Supporting information

Hydrogen/deuterium exchange memory NMR reveals structural epitopes involved in IgE cross-reactivity of allergenic lipid transfer proteins

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Running title: Epitope mapping using H/D exchange memory NMR

List of materials included

Supplementary Methods

Production of recombinant allergens

Recombinant Art v 3.0201 (for simplicity termed Art v 3) was produced as described previously (25). For isotope labeled Art v 3 (^{15}N Art v 3, ^{13}C^{15}N Art v 3), bacteria were grown in M9 minimal medium containing either ^{15}N-labeled NH_4Cl (99% ^{15}N, Cambridge Isotope Laboratories) or ^{15}N-labeled NH_4Cl together with ^{13}C-labeled glucose (99% ^{13}C, Cambridge Isotope Laboratories), containing ^{15}N as well as ^{13}C as the sole nitrogen and carbon source, respectively. After induction with 0.4 mM isopropyl-b-D-thiogalactopyaranosid at OD_{600}= 0.6, the culture was incubated over night at 20°C. Cells were harvested by centrifugation and the protein was purified as previously described (25). The purity was monitored by denaturing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by Coomassie staining. Another isoform, i. e. Art v 3.0301 as well as Api g 2 (isoform Api g 2.0101) from celery stalks, Cor a 8 (isoform Cor a 8.0101) from hazelnut, Pru p 3 (isoform Pru p 3.0102) from peach were obtained as previously published (33,55,56,59,60). Recombinant Fra a 3 (isoform Fra a 3.0201) from strawberry, Mal d 3 (isoform Mal d 3.0101) from apple and Amb a 6 (isoform Amb a 6.0101) from ragweed pollen were expressed in *E. coli* and purified using affinity chromatography according to published data (57,58). The herein used allergen nomenclature is according to the WHO/IUIS Allergen Nomenclature Sub-Committee (http://www.allergen.org).

Art v 3 epitope variants were obtained by applying the stability predictor MAESTRO (61) on the crystal structure of Art v 3 (PDB 6FRR). The design goal for the Art v 3 variants was to change surface properties in the epitope regions while maintaining the stability of the protein. Therefore, we defined positions to be mutated based on the results of the NMR and ELISA experiments. The residues subjected to mutations in MAESTRO were V24, K33, G34, N36, N77 for mAb I, P43, R45, Q46, N89, K90, K92 for mAb II, and K33, K73, S72, V76, N77 for mAbIII. An additional mAbIII variant omitting K33 and N77 was investigated avoiding an overlap between epitope mAbI and mAbIII. As the monomeric structure of Art v 3 shows two distinct conformational states in the crystal structure (PDB 6FRR), chain A and B were used in the *in silico* experiment, which resulted in two lists of possible mutations for each epitope. The two lists were sorted by decreasing predicted stability, and only solutions that appeared for both conformations were subsequently considered. For further selection, we preferred solutions with a lower change in the net charge and solutions with larger changes in the size and polarity of the mutated side chains. We further inspected the remaining solutions for possible disruption of H-bonds or other interactions that we expect to be important for the fold. This finally led to four Art v 3 epitope variants, i.e. V1 (K33A, G34Q, N36L, N77R for mAb I), V2 (P43R, R45L, K90Q, K92V for mAb II), V3A (K33L, K73Q, V76L, N77K for mAbIII), and V3B (S72Q, K73Q, V76T for the reduced mAbIII- epitope). Corresponding gene constructs were obtained for recombinant protein expression in pHis Parallel2 (ATG Biosynthetics, Merzhausen, Germany). Recombinant expression and purification of epitope variants V1, V2, V3A, and V3B was conducted following established protocols for the production of non-tagged recombinant Art v 3 (25).

Mass spectrometry

For measurement of the intact protein mass Art v 3, ^{15}N labeled Art v 3 samples and variants V1, V2, V3A, V3B were diluted in 50% ACN + 0.10% FA to a concentration of 0.1 mg·mL^{-1}. For analysis of Art v 3 and ^{13}C^{15}N labeled Art v 3, samples were diluted in ddH_2O + 0.10% FA to a concentration of 0.1 mg·mL^{-1}. Chromatographic separation of control and ^{13}C^{15}N labeled Art v 3 samples was carried out on a Dionex™ UltiMate™ 3000 Rapid Separation system from Thermo Fisher Scientific (Germering, Germany) at a flow rate of 200 μL min^{-1} employing an XBridge

BEH C4 column (150 × 2.1 mm i.d., d_p 3.5 µm, 300 Å, Waters, Milford, MA, USA), operated at a temperature of 50°C. Mobile phase A was composed of ddH_2O + 0.10% FA, mobile phase B of ACN + 0.10% FA. The protein was separated employing a linear gradient of 5.0−70.0% B in 15 min. UV-detection was carried out at 214 nm.

Mass spectrometry of Art v 3 and ^{15}N labeled Art v 3 was conducted on a benchtop quadrupole-Orbitrap instrument (Q Exactive™ Plus) equipped with a Nanospray Flex™ ion source, both from Thermo Fisher Scientific (Bremen, Germany). Samples were directly infused using coated borosilicate emitters for static nanospray (ES387, Thermo Fisher Scientific™, San Jose, CA, USA) at a spray voltage of 1.4 kV. Measurements were performed in full scan mode in a range of *m/z* 500 – 2,000 at a resolution of 140,000 at *m/*z 200 and rolling averaging for five minutes. The instrument settings were as follows: capillary temperature of 320 °C, in-source CID of 50.0 eV, S-lens RF level of 60.0, AGC target of 1e6 and a maximum injection time of 50 ms.

The spectra of Art v 3, $^{13}C^{15}N$ labeled Art v 3 samples and variants V1, V2, V3A, V3B were acquired on a benchtop quadrupole-Orbitrap instrument (Q Exactive™) equipped with an Ion Max™ source with a heated electrospray ionization (HESI) probe, both from Thermo Fisher Scientific (Bremen, Germany). Measurements were conducted in full scan mode in a range of *m/z* 500 – 2,500 at a resolution of 140,000 at *m/*z 200. The instrument settings were as follows: spray voltage of 3.6 kV, capillary temperature of 350°C, S-lens RF level of 70.0, AGC target of 1e6 and a maximum injection time of 150 ms. Data acquisition and evaluation were conducted using Xcalibur 2.0 (Thermo Fisher Scientific, Sunnyvale, CA, USA). Theoretical masses were determined by GPMAW (Version 9.51) (63). For heavy isotope labeled proteins, the extent of labeling was calculated based on the shift of the isotope distribution of the most intense charge state (M+8H)8+ of non-labeled and ^{15}N labeled Art v 3 or $^{13}C^{15}N$ labeled Art v 3, respectively.

Production of monoclonal anti Art v 3 antibodies

For immunization, 6-8 week old female BALB/c mice (Charles River Laboratories, Wilmington, MA, USA) were immunized subcutaneously with 10 µg recombinant Art v 3.0201 adsorbed to Alu-gel S (Serva, Heidelberg, Germany) in sterile PBS on day 1, 3, 7, and 14. Art v 3-specific humoral immune responses were monitored by ELISA. After two final subcutaneous boosts on day 28 and 42 B-cells from the spleen were collected and used for hybridoma production. Hybridoma fusion was performed using the BALB/c myeloma cell line P3X63Ag8.653 (ATCC, LGC Promochem, Wesel, Germany) according to previously described protocols (62). The secreted antibodies in the supernatant were tested in ELISA for IgG (IgG1, IgG2a, IgG2b and IgG3) and IgE specificity. Animal experiments were conducted in accordance with EU guidelines 86/609/EWG and national legal regulations (TVG 2012) and all efforts were made to minimize or avoid suffering. Experiments were approved by the Austrian Ministry of Science, permission No. GZ 66.012/0047-II/3b/2017. For terminal analysis, mice were euthanized by cervical dislocation.

Purification of monoclonal anti Art v 3 antibodies

B-cell hybridoma cells of the respective monoclonal antibodies were kept 14 days in culture and every second day fresh medium (Opti-MEM™ supplemented with GlutaMAX, 5% FCS, 1x Pen-Strep and 30 units/ml IL-6) was added until each of them reached a final volume of 500 ml. Cells were separated from the antibody containing supernatant by filtration using a paper filter and a 0.45 µM syringe filter. The pH of the supernatant was adjusted with NaOH to pH 7.0. The antibodies were purified by affinity chromatography using a 1 ml Protein G HiTrap

column (GE Healthcare, Chalfont St. Giles, UK). After washing with 20 mM NaP pH 7.0, 0.5 mL fractions were collected into reaction tubes filled with 100 µl 1 M Tris HCl pH 9.0 buffer during a 20 ml linear gradient elution to 0.1 M glycine-HCl pH 2.7. Fractions containing the purified antibodies were pooled, analyzed by gel electrophoresis and stored at 4°C until further use.

MAb ELISA detection of native, reduced/alkylated and Art v 3 variants

Art v 3 was reduced and alkylated by iodoacetamide according to the manufacturer's instructions using the reducing and blocking agents of the ProteoExtract Trypsin Digestion kit (Calbiochem, Gibbstown, NJ, USA). Native, or reduced/alkylated proteins (4 µg/ml) (3 times technical replicates) were coated overnight at 4°C to the wells of Nunc-Immuno™ MaxiSorp ELISA plates (Nalge Nunc, Rochester, NY, USA). As a control, a buffer sample was treated with the DTT and iodoacetamide-containing buffers from the kit and coated simultaneously to the wells. Plates were washed with 137 mM NaCl, 2.7 mM KCl, 10 mM Tris, pH 7.5, 0.05% (v/v) Tween 20 (TBST) and blocked with TBST, 1% (w/v) BSA for several hours at room temperature. After the blocking steps, plates were washed five times with TBST and incubated with Art v 3-specific monoclonal antibodies overnight at 4°C. After washing with TBST, plates were incubated with 1:5000 diluted alkaline phosphatase-labeled rabbit anti-mouse IgG/IgM antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) for 1h at 37°C and 4°C, respectively. After four TBST washes, plates were developed with 10 mM 4-Nitrophenyl phosphate (Sigma-Aldrich, St. Loius, MO) in alkaline phosphatase detection buffer (1 M diethanolamin, 1 mM $MgCl*6H_2O$, pH 9.8). Triplicate measurements were performed at 405/492 nm using a microplate reader (SLT Spectra 1.1, TECAN, Grödig, Austria). Comparison of mAb reactivity with Art v 3 and variants (V1, V2, V3A, and V3B) was performed following the same protocol, ELISA measurements were performed as 4-times technical replicates.

