Title

Biochemical Characterization of *Echinococcus multilocularis* Antigen B3 Reveals Insight into Adaptation and Maintenance of Parasitic Homeostasis at the Host-Parasite Interface

Author names and affiliations

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Supplementary Figure S1. Comparison of nucleotide sequences of EmuJ_000381600 (EmAgB3b) and EmuJ_000381700 (EmAgB3c) retrieved from the draft genome database of *Echinococcus multilocularis* (ori) or empirically determined from the genomic DNA (seq). Coding DNA sequences and intervening introns are marked with red and yellow letters, respectively. Nucleotide sequences in 5'- and 3'-flanking regions were also included in the comparison. Primers used in genomic PCR are underlined.

Supplementary Figure S2. The mono-specific antibody reactivity of polyclonal antibodies generated against rEmAg1-5. rEmAgB1-5 were separated by 15% SDS-PAGE under reducing conditions and transferred to nitrocellulose membranes. The blots were incubated with 1:2000 diluted respective antibodies overnight followed by incubation with 1:4000 diluted HRP-conjugated anti-mouse IgG antibody for 2 h. Blots were developed with ECL. Lanes 1-5 contained rEmAgB1-5 (each of 200 ng). M_r, relative molecular mass in kDa.

Supplementary Figure S3. Identification of EmAgB3b/c specific peptide fragments of the native EmAgB3b/c purified by immunoprecipitation by LC-ESI-MS/MS. Four peptide peaks were detected: 52LVEVMKEVGSVCQMVR67 (A), 21DDDEVTQTK29 (B), 72MALKEYVR79 (C), and 41HFFQSDPLGK50 (D).

Supplementary Figure S4. Subunit composition of the multimerized rEmAgB3b/c determined by blue native-PAGE. The multimerized rEmAgB3b/c was electrophoresed on 4-20% native PAGE (first-dimension), after which the gel strip was further separated by 10% Tricine SDS-

PAGE (second-dimension). The protein was visualized by CBB staining. M_r, relative molecular mass in kDa.

Supplementary Figure S5. Changes of hydrophobic-ligand-binding activity of rEmAgB3b/c following delipidation process. Increasing doses (2-16 μg protein) of mock *E. coli* vector (negative control) (A), GST-removed precleared rEmAgB3b/c (B), lipid-depleted rEmAgB3b/c by Sephadex-LH beads (C), and delipidated rEmAgB3b/c through Octyl-Sepharose reverse-phase hydrophobic interactive chromatography (D) were reacted with 10 μM 16-(9-anthroyloxy) palmitic acid (16-AP) for 2 min. The fluorescence spectra (Ex_{max}/Em_{max} = 360/460 nm) was recorded at 25 °C using black 96-well plates. The delipidated TsM 150 kDa HLBP complex (2 μg) was used as a positive control. (E and F) Binding affinity of delipidated rEmAgB3 toward DAUDA and DACA. Increasing doses of rEmAgB3b/c (2-16 μg) were added to ligand solutions (each 10 μM) and fluorescence was detected after incubation at 25 °C for 5 min (Ex_{max}/Em_{max} = 345/519 nm for DAUDA and Ex_{max}/Em_{max} = 350/519 nm for DACA). The delipidated TsM 150 kDa HLBP (2 μg) was used as a positive control. DAUDA, 11-([5-dimethylaminonaphthalene-1-sulfonyl]amino) undecannoic acid; DACA, dansyl-DL-α-aminocaprylic acid.

Supplementary Figure S6. Displacement of 1,8-ANS and 16-AP from rEmAgB3b/c:fatty acid binary complexes. (A-G) rEmAgB3b/c or rEgAgB3 bound to 1,8-ANS and 16-AP was assayed for competitive displacement by linoleic acid (LA; 2-8 μM), palmitic acid (PA; 2-8 μM), and myristic acid (MA; 2-8 μM). The displacement effects are shown by histogram (n = 3-5, mean ± S.D.). 1,8-ANS, 1-anilinonapthalene 8-sulfonic acid; 16-AP, 16-(9-anthroyloxy) palmitic acid.

Supplementary Figure S7. Subunit compositions of EmAgB3b/c in individual anatomical compartments of *E. multilocularis* metacestode under different reducing conditions. Proteins (10 μg each) extracted from *E. multilocularis* hydatid fluid (HF), protoscolex (PSC), and germinal layer (GL) were mixed with the same volume of differently prepared 2× sample buffers, each of which contained 200 mM dithiothreitol (DTT), 20% β-mercaptoethanol (2-ME), 8% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 8 M urea, and 20 mM reduced glutathione (GSH). After boiling for 5 min, samples were separated by 10% Tricine SDS-PAGE and electroblotted onto nitrocellulose membranes. The membranes were incubated with anti-rEmAgB3b/c antibody (1:2000 dilution) at 4 °C overnight. The blots were subsequently incubated with HRP-conjugated anti-mouse IgG (1:4000 dilution) for 2 h and detected by ECL. M_r, relative molecular mass in kDa.

