

Site-Specific Conjugation/HIC Purification Strategy to Obtain Homogeneous ADCs: **AJICAP™** Technology

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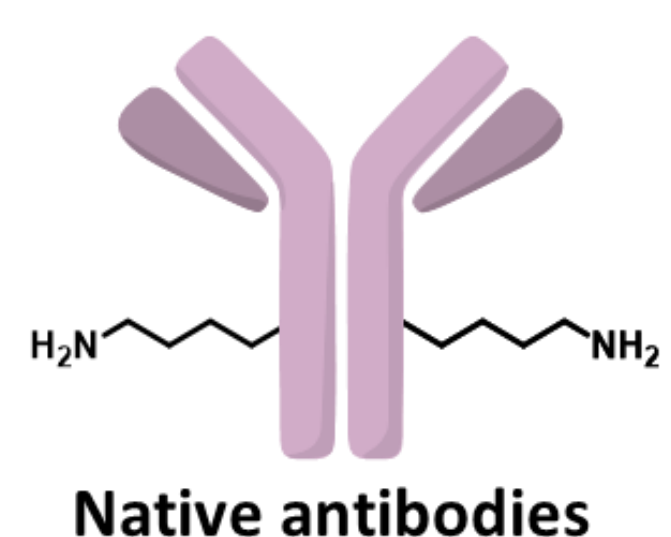
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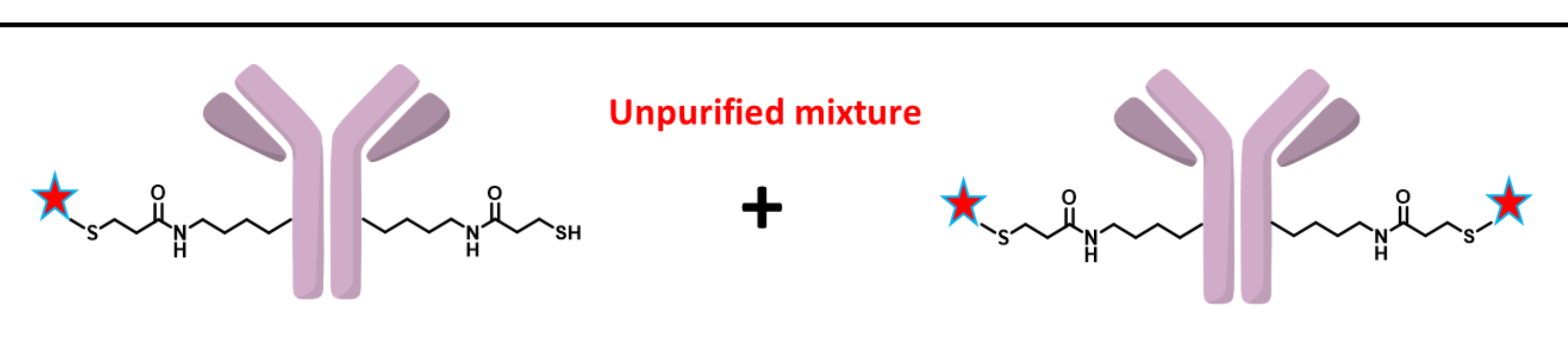
Objective

Promising results from early technology development studies necessitated further investigation of AJICAP™ ADC materials to obtain a “homogenous DAR” ADC.

Here we report our current efforts to propose site-specific conjugation followed by a preparative HIC purification strategy to obtain “DAR = 1.0” and “DAR = 2.0” AJICAP™ ADC materials respectively.



