Investigation of the Relationship of the Shape and Size of Glycoprotein and It Function

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Abstract: *The resaerch investigates the Investigation of the relationship of the shape and size of glycoprotein and it function. It was found from this test that fullness was not related particle size or the amount of GP but empty particles are more elliptical than normal or dense particles. So RNA or NP only explain some of the variability in virus shape, but not the relationship between shape and size. Since RNA and NP density does not change during fusion activation, RNA and NP are not good explanation for the sudden change in shape. Dense particles are round, Some empty particles are elliptical but fullness does not change with fusion activation. It was further revealed that The correlation coefficient for LCMV, TCRV and PICV were all below the level that would be considered statistically meaningful or should small contradictory effects (Table 4.1) Most virions were covered with GPs but a few did not appear to have any. From this data it was concluded that GP density does not affect virus shape.*
Keywords: *Investigation, Relationship, shape and size of glycoprotein, function*

4.1 Introduction:

In order to understand virus shape and size and the role of glycoproteins on arenavirus shape and size. GPs were counted and measured its space from one GP to another adjacent GP. Each of virion has glycoproteins (GPs) on the edge of lipid bilayer of virion of arenavirus. As it was discussed in previous chapter 3 that viral proteins play pivotal role in determining particle shape and size. In this chapter, the role of the glycoprotein in virus particle morphology is explored. To do this glycoproteins were counted from the virion of LCMV, PICV and TCRV. To help explain the role of GP in proteins inside the virion, underneath GP were refined.

4.2 Class Average (LCMV and JUNV)

In order to understand about the hypothesis that GPs have a role on structure of viruses. The cryo-EM was done to know about the role of GPs in virus shape and size. GPs were counted and picked using EMAN (boxer) program [156] of LCMV (See figure 4.1) and JUNV (see figure 4.2) and performed reference free alignment and clustering to produce a series of class averages, each representing the common structural features of part of the input dataset to see how they appear in virion. They are organized as complexes with GPs on the edge of virion then lipid bilayer and Z and NP aligned underneath.

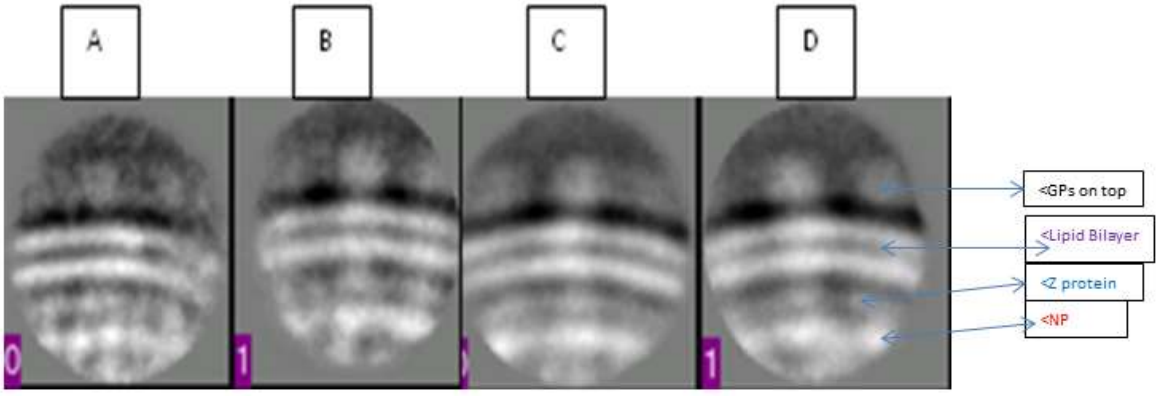

Figure 4.1. Surface features as well as inner densities. (A and B) Class averages of LCMV showing GP, LB, Z and NP attached from Top to bottom of the NR class generated images. (C and D) same as of A and B but different group of Classes.

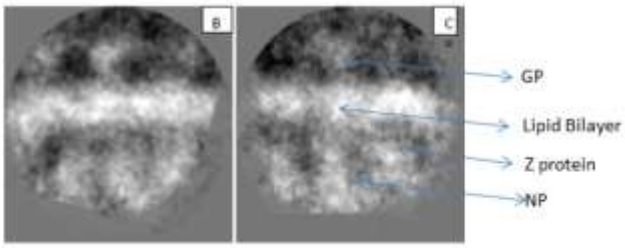

Figure 4. 2. First structural analysis on the Junin virus GP, NP and Z (B and C) From top to bottom NR class generated image of GP, Lipid Bilayer (LB), Z and NP attached in the virion.

