The Comparison of Reversal Activity and Mechanism of Action of UHRA, Andexanet and PER977 on Heparin and Oral FXa Inhibitors

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Supplemental Data

METHODS

Reagents

Unfractionated heparin (LEO Pharma Inc. Canada), enoxaparin (Lovenox®, Sanofi-Aventis Canada), fondaparinux (Arixtra®, GlaxoSmithKline Canada) were commercial products purchased from the manufacturers. Edoxaban and rivaroxaban were kindly provided by Portola Pharmaceuticals Inc. Human fibrinogen (Fibrinogen) and N-2-hydroxyethyl piperazine-N'-2-ethanesulfonic acid (HEPES) were obtained from Sigma-Aldrich. Human proteins such as human FXa, thrombin (IIa), FIX, FIXa and antithrombin were obtained from Haematologic Technologies Inc. Ciraparantag (PER977) and Andexanet alfa were provided by Portola Pharmaceuticals Inc. Methods for synthesizing PER977 are detailed below. The UHRA molecule (UHRA-7) used for this study was synthesized as described in Shenoi et al.[1] Polystyrene 96-well microplates (Costar) used for all clotting assays were purchased from Corning.

Synthesis of PER977

The synthesis of PER977, (2S,2'S)-N,N'-(piperazine-1,4-diylbis(propane-3,1-diyl)bis(2-amino-5-guanidinopentanamide) (L-enantiomer), was carried out as described in the patent US2013/0137702. PER977, (2S,2'S)-N,N'-(piperazine-1,4-diylbis(propane-3,1-diyl)-bis-(2-amino-5-guanidinopentanamide) was synthesized in two steps via coupling of 3,3'-(piperazine-1,4-diyl)-bis-(propan-1-amine) and (S)-5-(1,3-Bis((benzyloxy)carbonyl)guanidino)-2-((tert-butoxy-carbonyl)amino)pentanoic acid (S-Boc-DiBoc-Arg-OH) followed by removal of Boc protecting group. The product was characterized by proton NMR and mass spectrometry analysis to confirm the structural identity (Supplemental Figure S1), and the purity was determined by HPLC analysis.

Isothermal Titration Calorimetry (ITC)

All ITC experiments were performed in phosphate buffered saline (PBS) pH 7.40 (± 0.1) (Gibco). To enhance solubility, rivaroxaban and edoxaban titrations were performed in 1% DMSO solutions prepared from 10 mM stock solutions in DMSO. UFH, enoxaparin or fondaparinux samples were prepared by dilution into PBS of concentrated stock solutions in isotonic NaCl. Andexanet (Andexanet) was received as reconstituted frozen solution in formulation buffer (10 mM Tris, 45 mM arginine HCl, 2% sucrose, 5% mannitol, 0.01% Tween 80, pH 7.8). PER977 was received in saline. Stock solutions of andexanet and PER977 were buffer exchanged into PBS. Fibrinogen (Sigma F4883) was received as lyophilized powder and prepared in PBS, followed by gel filtration using a GE PD MidiTrap G25 column. Factor Xa, IX and Factor IXa (Haematologic Technologies Inc.) were prepared from stock solutions in 50% (v/v) glycerol/water by dialysis into PBS using a Pierce Slide-a-lyzer 10kD cassette. All solutions were degassed with stirring at room temperature before loading in the ITC cell.

ITC experiments were performed using a MicroCal iTC200 (Malvern Instruments). UHRA experiments were performed by injecting consecutive 2 µL aliquots of ligand solution (200 – 220 µM UFH, enoxaparin or fondaparinux) into the ITC cell (volume = 200 µL) containing UHRA (8 - 10 µM). Andexanet titrations were performed by injecting consecutive 2 µL aliquots of ligand solution (40 µM rivaroxaban, 100 µM edoxaban, or 400 µM UFH, enoxaparin or fondaparinux) into AnXa (5 –36 µM). PER977 titrations were performed by injecting consecutive 2 µL aliquots of PER977 solution (200 µM - 1.82 mM) into rivaroxaban, edoxaban, fibrinogen, FXa, FIX or FIXa (20 –182 µM). PER977 titrations were also conducted by injecting consecutive 2 µL aliquots of ligand solution (400 µM UFH, enoxaparin or fondaparinux; various concentrations

of rivaroxaban or edoxaban up to 1.5 mM) into PER977 (5 – 200 µM). The ITC data were corrected for the heat of dilution of the titrant by subtracting mixing enthalpies for 2 µL injections of ligand into buffer. At least 2 independent titration experiments were performed for each system at 25°C. The binding stoichiometry, *N*, and K_d for each measured interaction were determined by fitting the corrected data to a standard bimolecular interaction model.[2,3]

Fibrin Polymerization Assay

PER977 or andexanet (15 µL at various concentrations) was added to 135 µL of purified fibrinogen (3 mg/mL, initial concentration) in 20 mM HEPES (pH 7.4, 150 mM NaCl) buffer. Clotting at 37°C was initiated in 100 µL of this solution by adding 5 µL of $CaCl_2$ (3 mM, final) and 5 µL of IIa (2.5 NIHU/mL, final). Fibrin formation was monitored by recording changes in turbidity (A_{405}) every 30 seconds on a Spectramax microplate reader (Molecular Devices) for 1 hour at 37°C. Final turbidity of fibrin clots was also measured.

Scanning Electron Microscopy (SEM) of Fibrin and Whole-Blood Clots

The morphology of clots formed in the presence of andexanet or PER977 was determined by SEM. All samples were randomly coded and blinded to the individual performing imaging analyses to avoid bias. Fibrin clots were prepared in sterile, 5 mL round-bottom polypropylene tubes (BD Falcon) by mixing 200 µL of human fibrinogen (3 mg/mL, initial concentration) in 20 mM HEPES (pH 7.4 and 150 mM NaCl) buffer with 2.5 NIHU/mL thrombin (final), 3 mM $CaCl_2$ (final) and andexanet or PER977 (in HEPES buffer). Control clots were prepared in the absence of anticoagulant or antidote. After incubation for 1 hour at 37°C, clots were fixed using Karnovsky fixative (2.5% glutaraldehyde and 4% formaldehyde), repeatedly washed with 0.1 M sodium cacodylate buffer (pH=7.4), then subjected to post-fixation with 1% v/v osmium

tetroxide. The fixed samples were washed three times with distilled water and dehydrated with a gradient series of ethanol (20-95% v/v). Clots were then dried with CO_2 in a Tousimis Autosamdri 815B critical-point dryer, mounted onto stubs, and gold sputter-coated for SEM using a Hitachi S-4700 field emission microscope at different magnifications. Multiple images from different areas of each clot were captured. Fiber diameters of clots were measured with ImageJ.[4] For fibrin fiber diameter calculations, images from two independent experiments were analyzed. Fibrin fiber diameters (n = 60) from 8 separate areas of each clot were used to calculate the mean fiber diameter.

