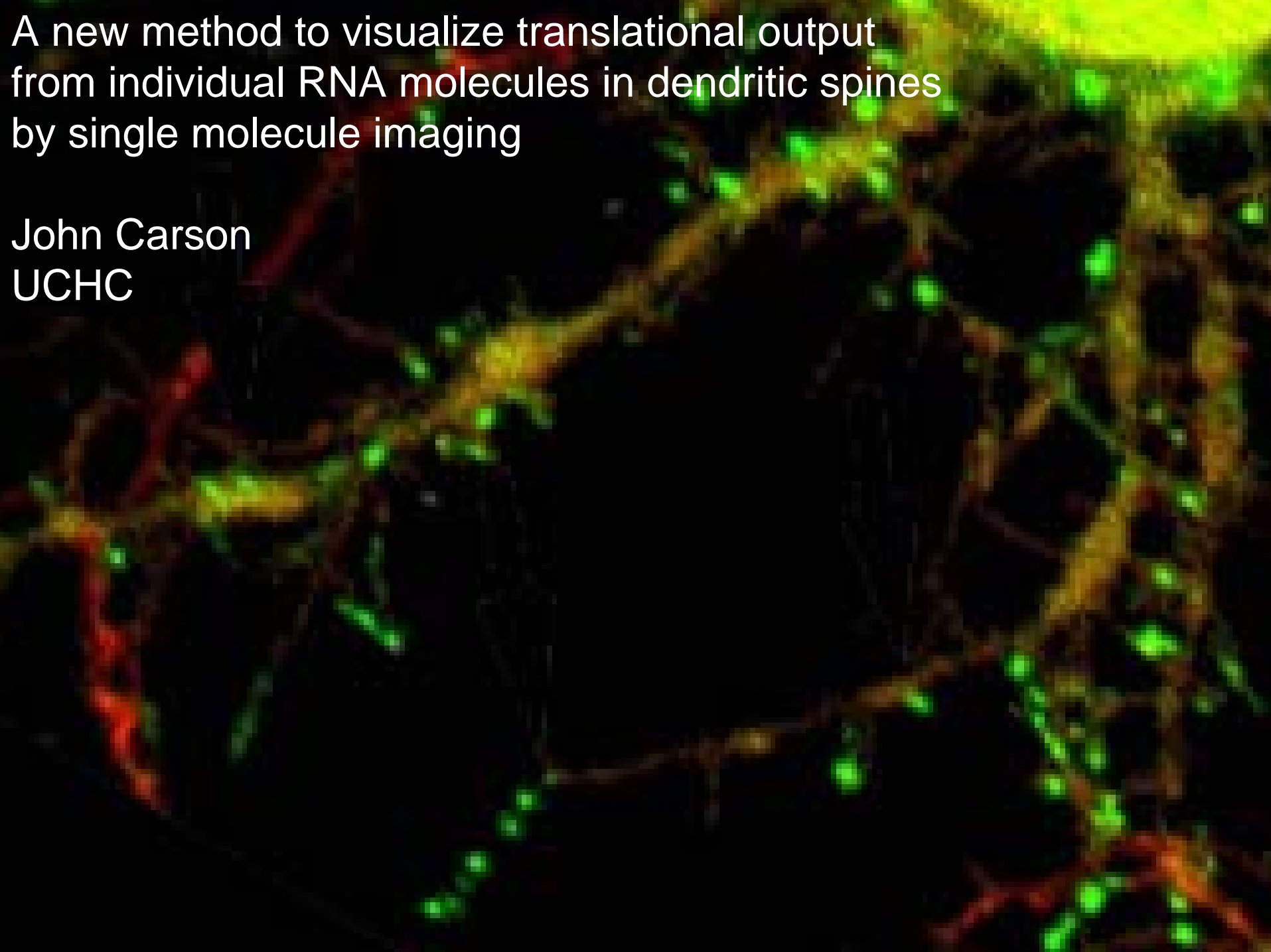
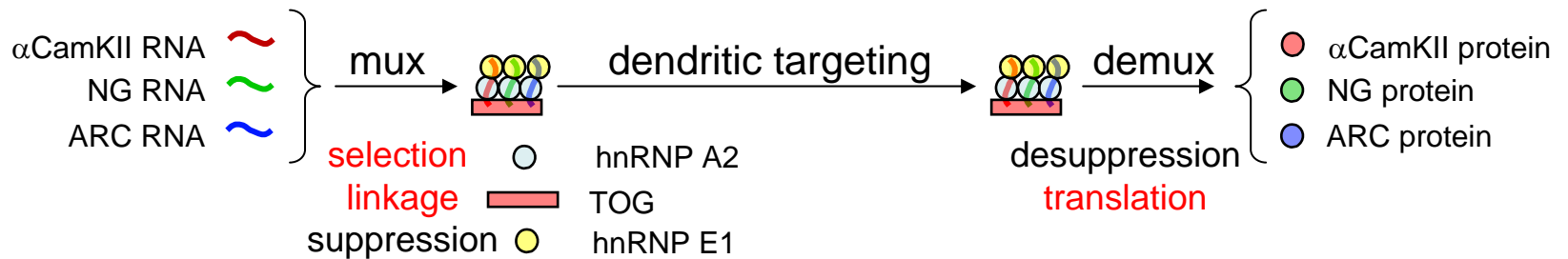
