Probing the Conformational Dynamics of Affinity-Enhanced T Cell Receptor Variants upon Binding the Peptide-Bound Major Histocompatibility Complex by Hydrogen/Deuterium Exchange Mass Spectrometry

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ABBREVIATIONS

β₂m, β₂-microglobulin; C, TCR constant domain; CDR, complementarity-determining region; FPLC, fast protein liquid chromatography; HDX, hydrogen/deuterium exchange; Kᵋ, dissociation constant; MHC, major histocompatibility complex; MS, mass spectrometry; PDB, Protein Data Bank; pMHC, peptide-bound major histocompatibility complex; QM, quadruple mutant; RT, room temperature; SM, single mutant; TCR, T cell receptor; TRAV, T cell receptor α variable; TRBV, T cell receptor β variable; V, TCR variable domain
ABSTRACT

Binding of the T cell receptor (TCR) to its cognate, peptide antigen-loaded major histocompatibility complex (pMHC) is a key interaction to trigger T cell activation and ultimately in elimination of the target cell. Despite the importance of this interaction for cellular immunity, a comprehensive molecular understanding of TCR specificity and affinity is lacking. Here, we conducted HDX-MS analyses on individual affinity-enhanced TCR variants and clinically relevant pMHC class I molecules (HLA-A*0201/NY-ESO-1157-165) to investigate the causality between increased binding affinity and conformational dynamics in TCR-pMHC complexes. Differential HDX-MS analyses of TCR variants revealed that mutations for affinity enhancement in TCR CDRs altered the conformational response of TCR to pMHC ligation. Improved pMHC binding affinity was in general observed to correlate with increased differences in HDX upon pMHC binding in modified TCR CDR loops, thereby providing new insights into the TCR-pMHC interaction. Furthermore, a specific point mutation in β-CDR3 loop of the NY-ESO-1 TCR associated with a substantial increase in binding affinity, resulted in a substantial change in pMHC binding kinetics (i.e. slow $k_{on}$, due to the detection of EX1 HDX kinetics), thus providing experimental evidence for a slow induced-fit binding mode. We also examined the conformational impact of pMHC binding on an unrelated TRAV12-2 gene-encoded TCR directed against the immunodominant MART-126-35 cancer antigen restricted by HLA-A*0201. Our findings provide a molecular basis for the observed TRAV12-2 gene bias in natural CD8$^+$ T cell-based immune responses against the MART-1 antigen; with potential implications for general ligand discrimination and TCR cross-reactivity processes.
INTRODUCTION

T cells (also known as T lymphocytes) play an essential role in adaptive immunity as they enable immune surveillance via the expression of T cell receptors (TCRs) on the cell surface. TCRs recognize short peptide antigens presented in the context of major histocompatibility complex,¹ which in turn allows T cells to discriminate between healthy and pathologic cells. The interaction between a TCR and its cognate peptide loaded major histocompatibility complex (pMHC) results in the activation of the T cell and ultimately elimination of the antigen-presenting target cell.² ³ The majority of T cells express heterodimeric TCRs that comprise an α- and a β-chain. Each polypeptide chain is composed of an N-terminal variable (V) and constant (C) immunoglobulin-like domain, followed by a membrane-spanning region, and a short cytoplasmic tail.⁴ ⁶ CD8⁺ cytotoxic T cells generally interact with pMHC class I molecules presenting endogenously-derived protein fragments. Upon activation, T cells eliminate the antigen-presenting target cell by apoptosis-inducing ligands or release of lytic granules.⁷ ⁹ The pMHC class I molecules share a conserved structural fold including a membrane-tethered heavy chain, which is arranged in three extracellular domains (α1, α2, and α3), as well as the nonpolymorphic β₂-microglobulin (β₂m) light chain.¹⁰ ¹² The α1 and α2 domains of the MHC scaffold shape a characteristic peptide-binding groove composed of a seven-stranded β-sheet and two α-helices into which the peptide antigen binds.¹²

Over the past two decades, crystallographic structures of individual TCR-pMHC complexes have greatly advanced our understanding of the immune system by providing fundamental structural insights on TCR-mediated recognition of pMHC.¹³ ¹⁴ The peptide antigen and both MHC α-helices form a contiguous binding surface for the three highly variable complementarity-determining regions (CDRs 1-3) of each TCR Vα/Vβ domain.¹⁵ ¹⁶ TCRs generally adopt an approximately diagonal orientation on top of pMHCs with the TCR CDR3α and CDR3β loops positioned over the peptide antigen amino- and carboxyl-
terminus, respectively. The TCR-pMHC interface is often dominated by contacts between CDRs and the MHC scaffold consistent with the experimental observation of an intrinsic specificity of αβ-TCRs towards MHC. The traditional view is that the germline-encoded CDR1 and CDR2 loops tend to form interactions with the MHC helices, while the hypervariable CDR3 loops contact the peptide antigen in the center of the binding interface, although molecular dynamics simulation reveals a more distributed and complex set of interactions.

Continuous advances in the mechanistic understanding of the TCR-pMHC interaction have spurred the development of innovative T cell-based therapies in the field of cancer medicine. As an example, a promising approach, termed genetically modified T cell immunotherapy, relies on altering the specificity and enhancing the effector function of T cells by mutational modification of the TCRs to achieve optimized binding characteristics for a given antigen employing in vitro technologies. First clinical successes of this approach were achieved by treating melanoma patients with genetically engineered T cells optimized for binding the MART-1 peptide. Likewise, tumor regression has been documented in patients with synovial cell sarcoma and melanoma following selective targeting of the NY-ESO-1 cancer testis antigen. Consequently, T cell-based immunotherapy would considerably benefit from an improved ability to proficiently engineer and evaluate TCRs with high specificity and avidity towards a given pMHC.

The exact molecular mechanism underlying TCR-mediated recognition of pMHC, however, has remained controversial. Despite identification of critical MHC contact residues along the peptide-binding groove potentially governing the initial formation of the TCR-pMHC complex, an accurate prediction of the molecular interaction pattern between a specific TCR and pMHC remains elusive.
Various structural and biophysical data support a two-step binding mechanism, in which the malleable TCR CDR3 loops interact with the antigen via induced-fit binding. However, the structural plasticity in the binding interface and the ability of TCRs to form compensatory interactions to pMHC has not yet allowed drawing of definite conclusions about the fundamental ‘rules of engagement’. Coordinated adjustments in conformation and dynamics of TCR CDR loop structures, the peptide antigen, and the MHC scaffold have been shown to cooperatively facilitate formation of the TCR-pMHC complex. Consequently, experimental and computational studies with focus on protein dynamics are required to complement the static structural framework provided by X-ray crystallography in order to uncover novel mechanistic features inherent to TCR function such as TCR recognition of pMHC and TCR cross-reactivity.

