

The Architect of Neurotransmission in *C. elegans*: How FLP-3 Neuropeptides' Structures Direct their Function

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Abstract

Neuropeptides direct functions in the nervous, endocrine, and immune systems of all animals by altering the activity at neural synapses. A single neuropeptide gene can be post-translationally modified to create multiple active peptides. These individual active peptides can have unique functions and drive discrete binding partners. We have previously shown that specific peptides encoded by the *C. elegans* neuropeptide gene, [flp-3](#), have sex-specific roles in response to a pheromone released by hermaphrodite *C. elegans*, ascaroside #8 ([ascr#8](#)). Using structural predictions of select [FLP-3](#) neuropeptides, we identify individual amino acids within specific neuropeptides that regulate specific behaviors suggesting structure-function relationships of neuropeptides in regulate sex-specific behaviors.

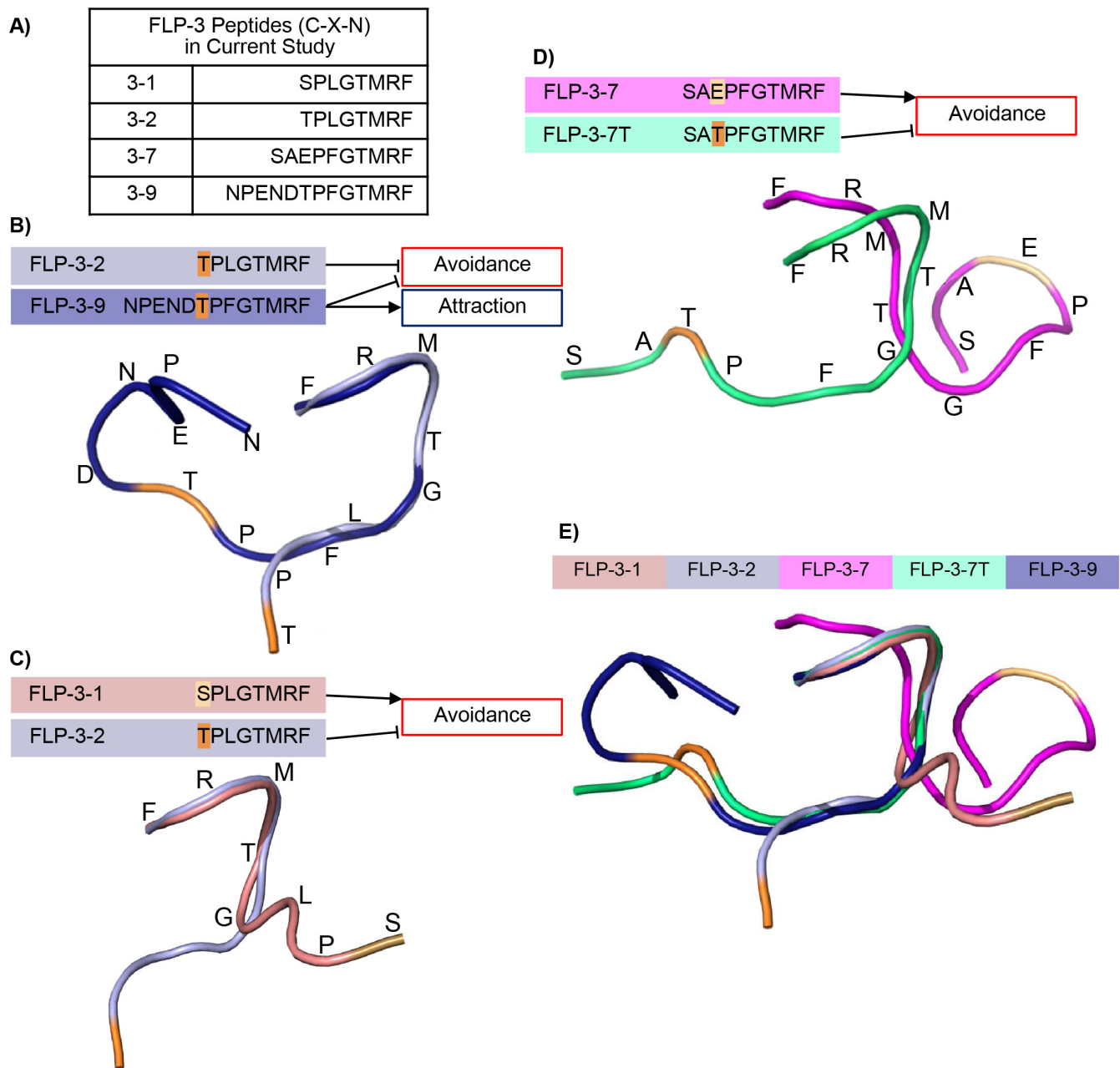


Figure 1. Neuropeptide conformation impacts behavioral response to *ascr#8* detection in male loss of *flp-3* function worms:

