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Site directed mutagenesis as a precision tool to enable synthetic biology with engineered modular polyketide synthases



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ARTICLE INFO ABSTRACT Keywords: Modular polyketide synthases (PKSs) are a multidomain megasynthase class of biosynthetic enzymes that have Polyketide synthase great promise for the development of new compounds, from new pharmaceuticals to high value commodity and Site directed mutagenesis specialty chemicals. Their colinear biosynthetic logic has been viewed as a promising platform for synthetic Rational design biology for decades. Due to this colinearity, domain swapping has long been used as a strategy to introduce Saturation mutagenesis molecular diversity. However, domain swapping often fails because it perturbs critical protein-protein interac-Synthetic biology tions within the PKS. With our increased level of structural elucidation of PKSs, using judicious targeted mu-Protein engineering tations of individual residues is a more precise way to introduce molecular diversity with less potential for global

domain communication, and promote more complex catalytic reactivity.

Introduction

Bacterial modular type I polyketide synthases (PKSs) are responsible for the generation of a diverse range of natural product therapeutics including antibiotics (e.g. erythromycin), antifungals (e.g. amphotericin), immunosuppressants (e.g. rapamycin), and antiparasidics (e.g. avermectin). The incredible potential of PKSs as pharmaceutical generators has lent considerable interest in their biosynthesis. They possess a tantalizing, colinear biosynthetic logic, meaning they generate polyketides via megasynthase molecular assembly lines organized into modules. Each module performs one round of elongation followed by optional reductive processing with discrete enzymatic domains correlating to chemical transformations. A module, corresponding to one round of elongation and processing, is minimally composed of a ketosynthase (KS) domain which catalyzes decarboxylative Claisen condensation, an acyltransferase (AT) domain which is responsible for extender unit selection (malonyl-CoA or derivatives thereof), and an acyl carrier protein (ACP) which anchors the elongating carbon chain through a phosphopantetheinyl prosthetic group. Optional processing domains include a C-methyltransferase (MT) that methylates at the α position as well as β-carbon processing domains such as a ketoreductase domain (KR) which reduces the keto moiety to a hydroxy moiety, a dehydratase (DH) responsible for dehydration of the hydroxy group to form an olefin, and an enoylreductase (ER) which forms a saturated carbon backbone. A canonical example of this colinearity is shown below with the 6-deoxyerythronolide B (6-dEB) synthase (DEBS) (Fig. 1).

disruption of the protein architecture. Here we review examples of targeted point mutagenesis to one or a few residues harbored within the PKS that alter domain specificity or selectivity, affect protein stability and inter-

> Because of issues with toxicity, analogs of natural products are often necessary for the development of a desired compound. However, due to the structural complexity of most natural product scaffolds, this can present some challenges when performed through traditional synthetic chemistry. Drawing inspiration from their colinear biosynthetic logic, the bioengineering community has had a longstanding interest in reengineering polyketide synthases. More recent efforts have expanded their potential utility. For example, Keasling and coworkers have noted that sequential polymerizations of C2 and C3 synthons of varying degrees of oxygenation and reduction present the chemical transformations necessary for producing many petrochemical derivatives including commodity chemicals, specialty chemicals, and fuel additives (Fig. 2) [1–3].

> The "legoization" [4] of PKS domains has been envisioned as a conceptual strategy for engineering since the discovery of their colinear

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Abbreviations: PKS, polyketide synthase; KS, ketosynthase; AT, acyltransferase; ACP, acyl carrier protein; KR, ketoreductase; MT, methyltransferase; ER, enoylreductase; DH, dehydratase; PS, pyran synthase; EI, enoylisomerase; SNAC, N-acetyl cysteamine; LM, loading module; DEBS, 6-deoxyerthronolide B synthase; Mod, module

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Fig. 1. The 6-deoxyerythronolide B synthase (DEBS) which produces the aglycone precursor to erythromycin, 6-dEB through priming with a loading module (LM) and undergoing six successive decarboxylative Claisen condensations followed by reductive processing to the β -carbon.



Fig. 2. Comparison of natural product scaffolds and petrochemically derived feedstocks such as adipic acid [3], 3-hydroxy acids [161,162], short chain ketones [97], and caprolactam [163] explored by Keasling and coworkers.

nature [5]. Because discrete domains catalyze transformations with specificity and selectivity, the idea of "domain swapping" to mix and match PKS biosynthetic machinery has been posited to realize the ramifications of colinearity [6-10] (Fig. 3A). A pioneering early example of the "molecular lego" approach that probed the modularity of domain swapping was performed by Menzella and coworkers. Restriction sites were introduced for the facile creation of both bimodular [11] and trimodular hybrid [4,12] polyketide synthases to generate model lactones with varying degrees of reduction and stereochemistry. While some success was observed, titers were low, often for reasons not easily rationalized. Further domain swapping experiments have been performed to alter selectivity of the AT [13-15], KR [16], and DH domains [3,17,18], of various PKSs, although typically with reduced or stalled activity. For example, more recently, Keasling and co-workers underwent an extensive study to engineer an enzyme from the borrelidin PKS that could produce adipic acid. Unfortunately, this study also produced trace amounts of active chimeric PKS with an accumulation of incompletely reduced product [3].

Over the past twenty years, significant advances have been made toward the structural characterization of PKS biosynthetic machinery [19–21]. These include the elucidation of PKS domain and didomain crystal structures [22–35], the characterization of interdomain and intermodular linkers [36-41], and the determination of a cryo-EM structure of a representative module [42-44]. The structural elucidation of PKSs has afforded us the ability to visualize the vast degree of evolutionary complexity and architecturally critical protein-protein interactions which ensure interdomain communication and stability of the dimer interface. When examining these characterized interactions, the rationale for why domain swapping has often failed to yield well folded or highly active protein [45,46] is apparent. However, when domain swapping is performed with critical consideration made to important interactions via judicious selection of domain boundaries, success can be observed. One of the most recent successful examples was performed by Yuzawa and coworkers who used a structure-based strategy to probe several AT domains and key associated linker regions to pinpoint boundaries for AT swaps. This study relied upon conserved motifs to install AT domains and suggested that the most successful boundaries preserved structurally distinct portions of the KS-AT linker and the post-AT linker, and were highly consistent between most ATs assayed. Indeed in one case, the kinetic data of the AT-swapped domain actually improved relative to wild type [47]. Nonetheless, examples of domain swapping with such success remain rare.

One alternative to domain swapping which minimizes global architectural disruption to the PKS involves engineering through



Fig. 3. Comparison of A) a domain swapping approach and B) a site directed mutagenesis approach to alter the selectivity of EryAT6.

individual point mutations (Fig. 3B). One or a few point mutations can drastically change the selectivity or specificity of domains harbored within the PKS, and in some circumstances, the communication between domains. Logically, making one or a few point mutations to a PKS is typically less globally destabilizing at the protein level than full domain swaps. One of the easiest transformations through site directed mutagenesis is to abolish activity through the mutation of a key active site residue. This has been performed numerous times for the purpose of mechanistic investigations, and loss-of-function mutations are frequently found in natural systems as a means of introducing molecular diversity. Furthermore, point mutations can be performed to alter the activity of a domain. This can be achieved through changing specificity (e.g. altering the extender unit choice in the AT domain), altering selectivity (shown in the KR and ER domains), or manipulating active site residues to perform new chemical transformations altogether (as is seen in the DH domain). Although not intended to be comprehensive, in this review we highlight examples of key amino acid residues identified through mutagenesis experiments which demonstrate the potential for precise manipulation of PKS biosynthesis. By capitalizing on our molecular-level structural understanding, this targeted "one at a time" approach is a powerful tool for creating chemical diversity.

Ketosynthase

Mutations to abolish activity: In nature, there are a few examples of KS domains lacking in catalytic function. Mostly commonly, these occur in *trans*-AT PKSs, which differ in that the AT domain is present on a separate polypeptide. These pathways have a number of other unique

features with regard to their modular organization relative to the canonical PKSs (also called cis-AT PKSs) and are reviewed extensively by Piel [48,49]. One distinct feature is that catalytically inactive KS domains (KS⁰) that nonetheless appear to have a structural role [50] are more prevalent in trans-AT pathways than cis-AT pathways. Three catalytic residues are present in the KS domain, two histidines and a cysteine. In the KS⁰ domains of *trans*-AT PKSs, typically the histidine from the HGTG decarboxylation motif is missing [51]. Loading KS domains that lack condensing activity are termed KS^Q domains and have a conserved glutamine residue replacing the catalytic cysteine. KS^Q domains catalyze decarboxylation of a malonyl or methymalonyl unit to an acetyl or propionyl priming unit, respectively [52,53]. Thus, nature has evolved roles for the KS domain for performing its canonical Claisen condensation. To purposefully inactivate the KS, mutation of either the active site cysteine or histidine of the HGTG motif to alanine has been successful [54,55].

Mutations to alter activity: In a study performed by Khosla and coworkers, amino acid sequences of assembly line and iterative PKSs were compared, and a 90% consensus sequence was generated for each subgroup. Of those residues conserved universally or within each subgroup, those within 10 Å of the active site cysteine were selected for mutation in EryKS1. In most cases the residues were replaced with alanine including T209A, S315A, N381A, H457A. The two exceptions include N455L where an isosteric change was introduced and Q386E where the corresponding residue found in the subgroup of iterative KSs was introduced [54]. Three mutants of the Module 1 KS including C211A, H346A, and K379A resulted in dramatically reduced activity. The H346A mutant produced \sim 1% of product compared to the wild



Fig. 4. A) Alignment of LovB and EryKS1 used in the mutagenesis experiment reported by Khosla and coworkers to identify conserved residues between modular and iterative PKSs essential for catalysis. Residues mutated in the report are indicated in yellow and residues found within 10 Å of the active site cysteine are in boxes. The alignment was performed with MUSCLE. B) Homology Model of EryKS1 with the residues mutated by Khosla and coworkers highlighted in blue. The homology model was made with Swiss PDB using EryKS3 as a template.

type, whereas the K379A mutant was added at 10-fold higher concentration and still produced only ~1% of product. These results suggest that both K379 and H346 are critical to catalyzing the formation of the C–C bond between the electrophilic and nucleophilic substrates, which differs from the mechanism of fatty acid synthesis regarding H346. H346 was also found to enhance decarboxylation of methylmalonyl-ACP by stabilizing the associated transition state. Similarly, K379 plays a role in C–C bond formation. Finally, the C211A mutant displayed no activity whatsoever, but was still a competent methylmalonyl-ACP carboxylase. Thus, the role of C211 is most likely to provide an extremely nucleophilic active site for anchoring the polyketide chain prior to decarboxylative Claisen condensation (Fig. 4) [54]. Considering that these residues appear to play an essential role in catalysis, homology-based methods may be used to uncover differences in the binding pocket affecting substrate selectivity.

One of the most powerful reasons to alter KS activity is that the KS possesses "gatekeeping" behavior towards substrates. It was revealed that the KS domain is inactive or kinetically slow towards unnatural substrates [56–58], often resulting in a kinetic bottleneck when the structures resulting from proceeding modules have been altered. This sequential selectivity of the KS is one of the biggest hurdles to PKS engineering. One classic example is that EryKS2 within DEBS1 appears to be sensitive to alterations in stereochemistry [56,57], and is thus unable to condense unnatural stereoisomers.

One of the most successful recent examples of altering the gatekeeping behavior of the KS domain was an investigation of EryKS3. Leadlay and coworkers performed a sequence alignment of EryKS3 with the mycolactone KS domains and discovered an exceptional degree of sequence similarity (> 97%) despite condensing units of differing lengths and stereochemistry [59]. As EryKS3 has been shown to have gatekeeping activity and a crystal structure is available [32], residues that deviated between the mycolactone KS domains and EryKS3 were targeted for mutagenesis. Indeed this approach proved successful; a simple mutation of alanine 154 in EryKS3 to tryptophan greatly enhanced the steric flexibility of the KS while allowing for the condensation of bulkier substrates (Fig. 5) [58]. The success of this simple mutation indicates that relatively conservative changes can be employed to address gatekeeping and lends credence to this homologybased approach. In addition to using pathways with high homology between modules, KSs from terminal modules (such as EryKS6) also tend to have looser substrate promiscuity [60,61]. Thus, targeting mutations to either KSs that accept large substrates or KSs that retain a great degree of homology from within their biosynthetic pathways may be the key to enabling the retention of structural changes including altered stereochemistry, degrees of reduction, and successful processing

of unnatural extender units.

The KS domain is also responsible for whether one round of elongation per module is performed, as is typical of type I PKSs, or iteration occurs via back-transfer to the ACP. Programmed iteration appears in a number of modular type I PKSs including the aureothin, borrelidin, and stigmatellin PKSs [62]. Therefore, altering residues that influence the kinetics of the KS towards back transfer of the substrate from the ACP may be a means to change the PKS structure for expansion of the carbon skeleton. Khosla and coworkers explored transforming EryMod3 from a modular enzyme to an iterative one, however such changes focused on altering regions of the ACP in contact with the KS [63]. To date, targeted mutation of the KS for reprogramming iteration is an underexplored area.

Acyltransferase

Mutations to abolish activity: The AT domain selects malonyl-CoA derivative extender units, most commonly methylmalonyl and malonyl-CoA. It is responsible for introducing functionality including heteroatoms, halogens, and alterations to the carbon chain length that can drastically affect the scaffold of the PKS carbon skeleton [64]. Mechanistically, the AT domain selects an extender unit through a serinehistidine catalytic dyad and either transfers the acyl enzyme intermediate through transthioesterification to the next ACP or hydrolyzes the extender unit from the acyl enzyme intermediate [13,65,66]. Inactivation of the AT can be achieved directly through mutation of the active site serine to alanine in the GHSxG motif (Fig. 6). One example of the utility of inactivating the AT domain to create molecular diversity was demonstrated via trans-AT complementation, a strategy wherein a trans-AT is introduced to deliver an extender unit to the module with the inactivated AT domain. This approach was originally employed by Khosla and coworkers using the disorazole pathway. It was then employed by Khosla and coworkers to introduce ethymalonyl-CoA through KirCII, a trans-AT of the kirromycin pathway, to Mod6 of DEBS [67], and later by Chang and co-workers [68,69] as a means of delivering fluoridated extender units.

Mutations to alter activity: Altering the selectivity of the acyltransferase domain was one of the earliest examples of targeted point mutations in PKS domains. The two most commonly utilized extender units, malonyl-CoA and methylmalonyl-CoA, are readily identifiable by diagnostic selectivity motifs, HAFH and YASH for malonyl-CoA and methylmalonyl-CoA, respectively, although occasionally VASH is observed instead of YASH for methylmalonyl-CoA. Early attempts to alter selectivity focusing on these motifs were performed in EryAT1 [70], EryAT4 [70], and EryAT6 [71]. In each of these domains, the mutations



Fig. 5. A) Overview of the ability of KS1 to extend unnatural N-acetyl cysteamine substrates. B) Panel of substrates tested. C) Structure of EryKS3 (pdb code 2QO3) with the residues mutated to explore the sequence space divergence in the mycolactone KSs. D) Sequence alignment of the KSs within DEBS and the those within the mycolactone PKS. The residue found by our laboratory in a common assembly of DEBS1-TE is highlighted (mutated to histidine to introduce a restriction site). The alignment was performed with MUSCLE.

increased promiscuity, although did not entirely reverse selectivity, indicating that mutations beyond this diagnostic "fingerprint" region are likely necessary to entirely reverse selectivity. This was later verified by Williams and coworkers *vide infra* [72]. Mixed selectivity motifs exist, including the HASH motif observed in epothilone AT3 which natively possesses a relaxed specificity and accepts a mixture of malonyl- and methylmalony-CoA [73]. Other motifs appear for rarer extender units including RPGH for SalAT1, a chloromalonyl-selective AT. Additionally, VASH is associated with a number of AT selectivities including methylmalonyl-, ethymalonyl-, and methoxymalonyl-CoA. Methoxymalonyl-CoA-selective ATs have a range of additional motifs including FAGH and HAGH. Finally, CPTH is observed in an allylmalonyl-selective AT in the FK506 pathway [74]. Thus, motifs correlating to ATs that accept rarer extender units tend to be much less conserved (Fig. 7).

A targeted approach was taken to expand the selectivity of the terminal AT domain of DEBS to accept varying extender units. Schulz and coworkers suggested that there are only subtle differences in the AT that afford discrimination between methylmalonyl-CoA and malonyl-CoA. In addition, they hypothesized that non-natural substrate exclusion is not strict but rather evolutionarily unimportant. This hypothesis is consistent with the observation that in pathways where a rare extender unit is employed, distributions of metabolites are often observed. For example, the salinosporamide PKS, SalAT1, predominantly accepts chloromethylmalonyl-CoA (salinosporamide A) but also accepts ethylmalonyl-CoA (salinosporamide B) and methylmalonyl-CoA (salinosporamide D) to a lesser degree, resulting in a distribution of metabolites

[64]. An analogous phenomenon is observed in the FK506 pathway where allylmalonyl-CoA is used in addition to propylmalonyl-CoA, ethylmalonyl-CoA, and methylmalonyl-CoA. As additional precedence, Williams and coworkers discovered that EryMod6 is more intrinsically promiscuous than anticipated toward a range of unnatural substrates including ethylmalonyl-CoA, propargylmalonyl-CoA, iso-propylmalonyl-CoA, butylmalonyl-CoA, methoxymalonyl-CoA, 2-azo-domalonyl-CoA, and phenylethylmalonyl-CoA *in vitro* [75].

To test their hypothesis, Shulz and coworkers used a directed mutagenesis approach to mutate EryAT6. Initial studies used a truncated substrate of the phosphopanetheinyl arm, 2-propargyl-malonyl-N-Acetylcysteamine (SNAC) to identify promising residues [71] and a follow-up study used 2-propargyl-malonyl-CoA as an extender unit [76,77]. These experiments identified a new mutation that expanded the promiscuity of EryAT6, V295A. Molecular dynamic (MD) simulations of wild type ErvAT6 suggested that the carboxylate of malonyl-CoA and methylmalonyl-SNAC interact with a few key residues in the active site such as Q198, M235, and V295 (Figs. 6 and 8). Q198 was found to have contact with the thioester of methylmalonyl-SNAC while the latter two residues appeared to be responsible for stereochemical control [76]. Among the mutations most successful for introducing new extender units, MD simulations revealed that an EryAT6 V295A mutation widens the active site to allow additional substrates to bind (Fig. 8). The V295A EryAT6 mutant was found to relax, but not switch, substrate specificity (Fig. 8) [76].

Williams and coworkers capitalized on the previously uncovered intrinsic promiscuity of ATs through a different approach. Rather than



Fig. 6. A) Example of a monomethylating MT domain harbored within in a PKS module. Frequently, other β -carbon processing domains are found, for example, a full reductive loop shown above. B) Example of a *gem*-dimethylating MT domain. C) Active site of CurMT3 (pdb code: 5THZ) from the curacin A pathway. Residues targeted for mutation are shown in orange and the SAH cofactor is shown in blue.

directed mutagenesis, they utilized saturation mutagenesis. Residues likely to form the binding pocket were identified via sequence alignment and homology modeling as L118, Y198, and S191 (Fig. 6). While Y198 and S191 correlate to the YASH motif, L118 does not correlate to any previously described specificity-conferring motif. However, it was chosen due to its likeliness to form interactions with the extender unit determined via homology modeling. Partially degenerate mutagenic oligonucleotides were used to introduce an average of 12 different amino acids at each targeted position, and the variants were incubated with a diketide-SNAC thioester and methymalonyl-CoA for an initial screen to determine their activity towards propargylmalonyl-CoA. Following this initial screen, the mutants were subjected to a more complex system utilizing a substrate mimic developed by Sherman and coworkers to determine if DEBS3 would extend. The most successful mutation discovered through this study was Y189R, a completely novel mutation which afforded enhanced propargylmalonyl-CoA selectivity. When a double mutant of Y189R in concert with the V187A mutation identified by the Schulz group was assayed, EryAT6's extender unit specificity was completely inverted. Rather than merely becoming more promiscuous, Y189R/V187A EryAT6 preferentially accepted unnatural extender units over methylmalonyl-CoA, particularly allylmalonyl-CoA, ethylmalonyl-CoA, propylmalonyl-CoA, and 2-azidomalonyl-CoA. Such an inversion of specificity was unprecedented in an engineered AT domain [72].

AT promiscuity was also uncovered by taking advantage of the S.

