



CD/LD spectropolarimetry Viscometry

InnoMol Molecular Interactions Workshop:
June 1-3, 2015.
Hands-on session

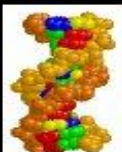
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Division of Organic Chemistry and Biochemistry

Ruđer Bošković Institute, Zagreb, Croatia



INNOMOL
Innovation Pipeline



RB



CD/LD spectropolarimetry

- a) DNA and protein structure characterisation
- b) Study of DNA/protein/ligand interaction – affinity, kinetics, structural aspects of complexes

Viscometry

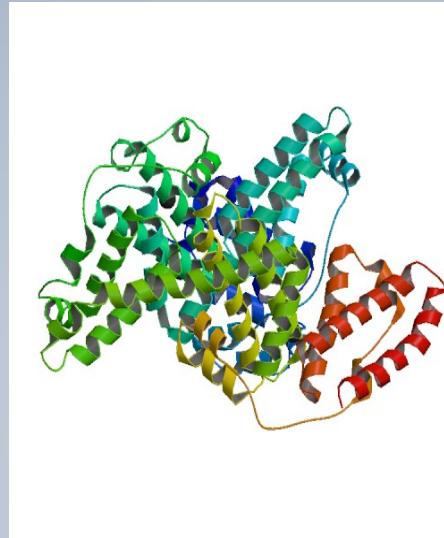
Ligand binding mode impact on viscosity of biomacromolecule solution



Biomacromolecular targets



polynucleotidesⁱ



proteinesⁱⁱ

and



protein-enzymesⁱⁱⁱ

i) <http://www.irb.hr/Istrazivanja/Zavodi-i-centri/Zavod-za-organsku-kemiju-i-biokemiju/Laboratorij-za-studij-interakcija-biomakromolekula> 29. 06 2014, 20:15

ii) <http://www.rcsb.org/> (crystal structure of Bovine serum albumine 29. 06. 2014, 23:50)

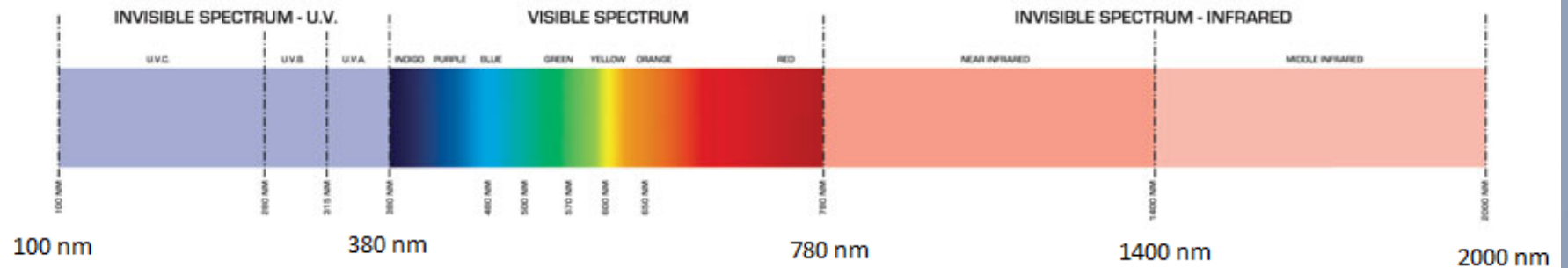
iii) <http://www.rcsb.org/> (structure of two different conformations of mRNA capping enzyme in complex with GTP 30. 06. 2014, 00:02)

CD/LD spectropolarimetry

DNA

Peptide
bonds,
Amino
acids

ligands



RADIATIONS

- UVC 100 to 280 nm
- UVB 280 to 315 nm
- UVA 315 to 380 nm
- Blue Light 380 to 780 nm
- Visible Light 380 to 780 nm
- Near IR 780 to 1400 nm
- Middle IR 1400 to 2000 nm

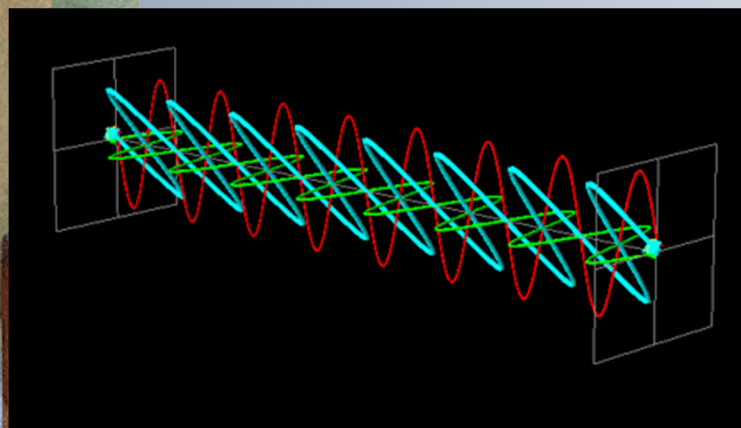
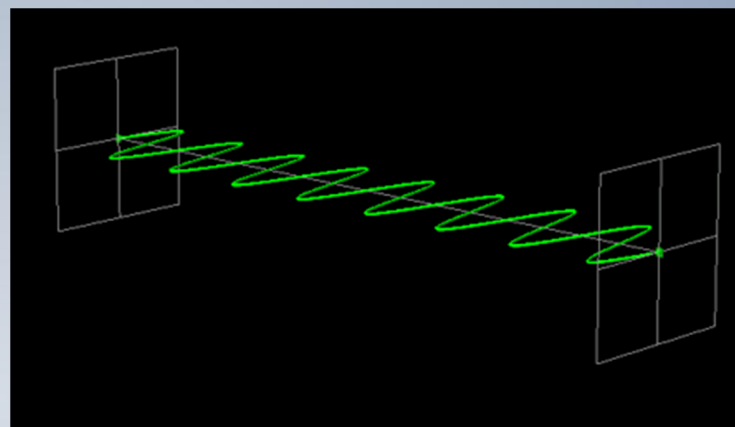
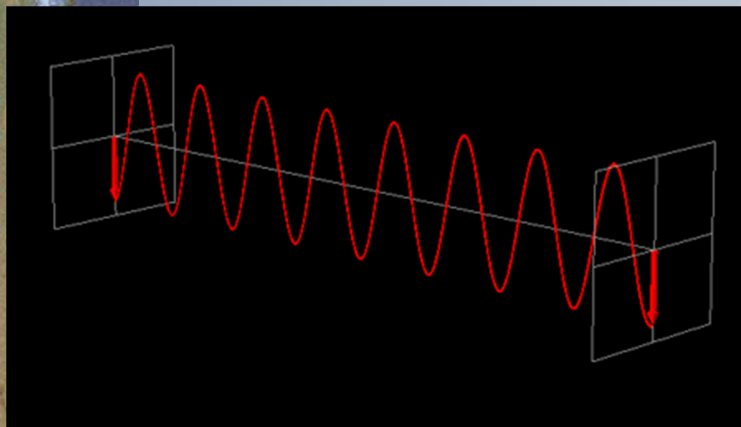


Circular Dichroism and Optical Rotational Dispersion Spectrometer (CD-ORD):

- Spectrometer **Jasco J-815** measures spectra of circular dichroism CD and optical rotational dichroism ORD
- simultaneous measurement of several variants of **UV/vis spectra**

