SUPPORTING INFORMATION

Functional expression and characterization of human myristoylated-Arf1 in nanodisc membrane mimetics

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EXPERIMENTAL PROCEDURES

S1. Production of the isotope labeled myr-Arf1

^{15}N and ^{13}C-methyl Isoleucine, Leucine, and Valine (ILV) labeled myr-Arf1: Cells were grown in M9 media supplemented with ^{15}N NH_4Cl and two ^{13}C-labeled keto acid precursors for Ile, Leu/Val [1]) (Sigma-Aldrich Cat # 571342 and 589063) were added to the cell culture 30 min before induction. The rest of the protocol was the same as for preparation of unlabeled myr-Arf1. The yields of unlabeled and labeled myr-Arf1 were equivalent.

S2. Disruption of the cells.

The frozen cells were thawed and resuspended in lysis buffer (Tris 20 mM, NaCl 150 mM, $MgSO_4$ 1 mM, DTT 1 mM, Imidazole 20 mM, pH8.0 (T20N150M1D1I20)). cOmplete™ EDTA-free cocktail protease inhibitor (Sigma-Aldrich, Ref # **1183617000**) was added prior to disruption. The cell suspension was passed through a Microfluidics model 110S microfluidizer at 4°C for 20 times to break the cells. Soluble and insoluble parts of the cells were separated by centrifugation at 48000 RCF for 30 min. Both the supernatant and pellets were used separately in the following purification procedure. Unlabeled and isotopically labeled samples shared the same protocol.

S3. Production of L8K-Arf1.

The plasmid containing the human L8K-Arf1 mutant sequence was a kind gift from Dr. Paul Randazzo. The vector was transformed into the *E. coli* competent cell line BL21 (star). The positive transformants were selected on the M9 agar plates containing carbenicillin.

For the production of the ^{15}N, ILV methyl labeled L8K-Arf1, BL21(star) cells with the L8K-Arf1 vector were cultured in ^{15}N NH_4Cl containing M9 media at 37C until OD_{600} = 1.0. Two ^{13}C labeled Ile, Leu/Val keto acid precursors (Sigma-Aldrich Cat # 571342 and 589063) were added to the cell culture 30 min before induction. After 30 min of growth, 1 mM IPTG was added to induce the protein expression and the cells were cultivated for another 4 h at 37C. The cells were pelleted by centrifugation at 7000 RCF for 30 min, and the pellets were collected and frozen at -80C. The samples used for backbone NH and ILV methyl assignments were produced by following the same protocol except that U^{13}C6-D7 Glucose (Cambridge Isotope Laboratory Cat # CDLM-3813) was used in the M9 media and the ILV keto acid precursors were substituted to Sigma-Aldrich Cat # 607541 and 596418.

Disruptions of the L8K-Arf1 frozen cells use the same protocol as myr-Arf1 except that the lysis buffer was substituted to T20N100M1D1, pH 8.0. Only the supernatant of the cell lysis was used in the following process: The supernatant was loaded on two 5ml HiTrap Q HP columns, connected in series, with the running buffer T20N100M1D1, pH 8.0. The flow through was collected and treated (on ice) with $(NH_4)_2SO_4$ up to a concentration of W/W 35%. The supernatant was collected after centrifugation at 48000 RCF for 30 min. Additional $(NH_4)_2SO_4$ was added on ice to a concentration of W/W 70%. After centrifugation at 48000 RCF for 30 min, the insoluble part was collected and resuspended in buffer T20N3000M1D1, pH 8.0. The sample was then purified using two series connected 5 ml Phenyl FF (HB) hydrophobic interaction columns. The desired protein was separated by gradient elution using elution buffer (T20N100M1D1, pH 8.0) from 0% to 100% in 10 column volumes. The eluted component was pooled and concentrated to 2ml volume. The sample then went through a Superdex S75 16/60 size exclusion column (running buffer T20N150M0.5D1, pH7.4) for further purification.

S4. L8K-Arf1 Nucleotide exchange.

