Supporting information for

Hydrogen Deuterium Exchange and other Mass Spectrometry-based Approaches for Epitope Mapping

Prashant N. Jethva and Michael L. Gross

Department of Chemistry, Washington University in St. Louis, St Louis, MO, USA.

Table 1: Summarizing the literature. Papers reporting HDX-MS based epitope mapping were retrieved by using SCOPUS and PUBMED databases with the search query "Epitope Mapping + Mass Spectrometry + Hydrogen Deuterium Exchange". Whenever an epitope type is not mentioned, the assignment is based on protection profiles observed in HDX-MS of peptides. Longer peptides and protection in multiple regions are considered as a signature of a conformational epitope. [$]Protease (O) and protease (C) indicate offline digestion and immobilized column digestion, respectively. *U and G indicate urea and guanidinium hydrochloride, respectively. [#]C and L indicate conformational and linear epitopes, respectively. We apologize to authors whose work was unintentionally overlooked in our literature searches.

Sr No.	Antigen	Size (Number of aa) Coverage (%)		Digestion enzyme[$]	Quench condition		Antibody	Type of antibody	Type of epitope[#]	Data analysis software	References
					TCEP	Denat*					
1	IL-6	184	100%	Infrared multiphoton dissociation (IRMPD)	-	-	MH166	IgG1	C	EIC analysis	(Yamada et al., 2002)
2	Thrombin	294	50%	Pepsin (O)	-	-	mAb		C	EIC analysis	(Baerga-Ortiz et al., 2002)
3	IL-1β	150	100%	Pepsin (O)	-	-	Hu007	IgG1	C	EIC analysis	(Lu et al., 2005)
4	Cytochrome C	104	100%	Pepsin (C)	-	-	E8	IgG1	C	Custom	(Coales et al., 2009)
5	Extracellular domain of human FasL protein	148	100%	Pepsin (O)	-	-	LA296	IgG4	C	EIC analysis	(Obungu et al., 2009)
6	IL17	152	100%	Pepsin (C)	1M	2M U	CAT-2200	IgG1	C	EIC analysis	(Gerhardt et al., 2009)
7	rAna o 2	457	100%	Pepsin, Fungal type XIII (O)	0.2M	8M U	1F5 2B5	IgG IgG	L C	Custom	(Zhang et al., 2011)
8	Cytocrome C, IL-13, IL17	104, 113, 152	100%	Pepsin (C)	1M	2M U 1.6M G	E8, CNTO607 CAT-2200	All IgG1	C C C	Custom	(Pandit et al., 2012)
9	Factor Hbinding protein (fHbp)	256	100%	Pepsin (C)	-		12C1	IgG2b	C	DynamX	(Malito et al., 2013)
10	rAna o 2	457	100%	Fungal type XIII (O)	0.2M	8M U	pAb	Polyclonal IgG	C	Custom	(Zhang et al., 2013)

11	Almond Prunin (Pru du 6)	540	100%	Fungal type XIII (O)	0.2M	8M U	4C10	IgG	C	Custom	(Willison et al., 2013)
12	Coagulation factor VIII	731	95%	Pepsin (C)	1.35M	-	KM33	IgG	C	DynamX	(Bloem et al., 2013)
13	Coagulation factor VIII C2 domain	162	86%	Pepsin (O)	-		BO2C11 I109 3E6 G99	IgG4 IgG1 IgG2 IgG2	C C C C	HX-Express	(Sevy et al., 2013)
14	TNFα (tumour necrosis factor α)	157	100%	Pepsin (C)	1M	2M U	AZD9773	Polyclonal	C	Custom	(Abbott et al., 2013)
15	Cp149.3CA from hepatitis B virus	149	100%	Pepsin (C)	-	4M G	E1 3120	Fabs	C C	DynamX	(Bereszczak et al., 2013)
16	Factor H binding protein (fHbp)	263	100%	Pepsin (C)	-		17C1 30G4	IgG1 IgG1	C C	HX-Express	(Faleri et al., 2014)
17	VP6f	322	100%	Pepsin (C)	-		RV6-25	Fab	C	DXMS	(Aiyegbo et al., 2014)
18	FtGroEL	541	100%	Pepsin (C)	-		Ab53, Ab64 N200 N30	All IgG2a	L L C L	DXMS	(Lu et al., 2014)
19	Vaccinia virus (VACV) L1	181	100%	Pepsin (C)	0.1M	1.4M G	M12B9	IgG2a	C	HDExaminer	(Kaever et al., 2014)
20	Neisseria adhesin A (NAD) 3 (24-170)	147		Pepsin (C)	-	4M G	33E8	Fab	C	DynamX	(Malito et al., 2014)
21	PD-L1	240	95%		0.2M	0.4M G	Anti-PD-L1	Fab	C	HDExaminer	(Hao et al., 2015)
22	Ana o 1	512	100%	Fungal type XIII (O)	0.2M	8M U	2G4	-	C	Custom	(Guan et al., 2015)
23	MDTCS domains	690	100%	Pepsin and Fungal XIII (O) parallel	0.09M	2M G	scVs	-	C	EXMS	(Casina et al., 2015)
24	Human CD1d	335	>90%	Pepsin (C)	1M	2M U	NIB.2	IgG4	C	Not defined	(Nambiar et al., 2015)
25	Duffy-binding–like (DBLb3_D4)	484	79%	Pepsin and nepenthesin -1 (C)	0.8M		24E9	IgG1	C	HDExaminer	(Lennartz et al., 2015)
26	Neisseria meningitidis adhesin A NadA	350	98%	Pepsin (C)	-	-	9F11	IgG2b	C	DynamX	(Caricchio et al., 2016)

