

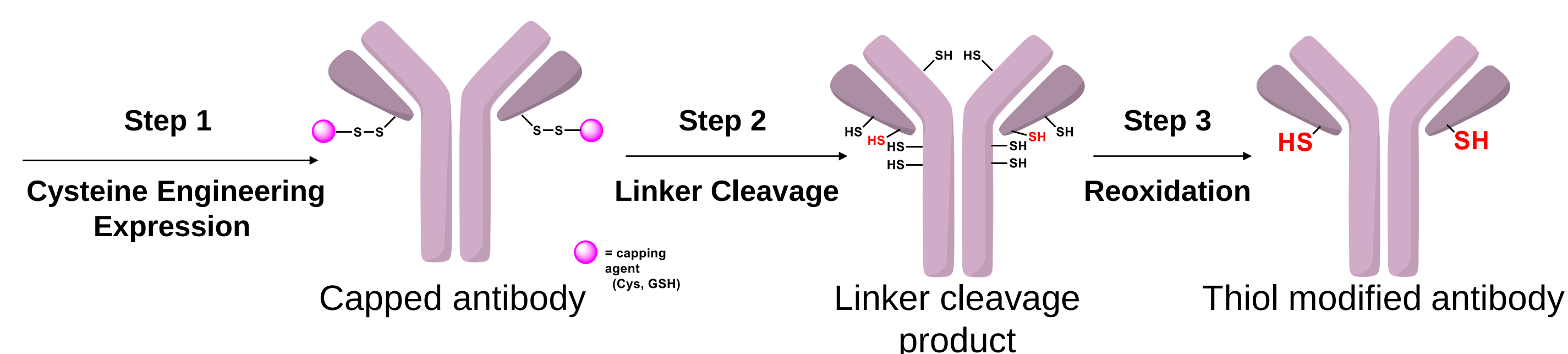
Robust Synthesis and Initial Evaluation of Stable Thiol-Modified Antibody Produced by Site-Specific Conjugation: **AJICAP™**

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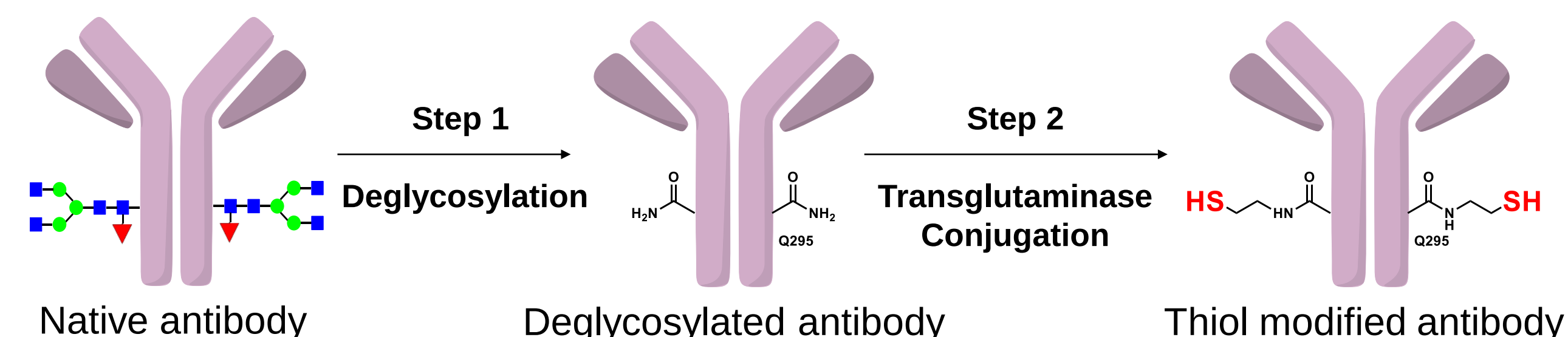
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Other Site-Specific Thiol mAbs

