

To:

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Subject:

PMF analysis of tendon material and binder (glue) from “Epitaph für Hans Stromer” (+1421): Jüngstes Gericht (Gemälde, Epitaph), inventory number Gm 124 from the Germanisches Nationalmuseum.

Background:

The backside of the epitaph has fibrous material imbedded in glue applied to panel joints, supposedly to strengthen the joints. A fiber sample was taken from a large fiber agglomerate and it visually contained little or none of the binding material. A second sample was taken from a nearby area of glue that was relatively free of fibrous material. Peptide mass fingerprinting (PMF)^{1,2,3,4} analysis was requested to determine the source(s) of the fibrous and binding materials. Beate Fücker supplied samples and accompanying documentation.

Summary of results:

The fibers/tendons are of cattle origin, and the binding material/glue is from sheep.

Analysis:

PMF analysis involves the enzymatic digestion of proteins followed by Matrix Assisted Laser Desorption-Ionization Time of Flight mass spectrometric (MALDI) analysis of the resultant peptide mixture. In the case of skin, hide, tendon, bone, and mammalian-based glues, collagen is the major constitutive protein, and for each mammalian source, the amino acid sequence of collagen is unique. Thus the mixture of peptides is unique—a “peptide mass fingerprint.” Marker ions⁵ in the MALDI spectra from known reference materials are compared with those from unknown samples for identification.

Figure 1 is the panel painting that was sampled: (“Epitaph für Hans Stromer”, Germanisches Nationalmuseum inventory number Gm 124); figure 2 shows the general sampling location on the backside; figure 3 shows the specific locations for fiber (Gm 124/1) and binding medium (Gm 124/2) samples.

Figure 4 is the PMF spectrum from the fiber sample with cattle markers indicated. The sample as received was sufficiently large that it could be sub-sampled. Thus, two small samples were taken that were visually free of binding material. The observed markers in figure 4 are specific for cattle/buffalo; other potential tendon sources, such as pig, horse, deer, sheep and goat, are confidentially excluded. Table 1 lists mammals that can be uniquely distinguished with our current PMF database.

Figure 5 is the PMF spectrum from the binding material. In this case, even with sub-sampling, a sample free of fibrous material could not be obtained. Thus the spectrum contains markers for both

cattle and sheep. These two sources are differentiated by A and F markers, which are shown clearly in figures 6 and 7 respectively. In both cases, the upper (blue) spectra are from the tendon-only sample; sheep markers are absent. The lower (red) spectra from the binder and fiber sample show both cattle and sheep markers.

The glue-free fiber sample allows the unambiguous identification of the tendon as cattle and the glue as sheep, and not vice versa.



Figure 1. "Epitaph für Hans Strome," Germanisches Nationalmuseum inventory number Gm 124.



Figure 2. Backside sampling location.

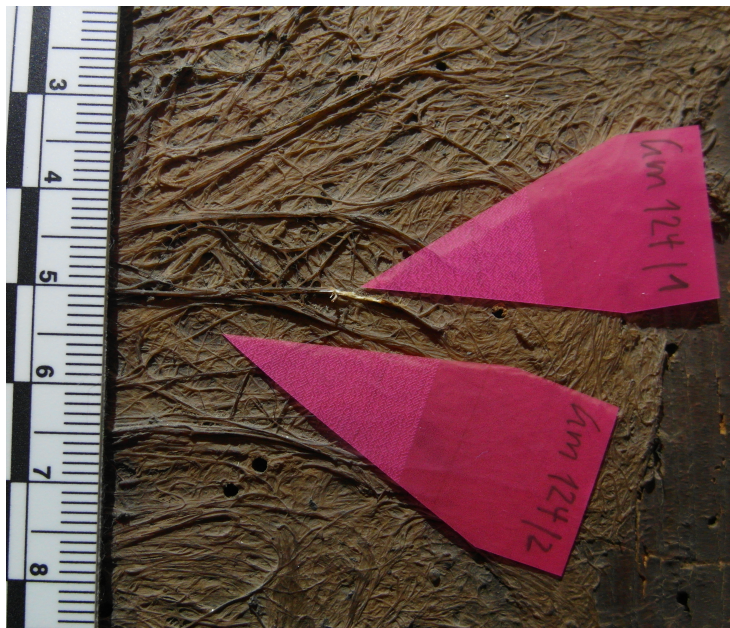


Figure 3. Fiber (Gm 124/1) and binding medium (Gm 124/2) sample locations.

