Exploring modular allostery via interchangeable regulatory domains

Yifei Fan, Penelope J. Cross, Geoffrey B. Jameson, and Emily J. Parker

Most proteins comprise two or more domains from a limited suite of protein families. These domains are often rearranged in various combinations through gene fusion events to evolve new protein functions, including the acquisition of protein allostery through the incorporation of regulatory domains. The enzyme 3-deoxy-o-arabino-heptulosonate 7-phosphate synthase (DAH7PS) is the first enzyme of aromatic amino acid biosynthesis and displays a diverse range of allosteric mechanisms. DAH7PSs adopt a common architecture with a shared (β/α)8 catalytic domain which can be attached to an ACT-like or a chorismate mutase regulatory domain that operates via distinct mechanisms. These respective domains confer allosteric regulation by controlling DAH7PS function in response to ligand Tyr or prephenate. Starting with contemporary DAH7PS proteins, two protein chimeras were created, with exchanged regulatory domains. Both engineered proteins were catalytically active and delivered new functional allostery with switched ligand specificity and allosteric mechanisms delivered by their nonhomologous regulatory domains. This interchangeability of protein domains represents an efficient method not only to engineer allostery in multidomain proteins but to create a new bifunctional enzyme.

Protein allostery is central to the regulation of many biological processes, including ligand transport and metabolic function and control. Allostery occurs when ligand binding at one site communicates with a remote functional site, resulting in a change in protein function. Allosteric regulation of protein functions often involves a complex network of interactions to deliver signals between distal sites. Signal communication is achieved via diverse mechanisms ranging from large conformational changes to subtle changes in protein dynamics (1–3). The understanding of these remote communications is of great interest, particularly in the fields of drug design and protein engineering (4, 5).

Most proteins contain two or more domains (6). These domains and their interactions govern the function of a protein and are considered evolutionary units for modular assembly of new protein architectures (7–9). Biological data suggest that only a limited number of protein folds exist in nature, and protein functions evolve from mutation, duplication, and recombination of ancestral genes under selective pressure (10). Domain recombination via gene fusion events represents one of the major pathways for the evolution of allostery (11–14). This principle has been recognized in many natural systems (12).

The enzyme 3-deoxy-o-arabino-heptulosonate 7-phosphate synthase (DAH7PS) is the first enzyme of the shikimate pathway for the biosynthesis of aromatic compounds. DAH7PS catalyzes the divergent metal-dependent condensation of two carbohydrate precursors, phosphoenolpyruvate (PEP) and d-erythrose 4-phosphate (E4P), to produce DAHP and phosphate. This enzyme is essential for most microorganisms because of its key role in the biosynthesis of the intermediate chorismate from which the pathway branches to allow formation of prephenate by chorismate mutase (CM) as well as a series of other important metabolites, including aromatic amino acids Phe, Tyr, and Trp (Fig. 1) (15). DAH7PS, situated at the first committed step of the pathway, is often precisely feedback regulated to control pathway flux in response to metabolic demand via a variety of allosteric strategies in different organisms. This allostery ranges from physical gating mechanisms undertaking large conformational changes to highly intricate through-protein dynamic networks (16). The levels of chorismate, prephenate, and the aromatic amino acids are important check points for feedback regulation of the DAH7PS function (16).

Most DAH7PS enzymes are tetrameric in solution, with each chain comprising a (β/α)8 catalytic barrel. This barrel is frequently decorated with additional structural elements, which are responsible for conferring allostery (16). One major class of DAH7PS enzymes (type Iip) share a catalytic domain of a structurally uninterrupted classic TIM barrel (β/α)8 fold, and are either unregulated or allosterically regulated (Fig. 24). The simplest form is composed solely of the barrel without any additional domain, and hence is unregulated. This form of DAH7PS enzymes has been characterized from Pyrococcus furiosus and Aeropyrum pernix (17–19). The regulated DAH7PSs in this group display discrete domains appended to the catalytic barrel at the N or C terminus. These domains are either an ACT-like domain (20) or a CM (AroQ) domain, which possesses CM enzymatic activity. The attached ACT-like and CM domains deliver allostery on a tetrameric DAH7PS scaffold via

Significance

Protein functions are often evolved from recombinations of a limited suite of protein folds. Engineering desired function into proteins via manipulation of the genes, mimicking natural evolutionary processes, represents a promising approach to synthesizing useful molecular tools. Allosteric proteins play important roles in many biological processes, often as regulatory switches or metabolic checkpoints. In this study, analysis of two natural allosteric proteins from different organisms revealed comparable yet distinct regulatory mechanisms via conformational rearrangement. Interchanging gene segments encoding for the nonhomologous regulatory elements of the two proteins results in new functional proteins with switched regulatory behavior. This modular approach represents a step toward a general scheme for construction of functional changes in proteins.

