

Biosensing

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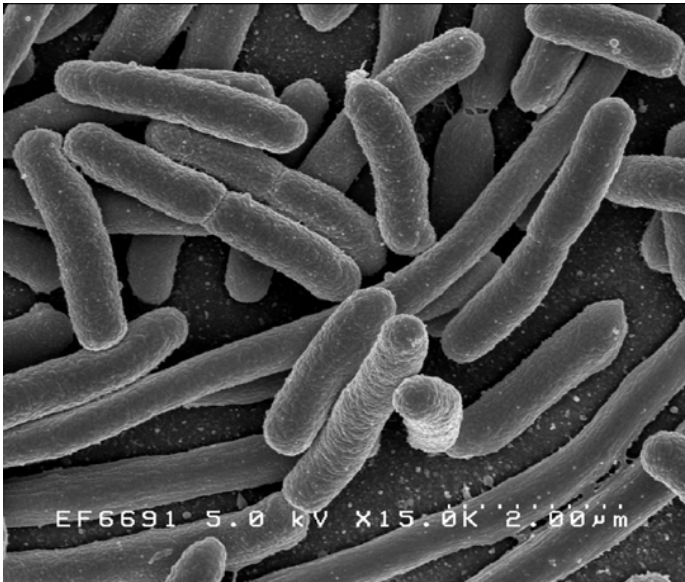
NIH 5R01HG003713-02

NSF NIRT # 0404030

5 μ m

E. coli—a living nanosystem

SEM of *E. coli*



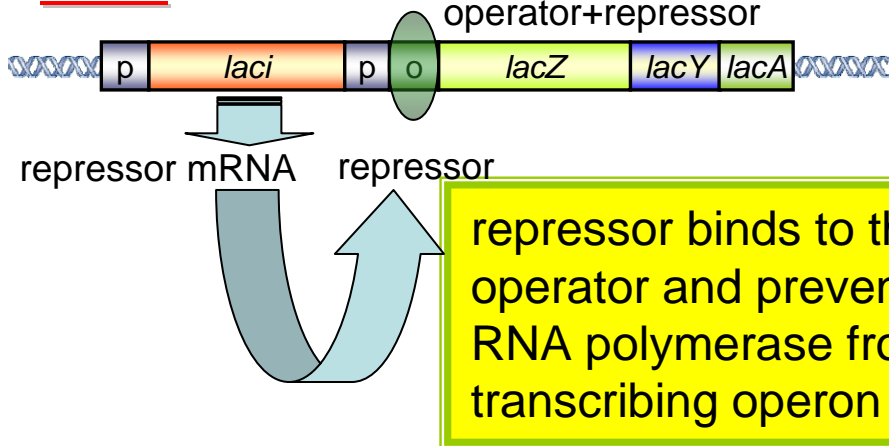
(1-2 μ m x 0.1-0.5 μ m dia.)

The “hydrogen atom” of biology

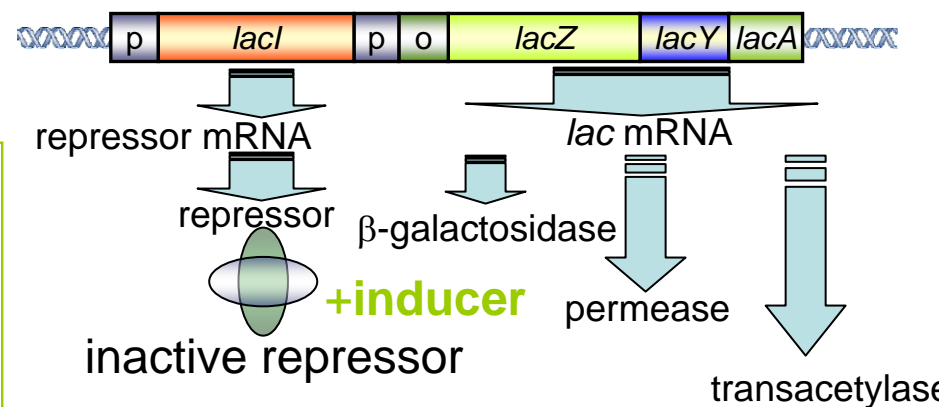
- **doubling time** \rightarrow 30min. to 4hrs.
 $\therefore 1 \rightarrow 10^8$ (12 hrs.)
- **dry mass** \rightarrow 4,000,000 proteins (5nm)
4,300 protein types(1nM)
- **diffusion** \rightarrow small molecule: 1ms
 \rightarrow protein: 100ms
- **protein transition** \rightarrow 1-100 μ s

