Efficacious vaccine against heroin contaminated with fentanyl

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Supplemental Information

Figure S1. MALDI-TOF MS spectrum of unconjugated BSA

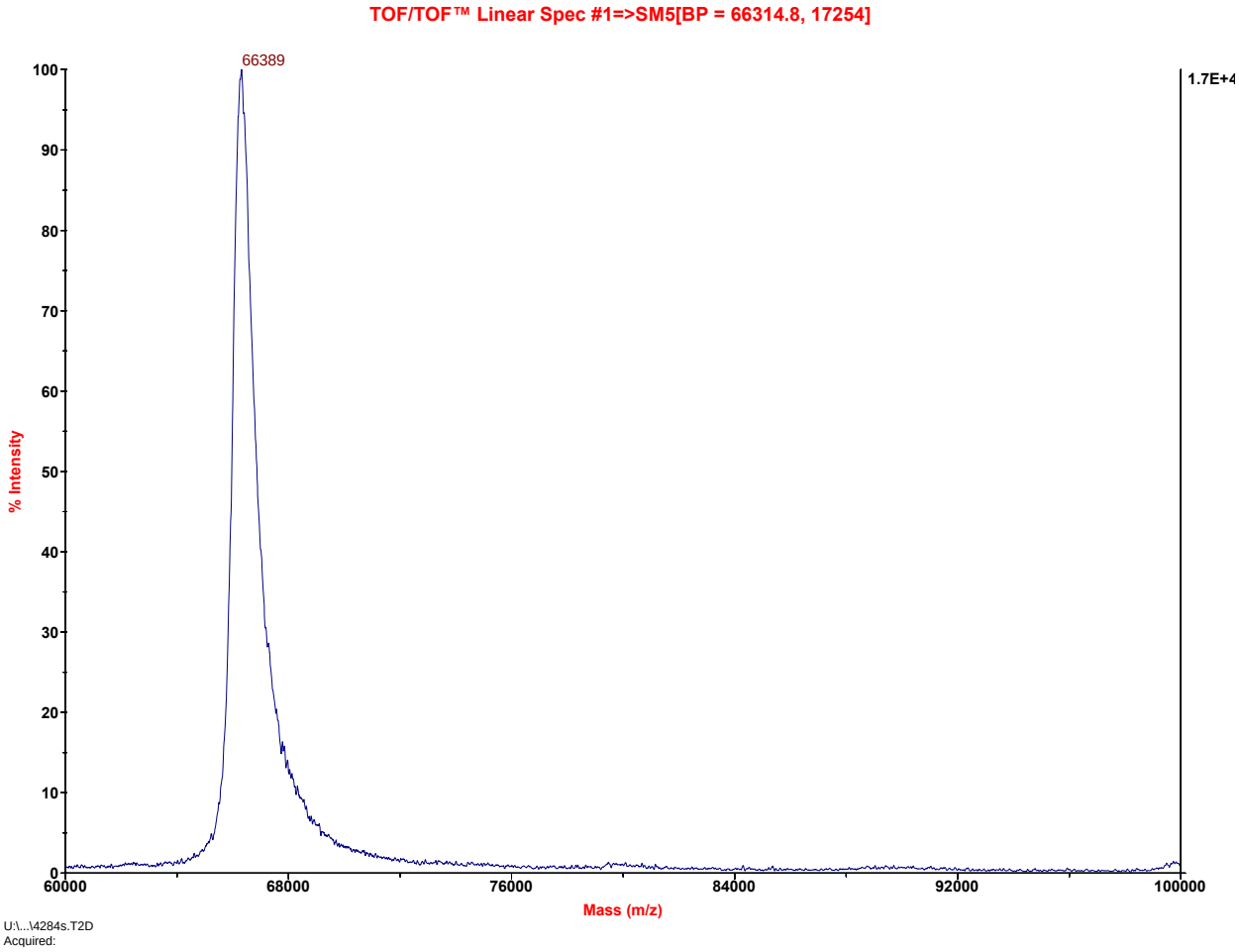


Figure S2. MS-ESI(+) spectrum of unconjugated BSA

Sample Name	JACH4284	Position	P1-A2	Instrument Name	Instrument 1
User Name		Inj Vol	8	InjPosition	
Sample Type	Sample	IRM Calibration Status	Success	Data Filename	092017_P_202_JACH4284.d
ACQ Method	routine_protein.m	Comment		Acquired Time	9/20/2017 5:33:54 PM (UTC-07:00)

