# The Secondary Structure and Antimicrobial Effectiveness of CE-03 and CE-05

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## Abstract

The escalating challenge of bacterial resistance to conventional antibiotics has spurred the exploration of alternative therapeutic strategies. Among these, antimicrobial peptides (AMPs) have garnered attention for their distinct mechanisms of action and broad-spectrum activity against bacterial infections. This study investigates the antimicrobial peptides CE-05 (16-mer) and CE-03 (12-mer), focusing on their structural properties and biocompatibility. We employed circular dichroism techniques to analyze the secondary structures of CE-05 and CE-03 in various lipid model membranes (LMMs) and concentrations. Our analysis revealed that CE-05 and CE-03 predominantly adopt random coil and beta-sheet structures across different lipid environments, underscoring their versatility in membrane interactions. Furthermore, in collaboration with Dr. Deslouches' lab at the University of Pittsburgh, we evaluated the biocompatibility of CE-05 and CE-03 with human red and white blood cells. Encouragingly, our findings demonstrate that both peptides exhibit negligible toxicity towards both cell types, highlighting their potential as a safe antimicrobial agent for human use. These results not only provide insights into the structural characteristics of CE-05 and CE-03 but also underscore the promising prospects of AMPs as effective and safe therapeutic agents against bacterial infections. By elucidating the interactions between AMPs and lipid membranes, this research lays the groundwork for the development of novel antimicrobial strategies with enhanced efficacy and reduced risk of resistance.

## What is circular dichroism?

- Circular dichroism (CD) is a spectroscopic technique used to analyze the structural properties of biomolecules and polymers.
- Biomolecules exhibiting chiral geometry demonstrate unique optical properties, as they absorb left and right circularly polarized light absorbed differently.
- Circularly-plane-polarized light is passed through a sample, allowing for the measurement of the difference in absorption of the left and right circularly polarized light, quantified as **ΔA** where

**ΔA = Left circularly polarized light - Right circularly polarized light**

- The ellipticity of the protein (θ) is calculated by

$$\theta = 2303\Delta A \cdot \frac{180}{4\pi}$$

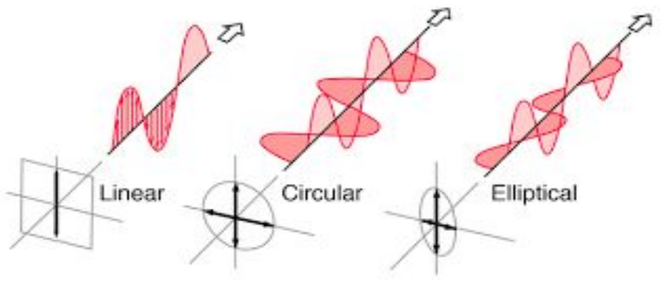


Figure 1: Diagrams of linear, circular, and elliptical polarization of light.[1]

## Protein Secondary Structure

- CE-03 Peptide Sequence (cyclic peptide): RRR RRR WW WW VV
- CE-05 Peptide Sequence (cyclic peptide): RRRR RRRR WWWW VVVV
- R = Arg, Arginine; W = Trp, Tryptophan; V = Val, Valine
- The most common types of secondary structure are: α-helix, β-sheet, β-turn, and random coil.

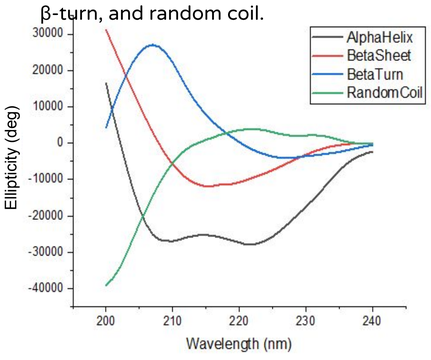


Figure 2 presents CD data for the four most common secondary structures in proteins. Computational fitting software (Levenberg-Marquardt) is employed to obtain the optimal linear combination of the four structural components, thereby determining the peptide's secondary structure.

Figure 2: CD data of common secondary structures

## Results

### Tables of CE-03 results of secondary structure in G(-), G(+) and Euk LMMs (L to R)

| Lipid-to-peptide Molar ratio | α-helix | β-sheet | β-turn | Random Coil | Adj. $R^2$ |
|---|---|---|---|---|---|
| 0:1 | 2±2 | 39±5 | 0±0 | 60±3 | 0.98±.02 |
| 5:1 | 1±2 | 39±3 | 0±0 | 60±2 | 0.96±0.01 |
| 10:1 | 0±0 | 39±1 | 0±0 | 61±1 | 0.99±0.01 |
| 20:1 | 0±0 | 39±4 | 0±0 | 61±4 | 0.96±0.04 |
| 30:1 | 0±0 | 35±3 | 0±0 | 65±3 | 0.95±0.04 |
| 50:1 | 0±0 | 41±1 | 0±0 | 59±1 | 0.98±0.03 |
| 70:1 | 0±0 | 27±6 | 0±0 | 73±6 | 0.98±0.02 |

