

Supporting Information for Journal of Proteome Research

Four-dimensional orthogonal electrophoresis system for screening of protein complexes and protein-protein interactions combined with mass spectrometry

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Table S1. List of protein complex candidates in the 2nd-DE identified by PMF and MS/MS

Spot No.	protein complex candidates name	PMF Coverage (%)	Number of peptides (MALDI-MS/MS)	Peptide sequences (Gene name/alternative name/ Accession number) ^a	MS/MS scores	Theoretical pI/MW (kDa) ^b	Experimental pI/ MW (kDa) ^c
<u>erythrocyte cytoplasmic extracts (HMW)</u>							
1	Proteasome		13	FILNLPFSVR (PSMB2/β4/P49721)	48	6.52/22.8	5.8~6.3 /668
				FVFDRPLVSR (PSMA1/α6/P25786)	59	6.15/29.6	
				AIHSLWTR (PSMB4/β7/P28070)	44	5.47/24.4	
				LDFLRPYTVPNK (PSMB7/β2/Q99436)	53	5.61/25.3	
				HVGMAVAGLLADAR (PSMA3/α7/P25788)	107	5.19/28.3	
				HITIFSPEGR (PSMA6/α1/P60900)	53	6.35/27.4	
				NQYDNDVTWSPQGR (PSMA1/α6/P25786)	95	6.15/29.6	
				LTVEDPVTVEYITR (PSMA7/α4/O14818)	66	8.60/27.8	
				TTIFSPEGR (PSMA4/α3/P25789)	58	7.58/29.5	
				LYIGLAGLATDVQTVAQR (PSMB3/β3/P49720)	141	6.13/22.8	
				SILYDER (PSMA2/α2/P25787)	49	7.12/25.8	
				GAVYSFDPVGSYQR (PSMB1/β6/P20618)	106	8.32/23.5	
				LSAEKVEIATLTR (PSMA4/α3/P25789)	54	7.58/29.5	
2	Hemoglobin (α ₂ β ₂)		2	VGAHAGEYGAEALER (HBA/α/P69905)	111	8.73/15.1	7.0/180
				LLVVYPWTQR (HBB/β/P68871)	50	6.81/15.9	
3	Hemoglobin (α ₂ β ₂)		3	VGAHAGEYGAEALER (HBA/α/P69905)	109	8.73/15.1	7.1/237
				TYFPHFDLSHGSAQVK (HBA/α/P69905)	59		
				LLVVYPWTQR (HBB/β/P68871)	49		

4	Hemoglobin ($\alpha_2\delta_2$)		2	VGAHAGEYGAEALER (HBA/ α /P69905)	90	8.73/15.1	7.5/352
				VNVDAVGGEALGR (HBD/ δ / P02042)	37	7.97/15.9	
5	Peroxiredoxi n-2	35	3	KEGGLGPLNIPLLDVTR (PRDX2/-/P32119)	53	5.67/21.8	4.9/159
				EGGLGPLNIPLLDVTR (PRDX2/-/P32119)	46		
				QITVNDLPVGR (PRDX2/-/P32119)	46		
6	Carbonic anhydrase 1	30	2	GGPFSDSYR (CH1/-/P00915)	36	6.63/28.7	6.2/442
				EIINVGHFSFHVNFEDNDR (CH1/-/P00915)	56		
<u>Raji cytoplasmic extracts</u>							
1 [#]	Heat shock protein 60	49	3	TLNDELEIIEGMKFDG (CH60/-/P10809)	55	5.24/58.0	4.4/403
				LVQDVANNTNEEAGDGTATVLR (CH60/-/P10809)	118		
				KPLVIAEDVDGEALSTLVLNR (CH60/-/P10809)	155		

^a Database searching was performed using the MASCOT program (<http://www.matrixscience.com>)

^b The theoretical pI/MW values were all calculated using the ExPASy Compute pI/MW tool (http://kr.expasy.org/tools/pi_tool/html.)

