Polyphosphate, Zn²⁺ and high-molecular-weight kininogen modulate individual reactions of the contact pathway of blood clotting

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Figure S1. Ability of polyP to accelerate FXII autoactivation depends on polyP concentration. In an endpoint assay (stopped at 20 min), 100 nM FXII was incubated with 5 μ M ZnCl₂ (but without HK) plus varying concentrations of polyP₁₂₀₀ (•) or polyP₇₉ (•). Timed aliquots (10 μ L) were removed and quenched in 70 μ L ice-cold Quench Buffer I. Data are mean ± S.E. ($n \ge 3$).

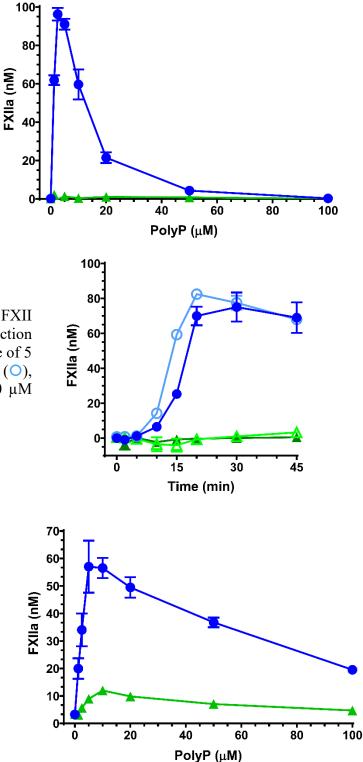
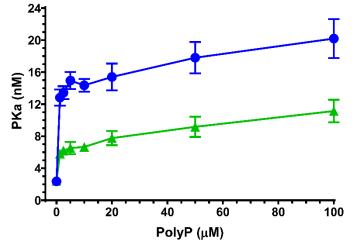


Figure S2. Progress curves of polyP-mediated FXII autoactivation. FXIIa levels were measured as a function of time after incubating 100 nM FXII in the presence of 5 μ M ZnCl₂ (but without HK) plus 2.5 μ M polyP₁₂₀₀ (\bigcirc), 10 μ M polyP₁₂₀₀ (\bigcirc), 10 μ M polyP₇₉ (\triangle), or 100 μ M polyP₇₉ (\triangle). Data are mean ± S.E. ($n \ge 3$).

Figure S3. Ability of long-chain and platelet-size polyP to accelerate FXII activation by PKa depends on polyP concentration. In an endpoint assay (stopped at 4 min), 100 nM FXII and 100 pM PKa were incubated with 10 μ M ZnCl₂, 100 nM HK and varying concentrations of polyP₁₂₀₀ (•) or polyP₇₉ (▲). Data are mean ± S.E. ($n \ge 3$).

Figure S4. Ability of long-chain and platelet-size polyP to accelerate PK activation by FXIIa depends on polyP concentration. In an endpoint assay (stopped at 3 min), 100 nM PK and 100 pM FXIIa were incubated with 10 µM ZnCl₂ (but without HK) plus varying concentrations of $polyP_{1200}$ (\bigcirc) or $polyP_{79}$ (\blacktriangle). Data in all *panels* are mean \pm S.E. ($n \ge 3$).



₹

100

80

PolyP (µM)

PK activation rate (nM/min/nM) 60-45-30-15 0-60 20 40 0

75-

Figure S5. Influence of EDTA on polyP-mediated PK activation by FXIIa. PKa levels were measured as a function of time after incubating 100 nM PK and 100 pM FXIIa (without HK) plus varying concentrations of polyP₁₂₀₀ in the presence of 0 (\bigcirc) or 5 (\blacklozenge) mM EDTA. Initial rates of PK activation (in nM/min) were divided by the FXIIa concentration. Data in are mean \pm S.E. ($n \ge 3$).