Developing Raman spectroscopy as a clinical diagnostic tool for hemoglobin disorders

Kevin C. Hewitt, Joel St. Aubin & Chelsea Nisbett

Dalhousie University
Department of Physics and Atmospheric Science
Halifax, NS Canada B3H 3J5

International Conference of Nanotechnology, Nov. 1, 2005
San Francisco, CA USA

NSERC CRSNG

Canada Foundation for Innovation
Fondation canadienne pour l'innovation

DALHOUSIE UNIVERSITY
Inspiring Minds

Outline

- Motivation
- Hemoglobinopathies, e.g. Sickle cell disease (HbS)
- Raman spectra of amino acids
  - L-Glutamic Acid
  - L-Valine
- Raman Spectra of HbA and HbS
- Conclusions

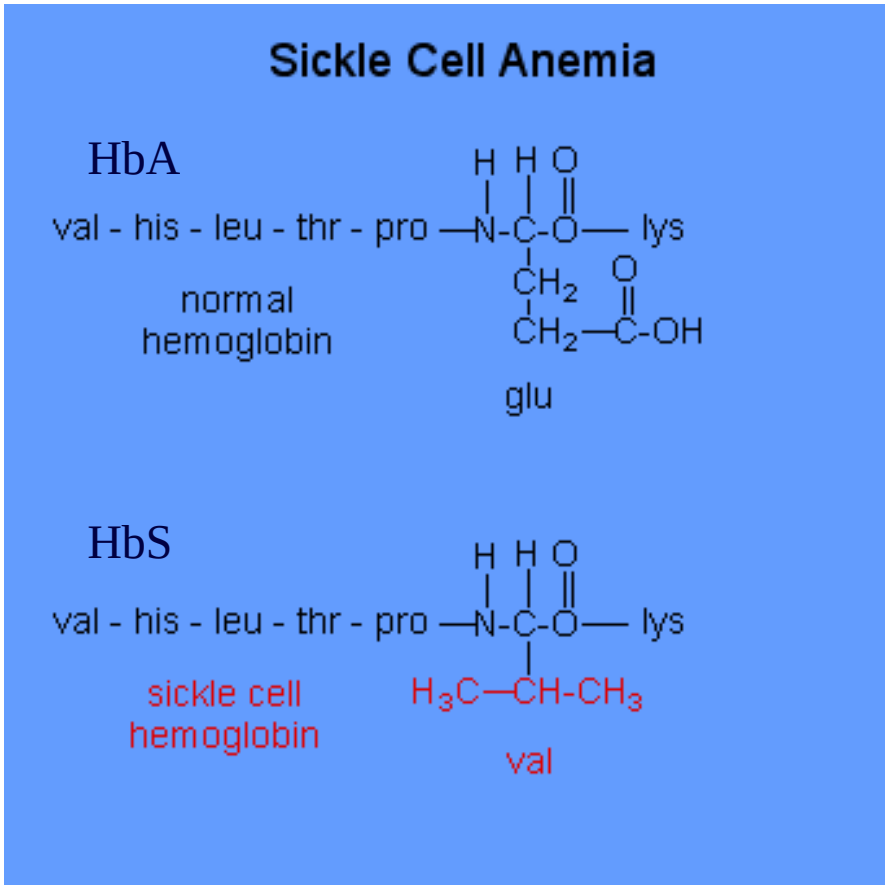
DALHOUSIE UNIVERSITY
Inspiring Minds

Hemoglobin disorders

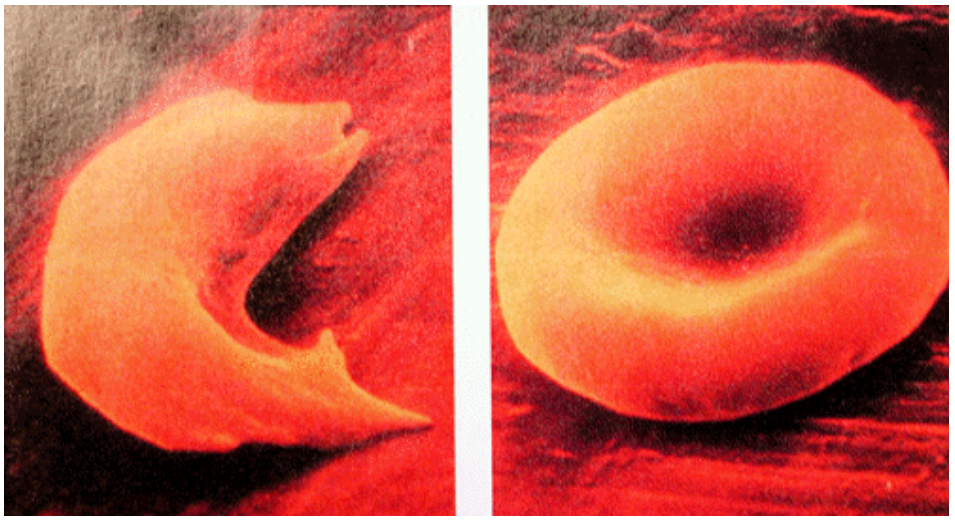
- Qualitative (e.g. amino acid substitutions) or quantitative (loss of portions of hemoglobin protein) changes in the oxygen-carrying protein hemoglobin (Hb)
- 75% of immigrant groups to Canada belong to at risk groups
- 270 million carriers worldwide
- Canadian Quality Management Program laboratory services survey (1990-2000) found "recurrent errors in diagnosis of carrier genotypes for serious hemoglobinopathies"
- A complementary technique is needed.
- Selectivity, sensitivity, cost, ease of use, turn-around time

DALHOUSIE UNIVERSITY
Inspiring Minds

Hemoglobinopathy, e.g. Sickle Cell disease



Oxygenated RBC with HbS looks normal but once deoxygenated it shows the characteristic sickle shape.



- Single Amino acid substitution on both beta chains.
- Valine is hydrophobic while Glutamic Acid is hydrophilic.

