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High-throughput SPR assay for FcγR binding to drug candidates on the new Carterra LSA^{XT}

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About me / About Dragonfly

- Graduated Northeastern with B.S. in Bioengineering in 2022
- Associate Scientist at Dragonfly for 2+ years
- Dragonfly founded in 2016
  - Multiple clinical-stage therapies using TriNKET and cytokine platforms
  - Partners include BMS, Merck, Gilead, AbbVie

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Outline

- Importance of FcγR characterization for Fc-containing molecules
- LSA^{XT} implementation for FcγR screening
- Human and cynomolgus assay design and results
- Comparison of LSA^{XT} to Biacore 8K
- Conclusions

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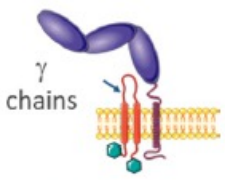
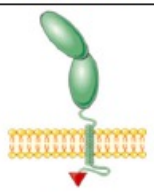
Fc gamma receptor (FcγR) binding is a critical component in antibody mediated immune activity

- FcγRs are essential in ADCC, ADCP, and CDC activity.
- FcγR binding should be characterized for all Fc-containing molecules.

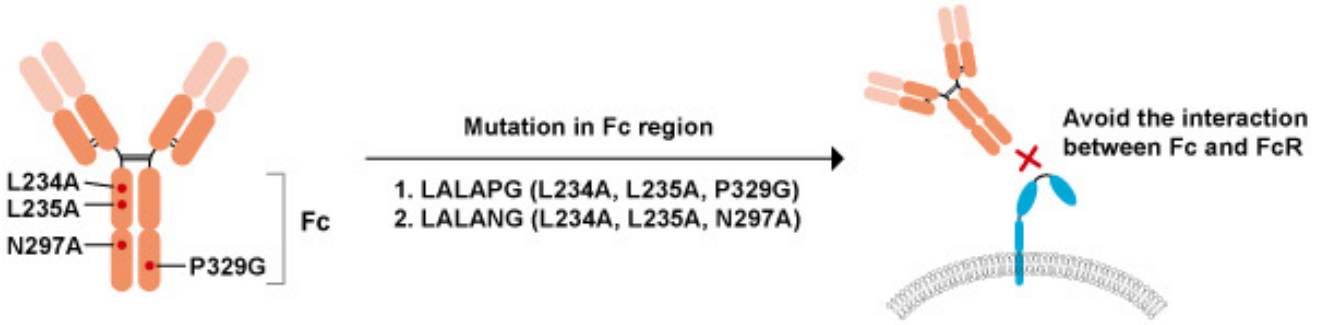
Receptor	FcγRI	FcγRII			FcγRIII	
		FcγRIIa	FcγRIIb	FcγRIIc	FcγRIIIa	FcγRIIIb
Structure						
Signal	ITAM	ITAM	ITIM	ITAM	ITAM	-
Polymorphism	-	R131H	I232T	Q57X	V158F	NA1, NA2, SH
Affinity for IgG	High (10^{-8}–10^{-9} M)		Low (10^{-6} M)		Low (10^{-6}–10^{-7} M)	

Forest-Nault *et al.* (Int J Mol Sci, 2021)

FcγR binding analysis is crucial in antibody and Fc-fusion therapeutic development

FcγR binding assessment provides critical information into wild-type or engineered Fc domains. Routine assessments include:

1. Wild-type FcγR binding determination
2. Cross-reactivity to relevant species FcγRs
3. Impact of Fc mutations (FcγR silencing, enhancing, etc.)



From Abnova

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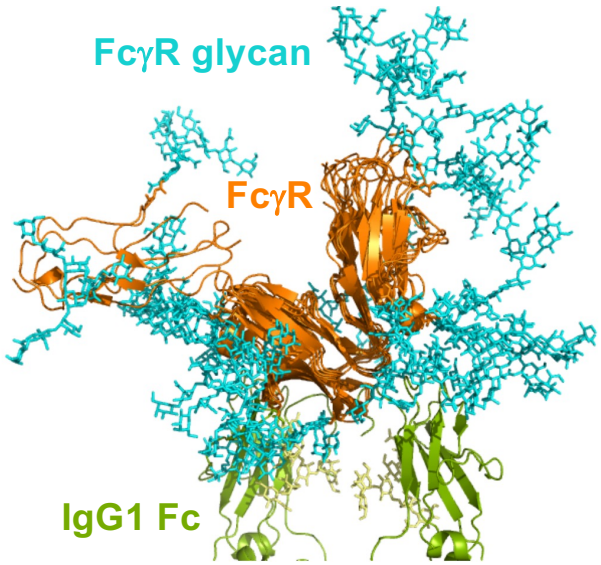
Mutations augmenting effector function modulate binding affinity for multiple FcγRs