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physical gating of the active site associated with the binding of an allosteric ligand (Fig. 2 B and C) (21–23). This is best exemplified in the well-characterized DAH7PS from Thermotoga maritima (Fig. 2 B), which undergoes a remarkable conformational change in response to the presence of Tyr, in which the ACT-like domains from opposing chains dimerize to form a binding site for the allosteric ligand and block substrate access to the active site (21). This enzyme is also inhibited by Phe, although to a reduced extent. Similarly, prephenate binding to the CM domain in DAH7PS from Geobacillus sp. (strain Y412MC61) is associated with the more intimate association between the CM and DAH7PS domains to form a more compact structure and limit catalysis (Fig. 2 C) (23).

Previously, we have demonstrated the transfer of the allosteric domain of the T. maritima DAH7PS onto the unadorned, unregulated DAH7PS of P. furiosus to confer an allosteric response in the latter DAH7PS (24). We propose that the recruitment of a regulatory domain is a general strategy that, by itself, is sufficient for providing allosteric control of enzymes. To validate this hypothesis and to demonstrate that the key information associated with delivery of allostery resides in the regulatory domain, we explore here the interchangeability of the two distinct regulatory strategies employed by different allosterically controlled DAH7PS enzymes through construction of protein variants that mix and match catalytic and regulatory domains of DAH7PSs from T. maritima and Geobacillus sp. These studies illustrate the remarkable ease with which functional allostery can be acquired by gene fusion events and provide insight into the evolution of modular allostery, in which existing ligand-binding domains or enzymes can be repurposed to provide allostery.

Results

Altered Regulatory and Catalytic Domain Combinations Deliver Functional Proteins. The wild-type parent proteins TmaDAH7PS and GspDAH7PS share similar homotetrameric quaternary structures and tertiary structures, with each chain composed of an N-terminal regulatory domain (respectively, ACT and CM) attached to a catalytic (β/α)₈ barrel housing the active site. The TmaDAH7PS and GspDAH7PS catalytic barrels share a moderate sequence identity of 56% (Fig. S1). Two protein variants were created with exchanged regulatory and catalytic domains based on the analysis of wild-type sequences and architectures. Both these domain-swapped variants delivered DAH7PS catalysis, albeit with some alterations in their catalytic efficiencies (Table 1). Compared with the wild-type TmaDAH7PS, GspCM-TmaDAH7PS, which shares the same catalytic core, exhibited impaired activity with significantly decreased kcat/KM values for both PEP and E4P substrates, implying there may be some restriction of access for substrates to the catalytic center introduced by the fused CM domains. The origin of this may lie in the fact that the CM domain is dimeric both in the absence and presence of the allosteric ligand, whereas the ACT-like domain only dimerizes upon ligand binding (21, 23). Hence, perhaps unsurprisingly, the adoption of the more restricted GspCM domain to the TmaDAH7PS catalytic core is accompanied by attenuation of catalysis, whereas TmaACT-GspDAH7PS displayed an approximately twofold boost in catalytic efficiency compared with the wild-type GspDAH7PS. That physical constraints restrict catalysis by the DAH7PS core barrel is supported by the higher activity displayed by truncated forms of

Fig. 1. The shikimate pathway leads to biosynthesis of aromatic amino acids Trp, Phe, and Tyr. DAH7PS catalyzes the first reaction. The pathway branches at chorismate, where CM catalyzes the conversion of chorismate to prephenate en route to formation of Phe and Tyr. The number of arrowheads represents the number of reactions.

