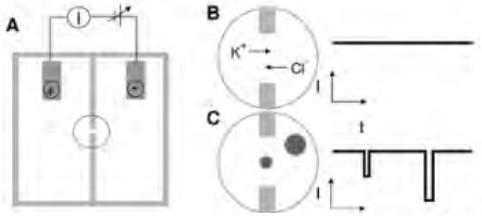
Investigating primary protein structure by nanopores

Coulter-counter



1953 - Patented
1970 - widely used for cell counting

- Current recorded through the pore
- Particles floating through the pore hinder the current flow:
  - #of particles=Fq of current drops
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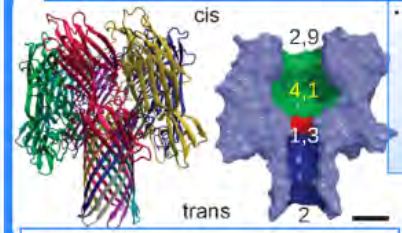
Nanopores

Howorka S, Siwy Z, Chem Soc Rev, 2009

Defining properties of NP analytics
- "blank" pores;
  no specificity to substrate
  detection is based on steric effects
- pores are in artificial membranes;
  organic/inorganic

Various types of Nanopores
protein, silicone, polymer (PET, PC), glass nanopipettes

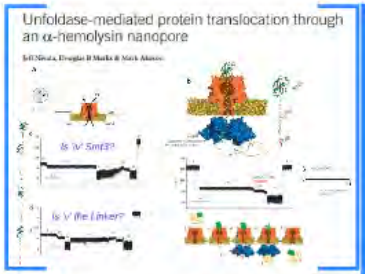
Protein Nanopores
- α-hemolysin (αHL);
  heptameric, robust
  blank
  no moving part
- OmpG;
  monomeric
  good for single mutations
  flexible loops
- OmpG; MspA, gramicidin, alamethicin

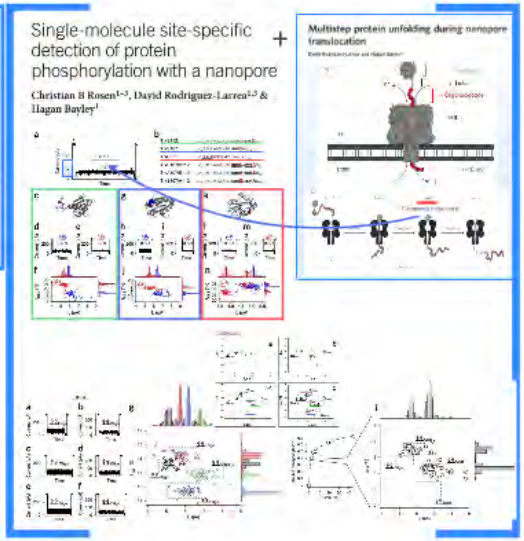


Engineering of protein Nanopores
- AA change
  Histidine for metal binding
  Hydrophobic ring for aromatic substrate
- ligands to bind DNA, Antibodies

Lipid bilayer
- 30-100µm orifices in hydrophobic polymers filled with electrolyte template the lipids, then pore solution is added
- membrane stabilizers

Detailed analysis of Proteins with Nanopores

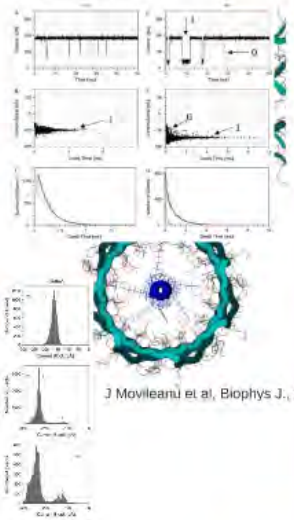




Analytes

Nucleotides

Peptides



J Movileanu et al, Biophys J., 2005

Folded/unfolded proteins

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- denaturing agents to sense unfolded proteins

Daniel Kirschenbaum, Technical Journalclub, Institute of Neuropathology, 01/07/2014

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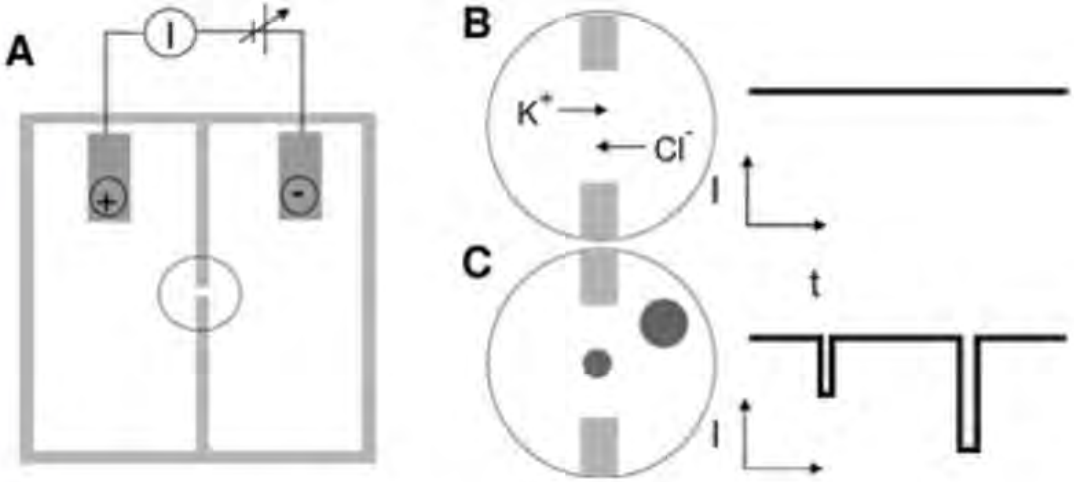