		Effector Function vs. WT		Binding Affinity vs. WT				
Fc Modification	ID	ADCC	ADCP	FcγRIIA^{H}	FcγRIIA^{R}	FcγRIIB	FcγRIIIA^{F}	FcγRIIIA^{V}
S298A/E333A/K334A	AAA	↑	n.d.	↓↓	n.d.	↓↓	↑↑	↑
Afucosylation (Potelligent)	Potelligent	↑↑	n.d.	n.d.	n.d.	n.d.	↑↑↑	n.d.
S239D/I332E	DE	↑↑	↑	n.d.	n.d.	↑↑↑	↑↑↑	↑↑↑↑
S239D/A330L/I332E	DLE	↑↑↑	↑	n.d.	n.d.	↑↑↑	↑↑↑	↑↑↑↑
G236A	G236A	–	↑	↑↑	↑↑	↑	–	–
G236A/S239D/I332E	ADE	↑	↑	↑↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑
G236A/A330L/I332E	GAALIE	d.n.s.	n.d.	↑↑	↑↑	↓↓	↑↑	↑↑
G236A/S239D/A330L/I332E	GASDALIE	n.d.	n.d.	↑↑↑	↑↑↑	↑	↑↑↑	↑
F243L/R292P/Y300L/V305I/P396L	LPLIL	↑↑	n.d.	↑↑	↑	↑	↑↑↑	↑↑↑
L235V/F243L/R292P/Y300L/P396L	VLPLL	↑	n.d.	↑	↓↓	↓↓	↑↑	↑

Hayes *et al.* (Journal of Inflammation Research, 2016)

Liu *et al.* (Antibodies, 2020)

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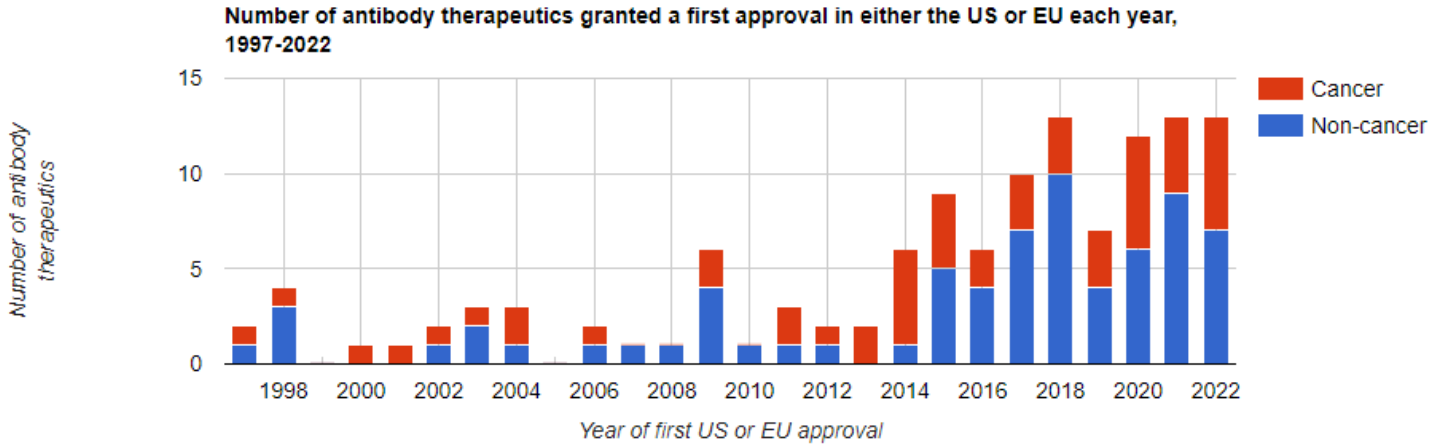
Fc-containing biologics are a substantial and growing class of drugs

- 175+ antibody therapeutics approved or under regulatory review globally
- US market alone valued at $180 billion (2021)
  - Compound annual growth rate of 14%



The Antibody Society (2023)

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Why consider improving the FcγR characterization workflow at Dragonfly?