Isothermal titration calorimetry (ITC)

ITC was performed on a VP-ITC microcalorimeter (MicroCal). Prior to the experiment, the buffer of both binding partners were extensively exchanged to 20 mM KH_2PO_4/ K_2HPO_4 buffer (pH 6.0)/10 mM NaCl using ultrafiltration devices (Amicon, 3.5 kDa cutoff). The sample cell was loaded with a solution of the mAb at a concentration of 7 to 10 µM, and the mircosyringe was loaded with a solution of Art v 3 at a concentration of either 0.38 mM (mAb I and mAb II) or 0.45 mM (mAb III). The titration was conducted at 25 °C using one initial injection of 2 µL with a duration of 4 s and 200 s spacing, followed by 35 identical injections of 3 µL with a duration of 6 s and 300 s spacing between injections. Slightly different parameters were used for the titration with mAb II in which one initial injection of 1 µL with a duration of 2 s and 200 s spacing, was followed by approx. 50 identical injections of 2 µL with a duration of 4 s and 300 s spacing between injections. Raw data were integrated, corrected for nonspecific heats, normalized to the molar concentration, and analyzed using the MicroCal ITC module of Origin 7.0 (OriginLab), applying a 1:2 binding model.

Surface acoustic wave (SAW) measurements

The sam®5BLUE biosensor instrument (NanoTemper Technologies, Munich, Germany) was used to determine the binding affinities of the different monoclonal antibodies to Art v 3. Art v 3 was coupled to the surface of a SAW CM-Dextran 3D sensor chip (NanoTemper Technologies). The surface of the chip was activated with a freshly prepared mixture of 100 mM N-hydroxysuccinimide (NHS) and 400 mM 1-(3-dimethylaminopropyl)-3-

ethylcarbodiimide hydrochloride (EDC). Art v 3 was diluted in 1x PBS to a concentration of 500 nM. Subsequently, 250 µl of the Art v 3 solution was injected to the chip. Residual activated groups on the surface of the chip were blocked by injecting 125 µl of 1 M ethanolamine pH 8.5. For the calculation of the affinity constant $K_{D,}$ increasing concentrations (0 nM – 300 nM) of the three different monoclonal antibodies in 20 mM imidazole pH 7.4, 100 mM NaCl, 2 % (w/v) BSA were injected. The coated chip was equilibrated with the identical buffer and affinity measurements were performed at 22°C and a flow rate of 40 µl/min. The different mAb solutions were injected for 4 min followed by a 4 min wait. Between each injection, residual mAbs were removed with regeneration buffer (40 mM citric acid, 40 mM NaCl pH 2.9). SAW phase changes were recorded and Trace Drawer 1.7 software (Ridgeview Instruments, Uppsala, Sweden) was used to calculate the affinities. K_D values obtained from 12 channels were determined from the evaluation of the kinetics using the provided uncoupled 1:1 binding model and standard deviations were calculated. Estimates of k_{off} values were extracted by fitting the decay by the function D Phase = A exp ($-k_{off}$ t) using OriginPro 2019 (OriginLab). The average and standard deviation of all usable decay curves for a particular mAb from different channels were determined.

Sera from mugwort pollen allergic patients

Twenty-one patients exhibiting clinical symptoms to mugwort pollen with positive *in vitro* reactivity to Art v 3 were included in this study (for details see Table S1). Diagnostic and clinical features have been recorded for each patient in an electronic allergy record (InterAll, Allergy Data Laboratories s.c., Latina, Italy). The study was approved by the Institutional Review Board (n. 106-CE-2005), and signed informed consents were obtained from all patients.

Patient ELISA with Art v 3, homologous LTPs and epitope variants

The wells of MaxiSorp ELISA plates (Nalge Nunc, Rochester, NY, USA) were coated overnight with 200 ng of Art v 3 of purified allergens (Art v 3.0201, Art v 3.0301, Amb a 6, Api g 2, Cor a 8, Fra a 3, Mal d 3 and Pru p 3). After washing and blocking steps, patients' sera were diluted 1:4 in TBST, 0.5% (w/v) BSA, and incubated overnight at 4°C. After four washing steps, bound IgE was detected with 1:2000 diluted alkaline phosphatase-conjugated monoclonal anti-human IgE (BD Biosciences, Franklin Lakes, NJ). 4-Nitrophenyl phosphate (Sigma-Aldrich, St. Loius, MO) was used as substrate and measurements were performed at OD 405/492. The measured OD values are depicted in Table S1. Patients' IgE reactivity to Art v 3 and epitope variants (V1, V2, V3A, and V3B) were conducted following the same protocol.

Inhibition ELISA

The wells of MaxiSorp ELISA plates (Nalge Nunc) were coated overnight with 200 ng of rArt v 3. After washing and blocking steps, the coated plates were pre-incubated with 2.5 µg/well Art v 3-specific monoclonal antibodies or with 2.5 µg/well of an unrelated monoclonal antibody (anti-Amb a 1 – the allergenic pectate lyase of ragweed pollen) as negative control overnight at 4°C. Patients' sera were diluted 1:2 or 1:4 in TBST, 0.5% (w/v) BSA, and incubated overnight at 4°C. IgE was detected with 1:2000 diluted alkaline phosphatase-conjugated monoclonal anti-human IgE (BD Biosciences, Franklin Lakes, NJ). 4-Nitrophenyl phosphate (Sigma-Aldrich, St. Loius, MO) was used as substrate and measurements were performed at OD 405/492. The remaining IgE reactivity of human sera was compared to buffer-treated samples and results are presented as the mean percentage of total inhibition. For the

calculation of the total inhibition different time points (measurements after 10, 30, 60 or 120 min) were used depending on the OD values of the individual sera.

NMR spectroscopy

All spectra were recorded on a 600 MHz Bruker Avance III HD spectrometer equipped with a QXI quadruple-resonance probe ($^{1}H/^{13}C/^{15}N/^{13}C$) at 278 K unless stated otherwise. Sample volumes of 500 µl in standard 5 mm NMR tubes were used. Standard 2D [^{1}H, ^{15}N]-HSQC, 3D HNCA, 3D CBCA(CO)NH, 3D HNCACB, 3D HNCO, 3D HN(CA)CO and 3D ^{15}N-edited NOESY spectra were recorded. All spectra were processed with Topspin 3.2 (Bruker Biospin), referenced to 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS) and further analyzed in Sparky (T. D. Goddard and D. G. Kneller, SPARKY 3, University of California, San Francisco, USA). Chemical shift assignments were deposited in the BioMagRes databank under the accession number 28092.

Epitope mapping by H/D exchange memory (HDXMEM) detected by NMR spectroscopy

The sample of 500 µL of an Art v 3 solution at a concentration of 160 µM (without mAb) was lyophilized. Similar samples additionally containing 0.25 equivalents of mAb were prepared by mixing 500 µL of 160 µM Art v 3 in 20 mM KH_2PO_4/ K_2HPO_4 buffer (pH 6.0)/10 mM NaCl with 0.25 equivalents of mAb in identical buffer at a concentration of 37 µM. For the interaction studies with NMR spectroscopy the buffer of each mAb was exchanged with an ultrafiltration device (Amicon, 100 kDa cutoff). Complex samples were concentrated using an ultracentrifugation device (Amicon, 100 kDa cut-off) to a concentration of approximately 150 µM Art v 3 and 75 µM mAb, flash frozen in liquid nitrogen and afterwards lyophilized. The Art v 3 signals in a ^{15}N-HSQC spectrum measured in the presence of mAb showed a drop in intensities to approximately 50%. H/D exchange was monitored by dissolving lyophilized samples in D_2O and measuring ^{1}H-^{15}N HSQC spectra at certain time points. For the measurement of fast exchanging protons we measured at 278 K to slow down the H/D exchange. Very slowly exchanging protons were measured at 298 K. We analyzed individual exchange rates of all observed signals as found in the Supplementary Figures S6-S14. After dissolving the lyophilized samples in D_2O the first ^{15}N-^{1}H HSQC spectra were measured with 8 scans allowing sampling spectra every 21 min in order to detect fast exchanging protons. After the first 4-6 spectra (approximately 2 hours) the number of scans was increased to 16 and later to 32 and 64 to get a better signal-to-noise ratio for slow exchanging amides. All spectra were processed with identical nc_proc. For plotting the intensities versus time, the intensities of spectra with double the scans were divided by a factor of 2, spectra with four times the scans were divided by 4 and so on to have comparable scales. Accessible surface areas for each residue of both chains of the crystal structure (PDB 6FRR) were calculated using VADAR (64). Residues with a fractional accessible surface area >0.1 were considered accessible.

Titer determination of mAbs by direct ELISA

For determination of monoclonal antibody titers, 200 ng purified Art v 3 or homologous proteins (Art v 3.0301, Amb a 6, Api g 2, Cor a 8, Fra a 3, Mal d 3 and Pru p 3) were coated overnight to the wells of Maxisorp ELISA plates (Nalge Nunc). After washing and blocking steps (further details see ELISA above), the proteins were incubated overnight with titrated monoclonal antibodies in concentrations ranging from 8 ng/well to 2.56 pg/well. Bound antibodies were detected using horseradish peroxidase labeled goat anti-mouse IgG (Biorad) diluted 1:1000. As substrate 4 mM 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) and 0.12% (v/v) hydrogen peroxide in

0.1 M citrate buffer pH 4.5 was used. For the titer calculation, the LOD (Limit of Detection) was used as cutoff value, which is defined as mean of the background plus three times the standard deviation of the background.

Statistical analyses

Statistical analyses were performed using GraphPad Prism 8.0.2. Comparison of mAb binding to native and reduced/alkylated Art v 3 was conducted by unpaired t-tests. Inhibition ELISA results were analyzed by the Friedman's test, using the Dunn's multiple comparison test. Comparison of mAb reactivity to Art v 3 and four epitope variants was performed by the Kruskal-Wallis test, multiple comparison to the reference Art v 3 using Dunn's correction. The IgE ELISA testing Art v 3 and variants was analyzed by the Friedman's test followed by Dunn's correction for multiple comparisons. Results were considered statistically significant for $p \leq 0.05$.

Supplementary table

Table S1. Patients' table and IgE reactivity to LTPs

Patient ID	Sex	Age	Symptoms on Mugwort Exposure	Art v 3	ELISA OD $_{405/492}$							
				ISAC kU/l	Art v 3.0201	Art v 3.0301	Cor a 8	Fra a 3	Mal d 3	Pru p 3	Api g 2	Amb a 6
P1	F	44	RCA	2.33	1.52	1.82	0.03	0.19	0.03	0.00	0.43	0.00
P2	M	34	R	20.17	3.83	3.75	0.84	1.10	0.86	0.82	0.05	0.02
P3	M	34	R	52.16	3.83	3.78	1.01	1.63	0.35	0.55	0.47	0.01
P4	F	54	R	1.15	0.15	0.20	0.44	0.70	0.55	0.90	0.25	0.00
P5	M	31	RC	3.25	2.11	1.21	1.60	3.68	3.93	3.75	2.77	0.30
P6	M	24	R	3.23	0.57	0.96	1.69	3.04	2.75	3.27	1.94	0.11
P7	F	38	RC	0.80	0.28	0.10	2.57	3.03	1.55	2.46	0.66	0.11
P8	F	36	RC	0.00	0.43	0.34	0.12	0.72	0.48	0.49	0.29	0.08
P9	M	15	R	5.17	1.10	0.48	1.86	3.63	3.60	3.69	0.12	0.00
P10	F	33	RC	5.60	3.73	3.67	3.43	3.67	3.89	3.73	3.72	1.02
P11	M	19	R	6.05	1.85	0.29	3.41	3.31	2.42	1.15	3.01	0.06
P12	F	18	R	5.27	3.73	3.25	3.81	3.66	3.89	3.72	3.78	0.00
P13	F	30	RC	6.21	1.59	0.07	2.94	3.39	1.29	1.86	1.59	0.00
P14	M	14	R	9.23	3.53	1.84	3.81	3.66	3.88	3.75	2.43	0.02
P15	M	26	R	7.62	3.80	3.72	3.20	3.65	3.84	3.68	3.76	0.24
P16	M	27	R	6.23	3.77	3.13	3.20	3.69	3.83	3.71	3.48	0.06
P17	F	28	RCA	23.50	3.18	2.91	0.15	0.63	2.63	0.27	0.09	0.00
P18	M	24	R	15.93	3.67	3.19	3.55	3.65	3.78	3.66	2.96	0.33
P19	F	48	R	6.31	3.50	2.21	2.72	3.67	3.79	3.64	3.78	1.00
P20	F	44	RC	14.58	2.44	1.76	2.16	3.10	2.60	3.24	2.39	0.03
P21	F	23	R	18.30	2.09	2.00	0.35	1.17	0.69	0.48	0.39	0.20

R, rhinitis; C, conjunctivitis; A, asthma
Intensity of IgE reactivity is indicated from low (light green) to high (dark green) reactivity.

