00

Genetic studies of an essential NTPase motor system in pathogenic parasites & Computational approaches in drug discovery.

Joshua Meehan, Zachary Goodall, Sai Vamshi R. Jonnalagadda, Asuka A. Orr, Andrew Hillhouse, James J. Cai, Achim Schnaufer, Phanourios Tamamis, and Jorge Cruz-Reyes

T3: TEXAS A&M TRIADS FOR TRANSFORMATION
A President's Excellence Fund Initiative

Abstract

Trypanosoma brucei and related single-celled Kinetoplastid organisms cause maladies of global impact that the WHO catalogue as neglected diseases. The unique biology of essential processes in these organisms offer a number of potential targets for therapeutic drug development. One such process termed U-indel RNA editing in the mitochondrial genome entails site-specific insertions and deletions of uridines (Fig.1). Mitochondrias cryptogenes generate primary transcripts that lack an open-reading frame (ORF). Extensive U-indel editing of these transcripts is needed to create mature translatable sequences. The holo-enzyme known as the "editosome" has several multiprotein editing complexes, and the U-indels are directed by small anti-sense guide RNAs. One of us (Cruz-Reyes lab) identified the editing complex termed REH2C (Figure.1). This complex contains an ATP-dependent DExH/RHA RNA helicase (KREH2), and the KREH2-associated protein factor with eight zing fingers (KH2F1) (Fig.2). T3 funds supported basic studies to dissect the essential function(s) of REH2C by using three high-throughput technologies: (A) RNA-seq studies in loss-of-function knockdowns (via RNAi) showed that the REH2C controls editing fidelity (Fig. 3); (B) Structural proteomics by Crosslinking Mass Spectrometry (CXMS) to examine protein conformational changes of the RNA helicase in the editosome (Fig. 2); and (C) State-of-the-art DMS-MapSeq methodology examine RNA conformational changes controlled by REH2C (Fig. 4). We are combined structural models of the ATP-binding site with a virtual screening to search for compounds that may inhibit the helicase. Our Our virtual screen using a powerful computational approach led to candidate inhibitors base on a docked ADP-REH2 structure. Overall, our T3-funded project combined expertise in enzyme kinetics, structure-based drug design, computational ligand-binding predictions, and molecular parasitology to better understand an essential editing factor discovered by us at TAMU, and to develop potential inhibitors of its essential RNA helicase in trypanosome protozoa. This work has contributed to multiple publications so far. Our powerful interdisciplinary approach may lead to new information to combat neglected diseases by parasites of global importance.

Figure 1: RPS12 sequence, the editosome, and RNA editing

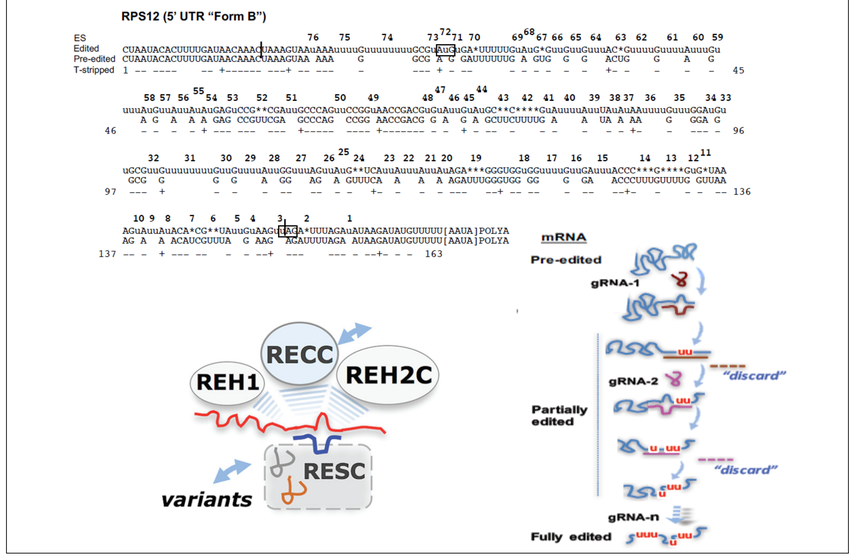


Figure 2: CXMS analysis of KREH2C recombinant protein reveals KH2F1 stabilizes KREH2 in complexes

