Protein stoichiometry of a multi-protein complex, the human spliceosomal U1 snRNP: Absolute quantification using isotope-coded tags and mass spectrometry

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Running Title: Stoichiometry determination of the U1 spliceosomal snRNP

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Summary

The human U1 snRNP (small nuclear ribonucleoprotein), which is a part of the spliceosome, consists of U1 snRNA and ten different proteins: seven Sm proteins B/B', D1, D2, D3, E, F and G and the three U1-specific proteins U1-70 K, U1-A, U1-C. In order to determine the stoichiometry of all ten proteins, the complex was denatured, digested completely with an endoprotease and labelled with an amine-specific tag. Corresponding peptides were synthesized and labelled with the same tag containing heavier isotopes. The digest was then spiked with defined amounts of the synthetic peptides and the resulting isotopic peptide pairs were analysed quantitatively by mass spectrometry. The mass spectra provided information about the absolute amount of each component in the starting protein mixture. The use of the isotope-coded, amine-specific reagents propionyl-N-oxysuccinimide and nicotinoyl-N-oxysuccinimide was evaluated for stoichiometry determination; the nicotinoyl reagent was found to be advantageous owing to its greater mass spectrometric sensitivity. Absolute quantities of all ten proteins were measured, showing equal numbers of all ten proteins in the U1 spliceosomal snRNP. These data demonstrate that quantitative mass spectrometry has great potential for the determination of the stoichiometry of multi-protein complexes.
Introduction

Many cellular functions are regulated by multi-protein complexes. Mass spectrometry based methods allowed significant advances in the discovery and identification of the interacting proteins. Furthermore, this technique has also been used to study the (relative) dynamics of protein complexes in different states by the incorporation of stable isotopes [1, 2], a method widely used in quantitative proteomics. Labeling with stable isotopes can be achieved either by chemical modification of side-chain amino groups with a reagent containing heavier isotopes (2H, 13C, 15N) [3, 4] or by incorporation of isotope coded amino acids from the growth medium [5, 6]. To understand the function of individual proteins in a complex, it is necessary to analyse the interacting proteins in a quantitative way, which reveals whether a certain protein is present in defined stoichiometric amounts or forms only a weak substoichiometric interaction. To gain a knowledge of the stoichiometry of a protein complex, however, determination of the absolute amounts [7,8] of the complex constituents is required.

We recently described a method for the determination of the stoichiometry of protein complexes, which is based on proteolytic digestion of the complex, labelling with a fluorescent reagent specific for amino or sulphhydryl groups, and separation by liquid chromatography with fluorescence and mass-spectrometric detection. The strength of the fluorescence signal of the labelled peptides from different proteins is directly proportional to the stoichiometry of these proteins in the complex [9]. For large multi-protein complexes a potential limitation of this method is the difficulty of the separation of very complex peptide mixtures: poor resolution of these may lead to an overlap of the peptide peaks in the fluorescence chromatogram, preventing correct quantification. Here we describe the application of a similar approach that overcomes this limitation.
We applied the new method to the determination of the protein stoichiometry of the spliceosomal multi-protein complex U1 snRNP. The spliceosome is formed by four small nuclear ribonucleoproteins, the snRNPs U1, U2, U5 and U4/U6 (named after their uridine-rich snRNAs) and numerous additional proteins [10]. The snRNP proteins can be divided into two groups: the U-snRNP-specific proteins and the Sm proteins [11]. The Sm proteins, named SmB, SmD1, SmD2, SmD3, SmE, SmF and SmG, are associated with the Sm binding site of the snRNAs, except for U6, which contains homologous “Sm-like” proteins. Some arginine residues of SmB and SmD3 have symmetrical dimethyl modifications [12]. In humans, some tissues, as well as HeLa cells, contain a second SmB protein called SmB', which is a splicing variant in which the last two amino-acid residues are replaced by a new sequence of eleven residues [13]. When bound to the snRNA, the Sm proteins form a very stable core RNP structure. In vitro, Sm proteins form specific protein complexes, and crystal structures have been obtained from the two dimeric SmD1/D2 and SmD3/B complexes. These results prompted the proposal of a heptameric circular model for the Sm core RNP [14]. Furthermore, co-precipitation experiments with genetically labelled yeast Sm proteins have suggested that one copy of each Sm protein is present in the core RNP [15]. U1 snRNP, purified from HeLa cells, is the smallest and best characterised human spliceosomal RNP subunit. Beside the Sm proteins, it contains only three specific proteins: U1-70K, U1-A and U1-C. Electron cryomicroscopy of U1 snRNP revealed a 3D structure consisting of a circular core domain quite similar to the proposed heptameric Sm ring structure and individual additional domains for U1-70 K and U1-A proteins with sizes compatible with one copy of each of these proteins [16]. The U1-C protein could not be recognised as an individual domain. Although these results suggested the presence of one copy of U1-C, other biochemical experiments suggested that U1-C in the U1 snRNP could be
present as a dimer [17].

