Published in final edited form as:

J Phys Chem Lett. 2020 November 05; 11(21): 9501–9506. doi:10.1021/acs.jpcllett.0c02094.

On the Aggregation State of Synergistic Antimicrobial Peptides

Jacob M. Remington^a, Chenyi Liao^a, Mona Sharafi^a, Emma Ste. Marie^{a,b}, Jonathon B. Ferrell^a, Robert Hondal^{a,b}, Matthew J. Wargo^c, Severin T. Schneebeli^a, Jianing Li^{a,*}

^aDepartment of Chemistry, University of Vermont, Burlington, VT 05405

^bDepartment of Biochemistry, University of Vermont, Burlington, VT 05405

^cDepartment of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT 05405

Abstract

By integrating various simulation and experimental techniques, we discovered that antimicrobial peptides (AMPs) may achieve synergy at an optimal concentration and ratio, which can be caused by aggregation of the synergistic peptides. On multiple time and length scales, our studies obtain novel evidence of how peptide co-aggregation in solution can affect disruption of membranes by synergistic AMPs. Our findings provide crucial details about the complex molecular origins of AMP synergy, which will help guide the future development of synergistic AMPs as well as applications of anti-infective peptide cocktail therapies.

Graphical Abstract

*Corresponding Author Jianing Li (jianing.li@uvm.edu).

Supporting Information. Simulation methods including model preparation, simulation protocols, and data analysis are included in the supporting information. Experimental methods describing peptide synthesis, dynamic light scattering, and the optical density measurements of *E. coli* inhibition are included as well.

The following files are available free of charge.

AMP_JPCL_SI.pdf file (PDF)

The authors declare no competing financial interests.

Keywords

Peptide co-aggregation; antimicrobial activity; membrane poration; antibiotic resistance; and molecular dynamics

Antibiotic resistance is one of the biggest threats to global health and food production. It leads to dangerous infections, long hospital stays, high medical costs, and significantly increased mortality.^{1,2} Additional to solutions like conventional antibiotics and vaccines, antimicrobial peptides (AMPs), especially broad-spectrum antibacterial ones, have emerged to aid our battle against challenging bacterial infections and multi-drug resistance. Over 12,000 antimicrobial AMPs have been identified from natural sources or via bioorganic synthesis.^{3–5} Despite great variation in the sequence, length, and structure, many AMPs have their antimicrobial activity largely attributed to the ability to induce membrane disruption or poration.^{6,7} Several mechanisms of action have been proposed, based on experimental evidence,^{8,9} as well as molecular dynamics (MD) simulations^{10–12} and accelerated techniques^{13–16} using either all-atom or coarse-grained models.¹⁷

While earlier studies almost exclusively focused on individual peptides, most natural AMPs exist in mixtures.^{18,19} It has been long hypothesized that combinations of two or more types of AMPs may generate synergism,^{20,21} where the effect of combined AMPs is stronger than that of each component in the equivalent dose. However, only few examples of synergistic AMPs have been identified,²² mainly due to limited knowledge about the synergy mechanisms. In this work, we obtained novel evidence from simulations and experiments to show how peptide aggregation in solution can affect disruption of membranes by synergistic AMPs, with a focus on two prototypical AMPs — Magainin 2 (MG2) and Tachyplesin 1 (TP1) — which are similar in the molecular mass but distinct in sequence and structure (Figure 1). The drastic structural differences between these two peptides contrasts MG2 and TP1 synergy from recent^{23,24} and past work^{25–27} on MG2 and PGLa synergy wherein both peptides are helical and found to self-assemble on the membrane surface to form

transmembrane pores. Our findings are significant in advancing current knowledge of AMP synergy mechanisms between peptides with different secondary structure elements, and in providing a new route for seeking synergistic AMPs.

