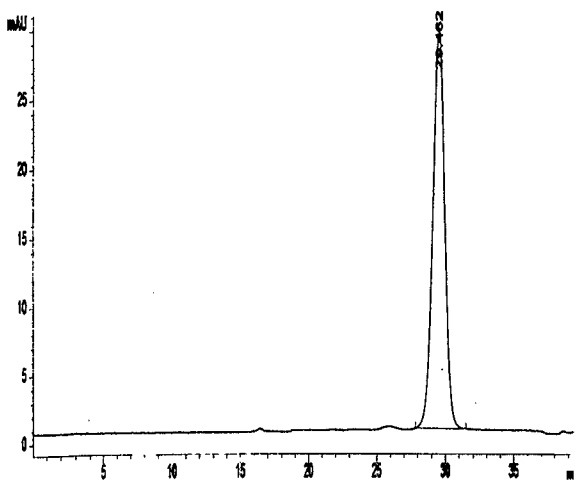
Bioconjugate Chemistry

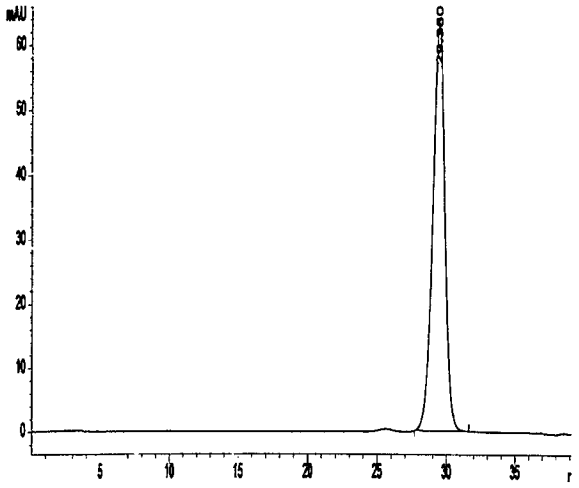
Bioconjugate Chem., 1998, 9(1), 100-107, DOI:10.1021/bc970152s

Size exclusion HPLC chromatograms of the streptavidin proteins evaluated. HPLC equipment and conditions were as described for non-labeled proteins in manuscript. All protein evaluations were conducted on the same day under identical HPLC conditions to obtain relative retention times (t_R). Differences in t_R observed for r-SAv proteins and commercial SAv proteins appear to be real as co-injection of r-SAv (wild type) with SAv-CS1 had a clearly discernible shoulder at about 30 min on a major peak at 29.5 min.

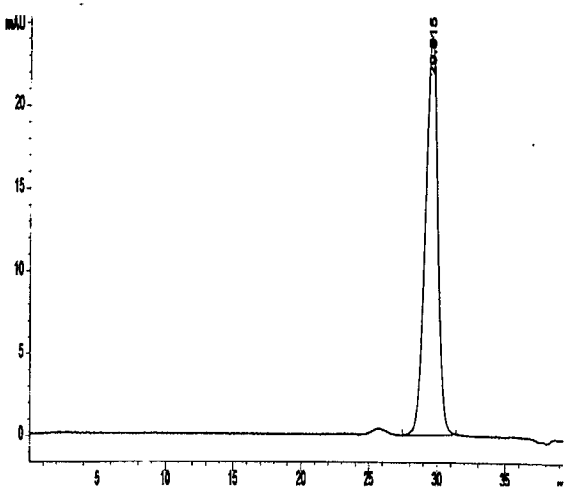
r-Streptavidin (wild type; t_R = 29.5 min)



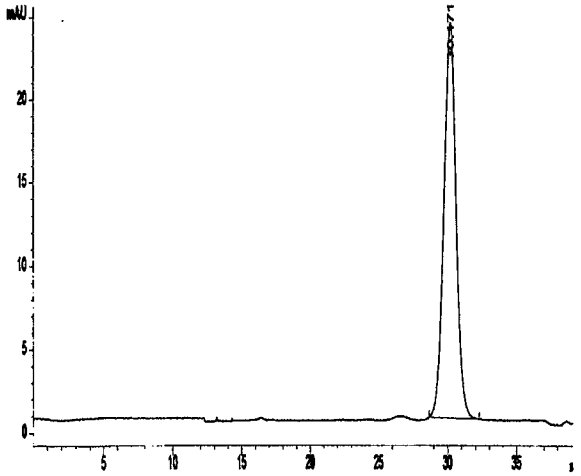
r-SAv-H127C (t_R = 29.4 min)