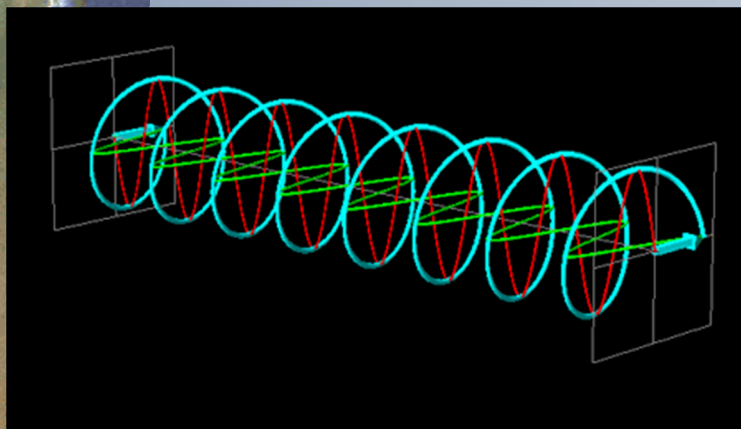


Linearly polarized light

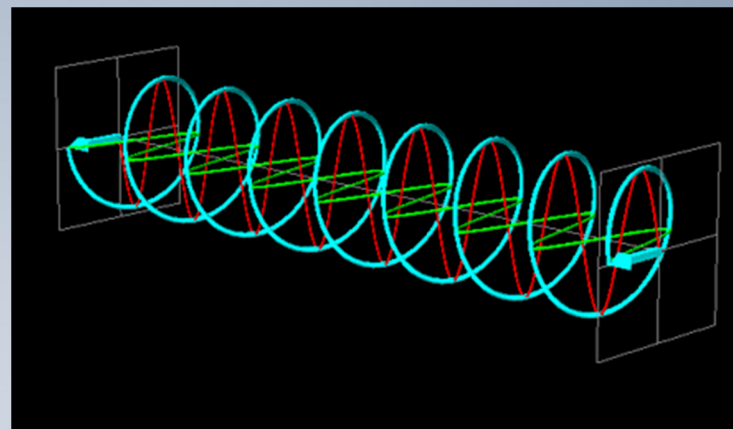


→ quarter-wave plate

Circularly polarized light



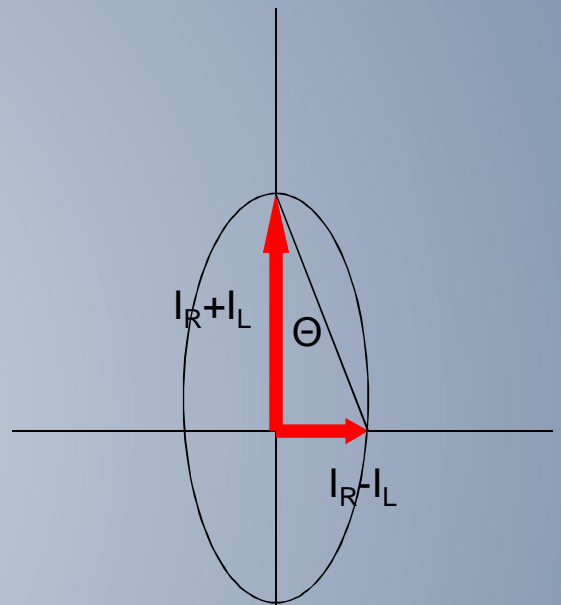
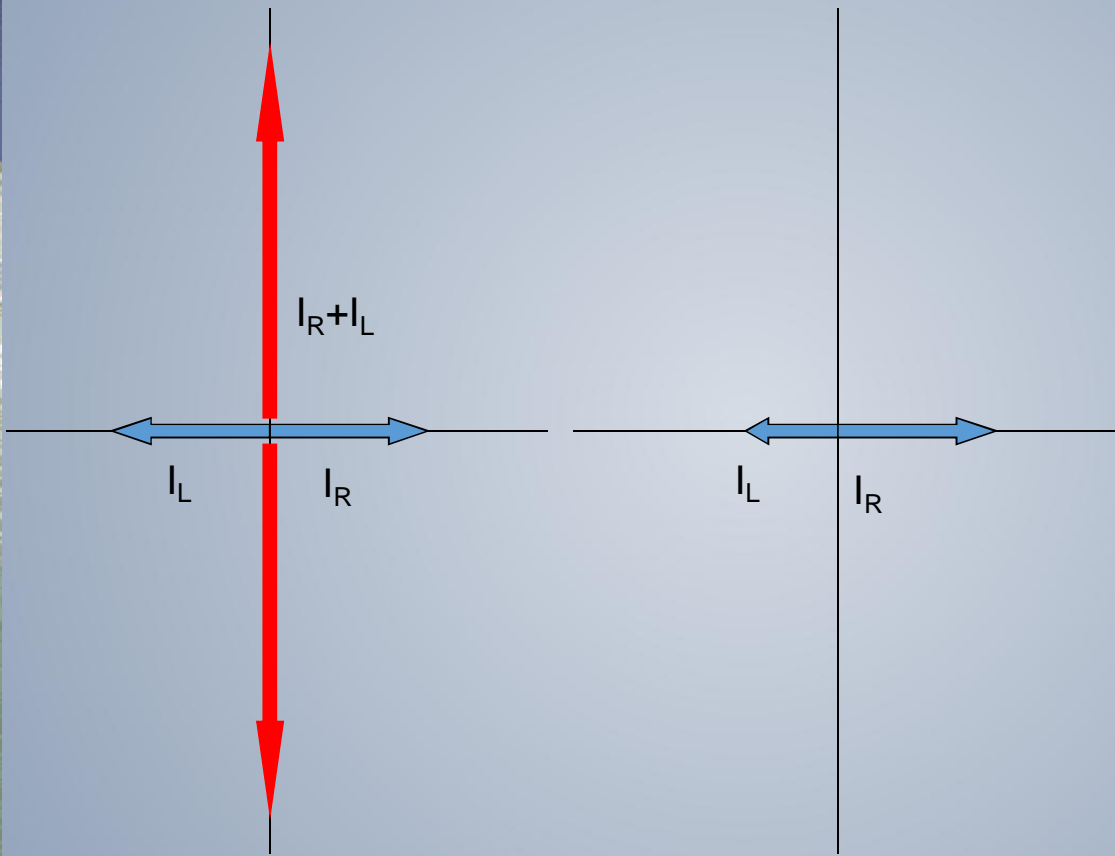
LCP light



RCP light

$$\Delta A = A_R - A_L = (\epsilon_L - \epsilon_R)/c = \Delta\epsilon/c \quad \sim \ll 1/1000 \text{ of total}$$

