Expressed Lanthanide Binding Tags as Visualization Tools
Technology #9382

Applications

Together with luminescence enhanced photography, lanthanide binding tags conjugated to proteins of interest can be used as fluorescent probes in 1D or 2D gel electrophoresis. They may also be used to directly analyze protein-protein interactions in place of traditional GFP-based protein-protein interaction assays or static and time-dependent variation in protein-protein distances in Forster Resonance Energy Transfer (FRET) experiments. In addition, lanthanide binding tags may be useful as heavy-atom derivatives in macromolecular X-ray crystallography.

Problem Addressed

Traditional luminescent protein probes, such as fluorescent tags, have a relatively short lifetime of emission and are susceptible to photobleaching, leading to lowered signal quality due to the interference of strong background signals and the overtime loss of probe signal. To address these issues, lanthanide ions (Ln³⁺), which have a long emission lifetime, have been incorporated in indirect probing methods such as dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA). However, these methods involve many time-consuming steps. On a separate issue, macromolecular crystallography traditionally relies on heavy-atom derivatization using the MIR method and constraints of the primary peptide sequence to resolve the phase problem. These methods are often fraught with difficulty because heavy atom soaking often alters the crystal cell structure. New tagging methods that increase fluorescent emission lifetime, as well as decrease heavy atom interference with target protein folding and crystallization, would greatly improve localization and imaging techniques.

Technology

This invention involves the incorporation of short oligopeptide motifs designed to complex trivalent Ln³⁺ ions into native amino acid sequences. Thus far, these motifs have been successfully fused with the protein RNAse at both the C- and N-terminals along with a polyhistidine tag for purification of the target protein. The complexes these motifs form with Ln³⁺ ions show physical properties including fluorescence and anomalous X-ray scattering. They exhibit high binding affinity for Ln³⁺ within nM and pM concentrations and low binding affinity for trace essential transition metal ions. They have low susceptibility to endogenous proteases. Their short sequence means that they are less likely to interfere with native protein function than traditional fluorescent constructs. Finally, they have a long emission lifetime that allows for the elimination of background signals from intrinsic fluorescent material, which means they can be used for highly selective detection and quantitation of the expression of tagged proteins. In terms of their potential in x-ray crystallography, they have been shown to absorb edges for anomalous scattering within the tunable range of synchrotron light sources (7-12 keV). The high binding affinity between the motifs and Ln³⁺ ions prevents Ln³⁺ ions from interfering with target protein folding and crystallization, and the low thermal motion of the Ln³⁺ ion within the peptide-binding site allows for reliable experimental results.
Advantages

- Maximum signal intensity for optimal sensitivity
- Long-lived fluorescent species that can be observed in time-resolved experiments
- Selective binding to lanthanide and low affinity for trace essential transition metal ions
- Low susceptibility to endogenous proteases
- Good stability

Categories For This Invention:

- Life Sciences
- Imaging
- X-ray, CT, PET
- Research Tools
- Protein & Protein Chemistry
- Transgenic
- Vector & Plasmid

Intellectual Property:

Lanthanide binding tags
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Publications:

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