Quantification of Biomolecular Dynamics Inside Real and Synthetic Nuclear Pore Complexes Using Time-Resolved Atomic Force Microscopy

Supporting Information

George J. Stanley[a,*], Bernice Akpinar[a,b,*], Qi Shen[c,d], Patrick D. Ellis Fisher[c,d], C. Patrick Lusk[c], Chenxiang Lin[c,d], Bart W. Hoogenboom[a,e,$]

[a] London Centre for Nanotechnology, University College London, 17-19 Gordon Street, London WC1H 0AH, United Kingdom

[b] Department of Chemistry, Imperial College London, London, SW7 2AZ, United Kingdom

[c] Department of Cell Biology, Yale School of Medicine, New Haven, Connecticut 06520, United States

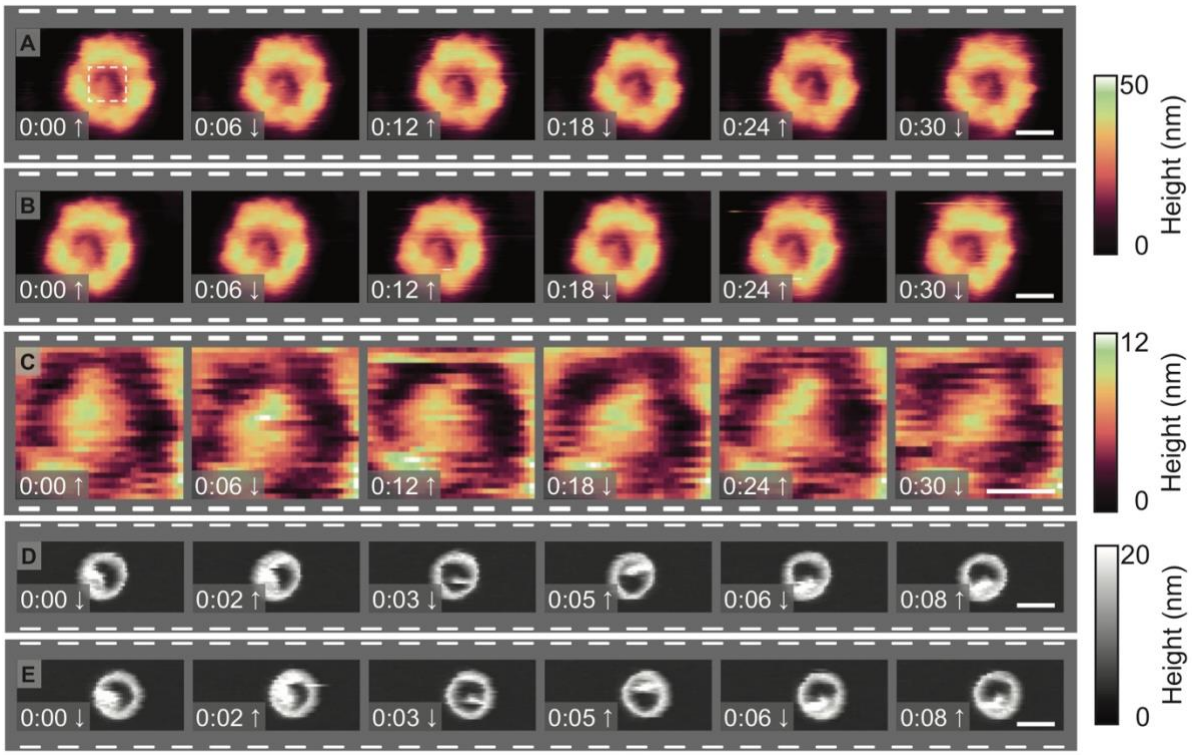
[d] Nanobiology Institute, Yale University, West Haven, Connecticut 06516, United States

[e] Department of Physics and Astronomy, University College London, Gower Street, London WC1E 6BT, United Kingdom