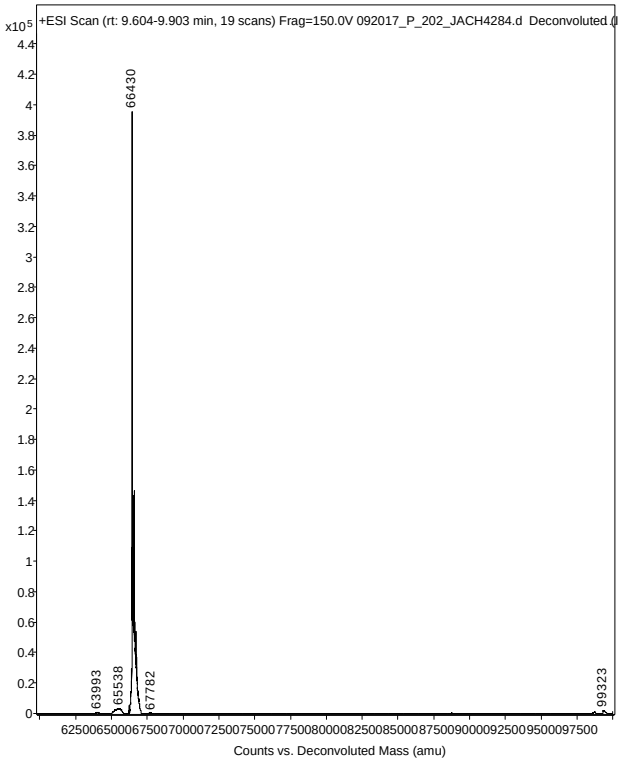


Figure S3. MALDI-TOF MS spectrum of Fent-BSA, 0.25 h

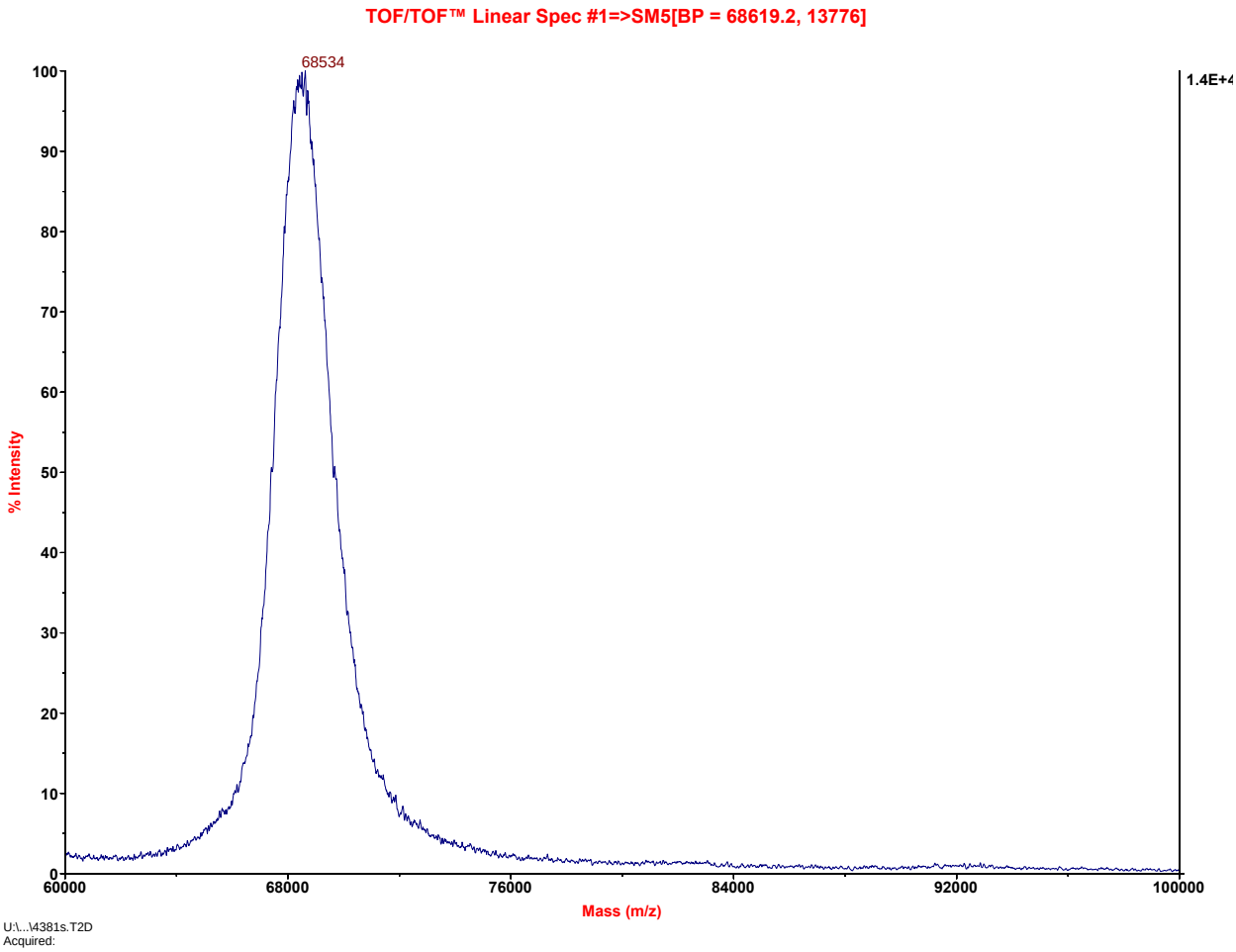


Figure S4. MALDI-TOF MS spectrum of Fent-BSA, 0.5 h

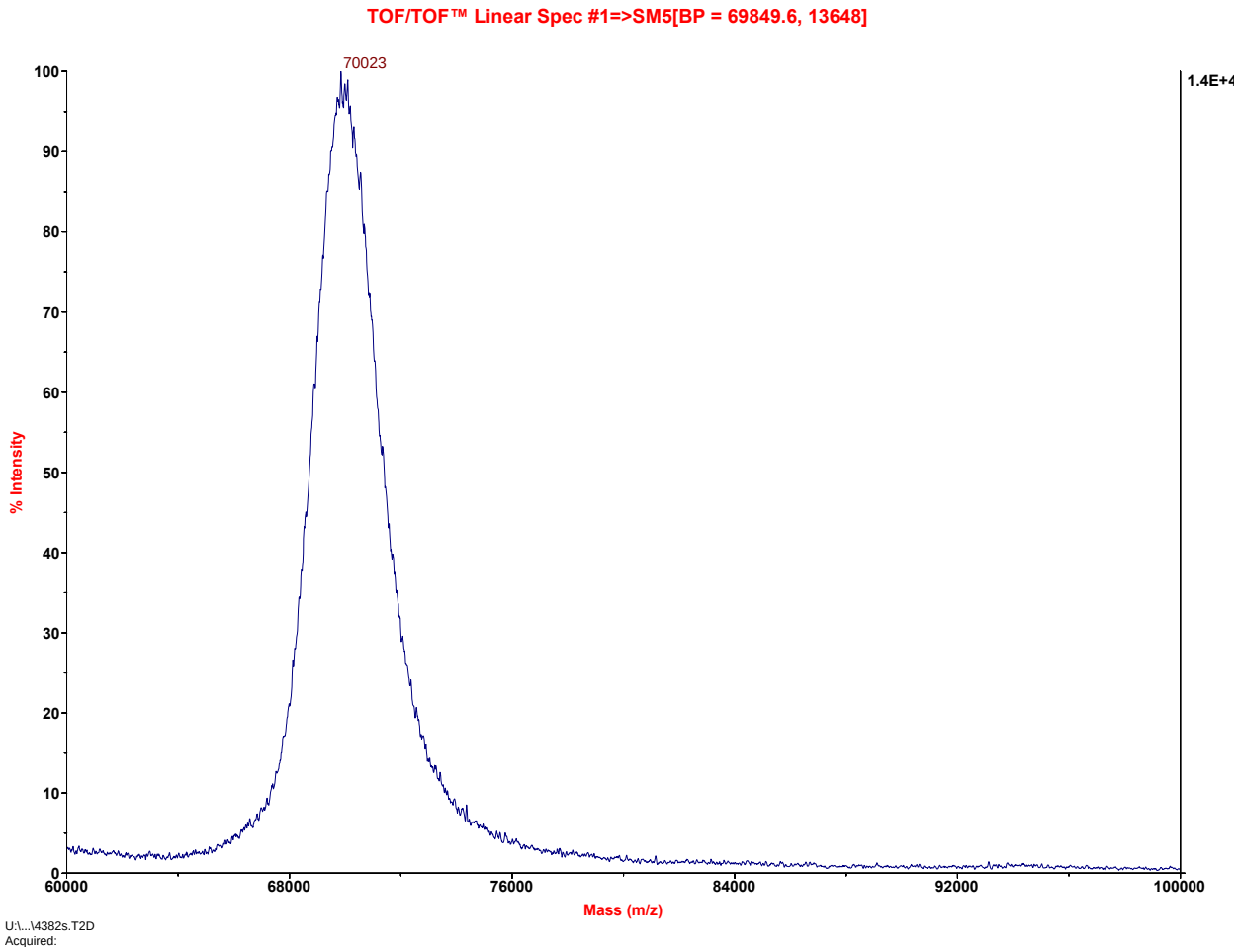


Figure S5. MALDI-TOF MS spectrum of Fent-BSA, 0.5 h (immunization batch)

