***Supplementary Information***

Computational Designing the Ligands of Protein L Afﬁnity Chromatography Based on Molecular Docking and Molecular Dynamics Simulations

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Table S1. The number of Na and Cl ions of MDs trajectories

|  |  |  |
| --- | --- | --- |
| **Proteins and complexes** | **Na** | **Cl** |
| 6B0 | 192 | 156 |
| 6B1 | 192 | 156 |
| 6B2 | 192 | 156 |
| WB | 45 | 45 |
| MB1 | 45 | 45 |
| MB2 | 45 | 45 |
| MB3 | 45 | 45 |
| MB4 | 45 | 47 |
| MB5 | 45 | 47 |
| MB6 | 45 | 45 |
| MB7 | 45 | 48 |

Table S2. A list of putative interacting residues in the protein-protein interface for B domain and Fab proteins using LigPlot software

|  |  |
| --- | --- |
| B domain interface | Fab fragment interface |
| Phe24, Glu25, Thr28, Ala29, Tyr32, Arg33, Asp36, Tyr45, Thr46, Ala47, Asp48, Leu49, Glu50, Gly52 | Ser7, Pro8, Ser10, Leu11, Ser12, Ala13, Asp17, Arg18, Thr20, Arg24, Ser65, Thr72, Thr74, Lys107 |

**Results:**

Table S3. Comparison of protein structure validation scores before and after MDs of target protein from PROCHEK, ProSA and MolProbility web servers

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Web servers | 6B0 (B MD) | 6B0 (A MD) | 6B1 (B MD) | 6B1 (A MD) | 6B2 (B MD) | 6B2 (A MD) |
| PROCHECK Favored region (%) | 50 | 84.7 | 50 | 83.9 | 50 | 85.4 |
| PROCHECK Additional allowed region (%) | 50 | 14.6 | 50 | 15.1 | 50 | 13.0 |
| PROCHECK Generously allowed region (%) | 0.0 | 0.5 | 0.0 | 0.3 | 0.0 | 0.5 |
| PROCHECK Disallowed region (%) | 0.0 | 0.3 | 2.2 | 0.8 | 0.4 | 1.1 |
| ProSA (Z‑score) | -4.55 | -4.67 | -4.28 | -4.93 | -4.52 | -4.67 |
| MolProbility score | 3.19 | 2.01 | 3.17 | 1.88 | 3.24 | 1.62 |

The modeled protein structure is considered to be a good model when: 1) favored region >98%, allowed region <2.0%, outlier~0%; 2) Value of Z‑score (in ProSA) is between -15 and 10; 3) value of MolProbility score should be <3. B MD: before MD, A MD: after MD.

Table S4. (A-P) Comparison of interactions including closest residues, distance, hydrogen bond, salt bridge, clash, surface complementarity and buried SASA between B domains and Fab before and after MDs using Schrodinger software. Chains C and E in complexes before MDs are Fab and B domains, respectively. Chains A and B in complexes after MDs are Fab and B domains, respectively.

(A) Complex of mutant MB1 (T865W) and Fab before MDs

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Residue** | **Closest** | **Distance** | **Specific Interactions** | **Surface Complementarity** | **Buried SASA** |
| C:7:Ser | E:855:Asp E:864:Tyr | 2.9 A  3.7 A | 1x hb to E:855:Asp | 0.69 | 0.885 |
| C:8:Pro | E:851:Tyr E:864:Tyr | 3.6 A  3.8 A |  | 0.74 | 0.966 |
| C:10:Ser | E:865:Trp E:866:Ala | 1.0 A  3.4 A | 1x hb to E:866:Ala | 0.28 | 0.978 |
| C:11:Leu | E:866:Ala E:865:Trp E:851:Tyr E:868:Leu | 3.0 A  3.1 A  3.4 A  3.5 A | 1x clash to E:866:Ala | 0.88 | 0.999 |
| C:12:Ser | E:868:Leu E:866:Ala E:867:Asp | 2.6 A  2.9 A  3.1 A | 1x hb to E:866:Ala 1x hb to E:868:Leu | 0.78 | 0.662 |
| C:13:Ala | E:868:Leu | 3.9 A | \_ | 0.89 | 1 |
| C:14:Ser | \_ | \_ | \_ | 0.44 | 0.065 |
| C:17:Asp | E:868:Leu E:871:Gly E:872:Gly | 3.4 A  3.7 A  3.9 A | \_ | 0.89 | 0.895 |
| C:18:Arg | E:871:Gly E:843:Phe E:868:Leu | 3.1 A  3.2 A  3.8 A | 1x hb to E:871:Gly | 0.86 | 0.513 |
| C:19:Val |  |  | \_ | 0.85 | 0.85 |
| C:20:Thr | E:851:Tyr E:844:Glu E:843:Phe E:847:Thr | 3.5 A  3.6 A  3.6 A  3.8 A | \_ | 0.77 | 0.987 |
| C:21:Ile |  |  | \_ | 0.29 | 0.339 |
| C:22:Thr |  |  | \_ | 0.47 | 0.925 |
| C:24:Arg | E:855:Asp E:852:Arg | 2.7 A  3.4 A | \_ | 0.62 | 0.493 |
| C:65:Ser | E:844:Glu | 3.2 A | 1x hb to E:844:Glu | 0 | 0.378 |
| C:72:Thr | E:848:Ala | 3.6 A | \_ | 0.61 | 0.729 |
| C:74:Thr | E:844:Glu | 3.5 A | \_ | 0.79 | 0.657 |
| C:107:Lys | E:867:Asp E:868:Leu E:869:Glu | 2.6 A  2.9 A  3.8 A | 1x clash to E:867:Asp 1x hb to E:868:Leu | 0.7 | 0.586 |
| C:143:Glu | E:878:Lys | 2.8 A | 1x salt bridge to E:878:Lys | 0 | 0.242 |
| E:843:Phe | C:18:Arg C:20:Thr | 3.2 A  3.6 A |  | 0.87 | 0.924 |
| E:844:Glu | C:65:Ser C:74:Thr C:20:Thr | 3.2 A  3.5 A  3.6 A | 1x hb to C:65:Ser | 0.87 | 0.655 |
| E:847:Thr | C:20:Thr | 3.8 A | \_ | 0.78 | 0.786 |
| E:848:Ala | C:72:Thr | 3.6 A | \_ | 0.48 | 0.706 |
| E:851:Tyr | C:11:Leu C:20:Thr C:8:Pro | 3.4 A  3.5 A  3.6 A | 1x clash to C:8:Pro | 0.69 | 0.989 |
| E:852:Arg | C:24:Arg C:70:Asp | 3.4 A  4.0 A | \_ | 0.4 | 0.334 |
| E:855:Asp | C:24:Arg C:7:Ser | 2.7 A  2.9 A | 1x hb to C:7:Ser | 0.49 | 0.658 |
| E:864:Tyr | C:7:Ser C:8:Pro | 3.7 A  3.8 A | \_ | 0.69 | 0.733 |
| **E:865:Trp** | C:10:Ser C:11:Leu C:9:Ser | 1.0 A  3.1 A  3.7 A | - | 0.1 | 0.445 |
| E:866:Ala | C:12:Ser C:11:Leu C:10:Ser | 2.9 A  3.0 A  3.4 A | 1x hb to C:10:Ser 1x clash to C:11:Leu 1x hb to C:12:Ser | 0.82 | 0.934 |
| E:867:Asp | C:107:Lys C:12:Ser | 2.6 A  3.1 A | 1x clash to C:107:Lys | 0.83 | 0.541 |
| E:868:Leu | C:12:Ser C:107:Lys C:17:Asp C:11:Leu C:18:Arg C:13:Ala | 2.6 A  2.9 A  3.4 A  3.5 A  3.8 A  3.9 A | 1x hb to C:12:Ser 1x hb to C:107:Lys | 0.84 | 0.958 |
| E:869:Glu | C:107:Lys | 3.8 A | \_ | 0.71 | 0.114 |
| E:871:Gly | C:18:Arg C:17:Asp | 3.1 A  3.7 A | 1x hb to C:18:Arg | 0.86 | 0.518 |
| E:872:Gly | C:17:Asp | 3.9 A | \_ | 0.84 | 0.426 |
| E:878:Lys | C:143:Glu | 2.8 A | 1x salt bridge to C:143:Glu | 0 | 0.343 |

(B) Complex of mutant MB1 (T865W) and Fab after MDs

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Residue** | **Closest** | **Distance** | **Specific Interactions** | **Surface Complementarity** | **Buried SASA** |
| A:7:Ser | B:855:Asp B:864:Tyr | 2.6 A 3.3 A | 1x hb to B:855:Asp | 0.71 | 0.859 |
| A:8:Pro | B:864:Tyr B:851:Tyr | 3.6 A 3.6 A |  | 0.75 | 0.993 |
| A:9:Ser | B:864:Tyr B:863:Glu | 2.7 A 3.6 A | 1x hb to B:864:Tyr | 0.71 | 0.607 |
| A:10:Ser | B:866:Ala B:865:Trp | 3.1 A 3.8 A | 1x hb to B:866:Ala | 0.83 | 0.564 |
| A:11:Leu | B:866:Ala B:851:Tyr | 3.4 A 3.6 A |  | 0.76 | 0.998 |
| A:12:Ser | B:866:Ala B:868:Leu B:867:Asp | 2.8 A 3.0 A 3.2 A | 1x hb to B:866:Ala 1x hb to B:868:Leu | 0.83 | 0.732 |
| A:13:Ala |  |  |  | 0.6 | 1 |
| A:17:Asp | B:871:Gly | 3.4 A |  | 0.63 | 0.836 |
| A:18:Arg | B:844:Glu B:871:Gly B:843:Phe | 2.7 A 3.0 A 3.6 A | 2x hb, 1x salt bridge to B:844:Glu 1x hb to B:871:Gly | 0.7 | 0.603 |
| A:19:Val |  |  |  | 0.74 | 0.824 |
| A:20:Thr | B:844:Glu | 3.4 A |  | 0.54 | 0.987 |
| A:21:Ile |  |  |  | 0.53 | 0.239 |
| A:22:Thr | B:851:Tyr | 3.7 A |  | 0.72 | 0.93 |
| A:24:Arg | B:852:Arg B:848:Ala B:855:Asp | 3.4 A 3.9 A 3.9 A |  | 0.75 | 0.659 |
| A:74:Thr | B:844:Glu | 3.7 A |  | 0.82 | 0.588 |
| A:107:Lys | B:867:Asp B:868:Leu | 2.6 A 3.2 A | 1x hb, 1x salt bridge | 0.79 | 0.516 |
| A:143:Glu | B:878:Lys | 2.7 A | 1x hb, 1x salt bridge to B:878:Lys | 0.61 | 0.564 |
| A:199:Gln | B:878:Lys | 3.4 A |  | 0.92 | 0.192 |
| B:843:Phe | A:18:Arg | 3.6 A |  | 0.84 | 0.609 |
| B:844:Glu | A:18:Arg A:20:Thr A:74:Thr | 2.7 A 3.4 A 3.7 A | 2x hb, 1x salt bridge to A:18:Arg | 0.71 | 0.767 |
| B:847:Thr |  |  |  | 0.52 | 0.925 |
| B:848:Ala | A:24:Arg | 3.9 A |  | 0.61 | 0.802 |
| B:851:Tyr | A:11:Leu A:8:Pro A:22:Thr | 3.6 A 3.6 A 3.7 A |  | 0.7 | 0.999 |
| B:852:Arg | A:24:Arg | 3.4 A |  | 0.78 | 0.22 |
| B:855:Asp | A:7:Ser A:24:Arg | 2.6 A 3.9 A | 1x hb to A:7:Ser | 0.61 | 0.483 |
| B:863:Glu | A:9:Ser | 3.6 A |  | 0.93 | 0.208 |
| B:864:Tyr | A:9:Ser A:7:Ser A:8:Pro | 2.7 A 3.3 A 3.6 A | 1x hb to A:9:Ser | 0.71 | 0.922 |
| **B:865:Trp** | A:10:Ser | 3.8 A |  | 0.79 | 0.438 |
| B:866:Ala | A:12:Ser A:10:Ser A:11:Leu | 2.8 A 3.1 A 3.4 A | 1x hb to A:10:Ser 1x hb to A:12:Ser | 0.84 | 0.987 |
| B:867:Asp | A:107:Lys A:12:Ser | 2.6 A 3.2 A | 1x clash to A:12:Ser 1x hb, 1x salt bridge | 0.84 | 0.675 |
| B:868:Leu | A:12:Ser A:107:Lys | 3.0 A 3.2 A | 1x hb to A:12:Ser | 0.7 | 0.971 |
| B:869:Glu |  |  |  | 0.93 | 0.213 |
| B:871:Gly | A:18:Arg A:17:Asp | 3.0 A 3.4 A | 1x hb to A:18:Arg | 0.78 | 0.657 |
| B:872:Gly |  |  |  | 0.38 | 0.869 |
| B:878:Lys | A:143:Glu A:199:Gln | 2.7 A 3.4 A | 1x hb, 1x salt bridge to A:143:Glu | 0.66 | 0.648 |

