Direct visualization of interactions the transcription factors for drug design

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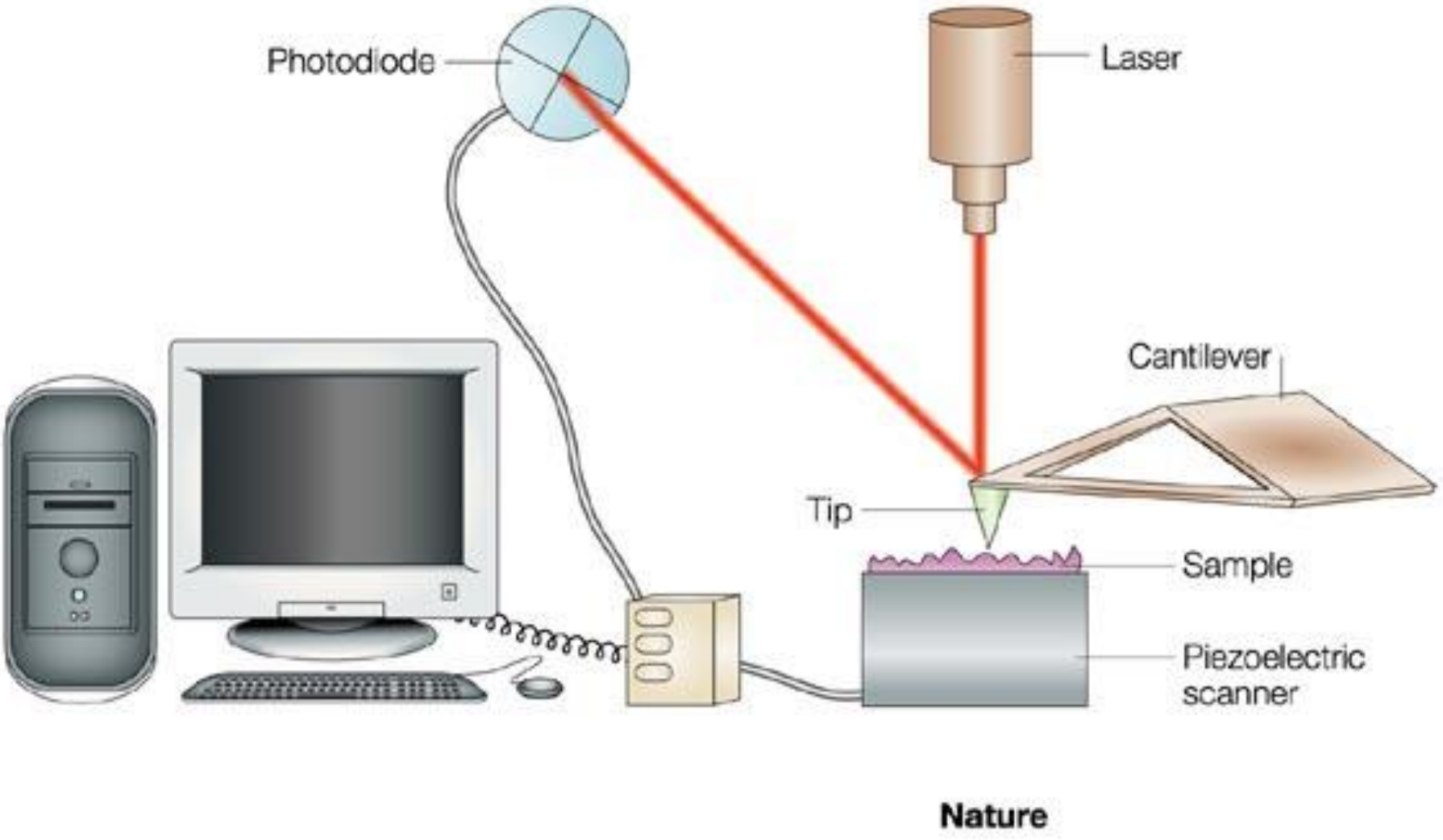
Atomic Force Microscopy



c-myc promoter

Background

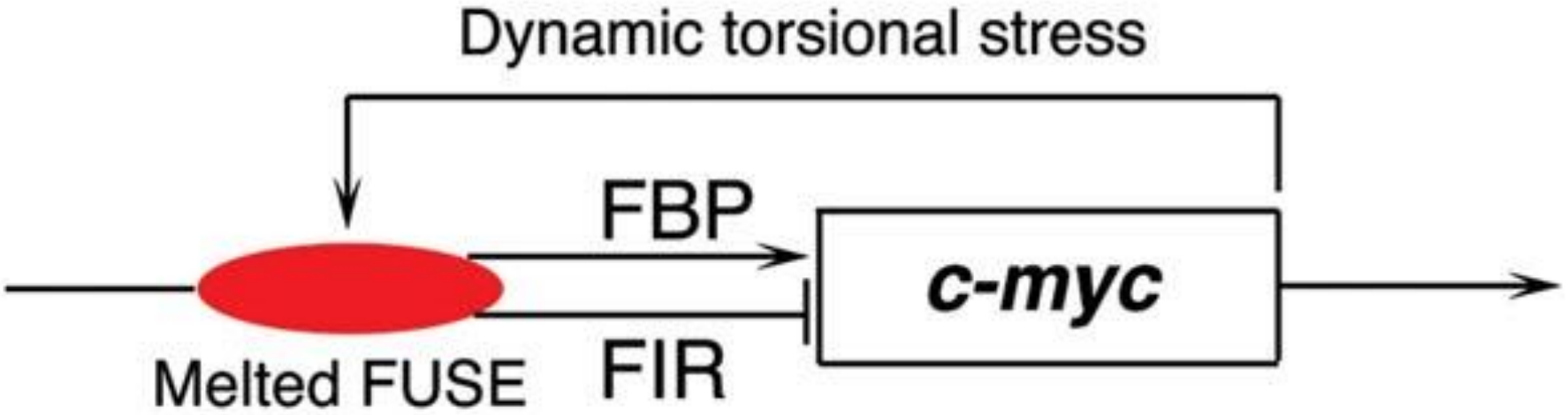
Transcriptional factor MYC binds to the promoters or regulates the expression of large number of genes controlling the metabolic processes, the macromolecular synthesis, the cell cycle and the apoptosis.

The c-myc promoter has architectural features such as the supercoiled-deformable **Far UpStream Element (FUSE)** as physical sensor of ongoing transcriptional activity, and the FUSE binding protein (FBP) as well as the FBP interaction repressor (FIR) as effectors to enforce normal transcription from the c-myc promoter.

FUSE element (100 bp) located 1.7 kb from the start of *c-myc* transcription

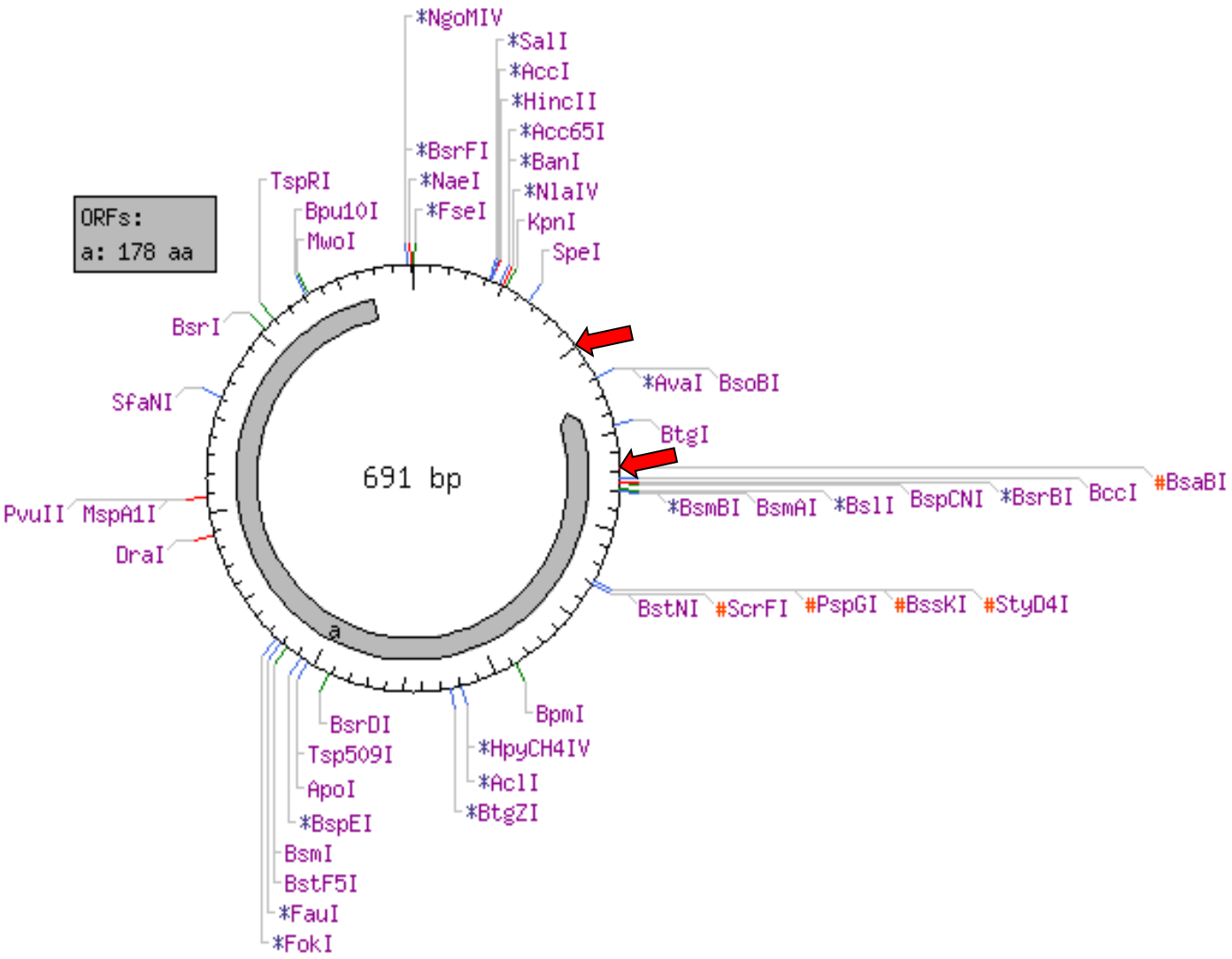
```
ACTAGAACTA GTGGATCTGC TCCCTGAAAT GATCTATATT TAATATATAA TGTATATTCC
CTCGGGATTT TTTATTTTGT GTTATTCCAC GGCATGAAAA ACAAGATCCC
```

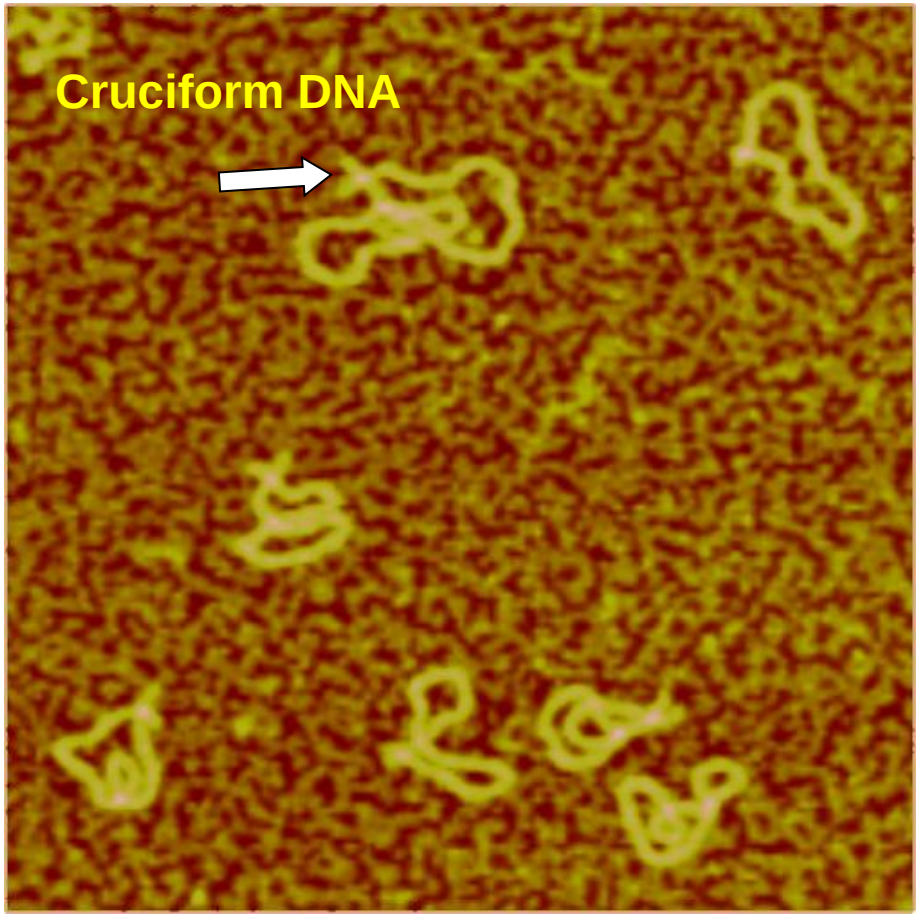
FUSE deformation authorizes binding of FBP (sequence-specific, single-strand DNA binding protein) and FIR (next) which control gene expression in real-time.

