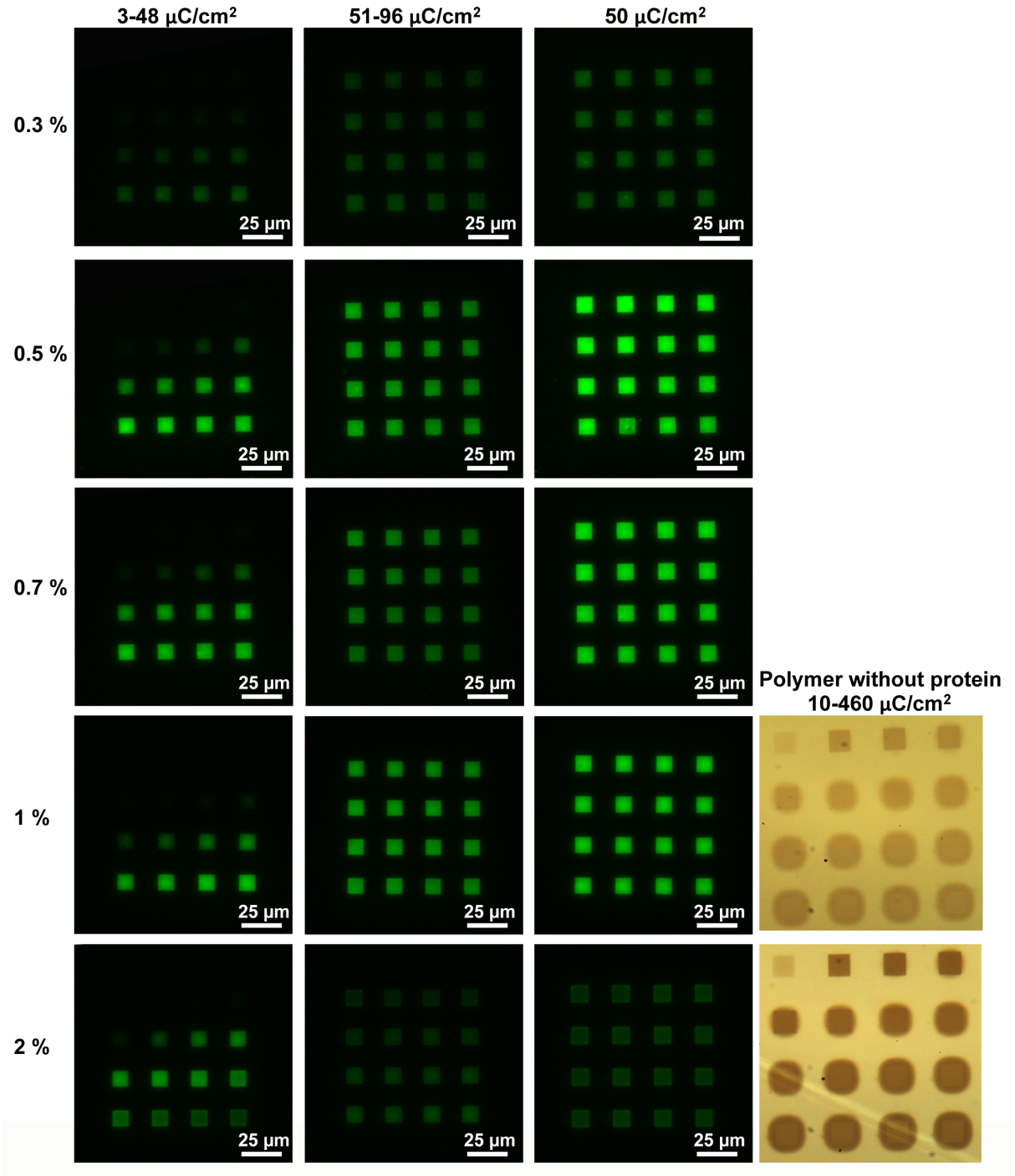
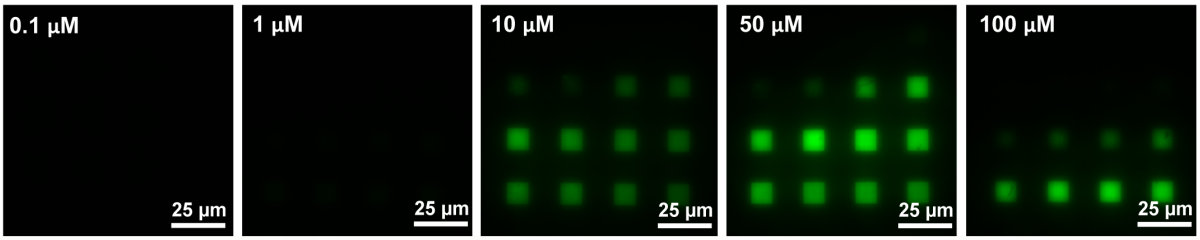
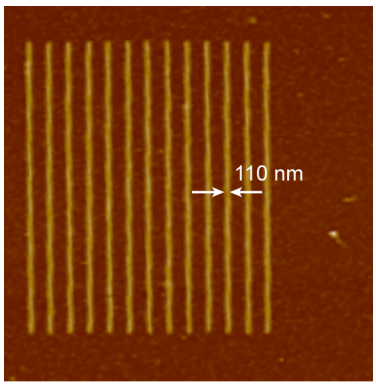


