Insights from molecular dynamics: the binding site of cocaine in the dopamine transporter and permeation pathways of substrates in the leucine and dopamine transporters

Bonnie A. Merchant and Jeffry D. Madura

Department of Chemistry and Biochemistry and Center for Computational Sciences, Duquesne University, 600 Forbes Avenue, Pittsburgh, PA 15282

April 24, 2012

Supporting Information

M1	**M2**	**M3**	**M4**
1R5	1N21	1F76	1W84
1A22	1A22	1D79	1Y88
1G24	1G26	1W84	1Y91
1G26	1R30	1R85	6F319
1N27	1G37	1Y88	10D475
1L29	il1H74	3V145	
1R30	il1T76	3V152	
2A65	il1T77	6F319	
2G73	3Y108	6G326	
2G75	6F253	6V327	
3Y108	6T254	el4D380	
el3G221	6S256	el4D384	
el3D225	6Y268	el4G385	
6F253	el4K316	8G425	
6T254	8D369	8E436	
6S256	10D404	10D475	
6S267	10G408		
7E290			
7L293			
7I297			
7S298			
7A301			
7F305			
el4F331			
8S355			
8D369			
10D404			

Table 1: Residues interacting with the substrate observed during the simulated permeation using the MCTI method are identified by their transmembrane (TM) helix, amino acid and residue number. That is, 1F76 indicates TM 1 phenylalanine 76. Setups are abbreviated by: M1, LeuT$_{Aa}$:Leu (Vacuum); M2, LeuT$_{Aa}$:Leu (Water Sphere); M3, DAT:DA (Vacuum); M4, DAT:COC (Vacuum).

