Supporting information for: AMASS: Algorithm for MSI Analysis by Semi-supervised Segmentation

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Supplementary Figures

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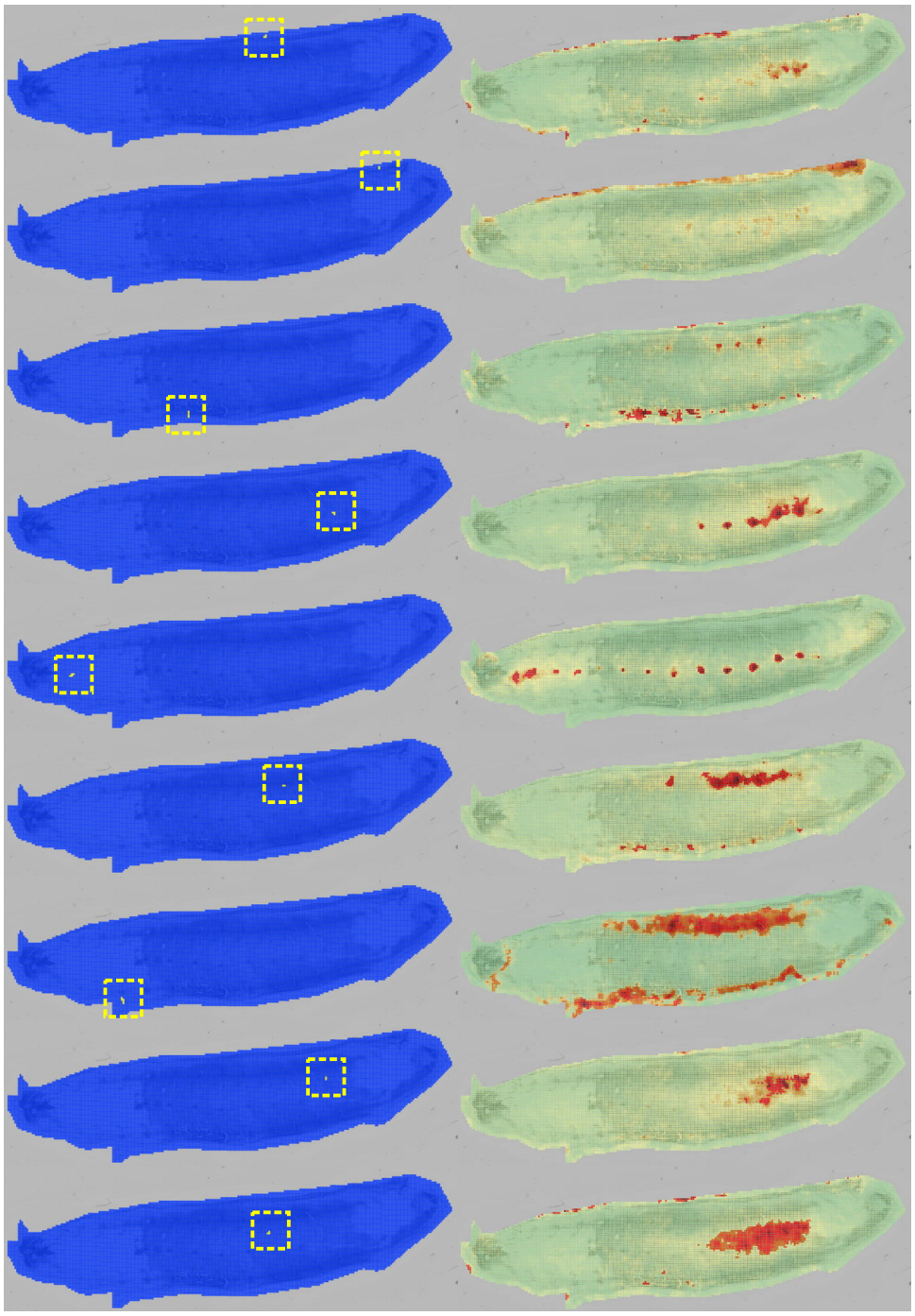


Figure 1: Querying with small random seeds also results in meaningful regions. Unlike the case of user-defined queries, many of the queries lead to similar results and some queries lead to lower quality results. Shown here are a few hand-selected random results. While the resulting log-odds images are in general not as specific as their user-defined counterpart, they still highlight different regions with specific molecular signatures. We can also expect the regions to gain specificity on the next iteration of the algorithm.