Supplementary figures

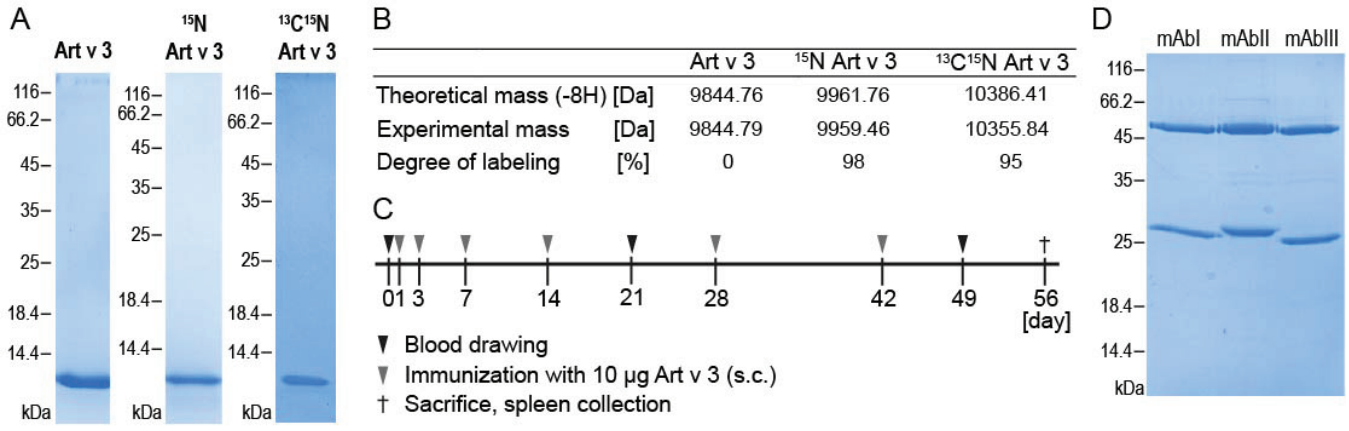
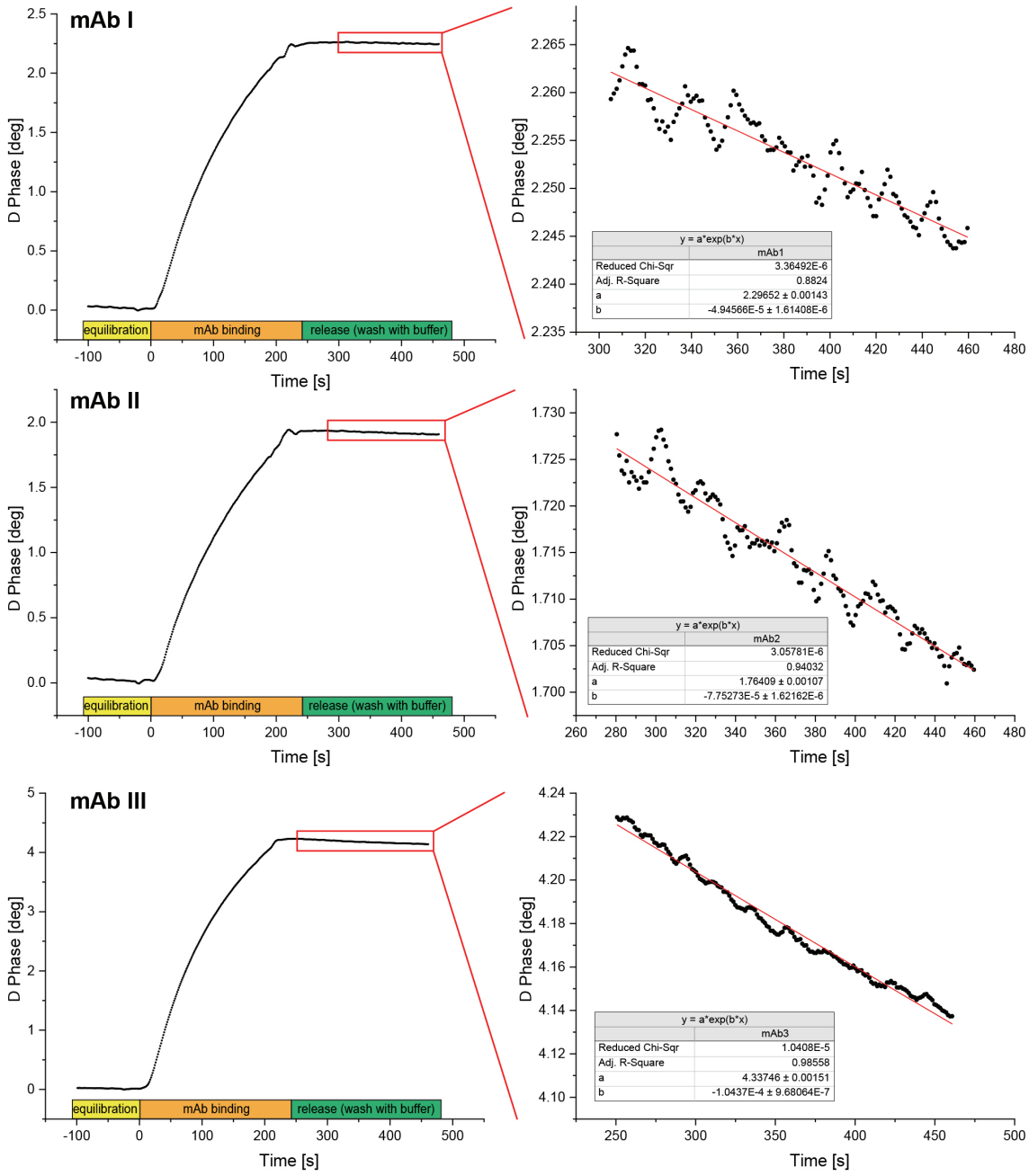


Figure S1. Characterization of recombinant Art v 3 and production of monoclonal antibodies. **A.** Purified recombinant non-labeled and isotope labeled Art v 3 monitored by denaturing gel electrophoresis. **B.** Intact mass spectrometry analyses of proteins. **C.** Scheme of murine immunization protocol for antibody production. **D.** Denaturing gel electrophoresis of purified Art v 3-specific monoclonal antibodies mAb I, mAb II and mAb III.

A



B

	Interpretable measurements	k_{off} [s^{-1}]	t_{off} [h]	$t_{1/2}$ [h]
mAb I	12	$(3.4 \pm 1.2)\cdot10^{-5}$	9.2 ± 4.0	6.4 ± 2.8
mAb II	9	$(7.8 \pm 2.1)\cdot10^{-5}$	3.8 ± 1.1	2.6 ± 0.7
mAb III	5	$(1.1 \pm 0.5)\cdot10^{-4}$	2.9 ± 1.1	2.0 ± 0.7

Figure S2. Extraction of k_{off} from the surface accoustic wave (SAW) data. **A.** Examples of data traces are shown on the left for all three mAbs and a zoom on the decay is shown on the right including the fit and fitting parameters. **B.** Extracted dissociation rate (k_{off}), dissociation time (t_{off}), and half-life ($t_{1/2}$) of all interpretable SAW measurements.

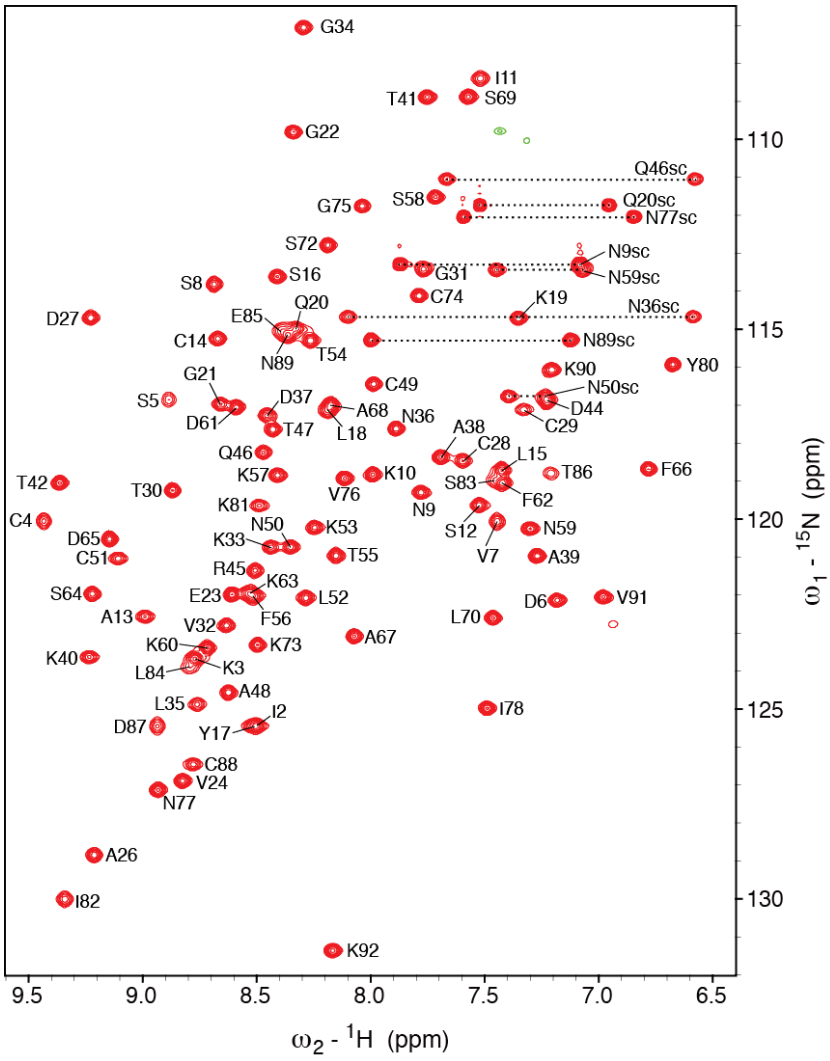


Figure S3. ^{15}N HSQC spectrum of Art v 3 with sequential assignment at a concentration of 1 mM measured at 278 K. Assignments of NH_2 groups of Asn and Gln are connected by dotted lines and indicated by "sc" (side chain).