Hydrogen/deuterium exchange coupled to mass spectrometry (HDX-MS) has emerged as a highly valuable experimental approach to probe the solution-phase conformation and dynamics of proteins in a sensitive and unperturbed manner. The HDX-MS technique relies on determining the incremental mass increase in individual segments of the target protein following time-dependent labeling of backbone amides with deuterium. The rate of isotopic exchange of a backbone amide is intimately correlated to hydrogen bonding status and stability, which in turn enables the use of HDX-MS to carefully probe the conformational dynamics of a protein in solution and, for instance, monitor local changes in dynamics as a function of protein/ligand and protein/protein interactions. A more detailed background on the HDX-MS technique, recent methodological advances, and its application areas has been covered in several reviews.
Here, we interrogated the solution-phase conformational dynamics of distinct affinity-enhanced TCRs in the presence and absence of their cognate pMHC by HDX-MS. Our measurements allow a delineation of the conformational impact of complex formation on individual TCR-pMHC constituents and comparison of the intrinsic conformational dynamics among unrelated TCRs in solution. The HDX-MS data indicate that mutations in TCR CDR loops attenuate local dynamics to give rise to increased binding affinity towards cognate pMHC. In conjunction, we observe a remarkable diversity in the conformational responses of closely related TCR variants to antigen binding. In summary, our results thus provide direct experimental evidence of the importance of conformational dynamics in TCR recognition of pMHC and document the impact of mutations in TCR CDR loop structures on pMHC binding affinity. Our HDX-MS measurements encompassing the T cell receptor α variable (TRAV) 12-2 gene-encoded TCR furthermore reveal unrecognized conformational dynamics in the Vα domain, which potentially play a role in TCR recognition of the clinically relevant, cognate MART-1/HLA-A*0201 complex.

EXPERIMENTAL PROCEDURES

Reagents and Recombinant Proteins — Soluble TCRs (TRAV23- T cell receptor β variable (TRBV) 13) variants specific for HLA-A*0201/NY-ESO-1\textsubscript{157-165} and others were expressed in \textit{Escherichia Coli} BL21(DE3) cells transformed with pGMT7 encoding TCR α- and β-chains. Upon resuspension of inclusion bodies and refolding of TCRs by dialysis,\textsuperscript{57} individual receptors were purified by a polyhistidine-tag based strategy using fast protein liquid chromatography (FPLC) essentially as described previously.\textsuperscript{58} A step imidazole gradient was applied to elute the His-tagged protein from a Ni\textsuperscript{2+} immobilized chelating sepharose packed column (GE Healthcare Life Sciences). Aggregates were removed by FPLC-based size-exclusion chromatography using an S200 column. The pMHC was either
produced as described previously\textsuperscript{59} or purchased from TCMetrix. The anchor-modified peptide sequence for the NY-ESO-1\textsubscript{157-165} analogue antigen was SLLMWITQA and for MART-1\textsubscript{26-35} it was ELAGIGILTV. All chemicals used were of the highest grade commercially available.

**Hydrogen/Deuterium Exchange** — A 20 minute equilibration period was introduced prior to the labeling reaction in order to allow formation of protein complexes at room temperature. A fractional pMHC occupancy of the TCR of 62\%, 97\% and 99.5\% was achieved in binding experiments using β-SM, αβ-QM and β-QM TCRs, respectively. Hydrogen/deuterium exchange was initiated by diluting the target protein, in the presence or absence of a molar excess of ligand, 10-fold into deuterated buffer (20 mM Tris, pDread 7.4, and 150 mM NaCl). Proteins were labeled at room temperature for the indicated time intervals (\textit{i.e.,} 0.25-240 min) and the isotopic exchange reaction quenched by acidification and cooling of the sample solution to pH 2.6 and 0 °C, respectively. Ice-cold quench buffer consisted of 75 mM phosphate buffer, pH 2.5, supplemented with 0.4 M tris(2-carboxyethyl)phosphine and was added in equal volume to the sample solution. Quenched protein samples were immediately frozen on dry ice and stored at -80 °C until mass spectrometric analysis.

**Liquid Chromatography and Mass Spectrometry** — Approximately 40-50 pmol of quenched target protein was typically injected onto a refrigerated (0 °C) UPLC chromatographic platform (nanoACQUITY-HDX technology, Waters) coupled to a Q-ToF Synapt G2 mass spectrometer (Waters, Milford, USA). Quenched protein samples were then passed through an in-house packed pepsin column (IDEX, Oak Harbor, USA) containing immobilized pepsin on agarose resin beads (Thermo Scientific Pierce, Rockford, USA) for proteolysis. The resulting peptide mixture was trapped on a C18 trap column (ACQUITY UPLC BEH C18 1.7 µm VanGuard column, Waters, Milford, USA) for efficient desalting.
with mobile phase A (0.2% (v/v) formic acid) at a constant flow rate of 200 µl/min for 3 min. Chromatographic separation was achieved by applying a 7 min linear gradient at a constant 40 µl/min flow rate and increasing concentrations (8% – 40% (v/v)) of mobile phase B (acetonitrile, 0.2% (v/v) formic acid) using a C18 analytical column (ACQUITY UPLC BEH C18 1.7 µm, 1x100 mm column, Waters, Milford, USA). The Synapt G2 mass spectrometer was interfaced with an ESI source and operated in positive ionization mode. Human Glu-Fibrinopeptide (Sigma-Aldrich, St. Louis, USA) served as an internal standard and was acquired throughout MS analysis. Peptide identification was performed on non-deuterated samples by data-independent tandem mass spectrometric analysis (MS^E).

