



Caractérisation par spectrométrie de masse des différentes étapes de la biosynthèse des acides mycoliques médiées par la polyketide synthase Pks13

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Plateforme Protéomique Toulouse Midi-Pyrénées

<http://proteomique.ipbs.fr>

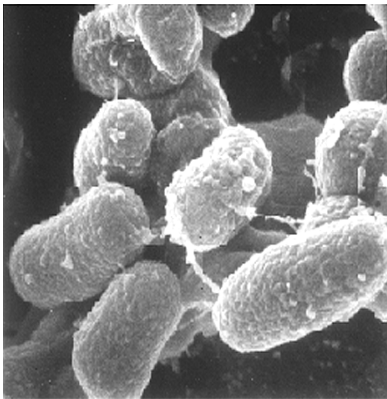
SMAP - Dijon - 14-17 septembre 2009



**Université
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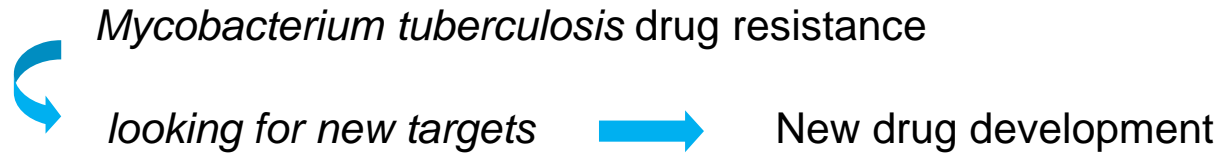


Tuberculosis Disease – The Mycobacteria cell wall



M. Tuberculosis
Tuberculosis disease agent

Collaboration Mamadou Daffé

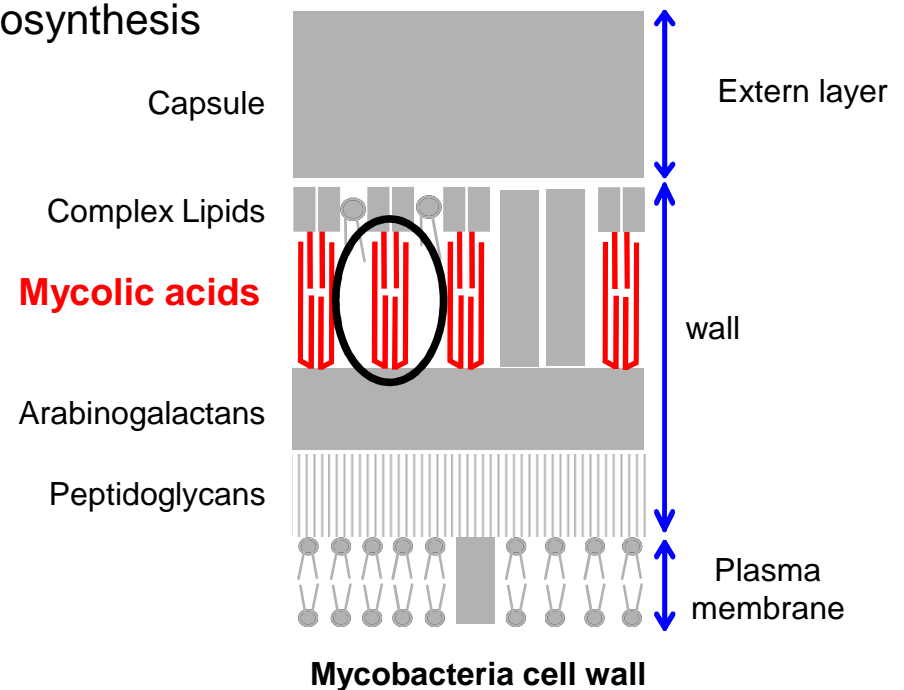


→ Antimycobacterial drugs target the cell wall biosynthesis

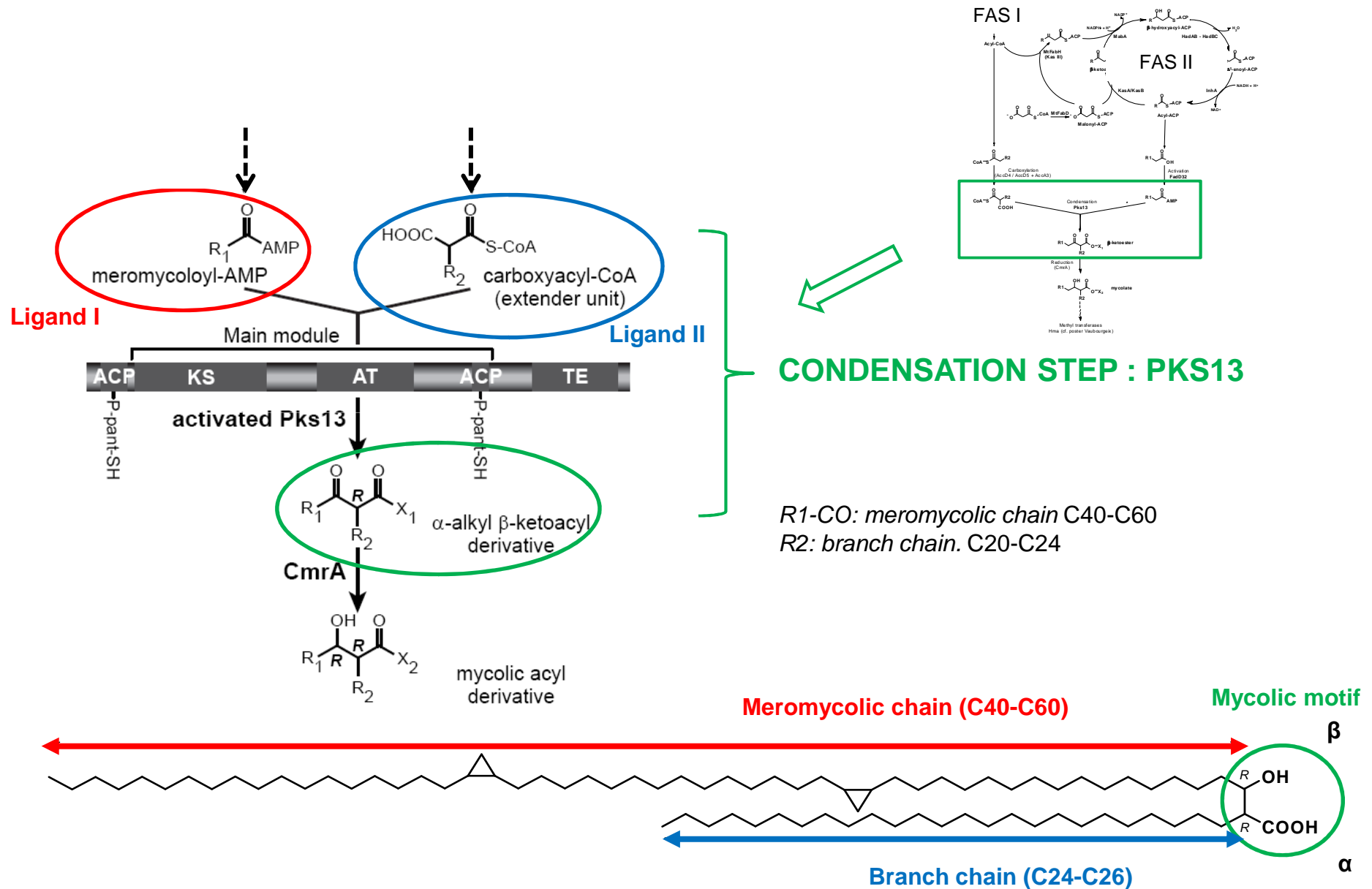
Major and specific constituents



Mycolic acids



The condensation step of mycolic acids biosynthesis

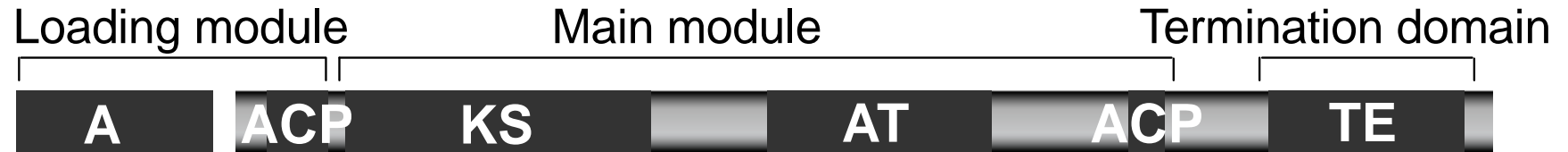


Objective of the study

- ▶ **Knowledge of the pks13 condensation mechanism**
 - To target novel antimycobacterial drugs

- ▶ **Description of the last steps of biosynthesis of mycolic acid**
 - To confirm and to characterize the PKS13 domains function
 - To localize by mass spectrometry catalytic amino acids corresponding to ligand binding sites

