Electronic Supplementary Information

Aptamer loaded superparamagnetic beads for selective capturing and gentle release of activated protein C

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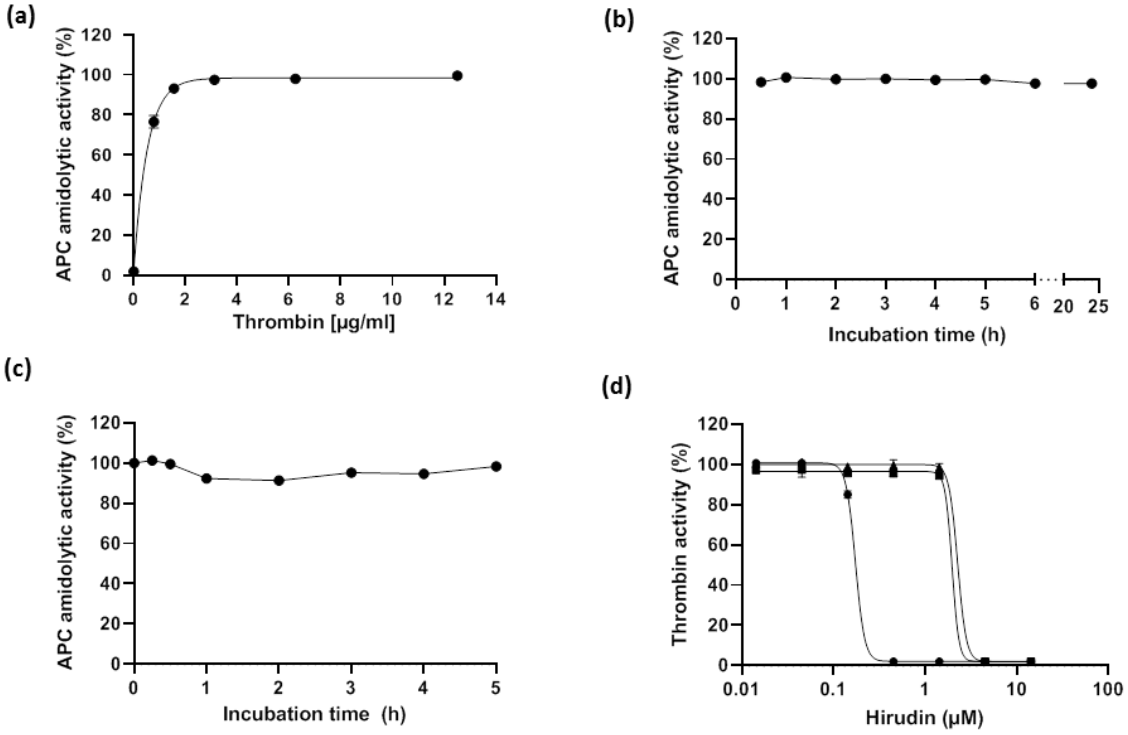

Figure S1. Optimization of PC activation. (a) Optimization of thrombin concentration. PC (62.5 µg/ml) was incubated with different concentrations of thrombin. After 1 h of incubation, hirudin was added to the mixture and APC activity was measured by mixing of 50 µl of a 1:50 dilution of the samples and 50 µl of PCa-5791 peptide substrate. (b) Incubation time optimization. PC (62.5 µg/ml) was mixed with 3.12 µg/ml thrombin. After the indicated incubation times, samples were diluted 1:50 and subjected to the APC activity assay. (c) APC stability in activation buffer. APC (160 nM in activation buffer) was incubated at 37°C for the indicated intervals followed by 1:10 dilution of the samples and activity measurement (d) Optimization of hirudin concentration. Different concentrations of thrombin (2.5 µg/ml solid circles; 12.5 µg/ml solid squares; and 20 µg/ml solid triangles) were incubated with increasing concentrations of hirudin (14 nM to 14 µM) in activation buffer. After incubation for 30 min, 50 µl of the mixture were transferred to the wells of a black F16 Fluoronunc module (Thermo Fisher Scientific, Nunc) containing 50 µl of 10 mM fluorogenic thrombin substrate (Boc-Asp(OBzl)-Pro-Arg-AMC) and thrombin catalysed substrate hydrolysis was monitored using a Synergy 2 microplate reader.

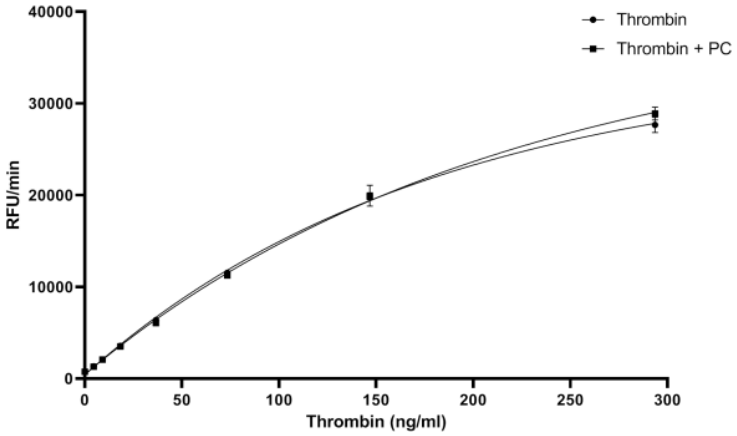

Figure S2. Monitoring the specificity of the PCa-5791 fluorogenic peptide substrate. Fifty microliters of of thrombin at indicated final concentrations which was pre-incubated with or without PC (5 µg/ml) were mixed with 50 µl of 600 µM PCa-5791 and the hydrolysis rate of the substrate was monitored at λ_{ex} 360 nm and λ_{em} of 460 nm using a microplate reader. Data are presented as mean ± s.d. of duplicate measurements.

