Supporting Information for:

Immobilisation of proteins at silicon surfaces using undecenylaldehyde: demonstration of the retention of protein functionality and detection strategies.

Qi Hong, Celia Rogero Jeremy H. Lakey, Bernard A. Connolly, Andrew Houlton and Benjamin R. Horrocks

1. Purification of his-tagged GFP and GFP-TolAIII fusion protein.

2. FTIR – undecene (liquid) and porous silicon modified with 1-undecenylaldehyde and urease/lysine (enlargement of fig 2a).

3. AFM of amyloid beta peptide Aβ(1-42) (enlargement of fig 4a & b).

4. Fluorescence data: GFP emission spectra, unfolding kinetics, thermal stability and patterning data (enlargement of fig 5).

5. AFM images showing typical porous silicon pore sizes.

1.

Purification of His tagged GFP:

The GFP used in this study was a UV-enhanced variant[1] with a hexahistidine tag. UV-enhanced GFP was amplified from a Clontech pBAD-GFP_{uv} expression vector by PCR and cloned in to a modified pET8c plasmid which adds a $His_{(6)}Ser_{(2)}$ tag to the N terminus.[2] The protein was overexpressed in *Escherichia coli* and purified by nickel-chelate affinity chromatography and gel filtration as previously described.[3]

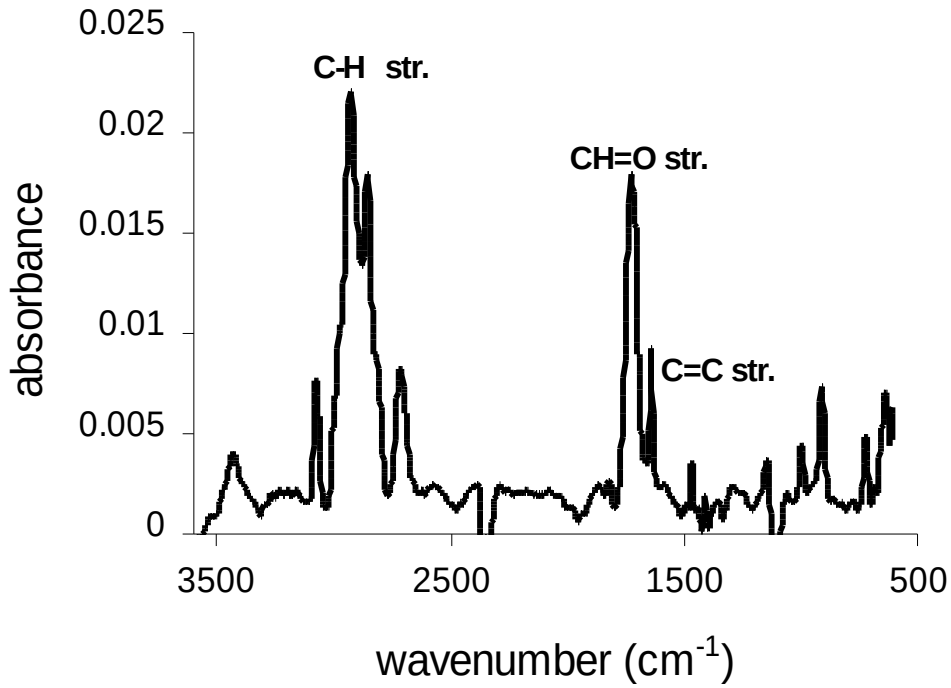
[1] A. Crameri, E. A. Whitehorn, E. Tate, W. P. C. Stemmer, Nature Biotechnology 1996, 14, 315.
[2] A. S. Politou, M. Gautel, M. Pfuhl, S. Labeit, A. Pastore, Biochemistry 1994, 33, 4730.
[3] S. L. Fridd, I. Gokce, J. H. Lakey, Biochimie 2002, 84, 477.

