SUPPLEMENTARY APPENDIX

# uPA-mediated plasminogen activation is enhanced by polyphosphate

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**Supplementary Methods**

**Materials**

Human plasminogen free fibrinogen, Glu-plasminogen, Lys-plasminogen and peroxidase conjugated goat anti-human FXII antibody were purchased from Enzyme Research Laboratories (Swansea, UK). Alexa-fluor 488 (AF488) conjugated fibrinogen from human plasma, Cascade Blue™ ethylenediamine, trisodium salt and DyLight kits were from Molecular Probes (Leiden, The Netherlands). Thrombin from human plasma, D-Val-Leu-Lys 7-amido-4-methylcoumarin (D–VLK-AMC) and polyphosphate (type 65; $polyP_{65}$) were purchased from Sigma-Aldrich (Dorset, UK). 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDAC), Slide-A-Lyzer dialysis cassettes G2 (2K MWCO 3ml), reacti-bind high capacity streptavidin coated microplates were from Thermo Scientific (Rockford, IL, USA). tPA was from Technoclone (Vienna, Austria) and high molecular weight uPA was from NIBSC (Potters Barr, UK). Chromogenic substrates S-2251 and S-2288, were from Chromogenix (Bedford, USA) and BIOPHEN CS-61(44) from Hyphen Biomed, (Neuville-sur-Oise, France).

**Labelling of Cascade Blue polyP**

Briefly, $polyP_{70}$ (1 mg/ml) was incubated overnight at 37 ˚C with Cascade Blue ethylenediamine (1 mM), $CaCl_2$ (1 mM), EDAC (100 mM), and 2-(N-morpholino)ethanesulfonic acid (100 mM), pH 6.5. CB-polyP adducts were purified using Slide-A-Lyzer dialysis cassettes.

**Protein binding assays to Biotin-labelled polyP**

Binding of tPA, uPA, plasmin, factor XII (FXII) and activated FXII (FXIIa) to biotin-labelled polyP (71 µM) were performed using an adaptation of the protocol described by Choi *et al*[13]. Streptavidin high binding capacity coated stripwells were coated with biotin-labelled polyP (71 µM) overnight at ambient temperature. Plates were washed with Tris-Tween (T/T; 50 mM Tris-HCl, 0.05 % Tween 20) containing 0.6% BSA plates and subsequently blocked for 3 h with T/T containing 5 % BSA. Various concentrations of tPA or uPA (0 – 400 nM), plasmin (0 – 100 nM), FXII (0 – 100 nM ) or FXIIa (0 – 25 nM) were added for 1 h. Bound tPA, uPA or

plasmin was detected with chromogenic substrates (1.2 mM S2288, CS-61 (44), or 0.6 mM S2251, respectively) by monitoring absorbance at 405 nm every 30 s for 200 min. FXII(a) was detected using a peroxidase conjugated goat anti-human FXII antibody. The antibody was incubated for 2 h before addition of 3,3',5,5'-tetramethylbenzidine substrate in 100 mM acetate/citrate buffer pH 6 containing 1 % urea $H_2O_2$. The reaction was stopped after 10 min using 2.5 M $H_2SO_4$ and the plate read at 450 nm with a reference wavelength of 620 nm.

**Supplementary Figure and Video Legends**

**Supplementary Figure 1 - PolyP binds with high affinity to FXII(a).** Binding of (A) FXII (0 - 100 nM) or (B) FXIIa (0 – 25 nM) to biotin-labelled polyP (71 μM) bound to streptavidin coated stripwells. Bound FXII(a) was detected with peroxidase conjugated goat anti-human FXII antibody. Data are expressed as baseline corrected nonlinear fit as mean ± SEM, *n* = 4.

**Supplementary videos 1 – uPA-mediated fibrinolysis in real-time.** Fibrinolysis by exogenous uPA (75 nM) was monitored by fluorescent confocal microscopy of clots containing fibrinogen (2.65 μM, 9% DyLight 488-labelled, green), plasminogen (1.25 μM, 20% DyLight 633-labelled, red), thrombin (0.25 U/ml), $CaCl_2$ (5 mM) in the (A) absence or (B) presence of 328 μM $polyP_{65}$. Images were taken every 15 s. Representative video of *n* = 3, scale bar = 10 μm.

**Supplemental Figure 1**