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220524 – Antibody discovery and engineering at Surrozen

Disclosure of Conflicts of Interest

- I herewith declare the following paid or unpaid consultancies, business interests or sources of honoraria payments for the past three years, and anything else which could potentially be viewed as a conflict of interest:
  - Surrozen, Inc.: shareholder and employee

- Forward Looking Statements
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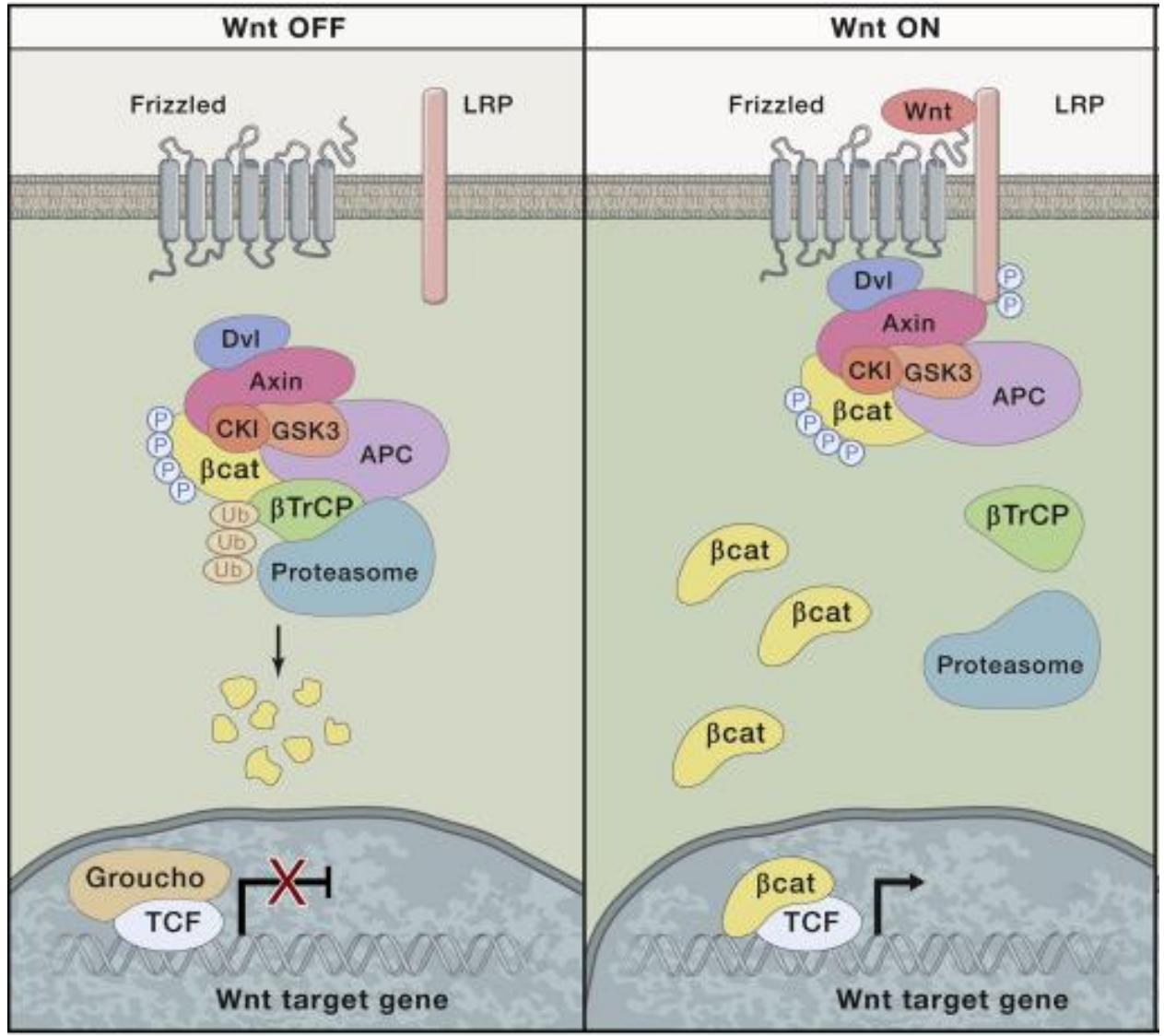
The WNT pathway – master regulator of proliferation and homeostasis

- WNTs are lipoglycoprotein growth factors
- 19 mammalian WNT proteins signal through 10 Frizzled receptors
- Lack of drug-like properties and controllable specificity have limited the utilization of WNTs as therapeutics



Adapted from Nusse and Clevers, 2017.
https://doi.org/10.1016/j.cell.2017.05.016

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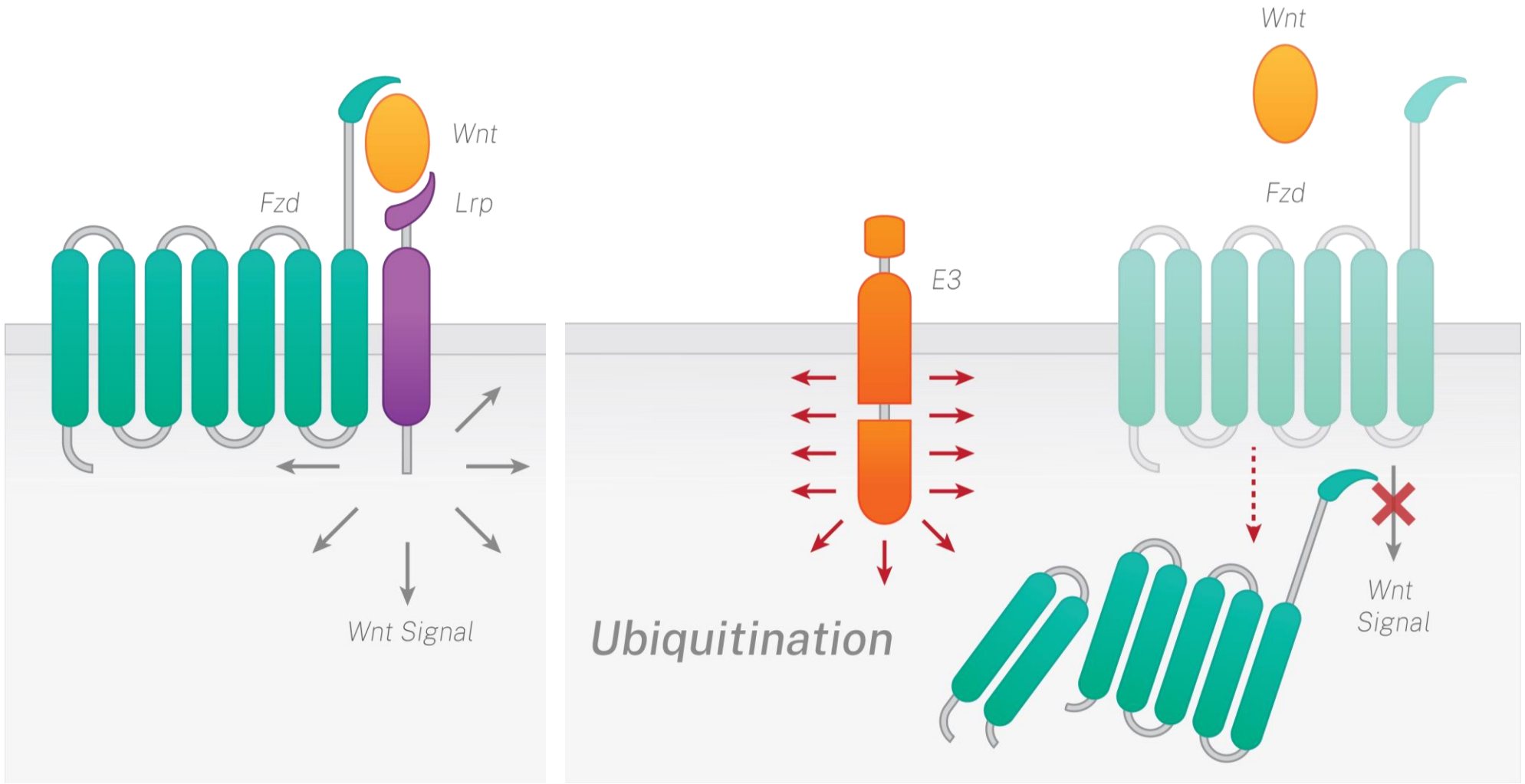
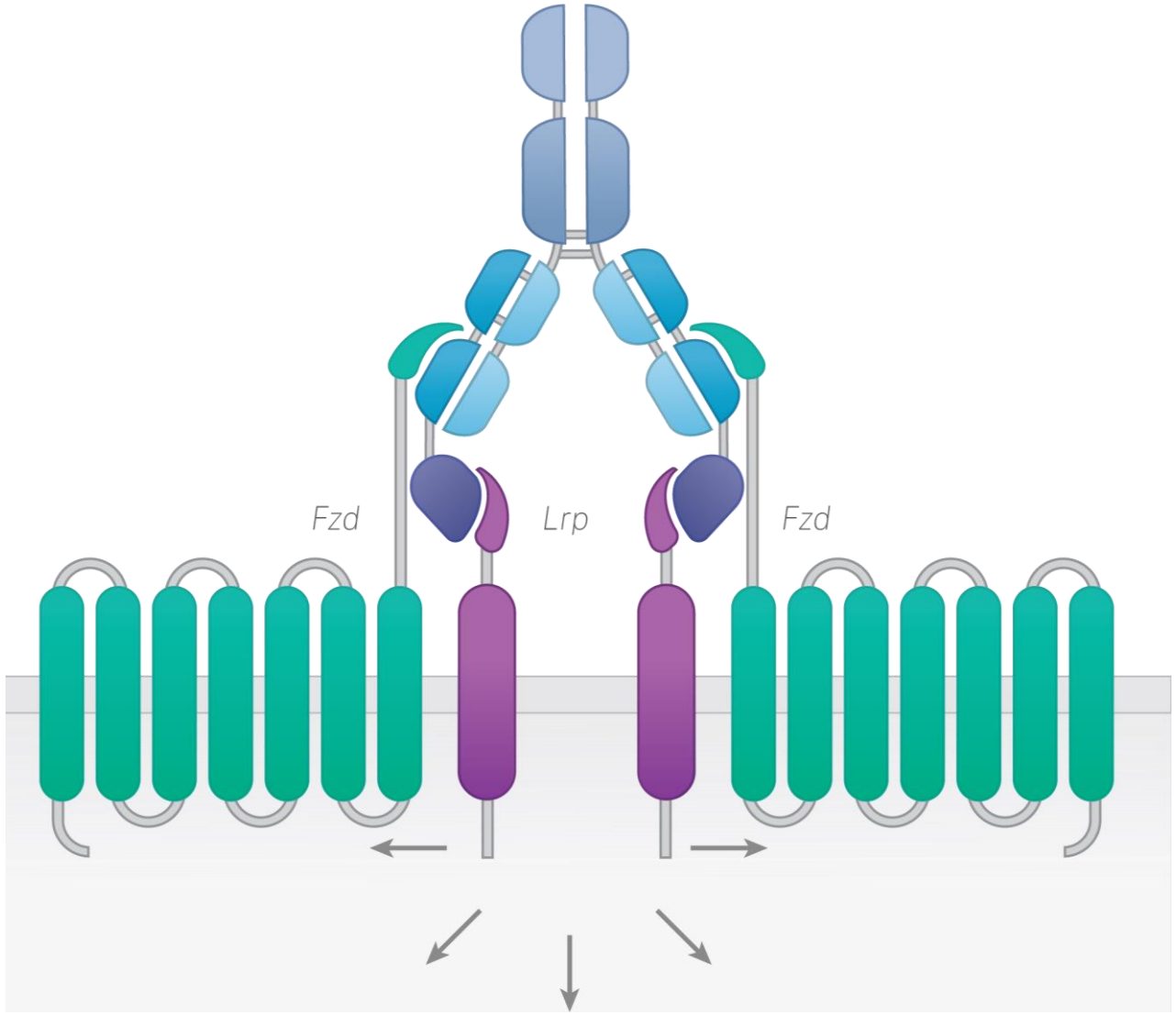
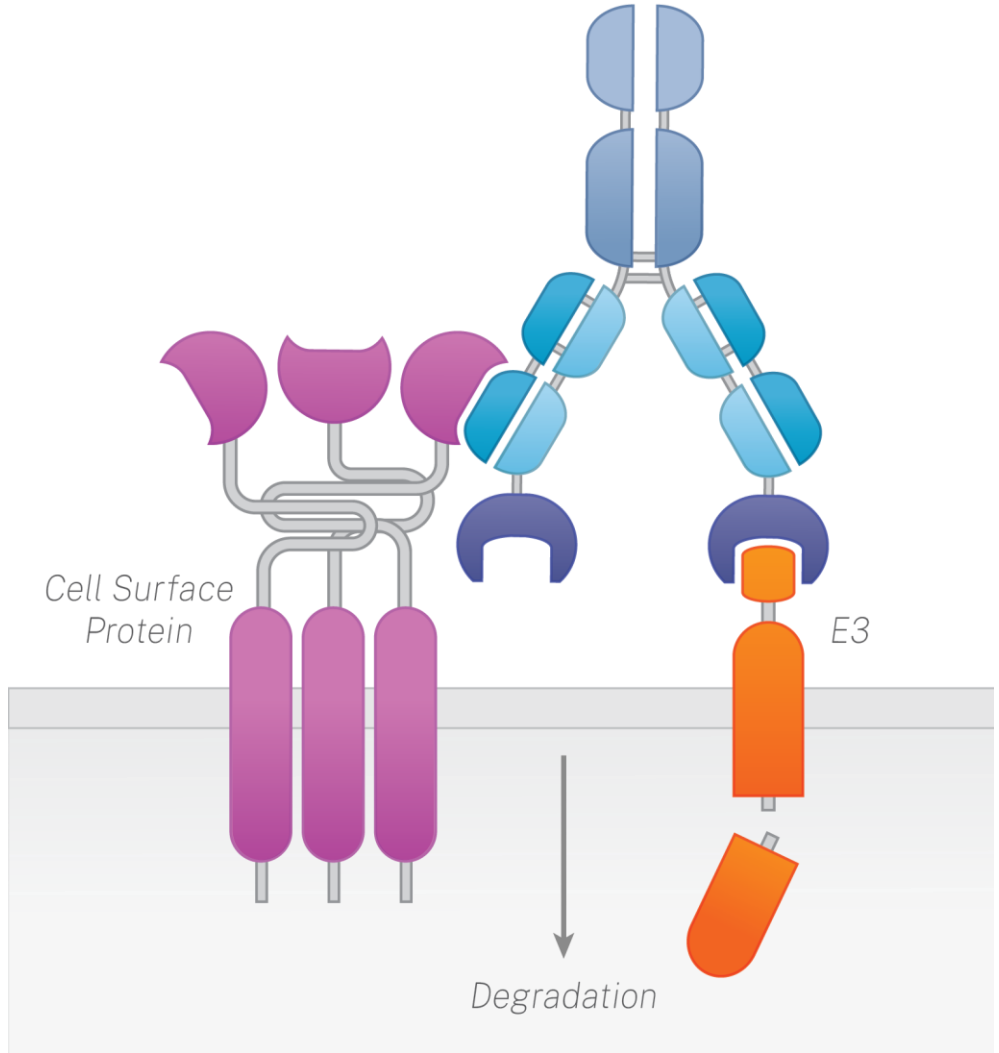
Overview of WNT signaling at the cell surface



