

Supporting information

Parallel imaging of coagulation pathway proteases activated protein C, thrombin, and factor Xa in human plasma

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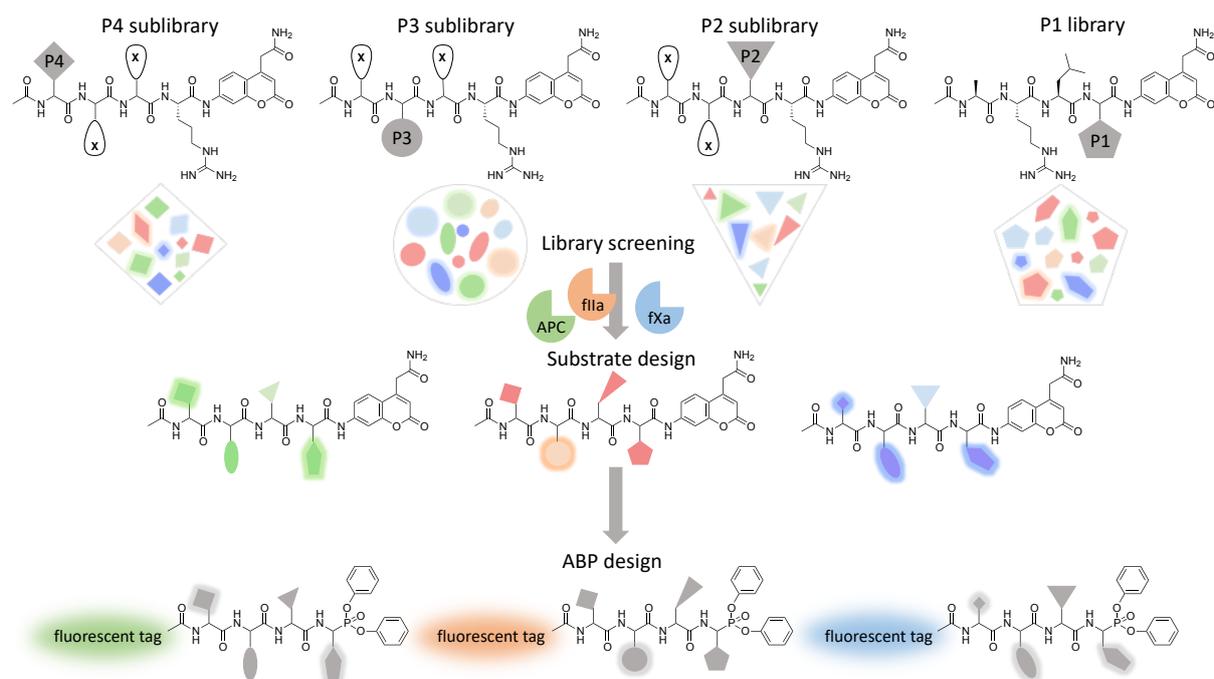


Figure S1. General scheme of library screening and its use for the design of selective substrates and activity-based probes.

Table S1. Purity and MS analysis of APC substrates.

