The use of surface plasmon resonance to guide the choice of nucleic acid oligomers for co-crystallisation with proteins

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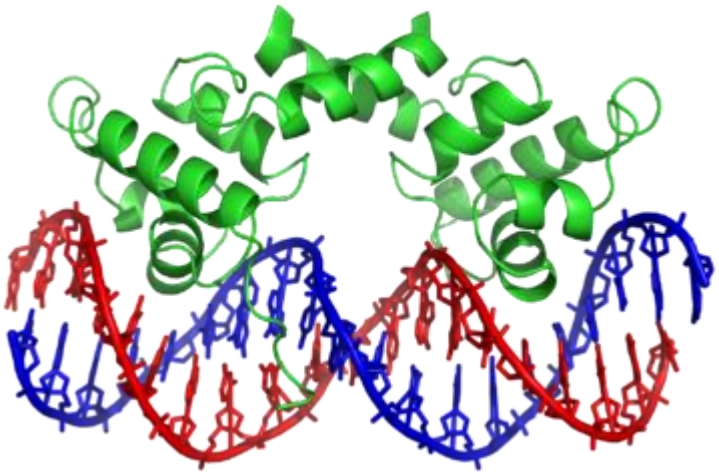
Introduction

- Methods to study Protein/DNA interactions
- SPR and how it works
- Explanation of ReDCaT chip

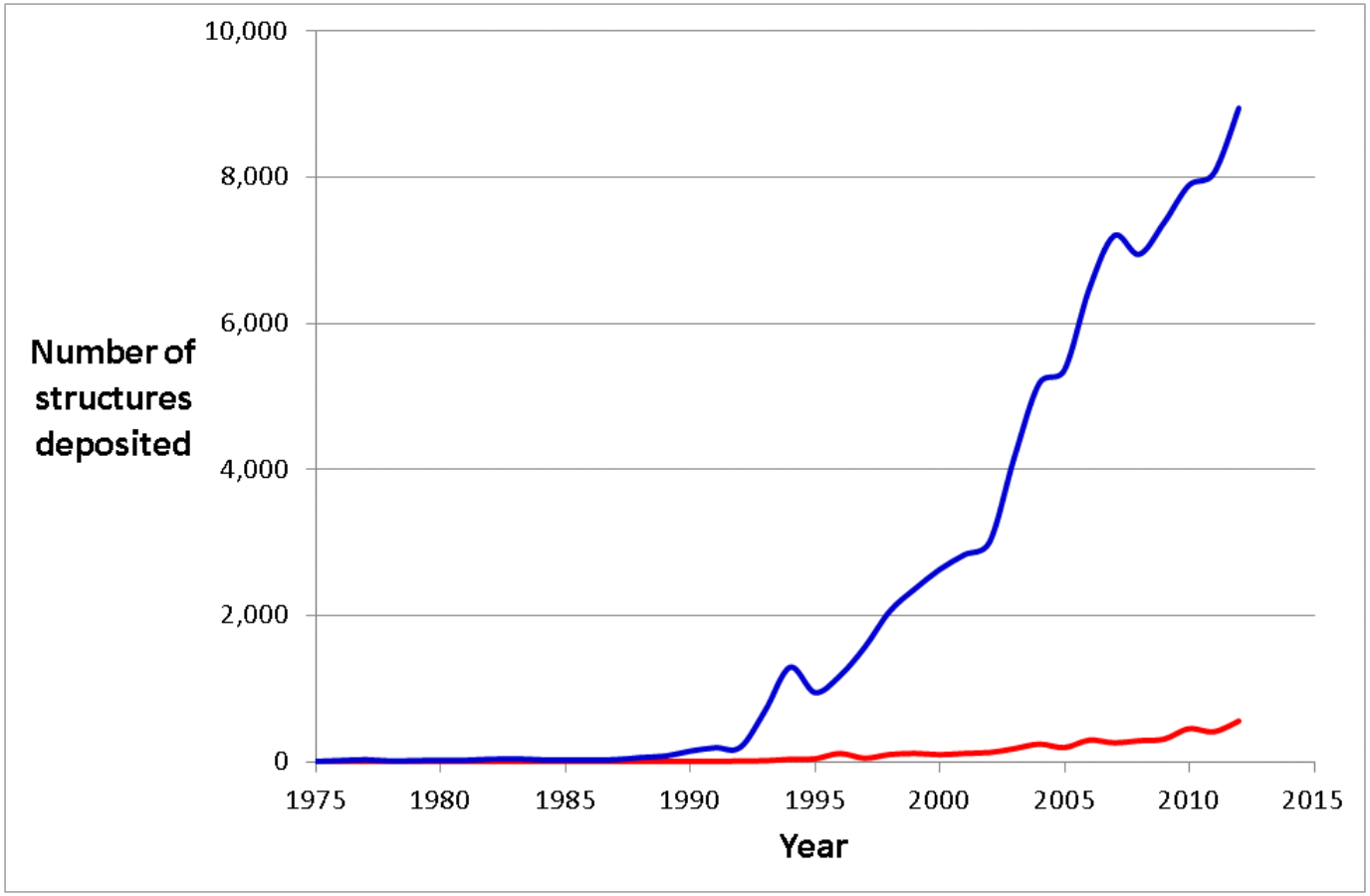
Case study

- Finding a binding site
- Footprinting a binding site
- Affinity of interaction
- Crystallisation
- Structure solution



Conclusions

Percentage of protein/nucleic acid complex structures is increasing



- Bottleneck is the production of well diffracting crystals
- Many variables in the crystallisation process
- Working with protein and nucleic acids introduces more variables!

Protein:DNA crystallisation

What sequence of DNA does the protein bind?

What length of DNA should be used?

Blunt or sticky ends?

Is the DNA sequence palindromic?

How tightly does your protein bind to the DNA?

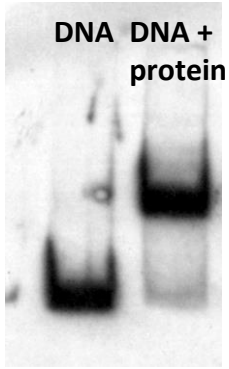
Selecting the right protein construct?

Often requires crystallisation attempts on multiple protein and DNA components

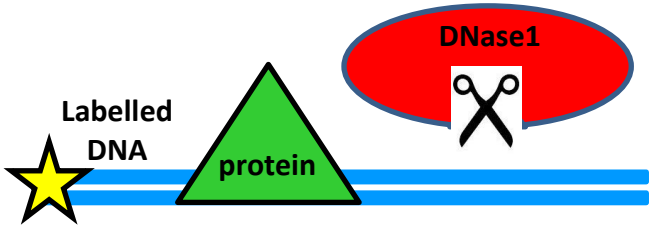
Biophysical tools can be used to guide the crystallisation and increasing the chance of finding crystals

Common methods used to study protein:DNA interactions

Electrophoretic mobility shift assay (EMSA)



DNase1 footprinting



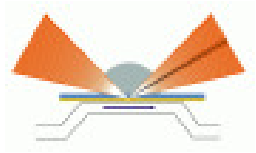
Chromatin immunoprecipitation

Isothermal titration calorimetry (ITC)

Surface Plasmon Resonance (SPR)

Surface Plasmon Resonance (SPR)

- Flexible technique for looking at interactions in real time
- Analyse a wide range of biomolecular interactions
- Requires no radiolabelling