Unique platforms at Surrozen for WNT activation and enhancement





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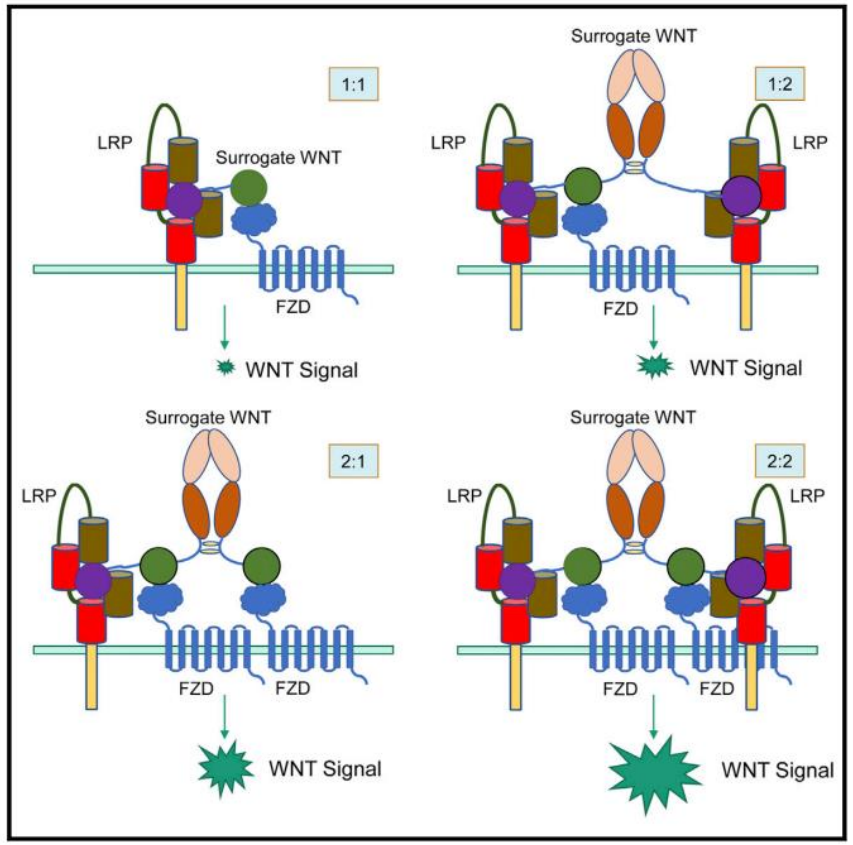
Unique platforms at Surrozen for WNT activation and enhancement

Cell Chemical Biology

Development of Potent, Selective Surrogate WNT Molecules and Their Application in Defining Frizzled Requirements

Graphical Abstract



Authors

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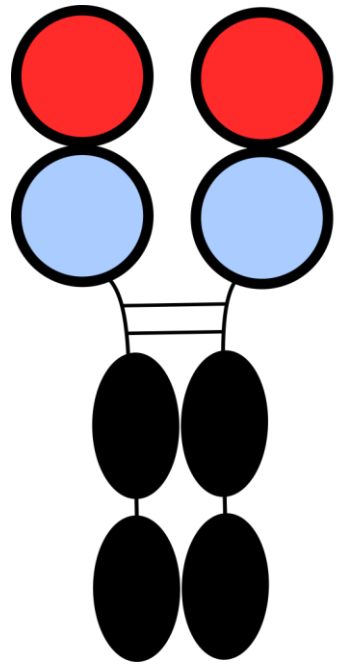
In Brief

WNT molecules have the potential to induce tissue regeneration and repair. However, their biophysical characteristics and lack of selectivity have hindered their application as therapeutics. Chen et al. have developed a platform for potent, selective WNT surrogate generation, and identified key requirements for maximal signaling.



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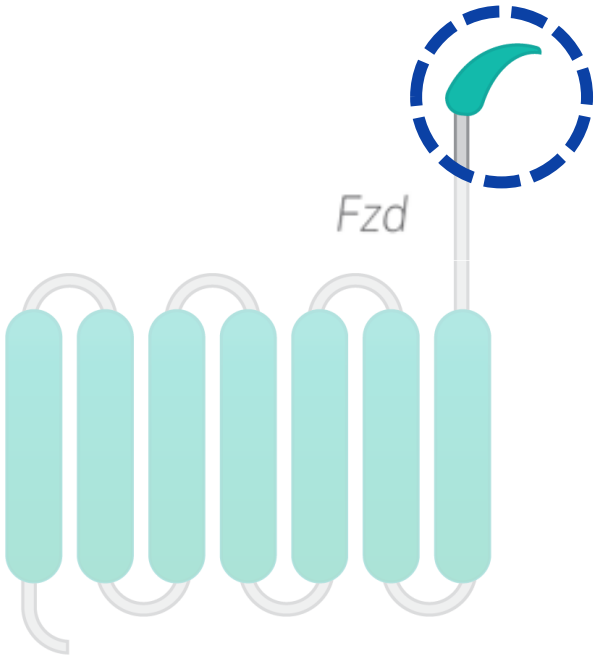
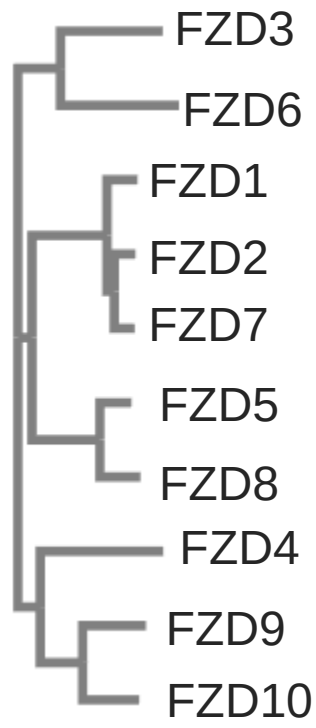
Conservation creates challenges for building targeted WNT activators

Cysteine Rich Domain



Fzd CRD Percent Identity Matrix

	FZD3	FZD6	FZD1	FZD2	FZD7	FZD5	FZD8	FZD4	FZD9	FZD10
FZD3	100	48.8	38.4	39.2	39.2	41.46	38.89	34.13	33.33	36.8
FZD6	48.8	100	33.33	35.71	35.71	35.25	34.65	31.75	30.71	33.06
FZD1	38.4	33.33	100	84.67	88.15	52.03	51.94	38.28	44.03	45.8
FZD2	39.2	35.71	84.67	100	90.37	53.66	49.61	39.84	44.03	45.04
FZD7	39.2	35.71	88.15	90.37	100	52.85	49.61	38.28	44.03	44.27
FZD5	41.46	35.25	52.03	53.66	52.85	100	83.2	37.6	46.4	45.6
FZD8	38.89	34.65	51.94	49.61	49.61	83.2	100	36.15	42.75	44.53
FZD4	34.13	31.75	38.28	39.84	38.28	37.6	36.15	100	46.92	48.44
FZD9	33.33	30.71	44.03	44.03	44.03	46.4	42.75	46.92	100	72.18
FZD10	36.8	33.06	45.8	45.04	44.27	45.6	44.53	48.44	72.18	100