AJICAP™ technology



HIC purification

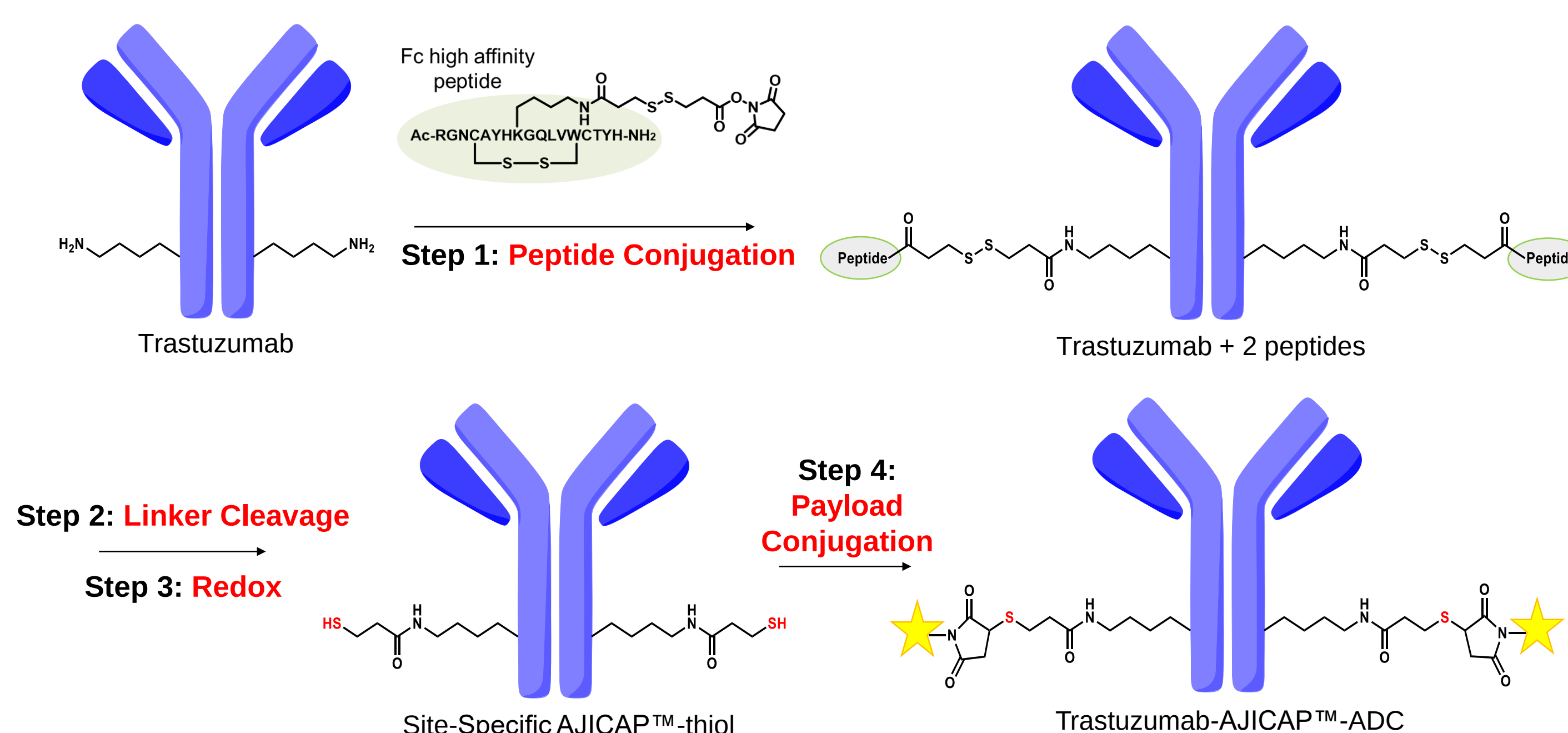


★ = highly hydrophobic payload

Based on this strategy, we can provide site-specific ADCs with upgraded homogeneity depending on their hydrophobicity and/or cytotoxicity

