Supporting Information For

Design, Synthesis, and Preliminary Evaluation of [^{68}Ga]Ga-NOTA-Insulin as a PET Probe in Alzheimer's Disease Mouse Model

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Description of Supporting Figures:

Figure S1: HPLC traces of different product during optimization of NOTA-insulin reaction time

Figure S2: Representative HPLC trace of different products after reaction optimization and PD-10 purification (one of the fractions)

Figure S3: Representative HPLC trace of one of the PD-10 purified fractions used for radiolabeling

Figure S4: r-TLC trace of [^{68}Ga]Ga-NOTA-insulin

Figure S5: MALDI-TOF spectrum of insulin

Figure S6: MALDI-TOF spectrum of NOTA-insulin before reaction condition optimization and after PD-10 purification

Figure S7: Uptake (SUV) of [^{11}C]PIB in Normal (B6SJL) and AD (APP/PS1) mice models at 30 min post intravenous administration measured via organ/tissue harvesting. P values have been adjusted for multiple testing

Figure S8: Brain (SUV) of (A) [^{18}F]AV1451 and (B) [^{11}C]PIB and Heart (SUV) (C) [^{18}F]AV1451 and (D) [^{11}C]PIB in AD (n=4 for each tracer) and normal (n=4 for each tracer) mice at 5, 10, 15 and 20 min post intravenous (i.v.) administration**.** The uptake (SUV) data for figure S8 was extracted from micro-PET/CT images by drawing region of interest (ROI) at different timepoints.

Figure S9: Uptake (SUV) and biodistribution of (A) [^{18}F]AV1451 and (B) [^{11}C]PIB in in different brain regions of AD (n=4 for each tracer) and normal (n=4 for each tracer) at 30 min post intravenous (i.v.) administration.

Figure S10: Uptake (SUV) and biodistribution of (A) [^{18}F]AV1451 and (B) [^{11}C]PIB in AD (n=4 for each tracer) and normal (n=4 for each tracer) at 30 min post intravenous (i.v.) administration.

Figure S11: Representative micro-PET/CT images of (A) [^{18}F]AV1451 and (B) [^{11}C]PIB in normal and AD mice at different time points post intravenous (i.v.) administration.

Description of Supporting Tables:

Table S1: Details of injected NOTA-insulin (nonradioactive mass) as [^{68}Ga]Ga-NOTA-insulin, specific activity(A_s) at time of injection and molar activity(A_m) at end of the synthesis

Table S2: Uptake (SUV) of [^{18}F]AV1451 in brain of AD (APP/PS1) and normal (B6SJL) mice post intravenous administration measured via micro PET/CT image analysis and drawing region of interest (ROI) on whole mice brain and whole heart at different timepoints.

Table S3: Uptake (SUV) of [^{18}F]AV1451 in different brain regions of B6SJL (normal) and APP/PS1 (AD) mice models at 30 min post intravenous administration measured via organ/tissue harvesting

Table S4: Uptake (SUV) of [^{18}F]AV1451 in Normal (B6SJL) and AD (APP/PS1)mice models at 30 min post intravenous administration measured via organ/tissue harvesting.

Table S5: Uptake (SUV) of [^{11}C]PIB in brain of AD (APP/PS1) and normal (B6SJL) mice post intravenous administration measured via micro PET/CT image analysis and drawing region of interest (ROI) on whole mice brain and whole heart at different timepoints.

Table S6: Uptake (SUV) of [^{11}C]PIB in different brain regions of Normal (B6SJL) and AD (APP/PS1) mice models at 30 min post intravenous administration measured via organ/tissue harvesting. P values have been adjusted for multiple testing.

Table S7: Uptake (SUV) of [^{11}C]PIB in normal (B6SJL) and AD (APP/PS1) mice models at 30 min post intravenous administration measured via organ/tissue harvesting. P values have been adjusted for multiple testing.

Figure S1: HPLC traces of different product during optimization of NOTA-insulin reaction time

