

Monitoring Transthyretin Protein Aggregation using yTRAP

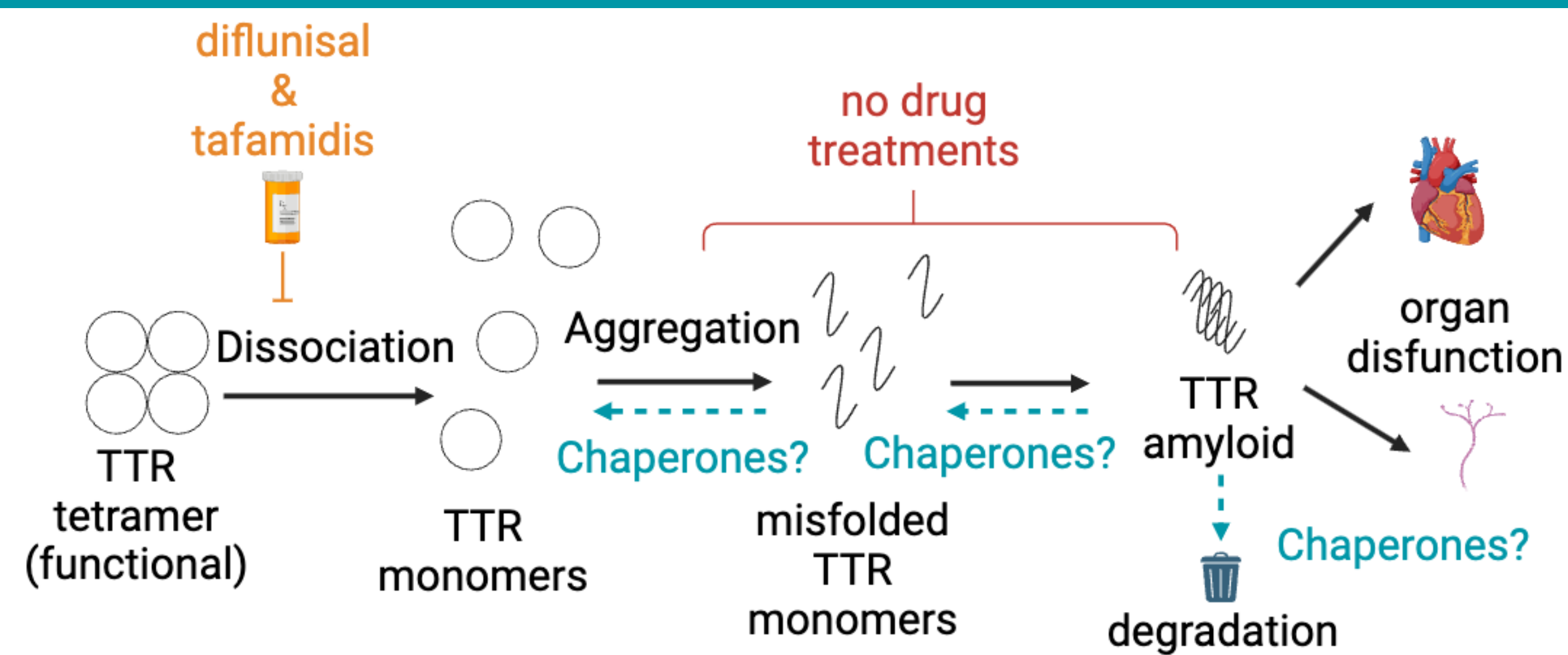
Sean Martin and Anita L. Manogaran

Department of Biological Sciences, Marquette University, Milwaukee, WI

Transthyretin Amyloidosis (ATTR)