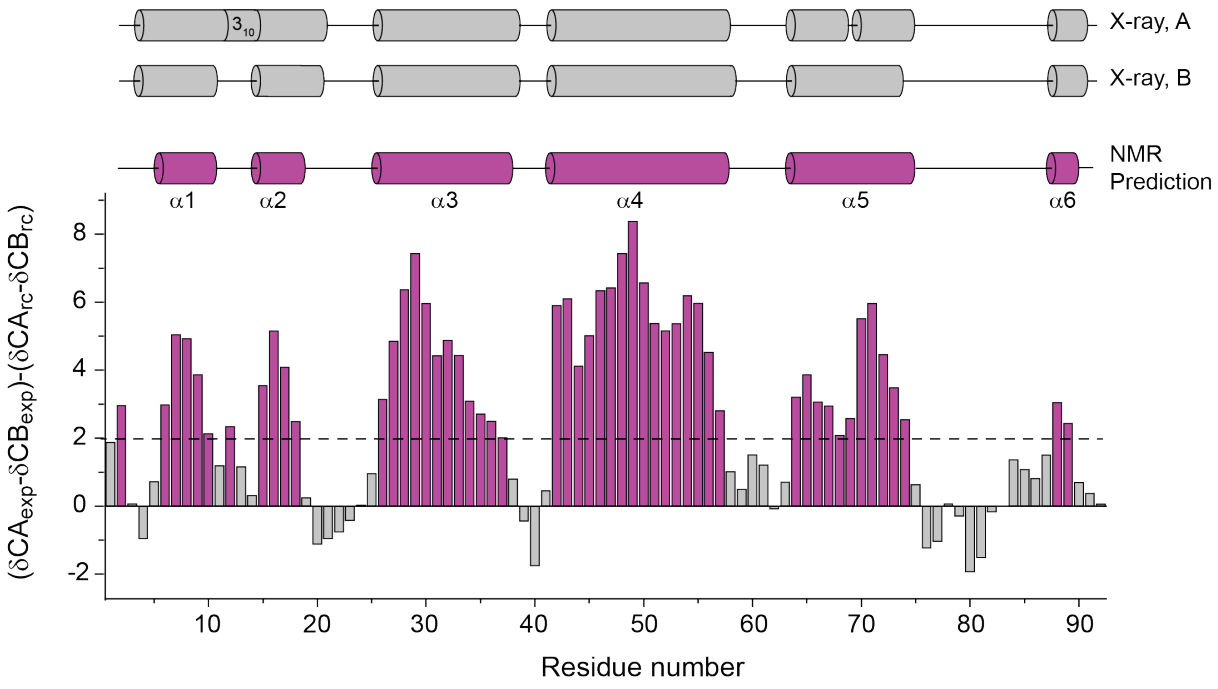


Figure S4. Secondary structure derived from chemical shift assignment at 298 K in comparison with the crystal structure (PDB 6FRR). Shown is the smoothened deviation of the observed chemical shift difference CA-CB from the random coil values for each residue number. Smoothening was obtained by averaging three consecutive residues by using $(\Delta_{i-1} + 2\Delta_i + \Delta_{i+1})/4$. Values above the 2 ppm cut-off (dotted line) indicate a helical secondary structure. The summarized secondary structure derived from the NMR data is shown above (magenta) and compared with both chains of the crystal structure (grey).

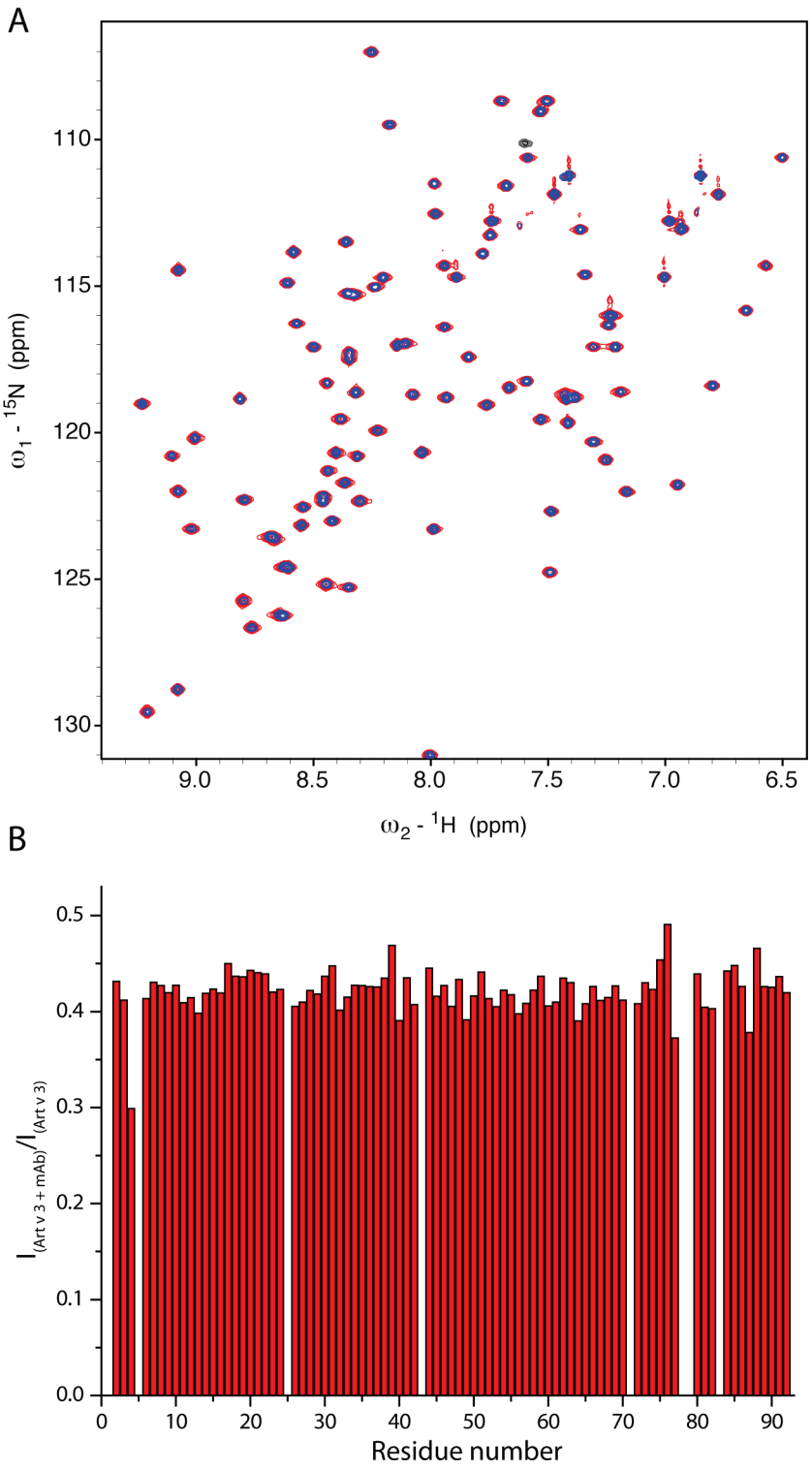


Figure S5. 2D ^{1}H-^{15}N HSQC spectra of Art v 3 in the absence and presence of mAb I. **A.** Overlay of ^{1}H-^{15}N HSQC spectra of Art v 3 in the absence of antibody (in red) and in the presence of 0.25 equivalents of mAb I (blue). The same processing and contour plot intensities were applied. **B.** Intensity ratios of the Art v 3 signal in the presence of mAb I divided by the Art v 3 signal in absence of mAb I is plotted as a function of the residue number. The intensity ratios were corrected with a factor of 1.23 correcting for a dilution effect after addition of mAb.

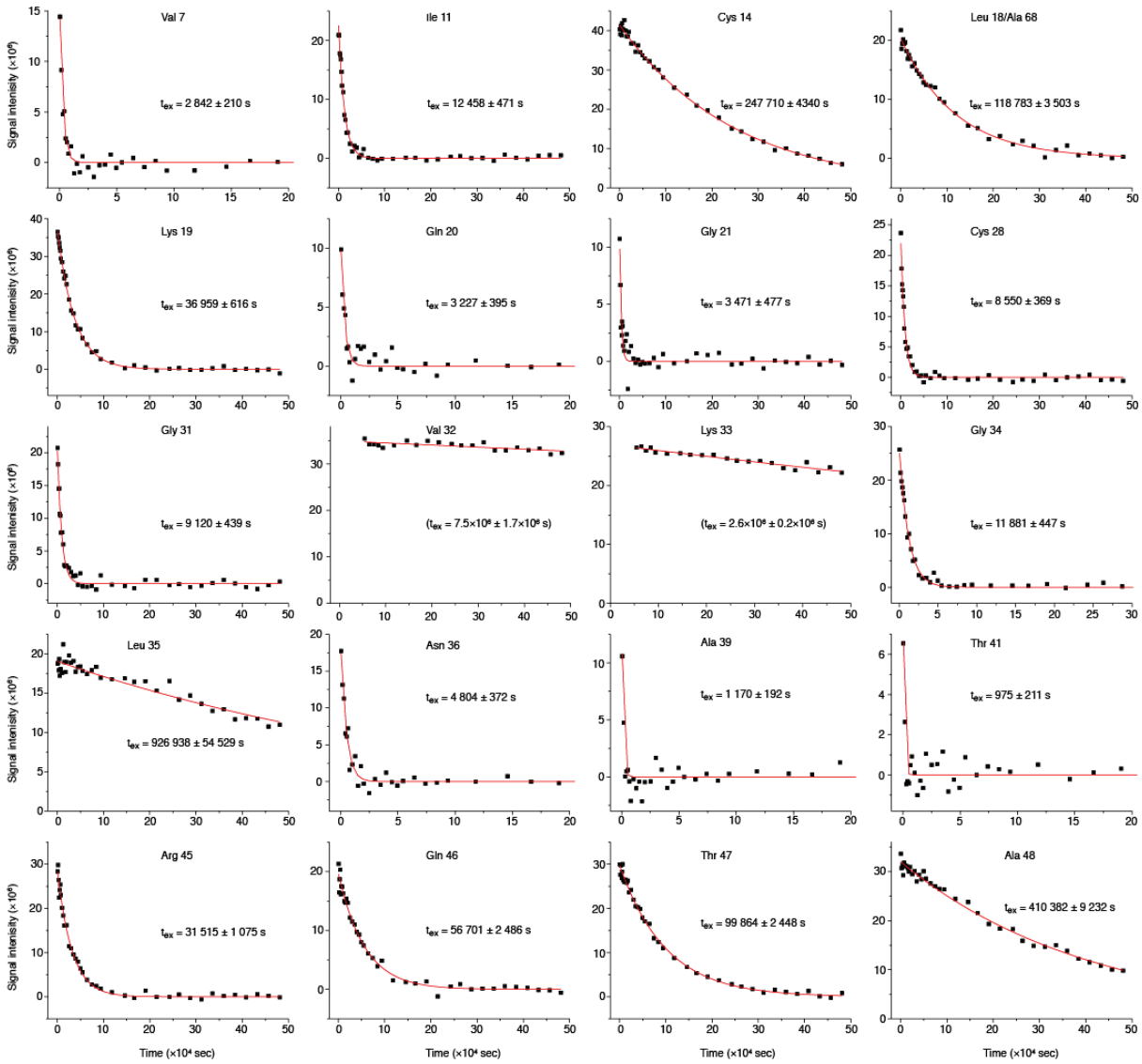


Figure S6. H/D exchange curves of Art v 3 **in the absence of antibody** measured at **278 K**. The signal intensity plotted over time was fitted using $y=A*\exp(-x/t_{ex})$. Residues marked with an asterisk gave a better fit using a small offset: $y=A*\exp(-x/t_{ex})+y_0$. The fitting parameter t1 is given in brackets if the exchange was too slow at 278 K and values at higher temperature were used.

