Supporting Information

Boronate-Mediated Covalent and Oriented Immobilization of Antibodies on PDMS surface Toward Improved Capture of Circulating Tumor Cells

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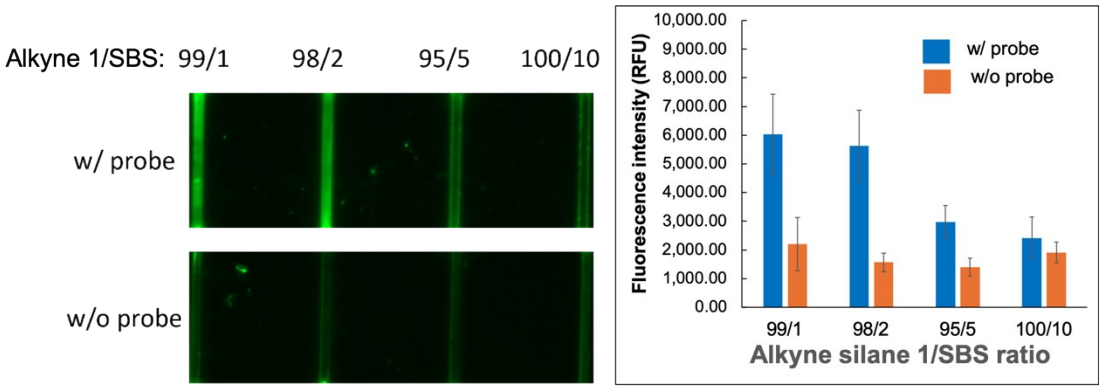

Fig. S1 Optimization of zwitterion concentration. PDMS microchannels were silanized with different molar ratios of alkyne 1/SBS (1–10 mol%) and subsequently functionalized with BA-Tosyl. After incubation with Herceptin and detection using anti-human IgG (Fab′-specific) Ab-Cy3, fluorescence intensity was quantified. The fluorescence intensity indicated optimal surface modification at 2 mol% SBS, yielding high fluorescence and low nonspecific adsorption. Error bars represent standard deviation (SD) from three independent experiments. Representative fluorescence images (with and without probe) are shown.

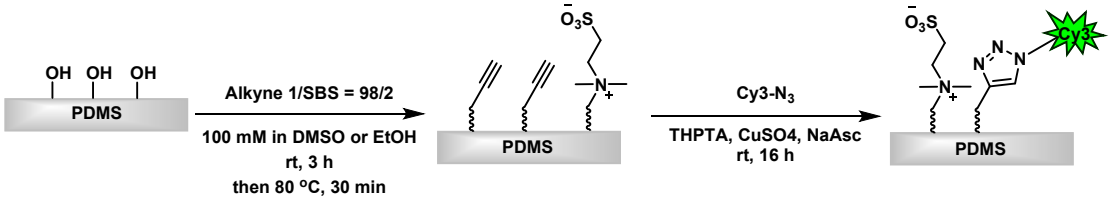

Fig. S2 Evaluation of alkyne-surface functionalization. Schematic representation of the mixed monolayer-protection and silanization on PDMS microfluidic channels using alkynyl triethoxysilane **1**, and SBS and the CuAAC reaction.