The average spacing between adjacent GPs at the virion edge was reported previously by Neuman *et al* [120]. This spacing was used, together with the length of the virion parameter to calculate the number of GPs expected at the virion edge for each virion. This quantity was termed GP% and is described below in table 4.1.

Table 4.1. Standard deviation and correlation coefficient of arenavirus shape, size, core edge is shown in table below.

Virus		Average	No of GP	Correlation					
						Shape		Size	
					SD	R^2	P	R^2	P
PICV	All	0.70	475	±	0.16	<0.01	0.49	<0.01	0.54
	0-900	0.71	80	±	0.26	8E-05	0.87	0.01	0.13
	900+	0.66	395	±	0.22	0.02	<0.01	0.02	<0.01
TCRV Native	All	0.79	683	±	0.15	0.01	0.01	0.016	<0.01
	0-900	0.76	239	±	0.15	<0.01	0.79	0.05	<0.01
	900+	0.80	444	±	0.14	0.01	0.03	<0.01	0.55
LCMV Native	All	0.93	2230	±	0.44	0.01	<0.01	<0.01	0.01
	0-900	0.92	1373	±	0.42	0.00	0.15	0.01	<0.01
	900+	0.96	857	±	0.46	0.02	<0.01	0.01	0.01

From this data it was concluded that coverage was not significantly related to virus shape or size across the Arenaviridae. The correlation coefficient for LCMV, TCRV and PICV were all below the level that would be considered statistically meaningful or should small contradictory effects (Table 4.1) Most virions were covered with GPs but a few did not appear to have any. From this data it was concluded that GP density does not affect virus shape.

4.3 Spacing of GP complexes.

In order to understand actual distance between two adjacent GPs at the edge of the native three kinds of arenavirus i-e LCMV, TCRV and PICV, the boxer program was used to measure the distance between two of them. It was found that GPs are spaced about 90Å apart. Results are shown in figure 4.3 and 4.4.

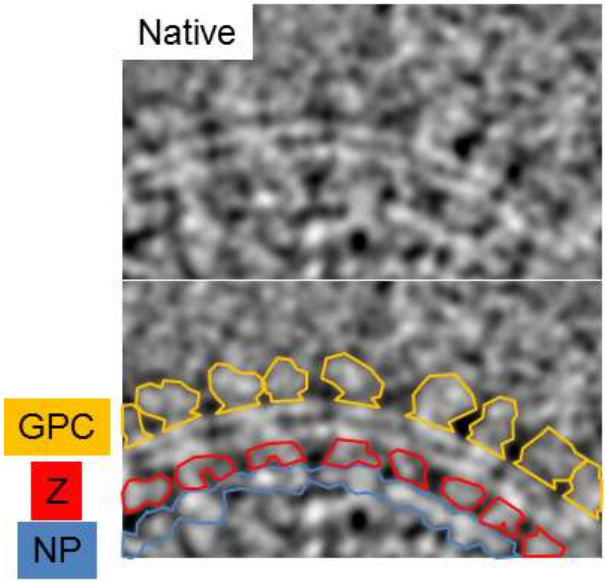

Figure 4.3 Image of LCMV particles with evenly spaced GPC projections highlighted.

