

The samples were resuspended in 20 uL 7M urea, 2M thiourea, 2% chaps, sonicated during 1 minute and divided into 2 aliquots. One fraction (1/2) was separated by SDS-PAGE and analysed by MALDI-TOF/TOF. The other (1/2) was analyzed by LTQ (Thermo).

1st approach (#16131): SDS-PAGE samples were mixed with loading buffer and incubated at 95 °C, 5 min and 4°C, 5 min. Then, they were applied in 10% SDS-PAGE gel (approximately 2.5 ug of sample A and 5 ug of sample B). The gel was stained following the Colloidal Coomassie blue protocol. Different bands were visualized (see Figure 1). Most of them appeared in both samples excepting 2 extra bands that only appeared in sample B (#2 and #3). All of the bands were excised, reduced, alkylated and digested with bovine trypsin. Peptides were analyzed by 4700 Analyzer MALDI-TOF/TOF (Applied Biosystems, AB). MS/MS spectra were used to search the Uniprot database with the Mascot search engine. Mass tolerance was set to ± 100 ppm for parent ion masses and ± 0.35 Da for fragment ion masses. Fixed modifications: Carbamidomethyl (C) and Variable modifications: Oxidation (M). RAGE_HUMAN (Q15109) Advanced glycosylation end product-specific receptor precursor (Receptor for advanced glycosylation) was identified in all the cases, being 38% the highest coverage obtained in both samples (band #7 and bands #2,5 for sample A and B, respectively). The peptide assigned for the identification correspond to the N-terminal half of the sequence protein.

2nd approach (#16132): The middle of each sample was subjected to the LC-MS/MS approach. Samples were in solution reduced, alkylated and digested with recombinant trypsin (1:50) and cleaned-up with a home made column filled with Poros R2 (AB). Then, both samples were resuspended in 0.5% FA. Approximately, 0.625 ug and 1.25 ug from samples A and B, respectively (1/8 of the initial amount) were injected. The samples were analyzed using a LTQ linear trap tandem mass spectrometer (Thermo Finnigan) with a nanospray ionization source and a reversed-phase PicoFrit™ column (BioBasic C18, 75 mm x 10cm, tip = 15 μ m, New Objective). The peptides were loaded onto the traps. After washing with 0.1% formic acid, the peptides were eluted by 0–70% solvent B in solvent A (A = 0.1% formic acid; B = acetonitrile) in 60 min at a flow rate of about 200 nl/min. The raw datafiles were searched against the IPI human protein database and Uniprot using Peaks software. The search parameters included the following: precursor-ion mass accuracy 200 ppm ; fragment-ion mass accuracy 0.5 Da; Fixed modifications: Carbamidomethyl (C) and Variable modifications: Oxidation (M); and 1 missed cleavages allowed

In sample A, RAGE1 protein was identified with a coverage of 24%, covering the N-terminal part. LTQ also identified RAGE_HUMAN Isoform 2 of Q15109. We obtained one differential peptides that were de novo sequenced with Peaks software. A 30% of coverage was obtained. However, in sample B, only RAGE1 was identified with a coverage of 41%, including a low confident peptide m/z 1763 (229-243). In both samples, A and B, some of the peptides found with MALDI such as 1181.65 (44-52) were missed in this approach. LTQ detected a lot of non tryptic peptides that were not detected in MALDI. This observation supports that both ionization sources are complementary.