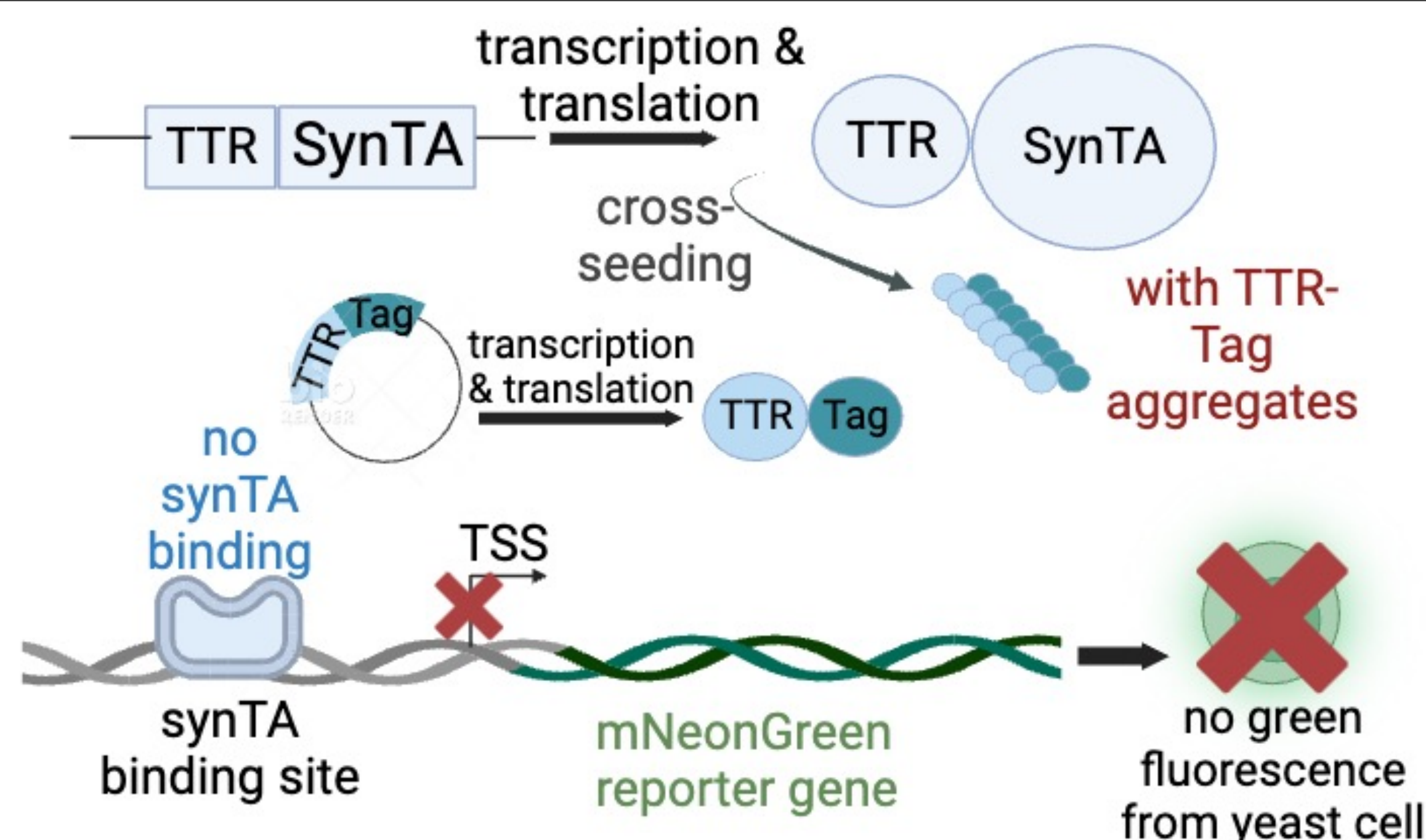
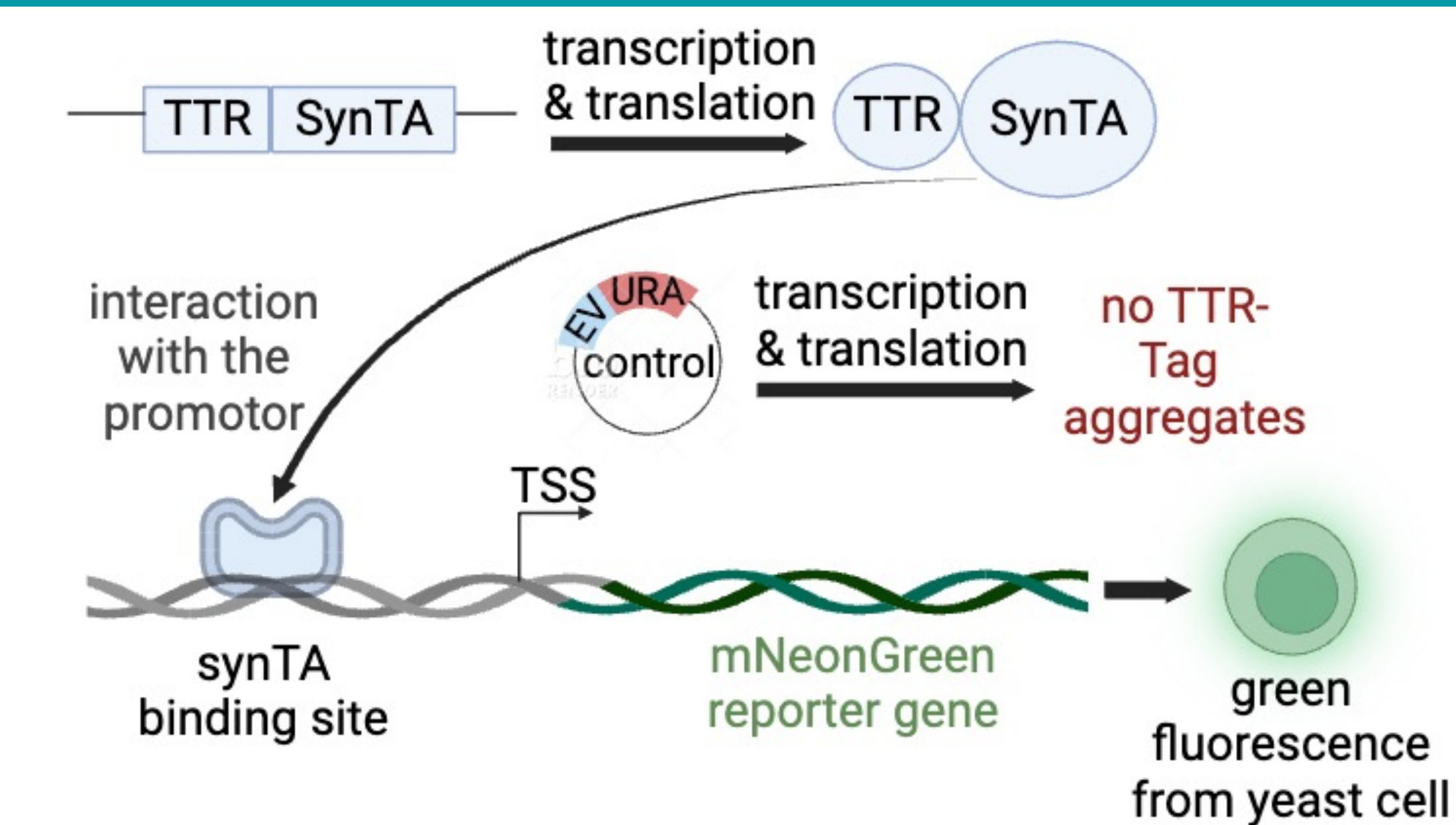
- Age Driven High Prevalence: 1 in 4 over the age of 80 are estimated to have disease
- Symptoms: hypotension, chronic wasting, & muscle weakness
 - Treatment: symptom management, but no cure
 - Diagnosis: poor diagnosis tools, leading to common misdiagnosis as heart failure
 - Cause: Transthyretin (TTR) protein aggregation

