Supplementary Information

Analysis of replication by optical mapping in nanochannels

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We focused on the alkynyl nucleoside analog EdU (5-ethynyl-2'-deoxyuridine), which serves as template for the labeling of newly synthesized DNA by click-chemistry (Supplementary Fig. S1A). The most common co-activator of the reaction is copper (I), yet this compound is known for its strong genotoxicity associated to single strand breaks through oxidative damage. We wished to evaluate the balance between DNA damage and DNA labeling efficiency. DNA damage was measured in bulk by gel electrophoresis of phage-λ DNA (Supplementary Fig. S3B), showing that the rapid degradation of DNA due to the presence of copper in the click-reaction medium. Because oxidative stress is responsible for DNA damage, we evaluated the stabilizing role of several anti-oxidants (reducing agents) to preserve chromosomes during click chemistry protocols. Dithiotreitol (DTT), Glutathione (GSH), and the inorganic reducing agent $Na_2S_2O_4$ were tested. We report our results on GSH in Supplementary Fig. S1C, but similar data were obtained with DTT and Na_2SO_4. We first evaluated the concentration of DTT required to avoid λ-DNA degradation in the presence of GSH using our gel assay. Our results showed that 10 mM GSH at least was required to stabilize DNA during a click-reaction of 2 hours (gel in the right of Fig. S3C). We then evaluated the labeling efficiency with various proportion of GSH in fixed cells, and observed that GSH interfered with the reaction. Notably, we tried to perform the Huisgen's Click reaction by thermal activation at 70°C during one day (Soo et al. 2003) without copper, but the labeling efficiency in fixated cells was weak (not shown) and the reaction was poorly selective because agarose or gelatin, which are generally considered as unreactive matrices, appeared to be fluorescent after 12 hours in the Click reaction mix. Overall this attractive labeling technique was inefficient for optical mapping in nanochannels.

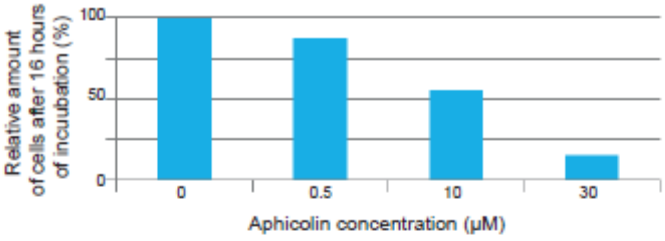
Supplementary Figure S1: Characterization of aphidicolin synchronization efficiency. (A) 700000 U2OS cells were seeded in 10 cm dishes and left in fresh medium during 24 hours. They were then exposed to a range of aphidicolin doses during 16 hours. The drug was rinsed, and fresh medium was supplemented with 10 µM BrdU during 15 min. Cells were harvested and living cells were counted with the TC10 cell counter (Biorad). **(B)** Cells were fixed in 70% ethanol, and BrdU was immunodetected with mouse anti-BrdU (Becton Dickinson) / anti-mouseAlexa-488 (Molecular probes). DNA was titrated with propidium iodide and cells were analyzed by flow cytometry (Fascalibur, BD Biosciences), as represented in the four graphs. **(C)** The graphs at the left correspond to the result of FACS analysis on untreated and dUTP-Cy3 treated cells (upper and lower plots, respectively). The plot in the right panel represents the fraction of fluorescent cells inferred from FACS.

Supplementary Figure S2: Calibration of DNA elongation, confocal microscopy of dUTP-Cy3 tracks, and analysis of SYTOX labeled chromosome fragments by DNA combing. (A) We prepared concatemers of λ-DNA using ligation reaction in the presence of PVP as crowding agent and elongated them in nanochannels as shown in the right panel. The length of 57 molecules smaller than 70 µm was measured and scored in one histogram (left panel) in order to estimate the stretching factor for a series of molecules of 50, 100, 150 kbp... The fit is obtained with a 4 species model each present in a different proportion and characterized by a Gaussian size distribution with an average length multiple set to 15 µm (the breadth is assumed to the same for each species). Note that the stretching factor of 15 µm/50 kbp~0.3 µm/kb is about 80% of the contour length of DNA labeled molecules of ~0.35 µm/kb. **(B)** The time series at the left shows confocal images of a chromosome fragment stained with YOYO-1 and presenting a single dUTP-Cy3 track during its uptake in one nanochannel. The time interval between two images is 350 ms. The graph in the right panel shows the intensity distribution along the contour indicated by the red arrow on the upper left image. Confocal microscopy was carried out with dual excitation of 458 and 561 nm lasers and fluorescence simultaneous collection in the 477-539 and 609-668 nm spectral windows. **(C)** The three fluorescence micrographs correspond to chromosomes fragments labeled with SYTOX Orange elongated on amino-coated glass coverslips by DNA combing. The spatial modulations of DNA fluorescence intensity are consistent with our observations in nanochannels. **(D)**Because our measurements are carried out "on the fly", we expect to be limited by the blurring associated to the passage of the molecule at a finite velocity of ~200 µm/s. Given that images are recorded during 15 ms, the blurring is expected to produce smears of ~3 µm. In order to validate this estimate, we selected one particular event in which a long molecule remained trapped in between two nanochannels in a U-shape (see the panel in the left). The trapped molecule exhibits a 3 µm feature in SYTOX concentration (indicated by the red triangle). This feature cannot be detected when the molecule travels, as shown by the fluorescence micrograph in the right which shows the same molecule traveling in the nanochannel after breakage in the U-shape region.

