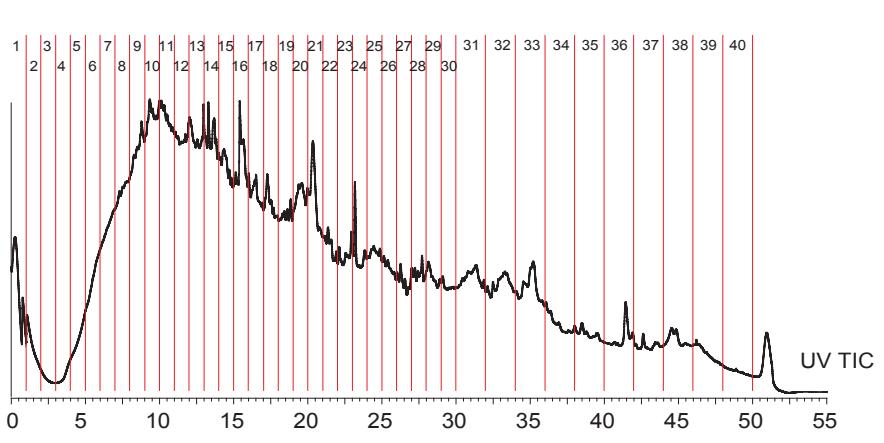


# LC-MS<sup>E</sup> analysis of human urine proteome

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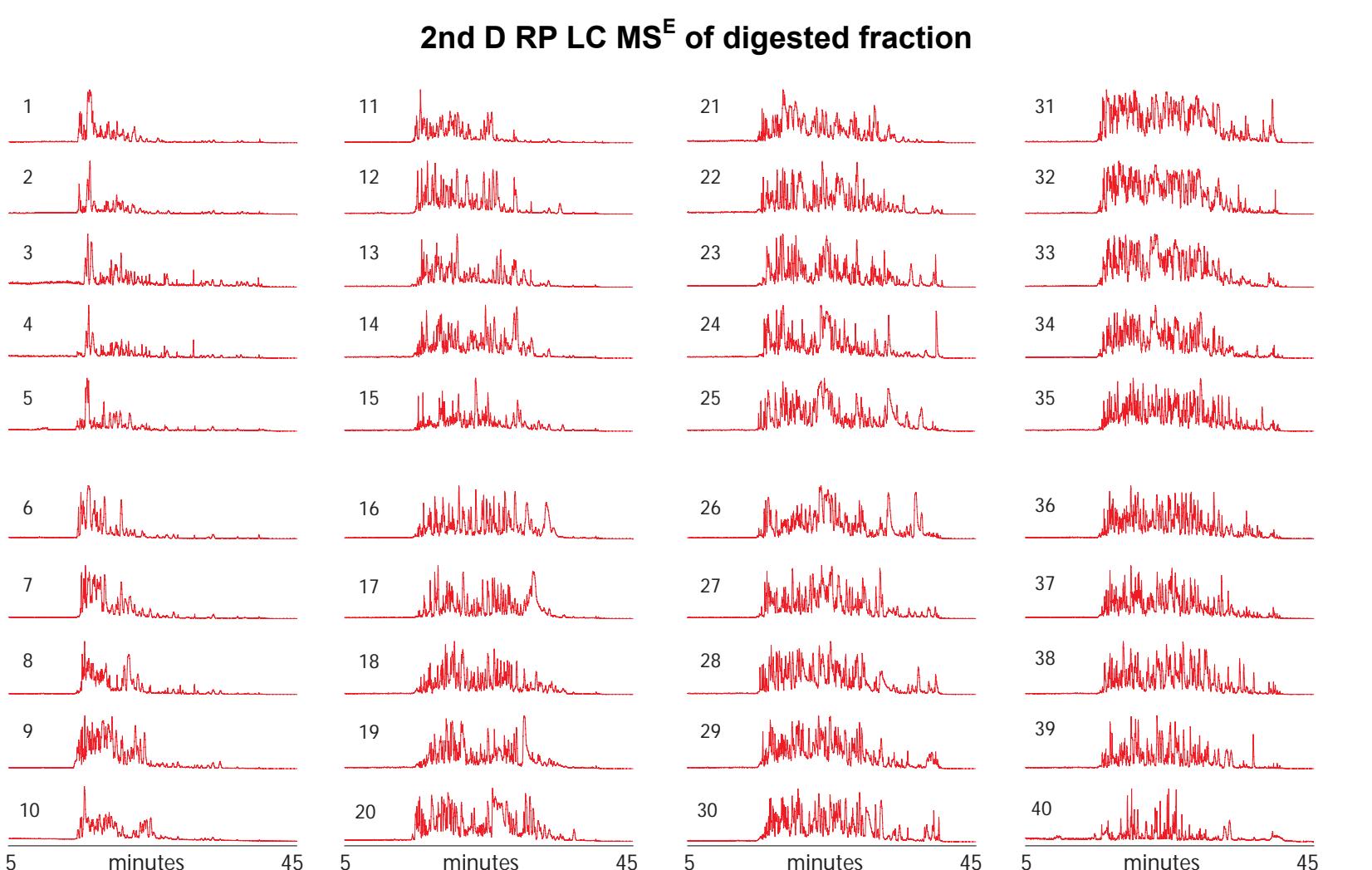
## OVERVIEW