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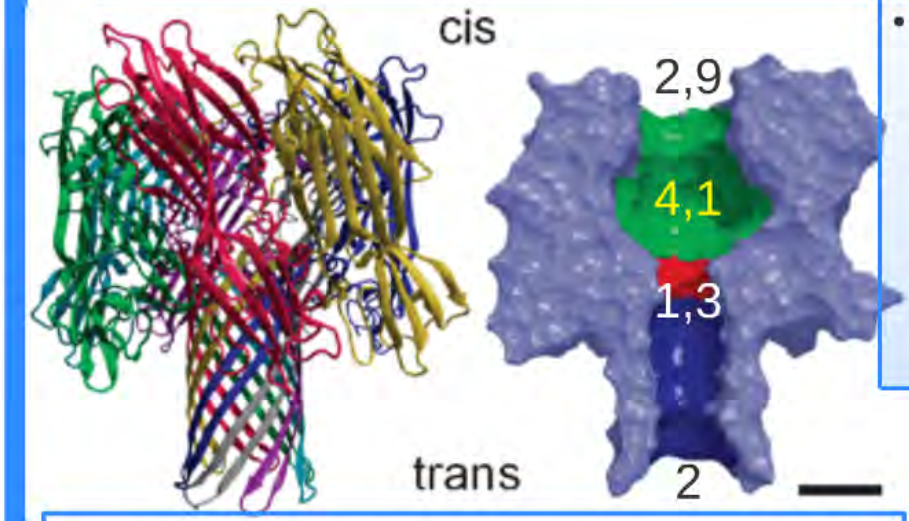

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Analytes

Nucleotides

Table 1 Sensing and c

Analyte
DNA and RNA
ssDNA and RNA, trans

Heterogeneity of ssDN

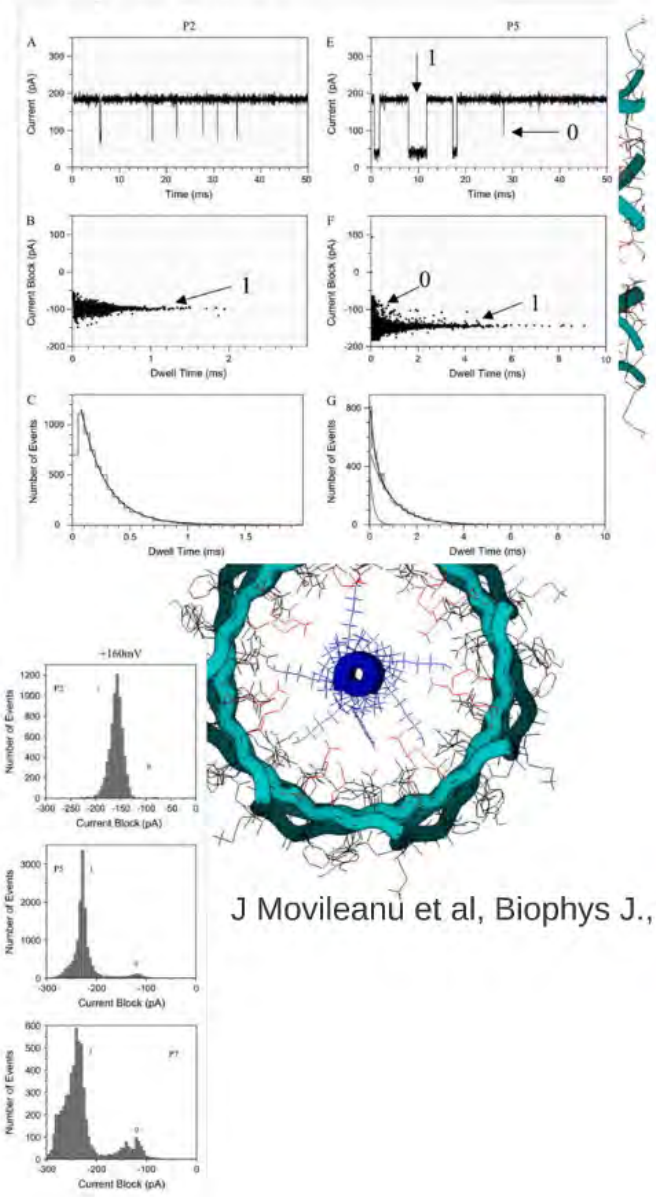
NATURE | NEWS

Nanopore genome sequencer makes its debut

Technique promises it will produce a human genome in 15 minutes.

Erika Check Hayden

Peptides



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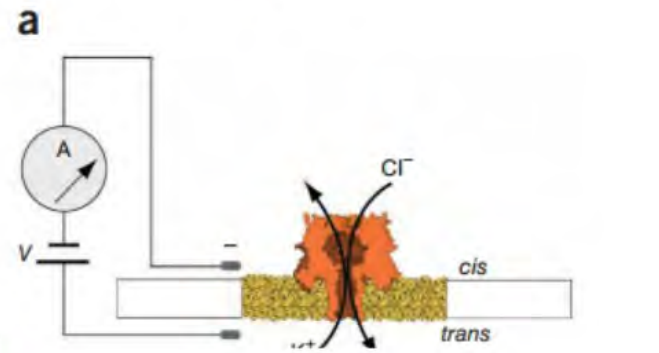
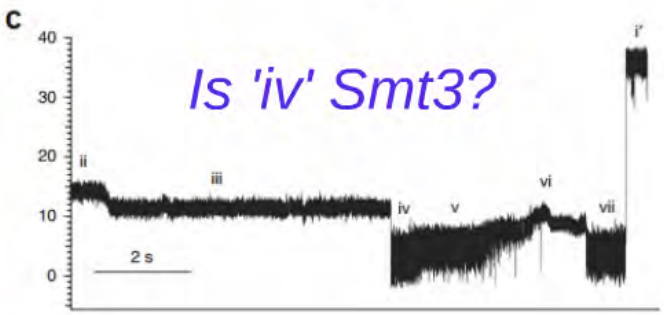
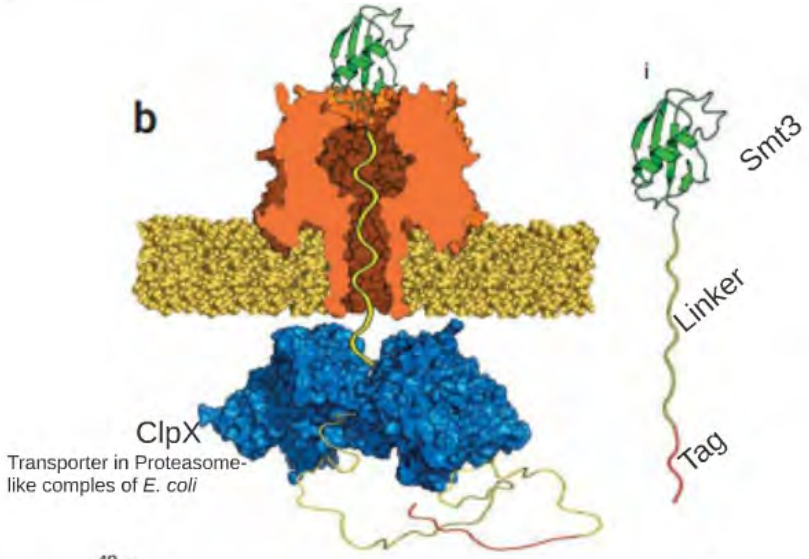
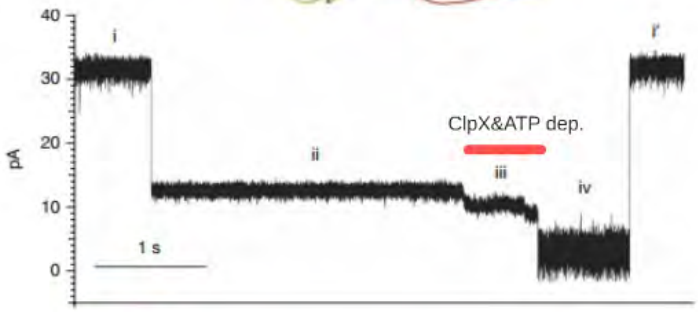
Unfoldase-mediated protein translocation through an α-hemolysin nanopore

