Tryptophan Fluorescence as a Research and Diagnostics Tool

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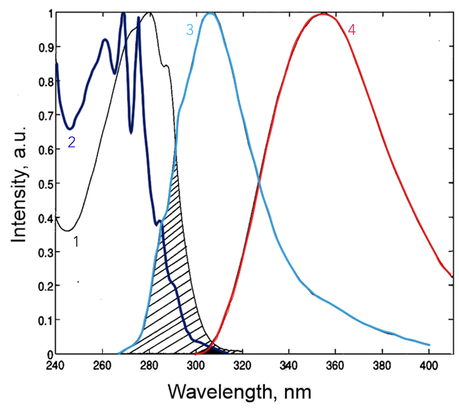
Tryptophan (Trp) amino-residues are natural reporters of protein structure and function due to the sensitivity of their electronic spectra to protein's photophysical and photochemical properties. The dependence of Trp emission spectrum on the polarity of the microenvironment can reveal conformational changes in proteins that can be used to diagnose diseases associated with protein misfolding. The spectral relaxation of Trp emission can be used to measure changes in the polarity and dynamics (viscosity) in proteins. In addition, proteins containing several Trp residues can exhibit non-radiative homo-energy transfer among the individual Trps, which causes pico- and nanosecond spectral relaxation and fluorescence depolarisation. Taking into account the sharp dependence of the efficiency of homo-energy transfer on the donor – acceptor separation distances, this method can be used to monitor relatively small conformational changes caused by protein-ligand interactions.[1] This method is especially useful when a protein cannot be crystallised due to a high mobility of its structural domains. Fluorescence of proteins is also indicative to photochemical modifications of Trp like Hydroxytryptophan, N-formylkynurenine, Kynurenine, Hydroxykynurenine, which also possess fluorescence. We demonstrated that a quantitative evaluation of fluorescent post-translational modifications in the eye lens proteins can be performed by decomposing the emission spectrum into its individual components. This method can be used for early diagnosis of cataracts.[2]

PROTEIN SCIENCE

Directed Homo - FRET



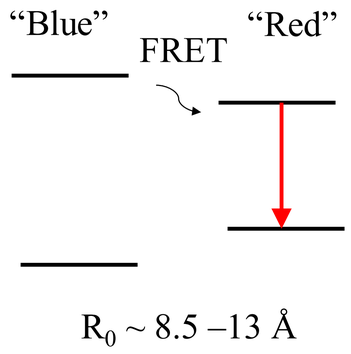


Fig.1 The excitation and emission spectra of Trp in solvents of different polarity

This difference in the spectral overlap of the Trp emission spectrum of in cyclohexane with its excitation spectrum in water (hatching) and the emission spectrum in water with the excitation spectrum in cyclohexane (filling) shows that the energy transfer proceeds with energy loss from the "blue-shifted" Trp residue toward the "red-shifted" one. The greater the polarity difference, the higher the dominance of the directed energy transfer.

Homo-FRET among Trp residues in human class I major Histocompatibility complex (HLA-A2)

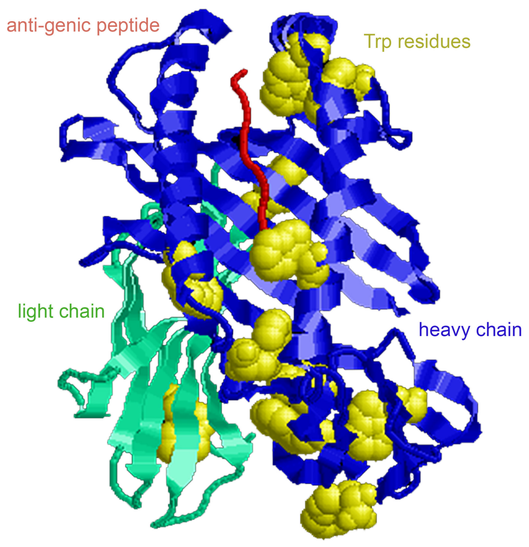


Fig.2 3D structure of peptide - HLA-A complex.[3]

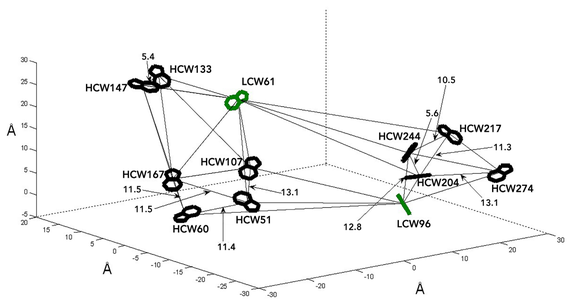


Fig.3 The network topology of Trp residues in peptide-HLA-A complex. The figure shows the separating distances between the Trp amino-residues in the heavy (black) and light (green) chain.

