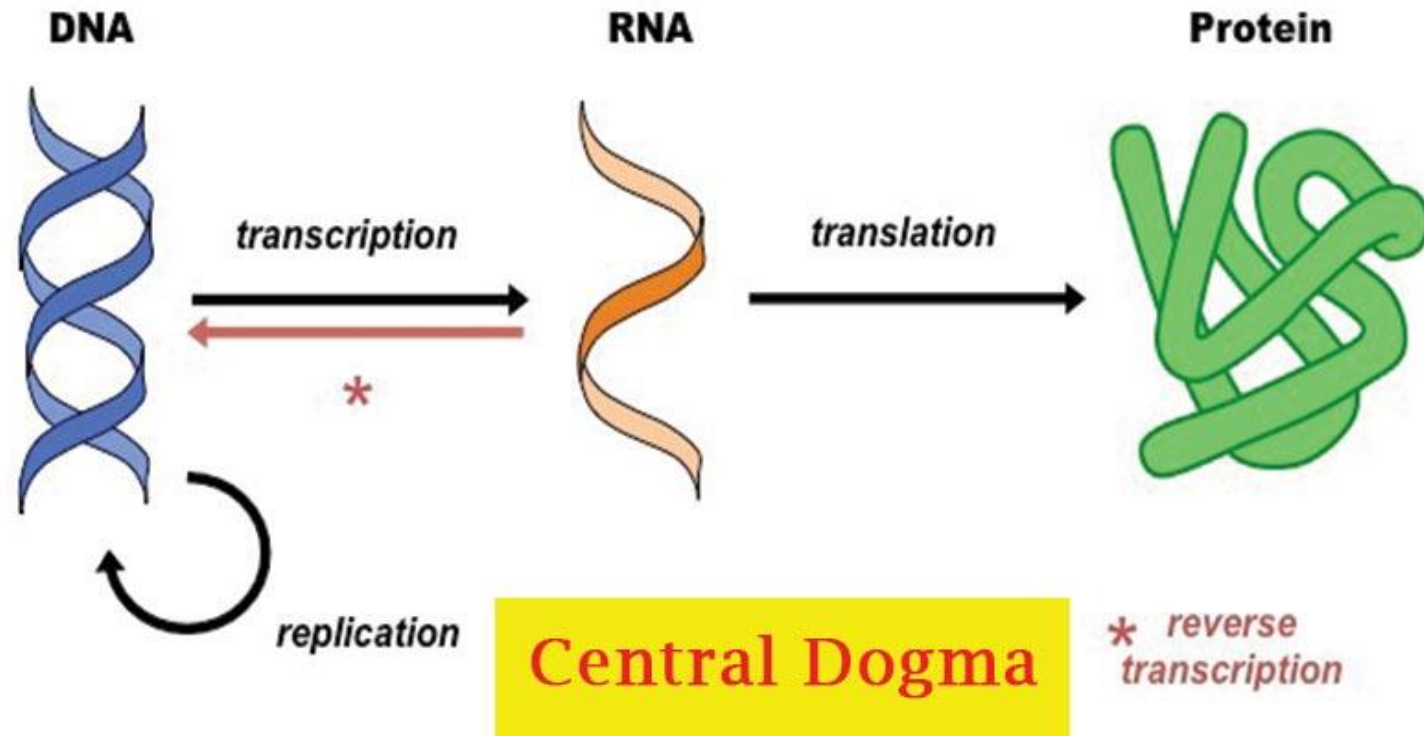




DNA, RNA and protein sequencing by biological nanopores



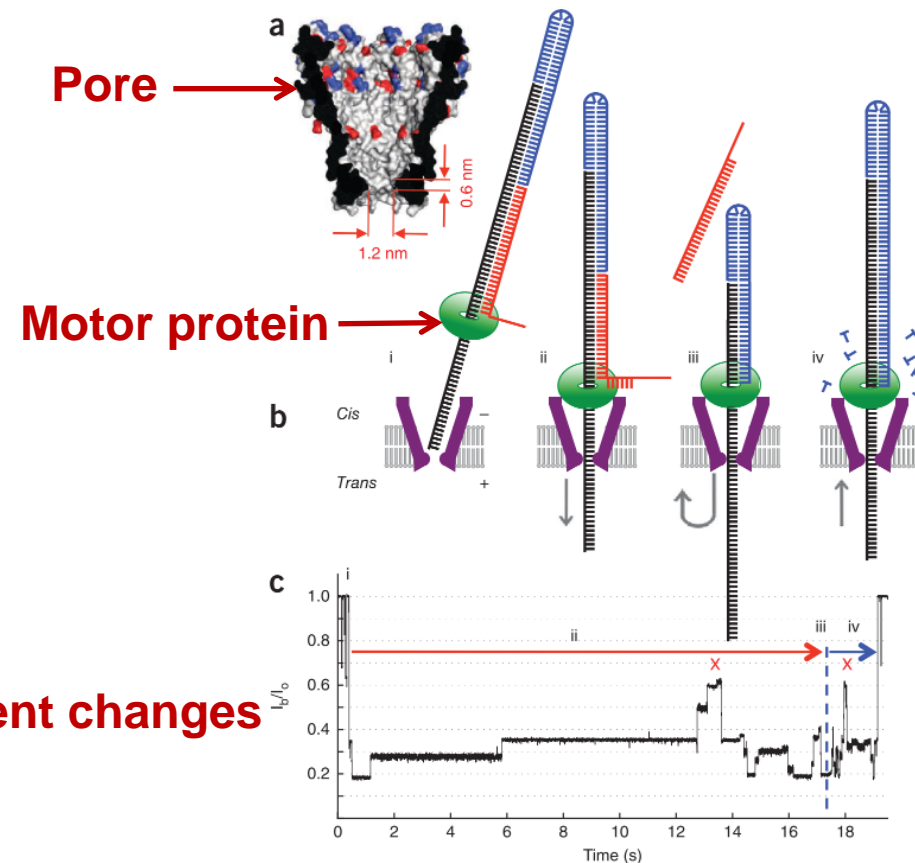
Yunhao Wang Ph.D.

August 21, 2020

Department of Biomedical Informatics, Ohio State University

Paper 1: Automated forward and reverse ratcheting of DNA in a nanopore at 5- Å precision**Gerald M Cherf @ UCSC; Mark Akeson @ UCSC; 2012****Paper 2: Reading DNA at single-nucleotide resolution with a mutant MspA nanopore and phi29 DNA polymerase****Elizabeth A Manrao @ U of Washington; Jens H Gundlach @ U of Washington; 2012**

- **Topics**
 - Nanopore DNA sequencing
- **Methods**
 - Akeson: α -hemolysin (pore) + phi29 DNA polymerase (motor)
 - Gundlach: MspA + phi29
 - ONT: CsgG for R9.4 flow cell
- **Results**
 - Four canonical bases (A, T, C, G)
 - Chemically-modified bases (e.g., 5mC, 5hmC)

**Ionic current changes**

Error rates for nanopore discrimination among cytosine, methylcytosine, and hydroxymethylcytosine along individual DNA strands

Jacob Schreiber, Zachary L. Wescoe, Robin Abu-Shumays, John T. Vivian, Baldandorj Baatar, Kevin Karplus, and **Mark Akeson**¹

Detection and mapping of 5-methylcytosine and 5-hydroxymethylcytosine with nanopore MspA

Andrew H. Laszlo^a, Ian M. Derrington^a, Henry Brinkerhoff^a, Kyle W. Langford^a, Ian C. Nova^a, Jenny Mae Samson^a, Joshua J. Bartlett^a, Mikhail Pavlenok^b, and **Jens H. Gundlach**^{a,1}

nature methods

Published: 20 February 2017

Mapping DNA methylation with high-throughput nanopore sequencing

Arthur C Rand, Miten Jain, Jordan M Eizenga, Audrey Musselman-Brown, Hugh E Olsen, **Mark Akeson** & Benedict Paten

nature methods

Published: 20 February 2017

Detecting DNA cytosine methylation using nanopore sequencing

Jared T Simpson, Rachael E Workman, P C Zuzarte, Matei David, L J Dursi, Winston Timp