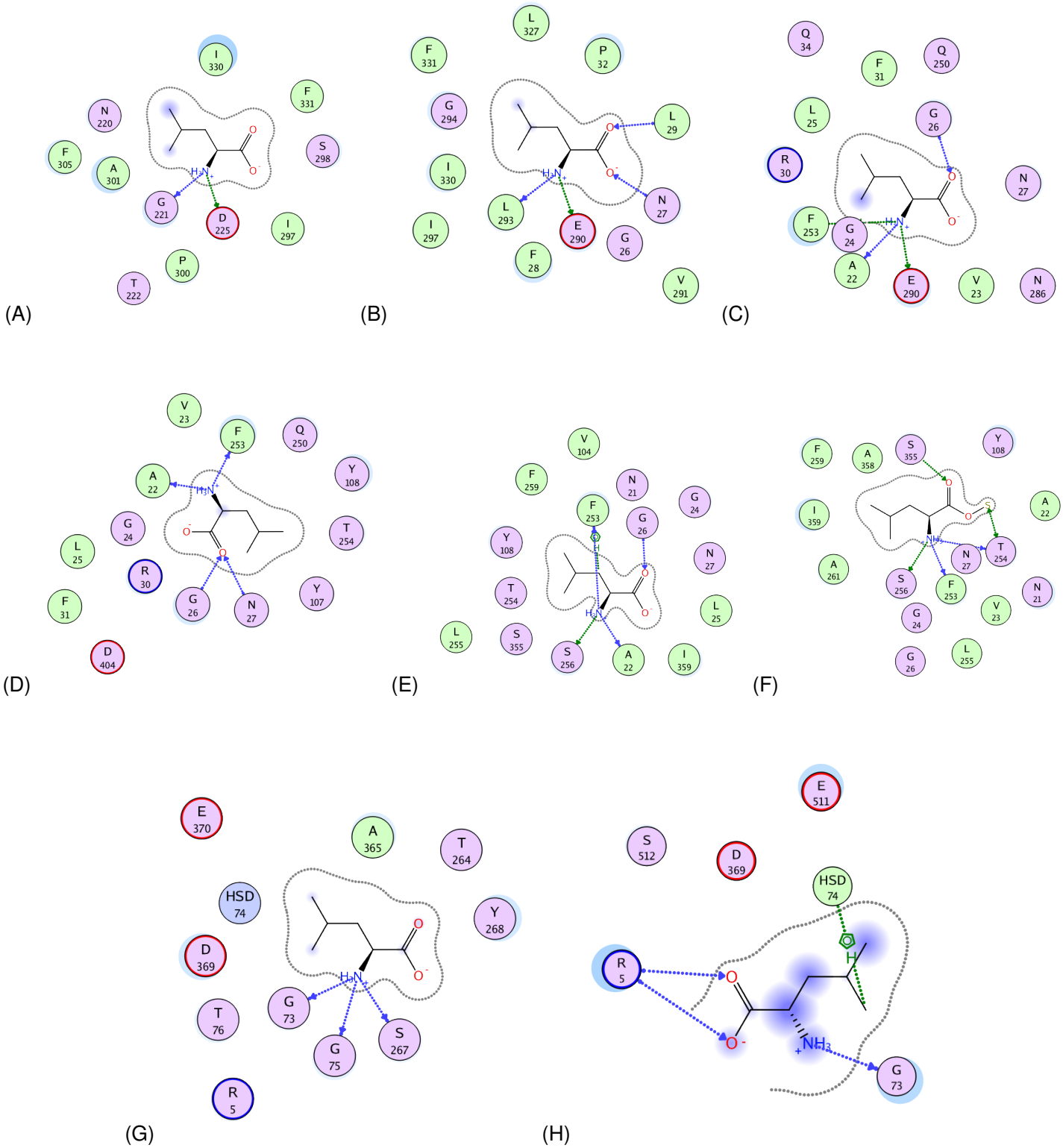

Figure 1: LEUT:LEU (in vacuum) stop over sites. 2D Interaction Plots generated using MOE 2009.10.

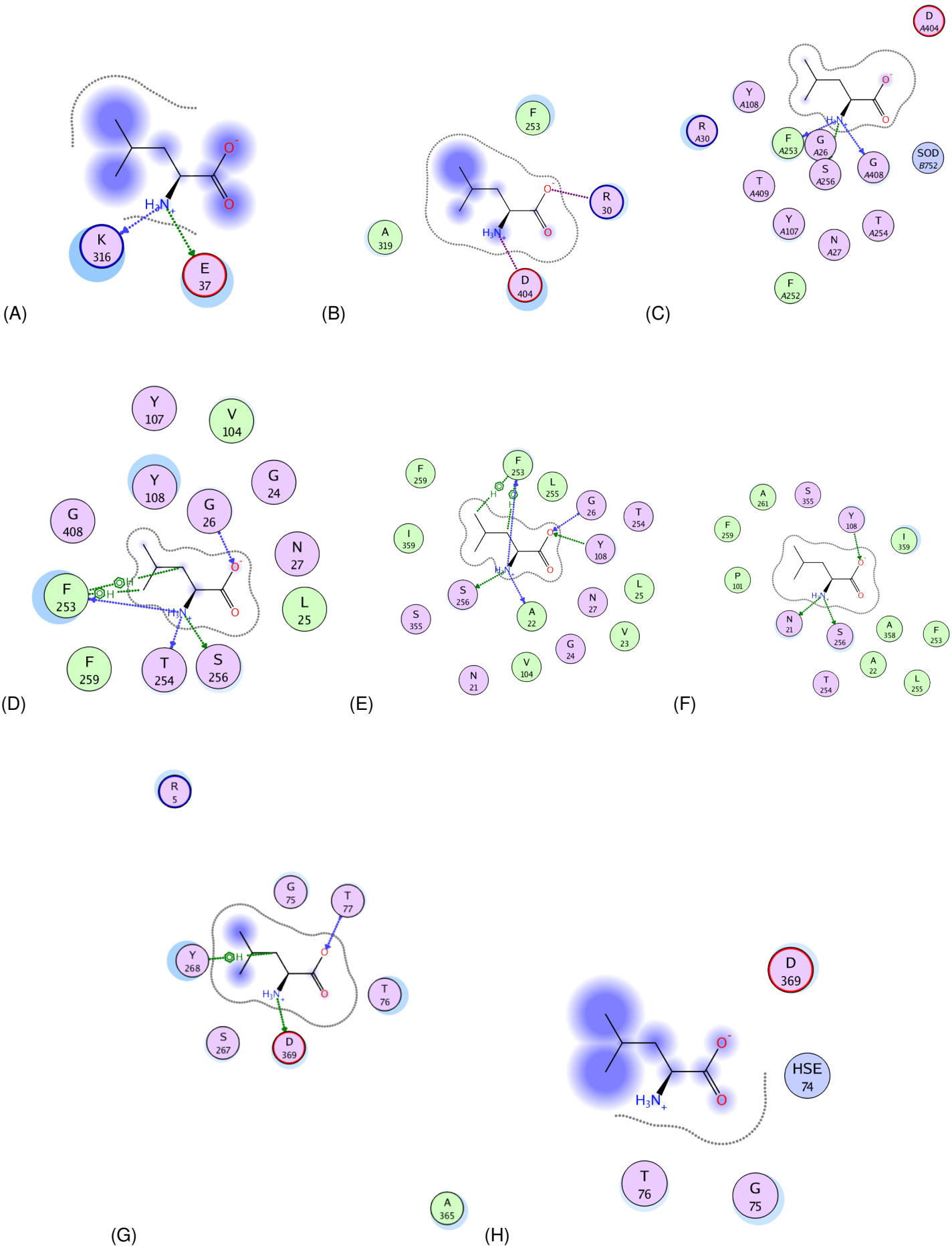

Figure 2: LEUT:LEU (in a water sphere) stop over sites. 2D Interaction Plots generated using MOE 2009.10.

