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Materials and Methods

Table S1

References and Notes

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Intensive Pre-Incan Metallurgy Recorded by Lake Sediments from the Bolivian Andes

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The history of pre-Columbian metallurgy in South America is incomplete because looting of metal artifacts has been pervasive. Here, we reconstruct a millennium of metallurgical activity in southern Bolivia using the stratigraphy of metals associated with smelting (Pb, Sb, Bi, Ag, Sn) from lake sediments deposited near the major silver deposit of Cerro Rico de Potosí. Pronounced metal enrichment events coincide with the terminal stages of Tiwanaku culture (1000 to 1200 A.D.) and Inca through early Colonial times (1400 to 1650 A.D.). The earliest of these events suggests that Cerro Rico ores were actively smelted at a large scale in the Late Intermediate Period, providing evidence for a major pre-Incan silver industry.

New World metallurgy emerged in the Andean region of South America between the Initial Period (1800 to 900 B.C.) and the Early Horizon (900 to 200 B.C.) (1). The oldest well-dated archaeological site containing metal artifacts is Mina Perdida (Lurín Valley) in coastal Peru, where hammered foils and gilded copper are preserved in contexts dating to 1400 to 1100 B.C. (2). The tradition of sheet-metal working (hammering, gilding, annealing, and *repoussé*) remained pervasive in the Andes throughout the Early Intermediate Period (200 B.C. to 600 A.D.) and the Middle Horizon (600 to 1100 A.D.) (3). By 1000 A.D., large-scale copper smelting and bronze production is evident at sites such as Batán Grande on the northern Peruvian coast (4). Beginning in the Late Intermediate Period (1100 to 1450 A.D.), intensive copper working became widespread on the Bolivian *altiplano*, with the production of materials of copper-tin alloy (i.e., bronze), in contrast to the copper-arsenic artifacts found in Peru (5). By this time, silver and gold were well-established as precious metals among Andean cultures. Although silver was

highly sought by royalty for symbolic and ritual purposes (6), the geographic distribution, intensity, and timing of Late Intermediate Period silver mining in the Andes remains unclear. Here, we infer a regional history of metallurgy from lake sediments retrieved adjacent to the largest silver deposit of the Bolivian tin belt.

Laguna Lobato (hereafter LL) (7) is small (0.2 km²), relatively deep (11 m), and occupies a nonglacial catchment of 3.9 km² (Fig. 1). The lake overflows only during the wet season (December to March), and it has no hydrological connection with surface waters draining Cerro Rico, 6 km west of the lake. Because westerly winds prevail for 8 months of the year (April to November) (8), LL is strategically located to record atmospheric deposition of metals volatilized during smelting or transported as fine-grain particulates. This study is based on a 74.5-cm core recovered from the deepest portion of the lake and dated by ²¹⁰Pb, ¹³⁷Cs, (table S1), and ¹⁴C analyses (9) (table S2).

Cerro Rico lies within a zone of xenothermal mineralization related to Middle Tertiary intrusions (10). In addition to native silver, the richest ores contain combinations of acanthite (Ag₂S), andorite (PbAgSb₃S₆), chlorargyrite (AgCl), matildite (AgBiS₂), miargyrite (AgSbS₂), pyrargyrite (Ag₃SbS₃), and tetrahedrite [(Ag, Cu, Fe, Zn)₁₂Sb₄S₁₃] (11). Tin is associated primarily with cassiterite

(SnO₂). To assess the history of smelting, we measured the concentrations in lake sediments of five metals (Ag, Bi, Pb, Sb, and Sn) associated with ore composition (9). Of these metals, Pb serves as the cornerstone of our interpretations for two reasons. First, the Incan smelting technology relied on argentiferous galena [*soroche* (Pb, Ag)S] as a flux during smelting, which was conducted in charcoal-fired, wind-drafted furnaces lined with clay (*huayras*) (12, 13). The use of *soroche* led to excessive Pb volatilization, resulting in lake sediment concentrations that are orders of magnitude higher than those of the other analyzed metals. Second, Pb is largely immobile once deposited in lake sediments (14, 15). Although molecular diffusion rates for Ag are higher than those for Pb (16, 17), in both cases they are insignificant in comparison to average sediment accumulation rates in LL (~1 mm year⁻¹). Thus, postdepositional mobility is not a confounding factor in the interpretation of the record, a conclusion supported by the largely parallel trends observed for each of the metals.