(C) Complex of mutant MB2 (847Met-865Trp) and Fab before MDs

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Residue** | **Closest** | **Distance** | **Specific Interactions** | **Surface Complementarity** | **Buried SASA** |
| C:7:Ser | E:855:Asp E:864:Tyr | 2.9 A 3.7 A | 1x hb to E:855:Asp | 0.69 | 0.885 |
| C:8:Pro | E:851:Tyr E:864:Tyr | 3.6 A 3.8 A | 1x clash to E:851:Tyr | 0.74 | 0.966 |
| C:9:Ser | E:865:Trp | 3.7 A |  | 0.3 | 0.367 |
| C:10:Ser | E:865:Trp E:866:Ala | 1.0 A 3.4 A | 39x clash to E:865:Trp 1x hb to E:866:Ala | 0.28 | 0.979 |
| C:11:Leu | E:866:Ala E:865:Trp E:851:Tyr E:868:Leu | 3.0 A 3.1 A 3.4 A 3.5 A | 1x clash to E:865:Trp 1x clash to E:866:Ala 2x clash to E:868:Leu | 0.88 | 0.999 |
| C:12:Ser | E:868:Leu E:866:Ala E:867:Asp | 2.6 A 2.9 A 3.1 A | 1x hb to E:866:Ala 1x clash to E:867:Asp 1x hb to E:868:Leu | 0.78 | 0.661 |
| C:13:Ala | E:868:Leu | 3.9 A |  | 0.89 | 1 |
| C:14:Ser |  |  |  | 0.44 | 0.065 |
| C:17:Asp | E:868:Leu E:871:Gly E:872:Gly | 3.4 A 3.7 A 3.9 A |  | 0.89 | 0.895 |
| C:18:Arg | E:871:Gly E:843:Phe E:868:Leu E:847:Met | 3.1 A 3.2 A 3.8 A 3.9 A | 1x hb to E:871:Gly | 0.86 | 0.516 |
| C:19:Val |  |  |  | 0.85 | 0.849 |
| C:20:Thr | E:851:Tyr E:847:Met E:844:Glu E:843:Phe | 3.5 A 3.5 A 3.6 A 3.6 A |  | 0.73 | 0.975 |
| C:22:Thr |  |  |  | 0.47 | 0.924 |
| C:24:Arg | E:855:Asp E:852:Arg | 2.7 A 3.4 A |  | 0.62 | 0.493 |
| C:65:Ser | E:844:Glu | 3.2 A | 1x hb to E:844:Glu | 0 | 0.378 |
| C:72:Thr | E:848:Ala | 3.6 A |  | 0.61 | 0.734 |
| C:74:Thr | E:844:Glu | 3.5 A |  | 0.79 | 0.65 |
| C:107:Lys | E:867:Asp E:868:Leu E:869:Glu | 2.6 A 2.9 A 3.8 A | 3x clash to E:867:Asp 1x hb to E:868:Leu | 0.7 | 0.586 |
| C:143:Glu | E:878:Lys | 2.8 A | 1x salt bridge to E:878:Lys | 0 | 0.242 |
| E:843:Phe | C:18:Arg C:20:Thr | 3.2 A 3.6 A |  | 0.86 | 0.93 |
| E:844:Glu | C:65:Ser C:74:Thr C:20:Thr | 3.2 A 3.5 A 3.6 A | 1x hb to C:65:Ser | 0.88 | 0.654 |
| E:847:Met | C:20:Thr C:18:Arg | 3.5 A 3.9 A |  | 0.73 | 0.754 |
| E:848:Ala | C:72:Thr | 3.6 A |  | 0.51 | 0.708 |
| E:851:Tyr | C:11:Leu C:20:Thr C:8:Pro | 3.4 A 3.5 A 3.6 A | 1x clash to C:8:Pro | 0.66 | 0.97 |
| E:852:Arg | C:24:Arg C:70:Asp | 3.4 A 4.0 A |  | 0.4 | 0.335 |
| E:855:Asp | C:24:Arg C:7:Ser | 2.7 A 2.9 A | 1x hb to C:7:Ser | 0.49 | 0.658 |
| E:864:Tyr | C:7:Ser C:8:Pro | 3.7 A 3.8 A |  | 0.69 | 0.734 |
| E:865:Trp | C:10:Ser C:11:Leu C:9:Ser | 1.0 A 3.1 A 3.7 A | 39x clash to C:10:Ser 1x clash to C:11:Leu | 0.1 | 0.445 |
| E:866:Ala | C:12:Ser C:11:Leu C:10:Ser | 2.9 A 3.0 A 3.4 A | 1x hb to C:10:Ser 1x clash to C:11:Leu 1x hb to C:12:Ser | 0.82 | 0.934 |
| E:867:Asp | C:107:Lys C:12:Ser | 2.6 A 3.1 A | 1x clash to C:12:Ser 3x clash to C:107:Lys | 0.83 | 0.541 |
| E:868:Leu | C:12:Ser C:107:Lys C:17:Asp C:11:Leu C:18:Arg C:13:Ala | 2.6 A 2.9 A 3.4 A 3.5 A 3.8 A 3.9 A | 2x clash to C:11:Leu 1x hb to C:12:Ser 1x hb to C:107:Lys | 0.85 | 0.944 |
| E:869:Glu | C:107:Lys | 3.8 A |  | 0.71 | 0.114 |
| E:871:Gly | C:18:Arg C:17:Asp | 3.1 A 3.7 A | 1x hb to C:18:Arg | 0.86 | 0.518 |
| E:872:Gly | C:17:Asp | 3.9 A |  | 0.84 | 0.645 |
| E:878:Lys | C:143:Glu | 2.8 A | 1x salt bridge to C:143:Glu | 0 | 0.343 |

(D) Complex of mutant MB2 (847Met-865Trp) after MDs

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Residue** | **Closest** | **Distance** | **Specific Interactions** | **Surface Complementarity** | **Buried SASA** |
| A:7:Ser | B:855:Asp B:864:Tyr | 2.9 A 3.1 A | 1x hb to B:855:Asp | 0.62 | 0.857 |
| A:8:Pro | B:851:Tyr B:864:Tyr | 3.7 A 4.0 A |  | 0.83 | 0.966 |
| A:9:Ser | B:864:Tyr | 3.2 A | 2x hb to B:864:Tyr | 0.81 | 0.619 |
| A:10:Ser | B:866:Ala B:865:Trp | 3.3 A 3.5 A | 1x hb to B:866:Ala | 0.81 | 0.883 |
| A:11:Leu | B:866:Ala B:851:Tyr B:867:Asp | 3.4 A 3.4 A 3.9 A |  | 0.78 | 0.996 |
| A:12:Ser | B:866:Ala B:867:Asp B:868:Leu | 2.9 A 3.0 A 3.3 A | 1x hb to B:866:Ala 1x hb to B:867:Asp 1x hb to B:868:Leu | 0.79 | 0.938 |
| A:18:Arg | B:843:Phe | 3.4 A |  | 0.5 | 0.518 |
| A:19:Val |  |  |  | 0.65 | 0.972 |
| A:20:Thr | B:851:Tyr B:843:Phe B:844:Glu B:847:Met | 2.8 A 3.3 A 3.4 A 3.8 A | 1x hb to B:851:Tyr | 0.8 | 0.997 |
| A:21:Ile |  |  |  | 0.89 | 0.365 |
| A:22:Thr | B:851:Tyr B:848:Ala | 3.4 A 3.7 A |  | 0.83 | 0.779 |
| A:24:Arg | B:855:Asp | 3.3 A |  | 0.88 | 0.252 |
| A:72:Thr |  |  |  | 0.77 | 0.553 |
| A:74:Thr | B:844:Glu | 3.9 A |  | 0.59 | 0.828 |
| A:107:Lys | B:867:Asp B:868:Leu B:869:Glu | 2.8 A 3.3 A 4.0 A | 1x salt bridge, 1x clash to B:867:Asp | 0.85 | 0.527 |
| A:141:Pro | B:865:Trp | 3.8 A |  | 0.64 | 0.573 |
| A:142:Arg |  |  |  | 0.34 | 0.263 |
| A:143:Glu | B:878:Lys B:865:Trp | 2.9 A 3.5 A | 1x hb, 1x salt bridge to B:878:Lys | 0.75 | 0.577 |
| A:199:Gln | B:878:Lys B:829:Phe B:830:Ala | 3.0 A 3.9 A 3.9 A | 1x hb to B:878:Lys | 0.88 | 0.405 |
| B:830:Ala | A:199:Gln | 3.9 A |  | 0.86 | 0.331 |
| B:843:Phe | A:20:Thr A:18:Arg | 3.3 A 3.4 A |  | 0.44 | 0.674 |
| B:844:Glu | A:20:Thr A:74:Thr | 3.4 A 3.9 A |  | 0.78 | 0.63 |
| **B:847:Met** | A:20:Thr | 3.8 A |  | 0.76 | 1 |
| B:848:Ala | A:22:Thr | 3.7 A |  | 0.64 | 0.653 |
| B:851:Tyr | A:20:Thr A:22:Thr A:11:Leu A:8:Pro | 2.8 A 3.4 A 3.4 A 3.7 A | 1x hb to A:20:Thr | 0.79 | 0.989 |
| B:855:Asp | A:7:Ser A:24:Arg | 2.9 A 3.3 A | 1x hb to A:7:Ser | 0.78 | 0.648 |
| B:864:Tyr | A:7:Ser A:9:Ser A:8:Pro | 3.1 A 3.2 A 4.0 A | 2x hb to A:9:Ser | 0.79 | 0.848 |
| **B:865:Trp** | A:143:Glu A:10:Ser A:141:Pro | 3.5 A 3.5 A 3.8 A |  | 0.7 | 0.765 |
| B:866:Ala | A:12:Ser A:10:Ser A:11:Leu | 2.9 A 3.3 A 3.4 A | 1x hb to A:10:Ser 1x hb to A:12:Ser | 0.8 | 1 |
| B:867:Asp | A:107:Lys A:12:Ser A:11:Leu A:140:Tyr | 2.8 A 3.0 A 3.9 A 4.0 A | 1x hb to A:12:Ser 1x salt bridge, 1x clash to A:107:Lys | 0.82 | 0.807 |
| B:868:Leu | A:107:Lys A:12:Ser | 3.3 A 3.3 A | 1x hb to A:12:Ser | 0.64 | 0.982 |
| B:869:Glu | A:107:Lys | 4.0 A |  | 0.86 | 0.178 |
| B:875:Met |  |  |  | 0.46 | 1 |
| B:878:Lys | A:143:Glu A:199:Gln | 2.9 A 3.0 A | 1x hb, 1x salt bridge to A:143:Glu 1x hb to A:199:Gln | 0.84 | 0.683 |

