

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

## (SUPPLEMENTAL INFORMATION)

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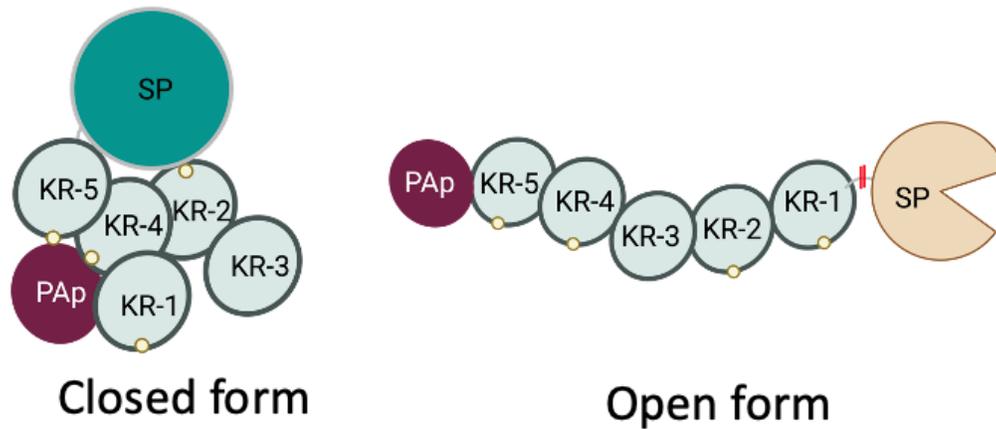
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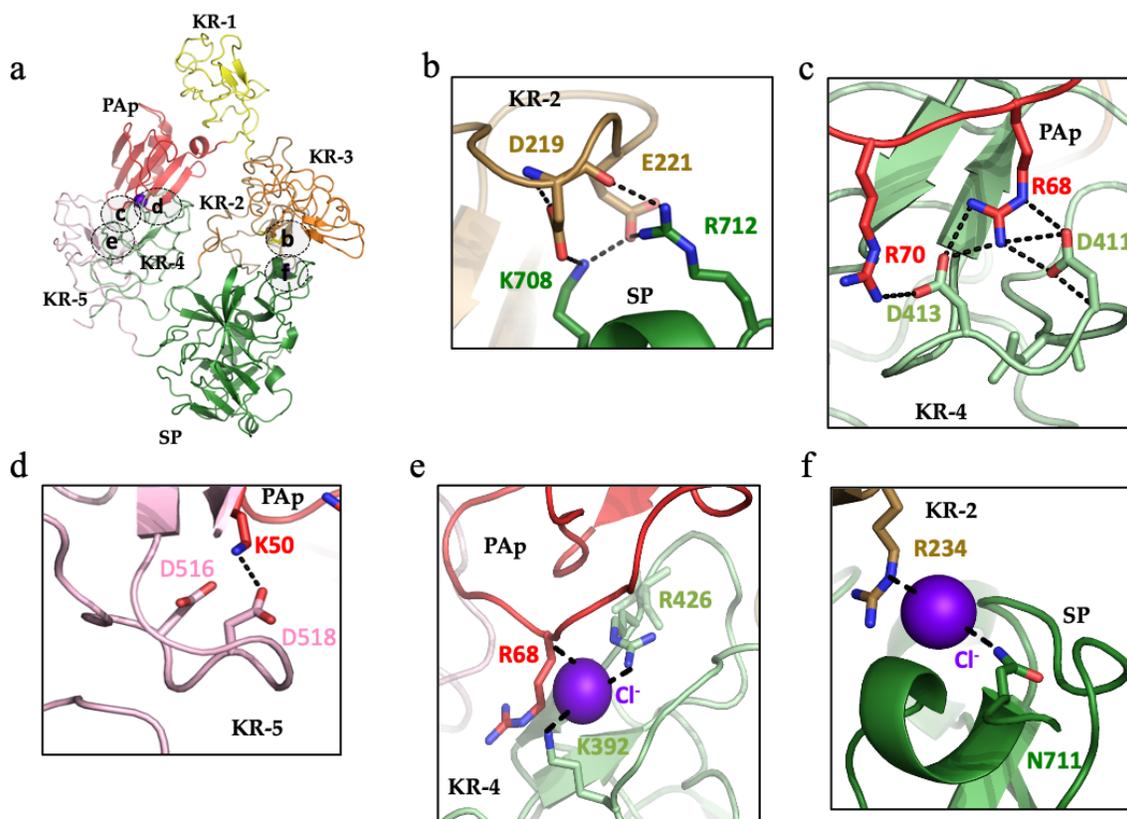
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**Running title:** Conformational change in plasminogen