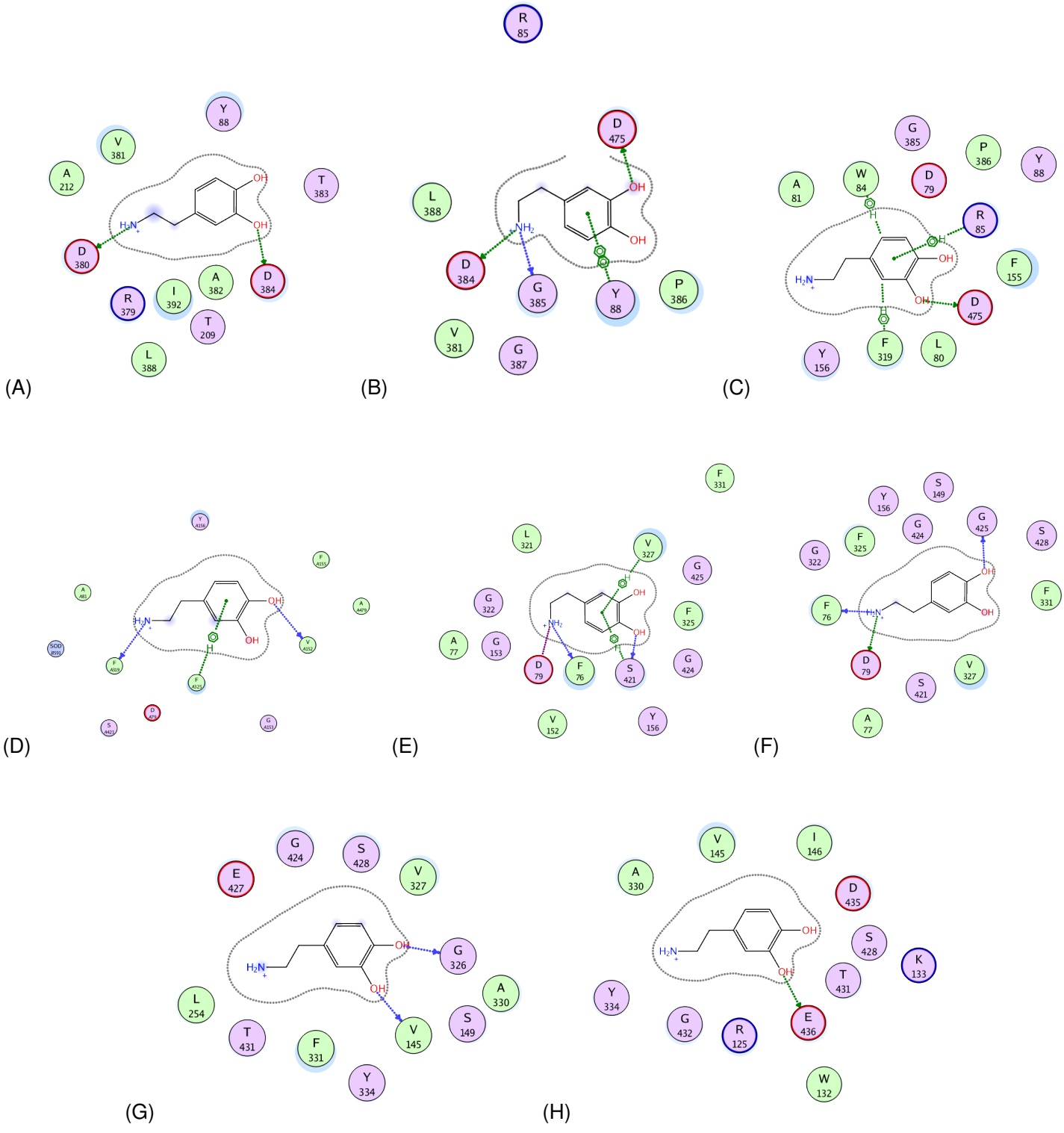

Figure 3: DAT:DA (in vacuum) stop over sites. 2D Interaction Plots generated using MOE 2009.10.

