Supporting Information

Direct and reversible immobilization and microcontact printing of functional proteins on glass using a genetically appended silica-binding tag

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Materials and Methods

DNA Manipulations

All DNA manipulations followed standard protocols and were conducted in *E. coli* Top 10 cells (F$^-$ *endA*1 *recA*1 *hsdR*17 (r^-_k,m^+_k) λ^- *supE*44 *thi*1 *gyrA*96 *relA*1 ϕ80 Δ*lac* Δ*M15* Δ(*lacZYA-argF*)U169 *deo*R). A plasmid encoding eGFP-Car9 was constructed by PCR-amplification of the eGFP (aka GFPmut2) gene from pKB100 (a generous gift from Prof. Beth Traxler, UW Microbiology) on a fragment flanked by *Nde*I and *Hin*dIII sites. Digestion of the amplicon with these enzymes produced 2 fragments (230 bp and 438 bp) due to presence of an internal *Nde*I cutsite. The larger 3' fragment was ligated in *Nde*I and *Hin*dIII digested pBLN200-Car9.[1] The resulting plasmid pBLN-GFPmut2part-Car9 was cleaved with *Nde*I, treated with shrimp alkaline phosphatase and the 230 bp *Nde*I fragment was inserted into the backbone to produce pBLN-GFPmut2-Car9. A DNA cassette encoding the Car15 sequence (RTYLPLPWMAAL) flanked by *Hind*III and *Xho*I restriction sites (5' –AAGCTTGGCGGCGGCTCTCGGACTTACCTTCC GCTCCCATGGATGGCCGCGCTGTAATAACTCGAG- 3') was digested with these enzymes and inserted into the same sites of pBLN-GFPmut2-Car9 to produce pBLN-GFPmut2-Car15. The sfGFP gene (a generous gift from Prof. Eric Klavins, UW Electrical Engineering) was subjected to site-directed mutagenesis to eliminate internal *Nde*I sites by introduction of the $T^{231}C$ and $T^{651}C$ silent mutations. The mutated gene was amplified on *Nde*I-*Xho*I fragment and inserted in the same sites of pBLN200,[2] yielding pBLN200-sfGFP. The same gene was

amplified on *Nde*I-*Hin*dIII fragment and ligated in the same sites of pBLN200-Car9 to produce pBLN200-sfGFP-Car9. All constructs were verified by DNA sequencing.

Protein Purification

Proteins were purified by modification of previously described methods.[1, 3] Briefly, seed cultures (25 mL) were used to inoculate 500 mL of LB medium supplemented with 50 μg/ml kanamycin and cells were grown to A_{600} ~ 0.5 at 37°C. Protein synthesis was induced by addition of 2% L-Arabinose. After 3.5h of cultivation at the same temperature, cells were harvested by centrifugation at 7,000*g* for 5 min, resuspended in 35 mL of 20 mM Tris-HCl pH 7.5 (Buffer A) supplemented with 2 mM EDTA, and disrupted by 6 rounds of sonication for 3 min at 30% duty cycle using a Branson sonifier. Lysates were clarified by centrifugation at 10,000*g* for 15 min. Clarified lysates of eGFP, eGFP-Car9, sfGFP and sfGFP-Car9 were heat-shocked at 75°C for 10 min and subjected to centrifugation at 10,000*g* for 15 min to remove thermolabile host proteins. eGFP-Car15 was not subjected to heat-shock due to its propensity to aggregate. Supernatants were subsequently purified by ion exchange chromatography on a Whatman DE52 column. mCherry-Car9 and the TrxA derivatives were purified as previously described.[1, 3] The typical concentration of purified proteins was 100 μM.

Confocal Microscopy

Glass microscope slides were washed with ethanol, air dried and subjected to UV-ozone treatment for 20 minutes in a UVO-cleaner (Jelight Co) to remove organic contaminants. Aliquots (20 μL) of 2.5 μM solutions of TrxA-eGFP and Trx::Car9-eGFP were deposited on individual microscope glass slides and incubated for 5 min before imaging by confocal microscopy. Images were captured using a Zeiss LSM 510 NLO Laser Scanning Confocal Microscope with excitation at 483 nm. The microscope was set to acquire z-slices through the solid-liquid interface. We reproducibly obtained images similar to those of Fig. 1.

