蛋白質解析の最新技術セミナー

1. 製品紹介

2. 蛋白質の翻訳後修飾の保存　Stabilizer T1

3. 安定同位体標識蛋白質を内部標準とする蛋白質定量および
翻訳後修飾部位同定法の開発

2011年7月29日

エーエムアール株式会社

株式会社バイオシス・テクノロジーズ

GELFREE™ 8100
FRACTIONATION SYSTEM

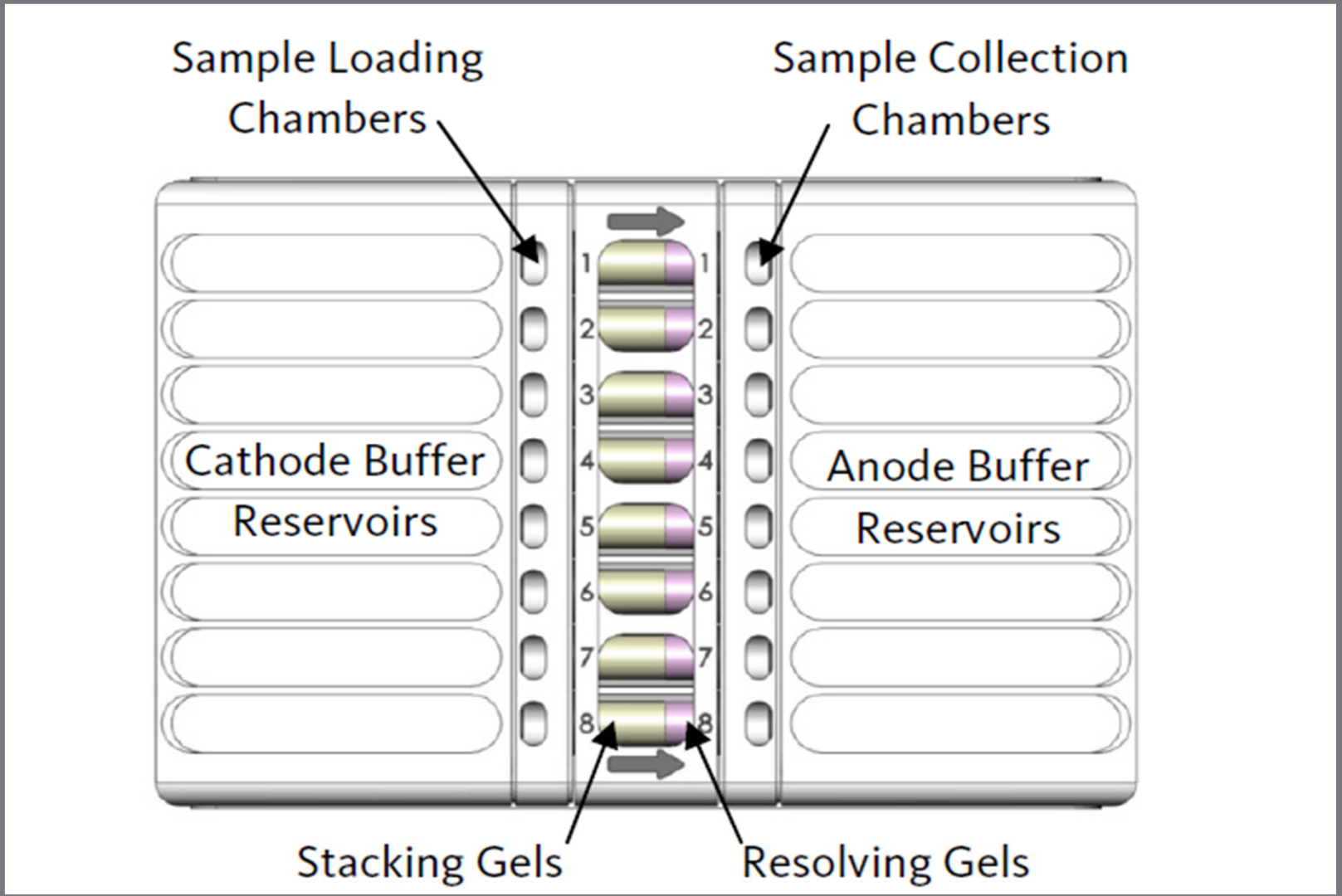
SYSTEM COMPONENTS

GELFREE 8100
FRACTIONATION STATION

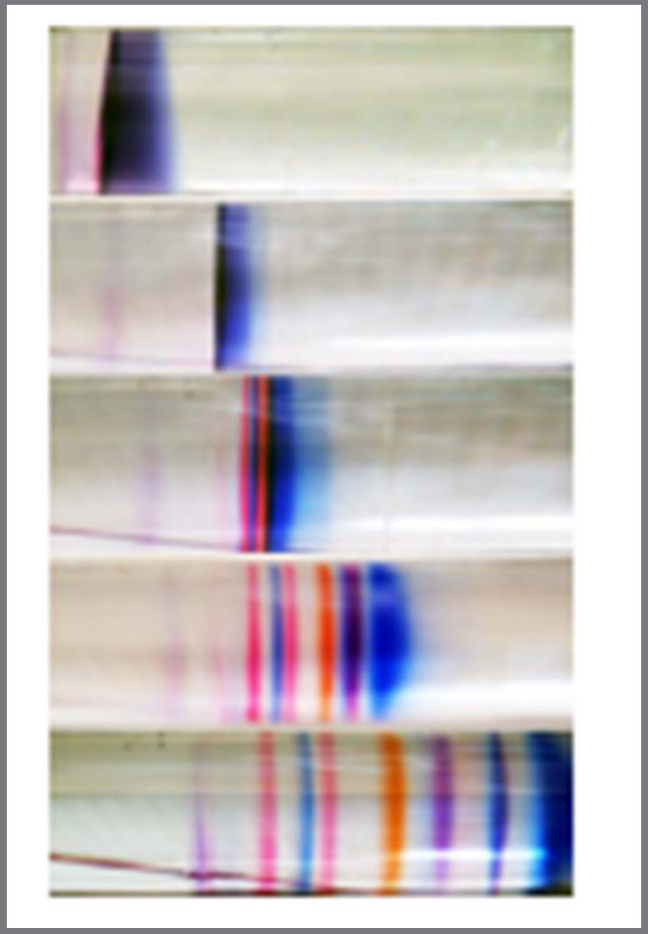
一度に8サンプルを処理
することが可能！！

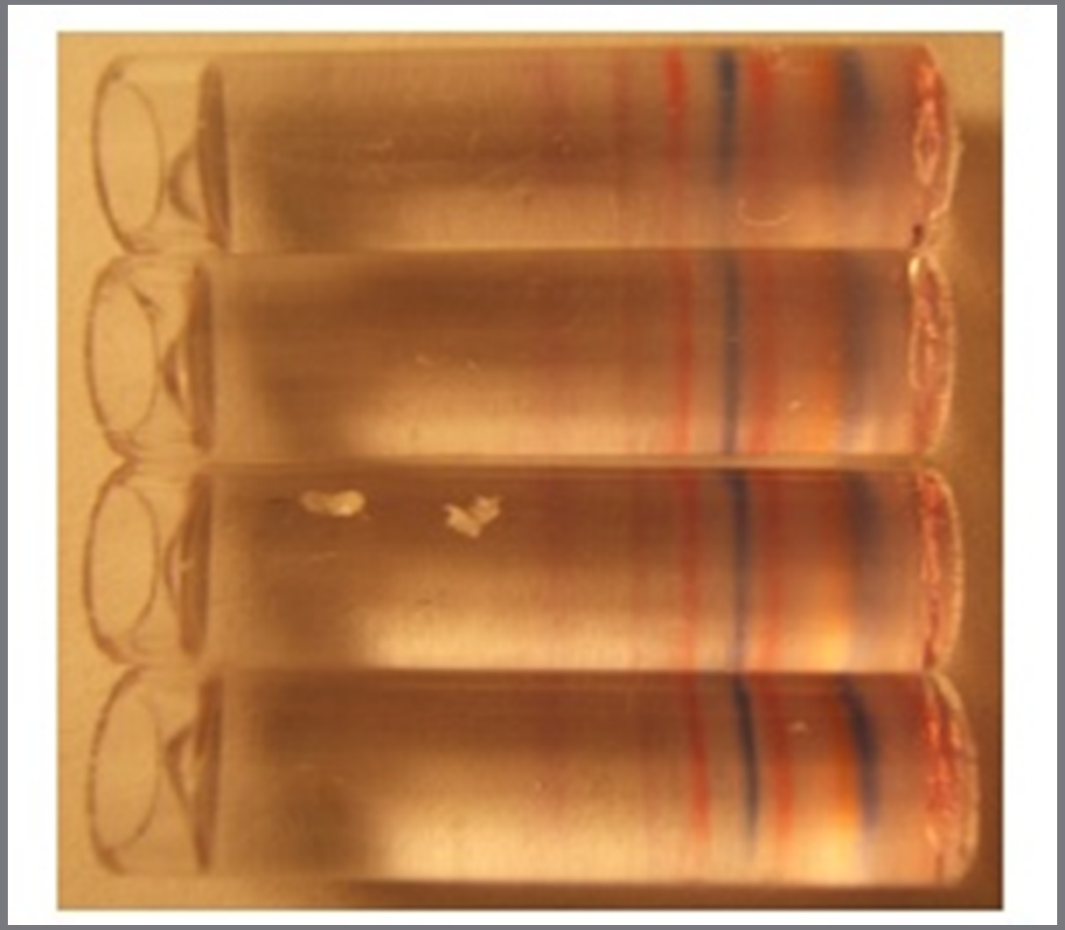
GELFREE 8100
CARTRIDGE

protein discovery



protein discovery





GELFREE™ 8100
FRACTIONATION SYSTEM

GELFREE 8100 CARTRIDGE KITS

- *Low Mass* Cartridge Kit: 3.5 – 50 kDa
- *Mid Mass* Cartridge Kit: 3.5 – 100 kDa
- *Mid Mass* Cartridge Kit : 3.5 - 150kDa
- *High Mass* Cartridge: 3.5– 300 kDa