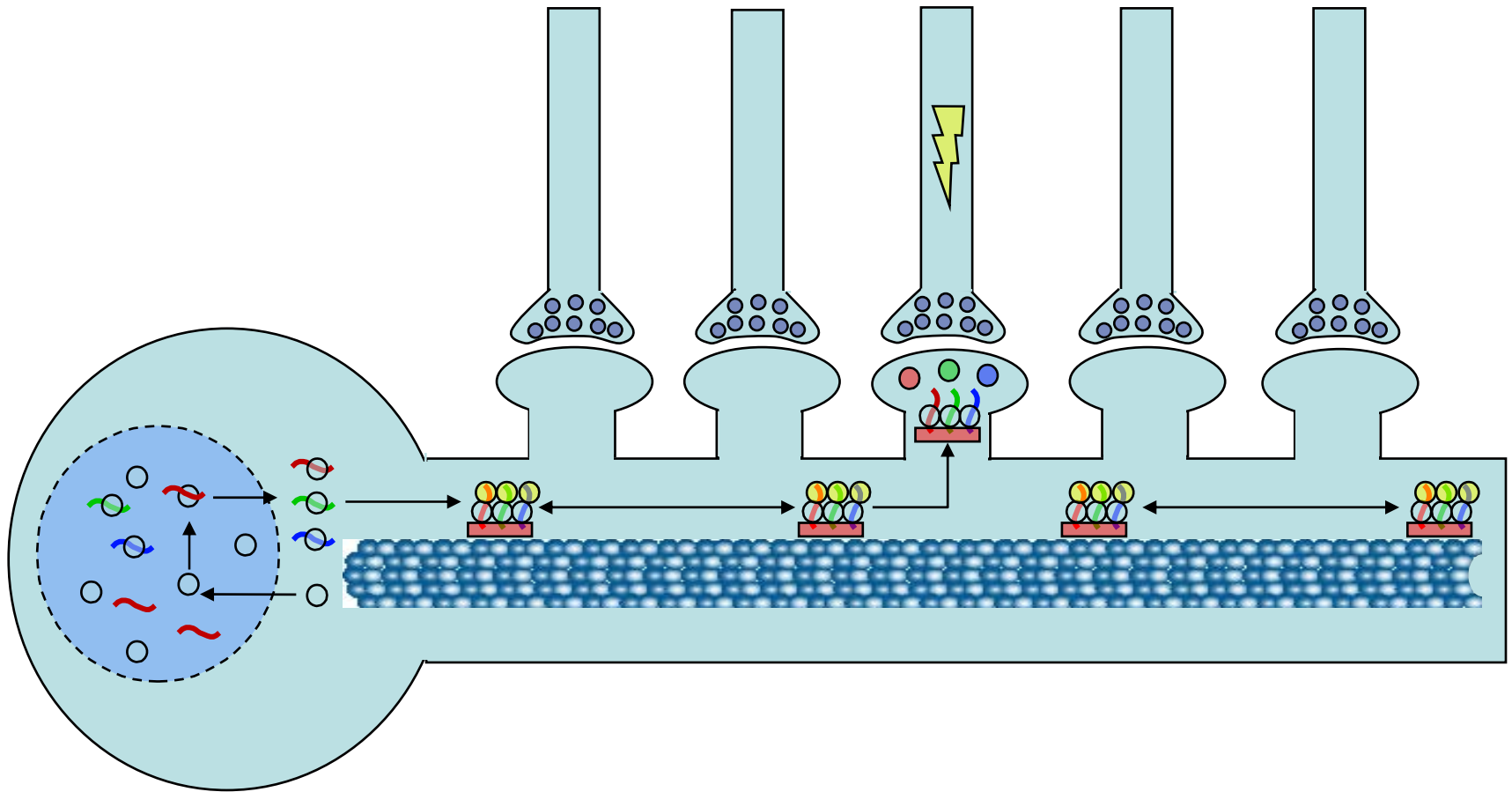