A) Peptides produced by the *flp-3* gene presented in the current study. **B)** *FLP-3-2* (light blue) and the threonine in position 8 (T8) from the N-terminus (orange) inhibits avoidance, *FLP-3-9* (dark blue) and T8 (orange) inhibits avoidance and promoted attraction. **C)** *FLP-3-1* (rose) promoted avoidance while *FLP-3-2* (light blue) inhibits avoidance, both contain the first 7 amino acids except for a serine in position 8 for *FLP-3-1* (tan). **D)** *FLP-3-7* (magenta) and E8 (tan) promotes avoidance, *FLP-3-7T* (cyan) and T8 (orange) inhibits avoidance. **E)** *FLP-3-7T* superimposed with, *FLP-3-1*, *FLP-3-2*, *FLP-3-7*, and *FLP-3-9*.

Description

Ascarosides are modular sugar derivatives produced by *C. elegans* for communication within their species. They are composed of 4 parts; head group, sugar, lipid side chain, and terminal group, with different moieties that produce distinct functions (Muirhead & Srinivasan, 2020). Previous work found that wildtype hermaphrodites secrete *ascr#8*, which attracts

male worms and repels other hermaphrodites (Narayan et al., 2016; Reilly et al., 2021). Our group further uncovered that loss of function in the *flp-3* gene causes male worms to avoid *ascr#8* rather than find it attractive (Reilly et al., 2021). The *flp-3* gene produces ten discrete *FLP-3* peptides named *FLP-3-1* to *FLP-3-10*. **Figure 1A** highlights the *FLP-3* peptides discussed in the current study. In prior studies, rescue of individual neuropeptides was accomplished by a peptide feeding approach where genetic information to produce a single neuropeptide was encoded in a vector which was transformed into *E. coli* bacteria that *C. elegans* consumed (Reilly et al., 2021). These bacteria encoding individual peptides were fed to *flp-3* loss of function worms. Any peptide activity was directed by the exogenous feeding of the bacterially-encoded individual peptides. Previous studies from our lab showed that restoration of *FLP-3-2* or *FLP-3-9* peptide caused loss of basal *ascr#8* avoidance in the *flp-3* mutant worms, **Figure 1B** (Reilly et al., 2021). *FLP-3-2* caused a loss of avoidance, yet there was no gain of attraction towards *ascr#8*, leaving the male worms neutral towards the ascaroside. Additionally, *FLP-3-9* was sufficient to restore native male attraction to *ascr#8*, suggesting that *FLP-3-9* is necessary for male worms to find *ascr#8* and therefore hermaphrodites attractive. The difference in behavior associated with individual *FLP-3* peptide rescue suggests that the neuropeptides' role in *ascr#8* attraction is related to peptide structure.

Our lab's previous behavioral work investigating the relationship between the *FLP-3* peptides and *ascr#8* responses spurs our current work to model the structures of the *FLP-3* neuropeptides and determine the role of the peptides' sequences and related structures. To determine any structure-function relationships between the rescuing peptides, we predicted the conformational structures of *FLP-3* neuropeptides using PEP-FOLD3 (Lamiable et al., 2016). We had particular interest in peptides *FLP-3-2* and *FLP-3-9* that resulted in behavioral changes in male *flp-3* loss of function worms in a previous study (Reilly et al., 2021). To assess for structural similarity between the peptides, we optimized conformations and then aligned them with one another along the α -carbon, starting at the amide end of the peptide by PyMol. Alignment of the predicted structures of *FLP-3-2* (light blue) and *FLP-3-9* (dark blue) suggests that they share the conformation structure with RMSD = 0.224, **Figure 1B**. *FLP-3-2* and *FLP-3-9* share the same sequence of the first eight N-terminal amino acids, with the replacement of lysine in *FLP-3-2* with phenylalanine in *FLP-3-9* at position six, suggesting this region may be connected with the loss of avoidance behavior seen when feeding occurred with these individual peptides. The five extra residues that *FLP-3-9* has may be responsible for attraction gain.

