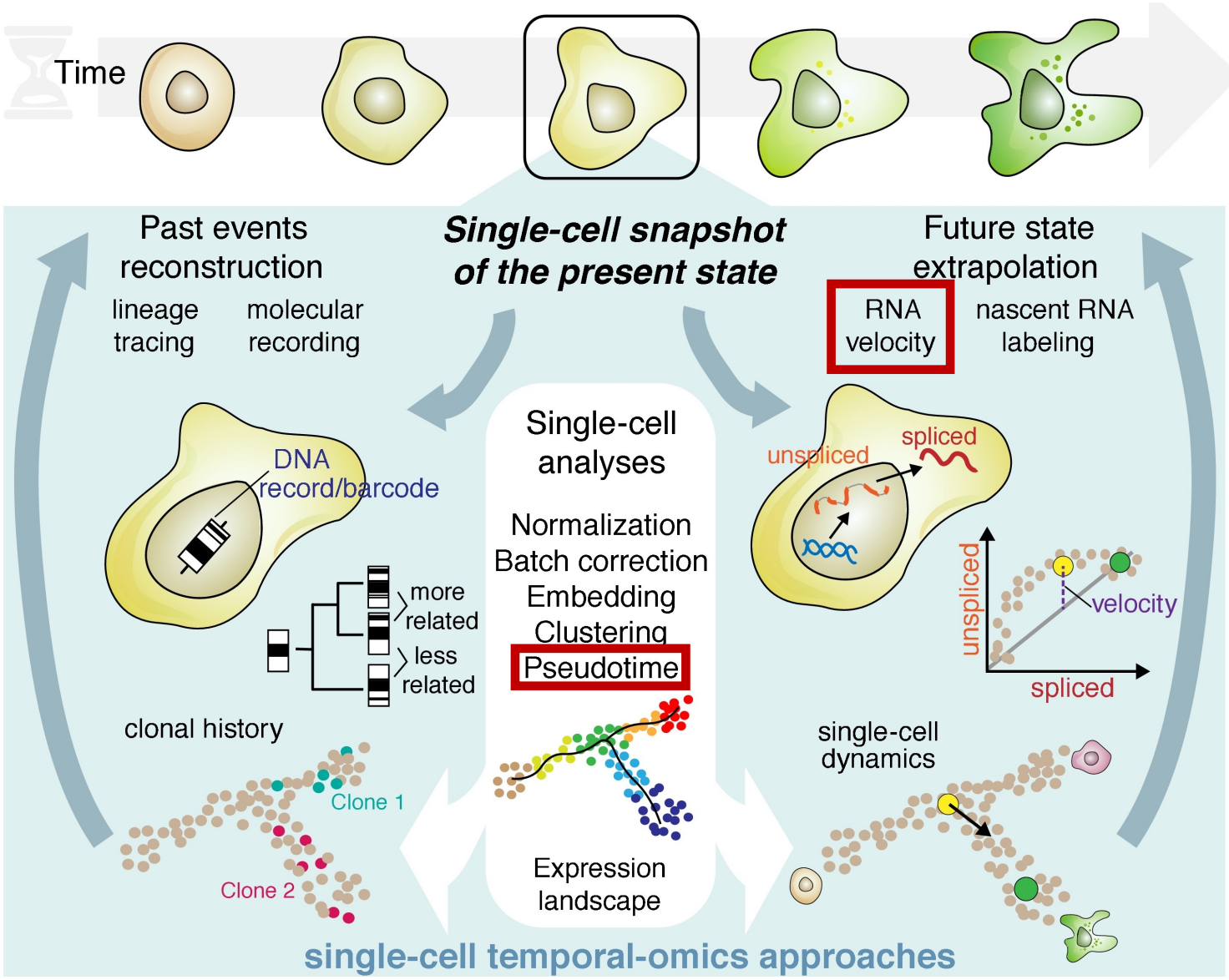
RNA velocity

Single Cell Transcriptomics in Python

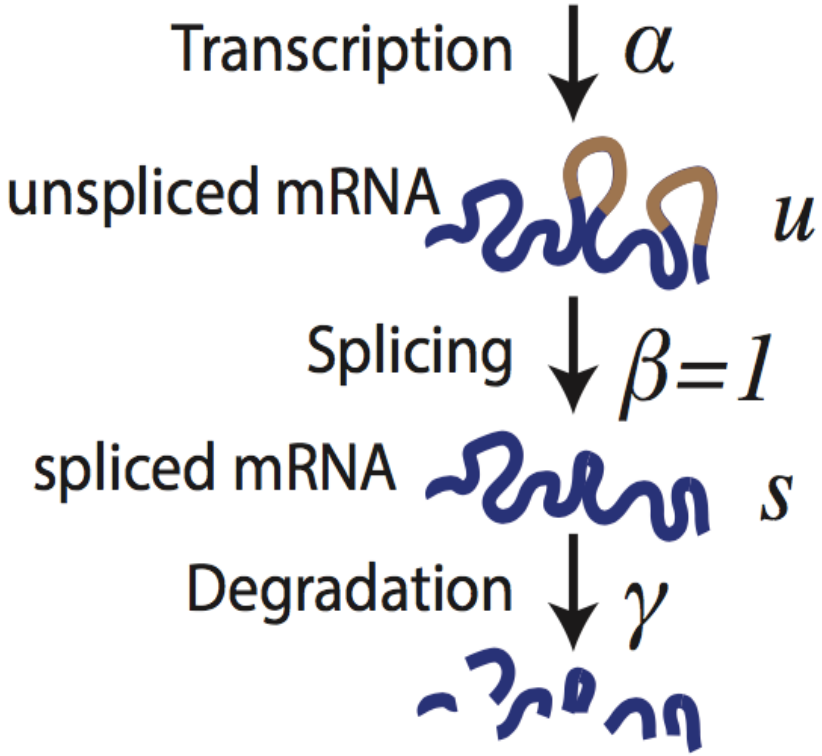
Alex Lederer

Emergence of single-cell temporal-omics approaches



Lederer and La Manno 2020

RNA velocity can be estimated from RNA metabolism



La Manno et al. 2018

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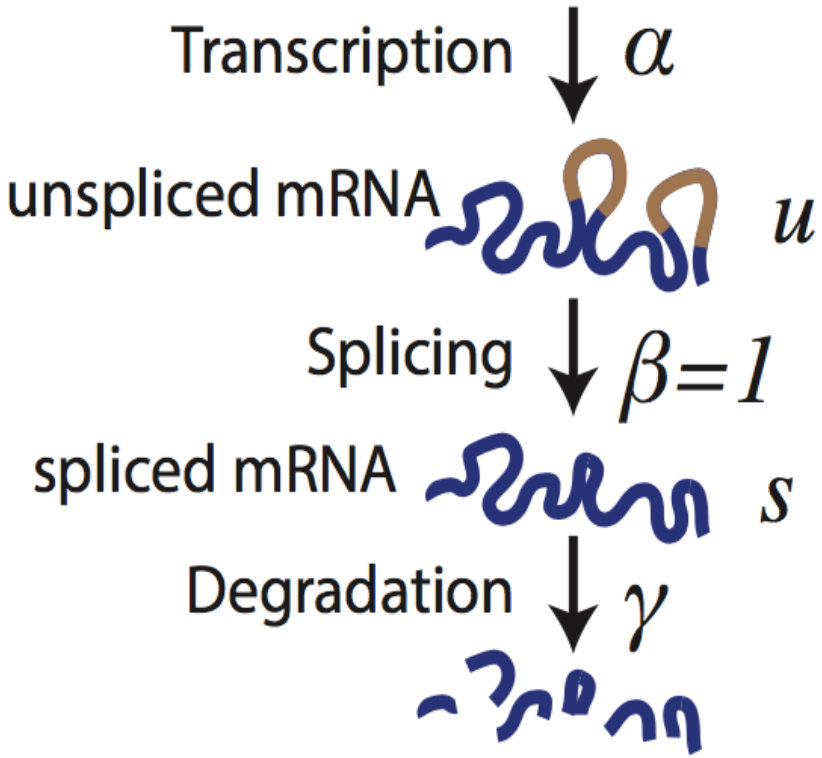

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La Manno et al. 2018

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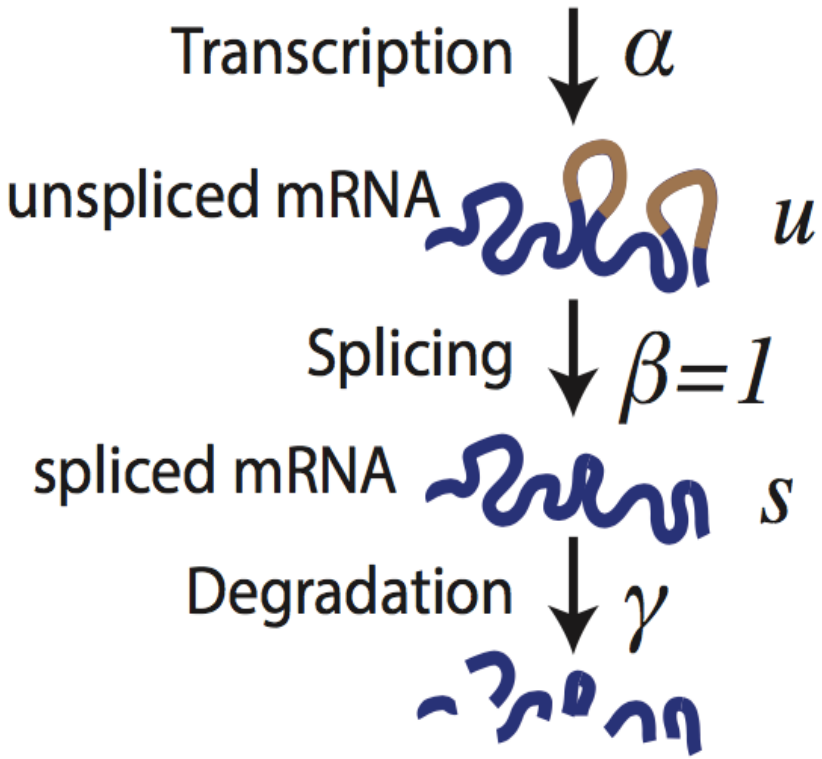

