Supplementary information for

"Modeling the three-dimensional structure of the right-terminal domain of pospiviroids"

Gerhard Steger¹

¹ Institut für Physikalische Biologie, Heinrich-Heine-University Düsseldorf, 40225 Düsseldorf, Germany

* Email: steger@biophys.uni-duesseldorf.de



Figure S1. Structure of Virp1. Plant BET (bromodomain(s) and extraterminal domain) proteins typically have only one bromodomain (marked in red; amino acids 192–290; Prosite entry PS50014), whereas they have mostly two bromodomains in animals; six amino acids (marked by red lines; aa 216, 223, 226, 265, 269, and 275) are responsible for the acetyl-lysine recognition of histones and other proteins.¹ The structure of the extraterminal domain (lightblue; aa 407–471; PS51525) has an acidic patch that may interact with other proteins or nucleic acids.² Furthermore, SCANPROSITE³ predicts an ATP/GTP-binding site (green; aa 21–28; PS00017) and a bipartite nuclear localization signal (orange; aa 328–344; PS50079). Virp1 Δ (aa 290–602) is sufficient for binding to PSTVd⁴.

	1	.70 I	180	190	
CEVd_EF488060	ag <mark>CG</mark> g A <mark>GAA</mark> -	AC <mark>A</mark> - GG	- agcUcgu c	u CCUUC - Cc UUC	- g c <mark>u G</mark> c u
CEVd_AJ564798	agCGg AGAA -		- agcUcgu c		- g c <mark>u G</mark> c u
CEVd_EF494685	agCGg AGAA -	gC <mark>A</mark> - GG	- agc Ucgu c	uccuuc - cuuuc	-gc <mark>uG</mark> cu
CEVd_EU877742	a g <mark>CG</mark> g A <mark>Gg A</mark> -	AC <mark>A - GG</mark>	- agc Ucgu c	u <mark>ccu</mark> uc - c <mark>uuuc</mark>	- g c <mark>u G</mark> c u
CEVd_EF488067	a g C G g - A G A A - a g C G g - A G A A - a g C G g - A G A A - a g A G A A - a - a g A G A A - a - a - a - a - a - a - a - a - a	ACA-GG	- agc Ucgu c		- g c <mark>u G</mark> c u
CEVd_S67438	ag <mark>CG</mark> g A <mark>GAA</mark> -	ACA - GG	- agcUcgu c	u CCUUC - CUUUC	-gU <mark>uG</mark> cu
CEVd_EF488068	a g <mark>CG</mark> g A <mark>GAA</mark> -	ACA - GG	- agcUcgu c	u CCUUC - CUUUC	-gc <mark>CG</mark> cu
CEVd_EF488058 CEVd_EF488059	g g C G g A G A A - g g C G g A G A A -	ACA - GG ACq - GG	- agc Ucgu c - agc Ucgu c		-gcuGcu -gcuGcu
CEVd_S79831	ag <mark>CG</mark> g A <mark>GAA</mark> -	AC <mark>A</mark> - GG	- ugc Ucgu c	u <mark>ccu</mark> uc-c <mark>uuuc</mark>	-gc <mark>uG</mark> cu