Antibody Engineering Technology¹

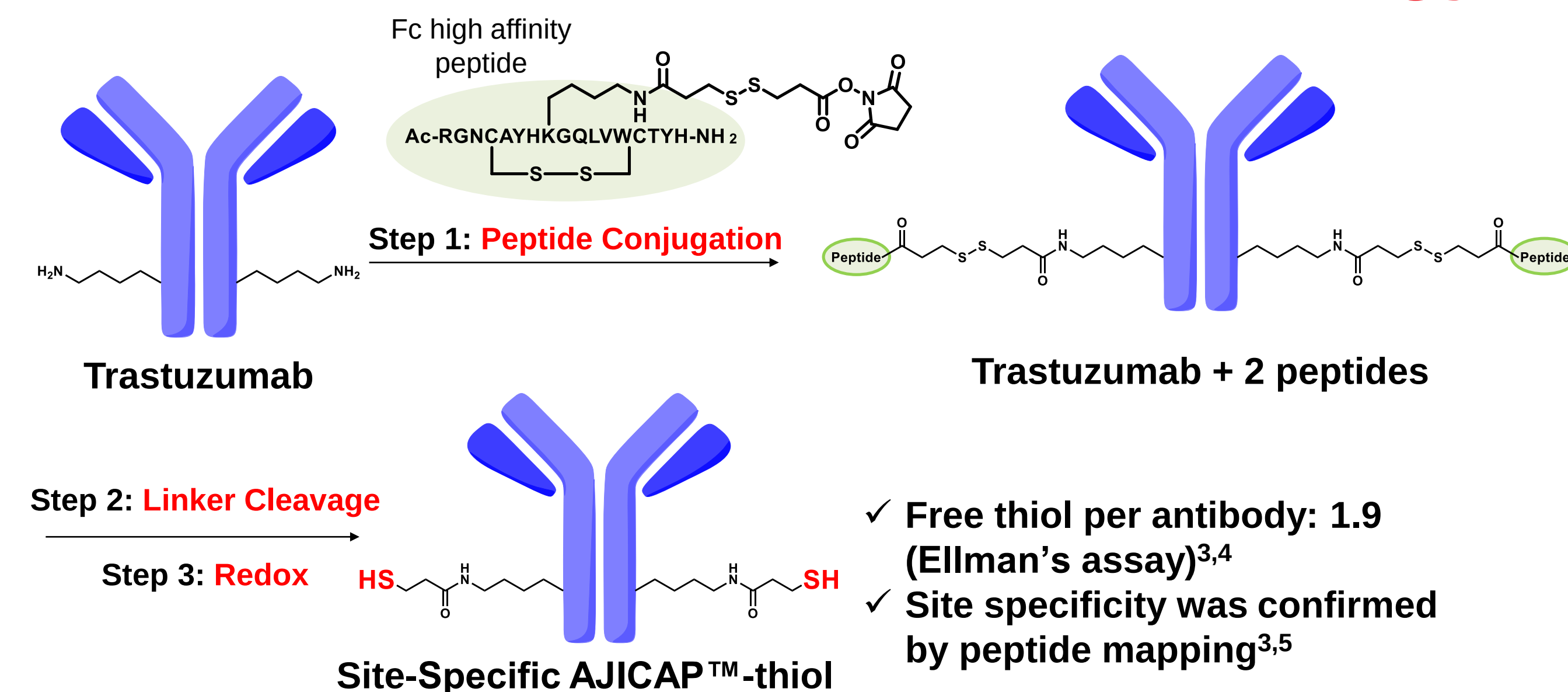


Enzymatic Conjugation Technology²



With either technology, the thiol intermediates are difficult to “isolate” due to their instability

