

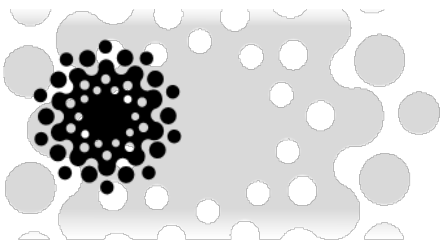
Workshop

Crosslinking Mass Spectrometry for Core Facilities

Berlin, Germany

23rd - 25th June, 2020

Workshop Program



Day 1		
Registration		8:30-9:00
Lecture 1: Introduction of CLMS ^{1-3, 4-23}		9:00-10:00
Principle of CLMS; What information can one learn from CLMS data; Advantages and limitations; Applications		
Lecture 2: Protein crosslinking ²³⁻²⁶		10:00-10:45
Sample preparation for CLMS analysis; Crosslinkers; Key factors that affect crosslinking reaction; Crosslinking titration		
Coffee Break		10:45-11:00
Lecture 3: Digestion of crosslinked proteins ²⁷⁻²⁹		11:00-11:45
In-gel or in-solution digestion; Sequential digestion; Peptide extraction		
Lecture 4: Enrichment of crosslinked peptides ^{8,23,27}		11:45-12:30
Why enrichment is necessary; Protein level enrichment; Peptide level: chromatography based enrichment (SEC, SCX or combined); Enrichable crosslinker		
Lunch		12:30-13:30
Lecture 5: LC-MS/MS analysis of crosslinked peptides ³⁰⁻³⁴		13:30-14:15
LC gradient; Importance of high-high acquisition; Charge based enrichment in gas phase; Fragmentation methods		
Seminar (Group 1): Crosslinking and sample preparation	Seminar (Group 2): LC-MS/MS acquisition of crosslinked peptides	14:30-15:30
Titration design; How to choose digestion methods; Enrichment setup (LC, StageTips)...	Instruments for CLMS; Tuning parameters for detection crosslinked peptides; Gas phase association?	
Coffee Break		15:30-15:45
Seminar (Group 1): LC-MS/MS acquisition of crosslinked peptides	Seminar (Group 2): Crosslinking and sample preparation	15:45-16:45
Instruments for CLMS; Tuning parameters for detection crosslinked peptides; Gas phase association?	Titration design; How to choose digestion methods; Enrichment setup (LC, StageTips)...	

Day 2

Lecture 6: Identification of crosslinked peptides using xiSEARCH ^{27,35}	9:00-10:00
Fragmentation of crosslinked peptides; Principle of xiSEARCH; Search CLMS data with xiSEARCH	
Lecture 7: False-discovery rates: xiFDR ^{36,37}	10:00-11:00
Principle of FDR for crosslinks; How xiFDR works; Alternative "auto-validation" function in xiSEARCH for small datasets	
Coffee Break	11:00-11:30
Lecture 8: Visualization of crosslink data in xiVIEW ^{38,39}	11:30-12:15
How normally crosslinking data were visualized and used in publication; Visualizing crosslinking data using xiVIEW.	
Lunch	12:15-13:15
Software workshop: xiSEARCH, xiFDR, xiVIEW	13:15-17:00
How to set up xi software on your own computer; Computational power and process speed/limitations; Understanding parameters; Using a model dataset to benchmark the setup	

Day 3	
Lecture 9: Quantitative CLMS ^{15,18,21,29,40–45}	9:00-10:00
QCLMS provide additional dynamic information to protein structures; Current applications of QCLMS; popular workflow and software tools	
Coffee Break	10:00-10:30
Lecture 10: Crosslinking as part of integrated structural biology (ISB) ^{15,46–50}	10:30-11:30
What is integrated structural biology; How crosslinking data used in ISB; Commonly used modelling tools	
Discussion: Communicating with collaborators	11:30-12:30
Experiment design for QCLMS analysis; Sample handover; Delivering results...	
Lunch	12:30-13:30
Data Analysis Workshop: Bring your own crosslinking data	13:30-17:00

Recommended reading

O'Reilly, F. J. & Rappsilber, J. Cross-linking mass spectrometry: methods and applications in structural, molecular and systems biology. *Nat. Struct. Mol. Biol.* **25**, 1000–1008 (2018).

