Criteria for the Mode of Binding of DNA Binding Agents

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Main Thrust

Overview of techniques that

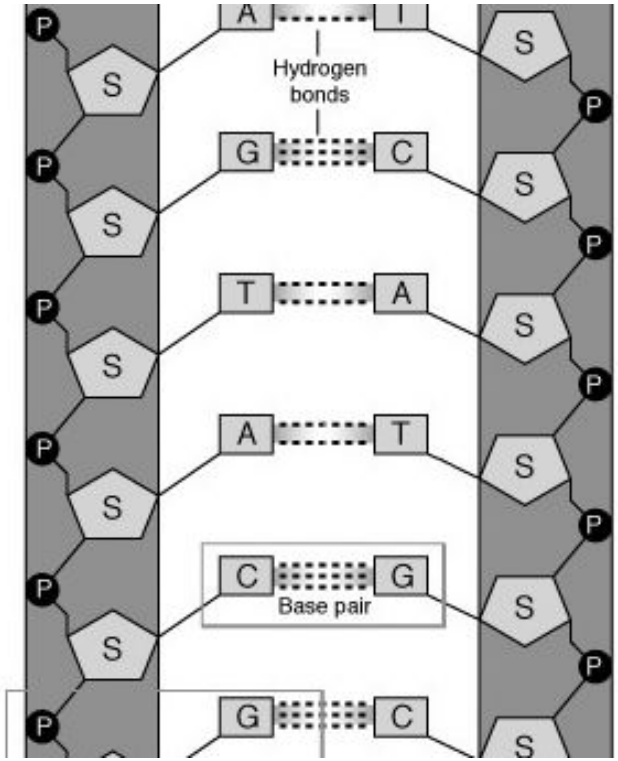
- *detect* binding of DNA binding agent to DNA
- *distinguish* groove-binding and intercalating DNA binding agents

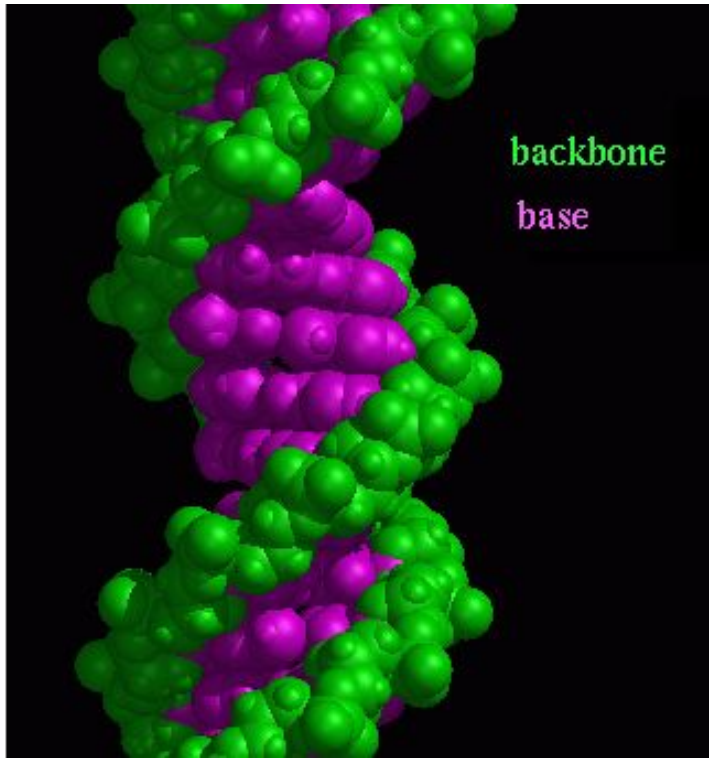
DNA Binding Agents

- compounds which bind DNA

Mode of Binding

- type of interaction with DNA
- *groove binding*
- *intercalation binding* (between bases)