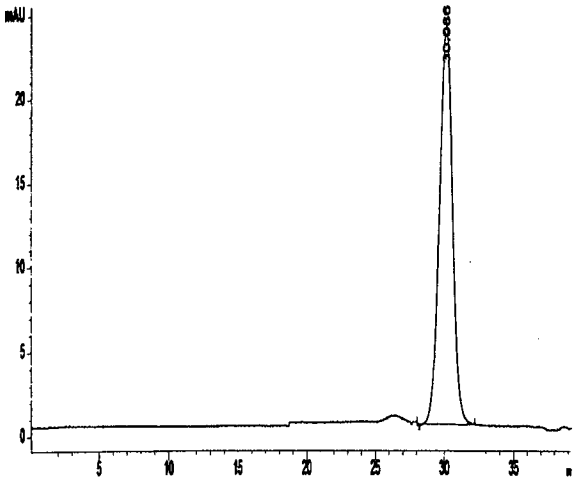
r-SAv-S139C (NEM capped; t_R = 29.5 min)



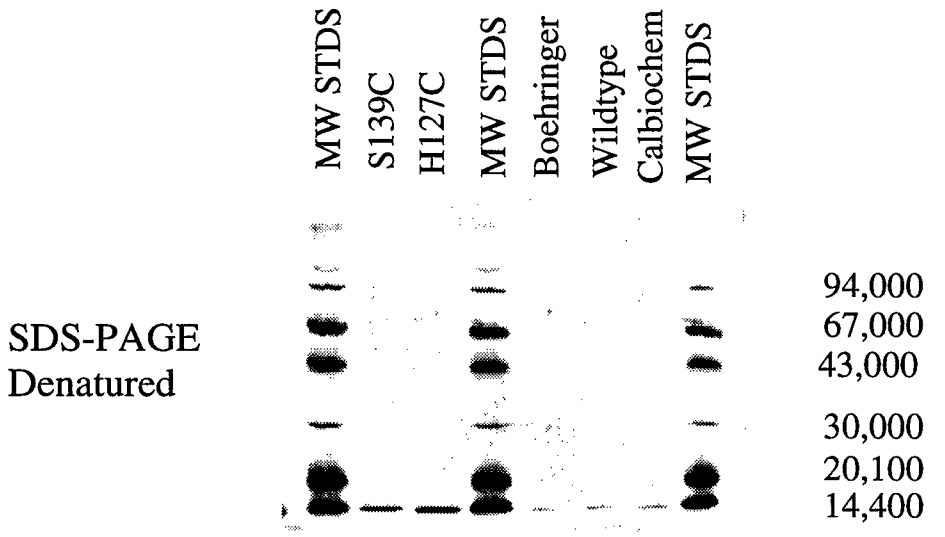
SAv-CS1 (Boehringer Mannheim; t_R = 30.2 min)