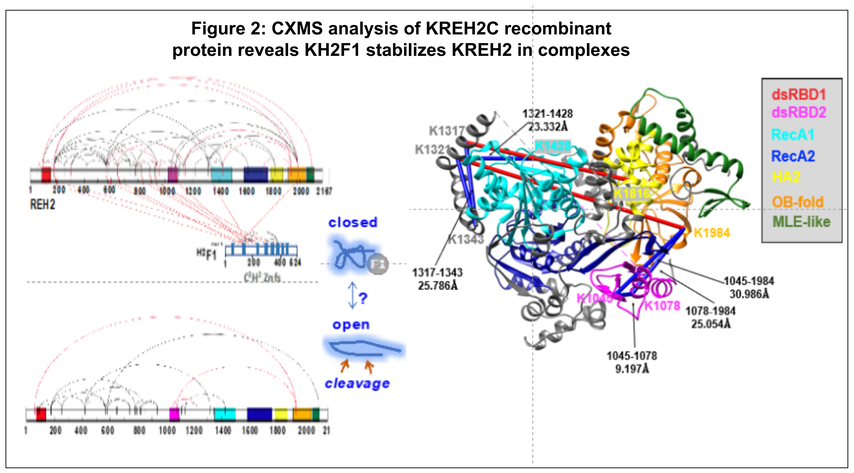


Figure 3: REH2C has site specific effects in editing accuracy

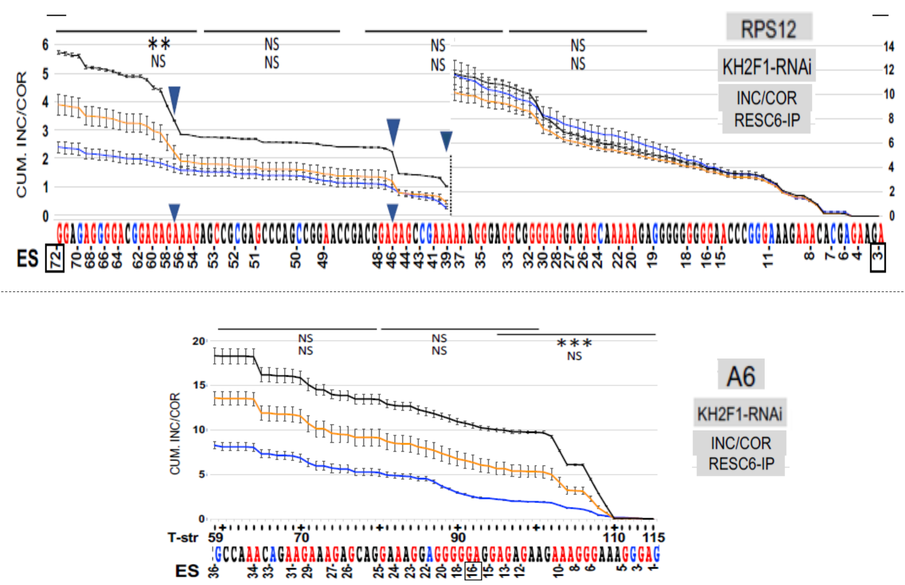
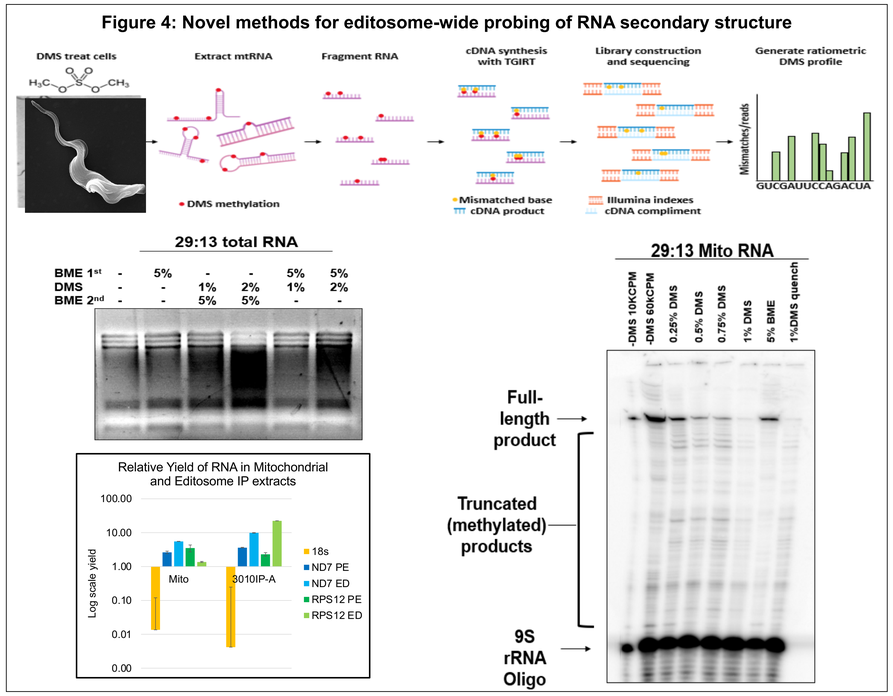