Hydrogen bonds
Base pair
P
S
A
T
G
C

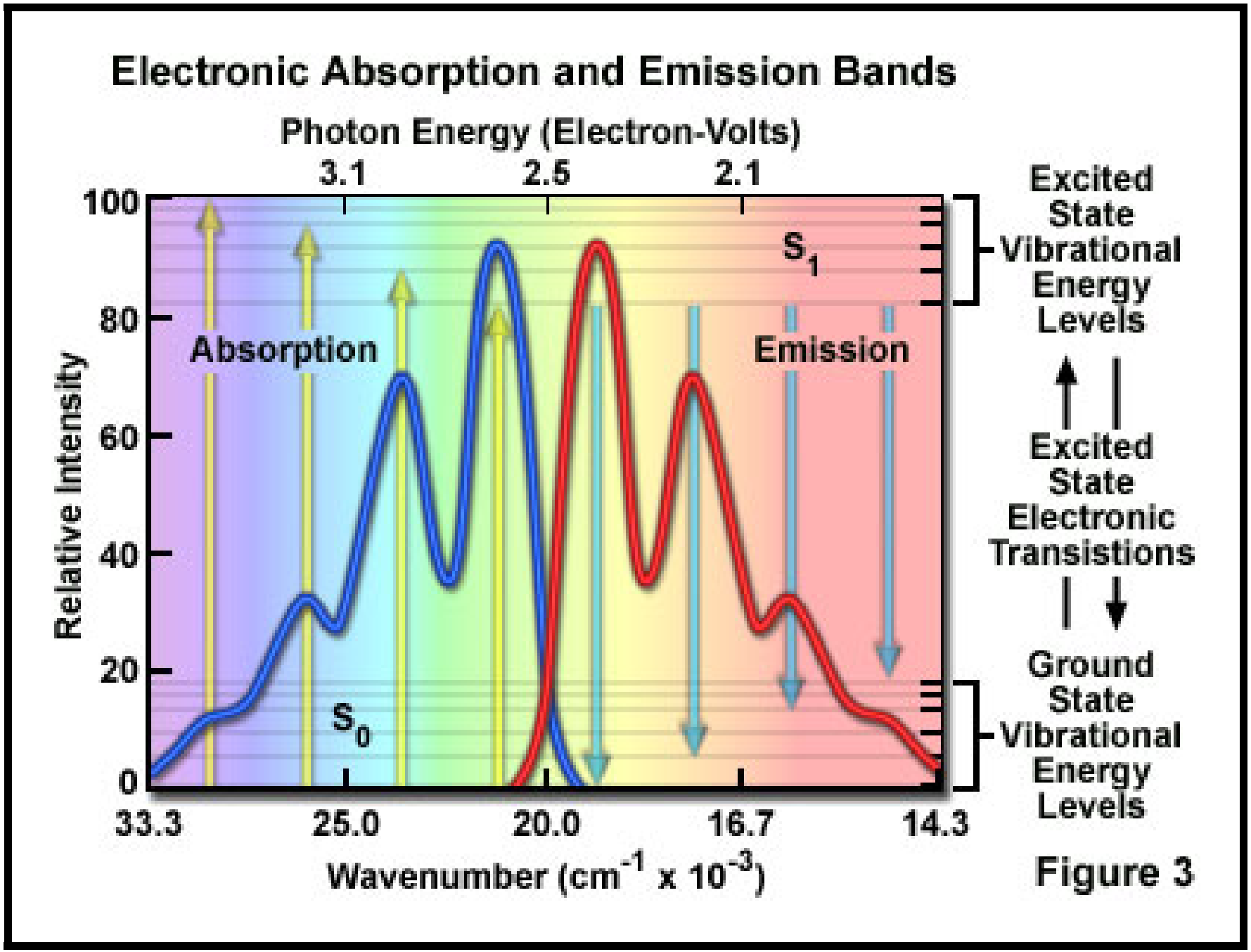

backbone
base

Techniques

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

Experimental setup

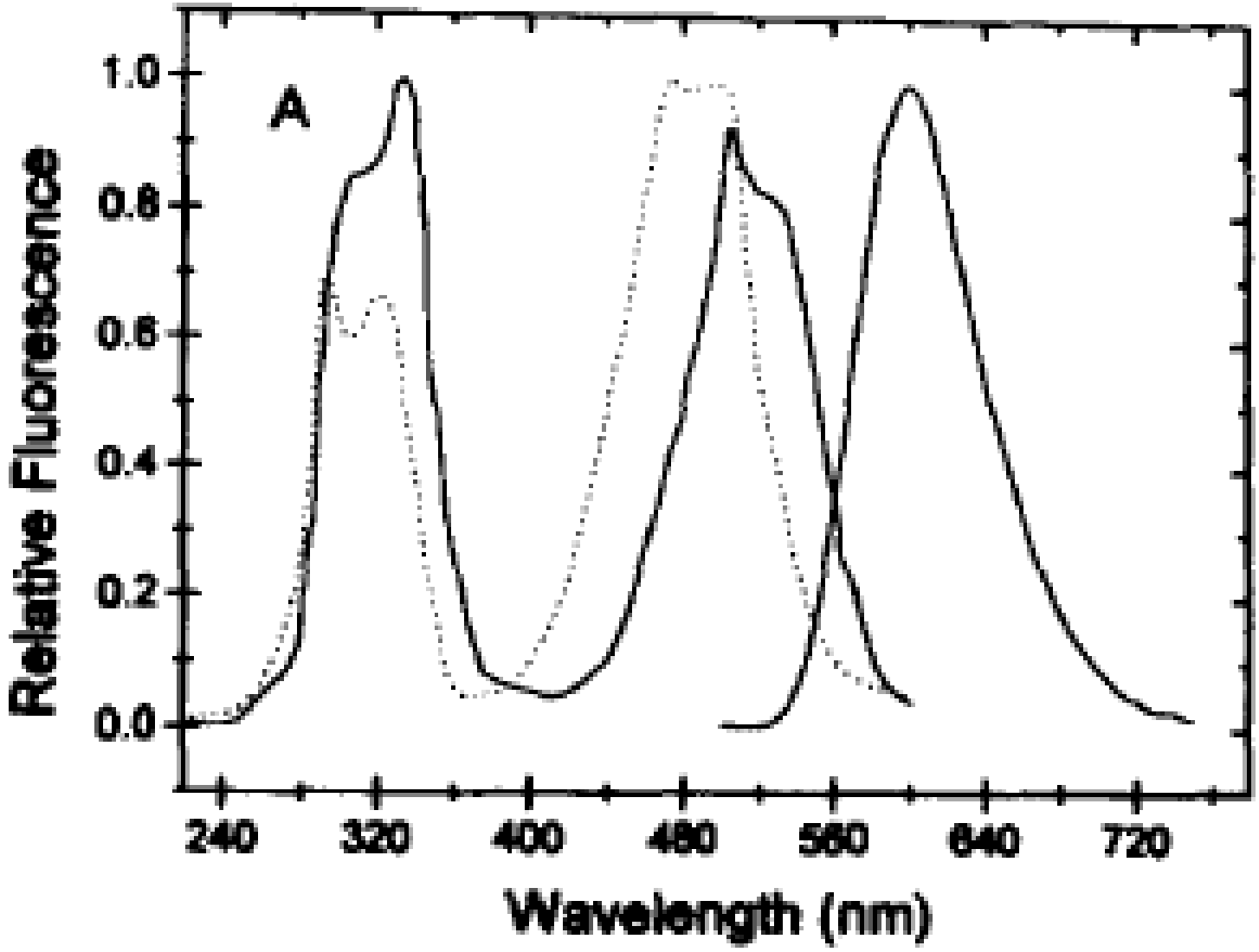
- *ethidium bromide*: standard intercalator
- *Hoechst 33258*: groove binding agent
- Calf thymus DNA


