

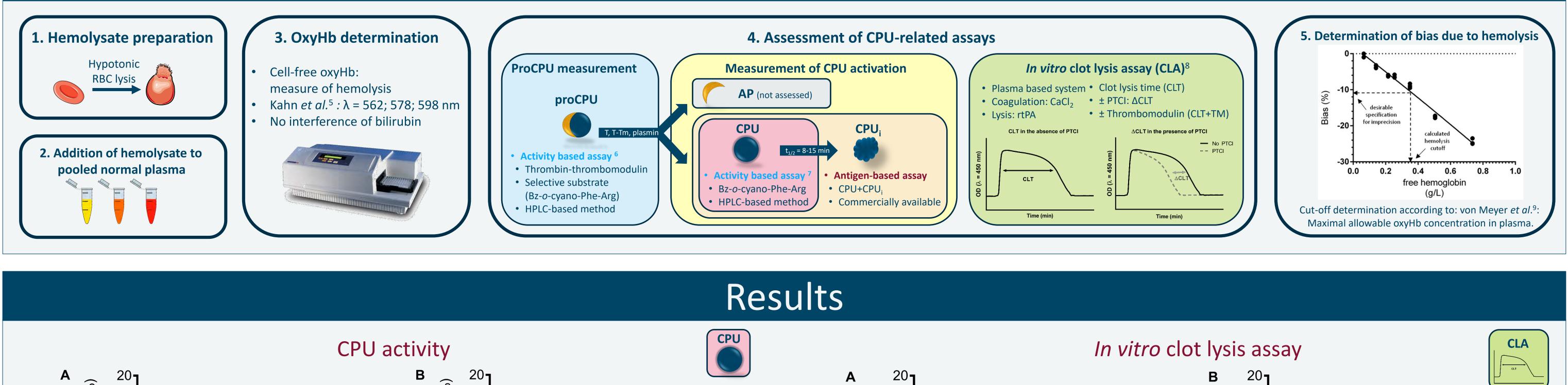


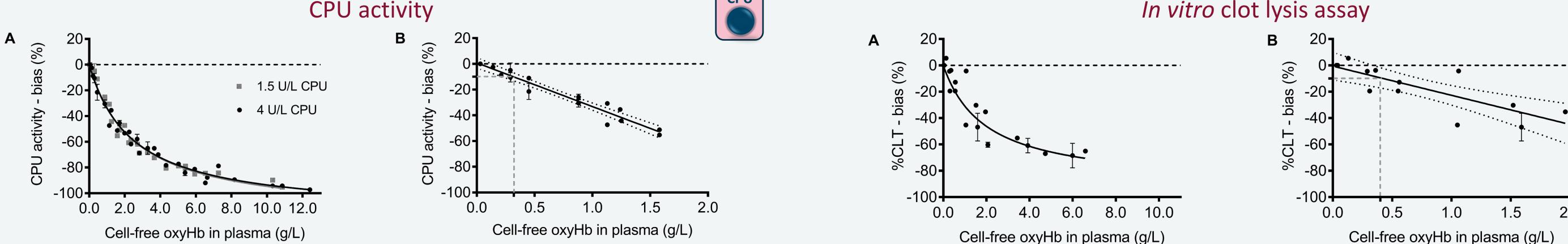
# Inhibition of the procarboxypeptidase U (proCPU, TAFI, proCPB2) system due to hemolysis.