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Methylmalonyl-Selectiv	re AT Domains
EryAT6_methylmal	SLERVDVVQPVLFSVMVSLARLWGA-CGVSPSAVIGHSQGEIAAAVVAGV
EryAT3_methylmal	GLDRVDVVQPVLFAVMVSLAELWRS-YGVEPAAVVG <mark>HS</mark> QGEIAAAHVAGA
StiAT4_methylmal	GFDRVEVVQPVLFAVQVALAALWRS-WGIEPGAVVG <mark>HSM</mark> GEVAAAHVAGA
EpoAT6_methylmal	QLERIDVVQPVLFAMEVALSALWRS-WGVEPEAVVGHSMGEVAAAHVAGA
SorAT8_methylmal	SLDRVDVVQPALFAVMVSLAALWRS-LGVEPAAVVGHSQGEIAAAFVAGA
Nuclm2 mothulmal	
MARKIS-meculatmat	BLERVDVVQFALEARIVGLSALWKS-NGVVFARVVG <mark>NO</mark> QGEIAAACVAGA
Malonyl-Selective AT D	Domains
AveAt2_mal	EDAAALLQQTRYAQPALFAFQVALHRLLTDGYHITPHYYAG <mark>HSL</mark> GEITAAHLAGI
AveAT8 mal	DTTTLEEAAALLQQTPYAQPALFAFQVALHRLLTDGYHITPHYYAG <mark>HSL</mark> GEITAAHLAGI
AveAT3_mal	DTTTLEEAAALLQQTRYAQPALFAFQVALHRLLTDGYHITPHYYAG <mark>HSL</mark> GEITAAHLAGI
NysAT3_mal	LLNDTGWAQPALFAVEVALYRLVAS-LGVTPDFVGG <mark>HSI</mark> GELAAAHVAGV
GdmAT6_mal	TAALLDRTEFTQPALFALEVALFRLVES-WGVRPAYVLG <mark>HSI</mark> GEVAAAHVAGV
HbmAT6_mal	RAALLDRTEFTQPALFALEVALFRLVES-WGVRPAYVLG <mark>HSI</mark> GEVAAAHVAGV
Thulmalanul Calestina	M. Demains
Ethyimalonyi-Selective	AT DOMAINS
TylAT5_ethylmal	SI DRUDUUODUI ETMALSIAACWRD-LGVHPAAVVGHSQGEIAAACVAGA
NIGAIS_echyimai	STDKADAAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
Methorylmalonyl_Select	ive AT Domains
CdmAT2 methoyumal	
HbmAT2 methoxymal	SLDBVDVLOPTTFAIMVSLAALWOA-NGTHPDAVIGHSOCFTAAACVACH
SorAT3 methoxymal	SLERIEVVOPALETVMVSLAALWRS-RGTEPDAVVGHSOCELAAAYVAGA
FkbAT7 methoxymal	PATHFAHOTALTALLRS-WGTTPHAVIGHSLCETTAAHAAGV
Unusual Extender Unit	Selective AT Domains
SalAT1_chloromal	SLDRIEILQPVLFTVMVSLAAVWQS-VGVTPDAVVG <mark>HSQ</mark> GEIAAAAVSGA
FkbAT4_ally1ma1	VTRRVDVVHPVCWAVMVSLAAVWEA-AGVRPDAVIG <mark>HSQ</mark> GEIAAACVAGA
Methylmalonyl-Selectiv	e AT Domains
EryAT6 methylmal	LSLEDGVRVVAL <mark>R</mark> AKAL-RALAGKGGMVSLAAPGERARAL <mark>I</mark> -APWEDRISVAAVNSP
EryAT3 methylmal	LTLEDAAKLVVGRSRLM-RSLSGEGGMAAVALGEAAVRERL-RPWQDRLSVAAVNGP
StiAT4 methylmal	LSLEDAARVIGLRSRLL-SRVRGRGGMALVELSLDOVRELI-RPYEGOLSVGASNGP
EpoAT6 methylmal	LSLEDAVAIICRRSRLL-RRISGOGEMALVELTLEEAEAAL-RGHEGRLSVAVSNSP
SorAT8 methylmal	LSLEDAARIAALRSKAL-TTVAGNGAMAAVELGASDLOTYL-APWGDRLSIAAVNSP
RifAT4 methylmal	LSLEDAAKVVALRSQAIAASLAGRGGMASVALSEEDATARL-EPWAGRVEVAAVNGP
NysAT2 methylmal	LSLADAARVVALRSQAL-POLSGRGGMMSVSAPVERVTALL-APWQEALSVAAVNGP
Malonyl-Selective AT I	Domains
AveAt2_mal	LTLTDATTLITQRATLM-QTMP-PGTMTTLHTTPHHITHHL-TAHENDLAIAAINTP
AveAT8 mal	LTLTDATTLITQRATLM-QTMP-PGTMTTLHTTPHHITHHI-TAHENDLAIAAINTP
AveAT3 mal	LTLTDATTLITQRATLM-QTMP-PGTMTTLHTTPHHITHHL-TAHENDLAIAAINTP
NysAT3_mal	LSLEDACTLVAARARLM-QALPRGGAMLAIRATEDEVTPHL-TDDVSIAAVNGP
GdmAT6_mal	LSLPDACTLVRARGRLM-QQLTATGAMVAVEAAEDEVAPLL-AGKEHKVSIAAVNGP
HbmAT6_mal	LSLPDACTLVRARGRLM-QRLTATGAMVAVEAAEDEVAPLL-AGKEHKVSIAAVNGP
mthan 1 and 1 and 1 and 1 and 1	All Developer
Ethylmalony1-Selective	AT Domains
TylAT5_ethylmal	AT DOMAINS LSLEDAARIVALRSRAW-LTLAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP
TylAT5_ethylmal NidAT5_ethylmal	AT DOMAINS LSLEDARIVALRSRAW-LTLAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLAVAAVNSP
TylAT5_ethylmal NidAT5_ethylmal Methoxylmalonyl-Select	AT DOMAINS LSLEDAARIVALRSRAW-LILLAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLAVAAVNSP
TylAT5_ethylmal NidAT5_ethylmal Methoxylmalonyl-Select GdmAT2_methoxymal	AT DOMAINS LSLEDAARIVALRSRAW-LILLAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLEDGARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLAVAAVNSP :ive AT Domains LILIDAARIVTLRSOTIAHHLTGHGAMMSVLAPHTWVOEAL-APWHGHLWIAAINGP
TylAT5_ethylmal NidAT5_ethylmal Methoxylmalonyl-Select GdmAT2_methoxymal HbmAT2_methoxymal	AT DOMAINS LSLEDAARIVALRSRAW-LTLAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLAVAAVNSP LYLDDAAKIVILRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAINGP LTIINAAKIVILRSQTIAHHLTGHGAMMSVLASPTWVQETL-APWHGHLWIAAINGP
TylA75_ethylmal NidAT5_ethylmal NidAT5_ethylmal Methoxylmalonyl-Select GdmAT2_methoxymal HbmA72_methoxymal SorA73 methoxymal	AT DOMAINS LSLEDARIVALRSRAW-LILAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLAVAAVNSP HIVE AT DOMAINS LILIDAAKIVTLRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APMHGHLWIAAINGP LILIDNAAKIVTLRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APMHGHLWIAAVNGP LSLDDAAKVVRRSRLL-STLSGQGAMMAVERPPALEPYUAETL-AFWHGHLSIAAINSP
tylaT5_ethylmal NidAT5_ethylmal Methoxylmalonyl-Select GdmAT2_methoxymal BoxAT2_methoxymal SorAT3_methoxymal FkbAT7_methoxymal	AT DOMAINS LSLEDARIVALRSRAW-LILLAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLAVAAVNSP LILTDAAKIVTLRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAINGP LILTINAKIVTLRSQTIAHHLTGHGAMMSVLASPTWVQETL-APWHGHLWIAAINGP LSLDDAAKVVARRSRLL-STLSGQGAMAAVERPPAALEPYL-ARFGRRLSIAAINSP LSLDDAALITTRTRLM-DQLPSGGAMVTVLJSEEKRAQVL-RPGRVEILANNGP
tylaT_ethylmal TylaT_ethylmal NidAT5_ethylmal Methoxylmalonyl-Select GdmAT2_methoxymal BhmAT2_methoxymal SorAT3_methoxymal FkbAT7_methoxymal	AT DOMAINS LSLEDAARIVALRSRAW-LILLAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLAVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAINGP LTLIDAAKIVILRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAINGP LSLDDAAKVVARRSRLL-STLSGQGAMAAVERPPAALEPYL-ARFGRRLSIAAINSP LSLRDAGALLTTRTRM-DQLPSGGAMVTVLTSEEKARQVL-RPGVEIAAVNGP
trylaT_ethylmal TylaT_ethylmal NidAT5_ethylmal Methoxylmalonyl-Select GdmAT2_methoxymal HbmAT2_methoxymal SorAT3_methoxymal FkbAT7_methoxymal Unusual Extender Unit Colami & Lorener	AT DOMAINS LSLEDARIVALRSRAW-LTLAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLAVAAVNSP LVLTDAAKIVTLRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAINGP LTLTURAKIVTLRSQTIAHHLTGHGAMMSVLASPTWVQETL-APWHGHLWIAAINGP LSLDDAAKVVARSRLL-STLSGQGAMAAVERPPAALEPYL-ARFGRRLSIAAINSP LSLDDAGALLTTTRIM-DQLPSGGAWVTVLTSEEKARQVL-RPGVEIAAVNGP Selective AT Domains
trylaT_ethylmal TylaT_ethylmal NidAT5_ethylmal Methoxylmalonyl-Select GdmAT2_methoxymal HbmAT2_methoxymal FkbAT7_methoxymal Unusual Extender Unit SalAT1_chloromal	AT DOMAINS LSLEDAARIVALRSRAW-LILLAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLEDBARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLAVAAVNSP LVE AT DOMAINS LITLTDAAKIVTLRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAINGP LSLEDDAKVVARSRLL-STLSGQGAMAAVERPPALEYTL-ARFGRRLSIAAINSP LSLEDDAALITTRTRLM-DLFPSGAMVTVLTSEEKARQVL-RPGVEIAAVNGP Selective AT DOMAINS LSLEDBAAQVVVLRSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP
trylmT_ethylmal TylAT5_ethylmal NidAT5_ethylmal Methoxylmalonyl-Select GdmAT2_methoxymal SorAT3_methoxymal SorAT3_methoxymal Unusual Extender Unit SalAT1_chloromal FkbAT4 allylmal	AT DOMAINS LSLEDAARIVALRSRAW-LILLAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLEDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLAVAAVNSP LSLEDDSARIVALRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAINGP LITLINAAKIVILRSQTIAHHLTGHGAMMSVLASPTWVQETL-APWHGHLWIAAINGP LSLEDDAAKVVARSSRLL-STLSQGGAMAAVERPPAALEPYL-ARFGRRLSIAAINSP LSLEDDAAKVVARSSLLS-STLSQGGAMAVERPPAALEPYL-ARFGRRLSIAAINSP LSLEDBAQUVVLRSQLFADDLVGGAVASVSLPAEVEARI-ARFNGDAEVLSIAGNNGP LSLEDDAQUVVLRSQLFADDLVGGAVASVSLPAEVEARI-ARFNGDAEVLSIAGNNGP LSLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA
tylaT_ethylmal TylaT_ethylmal NidAT5_ethylmal Methoxylmalonyl-Select GdmAT2_methoxymal BorAT3_methoxymal SorAT3_methoxymal FkbAT7_methoxymal SalAT1_chloromal FkbAT4_allylmal	AT DOMAINS LSLEDAARIVALRSRAW-LILLGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLAVAAVNSP LTUDAAKIVILRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAINGP LITLINAAKIVILRSQTIAHHLTGHGAMMSVLASPTWVQETL-APWHGHLWIAAINGP LSLDDAAKVVARRSRLL-STLSGGGAMAAVERPPAALEPYL-ARFGRRLSIAAINSP LSLRDAGALLTTRTRLM-DQLPSGGAWVTVLTSEEKARQVL-RPGVEIAAVNGP Selective AT Domains LSLEDAAQVVVLRSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA
EtylaTs_ethylmal TylaTs_ethylmal NidAT5_ethylmal Methoxylmalonyl-Select GdmAT2_methoxymal HbmAT2_methoxymal FkbAT7_methoxymal Unusual Extender Unit SalAT1_chloromal FkbAT4_allylmal	AT DOMAINS LSLEDBARIVALRSRAW-LILLAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLEDBARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLAVAAVNSP LVE AT DOMAINS LITLTDAAKIVTLRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAINGP LSLEDDAKVVARSKLL-STLSGQGAMAAVERPPAALEFYL-ARFGRRLSIAAINSP LSLEDDAALITTRTRLM-DLPFSGAMVTVLTSEEKARQVL-RPGVEIAAVNGP Selective AT DOMAINS LSLEDBAAQVVVLRSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA
EtylmElonyl-Selective TylAT5_ethylmal NidAT5_ethylmal Methoxylmalonyl-Select GdmAT2_methoxymal SorAT3_methoxymal SorAT3_methoxymal Unusual Extender Unit SalAT1_chloromal FkbAT4_allylmal Methylmalonyl-Selectiv	A T DOMAINS LSLEDAARIVALRSRAW-LILLAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLAVAAVNSP LSLDDSARIVALRSGTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAINGP LTLITDAAKIVTLRSGTIAHHLTGHGAMMSVLASPTWVQETL-APWHGHLWIAAINGP LSLDDAAKVVARRSRLL-STLSGGGAMAAVERPPAALEPYL-ARFGRRLSIAAINSP LSLRDAGALLTTRTRIM-DQLPSGGAMMAVERPPAALEPYL-ARFGRRLSIAAINSP LSLRDAGALLTTRTRIM-DQLPSGGAMVTVLTSEEKARQVL-RPGVEIAAVNGP Selective AT Domains LSLEDAAQVVVLRSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA
<pre>trylaTs_ethylmal NidATS_ethylmal NidATS_ethylmal Methoxylmalonyl-Select GdmAT2_methoxymal HbmAT2_methoxymal FkbAT7_methoxymal Unusual Extender Unit SalAT1_chloromal FkbAT4_allylmal Methylmalonyl-Selectiv EryAT6_methylmal</pre>	A T DOMAINS LSLEDAARIVALRSRAW-LILLAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLEDBARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGGRLAVAAVNSP LSLEDBARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGGRLWIAAINGP LTLTDRAKIVTLRSQTIAHHLTGHGAMMSVLAPPHTWVQEAL-APWHGHLWIAAINGP LSLEDBARIVTLRSQTIAHHLTGHGAMMSVLAPPHTWVQEAL-APWHGHLWIAAVNGP LSLEDBARLITTRRUM-DQLPSGGAMAVERPPAALEPYL-ARFGRRLSIAAINSP LSLEDBARLITTRRUM-DQLPSGGAMAVTVLISEEKARQVL-RPGVEIAAVNGP Selective AT Domains LSLEDBARQVVVLRSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA ENGEN
EtyjAR3_GHY_Selectiv TyjAR5_ethylmal NidAT5_ethylmal Methoxymalonyl-Select GdmAT2_methoxymal SorAT3_methoxymal SorAT3_methoxymal Vnusual Extender Unit SalAT1_ohloromal FkbAT4_allylmal Methylmalonyl-Selectiv EryAT5_methylmal	A T DOMAINS LSLEDAARIVALRSRAW-LILLAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLEDBARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGGRLAVAAVNSP LSLEDBARIVALRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAINGP LTLTNAAKIVTLRSQTIAHHLTGHGAMMSVLASPTWVQETL-APWHGHLWIAAINGP LSLEDDAAKVVARSSRLL-STLSGQGAMAAVERPPAALEPYL-ARFGRRLSIAAINSP LSLENDAAKVVARSSLLS-STLSGQGAMAVERPPAALEPYL-ARFGRRLSIAAINSP LSLENDAAKVVARSSLLS-STLSGQGAMAVERPPAALEPYL-ARFGRRLSIAAINSP LSLENDAAKVVARSSLLSTLSGGAMAVSLSPAWVQETL-APWHGHVWIAANNGP Selective AT Domains LSLEDDAQVVVLRSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA VONSGDPEALAELVARCEDEGVRAKTLP-DYASHSRHVEEIRETILADLDGISARRA RSVVVSGDPEALAELVARCEDEGVRAKTLP-DYASHSRHVEEIRETILADLDGISARRA
<pre>EtyInalonyl-Selectiv TyIAT5_ethylmal NidAT5_ethylmal Methoxylmalonyl-Select GdmAT2_methoxymal SorAT3_methoxymal SorAT3_methoxymal FkbAT7_methoxymal Unusual Extender Unit SalAT1_chloromal FkbAT4_allylmal Methylmalonyl-Selectiv EryAT6_methylmal StiAT4_methylmal</pre>	A T DOMAINS LSLEDAARIVALRSRAW-LILLAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLEDBARIVALRSRAW-LILLAGKGGMVAVPMPAEELRPRI-VTWGDRLWIAAINSP LSLEDBARIVALRSRAW-LIGLAGKGGMVAVPMPAEELRPRI-APWHGHLWIAAINGP LITLINAAKIVILRSQTIAHHLIGHGAMMSVLASPTWVQEAL-APWHGHLWIAAINGP LSLEDBARVVARRSRLL-SILSGGGAMAAVERPPAALEPYL-ARFGRRLSIAAINSP LSLEDBARVVARRSRLL-SILSGGGAMAVERPPAALEPYL-ARFGRRLSIAAINSP LSLEDBAAGVUVLRSQLFADELVGGGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LILEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA LILEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA SSVVVSGOPEALAELVARCEDEGVRAKTLP-DVASHSPQMEPILEELERTGJAPRPA RSVVVSGOPEALAELVARCEDEGVRAKTLP-DVASHSPQMEPILEELERGJAVLOPRAA
<pre>trylaT_ethylmal TylAT_ethylmal NidAT5_ethylmal Methoxylmalonyl-Select GdmAT2_methoxymal HbmAT2_methoxymal FkbAT7_methoxymal Unusual Extender Unit SalAT1_chloromal FkbAT4_allylmal EryAT6_methylmal EryAT6_methylmal StiAT4_methylmal StiAT4_methylmal</pre>	AT DOMAINS LSLEDAARIVALRSRAW-LILLAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLAVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAINOP LTLTDAAKIVTLRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAINOP LSLDDAAKVVARSRLJ-STLSQGGAMAAVERPPAALEYL-ARFGRRLSIAAINOP LSLDDAAKVVARSRLJ-STLSQGGAMAAVERPPAALEYL-ARFGRRLSIAAINSP LSLDDAAKVVARSRLJ-STLSQGGAMAAVERPPAALEYL-ARFGRRLSIAAINSP LSLDDAAKVVARSRLJ-STLSQGGAMAAVERPPAALEYL-ARFGRRLSIAAINSP LSLDDAAKVVARSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA SSVVVSGEPEALAELVARCEDEGVRAKTLP-DVASISPOIERVREELETTGDIAPRPA RSVVLSGEPAALGVLTSLRGQVECVENVOV-VVDNSISPOIERVREELETTGDIAPRPA RSVVLSGEPAALGEVLALLAKGVFWRQVK-VDVASISPOVDFLREELEGIAVLQPRA
Etylmalonyl-Selective TylAT5_ethylmal NidAT5_ethylmal Methoxylmalonyl-Select GdmAT2_methoxymal SorAT3_methoxymal SorAT3_methoxymal Unusual Extender Unit SalAT1_chloromal FkbAT4_allylmal Methylmalonyl-Selectiv EryAT5_methylmal StiAT4_methylmal SorAT5_methylmal	A T DOMAINS LSLEDAARIVALRSRAW-LILLAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLEDDAARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAINSP LSLEDDAARIVTLRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAINGP LTLTDAARIVTLRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAINGP LSLEDDAAKVVARSSRLL-STLSQGGAMAAVERPPAALEPYL-ARFGRRLSIAAINSP LSLEDDAAKVVARSSLL-STLSQGGAMAVERPPAALEPYL-ARFGRRLSIAAINSP LSLEDDAAQVVVLRSQLFADELVGGAVASVLSEKARQVL-RPGVEIAAVNGP Selective AT Domains LSLEDDAQVVVLRSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA ************************************
<pre>trylaTs_ethylmal NidATS_ethylmal NidATS_ethylmal Methoxylmalonyl-Select GdmAT2_methoxymal HbmAT2_methoxymal FkbAT7_methoxymal Unusual Extender Unit SalAT1_chloromal FkbAT4_allylmal EryAT6_methylmal EryAT6_methylmal EryAT6_methylmal EryAT6_methylmal SorAT6_methylmal NifAT4_methylmal</pre>	A T DOMAINS LSLEDAARIVALRSRAW-LILLAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLEDBARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLAVAAVNSP LSLEDBARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAINOP LITLTDAAKIVTLRSQTIAHHLTGHGAMMSVLAPPHTWVQEAL-APWHGHLWIAAVNOP LSLDDAAKVVARSRIL-STLSGQGAMAAVERPPAALEPYL-ARFGRRLSIAAINOP LSLDDAAKVVARSRIL-STLSGQGAMAAVERPPAALEPYL-ARFGRRLSIAAINOP LSLDDAAKVVARSSRLJ-STLSGQGAMAAVERPPAALEPYL-ARFGRRLSIAAINOP LSLDDAAKVVARSSLLSTLSGQGAMAAVERPPAALEPYL-ARFGRRLSIAAINOP LSLDDAAKVVARSQTIAHHLTGHGAMMSVLAPPHAVGENL-APWHGHLWIAAVNOP Selective AT DomainS LSLEDBAQVVVLRSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA SSVVVSGDFEALAELVARCEDEGVRAKTLP-DVASISPCJERVREELLETTGDIAPRPA RSVVVSGDFEALAELVARCEDEGVRAKTLP-VDVASISPCJERVREELLETTGDIAPRPA RSVVVSGDFEALAELVARCEDEGVRAKTLP-VDVASISPCJERVREELLETTGDIAPRPA RSVVLSGDFAALEQULTARKOEVFCRVV-VDVASISPCJERVREELAVLQPRA RSVVLAGEPAALEQULATAKOVFWRQVK-VDVASISPCJVDPLREELGAVLQPRA RSVVLAGEPAALEQULARTAQVFRAVK-VDVASISPCJVDPLAGLANLAPRFC TSVVLAGDFAALDALDDQGVRAVRUV-VDVASISPCJVDPLAGLANLAPRFC
<pre>trylaT_ethylmal TylAT_ethylmal NidAT5_ethylmal Methoxylmalonyl-Select GdmAT2_methoxymal BhmAT2_methoxymal SorAT3_methoxymal FkbAT7_methoxymal Unusual Extender Unit SalAT1_chloromal FkbAT4_allylmal EryAT6_methylmal EryAT6_methylmal StlAT4_methylmal SorAT8_methylmal SorAT8_methylmal NysAT2_methylmal</pre>	AT DOMAINS USLEDBARIVALRSRAW-LILLGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAINOP LTLTDAAKIVTLRSQTIAHHLTGHGAMMSVLASPHTWVQEAL-APWHGHLWIAAINOP LSLDDAAKVVARSRL-STLSGQGAMAAVERPPAALEPYL-ARFGRRLSIAAINSP LSLDDAAKVVARSRLI-STLSGQGAMAAVERPPAALEPYL-ARFGRRLWIAANNGP Selective AT Domains LSLEDAAQVVVLRSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA C e AT Domains SSVVVSGPERLAELVARCEDEGVRAKTLP-DVASHSPQIERVREELLETGIAPRPA RSVVLSGEPGALRAFSEDCAAEGIRVRDID-VDYASHSPQIERVREELLETGIAPRPA RSVVLSGEPGALRAFSEDCAAEGIRVRDID-VDYASHSPQIERVREELLETGIAGNPRAA
<pre>ktylmalonyl-selective TylAT5_ethylmal NidAT5_ethylmal Methoxylmalonyl-Select GdmAT2_methoxymal SorAT3_methoxymal FkbAT4_methoxymal FkbAT4_allylmal FkbAT4_allylmal Methylmalonyl-Selective EryAT5_methylmal StiAT4_methylmal SifAT4_methylmal RifAT4_methylmal RifAT4_methylmal</pre>	A T DOMAINS USLEDBARIVALRSRAW-LILIGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAINGP LTITDAAKIVILRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAINGP LTITNAKIVILRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAINGP LSLDDAAKVVARSSRLL-STLSQGGAMAVERPPAALEPYL-ARFGRRLSIAAINGP LSLDDAAKVVARSSLL-STLSQGGAMAVERPPAALEPYL-ARFGRRLSIAAINSP LSLRDAGALLTTRTRIM-DQLPSGGAMAVERPPAALEPYL-ARFGRRLSIAAINSP LSLEDBAQVVVLRSQLFADDLVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA "************************************
<pre>trylaT_ethylmal TylAT_ethylmal NidAT5_ethylmal Methoxylmalonyl-Selectiv GdmAT2_methoxymal HbmAT2_methoxymal SorAT3_methoxymal FkbAT7_methoxymal Unusual Extender Unit SalAT1_chloromal FkbAT4_allylmal EryAT6_methylmal StiAT4_methylmal StiAT4_methylmal SorAT8_methylmal SorAT8_methylmal NysAT2_methylmal NysAT2_methylmal Malonyl-Selective AT I Aveat2_methylmal</pre>	A T DOMAINS LSLEDAARIVALRSRAW-LILLAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAINOP LTLTDAAKIVTLRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAINOP LSLDDAAKVVARSRLJ-STLSQGGAMAAVERPPAALEYL-ARFGRRLWIAAINOP LSLDDAAKVVARSRLJ-STLSQGGAMAAVERPPAALEYL-ARFGRRLWIAAINOP LSLDDAAKVVARSRLJ-STLSQGGAMAAVERPPAALEYL-ARFGRRLWIAAINOP LSLDDAAKVVARSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA SSVVVSGDFEALAELVARCEDEGVRAKTLP-DVASISPOIERVREELETTGDIAPRPA RSVVLSGDFEALAELVARCEDEGVRAKTLP-DVASISPOIERVREELETTGDIAPRPA RSVVLSGDFEALAELVARCEDEGVRAKTLP-VDVASISPOVDFLREELETGDIAPRPA RSVVLSGDFEALAELVARCEDEGVRAKTLP-VDVASISPOVDFLREELETGDIAPRPA RSVVLSGDFEALAELVARCEDEGVRAKVS-VDVASISPOVDFLREELETGDIAPRPA RSVVSGDFEALAELVARCEDEGVRAKVLP-VDVASISPOVDFLAELAULAPRPA RSVVSGDFEALAELDVARDDQQWIRRVN-VDVASISPOVDFLAELAULAPRPA RSVVSGDFEALAELDVARDDQQWIRRVN-VDVASISPOVDFLAELAULAPRPA RSVVLSGDFAALDALIDSLTAAQVFARVN-VDVASISPOVDFLAELHGIAVAQPRA PSVVVSGDTDALDALHACQEQGVRARVS-VDVASISPOVDFLAELMGIALANIAPRTC TSVVISGDTDALDALHACQEQGVRARKVS-VDVASISPONDAUDELAALANIAPRTC TSVVISGDTDALDALHACQEQGVRARKVS-VDVASISPONDFLAELMGIAPAADPSSVVVSGDTDALDALHACQEQGVRARKVS-VDVASISPONDFLAELVALDGUPATITVPE
<pre>EtyInT_GetyInalonyI-Selective TyIAT5_ethyImal NidAT5_ethyImal MethoxyImalonyI-Select GdmAT2_methoxymal SorAT3_methoxymal SorAT3_methoxymal FkbAT7_methoxymal Unusual Extender Unit SalAT1_ohloromal FkbAT4_allyImal FkbAT4_allyImal FxyAT5_methyImal StiAT4_methyImal SorAT8_methyImal SifAT4_methyImal RifAT4_methyImal RifAT4_methyImal NysAT2_methyImal MalonyI-Selective AT II AveAT2 mal</pre>	A T DOMAINS USLEDBARIVALRSRAW-LILLGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAVNSP LTLTDAAKIVTLRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAVNSP LSLDDAAKVVARSSRLL-STLSGQGGAMAAVERPPAALEPYL-ARFGRRLWIAAINSP LSLDDAAKVVARSSLLS-TISGQGGAMAAVERPPAALEPYL-ARFGRRLWIAAVNSP LSLDDAAKVVARSSLLS-TISGQGGAMAAVERPPAALEPYL-ARFGRRLWIAANNSP LSLRDAGALLTTRTRLM-DQLPSGGAMVTVLTSEEKARQVL-RPGVEIAAVNGP Selective AT Domains LSLEDAAQVVVLRSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA ************************************
<pre>trylaTs_ethylmal NidATS_ethylmal NidATS_ethylmal Methoxylmalonyl-Select GdmAT2_methoxymal HbmAT2_methoxymal FkbAT7_methoxymal Vnusual Extender Unit SalAT1_chloromal FkbAT4_allylmal EryAT6_methylmal EryAT6_methylmal EryAT6_methylmal SorAT8_methylmal RifAT4_methylmal RifAT4_methylmal RifAT4_methylmal NysAT2_methylmal Malonyl-Selective AT I AveAT8_mal AveAT8_mal AveAT8_mal</pre>	A Y DOMAINS LSLEDAARIVALRSRAW-LILLGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLEDBARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAVNSP LSLEDBARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAVNSP LSLEDBARIVALRSRAW-LGLAGKGGMAAVERPPAALEPYL-ARFGRRLWIAAVNSP LSLEDBARIVTLRSQTIAHHLTGHGAMMSVLAPPHWQEAL-APWHGHLWIAAVNSP LSLEDBARIVTLRSQTIAHHLTGHGAMMSVLAPPHWQEAL-APWHGHLWIAAVNSP LSLEDBARIVTLRSQTIAHHLTGHGAMMSVLAPPHWQEAL-APWHGHLWIAAVNSP LSLEDBARIVTLRSQTIAHHLTGHGAMMSVLAPPHWQEEL-APWHGHLWIAAVNSP LSLEDBARIVTLRSQTIAHHLTGHGAMMSVLAPHWQEAL-APWHGHLWIAAVNSP Selective AT DomainS LSLEDBARUVVLRSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA SSVVVSGDFEALAELVARCEDEGVRAKTLP-DYASISPCJERVKEELLETTGDIAPRA RSVVVSGDFEALAELVARCEDEGVRAKTLP-DYASISPCJERVKEELLETTGDIAPRA RSVVVSGDFEALAELVARCEDEGVRAKTLP-DYASISPCJERVKEELLETGDIAPRA RSVVVSGDFEALAELVARCEDEGVRAKTLP-VDVASISPCJERVKEELLETGDIAPRA RSVVVSGDFEALAELVARCEDEGVRAKTLP-VDVASISPCJDERVKEELAVLQFRA RSVVVSGDFEALAELVARCEDEGVRAVN-VDVASISPCJDERVKEELAVLQFRA RSVVVSGDFEALAELVARCEDEGVRAKTLP-VDVASISPCJDERVKEELAVLGRAR SVVVSGDTALDALHTACQEQVVRAVN-VDVASISPCJDERVKEELAVLGRAR SVVVSGDTDALDALHACQEQVVRAVN-VDVASISPCJDERVKEELAVLGRARA SSVVSGDTDALDALHACQEQVVRAVN-VDVASISPCJDERVKEELAVLGRARA SSVVSGDTDALDALHTCQQQGIKKTKTP-TNHAFHSPHTNPLLNQLHOHTOTLTYHPP TSLVISGTPHTVQHITTLCQQQGIKKTKTP-TNHAFHSPHTNPLLNQLHOHTOTLTYHPP
