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Supporting Information

Coordination-Driven Reversible Surfaces with Site-Specifically immobilized Nanobody for Dynamic Cancer Cell Capture and Release

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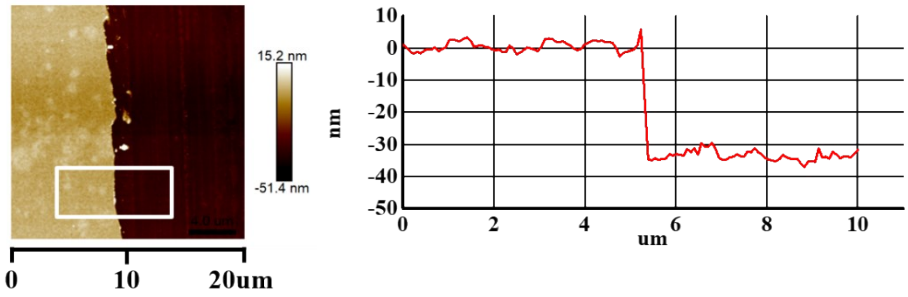

Figure S1. AFM image (right) of a PHEMA surface with a thickness profile (left). The thickness of the PHEMA brush characterized by AFM was 30 ± 2 nm, close to that measured by ellipsometry.

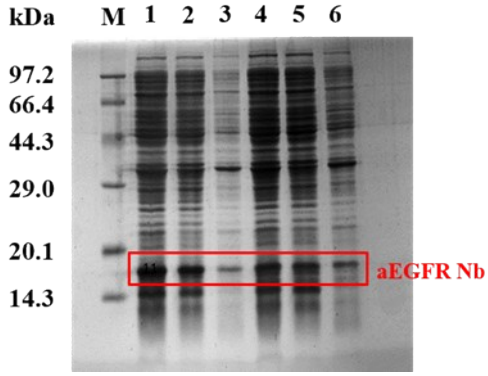

Figure S2. Expression and soluble detection of His-tagged anti-EGFR nanobody (aEGFR Nb) in *E. coli* Shuffle T7. Lane M, protein molecular weight markers; Lane 1, IPTG-induced total bacterial protein; Lane 2, IPTG-induced supernatant after the cell disruption and centrifugation; Lane 3, IPTG-induced precipitate after the cell disruption and centrifugation; Lane 4, uninduced total bacterial protein; Lane 5, uninduced supernatant after the cell disruption and centrifugation; Lane 6, uninduced precipitate after the cell disruption and centrifugation.

Isopropyl β-D-1-Thiogalactopyranoside (IPTG)-induced and non-IPTG-induced bacteria were collected separately. After sonication and subsequent centrifugation, electrophoresis samples were made from the collected bacteria, and centrifugated supernatant and precipitate, receptively, and evaluated under SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The expression of IPTG-inducted anti-EGFR nanobody (aEGFR Nb) was exhibited with a clear band at the theoretical molecular weight of 15 kDa. Moreover, the soluble aEGFR Nb was mainly present in the supernatant (Figure S2 lane 2 and lane 5).

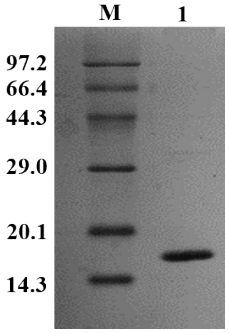

Figure S3. SDS image of purified His-tagged anti-EGFR nanobody (aEGFR Nb). Lane M, protein molecular weight markers; Lane 1, purified His-tagged aEGFR Nb. The resulting aEGFR Nb with a yield of 130 mg L^{-1} possessed a purity of more than 98%.

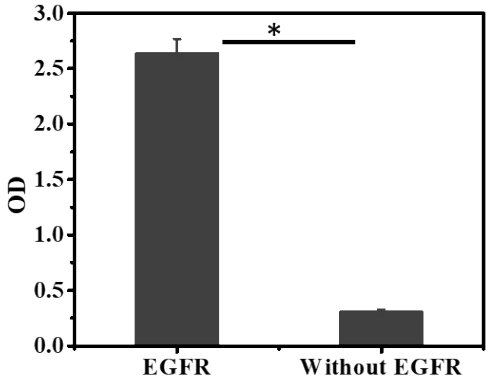

Figure S4. Binding activity of aEGFR Nb. Data are presented as mean ± standard deviation, n = 3. *p < 0.05.

The antigen-binding activities of the aEGFR Nb were detected by a cellular ELISA. First, 100 μL of A431(EGFR+) cell suspensions (1×10^4 cells mL^{-1}) were added to each well of a 96-well cell culture plate, and cultured for 12 h at 37°C in 5% CO_2 followed by washing three times with PBS. Afterward, 125 μL of 4% paraformaldehyde was added into each well for 30 min to fix the cells. After three washes with PBS, 200 μL of 5% (w/v) skim milk powder was added to block cells for 1 h at 37°C. Then 200 μL of aEGFR Nb (30 μg mL^{-1}) was used to incubate cells for 1 h at 37°C. Subsequently, the cells were incubated with a primary antibody solution (Anti-6×His antibody, diluted 1:2000 in PBS, Sangon Biotech, China) for 1 h at 37°C, and then stained with a secondary antibody (HRP-conjugated Goat Anti-Mouse IgG, diluted 1:2000 in PBS, Sangon Biotech, China) for 1 h at 37°C. Finally, after incubation with 200 μL of TMB working solution in the dark for 20 min, 50 μL of stop solution was added and OD value of the resulting solution in the well was measured under a microplate reader (TECAN, Switzerland) at 450 nm.