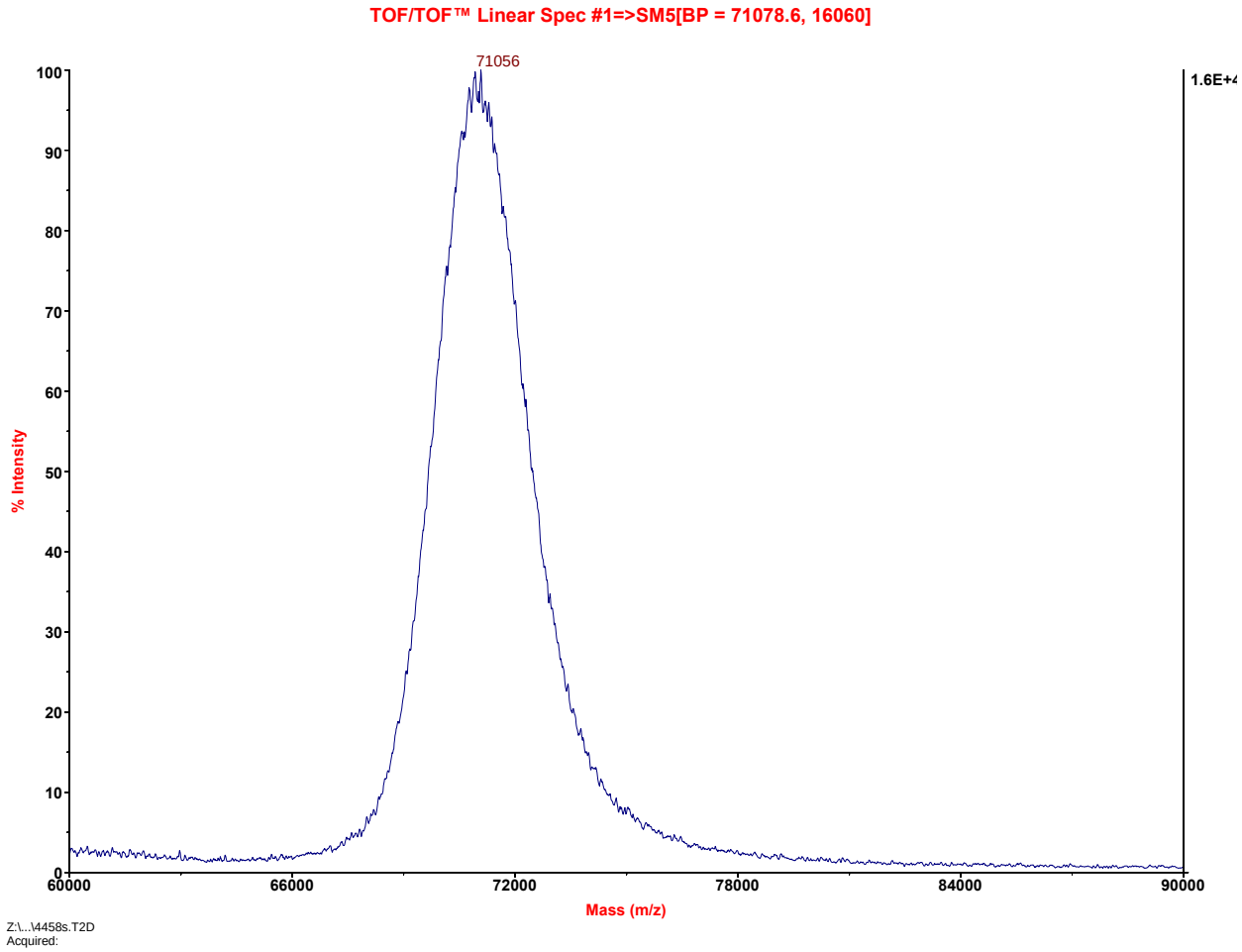


Figure S6. MALDI-TOF MS spectrum of Fent-BSA, 0.75 h

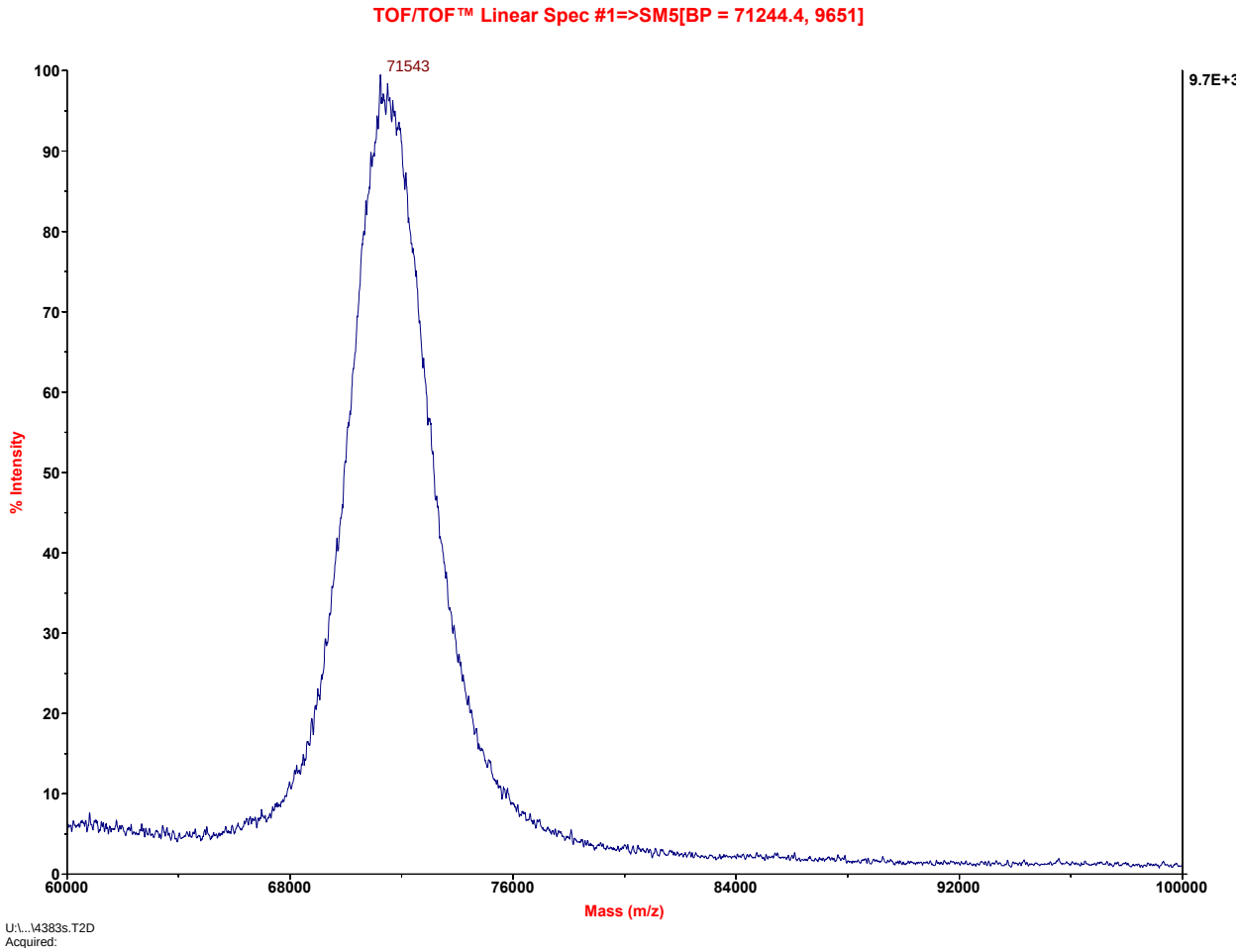


