Detection and structure characterization of proteins by surface enhanced Raman spectroscopy

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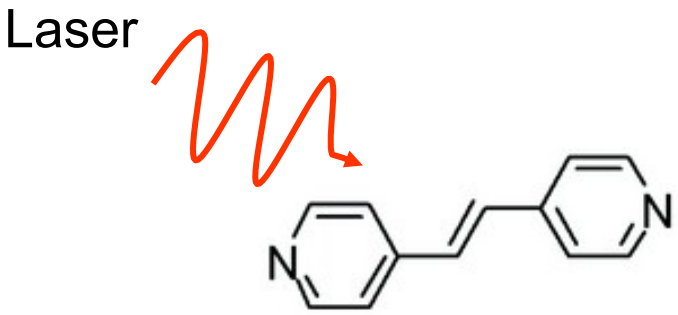
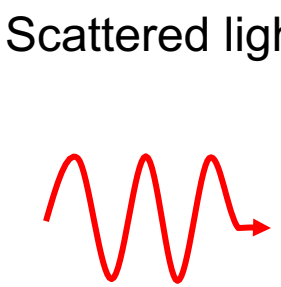
Outline

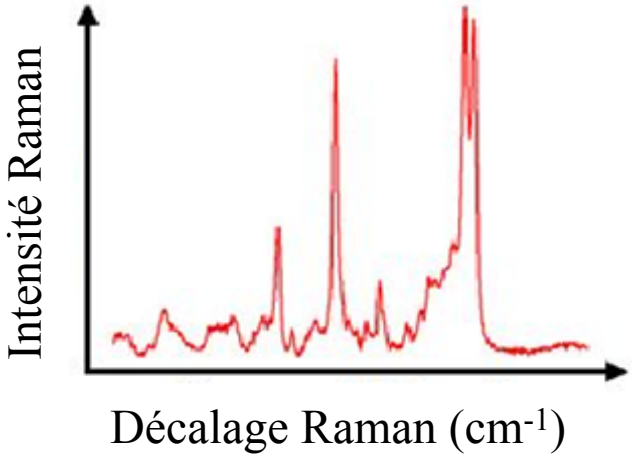
- Introduction
  - Raman and SERS spectroscopy
  - Substrates
- SERS application
  - Protein detection
    - Identification
    - Specific detection
    - Detection in real sample (serum and saliva)
  - Characterization of protein structure
    - Impact of mutation
    - Phosphorylation effect
- Conclusion

Raman spectroscopy

- Vibrational spectroscopy
- Non destructive technic
- one molecule = one spectrum

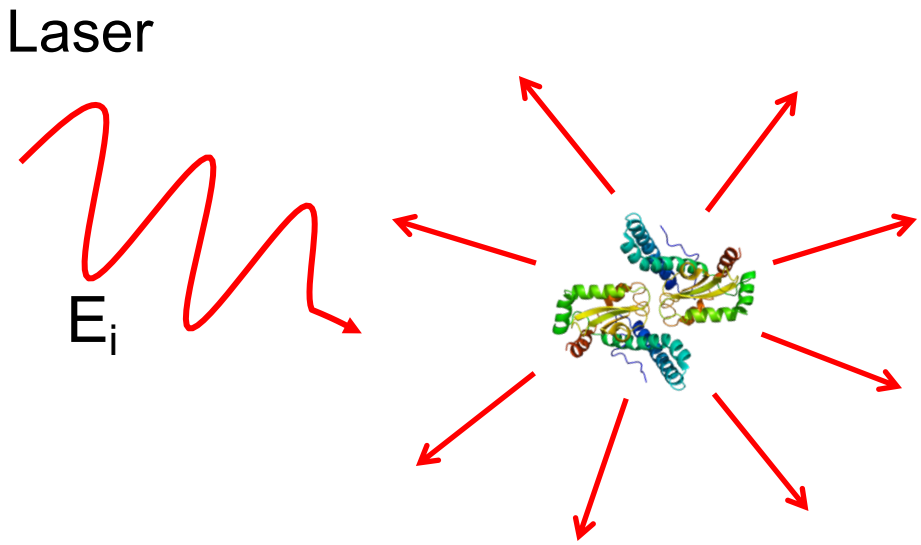






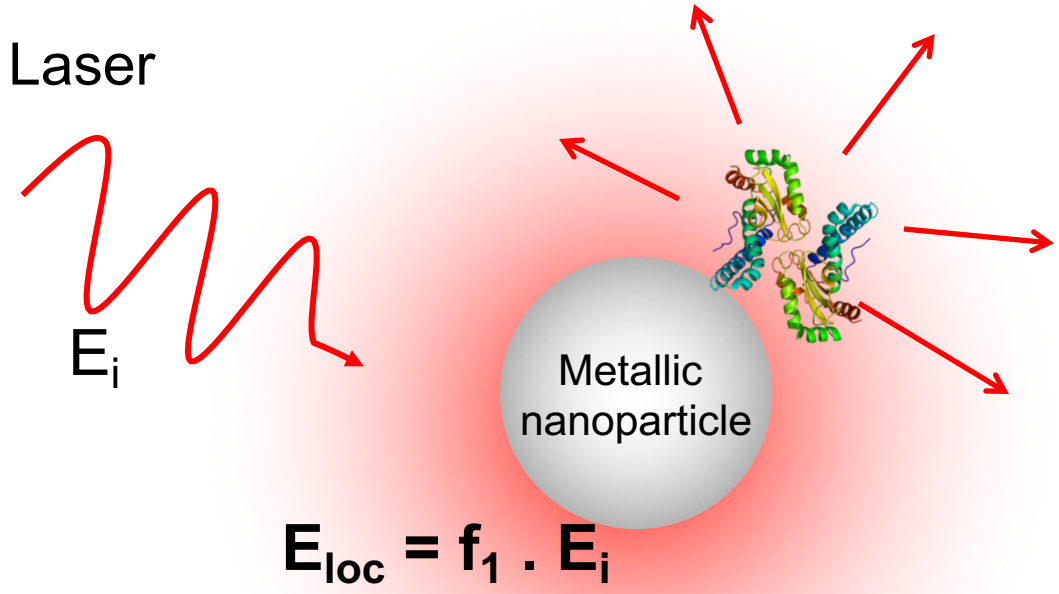
Raman intensity is very weak => need to enhance the signal

Surface enhanced Raman scattering



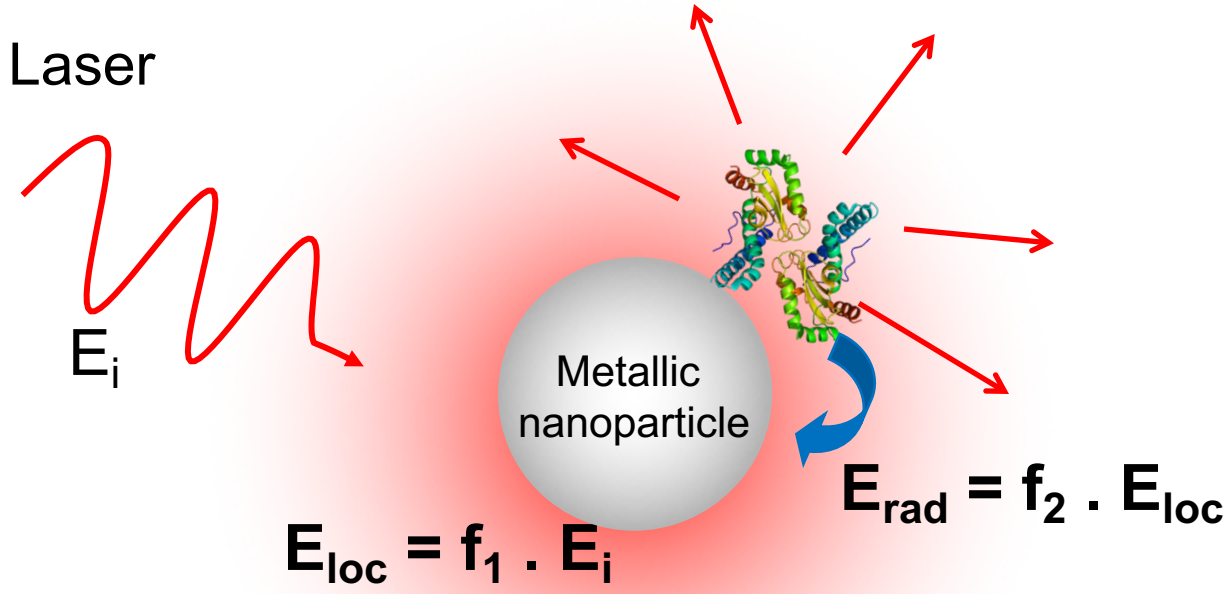
$$E_{Raman} \propto E_i$$
$$I_{Raman} \propto I_i$$

Surface enhanced Raman scattering



$$E_{SERS} \propto f_1 . E_i$$
$$I_{SERS} \propto f_1^2 . I_i$$

Surface enhanced Raman scattering

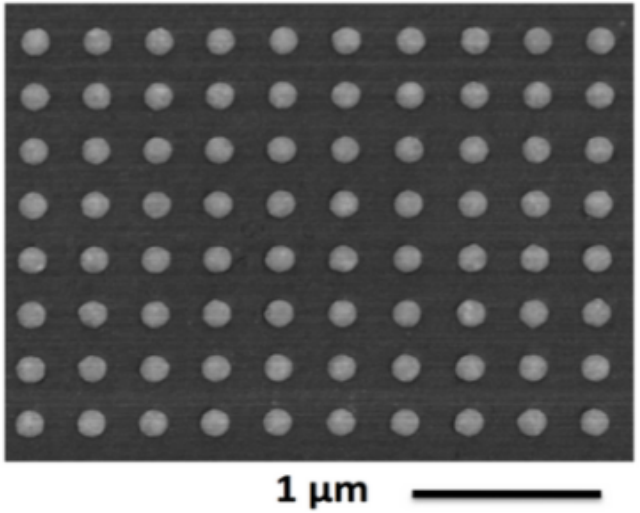


$$E_{SERS} \propto f_1 . f_2 . E_i$$
$$I_{SERS} \propto f_1^2 . f_2^2 . I_i$$

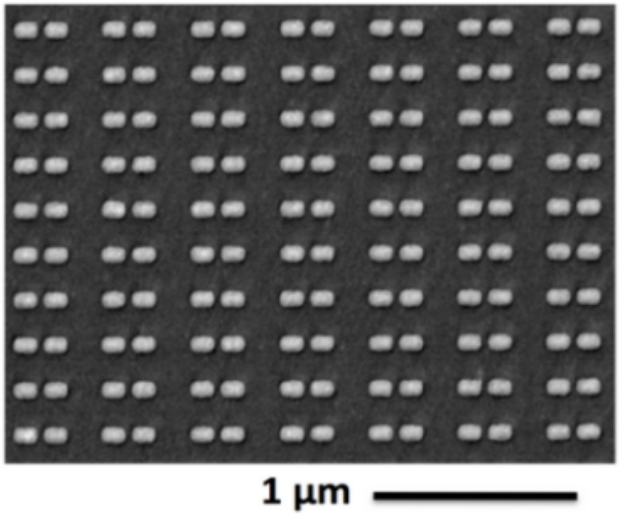
SERS substrates

- Gold nanostructures produced by electron-beam lithography (EBL) :

nanocylinders

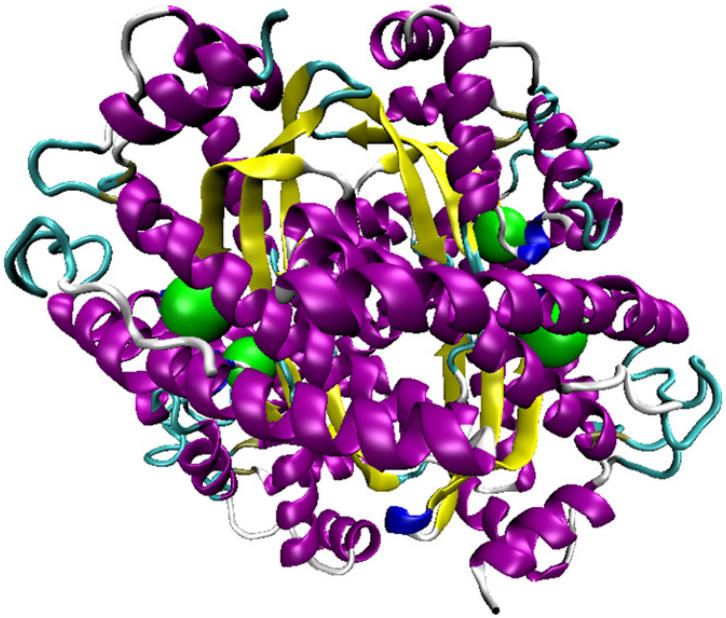


coupled nanorods



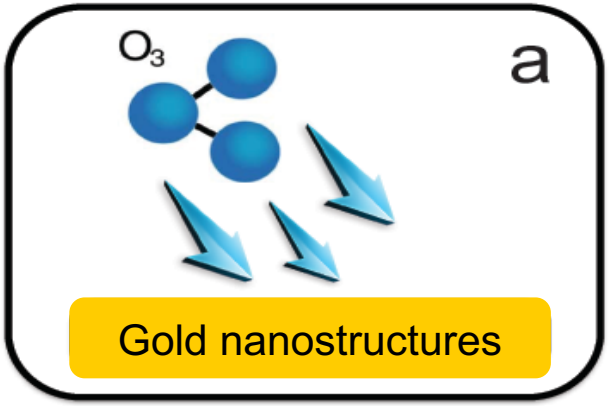
- Laser wavelength : 660 nm and 785 nm

Detection of protein : Manganese SuperOxide Dismutase (MnSOD)