Electronic Absorption and Emission Bands
Photon Energy (Electron-Volts)
3.1
2.5
2.1
100
80
60
40
20
0
Relative Intensity
Absorption
Emission
S_1
S_0
33.3
25.0
20.0
16.7
14.3
Wavenumber (cm^{-1} x 10^{-3})
Excited State Vibrational Energy Levels
Excited State Electronic Transistions
Ground State Vibrational Energy Levels
Figure 3

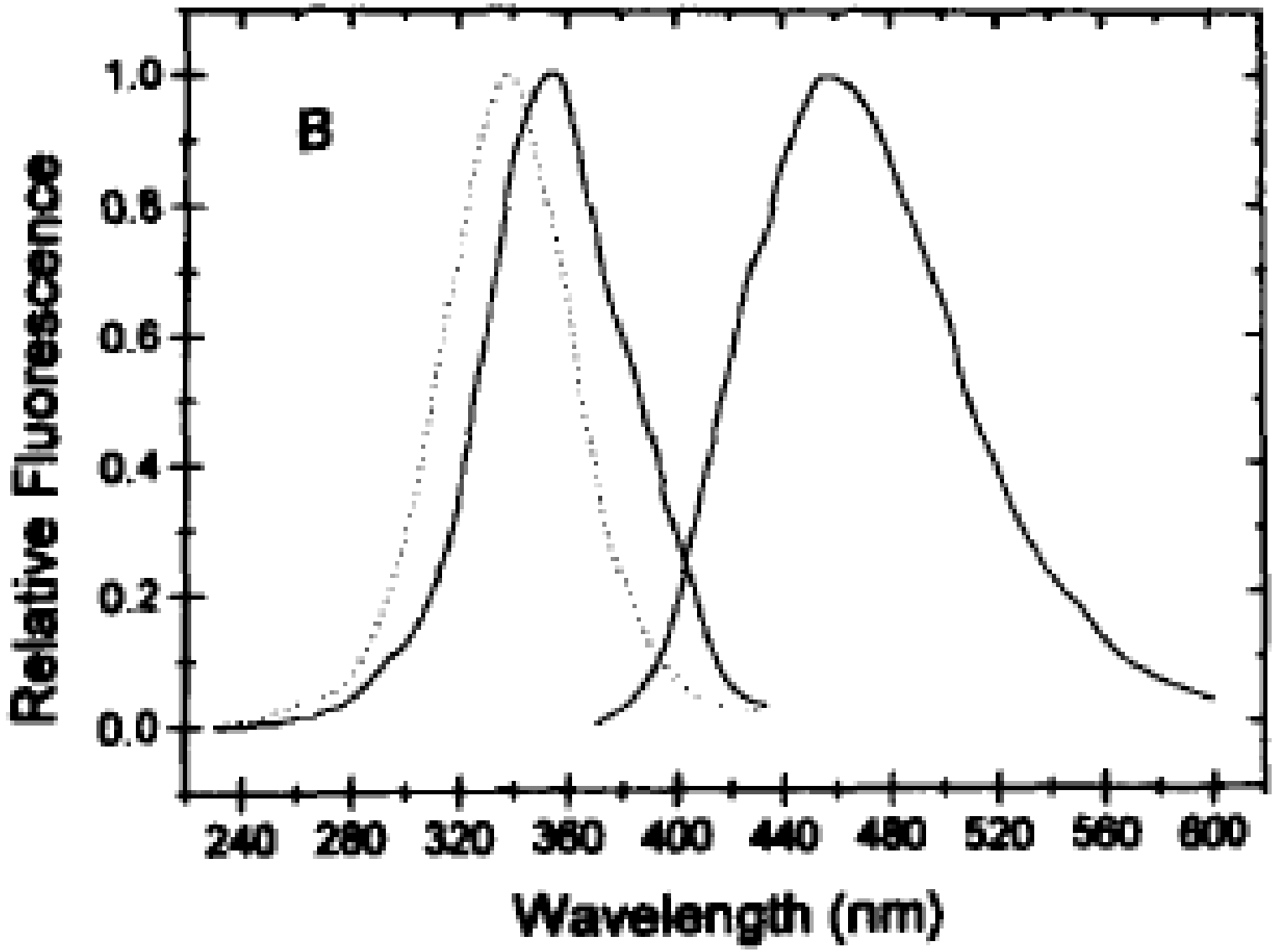
Fluorescence excitation/emission spectra