Supplementary Table S1. Oligonucleotide primers used in this study

Primer name	GeneDB no.	Sequence (5'-3')[a]	
		Gene cloning	qPCR
EmAgB1-f[b]	EmuJ_000381200	CGCGGATCCGATGATGGTCTCACCTCG	GATGATGGTCTCACCTCG
EmAgB1-r[c]		CGCCTCGAGCTATTTACCTTCAGCAAC	CTATTTACCTTCAGCAAC
EmAgB2-f	EmuJ_000381100	CGCGGATCCAAAGATGAGCCAAAAGCA	AAAGATGAGCCAAAAGCA
EmAgB2-r		CGCCTCGAGTTACTTTGAATCATCATC	TTACTTTGAATCATCATC
EmAgB3b/c-f	EmuJ_000381600/ EmuJ_000381700	CGCGGATCCGATGATGATGAAGTGACC	GATGATGATGAAGTGACC
EmAgB3b/c-r		CGCCTCGAGCTACTCATCCTCTTTAAT	CTACTCATCCTCTTTAAT
EmAgB3a-f	EmuJ_000381500	-	GTGGCTCGTGCTGAGGGT
EmAgB3a-r		-	CTACTCATCCTCTTTAAT
EmAgB4-f	EmuJ_000381400	CGCGGATCCAAAGCTGAACCCGAGAGA	AAAGCTGAACCCGAGAGA
EmAgB4-r		CGCCTCGAGTTACTTTAAATCATCCTC	TTACTTTAAATCATCCTC
EmAgB5-f	EmuJ_000381800	CGCGGATCCGAAGACGACATCGATTCG	GAAGACGACATCGATTCG
EmAgB5-r		CGCCTCGAGCTACTCCCCTTCATCCAT	CTACTCCCCTTCATCCAT
EgAgB3-f	EgrG_000381600	CGCGGATCCGATGATGATGAAGTGACA	-
EgAgB3-r		CGCTCGAGCTACTCATCCTCTTTAAC	-
EmAct-f	EmuJ_000407400	-	TCGAAGCGTGGTATTCTC
EmAct-r		-	TTGAGTGGTGCCTCAGTT

[a] *BamH*I and *Xho*I restriction sites on the primer sequences are underlined.
[b] forward
[c] reverse

Supplementary Table S2. Identification of proteins from hydatid fluid in *E. multilocularis* metacestode by MALDI-TOF MS[a]

Spot no.	MS[b]	SC[c]	MP[d]	M_r/pI	Accession no.[e]	Species	Protein name	SP[f]
1	155	46	24/98	70700/5.75	NP_033784	*Mus musculus*	Serum albumin precursor	+
2	75	34	10/51	49531/5.04	EmuJ_000317300	*E. multilocularis*	Vesicular amine transporter	-
3	323	67	37/88	70700/5.75	NP_033784	*M. musculus*	Serum albumin precursor	+
4	290	63	39/111	70700/5.75	NP_033784	*M. musculus*	Serum albumin precursor	+
5	222	54	31/97	70700/5.75	NP_033784	*M. musculus*	Serum albumin precursor	+
6	331	71	43/117	70700/5.75	NP_033784	*M. musculus*	Serum albumin precursor	+
7	303	65	36/90	70700/5.75	NP_033784	*M. musculus*	Serum albumin precursor	+
8	73	17	26/95	31345/5.72	EmuJ_000849600	*E. multilocularis*	Proteinase inhibitor I25	+
9	70	65	13/106	31345/5.72	EmuJ_000849600	*E. multilocularis*	Proteinase inhibitor I25	+
10	39	32	6/78	21359/5.18	EmuJ_000538000	*E. multilocularis*	Glutathione S-transferase	-
11	70	56	7/70	23961/5.36	EmuJ_000904500	*E. multilocularis*	Expressed conserved protein	-
12	129	65	14/93	22758/5.00	EmuJ_000315600	*E. multilocularis*	Expressed protein	+
13	50	69	7/94	13925/5.33	NP_035155	*M. musculus*	Oxytocin-neurophysin 1	+
14	35	44	4/95	8644/5.60	EmuJ_000121100	*E. multilocularis*	Hypothetical protein	-
15	266	53	35/87	85250/6.61	EmuJ_000908900	*E. multilocularis*	NAALAD2	+
16	309	56	37/80	85250/6.61	EmuJ_000908900	*E. multilocularis*	NAALAD2	+
17	346	54	41/80	85250/6.61	EmuJ_000908900	*E. multilocularis*	NAALAD2	+
18	188	45	26/80	85250/6.61	EmuJ_000908900	*E. multilocularis*	NAALAD2	+
19	169	37	25/80	85250/6.61	EmuJ_000908900	*E. multilocularis*	NAALAD2	+
20	189	37	27/80	85250/6.61	EmuJ_000908900	*E. multilocularis*	NAALAD2	+
21	126	32	21/80	85250/6.61	EmuJ_000908900	*E. multilocularis*	NAALAD2	+
22	204	45	28/80	85250/6.61	EmuJ_000908900	*E. multilocularis*	NAALAD2	+
23	156	35	24/80	85250/6.61	EmuJ_000908900	*E. multilocularis*	NAALAD2	+
24	329	51	38/80	78841/6.94	NP_598738	*M. musculus*	Serotransferrin precursor	+
25	188	42	27/80	78841/6.94	NP_598738	*M. musculus*	Serotransferrin precursor	+
26	136	33	23/80	78841/6.94	NP_598738	*M. musculus*	Serotransferrin precursor	+
27	67	31	14/89	61295/9.38	EDL37810	*M. musculus*	GABA-A	+
28	48	23	11/83	53827/8.51	EmuJ_000523300	*E. multilocularis*	Mechanosensory protein 2	-
29	112	41	17/80	55614/6.21	EmuJ_000184900	*E. multilocularis*	Glycoprotein Antigen 5	+
30	67	38	14/126	55614/6.21	EmuJ_000184900	*E. multilocularis*	Glycoprotein Antigen 5	+
31	39	40	6/81	15871/8.29	EmuJ_000360300	*E. multilocularis*	Thioredoxin, mitochondrial	-