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Successful in-house implementation of Carterra white papers

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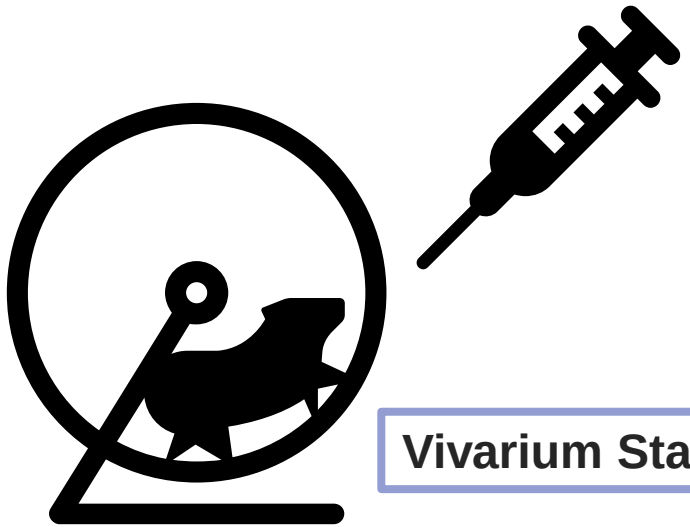
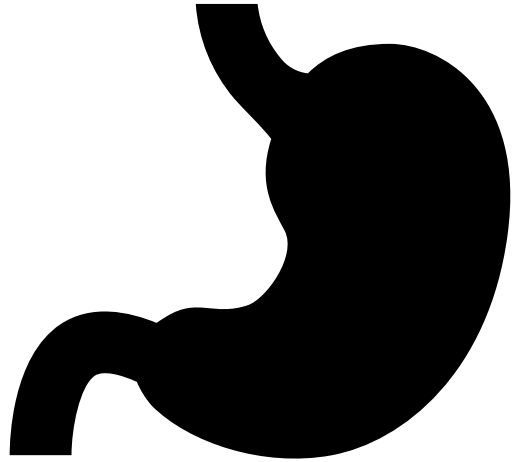
Collaborative pipeline at Surrozen for antibody discovery

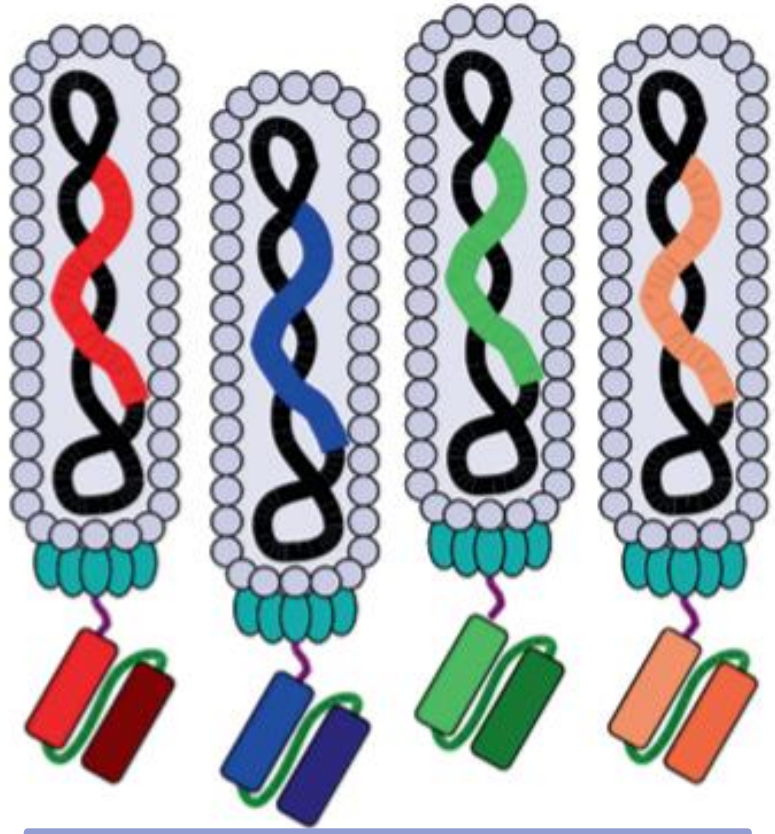
Antigen Construction



Vivarium Staff

Molecular Biology





Panning and PPE preparation

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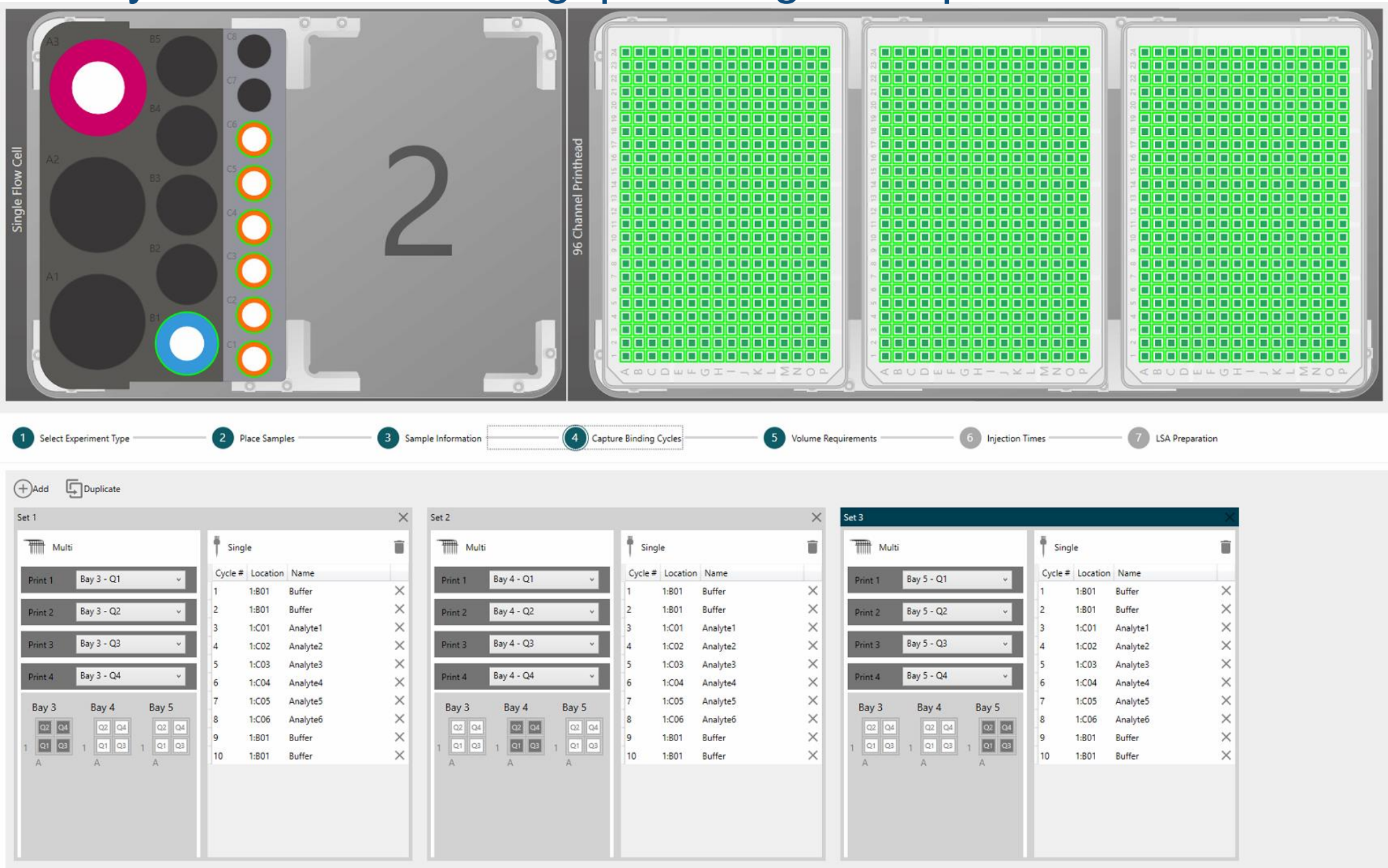
‘Walk away’ 1152-clone throughput using V5 capture



Clones of interest can be selected based on kinetics, specificity, etc

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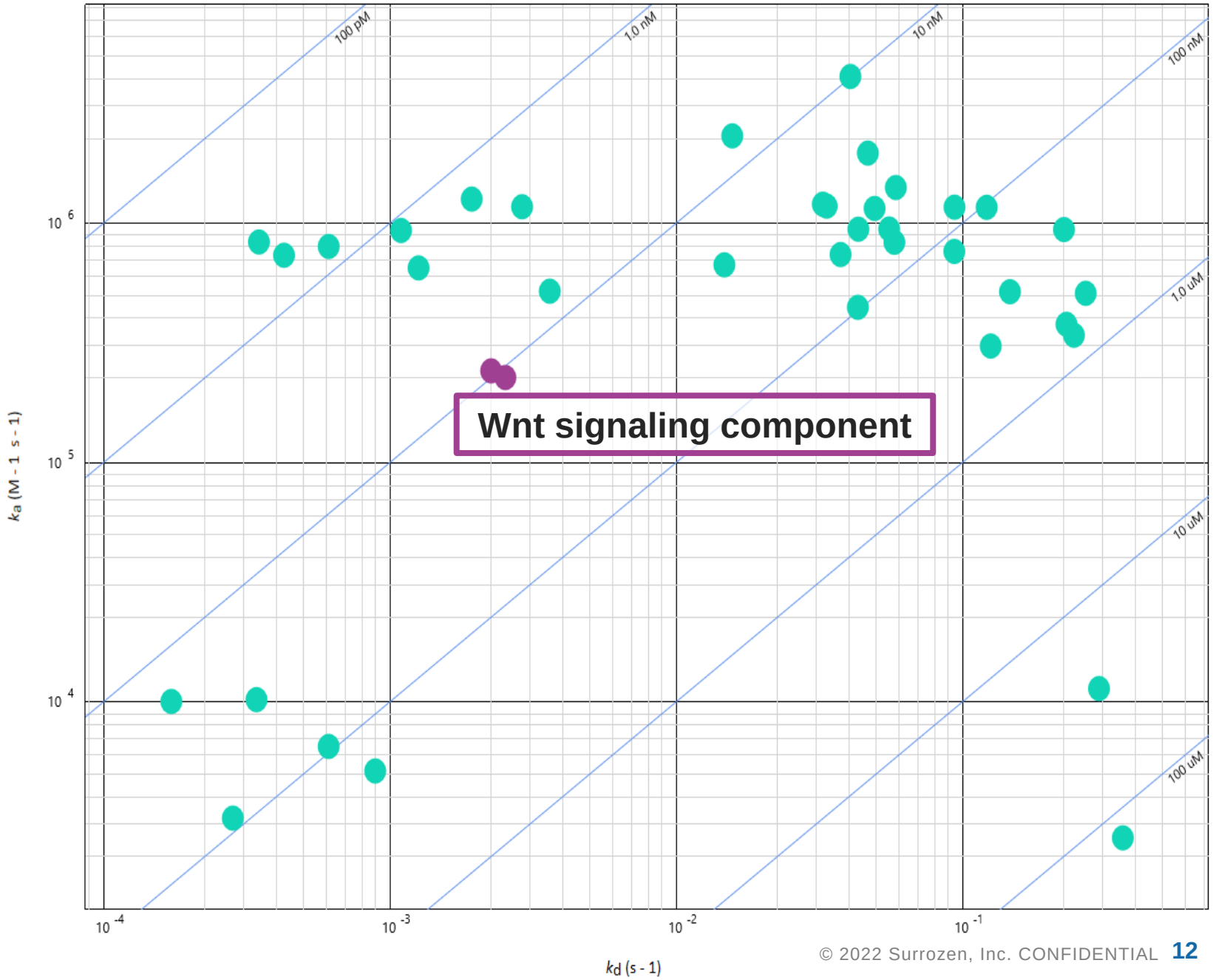
Reformat binders as IgG for confirmation of affinity and specificity

