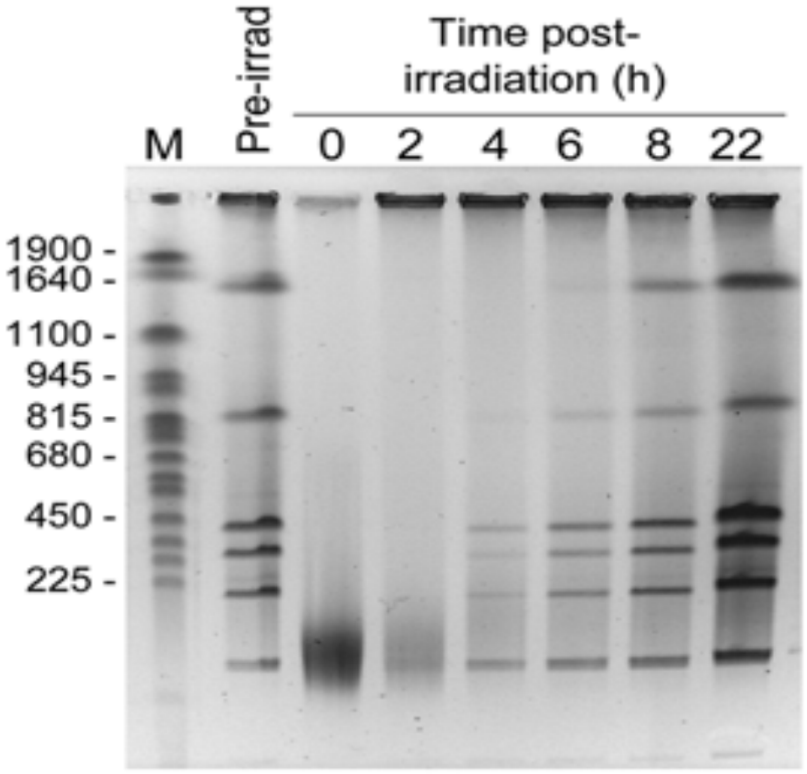
N-terminal protein acetylation is frequent in *Deinococcus deserti* and exclusive of serine and threonine residues.

Alain DEDIEU

CEA Centre de Marcoule - SBTN /Laboratory : »Biochemistry of Pertubated System » (LBSP)

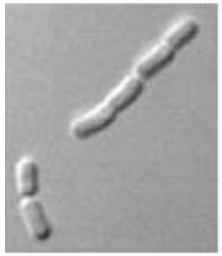
Deinococcus deserti : an exciting bacterium from the Sahara!

- Bacterium from the Deinococcus-Thermus phylum
- Isolated from sand of the Sahara desert
- Resistant to Gamma radiation and desiccation

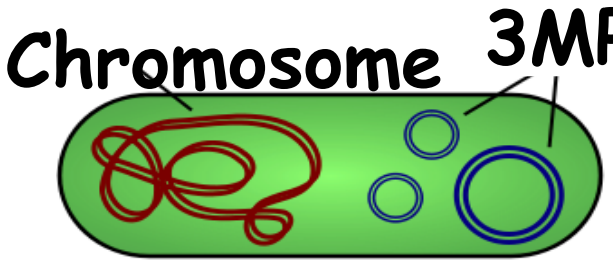


Our love story with *Deinococcus deserti*

The alliance of proteomics and genomics



D. deserti



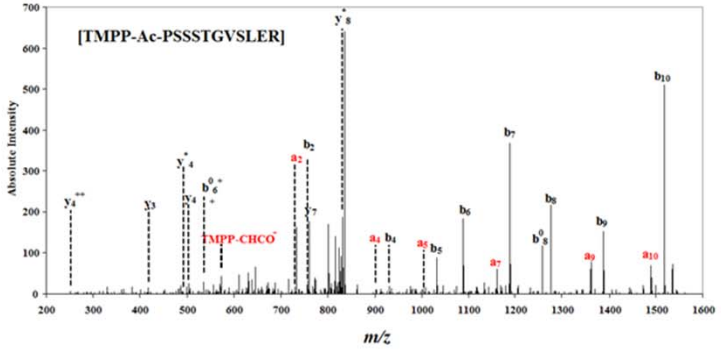
3 855 329 pb

3457 proteins

Alliance of Proteomics and Genomics to Unravel the Specificities of Sahara Bacterium Deinococcus deserti

Arjan de Groot, Rémi Dulermo, Philippe Ortet, Laurence **PLoS Genet. 2009 March; 5(3):**