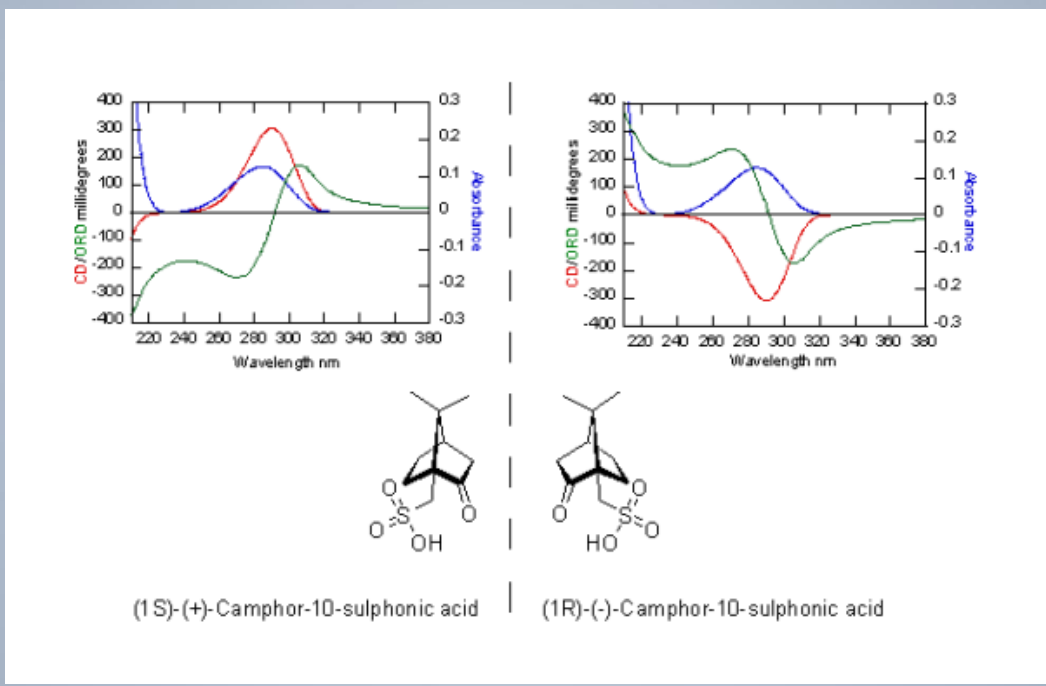
$\Delta\epsilon$ ~ typically $< 10 \text{ M}^{-1}\text{cm}^{-1}$ vs. ϵ ~ typically $20,000 \text{ M}^{-1}\text{cm}^{-1}$



Θ ellipticity!
 $\text{tg}\Theta = (I_R - I_L) / (I_R + I_L)$
 $\Delta A = \theta / 32.982$
 $[\theta] = 100 \times \theta / (C \times l)$

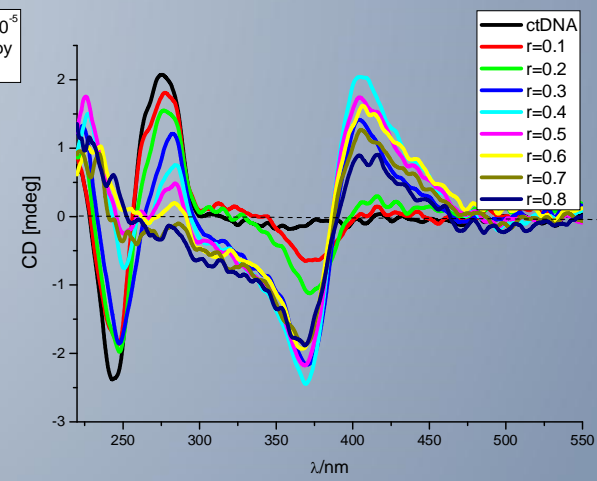
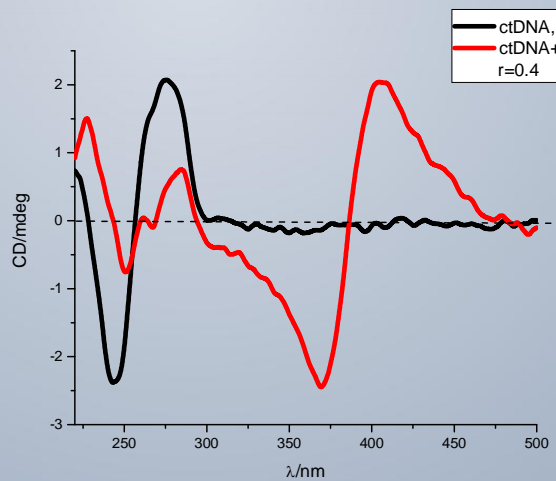
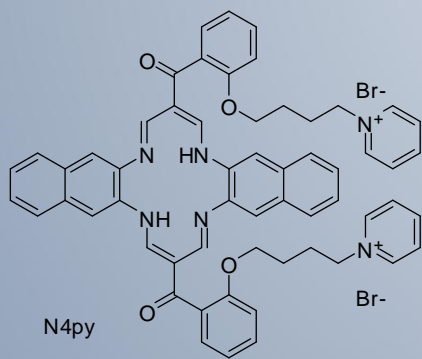
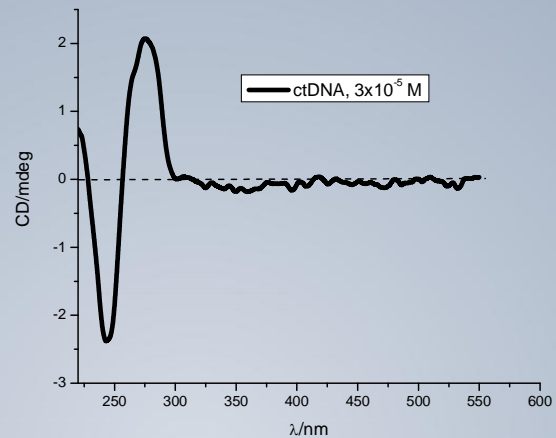
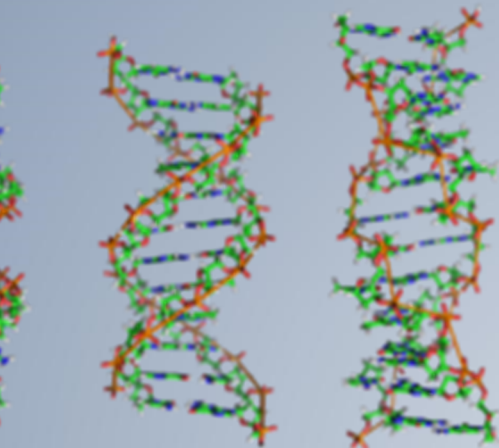
Circular dichroism