- GGA for high throughput reformatting
- Capture IgG's out of crude supernatant from 96-well 1mL transient transfections of Expi293 cells



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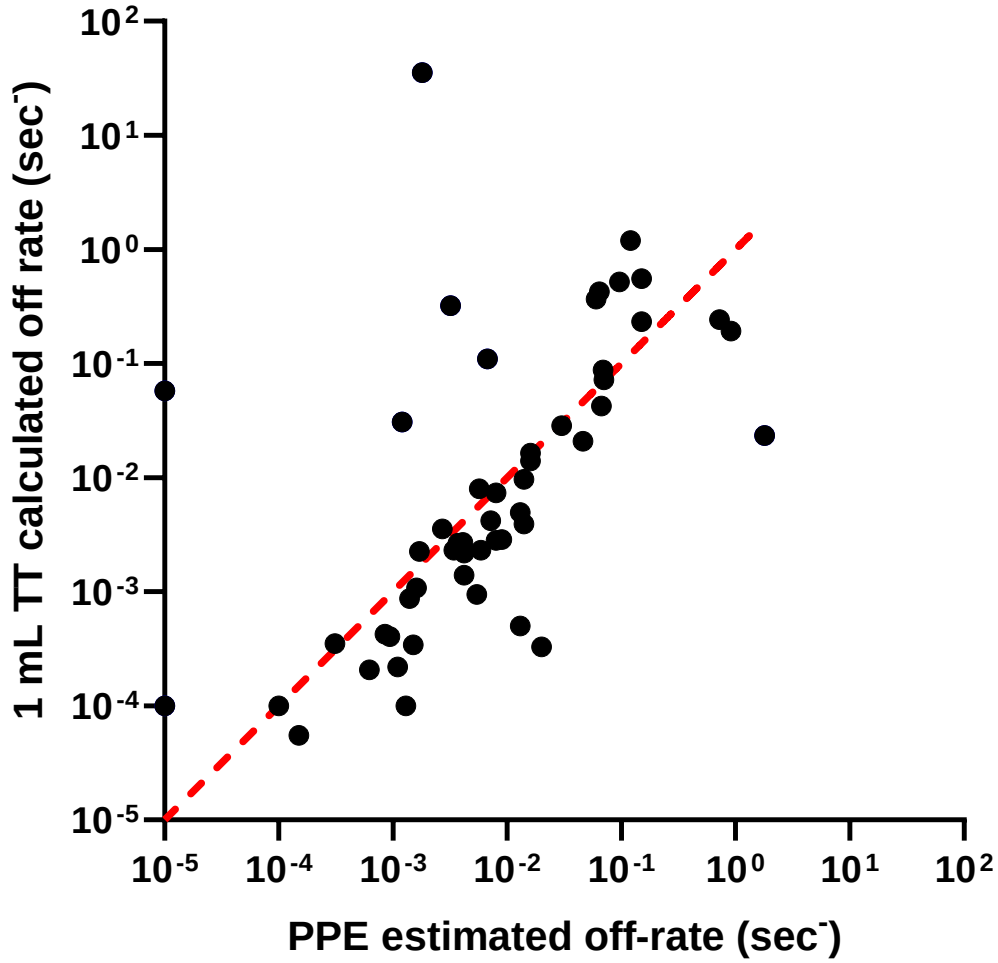
Comparison of off-rates between scFv from phage PPE and IgG



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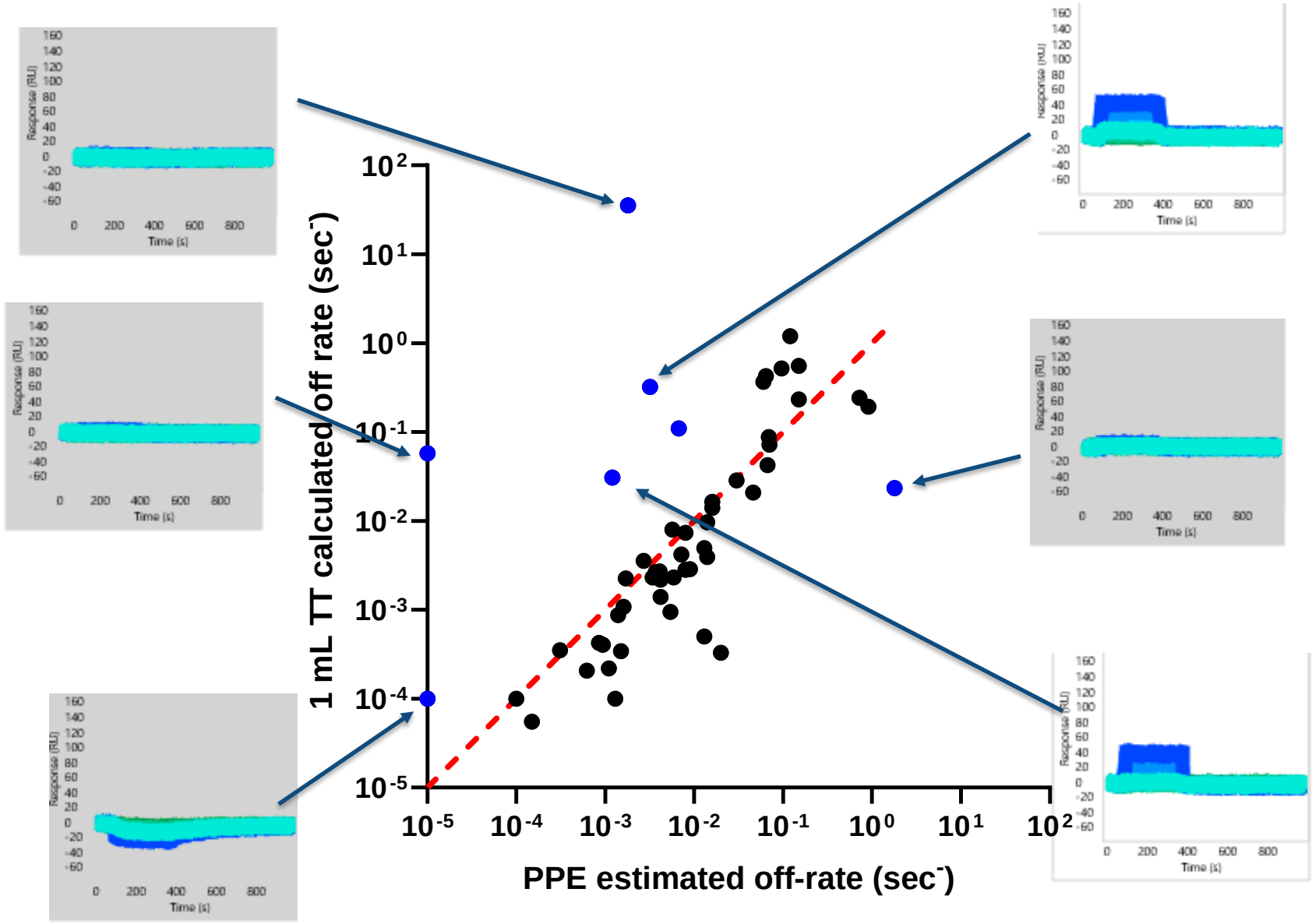
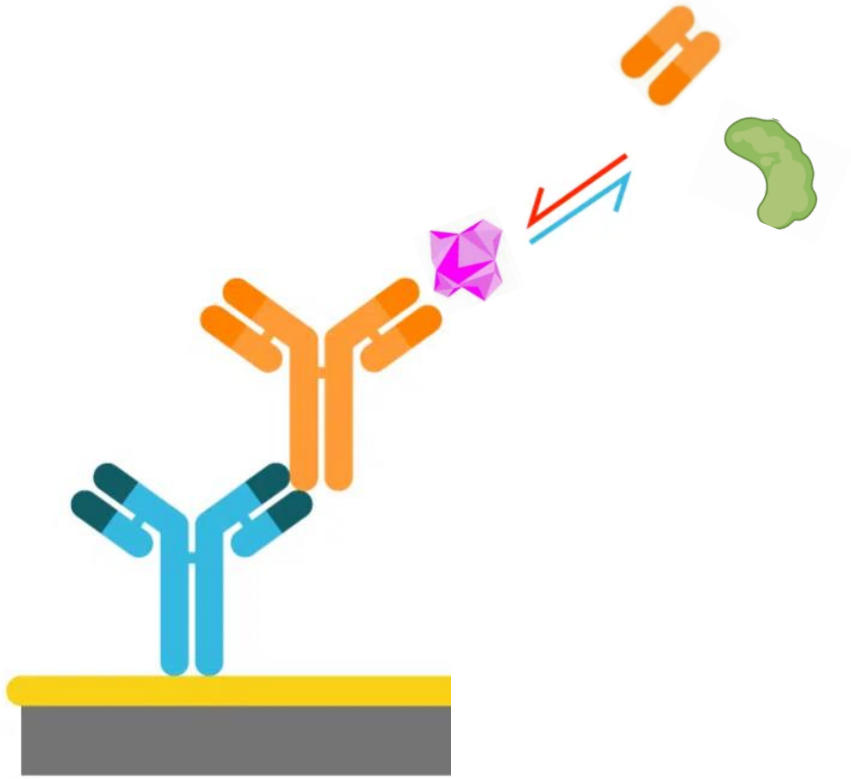
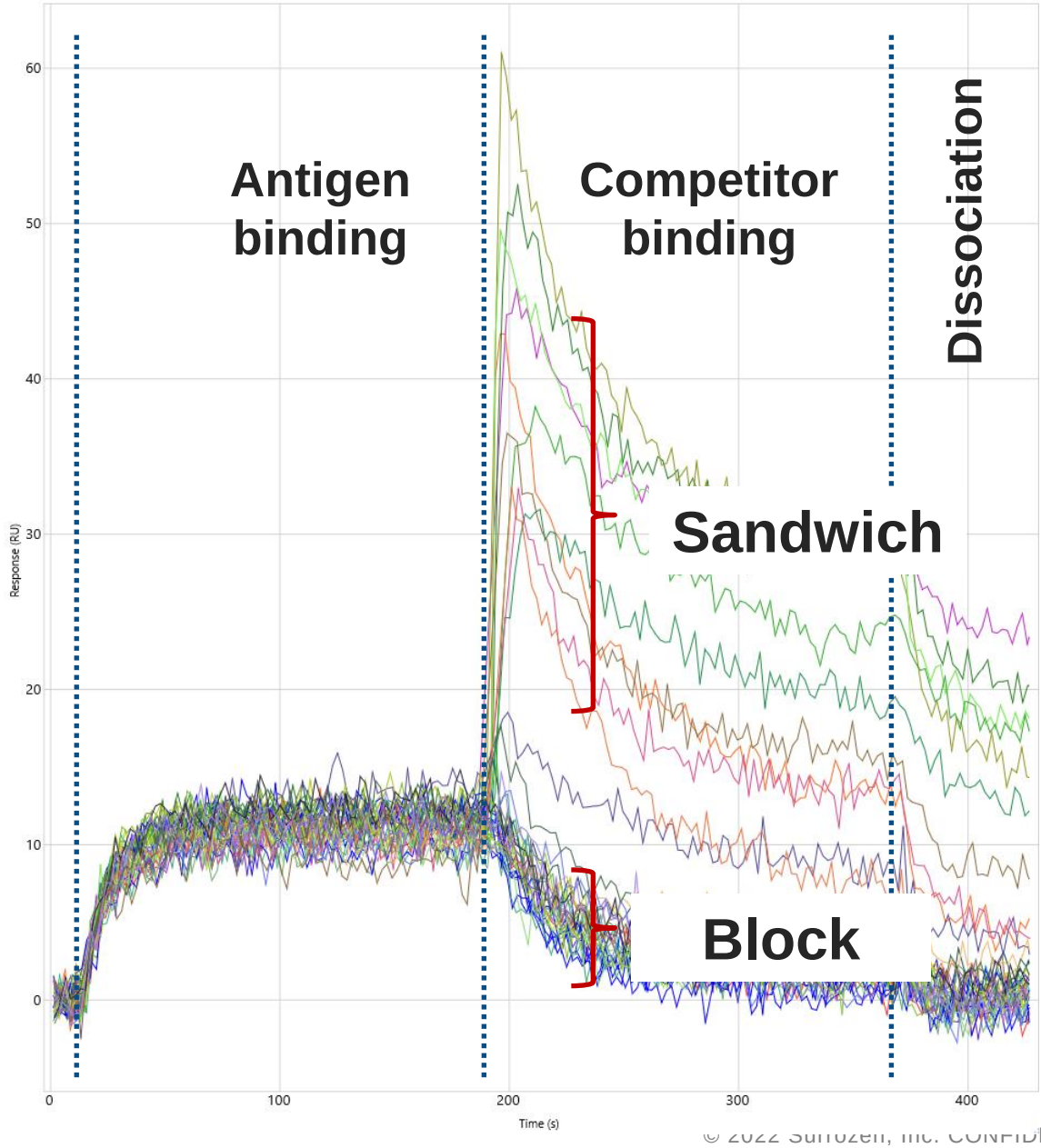
Many outliers turn out to be non-binders



