# Examining Cationic Antimicrobial Peptide Interactions with Lipoproteins

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## Abstract

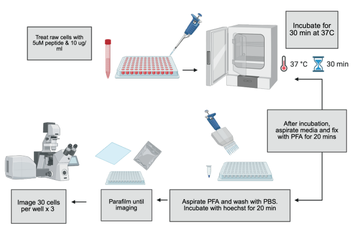
High cholesterol levels affect human health in more ways than one might initially expect. Lipo proteins are a category of fats, abbreviated as LDL, that carry cholesterol through the bloodstream to tissues in the human body. They are better known as the "bad fat" that humans are faced with. It is known that excess cholesterol in the bloodstream can be detrimental to human health, but the extent of its ramifications is still being researched today. LL-37 is a strain of peptides, or a protein, produced by the CAMP (cationic antimicrobial peptide) gene. As its name suggests, because LL-37 is produced by the CAMP gene, it is able to fight off bacteria as the cell's innate immune response detects pathogens and signals LL-37 production. LL-37's interactions with LDL are well studied; LL-37 increases LDL uptake into cells, suggesting that bacterial-killing activity is inhibited when cholesterol levels are elevated. The presence of LL-37 increases risk of inflammation, a direct link to neurological disorders. To investigate whether the other peptides shared a similar relationship with LDL, we used cell culture, confocal imaging, and image analysis to quantify the colocalization of LDL with the proteins. Our findings suggest that, despite peptides originating from the same gene, they do not share the same interactions with LDL as the widely studied LL-37.

## Introduction & Summary

The brain's synaptic functions are impacted by an increased presence of LDL in the brain. If LL-37 increases LDL uptake into the cell, the CAMP gene is important to analyze to determine whether this effect is consistent across the gene. Apart from the specificities of the larger implications of LL-37 and LDL's interactions, the additional question of how the 7 peptides produced by the same gene interact with LDL remains present. This question is addressed in the following research; its importance lies in discovering the implications of overpresent LDL in the bloodstream by analyzing whether LL-37's interaction is a pattern or a "fluke" in relation to the significance of the CAMP gene. LL-37, because of its recorded presence in the human antimicrobial pathway as a tool of innate immune response, has been studied over time in its interactions with bacteria and with lipoproteins. As a result, research is lacking in how the 7 other peptide strands interact with lipoproteins and with bacteria. These peptides share in their genetic sequencing, with 6 peptides being truncated, while FA-39 has an extended genetic sequence. With the data found and analysis conducted, the question of how these peptides interact will be thoroughly addressed. Based on prior data on the similarity between these proteins, these peptides are likely to interact in a manner similar to that of LL-37.

processed CAMP (cathelicidin)

| | | | |
|---|---|---|---|
| FALLGDFF**RK**SK**E**K**I**GK**E**F**KR**IVQ**R**IK**D**FL**R**NLVP**R**TES | FA-39 | 10.61 | +6 |
| --LLGDFF**RK**SK**E**K**I**GK**E**F**KR**IVQ**R**---------- | LL-23 | 10.55 | +5 |
| --LLGDFF**RK**SK**E**K**I**GK**E**F**KR**IVQ**R**IK**D**FL**R**-------- | LL-29 | 10.62 | +6 |
| --LLGDFF**RK**SK**E**K**I**GK**E**F**KR**IVQ**R**IK**D**FL**R**NLVP**R**TES | LL-37 | 10.61 | +6 |
| ------FF**RK**SK**E**K**I**GK**E**F**KR**IVQ**R**IK**D**FL**R**NLVP**R**TES | FF-33 | 11.04 | +7 |
| --------**RK**SK**E**K**I**GK**E**F**KR**IVQ**R**IK**D**FL**R**NLVP**R**TES | RK-31 | 11.04 | +7 |
| ---------K**S**K**E**K**I**GK**E**F**KR**IVQ**R**IK**D**FL**R**NLVP**R**TES | KS-30 | 10.62 | +6 |
| ------------------**KR**IVQ**R**IK**D**FL**R**NLVP**R**TES | KR-20 | 11.55 | +4 |