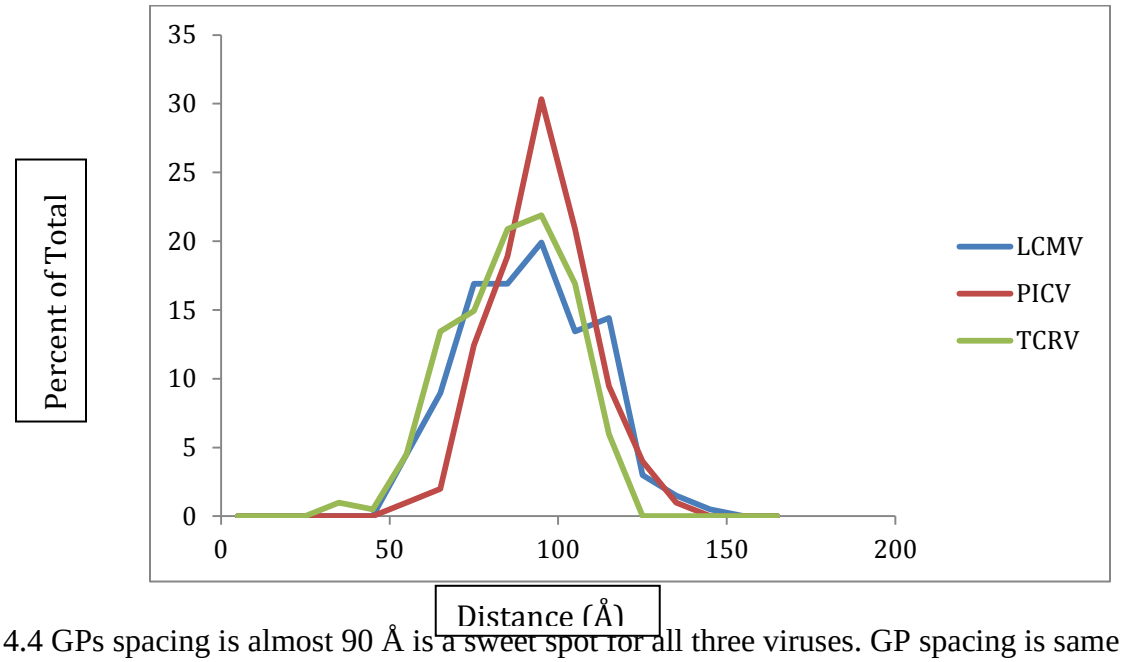

Figure 4.4 GPs spacing is almost 90 Å is a sweet spot for all three viruses. GP spacing is same in all three viruses.

4.4.Size vs GP all:

In order to illustrate the relationship between GP% and size, the data was selected and then plotted the scatter plots and concluded that there is no consistent relationship with size. GP% has consistant relationship over the size (see figure 4.5).

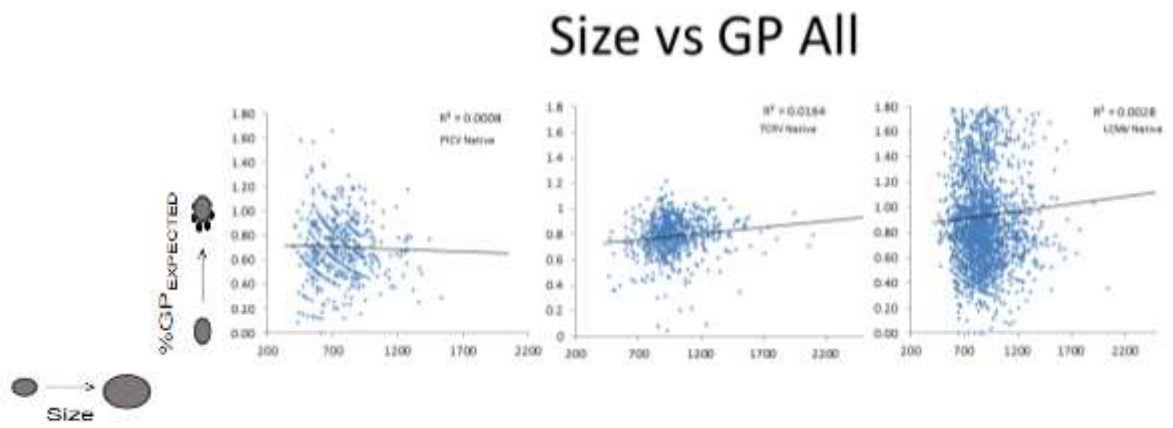

Figure 4. 5 Scatter plots are showing size vs glycoprotein % .

In order to understand whether GP% has a relationship with shape, the data was selected and then plotted a scatter plot and concluded that there is no consistent relationship with shape (figure 4.6). Slightly higher GP% on large, elliptical particles of LCM and TCRV were contradicted by the opposite result for PICV. However, it is possible that the PICV result is an error due to small sample size, because PICV particles were found to be uniformally round as reported in chapter 3.

