Enzyme Recognition

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Composite Aromatic Boxes for Enzymatic Transformations of Quaternary Ammonium Substrates**

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Abstract: *Cation–π interactions to cognate ligands in enzymes have key roles in ligand binding and enzymatic catalysis. We have deciphered the key functional role of both charged and aromatic residues within the choline binding subsite of CTP:phosphocholine cytidylyltransferase and choline kinase from Plasmodium falciparum. Comparison of quaternary ammonium binding site structures revealed a general composite aromatic box pattern of enzyme recognition sites, well distinguished from the aromatic box recognition site of receptors.*

Cation–π interactions with electrostatic ion-quadrupol and ion-induced dipol character are frequent key elements of molecular recognition.[1] Trimethyl ammonium moieties are of peculiar interest as diffuse distribution of their charge renders them highly polarizable.[2] For their accommodation, a sterically restricted protein environment has evolved, in which desolvation of the charged moiety enables its efficient binding through cation–π interactions from aromatic residues (receptor function), as reviewed recently.[3] Beyond binding and transporting such ligands, numerous enzymes catalyze their chemical transformation, involved in key cellular functions. We introduce a clear general framework that governs the architecture of the active sites of these enzymes. Our experimental results together with a full range protein data bank query establish a novel common structural epitope for enzymes, termed as "composite aromatic box", distinct from the previously described aromatic box/cage of receptor proteins.

As a case study, we choose a model system to decipher key elements of ligand binding and mechanism of action of two enzymes, catalyzing consecutive steps in the phospholipid biosynthetic process of *Plasmodium falciparum*. Choline kinase (*Pf*CK, E.C. 2.7.1.32) phosphorylates Cho (choline) to ChoP (choline-phosphate) using ATP, CTP:phosphocholine cytidylyltransferase (PfCCT, E.C.2.7.7.15) catalyzes the CTP + ChoP (choline-phosphate)→CDPCho (CDP-choline) + PP_i (pyrophosphate) reaction. These enzymes are targets for recently developed highly potent choline analogue antimalarial agents;[4,5] however, current structural and mechanistic insights still fail to fully explain how the aromatic/hydrophobic interactions at the choline subsite contribute to ligand binding and catalysis.

The choline binding cleft of *Pf*CCT is a partially hydrophobic pocket that accommodates quaternary ammonium moieties of ChoP and CDPCho (Figure 1 A). This subsite consists of strictly conserved residues of either charged or aromatic character, the latter providing cation–π interaction to the choline moiety of cognate ligands.[6] To investigate the role of these residues in ligand binding and enzyme action, we designed a series of point mutants conservative for structure in a construct encompassing the catalytic domain of *Pf*CCT (*Pf*CCT MΔK[6]) by modulating the character of charged (D623N, Y741F) as well as aromatic (W692F, W692Y, Y714F) residues. The introduced mutations do not perturb the overall

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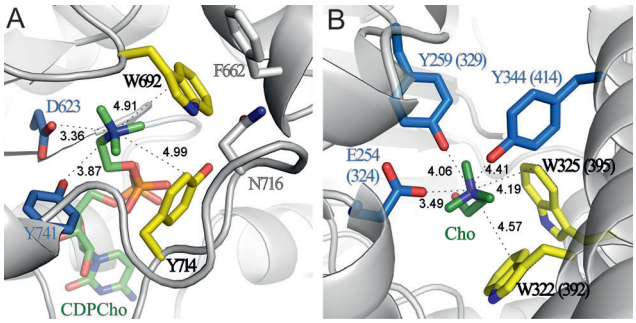


Figure 1. Coordination of the choline moiety at the active site of *Pf*CCT and *Pf*CK. A) Active site close-up of *Pf*CCT MΔK (PMDB ID: PM0078719). B) Active site close-up of choline kinase from *Plasmodium knowlesi*, a close homolog of *Pf*CK (PDB ID: 3C5I) Corresponding residue numbers of *Pf*CK are given in parenthesis. Structures are shown as a grey cartoon model, with the bound ligand shown as stick model, atomic coloring with green carbon. Side chains of charged and cation–π interactor residues within 5 Å and 6 Å of the quaternary nitrogen atom are displayed as sticks in atomic coloring with carbon is either blue or yellow for charged and aromatic interactions, respectively; O, red; N, blue; P, orange, with interaction distances indicated in Ångströms. Note the coordination partners of W692 in Figure 1 A, displayed as sticks, colored in atomic coloring, grey carbon.

quaternary structure of the enzyme (compare Figure S5 and Table S4 in the Supporting Information, SI). We performed enzyme kinetic investigations as well as a thermodynamic analysis of CDPCho binding by isothermal titration calorimetry as described in the SI.