^c The experimental pI/MW values were estimated according to Reference (22)

Table S2. Table S2: List of PMF and MS/MS results obtained from the 4th-DE

Spot No.	Gene name (alternative name) ^a	Accession number	Experimental MW ^b /Theoretical MW (kDa) ^c	Experimental pI ^b /Theoretical pI ^c	PMF Coverage (%)	Number of peptides (MALDI-MS/MS)	Peptide sequences	Mascot scores PMF/PMF_LIFT
CP subunit isoforms of erythrocytes								
1	PSMA5 (α 5)	P28066	26/26.4	4.3/4.74	59	1	GVNTFSPEGR	189/62
2	PSMB6 (β 1)	P28072	23/21.9	4.3/4.91	26	3	IFCCR LAAIAESGVER VTDKLTPIHDR	86/58
3	PSMB6 (β 1)	P28072	22/21.9	4.5/4.91	43	4	IFCCR LAAIAESGVER VTDKLTPIHDR QSFAIGGSGSSYIYGVDATYR	159/220
4	PSMB6 (β 1)	P28072	22/21.9	4.8/4.91	29	-	-	56
5	PSMA3 (α 7)	P25788	28/28.3	4.7/5.19	47	3	LYEEGSNKR HVGMAVAGLLADAR SLADIAREEASNFR	175/218
6	PSMA3 (α 7)	P25788	28/28.3	4.8/5.19	60	3	LYEEGSNKR HVGMAVAGLLADAR SLADIAREEASNFR	258/228

7	PSMA3 (α 7)	P25788	28/28.3	4.9/5.19	45	2	LYEEGSNKR HVGMAVAGLLADAR	200/154
8	PSMB4 (β 7)	P28070	26/24.4	5.2/5.47	34	2	AIHSWLTR QPVLSQTEAR	108/87
9	PSMB4 (β 7)	P28070	26/24.4	5.6/5.47	43	5	AIHSWLTR QPVLSQTEAR EVLEKQPVLSQTEAR FEGGVVIAADMLGSYGSLAR FEGGVVIAADMLGSYGSLAR+ Oxidation (M)	137/265
10	PSMB4 (β 7)	P28070	26/24.4	5.6/5.47	50	5	AIHSWLTR QPVLSQTEAR EVLEKQPVLSQTEAR FEGGVVIAADMLGSYGSLAR FEGGVVIAADMLGSYGSLAR+ Oxidation (M)	91/278
11	PSMA6 (α 1)	P60900	27/27.4	5.7/6.35	24	2	HITIFSPEGR ILTEAEIDAHLVALAERD	99/143
12	PSMA6 (α 1)	P60900	27/27.4	6.1/6.35	41	2	HITIFSPEGR ILTEAEIDAHLVALAERD	127/120

13	PSMA6 ($\alpha 1$)	P60900	28/27.4	6.1/6.35	35	2	HITIFSPEGR ILTEAEIDAHLVALAERD	125/123
14	PSMA6 ($\alpha 1$)	P60900	26/27.4	6.7/6.35	45	2	HITIFSPEGR ILTEAEIDAHLVALAERD	153/119
15	PSMB7 ($\beta 2$)	Q99436	29/25.3	5.5/5.61	13	1	LDFLRPYTVPNK	49/57
16	PSMB7 ($\beta 2$)	Q99436	29/25.3	5.7/5.61	31	2	LDFLRPYTVPNK LDFLRPYTVPNKK	122/70
17	PSMB7 ($\beta 2$)	Q99436	29/25.3	5.8/5.61	24	2	LDFLRPYTVPNK LDFLRPYTVPNKK	67/68
18	PSMA1 ($\alpha 6$)	P25786	31/29.6	6.1/6.15	33	2	FVFDRPLPVS R NQYDNDVTWSPQGR	85/106
19	PSMA1 ($\alpha 6$)	P25786	30/29.6	6.3/6.15	52	2	FVFDRPLPVS R NQYDNDVTWSPQGR	150/108
20	PSMA1 ($\alpha 6$)	P25786	30/29.6	6.7/6.15	65	2	FVFDRPLPVS R NQYDNDVTWSPQGR	148/104

21	PSMA1 (α 6)	P25786	30/29.6	6.9/6.15	27	2	FVFDRPLPVS R NQYDNDVTWSPQGR	67/106
22	PSMA7 (α 4)	O14818	28/27.8	6.5/8.60	72	2	LTVEDPVTVEYITR RPFGISALIVGFDFDGT R	249/118
23	PSMA7 (α 4)	O14818	27/27.8	7.7/8.60	75	3	ARVECQSHR LTVEDPVTVEYITR RPFGISALIVGFDFDGT R	317/131
24	PSMA7 (α 4)	O14818	27/27.8	7.7/8.60	79	5	VECQSHR ARVECQSHR GRDIVVLGVEK LTVEDPVTVEYITR RPFGISALIVGFDFDGT R	304/106
25	PSMA7 (α 4)	O14818	27/27.8	7.8/8.60	69	3	ARVECQSHR LTVEDPVTVEYITR RPFGISALIVGFDFDGT R	229/132
26	PSMA7 (α 4)	O14818	27/27.8	8.0/8.60	76	5	VECQSHR ARVECQSHR GRDIVVLGVEK LTVEDPVTVEYITR RPFGISALIVGFDFDGT R	336/143