	Structure	[M+H] ⁺²⁺ calculated	[M+H] ⁺²⁺ measured	Purity
SMA1	Ac-Lys-Dab(Z)-Lys(2-Cl-Z)-Arg-ACC	1075.4768	1075.4773	≥ 99%
SMA2	Ac-Lys-Agp-Lys(2-Cl-Z)-Arg-ACC	969.4462	969.4442	≥ 99%
SMA3	Ac-Lys-Glu(Bzl)-Lys(2-Cl-Z)-Arg-ACC	1060.4659	1060.4678	≥ 97%
SMA4	Ac-Lys-Nle(O-Bzl)-Lys(2-Cl-Z)-Arg-ACC	1060.5023	1060.5031	≥ 99%
SMA5	Ac-Lys-Dab(Z)-Igl-Arg-ACC	952.4681	952.4676	≥ 99%
SMA6	Ac-Lys-Agp-Igl-Arg-ACC	846.4375	846.4381	≥ 99%
SMA7	Ac-Lys-Glu(Bzl)-Igl-Arg-ACC	937.4572	937.4589	≥ 99%
SMA8	Ac-Lys-Nle(O-Bzl)-Igl-Arg-ACC	937.4936	937.4934	≥ 99%
SMA9	Ac-Lys-Dab(Z)-Val-Arg-ACC	878.4525	878.4521	≥ 99%
SMA10	Ac-Lys-Agp-Val-Arg-ACC	772.4218	772.4213	≥ 95%
SMA11	Ac-Lys-Glu(Bzl)-Val-Arg-ACC	863.4416	863.4434	≥ 95%
SMA12	Ac-Lys-Nle(O-Bzl)-Val-Arg-ACC	863.4780	863.4785	≥ 97%
SMA13	Ac-Lys-Dab(Z)-Oic-Arg-ACC	930.4838	930.4832	≥ 97%
SMA14	Ac-Lys-Agp-Oic-Arg-ACC	412.7305	412.7392	≥ 97%
SMA15	Ac-Lys-Glu(Bzl)-Oic-Arg-ACC	915.4728	915.4732	≥ 97%
SMA16	Ac-Lys-Nle(O-Bzl)-Oic-Arg-ACC	915.5093	915.5091	≥ 95%
SMA17	Ac-Lys-Dab(Z)-Lys-Arg-ACC	907.4790	907.4796	≥ 99%
SMA19	Ac-Lys-Glu(Bzl)-Lys-Arg-ACC	892.4681	892.4695	≥ 99%
SMA20	Ac-Lys-Nle(O-Bzl)-Lys-Arg-ACC	892.5045	892.5038	≥ 99%
SMA21	Ac-Lys-Dab(Z)-Cha-Arg-ACC	932.4994	932.4982	≥ 99%
SMA22	Ac-Lys-Agp-Cha-Arg-ACC	826.4688	826.4696	≥ 99%
SMA23	Ac-Lys-Glu(Bzl)-Cha-Arg-ACC	917.4885	917.4896	≥ 99%
SMA24	Ac-Lys-Nle(O-Bzl)-Cha-Arg-ACC	917.5249	917.5265	≥ 97%
SMA25	Ac-Lys-Glu(All)-Igl-Arg-ACC	887.4416	887.4402	≥ 99%
SMA26	Ac-Lys-βhLys-Igl-Arg-ACC	860.4783	860.4785	≥ 99%
SMA54	Ac-Lys-Gln-Lys-Arg-ACC	801.4371	801.4374	≥ 99%

Table S2. Purity and MS analysis of thrombin substrates.

	Structure	[M+H] ⁺ calculated	[M+H] ⁺ measured	Purity
SMII16	Ac-hCha-Cys(MeBzl)-Pro-Arg-ACC	888.4442	888.4429	≥ 99%
SMII17	Ac-hLeu-Cys(MeBzl)-Pro-Arg-ACC	848.4129	848.4133	≥ 99%
SMII18	Ac-2-Aoc-Cys(MeBzl)-Pro-Arg-ACC	862.4285	862.4281	≥ 99%
SMII19	Ac-hCha-Cys(MeBzl)-Aze-Arg-ACC	874.4285	874.4293	≥ 99%
SMII20	Ac-hLeu-Cys(MeBzl)-Aze-Arg-ACC	834.3973	834.3969	≥ 99%
SMII21	Ac-2-Aoc-Cys(MeBzl)-Aze-Arg-ACC	848.4129	848.4134	≥ 99%
SMII22	Ac-hCha-Cys(MeBzl)-Pip-Arg-ACC	902.4598	902.4587	≥ 99%
SMII23	Ac-hLeu-Cys(MeBzl)-Pip-Arg-ACC	862.4285	862.4279	≥ 99%
SMII24	Ac-2-Aoc-Cys(MeBzl)-Pip-Arg-ACC	876.4442	876.4443	≥ 99%
SMII25	Ac-hCha-Cys(4-MeOBzl)-Pro-Arg-ACC	904.4391	904.4390	≥ 97%
SMII26	Ac-hLeu-Cys(4-MeOBzl)-Pro-Arg-ACC	864.4078	864.4073	≥ 99%
SMII27	Ac-2-Aoc-Cys(4-MeOBzl)-Pro-Arg-ACC	878.4235	878.4227	≥ 99%
SMII28	Ac-hCha-Cys(4-MeOBzl)-Aze-Arg-ACC	890.4235	890.4224	≥ 97%
SMII29	Ac-hLeu-Cys(4-MeOBzl)-Aze-Arg-ACC	850.3922	850.3942	≥ 99%
SMII30	Ac-2-Aoc-Cys(4-MeOBzl)-Aze-Arg-ACC	864.4078	864.4067	≥ 99%
SMII31	Ac-hCha-Cys(4-MeOBzl)-Pip-Arg-ACC	918.4548	918.4554	≥ 99%
SMII32	Ac-hLeu-Cys(4-MeOBzl)-Pip-Arg-ACC	878.4235	878.4215	≥ 99%
SMII33	Ac-2-Aoc-Cys(4-MeOBzl)-Pip-Arg-ACC	892.4391	892.4382	≥ 99%
SMII34	Ac-hCha-Cys(4-MeOBzl)-Hyp(Bzl)-Arg-ACC	1010.4810	1010.4821	≥ 99%
SMII57	Ac-Chg-Cys(MeBzl)-Pip-Arg-ACC	874.4285	874.4277	≥ 99%
SMII59	Ac-Chg-Cys(MeBzl)-Hyp(Bzl)-Arg-ACC	966.4548	966.4547	≥ 97%
SMII60	Ac-2-Aoc-Cys(MeBzl)-Hyp(Bzl)-Arg-ACC	968.4704	968.4691	≥ 97%
SMII55	Ac-Nle-Lys-Pro-Arg-ACC	755.4204	755.4218	≥ 99%

Table S3. Purity and MS analysis of fXa substrates.

