Exploring the genetic robustness of ncRNA and protein

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Reviewer 2

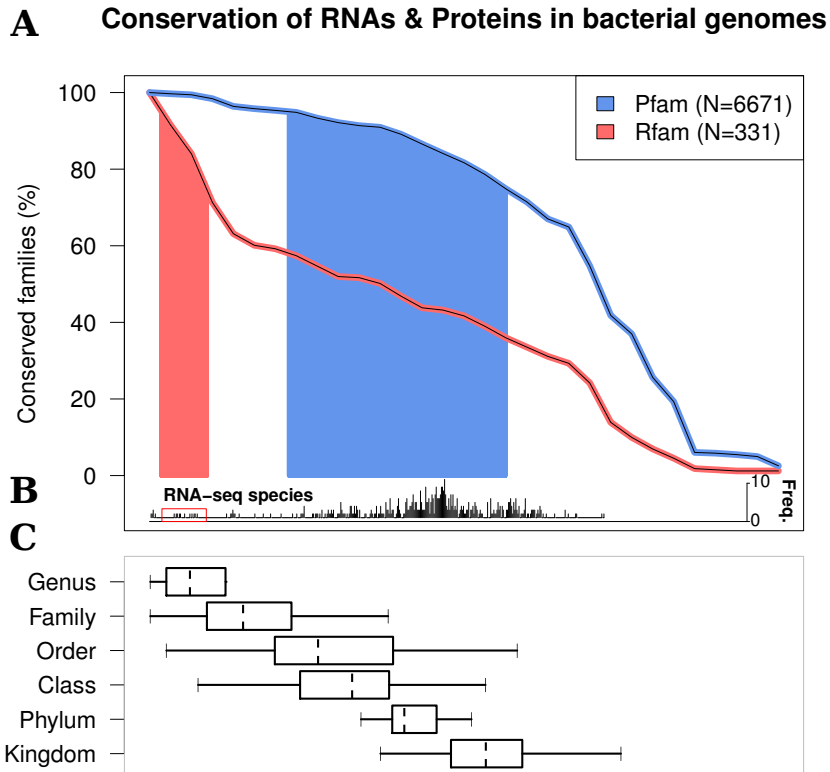
Me: RNAs, unlike proteins, are relatively robust to genetic variation.

Referee 2: Has the robustness to variation of RNAs ever been compared to that of proteins? I'm not convinced the comparative claim is true (or important to the paper).

Gardner & Eldai (2014) Annotating RNA motifs in sequences and alignments. *Nucleic acids research.*

I didn't think this was an unreasonable claim

- Proteins require extra steps to mature (translation, modification, ...)
- Proteins are generally more conserved than RNAs (one explanation is that RNA is tolerant of mutations)
- Many non-synonymous coding changes associated with disease than ncRNA ($> 30K$ in Humsavar), missing an equivalent resource for ncRNAs. Coding regions enriched in GWAS results.
- Neutral networks & genotype-phenotype maps

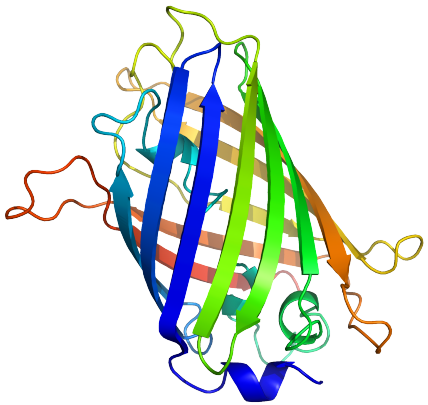


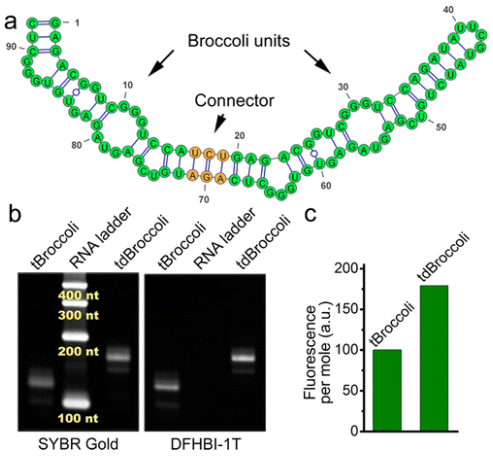
How can we test if protein is delicate and RNA is robust?