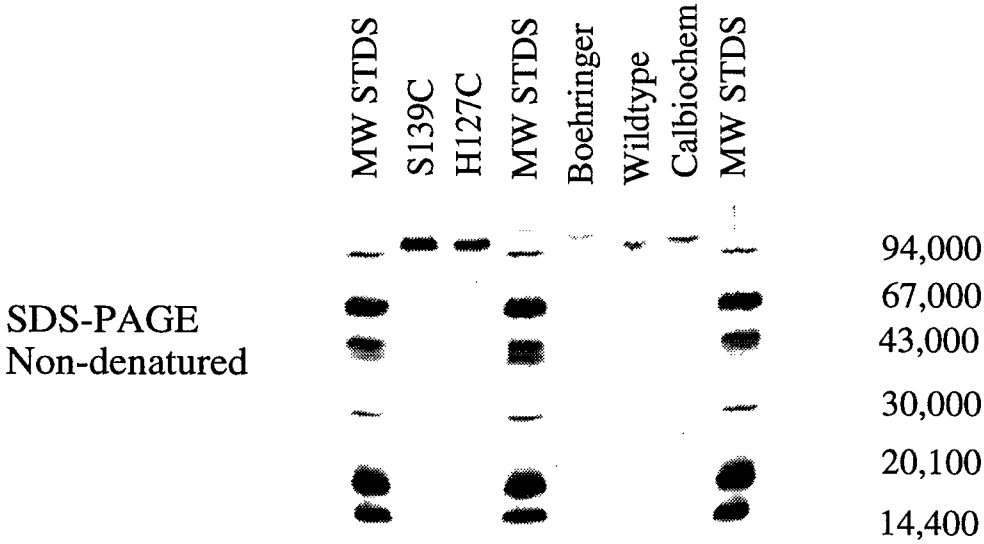


SAv-CS2 (CALBIOCHEM; t_R = 30.1 min)

