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Supplemental Information

Combining Transient Expression and Cryo-EM

to Obtain High-Resolution Structures

of Luteovirid Particles

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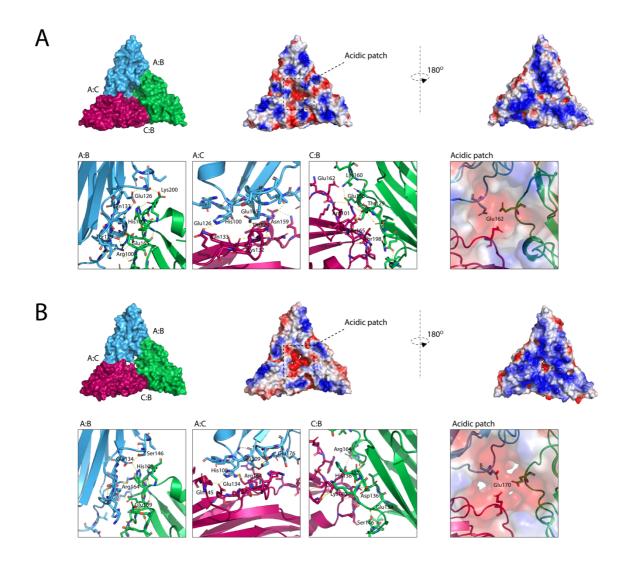


Figure S1. Luteovirid VLP surface charge and inter-subunit interactions (Related to Figure 3). The surface charge of luteovirid capsids proteins is conserved. **Left:** For clarity, CP trimers coloured according to quasi-conformer (A=blue, B=green & C=red), shown from the outer surface of the capsid. **Middle:** Vacuum electrostatic representation of the outer surface of the CP. **Right:** Vacuum electrostatic representation of the inner surface of the CP. **(A)** BYDV **(B)** PLRV. Labelled panels depict interactions at the A:B, A:C and C:B interfaces and central acidic patch. Key residues are labelled for clarity.

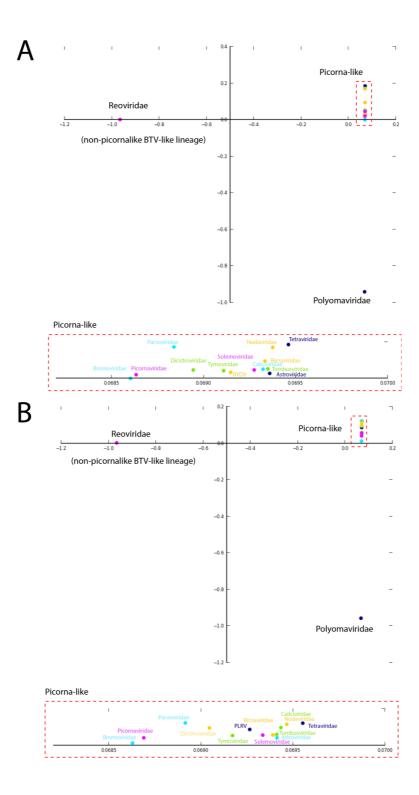


Figure S2. (Related to Figure 4) Correspondence analysis of BYDV/PLRV structural identity against representative members of picorna-like lineage families. A representative member of the structurally distinct BTV-like lineage is included for comparison. Analysis carried out with DALI server. Closely related families are expanded in the red dashed box for clarity. (A) BYDV (B) PLRV

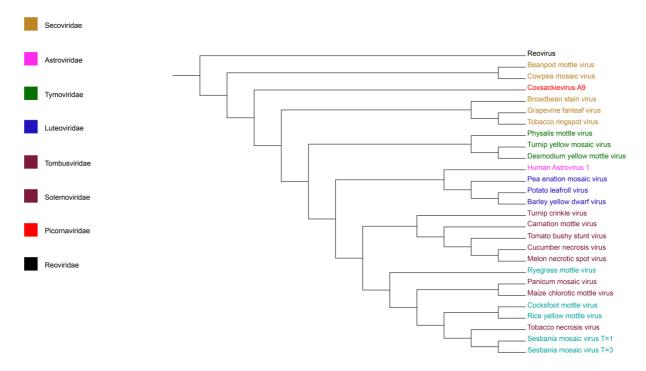


Figure S3. (Related to Figure 4 and 6) Structural dendrogram demonstrating the Luteoviridae are structurally similar to picorna-like viruses. Additionally, a picornaviridae member, Coxsackievirus A9, and a non-picorna-like virus, Reovirus, are included for comparison.

