# Bio-functional surfaces for smart entrapment of polysomes

L. Lunelli[a,b], L. Marocchi[a], L. Pasquardini[a,#], Lia Vanzetti[a], G. Viero[b], C. Potrich[a,b,*], C. Pederzolli[a]

[a]Fondazione Bruno Kessler - Center for Materials and Microsystems, Lab. Biomarker Studies and Structure Analysis for Health, Via Sommarive 18, I-38123, Povo, Trento, Italy; [b]CNR-Institute of Biophysics, Unit at Trento, via alla Cascata 56, 38123, Povo, Trento, Italy; [#]Present address: Indivenire s.r.l., via alla Cascata 56/C, I-38123 Trento, Italy.

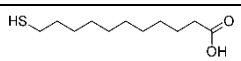
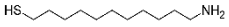
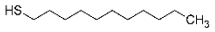
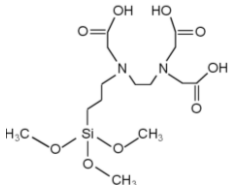
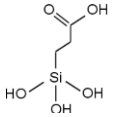
| acronym | molecule | Formula |
|---|---|---|
| MCOOH | 11-Mercaptoundecanoic acid |  |
| MOH | 11-Mercapto-1-undecanol |  |
| $MNH_2$ | 11-Amino-1-undecanethiol hydrochloride |  |
| $M11CH_3$ | 1-Undecanethiol |  |
| | | |
| triCAS | N-(trimethoxysilylpropyl)ethylene-diamine triacetic acid, trisodium salt |  |
| CST | Carboxyethylsilanetriol |  |

**Table S1**: List of undecanethiols used to functionalize gold substrates and of carboxysilanes used to functionalize silicon oxide surfaces.

| **surface** | **O 1s (%)** | **C 1s (%)** | **N 1s (%)** | **Si 2p (%)** |
|---|---|---|---|---|
| *plasma-treated TG-SO* | *68.0* | *6.2* | - | *25.8* |
| Silanization with triCAS | 58.5 | 15.9 | 0.7 | 24.9 |
| Silanization with CST | 57.8 | 16.0 | - | 26.3 |

**Table S2**: Chemical composition of bare and functionalized TG-SO surfaces, measured by XPS at 60° emission angle. The bare surface is reported in italics. Functionalizations are described in Section “Silicon oxide functionalization”.