Results indicate on one hand that the substitution of charged to noncharged residues yielded profound perturbation effects on catalytic efficiency (Figure 2, Table 1). This is mostly due to an altered choline coordination, as deduced from both $K_{M,ChoP}$ and the impaired CDPCho binding capability, characterized with diminished enthalpy of binding (Figure 2, Table 2, and Figure S7). On the other hand, partial conservation of the aromatic residues involved in cation–π interactions by tuning electrostatics, ring size, and quadrupole moment of the aromatic ring is still somewhat compatible with ligand binding and catalysis. The observed diverse performance of the mutants argue for the valid distinction of their charged versus cation–π interaction nature, and for

Table 1: Kinetic characterization of *Pf*CCT MΔK mutants.

Enzyme	k_{cat} [s^{-1}]	$K_{M,CTP}$ [μM]	$K_{M,ChoP}$ [mM]	rel. $k_{cat}/K_{M,CTP}$	rel. $k_{cat}/K_{M,ChoP}$
MΔK[a]	1.45 ± 0.05	168 ± 17	1.8 ± 1.1	1	1
$MΔK^{Y714F}$	0.21 ± 0.01	580 ± 60	10.2 ± 1.1	0.042	0.026
$MΔK^{W692F}$	0.13 ± 0.03	890 ± 380	7.5 ± 2.4	0.018	0.022
$MΔK^{W692Y}$	0.03 ± 0.003	191 ± 64	1.3 ± 0.2	0.017	0.029
$MΔK^{D623N}$	0.006 ± 0.001	460 ± 190	13.1 ± 3.1	0.0015	0.00057
$MΔK^{Y741F}$	0.019 ± 0.002	790 ± 180	8.5 ± 2.0	0.0028	0.0028
$MΔK^{W692A}$	6×10^{-4}	ND[b]	ND[b]	ND[b]	ND[b]

[a] Values reported previously.[6] [b] Values cannot be determined as seriously impaired ligand binding rendered results of Michaelis–Menten fit inaccurate.