ganglia 2-4		ganglia 13-15		images
m/z	weight	m/z	weight	
		2473.88	0.82	
		2475.03	0.77	
		2476.86	0.76	
		2478.69	0.78	
2523.77	0.75	2523.77	0.90	
		2524.92	0.73	
2525.62	0.75			
3091.63	0.77	3095.98	0.73	
3299.11	0.80			
3300.69	0.78			
		3488.03	0.77	
		3490.74	0.84	
3494.28	0.73	3497.00	0.82	
3501.62	0.88	3502.71	0.96	
		3504.35	0.95	
		3505.98	0.95	
3510.61	0.98	3511.16	0.93	
3511.70	0.98			
3512.79	0.98			
3513.89	0.97			
3514.98	0.97			
3516.07	0.93			
3517.16	0.88			
3518.25	0.75			
		3652.40	0.74	
		3654.07	0.72	
4006.51	0.74			
4008.26	0.81			
		4374.30	0.80	
		4376.12	0.82	
		4377.95	0.82	
		4379.17	0.75	

ganglia 2-4		ganglia 13-15		images
m/z	weight	m/z	weight	
5273.47	0.76			
		5414.12	0.78	
		5416.16	0.77	
5418.19	0.82	5418.19	0.80	
5422.26	0.78	5419.21	0.71	
5425.31	0.70			
5564.53	0.90	5567.96	0.94	
		5570.37	0.96	
		5571.40	0.94	
		5572.43	0.92	
5573.80	0.98	5573.46	0.82	
5574.83	0.98	5574.49	0.78	
5575.86	0.98	5576.21	0.71	
5576.90	0.97			
5577.93	0.93			
5578.96	0.90			
5581.71	0.93			
5582.74	0.87			
5587.90	0.82			
8423.80	0.90			
8427.60	0.90	8428.03	0.89	
8431.41	0.93	8429.29	0.80	
8432.68	0.89			
8433.95	0.88			
8435.22	0.77			
		8561.77	0.74	
		9461.98	0.77	
		9463.33	0.74	

Figure 2: Specific molecules from the same morphological segment sometimes have slightly different signatures. We look at the molecular signatures of two queries: anterior and posterior ganglia. While many molecules are expressed throughout the CNS, some m/z values (3299,4377,5293) have differentiated signatures, consistent with the rostricaudal gradient of leech.

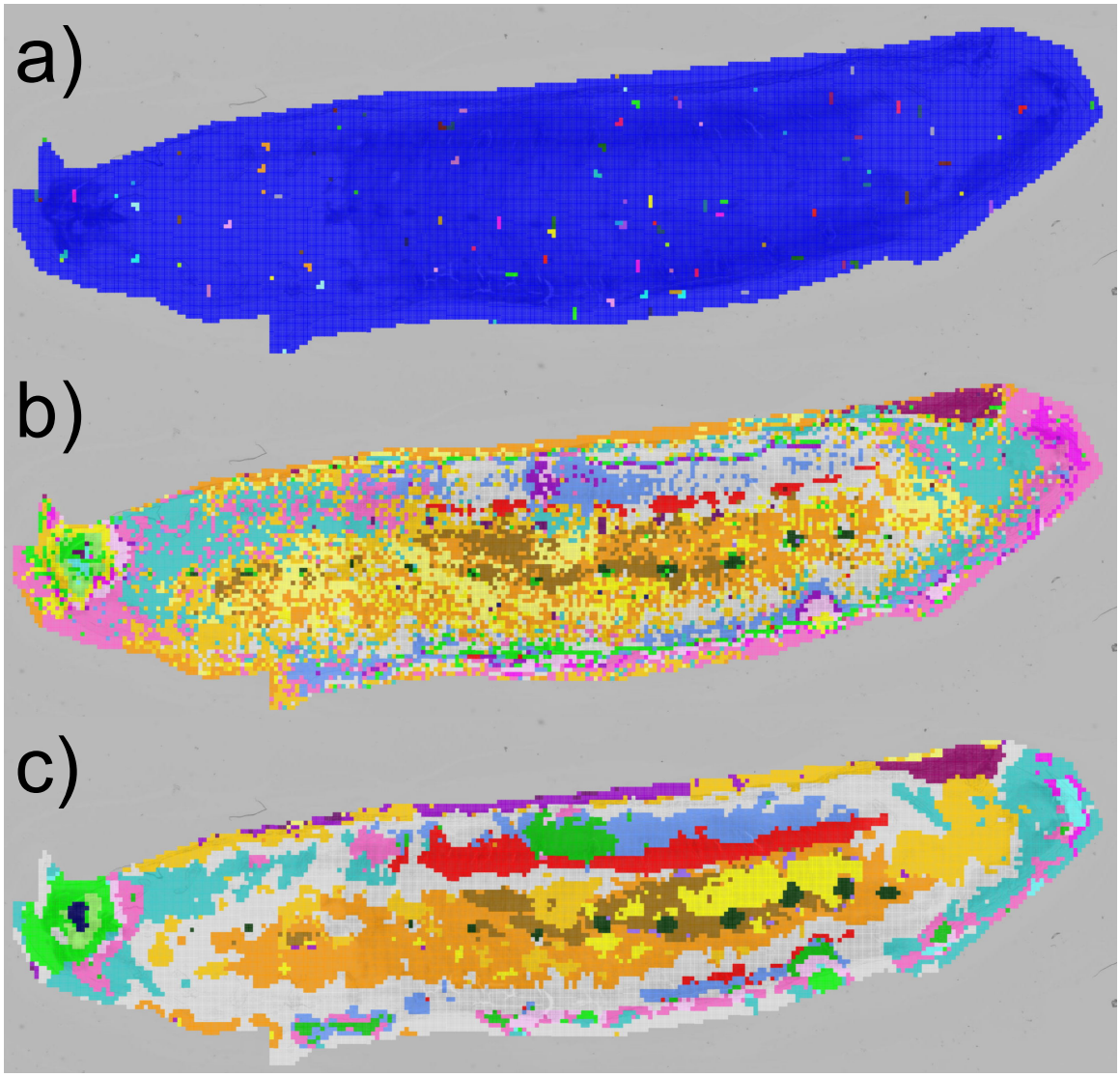


Figure 3: Segmentation results with and without smoothing after 10 iterations for random initial random segmentation in leech. a) Initial random segmentation. b) Resulting segmentation map without smoothing. c) Resulting segmentation map with 3x3 median smoothing.