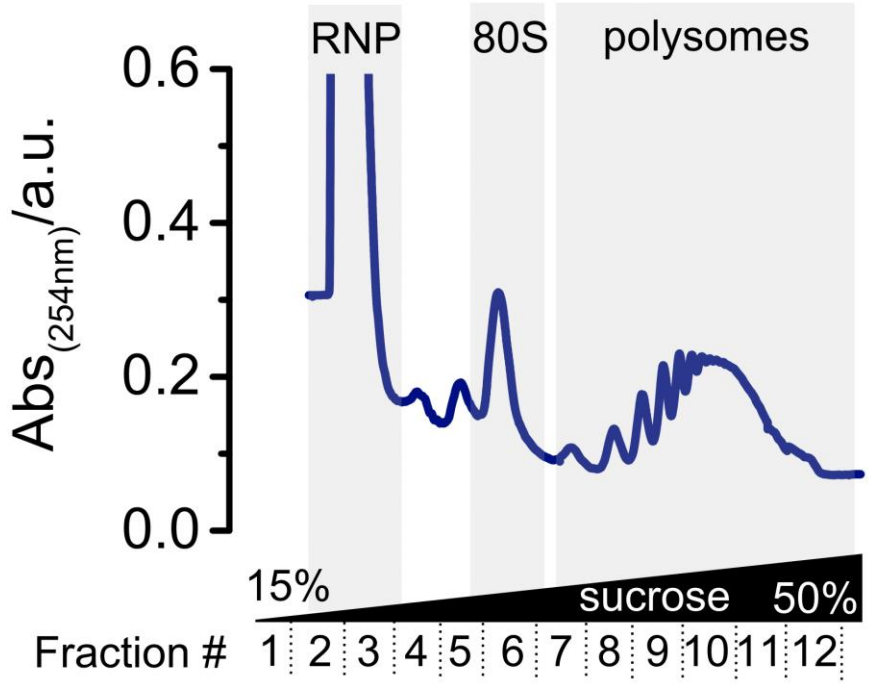



**Figure S1**: Typical polysome sedimentation profile of a MCF-7 cell lysate after sucrose gradient centrifugation and fractionation. The sample was collected in 1 mL fractions. The subpolysomal fractions 1 and 2 contains RiboNucleoParticles (RNP), the 3 and 4 the small and large ribosomal subunits (40S, 60S respectively). Fraction 5 corresponds to the ribosome (80S). Several polysomal peaks, corresponding to fractions with increasing weight (fractions 7 to 12) are clearly visible.

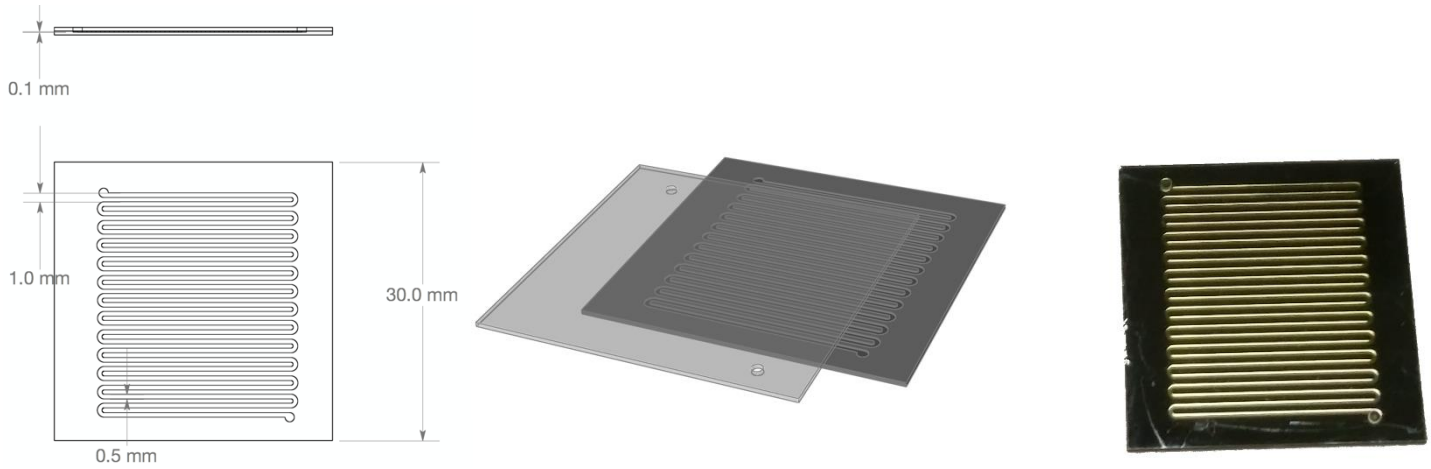



**Figure S2.** Scheme with dimensions (A), CAD rendering (B), and actual microdevice (C) employed for polysome purification. In panel (B), the Pyrex upper part was shifted to evidence inlet and outlet holes. Lateral size of the device is 3 cm, channel width 0.5 mm, channel step 1.0 mm, channel depth 0.1 mm. Total volume is 25 µL.

