

Amino acids binary barcodes for single point mutation detection in amyloid β peptide fragments

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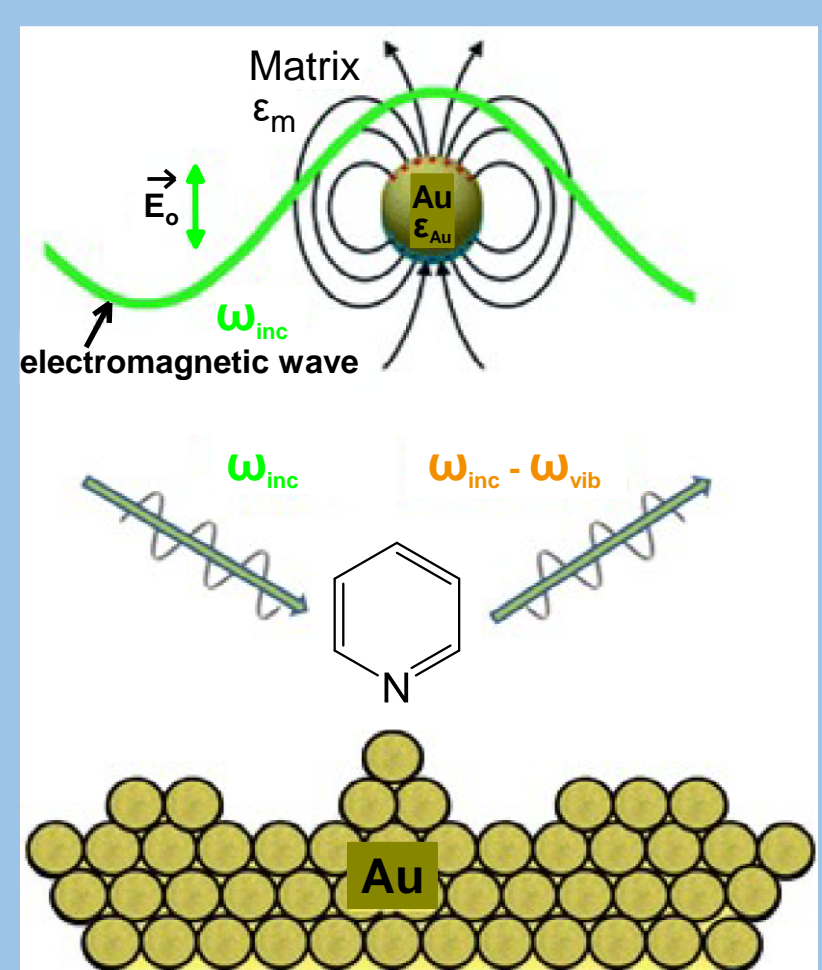
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Motivation

- Specific amino acid substitution in peptide can lead to formation of insoluble amyloid; which is linked to the cause of neurodegenerative diseases. Established experimental such as Edman degradation and microbeads coding¹ are either destructive or require rigorous molecular labeling.
- Non-invasive and label-free detection of amino acid mutation in peptide fragments using Raman spectroscopy can help reduce amount of sample and identify structural change effect on aggregation propensity of peptide.

Introduction

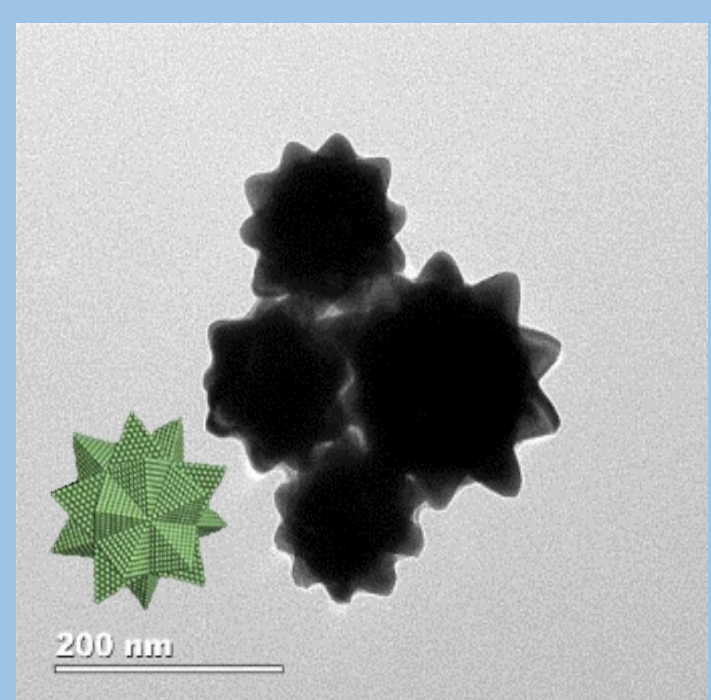


Surface enhanced Raman Scattering (SERS)