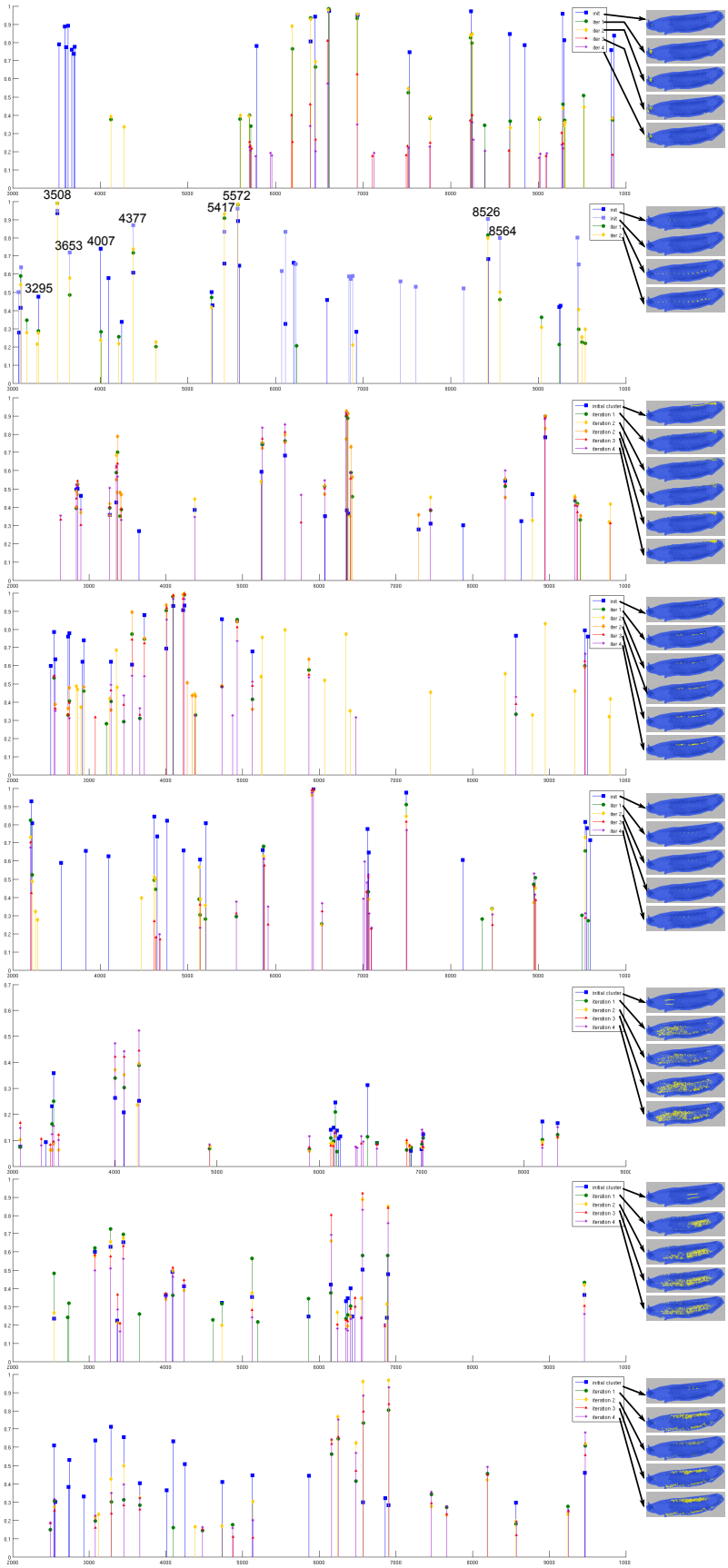


Figure 4: Top 20 score peaks at least 10 Daltons apart for segments in leech at successive iterations.

