# Bimetallic Nanostars (Ag@Au) with High Surface Enhanced Raman Scattering (SERS) Performance: Detection of β-Amyloid and Its Marker Thioflavin T

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SERRANO 121, 28006. MADRID-SPAIN

4th International Conference on
Nanotek and Expo
San Francisco, USA December 01-03, 2014

CSIC
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# Outline

**Introduction**

**Fabrication and Characterization of SERS substrates**
- **Nanostars**
  - **Synthesis**
  - **Characterization**

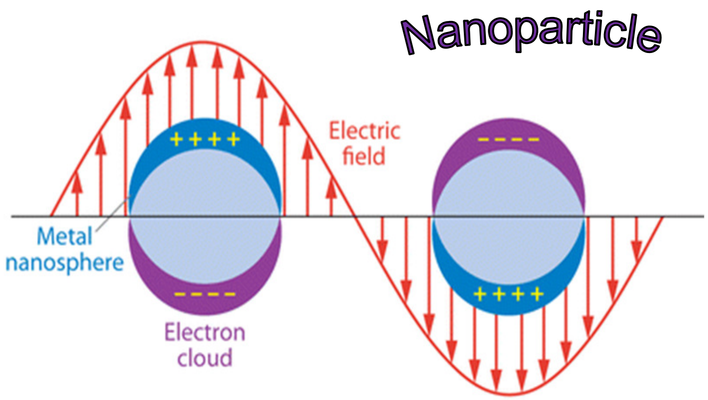
**Alzheimer Disease Markers**
- **β-amyloid**
  - **Direct SERS Detection**
- **Dyes (Congo Red and Thioflavin T)**
  - **SERS characterization**
  - **ThT - β-amyloid interaction**
- **Indirect SERS Detection of β-amyloid**

**Tailoring the size and shape of Silver Nanostars**

**Ag@AuNS using AgNS as seeds**

**Conclusions**

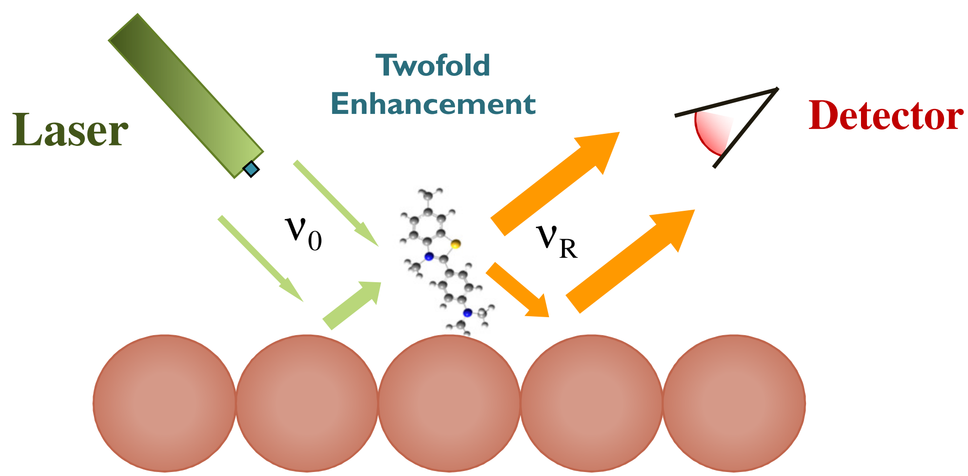
# Surface Enhanced Raman Spectroscopy



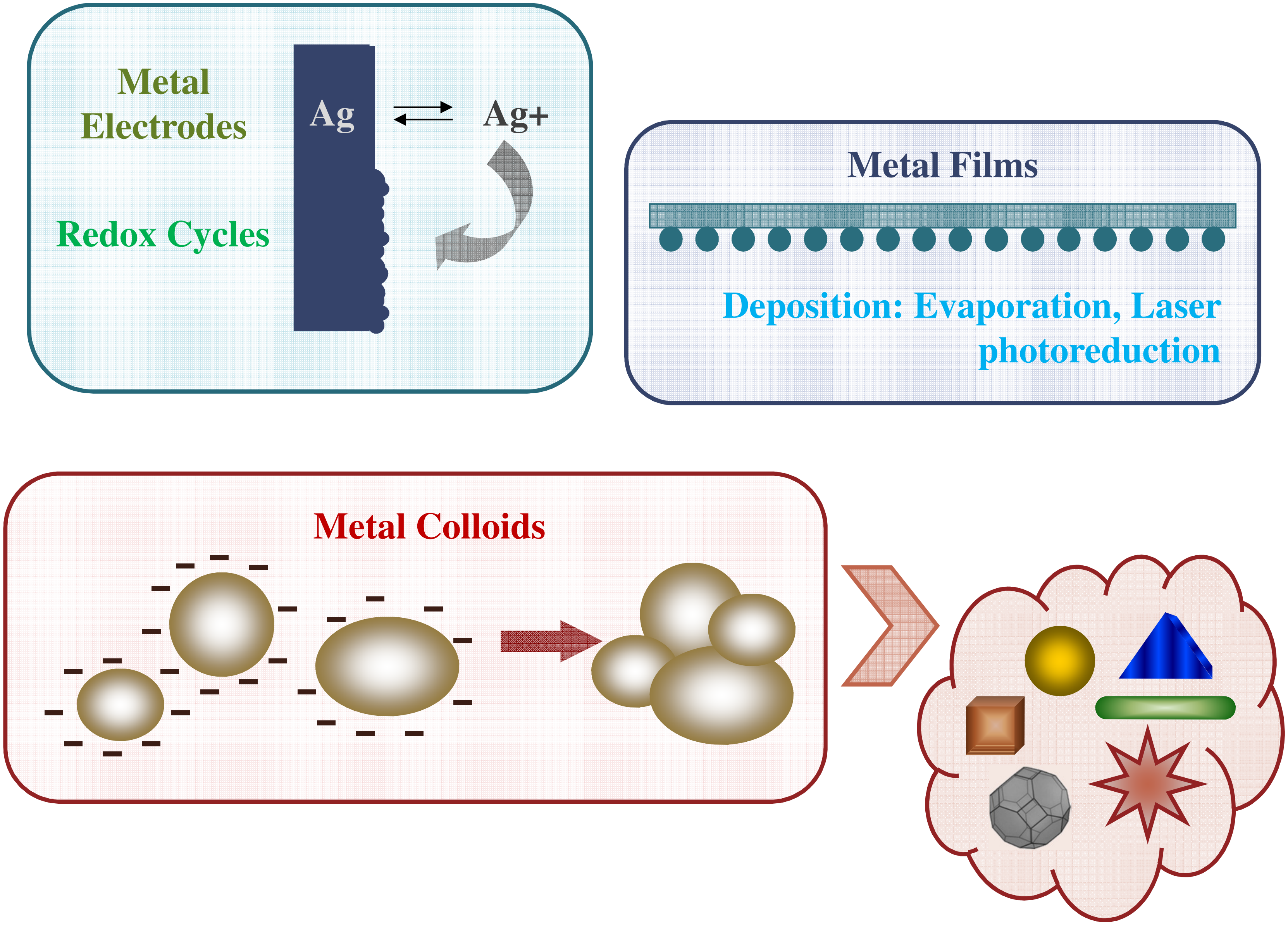

Localized Surface Plasmon Resonance

Ag Au Cu

Electromagnetic mechanism



# Metal nanostructures

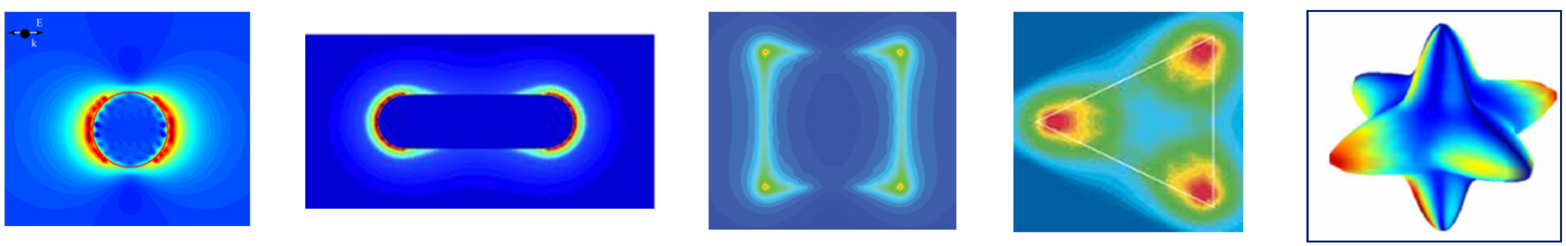


# Colloids

**Need external aggregation to detect low concentrations**

- **Non reproducibility**
- **Variation in signal/noise**

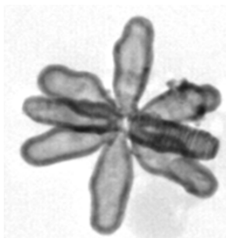
**SERS sensitive substrates with complex morphology**



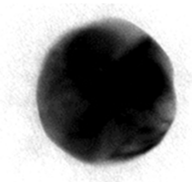
**Enhanced Electromagnetic Field**

# Nano-Stars and nano-spheres Fabrication

**$AgNO_3$ or $HAuCl_4$* $3H_2O$**



**Hydroxylamine**



**NEW!!!**

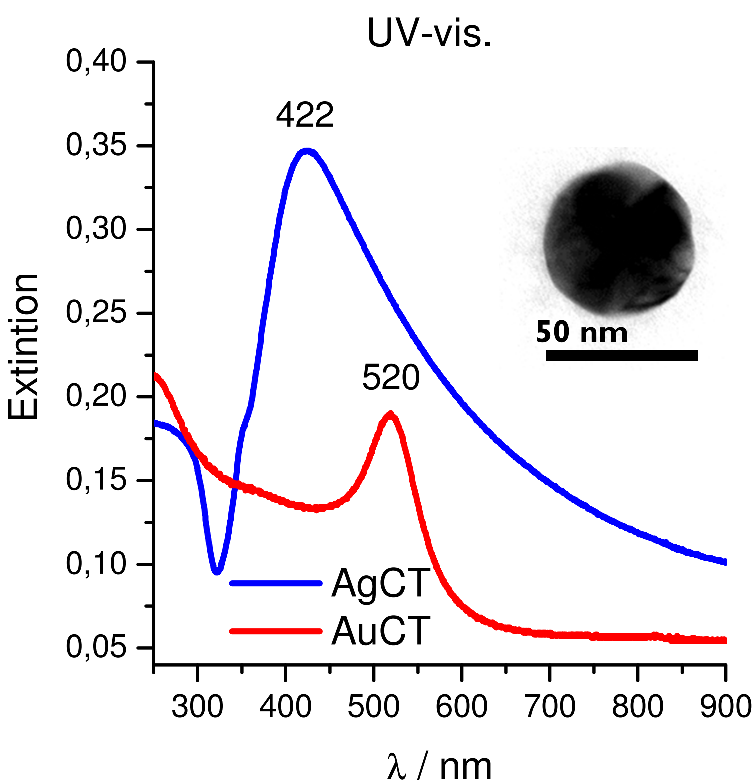
**Citrate**
**Borohydride**
**Hydrochloride Hydroxylamine**

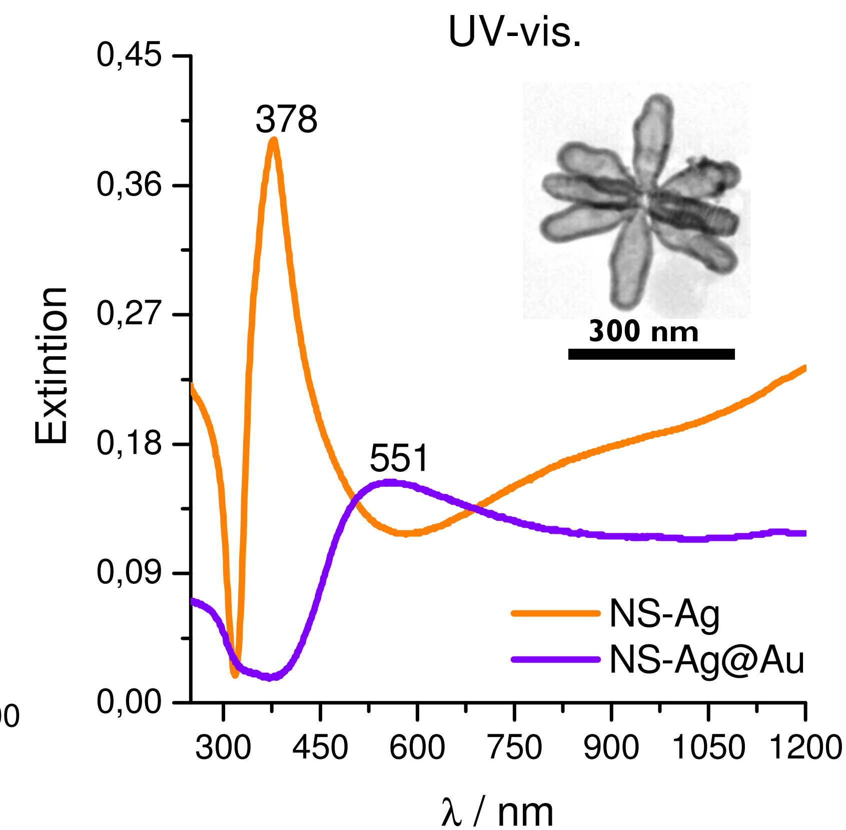
**Nano-Stars**
**NS-Ag / NS-Ag@Au**

**Nanospheres**

*A. Garcia-Leis, J.V. Garcia-Ramos and S. Sanchez-Cortes. JPC C. (2013) DOI: 10.1021/jp401737y*