**Data Analysis** — Peptides were identified by database searching in the PLGS 2.5 software and filtered in DynamX 2.0 by applying the following filtering criteria: mass error below 10 ppm and peptides should be identified in 3 out 4 MSe files. To verify the identified peptides all mass spectra were manually inspected. HDX-MS data was processed in DynamX 2.0 and all peptide assignments manually verified. Individual peptides displaying bimodal or broadened isotopic envelopes upon deuteration were further analyzed in HX-Express 2.0\textsuperscript{60} using standard settings for binomial fitting and bimodal deconvolution. Deuterium uptake for individual protein segments was compared and differences in HDX mapped onto relevant crystal structures of the target proteins using Pymol (http://pymol.sourceforge.net/). HDX experiments where performed in at least two biological replicates for each TCR, to confirm all findings at a qualitative level. Based on technical replicates of a limited data set, we estimated the standard deviation to 0.06D and the 95% confidence interval for significant differences in HDX between TCRs to ±0.57D.\textsuperscript{61}
To allow access to the HDX data of this study, the HDX Data Tables and the HDX Data Summary Table (Tables S1-S10) are included in the Supporting Information according to the community-based recommendations.62

RESULTS

Conformational Dynamics of the MHC Scaffold in the Absence and Presence of TCR — To examine the conformational dynamics of a representative MHC scaffold, we selected HLA-A*0201 bound to the cancer testis peptide NY-ESO-1157-165 and determined the time-resolved (0.25–240 min) deuterium uptake in the presence and absence of individual affinity-enhanced TCR variants. Online pepsin proteolysis yielded a total of 64 peptides suitable for local HDX analysis, which covered 93% of the MHC heavy chain and β2m sequence (cf. Figure S1).

HDX analysis of uncomplexed pMHC in solution revealed that structured protein segments (α-helices and β-sheets) of the MHC heavy chain and β2m were rather protected from HDX, whereas individual interconnecting loops displayed a more pronounced dynamic behavior (Figure 1, e.g. peptide 38-60, and Figure S2, teal curves). Notably, individual β-strands of the peptide-binding groove displayed only limited deuterium uptake even for the latest measured time-point (Figure 1, e.g. peptide 24-34) demonstrating a marked structural rigidity of the β-sheet, which is consistent with its primary role of accommodating the peptide.

Obtained HDX data for uncomplexed pMHC subsequently served as a reference for assessing the conformational impact of TCR binding on the MHC scaffold. For this purpose, we selected individual affinity-enhanced TCR variants (based on BC1 TCR, TRAV23-TRBV13) exhibiting optimized binding
The soluble TCR variants differed with regard to the expressed CDR loop sequences (cf. Table 1) and had previously been designed and characterized by structure-based modeling and several biological and biophysical techniques, respectively. Notably, TCR variants of increased binding affinity comprised amino acid replacements in α-CDR2 (αβ quadruple mutant (QM) TCR), β-CDR2 (αβ-QM and β-QM TCR), and β-CDR3 (αβ-QM and β single mutant (SM) TCR) as specified in Table 1.

Table 1. Overview of the sequence variation in CDR loop structures for selected BC1 TCR variants with a single mutation (SM) or quadruple mutation (QM). Mutations in CDR2 and CDR3 regions of the TCR α- and β-chain are indicated in red color. TCR variants are ordered according to their binding affinity towards cognate pMHC (HLA-A*0201/NY-ESO-1157-165).

<table>
<thead>
<tr>
<th>TCR</th>
<th>α-CDR2</th>
<th>β-CDR2</th>
<th>β-CDR3</th>
<th>K_d (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>Q S S Q</td>
<td>V G A G I</td>
<td>G A A G</td>
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</tr>
<tr>
<td>β-SM</td>
<td>Q S S Q</td>
<td>V G A G I</td>
<td>G L A G</td>
<td>2.69</td>
</tr>
<tr>
<td>αβ-QM</td>
<td>Q S W Q</td>
<td>V A E G I</td>
<td>G L A G</td>
<td>0.14</td>
</tr>
<tr>
<td>β-QM</td>
<td>Q S S Q</td>
<td>V A I Q T</td>
<td>G A A G</td>
<td>0.02</td>
</tr>
</tbody>
</table>

We observed a consistent decrease in HDX in discrete regions of the MHC heavy chain (e.g. peptides covering residues 34-60, 61-84 and 140-159) upon addition of a molar excess of any investigated TCR variant (Figure 1, teal vs. red curves). TCR-induced shifts in HDX were restricted to the peptide-binding groove and were most evident for both MHC α-helices (approximately 1-2 D). Our findings are consistent with crystallographic evidence for TCR-pMHC complexes and likely reflect the conserved TCR docking orientation on top of pMHC. A structural overview of the conformationally altered MHC segments and corresponding deuterium uptake plots for pMHC in the presence and absence of β-SM TCR is provided in Figure 1.
Figure 1. HDX of the HLA-A*0201 scaffold in the presence and absence of β-SM TCR. X-ray crystal structure (pdb entry 2BNR) of the HLA-A*0201 peptide-binding groove (teal) in complex with the NY-ESO-1 157-165 antigen (yellow) is shown. The MHC α3 domain, β2m, and the 1G4 TCR also present in the 2BNR crystal structure are omitted from the figure for clarity. MHC segments that displayed perturbed HDX rates in the presence of β-SM TCR are indicated in red in the crystal structure. Deuterium uptake plots for individual MHC peptides are presented based on a single measurement. Red and teal colored curves depict the time-resolved (0.25-240 min) HDX of the MHC scaffold in the presence and absence of β-SM TCR, respectively. Peptides 38-60, 61-82, and 62-84 cover residues of the MHC α1-helix, whereas peptides 140-156 and 140-157 constitute regions of the MHC α2-helix. Peptide 24-34 corresponds to the seven-stranded β-sheet of the peptide-binding groove and did not exhibit altered HDX rates upon formation of the TCR-pMHC complex in the investigated time window.

Despite a high similarity in the observed protection pattern along the MHC backbone, acquired HDX data hinted at subtle changes in TCR recognition of pMHC. That is, the relative contribution of each MHC α-helix to the overall observed protection varied for individual affinity-enhanced TCR variants in agreement with their optimized CDR sequences (data not shown). For instance, involvement of the MHC α1-helix in formation of the TCR-pMHC complex appeared to be a more prominent feature for β-QM TCR with its engineered β-CDR2 sequence (dissociation constant (Kd) ~ 0.015 μM, ratio of reduction in
HDX between $\alpha_1/\alpha_2 \sim 2.3$) than for $\beta$-SM TCR, which only contains a single point-mutation in $\beta$-CDR3 loop ($K_d \sim 2.7 \mu M$, ratio of reduction in HDX between $\alpha_1/\alpha_2 \sim 1.4$).

