Supporting Information Table of Contents

1 Materials

Ethanol (200 proof), tetraethyl orthosilicate (TEOS), and ammonia NH_4OH (28 wt%) were supplied by Sigma Aldrich. Polyphosphate was purified from P70 (BK Guilini GmbH, Germany). Deionized water was obtained using a Milli-Q water purification system (Millipore). Frozen citrated pooled normal plasma (PNP) and Factor XII-deficient plasma were purchased from George King Biomedical (Overland Park, KS) and handled according to package instructions. Phospholipid solutions in chloroform were purchased in Avanti Polar Lipids: L-α-phosphatidycholine (PC) and L-α-phosphatidylserine (PS). Sodium chloride, potassium chloride, sodium phosphate, dibasic and potassium phosphate were also purchased from BK Guilini GmbH (Germany).

Polyphosphate was obtained by solubilizing the P70 in 250 mM LiCl/50 mM $LiBO_3$, pH 10.5 at 100 °C for 10 min. The resulting crude material was then purified by isopropanol precipitation, with fractions characterized by Western blotting.

2 Synthesis of silica nanoparticles and polyphosphate coated silica nanoparticles

SNPs were synthesized following a modified Stöber method.[1] In a typical synthesis, TEOS and ammonia were added consecutively, dropwise into 57 mL of ethanol (EtOH) while stirring at 300 rpm at room temperature. Stirring was continued for 24 h. Differing amounts of TEOS (0.5 – 4 mL) and ammonia (0.5 – 4 mL) were used to produce a library of selectively sized nanoparticles with different diameters. To produce the desired 55 nm SNP, 5 mL TEOS were added dropwise to 57 mL EtOH followed by 3 mL NH_4OH (28 wt%). pH and particle size were measured directly after synthesis, in ethanol. The materials were recovered by centrifugation (14 k, 30 min) and washed three times with ethanol to remove impurities. After redispersing in ethanol by sonication (bath sonicator, Model FS20 Fisher Scientific), the products were dried overnight at 60 ºC. The powder was homogenized then calcined at 550 ºC for 4 h.

To prepare the polyP-coated SNP (polyP-SNP) the synthesized silica nanoparticles were first dispersed by sonication in Milli-Q water and placed at 30 ºC. Polyphosphate powder was added under vigorous stirring and the solution stirred overnight. The functionalized materials were recovered by two rounds of centrifugation (14 k, 30 min), each time washed with water and redispersed by sonication. PolyP-SNP products were dried overnight at 60 ºC. Successful modification was determined using dynamic light scattering (DLS) and zeta potential. Using PBS as the solvent at pH 7.4, functionalizing the SNP with P70 increased particle size roughly 15 nm.

[1] Stöber, W., Fink, A., Bohn, E. *Journal of Colloid and Interface Science.* **1968**, 26, 62–69.

3 Characterization of the particles

3.1 Zeta potential and particle size determination. Particle sizes and zeta potentials were measured by laser diffractometry using a Zetasizer Nano ZS instrument (ZEN 3600, Malvern Instruments) at 20 ºC with an incident wavelength of 633 nm and 173 º backscattering angle. Zeta potentials were measured in water at different pH values and in PBS buffer (approximately 137 mM NaCl, 2.7 mM KCl, and 12 mM phosphate, pH 7.4). Particle size was measured just after synthesis (with ethanol as a solvent) and again after the calcination step, in ethanol at 1 mg/mL. Disposable cuvettes were cleaned with ethanol and water prior to sample loading.

