

Novel Polymer Based Elemental Tags for Sensitive Bio-assays

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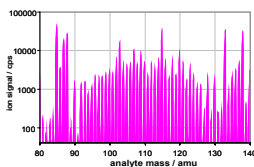
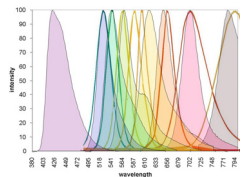
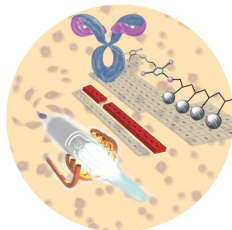
We are developing a highly sensitive bio-assay technology

- > Lanthanides as elemental tags.
- > Inductively coupled Plasma Mass Spectrometry (ICP-MS): an ideal technique for detecting and quantifying tags.
- > Simultaneous measurement of many proteins in a single sample.

Advantage of our method compared to currently available fluorescent tagging approaches

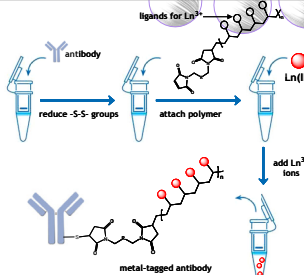
- Excellent resolution between the tags.
- Exceptional dynamic range (9 orders of magnitude).
- High sensitivity.
- Sample stability.

Our technique replaces fluorophores with elements/stable isotopes



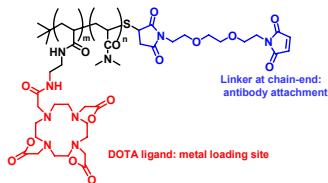
Our Experimental design:

- > Ligand-polymer conjugate attached to antibody.
- > Lanthanide metal loaded.
- > Metal-tagged antibody generated.
- > Different metal with different antibody.
- > Multiplex bio-assay experiment.
- > ICP-MS measurement of metal.

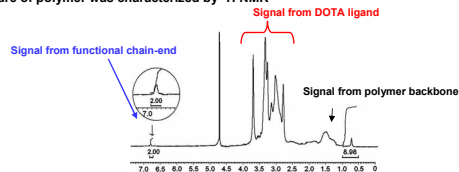


Polymeric reagent:

- A water-soluble polymer
- Containing many copies of ligand
- Functional chain-end which is reactive to antibody

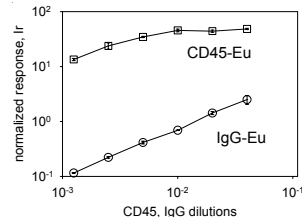


Structure of polymer was characterized by ¹H NMR



Specificity and titration of metal-tagged antibody performed on KG-1a cells.

- Polymer-antibody (CD45 and IgG) conjugates prepared.
- Europium loaded on conjugates (CD45-Eu and IgG-Eu).
- IgG-Eu is a control

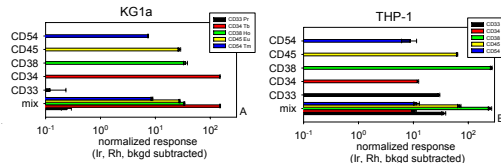


From this figure,

- Binding of CD45-Eu to KG-1a cell follows a saturation curve.
- Non specific binding of IgG-Eu displays a linear dependence.
- There is big difference between specific antibody binding and non-specific IgG binding.

Multiplex bio-assay experiment

- Five antibodies labeled with five metal elements.
- Two cell lines representing myeloid (KG-1a) and monocytic (THP-1) acute leukemia compared.
- Single antibody and mix experiments compared.



From these experiments,

- There is no signal interference between detection channels.
- Metal ions do not dissociate and re-associate with ligands on other polymer chains.
- Two cell types differ dramatically in the expression of CD33 and CD34.
- Quantitative information obtained in a single assay about two different protein markers that differ 500-fold in degree of expression.

CONCLUSIONS

We have developed a novel elemental tagging procedure which, in conjunction with ICP-MS analysis, allows the multiplexed detection of proteins on cells. The use of polymer-based elemental tags offers the important advantage that each tag carries many copies of a given element, leading to a large increase in assay sensitivity. Because of this sensitivity, we were able to detect in a single assay two different cell surface markers (CD33 and CD34 in KG1a), which differed by ca. 500-fold in their abundance. Our success in carrying out simultaneous assays for 5 cell surface markers opens the door for more massively multiplex analyses that will allow "fingerprint" detection of individual types of leukemia cell lines. This approach appears to have many advantages over conventional fluorescence-based detection.

ACKNOWLEDGEMENTS

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