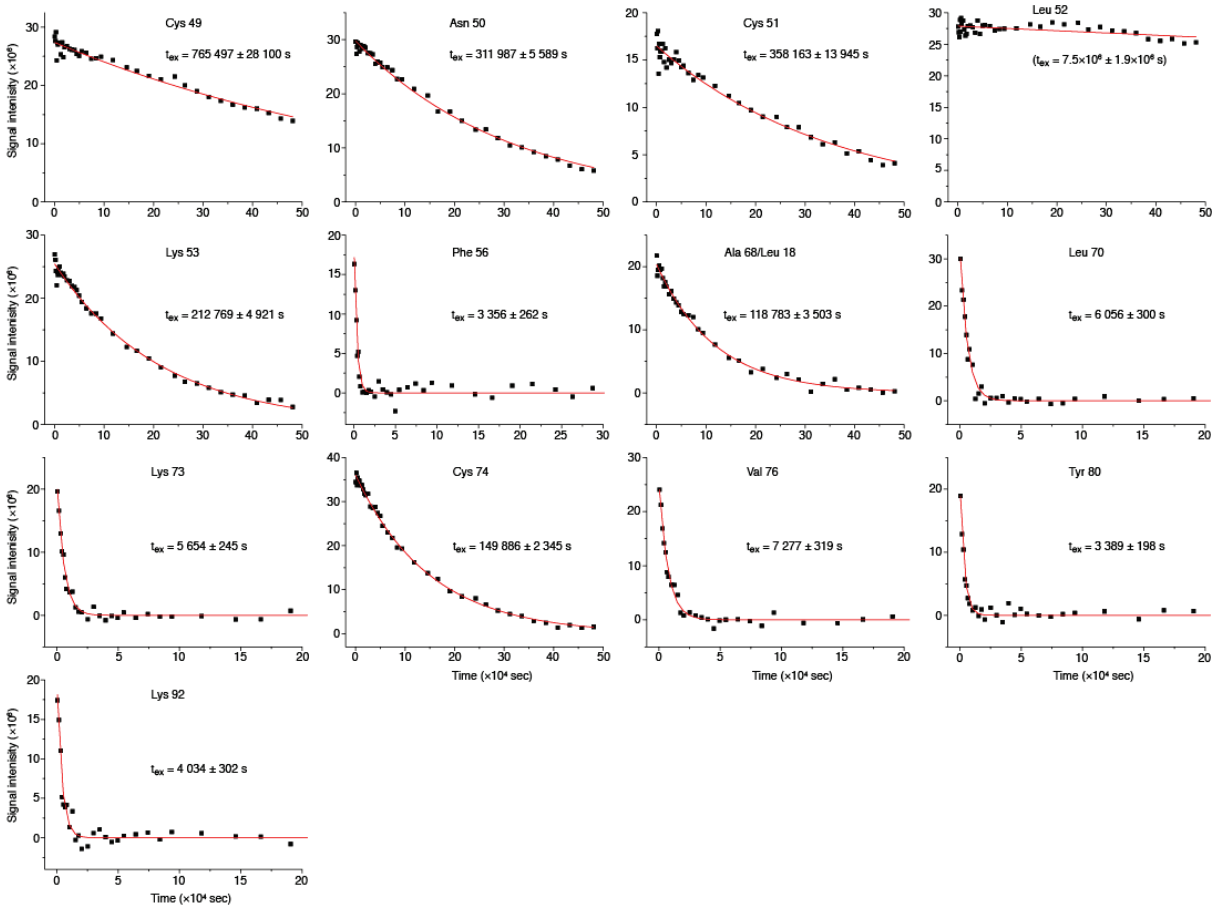


Figure S6. H/D exchange curves of Art v 3 **in the absence of antibody** measured at **278 K** (continuation).

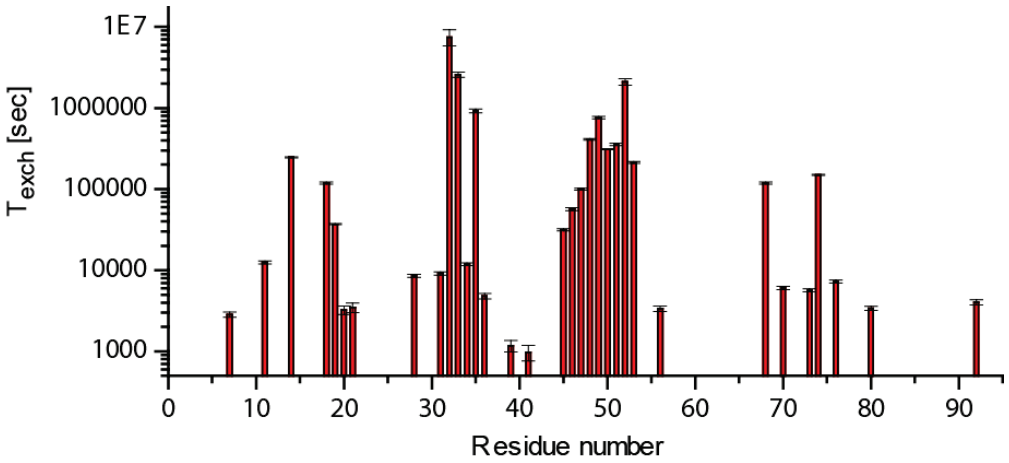


Figure S7. Absolute H/D exchange times of Art v 3 **in the absence of antibody** measured **at 278 K** obtained from the individual fits (Fig. S6). Error bars indicate the error obtained from the fitting procedure.

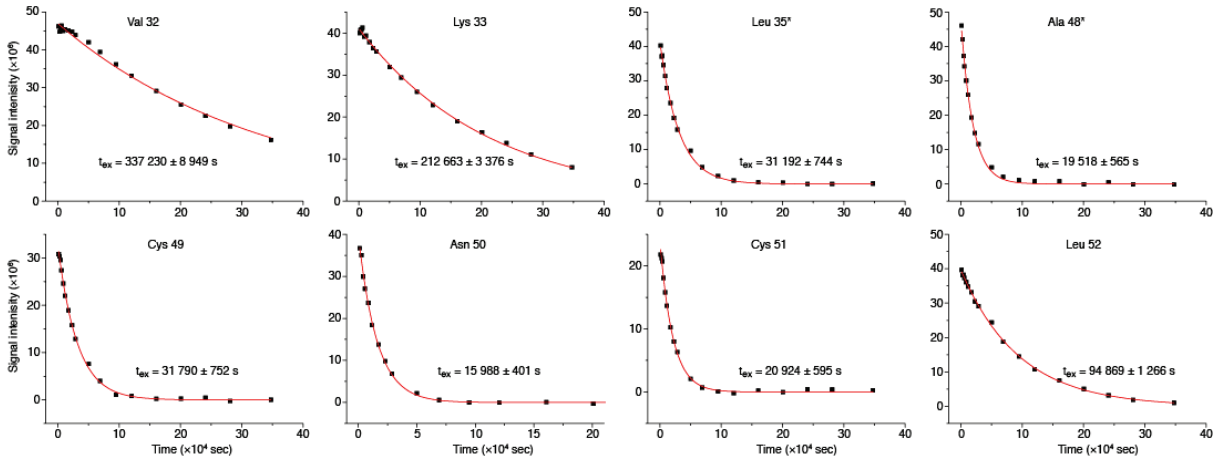


Figure S8. H/D exchange curves of Art v 3 **in the absence of antibody** measured at **298 K**. The signal intensity plotted over time was fit using $y=A*\exp(-x/t_{ex})$. Residues marked with an asterisk gave a better fit using a small offset: $y=A*\exp(-x/t_{ex})+y_0$.

