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Supporting Information

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Stability and Shape of Hepatitis B Virus Capsids *in vacuo*

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Supporting Information

Proteins

HBV capsids of cp149 $3^{C\rightarrow A}$ and cp149 61^{C} for mass spectrometry were purified as described previously [1].

Mass spectrometry

High resolution tandem mass spectra were recorded on a modified Q-ToF 1 instrument (Waters, Manchester, UK) [2, 3]. The voltages and pressures were optimized for large noncovalent protein complexes [4]. Ion mobility measurements were performed on a Synapt HDMS (Waters, Manchester, UK) [5]. Separation in the ion mobility device was adjusted to measure proteins below and above 100 kDa. To correlate the data, GroEL single ring (SR) and PCNA were analyzed with both settings. In all cases, the ion mobility chamber contained nitrogen. For proteins below 100 kDa Argon was used as collision gas, whereas Xenon was preferred for larger proteins [6]. Xenon was also used to measure HBV capsids with the modified Q-ToF 1. The measured drift times were corrected for the dead time before the pusher. The cross-section (Ω) is obtained from the absolute drift time according to equation (1) with calibration on known cross-sections [5, 7, 8].

$$\Omega = K \times t_D^{0.52} \times z \times \sqrt{\frac{1}{M_{ion}} + \frac{1}{m_{gas}}} \qquad (1)$$

Where, K is the calibration constant, t_D is the absolute drift time, i.e. corrected for the dead time before the pusher, z represents the charge of an ion, M_{ion} and m_{gas} are the

masses of the ion and the gas used in the ion mobility device. Mass and charge of an ion were determined from the corresponding mass spectra. Cross-sections were calculated for each charge state and then averaged.

A list of the analyzed proteins and their average cross-sections is given in Table S1. The cross-sections were determined from three independent measurements and for most proteins with two different separation settings, a fixed and ramped wave height in the ion mobility chamber. The fixed wave height resulted in larger cross-sections for high mass proteins, but no systematical error could be observed in the dataset. The cross-sections of fixed and ramped wave height show a good correlation (Figure S1) indicating both settings might be used for determination of cross-sections. Drift time peaks were narrower and the standard deviations in cross-sections smaller with a ramped wave height, especially for proteins which left the ion mobility chamber at the end of a duty cycle. Therefore, we used these settings for the large viral capsids. The deviation in cross-section over all charge states for small proteins is sometimes quite large because of the large difference between the conformers. Cross-sections of an individual charge state deviated usually less than 1 % indicating the good reproducibility of the results. The calibration constant and correlation coefficient for the small and large datasets showed a relative standard deviation of less than 4 %. The performance of the instruments was tested with CsI and a calibration was applied where necessary.

The HBV capsids formed of cp149 $3^{C\rightarrow A}$ and cp149 61^{C} were introduced to the mass spectrometer at 6 - 8 μM monomeric concentrations in 200 mM ammonium acetate, pH 6.8 [9]. All other proteins were sprayed from 50 mM ammonium acetate (pH 6.8) at a concentration between 2 and 10 μM regarding the monomers. Capillaries for electrospray

ionization were prepared in house from borosilicate glass tubes of 1.2 mm OD and 0.68 mm ID with filament (World Precision Instruments, Sarasota, FL) using a P-97 micropipette puller (Sutter Instruments, Novato, CA) and gold-coated using an Edwards Scancoat six Pirani 501 sputter coater (Edwards Laboratories, Milpitas, CA). Capillary tips were opened on the sample cone of the instrument. For ion mobility, capillaries purchased from Waters (Manchester, UK) were used.

