

## Supplementary Material For:

# Improved methodology for the affinity isolation of human protein complexes expressed at near endogenous levels

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## Procedure

**For the preparation and PMF analysis by MALDI-TOF MS of protein bands cut from SDS-PAGE gels containing RRP6-3xFLAG, RRP41-3xFLAG, and RBM7-LAP purified samples:**

**Procedure based upon:** <http://prowl.rockefeller.edu/protocols/in-gel-digestion.html>

(here modified by J. LaCava)

## Reagents

**Trypsin** – Trypsin Gold, Mass Spectrometry Grade, 100 µg, #V5280 (Promega, Madison, WI). Prepare a 100 µg/ml stock solution: dissolve contents of 1 vial (100 µg) of trypsin in 1 ml 1M HCl. Store as aliquots at -80°C. The working solution is 12.5 ng/µl – 1 part 100 µg/ml trypsin in 1 mM HCl, 4 parts 100 mM NH<sub>4</sub>CO<sub>3</sub> and 3 parts water (prepare just before use and keep on ice)

**4-HCCA matrix** – <http://prowl.rockefeller.edu/protocols/saturated-4HCCA-solution.html>

**RPC desalting pipette tips** – Omix 10 µl C18 tips - cat. #A5700310 (Agilent Technologies, Santa Clara, CA)

Prior to loading on the gel, samples were alkylated with iodoacetamide to block Cys residues from forming adducts. After staining and de-staining of the gel, bands were cut as thin slices from the gel, cubed, placed in a ~1.5 ml tube and fully destained with several washes of 50% v/v acetonitrile (ACN) in 50mM ammonium bicarbonate (AmBic) at 37°C with shaking. Destained gel pieces were dehydrated by washing with 100 µl ACN, and placed in a speed-vac for ~10' at RT. 15 µl trypsin working solution

were added to each tube and gel pieces were allowed to swell 45 min on ice. 5 µl additional 50mM AmBic was added to each, and all tubes incubated at 37°C for at least 6 hr to undergo tryptic proteolysis. 5 µl of 2% w/v trifluoroacetic acid (TFA) was added to each tube, and incubated 5 min at RT. The supernatant was recovered and transferred to a 0.5 ml microfuge tube (tryptic digest supernatant) — held at RT. 30 µl 0.1% w/v TFA was added to the gel pieces, which were extracted a further 45 min at RT with shaking. This supernatant removed and pooled with the appropriate tryptic digest supernatant. Pooled extracted peptides were desalted using RPC tips as per the manufacturers instructions. Peptide samples were directly eluted from the RPC tip in 4 µl 4-HCCA matrix solution. All 4 µl of elution were spotted on the MALDI target. Each spot was briefly washed with 4 µl ice cold 0.1% TFA, which was subsequently rapidly aspirated with vacuum.

## Data were collected on a PROTOF MALDI mass spectrometer with the following settings:

Laser shots 2400  
Laser energy 55%  
Declustering 30V  
Total time 2min 4sec  
Laser rate 20 Hz  
Cooling flow 190 ml/min

## Monoisotopic centroid masses calculated from these data using the software M/Z (Genomic Solutions) with the following settings:

\* centroid: 25  
\* s/n: 2.0  
\* resolution: 15000

\* smoothing: 5

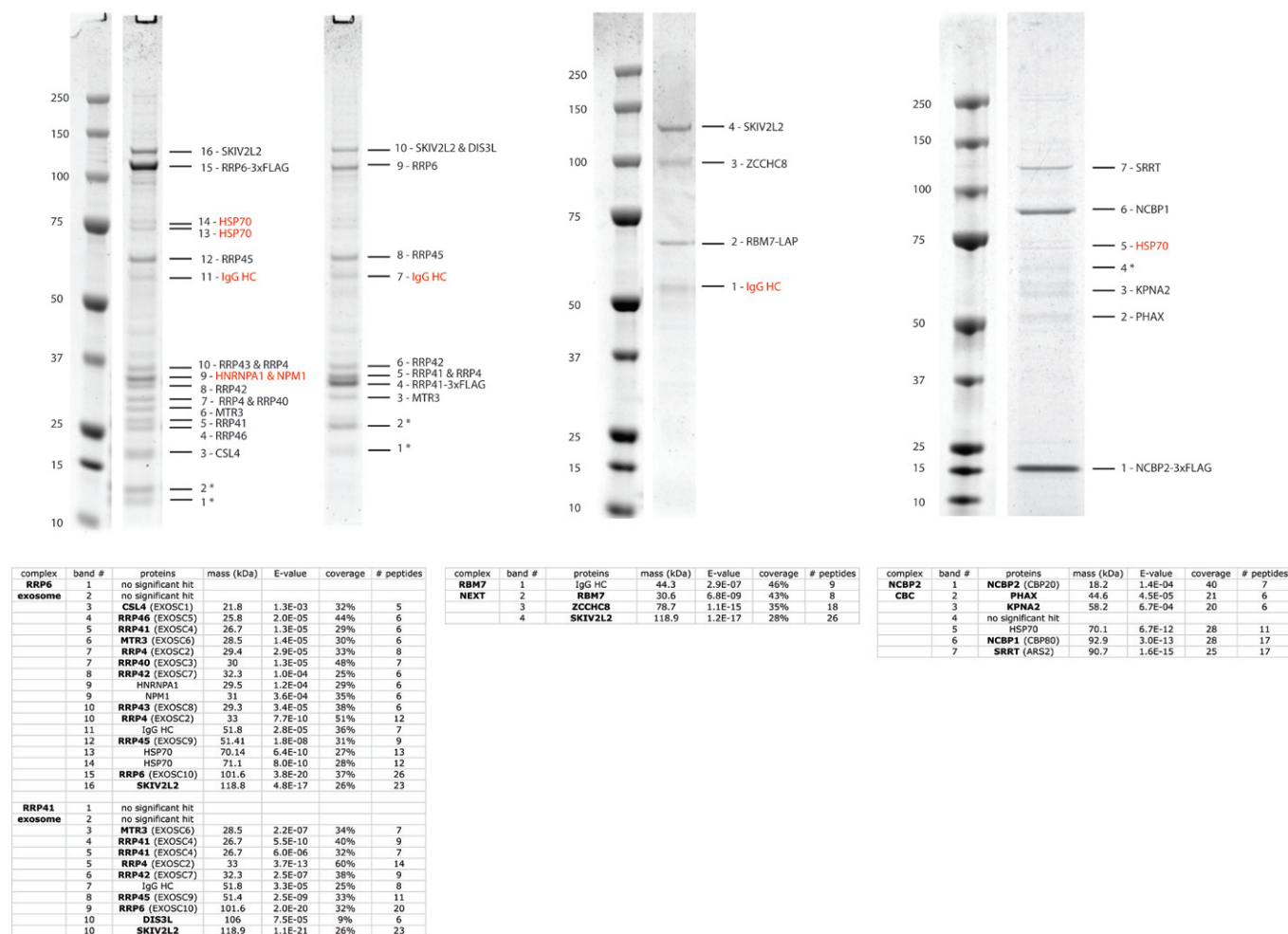
\* ABC filter: 100 (width), 5 (factor)

The raw data are provided as .massml files. These mass spectra were externally calibrated on two known masses (e.g. trypsin self-cleavage: 1045.564 Da, 2211.105 Da). Calibrated masses were filtered in PeakEraser (Eraser1.lst) at 50 ppm precision to remove common contaminants peaks, trypsin autocleavage products, matrix clusters and LAP-tag tryptic fragments (3xFLAG-tag fragments were below the m/z cut-off of data collected at the instrument). The remaining masses were searched in:

## ProFound with the following settings:

\* taxonomy: Homo Sapiens  
\* missed cleavages: 0  
\* modifications: iodoacetamide  
\* partial modifications: methionine  
\* tolerance unit: ppm  
\* mass tolerance (monoisotopic): 10  
\* charge state: MH+

All other settings were left as default. Protein IDs of expectation value lower than E-02 with at least 4 unique peptide sequence matches were taken as significant, and indicative of the presence of that protein in the excised band. When two significant results for proteins known to be stably associated with the complex under study were found in a search on a given mass list, the remaining masses were re-searched, respectively, to give the individual peptide mass finger print results. Bands identified as IgG HC (\*) were searched as above, but using the appropriate organism database (Mus Musculus or Other Mammalia for mouse and llama antibodies, respectively). The output of ProFound searches are included at .html files.



**MS supplement figure:** See MS preparation supplement for details of sample work up and analysis. All bands cut and analyzed are indicated. Significant results are presented next to each band, an \* indicates no significant result was obtained. Common and presumed contaminants colored in red. The results are summarized with added details in the table below each figure. The original data and search results are located in the folders named for each tagged, affinity isolated protein.