27	Neisseria meningitidis adhesin A NadA	350	98%	Pepsin (C)	-	-	6e3	IgG1	C	DynamX	(Bertoldi et al., 2016)
28	Plasmodium vivax Duffy binding protein pvDBP	525	100%	Pepsin (C)	-	-	2D10 2H2 2C6	IgG1, IgG1 IgG2b	C	HDX workbench	(Chen et al., 2016)
29	Neisserial Heparin Binding Antigen (NHBA)	600	70%	Pepsin (C)	-	-	31E10/E7	IgG2a	C	DynamX	(Domina et al., 2016)
30	Anganese Transporter MntC	291	100%	Pepsin (C)	-	-	305-72-5, 305-78-7, 305-101-8	All IgG	C C C	DXMS	(Gribenko et al., 2016)
31	Human cystatin C (hCC)	120	93%	Pepsin (C)	0.5M	3.3M U	Cys10, Cys28	IgG3 IgG1	C	HDExaminer	(Prądzińska et al., 2016)
32	Pneumococcal adhesin component (RrgA)	893	79%		0.2M	4M G	11B9/61		C	DynamX	(Amerighi et al., 2016)
33	CD73			Pepsin (C)	-		MEDI9447 Fab	IgG	C	DynamX	(Geoghegan et al., 2016)
34	Human IL-13	110	98%	Pepsin (C)	0.5M	4M G	Polyclonal antibodies (3 different serum samples)	IgG		Custom	(Yang et al., 2016)
35	Junctional adhesion molecule-A (JAM-A)	224	95%	Pepsin (C)	0.1M	2M G	hz6F4 F11 J10.4	IgG4	C C L	DynamX	(Terral et al., 2017)
36	Human Myoglobin	154	100%	Pepsin (O)	-	-	ab19607	IgG1	C	Custom	(Deng et al., 2017)
37	Human IL-23 p19	170	97%	Pepsin (C)	0.5M	1.5M G	bAb3, 7B7-Fab		C C	DynamX	(Li et al., 2017a)
38	Human IL-6R alpha extracellular region	338		Pepsin (C)	0.5M	4M G	adnectin1, adnectin2	-	L L	DynamX	(Li et al., 2017b)
39	Intact DENV particles			Pepsin (C)	0.25M	1.5M G	2D22		C	DynamX	(Lim et al., 2017)
40	Ricin toxin A subunit (RTA) (RiVax	267	100%	Pepsin (C)	-	-	JNM-C12, JNM-D1, V1B11	VH domain (VHH)	C C C	HDExaminer	(Vance et al., 2017)
41	Tau	441	75%	Pepsin (C)	0.5M	4M G	tau12, tau46	IgG1 IgG1	L L	DynamX	(Huang et al., 2018a)

42	Ricin toxin A subunit (RTA) (RiVax	267	100%	Pepsin (C)	-	-	PB10, R70, WECB2, SylH7, PA1, PH12, TB12, IB2, GD12, JD4	IgG2b Rest all IgG1	L L L C L C C C L L	HDExaminer	(Toth IV et al., 2017)
43	JEV E-DIII	102	100%	Pepsin (C)	0.5M	4M G	JEV-31 JEV-106 JEV-128 JEV-131 JEV-143	All IgG2c	C C C C C	HDX Workbench	(Fernandez et al., 2018)
44	TL1A, a tumor necrosis factor	253	80%	Pepsin (C)	0.4M	4M G	mAb1	IgG4	C	DynamX	(Huang et al., 2018b)
45	Glypican-3 (GPC3)	535	87%	Pepsin (C)	0.4M	4M G	mAb3.4, mAb1C2, SOMAmer	Aptamer	C	DynamX	(Duo et al., 2018)
46	Birch pollen allergen Bet v 1	159	94%	pepsin or type XIII protease (O)			5B4, 6H4	IgG1	C	HX-Express	(Brier et al., 2018)
47	IL-23R	373	100%	Pepsin (C)	0.32M	-	Macrocyclic Dodecapeptide Ligands	Macrocyclic compound	C		(Sayago et al., 2018)
48	Pollen allergen protein, birch Bet v1	159	100%	Pepsin (C)	0.2M	6M G	mAb1 mAb2 mAb3 mAb4	IgG	C & L	DynamX	(Zhang et al., 2018)
49	rFel d 1.mmh	199	-	-	-	-	REGN1908 REGN1909	IgG4	C L	-	(Orengo et al., 2018)
50	Diphtheria toxin (DTx)	527	90.70 %	Pepsin and fungal XIII dual enzyme (C)	0.5M	7.5M G	mAb 2−25, mAb 2−18		C	DynamX	(Zhu et al., 2019)

51	Hemagglutinin proteins of Infuenza A	522	>84%	Pepsin (C)	0.5M	4M G	Fab-CR6261, SD38, SD84, P3	IgG1 sdAb Cyclic peptide	C	DynamX	(Puchades et al., 2019)
52	PTH1R ECD		99%	Pepsin (C)	0.062 M	3.4M G	ECD-single chain Fv with human Fc fragment (scFvhFc)		C	DynamX	(Sarkar et al., 2019)
53	Recombinant SEB vaccine (STEBVax)			Pepsin (C)	0.5M	4M G	GC132a	IgG	C	HDExaminer	(Chen et al., 2019)
54	Major histocompatibility complex class I chain-related A and B	315	92%	Pepsin (C)	1M	8M U	mAb.2, mAb.39, mAb.40, mAb.36	All IgG1	C	DynamX	(Huang et al., 2020a)
55	Extracellular domain of mature PD-L1	290		Pepsin (C)	-	3M U	r22C3, r28-8	IgG	C	DynamX	(Lawson et al., 2020)
56	VEGF	121	80%	Pepsin (C)	EC*		mAb		C	DynamX	(Comamala et al., 2020)
57	VEGF	165		Pepsin beads	-		Avastin, ApoBev	IgG1	C	MS studio	(Brown et al., 2020)
58	Programmed cell death-1 (PD-1)	167	85%	Pepsin (C)	0.4M	4M G	Nivolumab	IgG4	C	DynamX	(Zhang et al., 2020)
59	Human cluster of differentiation 3 (CD3) and B-cell maturation antigen (BCMA).	174 and 80	98%, 84%	Pepsin (C)	0.4M	4M G	BsAb	Bispecific antibody	C	DynamX	(Huang et al., 2020b)
60	RBD-SARS-CoV2	223	84%	Pepsin and Fungal XII (C)	0.5 M	4 M U	REGN10987 REGN10934 REGN10989 REGN10977 REGN10933 REGN10954 REGN10986 REGN10964 REGN10984	All IgG	All C	HDExaminer	(Hansen et al., 2020)