Chiral light \longrightarrow Chiral molecule



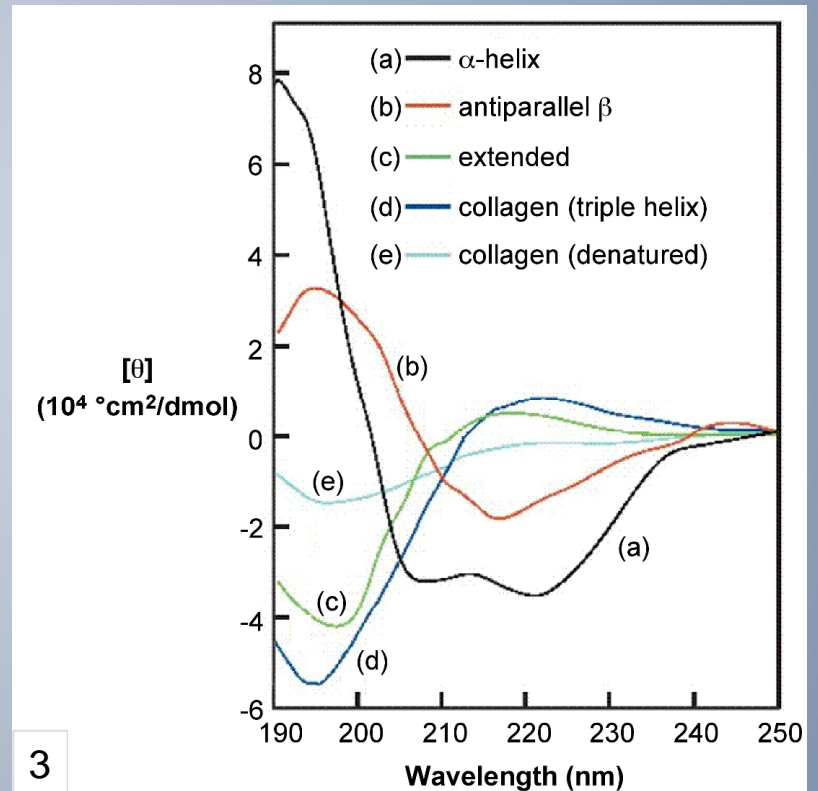
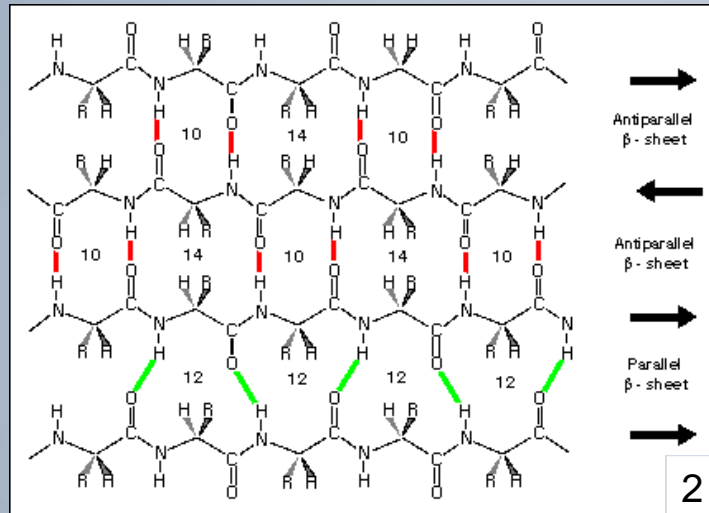
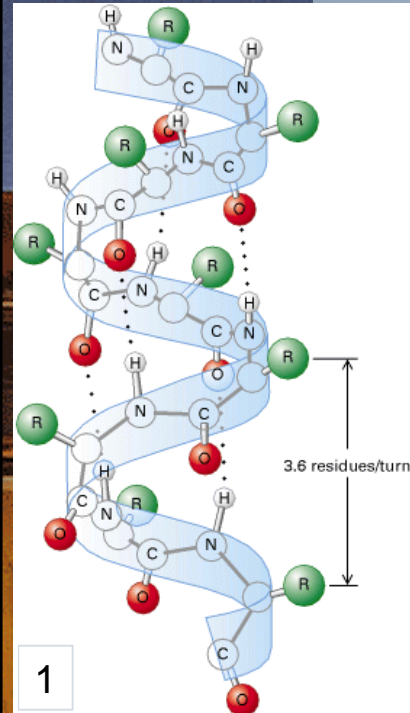


Side view of A-, B-, and Z-DNA.ⁱⁱ



ii) <http://en.wikipedia.org/wiki/A-DNA> 14.07.2014

Circular dichroism in protein science



- 1) http://www.nslc.wustl.edu/courses/bio2960/labs/02Protein_Structure/PS2011.htm
- 2) <http://www.nku.edu/~russellk/tutorial/peptide/peptide.html>
- 3) <http://cnx.org/content/m38277/latest/?collection=col10699/latest> 14.07.2014. 22:50

3

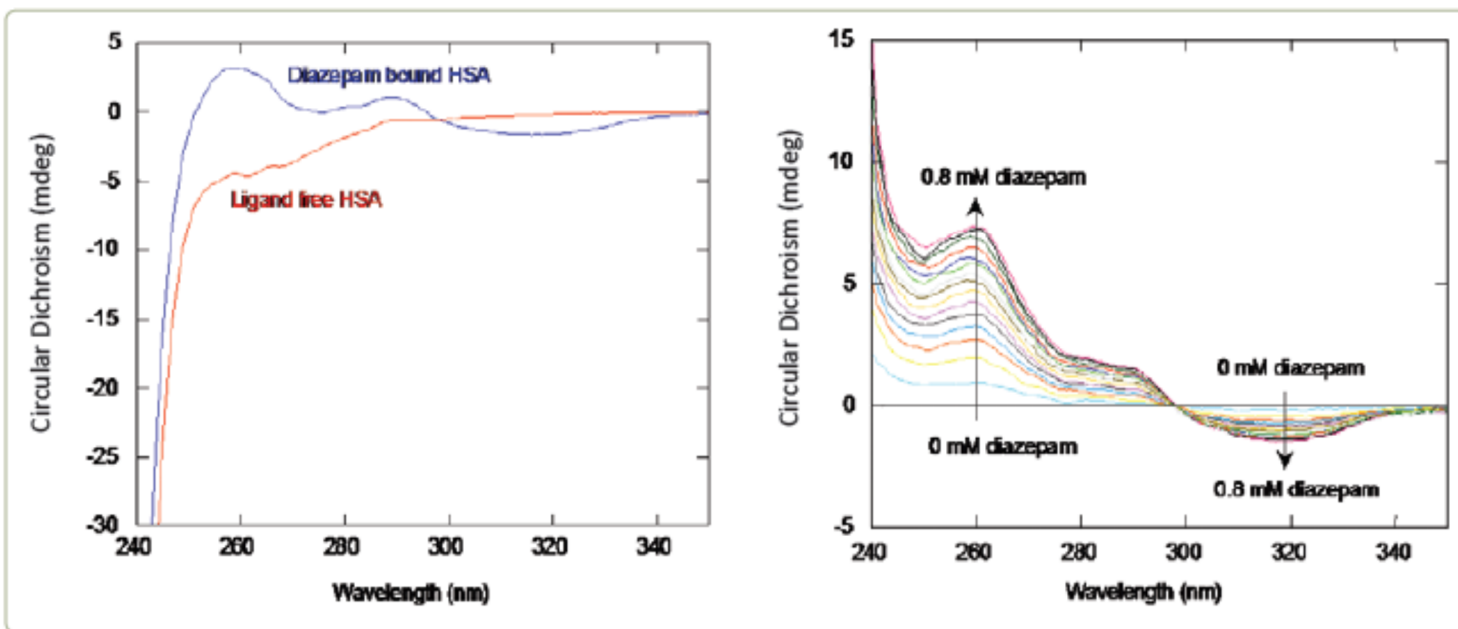
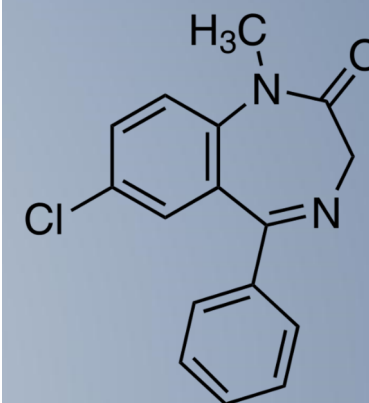
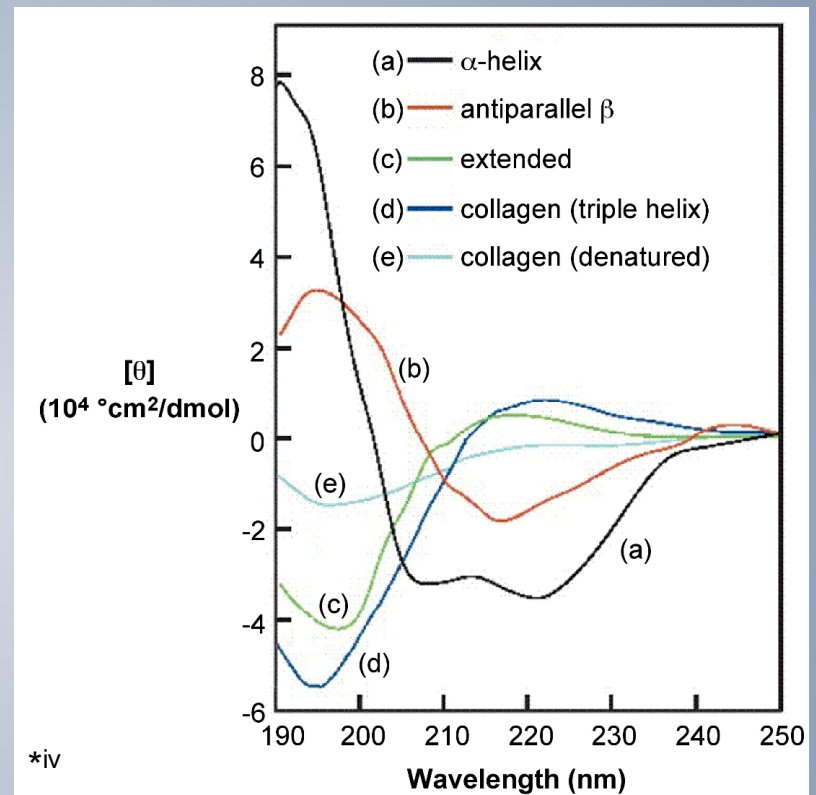
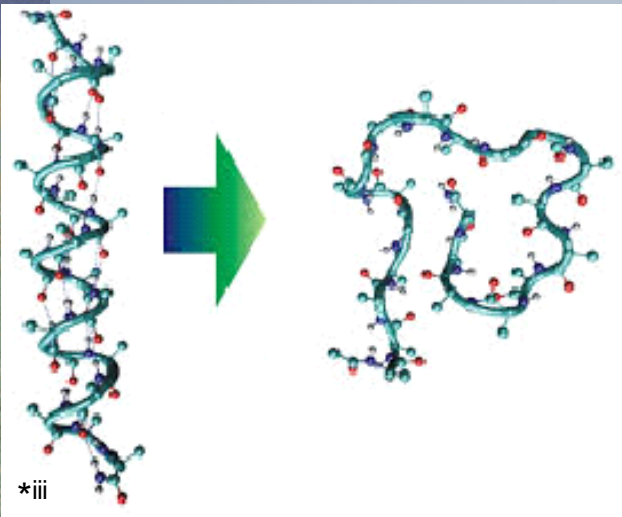


