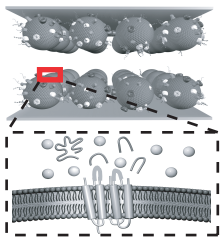
Nanoxis
Membrane protein solutions

Application Note

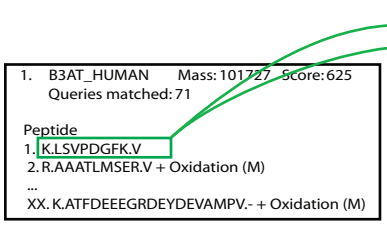
Improve your antigen epitope identification using LPI™ FlowCell

Straightforward experimental approach to isolate and identify surface-exposed linear epitopes of membrane protein targets

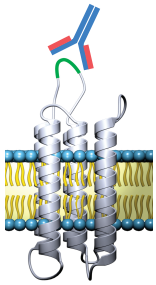
The Nanoxis way



Immobilized membrane proteins are digested for a short time. Peptides are created from surface exposed parts and identified by LC-MS/MS.



A peptide, highly accesible for the protease in the 3D structure of the protein, is chosen as antigen epitope.

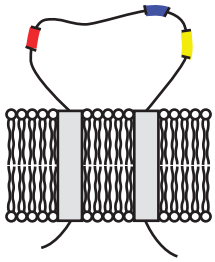


The identified epitope is located in an exposed loop region that is accessible by the antibody.

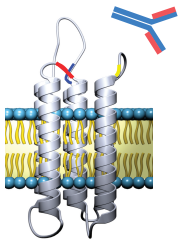
Only **one** antibody needs to be produced, showing high affinity and specificity.

The traditional way

Prediction tools deliver several potential epitopes based on hydrophobicity, similarity and statistics. In silico only.



Predicted structure of the protein. 2-5 epitopes are selected based on prediction algorithms.



The predicted epitope might be located in a loop region that is buried deep within the **actual** 3D structure of the protein. Not accessible by the antibody.

2-5 antibodies produced. Risk for no or very poor affinity and specificity of antibody.

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Use of LPI™ FlowCell to identify epitopes of a bacterial membrane protein

Summary

LPI™ FlowCell was used to identify surface-exposed epitopes of a target membrane protein in ANAMMOX (Anaerobic AMMonium Oxidation) bacteria*. A preparation of the anammoxosome (an internal organelle-like structure) was profiled using LPI™ FlowCell and the results revealed several interesting membrane proteins that could be involved in the anammox reaction. One of the identified membrane proteins was of special interest to the function of the anammoxosome and was chosen as target for immunogold stain. This was performed by raising antibodies against one of the peptides identified in the profiling study. The chosen peptide corresponds to an easily accessible and surface-exposed linear epitope of the target membrane protein.

Method

Profiling

A preparation of anammoxosomes was profiled using LPI™ FlowCell. In brief, the anammoxosomes were prepared according to protocols found in the literature. The anammoxosomes were sonicated to produce small vesicles and immobilized in LPI™ FlowCell. The immobilized membrane preparation was subjected to a tryptic digest and the resulting tryptic fragments were eluted from LPI™ FlowCell and analyzed using LC-MS/MS. The proteins of the anammoxosome were identified and some were classified as candidates for the anammox reaction.

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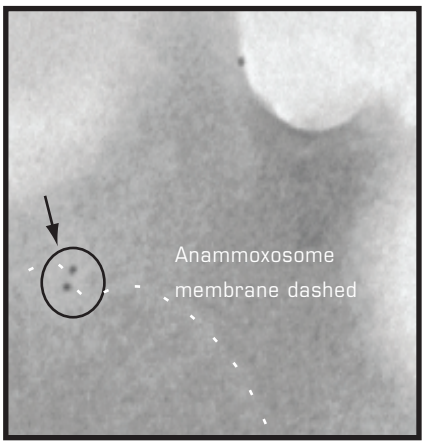
Epitope identification and antibody production

One of the membrane proteins identified in the preparation of anammoxosomes was chosen as target for immunogold stain and EM (electron microscopy) studies. Through the profiling study, a proteotypic peptide emanating from the target protein was chosen as a linear epitope for the antibody production. These types of linear epitopes are ideal as antibody targets. Antibodies were raised in rabbits against the target peptide and purified.

Results

Immunogold stain

Fixed bacteria were sliced in thin layers and subjected to the target antibody, followed by a secondary IgG antibody conjugated to gold particles. Visualization was performed using a transmission electron microscope. Interpretation of the images led to the conclusion that the target protein belonged to the anammoxosome.



Immunogold electron microscopy image showing a part of a thin-sectioned anammox bacterium. The subcellular location is shown by the gold labeled anti-rabbit IgG secondary antibody (black dots), bound to the epitope specific rabbit antibody, on the target membrane protein.

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