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NMR Structure Determination of KTM: A Rationally Designed Alpha-Conotoxin Targeting Parkinson's-Relevant Receptor Isoforms

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Abstract:
Validating Computational Results

KTM is a rationally designed alpha-conotoxin predicted to have optimal binding affinity for the rat α3β2 (rα3β2) nicotinic acetylcholine receptor (nAChR) isoform,[1] which has >80% sequence homology with the human α6α4β2β3 receptor isoform implicated in Parkinson's Disease.[2] Validation of computational accuracy will help adjust computational parameters to give more accurate predictions of receptor binding, which is critical to receptor understanding and effective drug development for neurodegenerative diseases such as Parkinson's.[3] The NMR structure of KTM is currently being solved in order to validate computational results. Current progress indicates that the NMR structure follows the predicted structure,[4] but is not as highly constrained as MII. Preliminary two-electrode voltage clamp electrophysiology (TEV) experiments confirm that KTM has affinity for rα3β2 on the order of MII,[5] supporting the reliability of computational results.

How was KTM designed?

KTM is based on alpha-conotoxin MII, which has the highest binding affinity for rα3β2 known.
The computational programs GAMPMS and Dockomatic were used to screen a peptide mutant library for optimal binding affinity for rα3β2.[1]

mutable residue	substitutable amino acids
G1	G A V L I M W F
S4	S T Y N Q D E K R H
N5	S T Y N Q D E K R H
V7	G A V L I M W F
H9	S T Y N Q D E K R H
L10	G A V L I M W F
E11	S T Y N Q D E K R H
H12	S T Y N Q D E K R H
S13	S T Y N Q D E K R H
N14	S T Y N Q D E K R H
L15	G A V L I M W F

Table 1. Mutant ligand library, defined as a base peptide and a set of mutation constraints.[1]

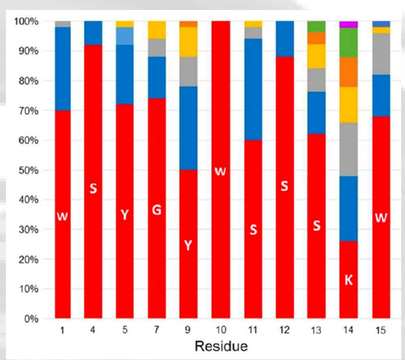


Fig 1. Relative frequencies of amino acid residues present in the top 50 sequences obtained from GAMPMS.[1]