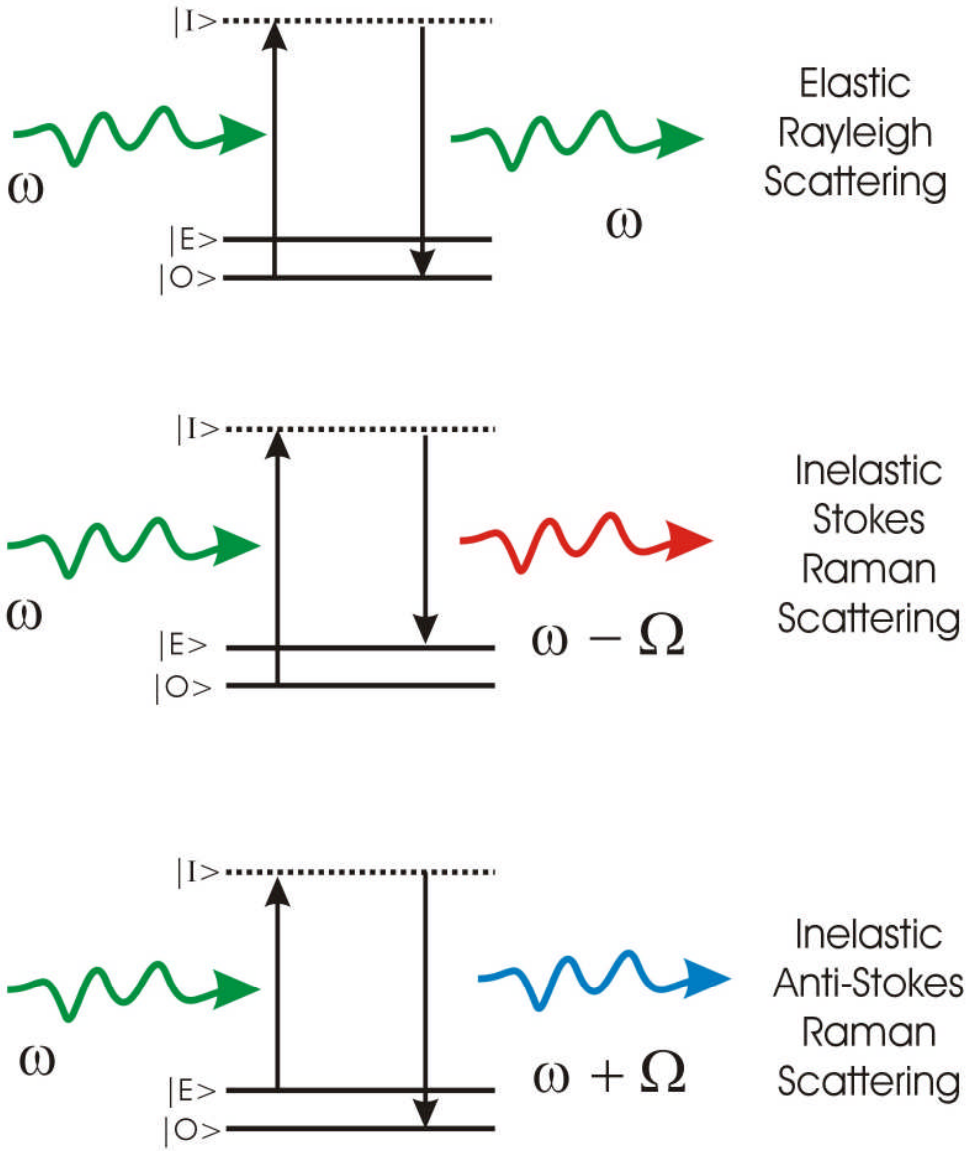
DALHOUSIE UNIVERSITY
Inspiring Minds

Raman Spectroscopy

- **Why Raman scattering?**
  - Inelastic light scattering.
  - Energy of the scattered photon changes due to its interaction with vibrational quanta (phonons) in the sample.
  - Vibrational modes determined by mass, bond type, and symmetry of the atoms in the solid.
  - Raman spectra gives molecular information - SPECTRAL FINGERPRINT of compound
  - No special sample preparation necessary.