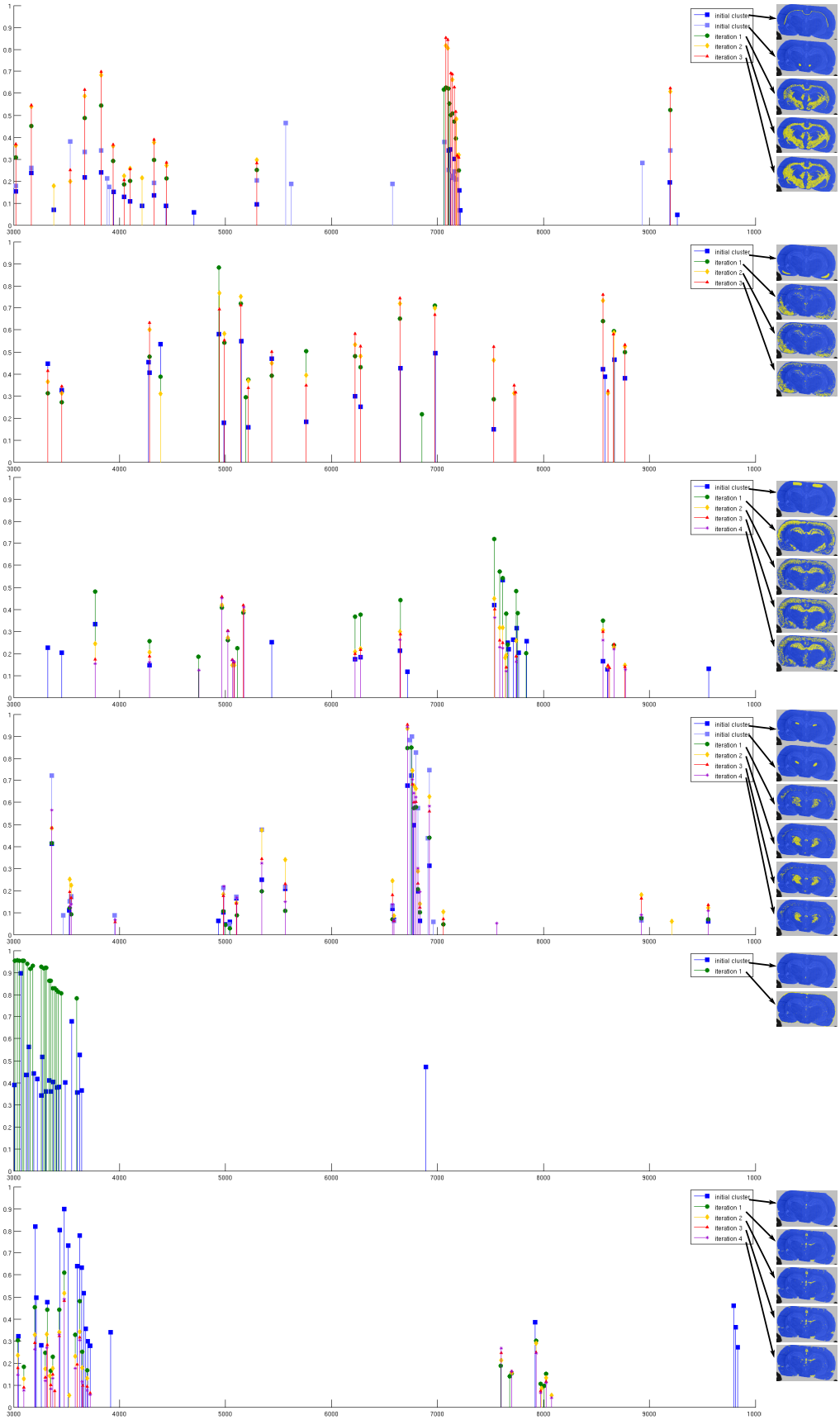
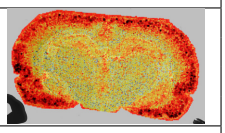
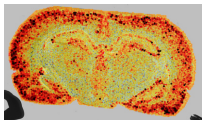
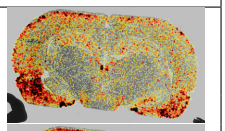
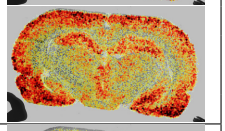
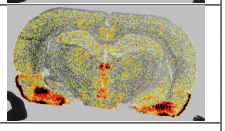
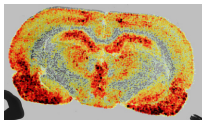
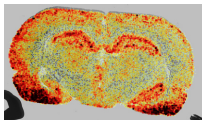
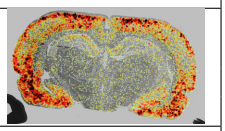
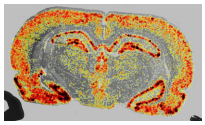
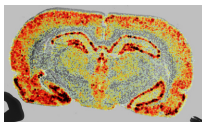


Figure 5: Top 20 score peaks at least 10 Daltons apart for segments in rat at successive iterations.

retrospenial cortex		**parietal association cortex**		**primary somatosensory cortex**		**auditory cortex**	
m/z	weight	m/z	weight	m/z	weight	m/z	weight
		3765.5	0.32				
		3767.5	0.33				
						6720.5	0.34
						6722.5	0.33
						6725.0	0.34
						6728.5	0.34
						6731.0	0.34
				7529.0	0.33		
		7532.5	0.42	7532.5	0.33		
7534.5	0.33	7534.5	0.42				
7537.5	0.34						
7541.0	0.34						
7543.0	0.33						
7546.5	0.31						
				7568.5	0.33		
				7571.5	0.36		
				7574.0	0.35		
				7580.0	0.31		
		7611.5	0.51				
		7614.0	0.53				
		7617.0	0.51				
		7619.5	0.46				
		7747.5	0.31				
7840.0	0.31						
7842.5	0.32						
7845.5	0.32						
9559.5	0.39						
9563.0	0.39						
9981.0	0.32						

