HMB-003, a long-acting peptide plasmin inhibitor, potently inhibits fibrinolysis and localizes with fibrin in a human whole blood microfluidic flow model

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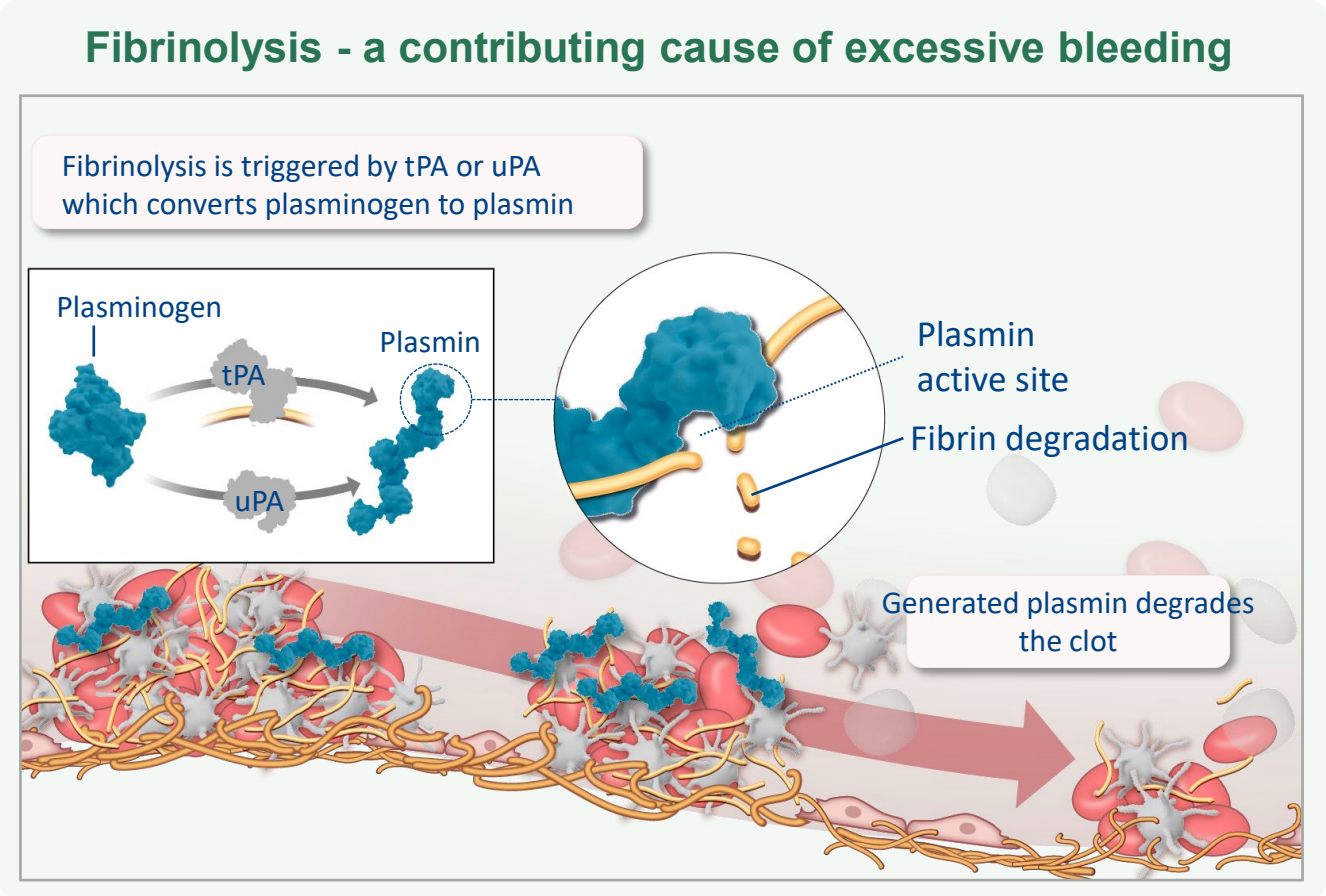
In compliance with COI policy, ISTH requires the following disclosures to the session audience

Shareholder	No relevant conflicts of interest to declare
Grant / Research Support	Research Support from Hemab Therapeutics paid to institution.
Consultant	No relevant conflicts of interest to declare
Employee	No relevant conflicts of interest to declare
Paid Instructor	No relevant conflicts of interest to declare
Speaker bureau	No relevant conflicts of interest to declare
Patents / other	No relevant conflicts of interest to declare

Presentation includes discussion of the following off-label use of a drug or medical device: N/A

Fibrinolysis and Mechanism-of-Action of HMB-003



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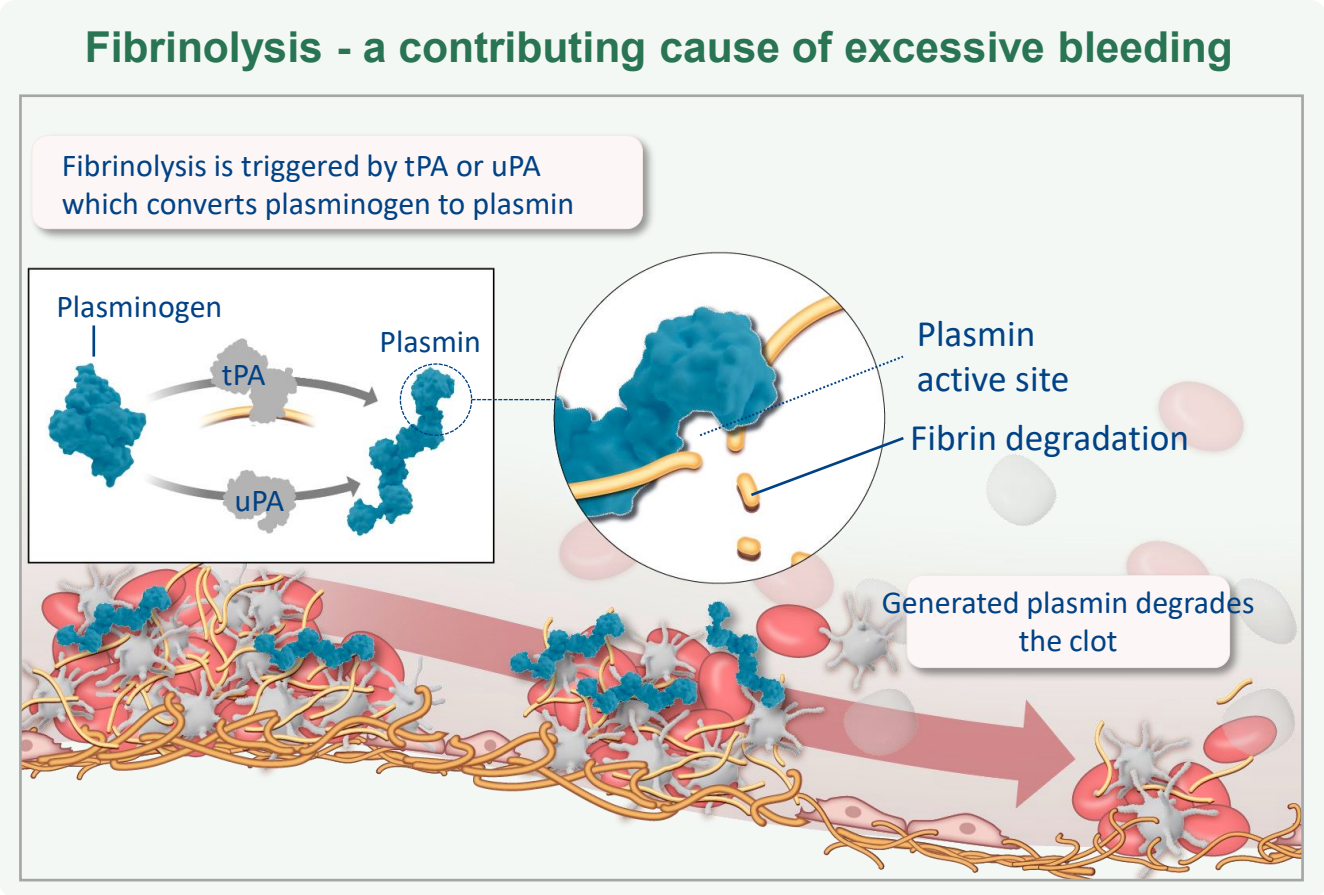

Imbalance of coagulation and fibrinolysis → excessive bleeding → range of bleeding orders, such as **heavy menstrual bleeding**