For protein stoichiometry determination up to three reference peptides were synthesized for each U1 snRNP protein, quantified and labelled with a $^{[13}\text{C}]$-coded tag while the peptides derived from U1 snRNP proteolysis were labelled with a $^{[12}\text{C}]$-coded tag. Mass spectrometric analysis of mixtures of both kinds of labelled peptides provided information about the absolute quantities of the constituents of the U1 spliceosomal snRNP.
Experimental

Materials

Acetonitrile (gradient grade), urea (analytical grade), dithiothreitol (DTT), ammonium chloride, trifluoroacetic acid (TFA, analytical grade) and guanidinium hydrochloride (analytical grade) were obtained from Merck (Darmstadt, Germany). 4-(2-hydroxyethyl) piperazine-1-ethane sulphonic acid (HEPES) was obtained from Sigma (St. Louis, MO, USA). Water from an ultra-pure water system (Millipore, Bedford, MA) was used for preparation of all aqueous solutions. Trypsin and endoproteinases Lys-C, Glu-C and Asp-N were from Roche (Mannheim, Germany). Tri-isopropylsilane and dimethylsulphoxide (DMSO) were obtained from Fluka Sigma-Aldrich GmbH (Seelze, Germany), Fmoc-amino acids from MultiSynTech GmbH (Witten, Germany), and NMM (N-methylmorpholine) from Biosolve BV (Valkenswaard, Netherlands).

Synthesis of reference peptides

Peptide synthesis was performed by using Fmoc (9-fluorenylmethyloxycarbonyl) chemistry (PyBop/NMM activation, Trt (trityl) / tBu (tert-butyl) / Pbf (2,2,4,6,7-pentamethyldihydrobenzofuran-S-sulfonyl) / Boc (tert-butoxyarbonyl) side-chain protection, pre-loaded Wang Resins (200–400 Mesh) on a multiple peptide synthesizer (AMS 422; Abimed GmbH) on a 20-μmol scale. Peptides were cleaved (92.5% TFA, 2.5% water, 5% tri-isopropylsilane) for 1.5 h, precipitated and washed three times with cold methyl t-butyl ether. Air-dried crude peptides were purified by using reversed-phase HPLC (solvents, water-acetonitrile gradients, 0.1% TFA; HPLC system, Sycam GmbH; column, GROM Sil ODS 2, 25 cm length, 2 cm diameter, 5
µm C18 silica beads). The purity of the peptide fractions was assessed by analytical RP-HPLC (solvents, water-acetonitrile gradient; HPLC system, Beckman System Gold; column, GE Healthcare SOURCE 5 RPC ST 4.6/150, 15 m length, 4.6 mm diameter, 5 µm C18 polystyrene/divinylbenzene). The sequence identity of the peptides was verified by MALDI-TOF mass spectrometry. Absolute quantification of the reference peptide amounts was performed gravimetrically (accuracy > 0.05 mg), carefully minimising errors caused by peptide hygroscopy or electrostatic effects of the sample cup.