The prototypical molecular switch: *lacI*

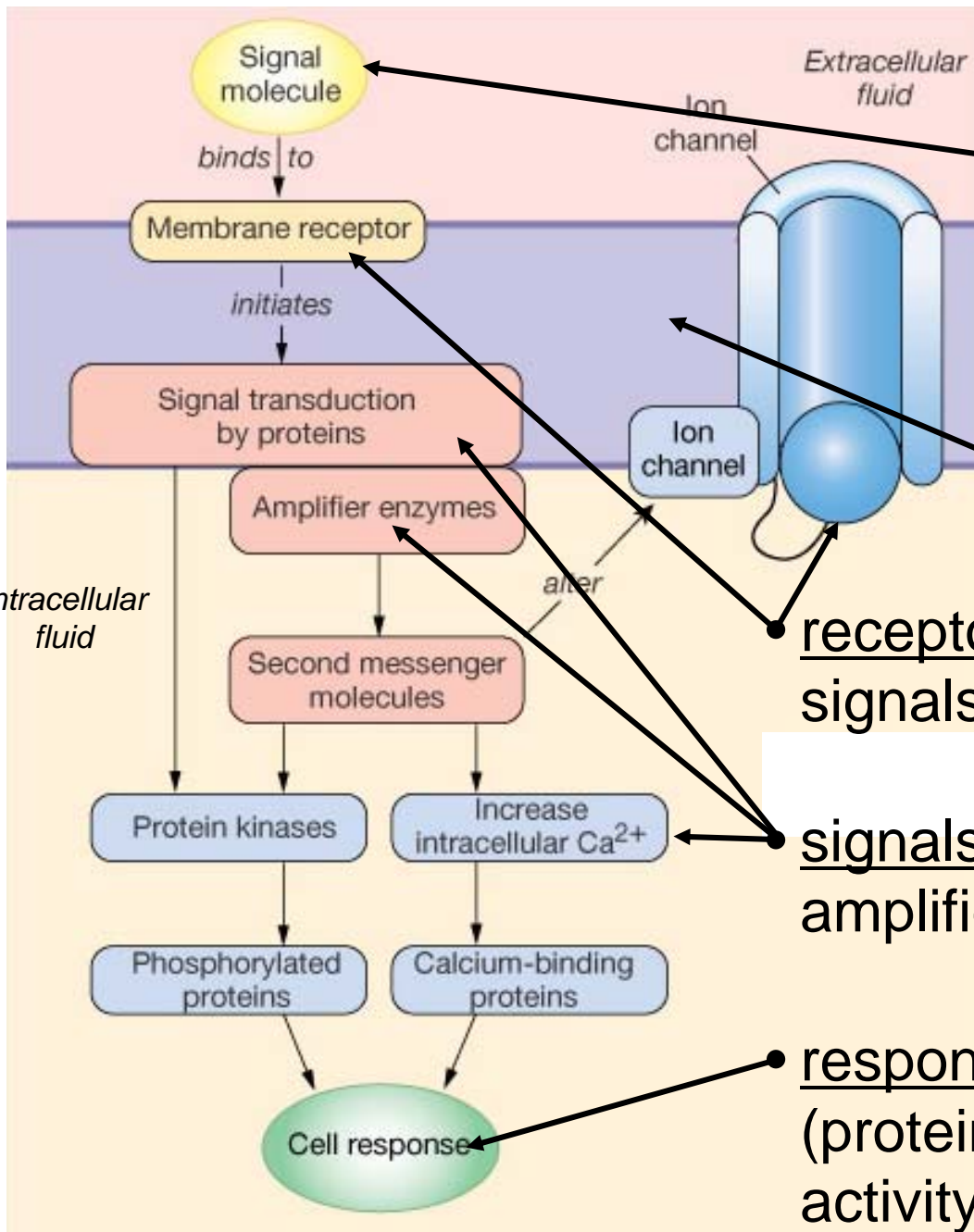
OFF: *lac*-operon \rightarrow no inducer
operator+repressor



ON: *lac*-operon \rightarrow inducer



Cell Signaling



• signals: ions (Ca²⁺), small molecules (sugar, lactones), proteins, DNA, temp., pH, mechanical, electrical

• membrane isolates cell from it's microenvironment

• receptors/ion channels convey signals across the membrane

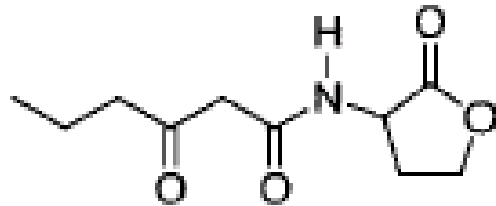
• signals trigger cascade or amplification resulting a response

• responses include gene activity (protein synthesis), enzyme activity, motor proteins....

lux bioluminescence genes

(*Vibrio fischeri* best-understood “quorum sensing” system)

N-acyl homoserine lactone (AHL) is the autoinducer—a signaling molecule



Transcription activator

AI synthase

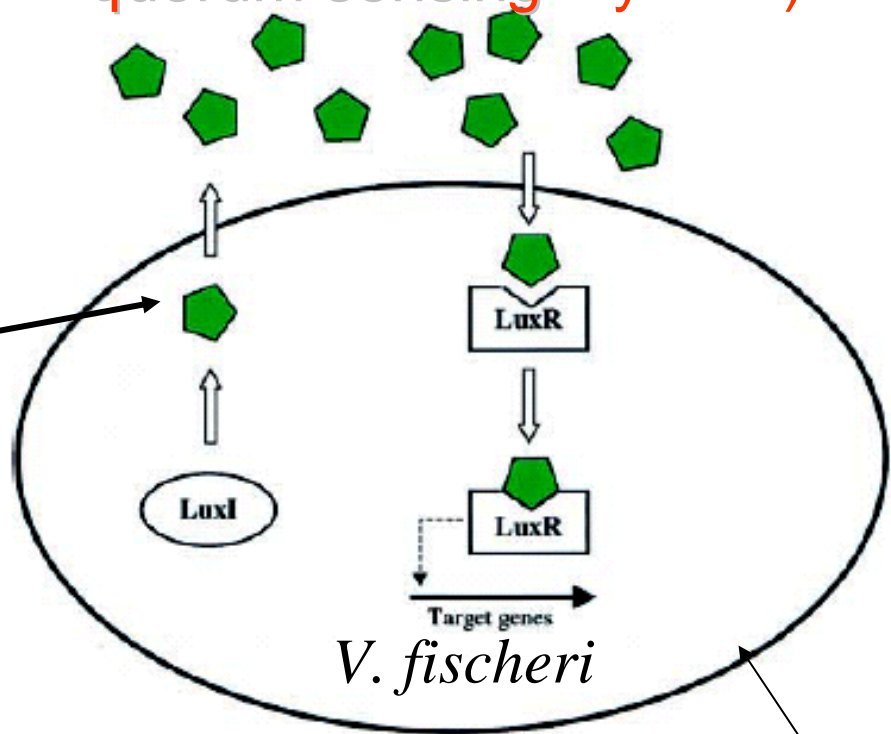
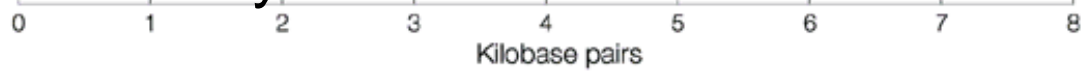
lux-operon

Luciferase

?