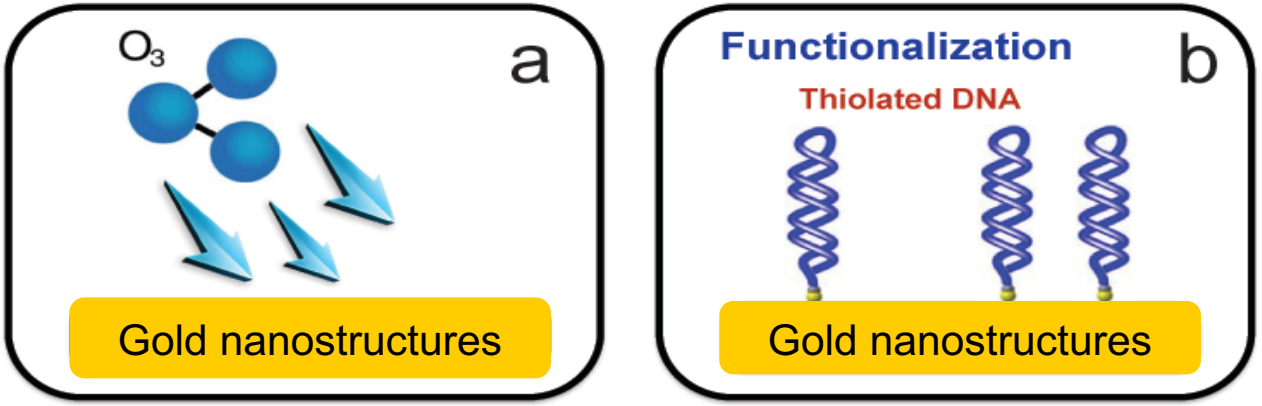
Cottat M. et al, JPCC, 2015

Gold surface functionnalization



Gold surface

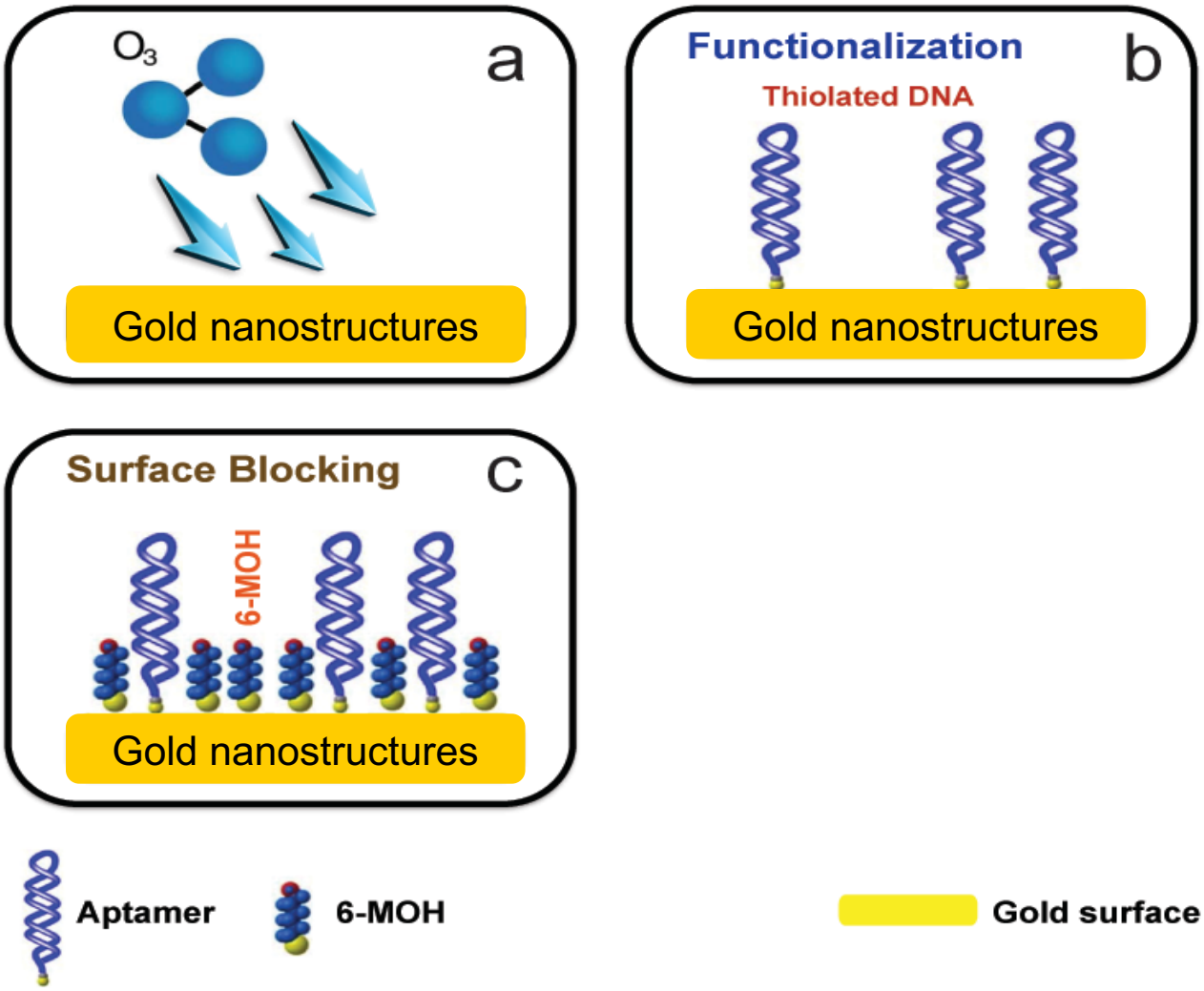
Gold surface functionnalization



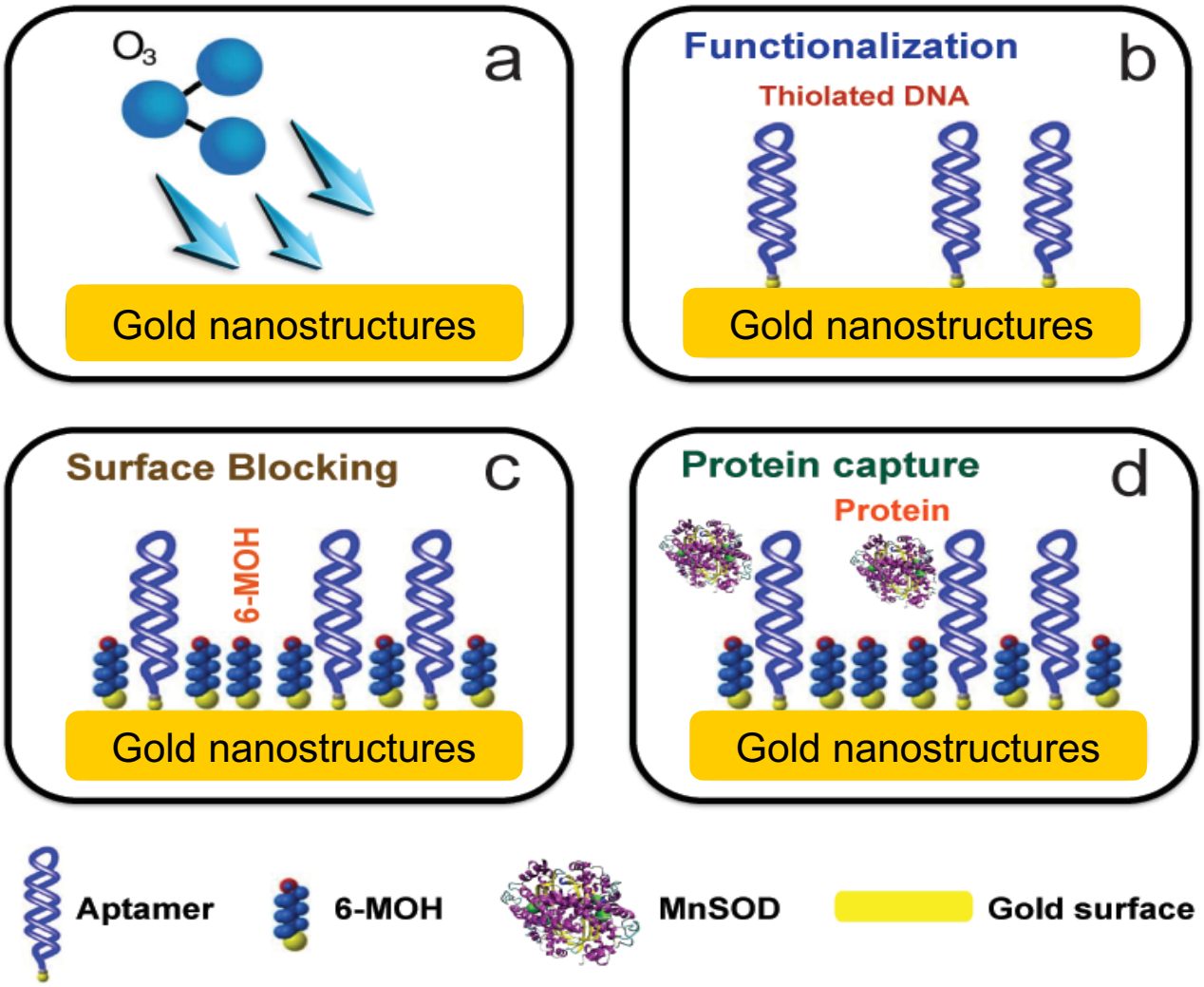
Aptamer

Gold surface

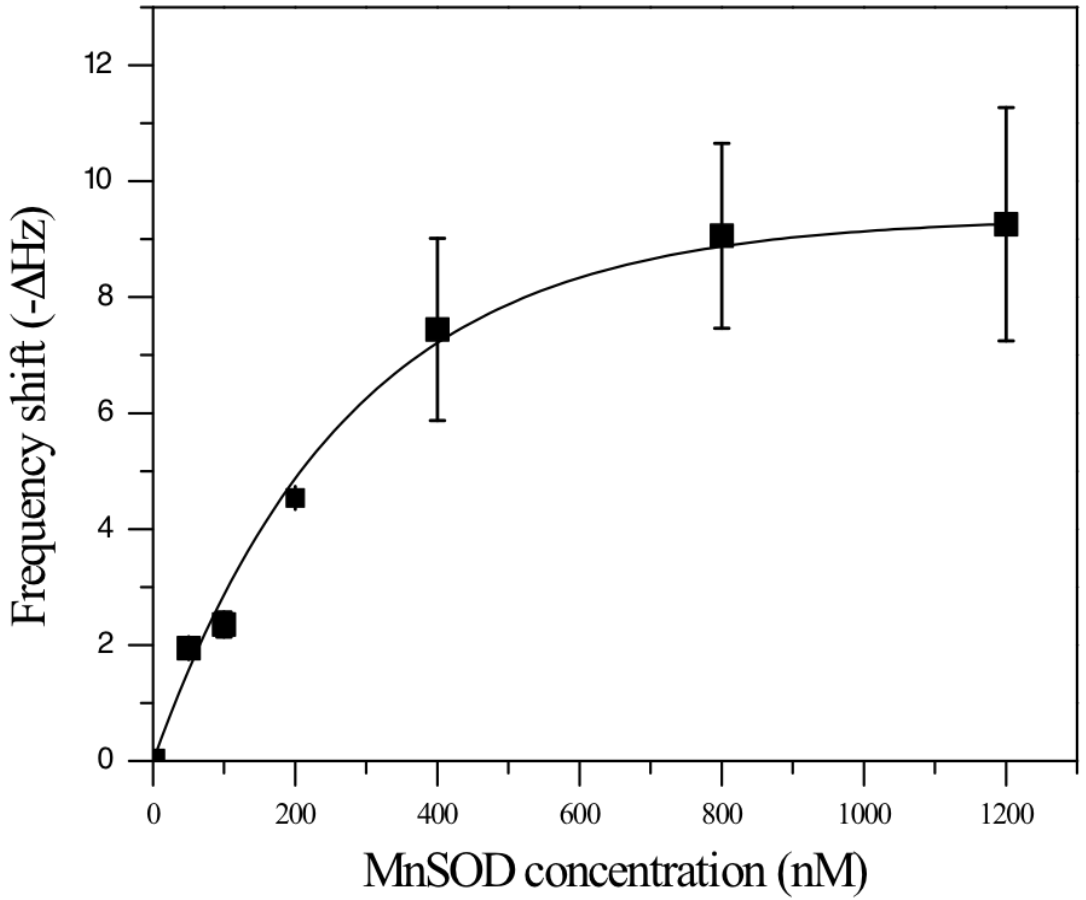
Gold surface functionnalization



Gold surface functionnalization

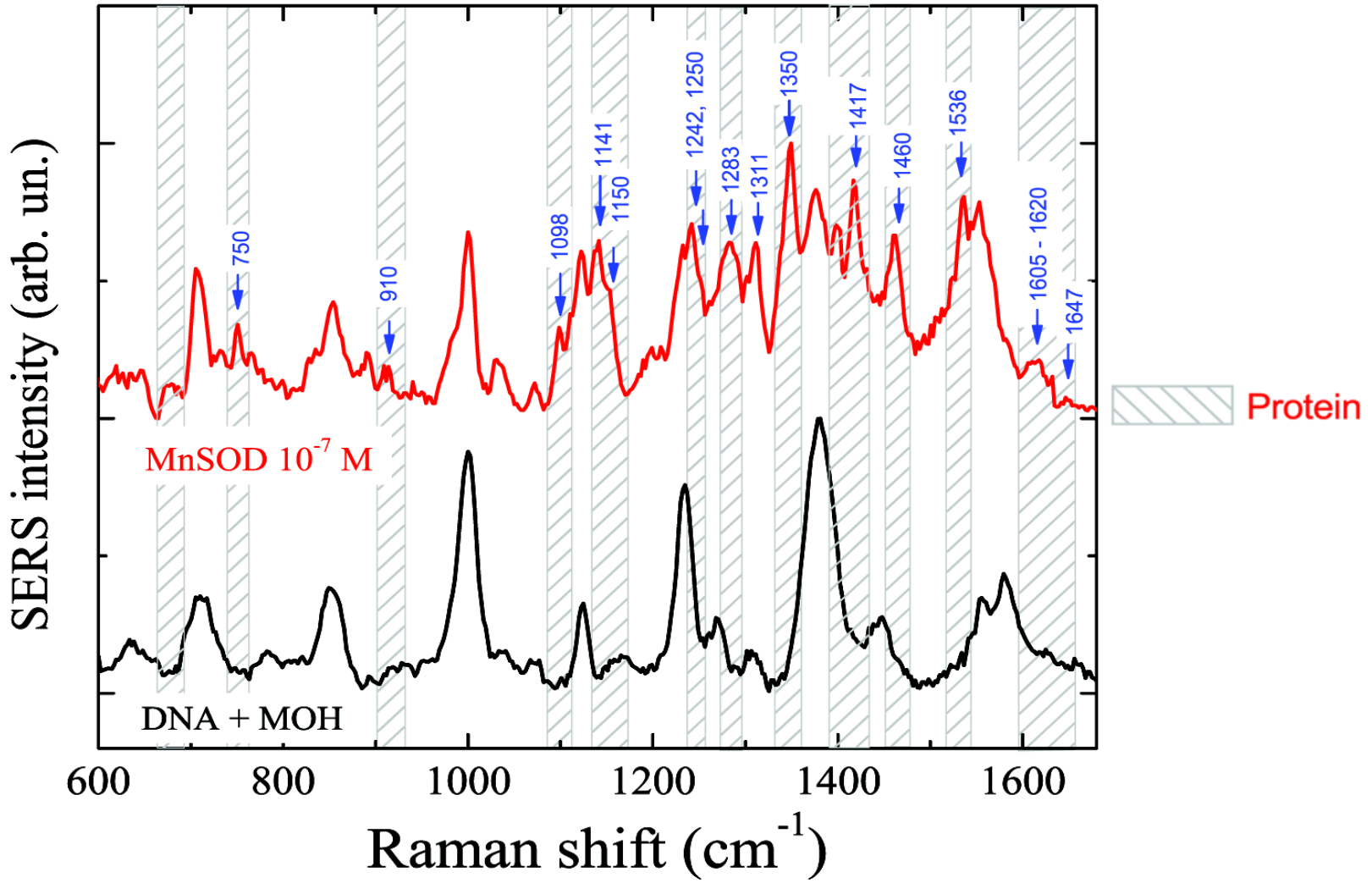


Aptamer-MnSOD affinity (by QCM)