MG2 and TP1 have been long known to kill bacteria via poration of bacterial membranes.^{28–32} Their synergy was previously suggested at 1.25 μ M MG2 and 0.1 μ M TP1, the minimum concentrations to inhibit *E. coli* growth.³³ Herein, we performed a systematic investigation, by directly measuring the percentages of *E. coli* growth inhibition at different concentrations and ratios. The quantitative nature of our results allowed us, for the first time, to investigate and optimize the actual ratios of the synergistic peptides, which has not been accomplished yet with the coarser measurements of minimum inhibition concentration. We discovered that maximum synergy was achieved with a 1:1 ratio of MG2 and TP1, while MG2 was present in large excess in the prior study.³³

We started with *E. coli* growth assays, which showed that both peptides inhibited bacterial growth when present in concentrations as low as 2 μ g/mL (<1 μ M). Furthermore, we found that *E. coli* growth decayed with increasing peptide concentration (Figure 2A), with TP1 appearing more potent than MG2. Synergy was then quantified by comparing experimentally measured *E. coli* growth (black bars in Figure 2B, with doses of 1:1, 1:4, and 4:1 MG2: TP1 mass ratio, raw data in Table S1-S3) to the expected *E. coli* growth ($G_{\text{calculated}}$) assuming the peptides act independently to inhibit bacterial growth (Eq. 1, orange bars in Figure 2B).

$$G_{\text{calculated}}([\text{MG2}], [\text{TP1}]) = G([\text{MG2}])G([\text{TP1}]) \quad \text{Eq. 1}$$

In Eq. 1 $G([\text{MG2}])$ and $G([\text{TP1}])$ are the measured concentration dependent *E. coli* growth of the individual peptides. While synergy was observed in nearly all of pairs of concentrations across all trials, (Tables S1-S3) only the 1:1 ratio with 8 μ g/mL of each peptide showed a statistically significant difference at a 95% confidence level (see Supporting Information). This combination had a $12.9 \pm 3.9\%$ higher activity than expected without synergy. However, excess of MG2 did not appear to enhance synergy, as becomes clear when one, for example, compares the *E. coli* growth at 32 μ g/mL MG2 and 8 μ g/mL TP1 versus at 8 μ g/mL MG2 and 32 μ g/mL TP1 (Figure 2B). This later finding contrasts findings from previous work that found higher MIC at higher ratios of MG2 to TP1.³³ Generally, these results reveal a dependence of MG2-TP1 synergy on peptide concentrations and ratios.

With the synergy confirmed, we next discovered that MG2 and TP1 co-aggregate in solution, using Dynamic Light Scattering (DLS) spectra and large-scale, all-atom MD simulations. At the tested concentrations (2.0×10^{-8} to 10^{-11} M), pure MG2 or TP1 aggregated into particles of hydrodynamic diameters $R_h > 80$ nm (Figures 3D and S1). However, our DLS data indicate that the formation of large peptide aggregates is generally reduced when the MG2 and TP1 peptides are mixed in a 1:1 ratio with the R_h of the particles formed in the range of 13 – 200 nm (Figure 3D), which differ from the aggregates formed by either MG2 or TP1 alone. It is noted that the aggregates larger than 200 nm in Figure 3D are

only present in very low numbers (since the DLS intensity depends on the 6th power of the particle size) and are thus unlikely to be the major active antimicrobial species in solution. These data suggest that within the sub-micromolar concentration range, MG2 and TP1 co-aggregate into smaller particles than the ones formed by individual peptides.

Consistent with the DLS evidence, our MD simulations show that the MG2 and TP1 peptides co-aggregate in solution on the microsecond timescale. We simulated four MG2 (either helical or coiled) and four TP1 (either cyclic or linear) peptides arbitrarily placed in the solution, and observed TP1 and MG2 forming hetero-oligomers of various sizes in all of our simulations (Figure S2 and S3). While MD simulations and DLS spectra agreed on the higher aggregation tendency of MG2 (compared to TP1) in pure peptide solutions, the helical MG2 and cyclic TP1 formed the most ordered and stable aggregates — with MG2 in the core and TP1 in the vicinity (Figure 3A). Loose complexes formed in the early stage with a radius of gyration, $R_g = 38 \text{ \AA}$, and then gradually turned into a stable hetero-oligomer (Figure 3A) toward the end of the simulations, with R_g reducing to as low as 18–25 $\text{\AA}$ and the solvent-accessible surface area (SASA) decreasing by 30 to 35% within 2 μs . Further simulation analysis provided more structural insight: like many amphipathic AMPs,³⁴ MG2 (Figure 1A) used one hydrophobic surface (F5, F12, and F16) for aggregation and the other surface (K4, K10, K11, K14, and E19) for solvent exposure; due to the alternating hydrophobic and cationic residues (Figure 1B), TP1 was inclined to bind E19 of MG2 and attach to the MG2 core in a nonspecific fashion (Figure 3A).

