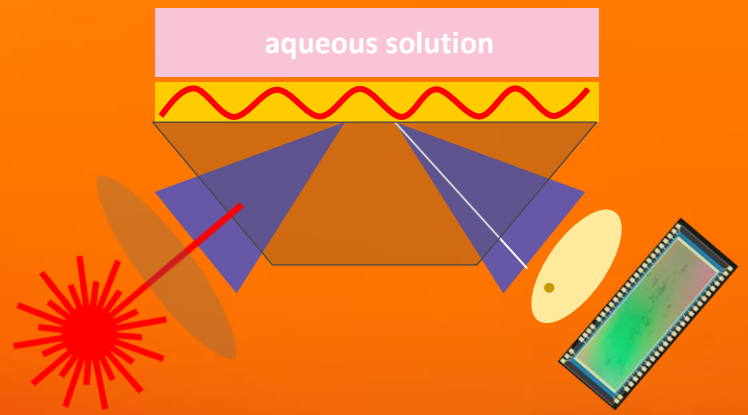


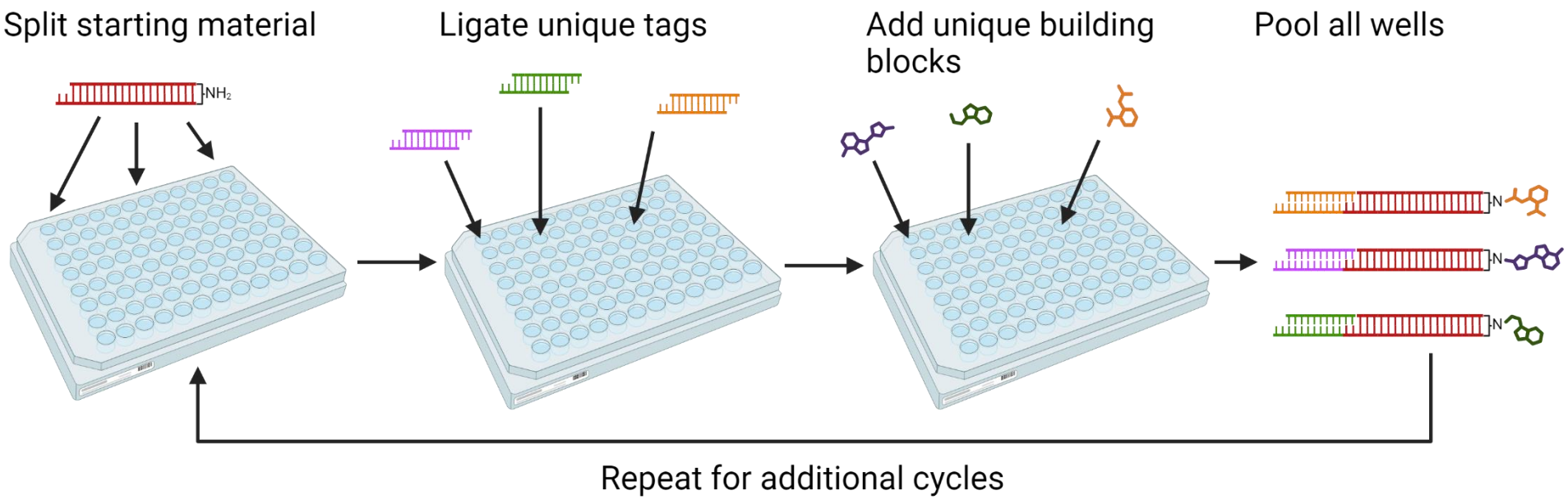
High throughput SPR in DNA-encoded library screening

Outline

- What is DNA-encoded library screening?
- What are our current workflows?
- Why Carterra LSA$^{XT®}$?

