Anti-idiotypic nanobody alkaline phosphatase fusion protein-triggered on-off-on fluorescence immunosensor for Aflatoxin in Cereals

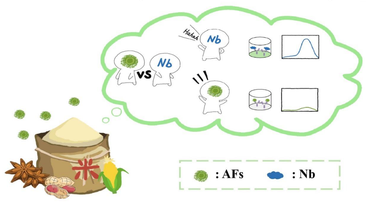
基于抗独特型纳米抗体-碱性磷酸酶融合蛋白触发的开-关荧光免疫传感器检测谷物中的黄曲霉毒素

小组成员：杨越凡

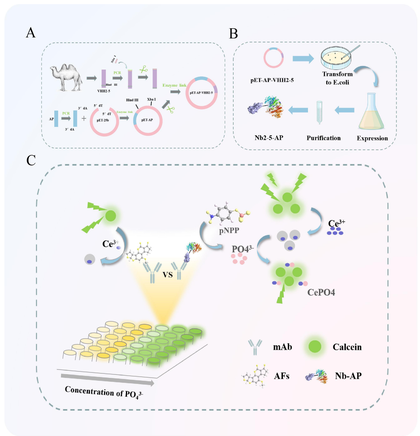
指导老师：王妍入（食品科学与工程学院 食品快速检测实验室）

Abstract

Nanobodies (Nbs) are widely used in immunoassays with the advantages of small size and high stability. Here, the nanobody employed as the surrogate of aflatoxin antigen and the recognition mechanism of anti-aflatoxin mAb with nanobody was studied by molecular modeling, which verified the feasibility of Nbs as antigen substitutes. On this basis, a nanobody-alkaline phosphatase fusion protein (Nb-AP) was constructed and highly sensitive “on-off-on” fluorescent immunosensor (OFO-FL immunosensor) based on the calcein/Ce3+ system was developed for aflatoxin quantification. Briefly, calcein served as a signal transducer, and its fluorescence can be quenched after being bound with Ce3+. In the presence of Nb-AP, AP catalyzed p-nitrophenyl phosphate to generate orthophosphate, which competes in binding with Ce3+ leading to fluorescence recovery. The method has a linearity range of 0.005–100 ng/mL, and the IC50 of the OFO-FL immunosensor was 0.063 ng/mL, which was 18-fold lower than that of conventional ELISA. The assay was successfully applied in food samples with a recovery of 88-121%.



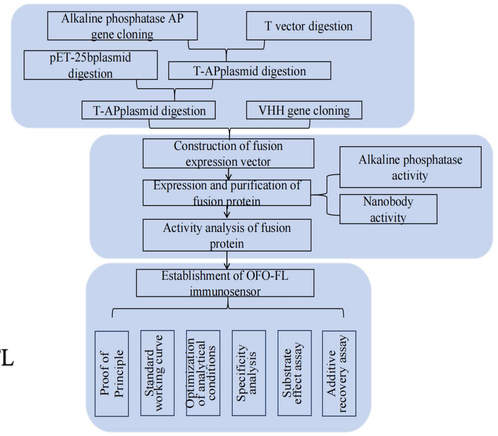
Schematic Diagram



Fusion vector construction

Fusion protein expression

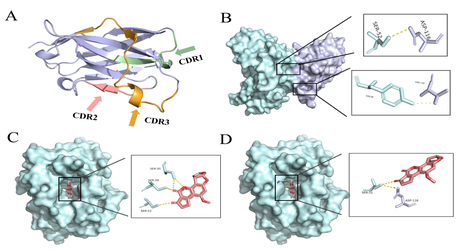
Establish OFO-FL immunosensor



Results

1.Homology modeling and molecular docking of Nb2-5 with mAb 1C11

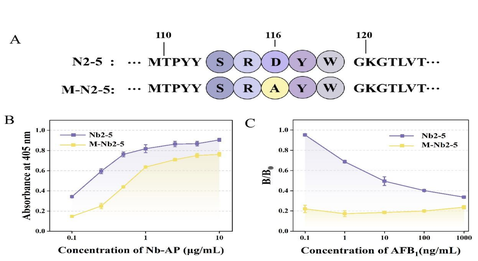
(1) Modeling prediction



·The homology modelling of mAb 1C11 and Nb2-5 was performed using the SWISS-MODEL server.

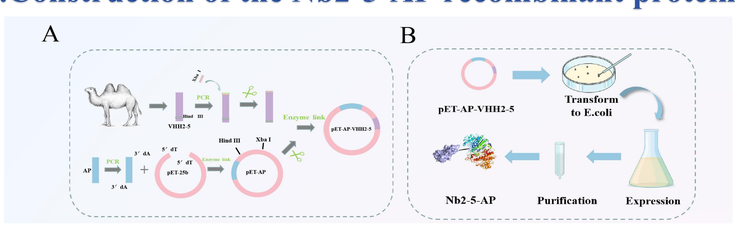
·The amino acid sequence is submitted, and the result with the highest Global Model Quality Estimate is selected as the target model.