Fig. 2. Structure and allostery of DAH7PS. (A) Monomeric units of DAH7PS from P. furiosus (left), T. maritima (center), and Geobacillus sp. (right) show an uninterrupted catalytic barrel. ACT domain in TmaDAH7PS is shown in red; CM domain in GspDAH7PS is shown in orange. Both TmaDAH7PS (B) and GspDAH7PS (C) display significant conformational changes upon ligand binding to the regulatory domain (Tyr is shown as green spheres, and prephenate is shown as purple spheres).
Table 1. Kinetics parameters for the chimeras and parent proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>DAH7PS activity</th>
<th>CM activity</th>
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<tbody>
<tr>
<td></td>
<td>$K_M^{PEP}$, $\mu$M</td>
<td>$K_M^{E4P}$, $\mu$M</td>
</tr>
<tr>
<td>$Gsp$CM-$Tma$DAH7PS</td>
<td>34 ± 2.7</td>
<td>19 ± 1.8</td>
</tr>
<tr>
<td>$Tma$ACT-$Gsp$DAH7PS</td>
<td>57 ± 1.6</td>
<td>54 ± 1.4</td>
</tr>
<tr>
<td>GspDAH7PS</td>
<td>45 ± 4</td>
<td>61 ± 8</td>
</tr>
<tr>
<td>$Tma$DAH7PS</td>
<td>8.4 ± 0.7</td>
<td>15 ± 1</td>
</tr>
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NA, not applicable for the specified enzyme activity.

$Tma$DAH7PS and GspDAH7PS in which, respectively, the ACT and CM domains have been removed (21, 23).

Unlike the ACT domain, which has only a ligand-binding role, the regulatory domain of GspDAH7PS has both catalytic and regulatory functions (23). The CM catalytic activity was comparably maintained when the domain was transferred to the alternative catalytic barrel in $Gsp$CM-$Tma$DAH7PS (Table 1), although this chimera displayed an approximately twofold increase in $K_M$ for chorismate compared with wild-type GspDAH7PS. As expected, with no CM domain, the variant $Tma$ACT-$Gsp$DAH7PS displayed only DAH7PS activity.

As with all wild-type DAH7PS enzymes characterized to date, the catalytic activities of the chimeras depend on the presence of a divalent metal ion (Fig. 3A). Mn$^{2+}$ is the most activating metal ion for the wild-type $Tma$DAH7PS, whereas Cd$^{2+}$ delivers maximal activity for GspDAH7PS (21, 23). A range of metal ions were tested with the protein chimeras. Largely as expected, each of the DAH7PS catalytic cores reflected its inherent metal preference, with $Tma$ACT-$Gsp$DAH7PS highly favoring Cd$^{2+}$, whereas $Gsp$CM-$Tma$DAH7PS showed more than 90% activity in the presence of Mn$^{2+}$ or Cd$^{2+}$.

Consistent with the thermophilic properties of the wild-type enzymes, both enzymes became more active at elevated temperatures, reaching their optimal activity at temperatures above 60 °C (Fig. 3B). Intriguingly, activity of $Tma$ACT-$Gsp$DAH7PS was enhanced significantly at elevated temperatures compared with the wild-type GspDAH7PS. On the other hand, $Gsp$CM-$Tma$DAH7PS appeared to be least active across all temperatures. Proteins with the GspCM domain, chimeric and wild-type, generally tend to display lower activities than proteins with the $Tma$ACT domain at the temperatures tested. This change in activity profile may relate to the structural difference in the regulatory domains and the different inherent optimal temperatures of the parent proteins.

Allosteric Inhibitor Preference Resides in the Regulatory Domain. To test the effect of potential allosteric ligands, inhibition assays were performed for both DAH7PS chimeras and compared with the response of the wild-type proteins (Fig. 4 and Fig. S2). Consistent with the presence of a regulatory domain in both chimeras, inhibition of both enzymes was observed. Presenting the ACT domain, the DAH7PS activity of $Tma$ACT-$Gsp$DAH7PS was reduced by 70% at high Tyr concentrations and by ~30% at high Phe concentrations. This difference in ligand sensitivity for Tyr and Phe is comparable with that of the wild-type $Tma$DAH7PS, for which Tyr exhibits more significant inhibitory effect than Phe, although the $IC_{50}$ value of Tyr for the chimera (210 μM) is almost 10-fold higher than that of the wild-type protein (22.5 μM). The presence of the CM domain in $Gsp$CM-$Tma$DAH7PS delivered sensitivity toward prephenate, although the inhibition was less profound than that for the wild-type GspDAH7PS. The presence of 77 μM prephenate reduced DAH7PS activity to 50% of its uninhibited value, compared with 20 μM required for the same attenuation of wild-type protein catalytic activity. The maximum level of inhibition was also altered; the chimeric protein retained 31% activity at higher prephenate concentrations in comparison with the 4% residual activity observed for the wild-type enzyme.