**Impact of Mutations in CDR Loops on TCR Binding Characteristics** — To investigate any dynamic origins of increased binding affinity for individual TCR variants listed in Table 1, we determined the time-resolved HDX (i.e., 0.25–60 min) of each receptor in the presence and absence of a molar excess of cognate pMHC (HLA-A*0201/NY-ESO-1157-165). Online pepsin proteolysis of TCR variants yielded a total of 56 peptides that were suitable for local HDX analysis covering approximately 80% of the sequence of both TCR chains (Figure S3).

The $\beta$-SM TCR represented an eligible starting point to establish general binding characteristics and to explore the mechanistic implications of further sequence optimization in CDR loop structures on TCR recognition of pMHC. The HDX-MS data-set of the $\beta$-SM TCR, which serves as a reference in the current study, was reported previously. Binding of pMHC to $\beta$-SM TCR in solution reduced the HDX of CDR loops of both V$\alpha$ (CDR1 and CDR3) and V$\beta$ (CDR2 and CDR3) with no pMHC binding-induced changes in HDX in TCR segments remote from the TCR-pMHC binding interface (Figure 2a and Figure S4). As reported earlier, reduced HDX was observed in parts of the TCR constant regions (C$\alpha$ C- and F-strand, e.g. $\beta$-SM TCR $\alpha$-chain peptides 143-157 and 192-201) shown to be involved in b$_2$m binding, indicating that a minor excess of b$_2$m was present in solution and bound to these parts in a similar manner as observed previously. This pattern was observed in all TCR-pMHC binding experiments reported here and will not be discussed further. The CDR loops generally form an elongated surface that contacts the peptide and the MHC scaffold. Individual TCR regions including $\alpha$-CDR2, $\beta$-CDR2, and $\beta$-CDR3 loop structures were of particular interest in this study as these receptor segments were subjected to affinity
enhancement by selective amino acid replacements. An overview of representative deuterium uptake plots for peptides covering these CDR loops is provided in Figure 2b for each TCR variant. We measured a changed conformational response in αβ-QM TCR upon pMHC ligation relative to the results obtained for β-SM TCR. We observed a change in conformational dynamics in both the free and complexed αβ-QM TCR relative to the results obtained for β-SM TCR. A reduction in HDX of αβ-QM TCR relative to the β-SM variant (approximately 0.5 D, n = 2) was observed in the two overlapping TCR segments comprising the engineered α-CDR2 sequence (α-chain peptides 48-62 and 49-62). Interestingly, this reduction was already observed in the uncomplexed state of αβ-QM TCR hinting at a pre-organization of this loop, which could decrease the entropy penalty upon ligation, thereby contributing to the increase in affinity when comparing β-SM TCR with αβ-QM TCR. Opposedly, mutations in β-CDR2 of the αβ-QM TCR made this region of the variant less sensitive to the presence of pMHC (e.g. β-chain peptide 42-52, grey vs. red curve) giving rise to a little to no effects on the β-CDR2 configuration of αβ-QM TCR upon ligation when compared to the ligated β-SM TCR. The measured HDX in β-CDR3 was unchanged between the αβ-QM and β-SM TCR consistent with the sequence conservation for these contact residues. Similarly, no difference in the HDX behavior was detected for the shared α-CDR2 region between the β-QM- and β-SM TCR. However, the mutations in β-CDR2 of β-QM TCR inferred an additional stabilization of this TCR segment upon complex formation at the earliest measured time-point (0.25 min) (e.g. β-chain peptide 42-50) as compared to the corresponding non-mutated region of β-SM TCR.
Figure 2. Impact of mutations in CDR loop structures on TCR recognition of pMHC. (a) Crystal structure (pdb entry 2BNR) of the 1G4 TCR (grey) in complex with the HLA-A*0201 scaffold (teal) and the NY-ESO-1157-165 peptide antigen (yellow). The TCR-pMHC interface is shown with corresponding TCR and MHC structures being only partially depicted. Differential HDX results for the β-SM TCR (i.e., in the presence and absence of cognate pMHC) are mapped onto the TCR crystal structure. TCR segments that displayed perturbed HDX upon pMHC binding (i.e., α-CDR1, α-CDR2, β-CDR2, β-CDR3, and/or neighboring regions) are colored in red in the 2BNR structure. Notably, pMHC-induced shifts in HDX along the TCR
backbone were confined to both variable domains. (b) Tabular overview of HDX results for individual affinity-enhanced TCR variants and different CDR loop structures. Each column and row contains deuterium uptake plots for a specific CDR region and TCR variant, respectively. Red and grey curves illustrate the time-resolved (0.25-60 min) HDX of the TCR in the presence and absence of cognate pMHC, respectively. The presented uptake plots are based on a single HDX-MS analysis but effects where reproducible in control replicate measurements. The presence of an introduced mutation in a given peptide is indicated for each TCR by the term “mutant”. Peptides that displayed exchange kinetics compatible with an EX1 regime are marked as “EX1”.

Most importantly, a comparison of mass spectra for β-CDR3 peptides (e.g. β-chain peptide 94-101) revealed that the β-chain A97L replacement (β-SM- and QM-TCR) induced a pMHC-dependent shift in the general exchange kinetics (EX1 vs. EX2 exchange regime). That is, the average mass of the peptide comprising the non-mutated β-CDR3 loop in the β-QM TCR increased gradually upon labeling of the TCR-pMHC complex (i.e., EX2 exchange regime) as indicated by a bell-shaped isotopic envelope (Figure 3b). In contrast, TCR variants bearing the β-chain A97L mutation (β-SM and αβ-QM TCR) followed an EX1-like, non-gradual increase in average mass in the presence of pMHC with characteristic broadening of the isotopic envelope (due to bimodality) at the earliest measured time-point (Figure 3a). Notably, bimodal isotopic envelopes were only observed in the presence of cognate pMHC signifying that the β-CDR3 loop structure of the β-SM and αβ-QM TCR assumed two coexisting and slowly exchanging configurations under these solution-phase conditions (an interpretation of the functional significance of such EX1 kinetics are described in more detail below and in the Discussion section).
Figure 3. Mutation-induced slowing of pMHC binding kinetics. (a) Representative mass spectra for the αβ-QM TCR in the absence (left) and presence (right) of cognate pMHC are depicted for different labeling time-points. The mass spectra signify the time-dependent mass increase due to deuterium incorporation into peptide 94-101, which covers the modified β-chain CDR3 loop structure (i.e., YVGLAGEL). In the unbound TCR state, this particular receptor segment of the αβ-QM TCR exchanged rapidly and followed an EX2 regime as indicated by bell-shaped isotopic envelopes. In the presence of cognate pMHC, however, the peptide signal at the 0.25 min time-point is best described by a bimodal isotopic distribution indicating that the β-CDR3 loop coexists in a protected (blue population) and a rather unprotected (red population) solution-phase configuration. The observation of EX1 kinetics in β-CDR3 is furthermore consistent with a mutation-induced, local slowing of pMHC binding kinetics (i.e., the $k_{on}$ rate constant). (b) Representative mass spectra for the β-QM TCR in the presence of cognate pMHC are shown. The mass spectra depict the time-dependent mass increase due to deuterium incorporation into peptide 95-101, which corresponds to the unmodified β-chain CDR3 loop structure (i.e., VGAAGEL). Notably, we observed a pronounced reduction in HDX at the earliest measured time-point together with an unimodal isotopic envelope. All spectra shown in (a) and (b) were computationally deconvoluted using the HX Express 2.0 software with standard settings.

