Low Fouling and Biodegradable Protein-Based Particles for Thrombus Imaging (supplementary information)

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SUPPORTING METHODS SECTION

Generation, expression and purification of PASK protein building blocks. The DNA sequence encoding the PASK protein (full amino acid sequence: MV(ASPAAPAPASPAAPAPSAPAK)$_{20}$LPETGGLE-His$_8$) codon optimized for bacterial expression, and flanked by the restriction sites NcoI and XhoI, was ordered from Geneart and transformed into NEB Turbo competent cells. DNA was prepped using a Qiagen plasmid mini kit and digested with NcoI and XhoI (NEB). The resultant 1382bp fragment was run on and excised from a 0.8% agarose gel, and purified using a QIAGEN plasmid gel extraction kit. pET20b+ (Novagen) was prepared equivalently. The construct was then ligated into the multiple cloning site of pET20b+ with T4 DNA Ligase (NEB) and subsequently transformed into NEB Turbo competent cells. Colony screening PCR was used to determine successful ligation, with GoTaq Green master mix (Promega). Primers flanked the multiple cloning site and annealed to the T7 promoter and terminator sequences (Novagen). A successful ligation was indicated by a 1567bp amplicon. Plasmid preparations of positive colonies were sent to AGRF for sequencing, with the same primers as for colony screening PCR, and the Emboss Needle sequence alignment tool was used to confirm a match with the expected sequence. DNA confirmed as MV(ASPAAPAPASPAAPAPSAPAK)$_{20}$LPETGGLE-His$_8$ correctly inserted into pET20b+ was transformed into One Shot® BL21 Star™ (DE3) cells for expression in *E. coli*. The cells were cultured in LB media containing 100 μg/mL ampicillin until the OD600 of 0.8 was reached. PASK production was induced with 1 mmol L^{-1} of isopropyl β-D-1-thiogalactopyranoside (IPTG) for 4 hours at 37^{o}C. Bacteria were then isolated by centrifugation at 6000 rcf for 10 min. Proteins were purified by fast liquid protein chromatography using Ni-NTA column (Invitrogen) followed by size exclusion chromatography (Superose 12, 10/300 GL, GE Healthcare), according to the

manufacturer's protocol. Protein concentration was determined with a Direct Detect Infrared Spectrometer (Merck, Millipore). The purity of the proteins was analyzed by SDS-PAGE gel stained with Coomassie Brilliant Blue visualized using a BioRad Gel-Doc system. Western blot analysis was performed to confirm the presence of the PASK protein by revealing the 8xHis-tag. After SDS-gel electrophoresis, the proteins were transferred on polyvinylidene difluoride (PVDF) membranes which were blocked with 5% skimmed milk at 4°C overnight then incubated for 1 hour with Anti-6xHis-tag antibody HRP (horse radish peroxidase). Detection was performed with SuperSignal West Pico chemiluminescent (ECL) substrate (Thermo Scientific) for the HRP enzyme. The ECL signal on membranes was visualized using a BioRad Gel-Doc system.

Assembly of PASKE particles. Mesoporous silica (MS) particles (3 mg) were washed with phosphate buffer (PB, 10 mM, pH 7.4) and incubated in 300 µL of PASK building blocks solution at 5 mg mL^{-1} in PB under constant shaking overnight. The MS@PASK particles were then isolated by centrifugation and washed three times with PB, followed by labeling with 5 µL of Cyanine 7 or Cyanine 5 succinimidyl ester (NHS-Cy7 or NHS-Cy5, Lumiprobe, 1 mg mL^{-1}) in 100 µL PB for 10 min. 100 µL Poly-L-glutamic acid (PGA, 1.5 kDa, 1.5 mg, Sigma Aldrich, US) was mixed with 100 µL of 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM, 4.5 mg) (Sigma Aldrich, Australia) and incubated for 15 minutes at room temperature while mixing. Crosslinking of PASK protein within the templates between the primary amines of lysine residues and activated carboxylate groups of glutamic acid residues was done by adding 200 µL of PGA and DMTMM mixture to PASK-loaded MS particle suspension and incubated overnight at room temperature. After three washing cycles with water, the MS@PASKE were resuspended in PB (10 mM). For the preparation of the PASKE particles, MS templates were removed with a 2 M Hydrofluoric acid/8 M Ammonium fluoride solution. Bovine serum albumin (BSA) based

particles were synthesized following similar method replacing PASK protein by BSA (Sigma Aldrich).

Mass spectrometry analysis. The samples were reduced and alkylated with DTT\Iodoacetamide and digested overnight with trypsin in a total of 50 uL of 20 mM ammonium bicarbonate buffer. Tryptic digests were analyzed by liquid chromatography coupled to mass spectrometry (LC-MS/MS) (LC: Ultimate 3000 nano RSLC, MS: QExactive mass spectrometer, ThermoFisher Scientific) by separation over a 30-minute gradient on a Thermo RSLC pepmap100, 50cm reversed phase nano column at a flow rate of 300 nL min^{-1}. The eluent was nebulized and ionized using the Thermo nano electrospray source. Peptides were selected for MS/MS analysis in full MS/dd-MS2 (TopN) mode with the following parameter settings: TopN 10, resolution 17500, MS/MS AGC target 1e5, 60ms Max IT, NCE 27 and 3 m z^{-1} isolation window. Underfill ratio was at 10% and dynamic exclusion was set to 15 seconds. Data from the LC-MS/MS run was exported to Mascot generic file format using proteo wizard open source software and searched against an in-house curated database of PASK protein sequence and the SwissProt database using the MASCOT search engine (version 2.4, Matrix Science Inc., London, UK) with all taxonomy selected. The following search parameters were used: missed cleavages, 1; peptide mass tolerance, ± 20 ppm Da; peptide fragment tolerance, ± 20 mmu; peptide charge, 2+, 3+ and 4+; fixed modifications, carbamidomethyl; Variable modification, oxidation (Met).