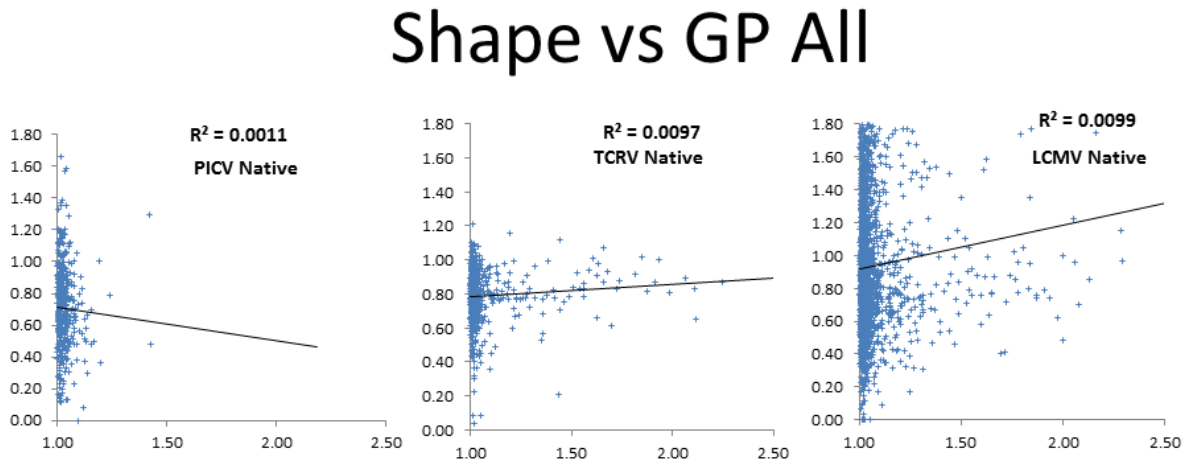

Figure 4.6. Scatter plot is showing shape versus glycoprotein all.

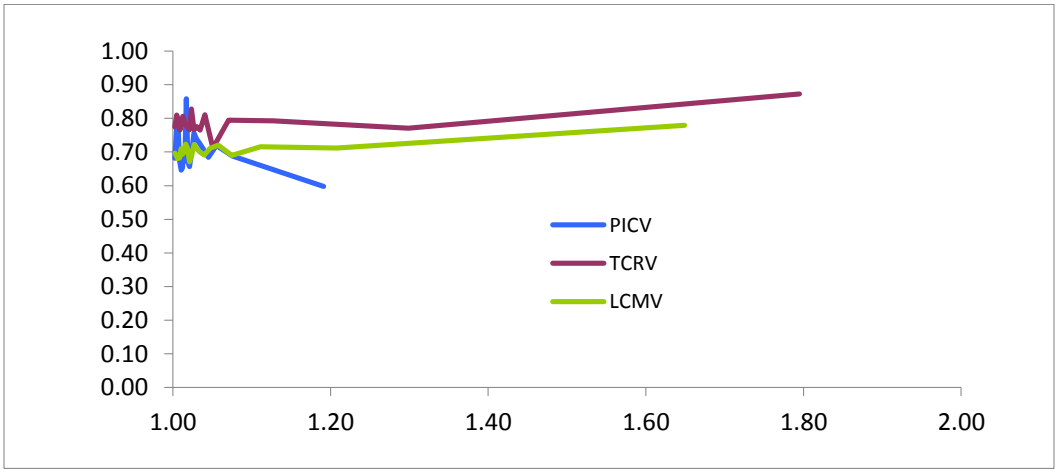

Figure 4.7. GP% there is no consistent relationship with shape . Slightly higher GP% on large, elliptical particles of LCM and TCRV predicted as non-specific effect from larger size.

In order to examine the number of GPs and size, the data was plotted to the next plot graph and concluded that they still spaced 90 angstroms and size of different particle was same.