32	100	76	11/80	10460/8.39	EmuJ_000381400	*E. multilocularis*	Antigen B4	+
33	42	63	6/69	10137/8.91	EmuJ_000381600	*E. multilocularis*	Antigen B3	+
34	45	64	8/87	10330/9.48	EmuJ_000381100	*E. multilocularis*	Antigen B2	+
35	96	70	10/80	15795/7.14	ABU63206	*Mus musculus*	Beta-globin	-
36	63	70	6/74	10460/8.39	EmuJ_000381400	*E. multilocularis*	Antigen B4	+
37	68	64	8/65	10460/8.39	EmuJ_000381400	*E. multilocularis*	Antigen B4	+
38	104	96	9/75	10460/8.39	EmuJ_000381400	*E. multilocularis*	Antigen B4	+
39	51	61	7/80	10460/8.39	EmuJ_000381400	*E. multilocularis*	Antigen B4	+
40	57	60	7/78	10330/9.48	EmuJ_000381100	*E. multilocularis*	Antigen B2	+
41	42	69	5/62	9844/9.25	EmuJ_000381200	*E. multilocularis*	Antigen B1	+
42	52	52	9/79	10137/8.91	EmuJ_000381600	*E. multilocularis*	Antigen B3	+
43	47	78	7/84	10066/9.36	EmuJ_000381500	*E. multilocularis*	Antigen B3	+
44	80	65	12/75	9844/9.25	EmuJ_000381200	*E. multilocularis*	Antigen B1	+
45	40	55	6/93	10330/9.48	EmuJ_000381100	*E. multilocularis*	Antigen B2	+
46	42	69	4/52	10330/9.48	EmuJ_000381100	*E. multilocularis*	Antigen B2	+
47	46	75	6/78	9844/9.25	EmuJ_000381200	*E. multilocularis*	Antgen B1	+
48	68	71	10/57	9920/9.36	EmuJ_000381600	*E. multilocularis*	Antigen B3	+
49	59	72	9/75	10066/9.36	EmuJ_000381500	*E. multilocularis*	Antigen B3	+
50	56	24	10/75	48792/5.47	AAA37190	*M. musculus*	Alpha-fetoprotein, partial	+
51	58	28	10/75	48792/5.47	AAA37190	*M. musculus*	Alpha-fetoprotein, partial	+
52	85	31	13/75	48792/5.47	AAA37190	*M. musculus*	Alpha-fetoprotein, partial	+
53	55	42	9/99	26314/5.08	NP_001025026	*M. musculus*	PAC	-
54	292	54	34/75	70700/5.75	NP_033784	*M. musculus*	Serum albumin precursor	+
55	323	56	36/75	70700/5.75	NP_033784	*M. musculus*	Serum albumin precursor	+
56	195	46	26/75	70700/5.75	NP_033784	*M. musculus*	Serum albumin precursor	+
57	58	39	9/75	40099/8.03	EmuJ_000905600	*E. multilocularis*	FBA	-
58	46	49	9/103	19779/8.33	BAB24719	*M. musculus*	Unnamed protein product	-
59	27	39	4/47	10066/9.36	EmuJ_000381500	*E. multilocularis*	Antigen B3	+
60	45	64	8/87	10066/9.36	EmuJ_000381500	*E. multilocularis*	Antigen B3	+

[a]Abbreviations used: NAALAD2, N-acetylated alpha-linked acidic dipeptidase 2; GABA-A, gamma-aminobutyric acid receptor, subunit alpha 4, isoform CRA_a; PAC, proteasome; FBA, fructose 1,6-bisphosphate aldolase. [b]Mascot score; protein scores greater than 43 are significant ($P < 0.05$). [c]Sequence coverage (%). [d]Matched peptides/Total peptides. [e]Accession numbers were identified from NCBI DB (http://www.ncbi.nlm.nih.gov/) and Sanger DB (http://www.genedb.org/Homepage/Emultilocularis). [f]Signal peptide.

Supplementary Table S3. Identity values (%) among 8 kDa EmAgB isoforms (RNA/protein)

Protein identification (GeneDB no.) (EmuJ_)	% identity (RNA/Protein)						
	EmAgB1 (000381200)	EmAgB2 (000381100)	EmAgB3a (000381500)	EmAgB3b (000381600)	EmAgB3c (000381700)	EmAgB4 (000381400)	EmAgB5 (000381800)
EmAgB1 (000381200)	100/100						
EmAgB2 (000381100)	53.9/44.6	100/100					
EmAgB3a (000381500)	72.5/56.5	55.9/40.7	100/100				
EmAgB3b (000381600)	72.6/56.6	54.5/38.1	96.9/93.0	100/100			
EmAgB3c (000381700)	72.6/56.6	54.5/38.1	96.9/93.0	100/100	100/100		
EmAgB4 (000381400)	56.9/42.7	77.8/67.4	58.5/43.5	57.5/43.4	57.5/43.4	100/100	
EmAgB5 (000381800)	62.0/42.2	57.1/39.5	64.4/51.2	64.3/50.0	64.3/50.0	57.8/37.7	100/100