Current therapies

- TXA and hormonal therapy

Limitations

- Short half-life of TXA
- Dissatisfaction with hormonal therapy

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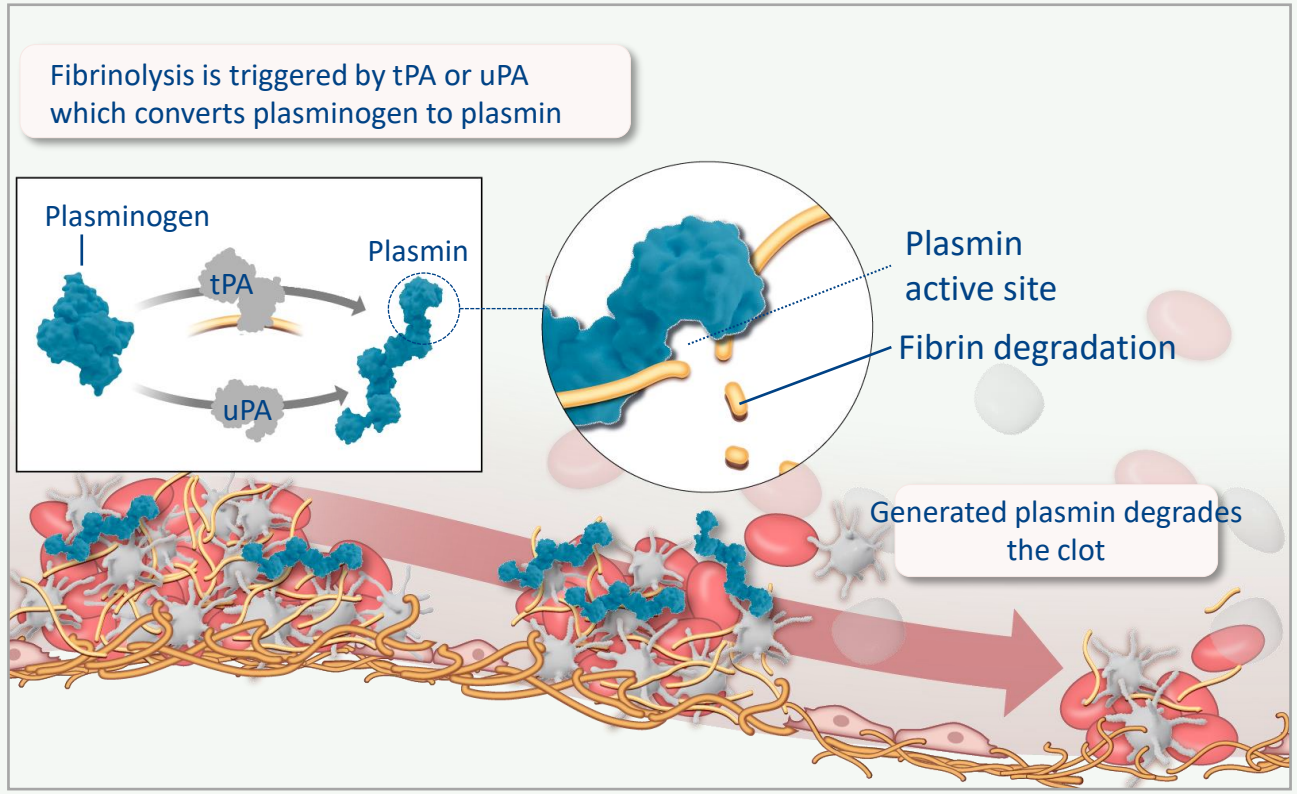
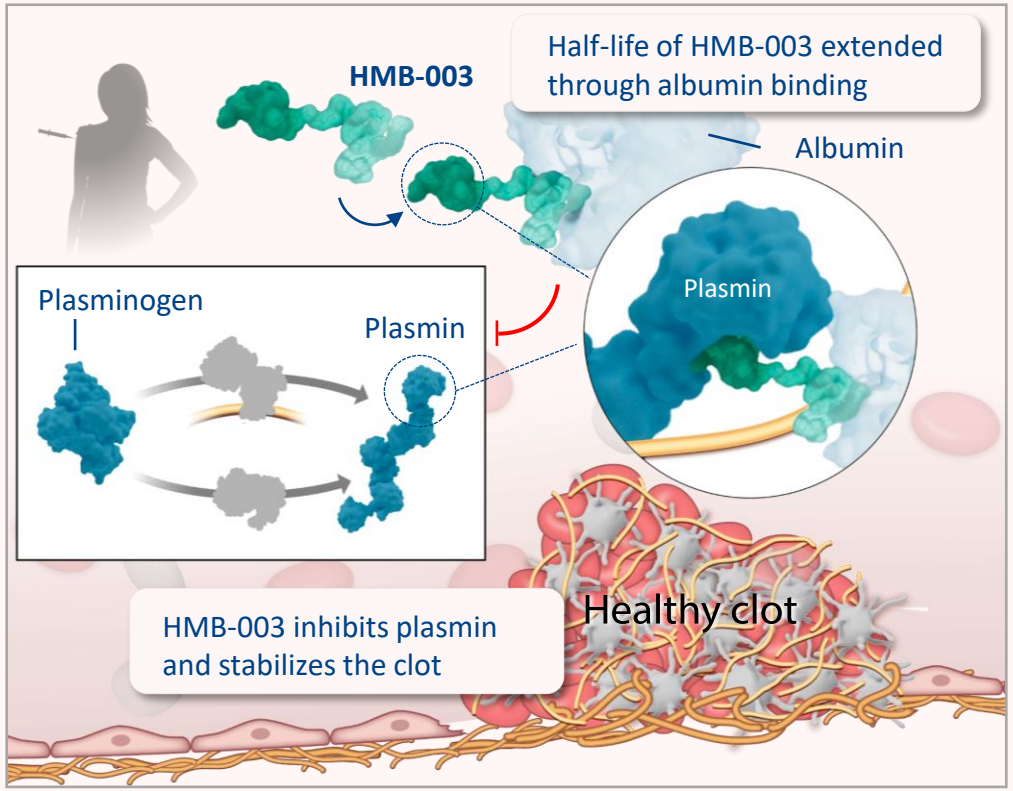
Fibrinolysis and Mechanism-of-Action of HMB-003

Fibrinolysis - a contributing cause of excessive bleeding



Inhibition of fibrinolysis by HMB-003



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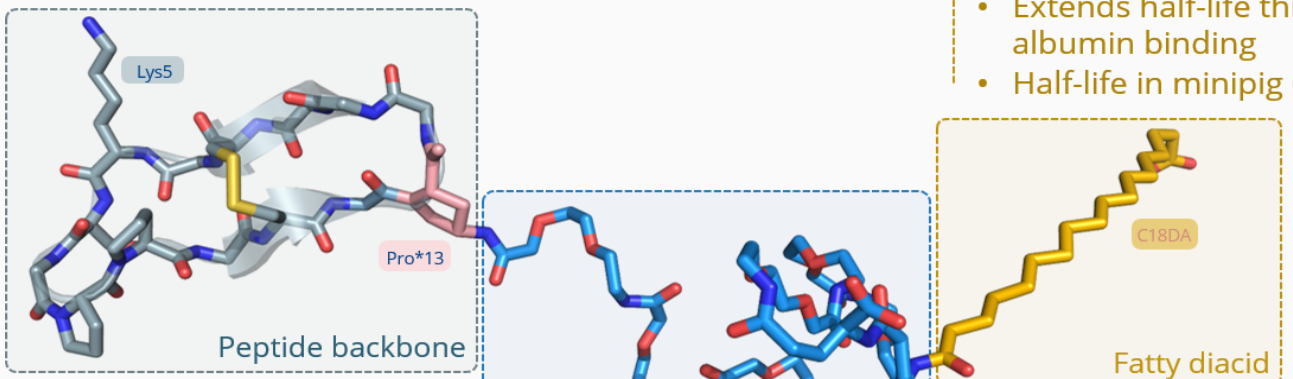
HMB-003 is a fatty acid-conjugated peptide-based plasmin inhibitor

HMB-003 was designed as a long-acting antifibrinolytic

Peptide-based plasmin inhibitor
- Active site inhibitor
- Potent and selective plasmin inhibition (IC_{50} = 2.5 µM[1])

C18 fatty acid
- Extends half-life through albumin binding
- Half-life in minipig 6 days[1]



Flexible linker
- Allowing for simultaneous binding of albumin and plasmin

[1] ISTH 2026. Urbanus, Rolf: A Long-Acting Peptide Plasmin Inhibitor with Extended Antifibrinolytic Activity Enabling Once-per-Cycle Subcutaneous Treatment for Heavy Menstrual Bleeding: Preclinical Development of HMB-003.

