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

Leanna Marquart
Boise State University

Lisa Warner
Boise State University

Matthew King
Boise State University

Joe Dumais
Boise State University

Owen McDougal
Boise State University

See next page for additional authors

Name

Leanna Marquart, Lisa Warner, Matthew King, Joe Dumais, Owen McDougal, and Jim Groome

Abstract: Validating Computational Results

KTM is a rationally designed alpha-conotoxin predicted to have optimal binding affinity for the rat $\alpha 3\beta 2$ ($\alpha 3\beta 2$) nicotinic acetylcholine receptor (nAChR) isoform,¹ which has >80% sequence homology with the human $\alpha 6\alpha 4\beta 2\beta 3$ 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 $\alpha 3\beta 2$ 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 $\alpha 3\beta 2$ known. The computational programs **GAMPMS** and **Dockomatic** were used to screen a peptide mutant library for optimal binding affinity for $\alpha 3\beta 2$.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

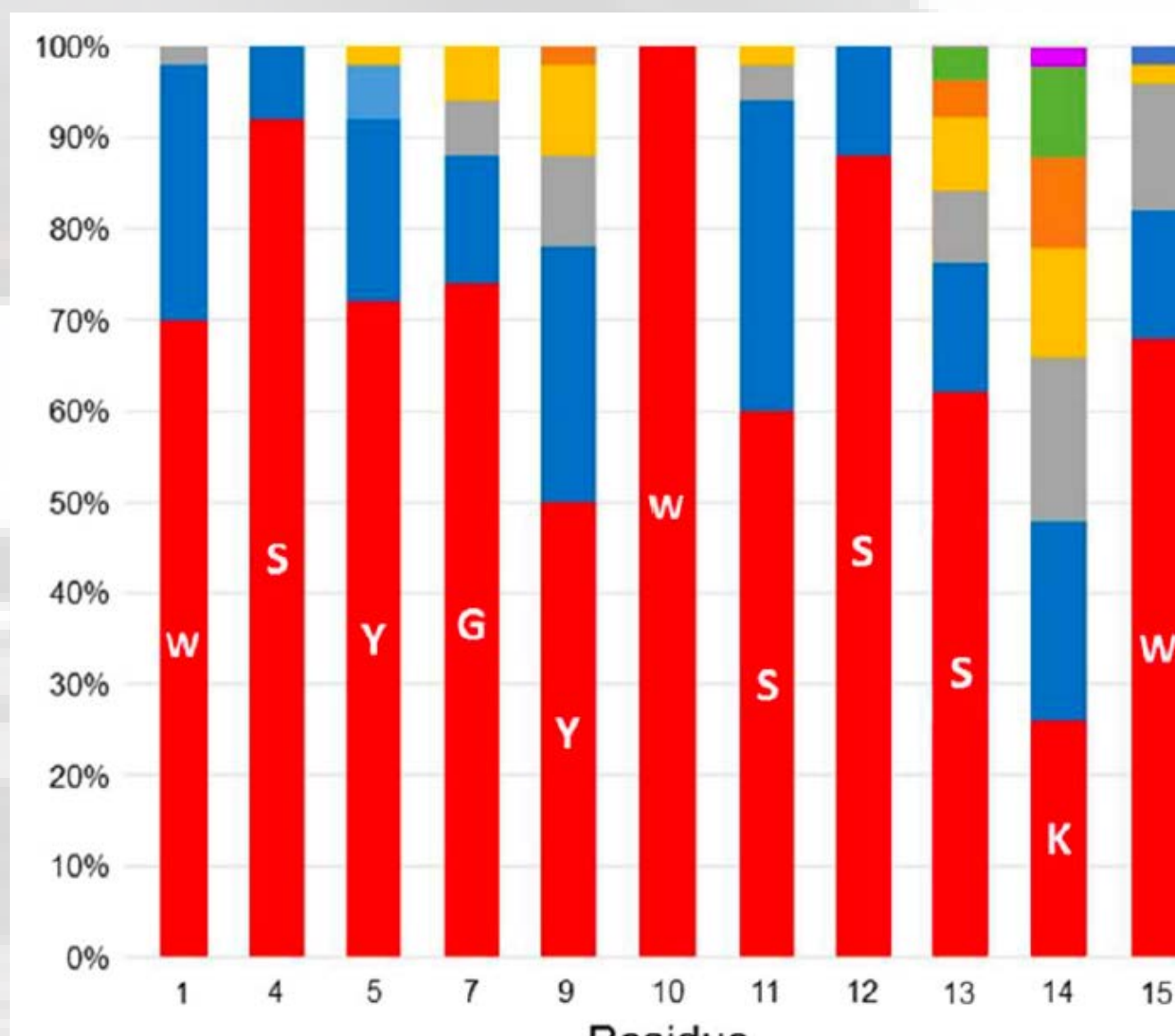
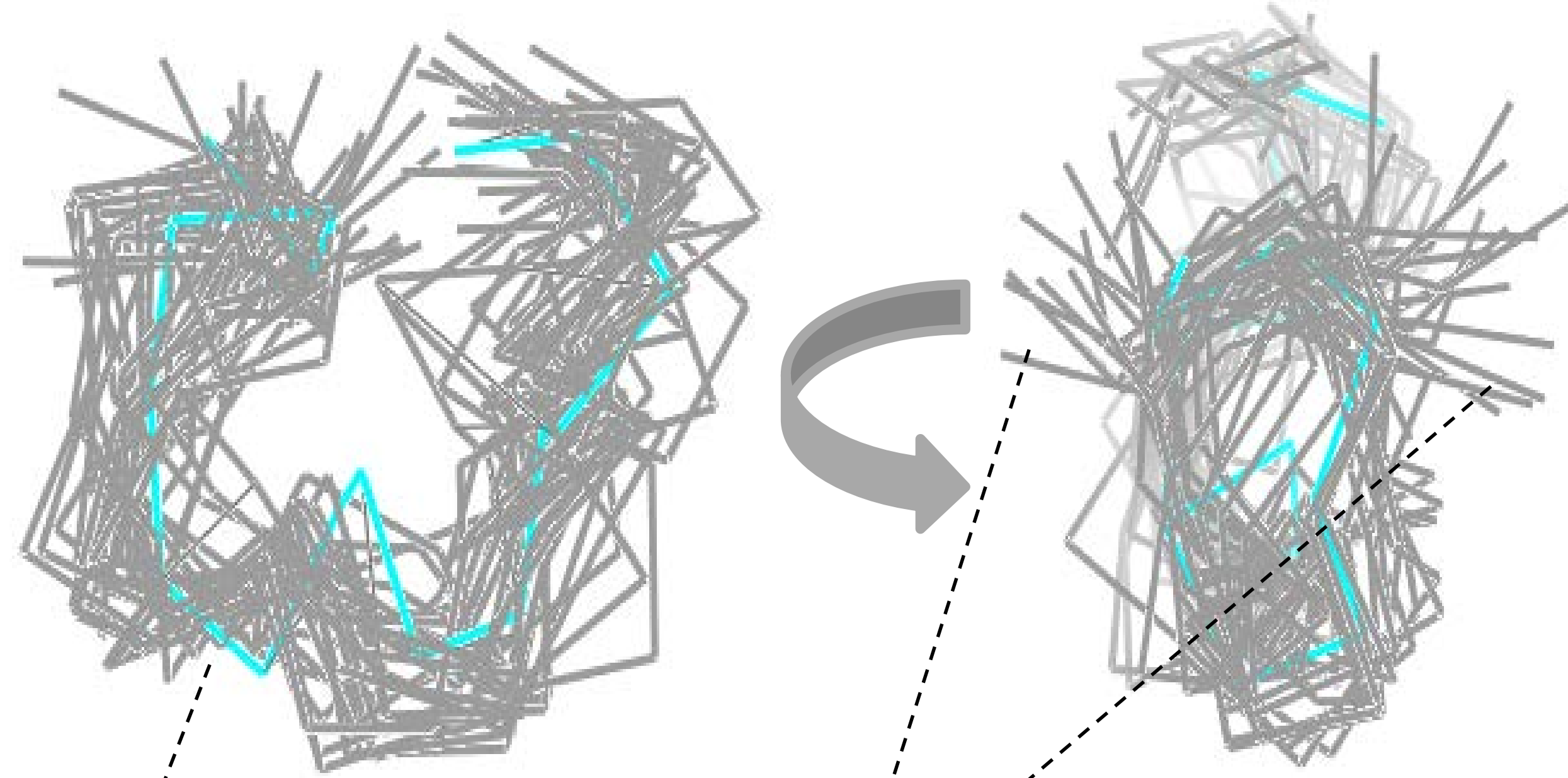