Transmission Electron Microscopy (TEM). A suspension containing the PASKE particles was cast on Formvar-coated copper grids, followed by overnight air-drying. The grids were then washed with pure water to remove salt and allowed to air dry. TEM imaging was conducted on a CM120 BioTWIN instrument (Philips, Germany) operating at an acceleration voltage of 120 kV.

Near Infra-Red (NIR) Confocal Microscopy. The NIR confocal images were taken on a customized confocal microscope. A Nikon (Tokyo, Japan) A1R Plus confocal microscope was modified to include a NIR solid-state continuous wave (CW) laser (Obis 30mW, 730-nm, Coherent Inc., Santa Clara, USA). This was mounted on a separate laser board (Nikon LU3, equipped with a manual laser attenuator). An 80/20 beam splitter was used as the primary dichroic. The emitted NIR fluorescent signal was reflected by a custom filter cube (secondary dichroic Mirror 801LP [Semrock FF801-Di02-25x36], Band Pass filter 796/41 [Semrock FF01-796-41-25]) and collected with a standard PMT as supplied with a Nikon A1R Plus confocal microscope.

Zetasizer measurements. The hydrodynamic diameter, scattering light intensity, and zeta(ζ)-potential of MS@PASKE, PASKE, BSA and polyethylene glycol (PEG) particles were measured using a Zetasizer Nano ZS instrument (Malvern Instruments, Malvern) equipped with a He−Ne ion laser (λ = 633-nm) as an incident beam. For the dynamic light scattering (DLS) measurements, a particle dispersion in PB was placed in a micro cuvette (ZEN0040, Malvern Instruments). The photon correlation function was analyzed by the cumulant method to derive the diffusion coefficients (DC) of the particles. The obtained DC was converted into the hydrodynamic diameter using the Stokes−Einstein equation: $DH = kBT/3\pi\eta DC$ (kB: Boltzmann constant, T: absolute temperature, η: viscosity of the solvent). For the ζ-potential measurements, a particle dispersion in PB (700 μL) was placed into a folded capillary cell (DTS1070, Malvern Instruments). The ζ-potential was obtained from the electrophoretic mobility and by using the Smoluchowski equation: $\zeta = 4\pi\eta\nu.\varepsilon^{-1}$ (η: viscosity of the solvent, ν: electrophoretic mobility, ε: dielectric constant of the solvent).

Cell association assays. THP-1 cells (ATCC) and RAW 264.7 cells (ATCC) were cultivated in Roswell Park Memorial Institute (RPMI) 1640 medium (Sigma Aldrich) or Dulbecco's Modified

Eagle's Medium respectively (DMEM) (Sigma Aldrich) supplemented with 10% Fetal Calf Serum (FCS), 1% L- Glutamine, and 1% penicillin/streptomycin. Particles (5×10^6 mL^{-1}) were incubated at 37C with 10^5 cells mL^{-1} (50:1 ratio) in 48-cell culture well plate (Corning, Sigma-Aldrich). PASKE-Cy5, PEG-Cy5, BSA-Cy5 and MS@PASKE-Cy5 particles were incubated for 1, 6 and 24 hours in the corresponding cell media or a mixture of cell media and human plasma (3:2 ratio). Cells were harvested, washed twice in phosphate buffer saline (PBS) and analyzed by flow cytometry (LSRFortessa, BD Biosciences). Cells association was identified from the fluorescence signal resulting from Cy5 labelled particles and percentage of association was measured from 10,000 events. For imaging flow cytometry (Amnis, ImageStreamX, Merck Millipore), THP-1 cells were labelled with LysoTracker Blue DND-22 (0.3 nM, ThermoFisher Scientific) and lysosomes were identified in 405-nm excitation 457/45 bandpass channel, Cy5 labelled particles were detected in the 642-nm excitation 702/85 bandpass channel and the cell structure was observed in the bright field channel. For confocal microscopy observation (A1R+, Nikon), RAW 264.7 cells were labelled with LysoTracker Blue DND-22 (0.3 nM, Life Technologies) and lysosomes were observed in the 405-nm channel, cell membranes were stained with wheat germ agglutinin Alexa Fluor 488 conjugate (WGA-AF488, 10 μg mL^{-1}, Life Technologies) and observed in the 488-nm channel and Cy5 labelled particles were detected in the 640-nm channel.

Biodegradability assays. PASKE and PEG particle degradation in tissue lysosomal fraction was tested *in vitro*. Freshly harvested mouse spleen, liver, kidney and lung tissue was homogenized (Ultra-turrax T10basic, IKA) and the lysosomal fraction was isolated from 200 mg mL^{-1} of tissue following a procedure described elsewhere.[1] PASKE-Cy5 and PEG-Cy5 particles (2×10^6 mL^{-1}) were incubated at 37°C with 50 μL of lysosomal fraction in 200 μL acetate buffer (100 mM sodium acetate, 5 mM calcium chloride, 1.33 mM EDTA and 2 mM DTT, pH 5.5) or in 50 μg mL^{-1}

Trypsin-EDTA (Gibco). The concentration of particles was measured at the beginning of the incubation (t=0) and at 0.5, 1, 6 and 24 hours incubations by flow cytometry (LSRFortessa, BD Biosciences) counting Cy5 positive event in a fixed volume and the percentage of degradation was calculated from the ratio of the concentration at t=0. Mean percentage of degradation ± SD was plotted over time (n=4). Additionally, PASKE-Cy7 and PEG-Cy7 particles were incubated at 37°C with the lysosomal fraction in acetate buffer (pH 5.5) or in 50 μg mL^{-1} Trypsin-EDTA overnight and were analyzed with SDS gel electrophoresis. The gel was scanned using an Odyssey CLx Infrared Imaging System (LI-COR, Biosciences) and particles or degradation products were detected in the 800-nm channel. Non-degraded particles were too big not migrate and were detected on top of the gel whereas degradation products migrated through the gel and were detected at the bottom of the gel.