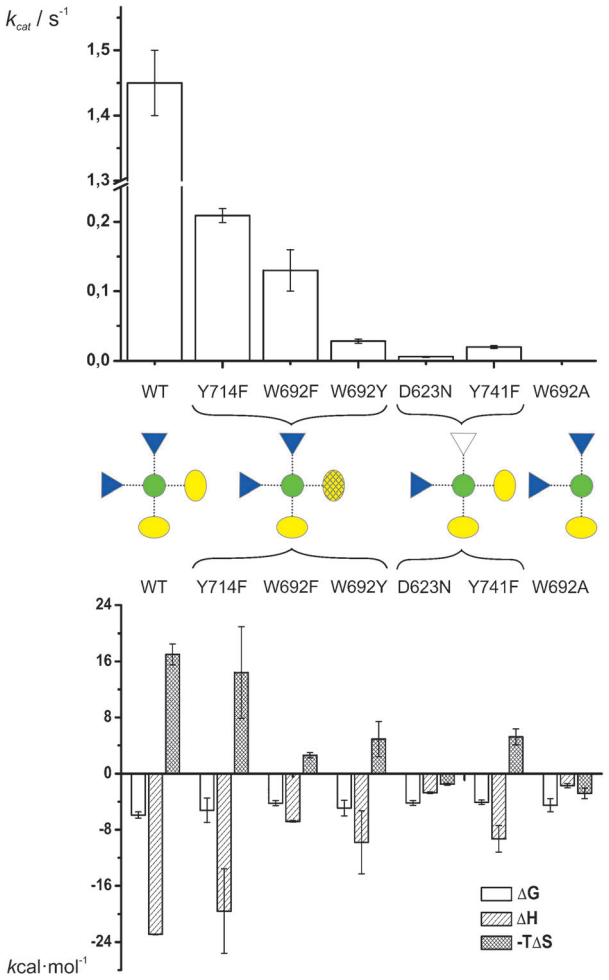


Figure 2. Effect of mutations on *Pf*CCT MΔK enzyme activity and ligand binding capability. Catalytic turnover and thermodynamic parameters of CDPCho binding (binding free enthalpy (Δ*G*), enthalpy (Δ*H*), and entropy (Δ*S*)) of *Pf*CCT MΔK and its point mutants are shown in top and bottom panels, respectively. The cartoon illustrates the quaternary ammonium binding site of *Pf*CCT with charged and aromatic interaction residues depicted as dark gray triangles and light gray ellipses, respectively. The functional effect of point mutation on a residue is indicated: altered aromatic character is depicted with a grid; exchange of charged to noncharged residue is marked by the empty triangle whereas alanine mutation of a given residue is depicted by deleting the respective symbol. Note the break on the *y* axis on the top panel.

Table 2: Thermodynamic analysis of CDPCho binding to *Pf*CCT MΔK mutants by ITC at 20 °C.

Enzyme	K_d [μM]	Δ*H* [kcal mol^{-1}]	Δ*S* [cal mol^{-1} K^{-1}]
MΔK[a]	44.4 ± 3.3	−22.9 ± 1.0	−58.1 ± 5.0
$MΔK^{Y714F}$	140 ± 50	−19.6 ± 6.0	−49.1 ± 22.3
$MΔK^{W692F}$	680 ± 60	−6.8 ± 0.8	−8.9 ± 1.3
$MΔK^{W692Y}$	220 ± 50	−9.8 ± 4.5	−16.8 ± 8.6
$MΔK^{D623N}$	790 ± 60	−2.7 ± 0.1	5.1 ± 0.5
$MΔK^{Y741F}$	1000 ± 80	−9.3 ± 1.9	−17.8 ± 3.9
$MΔK^{W692A}$	460 ± 100	−1.7 ± 0.3	9.6 ± 2.6

[a] Values reported previously.[6]

the key role of residues forming charged interactions with the choline moiety.

To assess the full contribution of interacting residues present at quaternary ammonium binding sites to enzyme action, we used alanine mutagenesis as an alternative investigation approach. Kinetic characterization was applied to the respective mutants of choline kinase from *Plasmodium falciparum*, a further example of an enzyme harboring a quaternary ammonium recognition site (Figure 1 B). Here, among the cognate charged interactors, as hypothesized based on the inspection of the CK crystal structure of *P. knowlesi*, a considerable role in enzyme action was only confirmed experimentally for E324, the equivalent of E254 of *P. knowlesi* CK (cf. Figure 3). In contrast, detrimental effects

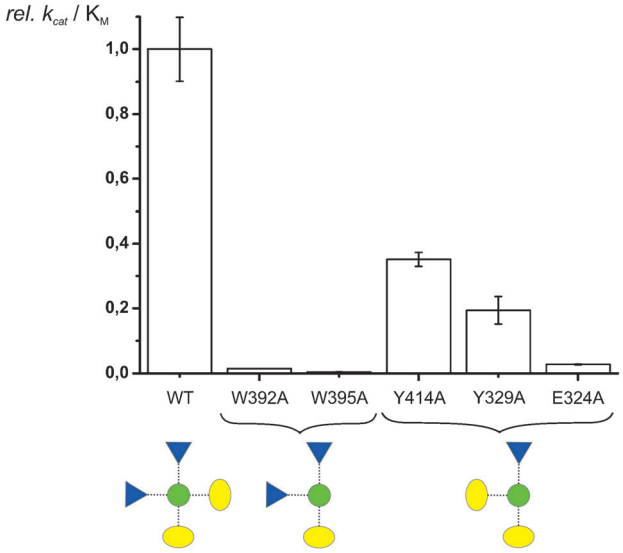


Figure 3. Kinetic parameters of wild-type *Pf*CK and its mutants. Relative catalytic efficiency of *Pf*CK mutants, compared to the wild type (WT) enzyme. The cartoon illustrates interaction types at the quaternary ammonium binding site of *Pf*CK; for the color code, see Figure 2. For the alanine mutation of a given residue, the respective symbol is deleted.

on enzyme action were also observed with the elimination of aromatic side chains (W392 and W395), which is mainly due to impaired ligand binding as indicated by K_M values. (Figure 3, Table 3, and Figure S8).