Biology of Transthyretin (TTR)

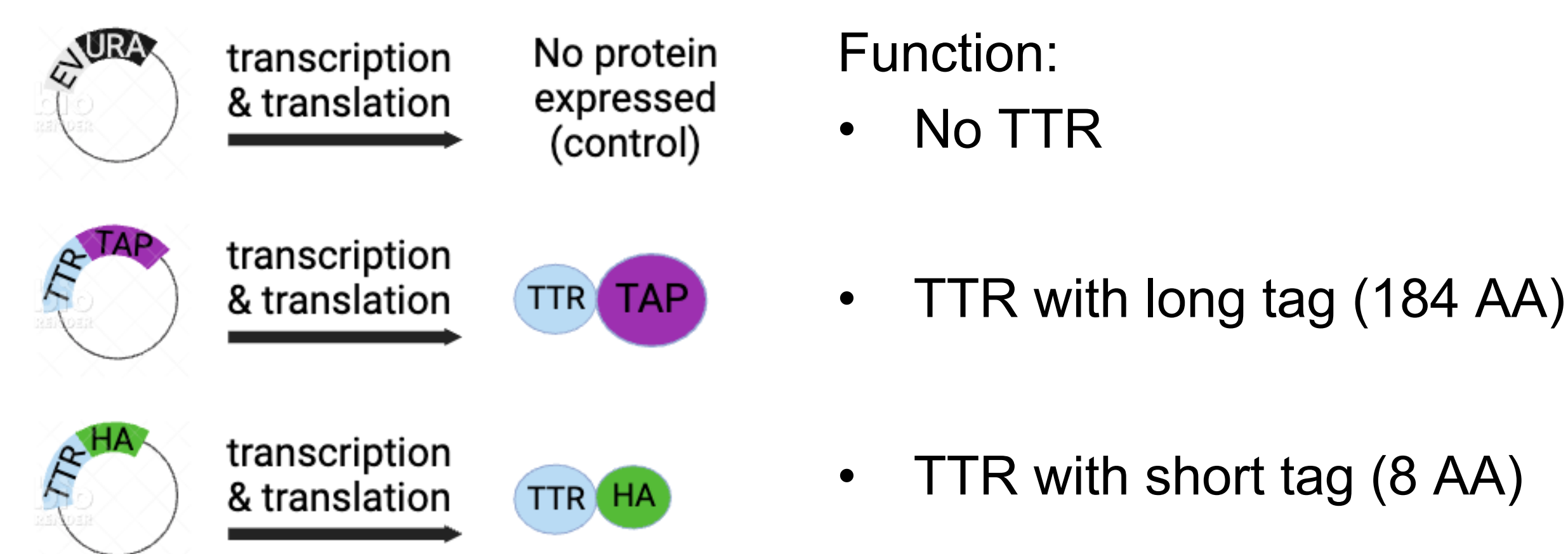


Currently, there are no drug treatments that target after tetramer dissociation. My project works to create a system to screen for drugs that reduce TTR aggregation.

yTRAP Assay



Approach



Function:

- No TTR
- TTR with long tag (184 AA)
- TTR with short tag (8 AA)

Flow Cytometry Results

Does TTR-tag (TAP or HA) influence mNeonGreen expression?