61	ZIKV-DIII	108	95%	Pepsin (C)	0.5M	4M G	ZV2 ZV47 ZV48 ZV54 ZV67 ZV68	All IgG2c	C C C C C C	HDX workbench	(Adhikari et al., 2021)
62	Human carbonic anhydrase (hCAIX),	255	82%	Pepsin (C)	0.5M	0.5M G	m9B6, m4A2, c2C7	IgG	C & L	MS Studio	(Sheff et al., 2021b)
63	SARS-CoV-2 HexaPro spike	1250	56.3%	Pepsin (C)	0.2 M	8M U	3A3	IgG	C	DynamX	(Huang et al., 2021b)
64	DENV Envelope protein	395	61%	Pepsin (C)	-	2.5M G	1C19	IgG	C	DynamX	(Fibriansah et al., 2021)
65	Annexin-A1 (ANXA1)	180	100%	offline solution and beads both	0.4M	0.8M G	anti-ANXA1	IgG	C	HDExaminer	(Gramlich et al., 2021)
66	C- terminal H chain (HC) BoNT/A1	458	85%	Pepsin (C)	-	4M U	TA12		C	DynamX	(Brier et al., 2021)
67	laforin	329	100%	Pepsin (C)		0.08M G	Nb40 Nb41 Nb50 Nb57 Nb72 Nb73	Nanobodies	C	HDExaminer	(Simmons et al., 2021)
68	mFcNKG2A-CD9	462		Pepsin (C)	0.4M	4M G	Fab1 Fab2		C	DynamX	(Huang et al., 2021a)
69	Neisserial adhesin A (NadA)	350	89%	Pepsin (C)	-		1C6, 7F11	IgG IgG	C	DynamX	(Grauslund et al., 2021)
70	Factor H-binding protein (fHbp)	258	99%	Pepsin (C)	-	4M G	Polyclo. Abs	IgG	C	DynamX	(Ständer et al., 2021)
71	Ectodomain of IGF1R	900	47%	Pepsin (C)	0.25M	2M G	VHH-IR5	sdAb	L	MS Studio	(Sheff et al., 2021a)
72	Powassan virus Envelope protein (POWV-E)	397	100%	Pepsin and Fungal type XIII (C)	0.5M	4M G	POWV-4, POWV-63	IgG2c	C	HDExaminer	(VanBlargan et al., 2021)
73	Eastern Equine Encephalitis Virus (EEEV E)	415		Pepsin and Fungal type XIII (C)	1M	4M G	DC2.112	IgG	C	HDExaminer	(Kim et al., 2021)
74	Sema domain of c-Met (SD c-Met)	489	>82%	Pepsin chip, PNGase chip	EC*		mAb		C	DynamX	(Comamala et al., 2021)

75	pertactin (PRN).	597	88%	Pepsin (C)	0.5 M	7.5M G	1-16 3–4, 3–16, 3-3, 3–21, 3–5,	-	L C C L C C	DynamX	(Zhu et al., 2022)
76	Bacillus anthracis Protective Agent (PA)	735	>90%	Fungal type XIII (O)	-	50% ACN, 1% FA	p1C03 p6C0 p1A06 p6C01	All IgG	C C C C	Custom	(Fang et al., 2022)
77	Yellow Fever Virus Envelope protein	415	95%	Pepsin and Fungal type XIII (C)	0.5M	4M G	YFV-136		C	HDExaminer	(Doyle et al., 2022)
78	Panton-Valentine Leukocidin (LukSF-PV) and α-hemolysin	292 and 294	100%	Pepsin (C) and Fungal type XIII (O)	0.2M	4M G	SA02, SA131, SA185, H5	IgG	C	HDExaminer	(Kailasan et al., 2022)
79	C-Reactive protein (CRP)	206	>84%	Pepsin (C)	0.5M	6M G	KAAb1, KAAb2, KSAb1, KBAb mAb KSAb2	polyclonal antibodies IgG		DynamX	(Sun et al., 2022)
80	Signal-regulating protein alpha (SIRPα)	320	86%	Pepsin (O)	0.1M	2M G	nanobody		C	HDExaminer	(Gramlich et al., 2022)
81	Ubiquitous surface protein A2 (UspA2)	480	97%	Pepsin (C)	0.8M	7M U and 0.4M G	FHUSPA2 /10	IgG2a	C	DynamX	(Donnarumma et al., 2022)
82	SARS-CoV RBD	591	76%	Pepsin and Fungal Type XIII (C)	-	-	B9-scFv	-	C	DynamX	(Burke et al., 2022)
83	S1-RBD and ACE2			Pepsin (C)	0.3M	3M G	mAbs 127, mAbs 150				(Lai et al., 2022)

84	outer surface protein A (OspA) from *B. burgdorferi*	273	100	Pepsin (C)	-	-	221-11 221-20 857-2 227-1 272-2 212-2 212-55 319-28 219-44 319-33 LA-2 3-24	IgG1	C C C C C C C C C C C C	HDExaminer	(Haque et al., 2022)
85	Ectodomain of insulin-like growth factor-1 receptor (eIGF1R)	913	93%	Pepsin (C) Nepen-I(C)	-	-	SdAb (VHH-IR4/5)	-	C	HDExaminer	(Sheff et al., 2022)
86	Outer surface protein A (OspA) from *B. burgdorferi*	273	100%	Pepsin (C)	-	-	212-55 3-24	IgG1	C C	HDExaminer	(Haque et al., 2023)
87	Cardiac troponin I (cTnI)	206	91%	Pepsin (C)	0.1 M	4M U	R195 S13 F12 D1 D2 pAb1 pAb1	mAbs and pAbs	L	DynamX	(Song et al., 2023)

References:

Abbott, W.M., Snow, M., Eckersley, S., Renshaw, J., Davies, G., Norman, R.A., et al. (2013). Characterization of the complex formed between a potent neutralizing ovine-derived polyclonal anti-TNFα Fab fragment and human TNFα. *Bioscience Reports* 33(4), 655-664. doi: 10.1042/BSR20130044.

