In vitro screening of bispecific antibody fragments against GABA-A receptors

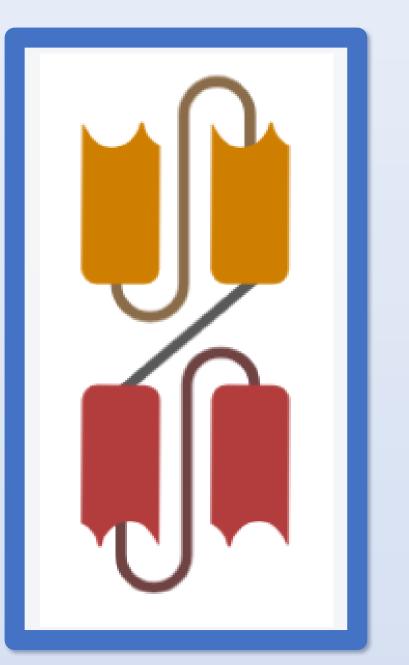


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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS



Schizophrenia is a mental disorder that affects person's perception of reality. Symptoms include hallucinations, delusions and paranoia. The pathophysiology is largely unknown.



STUDY AIM

 Produce bispecific antibody fragments that target GABA-A

A prevailing hypothesis is that loss of inhibitory pathways, mainly gammaaminobutyric acid (GABA) neurotransmitting pathways causes runaway signalling resulting in schizophrenia.

Fig.1 Visual representation of di-scFv antibody fragment

receptors and transferrin receptors (Tfr).

 Immuno-characterisation of antibody fragments and verifying their specific binding properties using dissociationenhanced lanthanide fluorescence immunoassay (DELFIA), fluorescent microscopy and confocal microscopy.

MATERIALS AND METHODS

Bispecific di-single chain variable fragment (GABA-A/Tfr di-scFv) antibody fragments are produces using Expi293™ mammalian cells. Produced antibody fragments are evaluated using DELFIA and

RESULTS AND CONCLUSIONS

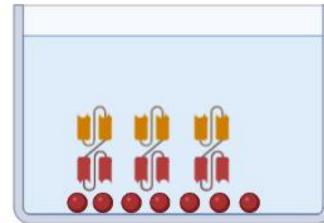
Immuno-characterisation of di-scFv construct revealed that the binding is specific against their intended target, extracellular parts of GABA-A receptor's alpha subunit. DiscFv's GABA-A binding part is working as intended but its

immunofluorescent microscopy.

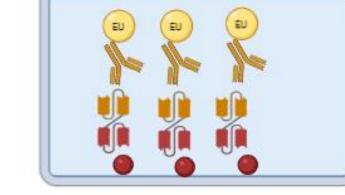
DELFIA Di-scFv specificity evaluation



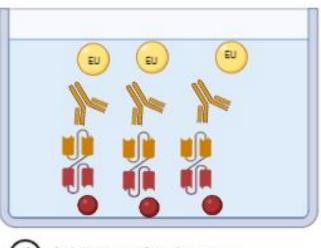
 Streptavidin coated wells are coated with biotinylated target antigens(GABA-A subunits)



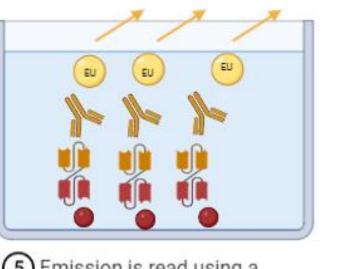
2 Di-scFv are added



Europium labeled antibody binds to the di-scFv



Addition of enhancer solution dissociates Europium from the secondary antibody



5 Emission is read using a Viktor plate reader

Immunofluorescent microscopy Transferrin receptor binding

affinity is less than we had hoped.

