Supporting Information for:

Multiple Enzymatic Digestions and Ion Mobility Separation Improve Quantification of Bacterial Ribosomal Proteins by LC-MS^E

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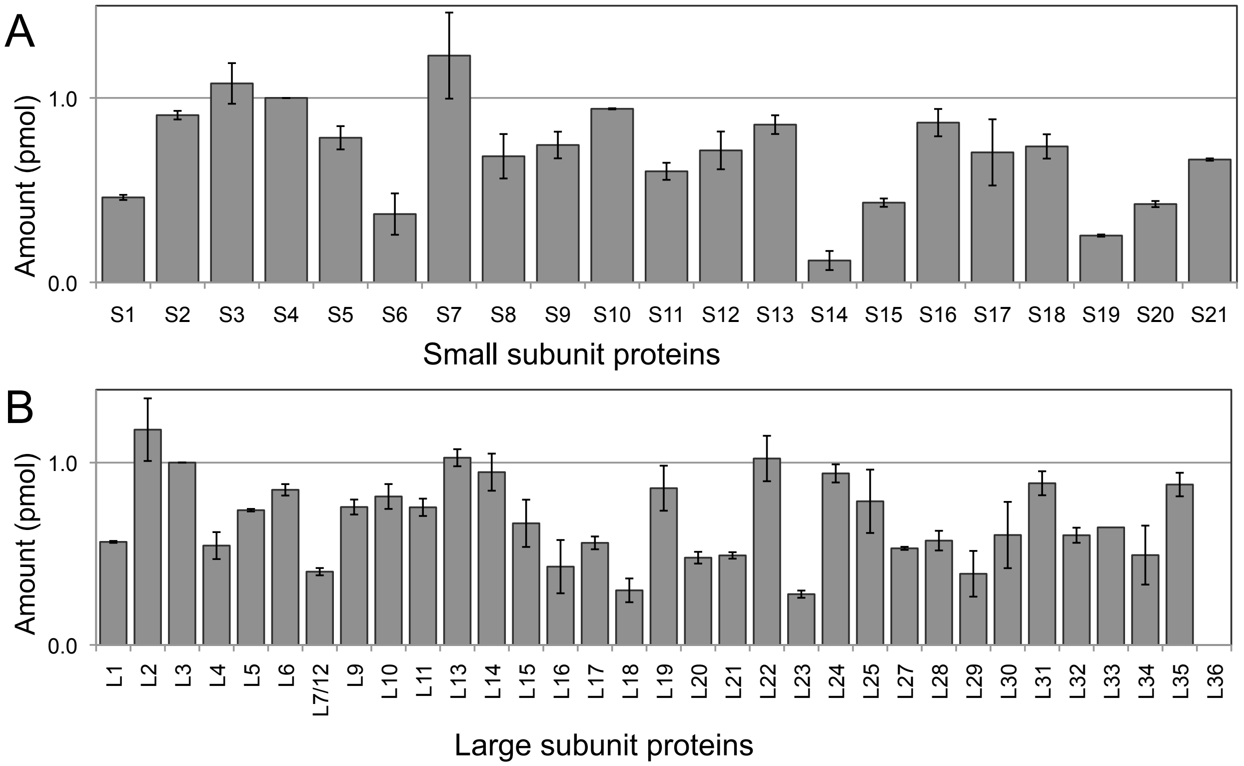
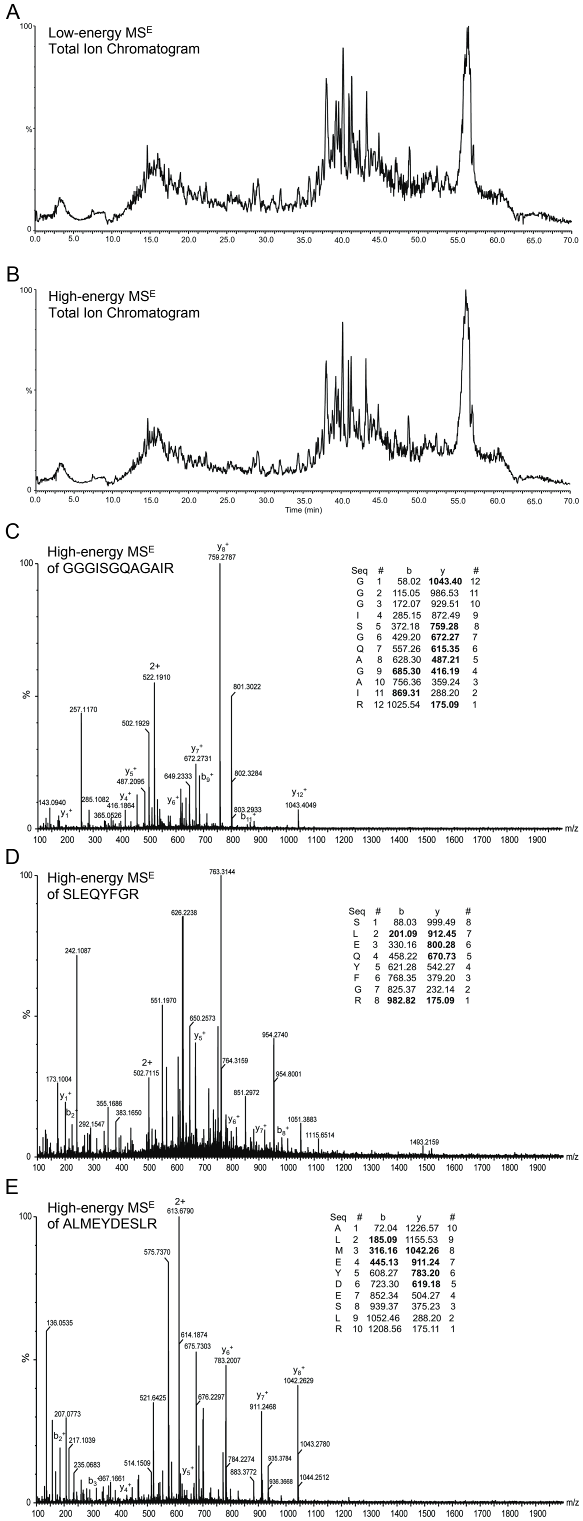
Supplemental Figures S1-S2

