

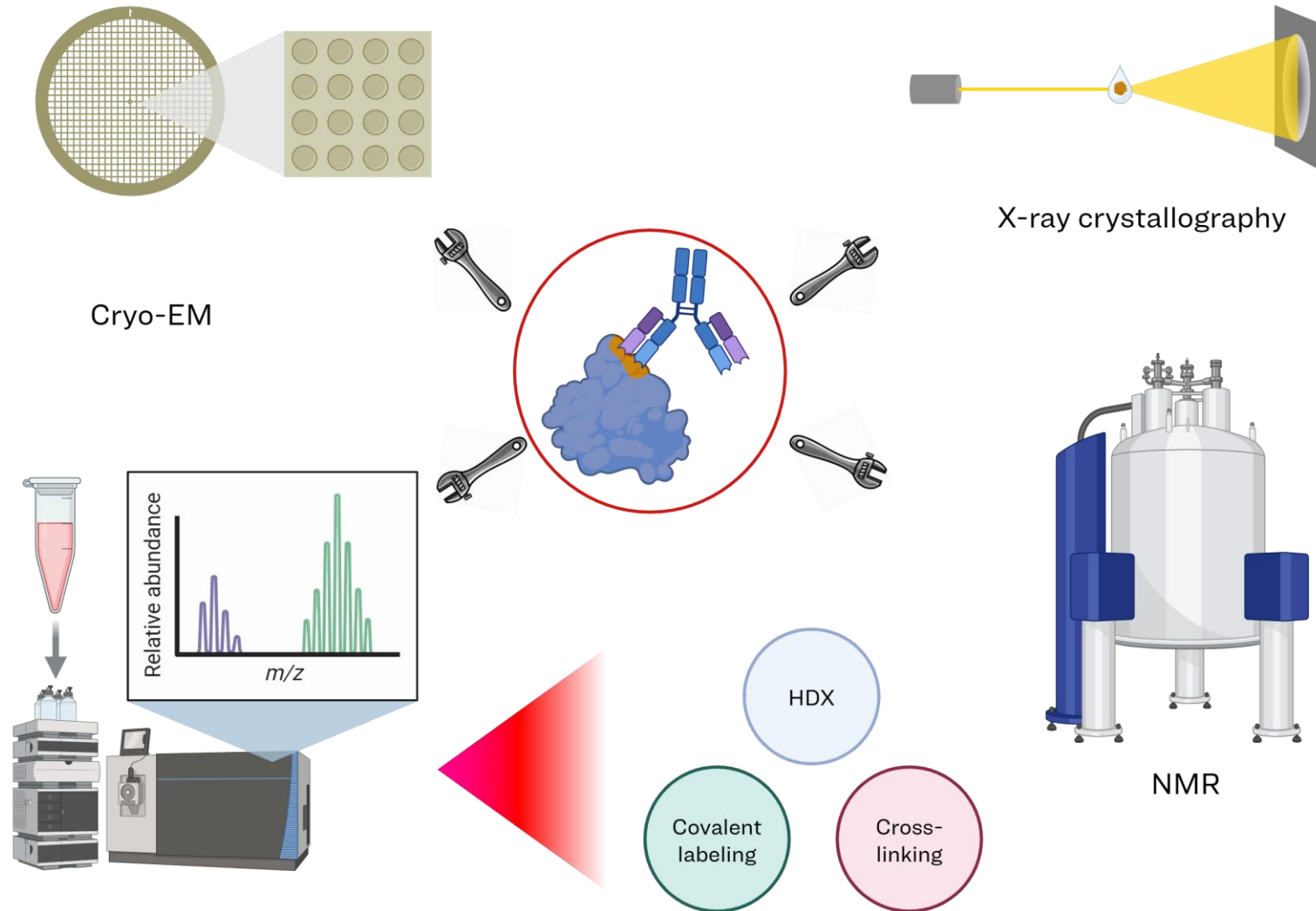


Evaluating Antigen-Antibody Interactions: A Comparative Study of Higher- Order Structural MS Methods

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Lilly

Techniques for characterizing protein-protein interactions



Overall aim of the project

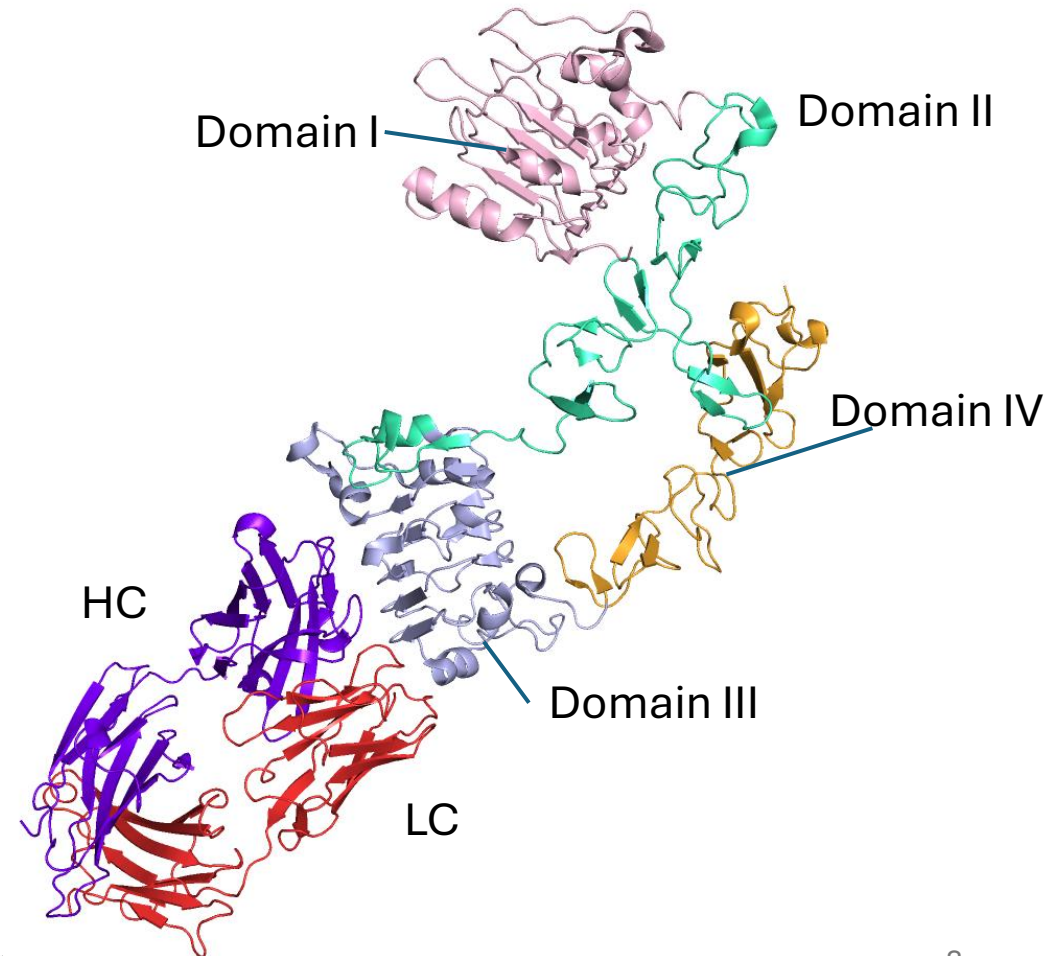
Goals

- Compare the applicability of hydrogen deuterium exchange (HDX), cross-linking (XL) and covalent labeling (CL) for epitope mapping from purified samples as well as directly from cells.