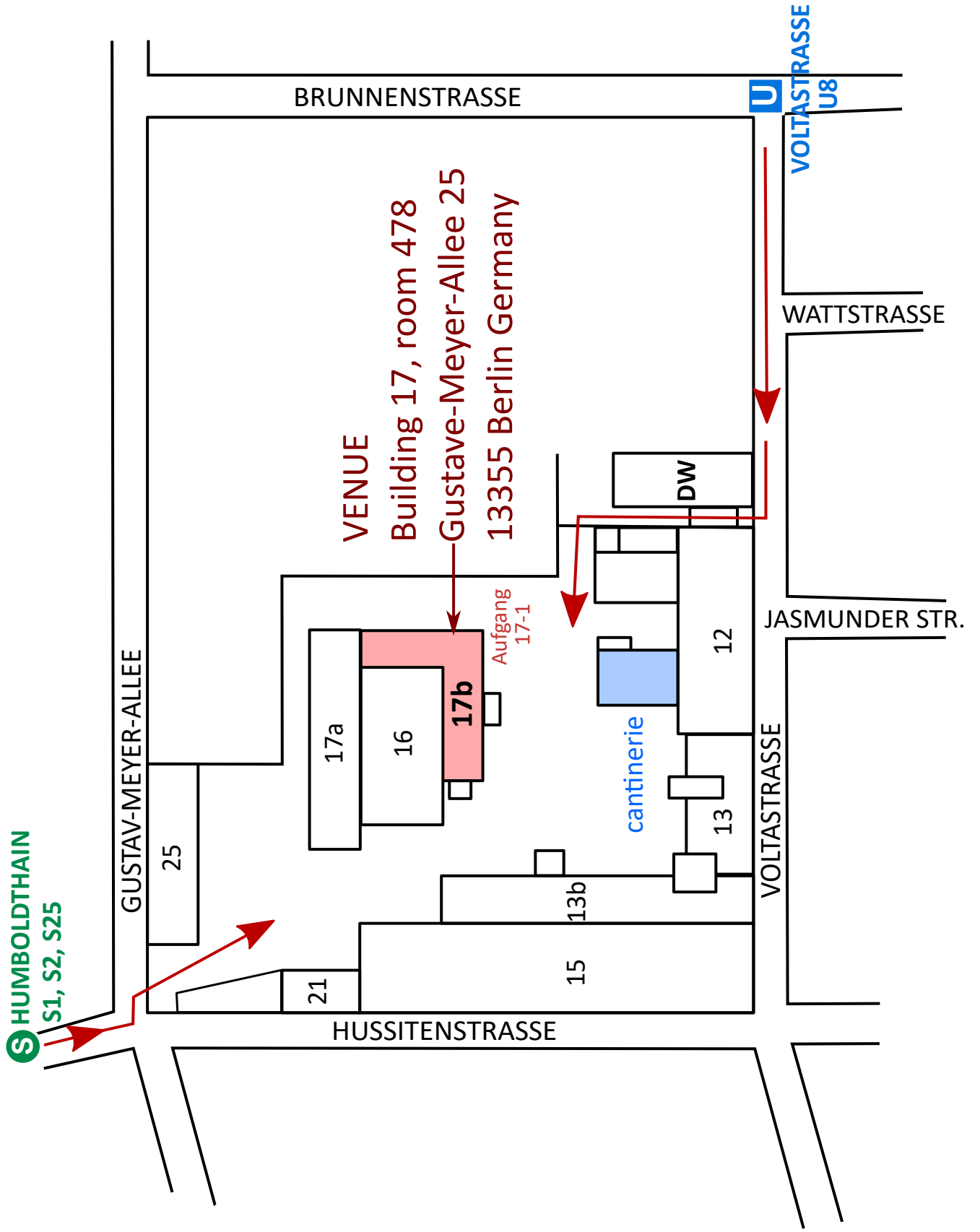
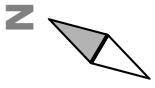
Optional reading

