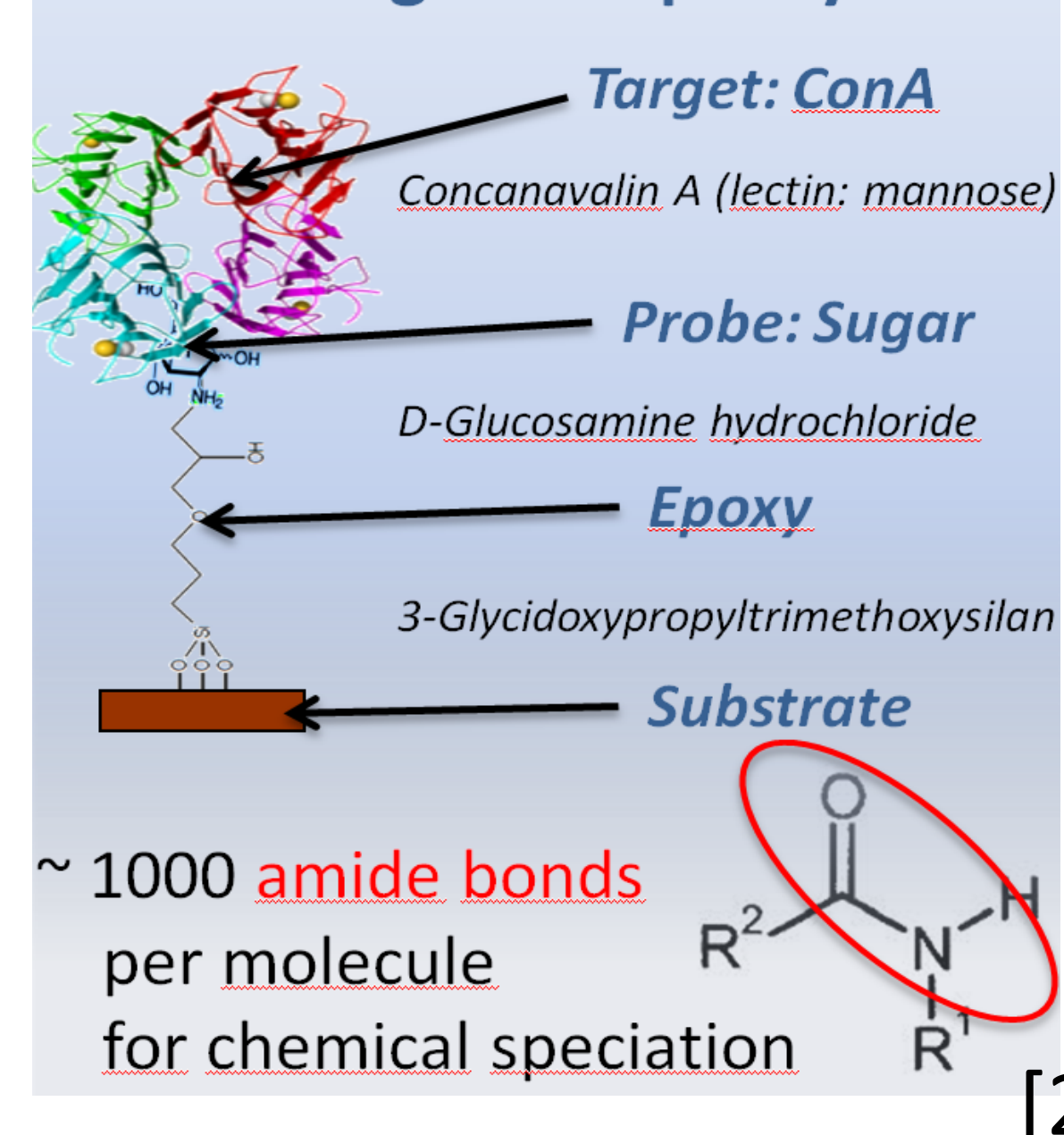


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Motivation

Capturing biochemical markers by biomolecular films is one of the most promising approaches for the development of highly sensitive and highly selective diagnosis. Future innovative tools for in vitro or point of care diagnostics are expected to rely on this principle. X-ray analysis has the potential to substantially contribute to the characterisation of biomolecular films. With this work we present first steps towards an in-situ preparation and successive in-situ chemical analysis of biomolecular films at liquid-solid interfaces.