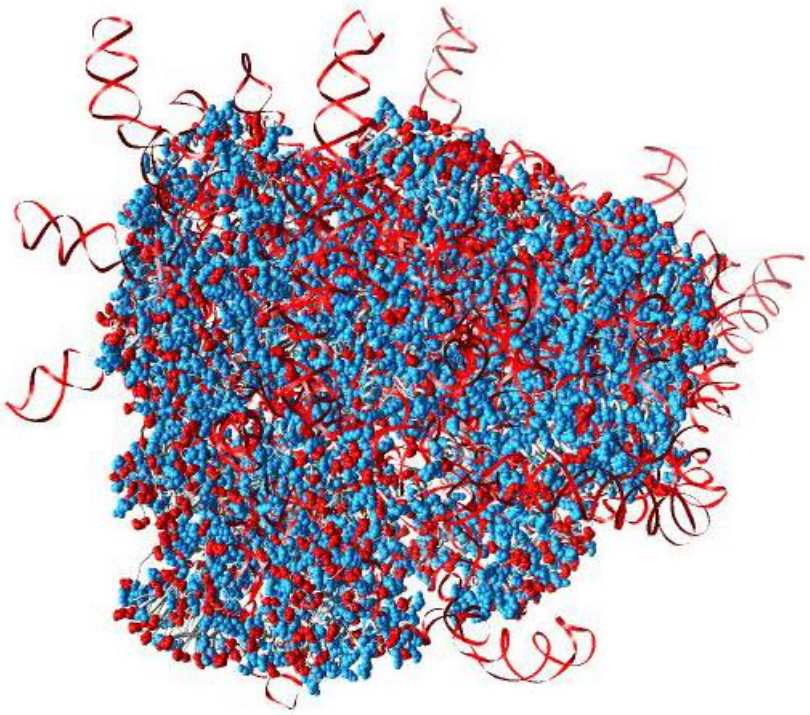


**Figure S3**. Human ribosome structure (PDF files 3J3A,3J3B, 3J3D and 3J3F from Anger2013), rendered with UCSF Chimera (Pettersen2004, www.rbvi.ucsf.edu/chimera/) showing charged residues. Blue: positively charged residues, red: negatively charged residues, ribbons: rRNA.

