Report MX-2341

Unraveling of nsp14 interactions with nsp10 and various ligands

Samples were measured in SEC-SAXS mode at BM29/ESRF, Grenoble France on 12.12.2020. (session ID MX2341). Samples (100 µL) were measured on Agilent AdvanceBio SEC 300 with 50mM Tris-HCl pH 8.5, 150mM NaCl, 5mM MgCl2, and 2 mM β-mercaptoethanol running phase at 0.16 mL/min flowrate. The measurements were performed at 0.99 Å wavelength. The sample to detector distance was set at 2.83 m with Pilatus2M detector for data acquisition.

Guinier analysis of scattering profiles established that the largest radii of gyration (Rg) characterized the ternary complex (40.3±1.0 Å). Nsp14 and the nsp14/nsp10 complexes were characterized by significantly smaller Rgs (28.0±0.5 and 30.1±1.8 Å, respectively); the nsp10/16 complex was characterized by the smallest (Rg = 21.0±1.5 Å). This data corresponds with the expected molecular weights of tested complexes at 1:1:1 stoichiometry, further supporting the stoichiometry of the ternary complex.

The estimated molecular weights of a scatterer differ from the expected values, perhaps the result of the flexibility of the tested system (SAXS averages all conformations). Nonetheless, the relative values follow the expected pattern, with nsp10/16 characterized by the lowest and the nsp10/14/16 ternary complex by the highest molecular weight, as determined by SAXS.

When reconstructed in real space using the indirect Fourier transform software GNOM, the scattering profiles present roughly Gaussian shapes with significant tailing for nsp10/14/16 and nsp10/14, indicating an elongated globular nature for the protein complexes, with peaks overlapping with radii of gyration obtained using Guinier analysis. The calculated maximal distances within scatterers support the trend established above, with nsp10/16 constituting the smallest complex at 80.0 Å, nsp14 at 95.8 Å, nsp10/14 at 122.0 Å, and the nsp10/14/16 at 140.0 Å (longest axis).

Molecular envelopes which best represent the scattering profiles were calculated using DAMMIF software. Crystal structures of nsp10/16, nsp14 and nsp10/14 and a model of the ternary complex, created assuming the “lid” hypothesis, were fitted into the envelopes using the SUPCOMB software. The crystal structures of nsp14 and nsp10/14 fit the molecular envelopes poorly, suggesting that these two remain flexible in solution. The structure of nsp10/16 fills the envelope tightly, suggesting complex rigidity in solution. The initial model of the ternary complex already filled the envelope relatively well and was further optimized via normal mode analysis using the SREFLEX software. SREFLEX rotates and translates rigid body domains of the input model within the constraints of flexible loops, optimizing the fit to the experimental envelope. The resulting heterotrimer model was characterized by a value of 1.08 for goodness-of-fit to the experimental SAXS data, suggesting a likely solution. The model fits the envelope tightly, suggesting rigidification of nsp14 structure upon ternary complex formation (compare nsp10/nsp14 envelope fit).The decomposition of the triplex scattering profile into volume fractions calculated from the binary complexes nsp10/14, nsp10/16 crystal structures or extracted individual protein in the software Oligomer suggest that the signal can be divided 1:1 into nsp10/16 and nsp14. This further implies that nsp10/16 interface within the triplex is retained, while it is nsp14 that

undergoes structural rearrangements upon the ternary complex formation.

