Our theory of facilitated diffusion
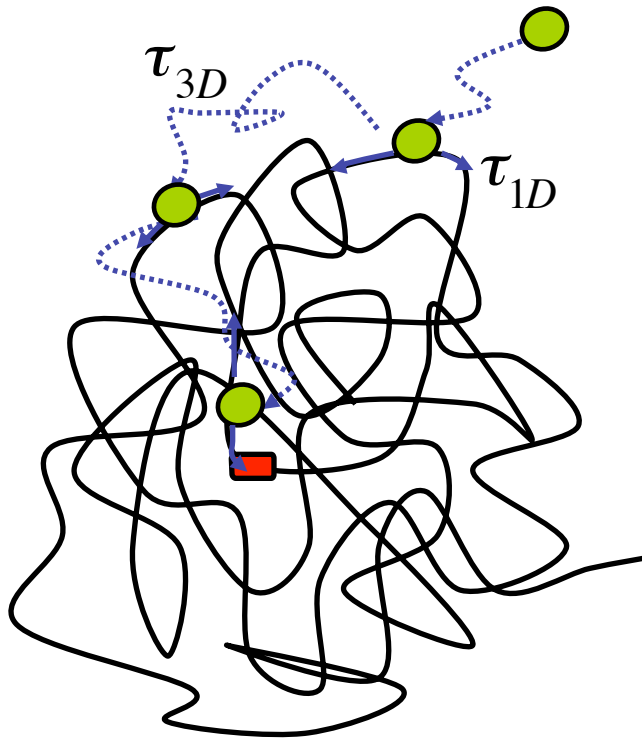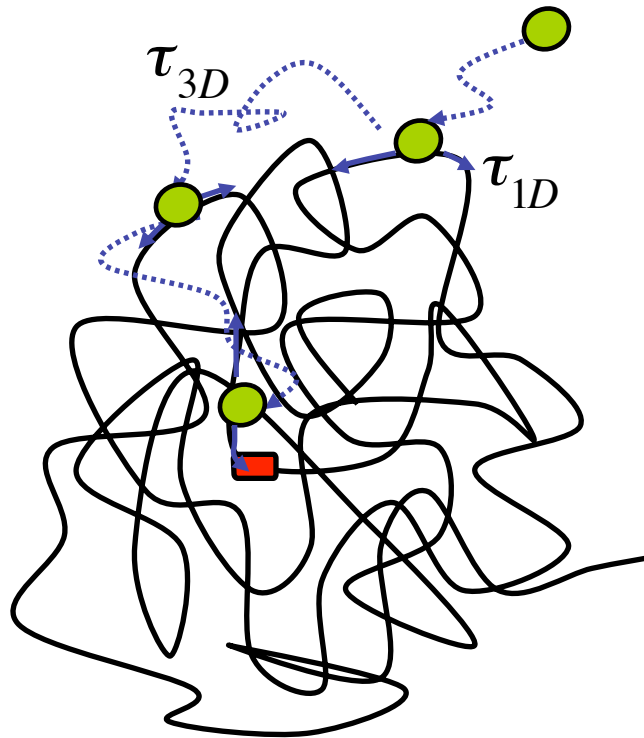

Total search time t_s

    one protein (TF), one site

    M base-pairs : genome length

τ_{3D}

τ_{1D}

Slutsky and Mirny 2004

Our theory of facilitated diffusion

Total search time t_s

M bp genome length
$\bar{n}$ bp scanned per round of sliding

$$t_s = \frac{M}{\bar{n}}(\tau_{1D} + \tau_{3D})$$
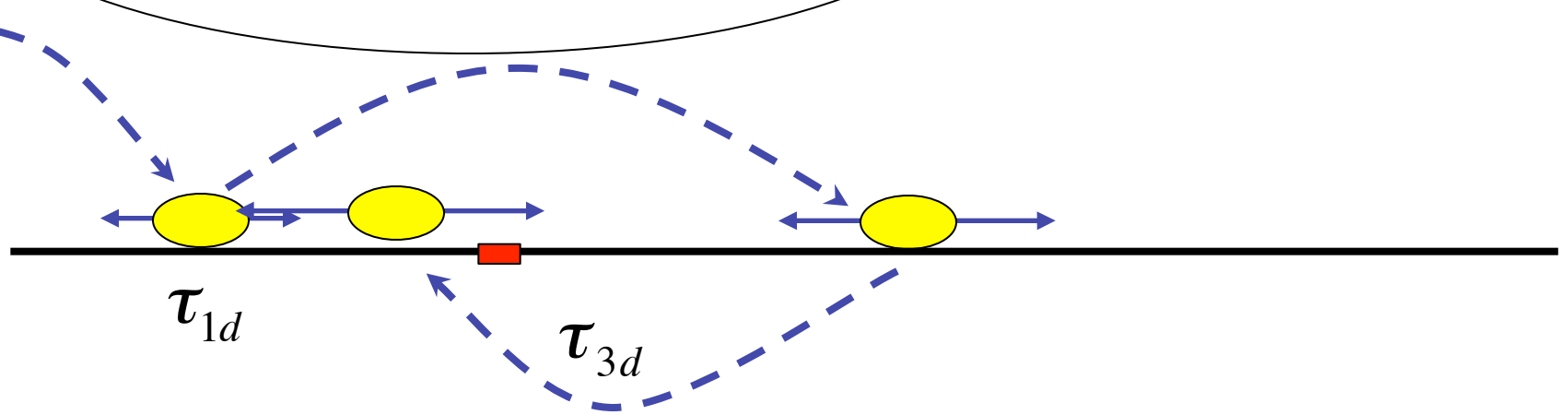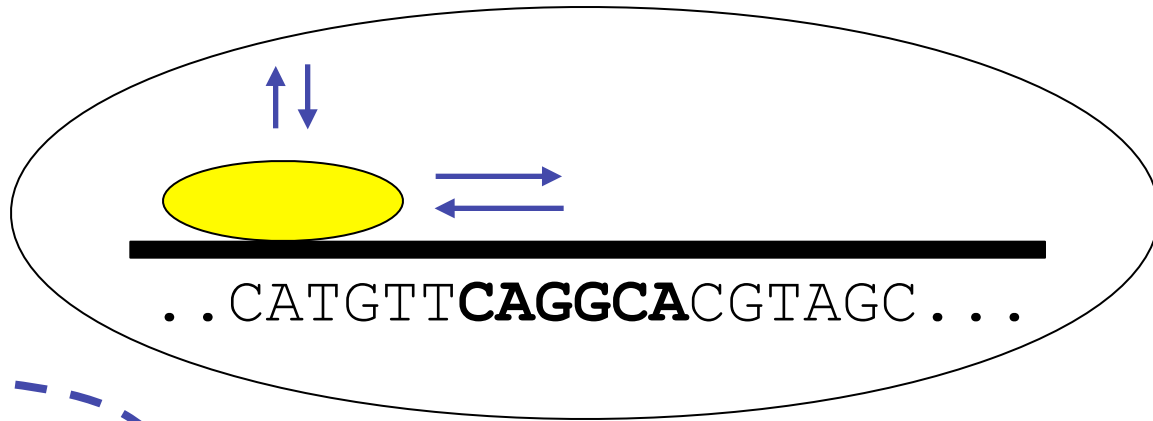
$$\bar{n} = 2\sqrt{D\tau_{1D}}$$