Probe-target sample system:

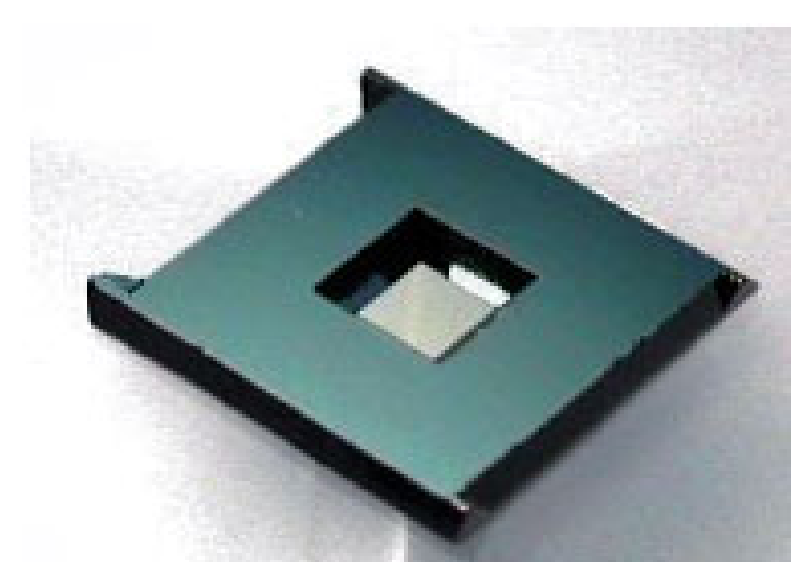


Sample System (3 layer)