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Table S1: Comparison of capsid conformers from ion mobility. Relative amounts of small conformer and difference in cross-section (ΔΩ) between large and small conformers for the different variants (in %). Values are averaged over all detected charge states at particular collision voltages (cv) and the standard deviation is given. The standard deviation for the amount of the geometry $T = 4$ small conformer at 200 V is high, due to overlap with fragmentation products of $T = 3$ capsids. In the case of $T = 3$ at 200 V the standard deviation is high, because highly charged ions fragment easier than less highly charged ions. As the stabilities of the small and large conformer differ, their ratio becomes charge state dependent upon fragmentation. This is also evident from the amount of the small conformer in $T = 3$ capsids missing a dimer. While increasing the collision energy from 175 V to 200 V the amount of small conformer over all charge states is reduced from 61 ± 9 % to 43 ± 11 % indicating that the small conformer has already lost another dimer.

cp149	cv/V	Small/%		ΔΩ/%	
		$T = 3$	$T = 4$	$T = 3$	$T = 4$
$3^{C\rightarrow A}$	75	79 ± 4	82 ± 4	4.4 ± 0.4	4.5 ± 0.3
	100	80 ± 5	79 ± 6	4.3 ± 0.5	4.6 ± 0.3
	150	74 ± 6	81 ± 5	4.4 ± 0.6	4.5 ± 0.3
61^{C}	100	75 ± 3	82 ± 4	4.1 ± 0.5	4.4 ± 0.5
	150	74 ± 2	82 ± 2	4.3 ± 0.4	4.4 ± 0.3
	175	69 ± 4	79 ± 4	4.4 ± 0.6	4.5 ± 0.6
	200	60 ± 11	77 ± 10	4.5 ± 0.4	4.2 ± 0.6

Table S2: Absolute cross-sections (Ω) of the proteins and protein complexes analyzed under near "native" *in vacuo* conditions.

Given are the protein name, the number of subunits (*N*), the determined mass (*m*) and cross-section (Ω) with standard deviation (± SD) as determined with fixed (fix) and ramped (ramp) wave height in the ion mobility chamber. HBV cross-sections are averaged over all measurements regardless of the applied acceleration energy. Synapt HDMS settings were different for small ([a]) and large ([b]) proteins and the cross-sections are therefore correlated on basis of the cross-section of GroEL SR. nd – not detected

Protein	*N*	*m*/kDa	Ω /nm^2			
			Fix	± SD	Ramp	± SD
GroEL SR, *E. coli* [b]	7	399.8	163.1	9.6	144.6	8.7
Holomyoglobin, horse heart [a]	1:1	17.6	22.9	3.1	20.7	4.4
Holomyoglobin, horse heart [a]	2:2	35.2	nd	nd	32.1	4.4
Apomyoglobin, horse heart [a]	1:1	17.0	20.5	4.8	20.5	3.7
Lysozyme, hen egg white [a]	1	14.3	14.1	2.1	14.8	2.3
Lysozyme, hen egg white [a]	2	28.7	nd	nd	20.3	0.9
Carbonicanhydrase II, bovine [a]	1	29.1	26.3	4.3	25.8	3.5
Ubiquitin, bovine [a]	1	8.6	10.5	2.1	11.3	2.6
Im9, *E. coli* [a]	1	9.6	10.8	1.6	12.0	2.0
Im9, *E. coli* [a]	2	19.2	18.8	1.2	19.2	0.9
E9, E. coli [a]	1	15.1	16.4	3.3	17.7	4.4
E9 (Zn^{2+}), E. coli [a]	1:1	15.2	17.2	3.5	17.6	3.9
E9 (Ni^{2+}), E. coli [a]	1:1	15.1	15.7	2.9	16.4	3.5
E9 (Ni^{2+}):Im9, E. coli [a]	1:1:1	24.7	22.9	2.9	22.6	3.6
E9:Im9, E. coli [a]	1:1	24.7	22.8	3.0	24.5	2.4
PCNA, human [b]	3	86.4	56.0	7.2	53.7	4.3
PCNA, human [a]	1	28.8	26.2	3.2	25.7	3.4
Hemoglobin, bovine [a]	2:2:4	64.5	58.7	5.2	52.4	5.5
Heme [a]	1	1.3	nd	nd	2.7	2.1e-2
Heme-Na [a]	1:1	1.2	2.5	1.4e-2	2.6	1.4e-2
P22 gp4 [a]	1	18.3	21.9	3.1	20.3	3.6
GroEL, *E. coli* [b]	14	801.0	284.3	5.4	229.5	5.0
VAO, *Penicillium simplicis.* [b]	8	508.5	207.7	4.9	173.9	5.4
V_1-ATPase (subunit A), yeast [b]	1	67.6	44.4	2.6	43.6	3.3
V_1-ATPase III, yeast [b]	11	427.9	172.4	3.7	144.6	5.7
V_1-ATPase I, yeast [b]	13	592.8	233.7	6.1	185.0	5.9
P22 gp1 [b]	1	69.2	44.2	4.1	43.1	3.5
$T = 3\ 3^{C\rightarrow A}$ (small conformer) [b]	120	3006.5			683.6	6.3
$T = 3\ 3^{C\rightarrow A}$ (large conformer) [b]	120	3006.5			713.1	6.4
$T = 4\ 3^{C\rightarrow A}$ (small conformer) [b]	240	4016.2			869.0	6.2
$T = 4\ 3^{C\rightarrow A}$ (large conformer) [b]	240	4016.2			908.6	6.5
HBV cp149 $3^{C\rightarrow A}$ [b]	1	16.7			17.8	3.4
$T = 3\ 61^{C}$ (small conformer) [b]	120	3012.4			687.4	8.4
$T = 3\ 61^{C}$ (large conformer) [b]	120	3012.4			717.3	9.0
$T = 4\ 61^{C}$ (small conformer) [b]	240	4018.9			875.4	5.7
$T = 4\ 61^{C}$ (large conformer) [b]	240	4018.9			913.5	6.8
HBV cp149 61^{C} [b]	2	33.4			30.0	3.4