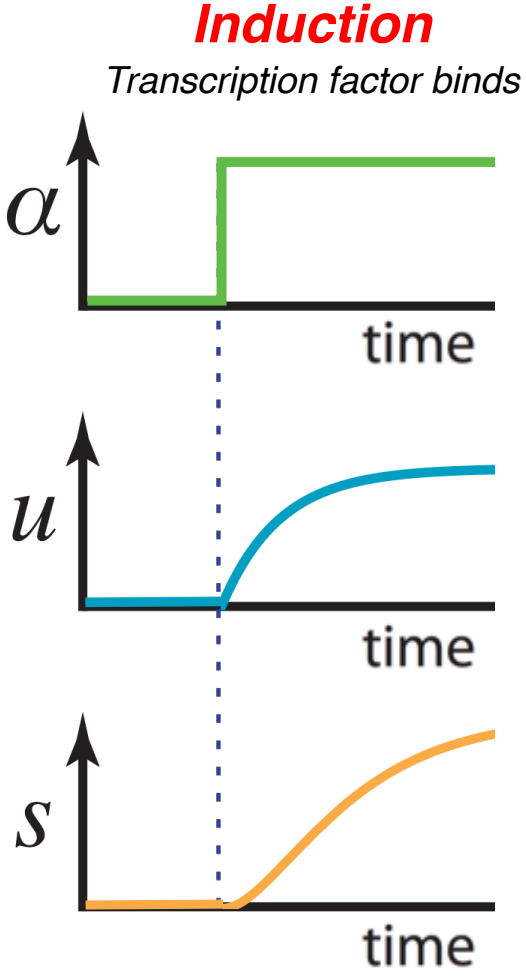

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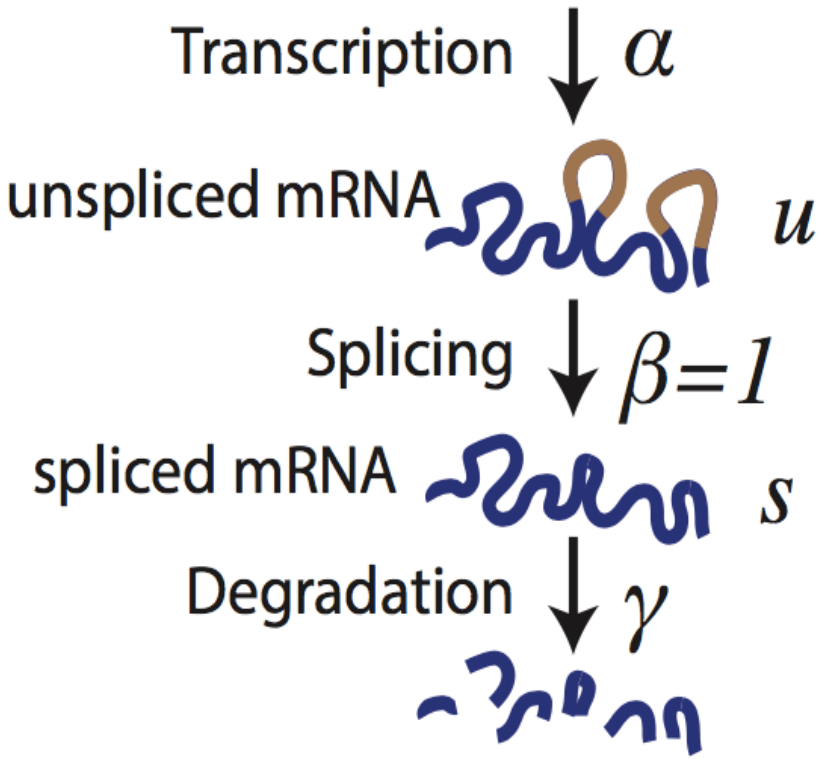

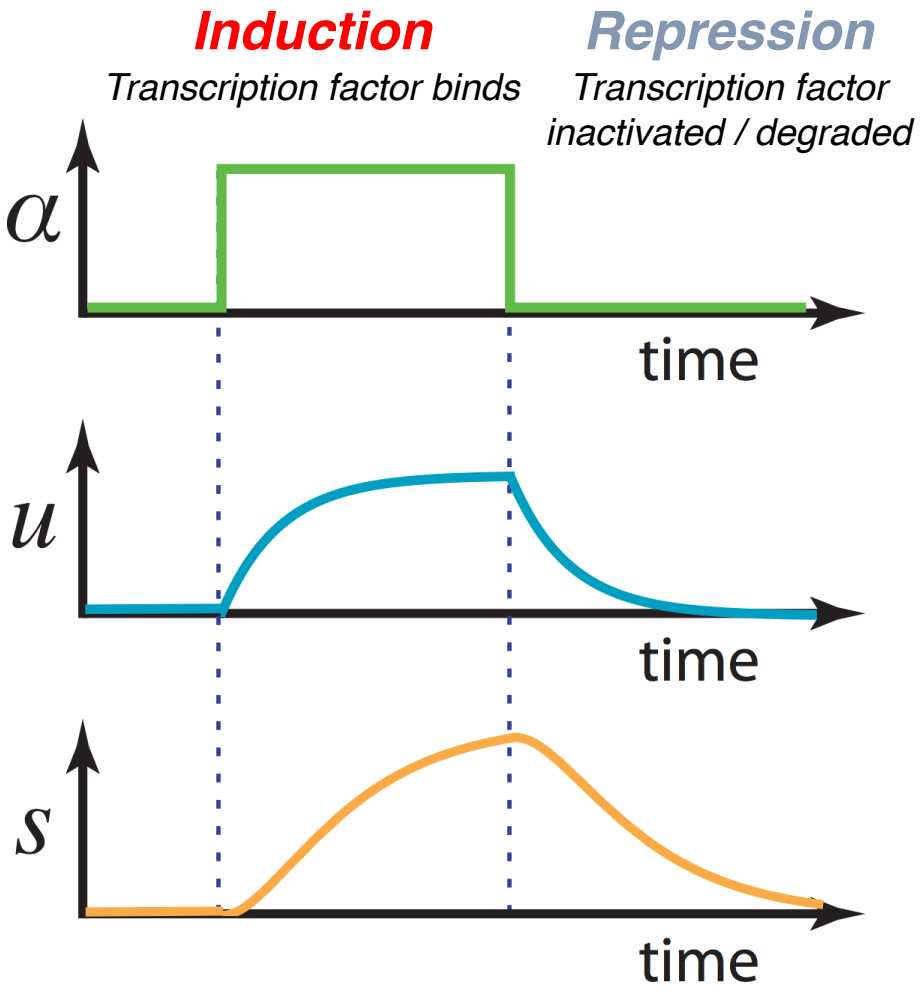

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La Manno et al. 2018

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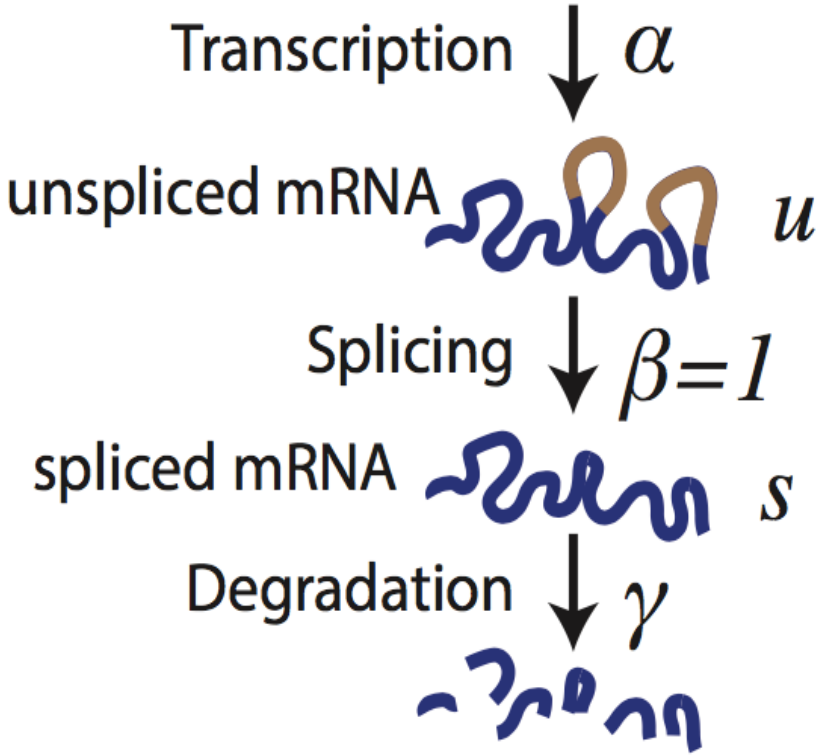

