Observation of single biomolecules and their dynamics in Surface Enhanced Raman Spectroscopy

E. Finot

eric.finot@u-bourgogne.fr

Outline

Motivation to single molecule techniques

Raman spectroscopy in biology

Fabrication of Nanostructured Sample enhancement

Sorting and identification of single molecules

Dynamics and counting of single molecules

Bourgogne Conseil régional

Liberté • Égalité • Fraternité
RÉPUBLIQUE FRANÇAISE
MINISTÈRE DE L'ENSEIGNEMENT SUPÉRIEUR ET DE LA RECHERCHE

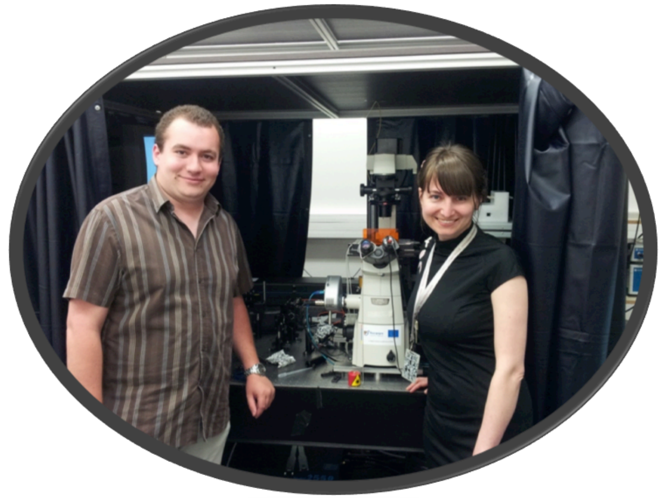
SEVENTH FRAMEWORK PROGRAMME

AGENCE NATIONALE DE LA RECHERCHE ANR

UMR 6303 ICB

UBFC UNIVERSITÉ BOURGOGNE FRANCHE-COMTÉ

Aymeric
Leray
Biology
microscopy



Thibault Brûlé
Physics

Hélène Yockell
Colloids

Alain Dereux
Director of Institute

Laurent Markey
Microfluidics

Jean Emmanuel
Clément

Alexandre Bouhelier
Optics

Why do we need single molecule techniques ?

Complexity

Challenges in biophysics

Context : existing sensors

- **Wireless mobile physical sensors (2000)**
  Environment monitoring or surveillance
  (light, heat , humidity, temperature)

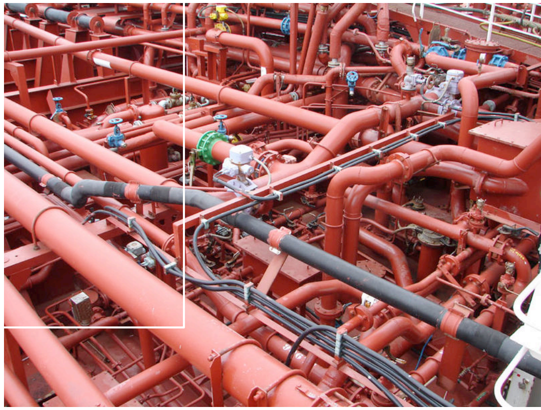
- **Operating system**
  On line collaborative sensor management plateform (Wikisensing platform, 2012)
  Operating systems for wireless sensor network nodes (TinyOS, 2009)

On board physical sensors

What is at stake today ?

- An increasingly complex networked world
- Exploring the unknown
- Decision support prescription tools

→ Give sense to sensors **in a real life**



Into the unknown : from complicated to complex issues

Target

Simple – Complicated – Complex – Chaotic

Simple

Sensitive Probe

↓

No analysis
Reflex

Complicated

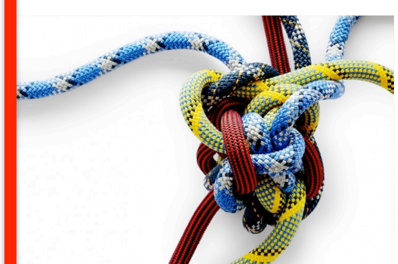
Sensing of selective parameters

↓

Expertise
SMART NETWORK
Correlation Model

↓

Prediction
Action



Complex

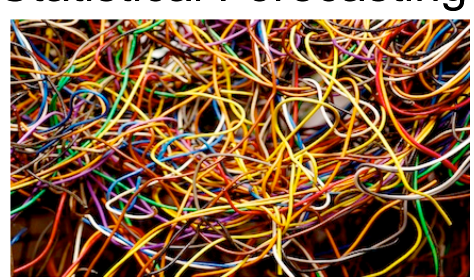
SMART Sensing

↓

No causal relationship
Learning process
SMART NETWORK

↓

Statistical Forecasting



Chaotic

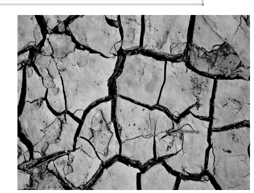
Unpredictable
Evolution of life

↓

Observations

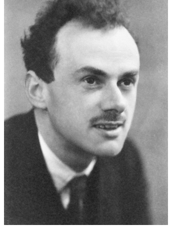
↓

a-priori decision



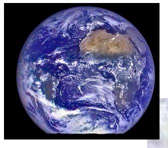
Target

« Pick a flower on Earth and you move the farthest star »
P. Dirac



MULTIDISCIPLINARiTY — ISSUE — SENSING TOOLS

m
10^6
10^3
1
10^{-3}
10^{-6}
10^{-9}



Ecology

Food

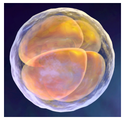
Ageing

Health

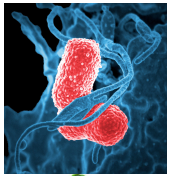
risk
rare
serious

risk
probable
but limited

Diagnostic



Human Health monitoring



Materials Health monitoring

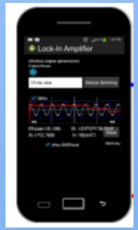
Human Health monitoring

Early-stage forecasting

Prognostic

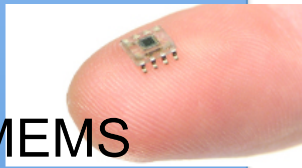
SMART NETWORK

drone



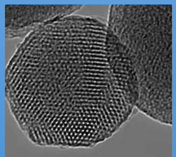
i-phone

SMART SENSORS

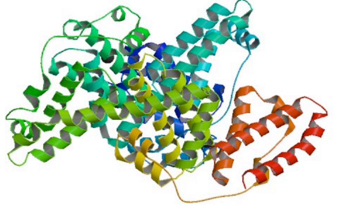


MEMS

Nanoparticle
Plasmonics

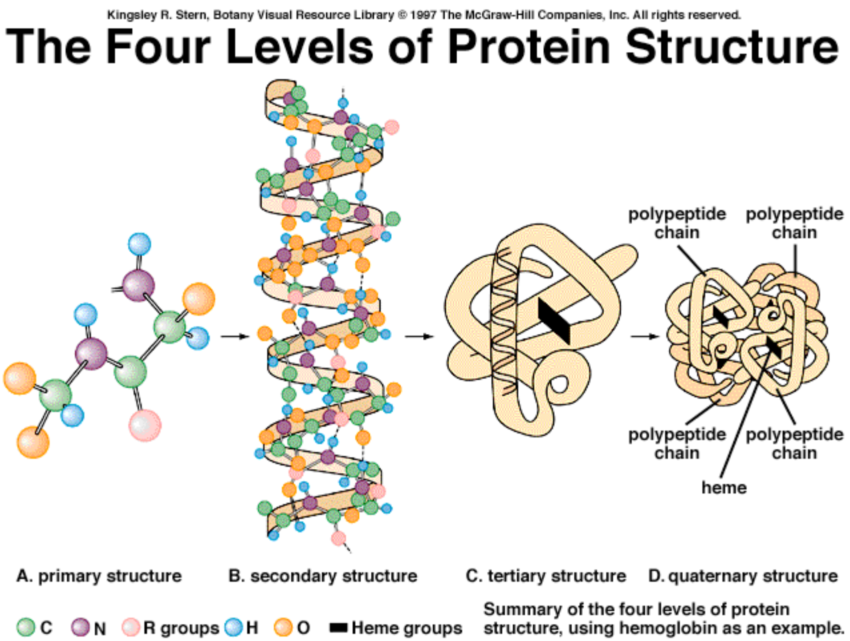


New tools for proteins



Determining dynamics of protein structure => Understanding of protein function

Design of new proteins



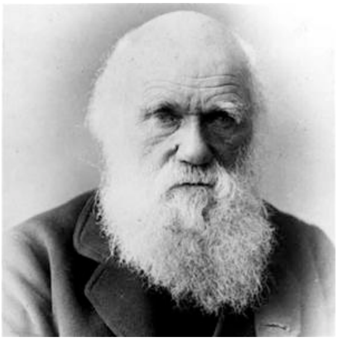
Primary structure : sequence 20 amino-acids connected by strong peptide bonds

Secondary structure : local folding
- α-helix
- β-sheet

Third structure : complex interactions
3-D conformation

"Species that survive are not the strongest or , the most intelligent but those that are the most adaptatable to their changing environment »

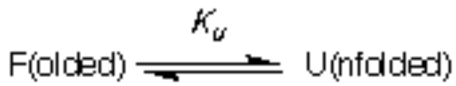
C. Darwing 1838



From chemical point of view

Phase transitions between unfolded and native forms

Assumption on reversibility : Linear polymer having thermodynamical stability



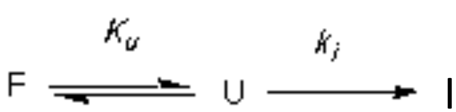
K_u is the equilibrium constant for unfolding.

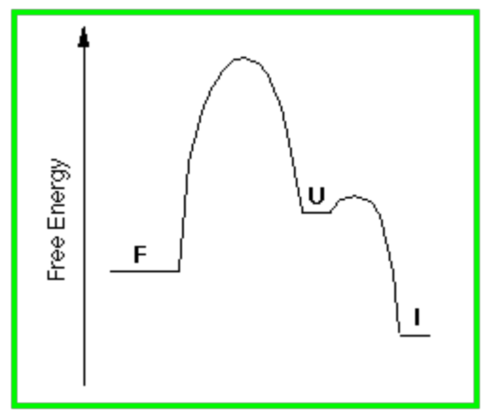
Activation by low difference in free energy G (5-15 kcal/mol)

Comparison to covalend bond (30-100 kcal/mol)

Irreversibility – unstability : not fully unfolded and/or folded

Kinetic unstability : multiple constant rates





Activation by - difference in free energy G

- surface properties (charge, surface energy, hydrophilicity)

Multiple states

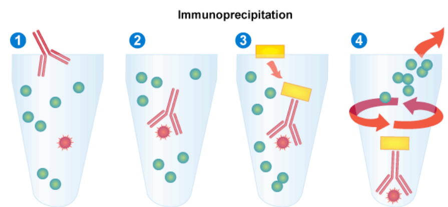
Challenges

Predicting reaction rates in complex protein dynamics

Environment fluctuations ⟷ Amino acids interaction

Protein identity : proteomics approaches

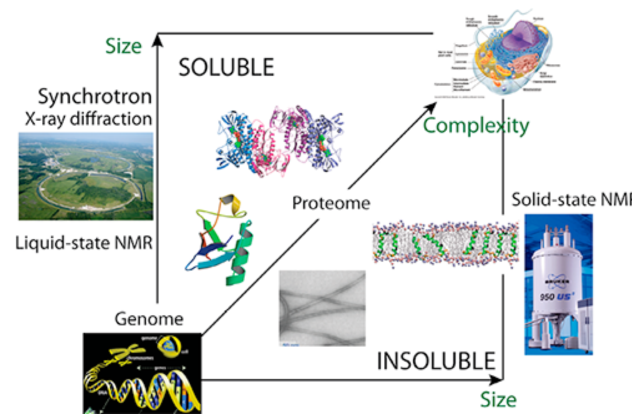
such as immunoassays based on antibodies
(Elisa, Western Blot, SDS-PAGE)



Surface Plasmon Resonance

High resolution structure

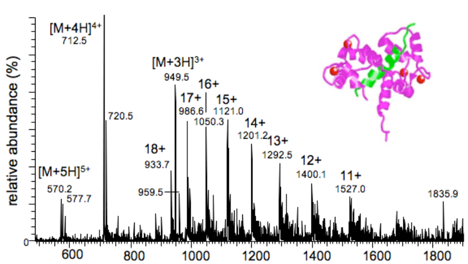
X-ray crystallography, TEM
neutron or nuclear magnetic resonance (NMR)



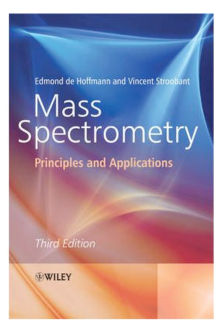
Limited <10 kDa

Protein structure : mass spectroscopy

Needs of large homogeneous sampling



Siuzdak (1994)

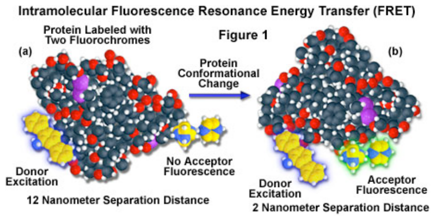


Single molecule techniques

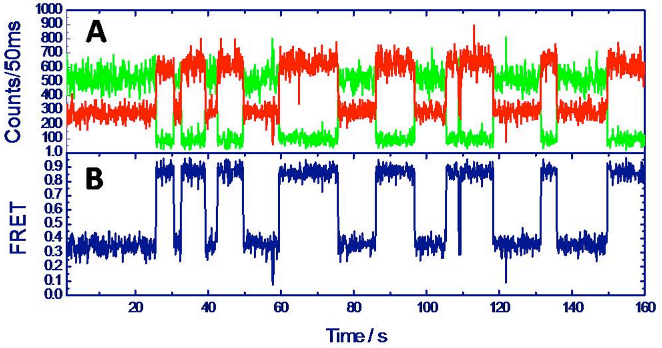
Direct observation of the dynamics of proteins

Fluorescence Correlation Spectroscopy

Förster resonance energy transfer (FRET)

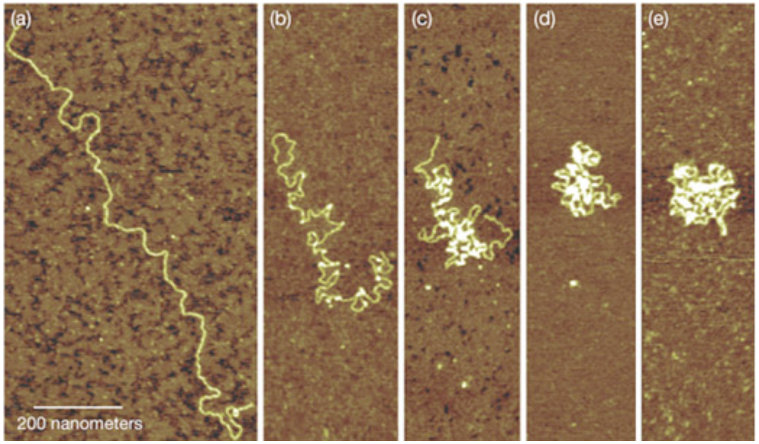


http://soft-matter.seas.harvard.edu/images/5/5e/FRET1.jpg



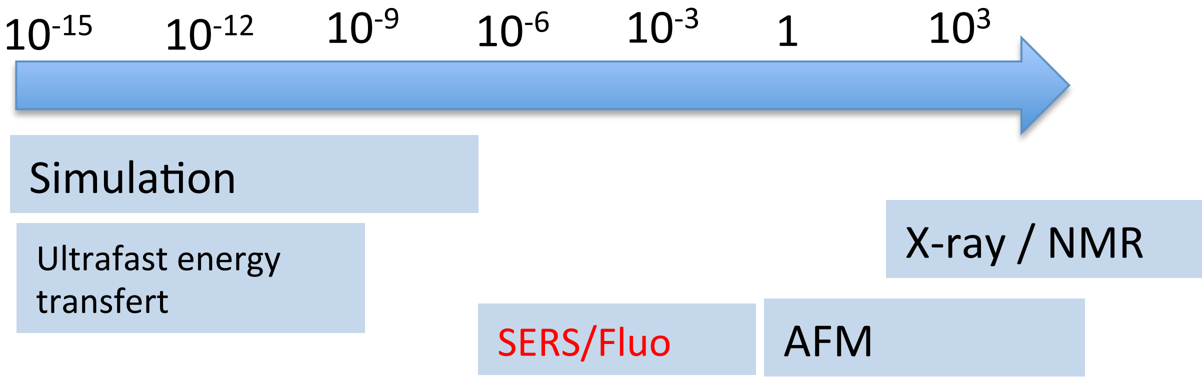
High Speed

Atomic Force Microscopy



This avoids the complexities of averaging over heterogeneous dynamics in bulk biochemical assays.

Time scale in protein dynamics



Numerical simulation for 1 μs

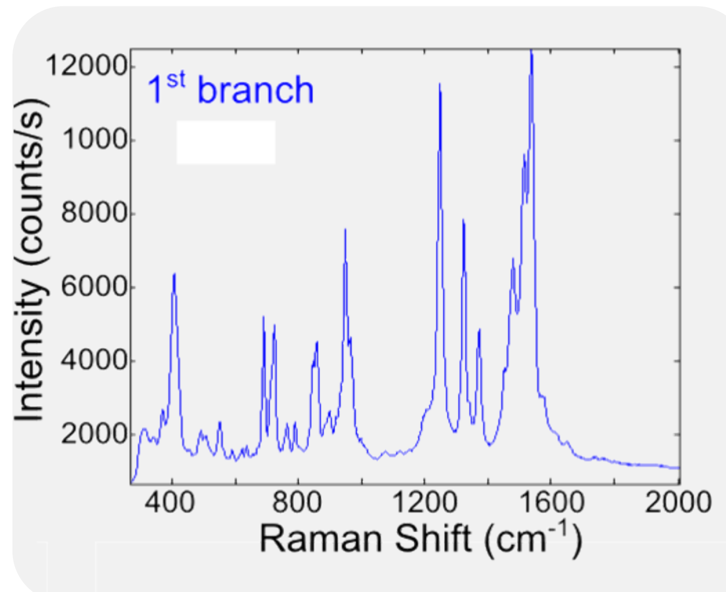
P. Senet , Dijon

Raman scattering in biology?

What information can we get from Raman ?