(E) Complex of mutant MB3 (847Met) and Fab before MDs

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Residue** | **Closest** | **Distance** | **Specific Interactions** | **Surface Complementarity** | **Buried SASA** |
| C:7:Ser | E:855:Asp E:864:Tyr | 2.9 A 3.7 A | 1x hb to E:855:Asp | 0.69 | 0.885 |
| C:8:Pro | E:851:Tyr E:864:Tyr | 3.6 A 3.8 A | 1x clash to E:851:Tyr | 0.74 | 0.966 |
| C:10:Ser | E:865:Thr E:866:Ala | 3.2 A 3.4 A | 1x hb to E:866:Ala | 0.66 | 0.502 |
| C:11:Leu | E:866:Ala E:851:Tyr E:868:Leu | 3.0 A 3.4 A 3.5 A | 1x clash to E:866:Ala 2x clash to E:868:Leu | 0.88 | 0.996 |
| C:12:Ser | E:868:Leu E:866:Ala E:867:Asp | 2.6 A 2.9 A 3.1 A | 1x hb to E:866:Ala 1x clash to E:867:Asp 1x hb to E:868:Leu | 0.77 | 0.646 |
| C:13:Ala | E:868:Leu | 3.9 A |  | 0.89 | 1 |
| C:14:Ser |  |  |  | 0.44 | 0.065 |
| C:17:Asp | E:868:Leu E:871:Gly E:872:Gly | 3.4 A 3.7 A 3.9 A |  | 0.89 | 0.895 |
| C:18:Arg | E:871:Gly E:843:Phe E:868:Leu E:847:Met | 3.1 A 3.2 A 3.8 A 3.9 A | 1x hb to E:871:Gly | 0.86 | 0.515 |
| C:19:Val |  |  |  | 0.85 | 0.85 |
| C:20:Thr | E:851:Tyr E:847:Met E:844:Glu E:843:Phe | 3.5 A 3.5 A 3.6 A 3.6 A |  | 0.73 | 0.975 |
| C:21:Ile |  |  |  | 0.29 | 0.33 |
| C:22:Thr |  |  |  | 0.47 | 0.925 |
| C:24:Arg | E:855:Asp E:852:Arg | 2.7 A 3.4 A |  | 0.62 | 0.494 |
| C:65:Ser | E:844:Glu | 3.2 A | 1x hb to E:844:Glu | 0 | 0.378 |
| C:72:Thr | E:848:Ala | 3.6 A |  | 0.61 | 0.729 |
| C:74:Thr | E:844:Glu | 3.5 A |  | 0.79 | 0.654 |
| C:107:Lys | E:867:Asp E:868:Leu E:869:Glu | 2.6 A 2.9 A 3.8 A | 3x clash to E:867:Asp 1x hb to E:868:Leu | 0.7 | 0.586 |
| C:143:Glu | E:878:Lys | 2.8 A | 1x salt bridge to E:878:Lys | 0 | 0.243 |
| E:843:Phe | C:18:Arg C:20:Thr | 3.2 A 3.6 A |  | 0.86 | 0.928 |
| E:844:Glu | C:65:Ser C:74:Thr C:20:Thr | 3.2 A 3.5 A 3.6 A | 1x hb to C:65:Ser | 0.88 | 0.655 |
| **E:847:Met** | C:20:Thr C:18:Arg | 3.5 A 3.9 A |  | 0.73 | 0.751 |
| E:848:Ala | C:72:Thr | 3.6 A |  | 0.51 | 0.707 |
| E:851:Tyr | C:11:Leu C:20:Thr C:8:Pro | 3.4 A 3.5 A 3.6 A | 1x clash to C:8:Pro | 0.66 | 0.97 |
| E:852:Arg | C:24:Arg C:70:Asp | 3.4 A 4.0 A |  | 0.4 | 0.334 |
| E:855:Asp | C:24:Arg C:7:Ser | 2.7 A 2.9 A | 1x hb to C:7:Ser | 0.49 | 0.657 |
| E:864:Tyr | C:7:Ser C:8:Pro | 3.7 A 3.8 A |  | 0.7 | 0.66 |
| E:865:Thr | C:10:Ser | 3.2 A |  | 0.52 | 0.401 |
| E:866:Ala | C:12:Ser C:11:Leu C:10:Ser | 2.9 A 3.0 A 3.4 A | 1x hb to C:10:Ser 1x clash to C:11:Leu 1x hb to C:12:Ser | 0.84 | 1 |
| E:867:Asp | C:107:Lys C:12:Ser | 2.6 A 3.1 A | 1x clash to C:12:Ser 3x clash to C:107:Lys | 0.82 | 0.569 |
| E:868:Leu | C:12:Ser C:107:Lys C:17:Asp C:11:Leu C:18:Arg C:13:Ala | 2.6 A 2.9 A 3.4 A 3.5 A 3.8 A 3.9 A | 2x clash to C:11:Leu 1x hb to C:12:Ser 1x hb to C:107:Lys | 0.85 | 0.944 |
| E:869:Glu | C:107:Lys | 3.8 A |  | 0.71 | 0.114 |
| E:871:Gly | C:18:Arg C:17:Asp | 3.1 A 3.7 A | 1x hb to C:18:Arg | 0.86 | 0.518 |
| E:872:Gly | C:17:Asp | 3.9 A |  | 0.84 | 0.648 |
| E:878:Lys | C:143:Glu | 2.8 A | 1x salt bridge to C:143:Glu | 0 | 0.348 |

(F) Complex of mutant MB3 (847Met) and Fab after MDs

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Residue** | **Closest** | **Distance** | **Specific Interactions** | **Surface Complementarity** | **Buried SASA** |
| A:7:Ser | B:855:Asp B:864:Tyr | 2.6 A 3.2 A | 1x hb to B:855:Asp | 0.81 | 0.887 |
| A:8:Pro | B:851:Tyr B:864:Tyr | 3.6 A 4.0 A |  | 0.77 | 0.996 |
| A:9:Ser | B:864:Tyr B:863:Glu | 3.0 A 3.5 A | 1x hb to B:864:Tyr | 0.85 | 0.628 |
| A:10:Ser | B:866:Ala B:865:Thr | 3.0 A 3.1 A | 1x clash to B:865:Thr 1x hb to B:866:Ala | 0.89 | 0.674 |
| A:11:Leu | B:866:Ala | 3.0 A | 1x clash to B:866:Ala | 0.57 | 0.974 |
| A:12:Ser | B:866:Ala B:868:Leu B:867:Asp | 2.8 A 3.0 A 3.2 A | 1x hb to B:866:Ala 1x hb to B:868:Leu | 0.83 | 0.631 |
| A:13:Ala |  |  |  | 0.7 | 1 |
| A:14:Ser |  |  |  | 0.7 | 0.033 |
| A:18:Arg | B:871:Gly B:868:Leu B:843:Phe | 3.2 A 3.4 A 3.5 A | 1x hb to B:871:Gly | 0.87 | 0.433 |
| A:19:Val |  |  |  | 0.65 | 0.942 |
| A:20:Thr | B:851:Tyr B:847:Met B:843:Phe B:844:Glu | 2.7 A 3.5 A 3.6 A 4.0 A | 1x hb to B:851:Tyr | 0.82 | 1 |
| A:21:Ile |  |  |  | 0.8 | 0.192 |
| A:22:Thr | B:851:Tyr B:848:Ala | 3.3 A 3.9 A |  | 0.82 | 0.781 |
| A:65:Ser | B:844:Glu | 2.8 A | 1x hb to B:844:Glu | 0 | 0.407 |
| A:70:Asp | B:852:Arg | 2.9 A | 1x hb, 1x salt bridge to B:852:Arg | 0 | 0.417 |
| A:72:Thr | B:848:Ala | 4.0 A |  | 0.56 | 0.702 |
| A:74:Thr | B:844:Glu | 3.7 A |  | 0.82 | 0.698 |
| A:107:Lys | B:867:Asp B:868:Leu | 2.6 A 3.0 A | 1x hb, 1x salt bridge, 1x clash to B:867:Asp 1x hb to B:868:Leu | 0.74 | 0.515 |
| B:843:Phe | A:18:Arg A:20:Thr | 3.5 A 3.6 A |  | 0.9 | 0.765 |
| B:844:Glu | A:65:Ser A:74:Thr A:20:Thr | 2.8 A 3.7 A 4.0 A | 1x hb to A:65:Ser | 0.85 | 0.595 |
| **B:847:Met** | A:20:Thr | 3.5 A |  | 0.8 | 1 |
| B:848:Ala | A:22:Thr A:72:Thr | 3.9 A 4.0 A |  | 0.42 | 0.79 |
| B:851:Tyr | A:20:Thr A:22:Thr A:8:Pro | 2.7 A 3.3 A 3.6 A | 1x hb to A:20:Thr | 0.78 | 0.992 |
| B:852:Arg | A:70:Asp A:24:Arg | 2.9 A 3.7 A | 1x hb, 1x salt bridge to A:70:Asp | 0 | 0.409 |
| B:855:Asp | A:7:Ser | 2.6 A | 1x hb to A:7:Ser | 0.81 | 0.301 |
| B:863:Glu | A:9:Ser | 3.5 A |  | 0.88 | 0.391 |
| B:864:Tyr | A:9:Ser A:7:Ser A:8:Pro | 3.0 A 3.2 A 4.0 A | 1x hb to A:9:Ser | 0.85 | 0.953 |
| B:865:Thr | A:10:Ser | 3.1 A | 1x clash to A:10:Ser | 0.89 | 0.486 |
| B:866:Ala | A:12:Ser A:10:Ser A:11:Leu | 2.8 A 3.0 A 3.0 A | 1x hb to A:10:Ser 1x clash to A:11:Leu 1x hb to A:12:Ser | 0.64 | 0.978 |
| B:867:Asp | A:107:Lys A:12:Ser | 2.6 A 3.2 A | 1x hb, 1x salt bridge, 1x clash to A:107:Lys | 0.88 | 0.626 |
| B:868:Leu | A:12:Ser A:107:Lys A:18:Arg | 3.0 A 3.0 A 3.4 A | 1x hb to A:12:Ser 1x hb to A:107:Lys | 0.59 | 0.998 |
| B:871:Gly | A:18:Arg A:17:Asp | 3.2 A 3.3 A | 1x hb to A:18:Arg | 0.77 | 0.674 |
| B:872:Gly |  |  |  | 0.42 | 1 |

(G) Complex of mutant MB4 (848His-865Arg) and Fab before MDs

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Residue** | **Closest** | **Distance** | **Specific Interactions** | **Surface Complementarity** | **Buried SASA** |
| C:7:Ser | E:855:Asp E:864:Tyr | 2.9 A 3.7 A | 1x hb to E:855:Asp | 0.69 | 0.885 |
| C:8:Pro | E:851:Tyr E:864:Tyr | 3.6 A 3.8 A | 1x clash to E:851:Tyr | 0.74 | 0.966 |
| C:10:Ser | E:865:Arg E:866:Ala | 3.1 A 3.4 A | 1x hb to E:866:Ala | 0.68 | 0.589 |
| C:11:Leu | E:866:Ala E:851:Tyr E:868:Leu | 3.0 A 3.4 A 3.5 A | 1x clash to E:866:Ala 2x clash to E:868:Leu | 0.89 | 0.996 |
| C:12:Ser | E:868:Leu E:866:Ala E:867:Asp | 2.6 A 2.9 A 3.1 A | 1x hb to E:866:Ala 1x clash to E:867:Asp 1x hb to E:868:Leu | 0.78 | 0.614 |
| C:13:Ala | E:868:Leu | 3.9 A |  | 0.89 | 1 |
| C:14:Ser |  |  |  | 0.44 | 0.065 |
| C:17:Asp | E:868:Leu E:871:Gly E:872:Gly | 3.4 A 3.7 A 3.9 A |  | 0.89 | 0.895 |
| C:18:Arg | E:871:Gly E:843:Phe E:868:Leu | 3.1 A 3.2 A 3.8 A | 1x hb to E:871:Gly | 0.86 | 0.514 |
| C:19:Val |  |  |  | 0.85 | 0.851 |
| C:20:Thr | E:851:Tyr E:844:Glu E:843:Phe E:847:Thr | 3.5 A 3.6 A 3.6 A 3.8 A |  | 0.76 | 0.987 |
| C:22:Thr |  |  |  | 0.47 | 0.913 |
| C:24:Arg | E:855:Asp E:852:Arg | 2.7 A 3.4 A |  | 0.62 | 0.493 |
| C:65:Ser | E:844:Glu | 3.2 A | 1x hb to E:844:Glu | 0 | 0.378 |
| C:72:Thr | E:848:Hid | 3.6 A |  | 0.72 | 0.863 |
| C:74:Thr | E:844:Glu | 3.5 A |  | 0.79 | 0.656 |
| C:107:Lys | E:867:Asp E:868:Leu E:869:Glu | 2.6 A 2.9 A 3.8 A | 3x clash to E:867:Asp 1x hb to E:868:Leu | 0.7 | 0.586 |
| C:143:Glu | E:878:Lys | 2.8 A | 1x salt bridge to E:878:Lys | 0 | 0.243 |
| E:821:Val |  |  |  | 0 | 0.106 |
| E:843:Phe | C:18:Arg C:20:Thr | 3.2 A 3.6 A |  | 0.87 | 0.924 |
| E:844:Glu | C:65:Ser C:74:Thr C:20:Thr | 3.2 A 3.5 A 3.6 A | 1x hb to C:65:Ser | 0.83 | 0.658 |
| E:847:Thr | C:20:Thr | 3.8 A |  | 0.78 | 0.786 |
| **E:848:His** | C:72:Thr | 3.6 A |  | 0.58 | 0.571 |
| E:851:Tyr | C:11:Leu C:20:Thr C:8:Pro | 3.4 A 3.5 A 3.6 A | 1x clash to C:8:Pro | 0.69 | 0.989 |
| E:852:Arg | C:24:Arg C:70:Asp | 3.4 A 4.0 A |  | 0.4 | 0.336 |
| E:855:Asp | C:24:Arg C:7:Ser | 2.7 A 2.9 A | 1x hb to C:7:Ser | 0.49 | 0.657 |
| E:864:Tyr | C:7:Ser C:8:Pro | 3.7 A 3.8 A |  | 0.68 | 0.745 |
| **E:865:Arg** | C:10:Ser | 3.1 A |  | 0.53 | 0.266 |
| E:866:Ala | C:12:Ser C:11:Leu C:10:Ser | 2.9 A 3.0 A 3.4 A | 1x hb to C:10:Ser 1x clash to C:11:Leu 1x hb to C:12:Ser | 0.83 | 0.92 |
| E:867:Asp | C:107:Lys C:12:Ser | 2.6 A 3.1 A | 1x clash to C:12:Ser 3x clash to C:107:Lys | 0.83 | 0.541 |
| E:868:Leu | C:12:Ser C:107:Lys C:17:Asp C:11:Leu C:18:Arg C:13:Ala | 2.6 A 2.9 A 3.4 A 3.5 A 3.8 A 3.9 A | 2x clash to C:11:Leu 1x hb to C:12:Ser 1x hb to C:107:Lys | 0.84 | 0.958 |
| E:869:Glu | C:107:Lys | 3.8 A |  | 0.71 | 0.114 |
| E:871:Gly | C:18:Arg C:17:Asp | 3.1 A 3.7 A | 1x hb to C:18:Arg | 0.86 | 0.518 |
| E:872:Gly | C:17:Asp | 3.9 A |  | 0.84 | 0.426 |
| E:878:Lys | C:143:Glu | 2.8 A | 1x salt bridge to C:143:Glu | 0 | 0.35 |