Results

optimum search time:

$$\tau_{1D} = \tau_{3D}$$

$$\bar{t}_{opt} = \frac{2M}{\bar{n}}\tau_{3D} = 2M\sqrt{\frac{\tau_{3D}}{D_{1D}}}$$
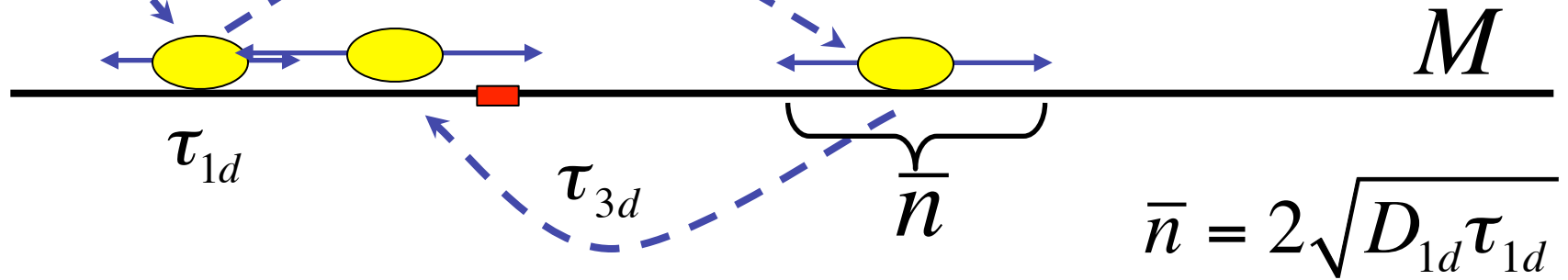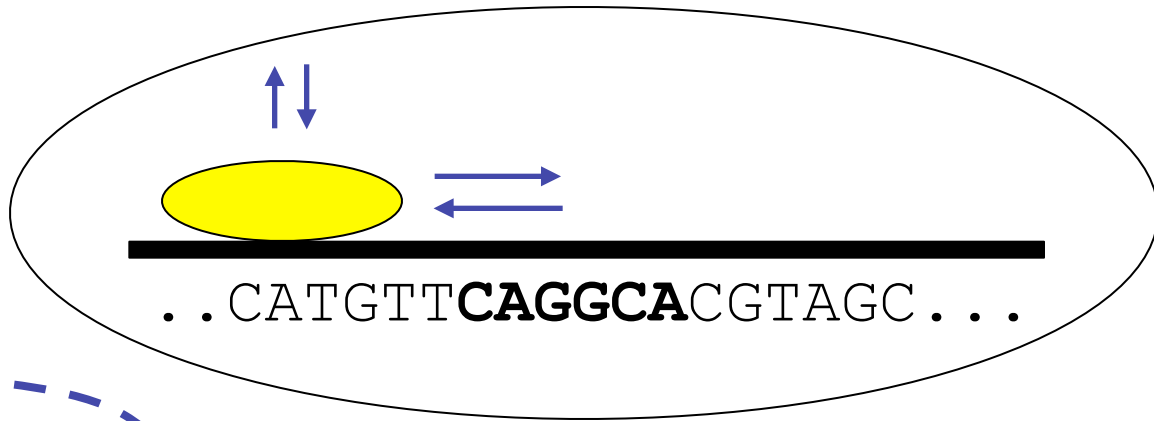
Optimum search time is $\bar{n}/2$ times faster than 3D alone and $M/\bar{n}$ times faster than 1D alone.