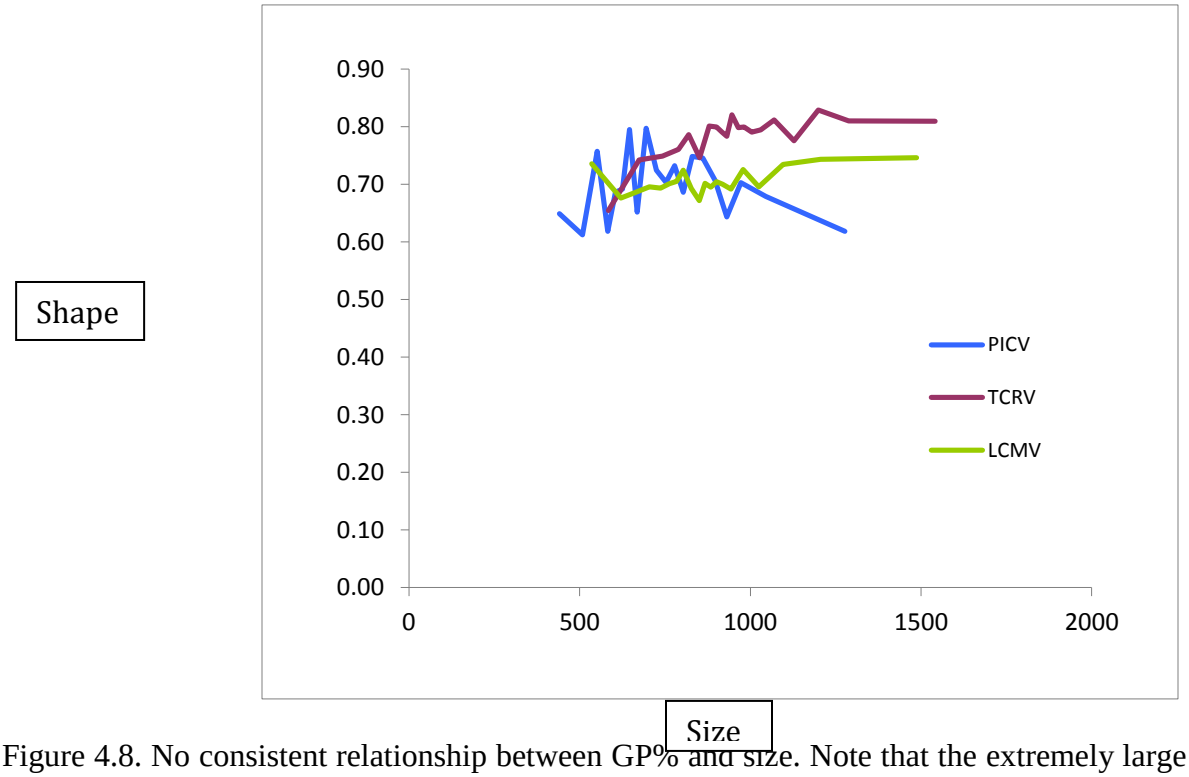

Figure 4.8. No consistent relationship between GP% and size. Note that the extremely large particles of LCMV and TCRV appear slightly higher GP%. This is predicted- lower curvature and larger diameter means more glycoproteins (GPs) are near perpendicular to the electron beam and so can be counted.

4.5 Core to edge ratio in arenavirus:

Another readily accessible variable in arenavirus was the apparent internal density of the virions, which ranged from essentially empty, GP decorated particles to super-dense particles (figure 4.9). To quantitate this variation, average density in a transect crossing the core and perpendicular to the membrane on either side of the virion was calculated. To account for differences in overall brightness of micrographs, the average density of protein-free ice from outside the particle was subtracted from all values. Then the core density was subtracted from the peak density of the viral membrane at the virion edge, which was observed to be relatively constant.

This core to edge ratio effects the relative density of the core regions of particles. Core to edge ratios for PICV, TCRV and LCMV are shown in table 4.2

Table 4.2. Core edge was calculated and got average and correlation of all of them as shown in this table in order to understand standard deviation, shape, GP% and size.

Core /Edge											
Virus		Avg (Å)	No of CE	Correlation							
						Shape		GP%		Size	
					SD	R^2	P	R^2	P	R^2	P
PICV1	All	0.78	303	±	0.20	9E-06	0.97	-	-	<0.01	0.51
	0-900	0.79	246	±	0.21	2E-05	0.95	-	-	<0.01	0.86
	900+	0.77	57	±	0.15	0.02	0.35	-	-	0.02	0.30
PICV2	All	0.69	445	±	0.16	0.03	<0.01	<0.01	0.30	0.08	<0.01
	0-900	0.68	76	±	0.15	0.05	<0.01	<0.01	0.85	0.013	0.03
	900+	0.76	369	±	0.15	<0.01	0.72	0.02	0.01	0.23	<0.01
PICVFA	All	0.82	127	±	0.15	-	0.88	-	-	-	0.62
	0-900	0.81	99	±	0.16	-	0.81	-	-	-	0.64
TCRV Native	All	0.82	533	±	0.13	0.01	<0.01	<0.01	0.47	0.01	0.01
	0-900	0.84	176	±	0.13	0.01	0.31	0.01	0.29	<0.01	0.75
	900+	0.81	357	±	0.13	0.01	0.04	<0.01	0.63	0.01	0.16
LCMV Native	All	0.73	633	±	0.15	0.01	<0.01	0.09	<0.01	<0.01	0.53
	0-900	0.73	420	±	0.15	0.01	0.01	0.08	<0.01	0.01	0.10
	900+	0.71	213	±	0.14	0.02	0.02	0.11	<0.01	<0.01	0.72
LCMV-LiCL	All	0.68	135	±	0.14	0.00	0.87	-	-	0.02	0.11
	0-900	0.68	96	±	0.14	0.01	0.37	-	-	0.01	0.34
LCMV-pH5	All	0.77	91	±	0.16	9.4E-05	0.93	-	-	0.01	0.37
	0-900	0.77	59	±	0.17	<0.01	0.79	-	-	6E-06	0.99
Vesicles Arv	All	0.33	39	±	0.20	<0.01	0.53		-	0.03	0.26

