

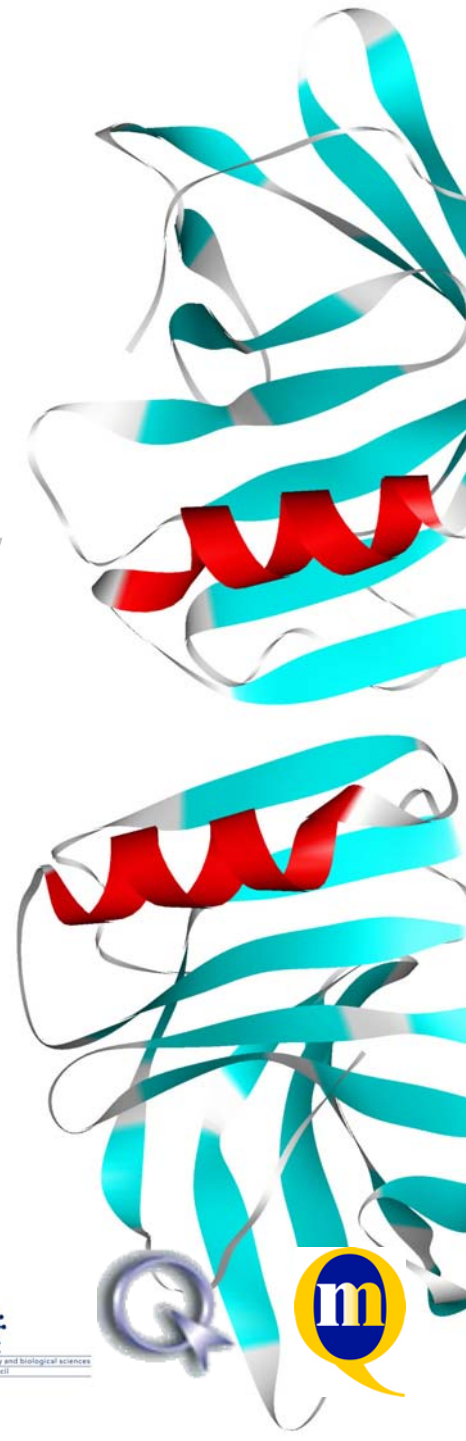
Designing an Evanescent Wave Optical Biosensor to Monitor Biochemical Activity

Gerry Ronan
Farfield Group Ltd

NW Biotechnology Company of the Year 2006
NW Exporter of the Year 2006

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Biochemistry & Molecular Machines

- Protein chemistry – molecular machinery of life
- Characterised by weak, reversible reactions
- Structure (or shape) determines function
- Structure changes as function happens
- Wrong structure causes malfunction

*Protein malfunction is at the heart of every **disease** & at the heart of **drug discovery***



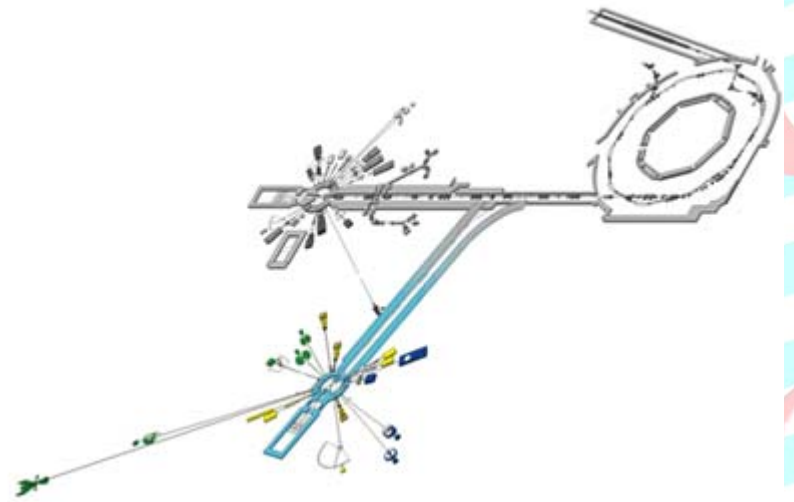
Structural Characterisation Methods

Daresbury



Synchrotron X-Rays

ISIS (Rutherford)



Neutrons

Beautiful 3D structures
but...

Static, Expensive, Time Consuming, Inaccessible

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Dual Polarisation Interferometry from Farfield

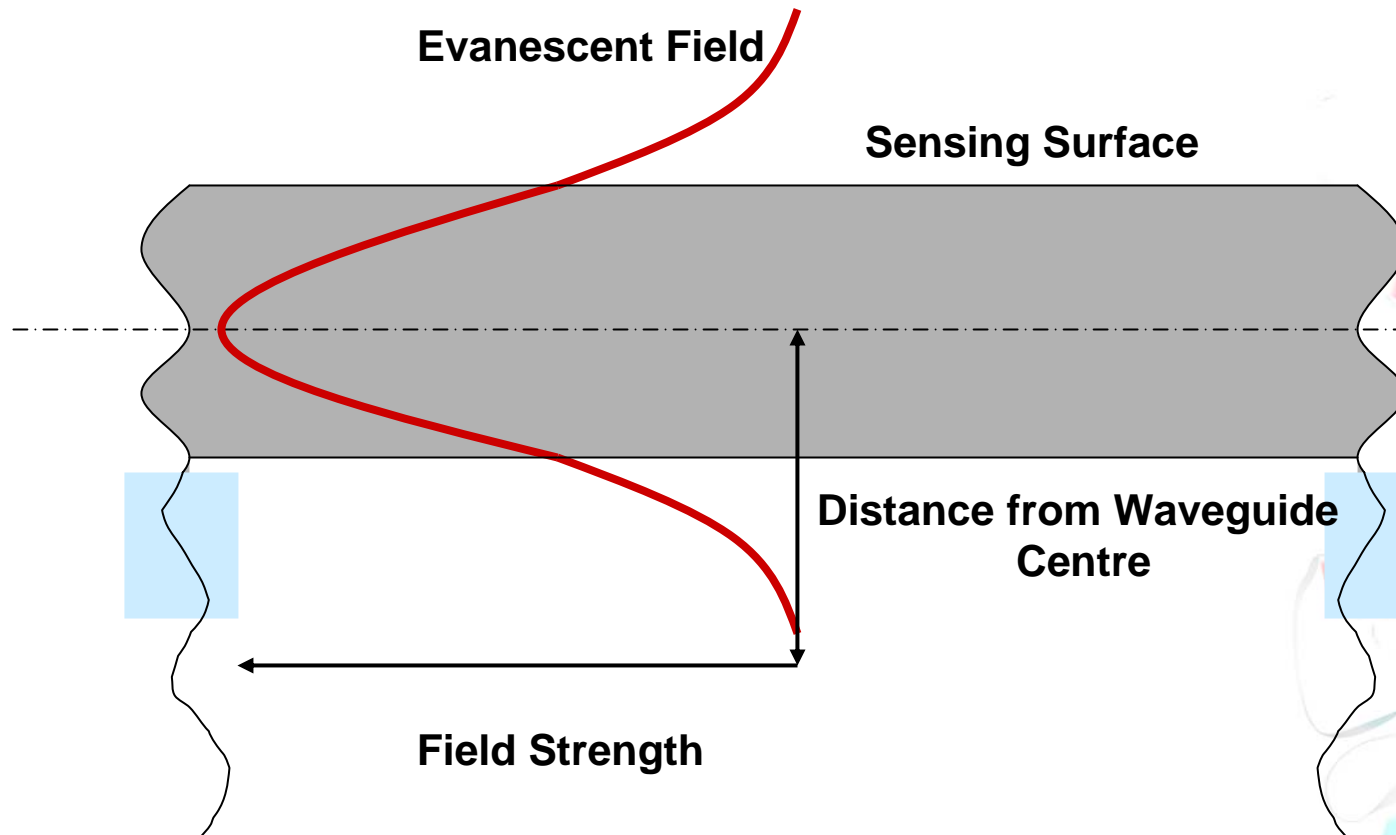
- Quantitative and absolute measurements of protein
 - *size, density* and *mass*
- Protein systems and their interactions
 - *real-time* changes in these parameters
- High resolution and sensitivity
 - *sub-picogram/mm²* in density & *sub-atomic* in dimensions