Model system

Epidermal growth factor receptor (EGFR) ECD bound to Cetuximab or Zalutumumab

EGFR ECD bound to Cetuximab



Hydrogen- deuterium exchange (HDX)

Cetuximab epitope on EGFR by HDXMS compared to published data

CETUX Epitope Regions found by HDXMS

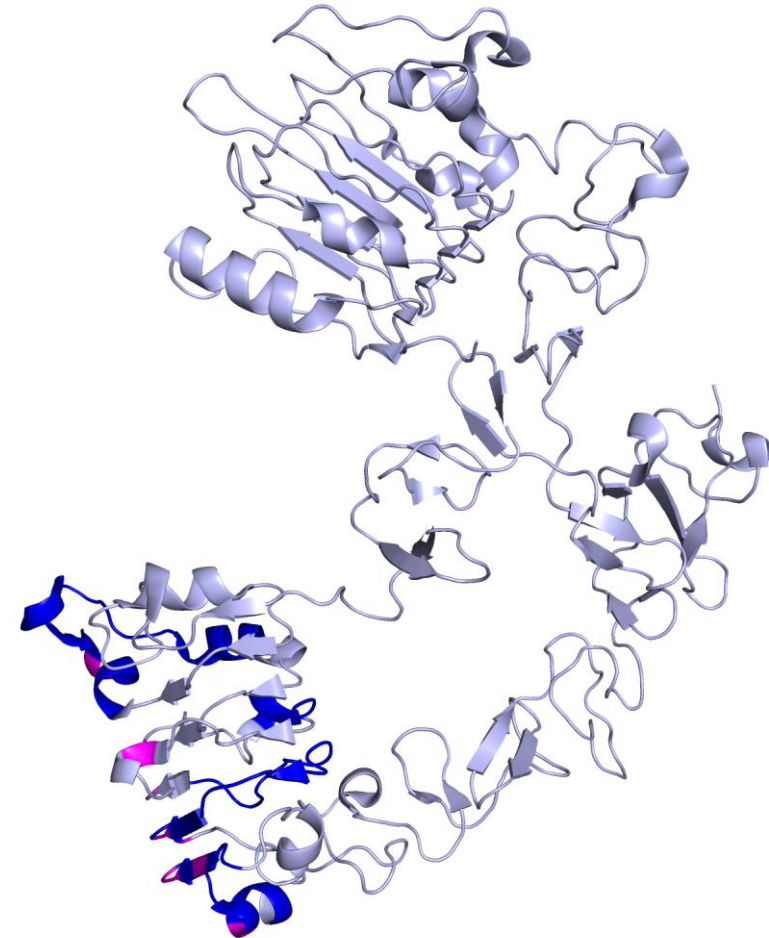
Residues	Sequence
350-355	VAFRGD
357-370	FTHTPPLDPQELDI
394-399	HAFENL
418-443	SLNITSLGLRSLKEISDGDVIIISGNK
461-476	GQKTKIISNRGENSCK

EGFR ECD: slate blue

HDXMS epitope: Dark Blue

Epitope from published literature: magenta

PDB:1YY9



Zalutumumab epitope on EGFR by HDXMS compared to published data

ZALU Epitope Regions found by HDXMS

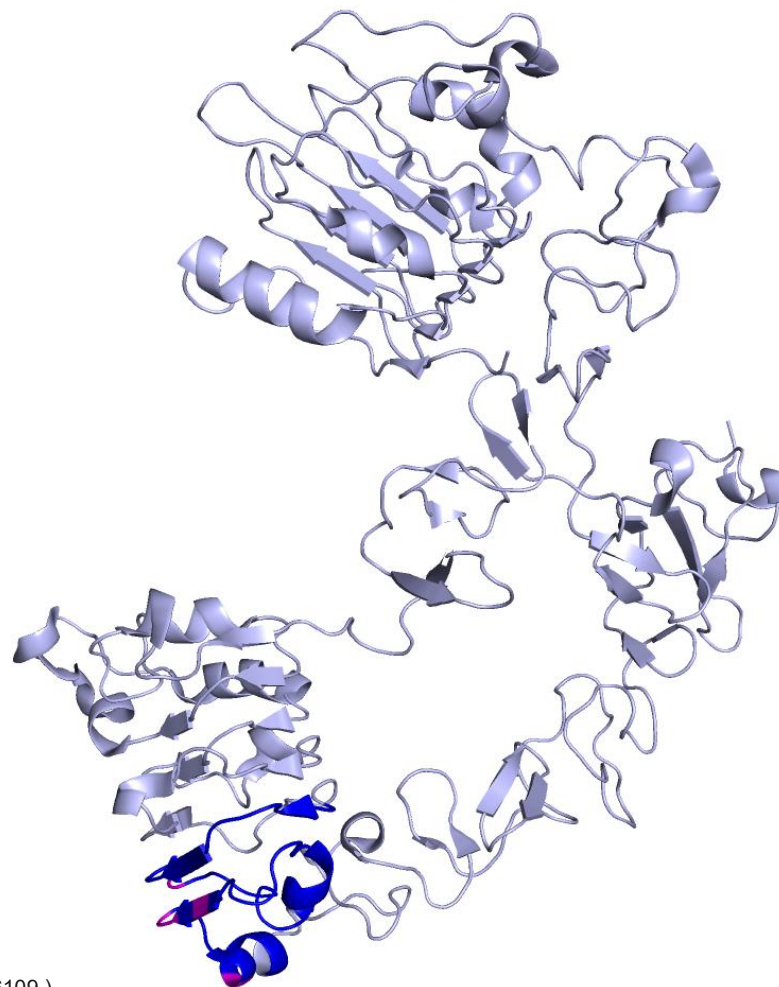
Residues	Sequence
430-443	KEISDGDV ^{II} ISG ^K
444-456	NLCYANTINWK ^{KL}
457-476	FGTSGQKT ^K ^{II} SNRGEN ^S SCK