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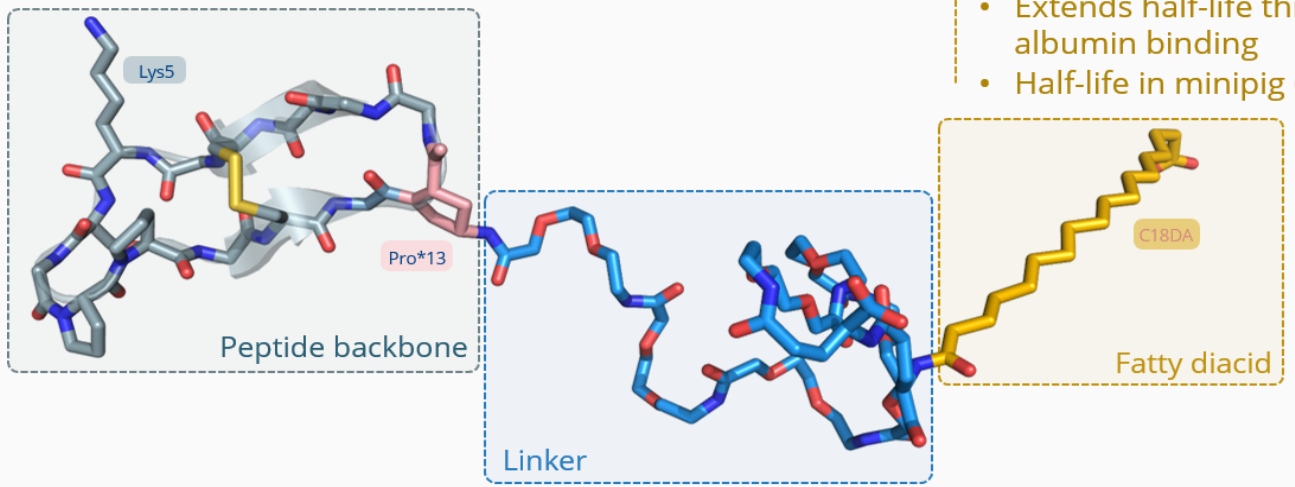
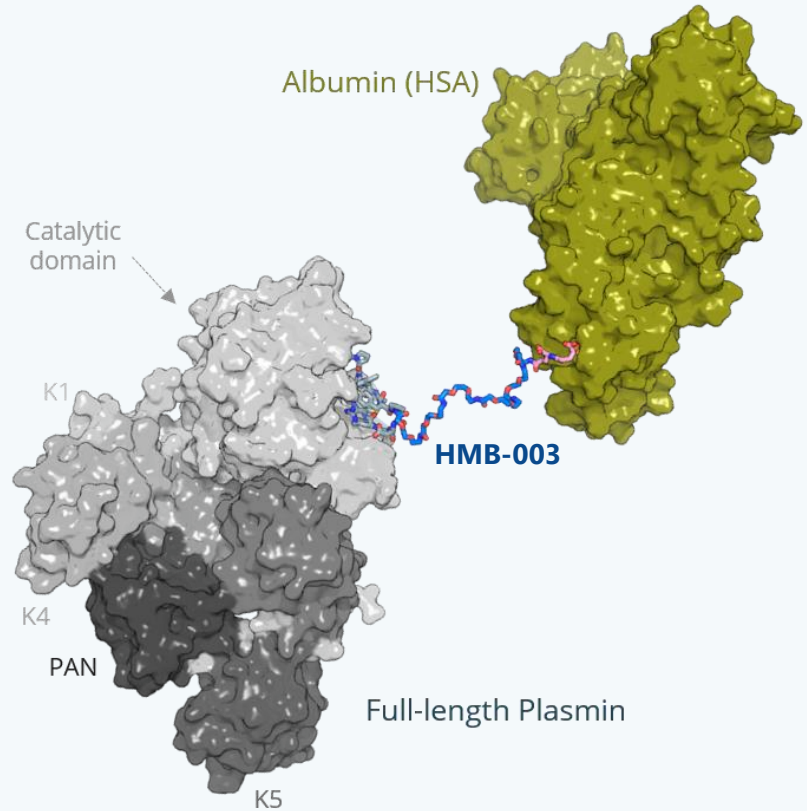


Flexible linker
- Allowing for simultaneous binding of albumin and plasmin

X-ray structure and molecular model confirms active site binding and ternary complex



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Objectives

1. Investigate the potency of HMB-003 in a physiological relevant model recapitulating key elements of the hemostatic system

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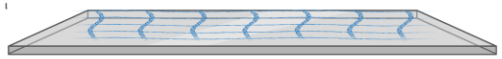
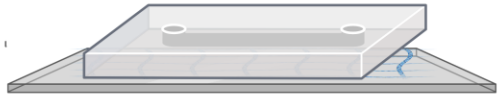
Whole blood microfluid flow model

1 Coat coverslip with TF+collagen



2 Apply PDMS flow chamber on coverslip

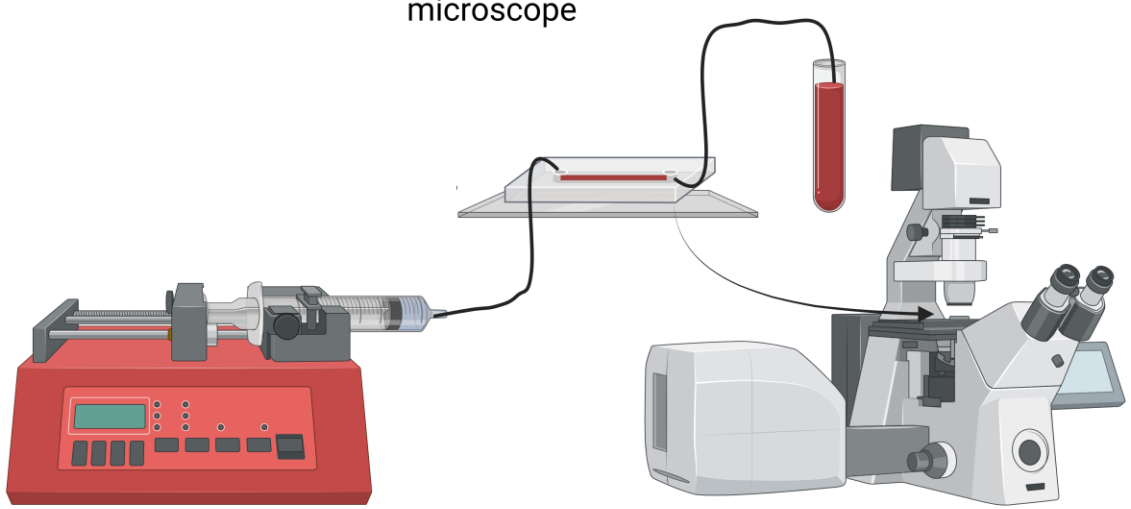


3 Prepare blood

30 nM tPA
anti-fibrin-AlexaFluor488
MitoTracker Orange
+/- (i)HMB-003
+/- TXA

$MgCl_2$ + $CaCl_2$

4 Flow blood over TF+collagen surface and measure fluorescence with fluorescence microscope



300 s^{-1}

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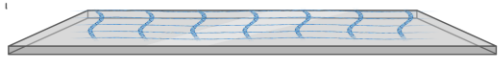

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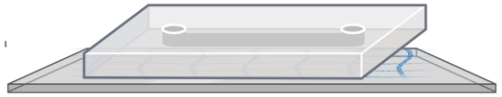

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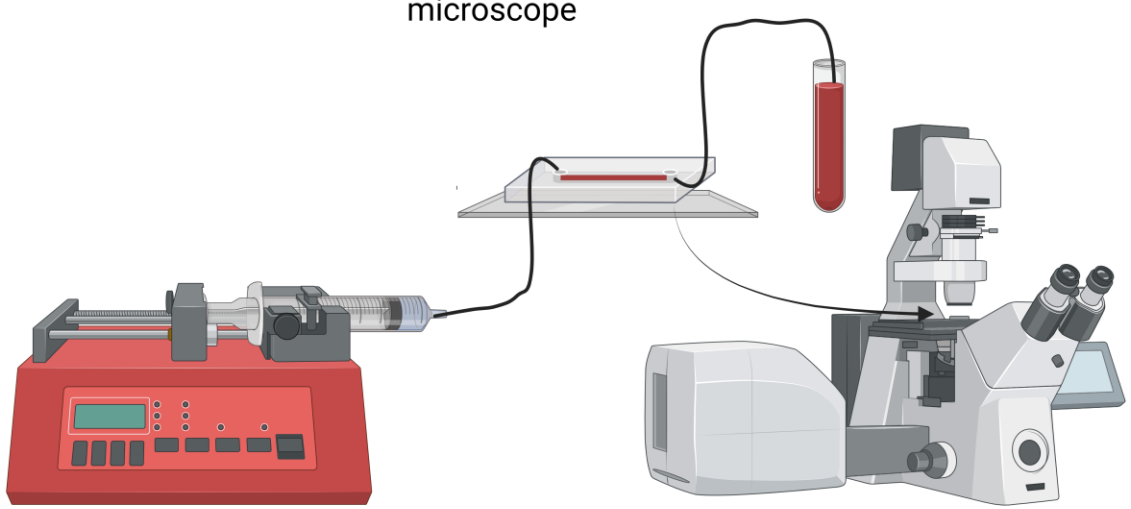