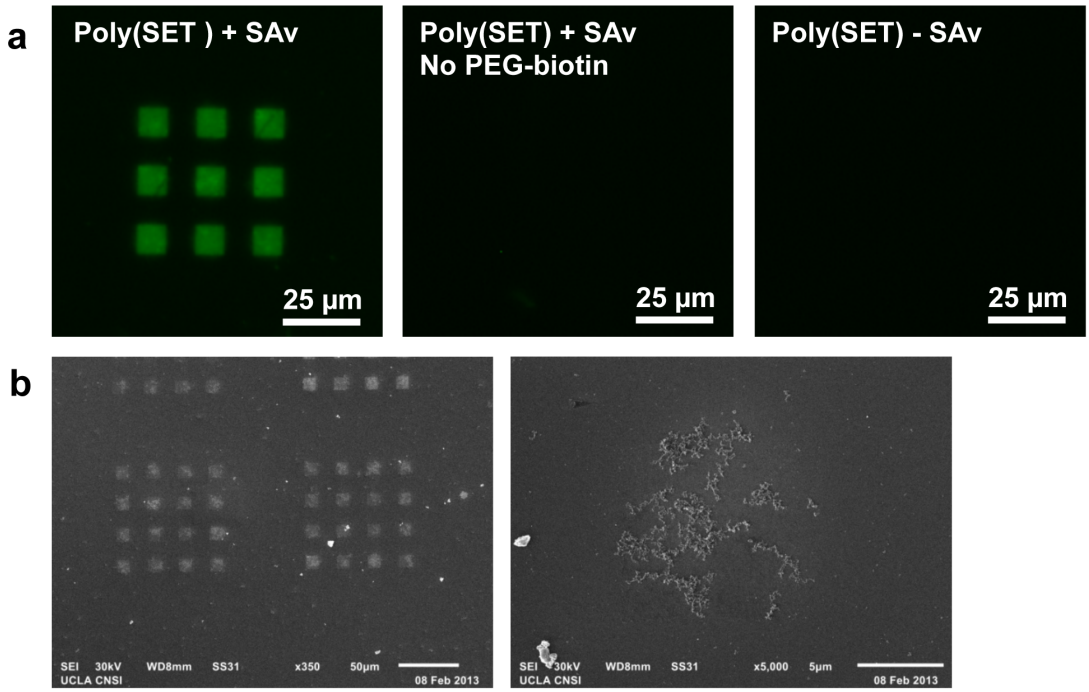
Supplementary Figure 1. Effect of poly(SET) concentration on protein patterning Fluorescence micrographs showing the effect of poly(SET) concentration on poly(SET)-HRP patterns. In the first two columns each square pattern was written at a different dose ranging from 3-96 µC/cm^2, therefore the patterns are visible only above a threshold dose. In the third column each square pattern was written at a constant dose of 50 µC/cm^2. HRP concentration in spin-coating solutions was 10 µM for all cases. The patterns have been stained with AlexaFluor® 488 goat anti-HRP antibody.