- We think of "robustness" as the insensitivity of phenotype/fitness to mutations
- There is (AFAIK) no ideal test for (genetic) robustness

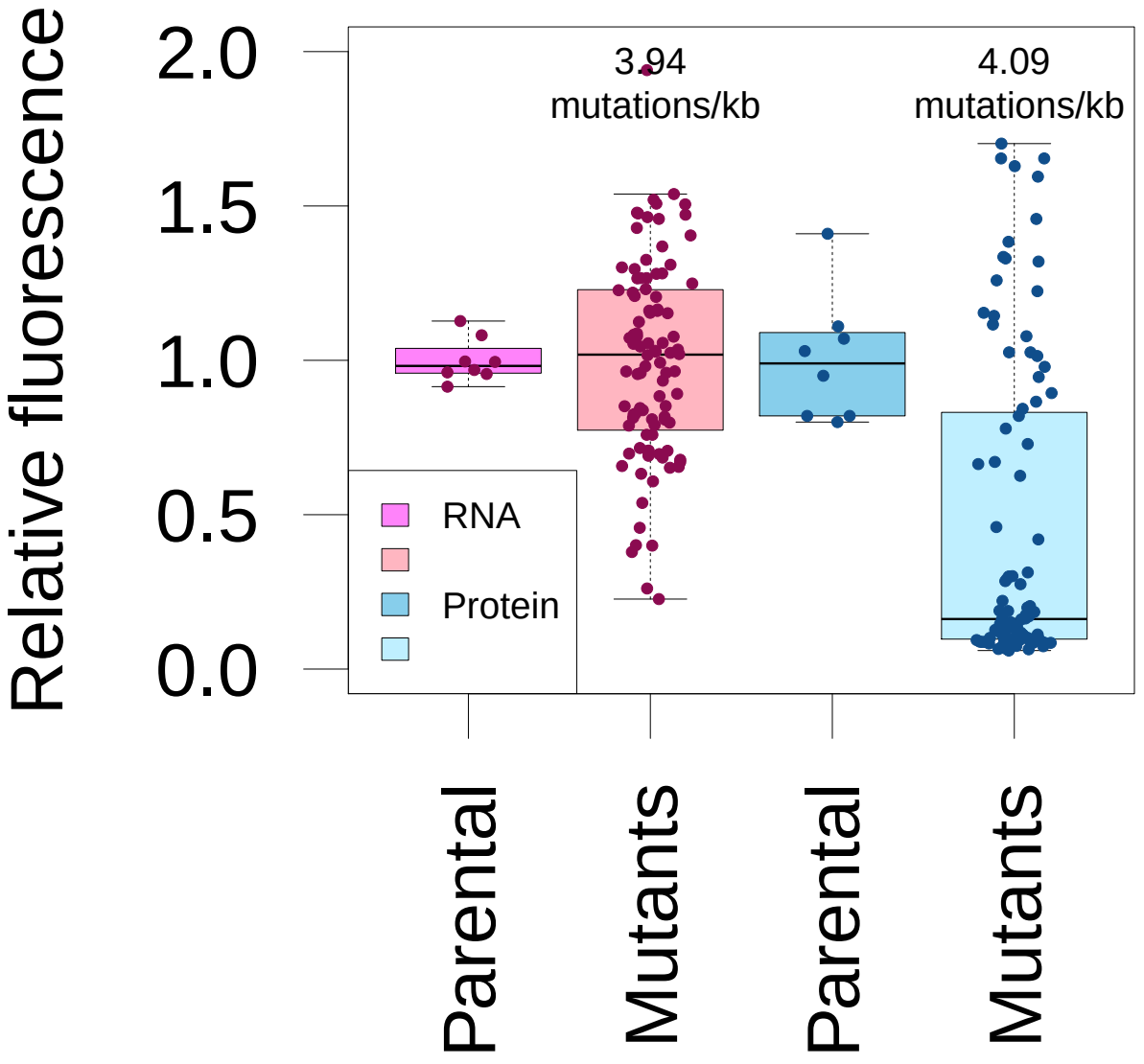
Fluoro test for robustness: error-prone PCR of a fluorescent protein & RNA