Adhikari, J., Zhao, H., Fernandez, E., Huang, Y., Diamond, M.S., Fremont, D.H., et al. (2021). Hydrogen–deuterium exchange mass spectrometry identifies spatially distinct antibody epitopes on domain III of the Zika virus envelope protein. *Journal of Mass Spectrometry* 56(1). doi: 10.1002/jms.4685.

Aiyegbo, M.S., Eli, I.M., Spiller, B.W., Williams, D.R., Kim, R., Lee, D.E., et al. (2014). Differential accessibility of a rotavirus VP6 epitope in trimers comprising type I, II, or III channels as revealed by binding of a human rotavirus VP6-specific antibody. *Journal of Virology* 88(1), 469-476. doi: 10.1128/JVI.01665-13.

Amerighi, F., Valeri, M., Donnarumma, D., Maccari, S., Moschioni, M., Taddei, A., et al. (2016). Identification of a monoclonal antibody against pneumococcal pilus 1 ancillary protein impairing bacterial adhesion to human epithelial cells. *Journal of Infectious Diseases* 213(4), 516-522. doi: 10.1093/infdis/jiv461.

Baerga-Ortiz, A., Hughes, C.A., Mandell, J.G., and Komives, E.A. (2002). Epitope mapping of a monoclonal antibody against human thrombin by H/D-exchange mass spectrometry reveals selection of a diverse sequence in a highly conserved protein. *Protein Science* 11(6), 1300-1308. doi: 10.1110/ps.4670102.

Bereszczak, J.Z., Rose, R.J., van Duijn, E., Watts, N.R., Wingfield, P.T., Steven, A.C., et al. (2013). Epitope-distal Effects Accompany the Binding of Two Distinct Antibodies to Hepatitis B Virus Capsids. *Journal of the American Chemical Society* 135(17), 6504-6512. doi: 10.1021/ja402023x.

Bertoldi, I., Faleri, A., Galli, B., Lo Surdo, P., Liguori, A., Norais, N., et al. (2016). Exploiting chimeric human antibodies to characterize a protective epitope of Neisseria adhesin A, one of the Bexsero vaccine components. *FASEB Journal* 30(1), 93-101. doi: 10.1096/fj.15-273813.

Bloem, E., Van Den Biggelaar, M., Wroblewska, A., Voorberg, J., Faber, J.H., Kjalke, M., et al. (2013). Factor VIII C1 domain spikes 2092-2093 and 2158-2159 comprise regions that modulate cofactor function and cellular uptake. *Journal of Biological Chemistry* 288(41), 29670-29679. doi: 10.1074/jbc.M113.473116.

Brier, S., Le Mignon, M., Jain, K., Lebrun, C., Peurois, F., Kellenberger, C., et al. (2018). Characterization of epitope specificities of reference antibodies used for the quantification of the birch pollen allergen Bet v 1. *Allergy: European Journal of Allergy and Clinical Immunology* 73(5), 1032-1040. doi: 10.1111/all.13364.

Brier, S., Rasetti-Escargueil, C., Wijkhuisen, A., Simon, S., Marechal, M., Lemichez, E., et al. (2021). Characterization of a highly neutralizing single monoclonal antibody to botulinum neurotoxin type A. *FASEB Journal* 35(5). doi: 10.1096/fj.202002492R.

Brown, K.A., Lento, C., Rajendran, S., Dowd, J., and Wilson, D.J. (2020). Epitope mapping for a preclinical bevacizumab (Avastin) biosimilar on an extended construct of vascular endothelial growth factor a using millisecond hydrogen–deuterium exchange mass spectrometry. *Biochemistry* 59(30), 2776-2781.

Burke, M.J., Scott, J.N., Minshull, T.C., Gao, Z., Manfield, I., Savic, S., et al. (2022). A bovine antibody possessing an ultralong complementarity-determining region CDRH3 targets a highly conserved epitope in sarbecovirus spike proteins. *Journal of Biological Chemistry* 298(12).

Cariccio, V.L., Domina, M., Benfatto, S., Venza, M., Venza, I., Faleri, A., et al. (2016). Phage display revisited: Epitope mapping of a monoclonal antibody directed against Neisseria meningitidis

adhesin A using the PROFILER technology. *mAbs* 8(4), 741-750. doi: 10.1080/19420862.2016.1158371.

Casina, V.C., Hu, W., Mao, J.H., Lu, R.N., Hanby, H.A., Pickens, B., et al. (2015). High-resolution epitope mapping by HX MS reveals the pathogenic mechanism and a possible therapy for autoimmune TTP syndrome. *Proceedings of the National Academy of Sciences of the United States of America* 112(31), 9620-9625. doi: 10.1073/pnas.1512561112.

Chen, E., Salinas, N.D., Huang, Y., Ntumngia, F., Plasencia, M.D., Gross, M.L., et al. (2016). Broadly neutralizing epitopes in the Plasmodium vivax vaccine candidate Duffy Binding Protein. *Proceedings of the National Academy of Sciences of the United States of America* 113(22), 6277-6282. doi: 10.1073/pnas.1600488113.

Chen, G., Karauzum, H., Long, H., Carranza, D., Holtsberg, F.W., Howell, K.A., et al. (2019). Potent Neutralization of Staphylococcal Enterotoxin B In Vivo by Antibodies that Block Binding to the T-Cell Receptor. *Journal of Molecular Biology* 431(21), 4354-4367. doi: 10.1016/j.jmb.2019.03.017.

Coales, S.J., Tuske, S.J., Tomasso, J.C., and Hamuro, Y. (2009). Epitope mapping by amide hydrogen/deuterium exchange coupled with immobilization of antibody, on-line proteolysis, liquid chromatography and mass spectrometry. *Rapid Communications in Mass Spectrometry* 23(5), 639-647. doi: 10.1002/rcm.3921.

Comamala, G., Krogh, C.C., Nielsen, V.S., Kutter, J.P., Voglmeir, J., and Rand, K.D. (2021). Hydrogen/Deuterium Exchange Mass Spectrometry with Integrated Electrochemical Reduction and Microchip-Enabled Deglycosylation for Epitope Mapping of Heavily Glycosylated and Disulfide-Bonded Proteins. *Analytical Chemistry* 93(49), 16330-16340. doi: 10.1021/acs.analchem.1c01728.