protein
discovery

ADVANCE Nanoflow UHPLC system

10000psiまで対応する高圧仕様のナノHPLC

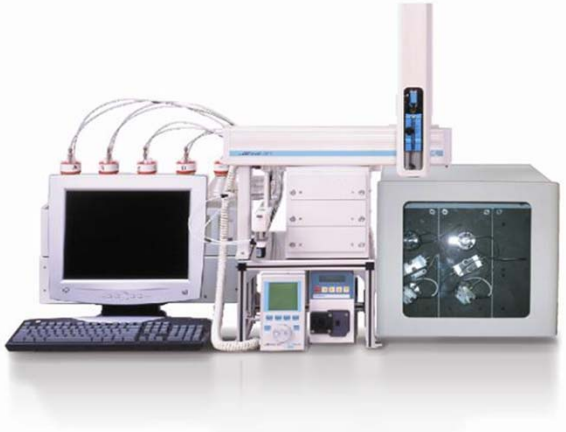
ダイレクトシリンジポンプによるスプリットレス送液

フローセンサーによる流量制御によりすぐれ再現性

デッドボリュームが少ないのでグラジエントディレイが少ない

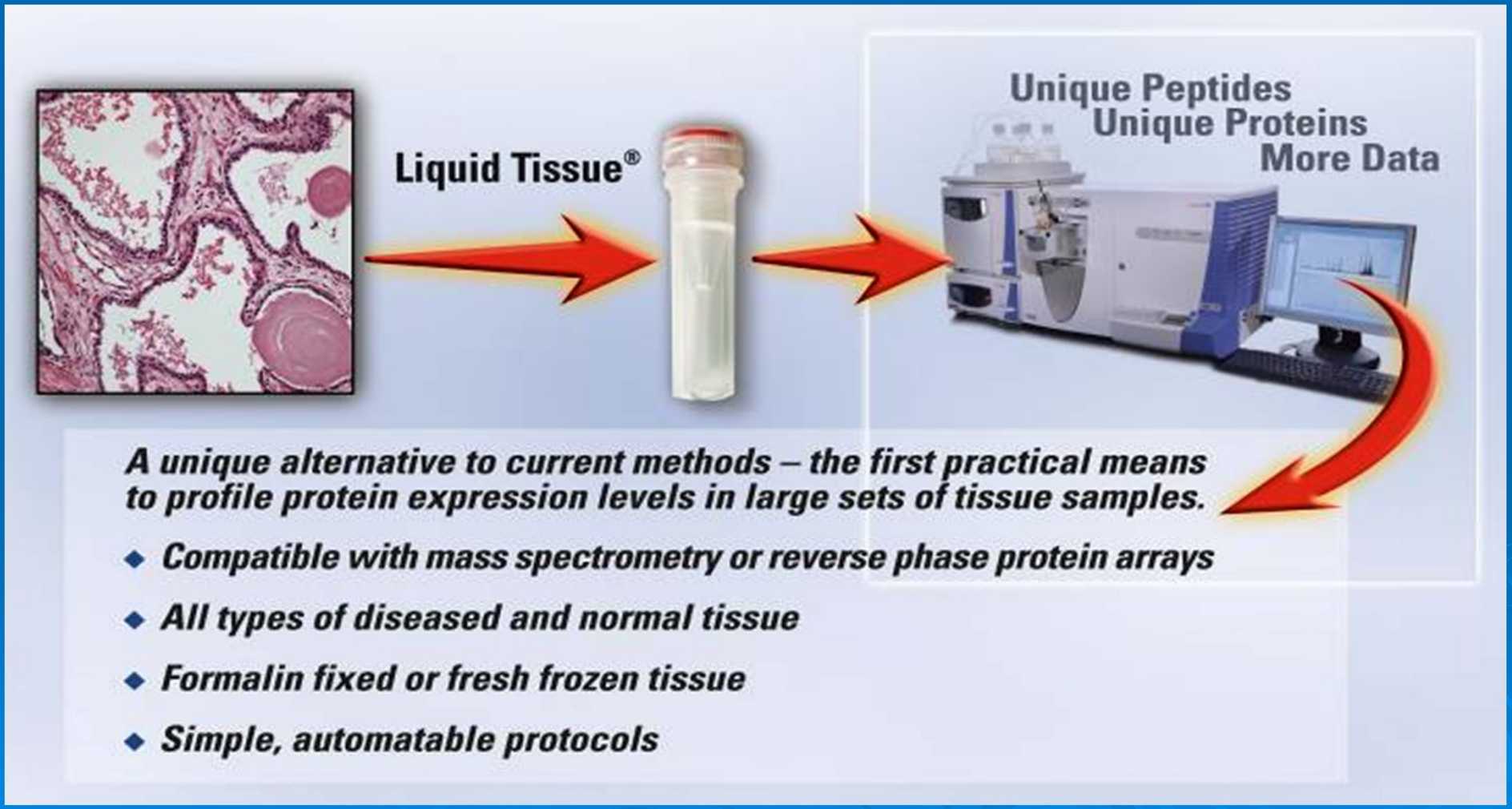
さまざまなカラムスイッチングが高圧仕様でも可能

高圧でもキャリーオーバーの少ないオートサンプラー仕様



2-Pump Binary
2-Pump + 1 Valve Binary
2-Pump + 2 Valves Binary
2-Pump + 1 Valve + Loading Pump Binary
3-Pump Ternary

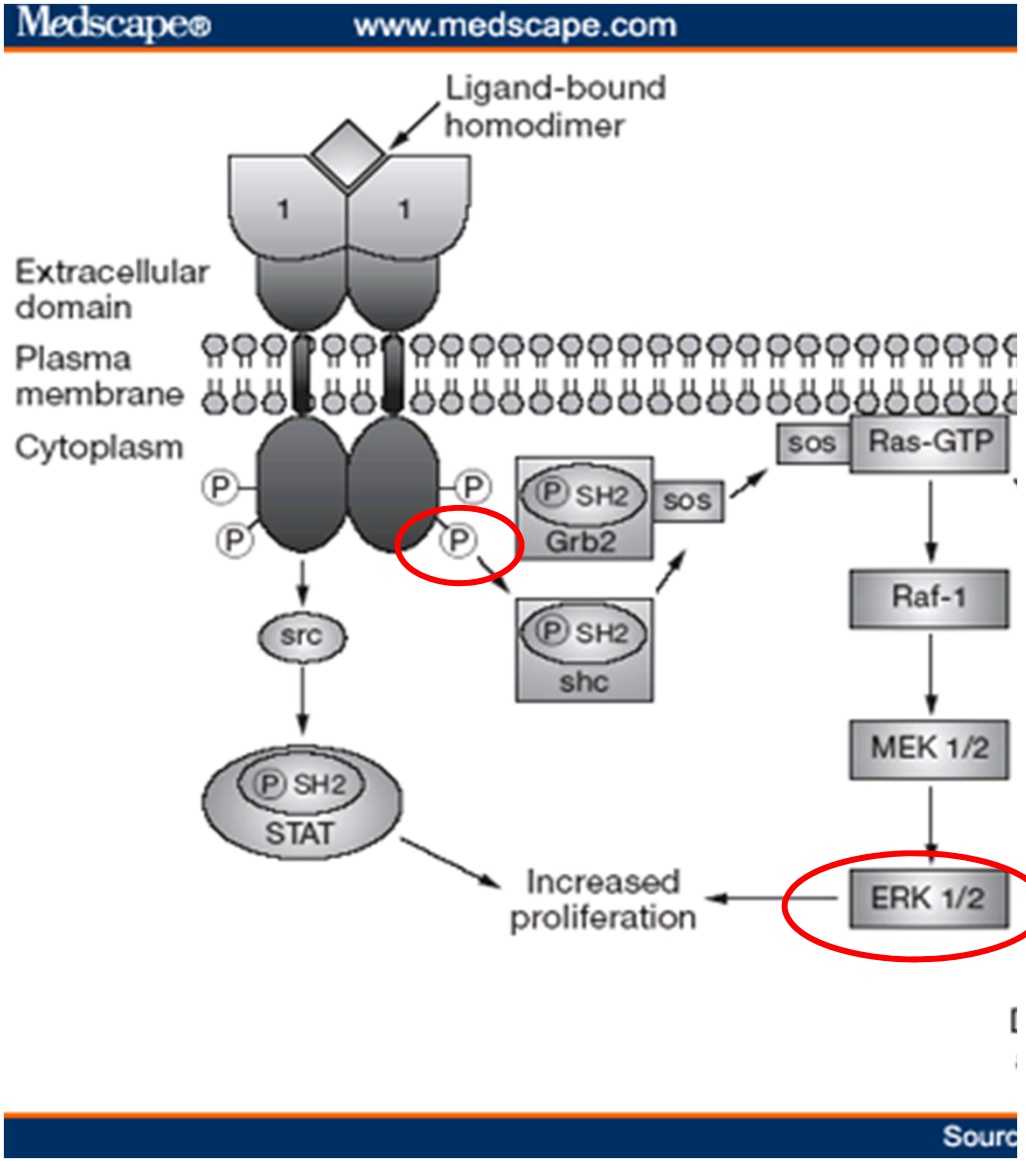
Proteomic Analysis of Formalin Fixed Tissue and Frozen tissue by Mass Spectrometry

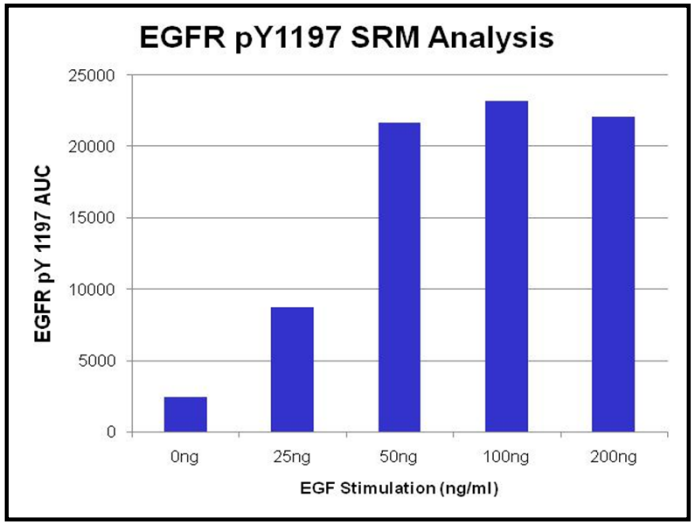


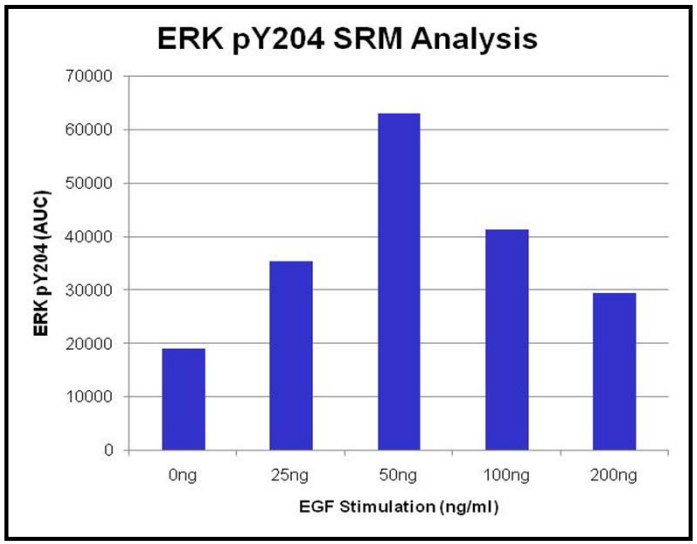
MS based Assay using Nanoflow LC and Quadropole MS with SRM