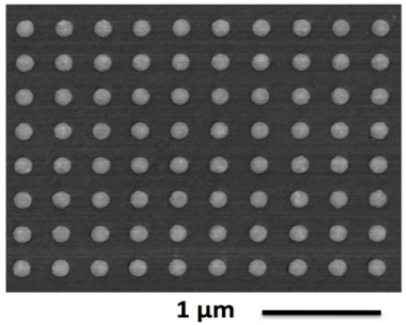


K_D (dissociation constant) : 313.10^{-9} M => strong affinity

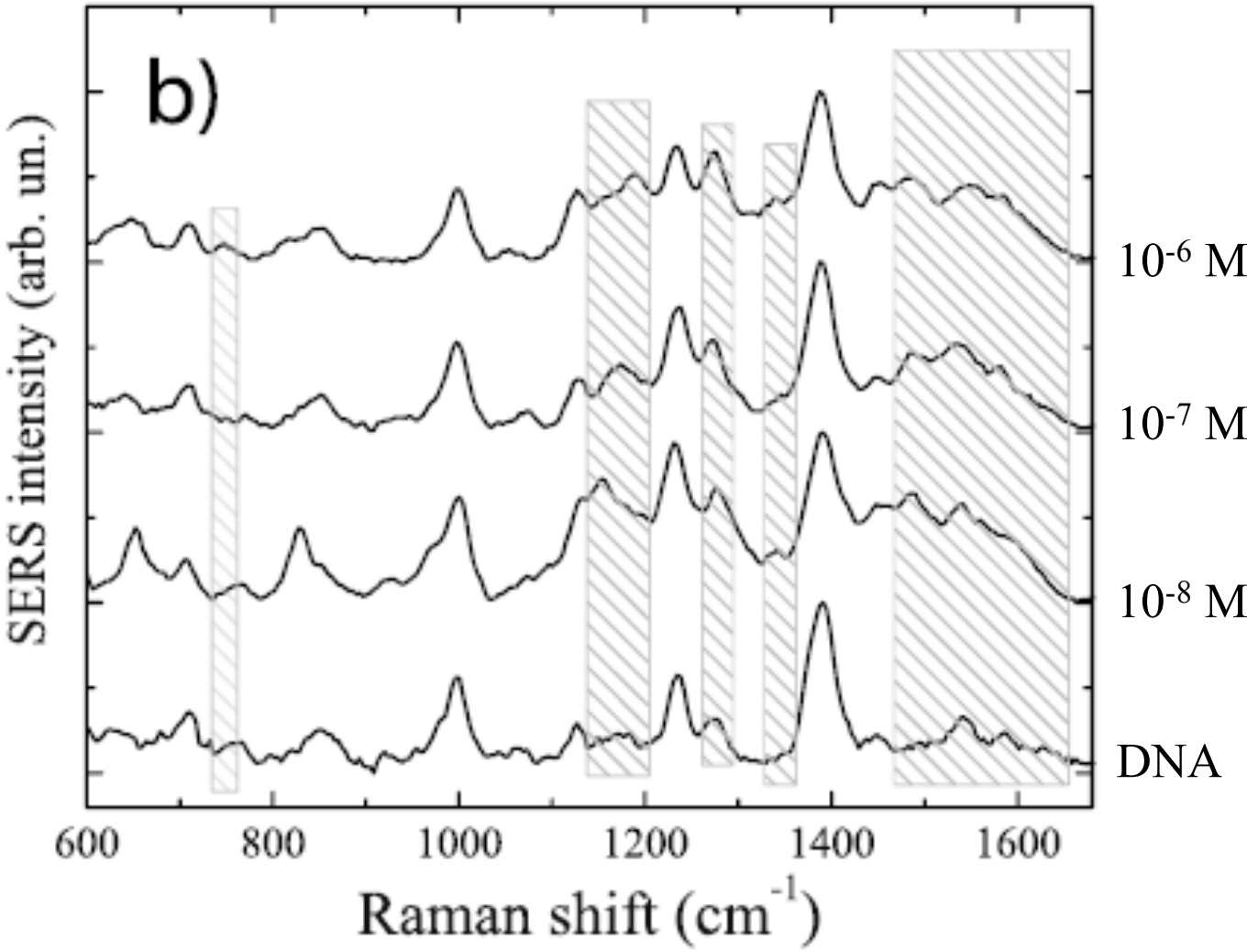
SERS detection of MnSOD



Concentration study (nanocylinders)

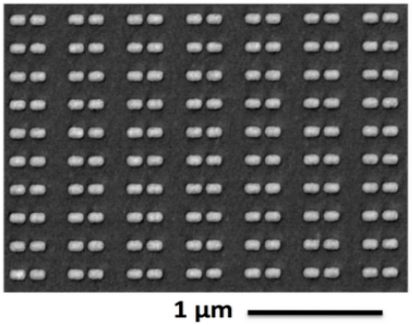


ϕ = 130 nm
λ = 660nm

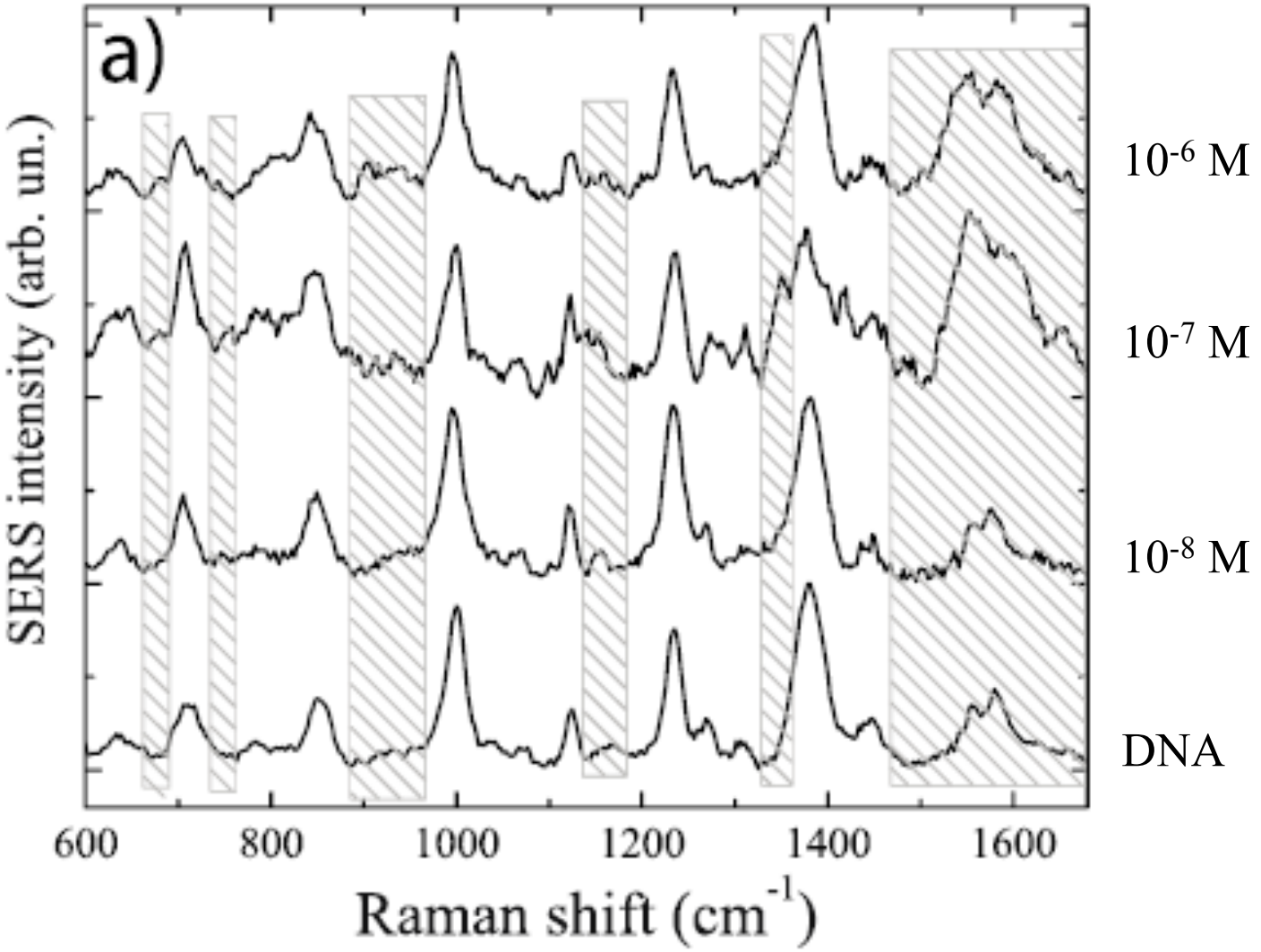


MnSOD detection from 10^{-8} M

Concentration study (nanorods)