Binding of allosteric ligands stabilizes the parent proteins and the chimeras (Fig. 5 and Fig. S3). In the presence of inhibitors, the melting temperatures of both chimeras increased by around 3 °C, in close agreement with the degree of stabilization for the wild-type proteins. Interestingly, the thermostability of $Tma$ACT-$Gsp$DAH7PS improved almost 20 °C compared with the wild-type GspDAH7PS. On the other hand, the stability of the $Gsp$CM-$Tma$DAH7PS appeared to be largely determined by the $Tma$DAH7PS core, with the denaturing event occurring at above 96 °C. The thermostability profiles exhibited by the chimeras largely reflect the difference in inherent thermostability of the wild-type proteins, remarkably, even when the transferred domain is only a fraction (approximately one-third) of the core DAH7PS barrel, as in $Tma$ACT-$Gsp$DAH7PS.

The Structural Changes of Allostery Are Transferred with the Regulatory Domains. The allosteric mechanisms of both parental proteins involve conformational rearrangements as observed in solution by small-angle X-ray scattering (SAXS) experiments (21, 23). To assess the allosteric mechanisms employed by the chimeras, SAXS experiments were performed. In the presence of Tyr, $Tma$ACT-$Gsp$DAH7PS became more compact, with a reduced $R_g$ value (derived from Guinier plot) of 32.8 ± 0.2 Å compared with the $R_g$ of the apo form 34.0 ± 0.2 Å (Fig. 6 and Table S2). The Kratky plot displayed a more defined curve in the presence of Tyr than without Tyr, consistent with the decreased flexibility of the protein. The $Gsp$CM-$Tma$DAH7PS also showed a conformational change with addition of prephenate, with $R_g$ decreasing from 36.9 ± 0.2 Å to 34.8 ± 0.2 Å, consistent also with the more defined Kratky plot.

To compare these conformational changes observed in the protein chimeras with those observed for the wild-type proteins, the SAXS profiles were fitted with the calculated theoretical scattering from crystal structures or homology model of each parent protein (Fig. 6 and Table S2). In the absence of Tyr, scattering profiles of $Tma$ACT-$Gsp$DAH7PS presented a good fit with the open form of the $Tma$DAH7PS crystal structure, but not the closed form. Conversely, in the absence of Tyr, the scattering profile

![Fig. 3. Catalytic activity of the parent proteins and chimeras. (A) DAH7PS activities of $Tma$DAH7PS (blue), GspDAH7PS (green), $Tma$ACT-$Gsp$DAH7PS (red), and $Gsp$CM-$Tma$DAH7PS (orange), in the presence metal ions or EDTA. (B) The effect of temperature on specific activity of the four enzymes in the same color coding as in A.](image-url)
Thermostability of the parent proteins and chimeras in the absence of prephenate, the scattering profile of $G_{\text{sp}}$DAH7PS($\square$) displayed sensitivity toward Tyr. (B) $G_{\text{sp}}$DAH7PS($\bullet$) and $C_{\text{CM}}$DAH7PS($\triangle$) displayed impaired catalysis with addition of prephenate.

Discussion

Design of proteins with new properties and functions is an important goal of biotechnology. The modular feature of many natural proteins suggests that common building domains and modules are likely to have the evolutionary advantages of being autonomous and portable, offering simplicity in recombination to generate new functions by allowing transfer of information through their interactions (25). Statistical coupling analysis revealed that the recombination of protein domains relies on the networks of coevolving amino acids involved in the allosteric communication and that these networks display strong connectivity, proposing the feasibility of engineering artificial allosteric systems by transfer of allosteric networks between proteins (5, 26). Although this transferability has not been extensively explored, a few studies indicate its plausibility. For example, a transferable molecular switches were built by modular recombination of multiple SH3–peptide autoinhibitory interactions on WASP to introduce strong cooperativity with respect to the ligand SH3 (30, 31). Also, an Escherichia coli dihydrofolate reductase (DHFR) was coupled with a light-sensing protein from plants to generate a light-sensitive DHFR (5). These studies demonstrate that methods of mix and match between modular components can be effective ways to create substantial functional changes associated with allosterol and to expand the repertoire of artificial proteins.

