Characterisation of an intrinsically disordered protein complex of Swi5-Sfr1 by ion mobility mass spectrometry and small-angle X-ray scattering

Kazumi Saikusa[1], Naoyuki Kuwabara[2], Yuichi Kokabu[1], Yu Inoue[1], Mamoru Sato[1], Hiroshi Iwasaki[3], Toshiyuki Shimizu[2], Mitsunori Ikeguchi[1], and Satoko Akashi[1]*

1. Graduate School of Nanobioscience, Yokohama City University
2. Graduate School of Pharmaceutical Sciences, University of Tokyo
3. Graduate School of Bioscience & Biotechnology, Tokyo Institute of Technology

Electronic Supplementary Information

1. **Building of a low-resolution model from X-ray structure**
2. **Examination of the CCS-calibration method**
3. **Analysis of CCS of the multiply-charged ions of Sfr1 and Sfr1C**

1. Building of a low-resolution model from X-ray structure

Table S1. Proteins subjected to construction of low-resolution dummy residue models

Protein name	Taxonomy	PDB entry	Molecular weight /kDa	# of Subunit
Ubiquitin	*Homo sapiens*	1UBQ	8.6	1
Cyrochrome C	*Equus caballus*	1HRC	12.4	1
Lysozyme	*Gallus gallus*	1DPX	14.4	1
Calmodulin	*Rattus rattus*	3CLN	16.9	1
Myoglobin	*Physeter catodon*	1VXG	17.9	1
Calmodulin	*Homo sapiens*	1WRZ	19.4	1
Swi5-Sfr1C	*Schizosaccharomyces pombe*	3VIR	23.9	2
Trypsinogen	*Bos taurus*	1TGN	24.0	1
Carbonic anhydrase I	*Homo sapiens*	1AZM	29.1	1
Lipase	*Burkholderia cepacia*	3LIP	33.2	1
Ovalbumin	*Gallus gallus*	1UHG	43.5	1
Transthyretin	*Homo sapiens*	1F41[a]	55.2	4
Avidin	*Gallus gallus*	1VYO[a]	58.6	4
Human serum albumin	*Homo sapiens*	1BJ5	67.7	1
Actin	*Oryctolagus cuniculus/ Bos Taurus*	1ATN	71.8	2
Conalbumin	*Gallus gallus*	1OVT	76.2	1

Protein name	Taxonomy	PDB entry	Molecular weight /kDa	# of Subunit
Creatine kinase	*Homo sapiens*	3B6R	86.2	2
Toroponin	*Homo sapiens*	1J1D	93.1	6
Concanavalin A	*Canavalia ensiformis*	1GKB[a]	103	4
Lactate dehydrogenase A	*Oryctolagus cuniculus*	3H3F	150	4

[a] Multimers were generated using symmetry information within a distance of 4 Å, obtained from the program PyMOL.