From Band intensity : The intensity of spectral features in solution is directly proportional to the concentration of the particular species and the time acquisition and laser power, the EM gain

From band position : the selectivity



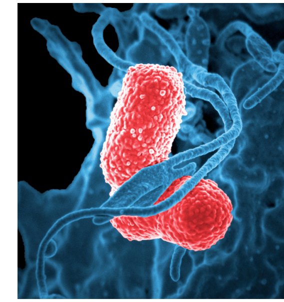
Bandwidth : time scale of vibration 10^{-13}s,
snapshot of conformers
=> hetereogenneous broadning
Flexible structures => broader bands
Quantification of the degree of freedom of molecules : entropic effect

Conformation selection

Spectroscopy
for medical diagnosis

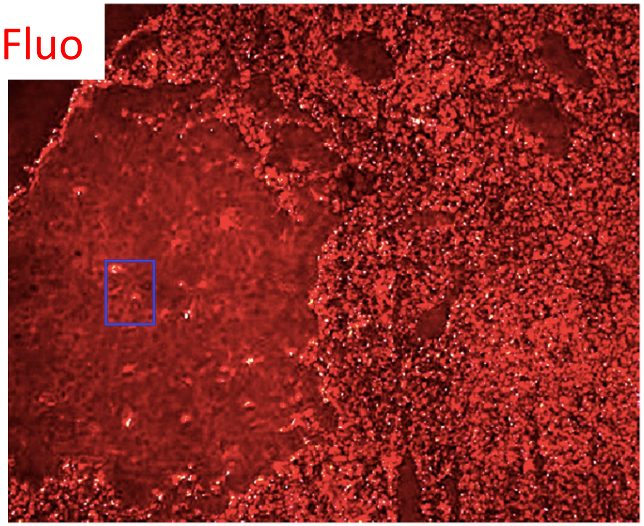
- Lipid
- Density of protein
- Solvent

Bacteria

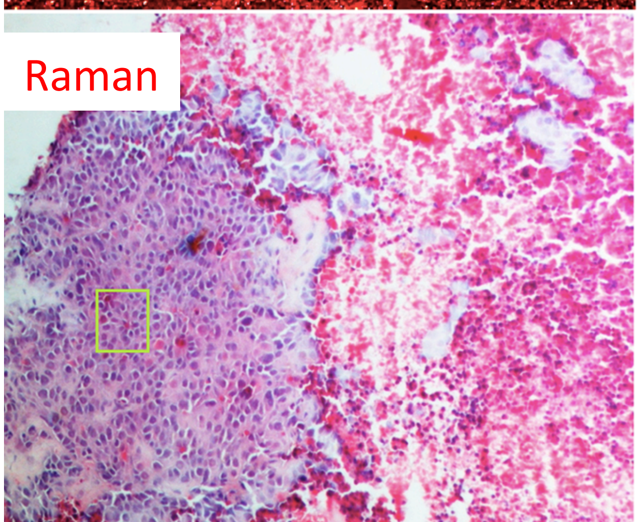


Optical microscopy
(Henkjan Gersen)

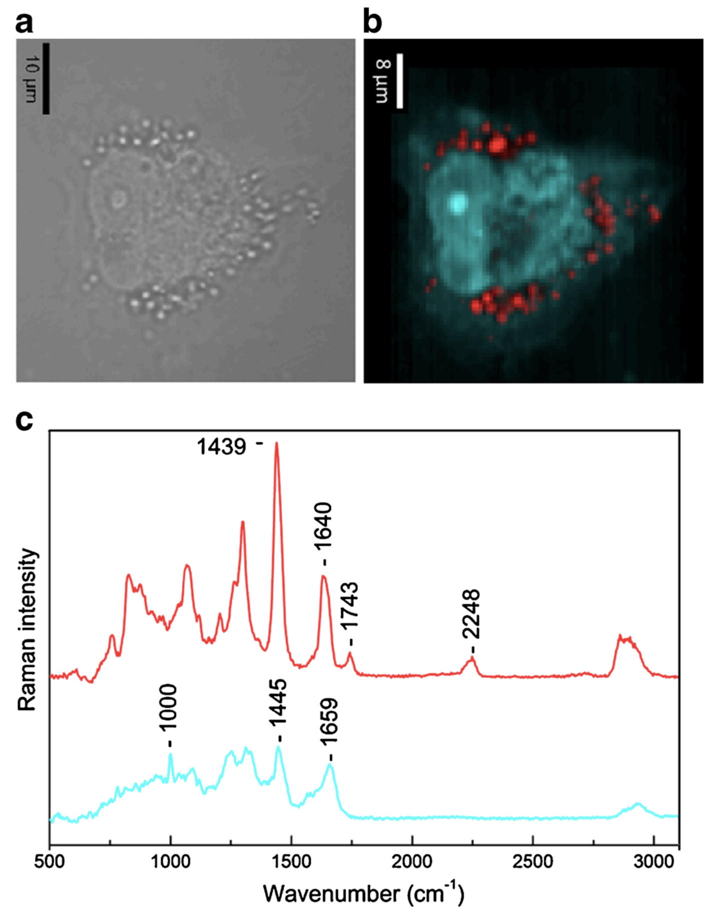
Fluo



Raman



Single cells

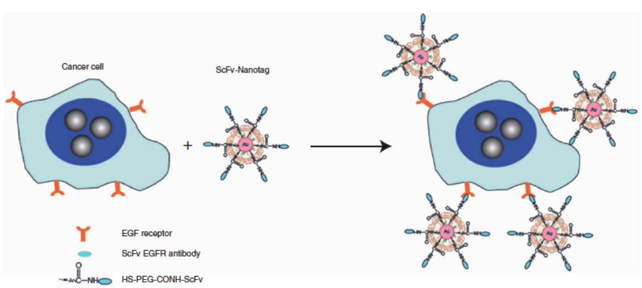


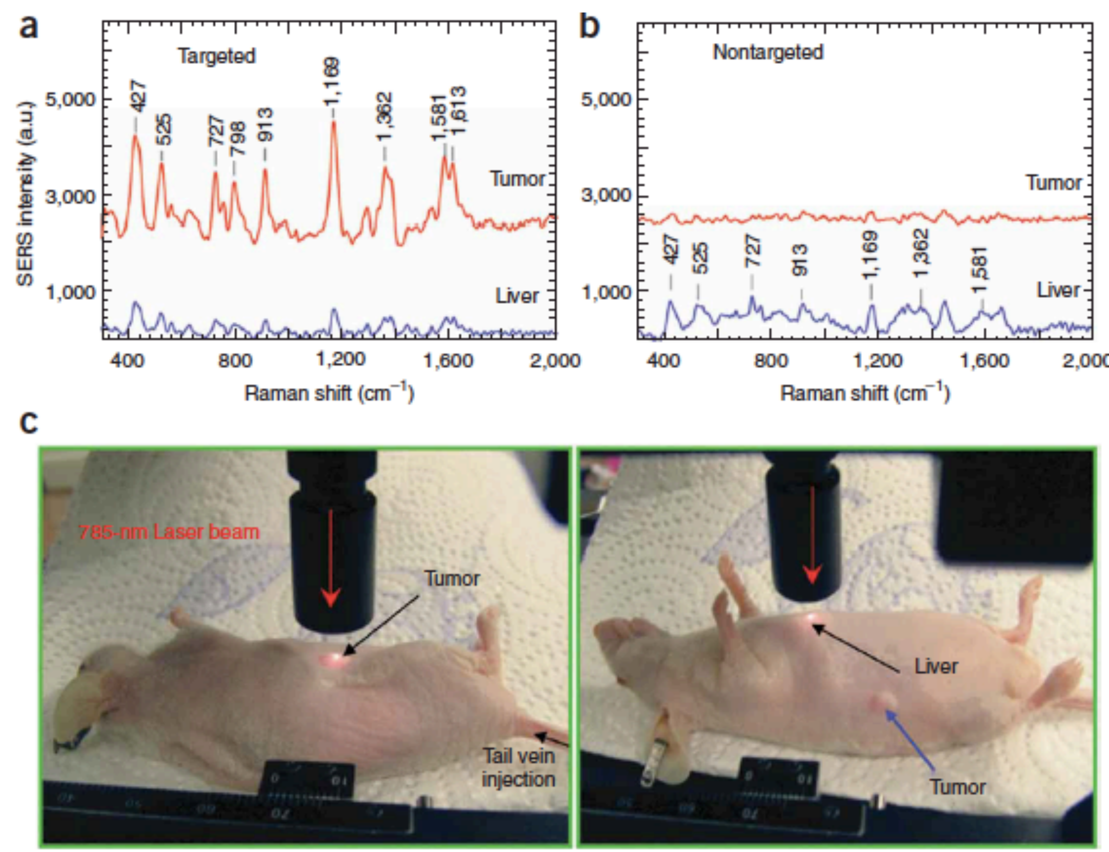
Krafft *et al. (2015, 2012)*

NATURE BIOTECHNOLOGY VOLUME 26 NUMBER 1 JANUARY 2008

In vivo tumor targeting and spectroscopic detection with surface-enhanced Raman nanoparticle tags

Ximei Qian[1], Xiang-Hong Peng[2], Dominic O Ansari[1], Qiqin Yin-Goen[3], Georgia Z Chen[2], Dong M Shin[2], Lily Yang[2,4], Andrew N Young[3], May D Wang[5] & Shuming Nie[1,2]

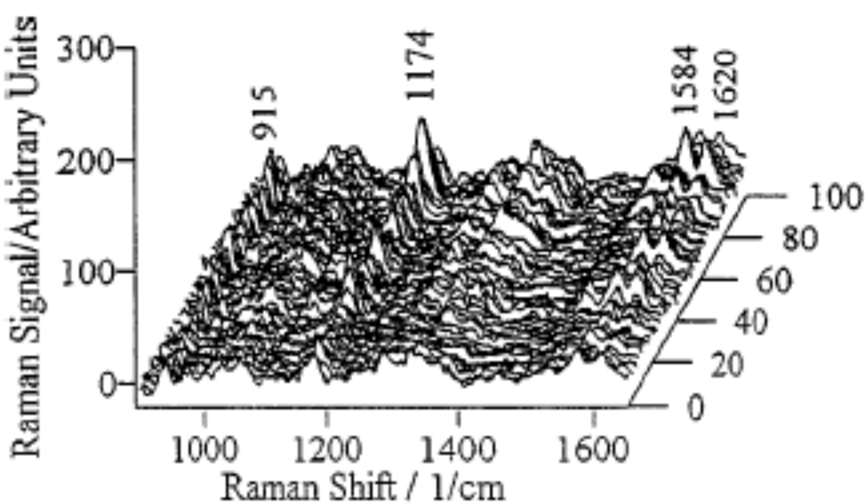


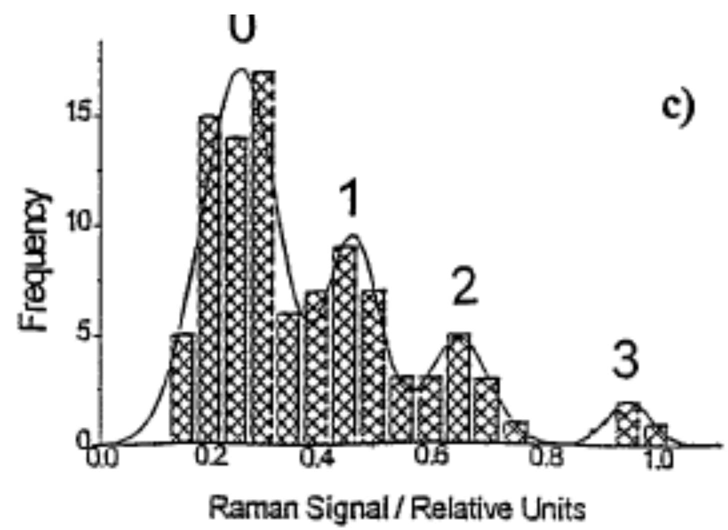


Single Molecule Detection Using Surface-Enhanced Raman Scattering (SERS)

Katrin Kneipp, Yang Wang,* Harald Kneipp,† Lev T. Perelman, Irving Itzkan,
Ramachandra R. Dasari, and Michael S. Feld

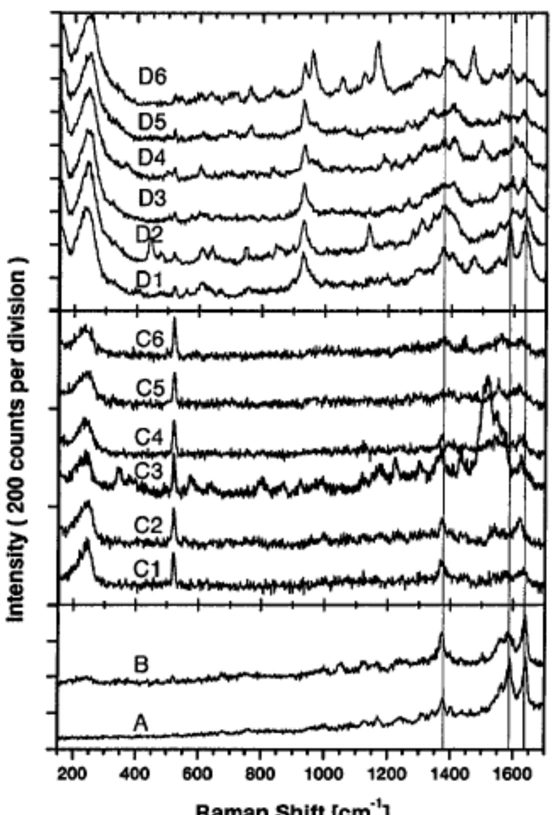
George R. Harrison Spectroscopy Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139
Department of Physics, Technical University of Berlin, D 10623 Berlin, Germany





Spectroscopy of Single Hemoglobin Molecules by Surface Enhanced Raman Scattering

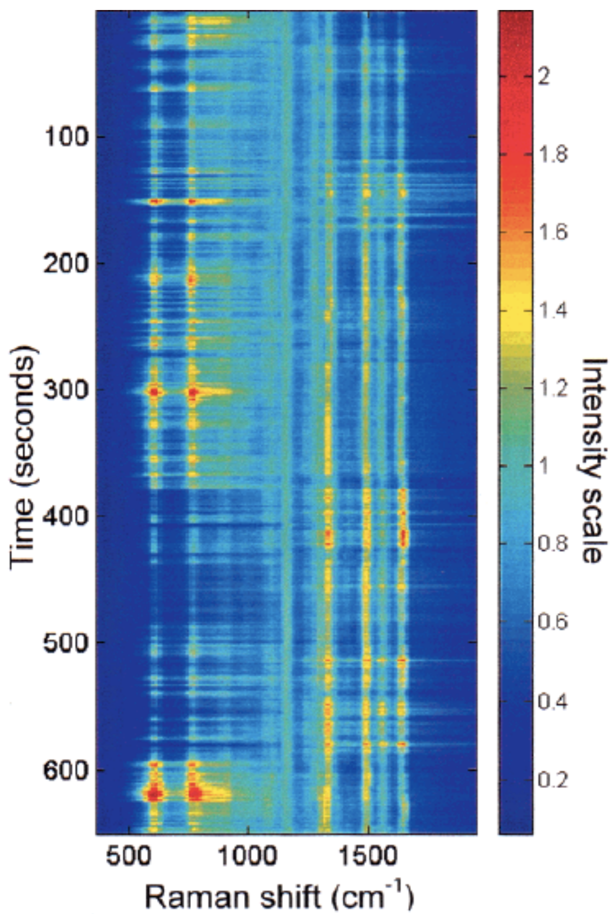
Hongxing Xu, Erik J. Bjerneld, Mikael Käll,* and Lars Börjesson
Department of Applied Physics, Chalmers University of Technology, S-412 96 Göteborg, Sweden
(Received 22 January 1999)



J. Phys. Chem. B **2001**, *105*, 12348−12354

Time-Dependent Single-Molecule Raman Scattering as a Probe of Surface Dynamics

Amir Weiss and Gilad Haran*
Chemical Physics Department, Weizmann Institute of Science, Rehovot 76100, Israel



EM Selection Rules and the Spectral Fluctuations.

orientation of a molecule on a surface

Charge Transfer and Spectral Fluctuations.

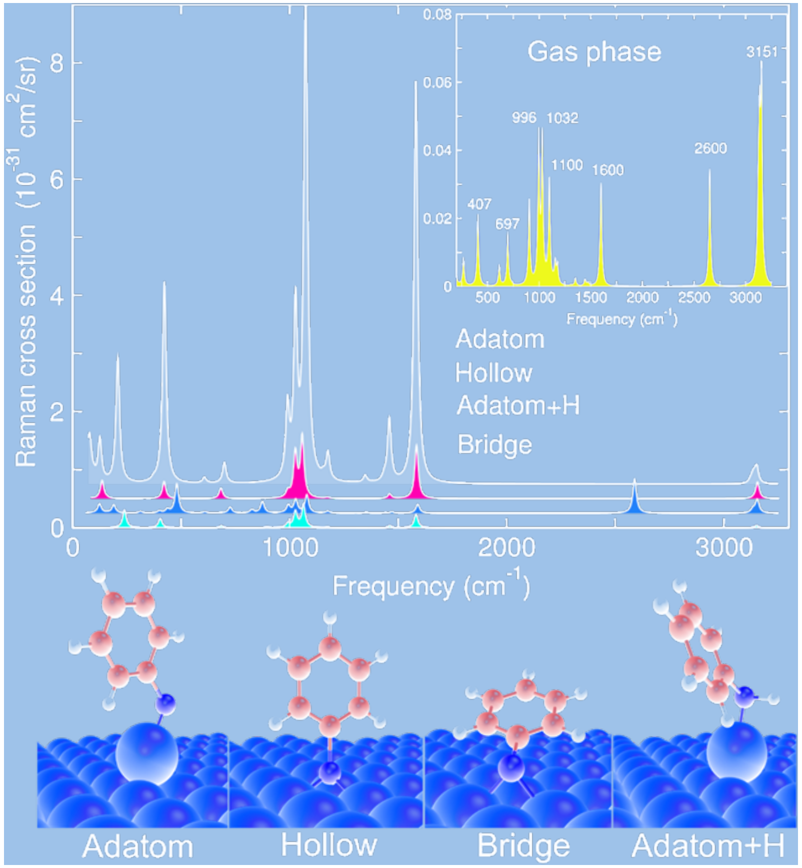
work function (or charge density) of the metal

WF Variations and the Diffuse Background.

role for electrostatic screening.

Diffusion of Silver Adatoms.