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Key elements of hemostasis:

- Collagen: platelet capture (*primary hemostasis*)
- Tissue factor (TF): coagulation initiation (*secondary hemostasis*)
- tPA: fibrinolysis

Whole blood microfluid flow model

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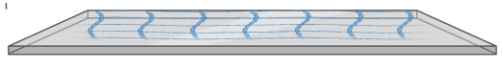

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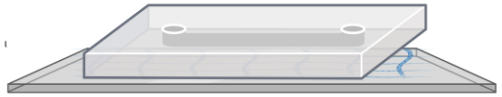

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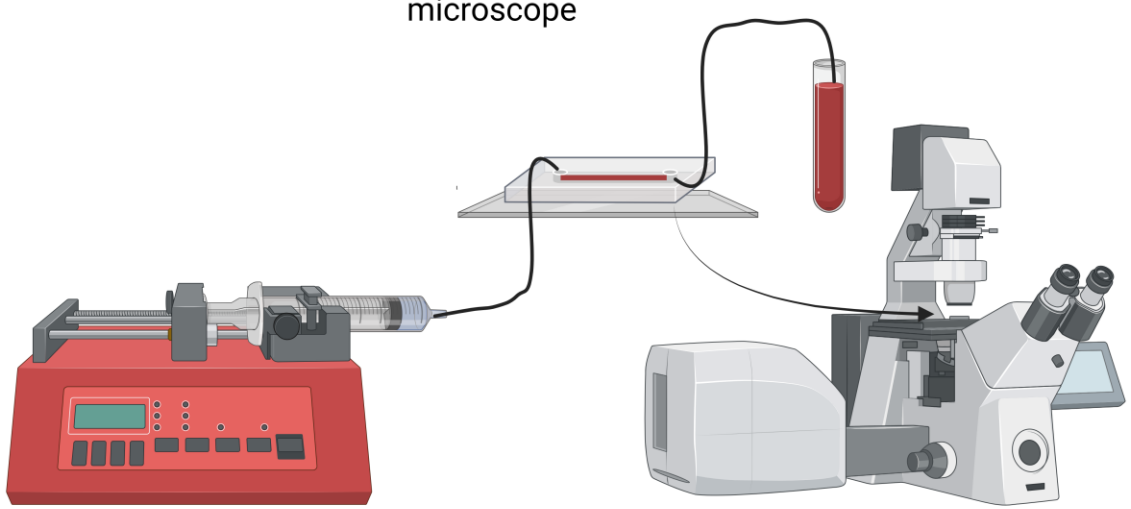

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Compounds:

- HMB-003: compound of interest
- TXA: benchmark control
- iHMB-003: negative control

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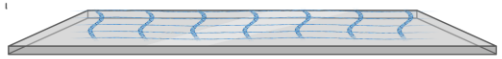

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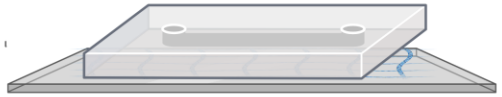

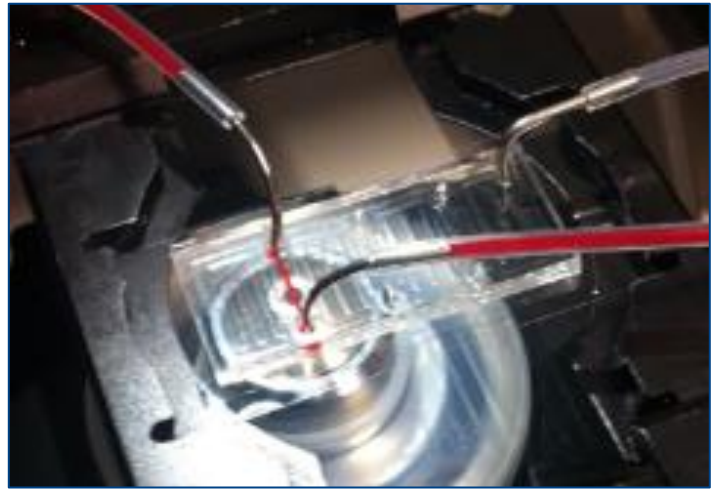

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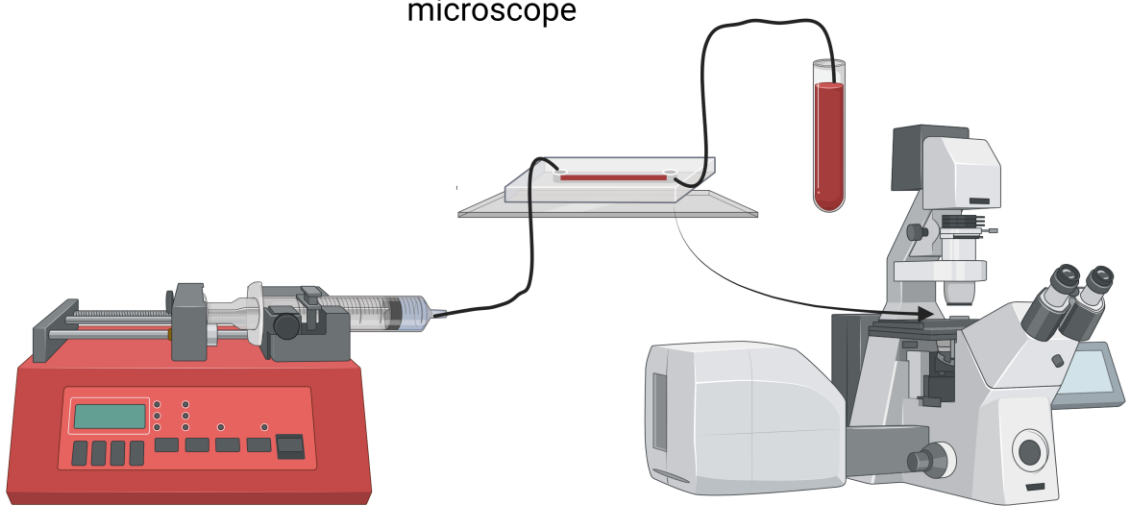

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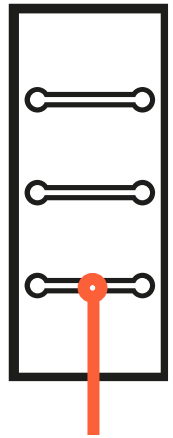
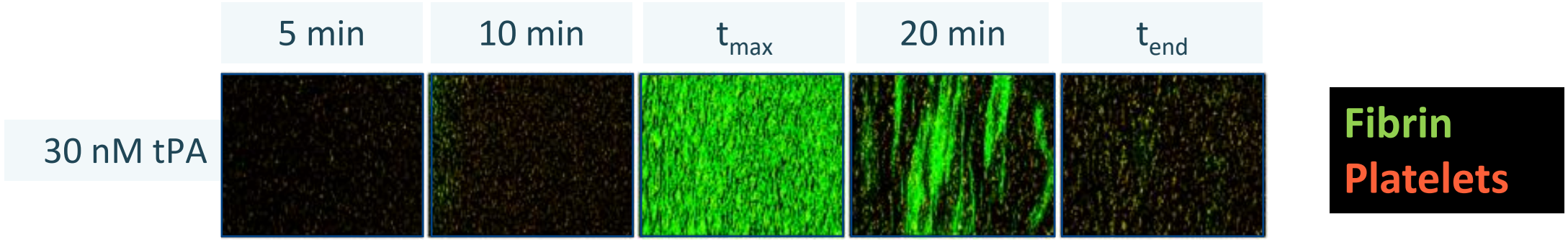