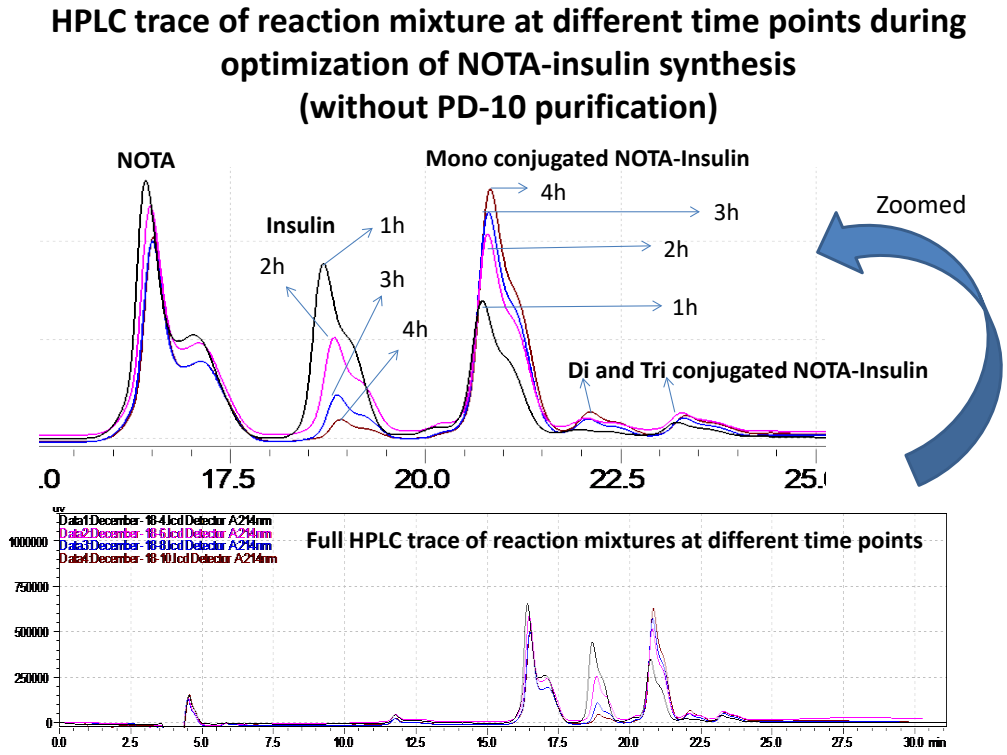
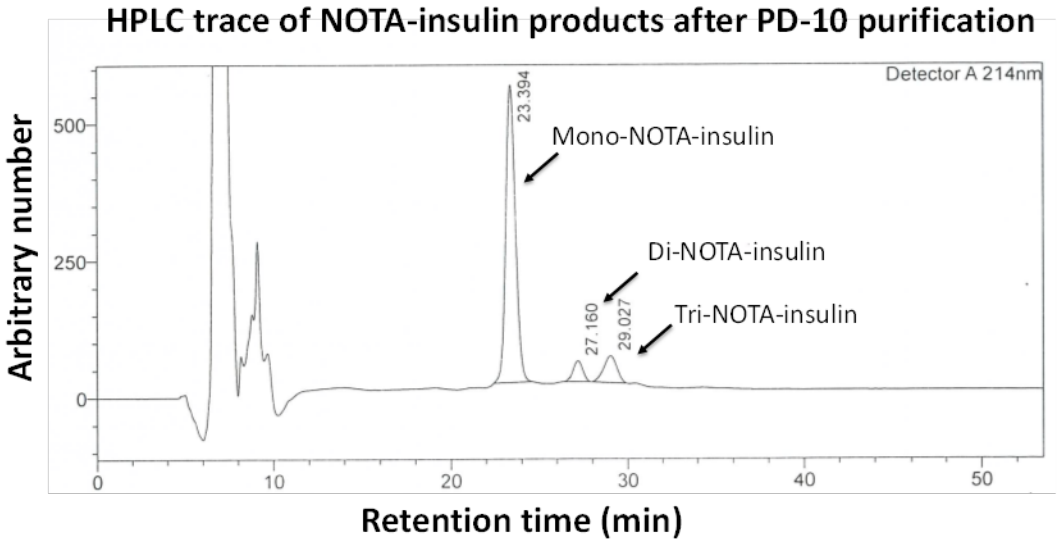
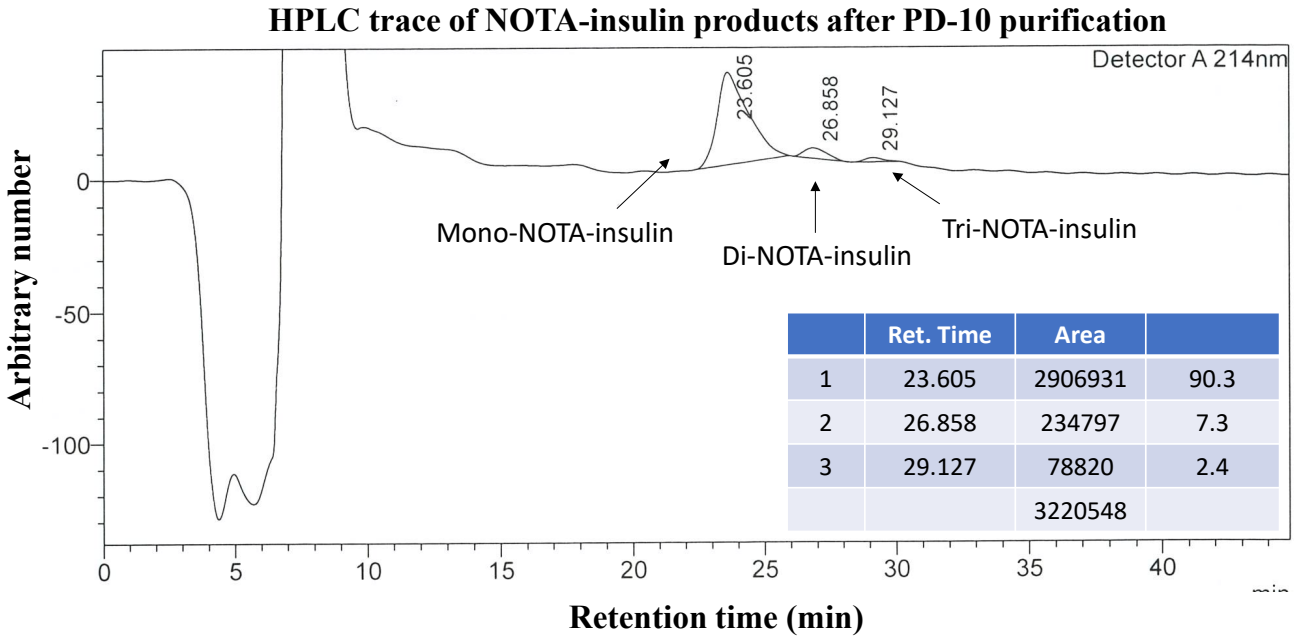


Figure S2: Representative HPLC trace of different products after reaction optimization and PD-10 purification (one of the fractions)