- Induced dipole moment in molecule²: $\vec{p} = \alpha \vec{E}_{local}$
- Local electric field (\vec{E}_{local}) is enhanced by excitation of localized surface plasmons
- SERS enhancement of Raman scattering signal 10^6 - 10^8
- Current challenges using Raman signal for peptide analysis:
 - Signal of aromatic components dominates the sequence spectrum³
 - Peptide spectrum is not linear composition of individual components' spectra⁴

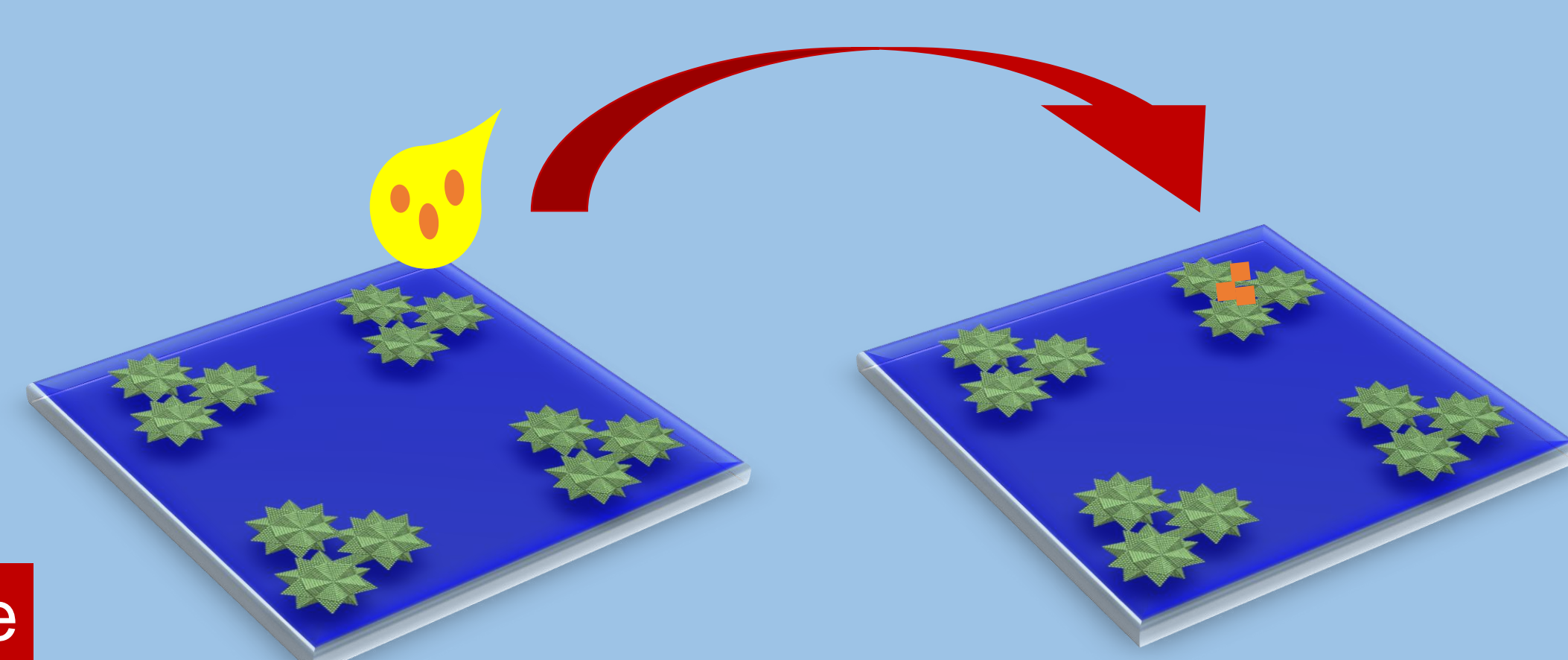
Method

