

## Preservation of sample content prior to mass spectrometry

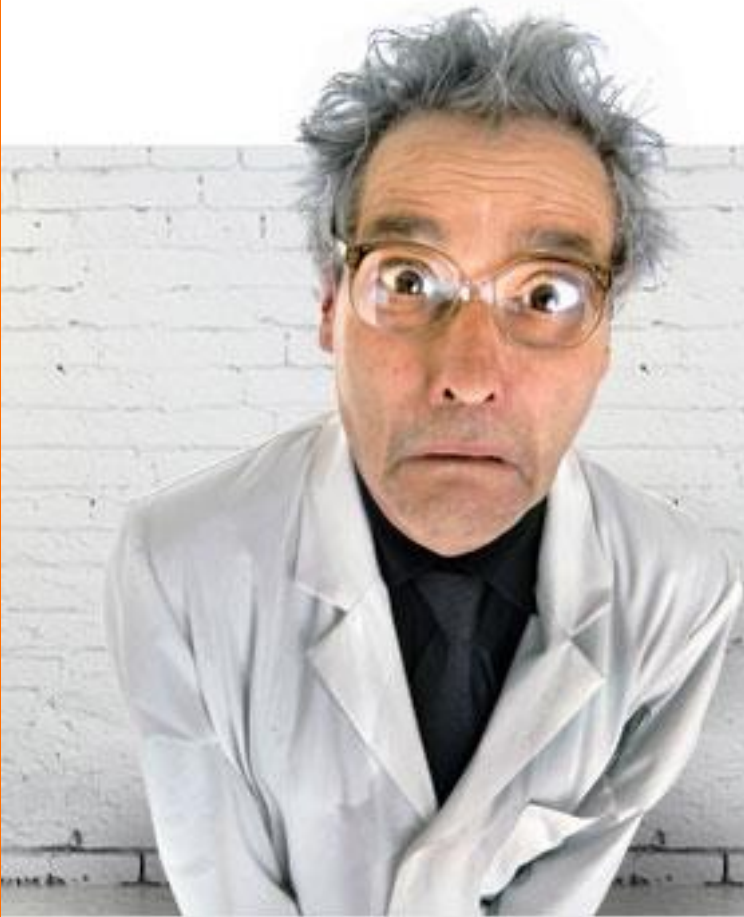
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デナーター  
スウェーデン

denator.com



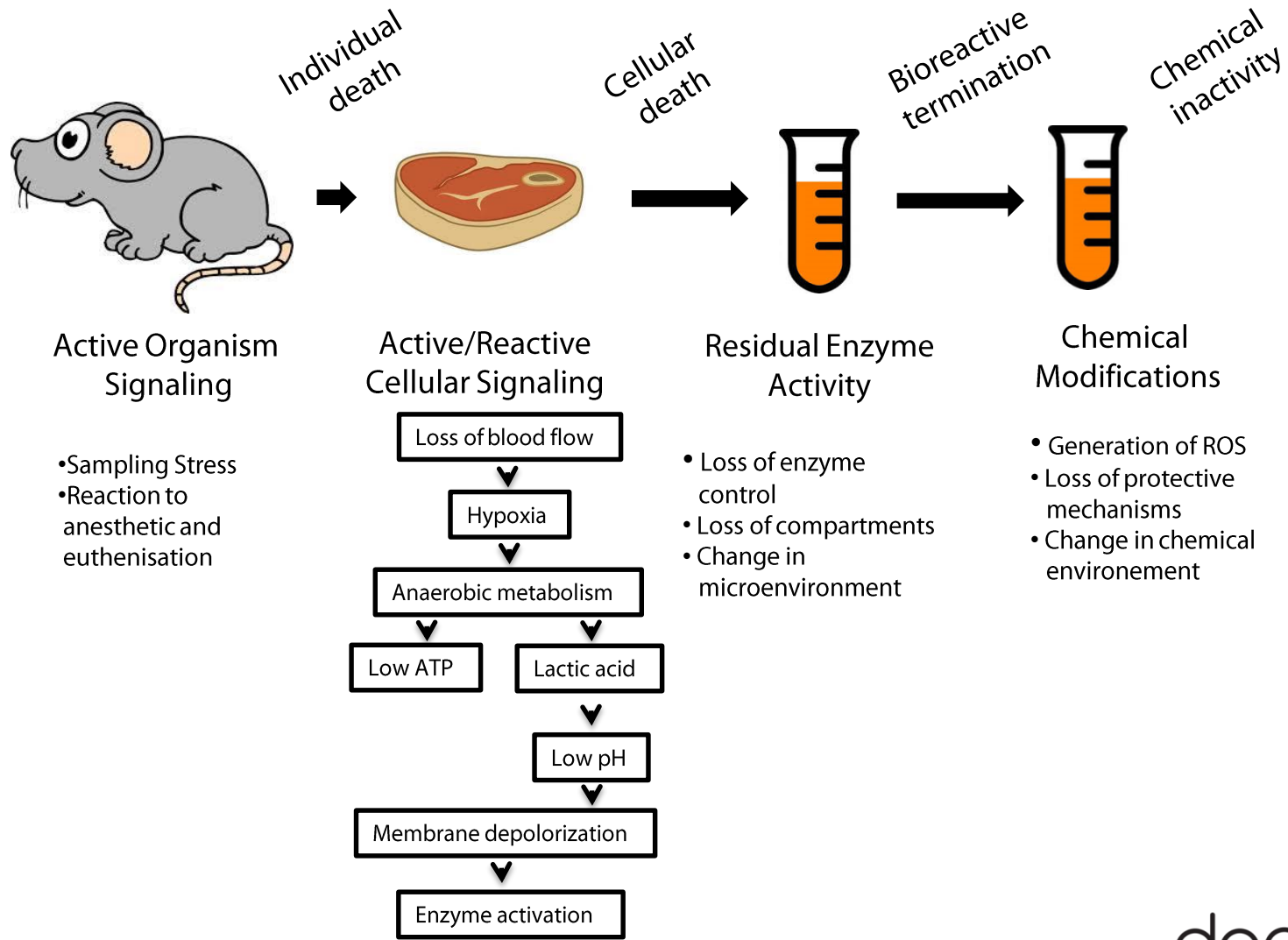
denator



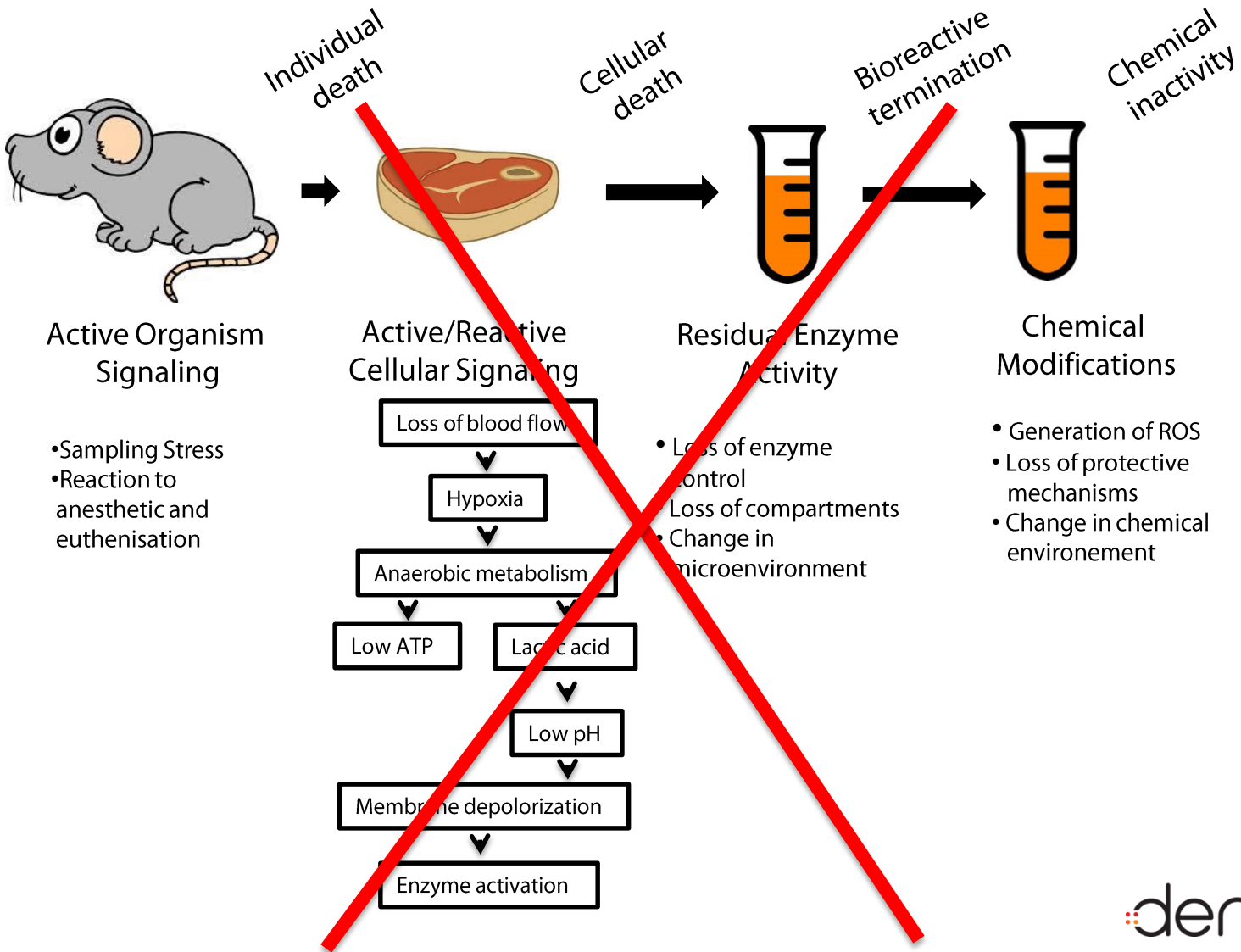
It's alive!

