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The protein–nanomaterial interface

Prashanth Asuri, Shyam Sundhar Bale, Sandeep S Karajanagi and Ravi S Kane

Developments in the past few years have illustrated the potentially revolutionizing impact of nanomaterials, especially in biomedical imaging, drug delivery, biosensing and the design of functional nanocomposites. Methods to effectively interface proteins with nanomaterials for realizing these applications continue to evolve. Proteins are being used to control both the synthesis and assembly of nanomaterials. There has also been an increasing interest in understanding the influence of nanomaterials on the structure and function of proteins. Understanding and controlling the protein–nanomaterial interface will be crucial for designing functional protein–nanomaterial conjugates and assemblies.

Addresses
Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180, USA

Corresponding author: Kane, Ravi S (kaner@rpi.edu)

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Introduction

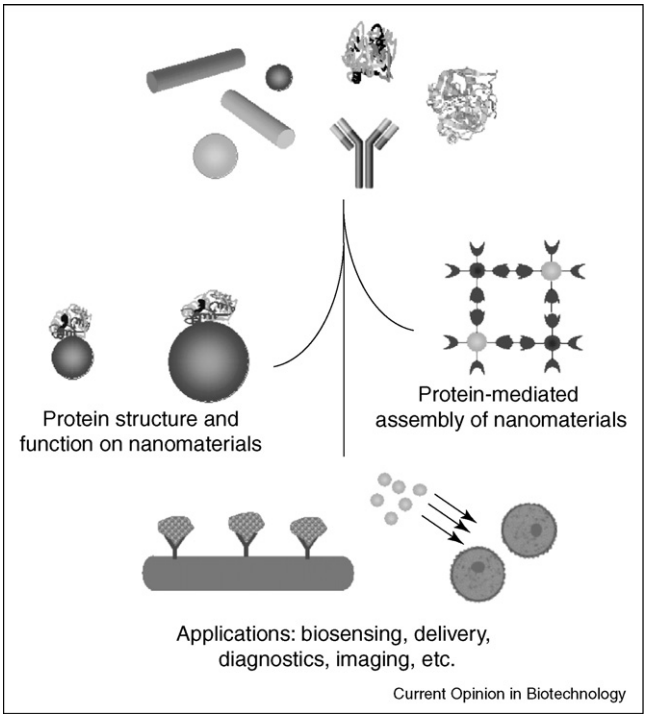
There has been considerable progress in the synthesis of nanomaterials with precise dimensions, geometries, and surface properties [1,2], and there is now an increasing interest in understanding and controlling the interactions of nanomaterials with biological molecules such as proteins [3,4••,5••,6]. Proteins have been used to functionalize nanomaterials and to influence their properties for applications ranging from sensing [7••,8,9••] and diagnostics [10,11•] to delivery [12••,13••], and for the design of nanocomposites [14–17] (see also Update). Nanomaterial properties in turn have a strong influence on the structure and function of proteins, and there has been increasing emphasis on obtaining a fundamental understanding of these effects [4••,5••,6,18•,19•,20••]. This review focuses on recent advances in understanding and applying protein–nanomaterial interactions, with particular emphasis on manuscripts published after 2003 (Figure 1).

Protein structure and function on nanomaterials

Although there have been numerous attempts to interface proteins with nanomaterials, many of these studies have focused on the modification and/or enhancement of nanomaterial properties to confer a specific biological function. It is, however, also important to understand how nanomaterial properties such as curvature and surface chemistry influence the structure and function of conjugated proteins [4••,5••,18•,19•,20••,21]. Vertegel *et al.* [18•] and Lundqvist *et al.* [19•] studied proteins adsorbed onto silica nanoparticles of varying sizes and demonstrated that differences in nanoparticle size strongly influence the secondary structure and activity of adsorbed proteins. These studies indicated that smaller nanoparticles, perhaps owing to higher surface curvature, promoted the retention of native-like protein structure and function when compared with larger particles, at least for the proteins studied (i.e. lysozyme [18•] and human carbonic anhydrase [19•]). In a recent study, Roach *et al.* studied the effects of curvature on two structurally different proteins — bovine serum albumin and fibrinogen [4••]. Although albumin retained more native-like structure on smaller particles, consistent with the previous work of Vertegel *et al.* [18•] and Lundqvist *et al.* [19•], fibrinogen was denatured to a greater extent on smaller particles; the influence of surface curvature on the structure of an adsorbed protein therefore seems to depend on the nature of the protein. A similar 'protein-dependent' behavior was reported by Karajanagi *et al.* [6] on single-walled carbon nanotubes (SWNTs). Spectroscopic measurements in conjunction with kinetic analysis revealed that soybean peroxidase (SBP) retained more of its native structure and activity when adsorbed onto SWNTs than chymotrypsin, which exhibited a nearly complete loss in activity and structure.