Figure 1. Binding of diazepam to HSA results in an observable change in CD signal. Left: near-UV CD spectra of free (red) and diazepam bound (blue) HSA. Right: change in the near-UV CD spectrum with increasing concentration of diazepam.

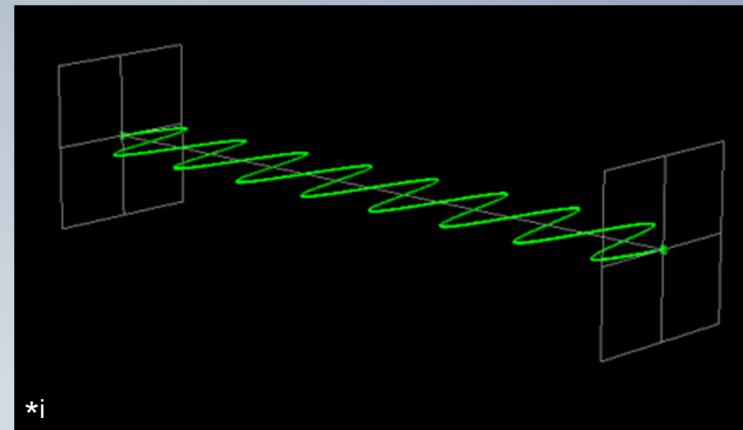
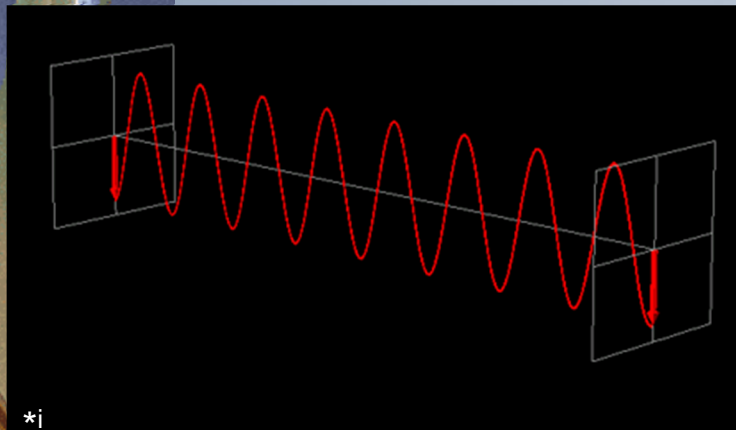




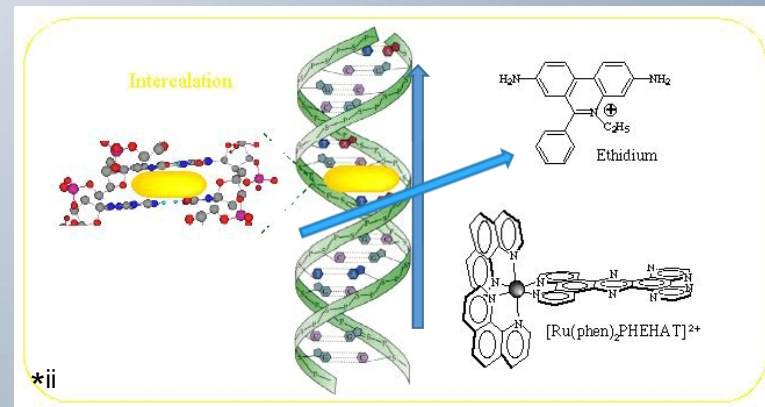
iii) <http://basys2.confex.com/acs/228nm/techprogram/P773650.HTM> 14.07.2014 22:53

iv) <http://cnx.org/content/m38277/latest/?collection=col10699/latest> 14.07.2014. 22:50