Functionalization of PASKE with SCE5 and Mut-scFv single-chain antibodies. Single-chain antibody (scFv) SCE5, specific for the activated glycoprotein complex IIb/IIIa (GPIIb/IIIa), Mut-scFv, the non-targeted version of the antibody and green fluorescent protein (GFP), all designed with a leucine, proline, glutamic acid, threonine and glycine tag (LPETG) at the C-terminus for Sortase A conjugation and the Sortase A enzyme were produced as previously described.[2] The glycine modified bicyclo[6.1.0]nonyne (G-PEG_3-BCN) Sortase substrate was prepared as previously described.[3] The scFv were then covalently attached to the PASKE particles *via* Sortase A bioconjugation and copper-free click chemistry (Figure S3 A), following previously established protocol.[4] Briefly, SCE5-LPETG, Mut-scFv-LPETG or GFP-LPETG (30 μM) were incubated 5 hours while shaking with Sortase A (90 μM) and G-PEG_3-BCN (90 μM) in 1 mL of Tris buffer (50 mM Tris(hydroxymethyl)aminomethane hydrochloride, 150 mM sodium chloride, 0.5 mM calcium chloride, pH 8). At the end of the reaction, the Sortase A enzyme, which has a His_8 tag at

the C terminus and the residual G-His_8 cleaved fragment were removed from the reaction with metal affinity resin beads (TALON). The modification of the scFv with the BCN group was confirmed by the addition of Cyanine7-azide dye and analysis by SDS gel electrophoresis scanned with an Odyssey CLx Infrared Imaging System (Figure S3 B and C). PASKE particles were functionalized with azide group (N_3) by incubation for 2 hours at room temperature under gentle mixing with N-hydroxysuccinimide-azide linker (N_3-PEG_8-NHS ester, Conju-probe) and washed by 5 min centrifugations at 6,000 g. SCE5-BCN, Mut-scFv-BCN or GFP-BCN were then mixed with PASKE-N_3 at a 2:1 molar ratio for 2 hours at room temperature under gentle mixing to enable a copper-free click reaction. An additional control group was obtained from non-modified PASKE particles incubated with GFP-BCN. PASKE-Cy5-N_3 and PASKE-Cy5 incubated with GFP-BCN were analyzed in flow cytometry (Figure S3 D) and in confocal microscopy (Figure S3 E) to verify successful conjugation of GFP-BCN detected in 488-nm channel to PASKE-Cy5-N_3 observed in 640-nm channel. PASKE-Cy5-SCE5, PASKE-Cy7-SCE5, PASKE-Cy5-Mut-scFv and PASKE-Cy7-Mut-scFv were then prepared following similar copper-free click procedure with PASKE-Cy5-N_3 or PASKE-Cy7-N_3 with SCE5-BCN or Mut-scFv-BCN.

Human blood and plasma preparation. The recruitment of participants and collection of blood specimens was approved by the Alfred Hospital Ethics Committee (Project 67/15). Signed informed consent was obtained prior to participation. Human blood was collected *via* venipuncture into tri-sodium citrate (3.2% w/v final). For plasma preparation, whole blood was centrifuged at 1,700 g for 15 minutes and plasma was collected from the supernatant and stored at -30 ^{o}C.

Flow chamber experiments. Microfluidic polydimethylsiloxane channels deposited on glass coverslip micro were incubated with bovine type 1 collagen (250 µg mL^{-1} in 10 mM acetic acid) for 30 minutes and then perfused with anticoagulated human whole blood labelled with 3,30-

dihexyloxacarbocyanine iodide ($DiOC_6$, 0.5 ug mL^{-1}) for 3 minutes to allow platelets to adhere to the collagen fibers and form micro-aggregates. PASKE-Cy5-anti-GPIIb/IIIa-scFv and PASKE-Cy5-Mut-scFv particles were flown over the top of the microthrombi at a shear rate fixed at 300 s^{-1} for 3 minutes. Microthrombi were observed with an inverted confocal microscope (A1R+, Nikon) in the 488-nm channel and PASKE-Cy5 particles in the 640-nm channel. Time-lapse Z-stack acquisitions were performed with a fast piezo nanopositioning system (Nano-Drive, Mad City Labs Inc.) over the first minute and then a range of images were taken along the flow channels for quantification. The amount of microthrombi was measured in the 488-nm channel images with Image J (NIH, USA) and expressed as the area of surface coverage (in mm^2). The PASKE-Cy5-anti-GPIIb/IIIa-scFv or PASKE-Cy5-Mut-scFv adherent to the micro thrombi were counted in the 640-nm channel images. Results are presented as mean values of particles per mm^2 of thrombi ± SD (n=17).

Blood clearance. Mice were anaesthetized and PASKE-Cy5, PEG-Cy5, BSA-Cy5 and MS@PASKE-Cy5 particles were injected intravenously at a dose of 10^6 g^{-1}. Drops of 5 µL of blood were harvested from the venous circulation at 2.5, 5, 10, 30 and 60 minutes post-injection and sampled in 200 µL 10 mM PB buffer. Samples to determine the injected dose were prepared by diluting injected suspensions at 10^6 in 60 µL (total blood volume was approximated at 6% of the body weight) then sampling this dilution 5 µL into 200 µL. Particle concentrations were determined by flow cytometry (LSRFortessa, BD Biosciences) counting Cy5 positive event in a fixed volume and percentage from the number of particles counted in the injected dose samples were calculated. Mean percentage of injected dose ± SD were plotted over time post-injection with nonlinear fit (n=4).

