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Selection of aptamers against Lactoferrin based on silver enhanced and fluorescence-activated cell sorting

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Abstract:

We report a novel method for efficiently screening aptamers from a complex ssDNA library based on silver decahedral nanoparticles (AgNP) and fluorescence activated cell sorting (FACS). In this method, target protein (lactoferrin) and negative proteins (α-lactalbumin, β-lactoglobulin, bovine serum albumin, casein) were respectively immobilized on polystyrene microspheres (PS) to form PS^{Lac}, $PS^{\alpha\text{-}Lac}$, $PS^{\beta\text{-}Lac}$, PS^{BSA} and PS^{Cas}. PS^{Lac} was firstly interacted with Cy5 labeled library (Lib), then hybridized with Cy5 modified silver decahedral nanoparticles ($AgNP^{Cy5}$) to form PS^{Lac}/Lib/$AgNP^{Cy5}$ conjugates. FACS was used to separate and collect PS^{Lac}/Lib/$AgNP^{Cy5}$ conjugates from complicated complex. AgNP was used to increase the fluorescence intensity in the selecting process and choose non-self-hybridization of Lib. Six aptamers (Ylac1, Ylac4, Ylac5, Ylac6, Ylac8 and Ylac9) were obtained after five-round of selection. These aptamers showed good specificity towards lactoferrin in the presence of negative proteins. The equilibrium dissociation constants (K_d) of six aptamers were calculated and all were in the nanomolar range. In a word, AgNP-FACS SELEX (AgFACS-SELEX) is a rapid, sensitive and highly efficient method for screening aptamers.

Graphical Abstract