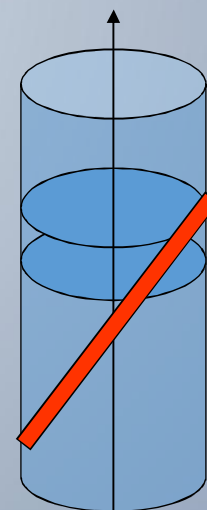
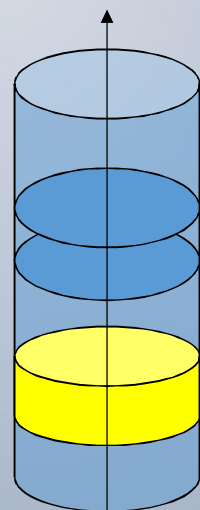
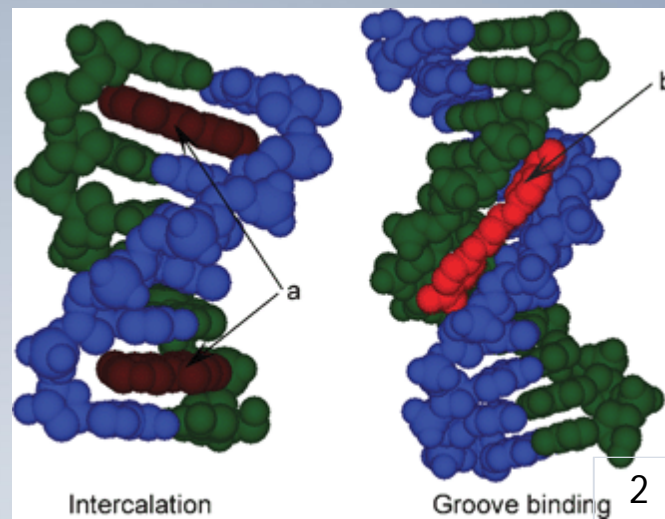
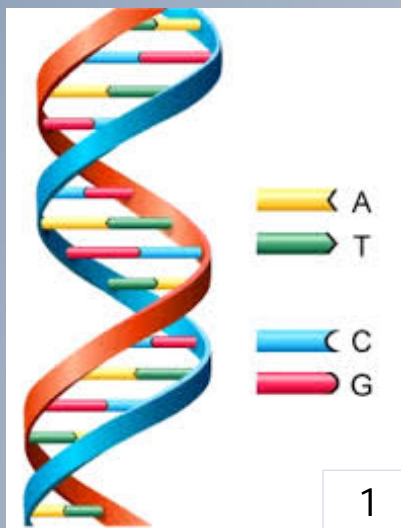
Linearly polarized light



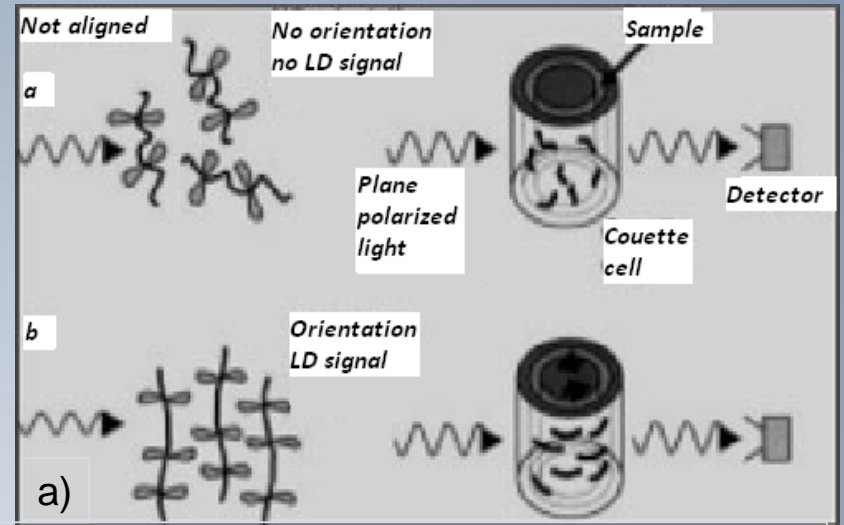
$$\Delta A = A_{\parallel} - A_{\perp}$$



- i) <http://www.photophysics.com/tutorials/circular-dichroism-cd-spectroscopy/1-understanding-circular-dichroism> 15.07.2014. 15.25
- ii) <http://www.photobiology.com/photoiupac2000/pierard/Interactionmain.html> 15.07.2014. 15.32



- 1) <http://www.astrochem.org/sci/Nucleobases.php>
- 2) *Chem. Soc. Rev.*, 2008, **37**, 2745-2757



a) <http://www.kromatek.co.uk/categories/Linear-Dichroism-Couettes/>

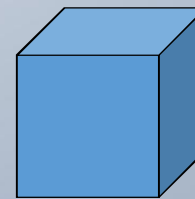
-molecules with high aspect ratio!



LD signal



polynucleotidesⁱ
(slide 3)

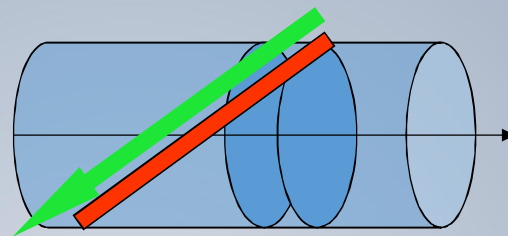
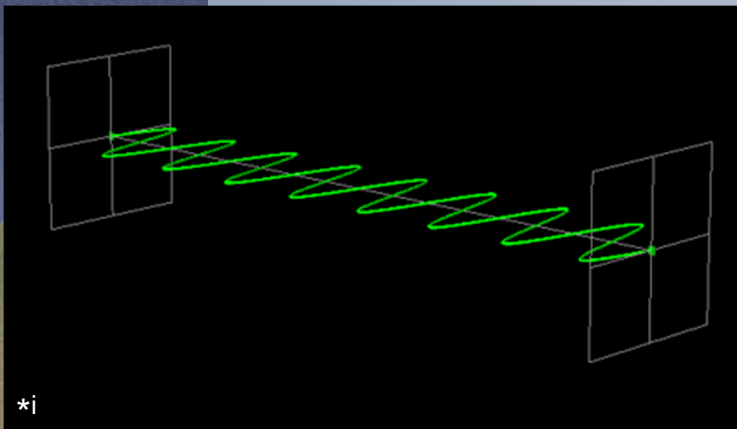


No LD signal

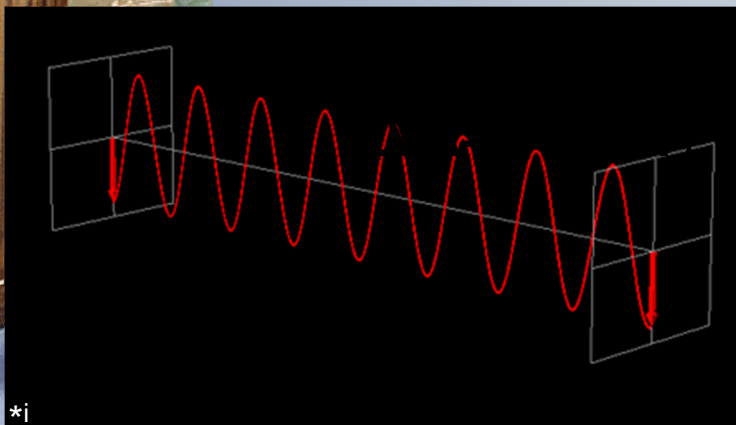
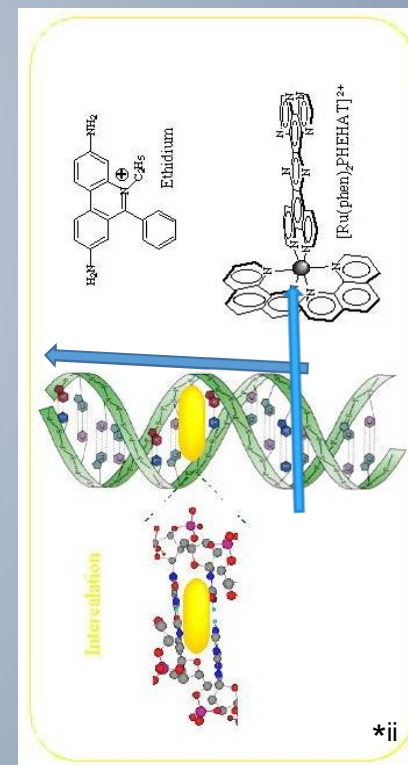
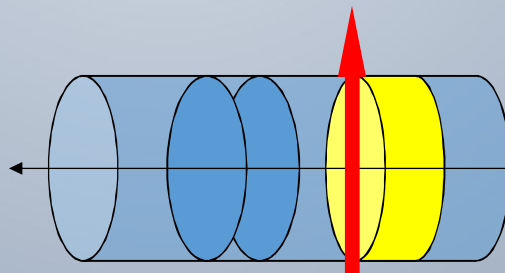