HMB-003 inhibits fibrinolysis in a whole blood flow model



Magnification: 200x
t_{max} (time at peak fibrin) and t_{end} (time at fibrin disappearance) defined by 30 nM tPA condition

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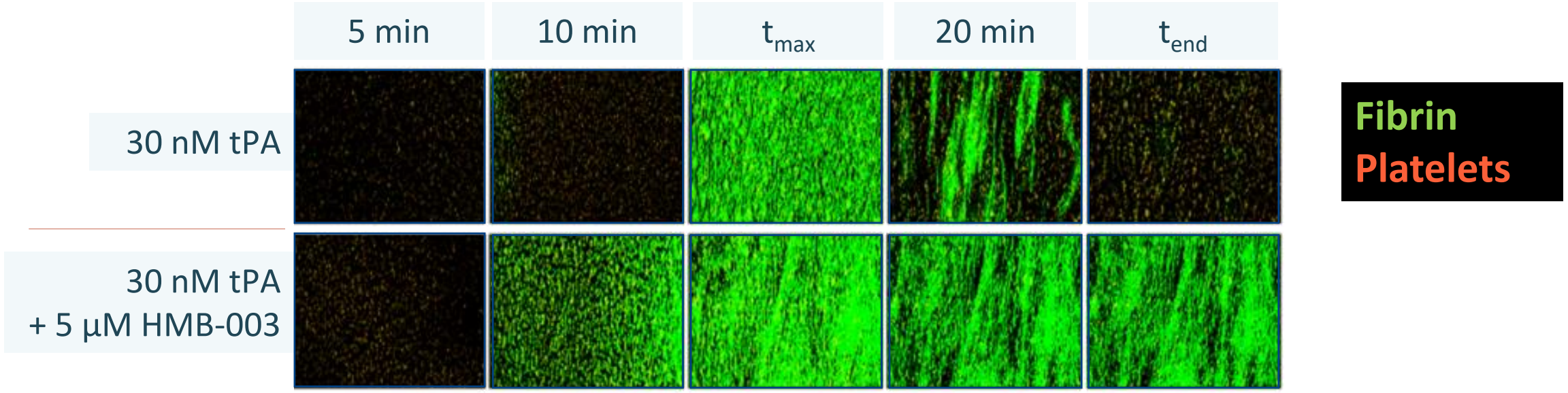

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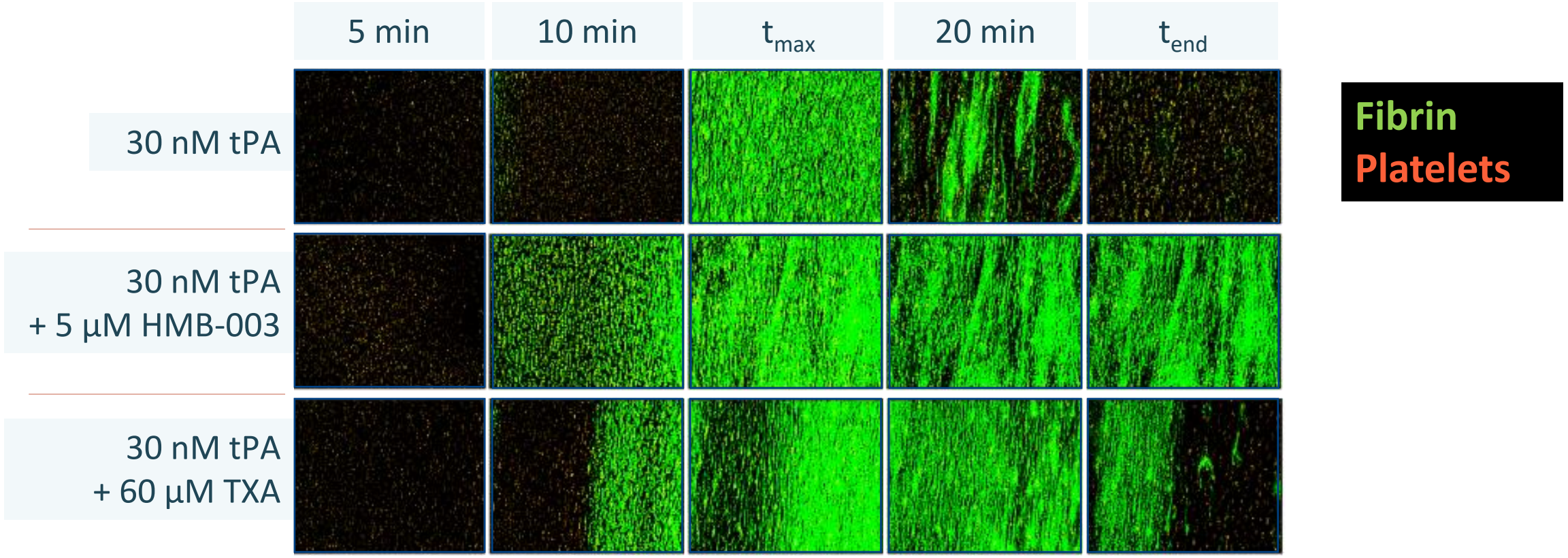

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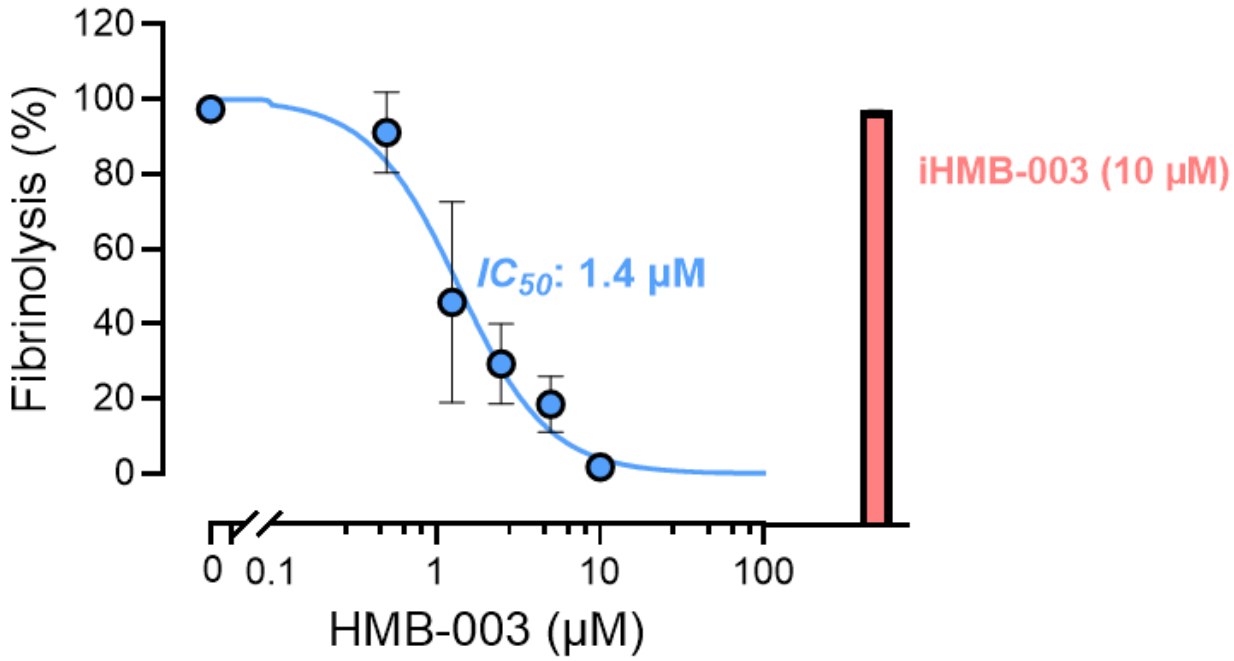