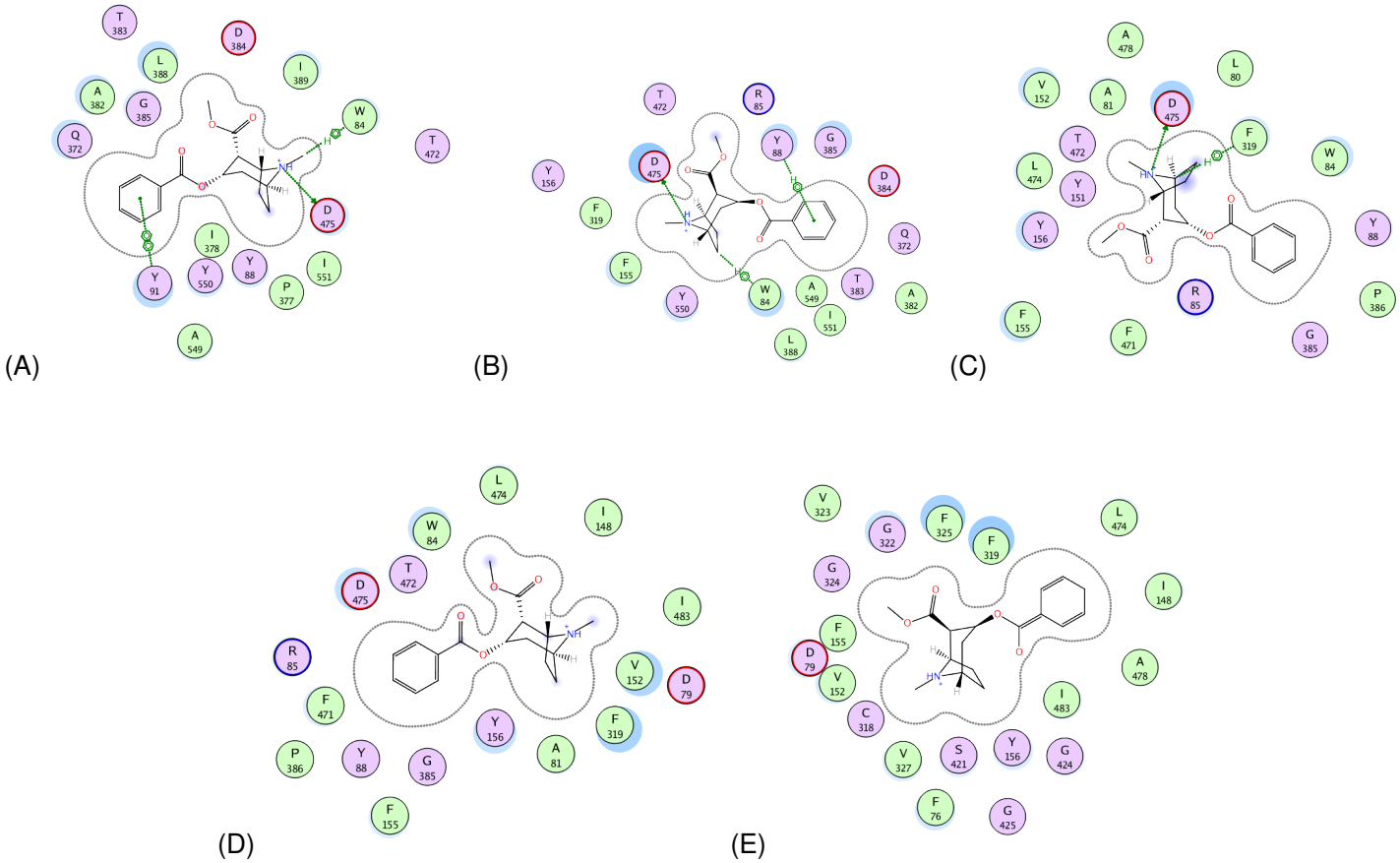

Figure 4: DAT:COC (in vacuum) stop over sites. 2D Interaction Plots generated using MOE 2009.10.