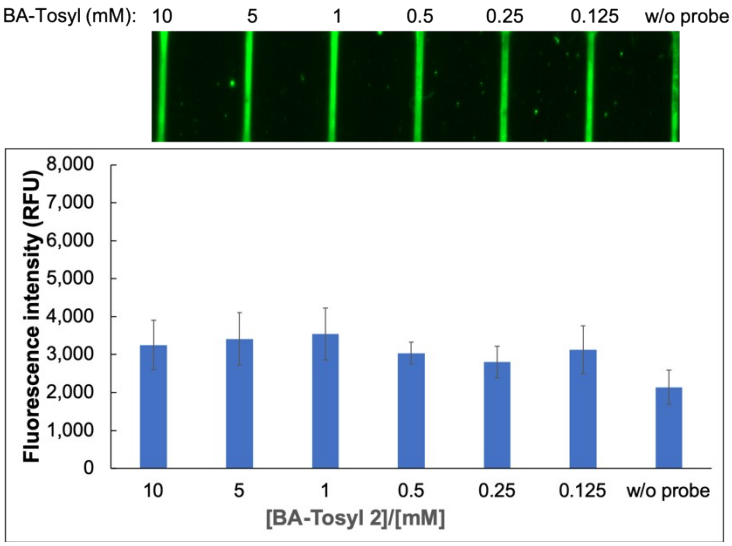

Fig. S3 Optimization of BA-Tosyl probe concentration. Alkynylated PDMS microchannels were modified with BA–Tosyl probes at concentrations ranging from 0.25 to 10 mM, followed by antibody (Herceptin) incubation and detection with anti-human IgG (Fab′-specific) Ab–Cy3. The fluorescence intensity reached saturation between 1 and 5 mM, with a slight decrease at 10 mM. Error bars represent SD from three replicate experiments. Representative fluorescence images at each concentration are shown.

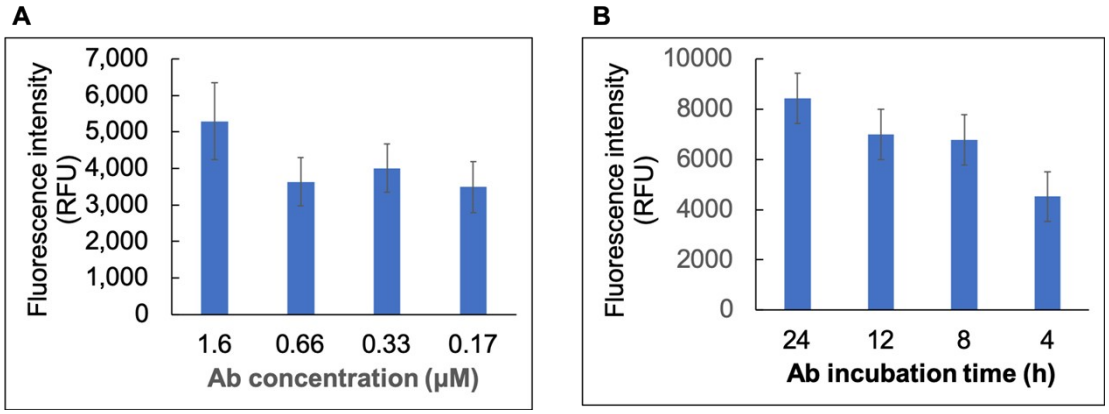

Fig. S4 Optimization of antibody concentration and incubation time. BA-functionalized PDMS microchannels were incubated with antibodies (a) at varying concentrations (0.17–1.6 μM) and (b) for different time periods (4–24 h). Fluorescence signals were measured using anti-human IgG (Fab′-specific) Ab–Cy3. (A) Antibody concentration. (B) Incubation time. Error bars represent SD from three replicate experiments.

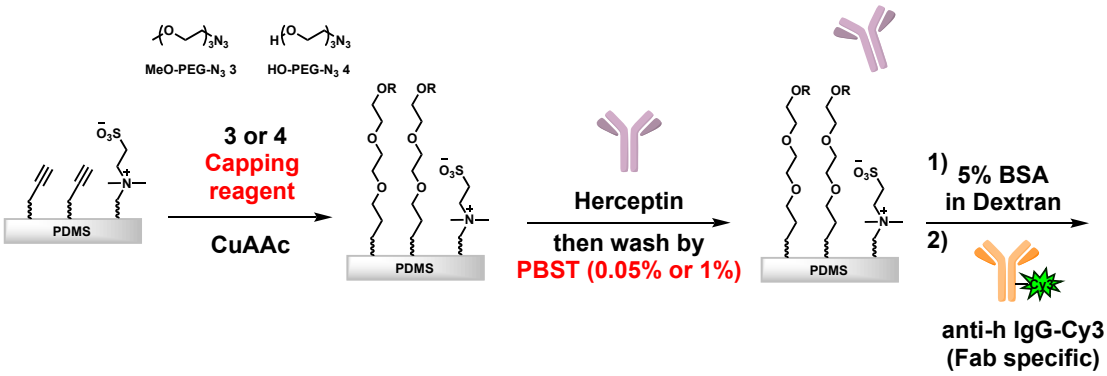

Fig. S5 Evaluation of capping reagents. Alkyne-modified surfaces were capped with capping reagents **3** or **4**. The representative fluorescence images with different washing buffers are shown in Figure 4B in the main text.