The PKS13 polyketide synthase A multi-domains protein



High molecular Protein : 186 kDa

A : protein-protein interaction domain

ACP : acyl carrier protein

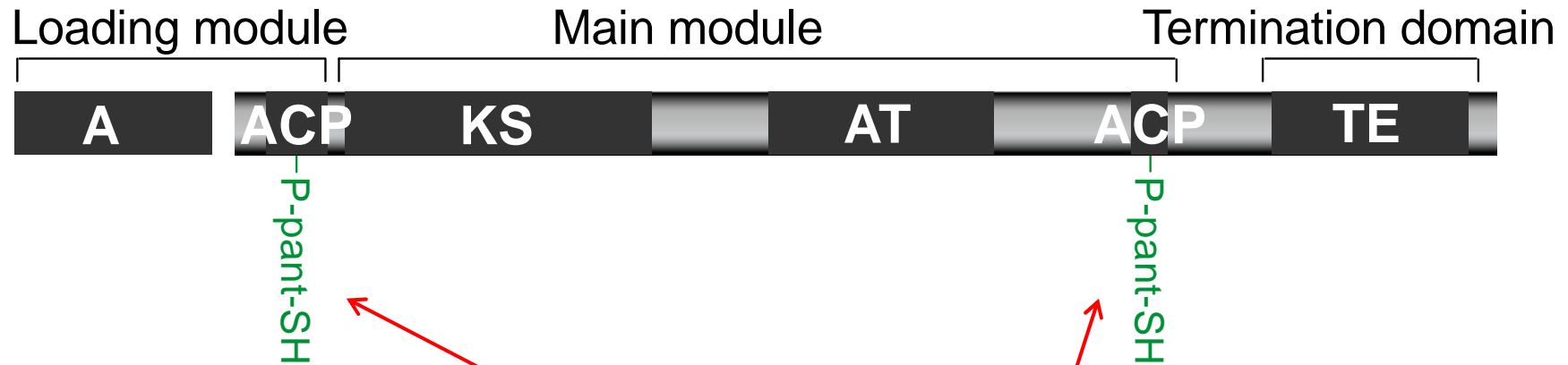
AT : acyl transferase

KS : ketosynthase domain

TE : thioesterase domain

The PKS13 polyketide synthase

A multi-domains protein



High molecular Protein : 180 kDa

A : protein-protein interaction domain

ACP : acyl carrier protein

AT : acyl transferase

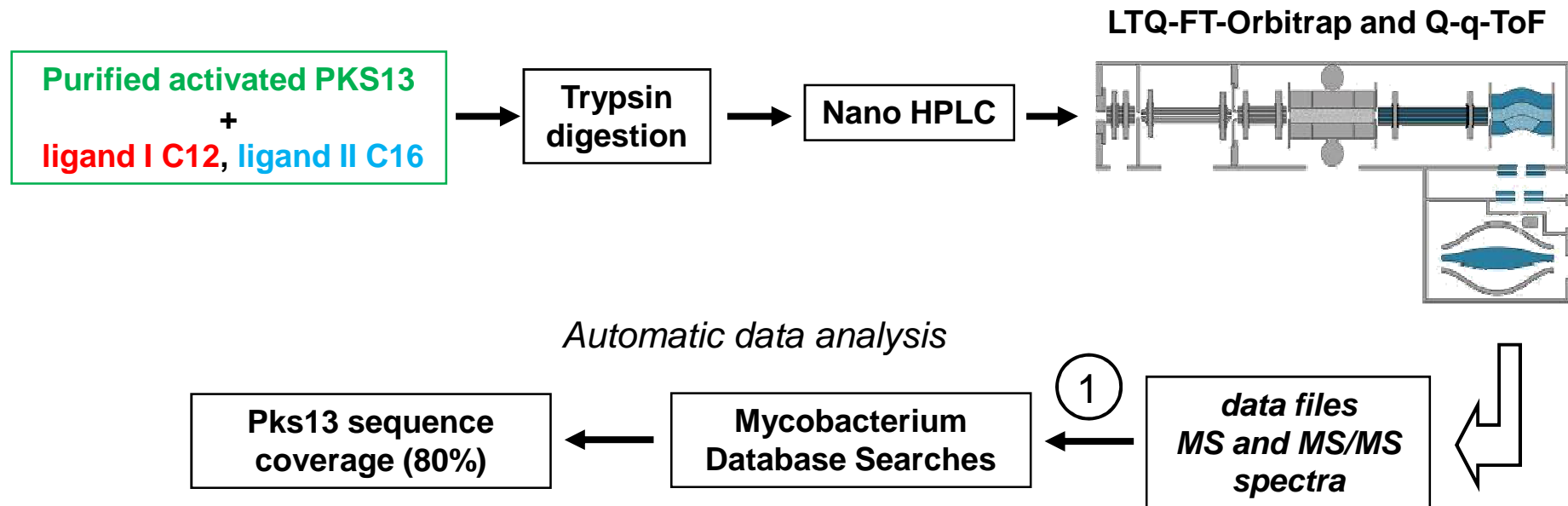
KS : ketosynthase domain

TE : thioesterase domain

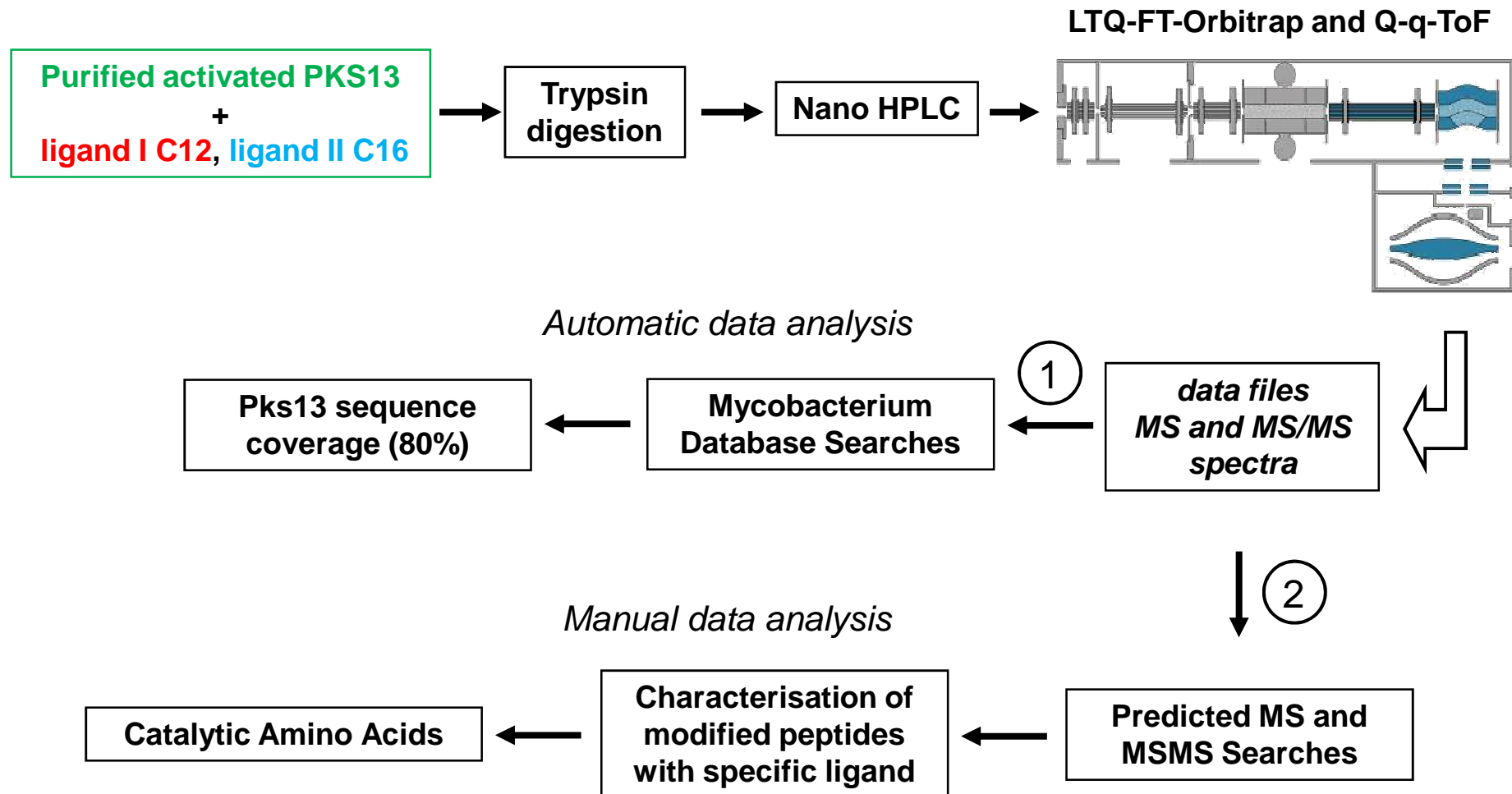
P-pant : 4'-phosphopantetheinyl
M = 339,07 g/mol