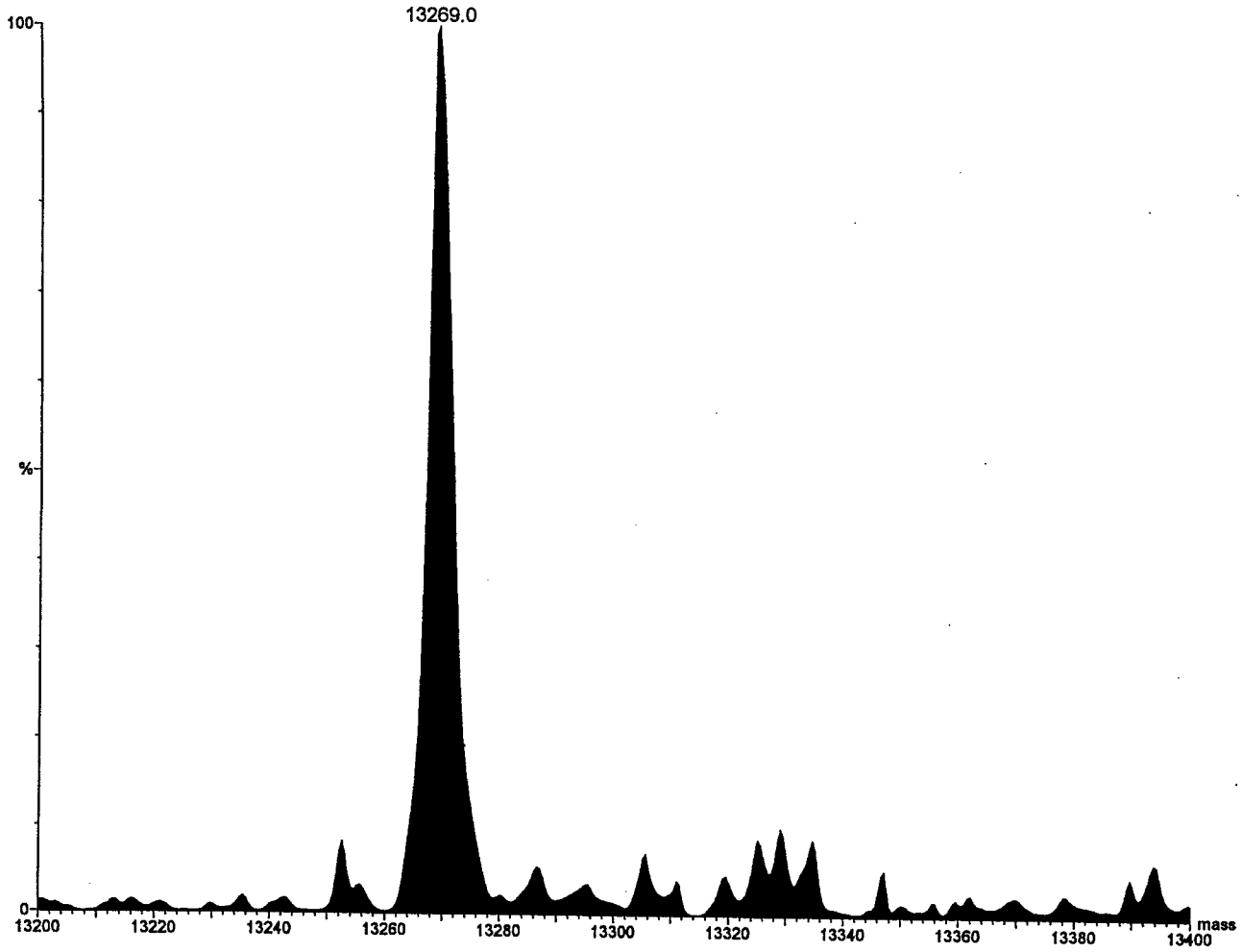


Digitized images of denatured and non-denatured (reducing, β-mercaptoethanol) SDS-PAGE gels of SAv proteins conducted on a PhastSystem™ electrophoresis apparatus (using PhastGel®, Homogeneous 12.5%). Lanes 1, 4, and 8; molecular weight standards (MW STDS; LMW Electrophoresis Calibration kit (Pharmacia)). Lane 2; r-SAv-S139C. Lane 3; r-SAv-H127C. Lane 5; SAv-CS1 (Boehringer Mannheim). Lane 6; r-SAv (wild type). Lane 7; SAv-CS2 (CALBIOCHEM). Gels were run by optimized method for Homogeneous 12.5% gel using "Separation Technique File #111. The protein band was developed by the silver staining method from "Development Technique File #210.