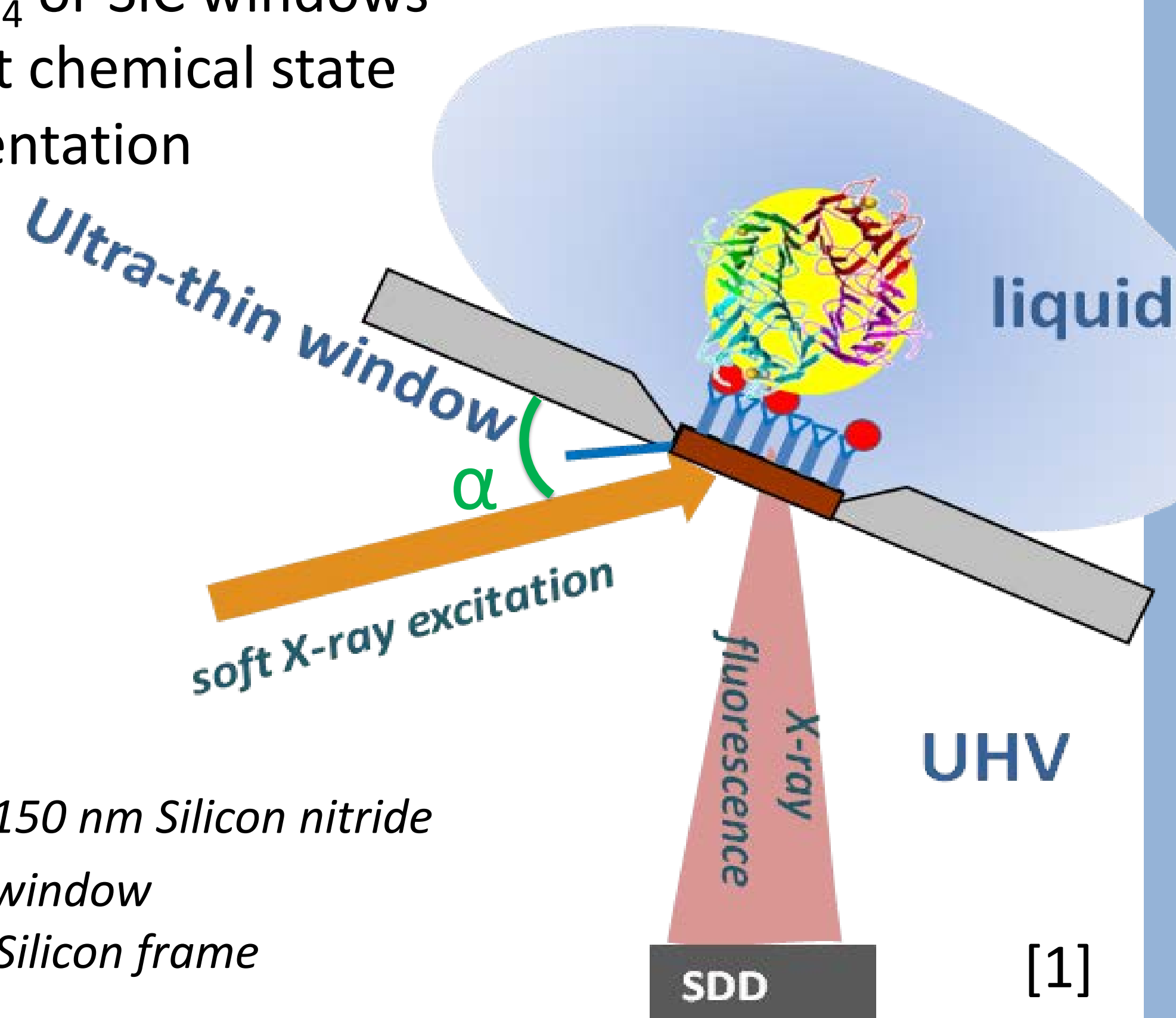
- epoxy-silane, functionalize the substrate surface
- D-Glucoseamine-hydrochloride, immobilize the epoxy layer and bind the target
- Convanavalin A (ConA), protein to be probed

Principle of measurement

- Near Edge X-ray Absorptions Fine Structure Spectroscopy (NEXAFS) of K-edges of C,O and N
- soft X-ray excitation (200...1000 eV)
- Experiment in ultra high vacuum
- requests thin Si₃N₄ or SiC windows
- information about chemical state and molecule orientation
- tuneable incidence angle (from 1° to 45°)



