

Spectroscopic detection of Z-DNA in short oligonucleotides

Presented by : Murtaza Shabbir-Hussain

Advisor: Dr. Milan Balaz

Department of Chemistry

University of Wyoming.

Background

- **DNA** (Deoxyribonucleic acid)
- Watson and Crick – First to discover the molecular structure of DNA in 1953.
- Many known conformations of DNA exist today.
 - A-DNA
 - B-DNA
 - C-DNA
 - Z-DNA

Background

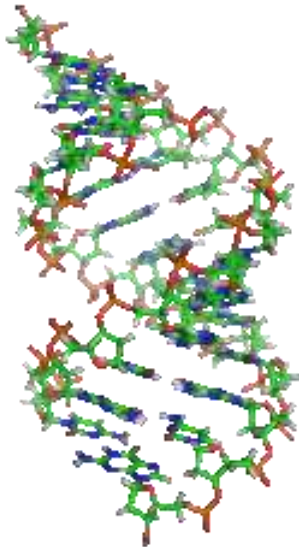
A-DNA

Right handed structure (clockwise rotation)

~11 base pairs per turn

Rise/base pair – 2.3 Å

Moderate amount of energy required to attain conformation.



B-DNA

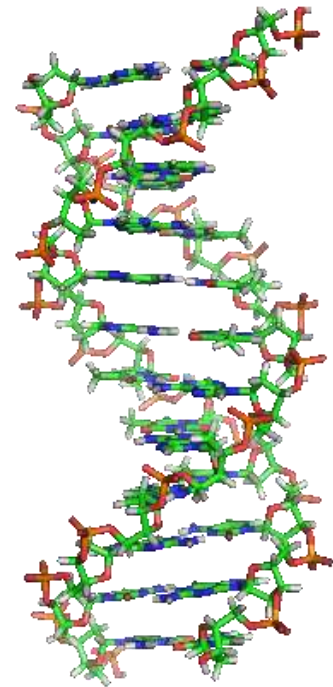
Predominant form in biological systems.

Right handed structure (clockwise rotation)

~10 base pairs per turn

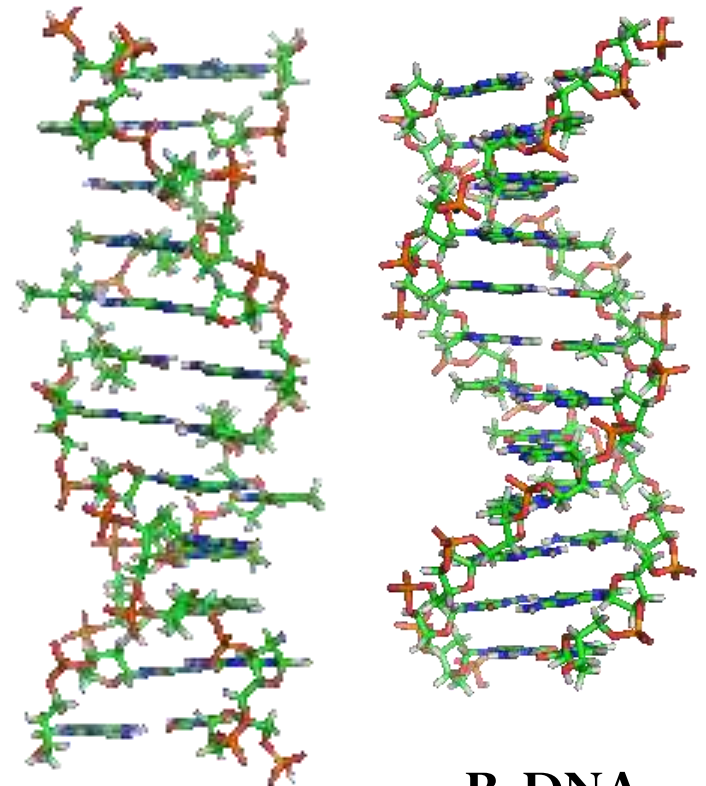
Rise/ base pair – 3.4 Å

Lowest amount of energy required to attain conformation.



