Finally, the collected supernatant was divided into two parts: one part was selected for direct proteomic analysis, and the other part was enriched with an immunoaffinity column before proteomic analysis.

### Proteomic analysis of the archaeological soil sample simulation sample

The same volume of simulated sample extraction solution I without enrichment of the immunoaffinity column and simulated sample extraction solution II enriched by the immunoaffinity column were individually added to the centrifuge tube, and an appropriate amount of 6 M guanidine hydrochloride was placed in the centrifuge tube with extraction solution, boiled in boiling water for 5 min, and centrifuged after cooling, after which the supernatant was extracted. Then, 200 μL of 8 M urea (pH 8.0) and 150 mM Tris-HCl mixture were added, the mixture was centrifuged at 12,000 × g for 15 min and then filtered, and an appropriate amount of 50 mM IAA was added. The mixture was allowed to react at room temperature for 30 min in the dark and centrifuged at 12,000 × g for 10 min. Then, 100 μL of UA buffer was added, and the mixture was centrifuged at 12,000 × g for 10 min; this process was repeated twice. Then, 100 μL of $NH_4HCO_3$ buffer was added, and the mixture was centrifuged for 10 min; this process was repeated twice. Then, 40 μL and 6 μg of trypsin were added for enzyme digestion, the mixture was shaken at 600 rpm for 1 min, and the mixture was subjected to enzyme digestion at 37 °C for 16–18 h. The liquid was transferred to a new centrifuge tube, the mixture was centrifuged at 12,000 × g for 10 min, and the filtrate was collected. The peptides were desalted using a C18 StageTip and dried under vacuum. After drying, the peptide was redissolved in 0.1% FA for LC–MS analysis.

The peptides were separated via Thermo Scientific Easy nLC 1200 chromatography. Appropriate polypeptides were isolated from each sample by gradient elution at a controlled flow rate of 300 nL/min. The peptide was isolated and analyzed by DDA mass spectrometry with a Q Exactive HF-X mass spectrometer. The primary mass spectrometry resolution was 60,000, the parent ion scanning range was 300–1800 m/z, and the analysis time was 60 min. The secondary mass spectrum resolution was 15,000, the secondary mass spectrum of the 20 highest-intensity parent ions was triggered after each full scan, and the analysis time was 25 min.

MaxQuant 1.6.1.0 software was used to compare the raw data with 18,488 protein sequences downloaded from the UniProt protein daTablease.

### SEM and X-ray 3D microscopy analysis

The longitudinal and cross-sectional morphologies of the mineralized fibers were observed by scanning electron microscopy (SEM, Sigma 300). The samples were sputtered and gilded for 60 s at an accelerating voltage of 15 kV. To assess the internal structure of the mineralized fibers, a Zeiss Xradia610Versa X-ray 3D microscope was used. A sample of the appropriate size was collected with a centrifugal tube and secured to a stand by a homemade device to ensure that it did not move when the stage is rotated. The resolution was 1.16 μm, the voltage was 80 kV, and 3DViewr software was used to extract two-dimensional microscopic high-definition images of longitudinal sections of fiber bundles.

The full experimental details and characterization of the compounds can be found in the Supplementary Information.

### Materials

The silk fibroin polyclonal antibody and monoclonal antibody were prepared by our laboratory, and other reagents were purchased from commercial suppliers (Hangzhou Hua'an Biotechnology Co., Ltd., McLean Biochemical Technology Co., Ltd., Aladdin Chemical Co., Ltd., Promega Biotech Co., Ltd., Merck Chemical (Shanghai) Co., Ltd., Sigma Aldrich (Shanghai) Trading Co., Ltd., Hangzhou Fengyu Biotechnology Co., Ltd.). The mulberry silk fibroin extraction solution was a $CaCl_2$:water: ethanol solution with a molar ratio of 1:8:2. Washing buffer (7.4 g of PBS) was prepared with 0.27 g of $KH_2PO_4$, 0.2 g of KCl, 1.42 g of $Na_2HPO_4$ and 8 g of NaCl. The antibodies and blocking solution were prepared by dissolving 2.5 g of BSA in 250 mL of 7.4 mL of PBS. The stopping solution was concentrated $H_2SO4$ diluted to a 2 M solution. Coupled buffer (pH 8.3) was prepared with $NaHCO_3$ and NaCl. The acid lotion (pH 4.0) was composed of 0.1 M NaAc and 0.15 M NaCl, and the alkali lotion (pH 8.0) was composed of 0.1 M Tris-HCl and 0.15 M NaCl.

## Data availability

All processed experimental data that support the findings of this study are available within the main text and its Supplementary Information, including the grid-like ware, experiments with mineralized fabrics, preparation and performance evaluation of immunoaffinity columns and mass spectrum. Proteomic analysis data for archaeological simulated soil samples and Sanxingdui ash layer sample has been deposited at theUniProt, the accession number SPIN200031796. Additional raw data of this study are available from the corresponding author upon request.

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## Competing interests
The authors declare no competing interests.

## Author contributions
H.Z. performed silk fibroin IAC methodology development, synthetic application and writing - original draft; R.Y., J.G., J.L., H.Y and L.J. performed the experimental work; F.T. and Y.Z. conceived the project; Z.X., J.Y. and Q.C. selected the samples, with contributions from all authors. The authors declare no competing interests.

## Declarations

## Competing interests
The authors declare no competing interests.

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