The significance of cation–π interactions to quaternary ammonium ligands, clearly demonstrated by these experiments with *Pf*CCT and *Pf*CK, is further underlined by the investigation of a *Pf*CCT MΔK mutant with fully abolished aromatic character, W692A. Elimination of the aromatic character at position 692 leads to a drastic disruption of catalytic function and also strongly perturbs the thermodynamics of CDPCho binding. Importantly, in this case the binding of CDPCho in the W692A mutant is now entropy-, rather than enthalpy-governed, which is in sharp contrast to its binding to other MΔK mutants, except for D623N (Figure 2, Table 2, and Figure S7). Such a thermodynamic pattern indicates that binding is accomplished in a much less directed and tight manner. Considering the 3D structural model of the CCT:CDPCho complex, we suggest that the W692 residue may have an additional role, beyond a catalytically competent accommodation of the substrate, in maintaining local structural integrity as well, by forming π–π interaction with the F662 and coordinating N716 (see Figure 1).

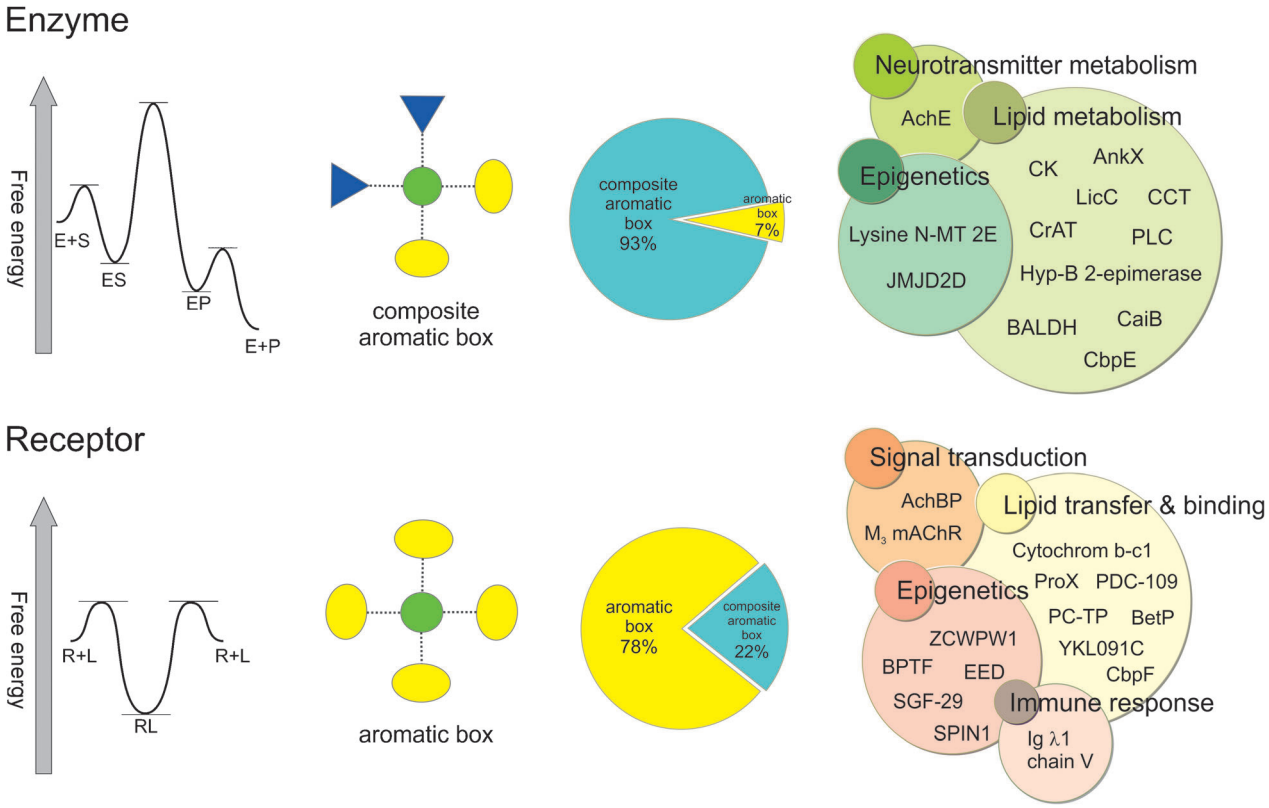
Table 3: Kinetic characterization of *Pf*CK mutants.[a]