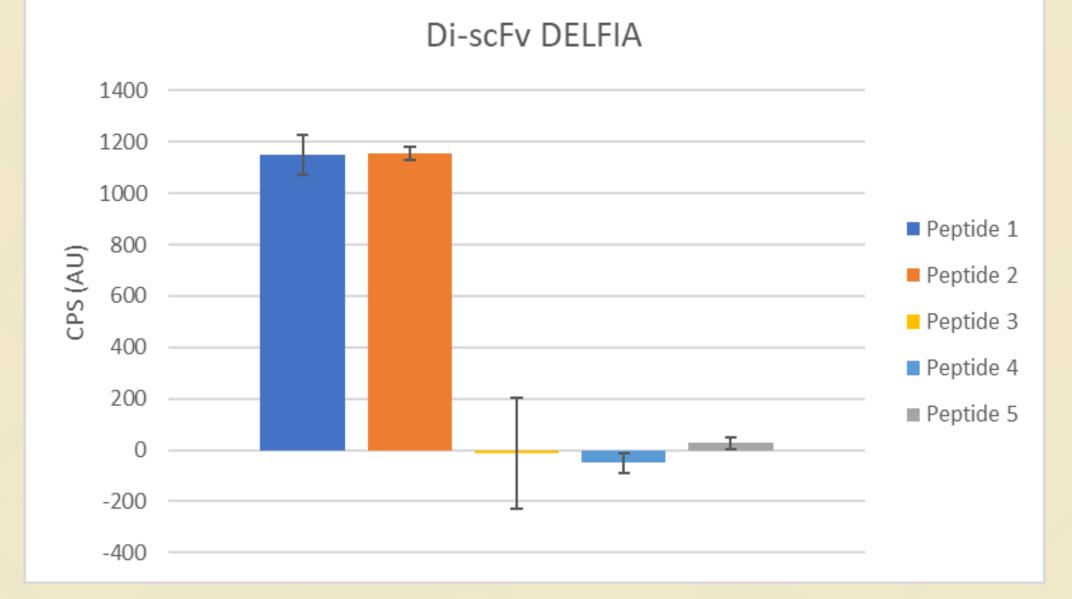


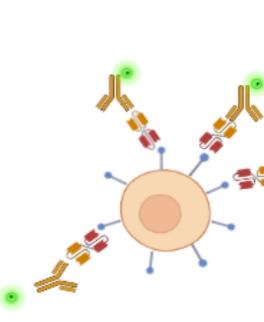
Fig.2 Immuno-characterisation of di-scFv antibody fragment using DELFIA. Peptides 1 and 2 are N-terminal extracellular parts of alpha 1 subunit of GABA-A receptor and are the intended targets. Peptides 2, 4 and 5 are other parts of the alpha 1 and 2 subunits that are not desirable targets.

Next step of this study is to optimize the immunofluorescence for the fluorescent microscopy in order to verify transferrin receptor binding in cells.

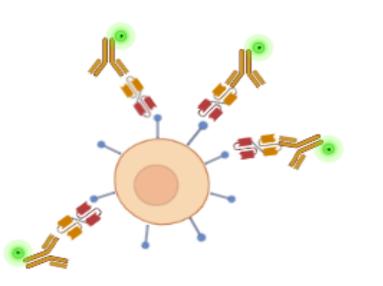
1. bEnd.3 mouse brain endothelial

cell transferrin

receptors bind with GABA-A/ Tfr di-scFv



 Fluorophore labeled secondary antibodies bind to the di-scFv



 Fluorescence microscope is used to verify antibody binding to the transferrin receptors

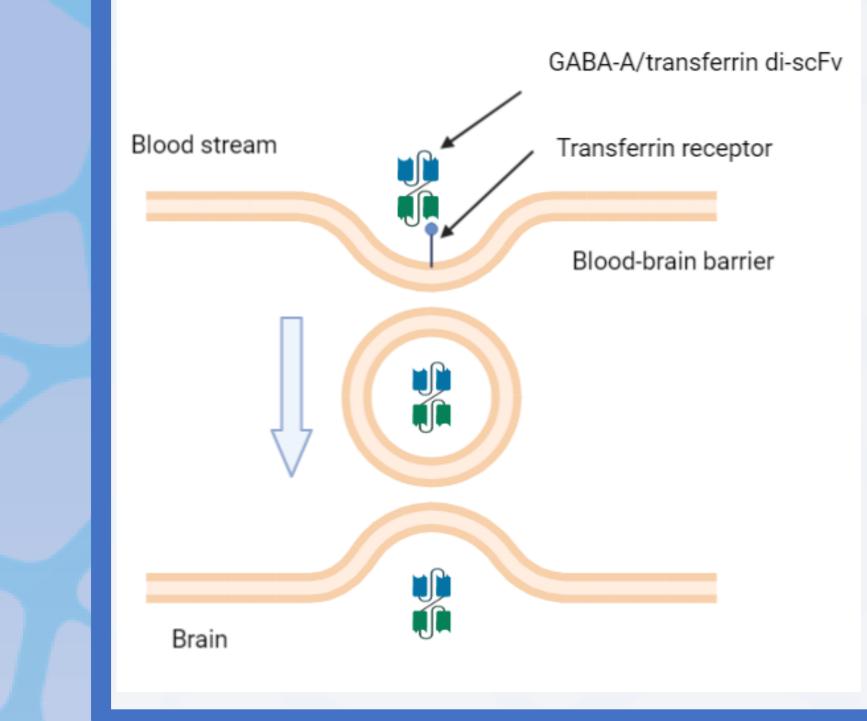


Fig.3 Bispecific antibody fragment transferrin induced transcytosis