With MG2-TP1 co-aggregation confirmed both experimentally and computationally, we simulated the detailed mechanism of membrane poration and provided evidence that the MG2-TP1 hetero-oligomers formed in the solution may damage bacterial membranes as an entity. AMP-induced pore formation — defined here as water channels that span the membrane — can be a central event of AMP mechanisms. Although it has been speculated that AMPs aggregate on the bacterial membrane surface to form membrane pores, MG2 and TP1 co-aggregation on the membrane surface likely occurs on a longer timescale than the corresponding co-aggregation in solution. We simulated four helical MG2 and four cyclic TP1 peptides dispersed on the surface of lipid bilayers (PE:PG 3:1, mass percent), but no consistent aggregation was observed on the membrane surface during the 2- μs simulations (Figure 3B), as indicated by the large R_g and SASA values of the peptides (Figure 3C). This finding contrasts the much faster MG2-TP1 hetero-oligomer formation in solution (Figure 3A), which supports the possibility that MG2 and TP1 act on the bacterial membrane in the hetero-oligomeric form.

To gain further evidence, we utilized an enhanced sampling protocol to (1) surmount the energy barrier associated with the MG2-TP1 oligomer embedding in the membrane and (2) sample relevant complex conformations in membrane-disrupted states. Indeed, the process of membrane poration often occurs on larger timescales than what has been simulated.^{35,36} It is even harder for complex systems with multiple peptide and lipid species in the bacterial membrane models. To obtain relevant conformations of peptide aggregates in a membrane-disrupted state, we have designed a protocol that employed alternating steered and unbiased MD simulations to mimic the process of peptides colliding into the elastic lipid bilayer. Mechanistically, this approach was used to reveal how the experimentally observed hetero-

oligomers may interact selectively with the bacterial membrane to form a pore. In each step, we pulled the hetero-oligomer along the negative Z-axis direction at a constant velocity applied to the peptide backbones for 40 ps (Figure S4), followed by an 8-ns relaxation of the peptide-lipid interactions. After ~20 steps a pore emerged in the membrane (indicated by water penetration), an unbiased MD simulation was run to sample stable MG2-TP1 conformations in the membrane pore (Figure 4). Two membrane models to mimic different bacterial membranes were tested (mass percent): (1) PE:PG 3:1 and (2) PE:PG:Cardiolipin 65:10:25, which displayed stable membrane pores under the simulation protocol. These pores remained during the final stage for over 100 ns and were observed in replica simulations (Figure S5), suggesting converged simulations with limited fluctuations of stable pore structures. Using similar protocols, however, we did not see pore formation in the simulations of MG2-TP1 oligomer in a mammalian membrane model (POPC:cholesterol 9:1, Figure S6) and a non-AMP peptide, FTA (Figure 1C) oligomer in the membrane (Figure 4A1). Both of these two results suggest that the mechanical forces alone (Figure S4) were insufficient to disrupt the membrane integrity. Hence, these results highlight the ability of our simulation protocol to differentiate between peptide oligomers which disrupt bacterial membranes and those that do not, providing evidence that the sampled conformations are biophysically relevant.