Model of surface diffusion



Gold adatom : 40% surface coverage

measured by STM

10^{-15} cm^2/s : a rate about nm/s

Thiol Chemisorbed to gold

10^{-18} cm^2/s : a rate of nm/hour

: non resonant adsorbate molecule

Thiophenol marker of Au adatome
Increase of the Raman cross section
With the orientation of ring towards the surface

Chemical Raman Enhancement of Organic Adsorbates on Metal Surface Zayak AT, Hu YS, Choo H, et al. PRL (2011)

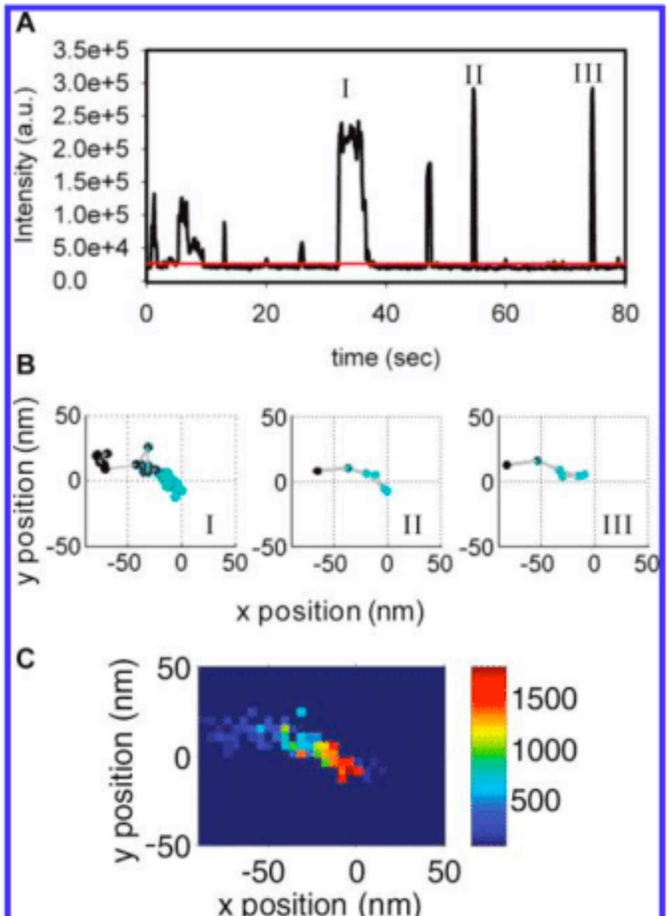
J. Marguerit at , J. Phys. Chem, 2012

NANO LETTERS
pubs.acs.org/NanoLett

Super-resolution Optical Imaging of Single-Molecule SERS Hot Spots

Sarah M. Stranahan, and Katherine A. Willets*

Department of Chemistry and Biochemistry, The University of Texas at Austin, 1 University Station A5300, Austin, Texas 78712



Challenges in Spectroscopy of protein

Bovine serum albumine

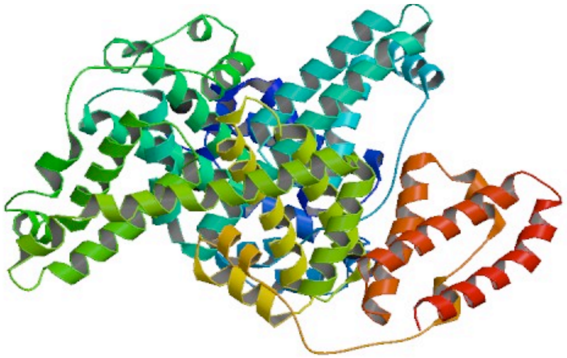
MW : 67 kDa
607 amino acids

15 atoms / amino acids

9100 atoms

3-6 degree of freedom

Around 30 000 normal modes !!!



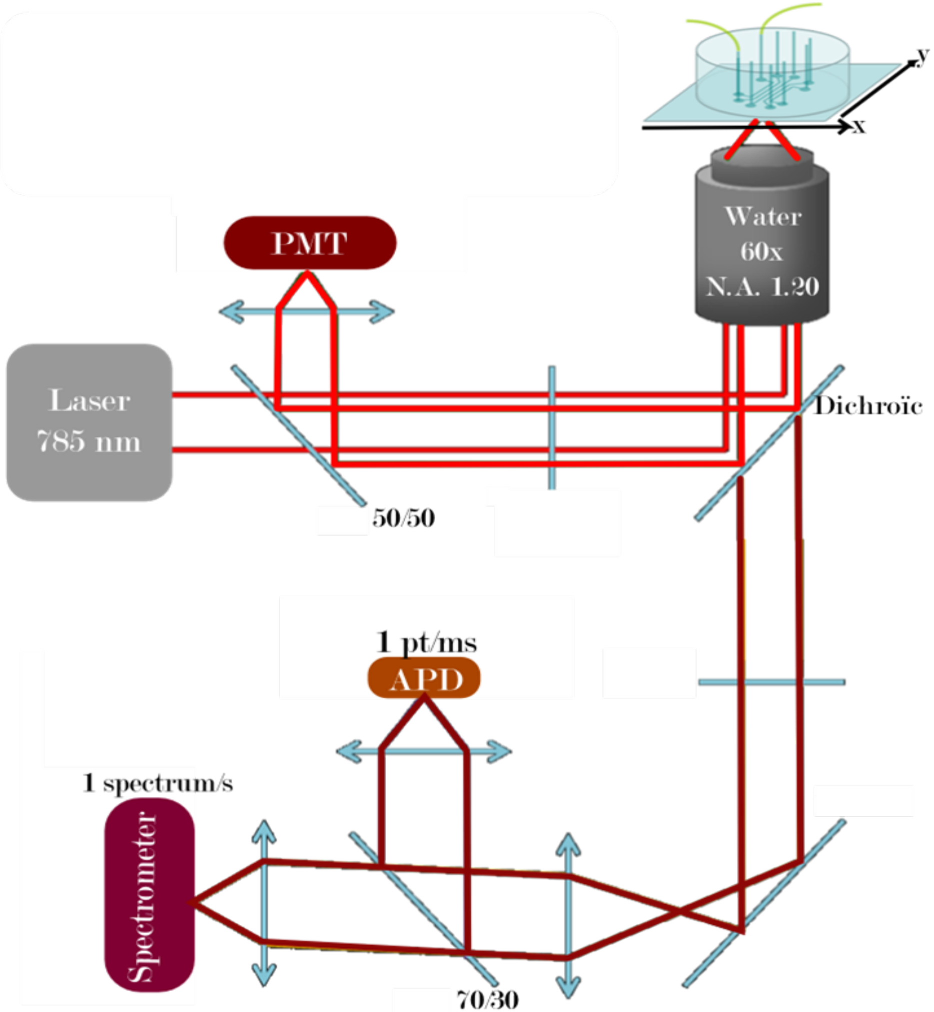
$5\ cm^{-1}$ <-> 0.02% of elongation

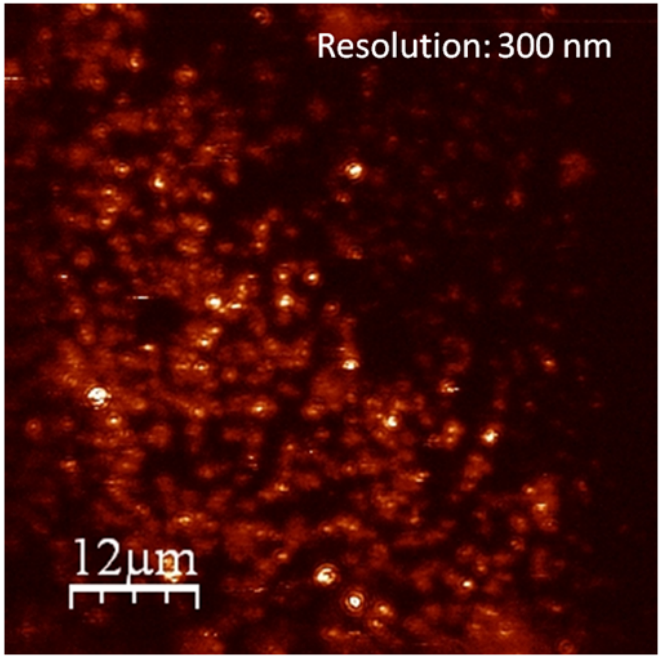
Enhancement using Nanostructured sample

Electromagnetic enhancement
Challenges in biophysics

Polarisation = polarisability of molecules x electrical field

High NA Raman microscopy

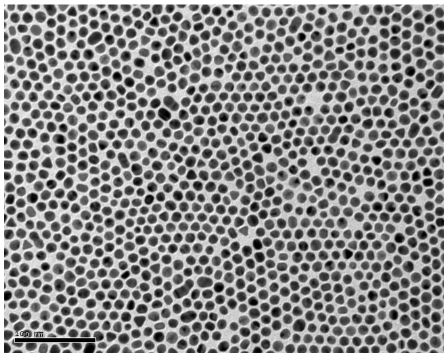




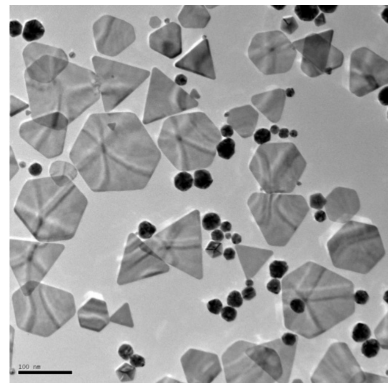
Nanoparticles

Bottom up approach : Cheap but ramdon

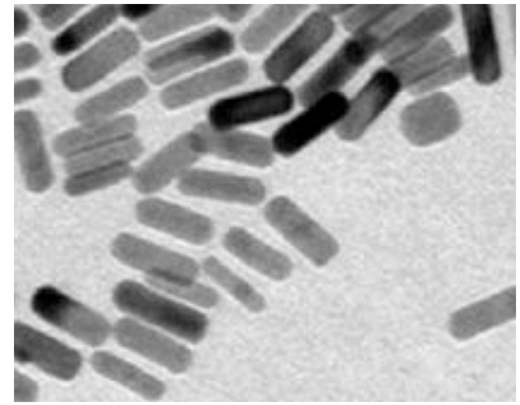
sphère ~10nm,
Turkevitch



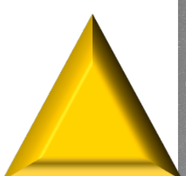
triangles & hexagones ~200nm,
(white tea)

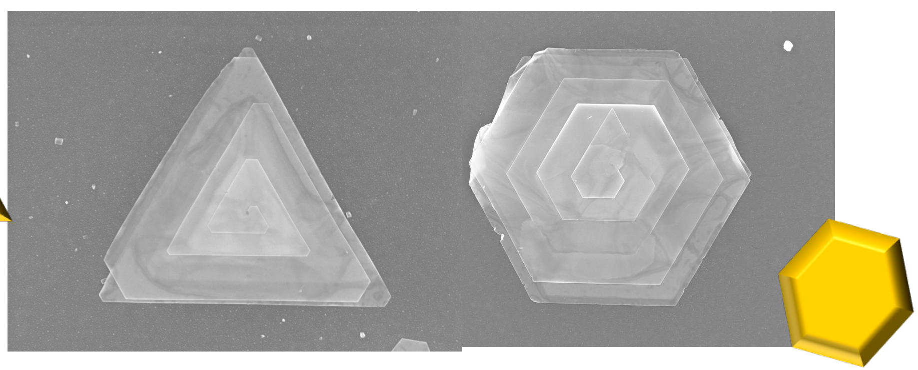


Nanorod

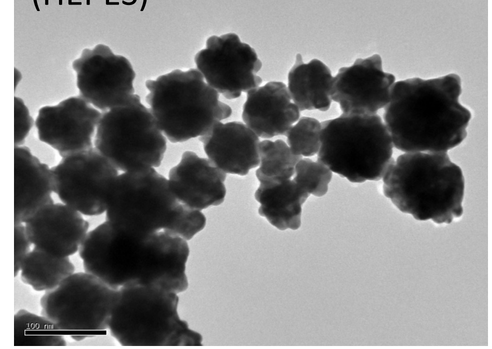


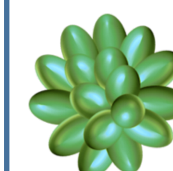
Flat spiral ~5μm
(grap juice)





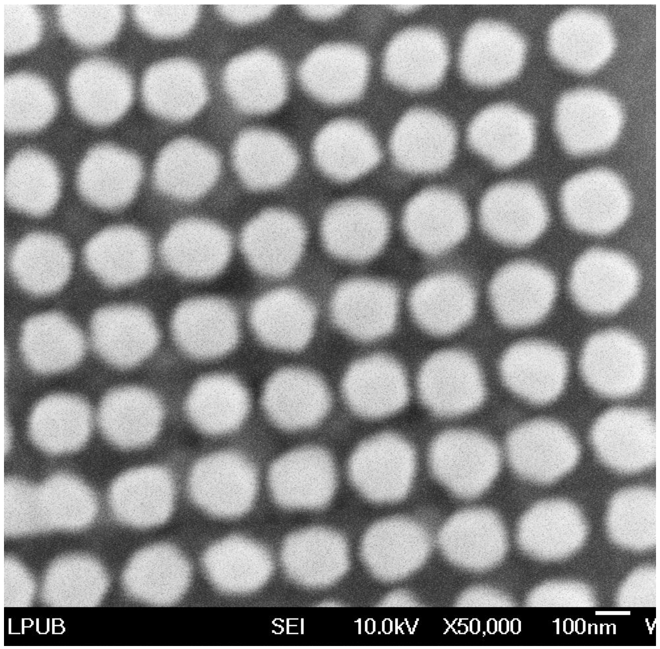
Nanoflowers ~100nm,
(HEPES) [1]



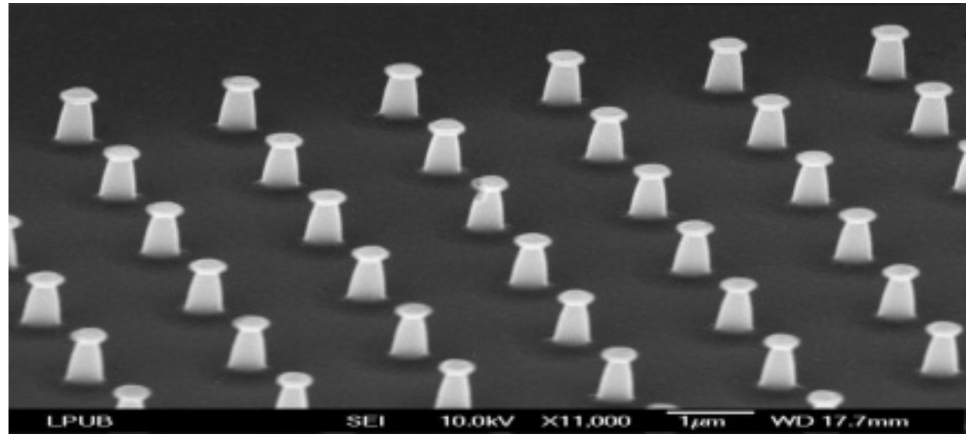


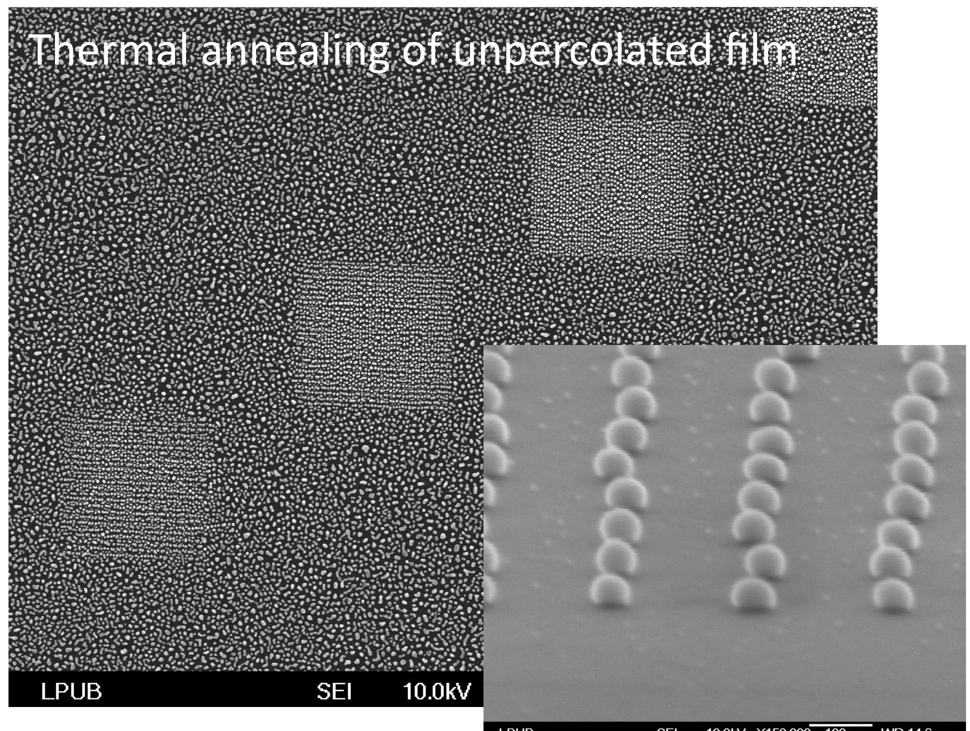
T. Brulé J. Phys. Chem C 2012

Nanostructured sample : expensive but more reproductible



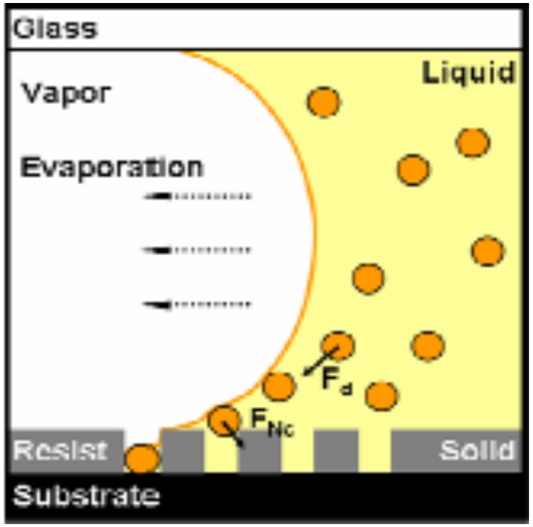
E-beam lithography

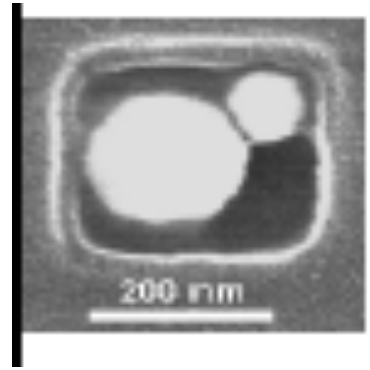


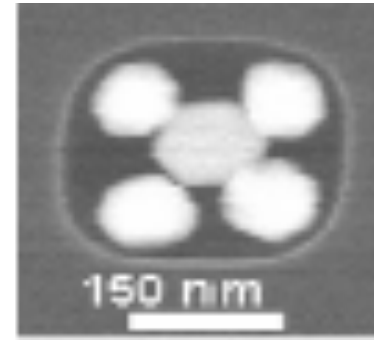


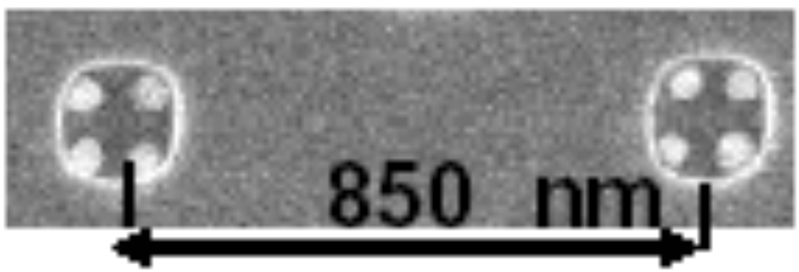
PM Adam , Troyes

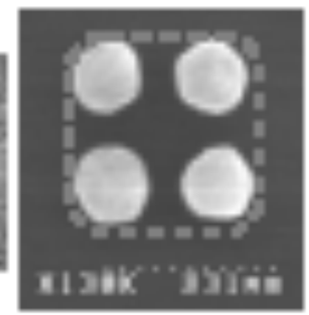
Capillary Force Assisted depositon (CFA)

