Biological and physiological properties of reverse ankyrin engineered for dimer construction to enhance HIV-1 capsid interaction

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Abstract

Assembly and budding in the late-stage of human immunodeficiency virus type 1 (HIV-1) production relies on the polymerization of Gag protein at the inner leaflet of the plasma membrane. We previously generated an ankyrin repeat protein (Ank1D4) that specifically interacts with the CAp24 protein. This study aimed to improve the binding activity of Ank1D4 by generating two platforms for the Ank1D4 dimer. The design of these constructs featured a distinct orientation of monomeric Ank1D4 connected by a linker peptide (G₄S)₄. The binding surfaces in either dimer generated from the C-terminus of the Ank1D4 monomer linked with the N-terminus of another monomer (Ank1D4_{NC-NC}) or its inverted form (Ank1D4_{NC-CN}), similar to monomeric Ank1D4. The interaction of Ank1D4_{NC-CN} with CAp24 from capture ELISA was significantly greater than that of Ank1D4_{NC-NC} and the parental Ank1D4. The bifunctional characteristic of Ank1D4_{NC-CN} was further demonstrated using sandwich ELISA. The binding kinetics of these ankyrins were evaluated using bio-layer interferometry analysis. The K_D of Ank1D4_{NC-CN}, Ank1D4_{NC-NC} and monomeric Ank1D4 was 3.5 nM, 53.7 nM, and 126.2 nM, respectively. The dynamics of the interdomain linker and the behavior of ankyrin dimers were investigated *in silico*. Upon the binding distance calculation from the candidate structures, the achievement in obtaining double active sites is more possible in Ank1D4_{NC-CN}. The CD spectroscopic data indicated that secondary structure of dimer forms resemble Ank1D4 monomer α -helical content. This finding confers the strategy to generate dimer from rigid scaffold for acquiring the binding avidity.

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Bifunctional ankyrin with sequence inversion.doc available at <https://authorea.com/users/441324/articles/541848-biological-and-physiological-properties-of-reverse-ankyrin-engineered-for-dimer-construction-to-enhance-hiv-1-capsid-interaction>

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Variants	$k_{on}(1/Ms)$	$k_{off}(1/s)$	$K_D(nM)$
Monomeric Ank1D4	2.1×10^5	2.7×10^{-2}	126.2 ± 3.6
Dimeric Ank1D4 _{NC-NC}	1.9×10^5	1.0×10^{-2}	53.7 ± 5.0
Dimeric Ank1D4 _{NC-CN}	5.8×10^4	1.5×10^{-4}	3.5 ± 2.9

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