Supplementary Information for

State-Targeting Stabilization of Adenosine A$_{2A}$ Receptor by Fusing a Custom-Made De Novo Designed α-Helical Protein

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Supplementary Figures

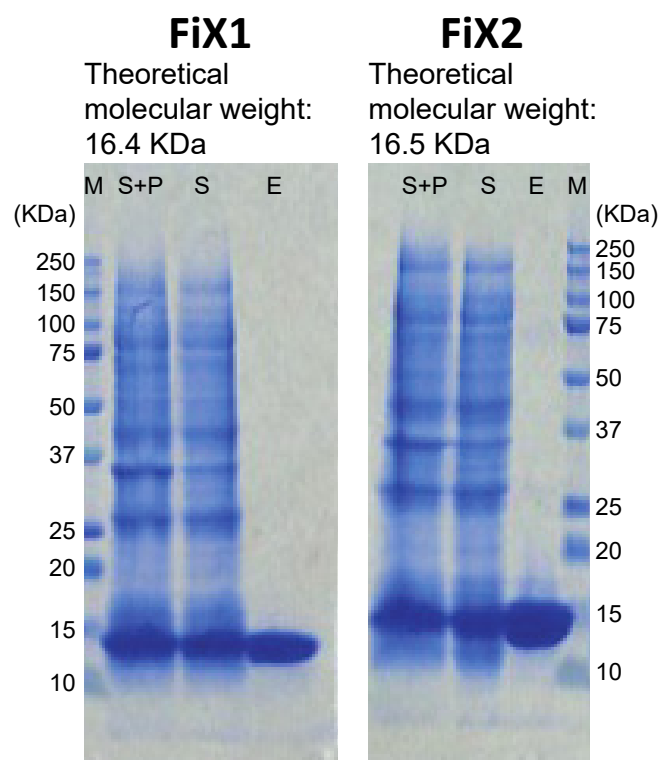1. **SDS-PAGE results for FiX1 and FiX2**

Supplementary Tables

1. **Sequence regions for A$_{2A}$R and fusion partner proteins in A$_{2A}$R chimeras**

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3. **Amino acid sequences of A$_{2A}$R fused with FiX1 and FiX2**

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Figure S1 | SDS-PAGE results for FiX1 and FiX2

M, S+P, S and E indicate marker, a mixture of supernatant (S) and pellet (P) after cell lysates; S, supernatant after centrifugation of cell lysates; and E, elution after Ni purification, respectively.

Table S1 | Sequence regions for A₂ₐR and fusion partner proteins in A₂ₐR chimeras

Construct	Truncated regions in A₂ₐR	Inserted regions in fusion partner proteins
A₂ₐR-BRIL	K209 - G218	A1 – L106
A₂ₐR-FiX1	K209 - R220	R10 - E119
A₂ₐR-FiX2	K209 - E219	R10 - E118

Table S2 | Amino acid sequences of FiX1 and FiX2

Computationally designed amino acid sequences are described in uppercase and residues added to allow expression and purification are described in lowercase.

Construct	Sequence
FiX1	mGEEEERRRRLLELLKRIAELLERGDLEEALKLVKKLAKEQGRQEIIDYIEEVLRLYQEGNREEARKLLE ELLRRLEKEGDTEFRELIRIILEFLELEERGDLEEAKKLARELKKQVDEQEKRLGlehhhhhh
FiX2	mGEEEERRRRLLELLERIARLLKRGDLEEALKLVKKLAKEQGEQEIIDFIEEVLRLYQEGNREQARELLE RLLRNLEKRGNQDFRNLIEIILRILELEQRGNQEEIKKLAEELRREVEERKRKLGlehhhhhh

Table S3 | Amino acid sequences of A₂ₐR fused with FiX1 and FiX2

Amino acid sequences of fusion partner proteins, FiX1 and FiX2, are highlighted by underlines. Glycine spacer + TEV protease recognition site + TagRFP + 8xHis-tag (lowercase) is added at the C-terminal of each designed protein.

Construct	Sequence
A₂ₐR-FiX1	MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNVTNYFVVSLAAADIAVGVLAIPFAITISTG FCAACHGCLFIACFVLVLTQSSIFSLLAIAIDRYIAIRIPLRYNGLVTGTRAKGIIAICWVLSFAIGLT PMLGWNNCGQPKEGKQHSQGCGEGQVACLFEDVVPMNYMVYFNFFACVLVPLLLMLGVYLRIFLAARRQ <u>LRLLELLKRIAELLERGDLEEALKLVKKLAKEQGRQEIIDYIEEVLRLYQEGNREEARKLLEELLRRLE KEGDTEFRELIRIILEFLELEERGDLEEAKKLARELKKQVDE</u>ARSTLQKEVHAAKSLAIIVGLFALCWL PLHIINCFTFFCPDCSHAPLWLMYLAIVLSHTNSVVNPFIYAYRIREFRQTFRKIIRSHVLRQQEPFKA genlyfqgvskgeelikenmhmklymegtvnnhhfkctsegegkpyegtqtmrikvveggpplpfafdil atsfmygsrtfinhtqgipdffkqsfpegftwervttyedggvltatqdtslqdgcliynvkirgvnfp sngpvmqkktlgweantemlypadgglegrsdmalklvgghlicnfkttyrskkpaknlkmpgvyyvd hrlerikeadketyveqhevavarycdlpsklghklnhhhhhhhh
A₂ₐR-FiX2	MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNVTNYFVVSLAAADIAVGVLAIPFAITISTG FCAACHGCLFIACFVLVLTQSSIFSLLAIAIDRYIAIRIPLRYNGLVTGTRAKGIIAICWVLSFAIGLT PMLGWNNCGQPKEGKQHSQGCGEGQVACLFEDVVPMNYMVYFNFFACVLVPLLLMLGVYLRIFLAARRQ <u>LRLLELLERIARLLKRGDLEEALKLVKKLAKEQGEQEIIDFIEEVLRLYQEGNREQARELLERLLRNLE KRGNQDFRNLIEIILRILELEQRGNQEEIKKLAEELRREVE</u>RARSTLQKEVHAAKSLAIIVGLFALCWL PLHIINCFTFFCPDCSHAPLWLMYLAIVLSHTNSVVNPFIYAYRIREFRQTFRKIIRSHVLRQQEPFKA genlyfqgvskgeelikenmhmklymegtvnnhhfkctsegegkpyegtqtmrikvveggpplpfafdil atsfmygsrtfinhtqgipdffkqsfpegftwervttyedggvltatqdtslqdgcliynvkirgvnfp sngpvmqkktlgweantemlypadgglegrsdmalklvgghlicnfkttyrskkpaknlkmpgvyyvd hrlerikeadketyveqhevavarycdlpsklghklnhhhhhhhh

Table S4 | Residues used for superposition between A₂ₐR and *de novo* designed fusion partner proteins

	TM5 and TM6 in A₂ₐR	N- and C-terminal regions in FiX1 and FiX2
A₂ₐR vs FiX1	R206-L208 and A221-S223	R7-R9 and Q120-K122
A₂ₐR vs FiX2	R206-L208 and R220-R222	R7-R9 and E119-K121