- Human urine proteome was analyzed in 1D and 2D LC-MS<sup>E</sup> setup.
- In 2D LC the intact proteins were resolved with RP UPLC (Figure 1).
- Digested fractions were analyzed in LC-MS<sup>E</sup> (Figure 2). The number of identified proteins per fraction is shown in Figure 3.
- Proteins found in urine are often clips (Table 1 and sequences)
- More informative "peptide maps" with greater coverage were constructed in 2D experiment compared to 1D LC (Table 1).
- LC-MS<sup>E</sup> provides for quantitative information about peptides and proteins in urine (Table 2, Figure 4).



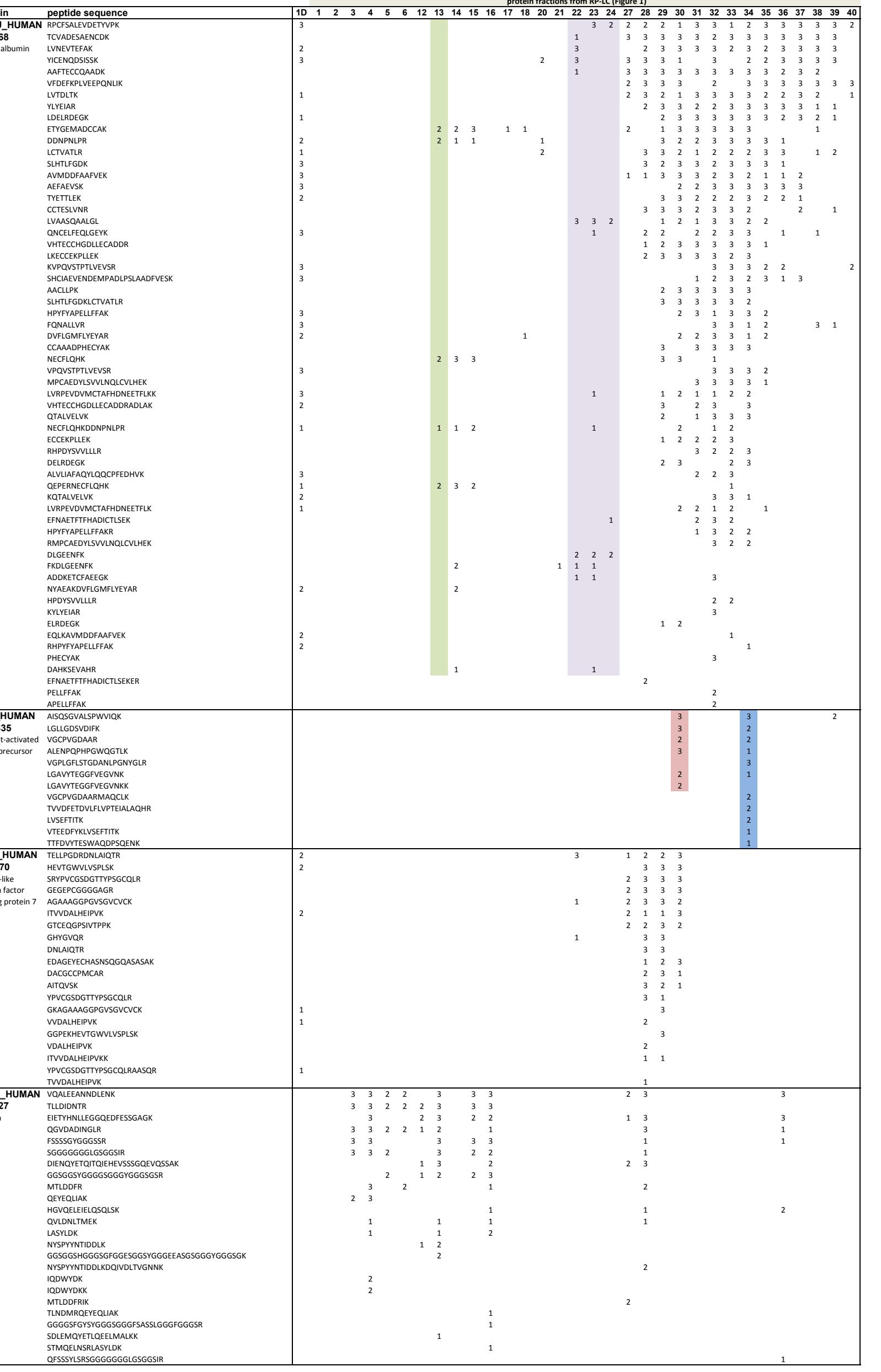
1D RP LC of intact urine proteins

**Figure 1:** Intact protein separation in RP-UPLC. Waters ACQUITY UPLC® System and ACQUITY UPLC® C18, 1.7µm, 2.1x50 mm column, 65 °C, UV TIC 210-350 nm. Mobile phase A: 0.1% TFA, B: 0.1% TFA in MeCN/IPOL (1:1). 5 min at 3% B, then gradient 3-55% B in 10 min, 55-90% B in 40-45 min. Flow rate 0.2 mL/min. Post column pressure restrictors, separation pressure 12000 psi.



**Figure 2:** Analysis of digested protein fractions. Waters nanoACQUITY UPLC® and ACQUITY C18, 75x100µm column, 1.7µm, 30 °C. Mobile phase A: aqueous 0.1% FA, B: 0.1% FA in MeCN. Gradient 3-40% B in 40 minutes, Flow rate 300 nL/minute.

## EVIDENCE OF PROTEIN CLIPPING IN URINE



**Table 1:** Peptides identified in digested fractions. Numbers in columns indicate how many times was each peptide identified in three repetitive analyses of the fraction.

