

Suppl. Figures to Joglekar et al. “Disruption of PF4/H multimolecular complex formation with a minimally anticoagulant heparin (ODSH)” (Thromb Haemost 2012; 107.4)

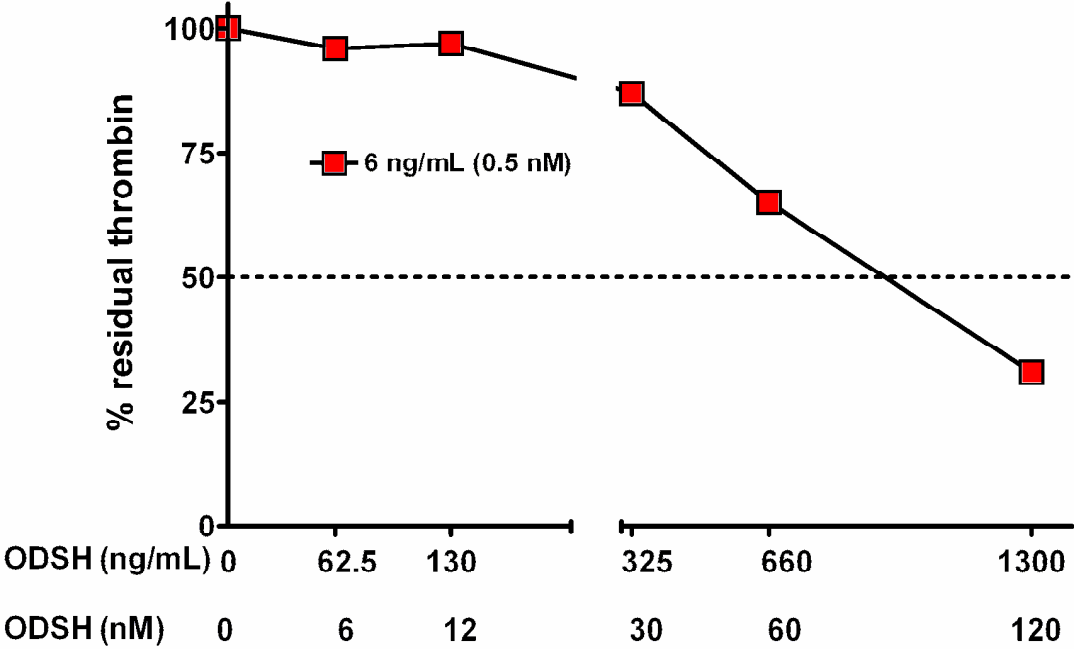


Figure S1: ODSH was added in increasing amounts (0-1300 ng/mL) to a fixed concentration of UFH (6 ng/mL) in a thrombin generation assay. Heparin neutralization was normalized to 100% in the absence of ODSH to demonstrate the effects of ODSH on residual thrombin generation. X-axis shows concentrations of ODSH in ng/mL and nM.

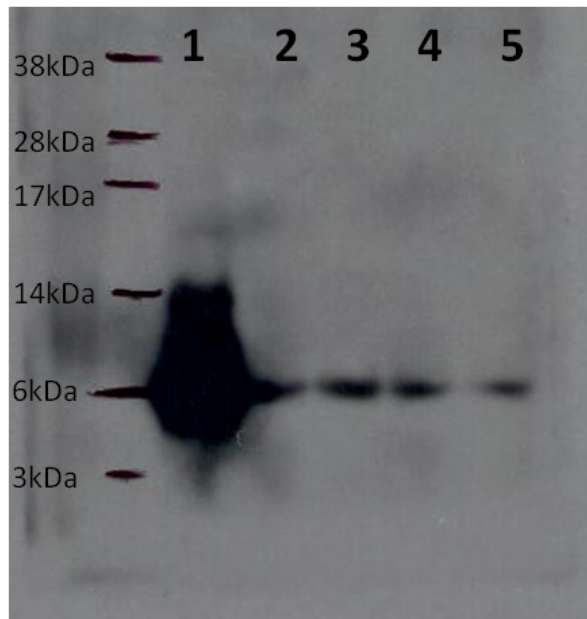


Figure S2: PF4 “eluates” from plate coated with PF4/ODSH or PF4/UFH. Microtiter plates were coated with PF4/ODSH or PF4/H as described in methods. After overnight incubation supernatant was collected and immunoblot of unbound PF4 or “eluate” was performed using goat anti-mouse PF4. Eluates from wells coated with PF4/ODSH (lane 4) and PF4/H (lane 5) were compared with solutions of PF4/ODSH (lane 2) or PF4/H (lane 3) used for coating. Recombinant mPF4 was used as positive control (24ug in Lane 1; Mw is ~7.8kDa for PF4 monomer).