<pre>EtyInT_GetyImalonyI-Selective TyIAT5_ethyImal NidAT5_ethyImal MethoxyImalonyI-Select GdmAT2_methoxymal HbmAT2_methoxymal SorAT3_methoxymal FkbAT7_methoxymal Unusual Extender Unit SalAT1_chloromal FkbAT4_allyImal EryAT6_methyImal EryAT6_methyImal SorAT8_methyImal SorAT8_methyImal SorAT8_methyImal NysAT2_methyImal MalonyI-Selective AT I AveAT2_mal AveAT3_mal NysAT3_mal</pre>	A T DOMAINS USLEDBARIVALRSRAW-LILLGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAINSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAINSP LTLTDAAKIVTLRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APMHGHLWIAAINGP LTLTDAAKIVTLRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APMHGHLWIAAINGP LSLDDAAKVVARSRL-STLSQGGAMAAVERPPAALEYL-ARFGRRLWIAAINGP LSLDDAAKVVARSRL-STLSQGGAMAAVERPPAALEYL-ARFGRRLWIAAINGP LSLDDAAKVVARSRL-STLSQGGAMAAVERPPAALEYL-ARFGRRLWIAANNGP SOLOCIVC AT DOMAINS LSLEDAAQVVVLRSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA F e AT Domains SSVVVSGPEALAELVARCEDEGVRAKTLP-DVASHSRVEEIRETILADLDGISARRA RSVVLSGEPGALRAFSEDCAAEGIRVRDID-VDYASHSPQIERVREELLETTGDIAPRPA RSTVLAGGPRALEQVLTSLRRQEVECRVD-VDVASHSPQMEPLLPELRGLAVLGPRAA RSTVLAGEPAALSEVLAALITAKGVFWRQV-VDVASHSPQMEPLDPELREALAGIRPRAP RSTVLSGEPAALDDLDALHTAKQVEQGVRAKVS-VDXSHSPQMDAVDDELAAGLANIAPRTC TSVVISGDTDALDALHTACQEQGVRAKVS-VDYASHSCHMEVARADALAHLGGIRAQAP SSVVSGDTDALDALHTACQEQGVRAKVS-VDYASHSPHTVPILNQLHQHT7LTYHPP TSLVISGTPHTVQHITTLCQQQGIKTKTLP-TNHAFHSPHTNPILNQLHQHT7LTYHPP TSLVISGTPHTVQHITTLCQQQGIKTKTLP-TNHAFHSPHTNPILNQLHQHT7LTYHPP TSVVSGTPHTVQHITTLCQQQGIKTKTLP-TNHAFHSPHTNPILNQLHQHT7LTYHPP
<pre>EtyIntS_etHyImal TyIATS_ethyImal NidATS_ethyImal MethoxyImalonyI-Select GdmAT2_methoxymal SorAT3_methoxymal SorAT3_methoxymal FkbAT4_methoxymal Unusual Extender Unit SalAT1_chloromal FkbAT4_allyImal EryAT3_methyImal StiAT4_methyImal SorAT8_methyImal SorAT8_methyImal SorAT8_methyImal NysAT2_methyImal MalonyI-Selective AT I AveAt2_mal AveAT5_mal AveAT3_mal SorAT3_mal GdmAT6_mal</pre>	<pre>N Domains LSLEDAARIVALRSRAW-LILIAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAINSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAINSP LTLTDAAKIVTLRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAINGP LTLTDAAKIVTLRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAINGP LSLDDAAKVVARSSRL-STLSQGGAMAVERPPAALEPYL-ARFGRRLSIAAINSP LSLRDAGALITTRTRIM-DQLPSGGAMAVERPPAALEPYL-ARFGRRLSIAAINSP LSLRDAGALUTTRTRIM-DQLPSGGAMAVERPPAALEPYL-ARFGRRLSIAAINSP LSLRDAGAUVVIRSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA VVSGDPEALAELVARCEDEGVRAKTLP-DVASHSRHVEEIRETILADLDGISARRA RSVVVSGPEALAELVARCEDEGVRAKTLP-DVASHSPOIERVREELETTGIAPRA RSTVLSGPFALAELVARCEDEGVRAKTLP-DVASHSPOIERVREELETTGIAPRA RSTVLSGPFALAEJVARCEDEGVRAKTLP-DVASHSPOIERVREELLETGDIAPRA RSTVLSGPFALAELVARCEDEGVRAKTLP-DVASHSPOIERVREELLETGDIAPRA RSTVLSGPFALAELVARCEDEGVRAKTLP-DVASHSPOIERVREELLETGDIAPRA RSTVLSGPFALAELVARCEDEGVRAKTLP-DVASHSPOIERVREELLETGDIAPRA RSTVLSGPFALAELVARCEDEGVRAKTLP-DVASHSPOIERVREELLETGDIAPRA RSTVLSGPAALESVLTSLRQEVRCRPVD-VDVASHSPOMEPLLEPLERGLAVLGARPRAMA RATLVSGEPAALEDLDLDDQGVKIRRVA-VDVASHSPAMEDLAENLGAIRPRAA RATLVSGETDAALEDLDLDDQGVKIRRVA-VDVASHSPAMPLARULAGHTPRATVIAGVPRQVVIAGTELAULAIDAUHTTLCQQQGIKKTTLP-TNHAFHSPHTNPILNQLHQHTQTLTYHPP TSLVISGTPHTVQHITTLCQQQGIKKTTLP-TNHAFHSPHTNPILNQLHQHTQTLTYHPP TSLVISGTPHTVQHITTLCQQQGIKKTTLP-TNHAFHSPHTNPILNQLHQHTQTLTYHPP TSLVISGTPHTVQHITTLCQQQGIKKTTLP-TNHAFHSPHTNPILNQLHQHTQTLTYHPP TSLVISGTPHTVQHITTLCQQQGIKKTTLP-TNHAFHSPHTNPILNQLHQHTQTLTYHPP TSLVISGTPHTVQHITTLCQQQGIKKTTLP-TNHAFHSPHTNPILNQLHQHTQTLTYHPP TSLVISGTPHTVQHITTLCQQQGIKKTLP-VHAFHSPHTNPILNQLHQHTQTLTYHPP TSLVISGTPHTVQHITTLCQQQGIKTTLP-NHAFHSPHTNPILNQLHQHTQTLTYHPP TSLVISGTPHTVQHITTLCQQQGIKTTLP-VHAFHSPHTNPILNQLHQHTQTLTYHPP TSLVISGTPHTVQHITTLCQQQGIKTTLP-NHAFHSPHTNPILNQLHQHTQTLTYHPP TSLVISGTPHTVQHITTLCQQQGIKTTLP-NHAFHSPHTNPILNQLHQHTQTLTYHPP TSLVISGTPHTVQHITTLCQQQIANTTLP-NHAFHSPHTNPILNQLHQHTQTLTYHPP TSLVISGTPHTVQHITTLCQQQIANTTLP-NHAFHSPHTNPILNQLHQHTQTLTYHPP TSLVISGTPH</pre>
<pre>trylaT_ethylmal TylAT_ethylmal NidAT5_ethylmal Methoxylmalonyl-Selectiv GdmAT2_methoxymal HbmAT2_methoxymal SorAT3_methoxymal FkbAT7_methoxymal Unusual Extender Unit SalAT1_chloromal FkbAT4_allylmal EryAT6_methylmal EryAT6_methylmal EryAT6_methylmal SorAT8_methylmal SorAT8_methylmal SorAT8_methylmal NysAT2_methylmal Aucent7_methylmal AvcAT8_mal AvcAT8_mal AvcAT8_mal AvcAT3_mal GdmAT6_mal HbmAT6_mal</pre>	AT DOMAINS USLEDBARIVALRSRAW-LILLGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAINOP LILDDARIVALRSRAW-LGLAGKGGMAAVERPPAALEFYL-ARFGRRLWIAAINOP LSLDDARVVARSRLJ-STLSGQGAMAAVERPPAALEFYL-ARFGRRLWIAAINOP Selective AT Domains LSLEDBAAQVVVLRSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWAGRNGA LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA SSVVVSGDFEALAELVARCEDEGVRAKTLP-DVASHSPQVEPLRELETGDIAPRPA RSVVSGEPGALARFSEDCAAGGIRVRDID-VDVASHSPQVEPLRELETGDIAPRPA RSVVSGEPGALAELVARCEDEGVRAKTLP-DVASHSPQVDPLREELUTGDIAPRPA RSVVSGEPGALAELVARCEDEGVRAKTLP-DVASHSPQVDPLREELUTGDIAPRPA RSVVSGEPGALAELVARCEDEGVRAKTLP-DVASHSPQVDPLREELUTGDIAPRPA RSVVSGEPGALAELVARCEDEGVRAKTLP-DVASHSPQVDPLREELUTGDIAPRPA RSVVSGEPGALAELVARCEDEGVRAKVLD-VDVASHSPQVDPLREELUTGDIAPRPA RSVVSGEPGALAELVARCEDEGVRAKVLD-VDVASHSPQVDPLREELUTGDIAPRPA RSVVSGEPGALAELOVARCHAAVGVKVVVVANABSAQMDAVQDELAAGLANIAPRTC TSVVIAGBEALDEALDALDDQCWXIRVA-VDVASHSPQVPLREELWAALGAIRPRAA RSTVLAGEPAALDEVILJDDQCWXIRVA-VDVASHSPTNPLLNQLHQHTQTLTYHPP TSLVISGTPHTVQHITTLCQQQGIKTKTLP-TNHAFHSPHTNPLLNQLHQHTQTLTYHPP TSLVISGTPHTVQHITTLCQQQGIKTKTLP-TNHAFHSPHTNPLLNQLHQHTQTLTYHPP TSVVVSGEDVYXAVETLARQCRRTKRUV-VSHAFBSPHMOGMLDAFREVAAGLAYALBYYA
<pre>EtyIntS_etyIntS_eterive TyIATS_ethyImal NidATS_ethyImal MethoxyImalonyI-Select GdmAT2_methoxymal BbmAT2_methoxymal SorAT3_methoxymal FkbAT7_methoxymal Unusual Extender Unit SalAT1_chloromal FkbAT4_allyImal FkbAT4_allyImal EryAT5_methyImal StiAT4_methyImal SorAT8_methyImal SorAT8_methyImal SorAT8_methyImal NysAT2_methyImal MalonyI-Selective AT II AveAT2_mal AveAT3_mal NysAT3_mal GdmAT6_mal HbmAT6_mal</pre>	A DOMAINS USLEDAARIVALRSRAW-LILIAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRI-VTWGDRLWIAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRI-VTWGDRLWIAAVNSP LSLDDAAKVVLRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAVNSP LSLDDAAKVVARSRLSTISSQGAMAAVERPPAALEYI-ARFGRRLWIAAINSP LSLDDAAKVVARSSLLS-TISGQGAMAAVERPPAALEYI-ARFGRRLWIAAINSP LSLDDAAKVVARSSLLSTISGQAMAAVERPPAALEYI-ARFGRRLWIAANNSP SOLOCIVA AT DOMAINS LSLEDAAQVVVLRSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA COMMONSE SSVVVSGDFALAELVARCEDEGVRAKTLP-DYXSHSRHVEEIRETILADLDGISARRA RSVVSGEPGALAELVARCEDEGVRAKTLP-DYXSHSRHVEEIRETILADLDGISARRA RSVVSGEPGALAELVARCEDEGVRAKTLV-VDXASISQUBELAGLAIGAIRPRA RSVVSGEPGALAELVARCEDEGVRAKTLP-DYXSHSRHVEEIRETILADLDGISARRA RSVVSGEPGALAELVARCEDEGVRAKTLP-DYXSHSRHVEELETTGIAPRPA RSVVSGEPGALAELVARCEDEGVRAKTLP-DYXSHSRHVEELETGIAPLGPRA RSVVSGEPGALAELVARCEDEGVRAKTLP-DYXSHSRHVEELLETGIAPPPA RSVVSGEPGALAELVARCEDEGVRAKTLP-DYXSHSRHVEELLETGIAPPPA RSVVSGEPGALAELVARCEDEGVRAKTLP-DYXSHSRHVEELLGTHQIAGAIRPRA RSVVSGEPGALAELVARCEDEGVRAKTLP-DYXSHSRHVEELLGIAALGAIRAPPA RSVVSGEPGAALSEVLAALTKAVFWRQVV-VDVXSISPQDELAAGAIRAPPA RSVVSGEPGAALSEVLAALTKAVFWRQVX-VDVXSISPQDELAAGAIRAPPA RSVVSGEPGAALSEVLAALTKAVFWRQVX-VDVXSHSHQMDAVQDELAAGAIRAPPA RSVVSGEPGAALSEVLAALTKAVFWRQVX-VDVXSHSHQMDAVDELAAGAIRAPPA SSVVSGDEDALDELDALDDQCWIRTRVX-VDVXSHSHQMDAVDELAAGAINAPPA SSVVSGDEDAALSEVLAALTAGVERGVX-VDXSHSHQMDAVDELAAGAINAPPA RSVVSGEPTALDALTAQQEGKTKTLP-TNHAFHSPHTNPILNQLHQHTQTLTYHPP TSLVSGFPHTVQHITTLCQQQGIKTKTLP-TNHAFHSPHTNPILNQLHQHTQTLTYHPP TSVVSGTEDAVAAIGARFTAQDRKTTRLP-VSHAFHSPHMDGMLDAFEVASALAGAIYAPP
<pre>trylaT_cetylmal TylAT_cetylmal NidAT5_cetylmal Methoxylmalonyl-Select GdmAT2_methoxymal HbmAT2_methoxymal FkbAT7_methoxymal FkbAT7_methoxymal FkbAT4_allylmal FkbAT4_allylmal EryAT6_methylmal EryAT6_methylmal EryAT6_methylmal SorAT8_methylmal RifAT4_methylmal RifAT4_methylmal RifAT4_methylmal RifAT4_methylmal NysAT2_methylmal AveAT8_mal AveAT8_mal AveAT8_mal GdmAT6_mal Ethylmalonyl-Selective</pre>	AT DOMAINS USLEDAARIVALRSRAW-LILLGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDAARIVALRSRAW-LGLAGKGGMVAVSPPEAELRPRL-VTWGDRLWIAAVNSP LSLDDAARIVALRSRAW-LGLAGKGGMVAVSPPEAELRPRL-VTWGDRLWIAAVNSP LSLDDAARVVARSGTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAVNSP LSLDDAARVVARSGTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAVNSP LSLDDAARVVARSGTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAVNSP LSLDDAARVVARSGTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAVNSP LSLDDAARVVARSGTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAVNSP SSLDDAARVVARSGTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAVNSP LSLDDAARVVARSGTIAHHLTGHGAMMSVLAPHTWQEAL-APWHGHLWIAAVNSP SSLDDAARVVARSGTIAHLTGHGAMMSVLAPHTWQEAL-APWHGHLWIAAVNSP SSLSLDDAARVVARSGTIATTRIL-DQLPSGGAMAVTVLTSEKARQVL-RPGVUAARNSP LSLEDAAQVVVLRSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA SVVVSGDFEALAELVARCEDEGVRAKTLP-DVASISPQUERVREELLETTGDIAPRA RSVVVSGEPGALARFSEDCAAEGIRVRDID-VDVASISPQUERVREELLETGDIAPRA RSVVVSGEPAALSQUTALRAQVERVRV-VDVASISPQUPELREELGAVLQPRA RSTVLAGEPAALSQUTALTAKGVEWRQVV-VDVASISPQUPELREELGAVLQPRAR SVVVSGEDTALDALHTACQEQQVRARVS-VDVASISPQUPELREELAALGANIAPRTC TSVVIAGAEALDEALDALDDQGVRIRRVA-VDVASISPQUPELREELAALGANIAPRTC TSVVISGETDALDALHTACQEQQVRARVS-VDVASISPMDAVDELARVLAGLANIAPRTC TSVVISGETPHTVQHITTLCQQGGIKTKTLP-TNHAFFISPHTNPILNQLHOHTQTLTYHPP TSVVVAGTEEAVAAIGARFTAQDRSKTKRLV-VSNAFGSPHTNPILNQLHOHTQTLTYHPP TSVVAGDEDVVTAVAETLARQGRKTKRLV-VSNAFGSPHTNPILNQLHOHTQTLTYHPP TSVVSGDEDVVTAVAETLARQGRKTKRLV-VSNAFGSPHDMEMLDAFREVAASLAYAPP TSVVVSGDEDVVTAVAETLARQGRKTKRLV-VSNAFGSPHDMEMLDAFREVAASLAYAPP TSVVVSGDEDVVTAVAETLARQGRKTKRLV-VSNAFGSPHDMEMLDAFREVAASLAYAPP
<pre>trylaT_cetylmal TylAT_cetylmal NidAT5_cetylmal Methoxymalonyl-Select HbmAT2_methoxymal HbmAT2_methoxymal SorAT3_methoxymal FkbAT7_methoxymal Unusual Extender Unit SalAT1_chloromal FkbAT4_allylmal EryAT6_methylmal EryAT6_methylmal StiAT4_methylmal EryAT6_methylmal SorAT8_methylmal SifAT4_methylmal SorAT8_methylmal NysAT2_methylmal AveAT6_mal AveAT3_mal GdmAT6_mal Ethylmalonyl-Selective TylAT5_ethylmal</pre>	A Domains SVVVSGPEALEEVALGAPGEVERVELPARCHEARSHARD CONSTRAINS SSVVSGPEALEEVALGAPGEVERVELPARCHEARSHARD CONSTRAINSE SVVVSGPEALEEVALGAPGEVERVELPARCHEARSHARD CONSTRAINSE SVVVSGPEALEEVALGAPGEVERVELVELTENALGAPAGEVERVELPARCHEARSHARD LTLEDAAKVVARSKIL-STLSGGGAMAAVERPPAALEFYL-ARFGRRLWIAAINGP LSLDDAAKVVARSKIL-STLSGGGAMAAVERPPAALEFYL-ARFGRRLWIAAINGP LSLDDAAKVVARSKIL-STLSGGGAMAAVERPPAALEFYL-ARFGRRLWIAAINGP LSLDDAAKVVARSKIL-STLSGGGAMAAVERPPAALEFYL-ARFGRRLWIAAINGP LSLDDAAKVVARSKIL-STLSGGGAMAAVERPPAALEFYL-ARFGRRLWIAANNGP LSLDDAAKVVARSKIL-STLSGGGAMAAVERPPAALEFYL-ARFNGDAEVLSIAGNNGP LSLDDAAKVVARSKIL-STLSGGGAMASVSLPAAEVERAI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA SVVVSGEPEALELVARCEDEGVRAKTLP-DYASHSPONEPLLEERTILADLDGISARRA RSVVVSGEPALAELVARCEDEGVRAKTLP-DYASHSPONEPLLEERTILADLDGISARRA RSVVVSGEPALAELVARCEDEGVRAKTLP-DYASHSPONEPLLEERTILADLDGISARRA RSVVSGEPALAELVARCEDEGVRAKTLP-DYASHSPONEPLLAELETGGIAAVLQPRA RSTVLSGEPAALESVLAALTAKVFWRVVF-VDVASHSPONEPLLAELETGGIAVLQPRA RSTVLSGEPAALESVLAALTAKVFWRVVF-VDVASHSPONEPLLAELETGGIAVLQPRA RSTVLSGEPAALDELDDLDQQVRIRKVA-VDVASHSPONEPLAALGALANIAPRTC TSVVISGDEALDEALDALDDQQVRIRKV-VDVASHSPONDELAALAANARPTC TSVVISGTPHTVQHITTLCQQQGIKKTKTLP-TNHAFHSPHTNPILNQLHQHTQTLTYHPP TSLVISGTPHTVQHITTLCQQQGIKKTKTLP-TNHAFHSPHTNPILNQLHQHTQTLTYHPP TSVVVSGEDEVVTAVAETLARQCRKTKRLV-VSHAFHSPHTNPILNQLHQHTQTLTYHPP TSVVVSGEDEVVTAVAETLARQCRKTKRLV-VSHAFHSPHTNPILNQLHQHTQLLYHPP TSVVVSGDEDVVTAVAETLARQCRKTKRLV-VSHAFHSPHTNPILNQLHARRAALTYHEP TSVVVSGDEDVVTAVAETLARQCRKTKRLV-VSHAFHSPHTNPILNQLHARFRAARLTYAPP AT Domains GTAAVAGOVDALRELLAELTAEGIRAKPIPGVDTAGHSAQVDGLKEHLEEVLAPVSRSLAALAFYPS
<pre>EtyIntS_etyInalonyI-Selective TyIATS_ethyImal NidATS_ethyImal MethoxymalonyI-Select GdmAT2_methoxymal HbmAT2_methoxymal SorAT3_methoxymal FkbAT7_methoxymal Unusual Extender Unit SalAT1_chloromal FkbAT4_allyImal FkbAT4_allyImal EtyAT5_methyImal SorAT8_methyImal SorAT8_methyImal SorAT8_methyImal SorAT8_methyImal NysAT2_methyImal MalonyI-Selective AT II AveAT2_mal AveAT3_mal HbmAT6_mal HbmAT6_mal NidAT5_ethyImal</pre>	A DOMAINS ISUBDARIVALRSRAW-LILAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVSHPAEELRPRI-VTWGDRLWIAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVSHPAEELRPRI-VTWGDRLWIAAVNSP LSLDDAKVVARSSRL-STLSGQGAMAAVERPPAALEYI-ARFGRRLWIAAINGP LSLDDAAKVVARSSRLS-STLSGQGAMAAVERPPAALEYI-ARFGRRLWIAAINGP LSLDDAAKVVARSSLLS-TISGQGAMAAVERPPAALEYI-ARFGRRLWIAAINSP LSLDDAAKVVARSSLLS-TISGQGAMAAVERPPAALEYI-ARFGRRLWIAANNSP SOLOCIVA AT DOMAINS LSLEDAAQVVVLRSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA CA AT DOMAINS SSVVVSGDPEALAELVARCEDEGVRAKTLP-DYASHSRHVEEIRETILADLDGISARRA RSVVVSGEPGALAELVTAKCEDEGVRAKTLP-DYASHSCHVERVELLETTGDIAPRPA RSVVJGGPFALAEVITSLRQEVFCRVD-VDVASHSPQUBELAGLAIGAIRPRA RSVVJSGEPGALAELVALTKAVFWRQVV-VVASSISQDMEPLLPELERGLAVLGPRAA RSVVSGEPGALAELVALLDGQCWIRKWV-VDVASHSPQUBELAAGLANIAPRTC TSVVISGDPAALEQULTSLRQEVFCRVD-VDVASHSPMDELAAGLANAIAPRTC TSVVISGDPAALEQULTSLRQEVGCRVD-VDVASHSPMDELAAGLANIAPRTC TSVVISGDPAALSEVIAALIDGQCWIRKWS-VDYASHSCHVEANRALAHLGGIRAQAP SSVVVSGDTDALDALHTACQQGIKTKTLP-TNHAFHSPHTNPILNQLHOHTQTLTYHPP TSLVISGTPHTVQHITTLCQQQGIKTKTLP-TNHAFHSPHTNPILNQLHOHTQTLTYHPP TSVVVSGDEDVYTAVAETLARQGRKTKRLV-VSHAFHSPHMDFMLDGLDAFREVASRLYAPP TSVVVSGDEDVYTAVAETLARQGRKTKRLV-VSHAFHSPHMDFMLDAFREVASRLYAPP
<pre>trylaT_cetylmal TylAT_cetylmal NidAT5_cetylmal Methoxylmalonyl-Selective GdmAT2_methoxymal HbmAT2_methoxymal FkbAT7_methoxymal FkbAT7_methoxymal FkbAT4_allylmal FkbAT4_allylmal FkbAT4_allylmal EryAT6_methylmal EryAT6_methylmal EryAT6_methylmal SorAT8_methylmal RifAT4_methylmal RifAT4_methylmal NysAT2_methylmal NysAT2_methylmal Malonyl-Selective AT I AveAT8_mal AveAT8_mal MysAT3_mal HbmAT6_mal Ethylmalonyl-Selective TylAT5_cethylmal NidAT5_cethylmal</pre>	A T DOMAINS LSLEDAARIVALRSRAW-LILLGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLEDBARIVALRSRAW-LGLAGKGGMVAVSHPEAELRPRI-VTWGGRLWIAAVNSP LSLEDBARIVALRSRAW-LGLAGKGGMVAVSHPEAELRPRI-VTWGGRLWIAAVNSP LILDTARAKIVTLRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAVNSP LTLTNAAKIVTLRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAVNSP LSLEDBALUTLRSQUIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAVNSP LSLEDBALUTLRSQUIAHHLTGHGAMMSVLASPHTWVQEAL-APWHGHLWIAAVNSP LSLEDBALUTLRSQUIAHHLTGHGAMMSVLASPHTWQEEL-AFFGRRLSIAAINSP LSLEDBALUTRTRIM-DGLEGGGAMAAVERPPAALEPYL-ARFGRRLSIAAINSP LSLEDBALUTTRTRIM-DGLEGGGAMAVERPPAALEPYL-ARFGRRLSIAAINSP LSLEDBAQVVVLRSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA SVVVSGDFEALAELVARCEDEGVRAKTLP-DYASISPCIERVREELLETTGDIAPRA RSVVVSGDFEALAELVARCEDEGVRAKTLP-DYASISPCIERVREELLETTGDIAPRA RSVVVSGDFEALAELVARCEDEGVRAKTLP-DYASISPCIERVREELLETTGDIAPRA RSVVVSGDFEALAELVARCEDEGVRAKTLP-VDVASISPCUPERVEELLETGDIAPRA RSVVVSGDFEALAELVARCEDEGVRAKTLP-VDVASISPCUPELREELGAVLQFRA RSVVVSGDFEALAELVARCEDEGVRAVN-VDVASISPCUPERVEELVALGAINAPRFC TSVVVSGDFAALEQUTTSIANQVFRAVK-VDVASISPCUPERVEELVALGAINAPRFC TSVVVSGDTAALDALHTACQEQCVRARKVS-VDVASISPCUPELREELAALGANIAPRFC TSVVSGDTAALDALHACQEQCVRARKVS-VDVASISPCUPELARALAEMLGGIRAQAP SSVVVSGDTDALDALHTACQEQCVRARKVS-VDVASISPCHVPELARALAEMLGGIRAQAP SSVVVSGDTDALDALHTACQEQCWRARKVS-VDVASISPCHVPELARALAEMLGGIRAQAP SSVVSGDTDALDALHTACQEQCWRARKVS-VDVASISPCHVPELARALAEMLGGIRAQAP SSVVSGDTDALDALHTACQEQCWRARKVS-VDVASISPCHVPELARALAEMLGGIRAQAP SSVVSGDEDVVVAATETARQCGRKTKRLV-VSINAFISPHMOGMLDAFREVASRLAYAPP TSVVSGDEDVVVAATETARQCGRKTKRLV-VSINAFISPHMOGMLDAFREVASRLAYAPP TSVVSGDEDVVVAATETLARQGRKTKRLV-VSINAFISPHMOGMLDAFREVASRLAYAPP SSVVSGDEDVVTAVAETLARQGRKTKRLV-VSINAFISPHMOGMLDAFREVASRLAYAPP SSVVAGDELAELVALLTGEGRHARPI PGVDTAGISPQVDALRAHLLEVLAPVSPRSA SGCAVAGDPALAELLARLTAEGIRARPI PGVDTAGISPQUALRAHLLEVLAPVSPRSA
<pre>trylaT_etHylmal TylAT_etHylmal NidAT5_etHylmal Methoxylmalonyl-Selective GdmAT2_methoxymal HbmAT2_methoxymal SorAT3_methoxymal FkbAT7_methoxymal Unusual Extender Unit SalAT1_chloromal FkbAT4_allylmal EryAT6_methylmal EryAT6_methylmal StlAT4_methylmal SorAT6_methylmal SorAT6_methylmal SorAT6_methylmal NysAT2_methylmal Malonyl-Selective AT E AveAT6_mal AveAT6_mal HbmAT6_mal Ethylmalonyl-Selective TylAT5_ethylmal NidAT5_ethylmal</pre>	AT DOMAINS USLEDAARIVALRSRAW-LILLAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRI-VTWGDRLWIAAIVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRI-VTWGDRLWIAAIVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRI-VTWGDRLWIAAIVNSP LTLTDAAKIVTLRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAIVNSP LSLDDAAKVVARSRLI-STLSGQGAMAAVERPPAALEYU-ARFGRRLWIAAINNSP LSLDDAAKVVARSRLI-STLSGQGAMAAVERPPAALEYU-ARFGRRLWIAAINNSP Selective AT Domains LSLEDAAQVVVLRSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA SSVVVSGDFEALAELVARCEDEGVRAKTLP-DVASHSPONEPLLPELTGDIAPRPA RSTVLSGPFAALEQVLTSLRQEVFCRVD-VDVASHSPOIERVKEELLETTGDIAPRPA RSTVLSGPFAALEQVLTSLRQEVFCRVD-VDVASHSPOIERVKEELLETGDIAPRPA RSTVLSGPFAALDALIDDQCWRIRKVA-VDVASHSPONDPLAKELLAALGAINPRC SSVVVSGDTDALDALHTACQEQGVRARKVS-VDVASHSPONDPLAKELLAALGAINPRA RSTVLSGFPANIDALIDSLTAAQVFARKVK-VDVASHSPONDPLAKELLAALGAINPRA RSTVLSGFPANIDALIDSLTAAQVFARKVK-VDVASHSPONDPLAKELLAALGAINPRC SVVVSGDTDALDALHTACQEQGVRARKVS-VDXASHSQMDAVDDELAAGLANIAPRC TSVVISGTPHTVQHITTLCQQQGIKKTKTLP-TNHAFHSPHTNPILNQLHQHTQTLTYHPP TSLVISGTPHTVQHITTLCQQQGIKKTKLP-TNHAFHSPHTNPILNQLHQHTQTLTYHPP TSVVSGDEDVYTAVAETLARQGRKTKRLV-VSHAFHSPHTNPILNQLHQHTQTLTYHPP TSVVSGDEDVYTAVAETLARQGRKTKRLV-VSHAFHSPHTNPILNQLHQHTQLTYHPP TSVVSGDEDVYTAVAETLARQGRKTKRLV-VSHAFHSPHTNPILNQLHQHTQLTYHPP TSVVSGDEDVYTAVAETLARQGRKTKRLV-VSHAFHSPHTNPILNQLHQHTQLTYHPP TSVVSGDEDVYTAVAETLARQGRKTKRLV-VSHAFHSPHTNPILNQLHQHTQLTYHPP TSVVSGDEDVYTAVAETLARQGRKTKRLV-VSHAFHSPHTNPILNQLHARAAGITYHEP SGCAVAGOPEALAELVALLTGEGINARPI GSVDTAGHSPQVDALREHLEVLAPAPAPAPA
<pre>EtyIntS_etyInalonyI-Selective TyIATS_ethyInal NidATS_ethyInal NidATS_ethyInal MethoxyInalonyI-Select GdmAT2_methoxymal SorAT3_methoxymal SorAT3_methoxymal FkbAT4_allyInal FkbAT4_allyInal FkbAT4_allyInal FkbAT4_allyInal FkbAT4_methyInal SorAT8_methyInal SorAT8_methyInal SorAT8_methyInal SorAT8_methyInal NifAT4_methyInal NifAT4_methyInal NifAT4_methyInal AveAT8_mal AveAT8_mal AveAT3_mal GdmAT6_mal BthyInalonyI-Selective TyIAT5_ethyInal MidAT5_ethyInal MethoxyInalonyI-Selective TyIAT5_ethyInal</pre>	<pre>Ar Domains LSLEDAARIVALRSRAW-LILLGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRI-VTWGDRLWIAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRI-VTWGDRLWIAAVNSP LTLTDAAKIVTLRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAINGP LTLTDAAKIVTLRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAANNSP LSLDDAAKVVARSSRLL-STLSGQGGAMAAVERPPAALEPYL-ARFGRRLSIAAINSP LSLRDAGALLTTRTRLM-DQLPSGGAMVTVLTSEEKARQVL-RPGCVEIAAVNGP Selective AT Domains LSLEDAAQVVVLRSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA SSVVVSGDPEALAELVARCEDEGVRAKTLP-DYXASHSPRVEELETTILADLDGISARRA RSVVVSGDPEALAELVARCEDEGVRAKTLP-DYXASHSPRVEELETTILADLDGISARRA RSVVUSGDPEALAELVARCEDEGVRAKTLP-DYXASHSPRVEELETTILADLDGISARRA RSVVUSGDPEALAELVARCEDEGVRAKTLP-DYXASHSPRVEELLETTGDIAPRRA RSVVUSGDPEALAELVARCEDEGVRAKTLP-VDVXSHSPQUPERKEUALGAIRPRRAR RSVVUSGDPEALAELVARCEDEGVRAKTLP-VDVXSHSPQUPELEELALGAIALGAIRPRARA RSVVUSGDPEALAELVARCEDEGVRAKTLP-VDVXSHSPQUPELEELALGAIALGAIRPRARA RSVVUSGDPEALAELVARCEDEGVRAKTLP-VDVXSHSPQUPELEELALGAIRAFRARA RSVVUSGDPEALAELVARCEDEGVRAKTLP-VDVXSHSPQUPELEELALGAIRAFRARA RSVVUSGDPEALAELVARCEDEGVRAKTV-VDVXSHSPQUPELEELALGAIRAFRARA RSVVUSGDPEALAELVARCEDEGVRAKTV-VDVXSHSPQUPELEELAUALGAIRPRARA RSVVUSGDPEALEDALDADDGVRIRRVA-VDVXSHSPQUPELEELAUALGAIRPRARA RSVVUSGDFDALAEGVCOQGIKTKTLP-TNHAFHSPHTNPILNOLHOHT0TLTYHPP TSUVISGTPHTVOHITTLCQQQGIKKTTLP-TNHAFHSPHTNPILNOLHOHT0TLTYHPP TSUVISGTPHTVOHITTLCQQQGIKKTTLP-TNHAFHSPHTNPILNOLHOHT0TLTYHPP TSVVVSGDEDVVTAVAETLARQCRRTKRV-VSHAFHSPHTNPILNOLHOHT0TLTYHPP TSVVVSGDEDVVTAVAETLARQCRTKTRVL-VSHAFHSPHTNPILNOLHOHT0TLTYHPP TSVVSGDEDVVTAVAETLARQCRTKRVL-VSHAFHSPHTNPILNOLHOHT0TLTYHPP TSVVSGDEDVVTAVAETLARQCRTKRVL-VSHAFHSPHTNPILNOLHOHT0TLTYHPP TSVVSGDEDVVTAVAETLARQCRTKRVL-VSHAFHSPHTNPILNOLHOHT0TLTYHPP TSVVSGDEDVVTAVAETLARGCRTKRVL-VSHAFHSPHTNPILNOLHOHT0TLTYHPP TSVVSGDEDVVTAVAETLARGCRTKRVL-VSHAFHSPHTNPILNOLHOHT0TLTYHPP TSVVSGDEDVVTAVAETLARGVTAGHSPQVDATKRILEVLFPVAPRAS SGCAVAGDPEALAELVALLTGECVHARPIPGVDTAGHSPQVDALRAHLLEVLAPVAPRAS SGCAVAGDPEALAELVALLTGECVHARPI</pre>
<pre>trylaT_cetylmal TylAT_cetylmal NidAT5_cetylmal NidAT5_cetylmal Methoxymalonyl-Select GdmAT2_methoxymal HbmAT2_methoxymal SorAT3_methoxymal TybAT7_methoxymal Unusual Extender Unit SalAT1_chloromal FkbAT4_allylmal FkbAT4_allylmal EryAT6_methylmal EryAT6_methylmal StiAT4_methylmal SorAT8_methylmal SorAT3_methylmal NysAT2_methylmal AveAT8_mal AveAT8_mal AveAT8_mal AveAT8_mal AveAT8_mal HbmAT6_mal Ethylmalonyl-Selective TylAT5_ethylmal NidAT5_ethylmal Methoxymal SorAT3_methoxymal SorAT3_methoxymal</pre>	A T DOMAINS USLEDBARIVALRSRAW-LILLGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAIVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAIVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAIVNSP LTLTDAAKIVTLRSQTIAHHLTGHGAMMSVLASPTWVQETL-APWHGHLWIAAIVNSP LSLDDAGKVVARSRLJ-STLSGQGGAMAAVERPPAALEYI-ARFGRRLWIAAIVNGP LSLDDAAKVVARSRLJ-STLSGQGGAMAAVERPPAALEYI-ARFGRRLWIAAINNSP Selective AT Domains LSLEDAAQVVVLRSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA eAT Domains SSVVVSGDFEALAELVARCEDEGVRAKTLP-DVASHSPQUEPLREELETGDIAPRPA RSVVVSGEPGALRAFSEDCAAGIKWRDID-VDVASHSPQUERVREELLETTGDIAPRPA RSVVLSGEPAALDEVLJALRGQEVCGRVD-VDVASHSPQUEPLREELEGLAVLOPRA RSVVSGEPAALDELJDLDQQUENRKVN-VDVASHSPQUEPLAELEKGLAVLOPRAP SSVVVSGDTDALDALHTACQEQGVRARVV-VDVASHSPQUELAAULAAIAINPTC TSVVIAGBEALDEALDALDDQQUKIKKV-VDVASHSGRHVEAVRDELARULAFVDPUPAP SSVVSGDTDALDALHTACQEQGKKKTLP-TNHAFHSPHTNPILNQLHQHTQTLTYHPP TSLVISGTPHTVQHITTLCQQQGIKKTEP-TNHAFHSPHTNPILNQLHQHTQTLTYHPP TSVVVSGDEDVYTAVAETLARQCRKTKRLV-VSHAFHSPHTNPILNQLHQHTQTLTYHPP TSVVVSGDEDVYTAVAETLARQCRKTKRLV-VSHAFHSPHTNPILNQLHQHTQTLTYHPP SVVVSGDEDVYTAVAETLARQCRKTKRLV-VSHAFHSPHTNPILNQLHQHTQTLTYHPP TSVVVSGDEDVYTAVAETLARQCRKTKRLV-VSHAFHSPHTNPILNQLHQHTQTLTYHPP TSVVVSGDEDVYTAVAETLARQCRKTKRLV-VSHAFHSPHTNPILNQLHQUTAGHSPVPAXAGLYAEPPA