GSK

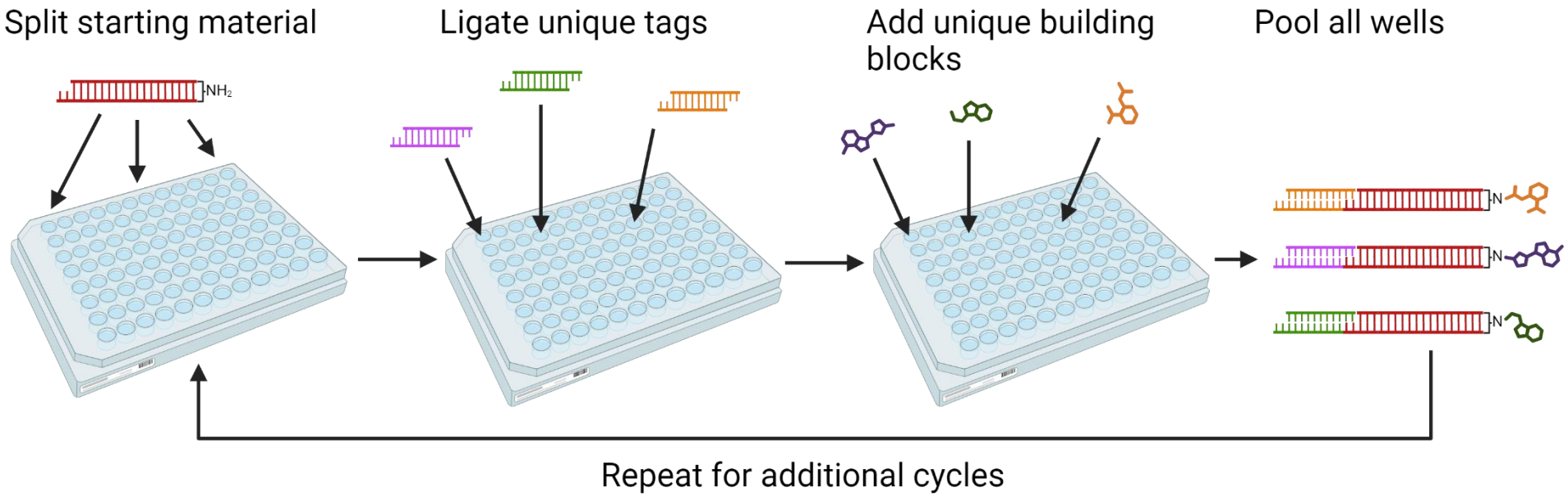
DNA Encoded library synthesis

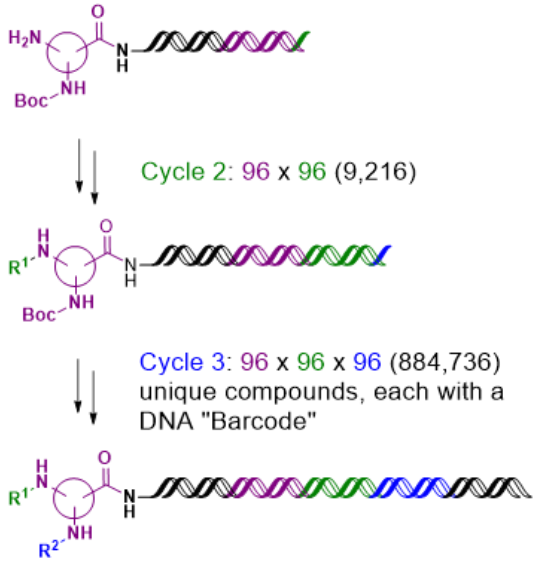


Adapted slide from Audrey Tolbert

Created with BioRender.com

DNA Encoded library synthesis

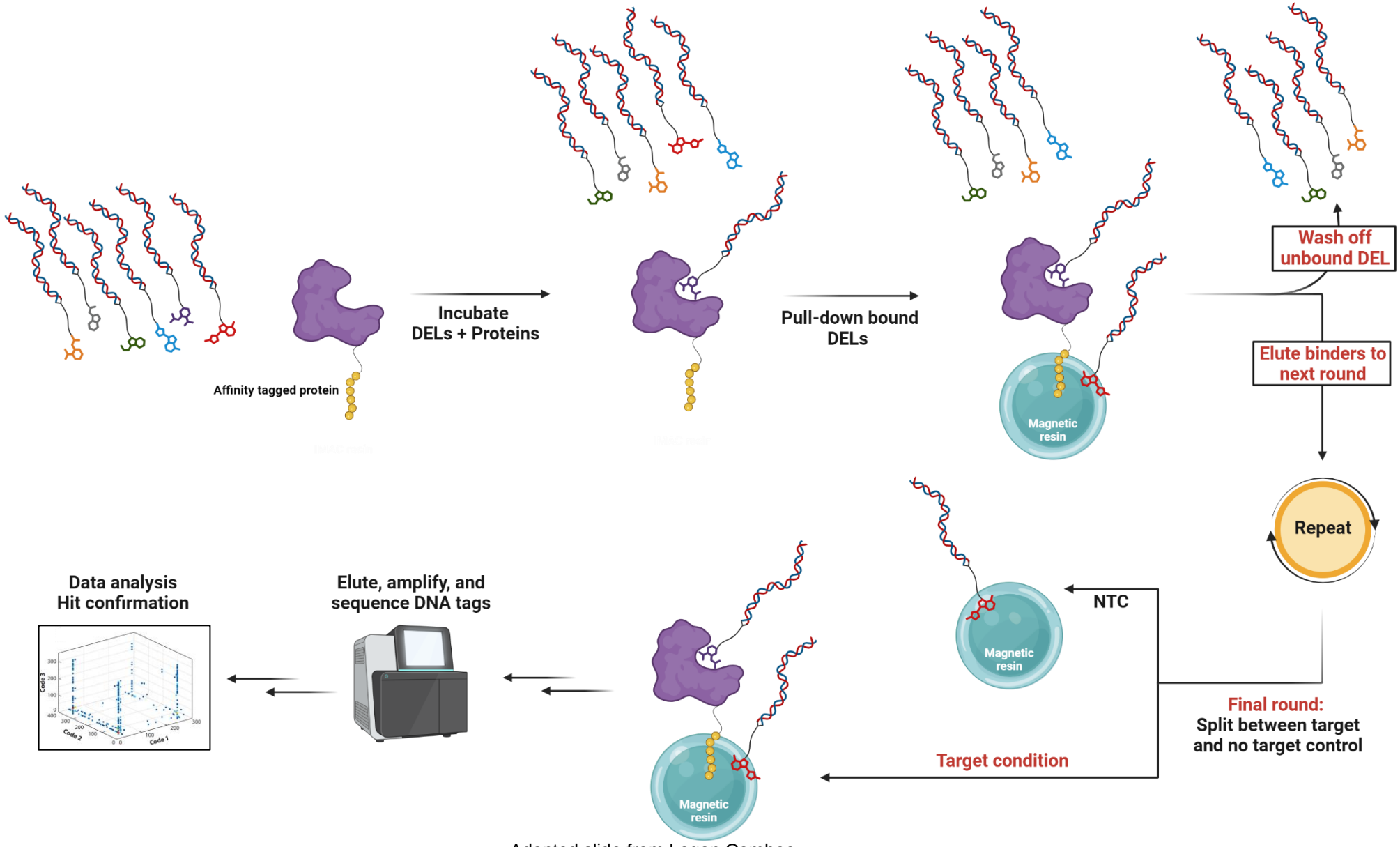




Adapted slide from Audrey Tolbert

Created with BioRender.com

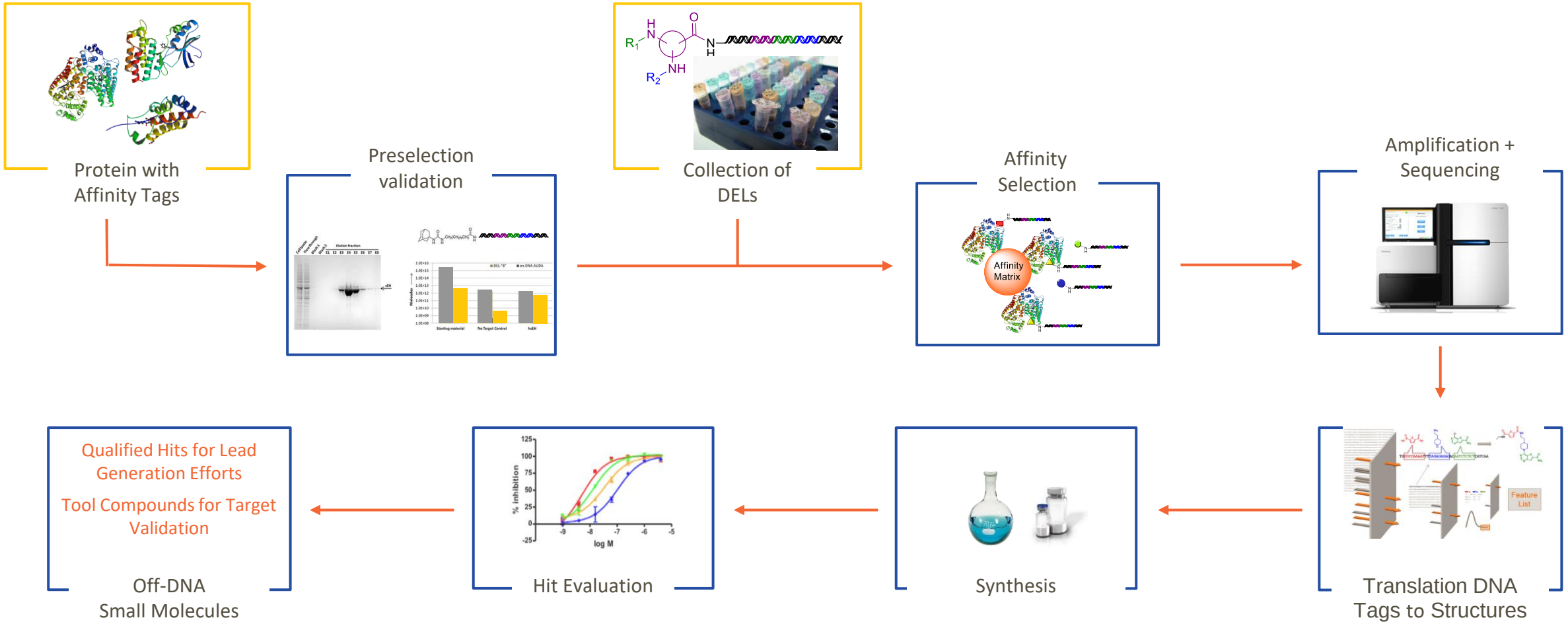
DNA Encoded library selections



Adapted slide from Logan Combee

Created with BioRender.com

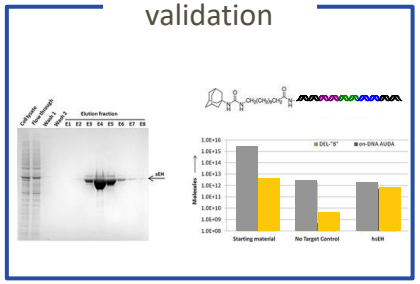
DNA Encoded Library workflow



Adapted slide from Melissa Grenier-Davies

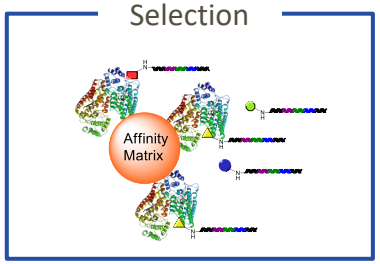
Biophysics in DNA Encoded Library screening

Preselection validation



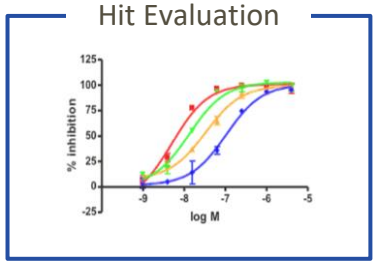
- Are the proteins folded?
  - CD
  - DLS
  - aSEC
  - DSF
  - HDx
- Do the proteins have expected function?
  - Tool binding?
  - Biochemical activity?