Labelling of the peptides with [13C]-propionyl-N-oxysuccinimide or [13C]-nicotinoyl-N-oxysuccinimide

0.8–6 mg of the reference peptides were dissolved in 1 mL of 10% acetonitrile or 10% acetonitrile and 10% formic acid in water. The sequences of the synthetic peptides matched those of the peptides expected to be created by endoproteinase cleavage of the U1 snRNP. All peptides that corresponded to cleavage products of a distinct proteinase (endoproteinase Lys-C, Asp-N, Glu-C or trypsin) were combined; this resulted in four peptide mixtures. These solutions were then diluted with water to a volume of 250 µL, resulting in concentrations between 0.03–0.13 µg/µl of each peptide. 5 µL of each of these solutions was combined with 45 µL HEPES buffer (50 mM, pH 8.5). Thereafter, 4 µL propionyl-N-oxysuccinimide (0.15 M in DMSO) or nicotinoyl-N-oxysuccinimide (0.15 M in DMSO) was added. The mixture was incubated at 25 °C for 1 h. To stop the reaction, 4 µL of ammonium chloride (1 M) was added, and the solution was incubated for 15 min at room temperature. To hydrolyse reaction side-products with hydroxyl groups [18, 19], the pH was raised to
12 with NaOH (1 M) for 10 min. The solution was then neutralised by the addition of the same molar amount of HCl (1 M). 0.5-2 µL of this solution was added to the digest of the human U1 snRNP.

Denaturation and digestion of the human U1 snRNP

2.5–4 µL of the U1 snRNP (0.5 mg/mL [20], dissolved in 20 mM Tris-HCl, pH 7.9, 1.5 mM MgCl₂, 150 mM KCl, 0.5 mM DTE, 0.5 mM phenylmethylsulfonyl fluoride), purified essentially according Bach et al. [21] and containing its complete set of proteins (see Figure 1 for a Coomassie stained electrophoresis gel) were mixed with 20 µL of 6 M guanidinium hydrochloride. 1 µL DTT (1 mg/mL) was added, and the solution was incubated at 60°C for 45 min. The solution was then diluted with 180 µL of 25 mM HEPES buffer (pH 8.5), whereafter 40 µL of endoproteinase Lys-C (0.05 µg/mL), trypsin, endoproteinase Glu-C or endoproteinase Asp-N were added and the digests were incubated at 37°C overnight.

Labelling of the digest with [12C]-propionyl-N-oxysuccinimide or [12C]-nicotinyl-N-oxysuccinimide

To 75 µL of the endoproteinase digest (see above), 4 µL of propionyl-N-oxysuccinimide or nicotinyl-N-oxysuccinimide (0.15 M in DMSO) was added and the solution was incubated at 25°C for 1h. Subsequently, 4 µL of ammonium chloride (1 M) was added and the mixture was incubated for a further 15 min. The pH was raised to 12 with NaOH (1 M) for 10 min and the solution was then neutralised with the same molar amount of HCl (1 M).
Capillary liquid chromatography and MALDI target preparation

Separation of the peptides was achieved with a capillary liquid chromatography system (Ultimate, LC Packings) and a reversed-phase column (LC Packings Pepmap reversed-phase C18 column, 75 µm i.d., 15 cm). 25 µL of the peptide solution was injected and the peptides were trapped on a short reversed-phase column. For the separation of the peptides, the solvents A (0.05% TFA) and B (80% acetonitrile, 0.04% TFA) were used and a linear gradient from 10 to 100% B in 30 min with subsequent 20 min isocratic at 100% B. The eluting peptides were spotted robotically onto a 192-well MALDI sample plate and mixed with a matrix solution (5 mg/mL of α-cyano-4-hydroxycinnamic acid dissolved in 50% acetonitrile, 0.1% TFA) during the spotting process. The sample spots were dried at room temperature.

