Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente, Ciclo XXXVIII

Studying fibrinogen activation into fibrin: structure and formation dynamics

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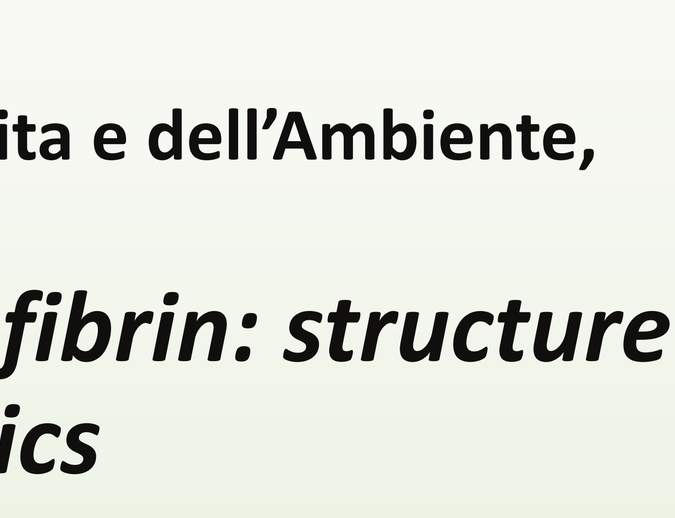
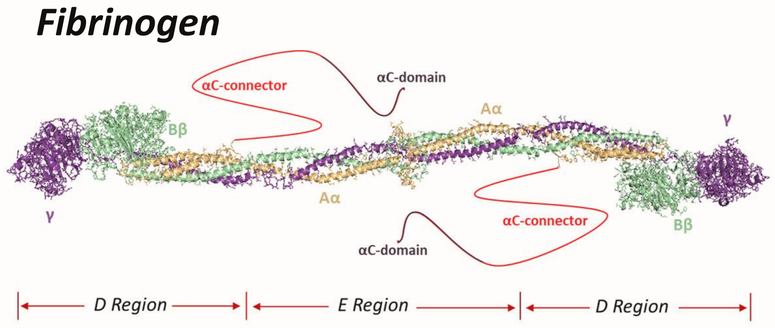
Background

The conversion of fibrinogen into insoluble fibrin and the formation of a stable clot are determinants in wound healing, tissue regeneration, and mediation of inflammatory responses that help the immune system fight invading pathogens [1]. However, clinical evidence points to fibrin(ogen) to contribute to pathological processes [1, 2]. These contributions may result from altered plasma concentrations, modified structural properties, or the impact of polymorphisms on clot permeability, stiffness, and resistance to lysis. Studying how environmental factors influence the properties of fibrin clots, particularly those mimicking chronic cardiovascular diseases (CVD), could help identify individuals at higher risk of thrombotic events and faster CVD progression [3]. Therefore, our research aims to investigate the detailed structure and mechanism by which thrombin triggers fibrinogen's self-assembly under different pathological associated conditions. Understanding how these aggregates form and the influence of their structural properties can provide valuable insights into the molecular processes involved and could ultimately lead to the development of new therapies.



What are we currently studying

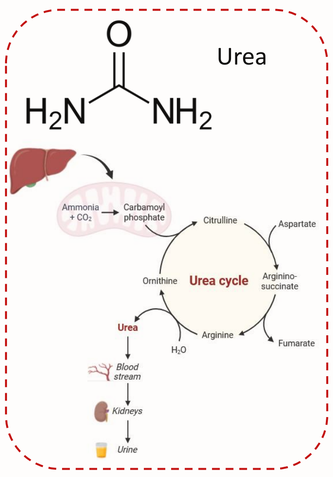
Fibrinogen



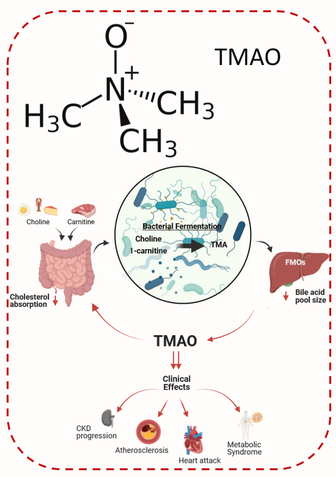
PDB ID: 3GHG

- Glycoprotein Synthesized in the liver
- Molecular Weight: 340 KDa
- Composed of three polipeptides chains (Aα, Bβ and γ) and three main regions: D-E-D
- When activated by Thrombin forms the fibrin clot

Pathogenic associated metabolites



- Metabolic waste produced by the liver and excreted by the kidneys
- Used as a biomarker to assess renal function on a regular basis
- Protein denaturant
- Recently linked to cardiovascular disease (CVD) mortality

