Binding Specificity of the Single Stranded DNA Binding Protein to the Target

Luda S. Shlyakhtenko, Alexander Y. Lushnikov, Atsushi Miyagi and Yuri L. Lyubchenko*

University of Nebraska Medical Center, 986025 Nebraska Medical Center, Omaha, NE 68198-6025

Supporting information



Purified restriction fragment with sticky end

Synthetic oligonucleotide annealed with adapter

Ligation of restriction fragment (1) with synthetic oligonucleotide annealed with adapter (2) Separation and purification of product (3) from agarose gel

Annealing of 1:1 mixture of 3' and 5' tail-DNA with bridge oligos Ligation of 3' and 5' tail-DNA

Separation of the bridge oligos from the gap-DNA in agarose gel

Supplement Fig.S1.A-Scheme for preparation tail-DNA. B- Scheme for preparation of gap-DNA.

Tail-DNA

5'-69ss-611bpDNA

94nt:

5'-AAA GAG AAA GTG AAA CCC AAA GAA TGA AAA CCC AAA TGT TAG AAT TGT TAA TGT GTG TGA TGA TGT TGA CAT ACG TGT AGG AAT TAT ATT CAT G-3' **21nt –adapter:** 5'-AAT ATA ATT CCT ACA CGT ATG-3'

Mixed tails-DNAs

5'-42ss-441bpDNA and 3'-42ss-231bpDNA

5'-42ss- 441bp-DNA

61nt: 5'- ATG AAA ACC CAA ATG TTA GAA TTG TTA AGT GAA ACC CAA AGA ATG TGT GTG ATG ATG TTG A -3' **23nt- adapter:** 5'- AGC TTC AAC ATC ATC ACA CAC AT -3'

3'-42ss-231bp-DNA

63nt: 5'- CCA GCA TAC GTG TAG GAA TTA AGT GAA ACC CAA AGA TAT TAA AGA GAA AGT GAA ACC CAA AGA -3' 25nt- adapter: 5'-TAA TTC CTA CAC GTA TGC TGG CAT G -3'

69ss gap-DNA Mixed 3'-27ss-231bpDNA and 5'-42ss-441bpDNA 3'-27ss-231bpDNA 48nt: 5'- CCA GCA TAC GTG TAG GAA TTA TAT TAA AGA GAA AGT GAA ACC CAA AGA -3' 25nt –adapter: 5'-TAA TTC CTA CAC GTA TGC TGG CAT G -3'

5'-42ss- 441bp-DNA 61nt: 5'- ATG AAA ACC CAA ATG TTA GAA TTG TTA AGT GAA ACC CAA AGA ATG TGT GTG ATG ATG TTG A -3' 23nt- adapter: 5'- AGC TTC AAC ATC ATC ACA CAC AT -3' bridge 5'- TGG GTT TTC ATT CTT TGG GTT T -3'





Zmin: -0.4028 r Rmax: 3.152 n Rq: 0.3296 nm Ra: 0.1522 nm Rsk: 5.067 Rku: 32.42

max: 3.714 r. q: 0.3507 nm a: 0.203 nm sk: 3.822

0.04 0.08 0.12 0.16 0.2 File: ssbi-69-4-11-11.121 Image data: Height





0.04 0.08 0.12 0.16 0.2 File: ssbi-69-5-9-11.39z Image data: Height





File: Copy 6 of ssbi-69-4-11-11.003 Image data: Height



Supplement, Fig. S2

Gallery of selected AFM images of SSB-69-tail-DNA complexes at low salt conditions: Images marked as (A)- are SSB clusters, (B) non specific end bound, (C)-middle bound. Images marked as (a), (b) and (c)- are the same images as (A), (B) and (C) with traces of DNA lengths. Bar size is 50 nm.



Low salt conditions- complex SSB-69ssDNA : End bound-20% Middle bound-11% Clusters-13%



High salt conditions- complex SSB-69ssDNA: End bound-7% Middle bound-2% Clusters-2%

Supplement Fig. S3 illustrates the yield of SSB-69ss-DNA complexes in low (A) and high (B) salt conditions. Yellow: end - bound specific complexes, dark blue: non specific end bound complexes, light blue: middle bound complexes and pink: clusters.

Α



Supplement Fig. S4 illustrates the yield of SSB-27ss-DNA complexes in low (A) and high (B) salt conditions. Yellow: end - bound specific complexes, dark blue: non specific end bound complexes, light blue: middle bound complexes and Pink: clusters.



Supplement, Fig S5. The distribution of volumes for the end-bound SSB protein complexes with 27-tail-DNA at different salt conditions: low salt (A), high salt (B).



Supplement, Fig. S6

Gallery of selected AFM images for complexes of SSB protein with 796 bp DNA fragment in low salt. Bar size 100nm.

SSB complex with 769bp PCR fragment in low salt (A) and in low salt with Mg (B).



Supplement, Fig. S7. SSB complex with 769bp DNA duplex in low salt (A) and in low salt with Mg (B). Bar size 200nm.





Movie 1 of SSB- gap DNA complex in standard conditions. Movie -0478asd



• Movie 3 of SSB-tail-DNA complex in standard conditions.



Movie 3. Association- dissociation