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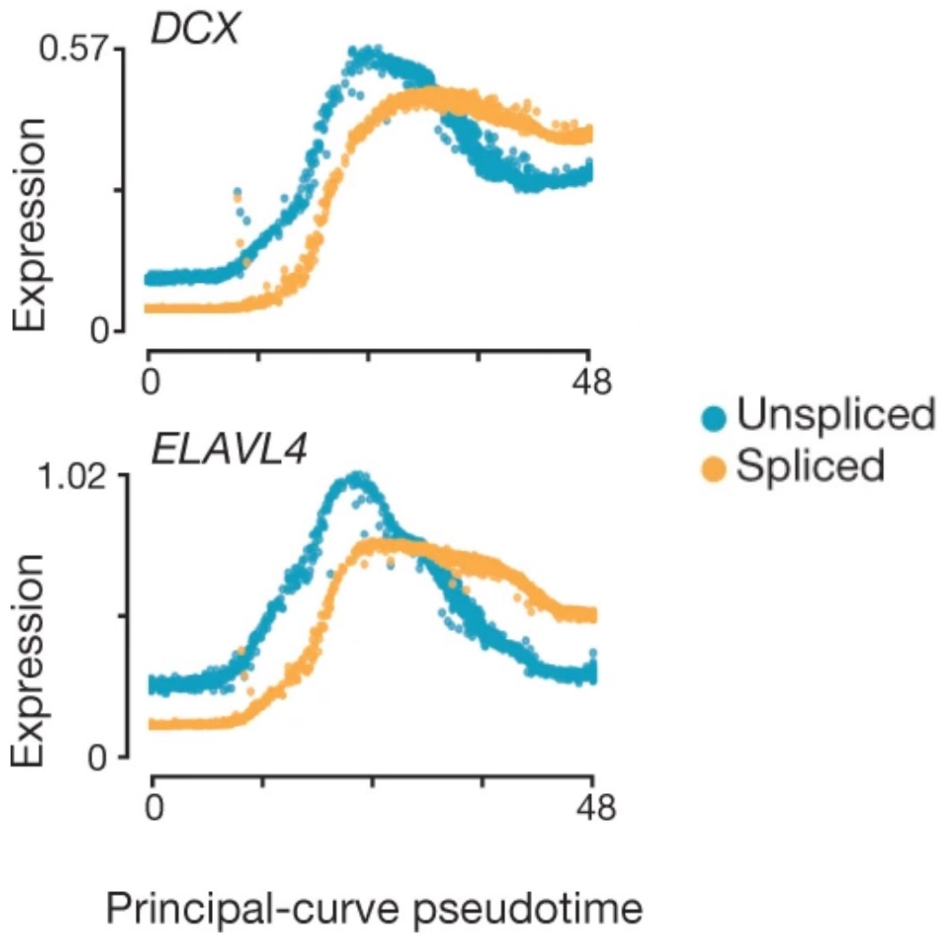
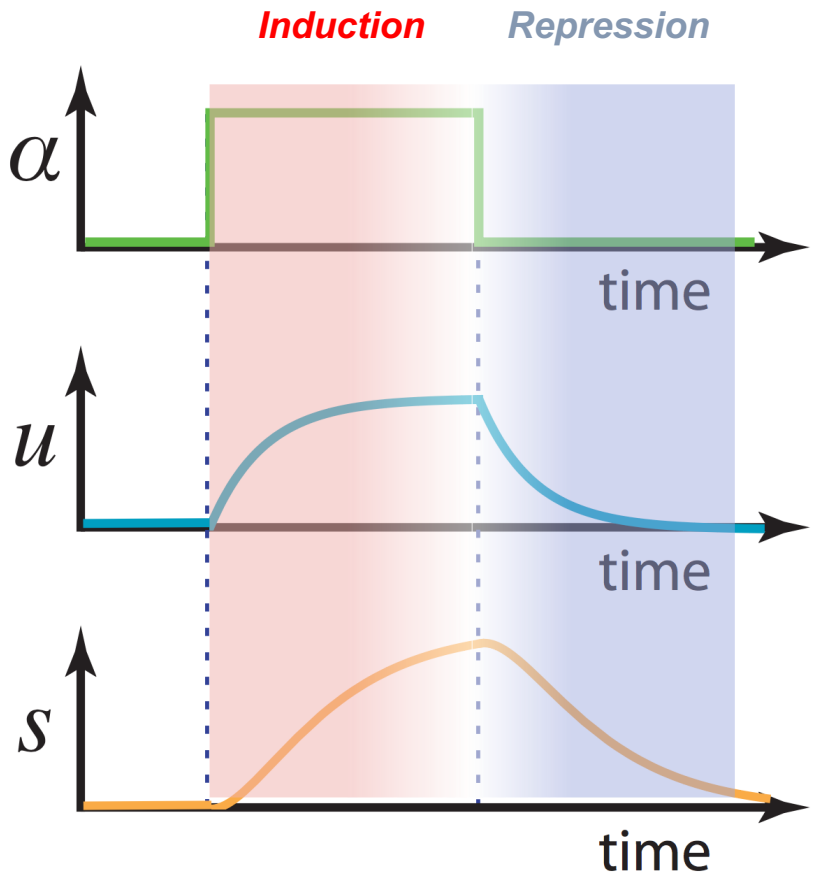
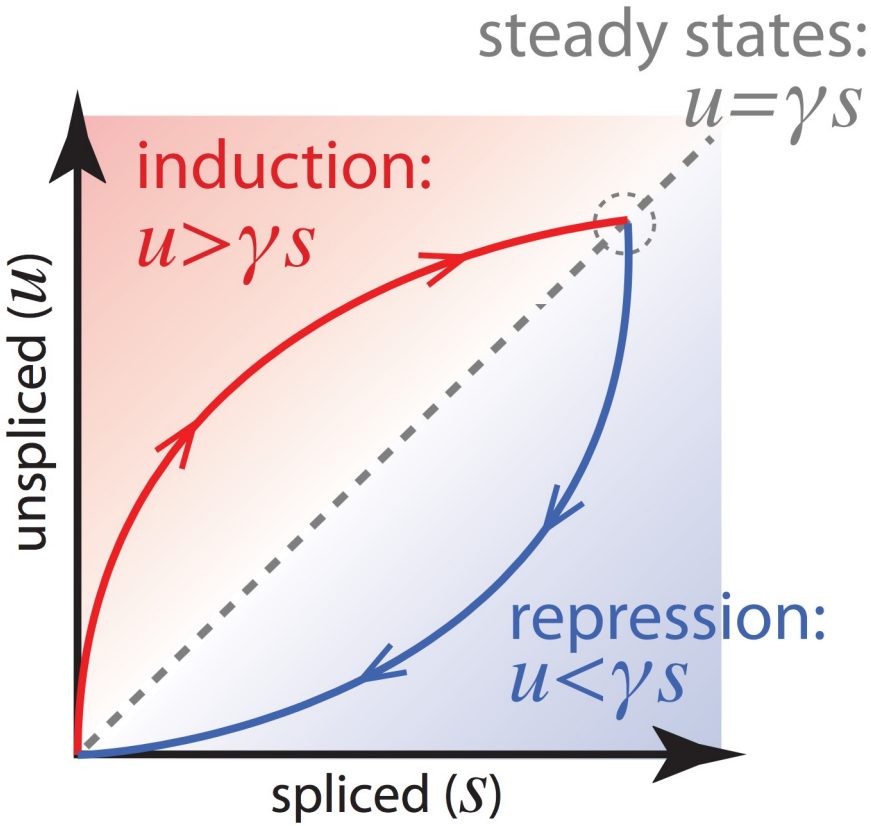


La Manno et al. 2018

Phase portraits show changes to relative unspliced and spliced RNA abundances





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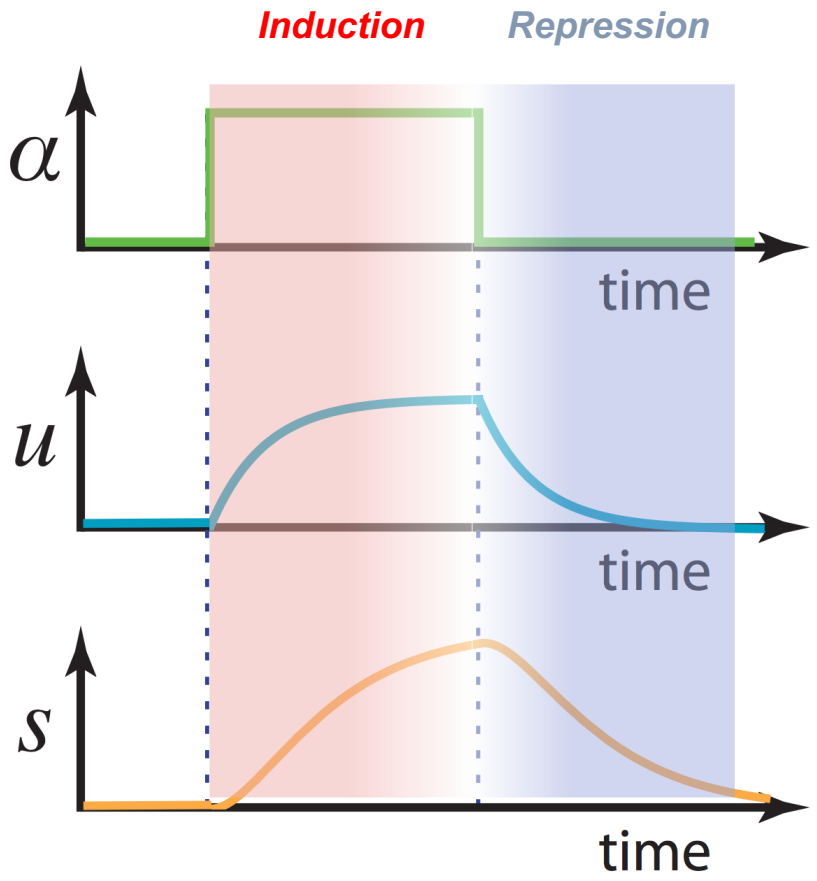
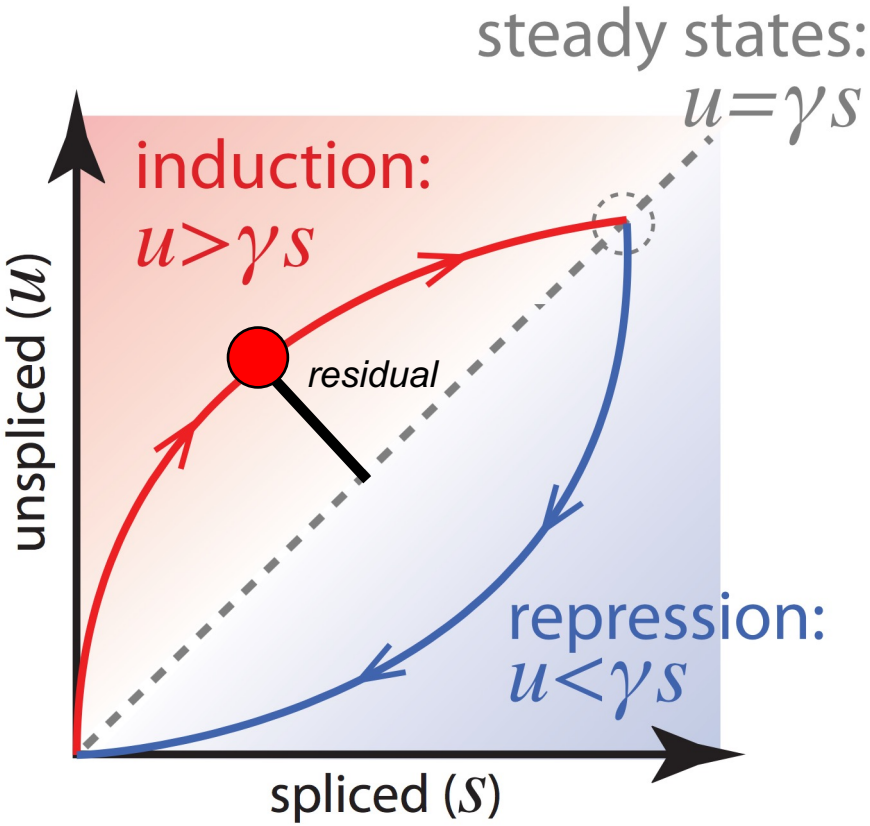
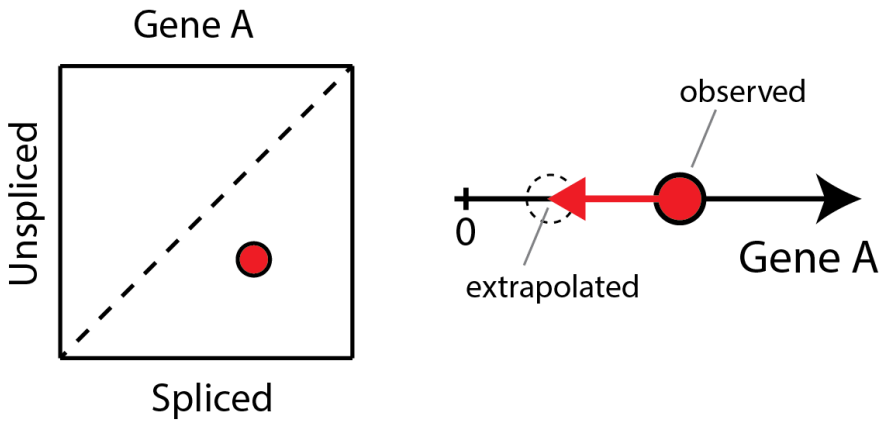




La Manno et al. 2018

RNA velocity is a gene-specific estimate



La Manno et al. 2018

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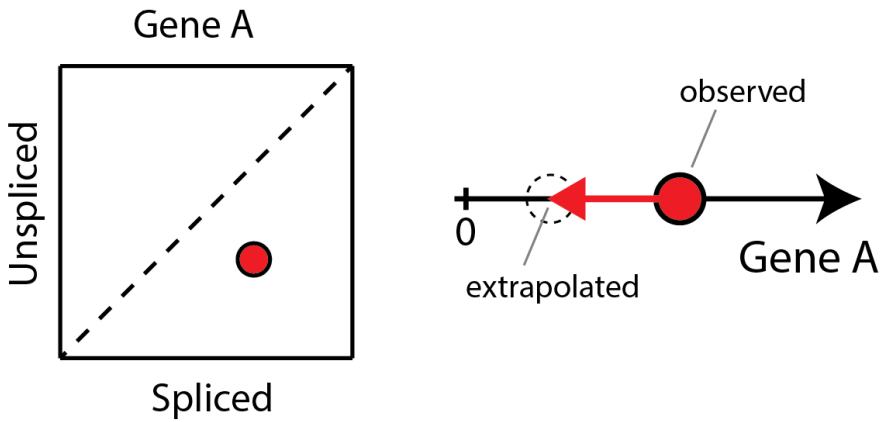