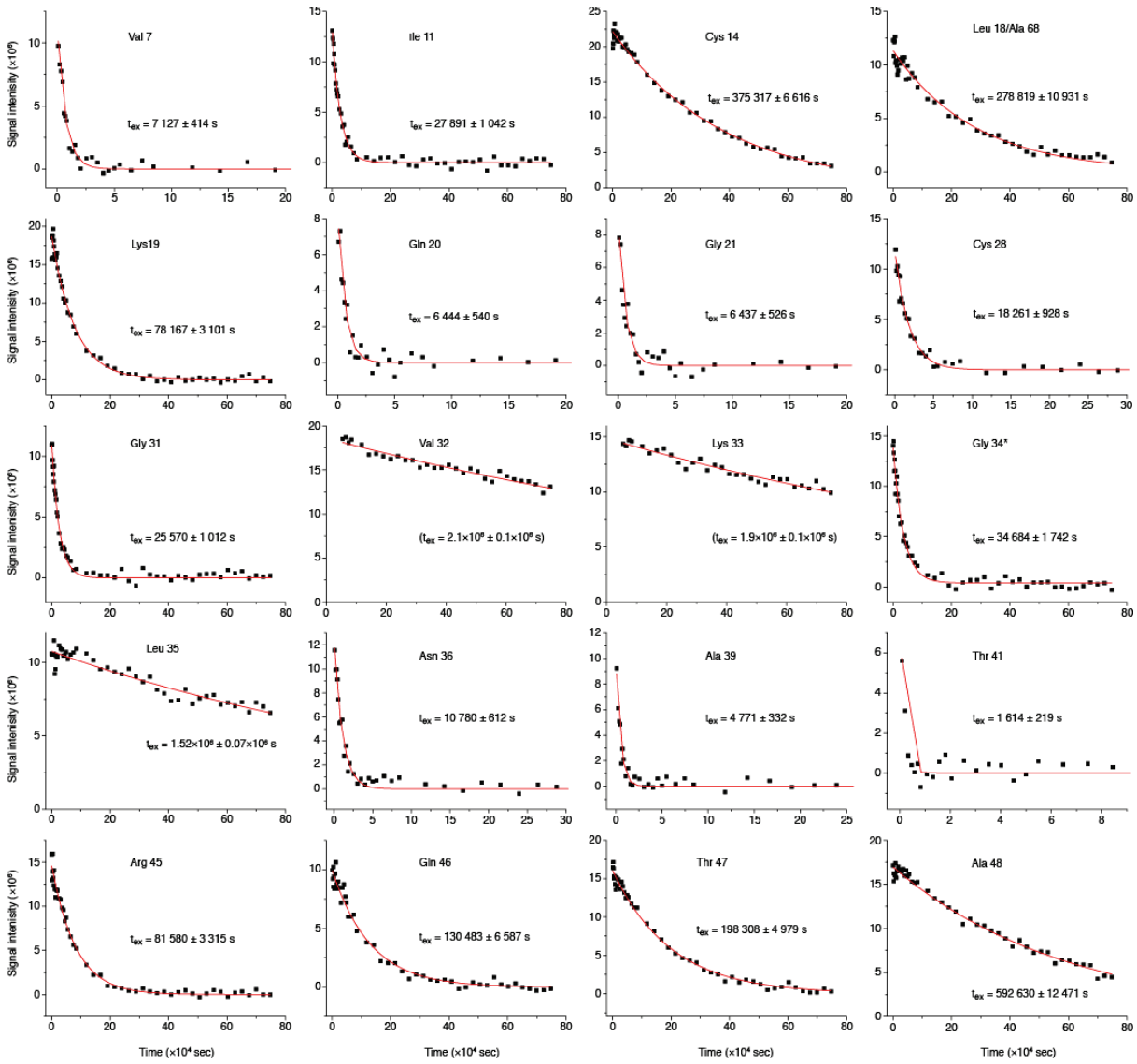


Figure S9. H/D exchange curves of Art v 3 bound ~50% to **mAb I** at **278 K**. The signal intensity plotted over time was fitted using $y=A*\exp(-x/t_{ex})$. Residues marked with an asterisk gave a better fit using a small offset: $y=A*\exp(-x/t_{ex})+y_0$. The fitting parameter t1 is given in brackets if the exchange was too slow at 278 K and values at higher temperature were used.

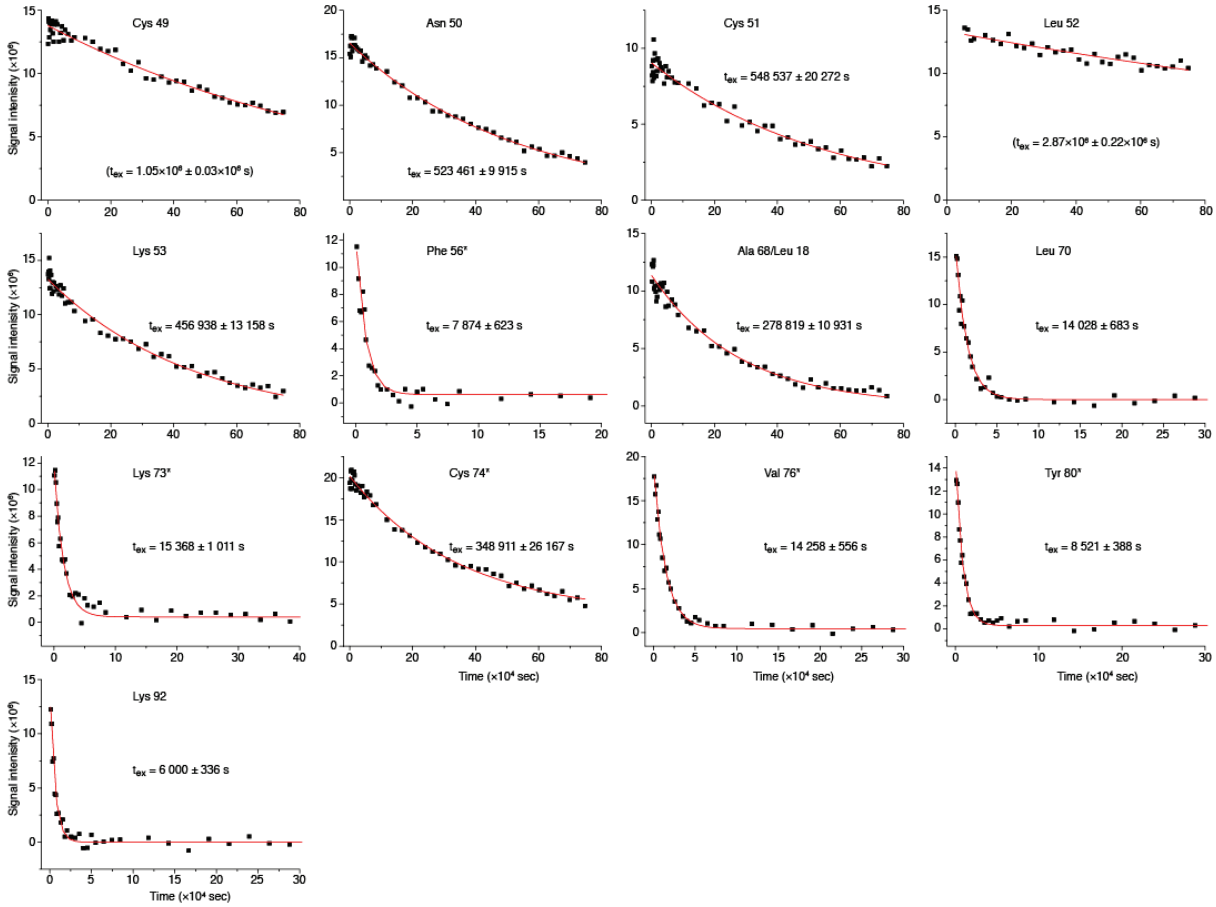


Figure S9. H/D exchange curves of Art v 3 bound ~50% to **mAb I** at **278 K** (continuation).

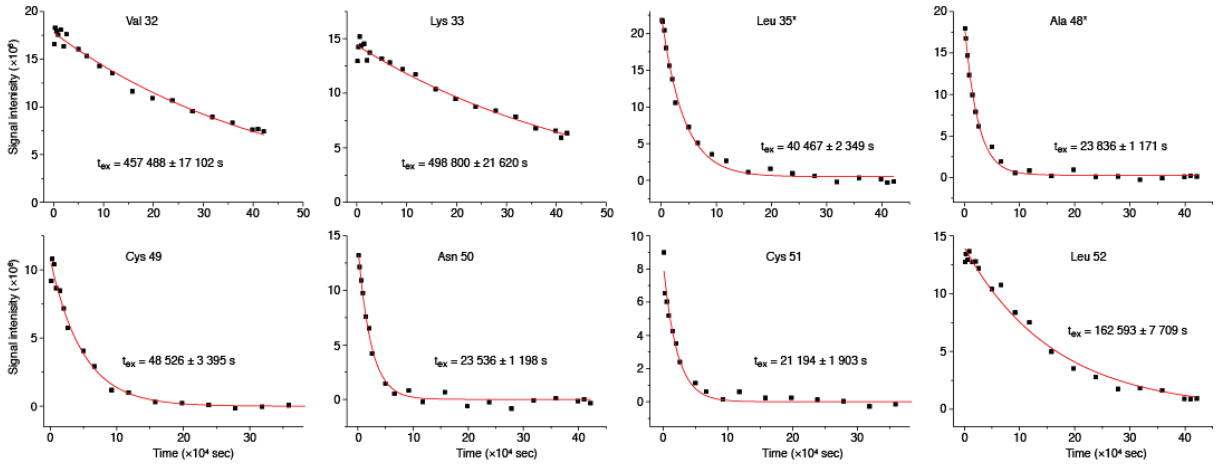


Figure S10. H/D exchange curves of Art v 3 bound ~50% to **mAb I** at **298 K**. The signal intensity plotted over time was fitted using $y=A*\exp(-x/t_{ex})$. Residues marked with an asterisk gave a better fit using a small offset: $y=A*\exp(-x/t_{ex})+y_0$.

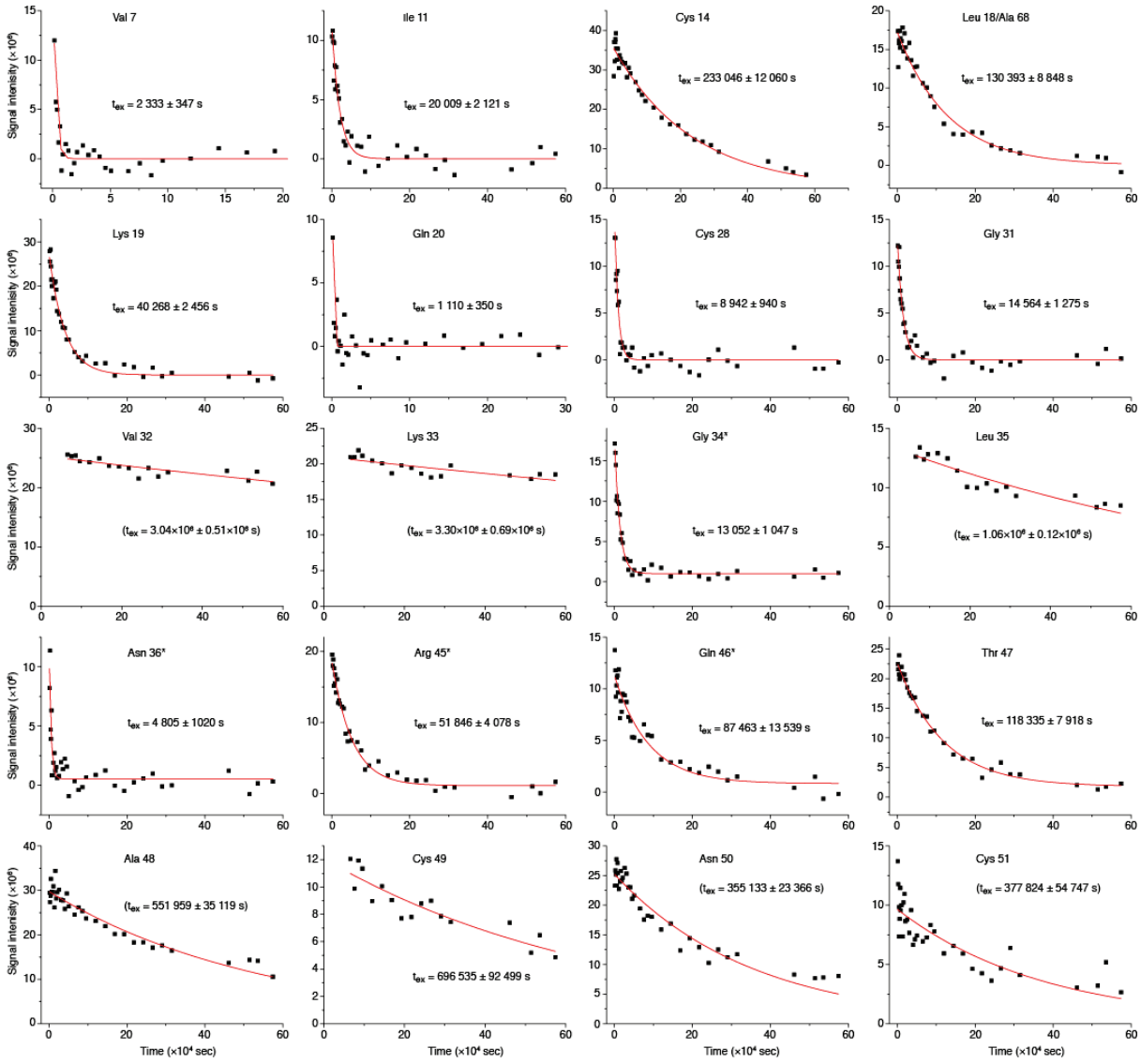


Figure S11. H/D exchange curves of Art v 3 bound ~50% to **mAb II** at **278 K**. The signal intensity (I) plotted over time was fitted using $y=A*\exp(-x/t_{ex})$. Residues marked with an asterisk gave a better fit using a small offset: $y=A*\exp(-x/t_{ex})+y_0$. The fitting parameter t1 is given in brackets if the exchange was too slow at 278 K and values at higher temperature were used.