SPR detection system

Biacore T200

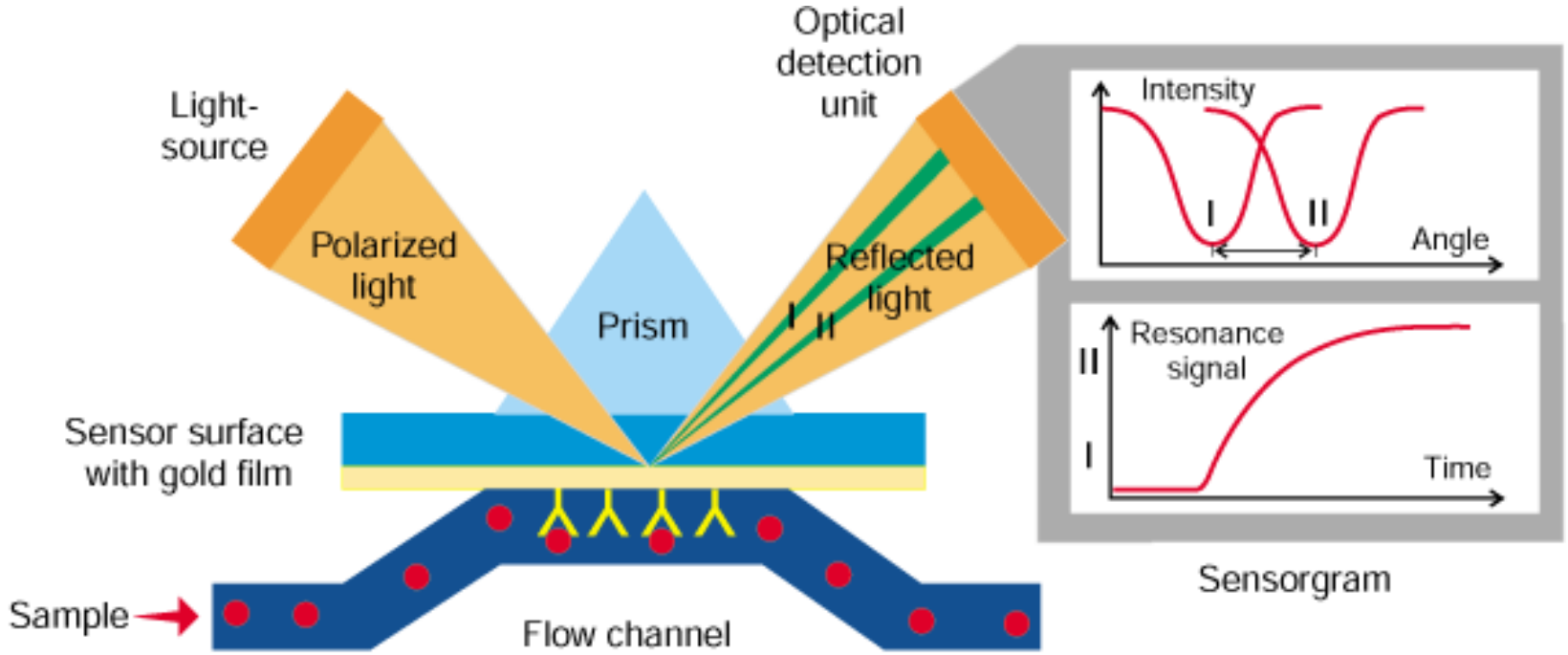
User-friendly software

Sensor chip

Microfluidic system

GE Healthcare

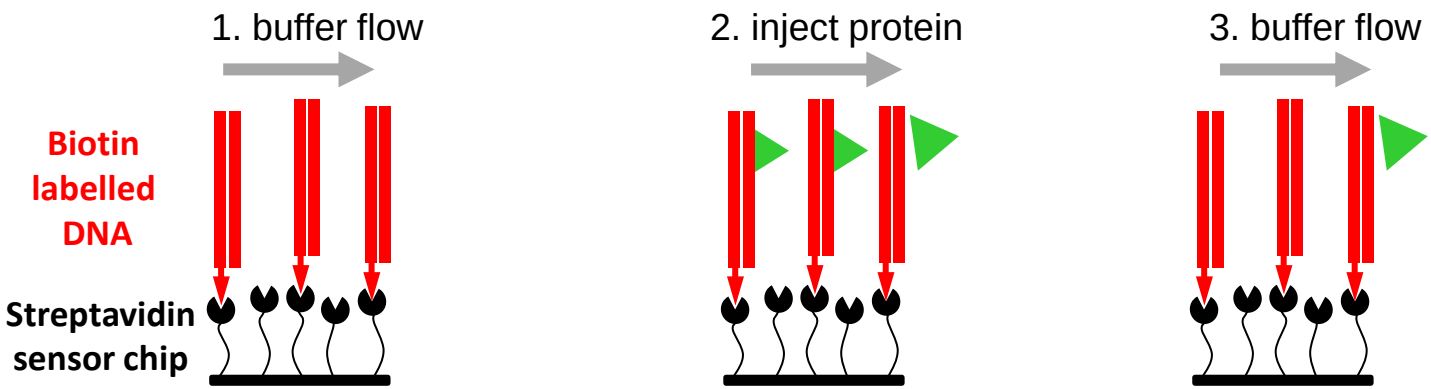
SPR detection system

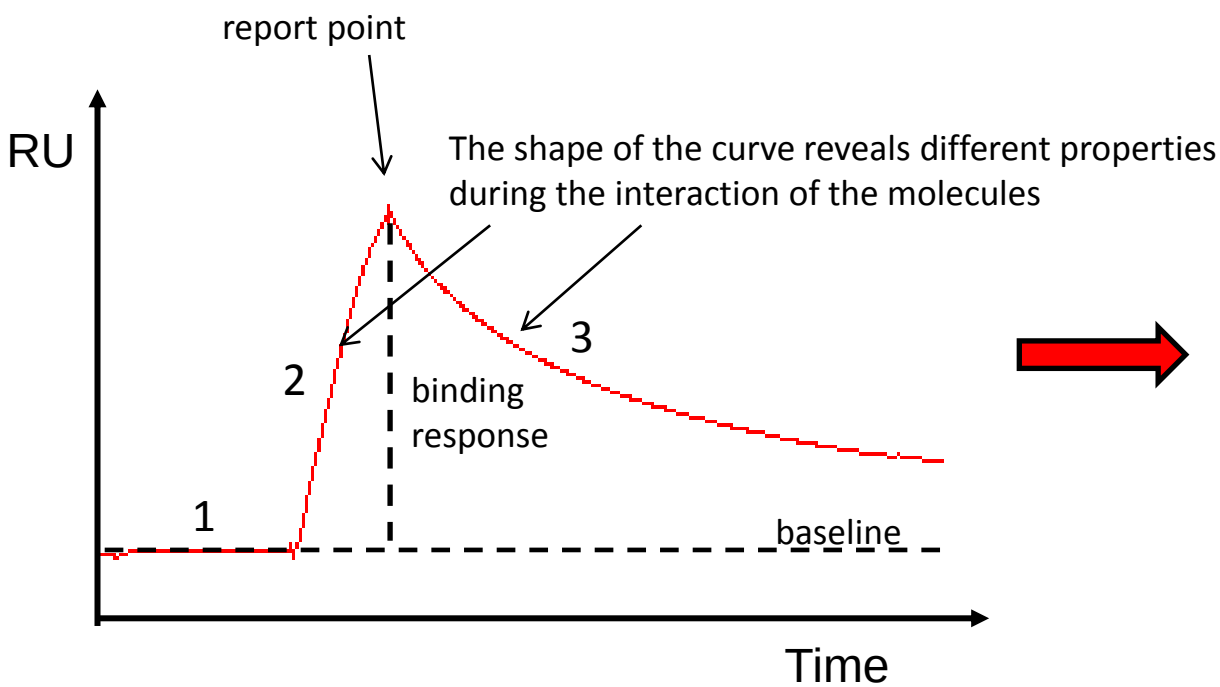


SPR is a refractive index sensor

- Measurements are dependent on surface concentration & temperature
- SPR response is a measure of changes in the resonance angle

The sensorgram

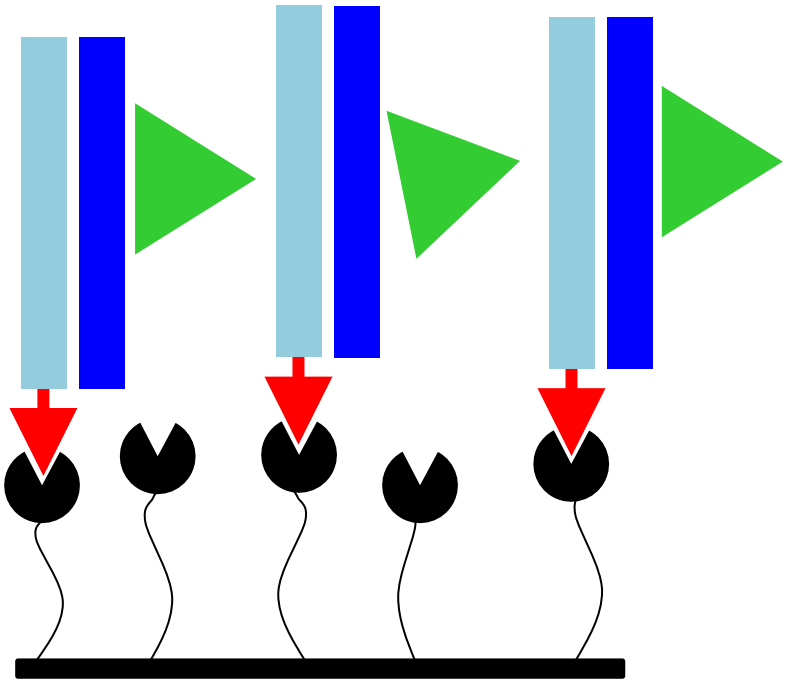




Yes/no binding?
Specificity of binding?
How strong?
How fast?
How much?