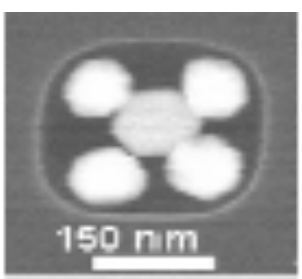








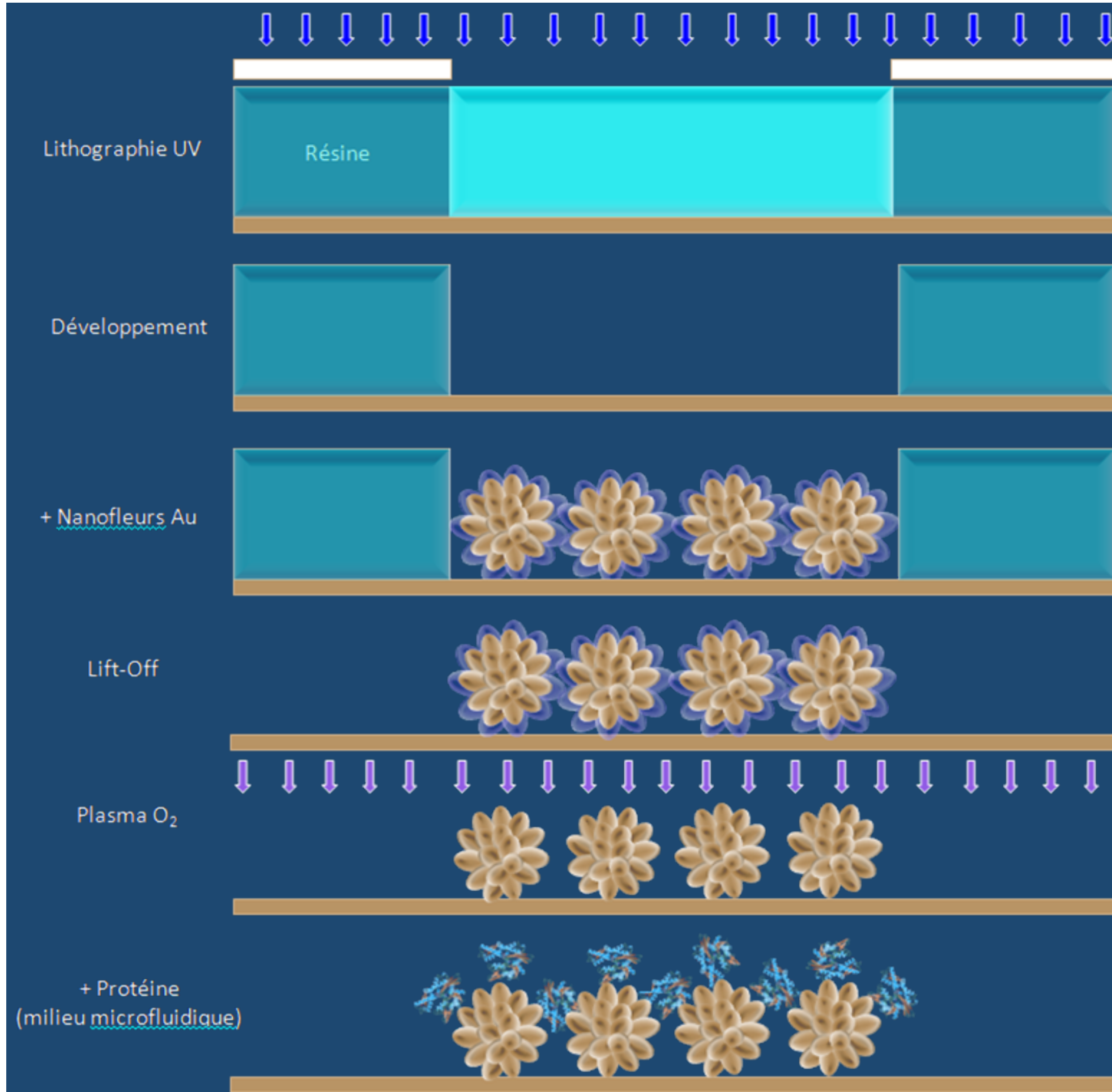




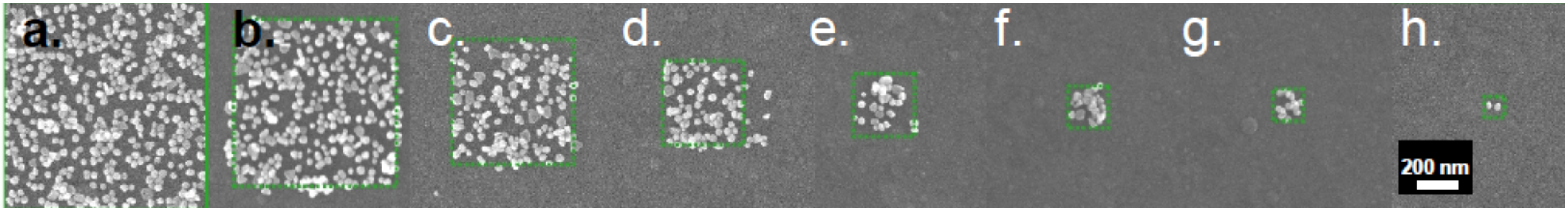
D. Peyrade, Grenoble

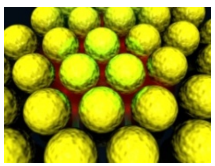
2 important steps :

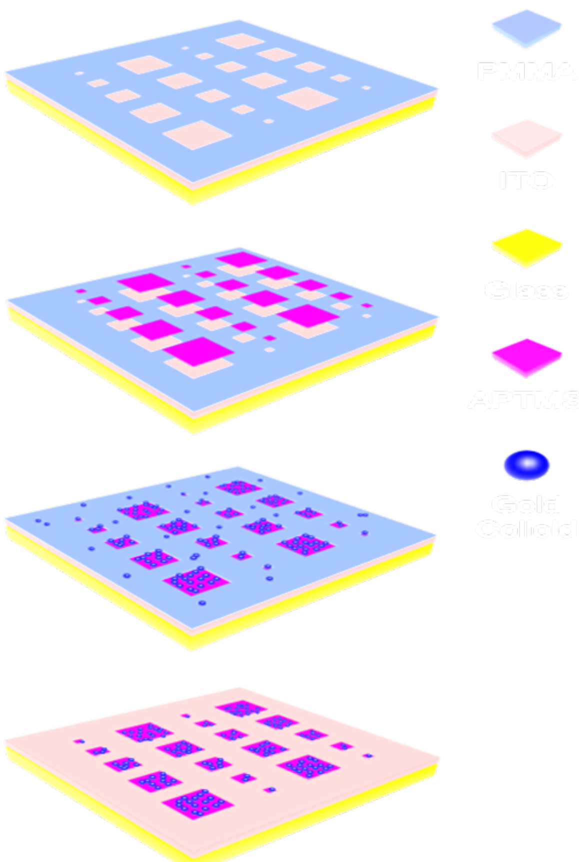
- Deposition of Functionalized Gold NanoParticles (GNPs) using UV lithography.
- Take off the functionalization using O_2 Plasma Cleaner

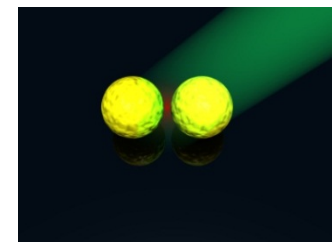


Nano assembly of colloids









well identified isolated SERS nanoarea

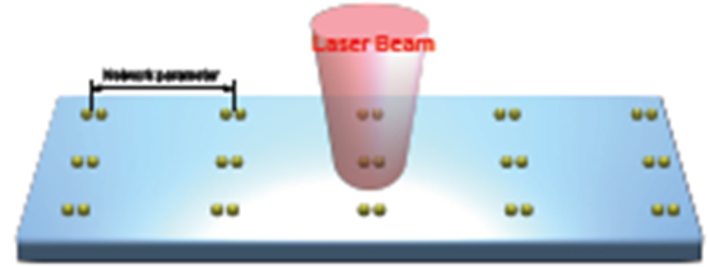
The e-beam lithography determines the SERS active area

Chemical immobilisation of colloids (25 nm in diameter) on glass template

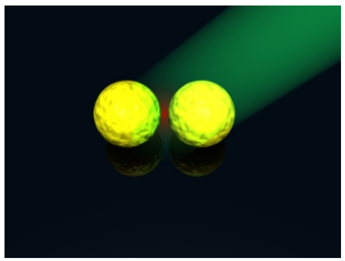
Nordin Felidj, Paris

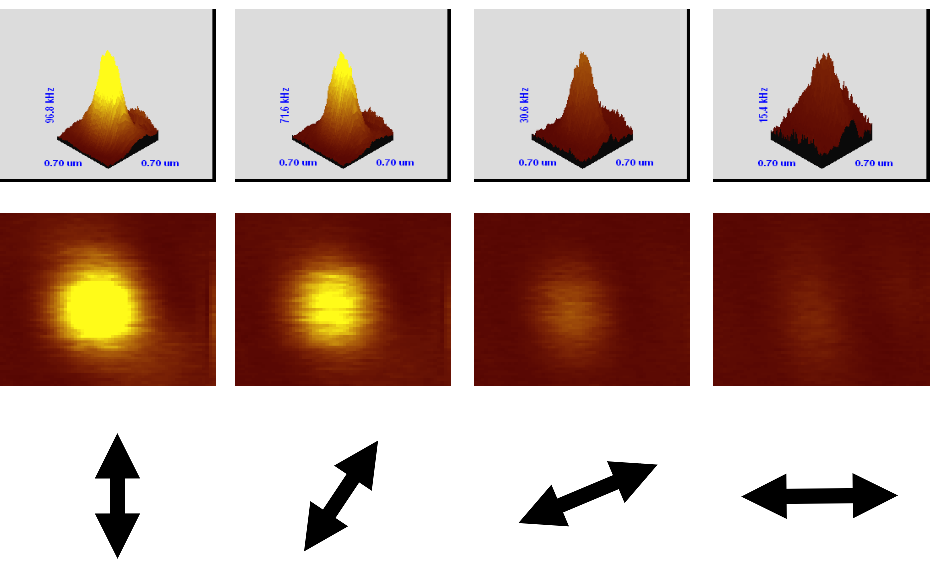
J. Margueritat, ACS Nano, 2011

Excitation EM field controlled by



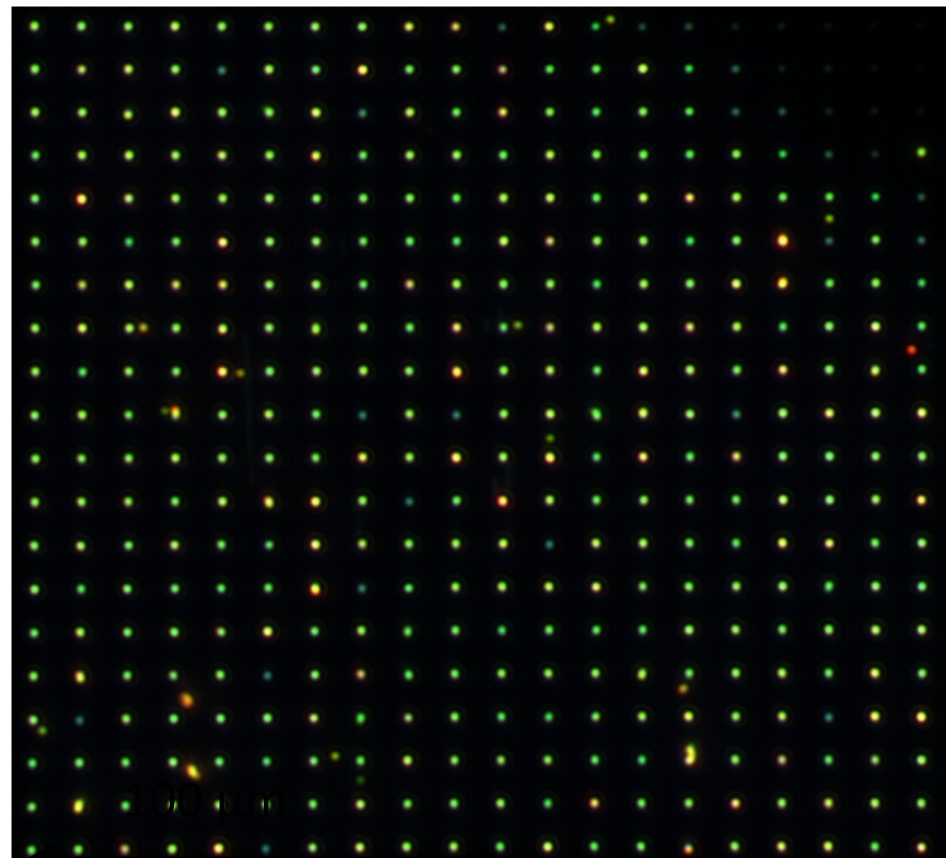
1. polarization



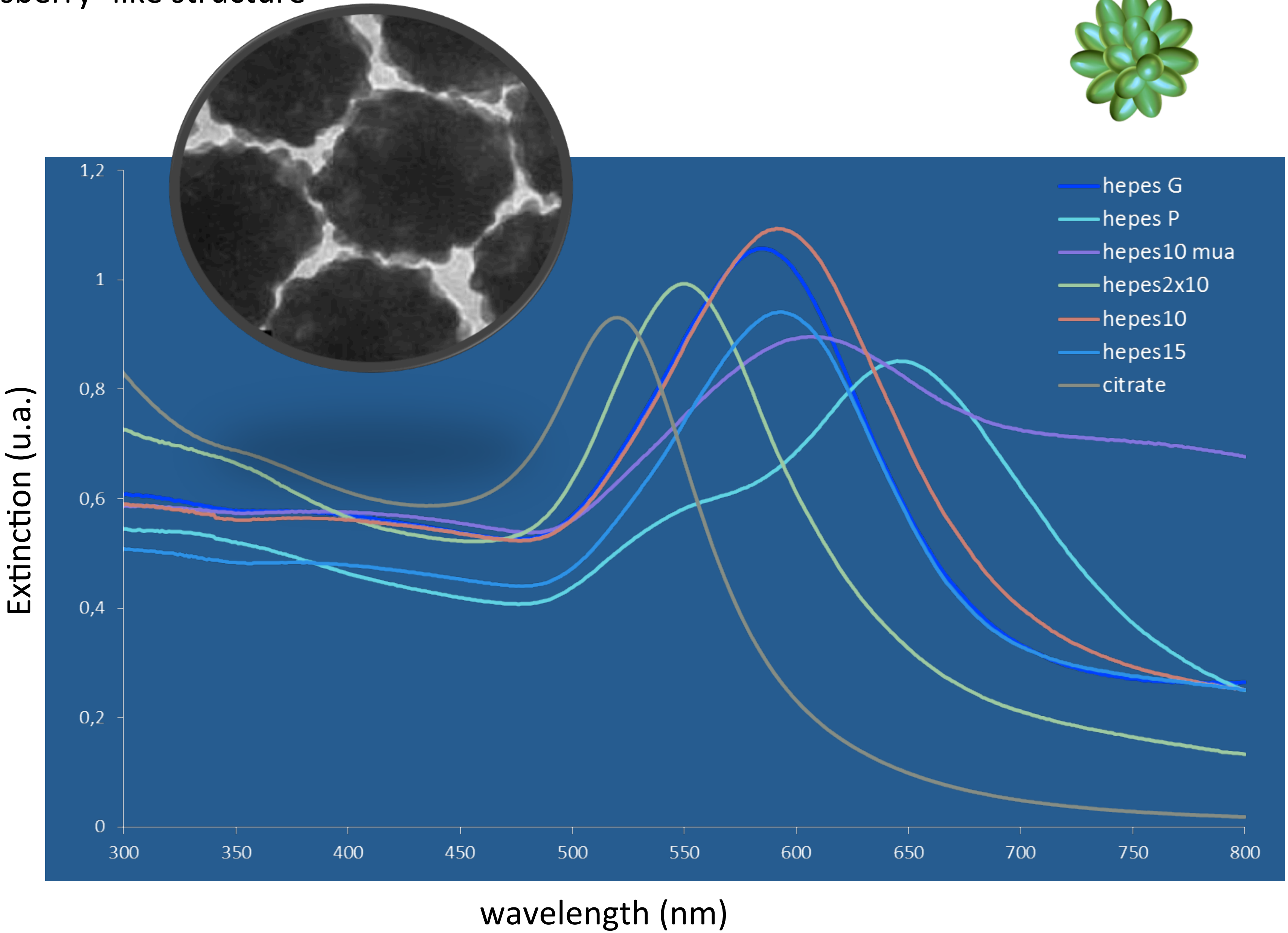


90° 60° 30° 0°

2. Colloid gap



Rasberry- like structure



Chemical and ElectroMagnetical enhancement

PHYSICAL REVIEW E VOLUME 62, NUMBER 3 SEPTEMBER 2000

Electromagnetic contributions to single-molecule sensitivity in surface-enhanced Raman scattering