Background – Z-DNA structure

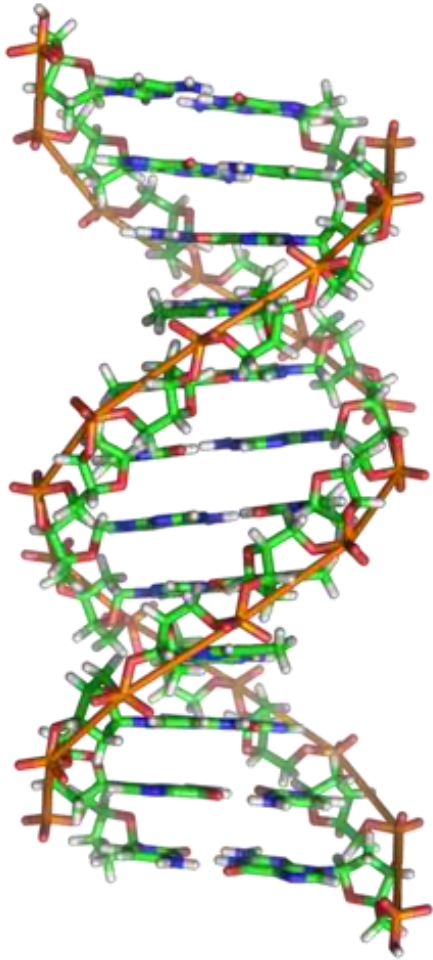
- Z-DNA – only known Left handed helical structure (counterclockwise rotation)- Discovered by Alexander Rich in 1972.
- ~12 base pairs/turn
- Rise/per base pair – 3.8 Å
- Longer and more elongated than the A or B form
- Requires the greatest amount of energy to assume conformation



