

Optimizing the Expression of Human Dopamine Receptors in *Escherichia coli*

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Supplementary Tables and Figures

Tables

Table S1. Oligodeoxynucleotides used for error prone PCR of D₃ and CPEC.

#	5'→3'
1	AAGACGCGCAGACTCATATG
2	CCTTGAAAATACAGGTTTTCAAGCTT
3	AAGCTTGAAAACCTGTATTTTCAAGGC
4	CATATGAGTCTGCGCGTCTTTCAG

Figures

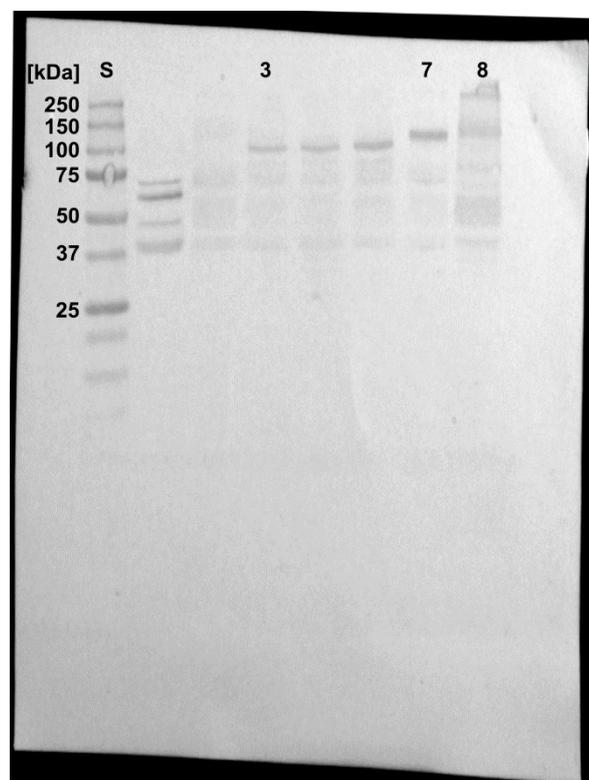


Figure S1. Expression analysis for D_{2S} expressed from different promoters and of a thermostabilized variant. Original image of the Western blot against the N-terminal MBP. S, molecular weight standard, the molecular weights of the standard proteins are indicated on the left. Numbers on top of the gel indicate construct numbers: **3**, *lac*-MBP-D_{2Swt}-mCherry; **7**, *tac*-MBP-D_{2Swt}-mCherry; **8**, *lac*-MBP-D_{2S}^{I122A,L346A,L350A}-mCherry.

