

Impact of processing stresses on enzymatic activity of lysozyme

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- **Enzymes** are frequently used in Sequential Batch Reactor to promote the biodegradability of waste activated sludge (WAS)
- Mixed enzymes (**protease:amylase** = 1:3) had great impact on promoting the sludge solubilisation
- Biodegradability is enhanced by enzyme like amylase or protease but **Lysozyme** has proven to be very effective to enhance the solubilisation of particulates
- Lysozyme has more effective ability to disrupt the microbial cell wall composed of peptidoglycans  Activity is measured in U/mg

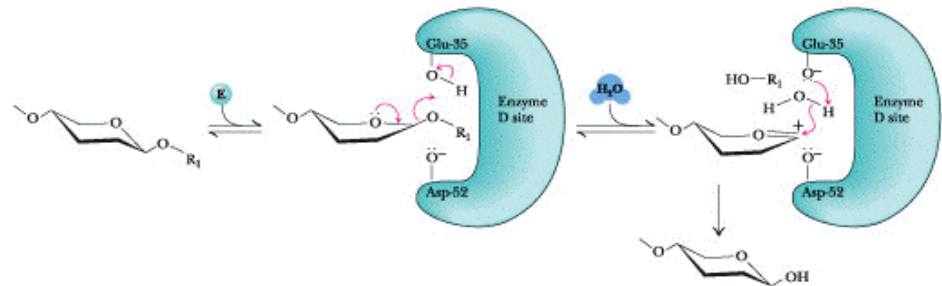
20000 U/mg < Activity (Commercial Lysozyme) < 100000 U/mg

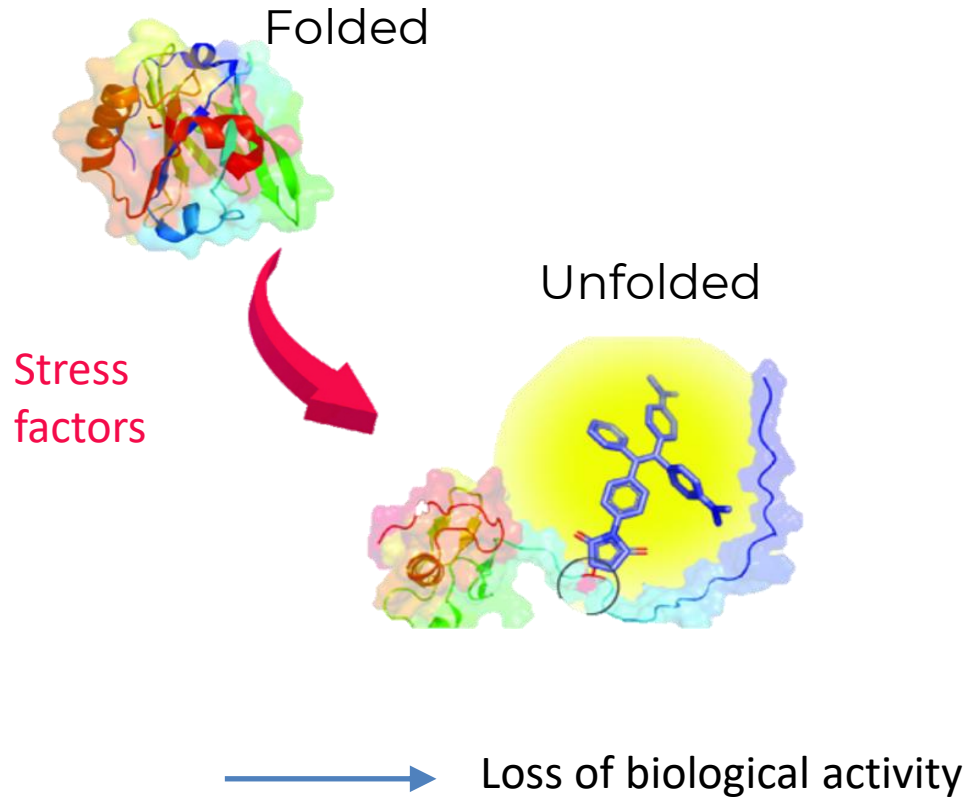
Teo, C. W et al. (2014) *Water Research*, 48(1), 335–344

He, J. G., Xin et al. (2014). *Bioresource Technology*, 170, 108–114



- Globular protein with molar mass of 14.3 kDa and isoelectric point of 11;
- Catalyse the hydrolysis of the $\beta(1-4)$ glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine residues of bacteria wall;
- Antibacterial action dependent of the 3D-structure.