Direct Capture

Streptavidin (SA) chip



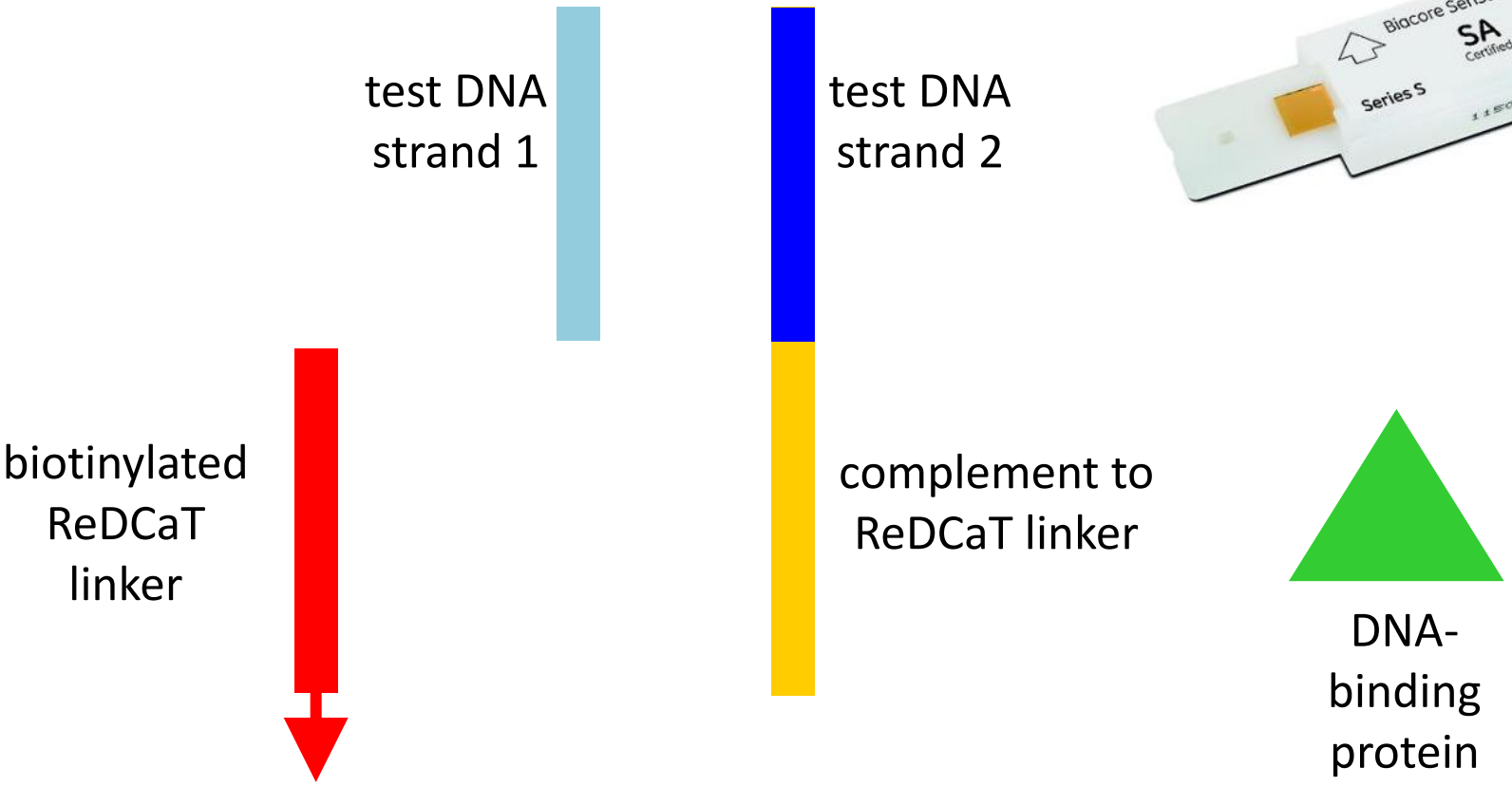
Streptavidin chip

Disadvantages

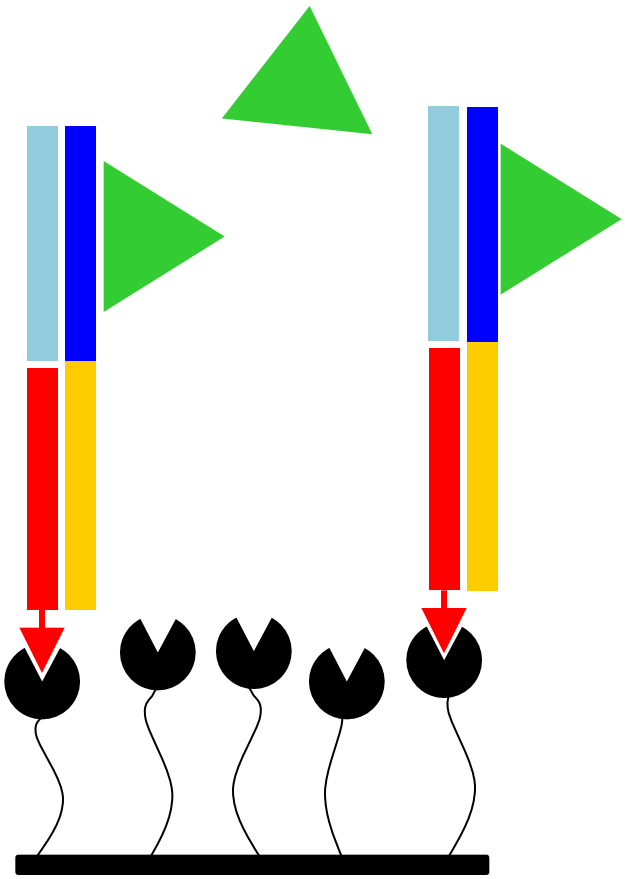
Regeneration conditions required

DNA capture irreversible

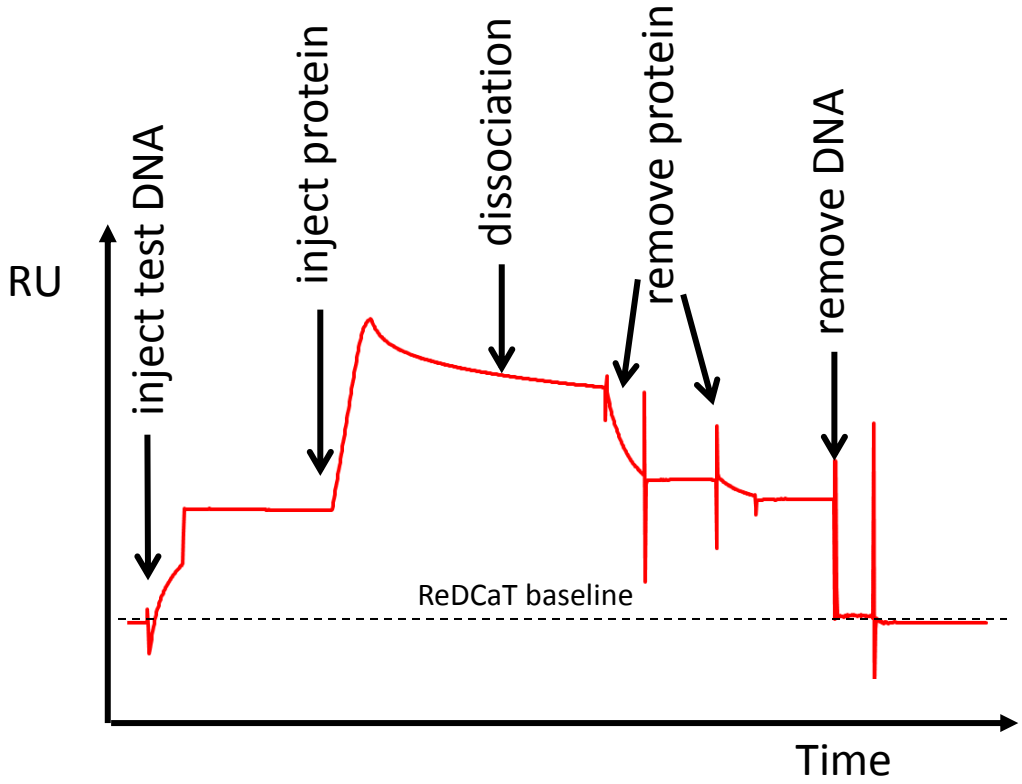
Reusable DNA Capture Technique



The method



Streptavidin chip



Typical ReDCaT SPR sensorgram

Advantages of ReDCaT

Chip can be used hundreds of times - Cheap

Procedure can be automated – Quick

Don’t need regeneration conditions, just re-capture nucleic acid every time

Simple to test many different DNA samples

Simple to test many different protein samples

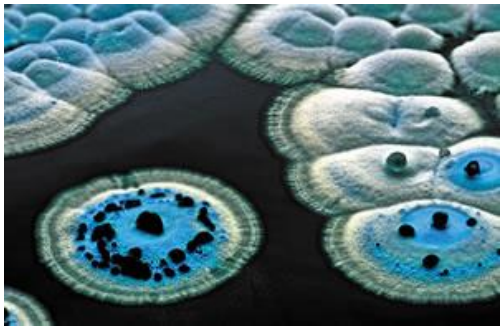
Simple to test the effect of ligands on protein/DNA interaction