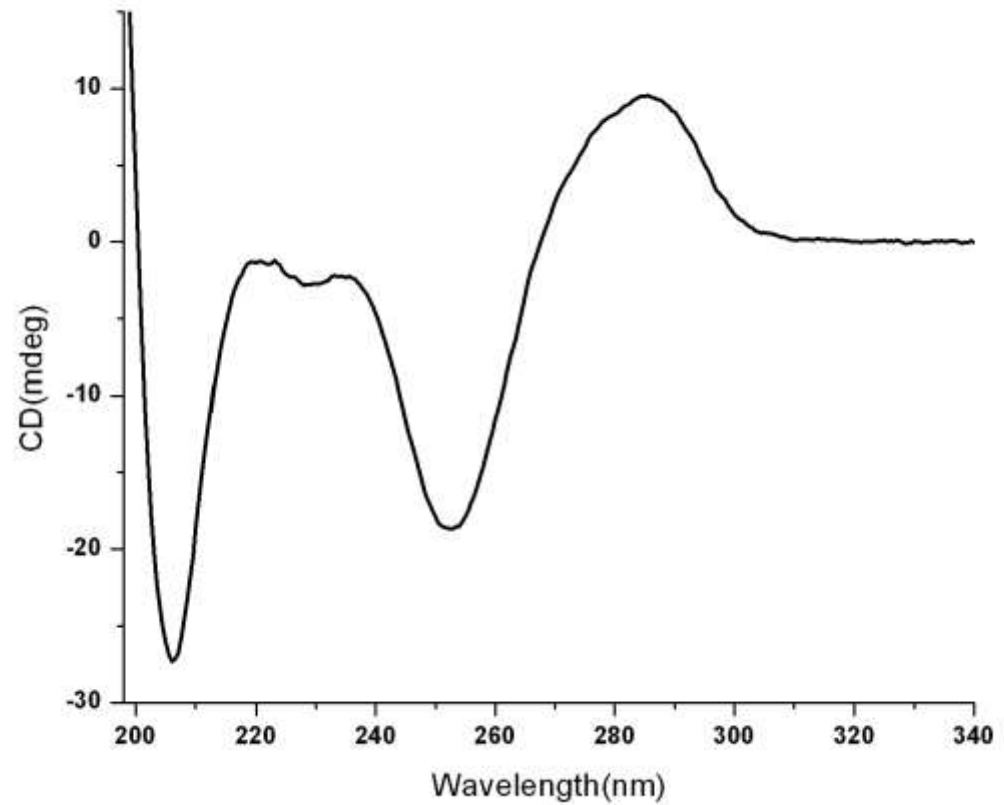
Z-DNA

B-DNA

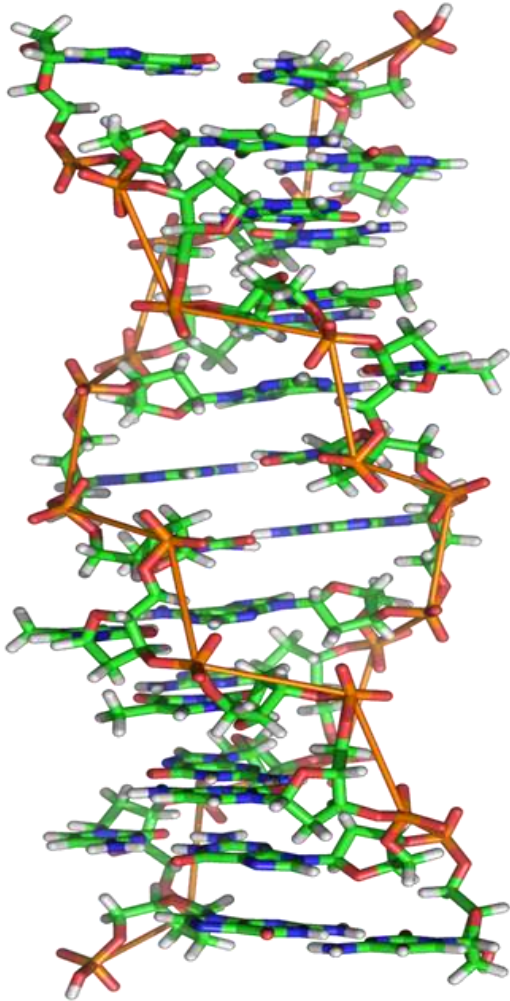
Background – Structure comparison



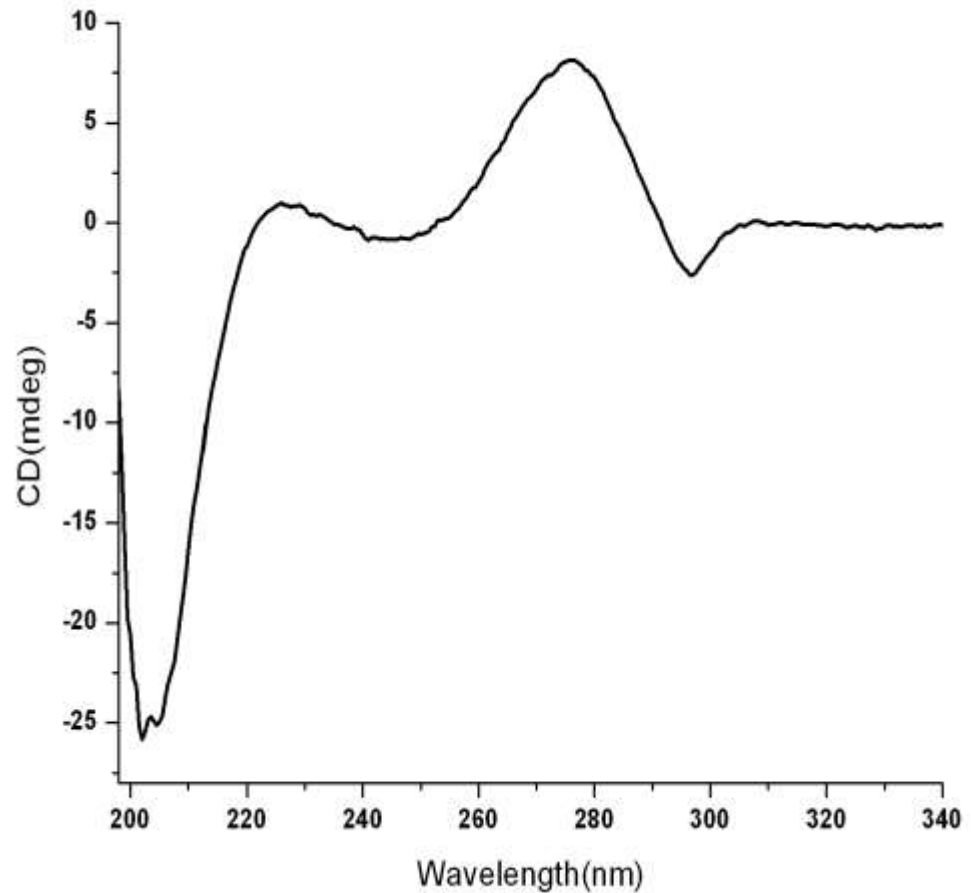
B-DNA



Background – Structure comparison



Z-DNA