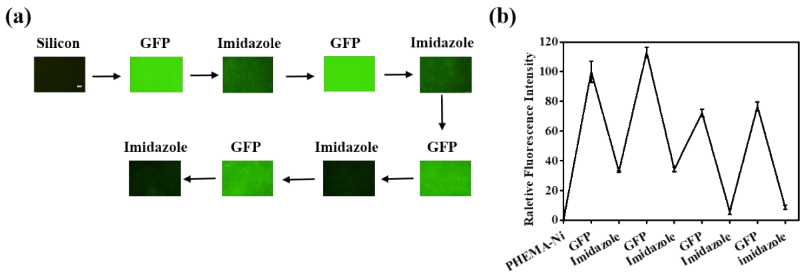

Figure S5. Site-specific immobilization and reversible release of His-tagged enhanced green fluorescent protein (EGFP). (a) Fluorescence images of the His-tagged EGFP immobilization and released on the PHEMA-Ni^{2+} surfaces *via* the treatment with a competitive agent imidazole. Scale bar = 200 μm. (b) Relative fluorescence intensity of different surfaces of site-specific immobilization and reversible release of His-tagged EGFP analyzed with ImageJ software. Prior to the normalization, the fluorescence intensity of the background PHEMA-Ni^{2+}surface was subtracted (that of the first immobilization is set as 100%). Data are presented as mean ± SD, n = 3.

His-tagged enhanced green fluorescent protein (EGFP, a gift from Li Xu Associated Professor in Dalian University of Technology, China) was used as a model protein to investigate the process of site-specific immobilization and reversible release of His-tagged protein on the PHEMA-Ni^{2+} surfaces. After incubation of the His-tagged EGFP solution (500 μg mL^{-1} in PBS) for 2 h, green fluorescence was observed on the PHEMA-Ni^{2+} surfaces (Figure S5a), indicating the successful immobilization of His-tagged EGFP. After the treatment of the imidazole solution (200 mM in PBS, 200 r min^{-1} for 1 h), the fluorescence intensity decreased significantly, showing the release of His-tagged EGFP. After the repeated immobilization-releasing process for three times, the His-tagged EGFP could still reversibly immobilize onto and release from the PHEMA-Ni^{2+} surfaces (Figure S5b).

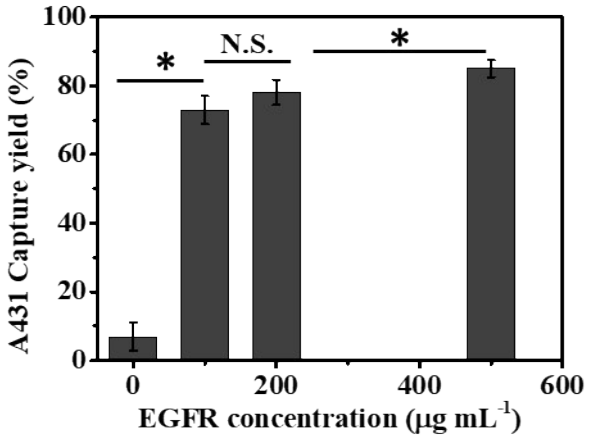

Figure S6. Capture yield of A431 cells as a function of immobilization concentration of aEGFR Nb. Different concentration of nanobody were immobilized to the PHEMA-Ni^{2+}. Data are presented as mean ± SD, n = 3.

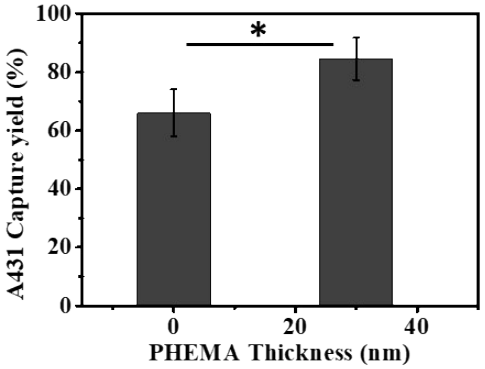

Figure S7. Influence of aEGFR Nb linker (w/o PHEMA brushes) on capture yield of A431 cells. PHEMA brushes with a thickness of 0 nm means direct immobilization of aEGFR Nb on the NTA-Ni^{2+}-modified silica surfaces. Data are presented as mean ± SD, n = 3.

Immobilization of aEGFR Nb on the NTA-Ni^{2+}-modified silica surfaces: First, APTES-modified silica surfaces were activated with CDI (20 mM in acetone) for 3 h at 25°C. After washes in acetone and water, the surfaces were incubated in a pH 8.5 PBS containing the mixture of 5 mg mL^{-1} NH_2-NTA and 11.63 mg mL^{-1} ethanolamine for 12 h. After washed with abundant water, the NTA-modified silica surfaces were placed into a $NiCl_2$ solution (100 mM in H_2O) for 2 h at 37 °C, and then immersed in an aEGFR Nb PBS solution (500 μg mL^{-1}) for 2 h at 37°C.

Table S1 Amino acid sequence of His-tagged anti-EGFR nanobody

Nanobody	Amino acid sequence
His-tagged anti-EGFR nanobody	AEFQVKLEESGGGSVQTGGSLRLTCAASGRTSRSYGMGW FRQAPGKEREFVSGISWRGDSTGYADSVKGRFTISRDNAK NTVDLQMNSLKPEDTAIYYCAAAAGSAWYGTLYEYDYW GQGTQVTVSSPRLCTPSRPRHHHHHH

Table S2 Molar ratios of surface elemental compositions obtained from XPS data

	%C 1s	%N 1s	%O 1s
PHEMA	68.11	_	31.89
PHEMA-CDI	62.71	10.95	26.35