Using ‘hallmark’ binders for epitope characterization

- Competition tested against binders with known epitopes after acquiring kinetics.





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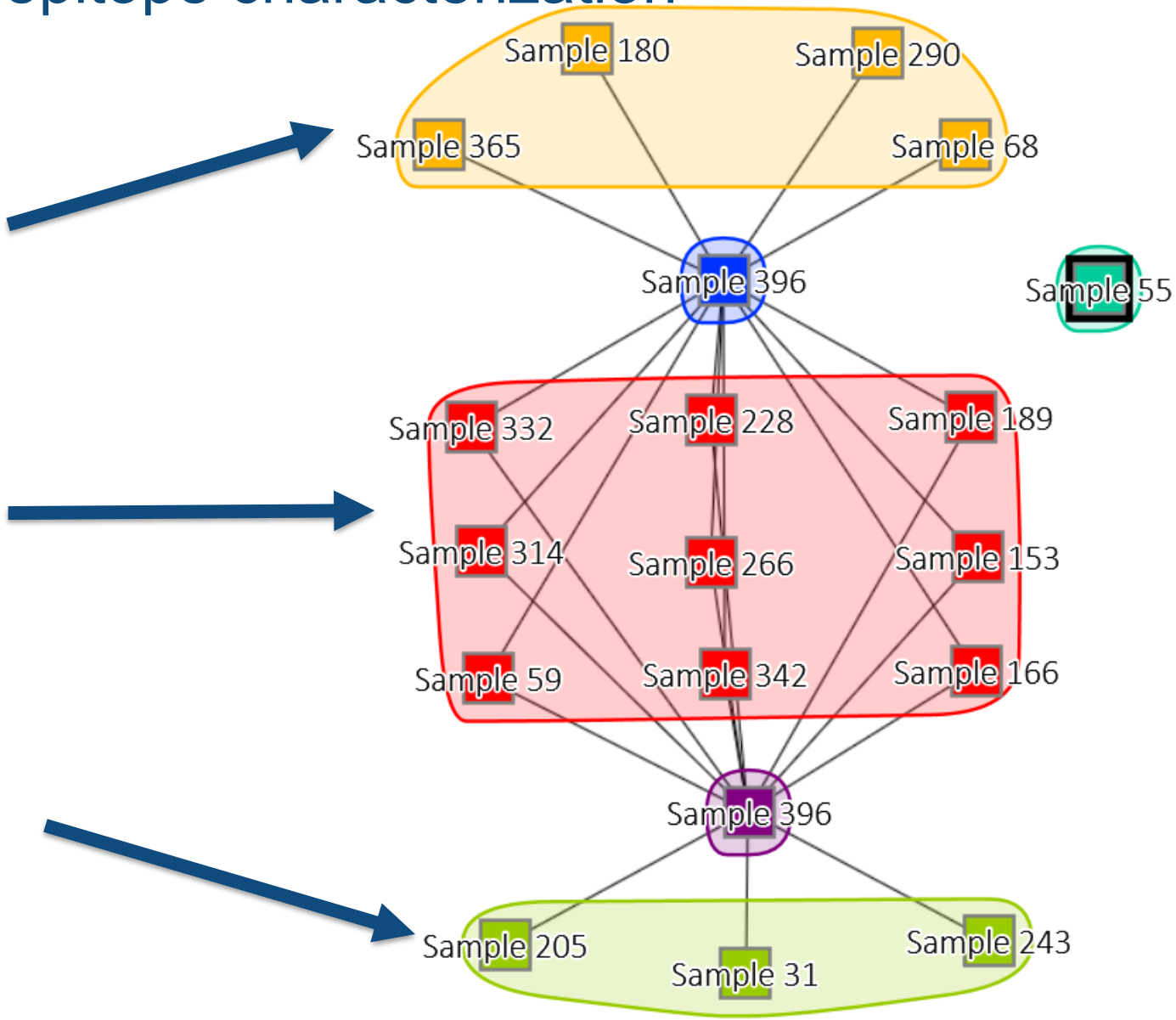
Using ‘hallmark’ binders for epitope characterization

- Binders that block Fab1 and sandwich Fab2
- Binders that block Fab1 and block Fab2
- Binders that sandwich Fab1 and block Fab2