(H) Complex of mutant MB4 (848His-865Arg) and Fab after MDs

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Residue** | **Closest** | **Distance** | **Specific Interactions** | **Surface Complementarity** | **Buried SASA** |
| A:7:Ser | B:855:Asp B:864:Tyr | 2.5 A 3.5 A | 1x hb, 1x clash to B:855:Asp | 0.66 | 0.83 |
| A:8:Pro | B:864:Tyr B:851:Tyr | 3.5 A 3.6 A |  | 0.83 | 0.993 |
| A:9:Ser | B:864:Tyr | 2.8 A | 1x hb to B:864:Tyr | 0.83 | 0.629 |
| A:10:Ser | B:866:Ala B:865:Arg | 2.9 A 3.4 A | 1x hb to B:866:Ala | 0.87 | 0.781 |
| A:11:Leu | B:866:Ala B:851:Tyr | 3.5 A 3.8 A |  | 0.87 | 0.999 |
| A:12:Ser | B:868:Leu B:866:Ala B:867:Asp | 3.0 A 3.1 A 3.6 A | 1x hb to B:866:Ala 1x hb to B:868:Leu | 0.85 | 0.646 |
| A:13:Ala | B:868:Leu | 3.9 A |  | 0.82 | 1 |
| A:17:Asp | B:871:Gly B:872:Gly B:868:Leu | 3.2 A 3.6 A 3.7 A |  | 0.82 | 0.925 |
| A:18:Arg | B:871:Gly B:843:Phe B:872:Gly | 2.8 A 3.5 A 3.8 A | 1x hb to B:871:Gly | 0.9 | 0.434 |
| A:19:Val | B:868:Leu | 4.0 A |  | 0.9 | 0.816 |
| A:20:Thr | B:851:Tyr B:847:Thr | 2.8 A 2.8 A | 1x hb to B:847:Thr 1x hb to B:851:Tyr | 0.75 | 0.974 |
| A:21:Ile |  |  |  | 0.6 | 0.001 |
| A:22:Thr | B:851:Tyr B:848:His | 3.5 A 3.5 A |  | 0.75 | 0.913 |
| A:24:Arg | B:852:Arg | 3.8 A |  | 0.6 | 0.273 |
| A:65:Ser | B:844:Glu | 2.7 A | 1x hb to B:844:Glu | 0.85 | 0.412 |
| A:70:Asp | B:848:His | 3.3 A |  | 0.66 | 0.35 |
| A:71:Phe |  |  |  | 0.4 | 1 |
| A:72:Thr | B:848:His B:844:Glu | 3.1 A 3.4 A |  | 0.85 | 0.866 |
| A:74:Thr | B:844:Glu | 3.5 A |  | 0.85 | 0.599 |
| A:107:Lys | B:868:Leu B:869:Glu | 2.9 A 3.5 A | 1x hb to B:868:Leu | 0.81 | 0.466 |
| A:143:Glu | B:865:Arg | 2.8 A | 1x hb, 3x clash to B:865:Arg | 0 | 0.392 |
| B:843:Phe | A:18:Arg | 3.5 A |  | 0.87 | 0.77 |
| B:844:Glu | A:65:Ser A:72:Thr A:74:Thr | 2.7 A 3.4 A 3.5 A | 1x hb to A:65:Ser | 0.86 | 0.65 |
| B:847:Thr | A:20:Thr | 2.8 A | 1x hb to A:20:Thr | 0.77 | 0.779 |
| B:848:His | A:72:Thr A:70:Asp A:22:Thr | 3.1 A 3.3 A 3.5 A |  | 0.76 | 0.871 |
| B:851:Tyr | A:20:Thr A:22:Thr A:8:Pro A:11:Leu | 2.8 A 3.5 A 3.6 A 3.8 A | 1x hb to A:20:Thr | 0.79 | 0.984 |
| B:852:Arg | A:24:Arg | 3.8 A |  | 0.63 | 0.354 |
| B:855:Asp | A:7:Ser | 2.5 A | 1x hb, 1x clash to A:7:Ser | 0.77 | 0.339 |
| B:864:Tyr | A:9:Ser A:8:Pro A:7:Ser | 2.8 A 3.5 A 3.5 A | 1x hb to A:9:Ser | 0.82 | 0.955 |
| B:865:Arg | A:143:Glu A:10:Ser | 2.8 A 3.4 A | 1x hb, 3x clash to A:143:Glu | 0.89 | 0.424 |
| B:866:Ala | A:10:Ser A:12:Ser A:11:Leu | 2.9 A 3.1 A 3.5 A | 1x hb to A:10:Ser 1x hb to A:12:Ser | 0.8 | 0.948 |
| B:867:Asp | A:12:Ser | 3.6 A |  | 0.86 | 0.542 |
| B:868:Leu | A:107:Lys A:12:Ser A:17:Asp A:13:Ala A:19:Val | 2.9 A 3.0 A 3.7 A 3.9 A 4.0 A | 1x hb to A:12:Ser 1x hb to A:107:Lys | 0.83 | 0.964 |
| B:869:Glu | A:107:Lys | 3.5 A |  | 0.8 | 0.211 |
| B:871:Gly | A:18:Arg A:17:Asp | 2.8 A 3.2 A | 1x hb to A:18:Arg | 0.87 | 0.581 |
| B:872:Gly | A:17:Asp A:18:Arg | 3.6 A 3.8 A |  | 0.85 | 0.861 |
| B:875:Met |  |  |  | 0.84 | 0.094 |

(I) Complex of mutant MB5 (843Arg-844Asn) and Fab before MDs

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Residue** | **Closest** | **Distance** | **Specific Interactions** | **Surface Complementarity** | **Buried SASA** |
| C:7:Ser | E:855:Asp E:864:Tyr | 2.9 A 3.7 A | 1x hb to E:855:Asp | 0.69 | 0.885 |
| C:10:Ser | E:865:Thr E:866:Ala | 3.2 A 3.4 A | 1x hb to E:866:Ala | 0.66 | 0.502 |
| C:11:Leu | E:866:Ala E:851:Tyr E:868:Leu | 3.0 A 3.4 A 3.5 A | 1x clash to E:866:Ala 2x clash to E:868:Leu | 0.88 | 0.996 |
| C:12:Ser | E:868:Leu E:866:Ala E:867:Asp | 2.6 A 2.9 A 3.1 A | 1x hb to E:866:Ala 1x clash to E:867:Asp 1x hb to E:868:Leu | 0.77 | 0.647 |
| C:13:Ala | E:868:Leu | 3.9 A |  | 0.89 | 1 |
| C:14:Ser |  |  |  | 0.44 | 0.065 |
| C:17:Asp | E:868:Leu E:871:Gly E:872:Gly | 3.4 A 3.7 A 3.9 A |  | 0.89 | 0.895 |
| C:18:Arg | E:871:Gly E:843:Arg E:868:Leu | 3.1 A 3.2 A 3.8 A | 1x hb to E:871:Gly | 0.84 | 0.502 |
| C:19:Val |  |  |  | 0.87 | 0.848 |
| C:20:Thr | E:851:Tyr E:844:Asn E:847:Thr | 3.5 A 3.6 A 3.8 A |  | 0.67 | 0.973 |
| C:22:Thr |  |  |  | 0.47 | 0.924 |
| C:24:Arg | E:855:Asp E:852:Arg | 2.7 A 3.4 A |  | 0.62 | 0.493 |
| C:72:Thr | E:848:Ala | 3.6 A |  | 0.6 | 0.747 |
| C:74:Thr | E:844:Asn | 3.8 A |  | 0.82 | 0.497 |
| C:107:Lys | E:867:Asp E:868:Leu E:869:Glu | 2.6 A 2.9 A 3.8 A | 3x clash to E:867:Asp 1x hb to E:868:Leu | 0.7 | 0.586 |
| C:143:Glu | E:878:Lys | 2.8 A | 1x salt bridge to E:878:Lys | 0 | 0.242 |
| **E:843:Arg** | C:18:Arg | 3.2 A |  | 0.85 | 0.902 |
| **E:844:Asn** | C:20:Thr C:74:Thr | 3.6 A 3.8 A |  | 0.76 | 0.684 |
| E:847:Thr | C:20:Thr | 3.8 A |  | 0.72 | 0.775 |
| E:848:Ala | C:72:Thr | 3.6 A |  | 0.48 | 0.708 |
| E:851:Tyr | C:11:Leu C:20:Thr C:8:Pro | 3.4 A 3.5 A 3.6 A | 1x clash to C:8:Pro | 0.69 | 0.989 |
| E:852:Arg | C:24:Arg C:70:Asp | 3.4 A 4.0 A |  | 0.4 | 0.334 |
| E:855:Asp | C:24:Arg C:7:Ser | 2.7 A 2.9 A | 1x hb to C:7:Ser | 0.49 | 0.657 |
| E:864:Tyr | C:7:Ser C:8:Pro | 3.7 A 3.8 A |  | 0.7 | 0.66 |
| E:865:Thr | C:10:Ser | 3.2 A |  | 0.52 | 0.401 |
| E:866:Ala | C:12:Ser C:11:Leu C:10:Ser | 2.9 A 3.0 A 3.4 A | 1x hb to C:10:Ser 1x clash to C:11:Leu 1x hb to C:12:Ser | 0.84 | 1 |
| E:867:Asp | C:107:Lys C:12:Ser | 2.6 A 3.1 A | 1x clash to C:12:Ser 3x clash to C:107:Lys | 0.82 | 0.569 |
| E:868:Leu | C:12:Ser C:107:Lys C:17:Asp C:11:Leu C:18:Arg C:13:Ala | 2.6 A 2.9 A 3.4 A 3.5 A 3.8 A 3.9 A | 2x clash to C:11:Leu 1x hb to C:12:Ser 1x hb to C:107:Lys | 0.85 | 0.959 |
| E:869:Glu | C:107:Lys | 3.8 A |  | 0.71 | 0.114 |
| E:871:Gly | C:18:Arg C:17:Asp | 3.1 A 3.7 A | 1x hb to C:18:Arg | 0.86 | 0.519 |
| E:872:Gly | C:17:Asp | 3.9 A |  | 0.82 | 0.637 |
| E:878:Lys | C:143:Glu | 2.8 A | 1x salt bridge to C:143:Glu | 0 | 0.348 |