Proteogenomic analysis of the N-terminal proteome.




```
>gi|226354810:754223-754855 Deinococcus deserti
TGATTTACTCTGCCTACCACCCAGCAGCTGCTCCGTAAGGGGCGCACC
1   *  F  T  L  P  T  T  Q  Q  L  L  R  K  G  R  T
2      M  P  T  T  Q  Q  L  L  R  K  G  R  T
```

73 N-terminal corrections

- **Proteomics-based Refinement of Deinococcus deserti Genome Annotation Reveals an Unwonted Use of Non-canonical Translation Initiation Codons**

Mathieu Baudet et al **Mol Cell Proteomics. 2010 February; 9(2): 415–426**

Post-Translational Modifications for Prokaryotes : the dawning of a new age!

Hindawi Publishing Corporation
Archaea
Volume 2010, Article ID 820681, 9 pages
doi:10.1155/2010/820681

Review Article

Protein Acetylation in Archaea, Bacteria, and Eukaryotes

Jörg Soppa

Institute for Molecular Biosciences, Goethe University, Max-von-Laue-StraBe 9, 60438 Frankfurt, Germany

Molecular Microbiology [2010] 77(1), 15–21 ■
doi:10.1111/j.1365-2958.2010.07204.x
First published online 18 May 2010

MicroReview

Bacterial protein acetylation: the dawning of a new age

Opinion TRENDS in Biochemical Sciences Vol.32 No.5
Full text provided by www.sciencedirect.com ScienceDirect

A newly discovered post-translational modification – the acetylation of serine and threonine residues

Sohini Mukherjee, Yi-Heng Hao and Kim Orth

Department of Molecular Biology, The University of Texas Southwestern Medical Center, Dallas, TX 75390, USA

Published in final edited form as:
Nat Protoc. 2008 ; 3(10): 1630–1638. doi:10.1038/nprot.2008.150.

The SCX/IMAC enrichment approach for global phosphorylation analysis by mass spectrometry

Judit Villén and **Steven P Gygi**
Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115, USA

The EMBO Journal Vol.19 No.6 pp.1176–1179, 2000

NEW EMBO MEMBER'S REVIEW

Acetylation: a regulatory modification to rival phosphorylation?