Before 1000 A.D., concentrations of all five metals in the sediments of LL were low and stable, representing natural background levels of metal accumulation (Fig. 2). An additional 15 samples spanning the earlier period from 2000 B.C. to 600 A.D. have similarly low Pb

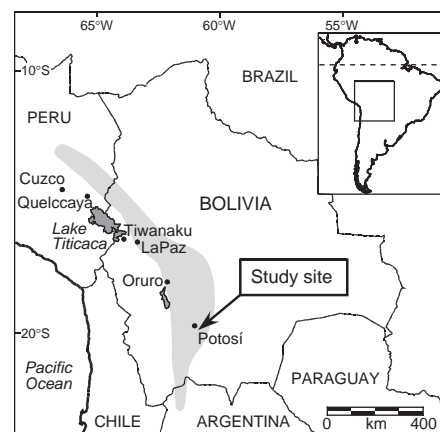


Fig. 1. Location map of the study site in relation to the Tiwanaku capital, Lake Titicaca, Potosí, and the Quelccaya ice core. The shaded area indicates the central Bolivian tin belt (27), which broadly corresponds to the crest of the Andean cordillera.

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concentrations ($\sim 20 \mu\text{g g}^{-1}$), despite stable isotopic evidence for pronounced hydrological changes in this time interval (18) (fig. S1).

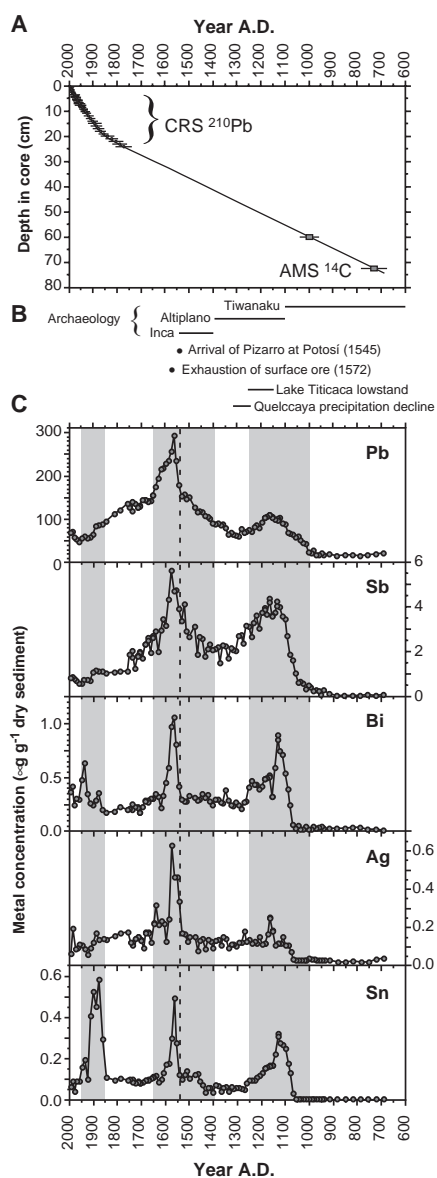


Fig. 2. Chronology of the core from LL, Andean archaeology and paleoclimate, and sediment metal concentrations. (A) Constant Rate of Supply (CRS) age model based on ^{210}Pb analyses of the upper core section (39 measurements) and macrofossil Accelerator Mass Spectrometer (AMS) ^{14}C dates (9). (B) Generalized archaeology [Peruvian chronology from (7) and (24), historical events from (12)], and regional paleoclimate from (23, 25)]. (C) Concentrations of Pb, Sb, Bi, Ag, and Sn from sediments of LL over the past 1300 years. Shaded zones identify three distinct metallurgical zones: Tiwanaku (1000 to 1250 A.D.), Inca-early Colonial (1400 to 1650 A.D.), and the rise and crash of tin mining (1850 to 1950 A.D.). The vertical dashed line (1545 A.D.) separates Incan and Colonial periods. Background concentrations (pre-1000 A.D.) are 20.2 ± 2.2 , 0.19 ± 0.15 , 0.02 ± 0.01 , 0.03 ± 0.01 , and $<0.01 \mu\text{g g}^{-1}$ for Pb, Sb, Bi, Ag, and Sn, respectively ($n = 15$).

