Supporting Information

Proteomic Evaluation of Plasma Membrane Fraction Prepared from a Mouse Liver and Kidney Using a Bead Homogenizer: Enrichment of Drug-Related Transporter Proteins

*Ryotaro Yagi[1], Takeshi Masuda[1,2,3], Seiryo Ogata[1], Ayano Mori[3], Shingo Ito[1,2,3], Sumio Ohtsuki[1,2,3]**

[1]Department of Pharmaceutical Microbiology, Graduate School of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Chuo-ku, Kumamoto 862-0973, Japan

[2]Department of Pharmaceutical Microbiology, Faculty of Life Sciences, Kumamoto University, 5-1 Oe-honmachi, Chuo-ku, Kumamoto 862-0973, Japan

[3]Department of Pharmaceutical Microbiology, School of Pharmacy, Kumamoto University, 5-1 Oe-honmachi, Chuo-ku, Kumamoto 862-0973, Japan

***Corresponding author: Sumio Ohtsuki, PhD.**

Department of Pharmaceutical Microbiology, Faculty of Life Sciences, Kumamoto University, 5-1 Oe-honmachi, Chuo-ku, Kumamoto 862-0973, Japan

TEL: +81-96-371-4323; FAX: +81-96-371-4329; E-mail: sohtsuki@kumamoto-u.ac.jp

Table S1. Amounts of Proteins in Subcellular Fractions from Mouse Liver and Kidney

tissue	homogenizer	fraction	protein amount (μg)	CV (%)
liver	Dounce	CM	217 ± 14	6.37
		PM	142 ± 74	52.0
	bead	CM	306 ± 2	0.672
		PM	143 ± 42	29.0
kidney	Dounce	CM	183 ± 12	6.50
		PM	117 ± 39	33.0
	bead	CM	192 ± 11	5.91
		PM	103 ± 24	23.0

Crude membrane (CM) and plasma membrane (PM) fractions were prepared from mouse liver (50 mg) and kidney (50 mg) using Dounce homogenizer, and from mouse liver (100 mg) and kidney (50 mg) using bead homogenizer. CM and PM fractions were prepared from 200 μL and 1,000 μL of tissue homogenate as described in the Materials and Methods. Values are shown as mean ± standard deviation (n = 3-6).

Supporting Information in Excel file

Table S2. Protein Concentrations in Crude and Plasma Membrane Fractions of Mouse Liver

Table S3. Protein Concentrations in Crude and Plasma Membrane Fractions of Mouse Kidney

Table S4. Comparison of Transporters' Protein Concentration (PM-to-CM Ratio < 2) in Kidney Plasma Membrane Fraction Obtained from Homogenates Prepared Using Bead and Dounce Homogenizers

	protein	bead-to-Dounce ratio
Abc transporter	Abce1	$1.34 \pm 0.20^{**}$
	Abcd3	1.45 ± 0.79
Slc transporter	Slc22a6 / Oat1	$1.91 \pm 0.69^{**}$
	Slc22a8 / Oat3	$1.76 \pm 0.66^{*}$
	Slc22a2 / Oct2	$2.47 \pm 0.92^{***}$
	Slc22a1 / Oct1	$2.07 \pm 0.89^{**}$
	Slc22a18	1.68 ± 0.92
	Na^{+}/K^{+}-ATPase $\alpha 1$	$2.02 \pm 0.68^{**}$

The ratio of each protein concentration in kidney plasma membrane fraction prepared with bead homogenizer to that with Dounce homogenizer is shown. Only transporters with PM-to-CM ratio < 2 in Table 4 are listed. Transporters with PM-to-CM ratio > 2 in Table 4 are shown in Table 5. Values are shown as mean ± standard deviation (n = 3–6). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$, significantly different from Dounce homogenizer.

