

A Specific Surface-coating Peptide for Rare Earth Nanomaterials

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ABSTRACT

Surface properties of nanoparticles play a predominant role in determining the biological effects, both desirable and adverse, elicited by engineered nanomaterials and can be tuned by surface modifications with a variety of covalent as well as non-covalent modifications. Using phage display, we have discovered an 11-amino acid peptide that binds to rare earth nanomaterials with high affinity and forms a stable coating layer on the surface of the nanocrystals. The peptide efficiently reduces sedimentation of the nanocrystals, decreases the interaction of nanocrystals with cells and surfaces, abrogates autophagy induction and toxicity, and acts as a linker in enabling biomedical applications for rare earth nanomaterials and formation of rare earth-based composite nanomaterials.

Keywords: rare earth nanomaterials, phage display, peptides, cell interaction, biomedical applications

1 RE-1 FORMS A COATING LAYER ON RE NANOCRYSTALS

Through phage display approach, we discovered a phage, designated as REOB-1, which exhibited high affinity binding towards rare earth (RE) oxide and RE-based upconversion nanomaterials. A synthetic peptide corresponding to the displayed sequence ACTARSPWICG also bound strongly to variety of RE oxide and RE upconversion nanocrystals (UCN), yet exhibited weak binding to diamond and titanium dioxide nanocrystals and minimal binding to SiO₂, SiO₃ and silver nanocrystals. The amino acid sequence, rather than the composition, of the displayed peptide was critical for the binding. Binding of RE-1 to RE nanocrystals was highly stable and was unaffected by changes in pH, temperature and ionic concentrations. TEM and SEM further revealed a peptide coating layer on the surface of nanocrystals.

2 RE-1 COATING REDUCES NANOCRYSTAL SEDIMENTATION AND NANOCRYSTAL-CELL INTERACTION

Compared to uncoated nanocrystals, RE-1-coated UCN exhibited significantly less interaction with the growing as well as the fixed HeLa cells. In principle, there are two main factors driving nanocrystals in solution to the surface of cells cultured at the bottom of the plate: sedimentation and diffusion. RE-1 coating effectively prevented the sedimentation of UCN and the much-bigger UCP nanoparticles. Through upright/inverted configuration system assay[1], we conclude that RE-1 coating has two prominent effects on nanocrystals: preventing sedimentation, likely achieved through decreasing nanocrystal-nanocrystal interactions, and reducing cell interaction. The first effect prevents the nanocrystals in solution from reaching the surface of cells, plastics or glass, while the second effect reduces nanoparticle adherence to a surface once the nanocrystals have reached the surface by either diffusion or sedimentation. For live cells, RE-1 coating may also decrease nanocrystal internalization.

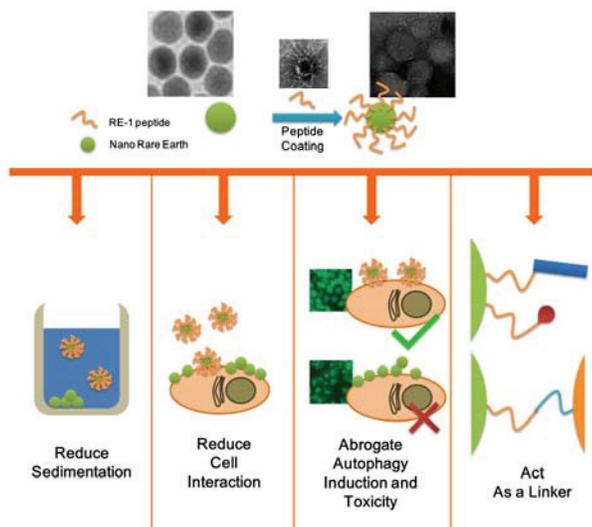


Figure 1: A specific surface-coating peptide for rare earth nanomaterials in bio-applications.

3 RE-1 COATING ABROGATES AUTOPHAGY INDUCTION AND TOXICITY FOR RE NANOCRYSTALS

Uncoated RE nanocrystals can induce autophagy and cytosolic vacuolization [2-3] in a dose-dependent manner, but coating with RE-1 dramatically reduced the autophagy and completely eliminated cytosolic vacuolization over the whole concentration range. RE-1 coating also reduced cellular uptake of nanocrystals. Uncoated UCN caused significant viability reduction and cell death in HeLa cells, but this toxicity was completely abolished by the RE-1 coating. UCP was more toxic than UCN and exhibited significant viability-reducing and death-promoting effects, and these effects were effectively abrogated by RE-1 coating as well. Moreover, RE-1 coating also dramatically reduced autophagy induction and toxicity in the liver tissue after in vivo administration of for UCN and UCP.

