A novel strategy for the purification of a recombinant protein using ceramic fluorapatite-binding peptides as affinity tags

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Abstract
In recent years, affinity fusion-tag systems have become a popular technique for the purification of recombinant proteins from crude extracts. However, several drawbacks including the high expense and low stability of ligands, their leakage during operation, and difficulties in immobilization, make it important to further develop the method. The present work is concerned with the utilization of a ceramic fluorapatite (CFT)-based chromatographic matrix to overcome these drawbacks. A heptapeptide library exhibiting a range of properties have been synthesized and subjected to ceramic fluorapatite (CFT) chromatography to characterize their retention behavior as a function of pH and composition of the binding buffer. The specific binding and elution behavior demonstrates the possible application of CFT-binding peptides as tags for enhancing the selective recovery of proteins by CFT chromatography. To materialize this strategy, a phage-derived CFT-specific sequence KPRSVSG (Tag1) with/without a consecutive hexalysine sequence, KKKKKKPRSVSG (Tag2), were fused at the C-terminus of an enhanced green fluorescent protein (eGFP). The resulting gene constructs H\textsuperscript{-eGFP}, H\textsuperscript{-eGFP-Tag1} and H\textsuperscript{-eGFP-Tag2} were expressed in Escherichia coli strain BL-21, and the clarified cell lysate was applied to the CFT column equilibrated with binding buffer (20-50. mM sodium phosphate, pH 6-8.4). Sodium phosphate (500. mM) or 1. M NaCl in the respective binding buffer was used to elute the fused proteins, and the chromatographic fractions were analyzed by gel electrophoresis. Both the yield and purity were over 90%, demonstrating the potential application of the present strategy. © 2014 The Authors.

SciVal Topic Prominence
Topic: Photoreceptors, Microbial | Chromophores | protein PYP
Prominence percentile: 87.101

Reaxys Database Information
View Compounds

Author keywords
Affinity chromatography; Ceramic fluorapatite; Peptide affinity tags; Peptide synthesis; Production and purification of recombinant proteins; Retention behavior

Indexed keywords
| Engineering controlled terms: | Affinity chromatography; Electrophoresis; Escherichia coli; Peptides; Phosphates; Purification; Recombinant proteins; Sodium compounds |
### Engineering uncontrolled terms
- Affinity tags; Chromatographic matrix; Enhanced green fluorescent protein; Fluorapatites; Gel electrophoresis; Peptide synthesis; Retention behavior; Selective recovery

### Engineering main heading:
- Ceramic materials

### EMTREE drug terms:
- Ceramic fluorapatite; fluorapatite; hybrid proteinre; combinant protein; unclassified drug

### EMTREE medical terms:
- Article; binding affinity; cell lysate; column chromatography; controlled study; gel electrophoresis; nonhuman; peptide analysis; peptide library; peptide synthesis; priority journal; protein purification; separation technique

### Species Index:
- Escherichia coli

### Medline keywords:
- Affinity chromatography; Ceramic fluorapatite; Peptide affinity tags; Peptide synthesis; Production and purification of recombinant proteins; Retention behavior

### MeSH:
- Affinity Labels; Apatites; Ceramics; Chromatography, Affinity; Escherichia coli; Green Fluorescent Proteins; Peptides; Recombinant Fusion Proteins

### Chemicals and CAS Registry Numbers:
- fluorapatite, 12525-40-5, 1306-05-4, 58051-82-4

### Funding details

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<td>Consejo Nacional de Investigaciones Científicas y Técnicas</td>
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### Funding text
This work was partially supported by the EU Project INTENSO (FP7-KBBE-2012-6 under GA 312004), Jacobs University Bremen, Germany, and Tecnológico de Monterrey, Monterrey, Mexico. Support was also provided by the Marie Curie-International Research Staff Exchange Scheme (IRSES) from the European Union. M. FernándezLahore is a member of the National Council for Research and Technology (CONICET), Buenos Aires, Argentina. Special thanks to the group members of Prof. Dr. Guy Cardineau, Centro de Biotecnología-FEMSA, Tecnológico de Monterrey, Mexico, for their help in protein expression. Thanks to Cera McDonald and Marcie Phillips for their assistance during the preparation of the manuscript.

- **ISSN:** 00219673
- **CODEN:** JCRAE
- **Source Type:** Journal
- **Original language:** English
- **DOI:** 10.1016/j.chroma.2014.02.079
- **PubMed ID:** 24661869
- **Document Type:** Article
- **Publisher:** Elsevier