Assembly of Alumina Nanoparticles Using Engineered Protein Linker

Nitin Chopra,[1*] Pramod Nednoor,[2] Ju Li,[2] Yinan Wei,[2] Vasilis G. Gavalas,[2] Leonidas G. Bachas[2**]
[1] Metallurgical and Materials Engineering Department, Center for Materials for Information Technology (MINT), The University of Alabama, Tuscaloosa, AL 35401
[2] Department of Chemistry, University of Kentucky, Lexington, KY 40506
* E mail: nchopra@eng.ua.edu, Tel: 205-348-4153; Fax: 205-348-2164
** E mail: bachas@uky.edu, Tel: 859-257-6350; Fax: 859-323-1069

Statement of Purpose: Biomolecules, such as proteins and nucleic acids, have unique size, shape, and chemical functionality that make them attractive for molecular recognition, chemical and biological sensing, and for developing complex self-assembled platforms.[1] Significant research has been focused on the use of oligonucleotides as linkers for the assembly of various nanomaterials.[2] However, there are only a few reports on the use of proteins as linkers for building bionanomaterials.[3] Nanomaterials can be conjugated with biological molecules through a variety of techniques such as physical adsorption, electrostatic attraction, specific recognition, and covalent binding.[1,4] Several peptides that bind selectively to metal and metal oxides have been identified.[1,5] Genetic engineering is being employed to prepare proteins and viruses that contain such peptides to promote assembly and/or organization of nanostructures.[6] In this regard, utilization of highly specific chemistry of proteins as suitable linkers to assemble nanostructures is a novel and challenging task. Dimensional similarity of biomolecules to nanostructures and their inherent molecular recognition properties will lead to well-controlled assembling of nanostructures. The major goal of this study is to use engineered protein (Glutathione S-Transferase, GST) to specifically bind to the surface of alumina nanoparticles (NPs) resulting in their controlled assembly.

Methods: A kinase recognition sequence was incorporated on the C-terminus of GST. The protein was phosphorylated at a single location by using protein kinase and ATP as described earlier.[7] Strong affinity of phosphate groups for alumina NPs allowed for the latter's controlled assembly (Figure 1 D). In order to achieve this, alumina NPs were first suspended in a 20 mM acetate buffer, pH 4.1 and sonicated for 24 h. Prior to the addition of phosphorylated GST (1 mg/ml), pH of the NPs suspension was adjusted to neutral with 1 M Tris solution. The sample was incubated with gentle shaking at 4°C for 2 h. Control samples containing just NPs or non-phosphorylated GST were prepared in a similar fashion. The assembly of alumina NPs is observed using transmission electron microscopy (TEM, JEOL 2010 F).

Results: GST (dimeric with subunits of 25-27 kDa) catalyzes conjugation of glutathione (GSH) to many electrophilic compounds. In addition, GST plays an important role in the detoxificaton of endogenous and xenobiotic electrophiles.[8] Here, phosphorylated GST has been specifically used to couple and assemble alumina NPs. Due to the strong affinity of phosphate groups for alumina NPs, the phosphorylated GST facilitated linking of nanoparticles. It was observed that specific chemistry precisely linked two, three or more (linear chains) of alumina NPs as evidenced in TEM (Figure 1). This was not observed in case of non-phosphorylated protein. Furthermore, in the presence of protein, aggregation of the NPs was nearly negligible as compared to that in the absence of protein. The protein functionalization onto NPs is carried out at pH 7.5 while the pI of GST is ~6 resulting in a negatively charged protein layer around individual NP in the suspension. This leads to electrostatic repulsion between the NPs that prevents aggregation and only allows for covalent linkage to occur. This is a unique approach to assemble NPs and is being demonstrated here for the first time.

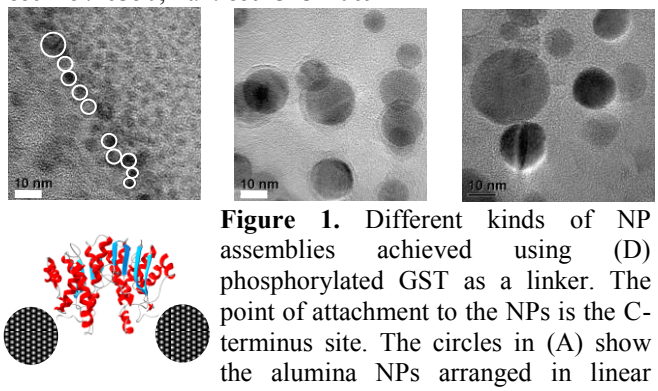

Figure 1. Different kinds of NP assemblies achieved using (D) phosphorylated GST as a linker. The point of attachment to the NPs is the C-terminus site. The circles in (A) show the alumina NPs arranged in linear chains, (B) show two NPs bound together, and (C) show three NPs bound together. Scale bar: 10 nm.

Conclusions: Well-controlled assembly of alumina NPs using phosphorylated GST is achieved in an approach coupling engineered GST protein and surface chemistry of alumina NPs. Such a well-controlled assembly of alumina NPs holds great promise in the field of novel chemical and biological sensors or analytical platforms.

References:

(1) Diamond, D. Principles of Chemical and Biological Sensors, John Wiley and Sons, Danwers, MA (1998).
(2) Niemeyer, C. M.; Adler, M. Angew. Chem. Int. Ed. 2002:41:3779-3783.
(3) Niemeyer, C. M. Angew. Chem. Int. Ed. 2001:40:4128-4158.
(4) Bruchez, J. M.; Moronne, M.; Gin, P.; Weiss, S.; Alivisatos, A. P. Science 1998:281:2013-2016.
(5) Sarikaya, M.; Tamerler, C.; Jen, A. K. Y.; Schulten, K.; Baneyx, F. Nat. Mater. 2003:2:577-585.
(6) Nam, K. T.; Kim, D. W.; Yoo, P. J.; Chiang, C.-Y.; Meethong, N.; Hammond, P. T.; Chiang, Y.-M.; Belcher, A. M. Science 2006:312:885-888.
(7) Kaelin, W.G.; Kreb, W.; Sellers, W. R.; DeCaprio, J.A.; Ajchenbaum, F.; Fuchs, C. S.; Chittenden, T.; Li, Y.; Farnham, P. J.; Blanar, M. A.; Livingston, D. M.; Flemington, E. K.. Cell 1992:70:351-364.
(8) Mctigue, M. A.; Tainer, J. A. J. Mol. Biol. 1995:246:21-27.