### For the preparation and PMF analysis by MALDI-TOF MS of protein bands cut from an SDS-PAGE gel containing a NCBP2-3xFLAG purified sample:

Procedure based upon: <http://prowl.rockefeller.edu/protocols/in-gel-digestion.html> (here modified by K. Molloy)

### Reagents

**Trypsin** – Sequencing Grade Modified Trypsin, 20 µg, #V5111 (Promega, Madison, WI). Prepare a 1 µg/µl stock solution: dissolve contents of 1 vial (20 µg) of trypsin in 20 µl 50mM acetic acid. Store as aliquots at -80°C. The working solution is 3.125 ng/µl – 1 part 1 µg/µl trypsin in 50mM acetic acid, 319 parts 25 mM NH<sub>4</sub>CO<sub>3</sub> (prepare just before use and keep on ice)

**4-HCCA matrix** – <http://prowl.rockefeller.edu/protocols/saturated-4HC->

CA-solution.html

**RPC desalting pipette tips** – 10 µl Millipore C18 Zip Tips

**POROS bead** – POROS 20 R2 (Applied Biosystems), 50 µg/µl slurry in 20% ethanol Dry stock POROS beads are washed in equal volumes of (1) methanol, then (2) 80% acetonitrile, then (3) 20% ethanol and then (4) resuspended in 20% ethanol at 50 µg/µl and stored at 4°C.

Prior to loading on the gel, samples were alkylated with iodoacetamide to block Cys residues from forming adducts. After staining and de-staining of the gel, bands were cut as thin slices from the gel, cubed, placed in a ~0.65 ml tube and destained with 50% v/v acetonitrile (ACN) in 25mM ammonium bicarbonate (AmBic) at 4°C with shaking. Aspirate the destaining

solution and replace with water and wash for 3 days. Aspirate the water and replace with 200 µl acetonitrile to dehydrate the gel pieces. Shake for 10 minutes. Aspirate the liquid. Air-dry. 10 µl trypsin working solution were added to each tube. 30 µl additional 25mM AmBic was added to each, and all tubes incubated at 37°C for 18 hr to undergo tryptic proteolysis. 40 µl POROS bead slurry in 5% formic acid, 0.2% trifluoroacetic acid was added to the gel pieces, which were extracted 24 hours at 4°C with shaking. Peptides were desalted using RPC tips. Tips washed with i) 40 µl 0.1% trifluoroacetic acid, ii) 40 µl 4-HCCA elution solution, and iii) 3 x 40 µl 0.1% trifluoroacetic acid. Transfer the 80 µl digest and bead mixture to the cleaned RPC tips and remove the liquid on a vacuum manifold. Wash gel pieces in microcentrifuge vial with 40 µl 0.1% trifluoroacetic acid, transfer wash

onto RPC tip and expel the wash solution as above. Thoroughly wash beads 3x with 80  $\mu$ l 0.1% trifluoroacetic acid. Expel wash solution as above. Peptide samples were directly eluted from the RPC tip in 3  $\mu$ l 4-HCCA matrix solution. All 3  $\mu$ l of elution were spotted on the MALDI target. Each spot was briefly washed with 10  $\mu$ l ice cold 0.1% TFA, which was subsequently rapidly aspirated with vacuum.

**Data were collected on a prOTOF MALDI mass spectrometer with the following settings:**

Laser shots 2400  
Laser energy 55%  
Declustering 30V  
Total time 2min 4sec  
Laser rate 20 Hz  
Cooling flow 190 ml/min

**Monoisotopic centroid masses calculated from these data using the software M/Z (Genomic Solutions) with the following settings:**

\* centroid: 7  
\* s/n: 1.8  
\* resolution: 12000  
\* smoothing: 5  
\* calibration on the 2211.105 m/z trypsin auto-digestion peak

Processed, calibrated, labeled data are provided as .massml files, and were further filtered in PeakErazor (Erazor2.lst) at 10 ppm precision to remove common contaminants. The remaining masses were searched in ProFound with the following settings:

\* taxonomy: Homo Sapiens  
\* Protein Mass: 0 – 600 kDa  
\* missed cleavages: 1  
\* modifications: iodoacetamide  
\* partial modifications: methionine  
\* tolerance unit: ppm  
\* mass tolerance (monoisotopic): 10  
\* charge state: MH+

The same criteria as described above were used to evaluate significant hits and the output of ProFound searches are included at .html files.

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