	Structure	[M+H] ⁺ calculated	[M+H] ⁺ measured	Purity
SMX1	Ac-Arg(NO ₂)-hArg-Trp-Arg-ACC	974.4709	974.4722	≥ 95%
SMX2	Ac-Gln-hArg-Trp-Arg-ACC	901.4433	901.4431	≥ 97%
SMX3	Ac-DPro-hArg-Trp-Arg-ACC	870.4375	870.4361	≥ 97%
SMX4	Ac-Arg(NO ₂)-hArg-Phe(3-Cl)-Arg-ACC	969.4210	969.4218	≥ 99%
SMX5	Ac-Gln-hArg-Phe(3-Cl)-Arg-ACC	896.3934	896.3934	≥ 99%
SMX6	Ac-DPro-hArg-Phe(3-Cl)-Arg-ACC	865.3876	865.3879	≥ 99%
SMX7	Ac-Arg(NO ₂)-hArg-Bta-Arg-ACC	991.4321	991.4320	≥ 99%
SMX8	Ac-Gln-hArg-Bta-Arg-ACC	918.4045	918.4065	≥ 97%
SMX9	Ac-DPro-hArg-Bta-Arg-ACC	887.3987	887.3996	≥ 99%
SMX10	Ac-Arg(NO ₂)-hArg-Phe-Arg-ACC	935.4600	935.4587	≥ 99%
SMX11	Ac-Gln-hArg-Phe-Arg-ACC	862.4324	862.4324	≥ 99%
SMX12	Ac-DPro-hArg-Phe-Arg-ACC	831.4266	831.4280	≥ 99%
SMX13	Ac-Arg(NO ₂)-hArg-1-Nal-Arg-ACC	985.4756	985.4747	≥ 99%
SMX14	Ac-Gln-hArg-1-Nal-Arg-ACC	912.4481	912.4468	≥ 99%
SMX15	Ac-DPro-hArg-1-Nal-Arg-ACC	881.4423	881.4412	≥ 97%
SMX61	Ac-Met-hArg-1-Nal-Arg-ACC	915.4299	915.4292	≥ 97%
SMX62	Ac-His(Bzl)-hArg-1-Nal-Arg-ACC	1011.4953	1011.4963	≥ 99%
SMX63	Ac-DPro-hArg-Gly-Arg-ACC	741.3796	741.3793	≥ 99%
SMX64	Ac-Met-hArg-Gly-Arg-ACC	775.3674	775.3679	≥ 99%
SMX65	Ac-His(Bzl)-hArg-Gly-Arg-ACC	871.4327	871.4340	≥ 99%
SMX56	Ac-Pro-Arg-Gly-Arg-ACC	727.3640	727.3647	≥ 99%

Table S4. Purity and MS analysis of ABPs and inhibitors.

	Structure	[M+H] ⁺²⁺ calculated	[M+H] ⁺²⁺ measured	Purity
P-SMA61	biotin-6-Ahx-Lys-Agp-Igl-Arg ^P (OPh) ₂	1131.5691	1131.6002	≥ 95%
P-SMA171	biotin-6-Ahx-Lys-Dab(Z)-Lys-Arg ^P (OPh) ₂	1192.6106	1192.6116	≥ 99%
P-SMA261	biotin-6-Ahx-Lys-βhLys-Igl-Arg ^P (OPh) ₂	1145.6099	1145.6179	≥ 99%
P-SMII221	biotin-6-Ahx-hCha-Cys(MeBzl)-Pip-Arg ^P (OPh) ₂	1187.5914	1187.5935	≥ 99%
P-SMII321	biotin-6-Ahx-hLeu-Cys(4-MeOBzl)-Pip-Arg ^P (OPh) ₂	1163.5551	1163.5557	≥ 99%
P-SMX91	biotin-6-Ahx-DPro-hArg-Bta-Arg ^P (OPh) ₂	1172.5303	1172.5311	≥ 97%
P-SMX151	biotin-6-Ahx-DPro-hArg-1-Nal-Arg ^P (OPh) ₂	1166.5739	1166.5748	≥ 97%
P-SMX611	biotin-6-Ahx-Met-hArg-1-Nal-Arg ^P (OPh) ₂	1200.5615	1200.5632	≥ 99%
P-SMA172	Cy5-6-Ahx-Lys-Dab(Z)-Lys-Arg ^P (OPh) ₂	715.9118	715.9117	≥ 95%
P-SMII222	Cy7-6-Ahx-hCha-Cys(MeBzl)-Pip-Arg ^P (OPh) ₂	746.4138	746.4142	≥ 99%
P-SMX152	Cy3-6-Ahx-DPro-hArg-1-Nal-Arg ^P (OPh) ₂	689.8856	689.8862	≥ 99%
I-SMA17	Ac-Lys-Dab(Z)-Lys-Arg ^P (OPh) ₂	895.4595	895.4594	≥ 97%
I-SMII22	Ac-hCha-Cys(MeBzl)-Pip-Arg ^P (OPh) ₂	890.4404	890.4404	≥ 95%
I-SMX15	Ac-DPro-hArg-1-Nal-Arg ^P (OPh) ₂	869.4227	869.4231	≥ 99%