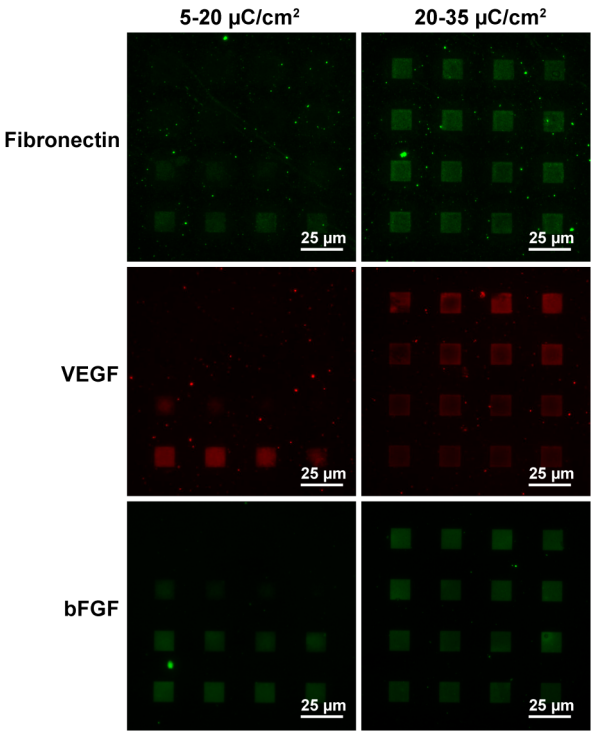
Supplementary Figure 2. Effect of HRP concentration on protein patterning by EBL. Fluorescence micrographs showing the effect of HRP concentration on poly(SET)-HRP patterns. Each square pattern on a single chip was written at a different dose ranging from 5-80 µC/cm^2, therefore the patterns are visible only above a threshold dose. A 0.5 wt % of poly(SET) solution was used for all cases. The patterns have been stained with AlexaFluor® 488 goat anti-HRP antibody.



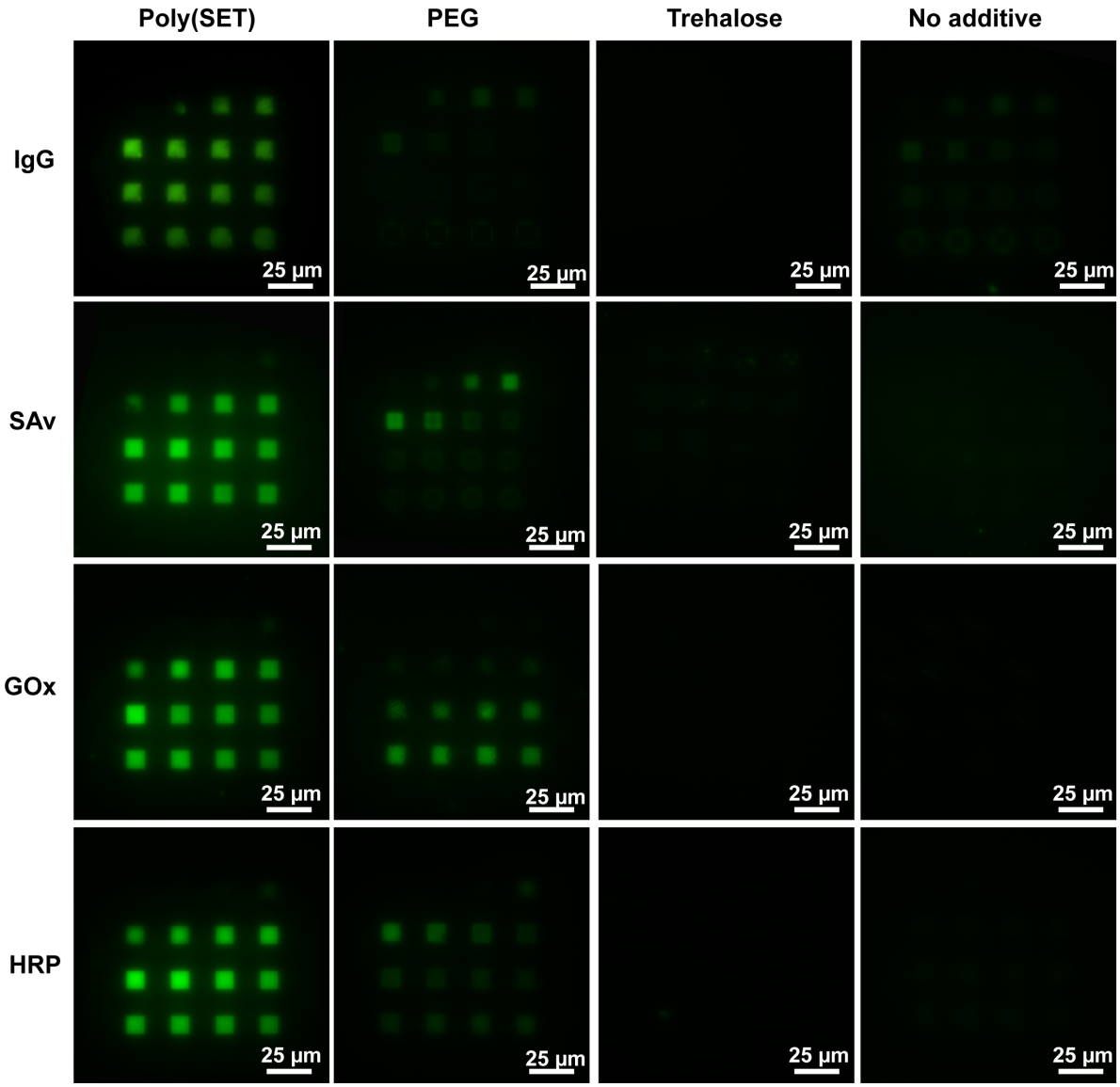
Supplementary Figure 3. Magnified view of AFM image in Figure 2e AFM image of HRP pattern with nanometer features.