AJICAP™ Technology¹

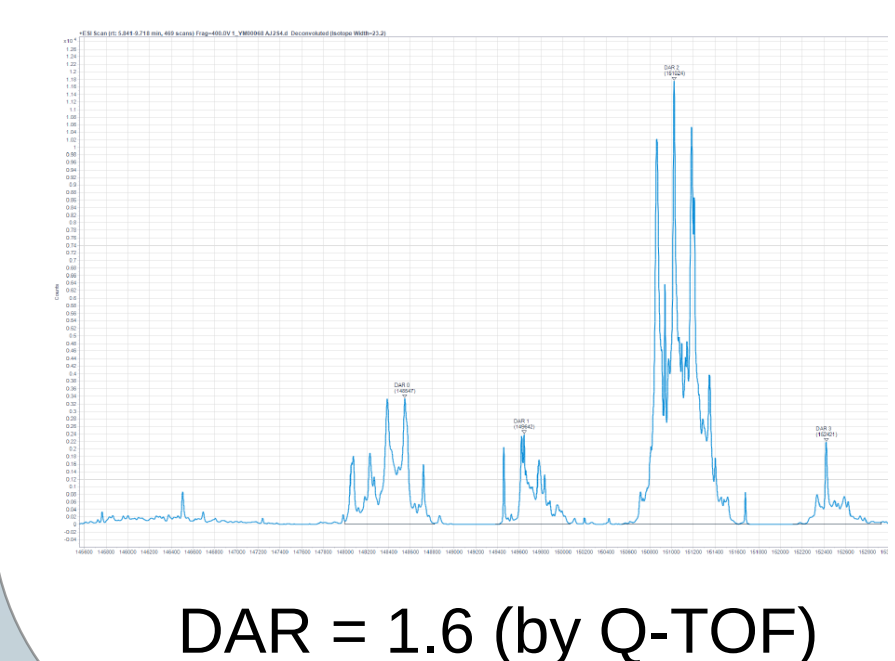
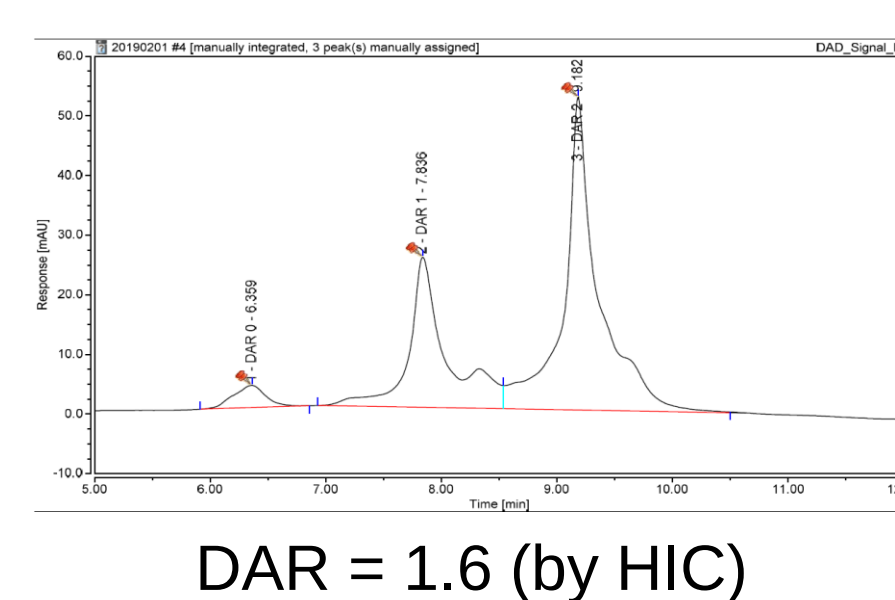
Ajinomoto Co. has developed an innovative direct chemical site-selective conjugation method intended for use with intact native antibodies. AJICAP™ technology is a novel platform for the site-selective conjugation of antibodies through the use of a class of Fc-affinity compounds to install payload-compatible linkers to well-defined amino acid residue(s).