- Step 1: Sensor fabrication and sample preparation

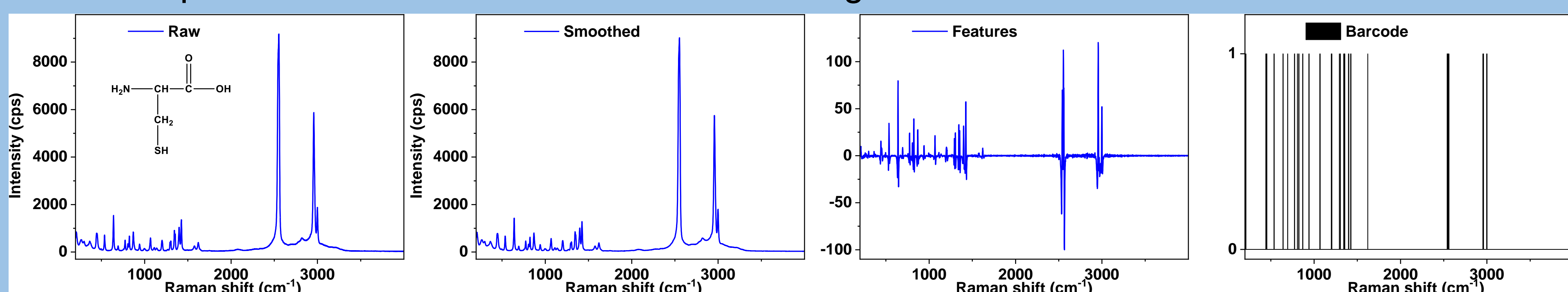


TEM image of 20-arm gold nanostars

Omniphobic substrate



- Step 2: Barcode transform from collected signal



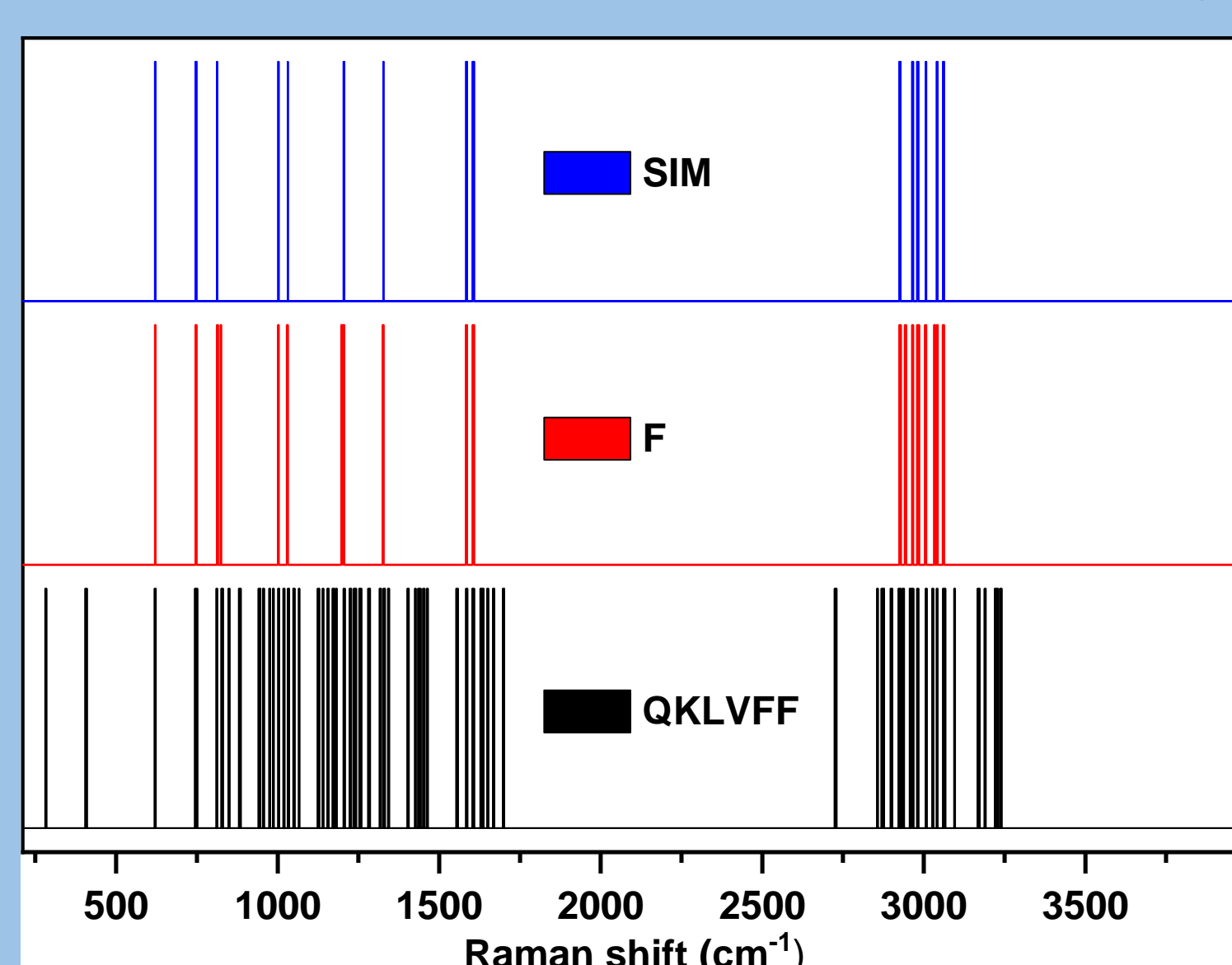
Raw spectrum

