Fantastic SSBs and where to find them

I. Cormack[1], E. Pohl[1], B. Striberny[2]

[1]Department of Chemistry, Durham University, DH1 3LE, UK. [2]ArcticZymes Technologies ASA, 9019 Tromsø, Norway.
isabel.g.cormack@durham.ac.uk

Metagenomic data can act as a "gold mine" of novel genes and enzymes. The research activities of the Virus-X project (Viral Metagenomics for Innovation Value, Horizon 2020) particularly focused on bacterial and archaeal viruses from extreme habitats as a source of novel enzymes for biotechnology applications [1]. Further technological progress within this field is reliant on the characterisation of viral enzymes from novel sources as key tools in biotechnology originate from very few bacterial viruses.

One such group of viral enzymes was single-stranded DNA-binding proteins (SSBs) which show potential to enhance loop-mediated isothermal amplification technologies. The SSBs were identified in metagenomic data collected from an Icelandic terrestrial hot spring. Here we present the characterisation of these SSBs through a wide range of biophysical and structural techniques, toward structure determination.

Thermal shift assays have been shown to be vital in optimising conditions during protein purification and storage. The use of the Durham Screens has been fundamental in deconvoluting the effects of cations and anions. This leads to increased protein stability long-term and an increase in the homogeneity of samples, therefore, improving the likelihood of obtaining diffracting crystals [2, 3]. For example, one SSB has been shown to have a melting temperature of 55 °C in water, which is increased to 69 °C under optimised conditions (Fig. 1). Other techniques used to currently characterise SSBs include electrophoretic mobility shift assays (EMSAs), alongside spectral shift and temperature-related intensity change (TRIC) to calculate binding constants of each SSB [4, 5]. By increasing the range of temperatures at which the SSBs are functional, alongside understanding their binding affinities, we aim to create a toolbox of proteins that can be used in various biotechnology applications.

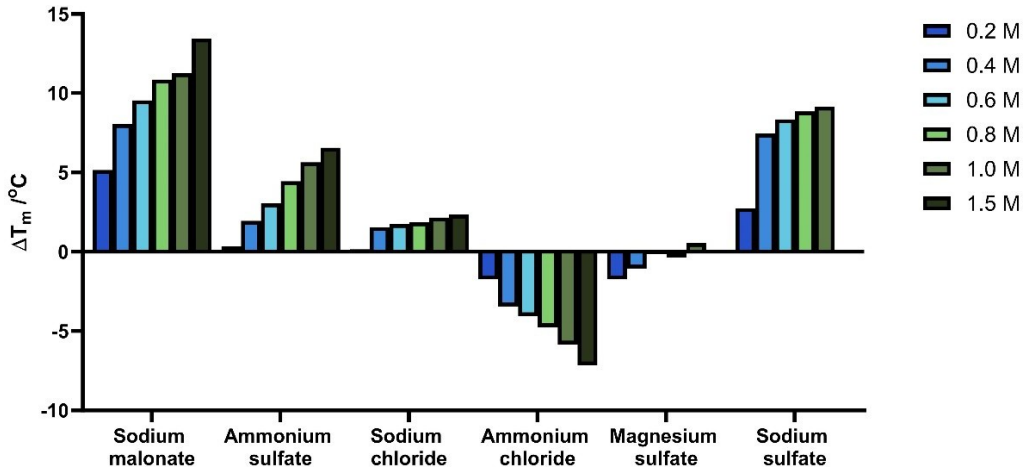

Figure 1. The effect of salt addition to the melting temperature of an SSB. The change in T_m is relative to the control experiment in water (T_m = 55 °C).

[1] Aevarsson, A., *et al.* (2021). *FEMS Microbiology Letters.* **368 (12)**.

[2] Bruce, D.. *et al.* (2019). *J. Vis. Exp.*, **144**.

[3] Groftehauge, M., (2015). *Acta Crystallogr D*. **71**. 36-44

[4] Hellman, L., and Fried, M. (2007). *Nat Protoc*, **8 (1)**. 1849-1861.

[5] Gupta. A., *et al. Encyclopaedia of Biophysics,* eds: Roberts, G., and Watts, A. Springer (2018) pp. 1-5.

The Virus-X project was funded by EU Horizon 2020, Grant/award number 685778. I. Cormack is also grateful to the EPSRC MoSMed CDT for their funding of this PhD project.