Enhancement of Binding Kinetics on Affinity Substrates
by Laser Point Heating Induced Transport -- Supplementary

Supplementary I. Temperature Calibration.
Temperature at the heating spot was measured using confocal microscopy and 50 mM 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF, Life Technologies, CA) in a 10 mM Tris buffer (Sigma Aldrich, WI). The fluorescence intensity of BCECF drops by 2.8% in response to an increase of 1 K in temperature.\textsuperscript{1,2} As shown in Figure S1, an input power of 12 mW generates a 10K temperature increase at the center of the microwell ceiling. This power was used for the nanoparticle experiments.

![Figure S1: Temperature calibration on the ceiling of the microwells in response to laser power. The fluorescence intensity of BCECF was measured on the ceiling to determine the temperature increase at the laser focal point in a 5 mm (L) × 5 mm (W) × 50 µm (H) microwell. For nanoparticle binding experiments, an input power of 12 mW was used to generate a 10K temperature increase from the bulk.](image-url)
Supplementary II. Reaction Time in Microwells of Different Heights

Figure S2 shows the COMSOL simulation result of the reaction half-life in a microwell with 5 mm × 5 mm footprint and various well heights (5 µm, 15 µm, 50 µm, 150 µm, 300 µm to 500 µm) used for the capture of 200 nm nanoparticles on the microwell floor. The bulk concentration of nanoparticles (0.05 nM) and surface concentration of receptors (1 × 10^{-8} mol/m^2) are the same in all cases at t=0, and the surface receptor concentration is sufficient to deplete all nanoparticles even in the deepest microwell studied here of 500 µm. Reaction rate constants corresponding to the NeutrAvidin-biotin pair are used. As the reverse rate constant is negligible for the NeutrAvidin-biotin reaction, all reactions reach equilibrium when the analyte is exhausted in the microwells. The reaction half-life is the time required for the surface concentration to reach half of the equilibrium concentration. As the height of the microwells increases stepwise from 5 µm to 500 µm, the reaction half-life increases significantly from 6 s to 12 hrs. The half-life scales with the square of the microwell heights (line in Figure S2), and the prefactor is on the same order as 1/2D, with D being the diffusion coefficient of the analyte. Since the microwell height is the characteristic length in most geometries studied here (wide and shallow wells), this relationship suggests that surface binding is diffusion limited despite microscale reactions. Diffusion limited reactions are also confirmed by dimensional analysis of the Damköhler number, which is on the order of 10^3 to 10^5 for the different chamber heights studied here.

S2

Figure S2: COMSOL simulation of reaction half-life of biotinylated 200 nm nanoparticles to surface immobilized NeutrAvidin in microwells with footprints of 500 µm × 500 µm and heights of 5 µm, 15 µm, 50 µm, 150 µm, 300 µm and 500 µm respectively. The reaction half-life (time required for the surface concentration to reach half of the equilibrium concentration, plotted as dots) scales with the square of the microwell height (solid line), indicating the reactions are diffusion limited.
References