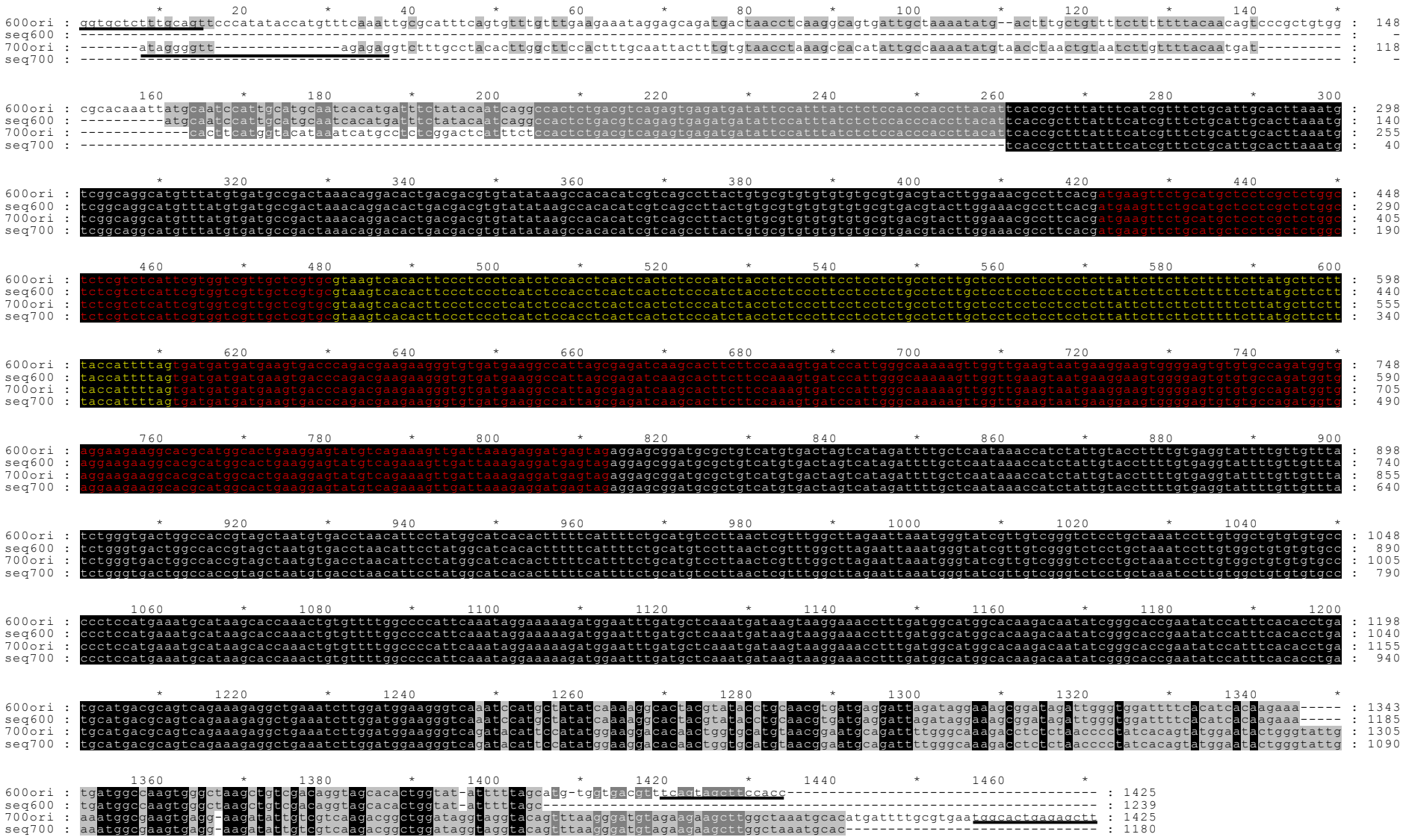
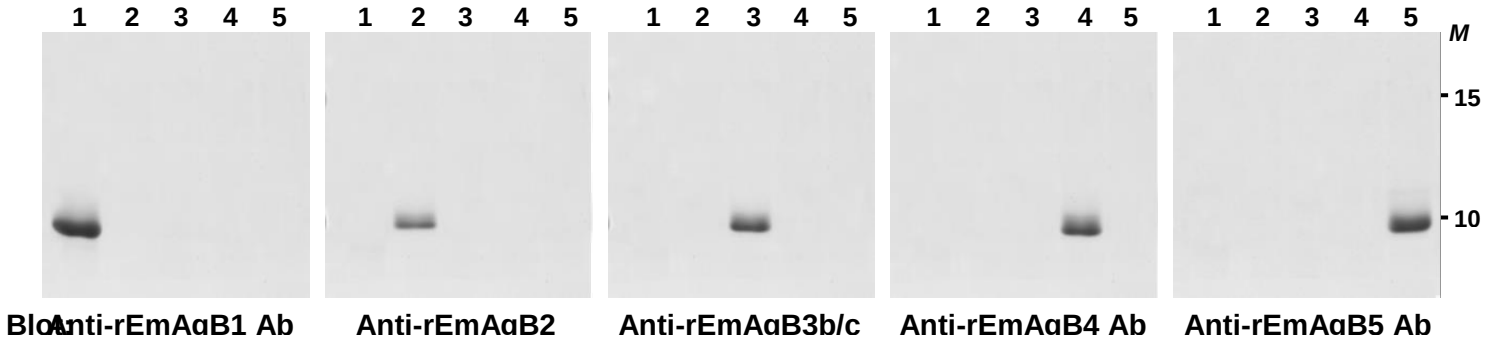


