Supplementary Information

Cryogenic optical localization provides three-dimensional protein structure data with Angstrom resolution

Siegfried Weisenburger[1,2], Daniel Boening[1], Benjamin Schomburg[3], Karin Giller[3], Stefan Becker[3], Christian Griesinger[3] & Vahid Sandoghdar[1,2*]

[1] Max Planck Institute for the Science of Light, Erlangen, Germany.
[2] Department of Physics, Friedrich Alexander University (FAU) Erlangen-Nürnberg, Erlangen, Germany.
[3] Max Planck Institute for Biophysical Chemistry, Göttingen, Germany.

*: Correspondence should be addressed to V.S. (vahid.sandoghdar@mpl.mpg.de).

This Supplementary Information contains:

Supplementary Protocol COLD experiments

Supplementary Protocol COLD measurements

A: Substrate cleaning

1) Rinsing using non-halogenated solvents (acetone and ethanol).
2) Incubation in H_2SO_4 and 30 % H_2O_2, ratio 1:1, for 2 days.
3) Ultrasonication in Helmanex (4 % at 60 °C) for one hour.
4) Thorough rinsing of glass substrates with DDH_2O and ethanol.
5) Additional cleaning step with oxygen plasma (O_2 plasma, 10 min).
6) Ultrasonication in HCl and H_2O_2 solution (ratio 3:1) at 60 °C for one hour.
7) Rinsing with DDH_2O and 2-propanol.
8) Another 10 min in the plasma cleaner (O_2 plasma).
9) Bleaching of residual fluorescence on substrate with UV-VIS mercury lamp for several days.

B: Sample preparation

1) Mixing 10 µl protein solution (Streptavidin-Biotin: approx. 10 µM stock solution resuspended in 1 ml Tris-EDTA buffer and diluted 1:100 in Tris-EDTA, or PASc: approx. 10 µM stock solution in H_2O diluted 1:10,000 in Tris-EDTA) with 90 µl Tris-EDTA Buffer, 10 µl of Trolox (20 mM stock solution in DMSO) and 20 µl of PVA (10 %w, filtered, degassed, stock solution in H_2O).
2) Spin coating of 5 µl of sample solution on 0.7 cm x 0.7 cm glass substrate (0.2 mm thickness) at 1000 rpm for 10 seconds, followed by 3000 rpm for 60 seconds.
3) Mounting of the sample to the cold finger of the cryostat by using a thin layer of Apiezon N grease to ensure thermal contact to the cold finger.

C: COLD measurement

1) Assembly of the cryostat vacuum chamber.
2) Evacuation of the chamber until it reaches a pressure of < 10^-6 mbar.
3) When pressure is reached, starting to cool down the sample with liquid Helium (cooling rate approximately 0.5-1 K per second) to 4.3 K.
4) Regulate helium flow to a minimum to hold the temperature.
5) Allowing the system to settle for 2 hours until the sample reached 4.3 K.
6) Adjusting the incident laser power to 200 µW.
7) Realign automated focusing system.
8) Focus laser to back focal plane of the objective to achieve wide-field illumination.
9) Recording fluorescence from the sample with an exposure time of 2 s.

Nature Methods: doi:10.1038/nmeth.4141