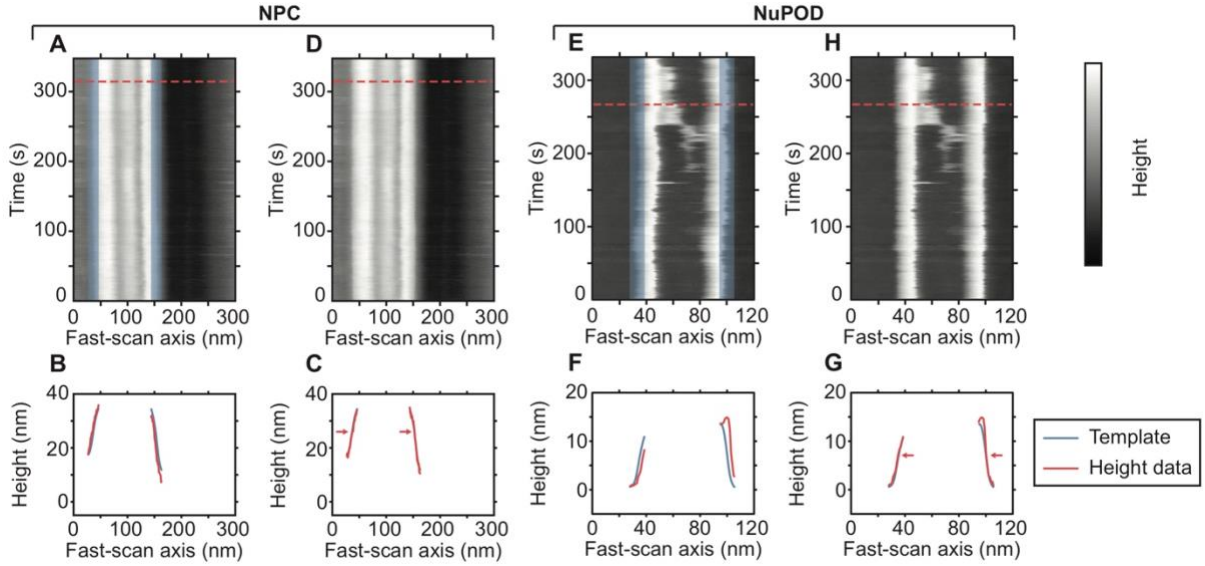
* These authors contributed equally to this work.