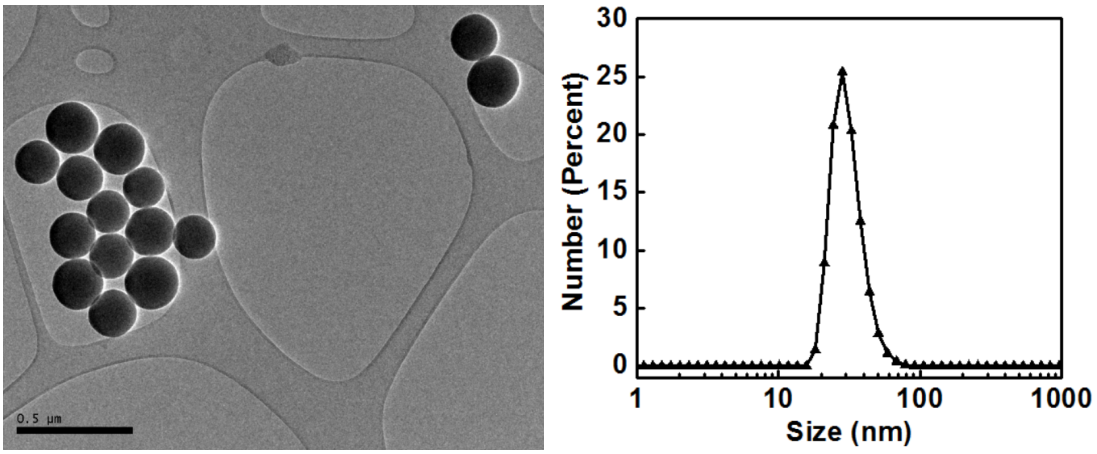

Figure 1. (Left) TEM images of SNPs;
(Right) Size of SNPs after synthesis in ethanol measured using DLS.

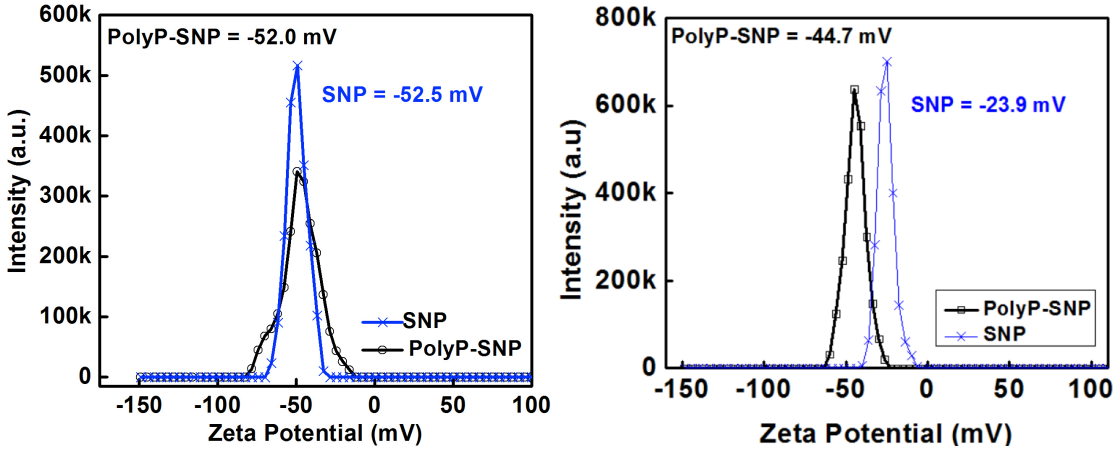

Figure 2. (Left) Both SNPs and polyP-SNP have a zeta potential of roughly -50 mV in PBS, pH 7.4;
(Right) Zeta potential of polyP-SNP and SNP in deionized water.

3.2 Morphology and structure of the particles. The size, morphology, and structure of representative samples were observed via transmission electron microscopy (TEM). TEM micrographs were obtained on a FEI Tecnai G2 Sphera electron microscope with an accelerating voltage of 200 kV.

3.3 Digestion of polyP-SNP particles into phosphate monomers for malachite green assay. PolyP content on the particles was quantified by hydrolysis to monophosphate. Calf intestinal alkaline phosphatase (CIAP, a potent exopolyphosphatase) was added to PolyP at 37 ºC, followed by phosphate analysis using malachite green microassay.[2] The polyphosphate is quantified in units of phosphate monomer (MW 102).