Fig 1. Relative frequencies of amino acid residues present in the top 50 sequences obtained from GAMPMS.¹



NMR Structure Determination⁴: KTM is dynamic and follows predicted structure

RMS: $2.6 \pm 0.3 \text{ \AA}$ Alignment to predicted: 2.5 \AA

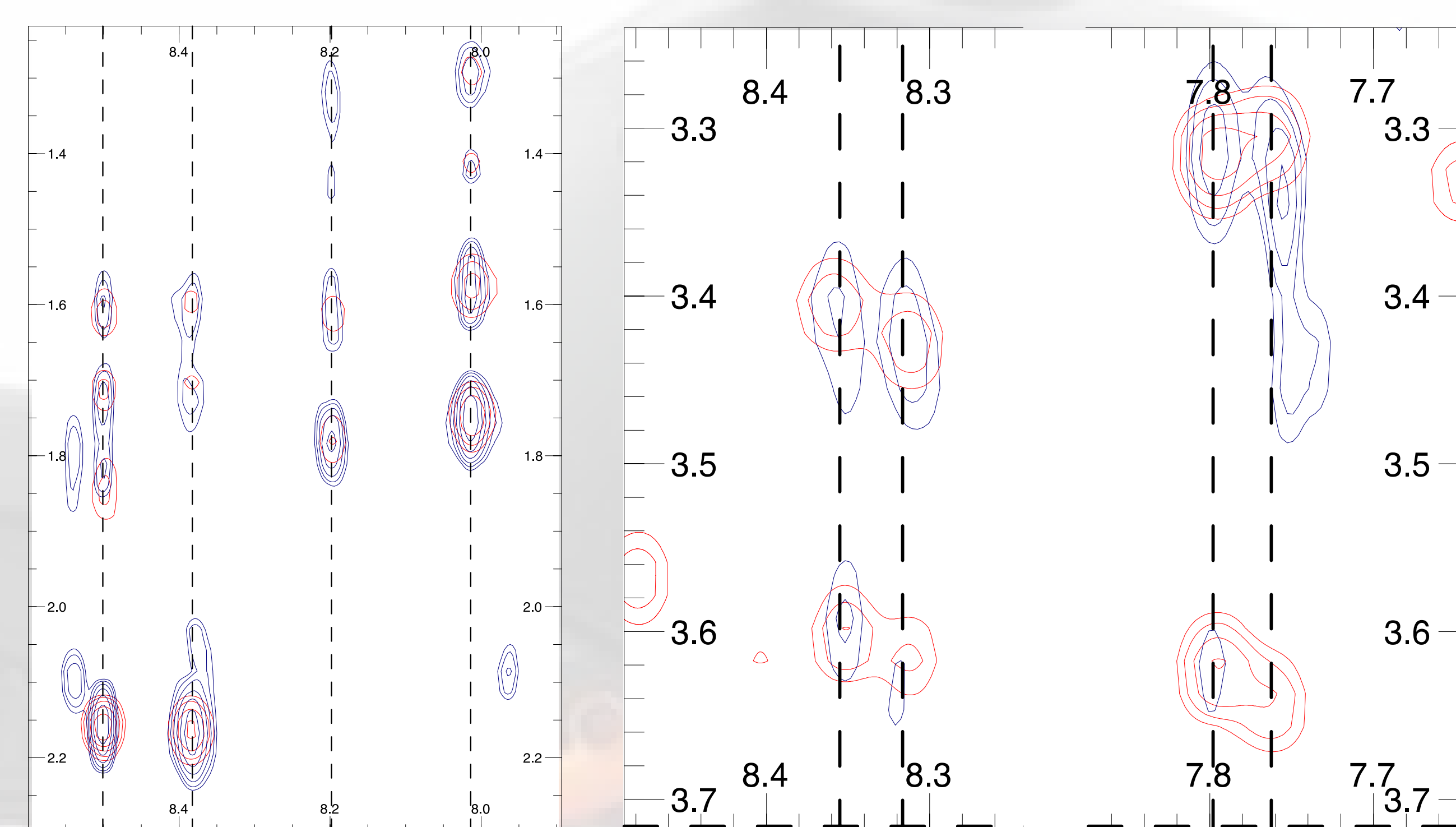


Two major conformations of N-terminus: inversion of P6?

