Abstracts

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Oligomerization of Concanavalin A in live cells detected by fluctuation analysis

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We report an experimental study on ConcanavalinA (ConA) aggregation in live cells. In vitro, close to physiological temperature, ConA readily forms fibrils involving secondary structure changes leading to β-aggregate structures. The effect of ConA on cell cultures and formation of protein aggregates were measured by confocal fluorescence microscopy. In particular, we monitored protein aggregation in live cells by means N&B analysis, Cross-N&B and RICS. N&B showed the aggregation kinetic and the progressive formation of ConA oligomers at cell surface. This suggests that, at cell membrane where local concentration is higher, nucleation sites for aggregation are provided. In parallel, the morphology of the cells changes indicating the progressive cell compaction and death. Aggregation and binding of small aggregates to the cell surface were assessed by RICS: it is possible to distinguish regions where small aggregates are diffusing and regions where they are bound to the cell. Oligomers formation may stimulate non-specific cellular responses due to the exposure of reactive regions of protein structure and of progressive formation of cross−β structures. Moreover, aggregates stoichiometry was measured during the kinetic by Cross-Variance N&B.