- environment of fluorophores changes when agent binds to DNA
- causes red-shift of *excitation* spectrum
- increase of *emission* intensity

Ethidium Bromide



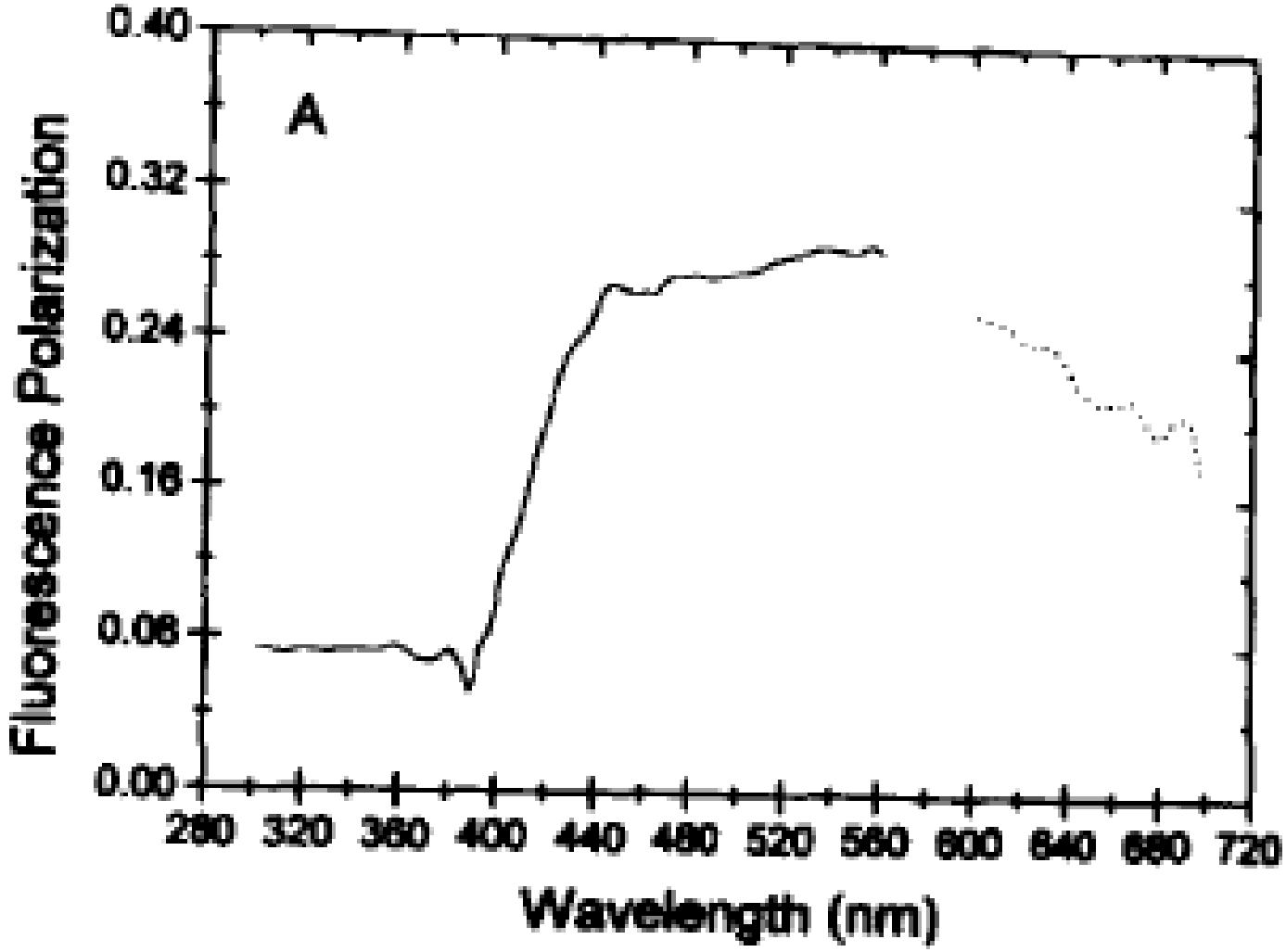
Hoechst 33258



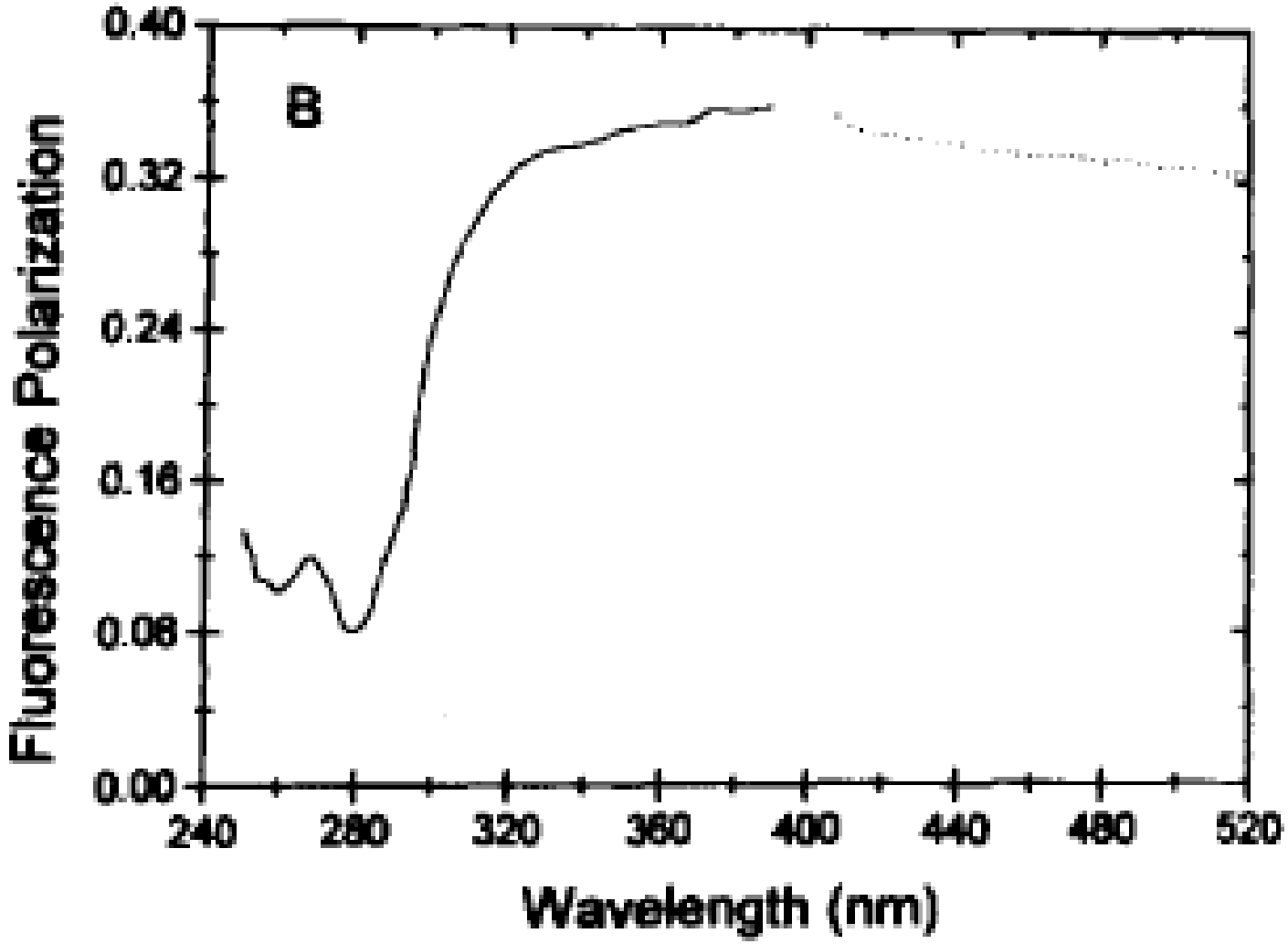
Fluorescence Polarization

- normally not polarized
- binding causes fluorescent ligand to be polarized

Ethidium


A
0.40
0.32
0.24
0.16
0.08
0.00
Fluorescence Polarization
280 320 360 400 440 480 520 560 600 640 680 720
Wavelength (nm)