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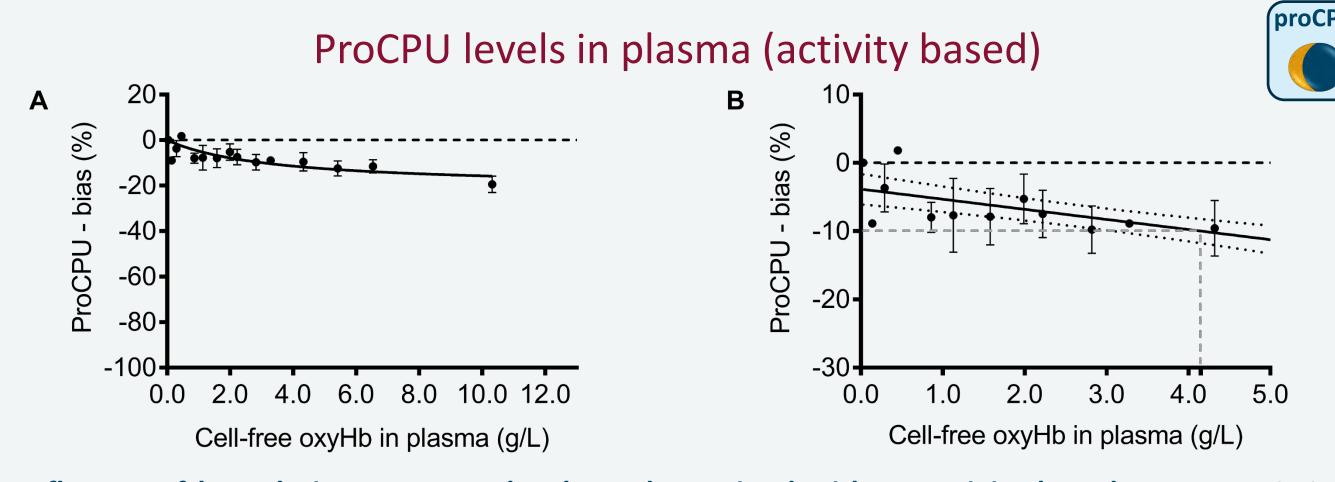
Background					
<ul> <li>Carboxypeptidase U (CPU, TAFIa, CPB2)</li> <li>Potent attenuator of fibrinolysis. <sup>1</sup></li> <li>Inactive precursor (proCPU, TAFI, proCPB2) in the blood: activated by thrombin, thrombin-thrombomodulin and plasmin.</li> <li>Very short half-life (8-15 min) due to thermal inactivation (CPU<sub>i</sub>). <sup>1</sup></li> </ul>	<ul> <li>Spurious hemolysis</li> <li>Leading cause of interference in hemostasis testing:<sup>2</sup> <ul> <li>Spectral and biological interference described.</li> </ul> </li> <li>Significantly enhances fibrinolysis in functional fibrinolysis assays (TEG).<sup>3</sup></li> <li>Inhibition of CPU-related effect in functional assays due to red blood cells (RBC)s.<sup>4</sup></li> </ul>				
Objectives					
A. Validation of activity based, functional and immunological assays of the CPU system in the presence of hemolysis.	B. Determination of maximal allowable oxyhemoglobin levels for all assays described.				

## Methods





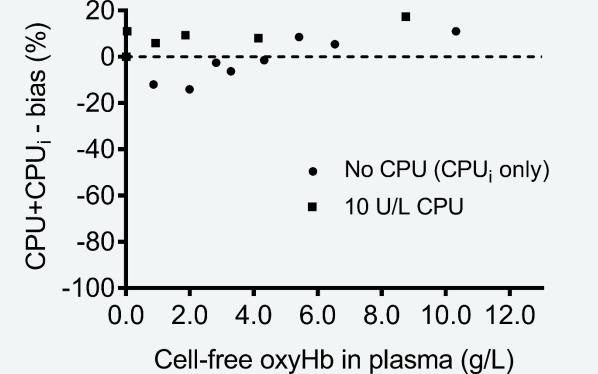
Influence of hemolysis on CPU activity levels. A. Decrease in CPU activity (mean ± SEM) observed in the presence of increasing concentrations of hemolysate. B. Cut-off determination based on linear regression (95% CI): 0.3 g/L OxyHb.



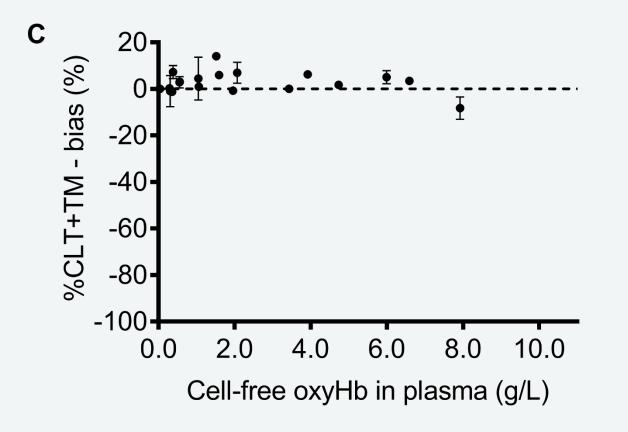
Influence of hemolysis on proCPU levels as determined with an activity based assay. A. Limited decrease in proCPU in the presence of increasing concentrations hemolysate (mainly due to sample dilution during sample preparation). B. Cut-off determination based on linear regression (95% CI): 4.2 g/L oxyHb.



#### Commercially TAFIa/ai Asserachrom<sup>®</sup> ELISA



Influence of hemolysis on Asserachrom<sup>®</sup> TAFIa/ai **ELISA.** No significant influence due to hemolysis was observed in the Asserachrom<sup>®</sup> TAFIa/ai ELISA. No difference was observed between samples without active CPU (19.2 ng/mL CPU+CPU<sub>i</sub>) and samples with 10 U/L active CPU added (84.2 ng/mL CPU+CPU<sub>i</sub>).