Purification of GFP-TolAIII fusion protein:

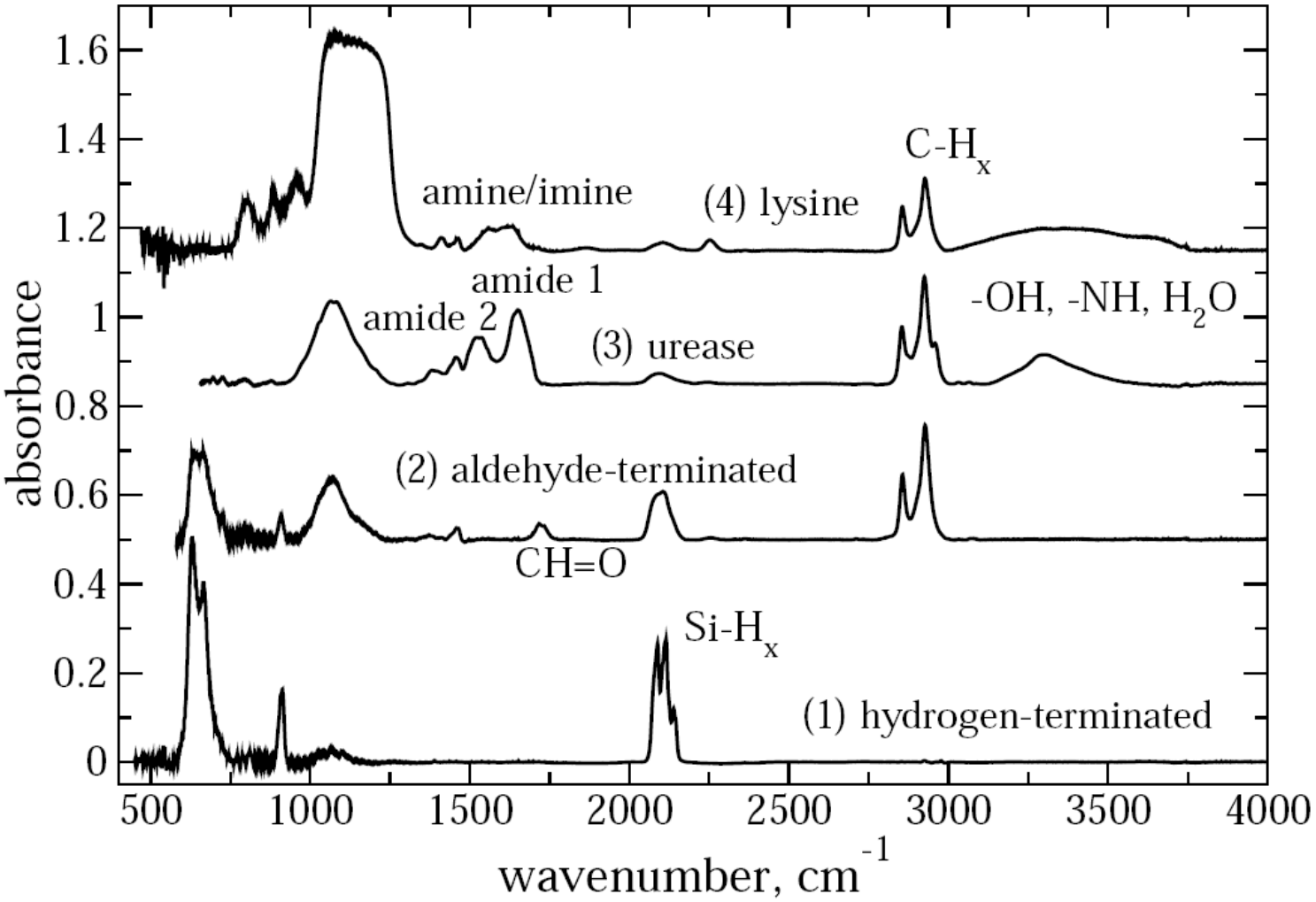
A fusion of GFP to the C-terminus of the positively charged *Escherichia coli* TolAIII protein (containing a His tag at its amino terminus) was created to enhance expression and provide additional lysine residues for immobilization. This was created by inserting the GFP gene in the pTol vector as previously described.[4] The fusion was expressed and purified as above using the His tag at the N-terminus of the TolAIII protein.

[4] G. Anderluh, I. Gokce, J. H. Lakey, *Protein Expression and Purification* **2003**, 28, 173-181.

2.