- **Advantages:** The protein (mCherry) and RNA (broccoli) have a similar function. The function is measurable!
- **Disadvantages:**
  - The protein evolved naturally, over millennia. The RNA evolved "unnaturally" in a few generations in a lab.
  - Different lengths
  - Didn't compare multiple proteins (e.g. GFP, luciferase) and RNAs (e.g. Spinach, iSpinach)



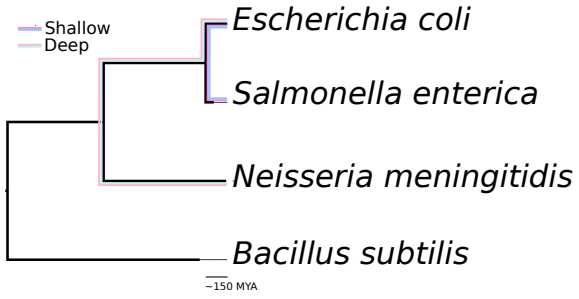


Fluoro test for robustness

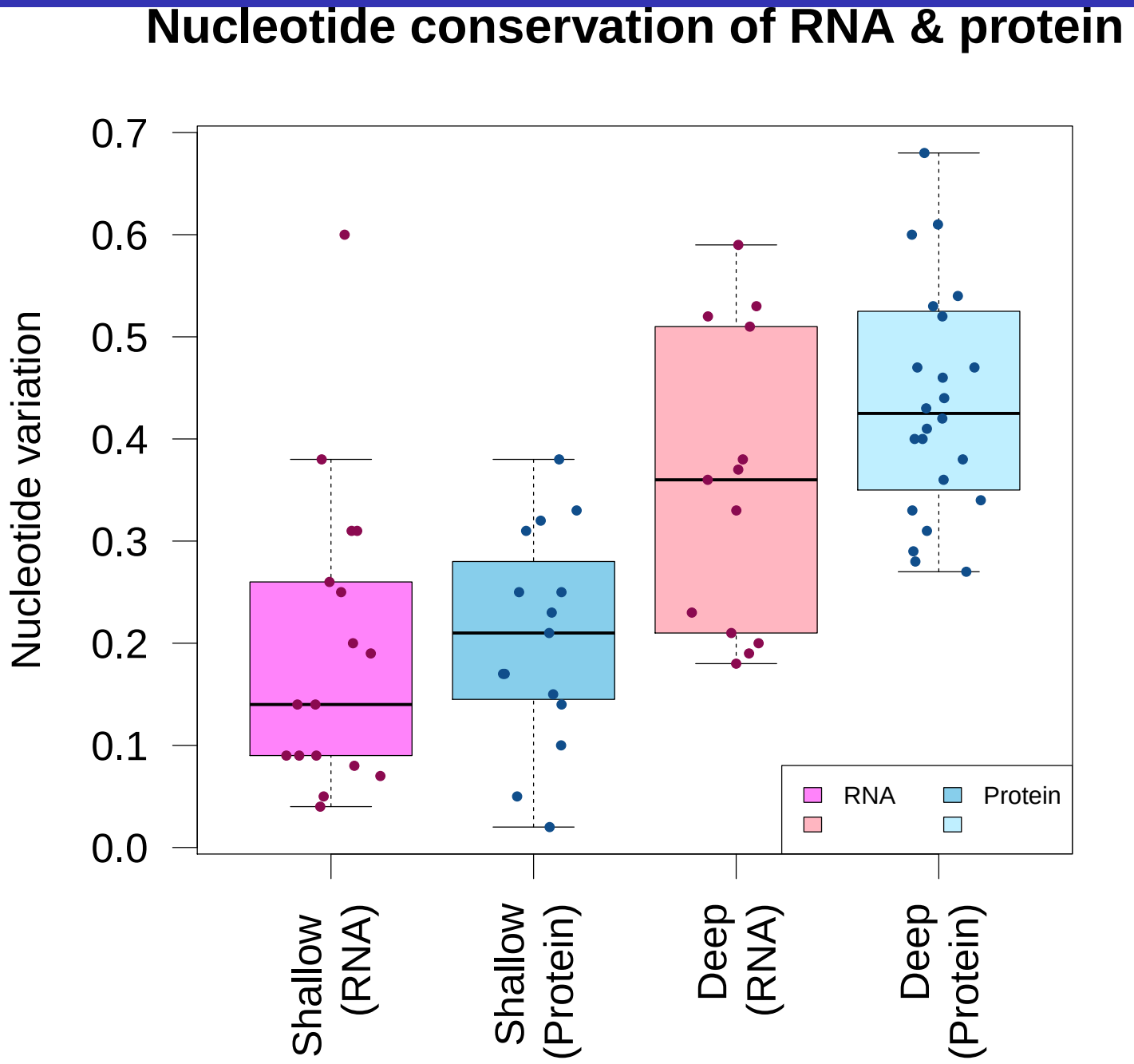


Mutation rates test for robustness: mutation rates in RnPs