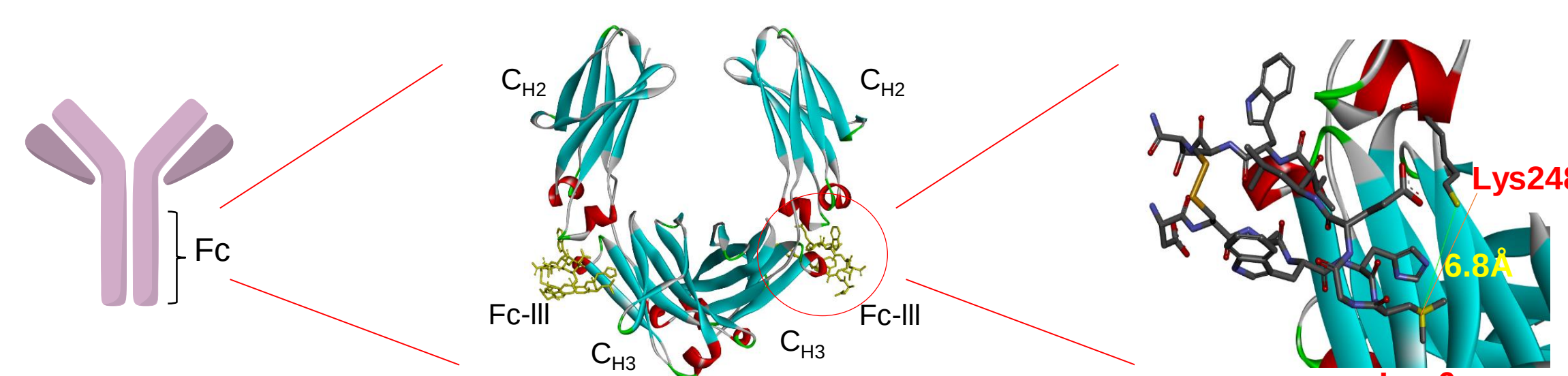
Introduction: **AJICAP™** Technology



✓ Free thiol per antibody: 1.9 (Ellman's assay)^{3,4}
✓ Site specificity was confirmed by peptide mapping^{3,5}

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 thiol linkers to well-defined amino acid residue(s).

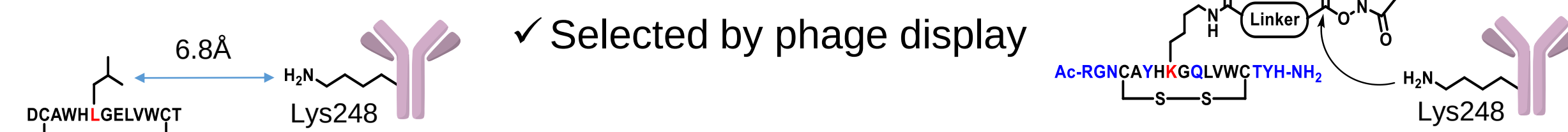
Human IgG Fc and Fc-III peptide co-crystal structure



