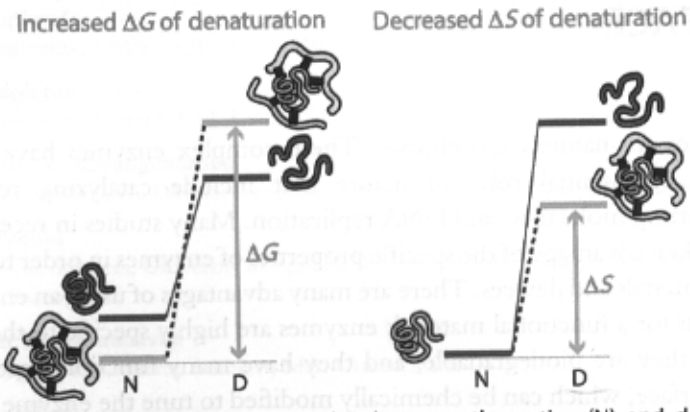
PREFACE

Enzymes are nature's workhorses. These complex enzymes have a wide variety of essential roles in nature that include catalyzing reactions, transporting molecules, and DNA replication. Many studies in recent years have taken advantage of the specific properties of enzymes in order to design new materials and devices. There are many advantages of using an enzyme as the basis for a functional material: enzymes are highly specific to their substrates, they are biodegradable, and they have many functional groups on their surface, which can be chemically modified to tune the enzyme's interactions with its environment. Compared to traditional organic synthesis reactions, enzymes are capable of catalyzing reactions in a more economic and green manner, and they also have very high activities and excellent selectivities. Enzymes, however, are often limited in their industrial and practical applications because of their poor stability—they are only stable in their physiological conditions (such as pH and temperature) and their poor recyclability and operational stability also limit their practical application. There are numerous methods developed over the decades to stabilize enzymes while maintaining their activity and selectivity.

Stabilization of enzymes is a fundamental thermodynamic issue. The free energy of denaturation (ΔG) of an enzyme decreases with increasing temperature. Above the denaturation temperature of the enzyme, the denatured state is thermodynamically more favored, and the enzyme unravels. Similarly, other factors such as pH and solvent also affect the free energy change and contribute to the enzyme's instability. In order to control the free energy of denaturation, one important thermodynamic component to consider is the entropy of denaturation (ΔS). When the enthalpy of denaturation is constant, ΔG can be increased if ΔS is decreased and stability of the enzyme enhanced (Scheme 1). Many methods have been developed for the stabilization of enzymes via this approach. One such method includes the conjugation of enzyme to a synthetic polymer, where the conformational entropy of the enzyme–polymer conjugate is reduced, and as a result the enzyme is armored with a polymer shell and its stability enhanced (Mudhivarthi et al., 2012; Riccardi et al., 2014; Thilakarathne, Briand, Zhou, Kasi, & Kumar, 2011). The nanoarmor provides the enzyme with protection from the harsh conditions encountered during various situations. Additionally, the armor also protects the enzyme from other degrading components such as bacteria