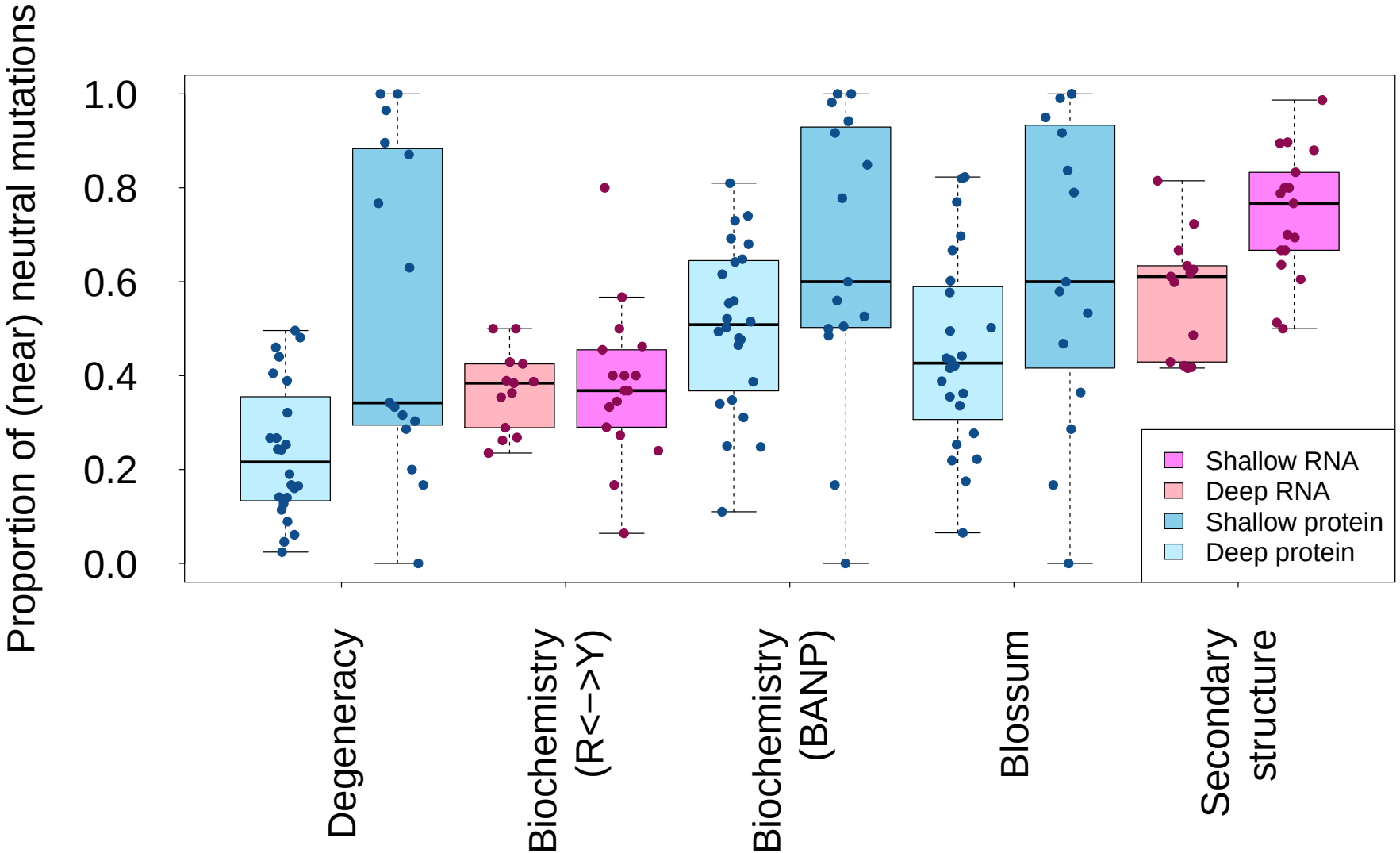
- Compare mutn rates of ribonucleoproteins (RNPs) and closely associated ncRNA & protein partners
- e.g. Ribosome, SRP, RNase P, tRNAs & aa transferases, cis-regs and downstream genes, sRNA & RNA-binding proteins.
- **Advantages:** Same phylogenetic distribution, shared process (e.g. translation).
- **Disadvantages:** The RNA and proteins components are involved in the same process but don't have the same function (e.g. catalysis vs structural support).
- Used 13 "deep" and 14 "shallow" RNA/protein pairs