- FcγR characterization time:
  - Screening human/cyno FcγR panel on 8K takes one week
- User lab time:
  - Each day a new experiment must be set up
- Protein requirements:
  - Nearly 16mg of each antibody required
- Sensor chips:
  - 6 chips required per antibody

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Could Carterra LSA^{XT} help with the FcγR workflow at Dragonfly?

100x the data | 10% the time | 1% the sample, *plus*:

- Enhanced signal-to-noise → Ability to reduce surface densities
- Increased signal uniformity → Confidence in replicates
- Faster data collection rate → Accurately describe rapid kinetics

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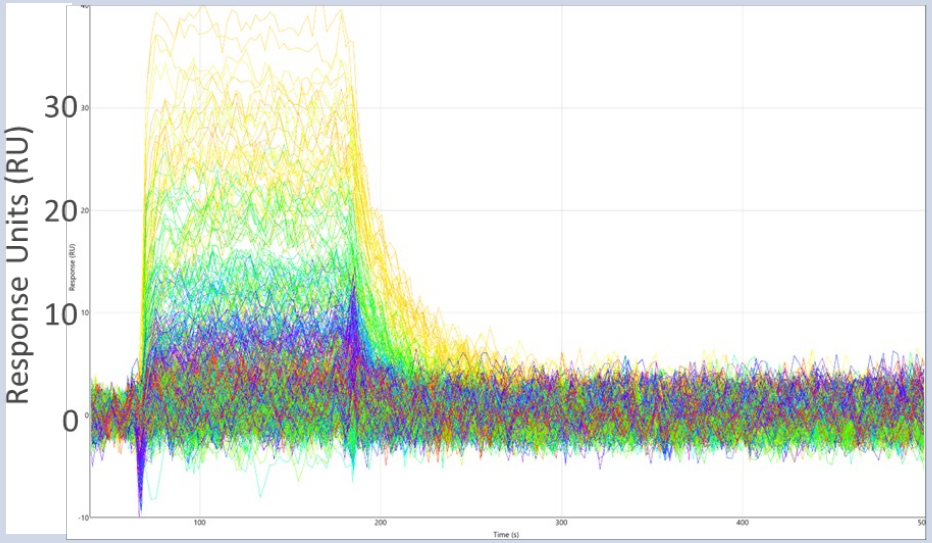
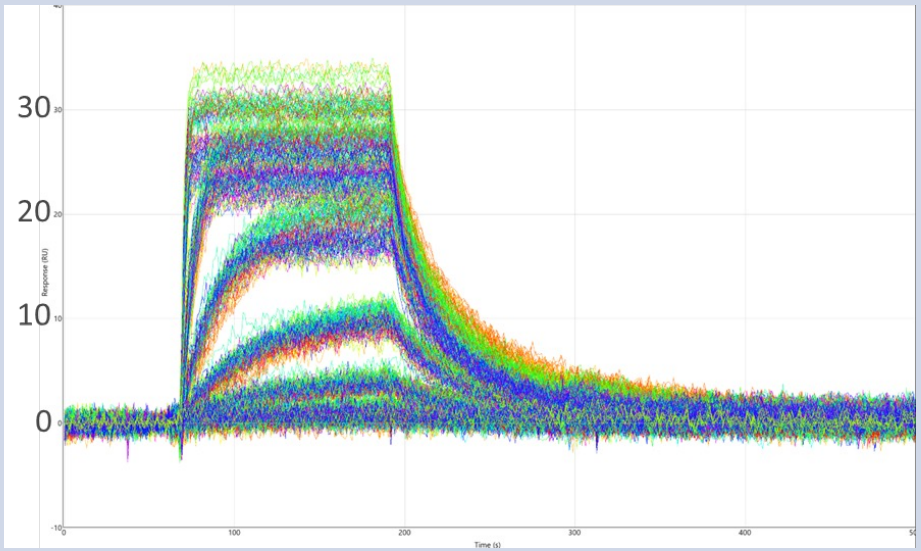
LSA^{XT} demonstrates increased data resolution and reproducibility over LSA