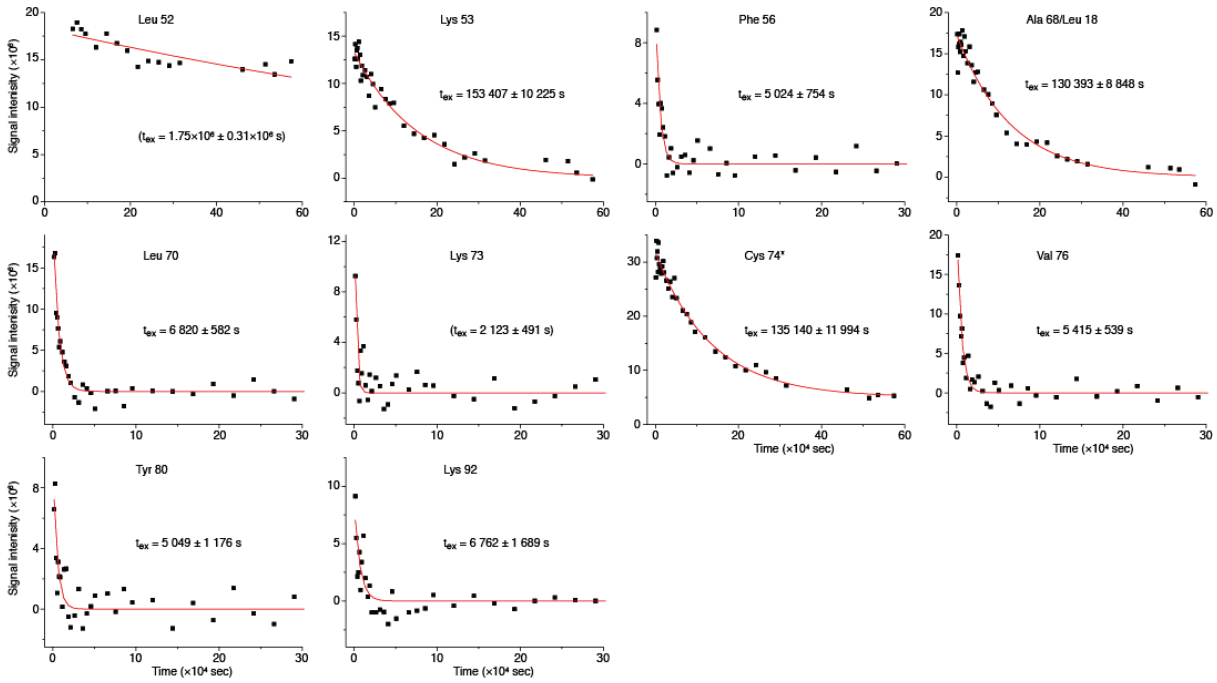


Figure S11. H/D exchange curves of Art v 3 bound ~50% to **mAb II** at **278 K** (continuation).

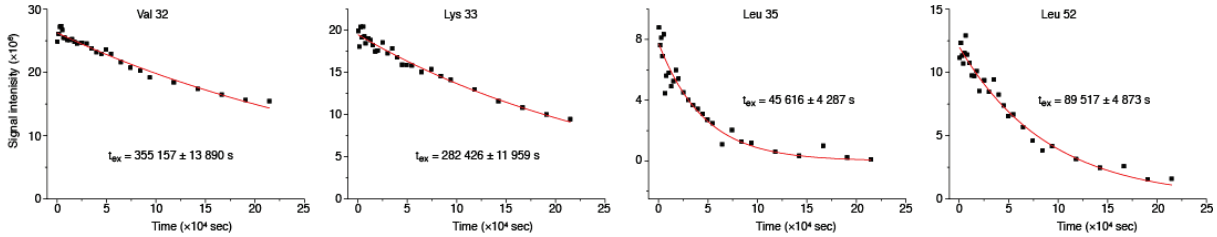


Figure S12. H/D exchange curves of Art v 3 bound ~50% to **mAb II** at **298 K**. The signal intensity (I) plotted over time was fitted using $y=A*\exp(-x/t_{ex})$. Residues marked with an asterisk gave a better fit using a small offset: $y=A*\exp(-x/t_{ex})+y_0$.

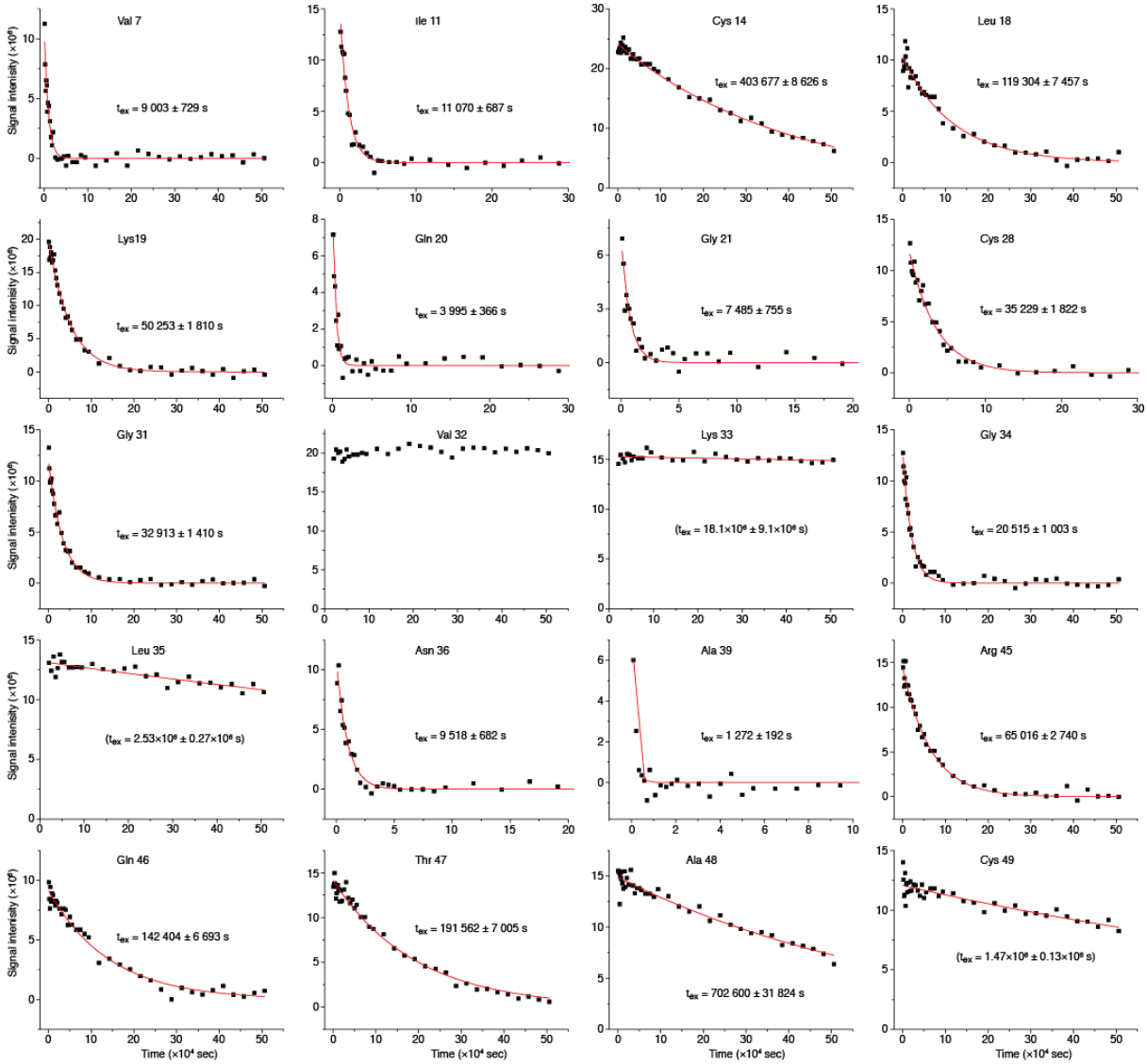


Figure S13. H/D exchange curves of Art v 3 bound ~50% to **mAb III** at **278 K**. The signal intensity (I) plotted over time was fitted using $y=A*\exp(-x/t_{ex})$. Residues marked with an asterisk gave a better fit using a small offset: $y=A*\exp(-x/t_{ex})+y_0$. The fitting parameter t1 is in brackets if the exchange was too slow at 278 K and values at higher temperature were used.

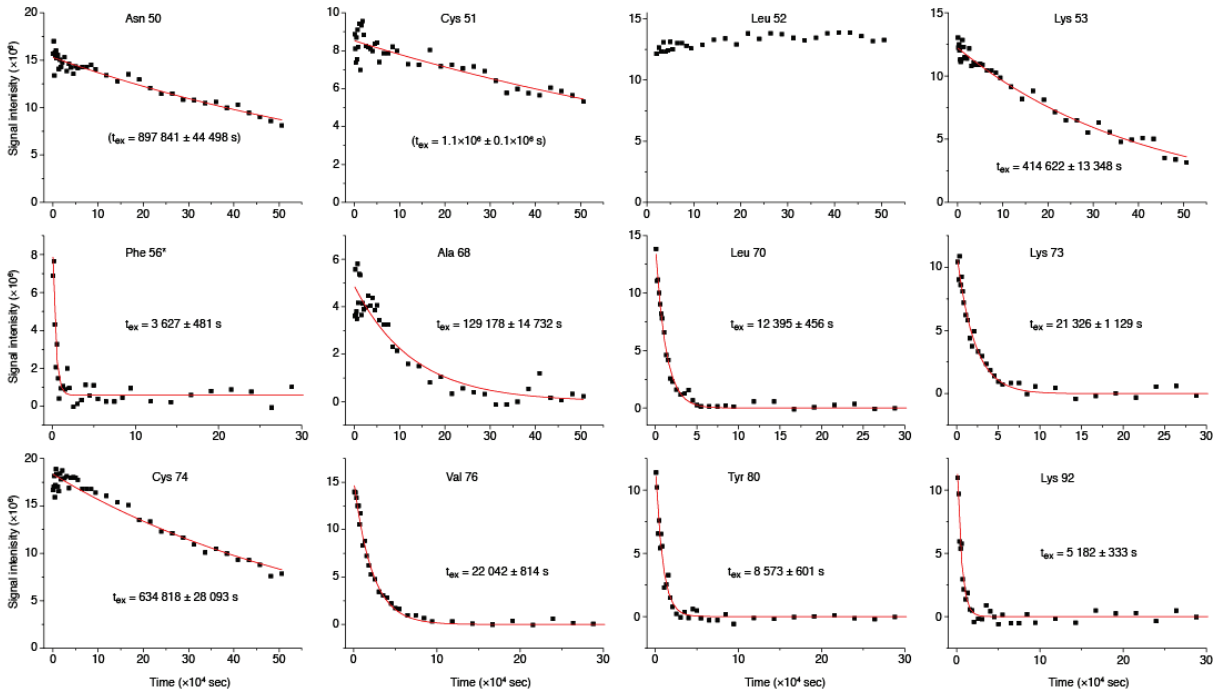


Figure S13. H/D exchange curves of Art v 3 bound ~50% to **mAb III** at **278 K** (continuation).