Noise reduction

Features selection

Signal encoding

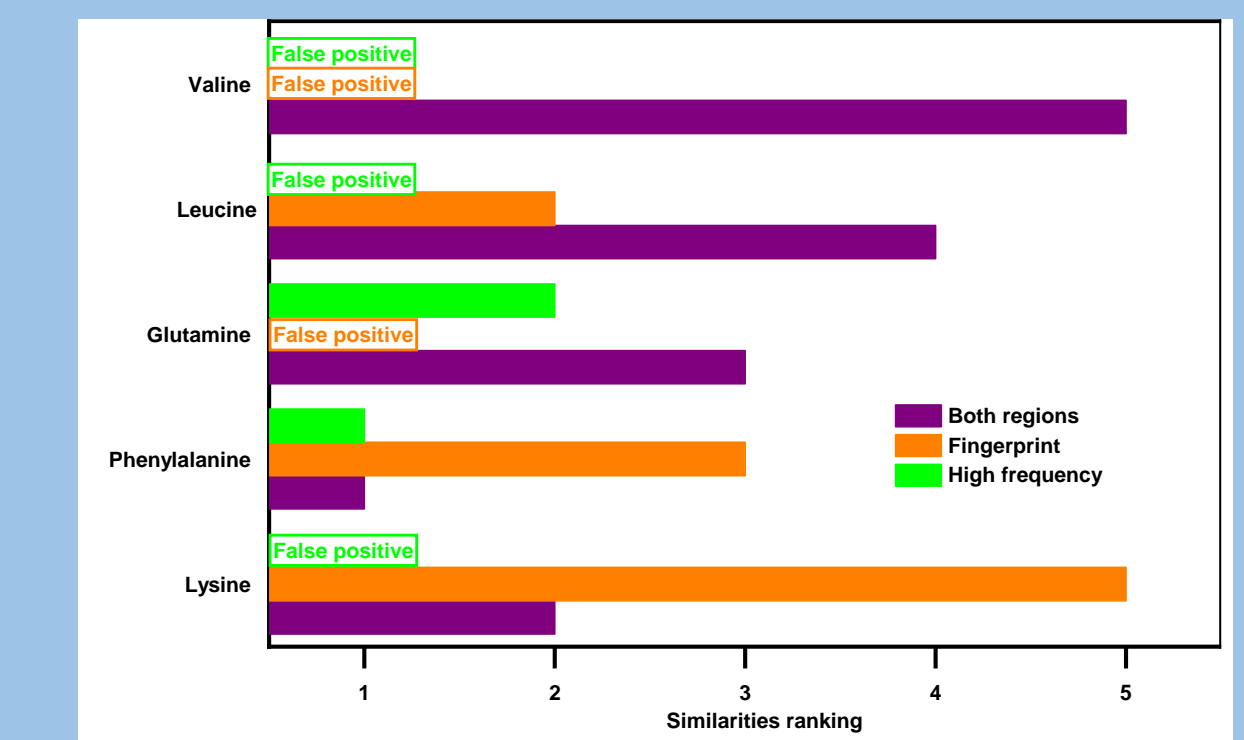
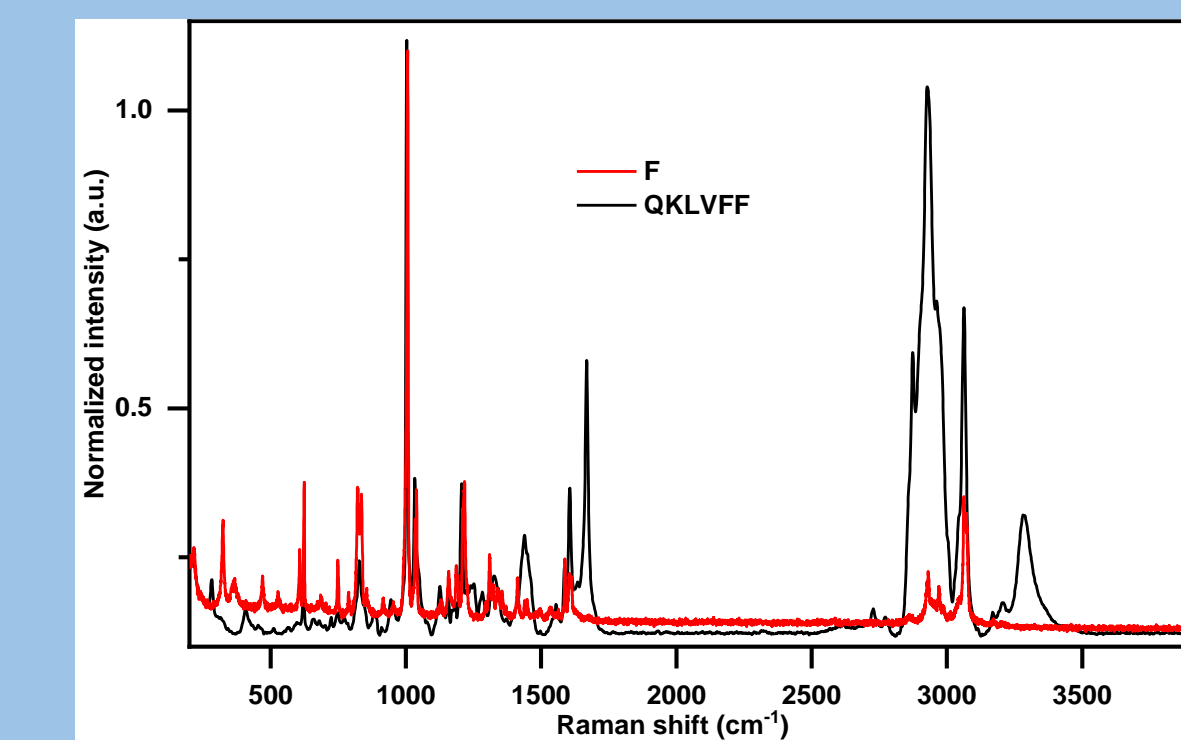
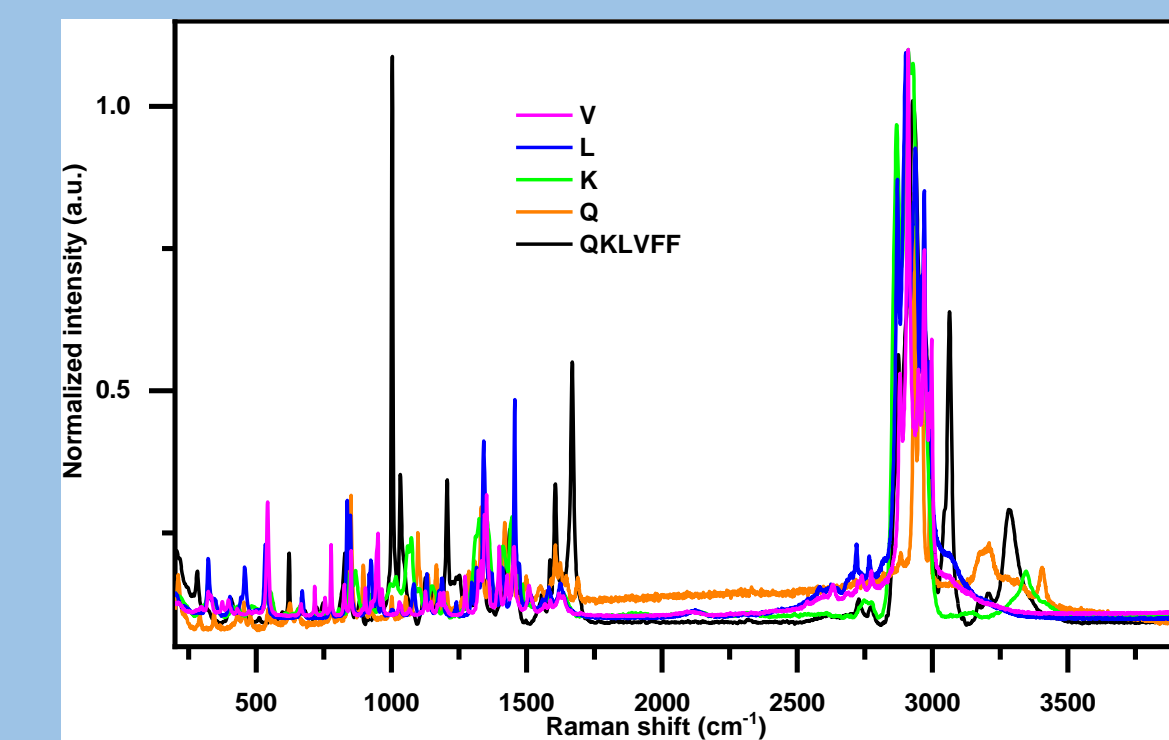
- Step 3: Detection of component by metric distance comparison