Delivers the *first* enabling tool for real-time *structure-function* studies of molecular behaviour

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Light in a Waveguide



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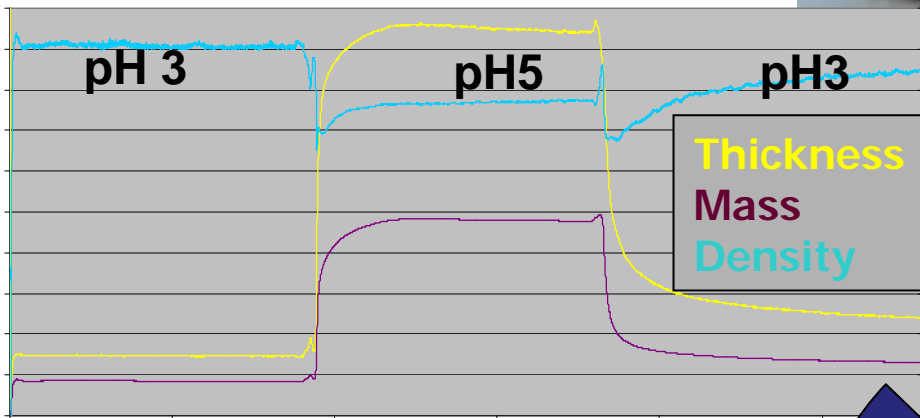
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Use the evanescent field to probe molecules on the sensing surface probe

The *AnaLight*® Product Range



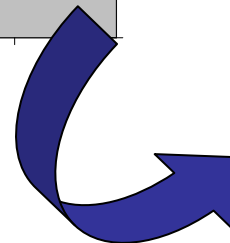
Sensor chips +
fluidics



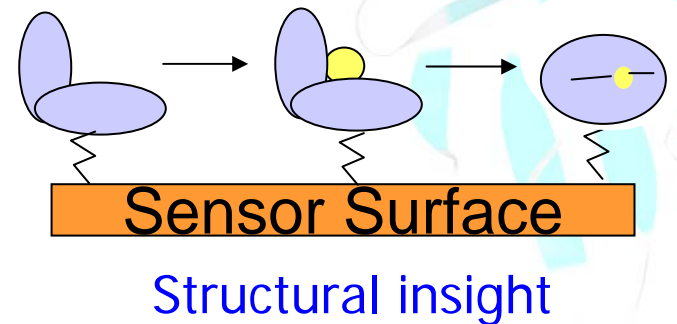
AnaLight® Software

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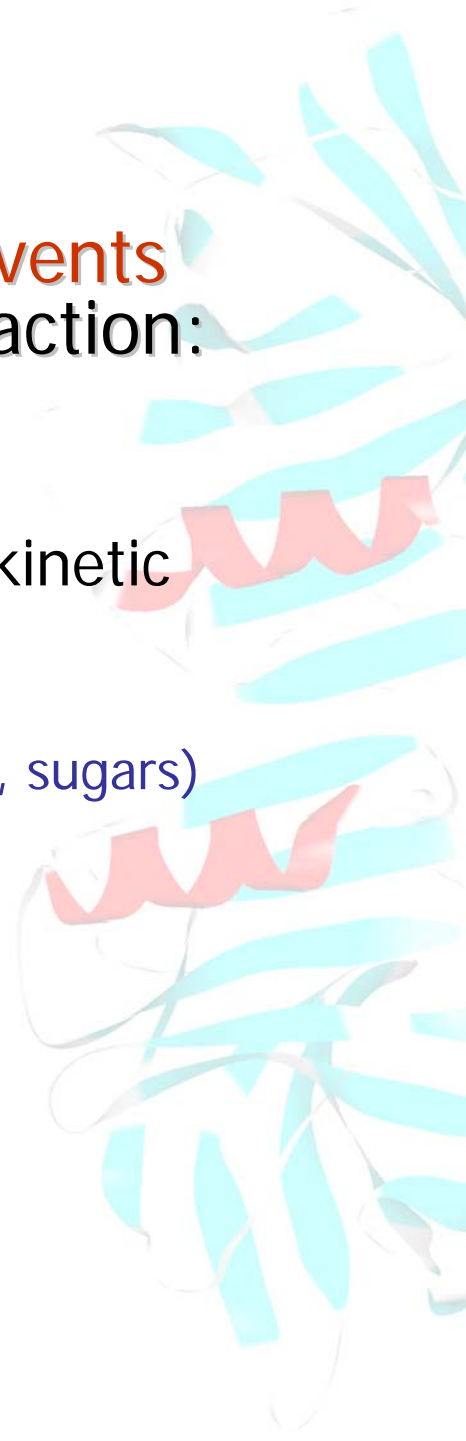
AnaLight® Flex



Application Areas

Real-time data following structural events during molecular function and interaction:

- Mechanisms of disease progression
- Structural characterisation with affinity and kinetic measurement
- Protein and biomolecule interactions
 - With other macromolecules (proteins, DNA, lipids, sugars)
 - With small molecules (inhibitors, metal ions)
- Aggregation & inhibition
- Protein folding and conformational changes
- Proteins on surfaces – diagnostics design
- Lipids and Membrane Proteins

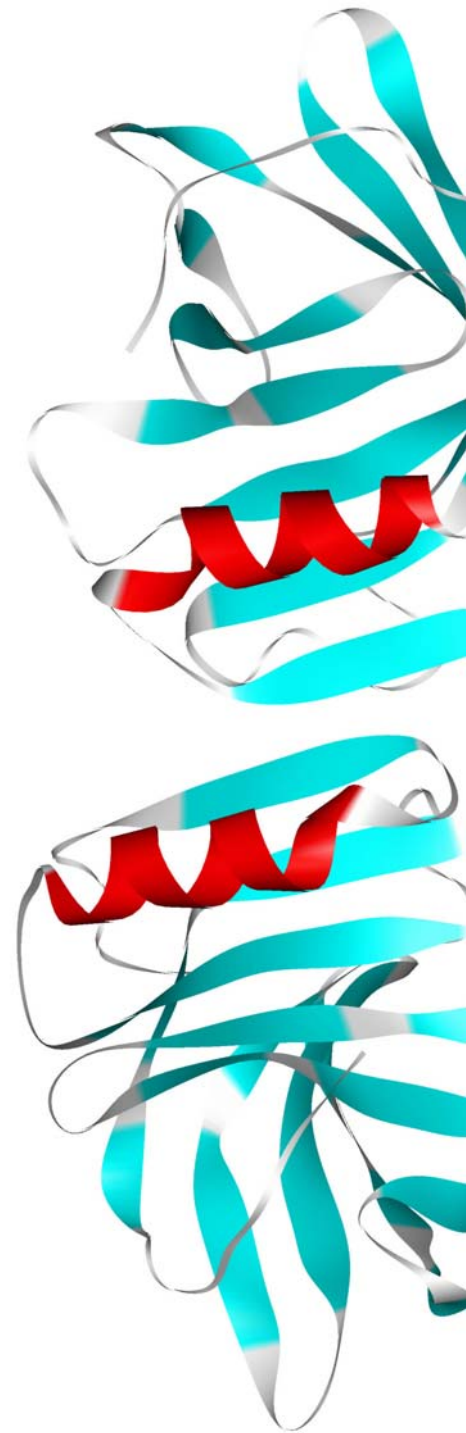


AnaLight® Applications

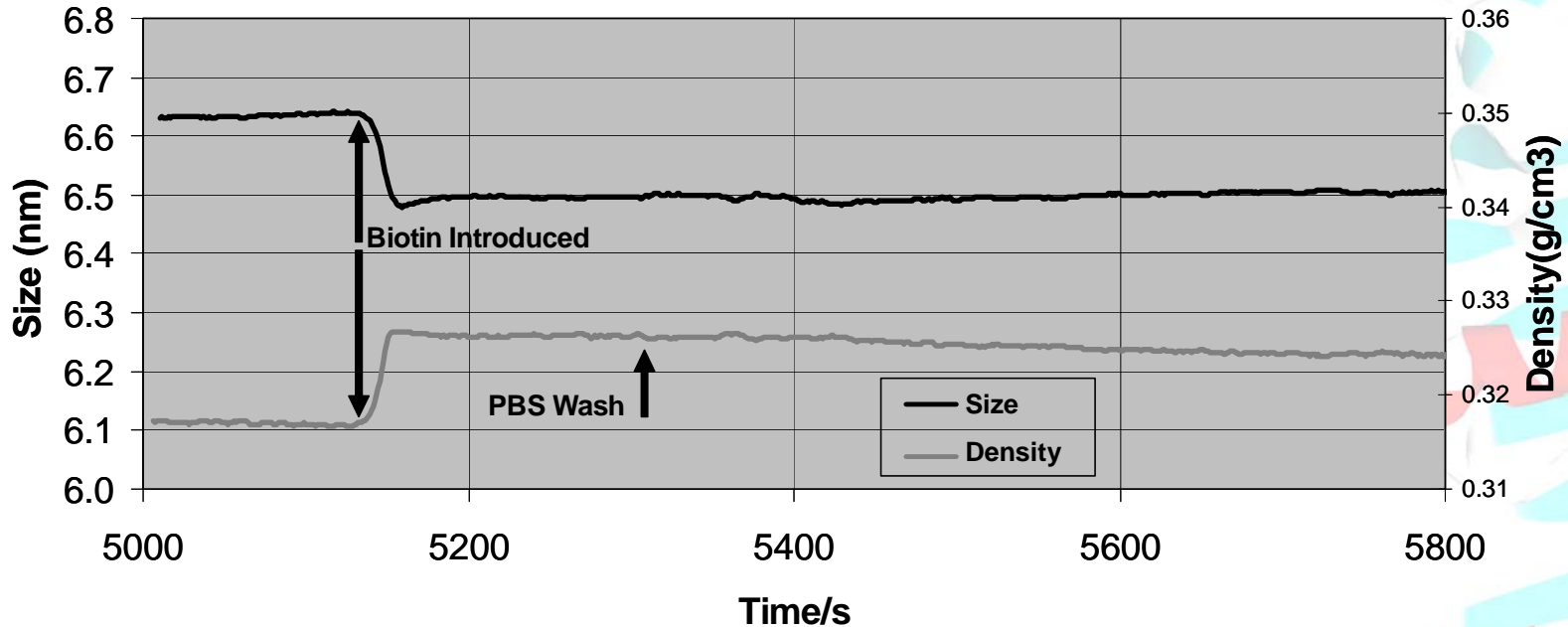
Protein – Small Molecule Interactions

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Compare *AnaLight*® & X Ray Crystallography



d-biotin (244 Da) causes thickness decrease & density increase:
Observe structural tightening in 55 kDa protein Streptavidin