27	PSMA4 (α 3)	P25789	28/29.5	7.3/7.58	34	2	TTIFSPEGR LSAEKVEIATLTR	164/129
28	PSMA4 (α 3)	P25789	28/29.5	7.6/7.58	65	3	TTIFSPEGR LSAEKVEIATLTR LLDEVFFSEK	214/151
29	PSMA4 (α 3)	P25789	28/29.5	7.9/7.58	60	3	TTIFSPEGR LSAEKVEIATLTR LLDEVFFSEK	194/152
30	PSMA4 (α 3)	P25789	28/29.5	8.3/7.58	36	3	TTIFSPEGR LSAEKVEIATLTR LLDEVFFSEK	137/155
31	PSMA4 (α 3)	P25789	28/29.5	7.0/7.58	60	3	TTIFSPEGR LSAEKVEIATLTR LLDEVFFSEK	218/152
32	PSMB3 (β 3)	P49720	25/22.8	5.6/6.13	44	2	FGPYYTEPVIAGLDPK LYIGLAGLATDVQTVAQR	104/207
33	PSMB3 (β 3)	P49720	23/22.8	6.3/6.13	50	2	FGPYYTEPVIAGLDPK LYIGLAGLATDVQTVAQR	142/205

34	PSMB2 (β4)	P49721	24/22.8	6.1/6.52	29	2	FILNLP T FSVR NGYELSP T AAANFTR	76/151
35	PSMA2 (α2)	P25787	24/25.8	6.2/7.12	55	2	SILYDER HIGLVYSGM G PDYR	147/75
36	PSMA2 (α2)	P25787	24/25.8	6.8/7.12	38	3	SILYDER HIGLVYSGM G PDYR HIGLVYSGM G PDYR + Oxidation (M)	140/90
37	PSMA2 (α2)	P25787	24/25.8	7.6/7.12	43	4	SILYDER HIGLVYSGM G PDYR HIGLVYSGM G PDYR + Oxidation (M) KLAQQYYLVYQEPIPTAQLVQ R	149/252
38	PSMA2 (α2)	P25787	24/25.8	8.0/7.12	39	3	SILYDER HIGLVYSGM G PDYR HIGLVYSGM G PDYR + Oxidation (M)	134/90
39	PSMB2 (β4)	P49721	22/22.8	6.7/6.52	37	3	YYTPTISR FILNLP T FSVR NGYELSP T AAANFTR	140/163

40	PSMB2 (β4)	P49721	23/22.8	7.4/6.52	29	4	YYTPTISR FILNLPTFSVR NGYELSPTAAANFTR IIDKNGIHDLDNISFPK	119/186
41	PSMB1 (β6)	P20618	24/23.5	8.2/8.32	38	3	LSEGFSIHTR EGIREETVSLR GAVYSFDPVGSYQR	95/168
<u>hemoglobin (α₂β₂) subunit isoforms of erythrocytes</u>								
1	HBB	P68871	11/15.9	6.0/6.81	84	3	LHVDPENFR LLVVYPWTQR VNVDEVGGEALGR	232/181
2	HBB	P68871	11/15.9	6.2/6.81	82	2	LHVDPENFR LLVVYPWTQR	211/70
3	HBB	P68871	10/15.9	6.2/6.81	84	2	LLVVYPWTQR VNVDEVGGEALGR	226/138
4	HBB	P68871	11/15.9	6.3/6.81	84	1	LLVVYPWTQR	240/71
5	HBB	P68871	10/15.9	6.3/6.81	80	1	VNVDEVGGEALGR	219/100
6	HBB	P68871	11/15.9	6.5/6.81	85	3	LHVDPENFR	263/159