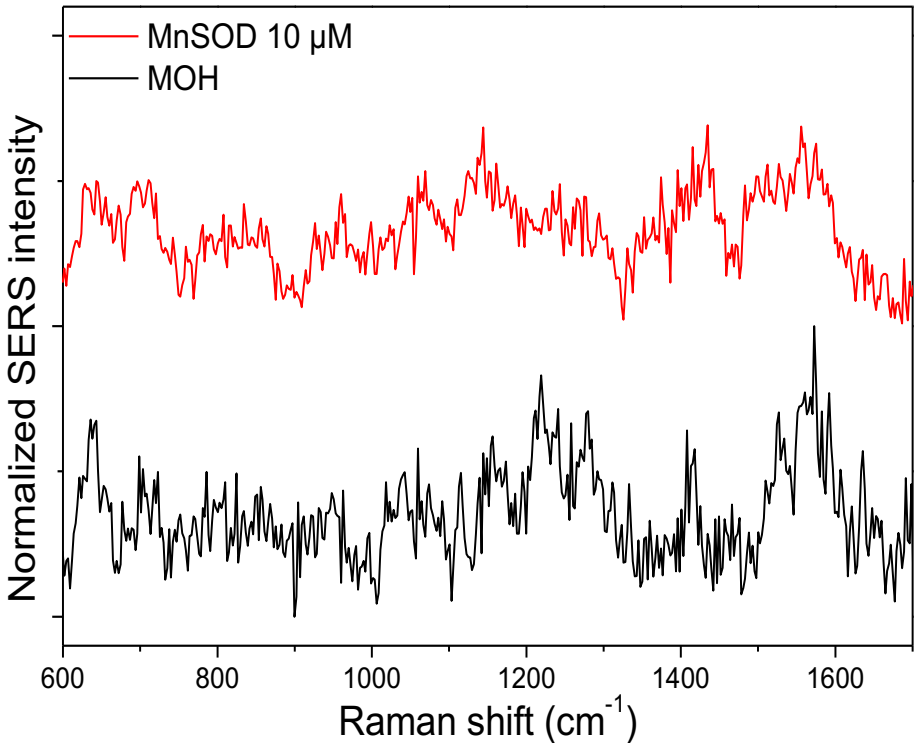


λ = 785nm



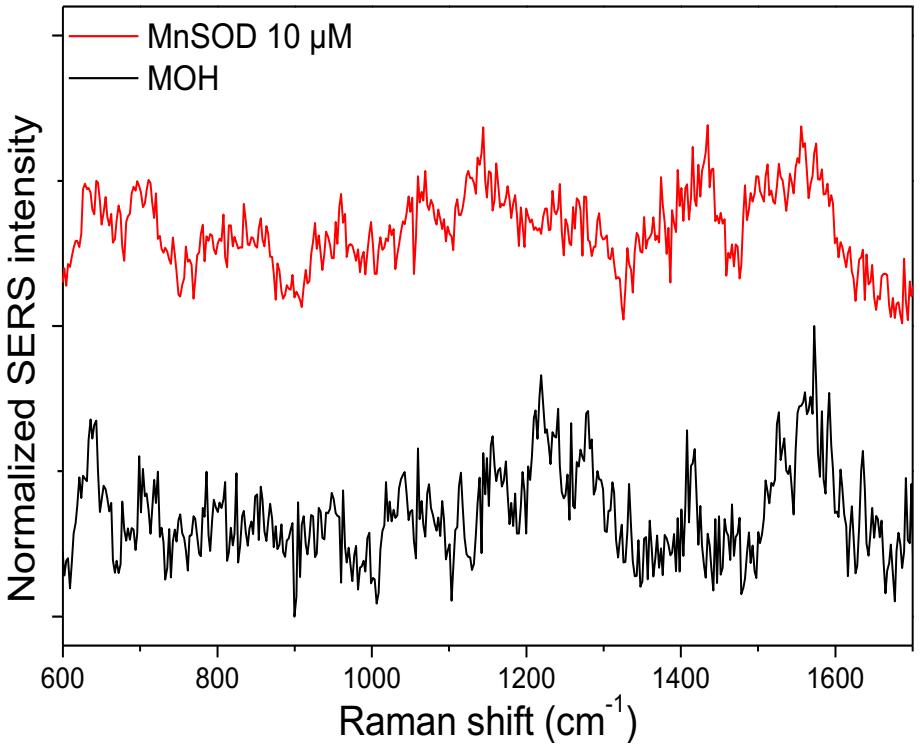
MnSOD detection from 10^{-7} M

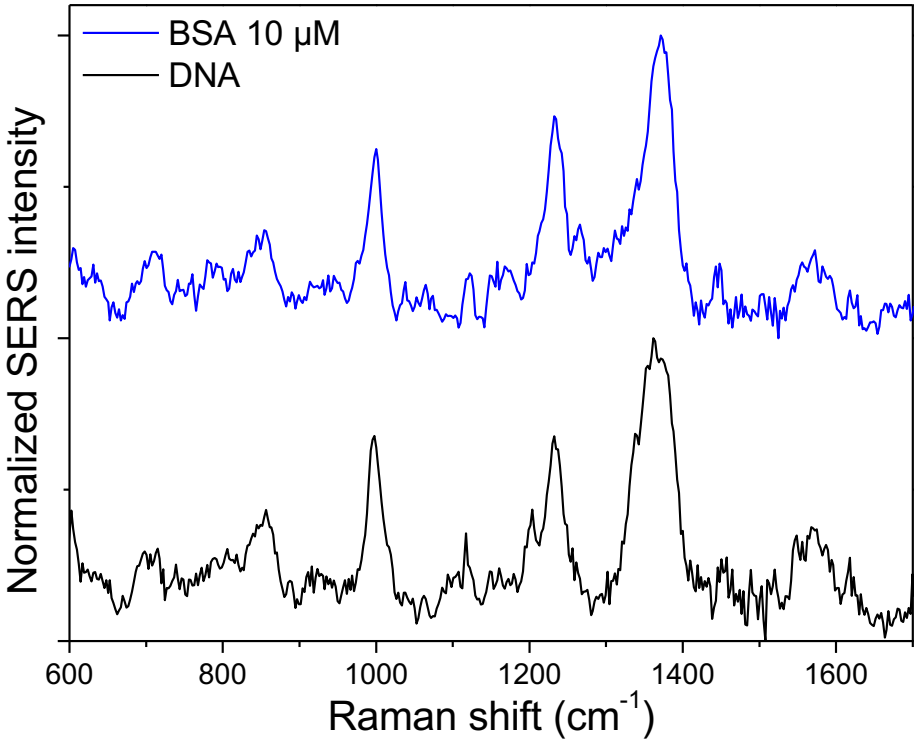
Specificity test



No binding between MnSOD and MOH

Specificity test



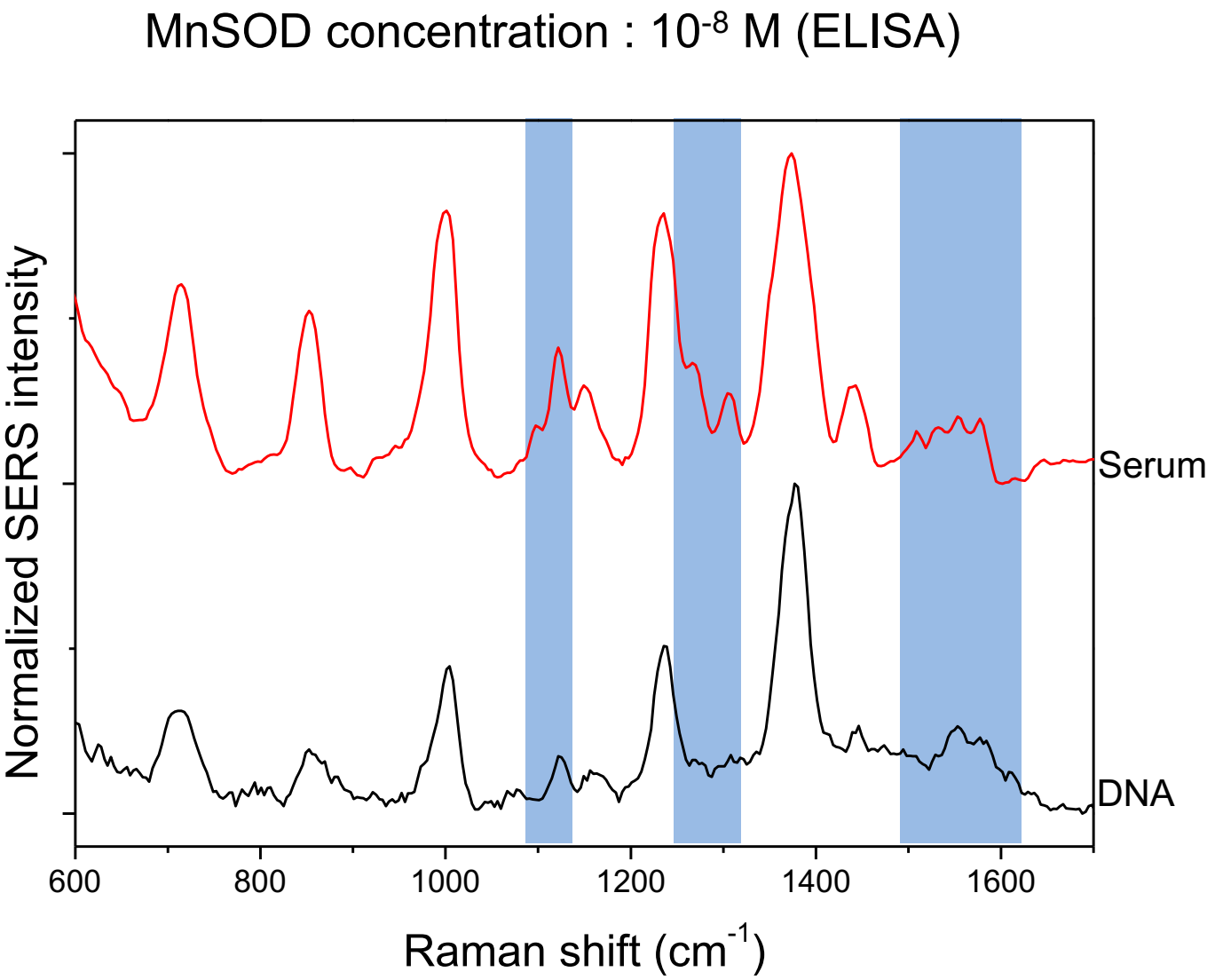


No binding between MnSOD and MOH

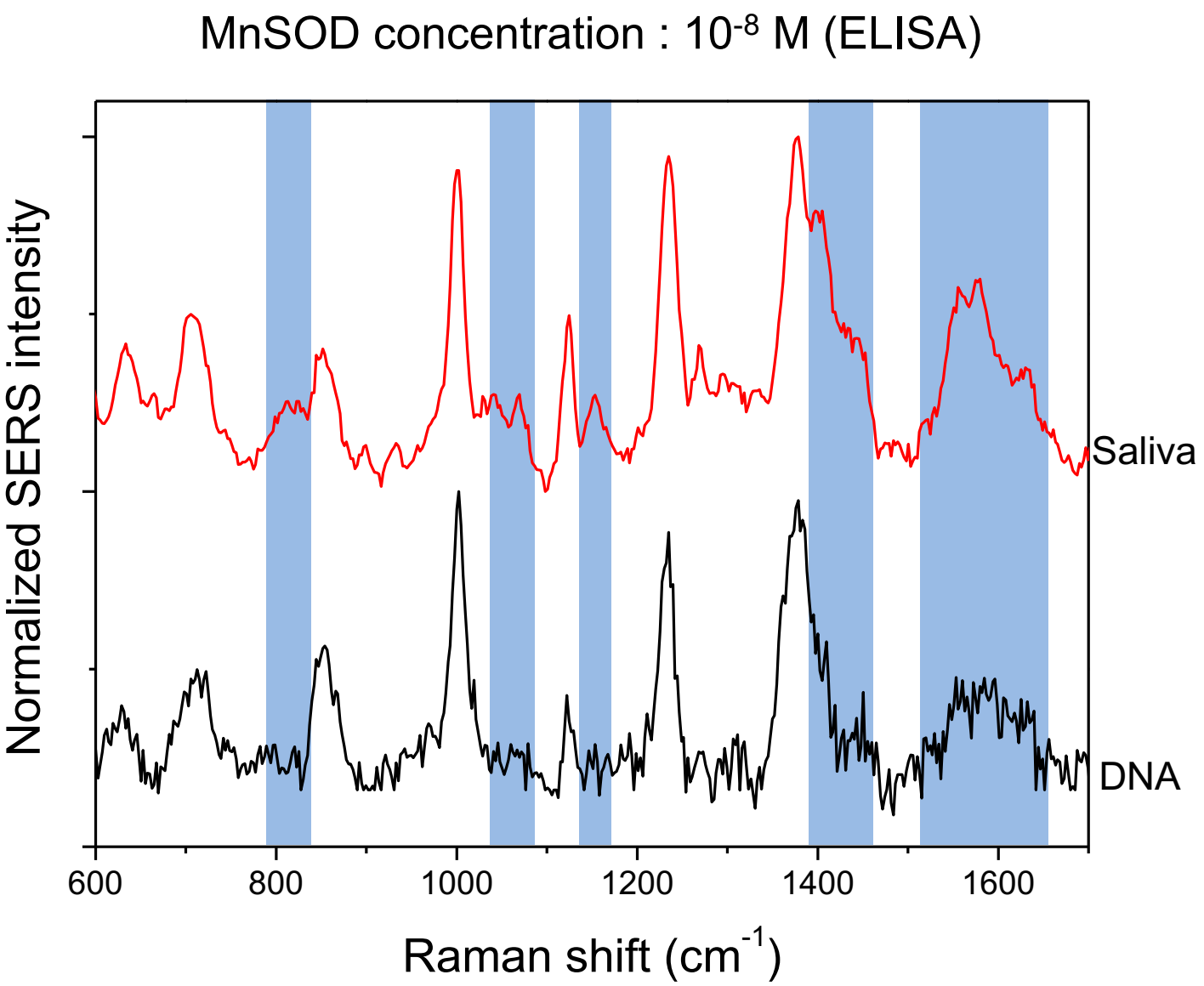
BSA does not bind to aptamer

Biosensor : specific detection of MnSOD

MnSOD detection in serum



MnSOD detection in saliva



Biosensor characteristic

- MnSOD detection from 10 nM.

Biosensor characteristic

- MnSOD detection from 10 nM.
- Specific interaction between MnSOD and aptamer

Biosensor characteristic

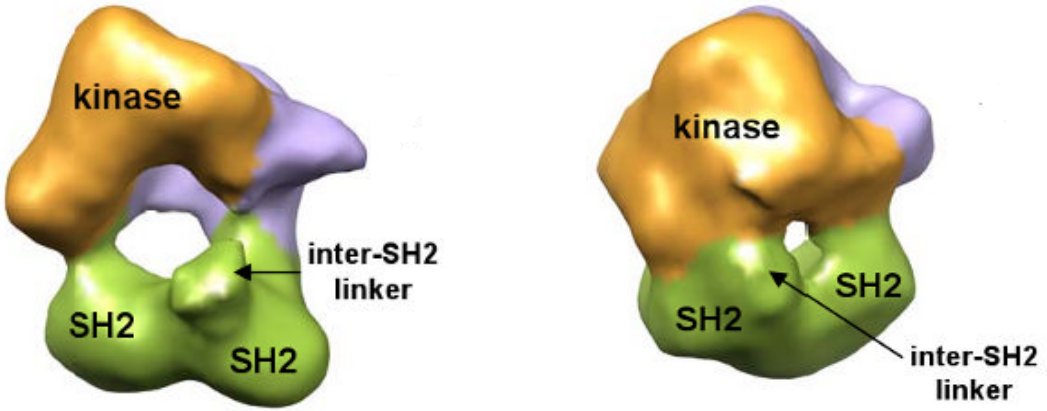
- MnSOD detection from 10 nM.
- Specific interaction between MnSOD and aptamer
- **Detection of protein of interest in physiocological media (serum and saliva).**