CEVd_EU564169	ag <mark>CG</mark> g AGAA -	ACA - GG	- agcUcga c		- g c <mark>u G</mark> c c
CEVd_HQ284017	agCGg AGAA -	ACA - GG ACA - GG	- <mark>G</mark> gcUcga c	uccuuc - cuuuc	- g c <mark>u G</mark> c u
CEVd_HQ284016	ag <mark>CG</mark> g A <mark>GAA</mark> -	ACA - GG	- agcUcga c	u <mark>CCU</mark> UC - C <mark>UUUu</mark>	- g c <mark>u G</mark> c u
CEVd_FJ773258 CEVd_DQ444473	agCGg AGAA -	ACA - GG ACA - GG	- agc Ucga c	u CCUUC - CUUUu	-gc <mark>uG</mark> c c
CEVd_DQ471994	ag <mark>CG</mark> g <mark>AGA</mark> A -	ACA - GG	- agc Ucga c	u CCUUC - CUa UC	- <mark>U</mark> c <mark>u G</mark> c u
CEVd_AY514444	ag <mark>CG</mark> g A <mark>GAA</mark> -	ACA - c G	- a c c U c g C c		- g c <mark>u G</mark> c u
CEVd_P00328 CEVd_DQ094297	agCGg AGAA - agCGg AGAA -	ACA-GG ACA-GG	- acc Uggu g - agc Ucguuuc		-gcuGcu -gcuGcu
CEVd_DQ094295	ag <mark>CG</mark> g AGAA -	ACA - GG	- agc Ucguauc	u <mark>ccu</mark> uc - c <mark>uuuc</mark>	- g c <mark>u G</mark> c u
CEVd_AF148717	agCGg AGAA -		- agc Ucga c		-gc-g <mark>uG</mark> cu
IrVd_X95734	ggCGga - AGAA -	ACA-GG	- agc Ucga c	uccuuc - cuuuc	ugU <mark>CG</mark> cc
IrVd_JQ889690	gg <mark>CG</mark> ga - A <mark>GAA</mark> -	AC <mark>A - G</mark> G	- agcUcgn c	u <mark>ccu</mark> uc - c <mark>uuuc</mark>	ug U - - <mark>CG</mark> c c
IrVd_DQ094294 CLVd DQ061193	ggCGga - AGAA - CCCGC AGAA -	ACA - GG ACA - GG	- a g c U c g u c - <mark>GU</mark> UU - U C A	CCCUBC - CUUUC	ug U CGc c - UU CGGC
PCFVd_JF742637	CCuGu AGAA -	ACA - GG	- <mark>GU</mark> UU - UC A	cccuuc - cuuuc	- <mark>UU CGGG</mark>
PCFVd_JF446907	CCCGu AGAA -	ACA - GG	- GUUU - UC A		- UU CGGG
CLVa_JF446930 CLVd_DQ022677	CCCGC AGAA -	ACA-GG	- GUUU - UC A - GUUU - UC A	CCCUUC-CUUUC	- 00 CGGG - <mark>U</mark> U CGGG
PSTVd_FM998549	CCCGC AGAA -	ACA - GG	- <mark>GU</mark> UU - UC <mark>A</mark>	cccuuc - c <mark>uu</mark> c c	- <mark>UU CGGG</mark>
PCFVd_JF742640	CCaGC AGAA -	uCA-GG	- GUUU - UC A		
CLVd_JF446929	CCaGC - AGAA -	ACA - GG	- <mark>GU</mark> UU - UC A	CCCUUC - CUUUC	- UU CGGG
PCFVd_JF446913	CCaGC <mark>AGA</mark> C -	AC <mark>A - GG</mark>	- <mark>GU</mark> UU - UC <mark>A</mark>	<mark>.cccu</mark> uc - c <mark>uu</mark> uc	- UU CGGG