# Characterization of nanoparticles

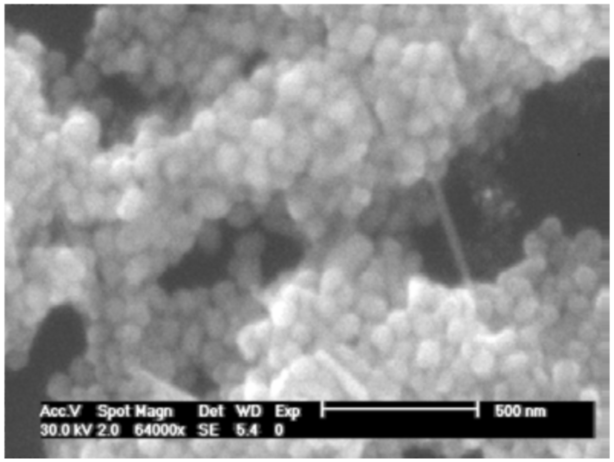


*Nanospheres*

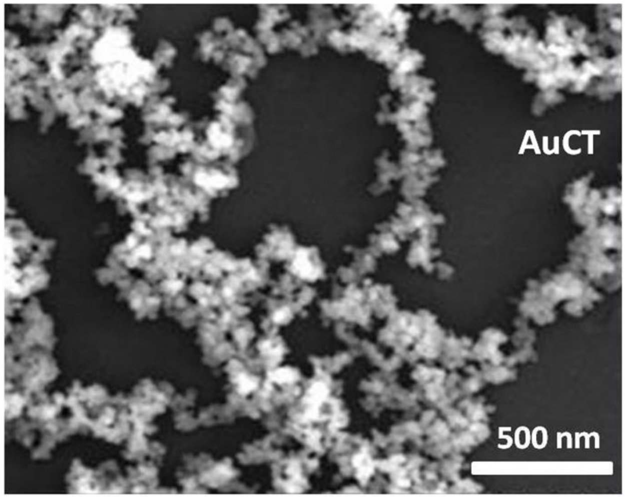
**AgCT**

(SEM)




**AuCT**

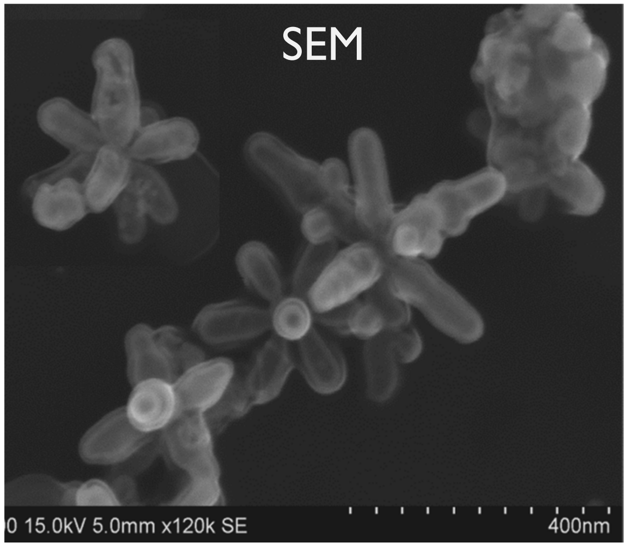
(SEM)



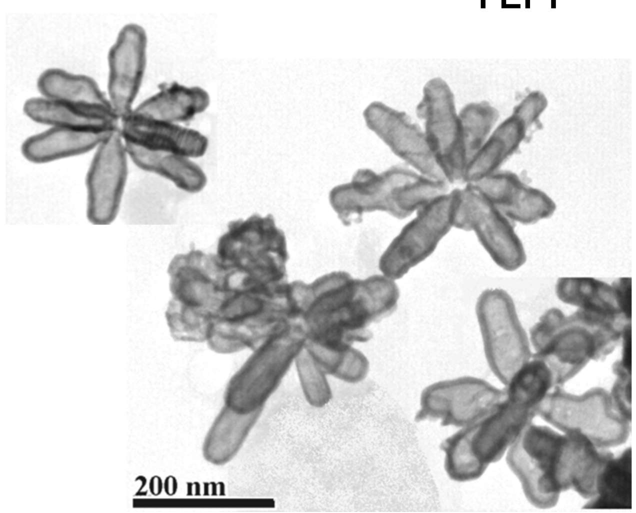
# *Nano-stars*

(TEM and SEM characterization)

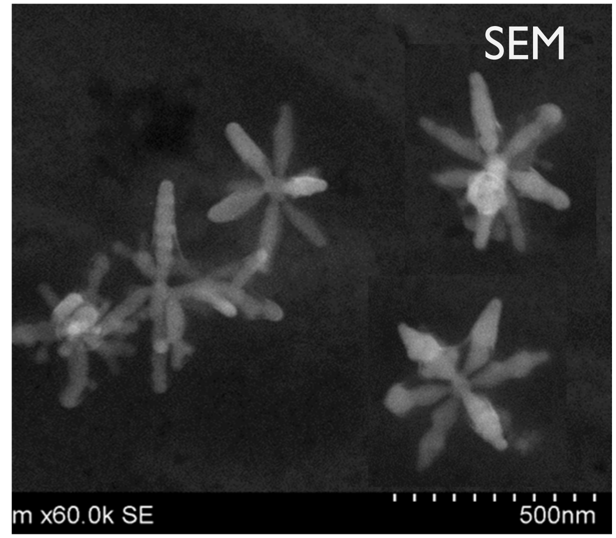
## NS-Ag@Au



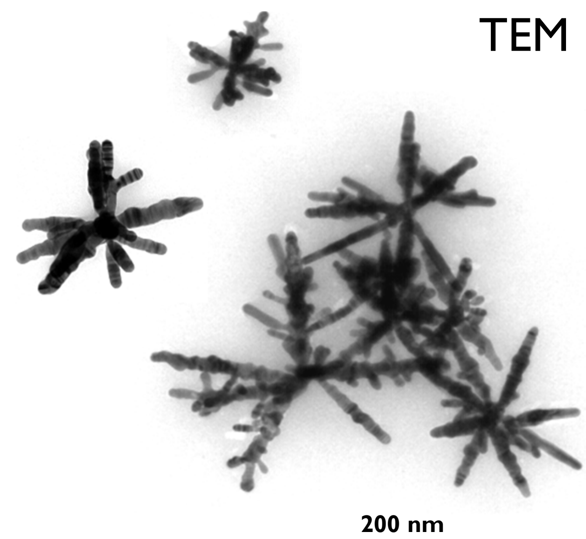

TEM




## NS-Ag

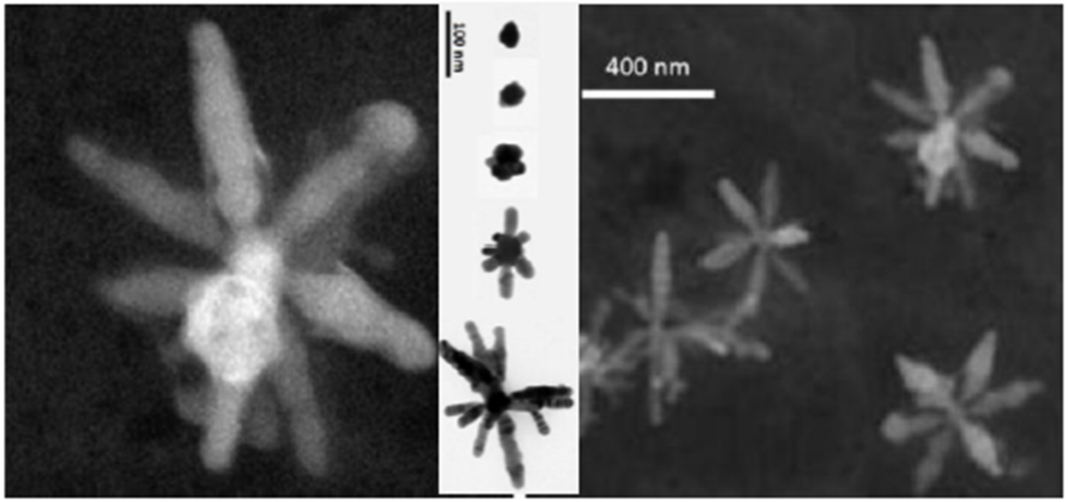



TEM



# *Nano-stars*

(TEM and SEM characterization)

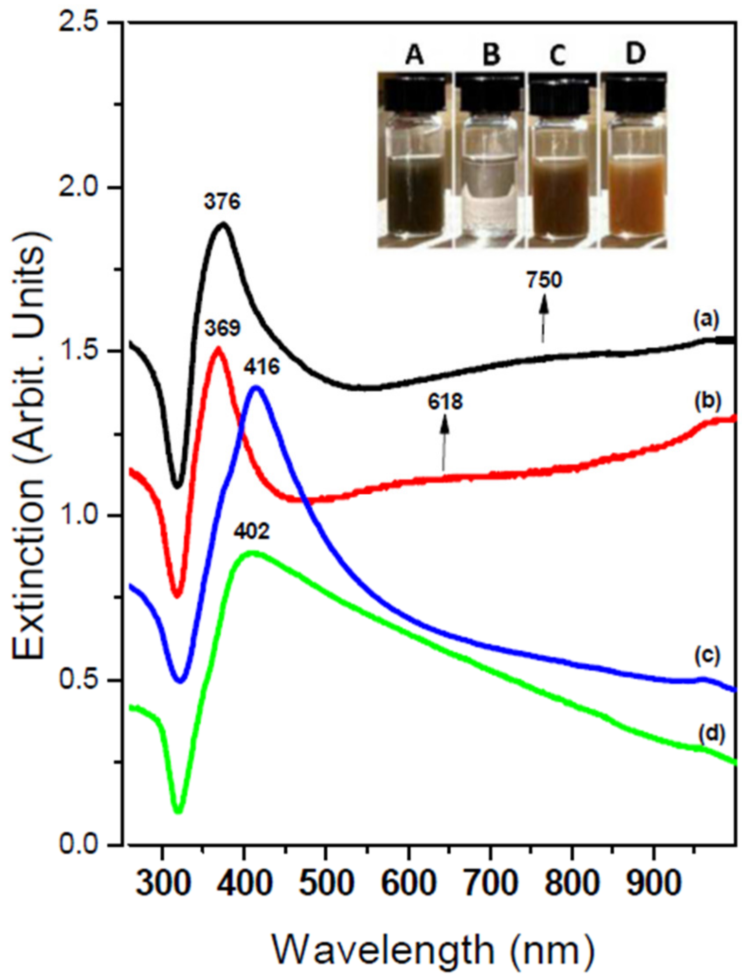



*SERS active substrates without aggregation!!*

*A. Garcia-Leis, J.V. Garcia-Ramos and S. Sanchez-Cortes. JPC C. (2013) DOI: 10.1021/jp401737y*

# *Nano-stars*

(Plasmon characterization)




**[$Ag^+$]/[HA]/[citrate]**

**A: 2.17/ 7.0 / 1.0**

**B: 0.21/ 7.0 / 1.0**

**C: 2.17/ 3.6 / 1.0**

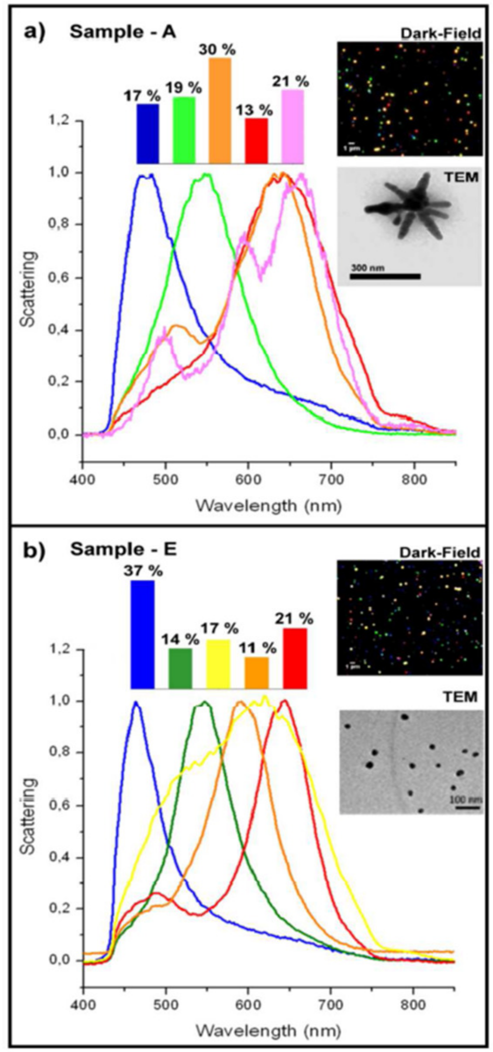
**D: 2.17/ 7.0 / 0.0**

*SERS active substrates without aggregation!!*

*A. Garcia-Leis, J.V. Garcia-Ramos and S. Sanchez-Cortes. JPC C. (2013) DOI: 10.1021/jp401737y*

# *Nano-stars*

(Dark-Field scattering)