SRM Analysis of EGFR Signaling Pathway

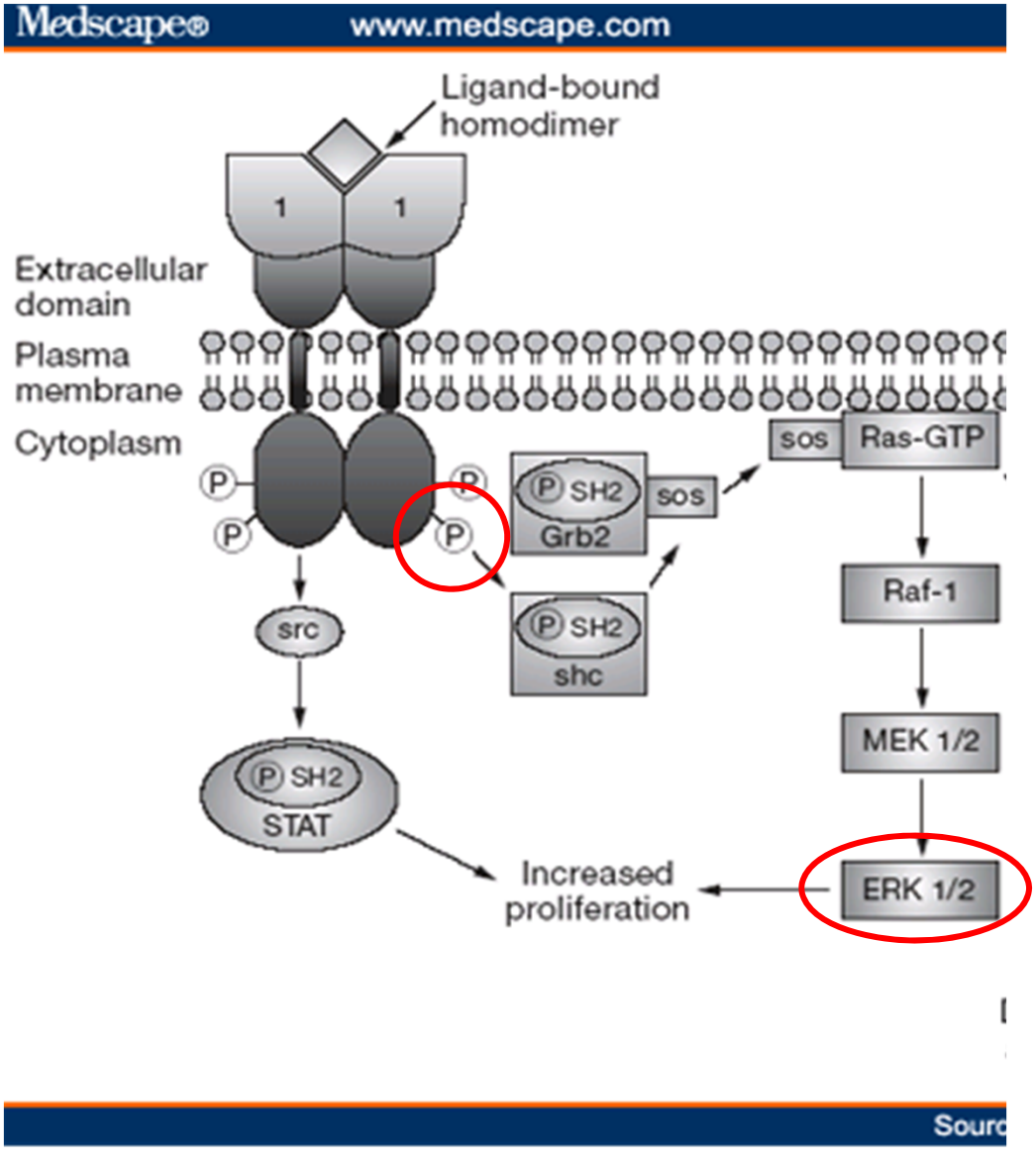
Measurement of EGFR pY1197 and ERK pY204 in FFPE A431 Cells

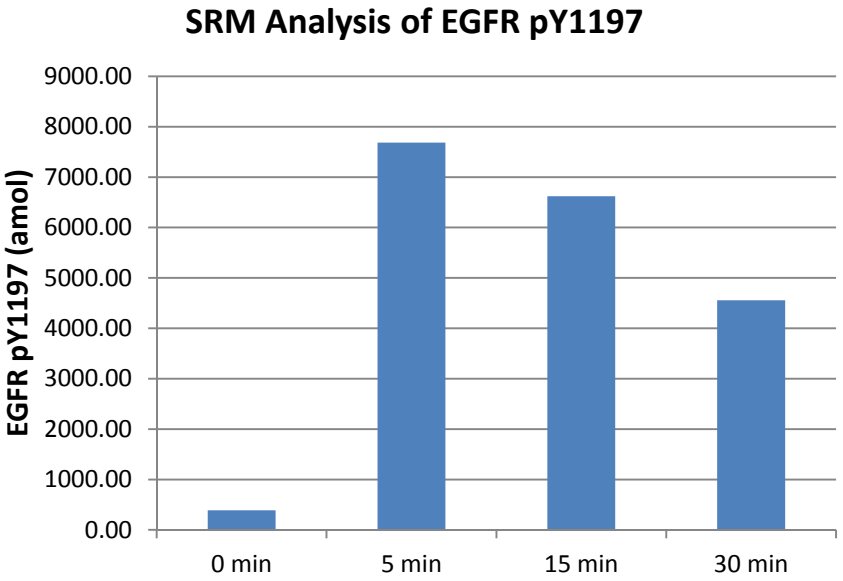


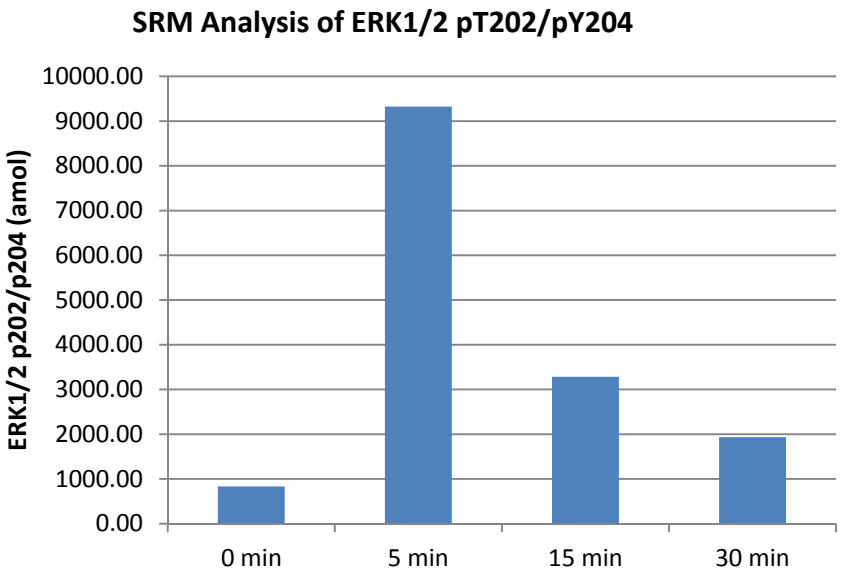




Timecourse Analysis of EGFR/ERK Pathway

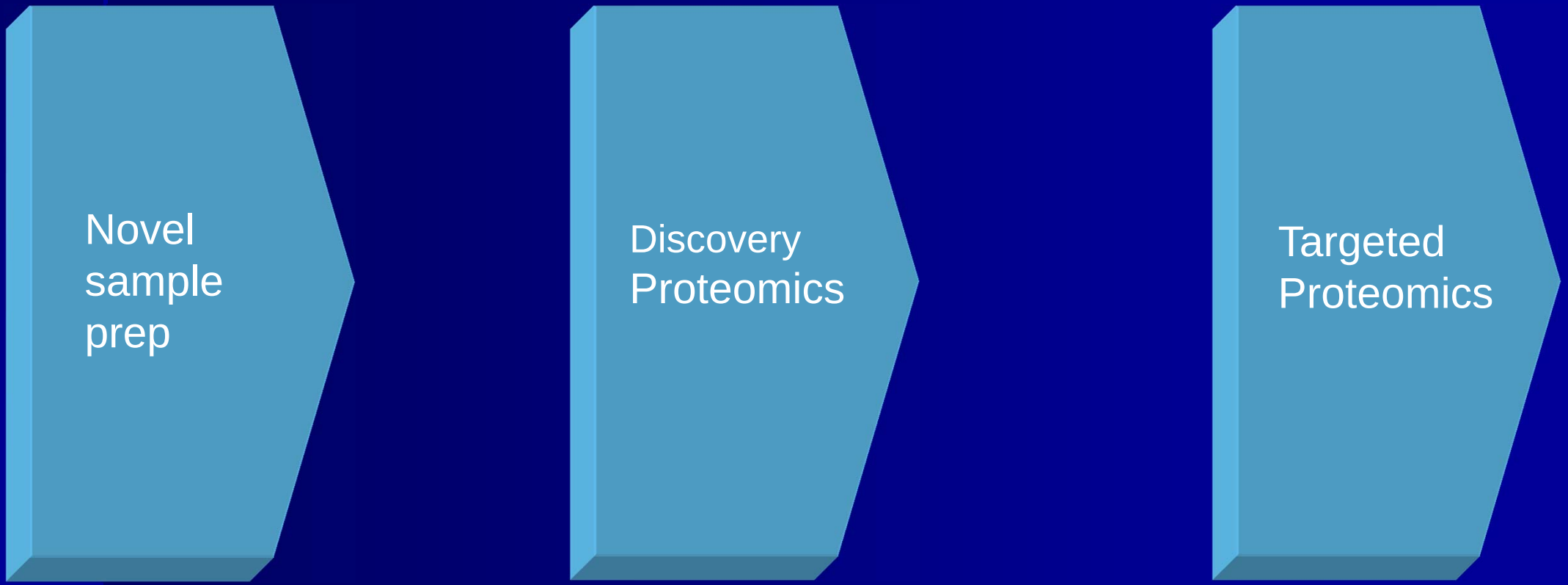






Bridging the gap on Clinical Proteomics for Biomarker discovery

Total solution for the handling of clinical sample and the following analysis platform



蛋白質のDegradationを防ぎ翻訳後修飾を保存するための新技術

A Method to Prevent Protein Degradation in Tissue Samples

– Key for high quality Peptidomics and Proteomics

Mats Borén *PhD, Head of Development, Denator AB, Sweden*

Biological challenge – revealing the true *in vivo* profile

生体サンプルのin vivo プロファイルを得るには？

- Proteins and peptides degrade/change rapidly post-sampling due to endogenous enzymatic activity
- Degradation products interfere with analysis such as 2D-gels, mass spectrometry or Western blot
- Results not representative of *in vivo* state

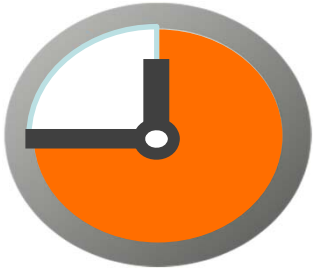
Post sampling changes in samples

生体サンプルのサンプリング後の変化

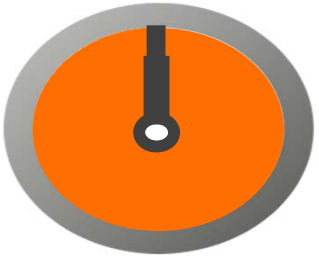
"The sample is alive"

- 15 seconds
  - 25-50% less ATP, ↓
  - 50% less glucose, ↓ 50% more lactate ↑

- 45 seconds
  - 75% less glucose, ↓ 150% more lactate ↑



- 1 min
  - 50% less ATP ↓
  - pH drops due to lactate accumulation ↓
  - 100% less glucose, ↓ 200% more lactate ↑
  - NA/K ATPase stops working
  - K-depolarisation
  - Cytoslic Ca increase