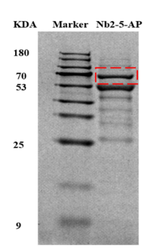
(2) Corroboration of the Predictions



·These results demonstrate that Asp 116 is responsible for the parent nanobody’s competition with AFB1 towards mAb 1C11.

2.Construction of the Nb2-5-AP recombinant protein





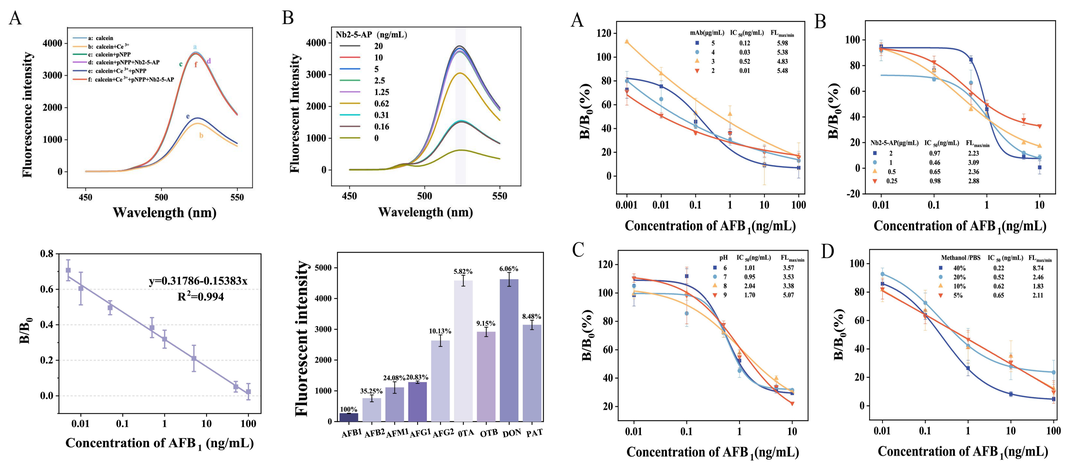
·Construction of the Nb2-5-AP recombinant plasmid

·Expression and purification of Nb2-5-AP fusion protein

·To verify the successful expression of the fusion protein, the Nb2-5-AP fusion protein was analyzed using SDS-PAGE after purification by the Ni-affinity chromatography column.

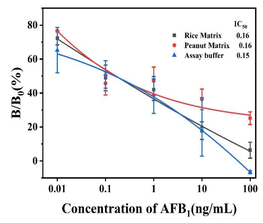
Results

3.Construction of Nb2-5-AP triggered OFO-FL immunosensor



4. Sample Analysis

Samples (n = 3)	This assay				HPLC			
	Added(ng/mL)	Found(ng/mL)	Recovery (%)	RSD (%)	Added(ng/mL)	Found(ng/mL)	Recovery (%)	RSD (%)
Rice	2.5	2.24	89.60	0.24	2.5	2.44	97.60	0.35
	1	1.20	120.00	0.51	1	1.10	110.00	0.28
	0.25	0.27	108.00	1.33	0.25	0.21	84.00	0.22
Peanut	2.5	2.79	111.60	0.67	2.5	2.37	94.80	0.39
	1	1.21	121.00	1.10	1	1.11	111.00	0.23
	0.25	0.22	88.00	1.35	0.25	0.26	104.00	0.34



Since the accuracy of an immunoassay can be affected by the sample matrix, it is essential to minimize or eliminate matrix effects prior.

HPLC analysis was employed to verify the accuracy of the OFO-FL immunosensor. The results detected by the two methods(OFO-FL immunosensor and HPLC) showed good consistency, indicating the excellent reliability of the proposed method.

Conclusion

In this work, the recognition mechanism of anti-idiotype nanobody Nb2-5 with primary antibody mAb1C11 was studied by molecular docking and verified through alanine scanning mutation. On this basis, a versatile “on-off-on” fluorescent immunosensor for AFs detection was developed by using an anti-idiotype nanobody fused with AP as an antigen surrogate. It exhibited high sensitivity, with an 18-fold improvement compared with the conventional ELISA. In addition, the assay showed good accuracy and reliability in real sample analysis. Attributed to the dual functions of Nb-AP, this one-step fluorescent immunosensor integrated the advantages of being rapid, sensitive, and environmental-friendly, offering a promising tool for mycotoxin detection in food and environment.

Acknowledgements

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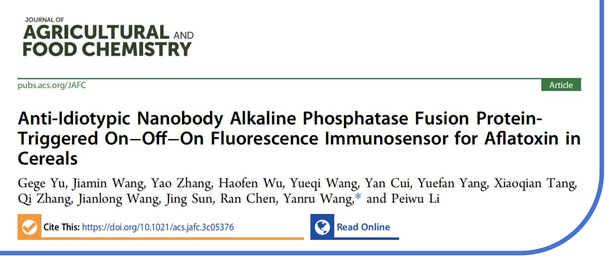

Communication

指导教师：王妍入老师，食品科学与工程学院，邮箱：yanruwang@nwafu.edu.cn
负责人：杨越凡，食品科学与工程学院，邮箱：18991329226@163.com