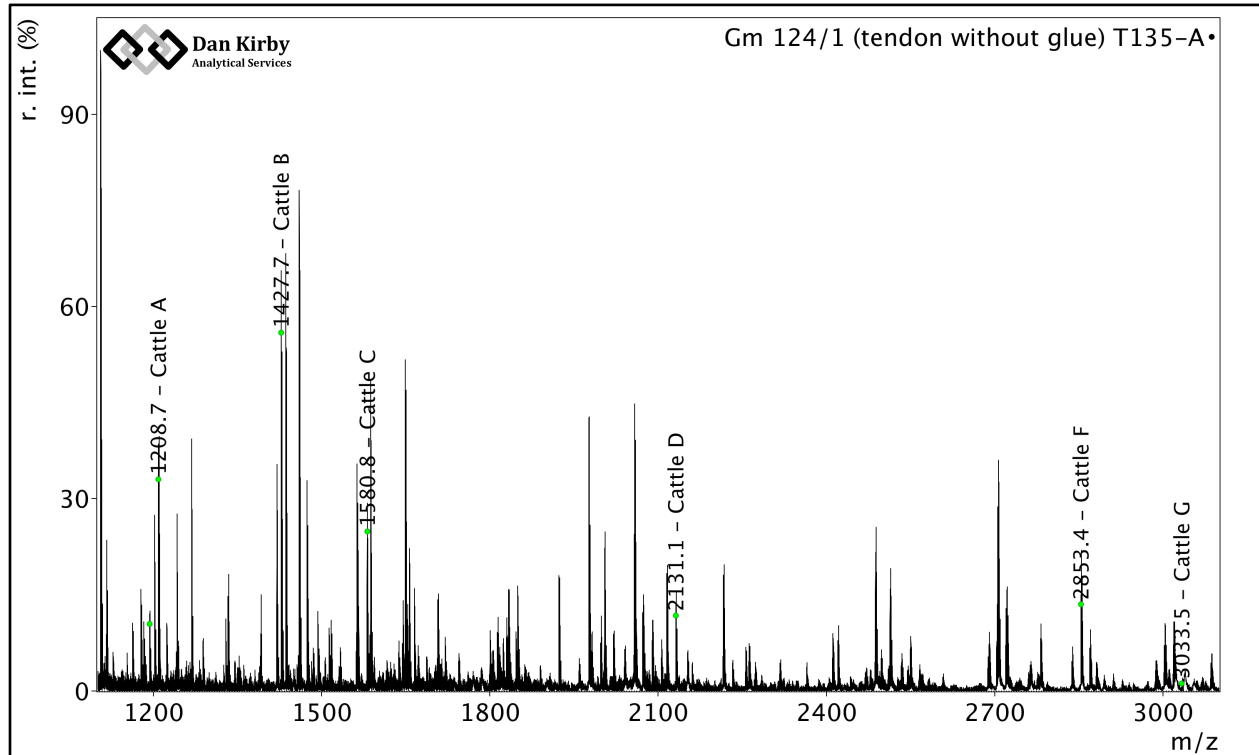


Figure 4. PMF (MALDI) spectrum: tendon sample (Gm 124/1) with cattle markers indicated.