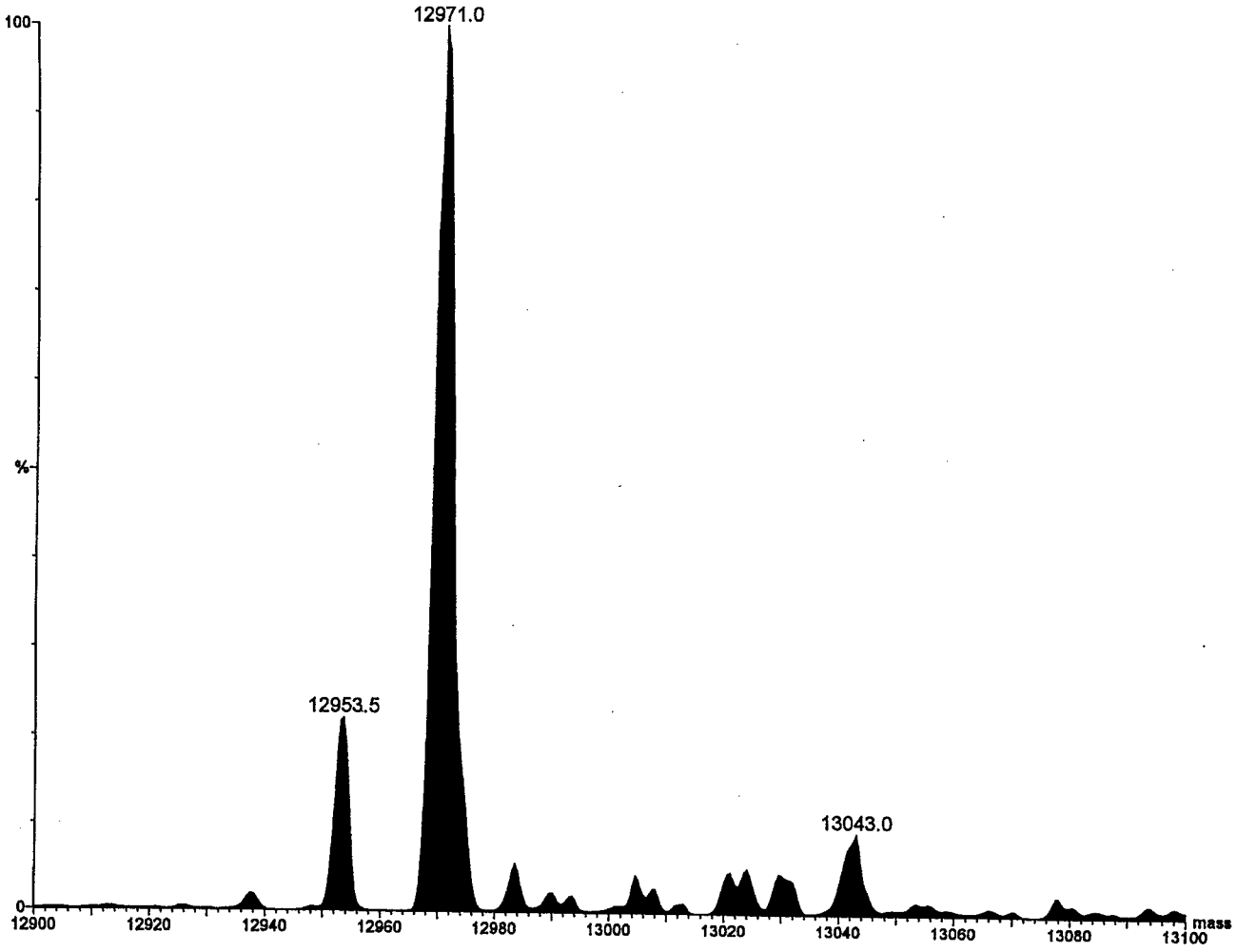




Electrospray Mass Spectrum (ESMS) of the wild type recombinant streptavidin (r-SAv) used in studies. Note that the minor peaks are thought to be Ca ion adducts or other background noise and are not considered to be impurities in the protein.



Electrospray Mass Spectrum (ESMS) of the streptavidin obtained from Boehringer Mannheim (SAv-CS1; lot number 133816721). Three proteins of different masses are readily apparent (with masses marked). Note that the minor peaks are thought to be Ca ion adducts or other background noise and are not considered to be impurities in the protein.



Electrospray Mass Spectrum (ESMS) of the streptavidin obtained from CALBIOCHEM (SAv-CS2; lot number B12651). Two proteins of different masses are readily apparent, with several other peaks likely to be attributed to different SAv proteins. Note that the smallest peaks are thought to be Ca ion adducts or other background noise and are not considered to be impurities in the protein.