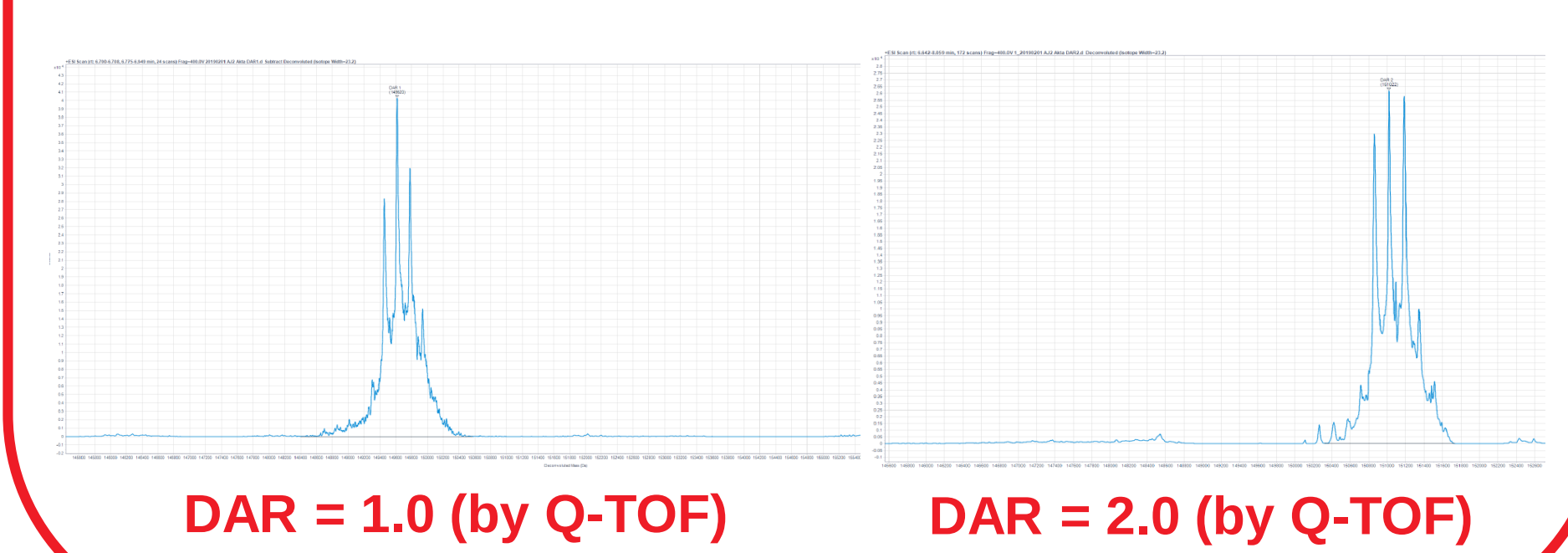
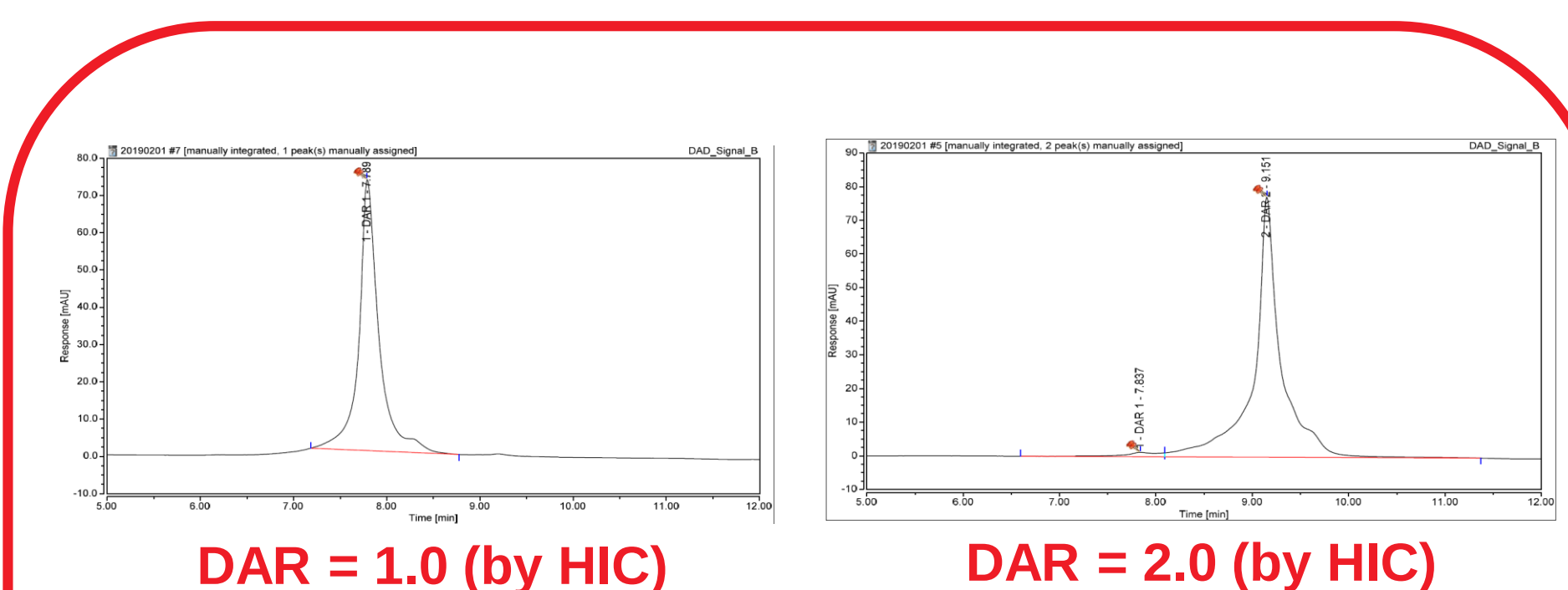
Experimental Results

	Conditions-A	Conditions-B	Conditions-C	Conditions-D
Resin	Phenyl-Resin	Phenyl-Resin	Butyl Resin	PPG Resin
Mobile Phases	Literature Conditions ²	Optimized Conditions	Literature Conditions ²	Optimized Conditions
Recovery Rate of ADCs	Mid	High	Not Eluted	High
DAR Separation	Separated	Separated	N.D.	Not Separated