Comparing the Conformational Dynamics of Unrelated TCRs in Solution — Evaluating the local HDX behavior along the protein backbone generally provides a sensitive and direct measure of the conformational dynamics of the target protein in solution. To explore the potential diversity in the intrinsic conformational dynamics among unrelated TCRs, we compared the local HDX characteristics of individual receptors in their unbound state. For this purpose, we compared the time-resolved HDX
(0.25–60 min) of the NY-ESO-1-specific TCR variants (cf. Table 1; HDX results presented in Figure 2b and Figure S4, S5 and S6, grey curves) to the results obtained for an unrelated TCR targeting the clinically relevant HLA-A*0201/MART-1\textsubscript{26-35} peptide complex. Inspection of the sequences of these two TCRs revealed a 70.5% and 89.2% sequence conservation for the α- and β-chain, respectively, between β-SM TCR (NY-ESO-1 specific) and the MART-1-specific TCR. Thus, the distinct specificity of these two TCRs for either the NY-ESO-1 or MART-1 originates mainly from sequence differences in the CDRs as well as in non-CDR regions of the V\textsubscript{α} domain. Notably, the constant domains of the α- and β-chain does not differ in sequence between these unrelated TCRs. Online pepsin proteolysis of the MART-1-specific TCR resulted in 45 identified peptides for which HDX was monitored, covering approximately 75% of both TCR chains (Figure S7). Differential HDX-MS analysis of the MART-1 TCR in the presence and absence of a molar excess of cognate pMHC indicated that the receptor is biologically functional and capable of binding pMHC through its CDR loops (α-CDR2, β-CDR2, and β-CDR3) as indicated by reductions in HDX (Figure S8). Notably, differential HDX results for the α-chain, however, markedly differed between the unrelated NY-ESO-1 and MART-1 TCRs upon formation of the TCR-pMHC complex. In contrast to NY-ESO-1 specific BC1 TCR variants (i.e., β-SM and β-QM TCR), a clear reduction in HDX in the α-CDR2 loop (peptide 48-62, Figure S8) of the MART-1-specific TCR was observed upon binding of cognate pMHC, indicating an important role of this CDR loop in the MART-1 TCR upon binding its cognate pMHC.

The earliest measured time-point most accurately sampled the HDX of fast exchanging, non-hydrogen bonded amide hydrogens\textsuperscript{46,47} and the observed deuterium incorporation after 0.25 min of labeling for all TCRs studied here generally correlated well with the expected TCR structural fold. That is, the structured immunoglobulin domains of the α- and β-chains of both TCRs exhibited partial protection from HDX
consistent with engagement of backbone amide hydrogens in intramolecular hydrogen bonds (Figure S4, S5, S6 and S8). One exception for both the MART-1-specific TCR and all variant NY-ESO-1 TCRs, was observed in the Cα domain, in which individual β-strands (Cα C- and F-strand, e.g. β-SM TCR α-chain peptides 143-157 and 192-201) displayed high HDX rates signifying a pronounced dynamic behavior in solution.

**Figure 4.** Slow and concerted solution-phase dynamics in the α-chain of the MART-1 specific TCR. Crystal structure (pdb entry 3HG1) of a TRAV12-2 gene-encoded TCR directed against the MART-126-35 antigen is shown. The MART-1 peptide antigen and the HLA-A*0201 scaffold also present in the 3HG1 structure are omitted from the figure for clarity. TCR regions
that exchanged according to an EX1 regime are indicated in orange color in the crystal structure. We repeatedly observed bimodal isotopic envelopes upon deuteration for individual peptides of both the Vα (peptides 1-22 and 76-87) and the Cα domain (peptide 124-138) of the MART-1-specific TCR. Affected regions encompassed individual β-strands and loop structures of the immunoglobulin-like TCR domains. Representative mass spectra for MART-1-specific TCR peptides displaying such bimodal isotopic envelopes are shown. The mass spectra illustrate the time-dependent mass increase due to deuterium incorporation into individual α-chain segments of the MART-1-specific TCR. Isotopic envelopes can be computationally resolved to a low-mass (blue) and high-mass (red) population indicating that the corresponding TCR segments coexist in at least two distinct solution-phase configurations that interconvert on a slow time-scale. Notably, all peptide mass spectra presented in this figure were obtained for the MART-1-specific TCR in the absence of cognate pMHC.

At longer exchange times, however, substantially dissimilar HDX kinetics in the α-chains of NY-ESO-1 and MART-1-specific TCRs became apparent. By comparison of individual mass spectra for the uncomplexed NY-ESO-1 and MART-1 receptors following prolonged HDX, discrete Vα (α-chain peptides 1-22 and 76-87) and Cα (α-chain peptide 124-138) segments of the MART-1-specific TCR was observed to undergo slow and cooperative unfolding/refolding motions in solution. The corresponding mass spectra of the labeled peptides exhibited all hallmarks of an EX1 exchange regime including the characteristic bimodal isotopic envelopes (Figure 4). These bimodal distributions can be resolved computationally to a low- (blue curve) and a high-mass (red curve) population as illustrated in Figure 4 for α-chain peptides 1-22 and 124-138, indicating that these segments coexist in at least two distinct solution-phase configurations that interconvert on a slow time-scale. The individual residues of the MART-1 TCR segments that underwent slow localized unfolding/refolding form two spatially neighboring β-strands (β-strands B and F) and a short helical stretch in the Vα domain as well as the AB loop and adjacent β-strands A and B of the Cα region. These unusual slow dynamics in the MART-1 TCR were observed in several independent measurements (n=3) and were also evident when the receptor was labeled in the presence of a molar excess of cognate pMHC (data not shown). Strikingly, TCR variants specific for the NY-ESO-1 peptide did not exhibit comparable dynamics in the TCR α-chain (Figure 5). In fact, individual peptides covering the respective β-strands and interconnecting loop structures in the Vα and Cα domains of the NY-ESO-1-specific TCR variants exchanged according to an
EX2 exchange regime displaying bell-shaped isotopic envelopes upon deuteration. This observation was surprising considering that the unrelated NY-ESO-1 and MART-1 TCRs have differences in their Vα (44% sequence identity) while sharing an identical Cα domain sequence. Thus, these findings suggested that the Vα and Cα domain in the MART-1 TCR are dynamically coupled. That is, sequence-related variations in conformational dynamics in the Vα domain appeared to be propagated to discrete structural motifs (e.g. AB loop) in the spatially distant constant domain.