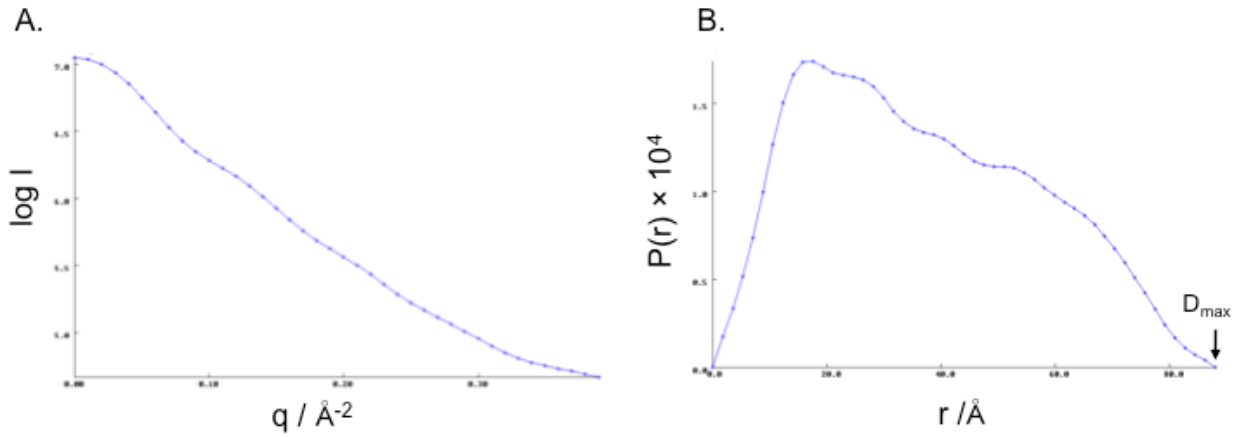

Fig. S1. Plots of (A) theoretical SAXS profile of the Swi5-Sfr1C complex obtained by CRYSOL and (B) pair-distance distribution function ([P(r)]) of the Swi5-Sfr1C complex.

2. Examination of the CCS-calibration method

Two calibration methods were examined. The first method used multiply-charged molecules of acid-denatured equine cytochrome c and equine myoglobin,[1-4] and the second method used multiply-charged protein molecules in native-like states, namely equine cytochrome c, bovine β-lactoglobulin A, egg avidin, bovine serum albumin, jack-bean

concanavalin A, human serum amyloid P, and yeast alcohol dehydrogenase, as reported by Bush et al.[4] A best fit power trendline ($y = ax^b$) of the corrected ATs versus the corrected published CCSs was obtained as a calibration curve for each calibrant set. The experimental CCSs of the Swi5-Sfr1 and the Swi5-Sfr1C complexes were calculated based on the equations of the calibration plots.

Table S2. Experimental and theoretical CCSs of the Swi5-Sfr1 and the Swi5-Sfr1C complexes.

	Experimental CCS/Å^2			Calculated CCS by MOBCAL			
	The calibration by		Structural analysis method	(a) PA method /Å^2	(b) Scaled PA value (a)×1.14/Å^2	(c) EHSS method /Å^2	(d) TM /Å^2
	Acid-denatured proteins	Native-like proteins					
Swi5-Sfr1C complex	2110 [a] (2640) [b]	1950 [a] (2430) [b]	X-ray	2401	2737	3034	2975
			SAXS	2428 ± 21	2768	– [c]	– [c]
Swi5-Sfr1 complex	3130 [a]	2880 [a]	SAXS	4879 ± 71	5562	– [c]	– [c]

[a] The experimental CCS values for the low charged ions (9+ for Swi5-Sfr1C and 13+ for Swi5-Sfr1) that were dominantly observed in the ESI mass spectra.
[b] The experimental CCS value for the 11+ charged ion (minor peak) of Swi5-Sfr1C, the intensity of which was about 40% of the main peak.
[c] Not available due to the absence of the atomic-level structure.

When a calibration curve obtained with globular protein calibrants was applied to the Swi5-Sfr1C and Swi5-Sfr1 complexes, ~10% smaller CCS values were obtained for the complexes compared with the CCS values obtained by calibration with acid-denatured proteins. Since SAXS analysis suggested elongated molecular shapes of the Swi5-Sfr1 and the Swi5-Sfr1C complexes (as discussed in text), we selected calibration with acid-denatured proteins.