iHMB-003 = HMB-003 incapable of binding to plasmin

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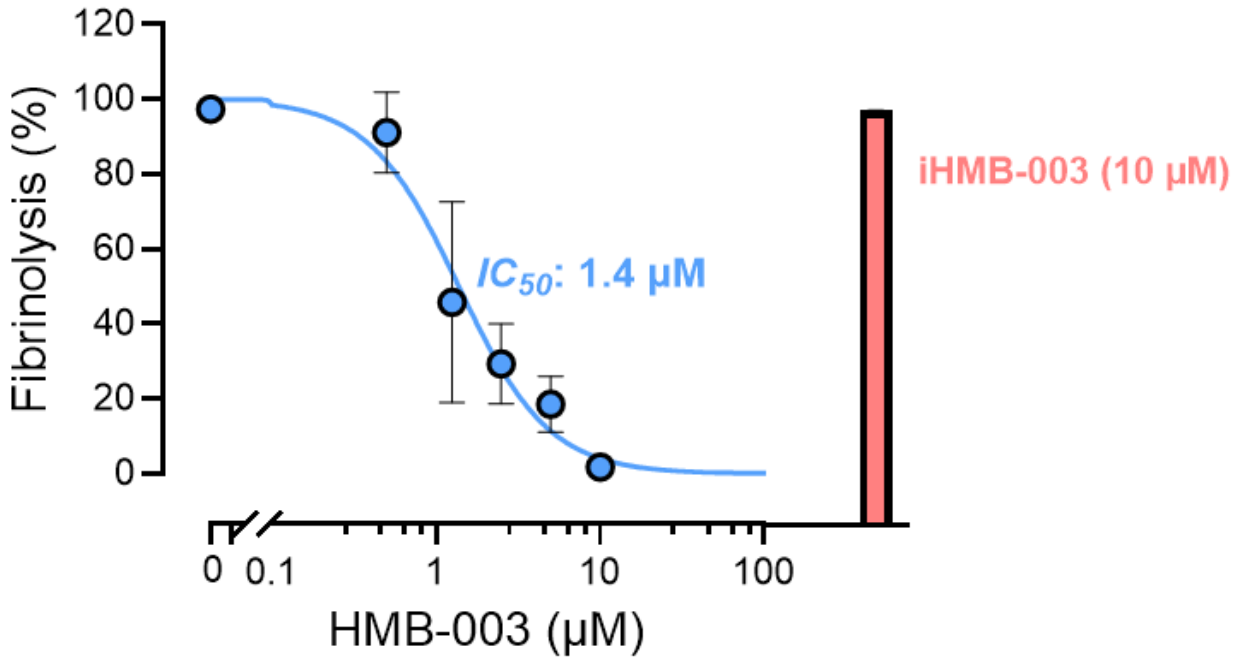

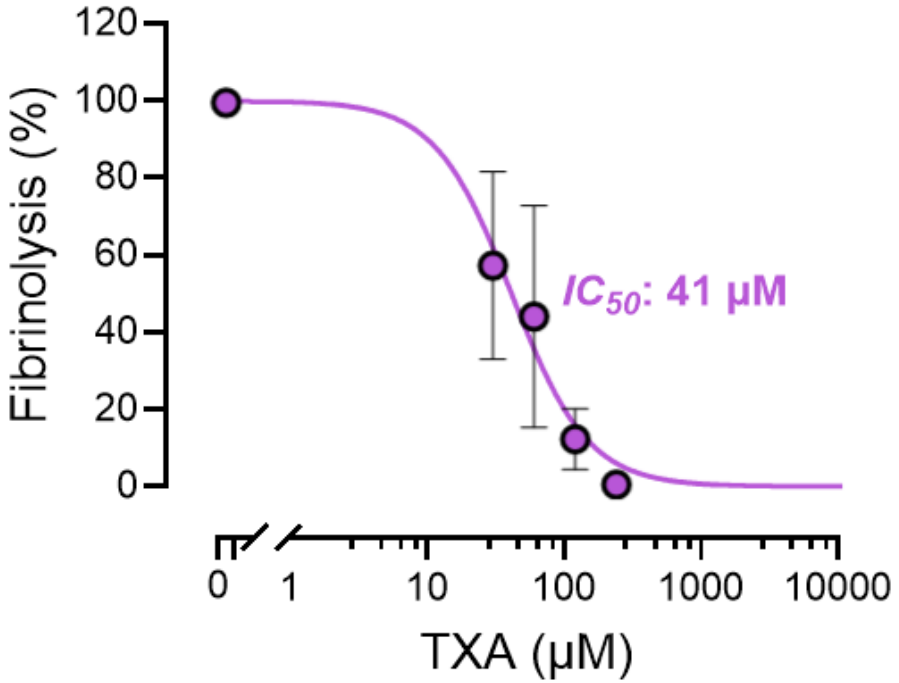

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IC_{50} is within therapeutic plasma levels of approved oral dose (1.3 g TID) of TXA
(Moore et al. 2012)

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Objectives

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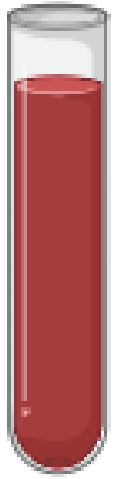
2. **Evaluate the spatial and temporal localization of HMB-003 in the context of an activated hemostatic system**

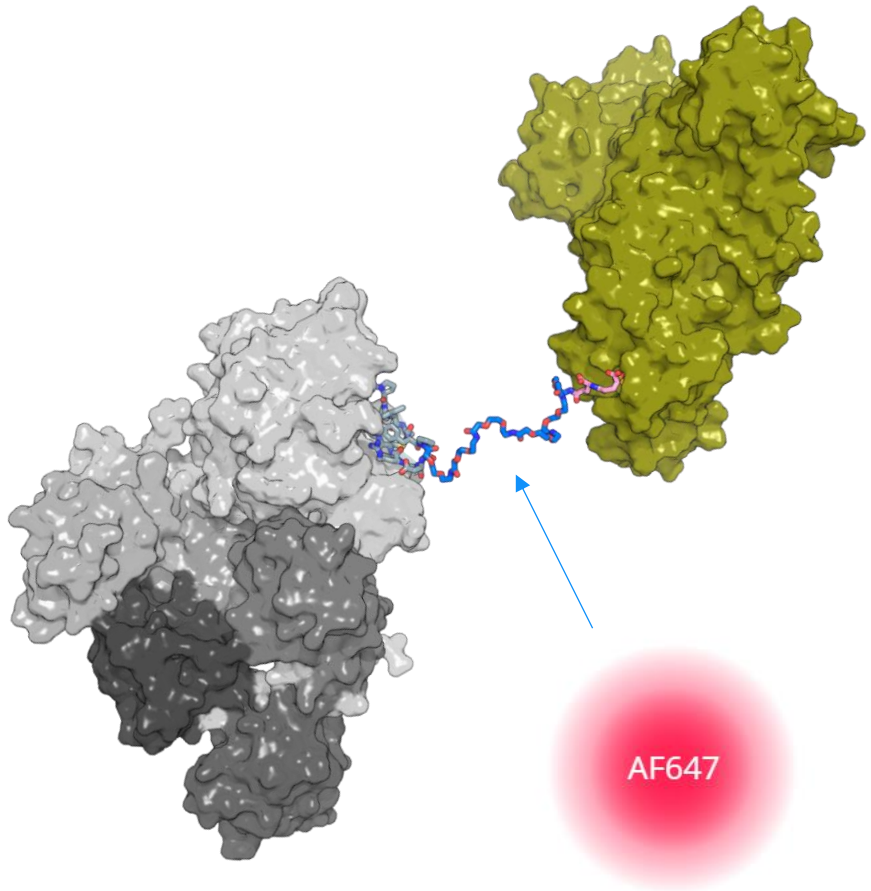
HMB-003 localisation study

Fibrin formation → plasmin(ogen) binding to fibrin → HMB-003 binding to active site of plasmin