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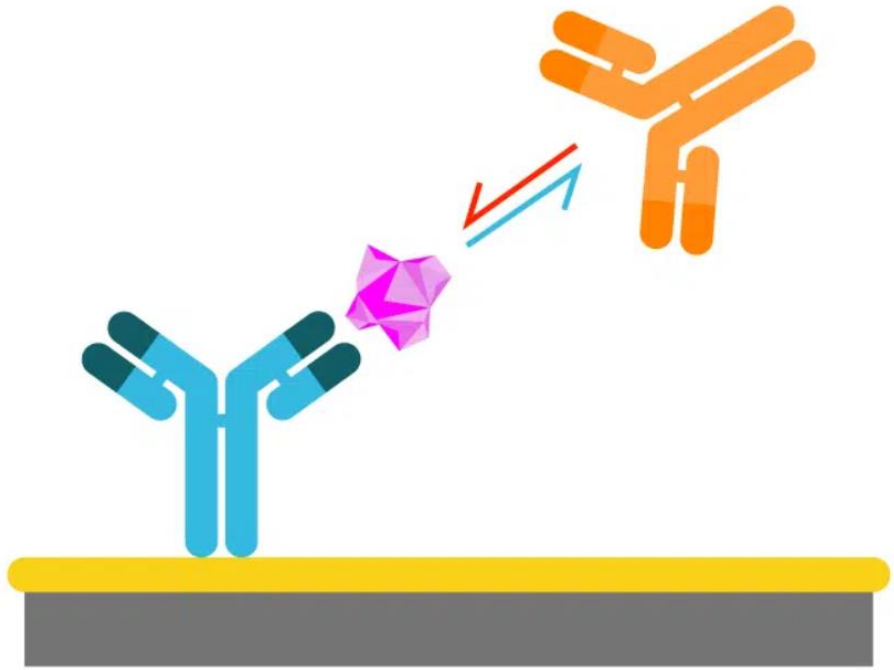
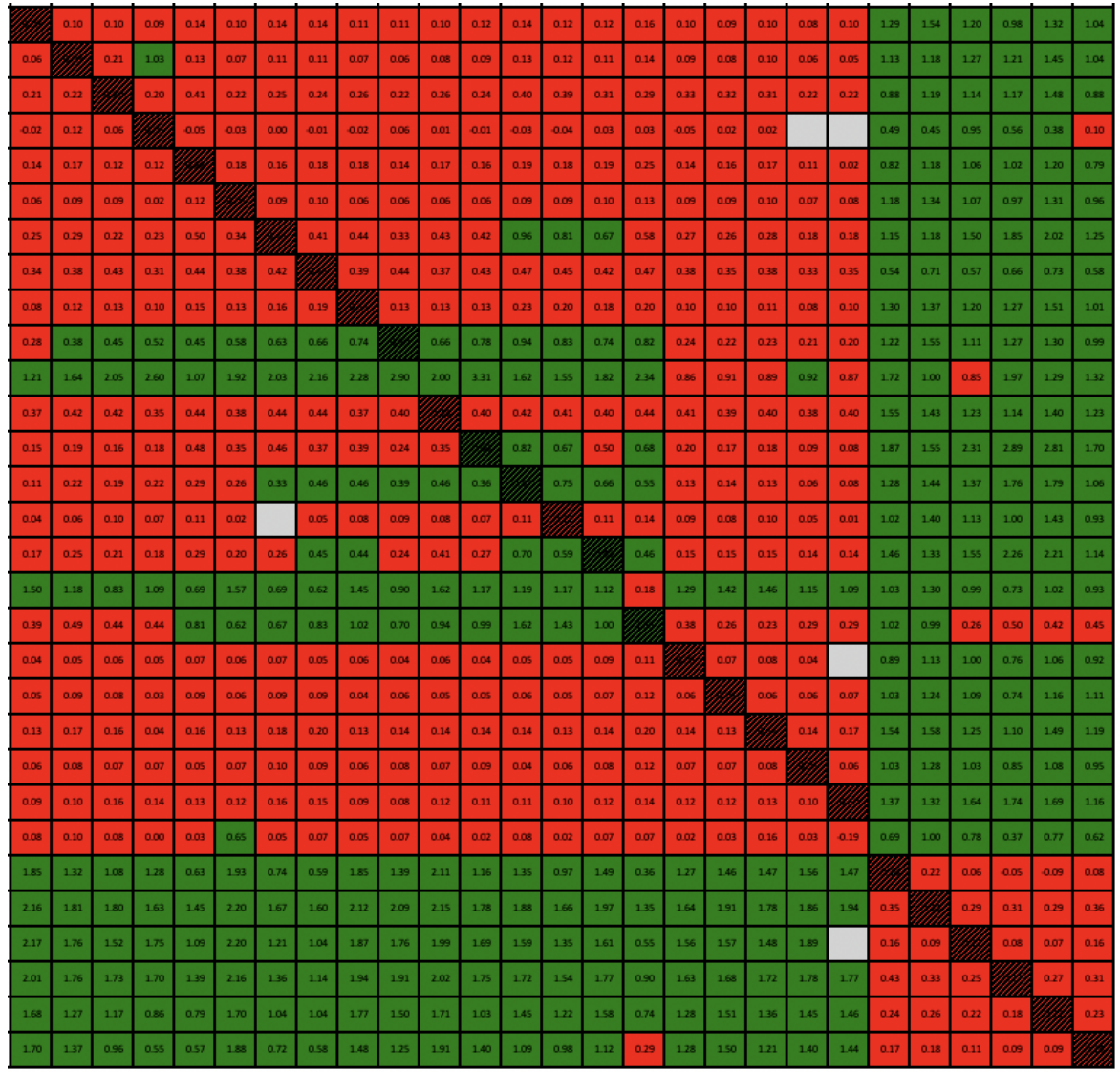
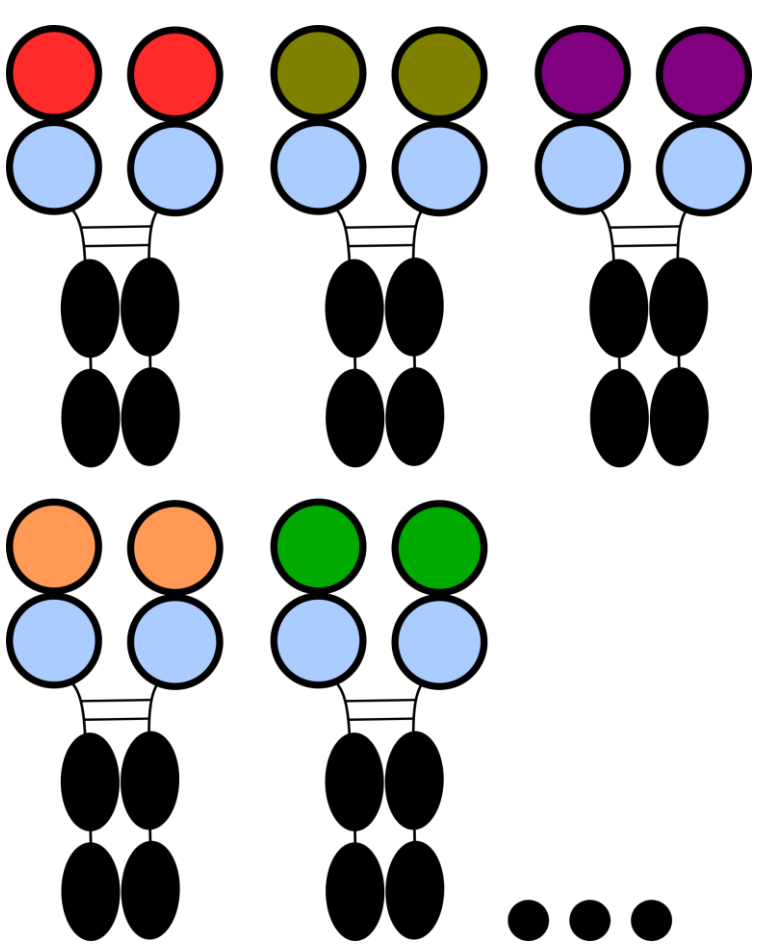
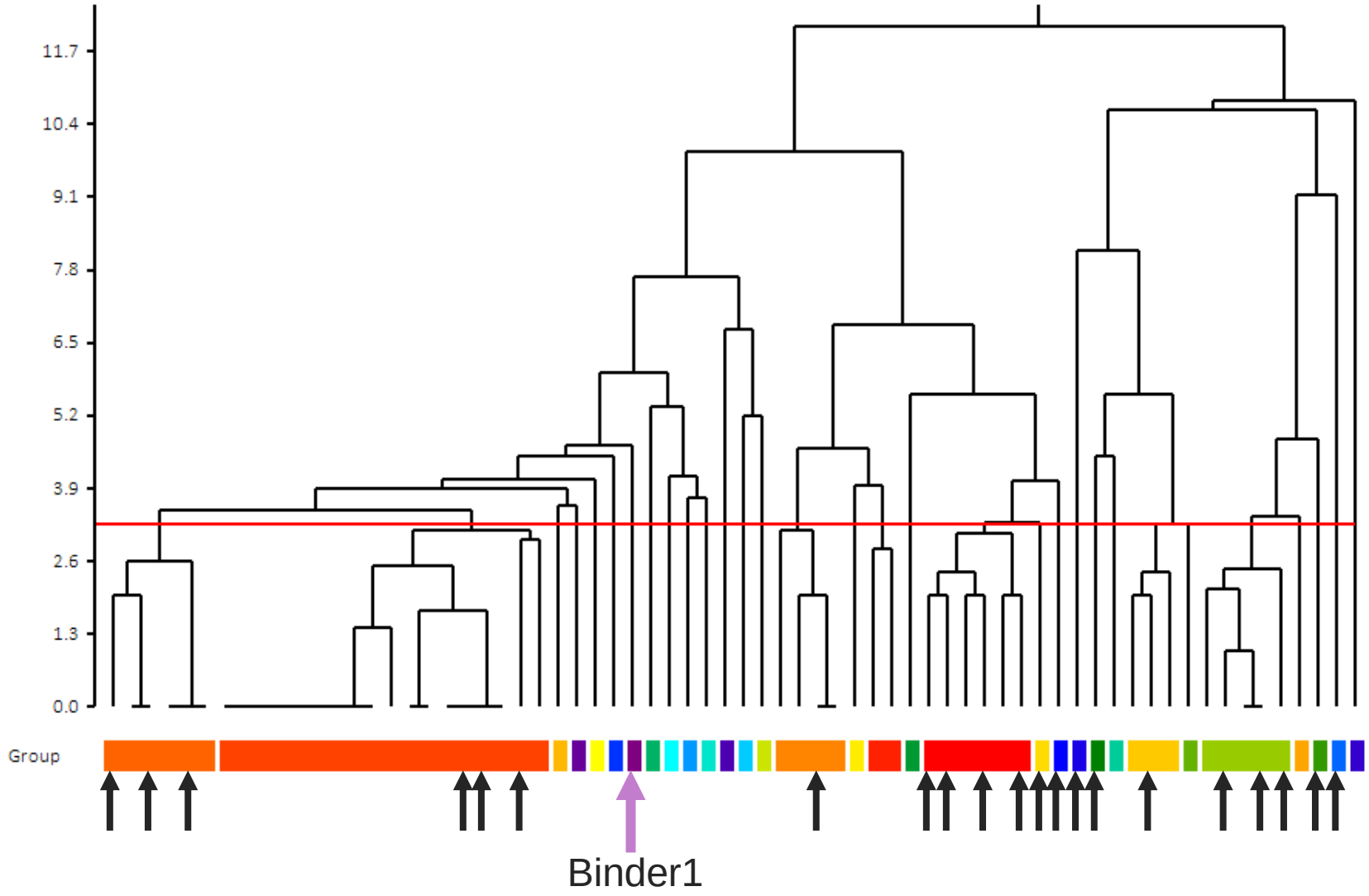
Follow up with “all against all” candidate binning





Designing surrogates aided by epitope and biophysical properties





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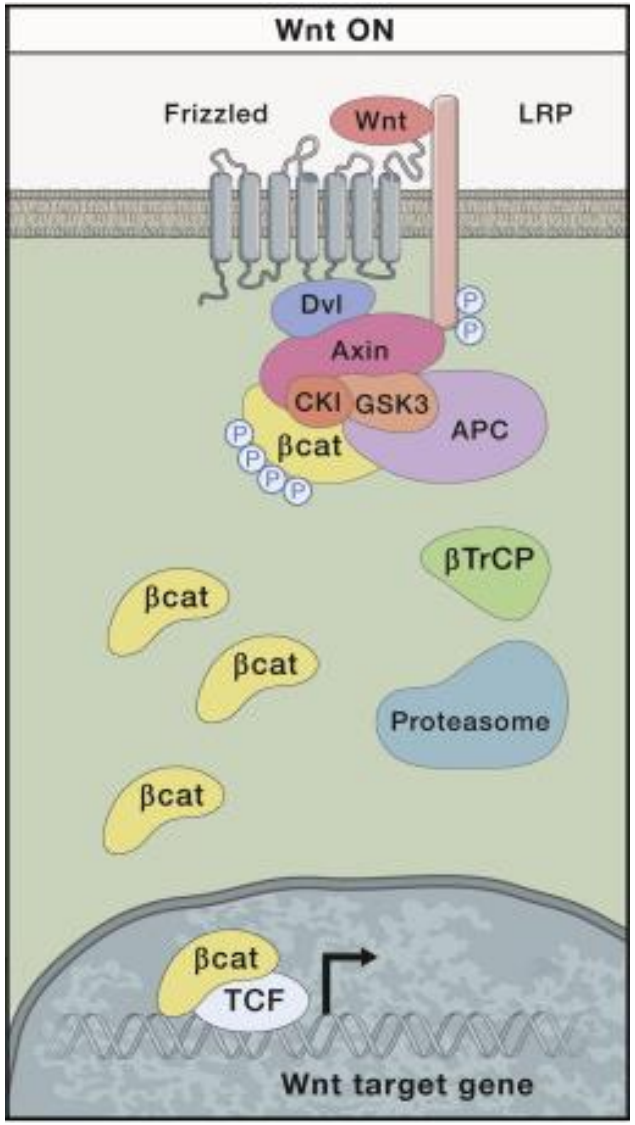
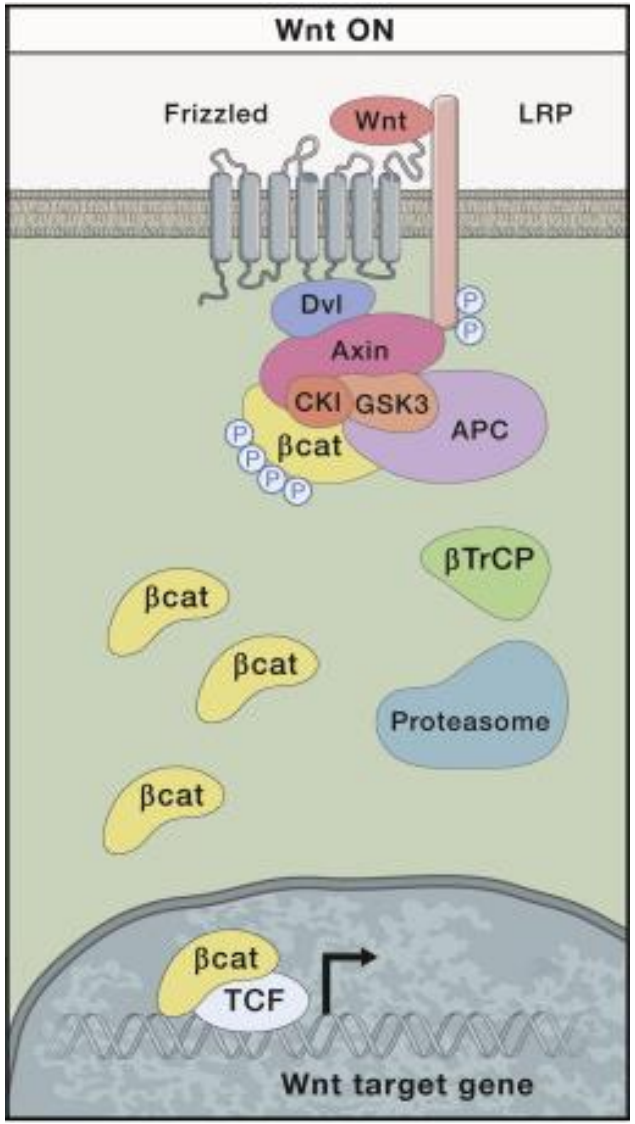
Testing WNT modulators