Liver and spleen cell analysis. Mice were anaesthetized and PASKE-Cy5, PEG-Cy5 and MS@PASKE-Cy5 particles were injected intravenously at a dose of 10^6 g^{-1}. At 3 hours post-injection, mice were culled, slowly perfused with warm PBS buffer and spleen and liver tissue was harvested. The protocol for spleen and liver cell suspensions preparation and subtype identification by flow cytometry was adapted from a method described elsewhere.[5, 6] Briefly, spleen tissue was gently dissociated into a single cell suspension using a 40 μm cell strainer, and divided into 3 suspensions labelled as follows: Suspension 1 was stained with a B220-FITC antibody to label B-cells and CD4/CD8-BV605 to label T-cells; suspension 2 was stained with CD41-FITC to label platelets and megakaryocytes and Ter119-PE to label red blood cells; suspension 3 was stained with F4/80-PE to label macrophages, Ly6C-BV605 to label monocytes and Ly6G-APC/Cy7 to label granulocytes. The gating strategy to identify cell subtypes in the spleen is presented in Figure S5. The liver tissue was incubated at 100 mg mL^{-1} with type IV collagenase (0.1%, Gibco), gently teased with forceps until a homogenous mixture was obtained, filtered through a 40 μm cell strainer and suspensions were labelled as follows; B220-FITC antibody to label B-cells, CD4/CD8-BV605 to label T-cells, CD31-PerCP/Cy5.5 to label endothelial cells, F4/80 PE and Mac1-V450 to identify inflammatory monocyte-derived (IMD) macrophages and Kupffer cells and the hepatocytes were identified as the non-labelled remaining cells. The gating strategy to identify cell subtypes in the liver is presented in Figure S6. The amount of Cy5 labelled particles in each cell subtype population was counted in the R-670/14 channel, expressed as the percentage of the amount of the cell subtype and presented as mean values ± SD (n=3).

Histology. Mice were anaesthetized and PASKE-Cy5, PEG-Cy5 and MS@PASKE-Cy5 particles were injected intravenously at a dose of 10^6 g^{-1}. At 30 minutes and 24 hours post-injection, mice were culled, slowly perfused with warm PBS buffer, the spleen and liver tissue was harvested and

fixed in 10% neutral buffered formalin for 24 hours. Processing to paraffin wax was carried out in a tissue processor (Leica Peloris II) and tissues were embedded in paraffin media. 4 μm thick paraffin sections of liver and spleen were prepared using a microtome (Leica RM2235 rotary), mounted on Superfrost Plus slides and allowed to dry prior to dewaxing and staining. Section were stained with wheat germ agglutinin Alexa Fluor 488 conjugate (WGA-AF488, 10 μg mL^{-1}, Life Technologies) to observe cell membrane in the 488-nm channel, with Hoechst 33342 (0.1 mg mL^{-1}, Life Technologies) to observe cell nuclei in 405-nm channel and Cy5 labelled particles were detected in the 640-nm channel.

Transmigration of PASKE particles across a human umbilical vein endothelial cell monolayer into collagen gels. PASKE-Cy5 particles were incubated at 10^6 particles mL^{-1} for 24 h on type I fibrous collagen gels overlaid with a tumour necrosis factor (TNF)-activated or non-activated primary human umbilical vein endothelial cell (HUVEC) monolayer prepared as previously described.[7] Following transmigration, gels were washed once with phosphate buffer saline (PBS) and fixed overnight in 2% formaldehyde. The next day, gels were stained with Hoechst 33342 (0.1 mg mL^{-1}, Life Technologies) for 30 mins, washed once with PBS and stained with wheat germ agglutinin Alexa Fluor 488 conjugate (WGA-AF488, 10 μg mL^{-1}, Life Technologies) for 20 mins. Gels were extracted and mounted on microscope slides as described elsewhere and analyzed by confocal microscopy (A1R+, Nikon).[7] Two 100 μm Z-stack observations were performed per gels from four gels per condition. Cy5 labelled particles localized within the collagen gel were counted in the 640-nm channel and expressed as mean number of particles per surface area of cell monolayer ± SD (n=8).

Statistical analysis. All results are expressed as mean values ± SD. Statistical analysis was performed with GraphPad Prism V7 (GraphPad Software). Cell association and tissue

biodistribution results were compared with two-way ANOVA with Tukey's multiple comparison post-tests. Flow chamber and particle migration data were compared with unpaired t-tests. A difference of $p<0.05$ was considered significant.

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Table S1. Physicochemical Properties of MS@PASKE, PASKE, PEG and BSA particles

Particles	Size[a,b] (nm)	PDI[a]	ζ-potential[a] (mV)	Cell association	
				media	plasma
MS@PASKE	1213	0.016	-37 ± 6	+++	+++
PASKE	731 ± 6	0.353 ± 0.057	-9 ± 4	-	+
PEG	1066 ± 457[c]	-	-9 ± 3	-	-
BSA	671 ± 20	0.085 ± 0.033	-39 ± 4	+++	+++

a) Determined in phosphate buffer (pH 7.4, 10 mM). The results are expressed as mean ± standard deviation; b) Size denotes hydrodynamic diameter measured by diffracted light scattering except for PEG; c) The approximate size of the PEG particles was measured from confocal microscope images using an image analysis software (Image J); d) +++ strong association, + slow association, - low association