Affinity Selection



- Conditions?
  - Conformational state
  - Homo – / hetero – multimer?
  - Buffer/ pH / +/- tools/ cofactors?
- Modality specific considerations?
  - What is the most relevant data?

Hit Evaluation

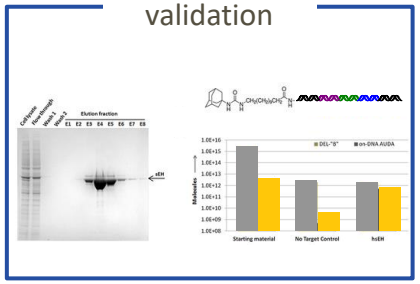


- What compounds get prioritized for off-DNA re-synthesis?
  - on-DNA synthesis
- Orthogonal binding assays
  - SPR
  - Thermal shift
  - MST
  - Proximity-based assays
  - FP
- Mechanism of action studies
  - Structure
  - Be-spoke biochemistry
- Cellular studies
  - Phenotypic readouts
  - CETSA

GSK

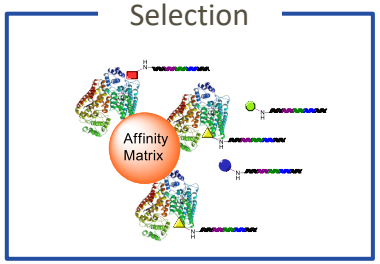
Biophysics in DNA Encoded Library screening

Preselection validation



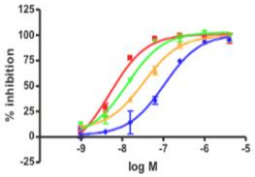
- Are the proteins folded?
  - CD
  - DLS
  - aSEC
  - DSF
  - HDx
- Do the proteins have expected function?
  - Tool binding?
  - Biochemical activity?

Affinity Selection



- Conditions?
  - Conformational state
  - Homo – / hetero – multimer?
  - Buffer/ pH / +/- tools/ cofactors?
- Modality specific considerations?
  - What is the most relevant data?

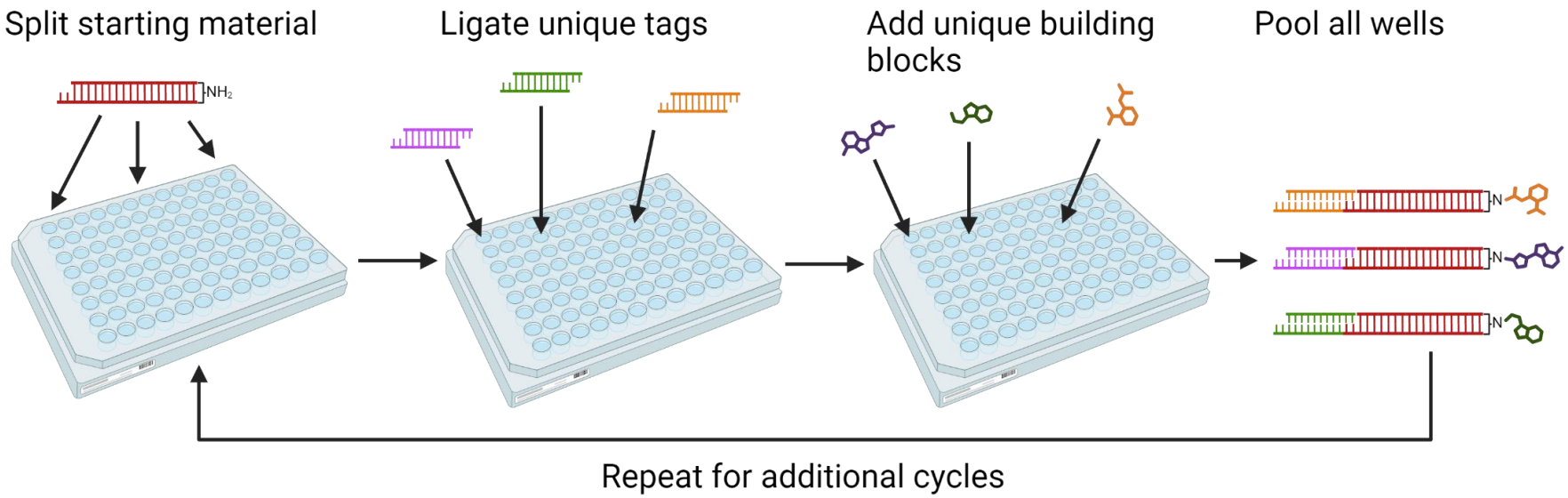
Hit Evaluation



- What compounds get prioritized for off-DNA re-synthesis?
  - on-DNA synthesis
- Orthogonal binding assays
  - SPR
  - Thermal shift
  - Proximity-based assays
  - MST
  - FP
- Mechanism of action studies
  - Structure
  - Be-spoke biochemistry
- Cellular studies
  - Phenotypic readouts
  - CETSA