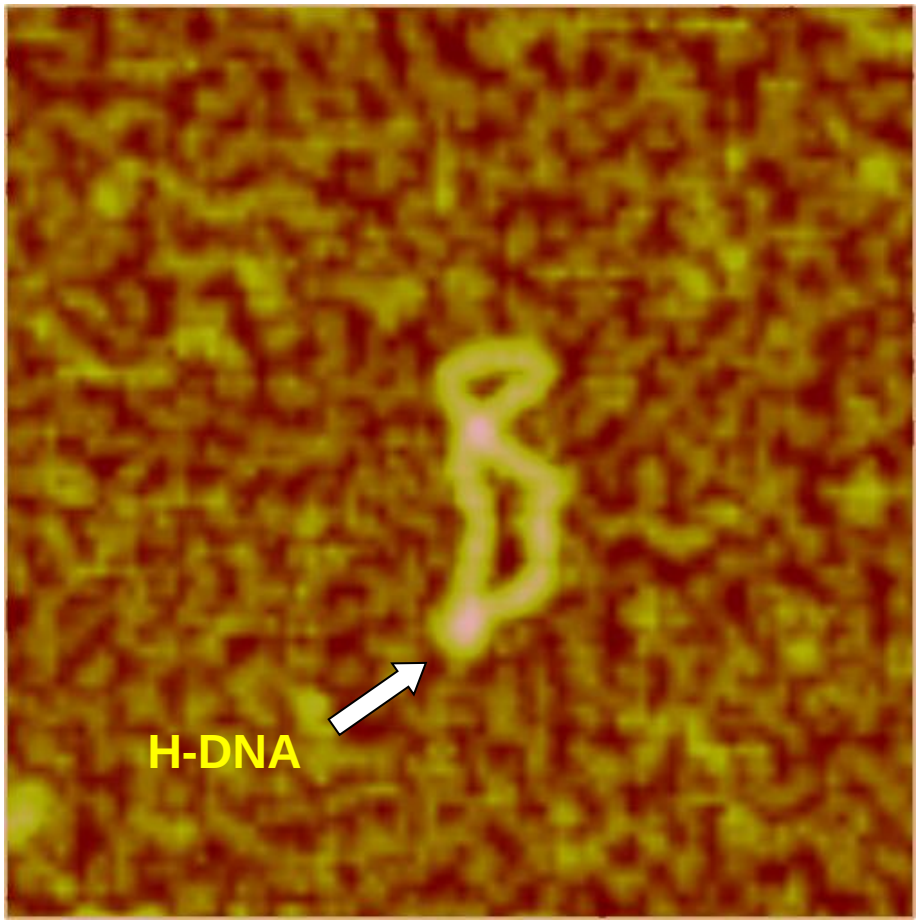

Dynamic torsional stress
FBP
c-myc
FIR
Melted FUSE

Questions

- What did the FUSE structure within DNA of mini-circles define the levels of supercoiling?
- What kinds of structure (melted DNA, cruciforms, H-DNA, etc) are targets for proteins binding?
- Do the FBP and FIR bind to FUSE separately, or together?

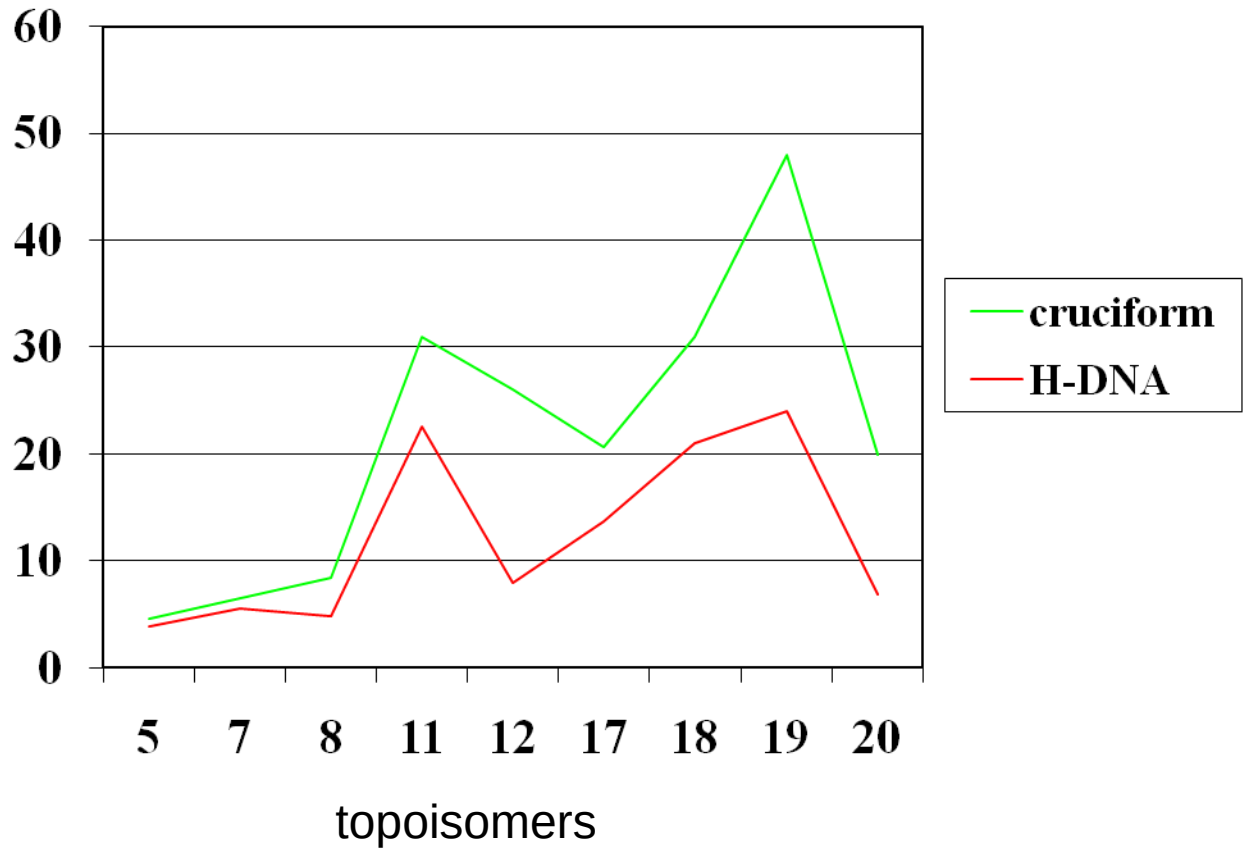

ORFs:
a: 178 aa
691 bp
a
*NgoMIV
*SalI
*AccI
*HincII
*Acc65I
*BanI
*NlaIV
KpnI
SpeI
*BsrFI
*NaeI
*FseI
TspRI
Bpu10I
MwoI
BsrI
SfaNI
PvuII
MspA1I
DraI
*AvaI
BsoBI
BtgI
#BsaBI
*BsmBI
BsmAI
*BsaI
BspCNI
*BsrBI
BccI
BstNI
#ScrFI
#PspGI
#BsskI
#StyD4I
BpmI
*HpyCH4IV
*AclI
*BtgZI
BsrDI
Tsp509I
ApoI
*BspEI
BsmI
BstF5I
*FauI
*FokI