ACTIVATION
Ppant-Tranferase

NanoHPLC-MSMS analysis to study PKS13 condensation mechanism



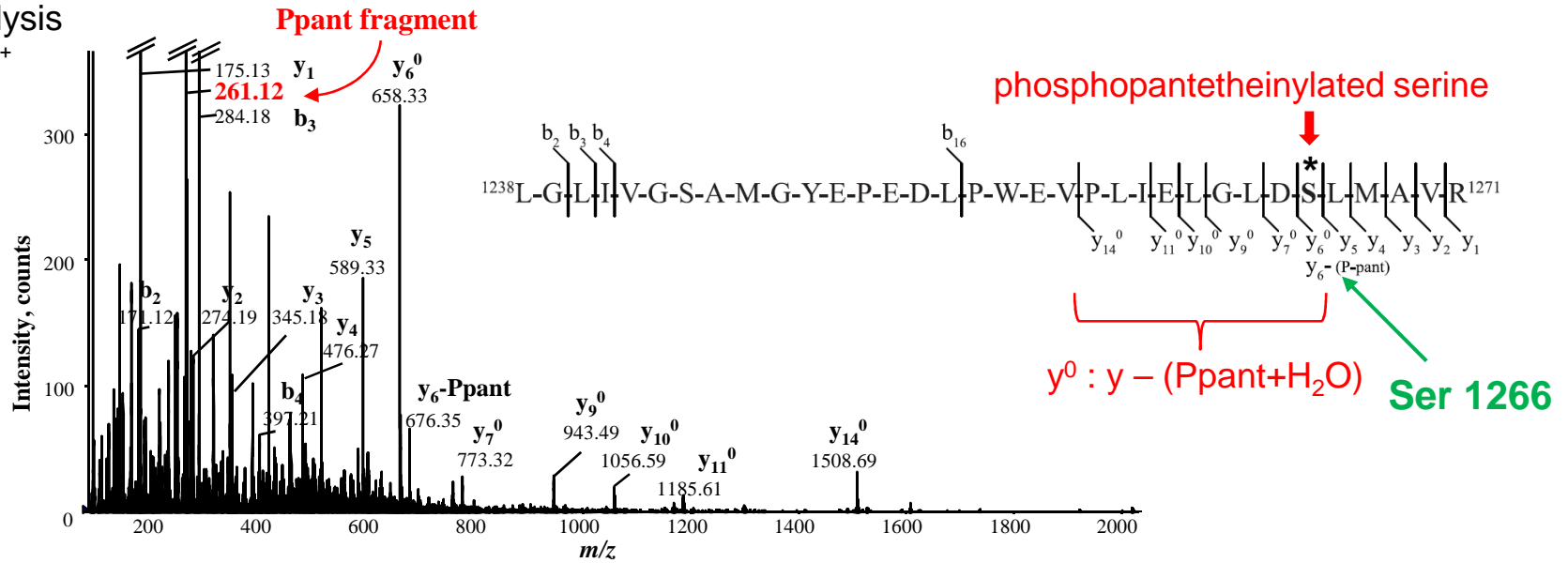
NanoHPLC-MSMS analysis to study PKS13 condensation mechanism