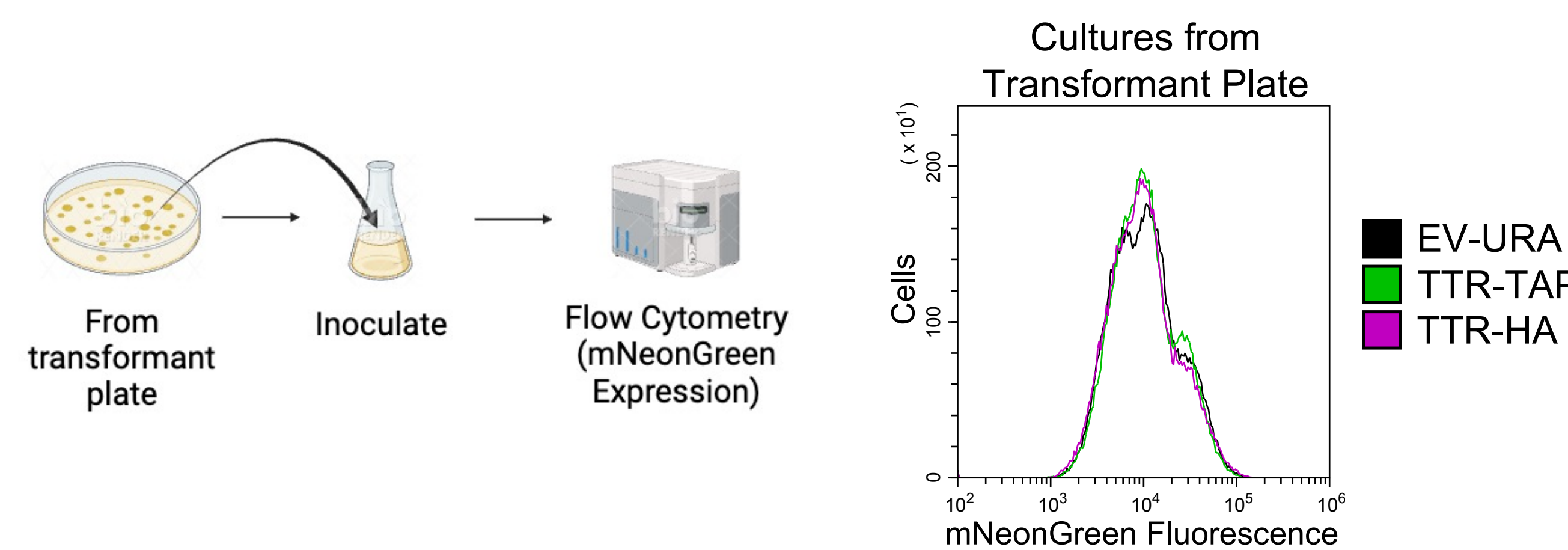


Figure 1: Cells taken directly from the transformant plate show no difference in mNeonGreen fluorescence. Transformants were inoculated into liquid media and incubated for 2 hours for 3 strains (EV, TTR-TAP, TTR-HA). 1 trial of each sample is shown.