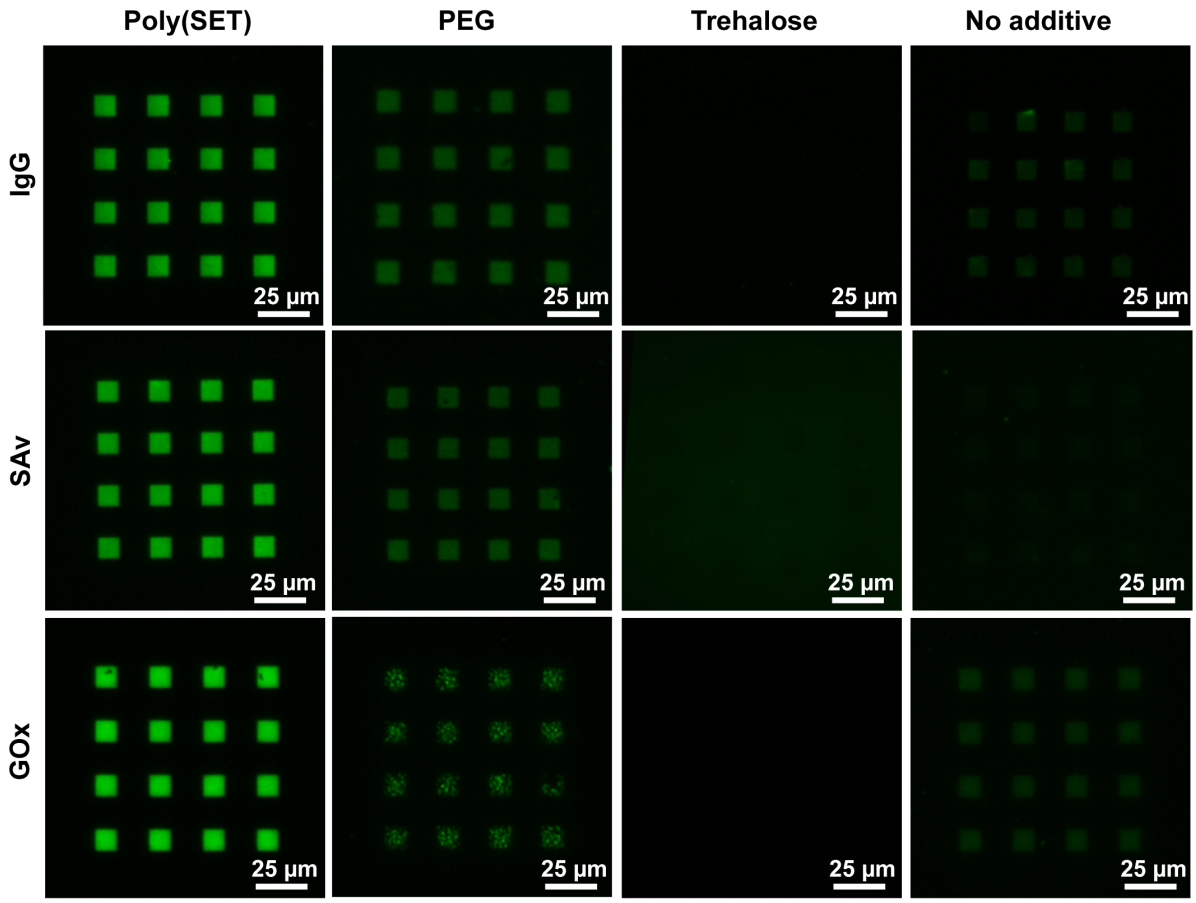
Supplementary Figure 4. Assessment of micropatterned SAv and GOx activity SAv and GOx patterns generated by EBL showed protein activity as assessed by biotin binding and gold nanoparticle formation respectively. **a**, Fluorescence micrographs showing poly(SET)-SAv patterns after incubation with 8-arm PEG-biotin (0.1 % in PBS for 1h) followed by incubation with AF488 conjugated SAv (10 µg/mL in PBS for 1h). No pattern was visible when PEG-biotin incubation step was skipped or only poly(SET) without SAv was patterned by EBL. **b**, SEM micrographs showing gold nanoparticles formed on an array of square poly(SET)-GOx patterns (left, scale bar 50 µm) and a magnified image of a single pattern (right, scale bar 5 µm) as a result of reduction of gold ions (3 mM) by glucose oxidase in the presence of glucose (12 mM in PBS, pH=7.4 for 8h).