Ppant binding to ACP-Cter domain

Q-q-ToF Analysis
 $m/z = 2012.01^{2+}$

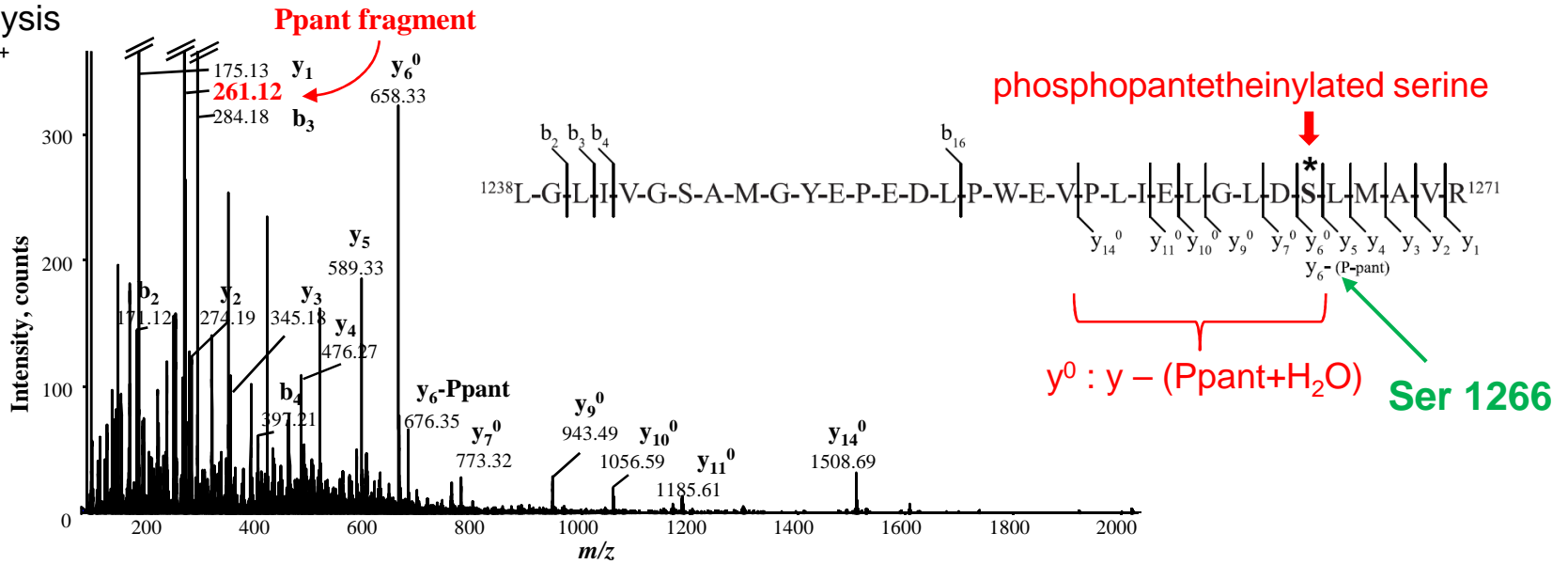
ACP C-ter



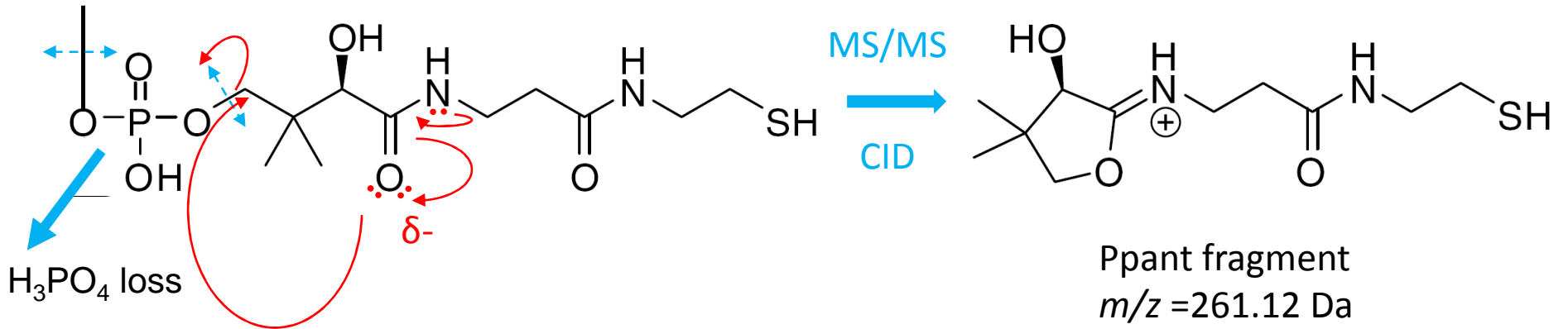
Ppant binding to ACP-Cter domain

Q-q-ToF Analysis
 $m/z = 2012.01^{2+}$

ACP C-ter



(Ser) Tryptic Peptide

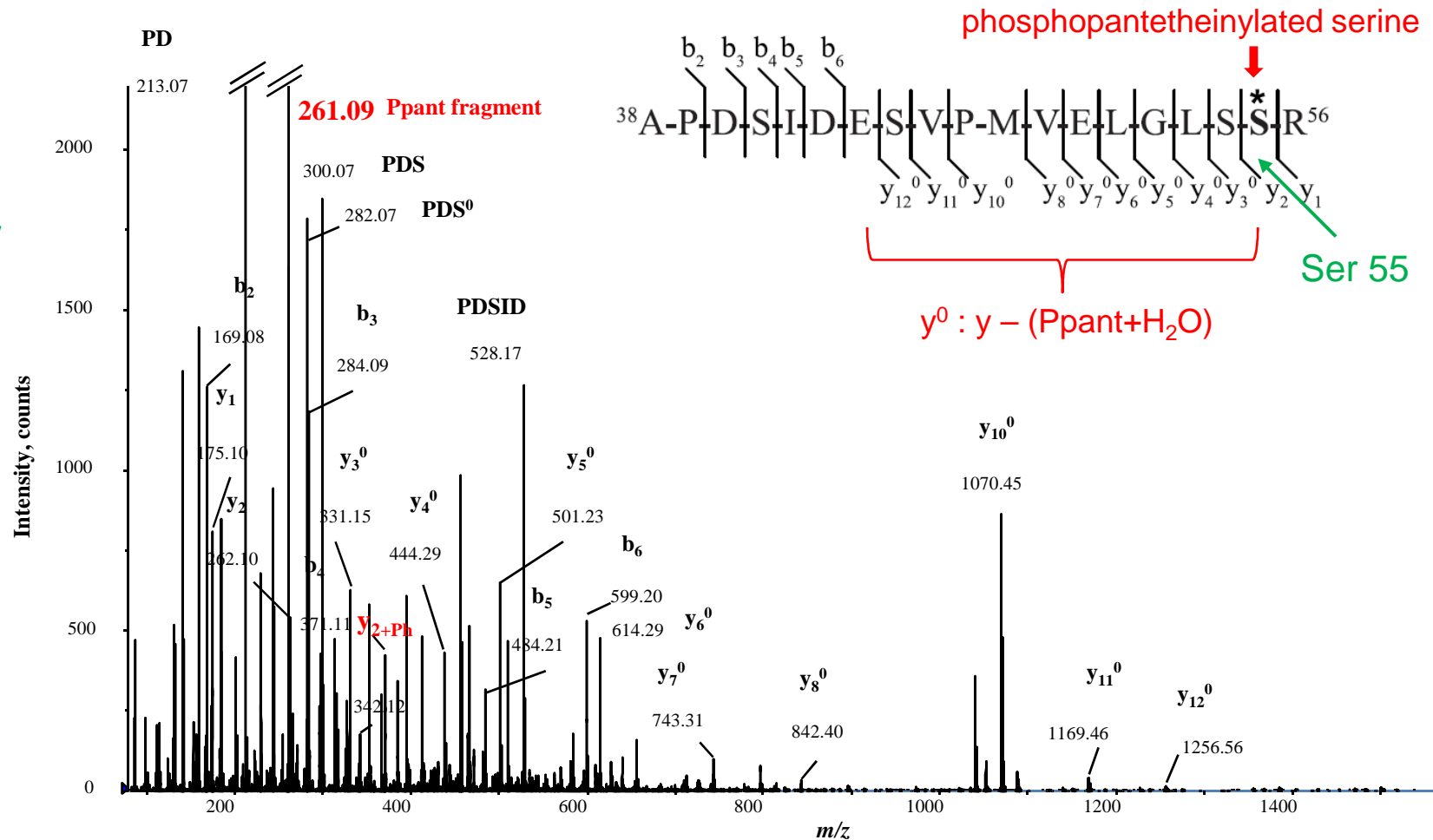


Modified Serine → DehydroAlanine

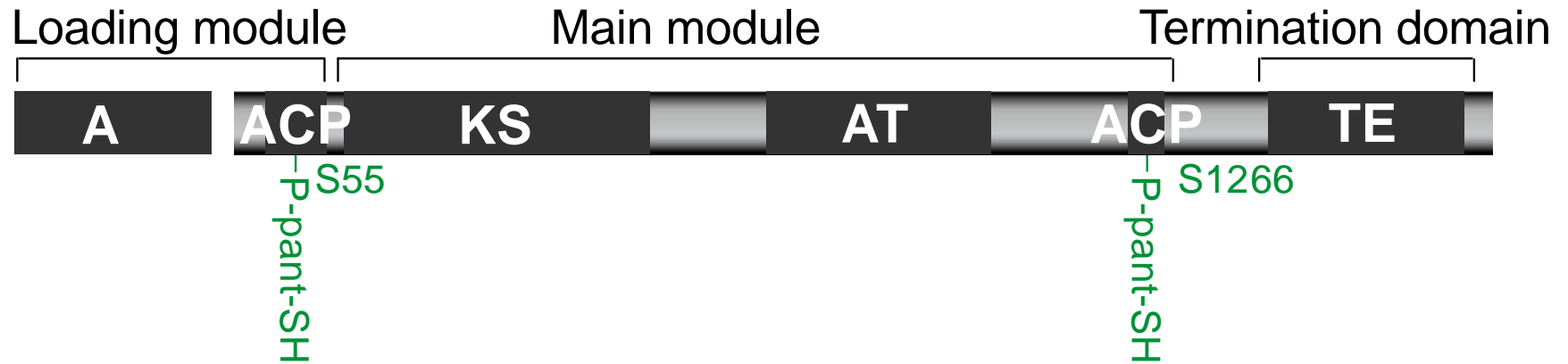