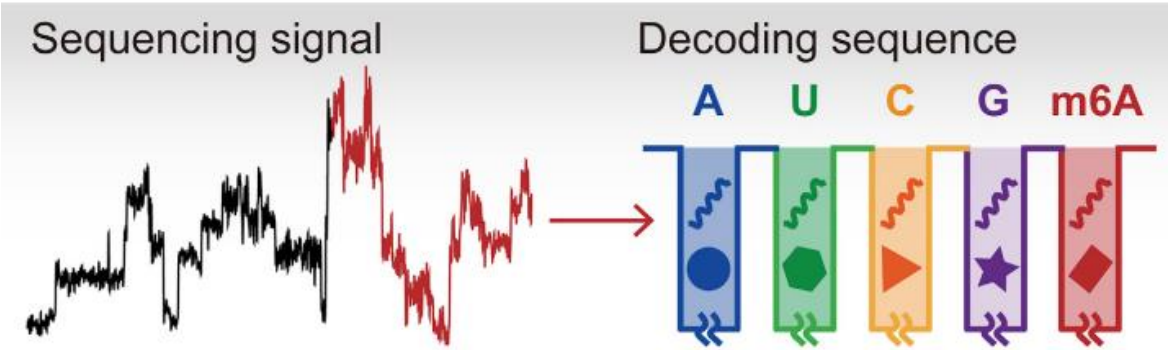
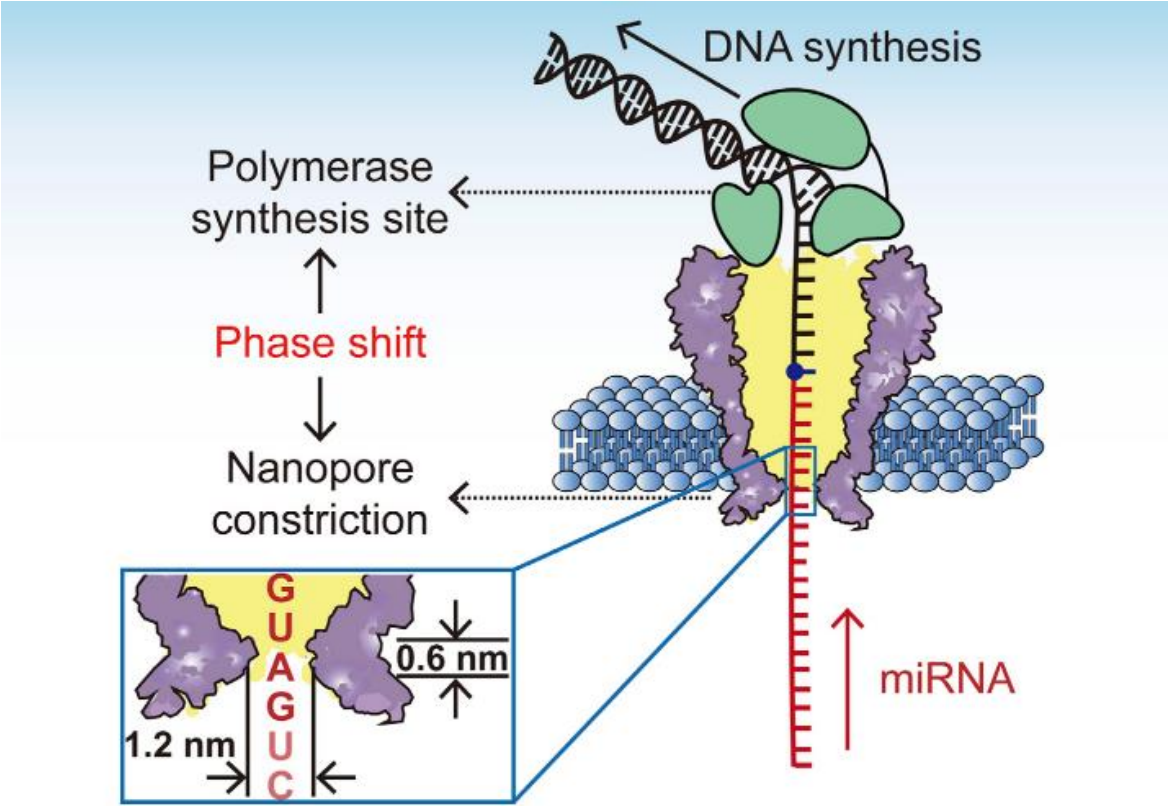
Paper 1: Highly parallel direct RNA sequencing on an array of nanopores