Further examination of the hetero-oligomer conformations and contacts with the lipids suggest that synergy between MG2 and TP1 may arise from forming active hetero-oligomers. (1) The hetero-oligomers provide the specific molecular scaffold for membrane disruption, similar to what was hypothesized about amyloid pores.^{37–40} Specifically, the MG2-TP1 hetero-oligomers remained compact during the simulated process of membrane poration, whereas in the control simulation, the FTA aggregate quickly dissociated on the membrane surface, indicated by the drastic increase in R_g (from 22 to 40 Å) and SASA (by 17%). Furthermore, the MG2 core readily intruded itself into the membrane, while TP1 stabilized the oligomer in the membrane (Figure 4 and S7). These events demonstrate how the arrangement of TP1 and MG2 within the aggregate structure can stabilize transmembrane pores. At the same time, inspection of Figures 4A3 and 4B3 established the formation of energetically stabilizing interactions between the positively charged sidechains of the AMP aggregates and the PG and CL headgroups. (2) The hetero-oligomers cause enhanced local disorder and facilitated membrane disruption. In the membrane-disrupted hetero-oligomer, MG2 readily adopted various orientations (Figure 4), which consequently altered the bound lipid orientations. Some lipid molecules can also be pulled out from the membrane by the MG2-TP1 oligomer (Figure 4A3).

It has been suggested that AMP oligomers at the nanometer scale provide a critical local concentration of peptides in functional conformations;⁴¹ however, large AMP aggregates increase the free-energy cost for membrane poration and reduce the antibacterial activity.⁴² In this respect, the electrostatic interactions between the MG2 and TP1 aggregates and the membranes observed in our simulations may provide stabilizing energy to enhance membrane disruption. Based on our computational and experimental results, we propose the following mechanism for the observed concentration dependence of the MG2-TP1 synergy: While the MG2-TP1 mixture at nanomolar to micromolar concentrations forms smaller, more active hetero-oligomers than individual peptides in solution, MG2 and TP1 — at a

concentration higher than the naturally occurring ones — can generate larger hetero-oligomers and thus become less effective to embed into the membrane. Further, because MG2 aggregates more readily than TP1, excessive MG2 does not improve the antibacterial activity. Thus, the observation that the size of aggregates formed by TP1 and MG2 mixtures depends on concentration provides a pathway to regulate the synergetic behavior of the two AMPs. Moreover, this can be a general mechanism underlying the concentration dependence of synergistic AMPs, as aggregation/co-aggregation is ubiquitous among peptides. With awareness of such concentration dependences, we will likely find that synergistic AMPs exist more commonly than what is currently known.

In summary, by means of MD simulations and experimental characterizations, we explored the molecular origins of MG2-TP1 synergy. These two AMPs likely form oligomeric structures before binding to the anionic membrane surface. The hetero-oligomers enhance recognition to the bacterial membranes. Also, the hetero oligomers' high stability and significant membrane disruption potential helps stimulate water channel formation. Our studies provide valuable insight into the mechanistic details of AMP synergism, using the MG2-TP1 combination as a model system. Specifically, we show that co-aggregation plays an important role in enabling molecular interactions that may lead to the observed synergy. While only few synergistic AMPs are known currently, our findings from the MG2-TP1 combination suggest several possible directions to pursue for future synergistic AMPs, which can give rise to higher efficacy and lower risk of adverse effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENT

We thank PSC-ANTON, XSEDE, and the Vermont Advanced Computing Core for supercomputing resources. J. L., J. M. R., and C. L. were partially supported by an NIH award (R01GM129431). S.T.S. was supported by the U.S. Army Research Office (Grant 71015-CH-YIP).

