A high-throughput small-angle X-ray scattering assay to determine the conformational change of plasminogen

(SUPPLEMENTAL INFORMATION)

Adam J. Quek[1,†], Nathan P. Cowieson[2,3,†], Tom T. Caradoc-Davies[2], Paul J. Conroy[1], James C. Whisstock[1,‡,*], Ruby HP. Law[1,‡,*]

[1] Department of Biochemistry and Molecular Biology, School of Biomedical Sciences, Monash University, Clayton, VIC 3800, Australia.
[2] Australian Synchrotron, ANSTO_Melbourne, 800 Blackburn Rd., Clayton, VIC 3168, Australia.
[3] Current address Diamond Light Source Ltd, Diamond House, Harwell Science and Innovation Campus, Didcot, Oxfordshire, OX11 0DE, England.
† These authors contributed equally to this work and are joint first authors.
‡ These authors contributed equally to this work.
* Correspondence: james.whisstock@monash.edu; ruby.law@monash.edu

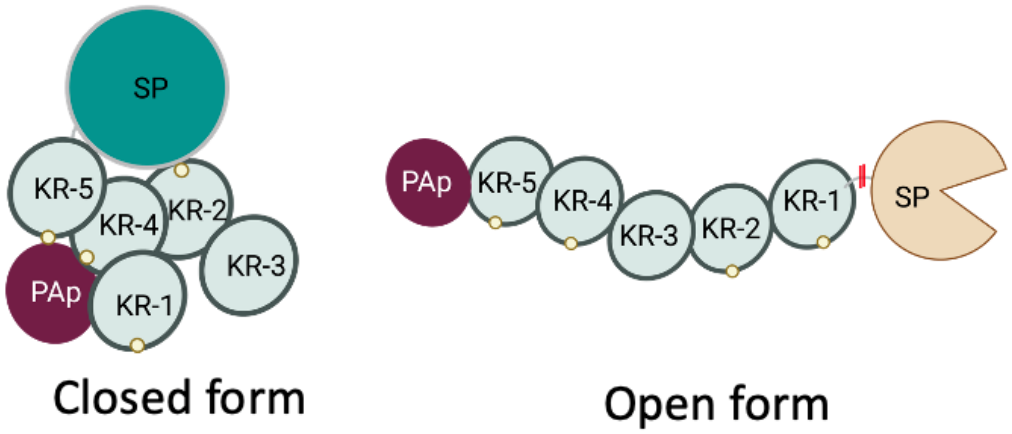


Figure S1. Cartoon representation of closed and open Plg. Plg is a 7-domain zymogen with an N-terminal PAN-domain (PAp), five kringle domains (KR1-5), and a serine protease domain (SP). In the closed conformation (**a**), the lysine binding site (D/EXD motif, LBS) found on KR-2, 4 and 5 (yellow circle) binds to SP, PAp and PAp domain, respectively; LBS in KR-1 is free. Here, the activation loop (grey line) is not exposed. The amino acid sequence corresponding to the KR-3 LBS is DXK, which does not bind to lysine or arginine residues. (**b**) Open Plg forms through an interaction between the LBSs and free lysine/analogues or surface lysine/arginine on target sites; it also exposes the activation loop, allowing activation by plasminogen activators. This illustration is generated using BioRender (Agreement number: MO25NKENPO).