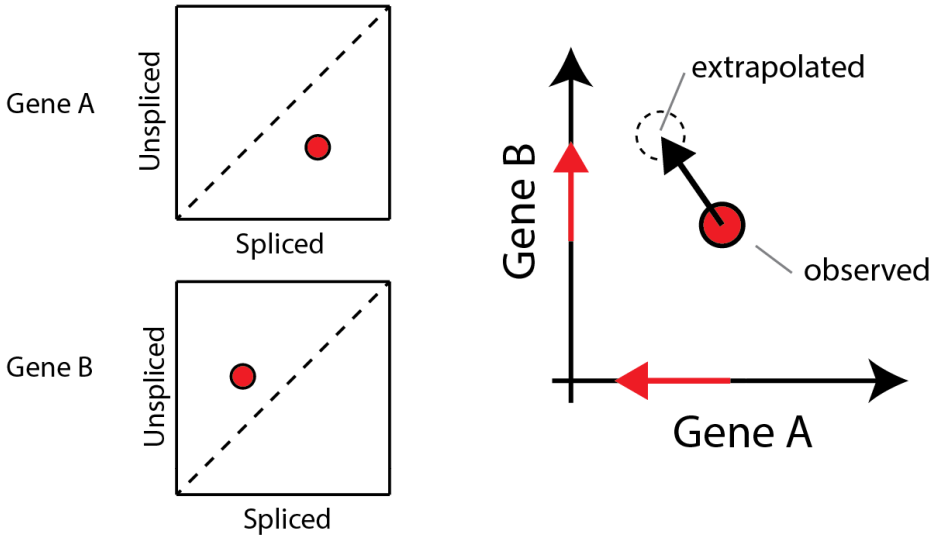

La Manno et al. 2018

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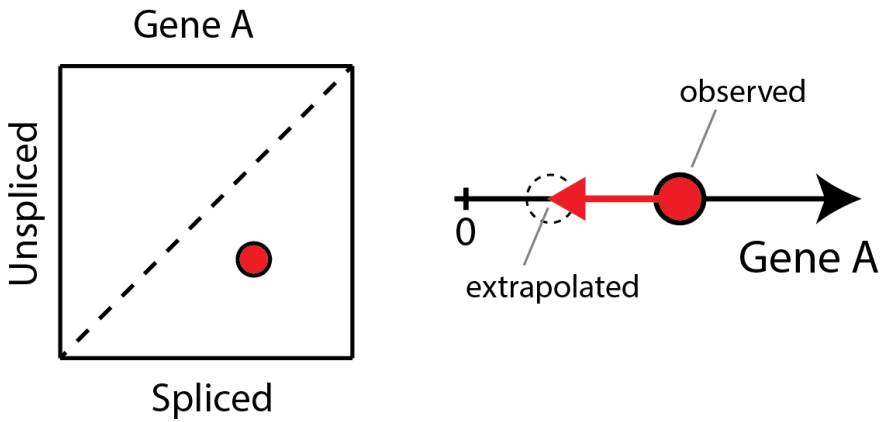
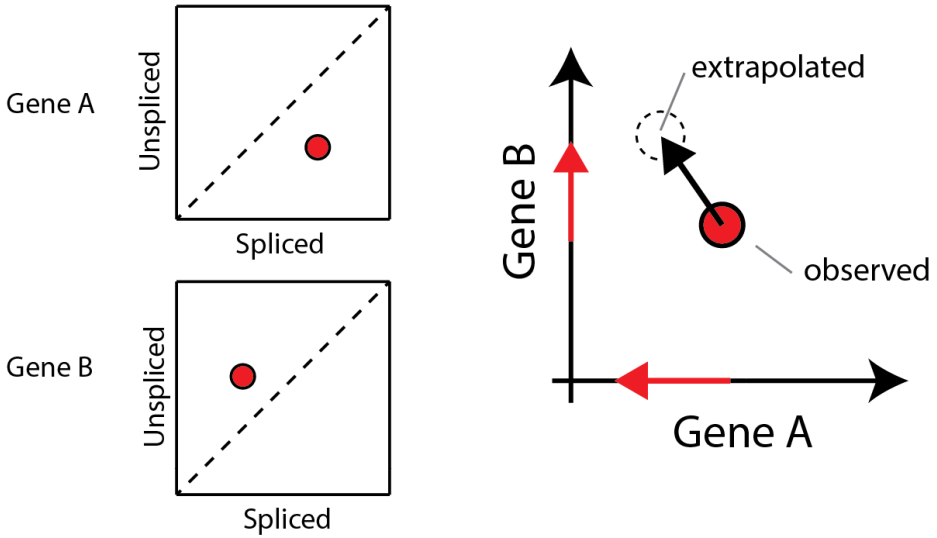


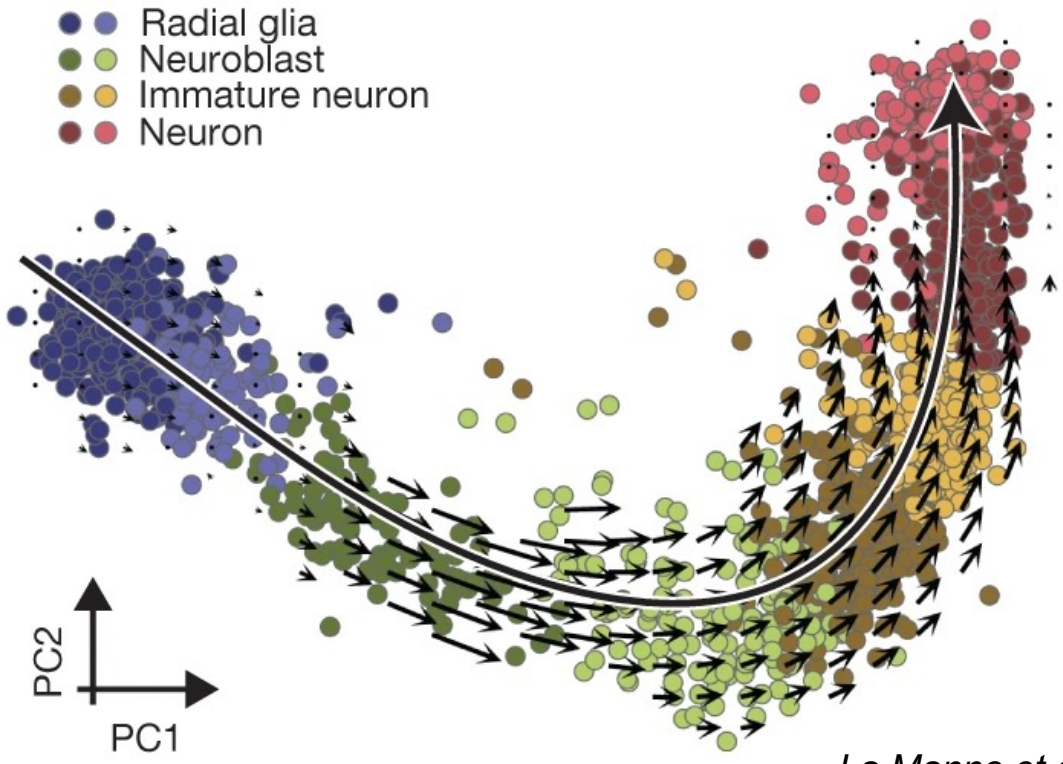


Problem: each gene is on a different time scale!

La Manno et al. 2018

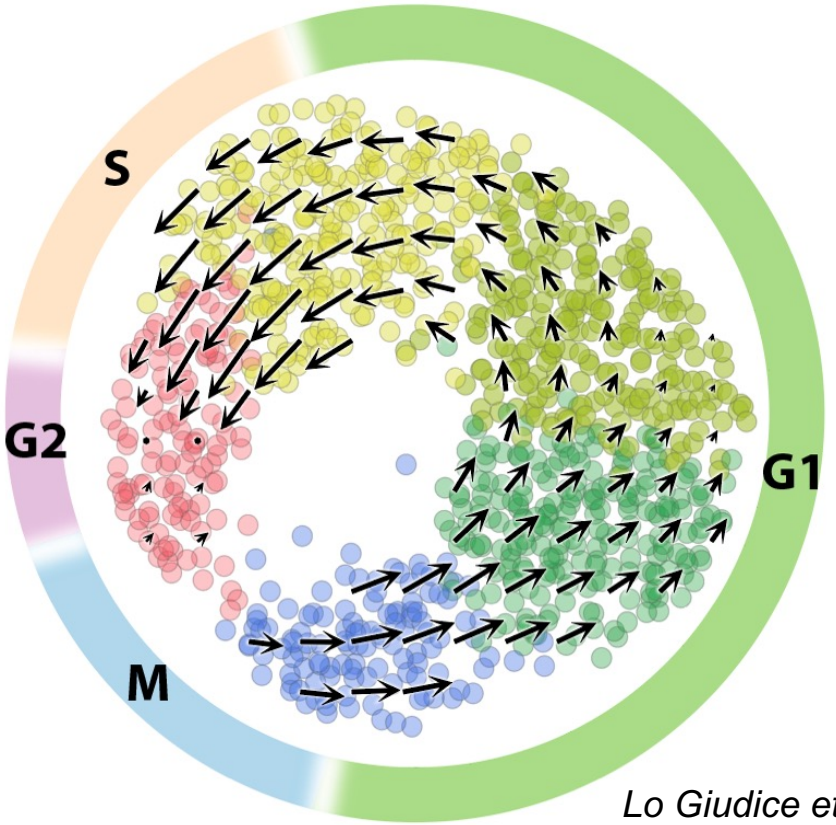
Examples of RNA velocity applied to trajectories