Figure 6: Molecules expressed in different regions of the rat brain cortex.

piriform cortex		amygdala		image
m/z	weight	m/z	weight	
3321.0	0.40			
3322.5	0.47	3322.5	0.52	
3325.0	0.45	3324.5	0.45	
3326.5	0.35	3327.0	0.33	
3451.0	0.33			
3453.5	0.53			
3455.0	0.52	3455.0	0.33	
3457.0	0.50	3457.5	0.30	
3459.0	0.35			
		4270.5	0.47	
		4273.0	0.45	
		4275.0	0.37	
4276.5	0.39			
4279.0	0.42			
4281.0	0.44	4281.5	0.42	
4283.0	0.40	4283.5	0.41	
4285.5	0.36			
		4383.5	0.42	
		4385.5	0.53	
		4388.0	0.50	
		4390.5	0.41	
4931.5	0.32	4931.0	0.44	
4933.5	0.37	4932.0	0.54	
4936.0	0.38	4936.0	0.59	
4938.0	0.34	4938.0	0.57	
		4940.5	0.52	
		5141.5	0.32	
		5143.5	0.42	
5146.0	0.38	5146.5	0.52	
5148.5	0.38	5148.0	0.55	
5151.0	0.33	5151.0	0.49	
		5434.5	0.36	
5436.5	0.31	5436.5	0.47	
		5439.0	0.46	
		5442.0	0.32	
6218.0	0.47			
6221.0	0.50			
6223.5	0.54			
6225.5	0.50			
6228.0	0.48			
6270.0	0.39			
6272.5	0.48			
6275.0	0.47			
6277.5	0.42			
6279.5	0.35			

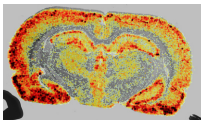
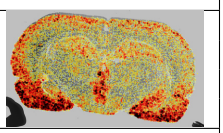
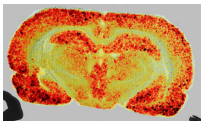
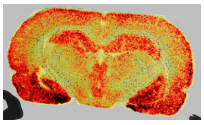
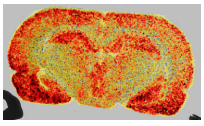
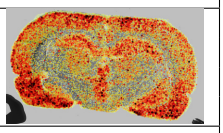
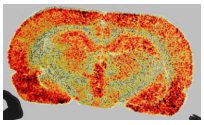
piriform cortex		amygdala		image
m/z	weight	m/z	weight	
6643.0	0.60	6643.0	0.35	
6646.0	0.65	6646.0	0.41	
6647.5	0.62	6648.5	0.43	
6651.0	0.61	6650.5	0.39	
6653.5	0.61	6654.0	0.33	
6854.0	0.33			
6972.5	0.39	6972.5	0.34	
6975.5	0.48	6976.0	0.48	
6978.0	0.46	6978.5	0.49	
6981.0	0.33	6981.0	0.42	
8558.0	0.34			
8561.0	0.37	8560.5	0.40	
8564.0	0.37	8563.0	0.46	
8566.5	0.34	8567.0	0.47	
		8569.5	0.41	
8581.5	0.33	8582.0	0.39	
		8660.0	0.40	
8663.0	0.30	8663.0	0.46	
8666.5	0.37	8666.0	0.46	
8669.0	0.35	8669.5	0.47	
8672.5	0.32	8672.5	0.41	
8767.0	0.31	8766.5	0.38	
8770.0	0.33	8769.5	0.38	
		8772.5	0.37	
8776.0	0.32	8776.0	0.38	
		8778.5	0.37	
		10942.0	0.33	
		10944.5	0.40	
		10948.0	0.38	
		10951.5	0.30	
		21860.5	0.46	
		21870.5	0.48	
		21875.5	0.47	
		21880.0	0.46	
		21885.0	0.44	
		21918.0	0.31	

Figure 7: Molecules expressed in the amygdala and piriform cortex of the rat brain.