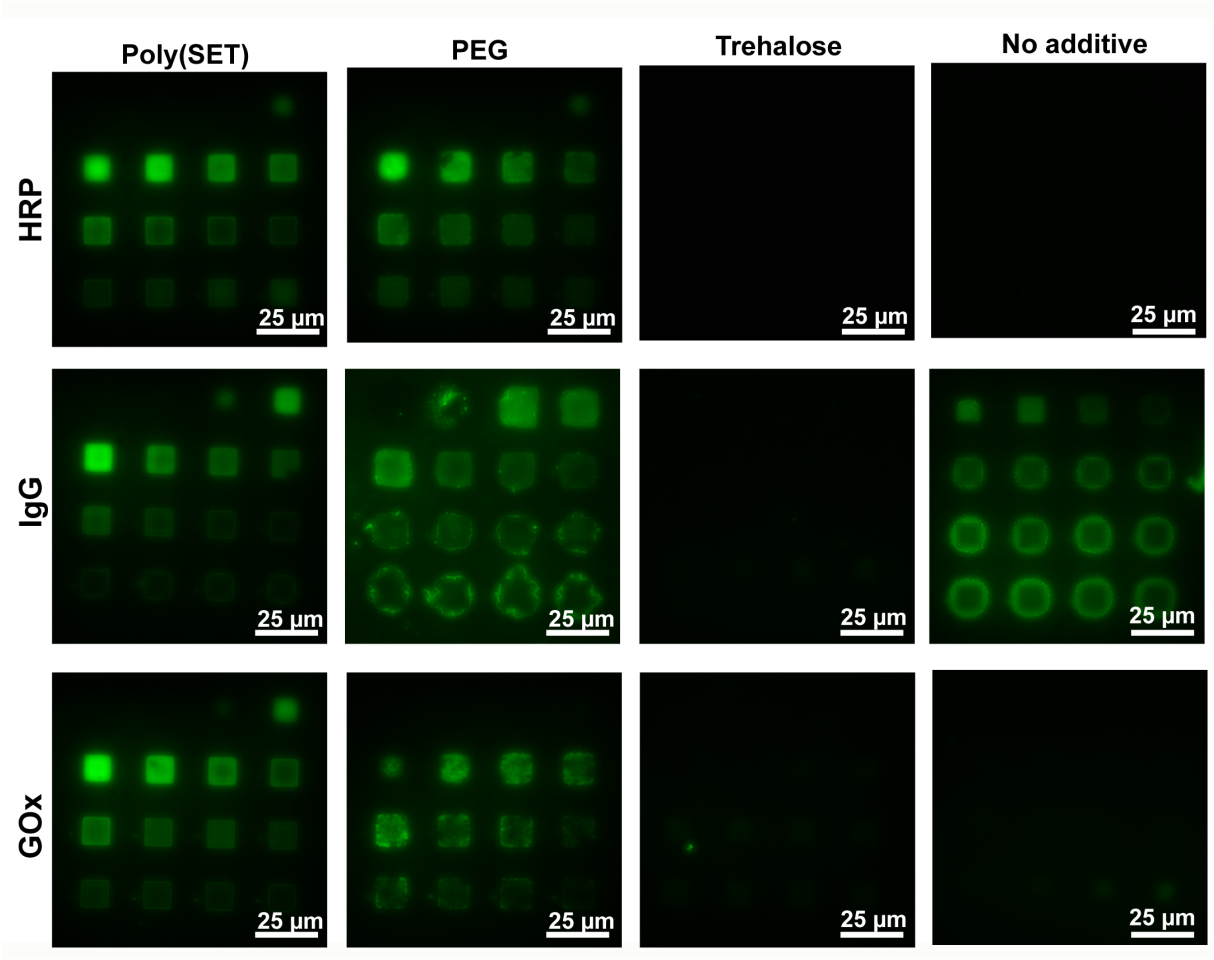
Supplementary Figure 5. Fibronectin, VEGF, and bFGF patterns. Fluorescent micrographs of generated patterns of fibronectin, VEGF, and bFGF with poly(SET). The doses range from 5-35 µC/cm^2. Fibronectin patterns were stained with mouse anti-fibronectin, followed by Alexa Fluor 488 anti-mouse. VEGF stained with mouse anti-VEGF, and Alexa Fluor 594 anti-mouse. bFGF was stained with biotinylated anti-bFGF, and streptavidin Alexa Fluor 488.