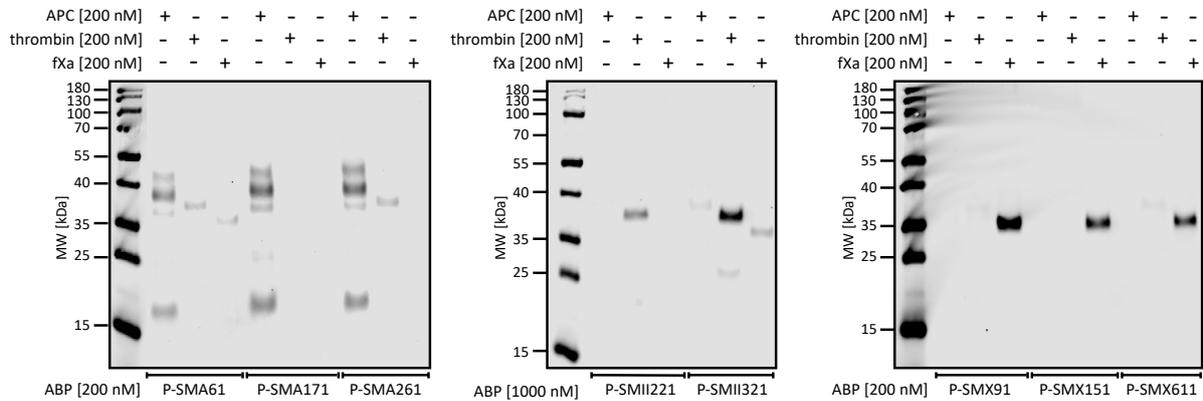


Figure S2. Labeling of purified coagulation factors (APC, thrombin, fXa) using biotinylated ABPs.

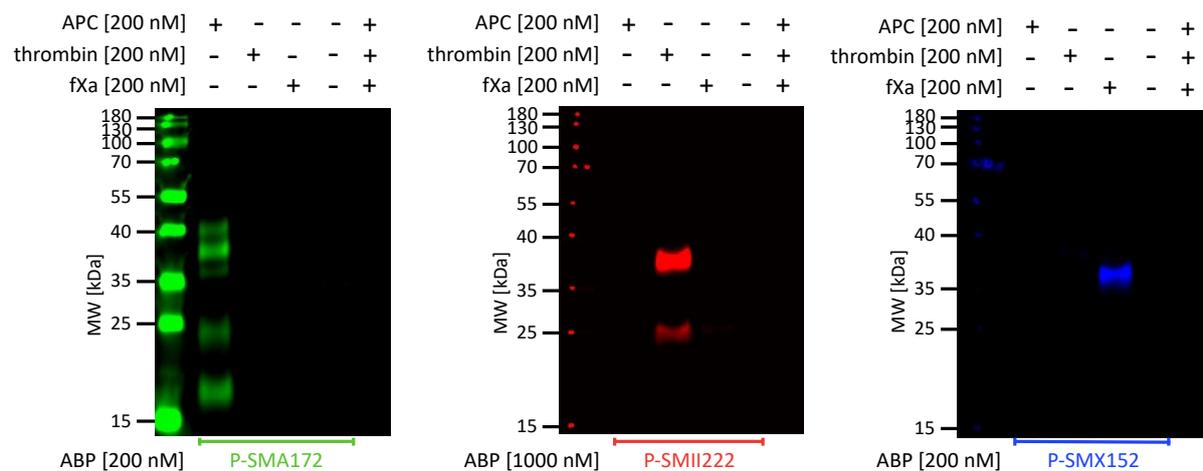


Figure S3. Labeling of purified coagulation factors (APC, thrombin, fXa) using fluorescent ABPs.