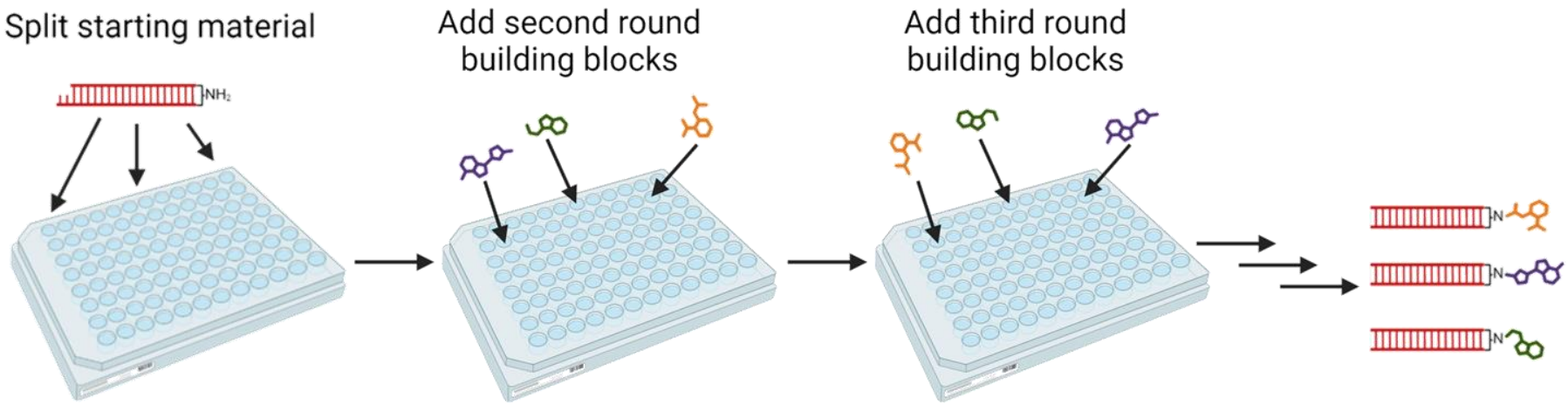
GSK

DEL-hits can be resynthesized with a DNA tag



Created with biorender.com

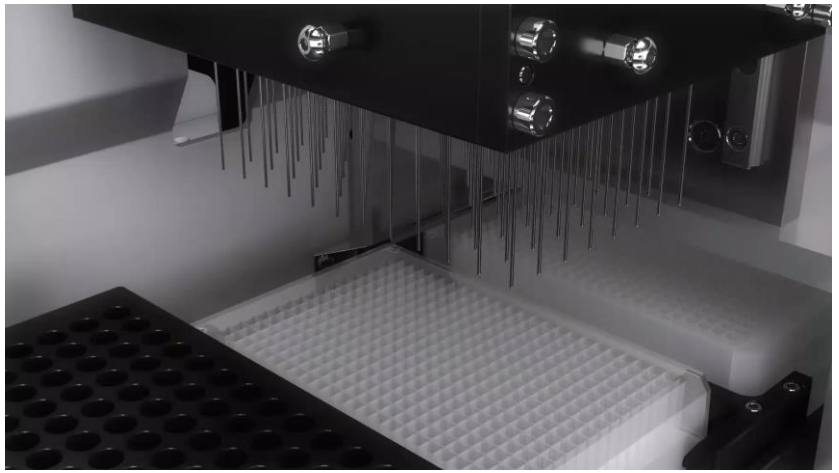
DEL-hits can be resynthesized with a DNA tag