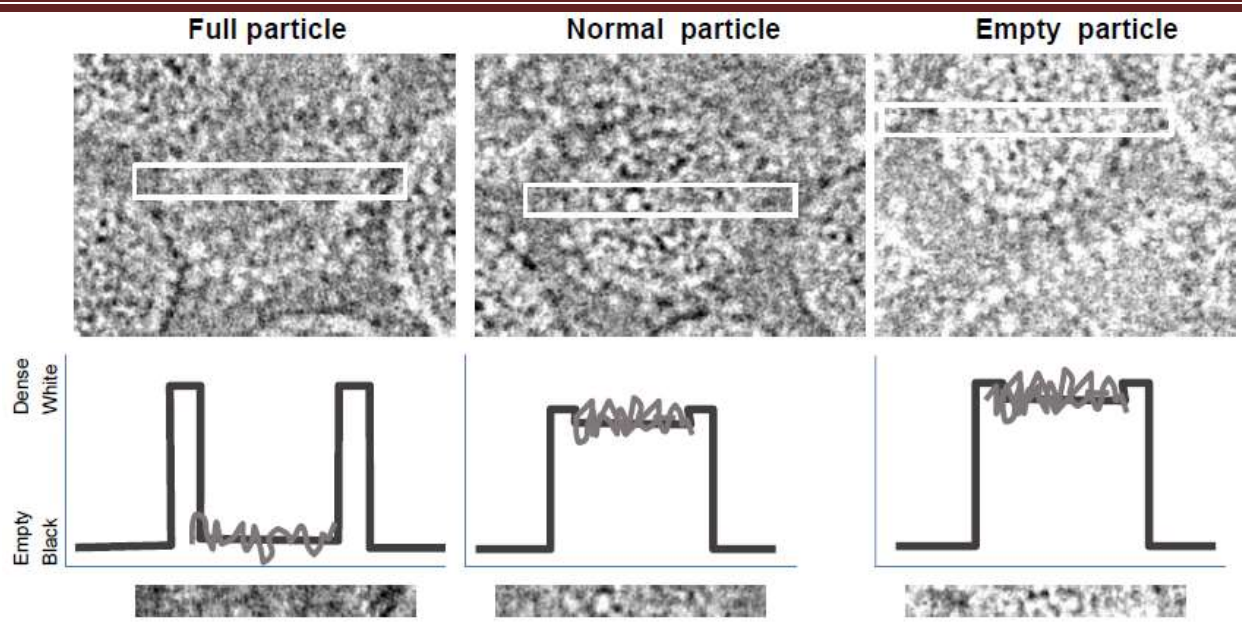

Figure 4.9 Full particle (left), normal particle in the middle and empty particles at the right corner.

It was found from this test that fullness was not related particle size or the amount of GP but empty particles are more elliptical than normal or dense particles. So RNA or NP only explain some of the variability in virus shape, but not the relationship between shape and size. Since RNA and NP density does not change during fusion activation, RNA and NP are not good explanation for the sudden change in shape. Dense particles are round, Some empty particles are elliptical but fullness does not change with fusion activation .

4.6 Core /Edge (NP+RNA) test Correlation Coefficient.

It is not known whether NP and RNA play role in virus shape and size. In order to understand we looked the role of NP and RNA in virus shape and size. As you can see some viruses have more or less RNP inside. To calculate how full a virus was we took the ratio of the brightness of the core, which is variable, to the envelope, which is constant as shown here. This works because electrons are scattered by density, so the whiter the image is, denser it is (figure 4.10).

