Optimization of Nanoparticle-mediated Brain Delivery of a Tetracationic Porphyrin with Potent Anti-Prion Activities: 24 Month Report

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Introduction:

Prion diseases are a neurodegenerative disorder caused by "prions" defined by pathogenic agents that are transmissible and can induce abnormal folding of a specific cellular protein, called PrP^{C}, most abundantly found in the brain. These diseases can affect both animals (e.g., sheep and cows) as well as humans. The misfolding of the naturally occurring PrP^{C} to the diseased PrP^{Sc} can be caused by exposure to exogeneous prions, inherited mutations in the gene encoding PrP^{C}, or can occur sporadically without a known genetic or environmental cause (**Figure 1**), leading to symptoms of prion diseases. Unfortunately, currently there are no effective treatments to block this transformation, and the self-propagating effect of the misfolded proteins makes late-stage diagnosis fatal. For this reason, new and improved therapeutics are being sought out.

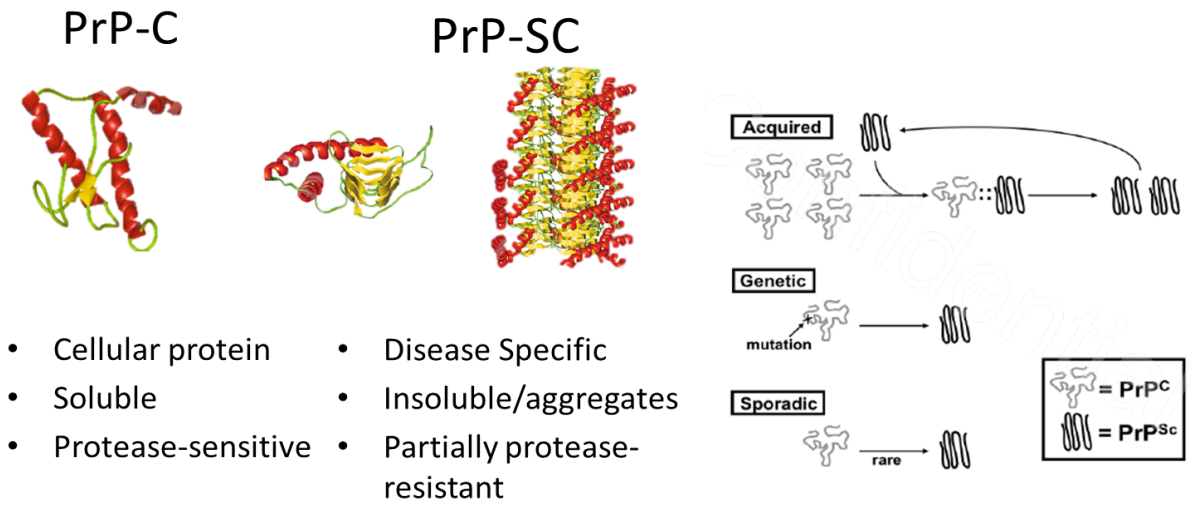

***Figure 1**: Misfolding of PrP^{C} to PrP^{Sc} and its causes.*

Results:

A previous project within our collaboration had discovered a porphyrin (**Fig 2.**) that can block the transformation of PrP^{C} into PrP^{Sc} and simultaneously lead to the degradation of PrP^{C}, reducing the precursor of PrP^{Sc}, giving a highly valuable possibility of both preventative and symptomatic treatments. Unfortunately, this porphyrin is not able to get into the brain where it is needed for treatment, making it ineffective. Our lab has previously designed safe biodegradable nanoparticles (NPs) allowing for the brain delivery of drugs thanks to the NPs functionalization with a peptide, named g7, targeting the blood-brain-barrier (BBB) [1-3]; however,

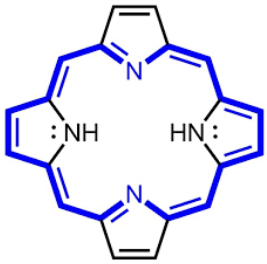

***Figure 2**: General core structure of porphyrins*

we found that when the g7-NPs were loaded with the porphyrin, therapeutic doses in the brain were not achieved. Therefore, to optimize our NPs for porphyrin delivery to the brain, our project was designed with two specific aims: 1) to analyze whether g7-NP delivery to the brain was blocked by detrimental interactions occurring between the porphyrin and the targeting peptide g7; and 2) to design and test NPs functionalized with new peptides targeting the BBB that don't interact with the porphyrin.

Specific aim 1). We identified the interactions between the porphyrin and the g7 peptide to find out which groups drive the interaction with the porphyrin. We discovered that the porphyrin and the g7 peptide bind to each other through the g7 phenylalanine and sugar groups (**Fig 3 Top**).

