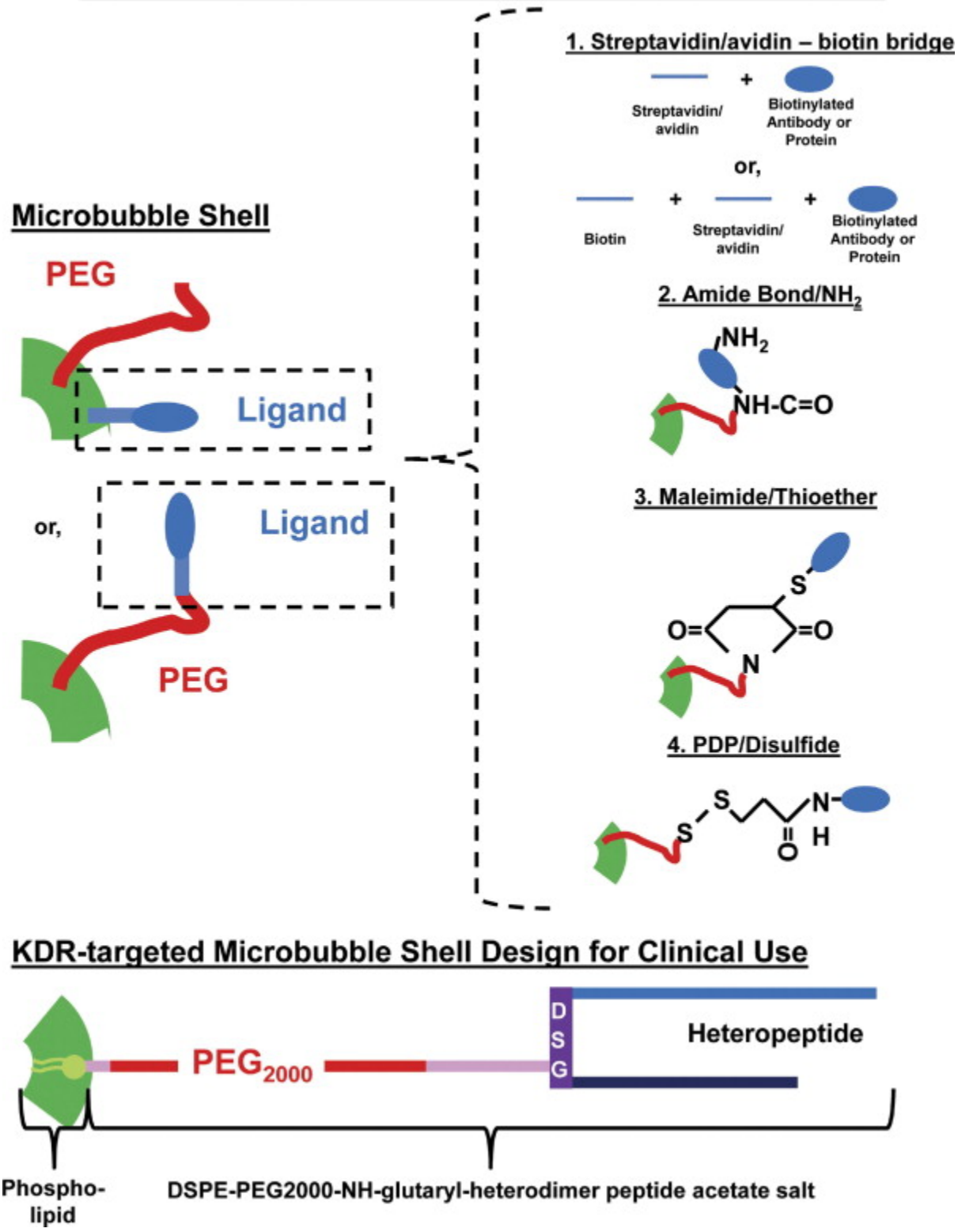