1. Rappsilber, J. The beginning of a beautiful friendship: cross-linking/mass spectrometry and modelling of proteins and multi-protein complexes. *J. Struct. Biol.* **173**, 530–540 (2011).
2. Rappsilber, J. Cross-linking/mass spectrometry as a new field and the proteomics information mountain of tomorrow. *Expert Rev. Proteomics* **9**, 485–487 (2012).
3. O'Reilly, F. J. & Rappsilber, J. Cross-linking mass spectrometry: methods and applications in structural, molecular and systems biology. *Nat. Struct. Mol. Biol.* **25**, 1000–1008 (2018).
4. Shakeel, S. *et al.* Structure of the Fanconi anaemia monoubiquitin ligase complex. *Nature* **575**, 234–237 (2019).
5. Bürmann, F. *et al.* A folded conformation of MukBEF and cohesin. *Nat. Struct. Mol. Biol.* **26**, 227–236 (2019).
6. Makarov, A. A. *et al.* Lamin A molecular compression and sliding as mechanisms behind nucleoskeleton elasticity. *Nat. Commun.* **10**, 3056 (2019).
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8. Ryl, P. S. J. *et al.* In Situ Structural Restraints from Crosslinking Mass Spectrometry in Human Mitochondria. *J. Proteome Res.* (2019) doi:10.1021/acs.jproteome.9b00541.
9. Yuan, Z. *et al.* Structural basis of Mcm2-7 replicative helicase loading by ORC-Cdc6 and Cdt1. *Nat. Struct. Mol. Biol.* **24**, 316–324 (2017).
10. Kastiris, P. L. *et al.* Capturing protein communities by structural proteomics in a thermophilic eukaryote. *Mol. Syst. Biol.* **13**, 936 (2017).
11. Chen, J. W. C. *et al.* Cross-linking mass spectrometry identifies new interfaces of Augmin required to localise the γ -tubulin ring complex to the mitotic spindle. *Biol. Open* **6**, 654–663 (2017).
12. Gupta, K. *et al.* Architecture of TAF11/TAF13/TBP complex suggests novel regulation properties of general transcription factor TFIID. *Elife* **6**, (2017).

13. McCaughan, U. M. *et al.* Pre-40S ribosome biogenesis factor Tsr1 is an inactive structural mimic of translational GTPases. *Nat. Commun.* **7**, 11789 (2016).
14. Sheppard, C. *et al.* Repression of RNA polymerase by the archaeo-viral regulator ORF145/RIP. *Nat. Commun.* **7**, 13595 (2016).
15. Chen, Z. A. *et al.* Structure of Complement C3(H₂O) Revealed By Quantitative Cross-Linking/Mass Spectrometry And Modeling. *Mol. Cell. Proteomics* **15**, 2730–2743 (2016).
16. Abad, M. A. *et al.* Ska3 Ensures Timely Mitotic Progression by Interacting Directly With Microtubules and Ska1 Microtubule Binding Domain. *Sci. Rep.* **6**, 34042 (2016).
17. Legal, T., Zou, J., Sochaj, A., Rappsilber, J. & Welburn, J. P. I. Molecular architecture of the Dam1 complex-microtubule interaction. *Open Biol.* **6**, (2016).
18. Tomko, R. J., Jr *et al.* A Single α Helix Drives Extensive Remodeling of the Proteasome Lid and Completion of Regulatory Particle Assembly. *Cell* **163**, 432–444 (2015).
19. Trowitzsch, S. *et al.* Cytoplasmic TAF2-TAF8-TAF10 complex provides evidence for nuclear holo-TFIID assembly from preformed submodules. *Nat. Commun.* **6**, 6011 (2015).
20. Borek, W. E. *et al.* Mto2 multisite phosphorylation inactivates non-spindle microtubule nucleation complexes during mitosis. *Nat. Commun.* **6**, 7929 (2015).
21. Herbert, A. P. *et al.* Complement Evasion Mediated by Enhancement of Captured Factor H: Implications for Protection of Self-Surfaces from Complement. *J. Immunol.* **195**, 4986–4998 (2015).
22. Barysz, H. *et al.* Three-dimensional topology of the SMC2/SMC4 subcomplex from chicken condensin I revealed by cross-linking and molecular modelling. *Open Biol.* **5**, 150005 (2015).
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36. Fischer, L. & Rappsilber, J. Quirks of Error Estimation in Cross-Linking/Mass Spectrometry. *Anal. Chem.* **89**, 3829–3833 (2017).
37. Fischer, L. & Rappsilber, J. False discovery rate estimation and heterobifunctional cross-linkers.

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38. Combe, C. W., Fischer, L. & Rappsilber, J. xiNET: cross-link network maps with residue resolution. *Mol. Cell. Proteomics* **14**, 1137–1147 (2015).
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Venue map



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Building 17, room 478
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S HUMBOLDTHAIN
S1, S2, S25