

This is a repository copy of Skp is a multivalent chaperone of outer membrane proteins.

White Rose Research Online URL for this paper: http://eprints.whiterose.ac.uk/101494/

Version: Supplemental Material

Article:

Schiffrin, B, Calabrese, AN, Devine, PWA et al. (4 more authors) (2016) Skp is a multivalent chaperone of outer membrane proteins. Nature Structural and Molecular Biology, 23 (9). pp. 786-793. ISSN 1545-9993

https://doi.org/10.1038/nsmb.3266

(c) 2016, Nature American, Inc. This is an author produced version of a paper published in Nature Structural & Molecular Biology. Uploaded in accordance with the publisher's self-archiving policy.

Reuse

Unless indicated otherwise, fulltext items are protected by copyright with all rights reserved. The copyright exception in section 29 of the Copyright, Designs and Patents Act 1988 allows the making of a single copy solely for the purpose of non-commercial research or private study within the limits of fair dealing. The publisher or other rights-holder may allow further reproduction and re-use of this version - refer to the White Rose Research Online record for this item. Where records identify the publisher as the copyright holder, users can verify any specific terms of use on the publisher's website.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.



eprints@whiterose.ac.uk https://eprints.whiterose.ac.uk/

OMP	No. of β- strands	Molecular weight* (kDa)	Function	
tOmpA	8	18.875	Adhesin/invasin/evasin	
PagP	8	19.110	Acyl transferase	
OmpT	10	35.284 [‡]	Protease	
OmpF	16	37.216	Porin	
tBamA	16	43.240	Insertase/secretase	

Supplementary Table 1: Model OMPs investigated have a range of functions, sizes and number of β-strands. *Molecular weight of the mature OMP sequences, lacking their cleavable N-terminal signal sequences. [‡] Molecular weight for the OmpT construct used here including an N-terminal hexahistidine tag and a TEV protease cleavage site.

ОМР	OMP:Skp ratio	k₁ (x10 ⁻³ s ⁻¹)	$k_2(x10^{-3} s^{-1})$		
tOmpA	OMP alone	15.7 ± 0.6	N/A		
	1:1	11.1 ± 1.4	1.3 ± 0.1		
	1:2	No folding	No folding		
PagP	OMP alone	3.8 ± 0.8	0.8 ± 0.1		
	1:1	1.2 ± 0.3	0.1 ± 0.02		
	1:2	No folding	No folding		
OmpF	OMP alone	10.5 ± 0.6	0.5 ± 0.2		
	1:2	1.8 ± 0.2	N/A		
	1:4	No folding	No folding		
tBamA	OMP alone	12.8 ± 2.4	1.6 ± 0.3		
	1:2	7.6 ± 0.6	1.4 ± 0.4		
	1:4	No folding	No folding		

Supplementary Table 2: Measured rate constants for OMP folding into liposomes after pre-incubation with varying molar ratios of Skp. Data are shown as the mean ± the standard deviation of the rate constants obtained from three separate folding experiments, each using independently prepared batches of liposomes. For each batch of liposomes at least three folding transients were fitted globally to obtain the rate constants shown. N/A: The kinetic traces for the condition were adequately described by a single exponential.

Complex	Expected Mass (Da)	Observed Mass (Da)
Skp	47,075	47,125 ± 10
1:1 Skp:tOmpA	65,818	65,898 ± 12
1:1 Skp:PagP	66,185	66,208 ±15
1:1 Skp:OmpT	82,355	83,304 ± 20
2:1 Skp:OmpT	129,430	130,542 ± 14
1:1 Skp:OmpF	84,220	84,210 ± 18
2:1 Skp:OmpF	131,295	131,337 ± 11
1:1 Skp:tBamA	90,315	90,183 ± 5
2:1 Skp:tBamA	137,390	137,303 ± 8

Supplementary Table 3: Observed and expected masses for the complexes studied. For mass measurements, mass spectrometry conditions were optimised empirically for each sample to decrease peak widths, by increasing the cone voltage and trap collision energy, (typically 100 V and 40 V, respectively), relative to the gentler conditions used to acquire IMS data.

	Skp	1:1	Skp:tOmpA	1:1	Skp: PagP	1:1	Skp:OmpT	1:1	Skp:OmpF
z	CCS								
13	36.4 ± 0.2	15	45.6 ± 0.1	15	45.8 ± 0.3	17	51.4 ± 0.8	17	51.8 ± 0.3
13	37.9 ± 0.6	16	46.1 ± 0.3	16	45.8 ± 0.1	18	53.0 ± 0.4	18	52.8 ± 0.6
14	37.1 ± 0.2	17	46.5 ± 0.6	17	45.8 ± 0.2	19	53.9 ± 0.7	19	54.2 ± 0.2
14	38.8 ± 0.3	18	47.3 ±0.2			20	57.1 ± 0.2	20	55.1 ± 0.2
15	39.3 ± 0.6								
16	40.8 ± 0.6								
1:1	Skp:tBamA	2:1	Skp:OmpT	2:1	Skp:OmpF	2:1	Skp:tBamA		
z	CCS	Z	CCS	Z	CCS	z	CCS		
18	54.2 ± 0.3	22	71.7 ± 0.04	22	71.2 ± 0.5	22	72.8 ± 0.2	-	
19	55.6 ± 0.8	23	72.7 ± 0.03	23	72.1 ± 0.9	23	74.1 ± 0.1		
20	57.4 ± 0.6	24	74.4 ± 0.02	24	73.4 ± 0.4	24	75.5 ± 0.5		
		25	76.2 ± 0.4	25	75.1 ±0.1	25	75.7 ± 0.3		
				26	76.9 ± 0.6	26	78.20 ± 0.1		

Supplementary Table 4: Experimentally determined modal collision cross-sections (CCSs) (nm²) for the observed charge states (*z*) of Skp and the Skp assemblies. CCS values are shown as mean ± standard deviation of three independent measurements.

Protein(s)	Calculated CCS from simulation (nm ²)	CCS measured by IMS-MS (nm ²)
tOmpA alone	19.0 ± 0.06	-
tBamA alone	37.9 ± 0.9	-
1:1 Skp-tOmpA (<i>in vacuo</i>)	43.7 ± 1.2	45.6 ± 0.1
2:1 Skp:tBamA (in vacuo)	74.4 ± 1.4	72.8 ± 0.2
Skp (<i>in vacuo</i>)	37.3 ± 1.9	37.9 ± 0.6
1:1 Skp-tOmpA (H ₂ O)	56.5 ± 0.3	-
2:1 Skp:tBamA (H ₂ O)	101.2 ± 6.0	-

Supplementary Table 5: Comparison of CCS values from MD simulations and IMS-MS measurements. CCS values from IMS-MS are shown as the mean ± standard deviation of the modal value of the lowest charge state ion from three independent measurements. CCS values from the simulations are the mean ± standard deviation for the endpoint structures at 100 ns from three independent simulations. For globular assemblies, the error between measured CCS values and those computed from atomic coordinates is below 3% (Benesch, J.L. & Ruotolo, B.T. *Curr Opin Struct Biol* **21** 641-9, 2011).