Tandem time-of-flight mass spectrometry

Mass-spectrometric analysis was performed on a 4700 Proteomics Analyser from Applied Biosystems (Framingham, MA) equipped with an Nd-YAG laser that produces pulsed power at 355 nm at pulse rates of 200 Hz. Mass analysis was performed using positive reflector mode with a deflection cut off range of m/z 700. 5000 laser shots were accumulated to produce a single spectrum. Subsequently, high-energy MALDI-TOF/TOF CID spectra were recorded on selected ions from the same sample spot. The collision energy was 2 keV. Air was used as collision gas.
Results

Peptide Selection

Figure 2 shows a schematic summary of the method, which we used for the determination of the stoichiometry of the U1 snRNP. The complex is denatured and digested completely with an endoproteinase. The proteolytic peptides are then labelled with an amine-specific $^{12}$C isotope coded tag [18, 19]. Reference peptides are selected from the database, synthesized and labelled with an $^{13}$C isotope coded tag. Mass spectrometric comparison of the intensities of the $^{12}$C and $^{13}$C labelled peptide pairs allows the absolute quantification of the peptides. The ratio of the absolute quantities of peptides resulting from different proteins corresponds to the stoichiometry of the proteins in the complex. In this study, we selected two peptides per protein from the protein sequences in the NCBI database for the absolute quantification of all ten U1 proteins. The sequences of the peptides are shown in Tables 1 and 2. We used the following selection criteria for the reference peptides. (i) No peptides starting with proline or glutamic acid were chosen, considering the possibility of incomplete cleavage by some endoproteinases (e.g. trypsin) before these amino acids. (ii) Amino acids that can be oxidised or transformed were avoided, such as methionine and tryptophan (which are easily oxidised) and N-terminal glutamine (which can be transformed into pyroglutamic acid). All the peptides chosen fullfill these requirements, except for the methionine-containing peptides 3–9 of SmD1, and peptides 73–80 and 13–23 of SmE. Peptides 10–20 and 66–86 of SmD1 and 81–92 of SmE, which would fit the criteria, could not be synthesized in sufficient purity. All reference peptides correspond to peptides that are expected to be produced from the spliceosomal proteins by endoproteinase
digestion. As shown in Tables 1 and 2, most peptides are created by endoproteinase Lys-C digestion. Alternatives were trypsin, endoproteinase Glu-C and Asp-N (Tables 1, 2).

Peptide derivatization

There are two possibilities for the incorporation of stable isotopes into peptides: peptide synthesis using isotope-coded amino acids, such as \([^{15}\text{N}]\)-, \([^{18}\text{O}]\)-leucine \([8]\), or derivatisation of their side chains with isotope coded reagents \([22]\). Munchbach et al. described the advantages of amine-specific isotope coded reagents, such as d0/d4 nicotinoyl-N-oxysuccinimide carrying a fixed positive charge and increase the mass-spectrometric response for labelled peptides significantly, compared with the acetylated peptide as well as with their unlabeled counterpart \([23]\). The increase in mass-spectrometric response is most pronounced for small peptides (up to 10 amino acids), which can also be synthesized more reliably. The use of nicotinoylation may therefore result in an increase in sensitivity for the absolute quantification and stoichiometry determination even for protein complexes that can only be isolated in low quantities.

Determination of optimal conditions for denaturation and digestion

In order to determine the correct stoichiometry with this method, complete denaturation and digestion needs to be achieved for the U1 snRNP. Initially, urea was used for the denaturation of the complex. Urea is tolerated by several endoproteinases up to a concentration of 4 M, which allowed the digestion of the
denatured protein complexes without prior dilution or dialysis [9]. However, even with a concentration of 8 M urea, complete denaturation of the U1 snRNP was not achieved (data not shown). Therefore, 5 M guanidinium hydrochloride (GHCl) was tested. Because most endoproteinases already show a significant loss of activity at a concentration of 1 M, the denatured complex was diluted to 0.5 M GHCl with HEPES buffer. Subsequently, the enzyme was added and incubated overnight. After labelling with a [12C] amine-specific tag, the digest was spiked with the synthetic peptides, which were derivatised with a [13C] amine specific tag. 0.36 µg of the complex was used in each experiment; assuming a hypothetical 1:1 stoichiometry of all complex constituents, this would correspond to ~ 1.7 pmol of the complex. Approximately the same molar amounts of the reference peptides were added, which should therefore theoretically result in a similar intensity of the [12C]- and [13C]-labelled peptides supposing the complex to have been pure, denaturation and digestion to have been complete. Figures 3 and 4 show the MALDI-TOF mass spectra of the peptides acquired after liquid-chromatographic separation, which demonstrate a similar intensity of the isotopic peptide pairs.