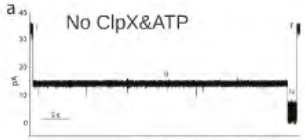
Jeff Nivala, Douglas B Marks & Mark Akeson



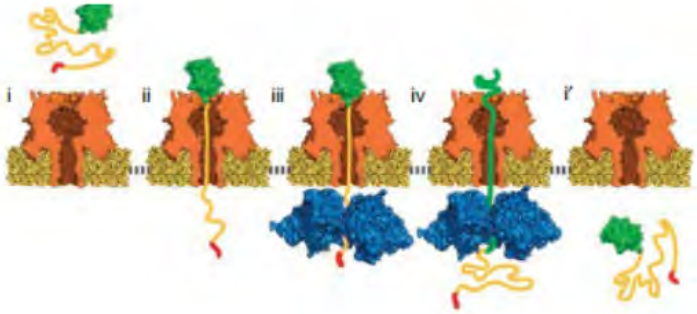


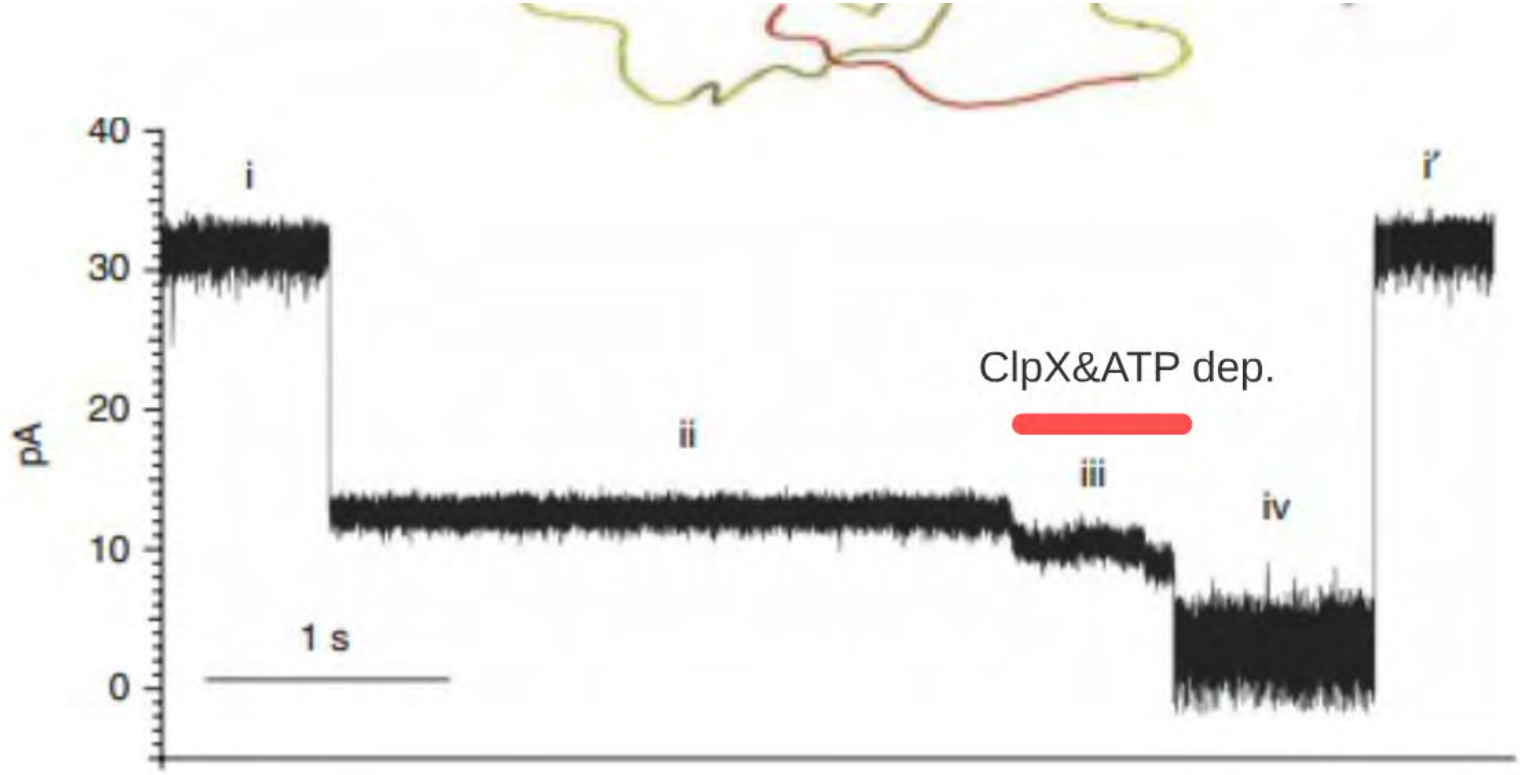


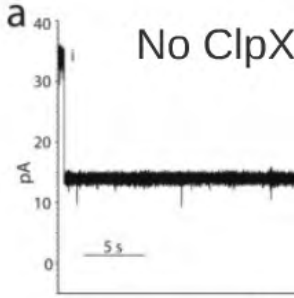




