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

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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

BACKGROUND

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

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

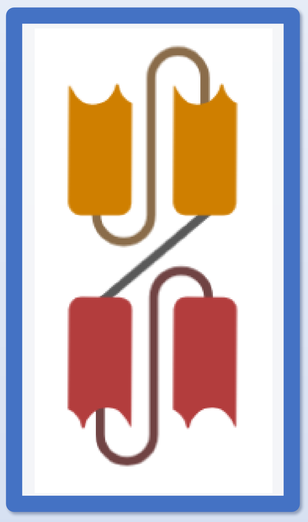


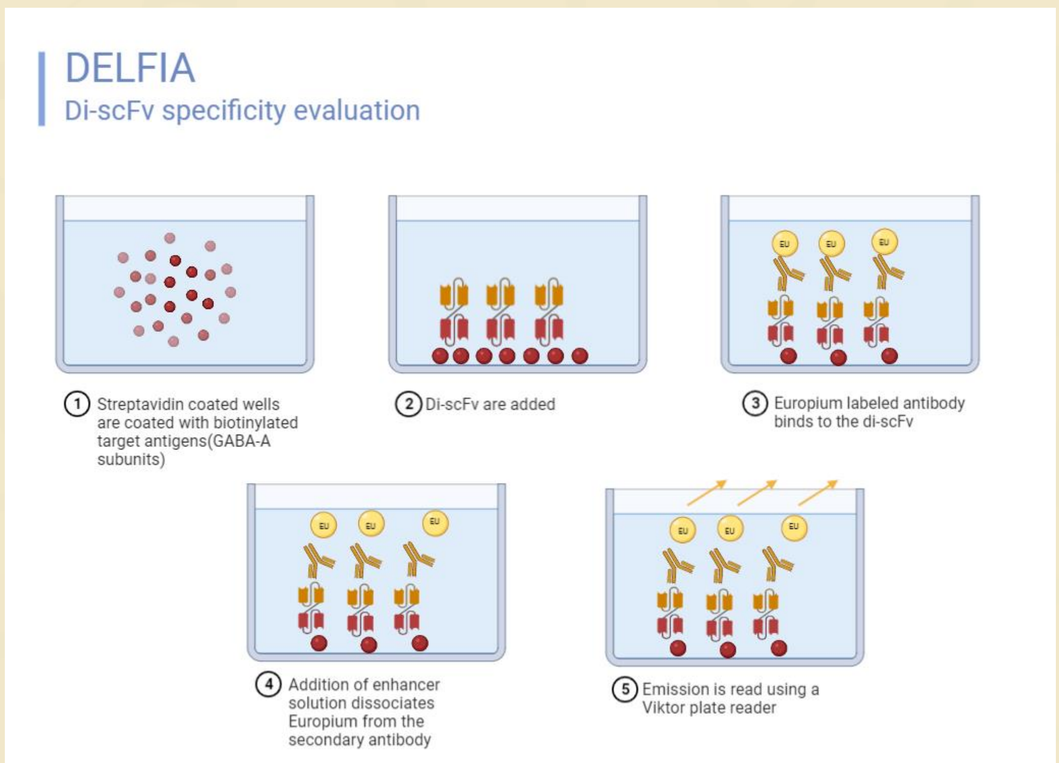
Fig.1 *Visual representation of di-scFv antibody fragment*