Model: 1D+3D

$$t_s = \sum_{i=1}^{N} \left(\tau_{1d,i} + \tau_{3d,i} \right)$$

Slutsky and Mirny 2004

Model: 1D+3D

$$\bar{n} = 2\sqrt{D_{1d}\tau_{1d}}$$

$$t_s\left(\bar{n}, M\right) = \frac{M}{\bar{n}}\left[\tau_{1d}\left(\bar{n}\right) + \bar{\tau}_{3d}\right]$$

Main results

1. Optimal search time reached at $(\tau_{1D} = \tau_{3D})$

$$t_s = \frac{2M}{\bar{n}} \tau_{3D}$$

$$t_s = 2M \sqrt{\frac{\tau_{3D}}{D_{1D}}}$$

t_s – search time
M – genome size

2. Mean number of bp scanned in one round
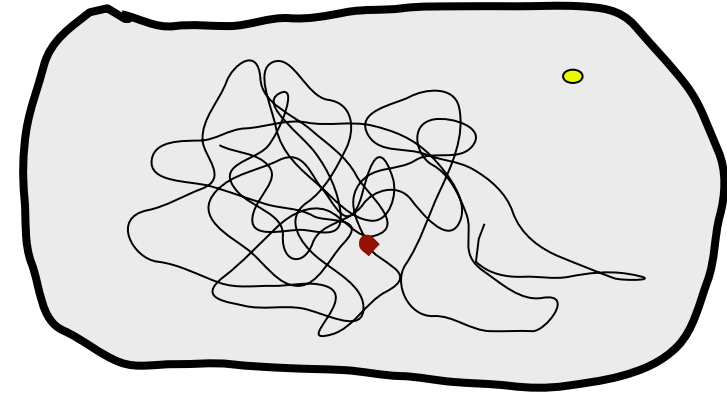
$$\bar{n} \sim 200 - 500 \text{ bp}$$

3. Optimal 1D/3D is

$n \sim 200$ times faster than 3D only

$M/n \sim 10000$ times faster than 1D only

Bacteria	$\tau_{1D} \approx 10\,\tau_{3D}$	Elf et al *Science* 2007
Yeast	$\tau_{1D} \approx \tau_{3D}$	Larson et al *Science* 2011

Reading

Slutsky M, Mirny LA
Kinetics of protein-DNA interaction: Facilitated target location in sequence-dependent potential
Biophysical Journal 87(6):4021-4035 (2004)

Slutsky M, Kardar M, Mirny L
Diffusion in correlated random potentials, with applications to DNA
Physical Review E 69(6)

Mirny L, Slutsky M, Wunderlich Z, Tafvizi A, Leith J, Kosmrlj A
How a protein searches for its site on DNA: The mechanism of facilitated diffusion
Journal of Physics A: Mathematical and Theoretical 42(43):434013 (2009)

Single-molecule experiments

Antoine van Oijen (Harvard Medical School)
Anahita Tafvizi (Harvard Physics and MIT)
Jason Leith (Harvard Biophysics)

Leith JS, Tafvizi A, Huang F, Uspal WE, Doyle PS, Fersht AR, Mirny LA, Oijen AM
Sequence-dependent sliding kinetics of p53
PNAS 109(41):16552-16557 (2012)

Tafvizi A, Mirny LA, Oijen AM **Dancing on DNA: Kinetic aspects of search processes on DNA**
ChemPhysChem 12(8):1481-1489 (2011)

Tafvizi A, Huang F, Fersht AR, Mirny LA, Oijen AM
A single-molecule characterization of p53 search on DNA
PNAS 108(2):563-568 (2011)