Blood clots were also prepared in 5 mL polypropylene tubes by incubating 180 µL of non-citrated blood with or without edoxaban (200 ng/mL, final) and with or without 20 µL of andexanet or PER977 in 20 mM HEPES buffer at 37°C. Blood clots were then processed for SEM. Fiber diameters of clots were measured with ImageJ. Fibrin fiber diameter calculations were performed as described previously.[5,6]

Anticoagulant Neutralization Assays

Activated Partial Thromboplastin Time (aPTT) assay

Andexanet, UHRA or PER977 solutions were prepared in 20 mM HEPES-buffered saline (150 mM NaCl, pH=7.4). In order to avoid potential interference of citrate with PER977 activity, non-anticoagulated whole blood was mixed with UFH or enoxaparin (2 IU/mL, final concentration) and then centrifuged at 3000 rpm (1735 xg) for 5 minutes to prepare heparinized plasma. The anticoagulant neutralization activity was examined by mixing 20 µL of andexanet, UHRA or PER977 solution with 180 µL of heparinized plasma (1:10 v/v). The final concentration of antidotes in plasma ranged from 25 to 200 µg/mL. aPTT reagent (200 µL) was then added to the

sample and 100 µL of this resulting mixture was transferred to cuvette-strips at 37°C. The clotting time was measured on a STart®4 coagulometer (Diagnostica Stago, France). 20 mM HEPES-buffered saline (150 mM NaCl, pH=7.4) added to heparinized plasma was used as a control for the experiments. All experiments were performed in triplicate and the average values (mean ± standard error of the mean) are reported.

Microplate-based whole blood clotting assay

In an eppendorf tube, 5 µL of antidote was mixed with 45 µL of heparinized whole blood (1 IU/mL). 5 µL of this mixture was then deposited to the bottom edge of the wells of a 96-well microplate, and the mixture is spread around the edge of the wells using a P20 pipette. After incubation at 37°C for 90 minutes, circular shaped clots were obtained. The preparation of halo/circular shaped blood clots in a 96-well microplate has been previously reported by Bonnard T *et al*.[7] The clotting reaction is halted by adding 95 µL of 20 mM HEPES buffer (150 mM NaCl, pH=7.4). After shaking the plate for 30 seconds in a Spectramax M3 plate reader, 50 µL of the mixture is transferred into wells of a 96-well microplate containing 50 µL of 20 mM HEPES buffer (150 mM, pH=7.4). The absorbance values of wells at 510 nm were measured. The percentage of stable clots formed was calculated using the equation:

$$\left[\mathbf{1} - \left(\frac{\mathbf{Absorbance_{UFH+antidote}} - \mathbf{Absorbance_{Buffer\ control}}}{\mathbf{Absorbance_{UFH\ control}} - \mathbf{Absorbance_{Buffer\ control}}}\right)\right] \times \mathbf{100}$$

Chromogenic Anti-FXa assay

The reversal of Anti-FXa activity of rivaroxaban or edoxaban by andexanet or PER977 was measured by a modified chromogenic Anti-FXa assay protocol previously described.[8] Bovine FXa

(Coamatic, DiaPharma) was reconstituted with distilled water (5 mL), and S2732 (FXa substrate; Coamatic, DiaPharma) was reconstituted with distilled water (2.5 mL). All experiments were performed in pooled citrated platelet-poor plasma (Affinity biologicals, Inc.). Plasma samples containing edoxaban or rivaroxaban were treated with increasing concentrations of antidotes, and incubated at room temperature for 5 minutes. 75 µL of this mixture was then transferred into the wells of the 96-well microplate. 25 µL of assay buffer and 25 µL of S2732 were then added. After incubation at room temperature for 15 minutes, 25 µL of bovine FXa was added. After incubation at room temperature for 5 minutes, the reaction was quenched by adding 20 % acetic acid (50 µL). The plate was then read at 405 and 490 nm, respectively. The absorbance of plasma samples without bovine FXa was used for background subtraction. The Anti-FXa activity of edoxaban or rivaroxaban (ng/mL) in plasma samples was calculated by interpolation of the standard curve generated with known concentrations of each anticoagulant.

Thromboelastography (TEG)

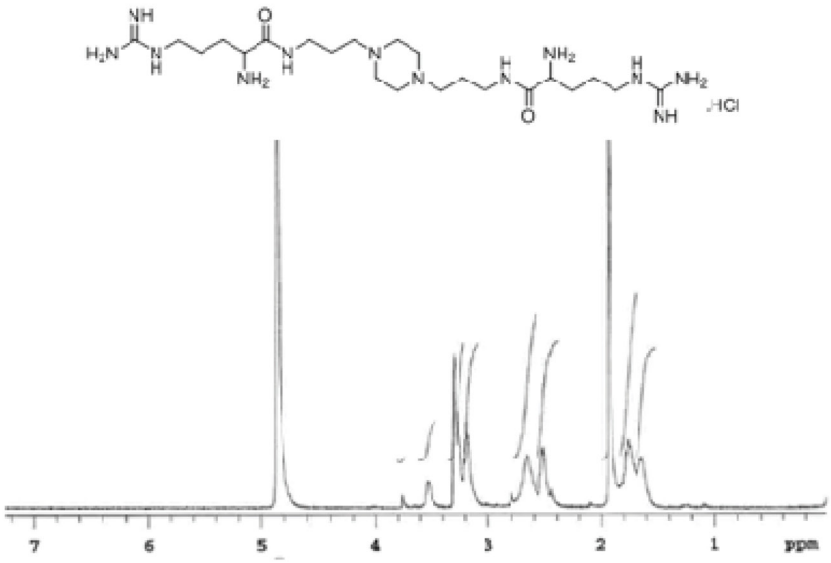
Non-citrated whole blood was mixed with edoxaban or rivaroxaban (200 ng/mL, final). The neutralization of anticoagulant activity of edoxaban or rivaroxaban by PER977 or andexanet was then analyzed using a Thromboelastograph Hemostasis System 5000 (TEG) (Haemoscope Corporation) at 37°C. Anticoagulated whole blood (360 µL) was mixed with each antidote (40 µL) to give a final concentration of 0.1 mg/mL. 360 µL of this blood mixture was transferred into a TEG cup within 5 minutes of blood draw and the coagulation then monitored. HEPES-buffered saline and edoxaban or rivaroxaban-anticoagulated blood were used as controls.

Antidote	Molecular weight	µg/mL	µM
UHRA	23 kDa	25	1.1
		50	2.2
		100	4.3
		200	8.7
Andexanet	41.6 kDa	25	0.6
		50	1.2
		100	2.4
		200	4.8
		500	12
PER977	512 Da	25	48.8
		50	97.6
		100	195.3
		200	390.6
		500	976.5

kDa= kilodaltons; Da =daltons

Supplemental Table S1. Concentration of antidotes expressed in µg/mL and µM, respectively.