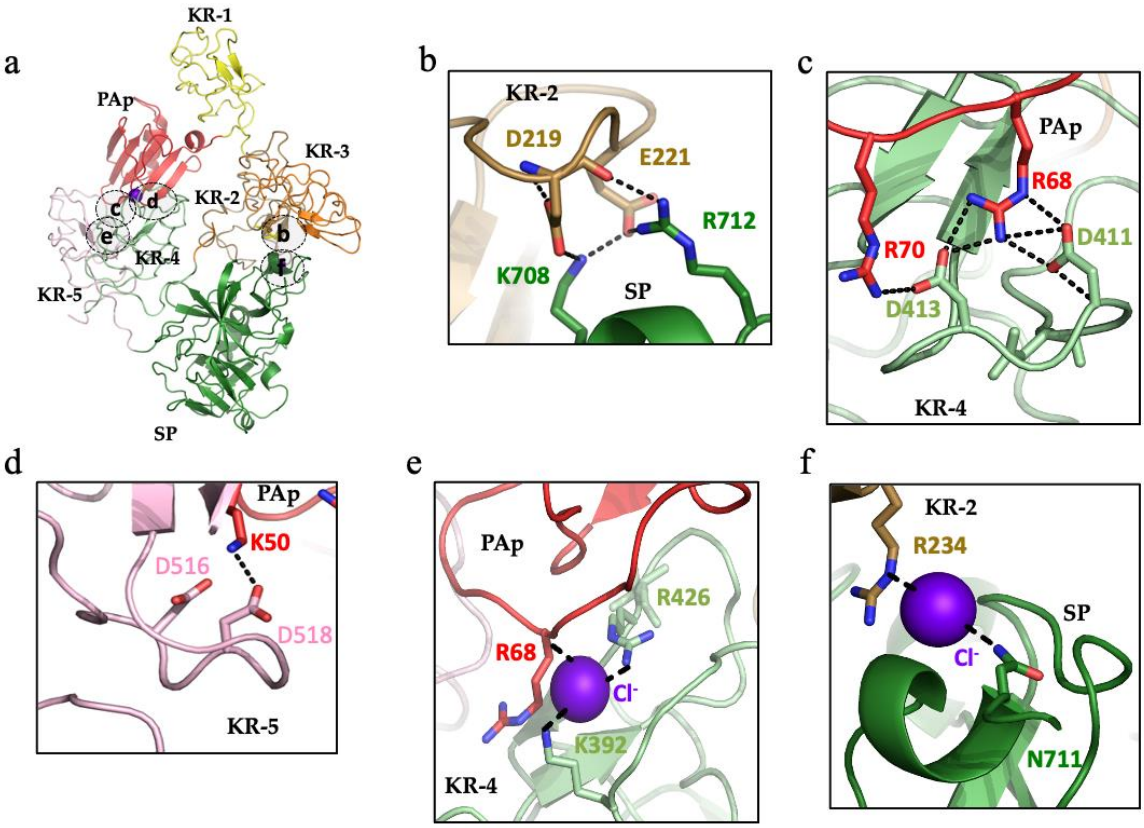


Figure S2. Interdomain interactions in closed Plg GII (**a**) Cartoon representation of Plg GII X-ray crystal structure. Letters enclosed in dotted

circles represent regions involved in inter-domain interactions shown in the corresponding panels **b-f**. (**b**) Inter-domain interactions between KR-2 LBS and SP domain. (**c**) Inter-domain interactions between KR-4 LBS and PAp domain. (**d**) Inter-domain interactions between KR-5 LBS and PAp domain. (**e-f**) Chloride (Cl^-) ions (depicted as purple spheres) also mediate inter-domain interactions between KR-2/SP and KR-4/PAp.

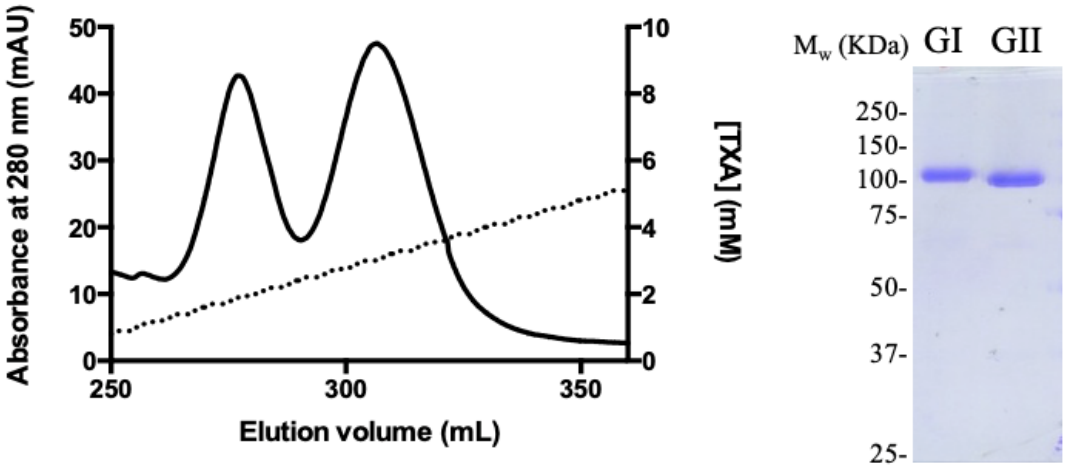
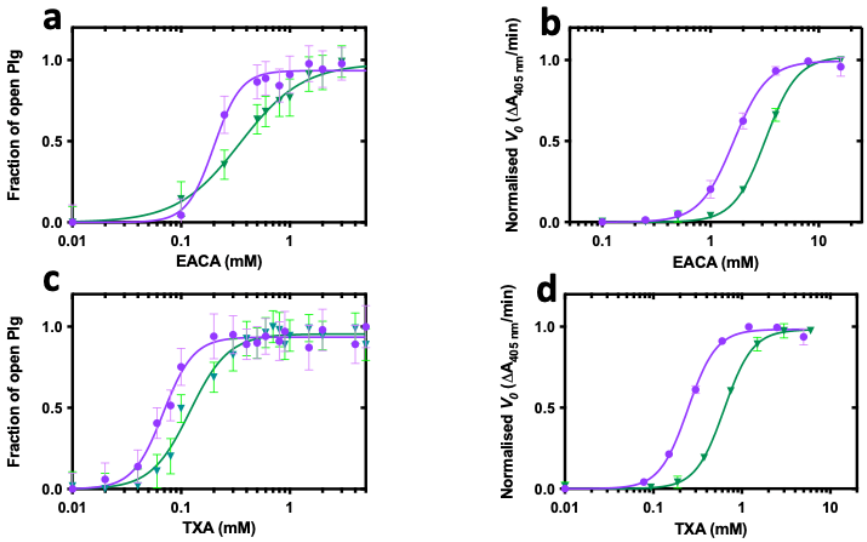