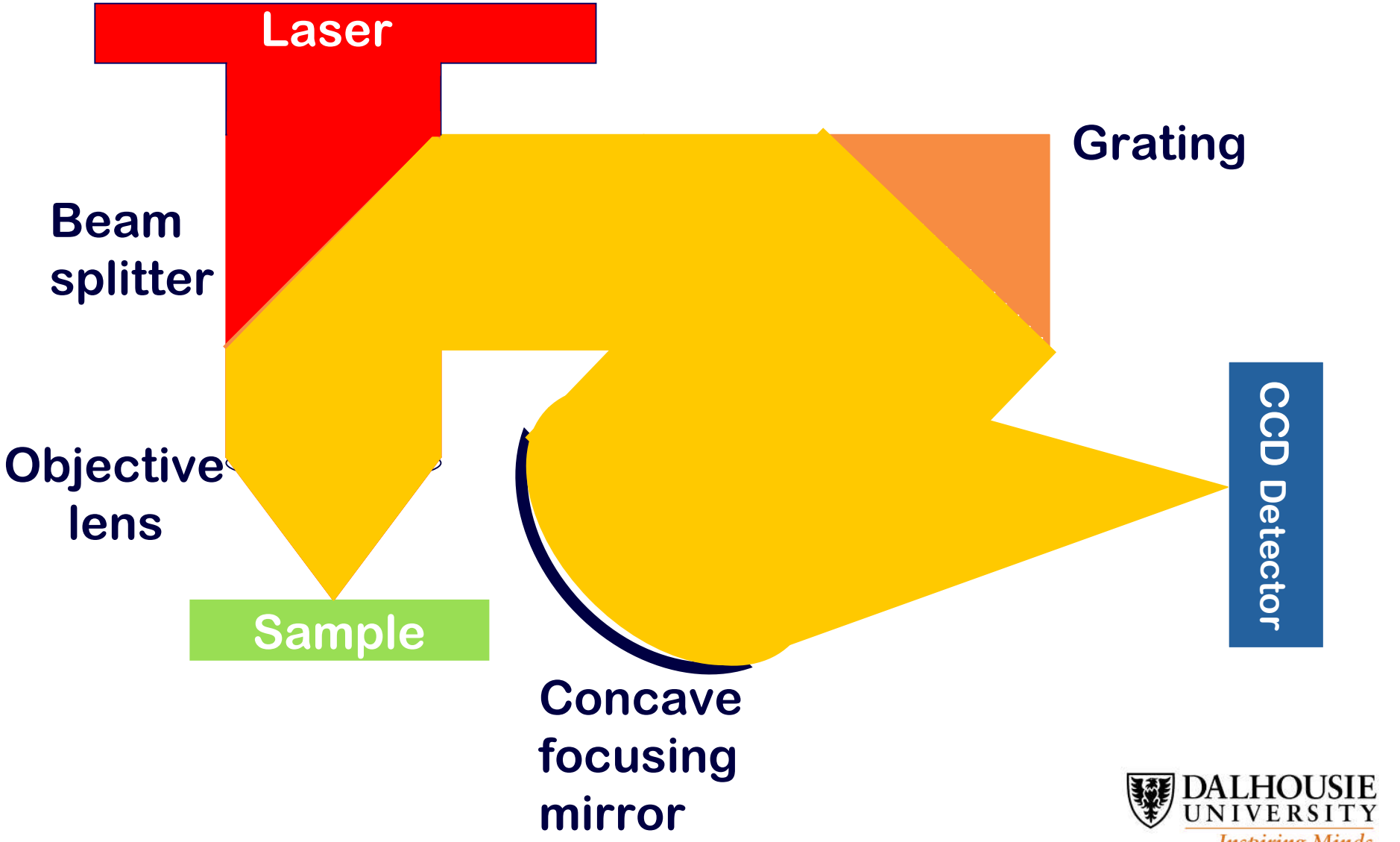
DALHOUSIE UNIVERSITY
Inspiring Minds

Raman Spectroscopy



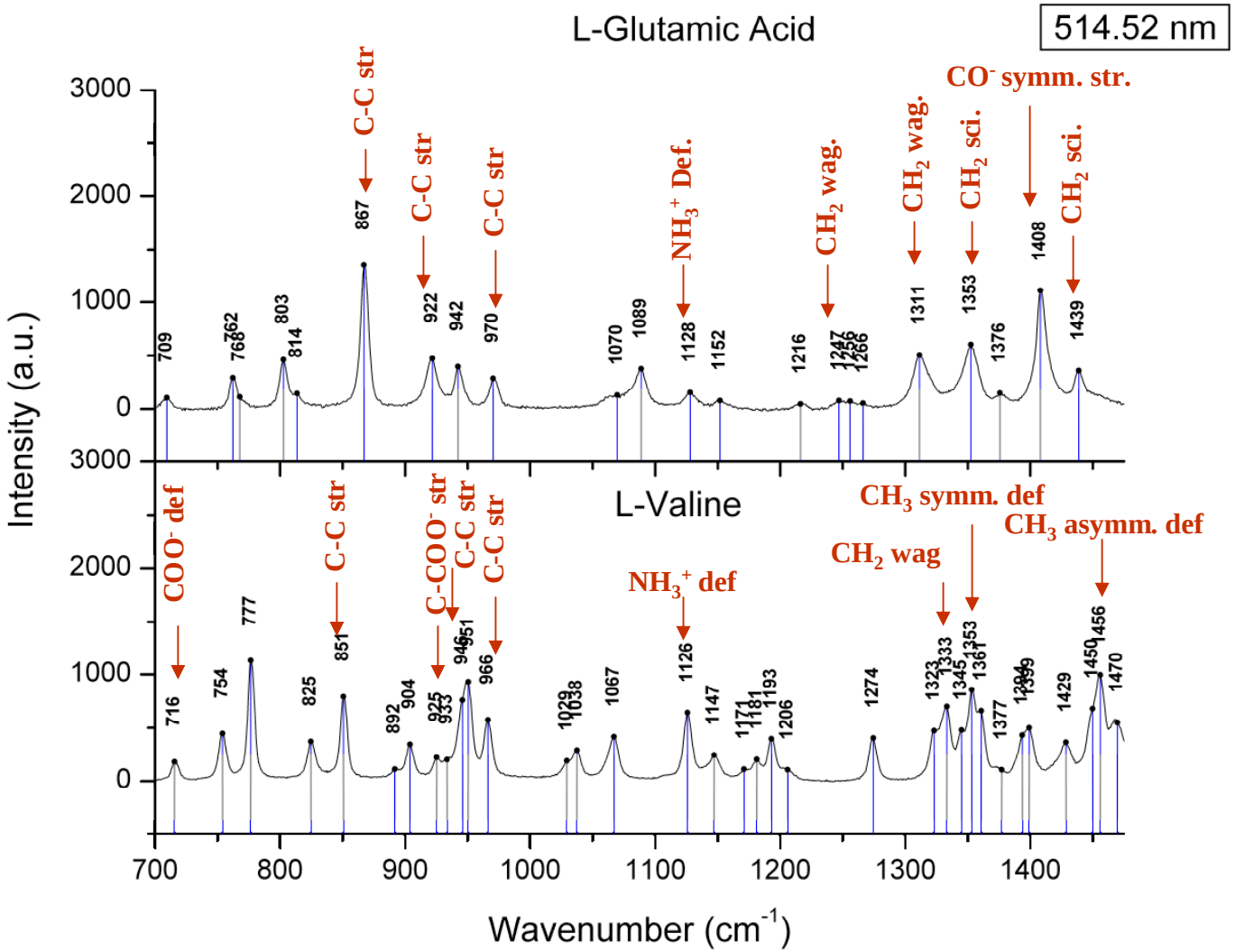
DALHOUSIE UNIVERSITY
Inspiring Minds