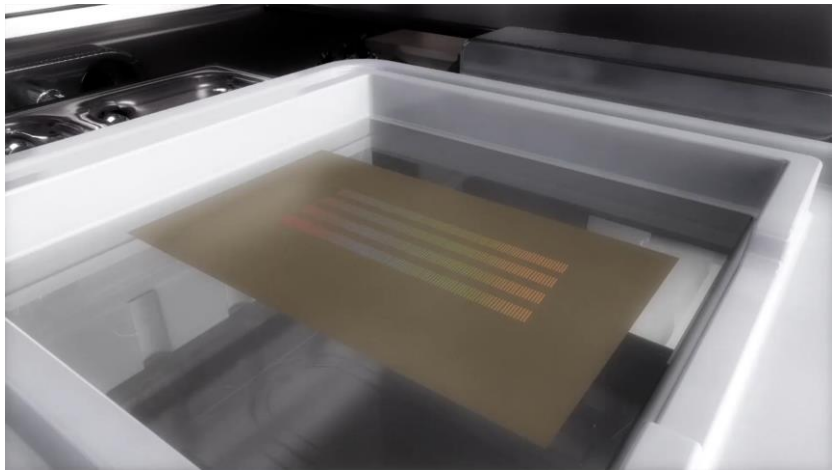
Created with biorender.com

Carterra LSA$^{XT®}$ allows massively parallel immobilization

Multi-channel mode

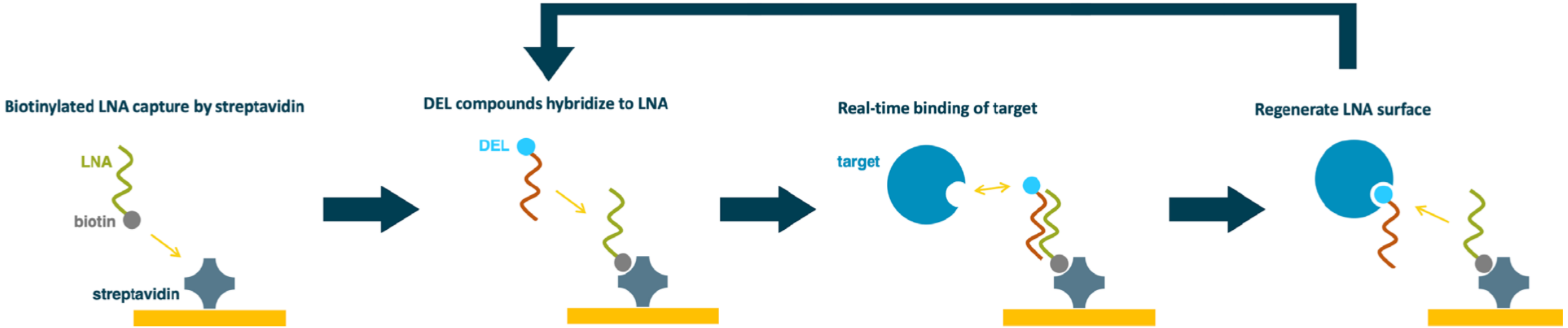


Single-channel mode



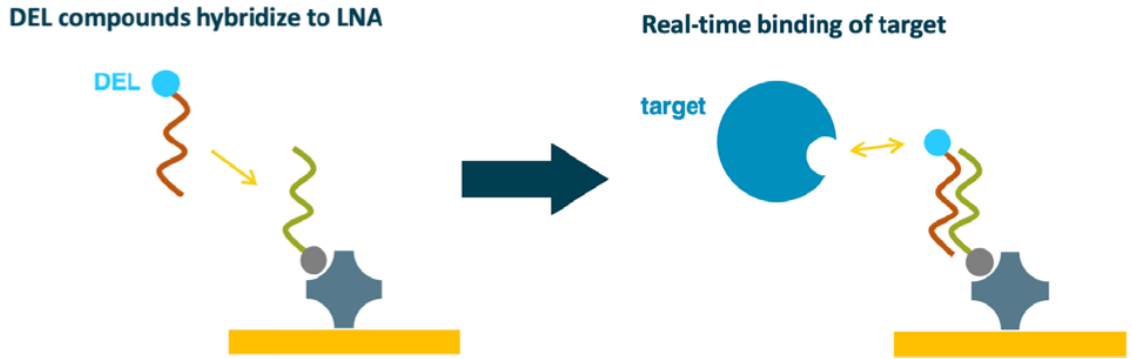
GSK

DNA barcode provides a handle for compound immobilization

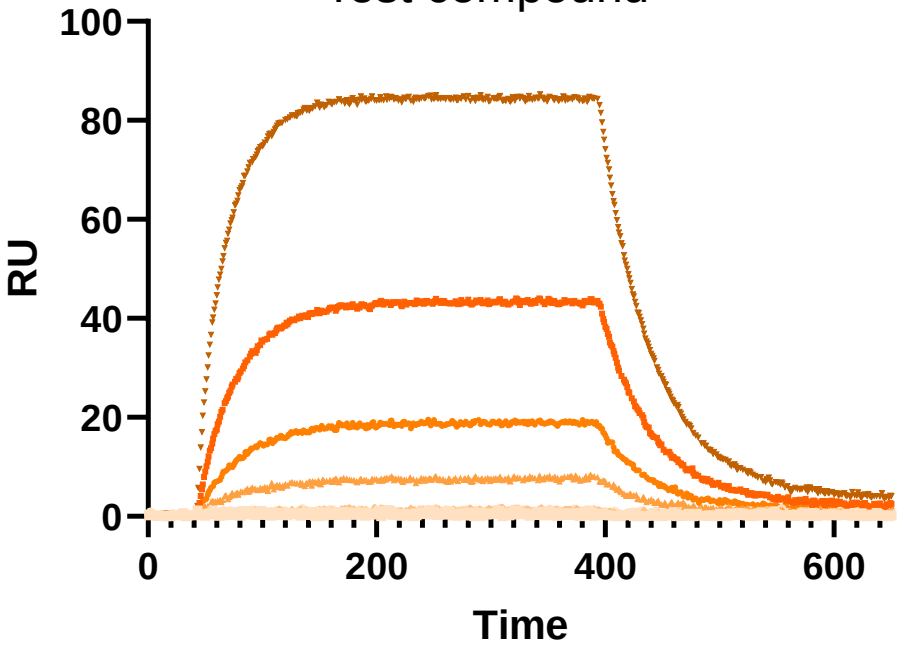


GSK

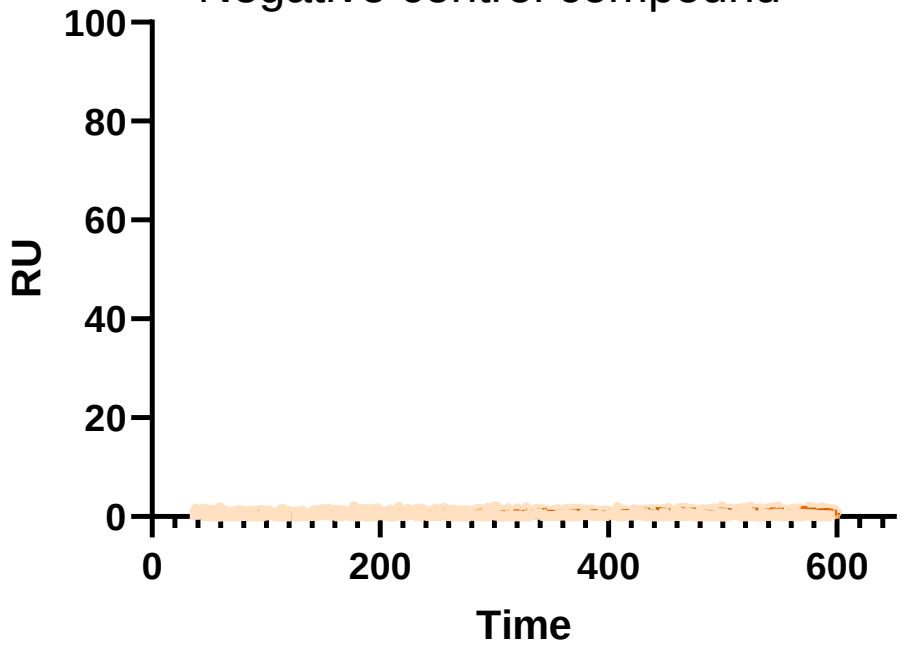
on-DNA compounds bind target



Test compound

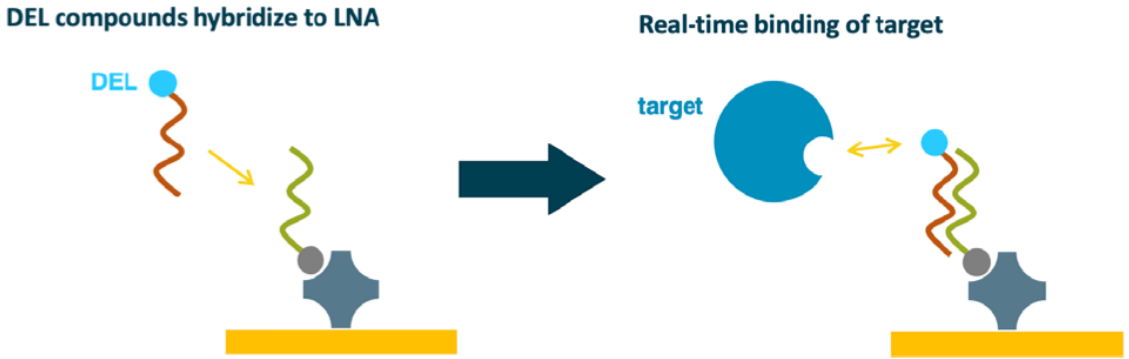


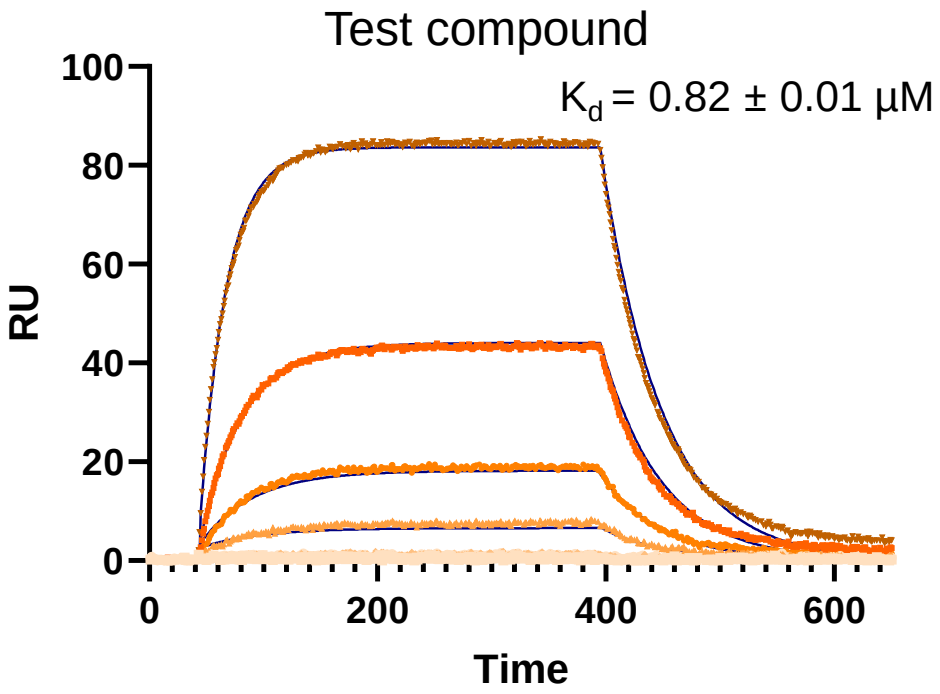
Negative control compound