Figure 3. Left panel: Chromatogram showing the separation of Plg GI and GII on Lysine Hyper D Resin. **Right panel:** SDS-PAGE showing purified glycoforms.



Ligand	K_{open} (mM)		$K_{activation}$ (mM)	
	Plg GI	Plg GII	Plg GI	Plg GII
EACA	0.20 ± 0.01	0.35 ± 0.02	1.63 ± 0.05	3.23 ± 0.06
TXA	0.068 ± 0.002	0.120 ± 0.008	0.25 ± 0.01	0.63 ± 0.01

Figure S4. Comparison of ligand-induced conformational change and tPA activation of Plg glycoforms. Experimental data presented in **Table 1** on the

conformational change of Plg GI (purple) and GII (green) are shown (**a**) EACA and (**c**) TXA. K_{open} is the ligand concentration required to induce the open conformation in 50% of the total Plg in solution. TXA exhibited lower Kopen (higher efficacy) for both glycoforms than EACA (**Table below**). tPA is used to determine the rate of Plg activation in the presence of EACA (**b**) and TXA (**d**). Normalized initial rates are plotted against their respective ligand concentration. $K_{activation}$ is the ligand concentration at which the initial velocity is 50% of the maximal rate. The $K_{activation}$ of TXA is also lower than that of EACA. Compared with K_{open}, the $K_{activation}$ concentration is significantly higher ($P < 0.0001$), >8-fold for EACA, and >3.5-fold for TXA, suggesting Plg activation by tPA may occur only on the fully open molecules.