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

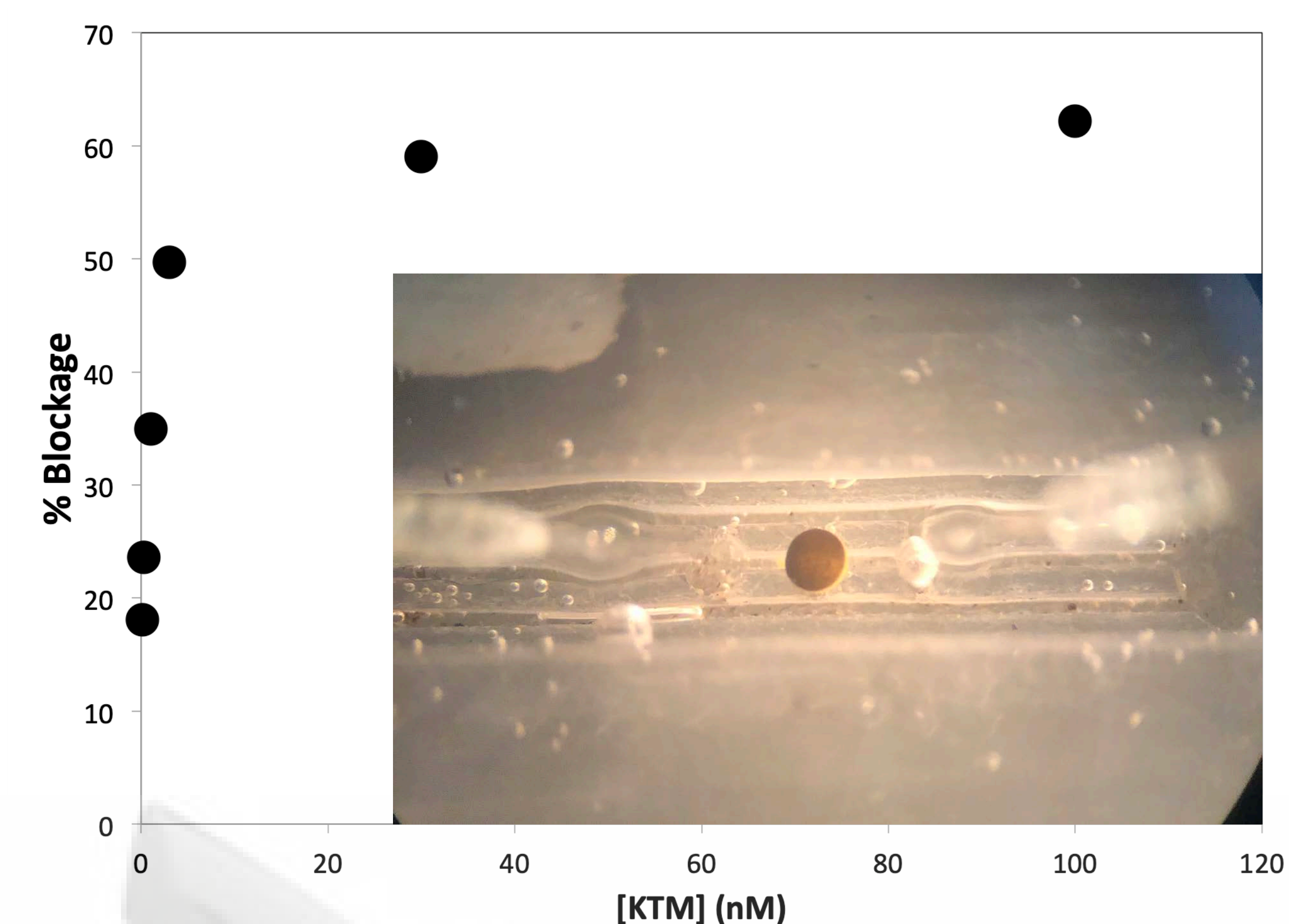
- TEV results indicate that KTM is a high-affinity antagonist of $\alpha 3\beta 2$
- TEV and NMR results indicate that computational results are reliable and can be used to predict lead compounds that will have high binding affinity for nAChR receptor isoforms

Future Work: How does KTM move?