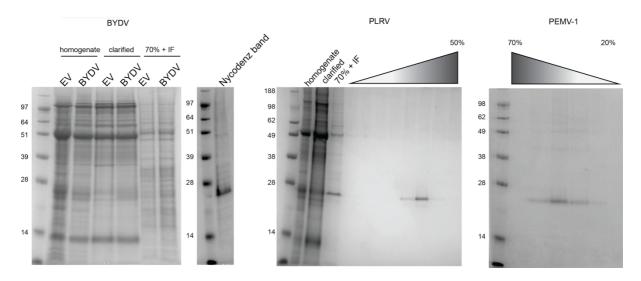


Figure S4. (Related to figure 2). SDS page analysis of purified Luteovirid VLPs.

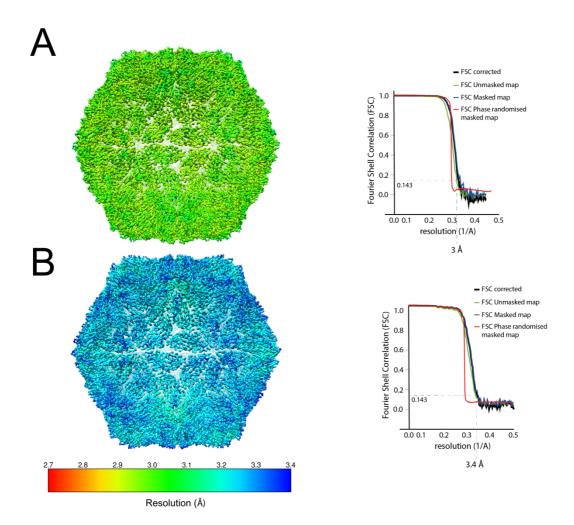


Figure S5. Local resolution and FSC curves for BYDV and PLRV (Related to Figure 3). Left: Local resolution of luteovirid capsid structures. Structures coloured according to the local resolution of the map. **Right:** Fourier shell correlation curves as a function of resolution. The resolution that corresponds to an FSC coefficient of 0.143 was 3.0 and 3.4 for BYDV and PLRV respectively. **(A)** BYDV **(B)** PLRV.