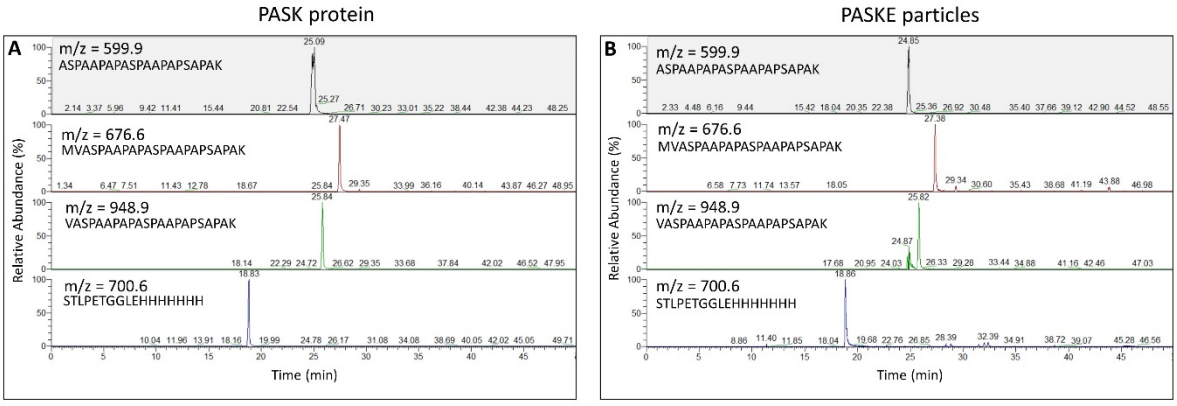

Figure S1: Ion chromatogram from liquid chromatography-mass spectrometry analysis of trypsin-digested PASK protein (**A**) and PASKE particles (**B**). The different peak corresponding to the 4 cleavage products ASPAAPAPASPAAPAPSAPAK, MVASPAAPAPASPAAPAPSAPAK, VASPAAPAPASPAAPAPSAPAK and STLPETGGLEHHHHHHHH were extracted at their corresponding mass to charge ratio. Data were exported and searched against an in-house curated database of PASK protein sequence and the SwissProt database using the MASCOT search engine;

matching scores of 1.87 10^4 and 1.22 10^4 were obtained for PASK protein and PASKE particles, respectively.

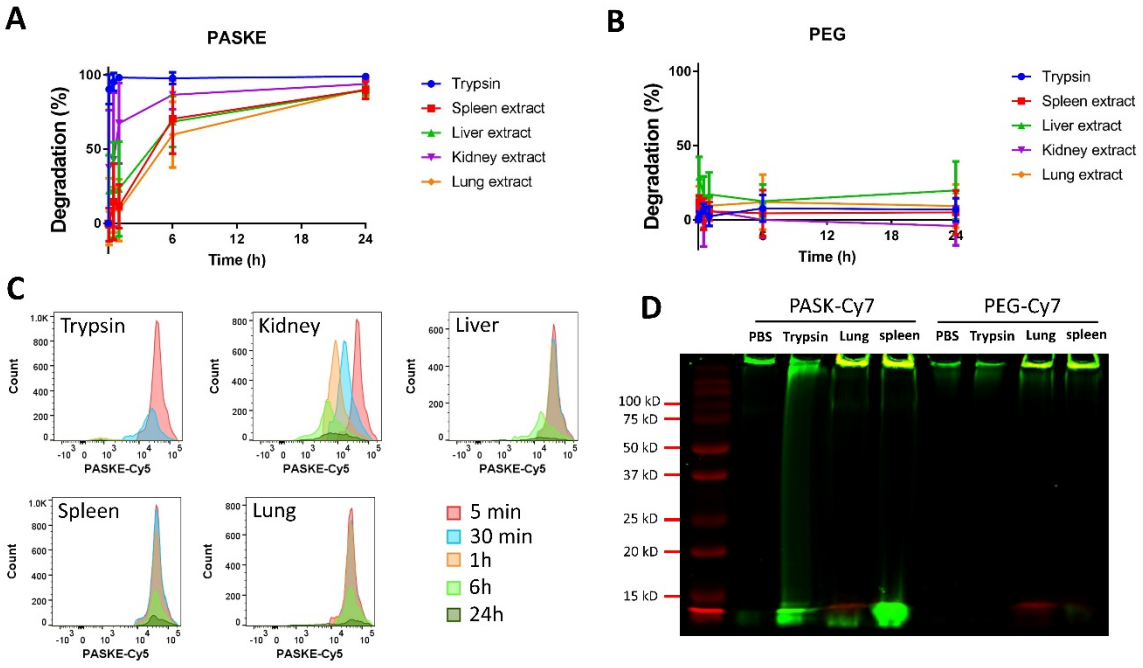

Figure S2: *In vitro* degradability of PASKE-Cy5 (**A**) and PEG-Cy5 (**B**) particles with lysosomal extract in acetate buffer (pH 5.5) or in 50 µg mL^{-1} Trypsin-EDTA over 24h as measured by flow cytometry. Mean percentage of degradation ± SD (n=4). (**C**) Overlay of flow cytometry sensograms obtained with PASKE-Cy5 incubated in Trypsin or in the lysosomal extract from mice kidney, liver, spleen or lung. (**D**) Degradability of PASKE-Cy7 or PEG-Cy7 in PBS, trypsin, lung or spleen lysosomal extract measured by sodium dodecyl sulfate (SDS) gel electrophoresis scanned with an Infrared Imaging System (Odyssey, LI-COR). Non-degraded particles were too big to migrate and were detected on top of the gel whereas degradation products migrated through the gel and were detected at the bottom of the gel.