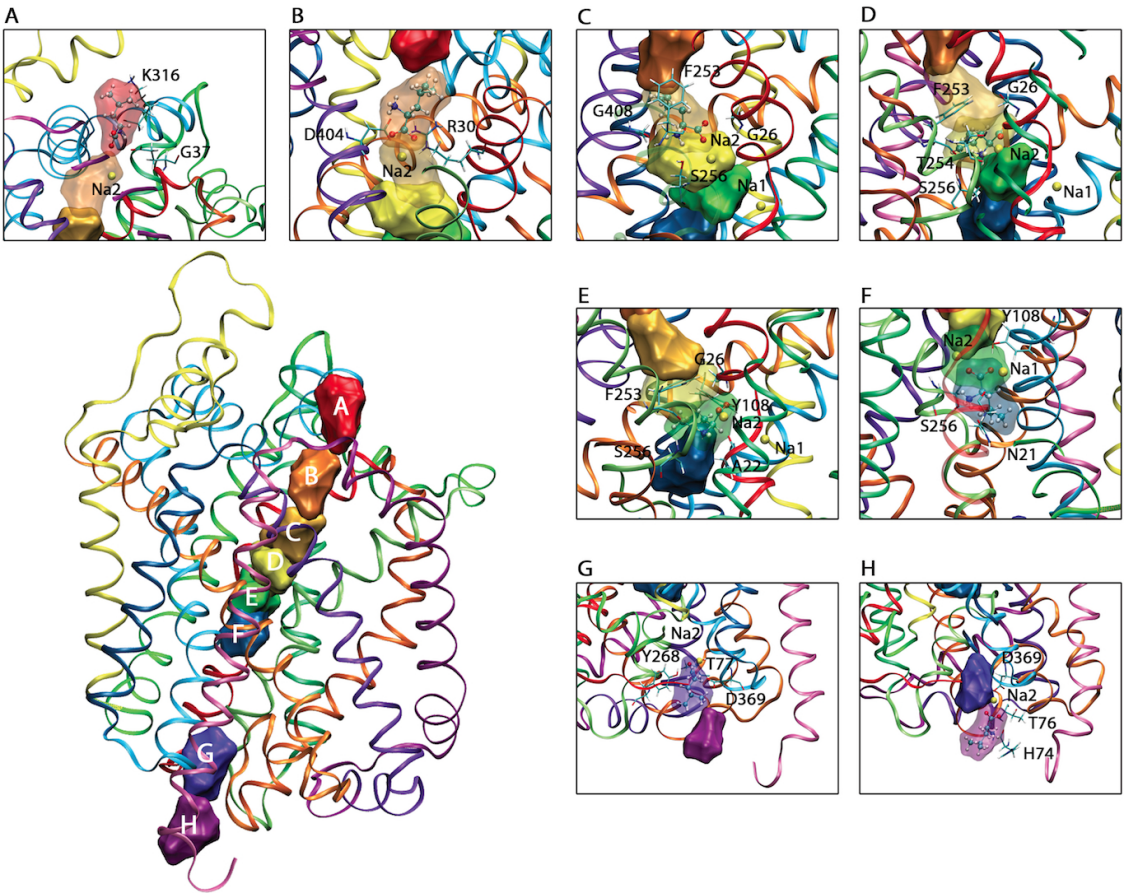

Figure 5: MCTI stop-over sites identified for *leucine in LeuT, vacuum environment*. Stop-over sites are labeled A-H, represented by a surface representation of the substrate at that particular site, with a 3.5 Å probe radius. The insets show a close up view of each site. The substrate, leucine is presented in a ball and stick representation, with the surface representation changed to transparent for clarity. (In some insets, nearby pockets are changed to transparent for added clarity and to highlight proximity and overlaps of the stop-over sites.) Key residues at each site are identified and labeled, drawn in a stick model. Note, site "E" is the primary (S1) substrate pocket while site "C" represents the secondary (S2) pocket of LeuT.

