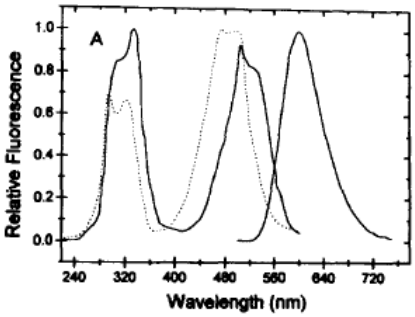
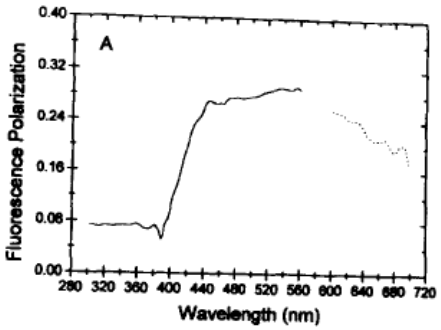
Criteria for the Mode of Binding of DNA Binding Agents by *D. Suh and J. B. Chaires*
Bioorganic & Medicinal Chemistry, Vol. 3, No. 6, pp. 723-728, 1995. Summary of paper by Warren Cheung.

Main Thrust: Overview of techniques that *detect* binding of DNA binding agent to DNA and *distinguish* groove-binding (e.g. Hoechst 33258) and intercalating (between base pairs e.g. ethidium bromide)

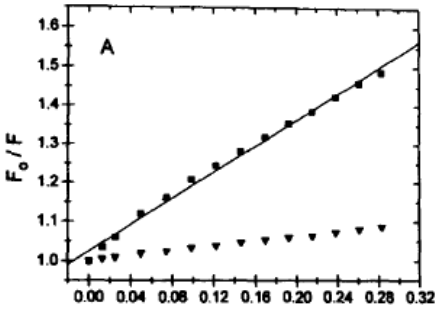
- fluorescence excitation/emission spectra
  - fluorophores are molecules that can emit light after absorbing
  - molecules tend to absorb and emit light at preferred wavelengths



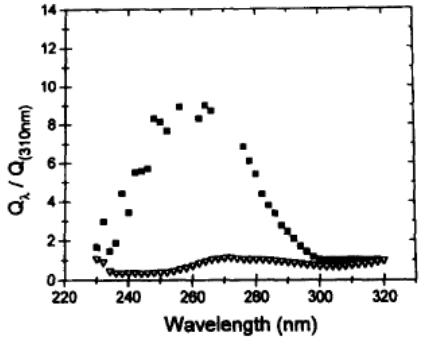
  - solid line=no binding, dotted line=binding
  - DNA binding causes red-shift (increased wavelength) of absorption spectrum (LHS of graph)
  - causes increase in intensity of emission spectrun (RHS of graph) — no wavelength shift (fluorescence normalised so no visible increase)
- fluorescence polarization



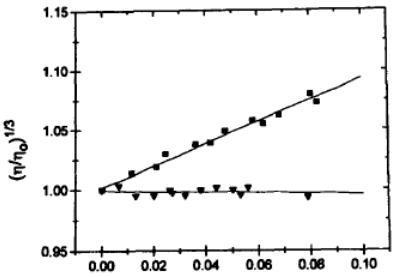
  - when not bound, DNA binding agent tumbles freely (polarization is < 0.03 at 480 nm, not shown on graph)
  - when bound, tumbles less freely (large polarization shown in graph)
- solute fluorescence quenching:



  - bottom axis = amount of quenching agent
  - quenching agent prevents fluorescence if DNA binding agent is free (rising line shows relative decrease in intensity)
  - binding to DNA makes fluorophore is inaccessible to quenching agent — fluorophore will have normal fluorescence (flat line shows fluorescence unaffected by quenching agent)
- fluorescence contact energy:



  - intercalator allow base to transfer UV energy to fluorophore — intercalator is in the right physical position near the base (squares show new peak in graph)
  - groove binding does *not* allow transfer of energy – wrong physical configuration far away from the base (inverse triangles have flat response in graph)
  - detectable difference between groove binding and intercalation
- viscosity:
  - Graph of viscocity vs. amount of binding agent



  - intercalator makes DNA longer by squeezing between base pairs and forcing the DNA to unwind
  - increased length of DNA causes increased viscocity as intercalator added (squares)
  - groove binding does not affect the length of DNA — no increased viscocity (triangles)
  - detectable difference between groove binding and intercalation