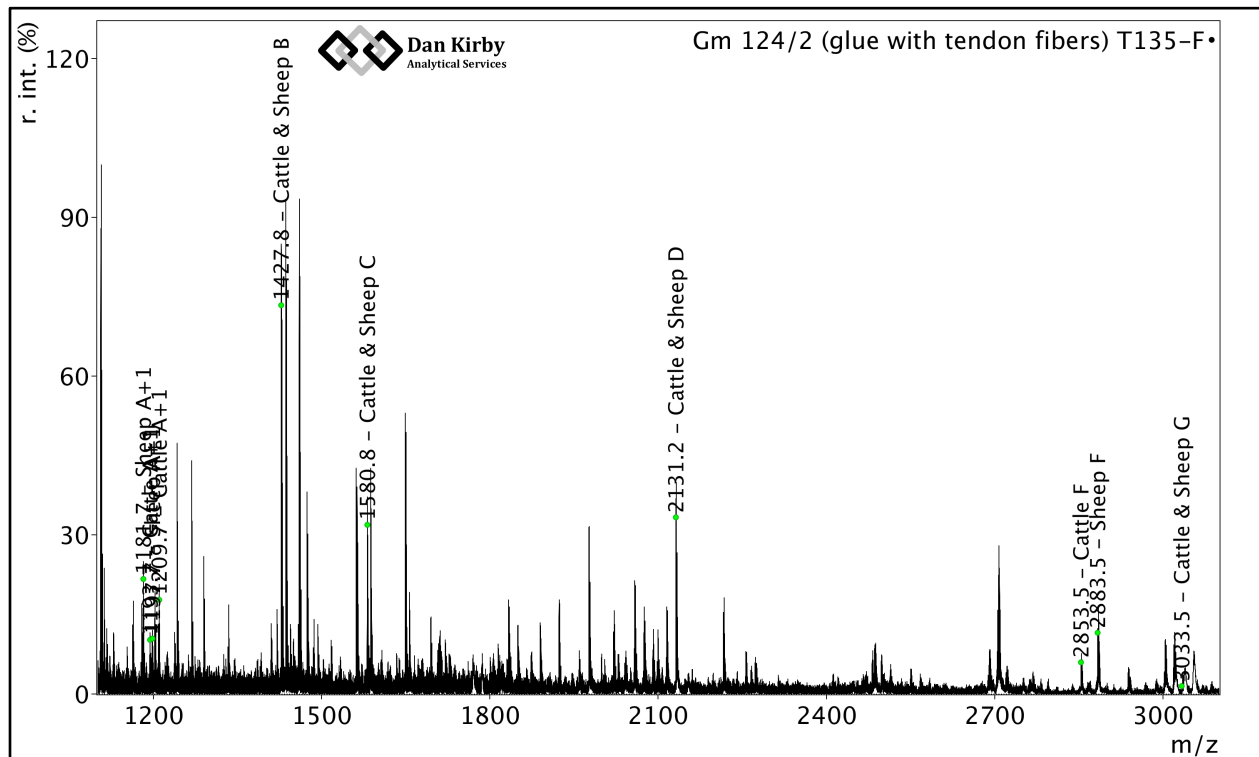


Figure 5. PMF (MALDI) spectrum: glue with tendon fibers sample (Gm 124/2) with cattle and sheep markers.

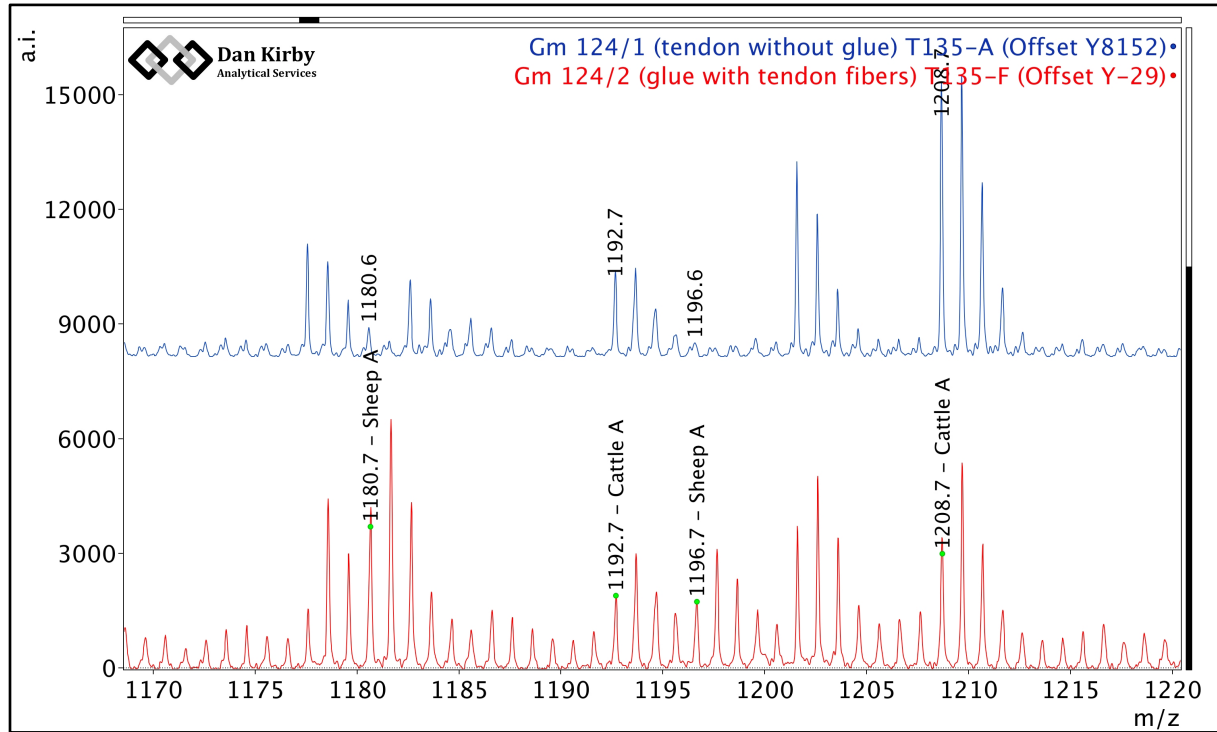


Figure 6. Detail of PMF spectra (tendon only, glue and tendon) showing A markers for cattle and sheep. The upper spectrum contains only the cattle markers whereas the lower shows both cattle and sheep.