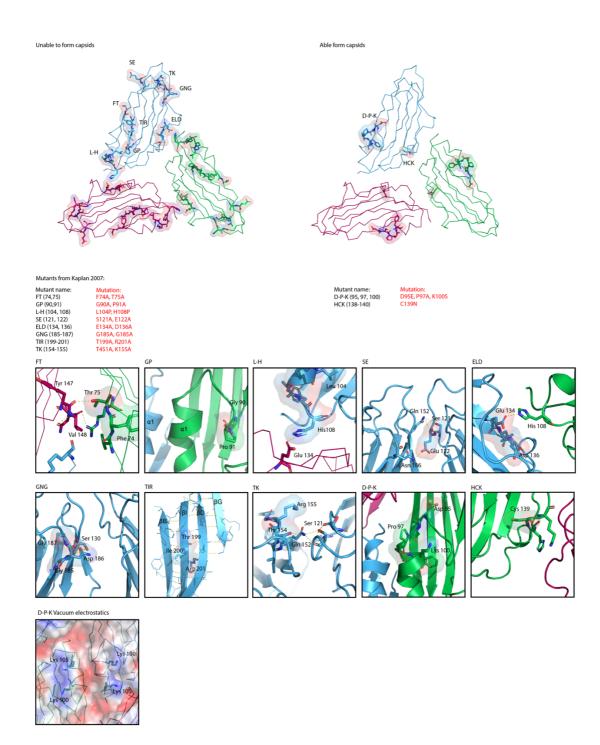


Figure S6. (**Related to Figure 7 and Table S2**). **PLRV mutant analysis. Top:** Mutations studied in Kaplan 2007 mapped onto experimental structure of PLRV CP from this study. Subunit backbones are shown in ribbon form, mutant regions are shown in stick and space filling representation, coloured according the subunit A (blue), subunit B (green), subunit C (red). Labels indicate mutation (nomenclature as in Kaplan 2007). **Bottom:** Panels showing detailed representations of mutant residues mapped onto the experimental PLRV CP structure.