APC [200 nM]	+	+	-	-	-	-
thrombin [200 nM]	-	-	+	+	-	-
fXa [200 nM]	-	-	-	-	+	+
I-SMA17 [5 μ M]	-	+	-	-	-	-
I-SMII22 [5 μ M]	-	-	-	+	-	-
I-SMX15 [5 μ M]	-	-	-	-	-	+

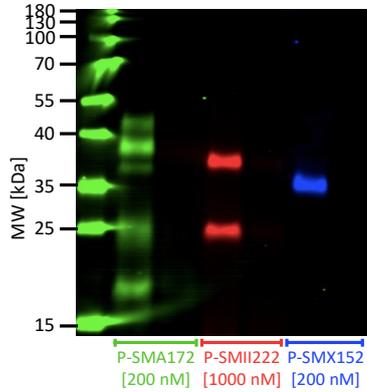


Figure S4. Simultaneous APC, thrombin, and fXa detection and inhibition.

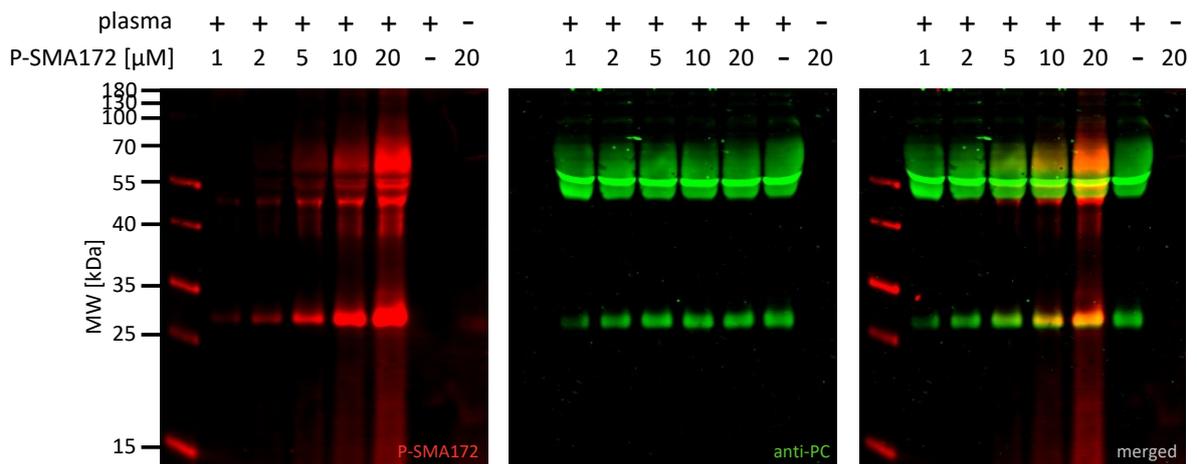


Figure S5. APC labeling in human plasma.

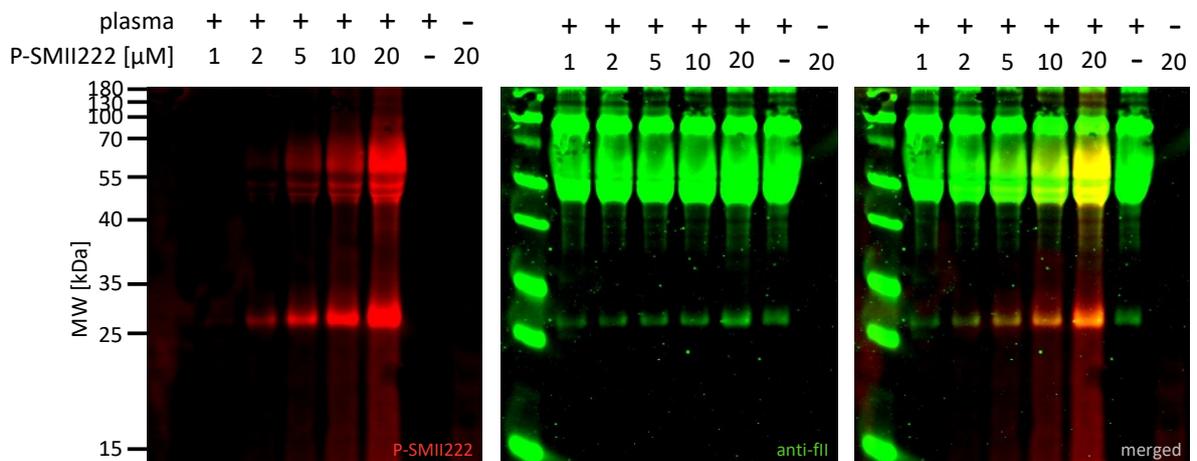


Figure S6. Thrombin labeling in human plasma.