CLVd_JF446916 CLVd_JF446934	CCCGC AGAA -	ACA - GG ACA - GG	- GUUU - g C A - GUUU - UC A	CCCUUC - CUUUC	- UU Cu GG - UU Cu GG
CLVd_JF446935	CCCGC AGAA -	ACA - GG	- <mark>GU</mark> UU - UC A	cccuuc - cuuuc	- UU Cu GG
CLVd_JF446923	CCUGC - AGAA -	ACA - GG	- GUUU - UC A		- UU Cu GG
CLVd_JF446937 CLVd_JF446920	CCaGC AGAA -	ACA-GG	- GUUU - UC A	cccuuc - cuuuc	- UU Cu GG
CLVd_JF446926	CCCGu u GAA -	ACA - GG	- <mark>GU</mark> UU - UC - - <mark>A</mark>	<mark>.cccu</mark> uc - c <mark>uuuc</mark>	- UU <mark>C</mark> u <mark>GG</mark>
CLVd_JF742635		ACA-GG	- GUUU - UC A		
CLVd_JF446928	CCaGC uGAA -	ACA - GG	- <mark>GU</mark> UU - UC A	cccuuc - cuuuc	- Uc <mark>Cu GG</mark>
CLVd_DQ923060	CCaGC uGAA -	ACA - GG	- GUUU - UC A		- UU <mark>Cu GG</mark>
CLVd_HM043812 CLVd_AY367350	CCaGC u GAA - CCaGC u GAA -	gCA-GG ACA-GG	- GUUU - UC A - GUUU - UC A	cccuuc - cuuuc	- UU CuGG - UU CGGG
TASVd_DQ144506	CCCGC u GAA -	ACA - GG	- a <mark>U</mark> UU - UC <mark>A</mark>	uccuuc-c <mark>uuuc</mark>	- UU <mark>CGGG</mark>
PSTVd_AY493559 PSTVd_EU879913	CCCGC C GAA -	ACg - GG ACA - GG	- GUUU - UC A - GUUU - UC A	CCCUUC - CUUUC	- UU <mark>CGGG</mark> - UU <mark>CGGG</mark>
PSTVd_AY372398	CCCGC c GAA -	ACA - GG	- <mark>GU</mark> UU - UC A	cccuuc - cuuun	- UU <mark>CGGG</mark>
TASVd_HQ667140	CCCGC C GAA -	ACA - GG	- GUUU - UC A		- UU <mark>CGGG</mark>
PSTVd_Y09886	CCCGC c GAA -	ACA - GG	- <mark>GU</mark> UU - UC A	CCCC UC - CUUUC	- UU <mark>CGGG</mark>
PSTVd_AY492079	CCCGCCGAA-	ACA - GG	- <mark>GU</mark> UU - UC <mark>A</mark>	cccuuc - c <mark>uuuc</mark>	- UU <mark>C</mark> a <mark>GG</mark>
PSTVd_AY492081 PSTVd DQ308556	CCuGC c GAA - CCuaC u GAA -	ACA - GG ACA - GG	- GUUU - UC A - GUUU - UC A	CCCUUC - CUUUC	- UU <mark>C</mark> a GG - UU Ca GG
PSTVd_KF049417	CCu <mark>G</mark> C uGAA -	ACA - GG	- <mark>GU</mark> UU - UC <mark>A</mark>		- UU <mark>Ca GG</mark>
PSTVd_AY492080	CCUGC c GAA -	ACA - GG	- GUUU - UC A		
CLVd_AM698094	CCCGC u GAA -	ACA - GG	- <mark>GU</mark> UU - UC A	cccuuc - cuuuc	- UU <mark>CGGG</mark>
PSTVd_HQ452399	CCCGC c GAA -	ACA - GG	- <mark>GU</mark> UU - UC A		- UU g <mark>GGG</mark>