Specific aim 2). Considering the information indicating the g7 groups do interact with the porphyrin, we designed and tested a new peptide with brain targeting potential (AAVF) that is devoid of the interfering sugar and phenylalanine moieties (**Fig 3 Bottom**). Interaction studies indicated a small interaction between the AAVF peptide and the porphyrin but much less than that of the g7 peptide. We therefore continued our rational peptide design to eliminate porphyrin interaction by synthesizing two new AAVF-based peptides (AAVF.2 and AAVF.3) which showed no binding with the porphyrin.

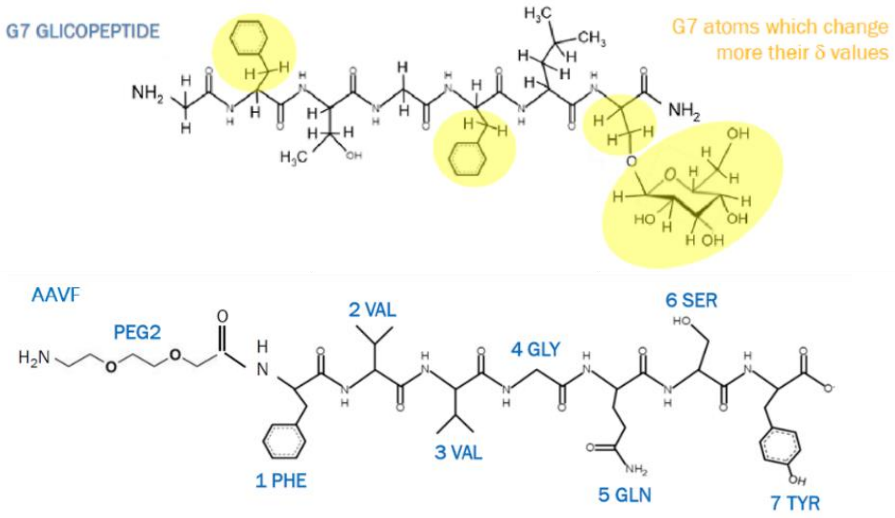

***Figure 3**: The sequence of the brain targeting peptides g7 with circles indicating interactive groups (Top) and AAVF (Bottom).*

Therefore, we analyzed these new peptides for their ability to target the BBB in mice. By functionalizing the NPs surface with the three AAVF peptides and injecting the NPs into the stomach cavity of mice, we demonstrated that two of the three new peptides (AAVF, and AAVF.3) were able to reach the brain and be taken up into various brain compartments and cells (**Fig 4**).

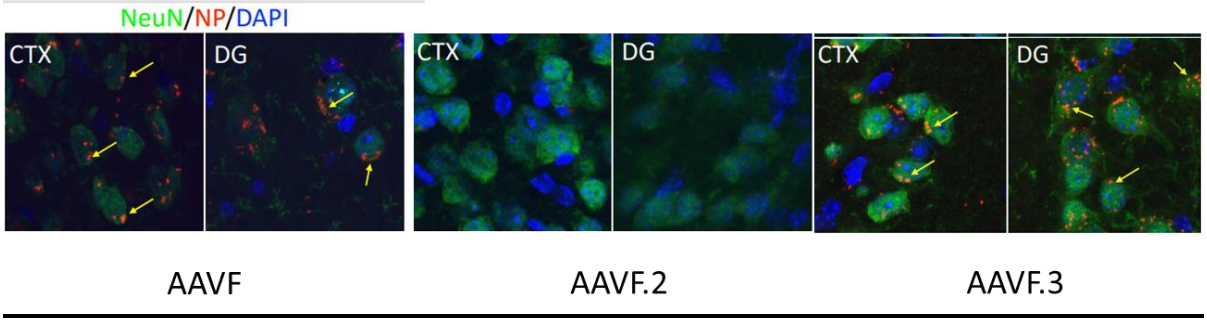

***Figure 4**:* Brain images of injected mice where Blue: cell nucleus, Green: neurons, and Red: Targeted NPs.

Conclusions and Future Experiments:

We performed a detailed binding analysis of the interactions occurring between our promising anti-prion drug (a porphyrin with dual anti-prion activity) and the peptides used to functionalize our safe biodegradable NPs for brain delivery of the porphyrin. In this way we were able to design peptide devoid of the chemical groups driving the detrimental porphyrin binding which hampered NP delivery across the blood-brain-barrier. We achieved promising *in vivo* results of brain delivery with NPs containing the new targeting peptides, demonstrating that we have successfully overcome the major obstacle to the curative effect of our new anti-prion drug.

The key to a functional treatment against prion diseases will be to take advantage of the powerful curative effect of the novel porphyrin with the innovative peptide targeted Nanomedicines. Ongoing experiments are being performed to demonstrate NPs carrying the new porphyrin and targeting peptides can degrade PrP^{C} and reduce the precursor of PrP^{Sc} in cell models and to find the dose needed for an optimal therapeutic effect. Furthermore, this newly optimized system will then be dosed in mice and the brain levels of PrP^{C} and PrP^{Sc}. Finally, studies in mice will be to look at the curative effect and determine if our new system can block the disease and increase life expectancy in the mice. We have high expectations and hope this new design will lead to an effective cure against prion diseases.

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