- Spectral mismatch is evaluated by Hamming distance
- $d^{HAD}(A,B) = \sum_{k=0}^{n-1} [y_{A,k} \neq y_{B,k}]$
 - k is the index of variable compared between spectrum A and B
 - y is the value of k-variable out of total n variables recorded in barcode
- Similarities score $SIM = (n - d^{HAD})/n$
- Amino acid presence in the sequence was confirmed with SIM score above threshold

Results

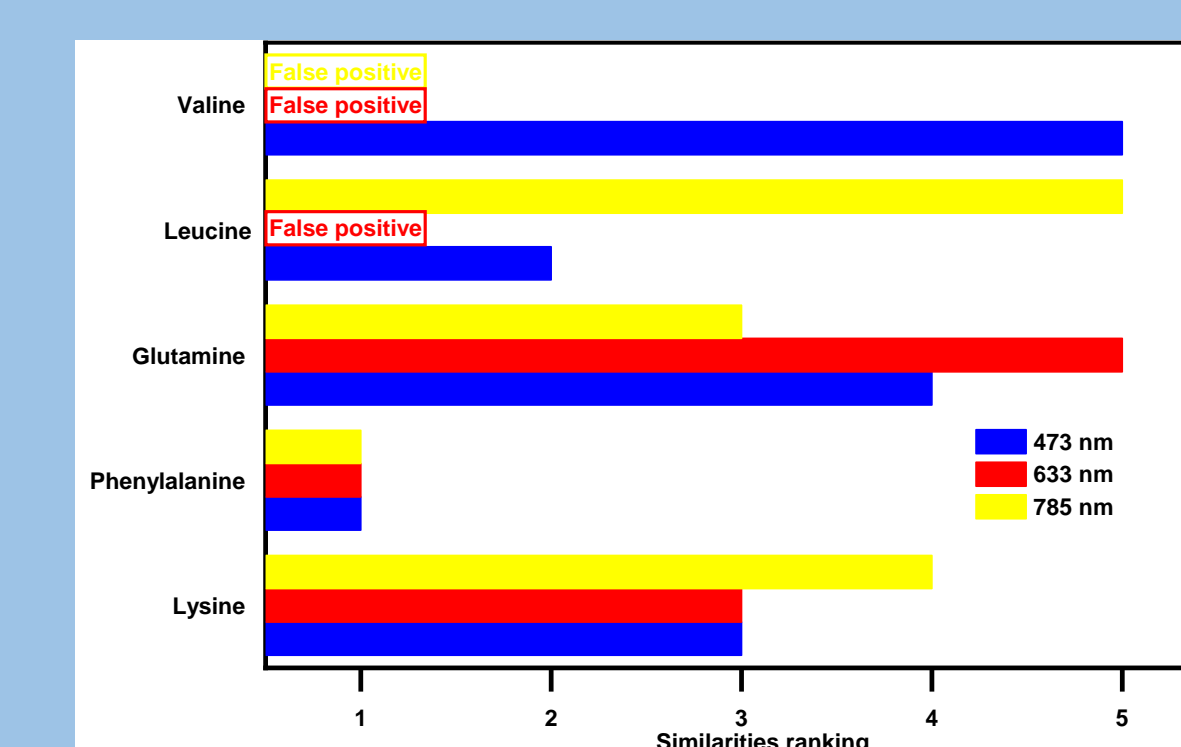
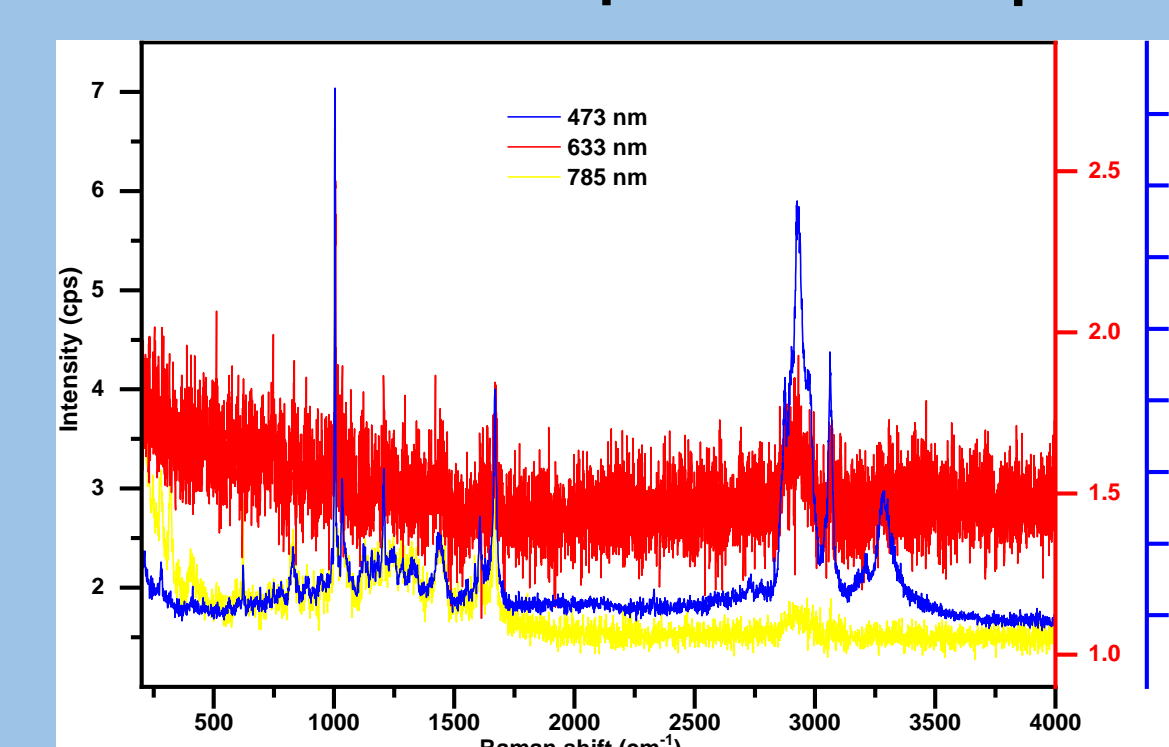
- 15-20 A β : all components were detected by extending the screening region.