Stoichiometry determination using propionyl-N-oxysuccinimide

Table 1 summarises the results of two independent experiments for the absolute quantification of the U1 proteins using propionyl-N-oxysuccinimide, using approximately 0.36 µg of the complex per experiment. Half of all peptides, however, could not be detected, owing to their low mass-spectrometric response. Peptides from the proteins U1-70 K, U1-A, SmD1, SmD2, SmD3, SmE and SmG showed absolute amounts in the range of 1.25-1.67 pmol suggesting 1:1 stoichiometries of these proteins in the complex. The observed deviations of approximately ±16% may
be due to variations in sample handling, such as pipetting of peptide samples and dilutions. Peptide 38–50 of SmD2 showed a very high amount (12.4 pmol), which may have been due to solubility problems of the reference peptide. Concentrations below 0.069 pmol were found for peptide 90–99 of SmD3, which was attributed to arginine dimethylation of R-97 [12].

Stoichiometry determination using nicotinoyl-N-oxysuccinimide

In order to quantify all the proteins in the complex, the experiments were repeated with the same absolute amounts of the U1 snRNP as described above, replacing propionyl-N-oxysuccinimide by nicotinoyl-N-oxysuccinimide (Table 2). In this case, all the peptides were detected; in particular, small peptides showed a significant increase in detectability as compared with propionyl-N-oxysuccinimide. One peptide of SmG (4–10, sequence: (K) AHPPELK), which is created by complete digestion with endoproteinase Lys-C or trypsin, was quantified in both digests. Quantification of this peptide in trypsin and endoproteinase Lys-C digests resulted in similar quantities (Table 2), which suggests that the digestion was complete. In similarity to the experiments with propionyl-N-oxysuccinimide, mean absolute quantities of 1.40-1.86 pmol (experiment 1) and 1.23-1.65 pmol (experiment 2) were measured for most peptides. For peptide 95–108 of SmB and 90–99 of SmD3, quantities below 0.01 pmol were determined. This is ascribed to dimethylation of arginine 97 of SmD3 and arginine 108 of SmB [12]. For peptide 9–17 of SmF, a quantity of 0.035–0.05 pmol was found. This peptide contains aspartic acid, which is also cleaved by endoproteinase Glu-C to a lesser extent. For peptide 38–50 of SmD2, very high quantities (11.5–13.5 pmol) were found. These data also demonstrate that one peptide per protein may be insufficient for the reliable absolute quantification of a
protein. A third peptide (104–118) from SmD2 was therefore quantified, resulting in 1.79–1.84 pmol, which is comparable to the quantity of peptide 93–98 (1.65–1.88 pmol).
Discussion