IL-2 binding to ILRα



LSA
12 replicates overlaid



LSA^{XT}
96 replicates overlaid

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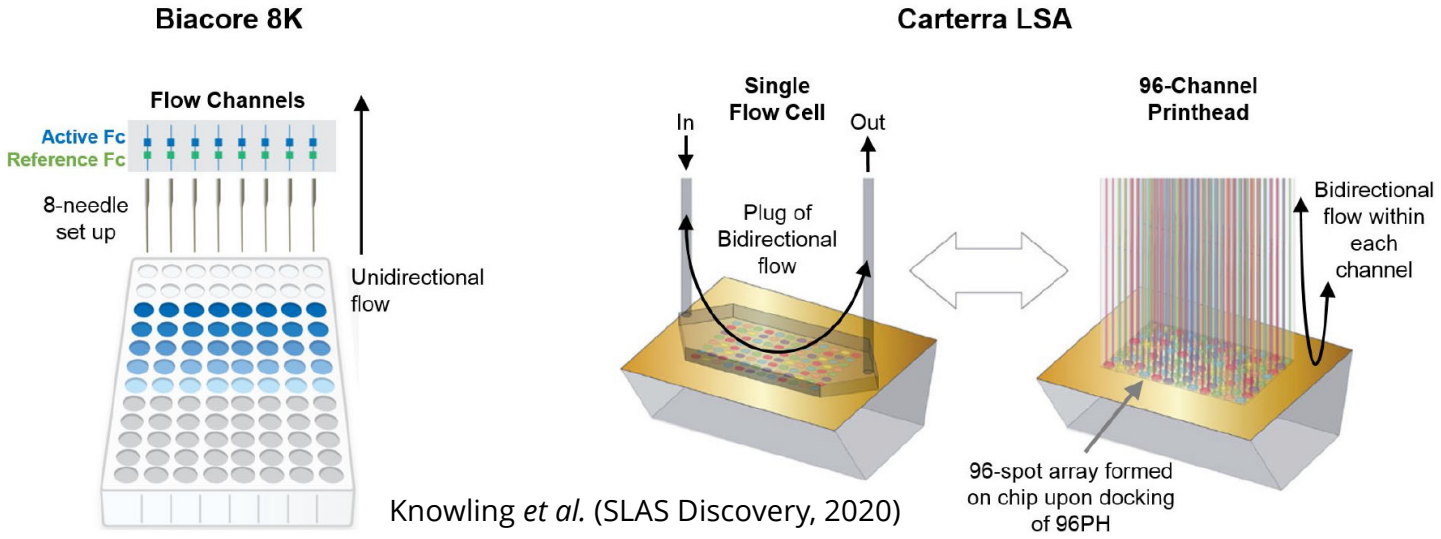
*Data provided by Carterra

Major differences in instrument design allow for higher throughput with Carterra LSA^{XT}

- Biacore 8K provides high-resolution kinetics, but flow cell design limits ligand capacity to 8.
- Carterra LSA^{XT}'s flow cell design can capture 384 unique ligands with now improved sensitivity.



Knowling *et al.* (SLAS Discovery, 2020)

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Similar assay conditions were established to directly compare between instruments

- Test trastuzumab (IgG1 mAb) binding against 11 FcγRs.
- Both instruments ran at 22˚C with streptavidin-coated chips.
- All receptors are biotinylated and provided by ACRO Biosystems.

Species	Receptor Tested
Human	CD16a (V & F)
Cynomolgus	CD16
Human	CD16b (NA1 & NA2)
Human	CD32a (H & R)
Human & Cynomolgus	CD32b
Human & Cynomolgus	CD64*

