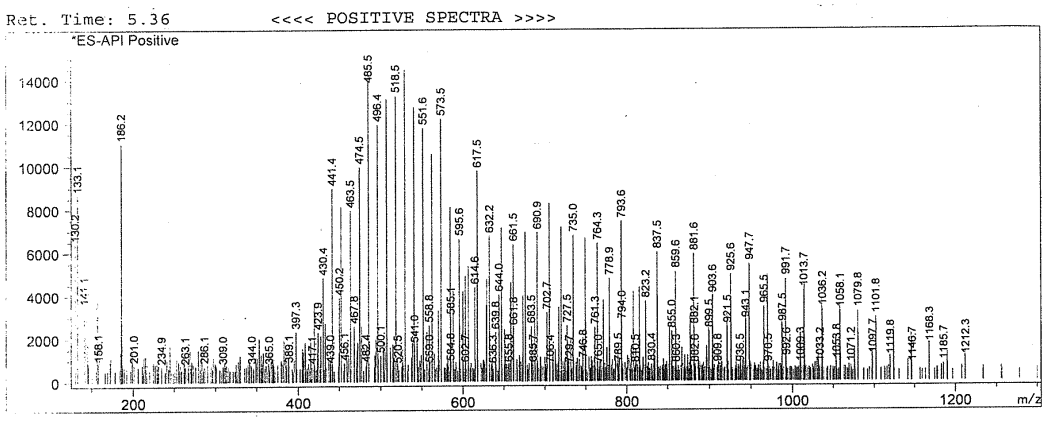
Supplemental Methods

Solid phase synthesis monomeric (F1A) and tetrameric (F4A) fibrin probes

Monomeric unit (F1A) was a bifunctional entity comprised of a fibrin-specific natural peptide analogue (QIWCLGYPCEWQ) [33] interconnected through a PEG_2 spacer to a technetium chelating amino acid sequence (HHE-PEG_2). (Figure 2A) PEG_{2000} tetramer was used to intercouple four F1A monomers to form F4A. (Figure 2B) More specifically, peptide precursors were synthesized with a CS136 peptide synthesizer at the 0.2mmol scale. Standard solid phase Fmoc deprotection and amino acid coupling reactions were used [34]. Disulfide cyclization was carried out with TECP reduction and DTNB coupling, followed by 1% TFA deprotection while the peptide intermediate remained on solid support [35]. After assembling the peptide, the resin was washed with DMF, and then cleaved with TFA/anisole/water (95/2.5/2.5) for 2h. The synthesis was repeated using HBTU coupling (≥99% purity) and Fmoc-Gln(tBu)-Wang resin (0.5 mmol/g). The final product was purified by HPLC and characterized by ESI-TOF mass spectrometer: ESI-TOF (positive mode): m/z $[C_{94}H_{126}N_{24}O_{26}S_2 + 2H]^+$ Calcd. 1036.4 Da.; Obsd. 1036.3 Da.

To obtain the tetramer, the side-chain protecting group, 4-{N-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]-amino}benzyl (Dmab) was selectively removed from glutamate and coupled with the 4-arm-PEG amine (PSB-431, 2K, Creative PEGWorks, Chapel Hill, NC). F4A was purified with size exclusion chromatography (Zeba Desalt Spin Column, 2 mL, ThermoScientific) using 10% acetic acid in water followed by three deionized water washes. The overall yield of F4A was < 10%. MALDI characterization of the product revealed an array molecular weights arising from an unexpectedly high polydispersity of the tetrameric PEG core.

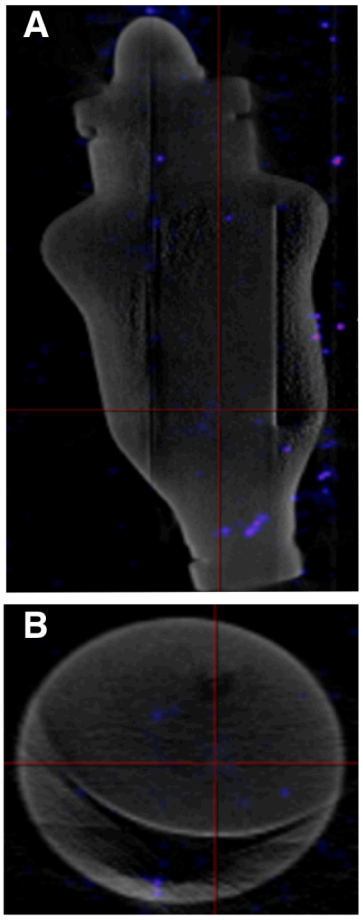


^{99m}Tc labeling of F1A and F4A

F1A and F4A were radiolabeled with ^{99m}Tc for gamma imaging. This was accomplished by adding $Na^{99m}TcO_4$ (~9 mCi; 1.0 ml) in saline to an IsoLink vial (Center for Radiopharmaceutical Sciences of PSI, ETH and USZ, Paul Scherrer Institute, Switzerland). The mixture was heated to 100°C for 20 min to form the intermediate $[^{99m}Tc(CO)_3(H_2O)_3]^+$, then cooled to ~25ºC and neutralized with 0.2 ml of a 1:2 mixture of 1 M phosphate buffer (pH 7.4) and 1 M HCl. This solution (0.16 M) was added into a 5 ml vial containing the peptide ligand in 0.15 ml of PBS. The reaction mixture was heated at 60°C for 30 min before cooling to ~25ºC. The resulting solution was purified by size exclusion column chromatography and analyzed by radio TLC. Radiochemical purity of 90 to 95% was repeatedly achieved with a specific activity of 40mCi/μmole or greater.