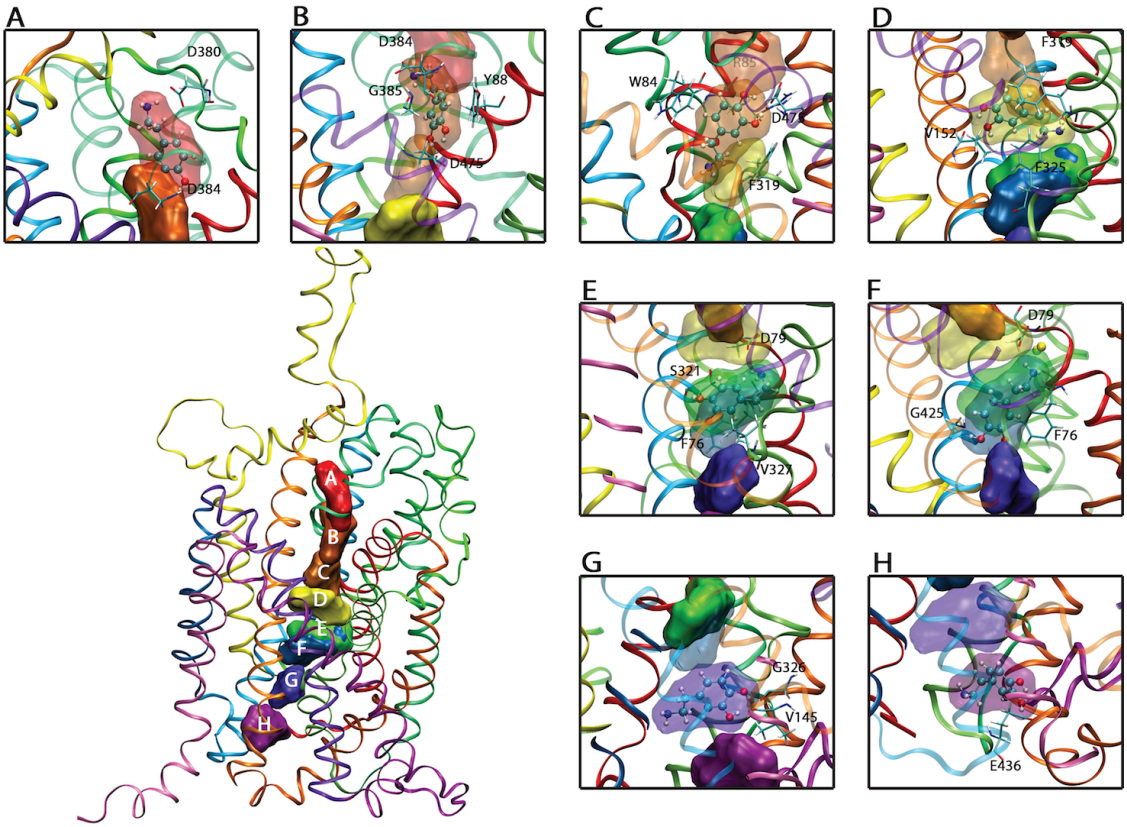

Figure 6: MCTI stop-over sites identified for *dopamine in DAT, vacuum environment*. Stop-over sites are labeled A-H, represented by a surface representation of the substrate at that particular site, with a 3.5 Å probe radius. The insets show a close up view of each site. The substrate, dopamine is presented in a ball and stick representation, with the surface representation changed to transparent for clarity. (In some insets, nearby pockets are changed to transparent for added clarity and to highlight proximity and overlaps of the stop-over sites.) Key residues at each site are identified and labeled, drawn in a stick model. Note that site "E" is the primary (S1) substrate pocket while site "D" represents the secondary (S2) pocket in DAT.

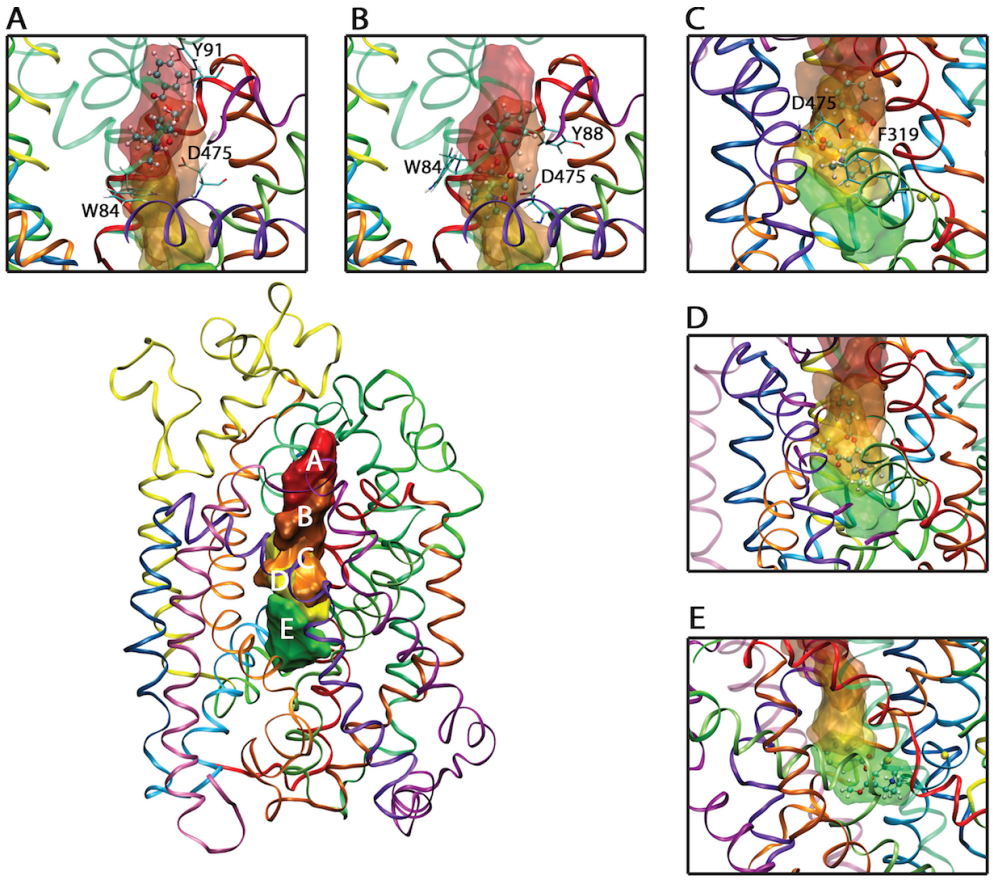

Figure 7: MCTI stop-over sites identified for *cocaine in DAT, vacuum environment*. Stop-over sites are labeled A-H, represented by a surface representation of the substrate at that particular site, with a 3.5 Å probe radius. The insets show a close up view of each site. The substrate, cocaine is presented in a ball and stick representation, with the surface representation changed to transparent for clarity. (In some insets, nearby pockets are changed to transparent for added clarity and to highlight proximity and overlaps of the stop-over sites.) Key residues at each site are identified and labeled, drawn in a stick model. Note that site "E" is the primary (S1) substrate pocket while site "D" represents the secondary (S2) pocket in DAT.