Structure characterization of Spleen tyrosine kinase (Syk) protein

Cottat M. et al, Scientific Report, 2017

Syk structures

Model obtained by electronic microscopy

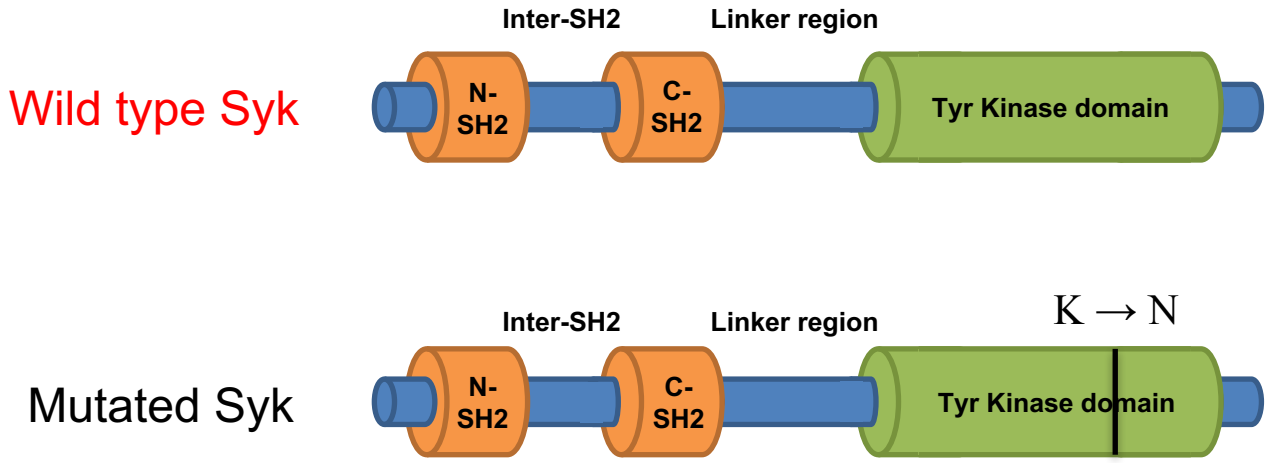


Arias-Palomo et al., Biochim Biophys Acta, 2009

Syk pSyk

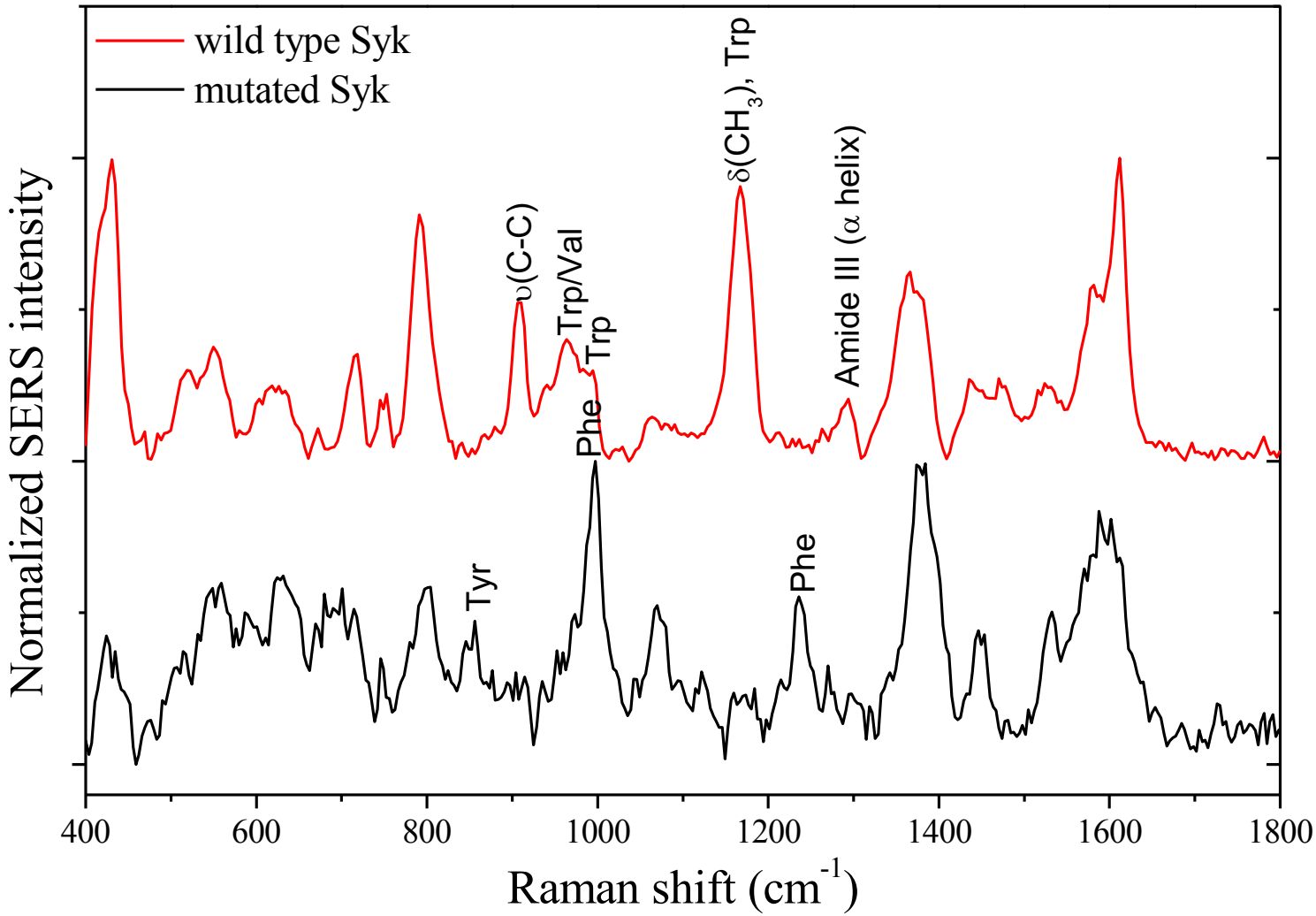
SERS analysis of sequence modifications :
- mutation
- phosphorylation

Mutation effect on Syk



Kinase domain mutated => ATP site affected => inactive

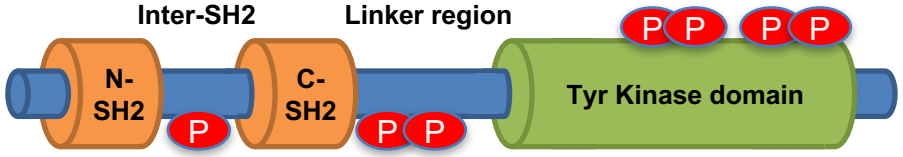
Mutation effect on SERS spectrum



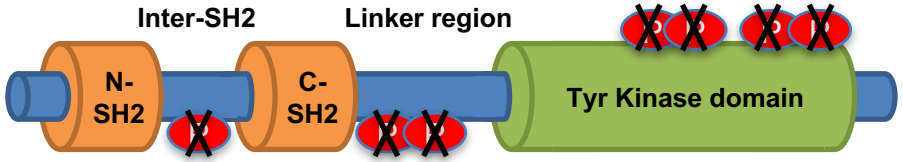
Mutation => different SERS spectra => structure modification

Phosphorylation impact

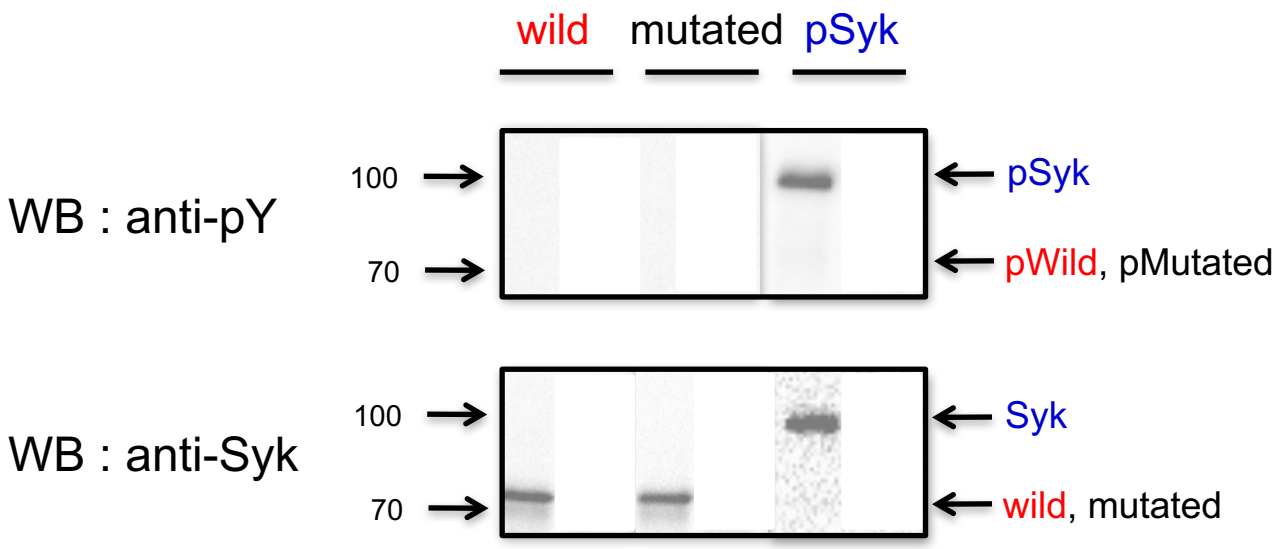
Active phosphorylated Syk



Unphosphorylated Syk

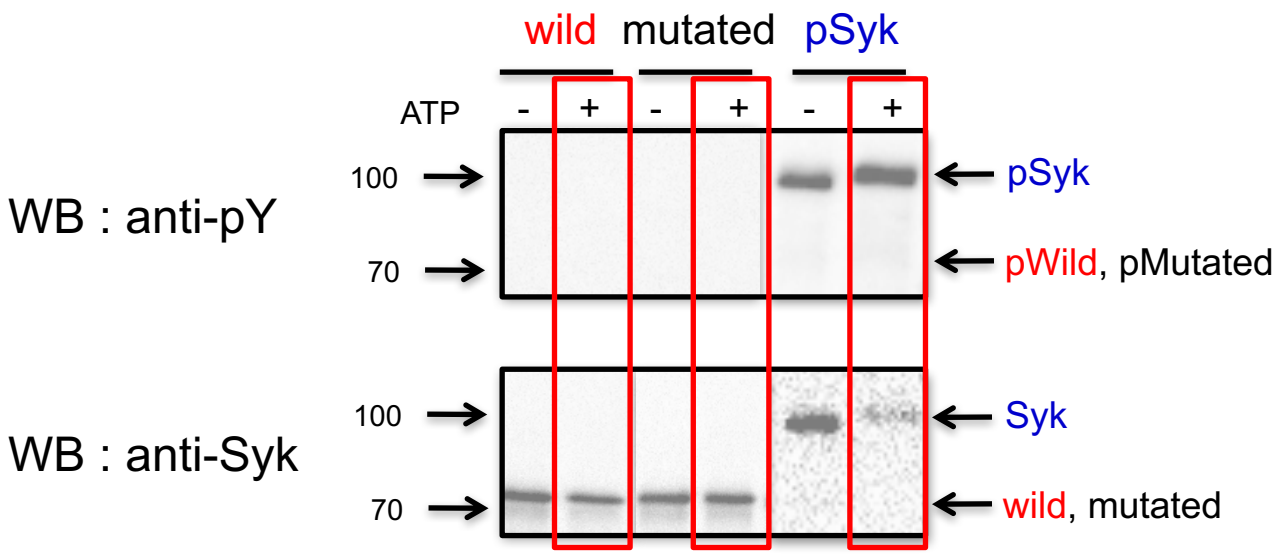


Phosphorylation level



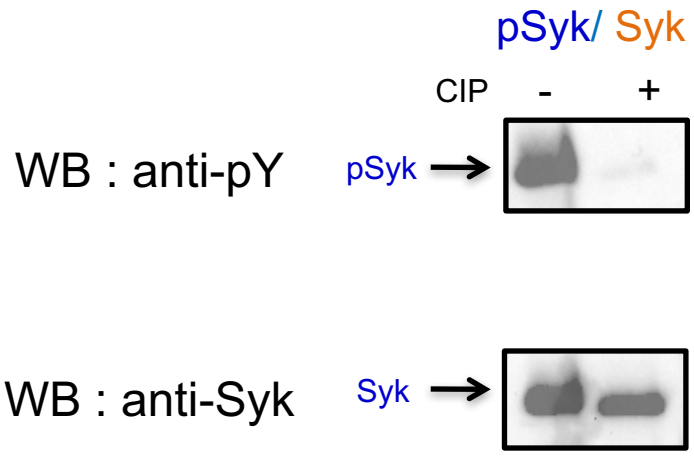
wild and mutated: unphosphorylated
pSyk : highly phosphorylated

Kinase activity



wild and mutated : unphosphorylated=> inactive
pSyk : highly phosphorylated=> active

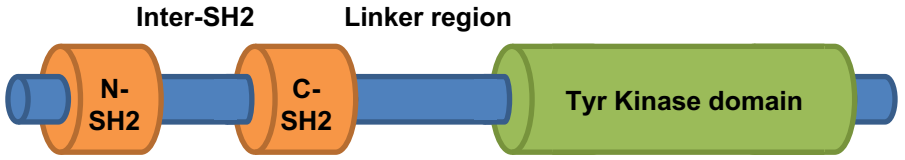
Phosphorylation fluctuation



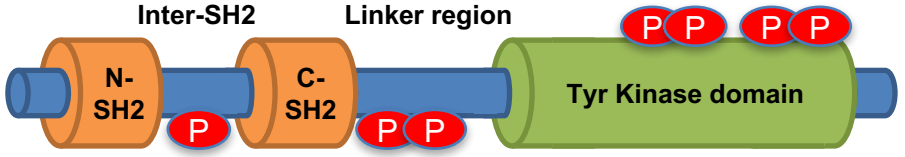
phosphatase CIP (Syk) => decrease of the phosphorylation level