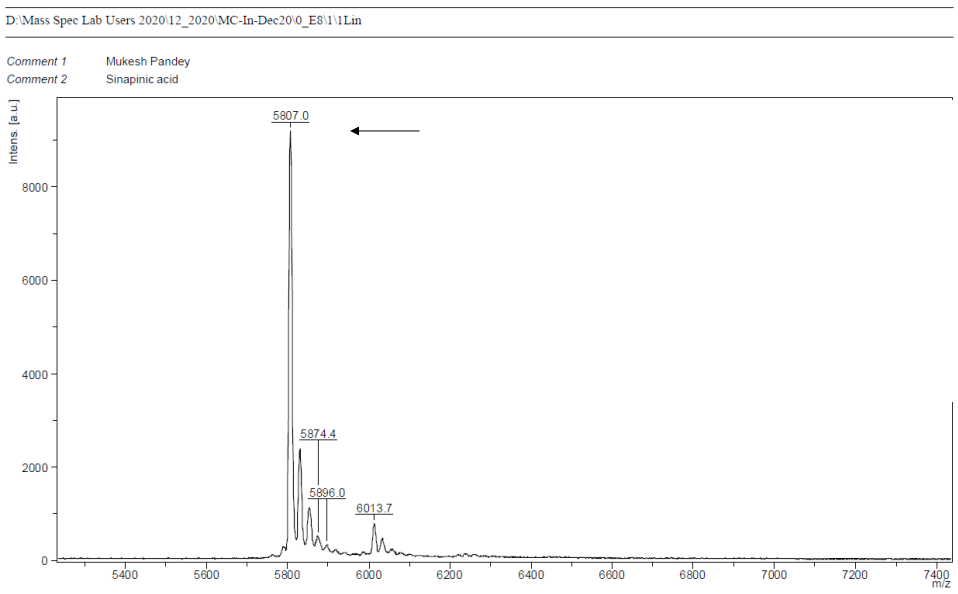
Note: Relative retention time may shift (±1.5 min) from day-to-day HPLC analysis and after PD-10 purification.

Figure S3: Representative HPLC trace of one of the PD-10 purified fractions used for radiolabeling



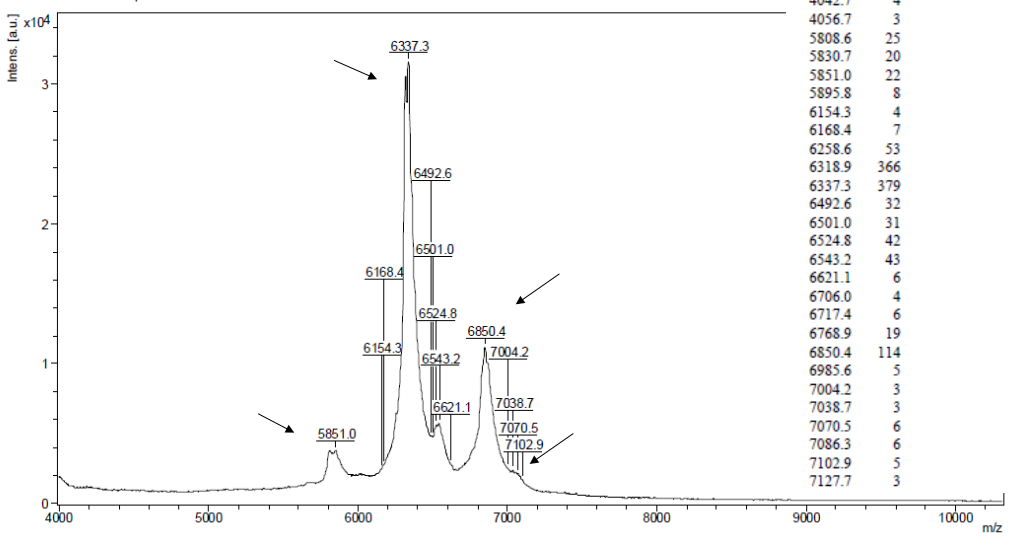
	Ret. Time	Area	
1	23.605	2906931	90.3
2	26.858	234797	7.3
3	29.127	78820	2.4
		3220548	