*Performed on Biacore biotin CAPture chip

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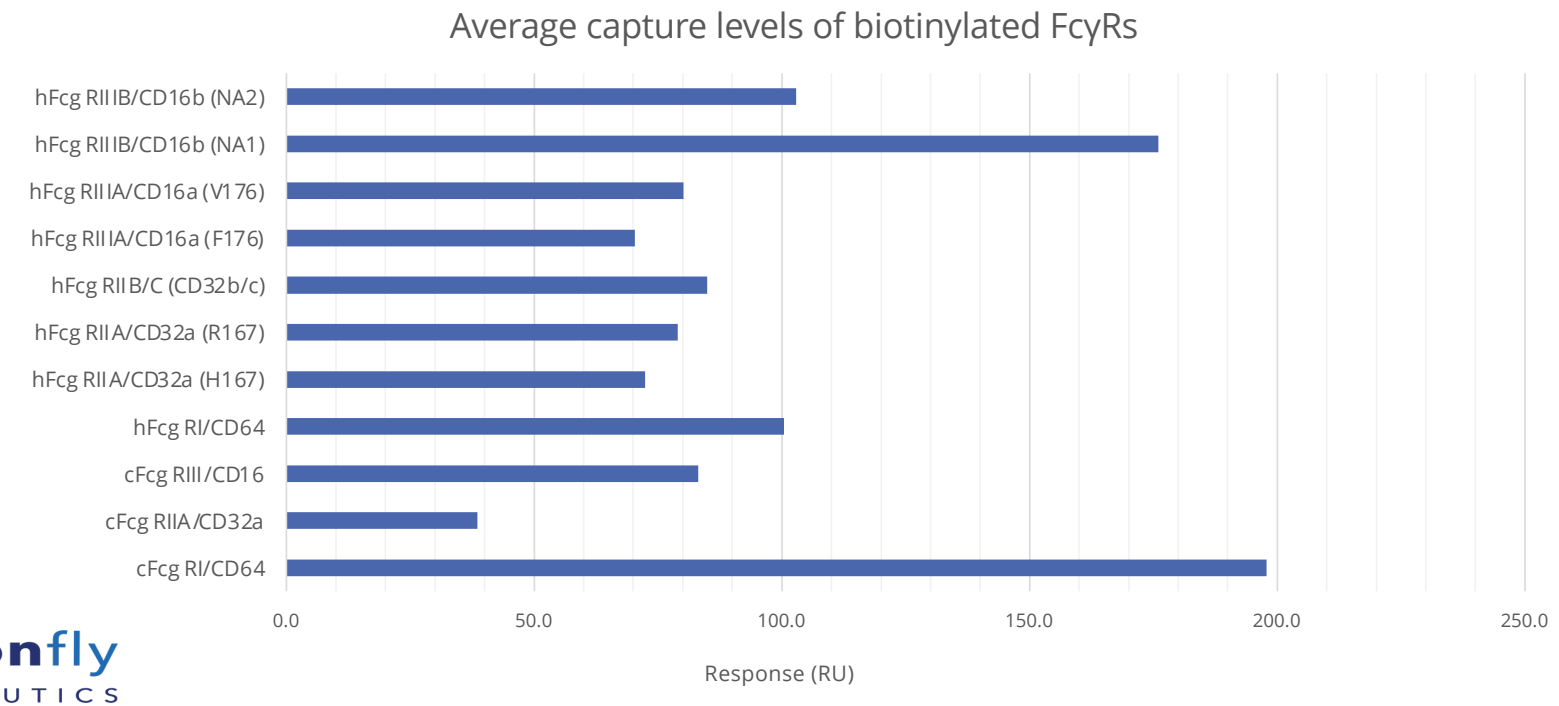
LSA^{XT} can capture full FcyR array at a consistent and low surface density

- 11 biotinylated receptors captured on SAHC30M sensor chip
- Standard deviation of capture response replicates ≤ 15% (n=8)



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Fc Gamma Receptor Affinity Characterization on LSA^{XT}

11 canonical human and cyno receptors screened against trastuzumab (n=8)

hu CD16aV

hu CD16aF

hu CD32aH

hu CD32aR

hu CD32b

hu CD16b (NA1)