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|---|---|---|---|---|---|
| 0:1 | 2±2 | 39±5 | 0±0 | 60±3 | 0.98±.02 |
| 10:1 | 0±0 | 46±0 | 0±0 | 55±0 | 0.81±0.02 |
| 20:1 | 1±2 | 43±2 | 1±2 | 55±3 | 0.93±0.09 |
| 30:1 | 0±0 | 40±5 | 0±0 | 60±5 | 0.95±0.01 |
| 50:1 | 4±0 | 42±0 | 0±0 | 54±2 | 0.92±0.01 |
| 70:1 | 0±0 | 22±2 | 0±0 | 78±2 | 0.68±0.11 |

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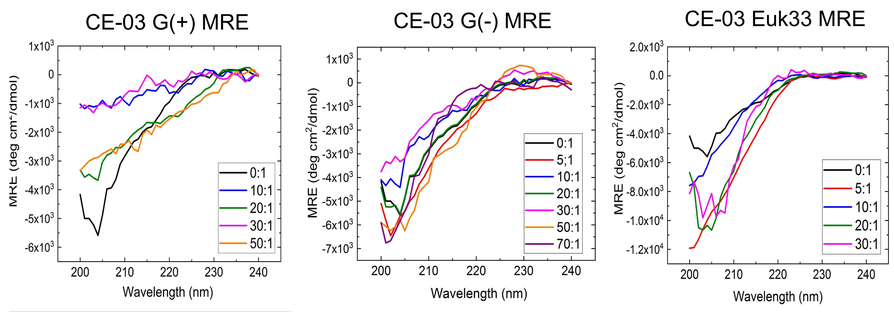
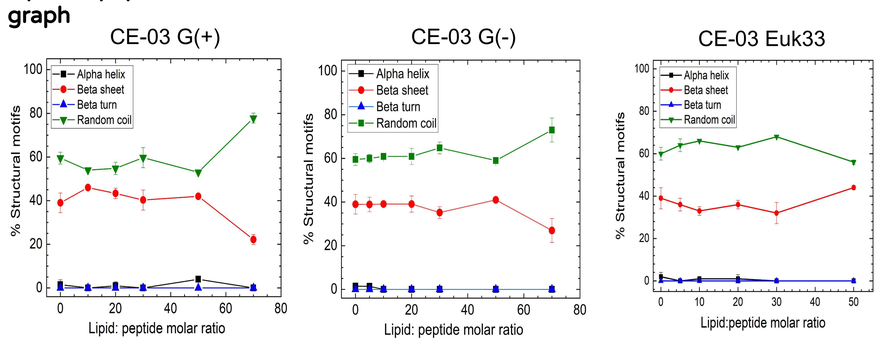
|---|---|---|---|---|---|
| 0:1 | 2±2 | 39±5 | 0±0 | 60±3 | 0.98±.02 |
| 5:1 | 0±0 | 36±3 | 0±0 | 64±3 | 0.97±0.01 |
| 10:1 | 1±1 | 33±2 | 0±0 | 66±0 | 0.98±0.01 |
| 20:1 | 1±2 | 36±2 | 0±0 | 63±0 | 0.94±0.05 |
| 30:1 | 0±0 | 32±5 | 0±0 | 68±0 | 0.90±0.09 |
| 50:1 | 0±0 | 44±1 | 0±0 | 56±1 | 0.96±0.01 |

### MREs




### Lipid-to-peptide ratio vs. % Structural motifs graph




### Tables of CE-05 results of secondary structure in G(-), G(+) and Euk LMMs (L to R)

| Lipid-to-peptide Molar ratio | α-helix | β-sheet | β-turn | Random Coil | Adj. $R^2$ |
|---|---|---|---|---|---|
| 0:1 | 0±0 | 38±1 | 0±1 | 62±1 | 0.98±.004 |
| 5:1 | 0±0 | 38±1 | 0±0 | 62±1 | 0.96±0.01 |
| 10:1 | 0±0 | 44±1 | 0±0 | 56±1 | 0.98±0.01 |
| 20:1 | 0±0 | 44±4 | 0±0 | 56±2 | 0.96±0.01 |
| 30:1 | 1±0 | 40±0 | 4±2 | 55±1 | 0.96±0.02 |
| 50:1 | 0±2 | 41±1 | 0±0 | 59±1 | 0.97±0.01 |

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| 20:1 | 0±0 | 31±3 | 0±0 | 69±3 | 0.95±0.01 |
| 30:1 | 0±0 | 36±2 | 0±0 | 64±2 | 0.92±0.06 |
| 50:1 | 0±0 | 31±1 | 0±0 | 69±1 | 0.99±0.01 |