PEMV RSDaV BYDV SCYLV CtRLV BLRV SbDV PeVYV TVDV MYDV_RMV MYDV_RMV MYDV PLRV CYDV_RPS	MPRSKSKANQRRBPRRAVWAPSMAPPTOSRPRRRSKRGCLNGSH-VDSSMVHGPFNCNALGTV 1 MPRSKSKRGCLNGSH-VDSSMVHGPFNCNALGTV 1 MSVGRCQRNNSRRNGQQQQGGSSQPNARPVVQVQPSNGRPPRRGGRSSBRGGSNMSSSBHVDSSMVHGPFNCNALGTV 1 MNSVGRCQRR
CYDV_RPV CLRDV SBMYV BChV BWYV TuYV CpSDaV CABYV PABYV MABYV SABYV	1 MSTVVLRSNGN-GSRRRORVARREPAARTOPVVVVASNGPARRGRR-RVG-PRRGRTPRSGGSRGTFVFSKDSLAGNSSGSI 1 MNTVVGRTIN-CRRPRORKRACKSOPVVVVASRTTORRERRRRGG-NTGGRIPGGASSTFVFSKDSLSGSSGSI 1 MNTVVGRTIN-CRRPROTRRACKSOPVVVVASRTTORRERRRRGN-NTGRIPGGASSTFVFSKDSLSGSSGSI 1 MNTVVGRTIN-CRRPROTRRACKSOPVVVVASRTTORRERRRRGN-NTGRTVFTRASSSTFVFSKDNLAGSSSGAI 1 MNTVVGRTIN-CRRPROTRRACKOPVVVASRTTORRERRRRGN-NTGRTVPTRASSSTFVFSKDNLAGSSSGAI 1 MNTVVGRTIN-CRRPROTRRACKOPVVVASRTTORRERRRRGN-NTGRTVPTRASSSTFVFSKDNLAGSSSGAI 1 MNTVVGRTIN-CRRPROTRRACKOPVVVASRTORRERRRRGN-NTGRTVPTRASSSTFVFSKDNLAGSSSGAI 1 MNTVVGRTIN-GRRPROTRRACKOPVVVASRTORRERRRRGN-NTGRTVPTRASSSTFVFSKDNLAGSSSGAI 1 MNTVVGRTIN-GRRPROTRRACKOPVVVASRTORRERRRRGN-NTGRTVPTRASSSTFVFSKDNLAGSSSGAI 1 MNTVAGRTIN-GRRPROTRRACKOPVVVASRTORRERRRRN-R-RRASGRSTGRGSSTFVFSKDNLAGSSSGAI 1 MNTVAVR-NNG-RRRRNRTVQRARRNPVVVVASRTORRERRRN-R-RRASGRSTGRGSSTFVFSKDNLAGSSSGSI 1 MNTVAVR-SSN-GTRRNRTVQRAKKNVVAIVOTSGPAGRGRRRN-R-RRASGRSTGRGSCTFVFSKDNLAGSSSGSI 1 MNTVAVR-SSN-GTRRNRRTKRSTRRVRVAIVOTSGPAGRGRRRN-R-RRASGRSTGRGSGSGSTFVFSKDNLAGSSSGSI 1 MNTVAAR-NON-GMGRRRNRRRTSKSNRVVVVOTGGPAGRGRRRN-P-RRSPRGGRAG-GRPGSTFVFSKDNLSGSSSGSI 1 MNTVAAR-NON-GMGRRRNRRRTSKSNRVVVQATGGPAGRGRRRN-P-RRSPRGGRAG-GRPGSTFVFSKDNLSGSSGSI 1 MNTVVR-NNN-GMGRRRNRRRNRSFSSRVVVQATGGPAGGRRRN-R-RRPSRGGRAG-GRPGSTFVFSKDNLSGSSGSI 1 MNTVVR-NNN-GMGRRRNRRRNRSFSSSRVVVQATGGPAGGRRRN-R-RRPSRGGRAG-GRPGSTFVFSKDNLSGSSGSI
PEMV RSDaV BYDV SCYLV CtRLV BLRV SbDV PeVYV TVDV MYDV_RMV MYDV_RMV MYDV_RMV MYDV_RPV CYDV_RPV CYDV_RPV CLRDV SBMYV BChV BChV BWYV TuYV CPSDaV CABYV PABYV MABYV SABYV	70 KFGPS-S.CQCI-KENLAANQKYKIVWIKUVICSEAAAIDRGCIAYHVUTSTTKKAADVVLUDTNIRSNESAIFGREIIGDQPNYESN- 91 KFGPSLSCCSALKSGIERSHEEKTININWKYIIHAASITSGAFYUUTSCTOILL-KYYUTVPIAKCGOFSTPECKIRTGALFTPD 80 KFGPSLSCCPALDGILKSYHEYKITSITCYNSCSDALPGAFFIELDTACKOSAL-CSYINSFTUKTSTFRSPAINGEFOST- 77 KFGPNLSQAAFNNCLLKAYHEYKITSITCYNSCSDALPGAFFIELDTACKOSAL-CSYINSFTUKTSTFRSPAINGKEFOST- 89 TFGKDLSCHAAFNNCLLKAYHEYKITSITCYNSCSDALPGAFFIELDTACKOSAL-CSYINSFTUKTSTFRSPAINGKEFOST- 80 TFGKDLSCHPAFSNGLLKAYHEYNITSITCYNSCSDALPGAFFIELDTACKOSAL-CSYINSFTUKSCSRFWFANNCKUVEFOST- 80 TFGKDLSCHLDGILKAYHEYNITSITCYNSCSSDALPGAFFIELDTACKOSAL-CSYINKFSTTKSEKKFSRKAINCOAHDTS- 81 TFGPSLSCKPLDGILKAYHEYNITNVEDAYIICSSSTSSGSIAYELDPHCKUTSL-CSTIRKFSTTKSECKFSRKAINCOAHDTS- 82 TFGPSLSCKPLDGILKAYHEYNITNVET VSESSTAEGSIAYELDPHCKUSL-CSTIRKFPUTKGGOAFFRACINCVEWHDTS- 83 TFGPSLSCKPLDGILKAYHEYNITNVERVSESSTAEGSIAYELDPHCKUSL-ASTINKFSTUKGGREVFANINGCOWRDTS- 84 TFGPSLSCKPLDGILKAYHEYNITNVERVSESSTAEGSIAYELDPHCKUSL-ASTINKFSTUKGGREVFANINGCGOVRDS- 85 TFGPSLSCAAFSGGILKAYHEYNITNI ILFISESSTAEGSIAYELDPHCKUSL-ASTINKFSTUKGGREVFANINGCGOVRDS- 86 TFGPSLSCAAFSGGILKAYHEYNISKI ILFFISEAASTAEGSIAYELDPHCKUSL-ASTINKFSTUKGGREVFANINGCGOVRDS- 87 TFGPSLSCAAFSGGILKAYHEYNISKI ILFFISEAASTAEGSIAYELDPHCKUSL-ASTINKFSTUKGGREVFANINGCGOVRDS- 87 TFGPSLSCAAFSGGILKAYHEYNISKI ILFFISEAASTAEGSIAYELDPHCKISSL-ASTINKFSTUKGGREVFANINGCGOVRDS- 87 TFGPSLSCAAFSGGILKAYHEYNISKI ILFFISEAASTAEGSIAYELDPHCKISSL-ASTINKFSTUKGGREVFANINGCGOVRDS- 87 TFGPSLSCPAFSNGUKAYHEYNISKI ILFFISEAASTAEGSIAYELDPHCKISSL-ASTINKFGUTKNGRAAFAASYINGACHWHOS- 87 TFGPSLSCPAFSNGUKAYHEYNISVILFFISEAASTAGSISYELDPHCKISSL-STINKFGUTKNGRAAFAASYINGACHWHOX- 87 TFGPSLSCPAFSNGUKAYHEYNISVILFFISEAASTAGSISYELDPHCKISSL-STINKFGUTKNGRAAFAASYINGACHWHOX- 87 TFGPSLSCPAFSNGUKAYHEYNISVILFFISEAASTAGSISYELDPHCKISSL-STINKFGUTKNGRAFAASYINGACHWHOX- 87 TFGPSLSCPAFSNGUKAYHEYNISVULFFISEASSTSSGSISYELDPHCKISSL-STINKFGUTKNGRAFAASYINGACHWHOX- 87 TFGPSLSCPAFSNGUKAYHEYNISVULFFISEASSTSGSISYELDPHCKISSL-STINKFGUTKNGRAFAASYINGACHWHOX- 87 TFGPSLSCPAFSNGUKAYHEYNISVULFFISEASSTSGSISYELDPHCKISSL-STINKFGUTKNGRAFAASYINGAC
PEMV RSDaV BYDV SCYLV CtRLV BLRV SbDV PeVYV TVDV MYDV_RMV MYDV_RMV MYDV_RPS CYDV_RPS CYDV_RPS CYDV_RPV CLRDV SBMYV BChV BChV BChV BWYV TuYV CQSDaV CABYV PABYV SABYV	157KDOFFFLYRGTCG-DVAGHWEICGIDMAASL 180 PDKTPVDK NOFFLYRGNGFS-SVAGCFVITACWFSPE 168