(J) Complex of mutant MB5 (843Arg-844Asn) and Fab after MDs

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Residue** | **Closest** | **Distance** | **Specific Interactions** | **Surface Complementarity** | **Buried SASA** |
| A:7:Ser | B:855:Asp | 2.6 A | 1x hb to B:855:Asp | 0.68 | 0.848 |
| A:8:Pro | B:851:Tyr | 3.7 A |  | 0.75 | 0.979 |
| A:10:Ser | B:866:Ala B:865:Thr | 3.6 A 4.0 A |  | 0.6 | 0.698 |
| A:11:Leu | B:866:Ala | 3.3 A |  | 0.71 | 0.968 |
| A:12:Ser | B:866:Ala B:868:Leu B:867:Asp | 2.9 A 3.1 A 3.6 A | 1x hb to B:866:Ala 1x hb to B:868:Leu | 0.7 | 0.738 |
| A:13:Ala |  |  |  | 0.71 | 1 |
| A:17:Asp | B:872:Gly B:871:Gly | 3.6 A 3.6 A |  | 0.76 | 0.85 |
| A:18:Arg | B:844:Asn B:871:Gly B:868:Leu B:872:Gly B:843:Arg | 2.7 A 3.0 A 3.2 A 3.4 A 3.6 A | 1x hb to B:844:Asn 1x hb to B:871:Gly | 0.71 | 0.857 |
| A:19:Val |  |  |  | 0.88 | 0.932 |
| A:20:Thr | B:851:Tyr B:844:Asn B:847:Thr | 3.3 A 3.6 A 3.6 A | 1x clash to B:851:Tyr | 0.69 | 0.939 |
| A:21:Ile |  |  |  | 0.63 | 0.594 |
| A:22:Thr |  |  |  | 0.64 | 0.978 |
| A:24:Arg | B:855:Asp | 2.8 A | 2x hb, 1x salt bridge to B:855:Asp | 0.53 | 0.474 |
| A:70:Asp | B:852:Arg | 2.9 A | 1x hb, 1x salt bridge, 2x clash to B:852:Arg | 0.52 | 0.505 |
| A:72:Thr | B:848:Ala | 3.9 A |  | 0.45 | 0.728 |
| A:74:Thr | B:844:Asn | 2.8 A | 1x hb to B:844:Asn | 0.55 | 0.723 |
| A:107:Lys | B:867:Asp B:868:Leu B:869:Glu | 2.8 A 3.0 A 3.5 A | 1x hb, 1x salt bridge, 1x clash to B:867:Asp 1x hb to B:868:Leu | 0.6 | 0.543 |
| A:143:Glu | B:878:Lys | 2.8 A | 1x hb, 1x salt bridge, 2x clash to B:878:Lys | 0.68 | 0.432 |
| **B:843:Arg** | A:18:Arg | 3.6 A |  | 0.69 | 0.405 |
| **B:844:Asn** | A:18:Arg A:74:Thr A:20:Thr | 2.7 A 2.8 A 3.6 A | 1x hb to A:18:Arg 1x hb to A:74:Thr | 0.61 | 0.655 |
| B:847:Thr | A:20:Thr | 3.6 A |  | 0.57 | 0.795 |
| B:848:Ala | A:72:Thr | 3.9 A |  | 0.57 | 0.969 |
| B:851:Tyr | A:20:Thr A:8:Pro | 3.3 A 3.7 A | 1x clash to A:20:Thr | 0.73 | 0.989 |
| B:852:Arg | A:70:Asp | 2.9 A | 1x hb, 1x salt bridge, 2x clash to A:70:Asp | 0.54 | 0.486 |
| B:855:Asp | A:7:Ser A:24:Arg | 2.6 A 2.8 A | 1x hb to A:7:Ser 2x hb, 1x salt bridge to A:24:Arg | 0.53 | 0.584 |
| B:864:Tyr |  |  |  | 0.66 | 0.701 |
| B:865:Thr | A:10:Ser | 4.0 A |  | 0.4 | 0.417 |
| B:866:Ala | A:12:Ser A:11:Leu A:10:Ser | 2.9 A 3.3 A 3.6 A | 1x hb to A:12:Ser | 0.7 | 0.946 |
| B:867:Asp | A:107:Lys A:12:Ser | 2.8 A 3.6 A | 1x hb, 1x salt bridge, 1x clash to A:107:Lys | 0.68 | 0.578 |
| B:868:Leu | A:107:Lys A:12:Ser A:18:Arg | 3.0 A 3.1 A 3.2 A | 1x hb to A:12:Ser 1x hb to A:107:Lys | 0.67 | 0.954 |
| B:869:Glu | A:107:Lys | 3.5 A |  | 0.47 | 0.245 |
| B:871:Gly | A:18:Arg A:17:Asp | 3.0 A 3.6 A | 1x hb to A:18:Arg | 0.81 | 0.559 |
| B:872:Gly | A:18:Arg A:17:Asp | 3.4 A 3.6 A |  | 0.78 | 0.817 |
| B:873:Asn |  |  |  | 0.4 | 0.117 |
| B:878:Lys | A:143:Glu | 2.8 A | 1x hb, 1x salt bridge, 2x clash to A:143:Glu | 0.74 | 0.378 |

(K) Complex of mutant MB6 (843Tyr-847Met-865Trp) and Fab before MDs

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Residue** | **Closest** | **Distance** | **Specific Interactions** | **Surface Complementarity** | **Buried SASA** |
| C:7:Ser | E:855:Asp E:864:Tyr | 2.9 A 3.7 A | 1x hb to E:855:Asp | 0.69 | 0.885 |
| C:8:Pro | E:851:Tyr E:864:Tyr | 3.6 A 3.8 A | 1x clash to E:851:Tyr | 0.74 | 0.966 |
| C:10:Ser | E:865:Trp E:866:Ala | 1.0 A 3.4 A | 39x clash to E:865:Trp 1x hb to E:866:Ala | 0.28 | 0.979 |
| C:11:Leu | E:866:Ala E:865:Trp E:851:Tyr E:868:Leu | 3.0 A 3.1 A 3.4 A 3.5 A | 1x clash to E:865:Trp 1x clash to E:866:Ala 2x clash to E:868:Leu | 0.88 | 0.999 |
| C:12:Ser | E:868:Leu E:866:Ala E:867:Asp | 2.6 A 2.9 A 3.1 A | 1x hb to E:866:Ala 1x clash to E:867:Asp 1x hb to E:868:Leu | 0.78 | 0.662 |
| C:13:Ala | E:868:Leu | 3.9 A |  | 0.89 | 1 |
| C:14:Ser |  |  |  | 0.44 | 0.065 |
| C:17:Asp | E:868:Leu E:871:Gly E:872:Gly | 3.4 A 3.7 A 3.9 A |  | 0.88 | 0.895 |
| C:18:Arg | E:843:Tyr E:871:Gly E:868:Leu E:847:Met | 1.9 A 3.1 A 3.8 A 3.9 A | 8x clash to E:843:Tyr 1x hb to E:871:Gly | 0.83 | 0.517 |
| C:19:Val | E:843:Tyr | 3.8 A |  | 0.87 | 0.867 |
| C:20:Thr | E:851:Tyr E:847:Met E:843:Tyr E:844:Glu | 3.5 A 3.5 A 3.6 A 3.6 A |  | 0.72 | 0.975 |
| C:21:Ile |  |  |  | 0.29 | 0.341 |
| C:22:Thr |  |  |  | 0.47 | 0.924 |
| C:24:Arg | E:855:Asp E:852:Arg | 2.7 A 3.4 A |  | 0.62 | 0.493 |
| C:65:Ser | E:844:Glu | 3.2 A | 1x hb to E:844:Glu | 0 | 0.378 |
| C:72:Thr | E:848:Ala | 3.6 A |  | 0.61 | 0.734 |
| C:74:Thr | E:844:Glu | 3.5 A |  | 0.79 | 0.652 |
| C:107:Lys | E:867:Asp E:868:Leu E:869:Glu | 2.6 A 2.9 A 3.8 A | 3x clash to E:867:Asp 1x hb to E:868:Leu | 0.7 | 0.586 |
| **E:843:Tyr** | C:18:Arg C:20:Thr C:19:Val | 1.9 A 3.6 A 3.8 A | 8x clash to C:18:Arg | 0.84 | 0.935 |
| E:844:Glu | C:65:Ser C:74:Thr C:20:Thr | 3.2 A 3.5 A 3.6 A | 1x hb to C:65:Ser | 0.88 | 0.653 |
| **E:847:Met** | C:20:Thr C:18:Arg | 3.5 A 3.9 A |  | 0.74 | 0.749 |
| E:848:Ala | C:72:Thr | 3.6 A |  | 0.51 | 0.708 |
| E:851:Tyr | C:11:Leu C:20:Thr C:8:Pro | 3.4 A 3.5 A 3.6 A | 1x clash to C:8:Pro | 0.66 | 0.97 |
| E:852:Arg | C:24:Arg C:70:Asp | 3.4 A 4.0 A |  | 0.4 | 0.335 |
| E:855:Asp | C:24:Arg C:7:Ser | 2.7 A 2.9 A | 1x hb to C:7:Ser | 0.49 | 0.658 |
| E:864:Tyr | C:7:Ser C:8:Pro | 3.7 A 3.8 A |  | 0.69 | 0.733 |
| **E:865:Trp** | C:10:Ser C:11:Leu C:9:Ser | 1.0 A 3.1 A 3.7 A | 39x clash to C:10:Ser 1x clash to C:11:Leu | 0.1 | 0.445 |
| E:866:Ala | C:12:Ser C:11:Leu C:10:Ser | 2.9 A 3.0 A 3.4 A | 1x hb to C:10:Ser 1x clash to C:11:Leu 1x hb to C:12:Ser | 0.82 | 0.934 |
| E:867:Asp | C:107:Lys C:12:Ser | 2.6 A 3.1 A | 1x clash to C:12:Ser 3x clash to C:107:Lys | 0.83 | 0.541 |
| E:868:Leu | C:12:Ser C:107:Lys C:17:Asp C:11:Leu C:18:Arg C:13:Ala | 2.6 A 2.9 A 3.4 A 3.5 A 3.8 A 3.9 A | 2x clash to C:11:Leu 1x hb to C:12:Ser 1x hb to C:107:Lys | 0.86 | 0.943 |
| E:869:Glu | C:107:Lys | 3.8 A |  | 0.71 | 0.114 |
| E:871:Gly | C:18:Arg C:17:Asp | 3.1 A 3.7 A | 1x hb to C:18:Arg | 0.87 | 0.507 |
| E:872:Gly | C:17:Asp | 3.9 A |  | 0.77 | 0.155 |
| E:878:Lys | C:143:Glu | 2.8 A | 1x salt bridge to C:143:Glu | 0 | 0.343 |