denator

# Drivers of molecular change post-sampling

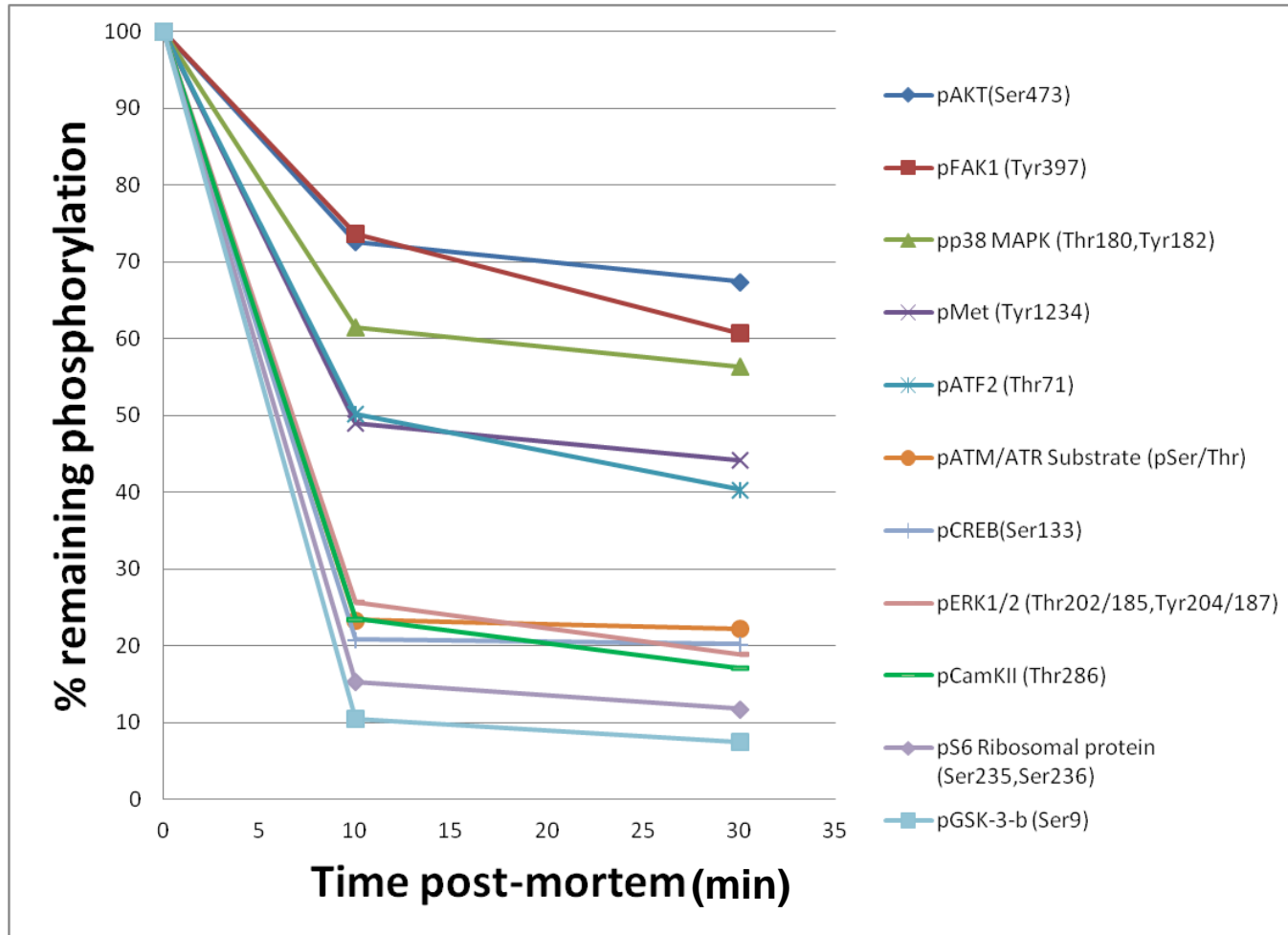


# Drivers of molecular change post-sampling



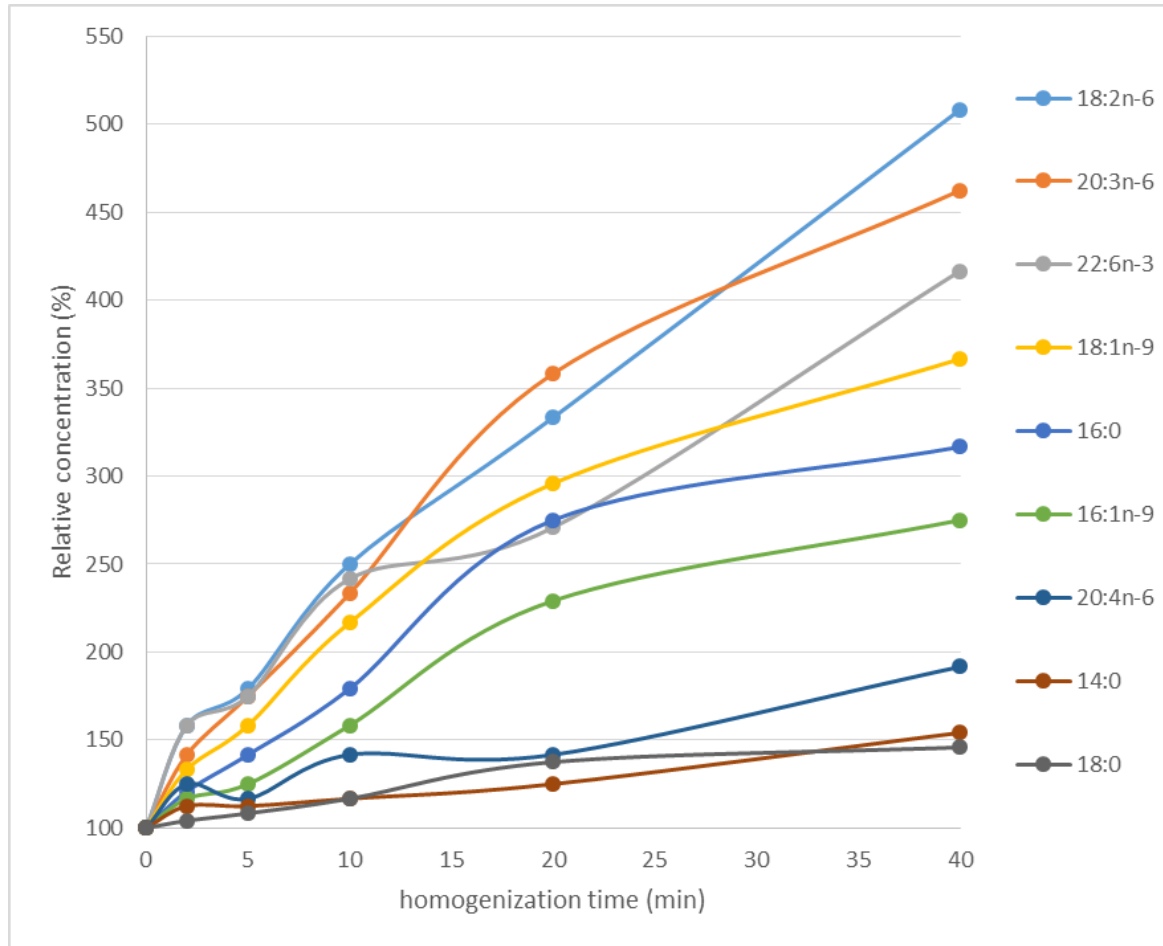
# Post-mortem change in phospho levels

Biosampling at three different time points



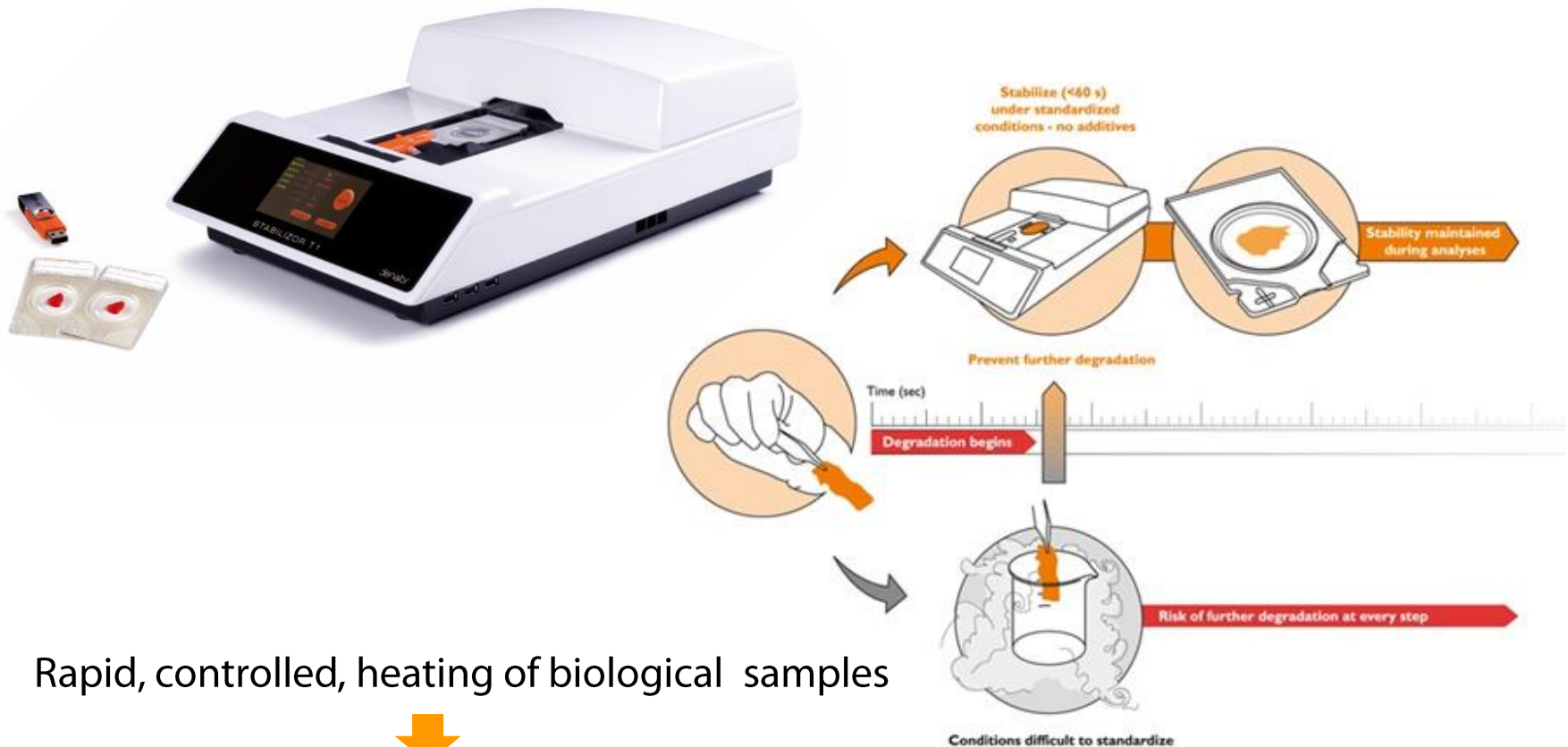
Mouse brain analysed using RPPA

# Levels of free fatty acids increases over time, 0-40 min



Levels of nine different free fatty acids (FFA) in brain homogenate

# Stabilizer™ system for improved tissue sample handling



Rapid, controlled, heating of biological samples