Recently, we described a method for the determination of the stoichiometry of protein complexes. Absolute quantification of the complexes constituents was achieved by comparing the areas of fluorescently labelled peptides resulting from different proteins in the fluorescence chromatogram. This method was used for the determination of the stoichiometry of the complex of hFc1 and sFcγRIII [9]. The method allows relatively fast stoichiometry determination of complexes consisting of a moderate number of proteins. Although cysteine-specific rather than amine-specific labelling reduced significantly the complexity of the fluorescently labelled peptide mixture resulting from hFc1 and sFcγRIII, the number of peptides resulting from very large multi-protein complexes may still be too high to allow a good separation. Therefore, we tested the strategy depicted in Figure 2 for stoichiometry determination of multi-protein complexes. Since reference peptides have to be synthesized for this approach, this method is more time-consuming than the fluorescence method; however, it overcomes several limitations for the analysis of large multi-protein complexes. First of all, it is absolutely compatible with multidimensional separations. Losses of peptides during the transfer from the 1st to the 2nd dimension have no influence on the absolute quantification, since the [13C]-labelled synthetic reference peptides show chromatographic behaviour identical to that of their 12C labelled counterparts. Furthermore, even poorly separated peptides in the chromatogram can be quantified, since the quantification is based on the comparison of the mass-spectrometric intensities belonging to the isotopic peptide pairs. A similar approach was suggested for absolute quantification in proteomics, using ICAT-labelled idiotypic synthetic reference peptides as internal standards [22].
However, several circumstances should be considered when using this approach for absolute protein quantification. Complete denaturation and digestion of the complex is a prerequisite for absolute quantification and stoichiometry determination. Furthermore, since unmodified synthetic peptides are used for quantification, unexpected post-translational modifications of the proteolytic peptides can falsify the result in quantification of the protein. The use of absolute quantification on a proteome-wide scale may therefore still be problematic. Multi-protein complexes, however, consist of a moderate number of proteins, which are usually better characterised. Another issue is that of miscleavage by endoproteinases, such as chymotryptic activity in trypsin or endoproteinase Glu-C cleavage at aspartic acid. Therefore, we used in this report two or more peptides per protein for the absolute quantification of most proteins. We found similar absolute quantities for all ten proteins, suggesting equal stoichiometries of all ten proteins in the U1 snRNP, including U1-C. For quantification of the SmB protein, only peptides derived from sequences common to both SmB and SmB’ were used. Consequently, there is only one type of SmB protein in the Sm core, and SmB can be replaced by SmB’ or vice versa. Because Coomassie-stained gels of electrophoretically separated snRNP proteins always show the two proteins SmB and SmB’ as two bands of equal intensity (see Figure 1), the two proteins are present in approximately similar amounts. The 1:1 stoichiometry of the Sm proteins is in good agreement with a model proposing that the Sm proteins form a heptamer ring with a single copy of each Sm protein in the snRNPs [14,15]. A 1:1 stoichiometry is also in best agreement with the three-dimensional structure of U1 snRNP derived from single-particle electron cryomicroscopy [16]. However, the stoichiometric analysis does not exclude the possibility that two kinds of U1 snRNP are present in equal amounts: one with two copies of the protein U1-C protein and the other with none. Nevertheless,
this possibility can be excluded, because the ion-exchange chromatography used here to purify U1 snRNP separates U1 snRNPs lacking protein U1-C from those containing it [21, and data not shown]. The method described here should therefore be well suited for the determination of the stoichiometric composition of a variety of snRNPs and (spliceosomal) multi-protein complexes.

Acknowledgement

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References


Figure Legends

Figure 1
Commassee stained 1D SDS gel of the U1 snRNP

Figure 2
Analytical strategy illustrated for a 2:1:1 complex

Figure 3
MALDI-TOF mass spectra used for the absolute quantification of spliceosomal proteins U1-70 K, SmE, D2, D1 and G of the U1 snRNP: The U1 spliceosomal snRNP was denatured, and digested completely with endoproteinase Lys-C, according to the chapter “Experimental”. The resulting peptides were labeled with $^{12}$C-propionyl-N-oxysuccinimide. Known amounts of synthetic peptides, labeled with the corresponding $^{13}$C isotope coded amine specific tag, were added (Reference *). MALDI-TOF mass spectrometry was used to absolutely quantify the proteins in the U1 spliceosomal snRNP.