Common stress factors affecting the activity

Heating

Stirring

pH changing

Surface contact

Shear stresses during protein processing and application

① Protein manufacturing

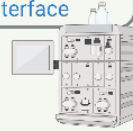


Extraction by precipitation

- pH changing
- Chemical interactions
- Ionic force

Separation by Chromatography

- Solid/liquid interface



Purification by filtration/ultrafiltration

- Membrane adsorption
- Air/liquid interface
- Cavitation
- Pressure
- Shear

② Drug manufacturing

Formulation mixing



- Air/liquid interface
- Cavitation
- Stirring
- Shear

Filtration



- Membrane adsorption
- Air/liquid interface
- Membrane/liquid interface

Sterilization



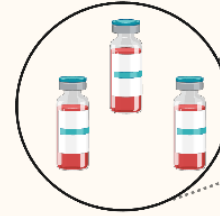
Temperature

Filling



- Pumping
- Surface contact
- Shear
- Cavitation

Shelf-life



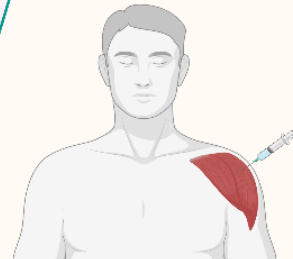
Transport
Air/liquid interface
Surface contact
Stirring



Storage
Temperature
Surface contact



Clinical use
Surface contact
Adsorption
Shear



Objectives

The main goal of the study is to evaluate the effect of stresses encountered in industrial plant on enzymes to avoid loss of material or decrease of activity.

→ Optimisation of production and storage to reduce waste.

→ Avoid loss of biological activity

Thermal

Ultrasonic

Chemical

Ultrafiltration

Experimental



- Turbidity of *Micrococcus Lysodeikticus* against lysozyme solutions;
- Measures done at 450 nm using a microplate reader;
- Absorbance decreasing is proportional to LSZ activity.

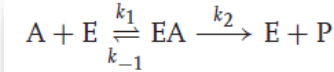
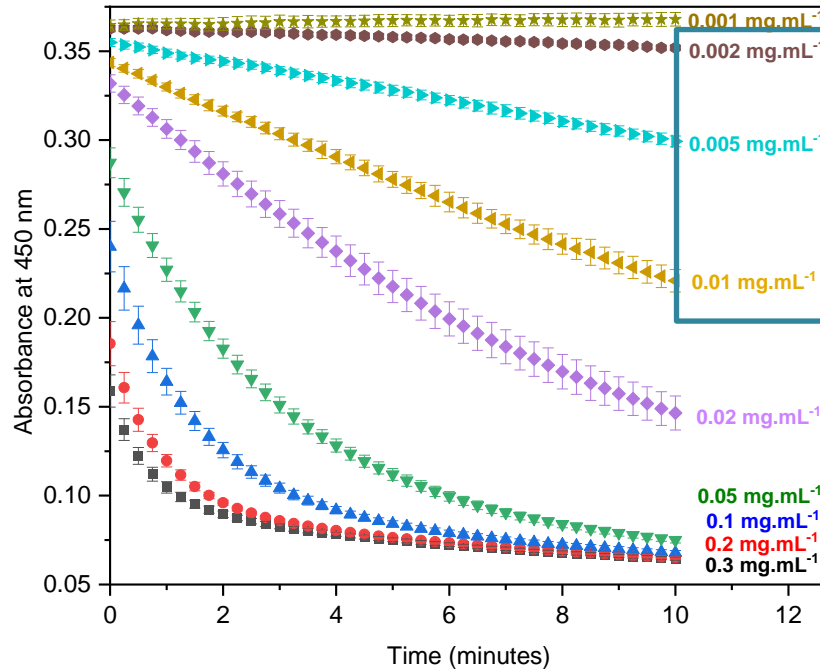
200μL of *ML* 0.3 mg/mL
solution in PBS 10 mM
+
20μL of LSZ solution in
water varying concentration



Incubation temperature: 30°C
Time of measure: 10 minutes
Intervals: 15 seconds
 λ : 450 nm
5 replicates in 3 different days