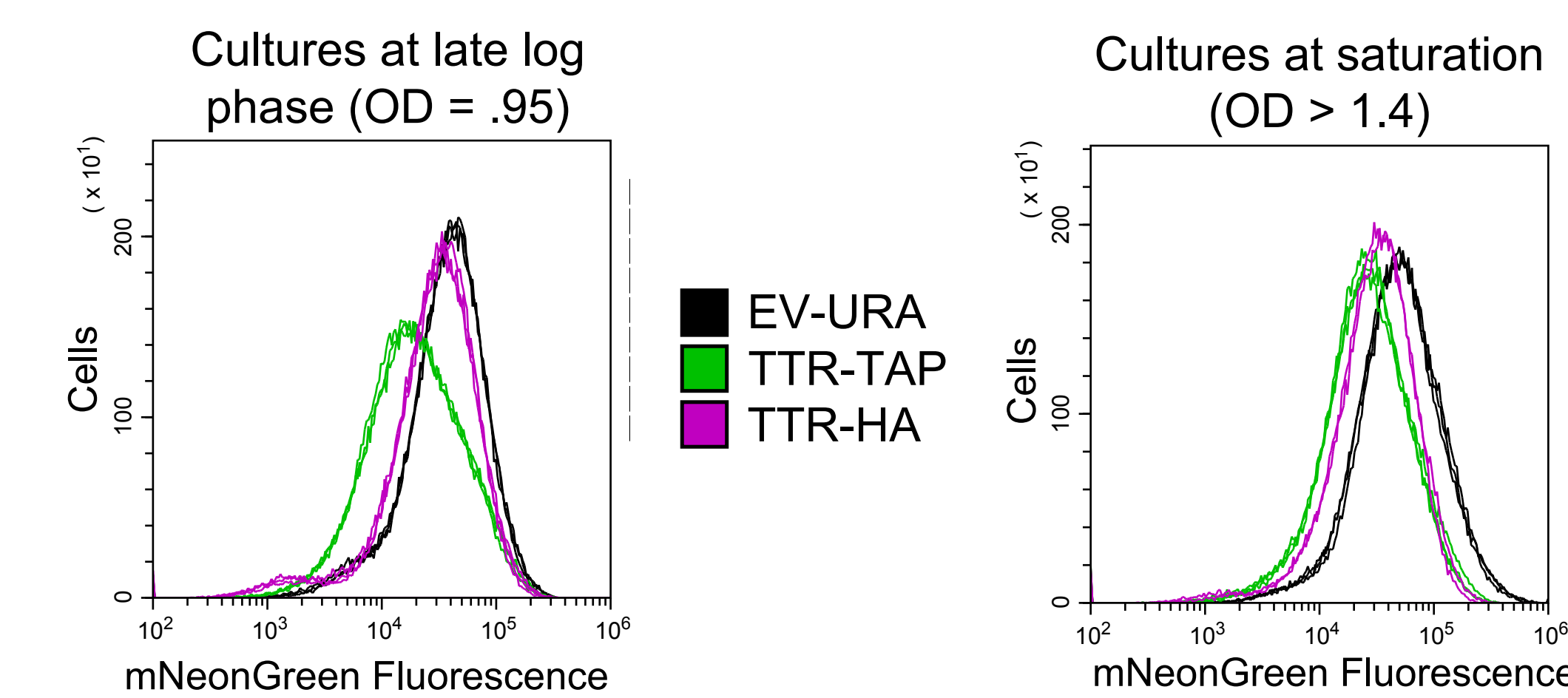
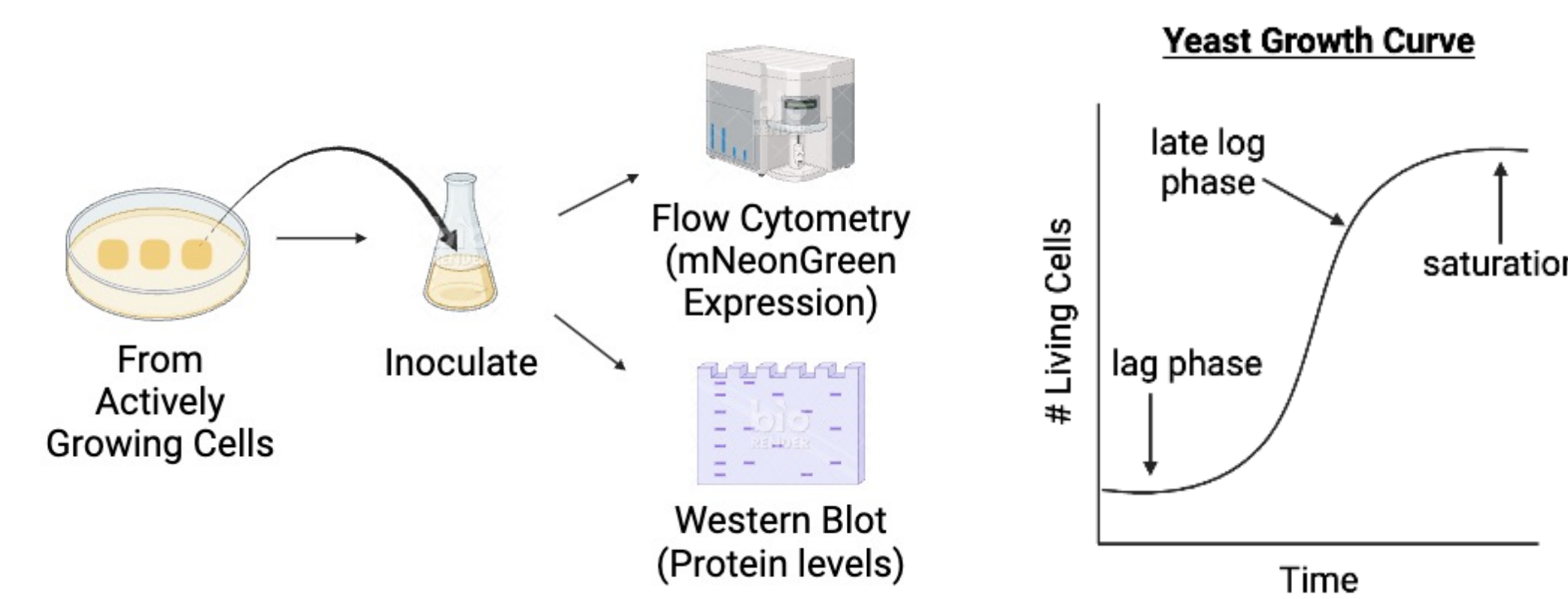


Figure 2: TTR-Tag at late log reduces mNeonGreen readout. 3 strains (EV, TTR-Tap, & TTR-HA) were grown to various ODs as indicated and subjected to flow cytometry. 3 trials of each sample are shown.