MPVd_FJ824844 TCDVd EF626530	CgCGC UGAA -	ACA - GU ACA - GG	- a UUU - UC A		- UU CGGG - UU CGc G
MPVd_L78460	CCCGC u GAA -	AC <mark>A</mark> - GG	- <mark>GU</mark> UU - UC <mark>A</mark>		- UU <mark>CGGG</mark>
TASVd_X06390	CCCGC u GAA -	ACA-GG	- GUUU - UC A		- UU CGGG
PCFVd_JF446895	CCCGC AGAA -	ACAu GG	- <mark>GU</mark> UU - UC A	ICCCUUC - CUUUC	- UU CGGG
PCFVd_JF446896	CCCGu AGAA -	ACAu GG	- <mark>GU</mark> UU - UC A	CCCUUC - CUUUC	- UU <mark>CGGG</mark>
CLVd_KC143293 CLVd_JF446927	CCaGC aGAA -	ACA-GG	- GUUU - UC A - GUUU - UC A	CCCUUC-CUUUC	- UU CuGG - UU CuGG
PCFVd_JF446902	CCCGu GAA -	ACA - GG	- <mark>GU</mark> UU - UC - - <mark>A</mark>	.cccuuc - c <mark>uuuc</mark>	- UU <mark>CGGG</mark>
PSTVd_EU257478 PCEVd_KC143301		ACA - GG	- GUUU - UC A	cccuuc - cuuuc	
PCFVd_KC143305	CCCGu u A A u	cg <mark>A</mark> - GG	- <mark>GU</mark> UU - UC A	cccuuc - cuuuc	- UU <mark>CGGG</mark>
PCFVd_KC143303	CCCGC a AAu	cgA-GG	- GUUU - UC A		- UU <mark>CGGG</mark>
TCDVd KF683201	CCGC CGAA - CCGC GU - AGAA -	ACA - GG ACA - GG	- GUUUa UC A - GUUU - UC A		- UU CGGG - UUcugcGG
TCDVd_EU625577	CCgugu - <mark>AGAA</mark> -	ACA - GG	- <mark>GU</mark> UU - UC <mark>A</mark>		- UUcugc <mark>GG</mark>
TCDVd_HG739070	CCgygu - AGAA -	ACA - GG	- <mark>GU</mark> UU - UC <mark>A</mark>		- UUcugc <mark>GG</mark>
TCDVd_AB329668	CCgag AGAA -	ACA - GG	- <mark>GU</mark> UU - UC <mark>A</mark>	ICCCUUC - CUUUC	- Ugauuc GG
TCDVd_FJ822878	CCgcgAGAA-		- GUUU - UC A		- Ugacgc <mark>GG</mark>
CSVd_E50939 CSVd_DQ094298	aau - C cuAA - aau - C cuAA -	ACA-GG	- GUUU - UC A - GUUU - UC A	CCCUUC - CUUUa	-gUcu -gUuu
CSVd_FN673554	aau - C cu <mark>AA</mark> -	ACg - GG	- <mark>GU</mark> UU - UC <mark>A</mark>	CCCUUC - CUUUa	- g U u u
CSVd_AB255880	aau - C cuAg -	ACA-GG	- GUUU - UC A	CCCUUC - CUc Ua	-gUuu -gUuu
CSVd_E13152	aau - C cuAA-	ACA - GG	- <mark>GU</mark> UU - UC A	CCCUUC - CUUUa	-gUau
CSVd_GQ229575	aau - C cuAA -		- GUUU - UC A		-gUuu
Cons.Seq	a a a - C CUAA - CCCGC AGAA -	ACA - GG ACA - GG	- <mark>GUUU</mark> - UC A	cccuuc - cuuuc	- g 0 Gu - <mark>UU</mark> <mark>CGG</mark> G
Cons.Struct	<mark>((((</mark> (<mark>(((</mark>	(. (. ((. <mark>((</mark>)))))))))	·)··· <mark>)))</mark>
cons.struct	aaaabccc. IL1	a. e. 11 IL2	HP	Igriedccc IL2	IL1