Introduction

- **Biological Significance of Z-DNA**

Biological function still unclear

BUT....

- **Regulation of Transcription in protein synthesis.**

- Blocks activity of RNA polymerase.

RNA polymerase is unable to transcribe through Z-DNA genes.

- Provides torsional strain relief while transcription occurs

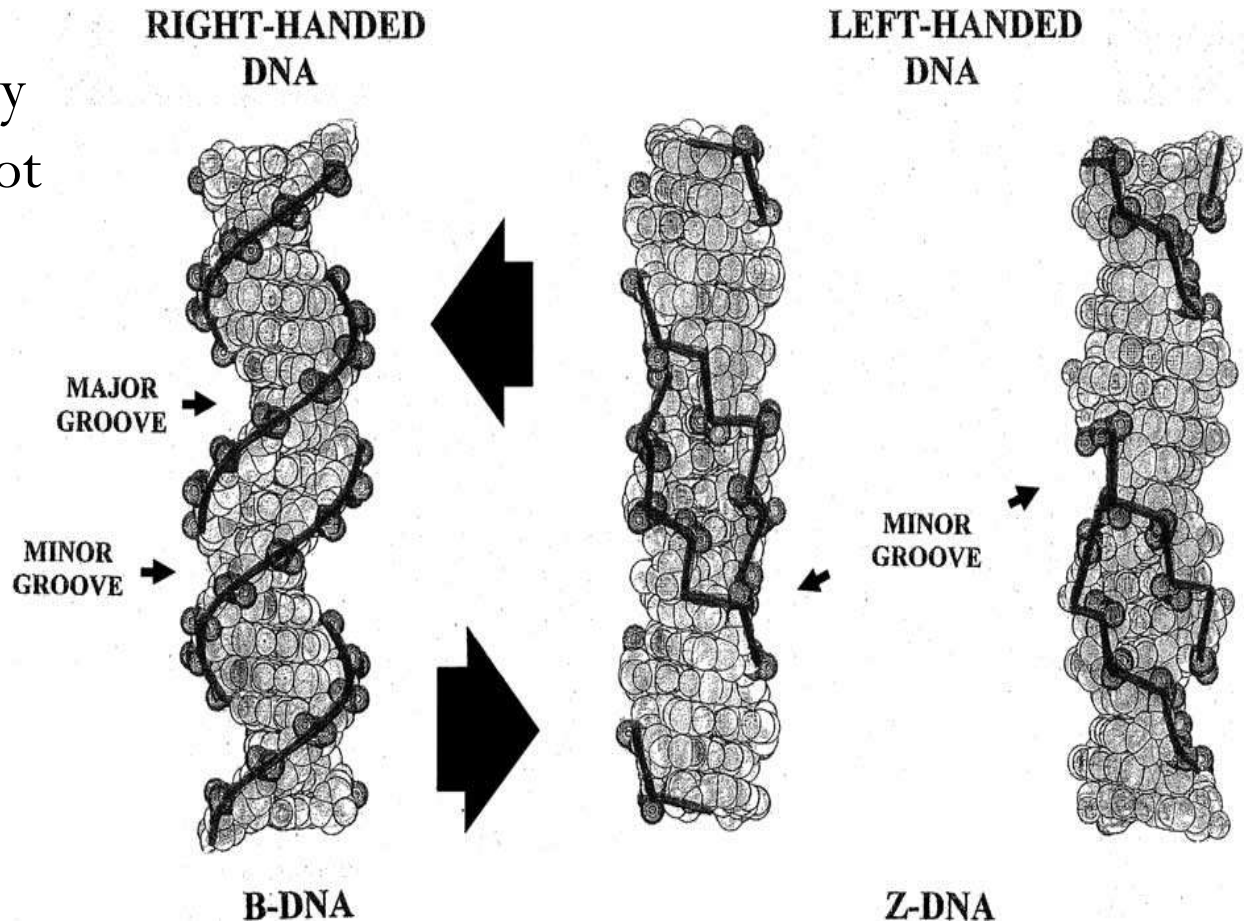
- **Chromosomal breakpoints for translocation-related human diseases mapped next to Z-DNA sequences.**