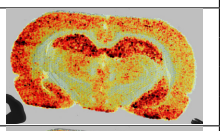
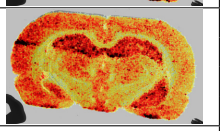
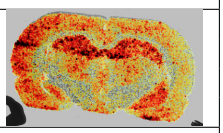
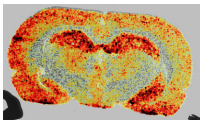
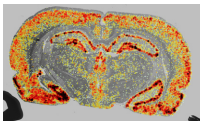
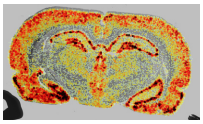
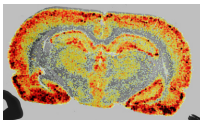
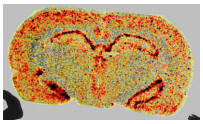
dentate gyrus		CA1-CA3 axons		CA1-CA3 cell bodies		CA3 cell bodies		images
m/z	weight	m/z	weight	m/z	weight	m/z	weight	
		4958.5 4961.5 4963.0 4966.0	0.44 0.47 0.46 0.44					
5000.0 5002.5	0.32 0.32	5000.5	0.43	5000.5 5003.0	0.23 0.22			
		5021.0 5023.5	0.38 0.38					
5039.0 5042.0	0.33 0.31	5037.5 5039.5	0.41 0.41	5037.0 5039.0	0.21 0.21			
		5057.5	0.33					
5080.5	0.32							
		5102.0 5104.5	0.33 0.34					
		5113.5	0.33					
		5165.0 5167.5 5169.5 5171.5 5174.0	0.43 0.46 0.47 0.46 0.44					
		5762.5 5765.0	0.37 0.34					
						6221.0 6223.5 6226.0 6228.5 6233.5	0.61 0.62 0.64 0.60 0.55	
						6272.5 6275.0 6277.0 6280.0 6282.5	0.58 0.63 0.59 0.55 0.53	
						6643.5 6645.5 6648.0 6650.5 6654.0	0.34 0.36 0.39 0.37 0.32	
						8444.0 8446.5 8449.5 8452.5 8456.0	0.32 0.60 0.54 0.53 0.36	

Figure 8: Molecules expressed in different regions of the rat brain hippocampus.

paraventricular thal. nucleus	
m/z	weight
3953.5	0.50
3956.0	0.61
4273.0	0.32
4383.5	0.61
4385.5	0.50
4388.0	0.56
4390.0	0.40
4392.0	0.38
4934.0	0.32
4936.5	0.34
4938.5	0.32
5765.0	0.39
6221.0	0.33
6223.0	0.35
6225.5	0.30
6228.5	0.30
6275.0	0.34
6277.0	0.36
6279.5	0.32
9537.5	0.47
9560.0	0.46
9563.0	0.62
9569.5	0.47
9573.0	0.46

lateral post. thal. nucleus	
m/z	weight
3356.5	0.32
3359.0	0.41
6717.5	0.68
6752.5	0.68
6755.0	0.72
6757.0	0.71
6760.0	0.69
6773.0	0.50
6924.5	0.31

ventral posteromedial thal. nucleus	
m/z	weight
3355.0	0.33
3356.5	0.62
3359.0	0.72
3360.5	0.54
5341.5	0.41
5343.5	0.48
5346.5	0.44
6739.0	0.88
6752.5	0.88
6754.5	0.89
6757.5	0.90
6760.5	0.89
6795.0	0.83
6802.5	0.59
6810.5	0.48
6813.5	0.57
6816.0	0.55
6911.0	0.44
6921.5	0.65
6924.5	0.75
6926.5	0.70
6929.5	0.53

ventral posteromedial thal. nucleus	
m/z	weight
6739.0	0.59
6744.5	0.57
6754.5	0.61
6757.5	0.57
6760.0	0.59
6792.5	0.41
6795.0	0.43
6926.5	0.31

lateral habenular nucleus	
m/z	weight
3530.5	0.34
3544.0	0.38
5562.5	0.43
5564.5	0.47
5567.0	0.59
5569.5	0.33
10600.50	0.41
10604.00	0.40
10607.50	0.54
10611.00	0.36
10614.50	0.41
12121.50	0.31

medial habenular nucleus	
m/z	weight
5346.0	0.39
5567.5	0.33
6215.5	0.30
6223.5	0.32
6270.0	0.30
6272.0	0.33
6277.0	0.36
6545.5	0.33
6595.5	0.35
6741.5	0.71
6747.0	0.74
6754.5	0.73
6760.0	0.78
6762.5	0.75
6794.5	0.53
6798.0	0.54
6889.5	0.46
6891.5	0.42
6924.0	0.59
6926.5	0.44
6929.5	0.54
6951.5	0.30
6986.0	0.40

medial habenular nucleus (cont'd)	
m/z	weight
7003.0	0.43
7014.0	0.64
7016.5	0.62
7025.0	0.40
8450.0	0.35
9563.5	0.38
9938.5	0.35
10607.00	0.40
10614.00	0.38
11256.50	0.37
11277.50	0.42
11301.00	0.49
11322.00	0.54
11336.50	0.50
11340.00	0.52
11342.50	0.51
11356.50	0.43
11378.00	0.55
11388.50	0.51
11409.00	0.34
11481.50	0.30
13758.00	0.40
13769.50	0.49
13773.50	0.51
13777.50	0.42
13781.50	0.57
13812.00	0.38
13823.50	0.41
13831.00	0.41
13862.00	0.47
13869.50	0.41
13873.00	0.42
13895.50	0.42
13900.00	0.45
13911.00	0.30
13934.00	0.36
13938.00	0.37
13957.00	0.35
13988.00	0.38
14000.00	0.36
14007.00	0.39
14011.00	0.36
14022.50	0.35
15281.50	0.32

Figure 9: Molecules expressed in different regions of the rat brain thalamus and epithalamus.