REFERENCES

- (1). Peterson E; Kaur P. Antibiotic Resistance Mechanisms in Bacteria: Relationships Between Resistance Determinants of Antibiotic Producers, Environmental Bacteria, and Clinical Pathogens. *Front Microbiol* 2018, 9, 2928–2928. 10.3389/fmicb.2018.02928. [PubMed: 30555448]
- (2). Blair JMA; Webber MA; Baylay AJ; Ogbolu DO; Piddock LJV Molecular Mechanisms of Antibiotic Resistance. *Nature Reviews Microbiology* 2015, 13 (1), 42–51. 10.1038/nrmicro3380. [PubMed: 25435309]
- (3). Wang G; Li X; Wang Z. APD3: The Antimicrobial Peptide Database as a Tool for Research and Education. *Nucleic acids research* 2016, 44 (D1), D1087–93. 10.1093/nar/gkv1278. [PubMed: 26602694]
- (4). Pirtskhalava M; Gabrielian A; Cruz P; Griggs HL; Squires RB; Hurt DE; Grigolava M; Chubinidze M; Gogoladze G; Vishnepolsky B; et al. DBAASP v.2: An Enhanced Database of Structure and Antimicrobial/Cytotoxic Activity of Natural and Synthetic Peptides. *Nucleic acids research* 2016, 44 (D1), D1104–12. 10.1093/nar/gkv1174. [PubMed: 26578581]

- (5). Mookherjee N; Anderson MA; Haagsman HP; Davidson DJ Antimicrobial Host Defence Peptides: Functions and Clinical Potential. *Nature Reviews Drug Discovery* 2020, 19 (5), 311–332. 10.1038/s41573-019-0058-8. [PubMed: 32107480]
- (6). Hamley IW Small Bioactive Peptides for Biomaterials Design and Therapeutics. *Chemical reviews* 2017, 117 (24), 14015–14041. 10.1021/acs.chemrev.7b00522. [PubMed: 29227635]
- (7). Xu C; Ma W; Wang K; He K; Chen Z; Liu J; Yang K; Yuan B. Correlation between Single-Molecule Dynamics and Biological Functions of Antimicrobial Peptide Melittin. *The Journal of Physical Chemistry Letters* 2020, 11 (12), 4834–4841. 10.1021/acs.jpclett.0c01169. [PubMed: 32478521]
- (8). Chan DI; Prenner EJ; Vogel HJ Tryptophan- and Arginine-Rich Antimicrobial Peptides: Structures and Mechanisms of Action. *Biochimica et biophysica acta* 2006, 1758 (9), 1184–1202. 10.1016/j.bbamem.2006.04.006. [PubMed: 16756942]
- (9). Lam KL; Wang H; Siaw TA; Chapman MR; Waring AJ; Kindt JT; Lee KY Mechanism of Structural Transformations Induced by Antimicrobial Peptides in Lipid Membranes. *Biochimica et biophysica acta* 2012, 1818 (2), 194–204. 10.1016/j.bbamem.2011.11.002. [PubMed: 22100601]
- (10). Sengupta D; Leontiadou H; Mark AE; Marrink SJ Toroidal Pores Formed by Antimicrobial Peptides Show Significant Disorder. *Biochimica et biophysica acta* 2008, 1778 (10), 2308–2317. 10.1016/j.bbamem.2008.06.007. [PubMed: 18602889]
- (11). Mihajlovic M; Lazaridis T. Antimicrobial Peptides in Toroidal and Cylindrical Pores. *Biochimica et biophysica acta* 2010, 1798 (8), 1485–1493. 10.1016/j.bbamem.2010.04.004. [PubMed: 20403332]
- (12). Irudayam SJ; Berkowitz ML Binding and Reorientation of Melittin in a POPC Bilayer: Computer Simulations. *Biochimica et biophysica acta* 2012, 1818 (12), 2975–2981. 10.1016/j.bbamem.2012.07.026. [PubMed: 22877705]
- (13). Rui H; Im W. Protegrin-1 Orientation and Physicochemical Properties in Membrane Bilayers Studied by Potential of Mean Force Calculations. *Journal of computational chemistry* 2010, 31 (16), 2859–2867. 10.1002/jcc.21580. [PubMed: 20589740]
- (14). Zhao J; Zhao C; Liang G; Zhang M; Zheng J. Engineering Antimicrobial Peptides with Improved Antimicrobial and Hemolytic Activities. *Journal of chemical information and modeling* 2013, 53 (12), 3280–3296. 10.1021/ci400477e. [PubMed: 24279498]
- (15). Sun D; Forsman J; Lund M; Woodward CE Effect of Arginine-Rich Cell Penetrating Peptides on Membrane Pore Formation and Life-Times: A Molecular Simulation Study. *Physical Chemistry Chemical Physics* 2014, 16 (38), 20785–20795. 10.1039/C4CP02211D. [PubMed: 25166723]
- (16). Mukherjee S; Kar RK; Nanga RPR; Mroue KH; Ramamoorthy A; Bhunia A. Accelerated Molecular Dynamics Simulation Analysis of MSI-594 in a Lipid Bilayer. *Physical Chemistry Chemical Physics* 2017, 19 (29), 19289–19299. 10.1039/C7CP01941F. [PubMed: 28702543]
- (17). Kirsch SA; Bockmann RA Membrane Pore Formation in Atomistic and Coarse-Grained Simulations. *Biochimica et biophysica acta* 2016, 1858 (10), 2266–2277. 10.1016/j.bbamem.2015.12.031. [PubMed: 26748016]
- (18). Vanhoye D; Bruston F; Nicolas P; Amiche M. Antimicrobial Peptides from Hyliid and Ranin Frogs Originated from a 150-Million-Year-Old Ancestral Precursor with a Conserved Signal Peptide but a Hypermutable Antimicrobial Domain. *European journal of biochemistry* 2003, 270 (9), 2068–2081. 10.1046/j.1432-1033.2003.03584.x. [PubMed: 12709067]
- (19). Hancock RE; Sahl HG Antimicrobial and Host-Defense Peptides as New Anti-Infective Therapeutic Strategies. *Nature biotechnology* 2006, 24 (12), 1551–1557. 10.1038/nbt1267.
- (20). Yu G; Baeder DY; Regoes RR; Rolff J. Combination Effects of Antimicrobial Peptides. *Antimicrobial agents and chemotherapy* 2016, 60 (3), 1717–1724. 10.1128/aac.02434-15. [PubMed: 26729502]
- (21). Hanson MA; Dostálová A; Ceroni C; Poidevin M; Kondo S; Lemaitre B. Synergy and Remarkable Specificity of Antimicrobial Peptides in Vivo Using a Systematic Knockout Approach. *Elife* 2019, 8, e44341. 10.7554/eLife.44341. [PubMed: 30803481]
- (22). Magana M; Pushpanathan M; Santos AL; Leanse L; Fernandez M; Ioannidis A; Giulianotti MA; Apidianakis Y; Bradfute S; Ferguson AL; et al. The Value of Antimicrobial Peptides in the Age

