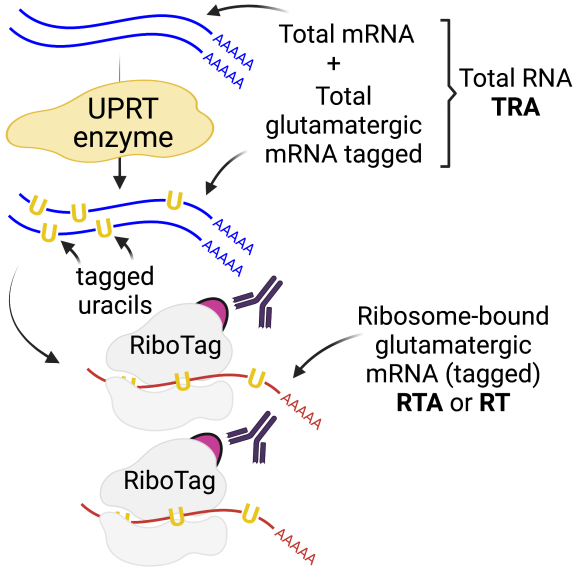
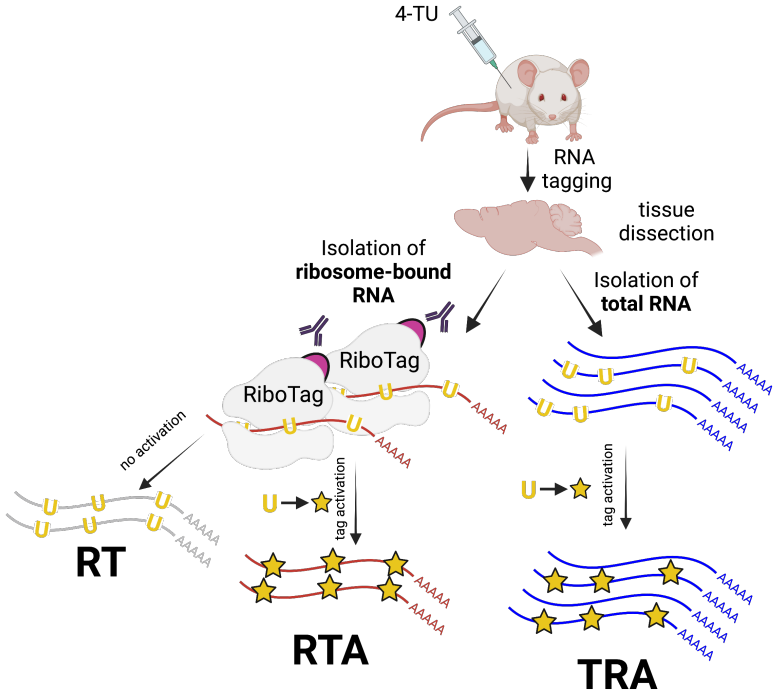
Tagger mice used in this experiment enable:

- labeling of RNA in glutamatergic cells of the brain (a subset of neuronal cells) by enzymatic tagging using **UPRT enzyme**
- purification of ribosome-bound RNA from specific cell types by ribosomal tagging using **Ribo-Tag protein**

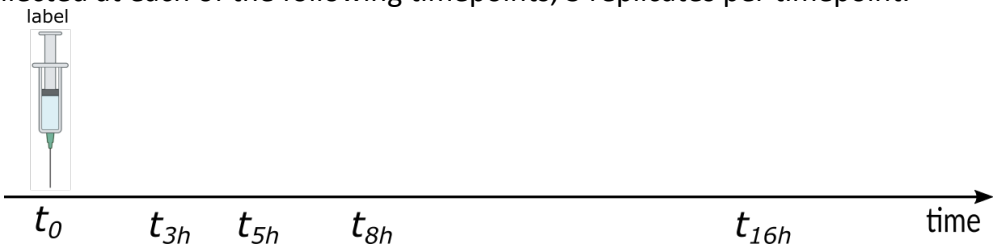
Both methods are combined and give quantifiable output (based on next generation RNA sequencing technologies) in form of **read count data** and **label (tagged uracil) count data**.