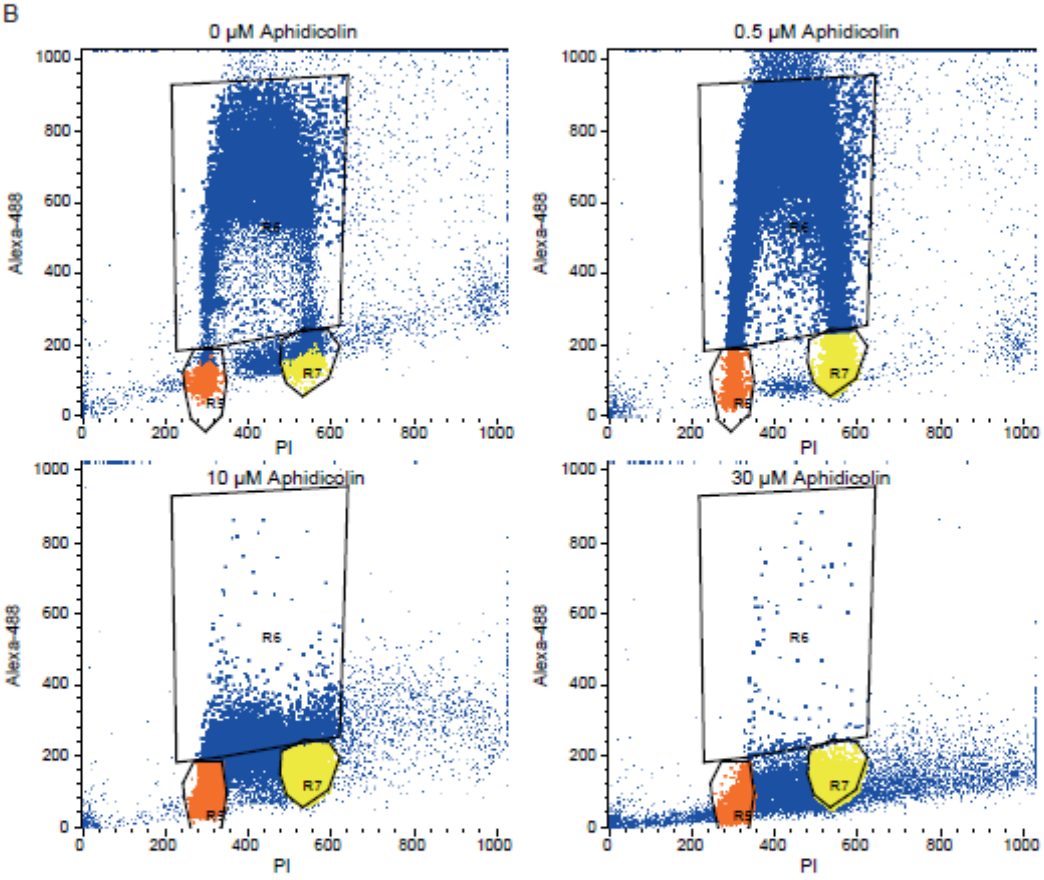
Supplementary Figure S3: Newly-synthesized DNA labeling with EdU. (A) Principle of the click reaction on EdU using azide Alexa-488 as label and Cu(I) as catalyst. **(B)** Gel electrophoresis of 0.5 µg λ-DNA in a click reaction mix composed of 100 mM de Tris-HCl (pH=7.5), various proportions of $CuSO_4$ as indicated in the legend, 10 or 100 µM azide and 100 mM ascorbic acid during 2 hour. The two images correspond to fluorescence micrographs of U2OS fixed cells after click reaction without $CuSO_4$

or with 100 µM $CuSO_4$. **(C)** The click reaction mix was supplemented with various proportion of Glutathione (GSH) during 1 hour, and the resulting DNA was electrophoresed (left panel). The same experiment was carried out during 2 hours in the right panel, showing that 10 mM GSH is required to preserve DNA. **(D)** The fluorescence micrographs from left to right correspond to U2OS fixed cells exposed to EdU and then placed in the Click reaction mix without GSH, with 1, 10, 20, and 40 mM GSH.