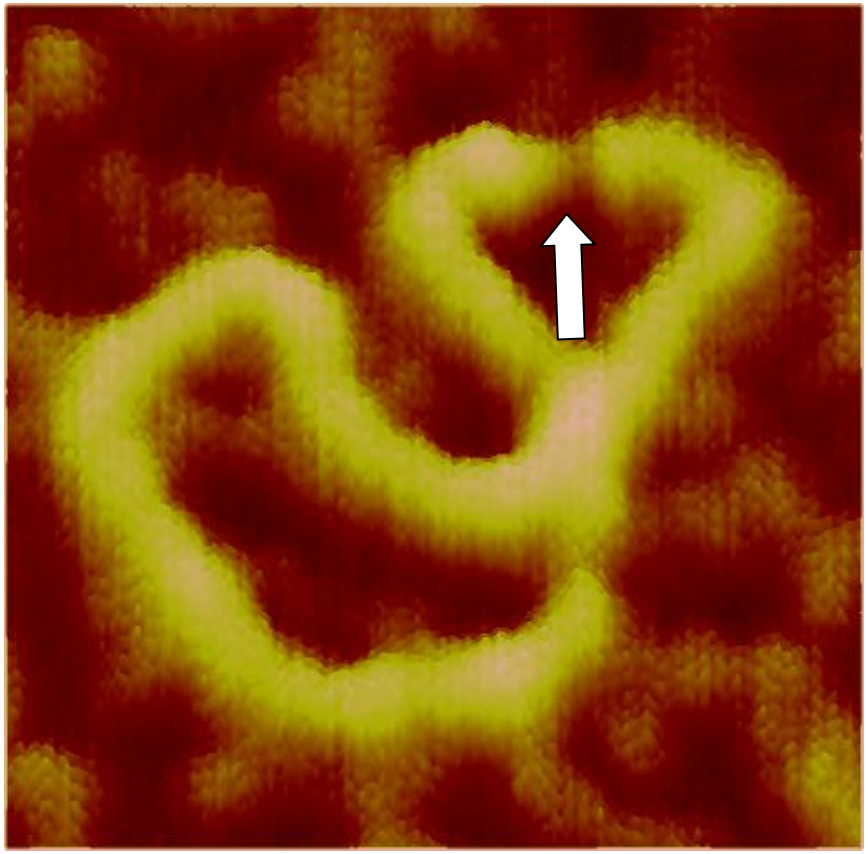


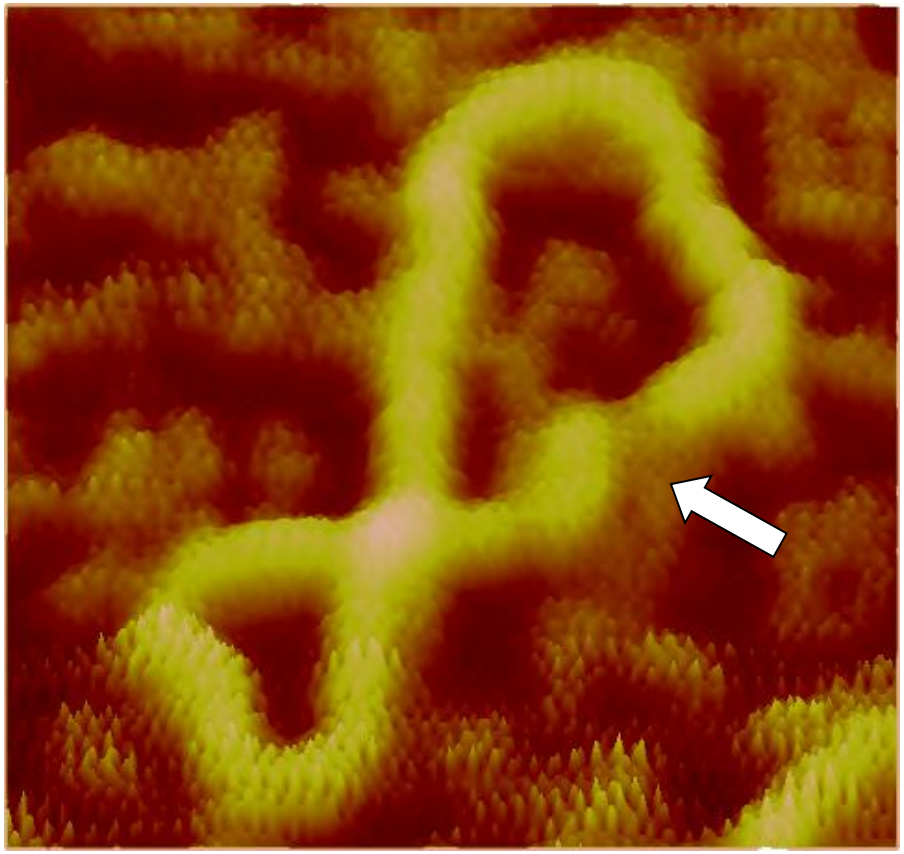
Height H-DND-1.2nm; B-DNA- 0.6nm

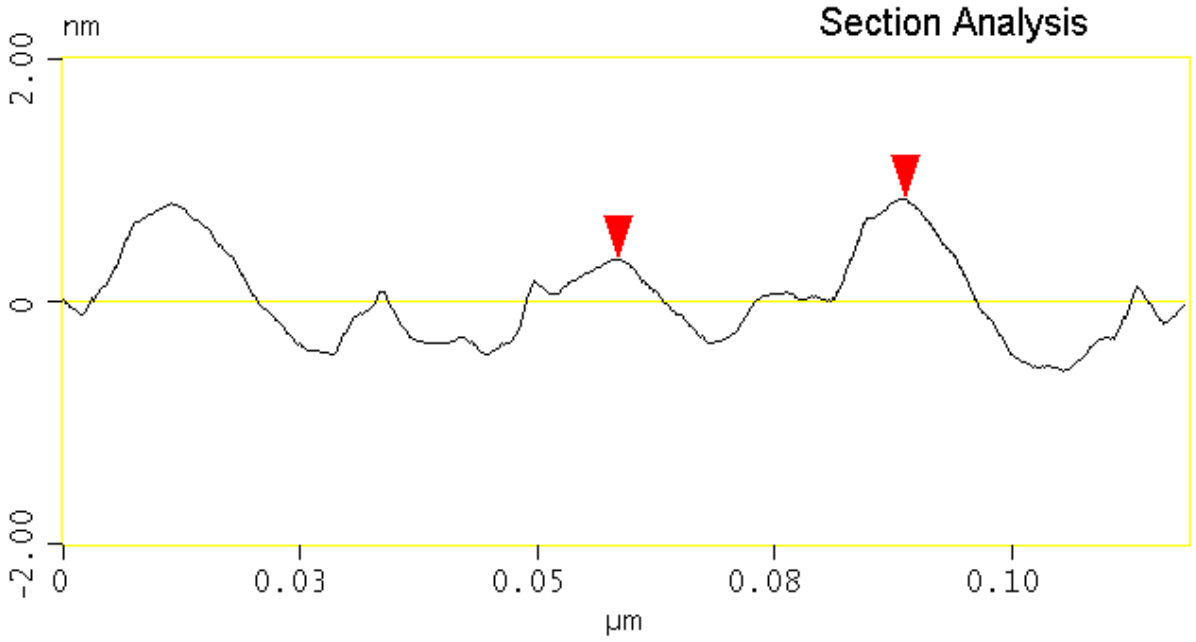
Percentage of the distribution of cruciform and H-DNA structures in the FUSE supercoiled mini-circles topoisomers

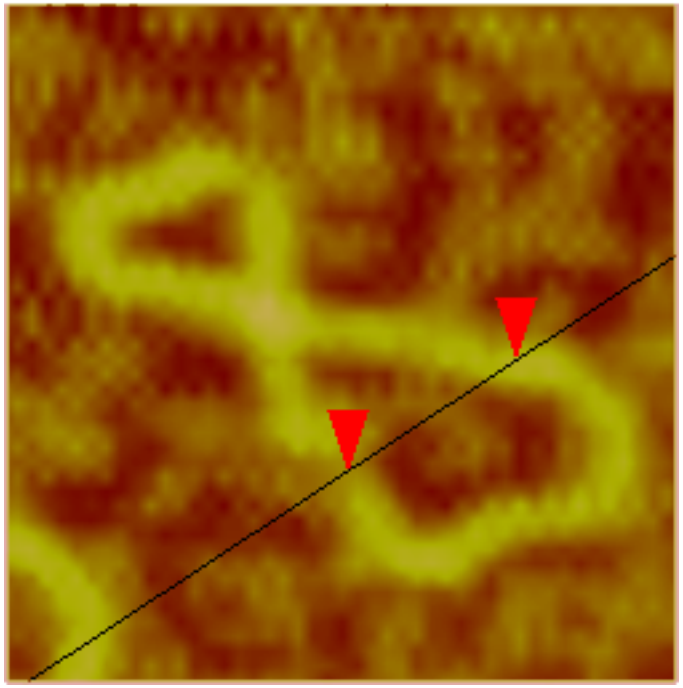


melted DNA structures









Length unpaired structure ≈ 30bp

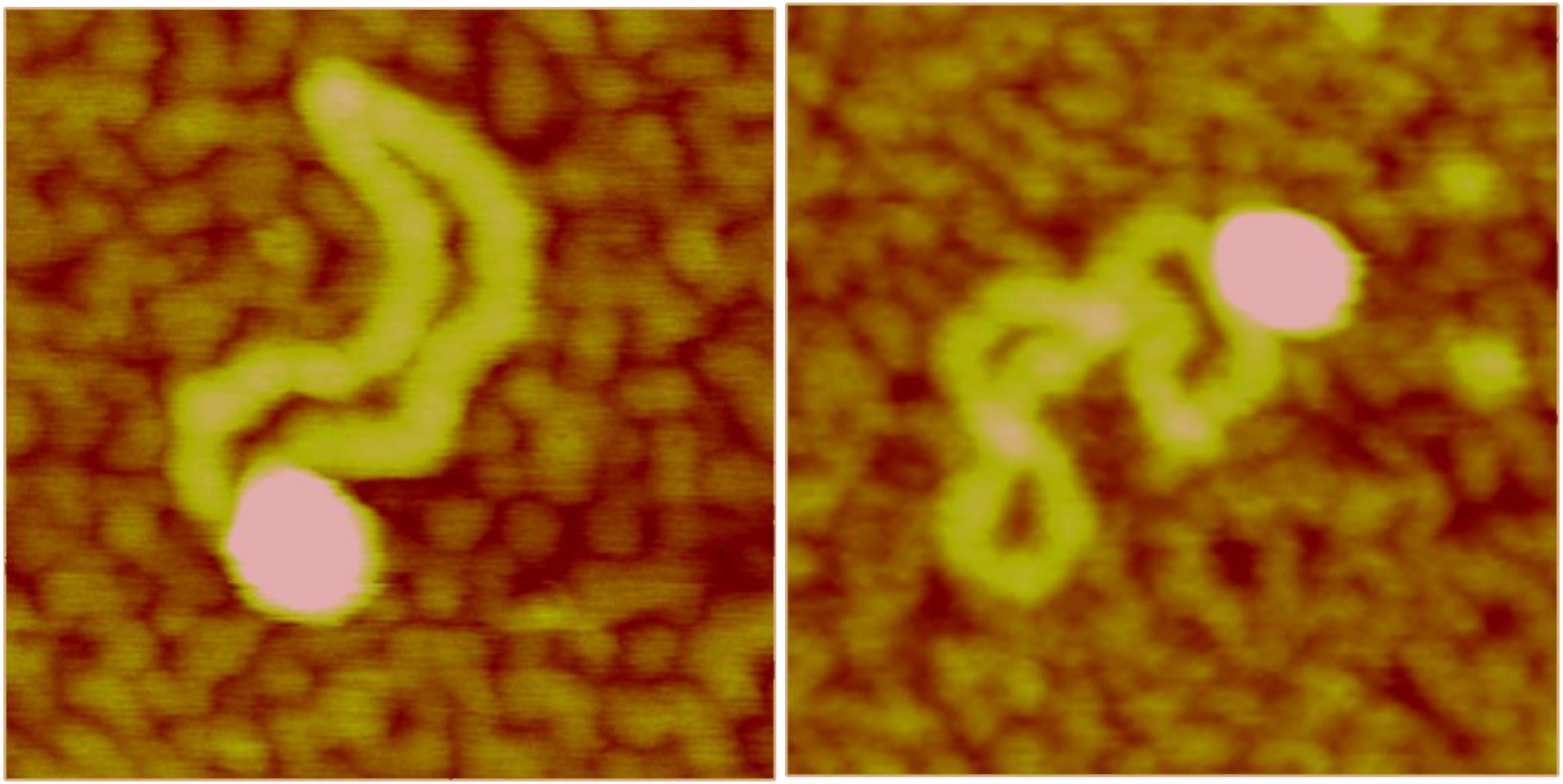
Surface distance	30.465 nm
Horiz distance(L)	30.292 nm
Vert distance	0.502 nm
Angle	0.949 °

Molecular structure FUSE binding protein (FBP)

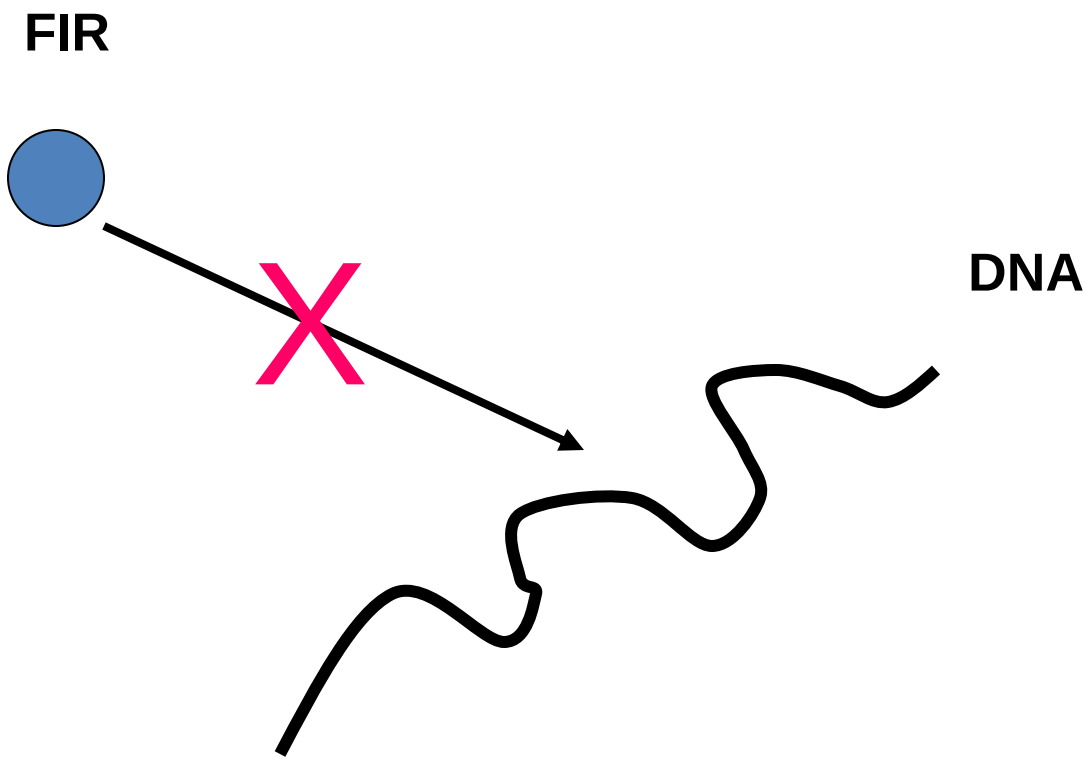
KH domain

FBP binds to FUSE in supercoiled but not linear DNA

FUSE binding protein (FBP)



FBP interaction repressor (FIR) protein



FBP interaction repressor (FIR) protein not direct binds to DNA

Subconclusion

- FUSE predominantly adopted in cruciform and H-DNA structures in mini-circles topoisomers

- FBP binds to cruciform and H-DNA structures in FUSE region

- FIR not directly interact with DNA

STAT3 protein

Signal Transducer and Activator of Transcription (STAT), is a group of latent transcription factors which have the ability to transduce signals from the cell membrane to the nucleus to activate gene transcription.