Can be used for screening and quantitative experiments

Generic protocols have been developed

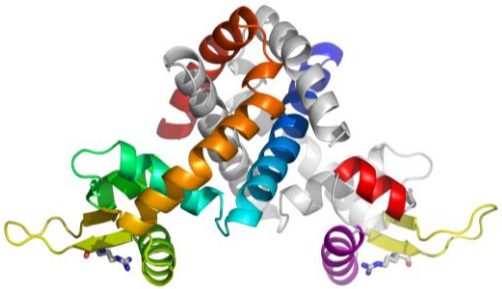
Case study – MarR family transcription factor

- MarR family widespread in Bacteria and Archaea
- 42 MarR proteins in *Streptomyces coelicolor*
- Homodimeric
- Majority act as transcriptional repressors
- Often negatively autoregulate
- Bind to pseudopalindromic DNA sequences

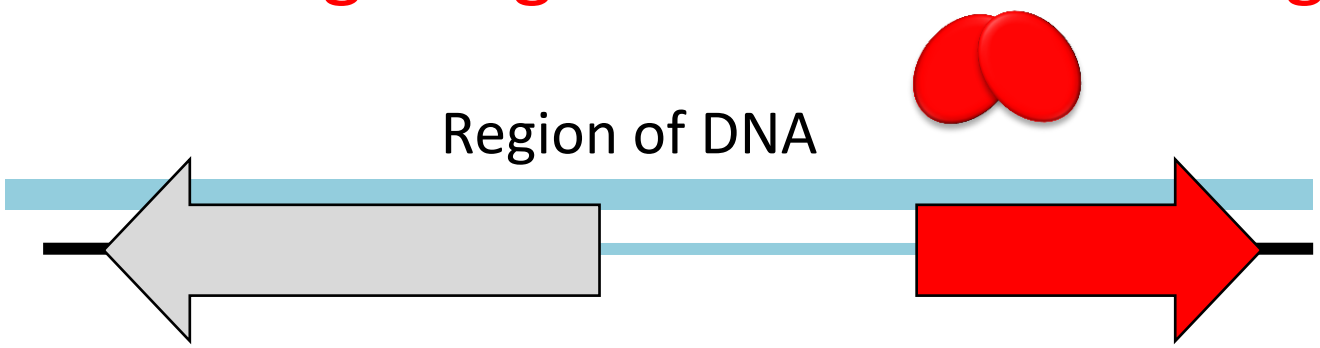


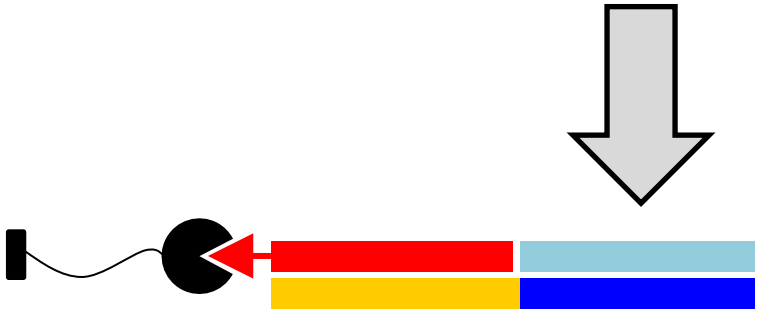
Aim

To understand the interaction of these proteins with DNA



1. Screening a region of DNA for binding





Generate overlapping fragments

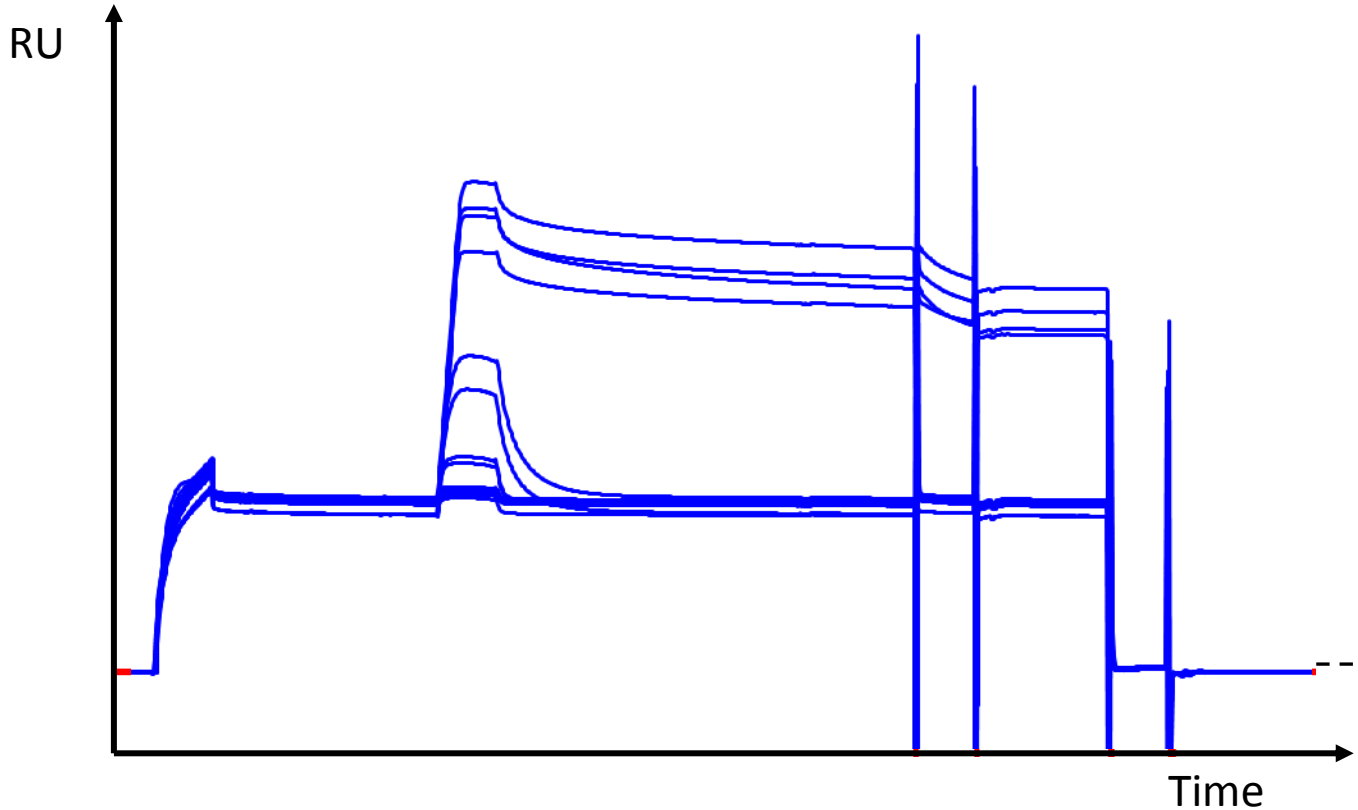
Complement has additional bases to attach to the chip