Fig. S1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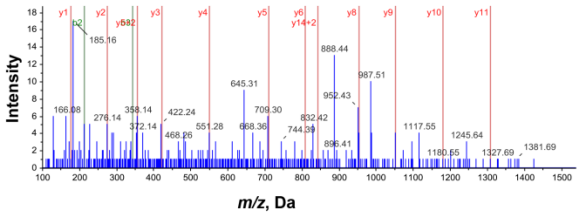
1 2 3 4 5
1 2 3 4 5
1 2 3 4 5
M
15
10
Blot
Anti-rEmAαB1 Ab
Anti-rEmAαB2
Anti-rEmAαB3b/c
Anti-rEmAαB4 Ab
Anti-rEmAαB5 Ab

Fig. S2

A **52LVEVMKEVGSVCQMVR67**

Spectrum	Time	Predicted MW	Predicted m/z	Predicted z	Modifications	Confidence	Theoretical MW	Z
1.1.1.2798.2	23.54	1894.929	632.6503	3	Oxidation(M)@5 and @14, Carbamidomethyl(C)@12,	99	1894.932	3

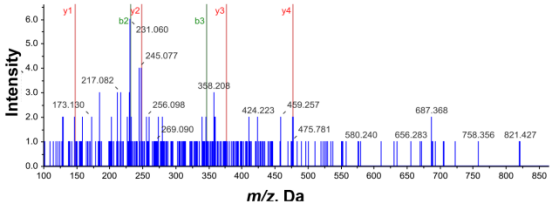
Residue	b	b+2	y	y+2
L	114.0913	57.5493	1895.939	948.473
V	213.1598	107.0835	1782.855	891.931
E	342.2023	171.6048	1683.786	842.3968
V	441.2708	221.139	1554.744	777.8755
M[Oxi]	588.3062	294.6567	1455.675	728.3413
K	716.4011	358.7042	1308.64	654.8236
E	845.4437	423.2255	1180.545	590.7761
V	944.5121	472.7597	1051.502	526.2548
G	1001.534	501.2704	952.4339	476.7206
S	1088.566	544.7864	895.4124	448.2099
V	1187.634	594.3207	808.3804	404.6938
C[CAM]	1347.665	674.336	709.312	355.1596
Q	1475.723	738.3653	549.2813	275.1443
M[Oxi]	1622.759	811.883	421.2228	211.115
V	1721.827	861.4172	274.1874	137.5973
R	1877.928	939.4677	175.119	88.0631



B **21DDDEVTQTK29**

Spectrum	Time	Predicted MW	Predicted m/z	Predicted z	Modifications	Confidence	Theoretical MW	z
1.1.1.1379.2	9.431	1049.451	525.7327	2		99	1049.451	2

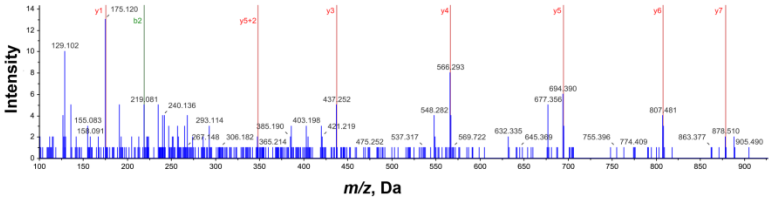
Residue	b	b+2	y	y+2
D	116.0342	58.5207	1050.459	525.7329
D	231.0612	116.0342	935.4316	468.2195
D	346.0881	173.5477	820.4047	410.706
E	475.1307	238.069	705.3777	353.1925
V	574.1991	287.6032	576.3352	288.6712
T	675.2468	338.127	477.2667	239.137
Q	803.3054	402.1563	376.2191	188.6132
T	904.353	452.6802	248.1605	124.5839
K	1032.448	516.7276	147.1128	74.06



C **72MALKEYVR79**

Spectrum	Time	Predicted MW	Predicted m/z	Predicted z	Modifications	Confidence	Theoretical MW	z
1.1.1.1993.2	15.96	1024.536	513.2753	2	Oxidation(M)@1	12	1024.538	2

Residue	b	b+2	y	y+2
M[Oxi]	148.0427	74.525	1025.545	513.2761
A	219.0798	110.0435	878.5094	439.7584
L	332.1639	166.5856	807.4723	404.2398
K	460.2588	230.633	694.3883	347.6978
E	589.3014	295.1543	566.2933	283.6503
Y	752.3647	376.686	437.2507	219.129
V	851.4332	426.2202	274.1874	137.5973
R	1007.534	504.2708	175.119	88.0631



D **41HFFQSDPLGK50**

Spectrum	Time	Predicted MW	Predicted m/z	Predicted z	Modifications	Confidence	Theoretical MW	z
1.1.1.2791.5	23.416	1174.577	588.2956	2		99	1174.577	2

Residue	b	b+2	y	y+2
H	138.0662	69.5367	1175.584	588.2958
F	285.1346	143.0709	1038.526	519.7664
F	432.203	216.6051	891.4571	446.2322
Q	560.2616	280.6344	744.3886	372.698
S	647.2936	324.1504	616.3301	308.6687
D	762.3206	381.6639	529.298	265.1527
P	859.3733	430.1903	414.2711	207.6392
L	972.4574	486.7323	317.2183	159.1128
G	1029.479	515.2431	204.1343	102.5708
K	1157.574	579.2905	147.1128	74.06