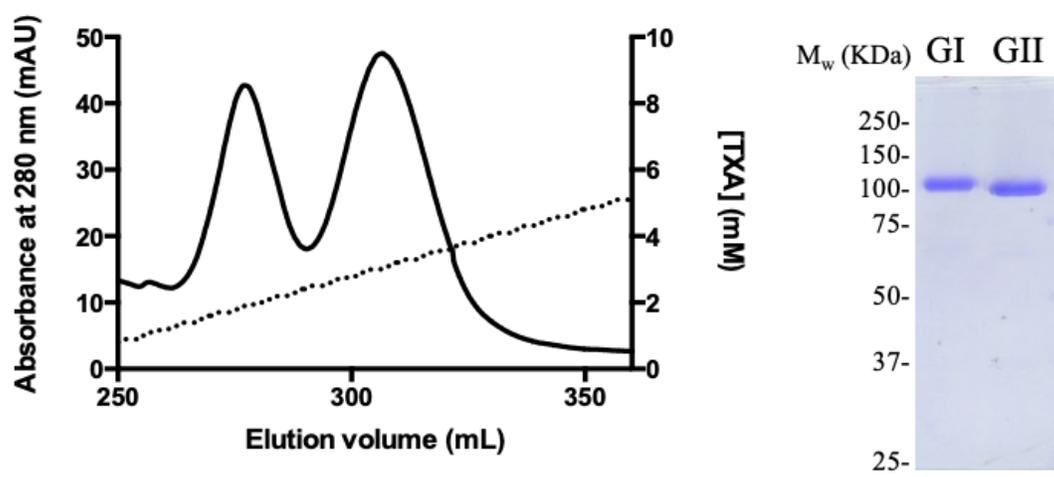
**Keywords:** SAXS, conformational change, plasminogen, fibrinolysis, structure-function, lysine binding site, lysine analogue, kringle domain



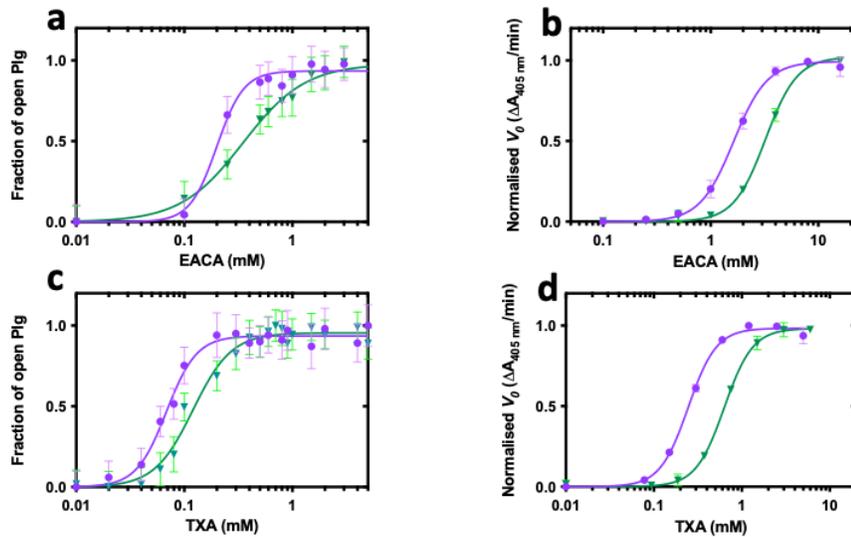
**Figure s1 Cartoon representation of closed and open Plg.** Plg is a 7-domain zymogen with an N-terminal PAN-domain (PAP), five kringle domains (KR1-5), and a serine protease domain (SP). In the closed conformation (**a**), the lysine binding site (D/EXD motif, LBS) found on KR-2, 4 and 5 (yellow circle) 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 ( $\text{Cl}^-$ ) ions (depicted as purple spheres) also mediate inter-domain interactions between KR-2/SP and KR-4/PAP.