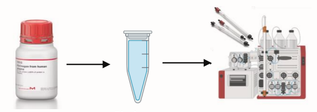


- Recently discovered to be present in humans
- Act as an osmolyte, shielding cellular proteins from the denaturing effects of urea and other perturbing osmolytes in some marine organisms, where it serves to protect them from osmotic stress, low temperatures, and high hydrostatic pressure
- Metabolite produced by gut microbiota
- Recently linked to cardiovascular disease (CVD) mortality

Current Results

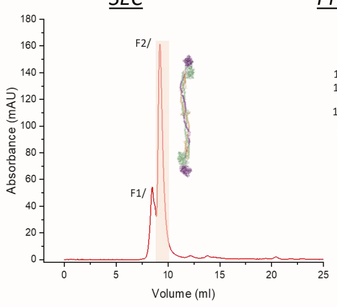
1 *Protein purification*
Size Exclusion Chromatography (SEC)

- ***Setup***

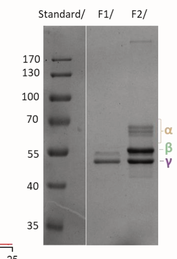


Human Fbrinogen — *Fbrinogen stock (Tris Buffer)* — *ÄKTA Pure Protein Purification System, Cytiva. Column: Superdex 200 Increase 10/300 GL*

SEC



Protein Electrophoresis



F1/. Fraction containing protein aggregates

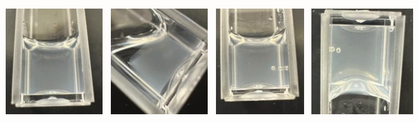
F2. Fraction containing the fibrinogen protein

2 *Dynamic Light Scattering (DLS)*

- *DLS measurements were performed to study the aggregation kinetics of fibrinogen under different conditions. Concentrations of TMAO and Urea range from the physiological to the pathological concentrations.*

Conditions:

- Buffer BTC pH 7.6
- NaCl - 150 mM
- Fibrinogen – 1 μM
- Thrombin – 1 nM
- TMAO – 15, 30, 60 μM
- Urea – 7, 20 mM



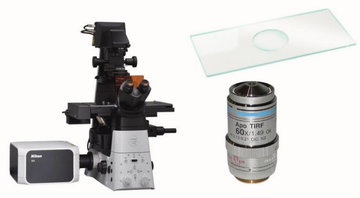
Fibrin gel after DLS measurements

3 *Confocal Microscopy*

- *Confocal Microscopy measurements were performed to study the structure of the fibrin clot under the different conditions.*

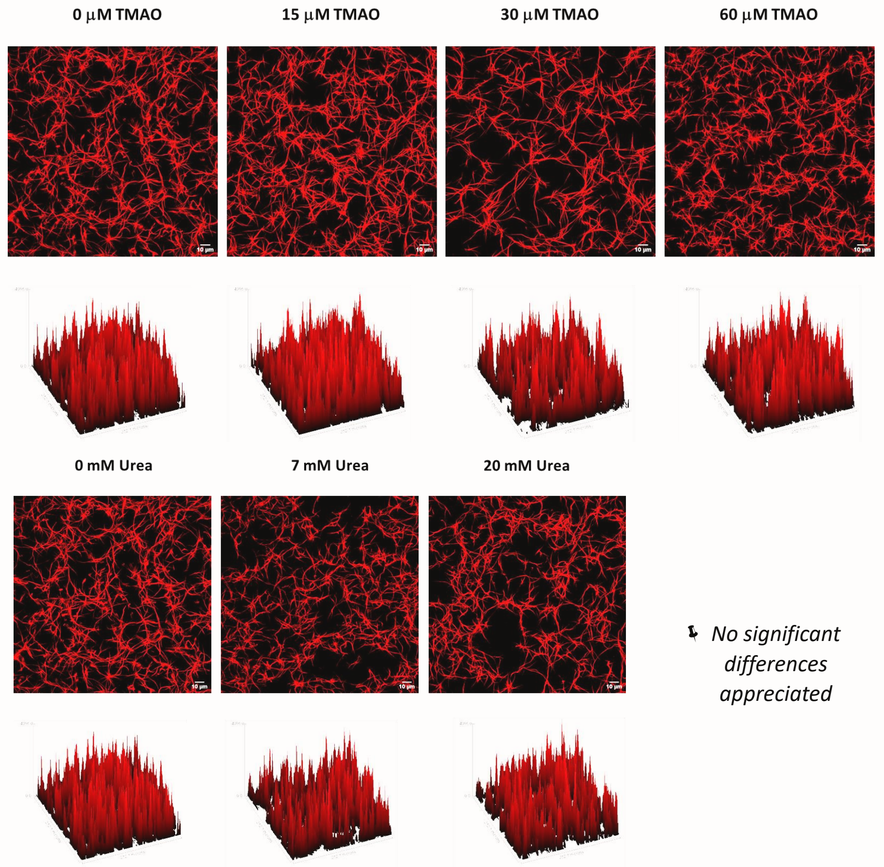
Conditions:

- Buffer BTC pH 7.6
- NaCl - 150 mM
- Fibrinogen – 1 μM
- Thrombin – 1 nM
- TMAO – 15, 30, 60 μM
- Urea – 7, 20 mM
- 1% v/v Fibrinogen Alexa Fluor 546



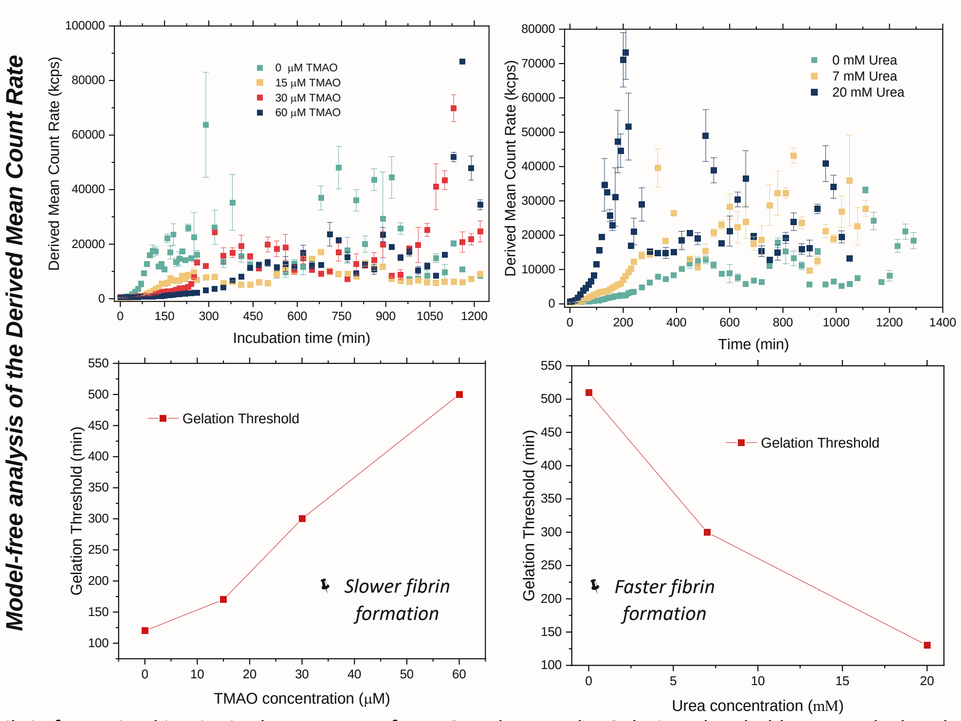
Clots were allowed to form on the concave glass slides at room temperature before measurements.

Images are a maximum Intensity projection of a Z-stack of 50 μm



- *No significant differences appreciated*

Structure of Fibrin Clot in the presence of TMAO and Urea. The images were taken at the final point of the Clot formation.



Fibrin formation kinetics in the presence of TMAO and Urea. The Gelation Thresholds were calculated from the derived mean count rate analyisis.

Take home message

- Both TMAO and Urea affected the kinetics of fibrin formation. While TMAO slows down fibrin clot formation, urea accelerates it. This may serve to raise the hypothesis that TMAO probably acts by counteracting the effect of urea, as has been shown to occur in marine organisms.
- Although TMAO and Urea affect the kinetics of fibrin formation, it seems they don't affect the final structure of the clot, as seen by confocal microscopy.
- Further studies are needed to understand better the mechanisms behind these phenomena.

References

1. *Gaule, T.G. and R.A. Ajjan Fibrin(ogen) as a Therapeutic Target: Opportunities and Challenges. International Journal of Molecular Sciences, 2021.* ***22****, DOI: 10.3390/ijms22136916.*
2. *Vilar, R., et al., Fibrin(ogen) in human disease: both friend and foe. Haematologica, 2020.* ***105****(2): p. 284-296.*
3. *Zqbczyk, M., R.A.S. Ariëns, and A. Undas, Fibrin clot properties in cardiovascular disease: from basic mechanisms to clinical practice. Cardiovasc Res, 2023.* ***119****(1): p. 94-111.*