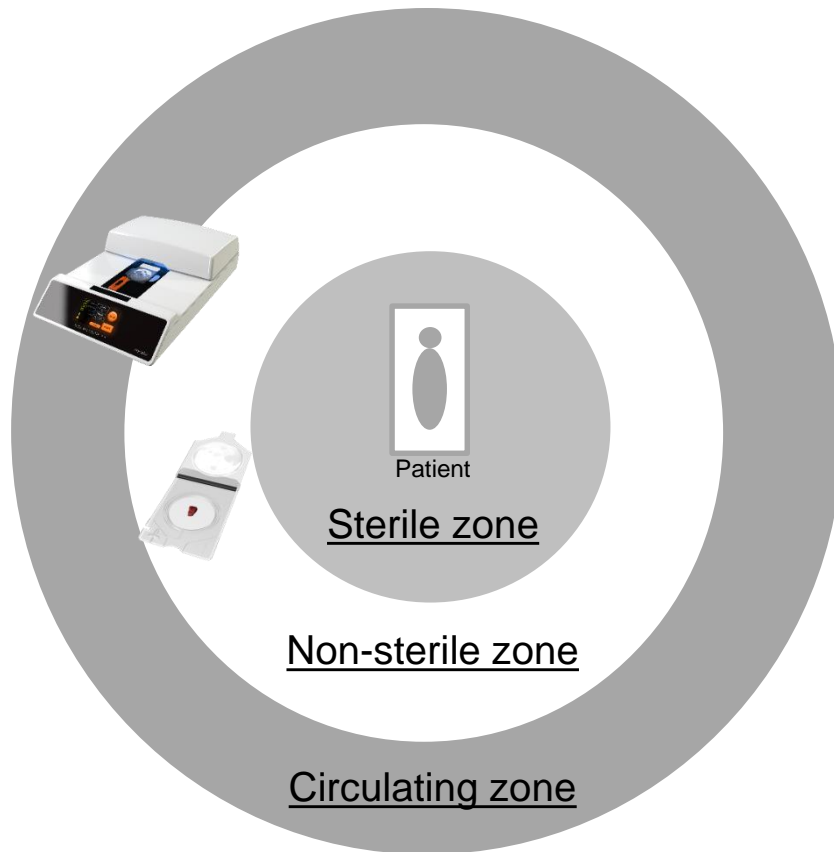
Inactivation of enzymes



Preservation of cellular components

# Use in practice

*Schematic view of zones in the operating room*

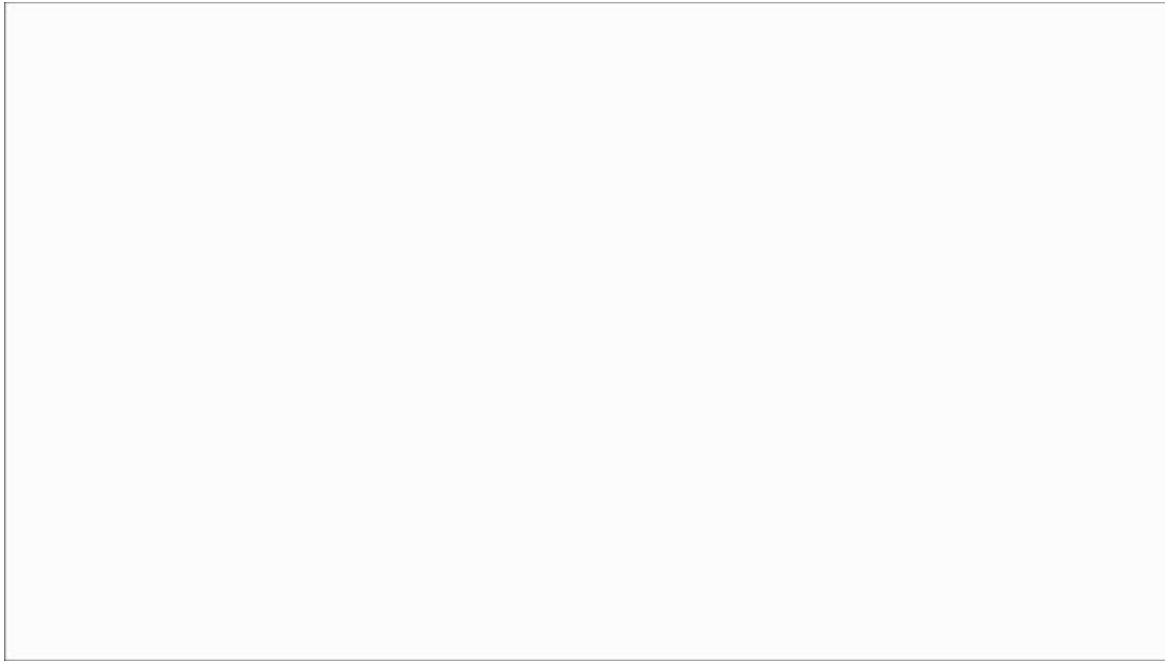


*The surgeon drops the sample in an opened card held by the assisting nurse or hands over the forceps with the sample (maximum 33 x 7 mm).*

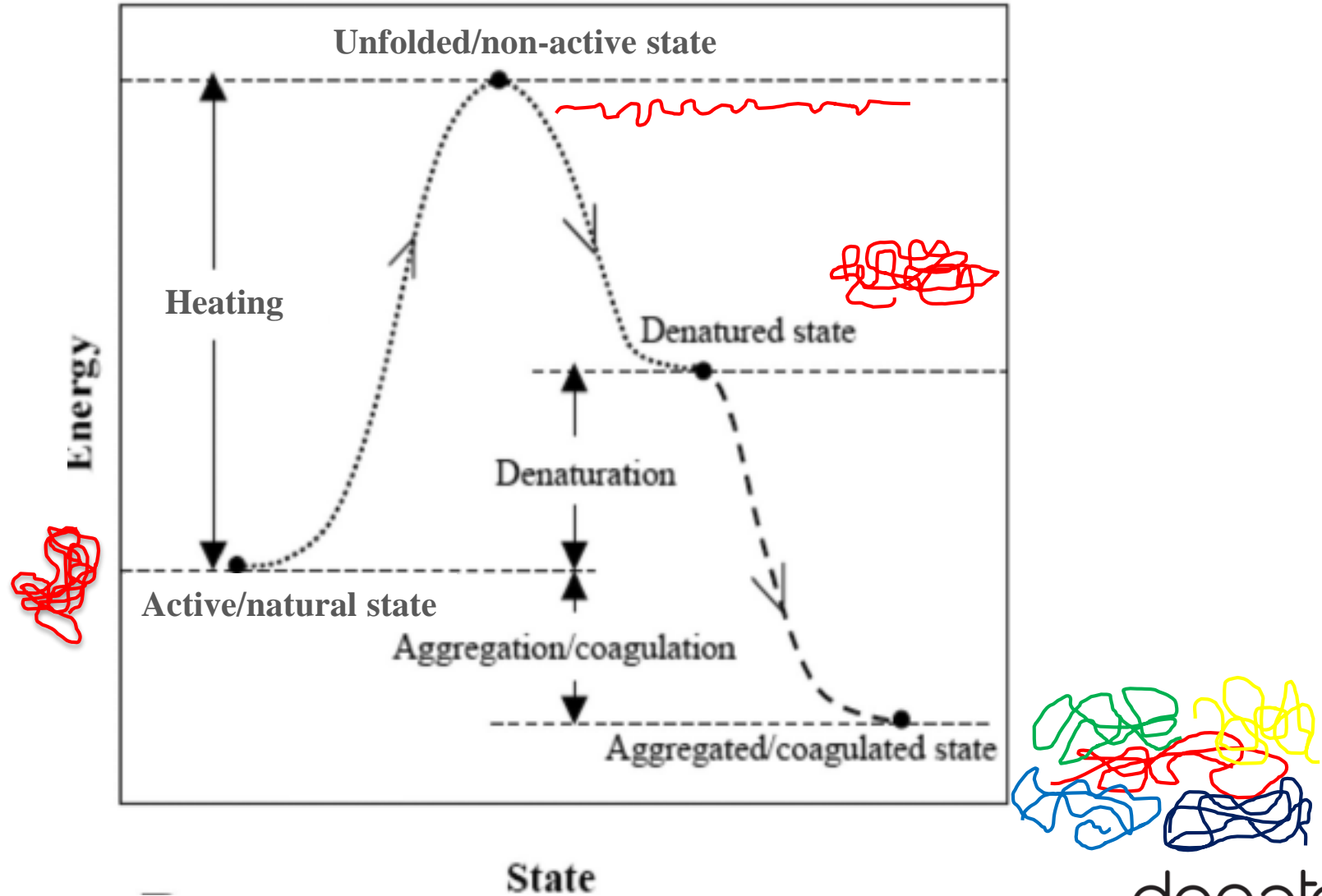




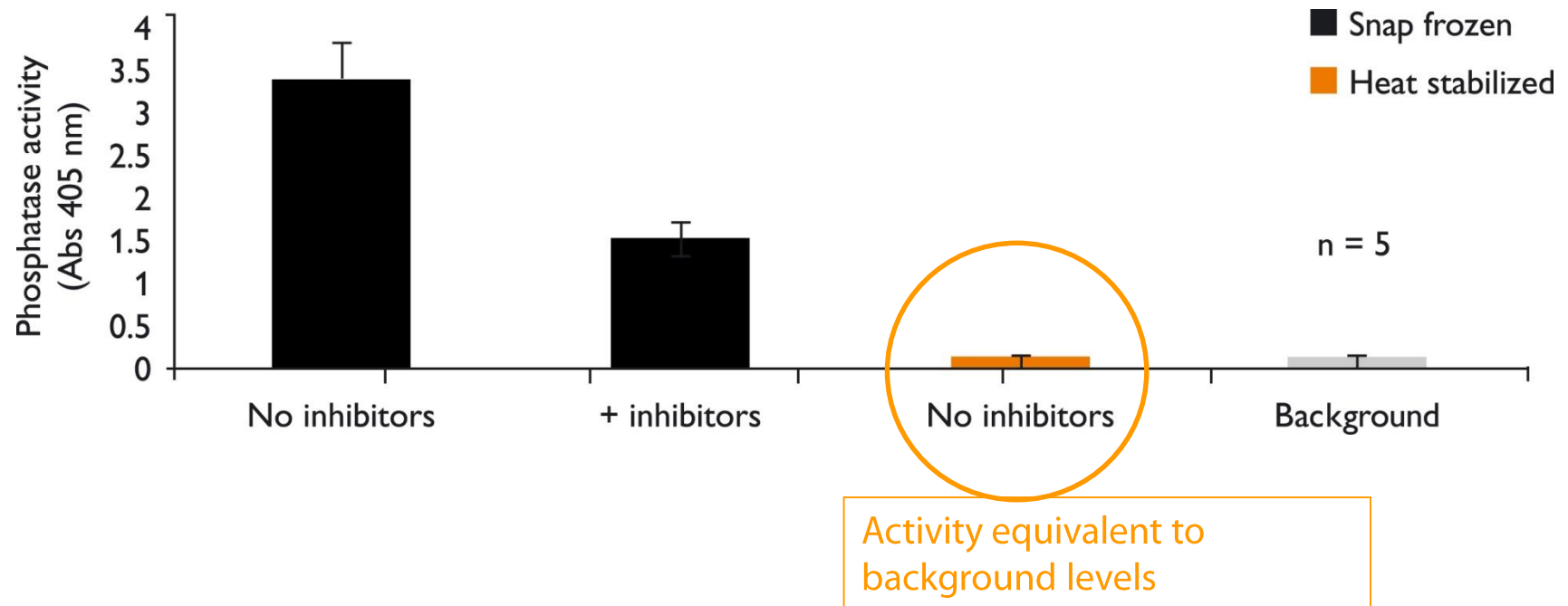
# Stabilizing a sample with the Stabilizer T1