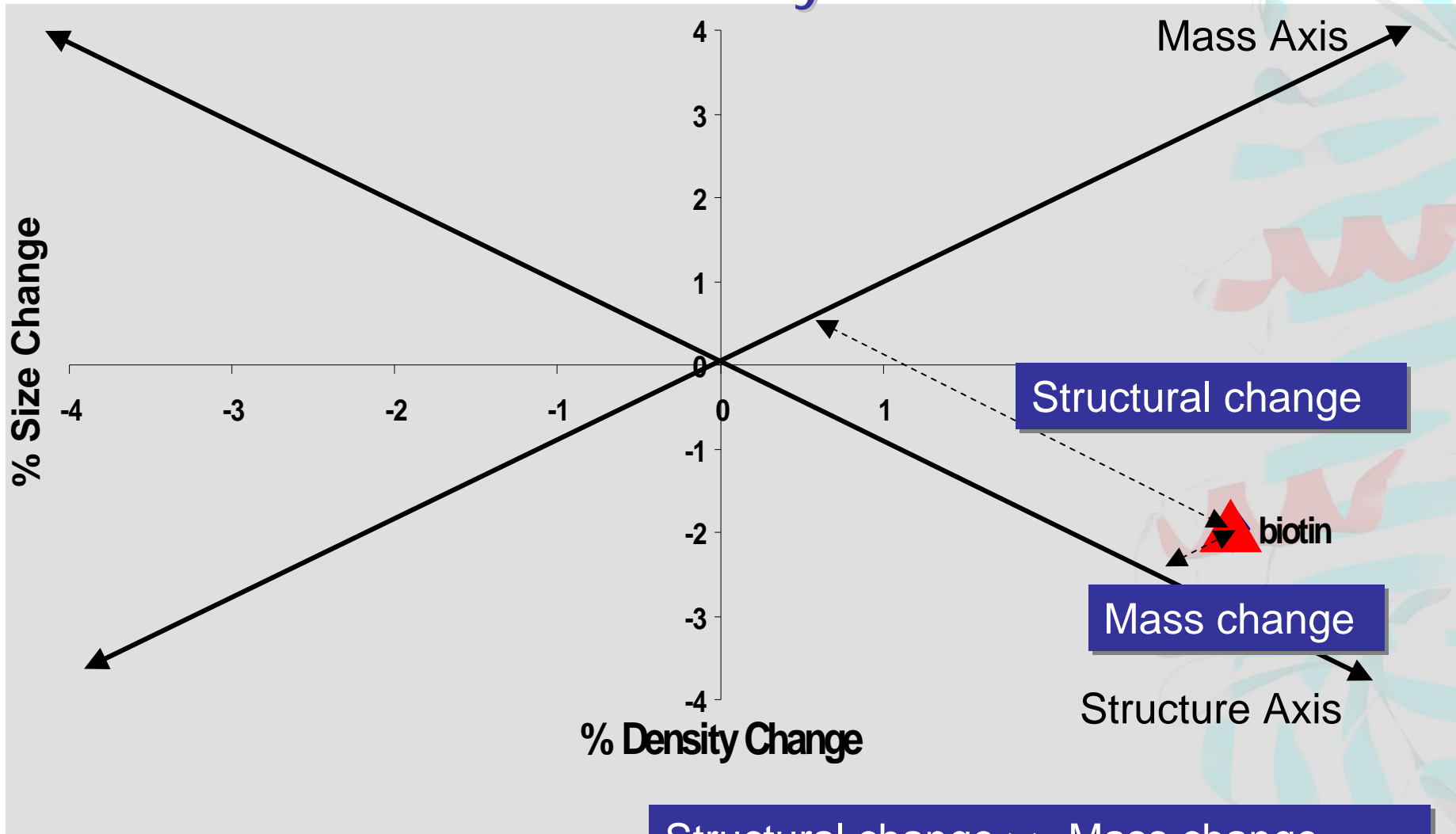
Protein	X Ray crystallography	5.6 – 6.8 nm
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	<i>AnaLight</i> ® measured	6.63 nm
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Conformational change	X Ray crystallography	0.08–0.4±0.2nm
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	<i>AnaLight</i> ® measured	0.13±0.01nm
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Streptavidin-Biotin Plotted on a Size-Density Matrix



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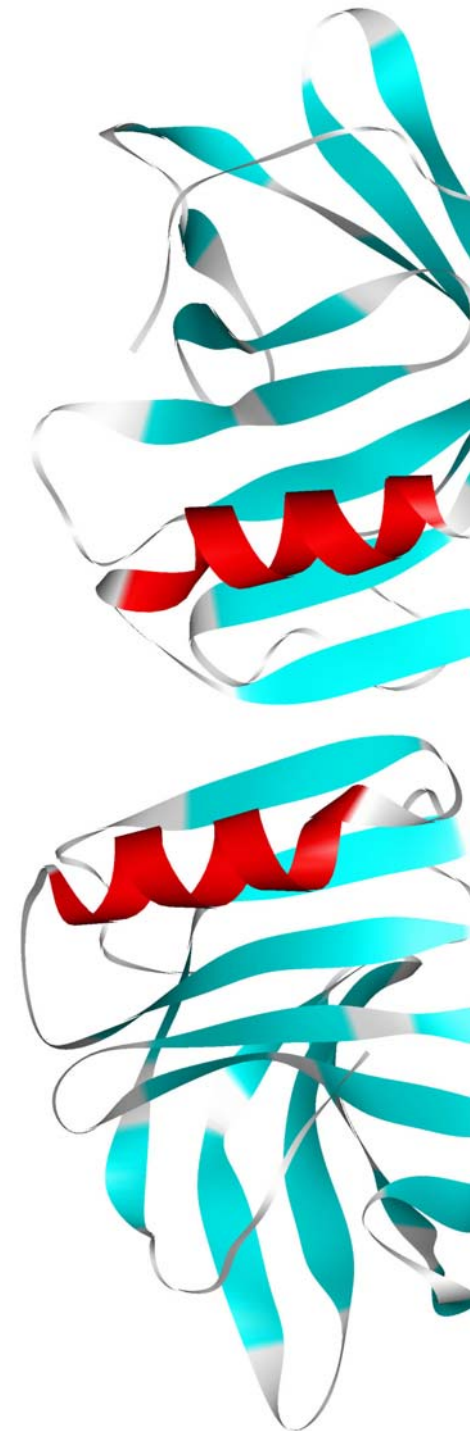
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AnaLight® Applications