<pre>trylaT_of+J=selective TylAT5_ethylmal NidAT5_ethylmal Methoxymalonyl-Select GdmAT2_methoxymal HbmAT2_methoxymal FkbAT7_methoxymal FkbAT7_methoxymal FkbAT4_allylmal EryAT6_methylmal EryAT6_methylmal EryAT6_methylmal SolAT8_methylmal SolAT8_methylmal SolAT8_methylmal SolAT8_methylmal Malonyl-Selective AT6AT8_methylmal Malonyl-Selective AT6AT8_methylmal Malonyl-Selective AT6AT8_mal AveAT8_mal AveAT3_mal BhmAT6_mal BhmAT6_mal Methoxylmalonyl-Selective GdmAT5_ethylmal Mat5_ethylmal Mat5_ethylmal Methoxylmalonyl-Selective GdmAT2_methoxymal BhmAT2_methoxymal SolAT3_methoxymal SolAT3_methoxymal</pre>	A T DOMAINS ISLEDBARIVALRSRAW-LILLGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAINSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAINSP LTLTDAAKIVTLRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAINGP LTLTDAAKIVTLRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAINGP LSLDDAAKVVARSRLJ-STLSGQGAMAAVERPPAALEPYL-ARFGRRLWIAAINSP LSLDDAAKVVARSRLJ-STLSGQGAMAAVERPPAALEPYL-ARFGRRLWIAAINSP LSLDDAAKVVARSRLJ-STLSGQGAMAAVERPPAALEPYL-ARFGRRLWIAAINSP LSLDDAAKVVARSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELGGGAMGSVALPAADVEADA-ARIDGVWVAGRNGA SOLVUSGEPEALAELVARCEDEGVRAKTLP-DYASHSRHVEEIRETILADLDGISARRA RSVVVSGEPEALAELVARCEDEGVRAKTLP-DYASHSPQIERVREELLETTGDIAPRPA RSVVVSGEPEALAELVARCEDEGVRAKTLP-DYASHSPQIERVREELLETTGDIAPRPA RSVVVSGEPEALSEVLAAINKAVENVPVVVVVVVVVVVVVVVVVVVVVVVVVVVVVVVVVV
<pre>trylaT_cetylmal TylAT_cetylmal NidAT5_cetylmal Methoxylmalonyl-Select GdmAT2_methoxymal HbmAT2_methoxymal FkbAT7_methoxymal FkbAT7_methoxymal FkbAT4_allylmal FkbAT4_allylmal FkbAT4_allylmal FkbAT4_methylmal FkbAT4_methylmal SorAT6_methylmal SorAT6_methylmal SorAT6_methylmal Maionyl-Selective AreAT6_mal MysAT2_methylmal Maionyl-Selective AreAT6_mal HbmAT6_mal FkbAT4_mal MysAT3_methylmal Mionyl-Selective TylAT5_ethylmal Mionyl-Selective TylAT5_ethylmal Mathoxylmalonyl-Selective TylAT5_methoxymal FkbAT7_methoxymal FkbAT7_methoxymal</pre>	<pre>AT DOMAINS LSLEDAARIVALRSRAW-LILLGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLEDBARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRI-VTWGGRLWIAAVNSP LSLEDBARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRI-VTWGGRLWIAAVNSP LSLEDBARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRI-VTWGGRLWIAAINOP LTLTDRAKIVTLRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAINOP LSLEDDAKVVARSSLI-STLSGQGAMAAVERPPAALEYI-ARFGRRLSIAAINSP LSLEDBARUVARSSLI-STLSGQGAMAAVERPPAALEYI-ARFGRRLSIAAINSP LSLEDBARUVARSSLI-STLSGQGAMAAVERPPAALEYI-ARFGRRLWIAAVNGP Selective AT Domains LSLEDBARUVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA SSVVVSGDPEALAELVARCEDEGVRAKTLP-DYASISSRHVEEIRETILADLDGISARRA RSVVVSGDPEALAELVARCEDEGVRAKTLP-DYASISSGNEPLIELEERGIAVLOPRA RSVVVSGDPEALAELVARCEDEGVRAKTLP-DYASISSGNEPLIELEERGIAVLOPRA RSVVVSGDPEALAELVARCEDEGVRAKTLP-DYASISSGNEPLIELEERGIAVLOPRA RSVVVSGDPEALAELVARCEDEGVRAKVLP-VDVASISSQUPERVEELLETTGDIAPRPA RSVVVSGDPEALAELVARCEDEGVRAVN-VDVASISSQUPERVEELLETTGDIAPRPA RSVVVSGDPEALAEUVARCEVCRVVD-VDVASISSQUPERVEELVALGAINPRPA RSVVVSGDPAALEQUTSIANQVPRAVN-VDVASISSQUPPERVEELAVLQPRA RSVVVSGDTDALDALHTACQEQCVRARVS-VDVASISSQUPPELEELRAGLAVLAPRPA RSVVVSGDTDALDALHTACQEQCVRARVS-VDVASISSQUPPELAELENGLAVLAPRPA SSVVVSGDTDALDALHTACQEQCVRARVS-VDVASISSQUPPELAELEVALGAINIAPRT SSVVSGDTDALDALHTACQEQCVRARVS-VDVASISSQUPPELAEVARDELARVLAPVDPRAP PSUJSGTPHTVQHITTLCQQQGIKKTKP-TNHAFHSPHTNPILNQLHQHTQTLTYHPP TSLVJSGTPHTVQHITTLCQQQGIKKTRLP-TNHAFHSPHTNPILNQLAGHTQLAVLPPAPARPA ASVVVSGDDVVXAVETLARQGRKTKRLV-VSHAFHSPHMOGMLDAFREVASALAYAPP TSVVVSGDEDVVXAVETLARQGRKTKRLV-VSHAFHSPHMOGMLDAFREVASALAYAPP TSVVVSGDEDVVXAVETLARQGRKTKRLV-VSHAFHSPHMOGMLDAFREVASALAYAPP TSVVVSGDEDVVXAVETLARQGRKTKRLV-VSHAFHSPHMOGMLDAFREVASALAYAPP TSVVSGDEDVVXAVETLARQGRKTKRLV-VSHAFHSPHMOGMLDAFREVASALAYAPP TSVVVSGDEDVVXAAVETLARQGRKTKRLV-VSHAFHSPHMOGMLDAFREVASALTYAPP SVSVSGDPDALAEFGVTLSKAVVRWQLPGVDFAGHSGVDTKDQLHAVLDQUATATFG ASVSVSGDPDALAEFGTTLSKAVVRWQLPGVDFAGHSGNVDTKDQLHRVLDQLHAVLDGTATAFS SATVSGEPDAINHLLAKLARGTFALKREVVDASKGGNDTKLDQLHNVLDGTATAFS SATVSGEPDAINHLAELLARGTAFACHVA</pre>
<pre>trylaT_6+tylmal TylAT_6+tylmal NidAT5_ethylmal Methoxymalonyl-Selective GdmAT2_methoxymal BhmAT2_methoxymal SorAT3_methoxymal FkbAT7_methoxymal Wnsual Extender Unit SalAT1_chloromal FkbAT4_allylmal EryAT6_methylmal StiAT4_methylmal EryAT6_methylmal SorAT6_methylmal SorAT6_methylmal SorAT6_methylmal NysAT2_methylmal Malonyl-Selective AT E AveAT6_mal AveAT3_mal GdmAT6_mal HbmAT6_mal Bthylmalonyl-Selective TylAT5_ethylmal NidAT5_ethylmal SorAT3_methoxymal Methoxymal_Selective TylAT5_ethylmal SorAT3_methoxymal SorAT3_methoxymal SorAT3_methoxymal SorAT3_methoxymal</pre>	AT DOMAINS LSLEDAARIVALRSRAW-LILLGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLEDBARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAINSP LSLEDBARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAINSP LTLTDAAKIVTLRSQTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAINSP LSLEDBARVVARSRLJ-STLSGQGAMAAVERPPAALEYL-ARFGRRLWIAAINSP LSLEDDARVVARSRLJ-STLSGQGAMAAVERPPAALEYL-ARFGRRLWIAAINSP LSLEDBARVVARSRLJ-STLSGQGAMAAVERPPAALEYL-ARFGRRLWIAAINSP LSLEDBARVVARSRLJ-STLSGQGAMAAVERPPAALEYL-ARFGRRLWIAANNGP LSLEDBARVVARSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA SVUVSGDFEALAELVARCEDEGVRAKTLP-DVASHSPONEPLLERTILADLDGISARRA RSVVVSGEPEALAELVARCEDEGVRAKTLP-DVASHSPONEPLLEERTILADLDGISARRA RSVVVSGEPEALAEVARCEDEGVRAKTLP-DVASHSPONEPLLEERTILADLDGISARRA RSVVVSGEPEALEQVLTSLRQEVFCRVD-VDVASHSPONEPLLPELERGLAVLQPRA RSTVLSGEPAALEGVLTSLRQEVFCRVD-VDVASHSPONEPLLPELERGLAVLQPRA RSTVLSGEPAALEGVLTSLRQEVGRVKV-VDVASHSPONEPLAELLENGLAVLQPRA RSTVLSGEPAALDELDDADDQGVRIRKVA-VDVASHSPONEPLAELLGGIRAQAP SSVVSGDTDALDALHTACQEQGVRARKVS-VDXASHSQMDAVQDELAACLANIAPRTC TSVVISGTPHTVQHITTLCQQQGIKKTKTLP-TNHAFHSPHTNPILNQLHQHTQTLTYHPP TSLVISGTPHTVQHITTLCQQQGIKKTKLP-TNHAFHSPHTNPILNQLHQHTQTLTYHPP TSLVISGTPHTVQHITTLCQQQGIKKTKLP-TNHAFHSPHTNPILNQLHQHTQTLTYHPP TSVVSGDEDVVTAVAETLARQGRKTKRLV-VSHAFHSPHTNPILNQLHQHTQLTLYHPP TSVVSGDEDVVTAVAETLARQGRKTKRLV-VSHAFHSPHTNPILNQLHQHTQLTYHPP TSVVSGDEDVVTAVAETLARQGRKTKRLV-VSHAFHSPHTNPILNQLHQHTQLTYHPP TSVVSGDEDVVTAVAETLARQGRKTKRLV-VSHAFHSPHTNPILNQLHQHTQLTYHPP TSVVSGDEDVVTAVAETLARQGRKTKRLV-VSHAFHSPHTNPILNQLHQLHQLFQARAXALTYPP AT DOMAINS GTAAVAGDVDALAELLAELTAEGIRAKPIPGVDTAGHSQVDALRAHLLEVLAPVAPRA ASVSSGDPDALAEFCVTLSKAKVYRWQLPGVDFAGHSGHVDTIKDQLHHVLDGVTASFG SAVSSGDPDALAEFCYTLSKAKVYRWQLPGVDFAGHSGHVDTIKDQLHAVLDUATTLTYHPF SSVSGDEDALEEFCVTLSKAKVYRWQLPGVDFAGHSGHVDTIKDQLHAVLDUATTLTYHPF SSVSSGDPDALAEFCHVLARLTGEVHSKYRWQLEGVDFAGHSGHVDTIKDQLHAVLDUATTLTYHPF SSVSSGDPDALAEFCHVLSKAKVYRWQLPGVDFAGHSGHVDTIKDQLHAVLDUATTLTYHPF SSVSSGDPDALAEFCHVLSKAKVYRWQLFGVDFAGHSGHVDTIKDQLHAVLDQTA
<pre>EtyIntS_etyInalonyI-Selective TyIATS_ethyImal NidATS_ethyImal MethoxymalonyI-Select GdmAT2_methoxymal BhmAT2_methoxymal SorAT3_methoxymal FkbAT7_methoxymal FkbAT4_allyImal FkbAT4_allyImal FkbAT4_allyImal FkbAT4_methyImal SorAT8_methyImal SorAT8_methyImal SorAT8_methyImal SorAT8_methyImal SorAT8_methyImal NysAT2_methyImal MalonyI-Selective AT II AveAt2_mal AveAT3_mal HomAT6_mal BthyImalonyI-Selective TyIAT5_ethyImal NidAT5_ethyImal NidAT5_ethyImal SorAT3_methoxymal SorAT3_methoxymal SorAT3_methoxymal SorAT3_methoxymal SorAT3_methoxymal SorAT3_methoxymal SorAT3_methoxymal</pre>	A DOMAINS ISTUALSSRAW-LILLGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LILLGKGGMAAVSLPEARLRERI-ERFGQRLWAAAVNSP LSLDDSARIVALRSRAW-LILGAGKGGMVAVPMPAEELRPRI-VTWGDRLWIAANNSP LSLDDARVVARSSRL-STLSGGGAMAAVERPPAALEPYL-ARFGRRLWIAANNSP LSLDDARVVARSSRLS-STLSGGGAMAAVERPPAALEPYL-ARFGRRLWIAANNSP LSLDDAARVVARSSLLS-TISGGGAMAAVERPPAALEPYL-ARFGRRLSIAINSP LSLDDAARVVARSSLLSTLSGGAMAAVERPPAALEPYL-ARFGRRLSIAINSP LSLDDAARVVARSSLLSTLSGGAMAAVERPPAALEPYL-ARFGRRLSIAINSP LSLDDAARVVARSSLFATLG-DJSGGAMVTVLTSEEKARQVL-RPGVWIAAVNGP SSLECTAAGLITTRTRLM-DQLSGGAMVTVLTSEEKARQVL-RPGVWIAAVNGP SSLEDDAARVVARSSLFADELVSKGAVSVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA CONTINUALSSULFADELVSKGAVSVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA SSVVVSGDPAALEUVTSLRRGEVECRVD-VDVASHSPUERELLALGAIRARAR STVLSGEPAALSEVLAALTKACVFWRQVV-VDVASHSVQUPERLELVALGAIRAPRA RSTVLSGEPAALSEVLAALTKACVFWRQVV-VDVASHSVQUPELAEVLALGAIRARAR RSTVJSGEPAALSEVLAALTKACVFWRQVV-VDVASHSVDVPLREEUVALGAIRAPRAR RSTVJSGEPAALSEVLAALTKACVFWRQVV-VDVASHSVDVPLREEUVALGAIRAPRAR RSTVJSGEPAALSEVLAALTKACVFWRQVV-VDVASHSVDVPLREEUVALGAIRAPRAR RSTVJSGEPAALSEVLAALTKACVFWRQVV-VDVASHSVDVPLREEUVALGAIRAPRAR RSTVJSGEPAALSEVLAALTAKOVFWRQVV-VDVASHSVDVPLREEUVALGAIRAPRAR RSTVJSGEPAALSEVLAALTAKOVFWRQVVVVVSGVDVARVELARVLARDARATVANANANANANANANANANANANANANANANANANANA
<pre>trylaT_6tylmalonyl-Selective TylAT5_ethylmal NidAT5_ethylmal Methoxylmalonyl-Selective GdmAT2_methoxymal HbmAT2_methoxymal FkbAT7_methoxymal FkbAT7_methoxymal FkbAT4_allylmal FkbAT4_allylmal FkbAT4_allylmal FkbAT4_methylmal SorAT8_methylmal SorAT8_methylmal SorAT8_methylmal SorAT8_methylmal NysAT2_methylmal AveAt2_mal AveAt2_mal AveAt2_mal AveAT8_mal BomAT6_mal Ethylmalonyl-Selective TylAT5_ethylmal BomAT2_methoxymal FkbAT7_methoxymal SorAT3_methoxymal FkbAT7_methoxymal</pre>	A DOMAINS USLEDAARIVALRSRAW-LILIAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDAARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRI-VTWGGRLWIAAVNSP LSLDDAARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRI-VTWGGRLWIAAVNSP LILDDARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRI-VTWGGRLWIAANNSP LSLDDAARVVARSRLJ-STLSGQGAMAAVERPPAALEYI-ARFGRRLWIAANNSP SELCTIVA AT DOMAINS LSLEDAAQVVVLRSQLFADELVGKGAVASVSLPAALEYI-ARFGRRLSIAAINSP LSLEDAAQVVVLRSQLFADELVGKGAVASVSLPAALEYI-ARFGRRLSIAAINSP LSLEDAAQVVVLRSQLFADELVGKGAVASVSLPAALEYA-ARFGGALVWAGRNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA SVVVSGDPEALAELVARCEDEGVRAKTLP-DVASISPOIERVREELLETTGDIAPRA RSVVVSGDPEALAELVARCEDEGVRAKTLP-DVASISPOIERVREELLETTGDIAPRA RSVVVSGDPEALAELVARCEDEGVRAKTLP-DVASISPOIERVREELLETTGDIAPRA RSVVSGDPEALAELVARCEDEGVRAKTLP-DVASISPOIERVREELLETTGDIAPRA RSVVSGDPEALAELVARCEDEGVRAKTLP-DVASISPOIERVREELLETTGDIAPRA RSVVSGDPEALAELVARCEDEGVRAKVLP-VDVASISPOIERVREELLETTGDIAPRA RSVVSGDPEALAELVARCEDEGVRAKVLP-VDVASISPOIERVREELLETTGDIAPRA RSVVSGDPEALAELVARCEDEGVRAKVLP-VDVASISPOIERVREELLAGALANIAPRA RSVVSGDPEALAELVARCEDEGVRAKVLP-VDVASISPOIPLEELEKELGAVLOPRA PSTULAGEPAALESVLALTAKGVERWROVV-VDVASISPOIPLEELEKELGAVLOPRA RSVVSGDPAALEQUTSLANGVECRVV-VDVASISPOIPLEELEKELGAVLOPRA RSVVSGDPAALEQUTSLANGVCCVVDVVDYDASISPOIPLAERELVAALGAINIAPRT TSVVISGDFHALDALHTACQEQGVRAKVS-VDYASISPANDALAENLGGIAAQDP SVVVSGDTDALDALHTACQEQGVRAKVS-VDYASISPANPINPLINQLHOHTOTLTYHPP TSLVISGTPHTVOHITTLCQQQGIKKKTLP-TNHAFISPHTNPLINQLHOHTOTLTYHPP TSLVISGTPHTVOHITTLCQQQGIKKKTLP-TNHAFISPHTNPLINQLHOHTOTLTYHPP TSVVSGDEDVYTAVAETLARQGRKTKRLV-VSHAFISPHMOGMLDAFREVASRLIXAPPT SVVVSGDEDVYTAVAETLARQGRKTKRLV-VSHAFISPHMOGMLDAFREVASRLIXAPPT SVVVSGDEDVYTAVAETLARQGRKTKRLV-VSHAFISPHMOGMLDAFREVASRLIXAPPT SVVVSGDEDVYTAVAETLARQGRKTKRLV-VSHAFISPHMOGMLDAFREVASRLIXAPPT SVVVSGDEDVYTAVAETLARQGRKTKRLV-VSHAFISPHMOGMLDAFREVASRLIXAPPT SVVVSGDEDVYTAVAETLARQGRKTKRLV-VSHAFISPHMOGMLDAFREVASRLIXAPPT SVVVSGDEDVYTAVAETLARQGRKTKRLV-VSHAFISPHMOGMLDAFREVASRLIXAPPT SVVVSGDEDVYTAVAETLARQGRKTKRLV-VSHAFISPHMOGMLDAFREVASRLIYAPPT SVVVSGDEDVYTAVAETLARQGRKTKRLV-VSHAFISPHMOGMLDAFREVA
<pre>trylaT_etHylmal TylAT_etHylmal NidAT5_etHylmal Methoxylmalonyl-Selective GdmAT2_methoxymal HbmAT2_methoxymal SorAT3_methoxymal FkbAT7_methoxymal FkbAT4_allylmal FkbAT4_allylmal EryAT6_methylmal SorAT6_methylmal SorAT6_methylmal SorAT6_methylmal SorAT6_methylmal SorAT6_methylmal Malonyl-Selective AT ff AveAT6_mal AveAT6_mal HbmAT2_methylmal Ethylmalonyl-Selective TylAT5_ethylmal NysAT3_mal GdmAT6_mal HbmAT6_mal HbmAT2_methoxymal HbmAT2_methoxymal SorAT3_methoxymal FkbAT7_methoxymal FkbAT7_methoxymal FkbAT7_methoxymal FkbAT4_allylmal</pre>	AT DOMAINS SLEDDARIVALRSRAW-LILLGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAINSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAINSP LITLTDAKIVTLRSQTIAHHLTGHGAMMSVLASPTWVQEIL-APFMGHLWIAAINSP LSLDDAKVVARSRLJ-STLSGQGAMAAVERPPAALEYL-ARFGRRLWIAANNGP LSLDDAAKVVARSRLJ-STLSGQGAMAAVERPPAALEYL-ARFGRRLWIAANNGP SSLDCIVA AT DOMAINS LSLEDAAQVVVLRSQLFADELVGKGAVASVSLPAALEYL-ARFGRGALWIAANNGP LSLEDAAQVVVLRSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA SVVVSGDFEALAELVARCEDEGVRAKTLP-DVASHSPOUEPLUPELTGDIAPRPA RSVVLSGDFEALEQVLTSLRQGVFCRVD-VDVASHSPOUEPLUPELERGLAVLQPRA RSVVUSGDFEALAELVARCEDEGVRAKTLP-DVASHSPOUPPLREELVALGAINPRA RSVVUSGDFALAEUVALRSALLDRELOGVRAKTLP-DVASHSPOUPPLREELVALGAINPRA RSVVUSGDFALAEUVALRSALLDQCVFCRVD-VDVASHSPOUEPLLPELERGLAVLQPRA RSVVUSGDFALEQVLTSLRQGVFCRVD-VDVASHSPOUPPLREELVAALGAINPRA RSVUSGDPAALDALLDDQCWRIRWA-VDVASHSPOUPPLREELVAALGAINPRA RSVUSGDFHAIDALDDQCWRIRWA-VDVASHSPOUPPLREELVAALGAINPRA RSVUSGDFHAIDALLDGLTAAQVFRKVK-VDVASHSQNDAVDDELAALANIAPRTC TSVVISGTPHTVQHITTLCQQQGIKTKTLP-TNHAFHSPHTNPILNQLHQHTQTLTYHPP TSLVISGTPHTVQHITTLCQQQGIKTKTLP-TNHAFHSPHTNPILNQLHQHTQTLTYHPP TSLVISGTPHTVQHITTLCQQQGIKTKTLP-TNHAFHSPHTNPILNQLHQHTQTLTYHPP TSVVVSGDEDVVTAVAETLARQGRKTKRLV-VSHAFHSPHMDGMLDAFREVASRLAALFYPP AT DOMAINS GTAAVAGOVDALEELLAELTAEQIGRKTKRLV-VSHAFHSPHMDGMLDAFREVASRLAALFYPP AT DOMAINS GTAAVAGOVDALEELLARLTAEQIGRKTKRLV-VSHAFHSPHMDGMLDAFREVASRLAYAPP TSVVVSGDEDVVTAVAETLARQGRKTKRLV-VSHAFHSPHMDGMLDAFREVASRLAYAPP AT DOMAINS GTAAVAGOVDALEELLARLTAEQIFAKRPI PGVDTAGHSQVDALRAHLLEVLAPVAPRPA ASVSSGDPDALAEFGVTLSKAKVYRWQLEGVDFAGHSGHVDTIKDQLHHVLDQVTASFG ASVSSGDPDALAEFGVTLSKAKVYRWQLEGVDFAGHSGHVDTIKDQLHVLDUARTLTYHQP SELCTIVG AT DOMAINS RSVVAGQVAALEELVALLREGERVAKVRGLEGVDFAGHSGHVDTIKDQLHAVLDUARTLTYHQP SUTVAGQVAALEELVAELAEAGUV-CHI-RHRD-TRHAGHSERMPLLVARVLDVARTLTYHQP SUTVAGDVALAEFGVTLSKAKVYRWQLEGVDFAGHSGHVDTIKDQLHAVLDUARTLTYHQP SUTVAGDVAALEELVAELAEGGRAKVSGOVRAGHSGRVDTIKDQLHAVLDQHAR
<pre>trylaT_cetylmal TylAT_cetylmal NidAT5_cetylmal Methoxylmalonyl-Selective GdmAT2_methoxymal HbmAT2_methoxymal FkbAT7_methoxymal FkbAT7_methoxymal FkbAT4_allylmal FkbAT4_allylmal FkbAT4_methylmal FkbAT4_methylmal FkbAT4_methylmal RifAT4_methylmal RifAT4_methylmal RifAT4_methylmal NysAT2_methylmal Malonyl-Selective AT I AveAT8_mal AveAT8_mal AveAT8_mal HbmAT6_mal Ethylmalonyl-Selective TylAT5_cethylmal MidAT5_methylmal MidAT5_methylmal MidAT5_methylmal MidAT5_methylmal MidAT5_methylmal MidAT5_methylmal MidAT5_methylmal MidAT5_methylmal MidAT5_methoxymal FkbAT4_allylmal</pre>	<pre>N* Domains LSLEDAARIVALRSRAW-LILLGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDAARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRI-VTWGDRLWIAAVNSP LSLDDAARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRI-VTWGDRLWIAAVNSP LSLDDAARVVARSRLI-STLSGQQAMAAVERPPAALEPYI-ARFGRRLWIAAVNSP LSLDDAAKVVARSGTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAVNSP LSLDDAAKVVARSGTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAVNSP LSLDDAAKVVARSGTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAVNSP LSLDDAAKVVARSGTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAVNSP LSLDDAAKVVARSGTIAHHLTGHGAMMSVLAPHTWVQEAL-APWHGHLWIAAVNSP LSLDDAAKVVARSGTIAHLTGHGAMMSVLAPHTWQEAL-APHGHLWIAAVNSP SSLDDAAKVVARSGTIAHLTGHGAMSVLAPHTWQEAL-APHGHLWIAAVNSP LSLDDAAKVVARSGTIATKELV-DQLPEGGAWVTVLTSEEKARQVL-RPCVWVAGRNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA TLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA SVVVSGDPEALAELVARCEDEGVRAKTLP-DYASISPQUERVREELLETTGDIAPRA RSVVVSGDFAALSQLTARGVECRVPU-VVXSISPQUPELREELTGDIAPRA RSVVVSGDFAALSQLTARGVECRVPU-VVXSISPQUPELREELGALVLAPRA RSVVVSGDFAALSQLTARQVEYRWRVX-VDXASISPQUPELREELGALVLAPRA RSVVVSGDTAALDALHTACQEQVKARKVS-VDXASISPQUPELREELGALVLAPRA RSVVSGDTAALDALHTACQEQVKARKVS-VDXASISPQUPELREELAALGANIAPRTC TSVVIGGTAALDALHTACQEQUKRRKVS-VDXASISPQUPELREELAALGANIAPRTC TSVVISGDTAALDALHTACQEQUKRKTLP-TNHAFHSPHTNPILNQLHOHTQTLTYHPP TSVVVSGDEDVVTAVAETLARQCRKTKRLV-VSHAFHSPHMDFMLALAPKAVAAGLAPAPDPRAPA NOMINS CTANAGUVDALKALLAELTAEGIRAKPI PGVDTAGHSPUDDELREVAALGAPTAPAPAPA SXVVSGDEDVVTAVAETLARQCRKTKRLV-VSHAFHSPHMDFMLADLAPREVAASLAYAPP TSVVVSGDEDVVTAVAETLARQCRKTKRLV-VSHAFHSPHMDFMLDALDAFREVAASLAYAPP TSVVVSGDEDVVTAVAETLARQCRKTKRLV-VSHAFHSPHMDFMLADLAPREVAASLAYAPP TSVVVSGDEDVVTAVAETLARQCRKTKRLV-VSHAFHSPHMDFMLDQLHOTLTYHPP TSVVVSGDEDVVTAVAETLARQCRKTKRLV-VSHAFHSPHMDFMLDALDAFREVAASLAYAPP TSVVVSGDEDVVTAVAETLARGCRKKRLV-VSHAFHSPHMDFMLDALDAFREVAASLAYAPP TSVVVAGDEEAVLALTGEGVHARPI PGVDTAGHSPUVDALLEVLAPVAPRPA SVSVSDDEDVVTAVAETLARGCRKKRLV-VSHAFHSPHMDFMLDALDAFREVAASLAYAPP TSVVAGDEBAVLEALELAELTAEGIRAKVIG-STVASHCAQVDGLKEHLEVLAPVAPRPA SVSVSGDEDVVTAVAETLARAGTAFKKRLV-VSHAFHSPHMDFMLDA</pre>
<pre>trylaT_cetylmal TylAT_cetylmal NidAT5_cetylmal NidAT5_cetylmal Methoxymalonyl-Select GdmAT2_methoxymal FkbAT7_methoxymal FkbAT7_methoxymal FkbAT4_allylmal FkbAT4_allylmal FkbAT4_methylmal EryAT6_methylmal StiAT4_methylmal StiAT4_methylmal StiAT4_methylmal StiAT4_methylmal StiAT4_methylmal NysAT2_methylmal Malonyl-Selective AT F AveAT5_mal AveAT5_mal AveAT5_mal HbmAT6_mal Ethylmalonyl-Selective TylAT5_cethylmal NidAT5_cethylmal SorAT3_methoxymal SorAT3_methoxymal SorAT3_methoxymal SorAT3_methoxymal FkbAT7_methoxymal SorAT3_methoxymal FkbAT4_allylmal</pre>	AT DOMAINS USLEDAARIVALRSRAW-LILIAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAINSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAINSP LSLDDSARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRL-VTWGDRLWIAAINSP LTTTNAKIVTLRSQTIAHHLTGHGAMMSVLASPTWVQEIL-APWHGHLWIAAINSP LSLDDAKVVARSSLL-STLSGQGAMAAVERPPAALEYL-ARFGRRLWIAAINSP LSLDDAKVVARSSLL-STLSGQGAMAAVERPPAALEYL-ARFGRRLWIAAINSP LSLDDAKVVARSSLLSTLSGQGAMAAVERPPAALEYL-ARFGRRLWIAAINSP LSLDDAAKVVARSSLLSTLSGQGAMAAVERPPAALEYL-ARFGRRLWIAAINSP LSLDDAAKVVARSSLLSTLSGQFGAWAVSLSEARAGVL-RPGVEIAAVNGP Selective AT Domains LSLEDAAQVVVLRSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA ************************************
<pre>trylaT_etHylmal TylAT_etHylmal NidAT5_etHylmal Methoxylmalonyl-Select GdmAT2_methoxymal BhmAT2_methoxymal SorAT3_methoxymal FkbAT7_methoxymal FkbAT4_allylmal FkbAT4_allylmal EryAT6_methylmal EryAT6_methylmal EryAT6_methylmal SorAT8_methylmal SorAT8_methylmal SorAT8_methylmal SorAT8_methylmal Malonyl-Selective AT I AveAT8_mal AveAT5_mal MysAT3_methylmal Bthylmalonyl-Selective TylAT5_ethylmal NysAT3_mal GdmAT6_mal HbmAT6_mal HbmAT6_mal SorAT3_methoxymal SorAT3_methoxymal SorAT3_methoxymal FkbAT4_allylmal FkbAT4_allylmal Fig. 7. Sequence alige</pre>	AT DOMAINS LSLEDAARIVALRSRAW-LILLAGKGGMAAVSLPEARLRERI-ERFGQRLSVAAVNSP LSLEDBARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRI-VTWGDRLWIAAINSP LSLEDBARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRI-VTWGDRLWIAAINSP LSLEDBARIVALRSRAW-LGLAGKGGMVAVPMPAEELRPRI-VTWGDRLWIAAINSP LTTNAAKIVTLRSQTIAHHLTGHGAMMSVLASPTWVQETL-APWHGHLWIAAINGP LSLEDDAAKVVARSSLI-STLSGQGAMAAVERPPAALEYI-ARFGRRLSIAAINSP LSLEDDAAKVVARSSLI-STLSGQGAMAVERPPAALEYI-ARFGRRLSIAAINSP LSLEDDAAKVVARSSLI-STLSGQGAMAVERPPAALEYI-ARFGRRLSIAAINSP LSLEDDAAKVVARSSLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LSLEDDAAVVVLRSQLFADELVGKGAVASVSLPAAEVEARI-ARFNGDAEVLSIAGNNGP LTLEDGARLVALRSALLLRELAGRGAMGSVALPAADVEADA-ARIDGVWVAGRNGA SVVVSGEPGALRSPECAAEGIRVRDID-VDYASHSPQIERVREELLETTGDIAPRPA RSTVLAGEPRALSEVLAAINKACEDEGVRAKTLP-DYASHSPQIERVREELLETTGDIAPRPA RSTVLGGPRALSEVLAINTAKGVEWRQVV-VDVASHSVQDPLAELAVALGAIRPRAA RSTVLAGEPRALSEVLAINTAKGVEWRQVV-VDVASHSVQDPLAELAUALGAIRPRAA RSTVLGGPAALEQUITSLARQEVECRVD-VDVASHSVQDPLAELAUALGAIRPRAA RSTVLGGPAALEQUITSLARQEVECRVD-VDVASHSVQDPLAELAUALGAIRPRAA RSTVLGGPAALEQUITSLARQEVECRVD-VDVASHSVQDPLAELAUALGAIRPRAA RSTVLGGPAALEQUITSLARQEVECRVD-VDVASHSVQDPLAELAUALGAIRPRAA RSTVLGGPAALEQUITSLARQEVECRVD-VDVASHSVDDPLAELAUALGAIRPRAA RSTVLGGPAALEQUITSLARQEVECRVD-VDVASHSVDDPLAELAUALGAIRPRAA RSTVLGGDAALEAUALEDGQVRARKVS-VDXASHSVDPLAELAVLAGAIRPRPA PSVVVSGDDDAALBALTACQEQGVRARKVS-VDXASHGRHVEAVRDELARVLAGITYHPP TSLVISGTPHTVQHITTLCQQQGIKTKTLP-TNHAFHSPHTNPILNQLHQHTQTLTYHPP TSLVISGTPHTVQHITTLCQQQGIKTKTLP-TNHAFHSPHTNPILNQLHQHTQLTYHPP TSVVSGDEDVVTAVAETLARQGRKKRLV-VSHAFHSPHMOGMLDAFREVASALAYAPP TSVVVSGDEDVVTAVAETLARQGRKKRLV-VSHAFHSPHMDGMLDAFREVASALAYAPP TSVVSGDEDVVTAVAETLARQGRKKRLV-VSHAFHSPHMDGMLDAFREVASALAYAPP TSVVSGDEDVVTAVAETLARQGRKKRLV-VSHAFHSPHMDGMLDAFREVASALAYAPP SSVVSGDEDALAEFGVTLSKAKVYRWQLEGVPARGSGHVDTIKDQLHNVLDGVTASPG ASVSSGDPDALAEFGVTLSKAKVYRWQLEGVPARGSGHVDTIKDQLHNVLDGVTASPG SSCAVAGDPEALAELVALLTGEGVARVIG-STVASHCAQVDPLHERILDLSFVQPREG SITVYSGPDALAEFGVTLSKAKVYRWQLEGVPARGSGRVDTIKDQLHVLDUARTITYHPP SSLUSGDEAVLEAARQLGIH-HRLP-TRHAGSERWQLVERLLELREIPESPS HSLVGSODAULAELLVALLAE