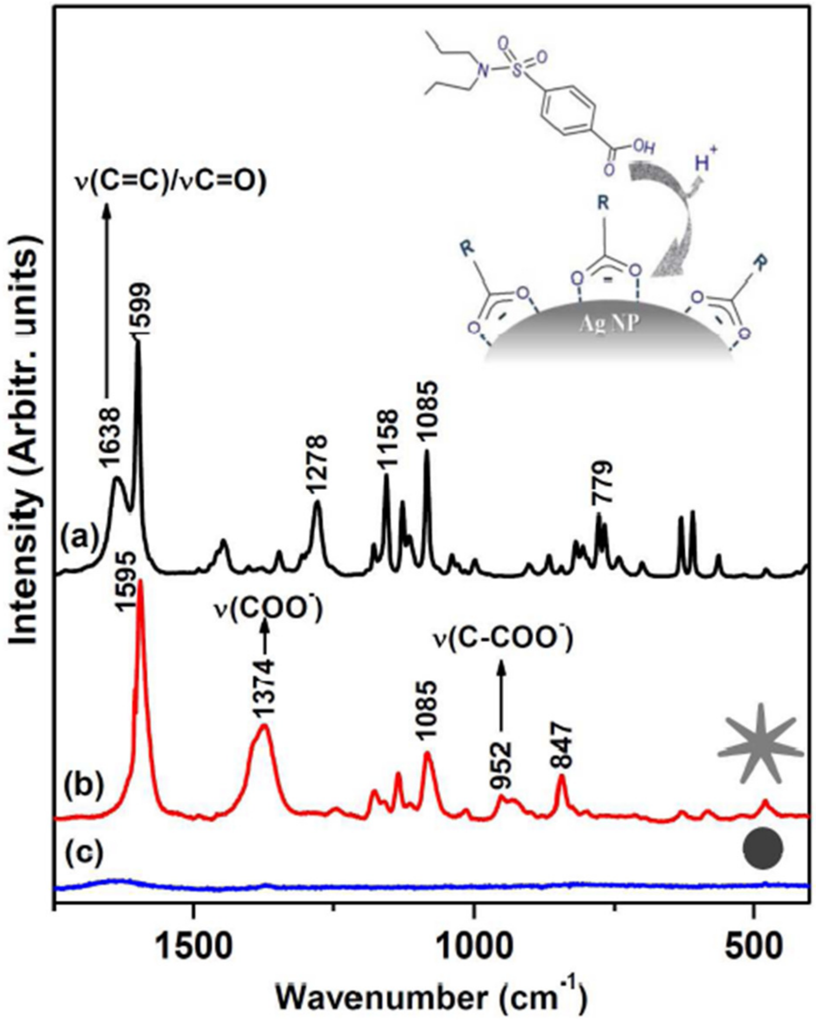

Nano-stars

Nanospheres

*A. Garcia-Leis, J.V. Garcia-Ramos and S. Sanchez-Cortes. JPC C. (2013) DOI: 10.1021/jp401737y*

# *Nano-stars*

SERS activity using probenecid (sulfamide) as probe molecule

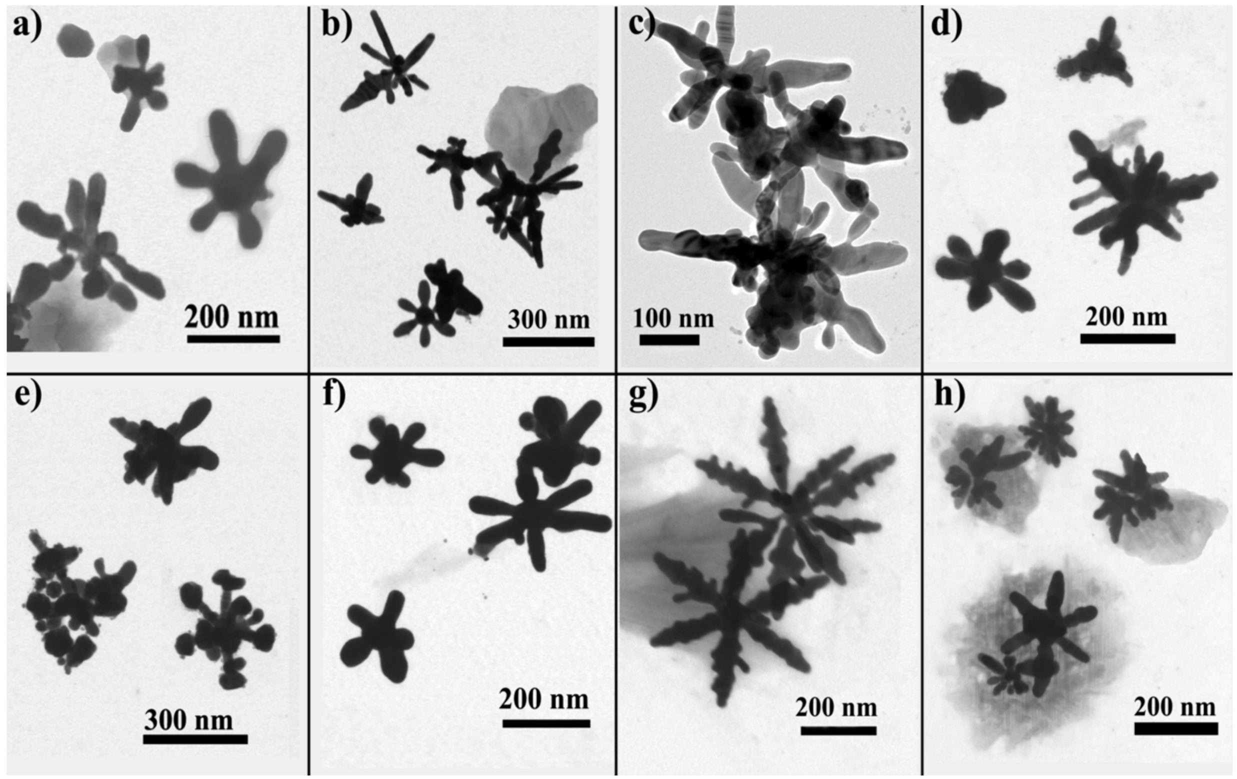



**Raman in solid state ($\lambda_{exc}$= 1064 nm)**

**SERS ($\lambda_{exc}$: 532 nm)**

*A. Garcia-Leis, J.V. Garcia-Ramos and S. Sanchez-Cortes. JPC C. (2013) DOI: 10.1021/jp401737y*

# Tailoring the size and shape of Silver Nanostars



## Preparation of a colloidal suspension of Silver Nano Stars

**Chemical reduction of $Ag^+$ in two steps:**

**Step 1: Reduction agent is a neutral Hydroxilamine solution**

**Step 2: After a time T1, a 1% citrate solution is added.**

The great novelty of this method is the use of neutral HA and the no use of strong surfactant agents.

**Experimental Conditions**
**24 different samples of AgNS**

[HA]
- ✓ 30.1 mM
- ✓ 60.2 mM
- ✓ 130 mM

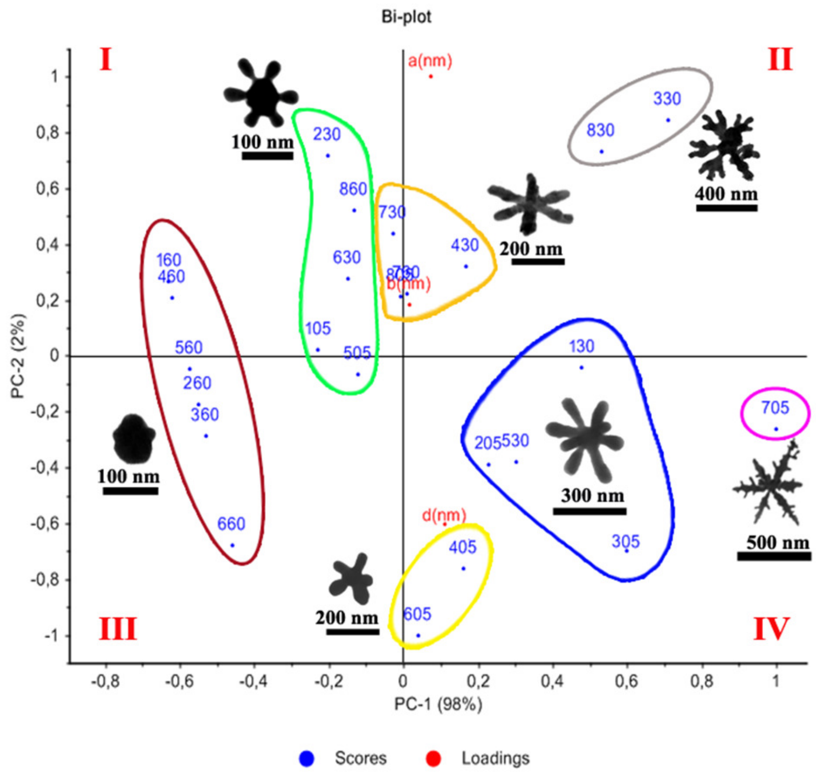
[Ag+]
- ✓1.11 mM

V(CIT)
- ✓100 µL

T1
- ✓ 5 min.
- ✓60 m0.11 mM
- ✓10 µL
- ✓30 min.
- ✓in.

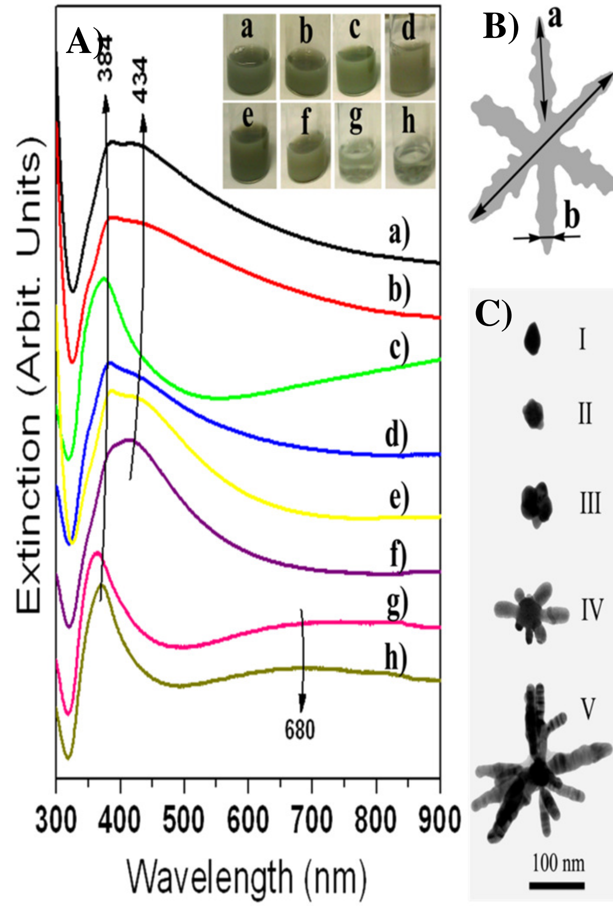
**[HA] = concentration of hydroxylamine solution**
**[$Ag^+$] = concentration of silver nitrate solution**
**V(CIT) = added volume of 1% citrate solution**
**T1 = the waiting time before adding the CIT solution**

## PCA graphic of scores and loadings of data obtained from morphological features of 24 AgNS samples.



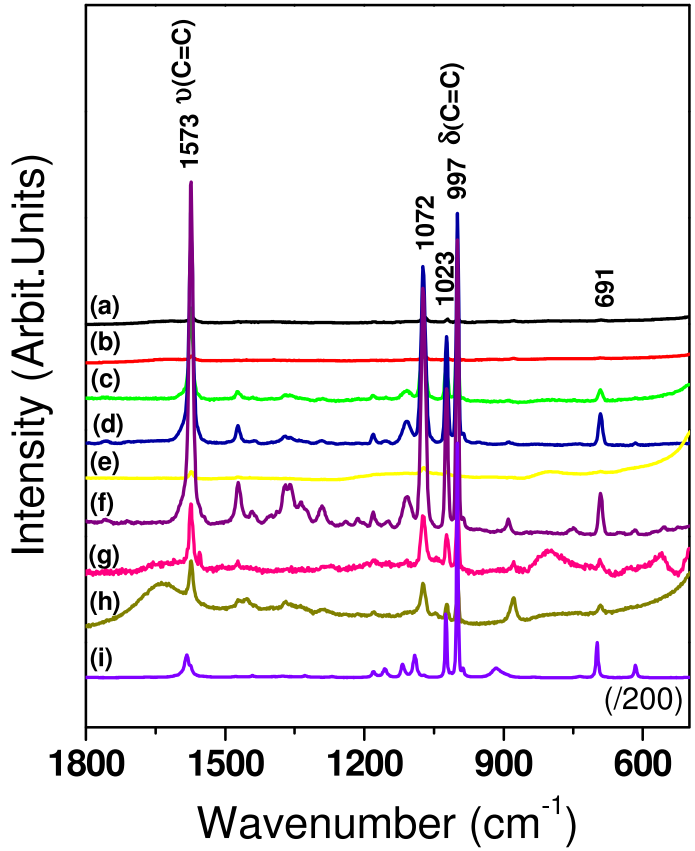

**PCA data can be classified in seven groups formed by samples with similar features depending of: size (bigger or smaller), type of arm (longer or shorter) and type of tip (spiky or rounded).**

# Extinction Spectra




Scheme of possible mechanism of growing nanoparticles based on TEM images.

