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Supplementary Materials

Bifunctional peptides as alternatives to copper-based formulations to control citrus canker

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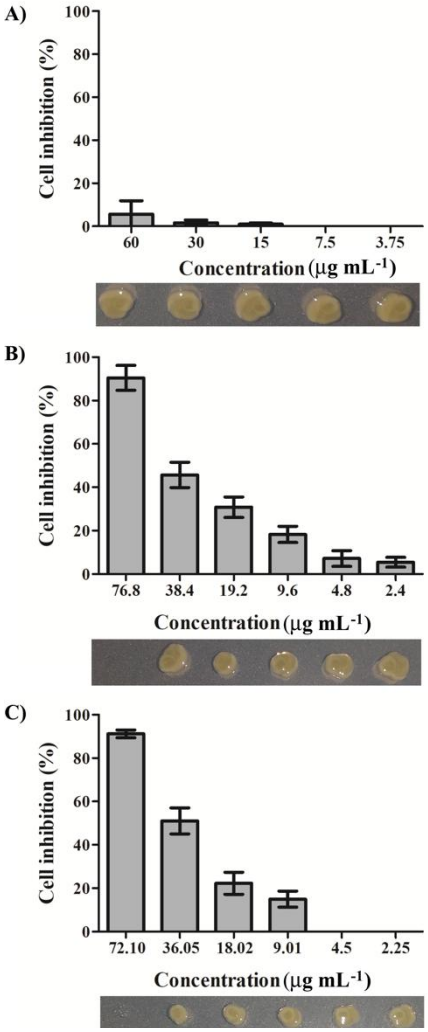

Fig. S1. Evaluating the antibacterial action of the BiFuProt modules used to construct Mel-CgDEF. The respiratory activity of *X. citri* cells was monitored by REMA following 16 hours of exposure to the BiFuProt modules at various concentrations. **A)** eGFP-CgDEF; **B)** eGFP-Mel, and **C)** Mel-eGFP. The average percentages of cells inhibited by the peptide are represented as bars and the standard deviation of the means by the vertical lines above the bars. Underneath the graph is shown a section of a NYG-plate in which samples from REMA were inoculated in order to evaluate if the BiFuProt modules had bactericidal or bacteriostatic effect. Yellow cell mass (colonies) indicate growth.

Table S1. Amino acid sequences, PDB and UniProt entries for all the peptides used.

Peptide	Abbr.	Amino acid sequence (from N- to C-terminus)	PDB	UniProt
Androctonin	Andt	RSVCRQIKICRRRGGCYYKCTNRPY	1CZ6	P56684
LCI	LCI	AIKLVQSPNGNFAASFVLDGTKWIFKSKYYDSSKGYWVGIYEVWDRK	2B9K	P82243
Plantaricin A	PlnA	KSSAYSLQMGATAIKQVKKLFKKWGW	1YTR	P80214
Tachystatin A2	TA2	YSRCQLQGFNCVVRSYGLPTIPCCRGLTCRSYFPGSTYGRCQRY	1CIX	Q9U8X3
Defensin	CgDEF	GFGCPGNQLKCNNHCKSISCRAGYCDAATLWLRCTCTDCNGKK	2B68	Q4GWV4
MBP-1	MPB1	RSGRGECRRQCLRRHEGQPWETQECMRRCRRRG		P28794
Melittin	Mel	GIGAVLKVLTTGLPALISWIKRKRQQ	2MLT	P01501

Table S2. Amino acid and DNA sequence of the bifunctional peptide and its five elements: melittin, 3xGGGS, StrepII-tag, domain Z, and CgDEF (from N- to C-terminus).

	N-Melittin-	-3xGGGS-	-StrepII-tag-	-Domain Z-	- CgDEF -C
AA	MGIGAVLKVLT TGLPALISWIKR KRQQ	GGGGSG GGGSGG GGS	SAWSHPQF EK	ADNKFNKEQQNAFYEILHLPNLNEEQ RNGFIQSLKDDPSQSANLLAEAKKLN DAQAPK	GFGCPGNQL KCNNHCKSIS CRAGYCDAA TLWLRCTCT DCNGKK
DNA	ATGGGAATTG GTGCTGTGTTG AAGGTTCTTAC TACCGGCCTGC CAGCGTTAATT TCCTGGATTAA ACGTAAGCGG CAA	CAGGGT GGTGGTG GTAGTGG TGGCGGT GGTTCAG GCGGTG GCGGTA GC	TCTGCATG GAGCCATC CGCAGTTC GAAAAG	GCCGACAACAAGTTTAACAAAGAA CAGCAGAACGCCTTCTATGAAATTC TGCATCTGCCGAATCTGAATGAAGA ACAGCGTAATGGTTTTATCCAGAGC CTGAAAGATGATCCGAGCCAGAGC GCAAATCTGCTGGCCGAAGCAAAA AAACTGAATGATGCGCAGGCACCG AAA	GGTTTTGGT TGTCCGGGT AATCAGCTG AAATGTAAC AATCATTGC AAAAGCATT AGCTGCCGT GCAGGTTAT TGTGATGCA GCAACCCTG TGGCTGCGT TGTACCTGT ACCGATTGT AATGGCAAA AAA

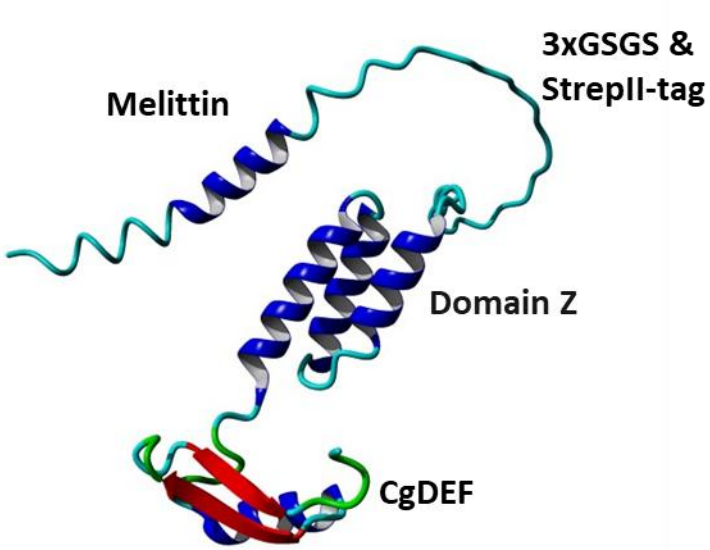

Fig. S2. 3D Protein Structure Model of the BiFuProt Mel-CgDEF. The model was generated using the established AlphaFold 2.0[1] structure database using the amino acid sequence from Tab. S2. The best ranked model is shown.

[1] Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R, Žídek A, Potapenko A, et al. (2021) Highly accurate protein structure prediction with AlphaFold. Nature 596:583–589.