Hongxing Xu,[1] Javier Aizpurua,[2] Mikael Käll,[1] and Peter Apell[2]
[1]*Condensed Matter Physics, Department of Applied Physics, Chalmers University of Technology, S-41296 Göteborg, Sweden*
[2]*Materials and Surface Theory, Department of Applied Physics, Chalmers University of Technology, S-41296 Göteborg, Sweden*

Nano Lett. **2010,** *10,* 4040–4048

NANO LETTERS

pubs.acs.org/NanoLett

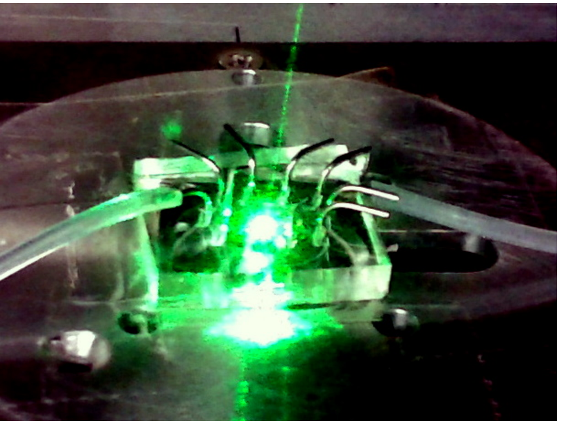
Charge Transfer Enhancement in the SERS of a Single Molecule

Won-Hwa Park, and Zee Hwan Kim*

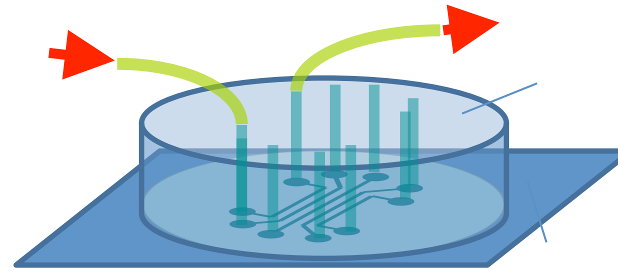
Department of Chemistry and BK21 Division of Chemistry, Korea University, Seoul 136-701, Korea

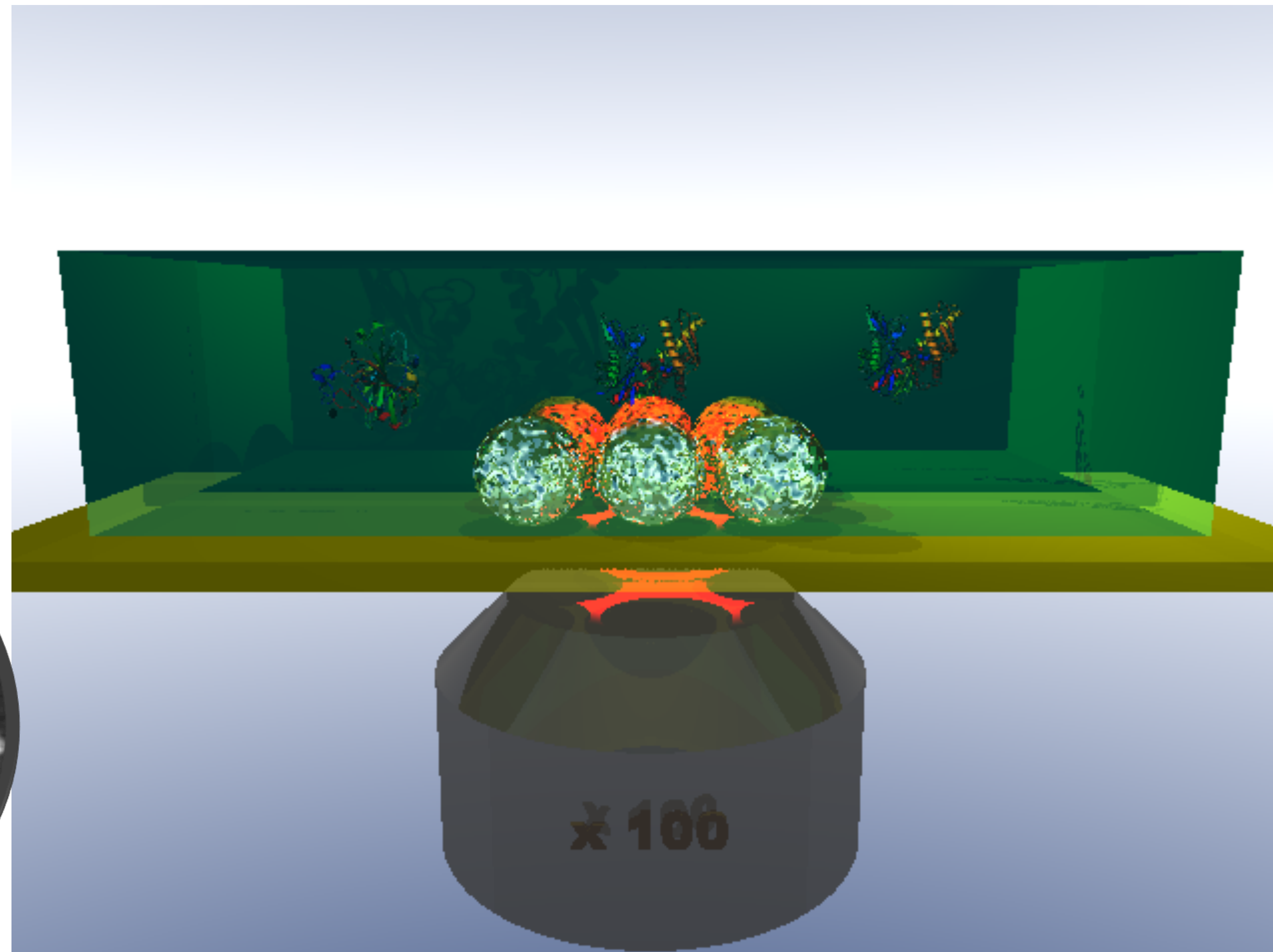
CT-enhancement of $10^1 \sim 10^3$,

(charge-transfer and electromagnetic) enhancement of $10^6 \sim 10^8$

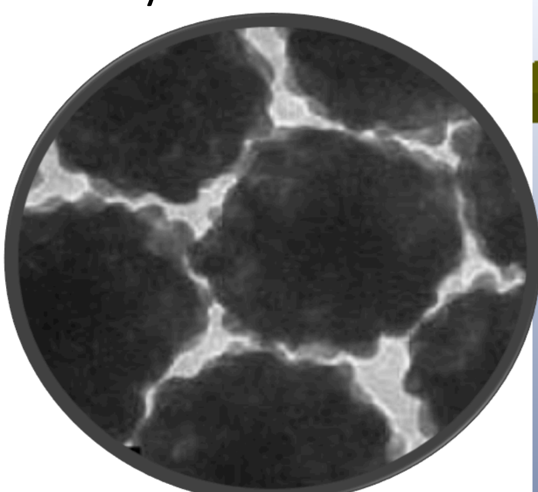


SERS for proteins

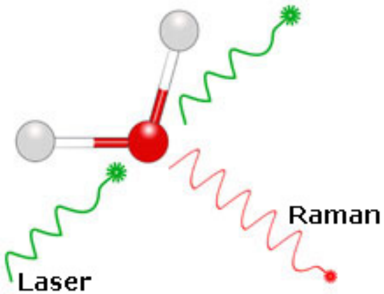




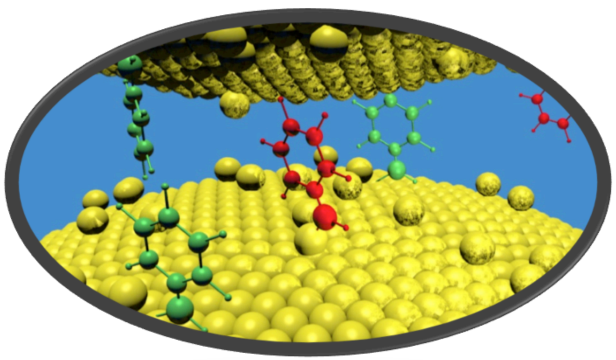
Rasberry- like structure



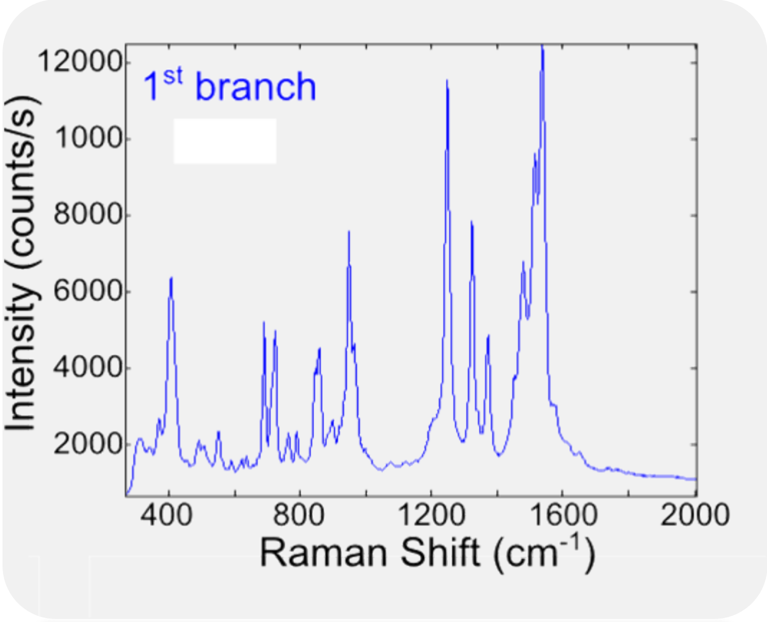
Optical fingerprint of proteins



Diffusion Raman

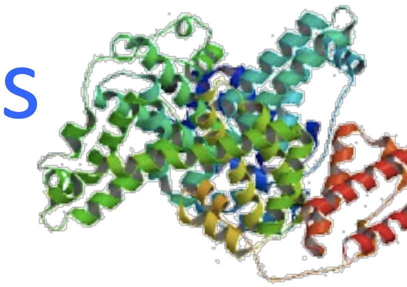


Exaltée par la surface d'or

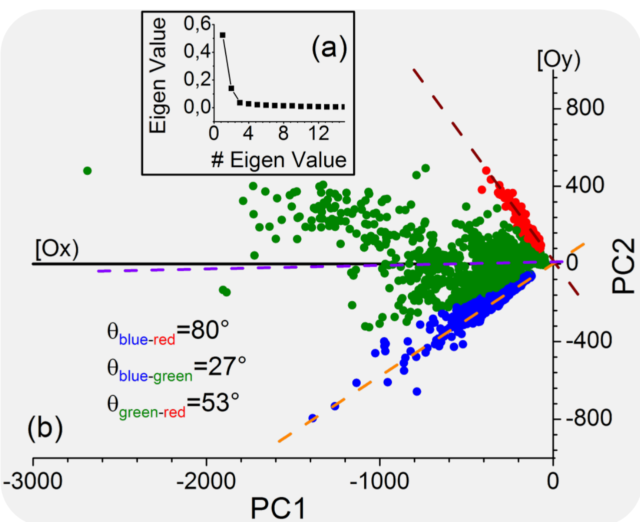


UMR 6303
ICB
UBFC

Sorting of molecules one by one

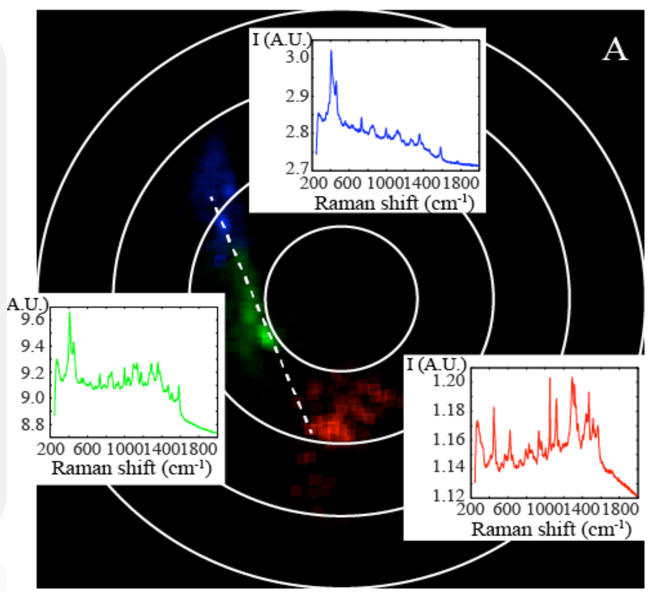


Principal component analysis



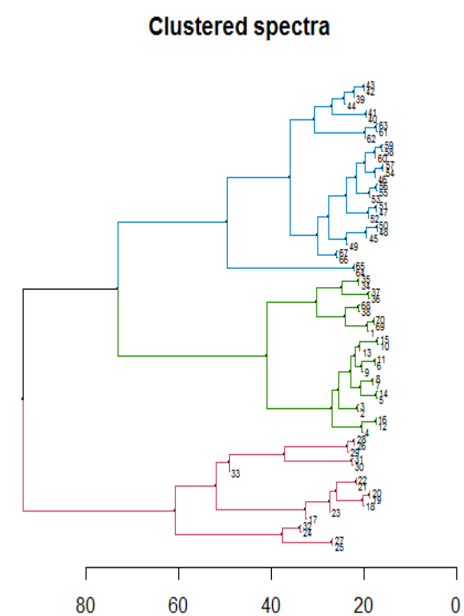
T. Brulé J. Phys Chem 2014

Fourier polar representation



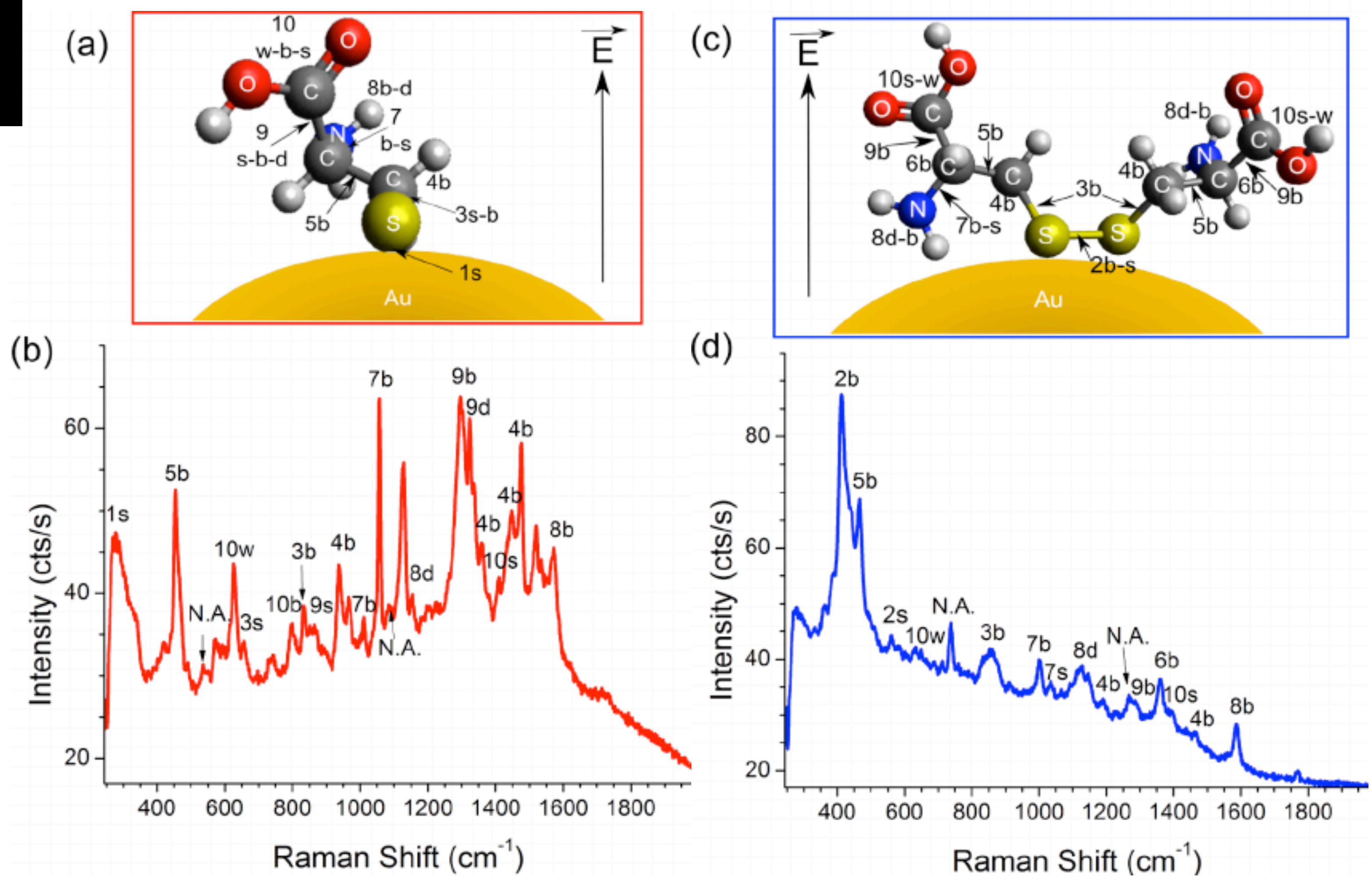
A. Leray Scientific Report 2016

Hierarchical clustering



JC Clement(in preparation)