(L) Complex of mutant MB6 (843Tyr-847Met-865Trp) and Fab after MDs

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Residue** | **Closest** | **Distance** | **Specific Interactions** | **Surface Complementarity** | **Buried SASA** |
| A:7:Ser | B:864:Tyr | 3.4 A |  | 0.49 | 0.715 |
| A:8:Pro | B:851:Tyr B:864:Tyr | 3.6 A 3.8 A |  | 0.88 | 0.993 |
| A:9:Ser | B:864:Tyr B:863:Glu B:865:Trp | 2.7 A 3.5 A 3.8 A | 2x hb to B:864:Tyr | 0.84 | 0.664 |
| A:10:Ser | B:866:Ala B:865:Trp | 3.0 A 3.2 A | 1x hb to B:866:Ala | 0.89 | 0.555 |
| A:11:Leu | B:866:Ala B:851:Tyr B:868:Leu | 3.4 A 3.5 A 3.7 A |  | 0.82 | 1 |
| A:12:Ser | B:866:Ala B:868:Leu B:867:Asp | 2.8 A 3.2 A 3.8 A | 1x hb to B:866:Ala 1x hb to B:868:Leu | 0.88 | 0.781 |
| A:13:Ala |  |  |  | 0.64 | 0.984 |
| A:17:Asp | B:871:Gly B:868:Leu | 3.5 A 4.0 A |  | 0.64 | 0.845 |
| A:18:Arg | B:871:Gly B:843:Tyr | 2.9 A 3.3 A | 1x hb to B:871:Gly | 0.75 | 0.532 |
| A:19:Val | B:868:Leu | 3.7 A |  | 0.79 | 0.763 |
| A:20:Thr | B:851:Tyr B:843:Tyr B:847:Met | 2.7 A 3.7 A 4.0 A | 1x hb to B:851:Tyr | 0.68 | 0.993 |
| A:21:Ile |  |  |  | 0.67 | 0.4 |
| A:22:Thr | B:848:Ala B:851:Tyr | 3.7 A 3.9 A |  | 0.69 | 0.939 |
| A:24:Arg | B:855:Asp B:852:Arg | 2.7 A 3.8 A | 1x hb, 1x salt bridge to B:855:Asp | 0.65 | 0.467 |
| A:65:Ser | B:844:Glu | 3.8 A |  | 0.4 | 0.302 |
| A:70:Asp | B:852:Arg | 4.0 A |  | 0.4 | 0.37 |
| A:72:Thr | B:844:Glu B:848:Ala | 3.8 A 3.9 A |  | 0.64 | 0.801 |
| A:74:Thr | B:844:Glu | 3.7 A |  | 0.7 | 0.738 |
| A:107:Lys | B:867:Asp | 2.8 A | 1x hb, 1x salt bridge to B:867:Asp | 0.37 | 0.417 |
| A:143:Glu | B:878:Lys B:865:Trp | 2.7 A 3.0 A | 1x hb to B:865:Trp 1x hb, 1x salt bridge, 1x clash to B:878:Lys | 0.69 | 0.685 |
| A:199:Gln | B:878:Lys | 3.5 A | 1x hb to B:878:Lys | 0.8 | 0.276 |
| **B:843:Tyr** | A:18:Arg A:20:Thr | 3.3 A 3.7 A |  | 0.76 | 0.891 |
| B:844:Glu | A:74:Thr A:65:Ser A:72:Thr | 3.7 A 3.8 A 3.8 A |  | 0.76 | 0.697 |
| **B:847:Met** | A:20:Thr | 4.0 A |  | 0.31 | 0.984 |
| B:848:Ala | A:22:Thr A:72:Thr | 3.7 A 3.9 A |  | 0.72 | 0.999 |
| B:851:Tyr | A:20:Thr A:11:Leu A:8:Pro A:22:Thr | 2.7 A 3.5 A 3.6 A 3.9 A | 1x hb to A:20:Thr | 0.68 | 0.998 |
| B:852:Arg | A:24:Arg A:70:Asp | 3.8 A 4.0 A |  | 0.68 | 0.592 |
| B:855:Asp | A:24:Arg | 2.7 A | 1x hb, 1x salt bridge to A:24:Arg | 0.56 | 0.52 |
| B:863:Glu | A:9:Ser | 3.5 A |  | 0.9 | 0.282 |
| B:864:Tyr | A:9:Ser A:7:Ser A:8:Pro | 2.7 A 3.4 A 3.8 A | 2x hb to A:9:Ser | 0.84 | 0.814 |
| **B:865:Trp** | A:143:Glu A:10:Ser A:9:Ser | 3.0 A 3.2 A 3.8 A | 1x hb to A:143:Glu | 0.88 | 0.53 |
| B:866:Ala | A:12:Ser A:10:Ser A:11:Leu | 2.8 A 3.0 A 3.4 A | 1x hb to A:10:Ser 1x hb to A:12:Ser | 0.81 | 0.998 |
| B:867:Asp | A:107:Lys A:12:Ser | 2.8 A 3.8 A | 1x hb, 1x salt bridge to A:107:Lys | 0.76 | 0.651 |
| B:868:Leu | A:12:Ser A:19:Val A:11:Leu A:17:Asp | 3.2 A 3.7 A 3.7 A 4.0 A | 1x hb to A:12:Ser | 0.64 | 0.967 |
| B:869:Glu |  |  |  | 0.5 | 0.144 |
| B:871:Gly | A:18:Arg A:17:Asp | 2.9 A 3.5 A | 1x hb to A:18:Arg | 0.65 | 0.639 |
| B:872:Gly |  |  |  | 0.53 | 0.799 |
| B:878:Lys | A:143:Glu A:199:Gln | 2.7 A 3.5 A | 1x hb, 1x salt bridge, 1x clash to A:143:Glu 1x hb to A:199:Gln | 0.63 | 0.588 |

(M) Complex of mutant MB7 (843Arg-844Arg) and Fab before MDs

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Residue** | **Closest** | **Distance** | **Specific Interactions** | **Surface Complementarity** | **Buried SASA** |
| C:7:Ser | E:855:Asp E:864:Tyr | 2.9 A 3.7 A | 1x hb to E:855:Asp | 0.69 | 0.885 |
| C:8:Pro | E:851:Tyr E:864:Tyr | 3.6 A 3.8 A | 1x clash to E:851:Tyr | 0.77 | 0.966 |
| C:10:Ser | E:865:Thr E:866:Ala | 3.2 A 3.4 A | 1x hb to E:866:Ala | 0.64 | 0.502 |
| C:11:Leu | E:866:Ala E:851:Tyr E:868:Leu | 3.0 A 3.4 A 3.5 A | 1x clash to E:866:Ala 2x clash to E:868:Leu | 0.9 | 0.996 |
| C:12:Ser | E:868:Leu E:866:Ala E:867:Asp | 2.6 A 2.9 A 3.1 A | 1x hb to E:866:Ala 1x clash to E:867:Asp 1x hb to E:868:Leu | 0.77 | 0.647 |
| C:13:Ala | E:868:Leu | 3.9 A |  | 0.89 | 1 |
| C:14:Ser |  |  |  | 0.45 | 0.065 |
| C:17:Asp | E:868:Leu E:871:Gly E:872:Gly | 3.4 A 3.7 A 3.9 A |  | 0.87 | 0.895 |
| C:18:Arg | E:871:Gly E:843:Arg E:868:Leu | 3.1 A 3.2 A 3.8 A | 1x hb to E:871:Gly | 0.84 | 0.502 |
| C:19:Val |  |  |  | 0.87 | 0.851 |
| C:20:Thr | E:851:Tyr E:844:Arg E:847:Thr | 3.5 A 3.6 A 3.8 A |  | 0.68 | 0.973 |
| C:22:Thr |  |  |  | 0.49 | 0.923 |
| C:24:Arg | E:855:Asp E:852:Arg | 2.7 A 3.4 A |  | 0.66 | 0.492 |
| C:65:Ser | E:844:Arg | 2.4 A | 4x clash to E:844:Arg | 0.5 | 0.731 |
| C:72:Thr | E:848:Ala | 3.6 A |  | 0.59 | 0.737 |
| C:74:Thr | E:844:Arg | 3.8 A |  | 0.82 | 0.616 |
| C:107:Lys | E:867:Asp E:868:Leu E:869:Glu | 2.6 A 2.9 A 3.8 A | 3x clash to E:867:Asp 1x hb to E:868:Leu | 0.72 | 0.586 |
| C:143:Glu | E:878:Lys | 2.8 A | 1x salt bridge to E:878:Lys | 0 | 0.243 |
| **E:843:Arg** | C:18:Arg | 3.2 A |  | 0.85 | 0.901 |
| **E:844:Arg** | C:65:Ser C:20:Thr C:74:Thr | 2.4 A 3.6 A 3.8 A | 4x clash to C:65:Ser | 0.79 | 0.523 |
| E:847:Thr | C:20:Thr | 3.8 A |  | 0.71 | 0.77 |
| E:848:Ala | C:72:Thr | 3.6 A |  | 0.48 | 0.706 |
| E:851:Tyr | C:11:Leu C:20:Thr C:8:Pro | 3.4 A 3.5 A 3.6 A | 1x clash to C:8:Pro | 0.69 | 0.989 |
| E:852:Arg | C:24:Arg C:70:Asp | 3.4 A 4.0 A |  | 0.48 | 0.336 |
| E:855:Asp | C:24:Arg C:7:Ser | 2.7 A 2.9 A | 1x hb to C:7:Ser | 0.56 | 0.658 |
| E:864:Tyr | C:7:Ser C:8:Pro | 3.7 A 3.8 A |  | 0.72 | 0.66 |
| E:865:Thr | C:10:Ser | 3.2 A |  | 0.54 | 0.402 |
| E:866:Ala | C:12:Ser C:11:Leu C:10:Ser | 2.9 A 3.0 A 3.4 A | 1x hb to C:10:Ser 1x clash to C:11:Leu 1x hb to C:12:Ser | 0.83 | 1 |
| E:867:Asp | C:107:Lys C:12:Ser | 2.6 A 3.1 A | 1x clash to C:12:Ser 3x clash to C:107:Lys | 0.83 | 0.568 |
| E:868:Leu | C:12:Ser C:107:Lys C:17:Asp C:11:Leu C:18:Arg C:13:Ala | 2.6 A 2.9 A 3.4 A 3.5 A 3.8 A 3.9 A | 2x clash to C:11:Leu 1x hb to C:12:Ser 1x hb to C:107:Lys | 0.85 | 0.958 |
| E:869:Glu | C:107:Lys | 3.8 A |  | 0.76 | 0.114 |
| E:871:Gly | C:18:Arg C:17:Asp | 3.1 A 3.7 A | 1x hb to C:18:Arg | 0.83 | 0.519 |
| E:872:Gly | C:17:Asp | 3.9 A |  | 0.82 | 0.637 |
| E:878:Lys | C:143:Glu | 2.8 A | 1x salt bridge to C:143:Glu | 0 | 0.349 |

(N) Complex of mutant MB7 (843Arg-844Arg) after MDs

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Residue** | **Closest** | **Distance** | **Specific Interactions** | **Surface Complementarity** | **Buried SASA** |
| A:7:Ser | B:855:Asp B:864:Tyr | 2.6 A 3.3 A | 1x hb to B:855:Asp | 0.65 | 0.832 |
| A:8:Pro | B:864:Tyr | 3.6 A | 1x clash to E:851:Tyr | 0.71 | 0.991 |
| A:9:Ser | B:864:Tyr B:863:Glu | 2.7 A 3.6 A | 1x hb to B:864:Tyr | 0.79 | 0.598 |
| A:10:Ser | B:866:Ala B:865:Thr | 3.2 A 3.5 A | 1x hb to B:866:Ala | 0.86 | 0.646 |
| A:11:Leu | B:866:Ala B:868:Leu | 3.4 A 3.9 A | 1x clash to E:866:Ala 2x clash to E:868:Leu | 0.84 | 0.965 |
| A:12:Ser | B:868:Leu B:866:Ala B:867:Asp | 2.9 A 3.1 A 3.3 A | 1x hb to B:866:Ala  1x clash to E:867:Asp 1x hb to B:868:Leu | 0.86 | 0.768 |
| A:13:Ala |  |  |  | 0.71 | 1 |
| A:17:Asp | B:871:Gly B:872:Gly B:868:Leu | 3.2 A 3.5 A 3.7 A |  | 0.81 | 0.928 |
| A:18:Arg | B:871:Gly B:843:Arg B:872:Gly | 3.2 A 3.8 A 3.9 A | 1x hb to B:871:Gly | 0.86 | 0.397 |
| A:19:Val | B:868:Leu | 3.5 A |  | 0.88 | 0.688 |
| A:20:Thr | B:847:Thr B:844:Arg | 3.3 A 3.6 A |  | 0.85 | 0.951 |
| A:21:Ile |  |  |  | 0.47 | 0.185 |
| A:22:Thr | B:848:Ala | 3.7 A |  | 0.67 | 0.856 |
| A:24:Arg | B:852:Arg | 3.7 A |  | 0.88 | 0.233 |
| A:63:Ser | B:844:Arg | 3.1 A | 1x hb to B:844:Arg | 0.71 | 0.368 |
| A:64:Gly |  |  |  | 0.69 | 0.182 |
| A:65:Ser | B:844:Arg | 3.5 A |  | 0.68 | 0.427 |
| A:70:Asp | B:852:Arg | 2.7 A | 2x hb, 1x salt bridge to B:852:Arg | 0.7 | 0.552 |
| A:72:Thr | B:848:Ala | 3.5 A |  | 0.76 | 0.811 |
| A:74:Thr | B:844:Arg | 3.4 A |  | 0.87 | 0.749 |
| A:100:Gln | B:863:Glu | 2.9 A | 1x hb to B:863:Glu | 0.7 | 0.291 |
| A:107:Lys | B:867:Asp B:868:Leu | 2.7 A 3.0 A | 1x hb to B:867:Asp  1x clash to E:867:Asp 1x hb to B:868:Leu | 0.77 | 0.512 |
| A:143:Glu | B:878:Lys | 3.0 A | 1x hb to B:878:Lys | 0 | 0.409 |
| B:843:Arg | A:18:Arg | 3.8 A |  | 0.83 | 0.397 |
| B:844:Arg | A:63:Ser A:74:Thr A:65:Ser A:20:Thr | 3.1 A 3.4 A 3.5 A 3.6 A | 1x hb to A:63:Ser | 0.81 | 0.662 |
| B:845:Glu |  |  |  | 0.18 | 0.025 |
| B:847:Thr | A:20:Thr | 3.3 A |  | 0.81 | 0.769 |
| B:848:Ala | A:72:Thr A:22:Thr | 3.5 A 3.7 A |  | 0.72 | 0.995 |
| B:851:Tyr | A:8:Pro | 3.5 A | 1x clash to A:8:Pro | 0.64 | 0.992 |
| B:852:Arg | A:70:Asp A:24:Arg | 2.7 A 3.7 A | 2x hb, 1x salt bridge to A:70:Asp | 0.88 | 0.533 |
| B:855:Asp | A:7:Ser | 2.6 A | 1x hb to A:7:Ser | 0.69 | 0.431 |
| B:863:Glu | A:100:Gln A:9:Ser | 2.9 A 3.6 A | 1x hb to A:100:Gln | 0.7 | 0.288 |
| B:864:Tyr | A:9:Ser A:7:Ser A:8:Pro | 2.7 A 3.3 A 3.6 A | 1x hb to A:9:Ser | 0.8 | 0.9 |
| B:865:Thr | A:10:Ser | 3.6 A |  | 0.85 | 0.462 |
| B:866:Ala | A:12:Ser A:10:Ser A:11:Leu | 3.1 A 3.2 A 3.4 A | 1x hb to A:10:Ser  1x clash to A:11:Leu 1x hb to A:12:Ser | 0.82 | 0.857 |
| B:867:Asp | A:107:Lys A:12:Ser | 2.7 A 3.3 A | 1x hb, 1x clash to A:107:Lys  1x clash to A:12:Ser | 0.92 | 0.593 |
| B:868:Leu | A:12:Ser A:107:Lys A:19:Val A:17:Asp A:11:Leu | 2.9 A 3.0 A 3.5 A 3.7 A 3.9 A | 1x hb to A:12:Ser 1x hb to A:107:Lys  2x clash to A:11:Leu | 0.77 | 0.938 |
| B:869:Glu |  |  |  | 0.77 | 0.115 |
| B:871:Gly | A:18:Arg A:17:Asp A:16:Gly | 3.2 A 3.2 A 4.0 A | 1x hb to A:18:Arg | 0.82 | 0.536 |
| B:872:Gly | A:17:Asp A:18:Arg | 3.5 A 3.9 A |  | 0.81 | 0.724 |
| B:878:Lys | A:143:Glu | 3.0 A | 1x hb to A:143:Glu | 0 | 0.364 |