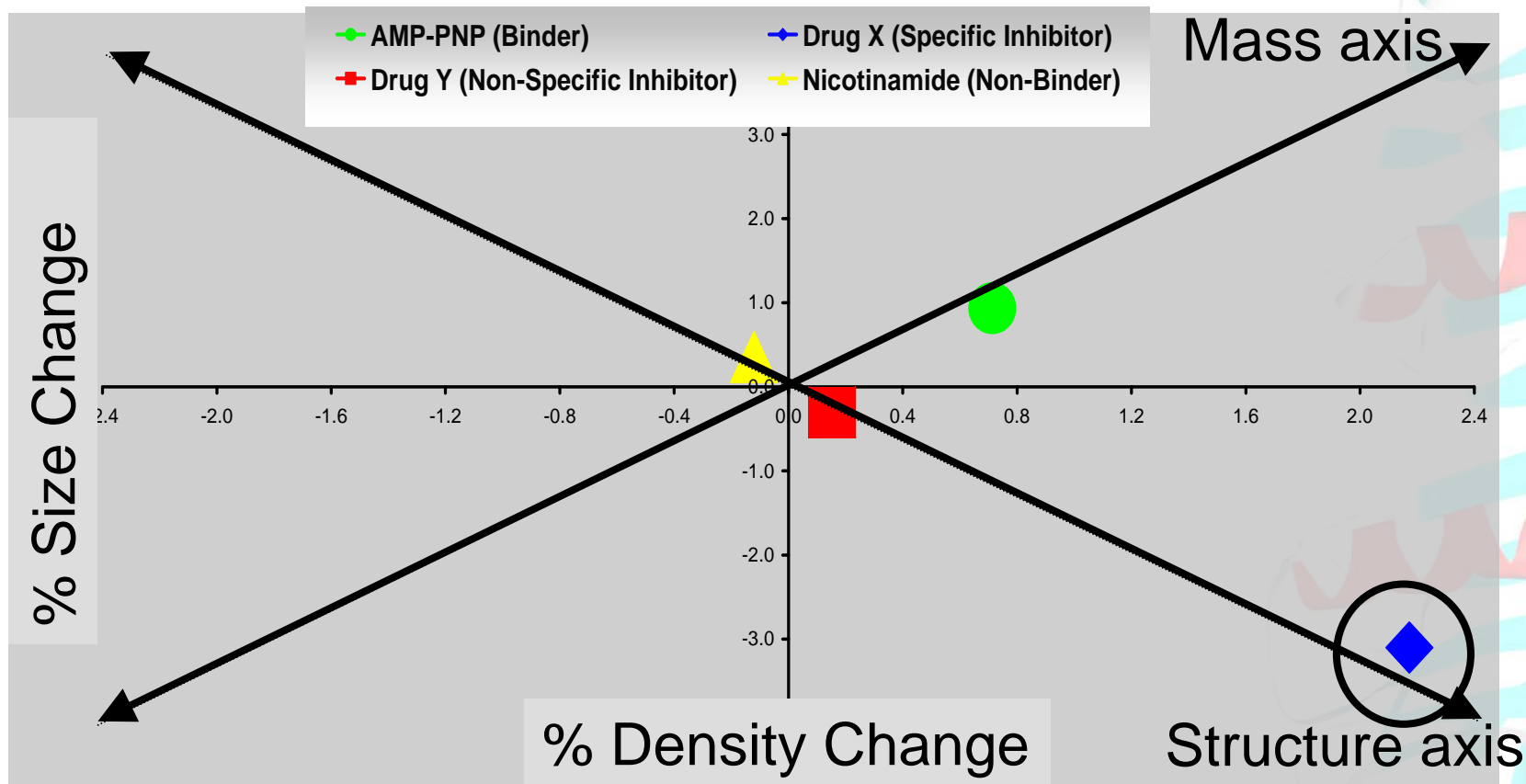
Target – Drug Screen
MAP Kinase

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MAP Kinase Size-Density Matrix

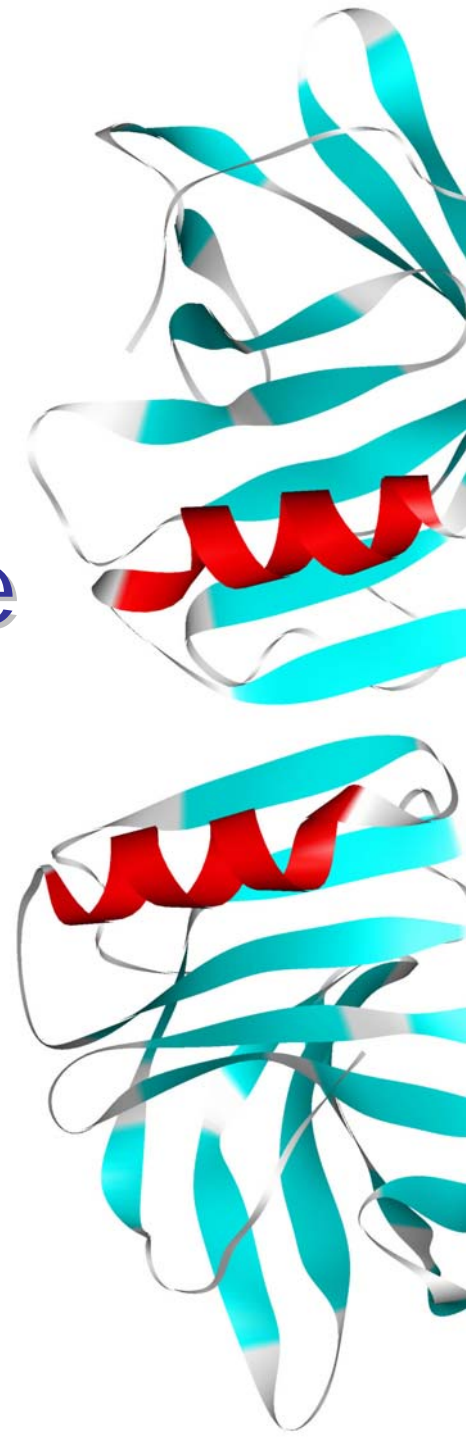


Clearly resolve structure change and mass change
-identifies structurally relevant binders

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Designing Antibody Capture Surfaces