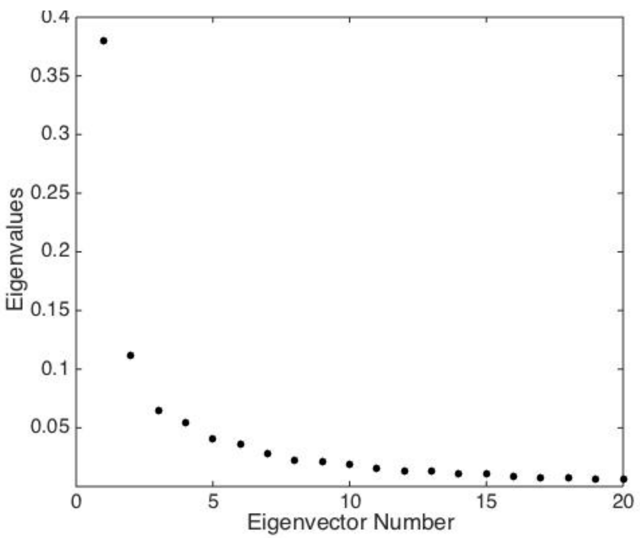
Cysteine fingerprints

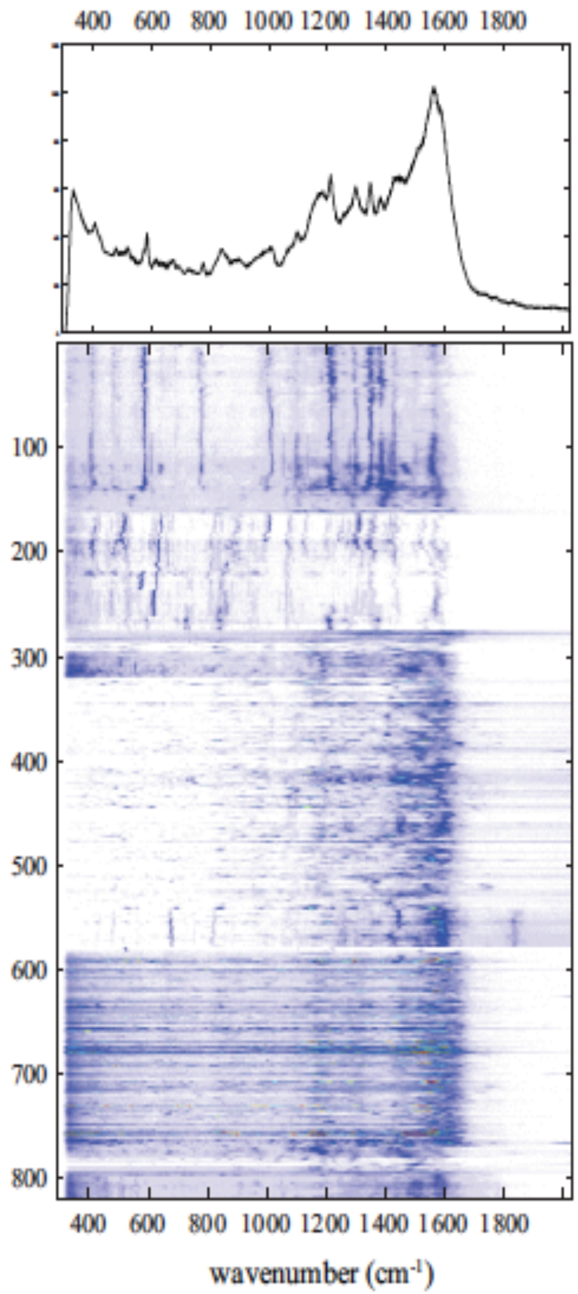


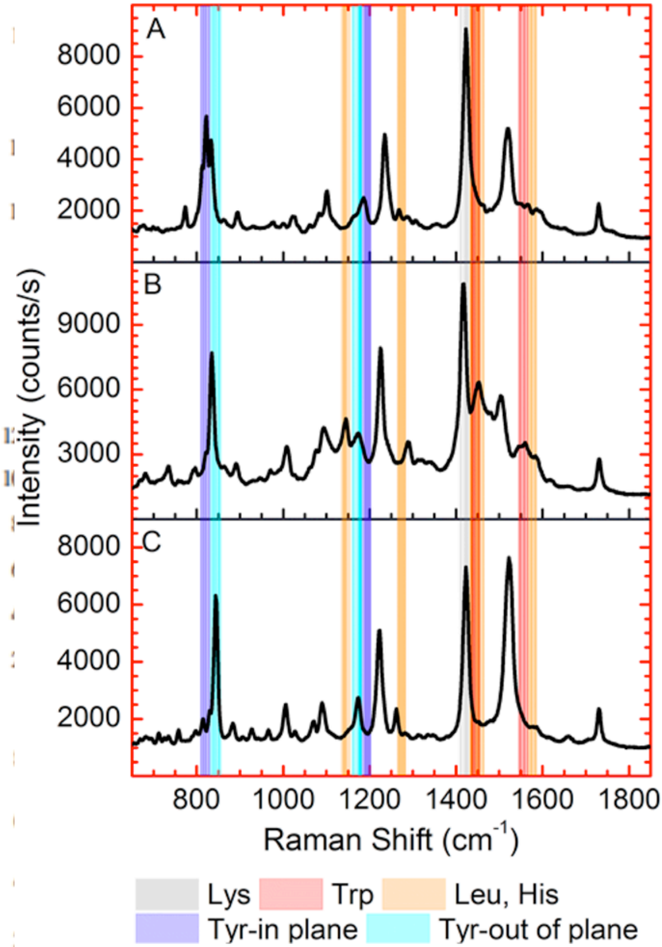
T. Brulé J. Phys Chem 2014

Higher level of complexity

Protein

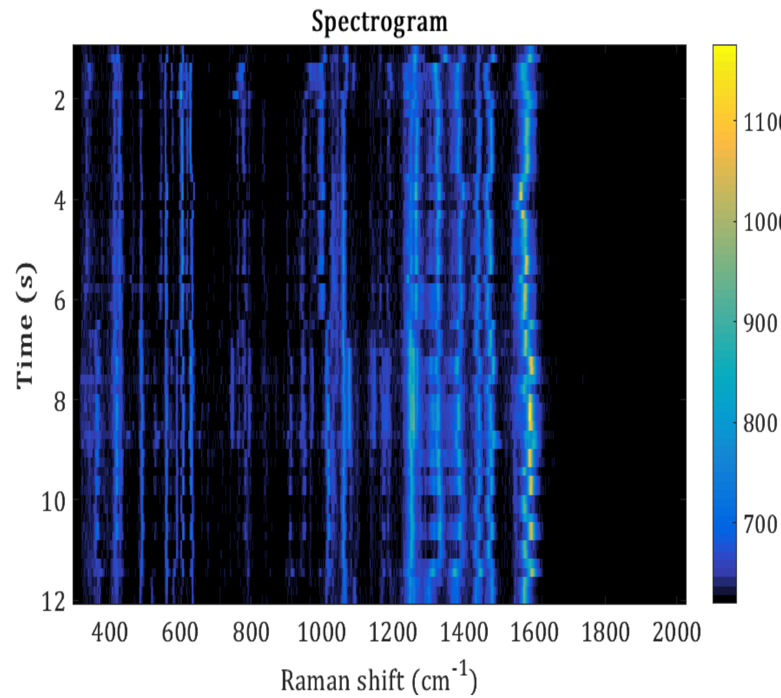




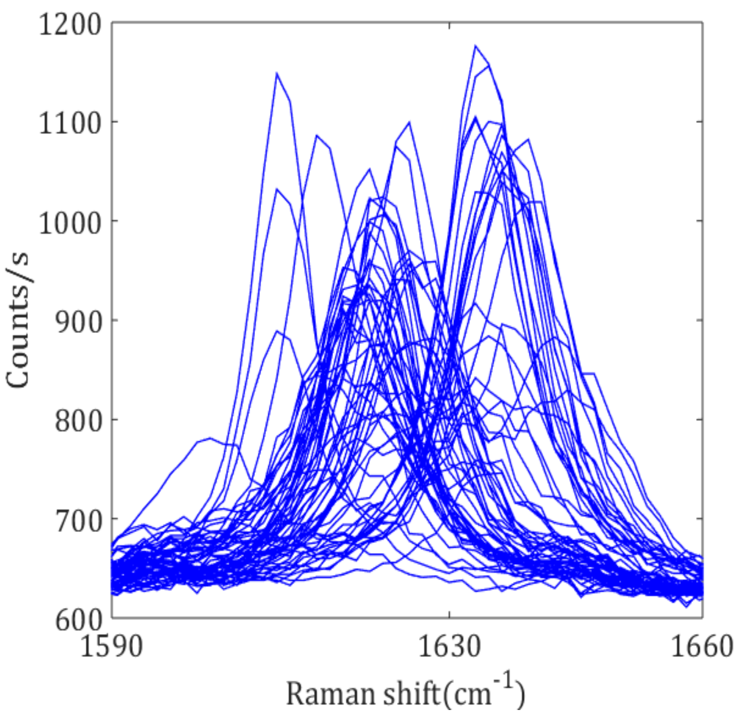


T Brulé · ACS sensors 2016

Spectral diffusion



Raman Band diffusion



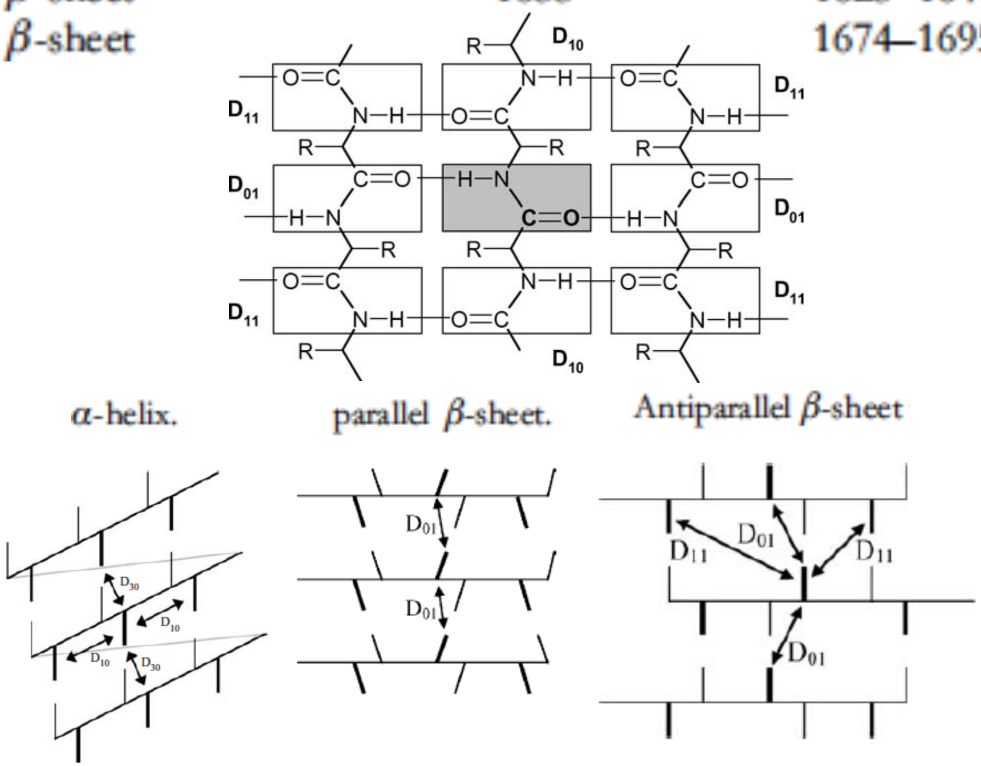
3-Stepwise Hetereogeneous broadning

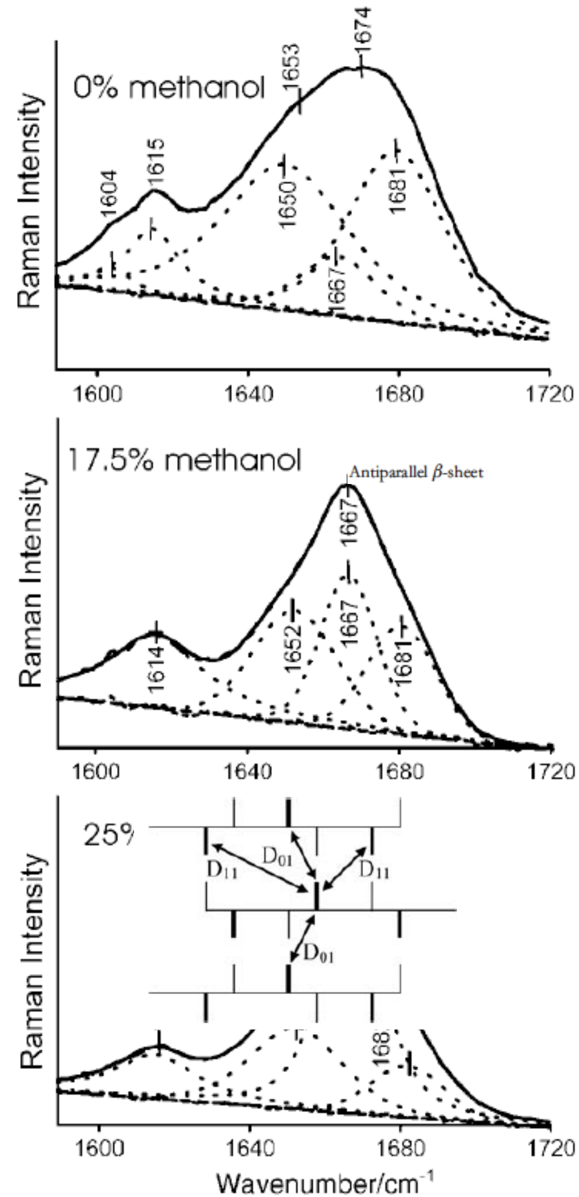
ENTROPY ?

Degree of freedom of molecules ?

Amide I of proteins

Secondary structure	Band position in $^1H_2O/cm^{-1}$ Average	Extremes
α-helix	1654	1648–1657
β-sheet	1633	1623–1641
β-sheet		1674–1695



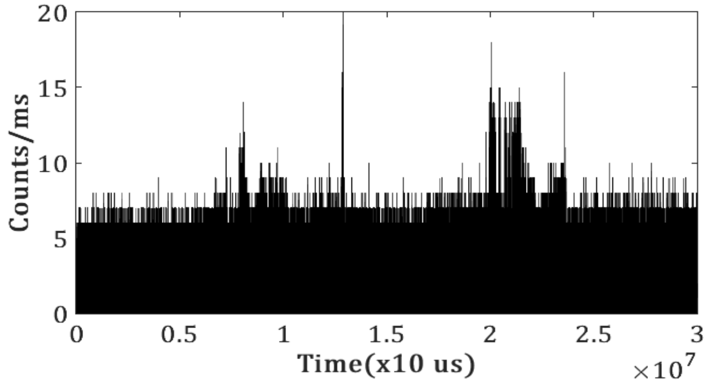


Dynamics

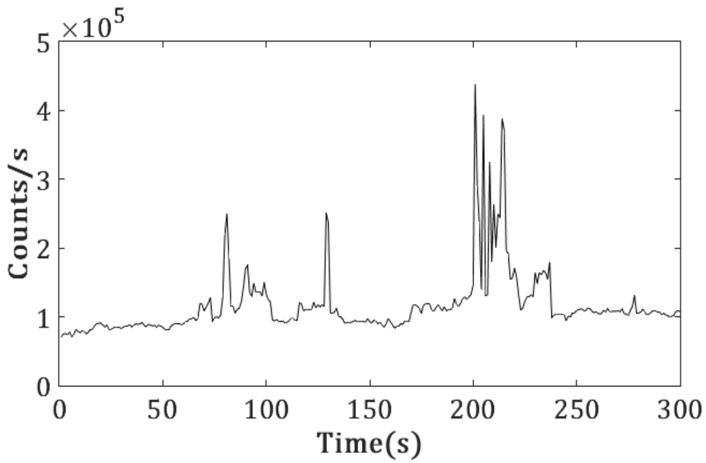
Small molecule

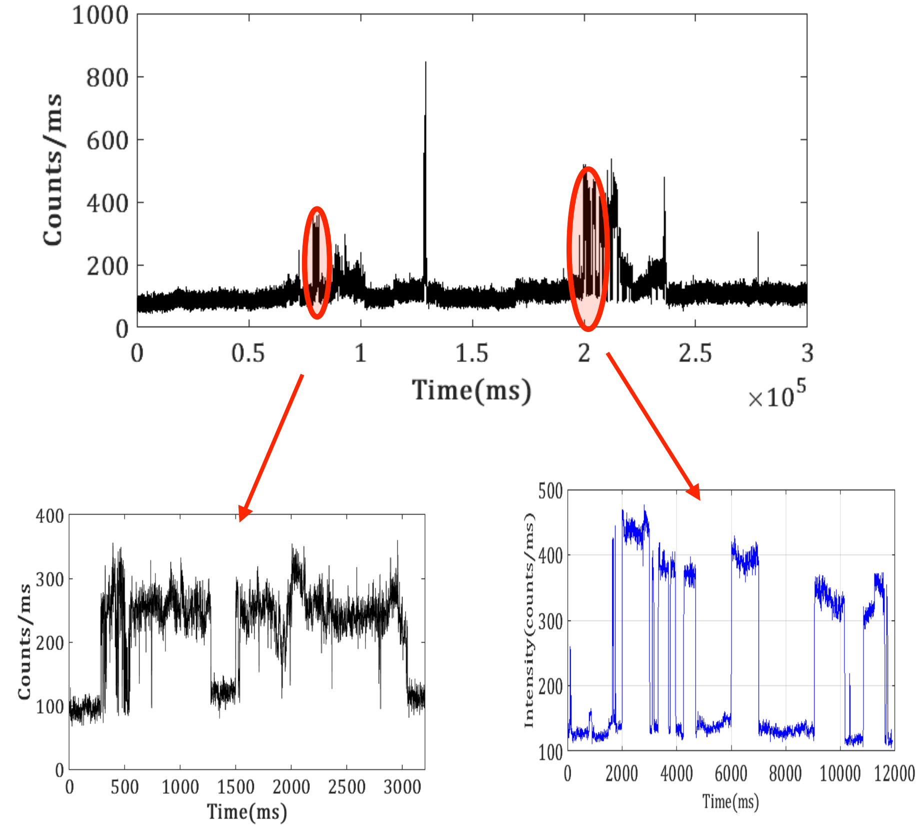
Stationary process ?