- of Resistance. *The Lancet Infectious Diseases* 2020, 20 (9), e216–e230. 10.1016/S1473-3099(20)30327-3. [PubMed: 32653070]
- (23). Ma W; Sun S; Li W; Zhang Z; Lin Z; Xia Y; Yuan B; Yang K. Individual Roles of Peptides PGLa and Magainin 2 in Synergistic Membrane Poration. *Langmuir* 2020, 36 (26), 7190–7199. 10.1021/acs.langmuir.0c00194. [PubMed: 32529830]
- (24). Aisenbrey C; Amaro M; Pospíšil P; Hof M; Bechinger B. Highly Synergistic Antimicrobial Activity of Magainin 2 and PGLa Peptides Is Rooted in the Formation of Supramolecular Complexes with Lipids. *Scientific Reports* 2020, 10 (1), 11652. 10.1038/s41598-020-68416-1. [PubMed: 32669585]
- (25). Strandberg E; Zerweck J; Horn D; Pritz G; Berditsch M; Bürck J; Wadhwani P; Ulrich AS Influence of Hydrophobic Residues on the Activity of the Antimicrobial Peptide Magainin 2 and Its Synergy with PGLa. *Journal of Peptide Science* 2015, 21 (5), 436–445. 10.1002/psc.2780. [PubMed: 25898805]
- (26). Zerweck J; Strandberg E; Kukharensko O; Reichert J; Bürck J; Wadhwani P; Ulrich AS Molecular Mechanism of Synergy between the Antimicrobial Peptides PGLa and Magainin 2. *Scientific Reports* 2017, 7 (1), 13153. 10.1038/s41598-017-12599-7. [PubMed: 29030606]
- (27). Zerweck J; Strandberg E; Bürck J; Reichert J; Wadhwani P; Kukharensko O; Ulrich AS Homo- and Heteromeric Interaction Strengths of the Synergistic Antimicrobial Peptides PGLa and Magainin 2 in Membranes. *European Biophysics Journal* 2016, 45 (6), 535–547. 10.1007/s00249-016-1120-7. [PubMed: 27052218]
- (28). Matsuzaki K; Fukui M; Fujii N; Miyajima K. Interactions of an Antimicrobial Peptide, Tachyplesin I, with Lipid Membranes. *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1991, 1070 (1), 259–264. 10.1016/0005-2736(91)90173-6. [PubMed: 1751532]
- (29). Ludtke SJ; He K; Heller WT; Harroun TA; Yang L; Huang HW Membrane Pores Induced by Magainin. *Biochemistry* 1996, 35 (43), 13723–13728. 10.1021/bi9620621. [PubMed: 8901513]
- (30). Tamba Y; Ariyama H; Levadny V; Yamazaki M. Kinetic Pathway of Antimicrobial Peptide Magainin 2-Induced Pore Formation in Lipid Membranes. *J. Phys. Chem. B* 2010, 114 (37), 12018–12026. 10.1021/jp104527y. [PubMed: 20799752]
- (31). Edwards IA; Elliott AG; Kavanagh AM; Blaskovich MAT; Cooper MA Structure-Activity and -Toxicity Relationships of the Antimicrobial Peptide Tachyplesin-1. *ACS Infectious Diseases* 2017, 3 (12), 917–926. 10.1021/acsfecdis.7b00123. [PubMed: 28960954]
- (32). Marín-Medina N; Mescala A; Alessandrini A. Effects of the Peptide Magainin H2 on Supported Lipid Bilayers Studied by Different Biophysical Techniques. *Biochimica et Biophysica Acta (BBA) - Biomembranes* 2018, 1860 (12), 2635–2643. 10.1016/j.bbamem.2018.10.003. [PubMed: 30292399]
- (33). Kobayashi S; Hirakura Y; Matsuzaki K. Bacteria-Selective Synergism between the Antimicrobial Peptides Alpha-Helical Magainin 2 and Cyclic Beta-Sheet Tachyplesin I: Toward Cocktail Therapy. *Biochemistry* 2001, 40 (48), 14330–14335. 10.1021/bi015626w. [PubMed: 11724544]
- (34). Liao CY; Selvan ME; Zhao J; Slimovitch JL; Schneebeli ST; Shelley M; Shelley JC; Li J. Melittin Aggregation in Aqueous Solutions: Insight from Molecular Dynamics Simulations. *J. Phys. Chem. B* 2015, 119 (33), 10390–10398. 10.1021/acs.jpcc.5b03254. [PubMed: 26208115]
- (35). Wang Y; Zhao T; Wei D; Strandberg E; Ulrich AS; Ulmschneider JP How Reliable Are Molecular Dynamics Simulations of Membrane Active Antimicrobial Peptides? *Biochimica et biophysica acta* 2014, 1838 (9), 2280–2288. 10.1016/j.bbamem.2014.04.009. [PubMed: 24747526]
- (36). Matyus E; Kandt C; Tieleman DP Computer Simulation of Antimicrobial Peptides. *Current Medicinal Chemistry* 2007, 14 (26), 2789–2798. 10.2174/092986707782360105. [PubMed: 18045125]
- (37). Jang H; Arce F; Ramachandran S; Kagan B; Lal R; Nussinov R. Disordered Amyloidogenic Peptides May Insert into the Membrane and Assemble into Common Cyclic Structural Motifs. *Chemical Society Reviews* 2014, 43. 10.1039/c3cs60459d.
- (38). Nguyen PH; Campanera JM; Ngo ST; Loquet A; Derreumaux P. Tetrameric A β 40 and A β 42 β -Barrel Structures by Extensive Atomistic Simulations. II. In Aqueous Solution. *J. Phys. Chem. B* 2019, 123 (31), 6750–6756. 10.1021/acs.jpcc.9b05288. [PubMed: 31296000]