3.4 Quantification of the polyphosphate on the nanoparticles. Further quantification used inductively coupled plasma atomic emission spectroscopy (ICP-AES). 170.35 mg polyP-SNP particles were digested in 1M hydrochloric acid at 100 ^{o}C for several hours to break polyP into phosphate ions. The resulting solution was decanted to remove the phosphorous-rich supernatant from solid SNPs. ICP-AES tests on the supernatant concentration revealed 1.5624 ppm P. The ppm ratios were converted to nmol PO_3/mg SNP and the resulting ratio was found to be 29.6 nmol PO_3/mg SNP, which correlates to the 26 nmol PO_3/mg SNP determined through malachite green assay. Digestion of other polyP-SNP samples in hydrofluoric acid also identified the presence of phosphorous in the silica nanoparticles (Table 2). However, the samples digested in HF were too dilute to reliably report the phosphorous concentration quantitatively.

Table 1. Concentration of P (20x dilution) in a 170.35 mg polyP-SNP sample digested in 1 M HCl as measured by ICP-AES

Element	Wavelength (nm)	Units	Avg	Std Dev	RSD
Phosphorous	177.495	ppm	1.574	0.0105	0.6675
Phosphorous	178.284	ppm	1.563	0.0038	0.2463
Phosphorous	178.766	ppm	1.56	0.0054	0.344
Phosphorous	213.618	ppm	1.565	0.0123	0.7855
Phosphorous	214.914	ppm	1.55	0.0066	0.4243
Average		ppm	**1.562**		

[2] Zhou, X., Arthur, G. *Journal of Lipid Research.* **1992**, 33, 1233–1236.
Carter, S. G., Karl, D. W. *Journal of Biochemical and Biophysical Methods.* **1982**, 7, 7–13.

Table 2. Concentration of P (9x dilution) in a 79.10 mg polyP-SNP sample digested in 1 M HCl as measured by ICP-AES

Element	Wavelength (nm)	Units	Avg	Std Dev	RSD
Phosphorous	177.495	ppm	11.06	0.0105	0.6675
Phosphorous	178.284	ppm	11.08	0.0038	0.2463
Phosphorous	178.766	ppm	11.08	0.0054	0.344
Phosphorous	213.618	ppm	11.06	0.0123	0.7855
Phosphorous	214.914	ppm	11.04	0.0066	0.4243
Average		ppm	**11.06**		

3.5 ^{31}P NMR (Nuclear Magnetic Resonance). The decanted phosphate sample was dried and resuspended in deuterium oxide. The resulting suspension was measured using an Agilent Technologies 400 MHz, 400-MR DD2 Spectrometer. A ^{31}P NMR spectra showed evidence of phosphate from the digested polyP-SNP sample.

3.6 Determination of the clotting activity. The clotting activity was determined by two methods: standard coagulometry and rotational thromboelastometry (TEG) (Thromboelastograph model TEG® 5000, Haemonetics). These tests measure several parameters that are relevant to coagulation, including initial time for clot formation (R, min), rate of clot formation (α, deg), time until clot reaches 20 mm (K, min) and clot strength (maximum amplitude (MA), mm). For these tests, the particles were dispersed in HEPES buffered saline (HBS) containing phospholipid (PL) vesicles and sonicated. The phospholipids were a mixture of 80 % phosphatidycholine (PC) and 20 % phosphatidylserine (PS) prepared by sonication of chloroform stocks in HBS, following the protocol by Morrissey et al.[3] All subsequent dilutions were made by diluting the stock dispersion in this same solvent. HBS is 100 mM HEPES (Sigma-Aldrich), 20 mM HEPES/NaOH buffer (pH 7.5), and 0.02 % (w/v) sodium azide (Sigma-Aldrich).

For the coagulometry tests, 50 µL of the particles were placed into a pre-warmed coagulometer cuvette followed by 50 µL of pooled normal plasma. After incubating for 33 minutes at 37 ºC, the contact pathway was activated and the mixture equilibrated to the chosen temperature, then 50 µL of pre-warmed 25 mM $CaCl_2$ was added into the cuvette. The results are the average values from duplicate runs.

[3] Morrissey, J. H. Morrissey lab protocol for preparing phospholipid vesicles (SUV) by sonication. 2001, 8–10. http://tf7.org/suv.pdf

In the TEG experiments, first 340 μL of plasma and 10 μL of the clotting agent were added in the TEG cup and incubated at 37 ºC. After 3 minutes, 20 μL of 0.2 M $CaCl_2$ were added to the cup and the test was started immediately. Concentration- and size-dependent analyses were performed. The results shown are the average value of, typically, 4 to 6 replicates.