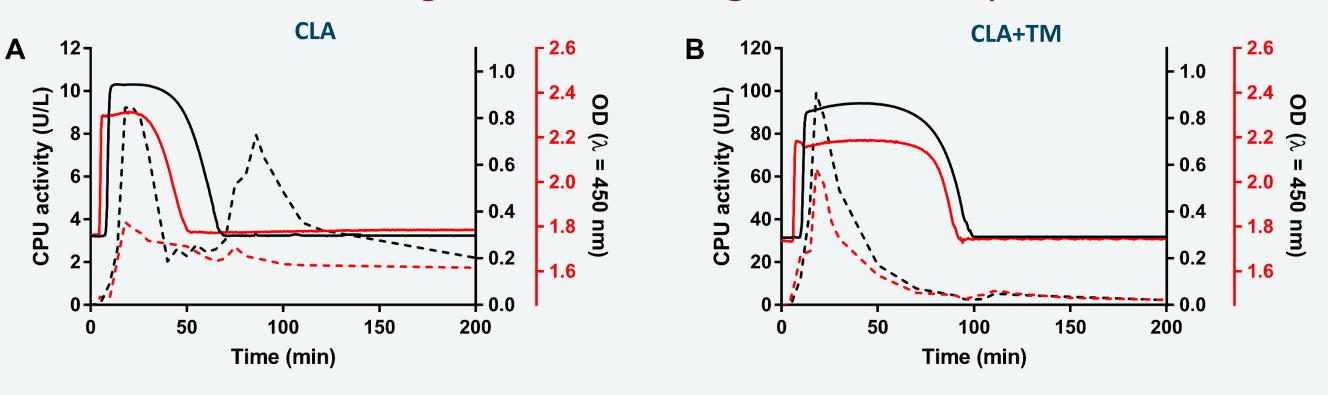


Influence of hemolysis on *in vitro* clot lysis assays. A. Reduction of the CLT (mean ± SEM) observed in the presence of increasing concentrations of hemolysate. B. Cut-off determination based on linear regression (95% CI): 0.4 g/L oxyHb. C. No significant influence of hemolysis observed on the CLT+TM in the presence of thrombomodulin (0.5 nM). OxyHb concentrations > 8.0 g/L resulted in a distortion of the clot lysis profile.

1.5

2.0

#### CPU generation during *in vitro* clot lysis



--- CLT(+TM) + hemolysate --- CPU activity --- CPU activity + hemolysate — CLT(+TM) Influence of hemolysis (5.3 g/L oxyHb) on CPU generation during in vitro clot lysis. A. Reduction of the CLT (- vs. -) was observed due to a significant reduction of the generated CPU during the coagulation phase in the presence of hemolysate (--- vs. ---). **B.** No significant influence of hemolysis was observed on the CLT+TM in the presence of thrombomodulin (0.5 nM). Although, a 35.7% reduction of the generated CPU-peak was observed. Based on the

#### CPU t<sub>1/2</sub> at 37 °C, a reduction of 2-3 min in CLT+TM was expected.

### Conclusions

- The CPU system is inhibited by hemolysis
- Activity based assays affected.
- Functional assays also affected.
  - Influence due to inhibition of active CPU during analysis.
- Commercial CPU+CPU<sub>i</sub> ELISA not affected.

Parameter	Bias	oxyHb cut-off (g/L)	Hemolysis category <sup>*</sup>	Visual assessment <sup>*</sup>
CPU activity	-10 %	0.3	Slightly hemolyzed	Yellow to slightly pink
ProCPU <sup>†</sup>	-10 %	4.2	Grossly hemolyzed	Red to browr
Clot lysis assay (CLA)				
CLT <sup>‡</sup>	-5 %	0.4		Pink to
$\Delta CLT$	-10 %	0.4	Mildly hemolyzed	slightly red
%CLT	-10 %	0.4		
CLA with TM	N/A	8.0	Grossly hemolyzed	Red to browr
Asserachrom® TAFIa/ai	N/A	10.3 <sup>§</sup>	Grossly hemolyzed	Red to brown



1.	Leurs et al. Thromb Haemost 2005; 94: 471–87.
2.	Lippi <i>et al.</i> Semin Thromb Hemost 2013; 39: 258–66.
3.	<b>Moore <i>et al.</i></b> Shock 2015; 43: 39–46.
4.	Carrieri et al. J. Thromb. Haemost 2012; 10(6) E21-22
5.	Kahn <i>et al.</i> Ann Clin Lab Sci 1981; 11: 126–31.
6.	Heylen et al. Anal Biochem. 2010; 396(1): 152–4.
7.	<b>Heylen <i>et al.</i></b> Anal Biochem 2010; 403: 114–6.
8.	Leenaerts et al. J Thromb Haemost 2018; 16: 2057–69
9.	<b>von Meyer <i>et al</i>.</b> Clin Chim Acta 2018; 484: 328–32.

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