for methylmalonyl-CoA, HAFH for malonyl-CoA, TAGH for ethylmalonyl-CoA, and less conserved motifs for rarer extender units as well as the diagnostic Q versus I/L motif indicating methylmalonyl versus malonyl-CoA are highlighted in cyan. The active site histidine/serine dyad is highlighted in yellow. Mutations introduced by Schulz and coworkers in EryAT6 are indicated in magenta [76,77,115], those introduced by Williams and coworkers [72] are indicated in green, and those explored by both the Williams and Schulz groups are indicated in grey. The alignment was performed with MUSCLE.



Fig. 8. Snapshots of molecular dynamics simulations of the wild type EryAT6 (rose) and EryAT6 V295A (ochre). A) Depiction of an alanine mutation which allows for greater flexibility of the binding pocket compared to valine. B) Allylmalonyl-SNAC placed in A295A EryAT6 which can be accommodated by the increased binding pocket of the alanine mutation. C) Propargylmalonyl-SNAC does not readily fit into the active site of wild type EryAT6. D) Propargylmalonyl-SNAC fits better in the V295A mutant than the wild type. E) V295A with isopropylmalonyl-SNAC. F) Hexanoylmalonyl-SNAC. Reproduced with permission from ref. [76].

cinnamonensis malonyl-CoA ligase (MatB) enzyme which ligates a range of malonate derivatives to CoA to then be used as extender units by AT domains. Such substrates include propanenitrile, phenyl, and thiophene substituted malonyl-CoAs [78]. MatB enzymes have been extensively engineered for enhanced AT promiscuity toward a range of malonate derivatives by the group of Gavin Williams [79,80].