All can be generated using the program POOP

Test all the sequences one by one

1. Screening a region of DNA for binding

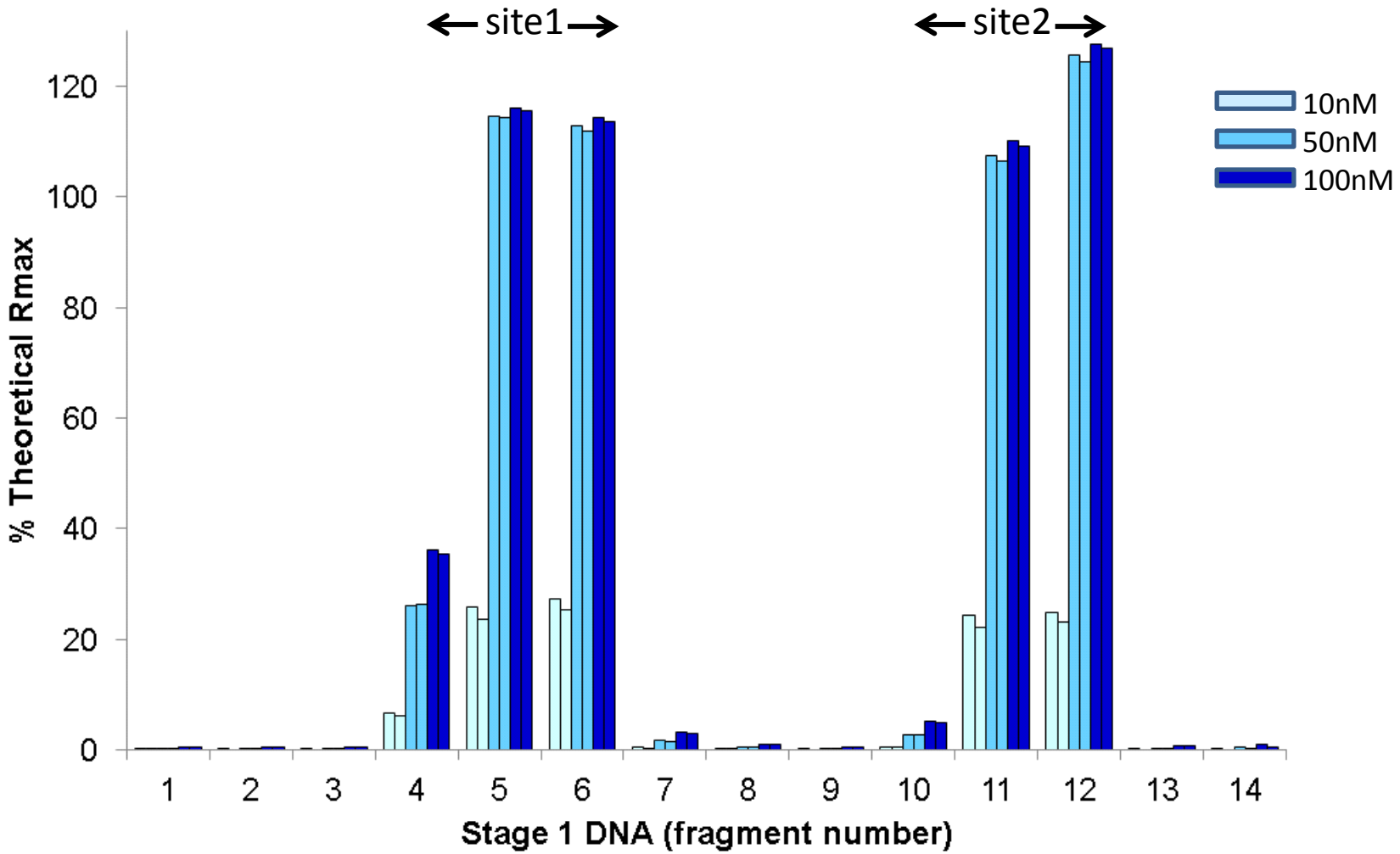
14 sequences tested, 3 protein concentrations, in duplicate = 84 cycles



$$\text{Rmax} = \frac{\text{Molecular weight of protein}}{\text{Molecular weight of DNA}} \times \text{Response DNA immobilised} \times \text{Stochiometry} \times 0.78$$

$$\%\ \text{Rmax} = \frac{\text{Experimental response}}{\text{Theoretical Rmax}} \times 100$$

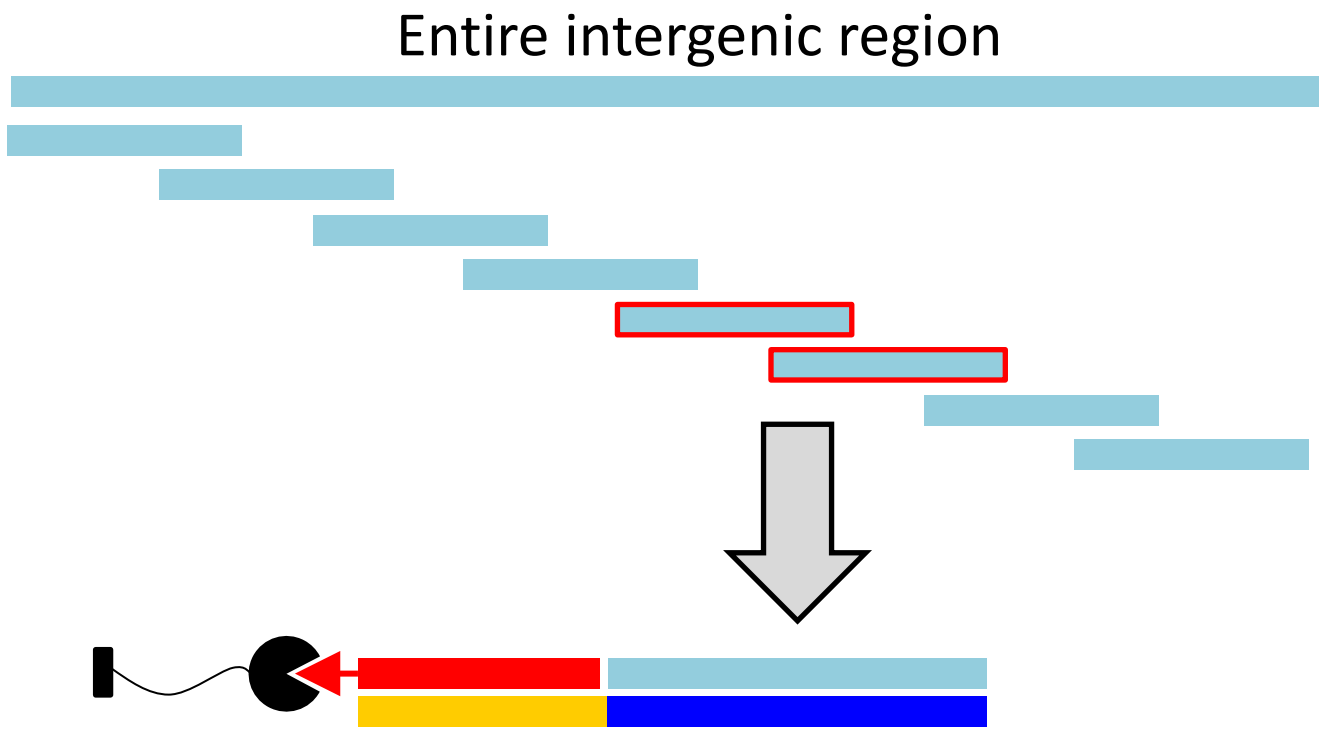
1. Screening a region of DNA for binding