Ppant binding to ACP-Nter domain

Q-q-ToF Analysis
 $m/z = 1171.54^{2+}$

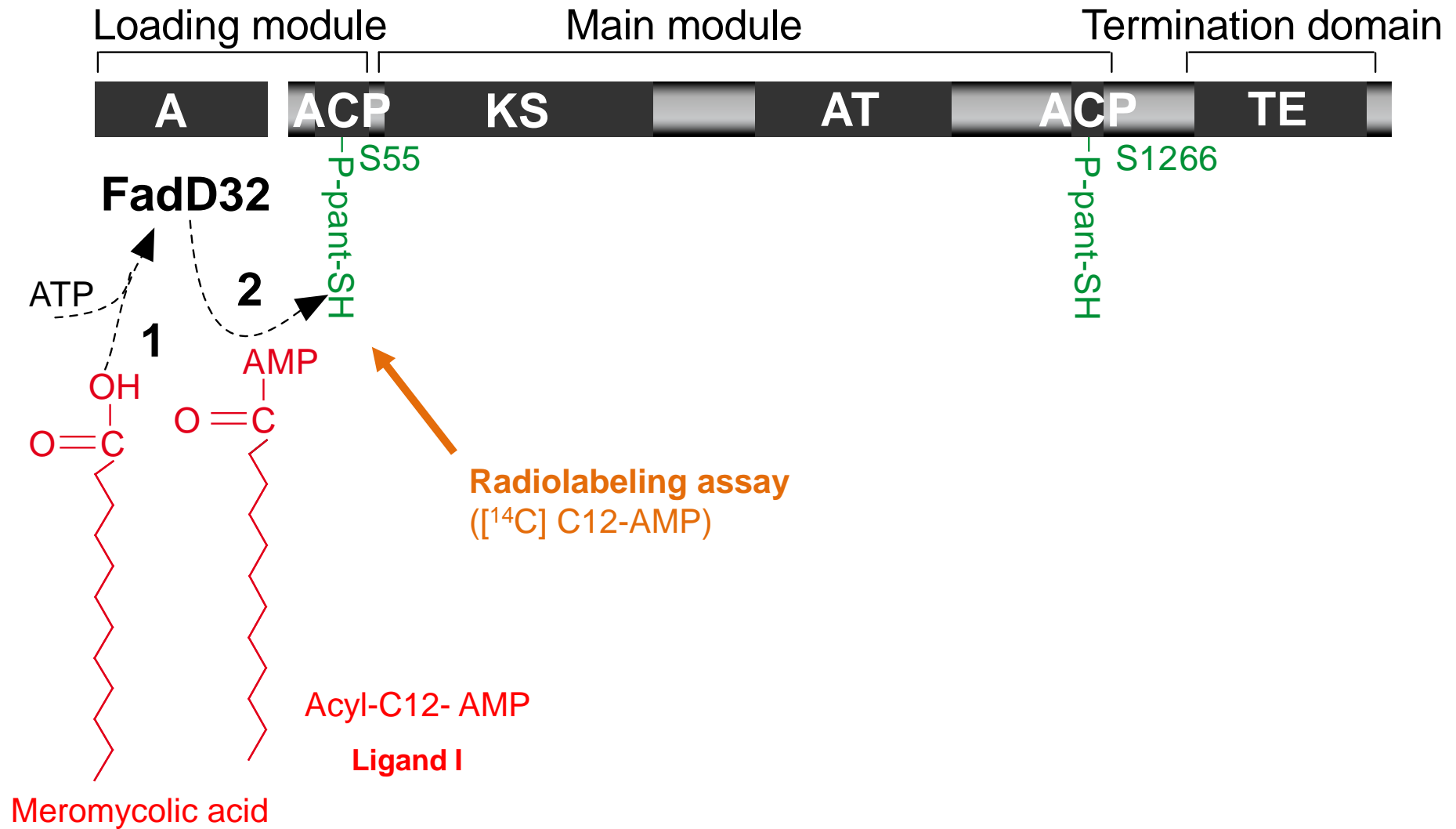
ACP N-ter



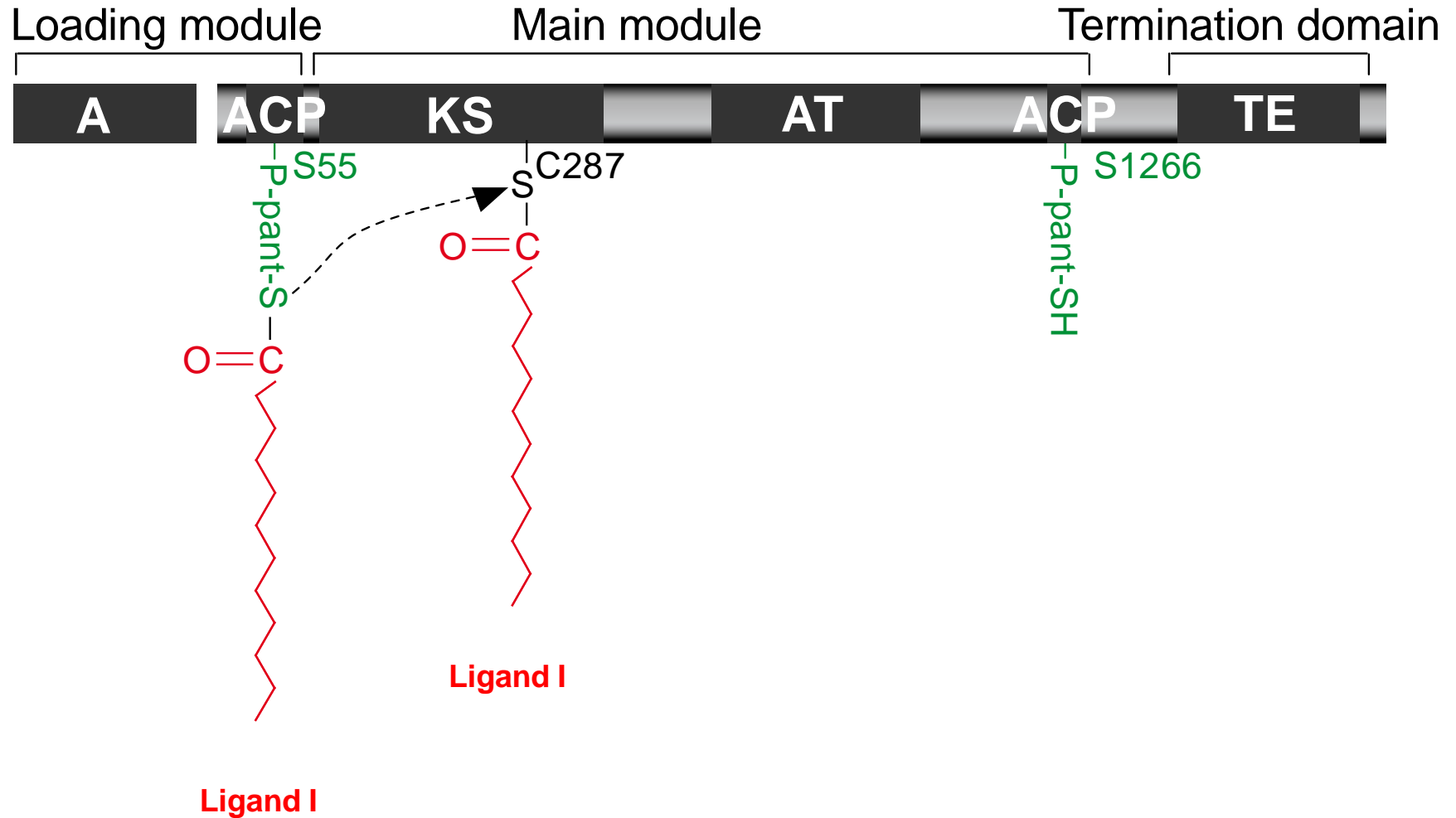
Stepwise activity of PKS13 and its domains organisation



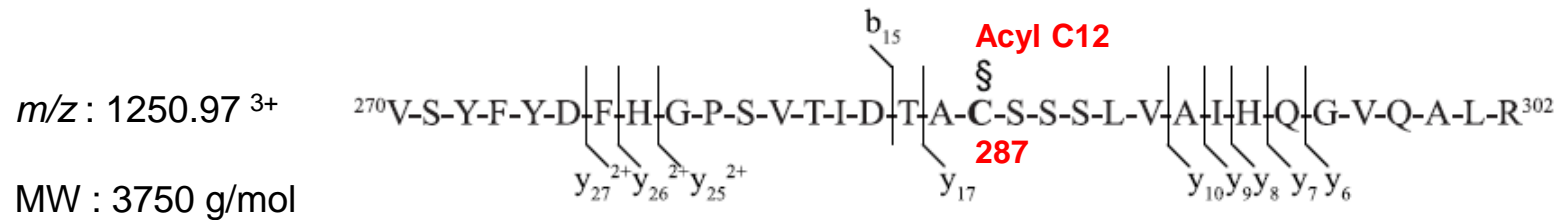
Stepwise activity of PKS13 and its domains organisation



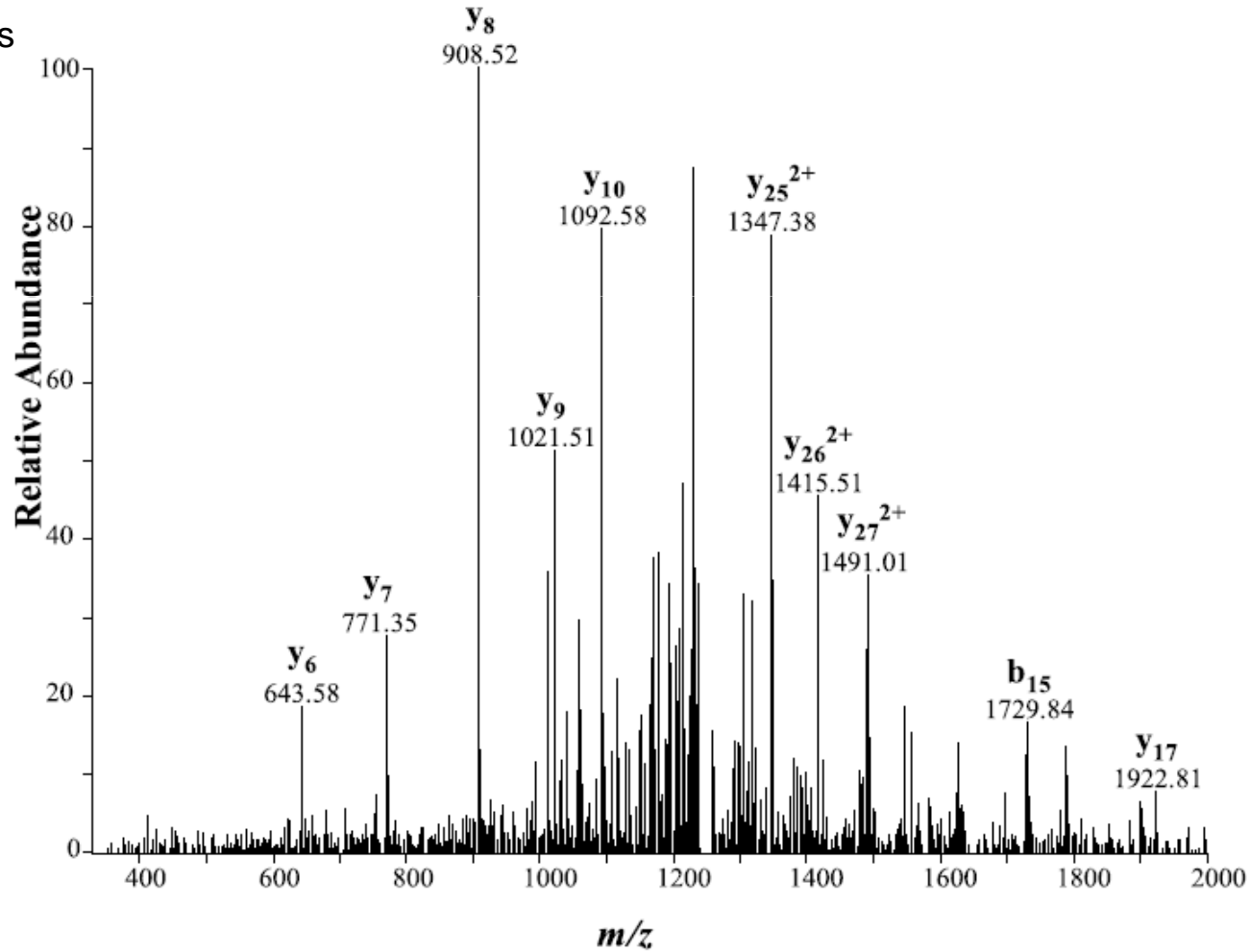
Stepwise activity of PKS13 and its domains organisation