Switch from aerobic to anaerobic metabolism

好気的代謝から嫌気的代謝にスイッチ

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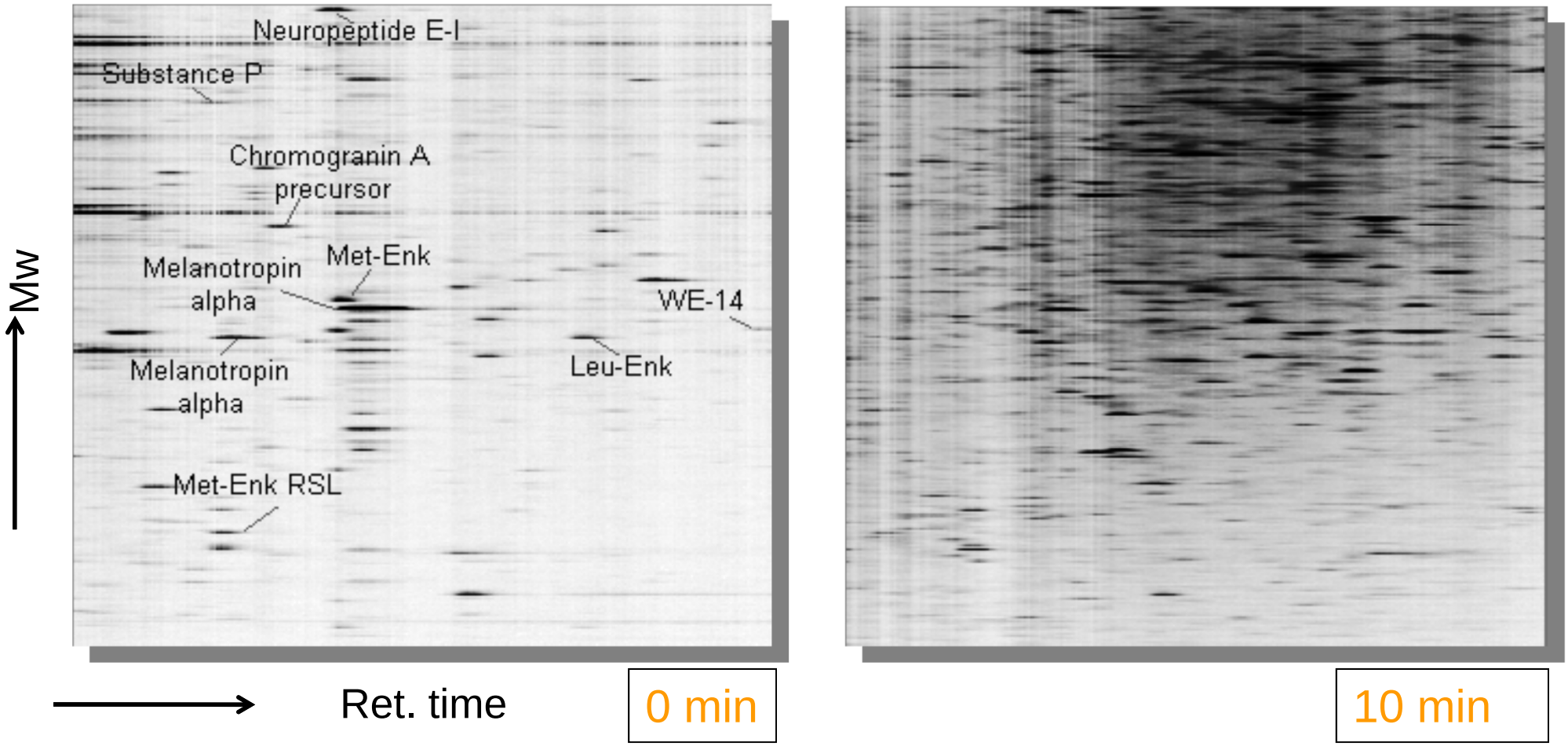
Protein degradation -> *peptides* post sampling

蛋白質のデグラデーション

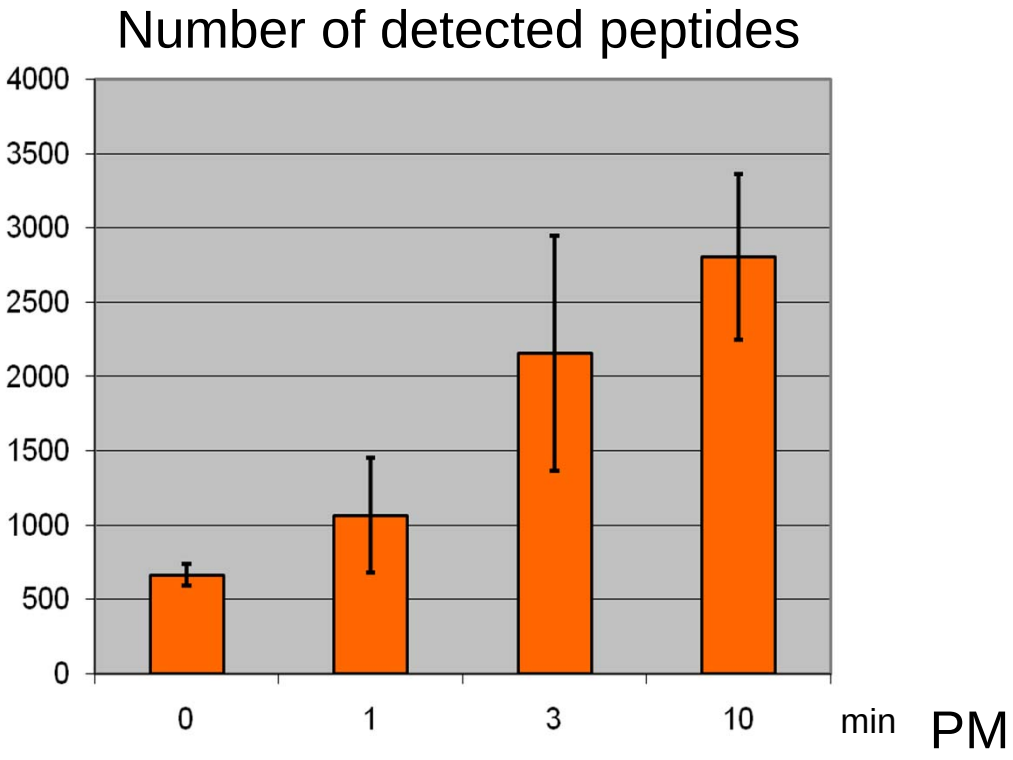


***Source:* Sköld, et al.,** *Proteomics 2007, 7, 4445–4456*

denator

Degradation

- Rapid increase of peptides -> the result of degradation
- “New” peptides are fragments from high abundant proteins