Figure 4
MALDI-TOF mass spectra used for the absolute quantification of spliceosomal proteins SmG, U1-C, SmE and SmB of the U1 snRNP: The U1 spliceosomal snRNP was denatured, and digested completely with endoproteinase Lys-C (U1-C, SmG), trypsin (SmG, SmE) and endoproteinase Glu-C (SmB), according to the chapter “Experimental”. The resulting peptides were labeled with $^{12}$C-nicotinyl-N-oxysuccinimide. Known amounts of synthetic peptides, labelled with the corresponding $^{13}$C isotope coded tag were added (Reference *). MALDI-TOF mass
spectrometry was used to absolutely quantify the proteins in the U1 spliceosomal snRNP.
Table 1: This table summarizes the results of two independent experiments for the absolute quantification of the human U1 snRNP denatured, digested with endoproteinase Lys-C, Glu-C or trypsin and labeled with $^{12}$C propionylsuccinimide. Absolute quantification was achieved by the addition of synthetic peptides labeled with $^{13}$C propionyl-N-oxysuccinimide. Two peptides were used for the quantification of most proteins. Quantification was compromised for some peptides of the Sm proteins because of methylation of arginine residues (SmB, SmD3).

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Table 2  This table summarizes the results of two independent experiments for the absolute quantification of the human U1 snRNP denatured, digested with endoproteinase Lys-C, Glu-C, trypsin or Asp-N and labeled with $^{12}$C nicotinyl-N-hydroxysuccinimide. Absolute quantification was achieved by the addition of synthetic peptides labeled with $^{13}$C nicotinyl-N-oxysuccinimide. Two peptides were used for the quantification of most proteins. Quantification was compromised for some peptides of the Sm proteins because of methylation of arginine residues (SmB, SmD3).

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<tr>
<td>U1-C</td>
<td>TTAIFQQQK (53-61)</td>
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<td>1.59</td>
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<td>U1-A</td>
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<td>SQETPATK (115-122)</td>
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<td>SmB</td>
<td>KRVLGLVLLRGE (64-75)</td>
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<td>VLGLVLLR (66-73)</td>
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<td>VPLAAGGPGIGR (95-108)</td>
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<td>DRYISK (93-98)</td>
<td>1.88</td>
<td>1.86</td>
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<td>NNTQVLINCRNNK (38-50)</td>
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<td>DSVIVVLRNLPIAGK (104-118)</td>
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<td>NQGSGAGRGK (90-99)</td>
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<td>VAQLEQVYIR (55-64)</td>
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<td>SmE</td>
<td>QLGRIMLK (73-80)</td>
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<td></td>
<td>VMQPINLFR (13-23)</td>
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<td>1.48</td>
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<td></td>
<td>HVQGILR (26-32)</td>
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<td>1.46</td>
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</table>
2:1:1 Complex

Selection of peptides from database

Weighing for exact mass determination

Labeling of free amines with a isotope coded tag (C13)

Cleave proteins into peptides

2:1:1 Molar Ratio of Peptides

Labeling of free amines with a isotope coded tag (C12)

Combination

Δm

m/z

1D Separation and MALDI-TOF/TOF mass spectrometry

Ratio of absolute quantity of peptides = Stoichiometry of proteins in the complex

Stoichiometry determination

Figure 2
Figure 3

Mass (m/z)

U1-70K

*Reference: 1.25 pmol
Spliceosome: 1.46 pmol
Endoproteinase Lys-C

SmE

*Reference: 1.27 pmol
Spliceosome: 1.50 pmol
Endoproteinase Lys-C

SmD2

*Reference: 1.52 pmol
Spliceosome: 1.46 pmol
Endoproteinase Lys-C

SmD1

*Reference: 0.91 pmol
Spliceosome: 1.57 pmol
Endoproteinase Lys-C

SmG

*Reference: 1.09 pmol
Spliceosome: 1.60 pmol
Endoproteinase Lys-C
Figure 4
Protein stoichiometry of a multi-protein complex, the human spliceosomal U1 snRNP: Absolute quantification using isotope-coded tags and mass spectrometry
Elisabeth O. Hochleitner, Berthold Kastner, Thomas Fröhlich, Alexander Schmidt, Reinhard Lührmann, Georg Arnold and Friedrich Lottspeich

J. Biol. Chem. published online November 3, 2004

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