$ E-mail: b.hoogenboom@ucl.ac.uk

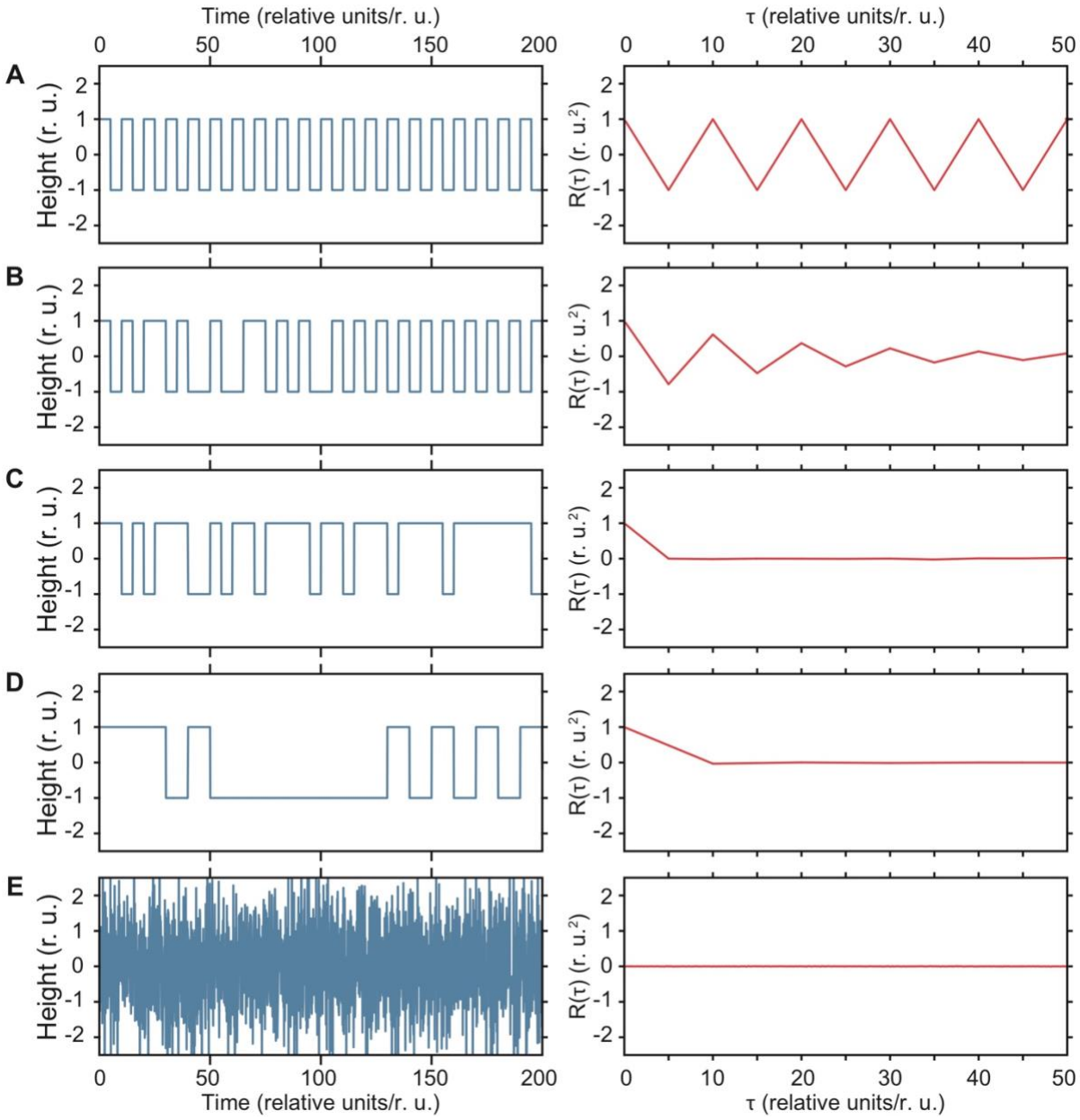
Supplementary Figures



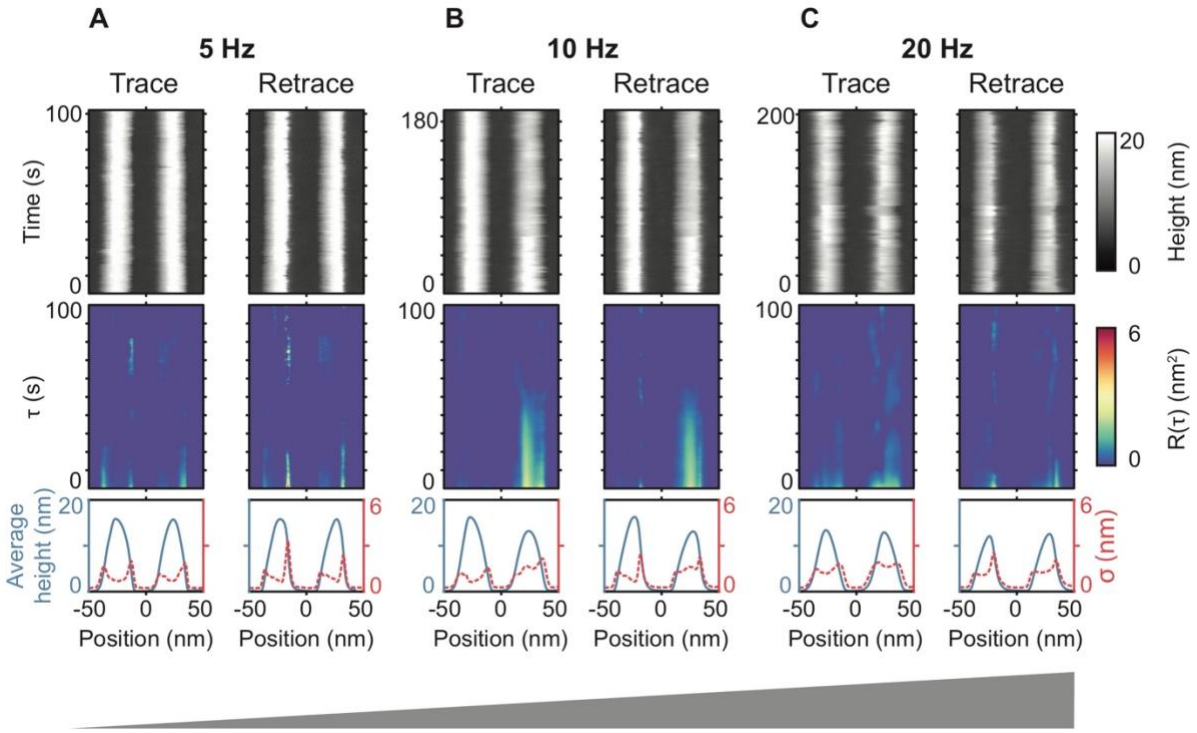
Supplementary Figure 1| AFM video sequence of NPCs and NuPODs. (A) Consecutive AFM images of the cytoplasmic face of an NPC, recorded at ~6 s/frame. The NPC's transport channel shows a stable protrusion. **(B)** Retrace images represent data acquired during the same scans as in **(A)**, but refer to the right-to-left line scans instead of the left-to-right line scans ('trace'). These data faithfully reproduce the video sequence shown in **(A)**, but are slightly offset due to scanner hysteresis. **(C)** A digital zoom of the inside of the pore lumen, cropped from (**A**, see white-dashed box). A 2nd order plane subtraction has been applied to each cropped image to better highlight any height differences within the pore lumen itself. **(D and E)** Trace and retrace images of a NuPOD containing 48 Nsp1 molecules, captured at ~1.6 frames/sec. Arrows indicate capture direction. These images represent snapshots extracted from data that have previously been published elsewhere in video format [1]. Scale bars: 50 nm **(A, B, D, E)**, 20 nm **(C)**.