Forebrain development



La Manno et al. 2018

Cycling retinal progenitors



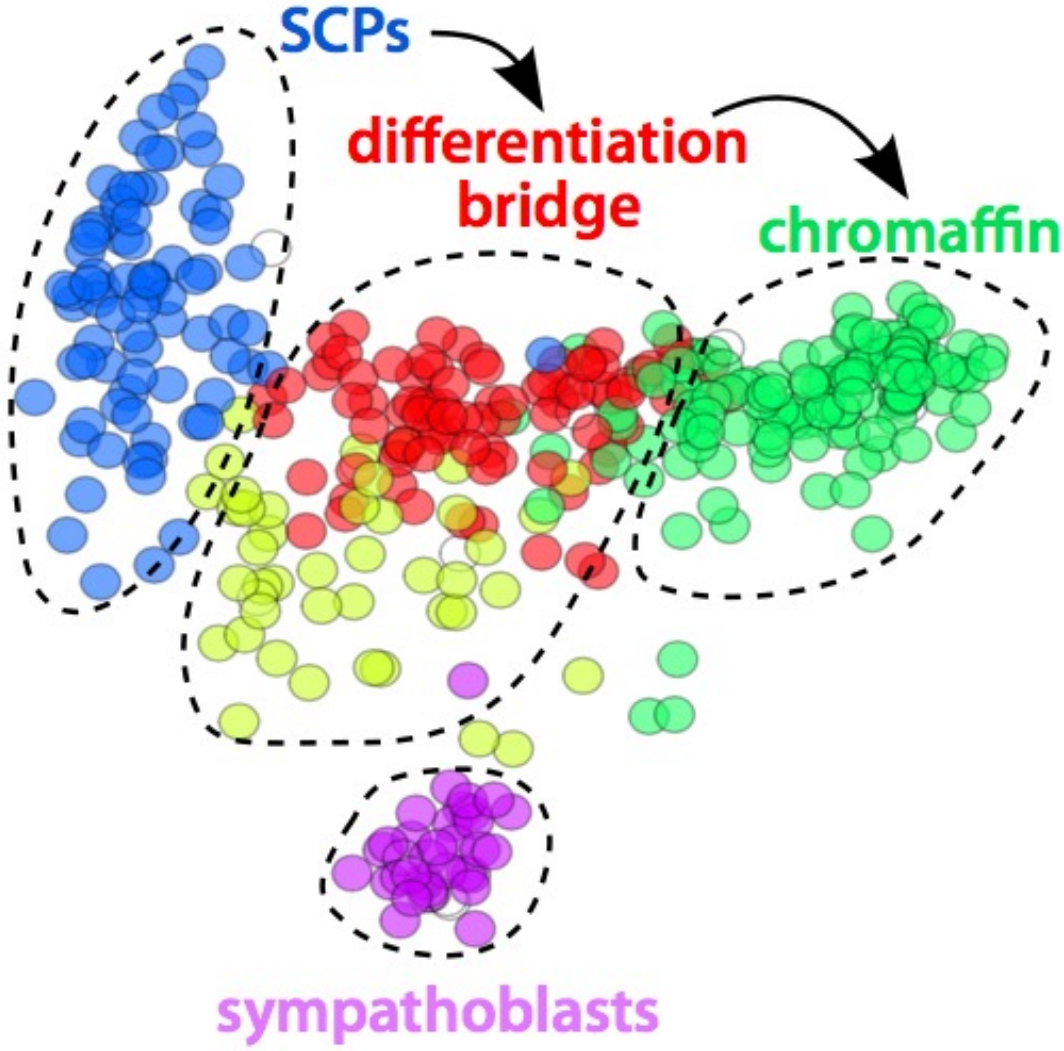
Lo Giudice et al 2019

When should I use RNA velocity?

- I expect there to be a trajectory in my single-cell dataset, but:
  - I do not know the direction along which cells should change
  - I expect the trajectories to change for a similar population of cells but in different dataset conditions (in response to gene perturbations, environmental stimuli, etc)

Note: the changes between cell states should unfold on a relatively short time scale (hours to days) and not a long time scale (weeks to months).

RNA velocity to describe a simple differentiation process



La Manno et al. 2018

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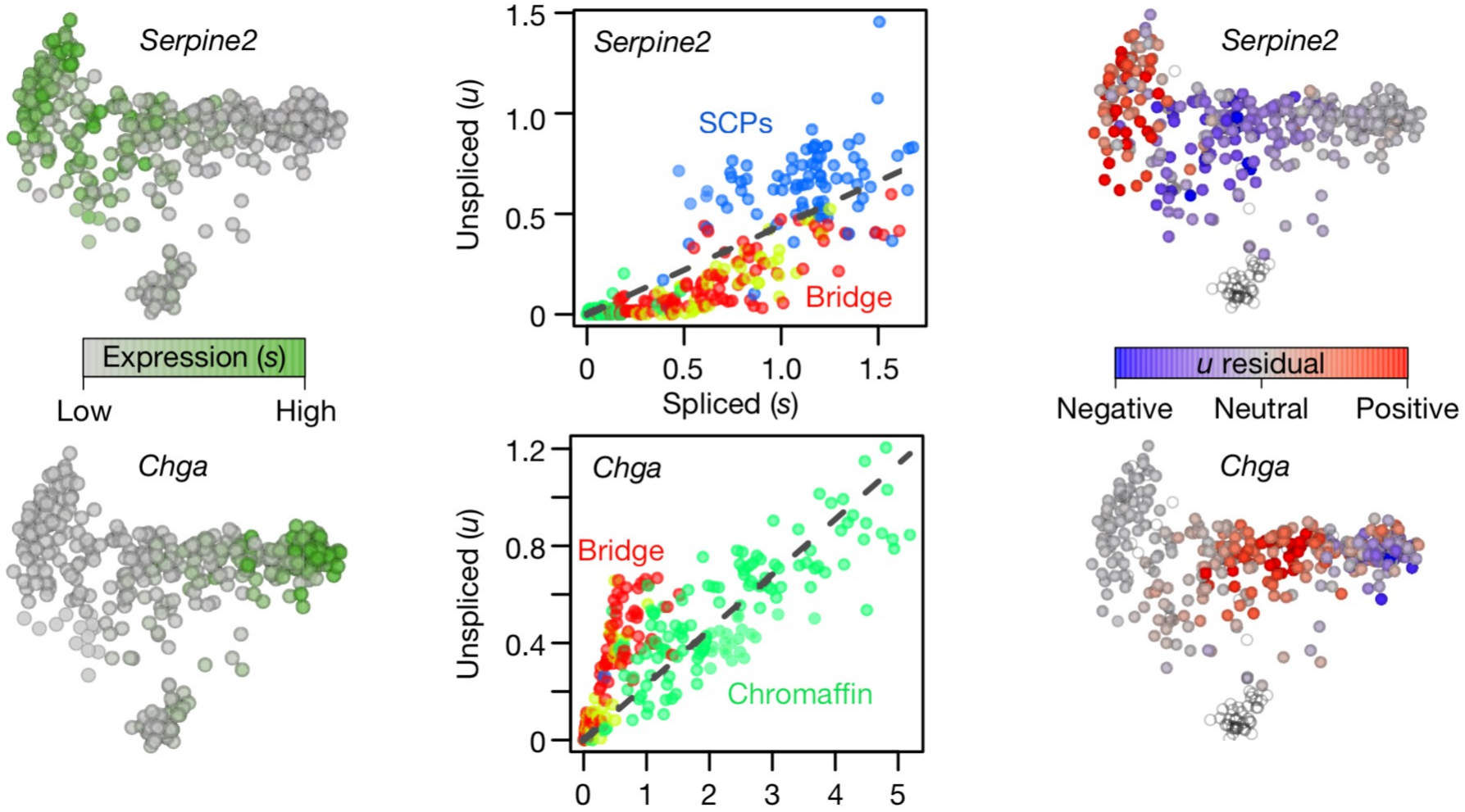

La Manno et al. 2018

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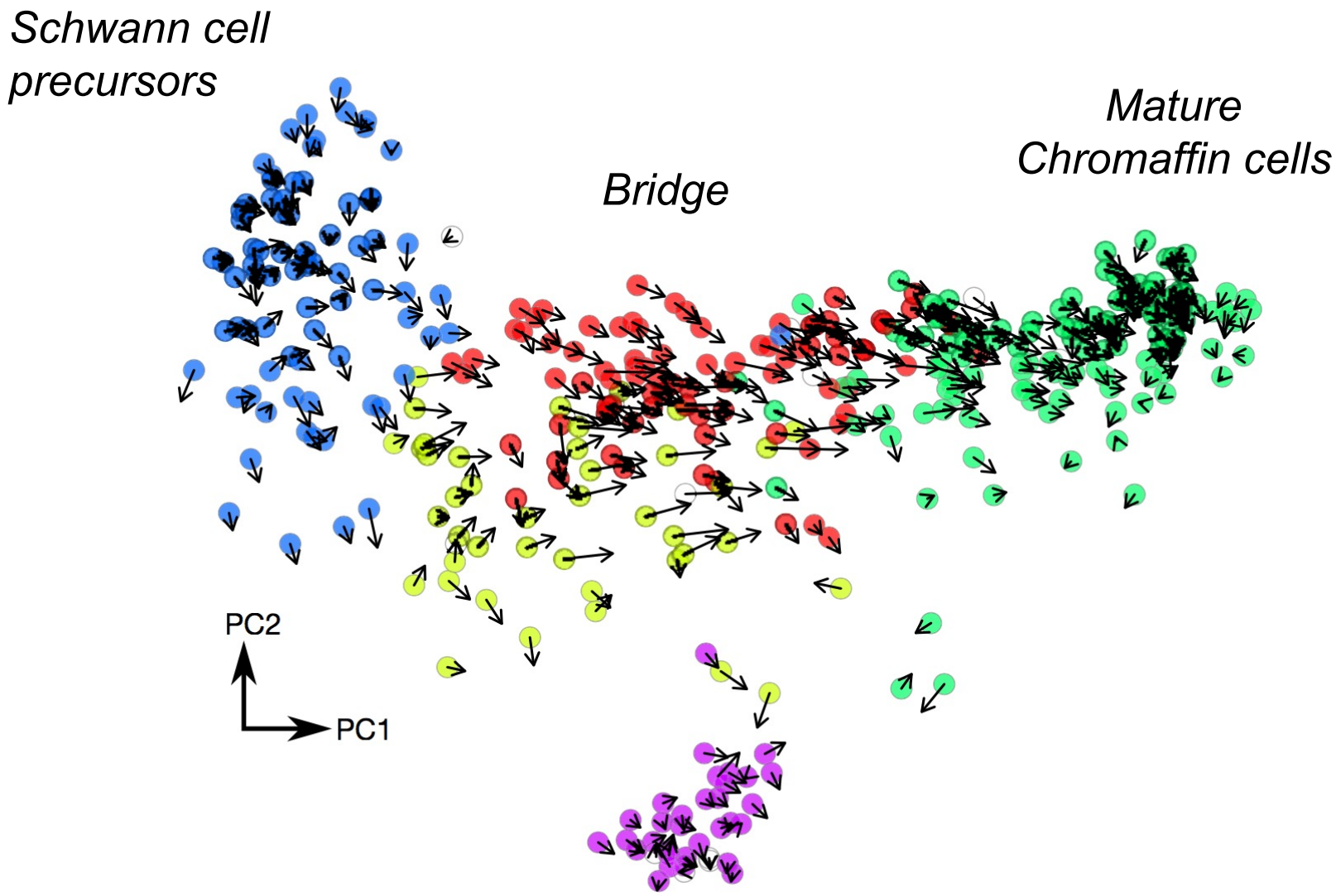
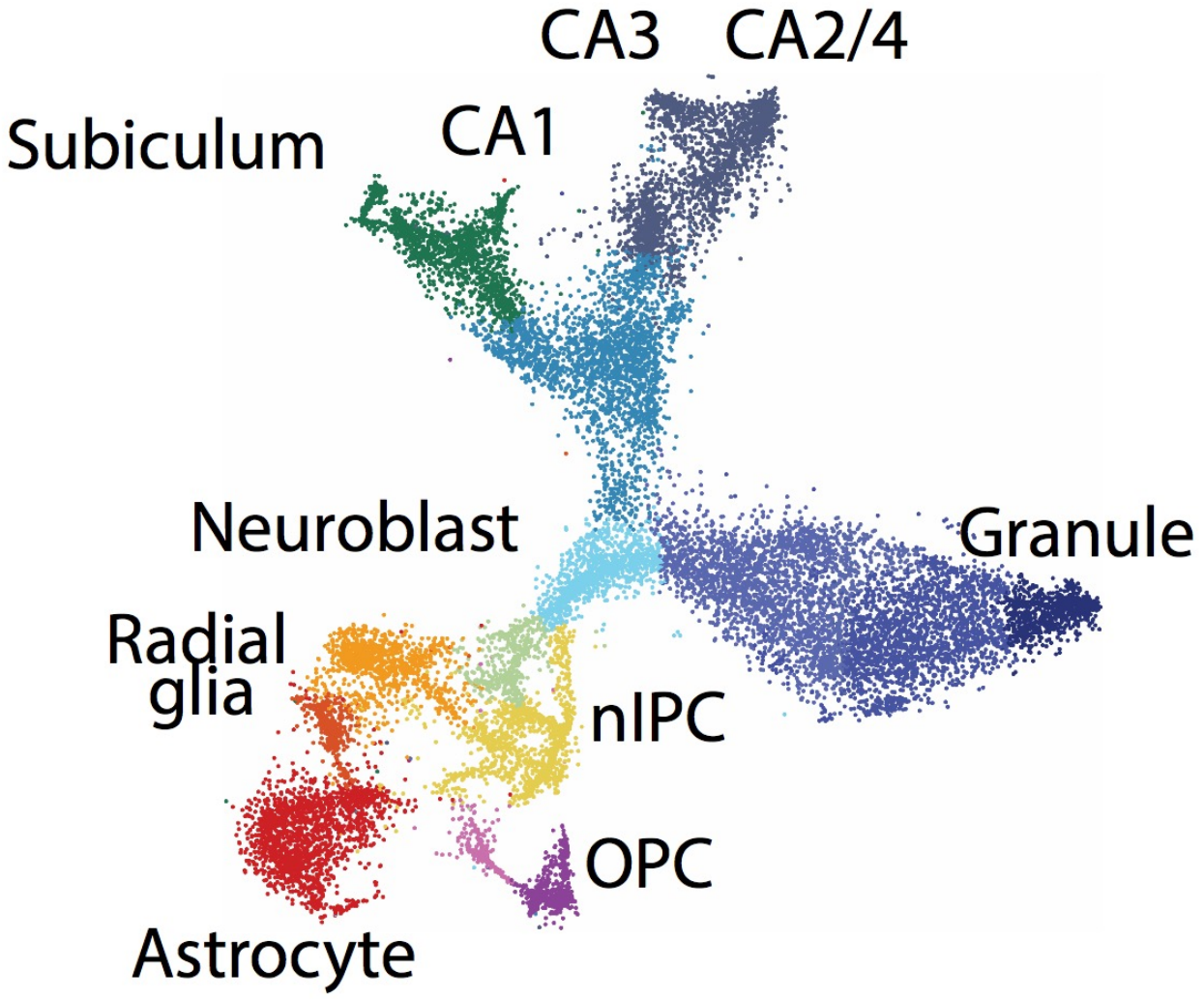