Acid reductase

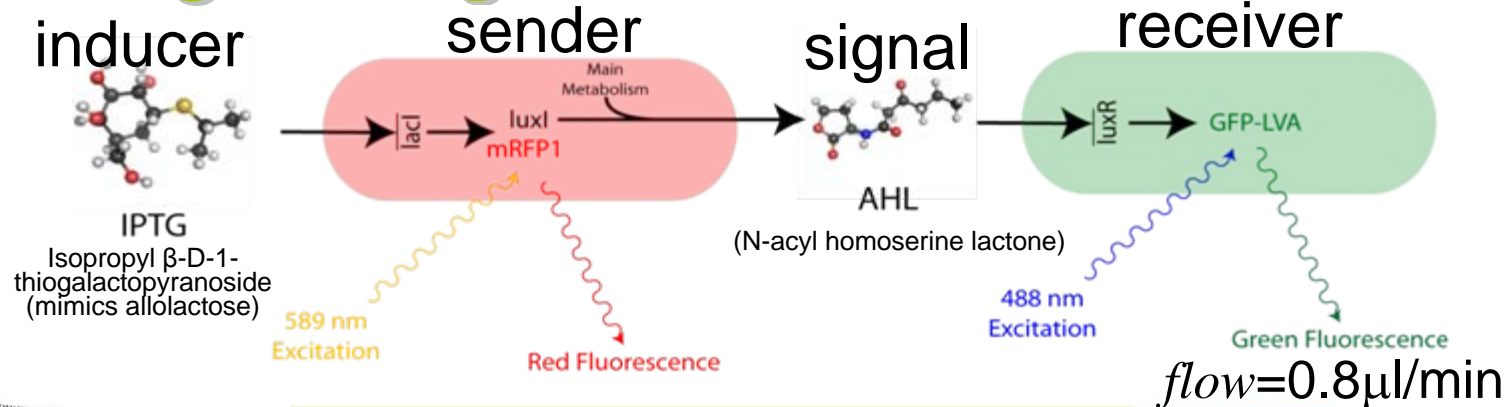
expressed constitutively



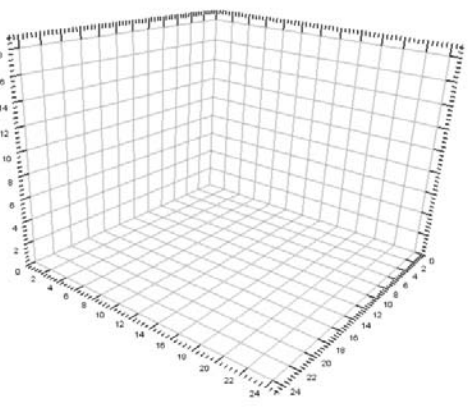
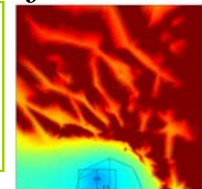
membrane

- Other Apps....**
- Bioluminescence
 - Swarming
 - Chemotaxis
 - ***Virulence***