One step-wise repeatable
but distribution of live time (t_{on})

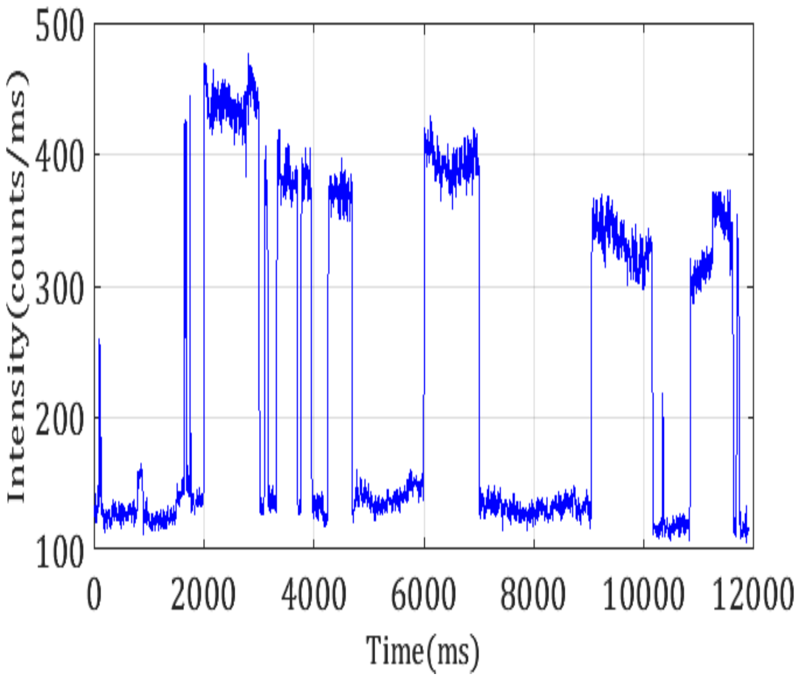


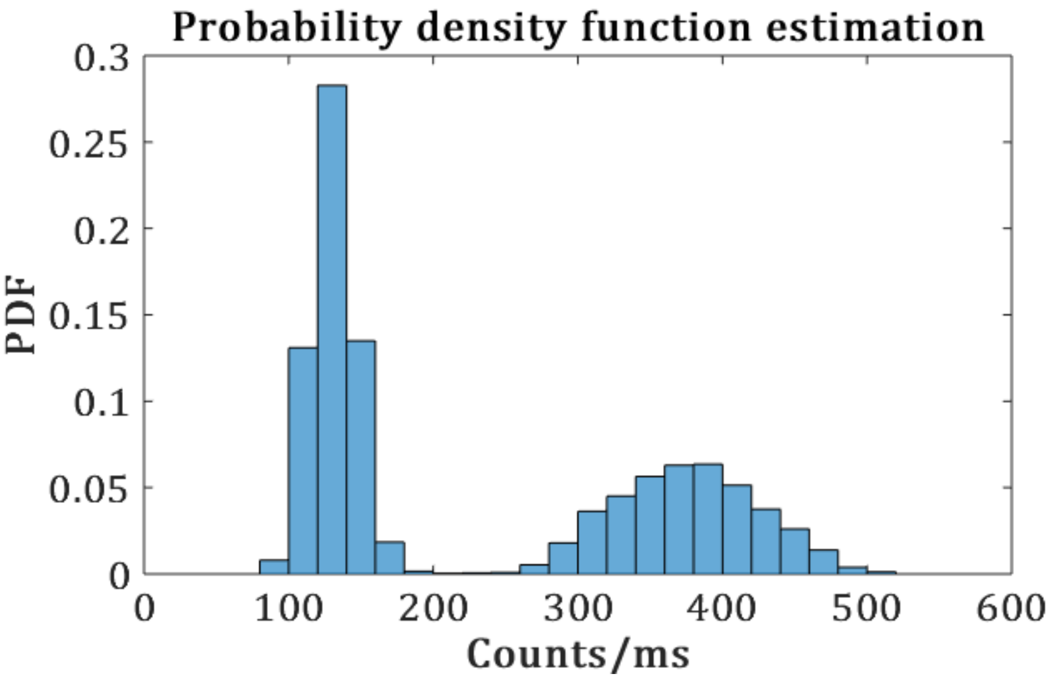
Rate : microseconde





Two step process





Stationnary process / ERGODICITY

Time average

$$\overline{x(t)^n} = \lim_{T\to\infty} \frac{1}{T} \int_{-T/2}^{T/2} x(t)^n \mathrm{d}t$$

Average over the entire protein sample

Probability density $p_X(x, t_0)$

$$E[X(t_0)^n] = \int_{-\infty}^{\infty} x^n p_X(x, t_0) \mathrm{d}x$$

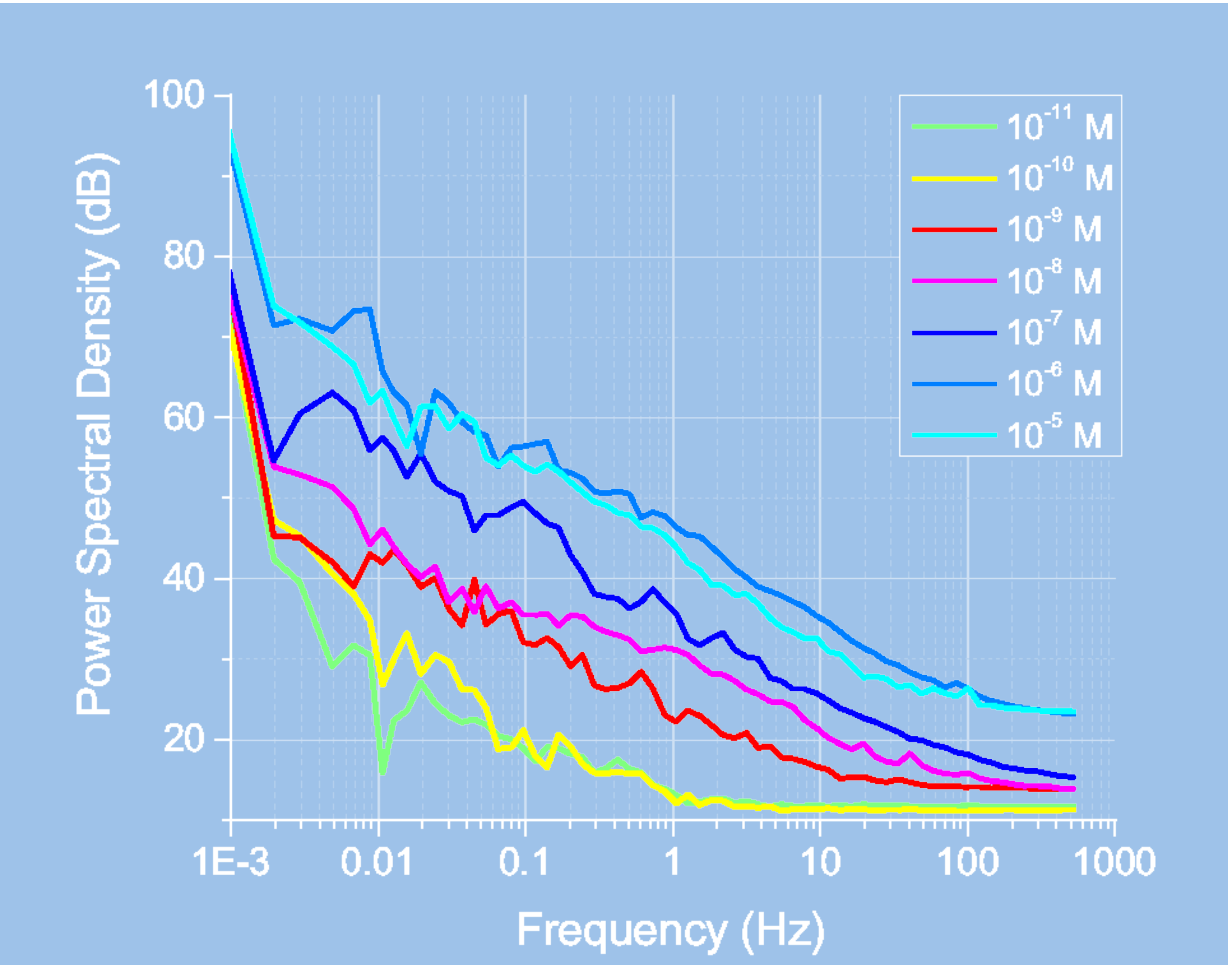
Stationnary process : p is independant of t_o

Ergodicity : the time average = the average over the entire protein volume

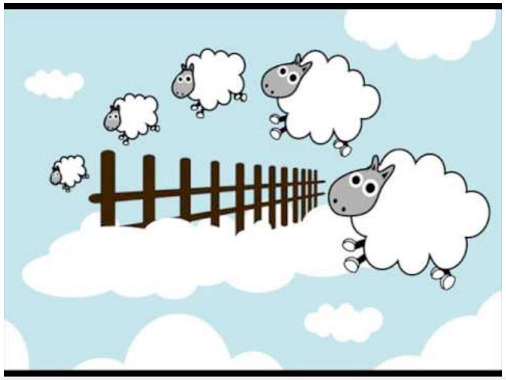
Noise analysis : Fourier Transform of time series

White noise : a constant PSD ; mostly a Poissonian process (diffusion , PL)

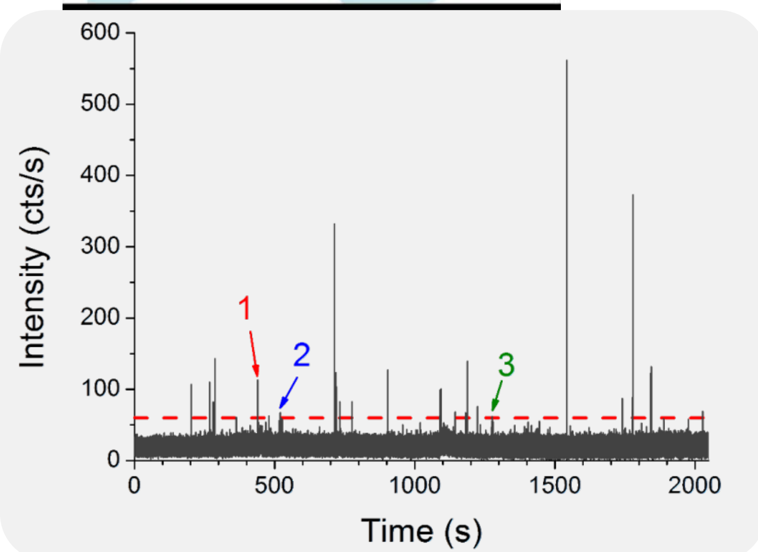
Pink noise : loss of - 10 dB per frequency decade ; Raman events

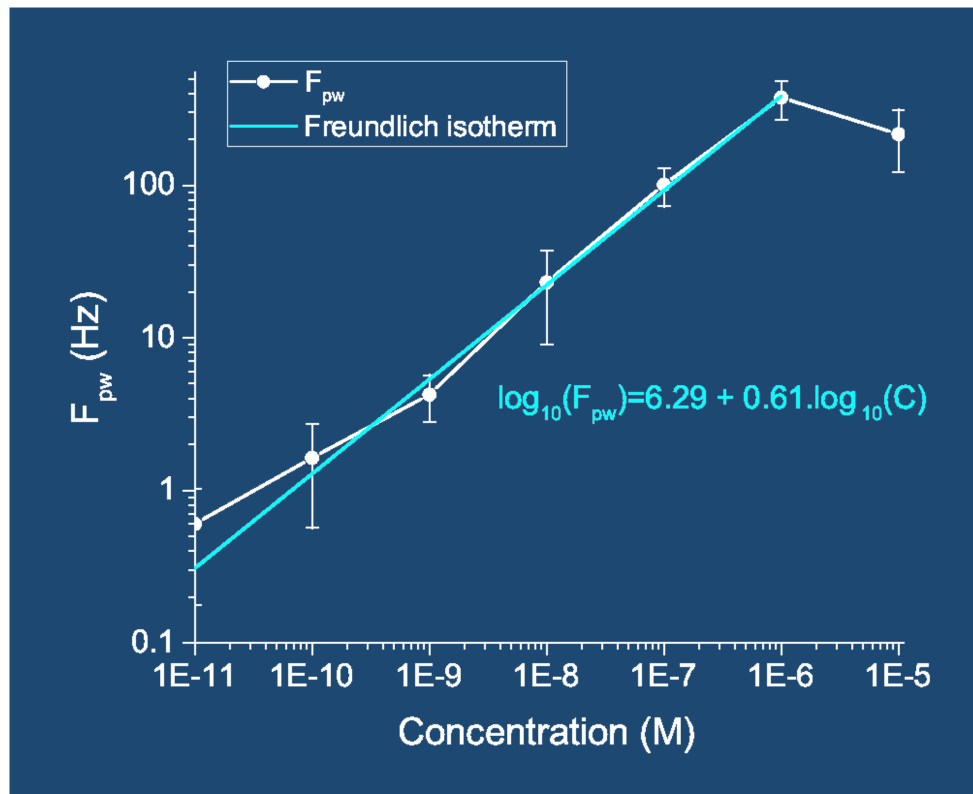


Cut off frequency F_{pw}



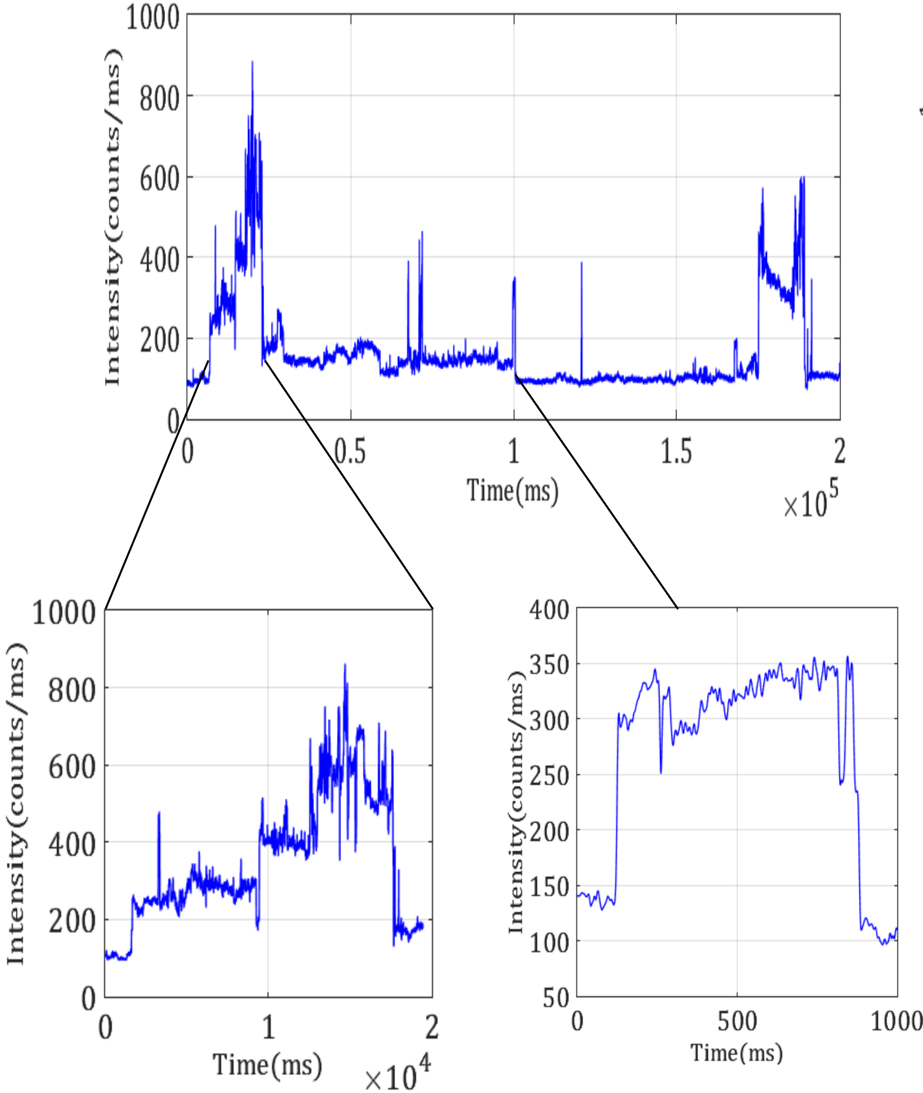
Counting molecules over 6 decades of concentrations