La Manno et al. 2018

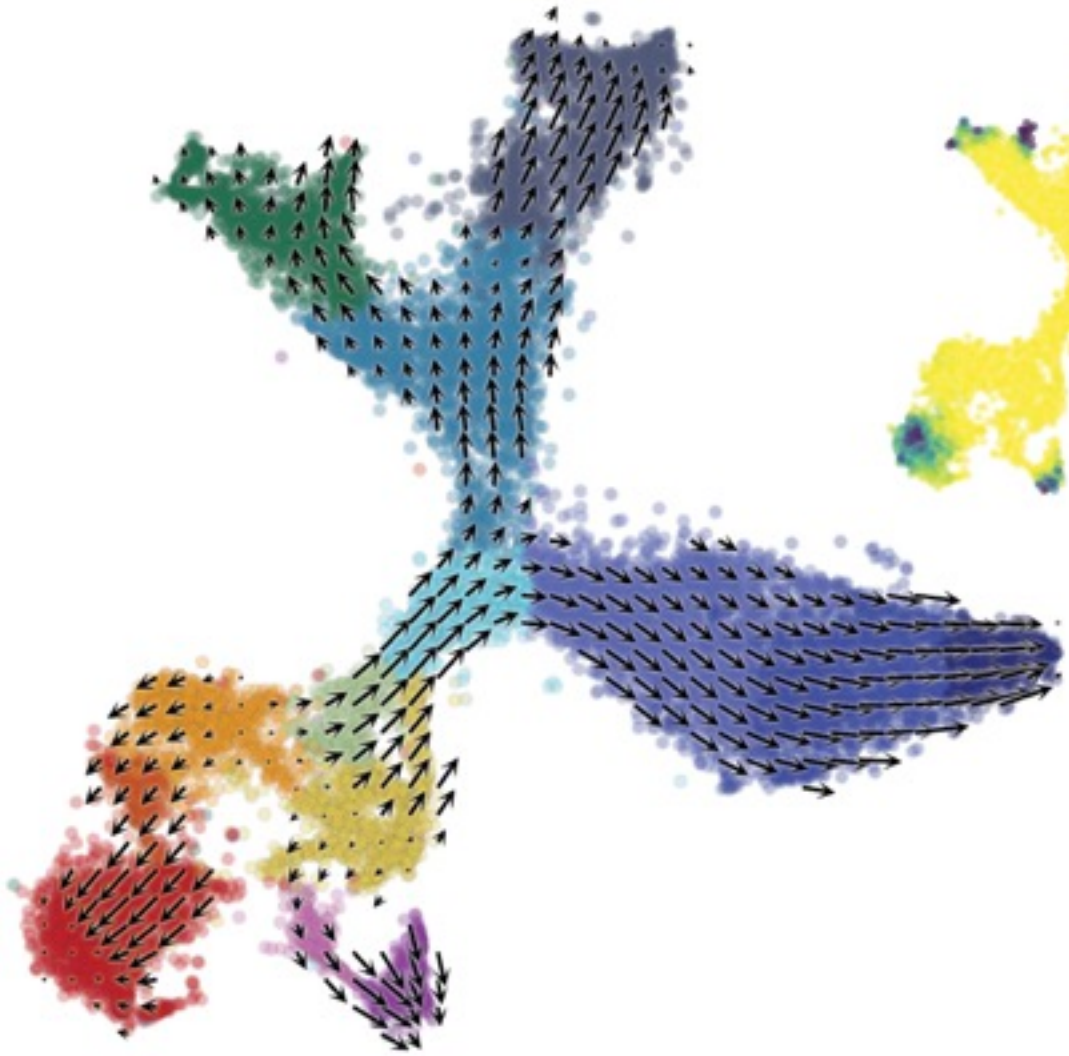
A more complex application of RNA velocity

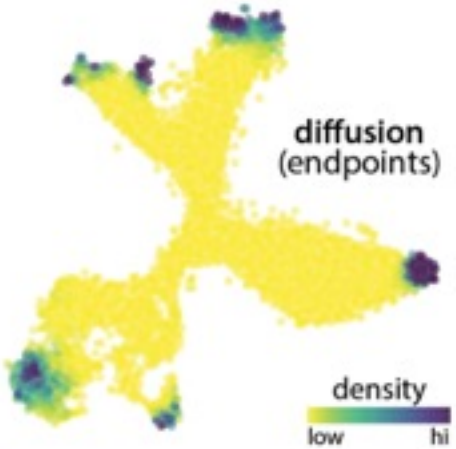


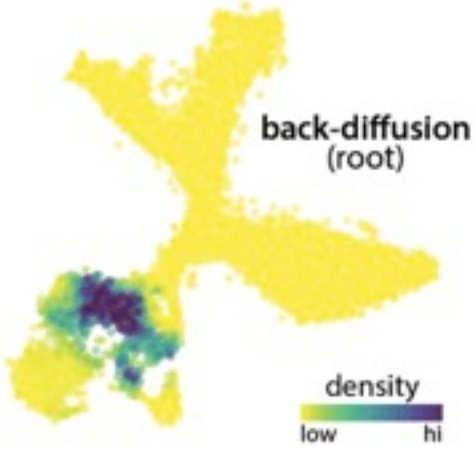
Pseudotime analysis could be misleading!

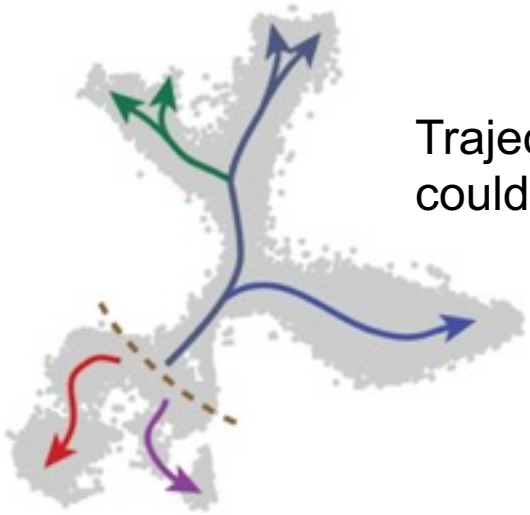
La Manno et al. 2018

A more complex application of RNA velocity









Trajectory inference alone could be misleading!

La Manno et al. 2018

Other recent velocities!

Generalizing RNA velocity to transient cell states through dynamical modeling

Chromatin Potential Identified by Shared Single-Cell Profiling of RNA and Chromatin

UniTVelo: temporally unified RNA velocity reinforces single-cell trajectory inference

Protein velocity and acceleration from single-cell multiomics experiments

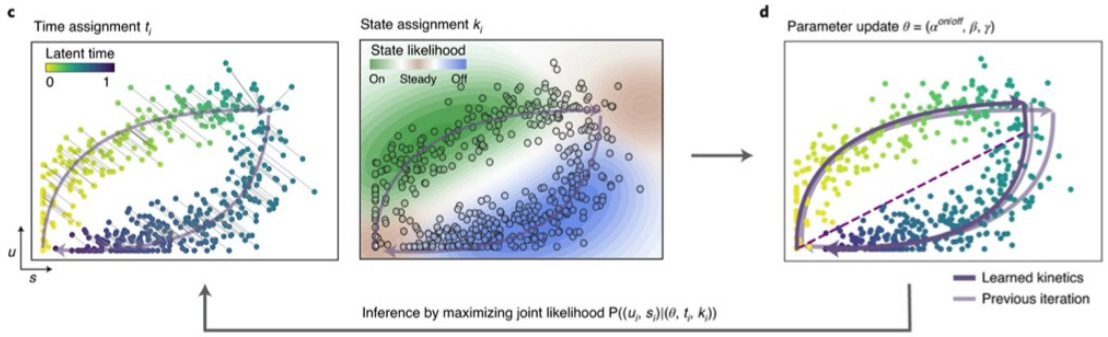
Representation learning of RNA velocity reveals robust cell transitions