Figure S7. MALDI-TOF MS spectrum of Fent-BSA, 2h

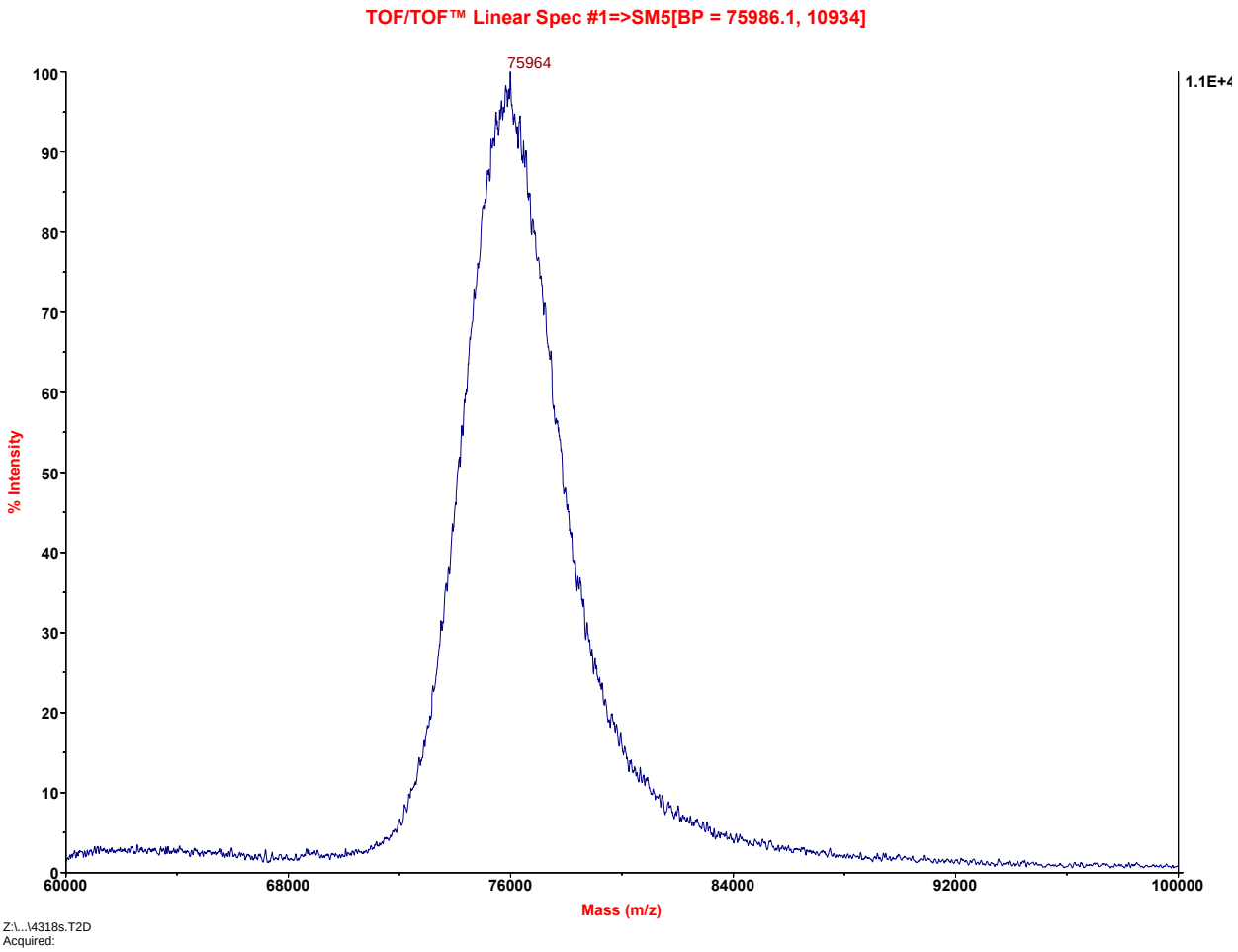


Figure S8. MALDI-TOF MS spectrum of Fent-BSA, 3h

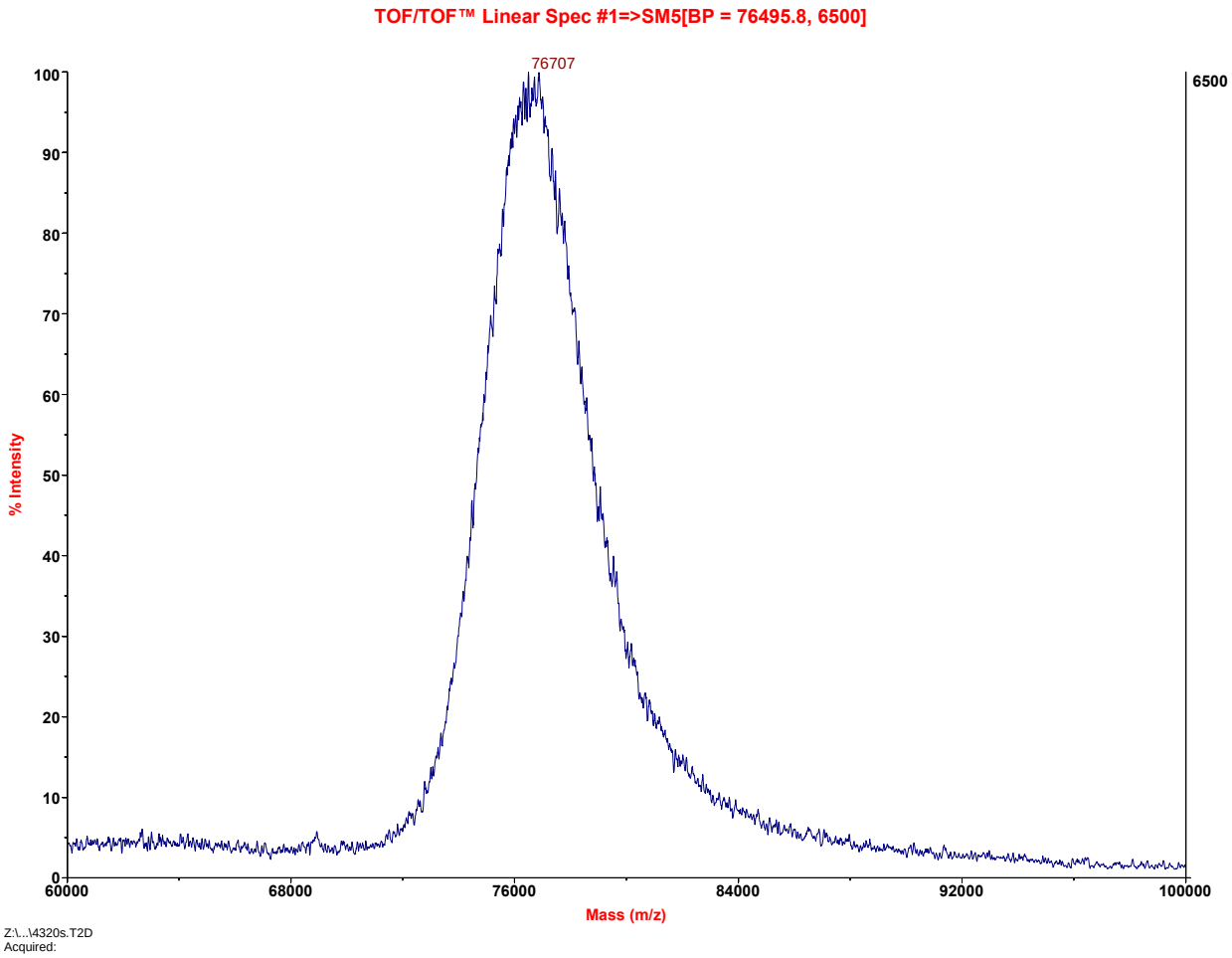