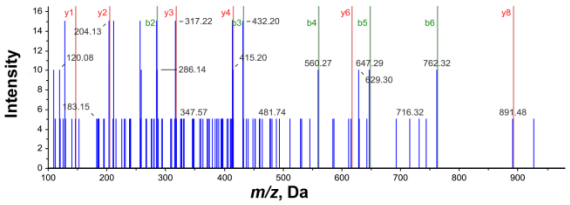


Fig. S3

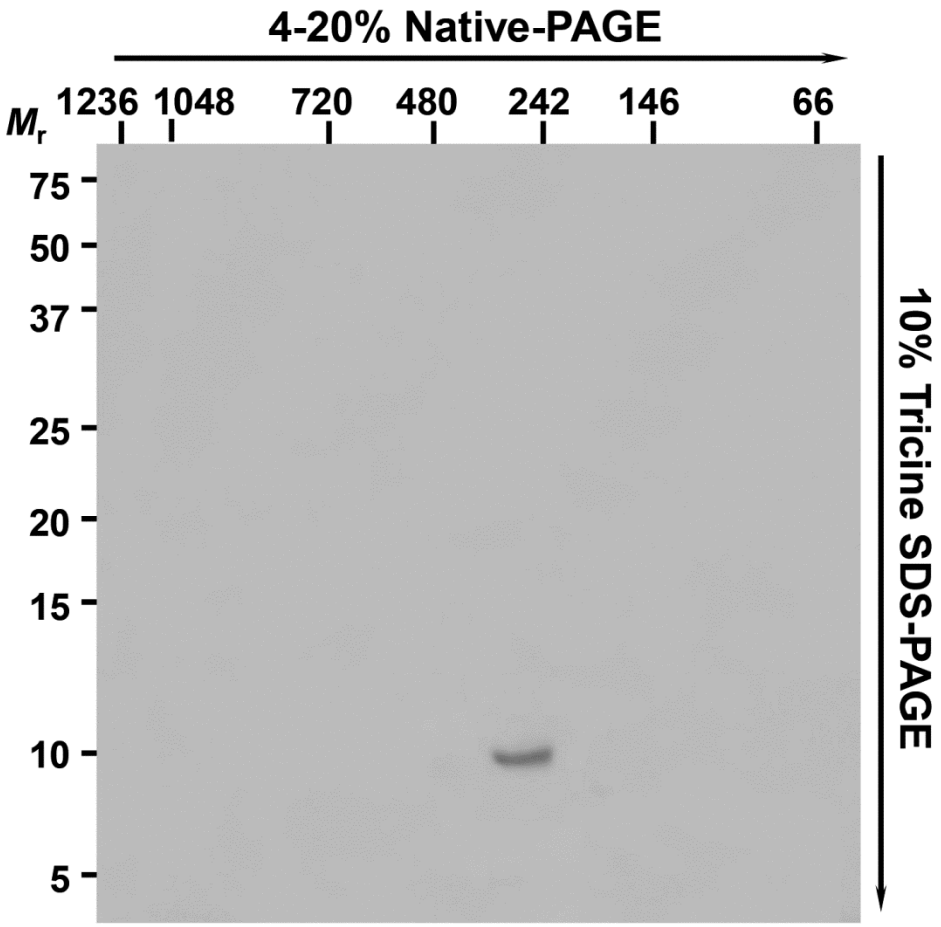

4-20% Native-PAGE
M_r
1236
1048
720
480
242
146
66
75
50
37
25
20
15
10
5
10% Tricine SDS-PAGE