Table S5. PM-to-CM Ratios of Metabolizing Enzymes in Mouse Liver

		PM-to-CM ratio of total peak area	
	protein	Dounce	bead
Cyps	Cyp3a13	0.663 ± 0.204**	0.281 ± 0.148$^{***, ##}$
	Cyp2f2	0.630 ± 0.178**	0.286 ± 0.143$^{***, ##}$
	Cyp2a12	0.642 ± 0.189***	0.289 ± 0.137$^{***, ##}$
	Cyp4a14	0.601 ± 0.178	0.292 ± 0.142$^{**, #}$
	Cyp2c70	0.657 ± 0.191*	0.301 ± 0.138$^{***, ##}$
	Cyp3a11	0.594 ± 0.238**	0.302 ± 0.137$^{***, #}$
	Cyp1a2	0.655 ± 0.198**	0.305 ± 0.137$^{***, ##}$
	Cyp4a12b	0.620 ± 0.192**	0.308 ± 0.144$^{***, #}$
	Cyp2c23	0.632 ± 0.180**	0.309 ± 0.136$^{***, ##}$
	Cyp2c67	0.663 ± 0.150**	0.310 ± 0.136$^{***, ##}$
	Cyp2e1	0.653 ± 0.203**	0.313 ± 0.139$^{***, #}$
	Cyp2c37	0.644 ± 0.184**	0.324 ± 0.144$^{***, #}$
	Cyp2c54	0.646 ± 0.191**	0.325 ± 0.127$^{***, #}$
	Cyp2c29	0.626 ± 0.202**	0.326 ± 0.139$^{***, #}$
	Cyp2c50	0.597 ± 0.158**	0.330 ± 0.128$^{***, #}$
	Cyp2c69	0.665 ± 0.195*	0.334 ± 0.154$^{***, #}$
	Cyp27a1	0.975 ± 0.278	0.334 ± 0.248$^{***, ##}$
	Cyp2a5	0.659 ± 0.196**	0.340 ± 0.132$^{**, #}$
	Cyp2d22	0.640 ± 0.177**	0.345 ± 0.145$^{***, #}$
	Cyp2c68	0.678 ± 0.221*	0.347 ± 0.211$^{***, #}$
	Cyp7b1	0.643 ± 0.189**	0.347 ± 0.120$^{**, #}$
	Cyp4f14	0.841 ± 0.306	0.349 ± 0.183$^{***, #}$
	Cyp2j5	0.638 ± 0.184***	0.350 ± 0.093$^{***, #}$
	Cyp4a10	0.626 ± 0.181*	0.356 ± 0.127$^{***, #}$
	Cyp4a12a	0.655 ± 0.190**	0.358 ± 0.136$^{***, #}$
	Cyp4v2	0.679 ± 0.211**	0.359 ± 0.129$^{***, #}$
	Cyp2d10	0.675 ± 0.170**	0.420 ± 0.106$^{***, #}$
	Cyp2d9	0.669 ± 0.165**	0.424 ± 0.091$^{***, #}$
	Cyp2d26	0.677 ± 0.161**	0.479 ± 0.110$^{***, #}$
	Cyp2d34	0.697 ± 0.210**	0.483 ± 0.100***

Continued on next page

Table S5. – PM-to-CM Ratios of Metabolizing Enzymes in Mouse Liver-Continue

		PM-to-CM ratio of total peak area	
	protein	Dounce	bead
Ugts	Ugt2a3	0.575 ± 0.187***	0.201 ± 0.131$^{***, ##}$
	Ugt1a1	0.605 ± 0.202***	0.222 ± 0.132$^{***, ##}$
	Ugt1a6b	0.612 ± 0.193***	0.223 ± 0.132$^{***, ##}$
	Ugt2b1	0.599 ± 0.215***	0.233 ± 0.114$^{***, ##}$
	Ugt2b36	0.651 ± 0.211**	0.243 ± 0.137$^{***, ##}$
	Ugt1a6	0.621 ± 0.177***	0.248 ± 0.148$^{***, ##}$
	Ugt2b17	0.653 ± 0.192**	0.250 ± 0.138$^{***, ##}$
	Ugt2b34	0.651 ± 0.213***	0.250 ± 0.126$^{***, ##}$
	Ugt1a9	0.571 ± 0.177***	0.255 ± 0.131$^{***, ##}$
	Ugt3a2	0.611 ± 0.194***	0.287 ± 0.143$^{***, #}$
Ces	Ces1d	0.550 ± 0.167***	0.292 ± 0.099$^{***, #}$
	Ces1	0.570 ± 0.177***	0.296 ± 0.090$^{***, #}$
	Ces3b	0.498 ± 0.152***	0.305 ± 0.092$^{***, #}$
	Ces3a	0.551 ± 0.149***	0.316 ± 0.090$^{***, #}$
	Ces1c	0.613 ± 0.201***	0.310 ± 0.091$^{***, #}$
	Ces1f	0.559 ± 0.164***	0.334 ± 0.103$^{***, #}$
	Ces1e	0.512 ± 0.155***	0.341 ± 0.089***
	Ces2c	0.596 ± 0.095***	0.346 ± 0.076$^{***, ##}$
	Ces2a	0.518 ± 0.137***	0.366 ± 0.078***
	Ces2e	0.554 ± 0.134***	0.372 ± 0.062$^{***, #}$
	Cox4i1	1.01 ± 0.31	0.323 ± 0.290$^{***, ##}$