Setup	Path	Sodium?	# Times	Residues
R1	E1	No	7	1N21, 1A22, 1G26, (1R30), 3Y108, 6T254, 6S256, 8S355, (8S356), (10D404)
	I1	Yes	14	1N21, 1A22, 1G26, (il1G73), (il1G75), il1T77, (3P101), 3Y108, 6F253, 6T254, 6S256, (6F259), 6G260, 6A261, 6T264, 8S355, 8A358, (8I359), (8E370), (12F496), 12L500, (12V501), (12E505), (12R508), (12N509)
	I2	No	14	1R5, 1N21, 1A22, 1G26, (2G69), (2G70), (il1Q72), (il1G73), (il1H74), (il1G75), (il1T77), 3Y108, 6F253, 6T254, 6S256, 6F259, 6G260, (6A261), 6T264, 6S267, 6Y268, 8S355, 8A358, 8Q361, (8D369), (8E370), (12F497), (12V504)
R2	I1	Yes	8	1N21, 1A22, (2M59), 2E62, 2W63, (2G66), 6T254, 6L255, 6S256, (6A266), (6V269), (6K271), (7Q273), 7D274, (7I275), (7S278), 8S355, (il5E435)
	I2	No	9	1N21, 1A22, (1G24), (2E62), 2W63, (2A64), (2R67), (3Y108), 6T254, 6L255, 6S256, (6A261), (6I262), (6I263), (6T264), (6A266), (6Y268), (6R270), 7Q273, (7N286), (7E290), 8S355
R3	I1	Yes	31	(1R5), 1N21, 1A22, 1G24, 1G26, 1N27, il1G73, il1H74, 3Y108, 6F253, 6T254, 6S256, 6F259, 6A261, 6T264, 6Y265, 6Y268, (7N286), 8S355
R4	I1	Yes	9	1N21, 1A22, (1V23), (1G24), 1G26, (il1H74), (il1G75), (il1T77), 3Y108, 6F253, 6T254, 6S256, (6A261), 6T264, 8S355, (8D369)
	I2	No	23	1N21, 1A22, (1L25), 1G26, il1G73, il1G75, il1T76, (il1T77), 3Y108, 6F253, 6T254, 6S256, 6F259, (6G260), (6A261), 6T264, 6Y265, 8S355, (8A358), (8P362), 8E368, (8D369)
R5	I1	Yes	24	(1M18), 1N21, 2W63, 6T254, 6S256, (6A261), (6Y265), 6R270, 6F259, (6G260), (6A261), 6T264, 6Y265, 8S355, (8A358), (8P362), 8E368, (8D369)
	I2	No	18	(1R11), 1A21, (1W63), (3Y108), 6T254, 6S256, 6R270, 7Q273, 8S355

Table 2: Observed residues interacting via hydrogen bonding for each pathway cluster of LEUT RAMD simulations. We denote the transmembrane (TM) helix the residue belongs to i.e.,1N21 specifies the N21 residue, belonging to TM1. Clusters are identified as intracellular (**I**) or extracellular (**E**), followed by a number denoting a specific pathway. Observation of sodium movement with the substrate is also indicated, as well as how many paths belong to the cluster. Residues observed to participate only once in a hydrogen bond pair with the ligand of interest are denoted by placing them inside a set of parenthesis.