What we demonstrate here is the interchangeability of regulatory domains in a homooligomeric protein. By simple gene recombination of contemporary sequences, we can interchange the Tyr-binding ACT domain on one DAH7PS with a structurally nonhomologous prephenate-binding CM domain from another DAH7PS. This interchange functionally swaps allosteric regulation in both DAH7PS enzymes elicited by formation of, or changes to, a dimeric structure upon binding of the appropriate allosteric effector. What is somewhat surprising is that gene fusion to functionally link these contemporary domains requires no modifications to the core catalytic barrels. Fusion of the regulatory domain nevertheless inflicts some catalytic penalty on the barrel, and the degree of compensated activity may be associated with the nature of the extended domains. We note that the functional domain-swapped chimeras developed here are quite distinct from other examples where chimeras have been generated by the modification of discrete structural elements on structurally homologous domains to alter allosteric ligand specificity (32–35). We have interchanged two structurally and functionally nonhomologous regulatory modules of contemporary DAH7PS scaffolds. That fully functional enzymes are generated highlights the conservation of allosteric strategy in these contemporary proteins, which is delivered by structurally diverse solutions (or gene fusions) (21, 23).

Despite the ease of functional allostery interchange, there are enabling features of the contemporary DAH7PS scaffold that are important in accommodating the recombination and for maintaining allosteric networks. Core barrel oligomerization is a prerequisite for allosterol that involves dimerization of the regulatory module (17, 21, 23, 36). The DAH7PS barrel homotetramer adopts an overall conformation that supports the allosteric function, with diagonally opposite chains delivering the regulatory domain on either side of the tetramer plane (Fig. 2). This tetrameric assembly is a feature of type II DAH7PS, which is shared by both regulated and unregulated DAH7PSs (19, 21, 23, 37) and a related enzyme, 3-deoxy-d-manno-2-octulosonate 8-phosphate synthase (38). Intriguingly, quaternary structure is delicately balanced in this DAH7PS enzyme class. Removal of the ACT-like domain from $T_{\text{ma}}$DAH7PS results in dimerization and the (unregulated) $P_{\text{fu}}$DAH7PS is rendered dimeric by a single amino acid substitution (19, 21). Association of dimers into tetramers was probably followed by acquisition of terminal regulatory domains (17). Thus, it appears that the functional gene fusion to deliver allosterol was facilitated serendipitously by the adoption of the appropriate homotetrameric catalytic template.

The barrels are not highly conserved overall, but there do appear to be some sequence elements that are associated with delivering allosterol in both systems that are shared between both $G_{\text{sp}}$DAH7PS and $T_{\text{ma}}$DAH7PS. The key hydrogen-bond contacts between either the ACT domain or the CM domain with the catalytic DAH7PS domain reside mostly at the C terminus of the diagonally adjacent barrel (Fig. S4). In the ligand-bound form of $T_{\text{ma}}$DAH7PS, major hydrogen bonds are formed between an ACT domain and the barrel from the adjacent chain, including Asp51 and Asp309, Ser55 and Arg277, and Asp57 and Glu304. Due to the asymmetrical nature of the domain arrangement across the vertical plane in the crystal structure of

![Fig. 4](https://www.pnas.org/cgi/doi/10.1073/pnas.1717621115)

**Fig. 4.** Inhibition of DAH7PS activity for the parent proteins and chimeras. (A) $T_{\text{ma}}$DAH7PS ($\square$) and $T_{\text{ma}}$ACT-DAH7PS ($\triangle$) displayed sensitivity toward Tyr. (B) $G_{\text{sp}}$DAH7PS ($\bullet$) and $C_{\text{CM}}$DAH7PS ($\triangle$) displayed impaired catalysis with addition of prephenate.

![Fig. 5](https://www.pnas.org/cgi/doi/10.1073/pnas.1717621115)

**Fig. 5.** Thermostability of the parent proteins and chimeras in the absence (red) and presence of Tyr (orange) or prephenate (blue).
In addition to the transfer of allostery, enzymatic activity of the CM domain was also transferred into the C27-CM-TmaDAH7PS chimera. Remarkably, thermostability was determined by the more thermophilic component of the chimera, even when that component, as in the case of the ACT domain of the DAH7PS from the hyperthermophilic T. maritima, was transferred onto the much larger DAH7PS domain from the less thermophilic Geobacillus sp.—the proverbial tail wagging the dog. The generality of this phenomenon as a mechanism for inducing thermostability into more mesophilic enzymes remains to be elucidated.

From one enzyme and two distinct regulatory domains we have four allosterically regulated enzymes, two occurring in nature and two created in the laboratory, one of which is additionally a bifunctional enzyme. The engineering of allosteric control, enzymatic activities, and thermostability by domain swapping illustrates that homologous (β/α)8 structures can tolerate new combinations with structurally and functionally nonhomologous regulatory domains. Future investigations into the details of the corresponding allosteric network experimentally and computationally are important for the design and optimization of allosteric systems with high performance.

