

Structure/Cross Linking in Real Time Updates in XL-MS workflow

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1

Sr. Manager, Omics Marketing April, 2023





Outline

Introduction

RLTS in XL analysis

- Method development to reject monolinks
- Enhancement in XL identification and CSMs.
- XMAS for XL visualization
 - View XLs using XMAS
 - Confirmation of dimer structure using XMAS
- NuXI
 - NA-protein crosslinkers
 identification





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2

Crosslinking overview- measuring distance constraints

Protein structure analysis at different levels of complexity

Single protein:

- Secondary structure elements
- Conformational changes
- 3D structure
- Small molecule interactions



- Topology of the complexes
- Orientation of subunits
- Interaction interfaces

Protein interaction networks

Identity of interacting proteins

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- Composition of the complexes
- Transient interactions





Crosslinking overview

Basic crosslinking workflow





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Which crosslinker to choose?

Crosslinkers selection tool

Conjugation	to	Cleavability	Water soluble*	Pegylated	Membrane 1 permeable			
Amines (e.g., lysine)	Amines (e.g., lysine)	By CID using MS	Алу	Any	Any			
Alkyne	Alkyne	Any	Yes	No	Yes			
Azide	Azide		No	Vez	Ma			
Carbohydrates (Oxidized sugars)	Carbohydrates (Oxidized sugars)	Not cleavable	NU	Tes	+			
Carboxyls (e.g., C-terminus)	Carboxyls (e.g., C-terminus)	By Thiols	Spacer arm lengt	1**	Packaging ¹			
NA (Metabolic labeling)	NA (Metabolic labeling)	By Base	Any	-	Апу			
Nonselective (Photoreactive)	Nonselective (Photoreactive)		Medium		Premium grade			
Phosphine	Phosphine	By Periodate	Short		Single use			
Sulfhydryls (e.g., cysteine)	Sulfhydryls (e.g., cysteine)	By Hydroxylamine	Long		Standard			
🛓 Download								
Catalog Number(s)	A35459		A33545					
Reagent name	DBSU		DSSO					
Spacer arm length**	Medium (12.5 Å)		Medium (1	Medium (10.1 Å)				

• https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-labeling-crosslinking/protein-crosslinking/crosslinker-selection-tool.html

5

Challenges in the XL-MS workflow

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Introducing New Family of Trifunctional Crosslinkers

PhoX: An IMAC-Enrichable Cross-Linking Reagent Barbara Steigenberger,^{†,‡,§} Roland J. Pieters,[§][®] Albert J. R. Heck,^{*,†,‡}[®] and Richard A. Scheltema^{*,†,‡}[®] A52283 High-Select™ Fe-NTA Magnetic Phosphopeptide Enrichment Kit, 20rxns

- A52284 High-Select™ Fe-NTA Magnetic Agarose, 5mL
- A52286 DSPP (Disuccinimidyl Phenyl Phosphonic Acid, PhoX), 50 mg
- A52287 TBDSPP (tert-Butyl Disuccinimidyl Phenyl Phosphonate, tBu-PhoX), 50 mg



COMMUNICATION

ingewandte Chemie International Editio

A Membrane-Permeable and Immobilized Metal Affinity Chromatography (IMAC)-Enrichable Cross-Linking Reagent to Advance In Vivo Cross-Linking Mass Spectrometry Pin-Lian Jiang^{1[a]}, Cong Wang^{1[a]}, Anne Diehl^[a], Rosa Viner^[b], Chris Etienne^[b], Prenchendar Nandhikonda^[c], Leigh Foster^[c], Ryan D. Bomgarden^[c], Fan Liu*^{[a][d]}



TBDSPP/tbPHoX



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10.1002/anie.20211393

WILEY-VCH

DSPP (Phox) Workflow

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TBDSPP(tbPhox) Workflow



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ER proteins are found with PhoX modifications in CHO cells