Figure 4: Novel methods for editosome-wide probing of RNA secondary structure



Peer-reviewed publications acknowledging this T3 Award

Site-specific and mRNA-specific control of accurate mRNA editing by a helicase complex in trypanosomes. Kumar V, Ivens A, Goodall Z, Meehan J, Doharey PK, Hillhouse A, Hurtado DO, Cai JJ, Zhang X, Schnaufer A, Cruz-Reyes J. RNA. 2020. rna.076513.120. PMID: 32873716

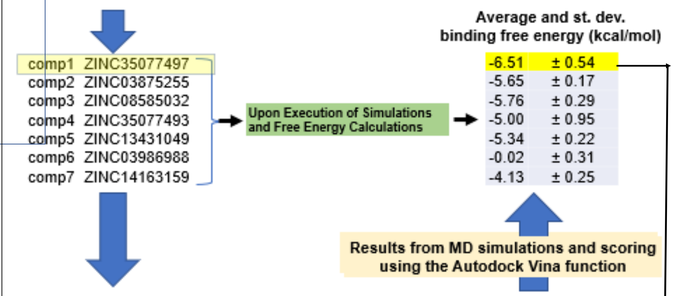
Lexis and Grammar of Mitochondrial RNA Processing in Trypanosomes. Aphasizheva I, Alfonzo J, Carnes J, Cestari I, Cruz-Reyes J, Göringer HU, Hajduk S, Lukeš J, Madison-Antenucci S, Maslov DA, McDermott SM, Ochsenreiter T, Read LK, Salavati R, Schnaufer A, Schneider A, Simpson L, Stuart K, Yurchenko V, Zhou ZH, Zíková A, Zhang L, Zimmer S, Aphasizhev R.Trends Parasitol. 2020, 6(4):337-355. PMID: 32191849

Protein features for assembly of the RNA editing helicase 2 subcomplex (REH2C) in Trypanosome holo-editosomes. Kumar V, Doharey PK, Gulati S, Meehan J, Martinez MG, Hughes K, Mooers BHM, Cruz-Reyes J. PLoS One. 2019;14(4):e0211525. PMID: 31034523

Peptidomimetic Vinyl Heterocyclic Inhibitors of Cruzain Effect Antitrypanosomal Activity. Chenna BC, Li L, Mellott DM, Zhai X, Siqueira-Neto JL, Calvet Alvarez C, Bernatchez JA, Desormeaux E, Alvarez Hernandez E, Gomez J, McKerrow JH, Cruz-Reyes J, Meek TD. J Med Chem. 2020;63(6):3298-3316. PMID: 32125159

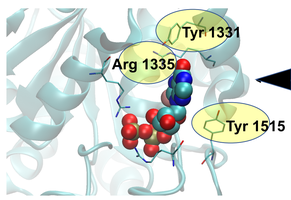
- **Figure 5: Modeling of the structure of the ATP-binding site using I-TASSER.**
Docking of ADP to the modeled structure based on superposition.
- We identified the closest homologous structure of a protein bound to ADP Prabu JR, et al. *Mol Cell*. 2015;60(3):487-499. doi:10.1016/j.molcel.2015.10.011
- We used structural superposition in UCSF Chimera to dock ADP to our modeled structure.
- The resulting structure was used as input in the Virtual screening search.

- **Virtual screening search for novel compounds based on docked ADP-.REH2 structure**
- We introduced pharmacophore points in ZINCPharmer.
- We ran ZINCPharmer and identified a list of promising compounds for further investigation in simulations.
- The compounds identified are presented below, using their ZINC IDs.



- **Molecular Parametrization of the compounds, MD simulations, and Free Energy Calculations**
- We parametrized the compounds using CGENFF,
- We ran simulations using CHARMM using analogous approaches to our published studies.
- We estimated the binding free energy of each of the simulated compounds in complex with the modeled protein, using Autodock Vina Scoring function.

Figure 5:



- The enhanced binding free energy of compound (COMP1) ZINC35077497, can be attributed to its ability to form additional interactions with Arg 1335, Tyr 1331, and Tyr 1515.
- This interactions, and the specific compounds, can serve as a basis for the discovery of novel compounds with improved binding properties.
- Importantly, MD simulations help to improve the interactions between the compound and the protein, which demonstrate their beneficial role in improving the structure of the initially virtually screened-docked compound.
- The RMSD of the compound with respect to its average structure is equal to 1.05 Å, which shows that the ligand's binding is fairly stable in the pocket.