Tony Kouzarides

ask the question: is acetyl

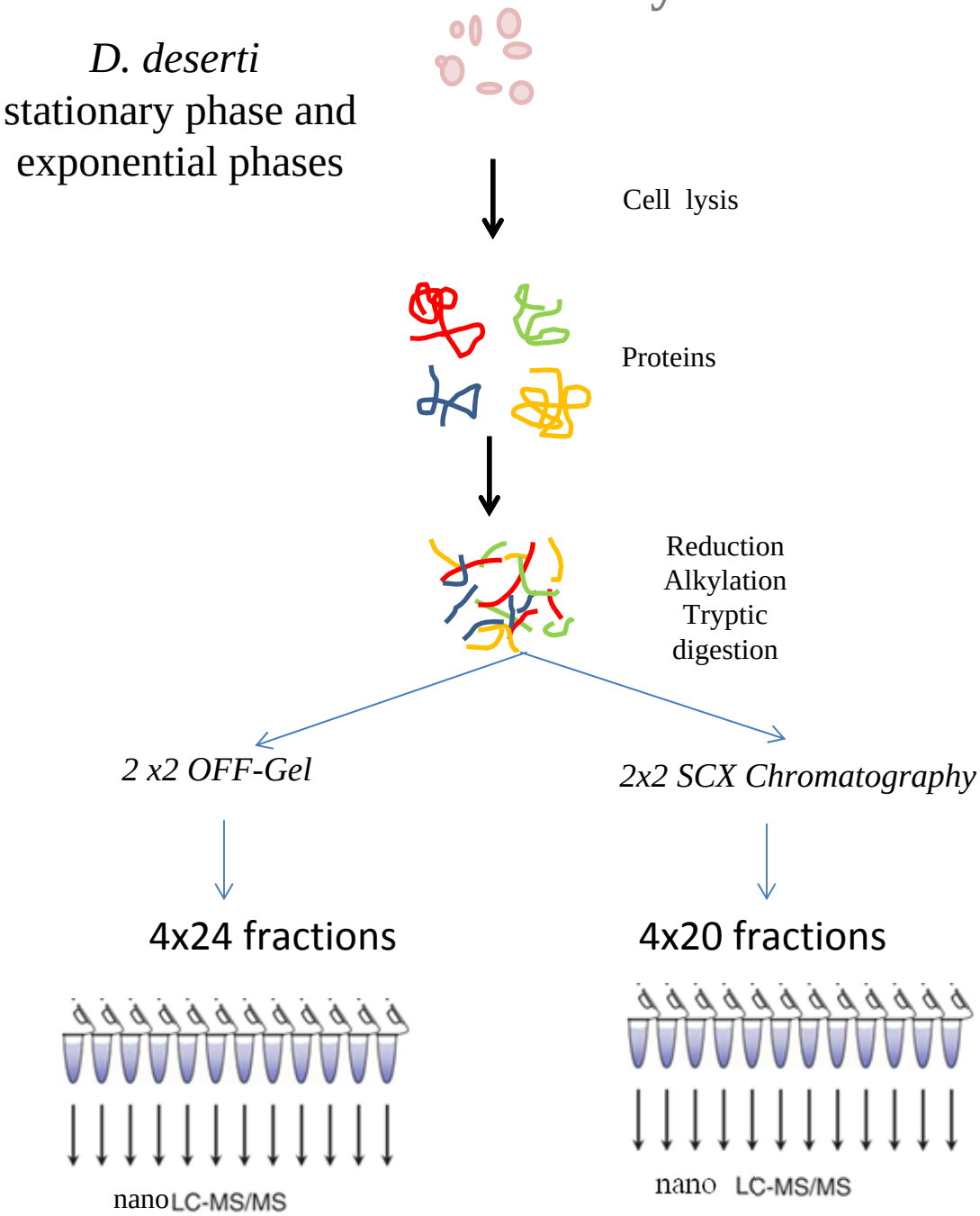
Science AAAS

N-Terminal Acetylation of Cellular Proteins Creates Specific Degradation Signals
Cheol-Sang Hwang, *et al.*
Science **327**, 973 (2010);
DOI: 10.1126/science.1183147

Review TRENDS in Biochemical Sciences Vol.32 No.2
Full text provided by www.sciencedirect.com ScienceDirect

Tyrosine phosphorylation: an emerging regulatory device of bacterial physiology

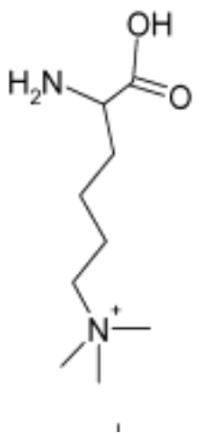
Strategy used to detect N-terminal acetylation in *Deinococcus deserti*:



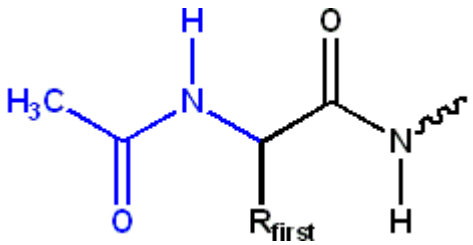
Acetylation VS Methylation: a need for high accurancy measurements

- For a peptide with Mr 1000 the mass difference between an acetylated or a tri-methylated peptide is of 36ppm.
- Far above the limit of 5 ppm tolerated for the selection of an N-terminal acetylated parent ion.
- **The high accuracy MS is able to differentiate unambiguously a tri-methylated peptide from an acetylated one.**

