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COMMUNICATION

# Membrane bound COMT isoform is an interfacial enzyme: general mechanism, new drug design paradigm

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**The enzyme Catechol-O-Methyltransferase (COMT) has water soluble (S-COMT) and membrane associated (MB-COMT), bitopic, isoforms. Of these MB-COMT is a drug target in relation to the treatment of Parkinson's disease. Using a combination of computational and experimental protocols, we have determined the substrate selection mechanism specific to MB-COMT. We show: 1) substrates with preferred affinity for MB-COMT over S-COMT orient in the membrane in a fashion conducive to catalysis from the membrane surface and 2) binding of COMT to its cofactor ADOMET induces conformational change that drives the catalytic surface of the protein to the membrane surface, where the substrates and $Mg^{2+}$ ions, required for catalysis, are found. Bioinformatics analysis reveals evidence of this mechanism in other proteins, including several existing drug targets. The development of new COMT inhibitors with preferential affinity for MB-COMT over S-COMT is now possible and insight of broader relevance, into the function of bitopic enzymes, is provided.**

The enzyme Catechol-O-methyltransferase (COMT) is found in a wide range of tissue types[1] (e.g., kidneys, liver, intestinal tract and brain tissue). It methylates the hydroxyl oxygen in the catechol moiety of a range of substrates, including catecholamines (dopamine, epinephrine and norepinephrine), catechol estrogens and catechol containing xenobiotics,[2] resulting in the deactivation of the substrate. Its catalytic mechanism involves 1) binding of the S-adenosyl methionine (ADOMET) cofactor, which 2) induces the formation of a chelation site, where 3) an $Mg^{2+}$ ion binds, 4) activating the catalytic site.[1] Two separate isoforms of COMT exist: a water soluble form, S-COMT, and a membrane associated form, MB-COMT, that differs from S-COMT in the addition of a 51 residue long segment, composed of a linker and a trans-membrane helix that anchors the protein to a lipid membrane.[3] While the two isoforms share an identical catalytic domain, their enzyme kinetics differ; for example, MB-COMT has a higher affinity but lower capacity towards monoamine substrates than S-COMT.[4] Recently, Robinson et al.[5] reported the affinity of a variety of known COMT substrates and inhibitors for both isoforms; they identified a subset with preferred affinity for MB-COMT, that ceases in absence of the lipid membrane.[6].

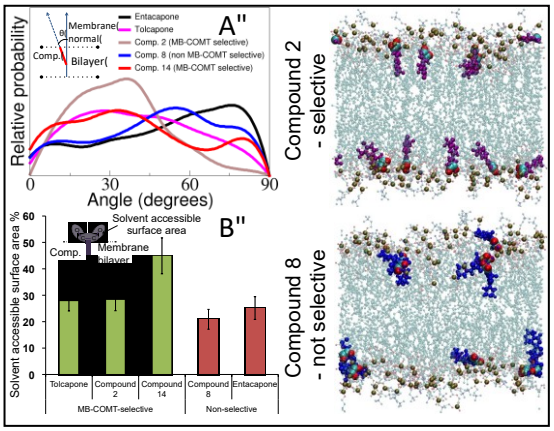


**Figure 1: Visualization of membrane with selective and non-selective compounds and orientation (A) and SASA (B) for all five compounds simulated with membrane.**

Substrate differentiation between the two COMT isoforms has important implications: MB-COMT is prevalent in brain tissue, thus predominantly involved in its neurological role,[7] while S-COMT is more prevalent elsewhere, thus mostly involved in the other roles the enzyme plays. Currently MB-COMT is an important drug target for inhibition,[1] regarding its role in neurochemistry.[2] For example, dopamine, the neurotransmitter and the drug L-dopa, the leading treatment

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for Parkinson's disease, [2,8] are methylated by COMT; selective targeting of MB-COMT is thus desirable. Two COMT inhibitors are worthy of special attention: entacapone[9] and tolcapone[10]. In clinical trials tolcapone has shown superior performance to entacapone,[11] however it has been withdrawn due to toxicity issues.[12,13] Neither of these, nor, so far, any other drug candidate molecule, has been developed with selective targeting of MB-COMT in mind.