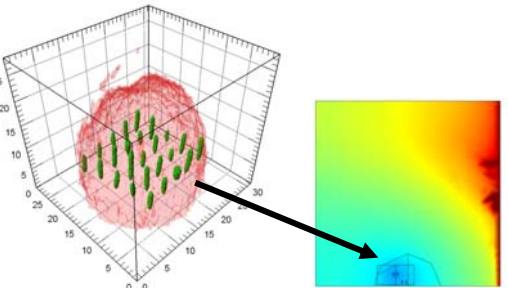
Cell Signaling: Sender → Receiver



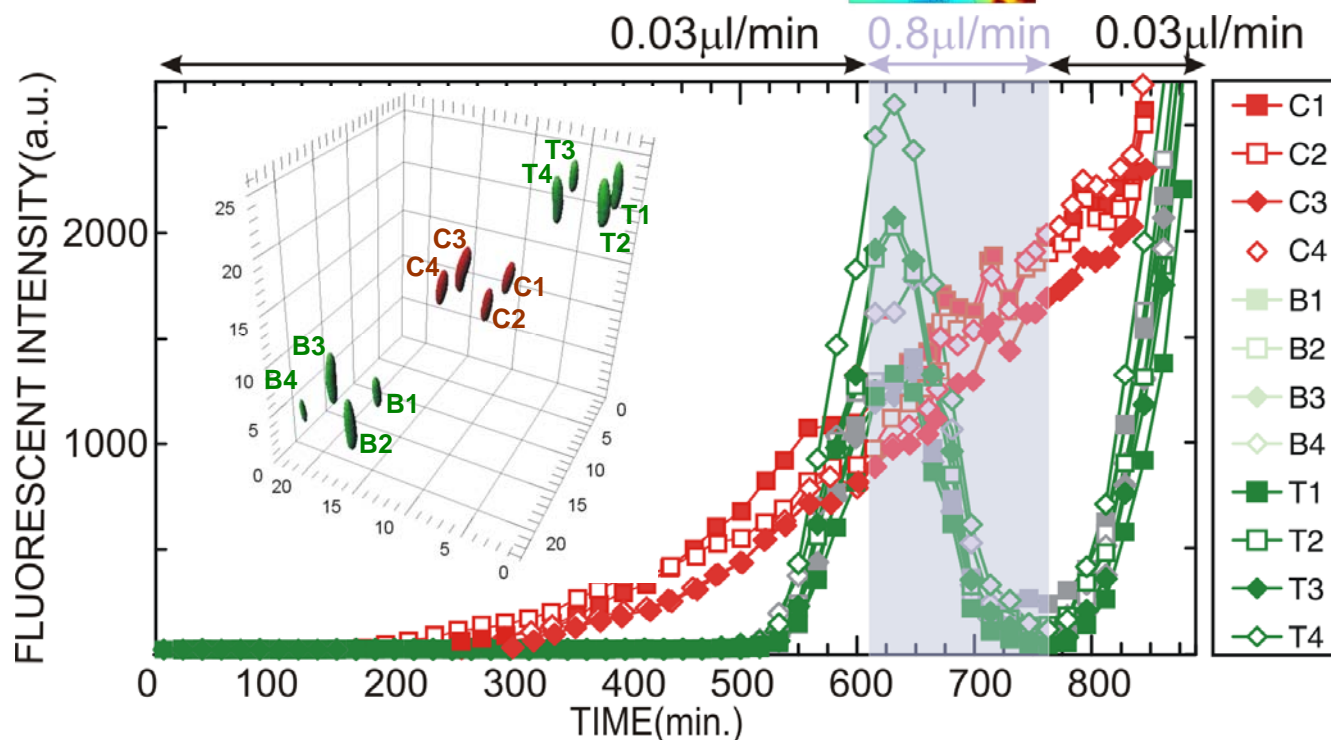
• signaling affected by physical, chemical, biologic. environment



cell microarray in hydrogel

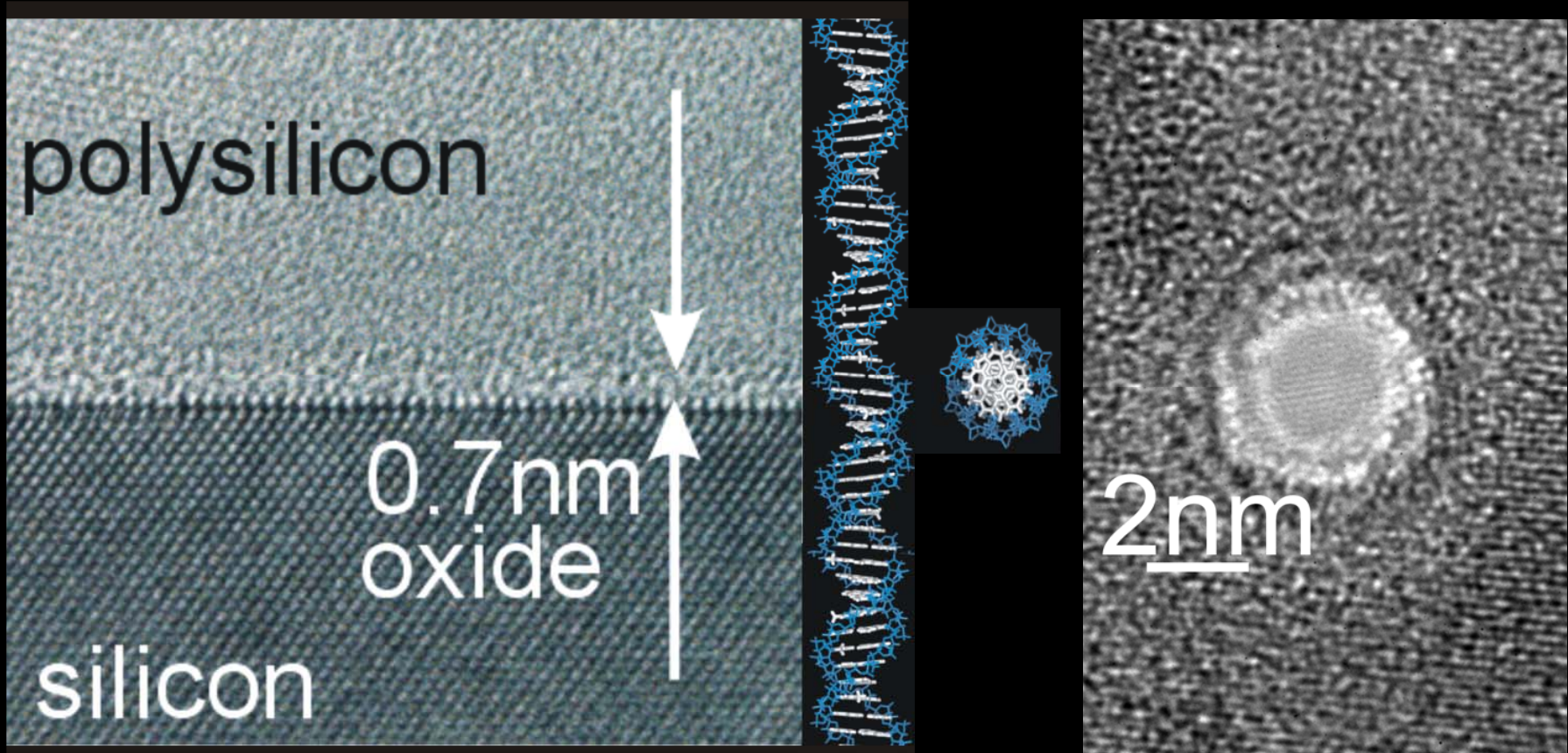


flow=0.03 μl/min



Si Nanotechnology for Sensing DNA

- ultra-thin MOS capacitors for membranes
- sub-nm, bright, high eV e-beam for lithography



