Title: **Screening of compounds affecting the interaction between domains of Src protein**

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Date: June 2019

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The c-Src protein is the leading member of the Src family non-receptor tyrosine kinases (SFKs) involved in many signalling pathways. The SH3, SH2 and kinase domains of SFK members display large sequence and structural similarity. But each SFKs has unique sequences called them as Unique domain (UD). This is an intrinsically disordered region and it is not clear its function. However, several studies demonstrated that UD is crucial for c-Src regulatory activity.

The aim of this study is discovering drugs that can bind to Unique or adjacent domains of c-Src. To do that, a Förster resonance energy transfer (FRET) biosensor composed with SH3, SH4 and Unique domain of c-Src has been obtained by plasmid transformation, protein expression and chromatography purification. FRET pair fluorophores for the biosensor were mClover3 (green) and mRuby3 (red). The binding of some drugs caused a change between the distance between the two fluorophores in FRET-biosensor leading to measure changes in fluorescence spectra. Drugs were classified according to their FRET's effect. Only two drugs presented a pronounced FRET signal (L13B-C2 and L10B-C9). Finally, binding assays were performed with the best drugs in order to determine the binding association constant assuming that they followed a model of binding 1:1 of protein:ligand. Based on the values obtained, L10B-C9 presents a higher affinity for the FRET-biosensor than L13B-C2 (mean of 0,0075 vs 0,006μM^{-1}, respectively).

Keywords: Src kinase, drug screening, Förster Resonance Energy Transfer (FRET), fluorescence, binding constant.