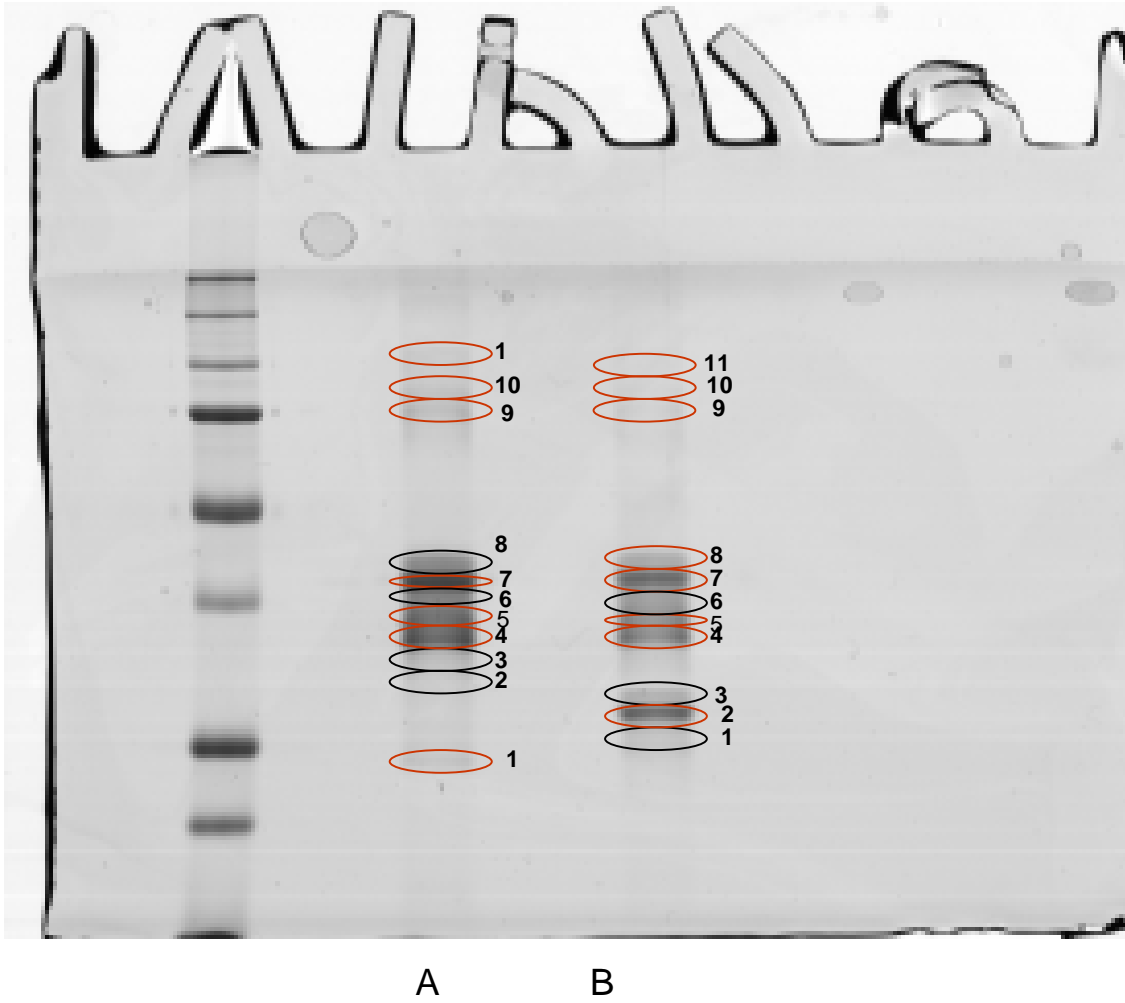
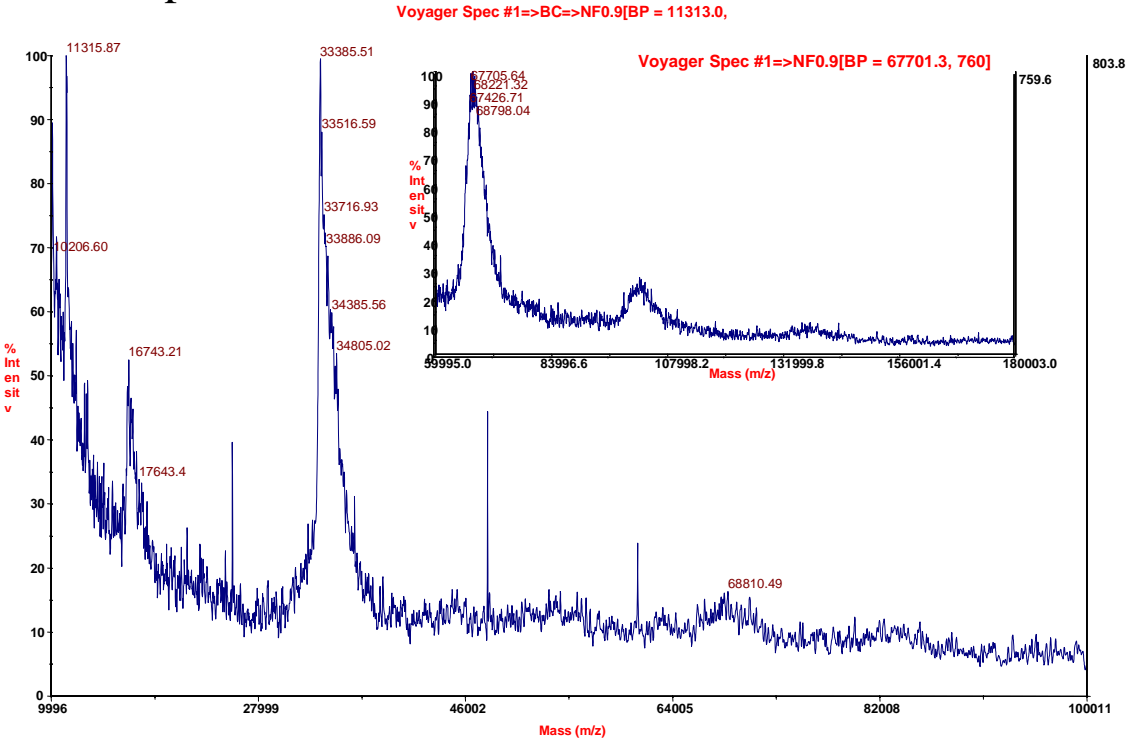


Figure 1. SDS-PAGE gel.

We also analyze the sample by MALDI-TOF to know the complexity and the differences between both samples. The results indicate that there are different signals in the samples, but are not according with the data from SDS-PAGE.

Sample A



Voyager Spec #1=>NF0.7[BP = 12662.6, 25515]

Sample B (5-120 Kda)