Tumor suppressor p53 as a model system

In 50% of all human cancers mutations of p53 have been observed.
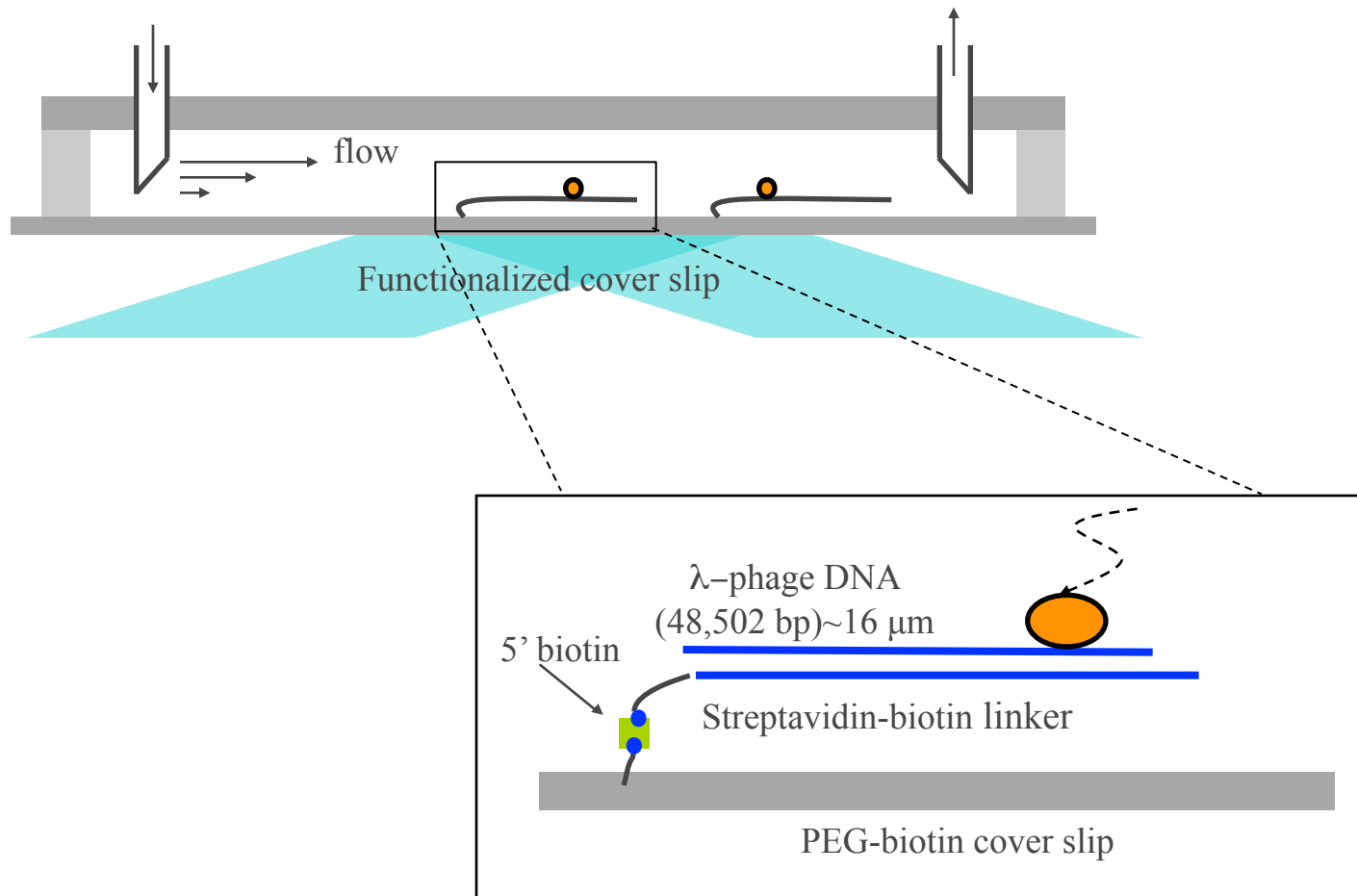
"Guardian of the genome" (Lane, 1992)
"Death star" (Vousden, 2000)
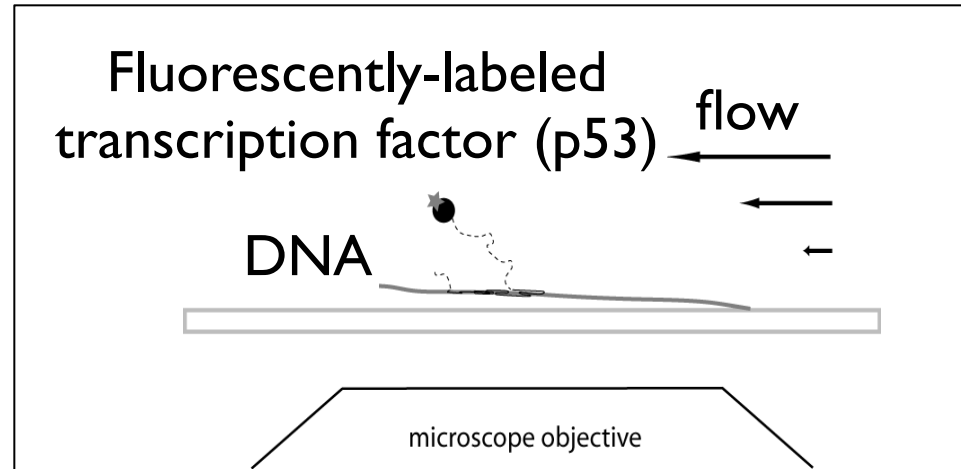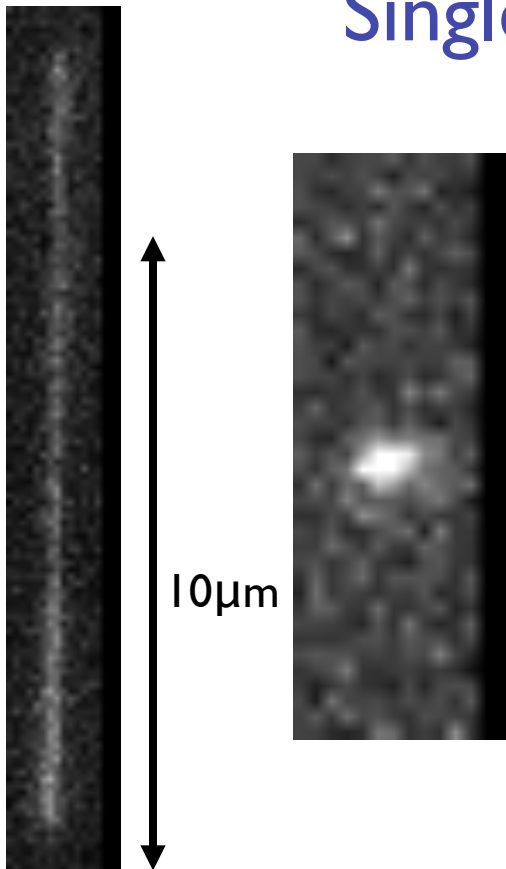"Good and bad cop" (Sharpless and DePinho, 2002)
"An acrobat in tumorigenesis" (Moll and Schramm, 1998)
"Molecule of the year" attributed by Science, in 1993 (Harris, 1993)
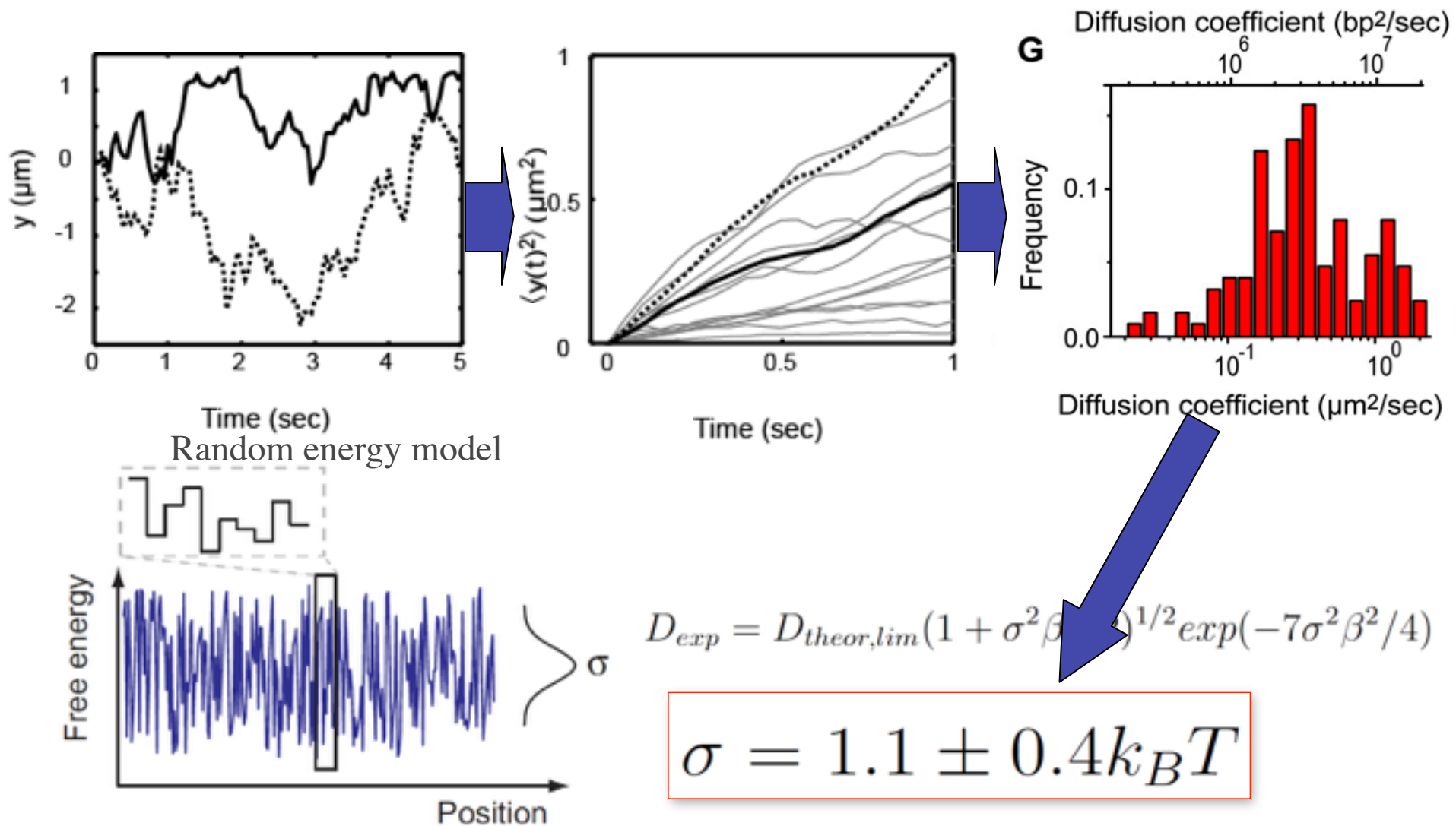
Single-molecule studies of p53-DNA sliding