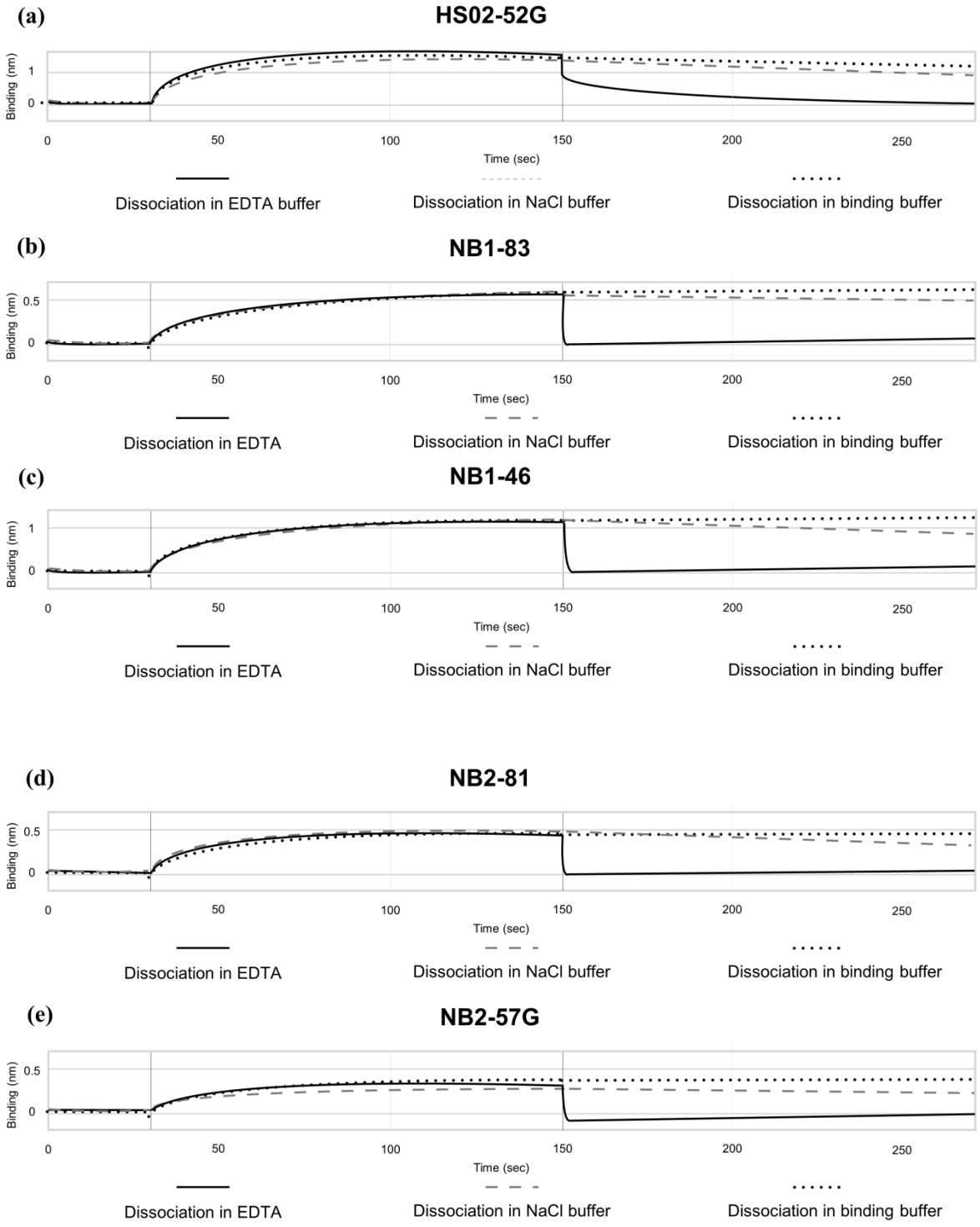

Figure S3. Dissociation of APC from immobilized aptamers using biolayer interferometry (BLI). Biotinylated aptamers (a) HS02-52G, (b) NB1-83, (c) NB1-46, (d) NB2-81, (e) NB2-57G were immobilized on the surface of streptavidin-coated biosensors by immersing of the hydrated biosensor in binding buffer containing 500 nM of each aptamer followed by association of 500 nM APC. The APC dissociation was performed in binding buffer either without or with 5 mM EDTA or 1 M NaCl.

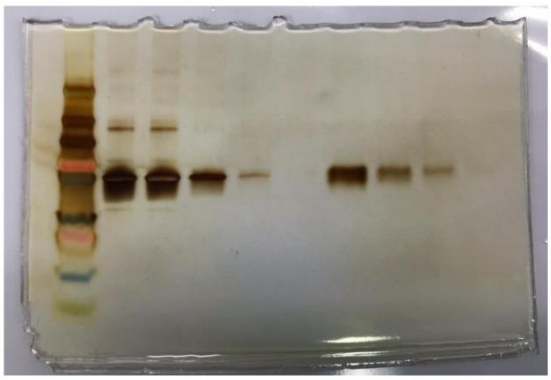

Figure S4. The original gel image corresponds to figure 5 which was run to qualitatively analyse the purity of the captured APC using MyOne super paramagnetic beads loaded with HS02-52G aptamer.

Table 1. Characterization of two different types of magnetic beads modified with HS02-52G aptamer

	Amount of beads, mg	Total served surface, cm^2	Coupled aptamer, µg/mg beads (nmol/mg beads)
MyOne™ Carboxylic Acid	5	142	13.3 (0.82)
M-270 Carboxylic Acid	15	246	5.38 (0.22)