# SERS Study



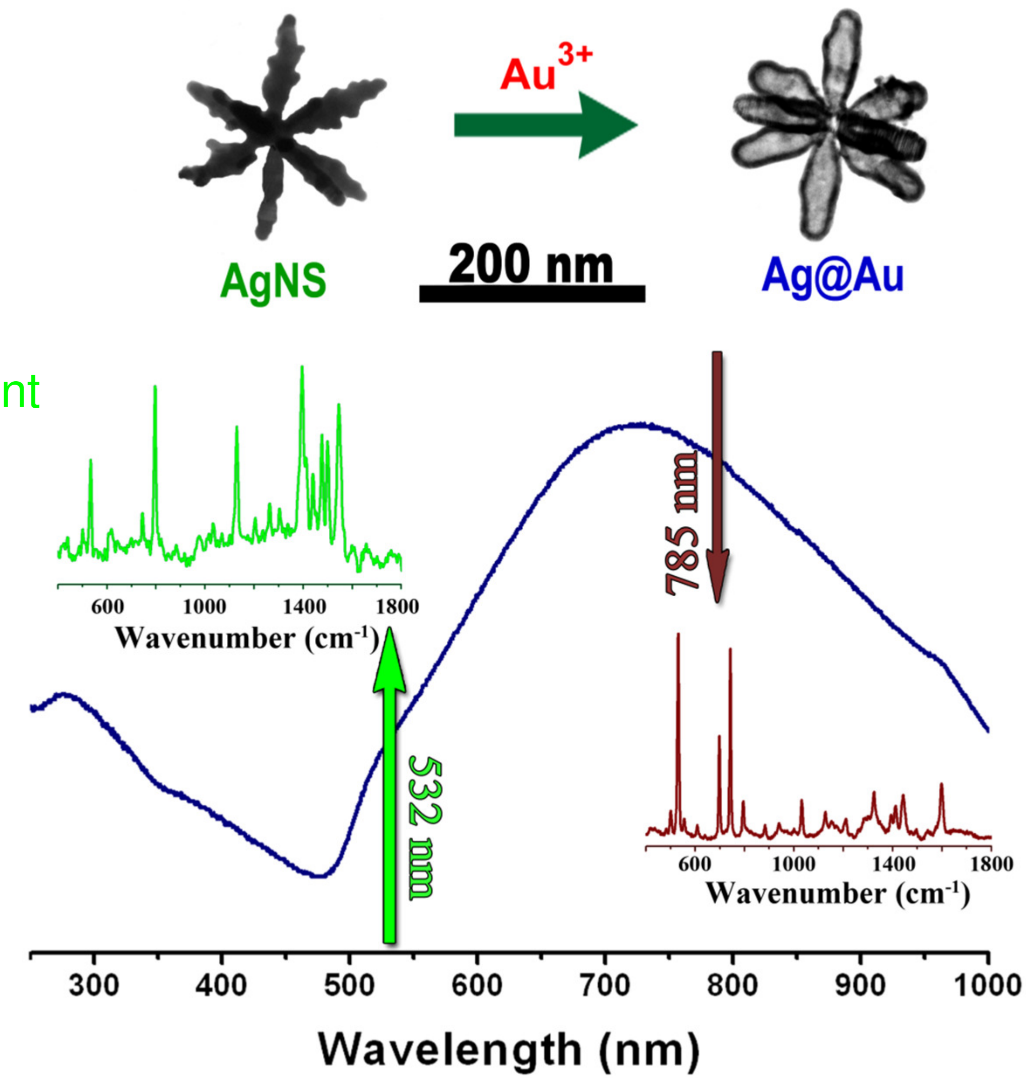

**E.F. (x10$^5$)**
a) 3.10, b) 3.93, c) 11.6, d) 35.4, e) 3.52, **f) 42.5**, g) 12.1 and h) 15.8

**SERS spectra of thiophenol at 1µM on different colloids samples of AgNS: a) 105, b) 205, c) 305, d) 405, e) 505, f) 605, g) 705, h) 805 and i) Raman spectra of pure thiophenol. Excitation 532 nm**.

- **CONCLUSIONS:** The reported fabrication method gives rise to silver star-shaped nanoparticles with good plasmonic properties to afford a large SERS intensification. The PCA study allowed the lump together of NPs by different groups, taking account the morphological parameters. The best Ag NS according to SERS EF are those bearing an intermediate size (200 nm) displaying a moderate number of arms.

# Final morphology of Ag@AuNS using AgNS as seeds

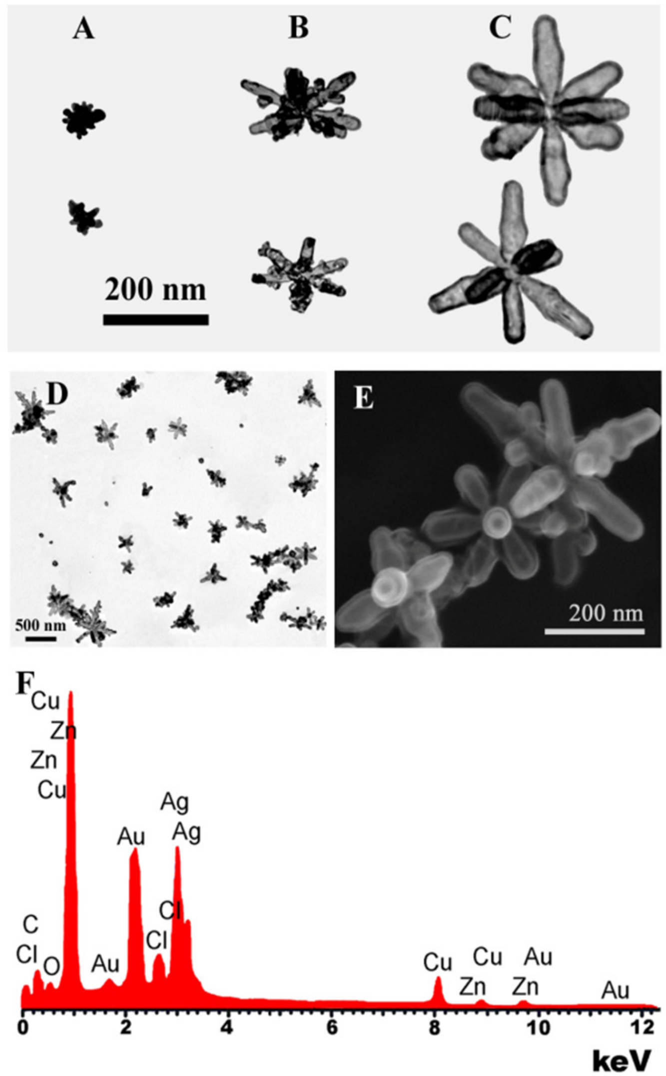
532 nm
(ThT resonant
wavelength)



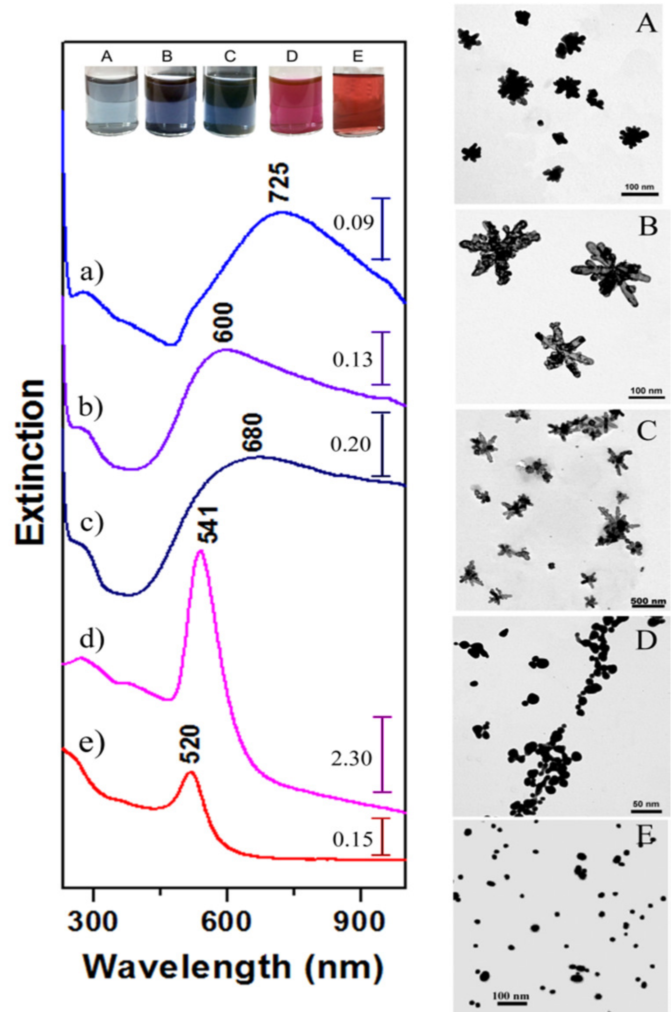

785 nm
(ThT non-
resonant
wavelength)

**Concentrations of reagents employed for the preparation of samples A-E and final pH of colloids solutions.**

| [Reagent]/ mM | A | B | C | D | E |
|---|---|---|---|---|---|
| $[AgNO_3]$ | 0.073 | 0.37 | 0.73 | 0 | 0 |
| $[HAuCl_4 \cdot 3H_2O]$ | 0.27 | 0.27 | 0.27 | 0.4 | 0.2 |
| [HA] | 3.0 | 3.0 | 3.0 | 3.0 | 0 |
| [CIT] | 0.26 | 0.26 | 0.26 | 0.26 | 0.77 |
| pH | 5.0 | 5.0 | 4.7 | 4.4 | 5.5 |




Micrograph of Ag@Au NS obtained by TEM of A (A), B (B), C (C) and (D) is a general view of sample C. (E) SEM micrograph of Sample C. (F) EDX spectra of Sample C.

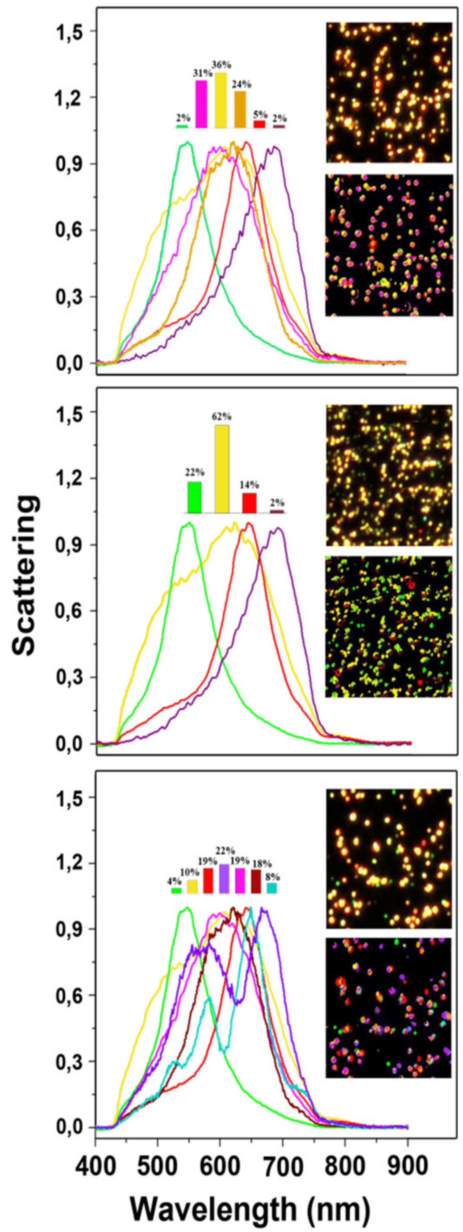



Extinction spectra (left) and TEM images (right) corresponding to the samples: A (a), B (b), C (c), D (d) and E (e).

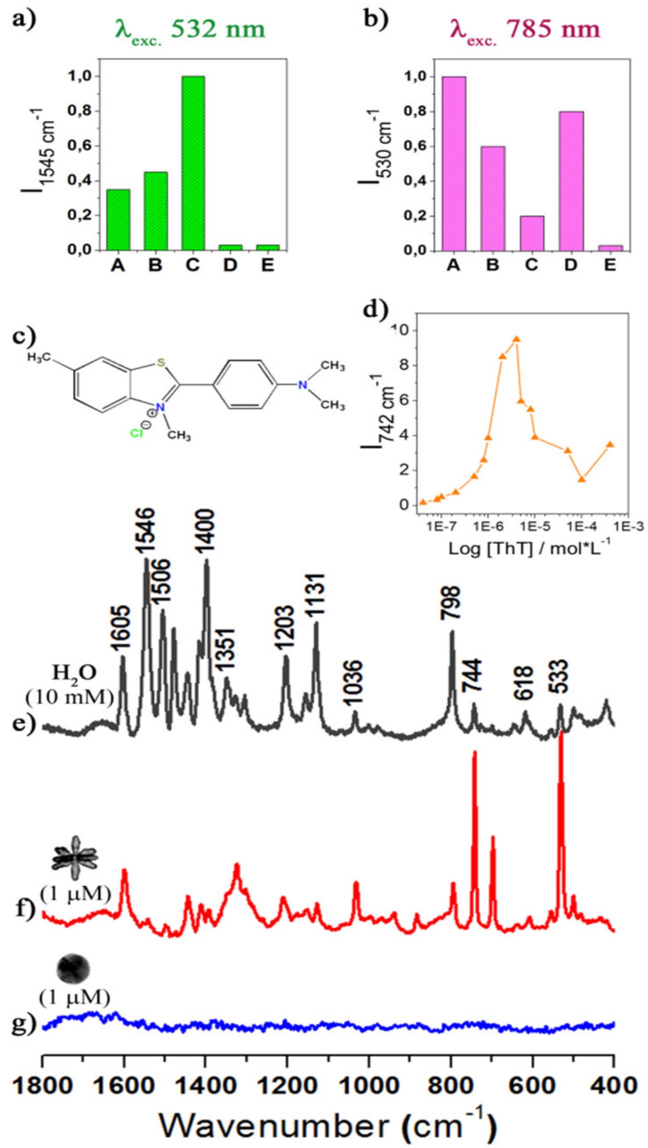
*Inset:* Pictures of the colloids obtained by methods A, B, C, D and E.