Similar result without ClpX-Tag Smt3

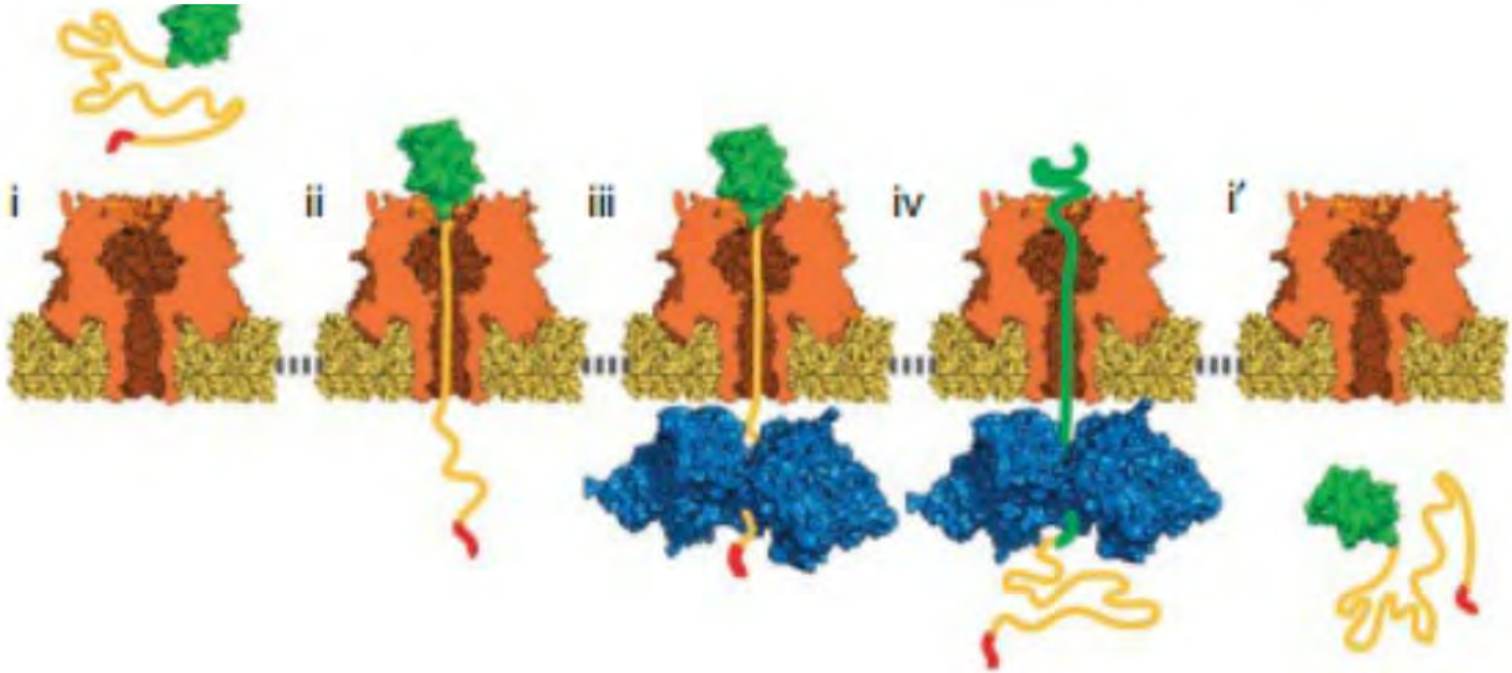


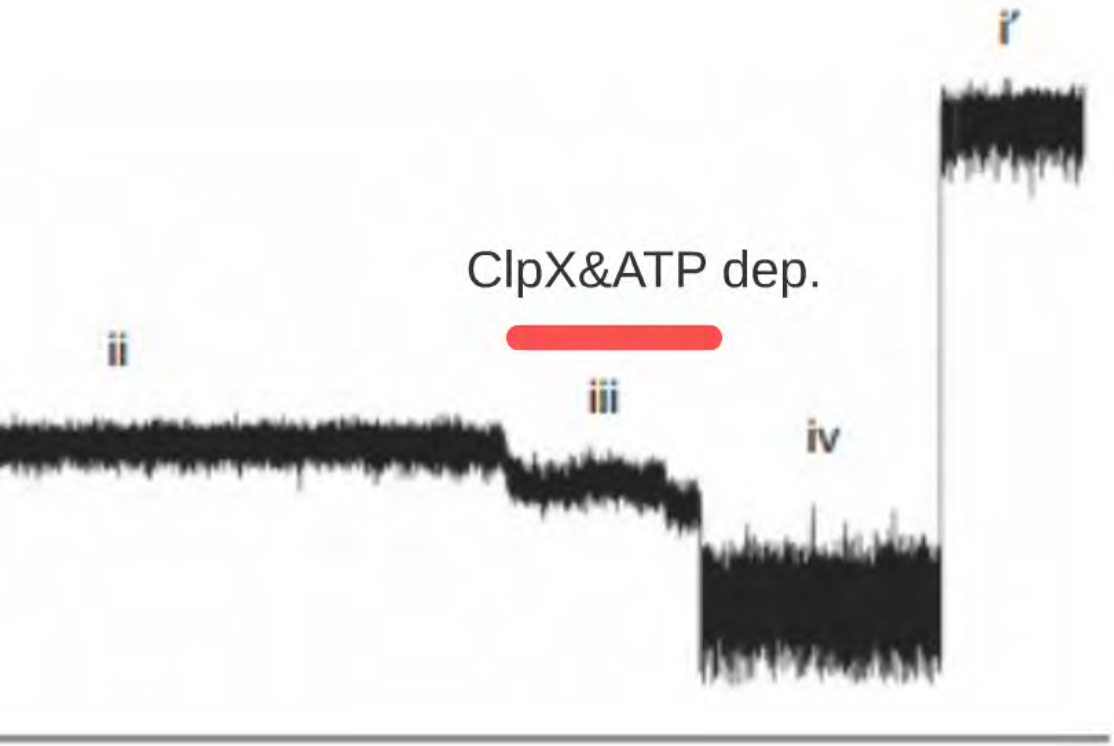


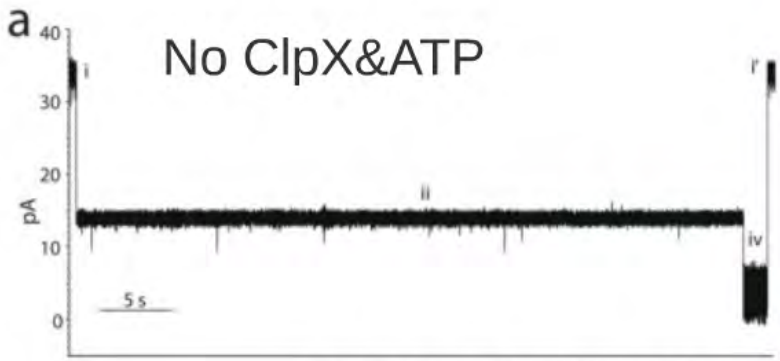


No ClpX

Sin
Tag