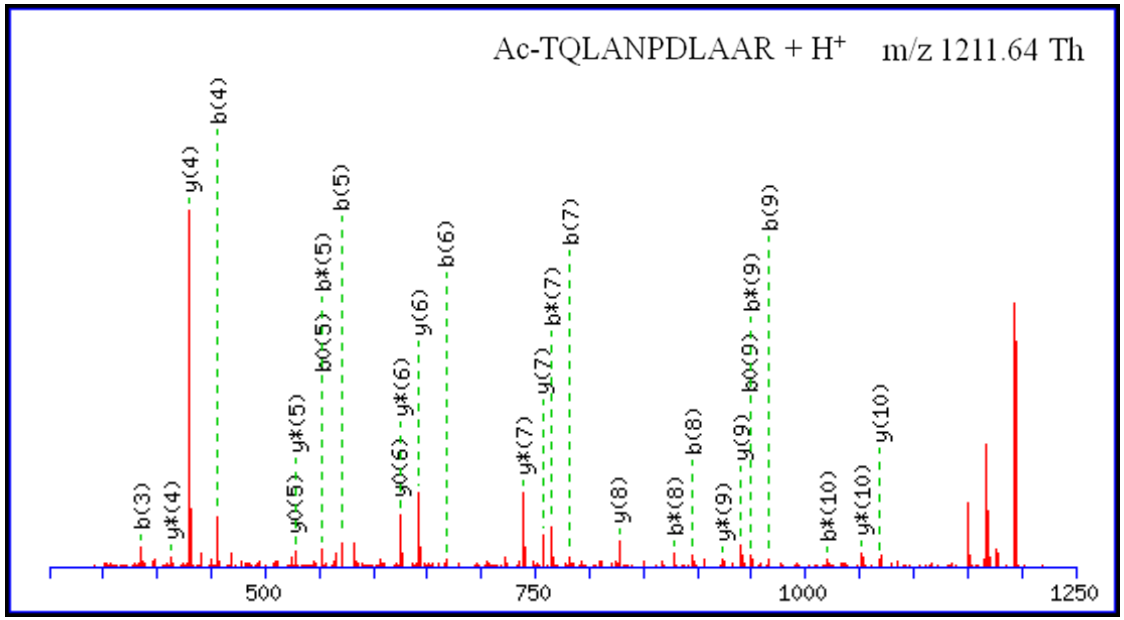
Tri-methylated Lysine

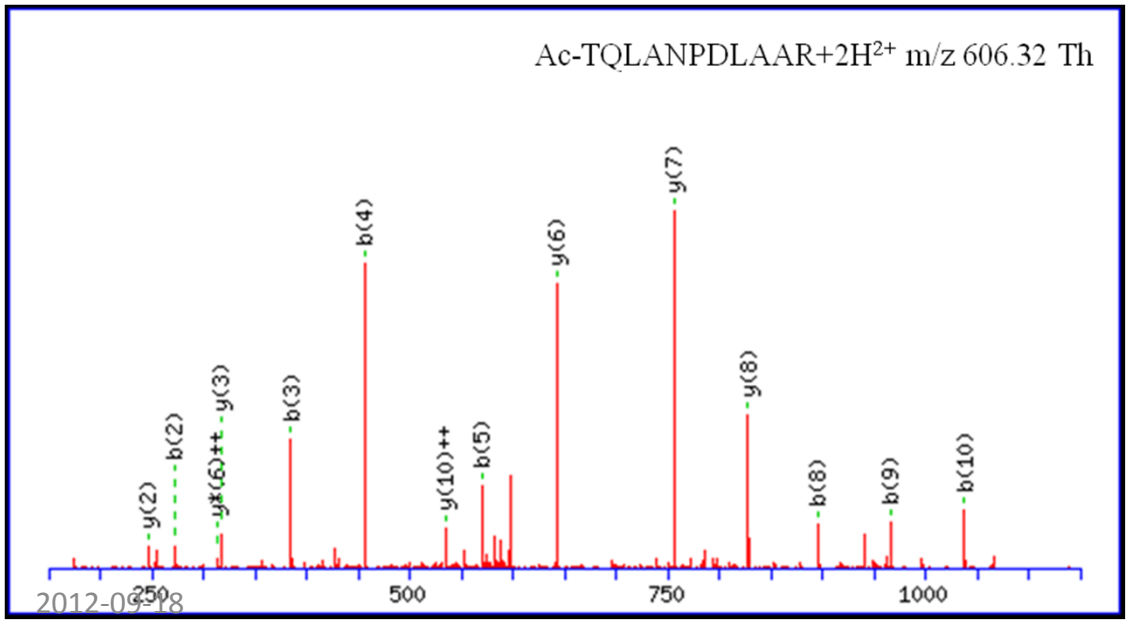


Acetylated Lysine



Mono or di-charged peptides ?





O versus N terminal acetylation

Peptides displaying an acetylated serine/threonine residue located at N-terminus, may exist as two different isomeric forms through an acetyl shift between the nitrogen and the oxygen atoms depending on the sample pH, Under acidic conditions which is the case during the ionisation step of the peptide in the Orbitrap, the N-acetylated form is favored. The recorded m/z may thus correspond to any of the two isobaric ions .

