

Expression regulation by a methyl-CpG binding domain and the incorporation of non-canonical amino acids in an *E. coli* based, cell-free TX-TL system

M. Schenkelberger¹, S. Shanak^{2,4}, M. Finkler¹, E. G. Worst¹, V. Noireaux³, V. Helms⁴, and A. Ott^{1,*}

¹Saarland University, Department of Experimental Physics, Saarbrücken, 66041, Germany

²Arab American University-Jenin, P.O.Box 240, Palestine

³University of Minnesota, School of Physics and Astronomy, Minneapolis, 55455, USA

⁴Saarland University, Center for Bioinformatics, Saarbrücken, 66041, Germany

Cytosine methylation plays an important role in the epigenetic regulation of eukaryotic gene expression. The methyl-CpG binding domain (MBD) is common to a family of eukaryotic transcriptional regulators. How MBD, a stretch of about 80 amino acids, recognizes CpGs in a methylation dependent manner, and as a function of sequence, is only partly understood. Here we show, using an *E. coli* cell-free expression system, that MBD from the human transcriptional regulator MeCP2 performs as a specific, methylation-dependent repressor in conjunction with the BDNF (Brain-Derived neurotrophic factor) promoter sequence [1]. We give a simple kinetic model that describes the repression and fits the experimental data [1]. The *E. coli* cell-free expression system can also be used for the incorporation of non-canonical amino acids [2]. We present first results as well as future plans.

- [1] M. Schenkelberger, S. Shanak, M. Finkler, E. Worst, V. Noireaux, V. Helms, and A. Ott, "Expression regulation by a methyl-CpG binding domain in an *E. coli* based, cell-free TX-TL system," *Phys. Biol.*, vol. 14, no. 2, 2017.
- [2] E. G. Worst, M. P. Exner, A. De Simone, M. Schenkelberger, V. Noireaux, N. Budisa, and A. Ott, "Cell-free expression with the toxic amino acid canavanine," *Bioorg. Med. Chem. Lett.*, vol. 25, no. 17, pp. 3658–3660, Jun. 2015.