

## **Newly developed Laboratory-based Size exclusion chromatography Small-angle x-ray scattering System (La-SSS)**

Rintaro Inoue<sup>1\*</sup>, Tatsuo Nakagawa<sup>2</sup>, Ken Morishima<sup>1</sup>, Nobuhiro Sato<sup>1</sup>, Aya Okuda<sup>1</sup>, Reiko Urade<sup>1</sup>, Rina Yogo<sup>3,4</sup>, Saeko Yanaka<sup>3,4</sup>, Maho Yagi-Utsumi<sup>3,4</sup>, Koichi Kato<sup>3,4</sup>, Kazuki Omoto<sup>5</sup>, Kazuki Ito<sup>5</sup> and Masaaki Sugiyama<sup>1\*</sup>

<sup>1</sup>*Institute for Integrated Radiation and Nuclear Science, Kyoto University, Kumatori, Sennan-gun, Osaka 590-0494 JAPAN.*

<sup>2</sup>*Unisoku Co. Ltd., 3-4-2 Kasugano, Hirakata City, Osaka 573-0131 JAPAN.*

<sup>3</sup>*Exploratory Research Center on Life and Living Systems (ExCELLS) and Institute for Molecular Science (IMS), National Institutes of Natural Sciences, 5-1 Higashiyama, Myodaiji, Okazaki, 444-8787, JAPAN.*

<sup>4</sup>*Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya, Aichi, 467-8603, JAPAN.*

<sup>5</sup>*Rigaku Corp. 12-9-3 Matsubara, Akishima City, Tokyo 196-8666 JAPAN.*

\*Corresponding authors. R. I. [rintaro@rri.kyoto-u.ac.jp](mailto:rintaro@rri.kyoto-u.ac.jp), M. S. [sugiyama@rri.kyoto-u.ac.jp](mailto:sugiyama@rri.kyoto-u.ac.jp)

### **This PDF file includes:**

Fig. S1, Fig. S2, Fig. S3, Fig. S4 and Table S1.