- (39). Ngo ST; Derreumaux P; Vu VV Probable Transmembrane Amyloid α -Helix Bundles Capable of Conducting Ca^{2+} Ions. *J. Phys. Chem. B* 2019, 123 (12), 2645–2653. 10.1021/acs.jpcc.8b10792. [PubMed: 30831027]
- (40). Ghio S; Camilleri A; Caruana M; Ruf VC; Schmidt F; Leonov A; Ryazanov S; Griesinger C; Cauchi RJ; Kamp F; et al. Cardiolipin Promotes Pore-Forming Activity of Alpha-Synuclein Oligomers in Mitochondrial Membranes. *ACS Chem. Neurosci* 2019, 10 (8), 3815–3829. 10.1021/acschemneuro.9b00320. [PubMed: 31356747]
- (41). Petkov P; Lilkova E; Ilieva N; Litov L. Self-Association of Antimicrobial Peptides: A Molecular Dynamics Simulation Study on Bombinin. *Int J Mol Sci* 2019, 20 (21), 5450. 10.3390/ijms20215450.
- (42). Zou R; Zhu X; Tu Y; Wu J; Landry MP Activity of Antimicrobial Peptide Aggregates Decreases with Increased Cell Membrane Embedding Free Energy Cost. *Biochemistry* 2018, 57 (18), 2606–2610. 10.1021/acs.biochem.8b00052. [PubMed: 29638118]