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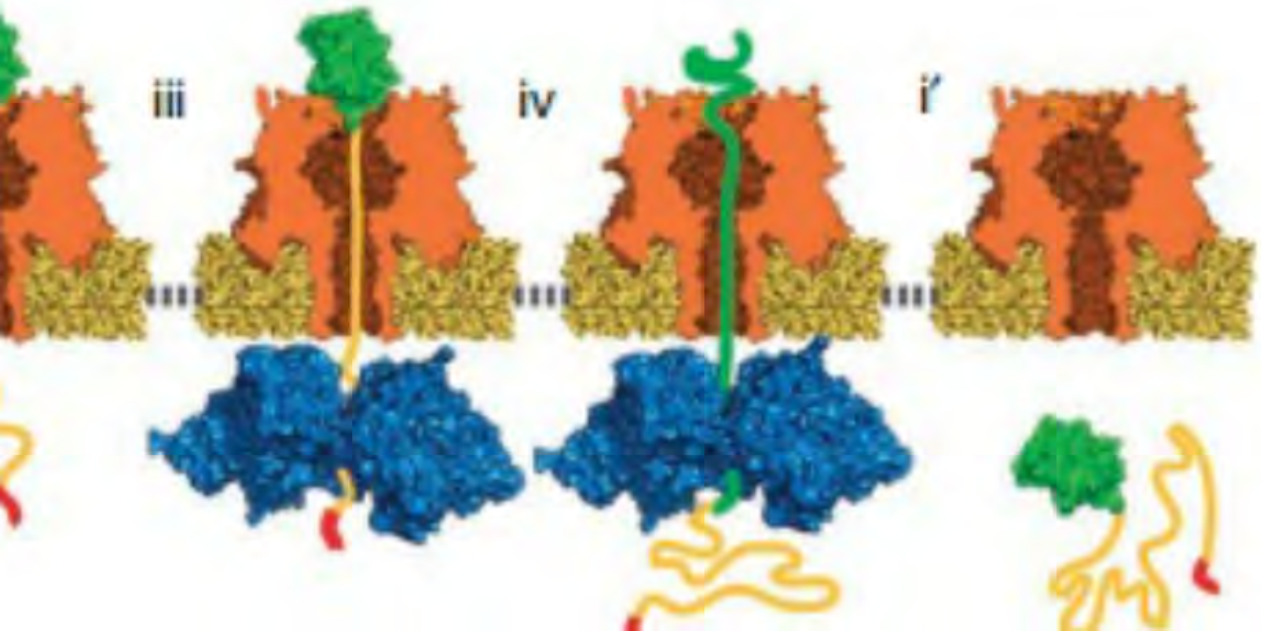


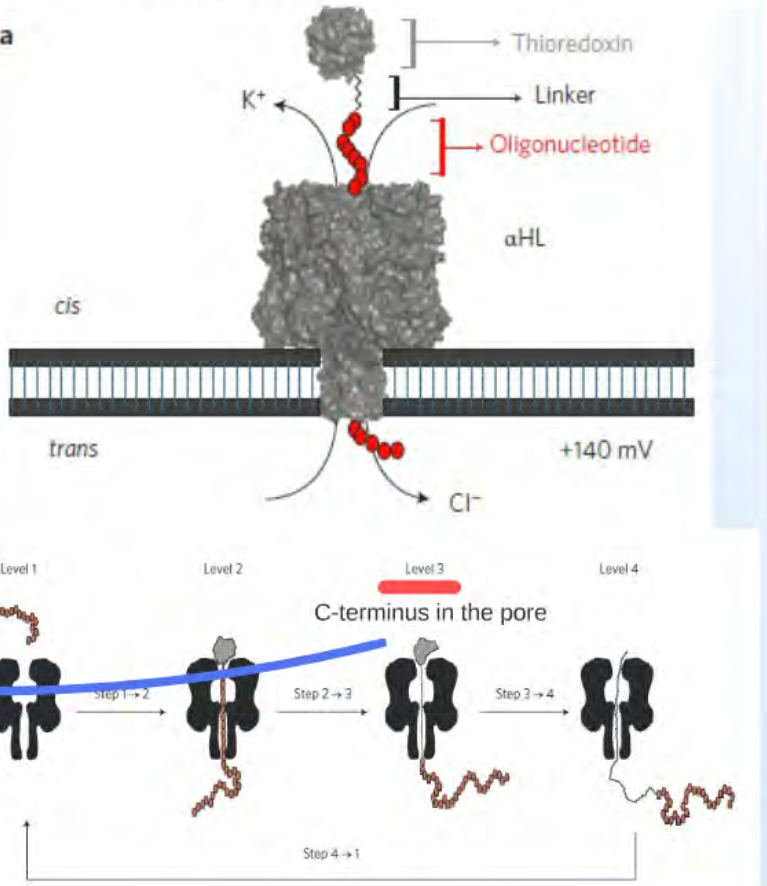
Single-molecule site-specific detection of protein phosphorylation with a nanopore