flow

Functionalized cover slip

λ–phage DNA
(48,502 bp)~16 μm

5' biotin

Streptavidin-biotin linker

PEG-biotin cover slip

Single-molecule sliding of p53

10μm

Fluorescently-labeled transcription factor (p53)

flow

DNA

microscope objective

0.5 sec

4μm

$D_{1D} = 1.5 \times 10^6 \ bp^2/sec$

$\tau_{1D} \approx 0.5$ sec for most TF in vivo(T.Mistelli)

$n \approx 10^3 \ bp$

Tafvizi et al Biophys J 2008
Tafvizi et al PNAS 2010

p53 slides fast over a relatively smooth landscape

Random energy model

$$D_{exp} = D_{theor,lim}(1 + \sigma^2 \beta^2)^{1/2} exp(-7\sigma^2\beta^2/4)$$

$$\sigma = 1.1 \pm 0.4 k_B T$$

Tafvizi et al *Biophys. J.* 2008

Challenges in determining the diffusion coefficient: effect of drift

n=162

drift= 262 bp/sec
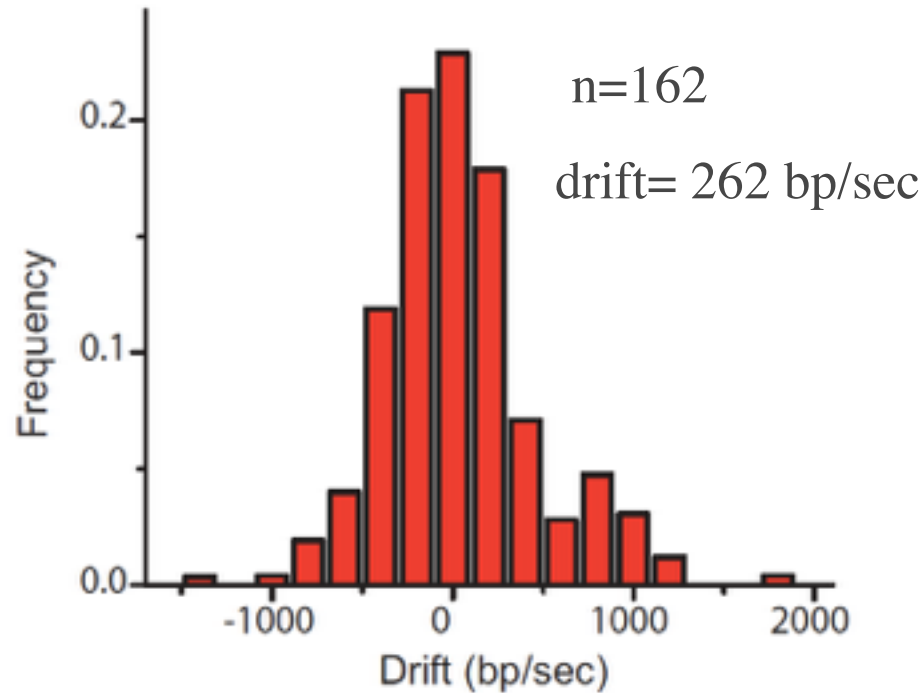
$$drift_{\text{mean,weighted}} = \frac{\sum_{i=1}^{N} l_i d_i}{\sum_{i=1}^{N} l_i}$$