Comamala, G., Wagner, C., de la Torre, P.S., Jakobsen, R.U., Hilger, M., Brouwer, H.J., et al. (2020). Hydrogen/deuterium exchange mass spectrometry with improved electrochemical reduction enables comprehensive epitope mapping of a therapeutic antibody to the cysteine-knot containing vascular endothelial growth factor. *Analytica Chimica Acta* 1115, 41-51. doi: 10.1016/j.aca.2020.04.014.

Deng, B., Zhu, S., Macklin, A.M., Xu, J., Lento, C., Sljoka, A., et al. (2017). Suppressing allostery in epitope mapping experiments using millisecond hydrogen / deuterium exchange mass spectrometry. *mAbs* 9(8), 1327-1336. doi: 10.1080/19420862.2017.1379641.

Domina, M., Cariccio, V.L., Benfatto, S., Venza, M., Venza, I., Donnarumma, D., et al. (2016). Epitope mapping of a monoclonal antibody directed against neisserial heparin binding antigen using next generation sequencing of antigen-specific libraries. *PLoS ONE* 11(8). doi: 10.1371/journal.pone.0160702.

Donnarumma, D., Giusti, F., Ysebaert, C., Hermand, P., Devos, N., Ferlenghi, I., et al. (2022). FHUSPA2/10 is a bactericidal monoclonal antibody targeting multiple repeated sequences of Moraxella catarrhalis UspA2. *Vaccine* 40(45), 6520-6527. doi: 10.1016/j.vaccine.2022.09.048.

Doyle, M.P., Genualdi, J.R., Bailey, A.L., Kose, N., Gainza, C., Rodriguez, J., et al. (2022). Isolation of a Potently Neutralizing and Protective Human Monoclonal Antibody Targeting Yellow Fever Virus. *mBio* 13(3), e00512-00522. doi: doi:10.1128/mbio.00512-22.

Duo, J., Chiriac, C., Huang, R.Y.-C., Mehl, J., Chen, G., Tymiak, A., et al. (2018). Slow off-rate modified aptamer (SOMAmer) as a novel reagent in immunoassay development for accurate soluble glypican-3 quantification in clinical samples. *Analytical chemistry* 90(8), 5162-5170.

Faleri, A., Santini, L., Brier, S., Pansegrau, W., Surdo, P.L., Scarselli, M., et al. (2014). Two cross-reactive monoclonal antibodies recognize overlapping epitopes on Neisseria meningitidis factor H binding protein but have different functional properties. *The FASEB Journal* 28(4), 1644-1653. doi: https://doi.org/10.1096/fj.13-239012.

Fang, M., Wang, Z., Norris, K., James, J.A., Wu, S., and Smith, K. (2022). Hydrogen-Deuterium Exchange Mass Spectrometry Reveals a Novel Binding Region of a Neutralizing Fully Human Monoclonal Antibody to Anthrax Protective Antigen. *Toxins* 14(2). doi: 10.3390/toxins14020092.

Fernandez, E., Kose, N., Edeling, M.A., Adhikari, J., Sapparapu, G., Lazarte, S.M., et al. (2018). Mouse and human monoclonal antibodies protect against infection by multiple genotypes of japanese encephalitis virus. *mBio* 9(1). doi: 10.1128/mBio.00008-18.

Fibriansah, G., Lim, E.X.Y., Marzinek, J.K., Ng, T.S., Tan, J.L., Huber, R.G., et al. (2021). Antibody affinity versus dengue morphology influences neutralization. *PLoS Pathogens* 17(2). doi: 10.1371/JOURNAL.PPAT.1009331.

Geoghegan, J.C., Diedrich, G., Lu, X., Rosenthal, K., Sachsenmeier, K.F., Wu, H., et al. (2016). Inhibition of CD73 AMP hydrolysis by a therapeutic antibody with a dual, non-competitive mechanism of action. *mAbs* 8(3), 454-467. doi: 10.1080/19420862.2016.1143182.

Gerhardt, S., Abbott, W.M., Hargreaves, D., Pauptit, R.A., Davies, R.A., Needham, M.R., et al. (2009). Structure of IL-17A in complex with a potent, fully human neutralizing antibody. *Journal of molecular biology* 394(5), 905-921.

Gramlich, M., Hays, H.C.W., Crichton, S., Kaiser, P.D., Heine, A., Schneiderhan-Marra, N., et al. (2021). Hdx-ms for epitope characterization of a therapeutic antibody candidate on the calcium-binding protein annexin-a1. *Antibodies* 10(1). doi: 10.3390/antib10010011.

Gramlich, M., Maier, S., Kaiser, P.D., Traenkle, B., Wagner, T.R., Voglmeir, J., et al. (2022). A Novel PNGase Rc for Improved Protein N-Deglycosylation in Bioanalytics and Hydrogen-Deuterium Exchange Coupled with Mass Spectrometry Epitope Mapping under Challenging Conditions. *Analytical Chemistry* 94(27), 9863-9871. doi: 10.1021/acs.analchem.2c01748.

Grauslund, L.R., Calvaresi, V., Pansegrau, W., Norais, N., and Rand, K.D. (2021). Epitope and Paratope Mapping by HDX-MS Combined with SPR Elucidates the Difference in Bactericidal Activity of Two Anti-NadA Monoclonal Antibodies. *Journal of the American Society for Mass Spectrometry* 32(7), 1575-1582. doi: 10.1021/jasms.0c00431.

Gribenko, A.V., Parris, K., Mosyak, L., Li, S., Handke, L., Hawkins, J.C., et al. (2016). High Resolution Mapping of Bactericidal Monoclonal Antibody Binding Epitopes on Staphylococcus aureus Antigen MntC. *PLoS Pathogens* 12(9). doi: 10.1371/journal.ppat.1005908.

Guan, X., Noble, K.A., Tao, Y., Roux, K.H., Sathe, S.K., Young, N.L., et al. (2015). Epitope mapping of 7S cashew antigen in complex with antibody by solution-phase H/D exchange monitored by FT-ICR mass spectrometry. *Journal of Mass Spectrometry* 50(6), 812-819. doi: 10.1002/jms.3589.