Uniquely among PKS domains, mutations to the AT domain have the most potential for metabolic engineering to alter metabolite scaffolds. Both in engineered and natural contexts, AT promiscuity is common and metabolite structure is dictated by precursor availability. One common biosynthetic pathway for uncommon malonyl-CoA derivatives is through a crotonyl-CoA carboxylase/reductase (CCR) enzyme reviewed by Moore [81,82]. An example of successful metabolic engineering of an extender unit was performed using the salinosporamide PKS. In this study, a strategy to engineer fluoromethylmalonyl-CoA production was implemented in *Salinospora tropica*. SalAT1 was able to accept this unnatural substrate and an analog of salinosporamide A, flourosalinosporamide, was successfully generated by replacing the chlorine atom with fluorine [83]. An example of utilizing engineered

MatB *in vivo* to supply unnatural precursors was demonstrated by the groups of Weber, Lee, and Williams to generate "bioderivativized" kirromycin analogs [84]. Although *trans*-AT domains typically utilize malonyl-CoA, KirCII is a *trans*-AT that natively accepts ethylmalonyl-CoA and has promiscuity towards a broad range of other analogs including azidomalonyl-CoA, allylmalonyl-CoA, propargylmalonyl-CoA, and others [80,84,85]. Thus, manipulation of the available precursor flux of extender units in concert with targeted changes to the AT domain ultimately determines the final metabolite structure [72,84,85].

Methyltransferase

Mutations to abolish activity: The MT is the most recently characterized domain harbored within modular bacterial PKSs and is somewhat uncommon in cis-AT PKSs, although common in trans-AT PKSs [86–90]. The MT introduces methyl branches to the α -carbon via S-adensoyl-methionine (SAM) as opposed to methylmalonyl-CoA. Notably, iterative fungal type I PKSs exclusively use MT domains to introduce methyl branching, however bacterial modular PKSs have evolved two distinct means of doing so. Both monomethylating MT domains and gem-dimethylating MT domains exist (Fig. 6A and B). The recent structural characterization of a monomethylating cis-AT PKS from the curacin biosynthetic pathway, CurMT3, has lent greater insight into the active site environment [86]. To understand gem-dimethylation, an MT from the disorazole PKS, DisMT3, was recently crystalized [87]. An invariant glutamine/histidine catalytic dyad is present in all MTs both from bacterial and fungal PKS pathways, and it is hypothesized that the imidazole of the histidine residue may act as a catalytic base to deprotonate the substrate α -carbon. Consistent with this hypothesis, alanine mutations to the active site histidine/glutamine dyad abolished activity in CurMT3. The melting temperatures of these mutants were comparable to the wild type, suggesting that the elimination of activity was not due to compromised stability of the enzyme, but rather the removal of active site residues [86]. Likewise, analogous mutations were made in DisMT3 to H288 (H288N and H288A) which resulted in inactivation of the MT. Thus to inactivate the MT, the conserved active site histidine appears to be a reliable route (Fig. 6C) [87].

Mutations to alter activity: In CurMT3, other mutations to conserved residues in the active site including Y169F and N277A decreased activity, suggesting that they play a role in substrate positioning (Fig. 6C). Although chemoenzymatic methods to create analogs of SAM have been developed [91], no research has been put forth involving the possible transfer of other unnatural analogs with varying sidechains in bacterial PKSs. However, such an approach has been demonstrated to allow for the incorporation of a propargyl moiety through a propargyl SAM analog in a fungal PKS. Thus, tuning the kinetics to incorporate unnatural SAM analogs may be a highly valuable future area of exploration to increase molecular diversity [92].

To determine the residues that impact catalysis for *gem*-dimethylating MTs, mutations were made to Y170F, E341A, N285A, and E341Q. None of these mutants substantially affected the rate of *gem*dimethylation. Other potentially relevant residues forming a hydrophobic tunnel were suggested including Y132, M166, L169, and Y170, although mutations to these residues were not performed [93]. For *gem*dimethylating MTs, an intriguing application would be the generation of quaternary carbons possessing different functional groups, for example, methylation to a domain extended with ethylmalonyl-CoA. Only one example of such a substitution through domain swapping has been explored by Abe and coworkers, and the experiment was unsuccessful [94]. In the future, targeting mutations that expand the hydrophobic tunnel for the accommodation of bulkier groups may be highly desirable to alter metabolite conformation [95].



Fig. 9. Example of strategic inactivation of KR residues to generate keto moieties. A) LipPKS, composed of the Lip1 gene (LM and Mod1) fused to EryTE. B) Inactivation of the KR to strategically generate short chain ketones [97].

Ketoreductase

Mutations to abolish activity: The active site of the KR is comprised of three residues including a serine, tyrosine (sometimes replaced by histidine), and asparagine [21]. Alteration of either the active site serine (typically to an alanine) or tyrosine (typically to a phenylalanine) can abolish activity [96]. One example of this strategy was used by Keasling and coworkers to manipulate the lipomycin PKS. The inactivated KR yielded a terminal β -keto acid which was readily converted to the corresponding ketone upon thermal decarboxylation. This method was utilized for the production of small branched chain ketones that could be used as gasoline additives (Fig. 9) [47,97].

KR domains are categorized twofold: first by the stereoselectivity conferred to the hydroxy group during reduction (A-type KRs generate L-configured hydroxy groups whereas B-type KRs generate D-configured hydroxy groups) and second by whether they are non-epimerizing or epimerizing (denoted by a 1 for non-epimerizing and a 2 for epimerizing). Additionally, reductase-incompetent KRs are termed C-type KRs. These are common in PKSs and lack the conserved active site asparagine, however some retain epimerizing function (Fig. 9) [26]. Epimerizing C2-type KRs harbor the catalytic tyrosine and serine, and it was determined that these residues are indeed responsible for catalyzing epimerization [96]. Examples of C2-type KRs are present in several common macrolide scaffolds including erythromycin, picromycin, tylosin, niddamycin, and meglomyicin [26]. Entirely inactive KRs (classified as C1-type) lacking both the active site serine and tyrosine are far rarer, but are occasionally found, such as oligiomycin KR4 [27].

Mutations to alter activity: Mutations to alter the stereoselectivity of the KR domain have been extensively studied, particularly by the groups of Leadlay and Keatinge-Clay (reviewed by Keatinge-Clay [98]). During biosynthesis, when α substitution is present, the AT domain typically only selects 2*S* configured units (e.g. 2*S*-methylmalonyl-CA). The condensation reaction catalyzed by the KS domain inverts the configuration, resulting in 2*R* stereochemistry (Fig. 9). While the exact mechanistic path for epimerization is unknown, one potential hypothesis is that differences in conformation allow varying access to water in the active site [99]. Congruent with that hypothesis, non-epimerizing KRs can be converted to epimerizing KRs with only a single point mutation, which need not be an active site base [22,100]. Diagnostic fingerprint motifs have been identified for all KR types (Fig. 10) [27,101,102].

The KR domain is one of the most thoroughly studied PKS domains. Biochemically, it is extremely stable when excised as a standalone domain and numerous studies have been performed on KRs with model substrates such as N-acetyl cysteamine (SNAC)-linked β -keto thioesters (Fig. 13). The residue numbering of standalone KRs (especially EryKR1) vary from report to report. The KR domain is composed of two Rossman folds: a catalytic subdomain and a structural subdomain that does not bind NADPH but is nonetheless part of the complete structure [24,98]. In some reports, only the catalytic subdomain is numbered, while in others the entire intact KR domain is numbered, and sometimes the gene numbering (e.g. EryKR1's position in DEBS1) is used. This inconsistency can cause confusion while comparing and analyzing mutations from different studies. For the sake of clarity, we refer the readers to Fig. 12 where we have used the standalone domain numbering for EryKR1 (including both catalytic and structural subdomains) to display the active site.

Seminal early investigations using excised KRs were performed by Leadlay and coworkers who examined EryKR1 and EryKR2. These were cloned as standalone domains and assayed for the reduction of truncated β -ketoacyl SNAC substrate mimics (Fig. 13) [103–105]. First, Leadlay and coworkers implemented a rationally designed directed mutagenesis approach based on apparent sequence differences between different KR types [104]. These motifs targeted two regions of the KR domain, first, the LDD motif diagnostic of B-type KRs, and second, the residues in a loop containing conserved tryptophan diagnostic of A type KRs. In EryKR1 a phenylalanine is present in this position (Fig. 11). At the time, only homology models were available for designing residue exchanges, however these predicted a reasonable structure for the Rossman fold and remain essentially in agreement with more recent structural characterization by X-ray crystallography [22-24,26,27,98,106,107]. In EryKR1, phenylalanine, proline, and glycine residues were determined to be part of a loop directly adjacent to the catalytic tyrosine, whereas the LDD motif was present in a loop adjacent to the active site. In EryKR2, the residues corresponding to the LDD motif were PQS. Therefore, up to six residues were mutated including the residues corresponding to the LDD motif (EryKR1 PQS and EryKR2 LDD) along with residues corresponding to tryptophan, proline, and glycine. For this motif, in EryKR1 and EryKR2, WGG and LPN triple mutants were generated, respectively. Finally, all six mutations were implemented (Fig. 12). All mutants probed in this study had comparable activity to the wild type on an SNAC β -keto thioester substrate, however a complete reversal of stereoselectivity to form the epimeric A2 product was observed with the EryKR1 WGG triple mutant. The EryKR2 PQS mutant also resulted in mixed stereoselectivity, with a close to equal mixture of the native A1 and epimeric A2 products. Upon the introduction of all six mutations, however, complete reversal to A2



Fig. 10. Schematic of chemical fates of the elongating polyketide by β-carbon processing domains.

stereoselectivity occurred in EryKR2 [104]. A complementary experiment involved a saturation mutagenesis approach on these motifs [105]. The libraries were screened against decalone, which had been shown previously to be an effective model substrate for screening activity [103,108,109]. Selected mutants with high activity towards decalone were then assayed with a β -keto thioester model substrate. Like the directed mutagenesis study, a handful of mutations affected both the B2-type EryKR1 and the A1-type EryKR2 through alterations in stereoselectivity, typically requiring at least 3 mutations for a complete reversal, but activity was reduced compared to wild type. Most frequently, the A2 isomer was observed, although for some mutations, trace amounts of other stereochemical alterations were also observed [105].