EGFR ECD: slate blue

HDXMS epitope: Dark Blue

Epitope from published literature: magenta

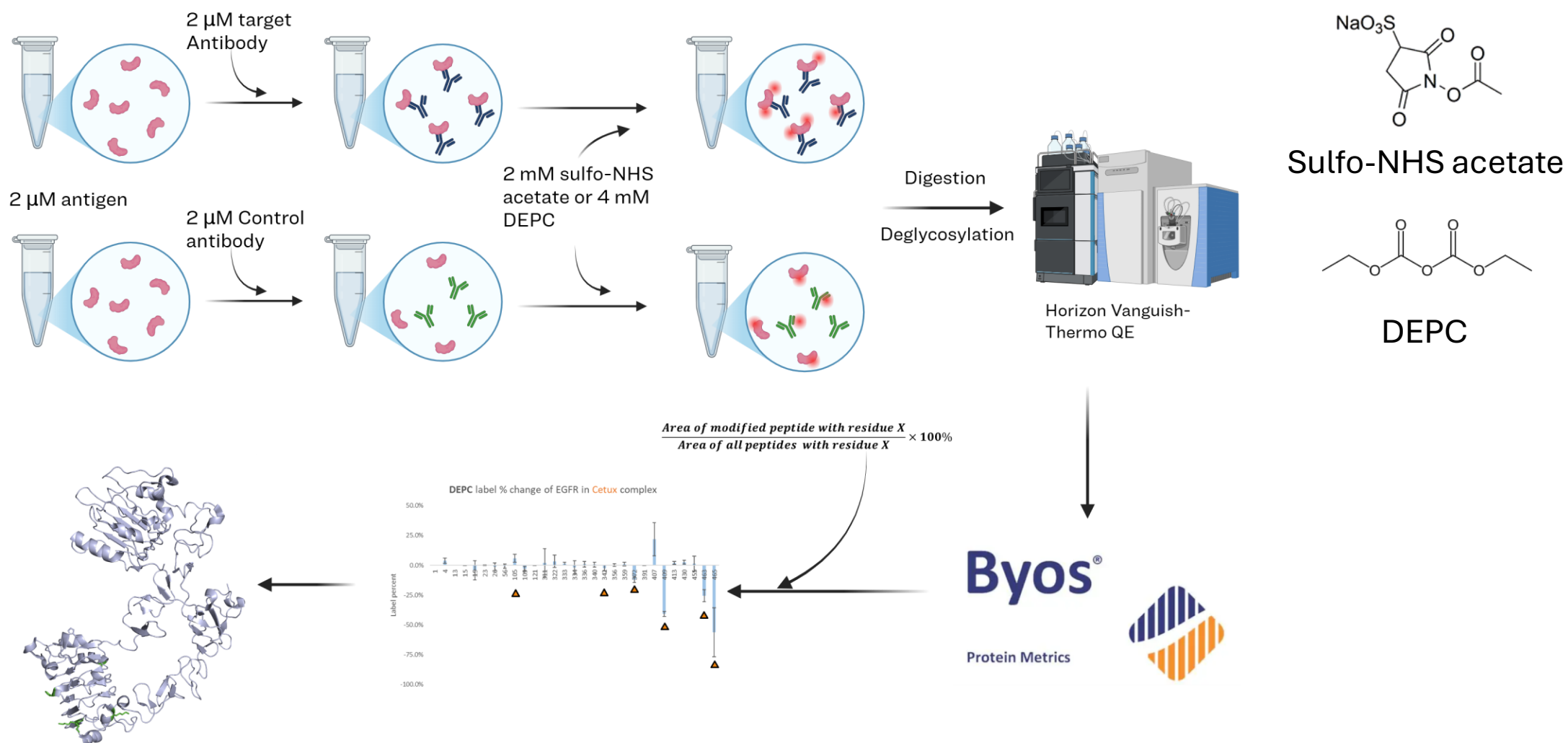
PDB:1YY9



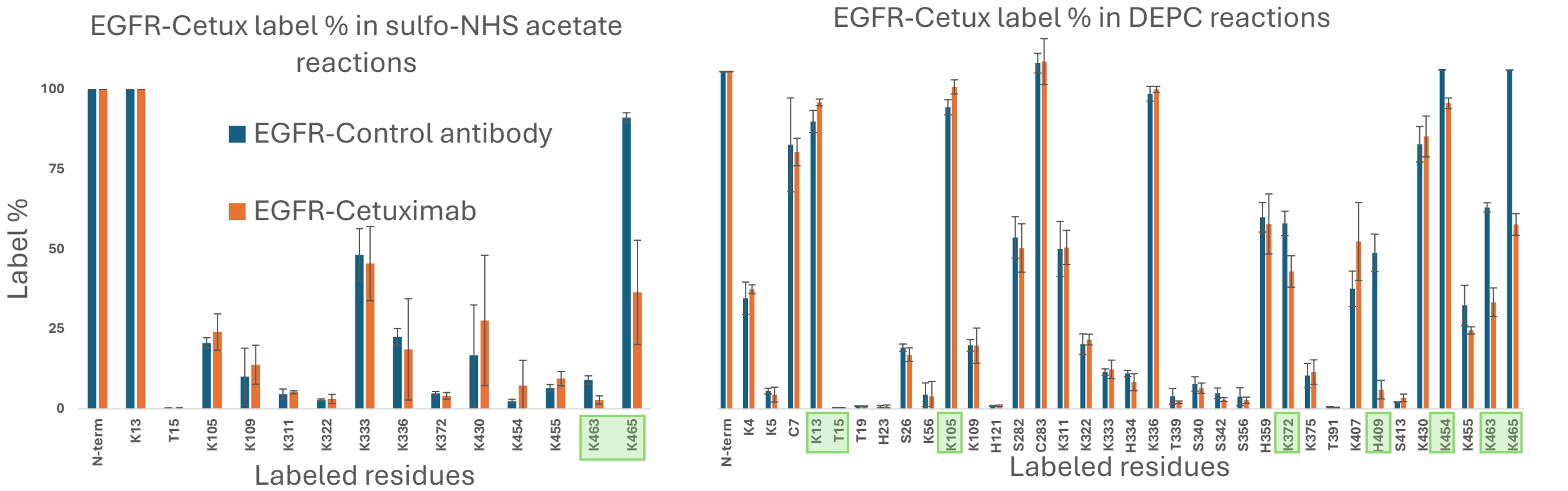
Lammerts van Bueren, J. J., et al. (2008). PNAS 105(16 (Retraction published PNAS U S A. 2012 Apr 3;109(14):5548. doi: 10.1073/pnas.1203736109.)

Covalent labeling (CLMS)

Covalent labeling with DEPC and NHS



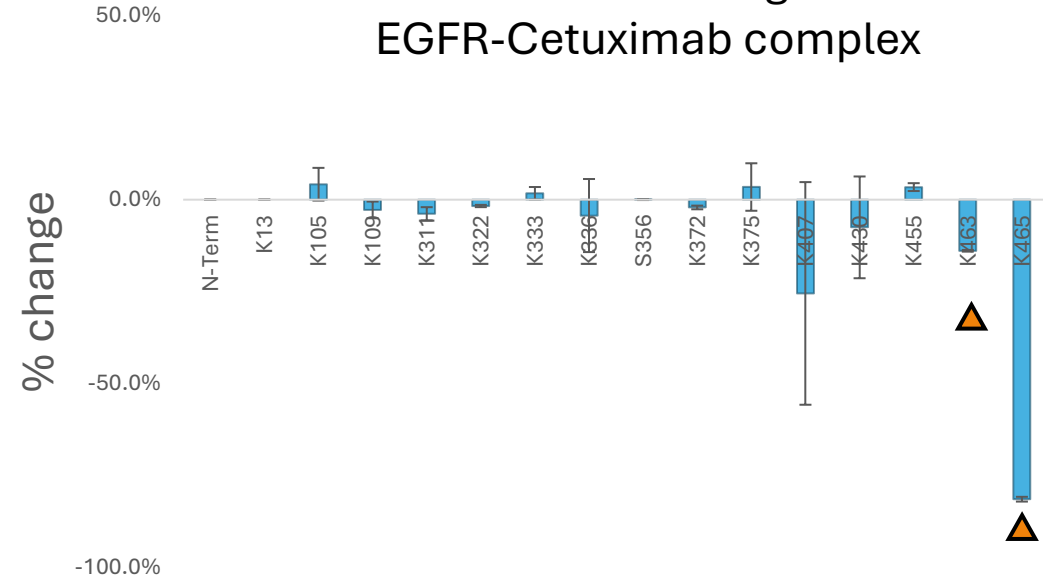
Label percentage in EGFR-Cetux complex



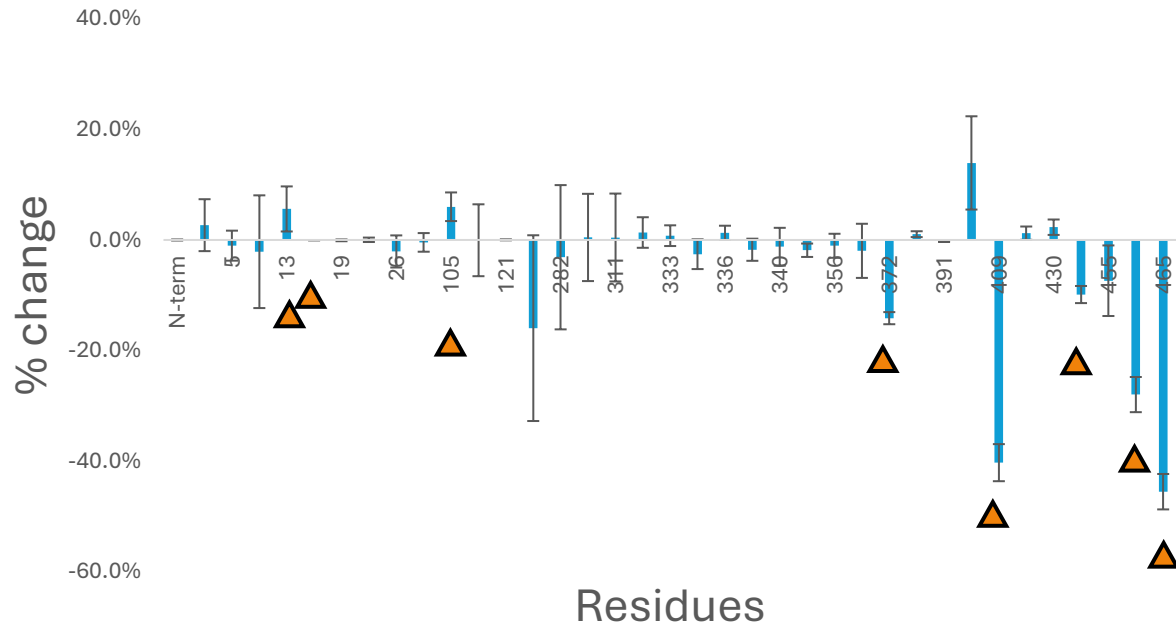
Residues that showed significant changes at 95% confidence