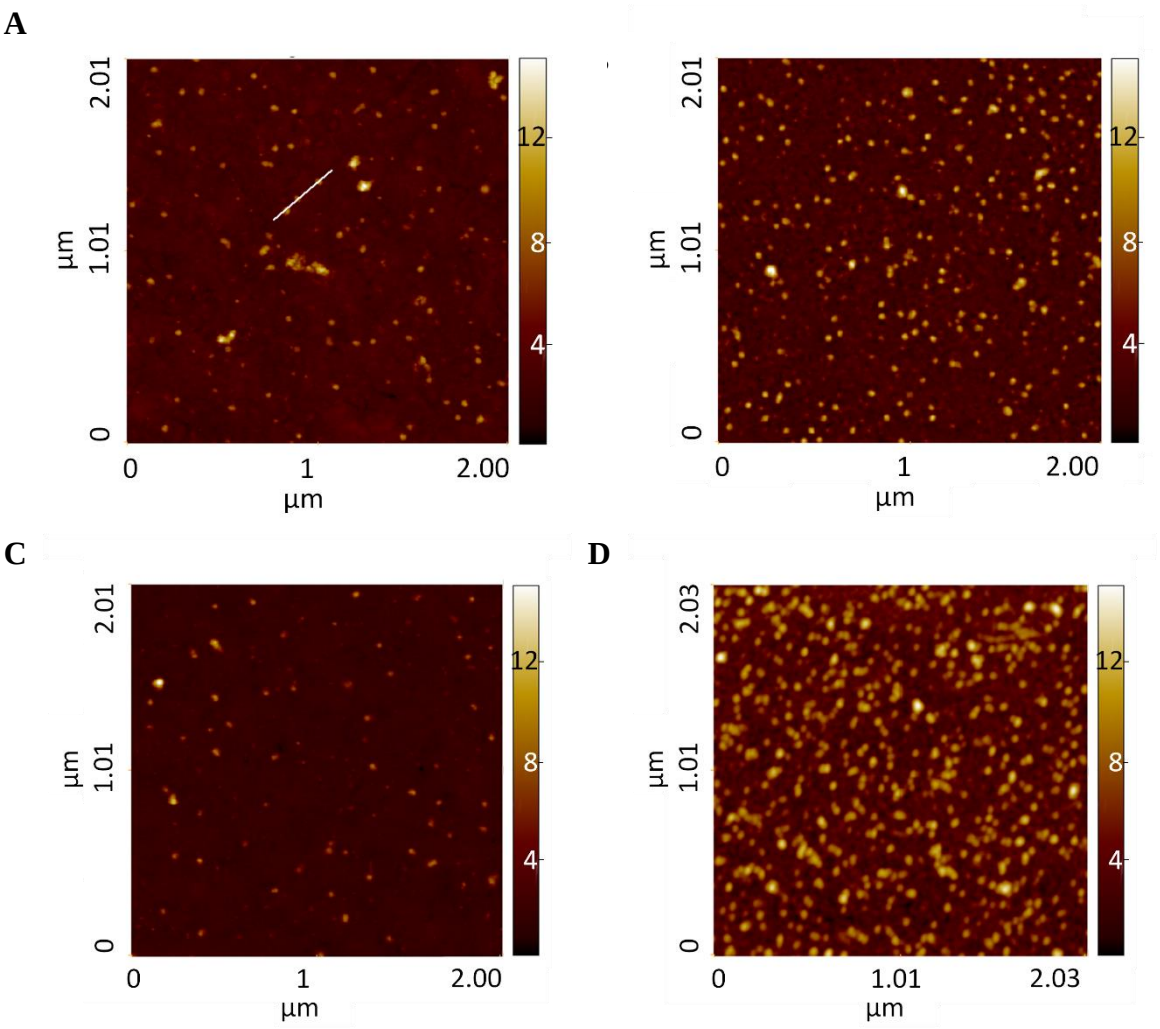



**Figure S4**: AFM images of ribosomes adsorbed on bare gold (panel A), on MCOOH (panel B), MOH (panel C) and $MNH_2$ (panel D) functionalized gold surfaces ($2 \times 2$ µm$^2$ images).