							LLVVYPWTQR VNVDEVGGEALGR	
7	HBB	P68871	10/15.9	6.5/6.81	85	4	LHVDPENFR LLVVYPWTQR VNVDEVGGEALGR EFTPPVQAAAYQK	231/217
8	HBB	P68871	11/15.9	6.7/6.81	84	3	LHVDPENFR LLVVYPWTQR VNVDEVGGEALGR	229/143
9	HBB	P68871	10/15.9	6.7/6.81	78	2	LHVDPENFR LLVVYPWTQR	222/57
10	HBB	P68871	11/15.9	7.0/6.81	84	2	LHVDPENFR LLVVYPWTQR	231/123
11	HBB	P68871	10/15.9	7.0/6.81	84	2	LHVDPENFR LLVVYPWTQR	206/84
12	HBA	P69905	10/15.1	7.0/8.73	57	1	VGAHAGEYGAEALER	75/122
13	HBA	P69905	10/15.1	7.3/8.73	74	3	<u>M</u> FLSFPTTK+ Oxidation (M) VGAHAGEYGAEALER TYFPHFDSLHGSQVK	105/214

14	HBA	P69905	10/15.1	7.8/8.73	63	3	<u>M</u> FLSFPTTK+ Oxidation (M) VGAHAGEYGAEALER TYFPHFDSLHGSAQVK	103/218
15	HBB	P68871	11/15.9	8.3/6.81	84	1	VNVDEVGGEALGR	219/85
16	HBB	P68871	10/15.9	8.3/6.81	78	1	LHVDPENFR	200/73
17	HBA	P69905	10/15.1	8.9/8.73	64	2	VGAHAGEYGAEALER TYFPHFDSLHGSAQVK	127/217

Peroxioredoxin-2 subunit isoforms of erythrocytes

1	PRDX2	P32119	22/21.8	4.8/5.67	42	1	QITVNDLPVGR	218/85
2	PRDX2	P32119	22/21.8	4.9/5.67	45	2	QITVNDLPVGR EGGLGPLNIPLLADVTR	258/194
3	PRDX2	P32119	22/21.8	5.2/5.67	40	2	EGGLGPLNIPLLADVTR KEGGLGPLNIPLLADVTR	213/256
4	PRDX2	P32119	21/21.8	5.5/5.67	42	3	QITVNDLPVGR EGGLGPLNIPLLADVTR KEGGLGPLNIPLLADVTR	180/320

HSP60 subunit isoforms of Raji cells

1	CH60	P10809	62/58.0	4.9/5.24	31	3	TLNDELEIIEG <u>M</u> KFDR + Oxidation (M) KPLVIIAEDVDGEALSTLVLNR LVQDVANNTNEEAGDGTSTA TVLAR	137/251
2	CH60	P10809	61/58.0	5.0/5.24	41	2	TLNDELEIIEG <u>M</u> KFDR + Oxidation (M) LVQDVANNTNEEAGDGTSTA TVLAR	202/132
3	CH60	P10809	61/58.0	5.0/5.24	56	2	KPLVIIAEDVDGEALSTLVLNR LVQDVANNTNEEAGDGTSTA TVLAR	315/303
4	CH60	P10809	58/58.0	5.1/5.24	48	3	TLNDELEIIEG <u>M</u> KFDR + Oxidation (M) KPLVIIAEDVDGEALSTLVLNR LVQDVANNTNEEAGDGTSTA TVLAR	259/329
5	CH60	P10809	58/58.0	5.0/5.24	48	2	KPLVIIAEDVDGEALSTLVLNR LVQDVANNTNEEAGDGTSTA TVLAR	268/258
6	CH60	P10809	59/58.0	4.9/5.24	45	1	KPLVIIAEDVDGEALSTLVLNR	283/181

7	CH60	P10809	59/58.0	4.9/5.24	26	1	LVQDVANNTNEEAGDGTTTA TVLAR	193/106
8	CH60	P10809	59/58.0	4.8/5.24	18	2	TLNDELEIIIEGMKFDR + Oxidation (M) LVQDVANNTNEEAGDGTTTA TVLAR	106/99

^a Database searching was performed using the MASCOT program (<http://www.matrixscience.com>)

^b The experimental pI/MW values were estimated according to Reference (22)

^c The theoretical pI/MW values were all calculated using the ExPASy Compute pI/MW tool (http://kr.expasy.org/tools/pi_tool/html.)