A



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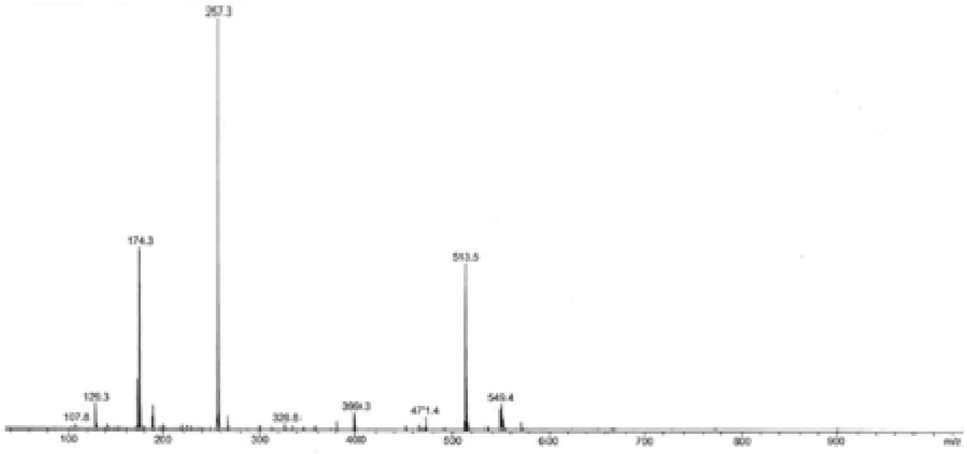
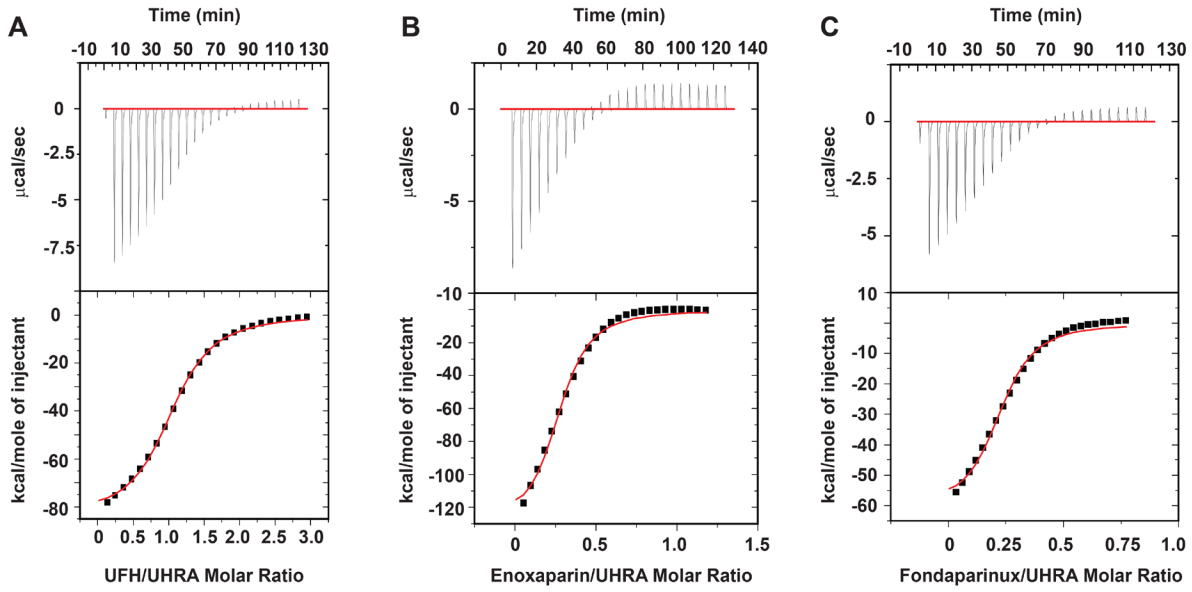
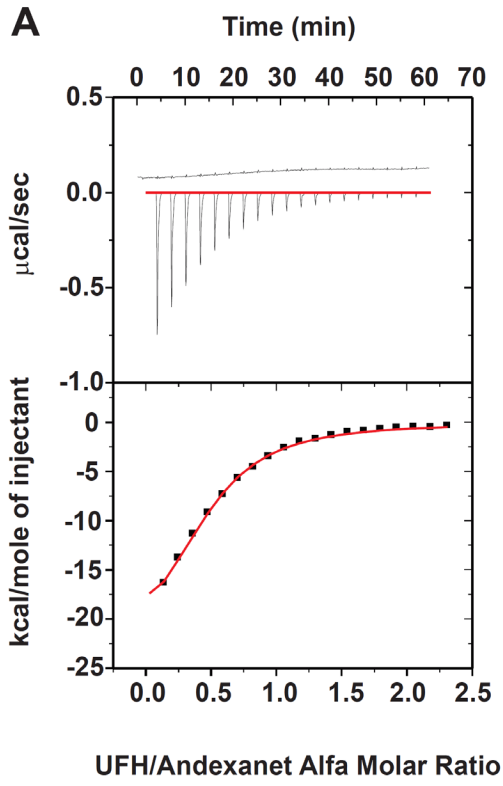


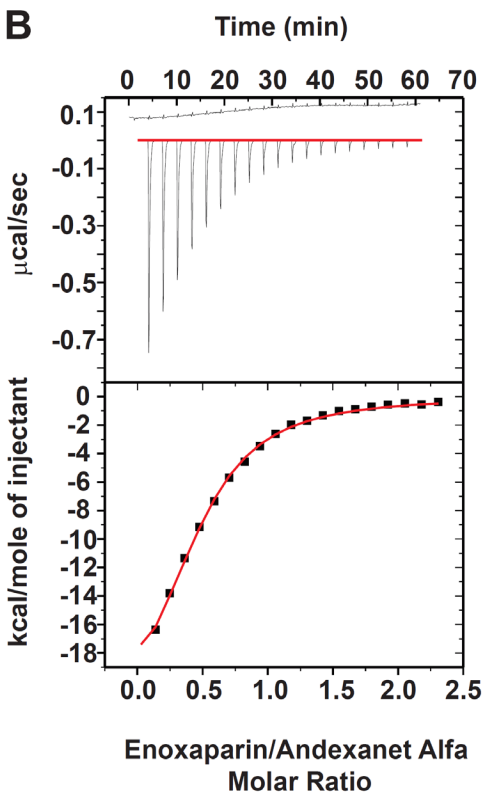
Supplemental Figure S1. Characterization of PER977 Structure: (A) ^{1}H NMR spectrum of PER977.HCl (PRT066919-7) in D_2O and **(B)** ESI-MS spectrum (positive mode) of PER977.HCl

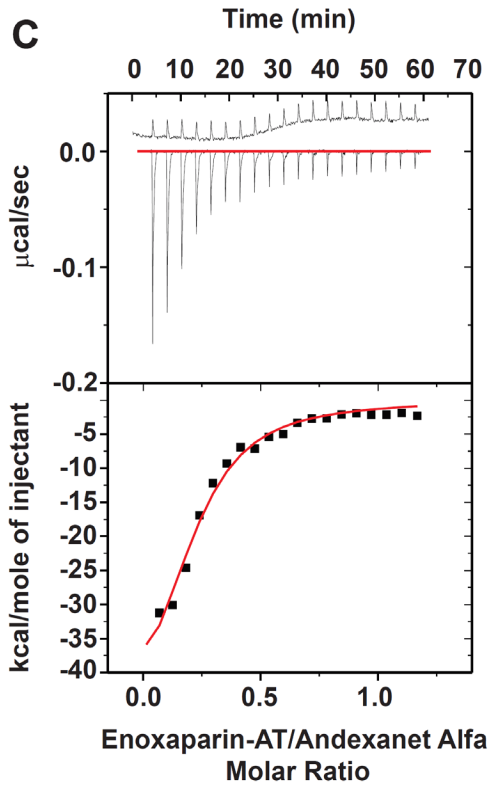
(PRT066919-7) confirming structure identity. The structure of PER977 molecule is reported by Ansell et al.[9]



Supplemental Figure S2. Isothermal Titration Calorimetry (ITC) for UHRA binding to heparin-based anticoagulants. Raw (top panel) and cumulative (bottom panel) heat data for titrations at 25°C. **(A)** 200-µM UFH into 10-µM UHRA; **(B)** 220-µM enoxaparin into 10-µM UHRA; and **(C)** 220-µM fondaparinux into 10-µM UHRA. All differential heat peaks for 2 µL injections; buffer: PBS with 1% DMSO. Molecular weights used for stoichiometry calculations were: UHRA: 25 kDa, UFH: 15 kDa, enoxaparin: 4.5 kDa, fondaparinux: 1728 Da.