Preliminary Western Blot Results

1. Do strains express SynTA similarly? → Measure using HA antibody
2. What are levels of TTR-tag protein? → Measure using TTR antibody

Expected Protein Sizes

| | |
|-----------|----------|
| TTR | 14 kDa |
| TTR SynTA | 46.5 kDa |
| TTR TAP | 35 kDa |
| TTR HA | 15 kDa |

Expected Western Blot Signals

| | EV-URA | TTR-TAP | TTR-HA | TTR-GFP | - control |
|-----------------|--------|---------|--------|---------|-----------|
| SynTA (α-HA) | + | + | + | + | - |
| TTR-Tag (α-TTR) | - | + | + | + | - |

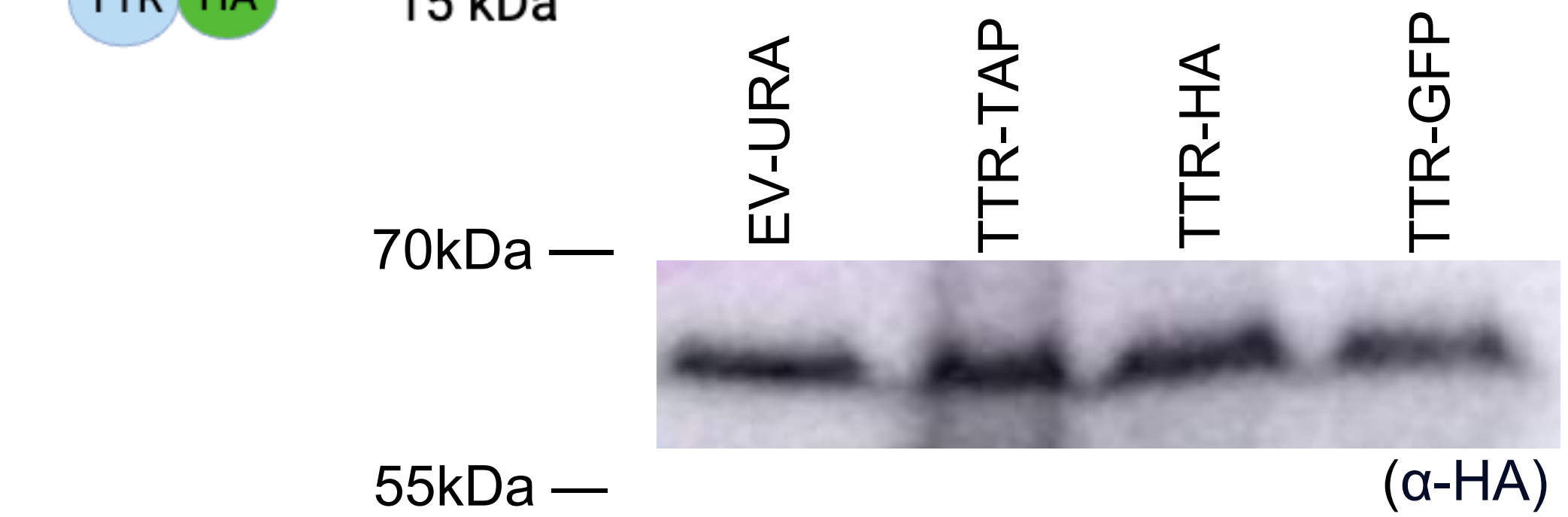


Figure 3: SynTA is expressed in all strains.