4 RE-1 ACTS AS A LINKER TO ENABLE VARIOUS NANOMATERIAL ENGINEERING

RE-1 can also act as a linker to enable various nanomaterial engineering. Here, we present three examples.

First of all, RE-1 linked RGD coating can enhance cell interaction and autophagy induction of UCN. While RE-1 coating provides an effective means for reducing cell interaction and autophagic response, it is also highly desirable to have a coating peptide that exhibits the opposite effect, that is, capable of promoting cell interaction and enhancing the autophagic response. The RGD cell adhesion sequence has been shown to promote cellular uptake for many different therapeutic agents and nanoparticles[4]. We thus synthesized a bifunctional peptide RE-1-RGD (CRGDCGGACTARSPWICG), combining the RE-1 sequence with the RGD motif via a short linker. RE-1-RGD had the similar activity as RE-1 in reducing UCN sedimentation. However, in contrast to RE-1, UCN coated by RE-1-RGD exhibited significantly more interaction with the cells as compared to uncoated UCN. Approximately equal UCN fluorescence was observed in the upright and in the inverted configuration for RE-1-RGD-coated UCN, indicating that the sedimentation effect was abolished by the peptide coating and that the enhanced cell interaction was manifested after the UCN nanocrystals have reached the cell surface through diffusion and likely through RGD interaction with integrins. Coating with RE-1-RGD significantly enhanced UCN-induced autophagic response and cellular toxicity.

Secondly, RE-1 linked AT-1 coating can enhance apoptosis cell targeting of UCN. UCN present a new technology for optical imaging/detection. Such upconverting nano-materials have highly unusual optical properties that are foreseen to overcome current limitations

of conventional fluorophores including photobleaching, short penetration depth and high autofluorescence background. Equally attractive is their near-zero background fluorescence since most other materials, including biological molecules, do not possess this upconverting property[5]. Thus, we use UCN to replace the easily quenched fluorescent dyes for apoptosis detection. We make RE-1 act like a bridge linker and synthesized a bifunctional peptide RE-AT-1 (CRGDCGGACTARSPWICG), combining the RE-1 sequence with the AT-1 peptide via a short linker for apoptosis cell targeting. To value the effect of RE-AT-1, we conduct RE-AT-1-coated UCN treated with the apoptosis cells and capture the results by Confocal Microscope. The apoptosis cells were induced by DOX. We can see the green fluorescence on the apoptosis cells in the confocal pictures (see figure 2) and RE-AT-1-coated UCN move to the apoptosis cells in the confocal movies but not in the results of uncoated UCN. The upconversion fluorescence has much longer photostability and lower background auto fluorescence than Annexin V- FITC. This provides a new approach for apoptosis testing and diagnosis in biomedical applications.

Finally, RE-1 linked CNT-1 coating can assemble UCN and carbon nanotubes into new composite nanocrystals. In biological and materials systems, organic molecules exert a remarkable level of the assembly of crystallites and other nanoscale building blocks. A variety of practically important materials has been assembled via peptide approach[6]. Therefore, in this part, we will discuss the ability of RE-1 as a linker for new composite nanocrystals assembling. Then we synthesized a bifunctional peptide RE-CNT-1(HWSAWWIRSNQSGGACTARSPWICG), combining the RE-1 sequence with the carbon nanotube binding peptide via a short linker. After incubate and wash treatment, the binding of UCN to the surface of carbon nanotube with RE-CNT-1 was directly visualized by transmission electron microscopy (TEM). The new composite nanocrystals is made of UCN and carbon nanotubes by the linker peptide RE-CNT-1. This work will expand the application of UCN and carbon nanotubes and provide a versatile technology platform to enable the new composite nanocrystals development applications of LN-based nanomaterials and nanodevices.

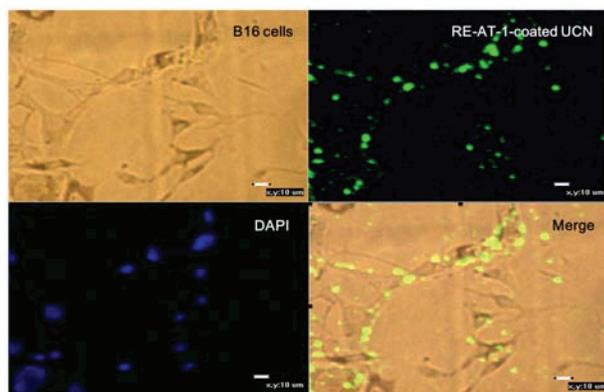


Figure 2: RE-AT-1 coating enhance apoptosis cell targeting of UCN.

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