Figure 5. Differences in intrinsic conformational dynamics of unrelated TCRs. (a) Sequence alignment of TCRs directed against either the NY-ESO-1 or MART-1 antigen is shown. The compared TCR sequences correspond to the Vα (residues 70-90, individual β-strands preceding the α-CDR3 loop) and Cα domain (residues 120-140, β-strands A/B and AB loop). Asterisks indicate shared residues between these unrelated TCRs. Orange colored residues specify protein segments undergoing EX1 exchange in the TRAV12-2 gene-encoded TCR specific for MART-1. (b) Representative mass spectra for
Individual peptides covering α-chain residues 76-87 and 124-138 are depicted for TCRs directed against either the NY-ESO-1 or MART-1 antigen. Individual TCR segments of the NY-ESO-1 TCR exchanged gradually as indicated by bell-shaped isotopic envelopes at the 1 min time-point. The corresponding MART-1 TCR segments in the Vα and Cα domain, however, exchanged according to an EX1 regime with peptide mass spectra exhibiting characteristic bimodal isotopic envelopes upon labeling. Thus, Vα regions (e.g. peptide 76-87) displaying EX1 exchange in the MART-1-specific TCR appear to undergo slow and cooperative unfolding/refolding motions that expose the amide groups of the TCR backbone facilitating correlated exchange. These unusual Vα domain dynamics furthermore seem to be propagated to the Cα domain (e.g. peptide 124-138).

DISCUSSION

Continuing efforts on elucidating the fundamental principles underlying TCR-mediated recognition of pMHC have paved the way for the development of novel, T cell-based therapies with promising clinical outcomes for a number of malignant and infectious diseases. Based on structural insights on the TCR-pMHC interaction, structure-based modeling represents a broadly applicable strategy to generate mutated TCRs with customized specificity and affinity for a disease-related antigen in silico. Affinity enhancement is often achieved by selectively altering the amino acid composition in CDR loop structures such that favorable intermolecular contacts are formed in the TCR-pMHC interface while target specificity is maintained. TCRs displaying such optimized binding characteristics can be expressed on the cell surface of T cells by employing in vitro technologies and the potentially changed effector function of the modified T cells assessed in biological assays. In solution studies, in which the molecular origin of increased binding affinity for genetically modified TCRs is re-evaluated, are rarely conducted. Furthermore, while a few other studies have been reported, an assessment of the role of conformational dynamics in TCR-pMHC complex formation, by experimental measurements, has not received widespread attention. Here, we performed reciprocal HDX-MS analyses on genetically engineered BC1 TCR variants (cf. Table 1) and cognate pMHC (i.e., HLA-A*0201/NY-ESO-1157-165) in solution with the aim to better understand the impact of conformational dynamics on complex formation and to revisit the molecular basis for affinity enhancement in these closely related, affinity-enhanced receptors. We
reasoned that amino acid substitutions in distinct TCR CDR loops potentially give rise to an altered TCR binding mode and that the accompanying changes in conformation and dynamics along the binding interface upon pMHC binding, if any, would be reflected in the local HDX-MS data for the individual constituents of the TCR-pMHC complex. It is well established that TCR CDR loops, the peptide, and the MHC scaffold are prone to undergo cooperative structural and dynamical adjustments in the course of pMHC ligation. In particular CDR loops are associated with a high degree of structural plasticity, which allows for the formation of compensatory interactions to the MHC scaffold in an antigen-dependent manner. Despite this structural malleability, critical MHC contact residues involved in TCR recognition of HLA molecules have been identified by site-directed mutagenesis and X-ray crystallography. That is, three MHC class I residues (Arg65, Ala69, and Gln155), commonly referred to as the restriction triad, appear to represent a generic interaction hotspot for the large majority of αβ-TCRs. Individual peptides covering these critical contact residues in the MHC (e.g. peptides 61-82 and 140-156) displayed decreased HDX in the TCR-bound pMHC relative to the unbound state (Figure 1). This observation is consistent with the perception of TCR-induced stabilization of both MHC helices upon binding of the receptor to pMHC. We emphasize that differences in HDX between the unbound and complexed pMHC were mainly confined to the peptide-binding groove and that structural stabilization was most apparent for both MHC α-helices. Overarching comparison of HDX-MS data for the MHC scaffold furthermore indicated that sequence variation in TCR CDR loop structures affected TCR recognition of pMHC on a molecular level consistent with the consensus binding polarity observed in TCR-pMHC complexes. That is, strategic mutations in the TCR α- and β-chain imposed an altered HDX rate in the MHC α2- and α1-helix in the TCR-pMHC complex, respectively (data not shown).
Differential HDX-MS analyses of the TCR variants allowed us to assess the conformational response of each TCR to pMHC ligation and to directly correlate the local HDX characteristics with the changed binding affinity (and binding kinetics) of these receptors. We would generally expect increased binding affinity for individual TCR variants to result in comparatively lowered deuterium uptake in peptides spanning the modified CDR loop structures upon formation of the TCR-pMHC complex.42,71 Surprisingly, strategic mutations in CDR loops affected the HDX of the respective TCR segments in an unequal manner upon pMHC binding (Figure 2). That is, we observed decreased HDX rates for peptides covering the modified α-CDR2 (αβ-QM TCR) and β-CDR2 loops (β-QM TCR) supportive of the notion that sequence variation in these TCR segments led to formation of new intermolecular interactions shielding the amide hydrogens from isotopic exchange. However, amino acid substitutions in the β-CDR2 region of the αβ-QM TCR did not further decrease the HDX rate in this segment when compared to the results for the ligated β-SM TCR with its wild-type β-CDR2 sequence. This may be due to an altered binding mode of the β-CDR2 loop to pMHC (mainly through TCR side-chains interactions) as well as increased flexibility of the engineered loop and/or adjacent structural motifs within the TCR-pMHC complex (β-chain peptide 42-52 also covered the preceding β-strand of the immunoglobulin fold). Furthermore, we cannot rule out the possibility that the performed mutations in the β-CDR2 region affected the binding affinity of the αβ-QM TCR for cognate pMHC in an unfavorable manner. Likewise, deuterium uptake plots for peptides spanning the modified β-CDR3 loop (Figure 2, e.g. β-chain peptide 95-101) did not mirror the enhanced TCR binding properties associated with the β-chain A97L mutation. Manual inspection and comparison of the corresponding peptide mass spectra for individual TCRs revealed a mutation-induced shift in the general exchange kinetics (Figure 3, EX2 vs. EX1 exchange regime). The manifestation of bimodal isotopic envelopes upon deuteration, as observed for all TCR variants bearing the A97L mutation, signifies the coexistence of an exchange-protected and an exchange-
unprotected configurations of the β-CDR3 backbone in the TCR-pMHC complex,\textsuperscript{67–69} which interconvert on a relatively slow time scale (typically several hundreds of milliseconds or more)\textsuperscript{72,73} in solution. Considering that this TCR segment forms a direct part of the TCR-pMHC binding interface, we postulate that the high- and low-mass population correspond to TCR molecules, where the β-CDR3 backbone, which exist in an exchange-protected state when complexed to pMHC, have or have not visited the uncomplexed (exchange-unprotected) configuration, respectively.\textsuperscript{74} The bimodal mass spectra furthermore imply that the pMHC binding kinetics for the β-CDR3 loop were considerably slowed-down upon mutation (\textit{i.e.}, observation of EX1 kinetics during binding signifies that the chemical exchange rate \(k_{\text{ch}}\) exceeds the association rate constant \(k_{\text{on}}\)).\textsuperscript{74} We hypothesize that slowed pMHC binding kinetics is indicative of a changed TCR binding mode, in which the engineered β-CDR3 loop engages pMHC via a slow induced-fit type of binding in accordance with the previously proposed two-step binding model for TCR recognition of pMHC.\textsuperscript{34} Intriguingly, a possibly similar binding mechanism has been proposed for the murine 2C TCR, which recognizes cognate pMHC (\textit{i.e.}, QL9/H-2L\textsuperscript{d} complex) by matching the β-CDR3 loop and QL9 peptide molecular flexibility within the TCR-pMHC complex.\textsuperscript{42} Thus, the HDX-MS technique may allow, in some instances, a spatially resolved evaluation of mutation-induced changes in pMHC binding kinetics as opposed to commonly employed global measurements of the \(k_{\text{on}}\) rate constant by surface plasmon resonance spectroscopy.