Figure 1.

The sequences and structures of MG2 (**A**), TP1 (**B**) (PDB IDs: 2MAG and 2RTV), and the sequence of a non-AMP called FTA (**C**). Positively charged residues are colored blue while negatively charged ones are red. The 23-residue MG2 is helical with a +4 charge, and the 17-residue TP1 with a +7 charge forms an antiparallel β -sheet with two S-S linkages. Non-AMP FTA was chosen as a control system given the similar length to TP1.

Figure 2.

E. coli growth assay to test MG2-TP1 synergy. **(A)** Plots of pure MG2 or TP1 concentration for *E. coli* inhibition. **(B)** Activity of MG2-TP1 combinations (black), compared with the calculated *E. coli* growth from independent application of MG2 and TP1 assuming no synergy (orange). Error bars (1σ) were determined from three independent trials.

Fig 3.

(**A-B**) Comparison of MG2 and TP1 co-aggregation in the solution and membrane environments. In panel B the initial frame for the AMP's associating on the membrane is shown. (**C**) The relative SASA and radius of gyration (R_g) for MG2 and TP1 co-aggregation in the solution (blue) and on the membrane (red). (**D**) DLS spectra of TP1 (blue), MG2 (brown), and a 1:1 TP1/MG2 mixture (magenta). The total AMP concentration ($[\text{AMP}]_{\text{total}}$) was 2.0 nM in each case. Error bands (1σ) are from three DLS measurements. The DLS results confirm that smaller aggregates are formed with the mixture of the peptides, compared to when the pure MG2 or TP1 is used with a comparable concentration.

Fig 4.

(A) Membrane thickness as judged by the minimal distance between waters above and below the POPE:POPG 3:1 ratio membrane during equilibrium (solid line) and SMD (dots) runs. The MG2-TP1 hetero-tetramer (blue) forms a stable pore at 350 ns while the control sequence (red) doesn't form a pore within the 600 ns. The final snapshot illustrating the membrane pore formed by MG2-TP1. The orientation of a MG2 monomer at the membrane/water interface exposes hydrophobic residues to the membrane tails and charged/polar ones to the water and membrane heads. (B) Membrane thickness for the MG2-TP1 hetero-tetramer in a cardiolipin- rich membrane model. Final snapshots of SMD and equilibrium MD runs for pore formation in a cardiolipin rich bacterial membrane model. Side chain interactions of TP1 with cardiolipin head groups.