EGFR label % changes in EGFR-Cetuximab complex

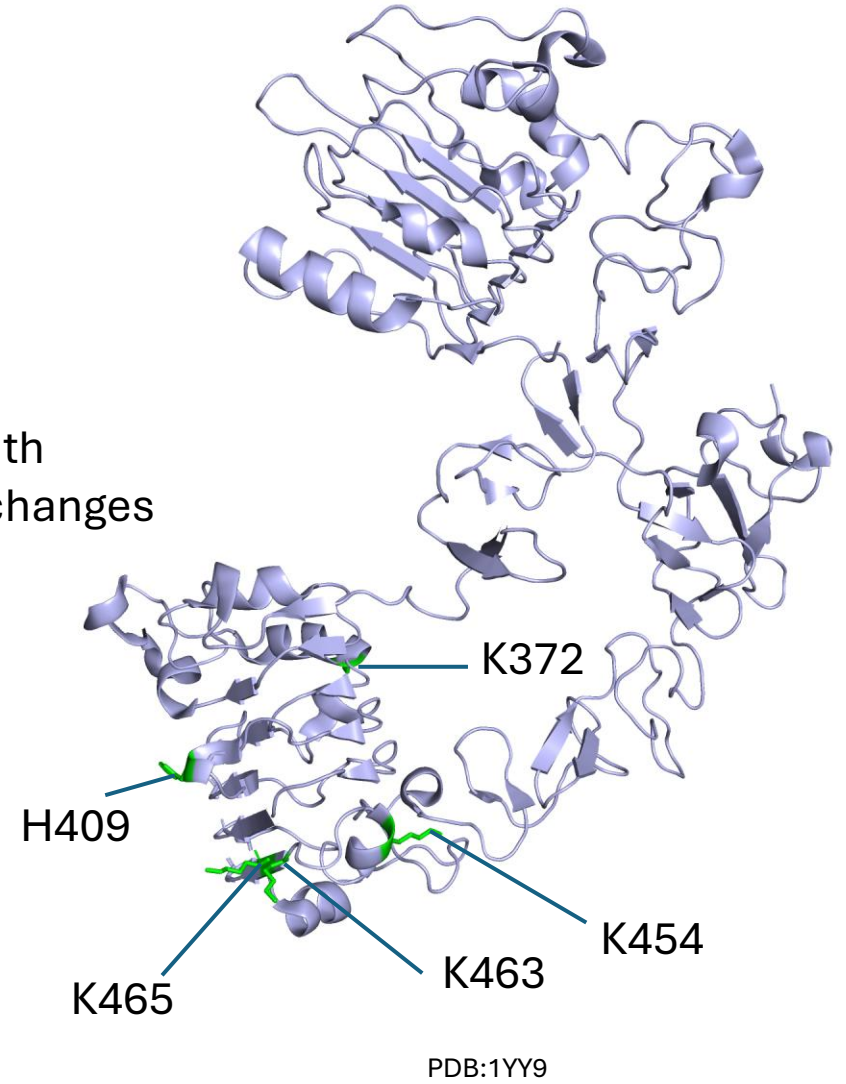
NHS acetate



DEPC



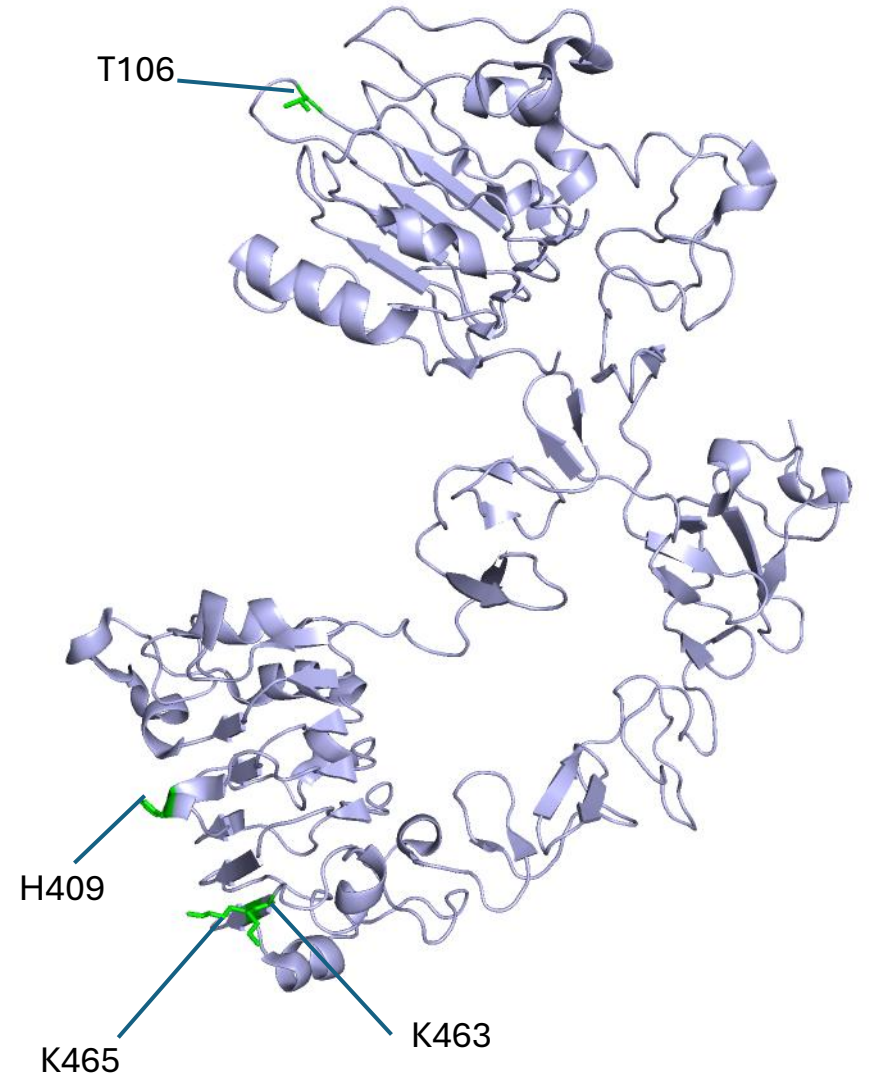
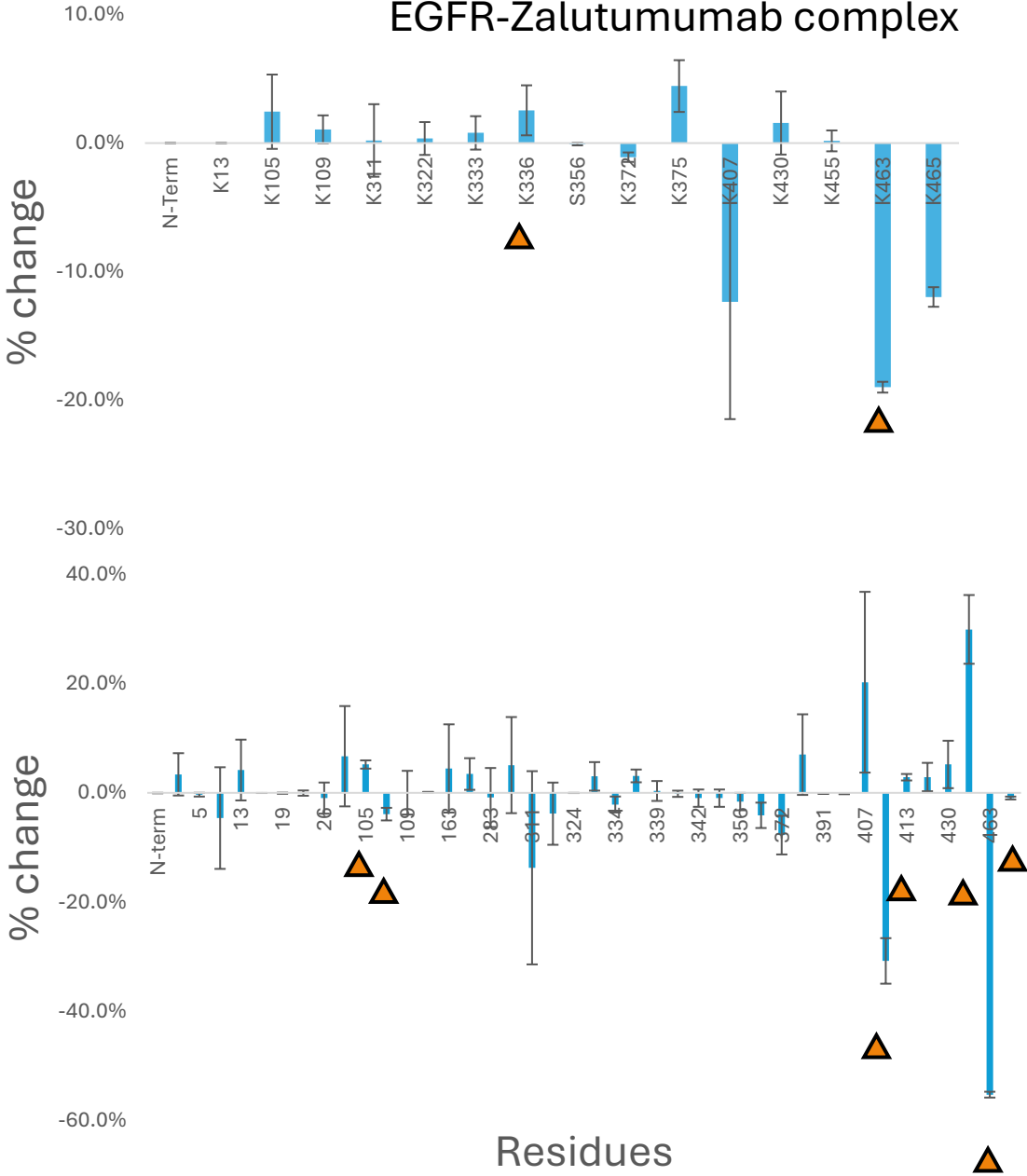
Residues with
significant changes