3. Analysis of CCS of the multiply-charged ions of Sfr1 and Sfr1C

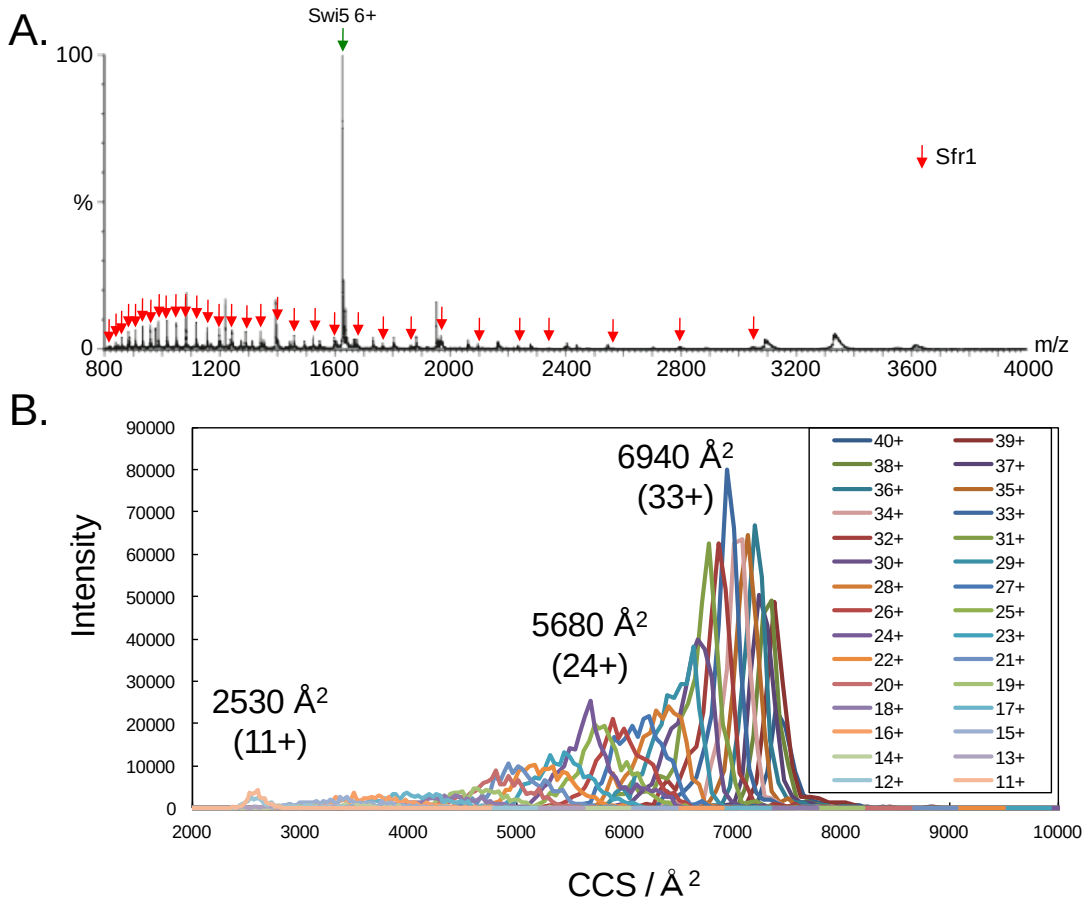

Fig. S2. ESI-IM-MS data of the Swi5-Sfr1 complex in 0.5 M NH_4OAc. (A) Mass spectrum of the Swi5-Sfr1 complex (*m/z* 800-4000), and (B) experimental CCS distributions of Sfr1. Red arrows in panel A indicate multiply-charged molecules of Sfr1. Ions of Sfr1 with charges in the range of 11+ to 40+ were observed. Panel B shows the CCS distribution of each ion of Sfr1. Three CCS values in panel B correspond to those for ions at the charge states of 11+, 24+ and 33+, respectively.

Since Sfr1 is expected to have a long disordered region, it is difficult to characterise the free-form structure by conventional analytical methods. However, in the present study, the CCS values of the free Sfr1 ions with various charges were obtained by ESI-IM-MS without isolation of the free Sfr1 protein. This presents a great advantage of ESI-IM-MS for the structural analysis of protein complexes containing ID regions.

