Confidential										
Title: Charaterization of AAV particles using cryp electron microscopy (CrypEM)										
Project #: Sample arrival: Analysis: Sa		Sample:	vi)							
2015-1117R-AR-VAB (S1) 2015-		2015-11-17	cryoEM	Sample 1						
Image a	nalysis: <u>Morph</u>	ology								
Sample pr	eparation (SOP V0074	4-1) and EM imaging (	SOP V2465-1)							
Dilution:	undiluted	Buffer:	n/a	Temperature:	-70 °C S	tain: 	n/a		5004	
Represen	tative Image (low	magnification)	Yes	TEIVI:	Representati	ve Image (	intermediat	e magnificat	ion)	
					50	D nm				
AMT-060_AAVUQ_RD_20k_qc_1x_140312_003.tif AMT-060_AAVUQ_RD_38k_qc_1x_140312_005.tif										
					Saba				Dioomorphic	
	000	200 0	o 8 (	86) Ø:	( ( ) ( m o m b o la	Sprier				Pleomorphic
	, 0. e		00,00		AAV morphoic	ogy 🔽				
e'	0° %0	0 20,60	00 0	0	Broken AAV	<1 _		50		>90
200	Quant		0.8	00	Small AAV clus	ster 🗸				
0			6	NO O	Large AAV aggregates					
10	0		Z °	do	Background de	ebris 🗸				
1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	6 00 000 0 000 0			6	Proteasomes					
	AMT-060_/	AAVUQ_RD_50k_qc_1x_140	200 312_006.tif							
Commen	ts									
Cryo-EM analysis showed a relatively high concentration of evenly distributed AAV particles with an approximate size of 22 nm. The majority of AAV particles displayed a										

Cryo-EM analysis showed a relatively high concentration of evenly distributed AAV particles with an approximate size of 22 nm. The majority of AAV particles displayed a distinct outer shell and minute internal density, characteristic of empty particles (white arrows), while a smaller subpopulation of particles displayed an inner density with no distinct boundary between the shell and the core, characteristic of filled particles (black arrows), see inset in special observation. Neither particle aggregation nor minor clustering could be detected on the grid. Some degree of ice contaminations (white dashed arrow), which likely arise during specimen preparation or transfer of the grid to the cryo-holder, are occasionally observed. From top image, scale bars represent 1000, 500 and 200 nm respectively.

Analysis Project Manager: Mathieu Colomb-Delsuc

Reviewed by: Emmanuel Tupin





## Comments

Cryo-EM images acquired at intermediate magnification (see original image versus detected image with the detected AAV particles over layered with red circles) were subjected to internal density analysis. Principal component analysis of each AAV particle's radial density profile revealed two separable clusters corresponding to the level of packaging i.e. full (red) versus empty (blue) particles. The dashed rings in the cluster plot correspond to the 99% confidence interval of the different particle classes. Scale bar represents 500 nm.

Analysis Project Manager: Mathieu Colomb-Delsuc

Reviewed by: Emmanuel Tupin