Sköld, et al., Proteomics 2007, 7(24), pp 4445

Stabilization thru Thermal Denaturation

熱による蛋白質の安定化

Maintainor®Tissue

Treatment/Storage

処理と保存

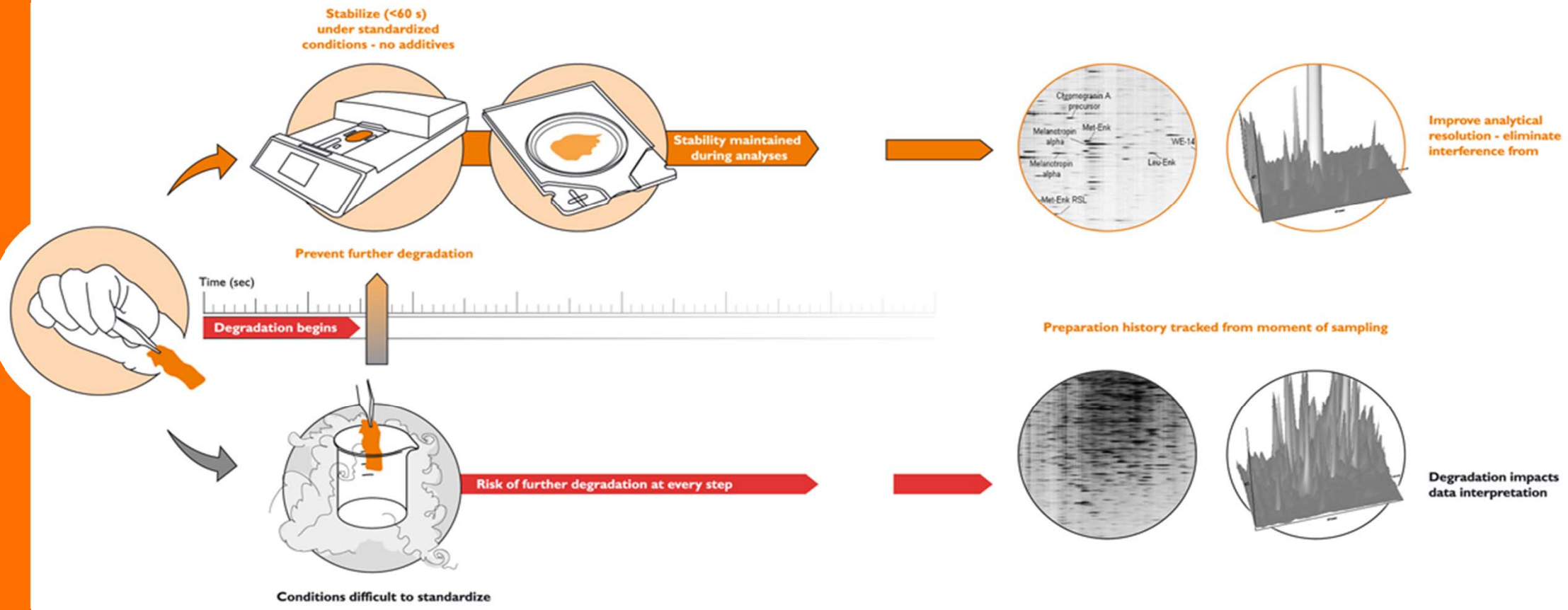
Stabilizor®T1

Stabilization

安定化

Stabilizor workflow

安定化処理のワークフロー

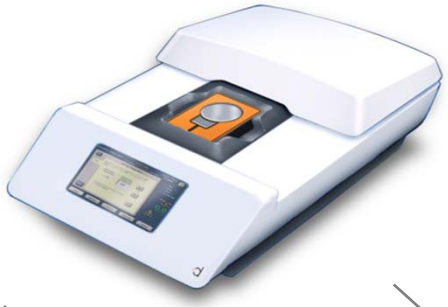


Multiple downstream applications

処理後のアプリケーション

MD LCMS

MALDI MS Imaging



ELISA

2D gels

LCMD

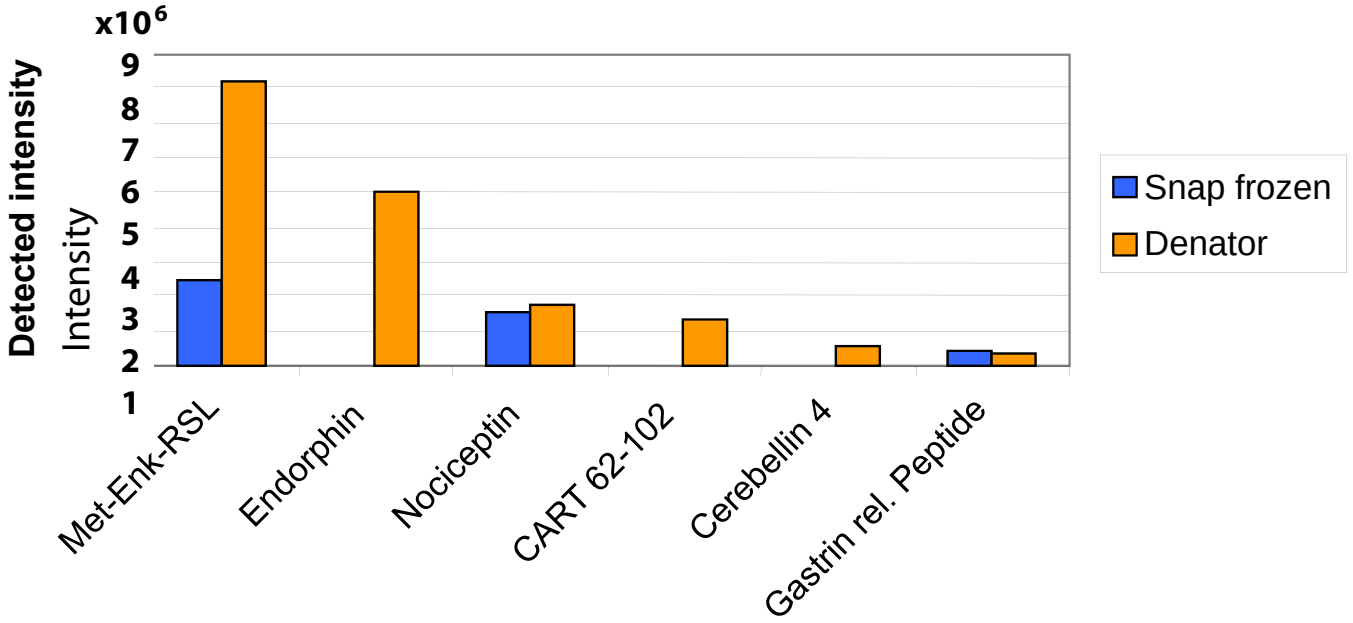
Western blot

LCMD-Laser Capture Micro Dissection

Peptidomics: Discovery and potential drugs

ペプチドミクス

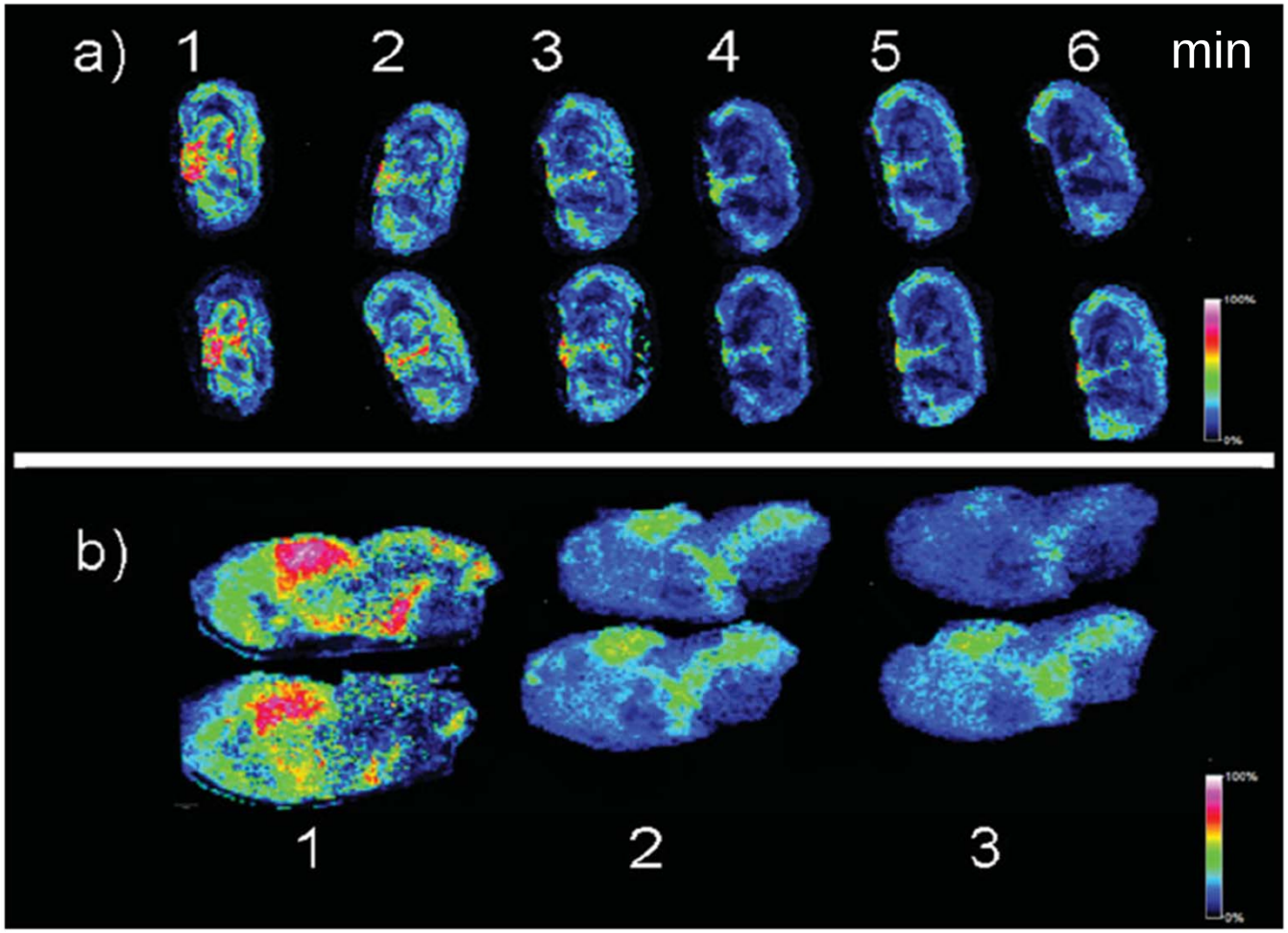
- Bioactive peptides as potential drugs
- Peptides of interest not seen when using conventional sample preparation
- *“Several potentially bioactive peptides where found when Stabilizor was used”*



Data courtesy: Sanofi-Aventis, France

MALDI Imaging: Peptides in mouse brain

MALDIイメージング



Stabilized, tissue Snap frozen Stabilized, on slide

m/z 4939 Da

Data courtesy of:

Goodwin et al, Proteomics 2008,8, pp 3785-3800

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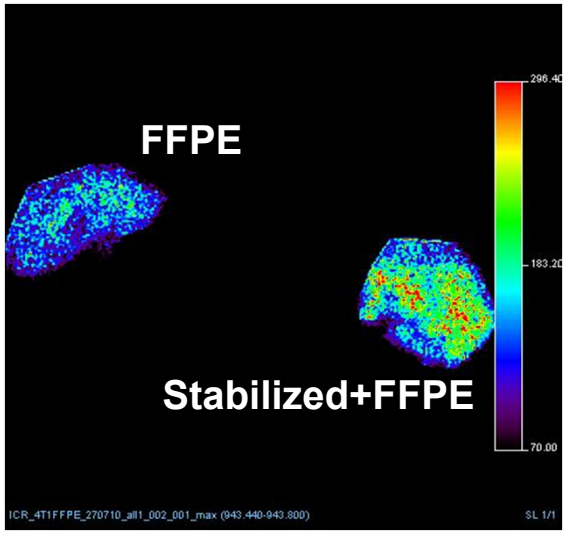
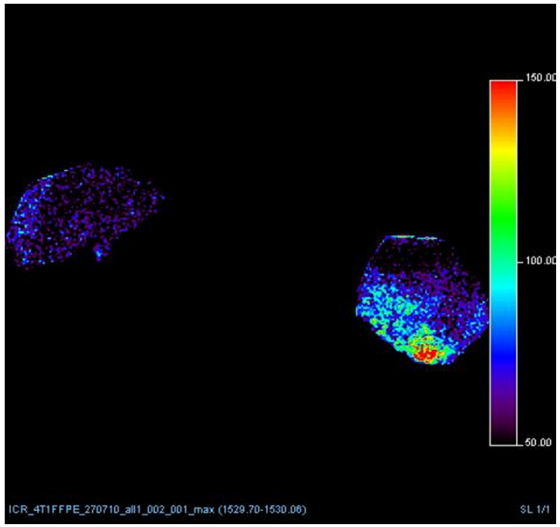
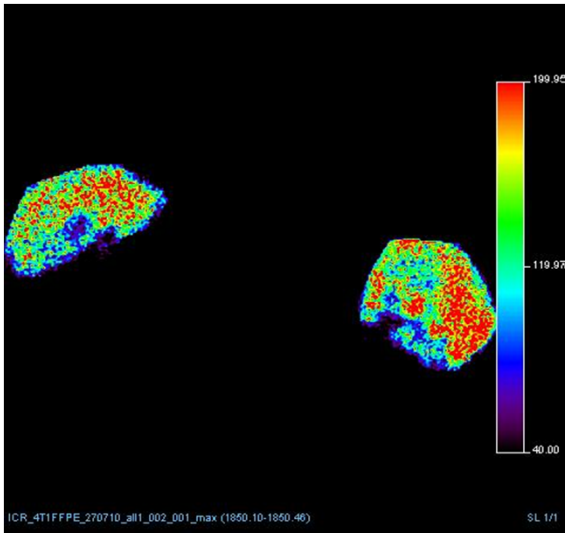
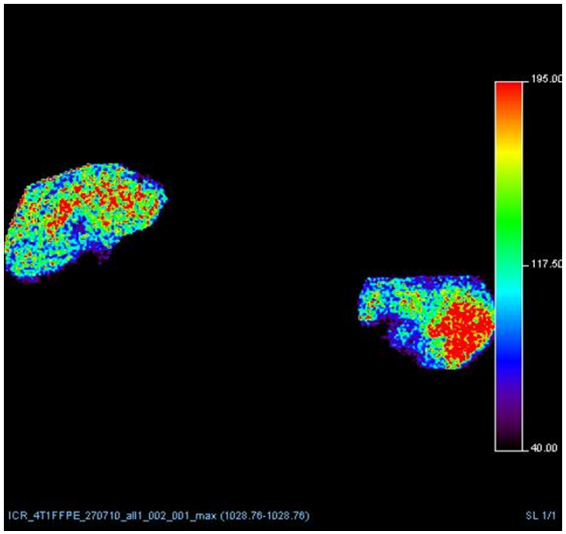
MALDI Imaging: Phospho biomarkers in xenografts

