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Supporting information for article:

Crystallization and X-ray diffraction of virus-like particles from a piscine betanodavirus

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We used commercially available kits to screen the crystallization conditions for DGNNV VLPs. Conditions 7, 20, 25, and 28 of PEG/Ion Screen kit gave a few very small crystal-like solids in the middle of brown precipitate after four weeks (Table S1). The PEG effects on the crystallization were further studied using the PEG3350 II and PEG II Suite (Table S1). Small crystals from DGNNV VLPs were observed under the conditions 31, 32, 35, and 56 of PEG II Suite kit. Under conditions 31, 35, and 56, crystals of DGNNV VLPs were 0.2 mm long and diamond-shaped, while crystals under condition 32 were much smaller. It was noted that the conditions 31 and 35 also contained Lithium Sulfate. Condition 47 of PEG3350 II kit resulted in crystals of 0.1 mm long needles, and crystals under condition 69 were diamond-shaped and very small. Some microcrystalline precipitates from DGNNV VLPs were observed under the conditions 38, 39, 42, and 45 of SaltRx1 kit (Table S1). Under conditions 42 and 45, crystals of DGNNV VLPs were 0.1 mm long and diamond-shaped, while crystals under conditions 38 and 39 were much smaller. These results suggested that Ammonium Nitrate was a useful salt for DGNNV VLP crystallization. The results from these trials suggested that the optimal molecular weight of PEG was in the range of 1500-8000, and that Lithium Sulfate would be a second choice for a salt in addition to Ammonium Nitrate. As Fig. S1 shows, the effects of adding divalent metal ions on the crystals growth of DGNNV VLPs were investigated at 277 K, with compounds $MgCl_2$, $CaCl_2$, $SrCl_2$, $BaCl_2$, $MnCl_2$, $CoCl_2$, $NiCl_2$, and $ZnCl_2$. Crystals were observed in two weeks. The concentrations of divalent ions (additives) were 2 mM. While $CaCl_2$ was our primary choice, crystals also grew to 0.3 mm in $ZnCl_2$ and $CoCl_2$ using the sitting-drop vapor diffusion method (Fig. S1). The salts Lithium Sulfate, Ammonium Sulfate, and Ammonium Nitrate were also factorially combined in various concentrations to improve the crystal quality. The crystallization of DGNNV VLPs was also carried out in quartz capillaries. The photos of crystals in different position within quartz capillary are illustrated in panel (Fig. S2H). The mother liquor solution was placed on the top (left in Fig.

S2H). The crystals is the bottom of capillary (Figs. S2F and S2G) grew bigger, likely due to the effects of protein concentration.

Table S1 Components of Kits and screening conditions for DGNNV VLP crystals.

Kits	No.	Crystallization reagent composition			Crystallization description
SaltRx1	38	2.5 M Ammonium Nitrate	0.1 M BIS-TRIS propane pH 7.0		Microcrystalline precipitates
	39	2.5 M Ammonium Nitrate	0.1 M Tris pH 8.5		Microcrystalline precipitates
	42	6.0 M Ammonium Nitrate	0.1 M Tris pH 8.5		Microcrystalline precipitates
	45	1.5 M Sodium Nitrate	0.1 M Tris pH 8.5		Microcrystalline precipitates
PEG/Ion Screen	7	0.2 M Calcium Chloride Dihydrate	20% w/v PEG 3350		Microcrystalline precipitates
	20	0.2 M Magnesium Formate Dihydrate	20% w/v PEG 3350		Microcrystalline precipitates
	25	0.2 M Magnesium Acetate Tetrahydrate	20% w/v PEG 3350		Microcrystalline precipitates
	28	0.2 M Calcium Acetate Hydrate	20% w/v PEG 3350		Microcrystalline precipitates
PEG3350 II	47	0.2 M Sodium Citrate,	20% w/v PEG 3350	0.1 M Bis Tris propane pH 8.5	Long needles

	69	0.2 M Sodium Formate	20% w/v PEG 3350		Small crystals
PEG II Suite	31	0.2 M Lithium Sulfate	16% w/v PEG 4000	0.1 M Tris pH 8.5	Small crystals
	32	0.2 M Sodium Acetate	16% w/v PEG 4000	0.1 M Tris pH 8.5	Small crystals
	35	0.2 M Lithium Sulfate	20% w/v PEG 4000	0.1 M Tris pH 8.5	Small crystals
	56	0.2 M Ammonium Sulfate	15% w/v PEG 4000	0.1 M tri Sodium Citrate pH 5.6	Small crystals