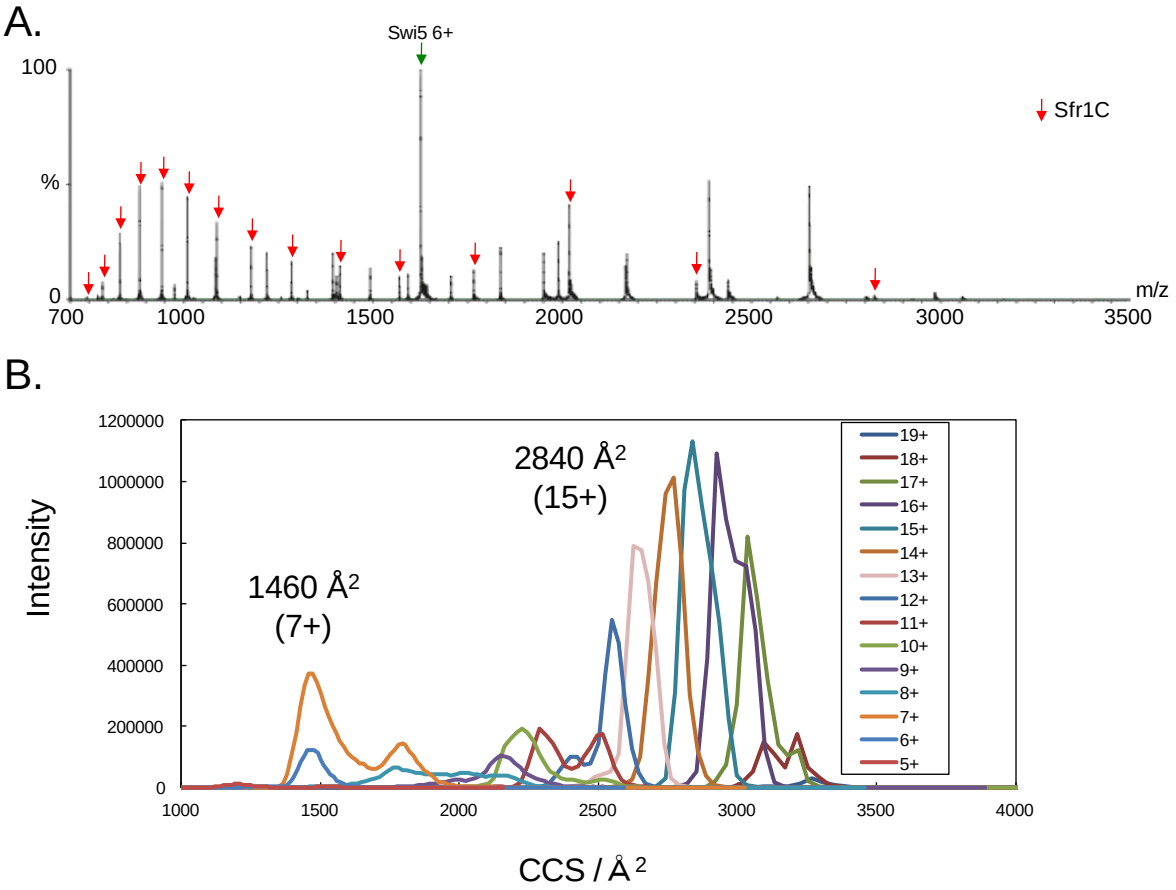

Fig. S3. ESI-IM-MS data of the Swi5-Sfr1C complex in 0.5 M NH_4OAc. (A) Mass spectrum of the Swi5-Sfr1C complex (*m/z* 700-3500), and (B) experimental CCS distributions of Sfr1C. Red arrows in panel A indicate multiply-charged molecules of Sfr1C. Ions of Sfr1C with charges in the range of 5+ to 19+ were observed. Panel B shows the CCS distribution of each ion of Sfr1C. Two CCS values in panel B correspond to those for ions at the charge states of 7+ and 15+, respectively.

References

1. B. T. Ruotolo, J. L. P. Benesch, A. M. Sandercock, S. J. Hyung, C. V. Robinson, *Nat. Protoc.*, 2008, **3**, 1139-1152.
2. D. E. Clemmer, *Cross Section Database*, http://www.indiana.edu/~clemmer.
3. K. B. Shelimov, D. E. Clemmer, R. R. Hudgins, M. F. Jarrold, *J. Am. Chem. Soc.*, 1997, **119**, 2240-2248.
4. M. F. Bush, Z. Hall, K. Giles, J. Hoyes, C. V. Robinson, B. T. Ruotolo, *Anal. Chem.*, 2010, **82**, 9557-9565.