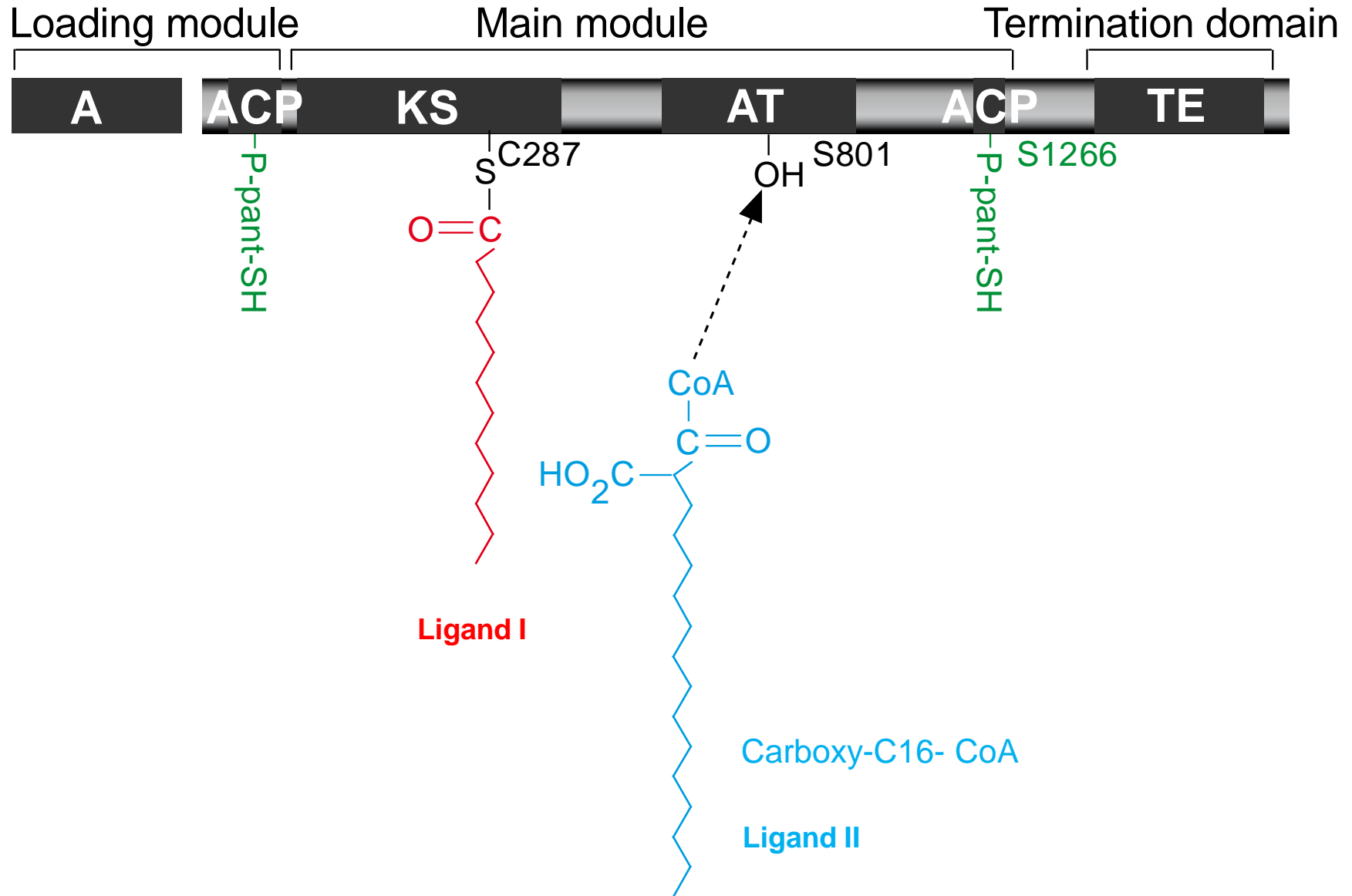
Acyl C12 binding on KS domain



Orbitrap Analysis



Stepwise activity of PKS13 and its domains organisation

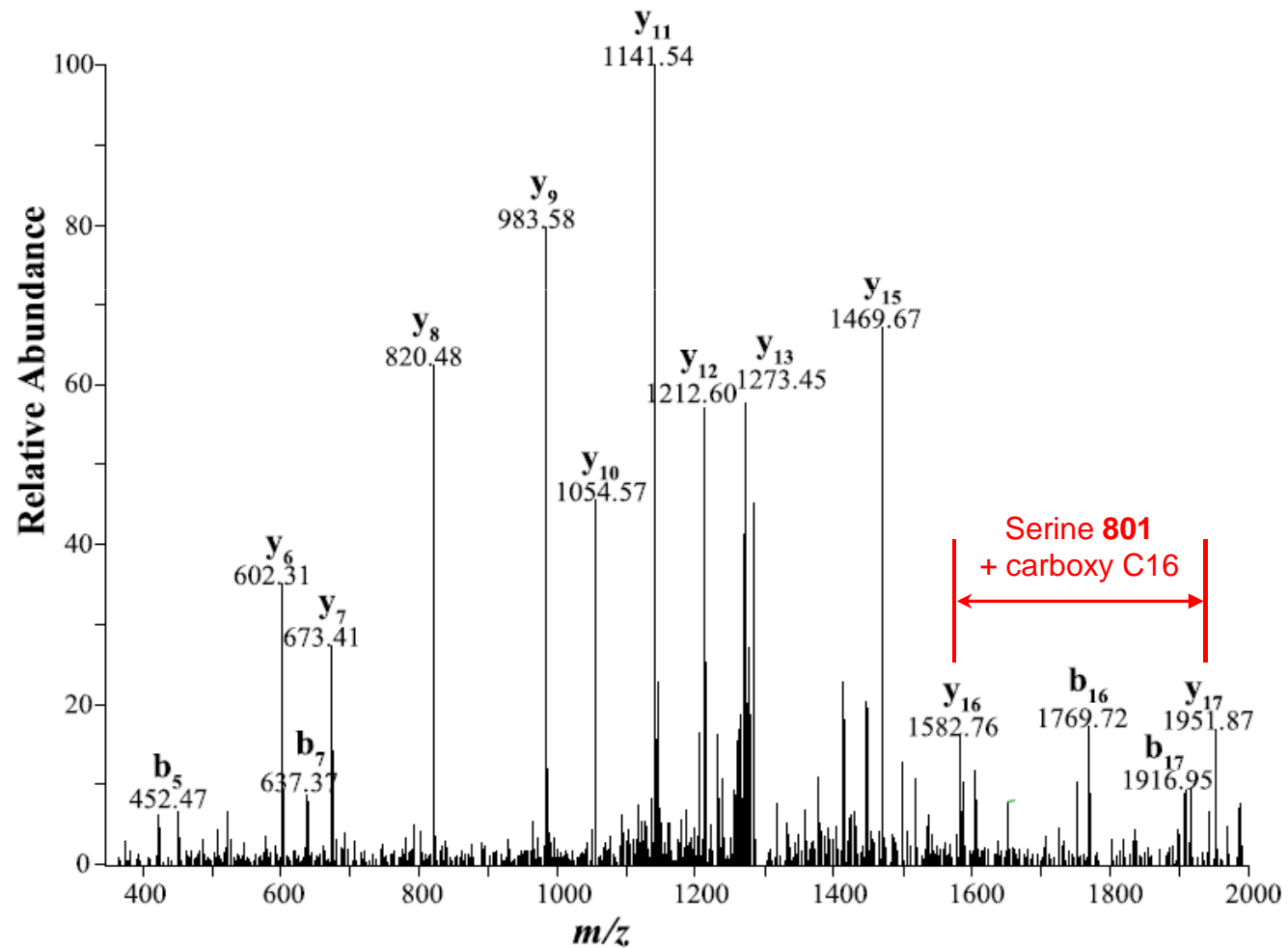
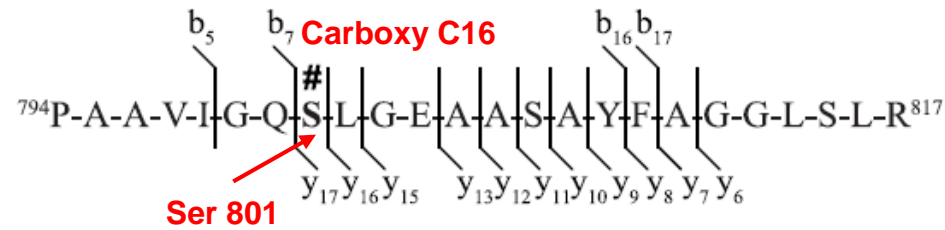