A
Time (min)
0 10 20 30 40 50 60 70
µcal/sec
0.5
0.0
-0.5
-1.0
kcal/mole of injectant
0
-5
-10
-15
-20
-25
0.0 0.5 1.0 1.5 2.0 2.5
UFH/Andexanet Alfa Molar Ratio


B
Time (min)
0 10 20 30 40 50 60 70
µcal/sec
0.1
-0.1
-0.3
-0.5
-0.7
kcal/mole of injectant
0
-2
-4
-6
-8
-10
-12
-14
-16
-18
0.0 0.5 1.0 1.5 2.0 2.5
Enoxaparin/Andexanet Alfa
Molar Ratio


C
Time (min)
0 10 20 30 40 50 60 70
µcal/sec
0.0
-0.1
-0.2
kcal/mole of injectant
-5
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-25
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-35
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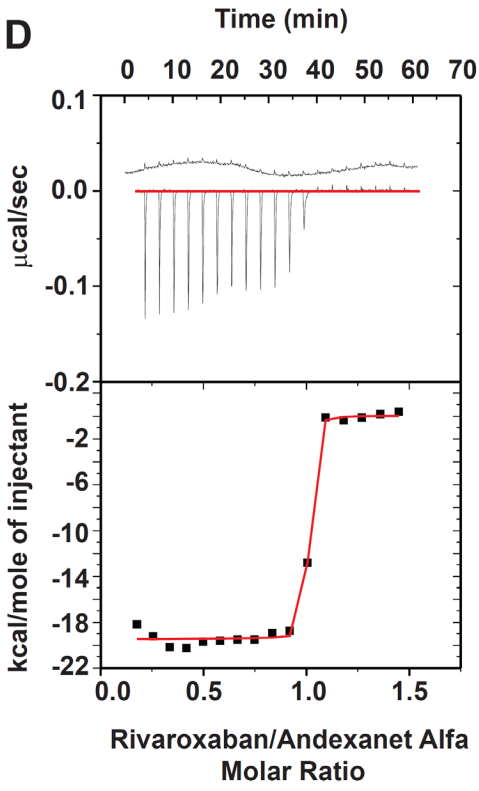
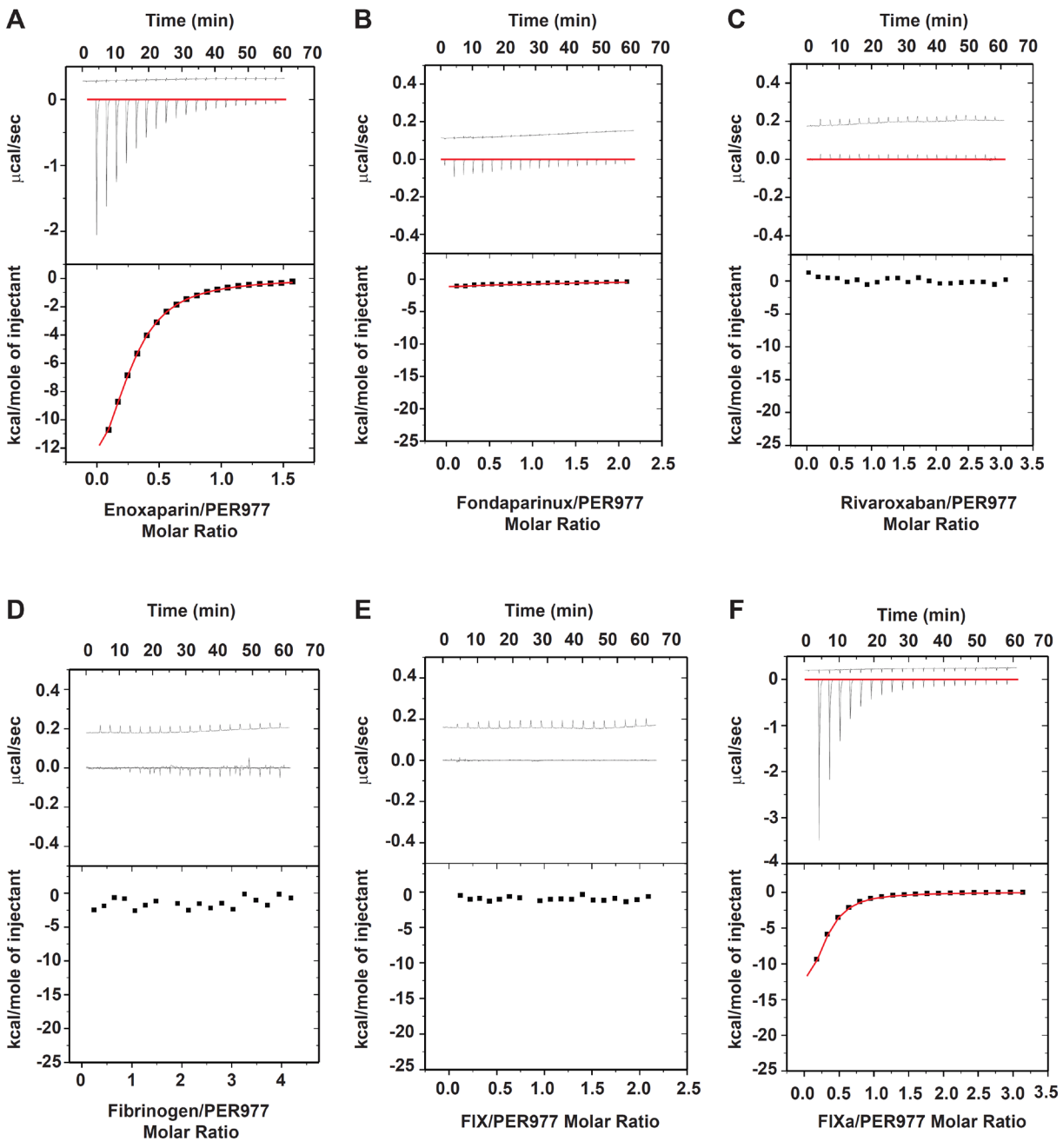
Enoxaparin-AT/Andexanet Alfa
Molar Ratio

