Supporting information

On-demand radiosynthesis of N-succinimidyl-4-[¹⁸F]fluorobenzoate on the electrowetting-on-dielectric microfluidic chip for ¹⁸F-labeling of protein

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Fig. S-1: Overlay TLC chromatogram of a crude samples of [¹⁸F]fluoride ion and ethyl-4-[¹⁸F]fluorobenzoate (blue trace) [¹⁸F]fluorobenzoic acid (red trace), and [¹⁸F]SFB (green trace)



Fig. S-2: A representative set of HPLC chromatograms (UV and γ -detection) of the purified and formulated [¹⁸F]SFB.



Fig. S-3: Representative images of reaction droplet at the end of the fluorination reaction using (a) 6 μ L or (b) 2 μ L droplet of precursor solution. The red circle outlines the reaction heater on the EWOD chip.



Fig. S-4: Reaction kinetics of the esterification reaction between the [¹⁸F]fluorobenzoic acid and the HSTU coupling agent. The optimal reaction time was selected to be 3.5 min.

Molecular characteristic of the diabody used in this study is a dimer of scFv with engineered C-terminal cysteine residues that form an inter-chain disulfide bridge with a molecular weight of 50.6 kDa. The Anti-PSCA Cys-Diabody 2B3 A2 (A2cDb) was generated by adding a C-terminal his-cys-tag (-H6-GGC) to the humanized and affinity-matured anti-PSCA 2B3 A2 diabody. Production and purification of A2cDb were previously described.¹

References:

1 G. A. Sonn, A. S. Behesnilian, Z. K. Jiang, K. A. Zettlitz, E. J. Lepin, L. A. Bentolila, S. M. Knowles, D. Lawrence, A. M. Wu and R. E. Reiter, *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.*, 2016, **22**, 1403–1412.