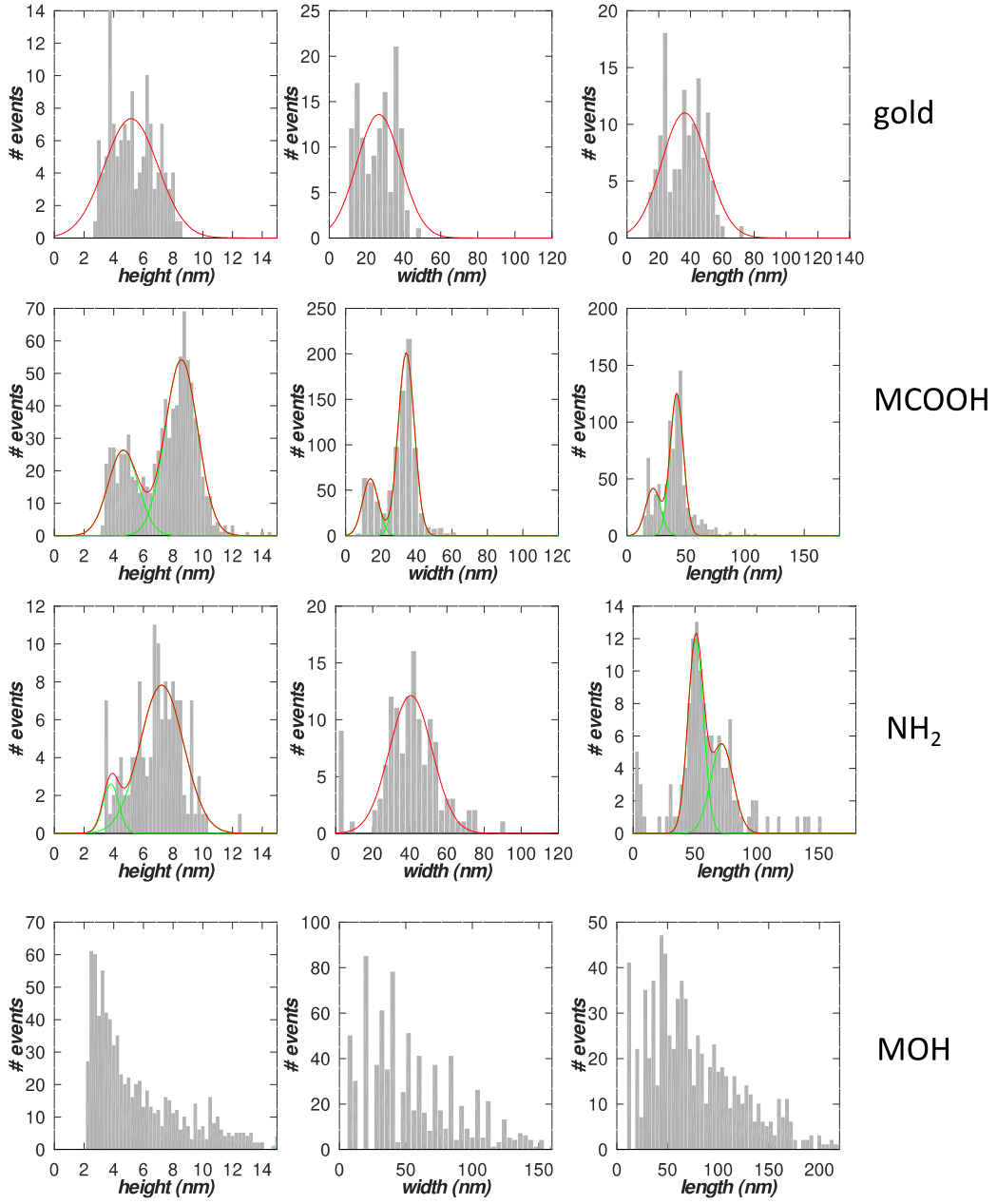



**Figure S5**. Height, minimum and maximum lateral size distributions obtained by grain-analysis on surfaces obtained depositing ribosomes on bare gold and MCOOH, $MNH_2$, and MOH functionalized gold (grey bars). Data were fitted (red lines) with 1 or 2 gaussian functions (green lines). The quite broad distributions obtained on MOH functionalized gold were not fitted.

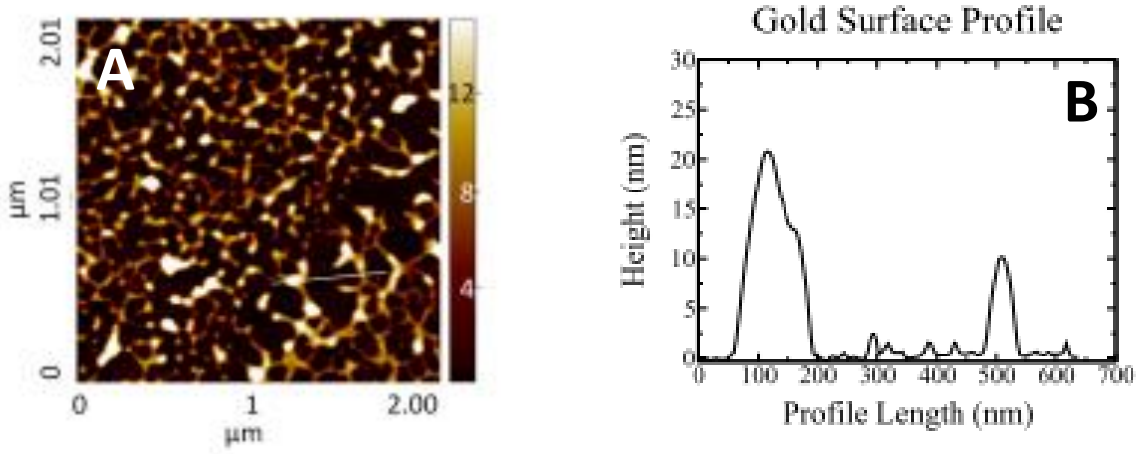



**Figure S6**: 2 × 2 µm$^2$ AFM image of M11CH$_3$ (A) functionalized gold surfaces treated with ribosomes (A). No intact ribosomes can be observed. The height profile traced along the white line in A is reported in panel B.

