Criteria for the Mode of Binding of DNA Binding Agents

_D. Suh and J. B. Chaires_
Presentation by _Warren Cheung_
Main Thrust
Overview of techniques that

- detect binding of DNA binding agent to DNA
- distinguish groove-binding and intercalating DNA binding agents
DNA Binding Agents

• compounds which bind DNA

Mode of Binding

• type of interaction with DNA

• *groove binding*

• *intercalation binding* (between bases)
Techniques

- fluorescence excitation/emission spectra [Detect]
- fluorescence polarization [Detect]
- solute fluorescence quenching [Detect]
- fluorescence contact energy [Detect/Distinguish]
- viscosity [Detect/Distinguish]
Experimental setup

- *ethidium bromide*: standard intercalator
- *Hoechst 33258*: groove binding agent
- Calf thymus DNA
Figure 3: Electronic Absorption and Emission Bands

- Photon Energy (Electron-Volts)
- Absorption
- Emission
- S₀
- S₁

Excited State Vibrational Energy Levels

Ground State Vibrational Energy Levels

Excited State Electronic Transistions

Wavenumber (cm⁻¹ x 10⁻³)
Fluorescence excitation/emission spectra

- environment of fluorophores changes when agent binds to DNA
- causes red-shift of *excitation* spectrum
- increase of *emission* intensity
Ethidium Bromide
Hoechst 33258
Fluorescence Polarization

- normally not polarized
- binding causes fluorescent ligand to be polarized
Ethidium
Hoechst 33258

![Graph showing fluorescence polarization vs. wavelength (nm) for Hoechst 33258](image-url)
Solute Fluorescence Quenching

- measure accessibility of fluorophore to quenching agent
- cannot access DNA binding agents
- possibly due to steric hindrance, charge repulsion, size of quenching agent
Ethidium
Hoechst 33258
Fluorescence Contact Energy Transfer

- UV energy transfer from base pair to fluorophore
- spectral overlap, within certain distance
- intercalators fulfil criteria, groove binding does not
- new band appears
Viscosity

- groove binding causes little change in DNA structure
- intercalators cause DNA to separate to bind the ligand
- detect increase in length of DNA
- can also use sedimentation
Conclusion

- fluorescence excitation/emission spectra and fluorescence polarization:
  - fluorophore environment changes
- solute fluorescence quenching:
  - binding makes fluorophore is inaccessible
- fluorescence contact energy:
  - intercalator allow base to transfer energy to fluorophore
- viscosity:
  - intercalator makes DNA longer
References

- [http://web.uconn.edu/gage/Media/229%20Pictures/1-DNA%20structure.JPG](http://web.uconn.edu/gage/Media/229%20Pictures/1-DNA%20structure.JPG)