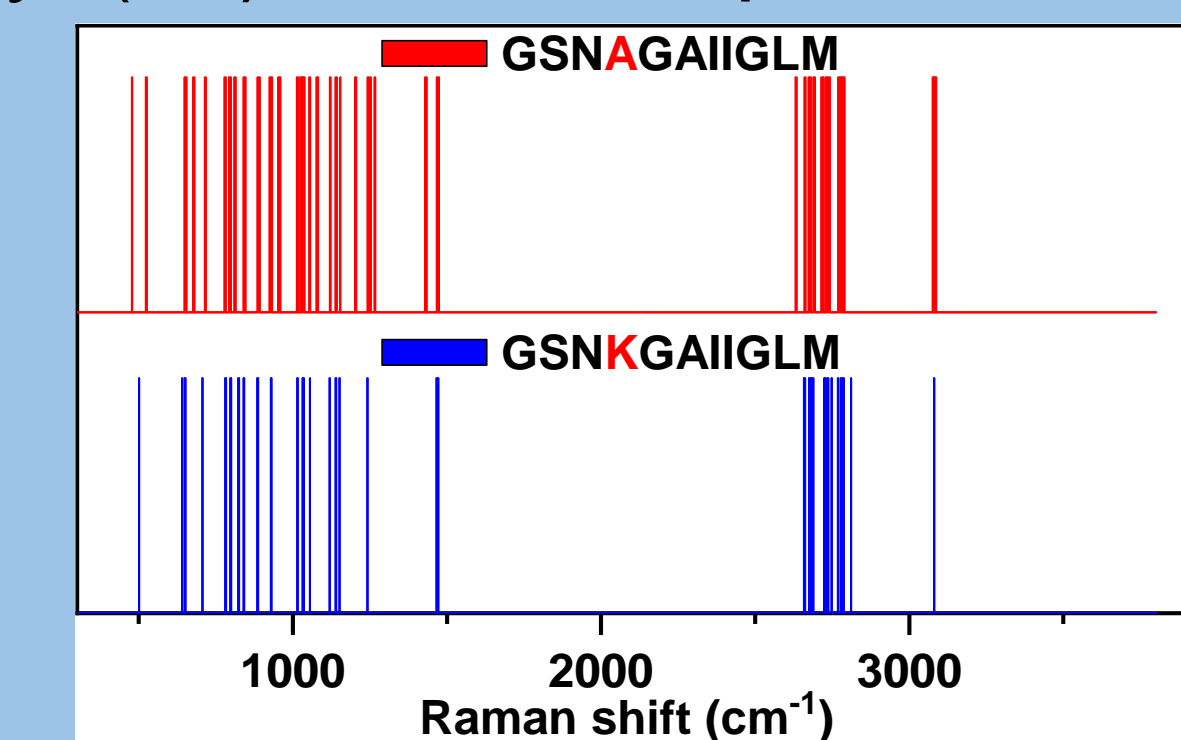
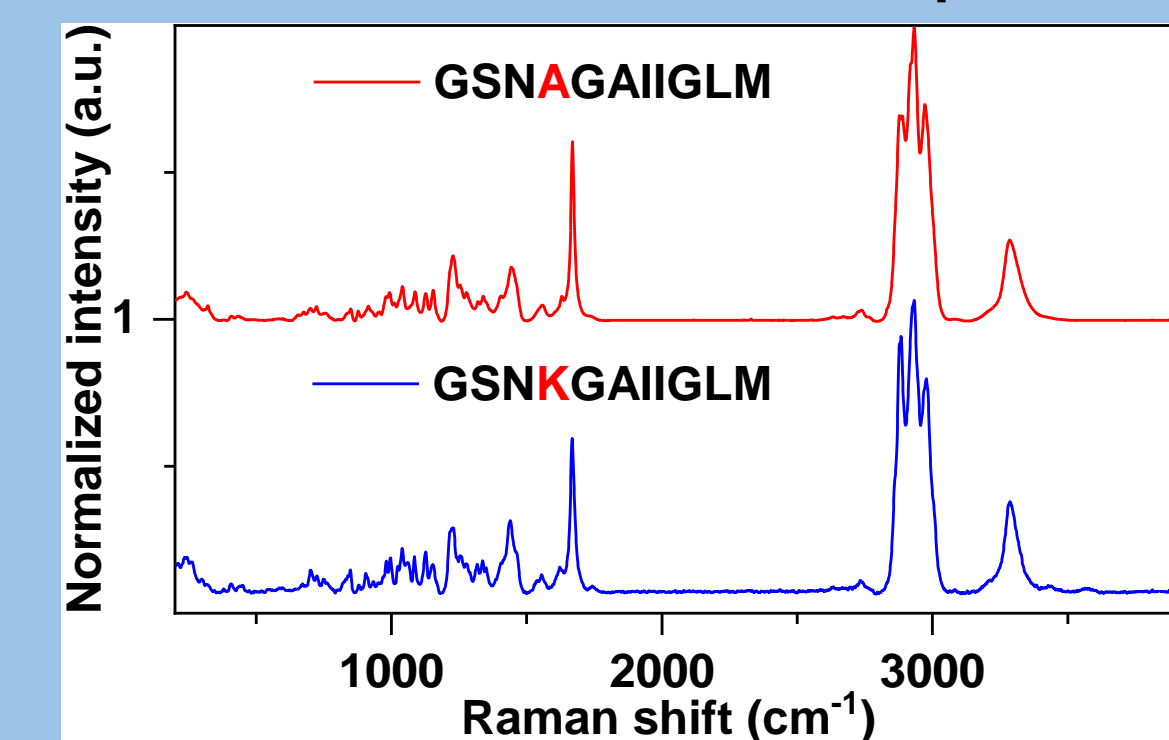
Non-aromatic AA

