Supplementary Information

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

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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 H₂SO₄ and 30 % H₂O₂, ratio 1:1, for 2 days.
   3) Ultrasonication in Helmanex (4 % at 60 °C) for one hour.
   4) Thorough rinsing of glass substrates with DDH₂O and ethanol.
   5) Additional cleaning step with oxygen plasma (O₂ plasma, 10 min).
   6) Ultrasonication in HCl and H₂O₂ solution (ratio 3:1) at 60 °C for one hour.
   7) Rinsing with DDH₂O and 2-propanol.
   8) Another 10 min in the plasma cleaner (O₂ 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 H₂O 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 H₂O).
   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.