Ratio of protein concentration in plasma membrane fraction (PM) to that in crude membrane fraction (CM) was calculated as PM-to-CM ratio. Each value represents the mean ± standard deviation (n = 5-6). $^{***}p < 0.001$, significantly different from CM. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$, significantly different from Dounce homogenizer.

Table S6. PM-to-CM Ratios of Metabolizing Enzymes in Mouse Kidney

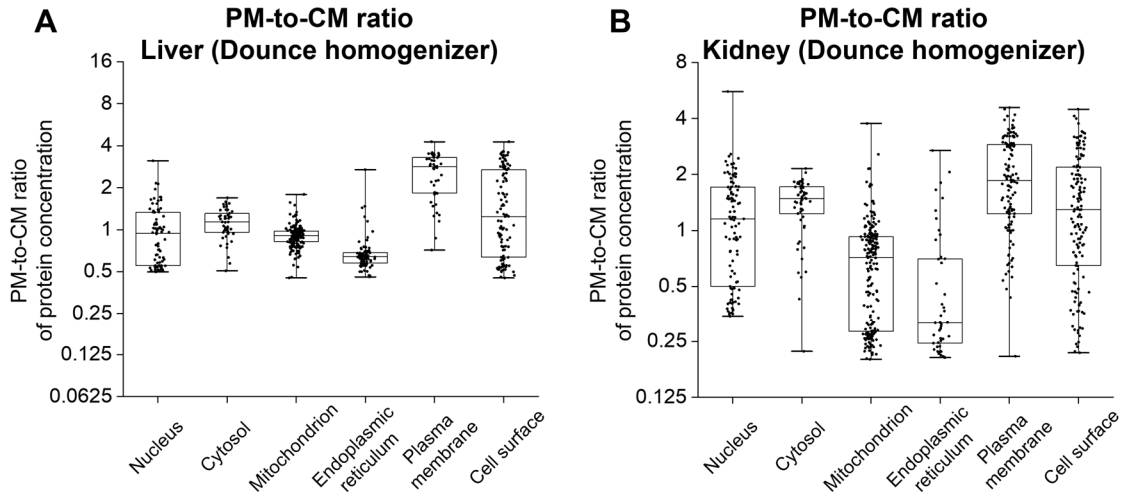
		PM-to-CM ratio of total peak area	
	protein	Dounce	bead
Cyps	Cyp2j5	0.253 ± 0.090***	0.256 ± 0.109***
	Cyp4a12a	0.278 ± 0.117***	0.257 ± 0.069***
	Cyp2e1	0.239 ± 0.087***	0.268 ± 0.096***
	Cyp4b1	0.213 ± 0.092***	0.273 ± 0.096***
	Cyp2a4	0.213 ± 0.093***	0.275 ± 0.093***
	Cyp51a1	0.268 ± 0.083***	0.323 ± 0.114**
Ugts	Ugt1a7c	0.218 ± 0.099***	0.229 ± 0.083***
	Ugt3a1	0.206 ± 0.093***	0.244 ± 0.090***
	Ugt3a2	0.218 ± 0.095***	0.261 ± 0.101***
	Ugt2b37	0.223 ± 0.102***	0.264 ± 0.104**
Ces	Ces1f	0.248 ± 0.075***	0.285 ± 0.060***
	Ces1d	0.331 ± 0.069***	0.323 ± 0.060***
	Ces2c	0.464 ± 0.118***	0.332 ± 0.055***
	Cox4i1	0.278 ± 0.141***	0.267 ± 0.095**

Ratio of protein concentration in plasma membrane fraction (PM) to that in crude membrane fraction (CM) was calculated as PM-to-CM ratio. Each value represents the mean ± standard deviation (n = 3-6). $^{**}p < 0.01$ and $^{***}p < 0.001$, significantly different from CM.