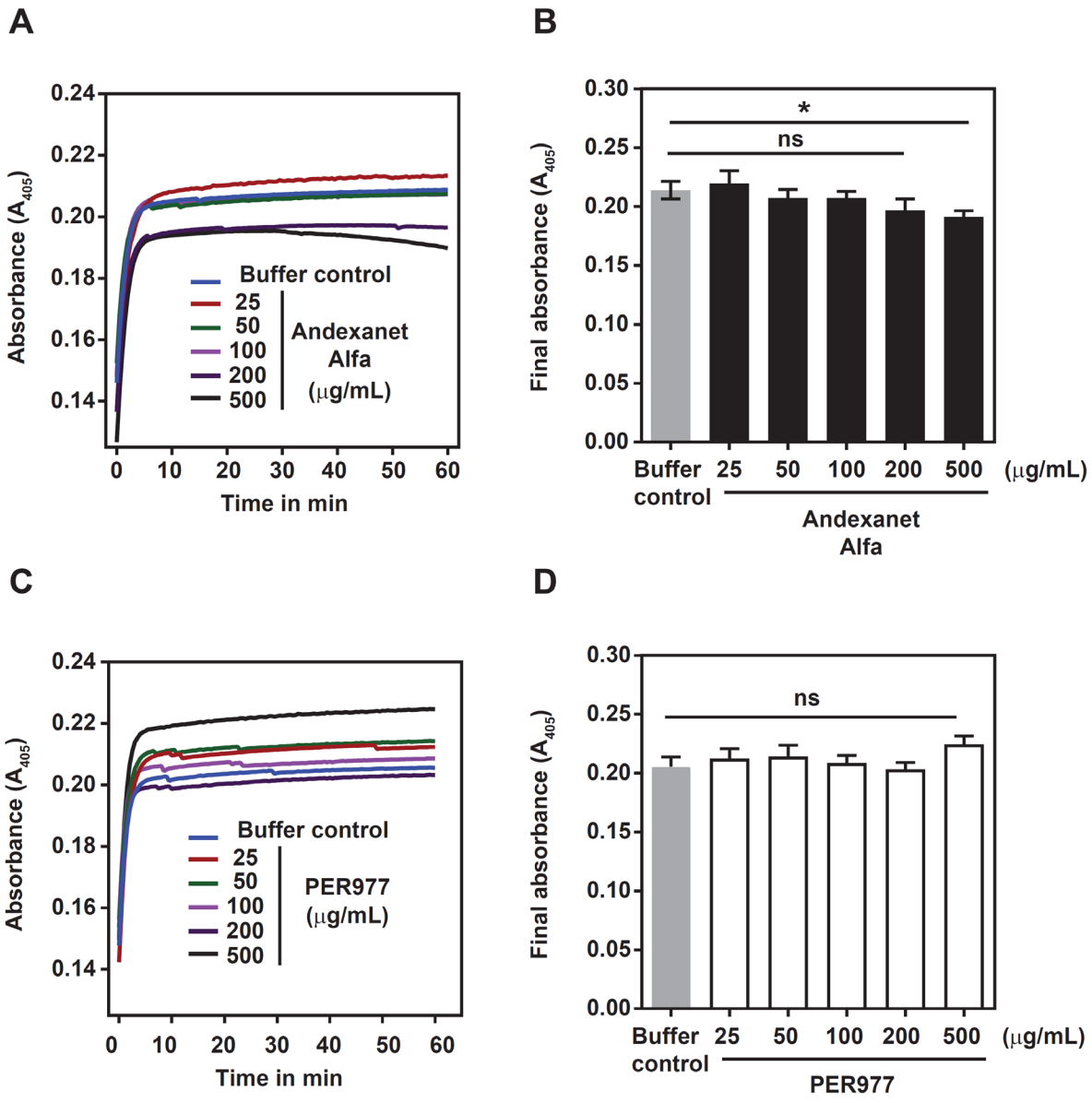

D
Time (min)
0 10 20 30 40 50 60 70
µcal/sec
0.1
0.0
-0.1
-0.2
kcal/mole of injectant
-2
-6
-10
-14
-18
-22
0.0 0.5 1.0 1.5
Rivaroxaban/Andexanet Alfa
Molar Ratio

Supplemental Figure S3. Isothermal Titration Calorimetry (ITC) for andexanet binding to various free anticoagulants or to the AT-enoxaparin complex. Raw (top panel) and cumulative (bottom panel) heat data for titrations at 25°C. **(A)** 400-µM UFH into buffer (top titration of upper panel) or 40-µM UFH into andexanet (bottom titration of upper panel); **(B)** 400-µM enoxaparin into buffer (top upper) or 36-µM andexanet (bottom upper); **(C)** 44.5-µM andexanet into buffer (top upper) or 8-µM each of enoxaparin and ATIII (bottom upper); **(D)** 40-µM rivaroxaban into buffer (top upper) or 5-µM andexanet (bottom upper). All differential heat peaks for 2 µL injections; buffer: PBS with 1% DMSO. Molecular weights used for stoichiometry calculations were: Andexanet: 41.6 kDa, UFH: 15 kDa, enoxaparin: 4.5 kDa, fondaparinux: 1728 Da.



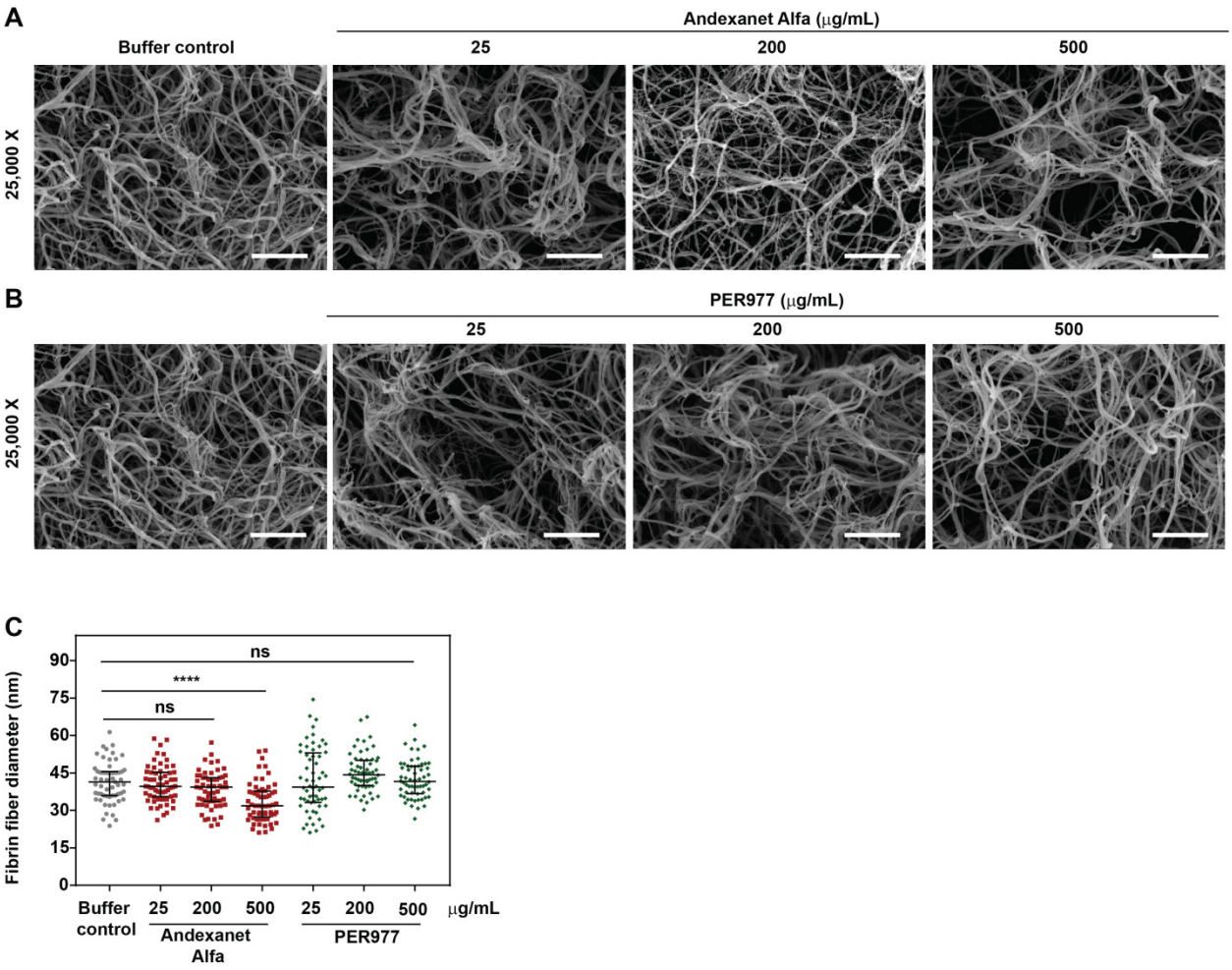
Supplemental Figure S4. Isothermal Titration Calorimetry (ITC) for PER977 binding to various anticoagulants. Raw (top panel) and cumulative (bottom panel) heat data for titrations at 25°C. **(A)** 1.5-mM enoxaparin into buffer (top upper panel) or 200-µM PER977 (bottom upper panel);

(B) 400-µM fondaparinux into buffer (top upper panel) or 40-µM PER977 (bottom upper panel); **(C)** 200-µM PER977 into buffer (top upper panel) or 20-µM rivaroxaban (bottom upper panel); **(D)** 200-µM PER977 into buffer (top upper panel) or 10-µM human fibrinogen (bottom upper panel); **(E)** 200-µM PER977 into buffer (top upper panel) or 20-µM human Factor IX (bottom upper panel); **(F)** 10-µM PER977 into buffer (top upper panel) or 200-µM human Factor IXa (bottom upper panel). All differential heat peaks for 2 µL injections; buffer: PBS with 1% DMSO. Molecular weights used for stoichiometry calculations were: PER977: 512 Da, enoxaparin: 4.5 kDa, fondaparinux: 1728 Da.