Figure S4: MALDI-TOF spectrum of insulin



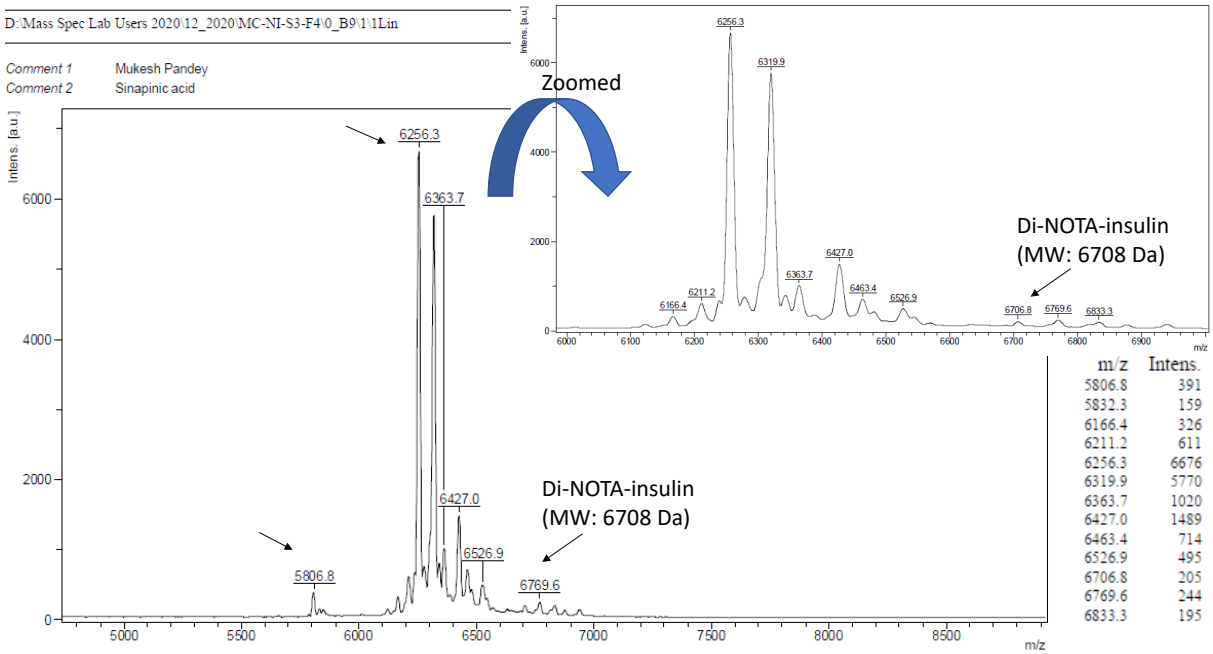
m/z	Intens.
5807.0	9192
5830.5	2391
5853.8	1131
5874.4	515
5896.0	344
5918.1	225
5988.3	183
6013.7	803
6033.9	475
6057.7	226

Figure S5: MALDI-TOF spectrum of NOTA-insulin before reaction condition optimization and after PD-10 purification



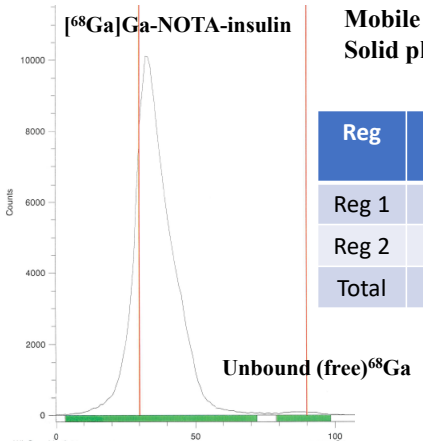
m/z	S/N	Quality Fac.	Res.	Intens.	Area
4002.8	8		1205	1918	5421
4008.5	8		849	1867	6703
4020.1	7		754	1749	6292
4031.4	5		879	1620	5057
4042.7	4		498	1501	4077
4056.7	3		853	1425	2490
5808.6	25		314	3744	64525
5830.7	20		964	3619	19455
5851.0	22		210	3790	75486
5895.8	8		1276	2616	5453
6154.3	4		576	2436	4764
6168.4	7		747	2747	7137
6258.6	53		279	6493	156211
6318.9	366		367	30557	842232
6337.3	379		175	31584	1.733630e6
6492.6	32		1541	4853	21322
6501.0	31		1435	4801	18615
6524.8	42		447	5661	68435
6543.2	43		181	5759	165917
6621.1	6		461	2850	5281
6706.0	4		1305	2738	2787
6717.4	6		1189	2879	4865
6768.9	19		360	3854	42224
6850.4	114		192	11121	428357
6985.6	5		888	2735	4102
7004.2	3		759	2525	3352
7038.7	3		1336	2352	1575
7070.5	6		603	2125	7701
7086.3	6		729	1898	6334
7102.9	5		950	1676	4374
7127.7	3		813	1342	3260

Figure S6: MALDI-TOF spectrum of NOTA-insulin after optimization of reaction condition and PD-10 purification



m/z	Intens.
5806.8	391
5832.3	159
6166.4	326
6211.2	611
6256.3	6676
6319.9	5770
6363.7	1020
6427.0	1489
6463.4	714
6526.9	495
6706.8	205
6769.6	244
6833.3	195

Figure S7: r-TLC trace of [^{68}Ga]Ga-NOTA-insulin



Mobile phase: 0.1 M sodium citrate solution (pH = 7)
Solid phase: iTLC-SG paper

Reg	Start (mm)	Stop (mm)	(mm)	RF	Region Counts	Region CPM		% of ROI
Reg 1	3.2	72.2	35.7	0.095	179565	384782.1		99.21
Reg 2	79.0	98.6	87.7	0.962	1434	3072.9	0.79	0.79
Total						387855.0		

Figure S8: Brain (SUV) of (A) [^{18}F]AV1451 and (B) [^{11}C]PIB and Heart (SUV) (C) [^{18}F]AV1451 and (D) [^{11}C]PIB in AD (n=4 for each tracer) and normal (n=4 for each tracer) mice at 5, 10, 15 and 20 min post intravenous (i.v.) administration**.** The uptake (SUV) data for figure 10 was extracted from micro-PET/CT images by drawing region of interest (ROI) at different timepoints.