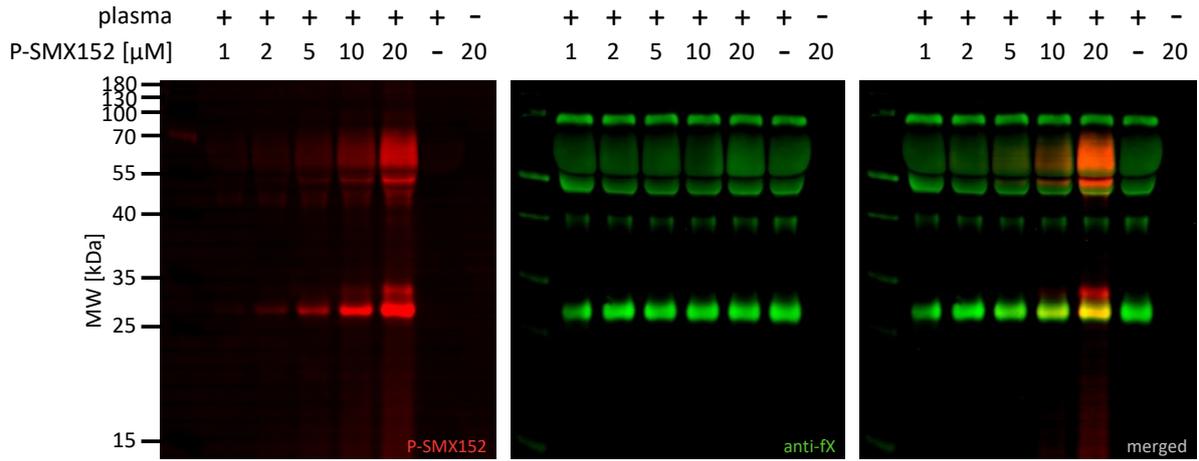


Figure S7. FXa labeling in human plasma.

