COMPUTATIONAL DESIGN OF NOVEL AMPAR AND NMDAR PEPTIDE MODULATORS.

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ABSTRACT

AMPA and NMDA ionotropic glutamate receptors are the most prominent therapeutic targets in the broad spectrum of neurological diseases. Here we design novel peptide binders to these receptors, which are able to modulate these receptors' physiological activity. We demonstrate that designed peptides have high affinity to selected targets in comparison to small molecules agonists by using molecular dynamics methods as well as modulator activity on rat cortical neuronal cell cultures by fluorescent calcium microscopy.

1 INTRODUCTION.

There has been a recent progress in developing powerful ML tools for de novo protein binder design. However, design of peptide binders which can affect cell physiology is still a difficult task.

We developed a generative neural network incorporated in the AF2seq protocol (Goverde et al. (2023)) to generate binder peptides to AMPA and NMDA ionotropic glutamate receptors. These receptors play crucial role in multiple neurological and neurodegenerative diseases, though specific modulation of their activity provide a major opportunity in developing novel therapeutics (Traynelis et al. (2010)).

2 RESULTS.

To generate novel peptide binders, we designed an AF2-generator pipeline as shown in Figure 1A. Our generator, consisting of several transposed convolutional blocks, takes a noisy vector z of representation 1×100 and transforms it to tensor $X \in \mathbb{R}^{N\times L\times A}$ representing a one-hot-encoded sequences of generated peptide sequences (where N is the number of sequences, L is the length of all sequences and A = 20 is amino acids alphabet size). Then peptide sequences from the generator, combined with the masked template of target protein (NMDAR or AMPAR) were used to predict peptide-receptor complex via AF2. Our primary goal was to generate binders targeting specific active sites of selected proteins, thus to update generator parameters we calculated loss based on the minimal distance of generated peptide structure to selected hotspot residues of the receptor and AF2 plDDT metric.

Using described pipeline, we generated 20 binders to AMPAR ligand binding domain (where its orthosteric site is located) and NMDAR N-terminal domain (which consists the number of its allosteric modulation sites) after training our model for 50 epochs. After that we sort generated peptides based on the minimal distance to targeted interface of selected peptide with plDDT of peptide-receptor complex > 80 and peptide pTM > 0.8.

We chose 4 best designs for each receptor and tested their affinity to selected receptors using Umbrella Sampling simulations. Our simulations revealed that all of our selected peptides can bind to target with higher affinity (with $\Delta G_{binding}$ ranging from -7 to -16 kCal/Mol) than glutamate - native agonist ($\Delta G_{binding}$ around -5 kCal/Mol) (Figure 1B) of AMPAR and NMDAR.

The best peptide binders for both AMPAR (AMPAp) and NMDAR (NMDAp) were synthesized (cell-free method) and used for in vitro experiments. AMPAR and NMDAR are ligand-gated ion

channels and their activation by agonists leads to increase of of intracellular Ca^{2+} concentration ($[Ca^{2+}]_{in}$) in neural cells. Thus to evaluate the activity of designed peptides on selected receptors we measured ($[Ca^{2+}]_{in}$) in co-cultures of neurons and astrocytes isolated from cortex of neonatal rats using fluorescent imaging as described in (Holmström et al. (2013)). Our experiments demonstrated that designed peptides increase $[Ca^{2+}]_{in}$ in neural cells in concentration-dependent manner. Effect of AMPAp was significantly decreased in the presence of AMPAR's orthosteric site antagonist – CNQX and in Ca^{2+}-free media, but wasn't dependent on the NMDAR channel blocker of MK-801. Similar results were observed with NMDAp – its effect was dependent on NMDAR inhibitor of MK-801 and Ca^{2+}-free media, but wasn't dependent on the AMPAR antagonist (Figure 1C).

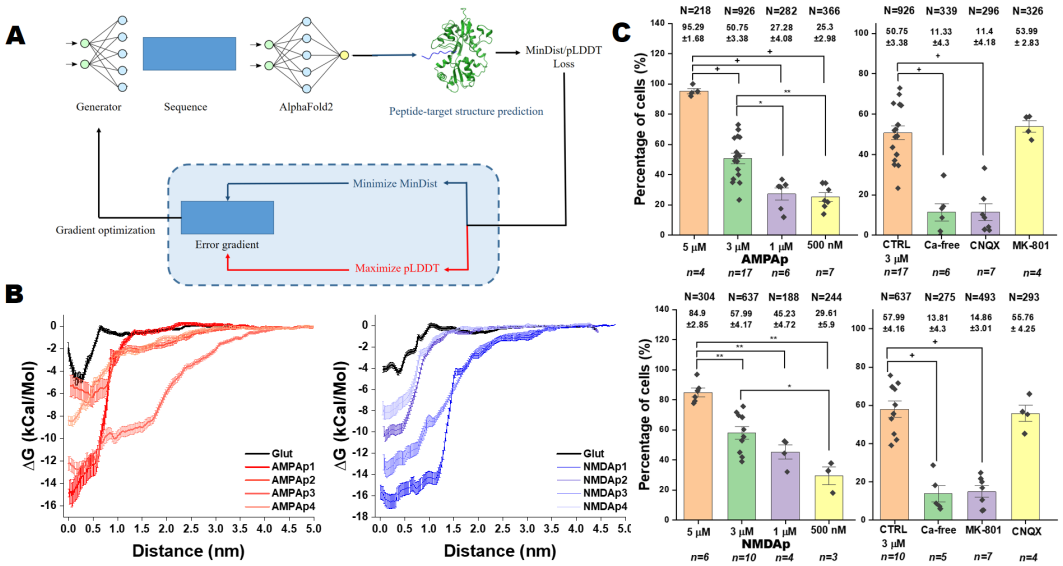

Figure 1: Generator based pipeline and overview of in silico and in vitro experimental characterization of the peptide designs. A) Structure of Generator-based AF2seq pipeline. B) Binding energy profiles of peptides to targets in comparison with classical agonists binding resulting from Umbrella sampling simulations. C) Functional Ca^{2+} fluorescent imaging experiments of peptides action on rat cortical neural cells cultures (+ - $p<1*10^{-6}$, ** - $p<1*10^{-3}$, * - $p<0.01$).

3 Conclusion.

In the current research we developed new neural network model for de novo peptide design and used it to generate new peptides which could selectively bind to ionotropic glutamate receptors AMPA and NMDA. The selectivity of the designed proteins was proved by in silico and in vitro experiments.

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