First single molecule microscopic visualization of the full-length human BRCA2 protein binding to DNA

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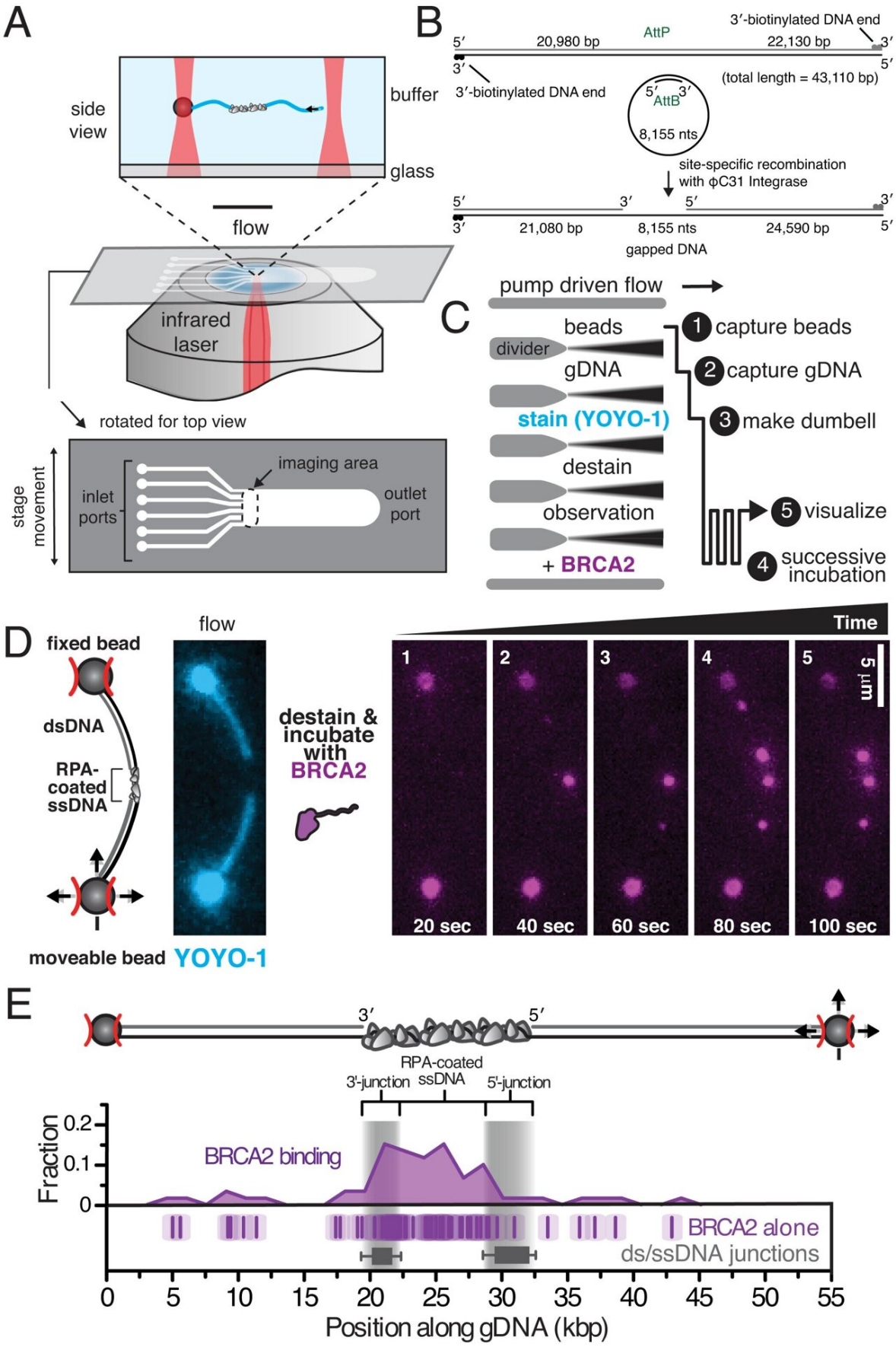
A
side view
buffer
glass
flow
infrared laser
rotated for top view
stage movement
inlet ports
imaging area
outlet port
B
3′-biotinylated DNA end
AttP
20,980 bp
22,130 bp
(total length = 43,110 bp)
AttB
8,155 nts
site-specific recombination with ϕC31 Integrase
21,080 bp
8,155 nts
24,590 bp
gapped DNA
C
pump driven flow
beads
divider
gDNA
stain (YOYO-1)
destain
observation
+ BRCA2
1 capture beads
2 capture gDNA
3 make dumbell
4 successive incubation
5 visualize
D
fixed bead
dsDNA
RPA-coated ssDNA
moveable bead
YOYO-1
flow
destain & incubate with BRCA2
Time
20 sec
40 sec
60 sec
80 sec
100 sec
5 μm
E
RPA-coated ssDNA
3′-junction
5′-junction
BRCA2 binding
Fraction
BRCA2 alone
ds/ssDNA junctions
Position along gDNA (kbp)