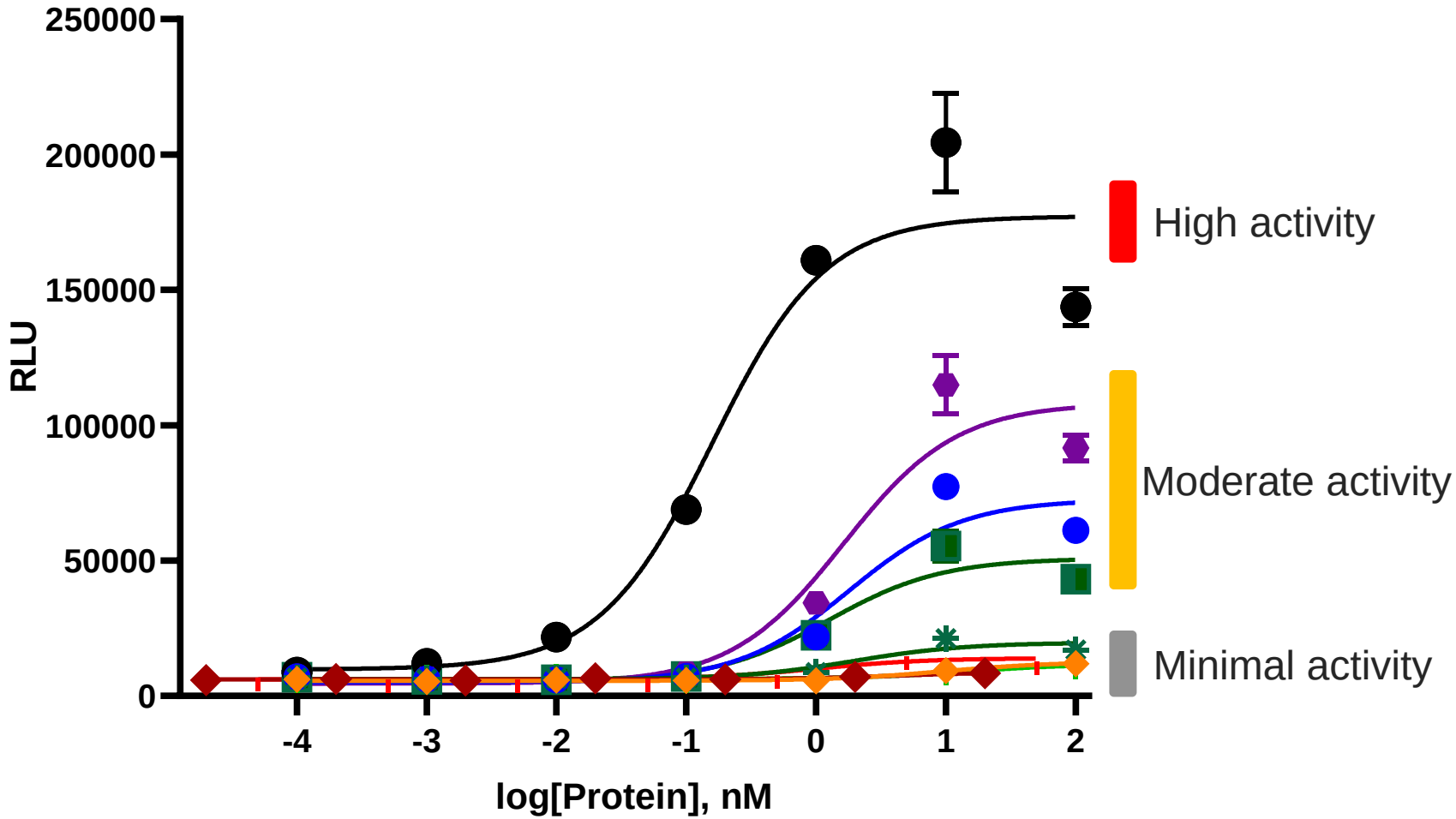


- TCF-Luciferase reporter constructs exist for canonical signaling
- Enables assaying for Fzd-specific signaling in different cellular backgrounds

Adapted from Nusse and Clevers, 2017.
https://doi.org/10.1016/j.cell.2017.05.016

Binders exhibit different potencies for wnt induction.





Adapted from Nusse and Clevers, 2017.
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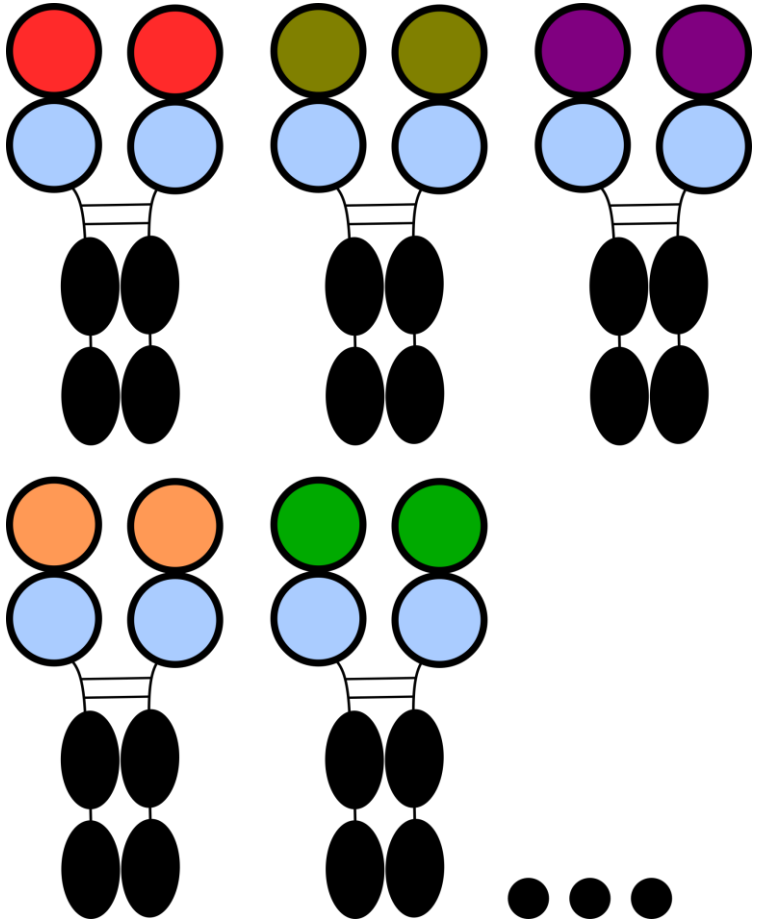
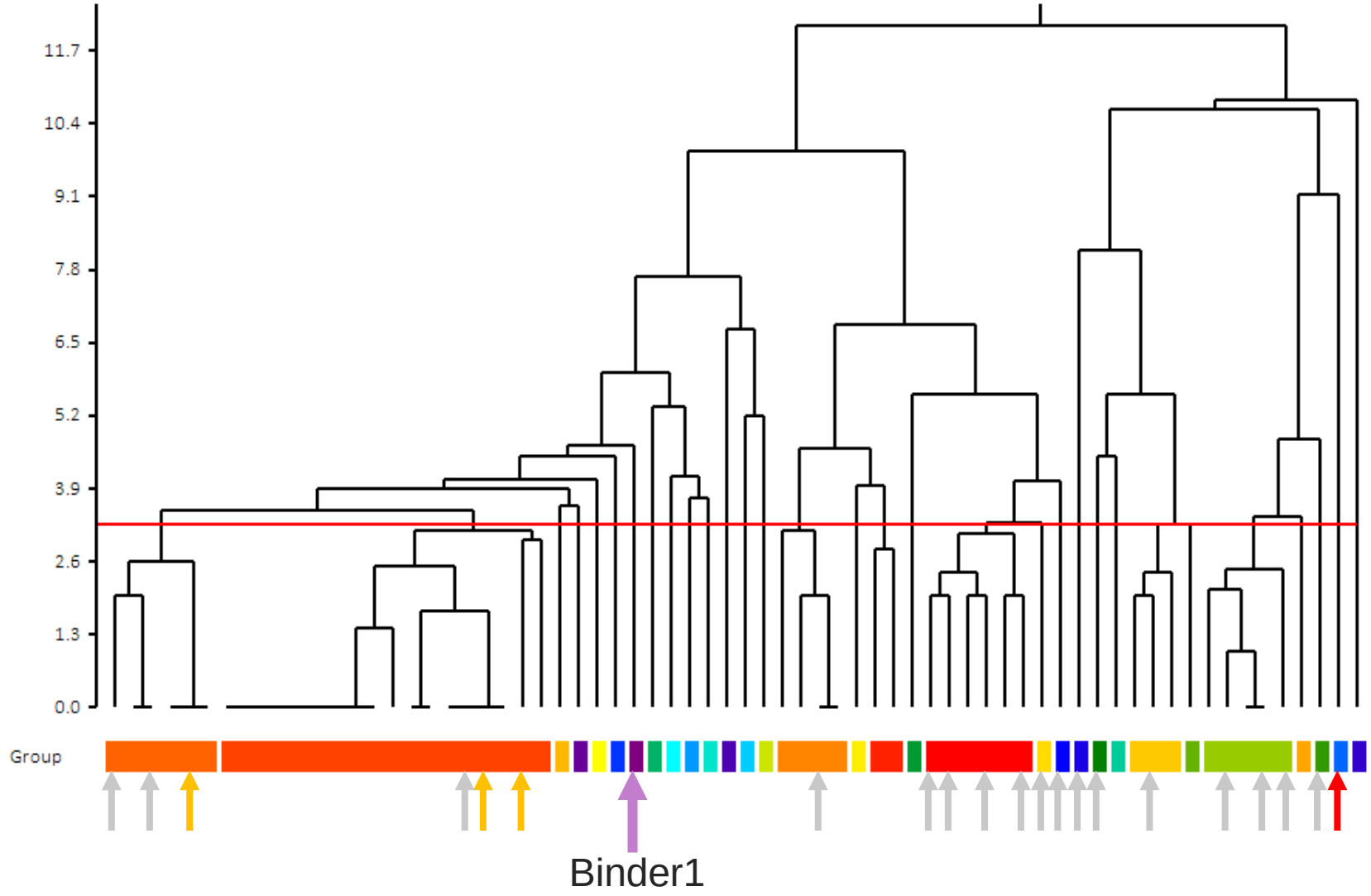
Community locations help understand activity ranks