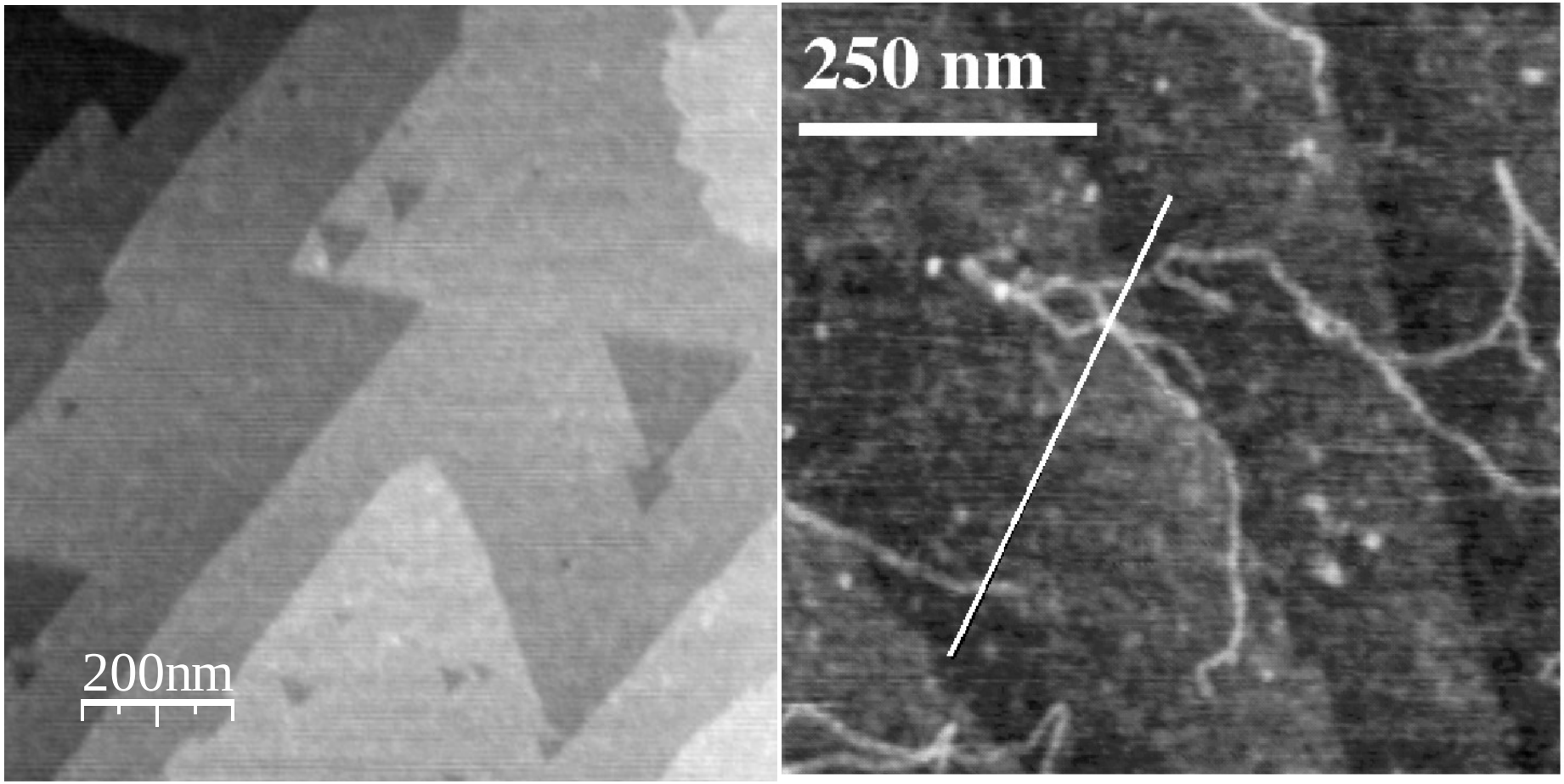


Infra red spectrum of 1-undecenylaldehyde (liquid drop on silicon wafer). Prominent peaks corresponding to C-H_x, CH=O and C=C stretches are shown.



