

Small summary of ABRF PRG 2008 from "46011"

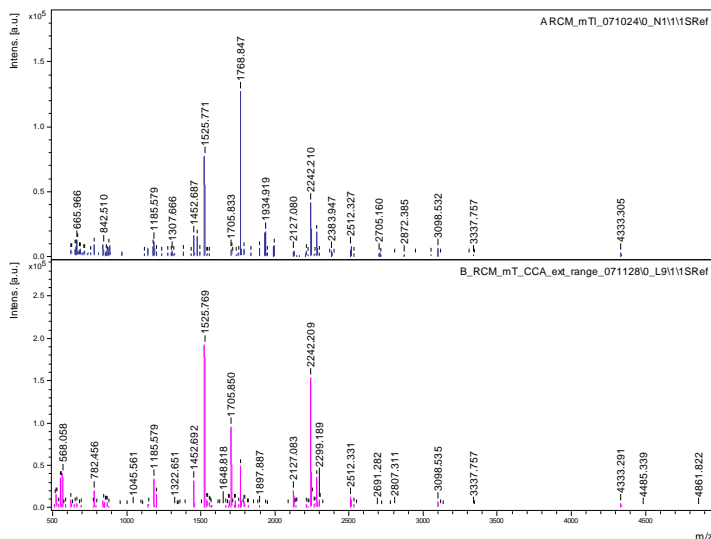
Sample A and B were dissolved in 0.1 % TFA/20 % ACN; 50 and 100 ul respectively, to obtain 0.1 ug/ul.

First attempt was to determine the mass of the intact proteins by linear MALDI TOF. Found about 32970 – same for A and B.

Then a part of native A and B were digested with trypsin – with the hope to later determine possible SS links.

Next, both samples were reduced (DTT) and alkylated (iodoacetamide), and again run in lin mode: To my surprise, I now found the mass of RCM A to be 19880 plus 13540 (which adds up to 33420, including 6x57 (I later found the id and could use the composition info). In sample B, an additional mass of 12638 was observed. That mass was not seen by lin MALDI of the intact samples. One could assume that the sample contains two linked polypeptides (which is not supported by info about the id'd protein)

Next, the red/alk samples were digested with trypsin, LysC and GluC. The tryptic digest easily identified the main component in A and B as human "advanced glycosylation end product-specific receptor". (Why did you have to select a protein with such a long name??).

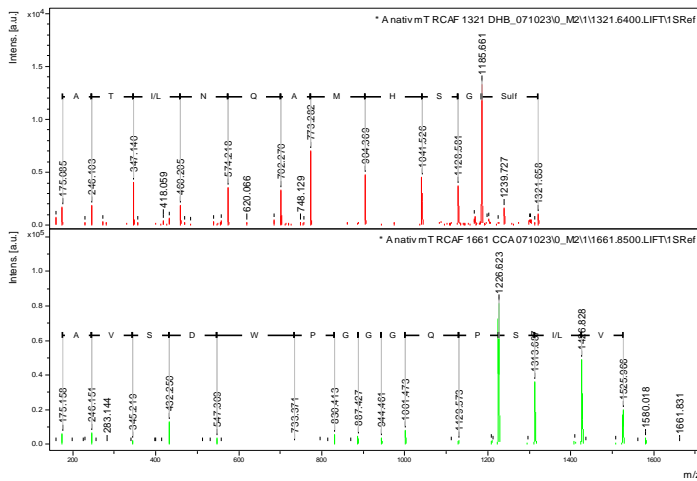


Spectra show tryptic dig of A and B.

The uses of additional proteases did unfortunately not add any useful info re the difference between A and B; but the id was of course confirmed.

All in all, the main protein was id'd, but I cannot find any strong evidence for any difference. I can only observe that ratios of tryptic peptides between sample A and B varies, and I suspect that isoform 1 and 2 are mixed; however, I could not find any peptide mass unique for isoform 2. There is however one unique peptide mass for isoform 1: VLSPQGGPWDSVAR, MH+ 1525.78, and this one varies slightly.

I used PSD after sulfonation to sequence several peptides, among them the N-terminal peptide (GSHMAQNITAR, MH+ 1185.58) that got modified after His-tag. Next figure shows PSD of that one and the peptide unique for isoform 1:



Searching for extra proteins (with the exception of keratins (which indeed were present in low ratios)) did not result in any significant match. In one attempt to detect another protein, I sequenced a His-tag in a peptide of MH+ 1818.79, but I assume it represents a "left-over" from the sample work-up? I therefore suspect that the difference between A and B is to be found in delicate modifications, which unfortunately I could not determine with the MALDI TOF/TOF used. I could have appreciated a bit longer time (in spite of two postponings of last date) due to heavy travelling this fall.