Ē	3	Checked	Master	Accessio	n Descriptio	n				rage [%]	# Peptides	# Crosslinks	# CSMs	# PSMs	# Uniqu	e Peptides	# AAs
16	-122		V	Q60446	Heat show	k protein 105 kDa [0	DS=Cric	etulus griseus]	6	45%	24	0	3	49		24	858
17	-		V	TP001	ToolmAb	heavy_chain	y_chain			32%	10	2	5	38	2	10	450
18	-12		1	P68361	Tubulin a	pha-1B chain [OS=0	1B chain [OS=Cricetulus griseus]			37%	14	٥	1	42		14	451
19	-12		4	P07156	High mob	ility group protein B1	group protein B1 [OS=Cricetulus griseus]			63%	15	10	33	41		15	180
20	÷2		×	Q8K3H7	Calreticul	in [OS=Cricetulus gr	S=Cricetulus griseus]			45%	12	5	11	11 33		12	417
21	-122	-	1	P69893	Tubulin b	eta-5 chain [OS=Crie	chain [OS=Cricetulus griseus]		1	43%	13	1	2	35		13	444
22	-		4	Q60432	Hypoxia u	p-regulated protein	julated protein 1 [OS=Cricetulus griseus]			27%	19	6	22	44		19	999
23	-12		1	Q7SIA2	Bifunction	al glutamate/proline	utamate/prolinetRNA ligase [OS=Cricetulus			19%	19	6	19	33	1	19	1511
24	-122		V	P48538	Galectin-	I [OS=Cricetulus gri:	=Cricetulus griseus]			54%	7	0	0	24		7	135
25	-12		V	P14851	Peptidyl-p	orolyl cis-trans isome	olyl cis-trans isomerase A [OS=Cricetulus griseu:				9	1	4	25		9	164
•						W-							.)	1			
Prote	ide As ein G	sociated T	ables Peptide	Groups	PSMs	MS/MS Spectrum	Info	Annotated Modificatio	ons	Annotati	on Protein Gr	ouds 😵	Cross	inks (CSMs	Crosslink	MS2
é	#	Checked	Confid	ence 🕶	dentifying No	ntifying Node PSM Ambiguity Annotated Sequence		tated Sequence	Mod	difications		# Proteins	Master Pr	otein Acc	essions	ons Protein Accessio	
1	÷₽.		(Sequest HT (B2) Unambiguous	EDE	DDRDEDEEDEDEKEEI				1	Q8K3H7		Q8K3H7		
2	-122		(Sequest HT (A2) Unambiguous	IDDP	TDSKPEDWDKPEHIPI	K24	(Phox:H2C))	1	Q8K3H7		1	Q8K3H7	
3	-12				Sequest HT (B2) Unambiguous	iguous EDEDDRDEDEEDEDEKEE					1	Q8K3H7			Q8K3H7	
4	-12			Sequest HT (A		A2) Unambiguous	KPE	DWDEEMDGEWEPPVI	K24	4(Phox:H2C))	1	Q8K3H7			Q8K3H7	
5	-12				Sequest HT (A2) Unambiguous	EDE	DDRDEDEEDEDEKEEI				1	Q8K3H7			Q8K3H7	
6	-12				Sequest HT (A2) Unambiguous	KPE	DWDEEMDGEWEPPVI	K24	(Phox:H2C))	1	Q8K3H7			Q8K3H7	

e#	Ch	hecked	Master	Accession	Description	Coverage [%]	# Peptides	# Crosslinks	#CSMs	# PS +	# Unique Peptides	# AAs
105 👳			1	G3I6P1	Glyceraldehyde-3-phosphate dehydrogenase OS=Cricetulu	28%	6	0	5	13	- 1	345
106 👳			¥	G3IEX2	Nuclease-sensitive element-binding protein 1 OS=Cricetulu:	46%	5	0	0	13	5	127
107 🗇		D	~	G3GXA9	protein disulfide-isomerase OS=Cricetulus griseus OX=100;	70%	9	1	2	13	9	184
108 🕂			4	G3HP69	Calnexin OS=Cricetulus griseus OX=10029 GN=Canx PE=:	24%	9	1	3	13	9	591
109 🗇			~	G3HLS2	14-3-3 protein beta/alpha OS=Cricetulus griseus OX=10029	32%	6	2	4	12	4	244
110 👳			1	G3HWC3	Ezrin OS=Cricetulus griseus OX=10029 GN=179_015278 PI	19%	9	1	1	12	7	586
111 🗇			V	G3IGQ3	Heat shock cognate 71 kDa protein OS=Cricetulus griseus (27%	9	1	1	12	5	207