Dark-field (DF) hyperspectral image

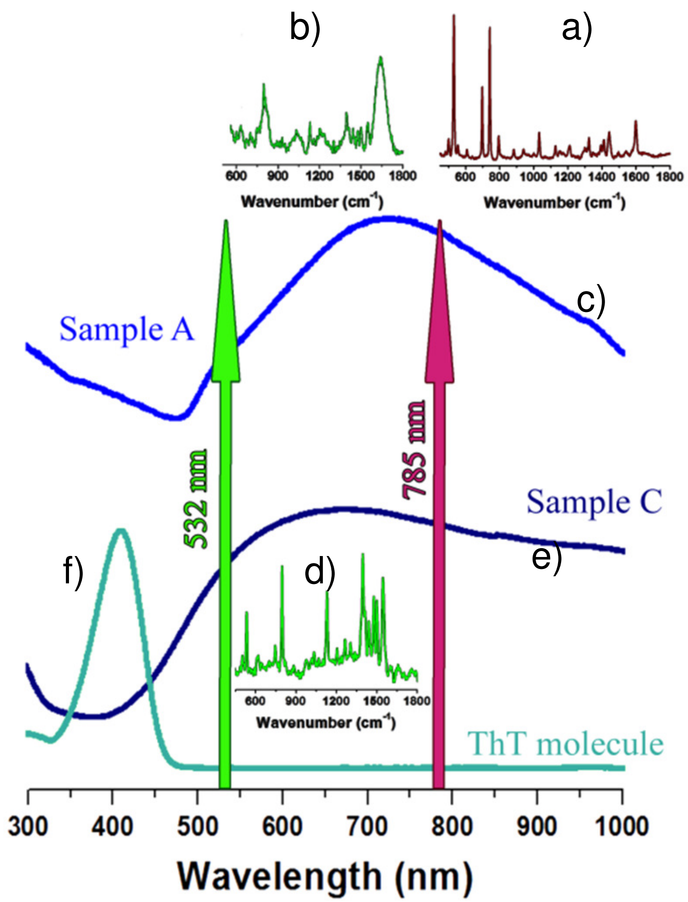
Spectral angle mapper (SAM) image




Dark-field scattering spectra observed in samples: A, C and D.



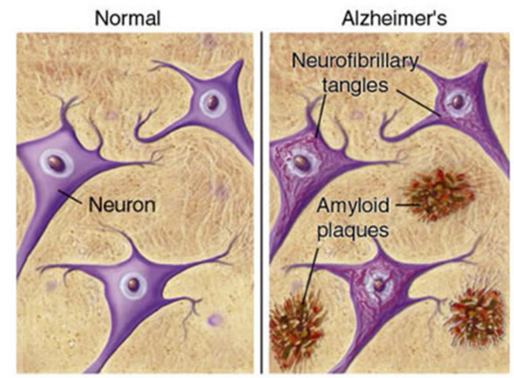

SERS enhancement dependence on the excitation wavelength and the substrate: 532 nm (a) and 785 nm (b). Chemical formula of ThT (c).
Adsorption isotherm of ThT on sample B (d). Raman spectrum of ThT 10 mM (e). SERS spectrum of ThT 1 µM on sample B (g) and sample E (g). Excitation 785 nm.



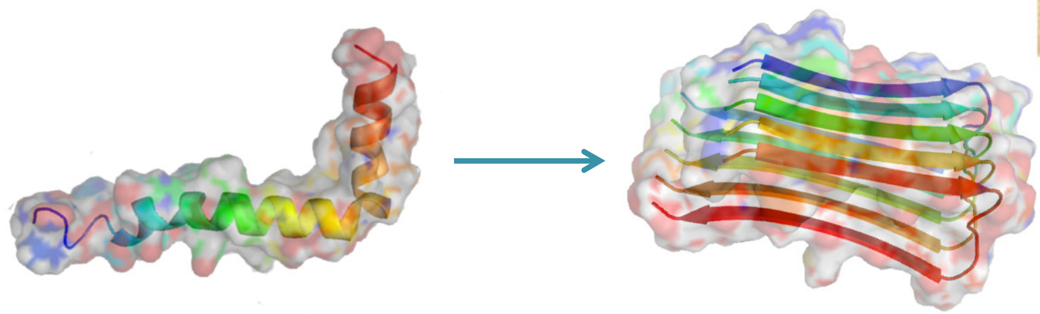

SERS spectra of ThT on Samples A and C exciting at 532nm (b and d, respectively) and 785nm on Sample A (a).
The extinction spectra of samples A (c) and C (e) and the absorption spectrum of ThT (0.1 M) in water (f) is also shown for comparison.