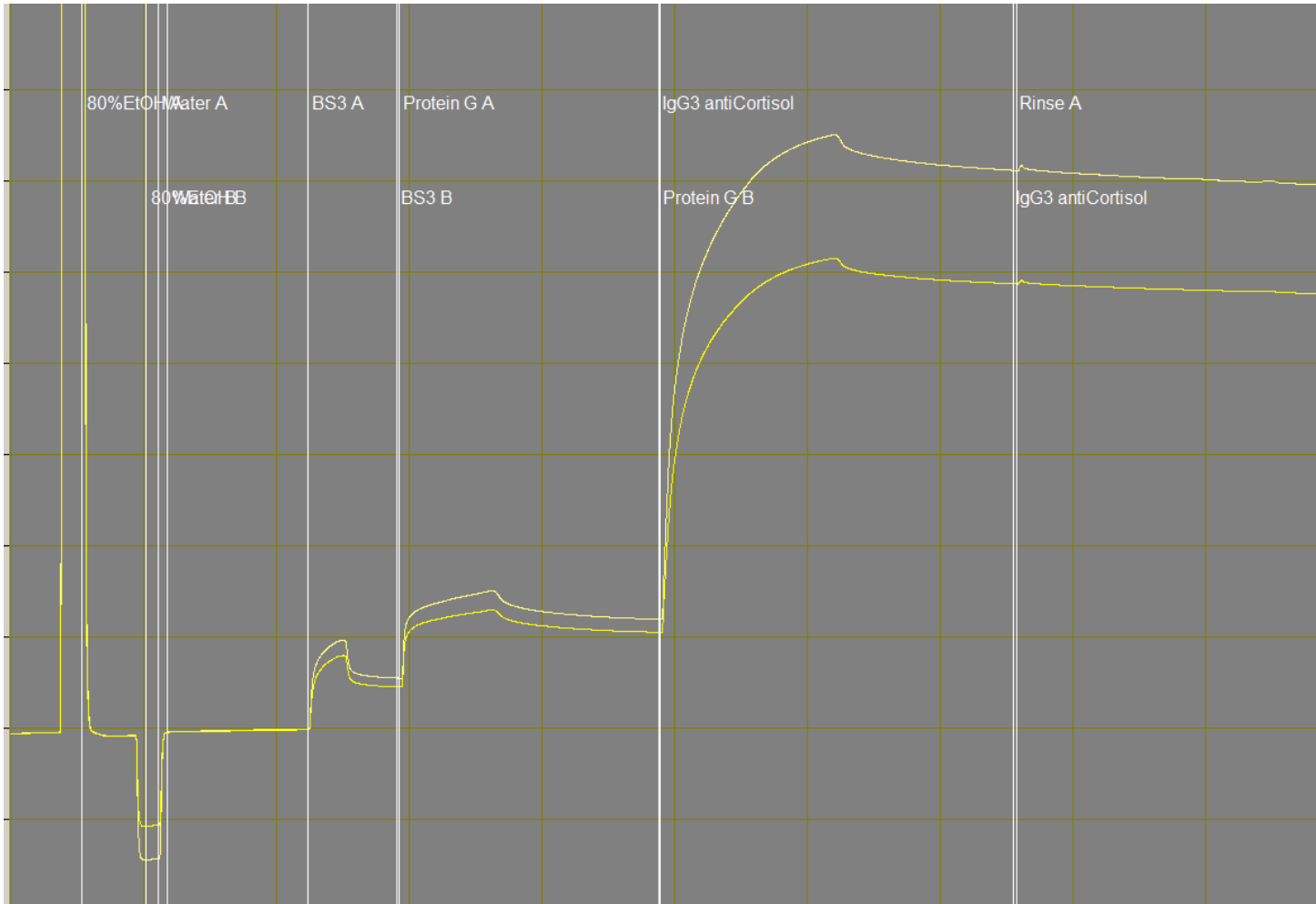


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Antibody-Antigen Interactions

Cortisol, MW 362Da



Experimental:

PBS, Thiol Chip

Injection sequence:

80% EtOH (calib.)

Water (buffer cal.)

GMBS(-SH/-NH₂)

Protein G

IgG3 (mouse anti-Cortisol)

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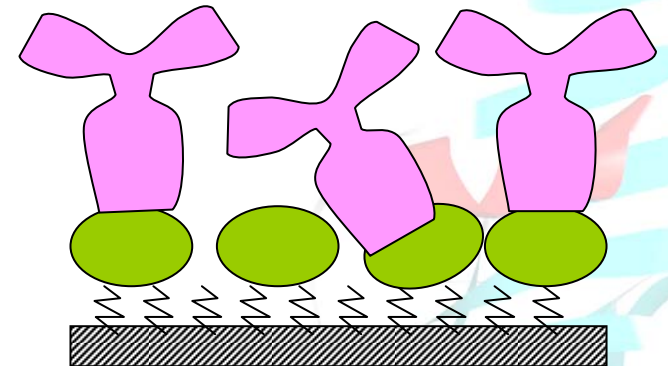
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DPI view of antibody capture

Antibody Immobilisation via Protein G

Name	Th / nm	Density/ gcm^{-3}	Mass/ ngmm^{-2}
Protein G	0.826	0.810	0.669
IgG3 a-Cortisol	15.126	0.181	2.743