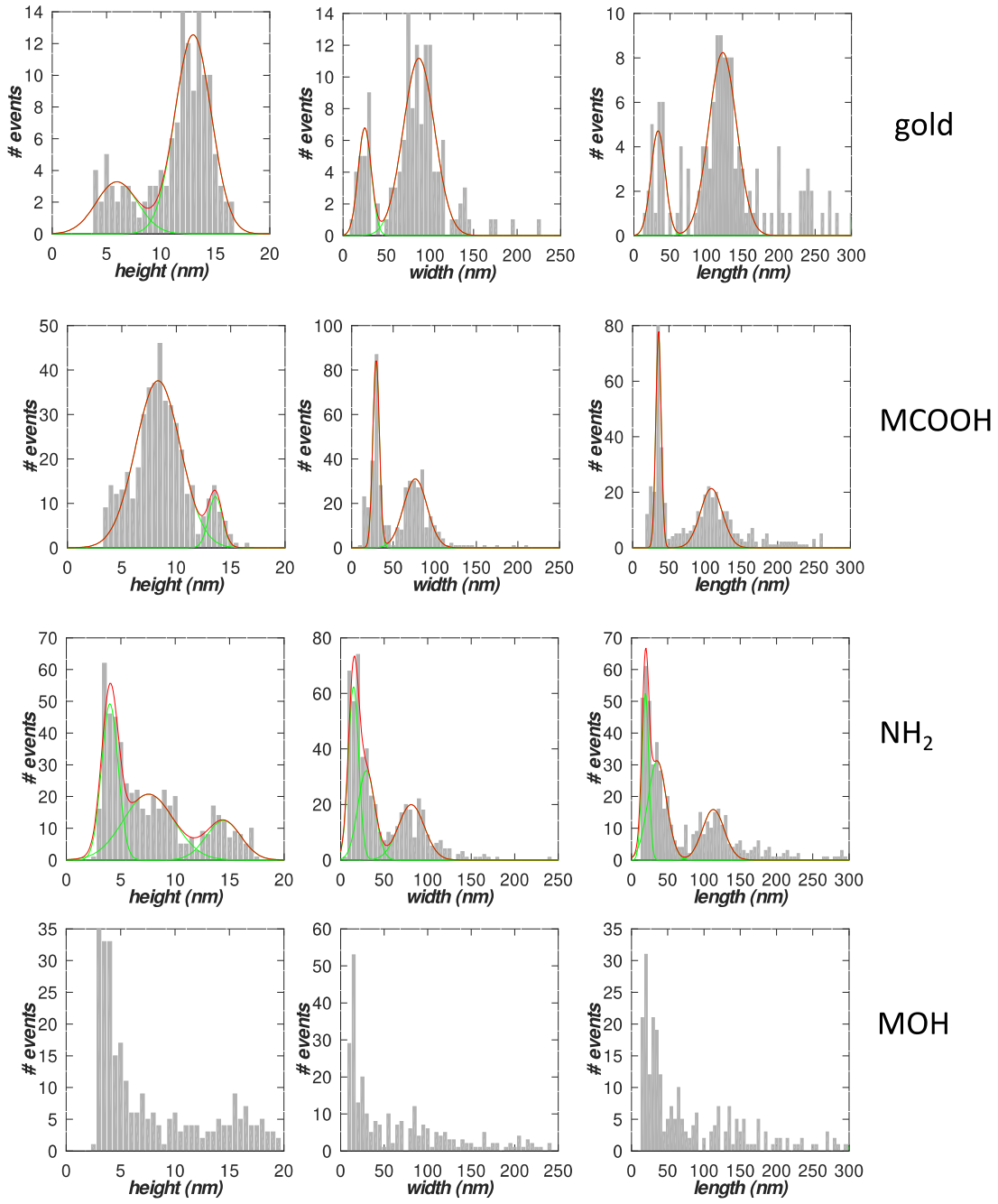



**Figure S7**. Height, minimum and maximum lateral size distributions obtained by grain-analysis on surfaces obtained depositing polysomes on bare gold, MCOOH, $MNH_2$, and MOH functionalized gold (grey bars). Data were fitted (red lines) with 2 or 3 gaussian functions (green lines). The quite broad distributions obtained on MOH functionalized gold were not fitted.