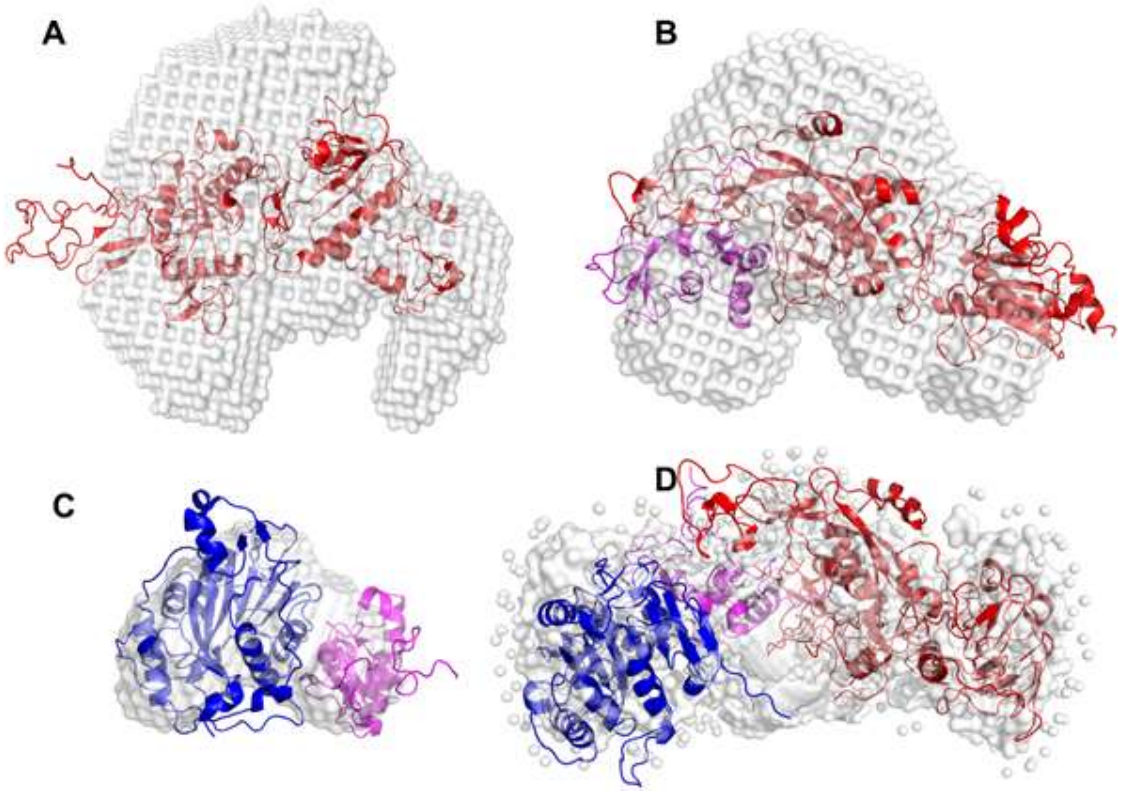

Figure 1 Molecular envelopes representing the experimental SAXS scattering profiles of nsp14 (A), nsp10/14 (B), nsp10/16 (C), nsp10/14/16 (D). Overlaid are best fits of crystallographic / theoretical models of relevant complexes. Color coding: Nsp10 in magenta, nsp14 in red, nsp16 in blue, molecular envelopes in grey.

a. Sample details				
	nsp14	nsp10/14	nsp10/16	nsp10/14/16
Source organism	*Severe acute respiratory syndrome coronavirus 2*			
Source	*E. coli BL21*			
UniProt sequence ID (residues in construct)	P0DTD1 (5926-6452)	P0DTD1 (5926-6452)	-	P0DTD1 (5926-6452)
	-	P0DTC1 (4254-4392)	P0DTC1 (4254-4392)	P0DTC1 (4254-4392)
	-	-	P0DTD1 (6799-7096)	P0DTD1 (6799-7096)
Molecular weight from chemical composition (kDa)	60	75	48.5	108.5
SEC–SAXS column	AdvanceBio Bio SEC 300 column			

Loading concentration (mg/mL)	4	3.5	2.6	2.8
Injection volume (µL)	100			
Flow rate (mg/mL)	0.16			
Running phase composition	50mM Tris, 150mM NaCl, 5mM $MgCl_2$, βME 2mM pH8.5			
b. SAXS data-collection parameters				
Instrument	BM29, ESRF, Grenoble France			
Detector	Pilatus2M			
Wavelength	0.99 Å			
Sample to detector distance	2.83 m			
c. Structural parameters				
	nsp14	nsp10/14	nsp10/16	nsp10/14/16
Guinier analysis				
I(0) (cm^{-1})	8.43±0.13	3.22±0.13	1.73±0.09	5.26±0.11
R_g(Å)	28.0±0.5	30.1±1.8	21.0±1.5	40.3±1.0
qR_g($Å^{-1}$)	0.59-1.51	0.59-1.32	0.52-1.39	0.76 – 1.4
P(r) analysis				
R_g(Å)/ I(0) (cm^{-1})	29.4/8.61	32.5/3.28	21.1/1.64	41.2/5.23
Guinier R_g(Å)/ I(0) (cm^{-1})	29.4/8.61	32.7/3.28	21.2/1.64	41.5/5.23
r_{max}(Å)	95.8	122	80	140
Total quality estimate	0.94	0.78	0.75	0.77
Molecular weight estimate/predicted (kDa)	33.1/60	28.09.1975	20.6/48.5	83.2/108.5
Oligomerization state	monomeric			
d. Shape model-fitting results				
DAMMIF (10 runs)	nsp14	nsp10/14	nsp10/16	nsp10/14/16
q_{max} range for fitting($Å^{-1}$)	0.26	0.26	0.21	0.17
Symmetry, anisotropy assumptions	P1, none			

NSD (standard deviation)	1.42(0.07)	1.10(0.07)	1.29(0.14)	0.80(0.08)
Chi-squared	1.16	1.09	1.07	1.08
Resolution (from SASRES) (Å)	43±3	39.3±3	33.3±3	38.3±3
SASDBD IDs	SASDKT6	SASDKU6	SASDKV6	SASDKW6
e. Oligomer volume fractions				
volume fractions Chi^2 1.06				
6yz1 (nsp10/16)	nsp16	nsp14	nsp10	5c8u (nsp10/14)
53%	0%	47%	0%	0%

Data is now used in a preprint:

https://doi.org/10.1101/2022.01.25.477673