NanoRING: Nanomaterials undergraduate Research in Germany

Impact of heparin on kinetic parameters of DNA endonuclease Cas9

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Motivation

- Cas9, a DNA cutting protein important in bacterial defense against viruses, has potential as a DNA-editing technology in humans.
- Experiments to date use a polymer called Heparin to reduce unspecific binding of Cas9 when studying its binding to target DNA
- We hypothesize Heparin interferes with the Cas9-DNA interaction and leads to inaccurately measured rate constants
- This work uses switchSENSE technology to show Heparin reduces the ability to characterize Cas9

Background

Figure 1. Cas9 uses a guide RNA to find complementary target DNA for cleavage.

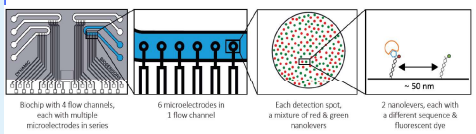

Figure 2. Layout of switchSENSE chip used in all experiments

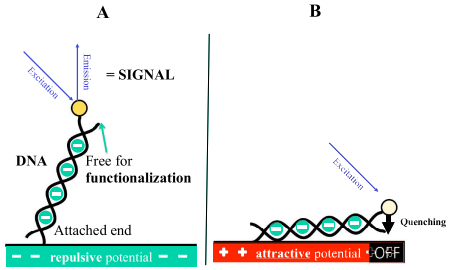

Figure 3. The DNA strand switches between a horizontal (A) and vertical (B) orientation based on the voltage applied to the gold surface.

Theory

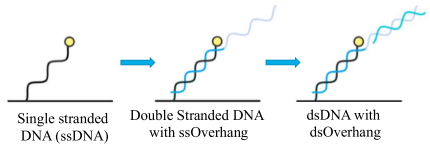

Figure 4. An overhang that is targeted by the Cas9-gRNA complex is attached to the surface tethered DNA.

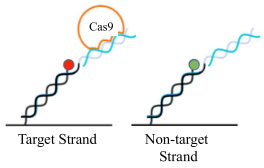

Figure 5. Red and green fluorophore are used to distinguish between specific and unspecific binding of Cas9.

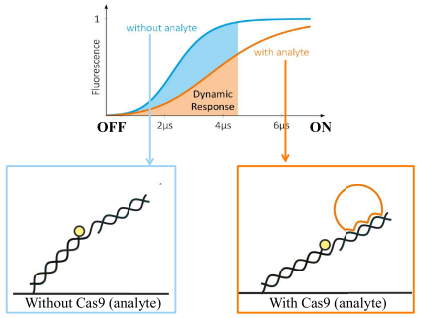

Figure 6: Upon analyte binding, DNA levers move more slowly, decreasing the dynamic response. The reverse occurs during unbinding.

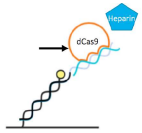

Figure 7. Heparin is used in standard Cas9 studies, but may interfere with the Cas9-DNA interaction.

Results

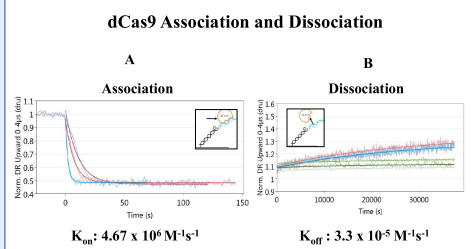

Figure 8. Decrease in dynamic response represents Cas9 association (a) while increase in dynamic response represent dissociation of Cas9 from DNA (b).

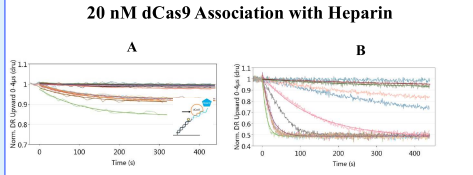

Figure 9. (a) Unspecific binding was monitored via the fluorescence of green fluorophore tagged non-target DNA levers. **(b)** Heparin concentrations of 375 ng/mL (black) and higher led to a decrease in K_{on} until no binding of Cas9 to target DNA could be observed.

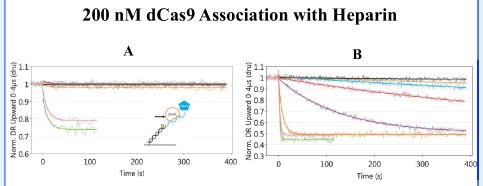

Figure 10. (a) Unspecific binding is observed in control samples (pink and green), but this is removed upon the addition of 3 ug/mL Heparin **(b)** At a Heparin concentration of 3 ug/mL (orange), specific binding of dCas9 decreases, shown by the lower K_{on} rate constants as the Heparin concentration increases.

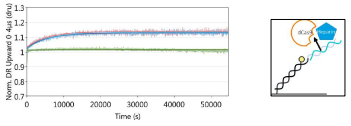

Figure 11. The average K_{off} (from 20, 40, 80 nM dCas9) for a dissociation that included Heparin was 1.8 x 10^{-4}Unbinding occurred on target strands only, as there was little unspecific binding of dCas9 even without the use of Heparin.

Conclusions

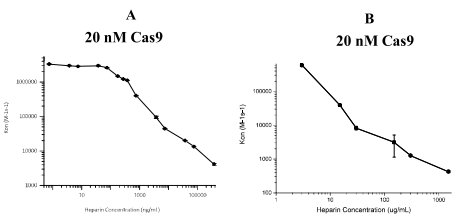

Figure 12. K_{on} values are shown to decrease as higher concentrations of Heparin are used in the association of 20 nM Cas9 **(a)** and 200 nm Cas9 **(b)**.

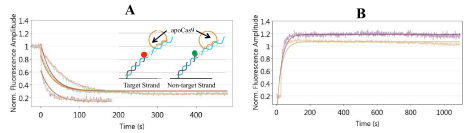

Figure 13. (a) Association of apoCas9 **(b)**.apoCas9 dissociates completely, so lack of complete dissociation in previous experiments is specific to dCas9.

- Heparin reduces the ability for kinetic parameters of Cas9 to be measured
- Unspecific binding should not be completely repressed
- The switchSENSE method is able to measure specific and unspecific binding of Cas9 without the need for Heparin

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Acknowledgment

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