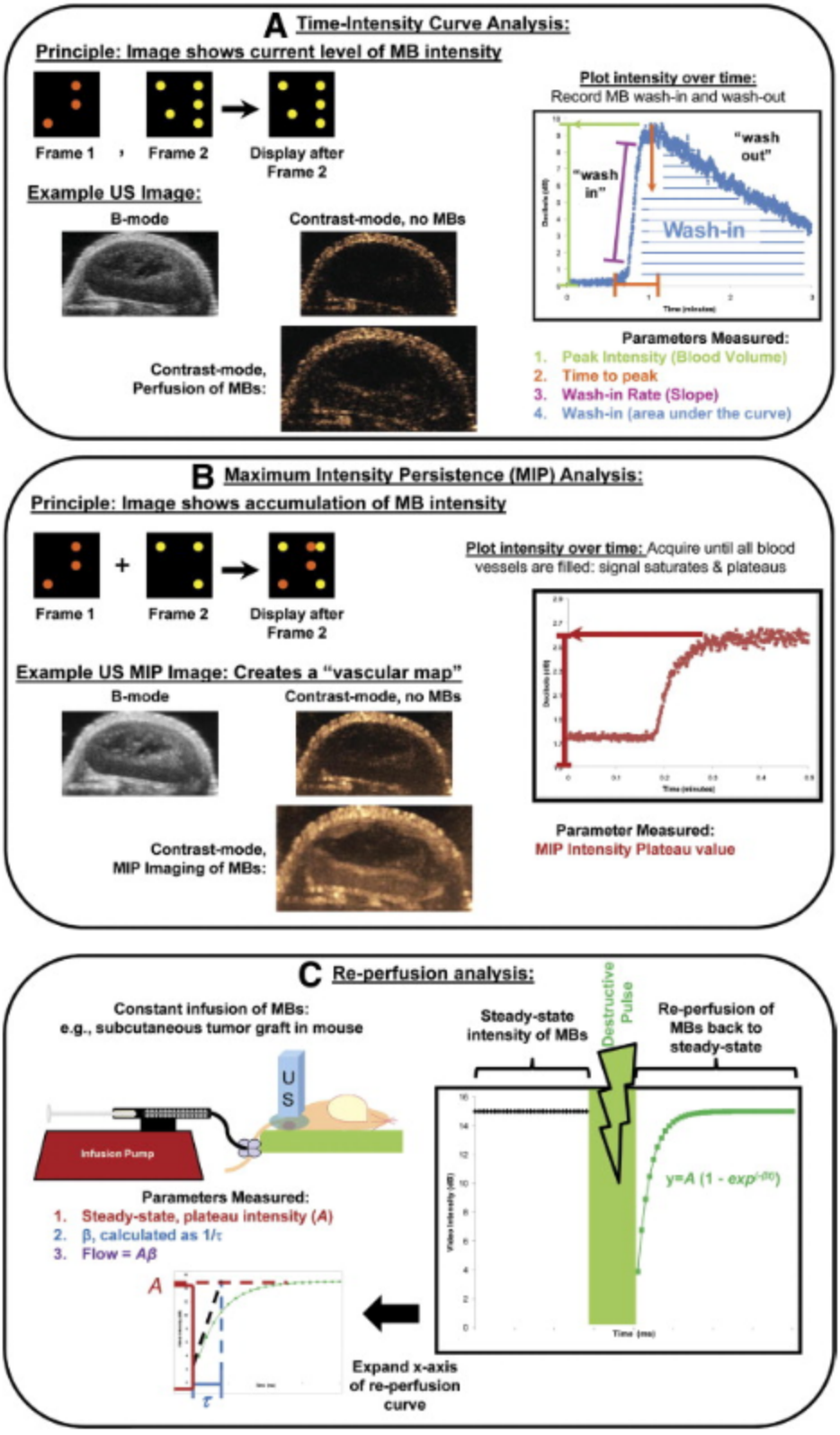