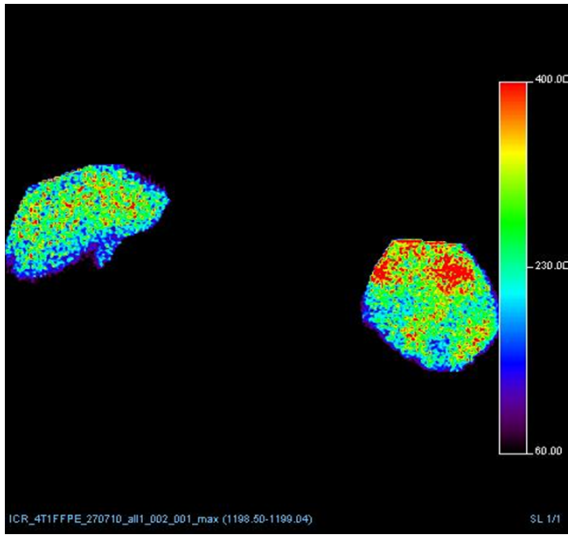
FFPE – Formalin Fixed Parafin Embedded

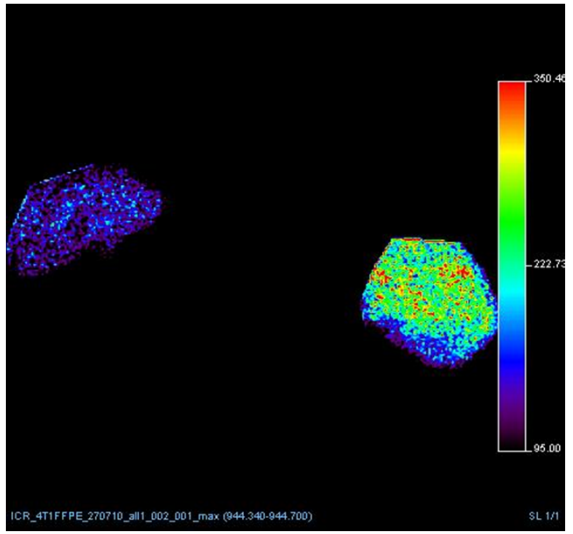












Data courtesy of The Institute of Cancer Research, UK

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Proteomics : Mouse brain

Stabilization results in:

- High molecular weight spots increase (green + orange)

  高分子量蛋白質のスポット増加ー緑とオレンジ

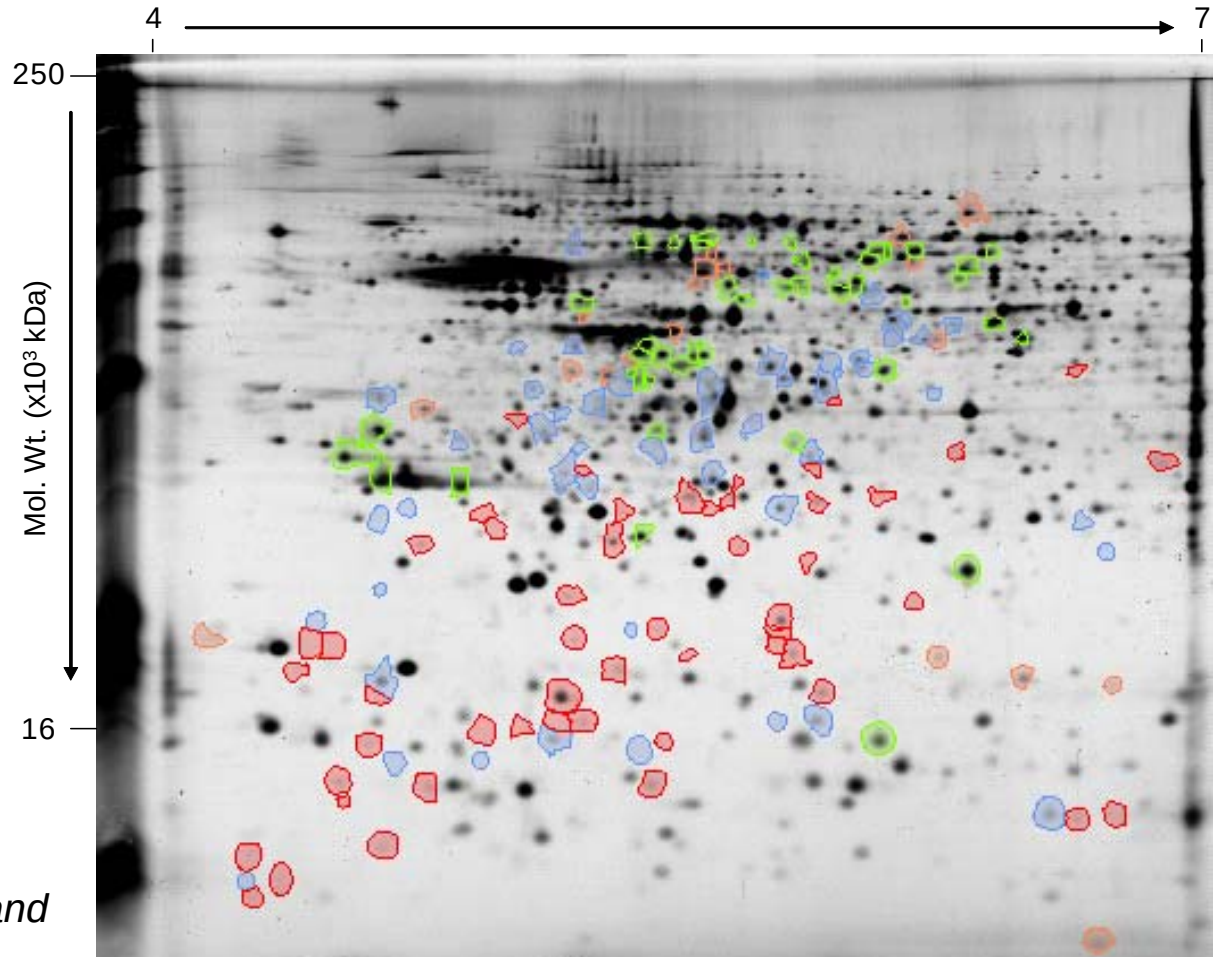
- Low molecular weight spots decrease (red + blue)

  低分子蛋白質スポットの減少ー赤と青

- Identifications show less degradation in stabilized samples

”Stabilization shows a favourable effect on the integrity of the mouse brain proteome affording increased abundance of several intact proteins and reduced fragmentation.”

Locations of differentially expressed proteins



Robinson et al, Proteomics 2009, 19(9):4433-4444

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Post translational modifications

翻訳後修飾

- **Phosphorylation**

  リン酸化修飾

- **Sumolation**

  Sumo化

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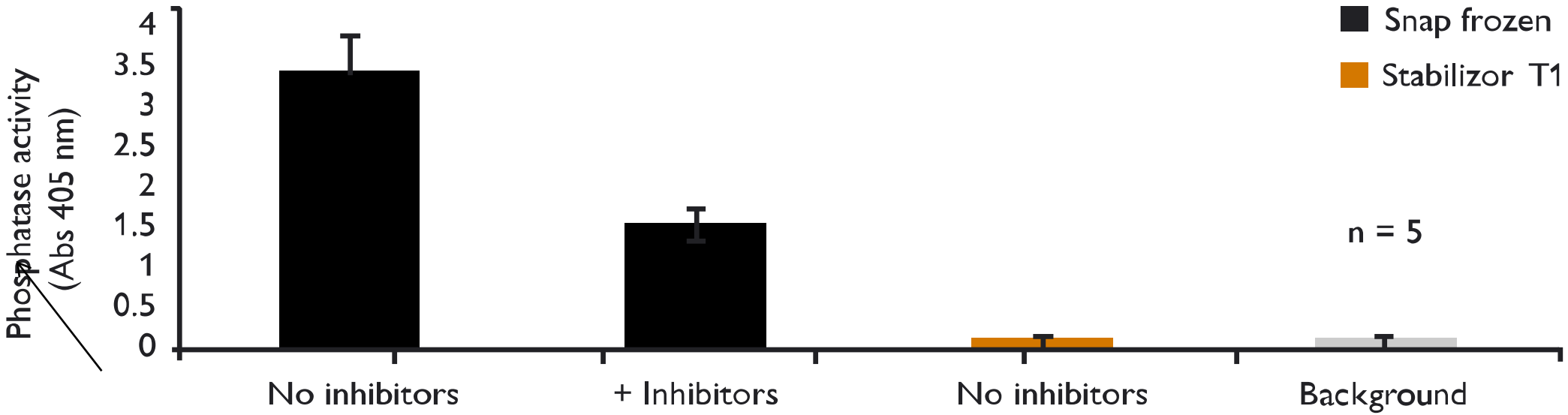
Inactivation of phosphatases