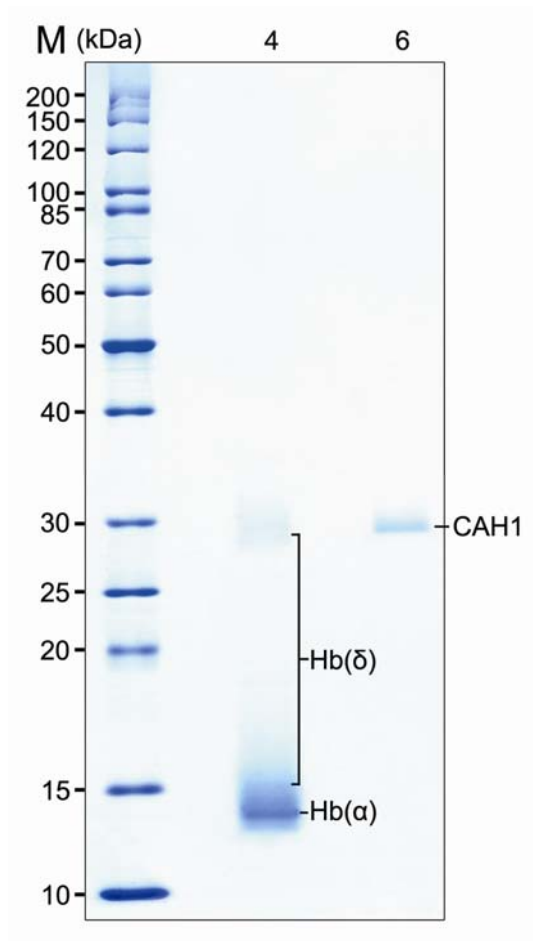


Figure S1. Hb ($\alpha_2\delta_2$) and CAH1 were analyzed by 12% SDS PAGE after alkaline-ultrasonic and solution extraction to inspect the efficiency of alkaline-ultrasonic and solution extraction. One spot of Hb ($\alpha_2\delta_2$) and two spots of CAH1 were excised from parallel 2nd-DE gels (Figure 6A), respectively.

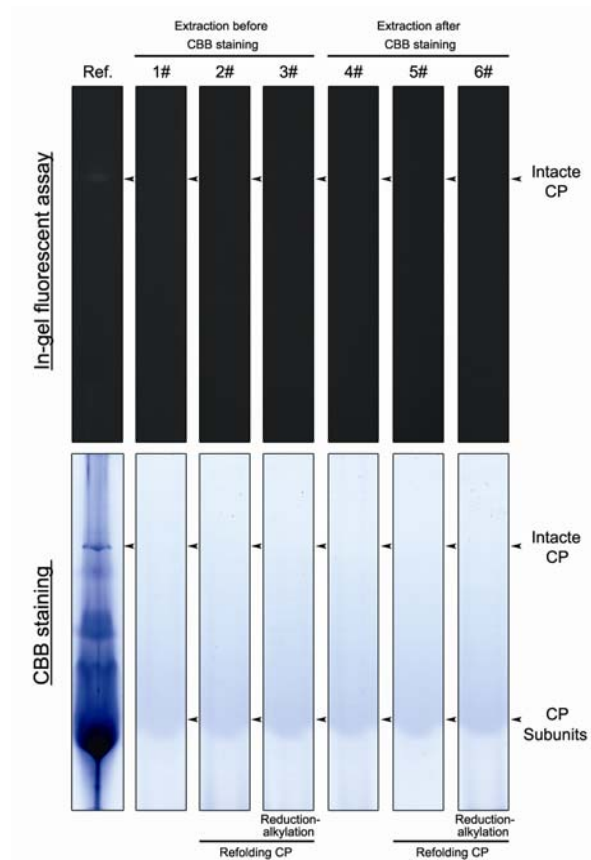


Figure S2. Activity assay of CP after alkaline-ultrasonic and solution extraction from 2nd-DE gels was carried out with help of 4%-17% linear gradient native-PAGE. The original sample of erythrocyte HMW cytoplasmic extracts was used as Ref.. The CP of lanes 1#, 2# and 3# were excised from 2nd-DE gels before CBB staining, on the contrary, the CP of lanes 4#, 5# and 6# were excised from 2nd-DE gels after CBB staining. The CP of lanes 3# and 6# were carried out reduction-alkylation before refolding. The CP of lanes 2#, 3#, 5# and 6# were refolded before native-PAGE separation.