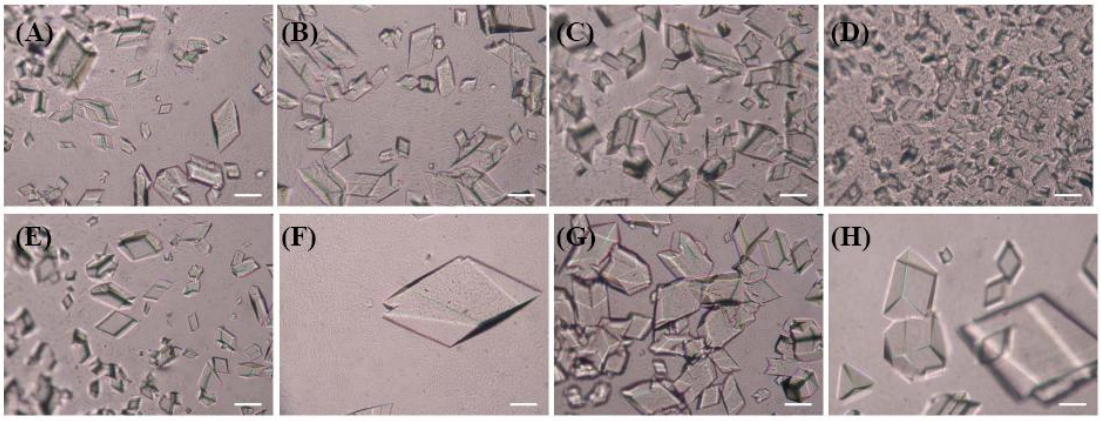

Figure S1 Effects of adding divalent metal ions on the crystals growth of DGNNV VLPs at 277 K. (A) $MgCl_2$, (B) $CaCl_2$, (C) $SrCl_2$, (D) $BaCl_2$, (E) $MnCl_2$, (F) $CoCl_2$, (G) $NiCl_2$, and (H) $ZnCl_2$ were added at the concentrating of 2 mM. Crystals were observed in two weeks. Bars represent 100 μm.

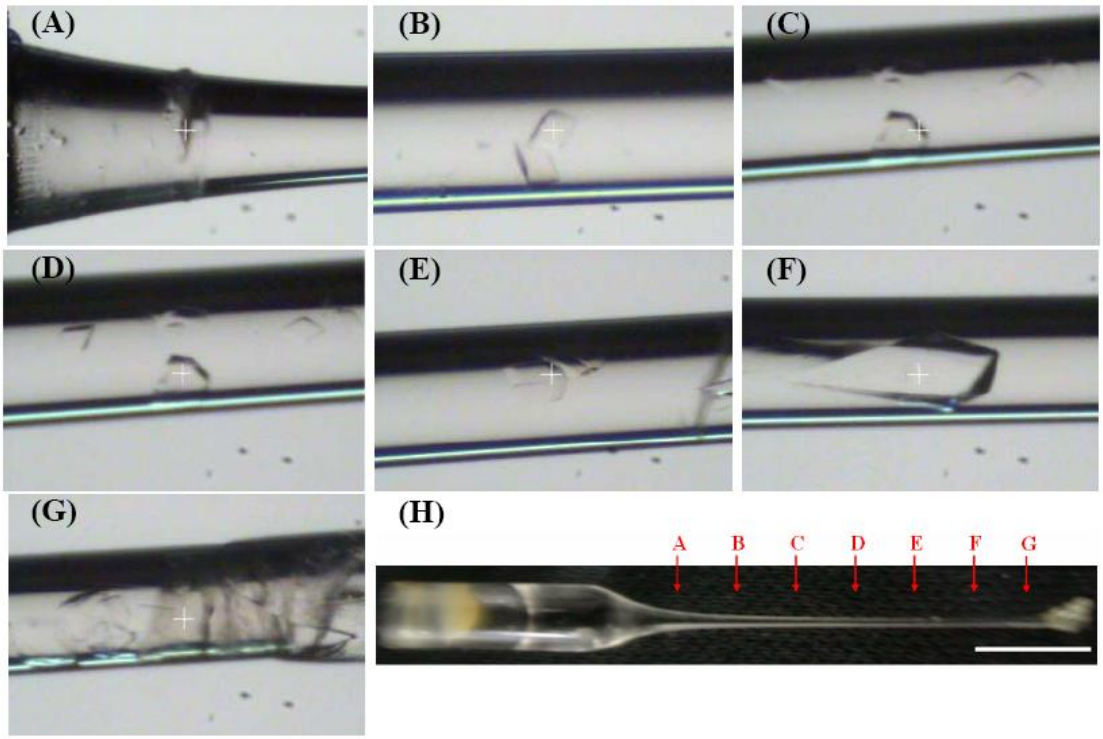

Figure S2 The crystallization of DGNNV VLPs in quartz capillaries. (A) to (G) show photos of crystals in different position within quartz capillary, illustrated in panel (H). Bar represents 0.5 cm.