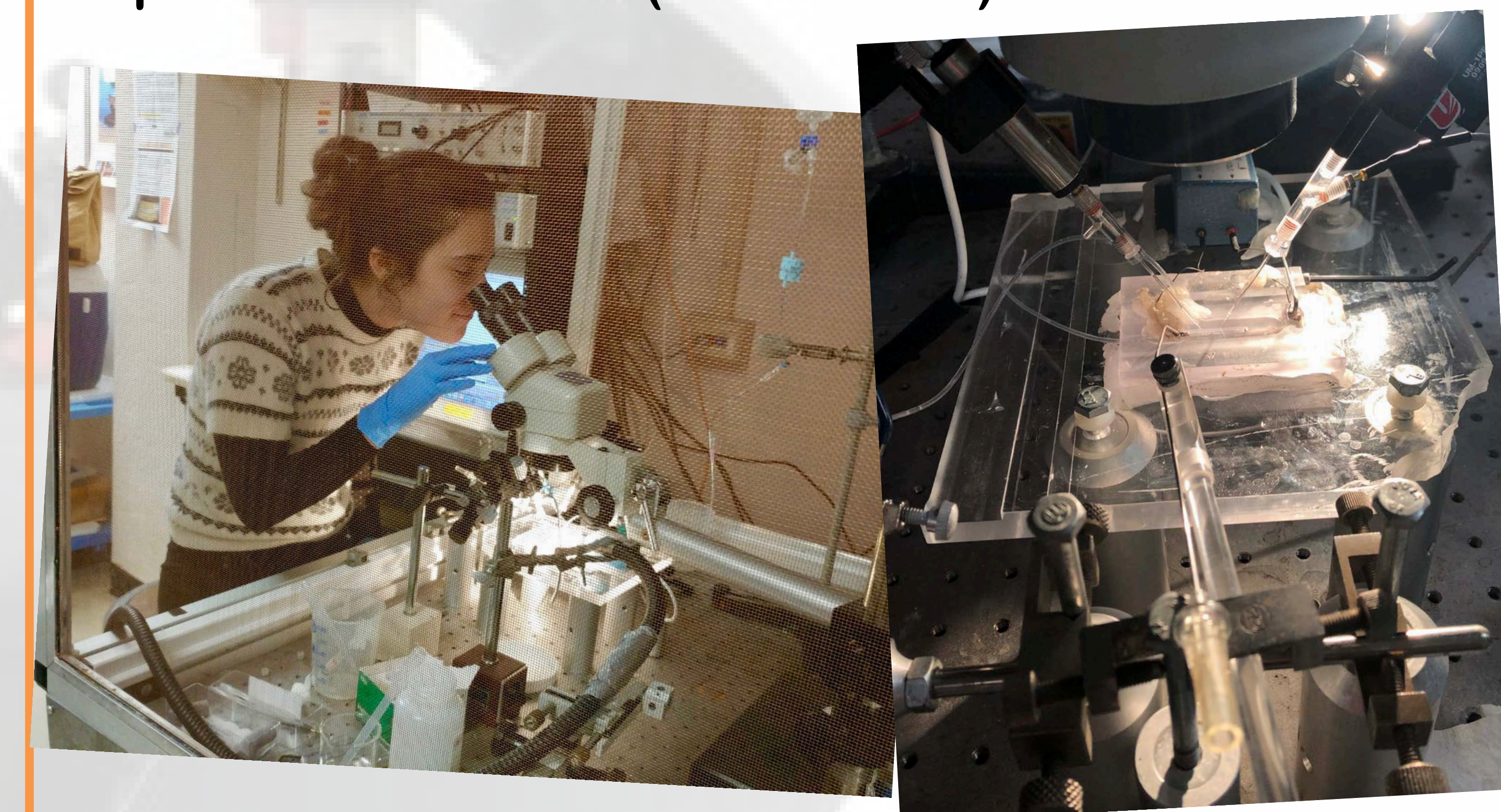
- MD simulations to identify peptide dynamics and key binding features
- Refine TEV and NMR data

TEV Results:

KTM is an antagonist of $\alpha 3\beta 2$ nAChR



Preliminary TEV experiments indicate that KTM has affinity for $\alpha 3\beta 2$ ($IC_{50} \sim 3nM$) on the order of alpha-conotoxin MII ($IC_{50} 2.2nM$).⁵



References

1. King, et al. *J. Chem. Inf. Mod.* 2016.
2. Quik, et al. *Pharm. Rev.* 2011.
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4. Güntert, et al. *Meth. Mol. Biol.* 2004.
5. Cartier, et al. *J. Biol. Chem.* 1996.

Acknowledgements

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