Enzyme	k_{cat} [s^{-1}]	$K_{M,ChoP}$ [mM]	rel. $k_{cat}/K_{M,ChoP}$
*Pf*CK^{WT}	23.6 ± 1.0	0.24 ± 0.01	1.0 ± 0.1
*Pf*CK^{W392A}	7.0 ± 0.1	6.8 ± 0.2	0.01
*Pf*CK^{W395A}	ND[a]	ND[a]	ND[a]
*Pf*CK^{Y414A}	25.9 ± 1.1	0.94 ± 0.05	0.26
*Pf*CK^{Y329A}	58.1 ± 1.2	3.3 ± 0.1	0.18
*Pf*CK^{E324A}	35.5 ± 4.7	15.9 ± 2.3	0.028 ± 0.002

[a] Values cannot be determined precisely as seriously impaired ligand binding rendered results of Michaelis–Menten fit inaccurate.

Our study showed that during catalysis performed by *Pf*CK and *Pf*CCT, enzyme function is governed by a combination of cation–π and charged interactions to the quaternary ammonium moiety. We extended our investigations to all other enzymes harboring such site, by performing a comprehensive search in the protein data bank for structures containing quaternary ammonium ligands using Relibase[7] and analyzing the accommodation patterns in each case, as detailed in the SI. We found quaternary ammonium ligands in enzymes and also in receptors without enzymatic function (Table S5). In overwhelming majority of enzyme:ligand complex structures, the quaternary ammonium ligand was coordinated by both aromatic and charged or polar interactions (86 hits, i.e., 93 %).

In these structures, only one or two aromatic rings coordinated the respective substrate or product, whereas further coordination was accomplished by polar or negatively charged residues (Scheme 1, Figure 4, and Figure S9). The respective aromatic residues in several instances were shown to have key contribution to ligand binding[8] and catalysis.[9–15] Noteworthy, this general architecture appears in a great number of protein families, belonging to 15 protein family clans thus providing another spectacular example for the concept of convergent evolution. Importantly, these enzymes are involved in neurotransmission, epigenetics, and lipid metabolism. We propose here the term "composite aromatic box" for this universal enzyme binding geometry. The idea that the biological recognition of quaternary ammonium ligands is accomplished by the involvement of hydrophobic and cation–π interactions instead of a fully anionic site was proposed as early as 1990.[16] Later, this concept was confirmed for acetylcholinesterase, the most well-discussed representative enzyme in which the aromatic ring of a tryptophan facing the positively charged ligand moiety in the active site can provide a suitable environment by means of cation–π interactions.[17] The energetic contribution of such interactions to ligand binding was estimated to be comparable to a hydrogen bond or an ion pair.[18] Nevertheless, the herein defined universal composite aromatic box recognition pattern is clearly distinct from the well-known aromatic box or aromatic cage architecture, abundantly found at receptor



Scheme 1. Model of widespread structural solutions for quaternary ammonium binding sites in enzymes (composite aromatic box) and in receptors (aromatic box). General quaternary ammonium recognition site pattern of enzymes (E, left) differs from that of receptors (R, right). The different types of interactions are represented schematically; for the color code, see Figure 2. Representatives of enzymes and receptors with quaternary ammonium ligands are listed (for more detailed information see Tables S6 and S7 in the SI). Their distinct biological functions are illustrated by free energy profiles showing substrate (S) to product (P) interconversion, catalyzed by enzymes, and ligand (L) binding by receptors.

proteins (78% of relevant receptor hits; Figure S10) in which three or four aromatic rings are positioned to face the bound charged moiety of the quaternary ammonium ligand (Scheme 1), with an additional accompanying acidic residue for charge compensation (Figure 4, panel F).

The multiple aromatic rings in the aromatic box recognition site are positioned to face the bound charged moiety of the ligand and thus provide an optimal geometric fit.[19] This geometry can ensure a tight ligand binding due to the multivalence effect.[20] Accordingly, deletion of aromatic residues was shown to produce severe effects on ligand binding affinity as described in several studies.[21–24] Trimethyllysine recognition by histones is a peculiar event, which in some cases is accomplished by two aromatic side chains as well as charged residues, referred to as "half aromatic cage" site.[25] However, in this specific interaction type, found in 20 of 30 composite aromatic box-like receptor hits (see Table S5), the efficient ligand binding is further stabilized by additional peptide–peptide interactions.