In addition to the reciprocal HDX-MS analyses encompassing affinity-enhanced BC1 TCR variants and cognate HLA-A*0201/NY-ESO-1\textsubscript{157-165}, we also measured the solution-phase HDX of an unrelated TCR (TRAV12-2-TRBV6-1) in the presence and absence of cognate pMHC (HLA-A*0201/MART-1\textsubscript{126-35}). The MART-1 protein is expressed by virtually all malignant melanoma tumors and thus represents a favorable target in immunotherapy of skin cancer.\textsuperscript{75–77} Initial attempts to selectively target the tumor-
related MART-127-35 immunodominant HLA-A*0201-restricted melanoma epitope, by adoptive T cell therapy showed promising clinical results with cancer regression reported for individual patients suffering from metastatic melanoma.\textsuperscript{25,26} Interestingly, naturally occurring CD8\textsuperscript{+} T cell-mediated immune responses against the MART-126-35 epitope are biased towards TRAV12-2 gene usage. That is, almost 90\% of CD8\textsuperscript{+} T cells specific for the MART-126-35 peptide express TCRs containing a TRAV12-2 gene-encoded α-chain.\textsuperscript{78} The unusual binding mode associated with these MART-126-35-specific TCRs, which allows “innate-style” recognition of the MART-1 antigen through germ line-encoded α-CDR1 and α-CDR2 loops,\textsuperscript{39,78,79} may provide a molecular explanation for the TRAV12-2 gene bias in corresponding T cells. Thus, measuring the differential HDX of a MART-126-35-specific TCR comprising a TRAV12-2 gene-encoded α-chain allowed us to (i) examine the molecular basis governing antigen recognition for this clinically-relevant TCR, (ii) to compare the conformational responses of unrelated TCRs directed against either the MART-1 or NY-ESO-1 antigen upon pMHC binding, and (iii) to assess potential differences in the intrinsic solution-phase dynamics of individual TCRs in their unbound state. As illustrated in Figure S8, binding of cognate pMHC led to structural stabilization in distinct CDR loop structures of the α- and β-chain of the MART-1-specific TCR. Our findings are consistent with the view that the MART-1-specific TCR interacts with cognate pMHC through α-CDR2, β-CDR2, and β-CDR3 segments. Notably, no HDX information could be retrieved for the α-CDR1 loop of the MART-1-specific TCR. The observed protection pattern for the TCR β-chain upon pMHC binding is thus highly similar between individual TCRs directed against either the NY-ESO-1 or MART-1 peptide antigen (compare Figure 2a with Figure S8). Differential HDX results for the α-chain, however, markedly differed between these unrelated TCRs upon formation of the TCR-pMHC complex. Our findings clearly support a critical role of the α-CDR2 loop of the MART-1-specific TCR in binding of cognate pMHC, whereas the same region in BC1 TCR variants (\textit{i.e.}, β-SM and β-QM TCR) did not display perturbed HDX in the TCR-
pMHC complex within the sampled time-frame. The reduced HDX rate and thus significant involvement in binding of the α-CDR2 segment of the MART-1-specific TCR thus appears to correlate with the uncommon TCR binding mode associated with the TRAV12-2 gene-encoded α-chain. Strikingly, we furthermore noted that discrete regions in the Vα and Cα region of the MART-1-specific TCR exhibited slow and correlated unfolding/refolding motions in solution (Figure 4). Cooperative, local unfolding events in the outer β-strands of the TCR Vα domain have, to our knowledge, not been described in the literature. TCR segments displaying EX1 kinetics were located remote from the TCR-pMHC binding interface encompassing individual loop structures and β-strands of the immunoglobulin-like domains. We emphasize that correlated exchange kinetics were pMHC-independent and thus were repeatedly observed for the MART-1-specific TCR in the unbound and bound solution-phase states. It is tempting to speculate that these long-lived fluctuations in the higher-order structure of the Vα domain represent a prerequisite for the MART-1-specific TCR to recognize cognate pMHC. That is, MART-1 TCR segments displaying EX1 kinetics are in close spatial proximity to the α-CDR1 and α-CDR3 loop. Conformational changes in these Vα regions potentially mediate global effects on the binding interface of the TCR α-chain and consequently impact ligand specificity in an indirect manner. The notion that residues remote from the TCR-pMHC interface have the potential to modulate TCR specificity has been experimentally established. Thus, our findings of the hitherto unrecognized structural malleability of the Vα immunoglobulin core could provide a molecular explanation for the TRAV12-2 gene-bias in T cells directed against the immunodominant MART-126-35 antigen and furthermore potentially exemplify an additional general mechanism implicated in ligand discrimination and TCR cross-reactivity. Remarkably, the unusual Vα domain dynamics in the MART-1-specific TCR appeared to be propagated to the AB loop and adjacent β-strand B of the Cα region. The Cα AB loop has been suggested to sample distinct conformational states in solution and is suspected to play a pivotal role in early signal
transduction events\textsuperscript{70,82} as well as in TCR dimerization processes.\textsuperscript{83} We again emphasize that binding of the MART-1-specific TCR to cognate pMHC did not alter the HDX behavior in this particular segment such that a direct correlation between pMHC ligation and structural transitions in the C\textalpha{} domain cannot be established, however residues close by did show an impact of pMHC binding e.g. segment 112-123. A dynamic coupling between the V\textalpha{} and C\textalpha{} domain as well as potential mechanistic implications of changed dynamics in the membrane-proximal C\textalpha{} region for TCR functioning remain to be further elucidated.