# Alzheimer disease

**Neurodegenerative disease**

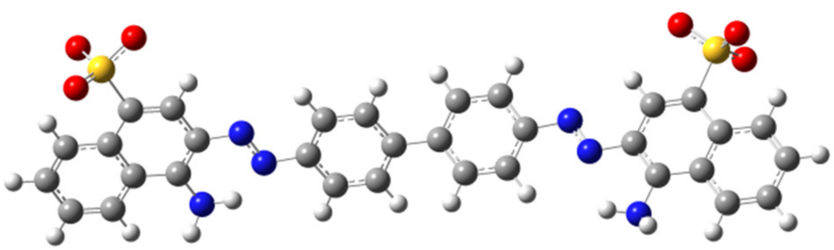



**β-amyloid**

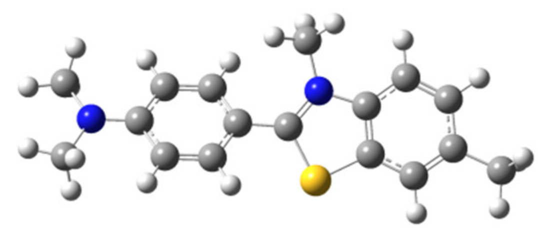


**Peptide with 36-43 aa**

**Histological dyes used to demonstrate the presence of amyloidal deposits in tissue**

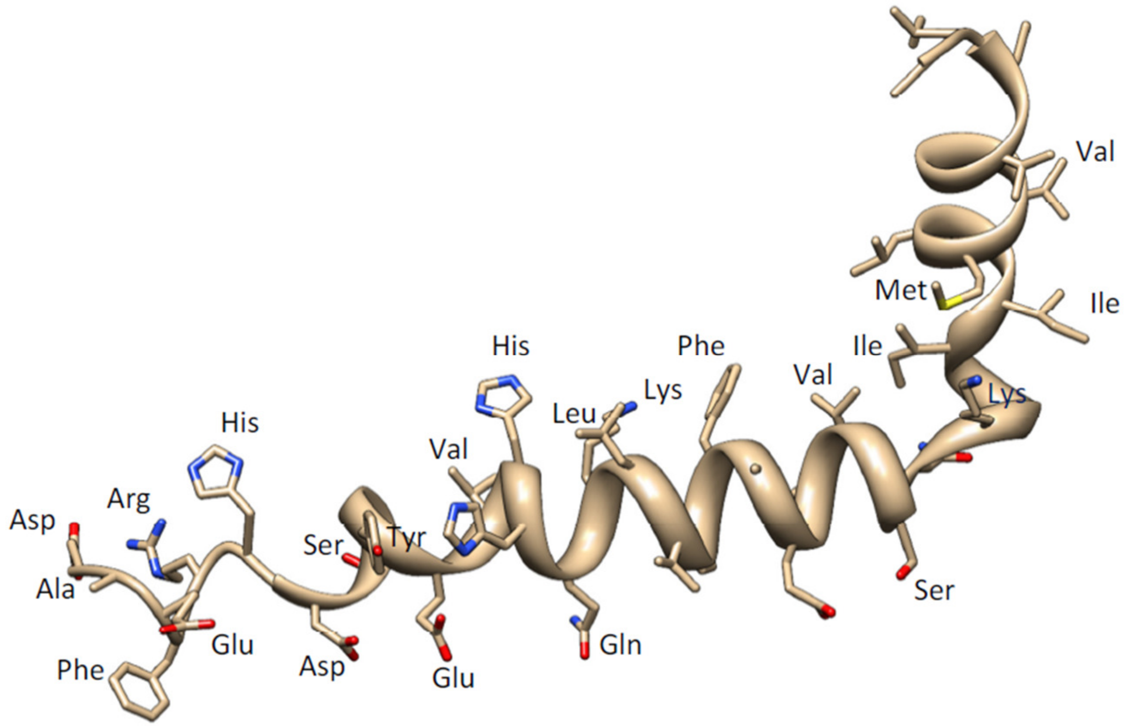


**Congo Red**



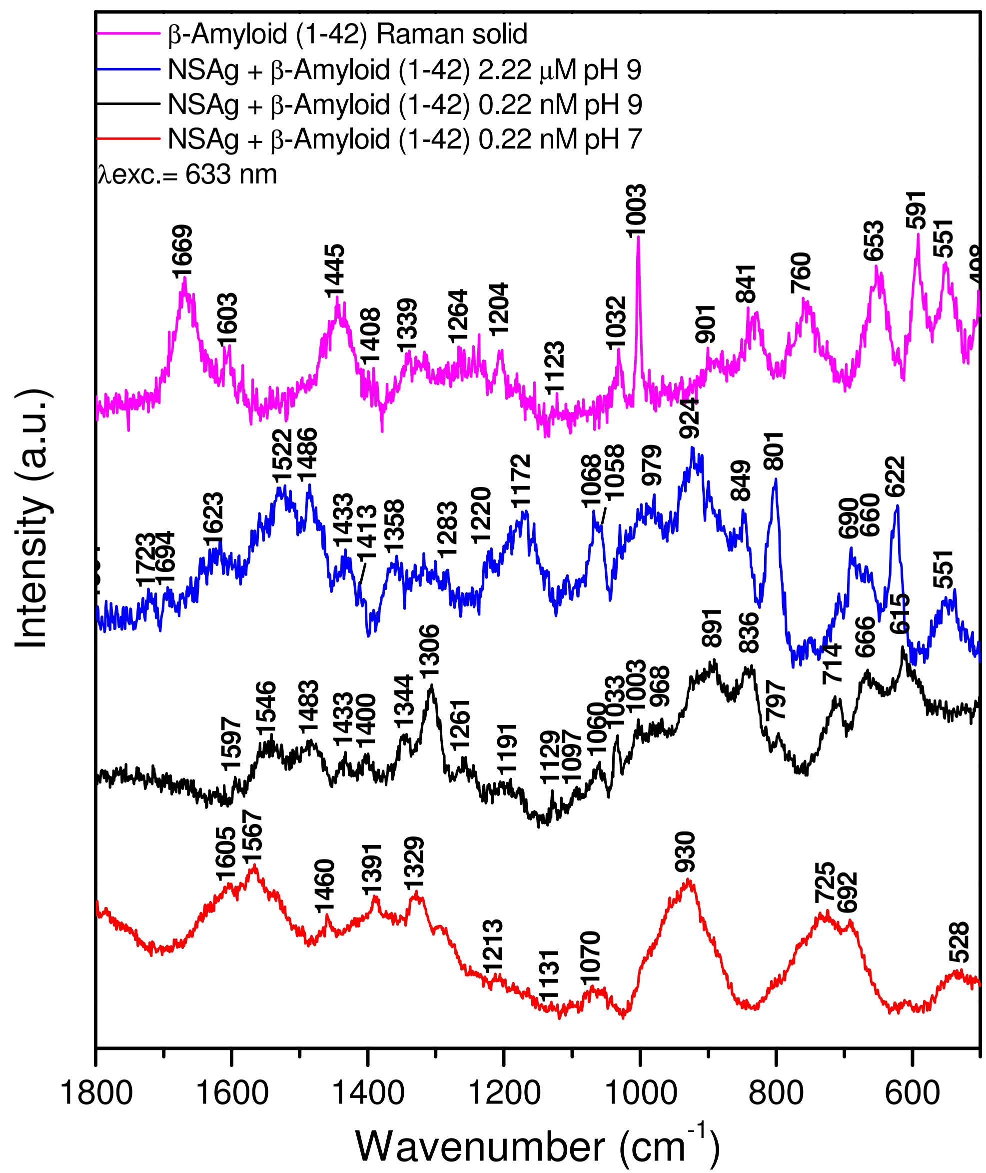
**Thioflavin T**

# β–amyloid (1-42)

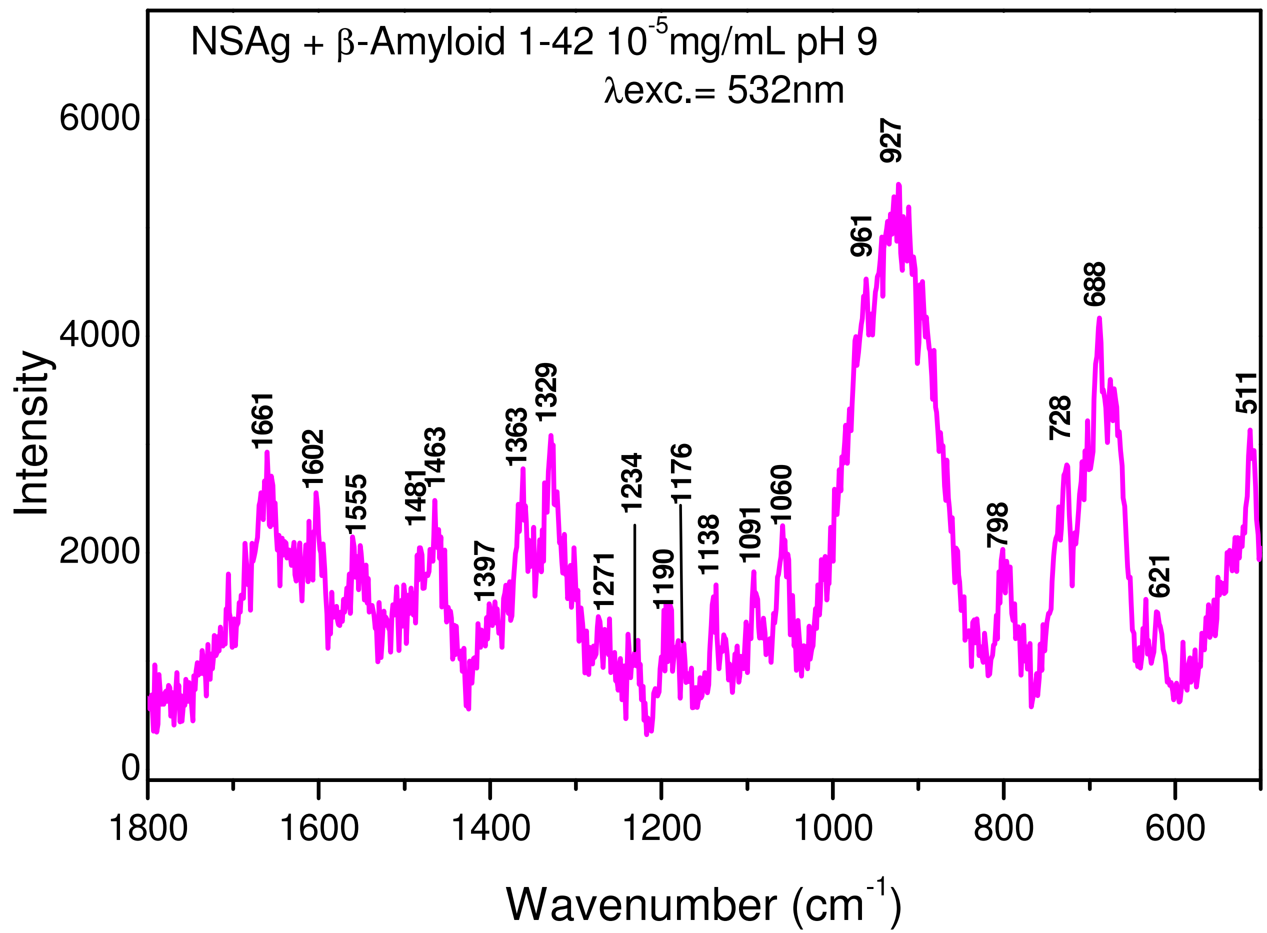



Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala

# SERS of β–amyloid(1-42) on Silver Nano-Stars



| Wavenumber / cm$^{-1}$ | | | Assignments |
|---|---|---|---|
| **Solid Raman** (λexc.= 633 nm) | **SERS NSAg** pH 9 (633nm) | **SERS AgCT** pH 9 (785nm) | |
| 1669 vs | - | - | Am I |
| - | 1623 | 1623 | $\nu(C{=}O)$ |
| 1606 m | - | - | Phe, $\nu(C{=}C)$ |
| 1585 w | 1595 | 1581 | Phe, Tyr |
| 1565 w | 1558 | 1567 | Am II |
| - | 1483 | 1494 | His |
| 1469 sh | 1463 | - | His (deprotonated) |
| 1444 s | 1433 | 1441 | $\delta(CH_2)$, $\delta(CH_3)$ |
| 1408 sh | 1413 | 1401 | $\delta(C_\alpha\text{-}H)$ |
| - | 1344 | 1347 | $t_w(CH_2)$ or $\rho(CH_2)$ |
| 1264 w | 1261 | 1268 | Am III (β-sheet) |
| 1236 w | 1237 | - | |
| 1185 vw | 1191 | - | Phe, Tyr |
| 1179 w | 1172 | 1171 | $\omega(C\text{-}C)$ |
| 1123 w | 1127 | 1128 | $\nu$ (C-C) |
| 1091 vw | 1091 | - | His, Lys, Arg |
| - | 1068 | 1064 | $\nu(C\text{-}C)$ aliphatic side chains |
| 1031 m | - | 1034 | Phe |
| 1003 vs | - | 1001 | Phe |
| 969vw | 968 | 961 | $\delta_{op}(=C\text{-}H)$ |




**Poor SERS spectra to be used for β-amyloid direct detection!!!**

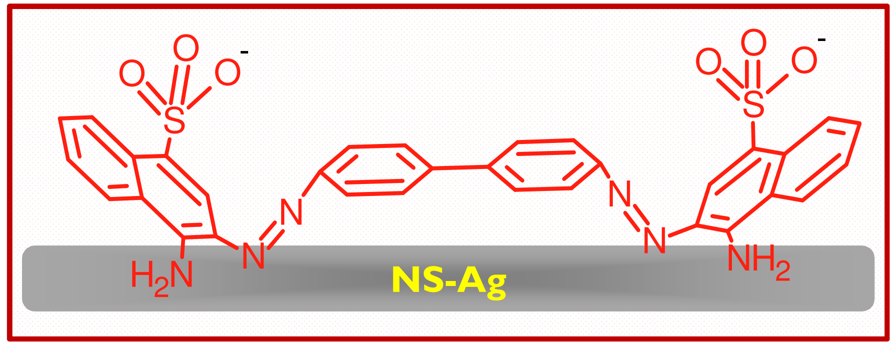
*Nano-stars*

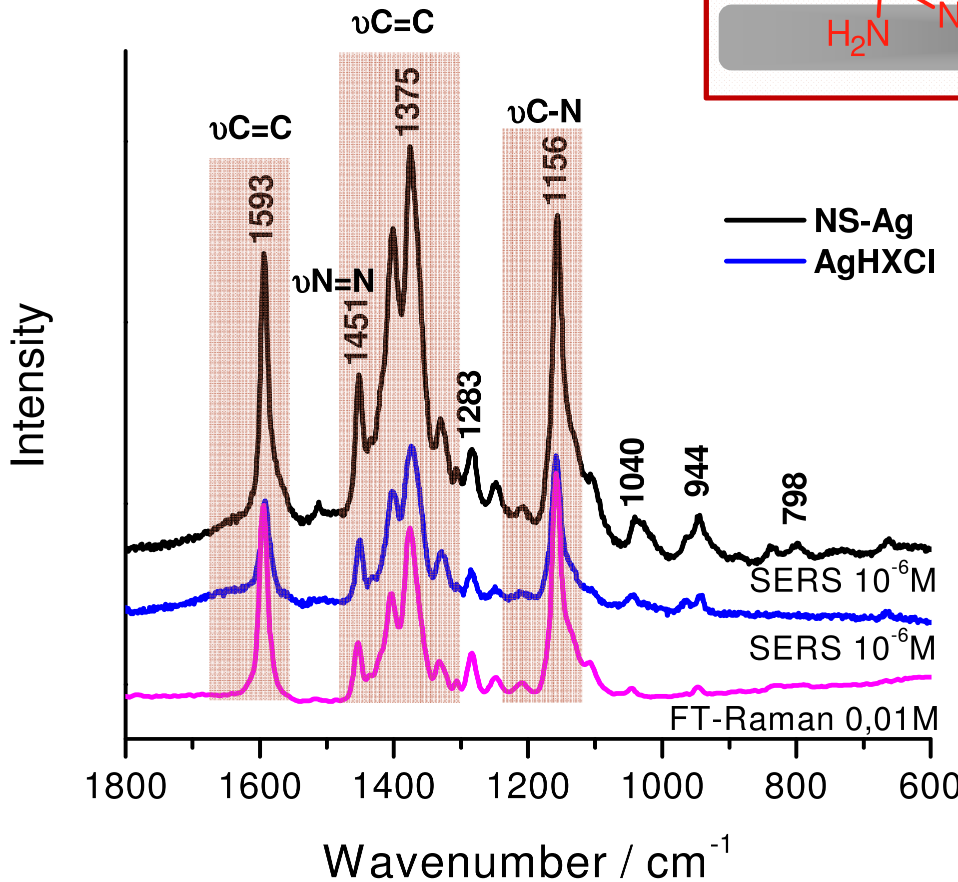
Use of Dyes to detect amyloid fibril formation