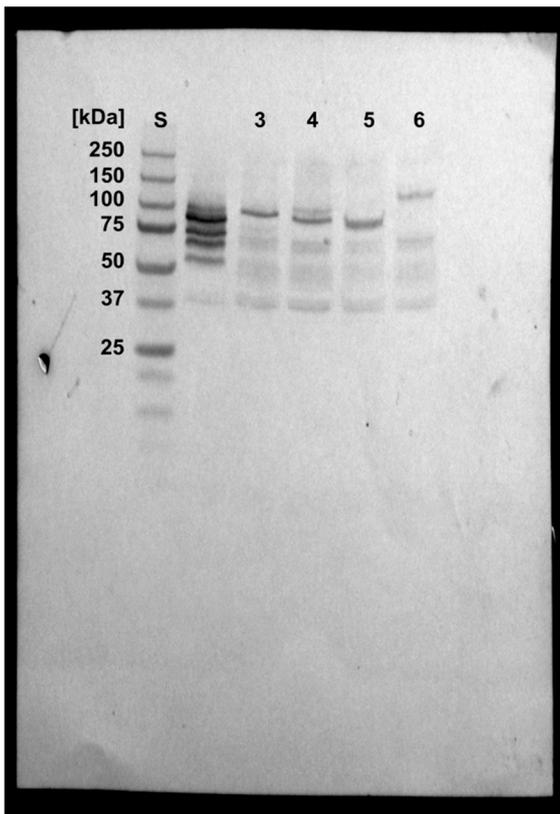
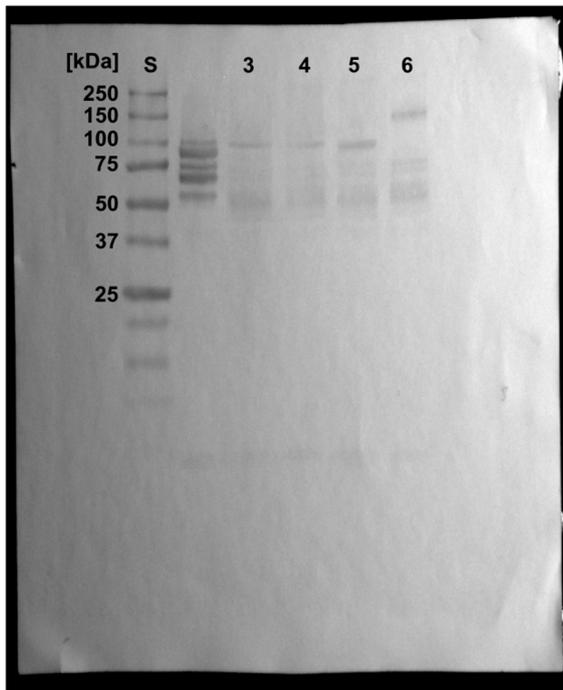


Figure S2. Original image of the Western Blot presented in Figure 2. Western Blot against the N-terminal MBP. S, molecular weight standard, the molecular weights of the standard proteins are indicated on the left. Numbers on top of the gel indicate construct numbers: **3** *lac*-MBP-D_{2Swt}-mCherry, **4** *lac*-MBP-D_{2Swt}-sfGFP, **5** *lac*-MBP-D_{2Swt}-TrxA, **6** *lac*-MBP-D_{2Swt}-G_{ai1}.

(a)



(b)

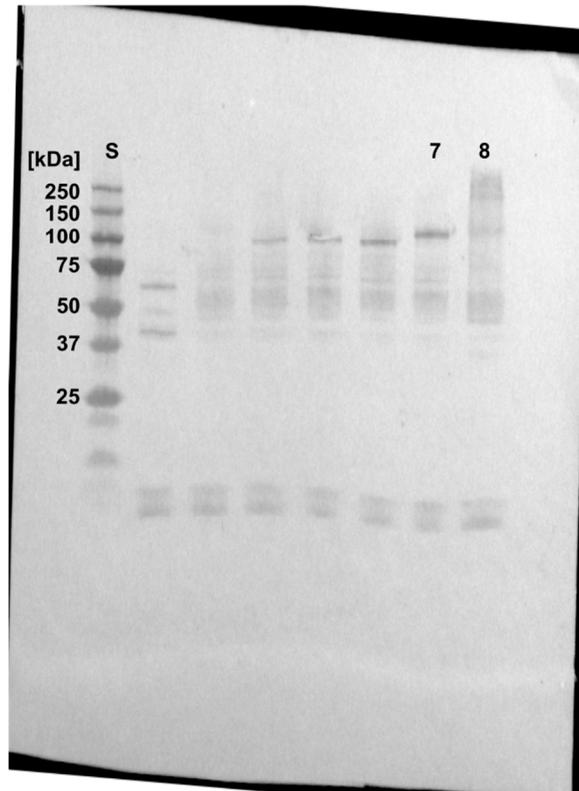


Figure S3. Original images of the Western Blots presented in Figure 4. Western Blots against the N-terminal MBP. S, molecular weight standard, the molecular weights of the standard proteins are indicated on the left. Numbers on top of the gel indicate construct numbers. **(a)** **3** *lac*-MBP-D_{2Swt}-mCherry, **4** *lac*-MBP-D_{2Swt}-sfGFP, **5** *lac*-MBP-D_{2Swt}-TrxA, **6** *lac*-MBP-D_{2Swt}-G_{ai1}. **(b)** **7** *lac*-MBP-D_{2S}I122A,L346A,L350A-mCherry, **8** *lac*-MBP-D_{2Swt}-mCherry.

