Analysis of Protein-DNA Interactions by SPR

Simple and cheap quantitation using the ReDCaT Chip

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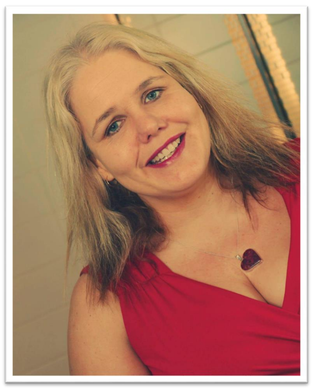
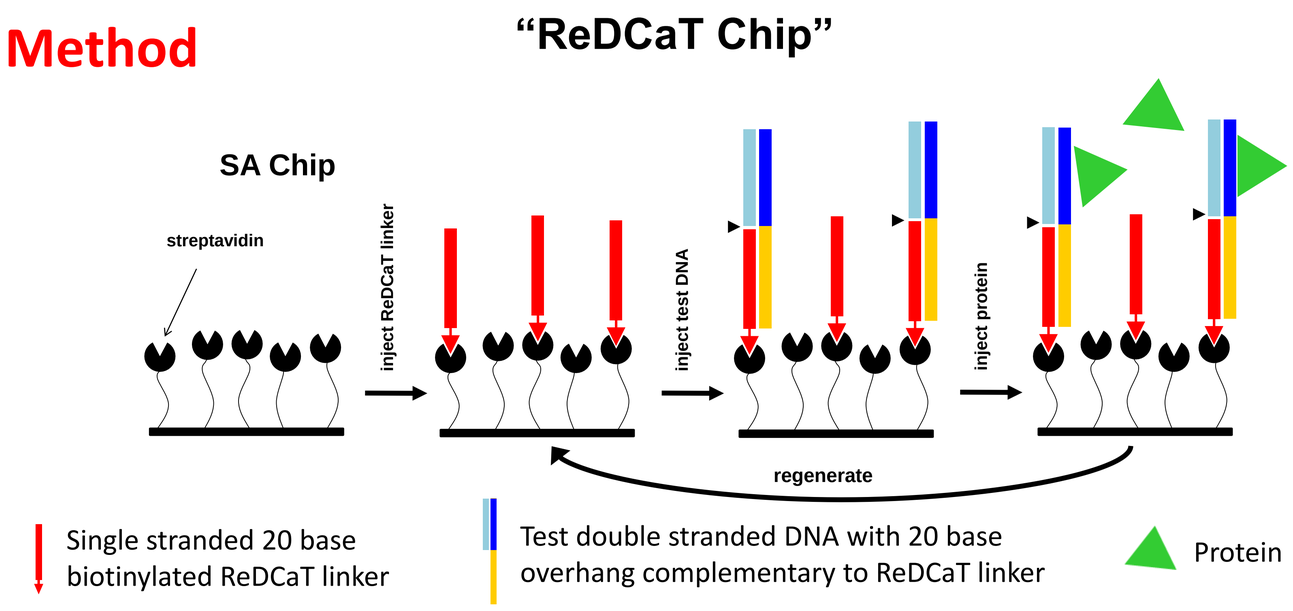
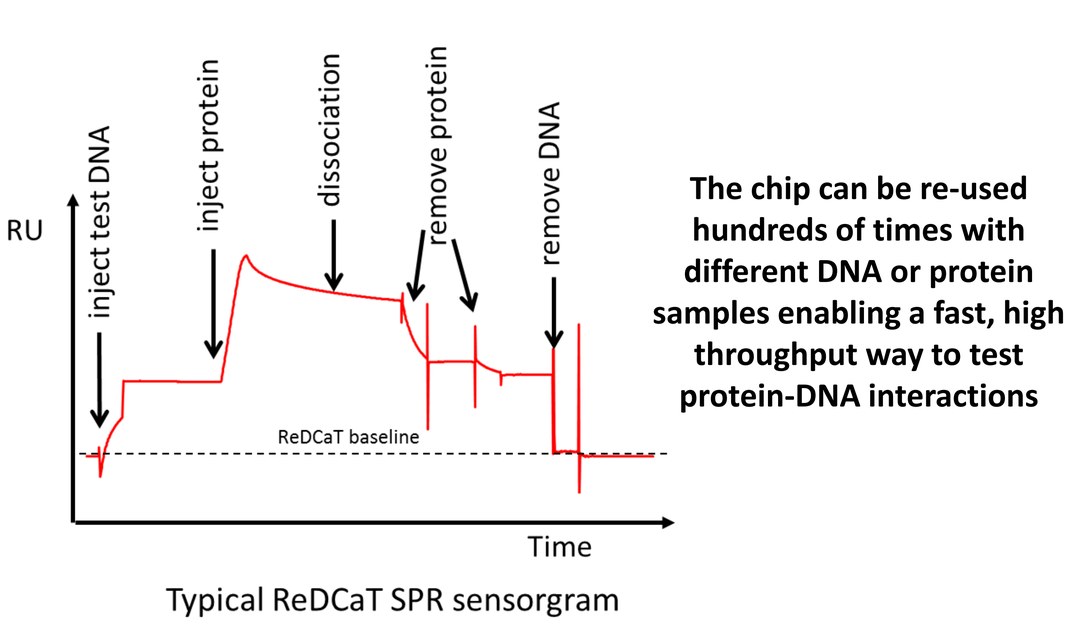