## SELECTED PROTEIN CLIPS FOUND IN URINE

### ALBU\_HUMAN, fraction 13

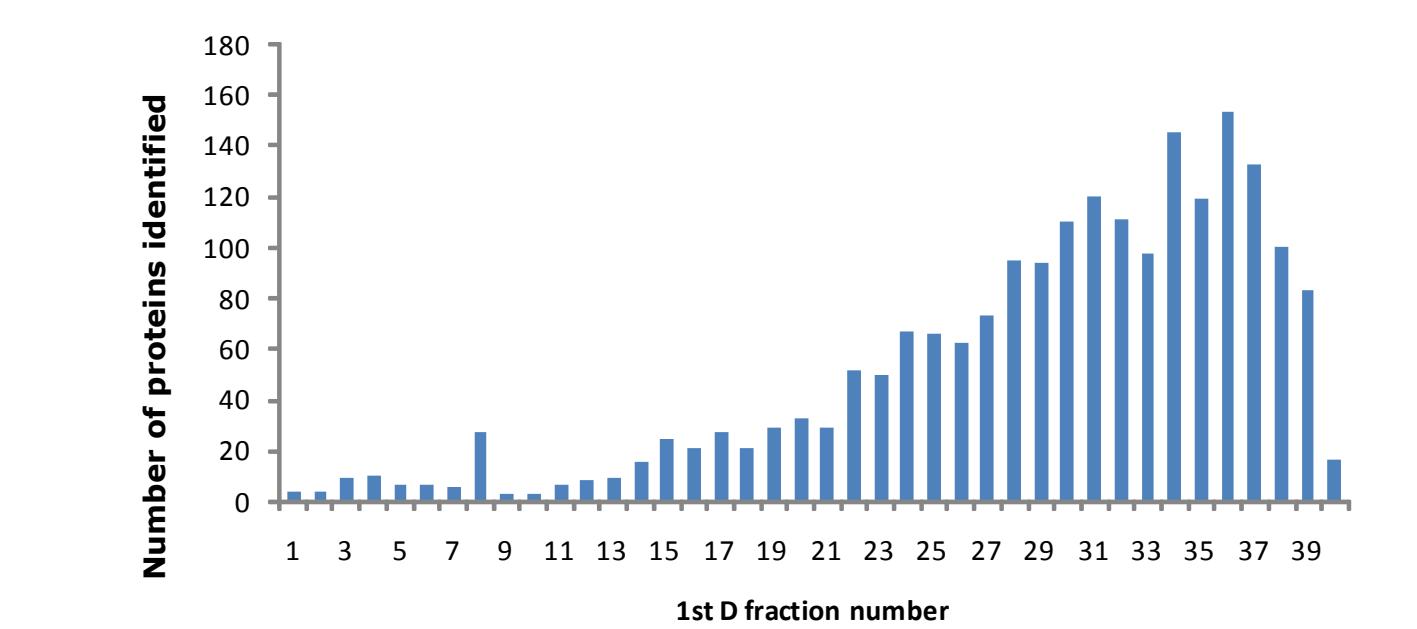
MKWVTFISLLFLFSSAYSRGVFRDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCP FEDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKL**LCTVATLRETYGEMADCCAK** QEPEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKLYEIARRHPYFYA PELLFFAKRYKAATTECCQQAADKAACLLPKLDELRDEGKASSAKQLRKCASLQKFCGER AFKAWAVARLSQRFPKAEEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICEN QDSISSKKLKECEKPLLEKSHCIAEVENDEMPADPLSLAADFVESKDVCKNYAEAKDVF LGMFLYEYARRHPDYSVLLRLRAKTYETTLEKCCAADPHECYAKVFDEFKPLVEEP QNLIKQNCLFEQLGEYKFQNALLVRYTCKVPQVSTPTLVEVSRLNGKVGSKCCKHPE AKRMPCAEDYLSVLNQLCVLHEKTPVSDRVTKCCTESLVRNRRPCFSALEVDETYVPK EFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCC KADDKETCFAEEGKKLVAASQAALGL

### ALBU\_HUMAN, fractions 22-24

MKWVTFISLLFLFSSAYSRGVFRDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCP FEDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKL**CVATLRETYGEMADCCAK** QEPEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKLYEIARRHPYFYA PELLFFAKRYKAATTECCQQAADKAACLLPKLDELRDEGKASSAKQLRKCASLQKFCGER AFKAWAVARLSQRFPKAEEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICEN QDSISSKKLKECEKPLLEKSHCIAEVENDEMPADPLSLAADFVESKDVCKNYAEAKDVF LGMFLYEYARRHPDYSVLLRLRAKTYETTLEKCCAADPHECYAKVFDEFKPLVEEP QNLIKQNCLFEQLGEYKFQNALLVRYTCKVPQVSTPTLVEVSRLNGKVGSKCCKHPE AKRMPCAEDYLSVLNQLCVLHEKTPVSDRVTKCCTESLVRNRRPCFSALEVDETYVPK EFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCC KADDKETCFAEEGKKLVAASQAALGL