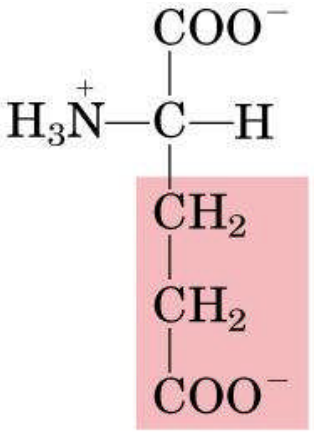
Experimental arrangement

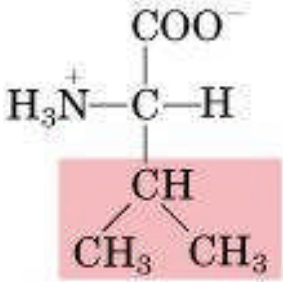


DALHOUSIE UNIVERSITY
Inspiring Minds

Raman spectra – valine & glutamic acid

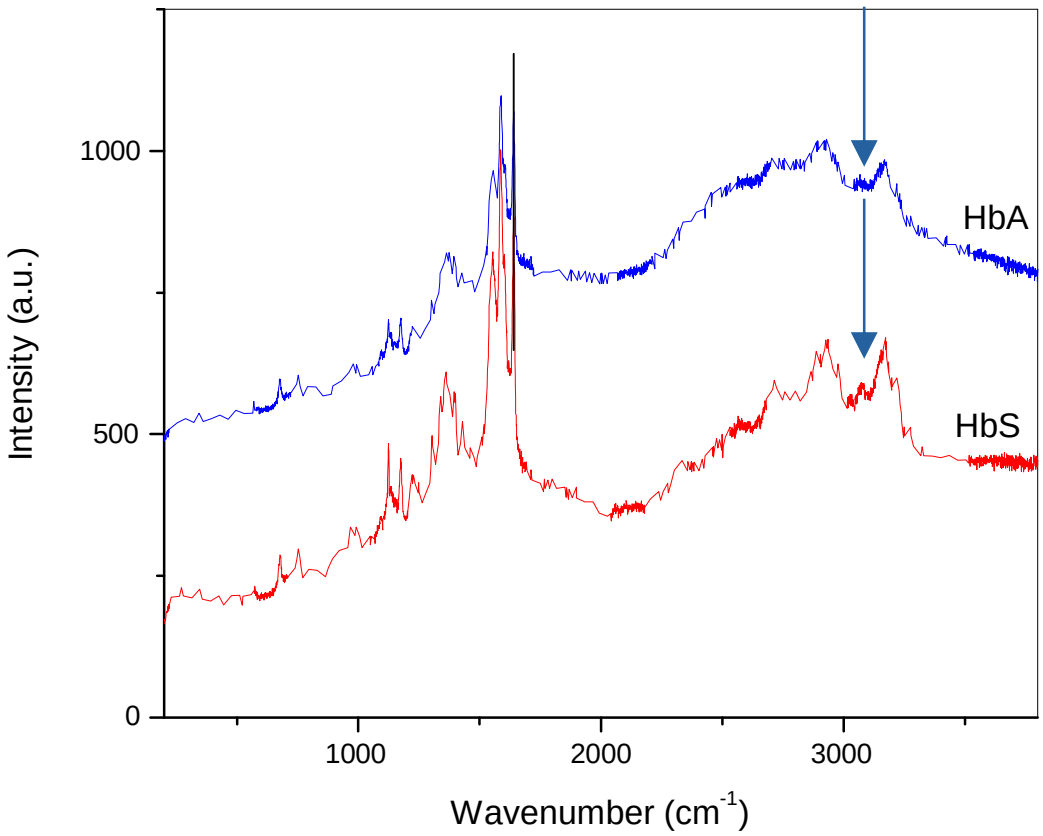






DALHOUSIE UNIVERSITY
Inspiring Minds

Conventional Raman spectra - HbA & HbS



Change of 2 in 574 amino acids (0.03%) not easily detected.