Supplemental Figures.

FIGURE S1. Negligible binding of the probe in a control reference Heartmate II LVAD. **A** Top image is a longitudinal view in line with blood flowing from the bottom to the top. **B** The bottom image is a transaxial view.



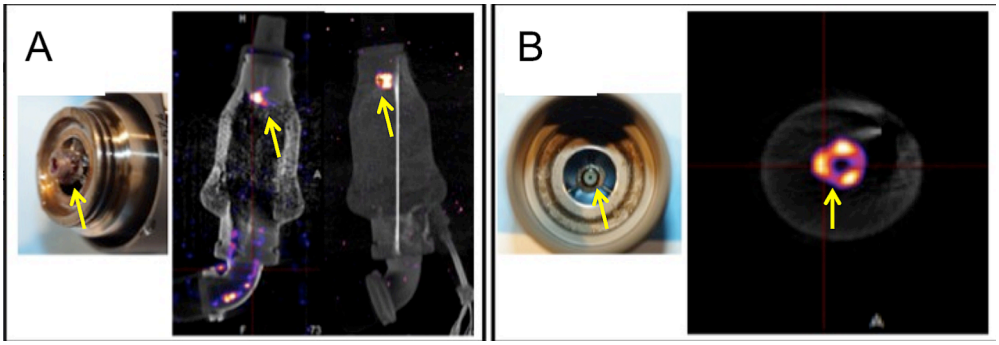


FIGURE S2. A LVAD from a patient with suspected pump thrombus showing ^{99m}Tc-F4A detection of fibrin on the bearing/stator assembly that was confirmed independently following disassembly by the manufacturer. (Implanted 519 days; LDH 9560 U/L; Exchanged). **B** Second example LVAD from a patient with suspected pump thrombus showing ^{99m}Tc-F4A detection of fibrin on the bearing/stator assembly that was confirmed following disassembly by the manufacturer. (Implanted 413 days; LDH 4960 U/L; Exchanged)

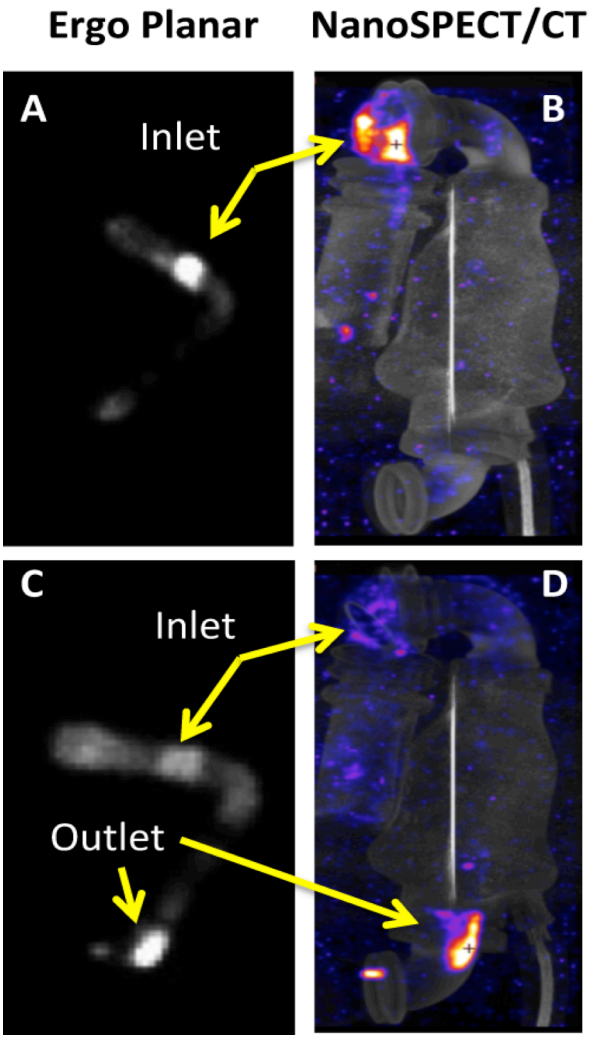


FIGURE S3. A. Digirad Ergo planar ^{99m}Tc-F4A image of excised LVAD (GIB, no hemolysis; implanted 230 days). Parameter settings: 256 x 256 matrix, LEAP collimator at 1.0 zoom, 15 min acquisition. **B**. Bioscan NanoSPECT/CT image of LVAD in **A**. **C**. Digirad Ergo planar ^{99m}Tc-F4A image of excised LVAD. (GIB, no hemolysis, implanted 1370 days). Parameter settings as above. **D**. NanoSPECT/CT image of LVAD in **C.** These data illustrate the potential of ^{99m}Tc-F4A enhanced native LVAD thrombus to be visualized with a clinical planar camera, suggesting adequate sensitivity for detection. A current clinical scanner should localize a medically significant “hot-spot” to pump regions impacted thrombus accumulation.