The activation of STAT3 is observed in many human tumors, including various carcinomas, lymphomas, leukemias and melanomas.

Upon receptor activation, STAT3 is phosphorylated. Once phosphorylated, STATs form homo- or heterodimers, translocate into the nucleus, and activate gene transcription.

Unphosphorylated STATs have been reported to drive gene transcription independent of phosphorylation, possibly by indirect binding to DNA as transcriptional co-regulators or perhaps by direct interaction with half-site GAS or unidentified DNA structures.

The activity of STAT proteins is negatively regulated by a variety of different mechanisms:

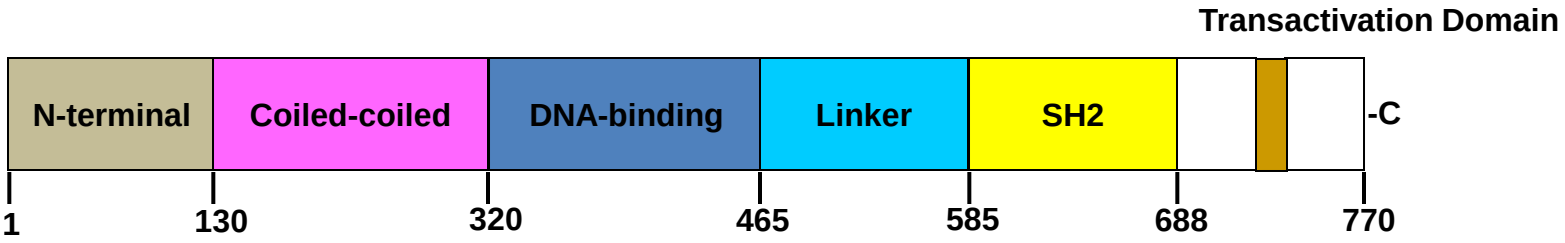
-alternative splicing,

-covalent modifications,

-protein–protein interactions with negative regulatory proteins,

-proteolytic processing by proteases.

Molecular structure of STAT3α protein (88 kDa)



The *N-domain* is involved in dimerization and tetramerization, and protein–protein interaction.

The *coiled-coil domain* is an interacting domain with other proteins including IRF-9/p48 for STAT1, c-Jun, StIP1, and GRIM-19 for STAT3, and SMRT with STAT5A and STAT5B, and for receptor
binding.

Proteolytic processing:

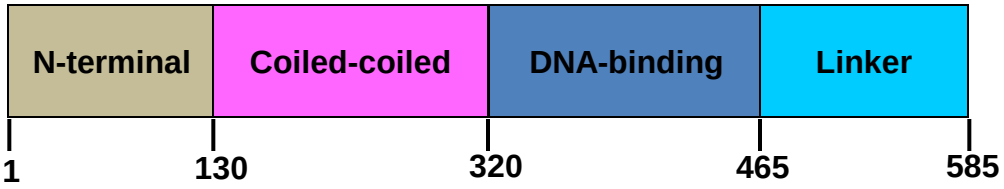
Cleavage of STAT proteins by serine proteases, caspases and calpain, results in the generation of C-terminally truncated isoforms.

Stat3 undergoes proteolytic processing by caspases. This processing is associated with a reduction in Stat3 expression, the formation of discrete cleavage fragments, and alternations in Stat3 transcriptional activity.

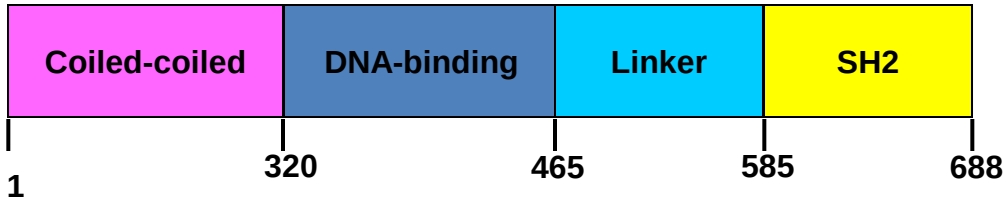
Caspase-mediated processing may represent another mechanism to modulate Stat3 signaling under apoptotic and non-apoptotic conditions.

STAT isoforms have been identified for Stat3, Stat5a, Stat5b and Stat6 in different cellular contexts and biological processes.

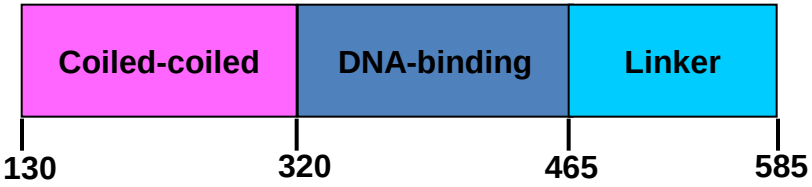
Proteolytic isoforms of STAT3



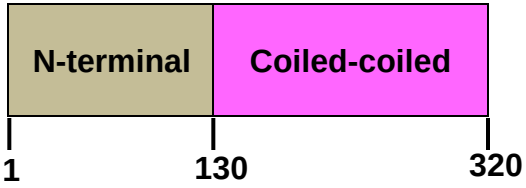
67.5 kDa



63.9 kDa

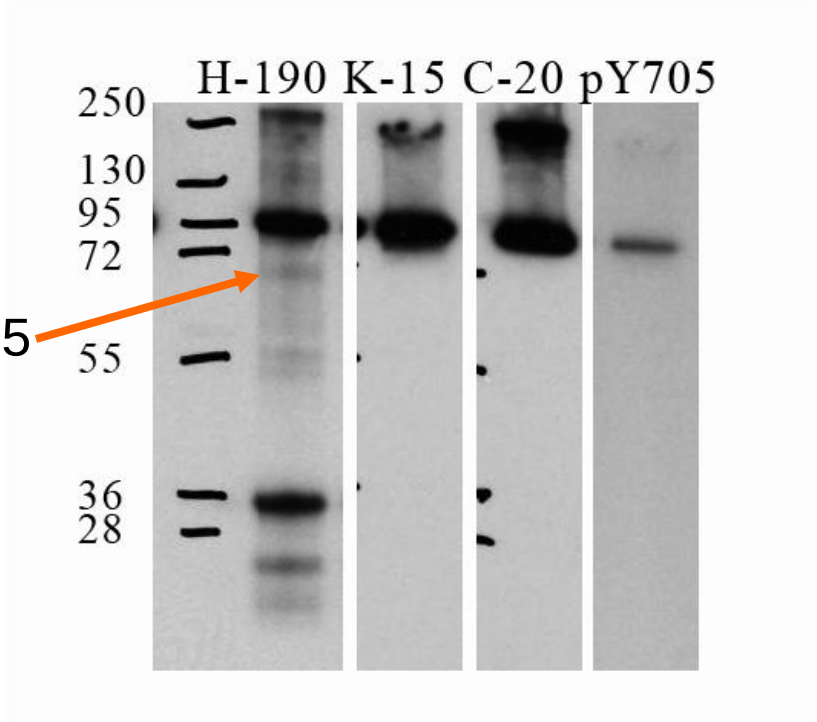


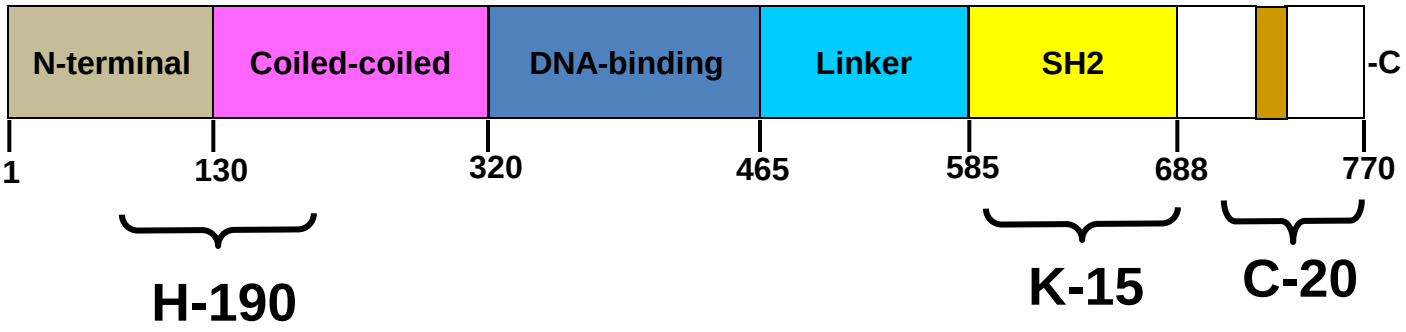
52.4 kDa



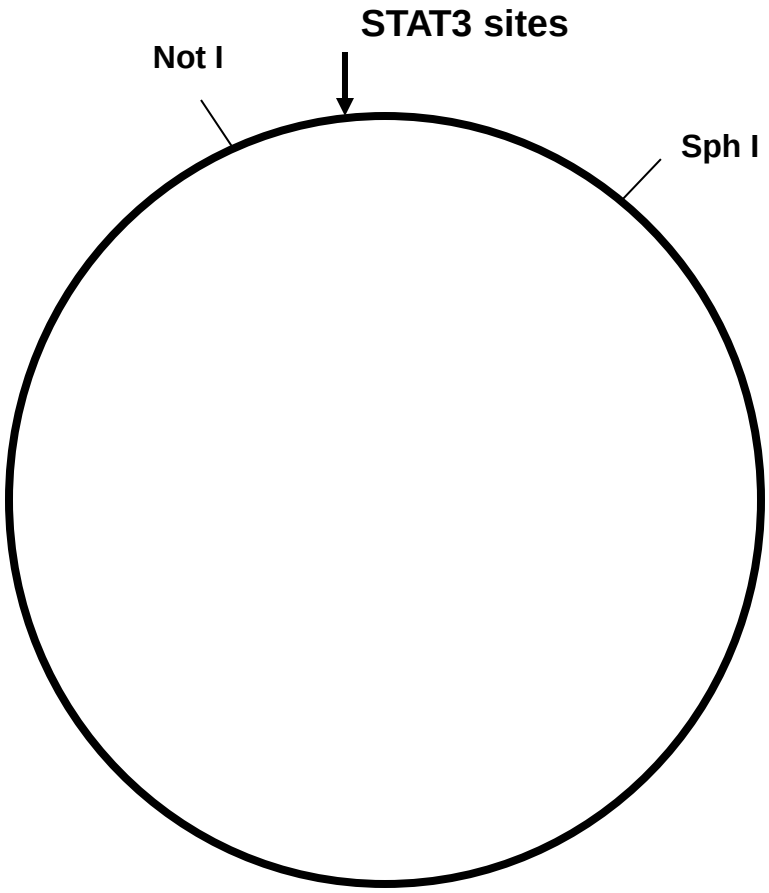
30.4 kDa

Recombinant STAT3a protein (88 kDa)