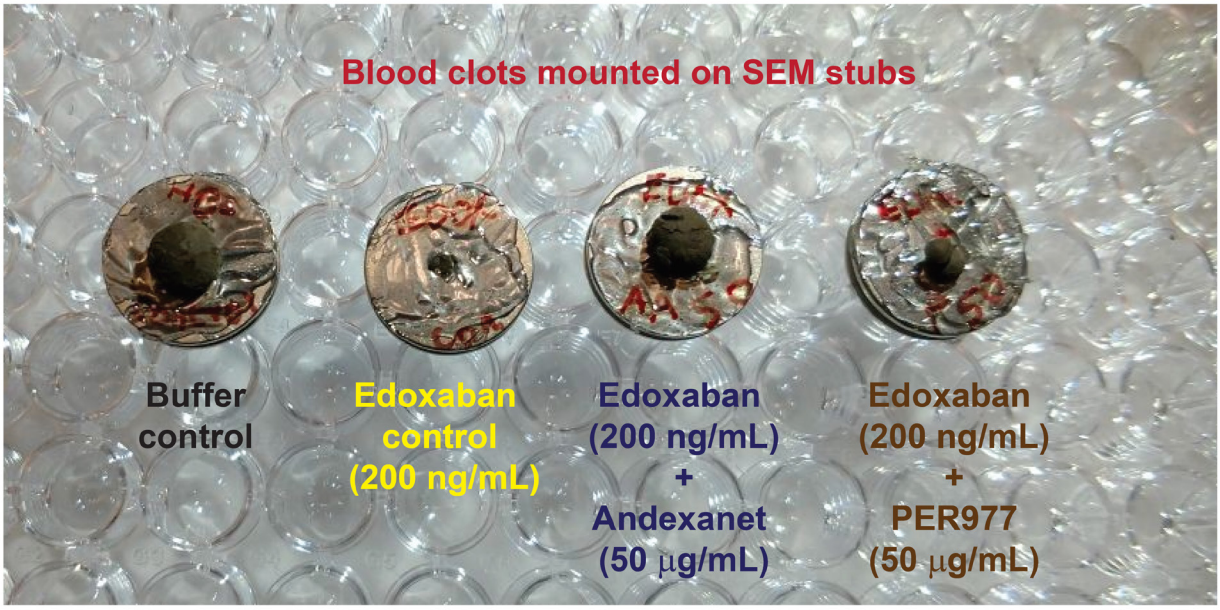
Supplemental Figure S5. Antidotes alone at therapeutic doses do not affect fibrinogen polymerization. Clotting was initiated by adding 2.5 NIHU/mL of thrombin and 3 mM $CaCl_2$ to 3 mg/mL fibrinogen solution, incubated with andexanet or PER977 for 10 minutes at 37^{O}C. Fibrin formation was monitored by measuring turbidity at 405 nm (A405nm) for 1 hour. **(A, C)** Absorbance changes following fibrin fiber formation in the presence of andexanet or PER977. For clarity, error bars are avoided in the raw turbidity traces. **(B, D)** The final turbidity of mature

fibrin clots produced from fibrinogen in the presence of andexanet or PER977. Even though, PER977 (500 μg/mL) showed a subtle increase in fibrin turbidity the value did not reach statistical significance compared to buffer control. However, fibrin turbidity was significantly reduced with andexanet (500 μg/mL), compared to the buffer control. All experiments were performed in triplicate. Results are expressed as the mean ± SE of six measurements from two independent experiments. Unpaired 2-tailed *t* tests were performed to determine significance, with $p < 0.05$ indicating a statistically significant change ($*p < 0.035$).