Thus, Holocene climate variability exerted little influence on nonpollution metal fluxes to the lake's sediments. Metal concentrations initially rose well above background shortly after 1000 A.D., reaching a first peak around 1130 to 1150 A.D. (Fig. 2). Concentrations of Pb exceed $100 \mu\text{g g}^{-1}$ in this interval, approximately one-third of the peak Pb burden reached subsequently in early Colonial times. Such enrichment trends are directly comparable to those reported from lakes proximal to major Medieval mining sites in Europe (19). Because we know of no natural processes capable of inducing this degree of Pb enrichment, and given the proximity of LL to a major source of metal pollution, we associate the magnitude of metal enrichment in the lake's sediments with the intensity of ore smelting.

This initial rise in metal concentration coincides with the late stages of the Tiwanaku Empire that controlled the Lake Titicaca basin and extended beyond Oruro in the southern *altiplano* (Fig. 2). Very few Tiwanaku silver artifacts appear to have survived, except for early discoveries at Chucaripupata, on Lake Titicaca's Island of the Sun (20). Therefore, it is difficult to ascertain which cultural group was smelting Cerro Rico ores before the Tiwanaku collapse, or where artifacts were being fabricated and traded. However, the synchrony between declining LL metal concentrations and the collapse of the Tiwanaku state suggests that metallurgy at Cerro Rico was directly linked to the larger Tiwanaku polity. Although the demise of the Tiwanaku is a subject of debate (21), it has been linked in part to pervasive droughts that forced the abandonment of raised-field agriculture (22). The level of Lake Titicaca dropped by as much as 6 m between 1100 and 1250 A.D. (23). The corresponding reduction of smelting intensity at Cerro Rico suggests some degree of state level administration over the extraction of silver from the mountain.

During the subsequent Altiplano Period (~ 1100 to 1400 A.D.), which separated Tiwanaku and Inca imperial regimes, decentralized polities arose across the Titicaca basin (24). Metal concentrations from LL sediments suggest that smelting at Cerro Rico continued during this interval, although at greatly reduced levels of activity. Decreased social organization after the fall of Tiwanaku likely curtailed the demand for ceremonial metals. Furthermore, regional population may have declined as a consequence of crop failures, given the strong evidence from the Quelccaya ice core for persistent drought in the 1250 to 1310 A.D. interval (25).

By 1400 A.D., a renewed intensification of smelting at Cerro Rico is indicated by increases in the concentrations of Pb and Sb in LL sediments. We attribute this to the rise of Incan metallurgy, which lasted until the Spanish conquest. At the height of this interval, Pb concentrations reached $150 \mu\text{g g}^{-1}$ (Fig. 2). During the Inca period however, Ag, Bi, and Sn con-

centrations did not return to levels recorded during the earlier smelting peak, suggesting technological advances by Incan metallurgists aimed at minimizing volatile losses of silver. This notion is supported by distinctive spikes of these three metals immediately after the Spanish arrival at Potosí (1545 A.D.). Early Colonial smelting used bellowed Castilian stone furnaces that had proven successful elsewhere in the Andes, but repeatedly failed at Cerro Rico (12). These furnaces overheated the ore, thus volatilizing metals, including the silver targeted for extraction. Accordingly, the maximum sediment Ag enrichment of the last millennium occurs in conjunction with colonial experimentation. Due to the failure of Spanish extractive techniques, the indigenous *huayra* technology was retained at Cerro Rico, leaving the smelting process largely in the hands of Incan metallurgists under colonial rule. In the 27 years that followed the Spanish arrival, thousands of active *huayras* adorned the mountain at any given time (11, 12). Their collective atmospheric Pb emission, largely associated with *soroche* use, raised sediment Pb concentrations in LL to nearly $300 \mu\text{g g}^{-1}$ (Fig. 2).

With the depletion of silver-rich surface ores in 1572 A.D., smelting was replaced by mercury amalgamation as the primary extraction process, a technology brought to Bolivia from Mexico (26). Amalgamation facilitated silver extraction from the lower grade ores recovered in subterranean adits. Atmospheric fluxes of metals progressively declined, as registered by their decreasing concentrations in LL sediments (Fig. 2). Silver production from Cerro Rico declined until its eventual abandonment in 1930 A.D. (10, 11). As the silver supply dwindled, large-scale tin production became important at Potosí in the late 19th and early 20th centuries A.D., ultimately fueled by high demands during World War I. Peak sediment Sn concentrations faithfully track both the transition to tin production and the industry's subsequent crash (1950 A.D.).