We also monitored the formation of thrombin by fluorescence plate assay, using a thrombin-sensitive dye T-butyloxycarbonyl-b-benzyl-L-aspartyl-L-prolyl-L-arginine-4-methyl-coumaryl-7-amide (Boc-Asp(OBzl)-Pro-Arg-MCA)Boc-Asp(OBzl)-Pro-Arg-MCA (Peptides International, Louisville, KY).[4] This assay is used to calculate the thrombin burst time, which is related to clot formation time.

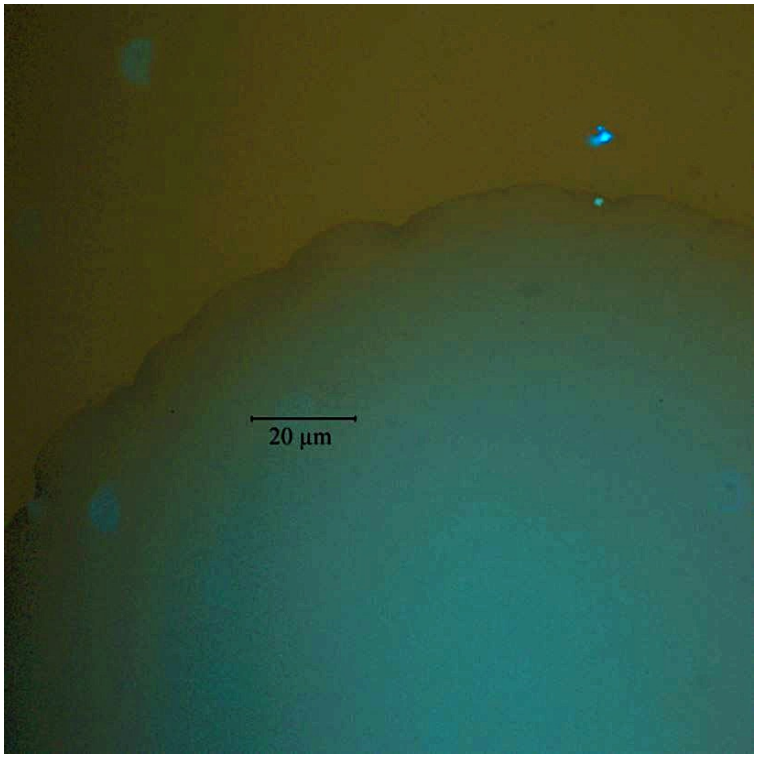

Figure 3. Upon addition of polyP-SNP to recalcified plasma, rapid thrombin generation is seen. The blue color indicates cleavage of the fluorescent dye by thrombin, signifying active coagulation.

[4] Kawabata, S.-I., Miura, T., Morita, T., Kato, H., Fujikawa, K., Iwanaga, S., Takada, K., Kimura, T., Sakakibara, S. *European Journal of Biochemistry / FEBS.* **1988**, 172, 17–25.
Kastrup, C. J., Shen, F., Runyon, M. K., Ismagilov, R. F. *Biophysical Journal.* **2007**, 93, 2969–77.