Fig. S4

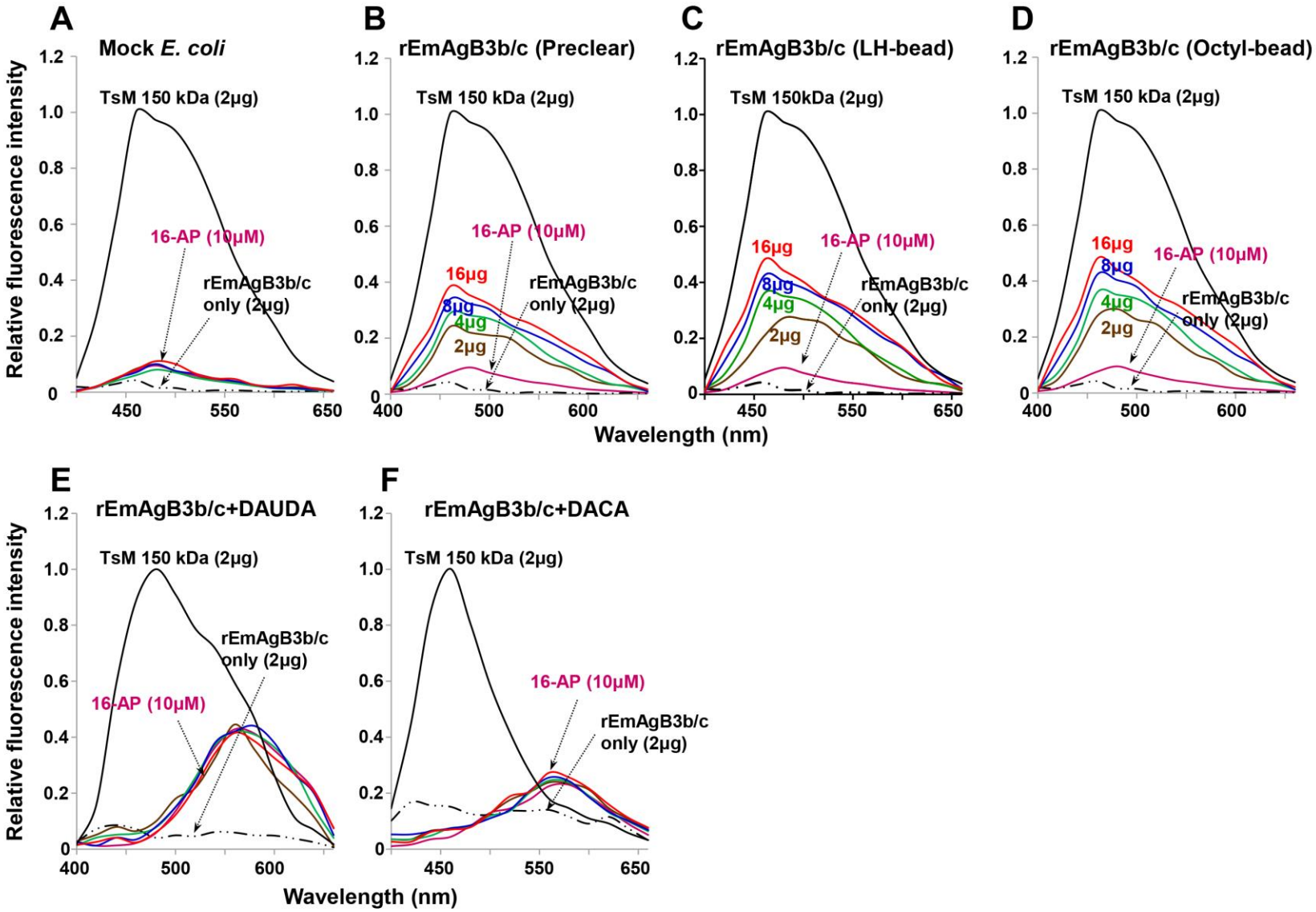

A
Mock E. coli
TsM 150 kDa (2μg)
16-AP (10μM)
rEmAgB3b/c only (2μg)
B
rEmAgB3b/c (Preclear)
TsM 150 kDa (2μg)
16-AP (10μM)
16μg
8μg
4μg
2μg
rEmAgB3b/c only (2μg)
C
rEmAgB3b/c (LH-bead)
TsM 150kDa (2μg)
16μg
16-AP (10μM)
8μg
4μg
2μg
rEmAgB3b/c only (2μg)
D
rEmAgB3b/c (Octyl-bead)
TsM 150 kDa (2μg)
16μg
8μg
16-AP (10μM)
4μg
2μg
rEmAgB3b/c only (2μg)
E
rEmAgB3b/c+DAUDA
TsM 150 kDa (2μg)
rEmAgB3b/c only (2μg)
16-AP (10μM)
F
rEmAgB3b/c+DACA
TsM 150 kDa (2μg)
16-AP (10μM)
rEmAgB3b/c only (2μg)
Relative fluorescence intensity
Wavelength (nm)