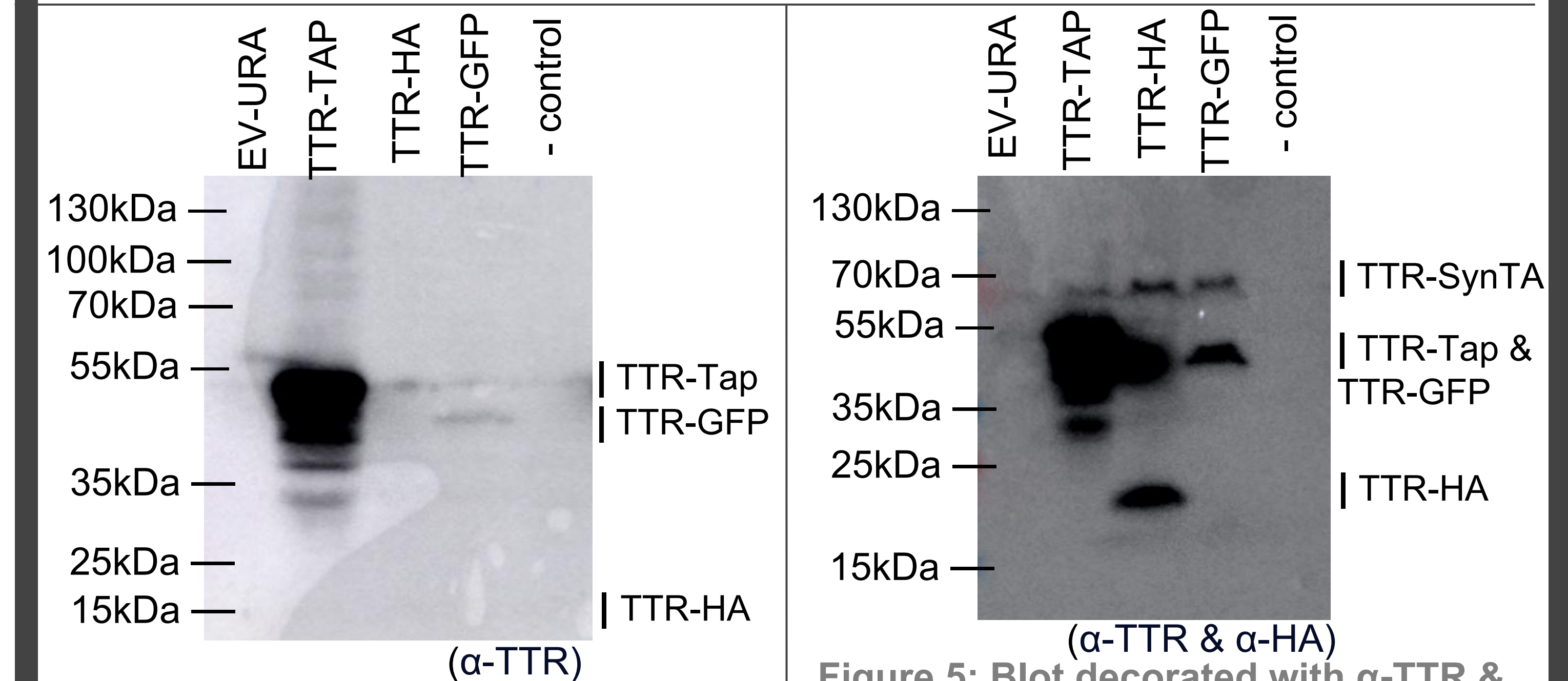


Figure 4: TTR-Tag has high steady state levels. Figure 5: Blot decorated with α-TTR & α-HA give off signal for SynTA, TTR-Tap, TTR-HA, & TTR-GFP.

Conclusions

- The system appears to be working as expected:
 - TTR-Tag influences mNeonGreen expression at late log
 - Strains express SynTA levels at similar levels
 - TTR-Tag protein is expressed in expected strains

Future Outlook

- Confirm TTR-Tag aggregates (Western Blot)
- HSP104 overexpression has been shown to increase TTR Aggregation (Knier et al., 2022). This yTRAP system will be tested to determine if HSP104 overexpression leads to changes in yTRAP output.

Acknowledgements

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