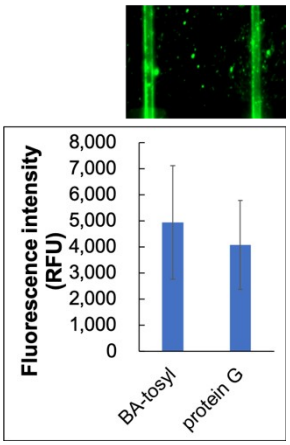

Fig. S6 Comparison of boronate-mediated antibody immobilization with Protein G-based Fc-directed assembly. Herceptin was immobilized on PDMS microchannels either through BA-Tosyl functionalization (boronate-affinity covalent coupling) or via Protein G attached to NHS-activated surfaces (Fc-directed immobilization). Following incubation with biotinylated HER2 antigen and detection using streptavidin-Cy3, the fluorescence signal was quantified. Boronate-mediated immobilization exhibited ~1.2-fold higher antigen binding signal relative to Protein G, indicating improved antibody orientation and covalent stability. Error bars represent SD. Representative fluorescence images for both strategies are shown.

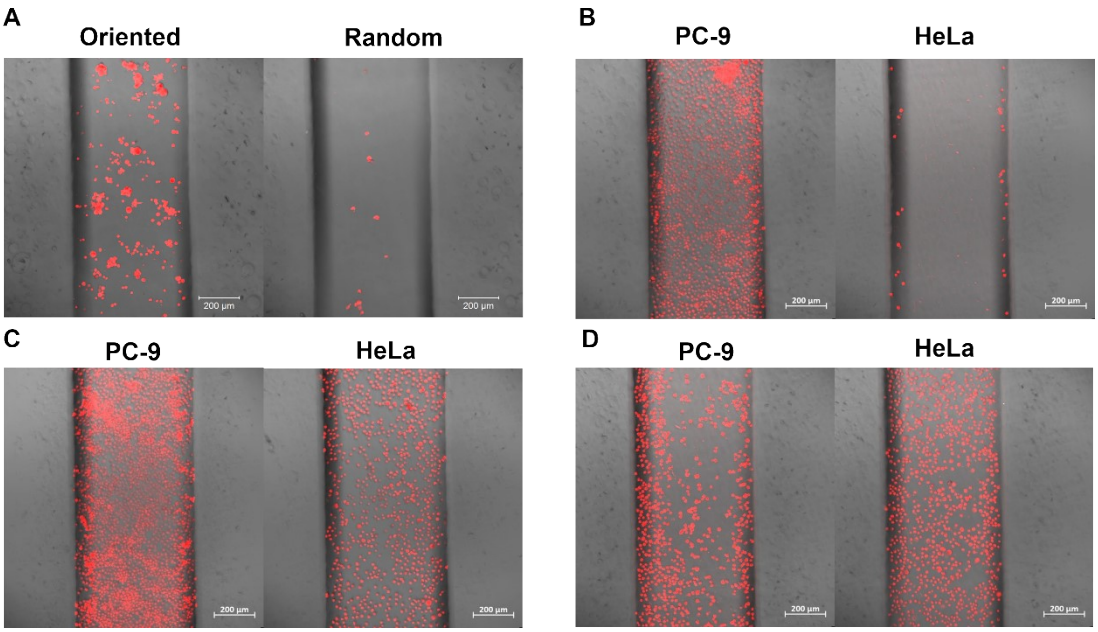

Fig. S7 Fluorescence microscopy images of the microfluidic channels (A) Figure 6A. (B) Figure 6B. (C) Figure 6C. (D) Figure 6D in the main text.

Chemical compounds.

Compound **1**[1], **2**[2], **3**[3], **4**[4], **5**[5], and **6**[2] were synthesized according to previously reported procedures.

References

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