Supplemental Tables S1-S4



Supplemental Figure S1. Quantification of small subunit 30S proteins (A) and large subunit 50S proteins (B) by LC-MS^E with additional dimension of gas-phase separation through ion-mobility. Although quantification of proteins is improved, a non-uniform distribution of proteins for both subunits is still observed. Amounts of small subunit proteins and large subunit proteins are normalized to protein S4 and protein L3, respectively. Error bars represent the standard deviation of 3 multiple measurements.



Seq	#	b	y	#
G	1	58.02	**1043.40**	12
G	2	115.05	986.53	11
G	3	172.07	929.51	10
I	4	285.15	872.49	9
S	5	372.18	**759.28**	8
G	6	429.20	**672.27**	7
Q	7	557.26	**615.35**	6
A	8	628.30	**487.21**	5
G	9	**685.30**	**416.19**	4
A	10	756.36	359.24	3
I	11	**869.31**	288.20	2
R	12	1025.54	**175.09**	1

Seq	#	b	y	#
S	1	88.03	999.49	8
L	2	**201.09**	**912.45**	7
E	3	330.16	**800.28**	6
Q	4	458.22	**670.73**	5
Y	5	621.28	542.27	4
F	6	768.35	379.20	3
G	7	825.37	232.14	2
R	8	**982.82**	**175.09**	1

Seq	#	b	y	#
A	1	72.04	1226.57	10
L	2	**185.09**	1155.53	9
M	3	**316.16**	**1042.26**	8
E	4	**445.13**	**911.24**	7
Y	5	608.27	**783.20**	6
D	6	723.30	**619.18**	5
E	7	852.34	504.27	4
S	8	939.37	375.23	3
L	9	1052.46	288.20	2
R	10	1208.56	175.11	1

Supplemental Figure S2. (A) Representative low energy CID total ion chromatogram (TIC) and (B) high energy CID TIC of multiple enzymatic digests of *E. coli* polysome proteins showing preservation of the chromatographic profile during alternate low energy CID and high energy CID scanning mode in LC-MS^E data acquisition. (C) High-energy mass spectrum of GGGISGQAGAIR peptide (2+, *m/z* 522.19). (D) High-energy mass spectrum of SLEQYFGR peptide (2+, *m/z* 502.71). (E) High-energy mass spectrum of ALMEYDESLR peptide (2+, *m/z* 613.67).