Why are binding sites for quaternary ammonium ligand characteristically different in enzyme versus receptor proteins? We suggest that these differences are tuned to the different kinetic and thermodynamic requirements of enzymes and receptors. On the one hand, the physiological function of a receptor without enzymatic function is to provide specific high affinity binding in which both fast association and slow dissociation of the ligand is required. An aromatic box with its sterically restricted and in many cases rigid environment seems to be optimally suited to perform such a role.[26] Receptor proteins possessing aromatic boxes are associated with widespread physiological function from signaling to ligand binding or transfer and cellular defense mechanisms (Scheme 1). On the other hand, enzymatic functions have distinct requirements. The active site of an enzyme is of course responsible for ligand accommodation, but a sufficiently high conformational dynamics is needed to assist activation, chemical transformation, and release of the transformed ligand(s). For efficient catalysis, the ability of fast ligand binding and release is an absolute requirement; for example, in G-proteins, GTP hydrolysis is limited by slow dissociation of the GDP product, rendering these enzymes to be inefficient catalysts on their own.[27] We propose that the presence of charged or polar residues within the composite aromatic boxes of enzymes allow a less hydrophobic character for the quaternary ammonium binding cleft, leading to reduced desolvation of the bound ligand moiety. This in some cases may also contribute to increased local conformational dynamics, for instance, as shown in reference [28]. The increased conformational dynamics required for enzymatic function may also be provided by the pronounced flexibility of aromatic side chains with a partially polar character (e.g., tyrosine). Actually, for betaine aldehyde dehydrogenase,

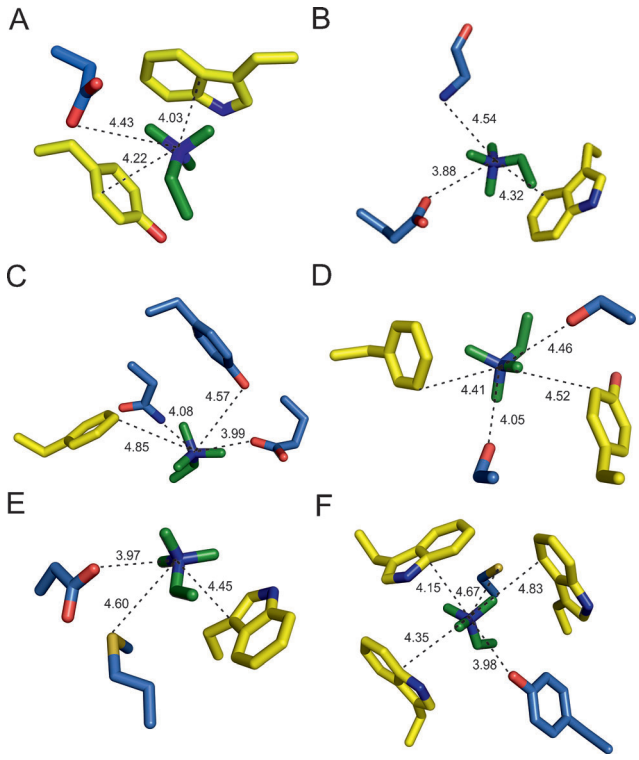


Figure 4. Quaternary ammonium recognition sites in enzymes (panels A–E) versus receptors (panel F). Selected examples show the characteristic composite aromatic box-type ligand binding site in enzymes. A) LicC bacterial CTP:phosphocholine cytidylyltransferase, PDB ID: 1JYL; B) Acetylcholinesterase (AchE), PDB ID: 2A3; C) Phospholipase C (PLC), PDB ID: 1P6D D) Carnitine *O*-acetyltransferase (1S5O); E) histone lysine *N*-methyltransferase, PDB ID: 4I58; F) Glycine betaine-binding protein (proX), PDB ID: 1R9L, is shown as illustration of the aromatic box structure present in receptors. Only the quaternary ammonium moiety is shown from the ligand structure as well as the side chain of residues. Charged and cation–π interactors are displayed within 5 Å and 6 Å of the quaternary nitrogen atom, respectively. Residues are shown as sticks, with atomic coloring based on the type of interaction in that they are involved (cation–π: yellow carbon; polar or charged: blue carbon; quaternary ammonium ligand, green carbon; O, red; N, blue; P, orange. For other examples of enzyme and receptor recognition, see the SI, Figures S9 and S10 and Tables S6 and S7.

computational studies proposed an accommodation of the quaternary ammonium ligand within the active site by four aromatic residues with significant static and dynamic disorder.[29] To gain in-depth insight in how residues of the composite aromatic box epitope site play their role in enzyme action, further thorough kinetic and structural analyses are clearly needed for each individual enzyme utilizing a quaternary ammonium.