Congo Red

Thioflavin T

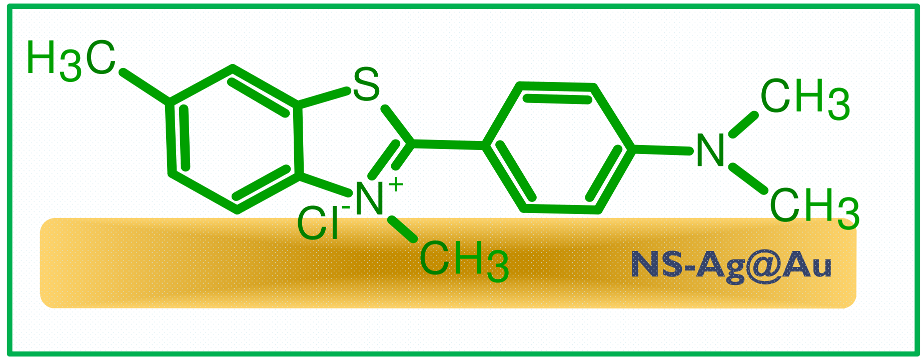
# Detection of **Congo Red** by SERS

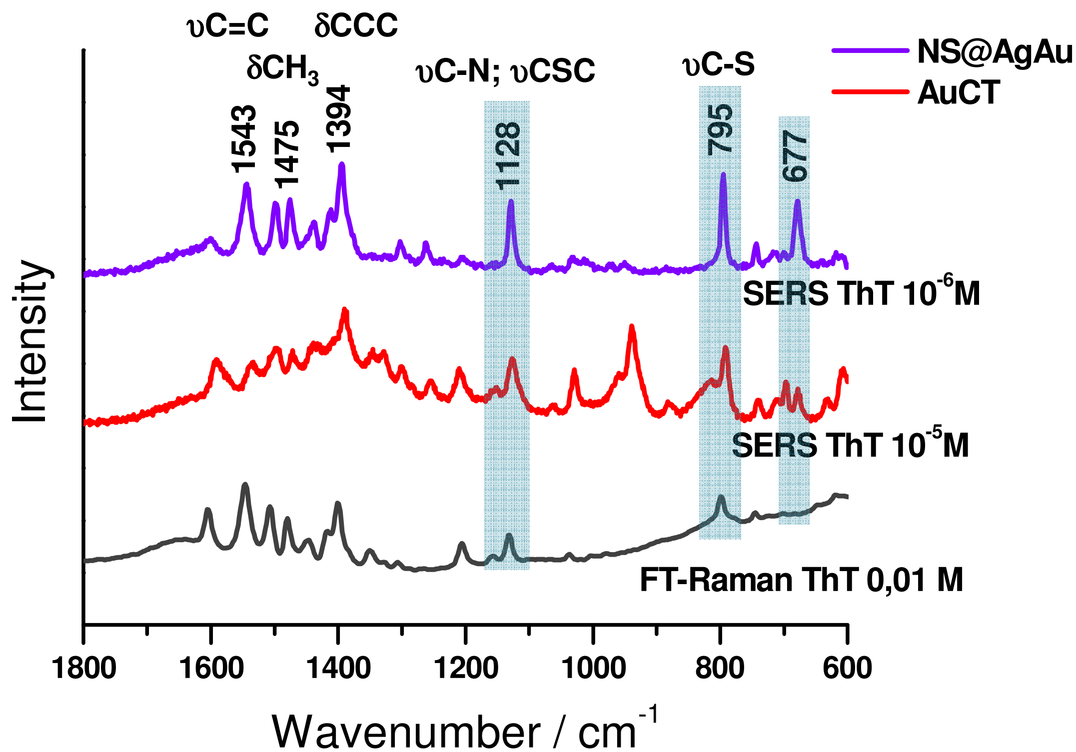


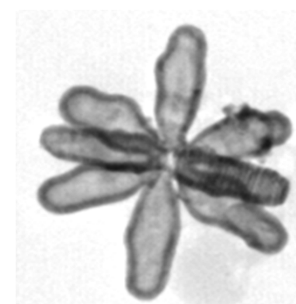




NS-Ag

# Detection of Thioflavin T by SERS

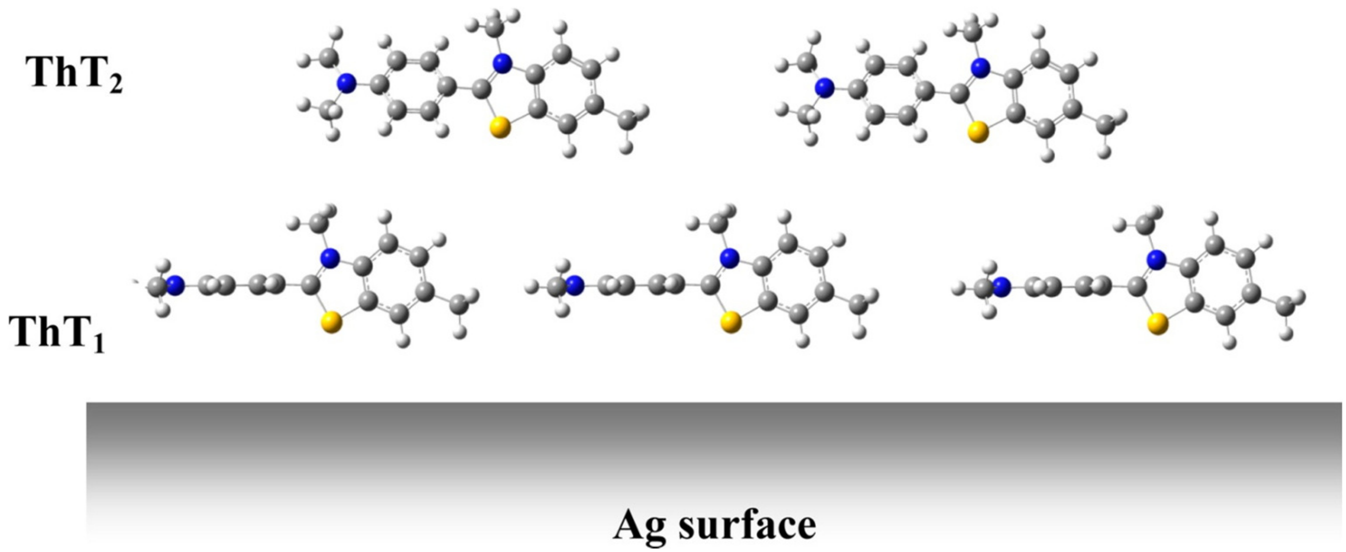


NS-Ag@Au

**Scheme of the adsorption of the ThT1 and ThT2 species on the Ag surface which implies a rotation of the φ angle to 90°**




Published in: Eduardo Lopez-Tobar; Marian Antalik; Daniel Jancura; Maria Vega Cañamares; Adianez García-Leis; Diana Fedunova; Gabriela Fabriciova; Santiago Sanchez-Cortes; *J. Phys. Chem. C* **2013,** 117, 3996-4005.
DOI: 10.1021/jp310619c

# Detection by SERS

**Langmuir Adsorption Isotherm**

$$I_S = \frac{K_{ad} I_{sm} [Analyte]}{1 + K_{ad} [Analyte]}$$

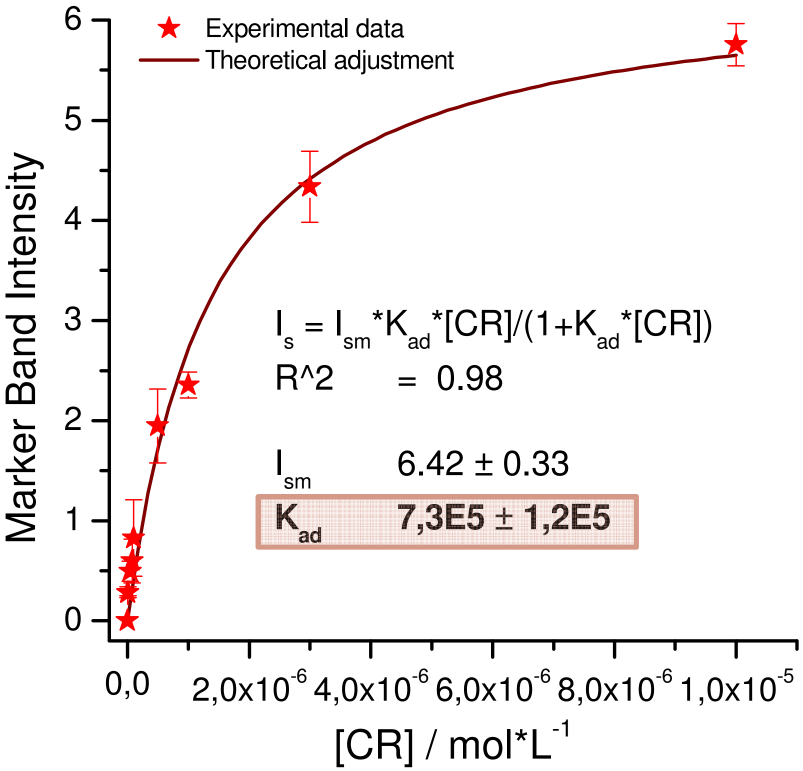
$I_s$ - SERS Intensity
$K_{ad}$ - Adsorption constant
$I_{sm}$- Maximum concentration of adsorbed analyte
*[Analyte]*- Molecule concentration

- Concentration ($10^{-4}$ M - $10^{-9}$ M)
- $\lambda$ excitation (532 nm, 785 and 633 nm)