Two binding sites observed

1 protein dimer binding to 1 double stranded DNA oligo

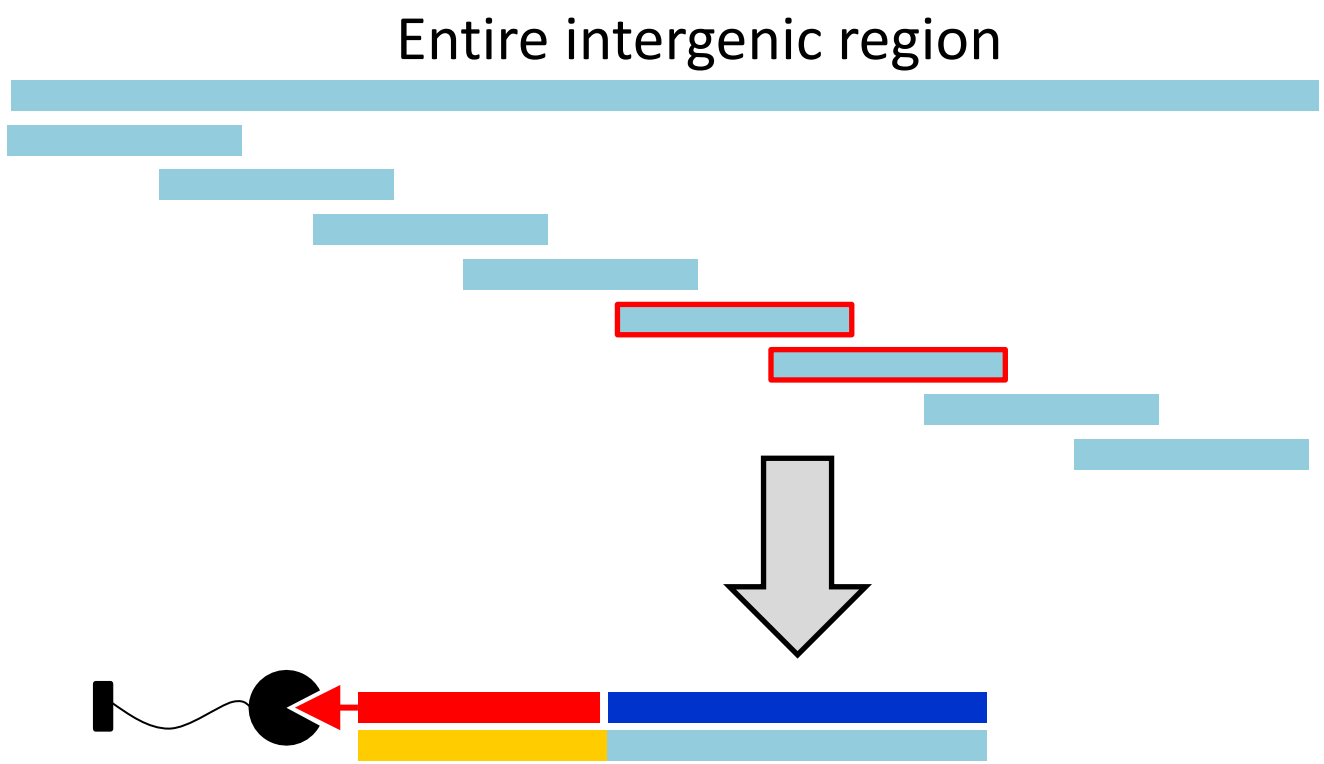
2. Footprinting binding site 1



Combine ‘hits’

Remove bases from RH end until binding is lost

2. Footprinting binding site 1



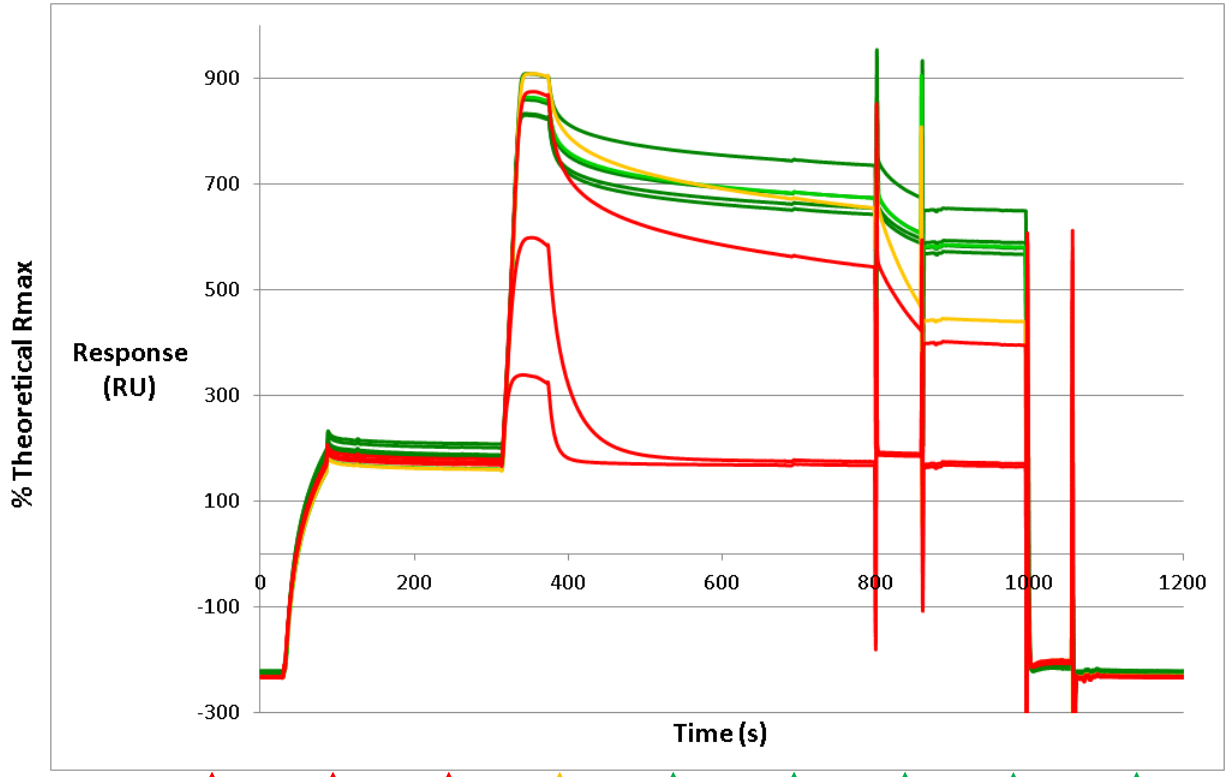
Take the reverse complement

Remove bases from RH end until binding is lost

2. Footprinting binding site 1

Site 1 – defining the RH boundary

- start from 36bp test sequence from merging fragments 5 and 6
- Shorten from RH boundary in 2bp steps