Conversion of L8K-Arf1 from GDP to GTPγS bound forms was done in the presence of 2mM EDTA and excess of GTPγS (GTPγS:L8K-Arf1 = 100:1) was added to the L8K-Arf1 sample. This process was conducted in an Amicon centrifugal device (10ml volume) with 10 kDa MWCO (Millipore). The mixture stirred at room temperature for 1 hour and buffer exchange to fresh buffer with EDTA and GTPγS. This process was repeated for 3 times and, subsequently, buffer exchanged to T20N150M0.5D1 pH 7.4.

S5. NMR sample conditions.

The GDP-bound myr-Arf1, GDP-bound Arf1 and ND-anchored GTPγS-bound myr-Arf1 (myr-Arf1ND) were buffered exchanged into 20 mM Tris, 150 mM NaCl, pH7.4 (T20N150, pH7.4) using Zeba™ 0.5ml spin desalting column (ThermoFisher, Cat # 89882). The myr-Arf1 and myr-Arf1ND samples were concentrated to final concentrations of 200 μM using Amicon ultrafiltration units with 10k cutoff membrane (Millipore). The concentration of free myr-Arf1 was measured by UV-spectroscopy at 280 nm using extinction coefficient 29450 $M^{-1}cm^{-1}$ determined by ProtParam [2]. The concentration of empty nanodisc was determined using UV absorption and an extinction coefficient of 18450 $M^{-1}\cdot cm^{-1}$. The concentration of anchored myr-Arf1 and the molar ratio between myr-Arf1 and nanodisc were measured using PAGE and scanning densitometry and a BSA standard curve [3]. Using a known concentration of MSPΔH5 in a reference lane, the concentration of ND and myr-Arf1 in the sample lane could be estimated from the densitometry. A Typhoon FLA 7000 imager was used to scan the Coomassie blue stained SDS-PAGE, and ImageQuant TL software was used to analyze the band densitometry. The GDP-bound and GTPγS-bound L8K-Arf1 were also buffer exchanged to the same NMR buffer and concentrated to 200 μM.

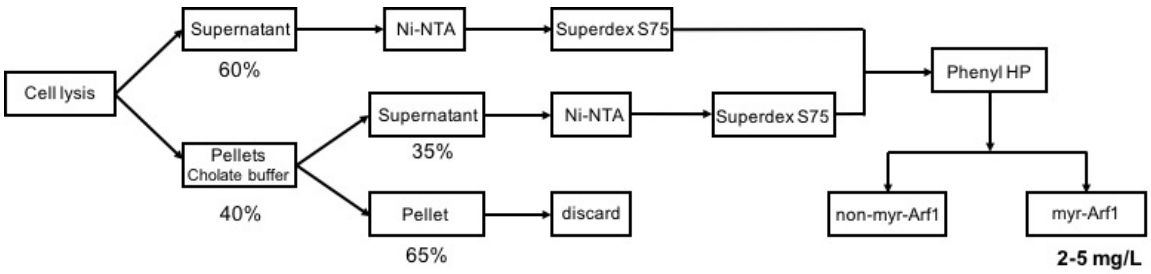
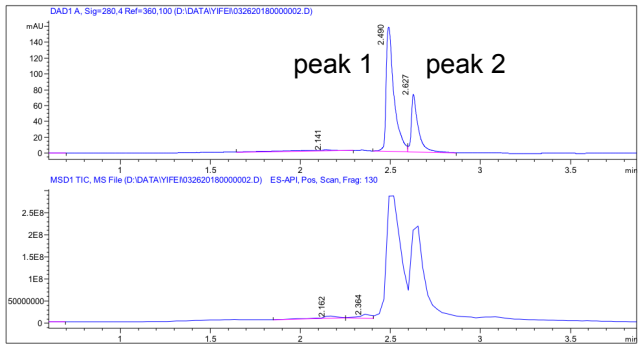


Chart S1: The schematically display of the purification of Arf1 and myr-Arf1. The percentages shown refer to the amount of material from the previous step. The net yield of myr-Arf1-HIS per Liter culture is shown as the average amount from many preparations.

Figure S1.