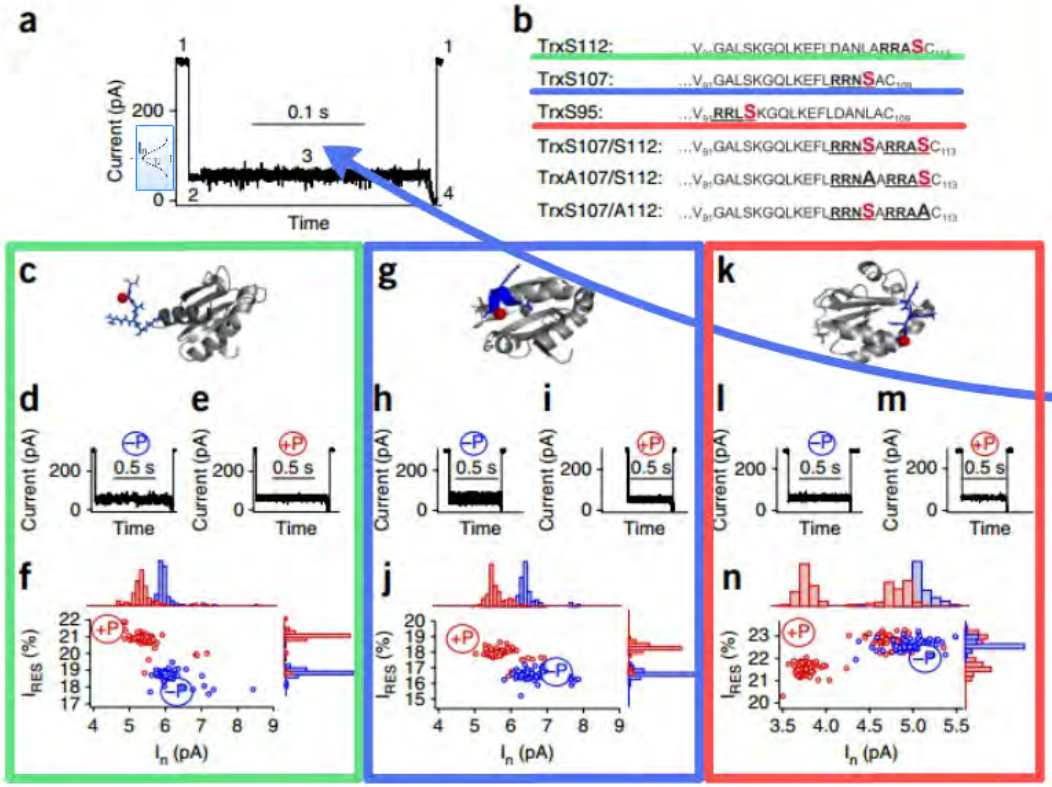
Christian B Rosen[1–3], David Rodriguez-Larrea[1,3] & Hagan Bayley[1]

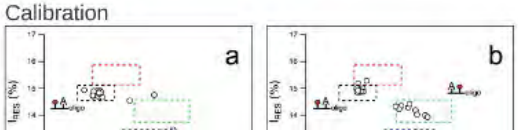
+

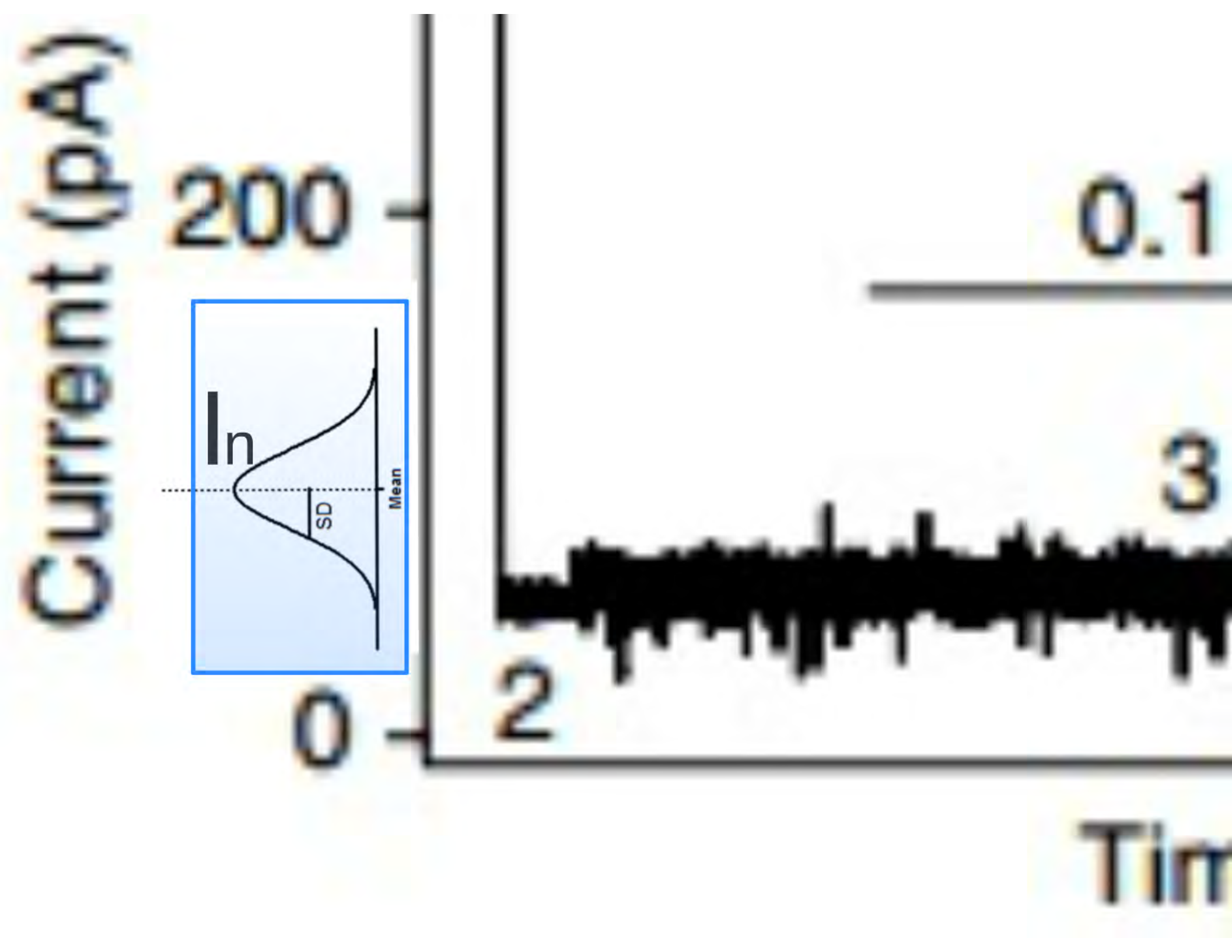
Multistep protein unfolding during nanopore translocation