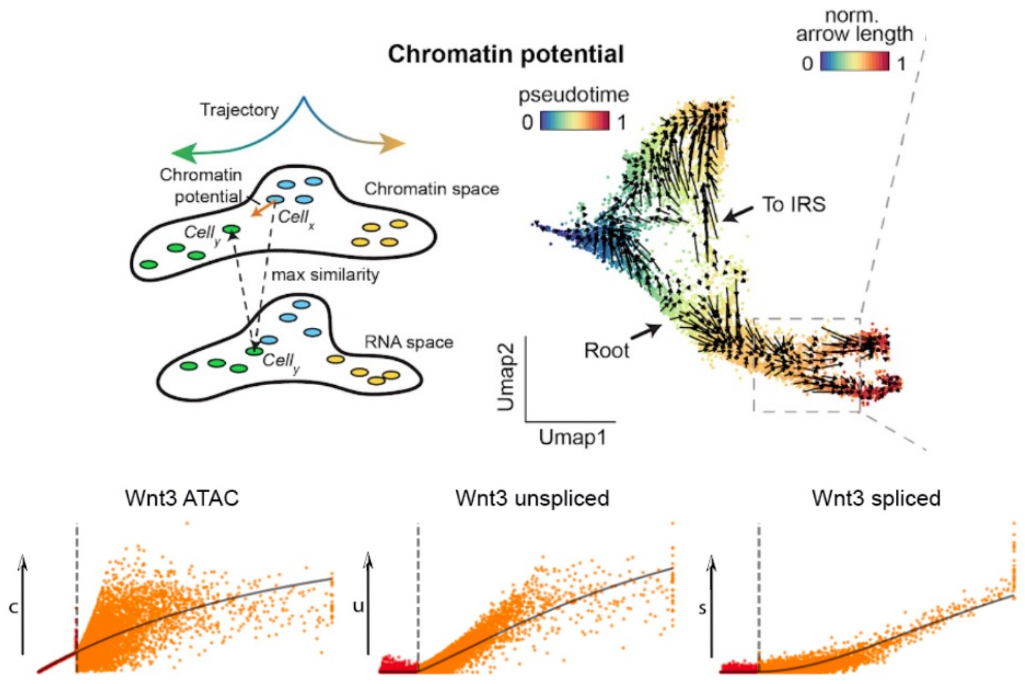
A relay velocity model infers cell-dependent RNA velocity

***DeepVelo*: Single-cell transcriptomic deep velocity field learning with neural ordinary differential equations**

Single-cell multi-omic velocity infers dynamic and decoupled gene regulation

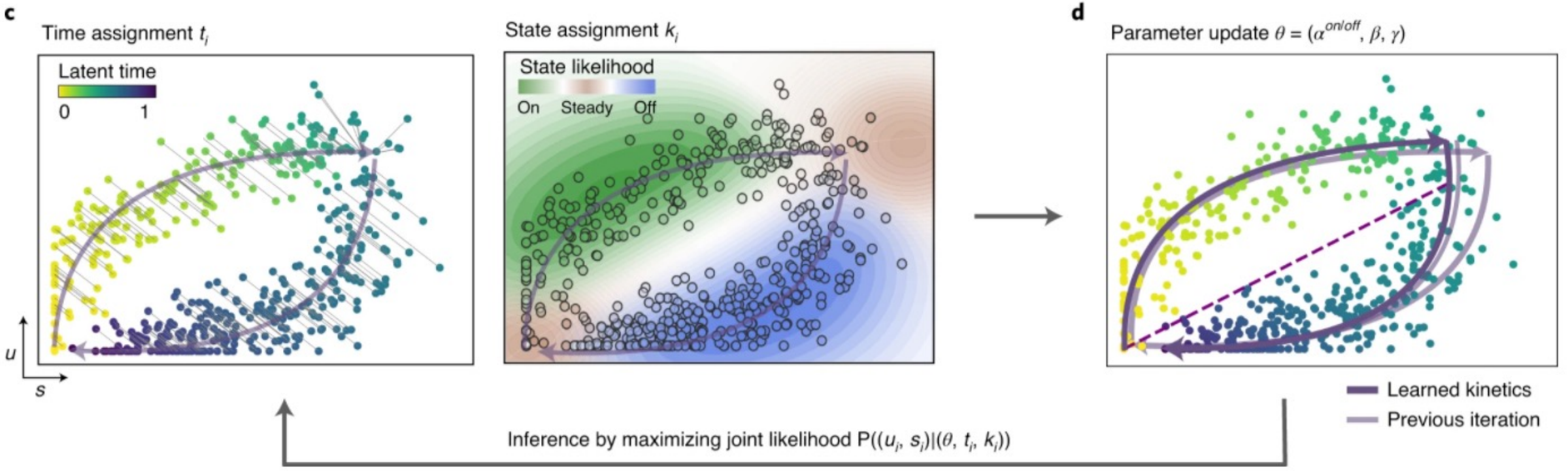
Multi-omic single-cell velocity models epigenome-transcriptome interactions and improves cell fate prediction





+ about 40-50 additional velocity methods! ***(it's not yet a category on scrna-tools.org)***

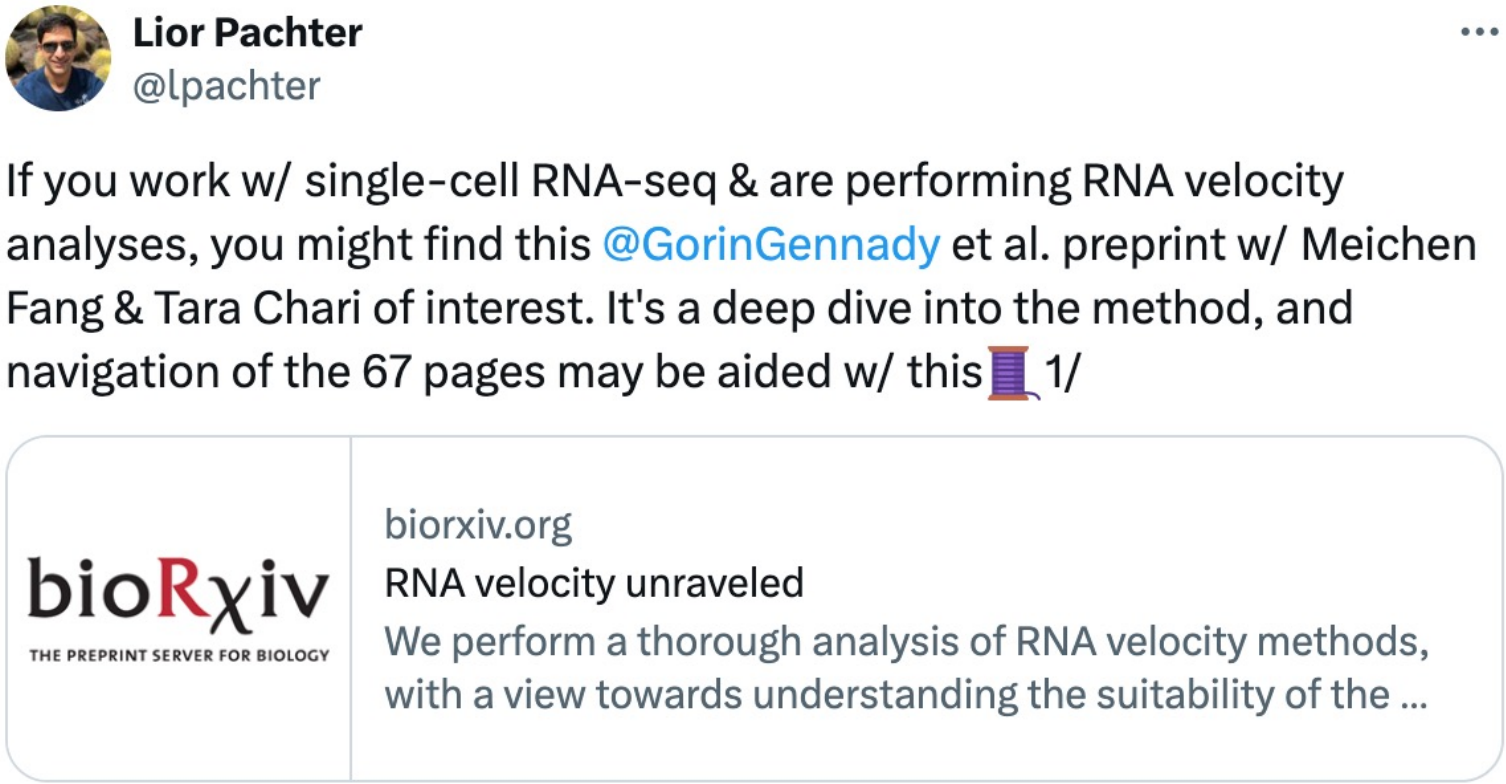
Using expectation-maximization to estimate velocity and RNA kinetics with scvelo



Bergen et al. Nat Biotech. 2020

Should you perform RNA velocity analysis on your data?

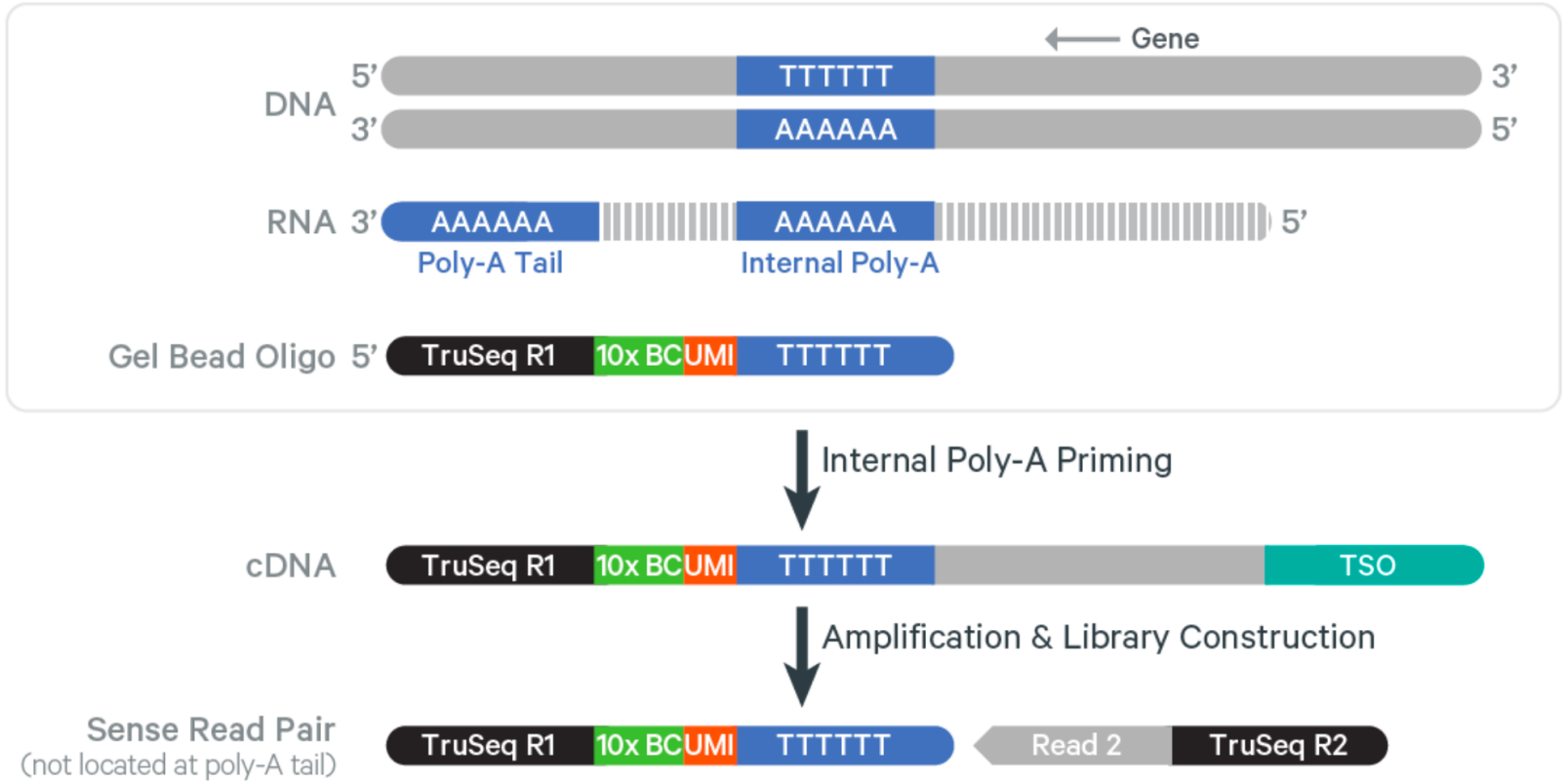
RNA velocity unraveled:
https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1010492



Should you perform RNA velocity analysis on your data?