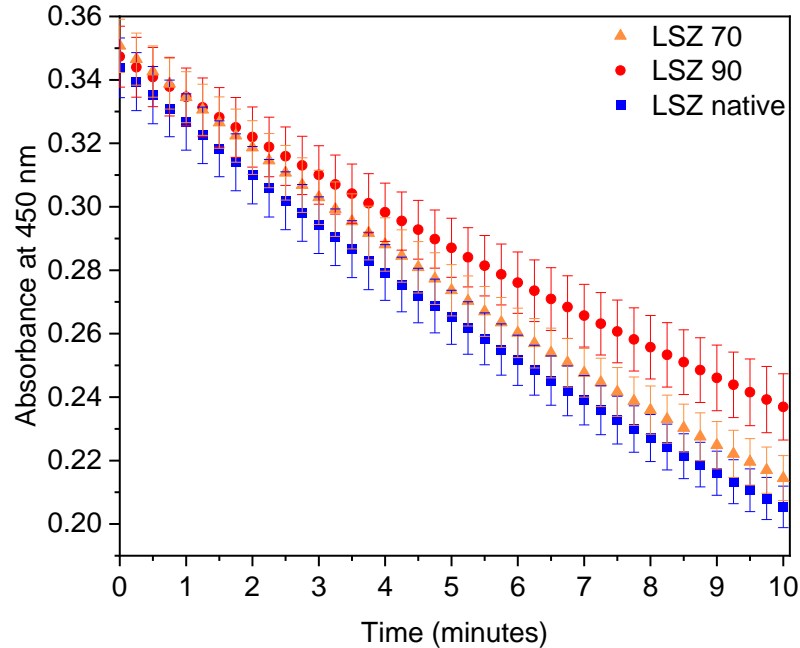
Experimental



- It is not a second order reaction because enzyme can participate more than once in the reaction
- Substrate conversion depends on enzyme amount
- Ideal condition: zero order reaction (linear during test period) when $[E] \ll [A]$.