Keatinge-Clay and coworkers demonstrated that individual point mutations could affect the stereoselectivity of the second KR from the amphotericin PKS, AmpKR2, an A1-type [22,25]. Intriguingly, two point mutations, Q364H and G355T, entirely resulted in the epimeric A2 product as well as a fourfold increase in catalytic activity [22]. Additionally, a single mutation from a glutamine to a histidine three residues away from the catalytic tyrosine changed the behavior of AmpKR2 from an A1-type to a nonspecific A-type KR [25]. Keatinge-Clay and coworkers later discovered that extremely truncated mimics of the phosphopantetheinyl arm, including ethanethiol thioesters could retain native stereoselectivity in a panel of representative KRs, which was somewhat corroborated by studies of TylKR1 by Lüdeke and coworkers [110,111]. With the goal of understanding why such poor mimics of the phosphopantethienyl arm retained near natural stereocontrol, Keatinge-Clay and coworkers examined motifs correlated with controlling α stereochemistry. They also sought to determine if subtle non-covalent interactions in the active site would dictate stereochemical outcome. To this end, a residue that correlates with α stereochemical outcome was identified three residues away from the catalytic tyrosine, typically leucine in B2-type, glutamine in A1-and B1type, and histidine in A2-type KRs (Fig. 11). In EryKR1, alteration of leucine to glutamine or histidine only resulted in a catalytically deficient enzyme. However, a leucine to alanine mutation in EryKR1

resulted in formation of the epimeric A2 product with increased catalytic activity [100]. Indeed, when a analogous mutations were made to the other KR types including TylKR1, AmpKR2, and RifKR7, a similar switch of selectivity to form the A2 product was observed in all but RifKR7, which is naturally an A2-type KR. From this, Keatinge-Clay and coworkers rationalized that the observed anti selectivity follows Felkin-Anh stereoselectivity, suggesting that when the active site is sterically flexible, an enzyme will default to the thermodynamically favored product [100]. Both this result and the results from directed mutagenesis of AmpKR2 [22,25] were consistent with Leadlay and coworkers' findings that the A2 isomer was most frequently observed in both directed mutagenesis [104] and saturation mutagenesis [108] experiments. However, the directed mutagenesis experiments performed by Keatinge-Clay and coworkers [22,25,100] achieved the same selectivity with fewer mutations compared to those by Leadlay and coworkers [104,105] (1-2 as compared to 3-6) and resulted in an enzyme with increased catalytic activity compared to the wild type rather than decreased.

As noted by Keatinge-Clay and coworkers, the difference required to generate an enantiomeric excess of 99% is only ~3 kcal/mol in $\Delta\Delta G^{\ddagger}$ at room temperature [100]. This suggests that effecting stereochemical changes through extremely subtle alterations of the active site should be achievable. It is currently unknown whether or not binding interactions of the phosphopantetheinyl arm play a large role in orienting the substrate, yet it is energetically possible to achieve total stereocontrol with a small energetic barrier. Thus, it is not implausible that minor changes to the active site from a single residue substitution, as was observed in model β -keto thioester substrates, could be retained in the context of a module fused to an ACP-linked substrate. The fact that kinetic parameters of β -keto SNAC thioester substrates and the more natural pantetheinyl thioester substrates remain comparable further supports this hypothesis [112]. The thermodynamic values for binding to the ACP compared to those of binding the KR to various mimics of the phosphopantethienyl arm have been probed by isothermal titration calorimetry. These K_d values are on the same order of magnitude for KRs binding to the ACP compared to increasingly truncated

Al Type	∇ ∇ ∇	
AmpKR2	QLGVRVTIAA-CDAADREALAALLAELPED-APLTAVFHSAGVAHDDAPVADLTLGQLDA	
EryKR2	RLGARVSVHA-CDVSSREPVRELVHGLIEQGDVVRGVVHAAGLP-QQVAINDMDEAAFDE	
PikKR5	ASGARVTIAA-CDVADPHAMRTLLDAIPAE-TPLTAVVHTAGAP-GGDPLDVTGPEDIAR	
A2 Type		
ConKR5	ALGTRVTVAA-CDVADRDRLAELVAGLEQDGPPIRTVIHAAGTG-LLVPLSDTDPDEFAD	
AmpKR1	AAGTEVRFAA-CDITDPDAVAALLADLKAEGRTVRTVVHTAAVI-ELAALADTTVDAFAD	
NysKR1	ESGTEVTVAA-CDITDRDAVAALLADLTADGRTLRTVIHAAAAI-ELSALADTTVAEFAD	
B1 Type	▼	
TylKR1	GHGCEVVHAA-CDVAERDALAALVTAYPPNAVFHTAGI <mark>L-DD</mark> AVIDTLSPESFET	
EryKR4	DLGASAEIAA-CDTADRDALSALLDGLPRPLTGVVHAAGV <mark>L-AD</mark> GLVTSIDEPAVEQ	
RapKR10	ELGAAVVDTAVCDVSDRAGLARVLAGVSPD-HPLTAVIHTAGV <mark>L-DD</mark> GVVESLTARRLDT	
B2 Type		
EryKR1	ALGARTTVAA-CDVTDRESVRELLGGIGDD-VPLSAVFHAAAT <mark>L-DD</mark> GTVDTLTGERIER	
MegKR1	DLGTRATVTA-CDVTDREQLRALLATVDDE-HPLSAVFHVAAT <mark>L-DD</mark> GTVETLTGDRIER	
OleKR1	DMGVRVTLAA-CDAADRHALETLLDSLRTDPAQLTAVIHAAGA <mark>L-DD</mark> GMTTVLTPEQMNN	
A1 Type AmpKR2 EryKR2 PikKR5	LMRAKLTAARHLHELTADL-DLDAFVLFSSGAAVWGSGGQPGYAAANAYLDALAEHRR VVAAKAGGAVHLDELCSDA-ELFLLFSSGAGVWGSARQGAYAAGNAFLDAFARHRR LLGAKTSGAEVLDDLLBGT-PLDAFVLYSSNAGVWGSGSOGVYAAANAHLDALAABBB	
Al Type AmpKR2 EryKR2 PikKR5	LMRAKLTAARHLHELTADL-DLDAFVLFSSGAAVWGSGGQPGYAAANAYLDALAEHRR VVAAKAGGAVHLDELCSDA-ELFLLFSSGAGVWGSAROGAYAAGNAFLDAFARHRR ILGAKTSGAEVLDDLLRGT-PLDAFVLYSSNAGVWGSGSQGVYAAANAHLDALAARRR	
A1 Type AmpKR2 EryKR2 PikKR5 A2 Type	LMRAKLTAARHLHELTADL-DLDAFVLFSSGAAVWGSGGQPGYAAANAYLDALAEHRR VVAAKAGGAVHLDELCSDA-ELFLLFSSGAGVWGSARQGAYAAGNAFLDAFARHRR ILGAKTSGAEVLDDLLRGT-PLDAFVLYSSNAGVWGSGSQGVYAAANAHLDALAARRR	
A1 Type AmpKR2 EryKR2 PikKR5 A2 Type ConKR5	LMRAKLTAARHLHELTADL-DLDAFVLFSSGAAVWGSGGOPGYAAANAYLDALAEHRR VVAAKAGGAVHLDELCSDA-ELFLLFSSGAGVWGSAROGAYAAGNAFLDAFARHRR ILGAKTSGAEVLDDLLRGT-PLDAFVLYSSNAGVWGSGSOGVYAAANAHLDALAARRR TLYAKVAGAENLDAVFDRD-DLDTFVLFSSISGVWGSGDHGAYAAANAHLDALADRRR	
A1 Type AmpKR2 EryKR2 PikKR5 A2 Type ConKR5 AmpKR1 NucKP1	LMRAKLTAARHLHELTADL-DLDAFVLFSSGAAVWGSGGOPGYAAANAYLDALAEHRR VVAAKAGGAVHLDELCSDA-ELFLLFSSGAGVWGSARQGAYAAGNAFLDAFARHRR ILGAKTSGAEVLDDLLRGT-PLDAFVLYSSNAGVWGSGSOGVYAAANAHLDALAARRR TLYAKVAGAENLDAVFDRD-DLDTFVLFSSISGVWGSGDHGAYAAANAHLDALADRRR VVHAKVTGARILDELLDDE-ELDDFVLYSSTAGMWGSGVHAAYVAGNAYLSALAEQRR	
Al Type AmpKR2 EryKR2 PikKR5 A2 Type ConKR5 AmpKR1 NysKR1	LMRAKLTAARHLHELTADL-DLDAFVLFSSGAAVWGSGGQPGYAAANAYLDALAEHRR VVAAKAGGAVHLDELCSDA-ELFLLFSSGAGVWGSARQGAYAAGNAFLDAFARHRR ILGAKTSGAEVLDDLLRGT-PLDAFVLYSSNAGVWGSGSQGVYAAANAHLDALAARRR TLYAKVAGAENLDAVFDRD-DLDTFVLFSSISGVWGSGDHGAYAAANAHLDALADRRR VVHAKVTGARILDELLDDE-ELDDFVLYSSTAGMWGSGVHAAYVAGNAYLSALAEQRR VVHAKVTGARILDELLDDA-ELDDFVLYSSTAGMWGSGVHAAYVAGNAYLSALAEQRR	
A1 Type AmpKR2 EryKR2 PikKR5 A2 Type ConKR5 AmpKR1 NysKR1 B1 Type	LMRAKLTAARHLHELTADL-DLDAFVLFSSGAAVWGSGGOPGYAAANAYLDALAEHRR VVAAKAGGAVHLDELCSDA-ELFLLFSSGAGVWGSAROGAYAAGNAFLDAFARHRR ILGAKTSGAEVLDDLLRGT-PLDAFVLYSSNAGVWGSGSOGVYAAANAHLDALAARRR TLYAKVAGAENLDAVFDRD-DLDTFVLFSSISGVWGSGDHGAYAAANAHLDALADRRR VVHAKVTGARILDELLDDE-ELDDFVLYSSTAGMWGSGVHAAYVAGNAYLSALAEQRR VVHAKVTGARILDELLDDA-ELDDFVLYSSTAGMWGSGVHAAYVAGNAYLSALAEQRR	
Al Type AmpKR2 EryKR2 PikKR5 A2 Type ConKR5 AmpKR1 NysKR1 B1 Type TylKR1	LMRAKLTAARHLHELTADL-DLDAFVLFSSGAAVWGSGGQPGYAAANAYLDALAEHRR VVAAKAGGAVHLDELCSDA-ELFLLFSSGAGVWGSARQGAYAAGNAFLDAFARHRR ILGAKTSGAEVLDDLLRGT-PLDAFVLYSSNAGVWGSGSQGVYAAANAHLDALAARRR TLYAKVAGAENLDAVFDRD-DLDTFVLFSSISGVWGSGDHGAYAAANAHLDALADRRR VVHAKVTGARILDELLDDE-ELDDFVLYSSTAGMWGSGVHAAYVAGNAYLSALAEQRR VVHAKVTGARILDELLDDA-ELDDFVLYSSTAGMWGSGVHAAYVAGNAYLSALAEQRR	
Al Type AmpKR2 EryKR2 PikKR5 A2 Type ConKR5 AmpKR1 NysKR1 Bl Type TylKR1 EryKR4	LMRAKLTAARHLHELTADL-DLDAFVLFSSGAAVWGSGGQPGYAAANAYLDALAEHRR VVAAKAGGAVHLDELCSDA-ELFLLFSSGAGVWGSARQGAYAAGNAFLDAFARHRR ILGAKTSGAEVLDDLLRGT-PLDAFVLYSSNAGVWGSGSQGVYAAANAHLDALAARRR TLYAKVAGAENLDAVFDRD-DLDTFVLFSSISGVWGSGDHGAYAAANAHLDALADRRR VVHAKVTGARILDELLDDE-ELDDFVLYSSTAGMWGSGVHAAYVAGNAYLSALAEQRR VVHAKVTGARILDELLDDA-ELDDFVLYSSTAGMWGSGVHAAYVAGNAYLSALAEQRR VVHAKVTGARILDELLDDIKGLDAFVLFSSVTGTWGNAGQGAYAAANAALDALAERRR VLRAKVDAAWNLHELTANT-GLSFFVLFSSAASVLAGPGQGVYAAANESLNALAALRR	
Al Type AmpKR2 EryKR2 PikKR5 A2 Type ConKR5 AmpKR1 NysKR1 Bl Type TylKR1 EryKR4 RapKR10	IMRAKLTAARHLHELTADL-DLDAFVLFS SGAAVWGSGGQPGYAAANAYLDALAEHRR VVAAKAGGAVHLDELCSDA-ELFLLFS SGAGVWGSAROGAYAAGNAFLDAFARHRR ILGAKTSGAEVLDDLLRGT-PLDAFVLYSSNAGVWGSGSQGVYAAANAHLDALAARRR TLYAKVAGAENLDAVFDRD-DLDTFVLFSSISGVWGSGDHGAYAAANAHLDALAARRR VVHAKVTGARILDELLDDE-ELDDFVLYSSTAGMWGSGVHAAYVAGNAYLSALAEQRR VVHAKVTGARILDELLDDA-ELDDFVLYSSTAGMWGSGVHAAYVAGNAYLSALAEQRR VRGAKVCGAELLHQLTADIKGLDAFVLFSSVTGTWGNAGQGAYAAANAALDALAERRR VRGAKVCGAELLHQLTADIKGLDAFVLFSSAASVLAGPGQGVYAAANASLDALAERRR VLRAKVDAAWNLHELTANT-GLSFFVLFSSAASVLAGPGQGVYAAANESLNALAALRR VLRPKADGAWNLHELTRDI-DLAAFVMYSSAAGVLGSAGQGNYAVANAFVDALAEQRR	
Al Type AmpKR2 EryKR2 PikKR5 A2 Type ConKR5 AmpKR1 NysKR1 B1 Type TylKR1 EryKR4 RapKR10	KARAKLTAARHLHELTADL-DLDAFVLFSSGAAVWGSGGOPGYAAANAYLDALAEHRR VVAAKAGGAVHLDELCSDA-ELFLLFSSGAGVWGSAROGAYAAGNAFLDAFARHRR ILGAKTSGAEVLDDLLRGT-PLDAFVLYSSNAGVWGSGSOGVYAAANAHLDALAARRR TLYAKVAGAENLDAVFDRD-DLDTFVLFSSISGVWGSGDHGAYAAANAHLDALAARRR VVHAKVTGARILDELLDDE-ELDDFVLYSSTAGMWGSGVHAAYVAGNAYLSALAEQRR VVHAKVTGARILDELLDDA-ELDDFVLYSSTAGMWGSGVHAAYVAGNAYLSALAEQRR VRGAKVCGAELLHQLTADIKGLDAFVLFSSVTGTWGNAGOGAYAAANAALDALAERRR VLRAKVDAAWNLHELTANT-GLSFFVLFSSAASVLAGPGOGVYAAANSINALAALRRR VLRAKVDGAWNLHELTRDI-DLAAFVMYSSAAGVLGSAGOGNYAVANAFVDALAEQRR	
Al Type AmpKR2 EryKR2 PikKR5 A2 Type ConKR5 AmpKR1 NysKR1 B1 Type TylKR1 EryKR4 RapKR10 B2 Type EryKB1	K K	
Al Type AmpKR2 EryKR2 PikKR5 A2 Type ConKR5 AmpKR1 NysKR1 B1 Type TylKR1 EryKR4 RapKR10 B2 Type EryKR1 MegKB1	V MRAKLTAARHLHELTADL-DLDAFVLFS SGAAVWGSGG PGYAAANAYLDALAEHRR VVAAKAGGAVHLDELCSDA-ELFLLFS SGAGVWGSAR GAYAAGNAFLDAFARHRR ILGAKTSGAEVLDDLLRGT-PLDAFVLYS SNAGVWGSGS GVYAAANAHLDALAARRR V VIAKVAGAENLDAVFDRD-DLDTFVLFS SISGVWGSGD GAYAAANAHLDALADRRR VVHAKVTGARILDELLDDE-ELDDFVLYS STAGMWGSGVHAAYVAGNAYLSALAEQRR VHAKVTGARILDELLDDA-ELDDFVLYS STAGMWGSGVHAAYVAGNAYLSALAEQRR VHAKVTGARILDELLDDIKGLDAFVLFS SVTGTWGNAG GAYAAANAALDALAERRR VLRAKVDAAWNLHELTANT-GLSFFVLFS SAASVLAGPGO GVYAAANAALDALAERRR VLRAKVDAAWNLHELTRDI-DLAAFVMYS SAAGVLGSAG GNYAVANAFVDALAEQRR VLRAKVLGARNLHELTRDI-DLTAFVLFS SFASAFGAPGLGGYA GANAYLDGLAQQRR ASRAKVLGARNLHELTREL-DLTAFVLFS SFASAFGAPGLGGYA GANAYLDGLAQQRR ANAKVLGARNLHELTREL-DLTAFVLFS SFASAFGAPGLGGYA GANAYLDGLAQQRR	
Al Type AmpKR2 EryKR2 PikKR5 A2 Type ConKR5 AmpKR1 NysKR1 B1 Type TylKR1 EryKR4 RapKR10 B2 Type EryKR1 MegKR1 OleKB1	IMRAKLTAARHLHELTADL-DLDAFVLFS SGAAVWGSGGO PGYAAANAYLDALAEHRR VVAAKAGGAVHLDELCSDA-ELFLLFS SGAAVWGSGGO PGYAAANAYLDALAEHRR VUAAKAGGAVHLDELCSDA-ELFLLFS SGAGVWGSGGO GYAAANAHLDALAARRR ILGAKTSGAEVLDDLLRGT-PLDAFVLYSSNAGVWGSGSO GYYAAANAHLDALAARRR TLYAKVAGAENLDAVFDRD-DLDTFVLFSSISGVWGSGD GAYAAANAHLDALADRRR VHAKVTGARILDELLDDE-ELDDFVLYSSTAGMWGSGVHAAYVAGNAYLSALAEQRR VHAKVTGARILDELLDDA-ELDDFVLYSSTAGMWGSGVHAAYVAGNAYLSALAEQRR VHAKVTGARILDELLDDA-ELDDFVLYSSTAGMWGSGVHAAYVAGNAYLSALAEQRR VLRAKVDAANNLHELTANT-GLSFFVLFSSVTGTWGNAG GAYAAANAALDALAERRR VLRAKVDAANNLHELTRDI-DLAAFVMYSSAAGVLGSAG GNYAVANAFVDALAEQRR VRGAKVCGAELLHQLTADIKGLDAFVLFSSFASAFGAPGI GGYAFGNAYLDGLAQQRR ASRAKVLGARNLHELTRDI-DLAAFVMYSSAAGVLGSAG GNYAVANAFVDALAEQRR ASRAKVLGARNLHELTRDI-DLAAFVMYSSAAGVLGSAG GNYAVANAFVDALAEQRR ASRAKVLGARNLHELTRDI-DLAAFVMYSSAAGVLGSAG GNYAVANAFVDALAEQRR ASRAKVLGARNLHELTRDI-DLAAFVMYSSAAGVLGSAG GNYAVANAFVDALAEQRR ANRAKVLGARNLHELTRDI-DLAAFVMYSSAAGVLGSAG GNYAVANAFVDALAEQRR	

Fig. 11. Sequence alignment indicating diagnostic motifs in the KR domain. Active site residues are shown in cyan. Residues that are fingerprints for hydroxy stereochemistry are indicated in yellow (for A type KRs a W and for B type KRs an LDD motif). Residues associated with α stereochemistry are indicated in magenta. Residues mutated by Leadlay and coworkers are indicated by blue arrows [104,105]. Residues mutated by Keatinge-Clay and coworkers are indicated by red arrows [22,25,100]. The alignment was performed with MUSCLE.

phosphopantetheinyl arm mimics, suggesting that any binding contributions from the phosphopantetheinyl arm of the ACP to the KR likely only account for less than 1 kcal/mol in $\Delta\Delta G^{*}$ at room temperature [113].

Translation of standalone mutant KR activity towards β -keto thioester SNAC substrates in the context of a module has been tested in an investigation by Leadlay and coworkers. When the most effective mutations from their studies on EryKR1 and EryKR2 were introduced to DEBS1-TE and grown *in vivo* in *Streptomyces coelicolor*, the result was

either no metabolite formation or trace amounts of incompletely reduced ketolactone. These results suggest that the mutations were not retained in the more complex environment of the module [112] despite the kinetic arguments outlined above. However, from these experiments it is unclear whether the EryKR1 and EryKR2 mutants have not retained the altered selectivity possessed as standalone domains toward model small molecule substrates in the context of a full module on a covalently attached ACP-linked substrate, or if this is due to downstream effects such as gatekeeping from downstream domains. More



Fig. 12. Active site of EryKR1 (pdb code 2FR1). The active site tyrosine and serine are indicated in orange. The NADP⁺ cofactor is indicated in blue. Residues mutated by the Keatinge-Clay group are indicated in magenta [100]. Residues mutated by the Leadlay group are indicated in yellow [104,105]. D320 was mutated by both Keatinge-Clay and coworkers and Leadlay and coworkers.



Native substrate, β-keto acyl-ACP



β-keto acyl pantetheine



β-keto acyl N-acetyl cysteamine (SNAC)



β-keto acyl ethanethiol

Fig. 13. Native β -keto acyl-ACP and truncated small molecule substrate mimics for *in vitro* studies of decreasing complexity used by standalone KR domains.

work needs to be done to discriminate between these two possibilities. Nonetheless, the fact that small energetic differences are sufficient to alter stereoselectivity at a chemical level is extremely promising. These results suggest that point mutations can be used as a means to subtly alter the KR active site environment and reliably alter molecular stereochemistry through enzymatic asymmetric induction.