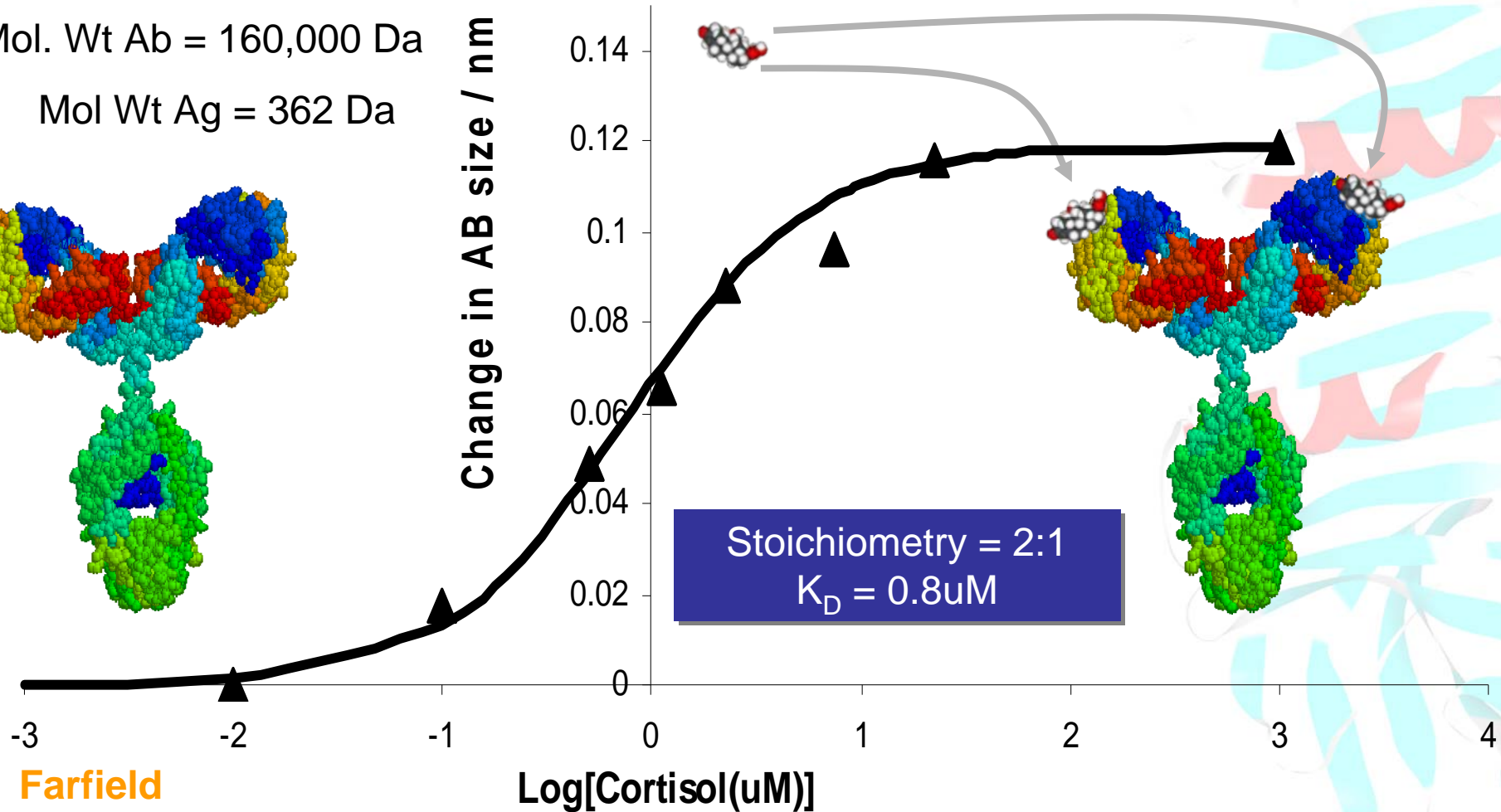
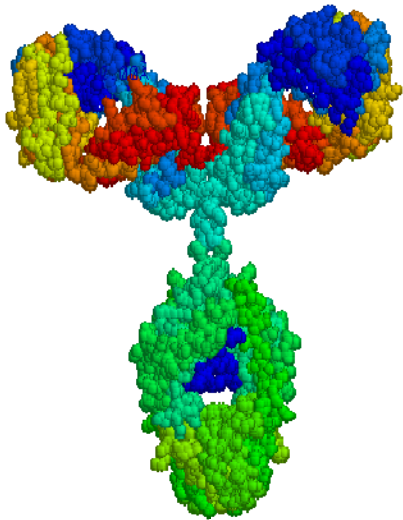
Antibody layer measured at 15nm:
Agrees with long axis from crystal structure
Reveals orientation of the antibody



Structure Change on Antigen (cortisol) capture

Mol. Wt Ab = 160,000 Da

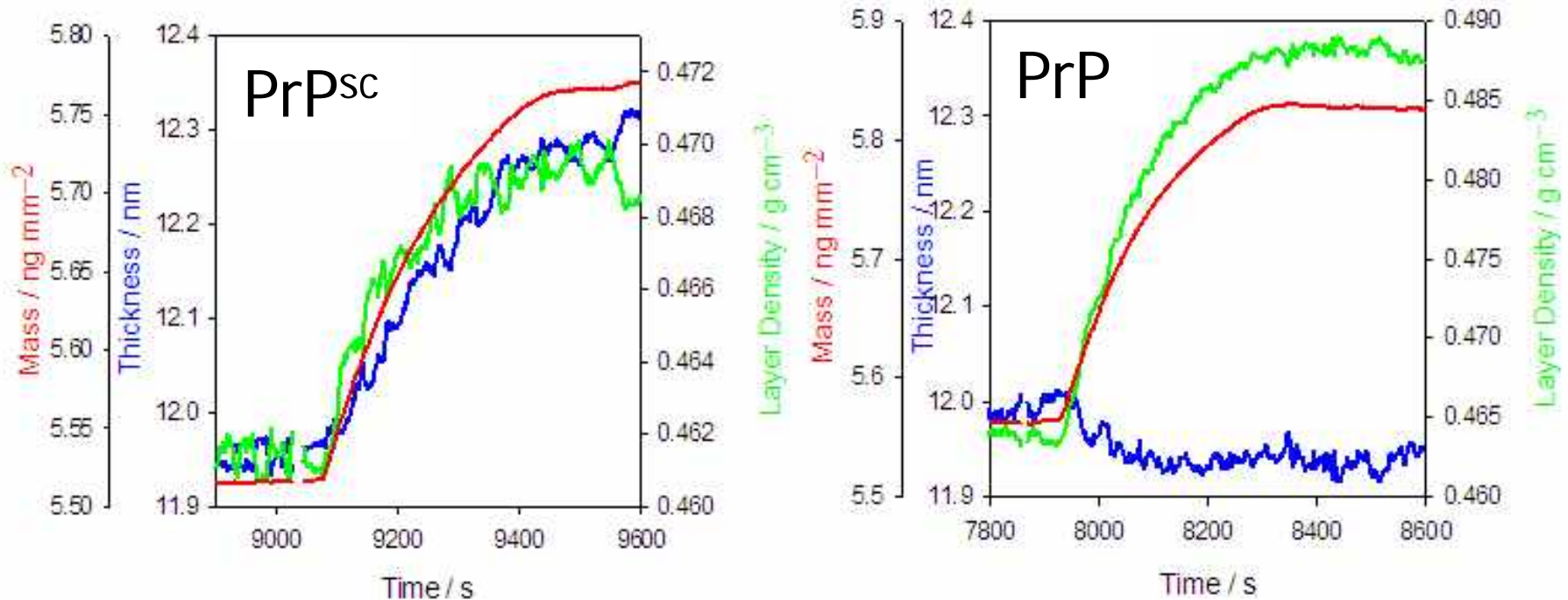
Mol Wt Ag = 362 Da



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Sometimes There is *Only* a Structural Difference, e.g. Prions