NHS acetate

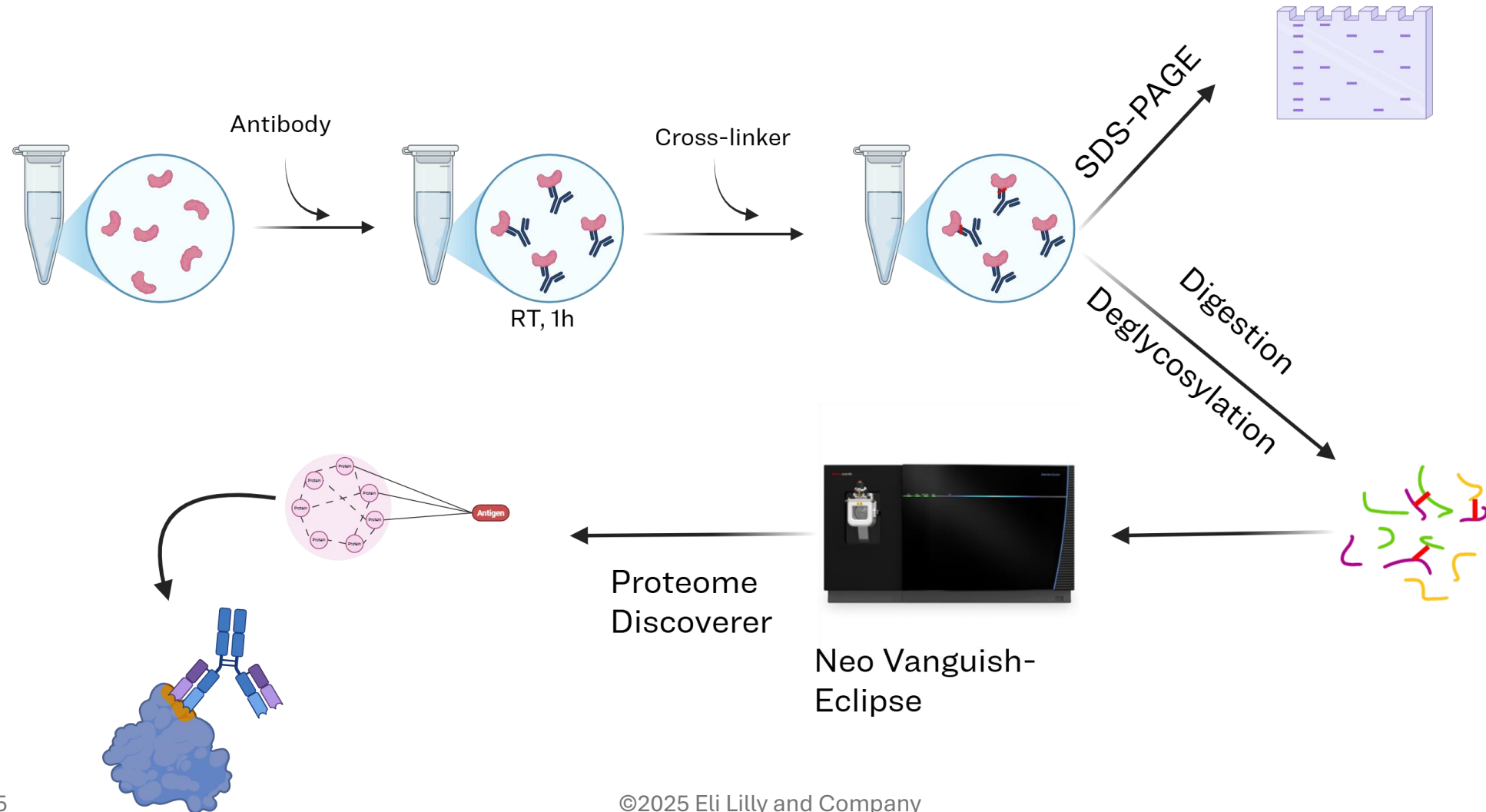
DEPC

EGFR label % changes in
EGFR-Zalutumumab complex

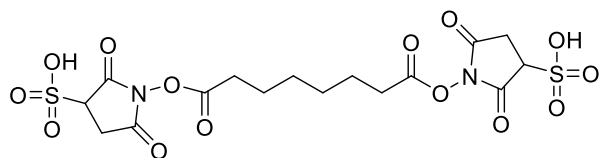


Cross- linking (XLMS)

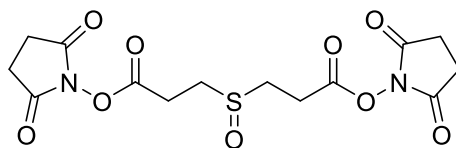
Cross-linking experimental procedure



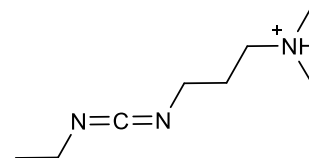
Cross-linkers



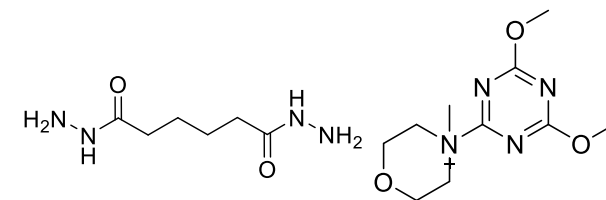
BS³



DSSO



EDC



ADH

DMTMM

Reactive sites

K, S

K, S

D, E

K, S, D, E

Ca-Ca limit

30Å

26Å

25Å

21Å - 25Å

Concentration

2 mM

2 mM

20 mM

20 mM

Reaction time

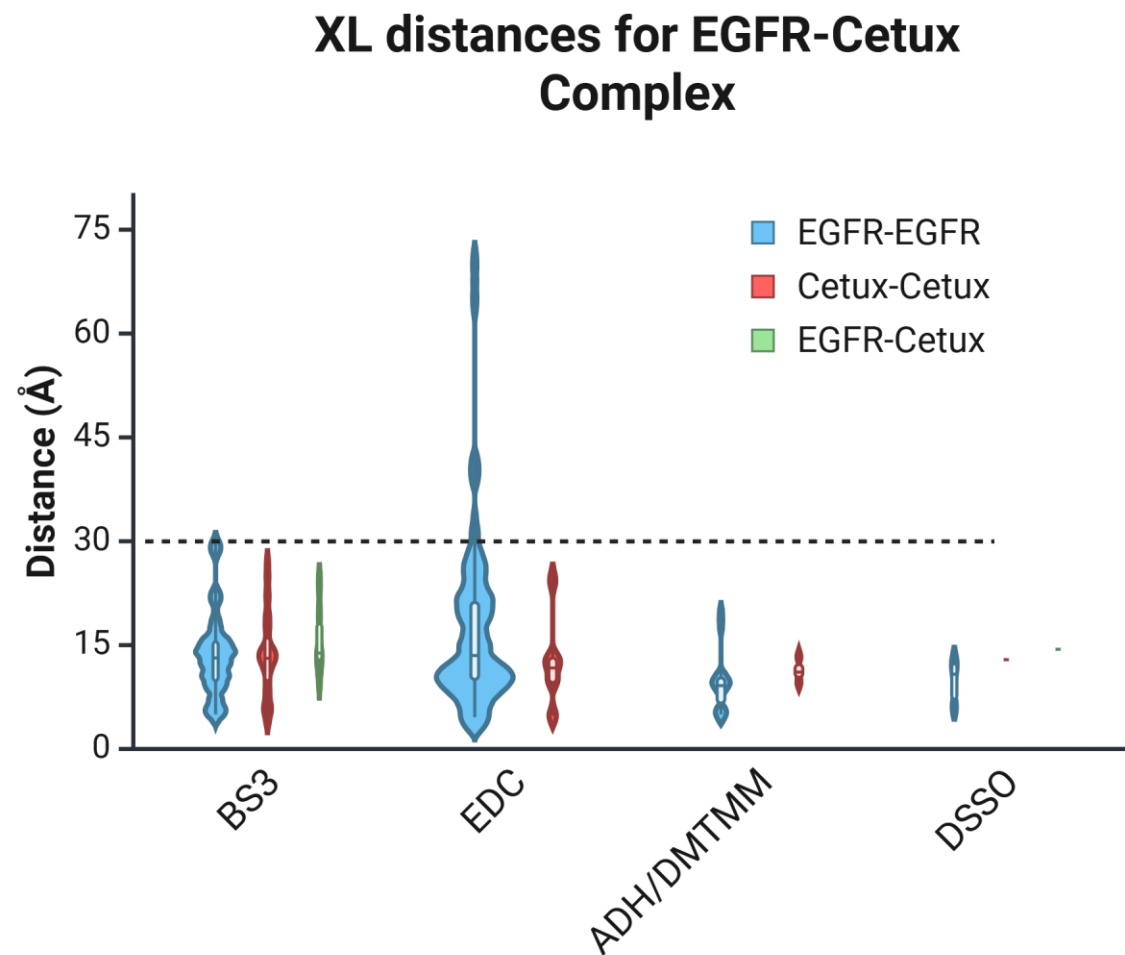
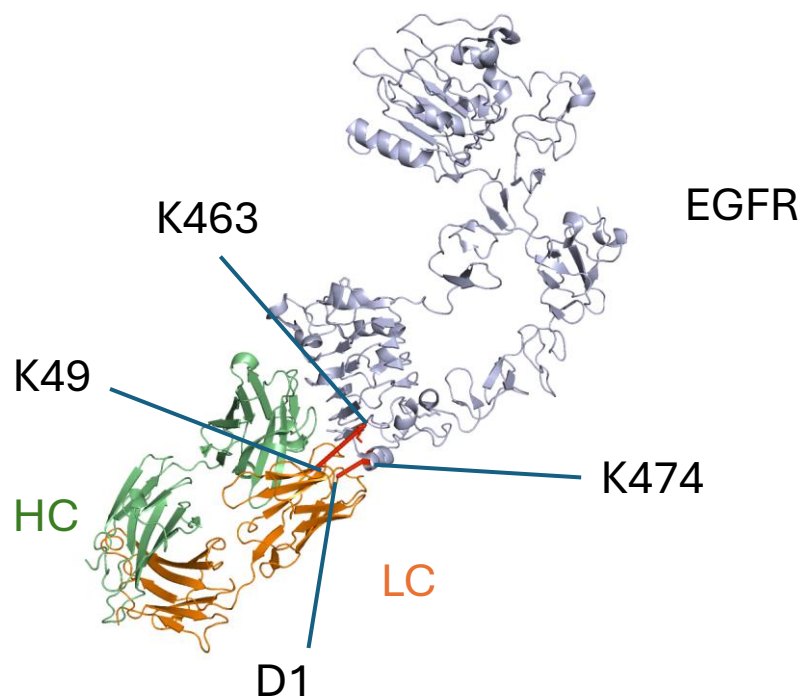
1.5h