Figure S9. MALDI-TOF MS spectrum of Her-BSA, 1h

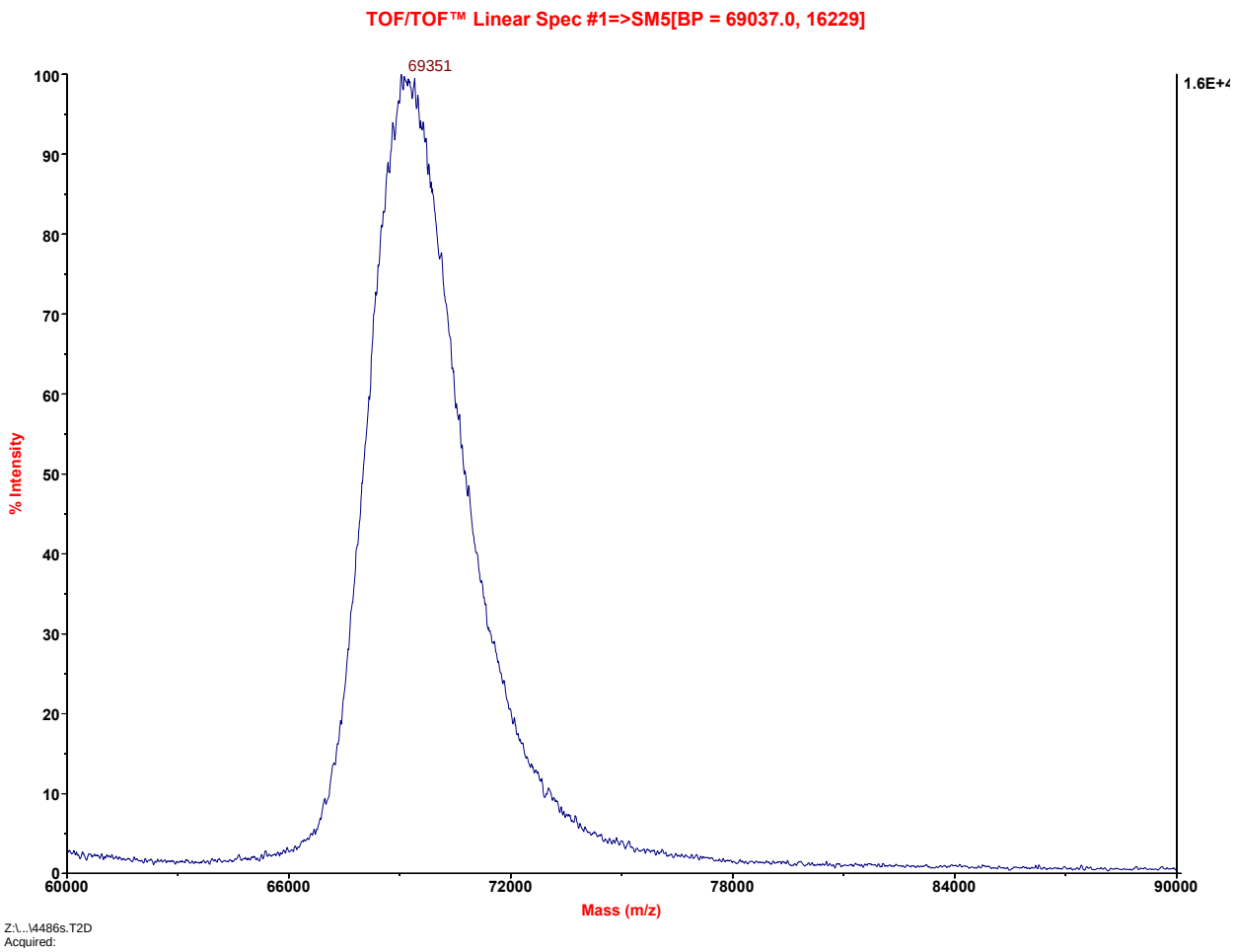


Figure S10. MALDI-TOF MS spectrum of Her-BSA, 1h (immunization batch)