RNA + NP density is estimated from the relative intensity of the core relative to the virus membrane and nearby ice.

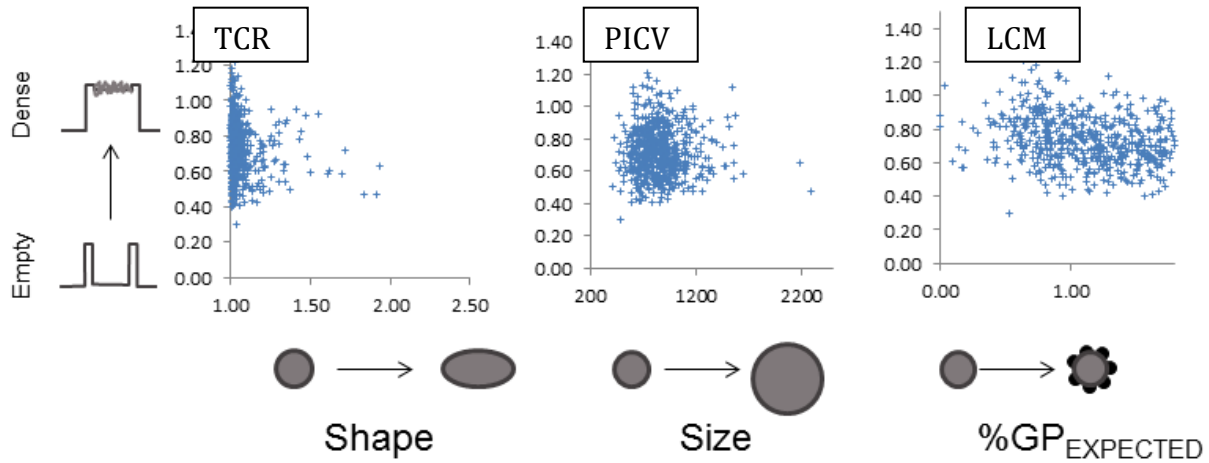

Figure 4.10 How is RNP density related to morphology? Ratio of Core to Envelope brightness is shown in above scatter plot.

4.7 Core edge vs Shape of GP:

In order to understand the relationship between Core edge and GP, the data was plotted and It was concluded that there is no consistent relationship with shape except when shape is >1.15 . Core edge for particle above 1.15 is lower than for rounder particles. These rounder particles are also smaller this efect goes against expectation, assuming all particles are filled to equal density, and against expectation that all particles contain same amount of genetic material (figure 4.11).