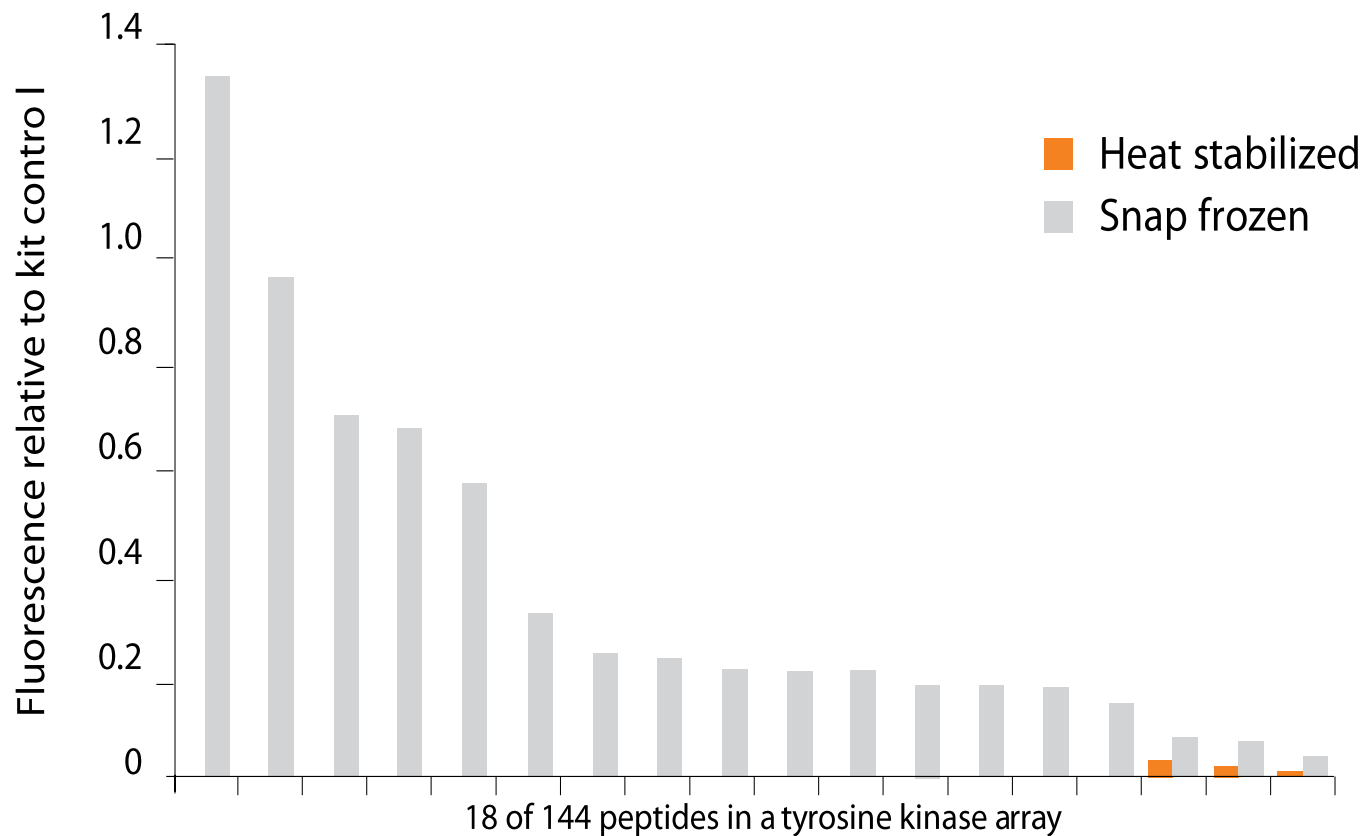
# Principal stages of heat induced inactivation



# Permanently inactivate phosphatases - avoid interference from inhibitors



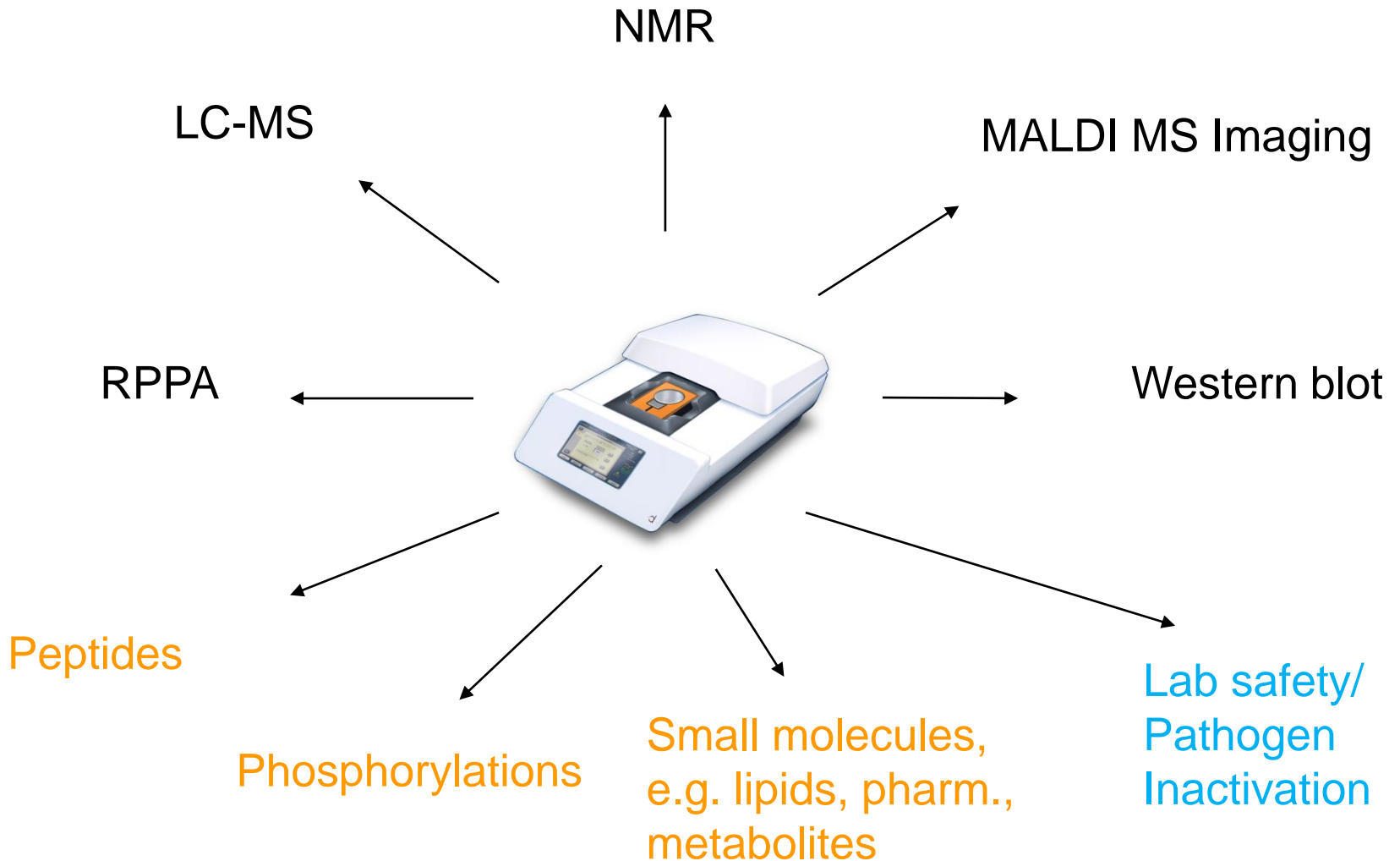
# Heat stabilization abolish 99.6% of kinase activity



Only 3 of 144 peptides produced measurable phosphorylation levels in heat stabilized samples.