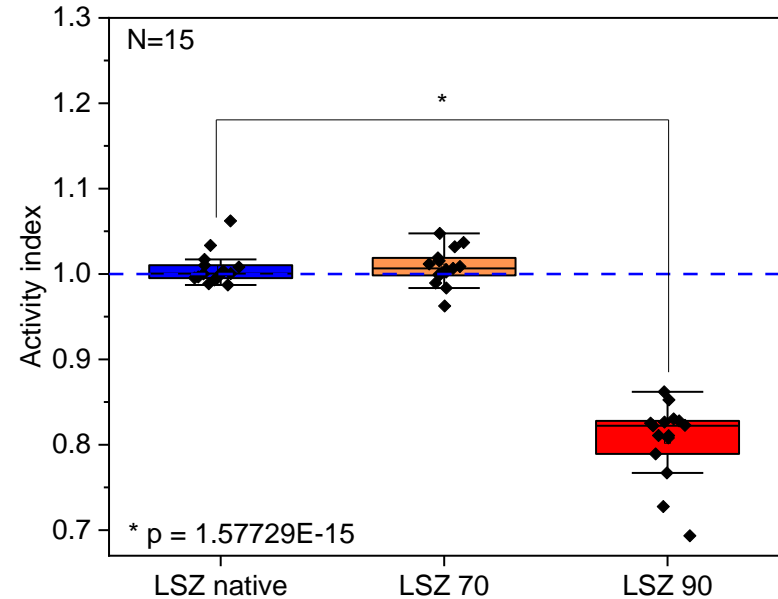
Thermal treatment

Antibacterial activity of LSZ after thermal treatment



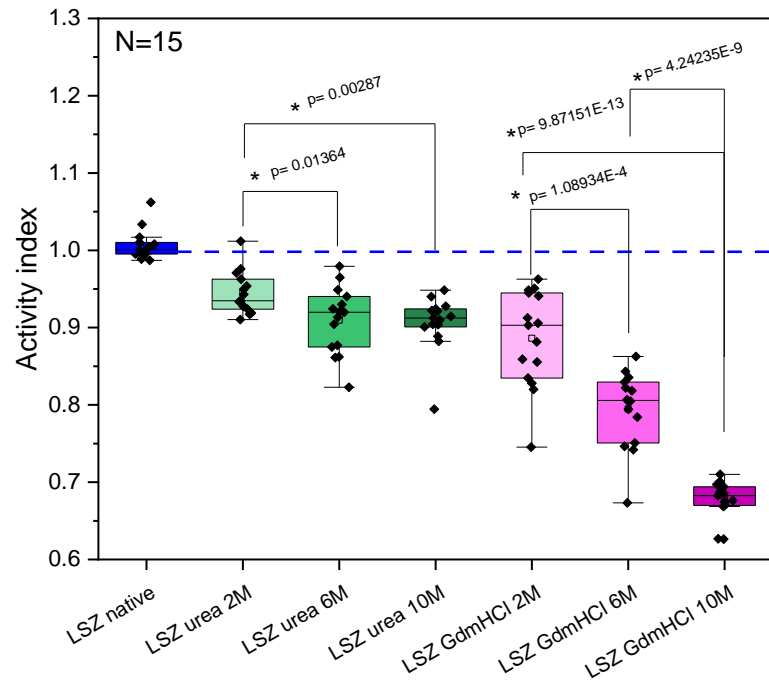
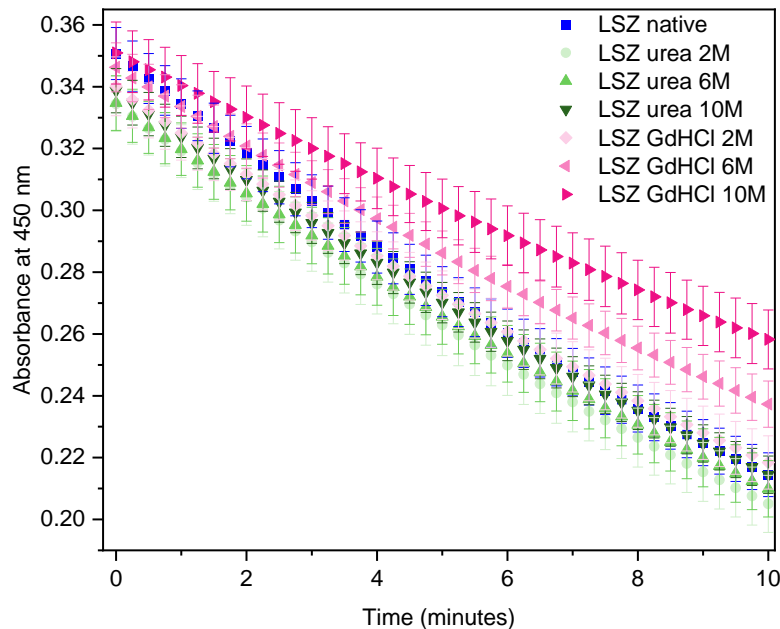
$$A_u = \frac{|slope|.1000}{m_{LZS}}$$

$$I_{Au} = \frac{A_u}{A_{u_{reference}}}$$



→ Temperature of denaturation around 78°C

Chemical denaturation



De Espindola, A., Dutournié, P., Ponche, A. (2023).

Impact of industrial stress factors on lysozyme enzyme: Role of denaturation processes and initial protein activity.

Sustainable Chemistry and Pharmacy, 31, 100964.