Figure S7. Luteovirid sequence alignment (Related to Figures 4 and 7). Alignment of BYDV, PLRV and PEMV CP sequences with that of other Luteoviridae family members. Conserved residues are indicated by a red asterisk for clarity.

Table S1. (Related to figure 3 and figure 4)

	PLRV	BYDV
Data collection and processing		
Sample applications to grid	3	1
Magnification	75, 000 x	75, 000 x
Voltage (kV)	300	300
Electron exposure (e–/Ų)	71.65	63.2
Defocus range of micrographs (µm)	-0.2 to -4.5	-0.2 to -4.2
Pixel size (Å)	1.065	1.065
Symmetry imposed	11	11
Initial particle images (no.)	91, 498	326, 364
Final particle images (no.)	74, 157	324, 235
Map resolution (Å)	3.4	3.0
FSC threshold	0.143	0.143
Number of frames	59	59
Refinement		
Map sharpening <i>B</i> factor (Ų)	-228.3	-198.1
Model composition		
Non-hydrogen atoms	0	0
Protein residues	1087	1070
Nucleic acids	0	0
R.m.s. deviations		
Bond lengths (Å)	0.01	0.01
Bond angles (°)	0.83	0.93
Validation		
Clashscore	2.36	2.02
Poor rotamers (%)	0.00	0.84
Ramachandran plot		
Favored (%)	88.66	94.52
Allowed (%)	11.34	5.48
Disallowed (%)	0.00	0.00

