Development of an AI Platform for Nanobody Design Targeting for Laser-induced Retinal Injury

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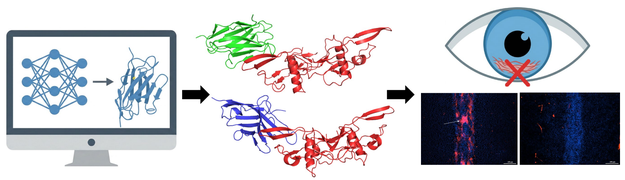
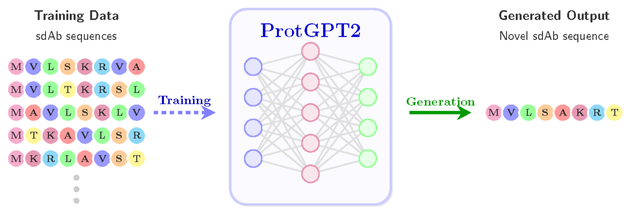
Using AI to develop protein binders to prevent vision loss from directed energy weapons



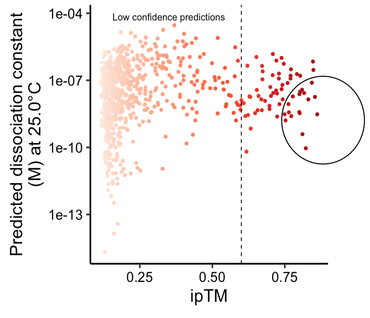
PLM-generated sequences | T38 and T381 sdAbs | Inhibit TGFβ2-induced fibrosis

Fine-tuning ProtGPT2 for sdAb Generation



ProtGPT2 was fine-tuned on a custom dataset of proteins known to bind TGFβ2 and similar proteins and used to predict single-domain antibody (sdAb) sequences..

Sequence selection prior to synthesis



Predicted sequences were modeled with AlphaFold and the characteristics of dissociation constants, structural integrity, sequence length, isoelectric point, and sequence similarity were used to select the top candidates.

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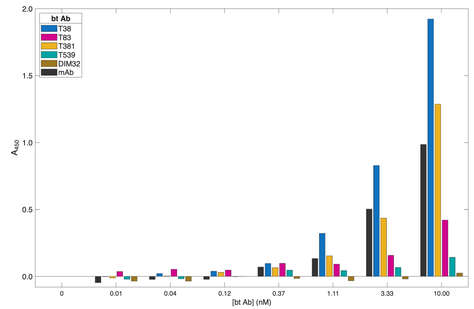
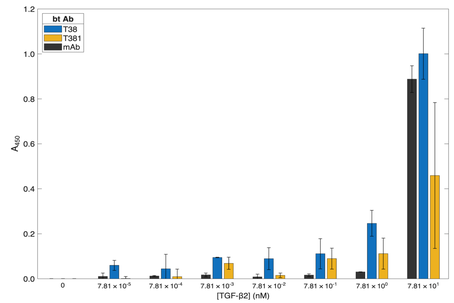
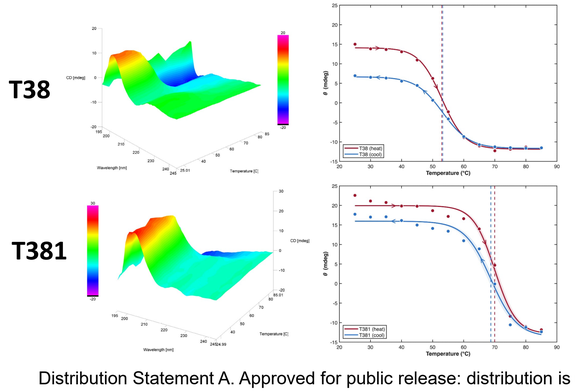
Direct binding assay for the predicted bt-sdAbs against TGFβ2