Introduction

Due to its high energy state, Z-DNA does not exist under normal conditions

Z-DNA induction has been studied:

- IN-VIVO
- IN-VITRO



Induction of Z-DNA

Induction of Z-DNA in vivo:

Negative super coiling of B-DNA strand → unwound double helical strand flips into Z-conformation.

Z-DNA binding proteins with Z-binding domains:

Example: **human dsRNA adenosine deaminase** (RNA editing enzyme)

Induction of Z-DNA in vitro:

- Micro molar concentrations of Spermine.
- **Millimolar concentrations of NiCl₂.**
- Molar concentrations NaCl and MgCl₂.
- 70% ethanol solution – not feasible under physiological conditions.

Z-DNA induction – in vitro

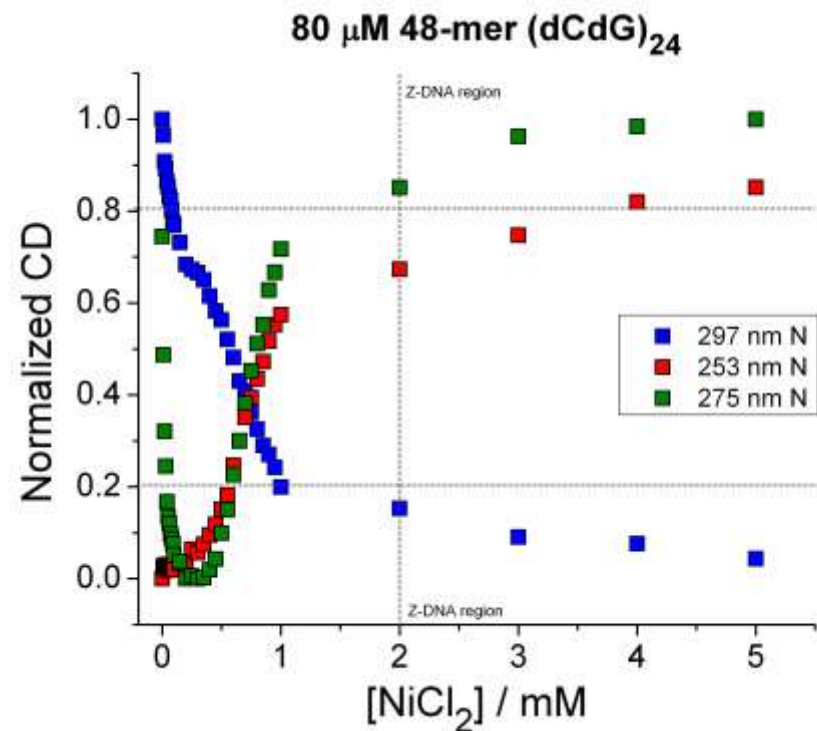
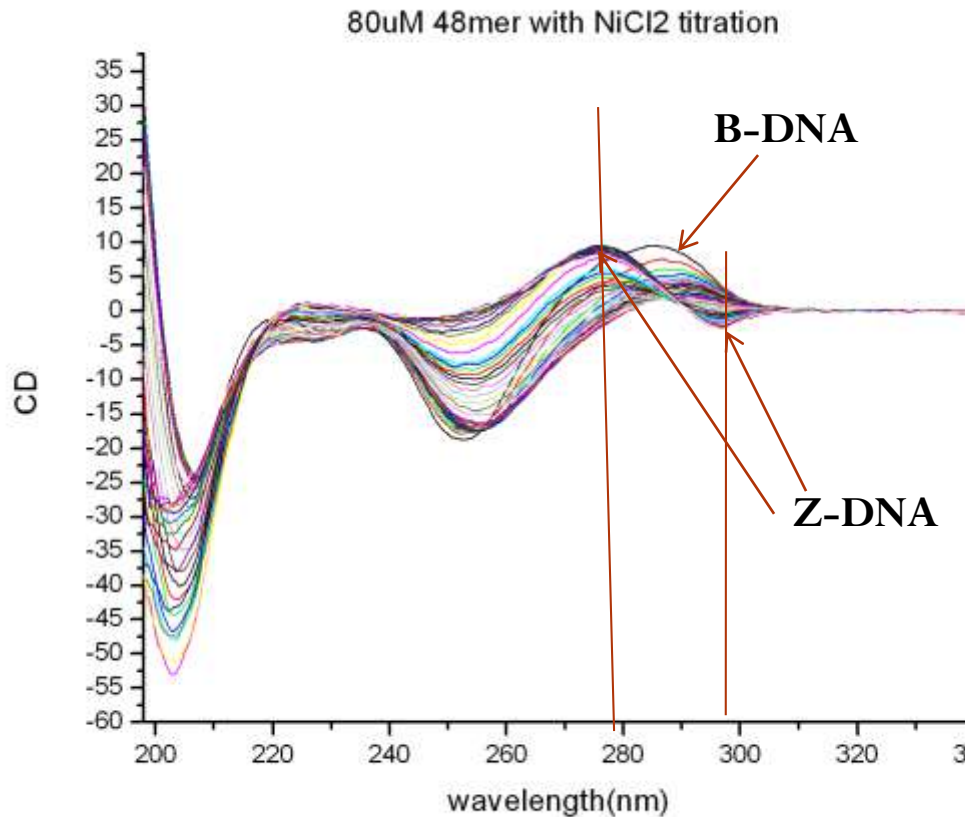
Reagents and conditions

- (dC-dG)₂₄ – 48mer oligonucleotide
 - 80μM
 - 20μM
 - 5μM
- Cacodylate buffer – 1mM (pH 7.0)
- Temperature (20 degrees Celsius)

Observed B-Z DNA induction using Circular dichroism (CD) spectroscopy

Results

- $(dC-dG)_{24}$ – 48mer oligonucleotide chain. – ($80\mu\text{M}$)
- Titration with NiCl_2 to induce B – Z-DNA formation – Circular dichroism