Fig. S5

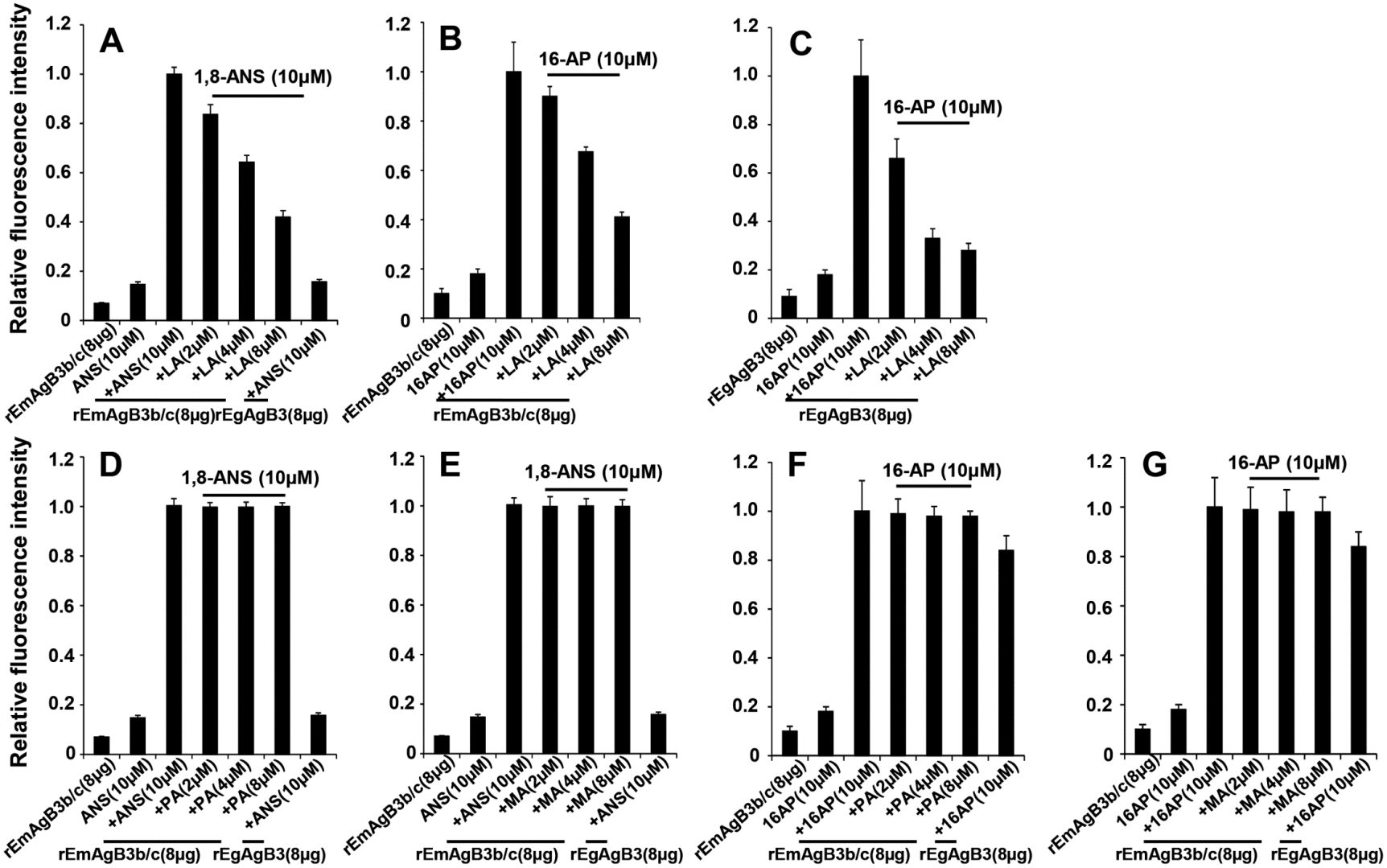

A
B
C
D
E
F
G
Relative fluorescence intensity
1,8-ANS (10μM)
16-AP (10μM)
rEmAgB3b/c(8μg)
ANS(10μM)
+ANS(10μM)
+LA(2μM)
+LA(4μM)
+LA(8μM)
rEmAgB3b/c(8μg)rEgAgB3(8μg)
16AP(10μM)
+16AP(10μM)
rEmAgB3b/c(8μg)
rEgAgB3(8μg)
+PA(2μM)
+PA(4μM)
+PA(8μM)
+MA(2μM)
+MA(4μM)
+MA(8μM)
rEmAgB3b/c(8μg) rEgAgB3(8μg)

Fig. S6

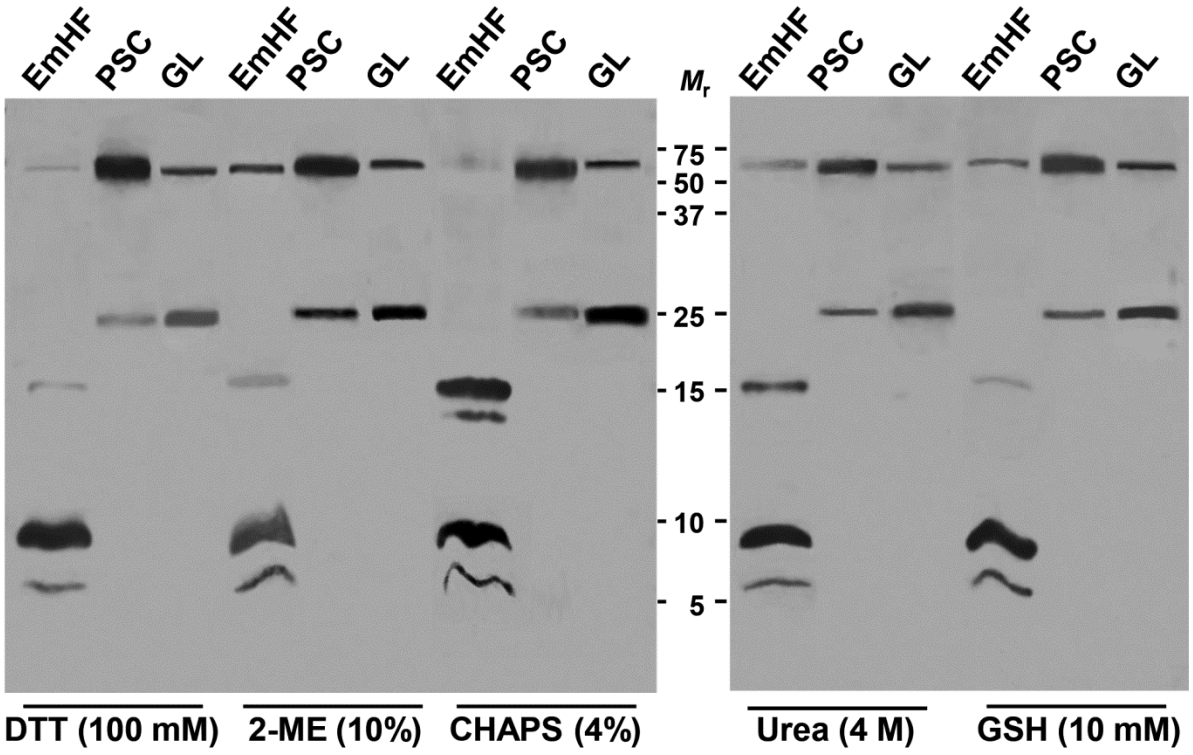

EmHF
PSC
GL
EmHF
PSC
GL
EmHF
PSC
GL
M_r
EmHF
PSC
GL
EmHF
PSC
GL
75
50
37
25
15
10
5
DTT (100 mM)
2-ME (10%)
CHAPS (4%)
Urea (4 M)
GSH (10 mM)

Fig. S7