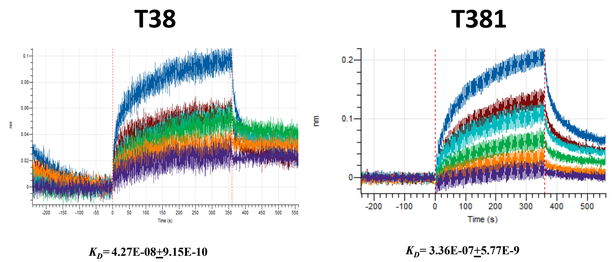
Limit of detection (LOD) of TGFβ2



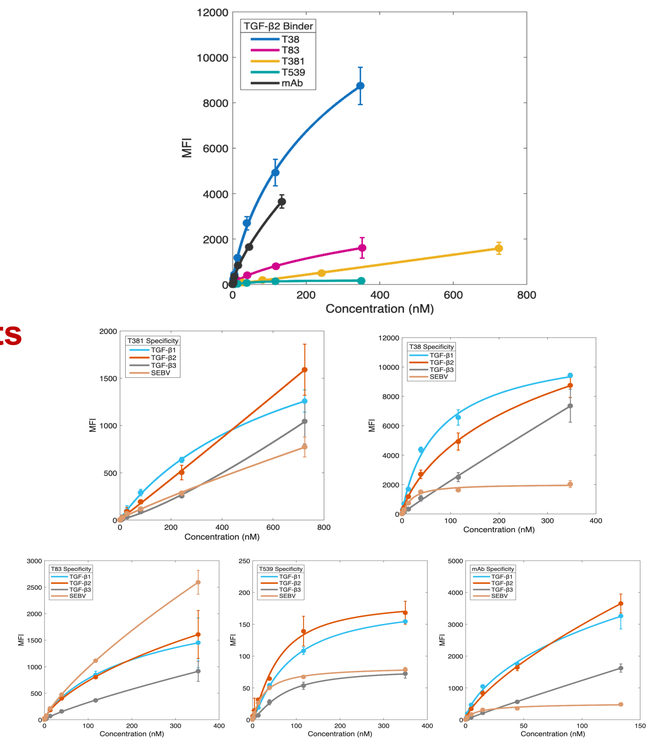
Circular Dichroism (CD) measurements for T38 and T381



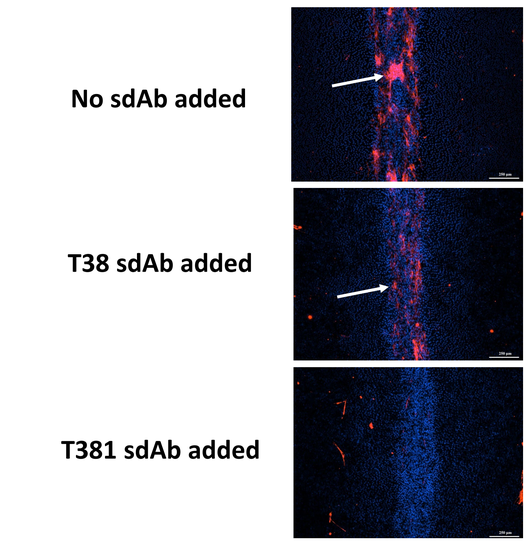
Measurement of the binding kinetics of T38 and T381 to TGFβ2 by Biolayer Interferometry (BLI)



Specificity and affinity of bt-sdAbs to TGFβ isoforms via MagPlex bead direct binding assay



Synthetic sdAb Inhibition of TGFβ2-Induced Fibrosis



The synthetic sdAbs were able to inhibit or totally eliminate TGFβ2-Induced fibrosis in a scratch wound that was introduced to a monolayer of human iPSC-RPE cells in the presence of 10 ng/mL of TGFβ2 for 14 days.

Conclusions

- A fine-tuned protein language model was used to predict potential binders for TGFβ2
- The binders were screened computationally to determine the most promising candidates
- Those proteins were synthesized and tested successfully against TGFβ2 *in vitro*
- The best binders *in vitro* were found to inhibit fibrosis in human iPSC-RPE cells

Acknowledgements

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