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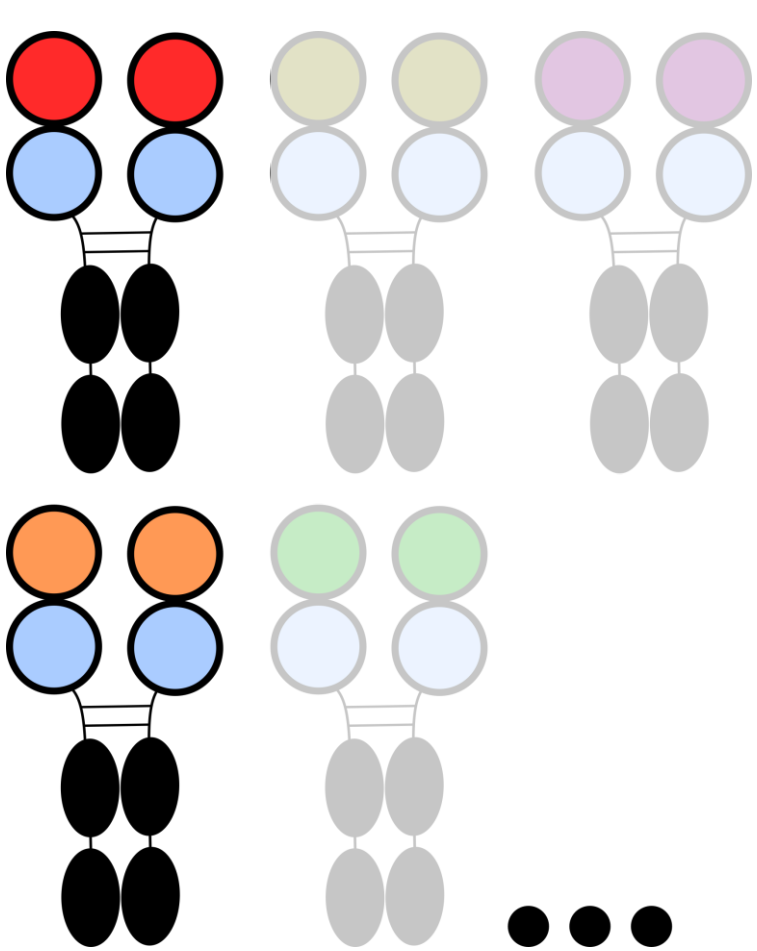
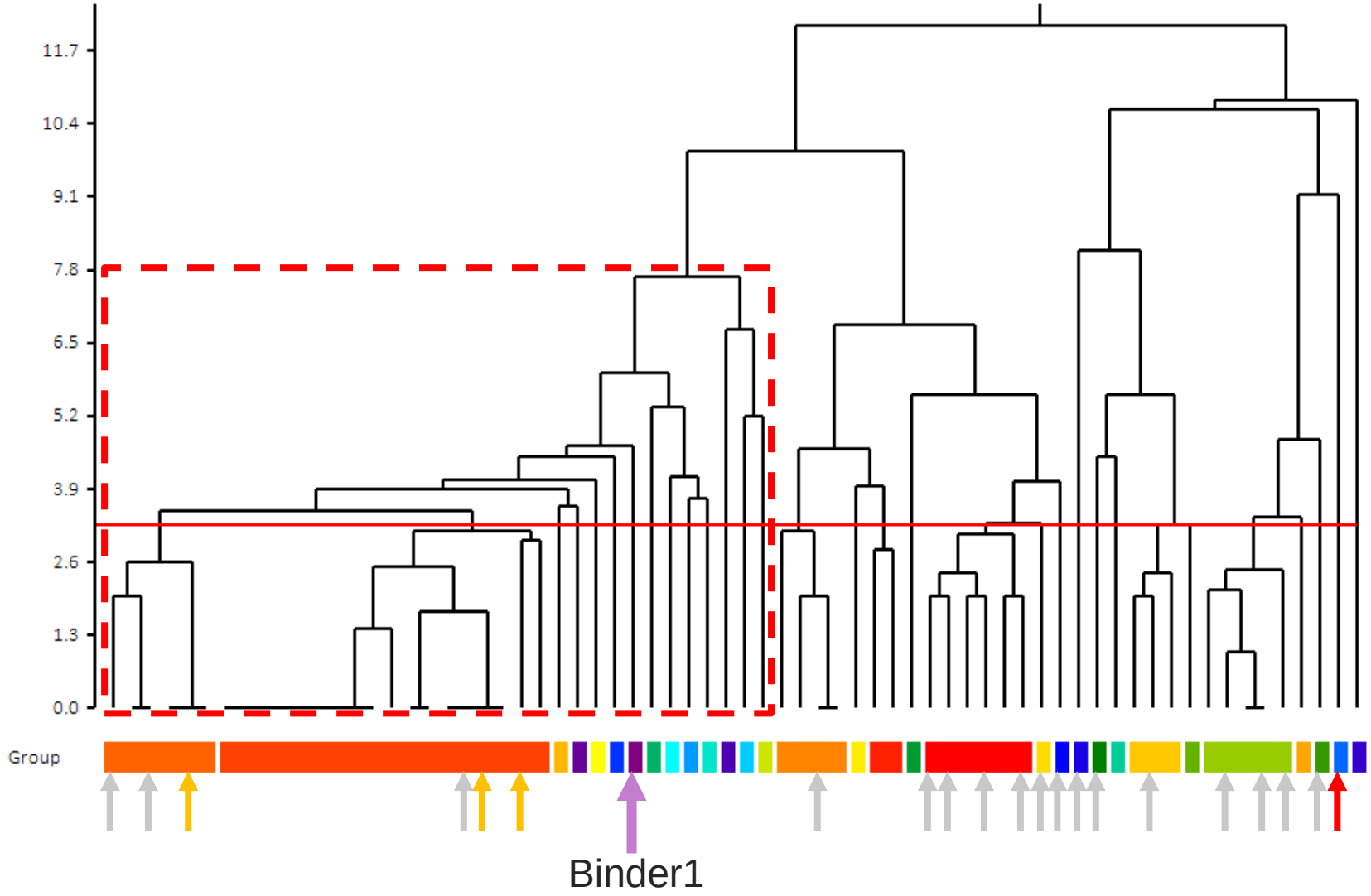
Focus future exploration on community that competes with “Binder1”





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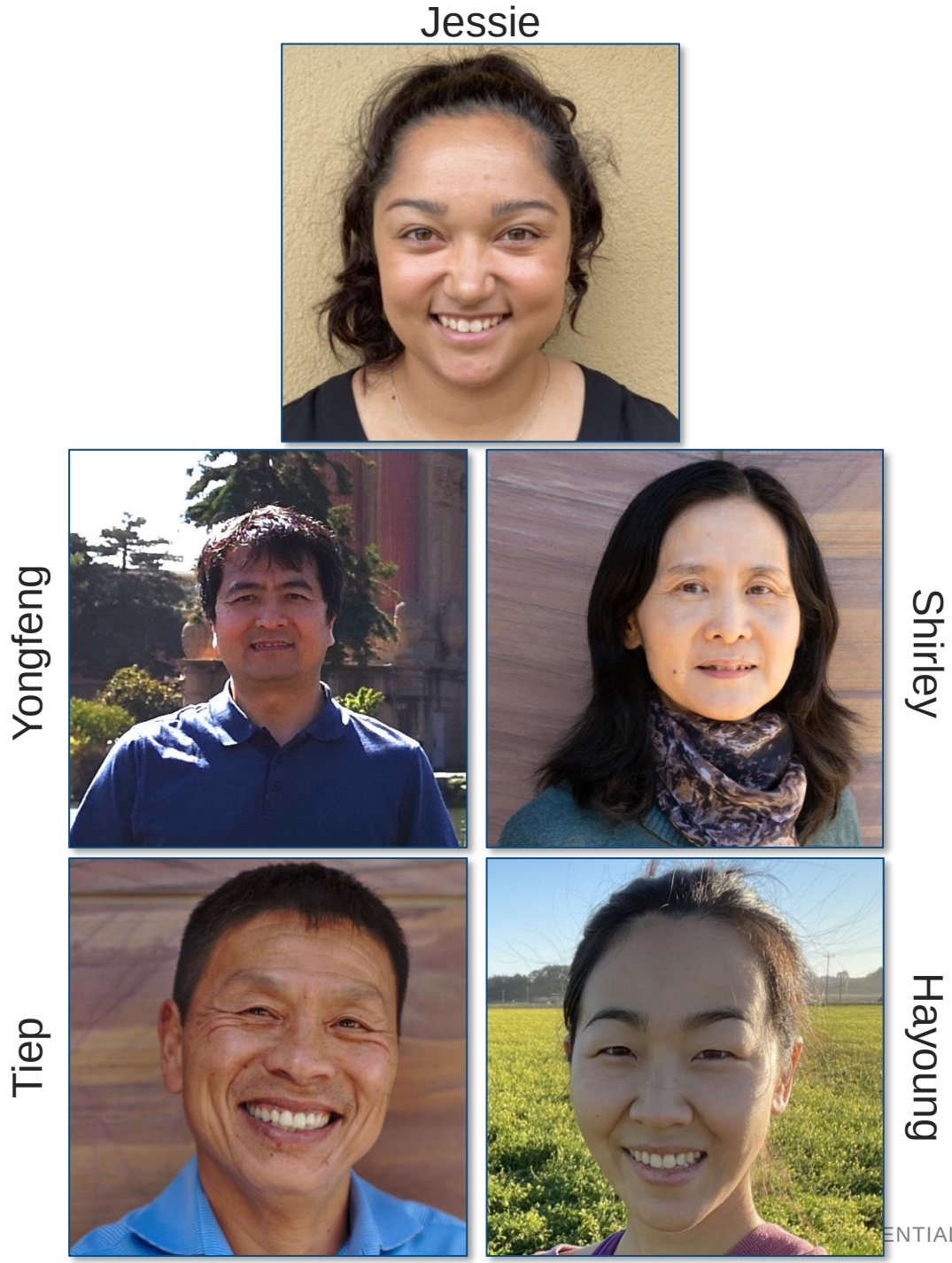
Thank you

- Surrozen is a regenerative medicine company focused on WNT pathway modulation
- High throughput SPR enables our molecule development pipeline
  - Binder discovery
  - Specificity characterization
  - Multi-specific format optimization
  - Developability polishing



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