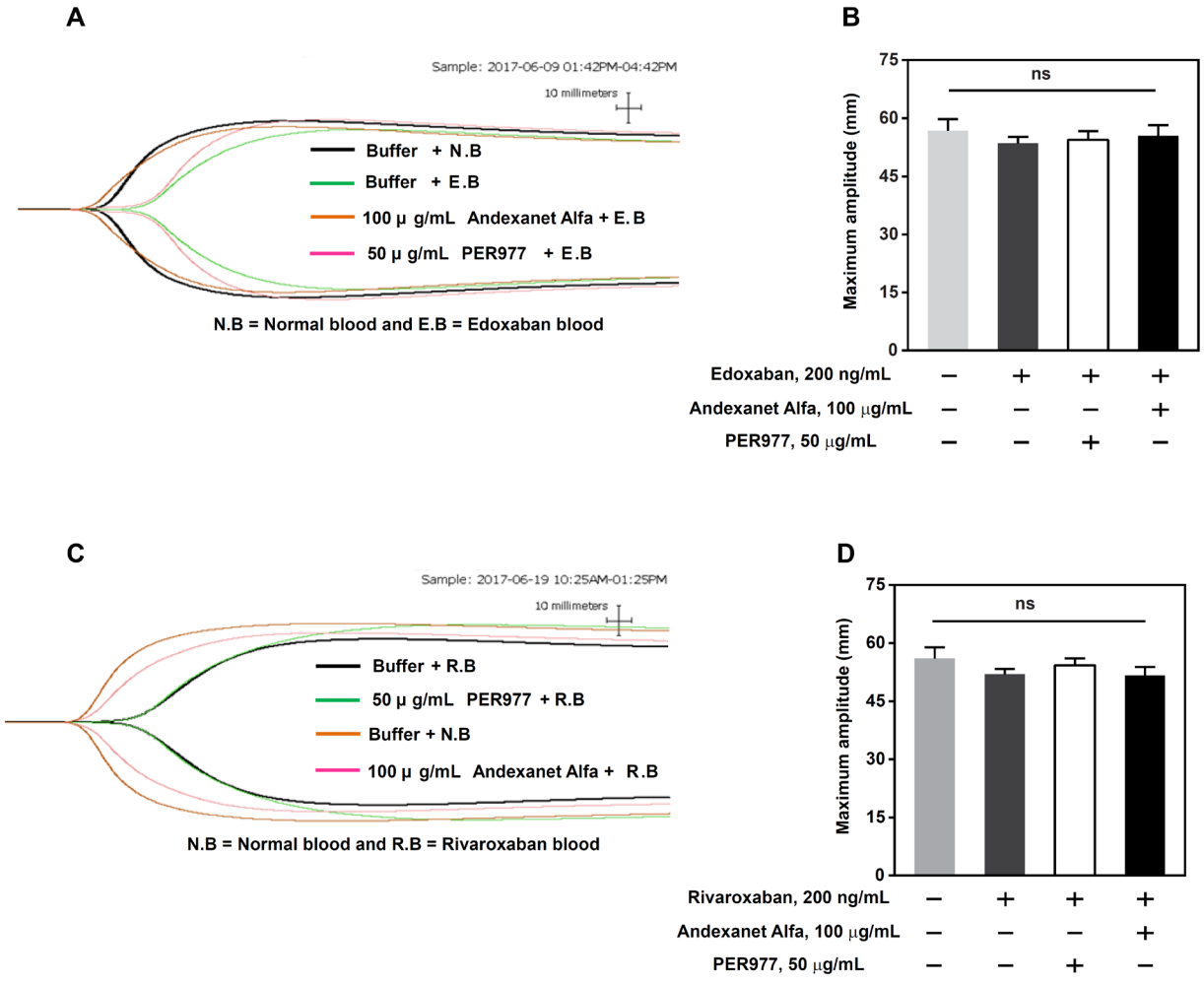


Supplemental Figure S6. Fibrin architecture and fiber size remain unaltered at therapeutic doses of antidotes alone. Fibrin clots were made by incubating 3 mg/mL human fibrinogen in 3.0 mM CaCl2 plus Andexanet Alfa or PER977, then initiating clotting with 2.5 NIHU/mL thrombin. Clots were then allowed to mature for 1 hour and processed for SEM imaging. Images were acquired using Hitachi S-4700 field emission scanning electron microscope **(A,B)** Scanning electron micrographs of fibrin clots formed in the presence of antidotes at different concentrations (25, 200 and 500 μg/mL) are depicted. Clot architectures formed in the presence of antidotes are comparable to buffer control. Clot images were taken at four

magnifications 2500 X, 5000 X, 10000 X and 25000 X, respectively. Only images from the 25000 X magnification are depicted (scale bar= 1 μm). **(C)** Fibrin fiber diameters of clots formed in the presence of Andexanet Alfa or PER977. Fiber diameter is measured from scanning electron micrographs using ImageJ software. A total of 60 fibers were analyzed. Fibers for size analysis were selected from four images by probing four different spots in each image. Data are median with interquartile ranges (n=60). Black horizontal lines represent median and error bars represent interquartile range. Statistical significance for fiber diameter was determined by comparing the antidote treated group to the buffer control using a Kruskal-Wallis test followed by a Dunn's post-test. Asterisks indicate significant differences in comparison to the buffer control. Fibers formed in the presence of 500 μg/mL Andexanet Alfa are significantly thinner than those in the control clot (****p <0.0001).

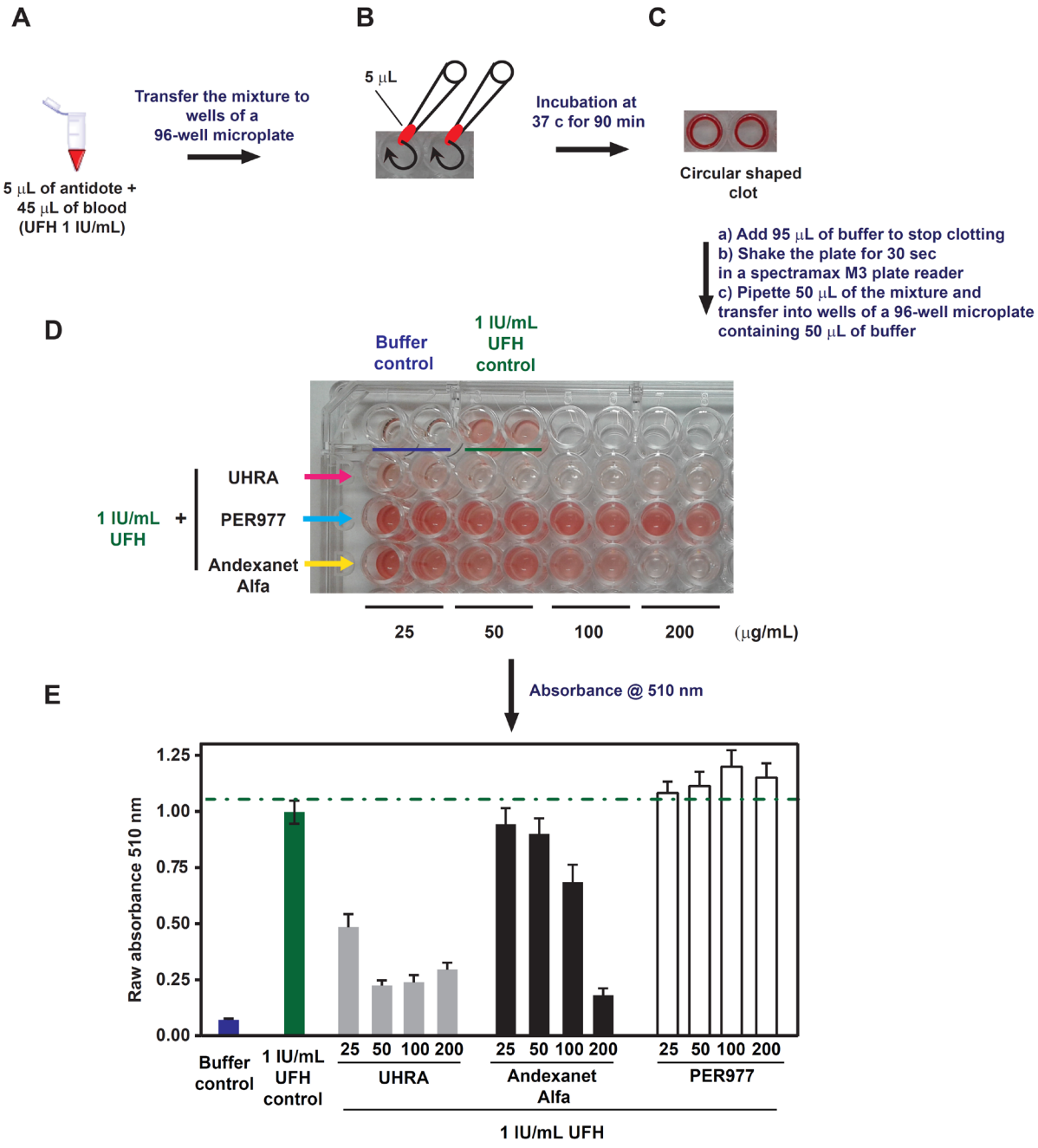


Supplemental Figure S7. Blood clots for SEM imaging. The image shows blood clots obtained after completing SEM processing steps as described in the methods section. Blood clots samples are mounted on stubs for SEM imaging. A tiny blood clot is formed in edoxaban (200 ng/mL)-anticoagulated blood in comparison to buffer control. In addition, electron micrographs and fibrin fiber analysis **(Figure 3)** shows significant reduction in fiber diameter in edoxaban (200 ng/mL)-anticoagulated blood in comparison to the buffer control. On the other hand, antidote treatment restores clot formation and fibrin fiber diameter in edoxaban-anticoagulated blood (Figure 3A-C). Blood clots obtained in edoxaban-treated blood in the presence of andexanet (50 μg/mL) and PER977 (50 μg/mL), respectively, are shown. The image is not to scale.