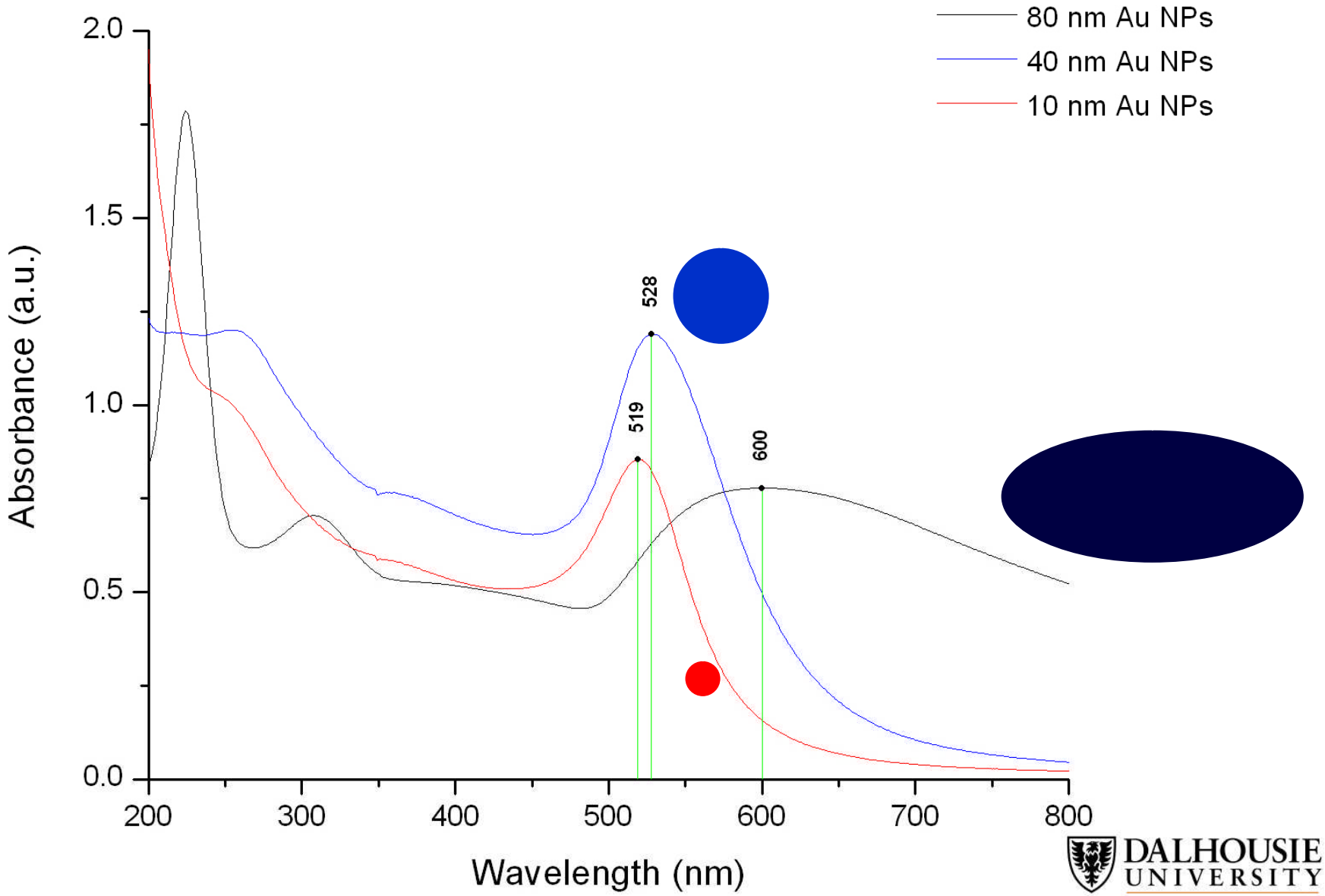
DALHOUSIE UNIVERSITY
Inspiring Minds

Surfaced Enhanced Raman Scattering (SERS)

- Enhancement in Raman signals obtained by adsorbing Au nanoparticles to site of interest.
- Plasmon resonance of Au nanoparticles create a large electric field.
- Plasmon resonance excitation wavelength can be found using UV-VIS of the Au nanoparticle.
- Plasmon resonance excitation wavelength depends on particle size and shape.