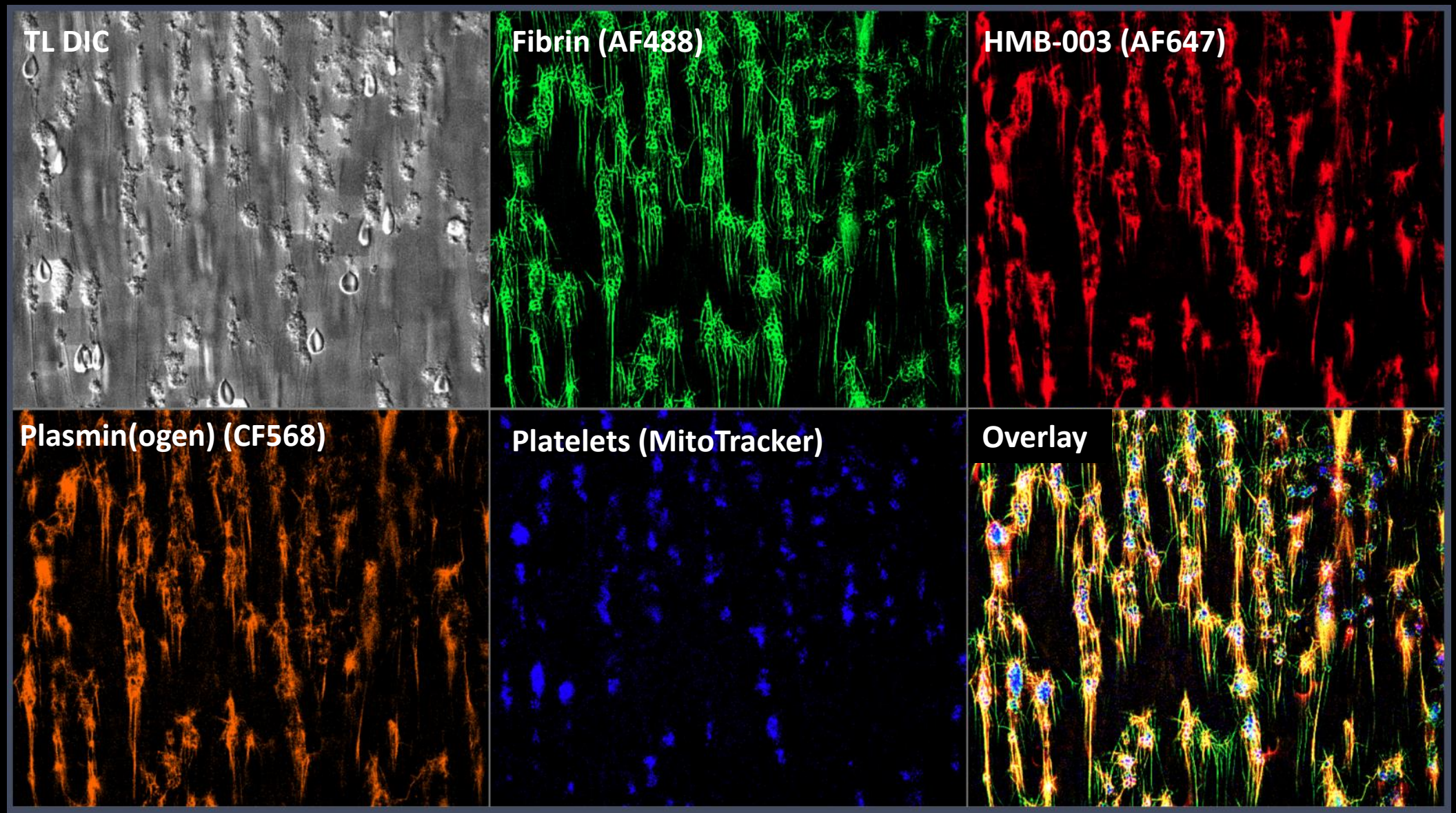


30 nM tPA
anti-fibrin-AlexaFluor488
anti-plasmin(ogen)-CF568
MitoTracker Blue
+/- (i)HMB-003-AlexaFluor647

$MgCl_2$ + $CaCl_2$



HMB-003 colocalizes with fibrin and plasmin(ogen)



t=15 min
Shear: 300 s^{-1}
Magnification: 200x

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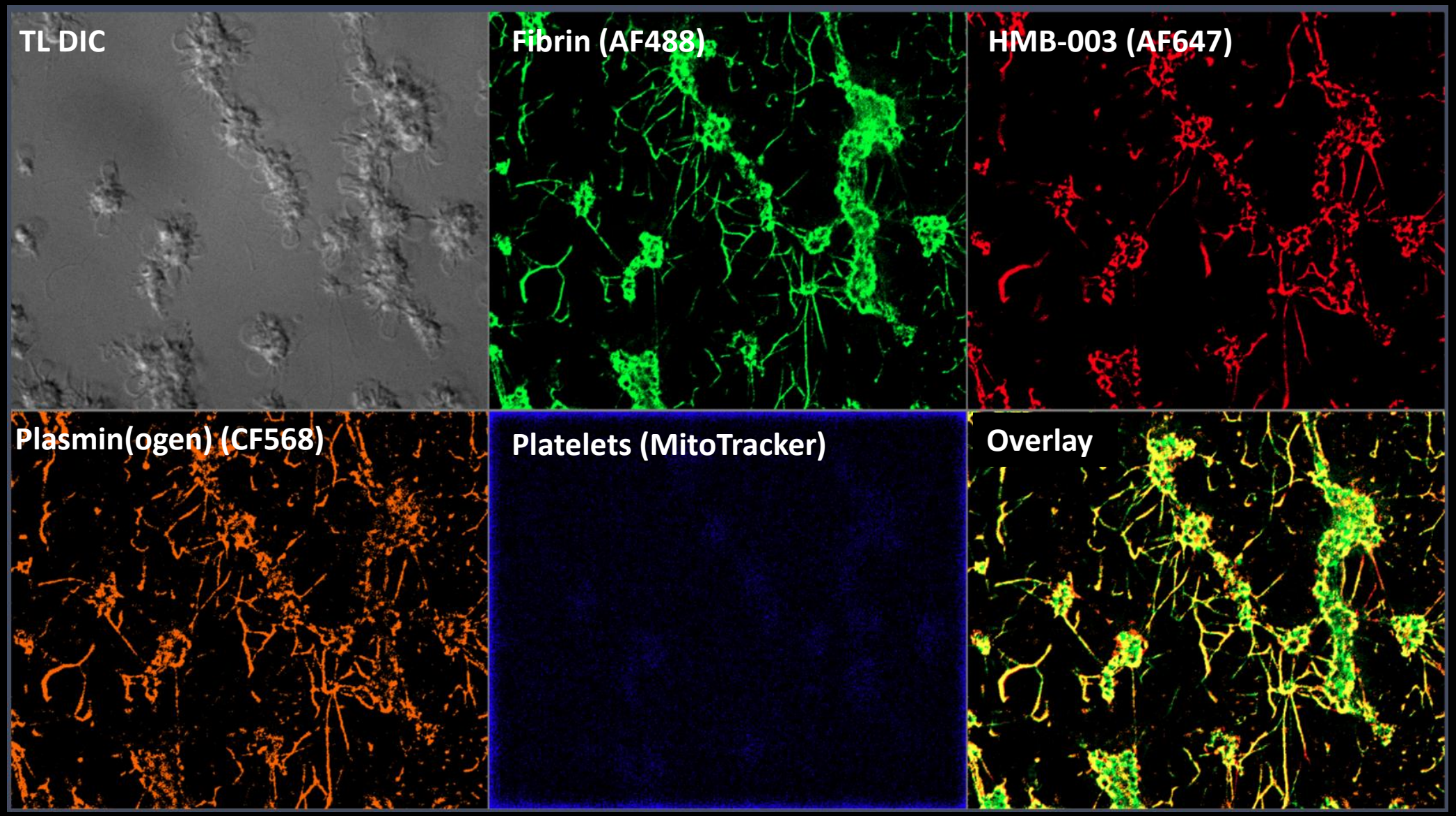
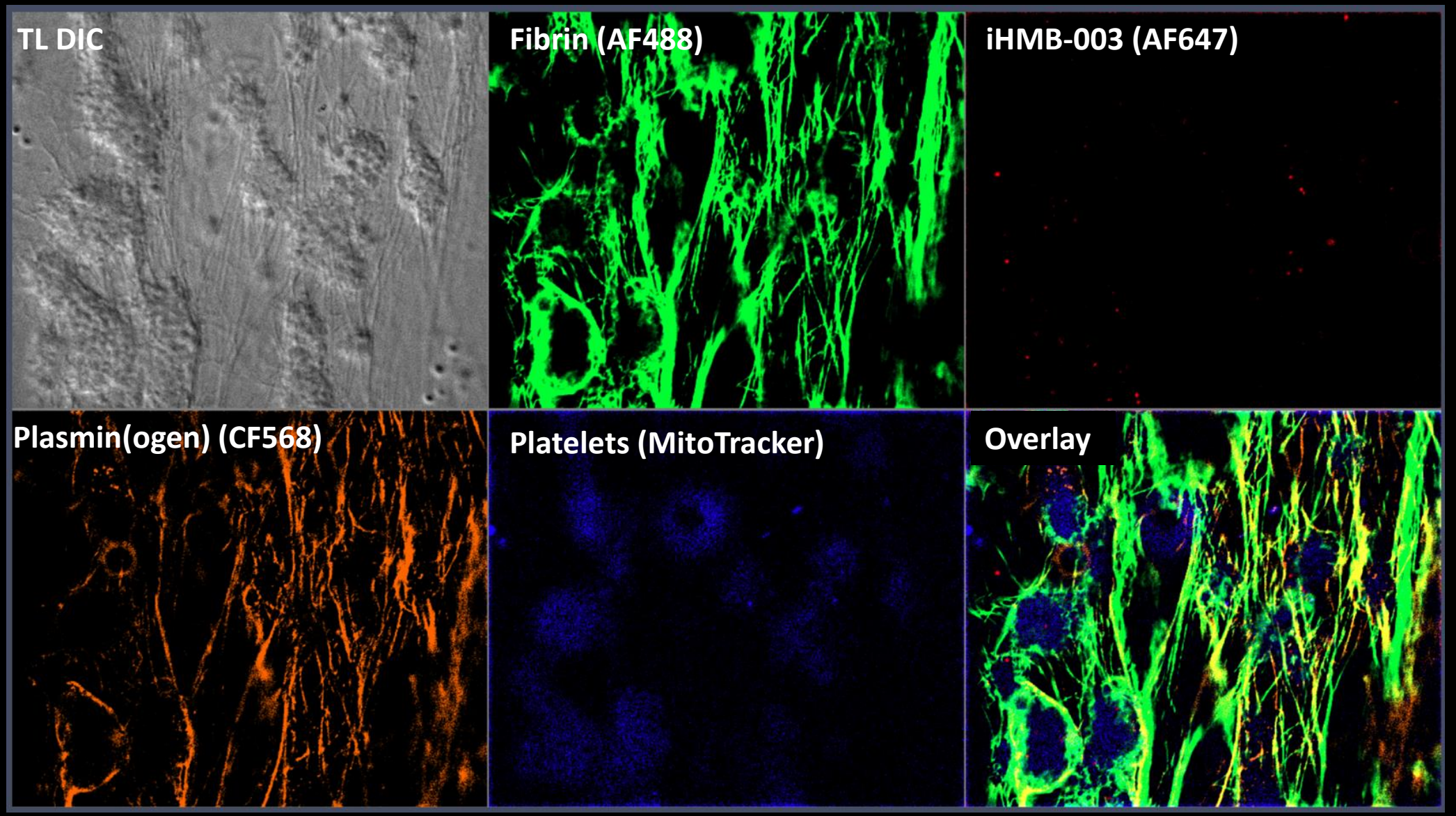