Carboxy C16 binding on AT domain

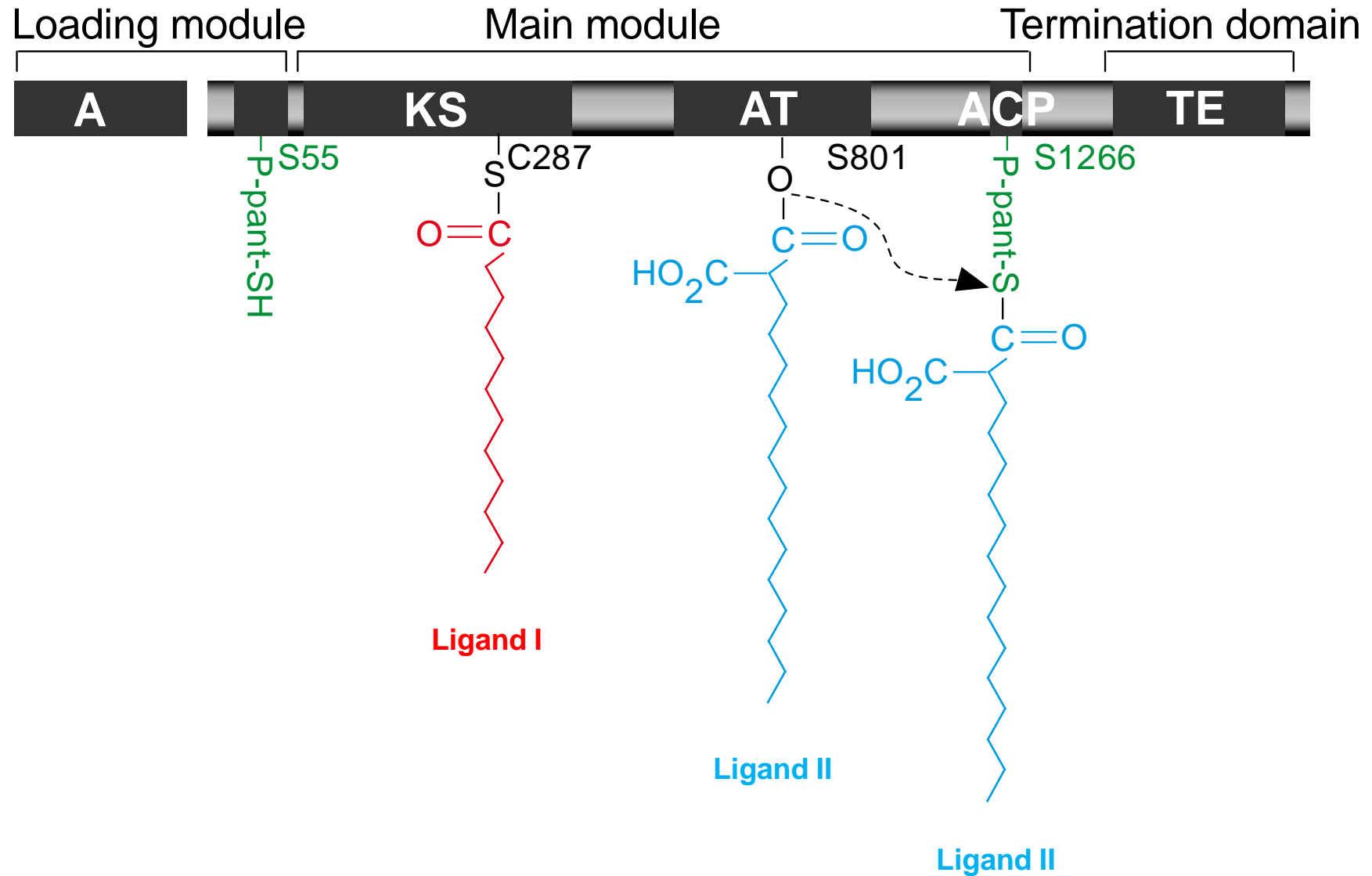
m/z : 1294.72 ²⁺

MW : 2587.44 g/mol

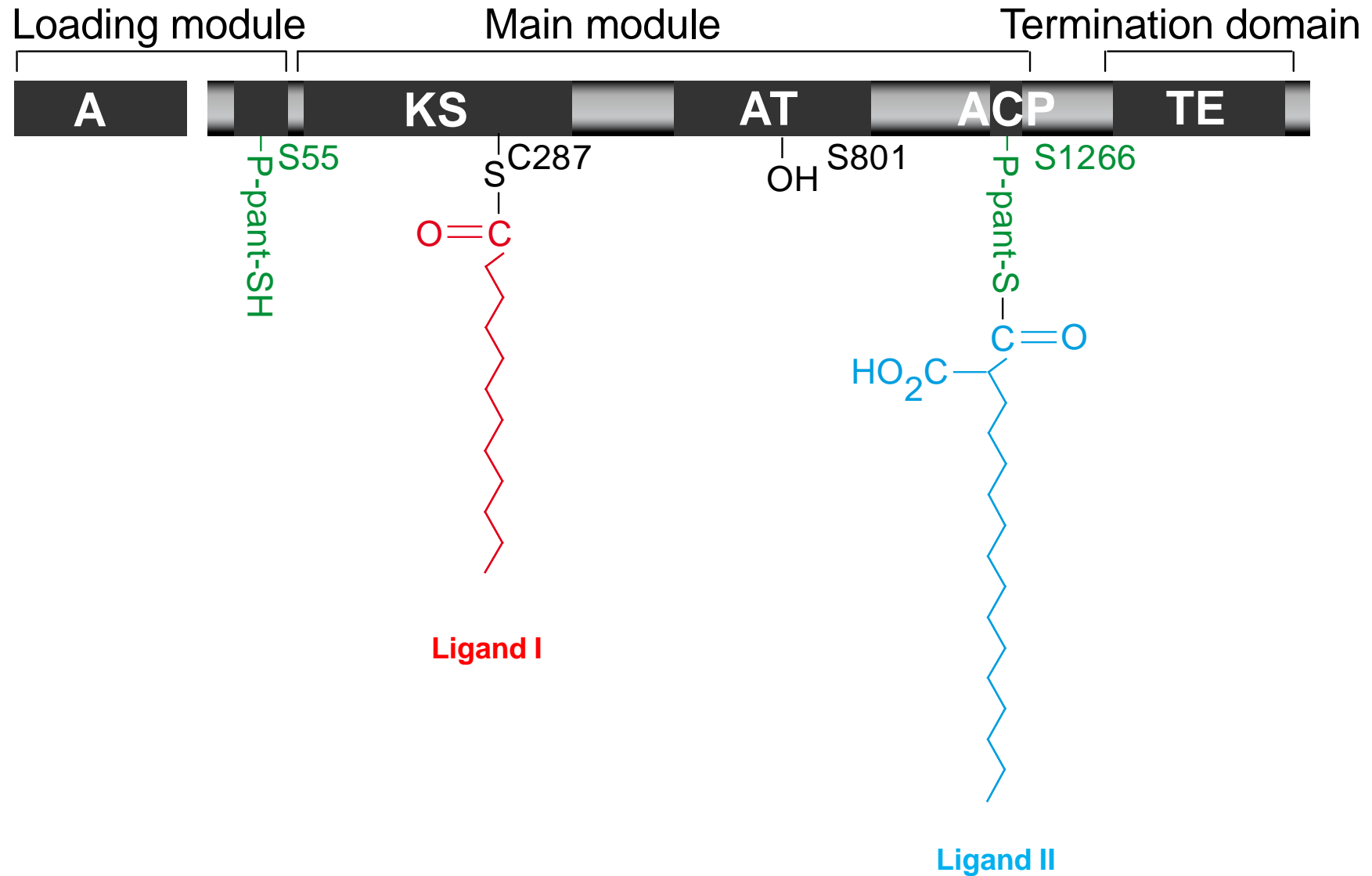
Orbitrap Analysis



Stepwise activity of PKS13 and its domains organisation



Stepwise activity of PKS13 and its domains organisation

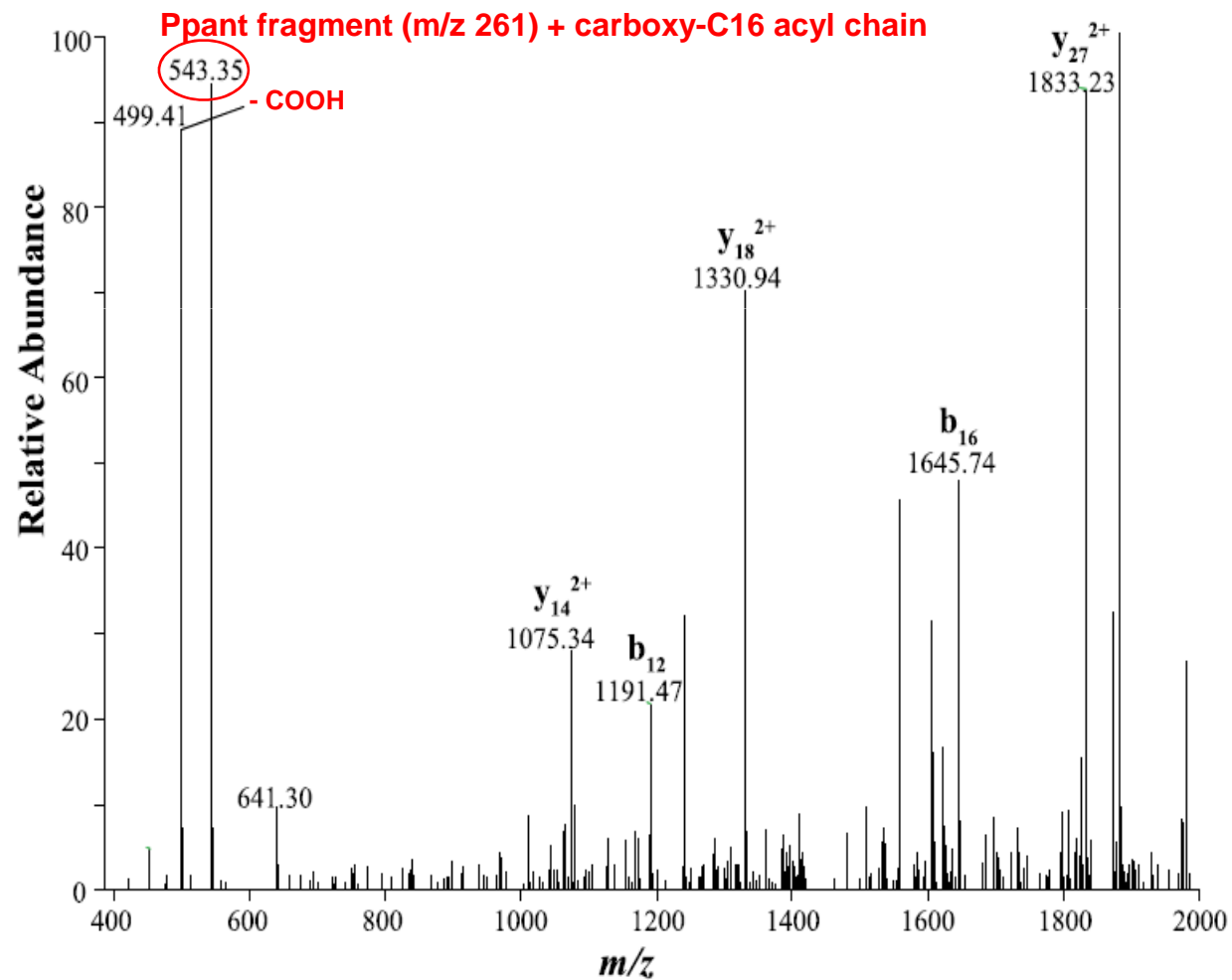
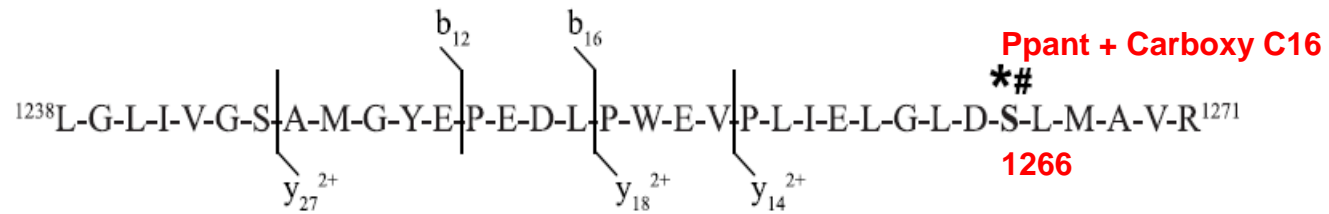


Carboxy-C16 binding on Ppant modified ACP-Cter domain

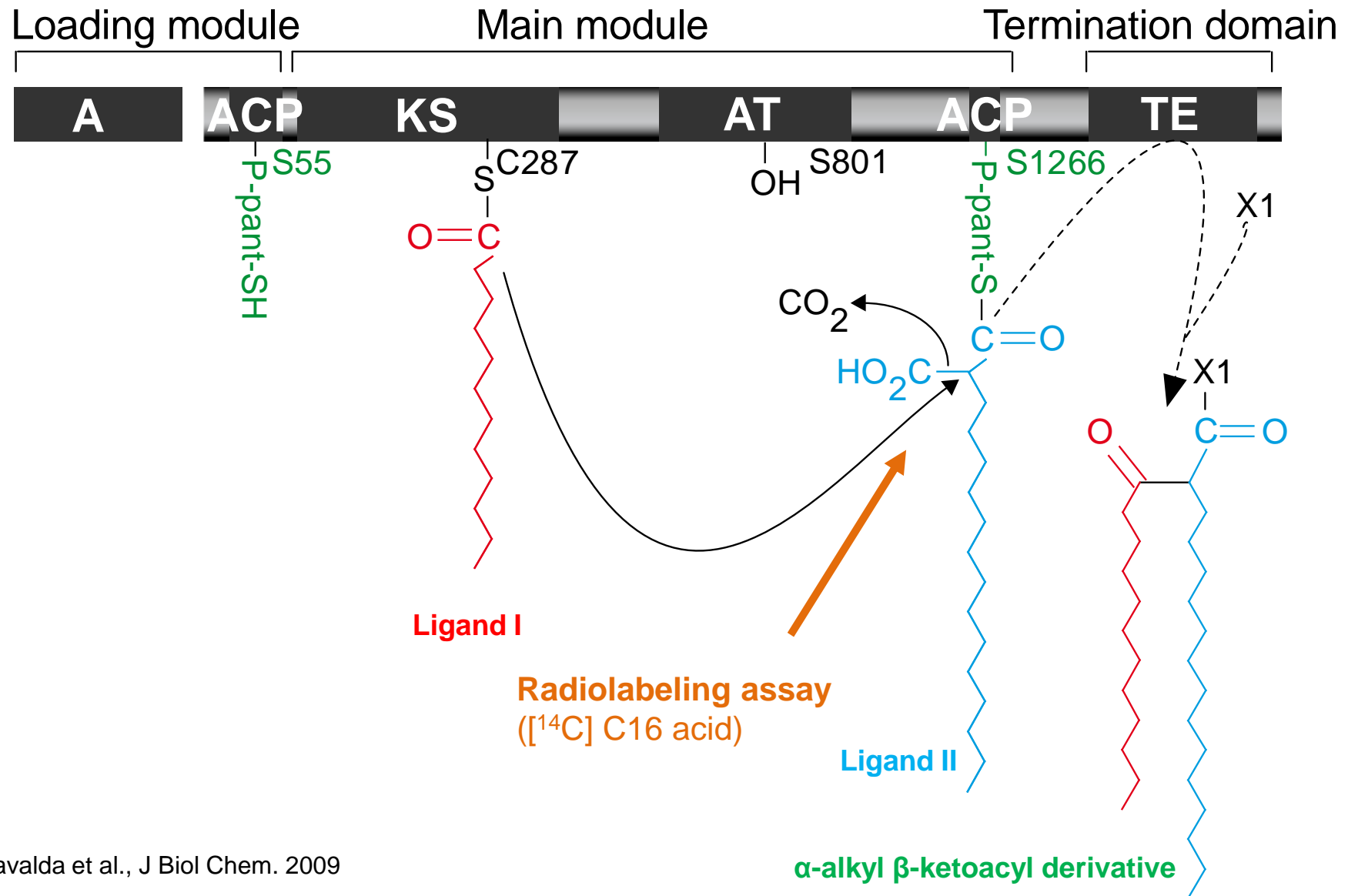
m/z : 1435.74 ³⁺
1-2 ppm

MW : 4304.22 g/mol

Orbitrap Analysis



Stepwise activity of PKS13 and its domains organisation



Conclusion

- ▶ Elucidation of stepwise PKS13 mechanism condensation
 - Mycolic acids precursors
- ▶ NanoHPLC-MS/MS is a powerful tool to confirm predicted catalytic amino acids
 - Helpful diagnostic ions to interpret MS/MS spectra
 - Hydrophobic and high mass peptides analyzed
 - Combination of automatic and manual data analysis
- ▶ Catalytic amino acids represent excellent targets for novel antimycobacterial drug design

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