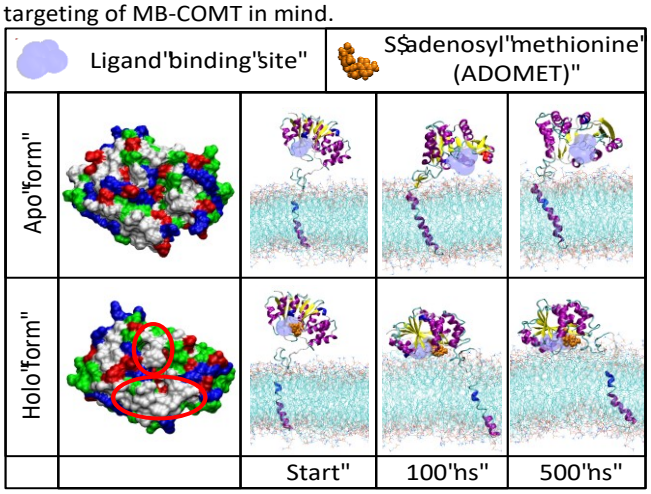


**Figure 2: Visualization of frames from trajectories of both holo and apo forms of MB-COMT bound to an intracellular membrane. The alteration to the surface around the catalytic site resulting from binding of the S-adenosyl methionine (ADOMET) ligand is also shown. The increase in the hydrophobicity of the surface of the protein in the region of the catalytic site is indicated. Also note the basic and polar residues that surround the hydrophobic surfaces, completing the membrane binding footprint.**

Bitopic enzymes, like MB-COMT, consist of a catalytic domain attached to the lipid membrane through only a linker segment and single membrane spanning helix;[14] they represent an important class of protein that has seen relatively little study. The only available complete structure of an individual bitopic enzyme[15] shows evidence that the transmembrane helix and linker segment orient the position of the catalytic domain with respect to the membrane; a direct role for the lipid membrane in the catalytic mechanism[15] and thus substrate selection, is suggested. Altogether, the evidence indicates interactions of both potential substrates and catalytic domain with the lipid membrane play a role in the catalytic activity of MB-COMT.

We used molecular dynamics simulation (MD) to study the interactions of both substrates and COMT with the lipid membrane. Four independent experimental techniques were used to verify our MD results: Surface Second Harmonic Generation (SSHG), Quartz crystal microbalance (QCM), Surface Plasmon Resonance (SPR) and Isothermal calorimetry (ITC). See Supplementary Information for all methodological details not found in the main text (MD simulation in Section 1, SSHG experiments in Section 2 and QCM, SPR and ITC in Section 3)

We performed MD simulations of five compounds interacting with an intracellular lipid membrane: three different COMT inhibitors studied by Robinson et al.,[5] two selective for MB-COMT vs. S-COMT, referred to as compound 2 (ratio of IC50 values ~17) and compound 14 (ratio of IC50 values ~19) and a