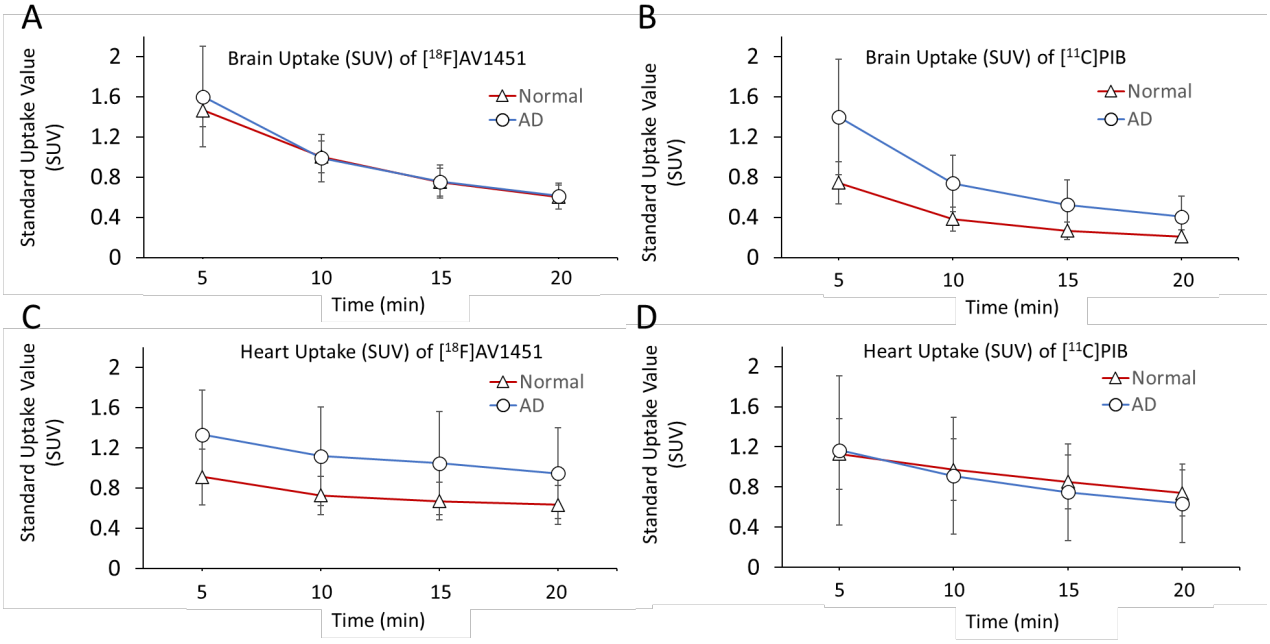


Figure S9: Uptake (SUV) and biodistribution of (A) [^{18}F]AV1451 and (B) [^{11}C]PIB in in different brain regions of AD (n=4 for each tracer) and normal (n=4 for each tracer) at 30 min post intravenous (i.v.) administration.

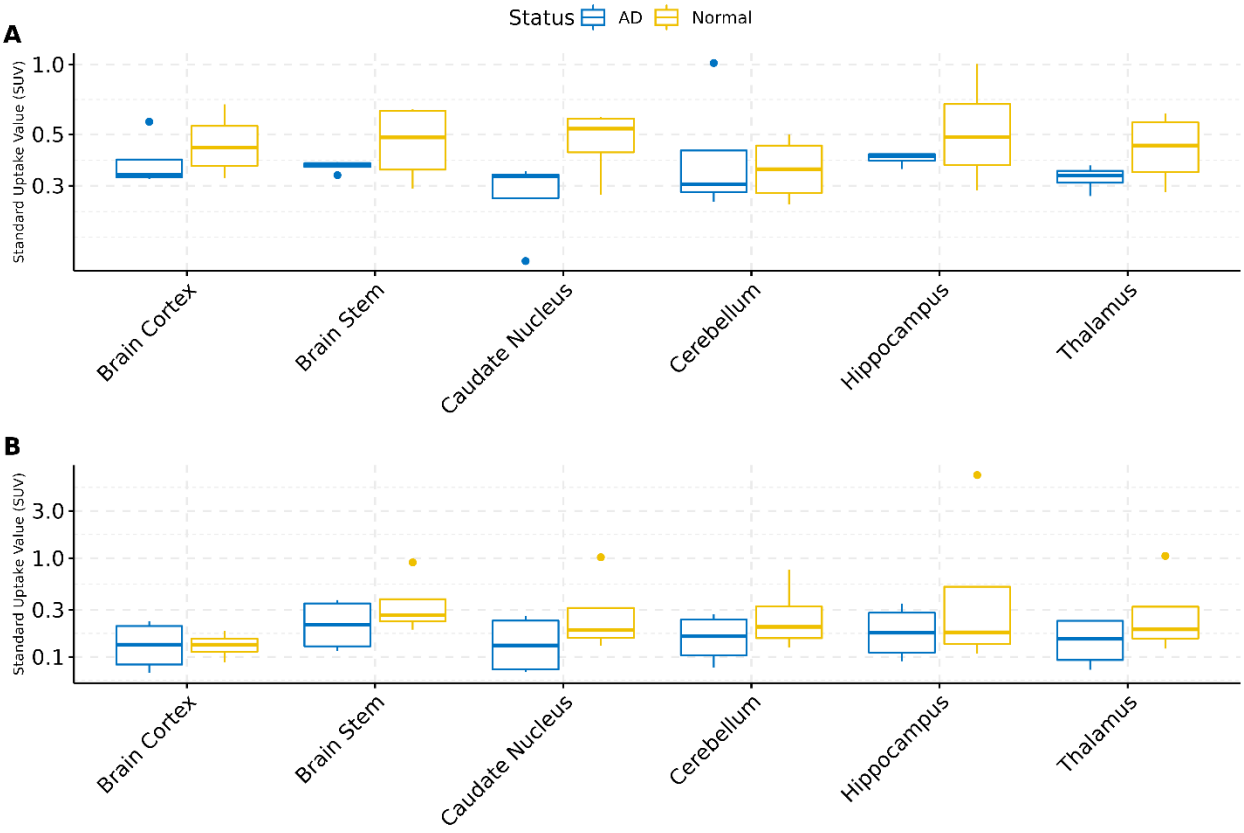


Figure S10: Uptake (SUV) and biodistribution of (A) [^{18}F]AV1451 and (B) [^{11}C]PIB in AD (n=4 for each tracer) and normal (n=4 for each tracer) at 30 min post intravenous (i.v.) administration.