Supplemental Table S1 Run-to-run reproducibility of the stoichiometric measurements of small 30S subunit proteins in wild-type polysomes (POLY).

r-protein	POLY 1 001	POLY 1 002	POLY 1 003	AVG	STDEV	%CV
S1	0.76	0.88	0.73	0.79	0.08	9.67
S2	0.92	0.89	0.85	0.89	0.03	3.94
S3	1.47	1.30	1.43	1.40	0.09	6.19
S4	1.00	1.00	1.00	1.00	0.00	0.00
S5	0.85	0.77	0.73	0.79	0.06	7.74
S6	-	0.99	1.03	1.01	0.03	3.34
S7	1.09	1.06	1.20	1.12	0.08	6.75
S8	0.71	0.66	0.68	0.68	0.03	3.69
S9	-	0.83	0.80	0.81	0.03	3.20
S10	0.99	0.98	1.00	0.99	0.01	1.13
S11	0.69	0.63	0.67	0.67	0.03	4.52
S12	0.72	0.73	0.77	0.74	0.03	3.53
S13	1.13	0.87	1.02	1.01	0.13	13.1
S14	0.96	0.77	0.82	0.85	0.10	11.5
S15	0.89	-	0.91	0.90	0.01	0.87
S16	1.07	0.95	1.19	1.07	0.12	11.1
S17	0.95	0.95	0.96	0.95	0.01	0.68
S18	0.51	0.49	0.48	0.49	0.02	3.11
S19	1.02	1.03	1.03	1.03	0.01	0.67
S20	0.86	0.87	0.91	0.88	0.03	3.27
S21	0.42	0.38	0.42	0.41	0.02	5.61

Supplemental Table S2. Run-to-run reproducibility of the stoichiometric measurements of large 50S subunit proteins in wild-type polysomes (POLY).

r-protein	POLY 1 001	POLY 1 002	POLY 1 003	AVG	STDEV	%CV
L1	0.72	0.76	0.72	0.74	0.02	3.38
L2	1.05	1.12	1.01	1.06	0.06	5.34
L3	1.00	1.00	1.00	1.00	0.00	0.00
L4	0.73	0.84	0.85	0.81	0.07	8.18
L5	1.05	1.01	1.08	1.05	0.03	3.28
L6	0.87	0.82	0.78	0.82	0.04	5.35
L7/12	1.74	1.89	1.91	1.85	0.09	4.97
L9	0.67	0.76	0.76	0.73	0.05	7.38
L10	0.91	0.89	0.82	0.87	0.05	5.50
L11	0.73	0.76	0.76	0.75	0.02	2.91
L13	0.99	1.08	-	1.04	0.06	5.58
L14	0.84	1.00	0.85	0.89	0.09	10.3
L15	0.87	0.92	0.87	0.88	0.03	3.13
L16	1.02	1.12	0.89	1.01	0.12	11.6
L17	0.95	0.92	0.83	0.90	0.06	6.53
L18	0.97	0.93	0.87	0.92	0.05	5.44
L19	0.89	0.99	0.99	0.96	0.06	5.85
L20	0.66	0.80	0.65	0.70	0.08	11.4
L21	0.96	1.00	0.90	0.95	0.05	5.41
L22	0.81	0.82	0.76	0.79	0.03	3.90
L23	0.79	0.80	0.71	0.76	0.05	6.64
L24	1.09	1.16	1.01	1.09	0.08	6.97
L25	0.68	0.68	0.60	0.65	0.04	6.71
L27	0.78	0.81	0.61	0.73	0.11	14.7
L28	0.65	0.58	0.61	0.62	0.04	5.97
L29	0.80	0.76	0.70	0.75	0.05	6.19
L30	0.46	-	0.55	0.51	0.06	12.5
L31	0.96	1.01	0.84	0.94	0.09	9.35
L32	0.82	0.84	0.80	0.82	0.02	2.64
L33	1.10	1.15	1.00	1.08	0.07	6.70
L34	0.00	0.00	0.00	0.00	0.00	-
L35	0.32	0.29	0.30	0.30	0.02	5.07
L36		0.27	0.29	0.28	0.02	5.89