Dehydratase

Mutations to abolish activity: The active site of the DH domain contains of a conserved catalytic histidine which acts as a general acid and general base [114] along with a conserved aspartate residue [30]. Mutation of the histidine residue to another abolishes the activity of the DH in nature. Examples of inactive DHs exist in antilid module 2, ansamitocin module 1, PMM110117 module 5, and nanchanomycin module 1. Schulz and coworkers successfully inactivated several DH domains in the monensin PKS via an active site histidine to phenylalanine mutation. Perplexingly, this approach appeared to be effective in only four out of six cases [115]. DH domains that retain the active site histidine but have substitutions to the active site aspartate tend to have new catalytic activities, a phenomenon which offers a potentially intriguing expansion of chemical space (*vide infra*).

Mutations to alter activity: The DH domain undergoes a syn coplanar elimination [28-30,116-120], thus the stereochemistry of the KR domain proceeding the DH domain enforces the stereochemical outcome of this reaction (Fig. 10). DH domains proceeding KR domains conferring L stereochemistry result in cis double bonds whereas DH domains proceeding KR domains that confer D stereochemistry result in trans double bonds [57]. The relationship between E and Z stereochemistry with α substitution is slightly more complex [29,118,121], but nonetheless appears to be dependent on KR stereochemistry. Thus, facile alterations to alter olefin geometry through DH domain engineering alone would likely be unsuccessful. However, DH domains do appear to be sensitive to the stereoelectronic geometry of the substrate they dehydrate [3,119,122,123]. Structural studies of the active sites of fluviricin DH1 and borrelidin DH3 suggest that this sensitivity is influenced by a diagnostic region of the substrate binding cavity [122]. Tuning this binding pocket with minor substitutions may be a feasible way to expand the substrates accommodated by DH domains. Similar to the KS domain, the DH domain may be a gatekeeper that prevents structural changes introduced in upstream modules. As the DH domain catalyzes a reversible, thermoneutral reaction that is further shifted towards the right by being in 55 M water, a poor or loose steric fit between substrate and binding pocket is likely to shift the equilibrium towards the hydroxy as opposed to the olefin, which poses a particular challenge for engineered systems [3,119,123].

Perhaps one of the most exciting prospects for DH engineering involves domains possessing the DH's characteristic double hot dog fold, but lacking activity as a dehydratase. These domains have been shown to perform multiple types of isomerization reactions when dehydration activity is absent, as the DH is the only non-reductive β -carbon processing domain harbored within PKSs. The general acid-general base chemistry catalyzed by the DH can be co-opted for other transformations. Specifically, this includes enoyl isomerization and the isomerization of a linear polyketide to form a pyran (Fig. 14). Enoyl isomerases possess an asparagine in place of the catalytic aspartate [124,125]. Pyran synthases typically have either histidine or asparagine at this location [126]. An unusual case where both the catalytic histidine and aspartate are present is found in the third dehydratase in the ambrucitin pathway [127,128]. AmbDH3 performs both dehydration and cyclization in dual steps. The typical H51 and D215 residues are essential for dehydration, but through examination and structural characterization, V173 was determined to be unique to the cyclizing activity of AmbDH3. In most dehydrating DHs, the residue homologous to V173 is a tyrosine. Homology models suggests that putting a hydrophobic branched residue in that location creates enough space in the active site to facilitate cyclization [128]. Hence, these studies provide both insight into the evolutionary path from a dehydratase to an isomerizing enzyme and clues for how subtle alterations to DH active sites enable alternative chemistry from the catalytic histidine.



Fig. 14. Types of reactions catalyzed by domains with DH double hot dog folds including A) cyclization to form a pyran and B) isomerization of the olefin.

Enoylreductase

Mutations to abolish activity: The ER domain stereospecifically reduces an enzyme-bound 2-enoyl intermediate. It is the least structurally and biochemically characterized β-carbon processing domain. To date, the exact active site residues are not entirely established, although they appear to be largely composed of an active site lysine, aspartate, and tyrosine [129]. As a member of the medium chain dehydrogenase (MDR) super family, it was assumed that, like the mammalian fatty acid synthase (FAS), the tyrosine would be a critical residue. Mutation of any one of the proposed active site residues does not entirely abolish catalytic activity, however in the spinosad enoylreductase, SpinER2, a lysine to alanine mutation resulted in the largest loss of activity [31]. For this reason, it is difficult to inactivate an ER domain through a catalytic residue. The most effective mutations of the ER domain appear to be in the NADPH binding pocket, as shown by Schulz and coworkers, who mutated the first two glycines in the TGGVG motif to S and P in the monensin PKS [115]. One example of an inactive ER domain in nature is fostreicin ER1 [130]. Fostreicin ER1 has a proline in place of the usual tyrosine or valine, however the NADPH binding site appears to be uninterrupted.

Mutations to alter activity: A study performed by Kwan and Leadley revealed key active site residues influencing the stereospecificity of enoylreduction in the rapamycin PKS. Additionally, several residues previously identified as important in the medium-chain dehydrogenase/reductase family of enzymes were found to be unimportant for enoylreduction in rapamycin biosynthesis [129]. For modular PKS ER domains in which the residue at position 52 is tyrosine, the stereochemical outcome consistently favors the formation of a (2L)alkyl-branched product, whereas for ER domains in which the residue at position 52 is valine, alanine, or phenylalanine, the (2R) product is almost always formed instead (Fig. 15) [131]. Following this discovery, amino acid mutations were performed in two different ER domains to determine whether stereochemistry of the product would be influenced. Mutagenesis of tyrosine to valine in EryER4 resulted in switching the stereochemistry of enoylreduction while mutatagenesis of valine to tyrosine in position 52 of RapER13 resulted in unchanged stereochemistry [129].

Further analysis of multiple sequence alignments of ER domains led to the investigation of additional residues, several of which were surprisingly found to have little to no effect on the stereochemistry of enoylreduction. These include V52, N53, V46, and V47. Additionally, different active site residues were mutated in a model triketide lactone synthase termed TKS Rap13 to test the effect on catalysis. TKS-Rap13 is a variant of DEBS1-TE where EryKR2 is swapped with the full set of reductive domains from rapamycin module 13. From sequence alignment data, several residues were chosen for mutagenesis studies including N41, D43, Thr122, Y124, Y125, and K236. Most of the strains of S. erythraea containing the amino acid mutations to TKS-Rap13 produced significantly less triketide lactone than the parent strain, the fully reduced triketide lactone, or in the case of the K236A mutant, both diastereomers. Each of the mutants also produced a portion of unreduced 3-keto triketide lactone in similar amounts. Although the amount of unreduced 3-keto triketide lactone was comparable between mutant strains, there were vast differences in the production of the fully reduced triketide lactone between different mutants. Surprisingly, mutagenesis of K236 in TKS-Rap13, which corresponds to the Lys1771 proposed as a catalytic residue in the mammalian FAS, had no effect on the production of fully reduced triketide lactone. However, the mutation resulted in a shift in enzyme stereospecificity with two different diastereomers produced in a ratio of 1:6. Therefore, K236 might act as a proton donor to C-2 [129]. This work serves as a valuable starting point for exploration into ER mutagenesis and its effect on stereoselectivity as well as catalytic activity.

Acyl carrier protein

Mutations to alter activity: While the ACP domain does not catalyze any chemical reactions, recognition between the ACP and the catalytic domains has been demonstrated as important for catalysis [34,56,85,132–135]. Thus, disrupting key interfaces along this region influences the kinetics of catalysis. In a study performed by Khosla and coworkers, the effect of protein-protein interactions on PKS activity was explored using chimeric bimodular and trimodular systems from DEBS. It was predicted that chain translocation from DEBS Mod1 to Mod3 in a bimodular PKS would be improved by applying an E23K mutation to EryACP1. This mutation was selected because a cationic residue also exists at the corresponding position of EryACP2. As a result, the turnover rate of the E23K mutant of DEBS Mod1+Mod3+TE was more than two-fold over the wild type bimodular PKS. This result shows the importance of ACP-KS interactions during PKS chain translocation [136]. Although a type II PKS rather than a type I modular PKS, studies of the actinorhodin PKS revealed critical insights regarding the apo and holo forms of the ACP's conformation. Conformational differences exist in the helix II residues including L45, M46, G47, and G53. The rearrangement of helix III is responsible for the observed apo-to-holo chemical shift differences for D62. The whole of D62 swings toward helix II and folds across the cleft. Additionally, the transition from the apo to holo form results in a subtle switch from Leu43 being solvent exposed to forming intramolecular interactions with the newly added phosphopantetheine side chain. Tryptophan fluorescence and FAS holosynthase (ACPS) assays revealed that apo-ACP has a twofold higher affinity than holo-ACP. Site-directed mutations to L43 and D62 were performed to examine the influence on binding and ACPS activity. Four point mutations were compared including L43A, L43R, D62A, and D62N. The mutation L43A produced a binding profile like that of the holo-ACP with twofold weaker binding. Conversely, the mutant L43R had tenfold weaker binding and was completely inactive in the phosphopantetheine transfer assay. Furthermore, the mutations D62A and D62N resulted in ~40% and 20% reductions in binding affinity, respectively. However, both mutants were completely activated after a period; the D62A mutant was completely activated after 2 h while the D62N mutant was activated after 1 h. Therefore, point mutations to L43 had a much greater effect on binding and activity than point mutations to A62 [132]. Both examples demonstrate the ability of the ACP to dramatically impact the efficiency of catalytic domains through protein-protein interactions.

Thioesterase

Mutations to abolish activity: The TE domain consists of a typical α/β hydrolase fold with a serine, histidine, aspartate catalytic triad. TE domains can either be hydrolyzing or lactonizing, where the acyl

A. 2L Configuration

LkmER4	-SLDALAFAAAPDAGRQPGPGEVRVELRSCGVNFRDV <mark>LL</mark> ALGM <mark>YP</mark> GAAVMGTEGAGV
EryER4	GAIDSVAFEPAPDVEQPLRAGEVRVDVRATGVNFRDV <mark>LL</mark> ALGM <mark>YP</mark> QKADMGTEAAGV
MegER4	GSIEAVAFAPVPDADRPLAPEEVRVAVRATGVNFRDV <mark>LL</mark> ALGM <mark>Y</mark> PEPAEMGTEASGV
PikER4	LESLTAAPGDAETLAPEPLGPGQVRIAIRATGLNFRDV <mark>LI</mark> ALGM <mark>YP</mark> DPALMGTEGAGV
TylER5	GTLEELSLAPAPDAEEPLAPGQVRIAVRAAGVNFRDA <mark>LI</mark> ALGM <mark>YP</mark> GKGTMGAEGAGV
MycER5	LADLCLEPADTATVSLLPGQVRIAVRAAGLIFRDT <mark>LI</mark> ALGV <mark>YP</mark> DQAAMGAEGAGV
ChmER5	GTLSDLALVPAQTDTVALAPGQVRIAVRAAGLNFRDT <mark>LI</mark> ALGM <mark>Y</mark> PGEGVMGAEGAGV
FkbER6	GTLDGLTAADSAEPRRPLAPAEVRIGVRAAGLNFRDV <mark>LI</mark> ALGT <mark>YP</mark> GQGVLGGEAAGI
FkbER7	GTLHDVALIADDTPRRALEAGEVRIDVRAAGLNFRDV <mark>LI</mark> ALGT <mark>YT</mark> GATAMGGEAAGV

2D Configuration

RapER1	GTLRDLALVPTDTAERPLQSGEVRVDVRAAGLNFRDVVVALGMVDDKRLA-GGEAAGV
RapER13	GTLQDLALVPTDTAEQPLRPGEVRIDVRAAGLNFRDV <mark>VV</mark> ALGM <mark>VN</mark> DNRPT-GGEAAGV
FkbER9	GSLDDLAVVPTDAPDRPLAAGEVRIAVRAAGLNFRDV <mark>TV</mark> ALGV <mark>VA</mark> DARPL-GSEAAGV
BorER5	GTFDNLTLGVYPHAEKTLADNEVRVAVHAGGLNFHDV <mark>VA</mark> ALGM <mark>VE</mark> DDLTL-GREAAGV
OlmER7	TIEGLTAVPYPQALEPLGPRQVRIAVRAAGINFKDV <mark>VV</mark> ALDL <mark>VP</mark> GLTGL-GGEVAGV
OlmER10	GTIENVALVPCPEVLDPLGPGQVRIAVHAVGLNFRDV <mark>VV</mark> ALGM <mark>VE</mark> GQNGI-GGDVAGT
GdmER1	GSLENLVLRPDPEATAPLATGQVRIEVRAAGQNFRDV <mark>LV</mark> ALGG <mark>VA</mark> GQEGL-GGEGAGV
HbmER1	GSLENLVLRPDPEATAPLATGQVRIEVRAAGQNFRDV <mark>LV</mark> ALGG <mark>VA</mark> GQEGL-GGEGAGV





enzyme intermediate is attacked by water or a hydroxy group on the polyketide chain, respectively [137–139]. There is little reason to inactivate the TE domain intentionally for synthetic biology engineering endeavors, as inactivating chain termination results in no catalytic turnover. One potential application for intentional TE inactivation

would be for a few PKSs where the TE domain is present in *trans* rather than at the C-terminus, such as the monensin and nanchangomycin PKSs. Inactivation of the TE and complementation with one in *trans* possessing distinct substrate selectivity, or changing a TE from lactonizing to hydrolyzing may be approaches to consider [140–142]. As one

Fig. 15. A) Sequence alignment of ER types by stereochemical outcome. The diagnostic tyrosine and valine are indicated in yellow. Other residues that correlate with stereochemical outcome but do not appear to have any impact in mutagenesis studies are indicated in yellow. The alignment was performed with MUSCLE. B) Homology model of EryER4 indicating a hypothesis for the role of the diagnostic tyrosine, which provides steric crowding and prevents approach from the re face. C) Homology model of RapER13 indicating a hypothesis for the role of the diagnostic valine, which sterically affords space for reduction from the re face. Panels B-C reproduced with permission from ref. [129,131].

would anticipate, mutation to the catalytic serine abolishes activity, and mutations to the other residues within the catalytic triad (e.g. an aspartate to asparagine mutation) compromise activity severely [143]. The most reliable inactivation to the TE domain is an active site serine to alanine mutation, which has been performed in mechanistic studies [143,144].

Mutations to alter activity: Although TE domains can be either hydrolyzing or lactonizing, many that are natively lactonizing such as EryTE or the pikromycin TE, PikTE, perform hydrolysis when a nucleophilic hydroxy group is absent [3,60,61,145]. Structural studies of the tautomycetin TE, a hydrolyzing TE, compared to lactonizing TEs indicate the presence of a constrained substrate tunnel that prevents macrocyclization [138,146–148]. Therefore, using mutagenesis to alter residues in the active site may be a means to toggle between chain termination via lactonization or hydrolysis. Other modes of chain termination exist including reductase (R) domains that reduce the thioester to a terminal alcohol [149–151] and sulfotransferase (SULT) domains which result in a terminal olefin [152,153]. However, introducing such chemistry would require domain swapping and is thus out of the scope of this review.

The TE domain is stereospecific for the configuration of the hydroxy group acting as a nucleophile for macrolactonization in both DEBS and the pikromycin PKS [60,145,154,155]. Because of steric sensitivity, the ability to alter stereochemical scaffolds is greatly hindered by the inability of the TE to cyclize varying stereochemical backbones. Inspired by proteases, Sherman and coworkers mutated the active site serine in PikTE to a cysteine which surprisingly resulted in the cyclization of an unnatural epimer (Fig. 16A). Additionally, the catalytic activity of the S148C mutant improved by approximately 4-6-fold in k_{cat}/K_m. The computationally verified mechanistic rationale suggested that the acyl enzyme intermediate was less kinetically stable, thus decreasing the barrier for the macrocylization reaction and affording a kinetically feasible path for the less sterically favorable macrolactonization (Fig. 17) [155,156]. Although not translated to another system, this method could be broadly applicable for the expansion of the stereochemical scaffolds of macrolides, as high stereospecificity is not unique to the pikromycin PKS.

Interdomain linkers

Mutations to alter activity: A striking example of the ability to alter catalytic efficiency through individual point mutations to interdomain linkers is revealed in a study by Khosla and coworkers involving DEBS Mod3. A crystal structure of the KS-AT didomain was available to visualize its interface [32]. It was assumed that mechanistically important residues in the KS-AT linker of DEBS are evolutionarily conserved. Thus, the amino acid sequence of the DEBS KS-AT linker was compared with other PKSs. The most conserved residues including V475, V476, S477, R479, L484, E487, I491, L509, R513, H516, H518, R519, L534, and I537 were individually replaced with alanine. The corresponding mutants were assayed, and the rate of triketide lactone formation was measured. Most mutants showed initial rates comparable to that of the wild type enzyme, however the R513A mutant had a 7fold decrease in activity at 23 °C and a 20-fold decrease in activity at 30 °C. The rates for individual KS- and AT-catalyzed self-acylation of the R513A mutant were comparable to those of the wild type enzyme, suggesting a lack of significant structural changes. Proteolysis experiments revealed that the R513A mutant has a more flexible structure than the wild type enzyme and two prototypically susceptible sites emerged as a result of the R513A mutation. As such, the significantly reduced turnover rate of the R513A mutant is not due to a direct role of R513 during interactions with the ACP domain, but is rather likely due to the role of the residue in stabilizing the KS-AT didomain quaternary structure. Thus, while not targeting an active site residue directly, the impact that key residues have on the stability of the overall fold can have ramifications on catalytic activity. The location of R513 in the KS-

AT linker is shown in Fig. 18 [157].

For protein-protein interactions among sequential modules that are not present on the same gene, N-terminal and C-terminal docking domains, termed NDD and CDD domains respectively, allow for the proteins between PKSs to associate. Charge complementarity has been observed between matching NDD/CDD pairings [37,38,40,41,158,159], and these linkers have also been used to promote binding in non-natural contexts [40,93,158]. Such critical linkers are key regions to exclude when performing random mutagenesis studies in order to retain critical protein-protein interactions.

Domain skipping and modular elasticity

More uncommonly, a single mutation can result in domain skipping which then alters the size and shape of the resulting metabolite. A key example of this phenomenon in the aureothin pathway was recently reported by Hertweck and coworkers. They noticed that the native Streptomyces thioluteus strain produces a minor truncated side product, luteoretculin (Fig. 19). Intriguingly, upon mutation of the AurKR2 active site tyrosine to phenylalanine, a keto moiety was produced instead of a fully reduced carbon. Therefore, AurKS3 skipped condensation, which was likely due to KS gatekeeping. Interestingly, mutations to the other β-carbon processing domains had different effects on product distributions. In AurDH2, mutation of the catalytic histidine to phenylalanine resulted in a combination of stalled but extended products, as one would expect from a typical domain inactivation. A null mutant of AurER2 was made by mutating the conserved NADPH binding motif of GGVGMA to SPVGMA. This also resulted in the expected stalled products. Only inactivation of the KR domain resulted in this reliable module skipping. Because the KS is often sensitive to the steric shape of its substrate, removal of functionality through a single point mutation can result in more dramatic alterations to the carbon-carbon backbone if condensation becomes kinetically stalled. The precedent for the formation of luteoretculin from the aureothin PKS indicates the need for caution, as a single point mutation can have more drastic effects on molecular structure than initially anticipated [160]. This kinetic phenomenon of module skipping at AurKS3 demonstrates the interplay between simple domain inactivation and more complex issues regarding interacting selectivities of sequential domains.

Synthetic biology ramifications

Due to the high GC content of many genes derived from actinobacteria and myxobacteria which average 75% and 70% GC, respectively, as well as the very large sizes of these genes, the assembly of PKS constructs has typically presented a challenge. Prior to the invention of scar-free strategies such as Gibson and Golden Gate cloning, typically, restriction sites were added to PKS genes to enable their assembly. To date, the secondary structure arising from high GC content presents a significant challenge for reliable assembly using modern scar-free strategies like Gibson cloning which are reliant on regions of homology. It is not always possible to introduce restriction sites resulting in silent mutations, thus historically, researchers have tolerated conservative mutations to the DNA sequence. Recently, our laboratory has discovered that a mutation commonly introduced into DEBS1 to add a BsJ1 site for assembly is in a site analogous to one that affects the substrate selectivity in DEBS KS3 [58]. This residue is homologous with K154 in DEBS3 which was mutated to alanine in a homology directed experiment guided by the mycolactone PKS (vide supra, Fig. 5D). Although the native DEBS1 sequence contains an asparagine, to introduce the restriction site it was altered to a histidine. While typically, mutations to introduce a restriction site are believed to be innocuous, as they are performed with the intention of avoiding key active site residues, much structural and biochemical elucidation has occurred since many of these classic constructs were first assembled. Many mutations introduced in early papers detailing domain swaps and functional assays

Fig. 16. Stereoisomers biocatalytically generated by the wild type and S148C mutant of PikTE through a biocatalytic extension of model substrates via methylmalonyl-SNAC [155,156].

Fig. 17. A) Energy diagram of and B) transition state calculations of wild type and S148C with the two epimeric substrates. Reproduced with permission from Ref. [156].

Fig. 18. Critical arginine salt bridge mediating contact between the KS-AT linker. Reproduced with permission from Ref. [157].

of DEBS remain unannotated. Therefore, the degree to which these "conservative" mutations affect stability or enzymatic function compared to the wild type is currently unknown. Some mutations may appear in residues near the active site, as the active site residues are not always close together in primary sequence. Prior to structural elucidation it was impossible to determine the proximity of distal residues to the active site. It is worth highlighting the need to rigorously sequence all plasmids commonly utilized in the PKS literature received from previous groups, as such restriction sites often remain unannotated.

Summary

Due to the wealth of available structural information on PKS domains, our ability to target mutations for specific purposes is fairly sophisticated. Both rational and random mutagenesis approaches can be utilized to effect desired changes in activity, selectivity, and kinetics among these complex multidomain systems. Targeted mutation has enormous potential to influence the catalytic activity of PKSs while being much more precise and less inherently globally disruptive than domain swapping, which often results in poor expression and low titers. So far, much mechanistic information on the catalytic efficiency, selectivity, and stereospecificity of PKS domains has been gleaned from point mutation experiments. Indeed, key active site residues, substrate binding motifs, and residues facilitating key didomain interactions have been identified. Additionally, the phenomenon of domain skipping has been explored, and is expected to be the focus of many future studies. Going forward, one of the biggest challenges will be using targeted point mutations that do not affect selectivity, but instead affect gatekeeping behavior due to stalled kinetics of downstream domains upon the introduction of unnatural substrates. Furthermore, recently characterized domains such as the MT and underexplored domains such as the DH and ER should be the subjects of future mutagenesis experiments. It is also important to note any "conservative" mutations used to insert restriction sites for genetic engineering, as these could influence enzyme structure and function more severely than expected. Taken together, point mutagenesis approaches are a powerful way to unleash the potential of PKSs for synthetic biology applications. This foundational work has provided the basis for expanding the scope from PKS mechanistic exploration to engineering application.

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Fig. 19. Example of domain skipping by a single KR inactivation in the aureothin pathway [160].

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