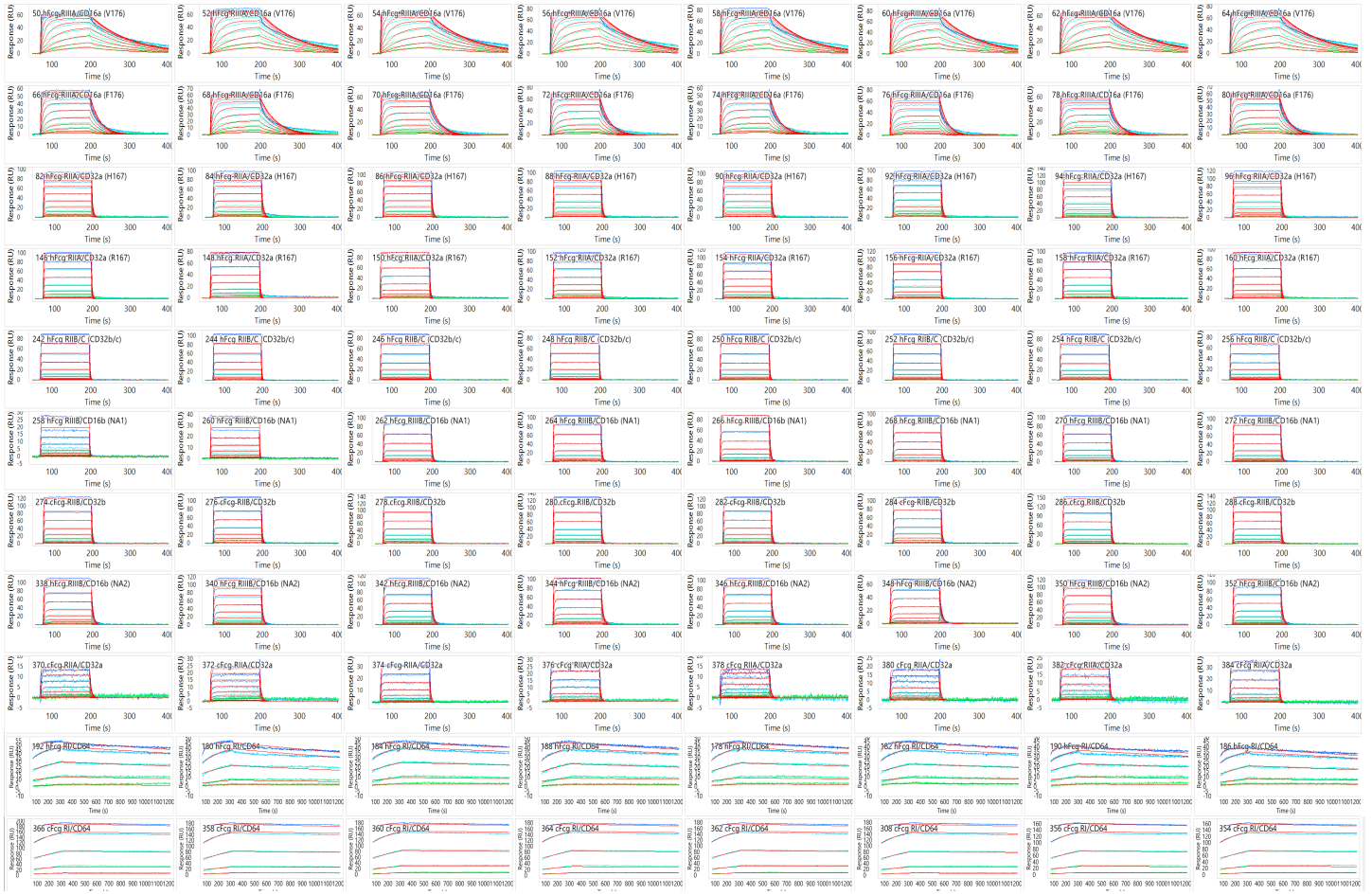
cy CD32b

hu CD16b (NA2)