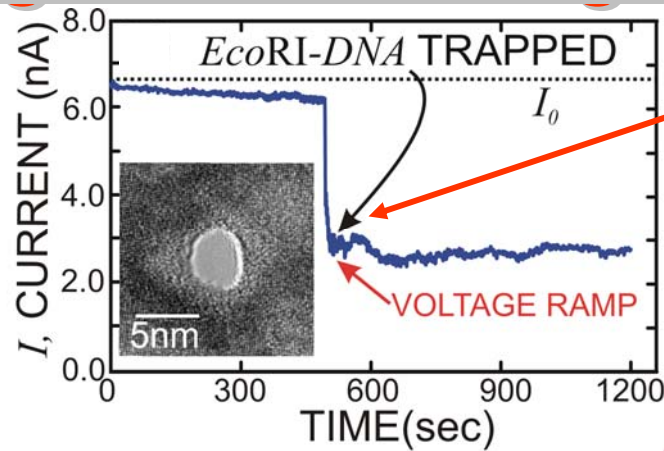
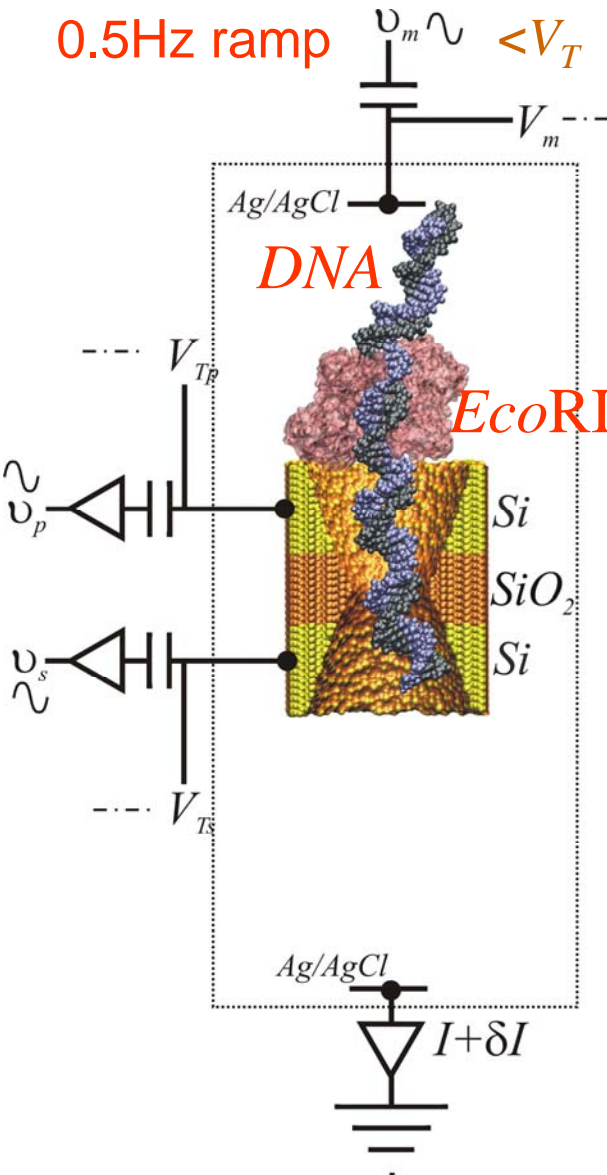
TEM X-section through a gate

DNA
(to scale)

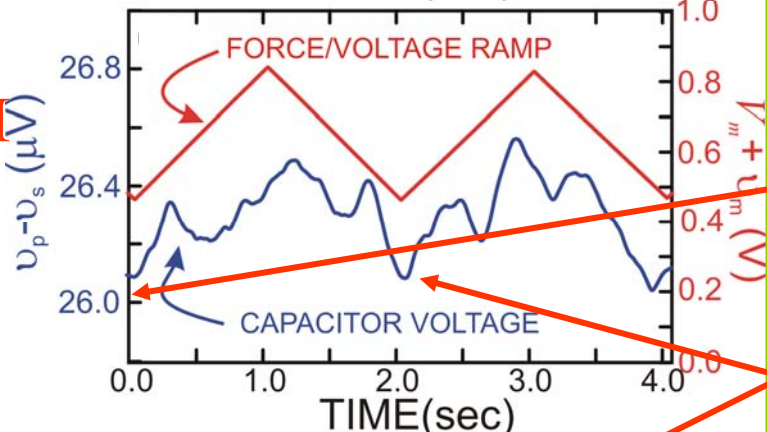
TEM (top-down)