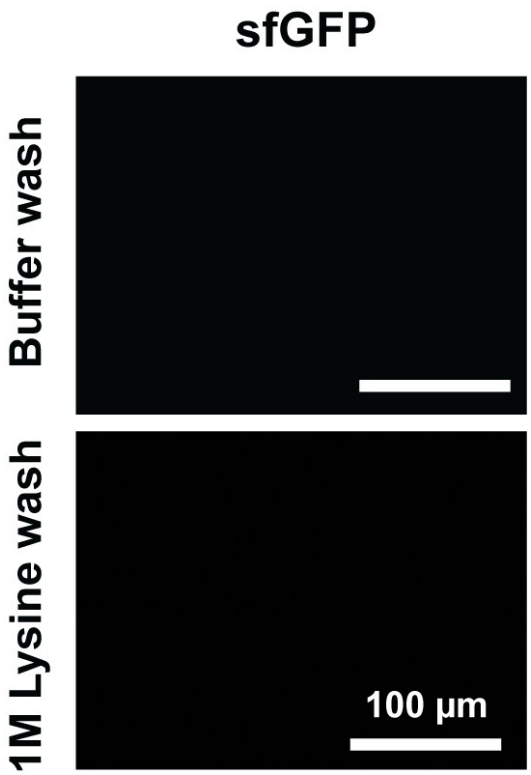
Microcontact Printing

The Sylgard 184 silicone elastomer kit (Dow Corning) was used according to the manufacturer's instructions. The polymer solution (10 mL) was poured onto a silicon master held in a Petri dish. The master pattern consisted of 50 μm diameter pillars with 70 μm center-to-center spacing. The dish and its contents were held for 2h under vacuum at 23°C to remove trapped air bubbles and

improve PDMS penetration and transferred to a 70°C oven to cure the polymer. The elastomer was peeled off from the master, producing a ~ 5 mm thick stamp that was cut into ~ 2 cm^2 squares with a razor blade. Before printing, the patterned side of each stamp was subjected to a 30 min UV-ozone treatment in a UVO-cleaner (Jelight Co) to increase hydrophilicity and remove organic contaminants. Proteins aliquots (50 µL of 10 µM sfGFP, sfGFP-Car9 and mCherry-Car9) were deposited onto the stamp and the excess material was wicked away with a paper towel after 10 min. Inked stamps were dried with compressed air. Glass microscope slides were washed with *dd*H2O, air dried and subjected to UV-ozone treatment for 30 min. Stamps were contacted with the glass slides and a 3 g weight was placed on their back to ensure conformal contact. The stamps were removed after 5 min and the slide was washed three times by immersion in Buffer A for 1 min. After washing, a coverslip was placed on the stamped area and the pattern was imaged by fluorescence microscopy at 30X magnification with excitation at 473 nm for sfGFP and sfGFP-Car9 and excitation at 587 nm for mCherry-Car9. For the experiments of Fig. 3, the slides were further washed 3 times by immersion in Buffer A supplemented with 1 M L-Lysine for 1 min and the patterns were imaged as above. For the experiments of Fig. 4, sfGFP-Car9 was printed as above, the stamp was removed and 10 µl of a 10 µM mCherry-Car9 solution was deposited on the printed area. After 5 min incubation, the slide was washed three times by immersion in Buffer A for 1 min, a cover slip was placed on the stamped area and the pattern was imaged by fluorescence microscopy at 30x magnification with excitation at 473 nm for sfGFP-Car9 and 587 nm for mCherry-Car9. Images were false colored and overlayed using Adobe Photoshop.

References

[1] B. L. Coyle, F. Baneyx, *Biotechnol. Bioeng.* **2014**, *111*, 2019-2026.
[2] B. L. Nannenga, F. Baneyx, *Protein Sci.* **2011**.
[3] B. L. Coyle, M. Rolandi, F. Baneyx, *Langmuir* **2013**, *29*, 4839-4846.



Supplementary Figure 1. µCP of sfGFP. PDMS stamps were inked with 10 µM of sfGFP solution, air-dried and contacted with microscope slides for 5 min. Slides were washed with buffer and imaged by fluorescence microscopy using 473 nm excitation (top panel). The bottom panel shows fluorescence images of the stamped region of the same slide after immersion in buffer containing 1M L-lysine. All bars correspond to 100 µm.