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Adaptation of Pt-DNA binding experiments into a multiweek upperlevel biochemistry laboratory activity

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Adaptation of Pt–DNA binding experiments into a multi-week, upper-level biochemistry laboratory activity

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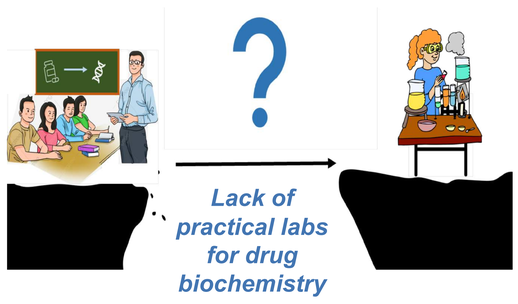
Significance & Problem

Undergraduate students are introduced to concepts of drug interactions and biomolecules while taking courses such as biochemistry and toxicology. However, few laboratory activities exist that provide students with hands-on approaches to explore this topic.



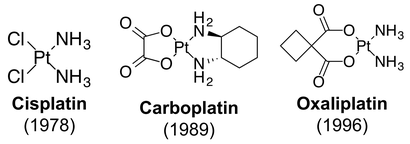
Well-known commercial drugs can be expensive, and the sophisticated instrumentation required for specific procedures is often inaccessible to a typical undergraduate lab.

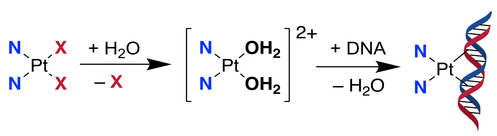
Our project focuses on providing upper-level biochemistry students with the opportunity to explore this real-world topic in the laboratory by investigating and quantifying DNA binding properties of several Pt-based anticancer therapeutics. Students will also gain experience using a molecular modeling software in order to visualize the effects of binding interactions between DNA and common drug complexes.

Background

There are currently three FDA-approved Pt-based drugs in use. These complexes are prime choices for cancer treatments because of their abilities to bind to DNA after ligand exchange, triggering apoptotic behavior in cells.

The structural differences of these drugs can result in varying rates and binding of DNA. The effects of these structural differences, as well as other tested variables, can be investigated and quantified using gel electrophoresis.





Learning Objectives

Conceptual: Nucleic acids and their structural features

- Recognize purine versus pyrimidine
- Describe nucleic acid, nucleotide, nucleoside, and nitrogenous base
- Name the major nucleotides, nucleosides, and nitrogenous bases from their structures
- Locate atoms in a nitrogenous base using the standard numbering scheme
- Describe the modes of DNA binding including electrostatic, intercalation, and alkylation
- Identify the major and minor grooves in a DNA structure
- Explain primary and secondary DNA structure

- Identify the three current Pt anticancer drugs
- Summarize the term structure–function relationship
- Relate the structure of a Pt drug to its reactivity
- Predict the reactivity of a Pt drug based on its structure

Practical: Laboratory skills

- Plan a drug–binding nucleic acid polyacrylamide gel electrophoresis experiment testing at least two experimental variables
- Execute PAGE analysis of nucleic acids
- Evaluate the binding of Pt complexes based on experimental data
- Contrast the binding of different Pt drugs based on experimental data
- Explain differences in drug binding based on drug structure (structure–function relationships)
- Evaluate predictions of drug binding compared to experimental results

- Generate publication-quality images of raw data and models
- Write detailed, information-rich figure captions for each image

Evaluation

Students will be evaluated based on a series of reading quizzes taken before beginning each week of laboratory work. In addition, students will be responsible for turning in two publication-quality images (as shown to the right) with descriptive figure captions.

