Characterizing the kinetics of receptor-antagonist interactions using a fluorescence polarization competition method on a plate reader

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A fluorescence polarization method has been developed using the Analyst plate reader to measure the association and dissociation rates of small molecule antagonists to the leukocyte receptor, LFA-1 (CD11a/CD18). Association curves are measured for a fluorescent probe binding to the receptor with and without a competing antagonist. The method enables data collection beginning 9 sec after reagent addition, using the minimal 5 sec interval between reads. The association curves are fit using global analysis to determine the probe and antagonists' on and off rates and affinities for LFA-1. These kinetic polarization assays have enhanced our understanding of the LFA-1/antagonist binding interaction and have potential for use with other purified receptor and cell bound receptor/ligand binding reactions.