Title

Fluorescence correlation and anisotropy approaches for the study of nuclear receptor/coactivator complexes

Journal

BIOPHYSICAL JOURNAL, 80(1)

ISSN

0006-3495

Authors

Margeat, E
Poujol, N
Chen, Y
et al.

Publication Date

2001

Emmanuel Margeat, Nicolas Poujol, Yan Chen, Joachim D Müller, Abdelhay Boulahtouf, Enrico Gratton, Vincent Cavailles, and Catherine A Royer.

Fluorescence correlation and anisotropy approaches the study of nuclear receptor/coactivator complexes.

45th Annual Meeting of the Biophysical Society, Boston, Massachusetts, 2001.
Biophys J. 2001; 80(1 Pt 2): 361a.

Abstract

Nuclear receptors act as ligand-inducible transcription factors. Agonist binding leads to interaction with coactivator proteins, and to the assembly of the general transcription machinery. In addition to structural information, a thorough understanding of transcriptional activation by the nuclear receptors requires the characterization of the thermodynamic parameters governing these protein / protein interactions. In this work, fluorescence spectroscopy has been used for the in-depth investigation of the interaction between Estrogen Receptor (ER) and the nuclear receptor coactivator SRC1570-780 labeled with Alexa 488. In a first step, photon counting histogram analysis has been applied on fluorescence correlation data for the elucidation of the stoichiometry of the complex. We show that, even at high concentration of fluorescent coactivator, outside the single molecule limits, one can directly measure the number of molecules in the complex, leading to a 1 SRC-1 molecule per ER dimer stoichiometry. Then, using fluorescence polarization, we address the influence of different ER agonists on the affinity between SRC-1 and ER α and β isoforms. This work emphasizes the use of photon counting histogram analysis for the resolution of the stoichiometry of biomolecular complexes, and provides a new tool for the discovery of new ligands for the nuclear receptor superfamily.