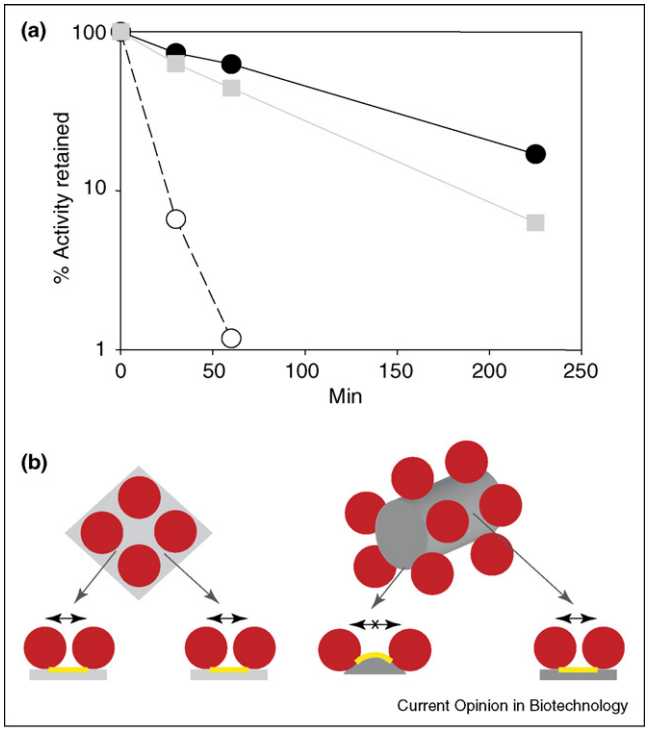
The surface chemistry of a nanoparticle also influences the structure and function of adsorbed proteins. Roach *et al.* [4••] reported a greater change in the secondary structure of both bovine serum albumin and fibrinogen on hydrophobic silica spheres than on hydrophilic ones. Moreover, Rotello and coworkers [5••,22] demonstrated the ability to control protein structure and function by tailoring the surface chemistry of nanoparticles. By controlling the surface chemistry, they achieved three distinct levels of interaction of chymotrypsin with CdSe nanoparticles: no interaction (i.e. no binding to the nanoparticles); enzyme inhibition with denaturation; and enzyme inhibition with retention of structure [5••] (see also Update).

Figure 1



Understanding and controlling the protein–nanomaterial interface. Areas of interest include understanding how protein structure and function is affected by attachment to nanomaterials, using proteins to control the assembly of nanomaterials, and applications of protein–nanomaterial conjugates.

Figure 2



Protein behaviour on single-walled carbon nanotubes (SWNTs). **(a)** Time-dependent loss of activity of soybean peroxidase (SBP) adsorbed onto SWNTs (black circles), SBP adsorbed onto graphite flakes (grey squares), and native SBP (open circles) at 95 °C (data taken from [20••]). **(b)** Schematic (drawn approximately to scale) depicting SBP molecules adsorbed onto a flat support (left) and on a nanoscale cylindrical support (right). The yellow line indicates the distance between adjacent proteins along the protein–substrate interface; for the same value of this average separation, curvature of the support might increase the average center-to-center distance and suppress unfavorable lateral interactions between adjacent proteins. (Part (a) was reproduced with permission from [20••]. Copyright 2006 American Chemical Society.)

Finally, Asuri and colleagues [20••,23] have uncovered a novel property of SWNTs — their ability to stabilize proteins under harsh conditions to a greater extent than conventional flat supports. For instance, the half-life of SBP adsorbed onto SWNTs at 95 °C was ~90 min, 10-fold greater than that of the native enzyme and ~1.9 times that of SBP adsorbed on graphite flakes or other flat supports (Figure 2a). Moreover, the enhanced stabilization on SWNTs was not unique to SBP and was also seen for the unrelated protease, subtilisin Carlsberg. Experimental and theoretical analyses suggested that lateral interactions between adjacent adsorbed proteins contribute to protein deactivation in harsh environments and that these unfavorable interactions are suppressed on highly curved supports such as SWNTs relative to flat surfaces (Figure 2b) [20••]. This work also suggests that enhancements in protein stability should not be unique to SWNTs, and could be obtained with other nanomaterials; enhanced protein stability on nanomaterials might therefore be a widely applicable phenomenon. However, application of this phenomenon requires that a protein retain significant activity during the initial adsorption step, a condition that is satisfied by several, but not all, proteins.

Collectively, these studies suggest that the structure, activity and stability of adsorbed proteins can be strongly influenced by both the surface chemistry of the nanomaterial and its curvature, but in a protein-dependent manner. Investigating the structure and function of proteins adsorbed onto different nanomaterials, as highlighted in this section, will be crucial for developing a better understanding of protein–material interactions at the nanoscale and for designing functional protein–nanomaterial conjugates.

Protein-mediated assembly of nanomaterials

Although the protein- and peptide-mediated synthesis of nanomaterials is itself an exciting field of research [24–27], this topic is beyond the scope of this review. We focus here only on reports concerning the protein-mediated assembly of nanomaterials (Figure 3). For instance, biotin–streptavidin [16,28,29] and antigen–