Mutation rates test for robustness: RnPs

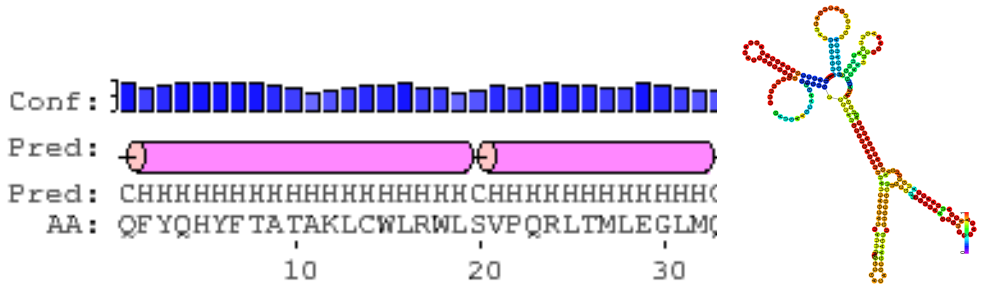


Proportion of "neutral" mutations: RnPs

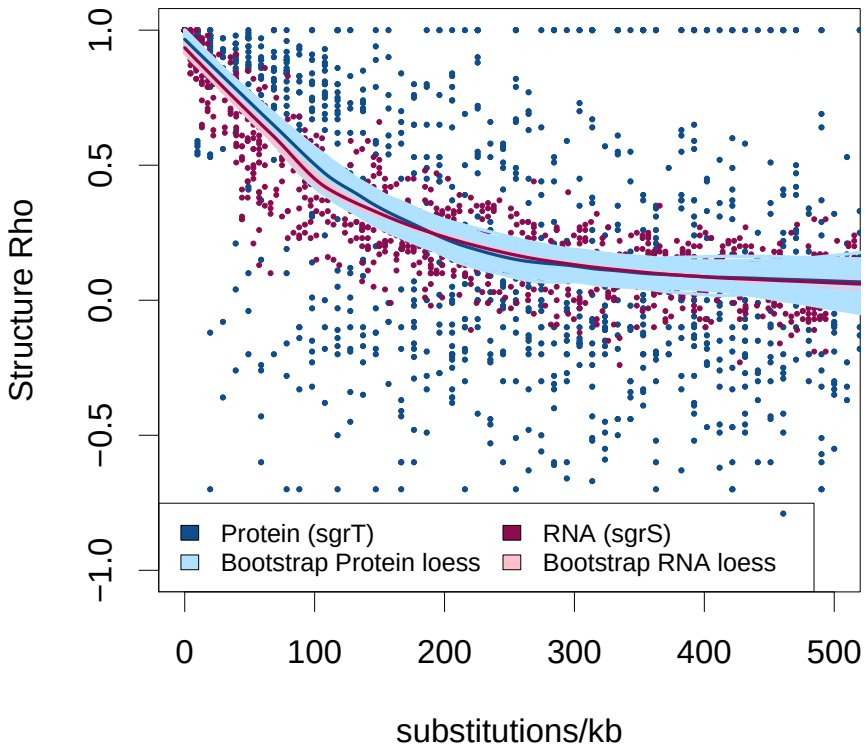


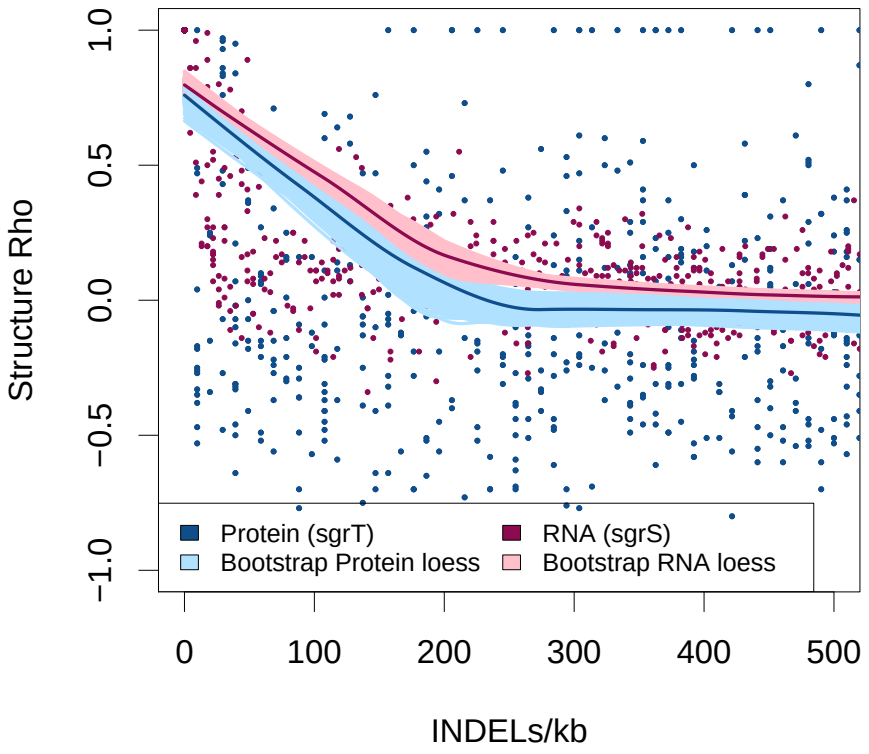
Structural robustness test

- Test "structural robustness" of RNA/protein pairs
- Use structure prediction tools (e.g. `RNAfold -p` & `PSSpred` (iTasser))
- Compute correlation between per-residue probabilities for native and randomly mutated sequences
- **Advantages:** if function is tied to structure, this may be a reasonable test
- **Disadvantages:**
  - the RNA and proteins components are involved in the same process but don't have the same function,
  - different methods for predictions, possible prediction errors
  - probabilities not necessarily comparable
  - issues with dependencies e.g. frame-shifts and truncations



Structural robustness test (SgrS/SgrT)





Other possible tests

- Use interaction networks (experimental/computational?). Test the "robustness" of these networks (i.e. if perturbed, how much will they change?).
- Structure comparisons with Boltzmann structure ensembles for proteins
- More comparable estimations of neutral mutations with e.g. FATHMM-like methods
- Explore robustness to thermal, pH, salt concentrations and other environmental gradients.
- Explore mutability between states with flow-reactor/SELEX

Summary/Conclusions

- Fluorescence test for robustness (**RNA wins!**)
- Mutation rates test for robustness (**no sig. difference**)
- Proportion of "neutral" mutations (**not comparable**)
- Structural robustness (**RNA/Protein tied**)
- There seems to be little evidence that I'm right or that Reviewer 2 is wrong!
  - I.e. can't reject the null hypothesis that there is no difference

Thanks

- Nellie Sibaeva
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- Dorien Coray

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