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### ACCEPTED MANUSCRIPT

# Sizing protein-templated gold nanoclusters by time resolved fluorescence anisotropy decay measurements\*

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#### Abstract

Protein-templated gold nanoclusters (AuNCs) are very attractive due to their unique fluorescence properties. A major problem however may arise due to protein structure changes upon the nucleation of an AuNC within the protein for any future use as *in vivo* probes, for instance. In this work, we propose a simple and reliable fluorescence based technique measuring the hydrodynamic size of protein-templated gold nanoclusters. This technique uses the relation between the time resolved fluorescence anisotropy decay and the hydrodynamic volume, through the rotational correlation time. We determine the molecular size of protein-directed AuNCs, with protein templates of increasing sizes, e.g. insulin, lysozyme, and bovine serum albumin (BSA). The comparison of sizes obtained by other techniques (e.g. dynamic light scattering and small-angle X-ray scattering) between bare and gold clusters containing proteins allows us to address the volume changes either induced by conformational changes (for BSA) or the formation of protein dimers (for insulin and lysozyme) during cluster formation and incorporation.

Keywords: Size, proteins, gold nanoclusters, fluorescence anisotropy, unfolding, oligomers

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