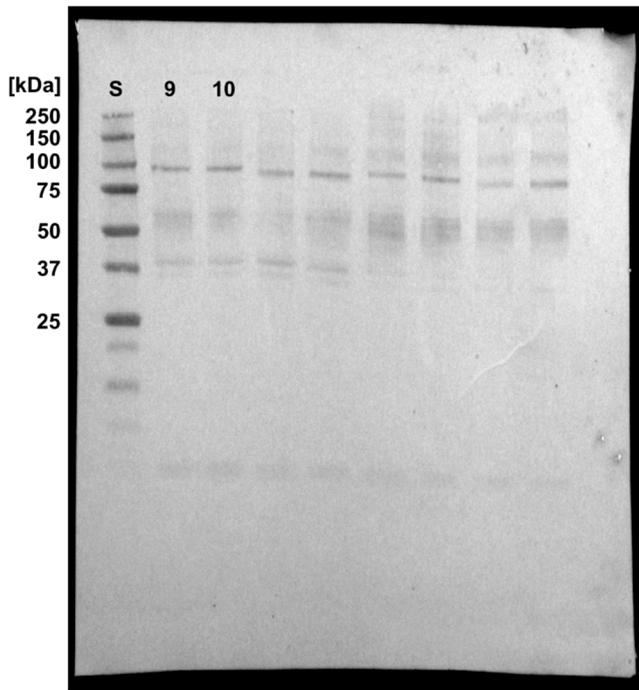


Figure S4. Original image of the Western Blot presented in Figure 5. Western Blot against the N-terminal MBP. S, molecular weight standard, the molecular weights of the standard proteins are indicated on the left. Numbers on top of the gel indicate construct numbers: **9** *lac*-MBP- $D_{3^{wt}}$ -mCherry, **10** *lac*-MBP- $D_{3^{L119W}}$ -mCherry.

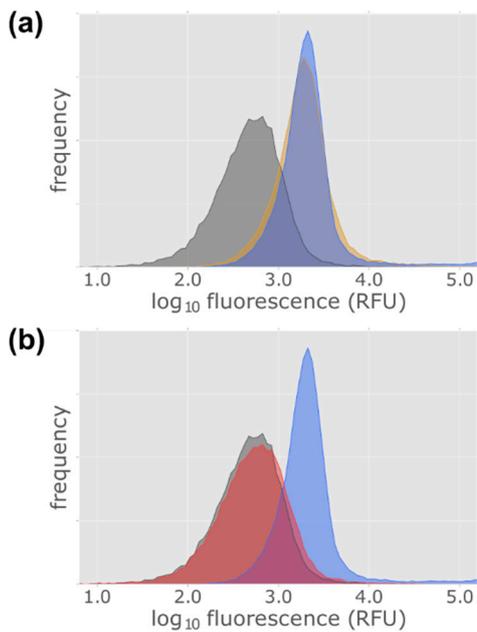


Figure S5. The D₃ ligand haloperidol can displace the fluorescent ligand NMP130. **(a)** FACS analysis at excitation and emission wave lengths of 488 and 525 nm showed that 8 % ethanol in the labeling reaction does not significantly alter binding of NMP130 to the D₃ receptor (control). Grey: without induction, yellow: after induction with 0.5 mM IPTG for 4 h, blue: after induction with 0.5 mM IPTG for 4 h and labeling reaction containing 8 % ethanol (see Material & Methods for further details). **(b)** Specific binding of NMP130 to the D_{3wt} receptor was confirmed by displacement with haloperidol. Grey: without induction, blue: after induction with 0.5 mM IPTG for 4 h and labeling reaction containing 8 % ethanol (Fig. S5a), red: after induction with 0.5 mM IPTG for 4 h and labeling reaction containing a 4000-fold excess of haloperidol.