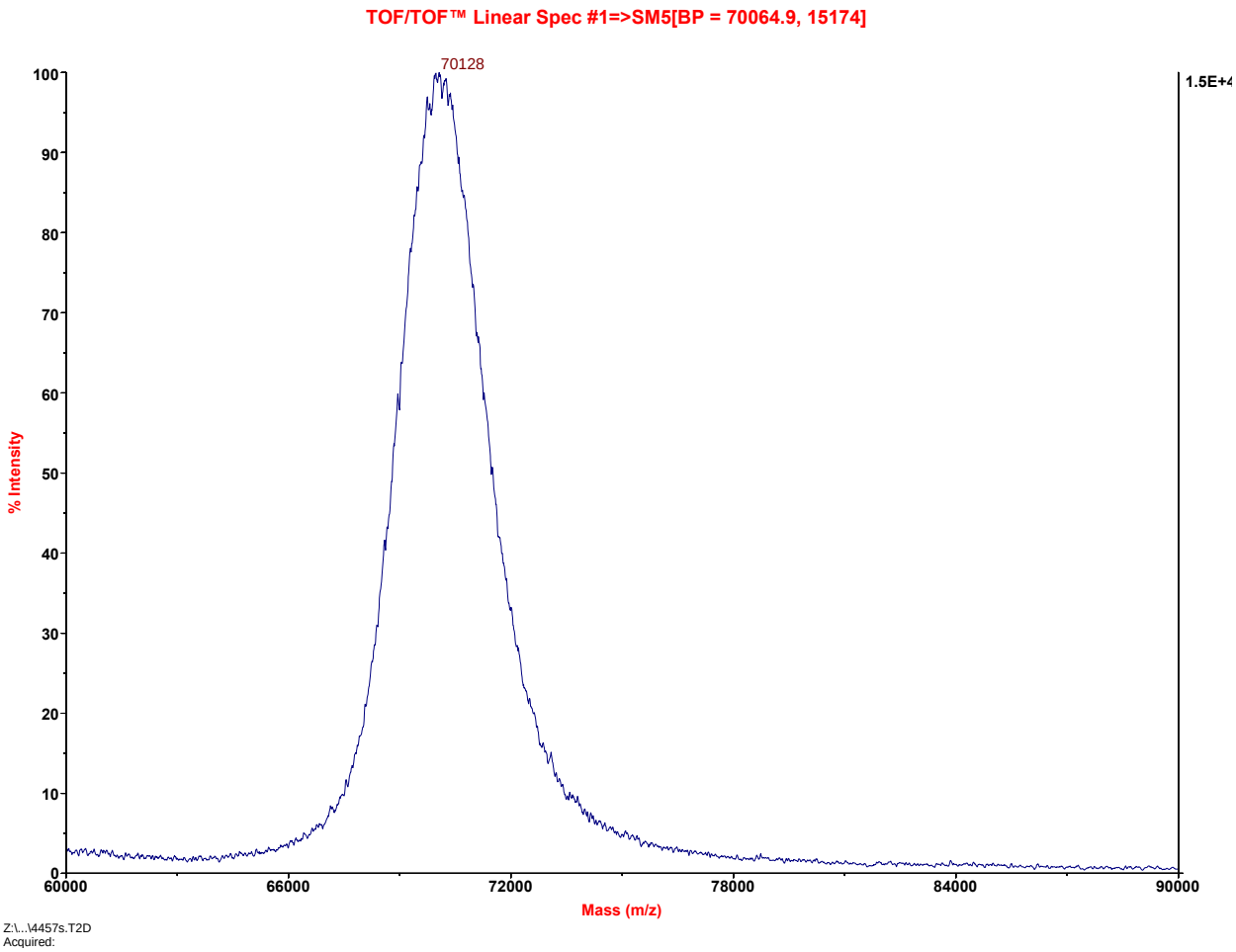


Figure S11. MALDI-TOF MS spectrum of Her-BSA, 2h

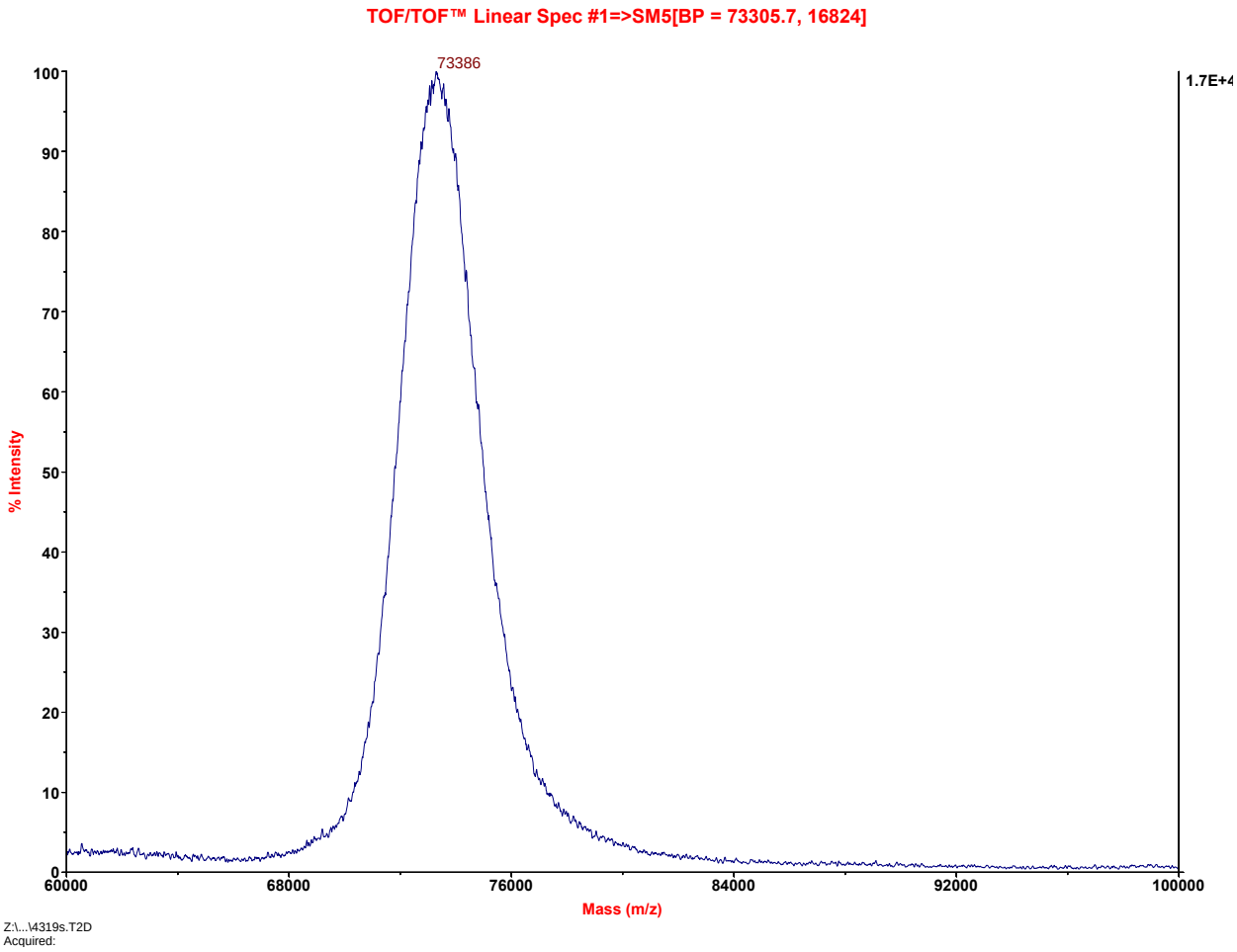


Figure S12. MALDI-TOF MS spectrum of Her-BSA, 3h

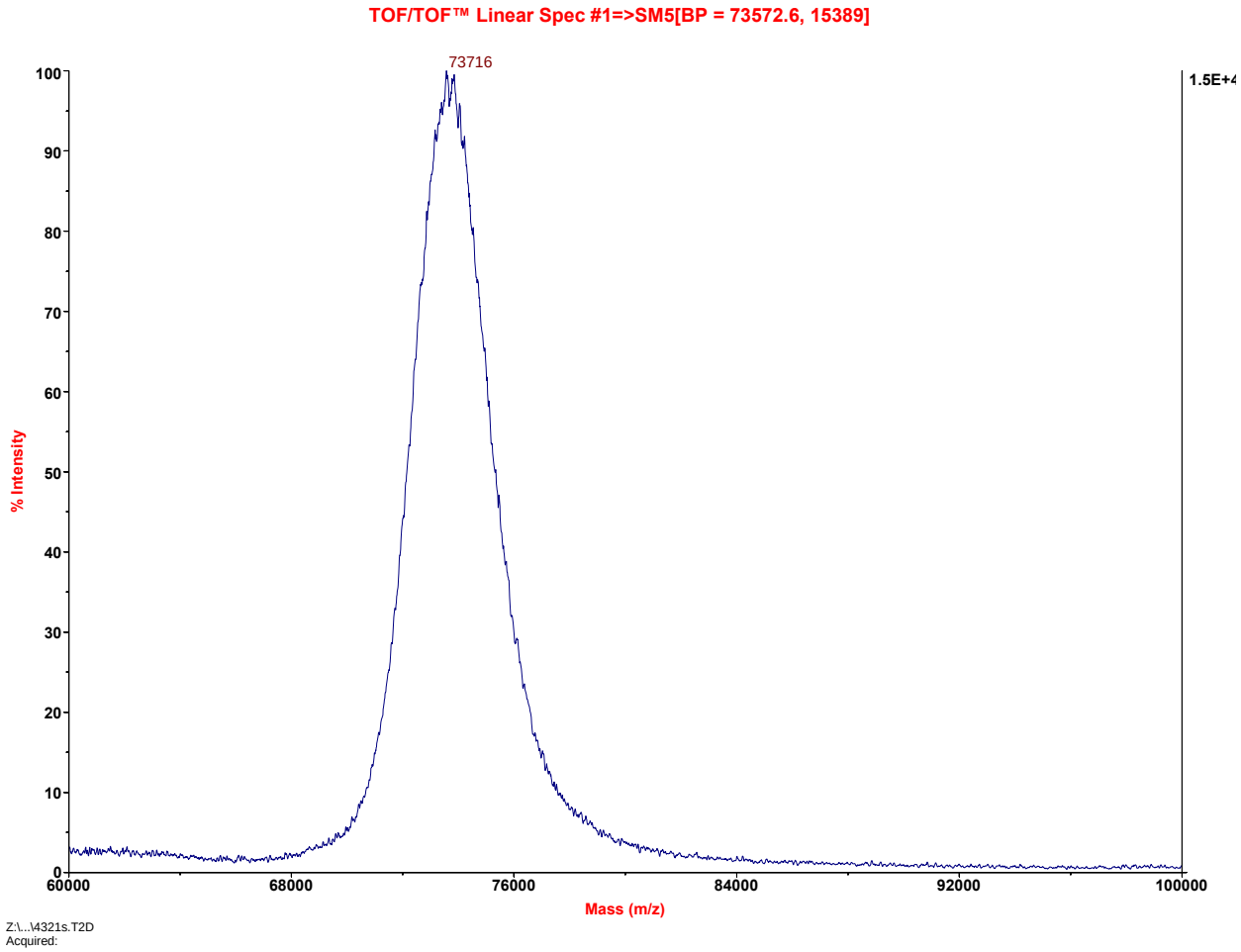


Figure S13. Relative hapten copy numbers vs. conjugation time

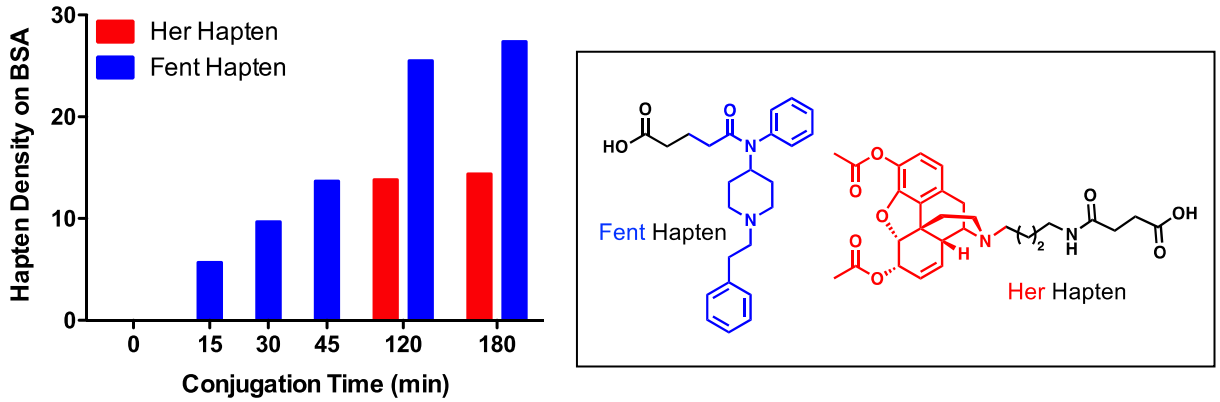


Table S1. Summary of MALDI-TOF results for heroin and fentanyl conjugates

Hapten	Conjugation Time (h)	MW	Conjugation Number
BSA only	N/A	66389	N/A
Fent	0.25	68534	5.7
Fent	0.5	70023	9.7
Fent	**0.5**	**71056**	**12.4**
Fent	0.75	71543	13.7
Fent	2	75964	25.5
Fent	3	76707	27.4
Her	**1**	**70128**	**7.4**
Her	2	73386	13.8
Her	3	73716	14.4

Conditions in **bold** indicate BSA surrogates that represent the KLH batch used for immunization.