The results from LL extend the record of precious metal smelting in the southern Bolivian Andes by several centuries. Although legend attributes the discovery of silver at Cerro Rico to the 11th Inca ruler, Huayna Capac, in the mid-15th century A.D. (11), our data suggests that the deposit was known and exploited as a source of silver in the Late Intermediate Period, as early as the 11th century A.D. The scarcity of coeval pre-Incan silver artifacts is, therefore, due neither to the absence of adequate extractive technologies nor to a limited knowledge of argentiferous ores. Rather, we propose that the earliest silver artifacts to originate from Cerro Rico were looted, likely both before and after European contact. Although it remains impossible to determine precisely the amount of pre-Colonial silver that was extracted from Cerro Rico, total production since 1545 A.D. is

estimated between 20,000 and 40,000 metric tons (10, 12). Assuming that pre-Incan technologies operated at efficiencies comparable to *huayras* and recognizing the initial presence of grades as rich as 25% Ag (11), our data imply that several thousand tons of silver were produced in pre-Incan times. Although major new archaeological discoveries in the Andes remain a distinct possibility, the likelihood seems equally probable that most of this silver was recycled and transported elsewhere in the Americas before conquest, or eventually exported overseas by the Spanish.

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Supporting Online Material

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Materials and Methods
Fig. S1
Tables S1 and S2

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Inactivation of TNF Signaling by Rationally Designed Dominant-Negative TNF Variants

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Tumor necrosis factor (TNF) is a key regulator of inflammatory responses and has been implicated in many pathological conditions. We used structure-based design to engineer variant TNF proteins that rapidly form heterotrimers with native TNF to give complexes that neither bind to nor stimulate signaling through TNF receptors. Thus, TNF is inactivated by sequestration. Dominant-negative TNFs represent a possible approach to anti-inflammatory biotherapeutics, and experiments in animal models show that the strategy can attenuate TNF-mediated pathology. Similar rational design could be used to engineer inhibitors of additional TNF superfamily cytokines as well as other multimeric ligands.

TNF is a proinflammatory cytokine that can complex two TNF receptors, TNFR1 (p55) and TNFR2 (p75), to activate signaling cascades controlling apoptosis, inflammation, cell proliferation, and the immune response (1–5). The 26-kD type II transmembrane TNF precursor protein, expressed on many cell types, is proteolytically converted into a soluble 52-kD homotrimer (6). An elevated serum level of TNF is associated with the pathophysiology of rheumatoid arthritis (RA), inflammatory bowel disease, and ankylosing spondylitis (1, 7, 8), and molecules that inhibit TNF signaling have demonstrated clinical efficacy in treating some of these diseases (9, 10).

We have engineered dominant-negative TNF (DN-TNF) variants that inactivate the native homotrimer by a sequestration mechanism that blocks TNF bioactivity (fig. S1). Protein design automation (PDA), an in silico method that predicts protein variants with improved biological properties (11–13), was used to introduce single or double amino acid changes into TNF (Fig. 1A) to generate the desired biological profile while maintaining the overall structural integrity of the molecule. Specifically, our goal was to design

homotrimeric TNF variants that (i) have decreased receptor binding, (ii) sequester native TNF homotrimers from TNF receptors by formation of inactive native:variant heterotrimers, (iii) abolish TNF signaling in relevant biological assays, and (iv) are easily expressed and purified in large quantities from bacteria. Variants were tested for TNF receptor activation in cell-based assays, and non-agonistic variants were then checked for their ability to antagonize native TNF in cell and animal models. Subsequently, we evaluated assembly state, receptor binding, and heterotrimer formation for several variants.

The computational design strategy used crystal structures of native and variant TNF trimers as templates for the simulations. Analysis of a homology model of the TNF-receptor complex revealed several distinct regions of the cytokine that make multiple direct contacts with its receptors (Fig. 1A), including interfaces rich in hydrophobic and electrostatic interactions. We ran simulations to select nonimmunogenic point mutations that would disrupt receptor interactions while preserving the structural integrity of the TNF variants and their ability to assemble into heterotrimers with native TNF (14). Many of the designed TNF variants displayed markedly reduced binding to TNFR1 and TNFR2, and several combinations of potent single mutations further decreased binding (Fig. 1B and fig. S2). As predicted by analysis of the TNF-TNFR structural complex, combinations of the most potent single mutations at different interaction domains (e.g., A145R and

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