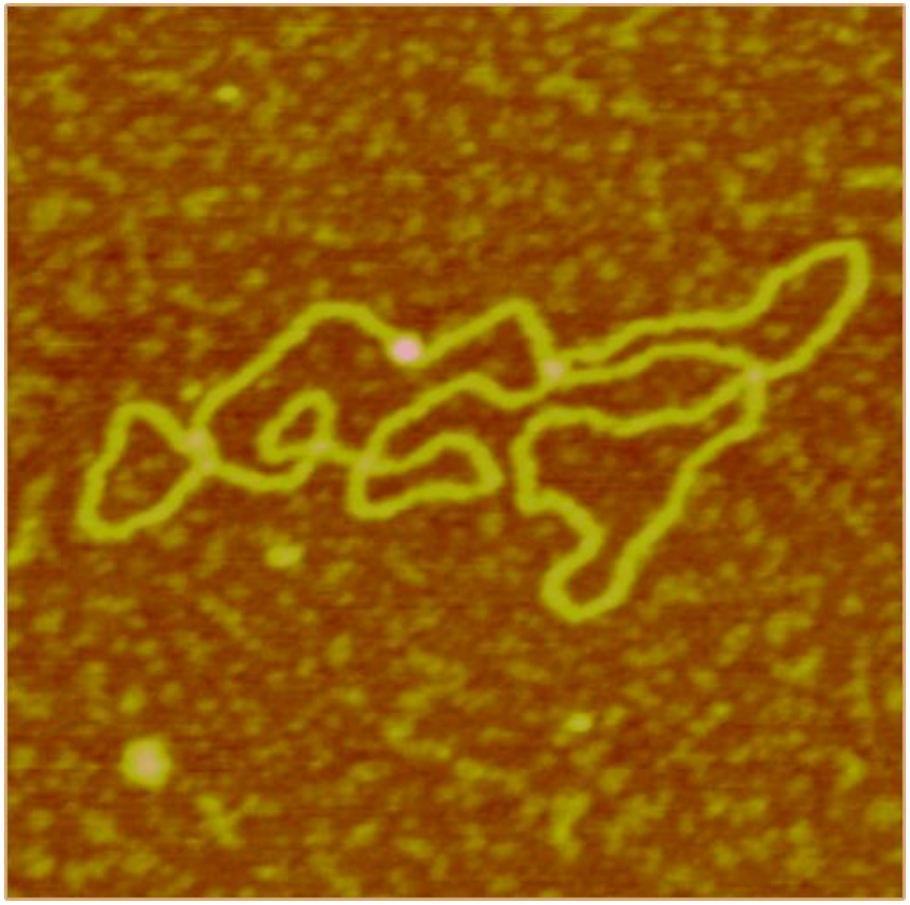
pGAS-TA-Luc Vector - 4,826 kb



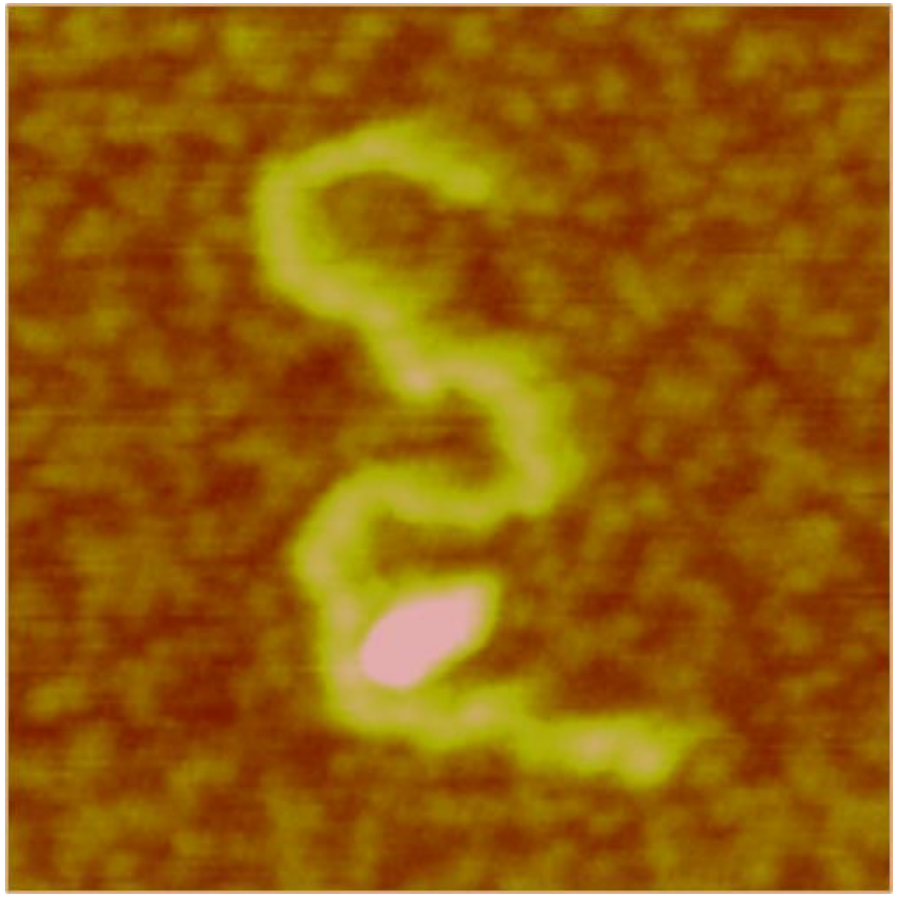
STAT3 binding sites (GAS element):