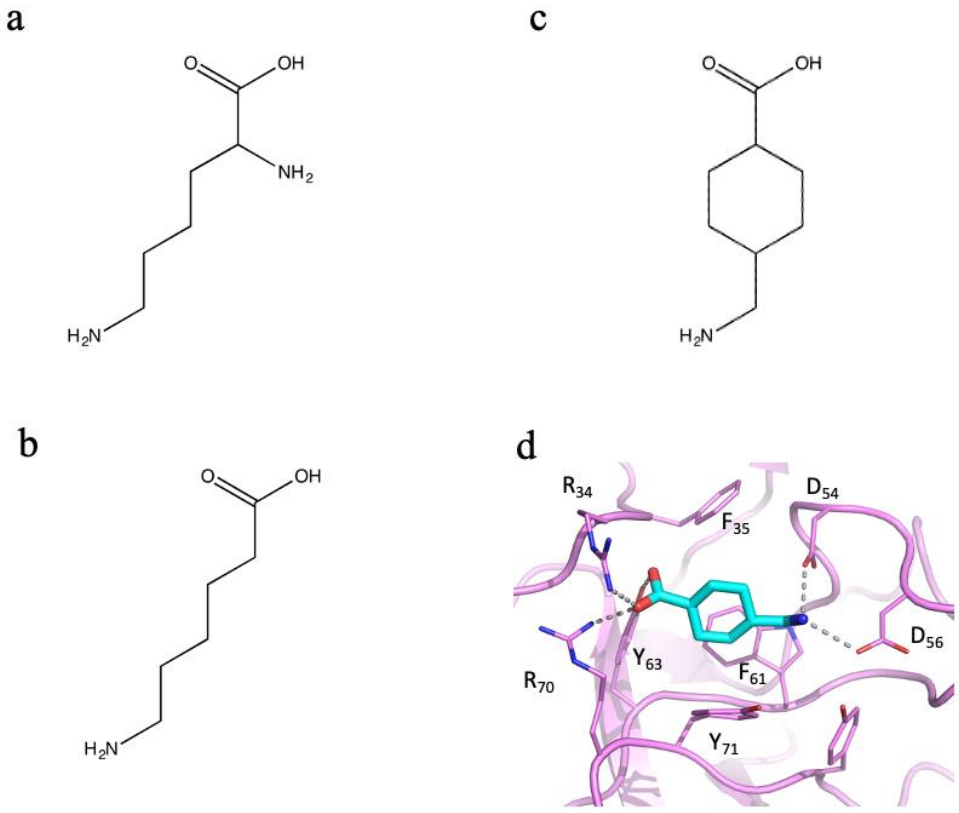
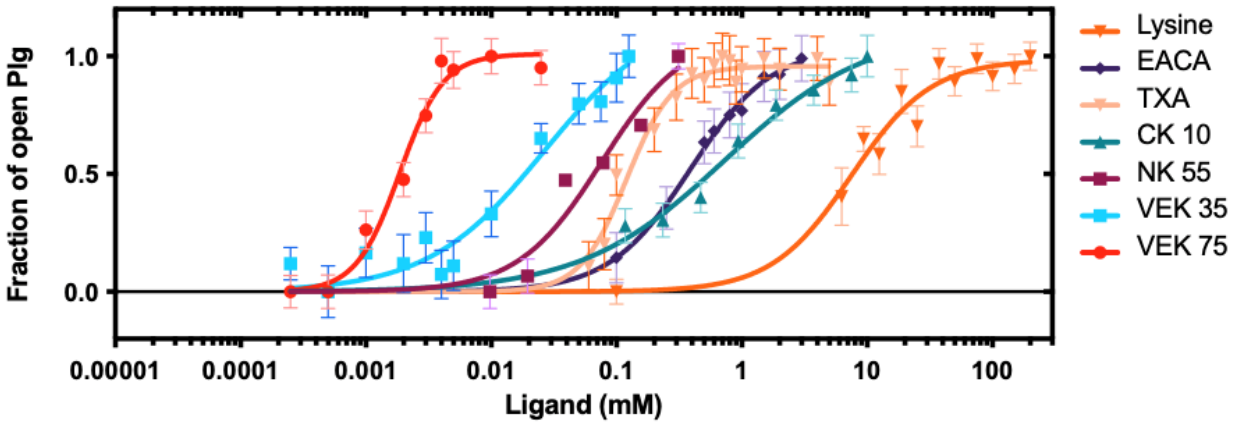


Figure S5. Lysine and analogues and molecular interaction with lysine binding site of KR-1. Chemical structures of (**a**) lysine, (**b**) ε-aminocaproic acid (EACA) and (**c**) Tranexamic acid (TXA). **(d)** Cartoon representation of the co-crystal structure of KR-1(light purple) and TXA (cyan) (PDB ID 1CEB) (39), where residues bind to TXA in the LBS, are labelled and shown in dotted lines.



Ligand/peptide	K_{open} (mM)
L-Lysine	7.47 ± 1.12
EACA	0.35 ± 0.02
TXA	0.120 ± 0.008
CK10	0.73 ± 0.24
MK12	*ND**
NK55	0.076 ± 0.046
VEK35[a]	***0.027 ± 0.016***
VEK75[a]	***0.0019 ± 0.00018***

Figure S6. Titration curves of ligands and peptides showing a concentration-dependent conformational change of Plg GII. The K_{open} determined is summarized in the Table. The order of ligand efficacy is MK12>L-lysine>CK10>EACA>TXA>NK55. Also shown is the side-by-side comparison with our previous work on VEK peptides derived from the plasminogen binding Group A Streptococcal M protein PAM, VEK35 (GSVE***K***LTADAELQ***R***L***K***NE***R***HEEAELE***R***L***K***SE***R***HDHDY) and VEK75 (GSEELQGL***K***DDVE***K***LTADAELQ***R***L***K***NE***R***HEEAELE***R***L***K***SE***R***HDHD***KK***EA E***RK***ALED***K***LAD***K***QEHLNGAL***R***YINE***K***EA). PAM has a much higher affinity for human Plg than the host receptors. The K_{open} for NK55 is 2.8-fold of VEK35 and 40-fold of VEK75. [a]refer to (30) for details.