

***Arabidopsis thaliana* plant natriuretic peptide active domain forms amyloid-like fibrils in a pH-dependent manner**

Georgia I. Nasi<sup>1,†</sup>, Foteini D. Aktypi<sup>1,†</sup>, Panagiotis M. Spatharas<sup>1,‡</sup>, Nikolaos N. Louros<sup>1,§,¶</sup>, Paraskevi L. Tsiolaki<sup>1,¶</sup>, Vassiliki Magafa<sup>2</sup>, Ioannis P. Trougkos<sup>1</sup> and Vassiliki A. Iconomidou<sup>1,\*</sup>

<sup>1</sup> Section of Cell Biology and Biophysics, Department of Biology, School of Sciences, National and Kapodistrian University of Athens, Panepistimiopolis, 157 01 Athens, Greece

<sup>2</sup> Department of Pharmacy, University of Patras, 265 04 Patras, Greece

\* Correspondence: veconom@biol.uoa.gr; Tel.: +30-210-7274871; Fax: +30-210-7274254

† These authors contributed equally to this work.

‡ Present Address: European Molecular Biology Laboratory, Hamburg Unit, Notkestrasse 85, 22607 Hamburg, Germany.

§ Present Address: KU Leuven, Department of Cellular and Molecular Medicine, VIB-KU Leuven, 3000 Leuven, Belgium.

" Present Address: VIB Center for Brain and Disease Research, Switch Laboratory, Herestraat 49, 3000 Leuven, Belgium.

¶ Present Address: Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143, USA.

[illegible]

The graphical chart represents the secondary structure prediction profile of AtPNP-A per residue (orange: turn, red: helix, green:  $\beta$ -strand). Yellow line illustrates a ‘consensus threshold’, where at least three (3) out of six (6) methods concur in secondary structure prediction. Bold letters depict the AtPNP-A36-69 peptide-analogue, tested experimentally. B-strands are the main secondary structure element of the AtPNP-A sequence, but there are regions that are predicted as “chameleon sequences” since, according to SECSTR prediction, they tend to adopt a helical or an extended conformation (H :  $\alpha$ -helix, B:  $\beta$ -strand, T:  $\beta$ -turn). (B) Secondary structure prediction of AtPNP-A from the PORTER algorithm. According to the prediction, AtPNP<sub>36-69</sub> adopts a turn –  $\beta$ -strand – turn –  $\beta$ -strand – helix – turn fold.

Antimicrobial peptide 2	1	10					
	----- -----						
	TESYFVFSVGM *****						
Defensin like protein 1	1	10	20	30	40	50	
	----- ----- ----- ----- ----- -----						
	QKLCERPSGTWSGVCNNACKNQCNLEKARHGSCNYVFPAHKCICYFPC *****						
Defensin like protein 2	1	10	20	30	40	50	
	----- ----- ----- ----- ----- -----						
	QKLCQRPSGTWSGVCNNACKNQCNIRLEKARHGSCNYVFPAHKCICYFPC *****						
Defensin like protein 16	1	10	20	30	40	50	
	----- ----- ----- ----- ----- -----						
	QKLCEKPSGTWSGVCNSNACKNQCNILEGAKHGSCNYVFPAHKCICYVPC *****						
AtPNP-A	1	10	20	30	40	50	
	----- ----- ----- ----- ----- -----						
	AQGKAVYYDPFYTRSACYGTQRETTLVGVKNNLWQNGRACGRRYVRVCIG *****						
	51	60	70	80	90	105	
	----- ----- ----- ----- ----- -----						
	ATYNFDRACTGRTVDVKVVDFCREPCNGDLNLSRDAFRVIANTDAGNIRVVYTP * *****						
Hevein-like preproprotein	1	10	20	30	40	50	60
	----- ----- ----- ----- ----- -----						
	QQCGRQGGGRTCPGNICCSQYGYCGTTADYCSPTNNCQSNCWGSGPSGPGESASNVRATY ***						
	61	70	80	90	100	110	120
	----- ----- ----- ----- ----- -----						
	HFYNPAQNNWDLRAVSAYCSTWDADKPYAWRSKYGWTAFCGPAGPRGQASCGKCLRVKNT *****						
	121	130	140	150	160	170	180
	----- ----- ----- ----- ----- -----						
	RTNAAVTVRIVDQCSNGGLDLDVAMFNQIDTDGFGYQQGHLIVDYQFVDCGNELIGQPDS *****						
	181	190					
	----- -----						
	RNMLVSAIDRV *****						
Pro-hevein	1	10	20	30	40	50	60
	----- ----- ----- ----- ----- -----						
	EQCGRQAGGKLCPNNLCCSQWGWCGSTDEYCSPDHNCQSNCKDSGEGVGGGSASNVLATY *****						
	61	70	80	90	100	110	120
	----- ----- ----- ----- ----- -----						
	HLYN SQDHGWDLNAASAYCSTWDANKPYSWRSKYGWTAFCGPVGAHQSSCGKCLSVTNT *****						
	121	130	140	150	160	170	180
	----- ----- ----- ----- ----- -----						
	GTGAKTTVRIVDQCSNGGLDLDVNVFRQLD TDGKGYERGHITVNYQFVDCGDSFNP LFSV *** *****						
	181						
	-----						
	MKSSVIN						

Supplementary Figure S4. Prediction of AMYLPRED [91] algorithm for Antimicrobial peptide 2 (P86706) of *Cocos nucifera*, Defensin-like protein 1 (P69241) and Defensin-like protein 2 (P30230)

of *Raphanus sativus*, Defensin-like protein 16, Hevein-like preprotein (P43082) and AtPNP-A (Q9ZV52) of *Arabidopsis thaliana* and Pro-hevein (P02877) of *Hevea brasiliensis*. Predicted “aggregation-prone” segments by AMYLPRED are shown in green and are marked with ‘\*’ under the sequence. The amyloidogenic profiles of Defensin-like protein 1, Defensin-like protein 2 and Defensin-like protein 16 are the same, implying that Defensin-like protein 16 has the ability to form amyloid fibrils. Additionally, the amyloidogenic profiles of Hevein-like preprotein and Pro-hevein are similar, suggesting that Hevein-like preprotein can form amyloid fibrils. Aggregation-prone regions are also predicted in AtPNP-A, with one of them located in the 36-69 region.