# Multiple downstream uses

## Multiple downstream analytical techniques

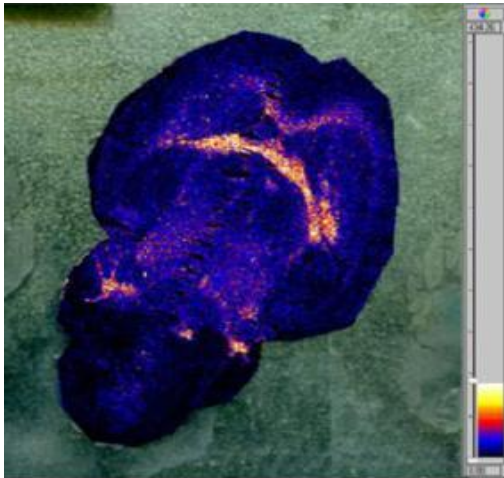


**Multiple downstream analytes**

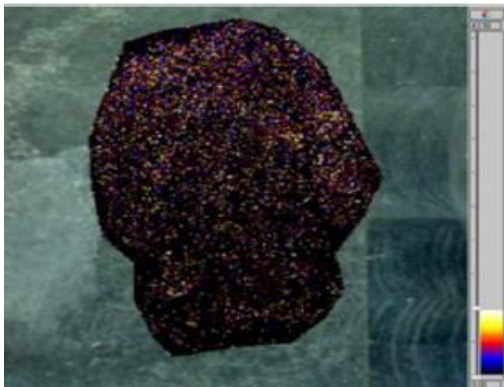
# Improve detection of small molecules

## Preservation of ATP

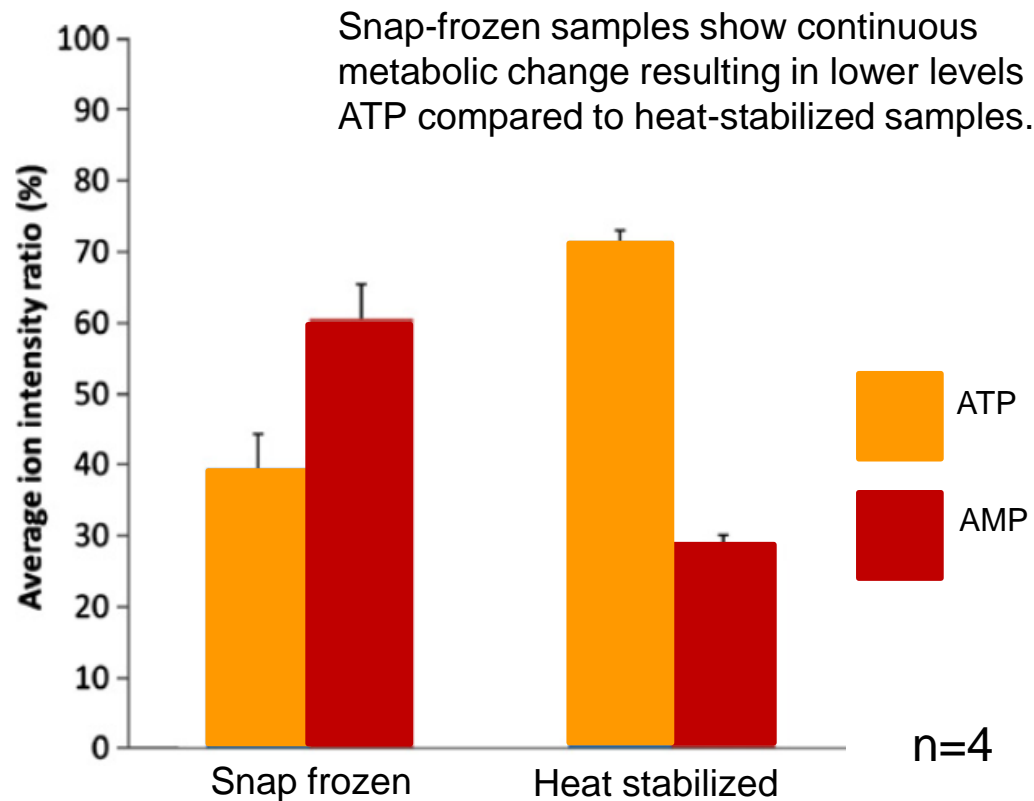
Heat stabilized



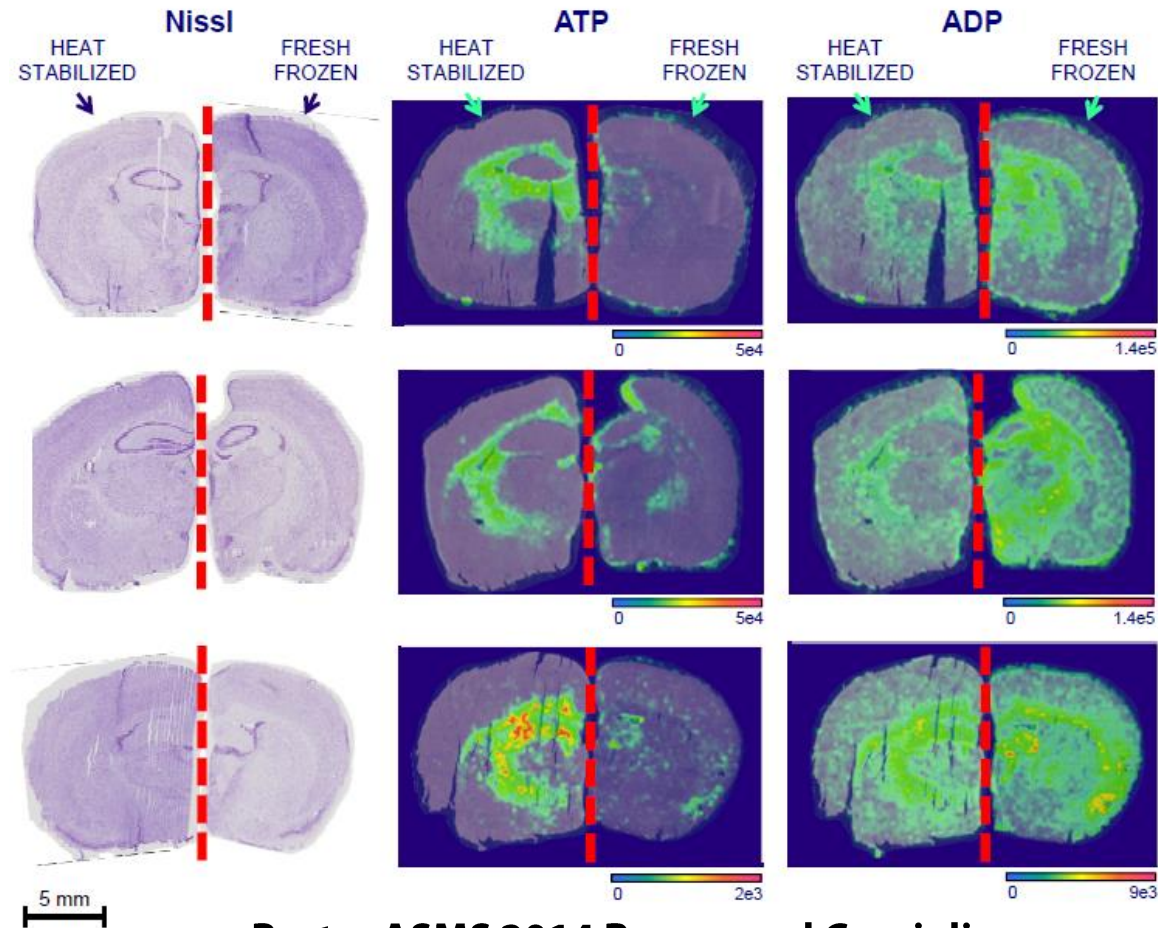
ATP



Snap frozen

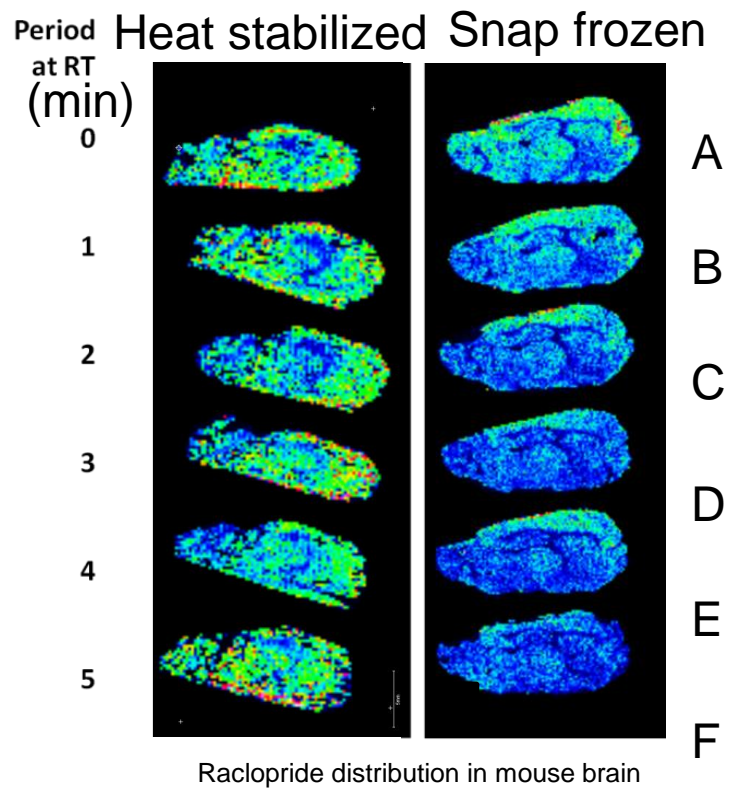


# Improve detection of small molecules: Preservation of ATP



Poster ASMS 2014 Reyzer and Caprioli

# Enables detection of unstable small molecules

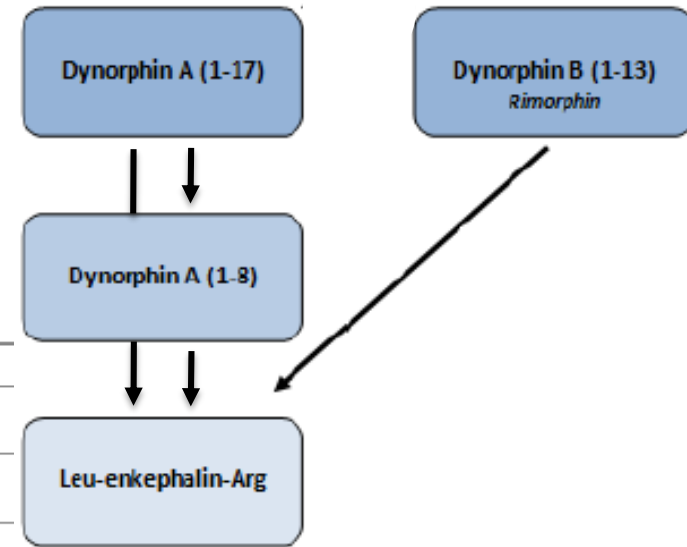
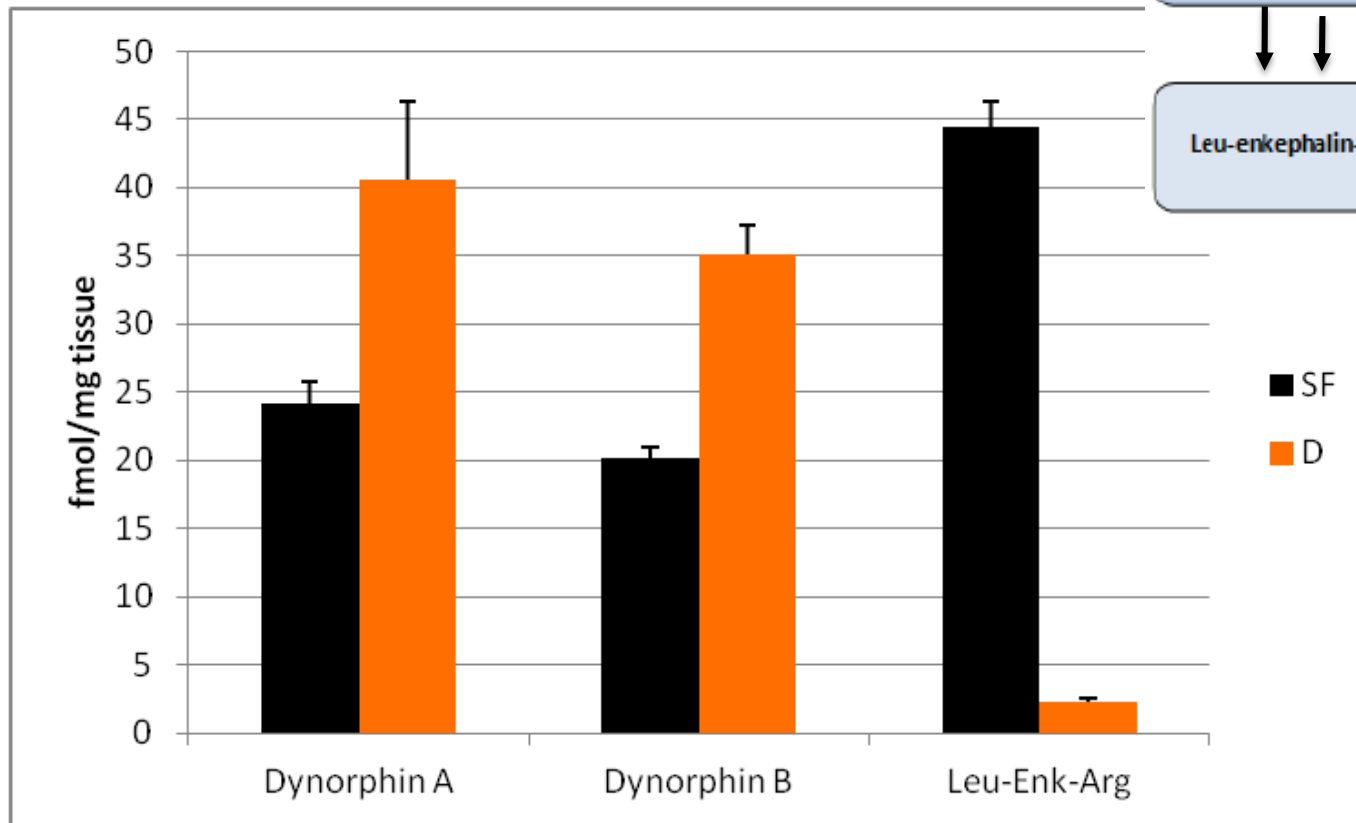


PI, Dr, R.Goodwin, Astra Zeneca, UK



# Studying peptides with RIA

Peptide levels in rat hypothalamus, study of endogenous peptides and their degradation products  
Ingrid Nylander, Sweden