A Hide Associated Tables

Protein Groups	Peptide Groups	PSMs	MS/MS Spectrum Info	Crosslinks	CSMs	Crosslink MS2 Scans
----------------	----------------	------	---------------------	------------	------	---------------------

E	ŧ.	Checked	Confidence -	Identifying Node	PSM Ambiguity	Annotated Sequence	Modifications	# Proteins	Master Protein Acce	Protein Accessions
1	4		۲	Sequest HT (A2)	Unambiguous	SDAEEDGGTGSQDEEDRkf	K18(Phox:H2O)	1	G3HP69	G3HP69
2	-12		•	Sequest HT (A2)	Unambiguous	LLSkTSELNLDQFHDK	K4(Phox:H2O)	1	G3HP69	G3HP69
3	-			Sequest HT (A2)	Unambiguous	QkSDAEEDGGTGSQDEEDI	K2(Phox:H2O)	1	G3HP69	G3HP69
4	-12		•	Sequest HT (A2)	Unambiguous	WkPPMIDNPNYQGVWKPR	K2(Phox:H2O)	1	G3HP69	G3HP69
5	-8		•	Sequest HT (A2)	Unambiguous	AkKDDTDDEIAK	K2(Phox:H2O)	1	G3HP69	G3HP69
								100		

RTLS methods for XL-MS analysis New

- Further reduction in monolinks
- PhoX labeled monolinks are also enriched.
- Monolinks are usually 5-8 times higher than crosslinks.

Crosslinked samples that can not be enriched

- Limited sample amount
- Other crosslinking labeling



Only on Eclipse and Ascend in Tune 4.0



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Real Time Library Search (RTLS) for PhoX crosslinked samples



Crosslinks

Monolinks

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 MS OT
 Library database

 MS OT
 Intensity

 Intensity
 Intensity

 Charge State
 Collision Energy Tolerance

RTLS method set up

Dynamic Exclusion

ddMS² IT HCD

Real-Time Library Search

> 1 scans

ddMS² OT HCD

5 sec

Real	-Time Library Search Propert	,	PEAK SELECTION AND THRESHOLD SETTINGS
-	LIBRARY SEARCH	H SETTINGS	Use as a Trigger Only
	Spectral Library	merged_cxms_v6.db	TMT SPS MS3 Mode
		Browse Clear	Consider Unmatched Peaks Only
	Collision Energy Tolerance	100	Add Matched Fragments to Dynamic Exclusion
	Similarity Search	✓	
	Maximum Search Time (ms)	50	Score Type Filter By Score
	Use Retention Time Filter		1 Cosine Score At least 40
	Isotope Error Correction	None (0) •	
	Reverse Library Search	✓	Define cosine score
	Use Multiple Precursors in Search		
•	PEAK SELECTION AND TH	IRESHOLD SETTINGS	
	Use as a Trigger Only		Kesword Promote/Paiert
	TMT SPS MS3 Mode		1 Monolink Reject
	Consider Unmatched Peaks Only		
	Add Matched Fragments to		Triggering mode



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Scheme of the "monolink reject" RTLS method



RTLS method optimization – IT vs OT for survey scan

E. coli Ovotransferrin 🔶 📥 cross-links 3000 loop-links of identifications mono-links 600 linear peptides 1 2000 Spectrum matche. eptide (Dalts) 400 1000 200 No. 0 0 600 4000 No. of identifications 400 2000 200 0 0 80 95 95 20 40 60 20 40 60 80 cosine score cosine score

Survey scans using IT provide • better results compared to OT

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High cosine score provide better • confidence in identification but low number of crosslinks

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RTLS method optimization - NCE levels in survey scans



