

## Supplemental Materials and Methods

### Radiochemistry

#### *HPLC*

Analytical liquid chromatographic analysis was performed with a Gilson HPLC system and a Dionex UVD170U photodiode array detector in series with a Scansys detector. Data collection and liquid chromatography control was performed with the use of Chromeleon 6.8 SR 10 Build 2818. All chemicals were used without further purification. HPLC analysis was performed using a C4 Grace Vydac column, 250 x 4.6 mm. A gradient system was used with mobile phase A, 1:9 MeCN:H<sub>2</sub>O, 0.1% TFA and B, 9:1 MeCN/H<sub>2</sub>O, 0.1% TFA, flow rate 1 mL/min (R<sub>t</sub> 16 min). The gradient was 0-68% B over 17 min, 68-100% B over 1 min, 100-0% B over 1 min, 0% B over 3 min.

#### *Protein precipitation*

500 μL human serum albumin (HAS) solution (10 mg/mL), 50 μL NaHCO<sub>3</sub> solution (0.1M) and 450 μL TCA solution (1.4M) was added to 1 μL <sup>64</sup>Cu-NOTA-FVIIai. The cloudy mixture was shaken and spun at 3500 rpm for 10 minutes before 500 μL supernatant was removed and both pellet and supernatant was counted on a gamma-counter (γ-Counter Cobra TM with auto-gamma, Packard). The protein-bound fraction as determined by the following formula:

$$\% \text{ Protein bound} = \frac{\text{Pellet} - \text{Supernatant}}{\text{Pellet} + \text{Supernatant} - 2 \cdot \text{Background}} \cdot 100\%$$

#### *Pull-down experiments*

Sepharose 4B-coupled TF(1–209) suspension (sTF-seph-4B) and sepharose 4B-coupled-anti-FVII monoclonal antibody F1A2 suspension (FVII-mcAb-Seph. 4B) were obtained from Novo Nordisk A/S. sTF-seph-4B and FVII-mcAb-seph-4B (400 μL) was equilibrated with 400 μL assay buffer (10 mM gly-gly, 150 mM NaCl, 10 mM CaCl<sub>2</sub>, 0.5% BSA, pH 7.5). The solution was spun down for 30 s, and the supernatant was removed. 800 μL assay buffer was added to the matrix. The washing step was performed a total of 3 times. After the last removal of supernatant, 200 μL assay buffer was added, and the matrices was divided into 4 x 40μL portions. To each vial, 40 μL assay buffer was added. 4 vials containing 40 μL of assay buffer was prepared as well as blank samples.

Nielsen et al. "PET Imaging of Tissue Factor in Pancreatic Cancer Using  $^{64}\text{Cu}$ -labeled Active Site Inhibited Factor VII"

The concentration of  $^{64}\text{Cu}$ -NOTA-FVIIai was determined by HPLC, and the sample diluted to 0.1  $\mu\text{g}/\text{mL}$ . 100  $\mu\text{L}$  of this solution was added to each vial. The samples was incubated for 2 h at room temperature, and centrifuged for 30 s. 90  $\mu\text{L}$  supernatant was removed from each vial and counted on a gamma.

Binding was calculated using the formula below.

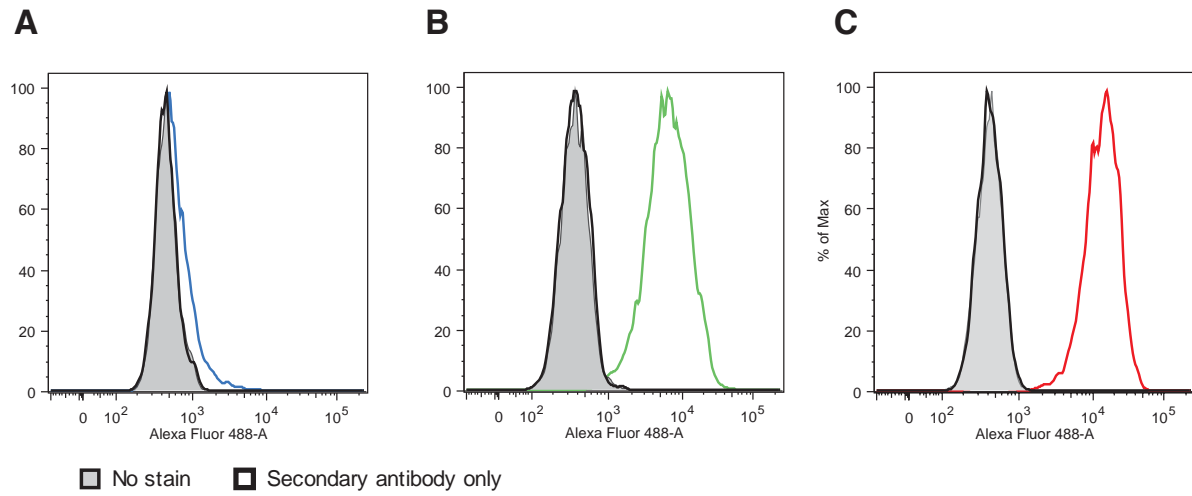
$$\%binding = \frac{Blank - Sample}{Blank} \cdot 100\%$$

Pull-down on untreated FVIIai was analysed using an FVIIa quantification assay from Dako as previously described (1).

## References

1. Erlandsson M, Nielsen CH, Jeppesen TE, et al. Synthesis and characterization of (18)F-labeled active site inhibited factor VII (ASIS). *J Label Compd Radiopharm.* 2015;58:196–201.

**Supplemental Figure**



**Supplemental Figure 1:** Flow cytometry of PANC-1 (A), ASPC-1 (B) and BxPC-3 (C) cells stained for TF. The PANC-1 cells were TF low expressing or negative. The ASPC-1 cells showed intermediate expression of TF and the Bxpc-3 cells showed high TF expression.