Conjugation Chemistries for Ligand Attachment to Microbubbles
1. Streptavidin/avidin – biotin bridge
Streptavidin/ avidin
Biotinylated Antibody or Protein
or,
Biotin
Streptavidin/ avidin
Biotinylated Antibody or Protein
Microbubble Shell
PEG
Ligand
or,
Ligand
PEG
2. Amide Bond/NH2
NH2
NH-C=O
3. Maleimide/Thioether
S
O=
=O
N
4. PDP/Disulfide
S
S
N
H
O
KDR-targeted Microbubble Shell Design for Clinical Use
PEG2000
D
S
G
Heteropeptide
Phospho-lipid
DSPE-PEG2000-NH-glutaryl-heterodimer peptide acetate salt

Fig. S1

Fig. S1. Ligands (eg, antibody, protein, or peptide; *blue oval*) can be conjugated either directly to the phospholipid/protein shell (*green arc*) of microbubbles or to the polyethylene glycol (PEG; *red line*) arm using several types of chemistries: (1) Streptavidin/avidin–biotin bridge; (2) amide/NH_2 linkage; (3) thioether/maleimide linkage; or (4) PDP/disulfide linkage (adapted from Klibanov [11] and [12]). A recently introduced clinical-grade KDR-targeted microbubble (BR55; not drawn to scale) was constructed as follows: (1) Two peptides were isolated by phage display and found to bind to human KDR with high affinity (K_Ds of 0.22 and 4.8 nmol/L) [59] and [61]; (2) the peptides were linked together via disuccinimidylglutarate (DSG) to form a heteropeptide (5.5 kDa molecular weight; K_D of 0.5 nmol/L; prepared as an acetate salt) [59], [60] and [61]; (3) the heteropeptide was combined to a 2000-unit polymer of PEG by mixing the 1,2-distearoyl-*sn*-glycero-3-phospho-ethanolamine-*N*-[amino PEG2000] ammonium salt, [DSPE-PEG2000-NH2]; (4) DSPE-PEG2000-NH-glutaryl-heterodimer peptide acetate salt was then linked to a phospholipid to form a heterolipopeptide that could be directly incorporated into microbubbles. [59] KDR-MBs (BR55; 1–3 µm diameter range) were constructed to contain 34,200 ± 1300 heteropeptides per square micrometer of MB. [2]



Supplementary Figure 2.

Fig. S2. Three different methods can be used for assessment of tissue perfusion or vascularity using ultrasound. (*A*) The time–intensity curve analysis method involves recording the real-time ultrasound signal intensity measurements immediately after intravenous microbubble injection. Within seconds, a rapid enhancement and wash-out pattern is observed, and parameters used for measurement include the peak enhancement intensity (*green bar*), time needed to reach peak intensity (*orange bar*), wash-in rate (*purple bar*, representing the slope of influx rate), and wash-in (*blue lines*, corresponding to the area under the curve). Example ultrasound images of a subcutaneous colon xenograft in a mouse include B-mode, contrast-mode acquired before microbubble (MB) injection, and contrast mode acquired after MB injection at peak enhancement intensity. (*B*) Maximum intensity persistence (MIP) imaging analysis also uses MB influx, but, instead records the cumulative "history" of ultrasound intensity in the imaging plane; therefore, the displayed image represents a sum of intensities over all frames acquired during a fixed time interval. The MIP imaging method effectively records MB tracks and creates a vascular map. After a few minutes, the MB tracks reach a saturation point, and the plateau or MIP intensity is recorded. Example ultrasound MIP images of a subcutaneous colon xenograft (same as in *A*) include B-mode, contrast-mode acquired before MB injection, and contrast mode acquired after MB injection where MIP imaging plateau has been reached. (*C*) Reperfusion analysis involves continuously injecting MBs (eg, via an injection pump) to obtain a steady-state concentration of MBs in the blood circulation; then, a high-powered pulse (as in Figure 1*B*) is delivered to destroy all the MBs within the beam elevation. The influx (reperfusion) of MBs into the field of view is then analyzed. The curve shape is given by the equation y (signal intensity) = $A\ (1 - \exp^{(-\beta\tau)})$, where A is the video intensity at steady-state, and β is the inverse of the time, τ, it takes to reach the steady-state intensity (with linear slope; *black dashed line*). Blood flow can then be measured as $A \times \beta$.