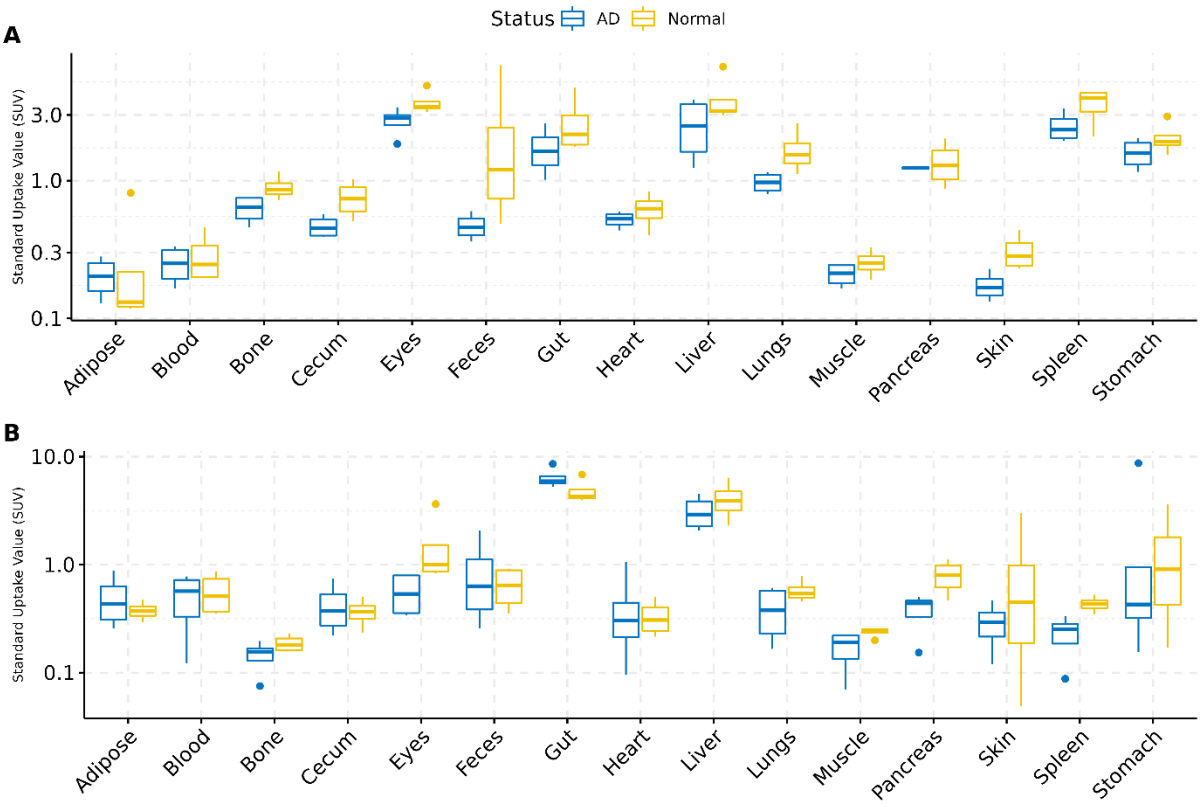


Figure S11: Representative micro-PET/CT images of (A) [^{18}F]AV1451 and (B) [^{11}C]PIB in normal and AD mice at different time points post intravenous (i.v.) administration.