Trends in Biochemical Sciences Vol.32 N°5
A newly discovered post-translational modification _ the acetylation of serine and threonine residues
S. Mukherjee et al

Tetrahedron Letters, Volume 45, Issue 6 2 February 2004, Pages 1173 1178
O–N-Acyl migration in N-terminal serine-containing peptides: mass spectrometric elucidation and subsequent development of site-directed acylation protocols
•L. Mouls, G. Subra, C. Enjalbal, J. Martinez, J.-L. Aubagnac_ ' _

Resolving peptides mixture

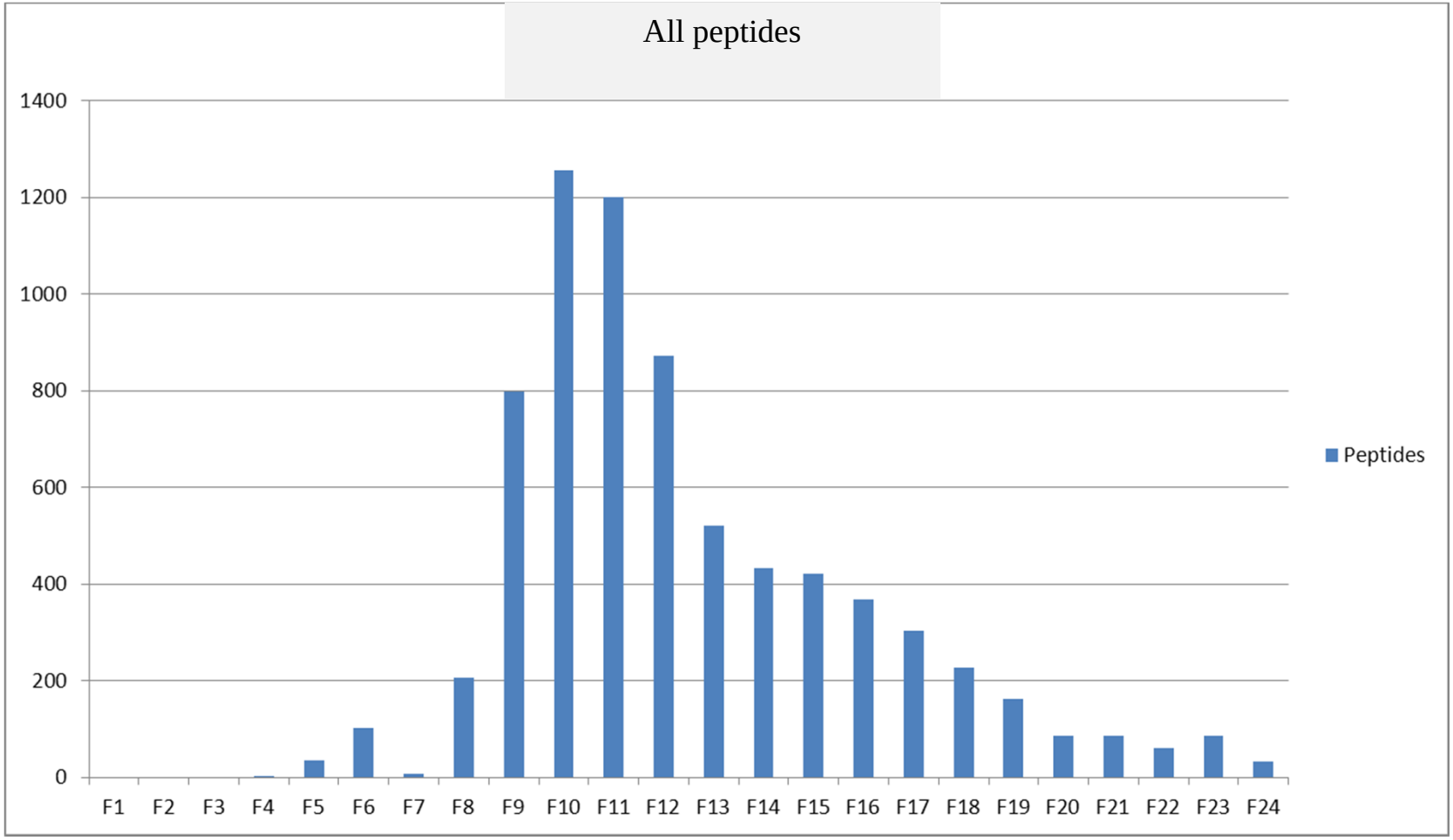
- Using an Off- gel separation of the peptides generated by tryptic digestion

1. Peptides are separated according to their pI.
2. N-terminal acetylated peptides are not particularly grouped together, but this approach will give us a broader view of the proteome.

- Using a cation exchange chromatography, SCX (Strong Cation eXchange).

1. The peptides are separated according to their charges.
2. N-terminal acetylated peptides come out primarily at the beginning of gradient.