Sample Data from Students (Fall '19)

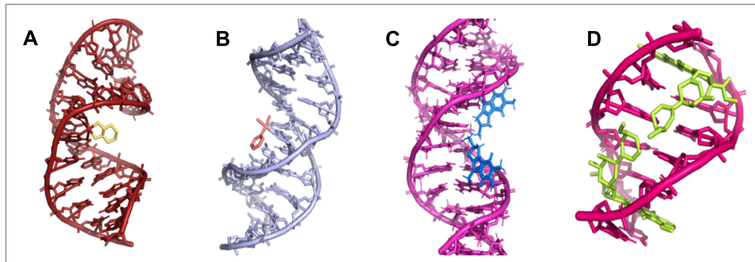


Figure 1. Several anticancer drugs interacting with DNA.

(A) Oxaliplatin bound to DNA obtained from murine cells. Platinum binds to N7 of purine nucleobases and distorts the secondary structure of DNA. Oxaliplatin is an example of alkylating-like agent; in this structure, PDB: 1ihh, Pt forms a GG intrastrand cross-link with a DNA duplex.

(B) Monofunctional platinum bound to DNA obtained from murine cells. In this structure, PDB: 3co3, Pt forms a single bond with DNA. Monofunctional platinum forms a covalent bond on N7 with purine base guanine. There is no intrastrand crosslinking with DNA in the presence of a monofunctional platinum drug. This monofunctional platinum is an example of an alkylating-like agent.

(C) 2,7-Diaminomitosene molecules bound to DNA obtained from murine cells. In this structure, PDB: 1jo1, C10 of the mitosene is covalently bound to the N7 of guanine and anchored within the major groove of DNA helical structure. 2,7-Diaminomitosene is another example of an alkylating-like agent.

(D) Doxorubicin molecules inserted between DNA strands obtained from murine cells. In this structure, PDB: 2des, doxorubicin molecule intercalates into DNA and pushes the DNA strands apart, the sugar structure of the doxorubicin sets into the minor groove of the DNA double helix.

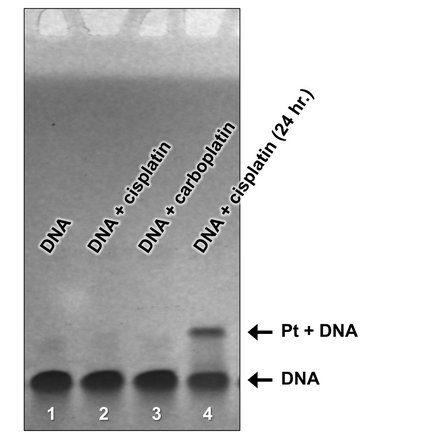


Figure 2. A PAGE gel was run to visualize the Pt-DNA binding experiment using cisplatin and a Pt complex, carboplatin. Pt binding conditions: 1 molar eq. Pt per DNA, 1 h, 37 ºC. DNA sequence: 20-mer hairpin duplex, 5' TATGGTATTTTTATACCATA–3'. In wells labeled "2" and "3" no bands were produced, displaying that no Pt is bound to DNA. However, well labeled "4" binding conditions were altered, and the binding time of cisplatin was extended to 24 hrs. Under these conditions, Pt was able to successfully bind to DNA. The Pt-DNA band is clearly visible in the gel and is labeled "Pt-DNA."

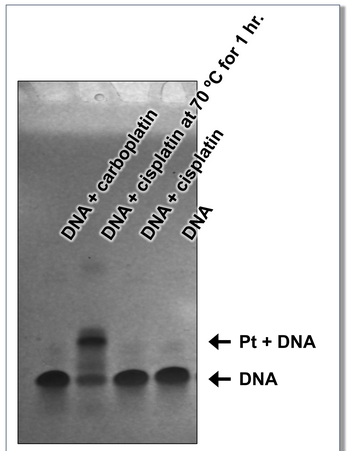


Figure 2. Carboplatin, cisplatin, and cisplatin binding at 70 ºC for 1 hr to DNA. Heating DNA and cisplatin at 70˚C for 1 hr had the best binding band. Pt binding conditions: 1 molar equivalent Pt per DNA, 1 hr. at 37º C. DNA sequence: 20 mer hairpin duplex, 5'-TATGGTATTTTTATACCATA–3'.

Acknowledgements

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