(O) Complex of the wild type and Fab before MDs

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Residue** | **Closest** | **Distance** | **Specific Interactions** | **Surface Complementarity** | **Buried SASA** |
| C:7:Ser | E:855:Asp E:864:Tyr | 2.9 A 3.7 A | 1x hb to E:855:Asp | 0.69 | 0.885 |
| C:8:Pro | E:851:Tyr E:864:Tyr | 3.6 A 3.8 A | 1x clash to E:851:Tyr | 0.74 | 0.966 |
| C:10:Ser | E:865:Thr E:866:Ala | 3.2 A 3.4 A | 1x hb to E:866:Ala | 0.66 | 0.502 |
| C:11:Leu | E:866:Ala E:851:Tyr E:868:Leu | 3.0 A 3.4 A 3.5 A | 1x clash to E:866:Ala 2x clash to E:868:Leu | 0.88 | 0.996 |
| C:12:Ser | E:868:Leu E:866:Ala E:867:Asp | 2.6 A 2.9 A 3.1 A | 1x hb to E:866:Ala 1x clash to E:867:Asp 1x hb to E:868:Leu | 0.77 | 0.646 |
| C:13:Ala | E:868:Leu | 3.9 A |  | 0.89 | 1 |
| C:14:Ser |  |  |  | 0.44 | 0.065 |
| C:17:Asp | E:868:Leu E:871:Gly E:872:Gly | 3.4 A 3.7 A 3.9 A |  | 0.89 | 0.895 |
| C:18:Arg | E:871:Gly E:843:Phe E:868:Leu | 3.1 A 3.2 A 3.8 A | 1x hb to E:871:Gly | 0.86 | 0.514 |
| C:19:Val |  |  |  | 0.85 | 0.851 |
| C:20:Thr | E:851:Tyr E:844:Glu E:843:Phe E:847:Thr | 3.5 A 3.6 A 3.6 A 3.8 A |  | 0.77 | 0.987 |
| C:21:Ile |  |  |  | 0.29 | 0.339 |
| C:22:Thr |  |  |  | 0.47 | 0.922 |
| C:24:Arg | E:855:Asp E:852:Arg | 2.7 A 3.4 A |  | 0.62 | 0.493 |
| C:65:Ser | E:844:Glu | 3.2 A | 1x hb to E:844:Glu | 0 | 0.378 |
| C:72:Thr | E:848:Ala | 3.6 A |  | 0.61 | 0.732 |
| C:74:Thr | E:844:Glu | 3.5 A |  | 0.79 | 0.654 |
| C:107:Lys | E:867:Asp E:868:Leu E:869:Glu | 2.6 A 2.9 A 3.8 A | 3x clash to E:867:Asp 1x hb to E:868:Leu | 0.7 | 0.586 |
| C:143:Glu | E:878:Lys | 2.8 A | 1x salt bridge to E:878:Lys | 0 | 0.243 |
| E:843:Phe | C:18:Arg C:20:Thr | 3.2 A 3.6 A |  | 0.87 | 0.924 |
| E:844:Glu | C:65:Ser C:74:Thr C:20:Thr | 3.2 A 3.5 A 3.6 A | 1x hb to C:65:Ser | 0.87 | 0.655 |
| E:847:Thr | C:20:Thr | 3.8 A |  | 0.78 | 0.785 |
| E:848:Ala | C:72:Thr | 3.6 A |  | 0.48 | 0.707 |
| E:851:Tyr | C:11:Leu C:20:Thr C:8:Pro | 3.4 A 3.5 A 3.6 A | 1x clash to C:8:Pro | 0.69 | 0.989 |
| E:852:Arg | C:24:Arg C:70:Asp | 3.4 A 4.0 A |  | 0.4 | 0.336 |
| E:855:Asp | C:24:Arg C:7:Ser | 2.7 A 2.9 A | 1x hb to C:7:Ser | 0.49 | 0.658 |
| E:864:Tyr | C:7:Ser C:8:Pro | 3.7 A 3.8 A |  | 0.7 | 0.659 |
| E:865:Thr | C:10:Ser | 3.2 A |  | 0.52 | 0.402 |
| E:866:Ala | C:12:Ser C:11:Leu C:10:Ser | 2.9 A 3.0 A 3.4 A | 1x hb to C:10:Ser 1x clash to C:11:Leu 1x hb to C:12:Ser | 0.84 | 1 |
| E:867:Asp | C:107:Lys C:12:Ser | 2.6 A 3.1 A | 1x clash to C:12:Ser 3x clash to C:107:Lys | 0.82 | 0.568 |
| E:868:Leu | C:12:Ser C:107:Lys C:17:Asp C:11:Leu C:18:Arg C:13:Ala | 2.6 A 2.9 A 3.4 A 3.5 A 3.8 A 3.9 A | 2x clash to C:11:Leu 1x hb to C:12:Ser 1x hb to C:107:Lys | 0.84 | 0.958 |
| E:869:Glu | C:107:Lys | 3.8 A |  | 0.71 | 0.114 |
| E:871:Gly | C:18:Arg C:17:Asp | 3.1 A 3.7 A | 1x hb to C:18:Arg | 0.86 | 0.518 |
| E:872:Gly | C:17:Asp | 3.9 A |  | 0.84 | 0.426 |
| E:878:Lys | C:143:Glu | 2.8 A | 1x salt bridge to C:143:Glu | 0 | 0.348 |

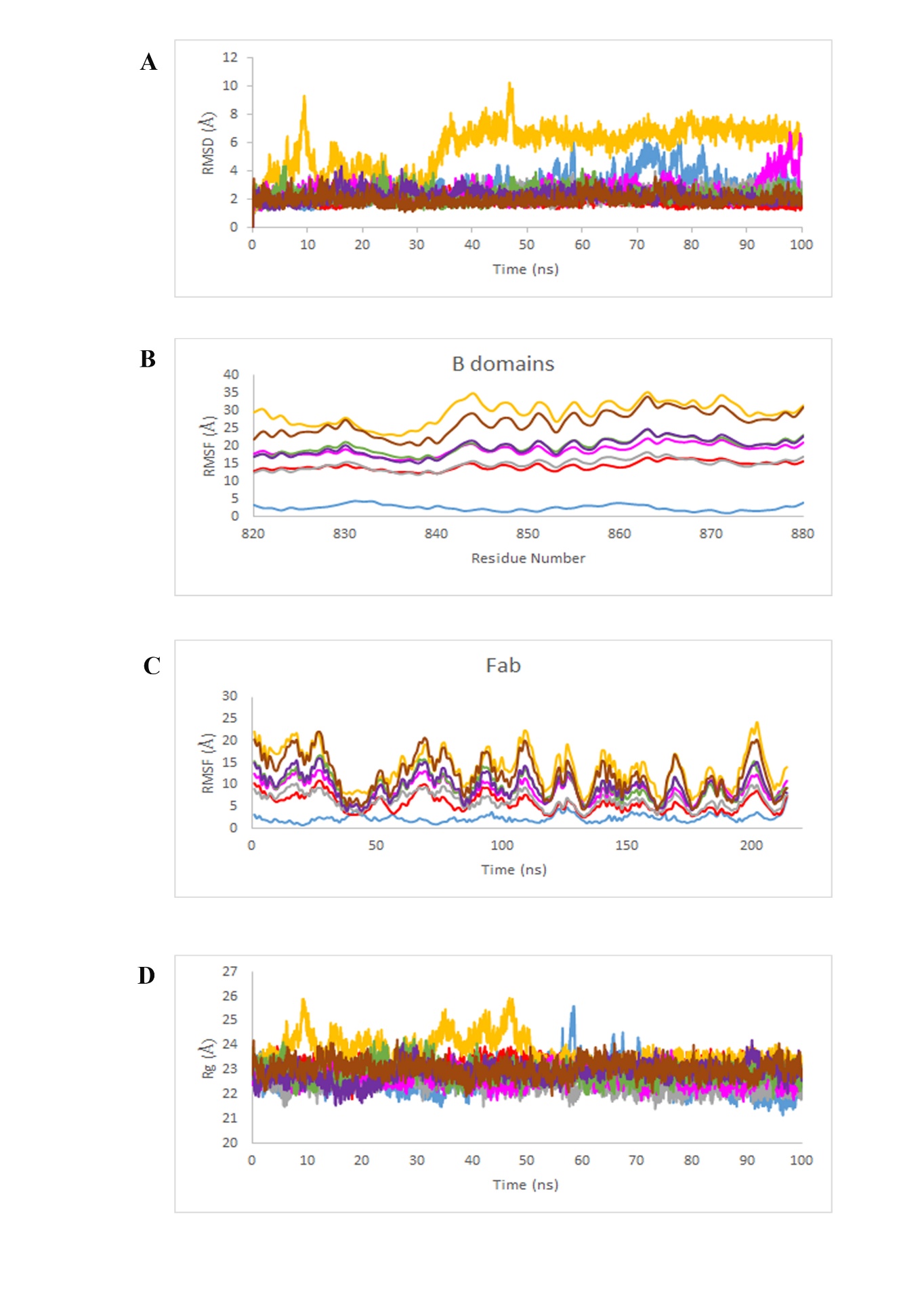
(P) Complex of the wild type and Fab after MDs