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Ecoli digest

Increase in XL spectrum matches for enriched samples



Ecoli digest

Thermo Fisher

Improvement in XL identification for unenriched samples

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BSA digest

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Improving structural models with additional XLs

А no enrichment enrichment В no enrichment RTLS standard chain A chain C 55 14 enrichment RTLS standard chain D 18 107 unique RTLS shared chain B unique standard

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Yeast alcohol dehydrogenase, PDB:4W6Z

References for FAIMS-XL-MS

RETURN TO ISSUE

< PREV ARTICLE NEXT >

\sim **Optimized TMT-Based Quantitative Cross-Linking Mass Spectrometry Strategy for** Large-Scale Interactomic Studies Max Ruwolt, Lennart Schnirch, Diogo Borges Lima, Michal Nadler-Holly, Rosa Viner, and Fan Liu* ♥ Cite this: Anal. Chem. 2022, 94, 13, 5265-5272 Article Views Altmetric Citations Share Add to Export Publication Date: March 15, 2022 ~ 1213 10 RIS https://doi.org/10.1021/acs.analchem.1c04812 Copyright © 2022 American Chemical Society LEARN ABOUT THESE METRICS **RIGHTS & PERMISSIONS** 月 PDF (3 MB) Read Online SI Supporting Info (1) » Dr. Fan Liu RETURN TO ISSUE < PREV ARTICLE NEXT > Group lead Expanding the Depth and Sensitivity of Cross-Link Identification by Differential Ion Mobility Using High-Field Asymmetric Waveform Ion Mobility Spectrometry **FMP** Berlin Lennart Schnirch, Michal Nadler-Holly, Siang-Wun Siao, Christian K. Frese, Rosa Viner, and Fan Liu* analytical. <u>GDCh</u> Angewandte Communications Edition Chemie Real-Time Library Search Increases Cross-Link Identification Depth www.angewandte.org across All Levels of Sample Complexity How to cite: Angew. Chem. Int. Ed. 2022, 61, e202113937 Max Ruwolt, Yi He, Diogo Borges Lima, William Barshop, Johannes Broichhagen, Romain Huguet Rosa Viner, and Fan Liu $^{\circ}$ International Edition: doi.org/10.1002/anie.202113937 Mass Spectrometry Hot Paper German Edition: doi.org/10.1002/ange.202113937 Cite This: Anal. Chem. 2023, 95, 5248-5255 Read Online ation, Ions, Mathem ACCESSI A Membrane-Permeable and Immobilized Metal Affinity La Metrics & More Article Recommendations Supporting Informatio ABSTRACT: Cross-linking mass spectrometry (XL-MS) is a universal tool for Chromatography (IMAC) Enrichable Cross-Linking Reagent to **Advance In Vivo Cross-Linking Mass Spectrometry**

Pin-Lian Jiang⁺, Cong Wang⁺, Anne Diehl, Rosa Viner, Chris Etienne, Premchendar Nandhikonda, Leigh Foster, Ryan D. Bomgarden, and Fan Liu* ARTERACT: Come laking must pretrometry (KLMS) is a universal tool for the second seco

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Analytical Chemistry

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Crosslinks Analysis in Proteome Discoverer XlinkX node

From raw files to interpreted results for all types of crosslinkers for protein-protein and protein-NA