Introduction

The recognition of specific DNA sequences by proteins is crucial to fundamental biological processes such as DNA replication, transcription and gene regulation. The technique of Surface Plasmon Resonance (SPR) is ideally suited for the measurement of these interactions because it is quantitative, simple to implement, reproducible, can be automated and requires very little sample. This typically involves the direct capture of biotinylated DNA to a streptavidin (SA) chip before flowing over the protein of interest and monitoring the interaction. However, once the DNA has been immobilised on the chip it cannot be removed without damaging the chip surface. Moreover, if the protein-DNA interaction is strong, then it may not be possible to remove the protein from the DNA without damaging the chip surface. Given that the chips are costly, this will limit the number of samples that can be tested. Therefore, we have developed a Reusable DNA Capture Technology, or ReDCaT Chip, that enables a single streptavidin chip to be used multiple times. Two projects will be used to illustrate the potential applications of the technology.

Method



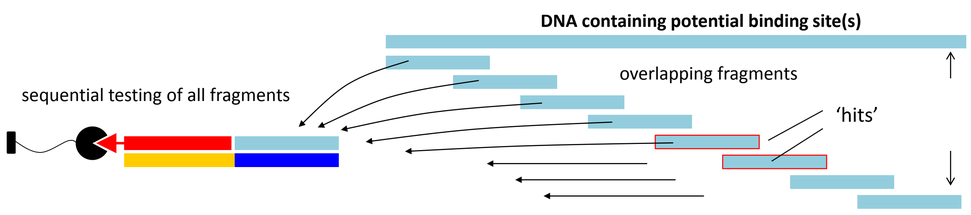


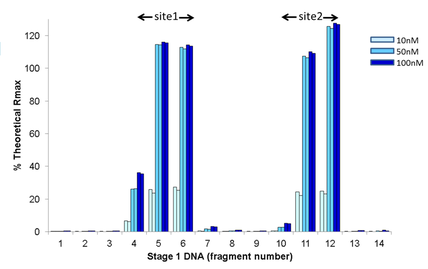
The chip can be re-used hundreds of times with different DNA or protein samples enabling a fast, high throughput way to test protein-DNA interactions

MarR transcription factor

1. Identification of DNA binding site

14 DNA sequences tested (from intergenic region) to identify sequence specific DNA that the transcription factor would bind
3 concentrations of protein used and all DNA sequences and concentrations tested in a single automated run

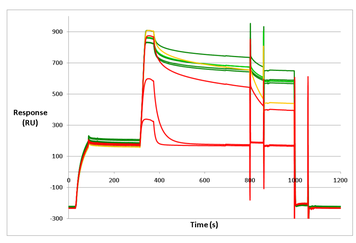




Two clear binding sites observed and one dimer binds one dsDNA

2. Footprinting the 2 DNA binding sites

Aim – To find the smallest possible piece of DNA that the protein still binds
A whole series of different DNA sequences were ordered truncating 2 bases at a time from either the left hand or right hand boundary of each site.
All sequences for both sites tested in one automated run

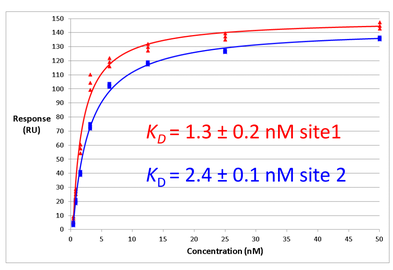


The optimum length of both sites was determined to be 24 basepairs

ACTCCAATACTTGAACTCTC AA TC TT TA CG TG CC GT

3. Affinity determination

Lower amount of DNA captured for accurate measurement
8 concentrations tested (triplicate) for each DNA sequence