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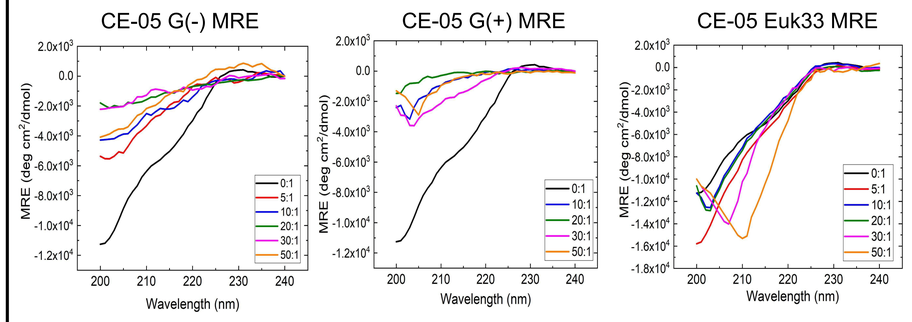


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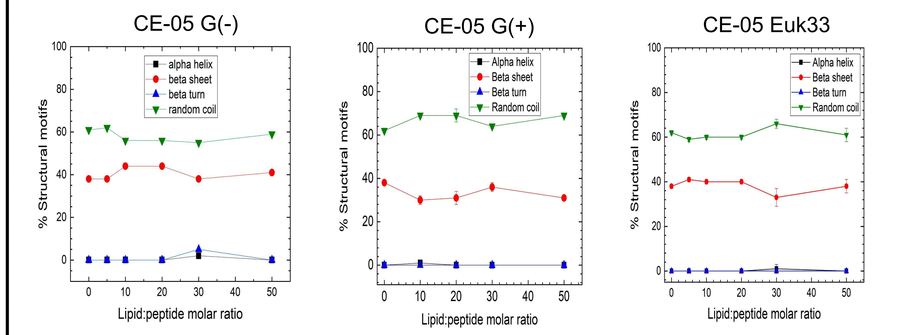


## How active are the peptides?

| | MIC (μM) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *PA* | *AB* | *KP* | *E .coli* | *Enterobacter* | *Average* | *Enterococci* | *SA* | *Average* | RBC | WBC |
| **CE-0003** | 7.1 | 2.2 | 8.2 | 1.5 | 3.7 | 4.6 ± 1.3 | 0.9 | 2.3 | 1.6 ± 0.7 | 0.00 | 0.00 |
| **CE-0005** | 6.1 | 1.1 | 6.7 | 2.5 | 3.2 | 3.9 ± 1.1 | 0.6 | 1.0 | 0.9 ± 0.6 | 0.00 | 0.00 |
| **Colistin** | 8.4 | 0.5 | 0.7 | 4.3 | 12.1 | 5.2±4.3 | 32.0 | 64.0 | 48.0±23.0 | -- | -- |
| **Tobramycin** | 32.0 | 32.0 | 2.1 | 28.0 | 24.5 | 23.7±3.4 | 25.0 | 13.1 | 19.0±10 | -- | -- |

**Table 1: Antibacterial activity and toxicity of CE-03 and CE-05 peptides.** Two peptides were examined for minimum inhibitory concentrations (MIC) against G(-) (blue)and G(+) (red) MDR isolates from UPMC. % RBC lysis at 32 μM and % toxicity at 16 μMagainst human WBCs are shown. The MICs are the average of four different strains of each type of bacteria. Data are representative of 2-3 experimental trials. The G(-) bacterial strains are: *Pseudomonas aeruginosa (PA231, PA235, PA239, PA249), Acinetobacter baumannii (AB78, AB83,AB273, AB275), Klebsiella pneumoniae (KP106, KP506, KP542, KP550), Escherichia coli(EC541, EC543, EC546, EC549) and Enterobacter (EA62, EC544, EC547, EA1042).* The G(+)bacterial strains are: *Enterococci (EF500, EF678, EF679, EF787) and Staphylococcus aureus (SA703, SA722, SA729).*

## Conclusions

We conclude that both CE-03 and CE-05 take on random coil and β-sheet structures in all three LMMs. We can also deduce that the random coil and beta-sheet structures of CE-03 and CE-05 have a positive impact on the antibacterial effectiveness and the lack of toxicity in human blood cells, unlike the toxicity that alpha-helical E2 peptides displayed[4] in red blood cells and white blood cells. **These findings suggest the potential for the clinical applications of antimicrobial peptides that do not harm human cells. Consequently, it is recommended that future research of antimicrobial peptides should prioritize peptides that exhibit random coil and β-sheet structures.**

## References

[1] "Classification of Polarization." *Gsu.edu*, 2019, hyperphysics.phy-astr.gsu.edu/hbase/phyopt/polclas.html.
[2] Lei, J., Sun, L., Huang, S., Zhu, C., Li, P., He, J., Mackey, V., Coy, D. H., & He, Q. (2019). The antimicrobial peptides and their potential clinical applications. *American journal of translational research*, *11*(7), 3919–3931.
[3] Tinoco, Ignacio, et al. "*Physical Chemistry: Principles and Applications in Biological Sciences*." Prentice-Hall, Inc, 1995.
[4] Mitra, S., et al (2023). Novel helical trp- and Arg-rich antimicrobial peptides locate near membrane surfaces and rigidify lipid model membranes. Advanced NanoBiomed Research, 3(5). https://doi.org/10.1002/anbr.202300013