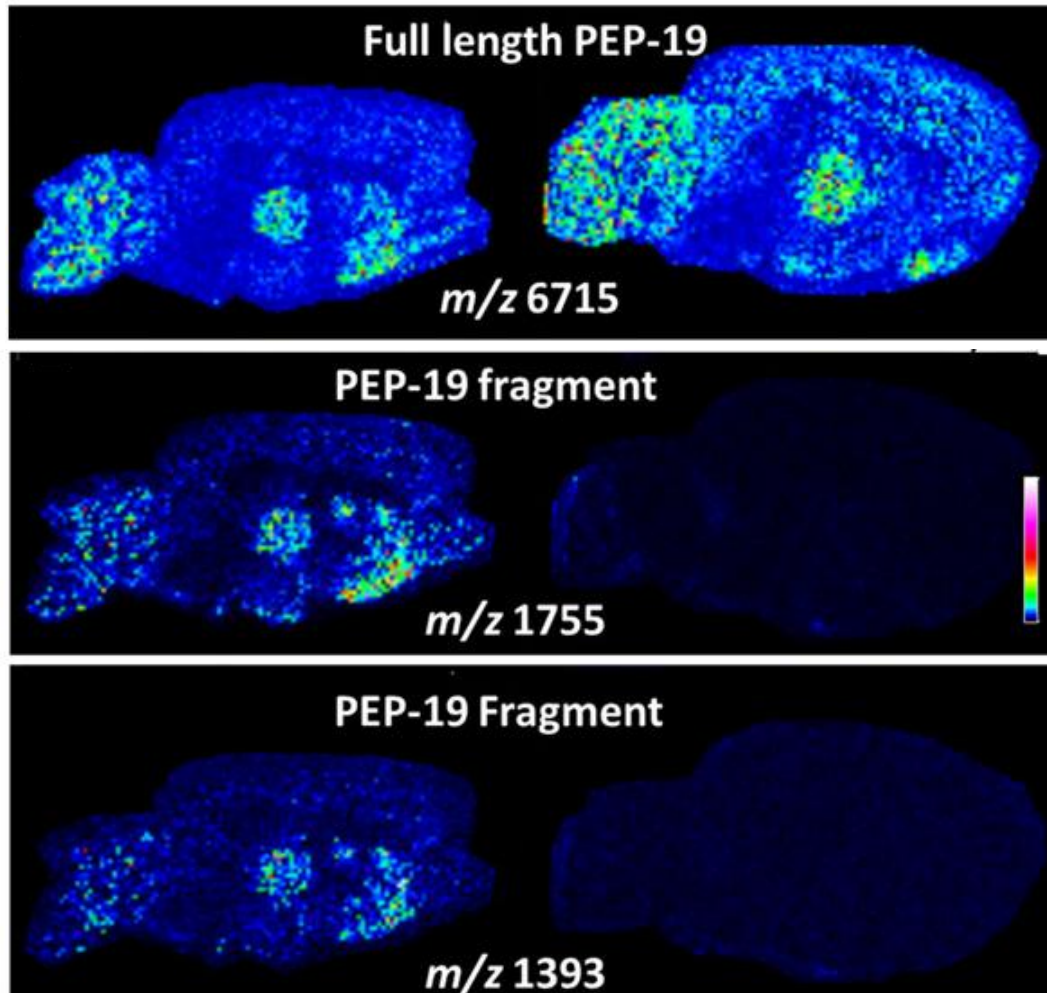
Arrows indicate enzyme driven conversion between peptides

In snap frozen (**SF**) samples, Dynorphins decrease due to continued metabolic conversion post-sampling into Leu-Enkephalin-Arg. This is prevented in heat stabilized samples (**D**) which show levels closer to *in vivo*

# IMS: Stabilizing PEP-19

Snap frozen

Stabilized

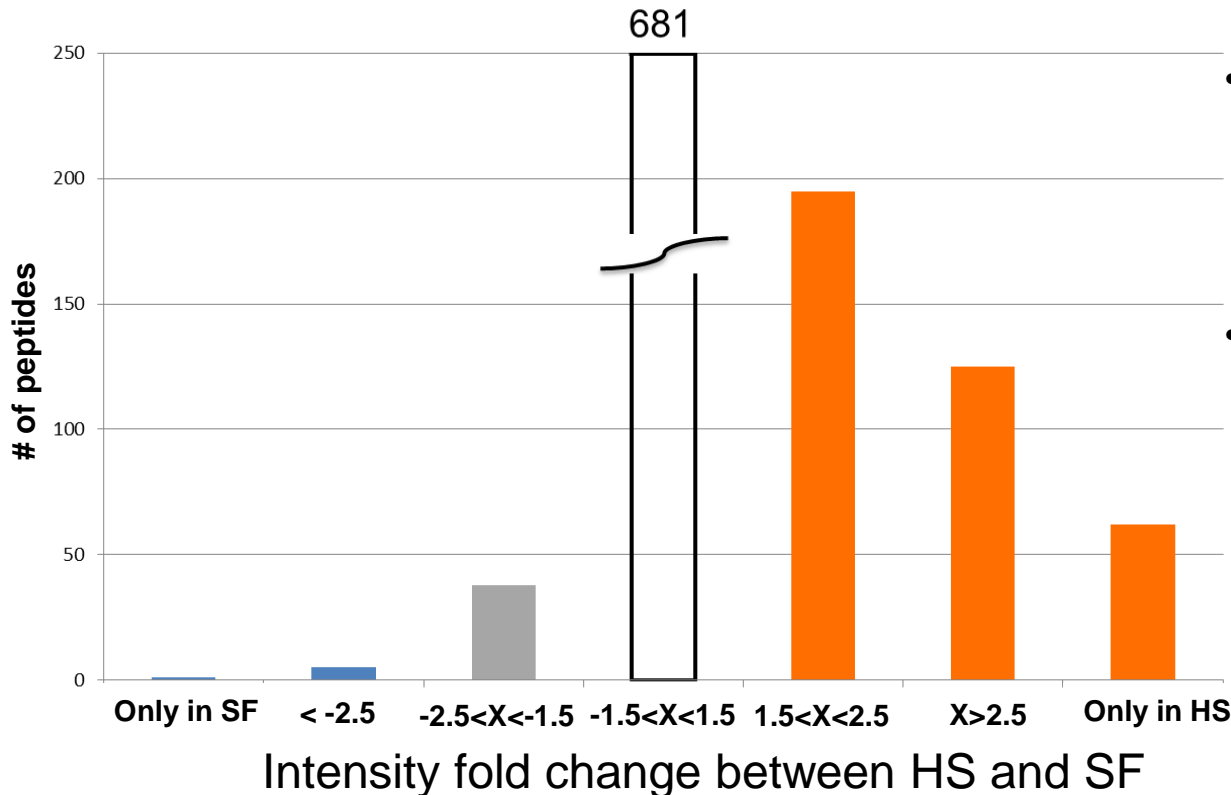


Intact PEP-19 peptide

*Ex vivo* fragment of PEP-19

*Ex vivo* fragment of PEP-19

# Intensity fold change distribution for peptides between HS and SF samples



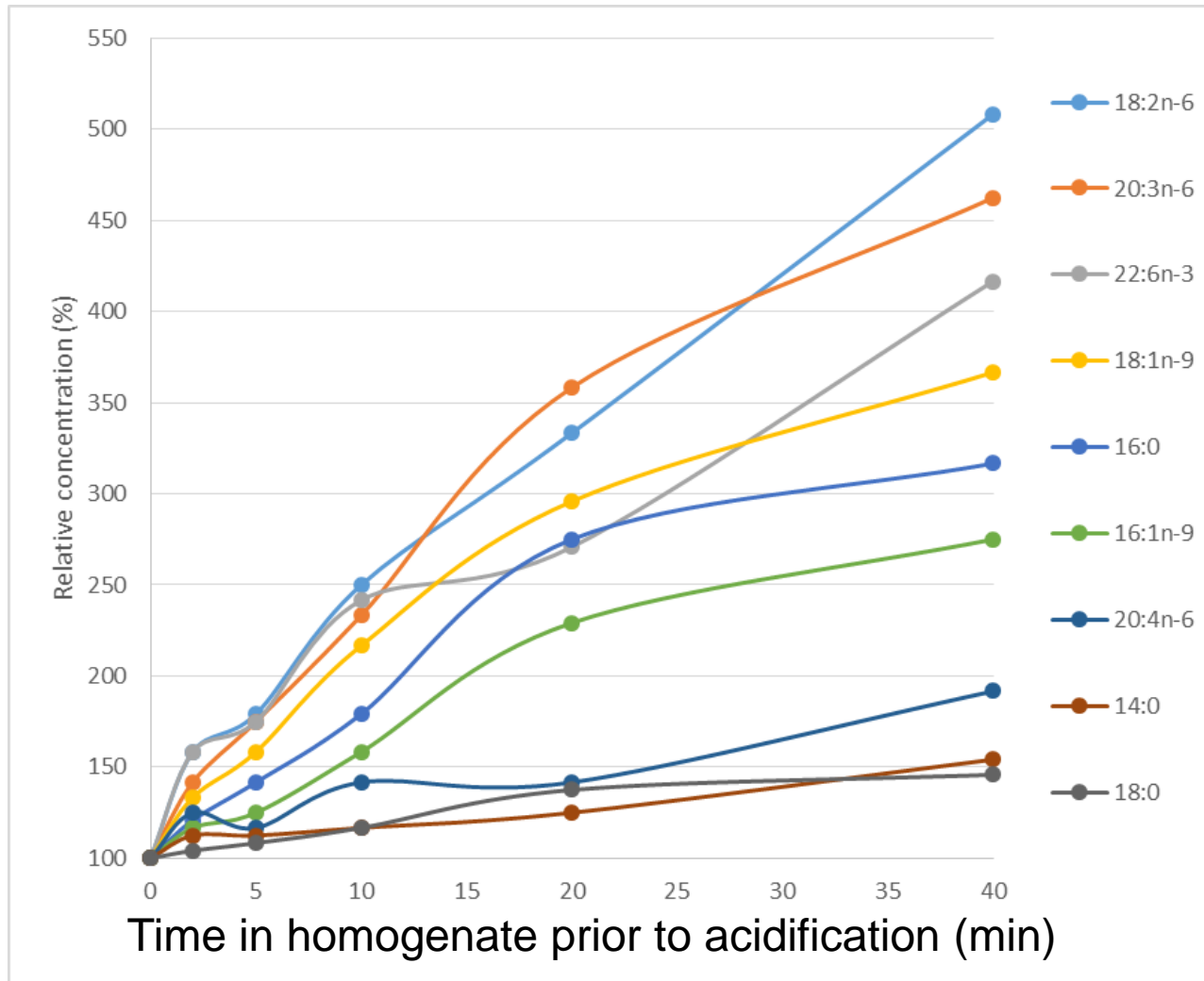
- Stabilizor T1 in the real test bench, Direct comparison with the best practice at Cell Signaling reputed lab.
- Out of 1107 identified Phospho-tyrosine peptides ~35% show higher signal on the stabilized ones in direct comparison with today's best practice.