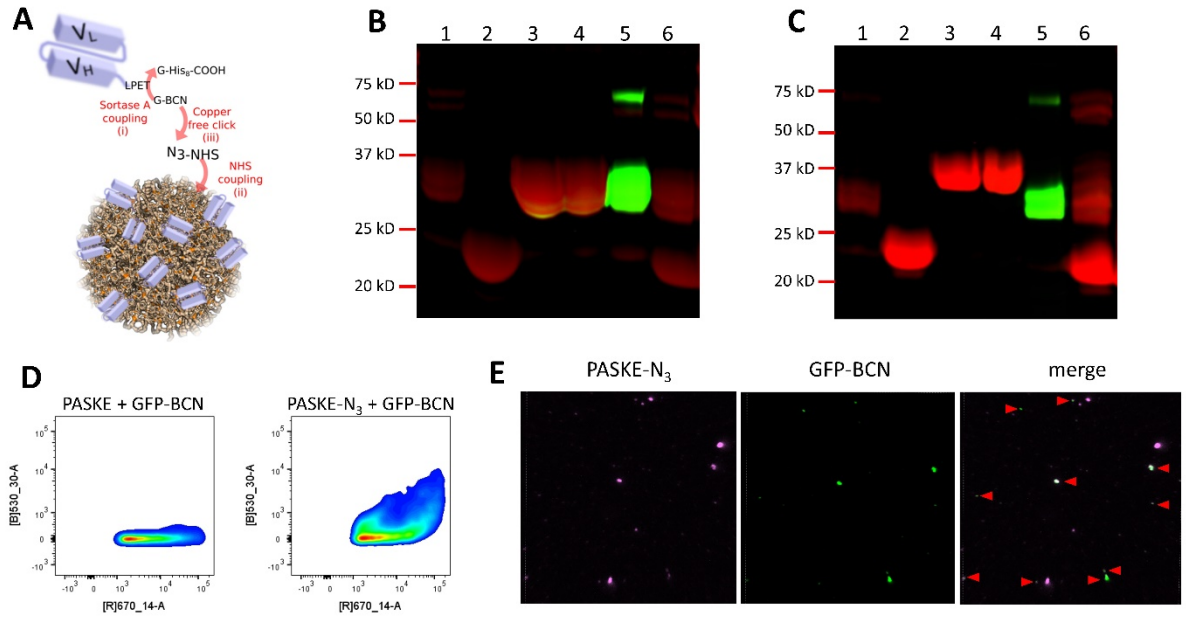

Figure S3: Functionalization of PASKE particles with single-chain antibodies using Sortase A conjugation coupled to copper-free click chemistry. (**A**) Scheme of the different step involved in the labelling; (i) the single-chain antibody is site-specifically modified with a C terminal bicyclo[6.1.0]nonyne (BCN) group *via* Sortase A conjugation, (ii) the remaining free primary amine groups from the lysines residues of PASKE particles are labelled with N-hydroxysuccinimide-azide linker (N_3-PEG_8-NHS) and (iii) BCN modified single-chain antibodies are attached on the surface of the PASKE-N_3 particles by copper-free click chemistry. Sortase conjugation of anti-GPIIb/IIIa-scFv (**B**) and Mut-scFv (**C**) single-chain antibodies was verified by Coomassie-stained sodium dodecyl sulfate (SDS) gel electrophoresis scanned with an Infrared Imaging System (Odyssey, LI-COR). Cyanine7-azide (N_3-Cy7) was used to confirm the presence of BCN group in the 800-nm channel (shown in green). Lanes for B; 1: anti-GPIIb/IIIa-scFv-BCN, 2: Sortase, 3: anti-GPIIb/IIIa-scFv, 4: anti-GPIIb/IIIa-scFv + Cyanine7-azide (N_3-Cy7), 5: anti-GPIIb/IIIa-scFv-BCN + N_3-Cy7, 6: anti-GPIIb/IIIa-scFv-BCN + Sortase (before harvesting Sortase with TALON beads). Lanes for C; 1: Mut-scFv-BCN, 2: Sortase, 3: Mut-scFv, 4: Mut-

scFv + N_3-Cy7, 5: Mut-scFv-BCN + N_3-Cy7, 6: Mut-scFv-BCN + Sortase (before harvesting Sortase with TALON beads). The success of the click conjugation on the surface of PASKE-Cy5 labelled with N_3-PEG_8-NHS (PASKE-Cy5-N_3) was then verified with the addition of green fluorescent protein functionalized with BCN group (GFP-BCN). The signal detected on the particles in the 488-nm channel indicated successful click conjugation. (**D**) Flow cytometry analysis of bare PASKE *versus* PASKE-N_3 incubated with GFP-BCN. (**E**) Confocal microscopy of PASKE-N_3-Cy5 particles shown in pink in the 640-nm channel and GFP-BCN signal shown in green in the 488-nm channel.