Intentional Defect DAR ADC



Purified **AJICAP™** ADCs



Optimized Hydrophobic ADC Conjugation

Antibody	Linker	Payload	DAR (after purification)
Trastuzumab	Thiol/maleimide	MMAE	2.0
Trastuzumab	Thiol/maleimide	DM1	2.0
Trastuzumab	Thiol/maleimide	PBD	1.8
Trastuzumab	Thiol/maleimide	Undisclosed payload	1.9
Trastuzumab	Azide/DBCO	MMAE	2.0
Rituximab	Thiol/maleimide	DM1	2.0
Undisclosed mAb	Undisclosed linker	Undisclosed payload	1.8

Several ADCs including undisclosed material have been tested

In Vitro Cytotoxic Assay

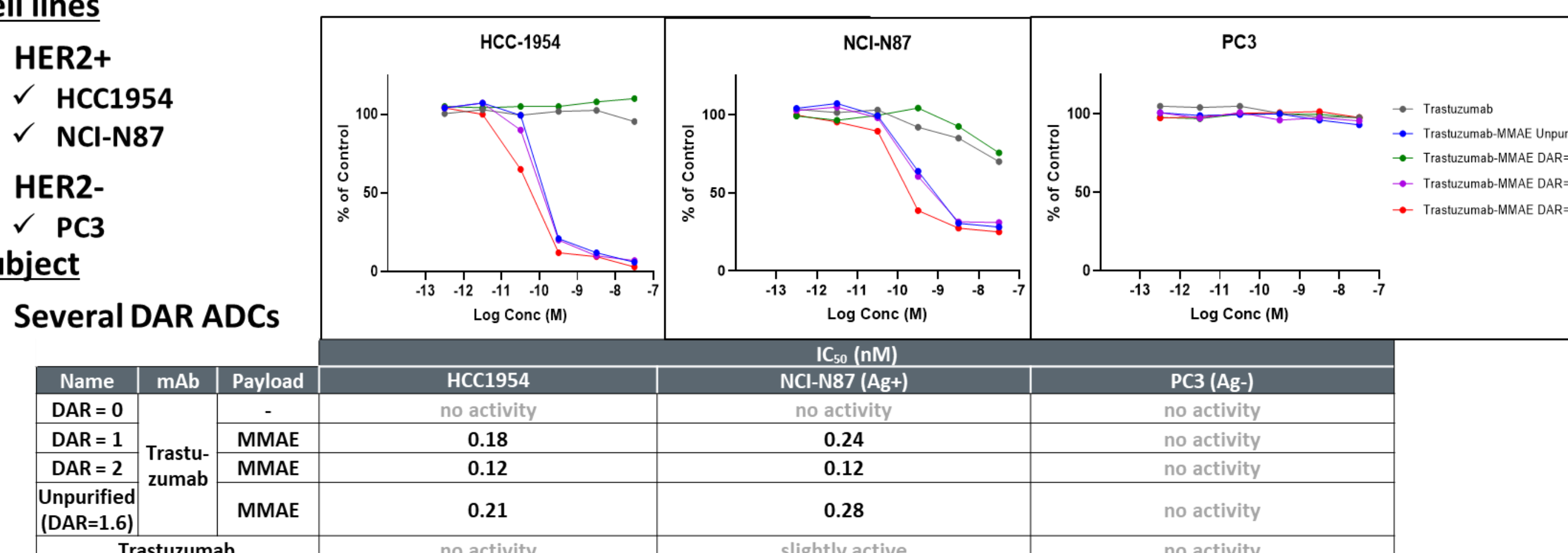
Cell lines

- HER2+
- ✓ HCC1954
- ✓ NCI-N87

- HER2-
- ✓ PC3

Subject

- Several DAR ADCs



- AJICAP™-ADCs have *in vitro* cytotoxic activities reflective of DAR
- Several DAR compounds showed considerable potency and target selectivity toward cancer cells after HIC purification

Conclusion

- Mobile phase/resin screening for DAR separation was completed
- AKTA/HIC purification resulted in homogeneous “DAR = 2.0”, “DAR = 1.0” ADCs
- DAR compounds showed considerable potency and target selectivity
- Site-specific conjugation/HIC separation strategy was accomplished

References

- Yamada, K.; Shikida, N.; Shimbo, K.; Ito, Y.; Khedri, Z.; Matsuda, Y.; Mendelsohn, B. A.; *Angew. Chem. Int. Ed.* **2019**, *58*, 5592–5597.
- Hamblett, K. J. *et al. Clin. Cancer Res.* **2004**, *10*, 7063–7070.

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