Supplementary Methods

Blood collection. Approval for research involving human subjects was obtained from the IRBs of Duke University School of Medicine, the Children's Hospital of Philadelphia, the Perelman School of Medicine, and the University of British Columbia Clinical Research Ethics Board. Blood was collected with informed consent from healthy donors and patients with suspected HIT, free of antiplatelet drugs into either lepirudin (50 μg/mL) or 0.32% citrate (final concentrations).

PPXbd and PPX1. Recombinant *Saccharomyces cerevisiae* exopolyphosphatase (PPX1), an enzyme that specifically degrades polyP via progressive hydrolysis of the phosphoanhydride bonds, was produced as described previously[1] using a plasmid that was a kind gift from Dr. Adolfo Saiardi, University College, London, UK. The recombinant polyP-binding domain from *Escherichia coli* exopolyphosphatase (PPXbd), which binds polyP with high affinity,[2] was produced containing an AviTag biotin acceptor peptide and then biotinylated as previously described.[3]

Effect of PF4 on the susceptibility of polyphosphates to digestion. $PolyP_{130}$ (2 μM), PF4 (5 μg/ml; 160 nM) or PF4/$PolyP_{130}$ complexes were incubated for 30 min at 37^0C in CutSmart Buffer (50 mM postassium acetate 20 mM Tris-acetate, 10 mM magnesium acetate, 100 μg/ml bovine serum albumin, pH 7.9, New England Biolabs) or NEBuffer 4 (as above but contains 1 mM DTT, New England Biolabs), with CIP (20 μg/ml) or BSA control for 30 min at 37^0C. In step 2, BSA, PF4 or CIP was added as indicated in Figure 1C for an additional 30 min at 37^0C. The mixtures were then used to coat Immulon 4 HBX plates for 20 min at RT and binding of 0.1 μg/ml KKO was measured.

Complement activation by PF4/heparin or PF4/polyP complexes. Activation of complement was detected using an ELISA-based capture assay. Briefly, fresh plasma from donors was incubated with buffer or antigen (25 μg/ml PF4 ± 0.25 u/ml UFH or ± 50 μM polyP) for 60 min at 37°C followed by addition of 10 mM EDTA to inhibit further complement activation. Plasma containing antigen and potential activated complement fragments were added to a microtiter plate coated with KKO for 1 hr at 4°C.

After three washes, complement was detected using a biotinylated Ab to C3c (Quidel Corporation, San Diego, CA) followed by streptavidin-HRP (BD Bio Sciences San Jose, CA). In other experiments, 50 μM polyP was preincubated ± scPPX1 (40 μg/ml) in Hepes buffer for 60 min at 37°C prior addition to plasma supplemented with 25 μg/ml PF4.

ELISA to measure IgG and C3 binding to PF4/polyanion complexes by KKO and HIT plasmas. ELISA reactions were performed as described[4]. Briefly, PF4 (5 μg/ml) mixed with various concentrations of $polyP_{14, 60, 130}$ or with a concentration of UFH (0.1 U/ml) optimized for antibody binding was added to wells overnight at room temperature (RT). Unbound protein was removed by washing. KKO (0.1 μg/ml) was added for 30 min at 37^{0}C, followed by washing and serial incubations with HRP-conjugated goat anti-mouse IgG and colorimetric substrate. To measure C3 fixation, experiments were performed as above except that $polyP_{130}$ was used at 3 μM and KKO was used at 3 μg/ml. Human plasma collected in lepirudin diluted 1:80 in 20 mM HEPES, 140 mM NaCl, 0.5 mM $MgCl_2$, 0.45 mM $CaCl_2$, pH 7.4 was added for 15 min at RT after the antibody step as a source of complement and binding of anti-C3 was measured. In a third set of experiments, plasmas from 12 patients with anti-PF4/UFH antibodies were added to wells coated with optimal concentrations of PF4/$polyP_{130}$ based on binding of KKO; in some experiments, human plasma was added as a source of complement as above. Binding of anti-human IgG or anti-C3 was measured using HRP-conjugated secondary antibodies.

Dynamic Light Scattering (DLS). PF4 (3-50 μg/mL) and various concentrations of $polyP_{130}$ or UFH, each in 500 μl HBSS, were incubated for 20 min at 25^0 C with gentle agitation (800 rpm, 25°C). HBSS was passed through a 0.22 μm filter and degassed prior to use. Size distribution analysis (DLS) was performed in standard plastic cuvettes (Fisher) as previously described[5] using a Malvern NanoZS Zetasizer (Malvern Instruments) immediately thereafter or at the indicated times. In other experiments, PF4/$polyP_{130}$ complexes were incubated with or without KKO for 1-120 hrs and the size analyses were repeated. In a third set of experiments, PF4/$polyP_{130}$ complexes were

incubated with KKO (1.6-16 μg/ml) for 1 hr followed by addition of 200 U/ml CIP and 10 mM $MgCl_2$ for 0-2 hrs with mild agitation prior to re-analysis.

Endocytosis of PF4/polyP-130 complexes. Endocytosis of PF4/$polyP_{130}$ complexes was assessed using confocal microscopy[6]. Peripheral blood monocytes ($1x10^6$ cells) adherent to fibronectin (Sigma)-coated glass coverslips were washed in PBS, 0.5% human serum albumin (Grifols, Los Angeles CA), 4 mg/mL IVIG (Gammunex-C, Grifols) and 0.1% Tween-20. The cells were incubated with pre-formed complexes containing AF647-PF4 (25 μg/mL) alone or with $polyP_{130}$ (10-100 μM) for 24 hrs at 37°C, washed, fixed with 4% paraformaldehyde, counterstained with anti-CD14 (monocyte marker) and DAPI (nuclear stain). Coverslips were photographed with a Leica SP5 inverted confocal microscope using a 100x oil immersion lens (Leica Microsystems) with a digital zoom (2-10x) and Leica LAS AF 2.6 software. Enumeration of PF4/$polyP_{130}$ complexes and intracellular vesicles were expressed as the mean ± standard deviation. Statistical analyses were performed using GraphPad Prism (Graph Pad Software Version 5.02).

References (Supplement).

1. Wurst H, Kornberg A. A soluble exopolyphosphatase of Saccharomyces cerevisiae. Purification and characterization. *J. Biol Chem.* 1994;269(15):10996-11001.
2. Bolesch DG, Keasling JD. Polyphosphate binding and chain length recognition of Escherichia coli exopolyphosphatase. *J Biol Chem.* 2000;275(43):33814-33819.
3. Zhu S, Travers RJ, Morrissey JH, Diamond SL. FXIa and platelet polyphosphate as therapeutic targets during human blood clotting on collagen/tissue factor surfaces under flow. *Blood.* 2015;126(12):1494-1502.
4. Sachais BS, Litvinov RI, Yarovoi SV, et al. Dynamic antibody-binding properties in the pathogenesis of HIT. *Blood.* 2012;120(5):1137-1142.
5. Suvarna S, Espinasse B, Qi R, et al. Determinants of PF4/heparin immunogenicity. *Blood.* 2007;110(13):4253-4260.
6. Joglekar M, Khandelwal S, Cines DB, Poncz M, Rauova L, Arepally GM. Heparin enhances uptake of platelet factor 4/heparin complexes by monocytes and macrophages. *J Thrombos Haemostas.* 2015;13(8):1416-1427.