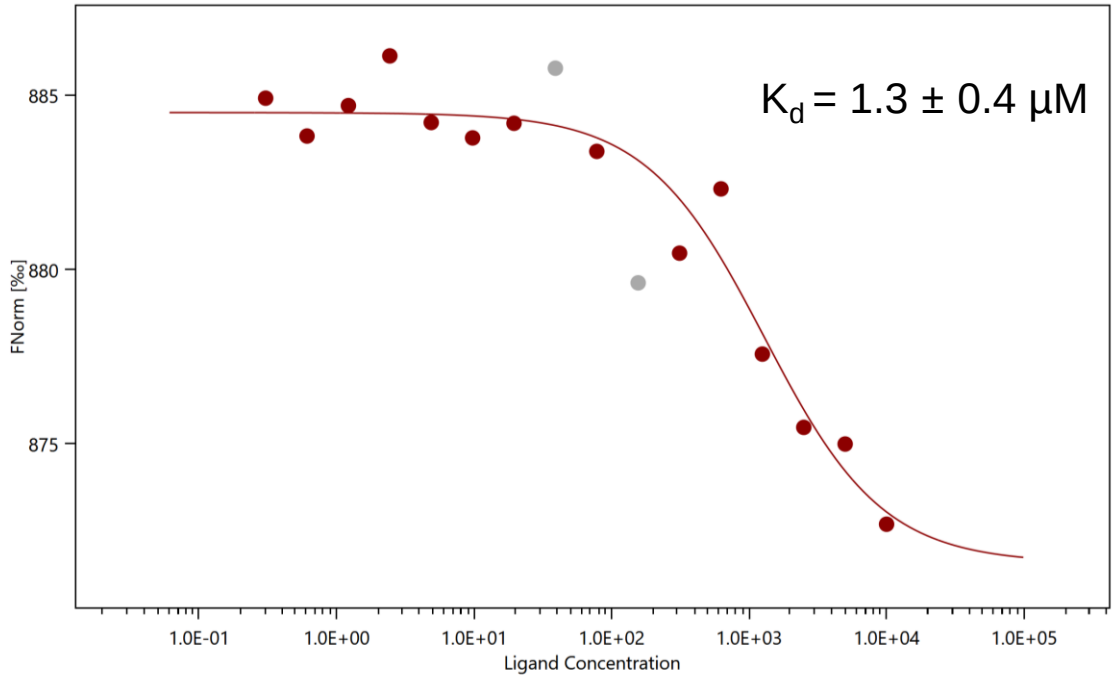


GSK

on-DNA compounds bind target

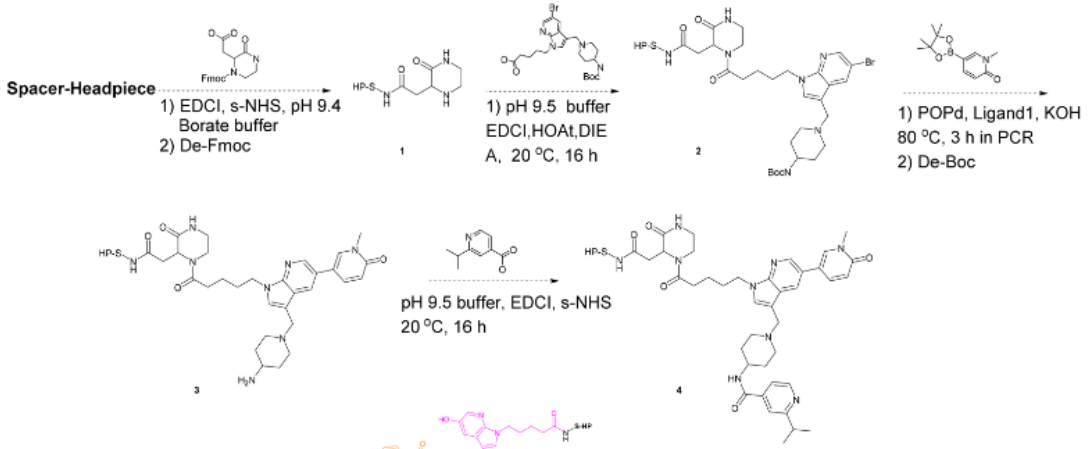






GSK

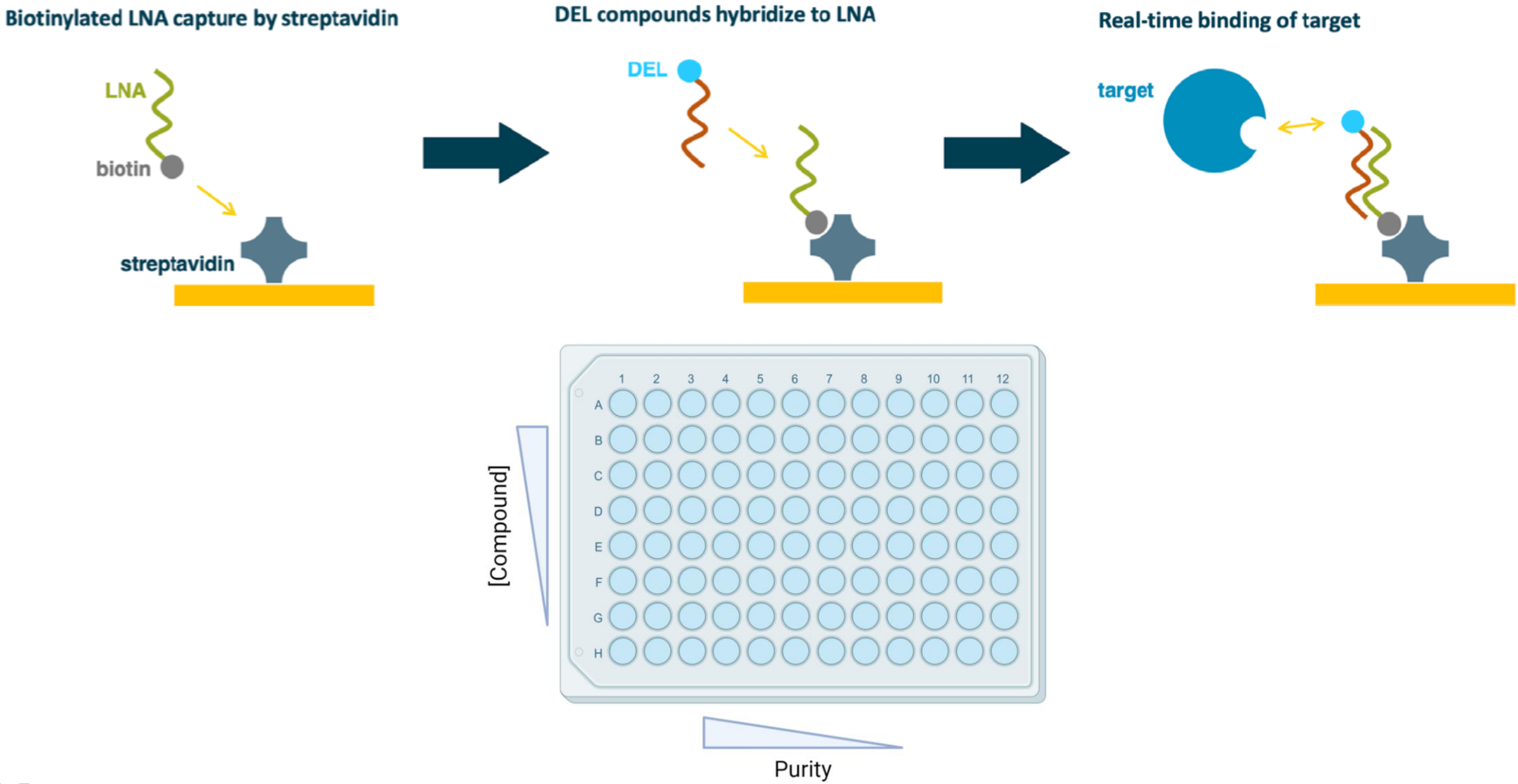
On-DNA compounds are impure mixtures



Product	Desired product	Intermediate /byproduct-1	Intermediate /byproduct-2	Intermediate /byproduct-3	Intermediate /byproduct-4
Retention time	2.14/2.34	1.37/1.48	1.65	1.86	2.63
Intensity	7.08%/35.96%	9.08%/30.67%	3.95%	4.15%	6.18%
M.W.	5890.4	5743.7	5659.8	5330.7	5843.4

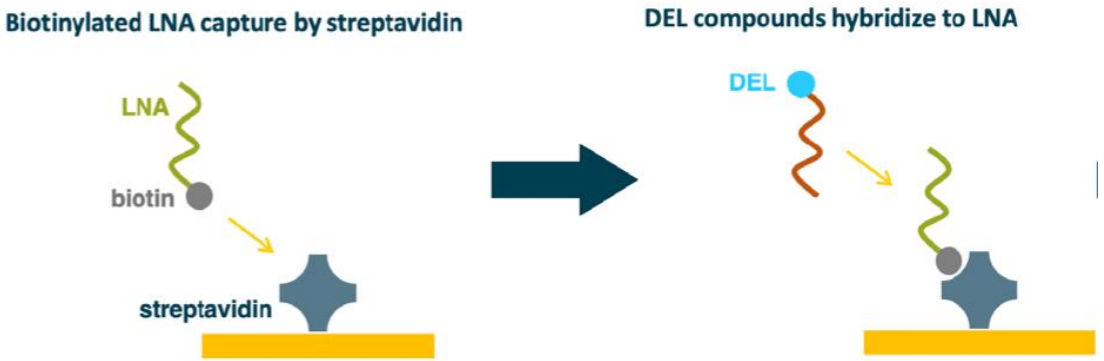
Su et al., Bioconjugate Chemistry, 2021, 32, 5, 1001-1007