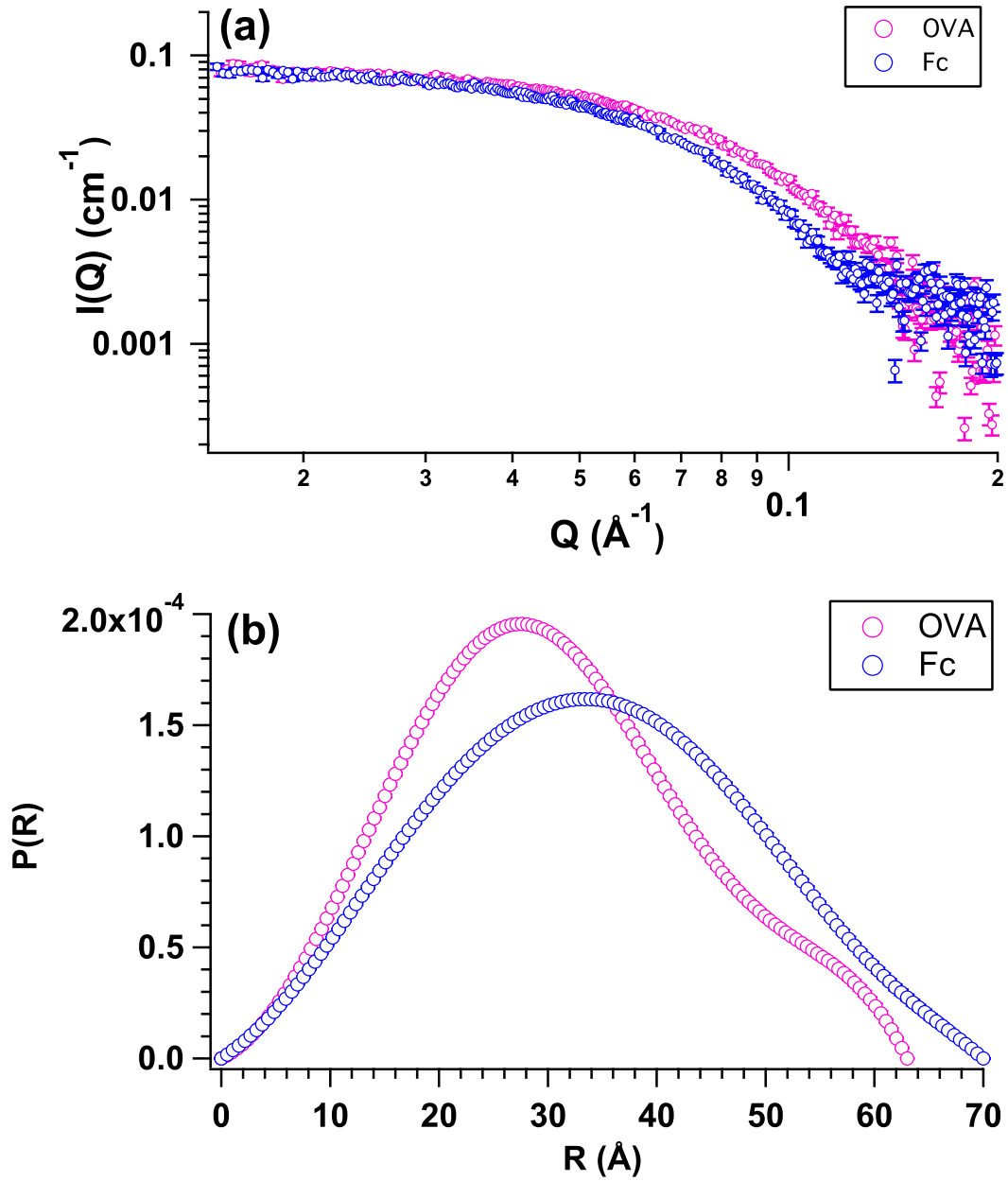


Fig. S1. (a) SAXS profiles of OVA and Fc. (b)  $P(R)$  functions of monomeric OVA and Fc.

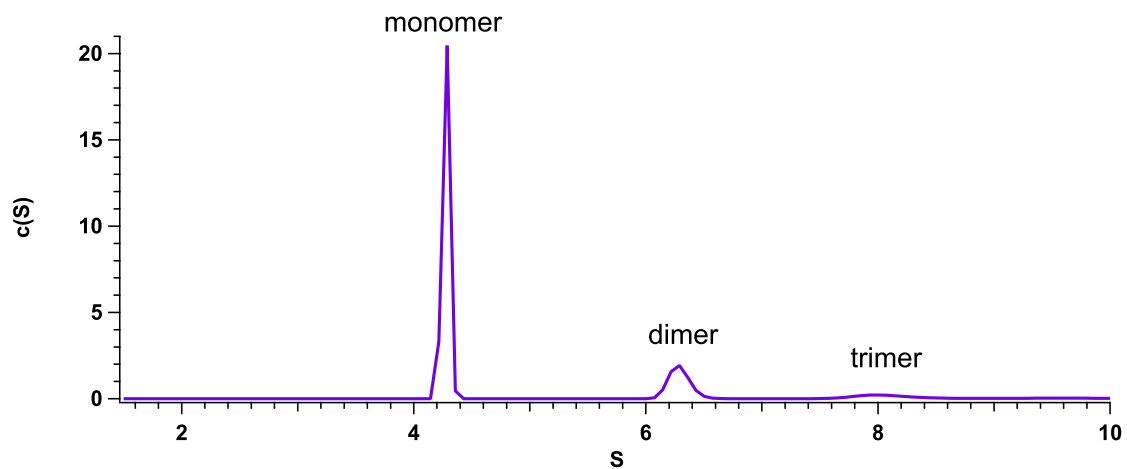


Fig. S2. The distribution of sedimentation coefficient ( $c(S_{20})$ ) of BSA solution at the concentration of 5.4 mg/mL. The peaks at around  $S_{20}=4$ ,  $S_{20}=6$  and  $S_{20}=8$  correspond to monomeric, dimeric and trimeric BSA oligomers, respectively.

