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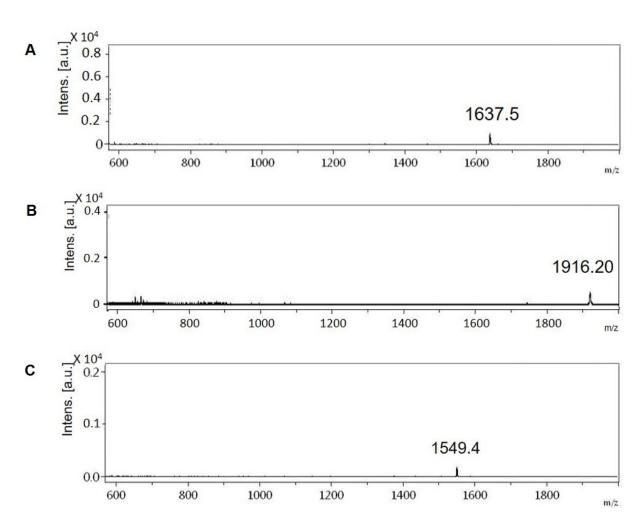
## **Supporting Information**

## Ligand design for cancer imaging with long blood circulation and enhanced accumulation ability in tumors

Elnaz Nakhaei, Chan Woo Kim, Daiki Funamoto, Hikari Sato, Yuta Nakamura, Akihiro Kishimura, Takeshi Mori, Yoshiki Katayama

## Characterization of folate-fluorophore conjugates (probe 1, 2 and 3)

Purity analysis of the purified probes was carried out on an analytical reversed-phase high performance liquid chromatography (HPLC) system using C18 RP ( $100 \times 4.6 \text{ mm } 5 \mu \text{m}$ ) column using a linear A-B gradient at a flow rate of 1 mL/min, where eluent A was 0.1% TFA in water and eluent B was 0.1% TFA in acetonitrile. B eluent with gradient of 25-70 in 45 minutes, 40-70% in 30 minutes and 20-50% in 30 minutes was run for the probe 1, 2 and 3, respectively. UV absorbance at 220, 552 nm and 740 nm was monitored. Calculated purity for the probes 1, 2 and 3 are 78%, 80% and 81%, respectively.



**Fig. S1** MALDI-TOF mass spectrum of the purified probes. (A) Probe 1 (MW: 1639.9) (B) Probe 2 (MW: 1915.3) (C) Probe 3 (MW: 1548.7)