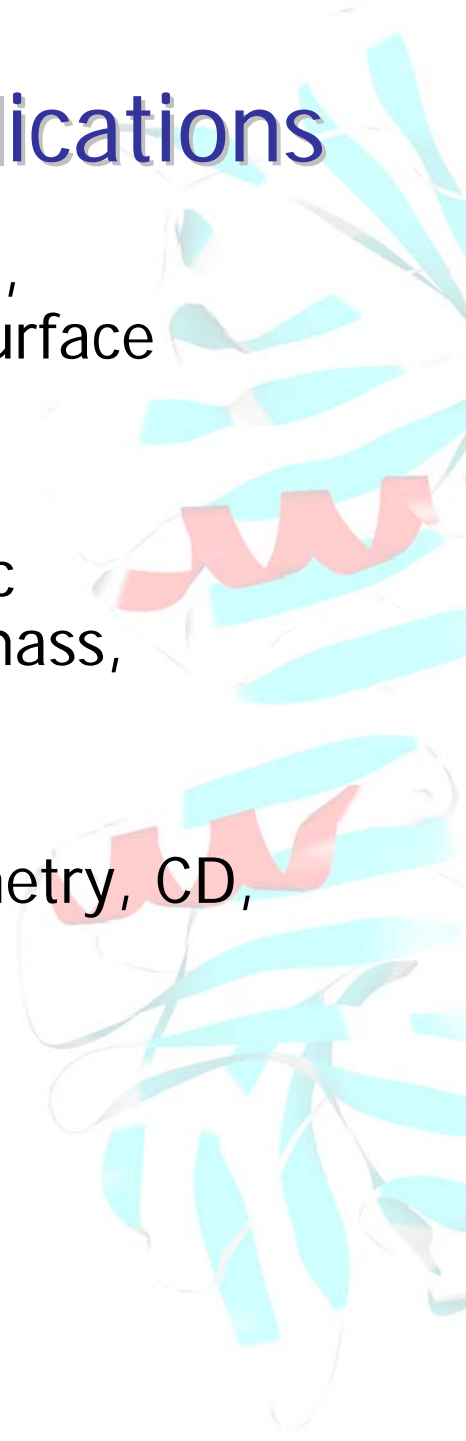
Clear discrimination between PrP and PrP^{sc}
on an antibody assay

Deployed across a range of applications

- Proteins, DNA, lipids, lectins, carbohydrates, surfactants, polymers, interfacial studies, surface characterisation and nanotechnology
- Provides real-time, high-resolution, dynamic measurements of molecular size, density, mass, affinities and kinetics
- Validated against X-Ray, Neutrons, ellipsometry, CD, QCM, SPR, etc
- Equivalent of a '*molecular microscope*'

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AnaLight® DPI Product Range



- Quantitative, absolute measurements traceable to international standards
- Sub-second measurements in real-time
- Sub-atomic resolution

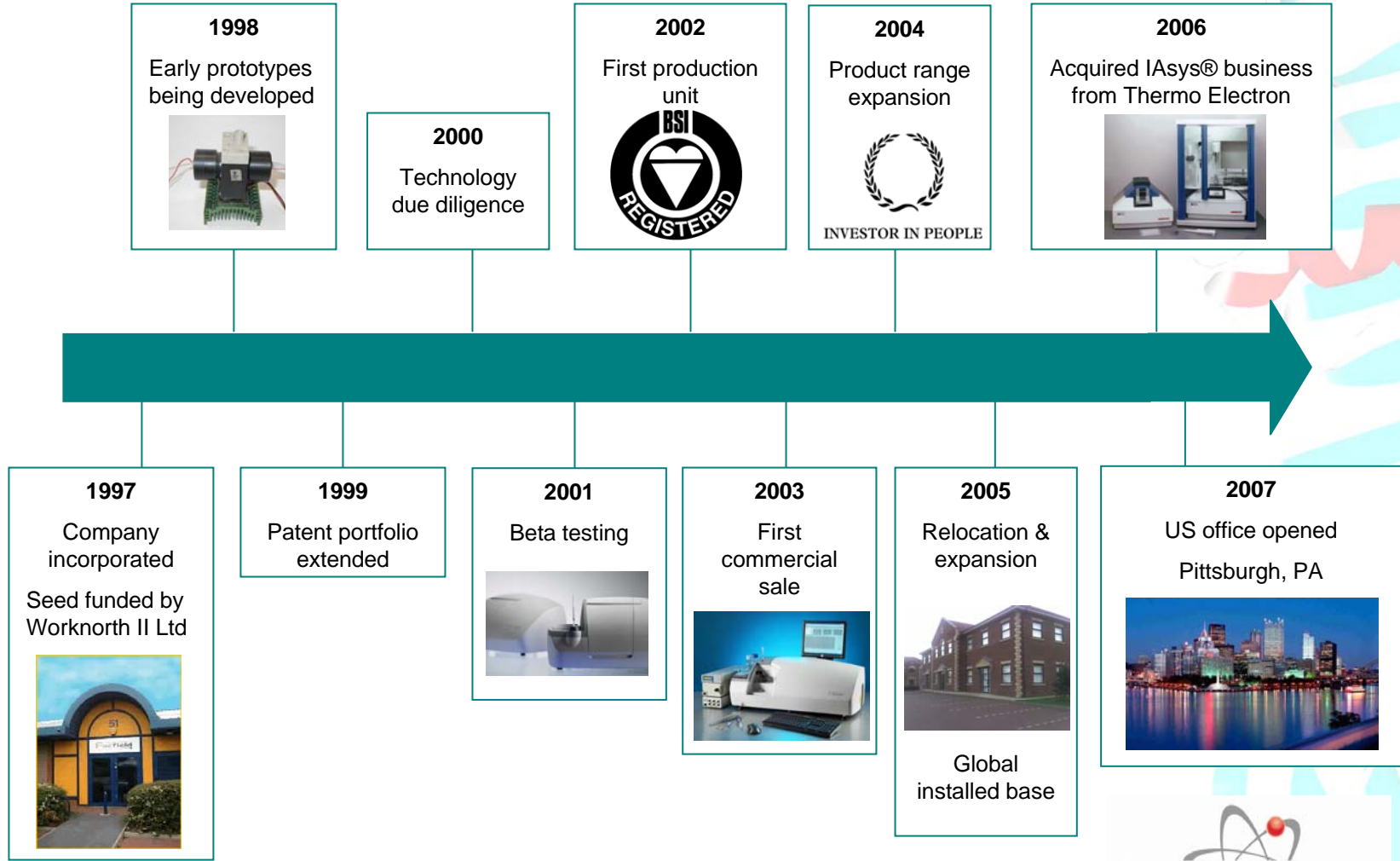
- User-friendly bench-top instruments
- Simple, fast and label-free experimental protocols
- IQ/OQ/PQ for GLP, GALP and cGMP



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History of Farfield



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Summary

- A completely new means of characterising biochemical activity
- Well validated against other biophysical methods
- Applications in understanding disease mechanisms and drug discovery
- Great insight into how molecules behave when captured on surfaces – design for diagnostics
- New capability for lipid-protein & membrane protein interactions by measuring order/ disruption in lipid bilayers

And it only took 10 years!

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INVESTOR IN PEOPLE