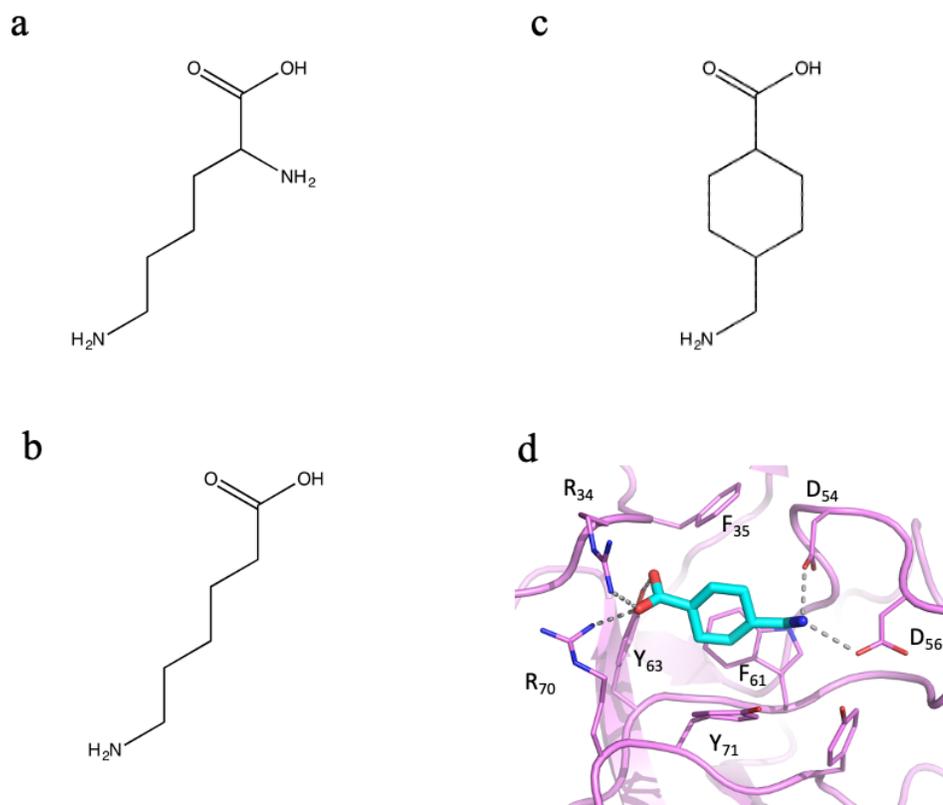


**Figure s3** Left panel: Chromatogram showing the separation of Plg GI and GII on Lysine Hyper D Resin. Right panel: SDS-PAGE showing purified glycoforms.

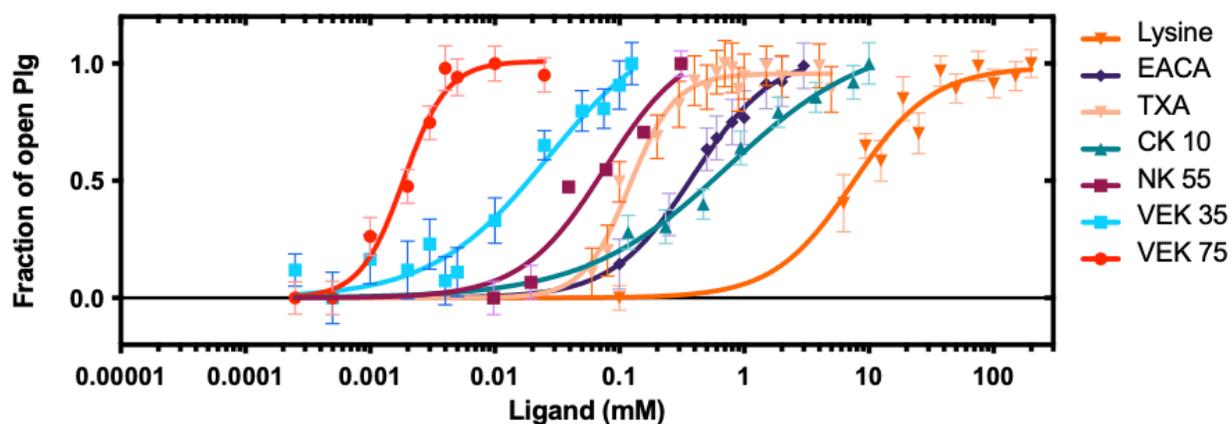


Ligand	$K_{open}$ (mM)		$K_{activation}$ (mM)	
	Plg GI	Plg GII	Plg GI	Plg GII
EACA	$0.20 \pm 0.01$	$0.35 \pm 0.02$	$1.63 \pm 0.05$	$3.23 \pm 0.06$
TXA	$0.068 \pm 0.002$	$0.120 \pm 0.008$	$0.25 \pm 0.01$	$0.63 \pm 0.01$

**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  $K_{open}$  (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 hydrogen bonds and salt bridges are shown in dotted lines



Ligand/peptide	$K_{open}$ (mM)
L-Lysine	$7.47 \pm 1.12$
EACA	$0.35 \pm 0.02$
TXA	$0.120 \pm 0.008$
CK10	$0.73 \pm 0.24$
MK12	ND*
NK55	$0.076 \pm 0.046$
<b>VEK35<sup>a</sup></b>	<b><math>0.027 \pm 0.016</math></b>
<b>VEK75<sup>a</sup></b>	<b><math>0.0019 \pm 0.00018</math></b>

**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 (GSVEKLTADAELQRLKNERHHEEAELERLKSERHDHDY) and VEK75 (GSEELQGLKDDVEKLTADAELQRLKNERHHEEAELERLKSERHDHDKKEAERKALEDKLDKQEH LINGALRYINEKEA). 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. <sup>a</sup>refer to (30) for details.