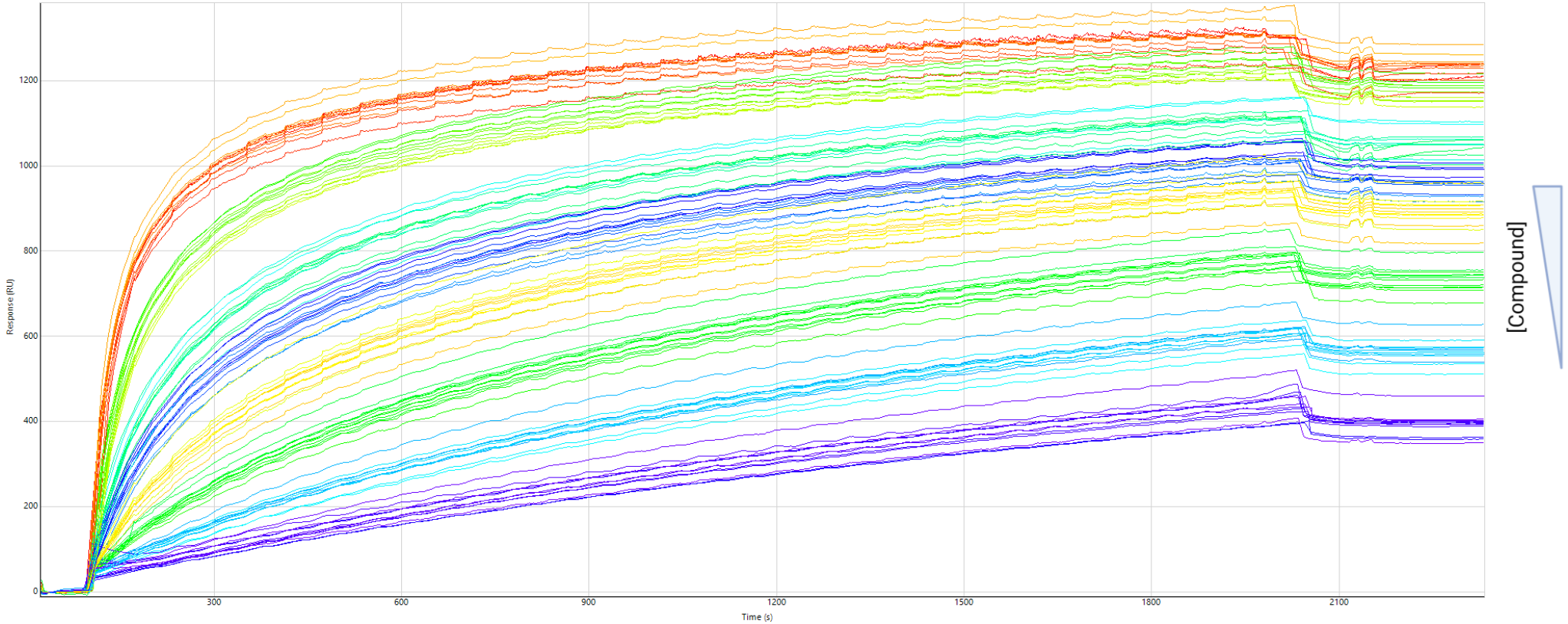
Can we get kinetics with impure immobilized ligands?



*data collected using a SAD200M chip; figure created with biorender.com

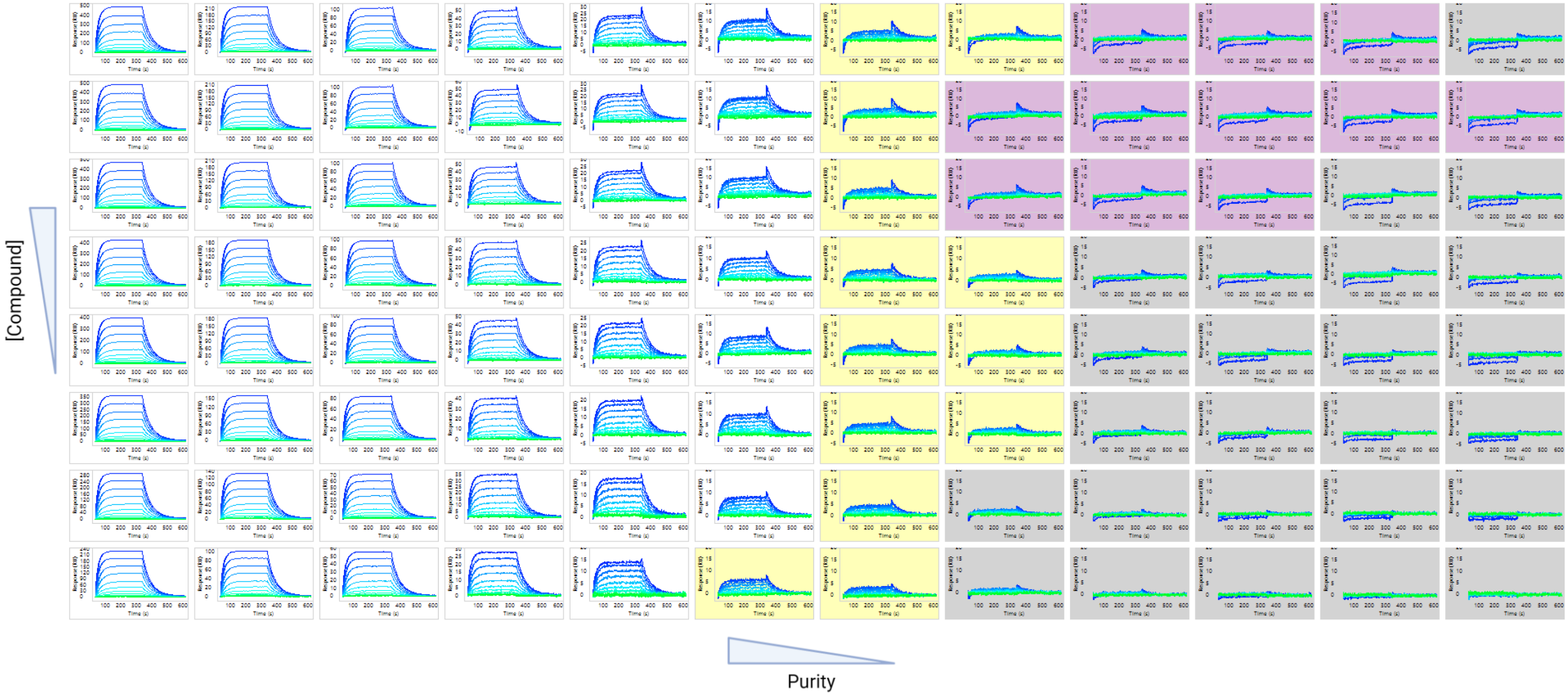
DNA barcode provides a handle for compound immobilization





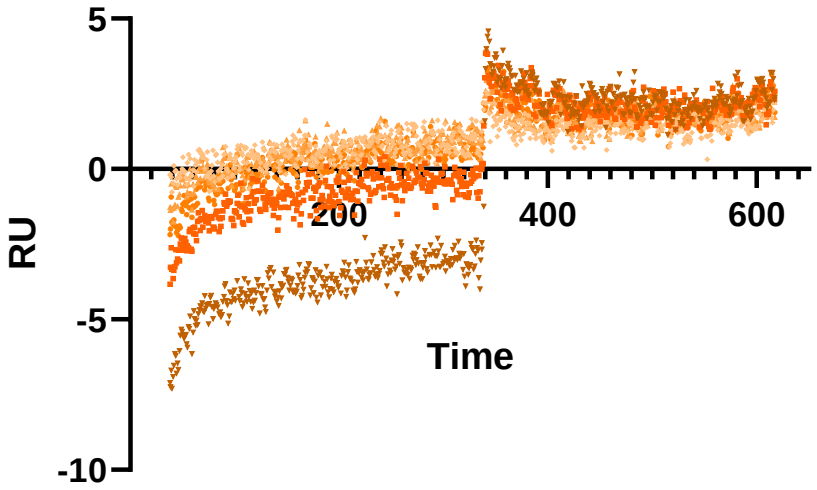
GSK

Can detect evidence of binding at < 1% purity!