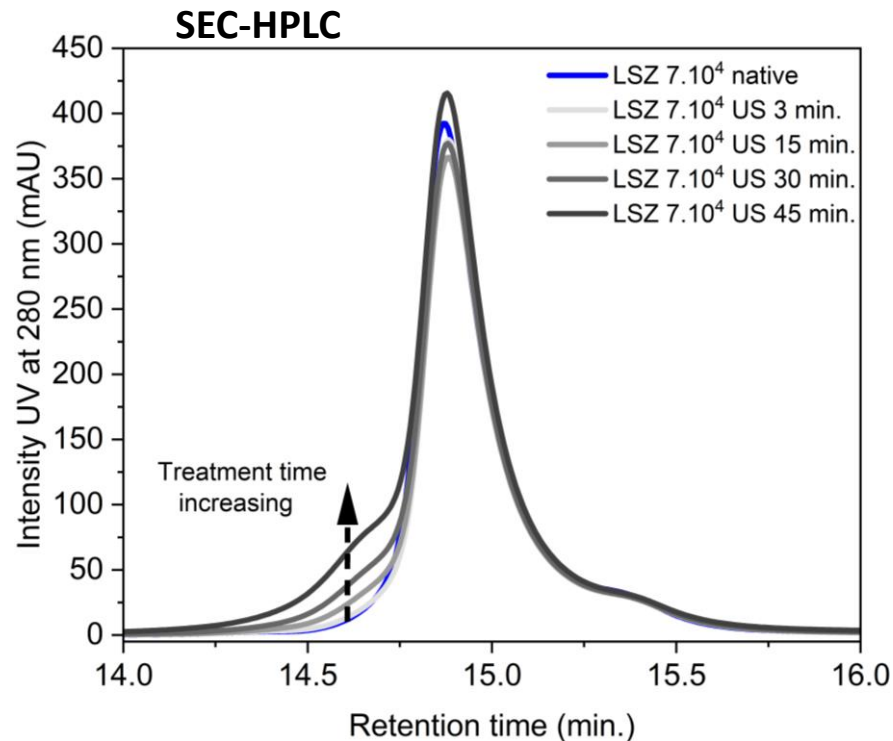
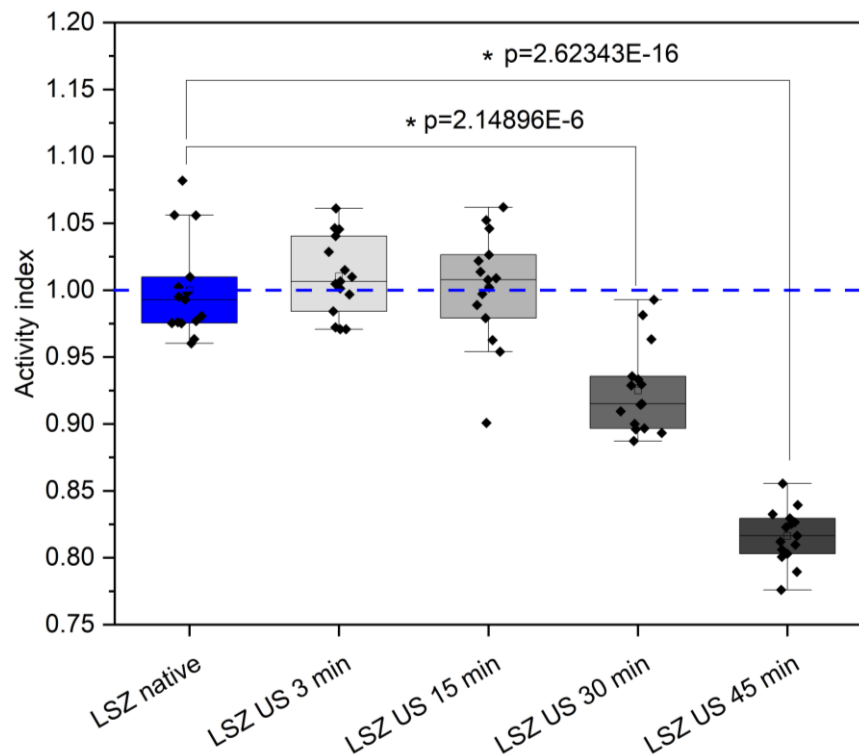
Ultrasonic shaking