Fc-III⁶
 K_D 20 nM
✓ 13 amino acids

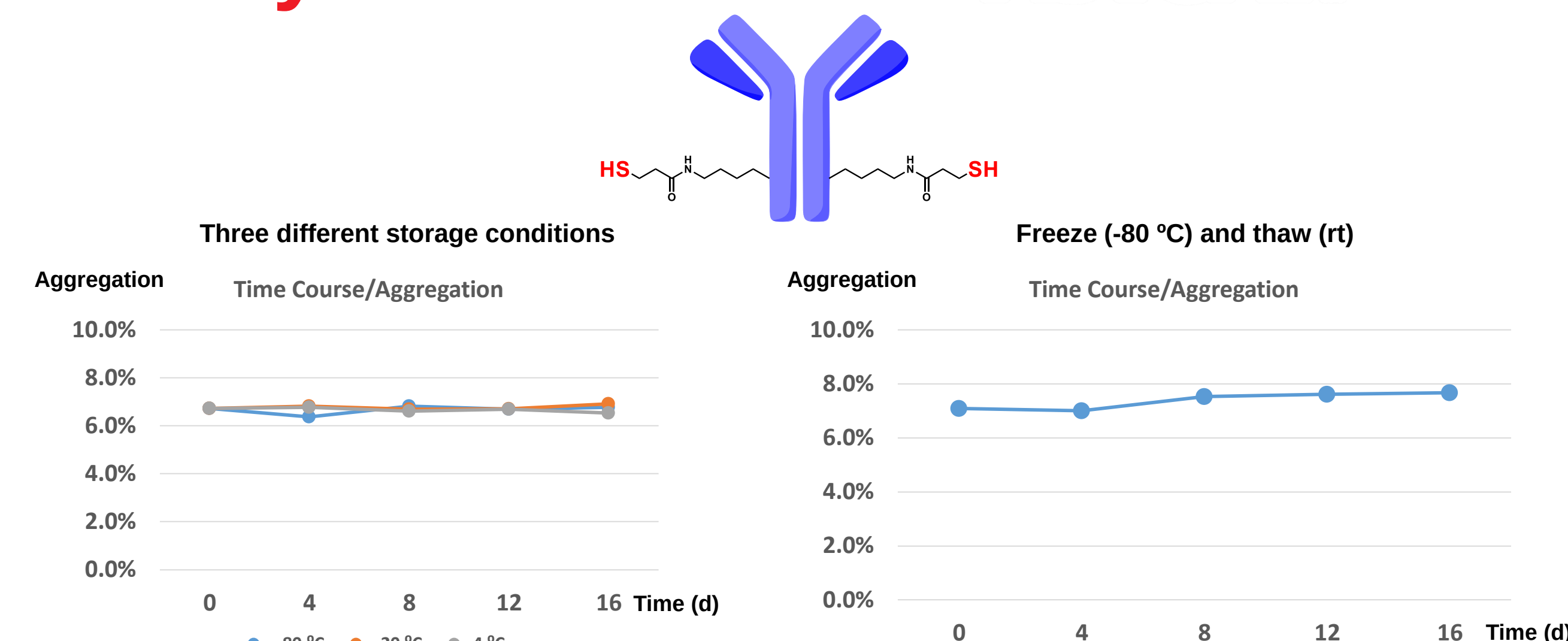
An example of peptides for **AJICAP™**

✓ K_D 4 nM
✓ 17 amino acids
✓ Selected by phage display



Promising results from early technology development studies necessitated the further investigation focusing on understanding of the AJICAP™-thiol chemistry. In this poster, we report our current efforts for the initial stability assessment of AJICAP™-thiol material.

Stability Assessment of **AJICAP™**-Thiol



AJICAP™-thiol is stable under stressed conditions

Compatibility and Scalability

Isotype	mAb	Isotype	mAb	Payload
IgG1	Trastuzumab	IgG1	A company-mAb a, b (client project)	Cytotoxic
	Rituximab		B company-mAb a, b (client project)	Fluorescent/Others
	Adalimumab		C company-mAb a-e (client project)	DM-1
	Ofatumumab		D company-mAb (client project)	MMAE
	Lifatumumab		E company-mAb (client project)	MMAF
	Infliximab		F company-mAb a, b (client project)	PBD
	Engineered-mAb A			α -amanitin
	Engineered-mAb B			Client payload A
IgG2	Engineered-mAb C	IgG4	Denosumab	Client payload B
			Dupilumab	Client payload C