In conclusion, we identified a potentially general architecture element termed as composite aromatic box within the active site of enzymes catalyzing the conversion of quaternary ammonium substrates. Harboring only one or two, instead of the three to four aromatic residues for cation–π interactions as seen in the aromatic box or cage, the composite aromatic box is proposed to be advantageous for assisting multiple steps of enzyme action. We hypothesize that the necessary conformational changes during the catalytic cycle may be poorly compatible with a fully hydrophobic rigid environment of an aromatic box. In a case study involving *Pf*CK and *Pf*CCT enzymes, we revealed the role of both charged/polar and aromatic residues within a composite aromatic box in ligand binding and catalysis. Together with literature data, our study points toward a general view of composite aromatic box sites in enzymes. This may lead to further pharmacological consequences, because many enzymes with quaternary ammonium ligands play important roles either in severe infectious diseases[30] or in diverse metabolic processes as lipid biosynthesis, epigenetics, or neurotransmitter metabolism. Their deficiencies lead to serious adverse phenotype effects in humans and therefore they themselves are well established drug targets.[31–34] The present study also contributes to a better understanding of the action of two malaria parasite enzymes, *Pf*CK and *Pf*CCT, that constitute potential antimalarial drug targets and may help in the ongoing structure-based pharmacophore design and validation.

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[1] A. S. Mahadevi, G. N. Sastry, *Chem. Rev.* **2013**, *113*, 2100 – 2138.
[2] D. A. Dougherty, *Acc. Chem. Res.* **2013**, *46*, 885 – 893.
[3] L. M. Salonen, M. Ellermann, F. Diederich, *Angew. Chem. Int. Ed.* **2011**, *50*, 4808 – 4842; *Angew. Chem.* **2011**, *123*, 4908 – 4944.
[4] H. J. Vial, S. Wein, C. Farenc, C. Kocken, O. Nicolas, M. L. Ancelin, F. Bressolle, A. Thomas, M. Calas, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 15458 – 15463.
[5] S. Wein, M. Maynadier, Y. Bordat, J. Perez, S. Maheshwari, P. Bette-Bobillo, C. Tran Van Ba, D. Penarete-Vargas, L. Fraisse, R. Cerdan, et al., *Br. J. Pharmacol.* **2012**, *166*, 2263 – 2276.
[6] G. N. Nagy, L. Marton, B. Krámos, J. Oláh, Á. Révész, K. Vékey, F. Delsuc, É. Hunyadi-Gulyás, K. F. Medzihradszky, M. Lavigne, et al., *FEBS J.* **2013**, *280*, 3132 – 3148.
[7] M. Hendlich, A. Bergner, J. Günther, G. Klebe, *J. Mol. Biol.* **2003**, *326*, 607 – 620.
[8] J. Lee, J. Johnson, Z. Ding, M. Paetzel, R. B. Cornell, *J. Biol. Chem.* **2009**, *284*, 33535 – 33548.
[9] B.-Y. Kwak, Y.-M. Zhang, M. Yun, R. J. Heath, C. O. Rock, S. Jackowski, H.-W. Park, *J. Biol. Chem.* **2002**, *277*, 4343 – 4350.
[10] C. Yuan, C. Kent, *J. Biol. Chem.* **2004**, *279*, 17801 – 17809.
[11] A. Ordentlich, D. Barak, C. Kronman, N. Ariel, Y. Segall, B. Velan, A. Shafferman, *J. Biol. Chem.* **1995**, *270*, 2082 – 2091.
[12] S. F. Martin, B. C. Follows, P. J. Hergenrother, B. K. Trotter, *Biochemistry* **2000**, *39*, 3410 – 3415.
[13] G. Jogl, L. Tong, *Cell* **2003**, *112*, 113 – 122.
[14] K. D. Green, V. R. Porter, Y. Zhang, S. Garneau-Tsodikova, *Biochemistry* **2010**, *49*, 6219 – 6227.
[15] V. Campanacci, S. Mukherjee, C. R. Roy, J. Cherfils, *EMBO J.* **2013**, *32*, 1469 – 1477.
[16] D. A. Dougherty, D. A. Stauffer, *Science* **1990**, *250*, 1558 – 1560.
[17] J. L. Sussman, M. Harel, F. Frolow, C. Oefner, A. Goldman, L. Toker, I. Silman, *Science* **1991**, *253*, 872 – 879.
[18] N. Zacharias, D. A. Dougherty, *Trends Pharmacol. Sci.* **2002**, *23*, 281 – 287.
[19] K. Schärer, M. Morgenthaler, R. Paulini, U. Obst-Sander, D. W. Banner, D. Schlatter, J. Benz, M. Stihle, F. Diederich, *Angew.*