KTM

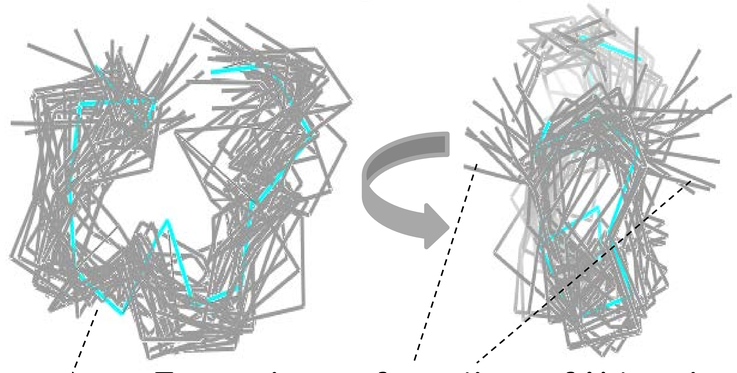
W C C S Y P G C Y W S S S K W C

G C C S N P V C H L E H S N L C

MII

NMR Structure Determination[4]: KTM is dynamic and follows predicted structure

RMS: 2.6±0.3 Å Alignment to predicted: 2.5 Å

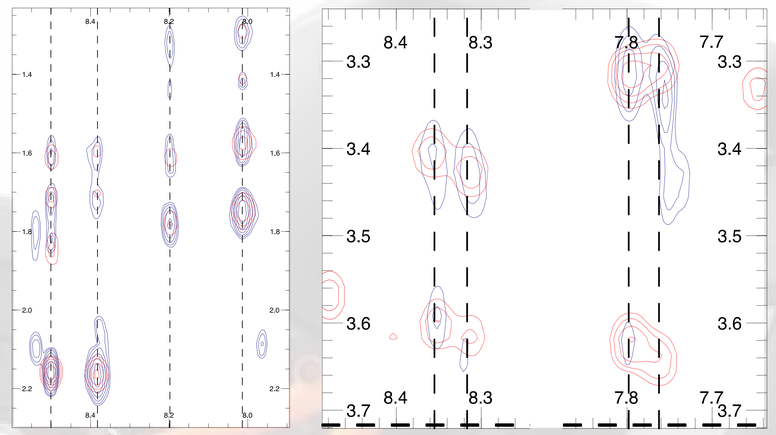


Two major conformations of N-terminus: inversion of P6?

Lack of defined α-helix supports CD data

Multiple dynamics observed in spectrum:

Two dominant K14 states Alternate aromatic states/flipping



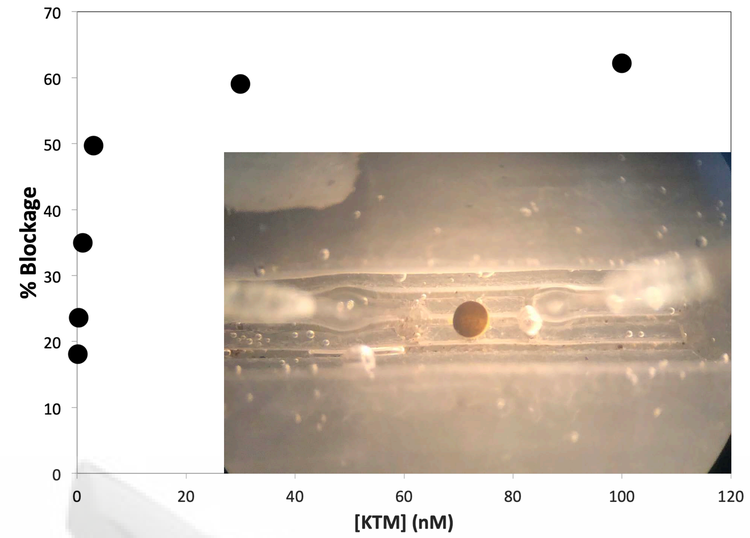
Conclusions:
Reliable Computational Predictions

- TEV results indicate that KTM is a high-affinity antagonist of rα3β2
- TEV and NMR results indicate that computational results are reliable and can be used to predict lead compounds that will have high binding affinity for nAChR receptor isoforms

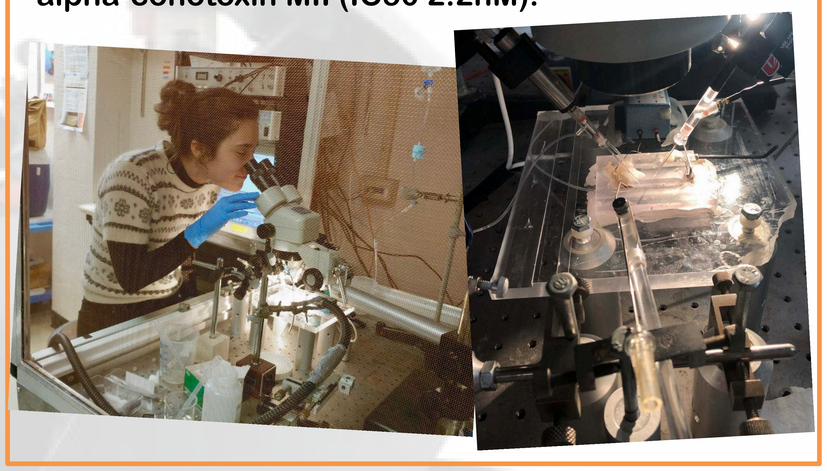
Future Work: How does KTM move?

- MD simulations to identify peptide dynamics and key binding features
- Refine TEV and NMR data

TEV Results:
KTM is an antagonist of rα3β2 nAChR



Preliminary TEV experiments indicate that KTM has affinity for rα3β2 (IC50 ~3nM) on the order of alpha-conotoxin MII (IC50 2.2nM).[5]



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