Detecting DNA Charge in Trap

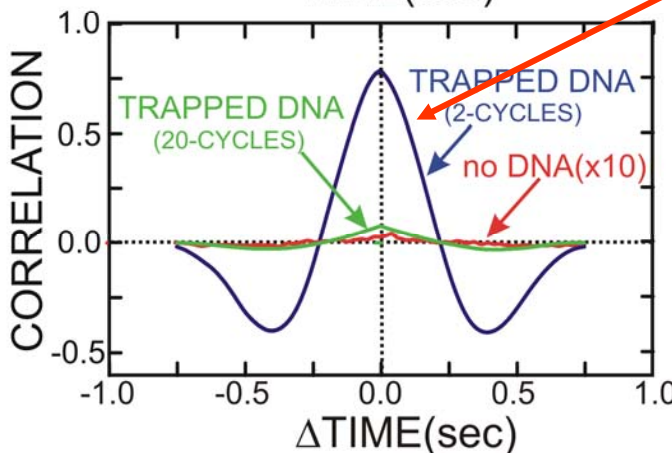
200kHz sine+
0.5Hz ramp



- trap DNA between E-field and *EcoRI*



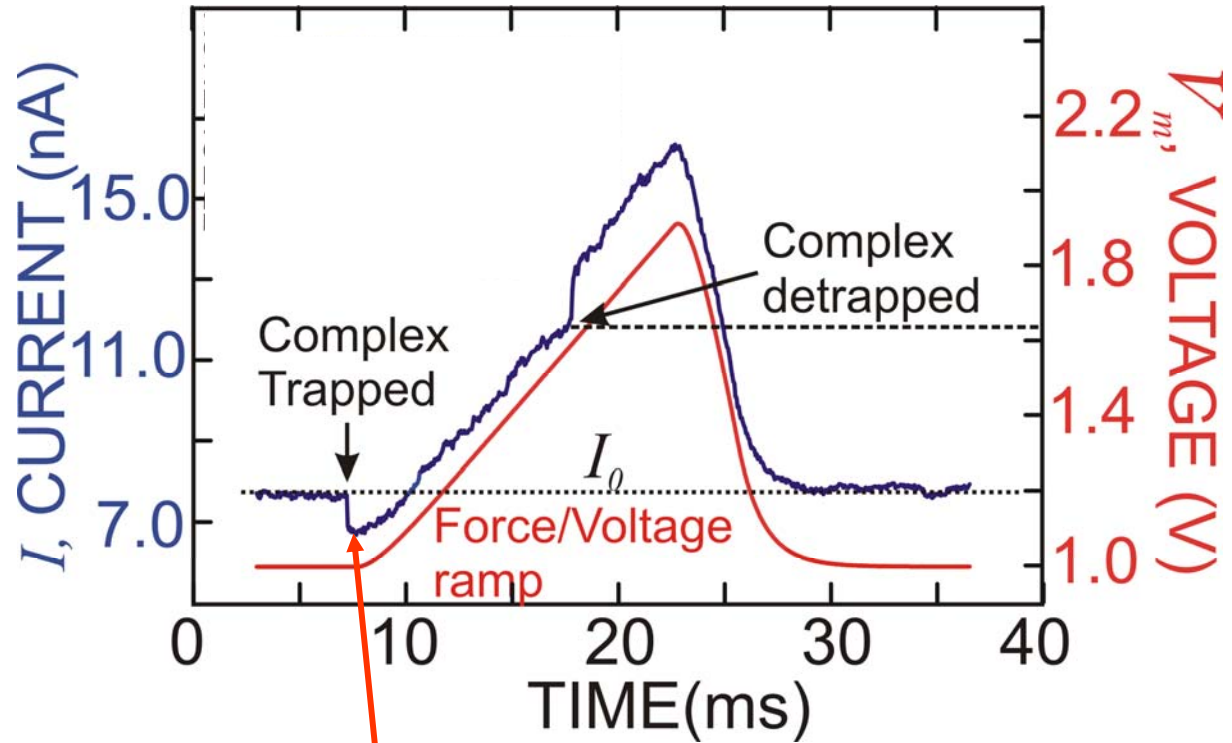
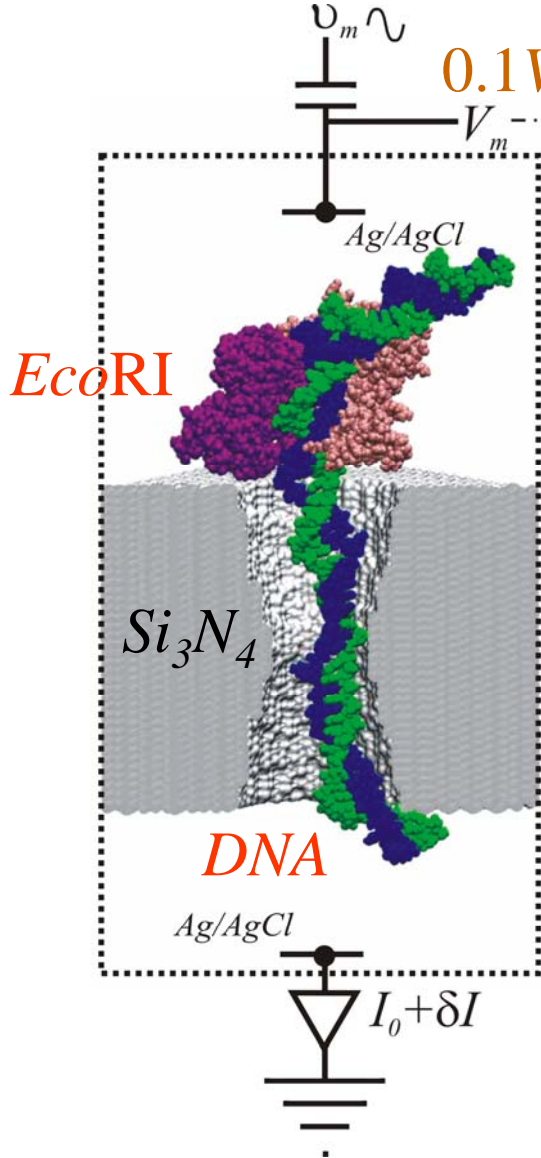
- use phase-sensitive (lock-in) detection to measure charge
- 26 μV comparable to prediction for phosphate
- fluctuations may indicate twisting