Direct imaging of BRCA2 binding to RPA-coated ssDNA on single molecules of gapped λ DNA. (A) Schematic of experimental approach combining fluorescence microscopy, a microfluidic flow cell, and optical trapping, as well as the micromanipulation used to capture and image BRCA2 on individual DNA molecules. (B) Illustration of the gapped λ DNA generated through in vitro recombination of circular ssDNA with an engineered λ DNA. (C) Schematic of experimental protocol: Each molecule of gapped λ DNA was captured and micromanipulated between two beads held in separately controllable optical traps. The molecule was moved between solutions in a six-channel flow cell and successively incubated in a solution containing BRCA2. (D) Cartoon and microscopic image of a single molecule of gapped λ DNA (Left, stained with YOYO-1, cyan) that was destained and then successively incubated with BRCA2 (5 nM) plus α-BRCA2 and α-IgGAF546. Montage shows BRCA2 (magenta) binding to the gapped λ DNA at increasing time intervals. (E) Cartoon representation of the gapped λ DNA between two beads (Top) and histogram (Middle) of binding positions of BRCA2 (number of foci, N = 60). Each data point is also plotted as a single tick (Bottom) where the semi-transparent box represents the SE associated with assigning position owing to the optical resolution of the microscope. Gray bars represent the 10 to 90th percentile range of the 5′- and 3′-termined junctions (N = 98). Credit: *Proceedings of the National Academy of Sciences* (2023). DOI: 10.1073/pnas.2221971120

Using a self-built inverted microscope complete with laser optical tweezers to capture DNA, Yale Cancer Center and University of California Davis researchers for the first time created a visualization of the full-length human BRCA2 protein at the single molecule level.

Mutations in the breast cancer susceptibility gene, BRCA2, can significantly increase an individual's lifetime risk of developing cancer. Approximately one in every 400 people carry a BRCA gene mutation accounting for a significant proportion of cancer that is heritable. The

study was published on March 28 in the *Proceedings of the National Academy of Sciences*.

"If you carry a BRCA mutation, you have this incredibly high risk for breast and ovarian cancer, and also for men, prostate and pancreatic cancer," said Yale Cancer Center member and co-author of the paper, Ryan Jensen, Ph.D., who is also an associate professor of therapeutic Radiology at Yale School of Medicine.

"It's critical that we understand at a molecular level when a mutation in BRCA2 is discovered in a patient. If the BRCA2 protein fails to do its job, why does that failure lead to cancer? My lab is completely invested in understanding the BRCA2 protein in all its facets."

The BRCA2 gene provides instructions for making a protein that acts as a tumor suppressor. Tumor suppressor proteins help prevent cells from growing and dividing too rapidly or in an uncontrolled way. Dr. Jensen said, "This experiment was designed to better understand how the BRCA2 protein binds and interacts with DNA, one molecule at a time. This 'visual biochemistry' approach is akin to sitting back in a theater and watching a movie of how proteins bind and interact with DNA in real time."

"BRCA2 is a DNA repair protein, so it repairs damage to our DNA. Specifically, double-strand breaks in the DNA, can be repaired by a few different pathways in human cells. BRCA2 in particular works in the homologous recombination pathway," said Dr. Jensen.

"By figuring out how BRCA2 works at the molecular level, that's going to give us more information to generate new strategies that could one day help BRCA mutation carriers who either don't respond or who relapse on current standard-of-care therapies. The more knowledge we have, the better," said Dr. Jensen.

More information: Jason C. Bell et al, BRCA2 chaperones RAD51 to single molecules of RPA-coated ssDNA, *Proceedings of the National Academy of Sciences* (2023). DOI: 10.1073/pnas.2221971120