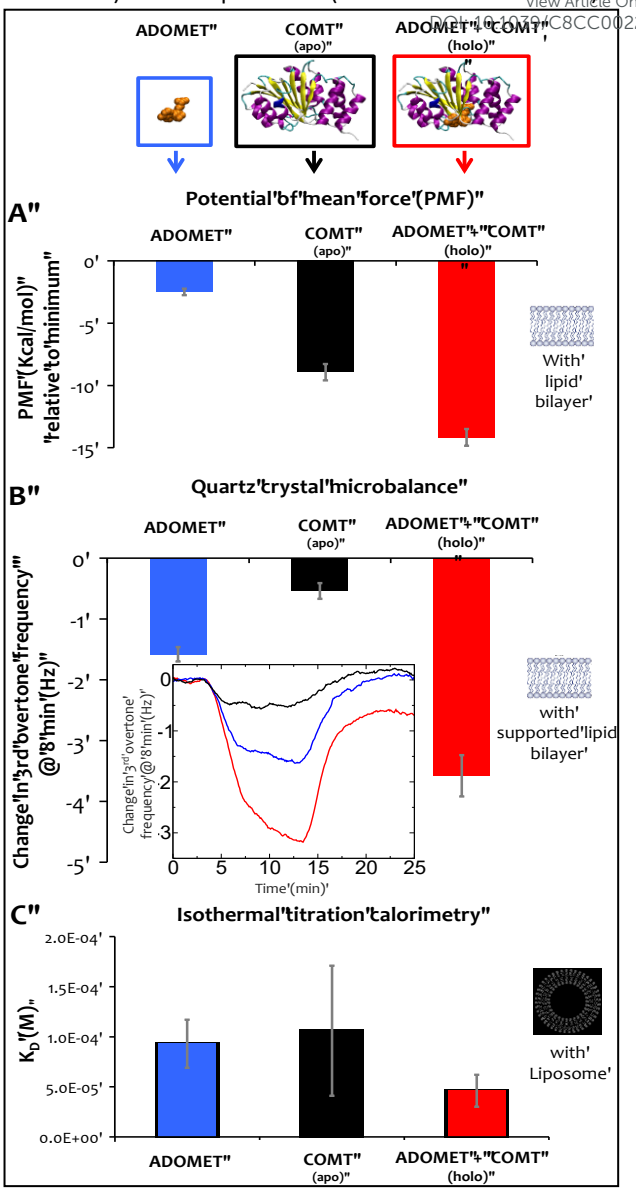


**Figure 3: Interaction of ADOMET (blue bar) and catalytic domain of COMT in apo (black bar) and holo (red bar) form with membrane: (A) PMF change when pulled away from lipid membrane, (B) QCM frequency responses (measured at the 3rd overtone) during interaction with the lipid bilayer. (C) Dissociation constant (inverse of affinity) from lipid bilayer (vesicle) determined by ITC.**

third that is non-selective, compound 8 (ratio of IC50 values close to one), in addition to entacapone and tolcapone, due to their relevance, as discussed above. In all five systems, the substrates partitioned to the membrane headgroups; closer observation of these (snapshots shown in Figure 1) however, reveals a key difference in behavior between compound 2, that is selective, and compound 8, that is not: compound 2 has a greater tendency to sit in the membrane with the OCCO group, involved in catalysis, oriented outwards from the membrane. In Figure 1A, the relative probability of the angle, between the major axis of the substrate molecule and the membrane normal, is plotted: the membrane selective compounds 2 and 14, along with tolcapone, have a greater probability of

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orientation outwards from the membrane (angles 0 to 45°) than that for the non-selective compound 8 and entacapone. The Solvent Accessible Surface Area (SASA) of the OCCO group for the five compounds, shown in Figure 1B, indicates a greater exposure of the compounds that are selective than those that are not, while the exposure of tolcapone is slightly greater than that of entacapone. Results for the mass density profiles of the OCCO groups vs. that of the entire substrate molecule (see Supplementary Information, Section 4, Figure 2) and the potential of mean force (PMF) change when a molecule is pulled off the membrane surface (see Supplementary Information, Section 4, Figure 3) also indicate this. In previous studies,[16-18] the interaction between dopamine, a natural substrate of COMT, with a lipid membrane, has been studied in greater detail. Using data from our previous work[16], we calculated the orientation of the dopamine molecules in the membrane and found (see Supplementary Information, Section 4, Figure 4) behavior similar to that of the selective inhibitors.

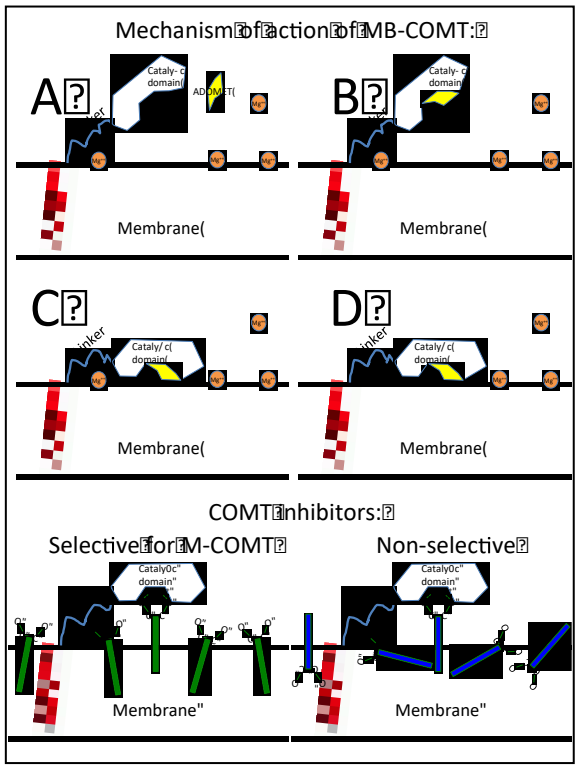


**Figure 4: Catalytic mechanism for MB-COMT, that differentiates it from S-COMT and the behavior of MB-COMT selective vs. non-selective inhibitors in the membrane.**

The SSHG technique[19,20] can be used to study the orientation of molecular probes at interfaces;[21] we investigated the orientation of both tolcapone and entacapone (the compounds studied by Robinson et al. [5] are no longer available) on a lipid membrane. Our results, described in greater detail in Supplementary Information, Section 5 and figures therein, show that there is indeed a difference between the respective orientations of the entacapone and tolcapone molecules in the membrane. A quantitative comparison with the MD simulation results was not attainable, however, this result indirectly excludes the possibility that both tolcapone and entacapone orient with their OCCO group directed outwards from the membrane.