- fluctuations correlated by time dependent

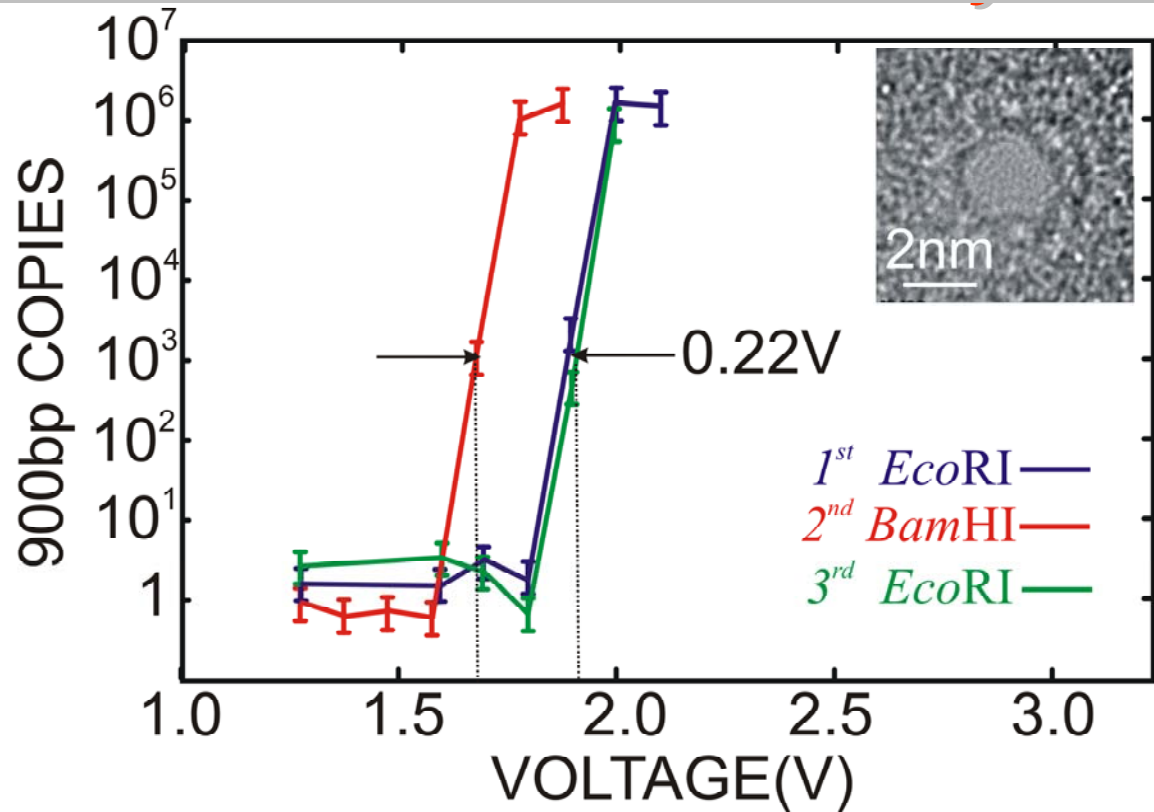
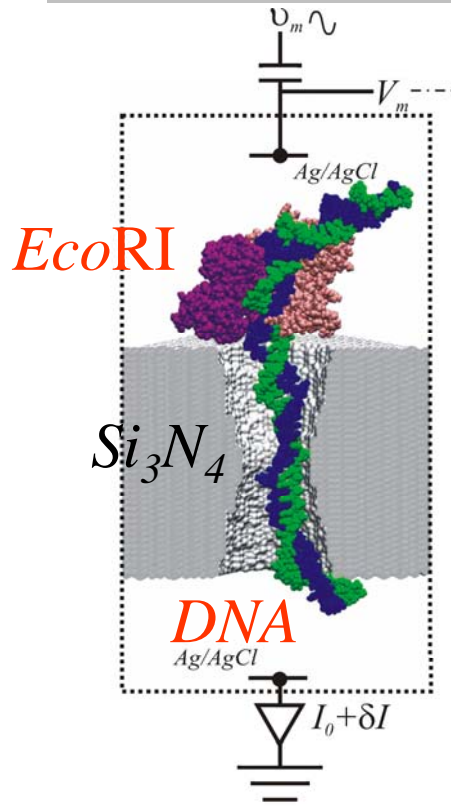
Detecting Threshold Electronically

