Metal Polymers, A Glue to Immobilise Proteins Onto Synthetic Surfaces

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Introduction

Many different applications such as bio-separations, biomaterials, drug delivery, biomarker discovery involve the immobilisation of biological materials onto synthetic surfaces. The challenge is that most biological materials such as proteins are easily damaged when attached to some synthetic surface. This challenge is further compounded by the variety of synthetic surfaces/materials as well as the variety of biological materials that need to be localised on such surfaces.

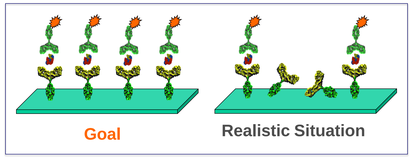

Fig.1. Immunoassays are one of the major techniques requiring attachment of proteins, e.g. antibodies to synthetic surfaces. In standard ELISA tests the damage done to a portion of the antibodies might not limit sensitivity because antibodies are present in vast excess. This, however, is no longer the case with increasing miniaturisation where the available surface area to bind proteins becomes limiting and every antibody counts.

The objective of our work was to develop a surface chemistry which maintains protein function and orientation per unit surface area, regardless of the surface used and in addition

- Improve sensitivity and dynamic range
- Reduce antibody usage
- Increase speed of assays
- Provide consistent performance even with “problem antibodies” that are sensitive to passive binding or existing covalent chemistry.

The approach we took was to use metal chelation with avidity binding concepts, i.e., create multiple, weak binding forces that together form strong but gentle binding interactions.

Methods

In Immobilized Metal Affinity Chromatography (IMAC), metal ions such as Ni(III) and Co(III) are used to isolate and purify His tagged proteins. In order to do so, the metal ion needs to bind to particular ligands (such as NTA) that are coupled to the synthetic surface. Even so, protein binding strength is relatively weak even for His tag proteins (μM binding strength).

Using high throughput screening methods, we identified various metal ion, counter ion combinations that exhibited far stronger binding potential to proteins (Anal. Biochem. 363 (2007) 97-107) even without the need for special ligands such as NTA (on the synthetic surface) or His tags (on the protein). One such combination is the use of metal ions in polymeric form. Metal ions can be linked together with the use of various multi-valent chelating ligands and some metal ions can naturally form hydrolytic polymers. **Mix&Go(TM)** is one such family of polymeric metal ions based around Cr(III).

Metal polymers such as Mix&Go are water soluble and because it can exist as di-mers, tri-mers and other longer polymers, its character gives Mix&Go multiple points of chelation whose number increases overall binding strength. In addition, polymer structure may potentially impart some degree of selectivity according to its shape.

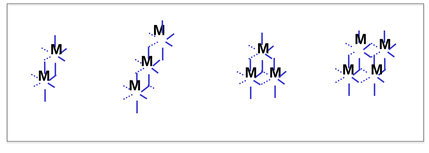

Fig 2. Different metal polymer shapes are possible and depending on metal ion number and shape, can give a large range of binding strengths and potentially selectivity to some target protein.

Since all sorts of basic, neutral and acidic groups can chelate with metal ions, these metal polymers were investigated as a generic “glue” to bind proteins to all sorts of synthetic surfaces. For ease of use, synthetic surfaces were first coated with these metal polymers to form an activated but stable surfaces. These Mix&Go activated surfaces were assessed for their potential to bind all sorts of proteins to many different surfaces.

Results

Key Characteristics of Metal Polymers

We studied the characteristics of metal polymers, such as Mix&Go, when used to attach proteins, e.g. antibodies, streptavidin, and Proteins A or G to synthetic surfaces with focus on characteristic that would improve immunoassay performance or ease-of use:

Reaction Kinetics: Binding Mix&Go to synthetic surfaces and binding proteins to Mix&Go activated surfaces are both very fast with maximum loading essentially achieved within 1 min.

Flexibility in Surfaces: Mix&Go will bind strongly to any surface having electron donating potential to form a stable activated surface for protein binding. Surfaces tested include:

- Acid, amine and non-functionalised polymer beads
- Bare silica beads or slides
- Sepharose, polyvinylalcohol, metal oxides, etc.
- Plain polystyrene plates
- Plasma or gamma treated materials that do not normally bind any proteins

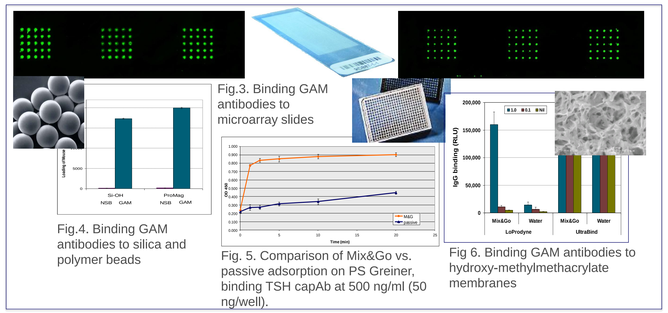

Fig. 3 Mix&Go on a variety of surfaces

Stable Binding of Proteins: MixGo has been proven to be stable in conditions such as:

- Boiling beads for 10 mins in 2% SDS-PAGE sample buffer (SDS, DTT, Glycerol,Coomassie Blue dye)
- Freeze-Drying Mix&Go-activated Beads for over a month
- Treating beads for 48 hours with 0.5M EDTA then boiling them in SDS-PAGE buffer
- Storing Mix&Go-activated beads in 200mM EDTA for 1 year
- Extremes of pH ~ 2.5 (Glycine or Citrate buffer elution) to pH ~ 12 (0.5M NaOH washing)

Good functionality of bound Proteins: Despite fast and strong binding, proteins bound to Mix&Go surfaces are gentle that nearly all immobilise proteins are functional.

Purification of human IgG with Protein A beads	Dynabead Control	**Mix&Go**
Binding Capacity for IgG, related to measured bead concentration (mg/gm bead)	8.5	12.2
Yield of IgG in elution step	67%	92%

Table 1. In immunoprecipitation studies >90% of immobilised Protein A bound and subsequently eluted IgG.

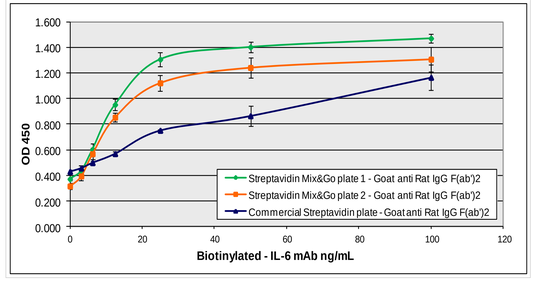

Fig. 7. Binding of biotinylated rat IL6 Ab to Streptavidin Mix&Go plates (Greiner and Nunc PolySorp) using anti-rat F_{ab} specific detection is far more efficient than with standard commercially available Streptavidin plates.

Mix&Go plates achieved detection of <2 ng/ml

Results, Cont.

Resulting Benefits of Metal Polymers in Immunoassays

Sensitivity and Dynamic Range: Under limited surface area for immobilising capture antibodies, e.g. FACS, significant sensitivities can be obtained

	Immunoassay Analyte					
Activation	IL-6*	HBsAg	TSH	PSA	TNFα	GM-CSF
Mix&Go	0.2	24	0.0006	3.5	0.5	12
Tosyl	2.4	1040	0.0017	200	0.9	67
Improvement Factor	60	43	2.8	57	1.8	5.6

Table 2 Loading efficiency for 2 different IgGs comparing amide vs. Mix&Go coupling on industry standard Tosyl Beads

Reducing Antibody Use: Because of rapid quantitative coupling there is less antibody wastage. In bead-based immunoassays we demonstrated up to 90% antibody savings, and 67% savings of beads at comparable sensitivity.

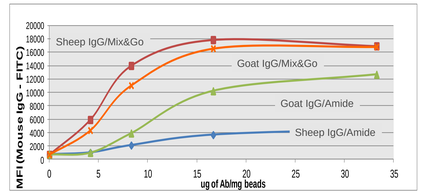

Fig.8 Loading efficiency for 2 different IgGs comparing amide vs. Mix&Go coupling on Dynal M270 beads.

Time to Result: Increased density of functional antibodies improves speed of binding cognate ligands, especially under limiting situations we observer far more functional, correctly orientated bound antibody

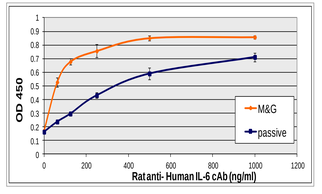

Fig.9. IL6 Ab (as detected by anti-rat F_{ab} specific Ab) on Mix&Go surfaces compared to passive adsorption on microtitre plates.

Linear Correlation: Under non-saturation conditions the amount of antibody added equals the amount of active antibody on the surface.

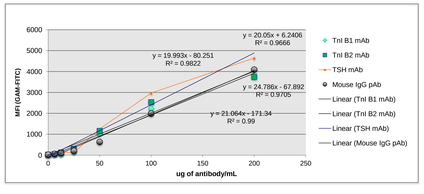

Fig.10. With Mix&Go, all 4 antibodies show a high linear correlation between what is added and what is immobilised on MyOne Dynal beads.

Conclusion

Mix&Go is a family of metal polymers of different sizes and shapes. The key benefits of using such metal polymers to attach proteins to synthetic surfaces are simplicity, as well as faster and simpler assays with greater sensitivity and improved dynamic range. Especially for miniaturized formats where maintaining correct antibody orientation, good antibody distribution, and activity of each antibody are critical, these metal polymer “glues” will provide enhanced performance and ease-of-use.