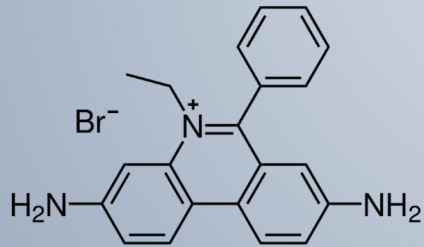
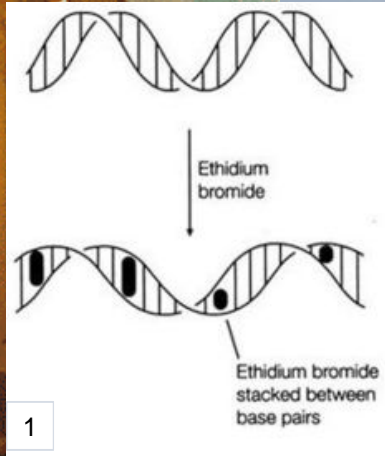
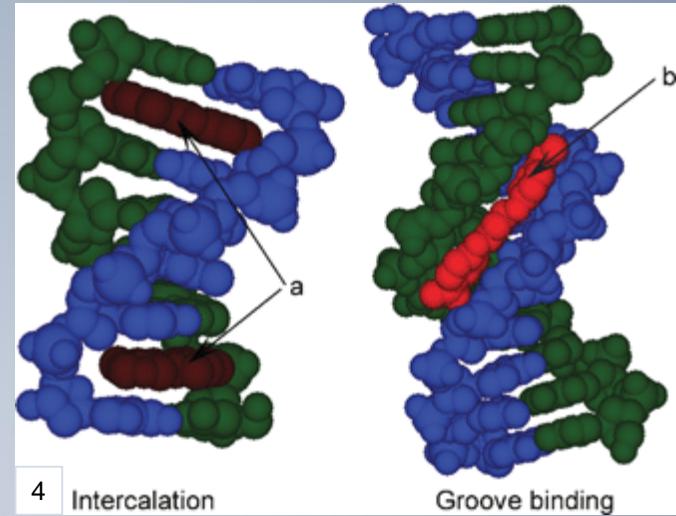
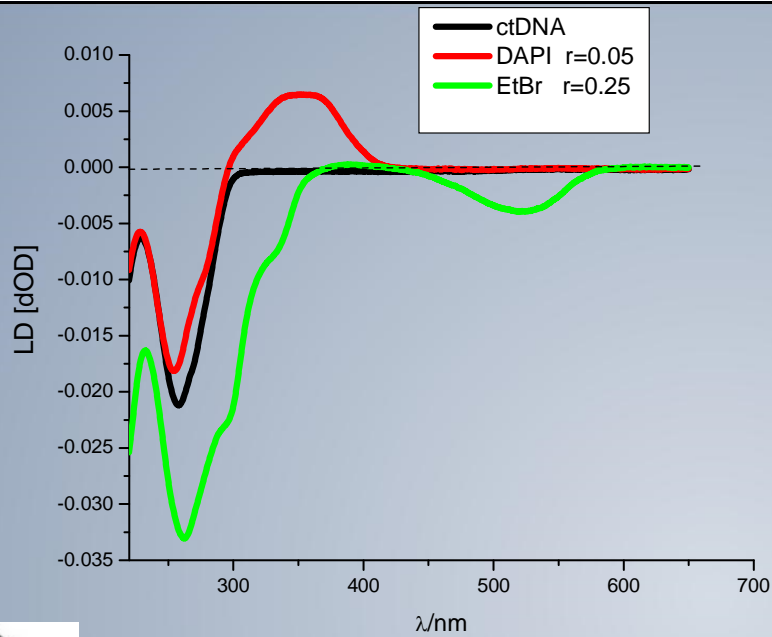
proteinesⁱ (slide 3)

- i) <http://www.photophysics.com/tutorials/circular-dichroism-cd-spectroscopy/1-understanding-circular-dichroism> 15.07.2014. 15.25
- ii) <http://www.photobiology.com/photoiupac2000/pierard/Interactionmain.html> 15.07.2014. 15.32

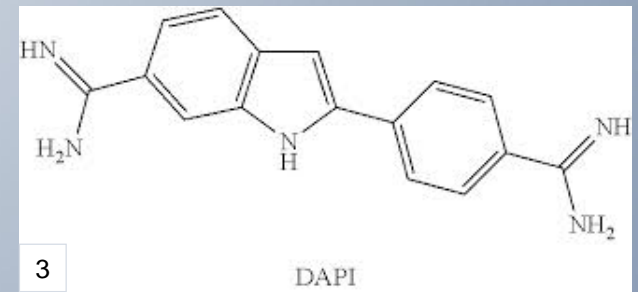


$$\Delta A = A_{\parallel} - A_{\perp}$$





2) Ethidium bromide



3

DAPI

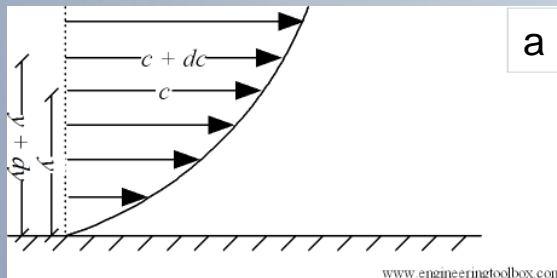
- 1) http://2008.igem.org/Team:Bologna/Biosafety_25.05.2015_14.35
- 2) http://commons.wikimedia.org/wiki/File:Ethidium_bromide.svg
- 3) <http://commons.wikimedia.org/wiki/File:DAPI.svg>
- 4) XiangDong Liu^{*a}, HongYan Diao^b and Norio Nishi^c, *Chem. Soc. Rev.*, 2008, **37**, 2745-2757

Viscometry

Ligand binding mode impact on viscosity of biomacromolecule solution

Viscosity





1. Viscosity of a fluid is a measure of its resistance to gradual deformation by shear stress or tensile stress.^c
2. Viscosity is the measure of a fluid's resistance to flow^c
3. -dynamic (or absolute)
-kinematic
-relative
-intrinsic




^{*)} at room temperature Liquid	Absolute Viscosity^{*)} (Pa s)	b
<u>Air</u>	1.983×10^{-5}	
Water	1×10^{-3}	
Olive Oil	1×10^{-1}	
Glycerol	1×10^0	
Liquid Honey	1×10^1	
Golden Syrup	1×10^2	
Glass	1×10^{40}	