Structures of the catalytic domain of COMT were obtained from the PDB database in both cofactor bound, holo (pdb id: 2CL5) and unbound, apo (pdb id: 2ZLB) forms. As shown in Figure 2 and, in greater detail, Supplementary Information, Section 4, Figure 5, binding of the cofactor induces conformational changes to the catalytic surface of COMT: in the holo form, when ADOMET is bound, the surface displays a hydrophobic patch (shown in white) surrounded by a ring of polar, acidic and basic residues (shown in green, red and blue respectively), reminiscent of the binding footprint of a peripheral membrane protein.[22,23]

To study the interaction between lipid membrane and COMT, six simulations of length 0.5 μ-seconds, three replica systems each for both holo and apo forms of MB-COMT, were performed. As shown in Figure 2, visualizations of the systems demonstrate increased affinity between the catalytic surface of the protein and the membrane surface, when the protein is in the holo form. We then performed force biased simulations of the membrane with the catalytic domain of COMT in both holo and apo forms, in addition to the ADOMET cofactor on its own, in order to calculate the potential of mean force (PMF) along the membrane normal; results are shown in Figure 3A. The change in PMF (free energy difference) when the holo form is removed from the membrane, is greater than the sum of the respective results for the apo form and ADOMET cofactor. While our result for the change in free energy when ADOMET is removed from the membrane is relatively small, it does locate preferentially to the membrane surface, as shown in Supplementary Information, Section 4, Figure 6. Additionally, the $Mg^{2+}$ ion, required for catalysis by COMT, has been shown to interact with the membrane headgroups (results shown in Supplementary Information, Section 4, Figure 7).

Properties that, like the PMF calculated from MD simulations, relate directly to the relative strengths of the interaction of the holo and apo forms of the catalytic domain of COMT and ADOMET cofactor with the membrane, can be measured using the QCM, SPR and ITC protocols. In our QCM experiments, the supported lipid bilayers (SLBs) used as a model of an internal cell membrane, were formed on SiO2 coated sensors using the vesicle rupture approach (described in Supplementary Information Section 3 and, section 6, Figure 13). The QCM frequency responses of the third overtone, induced after 8 minutes of interaction, is significantly larger for the holo form than the sum of the same quantity for the apo form and ADOMET cofactor (Figure 3B). Experiments performed using SPR on a similar system are in qualitative agreement with the QCM results (Supplementary Information, Section 6, Figure 14). Our ITC results indicate that the holo form exhibits higher affinity for the lipid bilayer in vesicles, as measured by a dissociation constant, KD, that is half that of the apo form (Figure 3C, dissociation constant is the inverse of affinity). All details regarding the QCM, ITC and SPR experimental results are found in Supplementary Information, Section 6.

In order to address the possible broader relevance of this phenomenon to all bitopic proteins, we carried out data mining

of the UniProt[24] database. The results are summarized in Supplementary Information, Section 7, Figure 19. We found 1) more than 1000 bitopic enzymes that require a cofactor and 2) more than 600 bitopic enzymes with a water-soluble isoform. The detailed results of our UniProt queries are included in the Supplementary Information file "Supplementary_data.xlsx". Furthermore, cross-referencing the protein hits with the DrugBank[25] database found 156 transmembrane catalytic proteins listed as drug targets; of these 110 are bitopic proteins and 65 are bitopic proteins that also have a water soluble isoform.

Altogether, our computational and experimental results show 1) substrates with greater affinity for MB-COMT over S-COMT orient in the membrane in a fashion conducive to catalysis from outside the membrane and 2) binding of the ADOMET cofactor increases the affinity of the catalytic domain of COMT for the lipid membrane. The mechanism of catalytic action specific to MB-COMT, that differentiates it from S-COMT thus emerges, shown in Figure 4: 1) the ADOMET cofactor binds to the catalytic site. 2) This forms the membrane binding footprint around the catalytic site, which 3) drives the catalytic domain to the membrane surface. Once at the membrane surface 4) the protein binds an $Mg^{2+}$ ion, present at the membrane surface. Finally, 5) the substrate at the membrane surface is bound and catalyzed. Thus (as also shown in Figure 4), if a substrate locates to the membrane with their OCCO groups oriented outwards, this will increase the affinity for MB-COMT but not affect affinity for S-COMT, possibly resulting in preferential affinity for MB-COMT over S-COMT. Tolcapone behaved in a manner more in line with the MB-COMT selective compounds than entacapone; this could be the cause of the greater efficacy observed for tolcapone. Our data mining results indicate this mechanism may be applicable to a broader range of proteins that possess both water soluble and membrane bound, bitopic, isoforms.

## Conflicts of interest

There are no conflicts to declare.

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