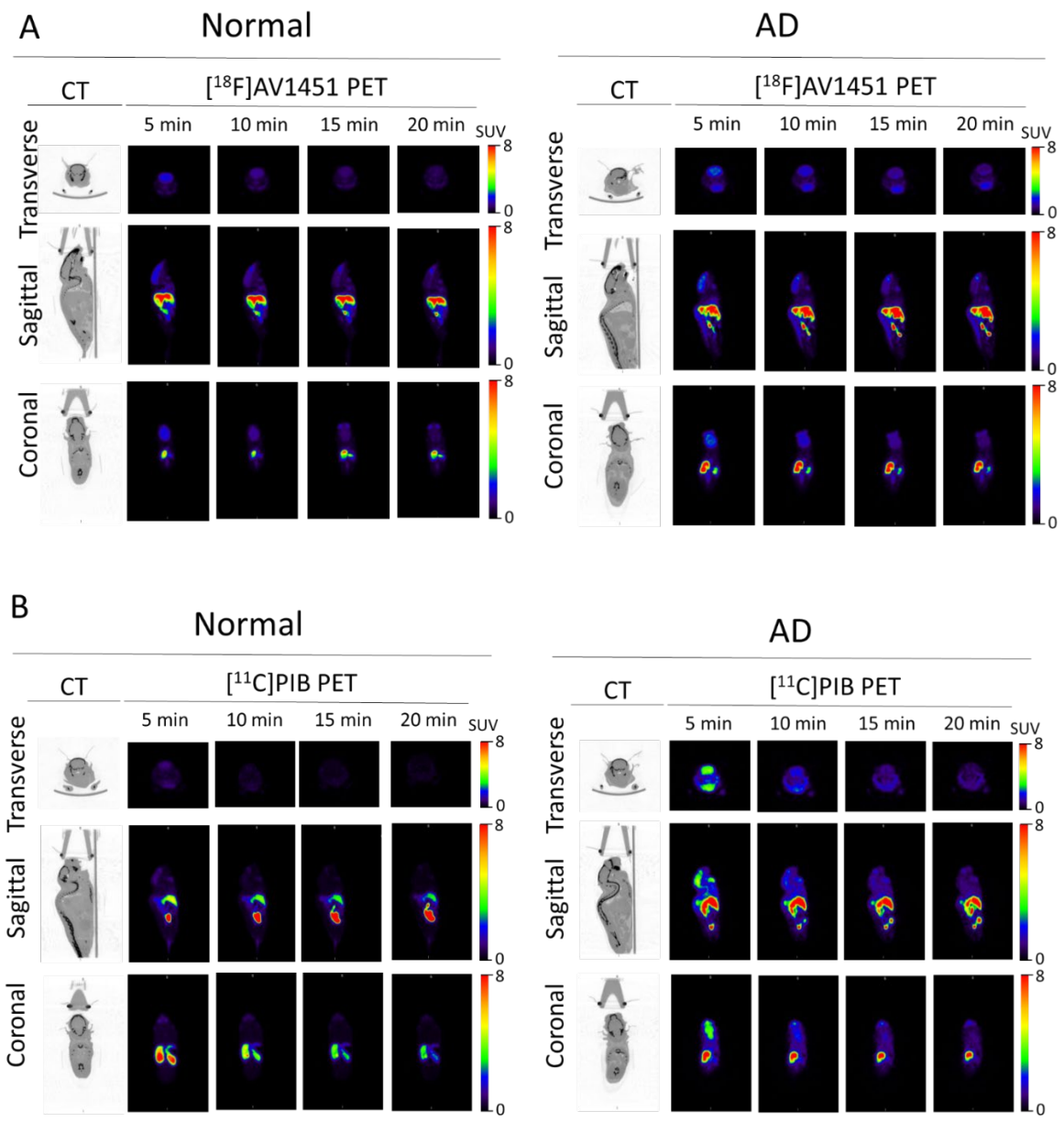


Table S1: Details of injected NOTA-insulin (nonradioactive mass) as [^{68}Ga]Ga-NOTA-insulin, specific activity(A_s) at time of injection and molar activity(A_m) at end of the synthesis

AD	Insulin injected /Body Wt (μg/g)	Specific activity (A_s) at Time of Injection (MBq/μg)	Molar activity (A_m) at End of synthesis (GBq/μmol)
AD Mouse 1	0.341	0.17	0.92
AD Mouse 2	0.318	0.13	
AD Mouse 3	0.176	0.36	1.17
AD Mouse 4	0.277	0.20	
AD Mouse 5	0.190	0.27	0.97
AD Mouse 6	0.297	0.17	
AD Mouse 7	0.170	0.34	1.21
AD Mouse 8	0.266	0.25	
AD Mouse 9	0.163	0.40	1.35
AD Mouse 10	0.297	0.29	
Normal			
Mouse 1	0.092	0.26	0.70
Mouse 2	0.197	0.22	
Mouse 3	0.085	0.48	1.11
Mouse 4	0.211	0.32	
Mouse 5	0.103	0.25	0.71
Mouse 6	0.210	0.18	
Mouse 7	0.116	0.36	0.96
Mouse 8	0.201	0.26	
Mouse 9	0.123	0.61	1.45
Mouse 10	0.256	0.43	
Mouse 11	0.098	0.61	1.40
Mouse 12	0.226	0.44	
		Average	1.1±0.26

Table S2: Uptake (SUV) of [^{18}F]AV1451 in brain of AD (APP/PS1) and normal (B6SJL) mice post intravenous administration measured via micro PET/CT image analysis and drawing region of interest (ROI) on whole mice brain and whole heart at different timepoints.

Time points (min)	AD Brain (Avg. SUV±SD, n=4)	Normal Brain (Avg. SUV±SD, n=4)	P value	AD Heart (Avg. SUV±SD, n=4)	Normal Heart (Avg. SUV±SD, n=4)	P value
5	1.601 ± 0.499	1.465 ± 0.164	0.311	1.329 ± 0.448	0.912 ± 0.278	0.082
10	0.989 ± 0.236	1.003 ± 0.158	0.464	1.118 ± 0.492	0.728 ± 0.190	0.095
15	0.754 ± 0.164	0.749 ± 0.138	0.483	1.047 ± 0.514	0.670 ± 0.187	0.108
20	0.611 ± 0.129	0.602 ± 0.118	0.461	0.948 ± 0.450	0.632 ± 0.193	0.123

Table S3: Uptake (SUV) of [^{18}F]AV1451 in different brain regions of Normal (B6SJL) and AD (APP/PS1) mice models at 30 min post intravenous administration measured via organ/tissue harvesting.

Mouse brain regions	**Alzheimer's disease mice (Avg. SUV±SD, n=4)**	**Normal mice (Avg. SUV±SD, n=4)**	**P value**
Caudate Nucleus	0.287 ± 0.097	0.483 ± 0.148	0.37
Cortex	0.390 ± 0.118	0.471 ± 0.155	0.58
Hippocampus	0.394 ± 0.027	0.572 ± 0.316	0.52
Thalamus	0.326 ± 0.041	0.453 ± 0.155	0.40
Brain stem	0.365 ± 0.021	0.484 ± 0.177	0.45
Cerebellum	0.471 ± 0.365	0.367 ± 0.117	0.76

Table S4: Uptake (SUV) of [^{18}F]AV1451 in Normal (B6SJL) and AD (APP/PS1)mice models at 30 min post intravenous administration measured via organ/tissue harvesting.