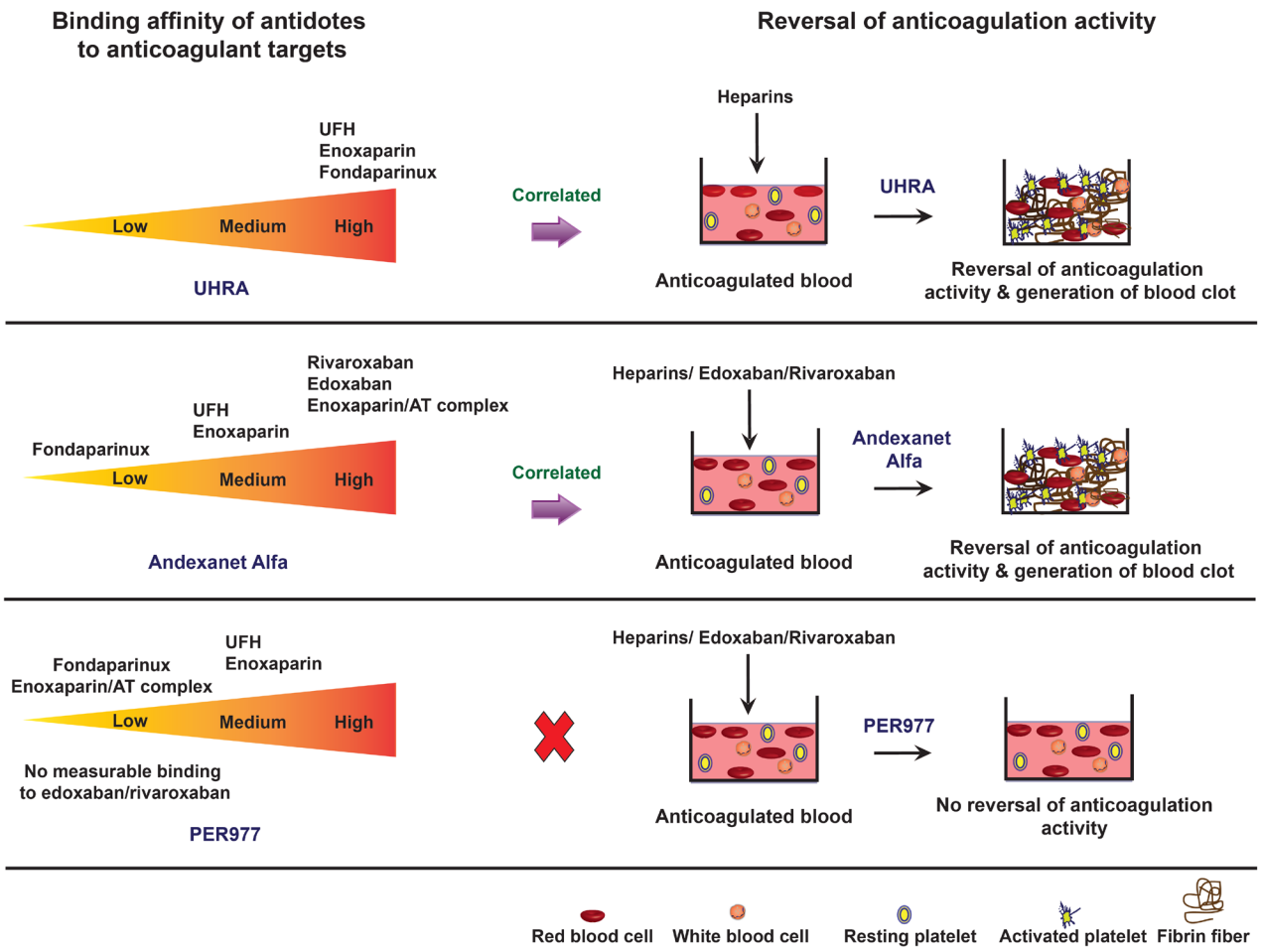
Supplemental Figure S8. Reversal of the anticoagulation activity of edoxaban and rivaroxaban. TEG assay was performed in non-citrated human blood. Andexanet neutralized the anticoagulation activity of edoxaban and rivaroxaban, respectively. However, PER977 did not neutralize the anticoagulation activity of edoxaban. **(A)** A representative TEG profile showing neutralization of edoxaban anticoagulation activity by antidotes. **(B)** No significant change was observed in the clot strength (maximum amplitude, mm) between antidote treated

group and the buffer control. **(C)** A representative TEG profile showing neutralization of rivaroxaban anticoagulation activity by antidotes. **(D)** No significant change was observed in the clot strength (maximum amplitude, mm) between antidote treated group and the buffer control. Data are mean ± SE (n=6). Mann-Whitney (Unpaired, 2-tailed) tests were performed to determine significance.



Supplemental Figure S9. Schematic illustration of the microplate-based whole blood clotting assay protocol. (A) 5 µL of antidote was mixed with 45 µL of heparinized whole blood (1 IU/mL). **(B)** 5 µL of this mixture was deposited to the bottom edge of the wells of a 96-well microplate, and the mixture is spread around the edge of the wells using a P20 pipette. **(C)**

After incubation at 37^{O} C for 90 minutes, circular shaped clots are obtained. **(D)** The clotting reaction is halted by adding 95 µL of 20 mM HEPES buffer (150 mM NaCl, pH=7.4). After shaking the plate for 30 seconds in a Spectramax M3 plate reader, 50 µL of the mixture is transferred into wells of a 96-well microplate containing 50 µL of 20 mM HEPES buffer (150 mM NaCl, pH=7.4). A representative microplate obtained following the protocol is shown. Both UHRA and Andexanet reverse the anticoagulation activity of UFH 1 IU/mL as evidenced by the reduction in the amount of free blood. **(E)** The raw absorbance values of wells at 510 nm (n= 5 experiments) corroborate the UFH reversal activity of UHRA and andexanet, respectively.



Supplemental Figure S10. Visual abstract

REFERENCES

1. Shenoi RA, Kalathottukaren MT, Travers RJ, et al. Affinity-based design and discovery of a synthetic universal reversal agent for clinically used parenteral heparin anticoagulants. *Sci Transl Med*. 2014; 6 (260): 260ra150.
2. Lai BFL, Creagh AL, Janzen J, Haynes CA, Brooks DE, and Kizhakkedathu JN. The induction of thrombus generation on nanostructured neutral polymer brush surfaces. *Biomaterials*. 2010; 31(26): 6710-6718.
3. Creagh AL, Koska J, Johnson PE, et al. Stability and Oligosaccharide Binding of the N1 Cellulose-Binding Domain of Cellulomonas fimi Endoglucanase CenC. *Biochemistry*. 1998; 37(10): 3529-3537.
4. Schneider CA, Rasband WS, and Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 2012; 9 (7): 672-675.
5. Lai BFL, Zou Y, Yang X, Yu X, and Kizhakkedathu JN. Abnormal blood clot formation induced by temperature responsive polymers by altered fibrin polymerization and platelet binding. *Biomaterials* 2014; 35: 2518-2528.
6. Kalathottukaren MT, Abraham L, Kapopara PR, et al. Alteration of blood clotting and lung damage by protamine are avoided using the heparin and polyphosphate inhibitor UHRA. *Blood* 2017; 129 (10): 1368-1379.
7. Bonnard T, Law LS, Tennant Z and Hagemeyer CE. Development and validation of a high throughput whole blood thrombolysis plate assay. *Sci Rep* 2017;7(1):2346.
8. Lu G, DeGuzman FR, Hollenbach SJ, Karbarz MJ, et al. A specific antidote for reversal of anticoagulation by direct and indirect inhibitors of coagulation factor Xa. *Nat. Medicine*. 2013; 19: 446–451.
9. Ansell JE, Bakhru SH, Laulicht BE, Steiner SS, et al. Use of PER977 to reverse the anticoagulant effect of edoxaban. *N Engl J Med*. 2014; 371 (22): 2141-2142.