A new method to visualize translational output
from individual RNA molecules in dendritic spines
by single molecule imaging

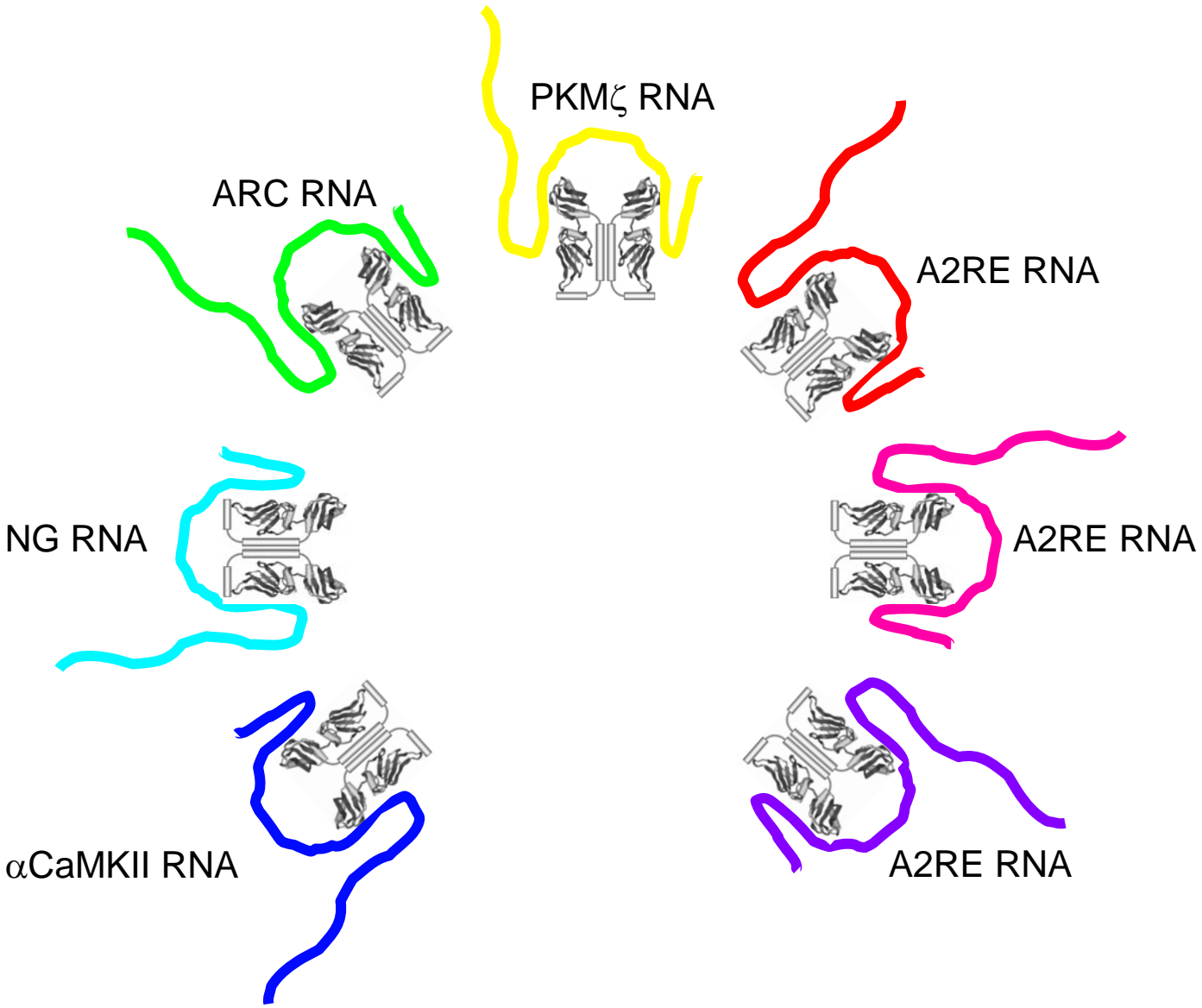
John Carson
UCHC



Multiplexed transmission of genetic information from nucleus to synapse