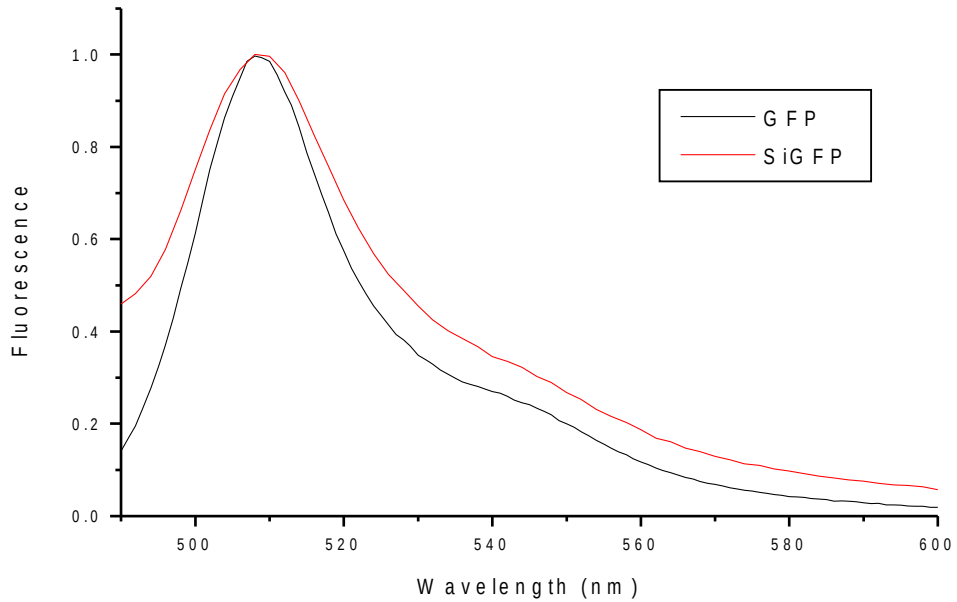
Enlargement of figure 2a in main paper

3.



Enlargement of figures 4a and b in main publication

4.

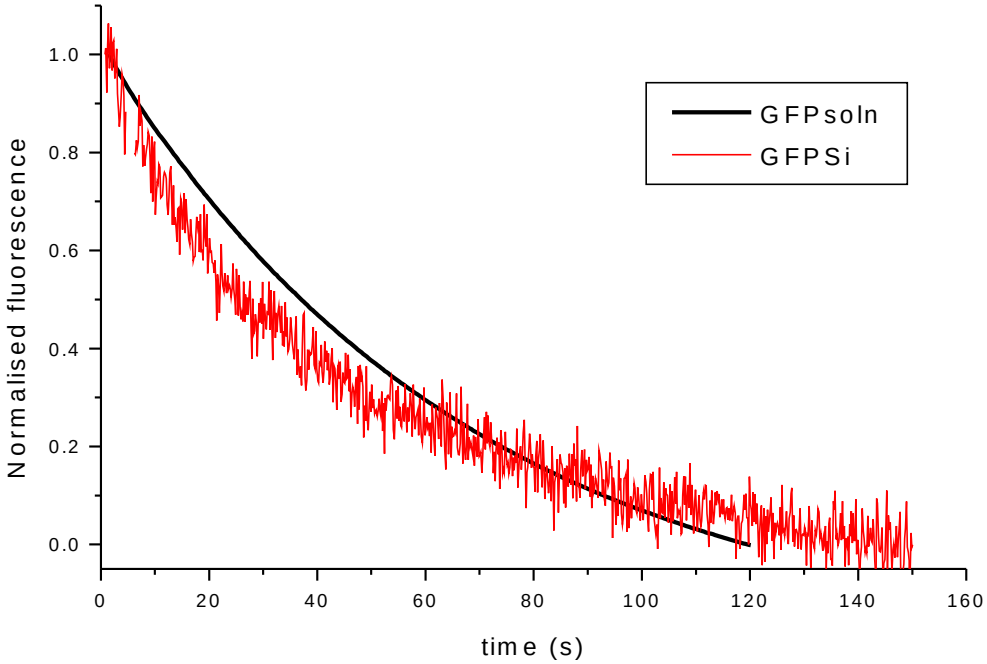


Comparison of emission spectra from GFP in solution (black) and attached to silicon surface (red) , data normalised to 1 at 508 nm due to difference in data collection conditions. All data was collected at room temperature in a Cary Eclipse fluorescence spectrometer. Excitation wavelength 395 nm. Band widths 5 nm for excitation and emission. 1 cm path length, 3ml volume Hellma QS-101 quartz cuvette.