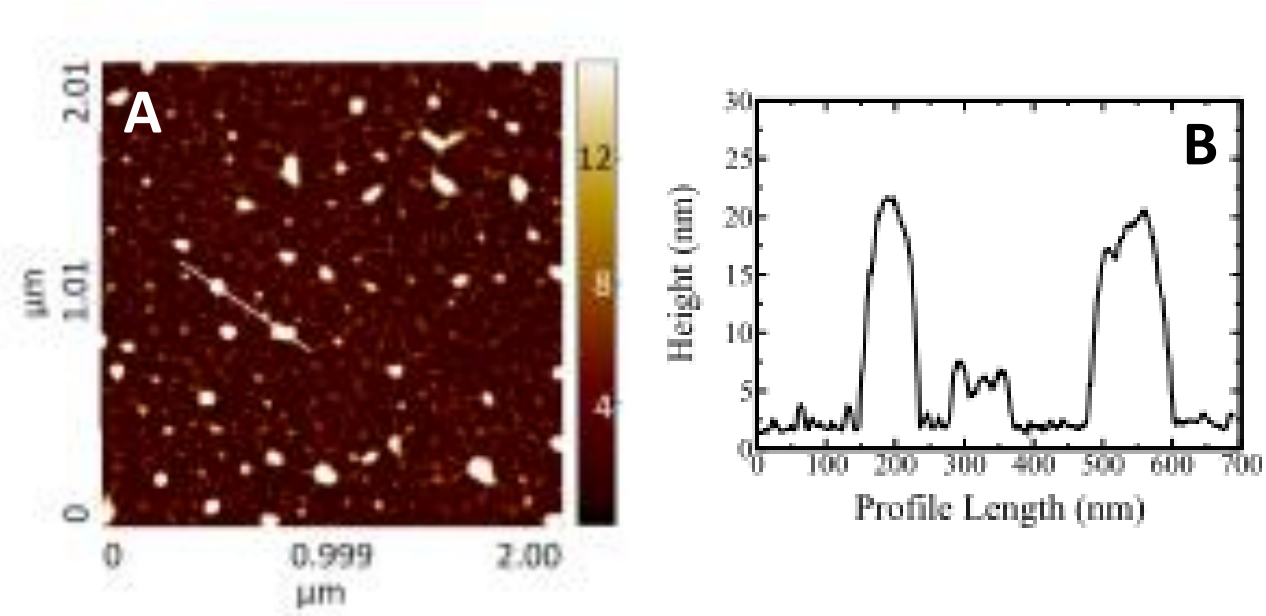



**Figure S8**: 2 × 2 µm$^2$ AFM image of M11CH$_3$ functionalized gold surfaces treated with polysomes (A). No intact polysomes (i.e. ribosome groups) can be observed. The height profile traced along the white line in A is reported in panel B as cross-section along the white line in panel A).