100V/sec ramp



• trap *DNA* between E-field and *EcoRI*

Permeation of dsDNA bound to Enzymes

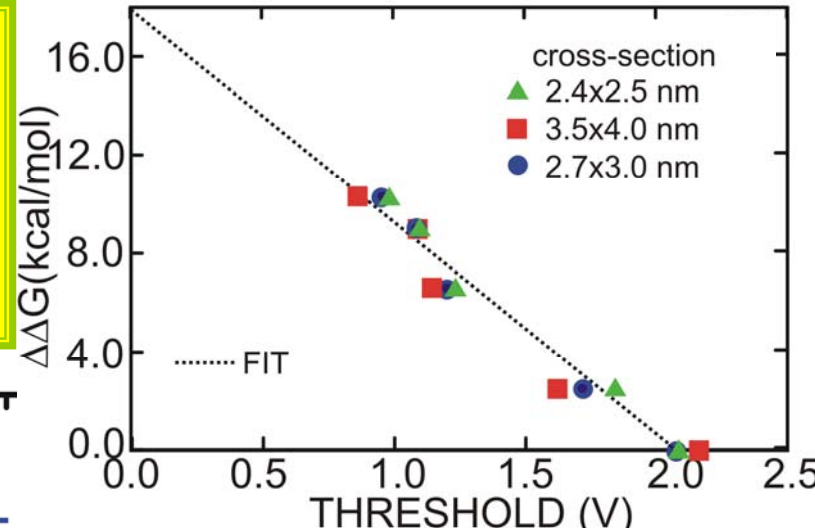
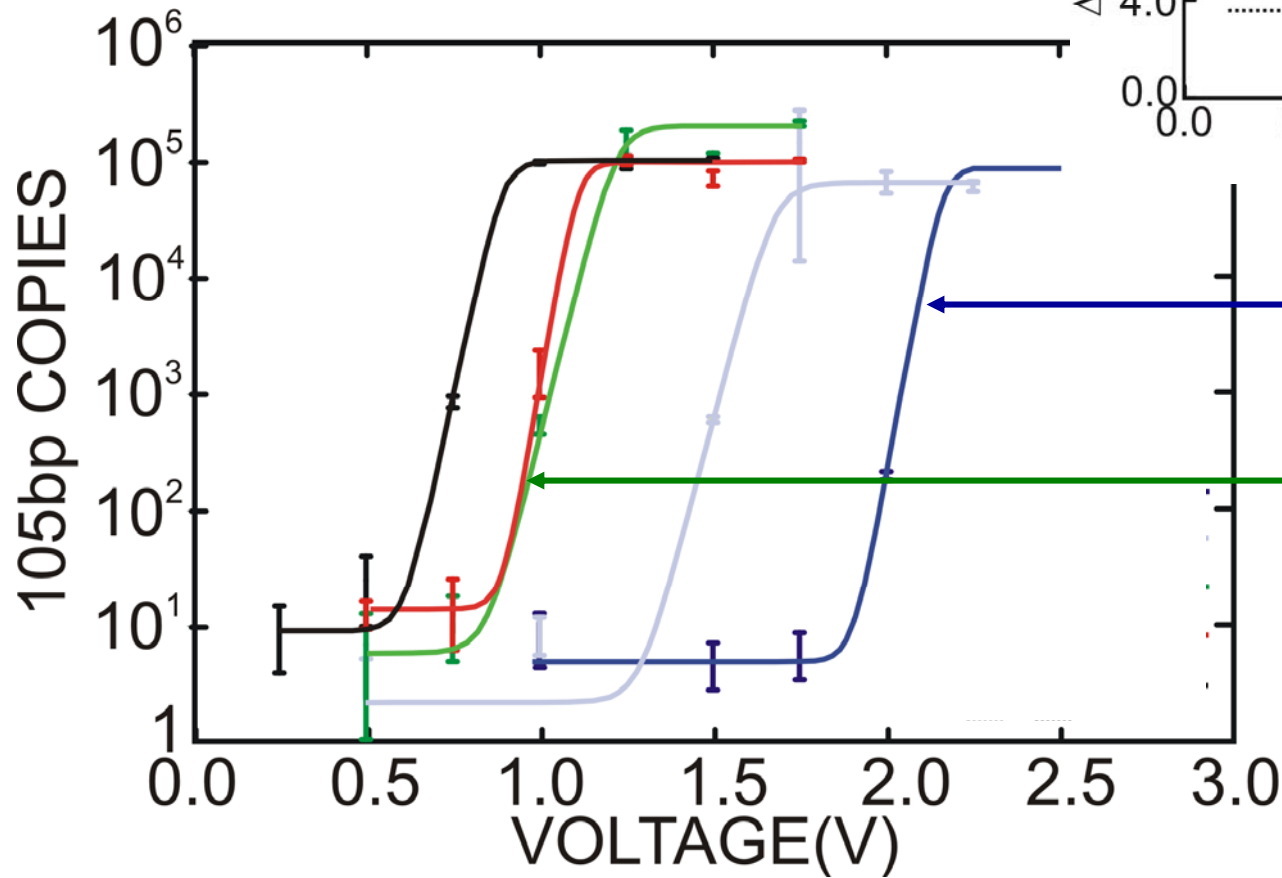


- restriction enzymes (like *EcoRI*) bind tenaciously to cognate sites on *DNA* (—GAATTC—).
 - ~6nm size frustrates translocation through <6nm pore.
- voltage threshold scales with the binding energy for proteins: i.e. *BamHI* ($\Delta\Delta G = -13.2 \text{ kcal/mol} \rightarrow 1.8 \text{ V}$) (—GGATCC—) while *EcoRI* ($\Delta\Delta G = -15.2 \text{ kcal/mol} \rightarrow 2.1 \text{ V}$ threshold) (—GAATTC—)

Detecting SNPs Using the Threshold

(reminiscent of *RFLP* but without fragments)

- voltage threshold scales with bulk dissociation energy
- **sensitive to single base mutation**
- insensitive to pore cross-section



TGAATTC —

AGAATTC —

TTAATTC —

TGACTTC —

TGCATTC —

Highlights

- ***Cell signals*** (including ions, small molecules, proteins, DNA, temp., pH, mechanical, electrical) ***affects gene activity*** (protein synthesis), enzyme activity, motor proteins, etc.
- ***In vivo sensing is mainly comprised of fluorescent probes*** (single molecule, FRET, ...), but toxicity and dynamic range compromise detection.
- ***Electrical detection offers advantages over fluorescence***: robust, simultaneous detection of multiple analytes, extreme sensitivity and improved dynamic range.
- ***Silicon Nanotechnology offers exquisite, sub-nanometer control of the electric field***. We intend to leverage this feature for sensing/sequencing single molecules translocating through a synthetic nanopore.