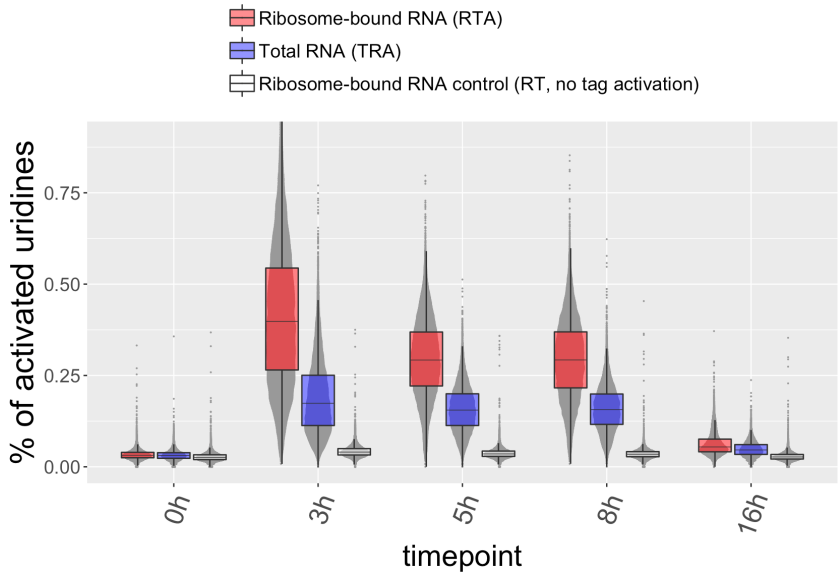
RNA is labeled by injection of 4-thiouracil (4-TU). Total RNA (**TRA**) and ribosome-bound RNA (**RTA**) are then isolated from the tissue of interest (brain), and the RNA is subjected to chemical activation of the label. Ribosome-bound RNA that is not chemically treated (**RT**) is used as a control for potential bias that may be introduced by the chemical treatment.



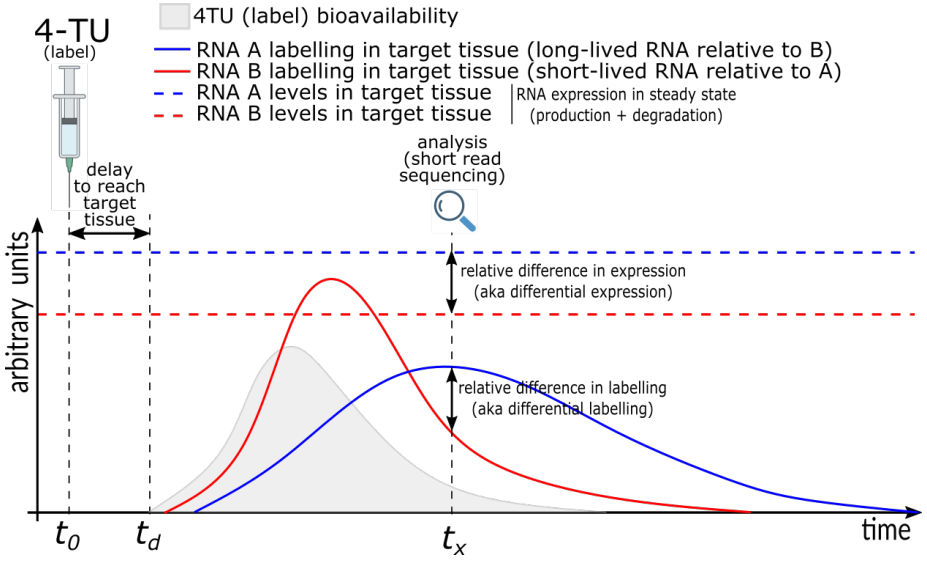
Total RNA (**TRA**), ribosome bound RNA (**RTA**), ribosome bound RNA witout tag activation (**RT**), were collected at each of the following timepoints, 3 replicates per timepoint.



An overview of the label detection is below. Grey quasi-violin plot beneath box plot visualizes the distribution of individual transcripts. Y axis represents the % fraction of activated uridines.



Simplified overview of RNA labelling kinetics below assumes a steady state system (RNA production rate = RNA degradation rate):



Visualization of the whole landscape of mismatches in the data (3h labeling time, mean of 3 replicates):