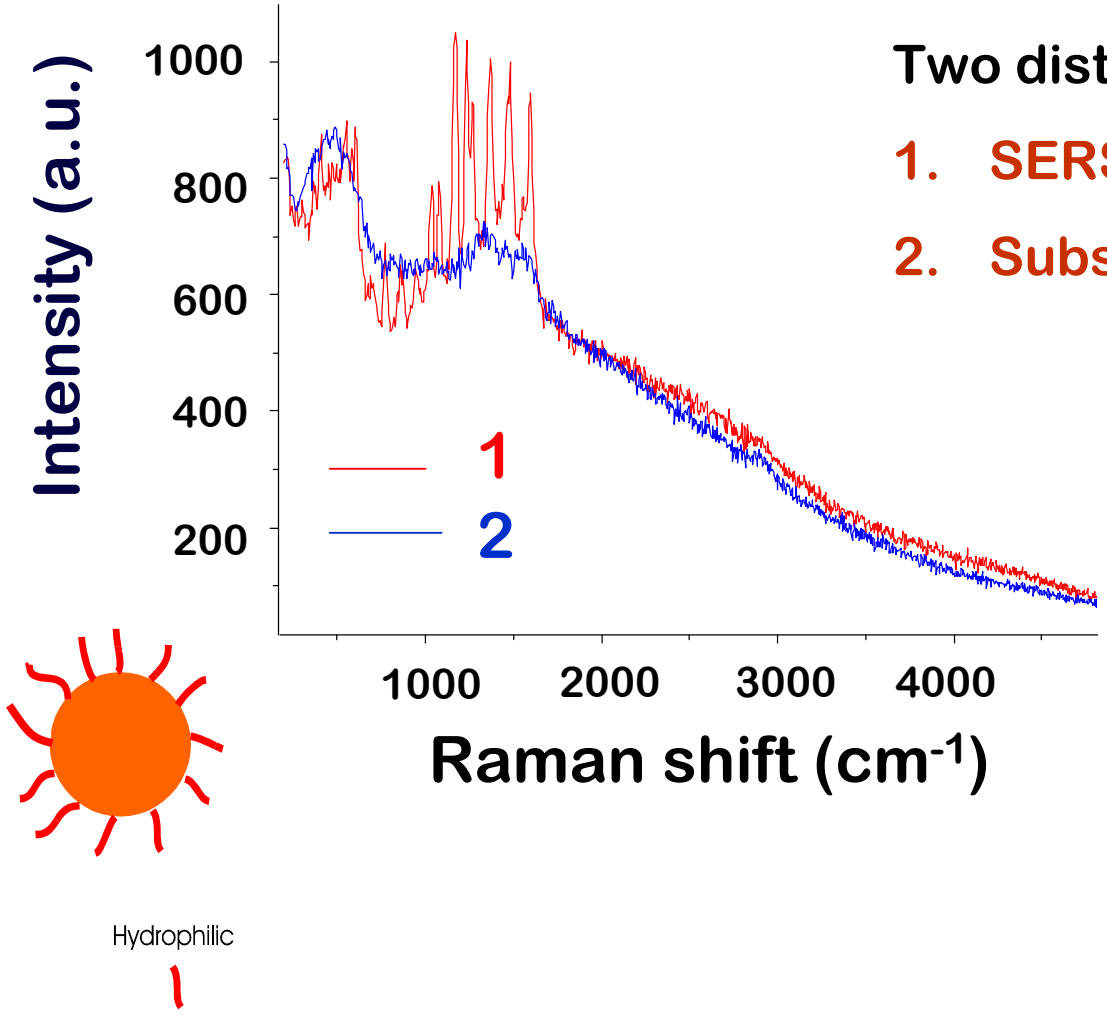
DALHOUSIE UNIVERSITY
Inspiring Minds

UV-VIS spectra – three particle sizes



DALHOUSIE UNIVERSITY
Inspiring Minds

SERS - hydrophilic ligand (10^{-4} M)