Reilly et al. 2021 suggested that the threonine closest to the C-terminus is critical for eliminating *ascr#8* avoidance. We investigated the importance of this threonine by comparing other *FLP-3* peptides that do not have this amino acid. *FLP-3-1* (rose) has the same structure as *FLP-3-2* (light blue) with serine instead of threonine. Previously it was shown that the uptake of *FLP-3-1* did not change the avoidance behavior to *ascr#8* in *flp-3* loss of function worms (Reilly et al., 2021), **Figure 1C**. When we aligned the structures of *FLP-3-1* and *FLP-3-2*, we found that there was divergent alignment starting at the glycine residue, despite the fact that the only difference is the C-terminal amino acid. *FLP-3-1* and *FLP-3-2* aligned with RMSD = 0.231. *FLP-3-7* also has a similar sequence, except with a glutamate at position eight rather than the threonine. Earlier investigation showed that when *FLP-3-7* was fed to *flp-3* loss of function male worms, they still avoided *ascr#8*, **Figure 1D**. Previous behavioral work by Reilly et al. modified the vector encoding *FLP-3-7* experimentally to produce a mutated version with threonine replacing the glutamate. The *FLP-3-7T* peptide was fed to *flp-3* loss of function male worms, resulting in non-avoidance to *ascr#8*. In contrast this peptide did not exhibit wildtype attraction to *ascr#8*. In our current work, we found that superimposing the structures of *FLP-3-7* and *FLP-3-7T* shows that *FLP-3-7* (magenta) changes conformation when a threonine is present in position eight, *FLP-3-7T* (teal), with RMSD = 2.323, **Figure 1D**. Compared to the original *FLP-3* peptides tested, *FLP-3-7* has a different conformation than *FLP-3-2* and *FLP-3-9* with RMSD = 2.350 and 2.387, respectively, **Figure 1E**. When we changed the glutamate at position eight from the N-terminus to a threonine (*FLP-3-7T*), the altered peptide conformation was similar to *FLP-3-2* and *FLP-3-9* with RMSD = 0.075, 0.208 respectively **Figure 1E**.

Overall, our computational results suggest a structure-function relationship among neuropeptides, influenced by single amino acid substitutions. Of the peptides discussed here, *FLP-3-1*, *FLP-3-2*, *FLP-3-7*, *FLP-3-9*, all are predicted to bind to target receptors NPR-10 and FRPR-16. All the peptides have a stronger affinity for FRPR-16 over NPR-10. Additionally, *FLP-3-1* has the highest affinity for each receptor and *FLP-3-9* the lowest (Reilly et al., 2021). The differences in receptor binding affinity to individual *FLP-3* peptides suggest that changes in behavior in response to *ascr#8* is directed by single amino acid changes to these neuropeptides. This comes while considering that the peptide *FLP-3-9* is the sole *FLP-3* peptide that overrides the basal *ascr#8* avoidance and restores male's attraction to hermaphrodite released *ascr#8* in the loss of *flp-3* function worms, yet it has the lowest binding affinity to NPR-10 or FRPR-16. The structural alignments presented in this work corroborates our previously described behavioral data (Reilly et al., 2021) further confirming the role of *FLP-3* peptides, especially *FLP-3-9*, in regulating male *C. elegans* attraction to hermaphrodite pheromone *ascr#8*. Our studies identify molecular-level conformational changes in amino acids strongly correlate to the overall function of the peptide suggesting that the dynamics of amino acids within a peptide/protein can dictate the overall function and activity.

Methods

The PEP-FOLD3 *de novo* technique (Lamiable et al., 2016; Maupetit, Derreumaux, & Tuffery, 2009; Maupetit, Derreumaux, & Tufféry, 2010; Shen, Maupetit, Derreumaux, & Tuffery, 2014; Thévenet et al., 2012) was used to predict the conformational tertiary structure of the neuropeptides. This platform is used for sequence predictions between 5-50 amino acids, a range in which most neuropeptides fit. Once the initial peptide sequences are inserted, the standard free modeling parameters were used. After running a short molecular dynamic simulation, the platform generates 100 models, sorts the clusters using sOPEP energy value or Apollo predicted TMscore, and ranks the top five models. The structure with the highest sOPEP value was chosen to represent the native or near native conformation of the neuropeptides comparison. [FLP-3-1](#), [FLP-3-2](#), [FLP-3-7](#), [FLP-3-7T](#), and [FLP-3-9](#) predicted structures were superimposed by the α carbon of residues 1 to 5 by PyMOL. The root mean square deviation (RMSD) reflects the square root of the average of the square of the distances between the complement atoms (Schrödinger).

Reagents

N/A

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