GGTTTCCGGGAAAGCAGTAGGTTTCCGGGAAAGCA

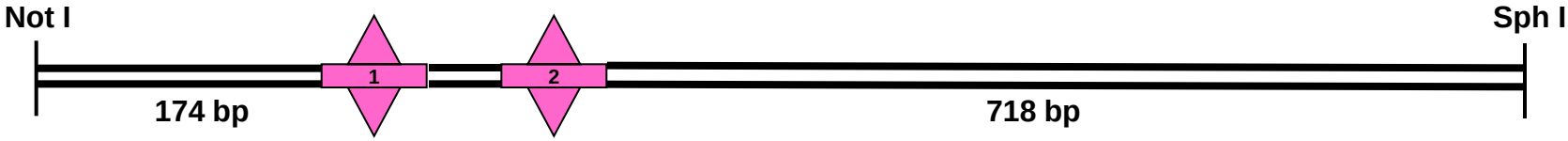
Monomeric 88 kDa STAT3-DNA complex



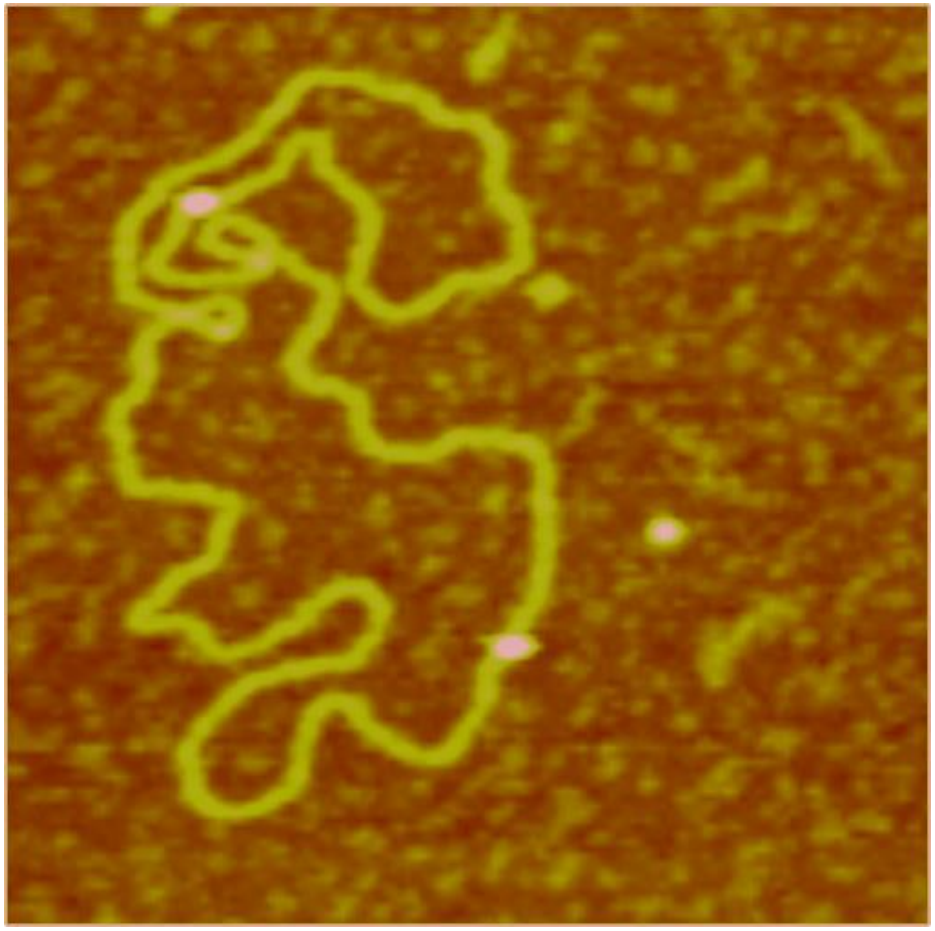
88 kDa STAT3 dimer on the GAS element



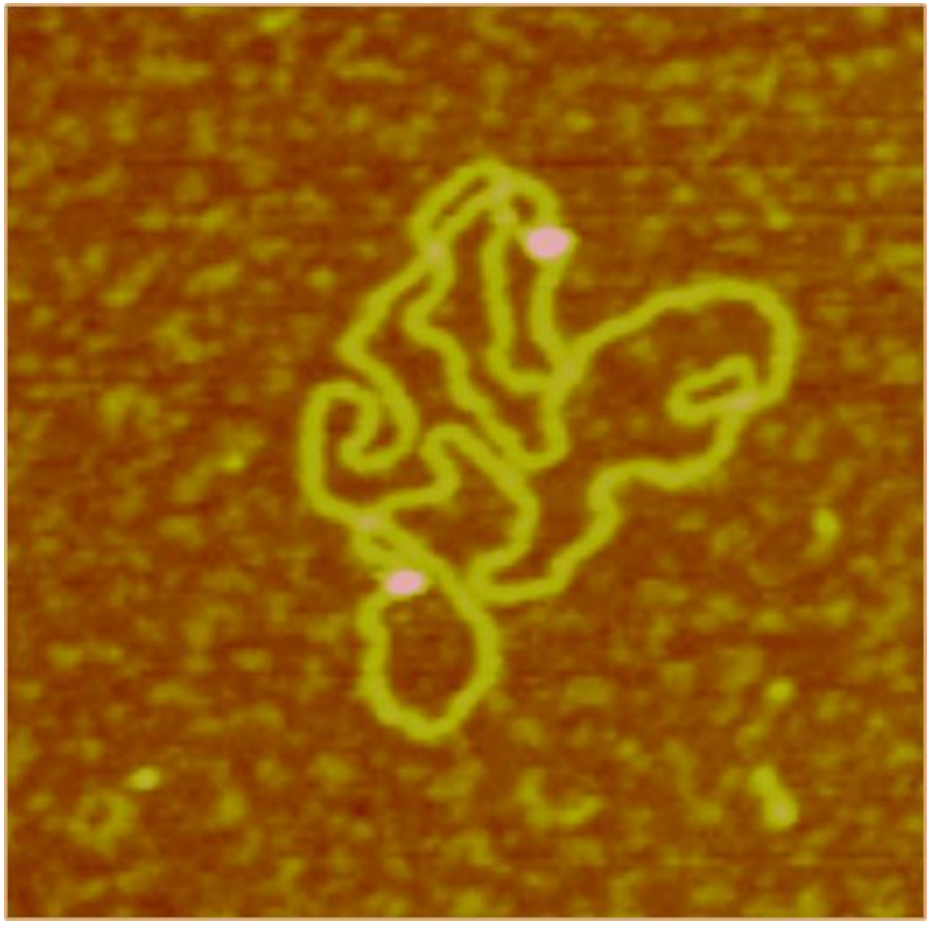
GAS element



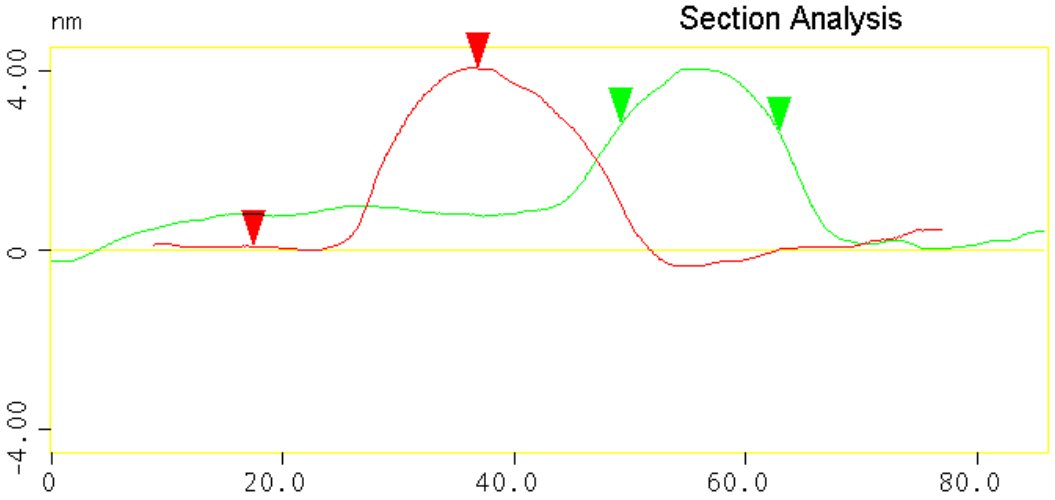
Monomeric 88 kDa STAT3-DNA complexs

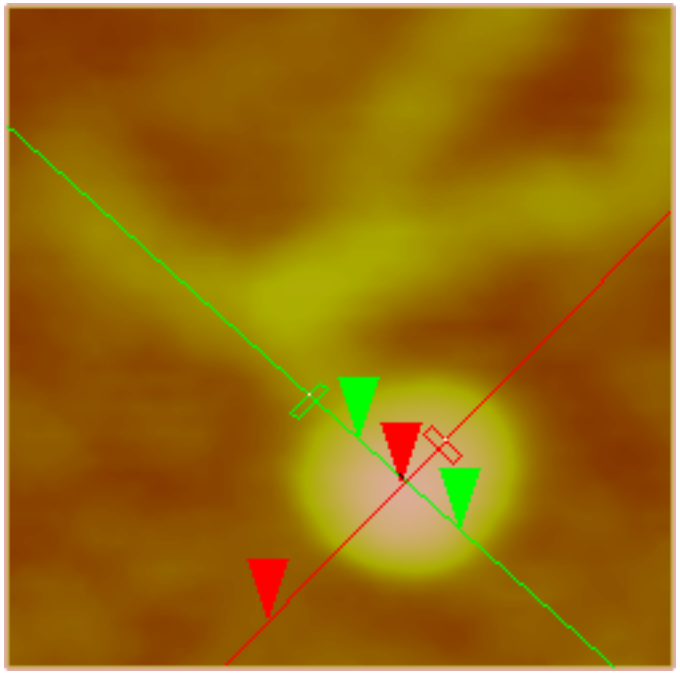


Dimeric 88 kDa STAT3-DNA complexs



Molecular STAT3 protein volume analysis



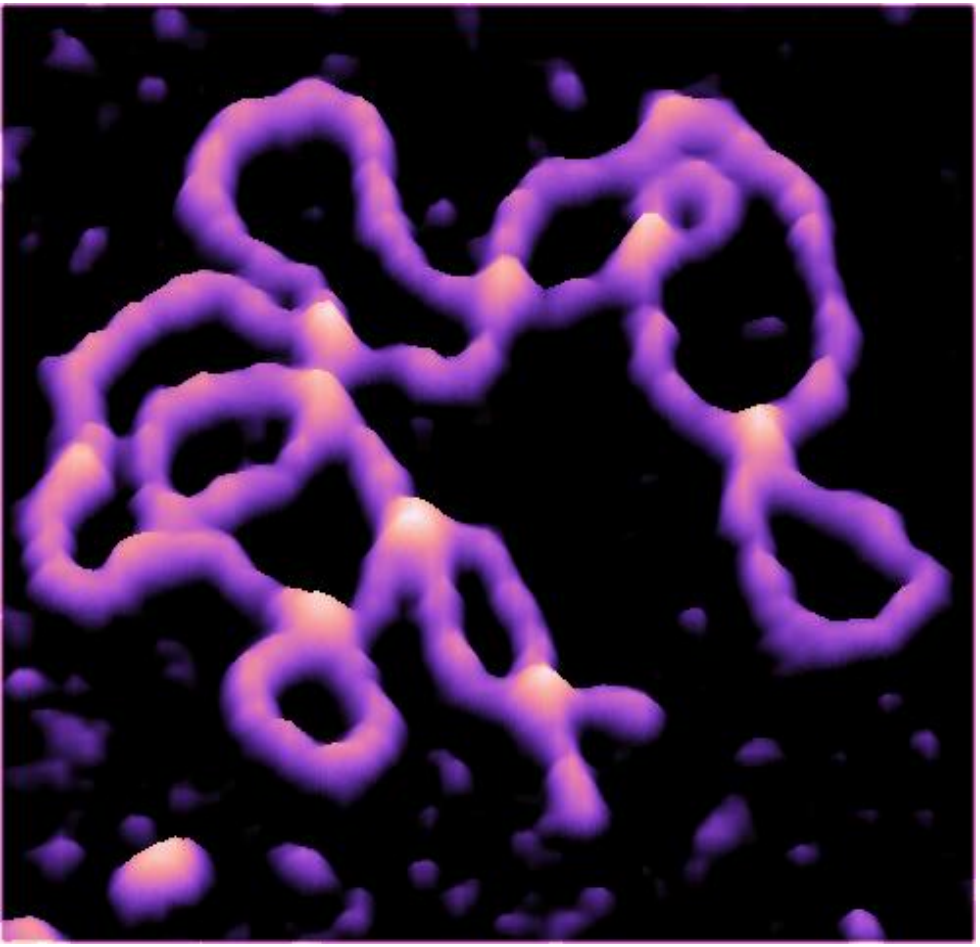


Vert distance 3.977 nm

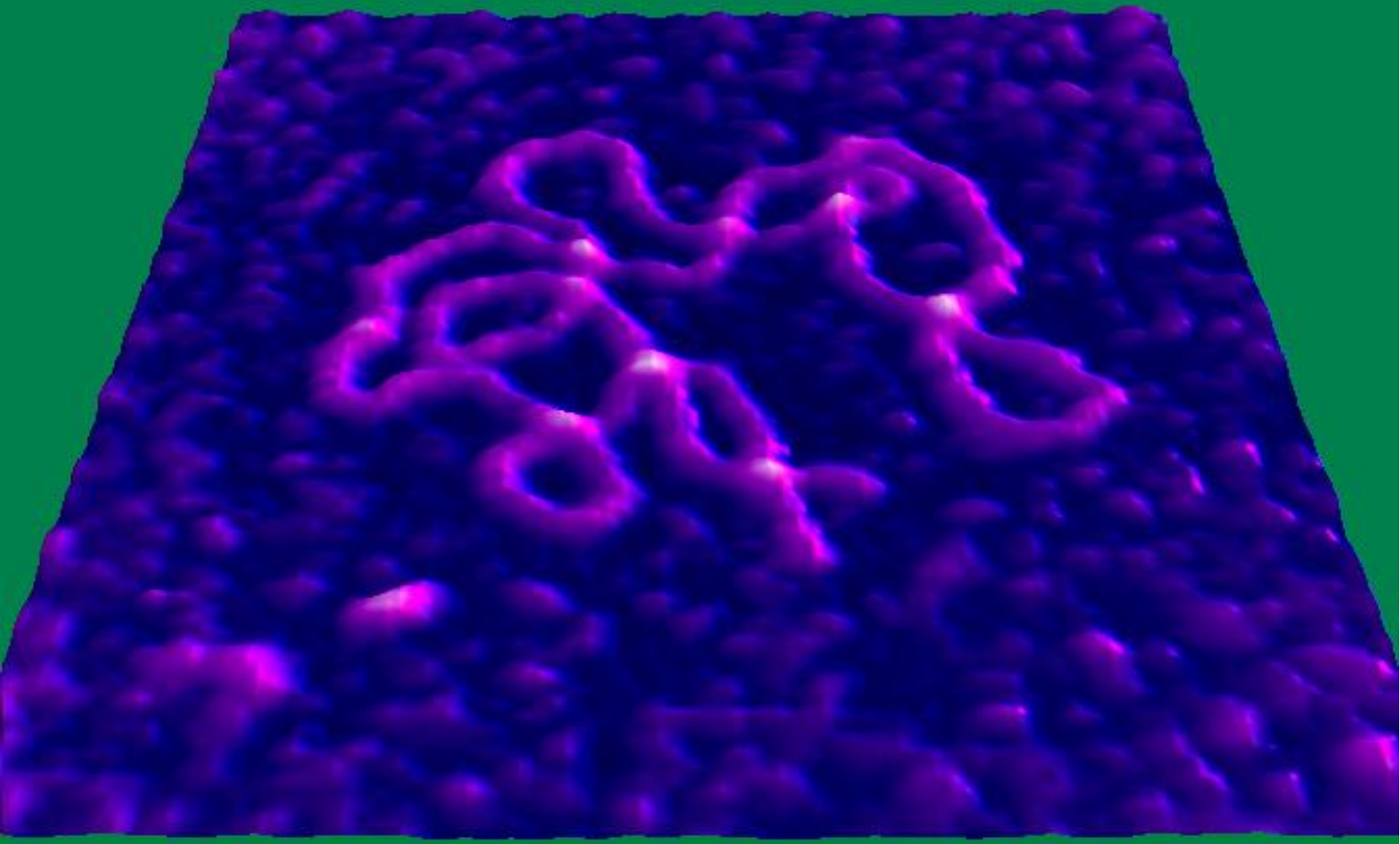
Horiz distance 13.698 nm

$$V = h\pi/6(3ab/4 + h^2)$$

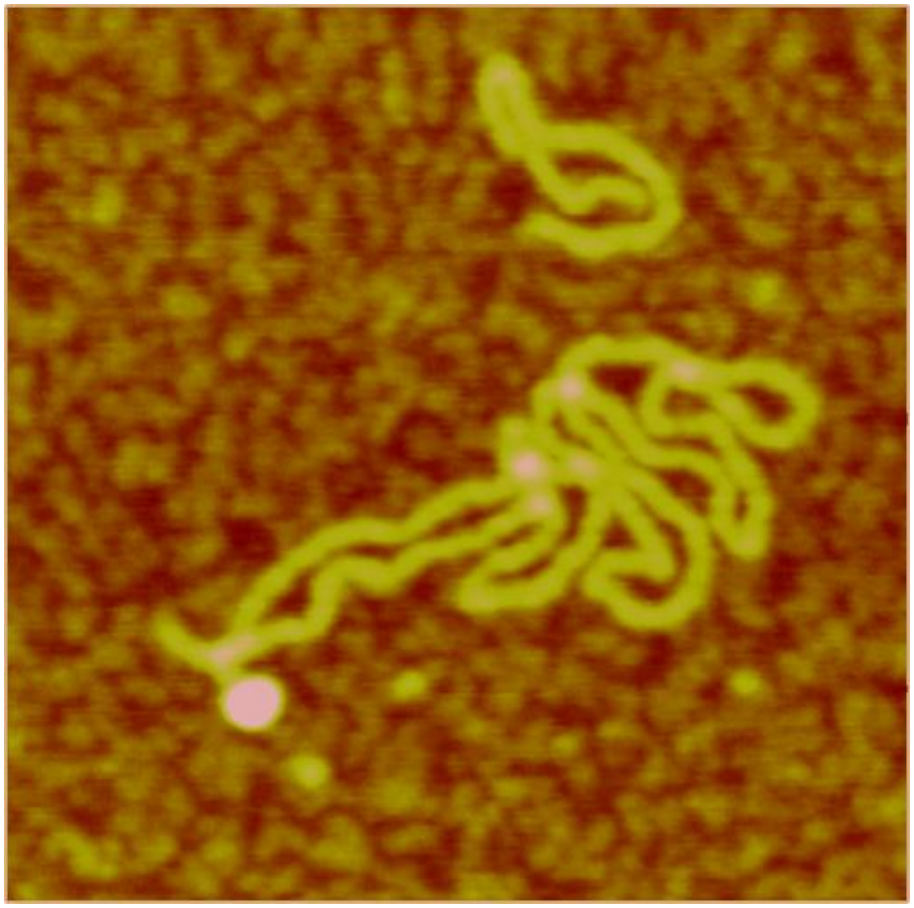
Full STAT3 and protein fragments binding to supercoiled DNA with cruciform structure