We note that an earlier study, Hawse et al. used HDX-MS to study the binding of two unrelated TCRs (A6 and DMF5) to their cognate pMHCs.\textsuperscript{49} The authors observed a surprising “global rigidification” throughout both TCRs, with reductions in HDX in almost all TCR peptides derived from either the variable or constant domains. Our results are, however, in some contrast to these findings, as we observed that most of the variable and constant domains in the NY-ESO-1 and MART-1 specific TCRs are unperturbed by the binding of cognate pMHCs, with pMHC-induced reductions in HDX being confined to the CDR loop regions of these two TCRs. While this could hint at a remarkable diversity in the conformational response of different TCRs to pMHC binding, significant differences in how HDX-MS analyses were conducted in the earlier study and present study exist which could also explain this discrepancy.\textsuperscript{66} These include the use of MALDI-MS vs. ESI-MS in our study and our use of LC-MS/MS performed under identical LC conditions to the HDX-MS experiment to optimize the accuracy of identification of the actual peptic peptides being monitored during HDX.

In summary, we performed reciprocal HDX-MS analyses on individual BC1 TCR variants and clinically relevant NY-ESO-1\textsubscript{157-165}-loaded HLA-A*0201 in order to examine the molecular basis of increased binding affinity for these genetically modified TCRs. Our results demonstrate that the HDX-MS
technique allows for detection of subtle, mutation-induced differences in conformational responses of each binding partner to TCR-pMHC complex formation on a peptide-length resolution. Overall, we observe distinct reductions in conformational dynamics (reduced HDX) upon TCR-pMHC complex formation, confined to the CDR loops of both unrelated TCR types studied, as well as the MHC regions encompassing the peptide-binding groove including previously reported critical MHC contact residues. In most instances, the measured differential HDX for peptides covering the modified CDR loop structures of affinity-enhanced TCRs reflected the increased TCR binding affinity and provided new mechanistic insights into the TCR-pMHC interaction. We envisage that pronounced shifts in general exchange kinetics, as observed for TCR variants having the β-chain A97L mutation (β-SM- and αβ-QM TCR), may relate to an improved induced-fit binding mode and thus could serve as diagnostic markers for increased binding affinity in the course of TCR engineering. Our HDX measurements encompassing a TRAV12-2 gene-encoded TCR directed against the immunodominant MART-1_26-35 epitope further support these arguments. In addition, we unexpectedly noted a substantial diversity in intrinsic conformational dynamics across both α-chain domains between the investigated TCRs. The relatively slow, concerted, and long-lived structural perturbations observed in the Vα domain of the MART-1-specific TCR could provide a molecular explanation why corresponding T cells are biased towards TRAV12-2 gene usage.

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**Author Contributions**

KDR and OM conceived and supervised the work. PSM, SH, ET and KDR performed and analyzed data from the HDX experiments. MI produced recombinant proteins. MI, VZ, IL, MF, TJDJ, and MAC contributed with equipment, reagents, and knowhow. PSM and KDR wrote the manuscript with input and comments from all authors.

**Notes**

The authors declare that they have no conflicts of interest with the contents of this article.

**SUPPORTING INFORMATION**

Effective sequence coverage map of pMHC (HLA-A*0201 bound to cancer testis antigen peptide NY-ESO-1\textsubscript{157-165}); HDX of pMHC (HLA-A*0201 bound to cancer testis antigen peptide NY-ESO-1\textsubscript{157-165}) in absence and presence β-SM TCR; Effective sequence coverage map of β-SM TCR; HDX of β-SM TCR in absence and presence pMHC (HLA-A*0201 bound to cancer testis antigen peptide NY-ESO-1\textsubscript{157-165}); HDX of αβ-QM TCR in absence and presence pMHC (HLA-A*0201 bound to cancer testis antigen peptide NY-ESO-1\textsubscript{157-165}); HDX of β-QM TCR in absence and presence pMHC (HLA-A*0201 bound to cancer testis antigen peptide NY-ESO-1\textsubscript{157-165}); Effective sequence coverage map of the MART-1-specific TCR; HDX of the MART-1-specific TCR in absence and presence of its cognate pMHC (HLA-A*0201 bound to antigen peptide MART-1); Tables of the HDX data described above.
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ACCESSION IDS

The data described in the current article contain experimental studies of multiple proteins. However, all the proteins are recombinant expressed and are as such synthetic proteins without correlating uniprot IDs.

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