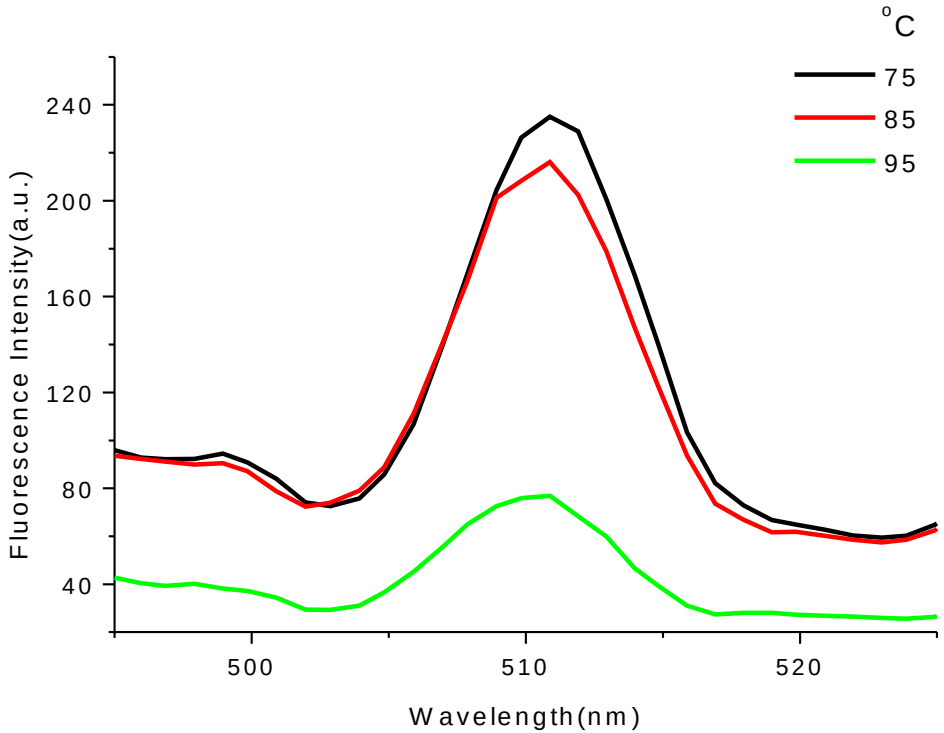
Solution conditions. His tagged GFP was dissolved in phosphate buffered saline (PBS; 10 mM sodium phosphate, pH 7.4 plus 150 mM NaCl), at a concentration of 0.05 mg/ml.

Silicon conditions. His-tagged GFP was immobilised on a silicon wafer as described in the text. Approx 1 cm square section of

silicon wafer was placed in the cuvette, facing the emission and excitation beams at 45 degrees, in PBS. An emission filter with a 430- 1100 nm pass was used to prevent overload with reflected excitation. The higher background is due to the silicon signal.