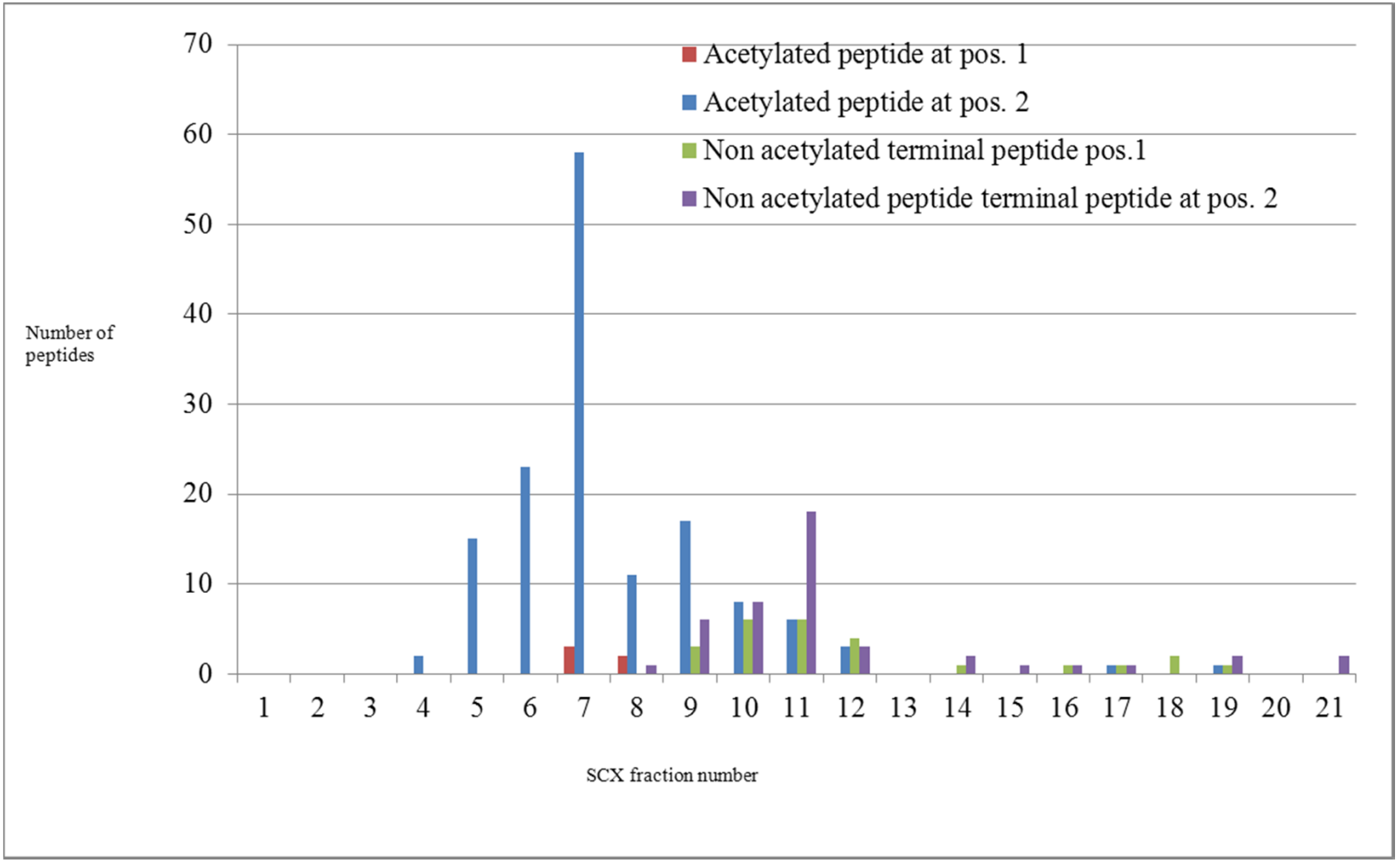
Distribution of peptides by SCX fractionation - *Deinococcus deserti*



From 17461 unique peptide sequences (out of 293 779 validated redundant peptides), **1754 proteins (51% of the total proteome)** could be detected (with 2 peptides assigned and **p = 0.001**).

A stringent value for **p**, choosen after manual checking of many spectra.