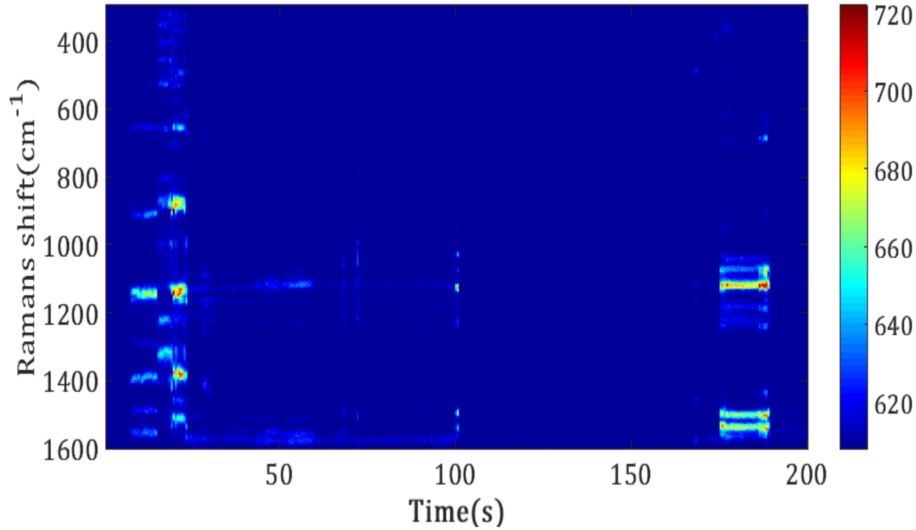


Single molecule spectroscopy : binary mixture

Atrazine chlorazidrone



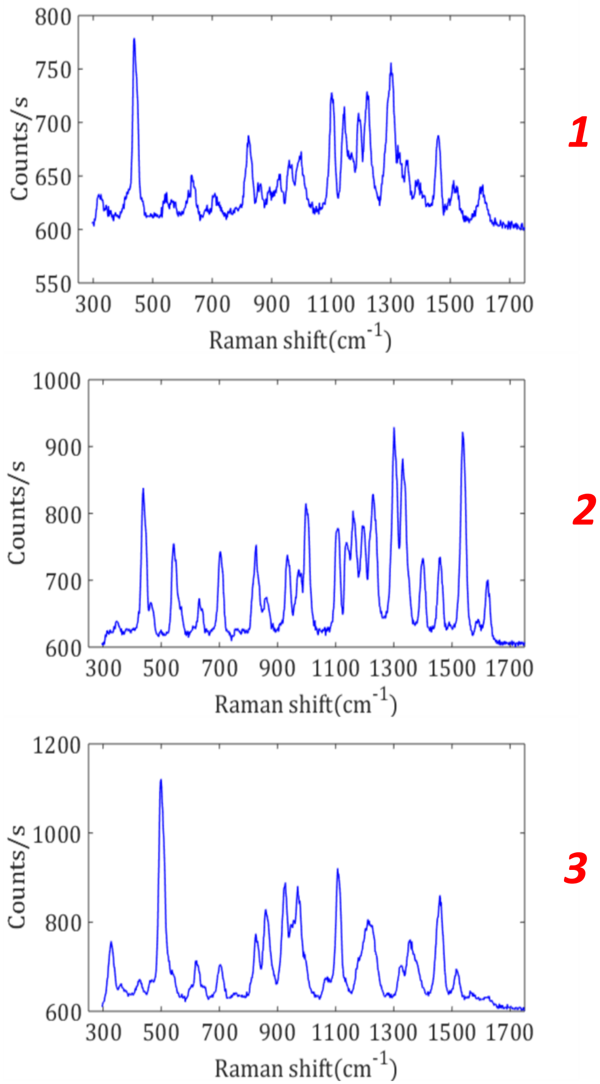
spectrogram

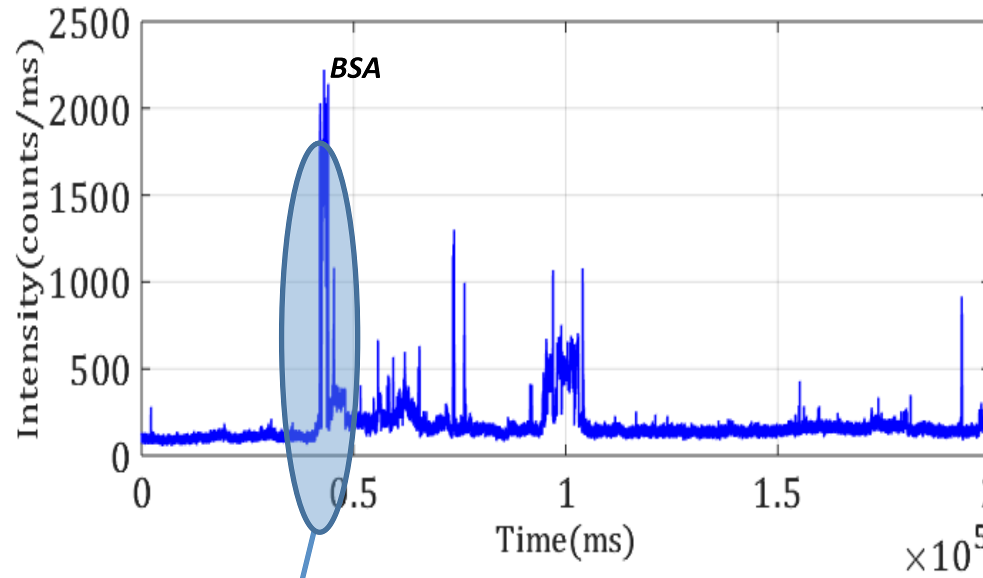


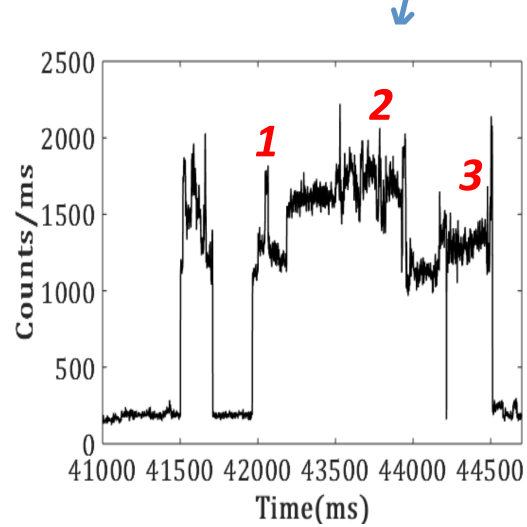
Different topologies of events
with different level of fluctuations

Is the level of noise unique for every molecules?

Higher level of complexity : Protein







Longer events ;

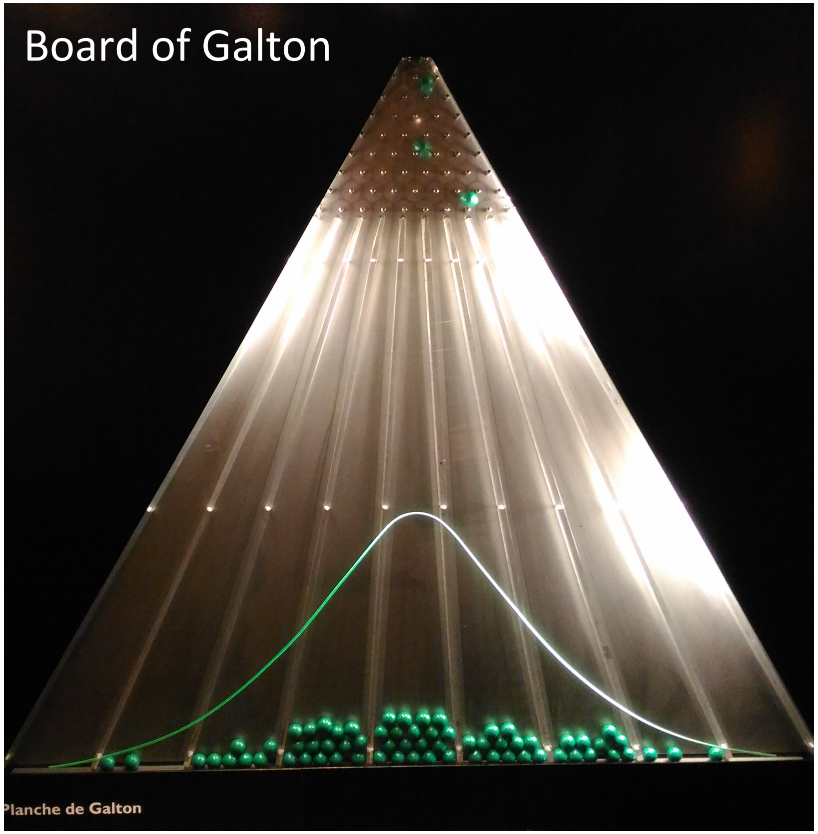
Higher volatility

More fluctuating ….

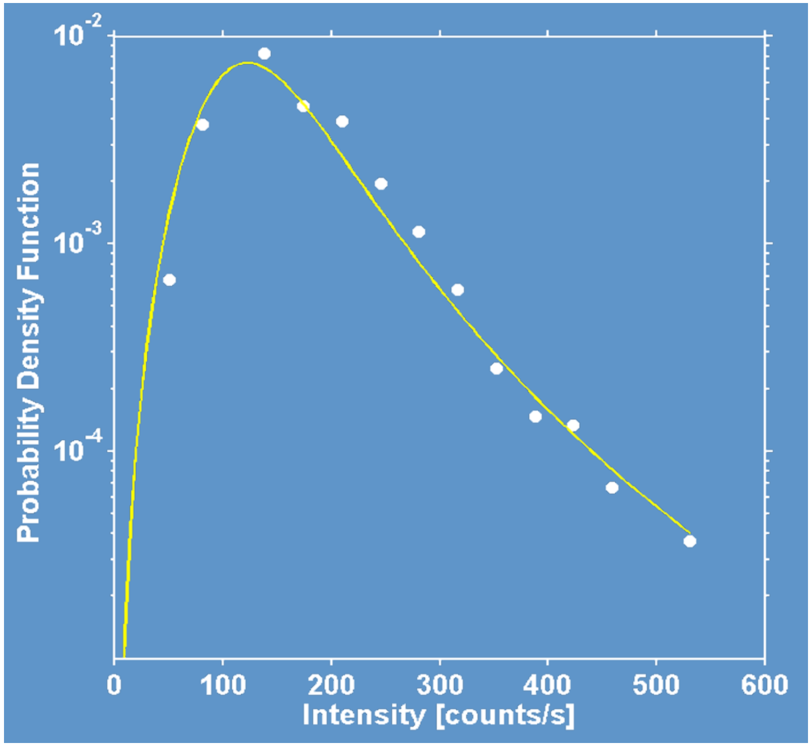
What is invariant event by event ?
How many events to consider a physical signal ?

What statistics for protein ?

$$p_{log,\lambda}(i) = \frac{\frac{\beta}{\alpha}\left(\frac{I_\lambda(i)}{\alpha}\right)^{\beta-1}}{\left(1+\left(\frac{x}{\alpha}\right)^{\beta}\right)^{2}}$$



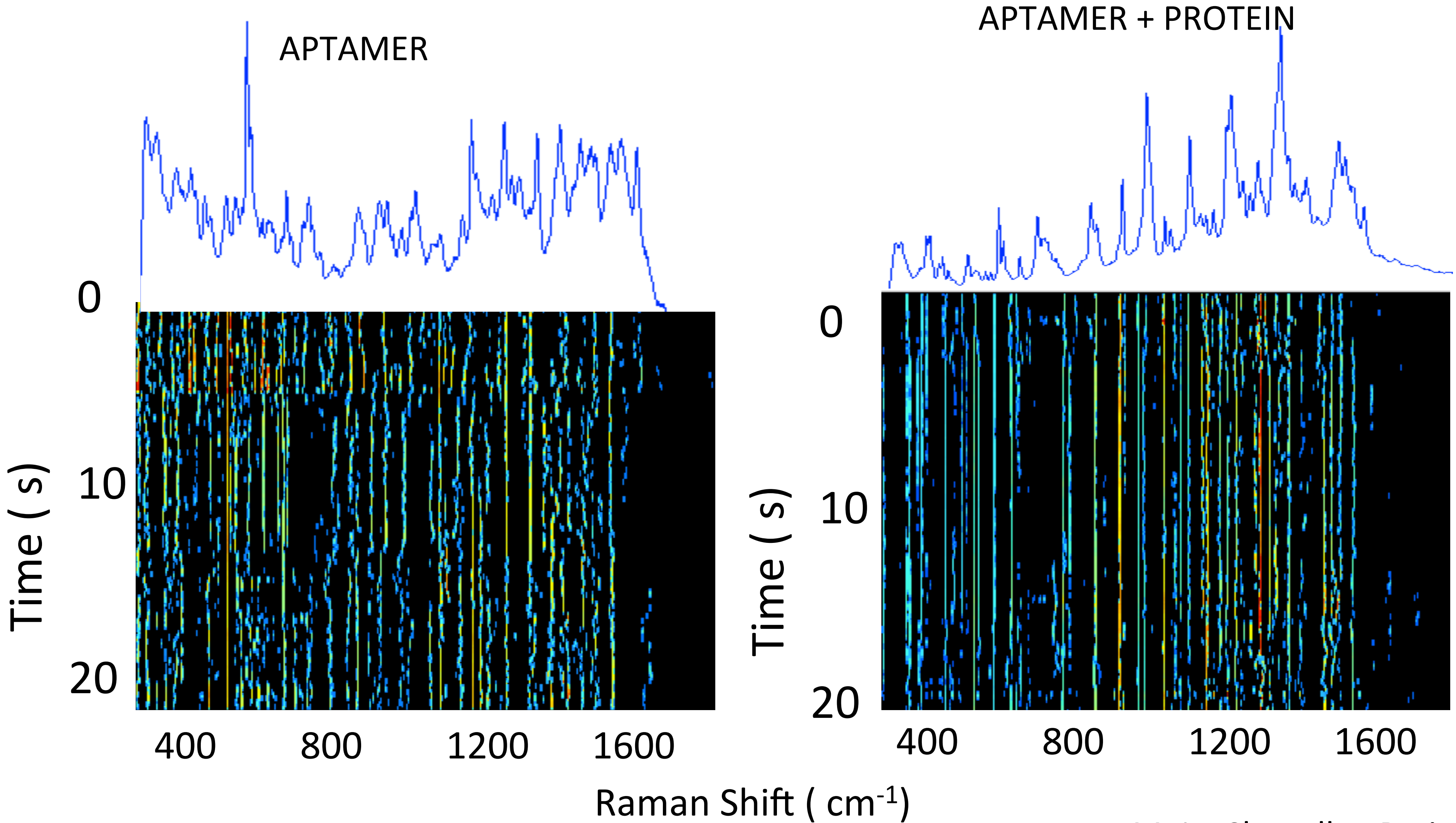
Extreme and rare statistics



Levy Jump

Law of Pareto (80–20 rule)

Blocking conformations using aptamer-protein interaction

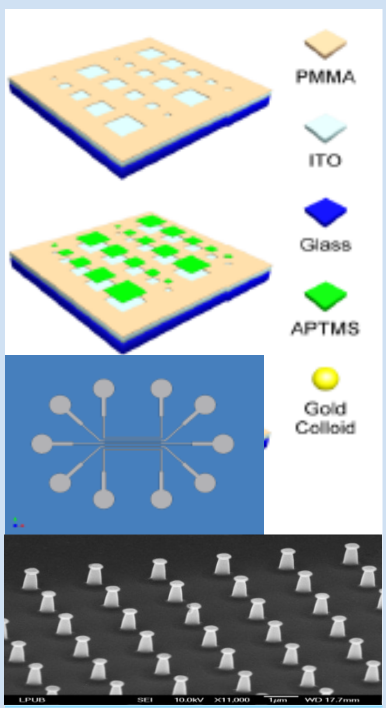


M. La Chapelle , Paris

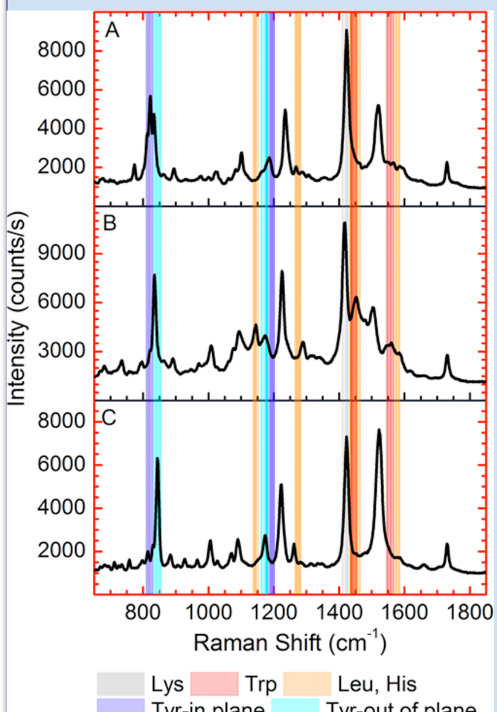
Conclusions

The story is far from over .....

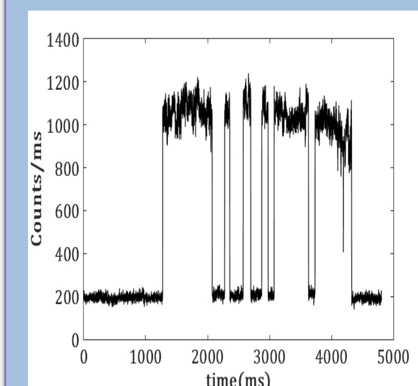
ENHANCEMENT

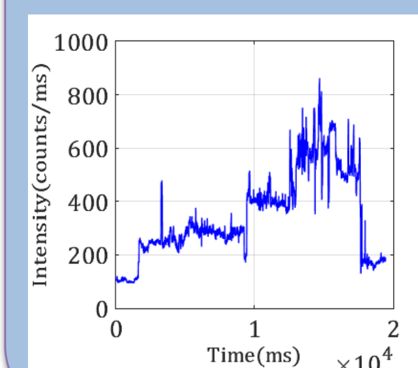


FINGERPRINTS OF SINGLE MOLECULE

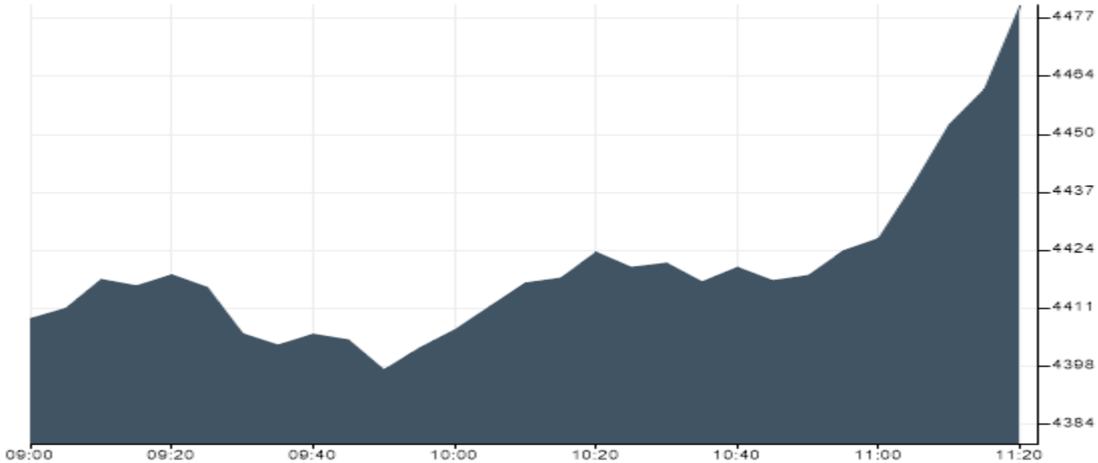


DYNAMICS

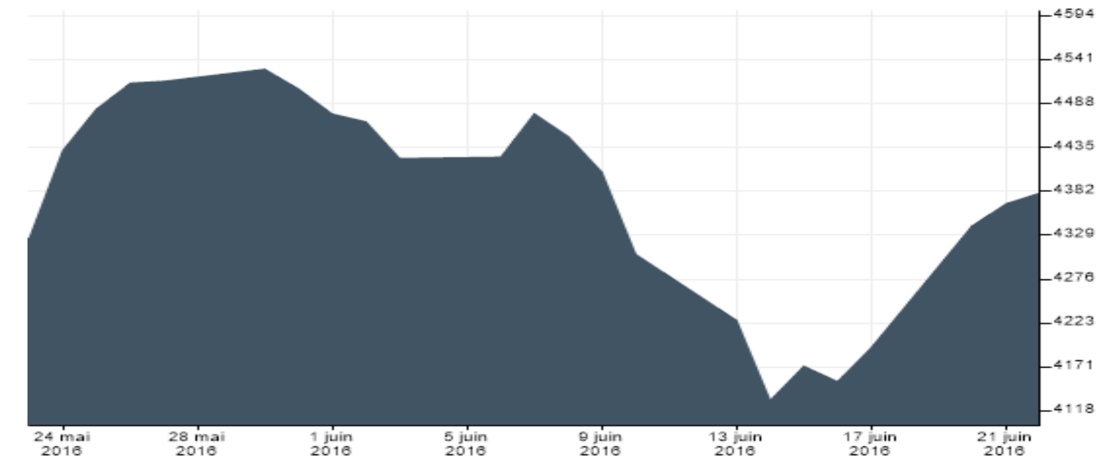




Analogy to CAC40



One hour



One month

One year