t=15 min
Shear: 300 s^{-1}
Magnification: 1000x

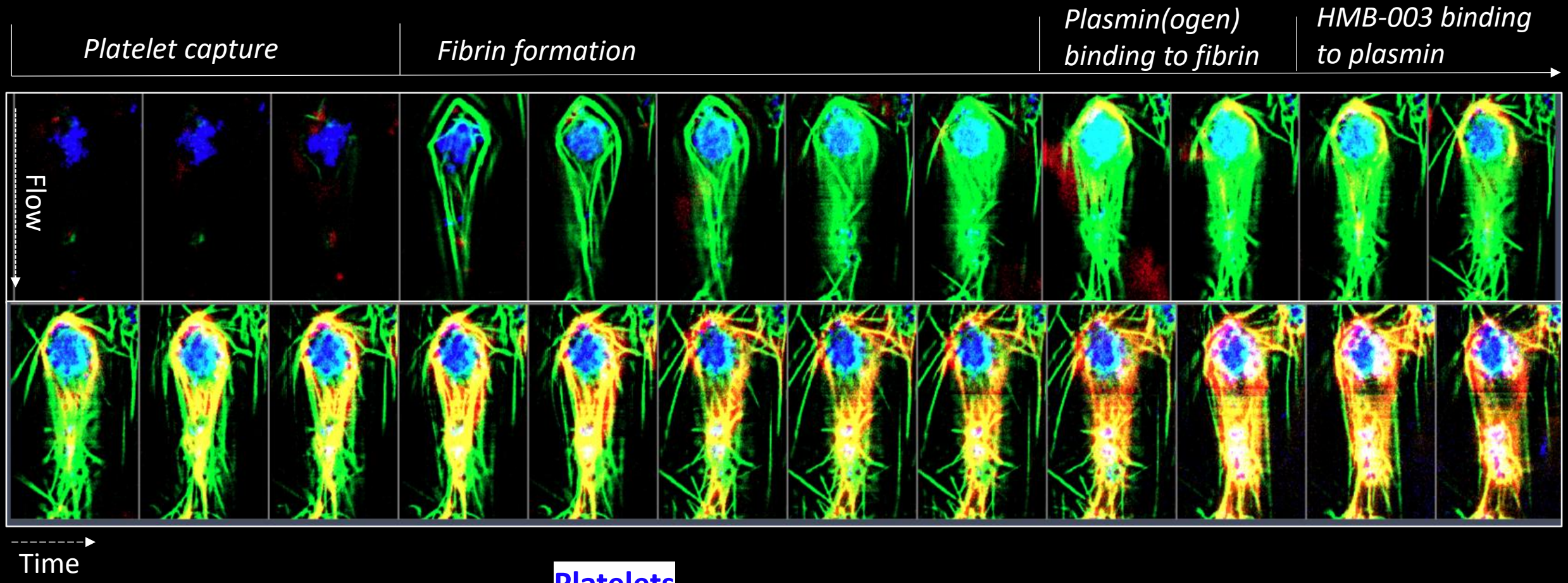
Inactivated HMB-003 (iHMB-003) does not colocalize with fibrin and plasmin(ogen)



iHMB-003 = lysine inhibited HMB-003

t=15 min
Shear: 300 s^{-1}
Magnification: 1000x

HMB-003 colocalizes with fibrin and plasmin(ogen)



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Platelets
Fibrin
Plasmin(ogen)
HMB-003

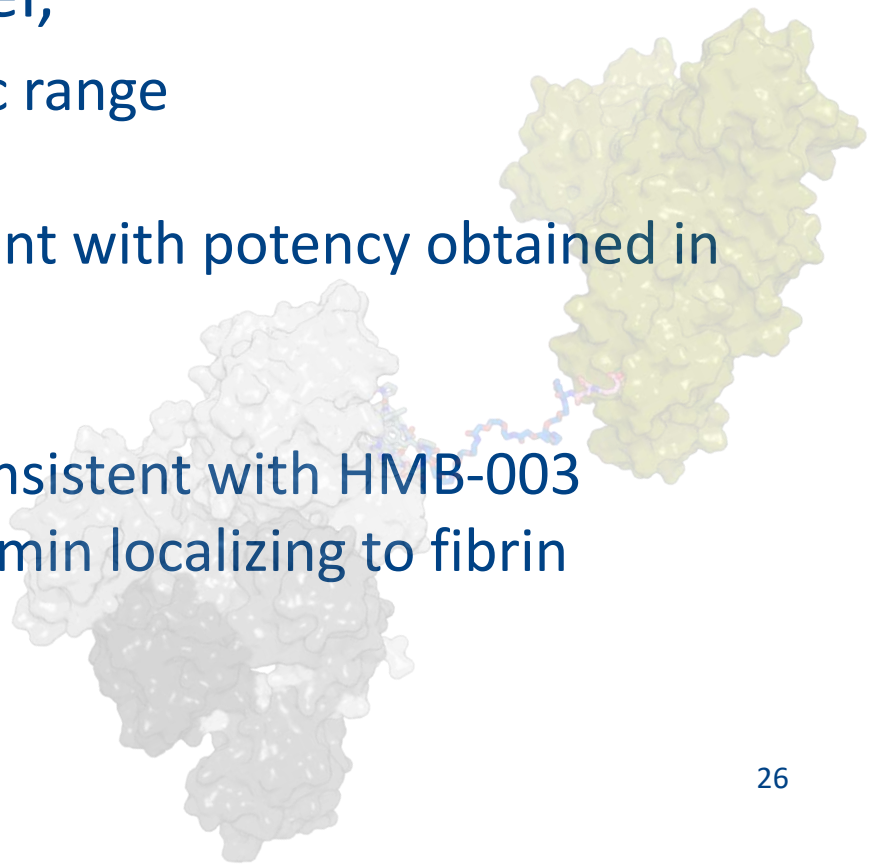
Shear: 300 s^{-1}
Magnification: 200x
1 image per 20 sec

Conclusion

HMB-003 potently inhibits fibrinolysis in a human whole blood microfluid model that recapitulates key elements of hemostasis under flow

In a whole blood tPA based microfluidic flow model,

- TXA potency (IC_{50} 41 µM) is aligned with its therapeutic range
- HMB-003 inhibits fibrinolysis with IC_{50} 1.4 µM, consistent with potency obtained in other *in vitro* assays
- HMB-003 colocalizes with plasmin(ogen) and fibrin, consistent with HMB-003 inhibiting plasmin through active site binding, and plasmin localizing to fibrin



Acknowledgements

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- Anne Weiss
- Henrik Østergaard
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- Mattias Häger
- Theresa Bak-Thomsen