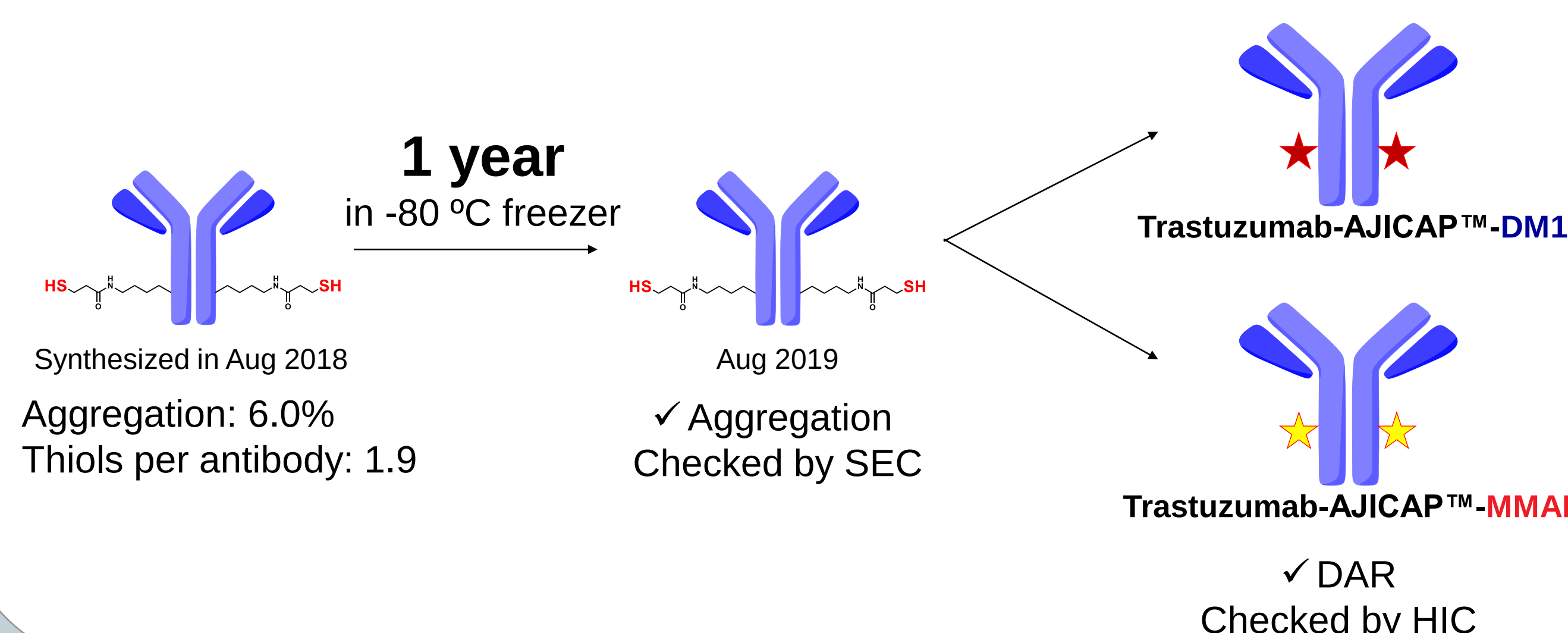
Compatibility

- **AJICAP™-technology** has great potential for mAb isotypes: IgG1, 2, 4
- **AJICAP™-thiol** has great compatibility to react with a wide variety of thiol reactive molecules

Scalability

- Gram-scale preparation of **AJICAP™-thiol** was successful (total **97% yield**)^{7,8}
- GMP strategy was established

ADC Synthesis from “1-Year-Old” Thiol

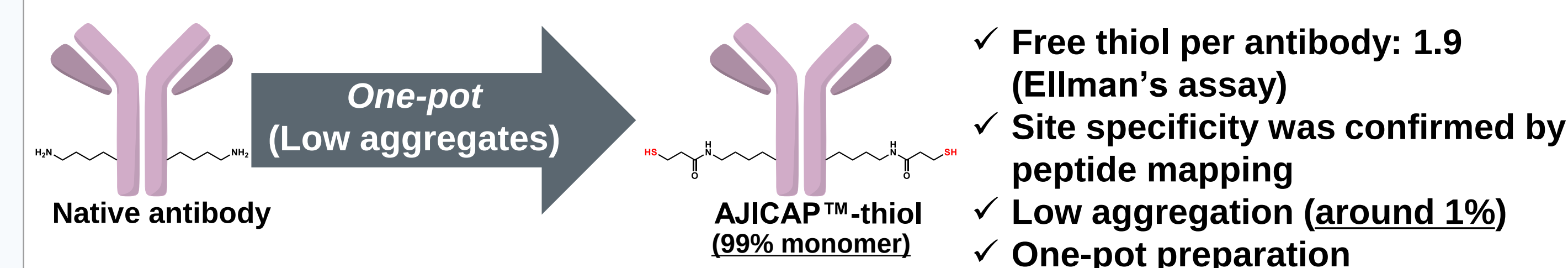


Analytical Results

“Fresh” AJICAP™-thiol		“1 Year-Old” AJICAP™-thiol	
Aggregation by SEC	6.0%	Aggregation by SEC	6.2%
DAR for DM1-ADC by HIC	1.9	DAR for DM1-ADC by HIC	1.9
DAR for MMAE-ADC by HIC	1.6	DAR for MMAE-ADC by HIC	1.6

AJICAP™-thiol is stable in -80 °C freezer for 1 year!

AJICAP™ 2nd Generation/Next Step



Aggregation by SEC

Thiol-modified mAb	1 st Generation	2 nd Generation
Trastuzumab-thiol	6.4%	0.8%
Rituximab-thiol	17%	1.4%
Client mAb A + thiol	40%	1.2%
Client mAb B + thiol	22%	1.4%

AJICAP™-thiol is a promising intermediate for many site-specific conjugates

Conclusion

- Wide compatibility and scalability were confirmed
- **AJICAP™-thiol** is stable for more than 1 year
- A variety of applications are ongoing

AJICAP™-thiol

- For research: can be a useful intermediate for payload screening
- For manufacturing: can be a “hold point” in an ADC manufacturing process

References

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