1.5h

2 h

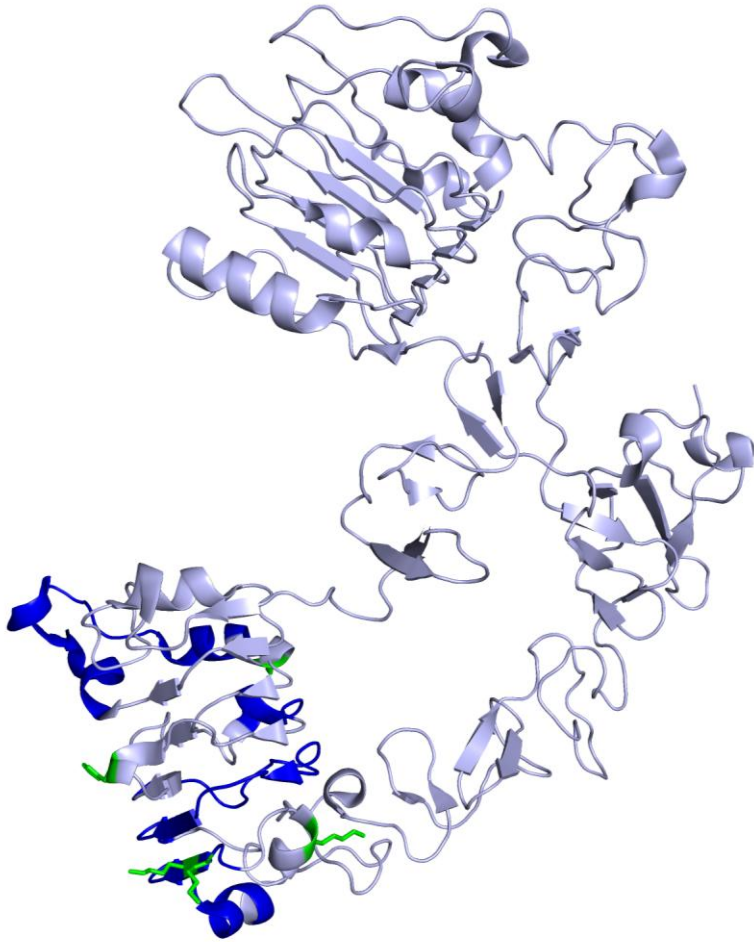
2 h

Cross-linking experimental Results



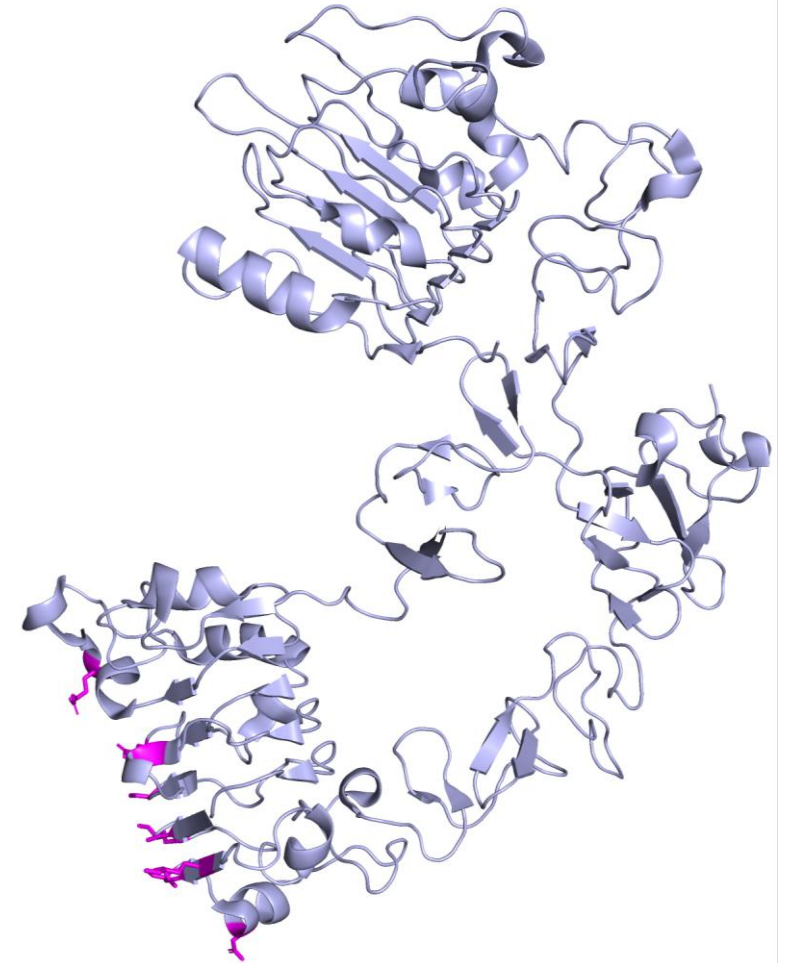
Comparing the structural MS techniques

EGFR- Cetux results from MS techniques matched published data



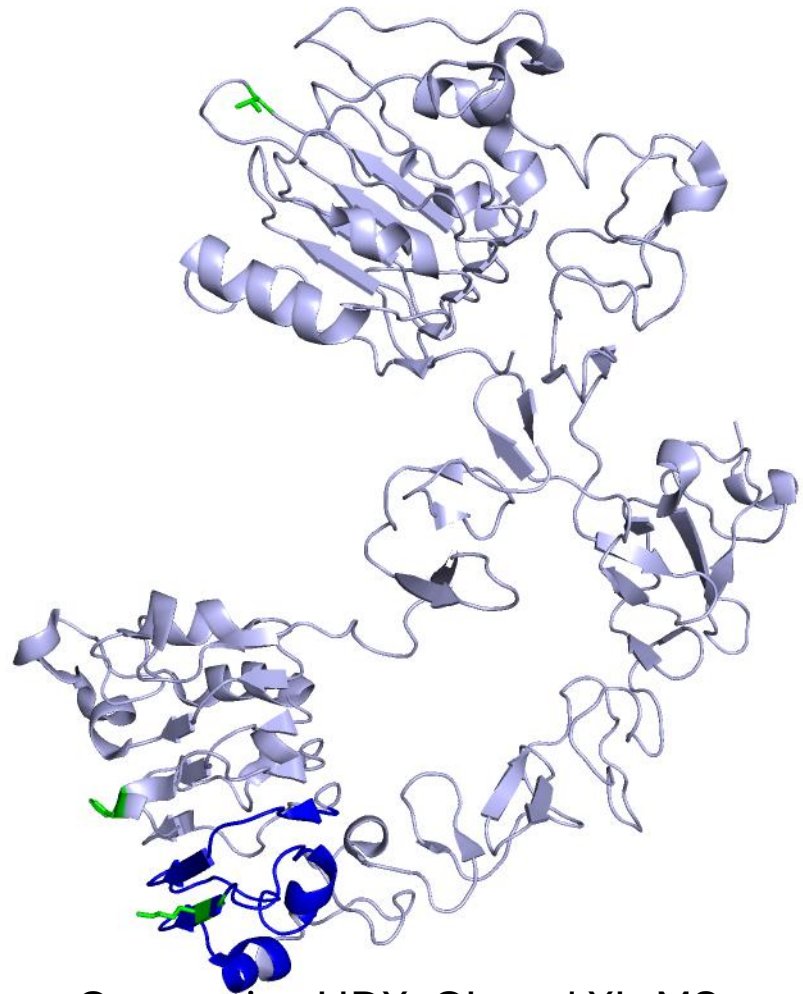
Comparing HDX, CL and XL-MS results

Magenta: Published epitope
Blue: epitope from HDX
Green: epitope from covalent labeling