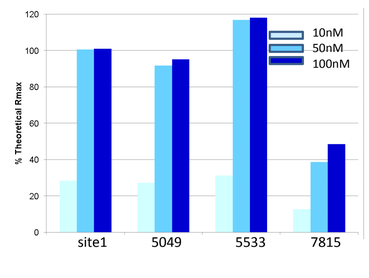


4. Identification of other binding sites

site1: CAATACTTGAACTCTCAATCTTTA
site2: ATTTTGTTTAATGTTCAAGGAACC
Consensus: TTxAAxxxTCAA

S. coelicolor genome

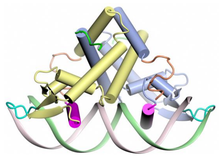
5049 AAGAAGTTCAAGCTTCAACCAAAA
5533 TTGATGTTGAATGTTCAACCAGTC
7815 GACCTGTTCAAAGCTCAAGCAGAA



5. Testing mutations

The key bases in the consensus sequence were mutated to the other options and tested using SPR to see what effect this had. In addition mutations in the protein were made and tested using SPR. R91W mutation abolished binding to DNA

6. Crystal structure determination

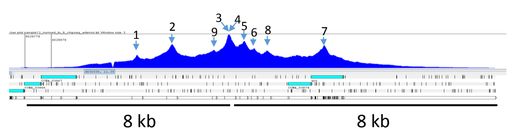


Structure determined bound to site 1 DNA validates SPR results

Reference - Stevenson C. E. M., Assaad A., Chandra G., Le. T.B.K., Greive S.J., Bibb M.J. and Lawson D.M. Nucleic Acids Research, 2013, issue 41 pages 7009-7022

ParB – Chromosome segregation in bacteria

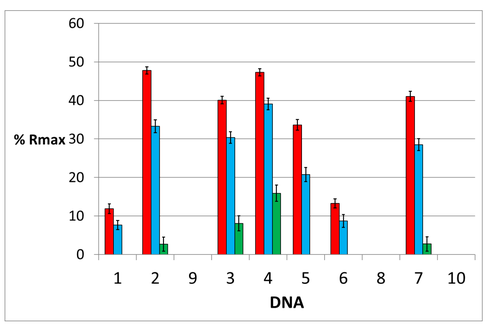
1. Identification of DNA binding sites by ChiP-seq



ChIP-seq identified 9 ParB binding sites

2. Confirmation of sites using ReDCaT SPR

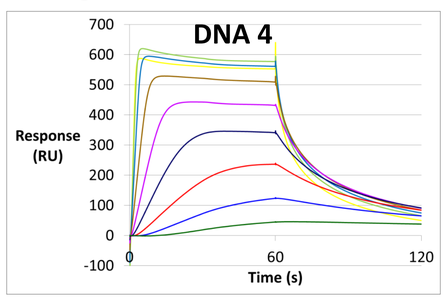
The 9 (+ 1 negative control) DNA sequences were ordered and binding tested, using ReDCaT SPR, at 3 concentrations (50, 200 and 500nM) of ParB protein

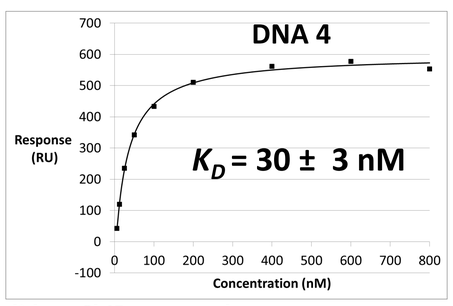


ParB bound to 7 sequences
No binding seen to 2 sequences or negative control

3. Affinity determination

The affinity was determined for DNA sequences 4, 3, 7, 2 and 5
These ranged from 30-170 nM





Quick, easy and cheap way to validate ChIP-seq results
For strong binders affinity was determined

Reference - Tran N. T., Stevenson C. E. M., Som N., Thanapipatsiri A., Jalal A., Le T. Nucleic Acids Research, 2018, issue 46, pages 1196–1209

Conclusions

ReDCaT Chip delivers results comparable to a direct capture approach

Label-free alternative to other techniques e.g. electrophoretic mobility shift assay

Uses small amounts of protein and easily automated

Can test different lengths of DNA

Different sizes and capture levels of DNA can be compared using %Rmax

Mutated proteins can easily be tested

Compounds interfering with the interaction can be screened (e.g. inhibitors or effector ligands)

Quick to test binding

Can do kinetics and affinity experiments

ReDCaT chip reusable hundreds of times meaning experiments are cheap