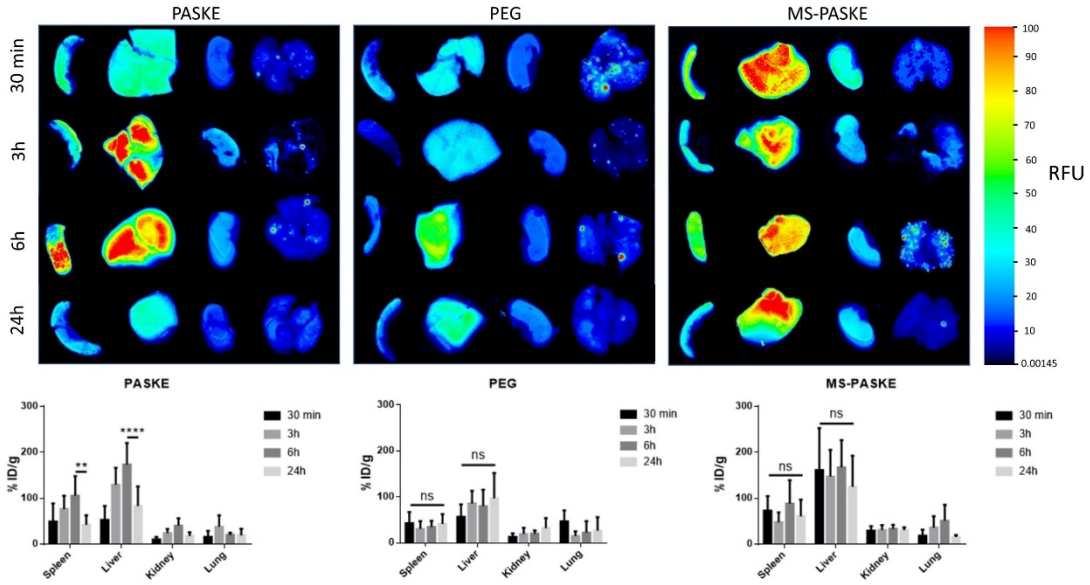

Figure S4: Tissue biodistribution study of PASKE, PEG and MS@PASKE particles labelled with Cyannine7 NHS ester. Animals were sacrificed at 30 min, 3h, 6h or 24h after intravenous injection, slowly perfused with saline, tissues of interest (the spleen, a part of the liver, the left kidney and the lung) were harvested and scanned using an Odyssey Infrared Imaging System. Representative scans showing the relative fluorescence intensity measured in the 800-nm channel are shown. The mean percentage of injected dose per gram of tissue values are presented as the mean ± standard deviation (n=4, two-way ANOVA, ****$p<0.0001$, **$p<0.01$, ns: non-significant).

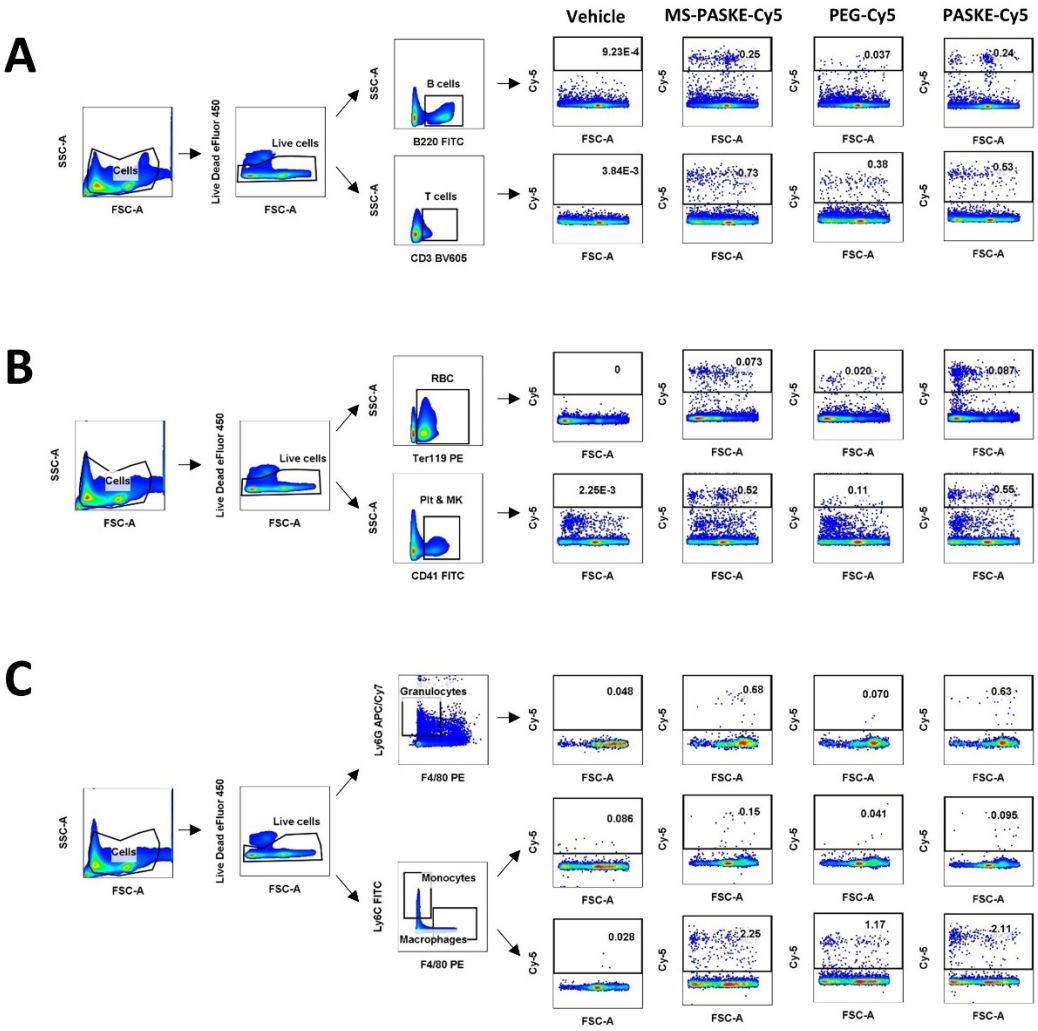

Figure S5: Gating strategy for spleen cell suspension analysis. (**A**) Suspension 1 stained with a B220-FITC antibody to label B-cells and CD4/CD8-BV605 to label T-cells. (**B**) suspension 2 stained with CD41-FITC to label platelets and megakaryocytes and Ter119-PE to label red blood cells. (**C**) suspension 3 stained with F4/80-PE to label macrophage, Ly6C-BV605 to label monocytes and Ly6G-APC/Cy7 to label granulocytes.

