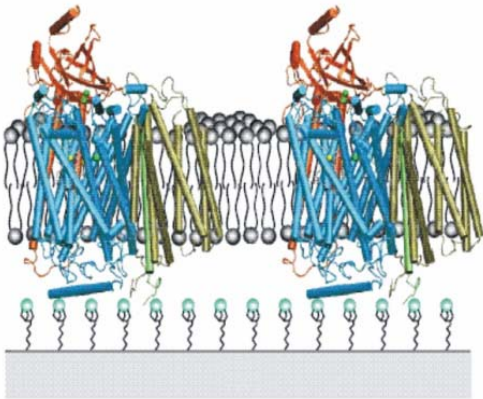


## Optimised binding of His-tagged proteins for DPI using an $M^{2+}$ chelating surface



The high level of sensitivity and precision achievable with Dual Polarisation Interferometry (DPI) makes it possible to dynamically characterise the assembly of His-tagged proteins using an  $M^{2+}$  **AnaChip™** surface. Proteins captured with such site specific tags offer a highly oriented and easily regenerated format for interaction assays.

### What is a His-tag?

A polyhistidine-tag is an amino acid motif in protein that consists of at least six histidine (*His*) residues, often at the N- or C-terminus of the protein. It is also known as hexa histidine-tag, 6x His-tag, and by the trademarked name His-tag.

## Introduction

A substructure of PEG has been shown to be beneficial in reducing non-specific binding (nsb). In this preliminary note, His-tagged molecules are thus immobilised via an  $M^{2+}$  chelation on a PEG substructure in an orientated fashion. The data shown demonstrates;

- the consistent loading of His-tagged protein
- the very low nsb of the PEG surface
- the correct orientation of the protein for its binding partner
- the ease of surface regeneration

## Binding to $M^{2+}$ -PEG surface

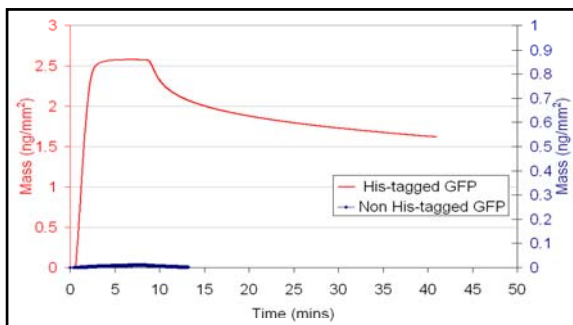
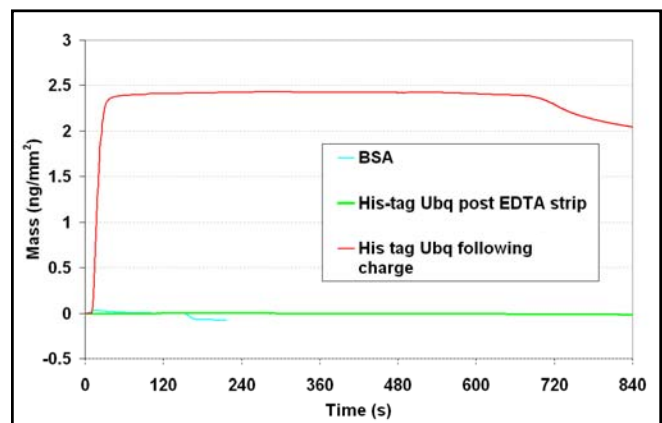


Figure 2. shows that when  $M^{2+}$  is stripped from chelating surface, His-tagged ubiquitin does not bind (green), compared to a stable layer formed with the same protein onto the charged  $M^{2+}$  surface (red). Importantly, very low nsb from a high concentration of sticky bovine serum albumin (cyan) is observed with this  $M^{2+}$  - PEG surface.

Figure 1 shows near zero binding of non His-tagged GFP (blue) compared with a stable layer for the same protein with a His-tag (red).



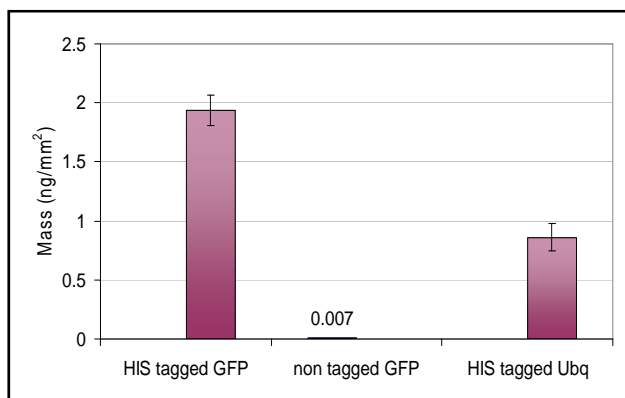


Figure 3 illustrates two different His-tagged proteins showing extremely reproducible loading.

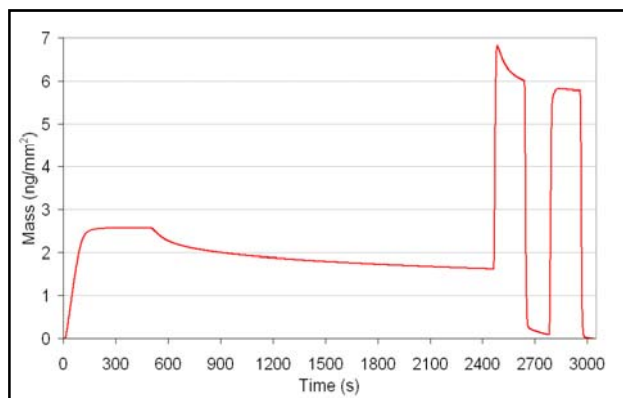


Figure 4 shows the simple and quick regeneration process using sequential EDTA + Tween injections.

	Change in Thickness (nm)	Surface coverage (ng/mm <sup>2</sup> )
Anti GFP to GFP layer	14.5	1.59
Anti GFP to control (non GFP) layer	2.5	0.28

Orientation of antibody and low nsb is shown in Table 1. The added anti GFP to GFP is bound in an orientated fashion showing the expected increase in thickness for antibody of circa 15 nm. A small amount of nsb is evident which from the thickness of 2.5 nm shows it is in a prone orientation to the substructure.

## Summary

The M<sup>2+</sup> His-tag capture surface is a highly orientated surface showing extremely low levels of non specific binding (nsb). The surface loading is easy to control, it demonstrates high signal-to-noise levels and additionally, is easy to regeneration. The potential of the surface for protein-protein as well as small molecule interactions makes it an excellent all round capture surface for DPI.

## The AnaChip™ His-tag Capture Surface

**Farfield** introduces a highly orientated capture system via the **AnaChip™** surface. The launch of this new sensor chip reflects Farfield's commitment to maintain a range of surface chemistries and capture formats to facilitate rapid migration of assays from other analytical techniques directly to the **AnaLight®** platform.

### Benefits:

- High degree of specificity and low non specific binding
- Optimal ligand orientation for efficient assay format
- Robust and stable surface that is amenable to regeneration
- Ideal for measuring conformation events during interactions
- Selectable surface loading for determining association / dissociation kinetics and affinity studies

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