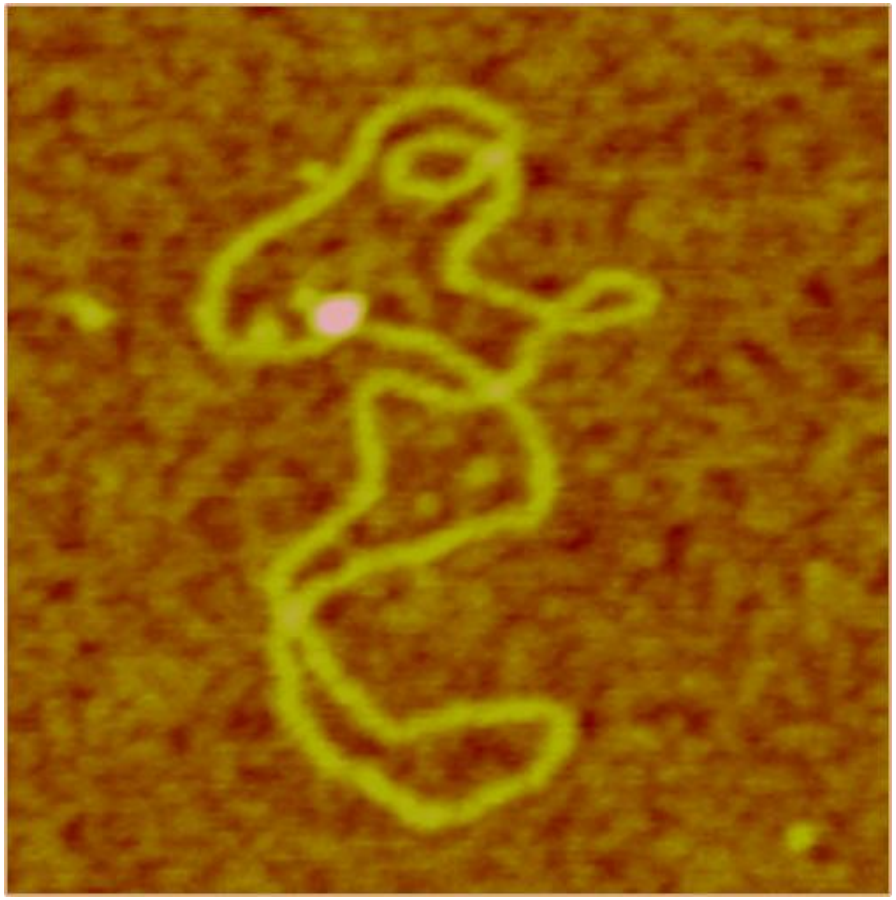


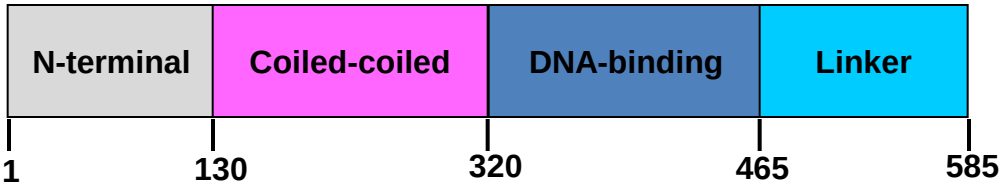
pUC8F14C plasmid DNA topoisomer with superhelical density σ=–0.040, containing a 106-bp F14C inverted repeat with an expected cruciform arm length of 53 bp. The AFM images revealed that cruciform structure in DNA molecules in 10 mM HEPES, 1 mM $MgCl_{2,}$, pH 7.3 buffer had an X-shaped conformation with the angle between the hairpin arms of 79±22.6.