Hopping or sliding

slide

hop

full-length p53
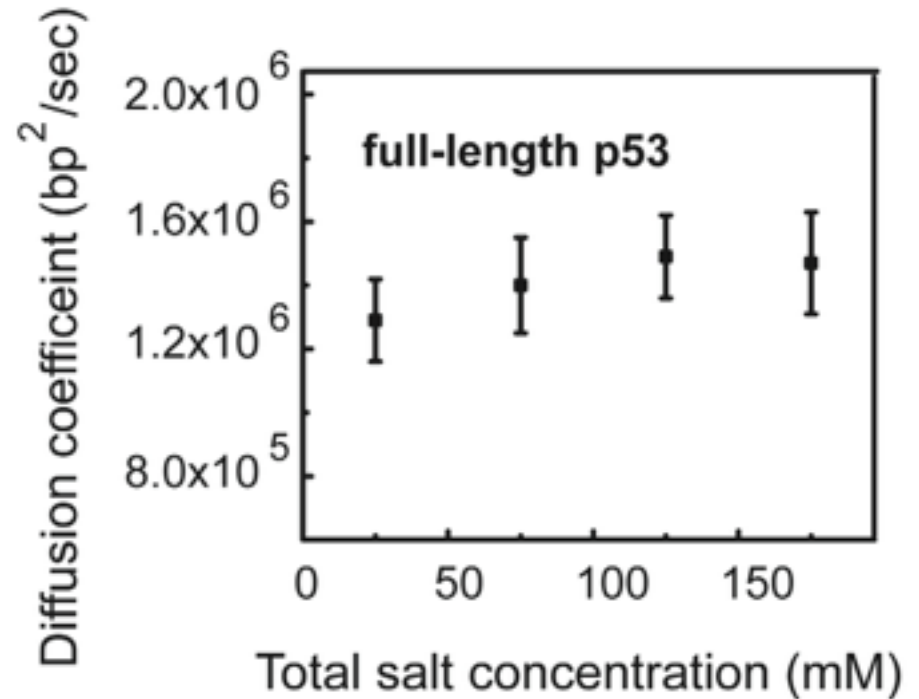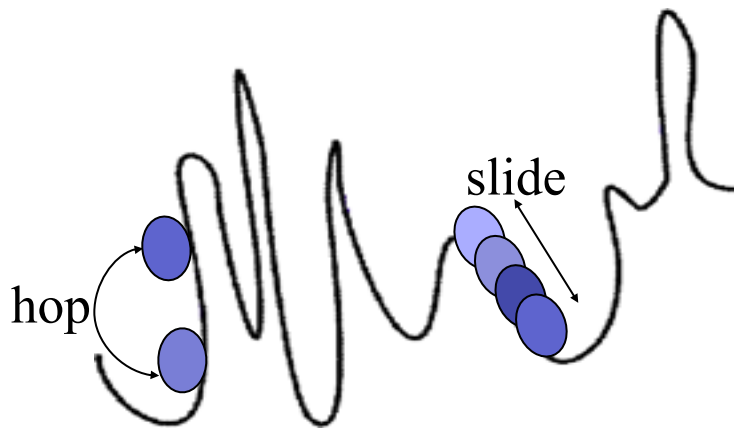
Sliding : indepe

Hopping : diffu
        higher

p53 slides along DNA
while keeping continues
contact with DNA.
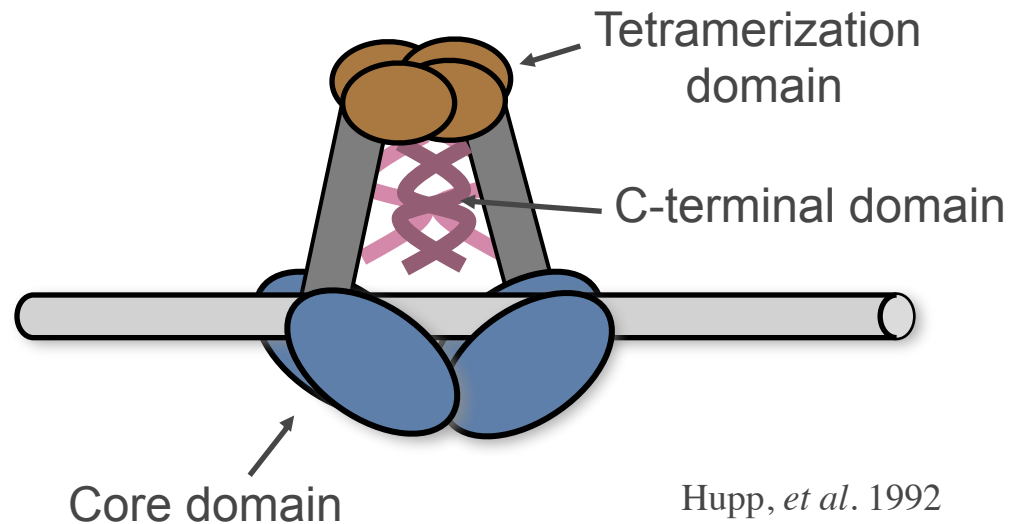
Tafvizi et al *Biophys. J.* 2008

Results

- p53 protein diffuses along DNA.
  First eukaryotic TF shown by single-molecule experiments to slide.

