# Hydroxyapatite Binding Peptide Genetically Fused to Green Fluorescent Protein for Monitoring Mineraliazation

Esra Yuca,[1,2,3] Urartu O.S. Seker[1,2] Mustafa Gungormus,[2] Ayten Y. Karatas,[1] Mehmet Sarikaya,[1,2] and Candan Tamerler [1,2*]

[1] Department of Molecular Biology and Genetics, Istanbul Technical University, Turkey

[2]Genetically Engineered Materials Science and Engineering Center, University of Washington, USA

[3] Department of Biology, Yildiz Technical University, Turkey

**Statement of Purpose:** Hydroxyapatite (HA) has been one of the most extensively studied biominerals, being the principal inorganic component of the human and other animal hard tissues such as bone and teeth ( Lowenstam H.A. Science. 1981:211:1126-1131.) Besides its wide use in hard tissue repair and replacement, HA has also attracted attention as a gene/drug and cell delivery agent and regulator of cell bioactivity. Therefore, it is essential to be able to label and track HA for its biomedical (gene/drug delivery, bone and teeth formation/ remodeling) and nano-technological importance (nano-particle composites). It is now possible to screen a large number of random peptides for for binding to any inorganic substance. In our group, we identified and characterized peptides that can bind inorganic materials specifically and with high affinity using cell surface and phage display technologies. (Tamerler C. ACS Nano. 2009:3:1606-1615). These peptides have applications as inorganic synthesizers, nanoparticle linkers, and molecular assemblers, or simply as molecular building blocks, in a wide range of fields from chemistry to materials science to medicine. In our previous studies, we have bio-combinatorially selected HA binding peptides (HABP). The effect of the two heptapeptides, a strong binder (HABP1, C-MLPHHGA-C) and a weak binder (HABP2, C-NAPFAQA-C) peptides, on the mineralization reaction and crystal morphology were demonstrated (Gungormus M. Biomacromolecules. 2008:9:966-973.). Various kinds of autofluorescent proteins with different spectral properties and enhanced brightness have been developed in recent years. Because of the nontoxicity, these genetically engineered fluorescent probes are safer than alternatives for labeling living subjects. (Chudakov D.M. Trends in Biotech. 2005:23:605.). In the present study, we report a simple and versatile method for fluorescent labeling of HA utilizing fusion protein of Green fluorescent protein (GFP) as probe and HABP as a molecular linker (Figure 1a).

**Methods:** We utilized GFPuv as a fluorescent probe together with HABP1 and HABP2 to construct peptide-fluorescent probe conjugates. Following PCR and subcloning, the constructs were cloned into pQE-1 (QIAGEN) expression vector and TOP10 *E. coli* competent strain was transformed. *E. coli* cells carrying the designed plasmids were cultured overnight at 37 $^{o}$C in 10 mL of Luria Bertani (LB) medium with 100 µg.ml$^{-1}$ ampicillin on the 200 rpm shaker (Figure 1c). GFPuv-HABP expressing bacteria were monitored using an epifluorescence microscope Following the expression step, purified GFPuv-HABP1 and GFPuv-HABP2 were incubated with HA powder, the binding affinities of the proteins on HA powder were compared by fluorescence microscopy.

**Results:** Bacteria expressing GFPuv and GFPuv-HABP constructs were monitored under fluorescence microscopy (Figure 1b) , and strong fluorescence was detected for all the three *E. coli* strains expressing GFPuv, GFPuv-HABP1 and GFPuv-HABP2. With the aim of analyzing the second function of the designed proteins, the HA binding properties of the construct were examined. The strong binder, GFPuv-HABP1 fusion structure can bind HA particles and has much more affinity to HA than GFPuv-HABP2 and than the negative control GFPuv.

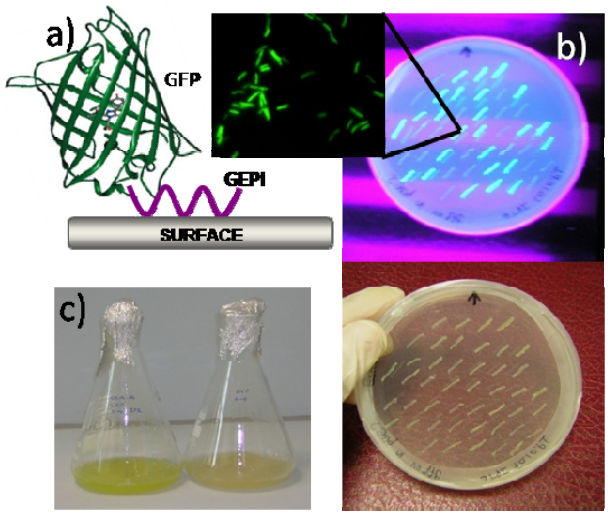


Figure 1: (a) GFP-GEPI based bifunctional conjugates. (b) After subcloning, *E. coli* TOP10 cells were transformed with the constructs. Transformed *E. coli* colonies were picked up from the solid media and observed under UV . c) GFPuv and GFPuv-HA binding peptide expressing *E. coli* cells were grown in liquid media, induced with IPTG and harvested for protein purification .

**Conclusions:** Our GFPuv-HABP1 construct may be utilized for real time monitoring of size, morphology and crystallization of hydroxyapatite during biomineralization process. HA particles conjugated with GFPuv-HABP1 fusion protein may be useful for in vitro and in vivo imaging studies in hard tissue engineering.

*The research supported by NSF-MRSEC and -IRES programs at the University of Washington. TR-SPO and TUBITAK-NSF Programs at Istanbul Technical University.*