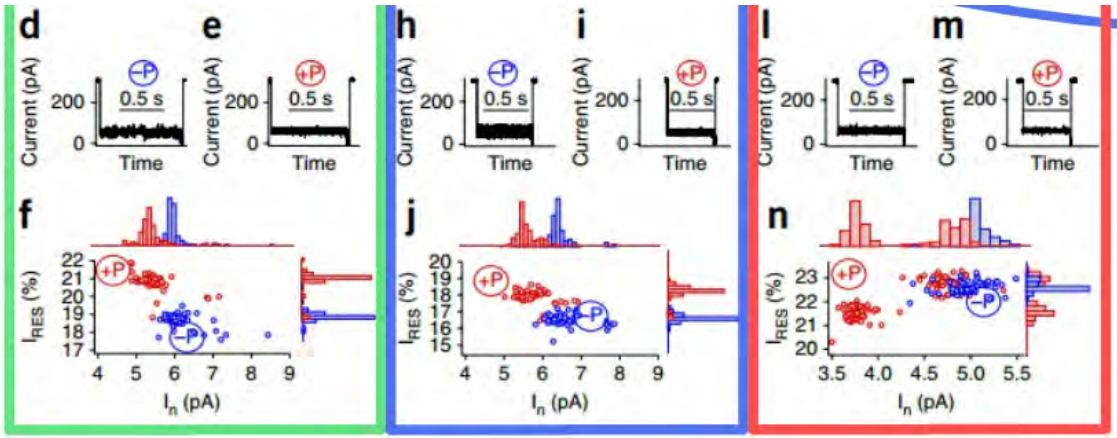
David Rodriguez-Larrea and Hagan Bayley*