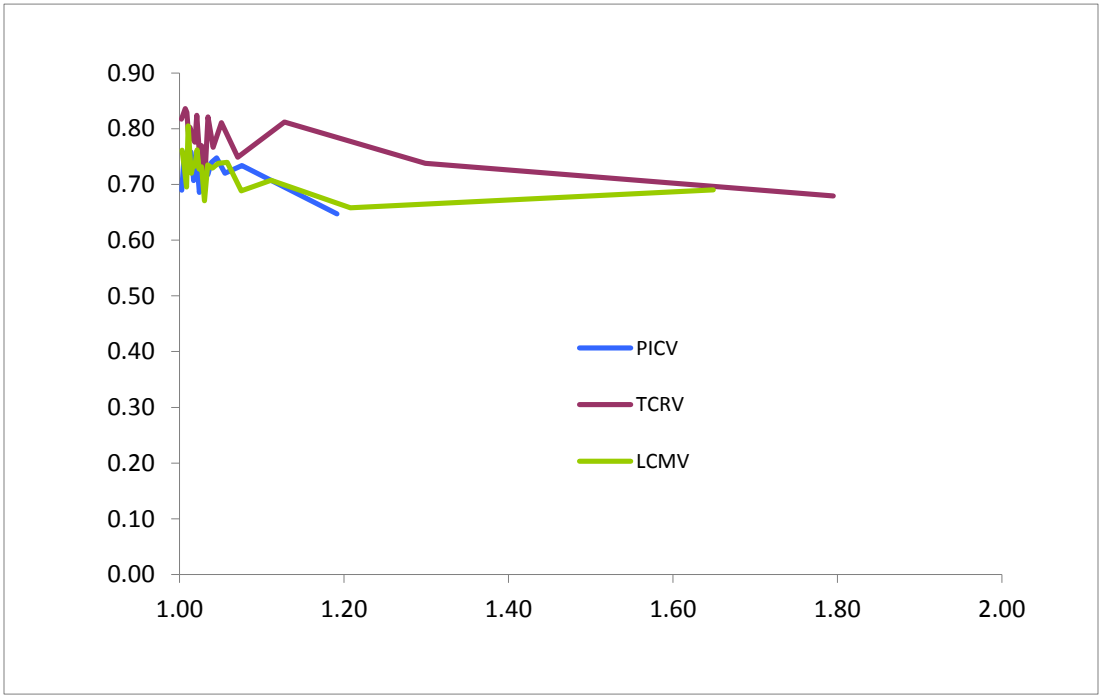

Figure 4.11. C/E No consistent relationship with shape except when shape is >1.15 . C/E for particle above 1.15 is lower than for rounder particles. These rounder particles are also smaller this efect goes against expectation, assuming all particles are filled to equal density, and against expectation that all particles contain same amount of genetic material.

4.8 Image comparison:

In order to understand how arenavirus particles look like in cryo electron microscopy image. Below is an example of a particle right cornered is dense and left sided above is empty at the top and normal one is below on left bottom. It clearly shows an example of arenavirus (figure 4.12).

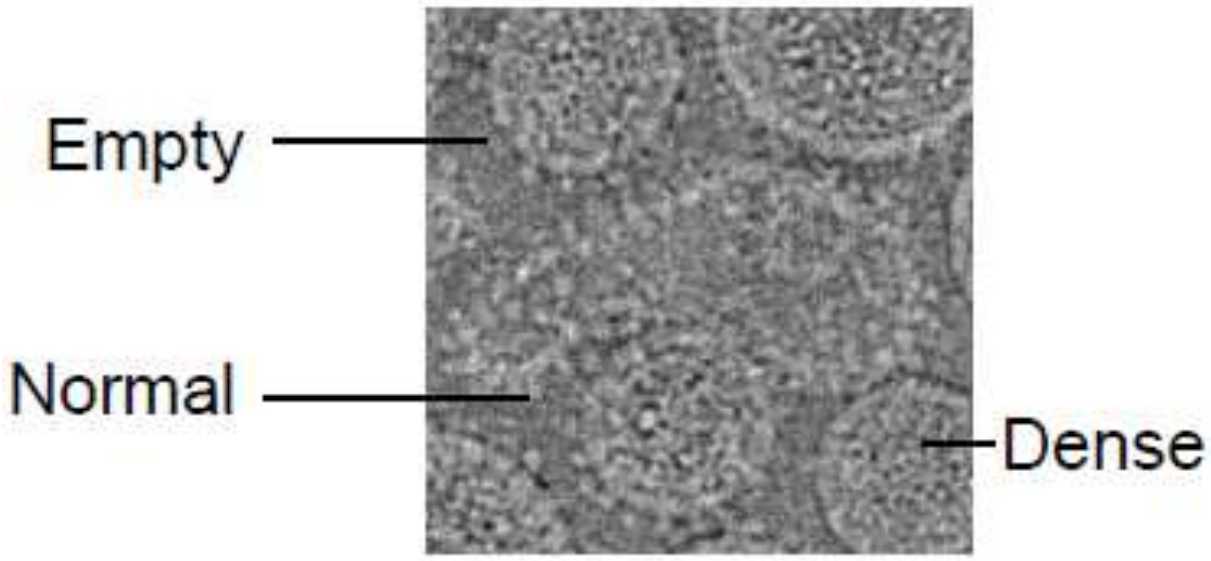

Figure 4.12 Image shows that particles are dense, normal and empty one.

4.9 Discussion:

From this chapter it is concluded that GP% effects on shape and size and core edge also effects on shape and size.

Conclusions

In order to understand whether GP% has a relationship with shape, the data was selected and then plotted a scatter plot and concluded that there is no consistent relationship with shape (figure 4.6). Slightly higher GP% on large, elliptical particles of LCM and TCRV were contradicted by the opposite result for PICV. However, it is possible that the PICV result is an error due to small sample size. It was found from this test that fullness was not related particle size or the amount of GP but empty particles are more elliptical than normal or dense particles. So RNA or NP only explain some of the variability in virus shape, but not the relationship between shape and size. Since RNA and NP density does not change during fusion activation, RNA and NP are not good explanation for the sudden change in shape. Dense particles are round, Some empty particles are elliptical but fullness does not change with fusion activation

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