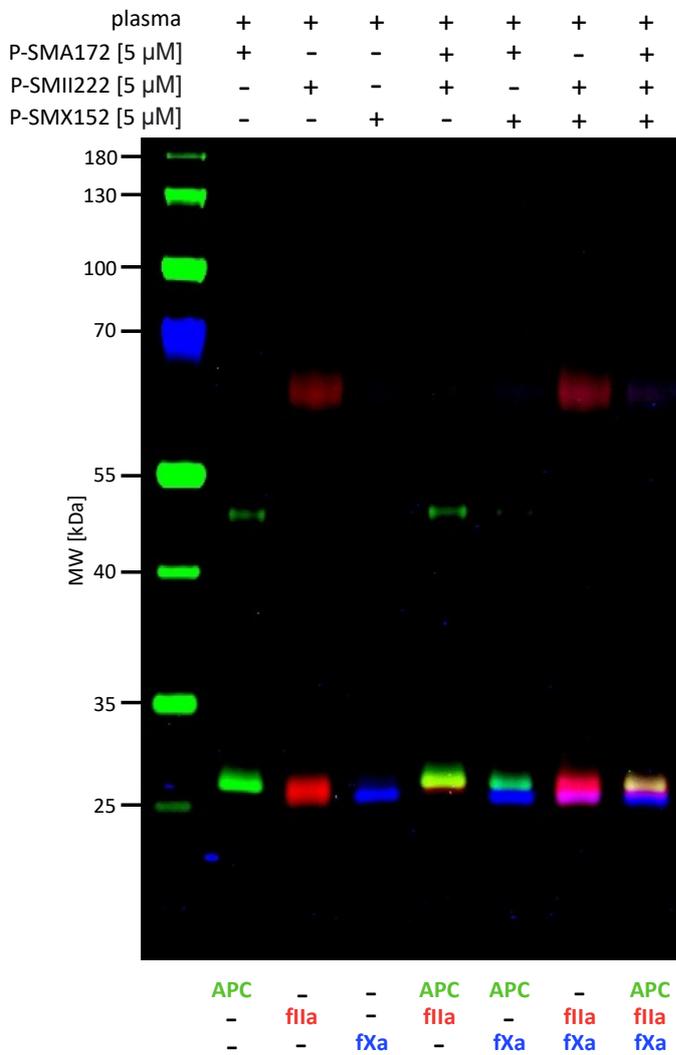
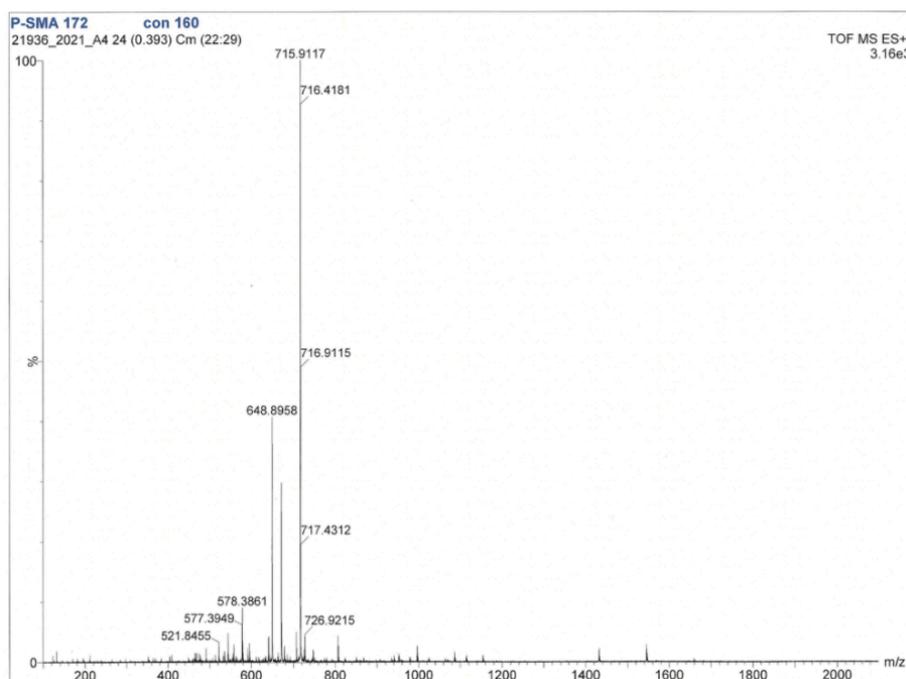
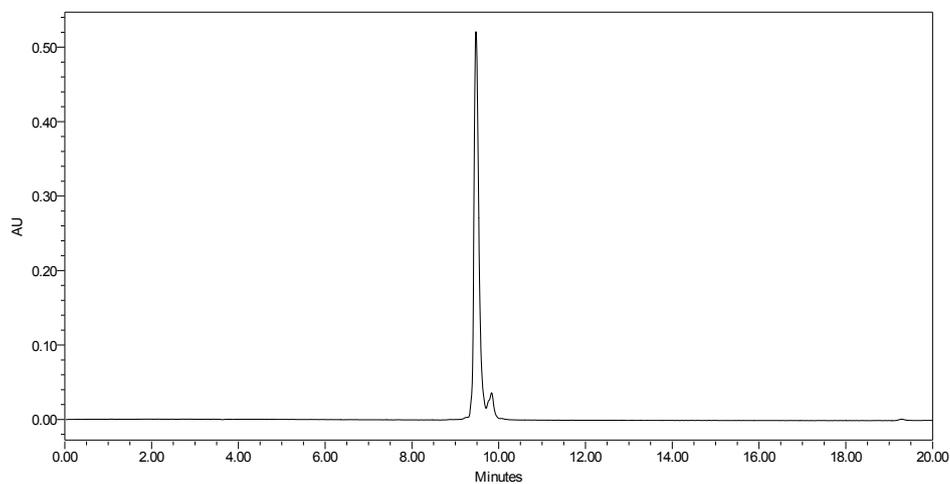
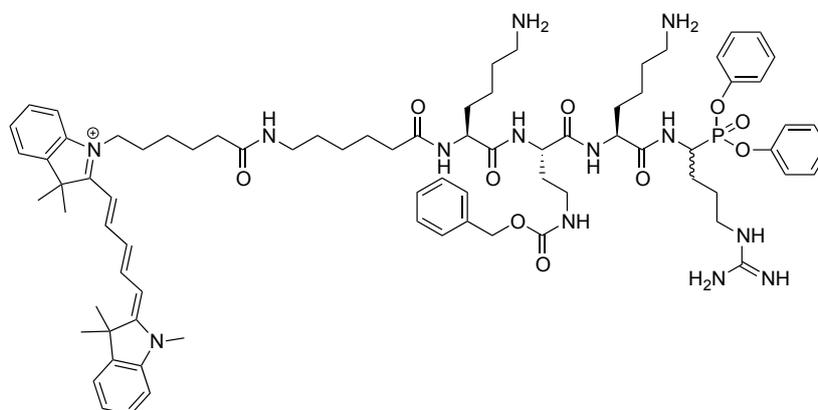


