Investigating a large human protein assembly by native MS: the SAGA complex

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Outline

• MS in structural biology
  ✓ Native MS
  ✓ Ion mobility-MS
  ✓ Top down proteomics
• The SAGA complex
• Interdisciplinary approach
MS-based strategy

Native MS → 2D interaction network of subunits

IM-MS → Shape of subcomplexes

MS-based proteomics → Map of PTMs
MS-based strategy

Native MS → 2D interaction network of subunits
Information from native MS

Composition

Stoichiometry

Core and peripheral subunits

Subunit interactions

Assembly pathway

Interaction network
How to maintain non covalent interactions in the gas phase?

- Aqueous buffer (e.g. ammonium acetate)
- Soft ionisation (electrospray)
- Modified instruments (Q-TOF, Q-IM-TOF)
Human SAGA complex

Spt–Ada–Gcn5–acetyltransferase

Collaborators:
Imre Berger (EMBL, Grenoble)
Christophe Masselon (EDyP, Grenoble)
Sarah Teichmann (MRC, Cambridge, UK)
Patrick Schultz and Laszlo Tora (IGBMC, Strasbourg)
Human SAGA complex

- Control of chromatin remodelling
- Transcriptional activation and elongation
- mRNA export
SAGA complex in diseases

- Mutations of ATXN7: spinocerebellar ataxia type 7
- USP22: predictor of tumour aggressiveness and metastatic probability (expression of 11 genes)
Human SAGA complex

- 18 subunits
- Incomplete interaction network: 14 subunits

Subunits:
- SGF29
- ADA3
- ADA2b
- GCN5
- USP22
- ATXN7L3
- ENY2
- TAF9
- TAF6L
- TAF5L
- TAF10
- Supt7L
- TRRAP

[Diagram showing the subunits of the SAGA complex]
Acetyltransferase submodule

mass (kDa)
Region of ADA3 interacting with SGF29 has been truncated
3mer: truncated ADA3

Building a 2D interaction network
MS-based strategy

IM-MS

Shape of subcomplexes
Information from IM-MS

Same mass, different shape: ring type and collapsed type
Information from IM-MS

Same mass, different shape: ring type and collapsed type

Collision cross section: the effective surface area of a macromolecular ion when it collides with a neutral gas molecule
Ion Mobility separation
Human eukaryotic initiation factor 3 (eIF3) complex
Zhou et al., PNAS, (2008), 105, 47, 18139–18144
Native MS

Ion Mobility

EM

Human eukaryotic initiation factor 3 (eIF3) complex
Zhou et al., PNAS, (2008), 105, 47, 18139–18144
Top down proteomics
The human SAGA complex

Collaboration with Christophe Masselon (EDyP, Grenoble)
Map of SAGA PTMs

• *S Cerevisiae*: 21 subunits

• 61 acetylated Lys

Map of SAGA PTMs

• *S Cerevisiae*: 21 subunits
• 61 acetylated Lys
• Acetylation regulates:
  ✓ binding affinity
  ✓ catalytic activity
Map of SAGA PTMs

- S *Cerevisiae*: 21 subunits
- 102 phosphorylation sites
- Share 4 subunits with Transcription factor II D

Map of SAGA PTMs

- *S Cerevisiae*: 21 subunits
- 102 phosphorylation sites
- Share 4 subunits with Transcription factor II D
- **Phosphorylation controls:**
  - location of shared subunits
HAT module of human SAGA

Detection of PTMs

- HAT module of SAGA (Coomassie)
- phosphorylated subunits (Pro-Q stain)
- acetylated subunits (α-AcetLys-WB)

• Simon Trowitzsch
Structure of SAGA complex: consortium

MS

2D interaction network and PTMs
Structure of SAGA complex: consortium

Patrick Schultz, IGBMC Strasbourg
Structure of SAGA complex: consortium

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Imre Berger, EMBL
### Structure of SAGA complex: consortium

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**Sarah Teichmann, MRC, Cambridge**
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3D architecture of subunits
Conclusions

• MS-based strategy:
  ✓ 2D interaction network of subunits
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• Complementary to electron microscopy and bioinformatics
Acknowledgments

• Imre Berger
• Simon Trowitzsch
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