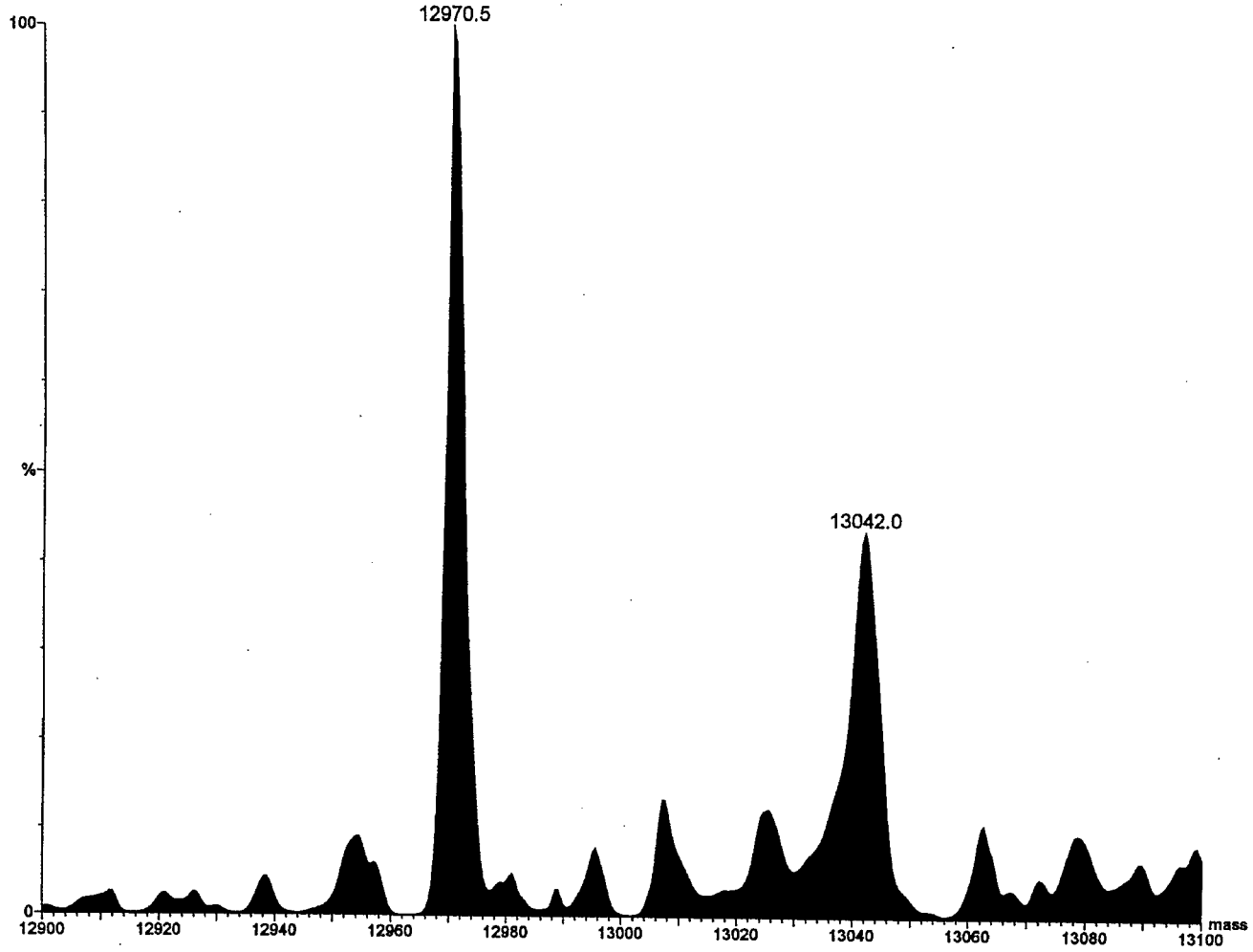


Table A: Distribution of Radioactivity at 4, 24 and 48 h Post Co-administration of r-SAv Mutant, r-[^{125}I]SAv-H127C, and r-[^{131}I]SAv in Athymic Mice[a]

Tissues	4 h[b,c]		24 h		48 h	
	I-125	I-131	I-125	I-131	I-125	I-131
blood	11.43 ± 0.40[e]	10.35 ± 0.35[e]	3.07 ± 0.27[e]	2.12 ± 0.23[e]	0.93 ± 0.22	0.58 ± 0.13
muscle	1.38 ± 0.09	1.24 ± 0.09	0.88 ± 0.08	0.69 ± 0.04	0.59 ± 0.04[e]	0.47 ± 0.03[e]
lung	7.52 ± 0.91	6.60 ± 0.84	2.88 ± 0.16[e]	2.16 ± 0.13[e]	2.08 ± 0.53	1.58 ± 0.41
kidney	18.92 ± 1.72[e]	26.43 ± 2.71[e]	17.21 ± 1.31	22.82 ± 1.47	20.17 ± 2.70	26.50 ± 3.21
spleen	3.96 ± 0.44	3.49 ± 0.39	3.12 ± 0.19	2.53 ± 0.19	2.64 ± 0.76	2.14 ± 0.63
liver	3.11 ± 0.30	2.77 ± 0.28	2.61 ± 0.07[e]	2.12 ± 0.07[e]	2.41 ± 0.31	1.96 ± 0.27
intestine	2.31 ± 0.26	2.05 ± 0.22	1.07 ± 0.09	0.82 ± 0.08	0.71 ± 0.13	0.55 ± 0.11
neck	4.51 ± 0.52	4.17 ± 0.48	3.92 ± 0.30	3.06 ± 0.22	6.48 ± 3.31	4.43 ± 1.93
stomach	2.69 ± 0.30	2.50 ± 0.29	1.78 ± 0.14[e]	1.24 ± 0.11[e]	1.44 ± 0.31	1.02 ± 0.19
urine[d]	15.76 ± 9.32	20.33 ± 8.93	11.90 ± 1.73	8.79 ± 1.44	7.24 ± 1.23	4.96 ± 0.79

[a]Values shown are % ID / g ± standard deviation. [b]Time of sacrifice from injection of radiolabeled r-SAv. [c]Data were obtained for n = 5 mice at each time point; average animal weight, 27.52 ± 2.04 g; Injectate for each animal had 15 µCi / 15 µg of r-SAv labeled with Na[^{131}I]I/ChT and 13 µCi / 15 µg of r-SAv mutant, r-SAv-H127C, labeled with Na[^{125}I]I/ChT in approximately 100 µL of 0.9% sterile saline. [d]Urine was collected by syringe bladder tap after sacrifice. [e]Statistical significance of difference in paired t test; $P<0.005$.