Multivalent interactions among A2RE RNAs, hnRNP A2 and TOG mediate granule assembly - each granule contains multiple different RNA molecules



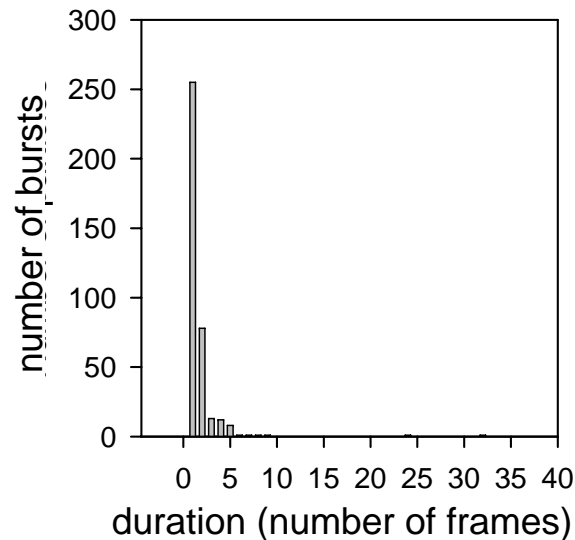
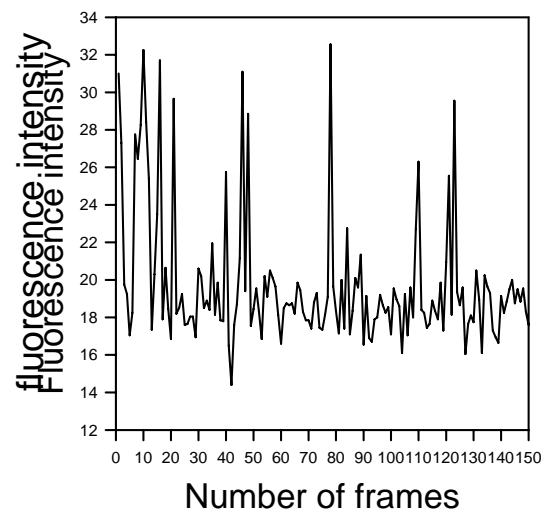
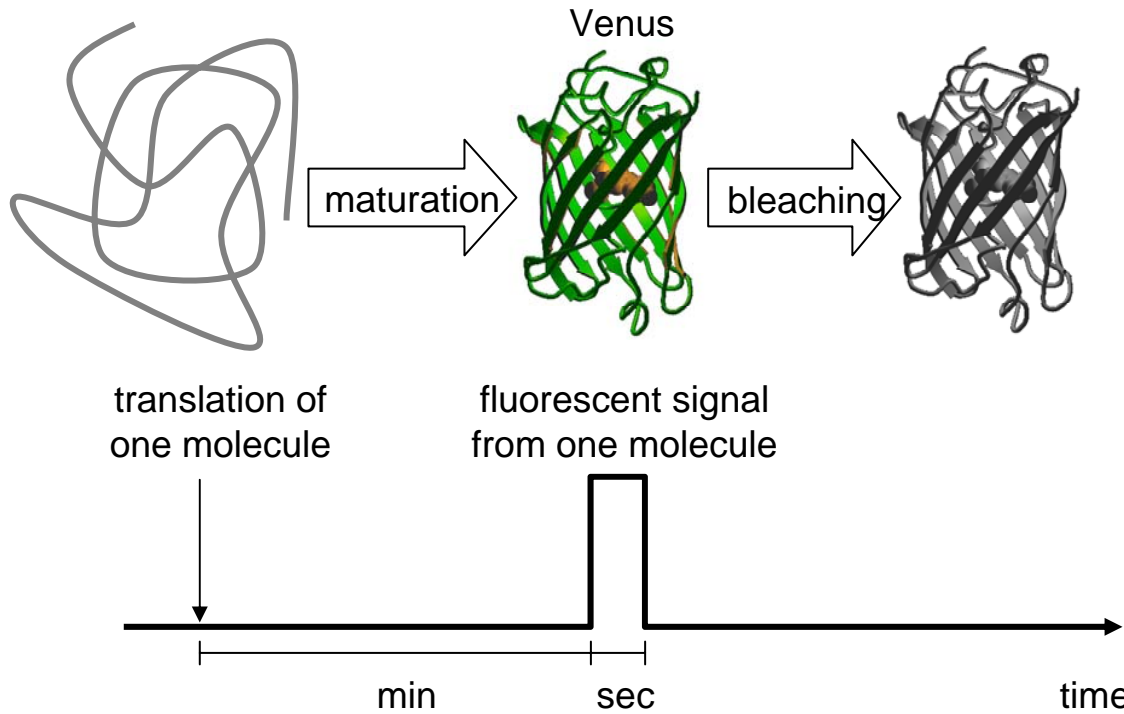
Most spines contain 1-2 polysomes