Daniel R Garalde; Daniel J Turner @ ONT; Nature Methods, 2018

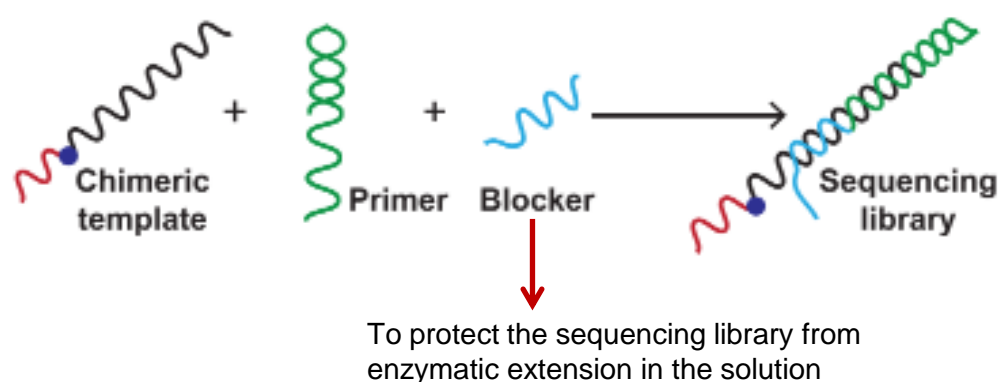
Paper 2: Direct microRNA sequencing using nanopore-induced phase-shift sequencing

Jinyue Zhang; Shuo Huang @ Nanjing University, China; iScience, 2020

- **Topics**
 - Nanopore direct RNA sequencing
- **Methods and data**
 - Turner/ONT: CsgG; long stretch
 - Nanopore configuration
 - Motor proteins
 - Huang: MspA; short stretch
 - miRNA (~22 nt)
- **Results**
 - Four canonical bases (A, U, C, G)
 - Chemically-modified bases (e.g., m6A)



Library preparation



Electrical recognition of the twenty proteinogenic amino acids using an aerolysin nanopore

Hadjer Ouldali; Abdelghani Oukhaled @ Université Paris-Seine (巴黎第十一大学), France; 2020

Topics

- Nanopore protein sequencing

Methods

- Aerolysin

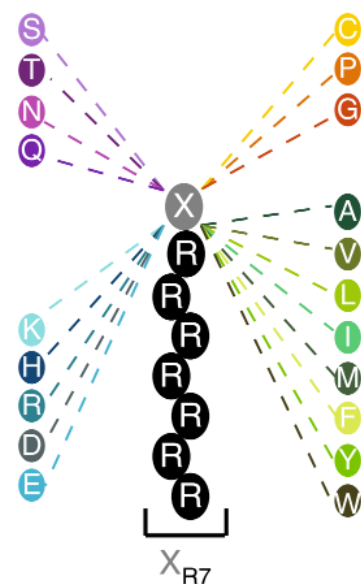
Results

- 13 of 20 natural amino acids with WT aerolysin
- All 20 amino acids with the help of chemical modifications, instrumentation advances and nanopore engineering

Outlook

- Residue-by-residue protein sequencing
- Residue-specific post-transcriptional modification detection
- A peptide chain continuously passes through the nanopore?
 - Tether a protein digestion enzyme to the aerolysin?

Library preparation



Sequencing