### CEL\_HUMAN, fractions 30 and 34

MGRLQLVGLTCCWAVASAAKL**GAVYTEGGFVEGVNKLLGLGSVDIFK**GIPFAAP TKALENPQPHGWQGTLKAKNFKKRLQATITQDSTYGDDEDCLYLNIVVPGMIWIYG GAFLMGSGHGANFLNNLYLDGEEIATGRNVIVTFNYRVGPLGLSTGDANLPGNYGL RDQHMAIAWVKRNIAFGGDPNNITLFGESAGGAVSLSQTLSPYNKGLIRRASQSGVA LSPWVIQKNPLFWAKVAEK**VGCPVGDAARMAQCLKVTDPRALTAYKVLPLAGEY**MLHVGFVPIVDGDFIPADPINLYANAADIYIAGTNMDGHIFASIDMPAINKGNKVTE EDFYKLVSEFTIKGLRGA**KTFDVTYTESWAQDPSQENKKTVDFETDVLFLVPTIEA** LAQHRAANAKSAKTYLFHSHPSRMPVYPKWVGADHDIQYVGPKFATPTGYRQD RTVSKAMIAWVNTNFAKTDGDPNMGDSAIVPTHWEPYTTENSYLEITKKGMSMMKRS RTNFLRYWLTLYALPLPTDQEATPVPPGDSEATPVPPGDSETAPVPPGDGAPPV PPPTGDGSGAPPVPTGDSGAPPVPTGDSGAPPVPTGDSGAPPVPTGDSGAPPV PPTGDGSGAPPVPTGDSGAPPVPTGDSGAPPVPTGDSGAPPVPTGDSGAPPV TGDSGAPPVPTGDSGAPPVPTGDSGAPPVPTGDSGAPPVPTGDSGAPPVPTGDSGAPPV