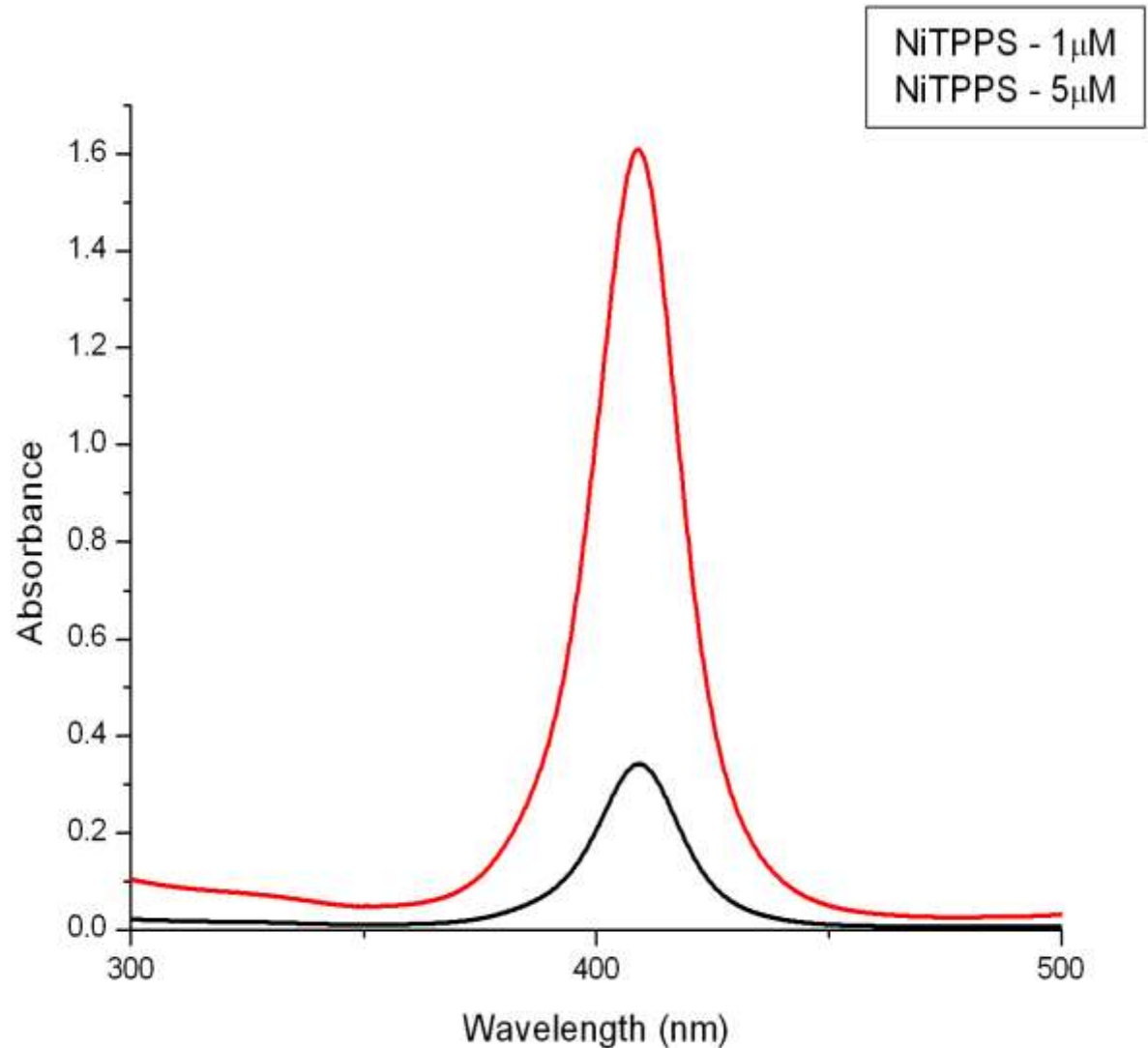


Z-DNA spectroscopic detection

Experimental Procedure

Spectroscopic detection of Z-DNA using the following metallo-porphyrins.