Translational output from individual granules

- most spines contain one RNA granule (with multiple RNA molecules) but only 1-2 polyribosomes (Bourne et al., 2007), which means that only 1-2 RNAs in each granule are being translated at any one time
- is translational output from individual RNA molecules continuous or “bursty”
- are specific RNAs in each granule translated preferentially
- does linkage affect translation
- how is translation output from each granule regulated
- translational output from individual RNA molecules in each granule can be analyzed by single molecule imaging

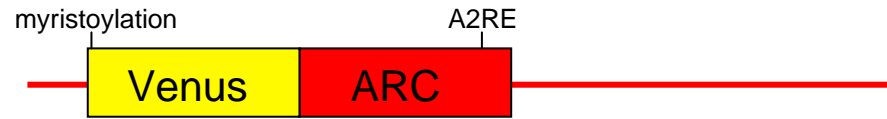
Venus photophysics facilitates single molecule imaging



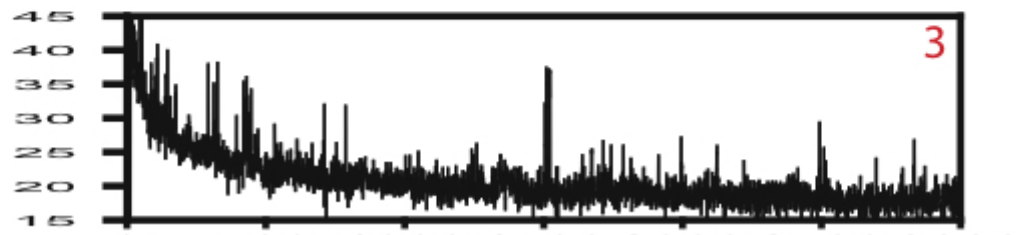
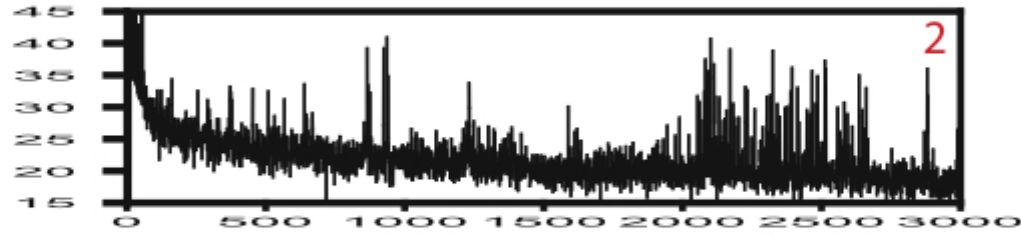
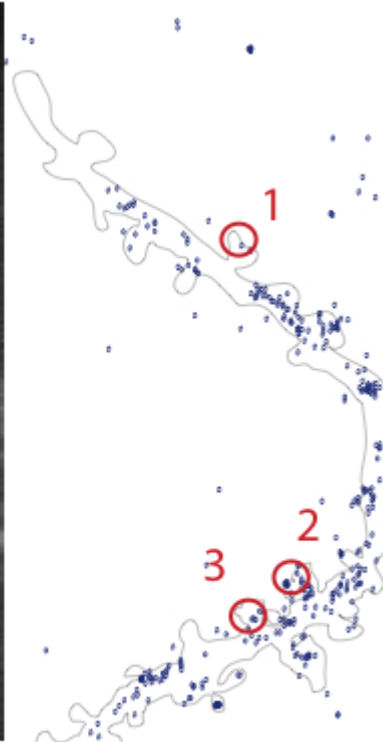
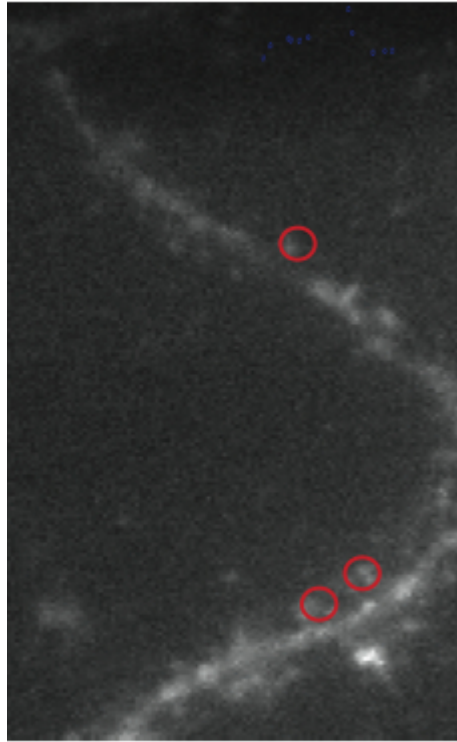
Criteria for single molecule imaging

- single step appearance and disappearance of fluorescent signal
- uniform amplitude of fluorescent peaks
- comparison of fluorescent signal to single molecule standard

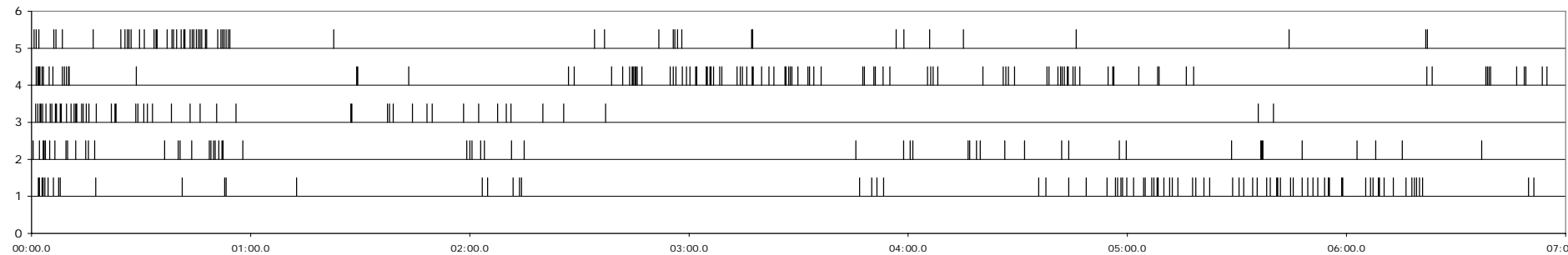
Microinject Venus-ARC RNA into hippocampal neurons



Translational output from individual granules is spatially clustered and temporally bursty