Hansen, J., Baum, A., Pascal, K.E., Russo, V., Giordano, S., Wloga, E., et al. (2020). Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. *Science* 369(6506), 1010-1014. doi: doi:10.1126/science.abd0827.

Hao, G., Wesolowski, J.S., Jiang, X., Lauder, S., and Sood, V.D. (2015). Epitope characterization of an anti-PD-L1 antibody using orthogonal approaches. *Journal of Molecular Recognition* 28(4), 269-276. doi: 10.1002/jmr.2418.

Haque, H.M.E., Ejemel, M., Vance, D.J., Willsey, G., Rudolph, M.J., Cavacini, L.A., et al. (2022). Human B Cell Epitope Map of the Lyme Disease Vaccine Antigen, OspA. *ACS Infectious Diseases*. doi: 10.1021/acsinfecdis.2c00346.

Haque, H.M.E., Mantis, N.J., and Weis, D.D. (2023). High-Throughput Epitope Mapping by Hydrogen Exchange-Mass Spectrometry. *Journal of the American Society for Mass Spectrometry* 34(1), 123-127. doi: 10.1021/jasms.2c00255.

Huang, R.Y.C., Iacob, R.E., Sankaranarayanan, S., Yang, L., Ahlijanian, M., Tao, L., et al. (2018a). Probing Conformational Dynamics of Tau Protein by Hydrogen/Deuterium Exchange Mass Spectrometry. *Journal of the American Society for Mass Spectrometry* 29(1), 174-182. doi: 10.1007/s13361-017-1815-8.

Huang, R.Y.C., Krystek, S.R., Felix, N., Graziano, R.F., Srinivasan, M., Pashine, A., et al. (2018b). Hydrogen/deuterium exchange mass spectrometry and computational modeling reveal a discontinuous epitope of an antibody/TL1A Interaction. *mAbs* 10(1), 95-103. doi: 10.1080/19420862.2017.1393595.

Huang, R.Y.C., Kuhne, M., Deshpande, S., Rangan, V., Srinivasan, M., Wang, Y., et al. (2020a). Mapping binding epitopes of monoclonal antibodies targeting major histocompatibility complex class I chain-related A (MICA) with hydrogen/deuterium exchange and electron-transfer dissociation mass spectrometry. *Analytical and Bioanalytical Chemistry* 412(7), 1693-1700. doi: 10.1007/s00216-020-02409-x.

Huang, R.Y.C., Wang, F., Wheeler, M., Wang, Y., Langish, R., Chau, B., et al. (2020b). Integrated Approach for Characterizing Bispecific Antibody/Antigens Complexes and Mapping Binding Epitopes with SEC/MALS, Native Mass Spectrometry, and Protein Footprinting. *Analytical Chemistry* 92(15), 10709-10716. doi: 10.1021/acs.analchem.0c01876.

Huang, R.Y.C., Wang, Y., Jhatakia, A.D., Deng, A.X., Bee, C., Deshpande, S., et al. (2021a). Higher-Order Structure Characterization of NKG2A/CD94 Protein Complex and Anti-NKG2A Antibody Binding Epitopes by Mass Spectrometry-Based Protein Footprinting Strategies. *Journal of the American Society for Mass Spectrometry* 32(7), 1567-1574. doi: 10.1021/jasms.0c00399.

Huang, Y., Nguyen, A.W., Hsieh, C.-L., Silva, R., Olaluwoye, O.S., Wilen, R.E., et al. (2021b). Identification of a conserved neutralizing epitope present on spike proteins from all highly pathogenic coronaviruses. *bioRxiv*, 2021.2001.2031.428824. doi: 10.1101/2021.01.31.428824.

Kaever, T., Meng, X., Matho, M.H., Schlossman, A., Li, S., Sela-Culang, I., et al. (2014). Potent neutralization of vaccinia virus by divergent murine antibodies targeting a common site of vulnerability in L1 protein. *Journal of Virology* 88(19), 11339-11355. doi: 10.1128/JVI.01491-14.

Kailasan, S., Kant, R., Noonan-Shueh, M., Kanipakala, T., Liao, G., Shulenin, S., et al. (2022). Antigenic landscapes on Staphylococcus aureus pore-forming toxins reveal insights into specificity and cross-neutralization. *mAbs* 14(1), 2083467. doi: 10.1080/19420862.2022.2083467.

Kim, A.S., Kafai, N.M., Winkler, E.S., Gilliland Jr, T.C., Cottle, E.L., Earnest, J.T., et al. (2021). Pan-protective anti-alphavirus human antibodies target a conserved E1 protein epitope. *Cell* 184(17), 4414-4429. e4419.

Lai, Y.C., Cheng, Y.W., Chao, C.H., Chang, Y.Y., Chen, C.D., Tsai, W.J., et al. (2022). Antigenic Cross-Reactivity Between SARS-CoV-2 S1-RBD and Its Receptor ACE2. *Frontiers in Immunology* 13. doi: 10.3389/fimmu.2022.868724.

Lawson, N.L., Dix, C.I., Scorer, P.W., Stubbs, C.J., Wong, E., Hutchinson, L., et al. (2020). Mapping the binding sites of antibodies utilized in programmed cell death ligand-1 predictive immunohistochemical assays for use with immuno-oncology therapies. *Modern Pathology* 33(4), 518-530. doi: 10.1038/s41379-019-0372-z.

Lennartz, F., Bengtsson, A., Olsen, R.W., Joergensen, L., Brown, A., Remy, L., et al. (2015). Mapping the binding site of a cross-reactive Plasmodium falciparum PfEMP1 monoclonal antibody inhibitory of ICAM-1 binding. *Journal of Immunology* 195(7), 3273-3283. doi: 10.4049/jimmunol.1501404.

Li, J., Wei, H., Krystek, S.R., Bond, D., Brender, T.M., Cohen, D., et al. (2017a). Mapping the Energetic Epitope of an Antibody/Interleukin-23 Interaction with Hydrogen/Deuterium Exchange, Fast Photochemical Oxidation of Proteins Mass Spectrometry, and Alanine Shave Mutagenesis. *Analytical Chemistry* 89(4), 2250-2258. doi: 10.1021/acs.analchem.6b03058.