Setup	Path	Sodium?	# Times	Residues
R6	E1	No	4	1F76, 1D79, (1R85), 3S149, 6F319, 6L321, (6G322), (6F325), (el4T383), (8S421), (8G425), 10D475
	E2	No	10	1F76, 1D79, (1W84), 1R85, (1Y88), I148, 3S149, 3Y156, 6F319, (6S320), 6L321, 6G322, 6F325, (7N352), (el4V381), el4A382, (el4T383), (el4D384), (el4G385), 8S421, (10L474), 10D475, (10A478)
	I1	No	15	1F76, 1D79, (2E126), il1G127, il1A128, (il1A129), (il1V131), (3V141), (3T144), (3V145), 3S149, 3Y156, 6F319, 6L321, 6G322, (6G324), 6F325, (6A330), (6S333), 8S421, 8G425, (8S428), (8E436), (10D475), (10A478), 10E490, (12T576)
	I2	No	7	1S72, (1V73), 1F76, 1D79, 3S149, 3Y156, 6F319, (6S320), 6L321,6G322, 6F325, (6V327), (6L328), (6F331), (7N352), (8G424), 8S428
	I3	No	2	1K66, 1F76, 1D79, (1R85), 3S149, 3Y156, 6F319, 6L321, (7N340), (7D344), (8G425), (10D475)
	I4	No	3	1F76, 1D79, (1Y88), (2E126), (il1A128), (il1G130), (il1W132), 3S149, (3Y156), 6F319, (6L321), (6F325), (6G326), (8S421), 8G425, (8S428), (8G432), (12T576), (12F579)
R7	E1	No	44	(1V73), (1F76), (1A77), 1D79, 1W84, (1Y88), 3V152, (3F155), 3Y156, 6F319, 6V327, (el4D380), (el4V381), el4A382, el4T383, el4D384, el4G385, (el4P386), 8S421, (8G424), (8S428), (10I468), 10F471, 10T472, 10D475
R8	E1	No	34†	1W84, 1R85, el4D384, el4G385, el4P386, (el4I389), (10Y469), (10T472), 10D475
R9	E1	No	16	1F76, (1A77), 1D79, (1Y88), 3I148, 3S149, 3Y156, 6F319, 6L321, (6G322), 6F325, (el4A382), el4T383, (el4G385), 8D420, 8S421, 8G425, (10F471), 10T472, (10L474), 10D475
	I1	No	3	(1W63), (1V73), 1F76, 1D79, 3S149, 3Y156, 6F319, (6S320), 6L321, (6F325), 8S421, (8S428)
	I2	No	3	(1K66), 1F76, (1A77), 1D79, 3S149, (3V152), 3Y156, (6C318), 6F319, 6L321, (6G322), 7N339, (7N340), 7D344, 7T348, 8S421, (8G424), 8G425, (8S428), (8V429)
	I3	No	3	1F76, 1D79, (il1W132), 3S149, 6F319, 6F325, 8G425, (8E436), (12T576)
	I4	No	4	1F76, 1D79, 3S149, 3Y156, 6F319, (6L321), (6G322), 6F325, 6V327, 8S421, (8A422), (8G425), (8S428), (8G432), (8D435), 8E436
R10	E1	No	9	1F76, 1D79, 1R85, 3I148, 3S149, 3Y151, (3V152), 3Y156, 6F319, (6S320), el4T383, (el4D384), (8G418), 8S421, (8A422), (8G425), (8M426), 9A442, (10L474), 10D475, 10A478, (10A479)
	E2	No	12	1F76, (1A77), 1D79, (1A81), 1R85, (1Y88), (1K92), 3I148, 3S149, (3V152), 3Y156, 6F319, (6S320), 6L321, 6F325, (el4V381), el4A382, 8S421, (8A422), (8G425), (8S428), (10L474), 10D475, (10H476), (11H546)
	I1	No	8	(1E61), (1W63), 1D68, 1S72, 1F76, (1A77), 1D79, (3I148), 3S149, (3V152), 3Y156, (5S253), 5L254, (5W255), (5K256), (5G257), (5T260), 6F319, 6S320, 6L321, 7N352, 8D420, 8S421, 8A422, 8G424, 8G425, 8S428
R11	E1	No	17	(1V73), 1F76, 1D79, 1R85, (1Y88), 3I148, 3S149, 3Y156, (4L254), 6F319, 6L321, 6F325, (el4A382), (el4G385), (8D420), (8S421), (8S428), (10Y469), (10F471), (10T472), (10L474), 10D475), (10A478)
	I1	No	4	1F76, 1D79, 3S149, (3Y156), (4L254), 6F319, 6L321, 6V327, (8G424)
	I2	No	7	1F76, 1D79, (2R125), il1A128, il1W132, (3V145), (3I148), 3S149, 3Y156, 6F319, (6S320), 6L321, 6F325, (6V327), (7D344), (7T348), 8S421, 8G425, 8S428, (8G432), 8E436, (12T576)

Table 3: Observed residues interacting via hydrogen bonding for each pathway cluster of DAT RAMD simulations. We denote the transmembrane (TM) helix the residue belongs to i.e.,1F76 specifies the F76 residue, belonging to TM1. Clusters are identified as intracellular (**I**) or extracellular (**E**), followed by a number denoting a specific pathway. Observation of sodium movement with the substrate is also indicated, as well as how many paths belong to the cluster. Residues observed to participate only once in a hydrogen bond pair with the ligand of interest are denoted by placing them inside a set of parenthesis. † In 16/50 DAT:COC RAMD runs, no hydrogen bonding between the protein and cocaine was observed.