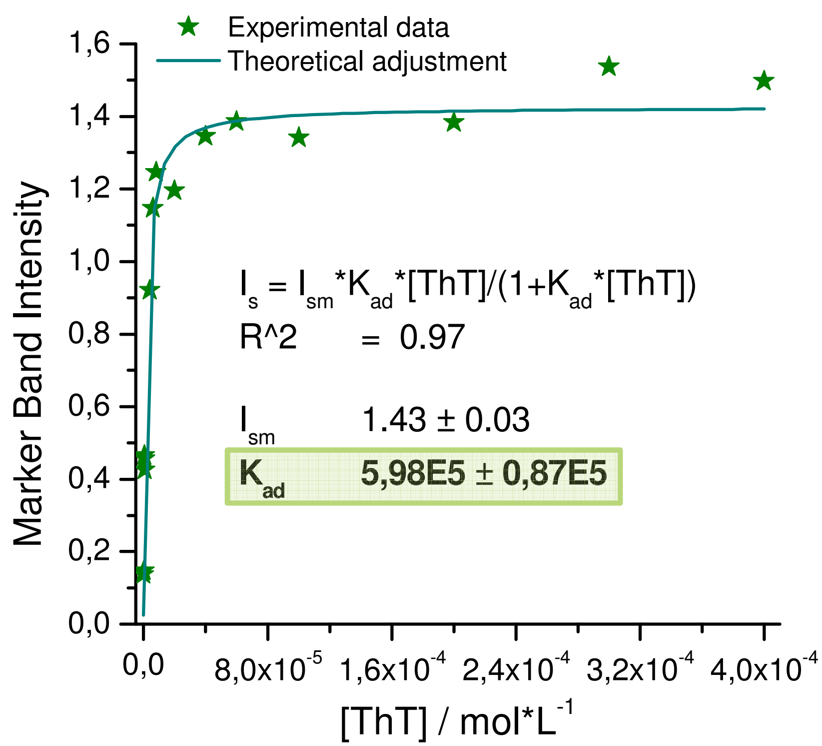
# Detection by SERS

## Langmuir adsorption isotherm

**CR**




**ThT**



# Detection by SERS

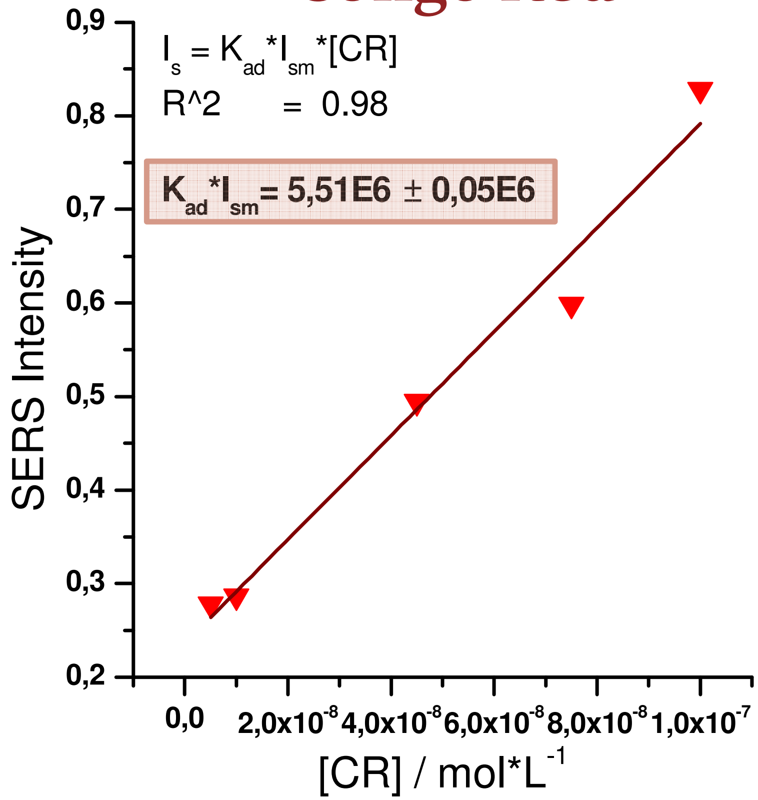
**Linear Region**

↓

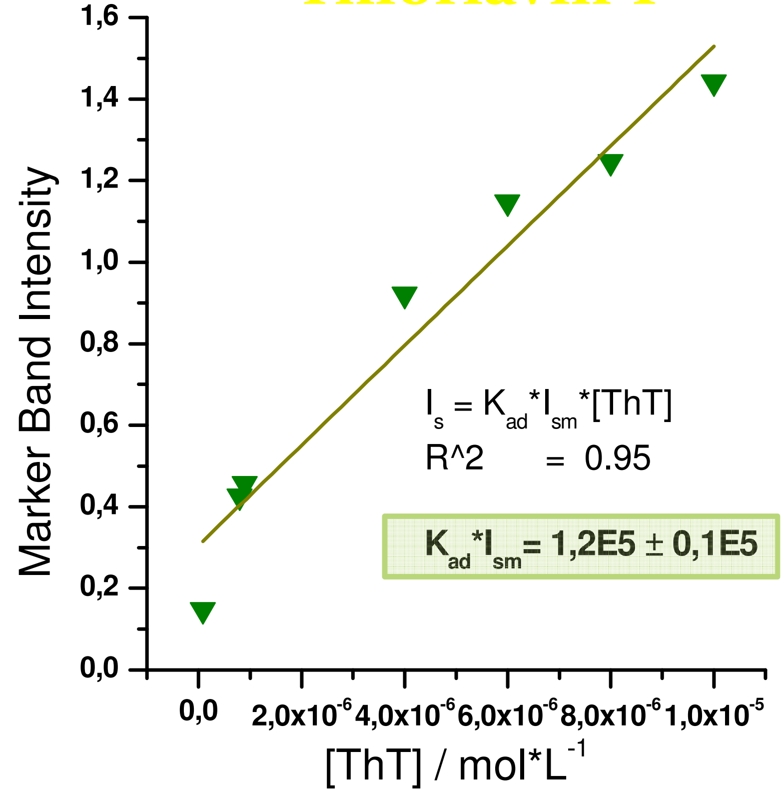
**SERS Sensitivity**

$$I_S = K_{ad} I_{sm} [Analyte]$$

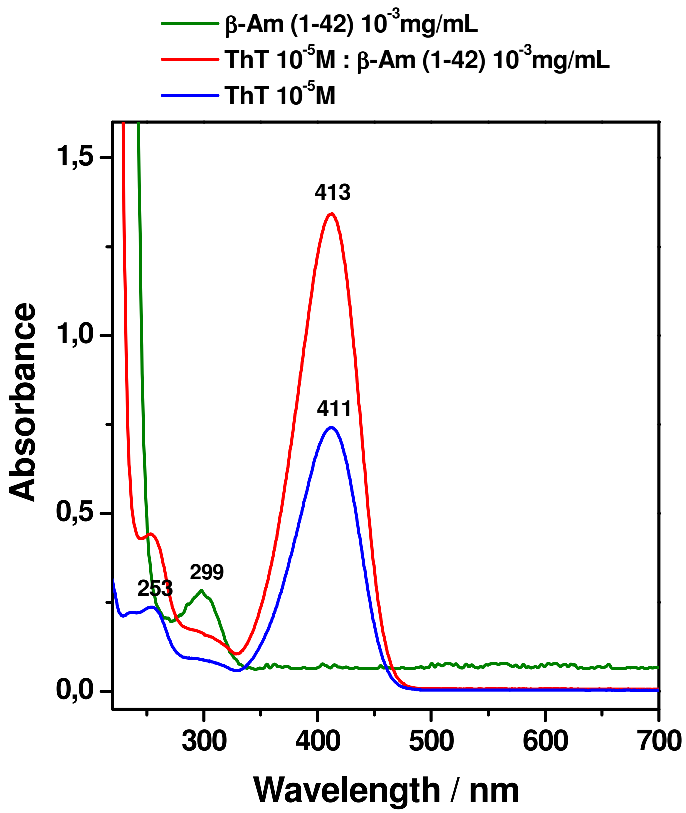
**Congo Red**

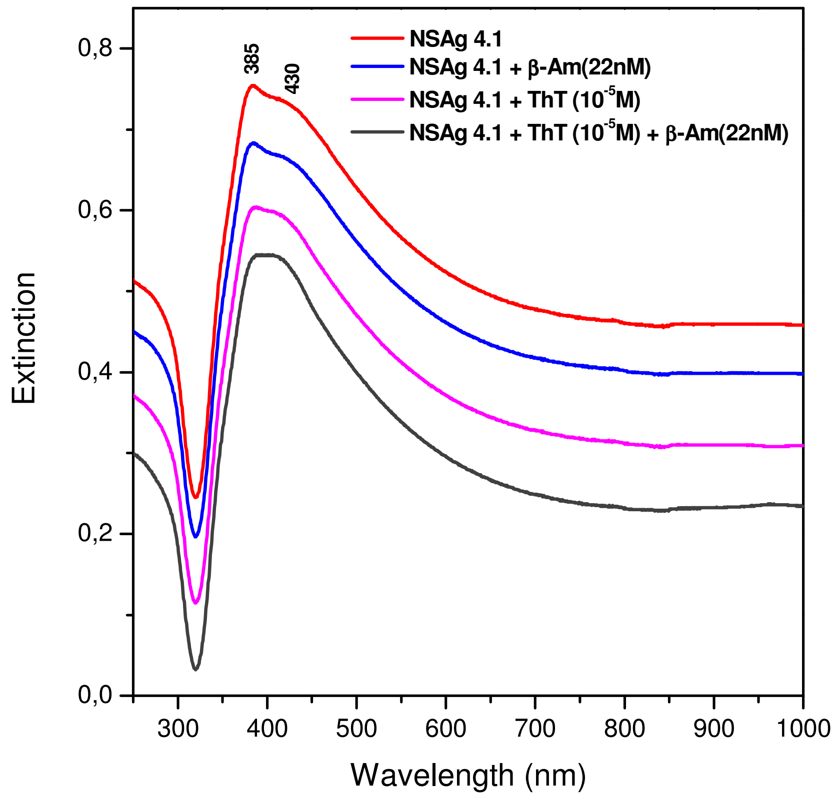


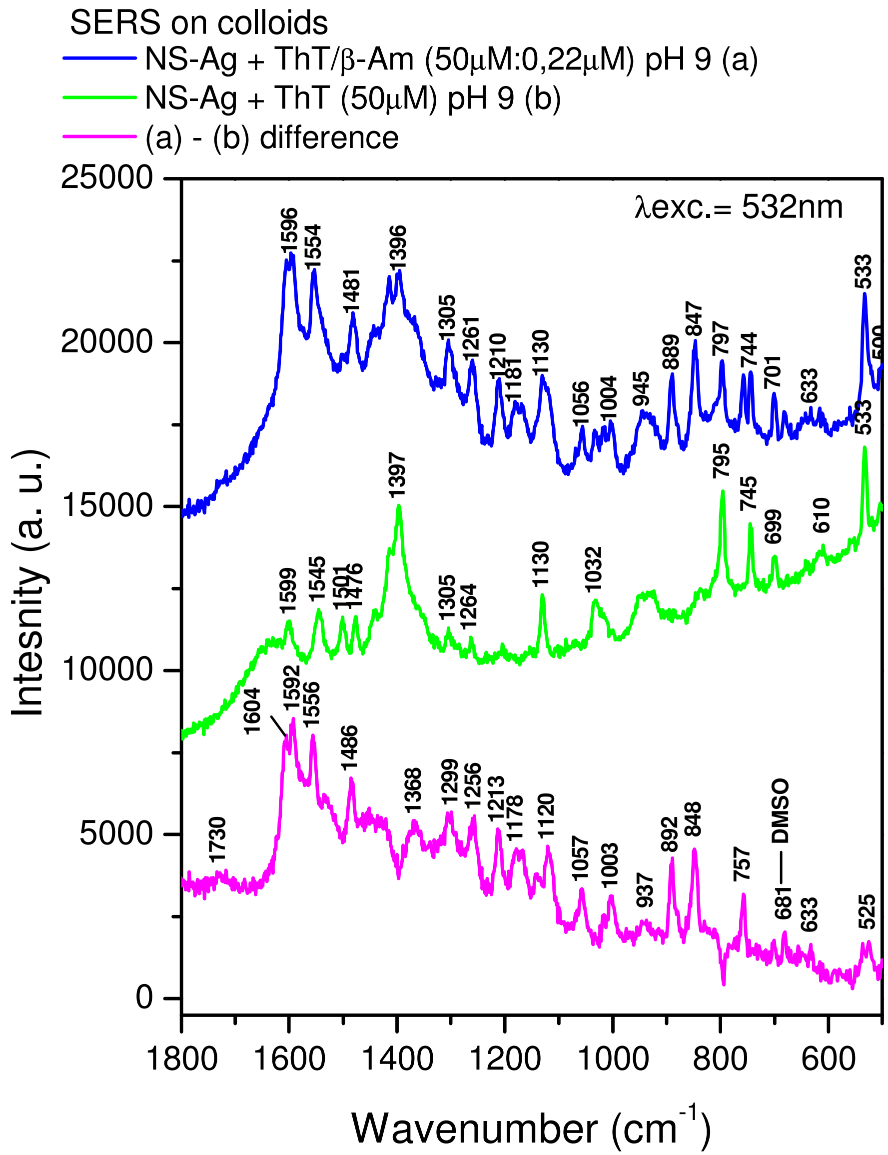

**Thioflavin T**

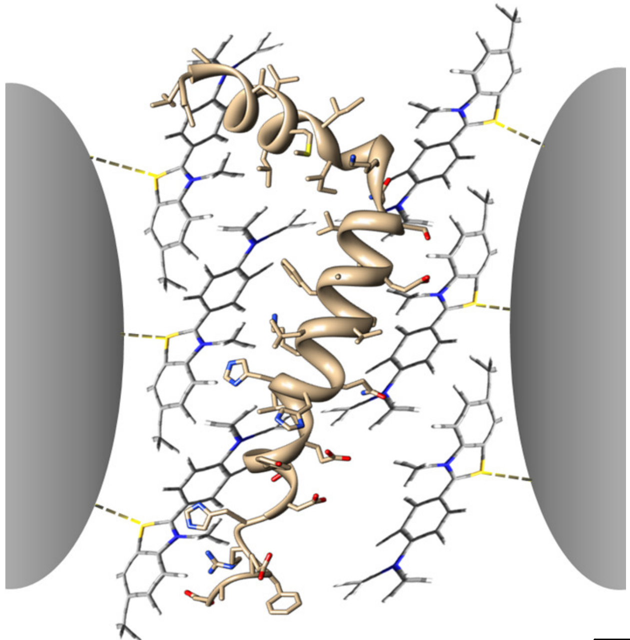


# Absorption spectra of the studied systems



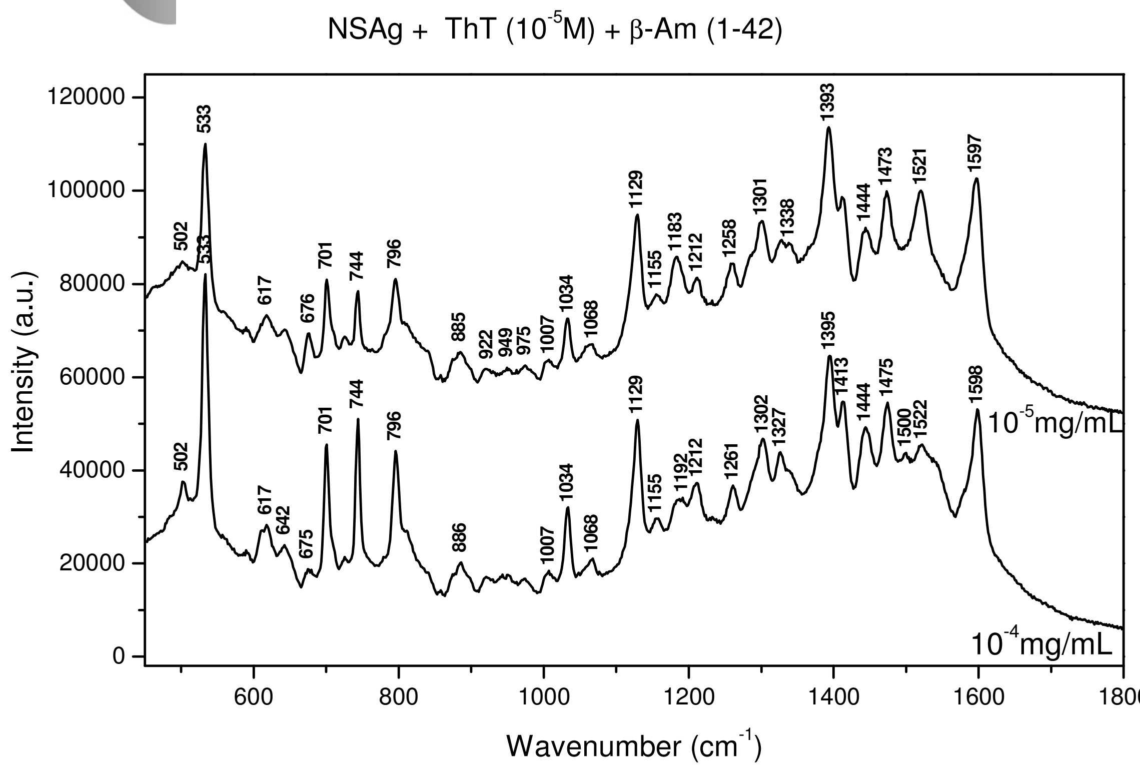




SERS on colloids
NS-Ag + ThT/β-Am (50μM:0,22μM) pH 9 (a)
NS-Ag + ThT (50μM) pH 9 (b)
(a) - (b) difference
λexc.= 532nm
Intesnity (a. u.)
Wavenumber (cm-1)
25000
20000
15000
10000
5000
0
1800
1600
1400
1200
1000
800
600
1596
1554
1481
1396
1305
1261
1210
1181
1130
1056
1004
945
889
847
797
744
701
633
533
1397
1599
1545
1501
1476
1305
1264
1130
1032
795
745
699
610
533
1604
1592
1556
1486
1730
1368
1299
1256
1213
1178
1120
1057
1003
937
892
848
757
DMSO
681
633
525



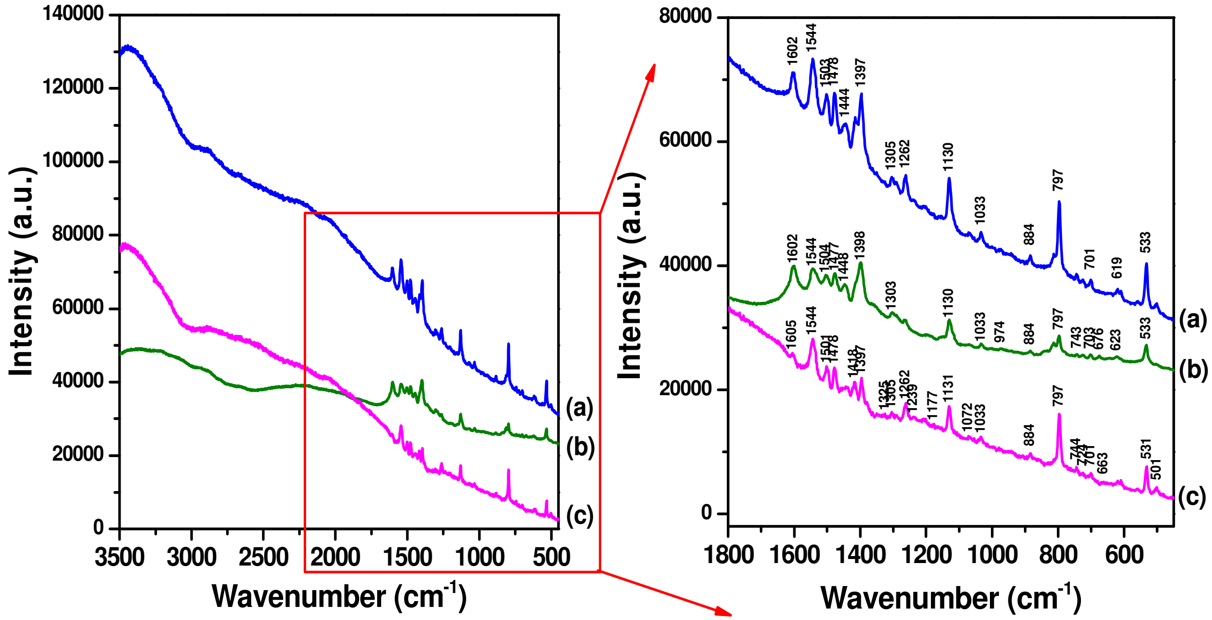
## ThT- β-amyloid complex

The effect of [β-amyloid] on the SERS spectra on Ag nanostars



(a) NSAg + [ThT:β-Am] (25µM:0,22µM) pH 6
(b) NSAg + [ThT] (25µM)
(c) [a-b] Difference

λexc.= 442 nm



# Conclusions

- ✓ A simple method of nanostructure fabrication with **high sensitivity** in SERS technique has been developed.
- ✓ This method allows higher reproducibility in SERS measurements **without aggregation.**
- ✓ Congo Red and Thioflavine T dyes were detected at **low concentrations**.
- ✓ The adsorption isotherm of ThT over nano-stars follows a **Langmuir model**
- ✓ **SERS of β-amyloid** peptide has been obtained through its interaction with ThT.

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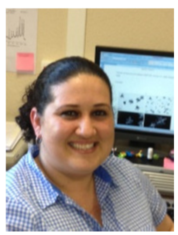
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# Acknowledgements

**Ministerio de Economía y Competitividad**

**Project FIS2010-15405**

EUROPEAN SOCIAL FUND

*Grant: JAE-Pre 2011 (A.G-L.)*