Chem. Int. Ed. **2005**, *44*, 4400 – 4404; *Angew. Chem.* **2005**, *117*, 4474 – 4479.
[20] H.-J. Schneider, *Angew. Chem. Int. Ed.* **2009**, *48*, 3924 – 3977; *Angew. Chem.* **2009**, *121*, 3982 – 4036.
[21] A. Schiefner, J. Breed, L. Bösser, S. Kneip, J. Gade, G. Holtmann, K. Diederichs, W. Welte, E. Bremer, *J. Biol. Chem.* **2004**, *279*, 5588 – 5596.
[22] S. Ressl, A. C. Terwisscha van Scheltinga, C. Vonrhein, V. Ott, C. Ziegler, *Nature* **2009**, *458*, 47 – 52.
[23] A. C. Kruse, J. Hu, A. C. Pan, D. H. Arlow, D. M. Rosenbaum, E. Rosemond, H. F. Green, T. Liu, P. S. Chae, R. O. Dror, et al., *Nature* **2012**, *482*, 552 – 556.
[24] J. A. Olsen, T. Balle, M. Gajhede, P. K. Ahring, J. S. Kastrup, *PLoS One* **2014**, *9*, e91232.
[25] M. Yun, J. Wu, J. L. Workman, B. Li, *Nat. Publ. Gr.* **2011**, *21*, 564 – 578.
[26] K. Haga, A. C. Kruse, H. Asada, T. Yurugi-Kobayashi, M. Shiroishi, C. Zhang, W. I. Weis, T. Okada, B. K. Kobilka, T. Haga, et al., *Nature* **2012**, *482*, 547 – 551.
[27] R. D. Vale, *J. Cell Biol.* **1996**, *135*, 291 – 302.
[28] B. Sanson, J.-P. Colletier, Y. Xu, P. T. Lang, H. Jiang, I. Silman, J. L. Sussman, M. Weik, *Protein Sci.* **2011**, *20*, 1114 – 1118.
[29] Á. G. Díaz-Sánchez, L. González-Segura, C. Mújica-Jiménez, E. Rudiño-Piñera, C. Montiel, L. P. Martínez-Castilla, R. A. Muñoz-Clares, *Plant Physiol.* **2012**, *158*, 1570 – 1582.
[30] J. A. Hermoso, L. Lagartera, A. González, M. Stelter, P. García, M. Martínez-Ripoll, J. L. García, M. Menéndez, *Nat. Struct. Mol. Biol.* **2005**, *12*, 533 – 538.
[31] T. A. Lagace, N. D. Ridgway, *Biochim. Biophys. Acta Mol. Cell Res.* **2013**, *1833*, 2499 – 2510.
[32] I. Anghelescu, I. Heuser, *MMW Fortschr. Med.* **2007**, *149 Suppl*, 76 – 78.
[33] D. Tousoulis, C. Bakogiannis, A. Briasoulis, N. Papageorgiou, E. Androulakis, G. Siasos, G. Latsios, A.-M. Kampoli, M. Charakida, K. Toutouzas, et al., *Curr. Pharm. Des.* **2013**, *19*, 1587 – 1592.
[34] C. Biancotto, G. Frigè, S. Minucci, *Adv. Genet.* **2010**, *70*, 341 – 386.