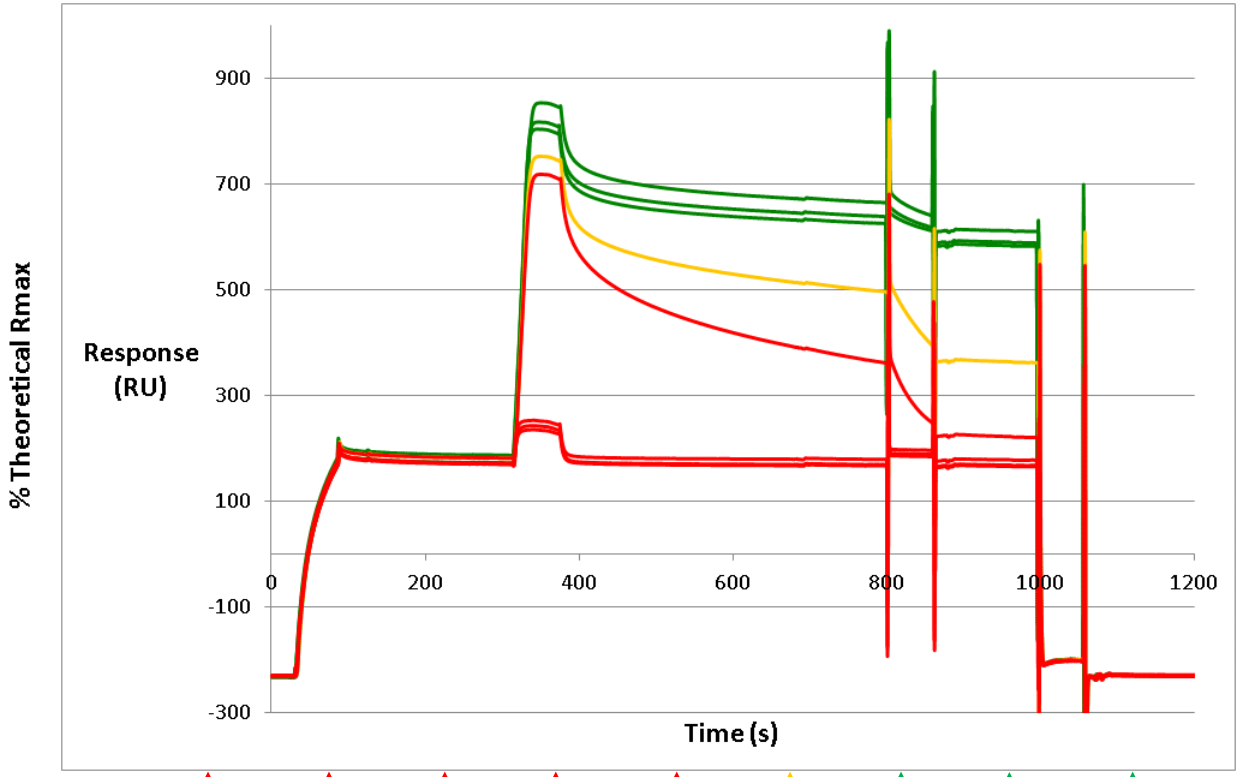


ACTCCAATACTTGAACTCTC AA TC TT TA CG TG CC GT

2. Footprinting binding site 1

Site 1 – defining the LH boundary

- start from reverse complement of 36bp test sequence from merging fragments 5 and 6
- Shorten RH boundary in 2bp steps (equates to original LH boundary)



ACGGCACGTAAAGATTGAGA GT TC AA GT AT TG GA GT

Site 1

ACTCCAATACTTGAACTCTCAATCTTTACGTGCCGT
TGAGGTTATGAACTTGAGAGTTAGAAATGCACGGCA

2. Footprinting both binding sites

Site 1

```
ACTCCAATACTTGAACTCTCAATCTTTACGTGCCGT
TGAGGTTATGAACTTGAGAGTTAGAAATGCACGGCA
```

Both sites
24 bp

Site 2

```
ACGCCGATTTTGTTTAATGTTCAAGGAACCGTCTCG
TGCGGCTAAAACAAATTACAAGTTCCTTGGCAGAGC
```

Footprinting both sites carried out in one SPR run

Total of 138 experiments were run unattended

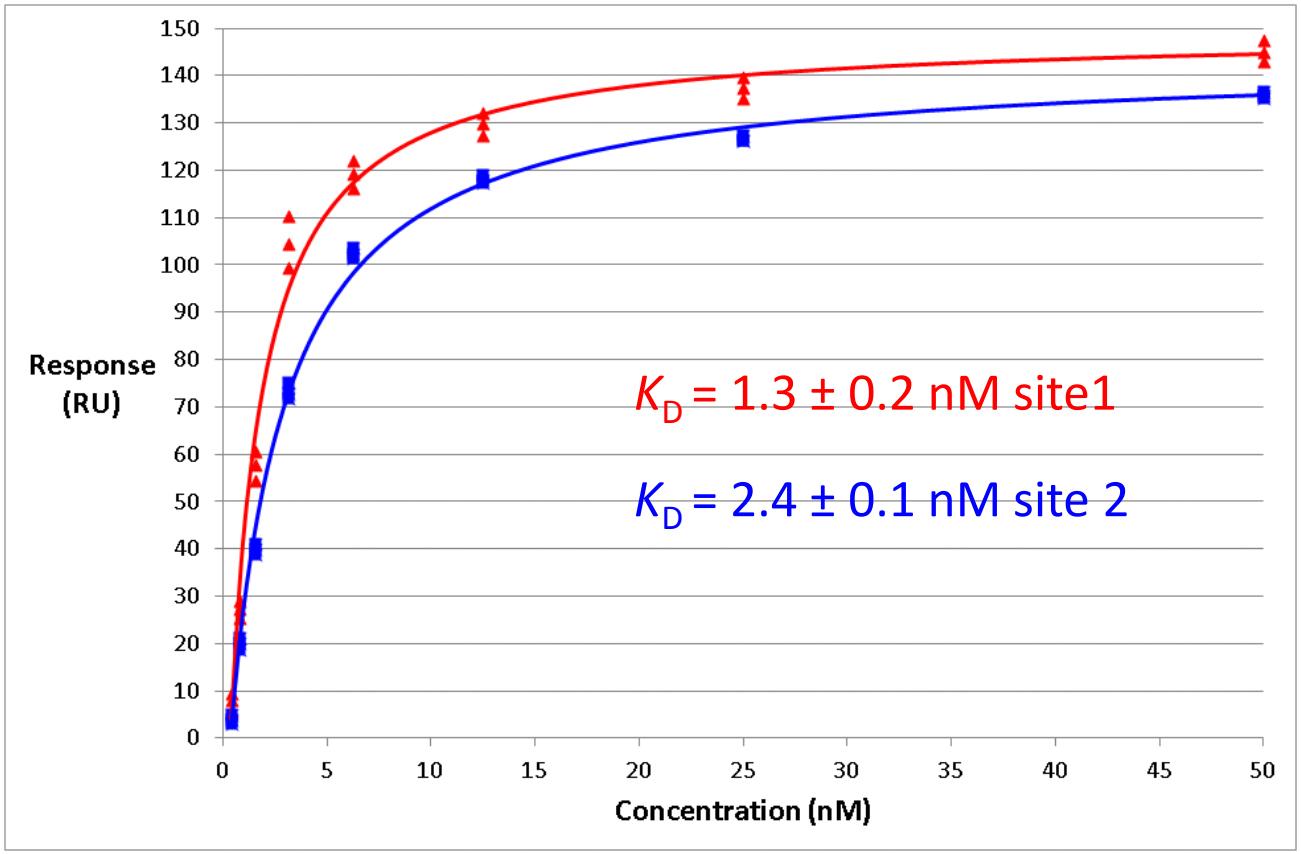
Results easy to interpret. Footprint determined to 2bp resolution

Knowing the accurate footprint guided the choice of DNA for crystallisation

3. Affinity of the interaction

Lower amount of DNA captured for accurate measurement

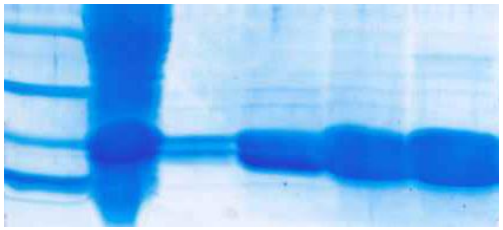
8 concentrations tested in triplicate for each DNA sequence



