CONFORMATIONAL SUBSTATES IN PROTEINS: TWO-DIMENSIONAL ANALYSIS OF THE POLARIZED INTENSITY DECAYS OF THE TRYPTOPHAN FLUORESCENCE EMISSION BYTHE MAXIMUM ENTROPY METHOD

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The heterogeneity of the tryptophan (Trp) fluorescence emission of several proteins containing a single tryptophan residue, either embedded in the interior of the protein matrix or freely accessible to the solvent, was studied by the time-correlated single photon counting technique. The data of the polarized components of the fluorescence emission were analyzed by the Maximum Entropy Method in one dimension (excited-state lifetimes t) and two dimensions (excited-state lifetimes t and rotational correlations times q). The 2D analysis of the Trp fluorescence emission clearly shows a correlation between the shortest excited state lifetime and the fastest motion. The existence of slowly exchanging conformational substates with different packing constraints affecting the indole subnanosecond mobility can be suggested in specific cases.

X-RAY INVESTIGATION OF A RECOMBINANT OUTER MEMBRANE PROTEIN FROM *Neisseria meningitidis*

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The P64K protein (MW = 64kDa) is present in the majority of pathogenic Neisseria strains and is capable of inducing immunologically active antibodies (bactericidal antibodies) in its natural host.

This protein contains 599 amino acid residues, including six cysteine residues and a flavin adenosine dinucleotide (FAD) prosthetic group. The first 111 amino acid residues of the N-terminal domain are homologous with the lipoyl domain of acetyltranferases. The other segment of the sequence, which represents almost the total protein, displays high homology with lipoamide dehydrogenases, the flavoenzymes containing a redox-active disulfide.The p64K crystallizes in the space group P43212. Unit cell parameters are a=b=140 Å, c=79 Å. The structure of dihydrolipoamide domain of the P64K is solved by Molecular Replacement with the Navazas's package (AMORE). The know crystal structure of dihydrolipoamide dehydrogenase of Pseudomonas putida was used as the starting model.

The molecule was rotated and traslated in the asymmetric unit until obtention of the best rotation/translation parameters. The model was first refined by rigid body refinement at low resolution, then refined against 3 Å data with the XPLOR program.The multiple isomorphous replacement method (MIR) is under evaluation to improve phases for location of the remaining 116 residues.

The optimization of the crystallization procedure (with NAD cofactor) will help to improve the diffraction and resolution.