

Magnetic force driven dissociation kinetics in case-mixed protein interaction assays

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Magnetic force driven dissociation kinetics in case-mixed protein interaction assays

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We quantify dissociation kinetics in assays with mixed specific and non-specific protein interactions. Ligand coupled superparamagnetic particles are incubated on surfaces coated with a mixture of specific receptors and non-specifically interacting proteins. Consequently, a case-mixed population of surface bound particles is formed with different binding strengths. Magnetic field gradients were used to apply translational forces on the bound complexes, either constant or increasing in time (applying a loading rate). Using a multi-component dissociation analysis, we observe case-dependent dissociation mechanisms of the particles. The classical Bell and Evans model successfully describes bond dissociation from the deep potential well of a specific bond. Bond characteristics in terms of rate constants, energy barriers and minima's in the dissociation pathway are revealed for the anti-biotin/biotin and streptavidin/biotin bond; and are in good agreement with values from SPR, other force clamp techniques, and molecular dynamics calculations. The particles bind non-specifically via interactions that show a force induced dissociation mechanism distinctly different from that of the specifically bound particles. The ability to rapidly differentiate and characterize specific and non-specific protein interactions in parallel, and affinity-rank different protein-ligand interactions on the basis of their binding pocket characteristics, will find various applications.