Li, K.S., Chen, G., Mo, J., Huang, R.Y.C., Deyanova, E.G., Beno, B.R., et al. (2017b). Orthogonal Mass Spectrometry-Based Footprinting for Epitope Mapping and Structural Characterization: The IL-6 Receptor upon Binding of Protein Therapeutics. *Analytical Chemistry* 89(14), 7742-7749. doi: 10.1021/acs.analchem.7b01748.

Lim, X.-X., Chandramohan, A., Lim, X.-Y.E., Crowe Jr, J.E., Lok, S.-M., and Anand, G.S. (2017). Epitope and paratope mapping reveals temperature-dependent alterations in the dengue-antibody interface. *Structure* 25(9), 1391-1402. e1393.

Lu, J., Witcher, D.R., White, M.A., Wang, X., Huang, L., Rathnachalam, R., et al. (2005). IL-1β epitope mapping using site-directed mutagenesis and hydrogen-deuterium exchange mass spectrometry analysis. *Biochemistry* 44(33), 11106-11114. doi: 10.1021/bi0505464.

Lu, Z., Rynkiewicz, M.J., Madico, G., Li, S., Yang, C.Y., Perkins, H.M., et al. (2014). B-cell epitopes in GroEL of Francisella tularensis. *PLoS ONE* 9(6). doi: 10.1371/journal.pone.0099847.

Malito, E., Biancucci, M., Faleri, A., Ferlenghi, I., Scarselli, M., Maruggi, G., et al. (2014). Structure of the meningococcal vaccine antigen nada and epitope mapping of a bactericidal antibody. *Proceedings of the National Academy of Sciences of the United States of America* 111(48), 17128-17133. doi: 10.1073/pnas.1419686111.

Malito, E., Faleri, A., Surdo, P.L., Veggi, D., Maruggi, G., Grassi, E., et al. (2013). Defining a protective epitope on factor H binding protein, a key meningococcal virulence factor and vaccine antigen. *Proceedings of the National Academy of Sciences of the United States of America* 110(9), 3304-3309. doi: 10.1073/pnas.1222845110.

Nambiar, J., Clarke, A.W., Shim, D., Mabon, D., Tian, C., Windloch, K., et al. (2015). Potent neutralizing anti-CD1d antibody reduces lung cytokine release in primate asthma model. *mAbs* 7(3), 638-650. doi: 10.1080/19420862.2015.1016693.

Obungu, V.H., Gelfanova, V., Rathnachalam, R., Bailey, A., Sloan-Lancaster, J., and Huang, L. (2009). Determination of the mechanism of action of anti-FasL antibody by epitope mapping and homology modeling. *Biochemistry* 48(30), 7251-7260. doi: 10.1021/bi900296g.

Orengo, J.M., Radin, A.R., Kamat, V., Badithe, A., Ben, L.H., Bennett, B.L., et al. (2018). Treating cat allergy with monoclonal IgG antibodies that bind allergen and prevent IgE engagement. *Nature Communications* 9(1), 1421. doi: 10.1038/s41467-018-03636-8.

Pandit, D., Tuske, S.J., Coales, S.J., Yen E, S., Liu, A., Lee, J.E., et al. (2012). Mapping of discontinuous conformational epitopes by amide hydrogen/deuterium exchange mass spectrometry and computational docking. *Journal of Molecular Recognition* 25(3), 114-124. doi: 10.1002/jmr.1169.

Prądzińska, M., Behrendt, I., Astorga-Wells, J., Manoilov, A., Zubarev, R.A., Kołodziejczyk, A.S., et al. (2016). Application of amide hydrogen/deuterium exchange mass spectrometry for epitope mapping in human cystatin C. *Amino Acids* 48(12), 2809-2820. doi: 10.1007/s00726-016-2316-y.

Puchades, C., Kűkrer, B., Diefenbach, O., Sneekes-Vriese, E., Juraszek, J., Koudstaal, W., et al. (2019). Epitope mapping of diverse influenza Hemagglutinin drug candidates using HDX-MS. *Scientific Reports* 9(1). doi: 10.1038/s41598-019-41179-0.

Sarkar, K., Joedicke, L., Westwood, M., Burnley, R., Wright, M., McMillan, D., et al. (2019). Modulation of PTH1R signaling by an ECD binding antibody results in inhibition of β-arrestin 2 coupling. *Scientific Reports* 9(1). doi: 10.1038/s41598-019-51016-z.

Sayago, C., Gonzalez Valcarcel, I.C., Qian, Y., Lee, J., Alsina-Fernandez, J., Fite, N.C., et al. (2018). Deciphering binding interactions of IL-23R with HDX-MS: Mapping protein and macrocyclic dodecapeptide ligands. *ACS medicinal chemistry letters* 9(9), 912-916.

Sevy, A.M., Healey, J.F., Deng, W., Spiegel, P.C., Meeks, S.L., and Li, R. (2013). Epitope mapping of inhibitory antibodies targeting the C2 domain of coagulation factor VIII by hydrogen-deuterium exchange mass spectrometry. *Journal of Thrombosis and Haemostasis* 11(12), 2128-2136. doi: 10.1111/jth.12433.

Sheff, J., Kelly, J., Foss, M., Brunette, E., Kemmerich, K., van Faassen, H., et al. (2022). Epitope mapping of a blood-brain barrier crossing antibody targeting the cysteine-rich region of IGF1R using hydrogen-exchange mass spectrometry enabled by electrochemical reduction. *The Journal of Biochemistry*. doi: 10.1093/jb/mvac088.

Sheff, J., Wang, P., Xu, P., Arbour, M., Masson, L., van Faassen, H., et al. (2021a). Defining the epitope of a blood–brain barrier crossing single domain antibody specific for the type 1 insulin-like growth factor receptor. *Scientific Reports* 11(1). doi: 10.1038/s41598-021-83198-w.

Sheff, J.G., Kelly, J.F., Robotham, A., Sulea, T., Malenfant, F., L'Abbé, D., et al. (2021b). Hydrogen-deuterium exchange mass spectrometry reveals three unique binding responses of mAbs directed to the catalytic domain of hCAIX. *mAbs* 13(1). doi: 10.1080/19420862.2021.1997072.