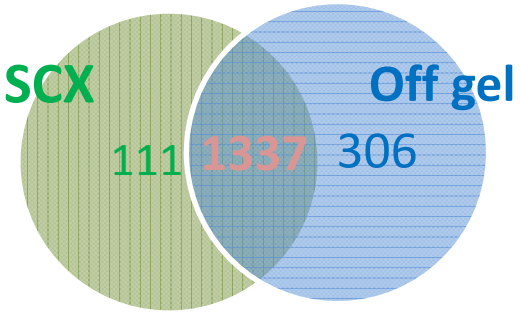
Distribution of N-term. acetylated peptides among the first fractions



Deinococcus deserti N-terminal acetylone

Results :

- **1754 proteins could be detected, 51%of the theorical proteomer. 1337 proteins were identified in both methods, 111 specifically with the SCX approach, and 306 specifically with the Off gel method.**



- **The Off gel strategy allow us to have a better coverage in term of N-terminal peptide of the proteome.**

- **The SCX strategy allow us to get a better coverage of the N-terminal acetylome.**

Deinococcus deserti N-terminal acetylone

Results :

- **Peptides : 17461 (unique sequences) among 293 779 assigned spectra.**

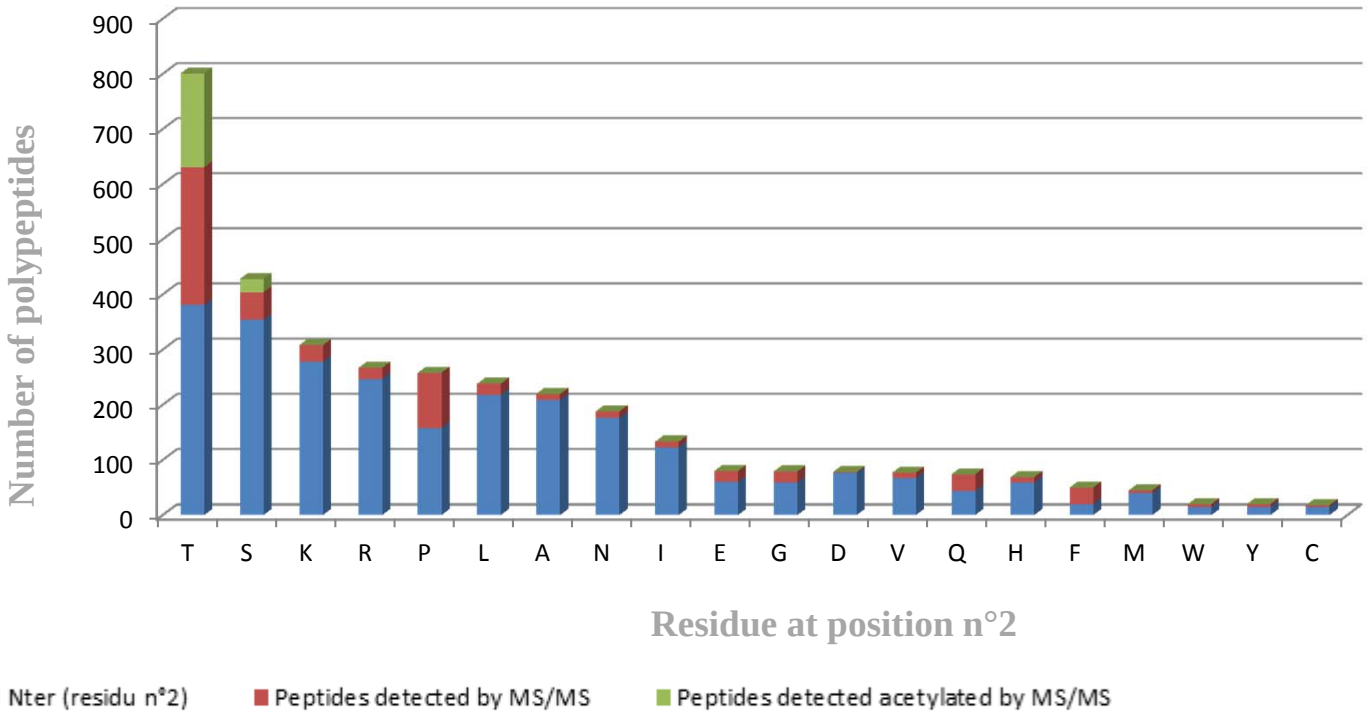
- **458 unique N-terminal sequences could be detected with a p< 0.001, leading to 440 proteins because one can observe some N-terminal peptides starting at position 1 and 2 ,**

- **Among these 458 unique N-terminal sequences, 230 (50%) are N-terminal acetylated.**