cy CD32a

hu CD64

cy CD64



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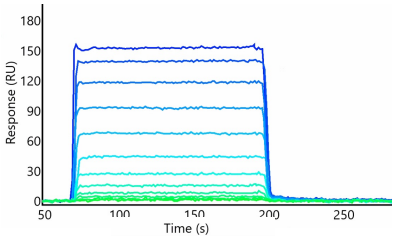
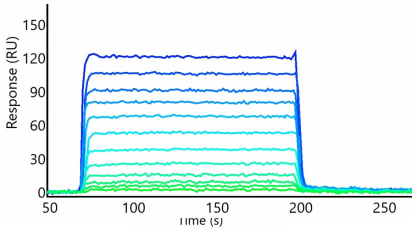
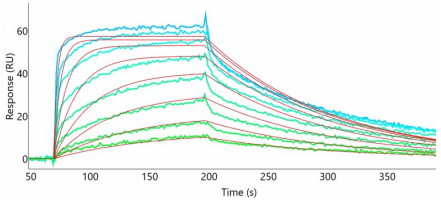
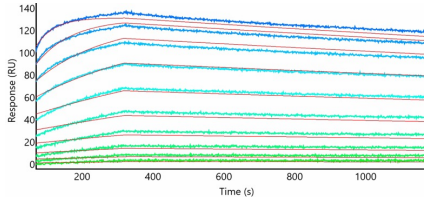
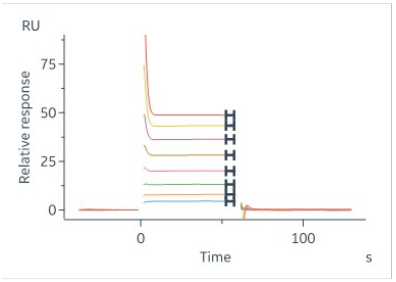
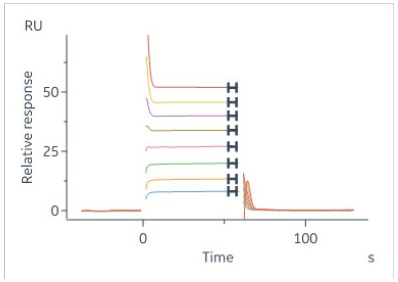
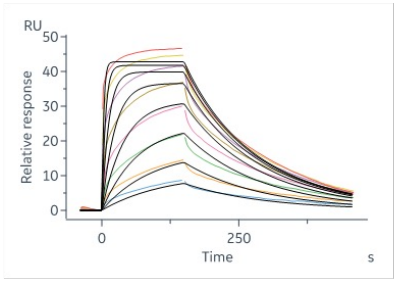
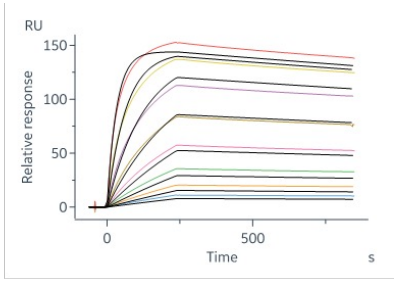
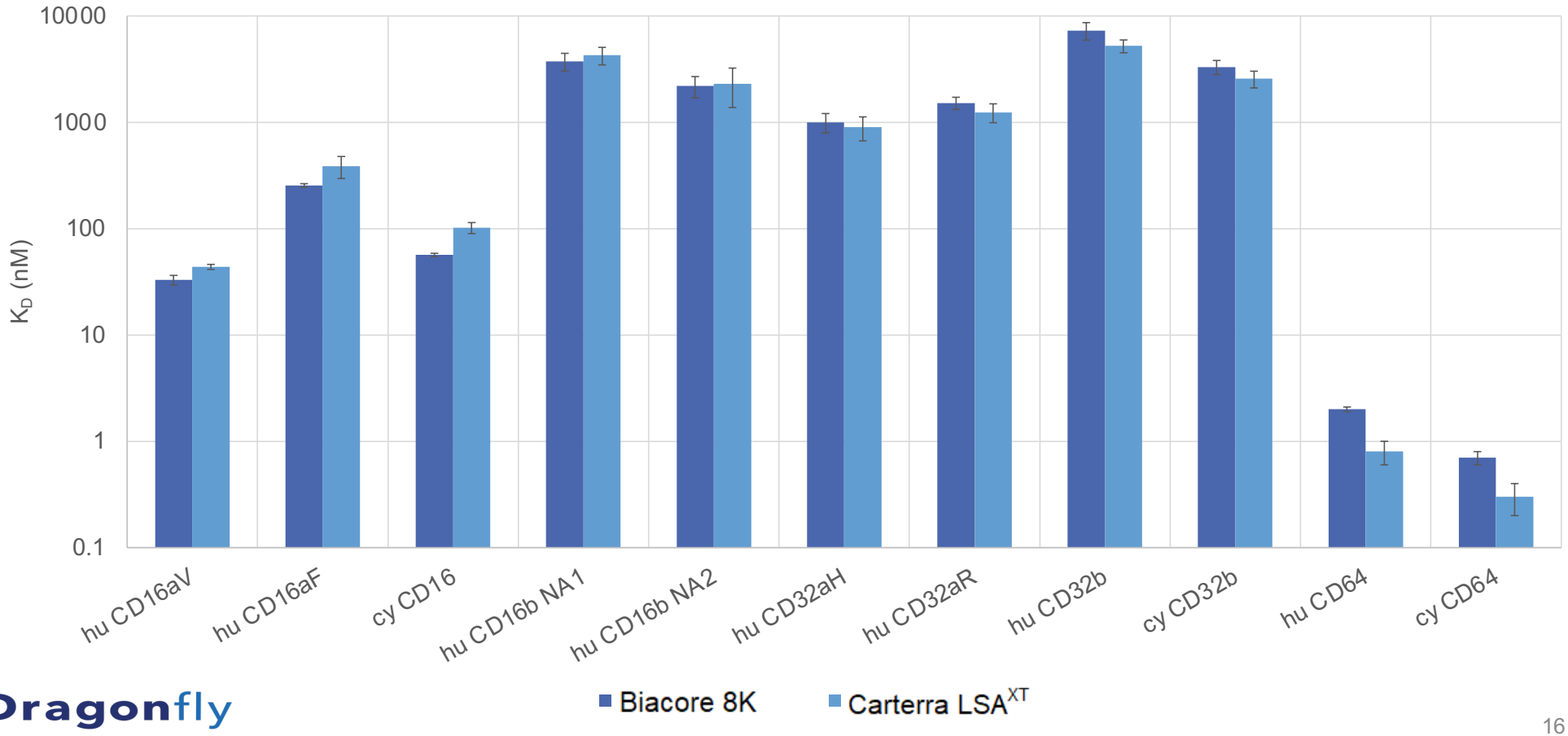
LSA^{XT} and 8K provide high-resolution sensorgrams