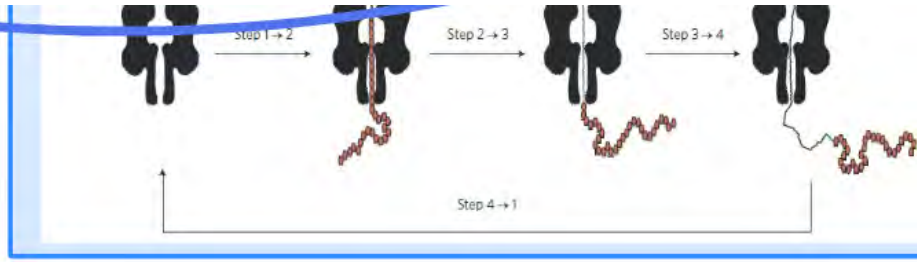


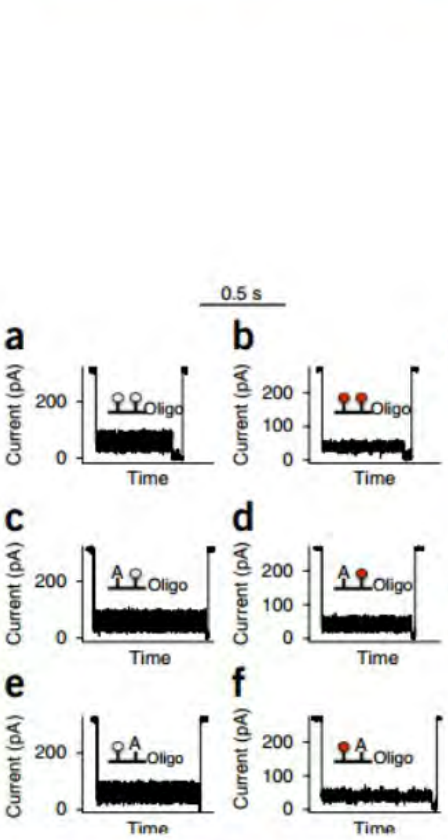


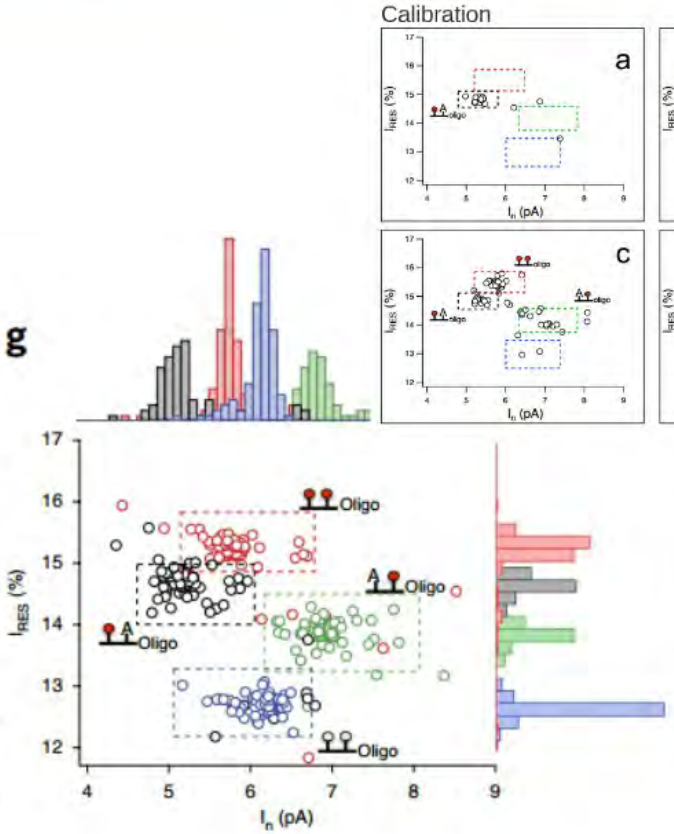



Current (pA)
200
0
In
SD
Mean
0.1
3
2
Tim

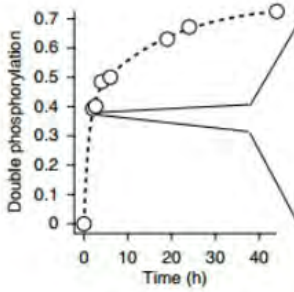

d
e
h
i
l
m
Current (pA)
200
0
0.5 s
Time
+P
−P
f
j
n
I_{RES} (%)
I_n (pA)

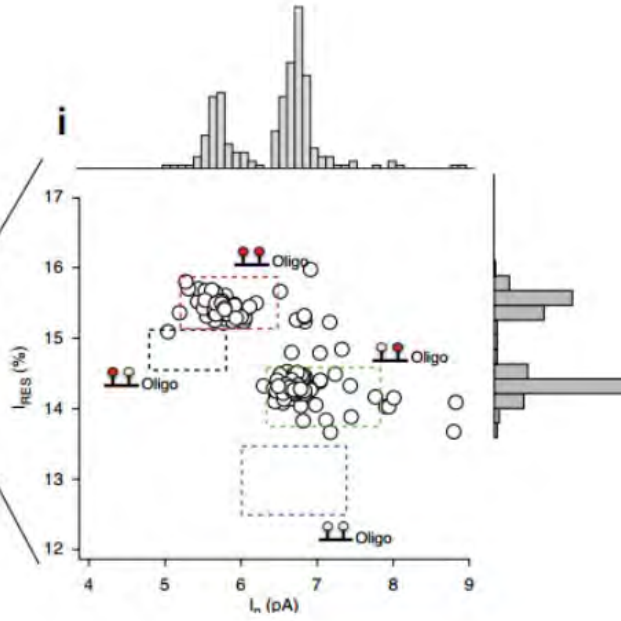

Step 1→2
Step 2→3
Step 3→4
Step 4→1


0.5 s
a
b
c
d
e
f
Current (pA)
200
100
0
Time
Oligo
A


g
I_{RES} (%)
I_n (pA)
Oligo
A

Calibration
a
b
c
d
I_{RES} (%)
I_n (pA)
Oligo


Double phosphorylation
Time (h)


i
I_{RES} (%)
I_n (pA)
Oligo

Conclusions

- It is possible to unfold proteins through a nanopore in a controlled manner
- It is possible to discriminate current traces of different AA sequences and PTM states (phosphorylation)

Advantages

In contrast to MS:

- No fragmentation
- Single polypeptide chain analysis

Drawbacks

- Needs calibration (no "fishing")
- Needs High purity
- Works only close to the c-term yet

Thank you for your attention!

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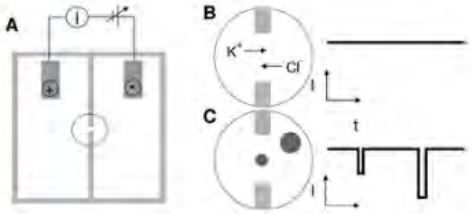

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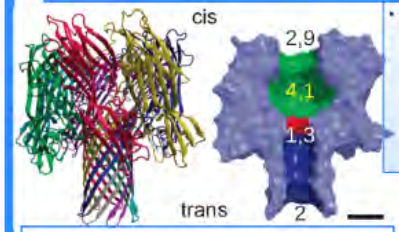

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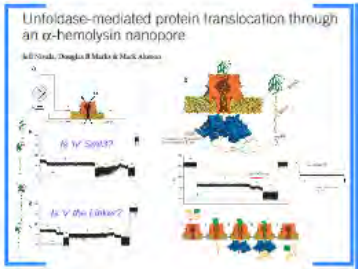

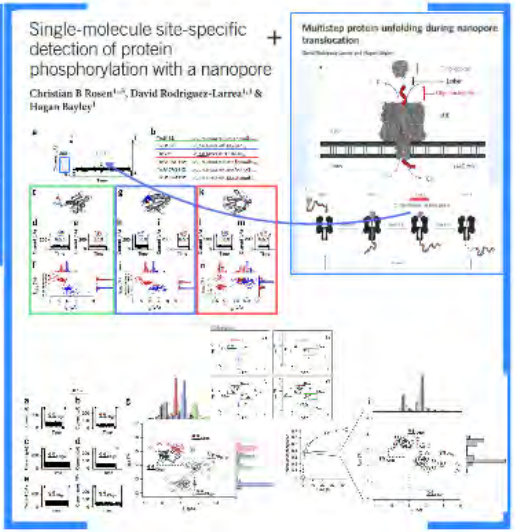

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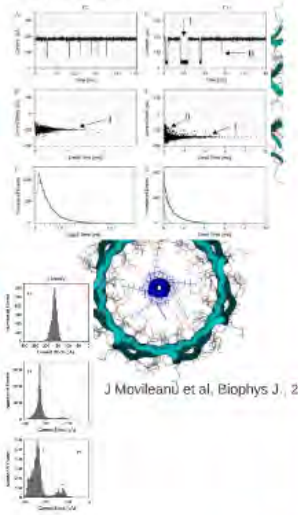

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