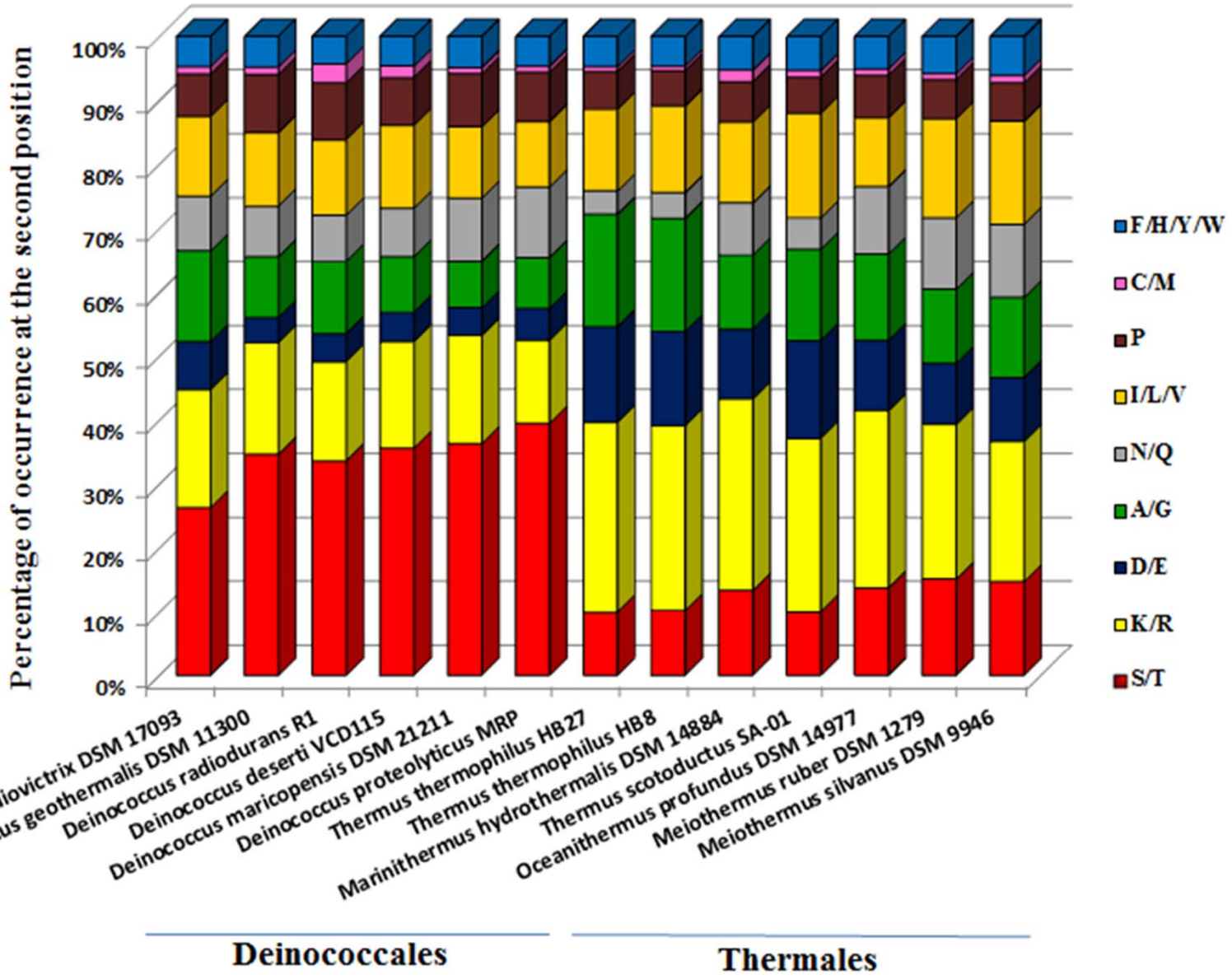
Deinococcus deserti an N-terminal acetylated specific pattern.

Position 1 : 17 on 122 (14%) are acetylated on the starting Methionine,
Position 2 : 213 on 336 (63.3%) are acetylated on this position.

- **Removal of the initiator methionine occurs in three-fourths of these bacterian proteins and requires a small penultimate residue.**
- **Among the 213 acetylated peptide at position 2, 189 are Threonine, 23 Serine a single Alanine**



Pattern for the first residues in the proteome of 12 sequenced representatives of Deinococcus-Thermus phylum.



It’s still a long way……..

Arjan de Groot
Laurence Blanchard
Thierry Heulin
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(Cadarache)

Jimmy Maevere
Jean Baptiste Boyer
Jean Charles Gaillard
Alain Dedieu
Jean Armengaud

iBEB-SBTN-LBSP
(Marcoule)

… and for your
kind attention !