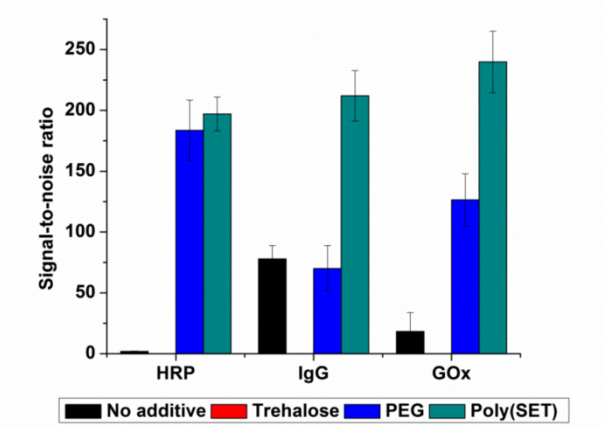
Supplementary Figure 6. Determination of optimal area dose for protein-excipient pairs Fluorescence micrographs showing the effect of area dose for four different proteins (IgG, SAv, GOx, and HRP) written with four different excipients (poly(SET), PEG, trehalose, and without excipient). Each square pattern on a single chip was written at a different dose ranging from 5-80 μC/cm^2, therefore the patterns are visible only above a threshold dose. The patterns have been stained with respective fluorescently labeled antibody for each protein. No pattern could be observed when poly(SET) or PEG patterns that did not contain any protein (controls) were stained with antibodies. The highest S/N ratio was obtained at an area dose of 45 μC/cm^2 for poly(SET)-HRP, poly(SET)-GOx, and poly(SET)-SAv while for poly(SET)-IgG the optimal dose was 25 μC/cm^2. Beyond these dosages S/N ratios decreased indicating protein damage.