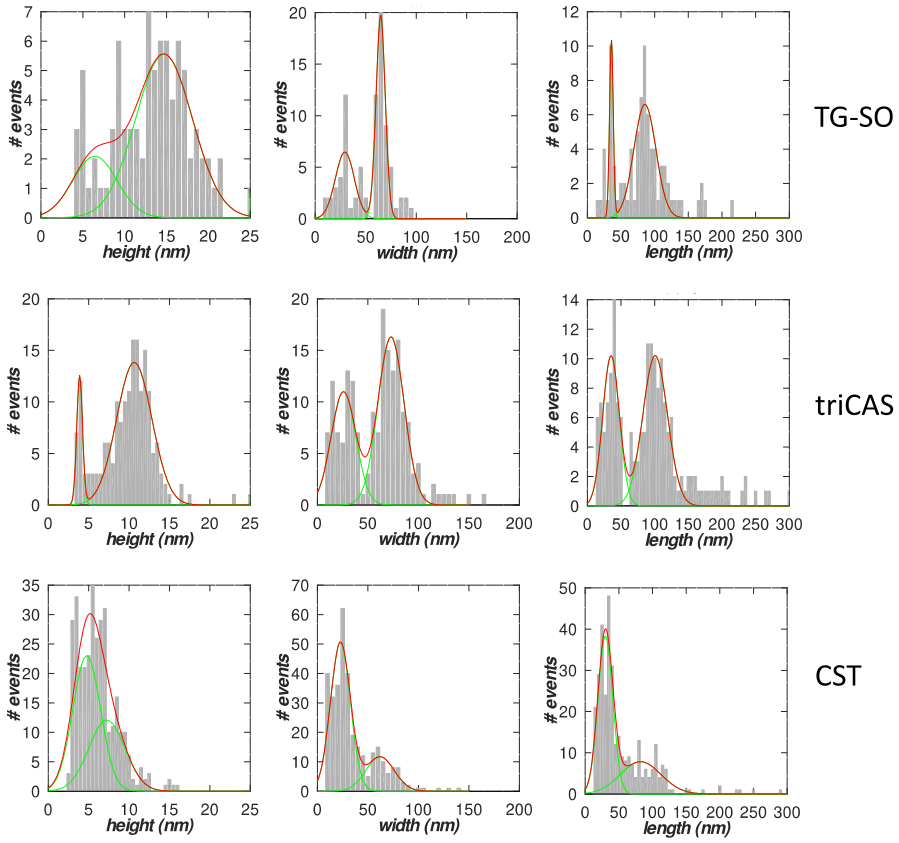



**Figure S9**. Height, minimum and maximum lateral size distributions obtained by grain-analysis on surfaces obtained depositing polysomes on plasma-treated TG-SO, triCAS treated silicon surfaces and CST treated TG-SO (grey bars). Data were fitted (red lines) with 2 gaussian functions (green lines).

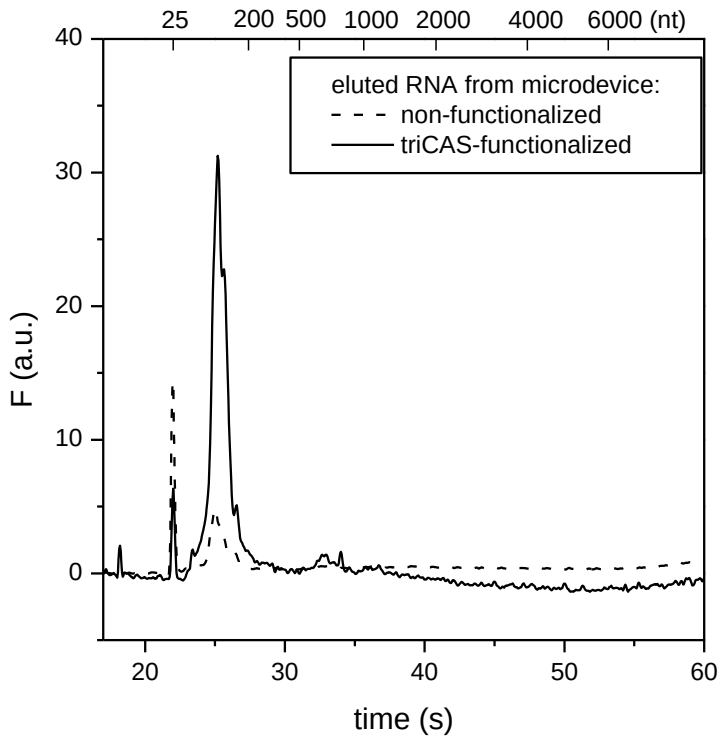



**Figure S10**. Electropherograms of RNA eluted from microdevices after incubation of cell lysate. Dashed line: non-functionalized microdevice; solid line: microdevice functionalized with triCAS. Peaks at 25 nt correspond to the marker. Cell lysate was inserted in the microdevice and let to adhere to the surfaces for 1 h at 4°C. Next, a wash with RNF-water was performed before elution with TE buffer for 10 min at 50°C. The eluted RNA was loaded on the RNA Pico chip and measured with a Bioanalyzer.

## References

Anger2013. A. M. Anger, J.-P. Armache, O. Berninghausen, M. Habeck, M. Subklewe, D. N. Wilson, and R. Beckmann. Structures of the human and drosophila 80S ribosome. Nature, 497(7447):80-85, 2013.

Pettersen2004. Pettersen EF, Goddard TD, Huang CC, et al. UCSF Chimera-a visualization system for exploratory research and analysis. J Comput Chem. 2004;25(13):1605-1612. doi:10.1002/jcc.20084