	Cy CD32b	Hu CD32aH	Hu CD16aV	Hu CD64
LSA^{XT}				
8K				

Weak interaction → Strong interaction

LSA^{XT} and 8K produce similar affinities in FcγR SPR binding assays



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LSA^{XT} and 8K produce similar on/off rates in trastuzumab FcγR binding assays

- Kinetic constants within 2-fold across instruments
- CD64 differences likely due to chip chemistry differences

k_a (1/Ms)		
	Biacore 8K	**Carterra LSA^{XT}**
Hu CD16a V	(2.6 ± 0.6) x 10^5	(2.2 ± 0.1) x 10^5
Hu CD16a F	(1.1 ± 0.0) x 10^5	(1.2 ± 0.2) x 10^5
Cy CD16	(2.1 ± 0.4) x 10^5	(1.2 ± 0.1) x 10^5
Hu CD64	(7.7 ± 0.1) x 10^4	(2.1 ± 0.2) x 10^5
Cy CD64	(1.1 ± 0.0) x 10^5	(1.4 ± 0.1) x 10^5

k_d (1/s)		
	Biacore 8K	**Carterra LSA^{XT}**
Hu CD16a V	(8.4 ± 1.1) x 10^{-3}	(9.4 ± 0.3) x 10^{-3}
Hu CD16a F	(2.8 ± 0.0) x 10^{-2}	(4.4 ± 0.6) x 10^{-2}
Cy CD16	(1.2 ± 0.2) x 10^{-2}	(1.2 ± 0.1) x 10^{-2}
Hu CD64	(1.5 ± 0.1) x 10^{-4}	(1.8 ± 0.3) x 10^{-4}
Cy CD64	(7.6 ± 0.5) x 10^{-5}	(4.5 ± 0.6) x 10^{-5}

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LSA^{XT} provides FcγR characterization with greater efficiency than 8K

	Biacore 8K	Carterra LSA^{XT}
Chip	6 total chips - $3924	1 chip - $1190
Analyte	15.9 mg	2.1 mg
# of experiments	6 separate experiments	2 combined experiments
Analyst Time	9 hours	3 hours
Replicates	4	32
Cost/replicate	$89.18	$3.10
Analyte/replicate	361.4 µg	5.5 µg

Conclusion

- Carterra LSA^{XT} can provide highly accurate and precise FcγR data using less overall analyte than Biacore 8K.
- Similar kinetic rate constants and K_D values were recorded between instruments.
- LSA^{XT} can perform FcγR assay faster and cheaper:
  1. Requires 7.5x less analyte
  2. Saves 6+ hours of analyst time
  3. Saves ~$3,000 in chip costs
  4. Produces 8x as many replicates

Acknowledgements

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ACRO Biosystems

- Larry Yu – Larry.Yu@ACROBiosystems.com

Dragonfly Therapeutics

- Dan Fallon

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Reference slides

FcγRs produced and provided by ACRO Biosystems

Target		Cat No.	Lot No.	Aliquot size
huCD16a V176	FcγRIIIa V176	CDA-H82E9	BV1899-21CRF1-16N	25 μg
huCD16a F176	FcγRIIIa F176	CDA-H82E8	BV1898-21BRF1-12S	25 μg
cyCD16	FcγRIII	FC6-C82E0	BV2520-79XF1-12P	25 μg
huCD16b NA1	FcγRIIIB	CDB-H82E4	BV2424-215KF1-12V	25 μg
huCD16b NA2	FcγRIIIB	CDB-H82Ea	BV2381-224XF2-163	25 μg
huCD32aH	FcγRIIaH	CDA-H82E6	BV1896-222QF1-13V	25 μg
huCD32aR	FcγRIIaR	CDA-H82E5	BV5439A-21BAF1-14Y	25 μg
huCD32b	FcγRIIB	CDB-H82E0	BV1900-2176F1-15H	25 μg
cyCD32b	FcγRIIB	CDB-C82E4	BV3105-93CF1-Y7	25 μg
huCD64	FcγRI	FCA-H82E8	CBV419P1-207EF1-14Y	25 μg
cyCD64	FcγRI	FCA-C82E8	CBV312P1-217FF1-12V	25 μg

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