Figure S2. Structural alignment of terminal right hairpin. Left: Sequence names (viroid abbreviation + GenBank UID). From top to bottom: sequences [with background colors green for loops, red for consensus base pairs, pink for consensus base pair changes (covarying pairs), and white for non-base pairs in paired regions], the consensus sequence, and the consensus structure in bracket-dot notation and character-encoded (both with background colors from white to red proportional to sequence conservation resp. pairing probability). At the bottom are named the respective structural regions, for which ROSETTA modelling was done: IL1, internal loop 1; IL2, internal loop 2; HP, hairpin loop.

 Table S1. Leontis & Westhof nomenclature⁵

cis	Watson-Crick/Watson-Crick	cWW	
trans	Watson-Crick/Watson-Crick	tWW	-0-
cis	Watson-Crick/Hoogsteen	cWH	●-■
trans	Watson-Crick/Hoogsteen	tWH	0-0
cis	Hoogsteen/Watson-Crick	cHW	■ -●
trans	Hoogsteen/Watson-Crick	tHW	
cis	Watson-Crick/sugar	cWS	●->
trans	Watson-Crick/sugar	tWS	\bigcirc - \triangleright
cis	sugar/Watson-Crick	cSW	←●
trans	sugar/Watson-Crick	tSW	�-0
cis	Hoogsteen/Hoogsteen	cHH	-8-
trans	Hoogsteen/Hoogsteen	tHH	-0-
cis	Hoogsteen/sugar	cHS	∎→
trans	Hoogsteen/sugar	tHS	
cis	sugar/Hoogsteen	cSH	← ∎
trans	sugar/Hoogsteen	tSH	<-□
cis	sugar/sugar	cSs	→
trans	sugar/sugar	tSs	-0-
cis	sugar/sugar	csS	-
trans	sugar/sugar	tsS	-<
		GC	=
		AU	—
stacking			



Figure S3. Results of ROSETTA modeling of HP loop. Results for a typical loop of most pospiviroids is shown in (**a**); a loop typical for CEVd, IrVd, and CBCVd is shown in (**b**); an exceptional loop of TCDVd is shown in (**c**). The numbering of the CEVd loop in (**b**) is arbitrary due the additional loop nucleotide; the numbering of the closing pair, however, fits to the consensus structure (Fig. 1c). Top: Interactions identified by X3DNA-DSSR. Middle: 3D view produced by PYMOL. Bottom: Logos produced by RNA REDESIGN.



Figure S4. Histone mRNA stem-loop. The overlay on the left is identical to Fig. 7a; each further overlay is turned by 90° along the vertical axis.



Figure S5. Structure of a hairpin loop from the *Triticum aestivum* 80 S ribosome. The model (PDB entry $4V7E^6$) is based on cryo-electron microscopy with a resolution of < 5 Å. The hairpin loop is identical in sequence to TCDVd (GenBank ID GQ169709), but the closing pair is C:G instead of an A:U in the TCDVd.

Table S2. Structures and infectivity of HP variants.⁷

Mutant ^a	R+	1	2	3
Structure(s) ^b	$ \begin{array}{c} {}^{6} {}^{AAA} {}^{C} {}^{A} {}^{C}$	۲۵۵ میں ۲۵۵ می ۲۵۵ میں ۲۵۵ میں ۲۵۵ میں ۲۵۵ میں	GAAACAGGGCAA 175 CUULUUCCUCU CUULUUCCUCU IV -5.50 IV -5.50 GAAACAGGCCAA 175 GAAACAGGCCAA 175 GAAACAGGCCAA 175 GAAACAGGCCAA 175 CUULUUCCUU GGGCAAA CUULUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUU	GAAACAGGGUAU 2000 2020 2020 1000 1000 2000 2020 2020
Root ^c	0	2	2	1
Root ^d Leaves ^d	0 0	3 6	1 0	1 0

^aThe synthetic variant R+ differs from PSTVd intermediate (WT; AC M88678) by an insertion +G₁₇₆ and two replacements $U_{177}U_{178} \rightarrow AA$; variants 1–3 are the progeny from *Agrobacterium tumefaciens*-mediated infections of tomato plants with a corresponding plasmid containing a R+ cDNA insertion in (+) orientation.

^bOn the left are given relevant structures and their $\Delta G_{37\,^{\circ}C}^{\circ}$ values (in kcal/mol) as predicted by RNAFOLD;⁸ mutations are marked in bold. On the right are give annotations by RNAVIEW of top HP models predicted by ROSETTA.