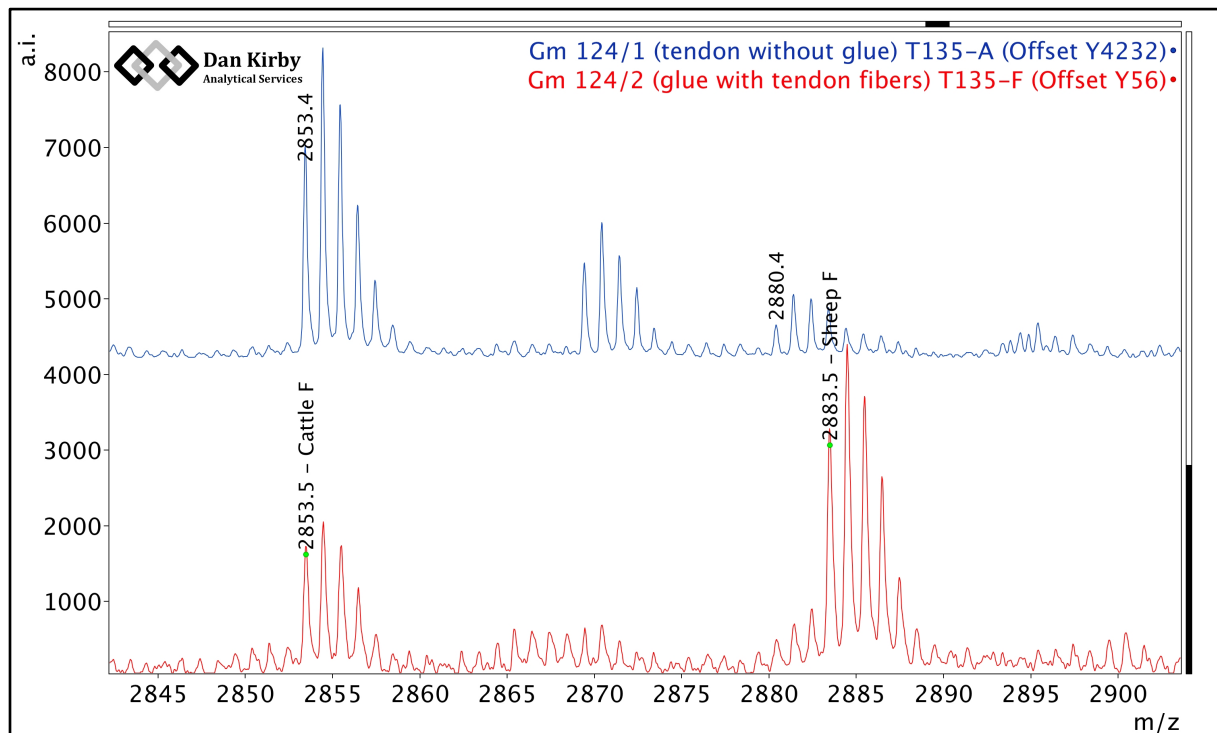


Figure 7. Detail of PMF spectra (tendon only, glue and tendon) showing F markers for cattle and sheep. The upper spectrum contains only the cattle marker whereas the lower shows both cattle and sheep.

- ¹ Kirby, D. P., N. Khandekar, J. Arslanoglu and K. Sutherland, "Protein Identification in Artworks by
² Kirby, D., M. Buckley, E. Promise, S. Trauger and T. R. Holdcraft 2013, "Identification of collagen-based materials in cultural heritage," *Analyst* (138) 4849-4858.
³ Promise, E., T. Rose Holdcraft, D. Kirby, and S. Haakanson, "Identifying collagen-based materials: A cross-cultural collaboration," Preprints, ICOM-CC 17th Triennial Conference, Melbourne, Australia (September, 2014).
⁴ Henzel, W. J., C. Watanabe and J. T. Stults 2003. "Protein identification: the origins of peptide mass fingerprinting," *J. Am. Soc. Mass Spectrom.* (14) 931-942.
⁵ M. Buckley and M. J. Collins, *Antiqua*, 2011, (1) 1-7.

Table 1. Mammals uniquely distinguished with PMF

Walrus	Sperm whale
Northern fur seal/ Steller sea lion	Bottlenose / Sowerby's whale
Bearded seal	Minke whale
Ringed seal	Fin Whale
Phocini seal: ribbon, spotted, grey, harbor, harp	Humpback whale
Hooded seal	Blue whale
Cattle / Bison	Gray whale
Sheep/Pronghorn	Sei whale
Goat	Right whale
Musk Ox	Elephant/Mastodon
Elk/red deer/fallow deer	Black rhino
Caribou/reindeer	Fox: red, Arctic
Roe deer	Gray fox
North American deer: mule, Sitka, whitetail	Cat
Horse	Pig
Dolphin: common, bottlenose, white-beaked, euphrosyne	Rabbit
Risso's Dolphin /pilot whale / false killer whale	Rat
Orca / White-sided dolphin	Mouse
Porpoise	Water buffalo
Narwhal	Dog/wolf
Beluga whale	Bear: brown, black, polar
	Lion
	Lynx
	Human
	Mustelidae (Badger)