フォスファターゼの不活化



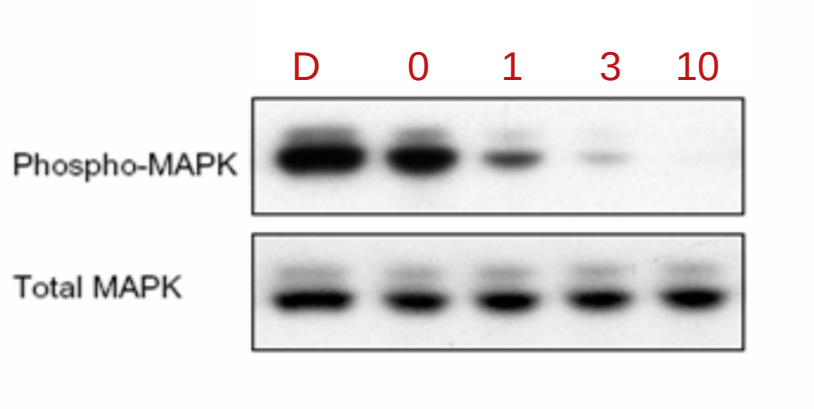
フォスファターゼ活性

Svensson et al., Journal of Proteome Research 2009, 8(2), pp 974–981

Phosphorylations change rapidly

リン酸化の迅速な変化

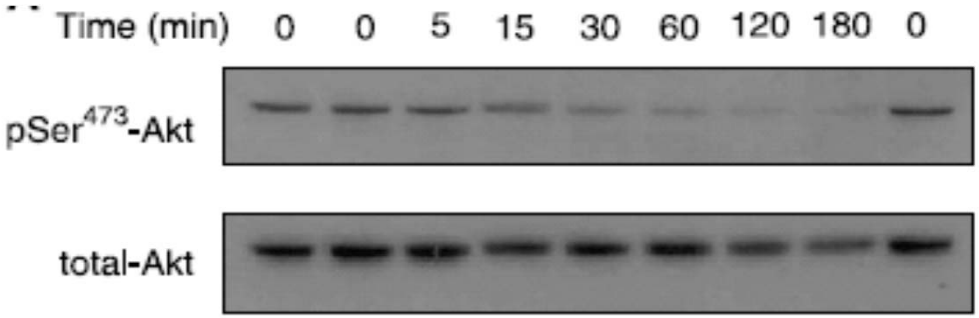
Western Blot – Brain tissue



Western Blot

Source:
Sköld, et al., Proteomics 2007, 7(24), pp 4445

Western Blot – HT29 Xenografts



Source:
Baker et al., Clin Cancer Res 2005, 11(12), pp 4339

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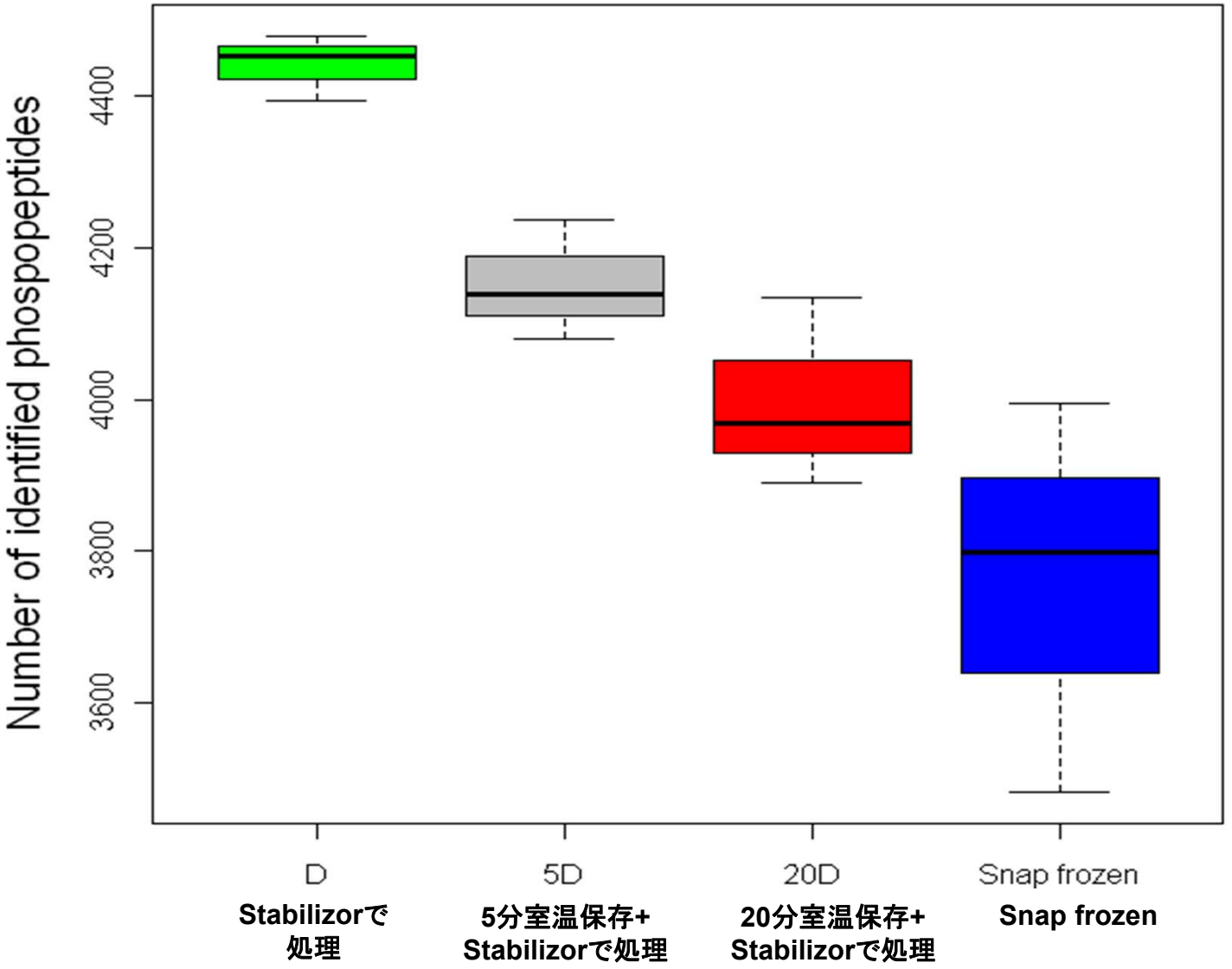
PhosphoShotgun

Number of identified phosphopeptides

リン酸化プロテオミクス　ショットガン解析でのリン酸化ペプチドの同定数

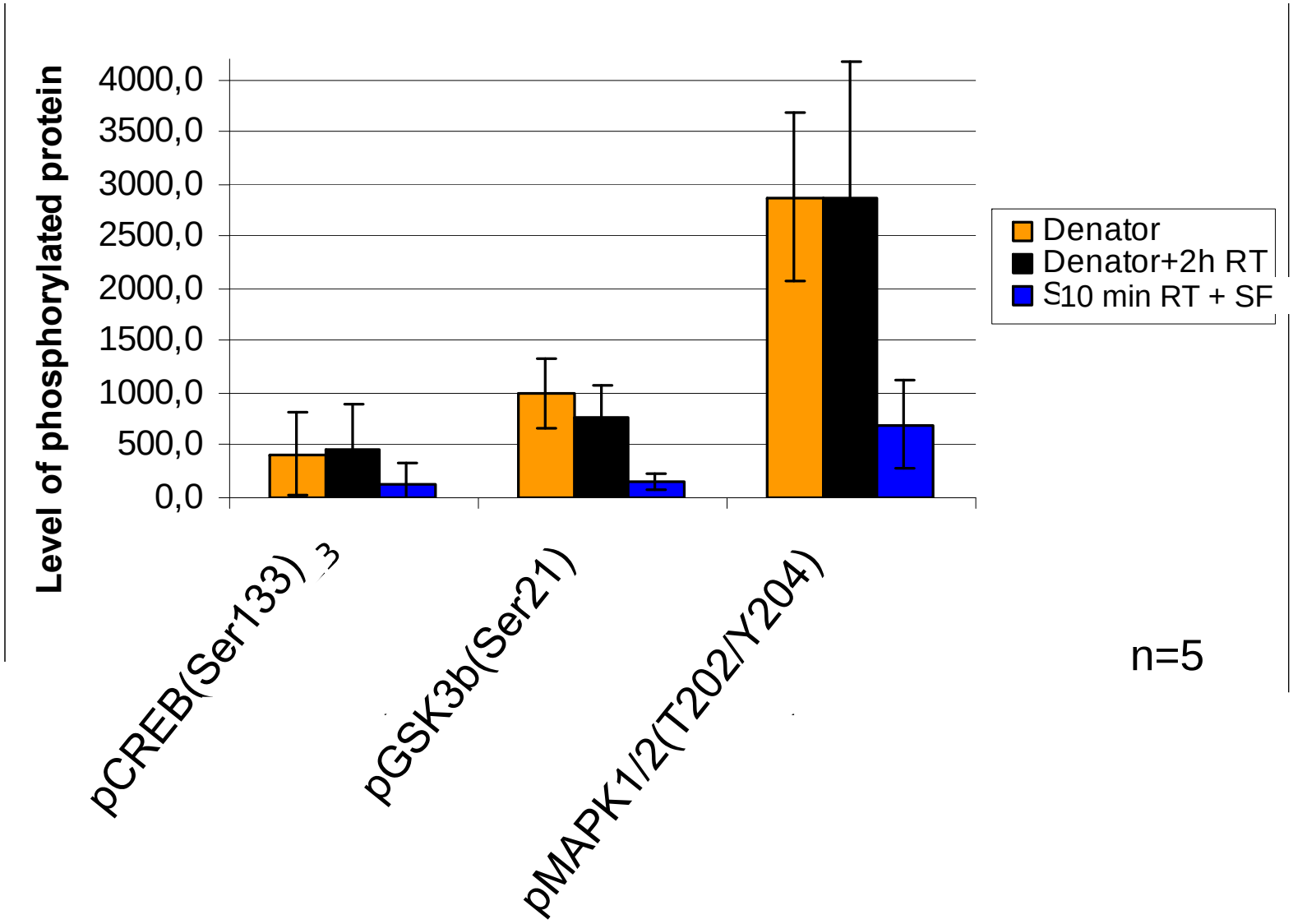


n=3

In collaboration **Jesper Olsson**, Denmark

Phosphorylated proteins – Stability after stabilization

リン酸化蛋白質—処理後の安定性



In collaboration with Prof. Per Svenningsson, Karolinska Institutet

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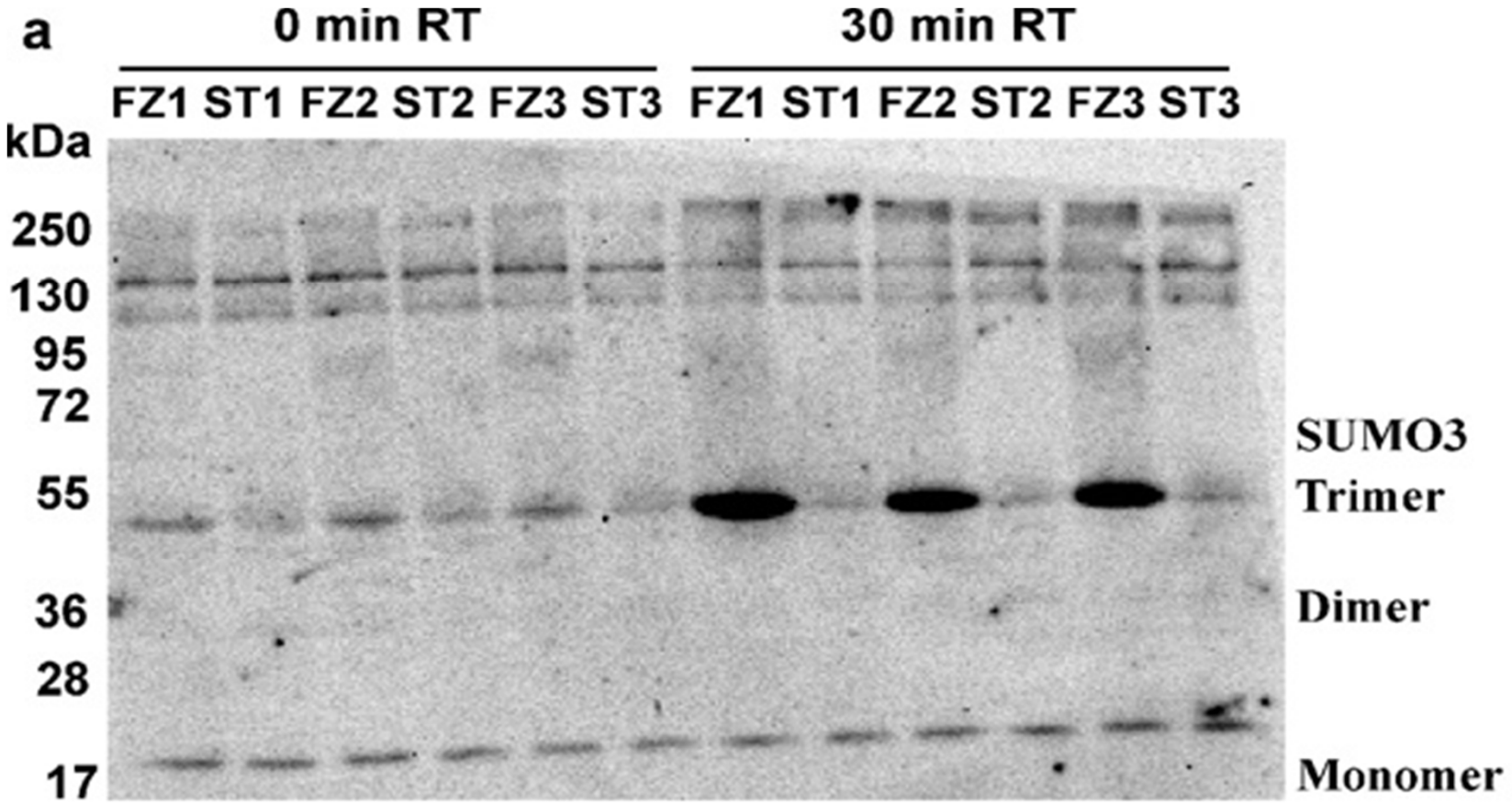
Stabilization of Protein Sumolation

SUMO化蛋白質の安定性



FZ – Snap Frozen ST - Stabilized

Ahmed M, Gardiner KJ. , Journal of Neuroscience Methods. 2011;196:99-106.