posterior hypothal. area	
m/z	weight
3066.0	0.40
3526.0	0.38
3528.0	0.45
3530.5	0.38
3542.0	0.39
3543.5	0.39
5564.5	0.43
5567.0	0.44
9557.0	0.30
10607.0	0.30

lateral hypothal. area	
m/z	weight
3528.0	0.31
3530.0	0.36
3532.0	0.38
3534.5	0.36
3536.0	0.32
3670.5	0.33
3826.0	0.32
3828.0	0.34
5562.0	0.33
5565.0	0.45
5567.0	0.47
5569.5	0.37
7057.0	0.33
7060.0	0.37
7063.0	0.38
7065.5	0.36
7068.0	0.34
9195.0	0.30
9197.5	0.34
9207.0	0.30
10597.5	0.44
10600.5	0.51
10604.5	0.54
10607.0	0.59
10611.0	0.53

lateral hypothal. area (cont'd)	
m/z	weight
13965.5	0.31
14108.0	0.39
14111.5	0.40
14115.0	0.39
14119.0	0.39
14123.5	0.39
14165.5	0.32
14177.0	0.32
14181.5	0.32
14189.0	0.32
14192.5	0.32
14215.5	0.32
14231.5	0.30
14277.5	0.33
14282.0	0.34
14286.0	0.34
14294.0	0.34
14313.0	0.34
14328.5	0.34
14332.0	0.36
14340.5	0.34
18347.0	0.31
18382.5	0.32
18386.5	0.31
18396.0	0.32

Figure 10: Molecules expressed in different regions of the rat brain hypothalamus.

zona incerta	
m/z	weight
3668.5	0.32
3670.5	0.32
5564.5	0.35
7046.0	0.34
7051.5	0.34
7055.0	0.33
7057.0	0.36
7060.0	0.34
7137.0	0.30
9195.0	0.33
9198.0	0.31
9201.5	0.31
10597.0	0.30
10600.5	0.38
10604.0	0.39
10607.0	0.40
10611.0	0.34
14088.5	0.35
14099.5	0.35
14104.0	0.35
14107.5	0.35
14119.5	0.35
14142.0	0.31
14154.0	0.31
14161.5	0.32
14165.5	0.31
14177.5	0.30
18342.5	0.36
18360.5	0.35
18373.5	0.36
18382.5	0.33
18387.0	0.34
18404.0	0.32

internal capsule	
m/z	weight
3165.5	0.42
3167.5	0.41
3169.0	0.34
3669.0	0.33
3671.0	0.44
3672.5	0.41
3823.5	0.43
3826.0	0.55
3827.5	0.49
3829.5	0.52
3834.0	0.32
4042.0	0.35
4044.5	0.32
4322.0	0.33
4324.0	0.36
4326.0	0.37
4699.5	0.36
5291.5	0.34
7044.0	0.52
7098.5	0.55
7101.5	0.61
7103.5	0.56
7123.5	0.55
7142.5	0.61
7153.5	0.51
7159.0	0.52
7161.5	0.52
7178.0	0.50
7197.0	0.36
7202.5	0.30
9191.5	0.43
9195.0	0.60
9198.0	0.49
9201.0	0.46
9204.5	0.50

internal capsule (cont'd)	
m/z	weight
11960.5	0.37
11964.5	0.35
11968.0	0.30
13850.0	0.32
13907.0	0.41
13961.5	0.46
13980.5	0.50
13991.5	0.48
14007.5	0.49
14050.0	0.53
14061.0	0.53
14072.5	0.55
14088.5	0.53
14095.5	0.52
14100.0	0.52
14177.0	0.61
14184.5	0.58
14189.0	0.57
14200.0	0.59
14208.0	0.57
14227.5	0.57
14239.0	0.61
14247.0	0.56
14258.5	0.62
14266.5	0.57
14285.5	0.59
14290.0	0.52
14305.5	0.58
14312.5	0.52
14321.0	0.52
14348.0	0.52
14351.5	0.53
14356.0	0.54
14363.5	0.66
14391.0	0.55

internal capsule (cont'd)	
m/z	weight
14403.0	0.38
14406.5	0.37
14414.5	0.38
14418.0	0.40
14441.5	0.34
14453.0	0.37
14461.0	0.45
14563.0	0.31
18303.0	0.64
18308.0	0.65
18316.0	0.64
18321.0	0.63
18352.0	0.67
18360.0	0.72
18374.0	0.66
18378.0	0.63
18382.0	0.67
18404.5	0.58
18409.0	0.58
18427.0	0.66
18431.0	0.63
18435.5	0.65
18457.0	0.57
18461.5	0.52
18483.5	0.54
18493.0	0.47
18502.0	0.50
18510.0	0.51
18528.5	0.41
18550.5	0.41
18554.5	0.54
18563.5	0.38
18576.5	0.45
18581.5	0.38
18607.5	0.36

Figure 11: Molecules expressed in other various regions of the rat brain.