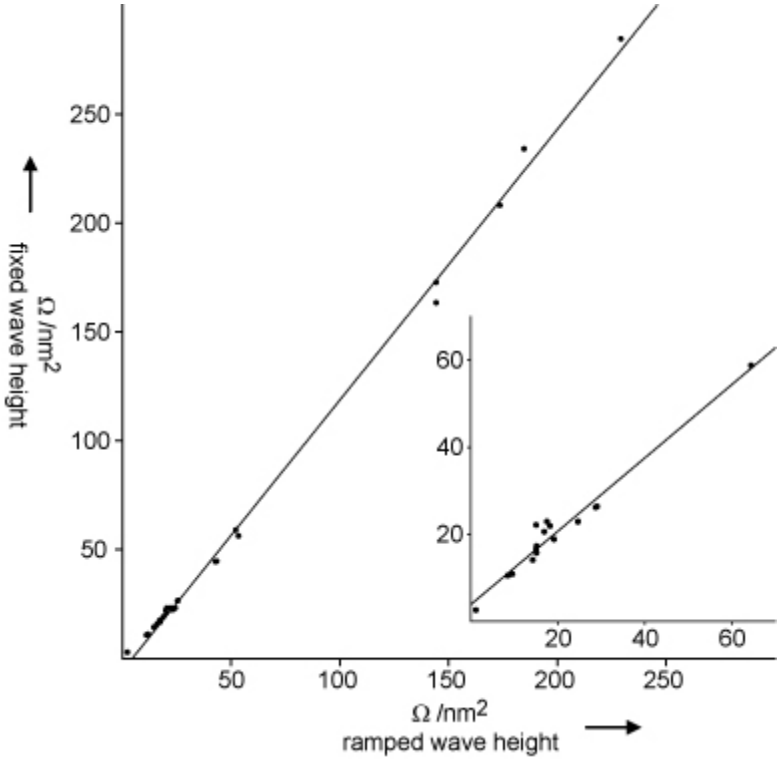

Fig. S1: Cross-sections (Ω) determined with a fixed or ramped wave height are comparable. They show a linear correlation. No systematical errors are observed. Cross-sections of large proteins measured with a fixed wave height are somewhat larger, as the cross-section for GroEL single ring, which was used for correlation of the small and large datasets, was already greater than in the ramped wave height dataset. The inset shows a zoom in on proteins below 100 kDa which were analyzed with identical fixed and ramped wave height settings.