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HARVARD MEDICAL SCHOOL

Phosphorylation states preserved in stabilised samples

Post mortem changes due to kinase activity was studied at Harvard Medical School

Inhibition of kinases is often overlooked or inadequate

The human genome codes for ~500 different kinases, no complete cocktail exists

Smejkal et al, Electrophoresis 2011, 32

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HARVARD MEDICAL SCHOOL

Phosphorylation states preserved in stabilised samples

The study:

2D GE followed by identification by nano LC MS/MS on 16 spots

Mouse cortex (n=6), randomised and divided in two groups

1. Stabilised: heat denatured within 60 seconds
2. Non-stabilised: immediately homogenised in 7M urea, 2M Thiourea and 4% CHAPS, and frozen in liquid N2 within 300 seconds of euthanasia

Smejkal et al, Electrophoresis 2011, 32

Hyperphosphorylated proteins evidence of post mortem kinase activity

- In stabilised samples

  5 unique phosphoprotein isoforms *"exemplifying the potential for loss of data from improperly preserved samples"*
- In unstabilised samples:

  4 unique spots, two of which was hyperphosphorylated isoforms of Proteasome subunit α type 3 and Glutathione-S-transferase P1

Smejkal et al, Electrophoresis 2011, 32

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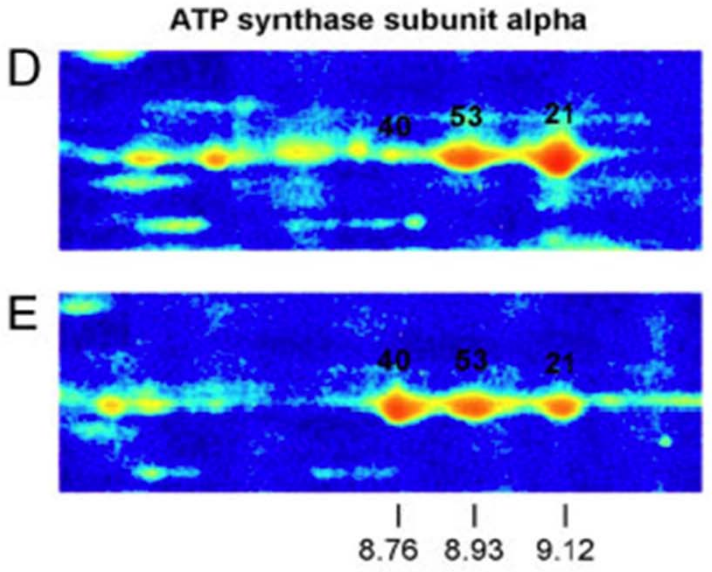
Detailed example 1:
a preserved protein

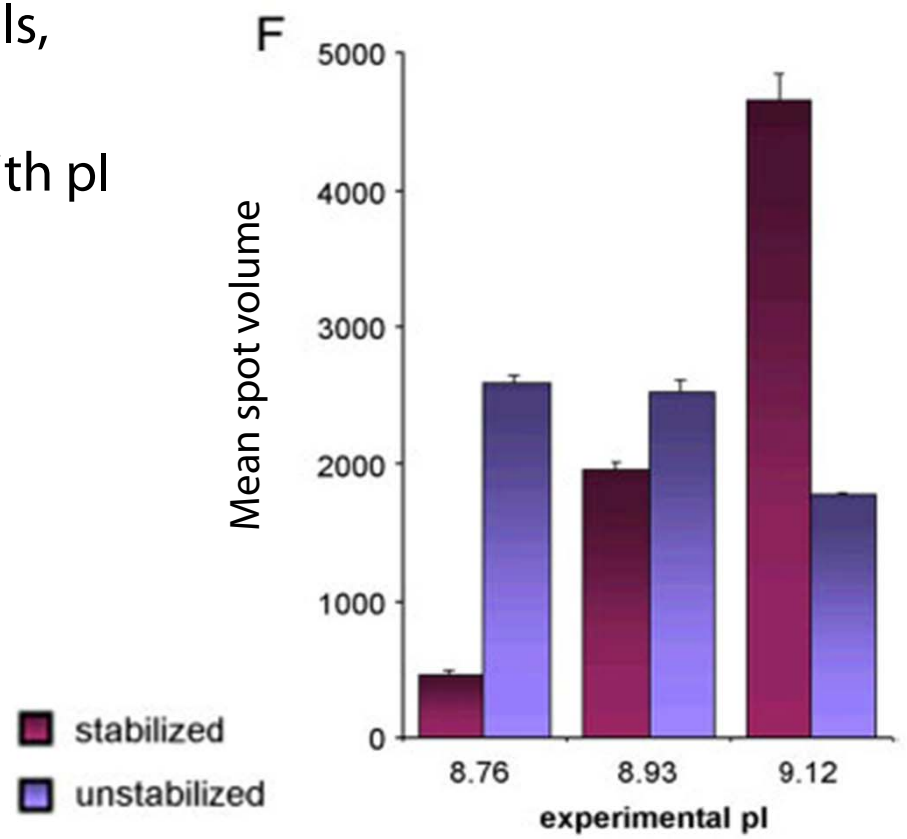
Stabilised

Unstabilised



ATP synthase subunit α

- Progressive changes in phosphorylation levels, shift towards more acid pI
- 550% increase in the phosporylation state with pI 8,76: hyperphosphorylation
- Sum of mean spot volume indentical in both samples.



Smejkal et al, Electrophoresis 2011, 32

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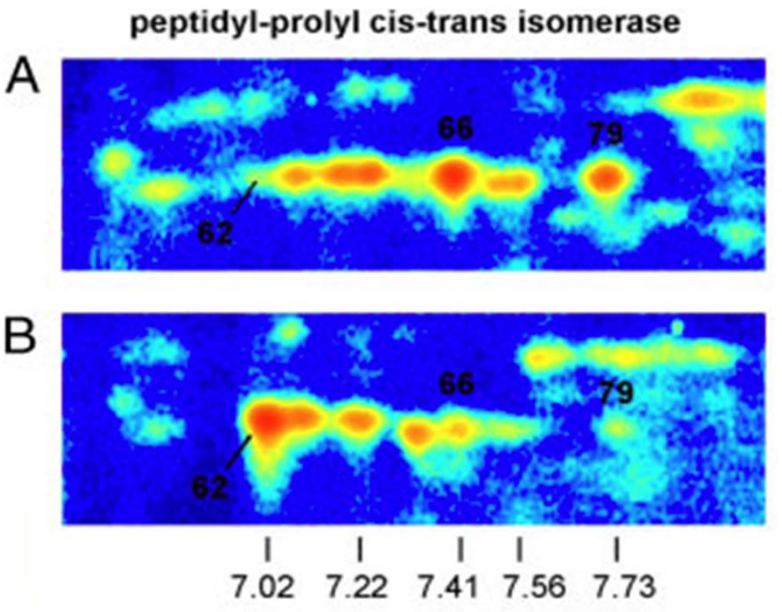
Detailed example 2:

a preserved protein

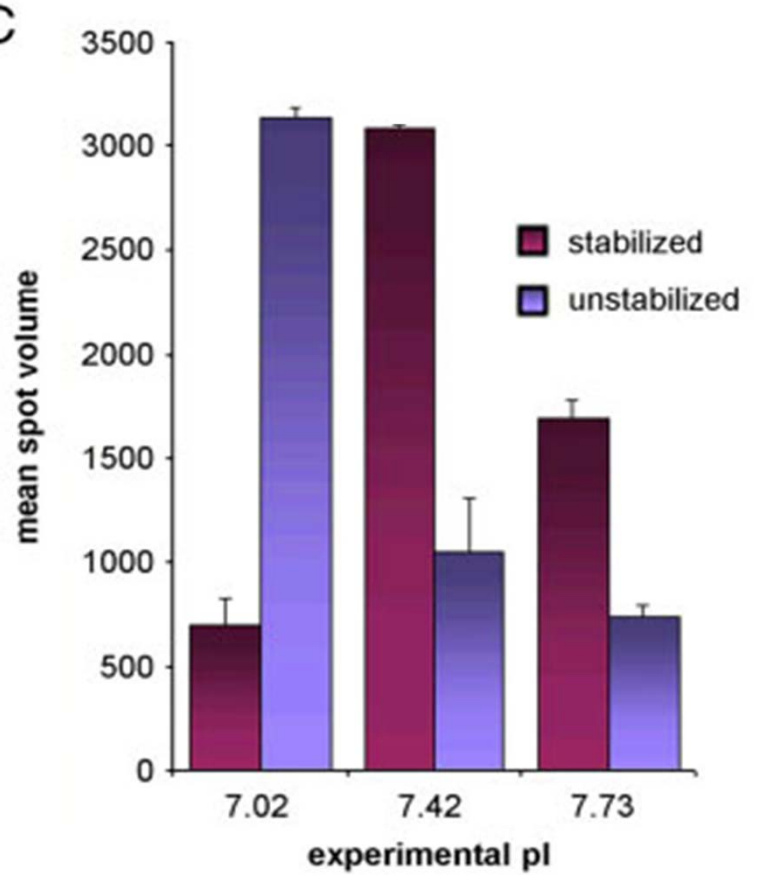
Stabilised

Unstabilised



Peptidyl-prolyl cis-trans isomerase

- Large progressive changes in phosphorylation profile
- Sum of mean spot volume indentical in both samples.



Smejkal et al, Electrophoresis 2011, 32

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Heat stabilisation preserves phosphorylation states

Summary of results:

- 99,6% of kinase activity was eliminated by heat stabilisation
- 53 of 588 spots were altered
- Two hyperphosphorylated isoforms were identified with nano LC MS/MS only in unstabilised samples, not present in stabilised
- An example: ATP Synthase subunit α had a 550% increase in a higher phosphorylation state

Smejkal et al, Electrophoresis 2011, 32

The importance of sample handling

サンプルの処理での重要事項

- Proteins, peptides and their modifications can change rapidly post sampling leading to loss of *in-vivo* information

蛋白質、ペプチドやその翻訳後修飾はサンプリング後迅速に変化し、***In-vivoの情報を失いやすい***。

- Standardization of sampling and rapid stabilization of molecules of interest is key for high quality protein studies

サンプリング方法の標準化と目的の蛋白質の迅速な安定化は蛋白質研究の鍵

- Stabilizor T1 effectively stops degradation permanently

スタビライザーT1は蛋白質のDegradationを恒久的にストップ

AMR

denator

Selected publications

主な文献

“Assessing the use of thermal treatment to preserve the intact proteomes of post-mortem heart and brain tissue” Aisling A. Robinson, Jules A. Westbrook , Jane A. English , Mats Borén , Michael J. Dunn, *Proteomics, 2009, 19 (9), pp 4433-4444*

“Heat stabilization of the Tissue Proteome: A New Technology for Improved Proteomics” Marcus Svensson, Mats Borén, Karl Sköld, Maria Fälth, Benita Sjögren, Malin Andersson, Per Svenningsson, Per E. Andrén, *Journal of Proteome Research, 2009, 8 (2), pp 974–981*

*“***Stopping the clock on proteomic degradation by heat-treatment at the point of tissue excision”** Richard J.A. Goodwin, Alastair M. Lang…Andrew R. Pitt, *Proteomics. 2010;10:1751-61.*

“Clinical application for the preservation of phospho-proteins through in-situ tissue stabilization” Rountree BC, Van Kirk CA, You H, Ding W, Dang H, VanGuilder HD*, et al. Proteome Science. 2010;8:61.*

Some Stabilizor T1 users worldwide

主な使用機関

- University College Dublin, **Ireland REFERENCE CENTER**
- Uppsala University, **Sweden**
- Karolinska Institutet, **Sweden**
- Leiden Medical University Center, **The Netherlands**
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