Figure S14. Change in mouse body weights throughout vaccination schedule

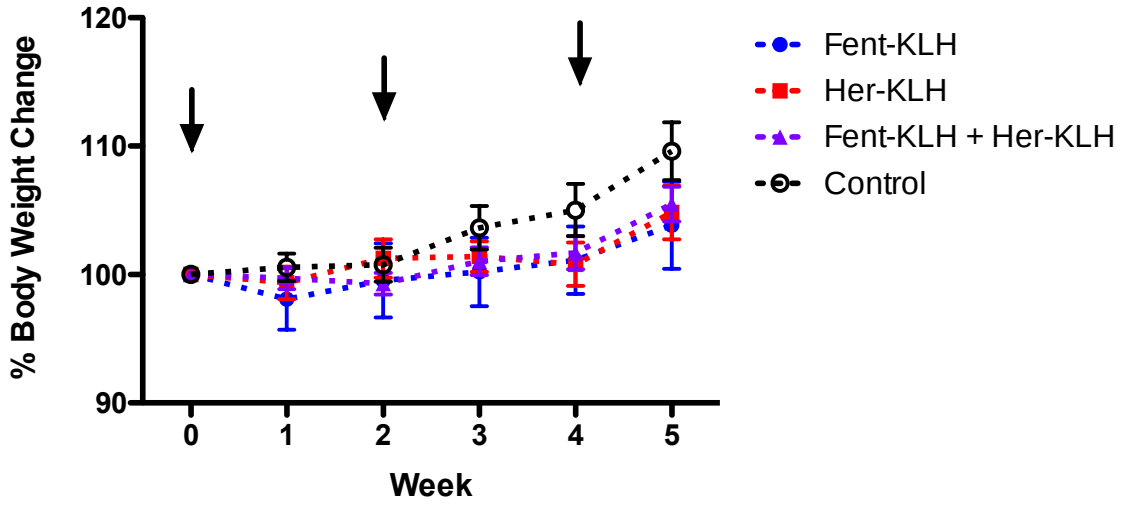


Figure S15. Injection site reactions for each vaccination group

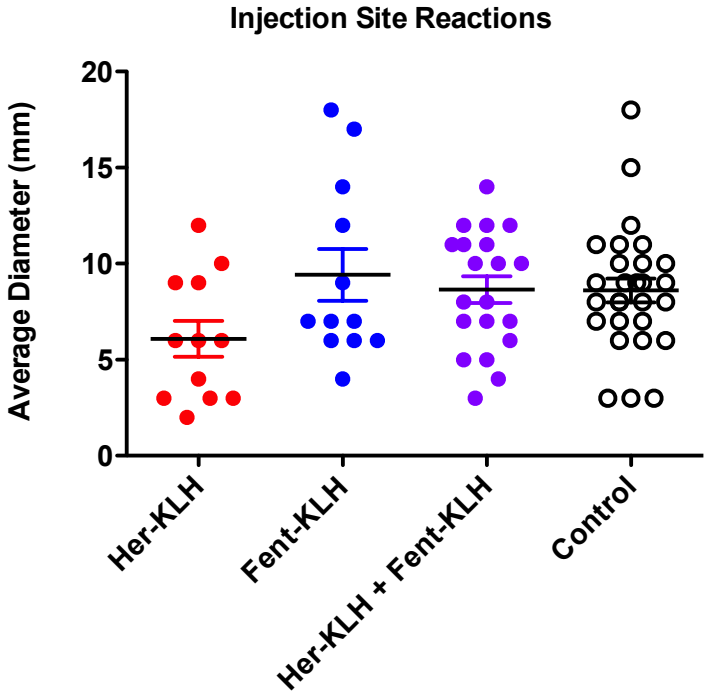


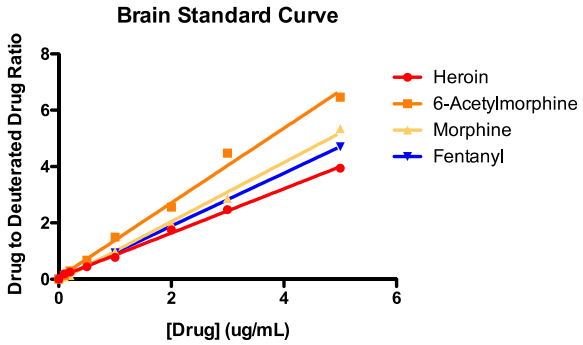
Table S2. Log(IC_{50}) values ± SEM of all vaccination groups over time determined by SPR

		Log(IC_{50}) ± SEM	
Vaccination Group	**Analyte**	**Week 3**	**Week 5**
Fent-KLH	6-AM[a]	–	–
	Fentanyl	-6.40 ± 0.12	-8.13 ± 0.03
Her-KLH	6-AM[b]	-6.50 ± 0.02	-6.66 ± 0.18
	Fentanyl[a]	–	–
Fent-KLH + Her-KLH	6-AM[b]	-6.55 ± 0.05	-6.57 ± 0.73
	Fentanyl	-6.63 ± 0.09	-8.03 ± 0.03
Control	6-AM[b]	n.d.[c]	n.d.[c]
	Fentanyl	n.d.[c]	n.d.[c]