150 nm Silicon nitride window
Silicon frame



several steps of preparation for biomolecular film

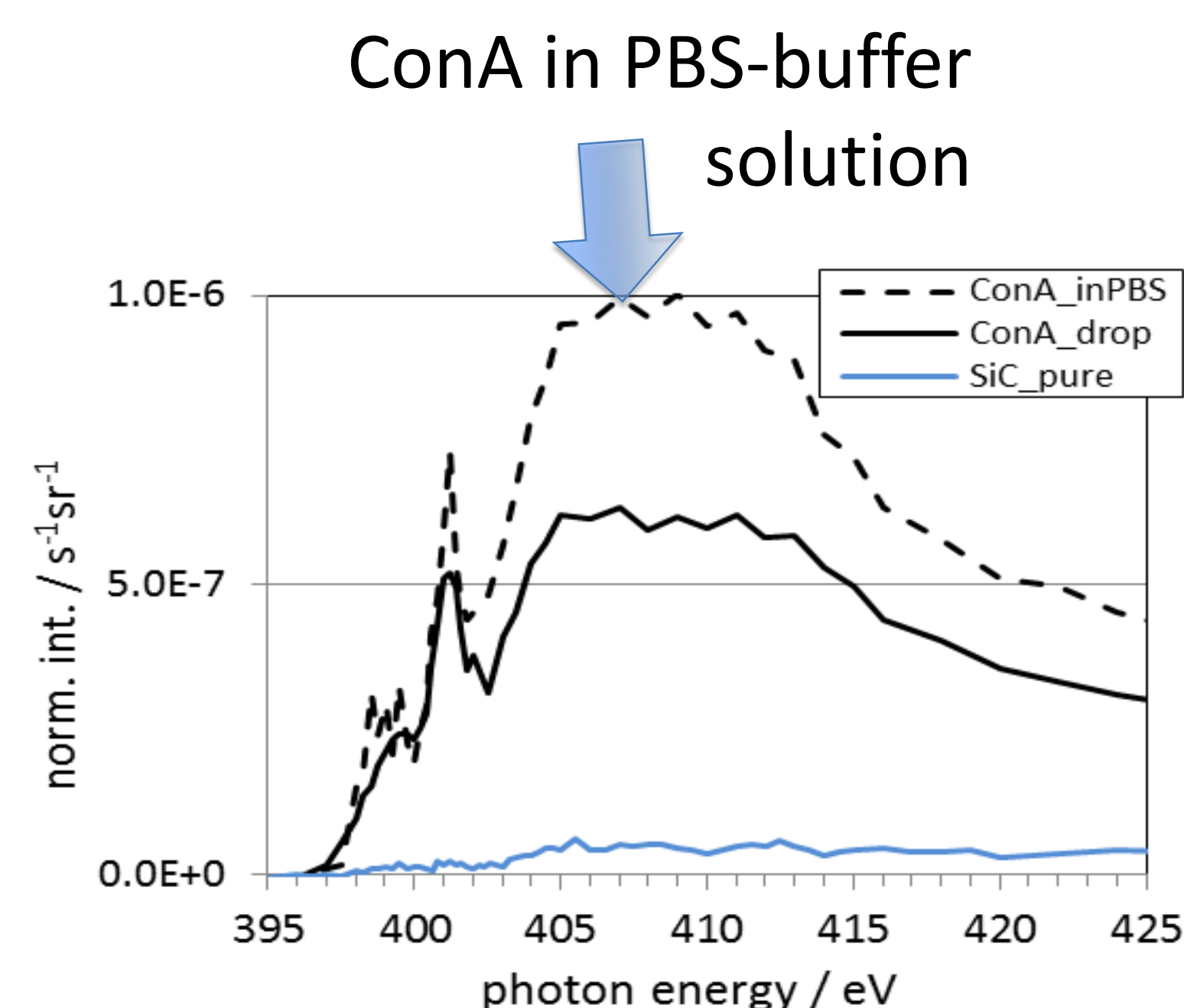
- coating of Si₃N₄ or SiC window
- mounting the window to a holder
- rinsing the the window through the holder with a solution of layer constituents
- complex functional layers can be formed
- holder will be sealed and transfered to the spectrometer
- during the preparation the functional layer is kept wet

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ConA in liquid environment spectra below show two different preparation methods:

- a drop of ConA solution dried on a prepared and immobilized substrate
- ConA solution behind the window



Window materials

Window material	C K-NEXAFS	N K-NEXAFS	O K-NEXAFS
Si ₃ N ₄	✓ Limited by carbon contaminations	✗ Limit of detection due to nitrogen in the window	✗ Limit of detection due to oxygen in the water
SiC	✗ Limit of detection due to carbon in the window	✓ Limited by count rate of nitrogen K α fluorescence line	✗ Limit of detection due to oxygen in the water

Conclusion

- sample holder for NEXAFS in-situ analysis of biomolecular films was built
- in-situ preparation and analysis of complex films
- window material must be carefully selected
- further analysis of films still in progress

References

- [1]: Cornelia Streeck, ECASIA 2013, *Session Solid liquid interfaces*
 [2]: Paul Dietrich, ECASIA 2013, *Sessions Life Science & Biosurface 2*

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