- Do I expect a temporal axis of variation in your data?
- Do I expect the time scale of temporal change to unfold in hours/days or weeks?
- Which single-cell technology do you use (single cell vs single nuclei) and how much detection of intronic reads do you expect?

Internal priming and measurement of additional intronic regions

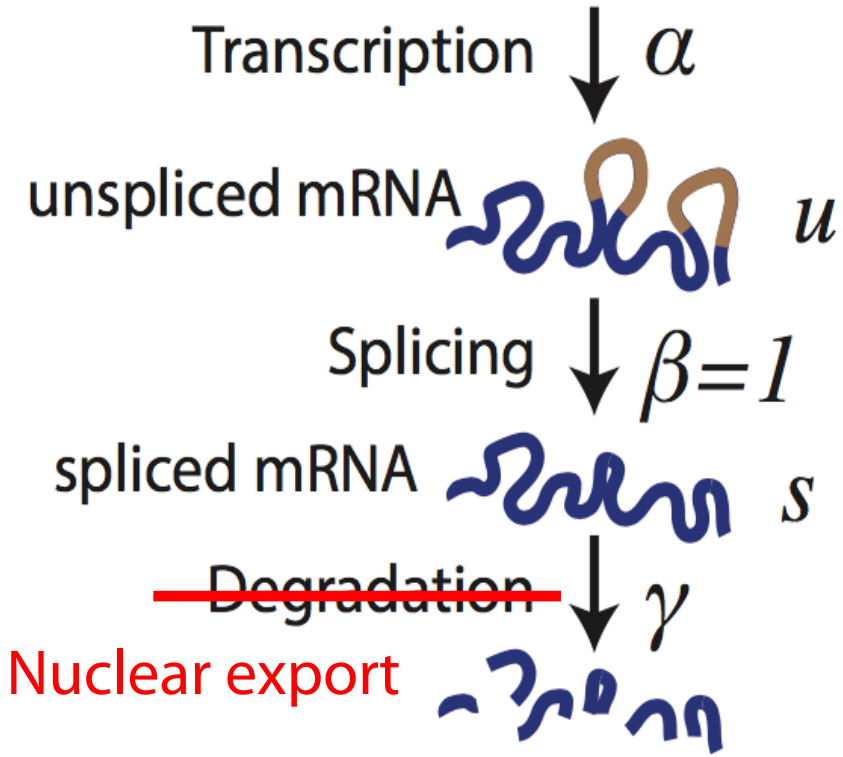


10X Genomics Technical Note, CG000376 (2021)

- The poly(dT) primer can prime to an internal poly-A stretch
- In humans, there are 21x more poly-A stretches found in introns than exons

Conclusion: there may be intron detection biases between different genes in the data!

The RNA life cycle captured by single nucleus RNA-seq is different



In single-nuclei data, it is not possible to measure the degradation rate for a gene.

The nuclear export rate may be instead described by changes to spliced abundance.

Nuclear export occurs much more quickly than the degradation – be careful!

Two steps for RNA velocity analysis

Step 1. Intron/exon counting

- Command line tool that takes the output bam files from CellRanger and generates separate count matrices for spliced and unspliced mRNA
- Original tool (*velocyto*): https://velocyto.org/velocyto.py/tutorial/cli.html
- Also possible with STARsolo and alevin-fry methods for read alignment

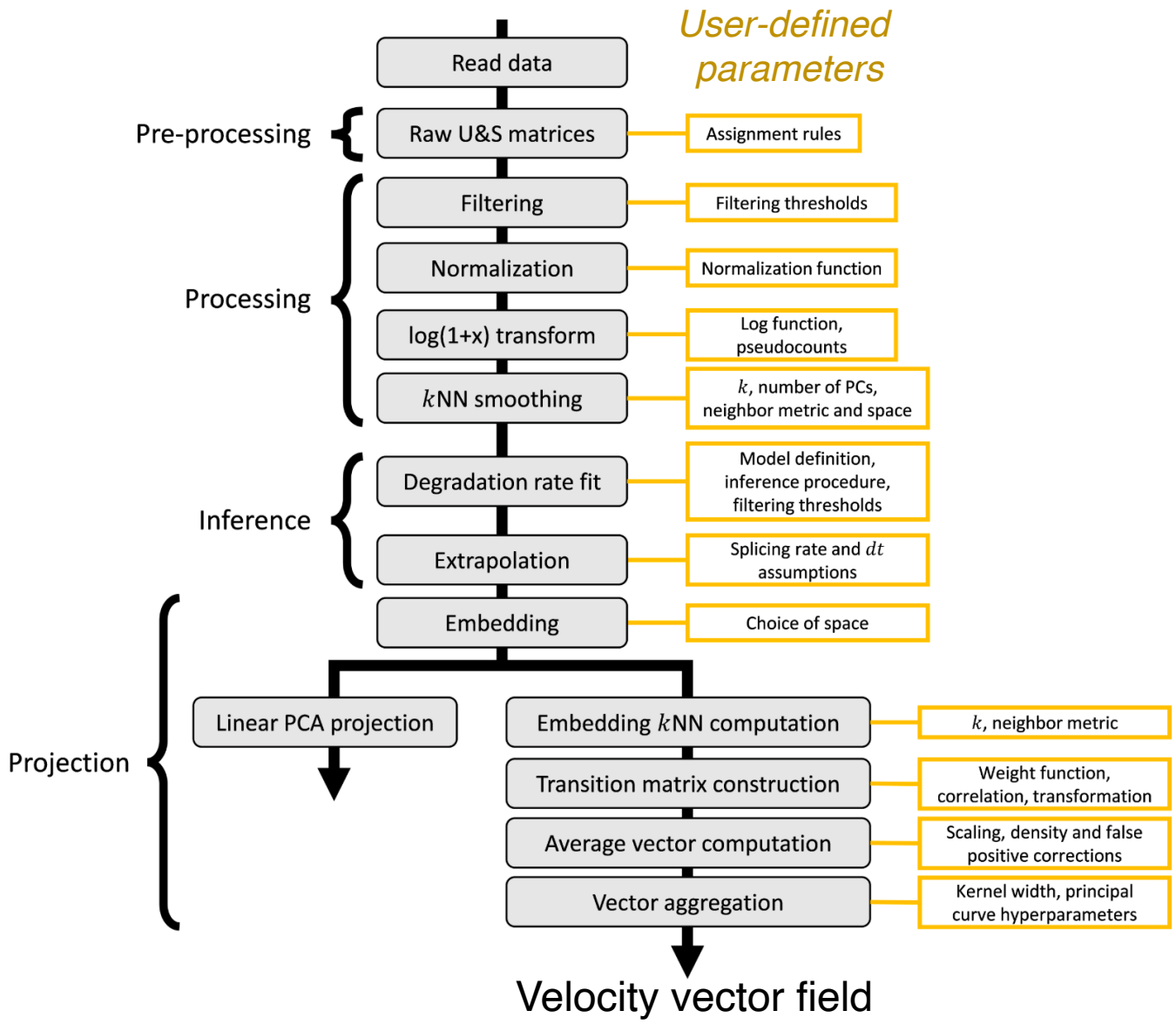
Step 2. Velocity estimation and visualization

Most widely-used tools are:

- *velocyto* (https://velocyto.org/velocyto.py/tutorial/analysis.html)
- *scvelo* (https://scvelo.readthedocs.io/)

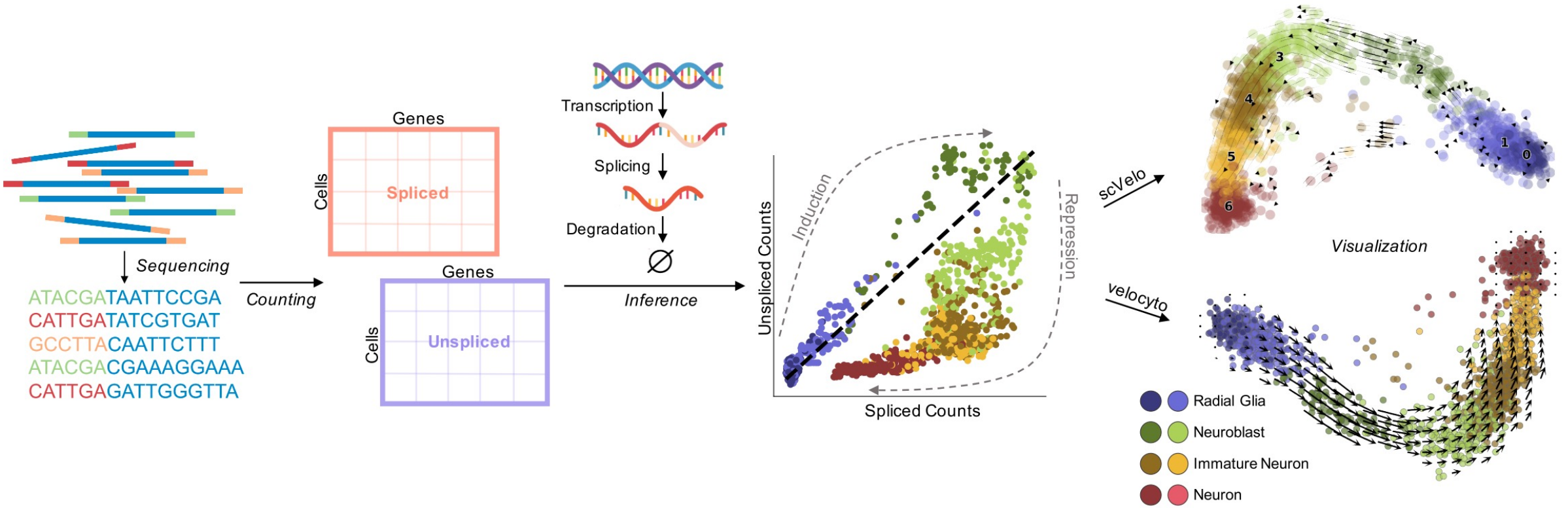
Most RNA velocity tools are implemented and better-supported in Python.

The RNA velocity workflow is complex!



"RNA velocity unraveled"
Gorin et al PLoS Comp Biol 2022

A recap of RNA velocity analysis



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