Materials and Methods

Design and Preparation of Protein Variants. Crystal structures of the TmaDAH7PS and GspDAH7PS suggest similar architecture and arrangement of the two domains, with the catalytic barrels connected to their respective N-terminal regulatory domains via a β-hairpin and a flexible linker region. The linker region is crucial for the interaction between regulatory and catalytic domains in both enzymes and to allow appropriate conformational change between active and inhibited states (21). The amino acid regions that incorporate regulatory domains, linker regions, and catalytic domains of TmaDAH7PS and GspDAH7PS are identified individually from sequence and structure alignments (Fig. S1). Two protein variants with exchanged regulatory and catalytic domains were designed: TmaACT-GspDAH7PS, with the regulatory domain and linker region from TmaDAH7PS (residues 1 to 93) and the catalytic domain from GspDAH7PS (residues 118 to 362); and the complementary chimera GspACT-TmaDAH7PS, with the regulatory domain and linker region from GspDAH7PS (residues 1 to 117) and the catalytic domain from TmaDAH7PS (residues 94 to 338). Both constructs were created by amplifying each segment from the corresponding parent wild-type gene with overlap and by fusing the amplified products (Table S1). The fused gene fragments encoding each chimeric protein were cloned using Gateway® technique. TmaACT-GspDAH7PS was transformed in pDEST14 and expressed in BL21(DE3)* cells, and its expression and purification were performed following previously published procedures for TmaDAH7PS (21). GspACT-TmaDAH7PS was transformed in pDEST15 and expressed in BL21(DE3)pLysS cells using the same expression conditions, and purified using an GSTrap HP column following published procedures for the truncated GspDAH7PS (23).

Enzyme Kinetics. Kinetics parameters for DAH7PS and CM activities of the chimeric proteins were determined following previously described procedures by measuring consumption of chorismate at 274 nm or PEP at 232 nm, with each reaction containing 0.05 μM enzyme (23). For DAH7PS assays, the concentration of PEP was varied between 5 μM and 400 μM while PEP was held at 295 μM, and the concentration of PEP was varied between 6 μM and 350 μM while E4P was fixed at 310 μM. Reactions contained enzyme, PEP, and 100 μM Mn+2 (for proteins containing the catalytic barrel from T. maritima) or Cd2+ (for proteins containing the catalytic barrel from Geobacillus sp.), and were equilibrated in 50 mM 1,3-bis[tris(hydroxymethyl)methylamino] propane (BTP) buffer at 60 °C (pH 7.4) before E4P was added to initiate the reaction. For CM assays, 9 to 400 μM chorismate was used to initiate the reaction in the same BTP buffer at 50 °C. Metal dependency studies were performed using the same DAH7PS assay described above in the presence of saturating PEP and E4P, with a range of metal ions or EDTA at 100 μM. For inhibition studies, assay solutions contained 283 μM PEP, 308 μM E4P, 100 μM Mn+2 or Cd2+, and 0 to 1 mM TyrPhe or 0 to 400 μL prephenate in 50 mM BTP (pH 7.4 at 60 °C).

Thermal Activity and Stability Measurements. Activity profiles of the chimeric proteins and parent proteins at elevated temperatures (30 to 80 °C) were assessed using the same methods as for kinetics measurements of DAH7PS activity. Each reaction contained 0.05 μM enzyme, 100 μM appropriate metal
SAXS. SAXS measurements were collected at the Australian Synchrotron SAXS/wide-angle X-ray scattering (WAXS) beamline using the setup previously described (21, 23). Scattering data were collected at 25 °C following the procedure described (21, 23, 29). Scattering intensity versus magnitude of scattering vector of each protein was fitted with a polynomial equation of the form $I(q) = I_0 + aq^n$. Theoretical scattering profiles were generated using the program CRYSOL (38) and aligned using SeaView 4 (37). A background scattering profile was fitted to the measured scattering data. Raw data were processed as described for wild-type proteins using Scatterbrain (21). Scattering intensity versus magnitude of scattering vector of each protein was fitted with a polynomial equation of the form $I(q) = I_0 + aq^n$. Theoretical scattering profiles were generated using the program CRYSOL (38) and aligned using SeaView 4 (37). A background scattering profile was fitted to the measured scattering data. Raw data were processed as described for wild-type proteins using Scatterbrain (21).