Impact of phosphorylation on SERS spectrum

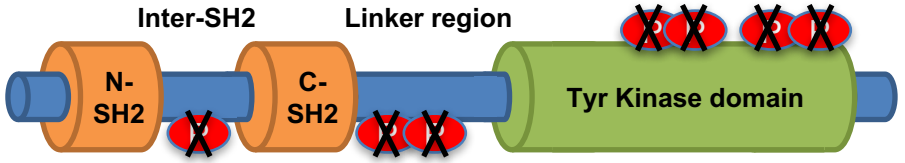
Unphosphorylated Syk, inactive



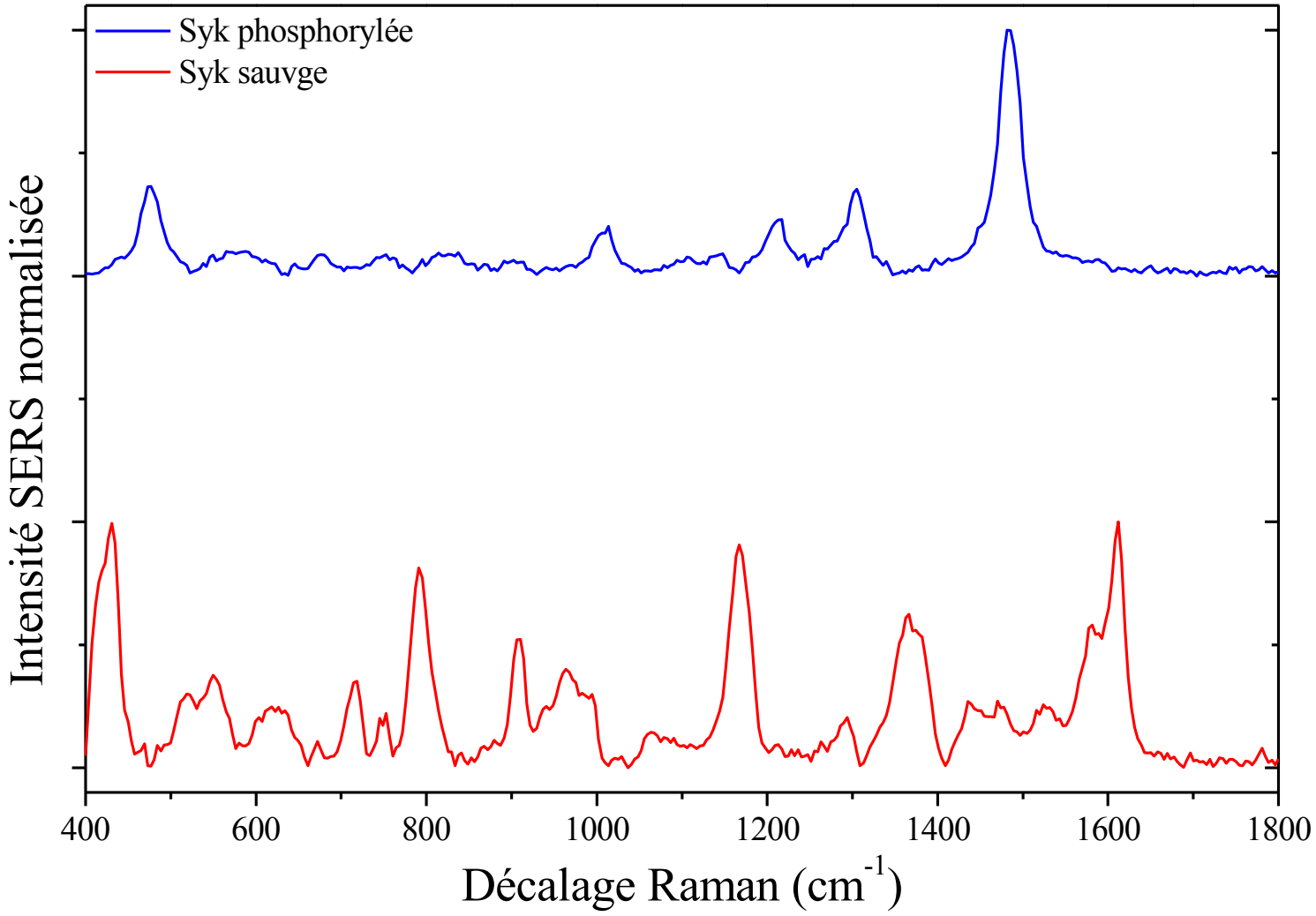
Phosphorylated Syk, active



Syk dephosphorylated

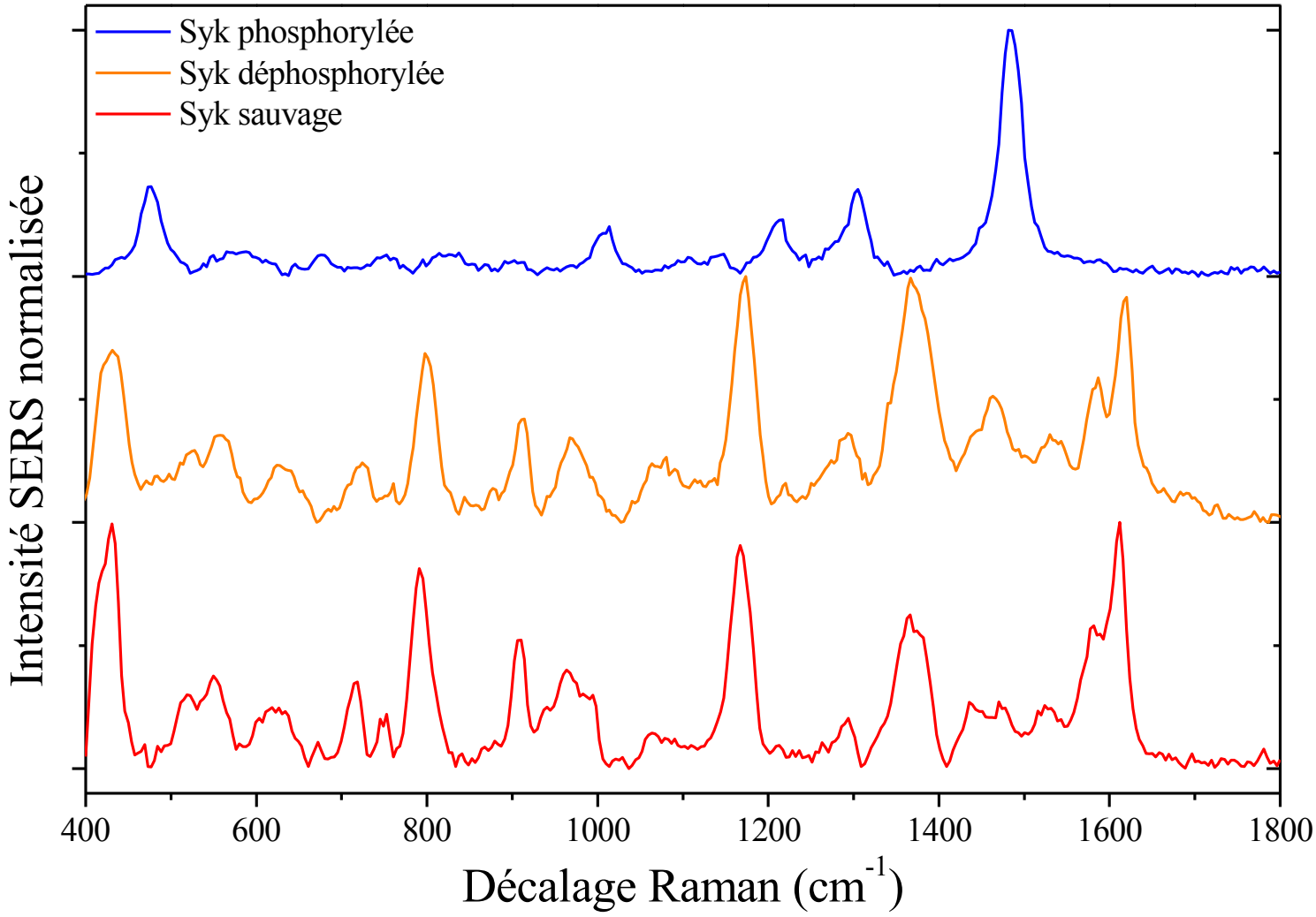


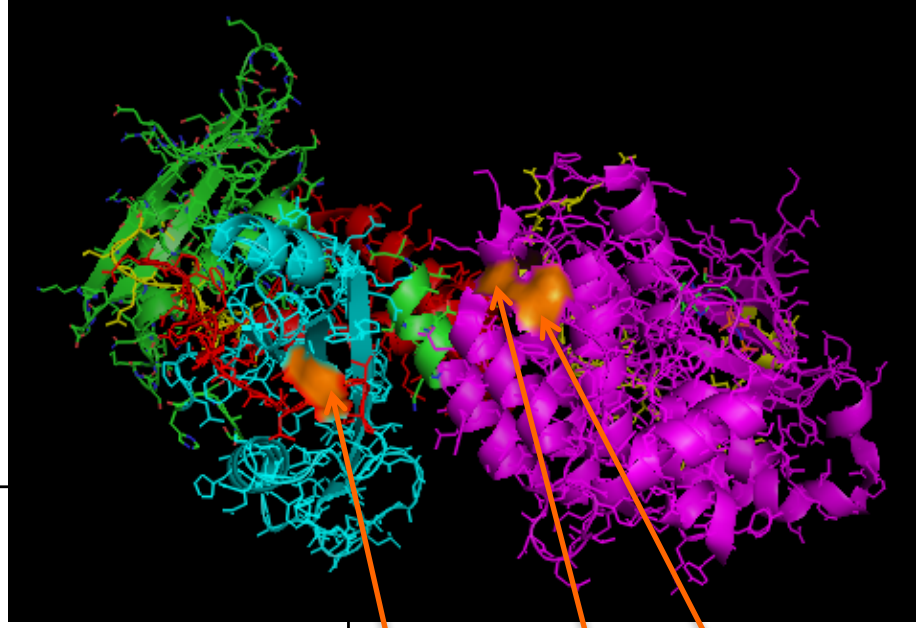
SERS spectra of various Syk forms

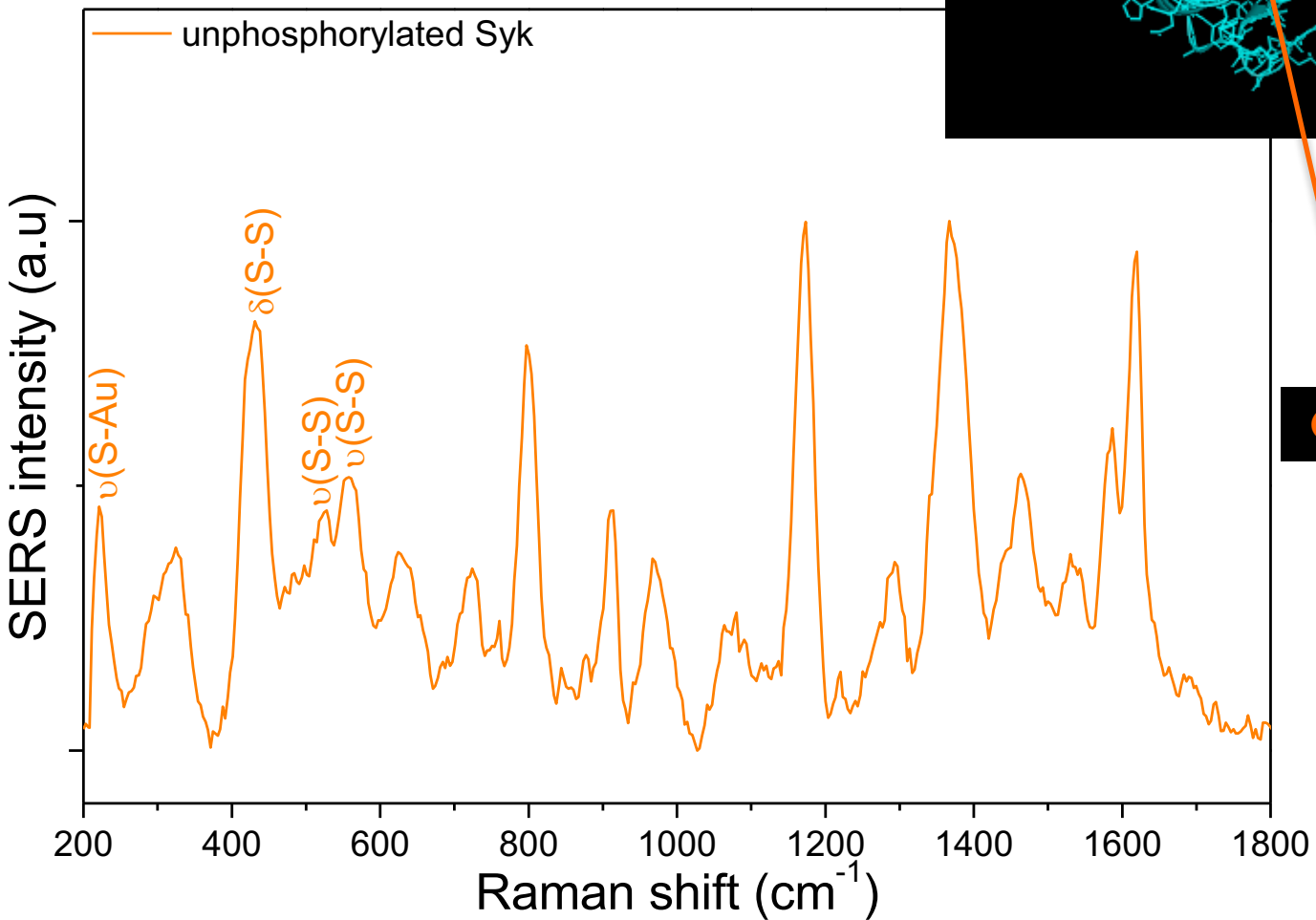


Different spectra signature: different protein structure

SERS spectra of various Syk forms

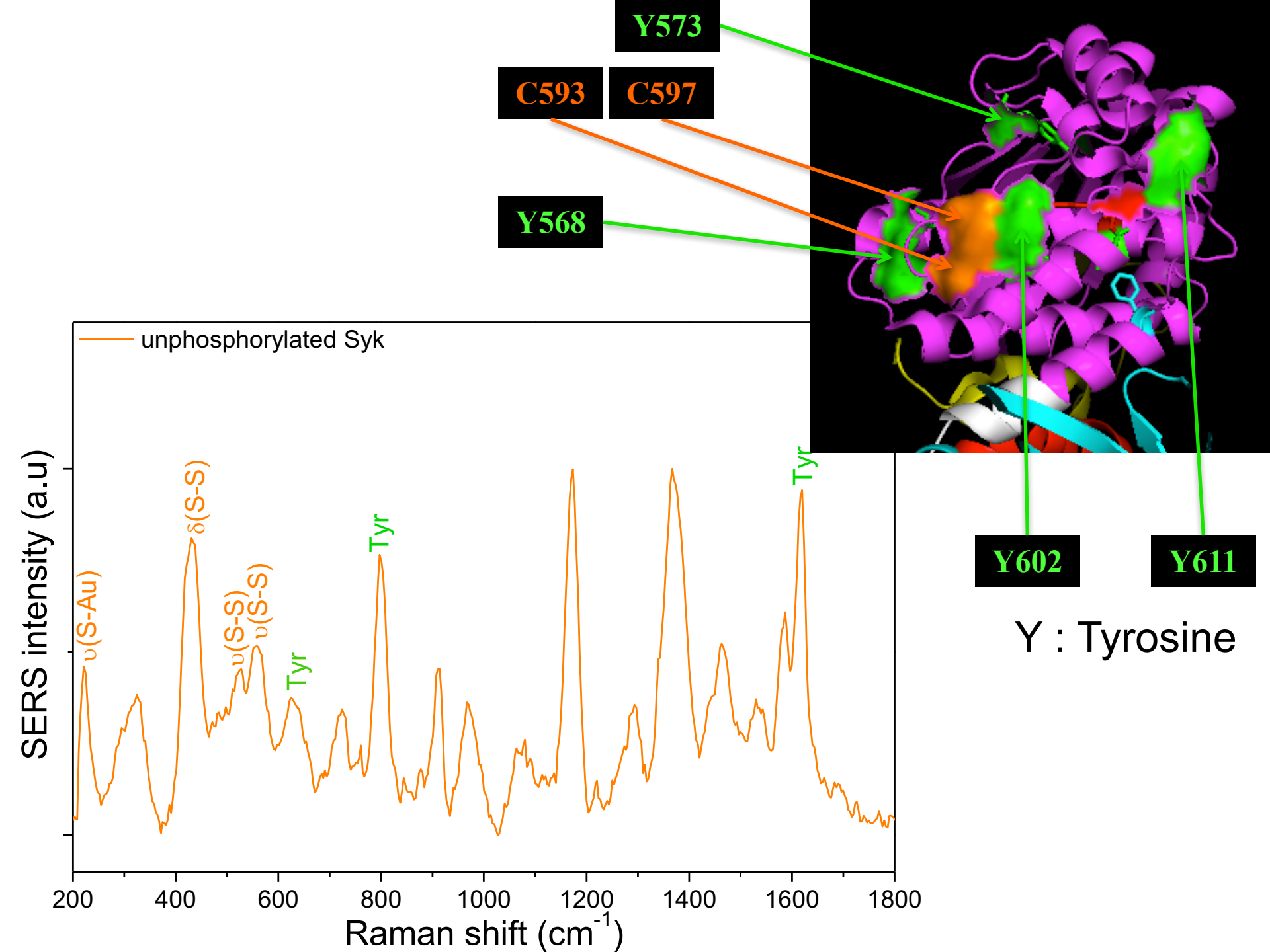


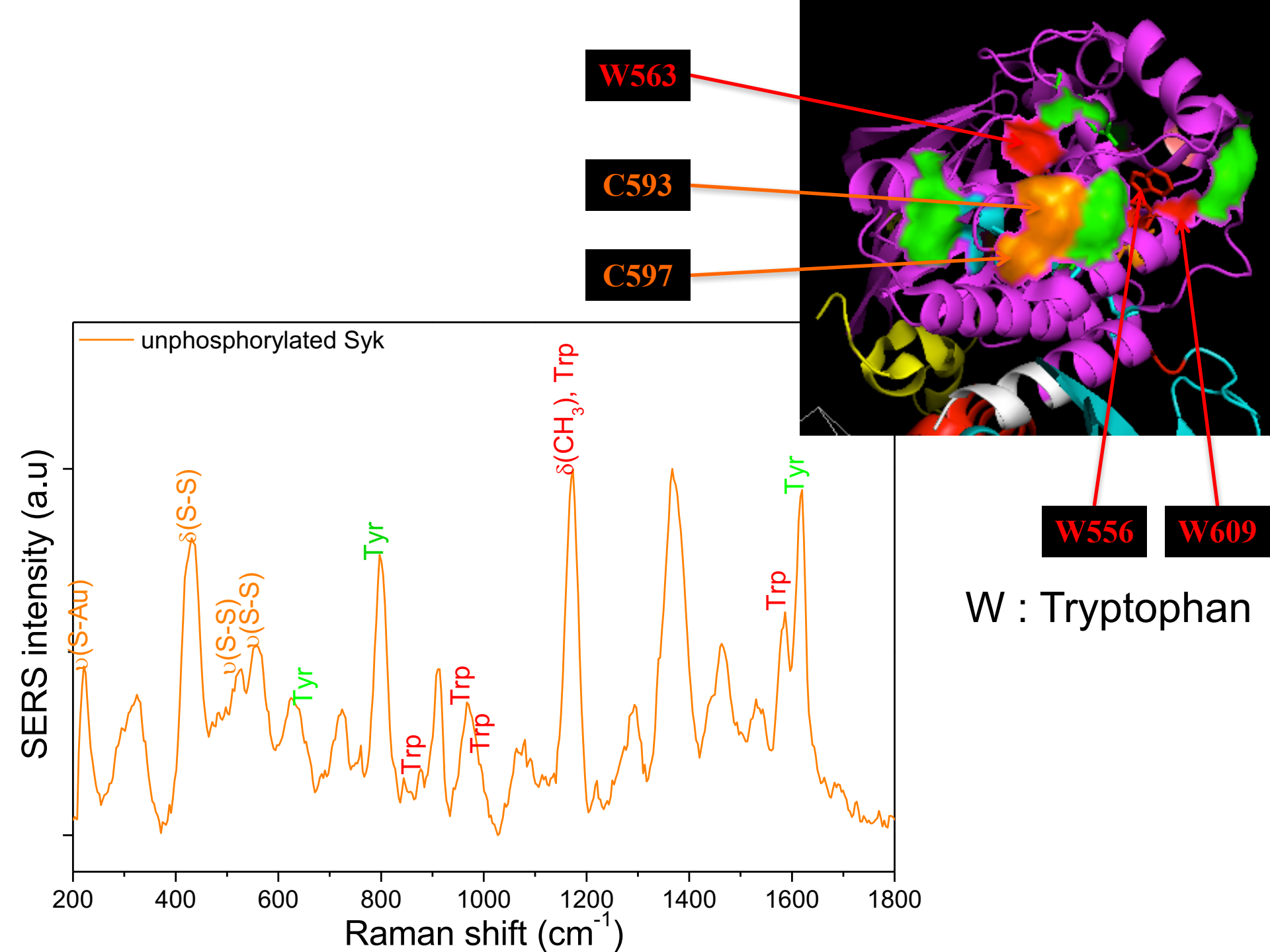


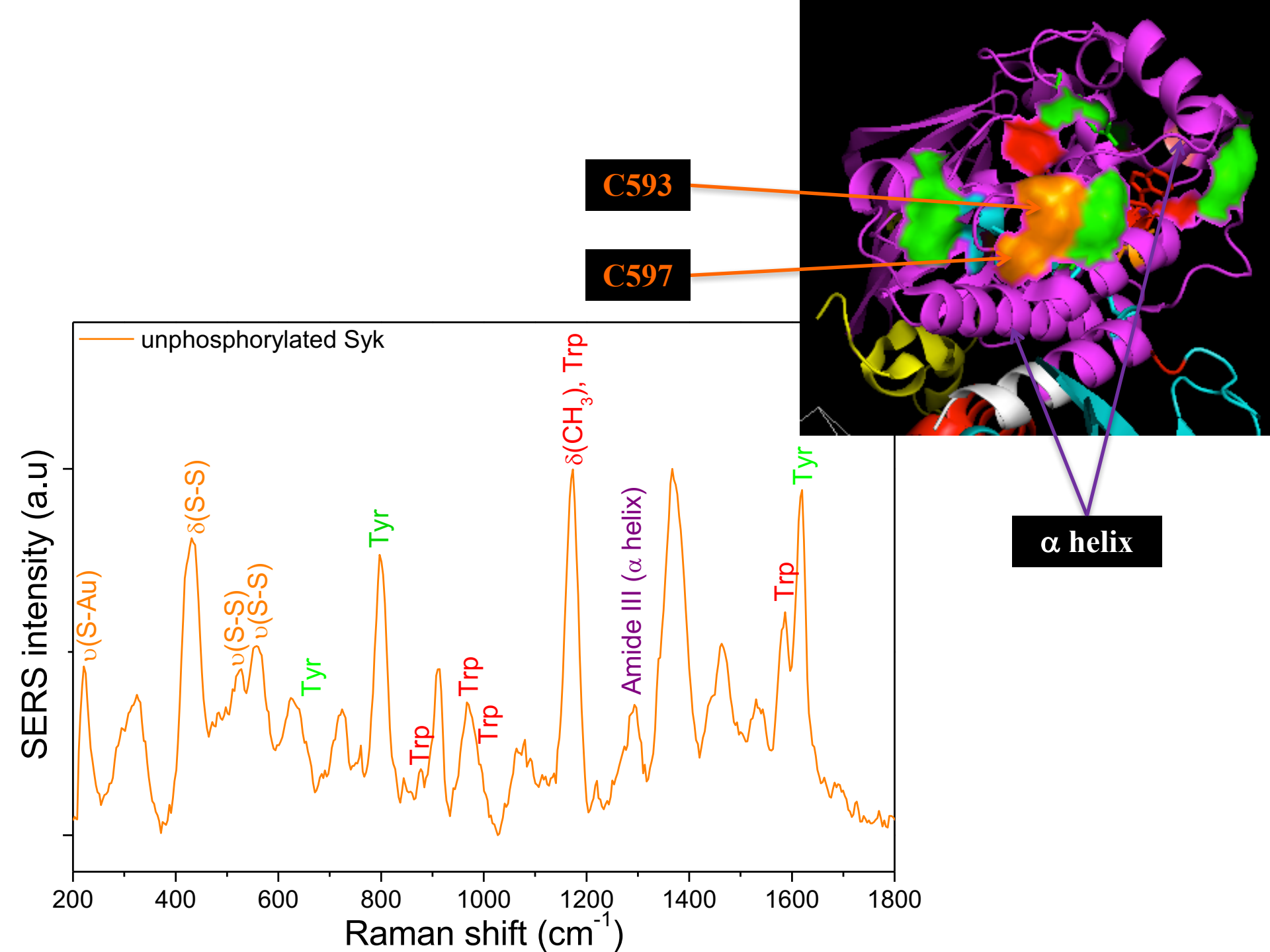


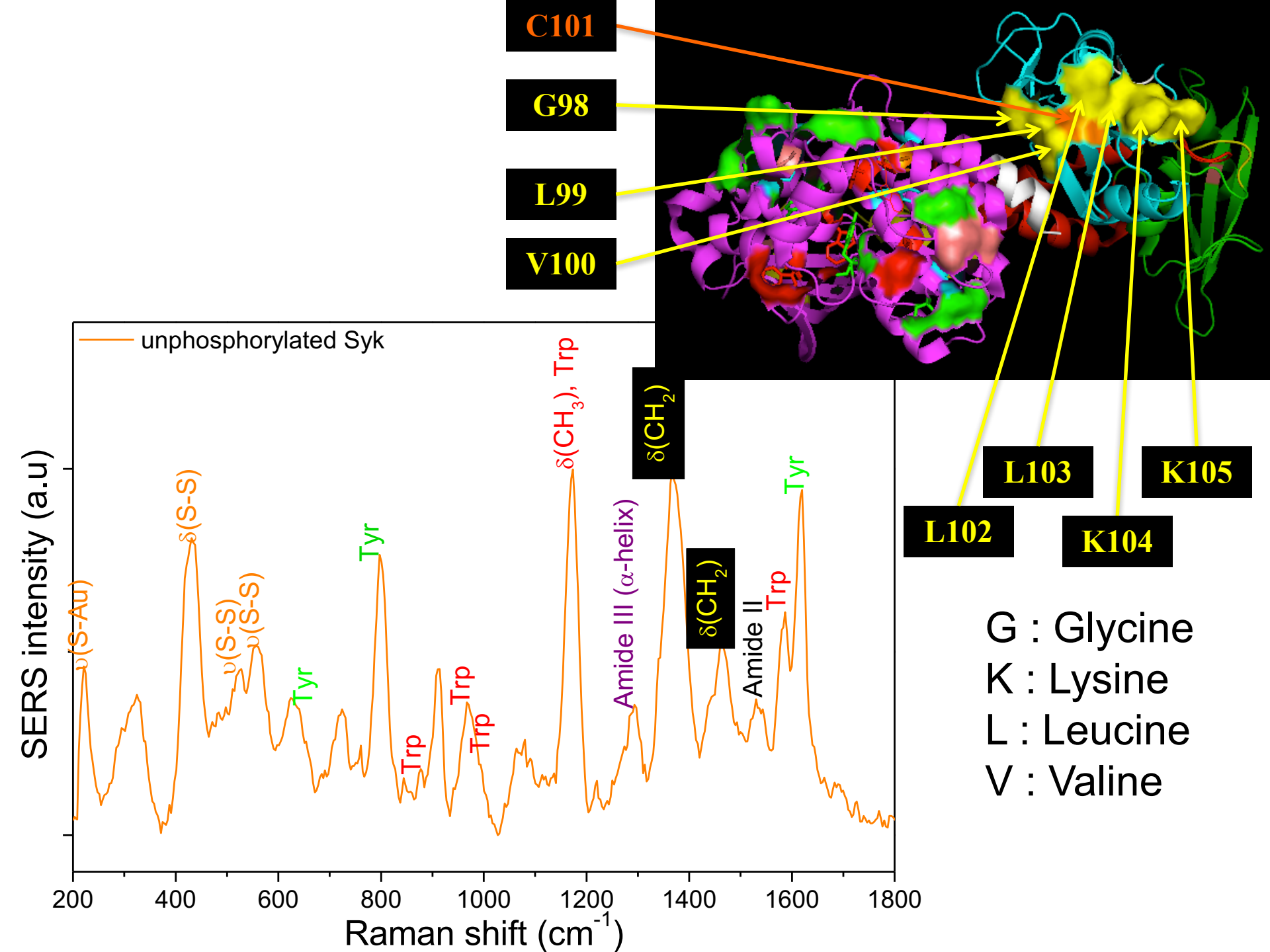
C101 C597 C593

C : Cysteine


Y573
C593