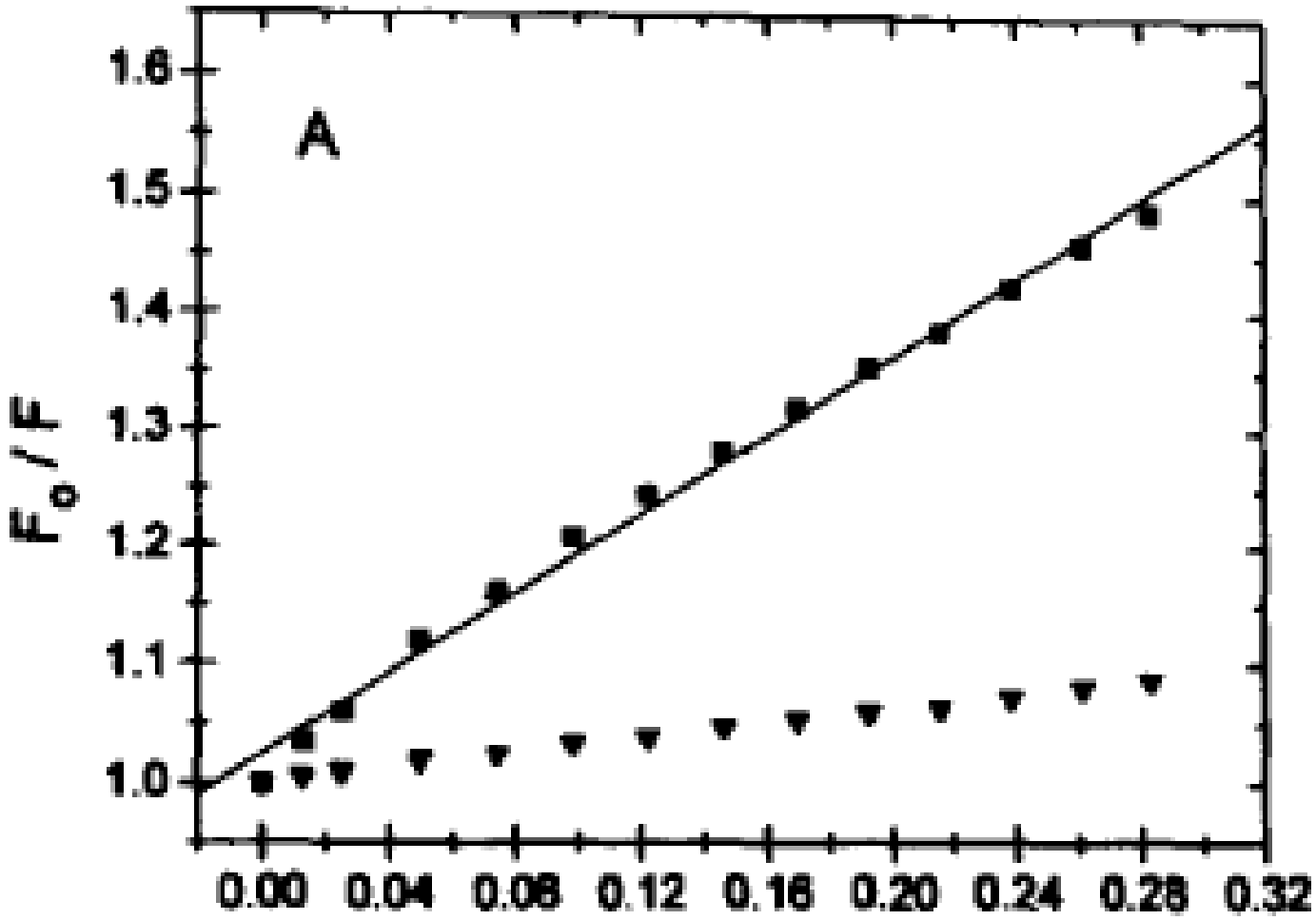
Hoechst 33258



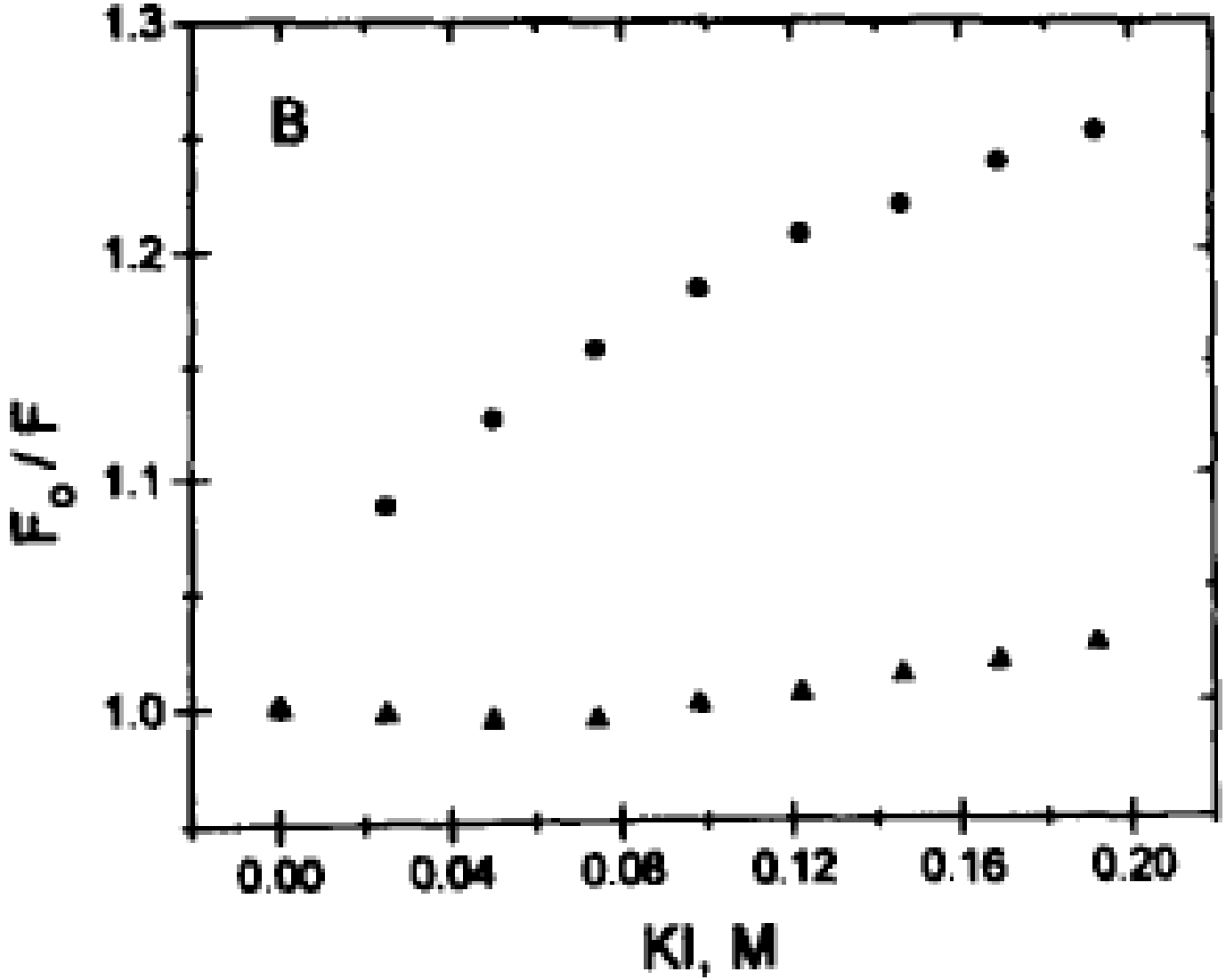
Solute Fluorescence Quenching

- measure accessibility of fluorophore to quenching agent
- cannot access DNA binding agents
- possibly due to steric hindrance, charge repulsion, size of quenching agent