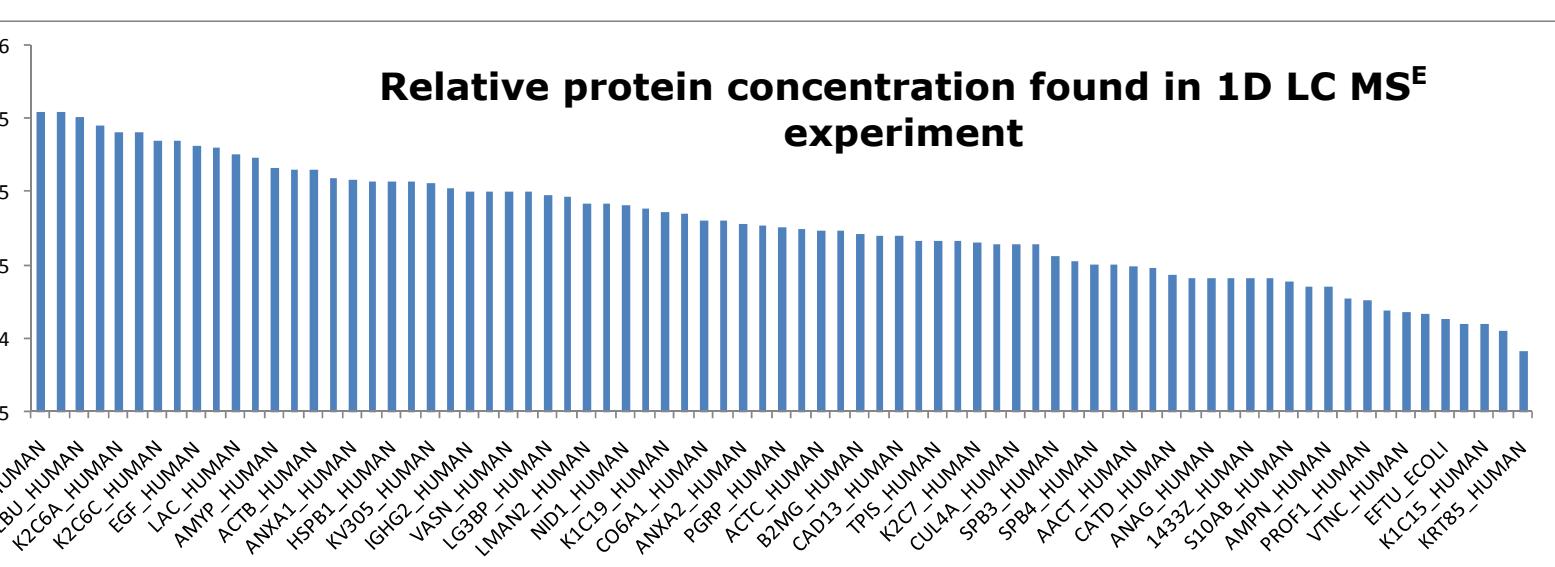


**Figure 4:** Relative protein concentration in 1D LC MS<sup>E</sup> was calculated as the average MS signal of the three most dominant peptides according to method published by Silva JC, Gorenstein MV, Li GZ, Vissers JP, Geromanos SJ, Mol Cell Proteomics. 2006, 5, 144-156.

## PEPTIDE/PROTEIN QUANTITATION

Pep. sequence	Protein	MS intensity
TVGSDTFSYFK	P01042 KNG1_HUMAN Kininogen-1 precursor	602467
AFIQLWFAFDVK	P02760 AMBP_HUMAN AMBP protein precursor	574362
DSTYLSLSTLTSK	P01834 KAC_HUMAN Ig kappa chain C region	394312
LVNEVTEFAK	P02768 ALBU_HUMAN Serum albumin precursor	389962
VILEIDNR	P13646 K1C13_HUMAN Keratin	349426
ILTATIENNR	P13646 K1C13_HUMAN Keratin	347989
ADTLDEINFLR	P02538 K2C6A_HUMAN Keratin	345847
LASYLEK	P13646 K1C13_HUMAN Keratin	343914
YELQVTAGR	P02538 K2C6A_HUMAN Keratin	343101
QGPVNLLSDPEQGVETQYER	Q14624 ITIH4_HUMAN Inter-alpha-trypsin inhibitor heavy chain H4 precursor	328495
YAASSYSLTPEQWK	P01842 LAC_HUMAN Ig lambda chain C regions	328186
LLTEHHGAGGPSR	Q16763 UBE2_HUMAN Ubiquitin-conjugating enzyme E2 S	321037
RPCFSALEVDETYVPK	P02768 ALBU_HUMAN Serum albumin precursor	291256
DPPQPVVPHVLDR	P10153 RNAS2_HUMAN Nonsecretory ribonuclease precursor	290090
VPOVSTPLTVEVS	P02768 ALBU_HUMAN Serum albumin precursor	286582