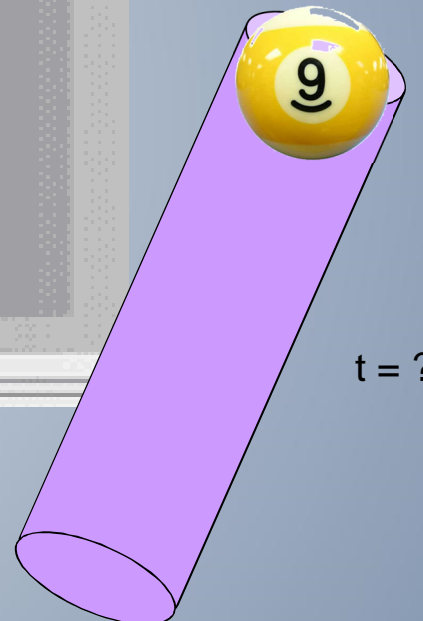
1

1		Water
2		Milk
3		Olive oil
4		Gasoline

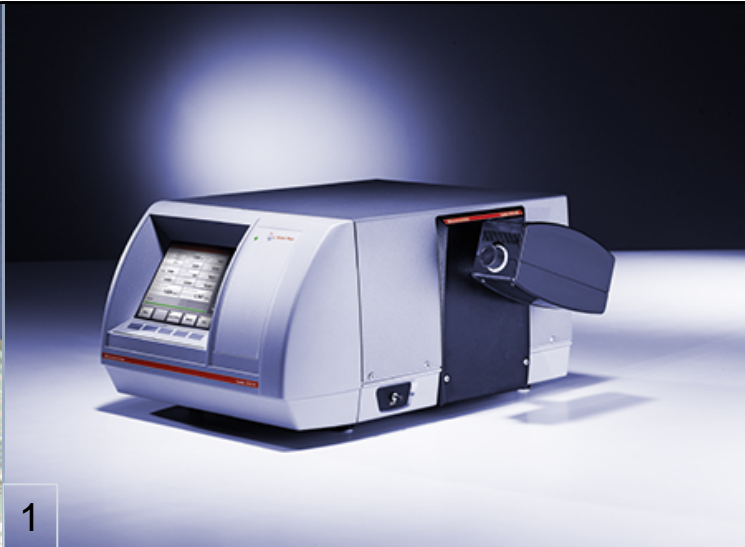
20 °C



Viscosity Exhibition - Discussing Viscosity - Viscosity Values



$t = ?$



1) <http://www.anton-paar.com/corp-en/products/details/rolling-ball-viscometer-lovis-2000-mme/viscometer/>

Lovis 2000 M/ME is intended for measuring the **rolling times** of a **ball in liquid** samples and calculating the samples' viscosities from the obtained times.

1

Viscosity of a solution and biointeractions?

L (free polynucleotide) ≠ L (polynucleotide +ligand)

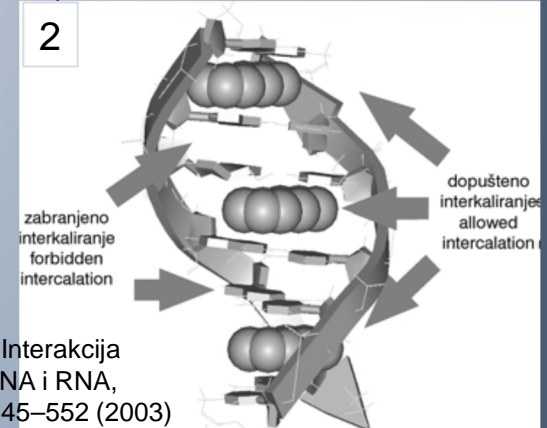
Visc. (free polynucleotide sol.) ≠ Visc. (polynucleotide +ligand sol.)

$$L/L_0 = [(t_r - t_0) / (t_{DNA} - t_0)]^{1/3} = 1 + \alpha^*r$$

α -the viscosity index

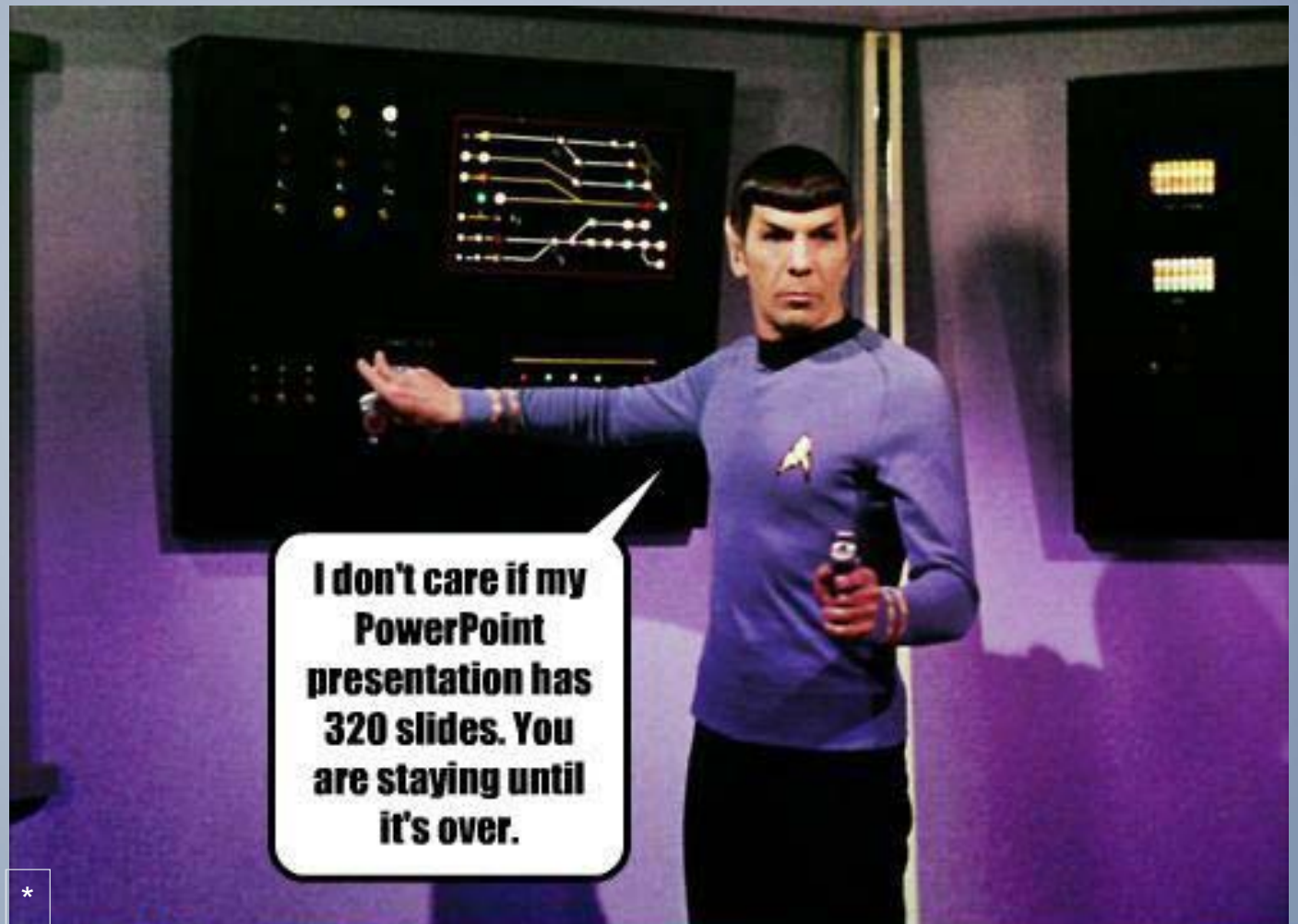
- t_0 buffer
- t_{DNA} free DNA
- t_r DNA complex at reagent / phosphate ratio r ,
- L/L_0 relative DNA lengthening

2



2) I. PIANTANIDA: Interakcija malih molekula s DNA i RNA, *Kem. Ind.* **52** (11) 545–552 (2003)

* <http://blog.flirtcommunications.com/end-30-slides-30-minutes-meetings/>



**I don't care if my
PowerPoint
presentation has
320 slides. You
are staying until
it's over.**

*