Experiment 18: CDS1 repeat #2 (2 fr)

Purpose: To perform another repeat of CDS1 refolding with a higher density. I think it worked better this time?

Major results and comments:

- Better density of molecules this time, managed to see a split peak distribution for last timepoint.
- Need to perform final third repeat to see whether this or the other repeat is more true to what's really going on.

Buffers used:

- 2x TROLOX buffer was made up in 2x refolding buffer (100 mM HEPES, 100 mM KCL, 10 mM MgCl2, 2 mM DTT, 0.05% T20 (pH 7.5)).
- FlucCDS1 was unfolded by flowing in chemical denaturant (4 M GdHCl, 50 mM HEPES, 50 mM KCl, 5 mM MgCl2, 0.05% (v/v) T20)

Concentration of proteins and stock origin:

- 3 uM A8 (127 uM stock origin- Shannon)
- 2 uM A2 (45 uM stock origin - Shannon)
- 0.5 Hsp110 (36.9 uM stock origin - Nicola)
- Fluc flowed on at 50 pM concentration for immobilisation.

Instrument/techniques/imaging settings: 532 laser imaging exposure = 0.2

532 nm laser = 1.6 ND

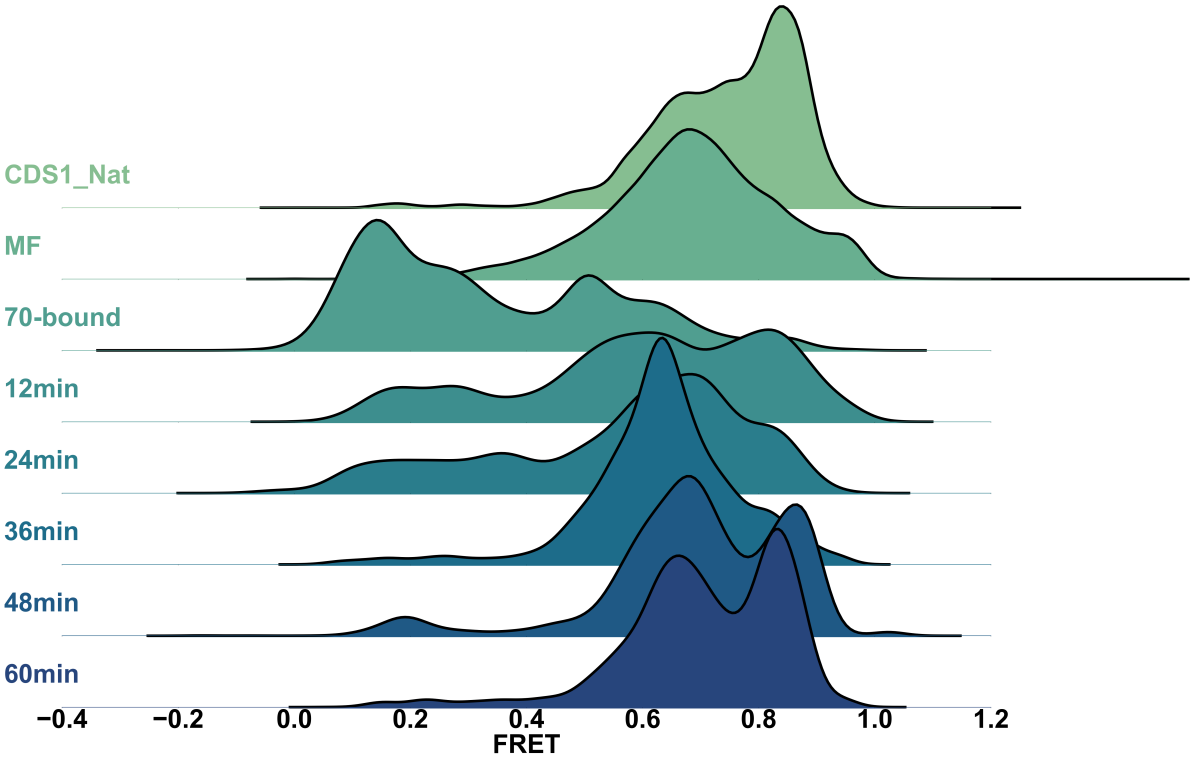
647 laser imaging exposure = 0.2

647 nm laser = 1.0 ND

Setup and workflow:

- CDS1 was immobilised at 5 nM concentration to achieve a high enough density for imaging. Could have pushed it up to 6 nM potentially, still not amazing density of molecule count.

Data:



- Again I got a strange 70-bound state which doesn't really appear to be fully captured by the chaperones.
- Interestingly I get a very prominent 0.6 peak at 36 minutes, but a split peak at 60 minutes this time.

The previous repeat for this mutant is shown below.

- I think I'll wait for the final repeat before starting to collate data.
- I also want to refold my sample for an hour with chaperones and then flow out chaperones to see what state the fluc exists in following refolding once chaperones are removed. Does it prefer the 0.6 or 0.8 conformation?

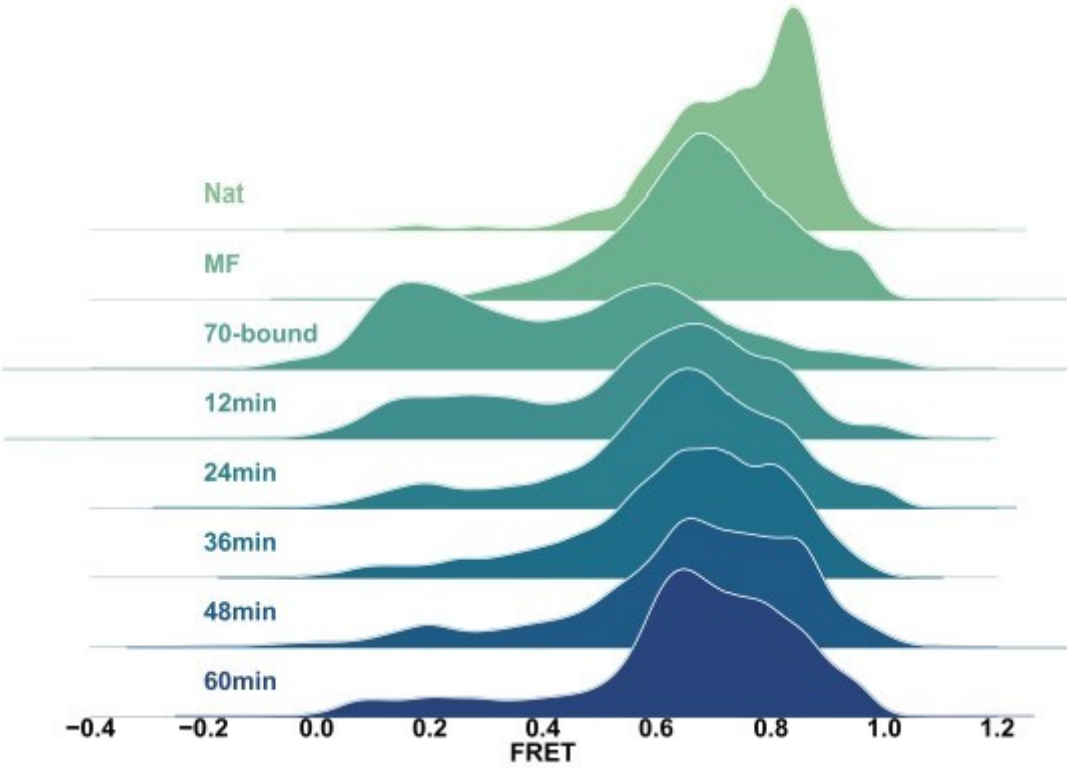


Figure 1: First real repeat of CDS1