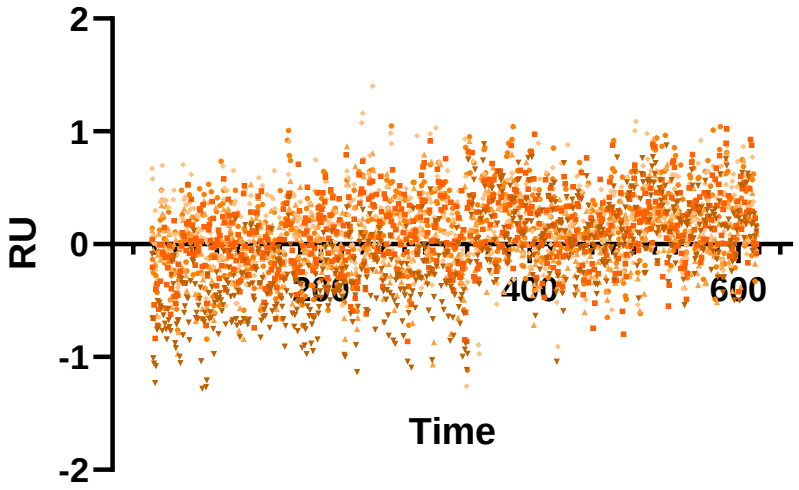


Higher surface densities allow flagging low-purity binders

0.04% purity, high density



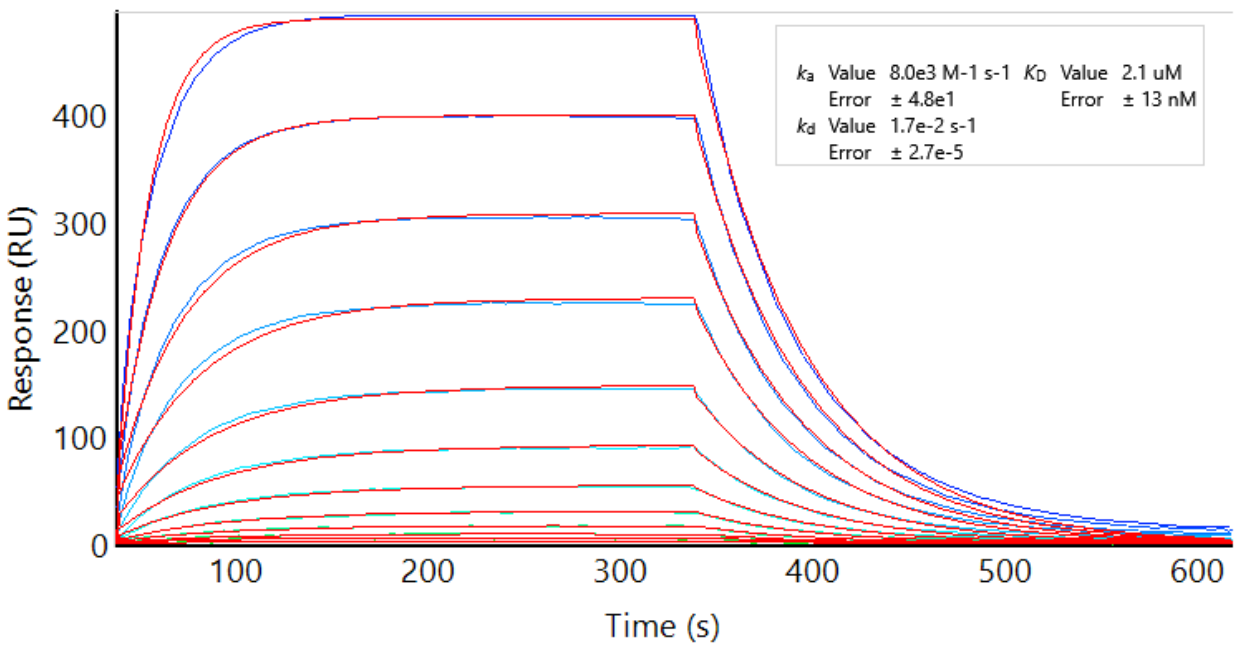
0.04% purity, low density



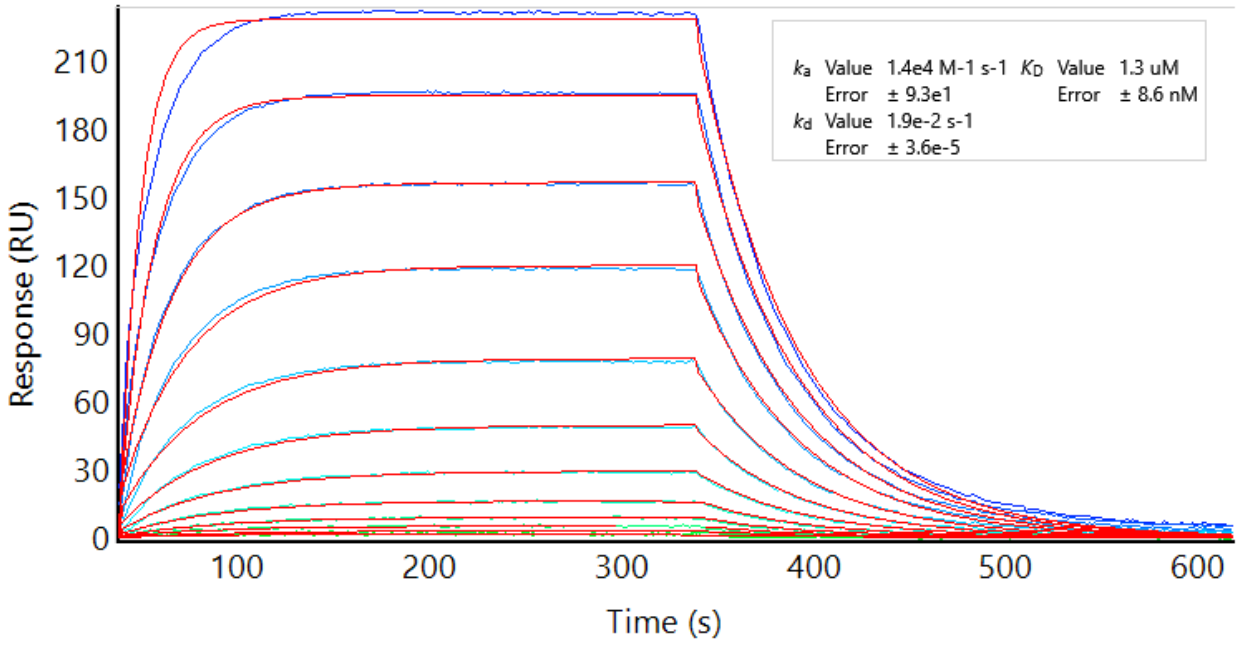
GSK

Lower surface densities enable better kinetics

80% purity, high density



80% purity, low density



GSK

Conclusions

- Biophysical assays play an important role in every step of a screening campaign
- On-DNA resynthesis and Carterra LSA$^{XT®}$ combine to allow high-throughput binding assays
- This enables us to make decisions on chemistry resource investments, including synthesis of compounds that may not have been obvious during data analysis
- “reversed” assay can improve signal-to-noise, opening the door to traditionally harder targets

GSK

Acknowledgements

GSK

Joshua Alper (now at Magnet)
Mark Mantell
Lisa Marcaurelle
Chris Dimitri
Robert Hale

Carterra

Nicholas Abuid
Perry Ripa

GSK

Thank you! Questions?

GSK

gsk.com