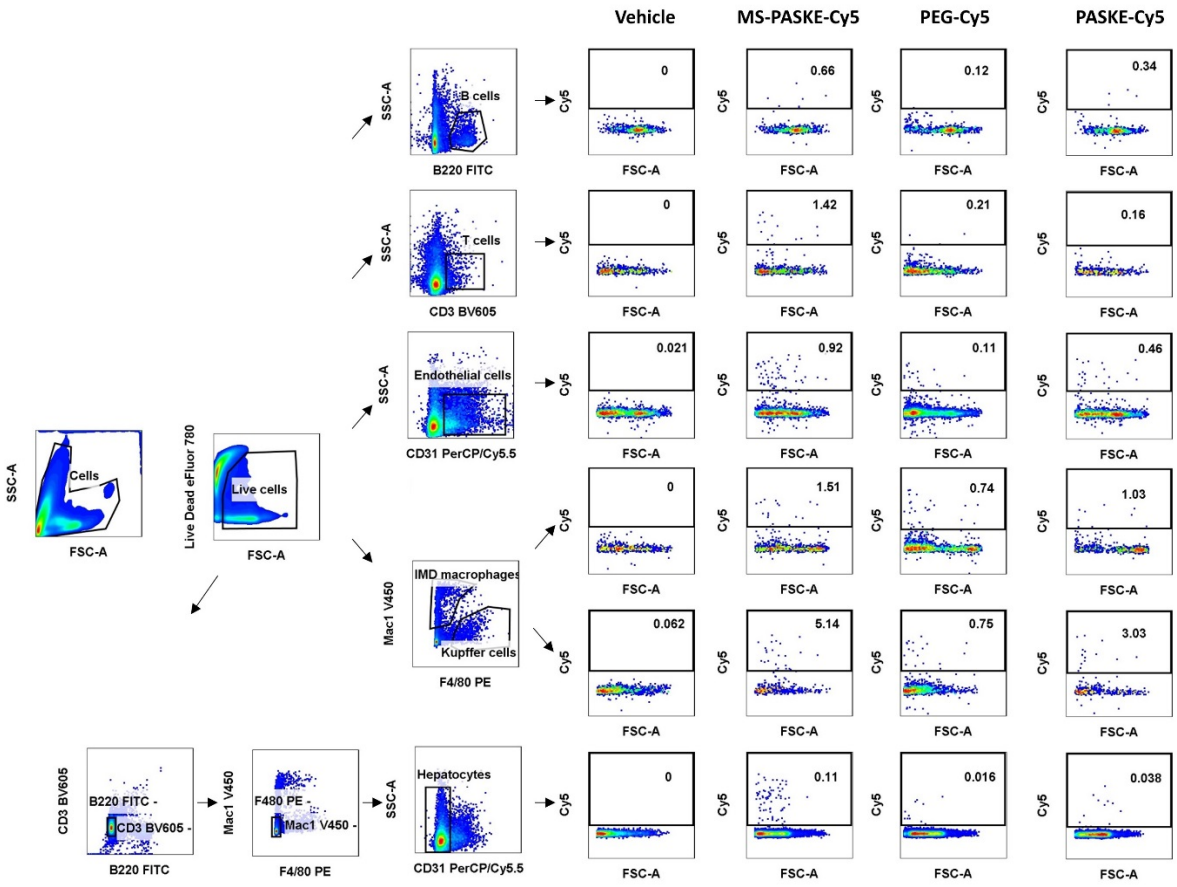

Figure S6: Gating strategy for liver cell suspension analysis. B-cells were labelled with a B220-FITC antibody, T-cells were labelled with CD4/CD8-BV605, endothelial cells were labelled CD31-PerCP/Cy5.5, F4/80 PE and Mac1-V450 were added to identify inflammatory monocyte-derived (IMD) macrophages and Kupffer cells. Hepatocytes were identified as the non-labelled remaining cells.

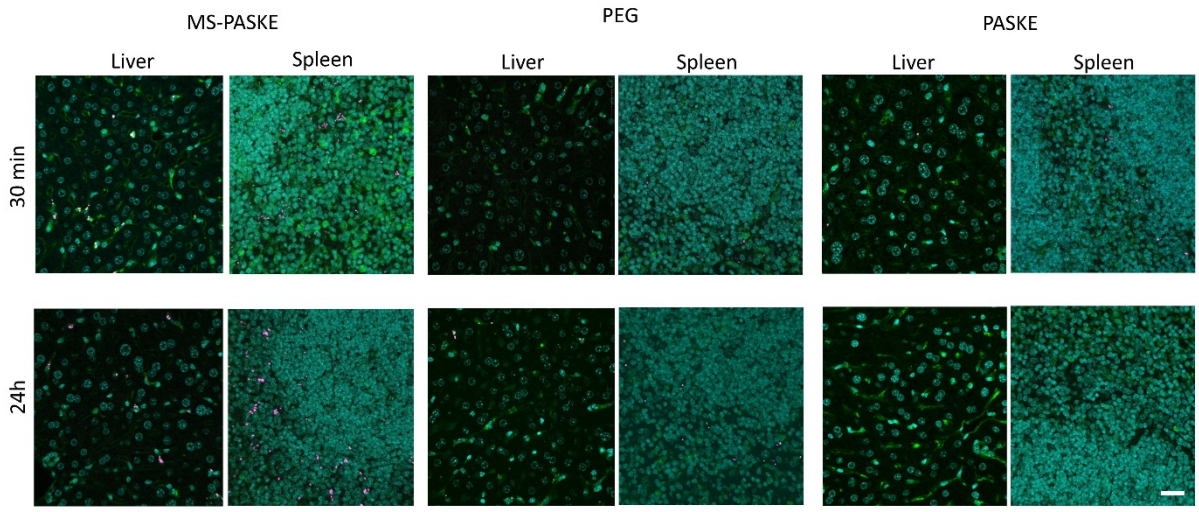

Figure S7: Histology observation of liver and spleen harvested at 30 min or 24h after injection of MS@PASKE-Cy5, PEG-Cy5 or PASKE-Cy5. Cell membrane and nuclei are stained with Alexa Fluor 488-conjugated wheat germ agglutinin (WGA-488, green) and Hoechst 33342 (cyan), respectively. Scale bar is 50 μm.