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Themo Proteome Discoverer 23:0:400 - 🛛 🔿								
Yew Administration Tools Window Help	» S	tudy Defin	ition	Input Files Samples Analysis Results				
	Erro	s Samp .	File	Sample Identifier	Sample Type		Quan Channel	Yeast St
				• •		•	· ·	۲
Annua Managan Managan Managan Managan Annua Managan Managan Managan Managan Managan Managan Managan Managan Man		S2	F2	TKOTT11_1ms3_1 - [126]	Sample	*	126	Met6
D Name File Type Sample Information		\$3	F2	TKOTT11 1ms3 1-1127NI	Sample		127N	Met6
E F E E		Q.4	E2	TKOTT11 1mm2 1 - (1930)	Samolo		1270	ALC: N
F2 SPM_DSB0_FAMB_80_65_120mm_ap2 new Sample Type (Sample)		04	F2	TROTTT_TMIS3_1*[127C]	Sample		1270	INNERO
		55	F2	IKO1111_1ms3_1 - [128N]	Sample	٠	128N	His4
		S6	F2	TKOTT11_1ms3_1 - [128C]	Sample	•	128C	His4
		S7	F2	TKOTT11_1ms3_1 - [129N]	Sample		129N	His4
		S8	F2	TKOTT11_1ms3_1 - [129C]	Sample	*	129C	Ura2
		59	F2	TKOTT11 1ms3 1-(130N)	Sample		130N	Ura2
		\$10	F2	TKOTT11 1mr3 1-0130C1	Sample		1300	Line2
		010	14		Cumpie		1000	- Ciuz
		SII	FZ	IKOTTT_Ims3_1-[131N]	Control	•	131N	Parenta
		S12	F2	TKOTT11_1ms3_1 - [131C]	Control	٠	131C	Parenta

Step 2: Select workflows, adjust parameters, run analysis Processing Consensus Run Queue MSF Files E PSM Grouper Reporter loss 12 Spectrum Salactor Ph. October Ph. Proceeds Ph. October Ph. O Feptide 2 Peptide and Protein Fiber Scan E offgeradus Processing Datings Oracle Sensors Millerature Millerature Millerature Oracle Sensors Oracle Fromin Grouping XinAXIPO Crosslink Crouping Tanget Decey Constant Fayort 11 --- Constants Validator

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21

Crosslinks Quantitation in Proteome Discoverer

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From raw files to interpreted results for all types of crosslinkers for protein-protein and protein-NA



Step 2: Select workflows and adjust parameters Spectrum Files 0 MST Files Masona Featana 2 Spectrum Selector Feature Mapper 8 FER control fo 😿 Sequest HT Processing Consensus Precursor loss Quantitier 9 Peptido Validator 2 Tanget Decoy PSM Vehicles Peptide and 3 Soctors (Inc. Protein Scorer 4 AMARTO Detect Protein Grouping 5 C XINKEFOFRM Consensus Validator 7 - Consensus Consensus Sinkkipo Maldatar a 🔶 🍪 Xinkk PD Search 6

Step 3: Choose ratios and run analysis



Step 4: Interpret results



Crosslinking Results Visualization: XiView Service

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Export mzldentML and mzML files for input into website to localize crosslinks inside the protein sequence/structure XiView Web Service



"xiView: A common platform for the downstream analysis of crosslinking mass spectrometry data", Graham, M., Combe, C.W., Kolbowski, L, Rappsilber, J., doi:10.1101/561829

CHOOSE FILE(S) 100% SUBMIT DATA Identification file: Xlink MS2 MS3 PD Contaminants LFO of DSSO crosslinked BSA data.mzid Xlink MS2_MS3 PD Contaminants LFQ of DSSO crosslinked BSA data.mzML 🗸 Peak list file(s): Xlink MS2 MS3 PD Contaminants LFQ of DSSO crosslinked BSA data.fasta 🗸 Sequence file: EXPORT HELP LOAD VIEWS PROTEIN-SELECTION ANNOTATIONS LAYOUT: LOAD V E 120 109 RESET ZOOM DOWNLOAD IMAGE AS SVG Drag to Pan Or Select SHOW PROTEIN PAIRING A LKLPSFMHFSQLDQR [8] Q6D0W9 - Q6D0W9 🗸 FAGR 1,000 800 600 400 200 200 400 600 800 1,000 NLY SHOW 2 TOP-SCO < > 1-2 of 2 Sele ONLY SHOW 2 TOP-SCORING MATCHES PER LINK ed across 1 Cross-Link 1 77.68 False NA 4755 F1 Agilent5 20151112 OK CRISPR 2-3 cid30-etd 2-(61)m pass fall Monolinks Ambig. Betw Pep Seq RESET >0 🐳 Min 20 21 2 118 🗟 21 🕏 Post-Filter: 11 of 11 TT Cross-Links