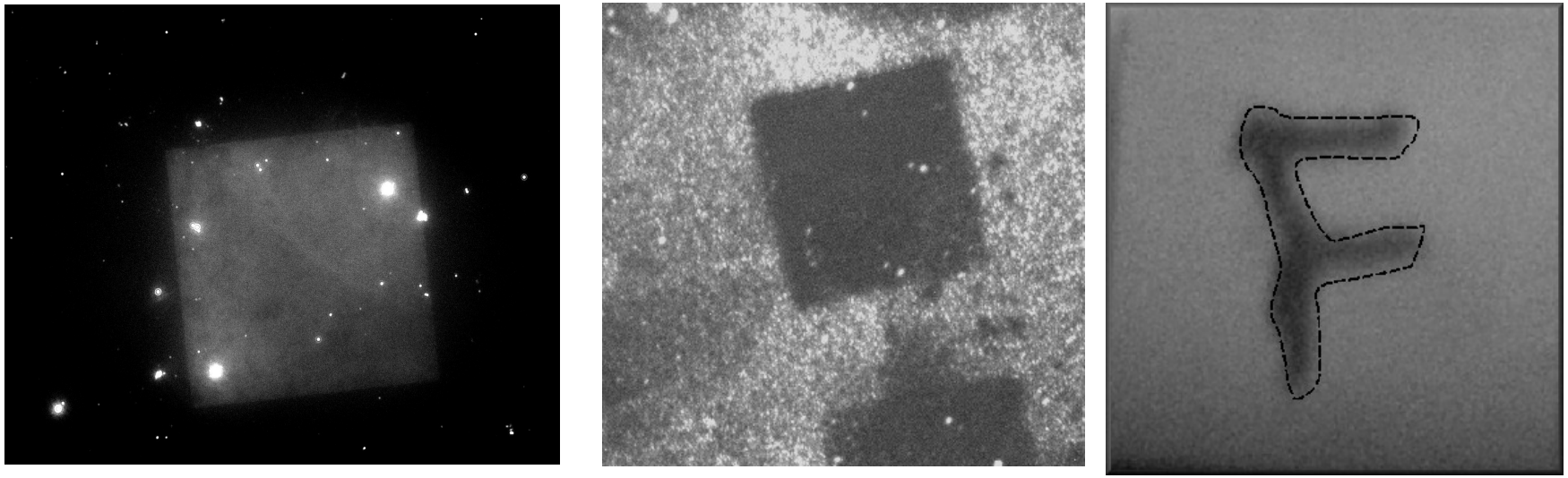


Unfolding kinetic data for GFP in solution and on silicon. Data collected as in the previous figure, but with 6M guanidine hydrochloride in the PBS. Time is after either addition of soluble GFP to guanidine hydrochloride or placing the silicon wafer in the guanidine hydrochloride solution. Data normalised after subtracting final baseline value.



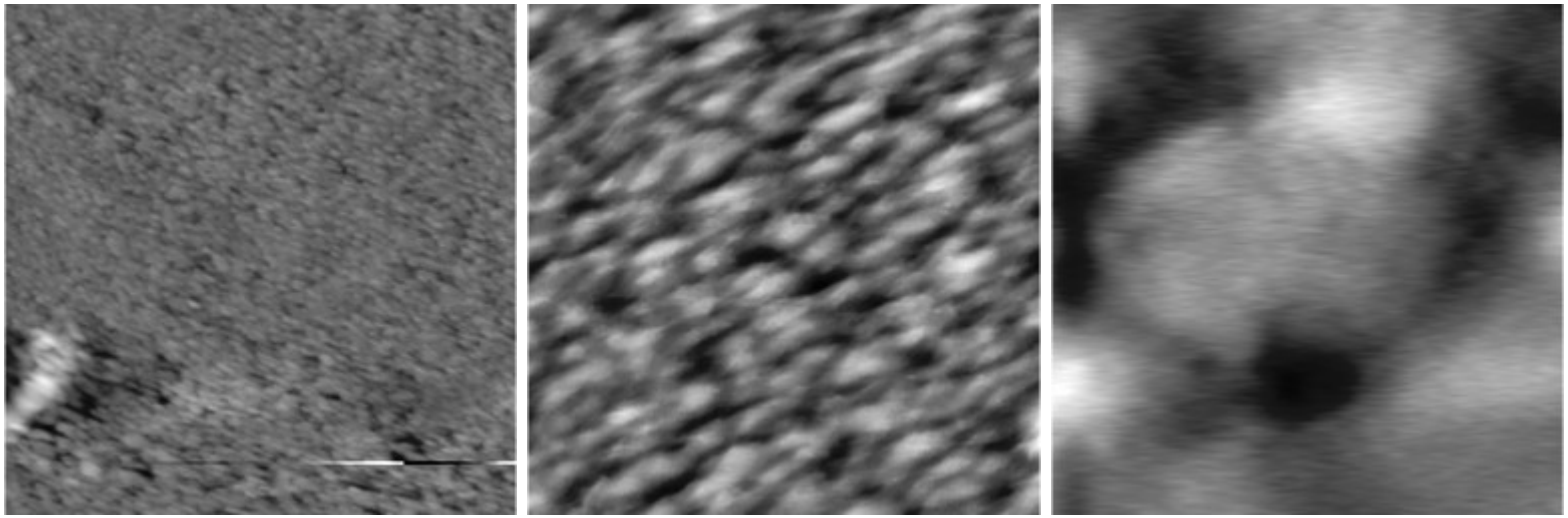
Thermal stability of GFP on silicon. GFP, immobilised on a silicon wafer as described in the text, was heated to the indicated temperatures for one hour prior to the fluorescence spectrum being collected. The protein retains its natural thermal stability.[5]

[5] S. Topell, J. Hennecke, R. Glockshuber, *FEBS Letters* **1999**, *457*, 283- 289.



Enlargement of figure 5 in main publication

5.



AFM images of porous silicon indicating the typical pore size.

Left image 10 x 10 μm, middle image 2 x 2 μm and right image 390 x 390 nm. An individual pore opening is visible in the middle of the lower half of the right hand image.