Expression and Purification of DNA Polymerase 1 (Klenow Fragment) for Single-Molecule Nanotube DNA Sequencing

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Introduction

Point mutations within a DNA sequence can cause mutations leading to disease. Examining these point mutations using single-molecule methods can advance our understanding of enzyme function at a finer scale and further improve DNA sequencing for commercial and clinical purposes. Conventional single-molecule DNA sequence detection methods have been developed using optical and force based techniques, such as single-molecule fluorescence and magnetic tweezers. Our research analyzes electronic transduction via single-walled carbon nanotube field effect transistors (SWCNT-FETs).

Expression and Purification

Methods

Expression and Purification

Variant Design

<table>
<thead>
<tr>
<th>Variant</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>L790C</td>
<td>Wild type with exonuclease activity and (Lys790Cys)</td>
</tr>
<tr>
<td>L790C (+)</td>
<td>Positively supercharged protein surface proximal to carbon nanotube in an effort to enhance signal to noise ratio</td>
</tr>
<tr>
<td>L790C (-)</td>
<td>Negatively supercharged protein surface proximal to carbon nanotube in an effort to enhance signal to noise ratio</td>
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</tbody>
</table>

Mutations were made on KF for testing attachment on SWCNT-FETs. Variants produced were expected to increase the signal to noise ratio observed at attachment with the nanotube.

Specific Aim

To examine mutagenic protein variants to improve SWCNT attachment efficiency and the signal to noise ratio- observed during deoxynucleotidetriphosphate (dNTP) incorporation.

Klenow Fragment

The Klenow Fragment (KF) domain of DNA Polymerase I catalyzes dNTP incorporation during DNA replication. KF lacks the 5’→3’ exonuclease domain, but retains its 5’→3’ polymerase and 3’→5’ nuclease activity.

Previous attachment study of KF has shown efficient attachment and measurement with the SWCNT-FET after mutating lysine at position 790 into cysteine.

Variant Design

Discussion

KF L790C was expressed, purified, and concentrated. The protein appeared active based on the gel band shift shown in lane 6 of the activity assay. This active protein was then given to the Collins lab for attachment studies.

Attachment study involves a SWCNT-FET placed between two electrodes, a source, and a drain. The protein is attached to the nanotube via a pyrene maleimide linker conjugated to a single cysteine. The signal produced was analyzed via a current amplifier.

In the future, experiments are planned with other variants of the Klenow Fragment as well as other DNA Polymerases to increase the signal to noise ratio.

Acknowledgements

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References