^cIn 90% of infected plants, viroid progeny was only detected in galls and roots, but not leaves. Numbers give sequence variants present in six cDNA clones from root.

^dIn 10% of infected plants, viroid progeny was detected in galls, roots, and leaves. Numbers give sequence variants present in six cDNA clones from root (upper row) and leaf (lower row), respectively.

Mutant ^a	WT	MT1	MT2	MT3	MT4	MT6
Structure ^b	169 A A C A G G I I I I U U U C C 190 C C U 183	169 A A C U G G U U C C 190 C U 183	169 A A C A G G U U C C 190 C U 183	169 174 A A C U G G U U A C C 10 C C 183	169 174 Å A ^C A G G U U U C C 190 ^G C U 183	168 174 A A U ^G U G G U U A A C C 191 G A 183
Structure ^c	169 174 A A C A G G / / U U / / U C C 190 C C U 183	169 174 A A C U GG ↓ ↓ UU/ ↓ C C 190 C C U U 183	169 174 174 174 174 174 174 174 174	169 A A C UGG // UU/ACC 190 C U 183	169 174 1 C A G G 1 6 U G / U C C 190 C U 183	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$
Infectivity ^d	infectious	non-infectious	reverts	non-infectious	non-infectious	non-infectious
Percentage of bound RNA ^e	100	13	16	25	17	16

Table S3. Predicted structure of IL2 mutants.⁹

^aWild-type (WT) and mutant (MT1–4, MT6) names; the TR fragments (nt. 145–223) are named R79-wt, R79-mt1–4, and R79-mt6, respectively, in Gozmanova *et al.*⁹

^bNucleotides mutated in comparison to WT are in red; these structures were used as input for ROSETTA modelling.

^cAnnotation by RNAVIEW of top models predicted by ROSETTA.

^dLonger-than-unit-length viroid (based on variant KF440-2, AC X58388) RNA transcripts were assayed for infectivity on tomato plants.⁹

^eThe binding of a Virp1 fragment to TR RNA fragments was determined by EMSA; the fraction of bound WT RNA was set to 100%.⁹

Mutant ^a	25 (IL1)	26 (IL2)	27 (HP)
Structure of WT ^b	163 168 └GCCG↓ ↓↓ GCUUCU 196 191	$ \begin{array}{cccc} {}^{169} & {}^{173} \\ {}^{4} A & C & AG \\ {}^{1} A & / \\ {}^{1} V & / \\ {}^{1} U & V & / VC \\ {}^{190} & C & U & {}^{1}_{184} \end{array} $	175 G U U H ↓ U C A C ✓ 182
Structure of mutant ^c	163 C G A A G A G C U U C U 196 191	169 A G G A A G I I II I I I I U U C C U U C 190 184	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$
Replication efficiency/% ^d Trafficking ^e	29 0/12	46 0/12	53 1/12 ^f

Table S4. Structures and ability to replicate and traffick of synthetic TR mutants.¹⁰

^aThe loops in the secondary structure of PSTVd (see Fig. 1a) were numbered from left to right in Zhong *et al.*;¹⁰ that is, loops 25, 26, and 27 correspond to IL1, IL2, and HP, respectively. The internal loops IL1 and IL2 were closed by canonical WC base pairs; in the HP loop a replacement $U_{176}U \rightarrow AA$ was made.

^bAnnotation by RNAVIEW of top models predicted by ROSETTA for the WT sequence.

^cAnnotation by RNAVIEW of top models predicted by ROSETTA for the mutant sequences. The red letters denote introduced mutations from the WT.

^dAccumulation level of circular genomic RNA in inoculated protoplasts of *Nicotiana benthamiana* cultured cells expressed as percentage of wild-type PSTVd.

^eNumber of plants showing systemic infection over total number of plants inoculated.

^f In the one plant that got systemically infected an additional mutation ($A_{181} \rightarrow U$; see structure on the right) occurred that restored the loop-closing basepair.

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