Scan of

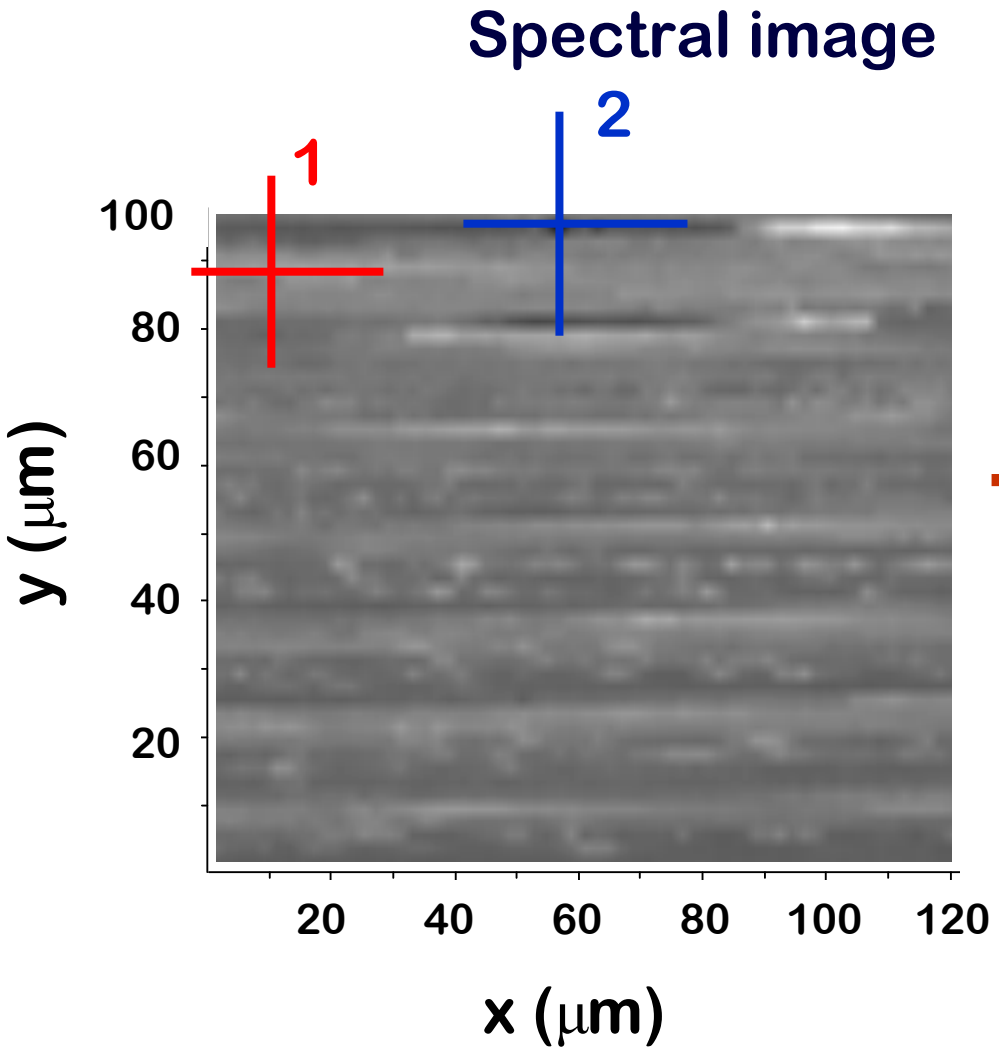
– 100 µm ×120 µm area reveals

Two distinct spectral signatures

1. SERS of ligand
2. Substrate background

DALHOUSIE UNIVERSITY
Inspiring Minds

SERS - hydrophilic ligand (10^{-4} M)

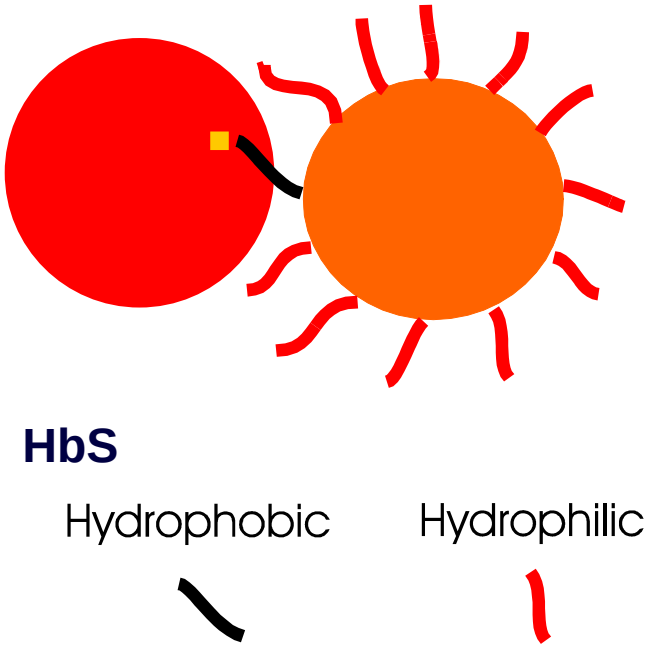


$$\frac{\int I_{(971\ cm^{-1} \le \omega \le 1749\ cm^{-1})}\, d\omega}{\int I_{(171\ cm^{-1} \le \omega \le 1628\ cm^{-1})}\, d\omega} = R$$

Wang *et al.* J. Am. Chem. Soc. 127, 14992 (2005)

DALHOUSIE UNIVERSITY
Inspiring Minds

Future experiment – SERS, HbS



DALHOUSIE UNIVERSITY
Inspiring Minds

Summary

- Observe clear differences in Raman spectra of valine and glutamic acid
- Observe very small differences in conventional Raman spectra of HbA and HbS
- Observe surface enhanced Raman spectroscopy of hydrophilic ligand adsorbed to gold nanoparticles -- at low concentrations
- Next step – attach to hemoglobin

DALHOUSIE UNIVERSITY
Inspiring Minds