C597
Y568
Y602
Y611
Y : Tyrosine
unphosphorylated Syk
SERS intensity (a.u)
Raman shift (cm-1)
υ(S-Au)
δ(S-S)
υ(S-S)
υ(S-S)
Tyr
Tyr
Tyr
200
400
600
800
1000
1200
1400
1600
1800

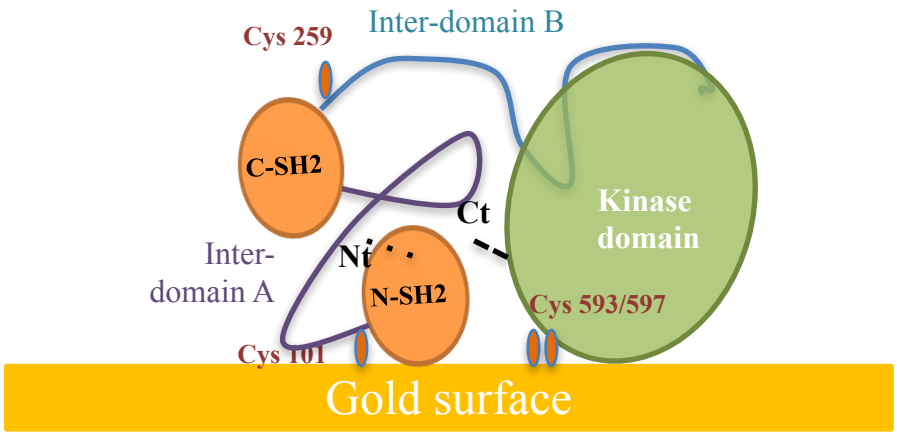

W563
C593
C597
W556
W609
W : Tryptophan
unphosphorylated Syk
SERS intensity (a.u)
Raman shift (cm-1)
200
400
600
800
1000
1200
1400
1600
1800
υ(S-Au)
δ(S-S)
υ(S-S)
υ(S-S)
Tyr
Tyr
Trp
Trp
Trp
δ(CH3), Trp
Trp
Tyr

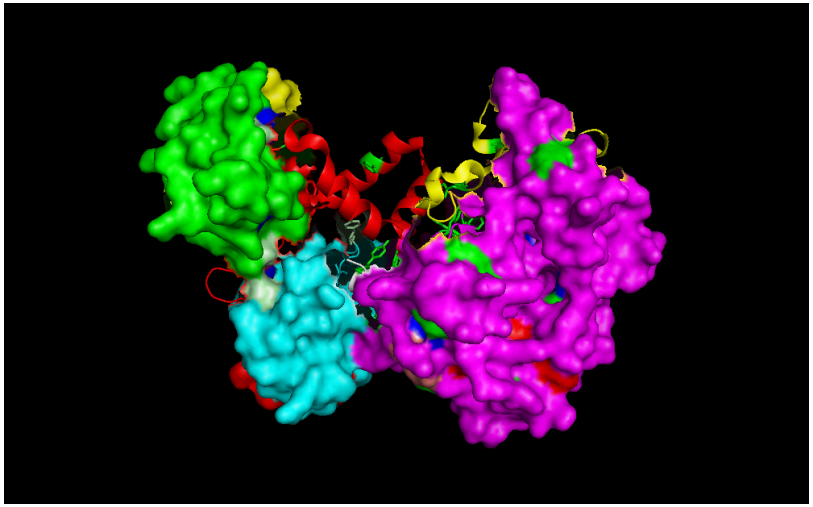

C593
C597
α helix
unphosphorylated Syk
SERS intensity (a.u)
Raman shift (cm-1)
200
400
600
800
1000
1200
1400
1600
1800
υ(S-Au)
δ(S-S)
υ(S-S)
υ(S-S)
Tyr
Tyr
Trp
Trp
Trp
δ(CH3), Trp
Amide III (α helix)
Trp
Tyr