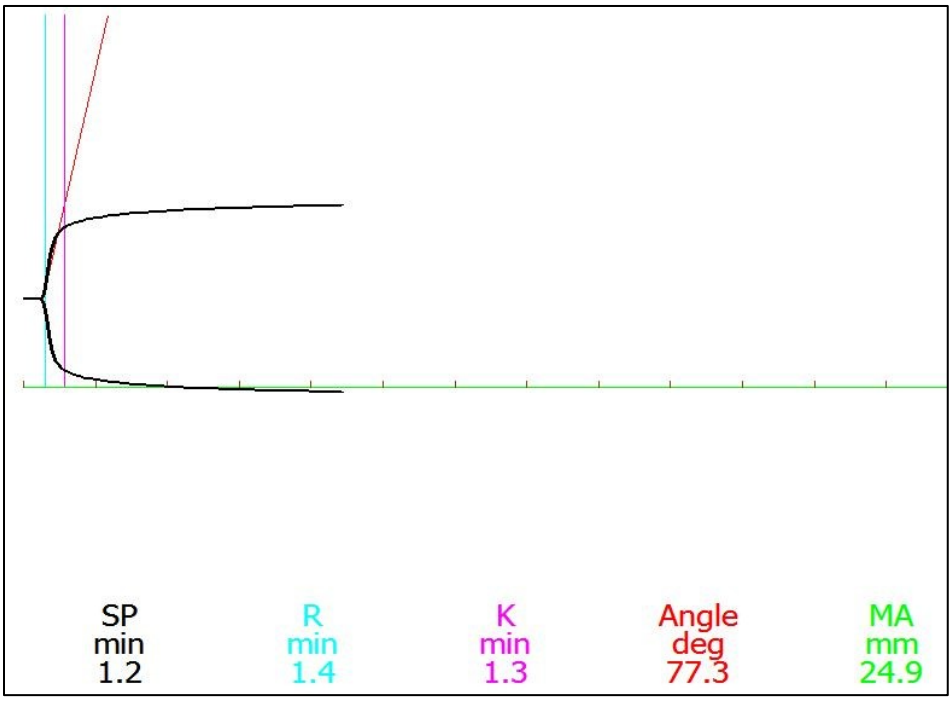

Figure 4. Sample thromboelastography curve (colors inverted from instrument output)

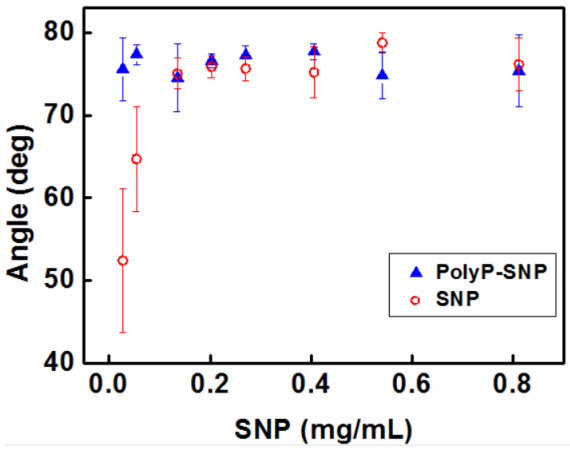

Figure 5. The angle value (α) for thromboelastography measurements correlates to the rate of clot growth. At low concentrations, polyP-SNP-induced clots grow faster than SNP-induced clots.

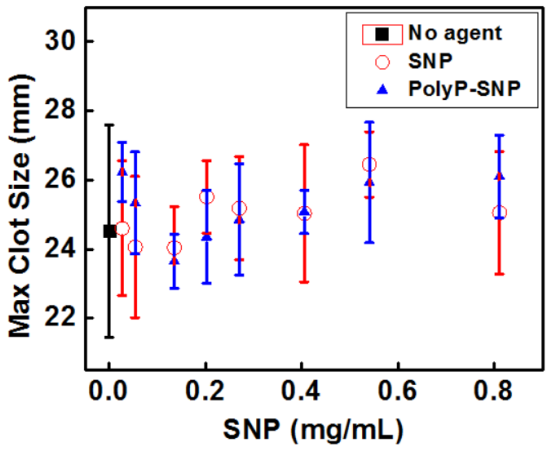

Figure 6. The agent used had minimal impact on the maximum size of the clot formed in plasma.

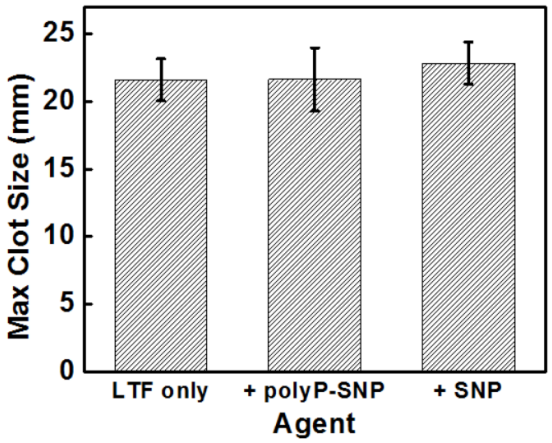

Figure 7. The maximum clot size formed in FXII deficient plasma was nearly identical regardless of the agent used.