Peptides with negative fold change in grey (snap frozen) and positive in orange (stabilized)

**More than 35% of the peptides are detected with more than 50% higher intensity in stabilized samples**

In collaboration with Cell Signaling Technologies

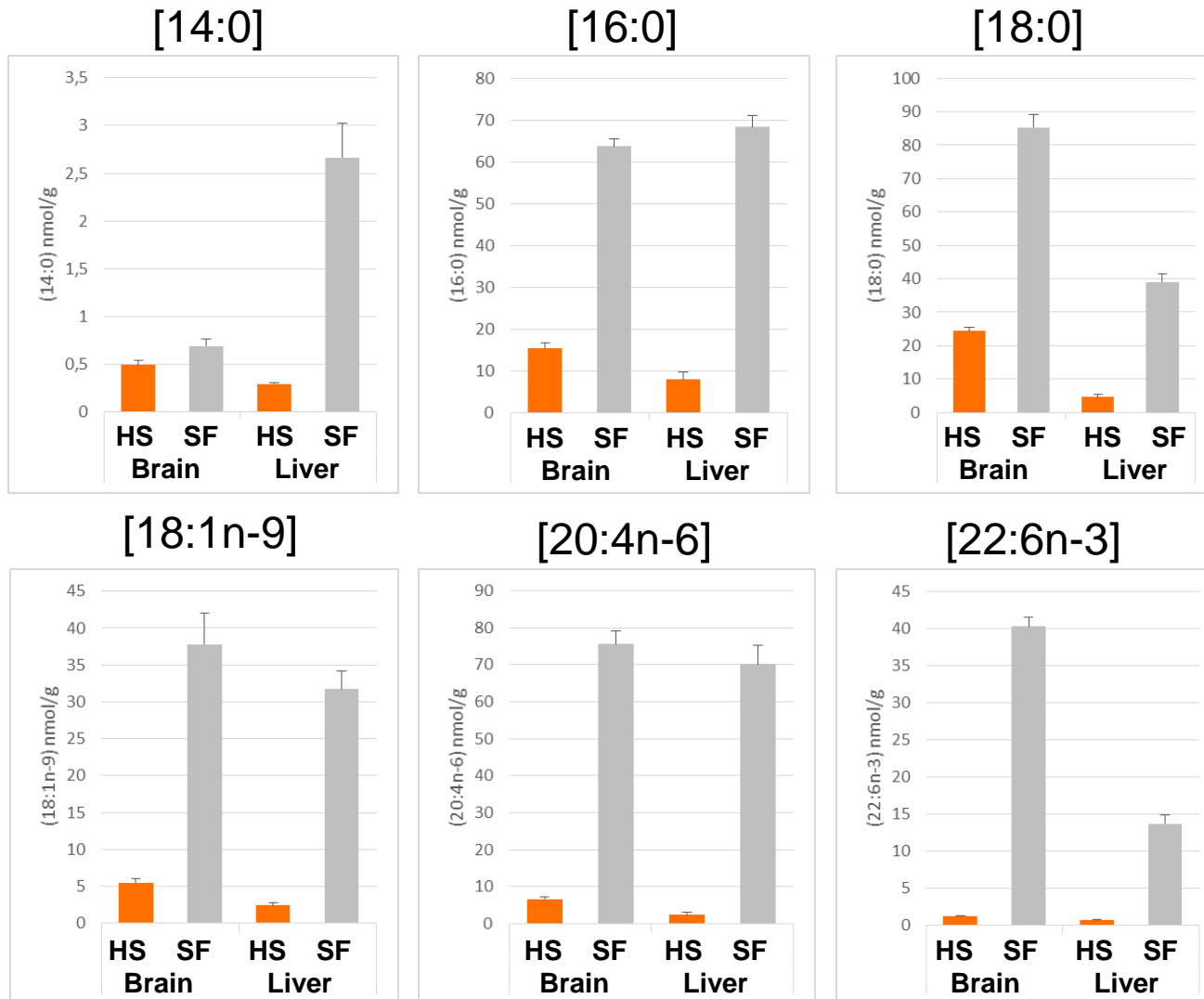
# Levels of FFA increases over time, 0-40 min



Levels of nine different free fatty acids (FFA) in brain homogenate

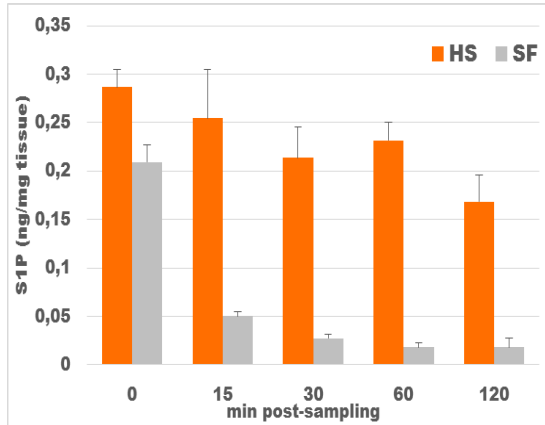
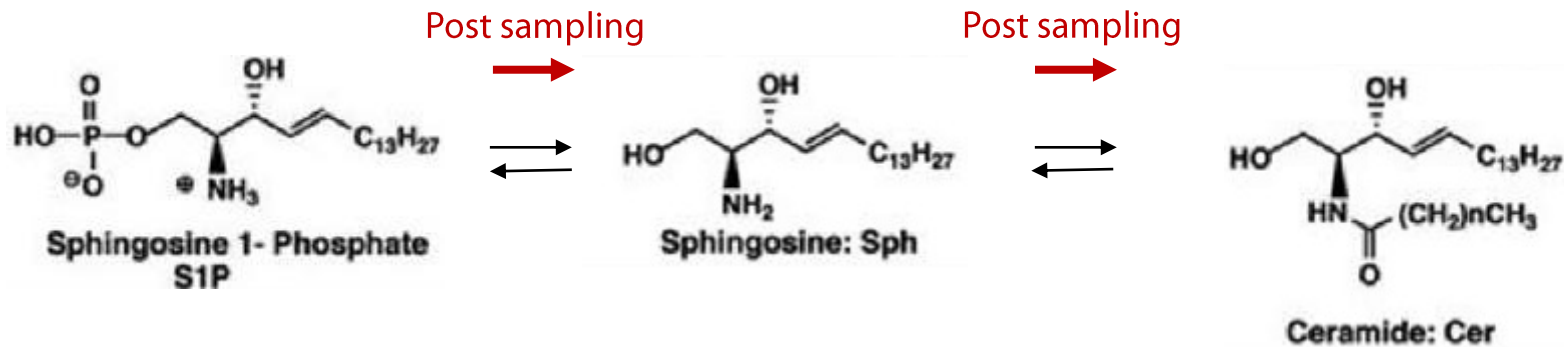
Ref: Jernerén, F., et al., Post-sampling release of free fatty acids—effects of heat stabilization and methods of euthanasia. *Journal of Pharmacological and Toxicological Methods* (2014)

# Levels of FFA, heat stabilized (HS) vs. snap frozen (SF)

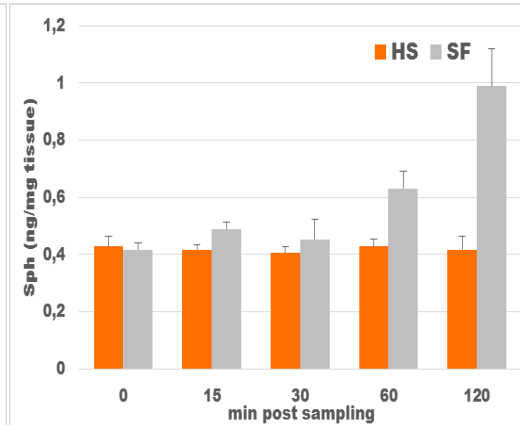


Ref: Jernerén, F., et al., Post-sampling release of free fatty acids—effects of heat stabilization and methods of euthanasia. *Journal of Pharmacological and Toxicological Methods* (2014)

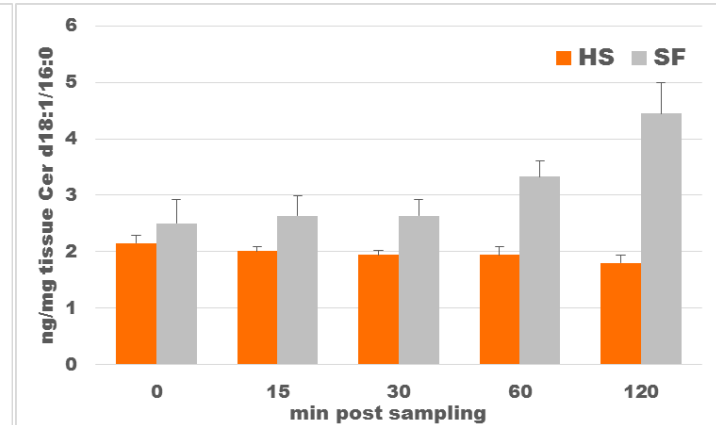
# Sphingolipid regulation, HS vs. SF samples



Phosphorylated Sphingosine (S1p)



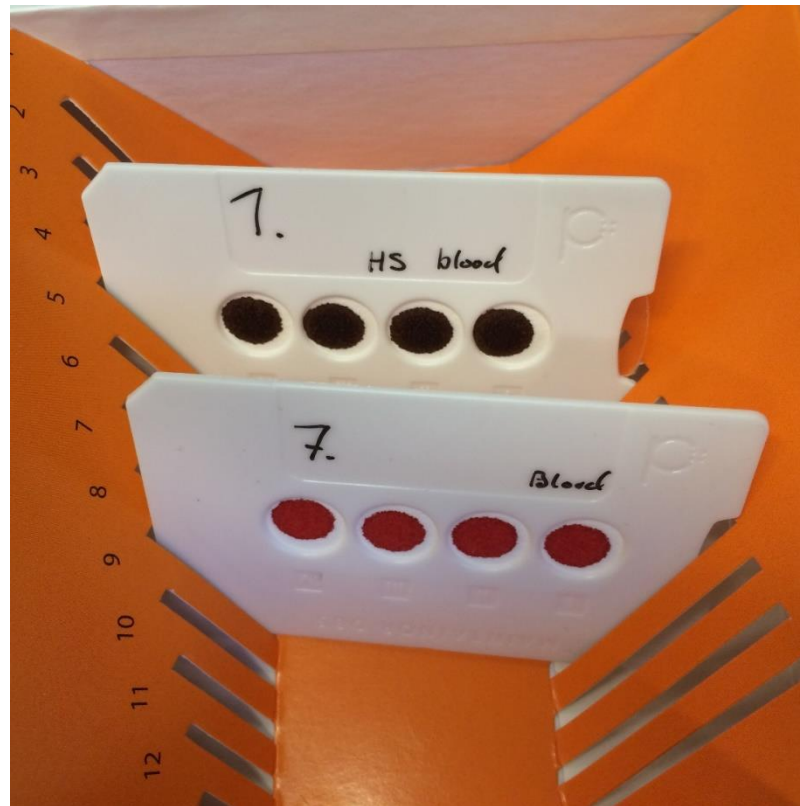
Sphingosine (Sph)



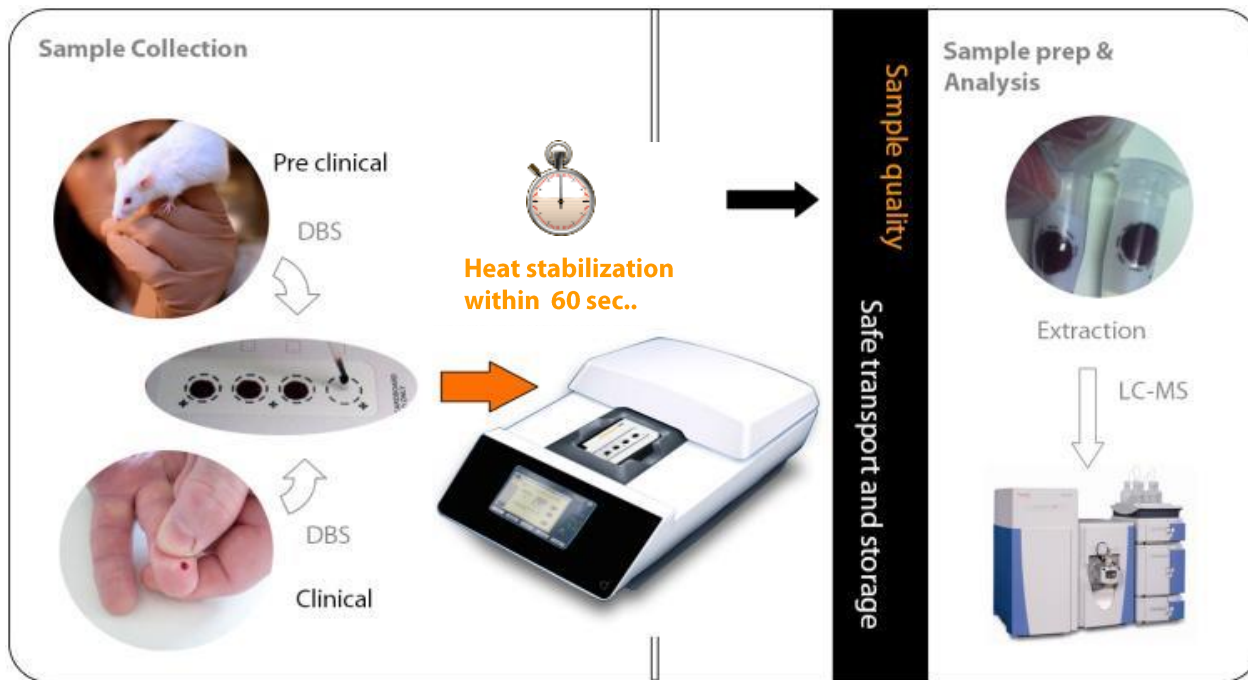
Ceramide d18:1/d16:0

# Maintainor DBS cards

- DBS card with four spots, ~25  $\mu$ l sample
- On going beta testing, one publication (Blessborn et al. 2013)
- Available for purchase/demo for select projects