Table S7. Comparison of Bead-to-Dounce Ratios of Transporters with and without Normalization in Liver Plasma Membrane Fraction

protein	membrane location	bead-to-Dounce ratio	
		without normalization	with normalization
Abcb6	unknown	3.58 ± 1.33***	1.83 ± 0.29***
Abcb4 / Mdr2	canalicular	2.27 ± 0.76***	1.18 ± 0.15*
Abcc2 / Mrp2	canalicular	2.02 ± 0.74***	1.07 ± 0.19
Abcb11 / Bsep	canalicular	2.01 ± 0.73***	1.05 ± 0.19
Abcc3 / Mrp3	sinusoidal	1.99 ± 0.81**	1.03 ± 0.15
Abcg2 / Bcrp1	canalicular	1.98 ± 0.79**	1.04 ± 0.20
Abcc6 / Mrp6	sinusoidal	1.60 ± 0.67*	0.800 ± 0.081***
Slco2b1 / Oatp2b1	sinusoidal	3.18 ± 1.31***	1.63 ± 0.20***
Slco1a1 / Oatp1a1	sinusoidal	3.05 ± 1.49**	1.52 ± 0.29***
Slco1b2 / Oatp1b2	sinusoidal	2.58 ± 1.01**	1.27 ± 0.15**
Slc22a1 / Oct1	sinusoidal	2.21 ± 1.06**	1.13 ± 0.16
Na^+/K^+-ATPase α1	canalicular, sinusoidal	1.96 ± 0.74**	

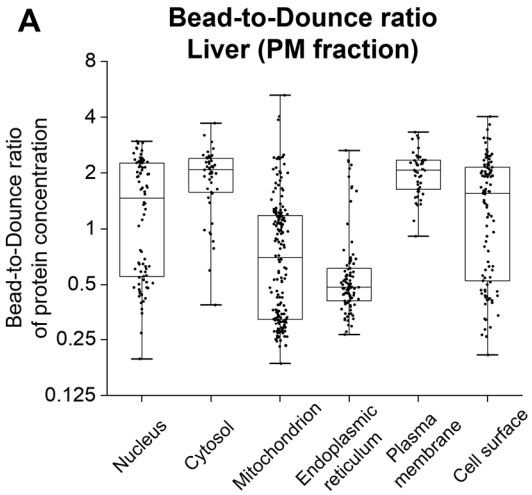
The ratio of each protein concentration in liver plasma membrane fraction prepared using bead homogenizer to that prepared using Dounce homogenizer (bead-to-Dounce ratio) with or without normalization by Na^+/K^+-ATPase α1. Only transporters with PM-to-CM ratios > 2 in Table 2 are listed. Each value represents the mean ± standard deviation (n = 5-6). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$, significantly different from Dounce homogenizer.



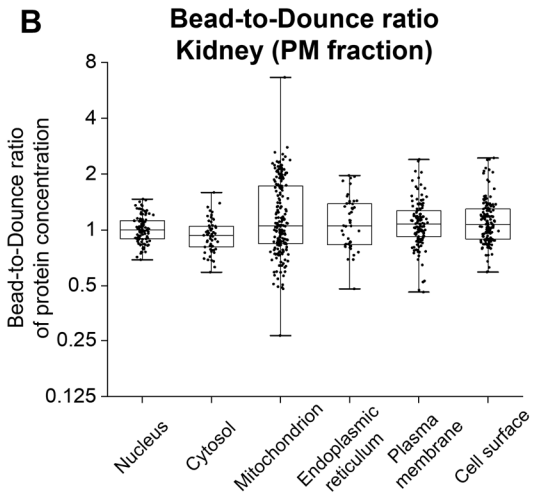
	Nucleus	Cytosol	Mitochondrion	Endoplasmic reticulum	Plasma membrane	Cell surface
N	82	47	182	91	45	105
Median	0.942	1.14	0.907	0.640	2.84	1.24
Mean	0.980	1.13	0.914	0.687	2.59	1.61
SD	0.491	0.272	0.172	0.270	0.877	1.07
25th percentile	0.553	0.964	0.822	0.583	1.83	0.635
75th percentile	1.31	1.29	0.975	0.684	3.29	2.69