Aromatic AA

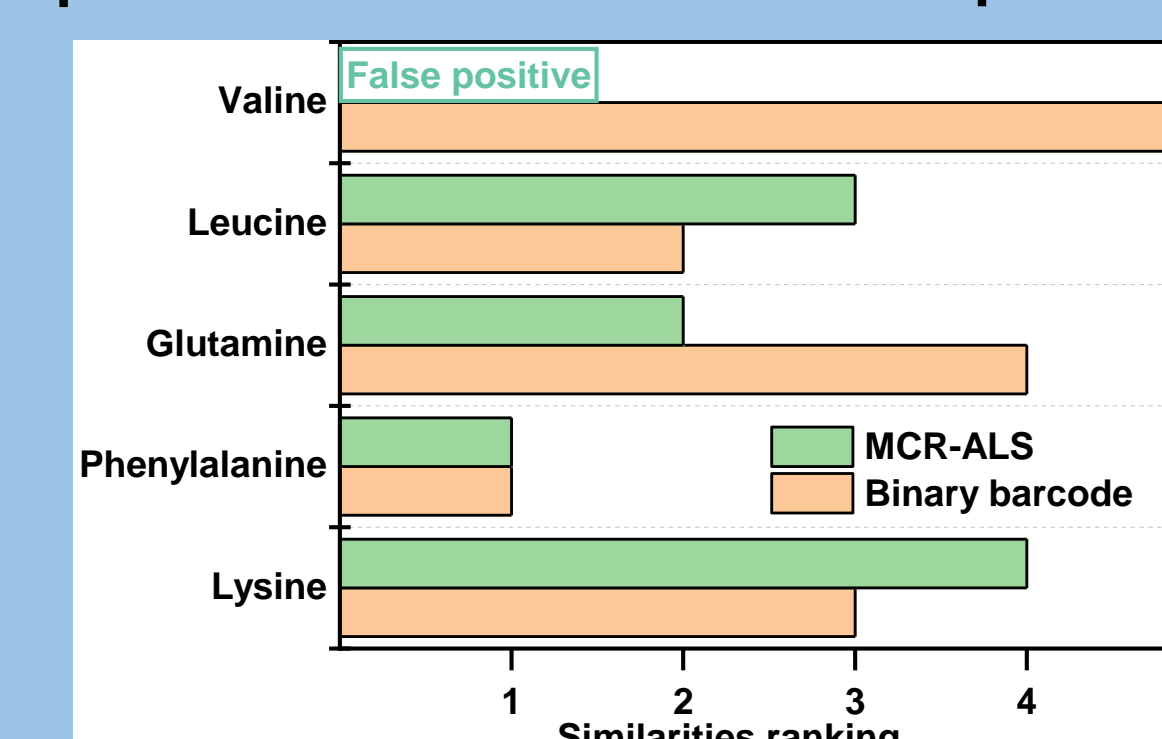
- 15-20 A β : all components were detected with 473 nm.



- Ala mutation at position Lys(28) of 25-35 A β was detected with limit of 10^{-12} mole



- Compare result of 15-20 A β with multivariate curve resolution (MCR)



$$\text{Data matrix } X(M \times N) = T(\text{real}) \times P(\text{real}) \\ \approx T(M \times C) \times P(C \times N)$$

- Alternate least squares (ALS) constraints
- $T, P \geq 0$
- $\|X - TP\|^2$ is minimized

Conclusion

- Provide a label free method to directly encode Raman library of amino acid compounds.
- Extended screening region to high frequency range helps identify non-aromatic amino acids in peptide fragments that contain aromatic components.
- Achieve picomole limit of detection for single point mutation of 10-monomer fragment.

Future works

- Detect missense mutation in A β fragments.
- Detect frameshift mutation in A β fragments.

References

- Kang, H.; et al. *Scientific Reports* 2015, 5.
- Procházka, M. *Springer International Publishing*: Cham, 2016.
- Gómez, J.; et al. *Journal of Physics: Conference Series* 2016, 687, 012112.
- Candeloro, P.; et al. *The Analyst* 2013, 138, 7331.

Acknowledgement

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