Mutant	Proposed impact			
FT	Thr75: Loss of H-bond with Tyr147 on			
	neighbouring subunit.			
	Phe74: disruption of hydrophobic core of			
	jelly roll.			
GP	Gly90, Pro91: displacement of α 1- β C			
	loop. Disruption of $\alpha 1-\alpha 1$ interface			
	between neighbouring subunits.			
L-H	His108: loss of H-bond with Glu134 of			
	neighbouring subunit.			
	Leu104: Disruption of hydrophobic core			
	of jelly roll fold.			
SE	Ser121: loss of H-bond with GIn152 on			
	neighbouring subunit.			
	Glu122: loss of hydrogen bond with Asn186.			
	Disruption of intersubunit interactions.			
ELD	Glu134: Loss of H-bond with His108 (as			
	in L-H mutant). Disruption of intersubunit			
	interactions.			
GNG	Asn186: Loss of H-bond with Ser130.			
	Potential displacement of β H- β I and β C-			
	βD loops.			
TIR	Thr199, Ile200, Arg201: Disruption of			
	BIDG β -sheet interactions.			
	Thr199, Arg201: Face to capsid interior,			
	may be involved in encapsidation of			
	RNA.			
	Ile200: Disruption of hydrophobic core of			
ТК	jelly roll.			
	Thr154: disruption of H-bond network			
	with GIn152 and Ser121 on neighbouring subunit at 5-fold and β -annulus.			
DPK	Lys100: Loss of charge at capsid			
	surface, may impact engagement of			
	receptor or other process involved in			
	vector transmission.			
НСК	Cis139: Sidechain facing jelly roll			
	interior. No apparent impact on capsid			
	formation or transmission.			
Table S2 DI DV mutants disrupt cansid formation and transmission efficienc				

Table S2. PLRV mutants disrupt capsid formation and transmission efficiency.(Related to Figure 7 and Figure S8). Using the experimentally derived structure ofthe PLRV capsid from our study, we attempt to rationalise the findings of Kaplan et al.2007.

	PLRV	BYDV	PEMV ^{BYDV}	PEMV ^{PLRV}	PLRV ^{BYDV}	BYDV ^{PLRV}
PLRV	-	0.772	0.716	0.100	0.840	1.127
BYDV		-	0.119	0.780	0.074	1.072
PEMV ^{BYDV}			-	0.829	0.110	0.923
PEMV ^{PLRV}				-	0.749	1.107
PLRV ^{BYDV}					-	0.923
BYDV ^{PLRV}						-

Table S3. (Related to Figure 4) Structural superposition RMSD matrix of all models presented in this study (RMSD in Å).

Analyses of protein:protein interfaces within the AU						
Virus CP	Chain 1	Chain 2	Buried surface			
			area at interface			
			(Ų)			
PLRV	А	В	438.3			
PLRV	А	С	457.7			
PLRV	В	С	452.6			
BYDV	BYDV A		416.3			
BYDV	BYDV A		406.8			
BYDV B		С	426.6			

Table S4. (Related to Figure 3) Analysis of protein:protein interfaces within the asymmetric unit of luteovirid capsid structures. AB:AB interfaces represent the buried surface area between the constituents of AUs opposed around the 5 and 3 fold symmetry axes. BC:BC interfaces represent the buried surface area between the constituents of AUs opposed at the 2-fold symmetry axis.

Analyses of protein:protein interfaces across neighbouring AUs					
Virus CP	Interface AU 1		Interface AU 2		Buried surface
					area at interface
					(Ų)
PLRV	А	В	А	В	1380.2
PLRV	С	В	С	В	1197.9
BYDV	А	В	А	В	1312.2
BYDV	С	В	С	В	1428.7

Table S5. (Related to Figure 3) Analysis of protein:protein interfaces across neighbouring asymmetric units in luteovirid capsid structures. AB:AB interfaces represent the buried surface area between the constituents of AUs opposed around the 5 and 3 fold symmetry axes. BC:BC interfaces represent the buried surface area between the constituents of AUs opposed at the 2-fold symmetry axis.