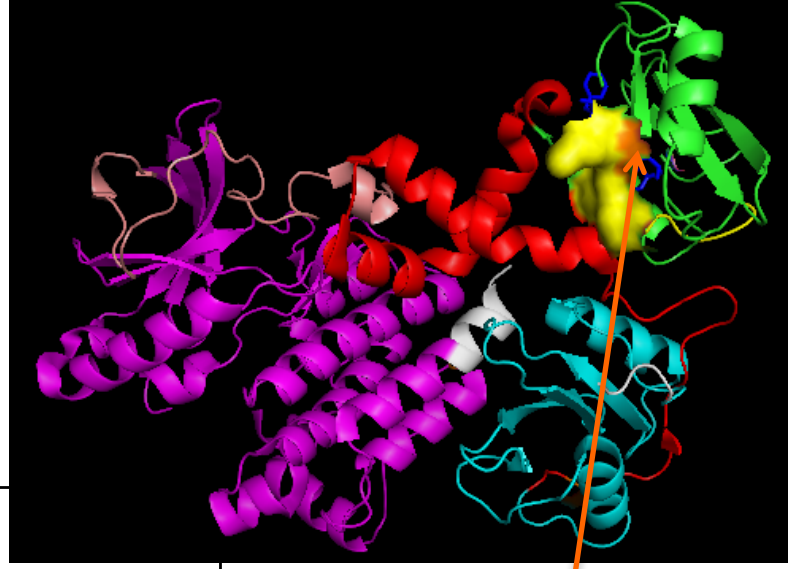

C101
G98
L99
V100
L102
L103
K104
K105
unphosphorylated Syk
SERS intensity (a.u)
Raman shift (cm-1)
200
400
600
800
1000
1200
1400
1600
1800
υ(S-Au)
δ(S-S)
υ(S-S)
υ(S-S)
Tyr
Tyr
Trp
Trp
Trp
δ(CH3), Trp
Amide III (α-helix)
δ(CH2)
δ(CH2)
Amide II
Trp
Tyr
G : Glycine
K : Lysine
L : Leucine
V : Valine

Model of wild type Syk structure

Wild type Syk







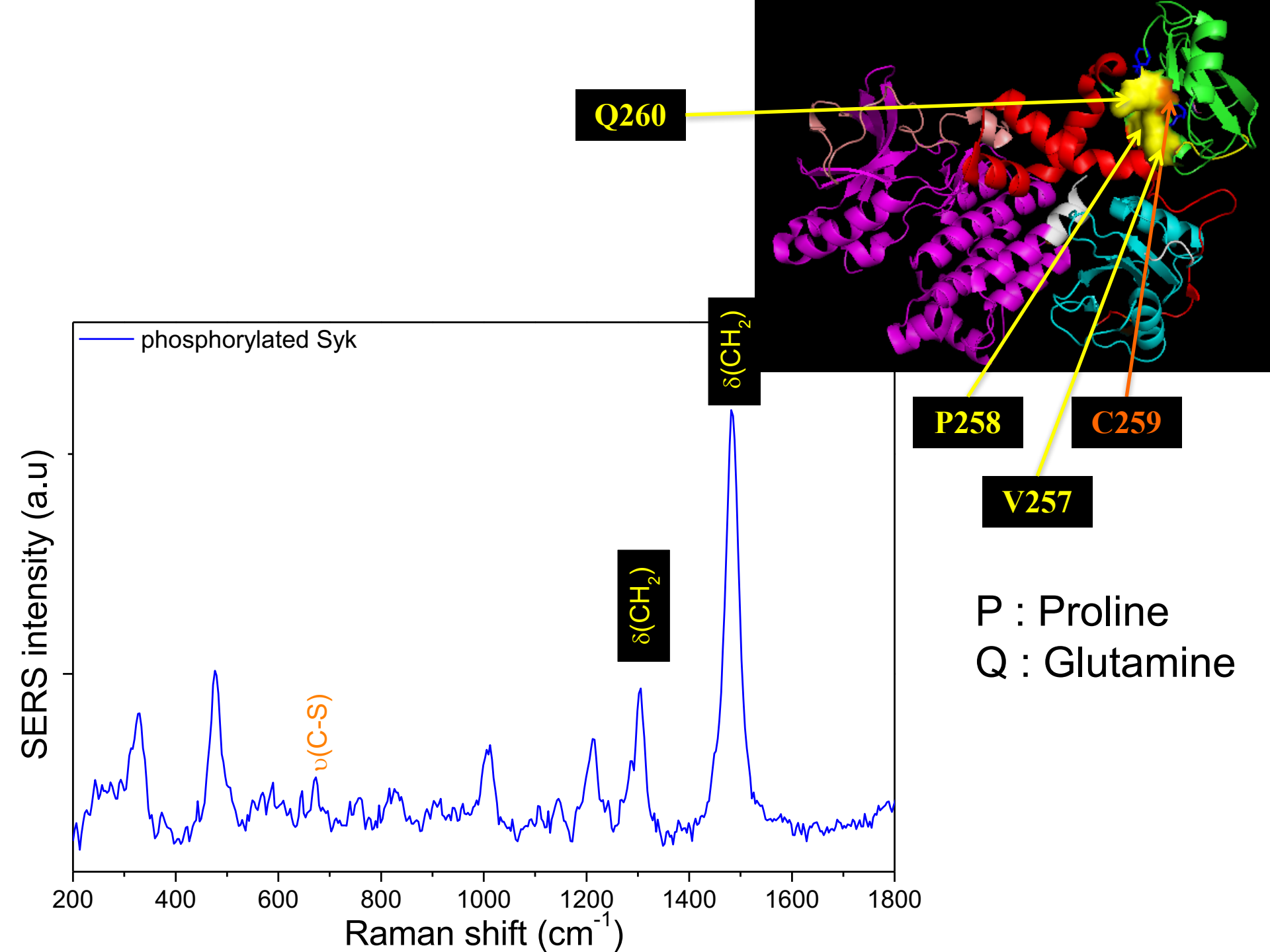
phosphorylated Syk

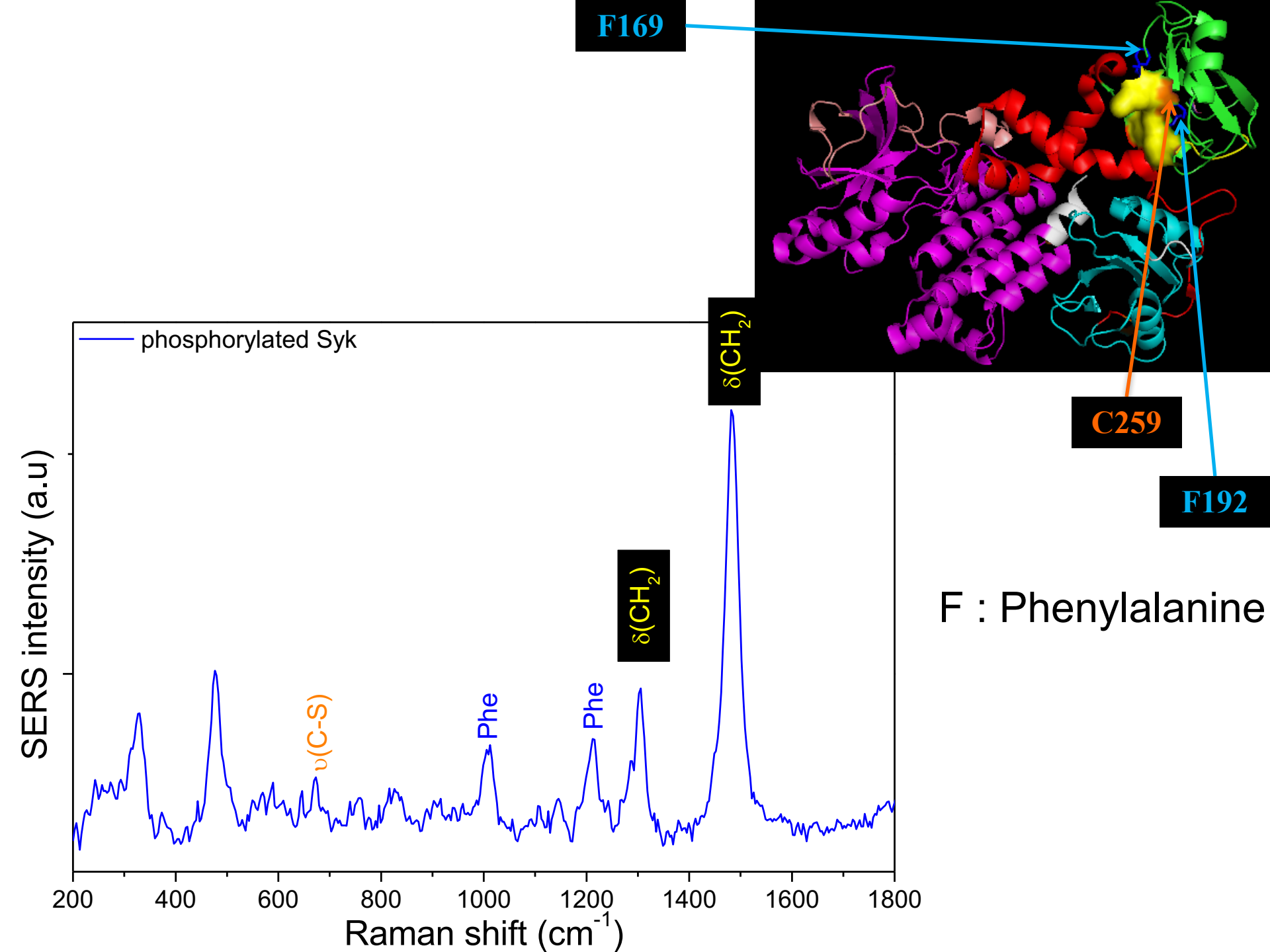
SERS intensity (a.u)

υ(C-S)

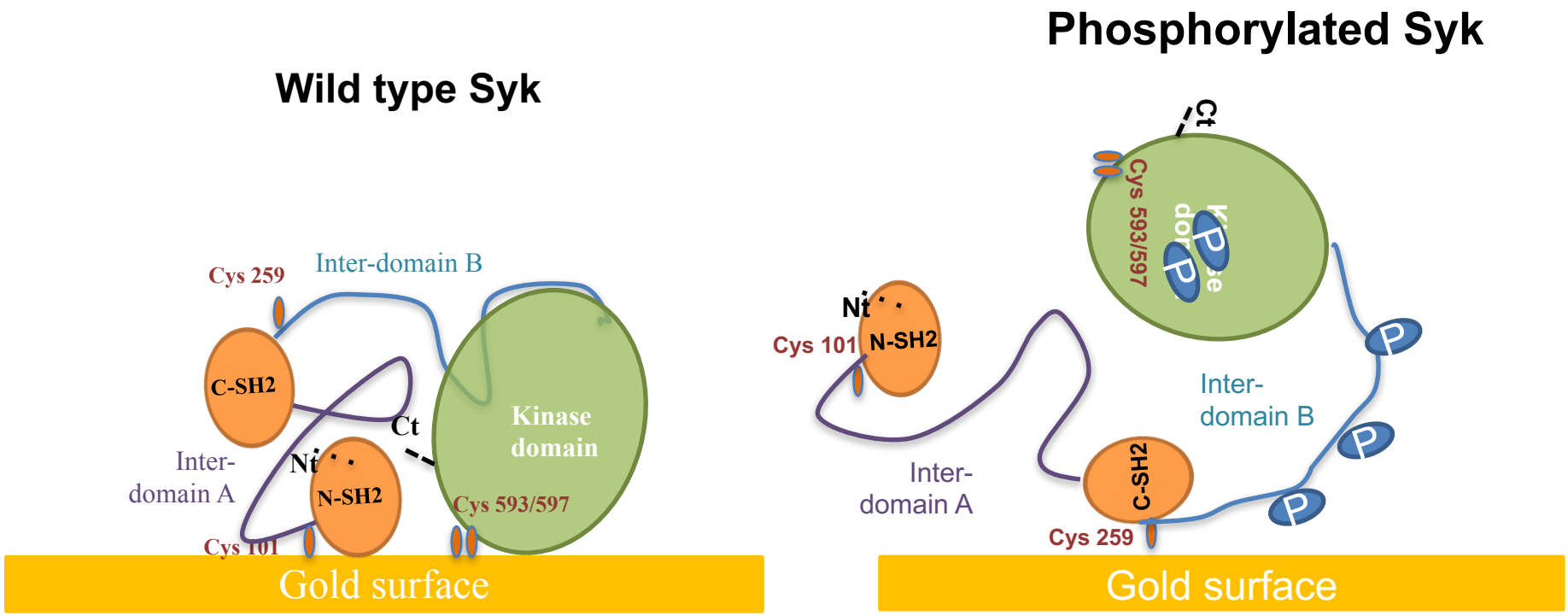
200 400 600 800 1000 1200 1400 1600 1800

Raman shift (cm^{-1})

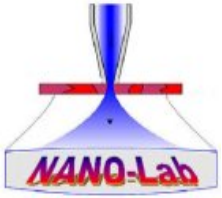

Q260
P258
C259
V257
phosphorylated Syk
δ(CH2)
δ(CH2)
υ(C-S)
SERS intensity (a.u)
200
400
600
800
1000
1200
1400
1600
1800
Raman shift (cm-1)
P : Proline
Q : Glutamine


F169
C259
F192
phosphorylated Syk
δ(CH2)
δ(CH2)
υ(C-S)
Phe
Phe
SERS intensity (a.u)
200
400
600
800
1000
1200
1400
1600
1800
Raman shift (cm-1)
F : Phenylalanine

Model of the phosphorylation impact on Syk structure



Variation of phosphorylation => important modification of Syk structure



Thank you for your attention