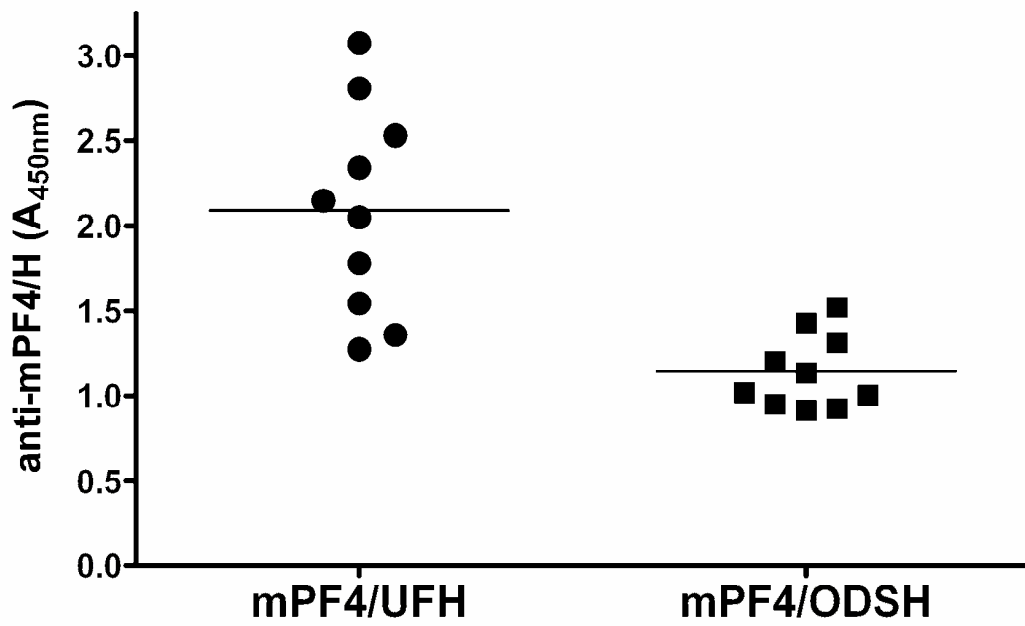


Figure S3: Immunogenicity of mPF4/H and mPF4/ODSH complexes. Mice (n=10/cohort) were injected with complexes of mPF4 (100 $\mu\text{g}/\text{mL}$) and UFH (36 $\mu\text{g}/\text{mL}$, 2.4 μM) or mPF4 (100 $\mu\text{g}/\text{mL}$) and ODSH (26 $\mu\text{g}/\text{mL}$, 2.4 μM) according to immunization protocol outlined in methods. On day 16 of immunization protocol, mice were bled and plasma analyzed for antibodies to mPF4/H. Mean anti-PF4/H ($A_{450\text{nm}}$) for mice injected with mPF4/UFH was 2.1 ± 0.61 as compared to mice injected with mPF4/ODSH 1.1 ± 0.22 .

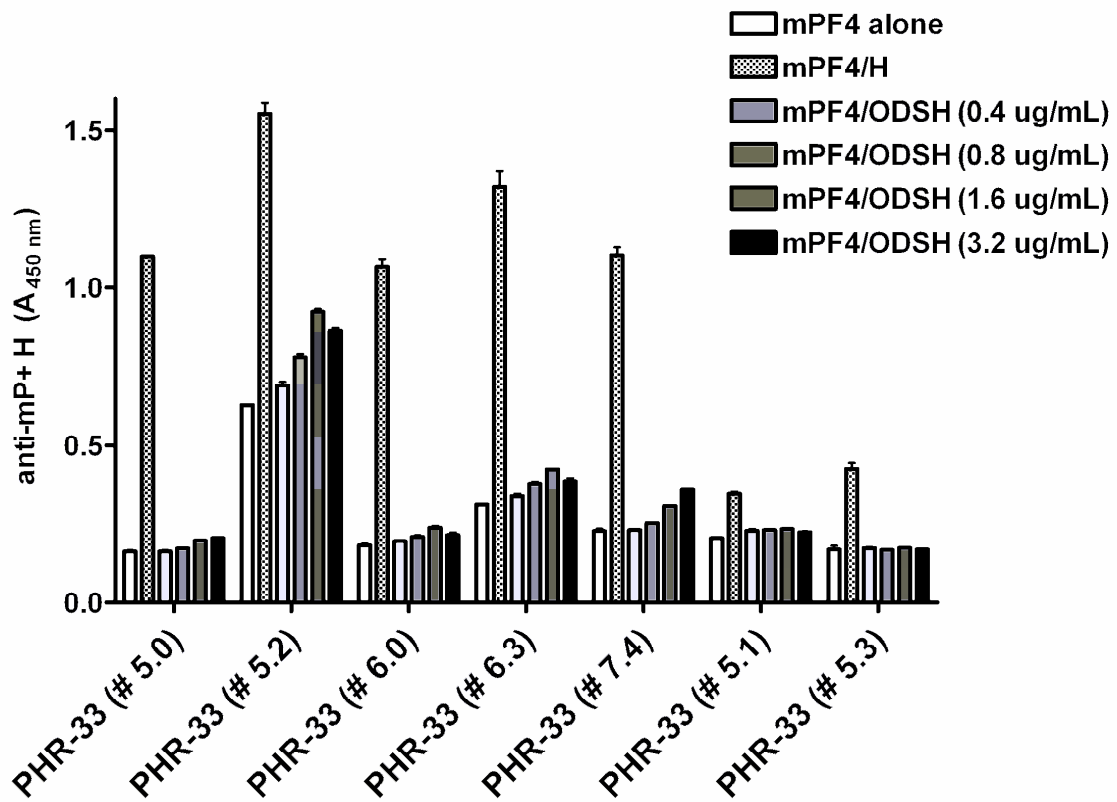


Figure S4: Cross-reactivity of anti-mPF4/H with mPF4/ODSH. Plasma from mice injected with mPF4+UFH were incubated with microtiter wells coated with mPF4/H or wells coated with varying concentrations of mPF4 and ODSH. Antibody binding was measured by ELISA. Each data point represents duplicate measurements.