TAHCIEVENDEMPADPLSLAADFVESK	P048666 K2C6_HUMAN Keratin	263749
NLDLSSIAEV	P02768 ALBU_HUMAN Serum albumin precursor	259255
P19013 K1C4_HUMAN Keratin	P19013 K1C4_HUMAN Keratin	246955

**Table 2:** MS<sup>E</sup> provides an information about precursor MS ion intensity. List of most intensive MS signals for 1D experiment suggests that albumin is not the most dominant protein in urine.



**Figure 4:** Relative protein concentration in 1D LC MS<sup>E</sup> was calculated as the average MS signal of the three most dominant peptides according to method published by Silva JC, Gorenstein MV, Li GZ, Vissers JP, Geromanos SJ, Mol Cell Proteomics. 2006, 5, 144-156.

## CONCLUSIONS

- Efficient fractionation of intact proteins with ACQUITY UPLC system was useful for deeper insight into urine proteomic.
- Various protein clips were detected in different fractions.
- Relatively few proteins were identified in earlier fractions when using strict tryptic specificity rule.
- When accepting peptide hits based on 3 b/y ions, 175 proteins and 1385 peptides were identified in 1D experiment. 536 proteins based on 4224 unique peptides were found in 2D LC MS<sup>E</sup>.
- Relative concentration of proteins in the urine is reported. Keratins, microglobulin, IgG's, pro-epidermal growth factor, and uromodulin are among the most abundant proteins.