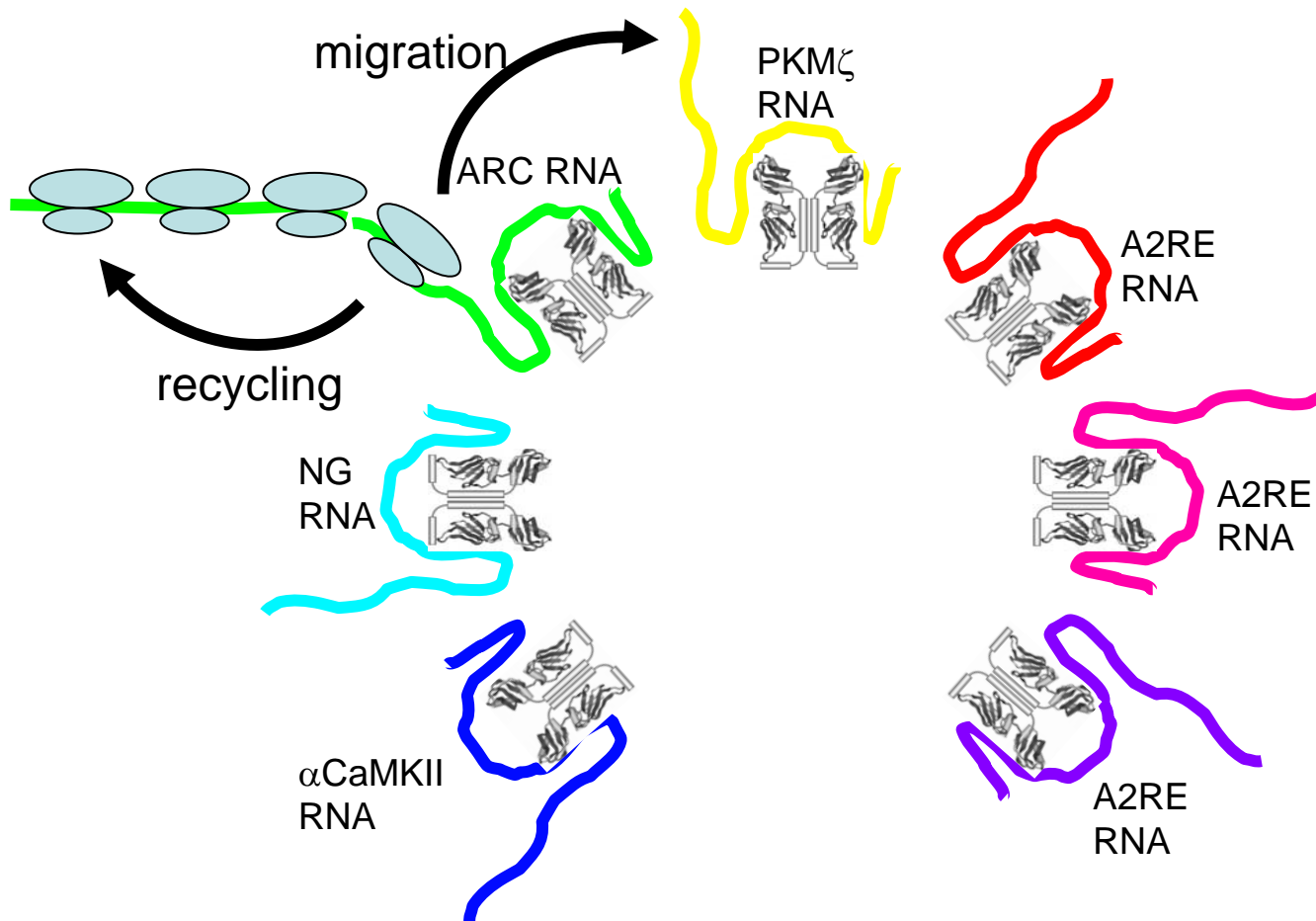


Number of frames (15/sec)



translational output parameters measured by single molecule imaging

- translational events per unit length of dendrite per unit time - overall translational activity
- translational events per granule per unit time - initiation rates on individual RNA molecules
- translational events per burst - recycling of ribosomal subunits on a single RNA molecule
- bursts per granule per unit time - migration of ribosomal subunits from one RNA molecule to a different RNA molecule in the same granule



Acknowledgements

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Ji Yu

TCNP