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Nanobody Affinity Optimization Through Site-Directed Mutagenesis

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Magistro baigiamasis darbas

Nanokūnų afiniteto optimizavimas taikant kryptingąją mutagenezę

SANTRAUKA

Nanokūnai, dar vadinami VHH domenais, yra mažiausi antigeną prisijungiantys fragmentai, kilę iš kupranugariniams gyvūnams būdingos sunkiųjų grandinių antikūnų klasės (angl. *heavy-chain-only antibodies, HcAbs*). Dėl kompaktiškos struktūros, stabilumo ir didelio specifiškumo nanokūnai vis dažniau naudojami kaip taikinių atpažinimo ir detekcijos molekulės biotechnologijoje. Vis dėlto, priklausomai nuo numatomos taikymo srities, šių baltymų jungimosi su taikiniu savybės ne visada yra pakankamos, todėl atsiranda afiniškumo optimizavimo strategijų poreikis.

Šio darbo tikslas buvo sukurti eksperimentinę kryptingos mutagenezės strategiją nanokūno afiniteto optimizavimui, leidžiančią įvertinti pasirinktų antigeno atpažinime dalyvaujančių baltymo sričių funkcinę reikšmę. Darbe buvo taikyta bioinformatinė sekos analizė, kryptinga pasirinktų aminorūgščių mutagenezė, rekombinantinė mutantinių nanokūno variantų ekspresija ir jų sąveikos su taikiniu vertinimas.

Šis principas suteikia pagrindą racionaliai nanokūnų inžinerijai, kai trūksta eksperimentiškai nustatytos struktūrinės informacijos apie nanokūną ir jo sąveiką su taikiniu. Apibendrinant, darbas prisideda prie sisteminių nanokūnų optimizavimo strategijų vystymo biotechnologinėms taikymo sritims.

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ABSTRACT

Nanobodies, also known as VHH domains, are the smallest antigen-binding fragments, derived from camelid heavy-chain-only antibodies (HcAbs). Due to their compact structure, stability, and high specificity, they are increasingly recognized as versatile affinity ligands in biotechnology. However, depending on the intended application, their binding properties may require further improvement, creating a need for targeted optimization strategies.

The aim of this study was to develop an experimental framework for site-directed mutagenesis-based nanobody affinity optimization, enabling the functional evaluation of selected protein regions involved in antigen recognition. The work included bioinformatic sequence analysis, site-directed mutagenesis of selected amino acid positions, recombinant expression of mutant nanobody variants, and assessment of their interaction with the target molecule.

This approach provides a framework for rational nanobody engineering when experimentally determined structural information is not available. Overall, the work contributes to the development of systematic strategies for nanobody optimization in biotechnological applications.

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