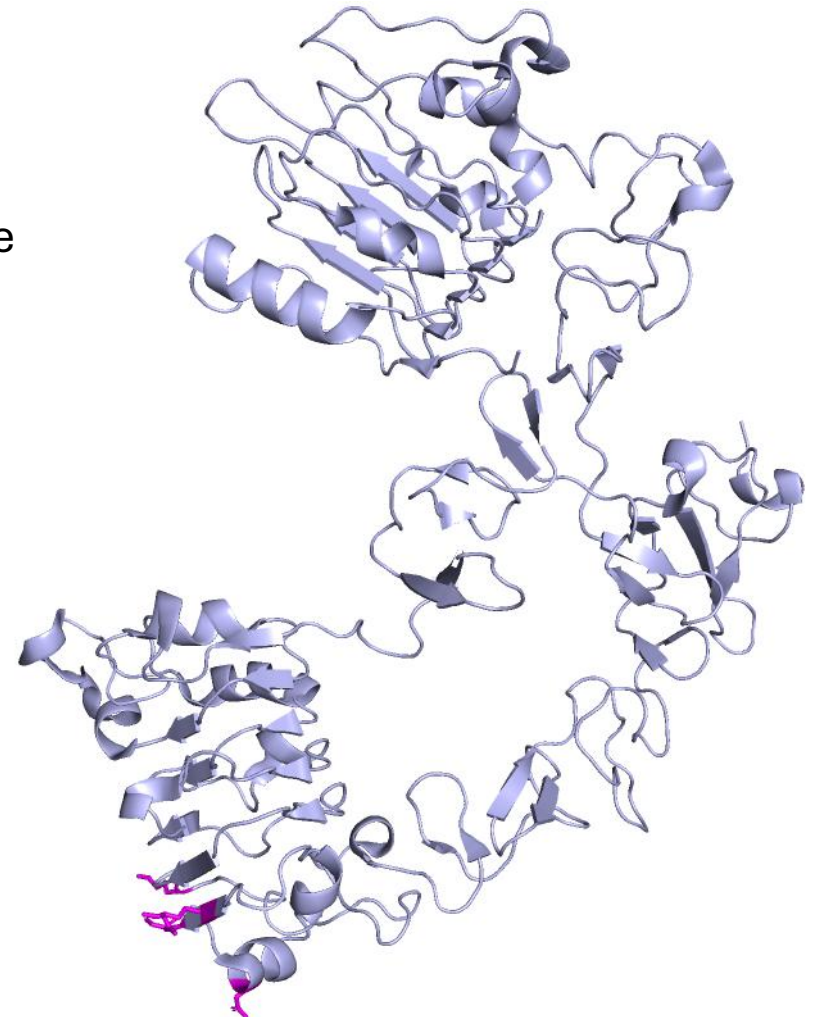
Epitope from published paper

EGFR- Zalu results from MS techniques matched published data



Magenta: Published epitope
Blue: epitope from HDX
Green: epitope from covalent labeling

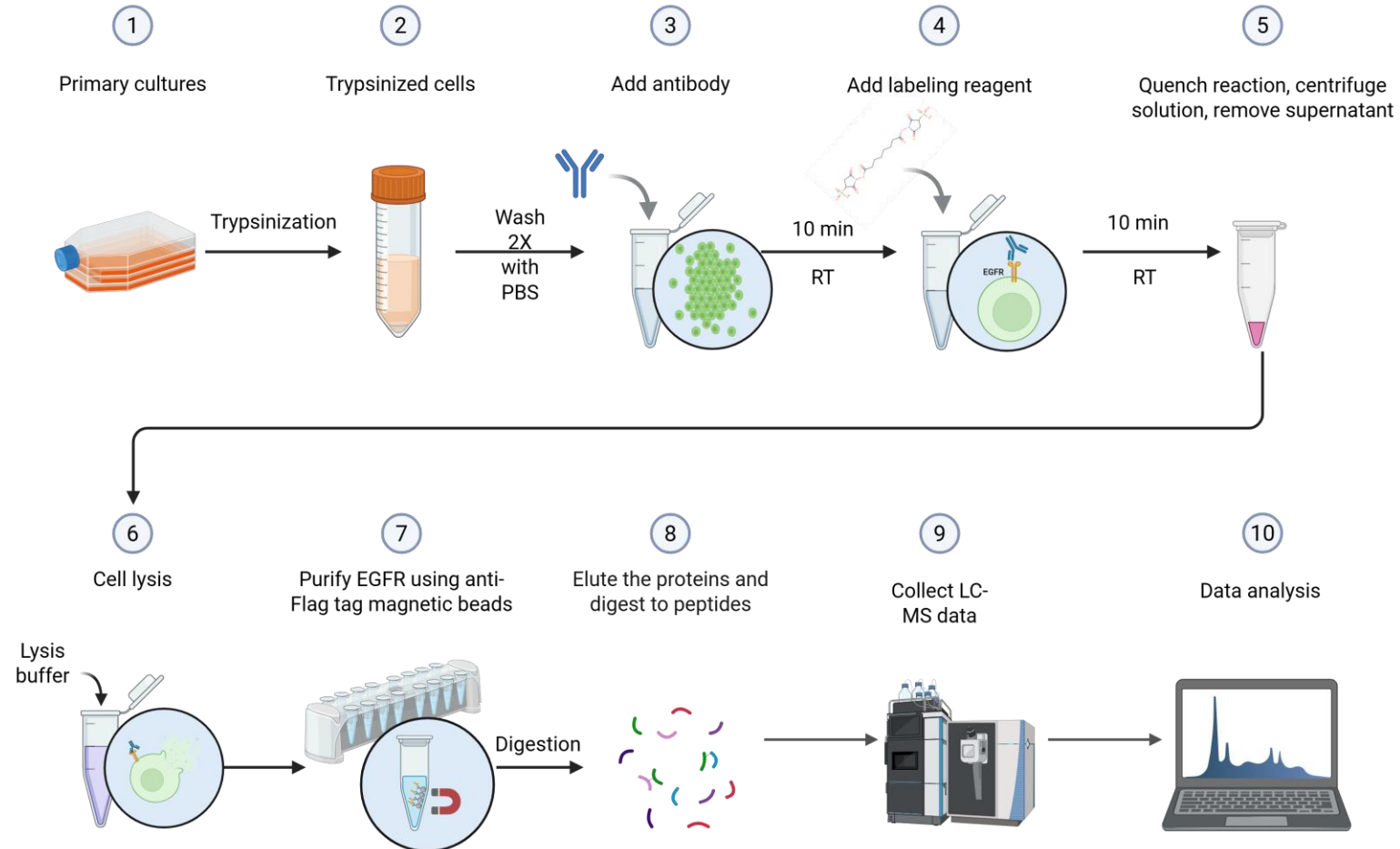
Comparing HDX, CL and XL-MS results



Epitope from published paper

Covalent labeling in cells

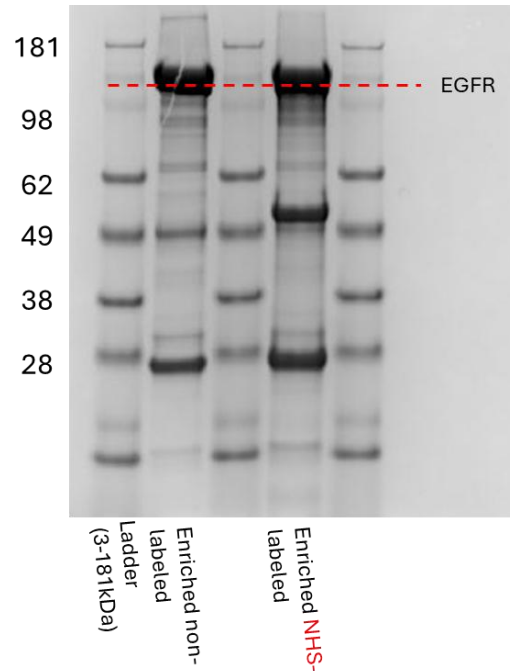
Labeling experiments in cell



HEK293T -flagged EGFR enrichment check

- Can EGFR be enriched with anti-flag tag magnetic beads before and after labeling reaction?
- Are there any impurities from unspecific binding of magnetic beads?