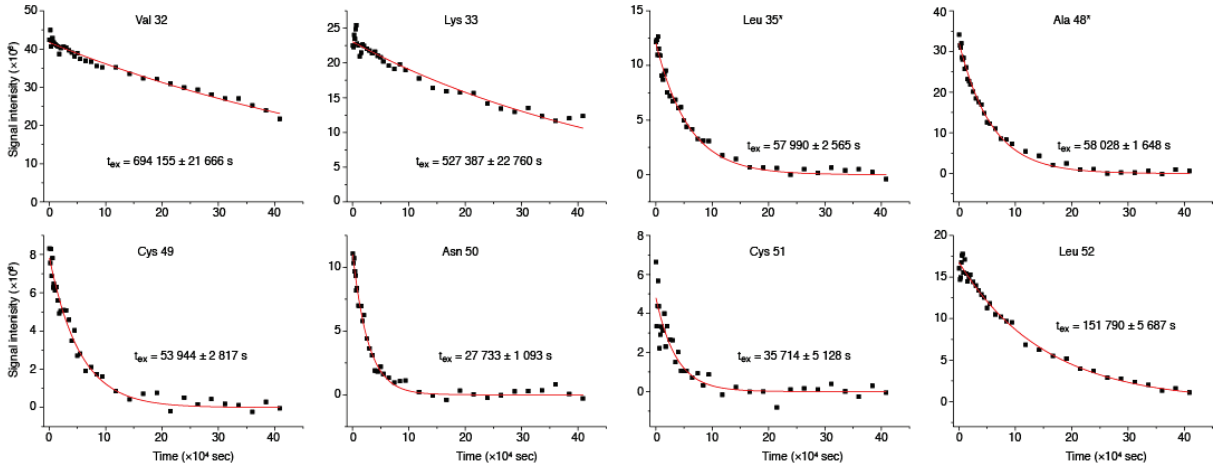


Figure S14. H/D exchange curves of Art v 3 bound ~50% to **mAb III** at **298 K**. The signal intensity (I) plotted over time was fitted using $y=A*\exp(-x/t_{ex})$. Residues marked with an asterisk gave a better fit using a small offset: $y=A*\exp(-x/t_{ex})+y_0$.

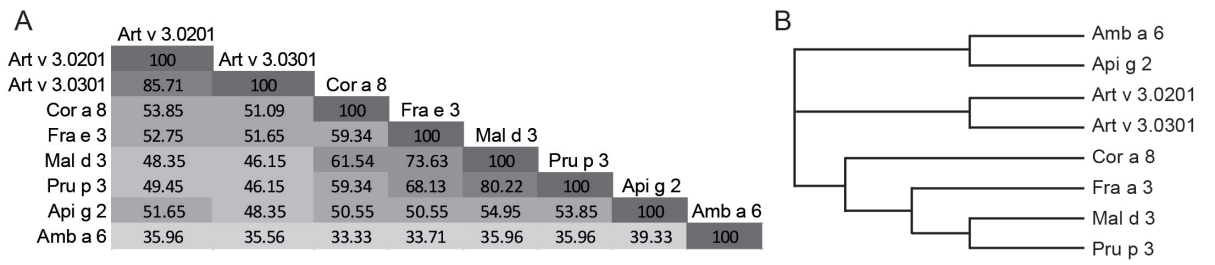


Figure S15. Sequence identity of Art v 3 and other allergenic LTPs. **A.** Percent identity matrix of Art v 3 and homologs based on the multiple sequence alignment using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/). Numbers represent percentage of sequence identity and the degree of identity is indicated by colors ranging from light grey indicating low identity to dark grey indicating high identity. **B.** The phylogenetic relations within the LTPs were elucidated based on the multiple sequence alignment.

A

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                1         10        20        30        40        50        60        70        80        90
Art v 3         MIKCSDVSNKISACLSYLKQGGEVPADCCTGVKGLNDAAKTTPDRQTACNCLKTTFKSNKDFKSDFAASLPSKCGVNIPYKISLETDCNKVK
V1              MIKCSDVSNKISACLSYLKQGGEVPADCCTGVAQLLDAAKTTPDRQTACNCLKTTFKSNKDFKSDFAASLPSKCGVRIPYKISLETDCNKVK
V2              MIKCSDVSNKISACLSYLKQGGEVPADCCTGVKGLNDAAKTTRDLQTACNCLKTTFKSNKDFKSDFAASLPSKCGVNIPYKISLETDCNQVV
V3A             MIKCSDVSNKISACLSYLKQGGEVPADCCTGVLGLNDAAKTTPDRQTACNCLKTTFKSNKDFKSDFAASLPSQCGLKIPYKISLETDCNKVK
V3B             MIKCSDVSNKISACLSYLKQGGEVPADCCTGVKGLNDAAKTTPDRQTACNCLKTTFKSNKDFKSDFAASLPQQCGTNIPYKISLETDCNKVK
                ********************************  * ****** * *************************.:** .************:*
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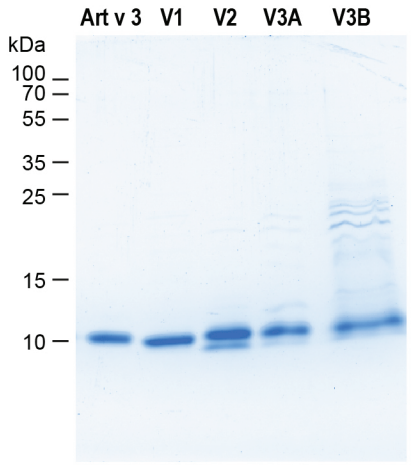
B



C

Art v 3 variant	Theoretical mass (-8H) [Da]	Experimental mass [Da]	Δ mass [Da]
V1	9899.84	9899.88	0.04
V2	9831.73	9831.76	0.03
V3A	9857.78	9857.82	0.04
V3B	9887.73	9887.76	0.03

Figure S16. Sequence and identity of Art v 3 and epitope variants. **A.** Clustal Omega amino acid sequence alignment of Art v 3 and epitope variants V1, V2, V3A, and V3B; residues shown in bold green represent exchanged amino acids for each variant. **B.** Gel electrophoresis of purified recombinant Art v 3 and variants V1, V2, V3A, and V3B. **C.** Mass spectrometry results of intact non-reduced Art v 3 and variants V1, V2, V3A, and V3B confirm the correct primary sequence and thus identity of each variant.

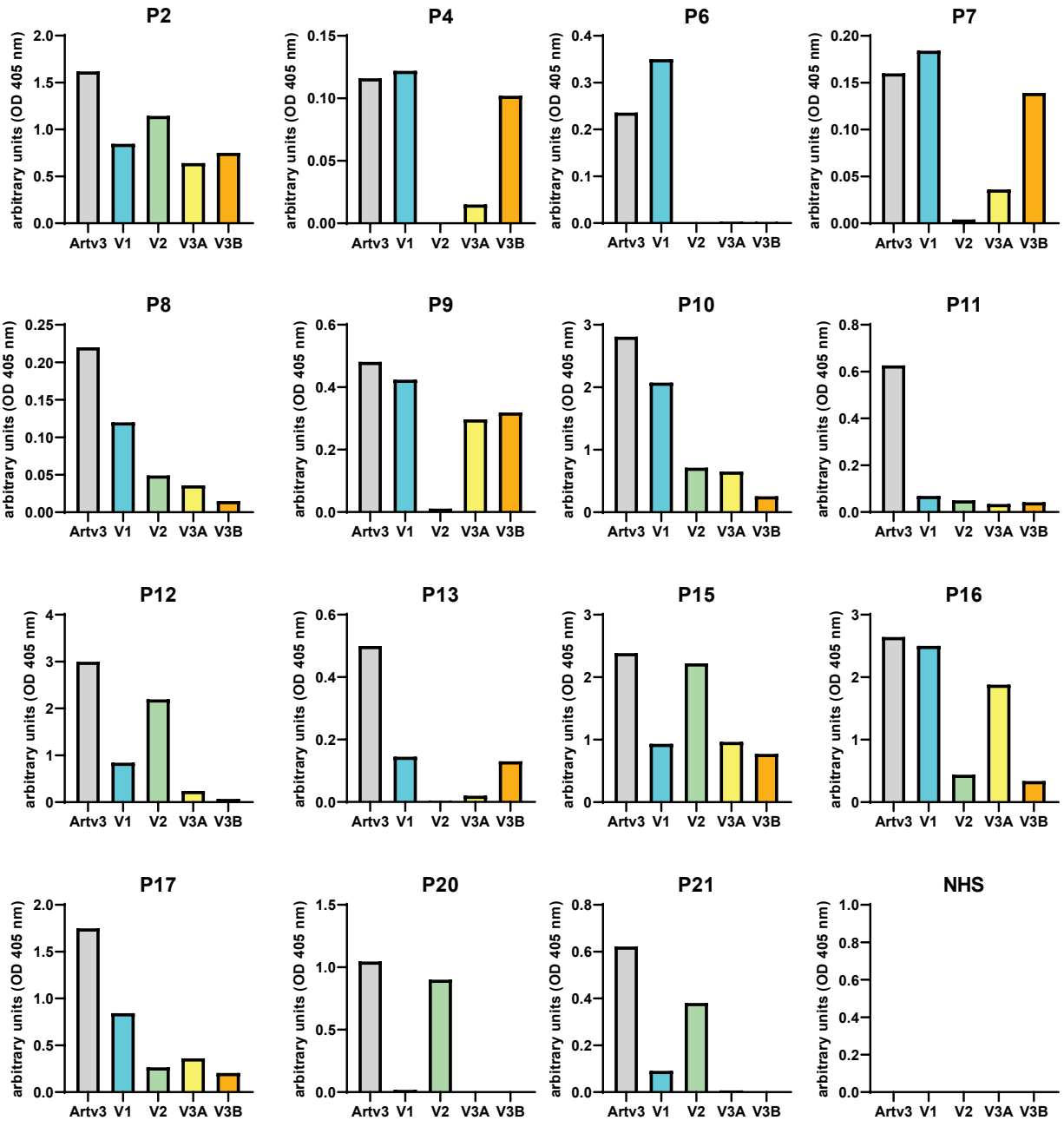


Figure S17. IgE reactivity profiles to Art v 3 and epitope variants. IgE reactivity to Art v 3 and epitope variants V1, V2, V3A, and V3B was determined by ELISA using individual sera from fifteen mugwort pollen allergic patients (P2 – P21). Non-atopic human serum (NHS) was used as negative control.