Table B: Distribution of Radioactivity at 4, 24 and 48 h Post Co-administration of r-SAv Mutant, r-[^{125}I]SAv-S139C, and r-[^{131}I]SAv in Athymic Mice[a]

Tissues	4 h[b,c]		24 h		48 h	
	I-125	I-131	I-125	I-131	I-125	I-131
blood	10.67 ± 0.30[e]	9.15 ± 0.37[e]	2.30 ± 0.44	1.90 ± 0.46	0.59 ± 0.05[e]	0.40 ± 0.03[e]
muscle	1.65 ± 0.20	1.46 ± 0.20	0.84 ± 0.22	0.74 ± 0.15	0.51 ± 0.03[e]	0.42 ± 0.03[e]
lung	6.72 ± 0.27[e]	5.59 ± 0.29[e]	2.53 ± 0.34	2.17 ± 0.35	1.44 ± 0.15	1.21 ± 0.13
kidney	19.84 ± 1.12	22.34 ± 1.14	19.53 ± 3.69	23.30 ± 4.60	17.10 ± 3.36	21.31 ± 3.70
spleen	3.98 ± 0.06[e]	2.99 ± 0.07[e]	3.69 ± 0.39[e]	2.59 ± 0.40[e]	2.96 ± 0.47	2.10 ± 0.27
liver	2.84 ± 0.15e	2.27 ± 0.13e	2.74 ± 0.43	2.22 ± 0.38	2.30 ± 0.23	1.85 ± 0.20
intestine	2.51 ± 0.26	2.14 ± 0.26	1.07 ± 0.15	0.87 ± 0.13	0.68 ± 0.05[e]	0.54 ± 0.04[e]
neck	4.91 ± 0.22[e]	4.01 ± 0.31[e]	4.75 ± 1.43	3.45 ± 0.91	5.81 ± 1.78	4.05 ± 1.07
stomach	2.46 ± 0.19[e]	1.96 ± 0.14[e]	1.81 ± 0.40	1.34 ± 0.32	1.62 ± 0.29	1.24 ± 0.21
urine[d]	25.86 ± 2.25	28.79 ± 3.12	9.54 ± 1.17	8.92 ± 1.21	6.02 ± 1.23	4.73 ± 0.92

[a]Values shown are % ID / g ± standard deviation. [b]Time of sacrifice from injection of radiolabeled r-SAv. [c]Data were obtained for n = 5 mice at each time point; average animal weight, 26.34 ± 1.36 g; Injectate for each animal had 18 µCi / 15 µg of r-SAv labeled with Na[^{131}I]I/ChT and 17 µCi / 15 µg of r-SAv mutant, r-SAv-S139C, labeled with Na[^{125}I]I/ChT in approximately 100 µL of 0.9% sterile saline. [d]Urine was collected by syringe bladder tap after sacrifice. [e]Statistical significance of difference in paired t test; P<0.005.

Table C: Distribution of Radioactivity at 4, 24 and 48 h Post Co-administration of SAv from a Commercial Source, [^{125}I]SAv-CS1[a], and r-[^{131}I]SAv in Athymic Mice[b]

Tissues	4 h[c,d]		24 h		48 h	
	I-125	I-131	I-125	I-131	I-125	I-131
blood	6.65 ± 0.48[f]	8.38 ± 0.65[f]	1.33 ± 0.19[f]	1.94 ± 0.24[f]	0.31 ± 0.06	0.48 ± 0.09
muscle	1.33 ± 0.15	1.38 ± 0.17	0.55 ± 0.12[f]	0.77 ± 0.03[f]	0.43 ± 0.11	0.60 ± 0.14
lung	5.14 ± 0.41	5.83 ± 0.44	1.79 ± 0.20	2.28 ± 0.25	1.02 ± 0.22	1.34 ± 0.28
kidney	18.69 ± 3.46	19.31 ± 3.23	15.68 ± 1.18[f]	22.09 ± 1.35[f]	15.48 ± 3.97	23.55 ± 5.41
spleen	1.92 ± 0.26	2.29 ± 0.32	1.68 ± 0.22[f]	2.29 ± 0.24[f]	1.45 ± 0.21	2.08 ± 0.37
liver	1.94 ± 0.21	2.23 ± 0.26	1.97 ± 0.22	2.42 ± 0.25	1.53 ± 0.30	1.96 ± 0.35
intestine	1.56 ± 0.27	1.73 ± 0.28	0.70 ± 0.12	0.87 ± 0.14	0.43 ± 0.05[f]	0.57 ± 0.06[f]
neck	4.22 ± 0.55	4.67 ± 0.70	3.87 ± 1.46	4.16 ± 1.20	2.84 ± 2.42	3.42 ± 2.48
stomach	1.87 ± 0.24	1.93 ± 0.26	1.56 ± 0.30	1.65 ± 0.26	1.25 ± 0.11	1.53 ± 0.13
urine[e]	36.96 ± 17.16	20.33 ± 8.93	10.91 ± 1.44	8.43 ± 0.52	4.62 ± 1.19	5.32 ± 1.19

