

Supplemental Material

Table S1. *In meso* crystallization conditions in three hosting MAGs and gramicidin solubility in the corresponding precipitant solutions.

	7.7 MAG	8.8 MAG	9.9 MAG
Precipitant ^a	25 % PEG 3.35k	30 % PEG 8k	20 % PEG 6k
Salt	0.2 M LiSO ₄	0.2 M NH ₄ SO ₄	-
Buffer	0.1 M Bis-Tris pH 5.5		0.1 M Bicine pH 9.0
gD solubility ^b	0.04 g/L, 21 μM	0.10 g/L, 53 μM	0.02 g/L, 11 μM

^a Percentage is reported as weight by volume. ^b Gramicidin solubility in precipitant solutions was determined in the absence of lipid.

Table S2 – Ramachandran analysis.^a

Structure	Residues in favoured regions, Number (%)	Residues in allowed regions, Number (%)	Residues in outlier regions, Number (%)
7.7 MAG	68 (87.2)	10 (12.8)	0 (0)
8.8 MAG	69 (88.5)	9 (11.5)	0 (0)
9.9 MAG	68 (87.2)	10 (12.8)	0 (0)

^a The ϕ and ψ torsion angles for all amino acids were calculated using RAMPAGE.¹ The torsion angle sign for the D-amino acids was reversed, as described by Glowka et al.,² moving them into the most favoured region of the Ramachandran plot. All glycines are in glycine allowed regions.

Table S3. Possible scenarios for the way in which gramicidin crystallizes in mesophases formed with different MAGs.

Steps in <i>in meso</i> crystallization	Scenario / conformation ^a				
	1	2	3	4	5
Solubilization/Reconstitution	DSDH	MIXED	MIXED	MIXED	MIXED
In mesophase	DSDH	DSDH	MIXED	MIXED	MIXED
In mesophase + precipitant	DSDH	DSDH	DSDH	MIXED	MIXED
Phase separation	DSDH	DSDH	DSDH	DSDH	MIXED
Final crystal	DSDH	DSDH	DSDH	DSDH	DSDH

^a DSDH refers to the specific double stranded, intertwined double helix conformation of gramicidin that appears in final crystals. MIXED indicates that gramicidin can adopt different conformations.

1. Lovell, S. C.; Davis, I. W.; Arendall, W. B., 3rd; de Bakker, P. I.; Word, J. M.; Prisant, M. G.; Richardson, J. S.; Richardson, D. C. *Proteins* **2003**, *50*, 437-450.
2. Glowka, M. L.; Olczak, A.; Bojarska, J.; Szczesio, M.; Duax, W. L.; Burkhart, B. M.; Pangborn, W. A.; Langs, D. A.; Wawrzak, Z. *Acta Crystallogr. Sect. D* **2005**, *61*, 433-441.