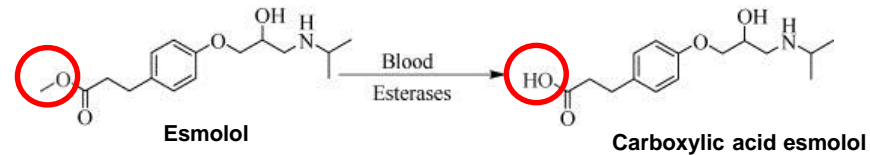
# Whole blood – workflow



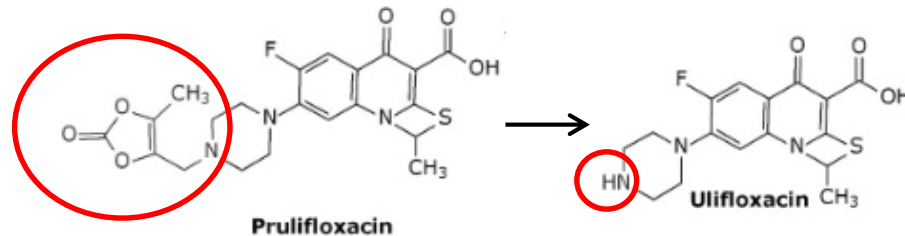


# Esmolol and Prulifloxacin - metabolism

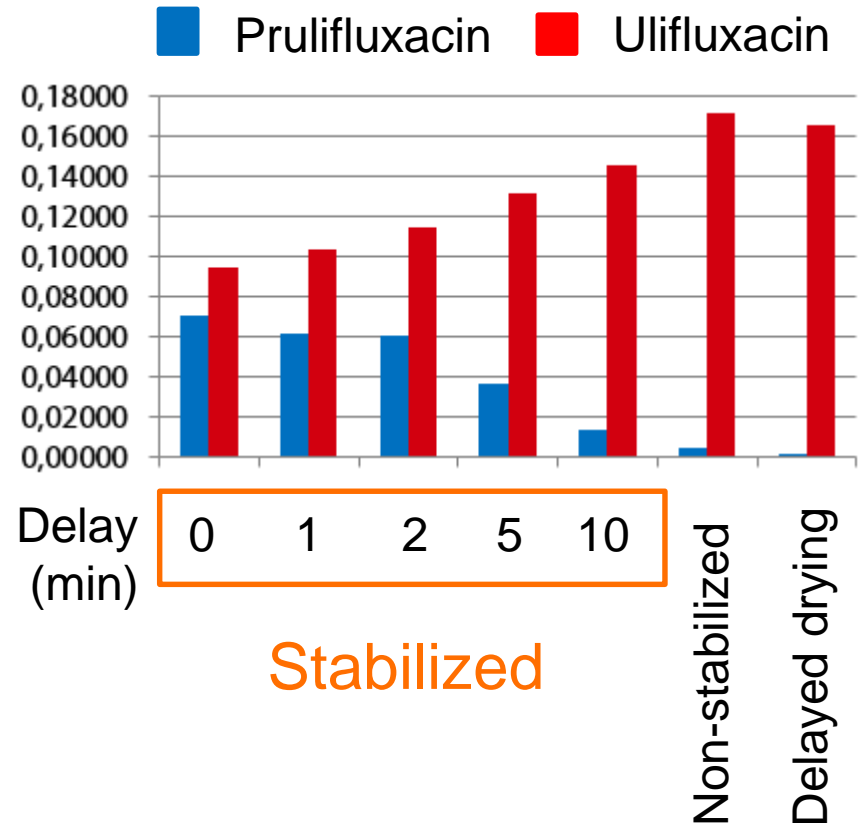
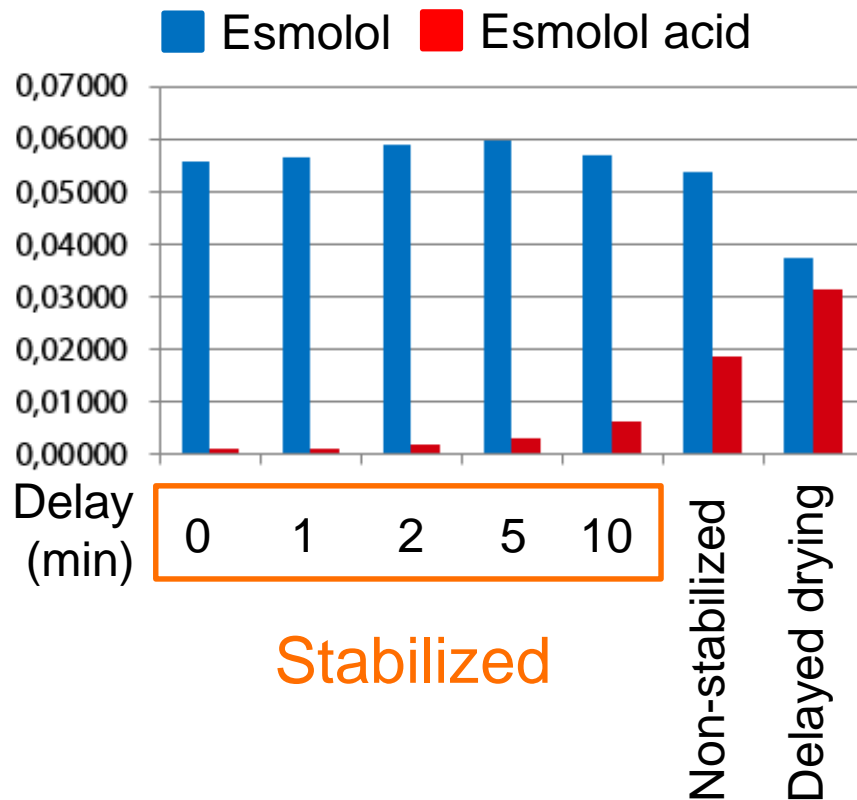
- Rapid hydrolysis in plasma inactivates esmolol and form a carboxylic acid esmolol metabolite



- Prulifloxacin is hydrolysed by esterases in plasma, mainly paraoxonase to form ulifloxacin, the active metabolite



# DBS Blood – spiked with esmolol and prulifluxacin



Quantification of parent compound (blue bars) and degradation product (red bars)  
 With increasing time prior to heat stabilization, the parent compound is degraded  
 on a minute scale. Standard drying gives high levels of degradation.

# Lab Safety and Pathogen Inactivation

Reduced risk when handling infected samples

- Enable removal of samples from BSL 3&4 for MS analysis



Dr. Lisa Cazares, US Army, Fort Detrick, Frederick, Maryland, USA

Ref: Heat fixation inactivates viral and bacterial pathogens and is compatible with downstream MALDI mass spectrometry tissue imaging. Lisa H Cazares et al., BMC Microbiology 2015, 15:101.

- Enable long range NGS and Optical genome mapping of *Mycobacterium tuberculosis* (BSL3 pathogen)  
Center for Disease Control and prevention (CDC), USA



- Reduction of potential pathogens in patient samples  
ISAS, Dortmund, Germany

## Result bacteria & virus inactivation experiment

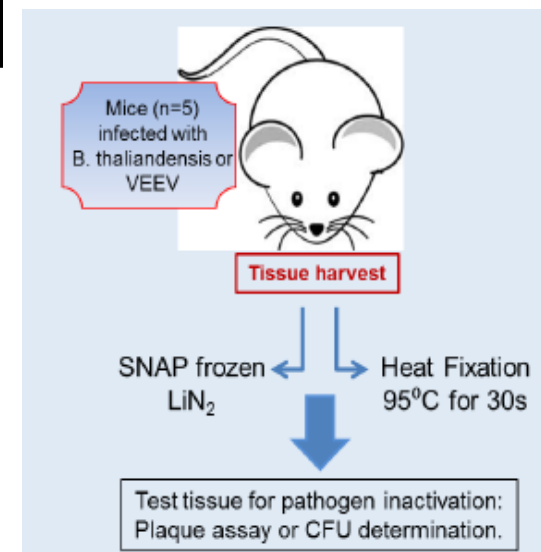
Dr. Lisa Cazares, US Army, Fort Detrick, Frederick, Maryland, USA

Pathogen	Tissue	Treatment	Replicates	Pathogens (u/ml)
VEE (TC83) vaccine strain	brain	SF	2	4E04
		HS	2	0
	spleen	SF	2	0
		HS	2	0
VEE (Trinidad donkey) virulent strain	brain	SF	4	3.1E08
		HS	4	0
	spleen	SF	4	6.3E05
		HS	4	0
<i>B. thailandensis</i>	lung	SF	3	>1E05
		HS	3	0
	spleen	SF	3	7.2E04
		HS	3	0
<i>B. mallei</i>	lung	SF	3	>1E05
		HS	3	0

**Conclusion:** "Heat fixation preserves tissue for proteomic analysis and has the added benefit of inactivating pathogens. This will enable the use of infected tissue from many studies which are performed at bio-safety levels 3 and 4 to be used safely for subsequent proteomic, small molecule drug detection, and imaging mass spectrometry analysis"

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# Users of the Stabilizor system

Over 150 systems installed worldwide (May 2016)

## **Academic labs**

- National Institute of Health (NIH), USA
- Karolinska Institute, Sweden
- Vanderbilt University, USA

## **Clinical Research**

- Childrens Hospital, Phoenix, USA
- M4I, Maastriche, The Neatherlands

## **Pharm companies**

- NovoNordisk, Denmark/USA/Schweiz
- Merck, USA

## **Biobanks**

- Integrated BioBank of Luxembourg
- CHTN, USA

## **Biosafety/Pathogen Inactivation**

- USAMRIID, USA
- CDC, USA

# The importance of sample handling

- Analytes in the sample can change rapidly post sampling leading to loss of *in-vivo* information
- Standardization of sampling and rapid stabilization of molecules of interest is key for high quality biological samples
- Stabilizer system can be a vital part of proper sample handling

ありがとうございます。

Thank you very much!