4. Crystallisation

Protein component

Gene was codon optimised and chemically synthesised



Designed to contain a His tag with TEV cleavage site

Purified by affinity and size exclusion chromatography

Dynamic Light Scattering confirmed that the protein was a dimer

Issues with precipitation of protein

Addition of either Stabil-PAC (Expedion) or 300mM arginine improves

Neither affected the DNA binding of the protein

Commercial screens set up but unable to obtain crystals

4. Crystallisation

DNA component

From SPR

Protein binds to the two different sites and an additional site

Both sites footprinted to 2 nucleotide resolution

Decided to use 22 bp DNA for screening

DNA ordered from Sigma, desalted and 1 µM scale

Made up to 4 mM with water

Mix equal volumes and anneal

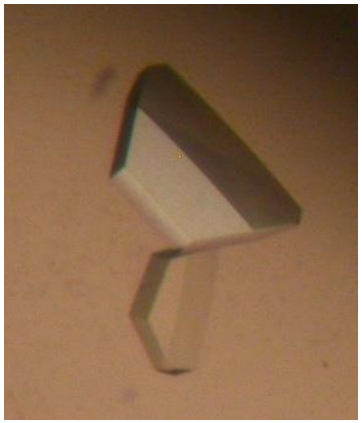
Ratio of protein to DNA 1/1.5

A range of commercially available screens were set up

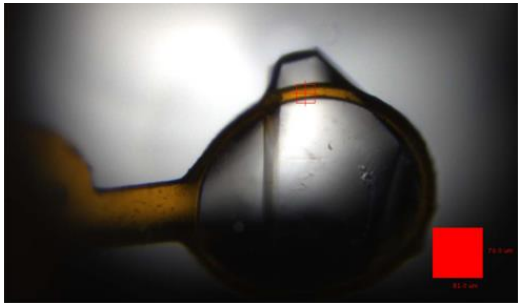
4. Crystallisation

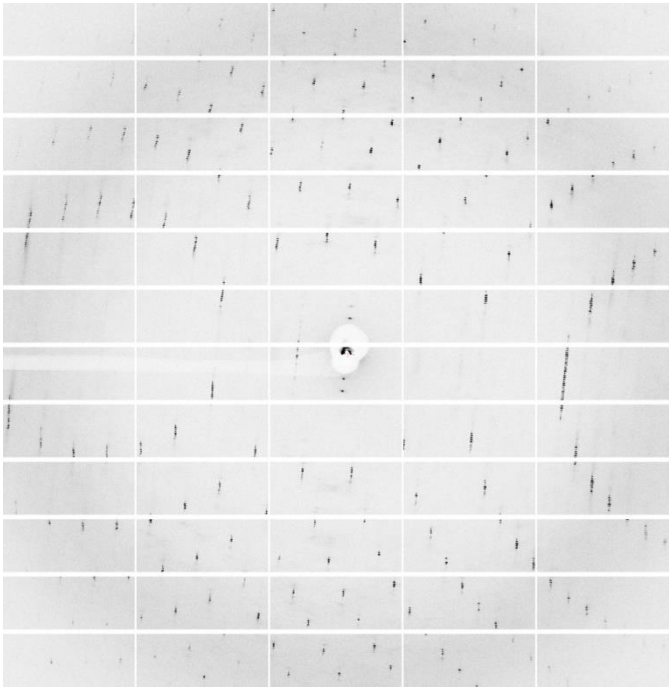
	Site 1	Site 2	Site 3
Wild type sequence		X	
RH symmetrical		X	X
LH symmetrical	X	X	X

4. Crystallisation



Crystallised from
20-40% MPD, 80-100 mM sodium acetate and
50 mM Mes pH 5.6





Protein with site 1 RHsymmetrical
22bp blunt-ended DNA to 2.8 Å

Spacegroup P6?
a=b= 70.80, c = 557.48 Å

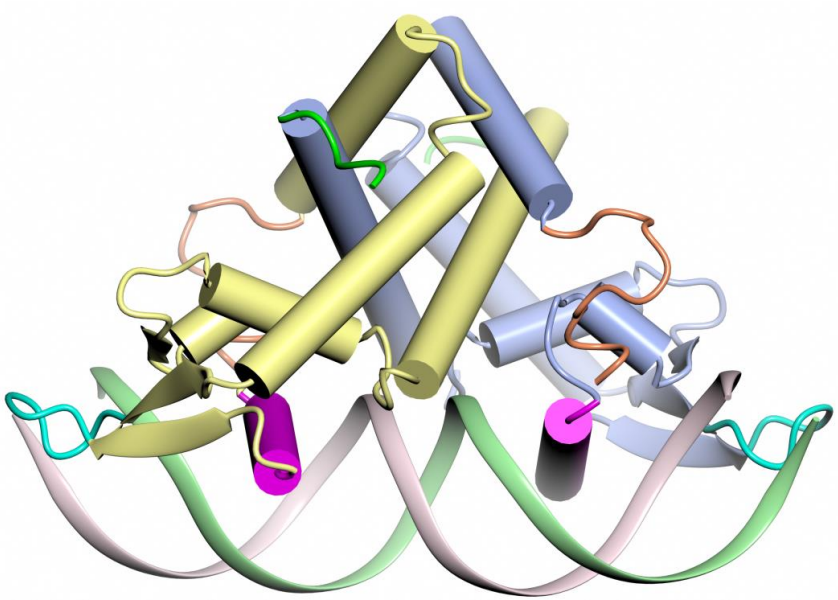
5. Structure solution

Careful data collection required due to long cell axis

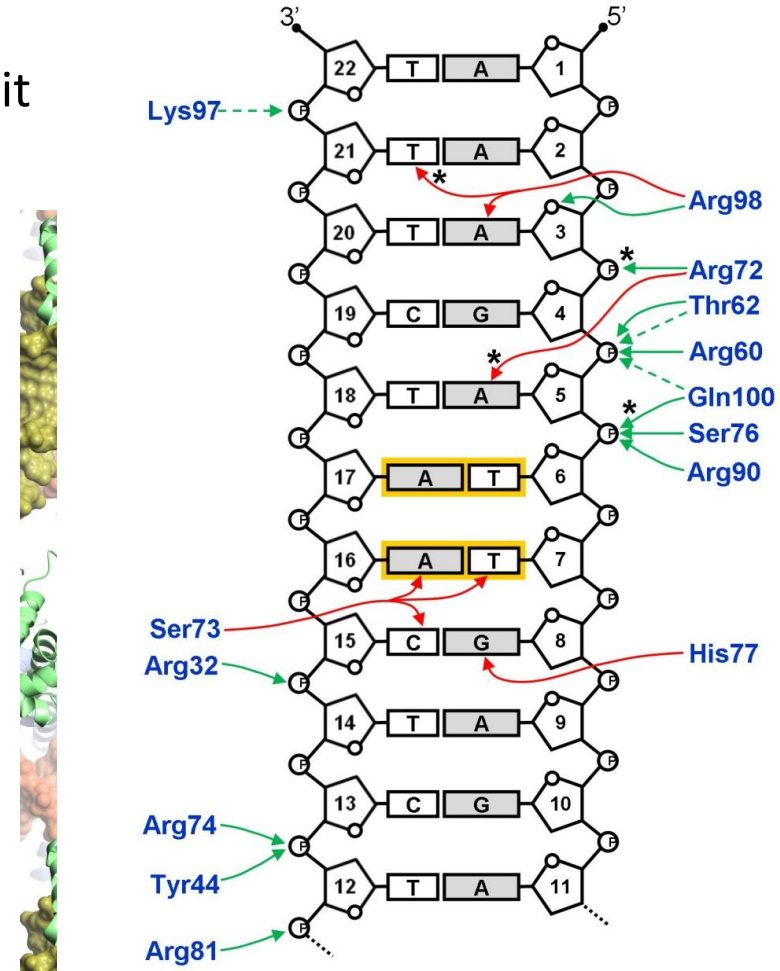
Space group confirmed at $P6_5$ but twinned

2 copies of the complex in the asymmetric unit

Structure solved by molecular replacement



R_{work} = 0.176
R_{free} = 0.196



Conclusions

SPR ideal technique for studying protein:DNA interactions

The ReDCaT chip can be used many times

Quick to screen multiple sequences/proteins or ligands in automated manner

Provides a real alternative to EMSA

Footprinting by SPR is accurate

Can be used for screening or accurate quantitative measurements

Uses a small amount of protein

Can be used to study any protein:oligonucleotide interaction

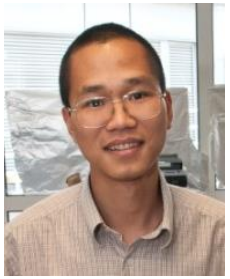
Can be used to guide choice of oligonucleotides for crystallisation

Cheap

Acknowledgements

- David Lawson

- Aoun Assaad
- Sandra Greive
- Merv Bibb
- Tung Le



GE Healthcare

- Catherine McDonnell

BBSRC bioscience for the future

diamond

Clare E. M. Stevenson, Aoun Assaad, Govind Chandra, Tung B. K. Le, Sandra J. Greive, Mervyn J. Bibb and David M. Lawson (2013). *Nucleic Acids Research,* Vol 41, No. 14