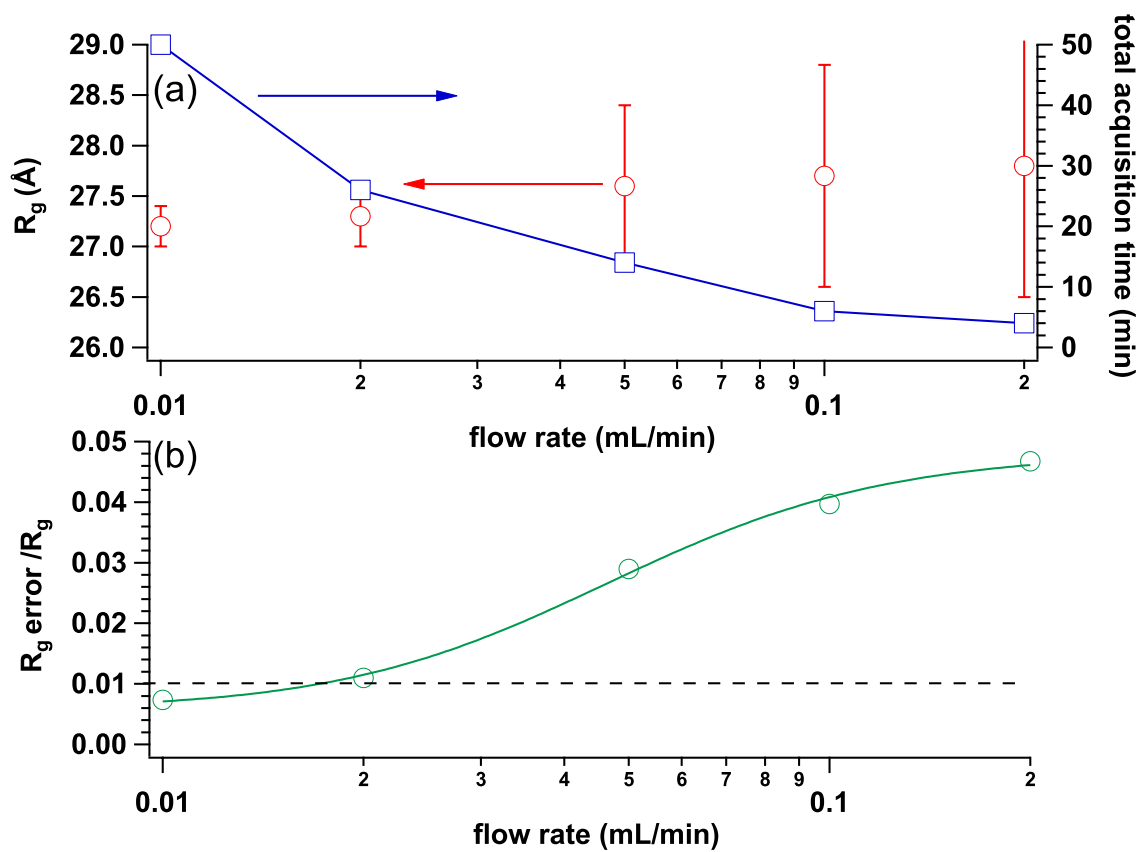


Fig. S3 (a) The dependence of  $R_g$  and total data acquisition time with flow rate. (b) The dependence of  $R_g$  error/ $R_g$  with flow rate and the solid curve was guided by eye.