Monomeric 67.5 kDa STAT3 fragment-cruciform DNA complexes

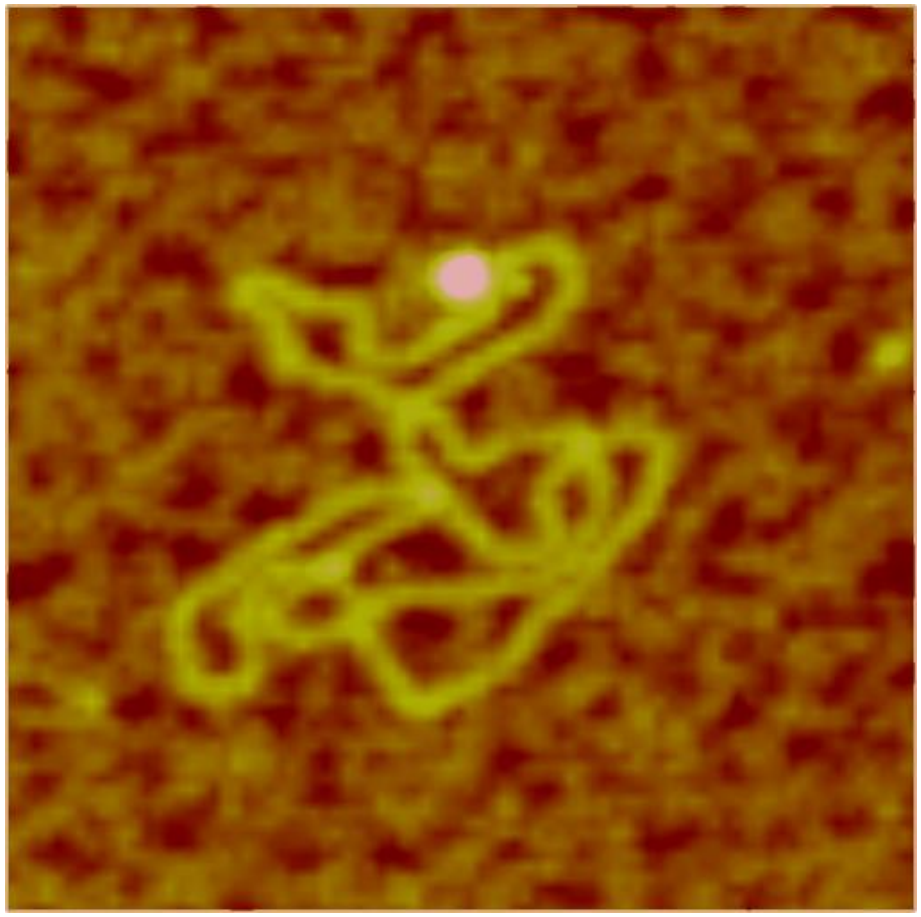




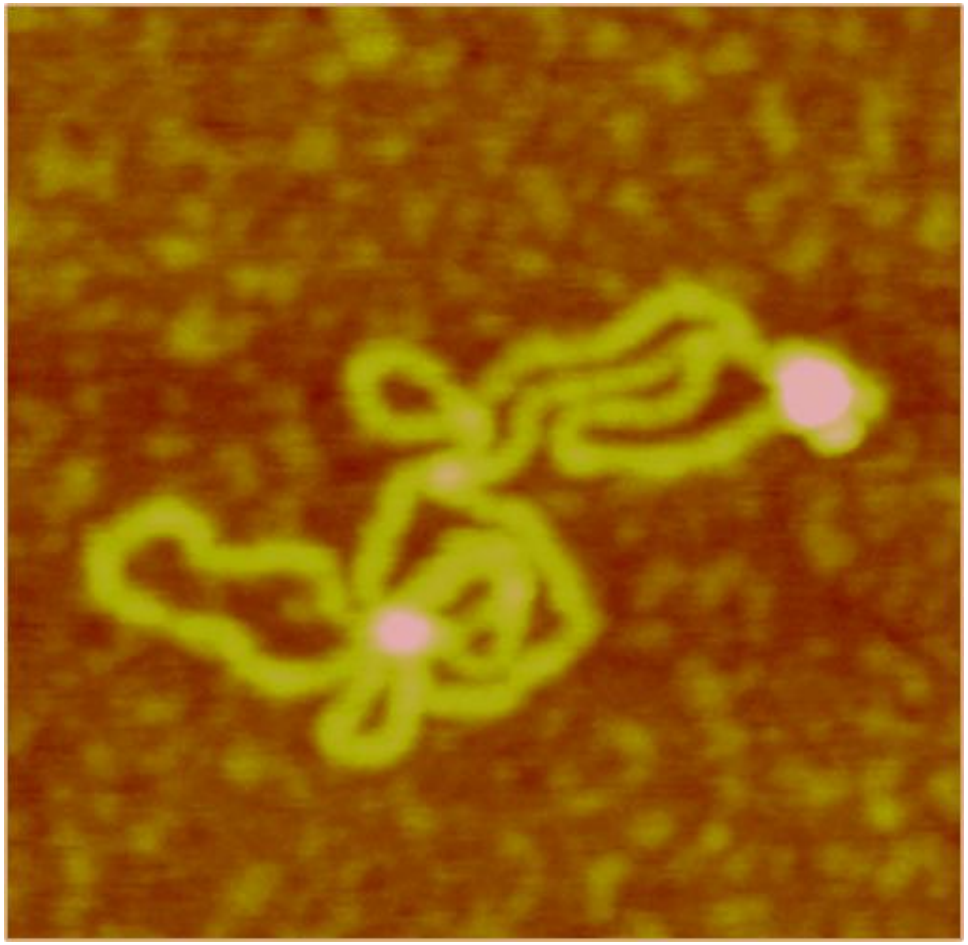


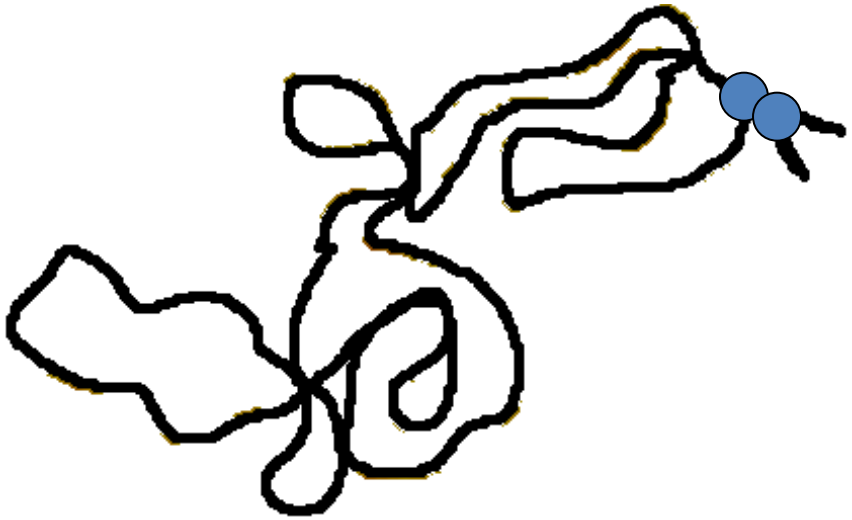
67.5 kDa

Full size STAT3-cruciform DNA interaction

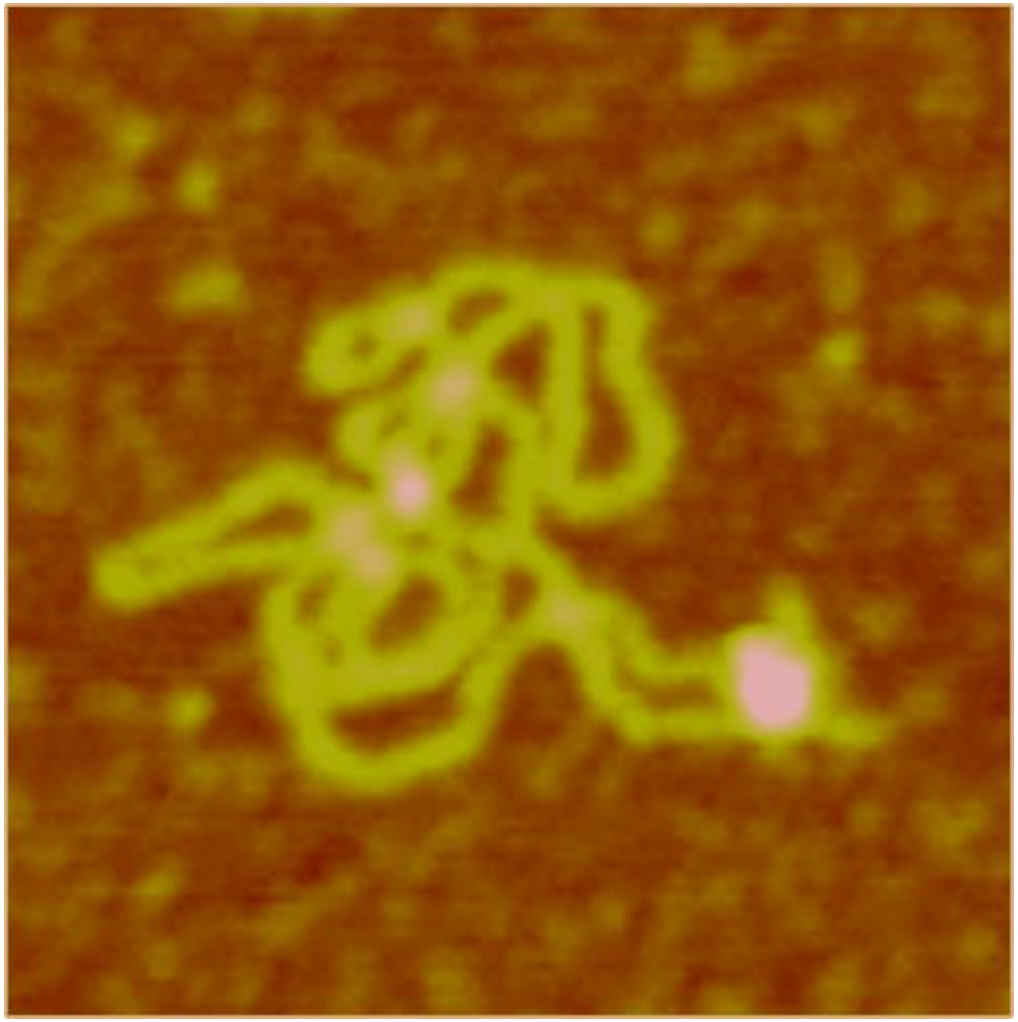


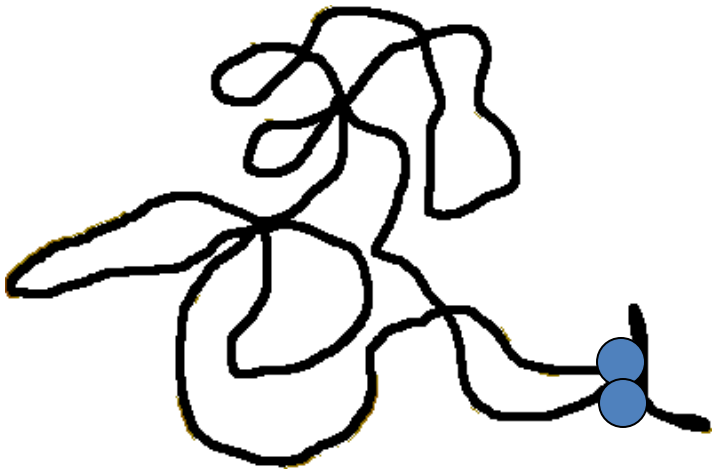
Full size STAT3-cruciform DNA interaction





Full size STAT3-cruciform DNA interaction





Inhibitor for STAT3 N-Domain as a Promising Anticancer Drug

N-domain of STAT3 is involved in interaction of two STAT dimers, the interaction of STAT3 with other transcription factors and the interaction with histone-modifier proteins to induce changes in chromatin structure.

These complex interactions provide for maximum STAT3-dependent transcriptional stimulation in normal and cancer cells.

NMR studies revealed that synthetic analog of the STAT4 second α-helix bind to the N-domain was used for the rational design of STAT3 helix 2 analogs with enhanced biological activity.

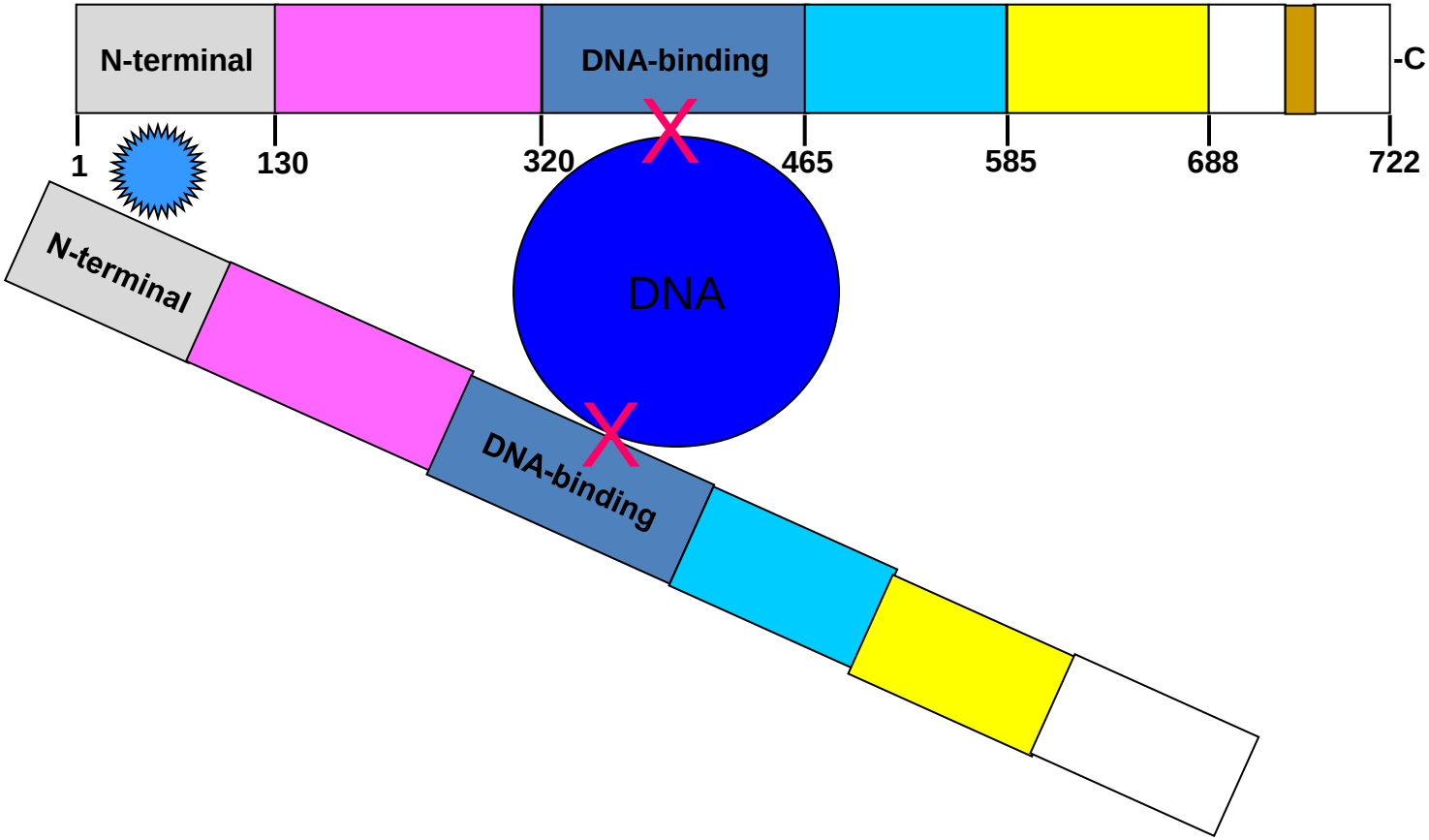
STAT3 protein

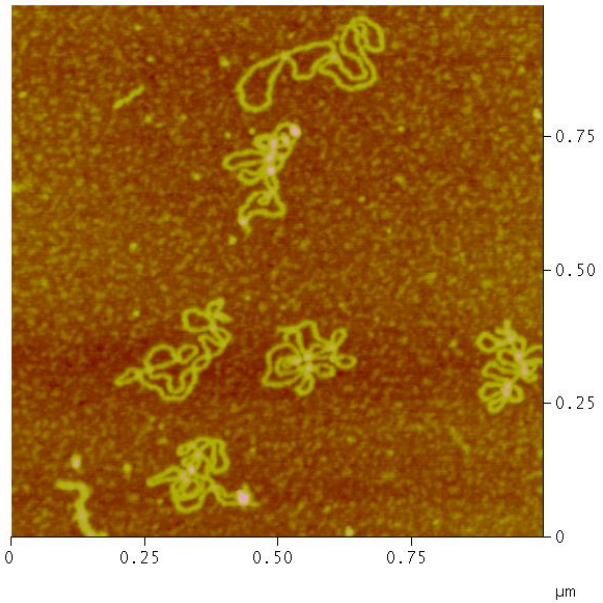
Amino acid sequence **hel2A-pen** inhibitor: **DTRYLEQLHKLYS**

To enable cell membrane penetration, the peptides were fuse on the C-terminus to penetratin, which is a 16-amino-acid-long peptide fragment thatis internalized by cells in a specific, non-receptor-mediated manner and can be used as an intracellular manner and can be used as an intracellular vehicle for the delivery of oligopeptidesand oligonucleotides. Penetratin sequence: ***RQIKIWFPNRR-Nle-KWKK-NH_2***

Cell-permeable derivatives of the STAT3 second helix were found to **direct and specifically binds to STAT3 and potently induced apoptotic death in breast cancer cells**, probably through changes in the mitochondrial membrane potential that result in activation of caspases, but not normal breast cells or STAT3-deficient fibroblasts. The molecular mechanisms of inhibitor-induced cell toxicity are not clear.

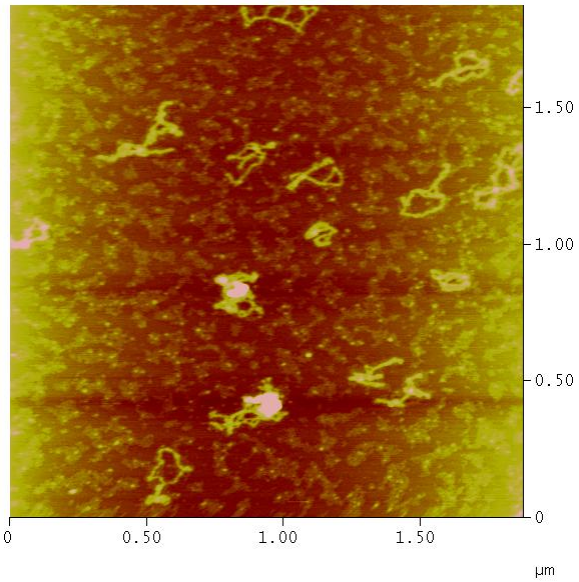
Hypothetical mechanisms of inhibition of **hel2A-pen** STAT3 dimerization across the N-terminal domain and interactions with DNA





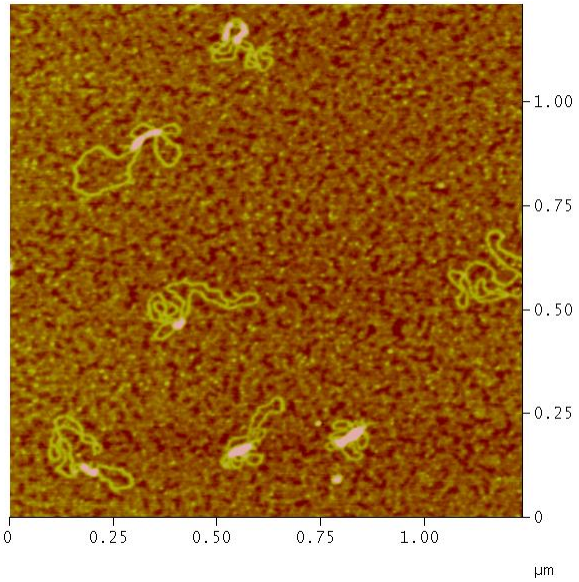
10x

hel2A-pen inhibitor



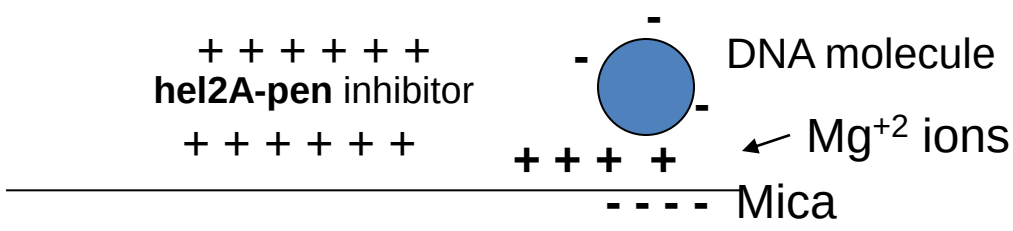
25x

hel2A-pen inhibitor



50x

hel2A-pen inhibitor



Conclusion

- Full size and 67.5 kDa fragment of STAT3 bind to GAS element and secondary structure of the DNA

- Full size and 67.5 kDa fragment of STAT3 bind to DNA as a monomer, dimer and tetramer

- STAT3 fragment (67.5 kDa) binds to cruciform structure and single-stranded DNA

- Hel2A-pen inhibitor of STAT3:

a) binds directly to DNA

b) activates process of oligomerization of STAT3 proteins on the supercoiled DNA