	Nucleus	Cytosol	Mitochondrion	Endoplasmic reticulum	Plasma membrane	Cell surface
N	103	57	206	46	123	138
Median	1.15	1.49	0.714	0.316	1.86	1.29
Mean	1.22	1.40	0.706	0.584	2.03	1.50
SD	0.775	0.427	0.471	0.565	1.03	1.02
25th percentile	0.513	1.23	0.284	0.245	1.24	0.652
75th percentile	1.71	1.72	0.926	0.703	2.89	2.17

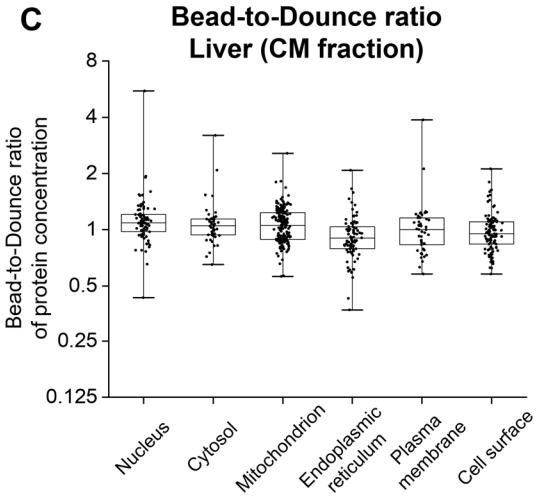
Figure S1. PM-to-CM ratio profiles of identified proteins with Dounce homogenizer in mouse liver (A) and kidney (B). Proteins, having only one corresponding GO term, were extracted for each subcellular group. The GO terms of identified proteins are shown in Tables S2 and S3. The cell surface proteins were extracted by cell surface protein atlas.[1] Each dot represents the mean concentration of each identified protein (n = 3-6). The band inside the box represents the median, while the box's bottom and top show the first and third quartiles, respectively. The whiskers indicate the minimum and maximum protein levels. N, number; SD, standard deviation.



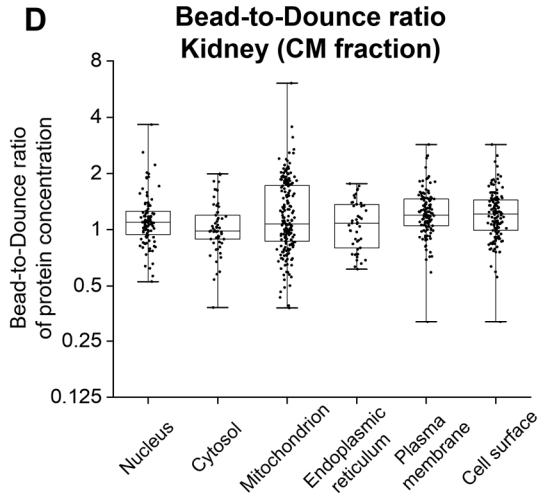
	Nucleus	Cytosol	Mitochondrion	Endoplasmic reticulum	Plasma membrane	Cell surface
N	82	47	182	91	45	105
Median	1.46	2.08	0.701	0.484	2.07	1.55
Mean	1.42	1.96	0.898	0.654	2.06	1.47
SD	0.853	0.676	0.744	0.503	0.541	0.914
25th percentile	0.555	1.61	0.326	0.409	1.64	0.524
75th percentile	2.24	2.40	1.18	0.609	2.35	2.15



	Nucleus	Cytosol	Mitochondrion	Endoplasmic reticulum	Plasma membrane	Cell surface
N	103	57	206	46	123	138
Median	1.00	0.935	1.05	1.05	1.08	1.07
Mean	1.02	0.945	1.27	1.14	1.13	1.15
SD	0.174	0.199	0.668	0.370	0.337	0.355
25th percentile	0.897	0.810	0.845	0.833	0.923	0.891
75th percentile	1.12	1.05	1.72	1.39	1.26	1.30