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Export to PyMOL

Export proteins containing crosslinkers from Proteome Discoverer software to multiple formats for visualization



... for pymol: Protein_chain.py ... for Xlink Analyzer: Protein_chain.txt ... generates Protein_chain.Distances.txt for view in text editor or Excel

PDB_id	Cha	in 1	Pos	sition	n_in	str	ucture_	1 Chain_2 Position	n_in_structure_2
Positi	on in	PDr	esul	lt 1	Pos	itio	n in PD:	result 2 Type	
Descri	ption	Dis	tand	ce (Ang	gstr	oem)		1.76	
6059	A	4	A	439	28	463	intra	intra K4 K439	51.92
6059	A	431	A	131	455	155	intra	intra K431 K131	34.84
6059	A	537	A	431	561	455	intra	intra K537 K431	26.1
6QS9	A	4	A	431	28	455	intra	intra K4 K431	49.87
6029	A	221	A	131	245	155	intra	intra K221 K131	35.93
6029	A	439	Α	431	463	455	intra	intra K439 K431	13.21
6059	A	4	A	221	28	245	intra	intra K4 K221	37.81
6059	A	132	A	131	156	155	intra	intra K132 K131	3.81
6059	A	439	A	221	463	245	intra	intra K439 K221	19.21
6029	A	4	A	239	28	263	intra	intra K4 K239	20.81
6029	A	12	A	4	36	28	intra	intra K12 K4	12.94
6029	A	12	A	131	36	155	intra	intra K12 K131	20.37
6059	A	242	A	431	266	455	intra	intra K242 K431	29.65
6059	A	221	A	431	245	455	intra	intra K221 K431	25.97
6059	A	413	A	535	437	559	intra	intra K413 K535	13.2



Analysis of Identified Crosslinks in ChimeraX by XMAS Color-coded display of Export To Text (tab delimited) ? × Path: crosslinks on*protein dimer G:\PD_3.0_YH\Enolase\enolasedss_hcd_s Map Markers **Right Mous** Intralinks (red) ٠ 5 Items to be Exported: Interlinks (yellow) Checked Items Only Proteins • Protein Groups Excluded Items Only **Peptide Groups** UCSF ChimeraX version: 1.4 (2022-06-03) © 2016-2022 Regents of the University of California. All co file **PSMs** Item Connections rights reserved. How to cite UCSE chimeraX MS/MS Spectrum Info enolasedss hcd secf-1 Cro. Generate R-friendly Headers Input Files Study Information Export IDs 2al1.pdb Hide Specialized Traces enolasedss_hcd_secf-1_Crossli... 2 enolasedss_hcd_secf-1_Crossli... 3 Show Crosslinks Enolase_shortest_intra.pb View CSMs Enolase shortest inter.pb Crosslink MS2 Scan The distance Crosslink Reporter Peak rt files Remove selected file Crosslink Summary distribution of identified Linked sslinks_1.pb ... for ChimeraX crosslinks slinks_1_shortest.pb crosslinks.txt Close Export

All positions aligned to

20

40 Distance (Å)

PDB structure with

distance information

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Lagerwaard et al. BioRxiv, 2022. https://doi.org/10.1101/2022.04.21.489026

Not overlap associated 52.62373172

Not overlap associated 52.6013682

Not overlap associated 10.22817392

Not overlap associated 10.48102181

Not overlap associated 61.18552011

Not overlap associated 61.10514645

Not overlap associated 10.50103885

Not overlap associated 12.86769599

Integrate

D

Distance (A) Not overlap associated 10.35076499 ise; 2-phospho-D- glycerate hydro-lyase