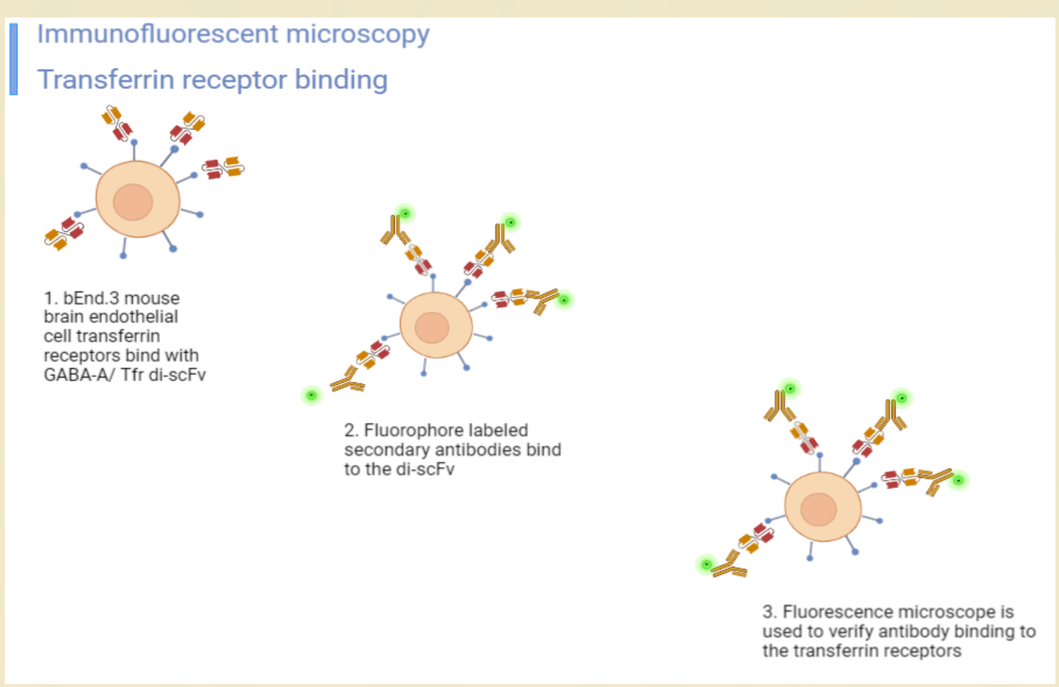
STUDY AIM

- Produce bispecific antibody fragments that target GABA-A receptors and transferrin receptors (Tfr).
- Immuno-characterisation of antibody fragments and verifying their specific binding properties using dissociation-enhanced 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 immunofluorescent microscopy.





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. Di-scFv's GABA-A binding part is working as intended but its affinity is less than we had hoped.

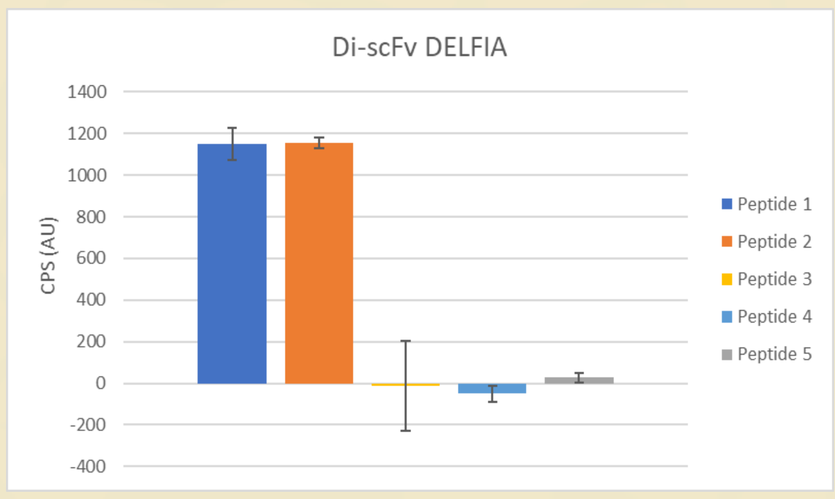


Fig.2 *Immuno-characterisation of di-scFv antibody fragment using DELFIA. Peptides 1 and 2 are N-terminal extracellular parts of alpha 1 subunit of GABA-A receptor and are the intended targets. Peptides 2, 4 and 5 are other parts of the alpha 1 and 2 subunits that are not desirable targets.*

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

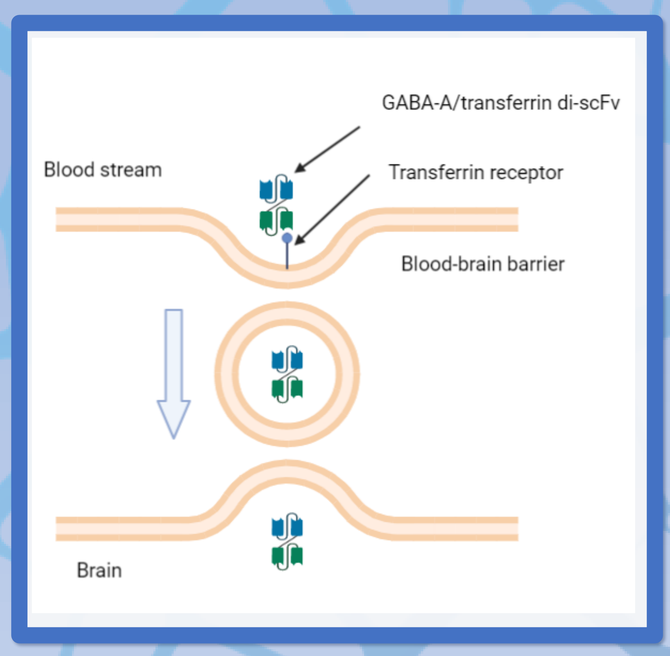


Fig.3 *Bispecific antibody fragment transferrin induced transcytosis*