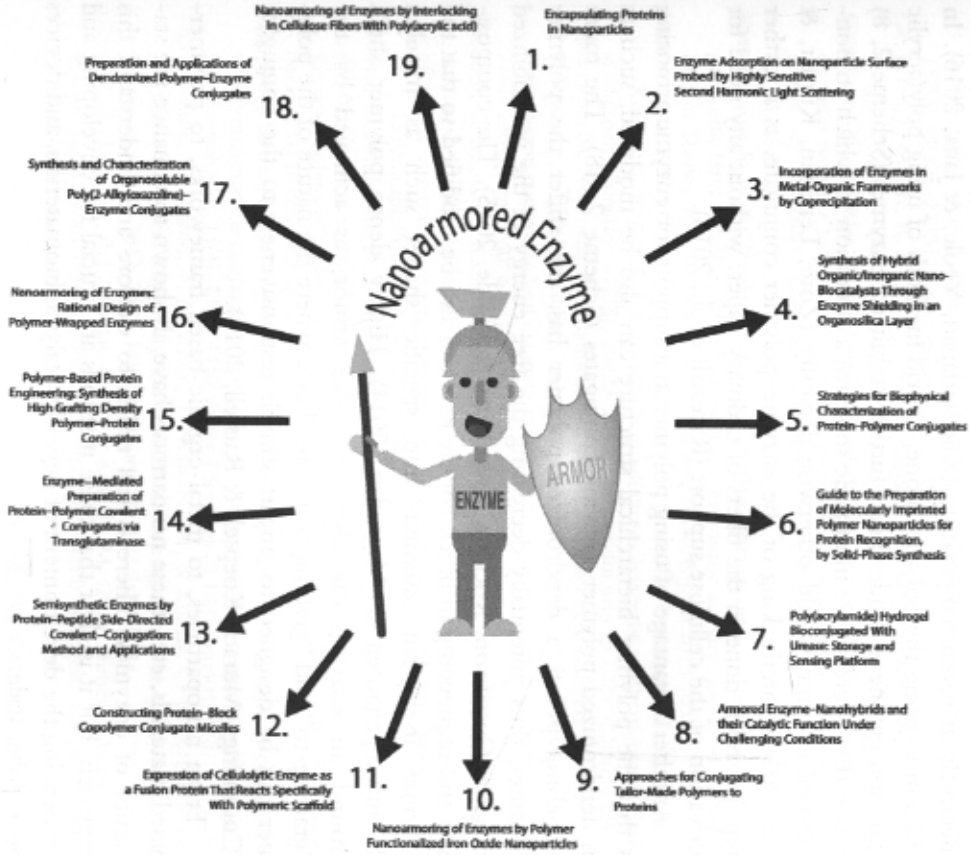


Scheme 1 Thermodynamic interconversion between the native (N) and denatured (D) states of an enzyme (*red lines*) are inhibited when the enzyme is conjugated to a nanoarmor such as a polymer (*green lines*). This is typically achieved by lowering the conformational entropy of the enzyme (*right graphic*).

and proteases as well as prevents the dissociation of the subunits of multimeric enzymes.

There are several experimental protocols, which have been reported for nanoarmoring of enzymes, such as encapsulation in nanoparticles, incorporation in metal-organic frameworks, modification with polymers, and immobilization in organosilica (Scheme 2). One example is to encapsulate enzymes inside nanoparticles, where the pore size of the nanoparticle can be tuned while also being able to tune the size of the overall particle for specific applications such as cellular imaging (Scheme 2, 1) (Cao et al., 2010). Immobilization of enzymes onto the surface of magnetic nanoparticles (Scheme 2, 10) is also used to impart stability and easy recovery of the enzyme and thus enhance its industrial value (Premaratne et al., 2016). Entrapment and immobilization are sometimes even used together, where the enzyme is first immobilized on the surface of one nanoparticle, and then other nanoparticles are then attached around it to create an enzyme armor (Correro et al., 2016). The enzyme–nanoparticle interactions are important in this design and several factors that may contribute are the binding free energy, binding stoichiometry, and structure distortion upon binding (Scheme 2, 2) (Das, Chakrabarti, & Das, 2016).

Similar to the encapsulation method in nanoparticles, metal-organic frameworks are also reported for armoring enzymes—as they are structurally diverse and can be tuned to the desired functionality (Scheme 2, 3–4) with retention of activity (Ge, Lei, & Zare, 2012).



Scheme 2 Nanoarmoring of enzymes by the various methods illustrated in this book. Each number corresponds to the chapter number and title.

Polymer-based armoring (Scheme 2, 5–9) is another widely studied method. Specific functional groups on the polymers are used to covalently conjugate to the enzyme, e.g., polyethylene glycol, poly(acrylic acid), poly(acrylamide), cellulose, and polyamide. The conjugation of enzyme to the polymer can tune the preferred properties of enzymes. For example, the operational temperature of urease was controlled so that it can be used effectively at room temperature (Kutcherlapati, Yeole, & Jana, 2016). In addition to using the polymer alone, hybrid methods of using poly(acrylic acid) and graphene oxide sheets further stabilized enzymes (Scheme 2, 8) so that they were active in organic solvents, a condition in which an non-armored enzyme would otherwise denature (Zore, Lenehan, Kumar, & Kasi, 2014). Interlocking of the enzyme–polymer conjugate, as a further step, has been done in the fibers of ordinary paper, without any need for activation of the cellulose support (Riccardi et al., 2016).

Another advantage of using polymer as a support for enzyme armoring is that the polymer hierarchical structure can also be modified, such as in dendronized polymer–enzyme conjugates (Scheme 2, 18). The more branching points a dendronized polymer has, the stiffer the polymer becomes, thus potentially decreasing the free energy of the immobilized enzyme (Gustafsson, Küchler, Holmberg, & Walde, 2015). The composition and sequencing of block copolymers can also be modified so that the polymer itself can assemble into specific shapes such as micelles (Suthiwangcharoen & Nagarajan, 2014). Highly dense polymer shells around an enzyme can also serve like armor, as achieved by the "grafting-to" and "grafting-from" methods, where the nature of the polymer can be designed to impart stimuli responsiveness to the conjugate (Cummings, Murata, Koepsel, & Russell, 2014).

From nanoparticles, to metal-organic-based frameworks, to polymer-based materials, etc., these nanoarmors have all shown to enhance the stabilities of enzymes. There is still much to explore and understand this approach, but it is clear that these methods are crucial for developing and influencing the development of new biocatalysts, biomaterials, and devices on a global scale.

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