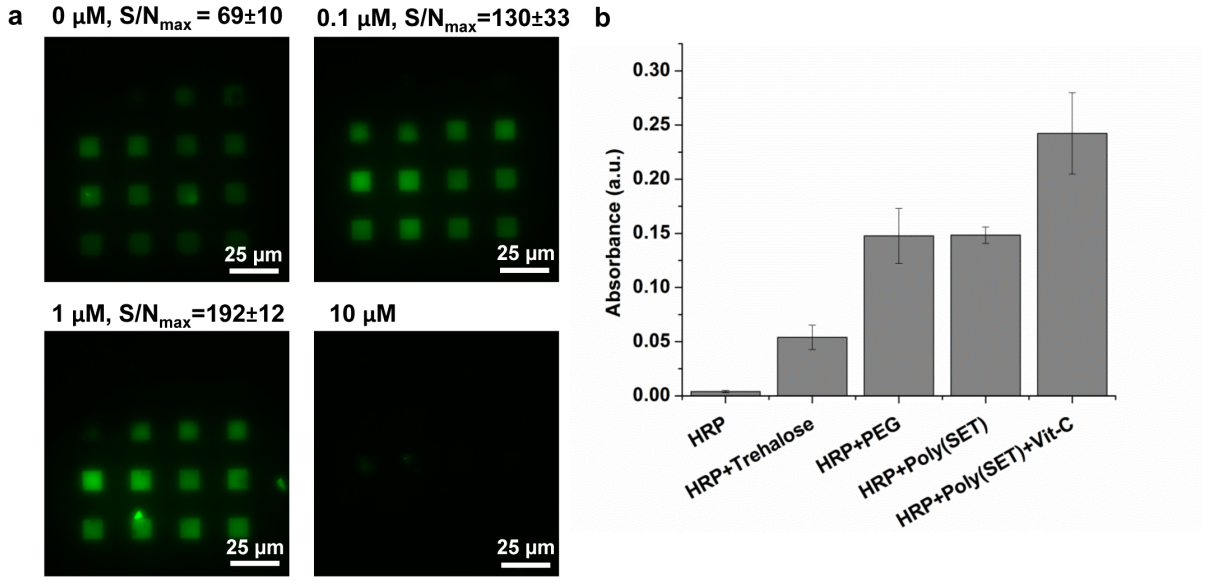
Supplementary Figure 7. Comparison of different excipients in stabilization of proteins Fluorescence micrographs showing IgG, SAv, and GOx patterned with either poly(SET), or PEG, or trehalose, or without any excipient. Substrates were prepared by spin-coating protein-excipient solutions.



Supplementary Figure 8. Comparison of vacuum dried films of proteins As a comparison to spin-coated chips, substrates were prepared by placing a 5 µL drop of protein-excipient solution onto the chips and let it spread over. After drying these substrates under vacuum, patterns were generated by EBL and stained with fluorescent antibodies as described above for spin-coated chips. Fluorescence micrographs showing HRP, IgG, and GOx patterned with either poly(SET), or PEG, or trehalose, or without any excipient. Each square pattern on a single chip was written at a different dose ranging from 5 to 80 µC/cm^2. Therefore, only the square patterns above a threshold dose are visible. Substrates were coated by spreading protein-excipient solutions and vacuum drying.



Supplementary Figure 9. Comparison of vacuum dried films of proteins Maximum signal-to-noise ratios for HRP, IgG, and GOx patterned with either poly(SET), or PEG, or trehalose, or without any excipient. Substrates were coated by spreading protein-excipient solutions and vacuum drying.



Supplementary Figure 10. Effect of ascorbic acid and high vacuum on protein stability a, Fluorescence micrographs showing patterns of poly(SET)-HRP containing different amounts of ascorbic acid (Vitamin C) and written at a dose range of 5 to 80 μC/cm^2. **b**, Activity of HRP after incubation under high vacuum conditions for 4h with different excipients. To investigate the effect of ascorbic acid, poly(SET)-HRP patterns were made in the same way as the single protein patterns were made as described above using solutions with 10 μM final HRP concentration, 0.5 wt % poly(SET) in H_2O and varying concentrations of ascorbic acid. The effect of high vacuum conditions on HRP with different excipients were assessed after placing 5 μL of poly(SET)-HRP solution (8.3 nM HRP, 0.5 wt % poly(SET)) onto the chips and keeping under high vacuum conditions in an SEM chamber for 4h. Then, the activity of HRP on the chips was measured using a tetramethylbenzidine (TMB) assay.

Supplementary Table 1. Spin-coated thickness of varying concentrations of HRP and poly(SET).

Measured Thickness (Å)				
	0.3 wt% poly(SET)	**0.5 wt% poly(SET)**	**1.0 wt% poly(SET)**	
10 µM HRP	184.0 ± 1.8	238.8 ± 2.9	388.1 ± 8.7	
	0.1 µM HRP	**1 µM HRP**	**10 µM HRP**	**100 µM HRP**
0.5 wt% poly(SET)	230.0 ± 2.9	222.8 ± 2.3	218.1 ± 1.6	367.6 ± 12.7

Supplementary Table 2. Drop-coated thickness of HRP with different additives.

Thickness (Å)			
PEG	Trehalose	Poly(SET)	HRP only
3335.6 ± 81.4	2449.5 ± 150	1583.4 ± 85.1	3269.1 ± 9.6