C

Overlap category

1 Row in evic Pseudobond

2 #1/A:357@CA #1/A:87@CA

2 #1/A:357@CA #1/B:87@CA

2 #1/A:87@CA #1/B:357@CA

2 #1/B:357@CA #1/B:87@CA

3 #1/A:328@CA #1/A:357@CA

3 #1/A:328@CA #1/B:357@CA

3 #1/A:357@CA #1/B:328@CA

3 #1/B:328@CA #1/B:357@CA

4 #1/A:138@CA #1/A:87@CA

2

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4

5 6

7

8

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10

Export to ChimeraX plugin–XMAS for integrative assisted

Thermo Fisher



visualization (DisVis) Interactive exclusion of "False positives" based on score and distance Export validated results for molecular docking to (HADDOCK) or IMP

Interaction interface



Xlink Mapping and AnalySis(XMAS)-Smooth Integrative Modeling in ChimeraX, https://doi.org/10.1101/2022.04.21.489026

26

Challenges in membrane protein XL-MS

Stability of membrane proteins

- Easy to aggregate
- Need lipid or detergent to keep in their native conformation.

Membrane proteins in SMALP

- Not compatible with MS analysis
- No established workflow for crosslinking analysis

• XL of membrane proteins in cell

- · What crosslinker to use
- Overcome poor sequence coverage



Zhuang, Q. et al. Curr. Opin. Struct. Biol. 2019, 58, 278-285



Refinement of S.enterica WbaP structure with XL-MS



In collaboration with G.Dodge and B.Impreiali, MIT

Mapping crosslinks onto the dimer structure in XMASS



54 DSS crosslinks

Lagerwaard et al. BioRxiv, 2022. https://doi.org/10.1101/2022.04.21.489026



48 tBuPhoX crosslinks

Dimer interface confirmation by XL-MS



Inter crosslinks

Residue 1	Residue 2	Distance – intra (Å)	Distance – inter (Å)
Lys81	Lys273	28.4	20.7
Lys148	Lys273	43.2	20.8
Lys232	Lys273	46.3	13.0
Lys222	Lys422	42.2	26.6

Observed in both DSS and tBuPhoX samples Unique for tbPhoX

New ! Nucleic Acid- Protein XL Workflow - NuXL

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Thermo Scientific[™] FAIMS Pro[™] interface, XL-MS workflow

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Unique workflow that is complete, easy to use, sensitive, and quantitative



Primers on XL-MS

Thermo Fisher

Study factors

Application note | 000928 IMAC-enrichable crosslinking reagents for structural biology and interactomics XlinkX Quantification Experimental design Nested & non-nested · Biological & technical replicates Thermo Fisher Setting up a study step-by-step · Label free Quantification TMT Quantification The world leader in serving scienc Getting start on XlinkX 3.0 for Proteome Discoverer 2.5 and 3.0 Thermo Scientific Center of Excellence The world leader in serving science

Home > Life Sciences > Structural Biology > Integrative Structural Biology

Integrative Structural Biology

High precision 3D analysis from structure to function

Contact us

Integrative Structural Biology https://www.thermofisher.com/u ology/integrative-structural-bi rosslinking mass spectrometr tps://www.thermofisher.com/u pectrometry/proteomics-mass halysis-mass-spectrometry/cro



Proteome Discoverer XlinkX Workshop February 15-16, 2023

Berlin	16:00 - 19:00
London	15:00 - 18:00
New York	10:00 - 13:00
Chicago	9:00 - 12:00
Denver	8:00 - 11:00

You are being invited to join a practical XlinkX workshop delivered via Microsoft Teams. Thermo Fisher Scientific experts will provide guidance to crosslinking (XL) analysis data processing in Thermo Scientific[™] Proteome Discoverer[™] 3.0 SP1 (PD) software and introduction to structural modelling using the generated PD results. The focus will be on handson sessions, including step-by-step experiment setup in PD, discussion of data processing parameter settings, and exporting the results in data formats suitable for third party modelling packages.

We will also use PD results to showcase xiView, PyMol and ChimeraX modelling packages. There are two interactive on-line sessions planned. The workshop should give ample time for discussing participants' questions. For detailed agenda please refer to the next page. Registration deadline is February 8, 2023. Links to the training sessions and for downloading the training materials will be send out by February 10, 2023. The sessions will be recorded and recordings made available to all registered participants.

The workshop is free of charge. Please register using the link below.

Registration link

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