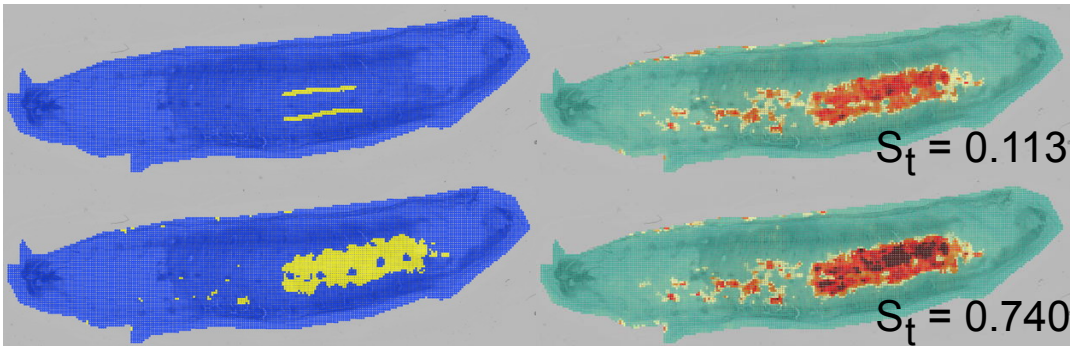


Figure 12: Query consistency scores. In the top panel, the original query (left) recruits may other spots (right) sharing a similar molecular signature, thus resulting in a low consistency score (0.113). In the bottom panel, the query and query-result are very similar indicating that all spots within the query have similar molecular signatures and spots outside the query have different molecular signatures. This results in a much higher consistency score (0.74).

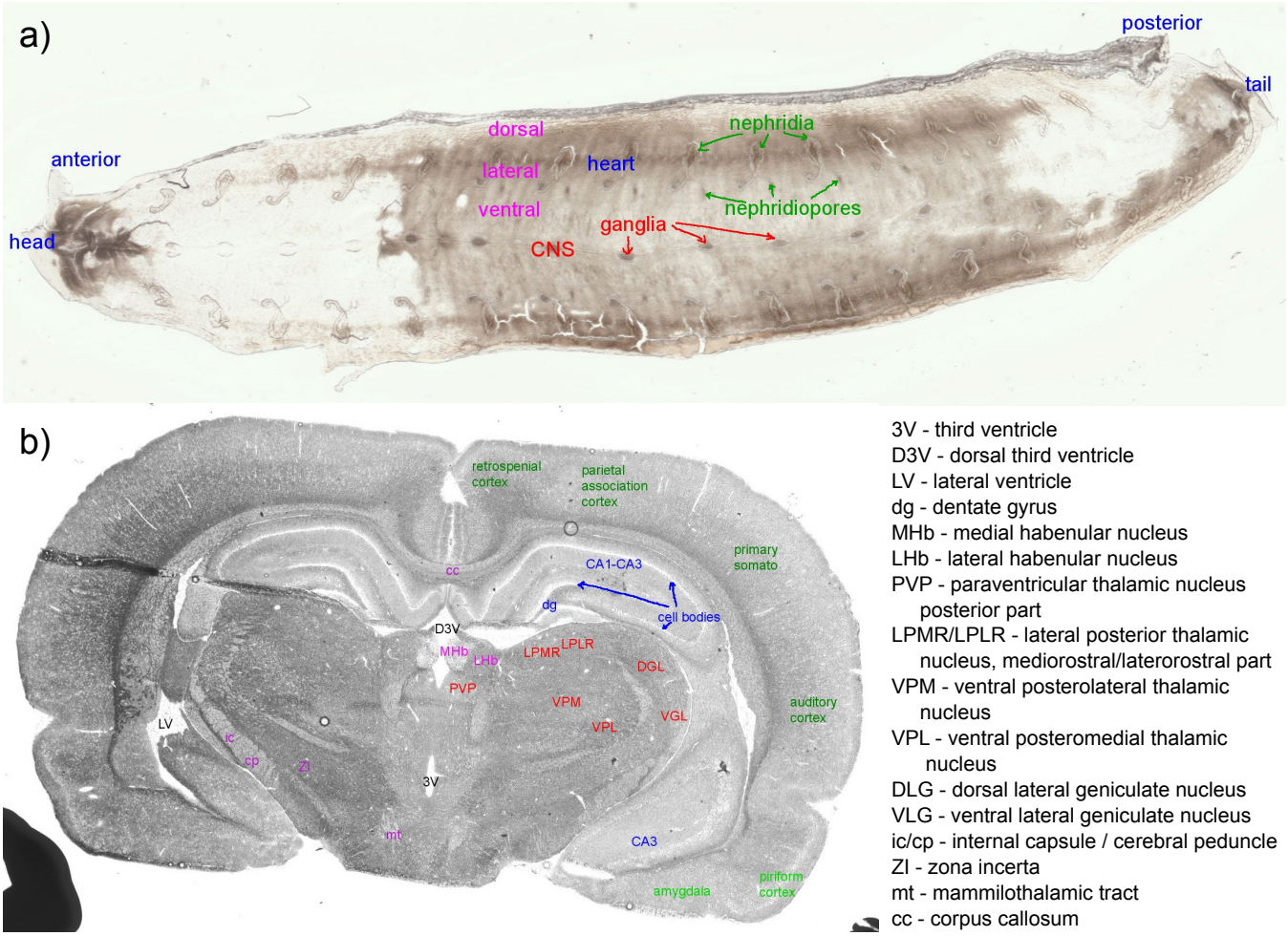


Figure 13: Basic anatomy for the a) the leech embryo and b) the rat brain slice.