Distance between Trp, Å	Number of Trp	Homo-FRET time range
~5.5	2	picosecond
11 to 15	5	nanosecond
>15	5	no FRET

Homo-FRET among Trp residues causes fluorescence depolarization

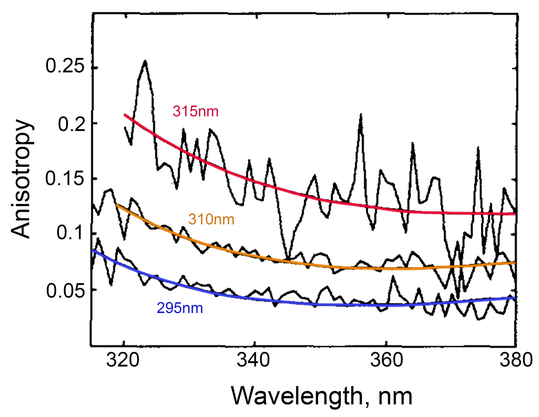


Fig.4 Trp anisotropy emission spectra of HLA-A2-peptide complex at different excitation wavelength.

- Stokes excitation (295nm) => all Trp residues are excited => the depolarisation is maximal
- Excitation on the red edge (310nm, 315nm) => only the "red" Trp sub-population is excited => the depolarisation is reduced because the transfer can operate only towards the residues having even more "red" position of their excitation spectrum.

Tryptophan Time-Resolved spectral shift as a function of 3D structure

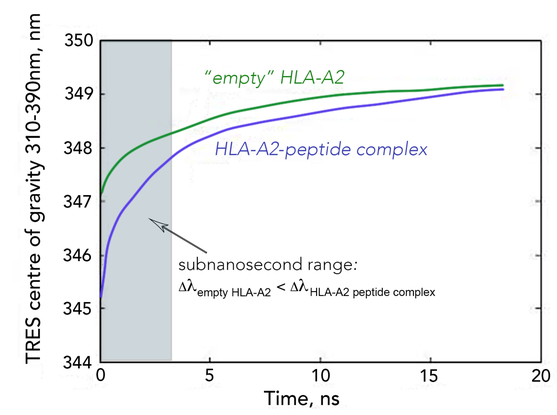


Fig.5 Time-courses of Trp emission spectral shift in the peptide-loaded and peptide-free HLA-A2 molecules.

The results suggest that the Trp pairs with the shortest separation distance are more separated in the peptide-free HLA-A2 than in the peptide loaded one, i.e. binding of the peptide causes conformational changes in the terminal regions of the MHC binding site, which supports the allosteric model of the peptide – MHC complex formation.[1]

Conclusions

1) Multi-tryptophan proteins can exhibit homo-energy transfer among the individual Trp residues, which causes pico- and nanosecond spectral relaxation and fluorescence depolarisation.
2) This phenomenon can be used to reveal conformational changes in proteins.

MEDICAL DIAGNOSTICS

Red-Edge Excitation of the Eye Lens

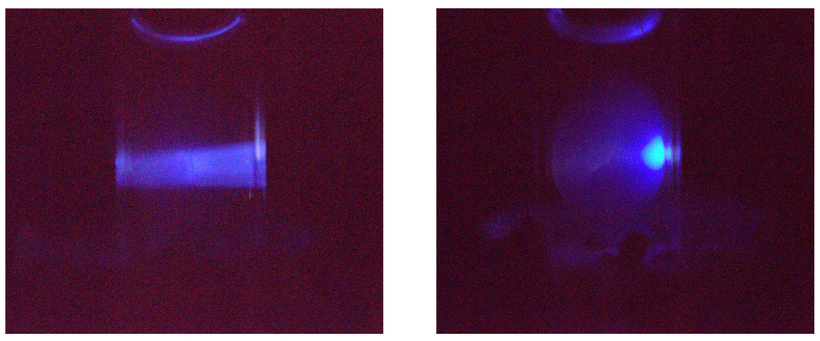


Fig.6 Excitation of the eye lens fluorescence in a normal (A) and UV-irradiated (B) porcine lens by a 317nm-LED. The excitation light passes through the normal lens without significant attenuation, whereas it is completely absorbed by the damaged site in the UV-irradiated lens, which leads to characteristic luminescence from the damaged region.