## Method

Quantitative data was collected to investigate the hypothesis of this experiment. RAW mouse cells were used in this experiment. The cells were passaged approximately 20 times before use, ensuring adequate growth before they were plated and treated. These raw cells were plated into 8 different columns on a lobind well plate, and treated with the eight different peptides produced by the CAMP gene with one peptide per column. After treating the raw cells and incubating, the media (food given to cells so they can survive over periods of time) was aspirated from the plate, and the cells were fixed (frozen in the position they were in) with PFA, since the experiment moved from a sterile environment to a non-sterile environment. When the cells were treated with peptides, the media in the wells already contained LDL-DIL, a lipoprotein that is hydrogen-bonded to DIL, a neon orange pigment. While imaging, the nuclei of the cells would have to be distinct, so the LDL surrounding the nucleus could be quantified, since the uptake of LDL into the cell was being categorized. Because of the hydrogen bonding of LDL-DIL, the nuclei could not be fluorescently stained, so hoechst, a bright blue pigment, was used to stain the nuclei. After treating with hoechst, the plate was parafilmed to ensure that no bacteria/dust was able to enter the wells while the plate was left overnight. Following the treatment, imaging began using a confocal microscope. A minimum of thirty cells per row - there were three rows for each peptide for a total of imaging ninety cells per column/peptide were imaged. While imaging, pictures of both the LDL-DIL channel and the hoechst channel overlapping one another were taken. To ensure that accurate data points were quantified from the images, an image analysis software called ImageJ/Fiji was used. Using this software, the amount of LDL-DIL around the nucleus of each individual cell was analyzed. To create a graph to analyze the data, the mean*average LDL of the data was calculated. Because some of the cells in certain peptides were significantly larger than others, and as a result had larger lipid uptake reported through image analysis, there was a possibility of innacuryes with the graph presenting the data. By using the mean to create a graph of the data, the discrepancies in LDL uptake caused by size are not visible, since each peptide had the same number of cells analyzed.

## References

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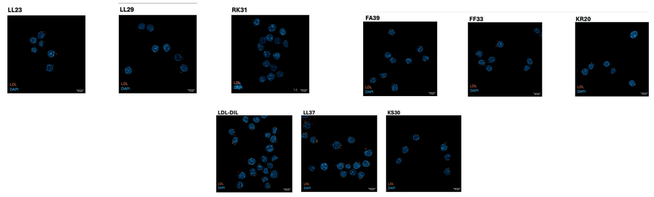
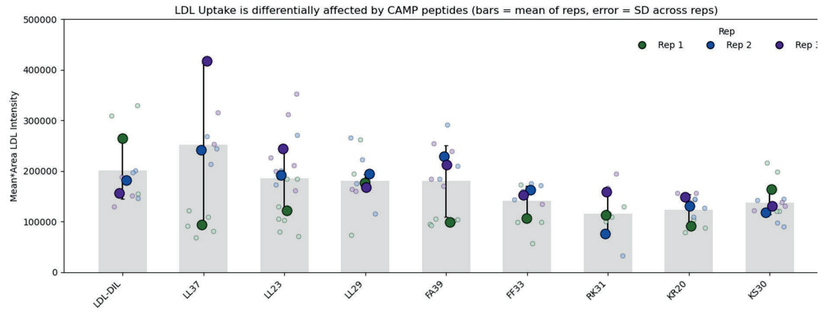
## Results




## Findings

From the quantified data converted into a graph, the difference in how LL-37 affects LDL uptake compared to the other seven peptides produced by the CAMP gene is clear. LL-37, on average, increased LDL uptake into the cell, though the result from the first set of analysis is surprising, as it goes against proven research that LL-37 increases LDL uptake. The following seven peptides did not increase uptake; in the case of these peptides, they were shown to decrease LDL uptake into the cell. These findings go against my original hypothesis that, because the peptides analyzed were from the same DNA, they would interact with LDL in a manner similar to LL-37. However, they are still reasonable considering that there are truncations in six peptides, apart from FA-39, which has an extended genetic sequence in comparison with LL-37. LL-37 is unique in its genetic sequence, so the seven peptides apart from LL-37 not interacting with LDL in the same way is a logical result. Despite the result of this experiment being logically supported, there is a large disparity in the averages across the three rounds of analysis for each peptide. The implications of this experiment were that similarities in genetics in the case of these CAMP peptides do not increase risk of neurological disease in relation to LDL uptake.

## Discussion

This experiment supports the assertion that peptides originating from the CAMP gene, other than LL-37, do not contribute significantly to neurodegenerative disorders. Multiple images were taken, and across each set of images, the data consistently showed that the peptides that were not LL-37 did not increase LDL uptake. Despite data collected indicating consistency, due to the variability between sets of each peptide, it would be beneficial to repeat this experiment, quantifying the number of puncta (dots) of LDL within cells rather than around the cell, to obtain more accurate data.