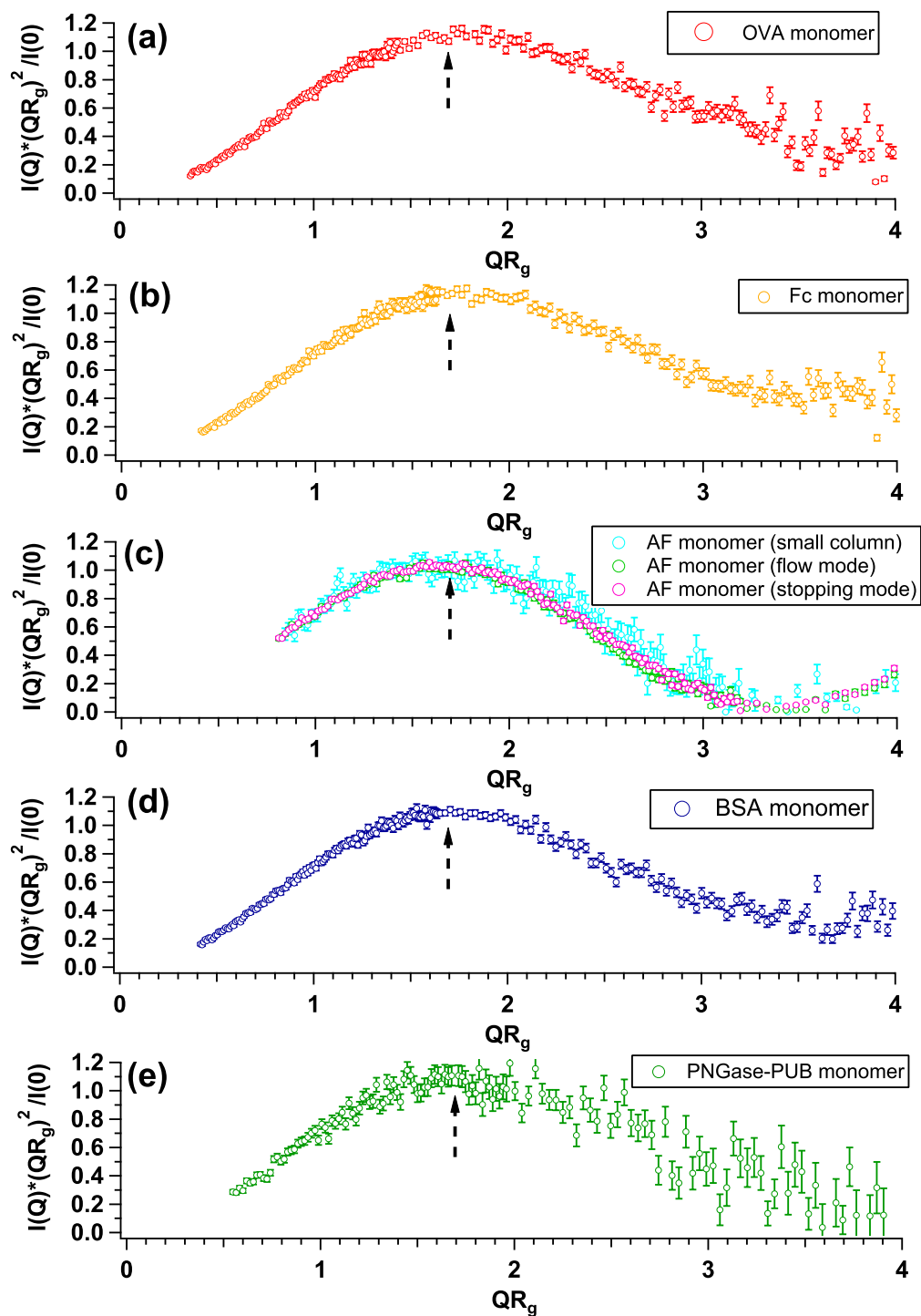


Fig. S4. Dimensionless Kratky plots of OVA monomer (a), Fc monomer (b), AF monomer (c), BSA monomer (d), PNGase-PUB monomer (e), respectively. In the panel (c), light blue, light green and pink circles measured by the small system, flow mode and stopping mode, respectively. Dotted arrows correspond to  $QR_g=1.7$ .

Detailed setup for La-SSS	Products and Manufacturer
HPLC-components	Degasser (DGU-20A, SHIMADZU Co., Ltd.) Pump (LC-20Ai, SHIMADZU Co., Ltd.) Column oven (CTO-20AC, SHIMADZU Co., Ltd.) Control bus unit (CBM-20A, SHIMADZU Co., Ltd.) UV detector (SPD-20A, SHIMADZU Co., Ltd.) Fraction collector (FRC-10A, SHIMADZU Co., Ltd.)
auto valve	VF21-1005 (FLOM Corp.)
SAXS cell	Flow cell (Unisoku. Co., Ltd.)
SAXS cell UV source	Deuterium light source for UV, SL3 (StellarNet Inc. )
SAXS cell UV detector	Compact UV spectrometer, Qmini2 UV (RGB Photonics GmbH)
Tubing	PEEK 0.13 mm (ISIS ltd.)

Table S1. Specifications of products concerned for La-SSS.