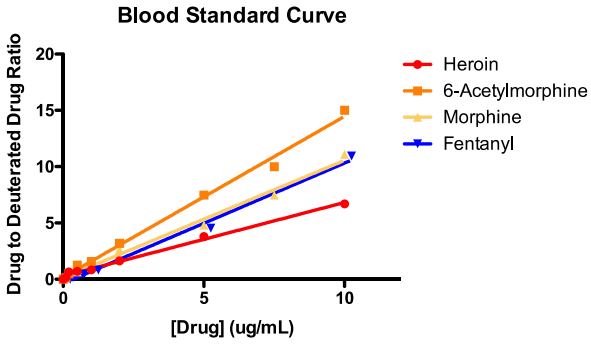
[a] 6-AM and fentanyl were not run for Fent-KLH and Her-KLH vaccination groups, respectively
[b] 6-AM was the primary analyte used in the SPR studies due to its longer half-life at pH 7.4 at rt compared to heroin. Heroin rapidly hydrolyzed during the experimental timeframe and therefore was not reported
[c] Control vaccine groups were run by SPR but did not converge due to lack of detected antibody binding

Figure S16. Standard curves for blood-brain barrier concentrations

Curves were generated using blank plasma or brain samples with known concentrations of heroin, 6-acetylmorphine, morphine, and fentanyl. The curves were used to quantify concentrations of drug in mice by LCMS analysis.



Drug	Standard Curve Equation	R^2
Heroin	$y = 0.785x + 0.0718$	0.998
6-Acetylmorphine	$y = 1.33x + 0.0594$	0.993
Morphine	$y = 1.05x - 0.0533$	0.996
Fentanyl	$y = 0.939x + 0.00885$	1.00



Drug	Standard Curve Equation	R^2
Heroin	$y = 0.655x + 0.270$	0.993
6-Acetylmorphine	$y = 1.42x + 0.118$	0.994
Morphine	$y = 1.04x + 0.118$	0.991
Fentanyl	$y = 1.07x - 0.104$	0.993

Figure S17. Blood-brain barrier concentrations of all drug metabolites

Bars show means + SEM. Significance is denoted by an asterisk from a two-way ANOVA and a Dunnett *post hoc* test when comparing vaccinated groups to vaccinated controls. *$P < 0.05$ and ****$P < 0.0001$ versus control.

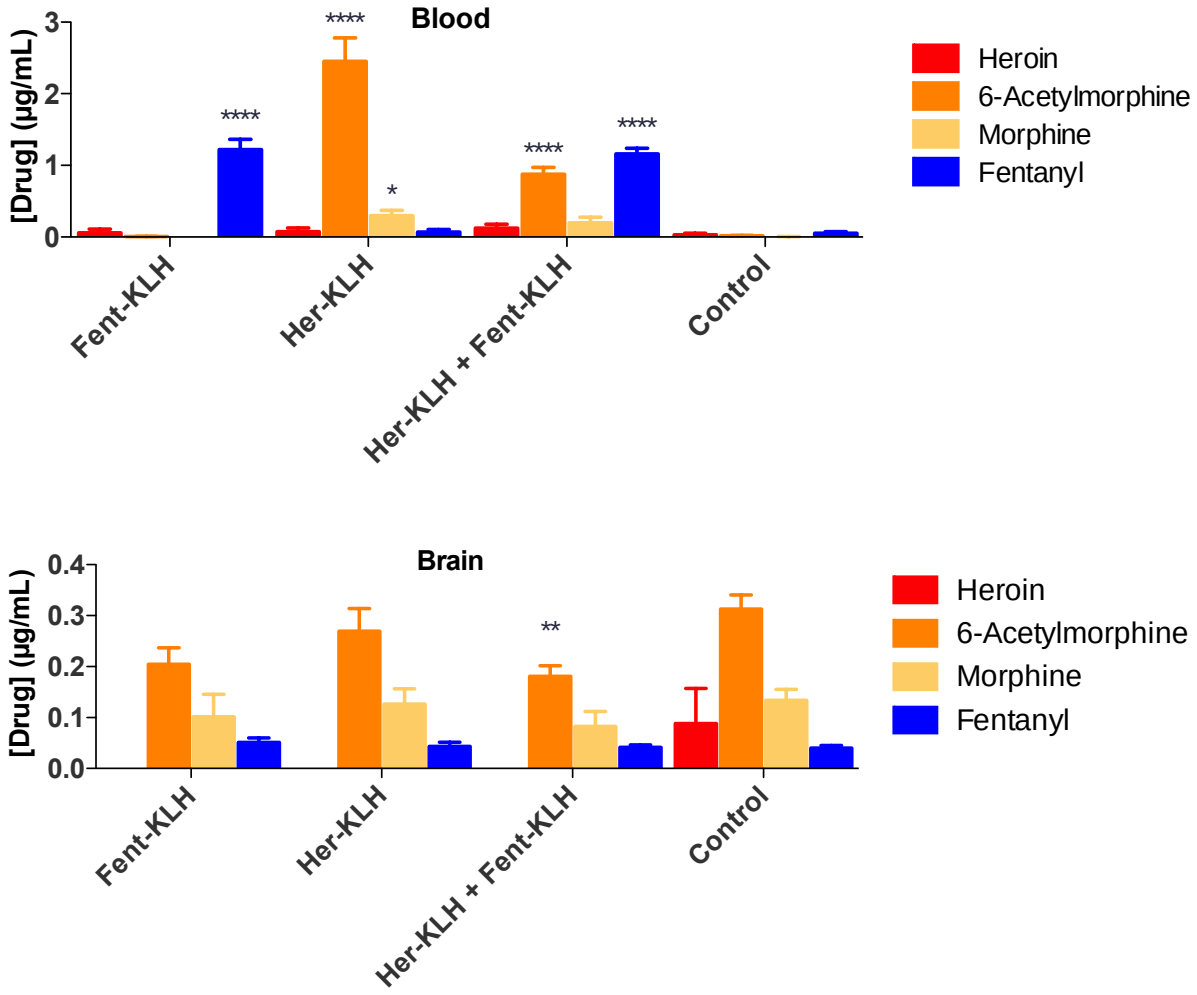
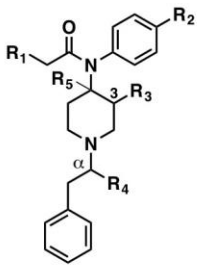


Figure S18. Log(IC_{50}) values ± SEM of Fent-KLH and admixture vaccinated mice against fentanyl and related analogues



A. Fentanyl hapten: R_1=$(CH_2)_2$COOH
B. Fentanyl: R_1=Me
C. Acetylfentanyl: R_1=H
D. Butylfentanyl: R_1=Pr
E. Tolylfentanyl: R_1=Me, R_2=Me
F. 3-Methylfentanyl: R_1=Me, R_3=Me
G. α–Methylfentanyl: R_1=Me, R_4=Me
H. Carfentanil: R_1=Me, R_5=COOMe
Unspecified R Groups = H

		Log(IC_{50}) ± SEM	
	Analyte	**Fent-KLH**	**Fent-KLH + Her-KLH**
B.	Fentanyl	-8.13 ± 0.03	-8.03 ± 0.03
C.	Acetylfentanyl	-8.30 ± 0.06	-8.08 ± 0.01
D.	Butylfentanyl	-8.26 ± 0.09	-8.16 ± 0.05
E.	Tolylfentanyl	-7.54 ± 0.05	-7.54 ± 0.04
F.	3-Methylfentanyl	-6.56 ± 0.06	-6.60 ± 0.04
G.	α-Methylfentanyl	-6.94 ± 0.03	-6.85 ± 0.07
H.	Carfentanil	-6.82 ± 0.09	-6.86 ± 0.08