Ethidium

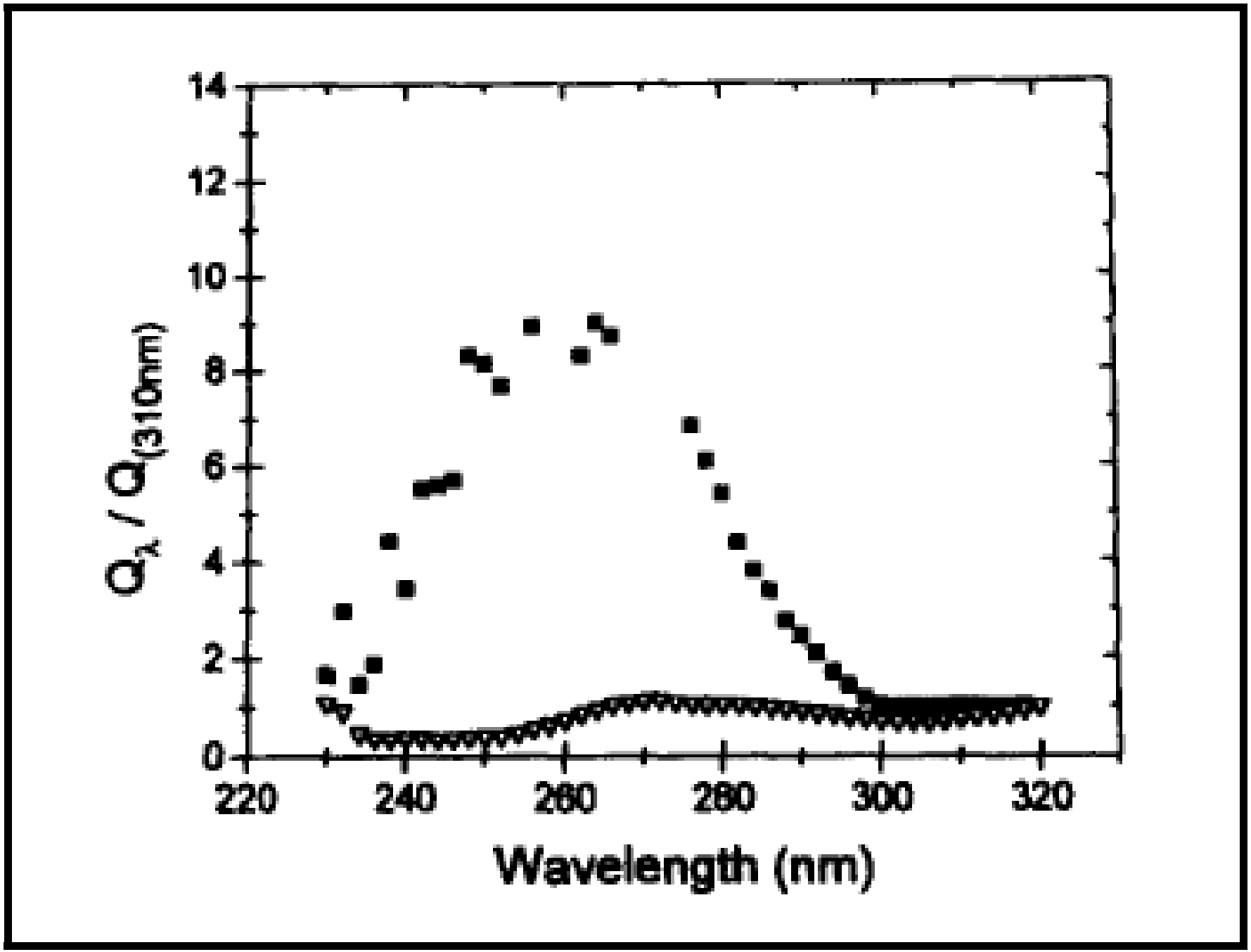


Hoechst 33258



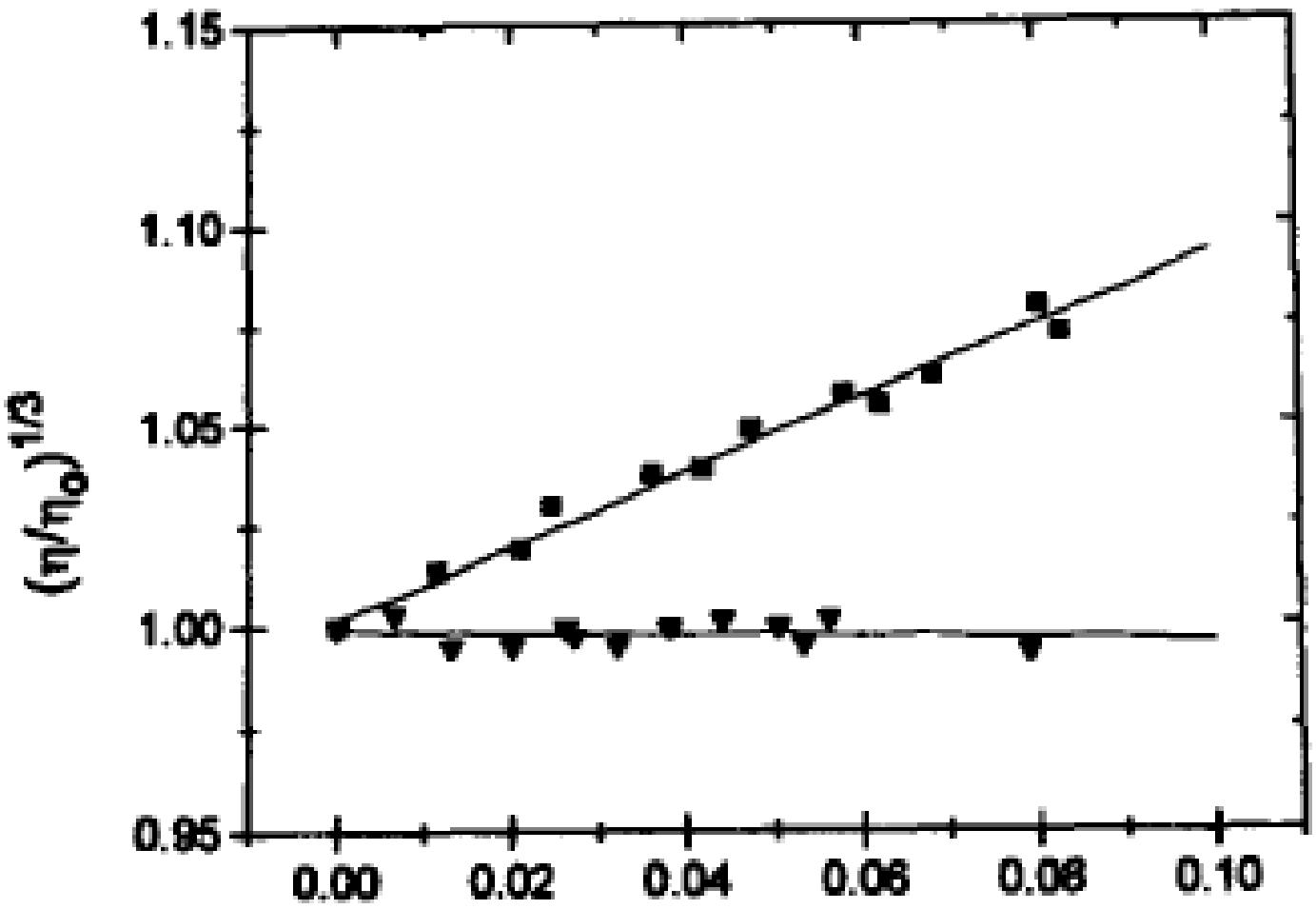
Fluorescence Contact Energy Transfer

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


14
12
10
8
6
4
2
0
220
240
260
280
300
320
Q_λ / $Q_{(310nm)}$
Wavelength (nm)

Viscocity

- 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


1.15
1.10
1.05
1.00
0.95
$(\eta/\eta_0)^{1/3}$
0.00
0.02
0.04
0.06
0.08
0.10

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%20stucture.JPG
- http://www.olympusfluoview.com/theory/fluoroexciteemit.html