A



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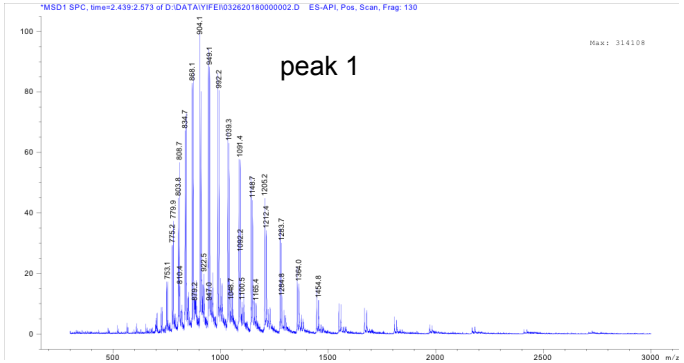

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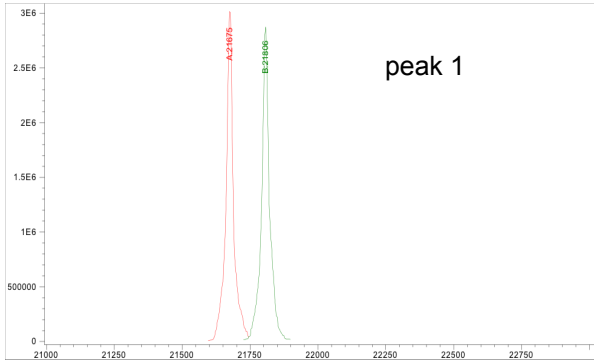

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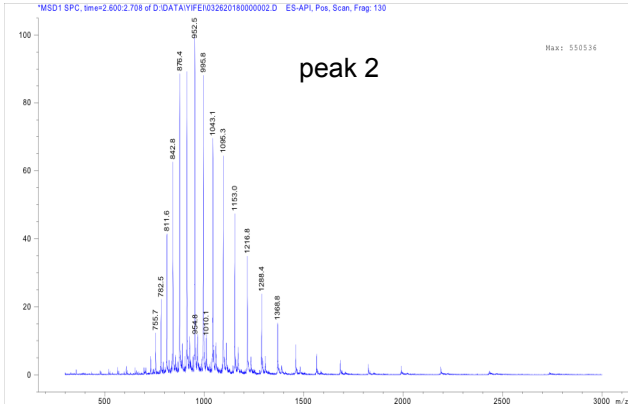

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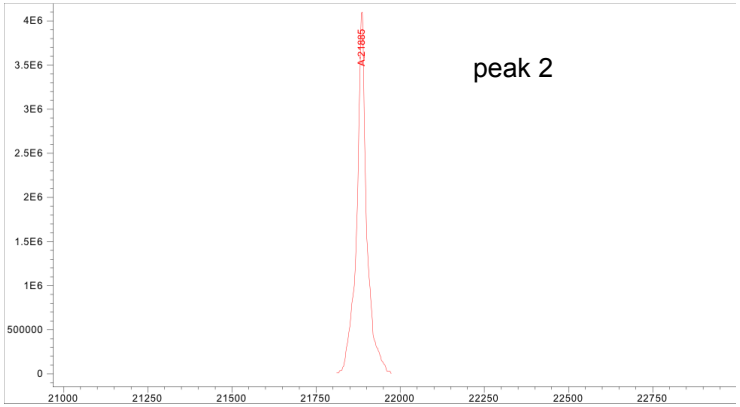
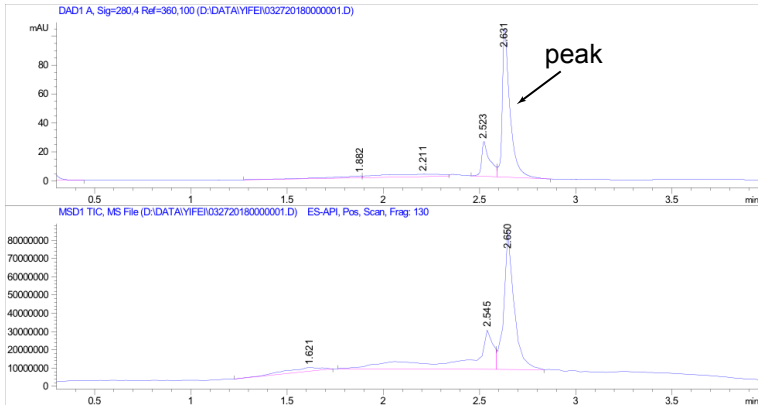