Organ/Tissue	Alzheimer's disease mice (Avg. SUV±SD, n=4)	Normal mice (Avg. SUV±SD, n=4)	P value
Blood	0.252 ± 0.080	0.289 ± 0.122	0.76
Heart	0.522 ± 0.071	0.625 ± 0.181	0.52
Lungs	0.976 ± 0.171	1.707 ± 0.647	0.37
Liver	2.593 ± 1.284	4.038 ± 1.796	0.40
Spleen	2.503 ± 0.635	3.616 ± 1.067	0.40
Pancreas	1.234 ± 0.033	1.379 ± 0.515	0.76
Bone	0.628 ± 0.146	0.903 ± 0.190	0.37
Gut	1.733 ± 0.685	2.727 ± 1.385	0.40
Feces	0.471 ± 0.105	2.486 ± 2.986	0.40
Adipose	0.206 ± 0.069	0.299 ± 0.344	0.76
Stomach	1.597 ± 0.404	2.074 ± 0.594	0.40
Skin	0.174 ± 0.042	0.311 ± 0.095	0.37
Muscle	0.209 ± 0.040	0.256 ± 0.057	0.40
Cecum	0.469 ± 0.087	0.756 ± 0.231	0.37
Eyes	2.738 ± 0.652	3.729 ± 0.791	0.40
Bladder	1.799 ± 1.093	2.529 ± 2.640	0.80
Kidneys	2.618 ± 1.931	4.753 ± 1.427	0.40
Urine	7.971 ± 8.091	6.788 ± 2.134	0.79

Table S5: Uptake (SUV) of [^{11}C]PIB in brain of AD (APP/PS1) and normal (B6SJL) mice post intravenous administration measured via micro PET/CT image analysis and drawing region of interest (ROI) on whole mice brain and whole heart at different timepoints.

Time points (min)	**AD Brain (Avg. SUV±SD, n=4)**	**Normal Brain (Avg. SUV±SD, n=4)**	**P value**	**AD Heart (Avg. SUV±SD, n=4)**	**Normal Heart (Avg. SUV±SD, n=4)**	**P value**
5	0.866 ± 0.386	0.743 ± 0.211	0.299	1.167 ± 0.743	1.131 ± 0.351	0.215
10	0.436 ± 0.172	0.381 ± 0.118	0.307	0.911 ± 0.582	0.972 ± 0.306	0.328
15	0.278 ± 0.099	0.266 ± 0.088	0.434	0.749 ± 0.482	0.854 ± 0.267	0.414
20	0.203 ± 0.067	0.210 ± 0.065	0.450	0.637 ± 0.388	0.740 ± 0.228	0.434

Table S6: Uptake (SUV) of [^{11}C]PIB in different brain regions of Normal (B6SJL) and AD (APP/PS1) mice models at 30 min post intravenous administration measured via organ/tissue harvesting. P values have been adjusted for multiple testing.

Mouse brain regions	**Alzheimer's disease mice (Avg. SUV±SD, n=4)**	**Normal mice (Avg. SUV±SD, n=4)**	**P value**
Cortex	0.147 ± 0.08	0.132 ± 0.049	0.90
Caudate Nucleus	0.158 ± 0.09	0.169 ± 0.041	0.64
Hippocampus	0.204 ± 0.12	0.157 ± 0.055	0.64
Thalamus	0.160 ± 0.08	0.168 ± 0.048	0.64
Brain stem	0.241 ± 0.13	0.241 ± 0.050	0.64
Cerebellum	0.173 ± 0.09	0.178 ± 0.061	0.64

Table S7: Uptake (SUV) of [^{11}C]PIB in Normal (B6SJL) and AD (APP/PS1) mice models at 30 min post intravenous administration measured via organ/tissue harvesting. P values have been adjusted for multiple testing.

Organ/Tissue	Alzheimer's disease mice (Avg. SUV±SD, n=4)	Normal mice (Avg. SUV±SD, n=4)	P value
Blood	0.516 ± 0.296	0.532 ± 0.294	0.86
Heart	0.441 ± 0.424	0.325 ± 0.158	0.86
Lungs	0.398 ± 0.220	0.607 ± 0.163	0.63
Liver	3.148 ± 1.159	4.076 ± 2.094	0.64
Spleen	0.232 ± 0.104	0.464 ± 0.057	0.55
Pancreas	0.384 ± 0.157	0.911 ± 0.217	0.55
Bone	0.146 ± 0.051	0.184 ± 0.042	0.63
Gut	6.440 ± 1.464	4.989 ± 1.604	0.63
Feces	0.922 ± 0.819	0.716 ± 0.316	0.86
Adipose	0.507 ± 0.279	0.406 ± 0.061	0.64
Stomach	2.433 ± 4.190	1.873 ± 1.570	0.98
Skin	0.295 ± 0.144	0.341 ± 0.317	0.64
Muscle	0.171 ± 0.072	0.232 ± 0.028	0.63
Cecum	0.435 ± 0.236	0.413 ± 0.082	0.86
Eyes	0.574 ± 0.258	1.886 ± 1.530	0.63
Bladder	11.353 ± 7.539	5.848 ± 4.365	0.63
Kidneys	6.853 ± 5.772	3.881 ± 0.803	0.98

Urine	64.293 ± 32.503	30.571 ± 27.148	0.63