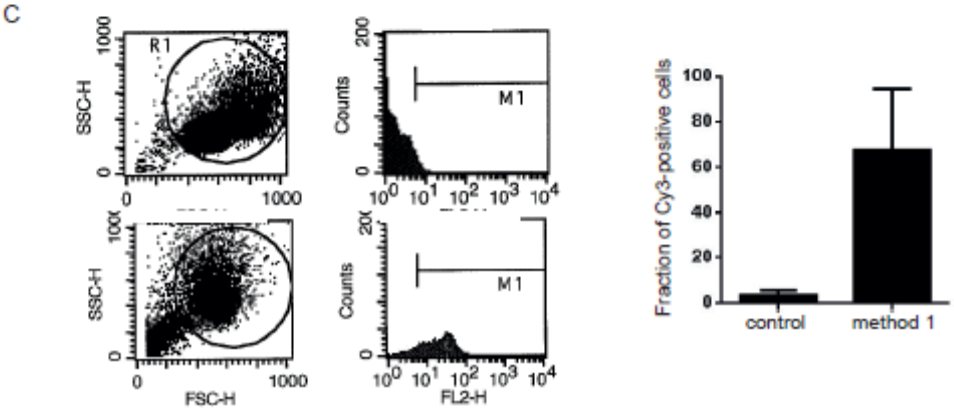
A


Relative amount of cells after 16 hours of incubation (%)
100
50
0
0
0.5
10
30
Aphicolin concentration (µM)

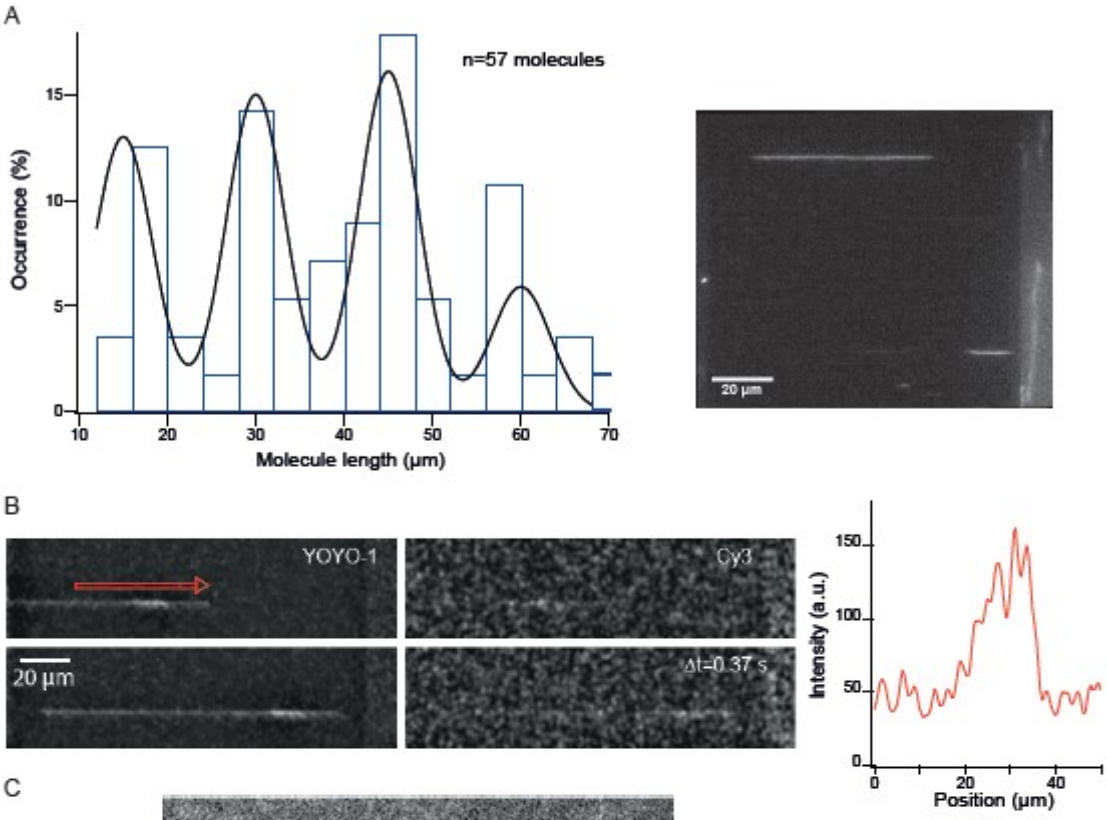
B


0 µM Aphidicolin
0.5 µM Aphidicolin
10 µM Aphidicolin
30 µM Aphidicolin
Alexa-488
PI
R6
R7
R8

C


R1
SSC-H
FSC-H
Counts
M1
FL2-H
Fraction of Cy3-positive cells
control
method 1

Supplementary Figure S2 Lacroix et al., 2016



Supplementary Figure S3 Lacroix et al., 2015