Simmons, Z.R., Sharma, S., Wayne, J., Li, S., Vander Kooi, C.W., and Gentry, M.S. (2021). Generation and characterization of a laforin nanobody inhibitor. *Clinical Biochemistry* 93, 80-89. doi: 10.1016/j.clinbiochem.2021.03.017.

Song, D., Sun, H., Ma, L., Liu, J., Gao, Y., Zhang, Q., et al. (2023). In-Vitro Diagnostic Reagent Evaluation of Commercially Available Cardiac Troponin I Assay Kits Using H/D Exchange Mass Spectrometry for Antibody-Epitope Mapping. *Analytical Chemistry* 95(4), 2278-2284. doi: 10.1021/acs.analchem.2c03946.

Ständer, S., R. Grauslund, L., Scarselli, M., Norais, N., and Rand, K. (2021). Epitope Mapping of Polyclonal Antibodies by Hydrogen-Deuterium Exchange Mass Spectrometry (HDX-MS). *Analytical Chemistry* 93(34), 11669-11678. doi: 10.1021/acs.analchem.1c00696.

Sun, H., Liu, J., Xiao, P., Zhou, Y., Li, H., Shen, M., et al. (2022). Epitope mapping of antibodies in C-reactive protein assay kits by hydrogen-deuterium exchange mass spectrometry explains differential results across kits. *Analytical and Bioanalytical Chemistry* 414(13), 3875-3884. doi: 10.1007/s00216-022-04029-z.

Terral, G., Champion, T., Debaene, F., Colas, O., Bourguet, M., Wagner-Rousset, E., et al. (2017). Epitope characterization of anti-JAM-A antibodies using orthogonal mass spectrometry and surface plasmon resonance approaches. *mAbs* 9(8), 1317-1326. doi: 10.1080/19420862.2017.1380762.

Toth IV, R.T., Angalakurthi, S.K., Van Slyke, G., Vance, D.J., Hickey, J.M., Joshi, S.B., et al. (2017). High-definition mapping of four spatially distinct neutralizing epitope clusters on RiVax, a candidate ricin toxin subunit vaccine. *Clinical and Vaccine Immunology* 24(12), e00237-00217.

VanBlargan, L.A., Errico, J.M., Kafai, N.M., Burgomaster, K.E., Jethva, P.N., Broeckel, R.M., et al. (2021). Broadly neutralizing monoclonal antibodies protect against multiple tick-borne flaviviruses. *Journal of Experimental Medicine* 218(5). doi: 10.1084/jem.20210174.

Vance, D.J., Tremblay, J.M., Rong, Y., Angalakurthi, S.K., Volkin, D.B., Middaugh, C.R., et al. (2017). High-resolution epitope positioning of a large collection of neutralizing and nonneutralizing single-domain antibodies on the enzymatic and binding subunits of ricin toxin. *Clinical and Vaccine Immunology* 24(12), e00236-00217.

Willison, L.N., Zhang, Q., Su, M., Teuber, S.S., Sathe, S.K., and Roux, K.H. (2013). Conformational epitope mapping of Pru du 6, a major allergen from almond nut. *Molecular Immunology* 55(3-4), 253-263. doi: 10.1016/j.molimm.2013.02.004.

Yamada, N., Suzuki, E.-i., and Hirayama, K. (2002). Identification of the interface of a large protein–protein complex using H/D exchange and Fourier transform ion cyclotron resonance mass spectrometry. *Rapid Communications in Mass Spectrometry* 16(4), 293-299. doi: https://doi.org/10.1002/rcm.579.

Yang, D., Frego, L., Lasaro, M., Truncali, K., Kroe-Barrett, R., and Singh, S. (2016). Efficient qualitative and quantitative determination of antigen-induced immune responses. *Journal of Biological Chemistry* 291(31), 16361-16374. doi: 10.1074/jbc.M116.736660.

Zhang, M.M., Huang, R.Y.C., Beno, B.R., Deyanova, E.G., Li, J., Chen, G., et al. (2020). Epitope and Paratope Mapping of PD-1/Nivolumab by Mass Spectrometry-Based Hydrogen-Deuterium Exchange, Cross-linking, and Molecular Docking. *Analytical Chemistry* 92(13), 9086-9094. doi: 10.1021/acs.analchem.0c01291.

Zhang, Q., Noble, K.A., Mao, Y., Young, N.L., Sathe, S.K., Roux, K.H., et al. (2013). Rapid screening for potential epitopes reactive with a polycolonal antibody by solution-phase H/D exchange monitored by FT-ICR mass spectrometry. *Journal of the American Society for Mass Spectrometry* 24(7), 1016-1025. doi: 10.1007/s13361-013-0644-7.

Zhang, Q., Willison, L.N., Tripathi, P., Sathe, S.K., Roux, K.H., Emmett, M.R., et al. (2011). Epitope mapping of a 95 kDa antigen in complex with antibody by solution-phase amide backbone hydrogen/deuterium exchange monitored by Fourier transform ion cyclotron resonance mass spectrometry. *Analytical Chemistry* 83(18), 7129-7136. doi: 10.1021/ac201501z.

Zhang, Q., Yang, J., Bautista, J., Badithe, A., Olson, W., and Liu, Y. (2018). Epitope Mapping by HDX-MS Elucidates the Surface Coverage of Antigens Associated with High Blocking Efficiency of Antibodies to Birch Pollen Allergen. *Analytical Chemistry* 90(19), 11315-11323. doi: 10.1021/acs.analchem.8b01864.

Zhu, S., Liuni, P., Chen, T., Houy, C., Wilson, D.J., and James, D.A. (2022). Epitope screening using Hydrogen/Deuterium Exchange Mass Spectrometry (HDX-MS): An accelerated workflow for evaluation of lead monoclonal antibodies. *Biotechnology Journal* 17(2), 2100358. doi: https://doi.org/10.1002/biot.202100358.

Zhu, S., Liuni, P., Ettorre, L., Chen, T., Szeto, J., Carpick, B., et al. (2019). Hydrogen-Deuterium Exchange Epitope Mapping Reveals Distinct Neutralizing Mechanisms for Two Monoclonal Antibodies against Diphtheria Toxin. *Biochemistry* 58(6), 646-656. doi: 10.1021/acs.biochem.8b01123.