[a]The SAv was purchased from Boehringer Mannheim (Indianapolis, IN). [b]Values shown are % ID / g ± standard deviation. [c]Time of sacrifice from injection of radiolabeled SAv. [d]Data were obtained for $n = 5$ mice at each time point; average animal weight, 24.54 ± 1.82 g; Injectate for each animal had 15 µCi / 15 µg of r-SAv labeled with Na[^{131}I]I/ChT and 15 µCi / 15 µg of SAv-CS1 labeled with Na[^{125}I]I/ChT in approximately 100 µL of 0.9% sterile saline. [e]Urine was collected by syringe bladder tap after sacrifice. [f]Statistical significance of difference in paired t test; $P<0.005$.

Table D: Distribution of Radioactivity at 4, 24 and 48 h Post Co-administration of SAv from a Commercial Source, [^{125}I]SAv-CS2[a], and r-[^{131}I]SAv in Athymic Mice[b]

Tissues	4 h[c,d]		24 h		48 h	
	I-125	I-131	I-125	I-131	I-125	I-131
blood	6.64 ± 0.23[f]	9.12 ± 0.40[f]	1.35 ± 0.14[f]	2.16 ± 0.31[f]	0.34 ± 0.04[f]	0.54 ± 0.05[f]
muscle	1.30 ± 0.07[f]	1.48 ± 0.07[f]	0.71 ± 0.16	0.98 ± 0.20	0.38 ± 0.02[f]	0.57 ± 0.05[f]
lung	4.61 ± 0.26[f]	5.73 ± 0.28[f]	1.83 ± 0.20[f]	2.53 ± 0.24[f]	1.00 ± 0.11[f]	1.45 ± 0.15[f]
kidney	29.23 ± 1.48[f]	21.65 ± 1.65[f]	24.27 ± 4.43	27.25 ± 4.64	20.33 ± 2.30[f]	26.56 ± 2.78[f]
spleen	2.17 ± 0.21	2.63 ± 0.23	1.77 ± 0.09[f]	2.48 ± 0.15[f]	1.62 ± 0.15[f]	2.45 ± 0.28[f]
liver	2.07 ± 0.13[f]	2.55 ± 0.18[f]	1.96 ± 0.20[f]	2.61 ± 0.27[f]	1.69 ± 0.15[f]	2.32 ± 0.22[f]
intestine	1.54 ± 0.30	1.86 ± 0.37	0.84 ± 0.31	1.07 ± 0.31	0.46 ± 0.03[f]	0.65 ± 0.05[f]
neck	4.59 ± 0.78	4.67 ± 0.70	2.63 ± 0.48	3.42 ± 0.62	3.64 ± 3.84	3.74 ± 2.37
stomach	3.49 ± 0.27[f]	1.93 ± 0.21[f]	1.79 ± 0.35	1.76 ± 0.31	1.45 ± 0.19	1.78 ± 0.19
urine[e]	33.77 ± 16.30	20.33 ± 8.93	8.78 ± 5.45	9.11 ± 7.59	4.02 ± 2.51	4.90 ± 2.92

[a]The SAv was purchased from CALBIOCHEM (La Jolla, CA). [b]Values shown are % ID / g ± standard deviation. [c]Time of sacrifice from injection of radiolabeled SAv. [d]Data were obtained for $n = 5$ mice at each time point; average animal weight, 23.38 ± 1.81 g; Injectate for each animal had 15 μCi / 15 μg of r-SAv labeled with Na[^{131}I]I/ChT and 11 μCi / 15 μg of SAv-CS2 labeled with Na[^{125}I]I/ChT in approximately 100 μL of 0.9% sterile saline. [e]Urine was collected by syringe bladder tap after sacrifice. [f]Statistical significance of difference in paired t test; $P<0.005$.