Supplementary Figure 2| Drift correction of kymographs. (A) A kymograph of the cytoplasmic side of an NPC (scanned at 5 Hz). The outer edges of the pore scaffold are selected, and windows (typically of ~20 nm width) centred around these areas are cropped (blue, transparent regions). These regions are height averaged to produce a template (**B and C**, blue lines). Each line in the kymograph is then compared against this template within the window regions (red lines, **B**, corresponding to the red-dashed line in **A**). The data is then shifted in 1 nm intervals to both the left and right, for ~10 nm, and the sum of absolute difference of height values (SAD score) is calculated at each position. Whichever shift results in the best height correlation (or minimum SAD score), is used to correct that particular line **(C)**. **(D)** This operation is performed on every line in the kymograph to correct for lateral drift in our experiments. **(E-H)** Same as for **(A-D)** but demonstrated on a NuPOD containing 48 Nsp1 molecules.



Supplementary Figure 3| Interpreting the auto-correlation factor *R*(τ). (A) A simulated step function that changes between the values -1 and 1 at intervals of 5, therefore having a period of 10 (left panel), and calculated *R*(τ) (right panel). *R*(τ) is maximum for lag times, τ, that match integer multiples of the period of the original signal (*i.e.*, for τ = 0, 10, 20, *etc.*), indicating high correlation. By contrast, for half-integer multiples of the period (τ = 5, 15, 25, *etc.*), there is maximum anti-correlation. **(B and C)** Increased randomness in this function reduces long-scale auto-correlation, here shown when the flipping probability at each interval is reduced from 100% to 90% **(B)** and 50% **(C)**. In the case of **(C)**, no correlation is observed beyond a single half-period. **(D)** Same as **(C)**, but the interval length is increased from 5 to 10. Now correlation is observed up until τ = 10 instead of τ = 5. **(E)** For white noise, *R*(τ) is zero.



Supplementary Figure 4| Kymographs and auto-correlation analysis of empty NuPODs. (A-C) Kymographs of NuPODs without FG-nups (top row), recorded at 5 **(A)**, 10 **(B)**, and 20 Hz **(C)**, showing both trace and retrace data. The light features are the DNA scaffold and the dark background the lipid bilayer. The auto-correlation heatmaps (second row) show background noise in the NuPOD lumens, and weak signatures of stochastic fluctuations at the edges of the DNA scaffold structure. Average height profiles of each pixel in the kymograph with time (bottom row, blue line), plotted with their respective standard deviation, σ (bottom row, red-dashed line), show maximum σ where the slope of the height profiles is steepest. Data shown in **(A)**, trace, is also printed in **Figure 3A.**