- The landscape is not very rugged $\sigma < 2kBT$

- Mostly sliding rather than hopping.

- How about the TWO-STATE MODEL?

p53 has two DNA-binding domains

N		Core		T		C
1	60	100	300	325	355	363	393

Activation domain — Sequence-specific DNA binding domain — Tetramerization domain — Non-specific DNA binding domain

Tidow *et al* 2007

Tetramerization domain
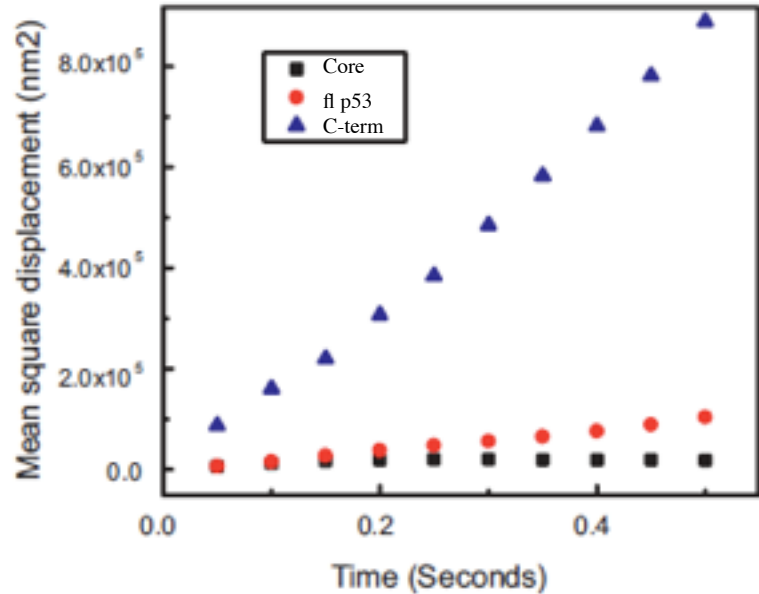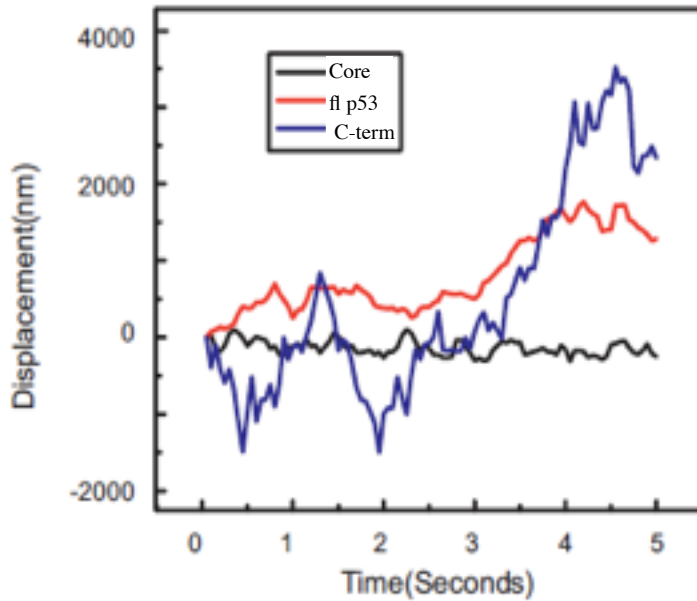
C-terminal domain

Core domain
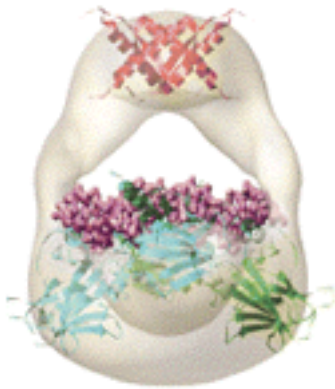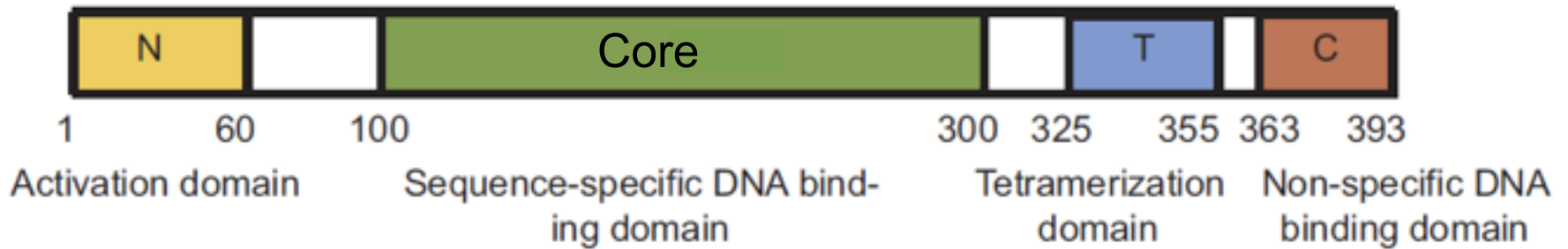
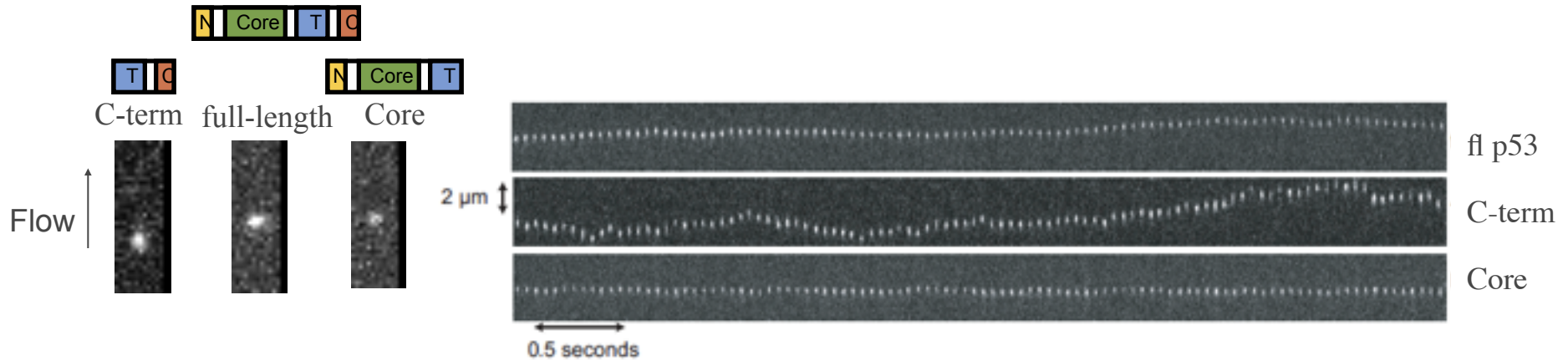Hupp, *et al*. 1992
Anderson, *et al*. 1997
McKinney, *et al*. 2004