Supplemental Table S3. Biological reproducibility of the stoichiometric measurements of small 30S subunit proteins in wild-type polysomes (POLY).

r-protein	POLY 1	POLY 2	AVG	STDEV	%CV
S1	0.50	0.79	0.64	0.21	32.0
S2	0.83	0.89	0.86	0.04	4.71
S3	1.28	1.40	1.34	0.09	6.35
S4	1.00	1.00	1.00	0.00	0.00
S5	0.82	0.79	0.80	0.02	2.96
S6	0.98	1.01	1.00	0.02	1.95
S7	1.32	1.12	1.22	0.15	12.0
S8	0.96	0.68	0.82	0.20	23.9
S9	1.03	0.81	0.92	0.15	16.5
S10	0.86	0.99	0.93	0.09	10.1
S11	0.89	0.67	0.78	0.16	20.1
S12	0.79	0.74	0.77	0.04	4.83
S13	1.06	1.01	1.03	0.04	3.63
S14	1.07	0.85	0.96	0.16	16.5
S15	1.07	0.90	0.99	0.12	12.3
S16	1.23	1.07	1.15	0.11	9.90
S17	1.27	0.95	1.11	0.22	20.0
S18	0.82	0.49	0.66	0.23	35.3
S19	0.99	1.03	1.01	0.02	2.36
S20	0.88	0.88	0.88	0.00	0.12
S21	0.55	0.41	0.48	0.11	21.9

Supplemental Table S4. Biological reproducibility of the stoichiometric measurements of large 50S subunit proteins in wild-type polysomes (POLY).

r-protein	POLY 1	POLY 2	AVG	STDEV	%CV
L1	0.87	0.74	0.80	0.09	11.5
L2	1.01	1.06	1.03	0.04	3.58
L3	1.00	1.00	1.00	0.00	0.00
L4	0.84	0.81	0.82	0.02	2.48
L5	1.07	1.05	1.06	0.01	1.22
L6	0.78	0.82	0.80	0.03	3.85
L7/12	1.36	1.85	1.60	0.34	21.3
L9	0.78	0.73	0.75	0.04	5.06
L10	0.78	0.87	0.83	0.06	7.82
L11	0.73	0.75	0.74	0.01	1.79
L13	0.93	1.04	0.98	0.07	7.58
L14	0.72	0.89	0.81	0.12	15.2
L15	0.83	0.88	0.86	0.04	4.58
L16	0.78	1.01	0.90	0.16	17.9
L17	0.77	0.90	0.83	0.09	11.2
L18	0.76	0.92	0.84	0.12	13.8
L19	0.84	0.96	0.90	0.08	9.41
L20	0.59	0.70	0.65	0.08	11.9
L21	0.89	0.95	0.92	0.04	4.84
L22	0.69	0.79	0.74	0.08	10.4
L23	0.70	0.76	0.73	0.04	5.99
L24	0.76	1.09	0.92	0.23	24.5
L25	0.51	0.65	0.58	0.10	17.0
L27	0.62	0.73	0.68	0.08	11.5
L28	0.71	0.62	0.66	0.07	10.4
L29	0.78	0.75	0.77	0.02	2.28
L30	0.33	0.51	0.42	0.12	29.3
L31	0.93	0.94	0.93	0.01	0.69
L32	0.75	0.82	0.79	0.05	5.83
L33	0.80	1.08	0.94	0.20	21.4
L34	0.00	0.00	0.00	0.00	-
L35	0.35	0.30	0.33	0.03	10.7
L36	0.37	0.28	0.32	0.06	19.3