# Fluorescence polarization reveals a possible displacement model of competition in PRC2:RNA:DNA interactions

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*The following is an excerpt from a longer piece. For full text, please visit https://scholar.colorado.edu/concern/undergraduate_honors_theses/5x21tg835*

**Abstract**

PRC2 is a histone methyltransferase that acts on histone subunit H3 at lysine 27 to repress chromatin state and inhibit gene expression. The interactions between PRC2 and RNA have been heavily studied in vivo and in vitro with conflicting results reported on the relationship, but there is less data on interactions between PRC2 and DNA. Fluorescence polarization-based methodology was used with various RNA and DNA species to study the binding kinetics of PRC2 with RNA and DNA. Previous data indicate that PRC2 has a relatively high affinity for DNA species that are rich in consecutive G and C nucleotides. Double-stranded DNA species with lengths of 50-60 bp were designed for this project. Fluorescence polarization binding experiments were used to identify the general binding affinity of the DNA and RNA species to PRC2 by calculating the Kd apparent for the binding curve. After the apparent binding affinities were determined, various FP-competition experiments were performed to determine if each DNA species could be competed off by an RNA species or itself, and vice versa. These results showed that the DNA species were more effective competitors although the RNA species were stronger binders. These results suggest a mechanism for RNA-mediated PRC2 regulation that could reconcile the conflicting experimental results and interpretations of past experiments. Further FP-based Kd experiments under varying salt concentrations revealed PRC2 has ionic interactions with DNA that are not seen with RNA. This suggests PRC2 has extra unique interactions with DNA, which could explain the differing effectiveness of DNA versus RNA as competitors. Altogether, the results imply that DNA has additional contacts with PRC2 that limit its displacement by RNA, allowing for RNA to guide PRC2 to its target genetic loci and then for PRC2 to deposit its methyl marks without being sequestered by RNA.

**Lay Summary**

PRC2 is a protein involved in gene expression. Its role is to repress chromatin state, inhibiting gene expression during development. This protein is vital to development
and has been linked to multiple diseases of the body including skin cancer and other forms. When the protein is doing its job correctly, it interacts with DNA in order to repress chromatin. RNA has been shown to be vital in anchoring PRC2 in the correct spot on chromatin, but studies have shown conflicting results

in how it interacts with PRC2. In order to study how PRC2 interacts with DNA and RNA, fluorescence polarization-based methodology was used with various RNA and DNA species to study their binding kinetics to PRC2 individually. After the apparent binding affinities were determined, various FP-competition experiments were performed to determine if each DNA species could be competed off by an RNA species or itself, and vice versa.

The DNA species were more effective competitors although the RNA species were stronger binders. These results along with other experiments revealed PRC2 has ionic interactions with DNA that are unique to DNA, which explains how the DNA species could compete off RNA. Altogether, the results imply that DNA has additional contacts with PRC2 that limit its displacement by RNA, allowing for RNA to guide PRC2 to its target genetic loci and then for PRC2 to deposit its methyl marks without being sequestered by RNA.