p53 has two DNA-binding domains

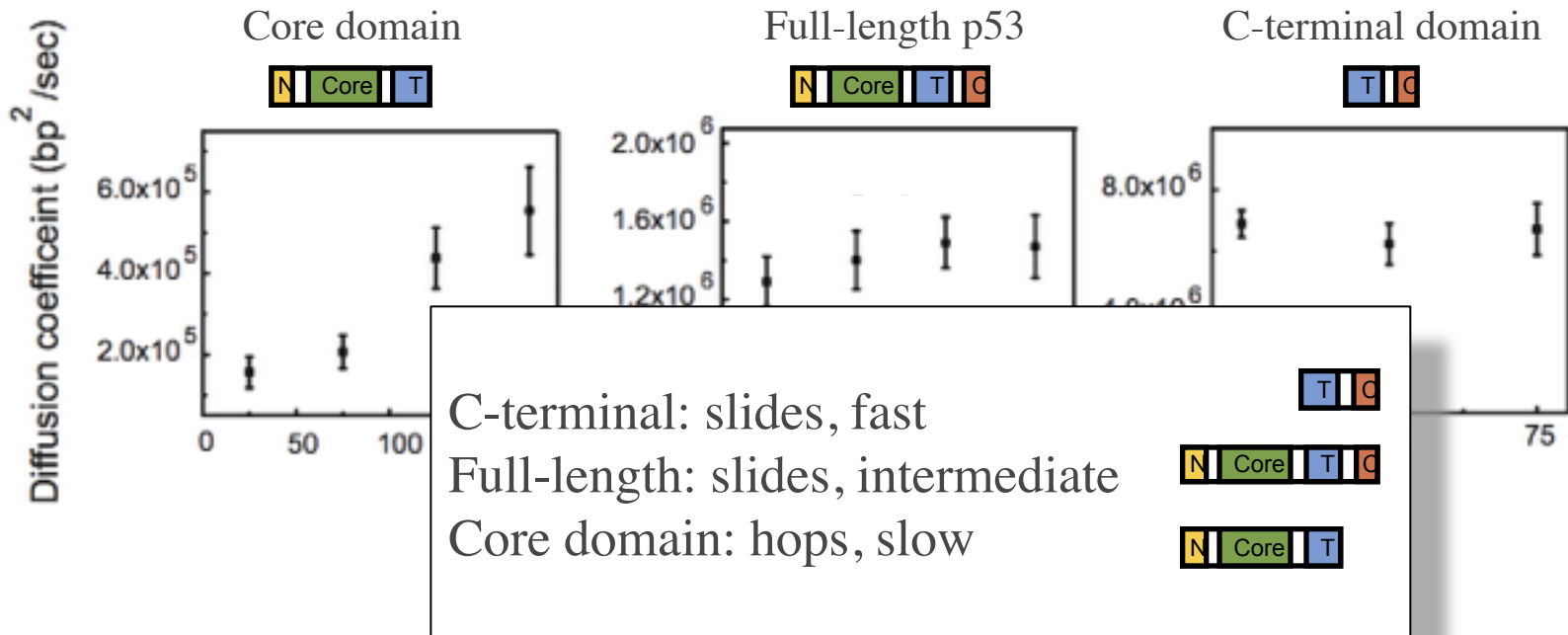## Three constructs

full-length

core (specific)

C-term (non-specific)
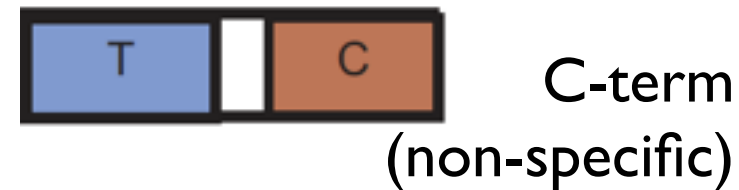
Different domains show different sliding speed

Diffusion coefficient of different constructs as a function of salt concentration

C-terminal: slides, fast
Full-length: slides, intermediate
Core domain: hops, slow

Tafvizi et al *PNAS* 2011

Single-molecule experiments

- Full-length p53 slides sufficiently fast
- Core domain (specific) is unable to slide
- Binding via C-terminal domain provides fast sliding mode
- ~50% of time sliding
- switching faster than 700 s^{-1}

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Tafvizi et al *Biophys. J. Letters* 2008
Tafvizi et al *PNAS* 2011