- NiTPPS
- ZnTPPS
- CoTPPS
- CuTPPS



Experimental Procedure

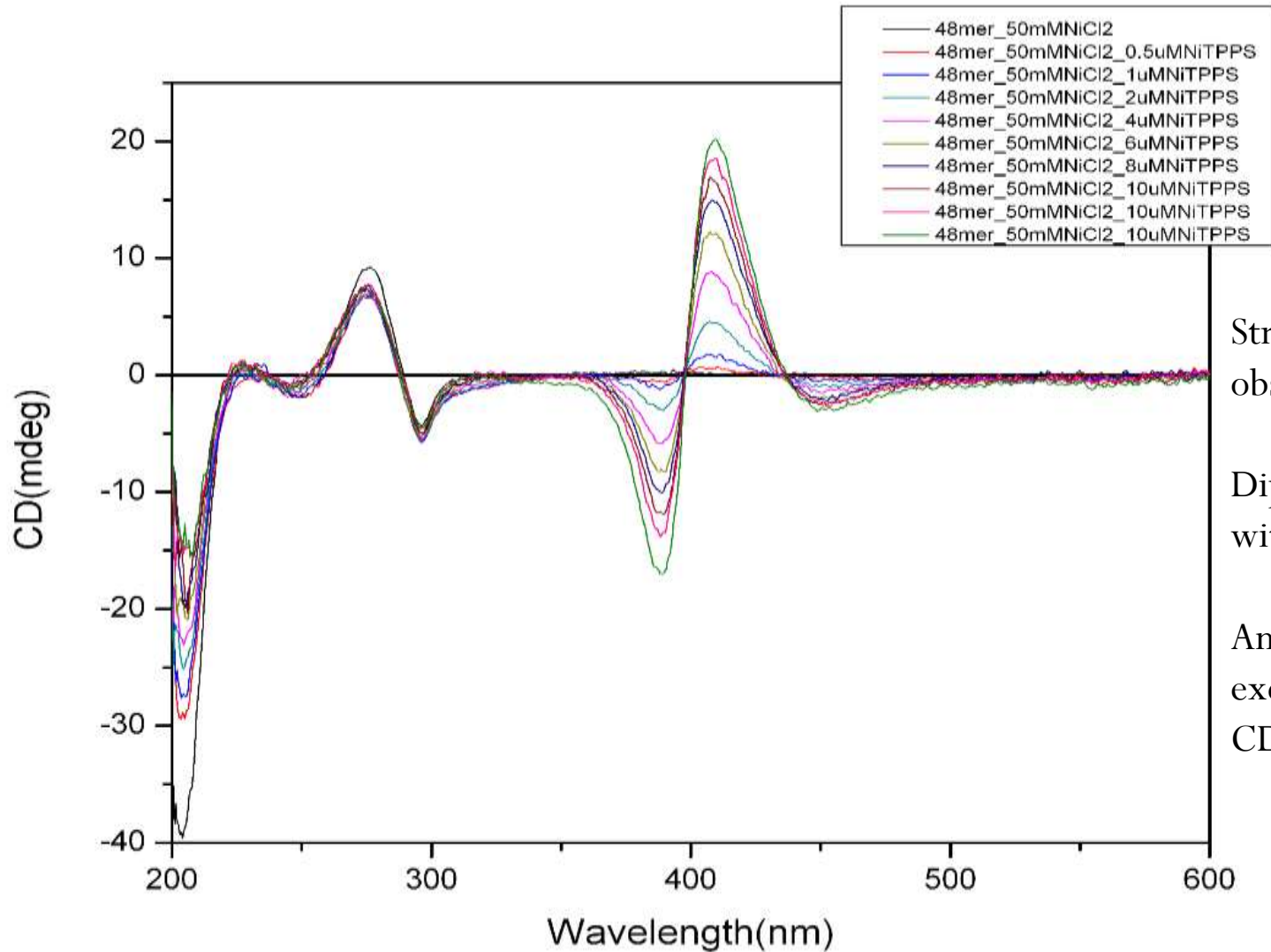
Reagents and conditions

- (dC-dG)₂₄ – 48mer oligonucleotide –(80μM)
- NiCl₂ – 50mM
- Titration with micromolar concentrations of NiTPPS

- Cacodylate buffer – 1mM (pH 7.0)
- Temperature (20 degrees Celsius)

Observed Z-DNA detection using Circular dichroism (CD) spectroscopy

Results



Strong binding
observed.

Dipole interactions
with the DNA

An intense positive
exciton coupled
CD signal appears

Conclusion

- The anionic nickel(II) porphyrin is able to selectively sense Z-DNA.
- Spectroscopic discrimination between the B-DNA and Z-DNA at micromolar concentrations.
- Therefore, NiTPPS is a very good spectroscopic sensor of left handed Z-DNA.

Acknowledgment

I would like to thank:

- **NSF – EPSCoR**
- **INBRE**
- Advisor : Dr. Milan Balaz



• Research group:



Jung Kyu Choi



Alessandro D'Urso



Fidelis Ngwa