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1. HPLC/MS of intact protein (10 % of sample) – QTOF; found sample A was heavily degraded, found sample B had three major peaks with masses of: Peak 1 = 12,537, Peak 2 = 8193 and 12,290 and peak 3 = 11,053 and 33,159 Da.
2. 1D SDS gel (50% of samples) showed two bands for B and none for A using Sypro Ruby stain. The entire lanes were cut into 8 sections, digest with trypsin, extracted and analyzed on two MS systems using cap HPLC/MS/MS. The samples were also analyzed using a tube gel (remainder of sample) cleaved/capped/trypsin/HPLC/MS/MS.
3. Data searching was carried independently for the MS/MS runs and the runs were merged and searched again for the 1D gel samples. All of the searches were compiled using Scaffold to get an overview and Peaks, Scaffold and Mascot were used to identify modifications.

Although the analysis of intact proteins indicated that three to five proteins were present, but the bottom up analysis only confidently identified two proteins, other than the massive amounts of keratin.