Sonics Vibra cell VCX 500

Amplitude 60%

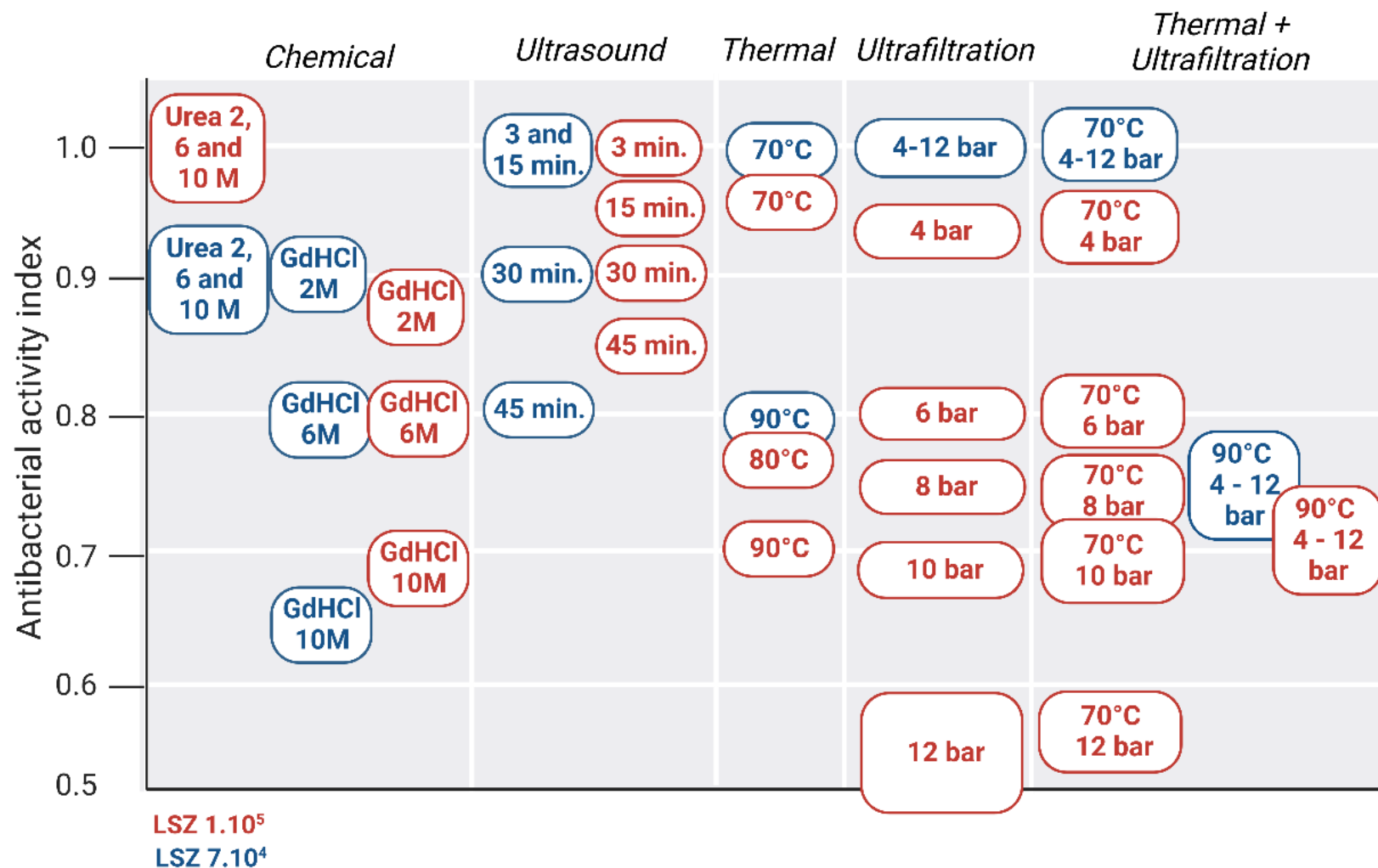
8000J/3min

150mL LSZ solution



Creation of soluble oligomers in the solution

Summary

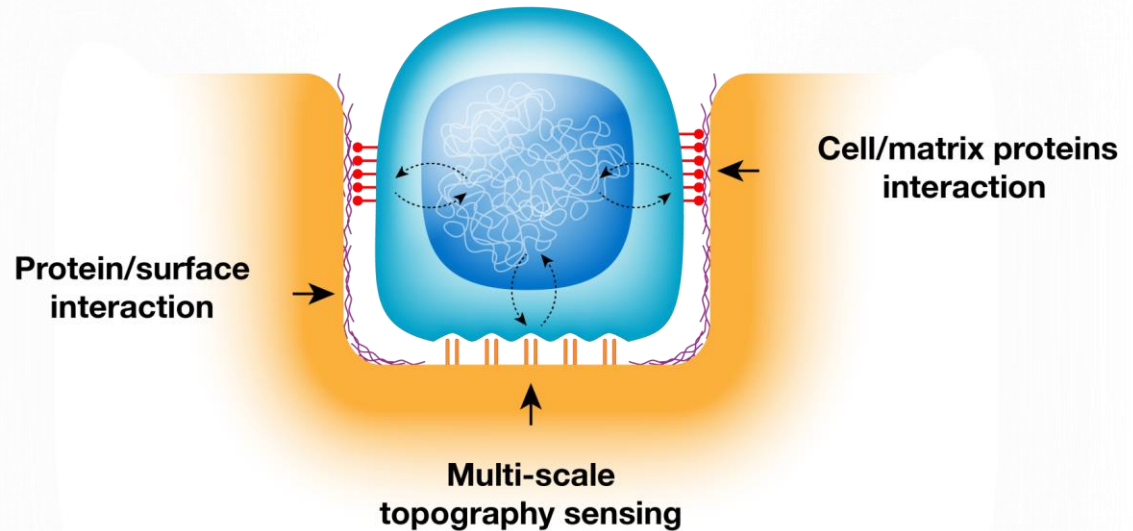


Thanks for your attention



Biointerface group
biointerfaces.wordpress.com

Fundamental questions & **Applied** research



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