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Residue** | **Closest** | **Distance** | **Specific Interactions** | **Surface Complementarity** | **Buried SASA** |
| A:8:Pro | B:864:Tyr B:866:Ala | 3.4 A 3.8 A |  | 0.77 | 0.967 |
| A:9:Ser | B:864:Tyr B:865:Thr | 3.1 A 3.4 A | 1x hb to B:864:Tyr | 0.94 | 0.513 |
| A:10:Ser | B:866:Ala B:865:Thr | 3.1 A 3.5 A | 1x clash to B:865:Thr 1x hb to B:866:Ala | 0.92 | 0.781 |
| A:11:Leu | B:866:Ala B:851:Tyr B:868:Leu | 3.1 A 3.2 A 3.9 A | 1x clash to B:866:Ala | 0.87 | 1 |
| A:12:Ser | B:868:Leu B:866:Ala B:867:Asp | 2.9 A 3.1 A 3.3 A | 1x hb to B:866:Ala 1x hb to B:868:Leu | 0.84 | 0.715 |
| A:13:Ala |  |  |  | 0.77 | 1 |
| A:17:Asp | B:871:Gly B:872:Gly | 3.6 A 3.8 A |  | 0.74 | 0.815 |
| A:18:Arg | B:871:Gly B:868:Leu B:843:Phe B:872:Gly | 3.0 A 3.1 A 3.5 A 3.8 A | 1x hb to B:871:Gly | 0.9 | 0.558 |
| A:19:Val | B:843:Phe | 3.8 A |  | 0.88 | 0.861 |
| A:20:Thr | B:847:Thr B:851:Tyr B:843:Phe | 2.8 A 2.9 A 3.2 A | 1x hb to B:847:Thr 1x hb to B:851:Tyr | 0.63 | 0.973 |
| A:21:Ile |  |  |  | 0.71 | 0.299 |
| A:22:Thr | B:851:Tyr | 3.6 A |  | 0.65 | 0.75 |
| A:24:Arg | B:855:Asp | 2.9 A | 1x hb, 1x salt bridge to B:855:Asp | 0.81 | 0.34 |
| A:72:Thr |  |  |  | 0.43 | 0.719 |
| A:74:Thr | B:843:Phe | 3.9 A |  | 0.64 | 0.601 |
| A:107:Lys | B:868:Leu B:869:Glu B:867:Asp | 2.9 A 3.2 A 3.2 A | 1x hb, 1x salt bridge to B:867:Asp 1x hb to B:868:Leu | 0.84 | 0.509 |
| A:143:Glu | B:878:Lys | 2.6 A | 1x salt bridge, 2x clash to B:878:Lys | 0.56 | 0.524 |
| B:843:Phe | A:20:Thr A:18:Arg A:19:Val A:74:Thr | 3.2 A 3.5 A 3.8 A 3.9 A |  | 0.85 | 0.926 |
| B:844:Glu |  |  |  | 0.61 | 0.566 |
| B:847:Thr | A:20:Thr | 2.8 A | 1x hb to A:20:Thr | 0.62 | 0.803 |
| B:851:Tyr | A:20:Thr A:11:Leu A:22:Thr | 2.9 A 3.2 A 3.6 A | 1x hb to A:20:Thr | 0.72 | 0.974 |
| B:855:Asp | A:24:Arg | 2.9 A | 1x hb, 1x salt bridge to A:24:Arg | 0.81 | 0.506 |
| B:864:Tyr | A:9:Ser A:8:Pro A:7:Ser | 3.1 A 3.4 A 3.6 A | 1x hb to A:9:Ser | 0.75 | 0.841 |
| B:865:Thr | A:9:Ser A:10:Ser | 3.4 A 3.5 A | 1x clash to A:10:Ser | 0.92 | 0.583 |
| B:866:Ala | A:12:Ser A:10:Ser A:11:Leu A:8:Pro | 3.1 A 3.1 A 3.1 A 3.8 A | 1x hb to A:10:Ser 1x clash to A:11:Leu 1x hb to A:12:Ser | 0.82 | 0.922 |
| B:867:Asp | A:107:Lys A:12:Ser | 3.2 A 3.3 A | 1x hb, 1x salt bridge to A:107:Lys | 0.89 | 0.564 |
| B:868:Leu | A:107:Lys A:12:Ser A:18:Arg A:11:Leu | 2.9 A 2.9 A 3.1 A 3.9 A | 1x hb to A:12:Ser 1x hb to A:107:Lys | 0.8 | 0.976 |
| B:869:Glu | A:107:Lys | 3.2 A |  | 0.86 | 0.2 |
| B:871:Gly | A:18:Arg A:17:Asp | 3.0 A 3.6 A | 1x hb to A:18:Arg | 0.85 | 0.588 |
| B:872:Gly | A:17:Asp A:18:Arg | 3.8 A 3.8 A |  | 0.87 | 0.832 |
| B:875:Met |  |  |  | 0.66 | 0.114 |
| B:878:Lys | A:143:Glu | 2.6 A | 1x salt bridge, 2x clash to A:143:Glu | 0.59 | 0.406 |

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Figure S1. LigPlot analysis to determine the molecular interactions between the wild and mutated B domains and Fab using LigPlot. Interactions of the MW-Fab (A), the MB1-Fab (B), the MB2-Fab (C), the MB3-Fab (D), the MB4-Fab (E), the MB5-Fab (F), the MB6-Fab (G), the MB7-Fab (H) were displayed using LigPlot. Residues of the wild and mutated B domains (pink), Fab fragment (red), hydrogen bonds (green bonds) and electrostatic interactions (red bond) were illustrated.



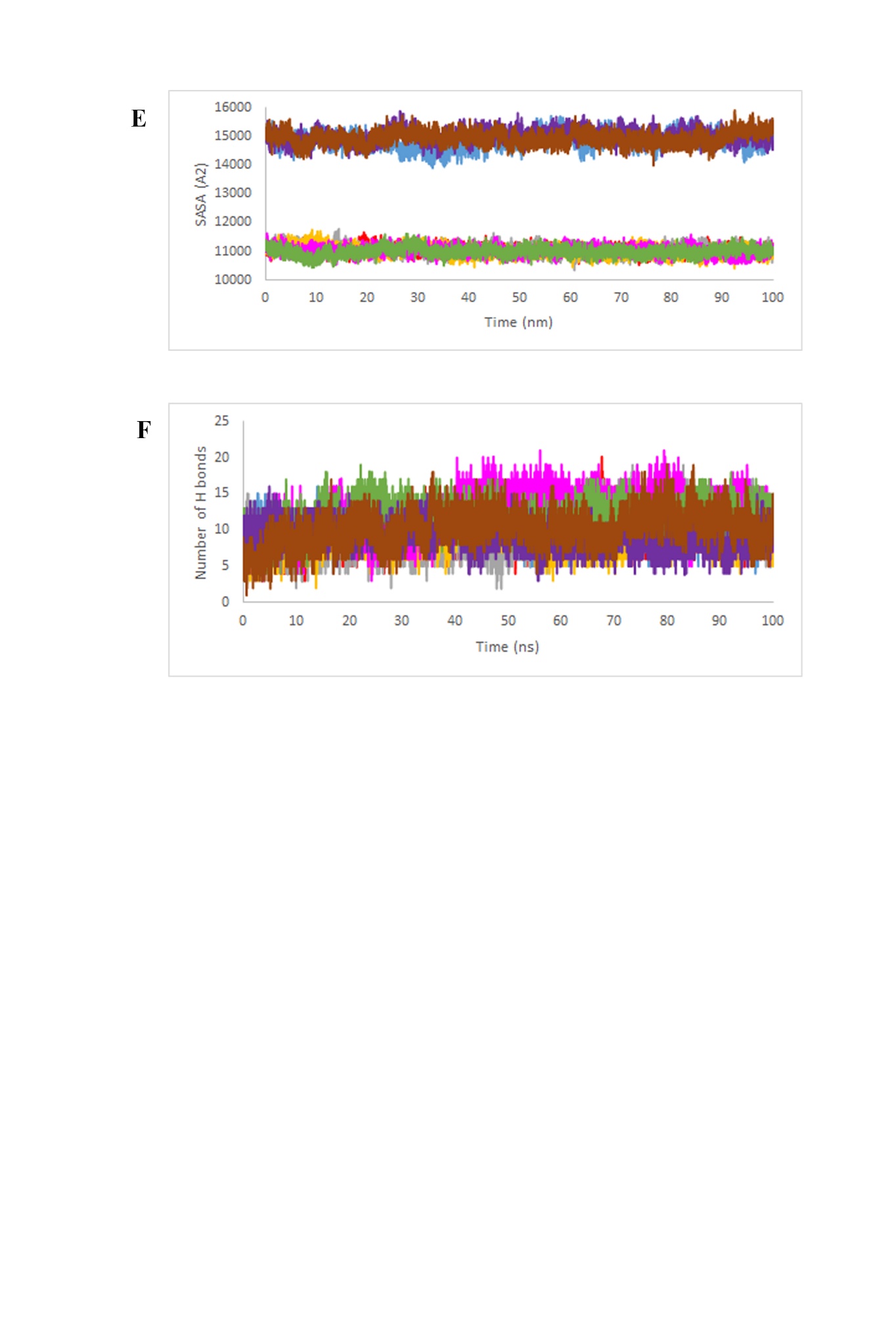


Figure S2. Molecular dynamics simulation of the wild B domain-Fab and the mutated B domains-Fab complexes for 100 ns. (A) The RMSD plot, (B) The RMSF plot, (C) The radius of gyration plot, (D) SASA plot, (E) Hydrogen bond analysis were illustrated. The WB-Fab complex (blue), the MB1-Fab complex (red), the MB2-Fab complex (gray), the MB3-Fab complex (yellow), the MB4-Fab complex (pink), the MB5-Fab complex (green), the MB6-Fab complex (purple), the MB7-Fab complex (brown) were represented during MD simulation.

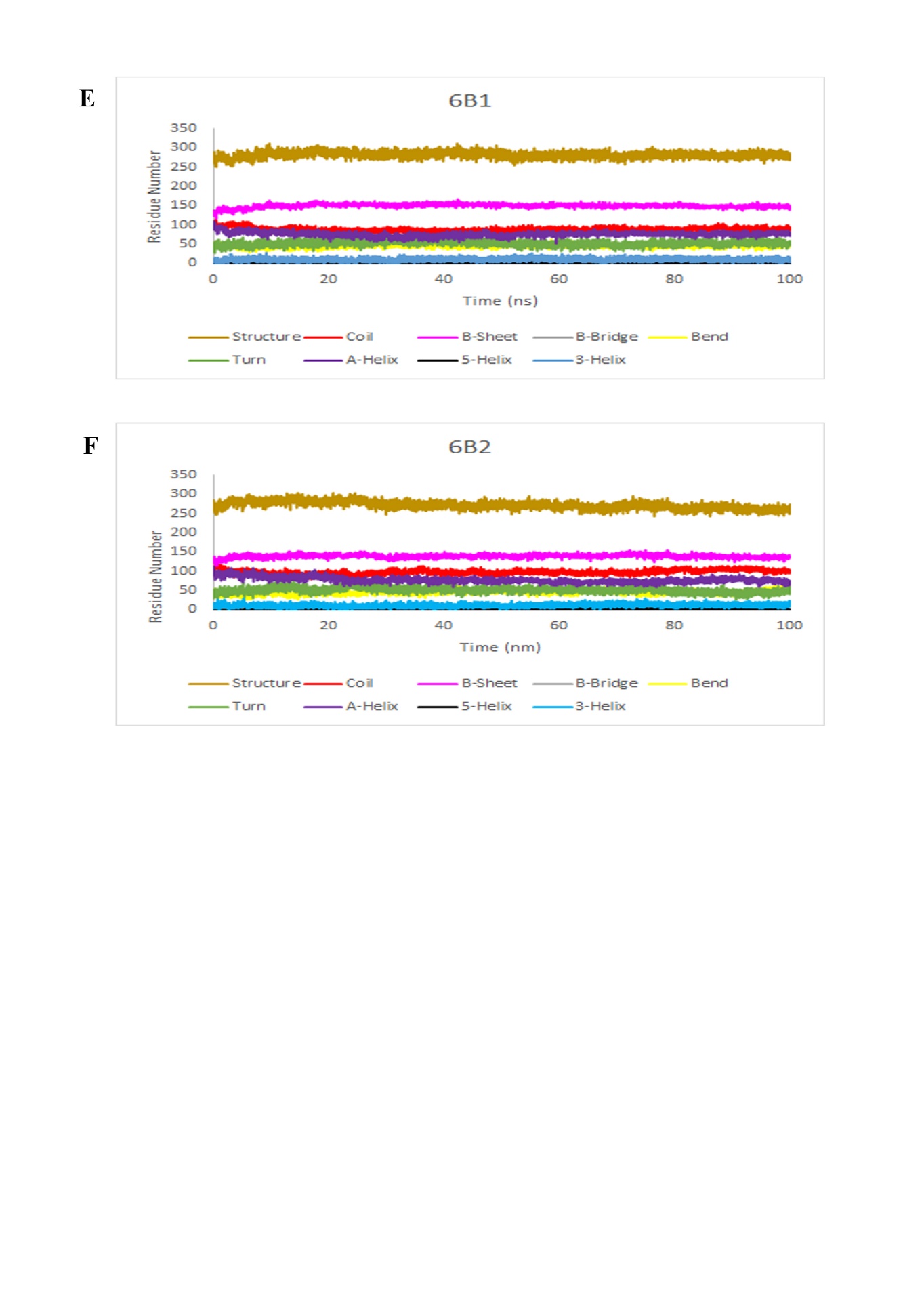


Figure S3. Molecular dynamics simulation of the designed Proteins L including 6B0, 6B1 and 6B2 for 100 ns. (A) The RMSD plot, (B) The RMSF plot, (C) The radius of gyration plot, (D,E,F) The secondary structure using DSSP were indicated. The 6B0 (blue), the 6B1 (purple) and the 6B2 (gray) showed the designed Proteins L during MD simulation.

Table S5. The participant residues in different structures of the modeled proteins L during 100ns of MDs (all values are in percent (%)).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Variant** | **Sructure** | **Coil** | **β-sheet** | **Β-bridge** | **Bend** | **Turn** | **Α-helix** | **3-helix** |
| **6B0** | 66 | 19 | 34 | 1 | 11 | 13 | 18 | 4 |
| **6B1** | 66 | 21 | 35 | 1 | 11 | 12 | 18 | 2 |
| **6B2** | 63 | 23 | 33 | 0 | 11 | 12 | 18 | 3 |