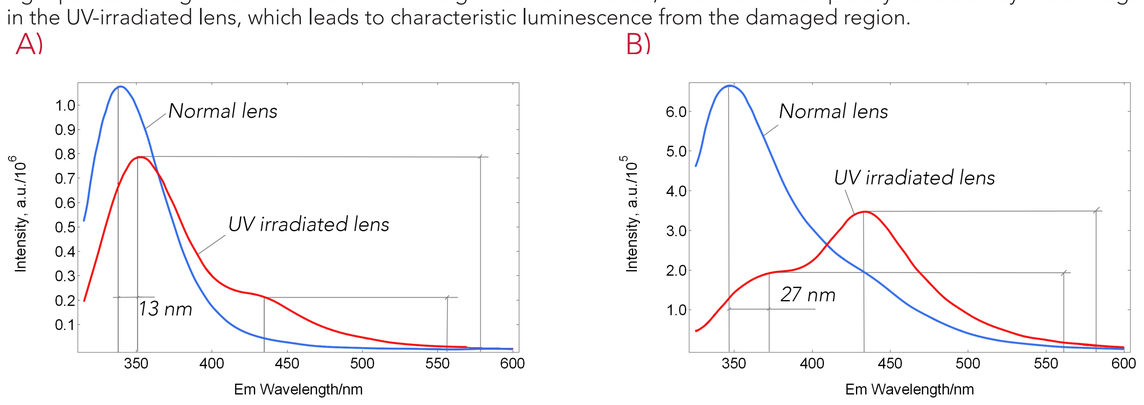
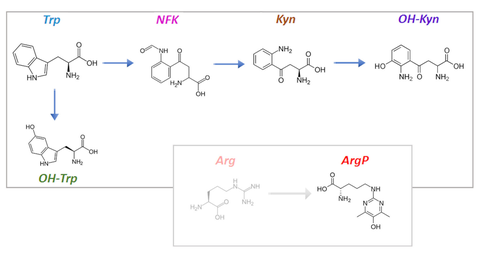


Fig.7 Corrected fluorescence spectra of a normal and UV-irradiated porcine eye lens at Stokes (A) and red-edge (B) excitation.

Excitation	Shift of Trp emission	Relative intensity of the additional band
305 nm	13 nm	$I_{440}/I_{Trp} < 1$
315 nm	27 nm	$I_{440}/I_{Trp} > 1$

Mass Spectrometry of the Eye Lens

Samples: Solubilised irradiated porcine eye lens proteins and emulsified human post-operational eye lens proteins.



Mass spectrometry results:

- Tryptophan residues
- Two primary products of Trp: hydroxytryptophan (OH-Trp) and N-formylkynurenine (NFK)
- Two downstream products: kynurenine (Kyn) and hydroxykynurenine (OH-Kyn).
- A fluorescent derivative of arginine argpyrimidine (ArgP)

Quantitative evaluation of fluorescent post-translational modifications in the eye lens proteins by spectral decomposition

Samples: a post-operational emulsified sample with the second grade of nuclear cataract

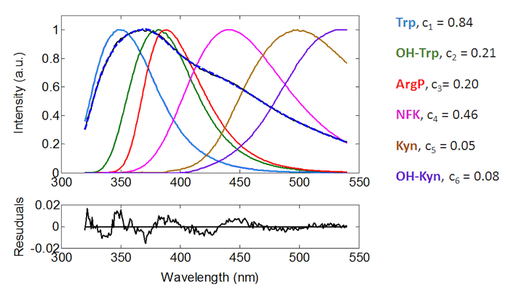


Fig.8 Fluorescent spectrum of the insoluble fraction of an emulsified cataractous sample (experimental data - black, fit - blue) and its decomposition calculated using the spectra of the identified Trp's fluorescent derivatives and ArgP.

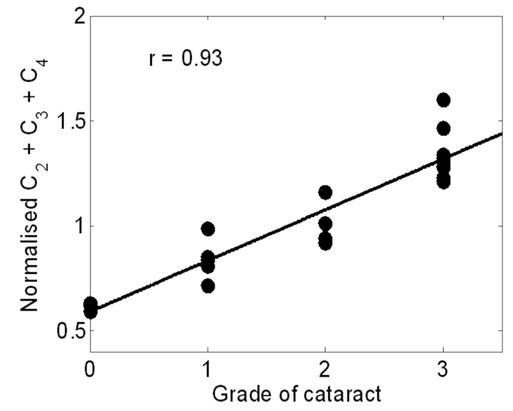


Fig.9 Correlation of cataract grade with the normalised cumulative emission of OH-Trp, ArgP and NFK for 21 emulsified eye lens proteins.

Conclusions

1) The method can be used for a non-invasive, quantitative cataract diagnostics, with greatly increased sensitivity over the current slit-lamp technique.
2) The method has the potential to diagnose diseases associated with protein misfolding as Alzheimer's and Parkinson's.

References

1. Gakamsky et al. Assembly and Dissociation of Human Leukocyte Antigen HLA-A2 Studied by Real-Time Fluorescence Resonance Energy Transfer. Biochemistry 39 (2000) 36:11163-9.
2. Gakamsky et al. Tryptophan and Non-Tryptophan Fluorescence of the Eye Lens Proteins Provides Diagnostics of Cataract at the Molecular Level. Sci Rep. 2017, 7:40375. doi: 10.1038/srep40375.
3. Madden et al. The antigenic identity of peptide-MHC complexes: a comparison of the conformations of five viral peptides presented by HLA-A2. Cell (1993) 75 p.693-708