SDS-PAGE

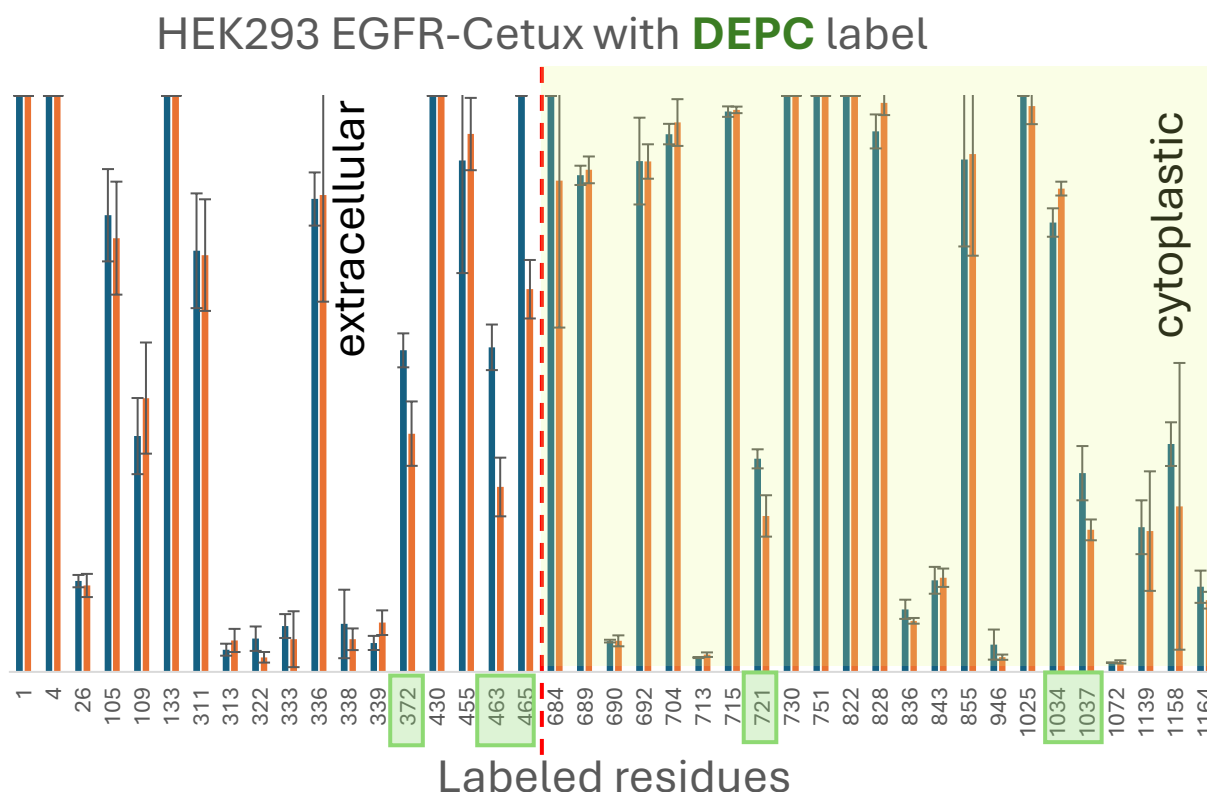
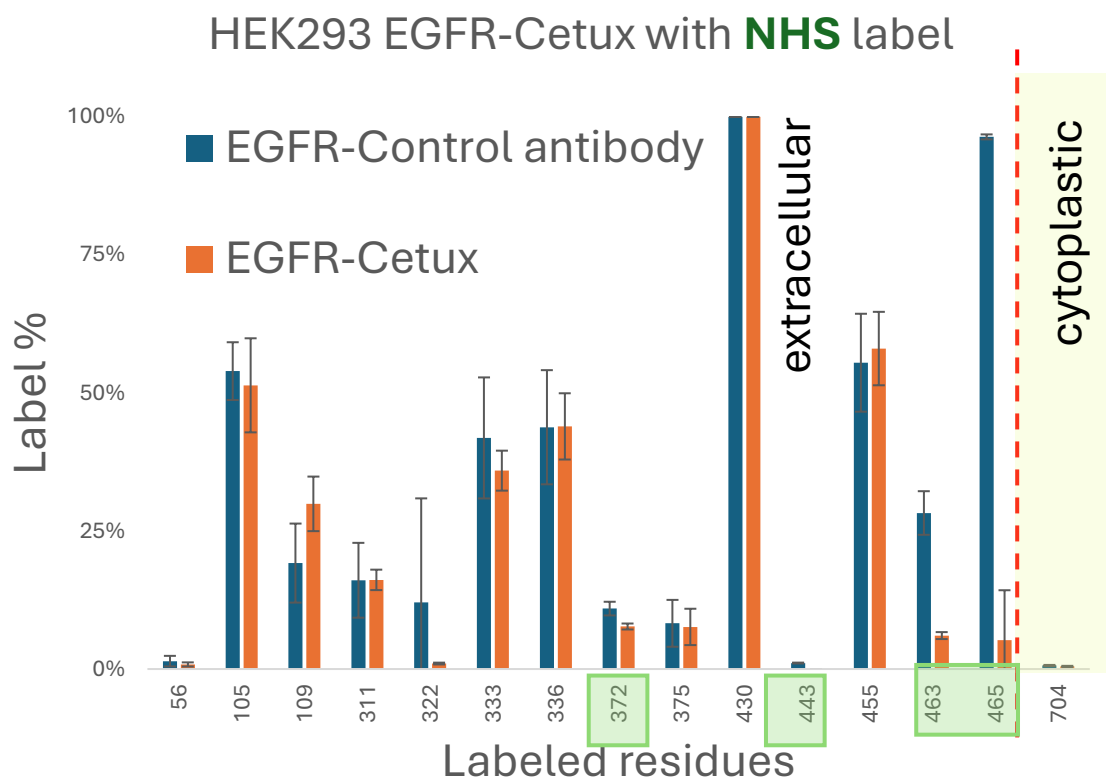


LC-MS

- Sample: digested non-labeled peptides from enriched proteins
- Data analysis: searched against all HEK293 cell line proteins
- Shorten the sequence lists for future CL search

Master	Accession	Description	Exp. q-value	Sum PEP Score	Coverage [%]	Sequence Coverage	# Peptides	# PSMS	# Unique Peptides	# AAs	MW [kDa]
✓	EGFR	T598S EGFR	0.000	280.506	75%		65	2338	65	1235	137.1
✓	Q9BVA1	Tubulin beta-2B chain OS=Homo sapien	0.000	31.931	43%		11	190	11	445	49.9
✓	P0DMV8	Heat shock 70 kDa protein 1A OS=Hom	0.000	20.198	21%		8	40	8	641	70.0
✓	P0C0S8	Histone H2A type 1 OS=Homo sapiens	0.000	10.437	44%		3	47	3	130	14.1
✓	P0CG47	Polyubiquitin-B OS=Homo sapiens OX=	0.000	5.229	41%		3	41	3	229	25.7
✓	Q07021	Complement component 1 Q subcompo	0.000	4.681	11%		1	1	1	282	31.3
✓	P84077	ADP-ribosylation factor 1 OS=Homo sa	0.000	1.220	6%		1	1	1	181	20.7
✓	Q13162	Peroxiredoxin-4 OS=Homo sapiens OX=	0.000	0.976	3%		1	11	1	271	30.5
✓	Q14257	Reticulocalbin-2 OS=Homo sapiens OX=	0.000	0.600	13%		1	1	1	317	36.9
✓	P07949	Proto-oncogene tyrosine-protein kinase	0.000	0.531	3%		1	9	1	1114	124.2
✓	Q99759	Mitogen-activated protein kinase kinase	0.000	0.513	7%		1	3	1	626	70.9

Label % for cell enriched EGFR in two labeling rxn



Residues that showed significant changes at 95% confidence

Summary

- The combined results from HDX, CLMS and XLMS were consistent with literature
- Resolution: HDX > CLMS > XLMS
- Time: CLMS (fastest) > HDX and XLMS (slow)
- Easy to work with/reliability: CLMS > HDX > XLMS
- Instrumentation requirement: CLMS (lowest) < XLMS < HDX (highest)
- All three techniques can be used in other ways to investigate protein-protein interactions

Acknowledgements

- Deepa Balasubramaniam
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THANK YOU!