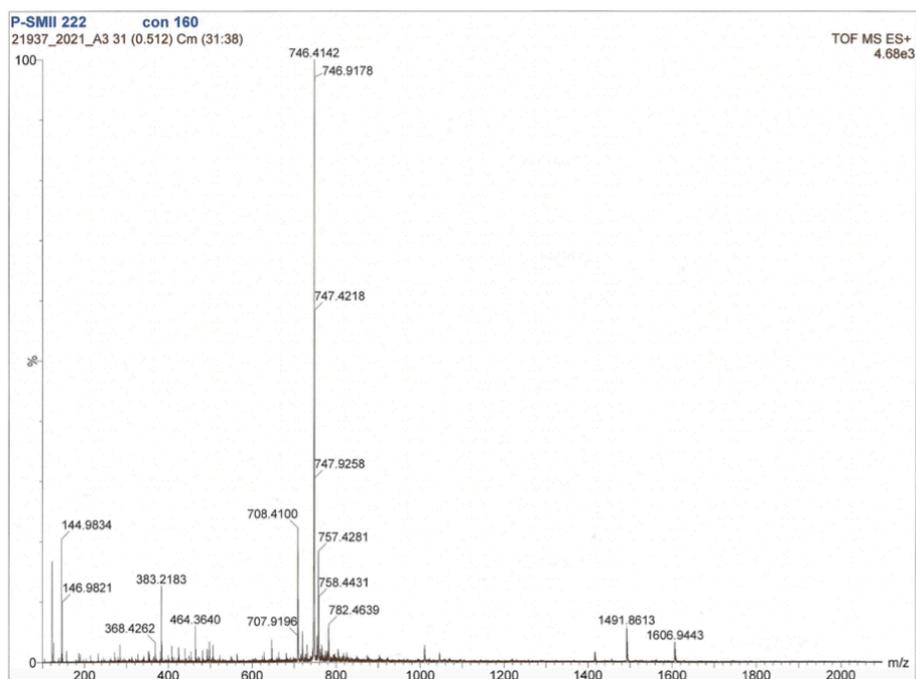
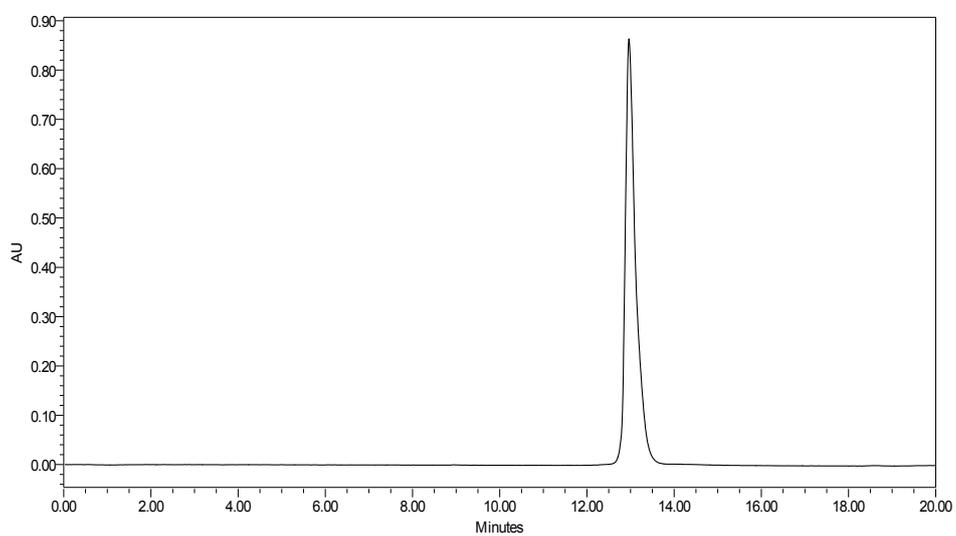
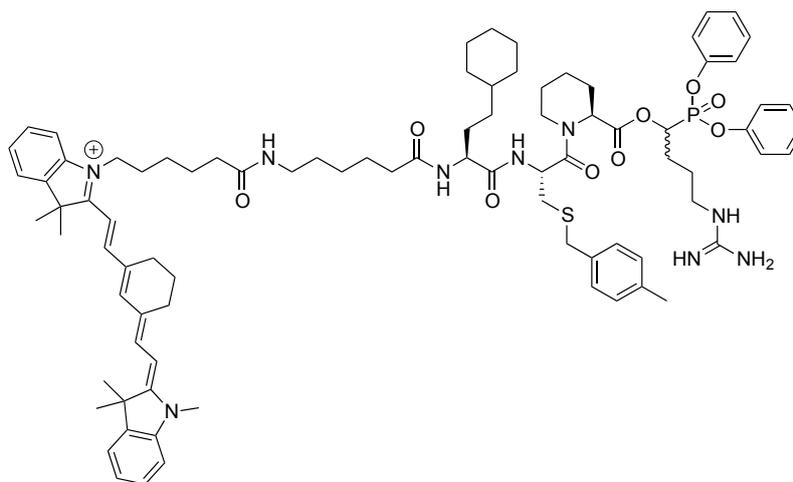
Figure S8. Simultaneous coagulation factors labeling in human plasma.

Purity and MS analysis of fluorescent ABPs

P-SMA172, Cy5-6-Ahx-Lys-Dab(Z)-Lys-Arg^P(OPh)₂



P-SMII222, Cy7-6-Ahx-hCha-Cys(MeBzl)-Pip-Arg^P(OPh)₂



P-SMX152, Cy3-6-Ahx-DPro-hArg-1-Nal-Arg^P(OPh)₂