Mass spectra collected on an LC-MS for the elutant from the Ni-NTA column. (A) UV (upper) and total mass (lower) traces of the LC/MS. (B) Raw mass spectral m/z data for the peak 1 identified in (A). (C) Deconvolution of panel B revealing two components with masses of 21675 Da and 21806 Da. (D) Raw mass spectral m/z data for the peak 2 identified in (A). (E) Deconvolution of panel (D) yielding the mass of 21885 Da.

Figure S2.

A



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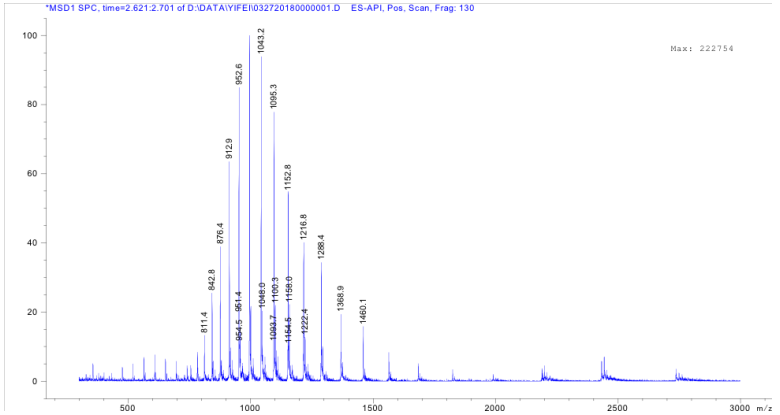

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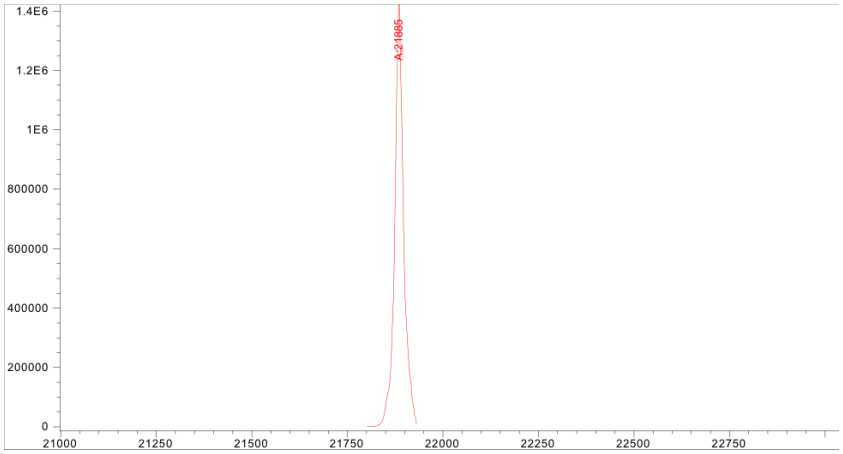

Mass spectra collected on an LC-MS for the elutant from the Phenyl HP column. (A) UV (upper) and total mass (lower) traces of the LC/MS. (B) Raw mass spectral m/z data for the peak identified in (A). (C) Deconvolution of panel B yielding the mass of 21885 Da.

REFERENCES

[1] Tugarinov, V., Kanelis, V., and Kay, L. E. (2006) Isotope labeling strategies for the study of high-molecular-weight proteins by solution NMR spectroscopy, *Nature protocols 1*, 749-754.
[2] Wilkins, M. R., Gasteiger, E., Bairoch, A., Sanchez, J. C., Williams, K. L., Appel, R. D., and Hochstrasser, D. F. (1999) Protein identification and analysis tools in the ExPASy server, *Methods in molecular biology 112*, 531-552.
[3] Vincent, S. G., Cunningham, P. R., Stephens, N. L., Halayko, A. J., and Fisher, J. T. (1997) Quantitative densitometry of proteins stained with coomassie blue using a Hewlett Packard scanjet scanner and Scanplot software, *Electrophoresis 18*, 67-71.