	Nucleus	Cytosol	Mitochondrion	Endoplasmic reticulum	Plasma membrane	Cell surface
N	82	47	182	91	45	105
Median	1.09	1.05	1.06	0.903	1.00	0.951
Mean	1.16	1.11	1.08	0.934	1.06	0.988
SD	0.544	0.386	0.244	0.255	0.499	0.251
25th percentile	0.977	0.940	0.887	0.790	0.832	0.837
75th percentile	1.20	1.14	1.23	1.03	1.16	1.10



	Nucleus	Cytosol	Mitochondrion	Endoplasmic reticulum	Plasma membrane	Cell surface
N	103	57	206	46	123	138
Median	1.10	0.986	1.08	1.08	1.20	1.21
Mean	1.17	1.07	1.29	1.10	1.26	1.24
SD	0.424	0.346	0.644	0.324	0.364	0.376
25th percentile	0.948	0.890	0.868	0.809	1.05	0.991
75th percentile	1.25	1.19	1.72	1.36	1.45	1.44

Figure S2. Bead-to-Dounce ratio profiles of identified proteins. Proteins, having only one corresponding GO term, were extracted for each subcellular group. The GO terms of identified proteins are shown in Tables S2 and S3. The cell surface proteins were extracted by cell surface protein atlas.[1] Each dot represents the mean concentration of each identified protein (n = 3-6). The band inside the box represents the median, while the box's bottom and top show the first and third quartiles, respectively. The whiskers indicate the minimum and maximum protein levels. N, number; SD, standard deviation.

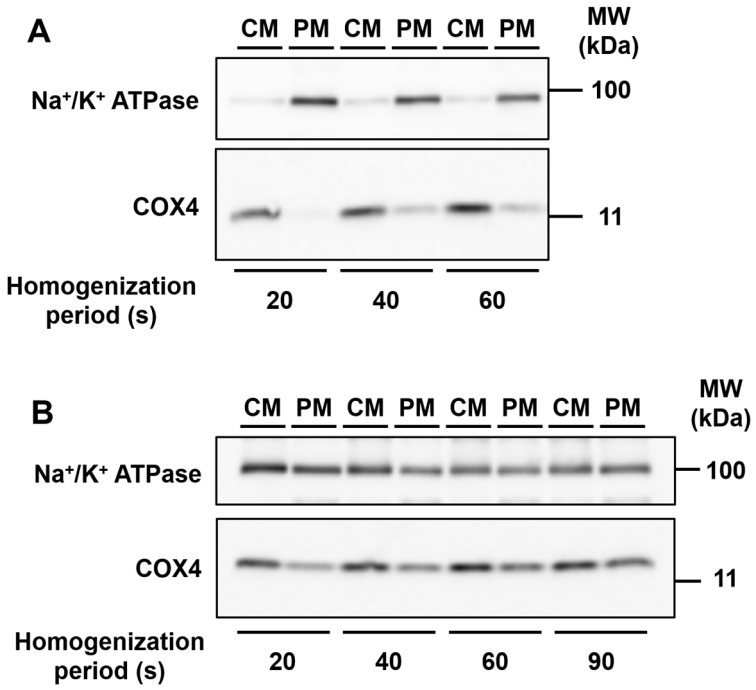


Figure S3. Western blot analysis of subcellular marker proteins in plasma and crude membrane fractions from mouse liver and kidney. Crude membrane (CM) and plasma membrane (PM) fractions were prepared from mouse liver (A) and kidney (B) tissues using the membrane protein extraction kit for indicated periods of homogenization using the bead homogenizer. MW, molecular weight; COX4, cytochrome c oxidase 4.

Reference

(1) Bausch-Fluck, D.; Hofmann, A.; Bock, T.; Frei, A. P.; Cerciello, F.; Jacobs, A.; Moest, H.; Omasits, U.; Gundry, R. L.; Yoon, C.; Schiess, R.; Schmidt, A.; Mirkowska, P.; Hartlova, A.; Van Eyk, J. E.; Bourquin, J. P.; Aebersold, R.; Boheler, K. R.; Zandstra, P.; Wollscheid, B. A mass spectrometric-derived cell surface protein atlas. *PLoS One* **2015,** *10* (3), e0121314.