## Supplementary Materials

## Ewald et al., A Combined NMR and Computational Approach to Investigate Peptide Binding to a Designed Armadillo Repeat Protein

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## 1. Sequence details



Figure S1 The sequence of proteins based on $Y_{I} M R{ }^{1} R^{2} R^{3} M A_{I I}$ and associated modifications and mutations. The proteins were either produced with a non-cleavable his tag as encoded in the vector pPANK (first line) or a cleavable his tag as encoded in the vector pLIC (second line). After cleavage, only a single Gly is left. For example, the sequence of $Y_{I} M R{ }^{1} R^{2} R^{3} M A_{I I}$ includes the non-cleavable $\mathrm{His}_{6}$-tag followed by the sequence of the capping and repeat sequences given in the subsequent lines.

Shorter versions, for example $\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{~A}_{\mathrm{II}}$, in turn include the sequences of the pPANK-based His $_{6}$-tag, the $Y_{I} N$-cap, the first unrandomized M-type repeat, randomized repeats $R^{1}$ and $R^{2}$ and the $\mathrm{A}_{\text {II }} \mathrm{C}$-cap. N-terminally truncated proteins (not containing an N-cap) are headed by a TEV-cleavable His $_{6}$-tag (pLIC-based), which leaves a single N -terminal Gly after cleavage, and include the repeats indicated by their name, e.g. $\mathrm{R}^{3} \mathrm{MA}_{\mathrm{II}}$. Due to the glycine residue left after TEV-cleavage (ENLYFQ/G) at the N -terminus of the fragment, N-terminally truncated proteins effectively start with the last glycine of the preceding repeat, which is naturally present in the sequence. To facilitate cloning, the $M R^{1} R^{2} \mathrm{R}^{3} \mathrm{MA}_{\text {II }}$ fragment starts two residues N -terminal of the glycine, adding " RD " to the sequence. C-terminally truncated proteins end at position 41 of their last respective internal repeat, e.g. $\mathrm{Y}_{\mathrm{I}} M \mathrm{R}^{1} \mathrm{R}^{2}$ ends with the glutamate in position 41 of the $\mathrm{R}^{2}$ repeat.

Helices 1, 2 and 3 of each repeat and the C-cap are indicated by light blue boxes. the N-cap only contains two helices, H1 and H2. Randomized positions in H3 of internal repeats are indicated in red. Spontaneous point mutations acquired during ribosome display are indicated in green. Sites of mutations introduced in different cap generations to improve protein stability are indicated in bold and underlined: V34, R37 and R42 (continuous numbering including pPANK-based His ${ }_{6}$-tag) are mutated to convert a $Y_{I} N$-cap into a $Y_{\text {II }} N$-cap (R34, S37, R42 4 ); The LQ motif at the end of the $A_{\text {II }} \mathrm{C}$-cap corresponds to QF in a $A_{I}$ C-cap. Conserved asparagine positions in helix 3 of each repeat are N 81 , N123, N165, N207 and N249.

Table S1: Peptides, uncapped (unless indicated otherwise).

| Peptide name | Sequence | Purpose |
| :--- | :--- | :--- |
| NT | pGlu-LYENKPRRPYIL | CSP and PRE-studies |
| NT7-13 | Ac-PRRPYIL | CSP |
| NTQ1C | CLYENKPRRPYIL | PRE-studies |
| NTK6C | pGlu-LYENKPRRPYIC | PRE-studies |
| NTL13C | pGlu-LYENCPRRPYIL | PRE-studies |
| NT $_{\text {biotin }}$ | Biotin-6ACA- $\beta$ A- $\beta$ A-NT | ELISA |

Ac, acetyl; 6ACA, 6-amino-caproic acid; $\beta$ A, beta-alanine; CSP, chemical shift perturbations; ELISA, enzyme linked immunosorbent assay; NT, neurotensin; pGlu, pyro-glutamate; PRE, paramagnetic relaxation enhancement.

Table S2 Details of Cloning: Oligonucleotides

| Name | Sequence 5'-3' direction | Purpose |
| :---: | :---: | :---: |
| 078_RMAm_FWD | GAA AAT TTA TAT TTT CAG GGT AAC GAA CAA CAA | Construction of $\mathrm{R}^{3} \mathrm{MA}_{\text {II }}$ from $\mathrm{pPANK} \mathrm{N}_{\mathrm{I}} \mathrm{M} 328 \mathrm{MC}_{\text {II }}$ |
| 077_R23MAm_FWD | GAA AAT TTA TAT TTT CAG GGT AAC GAA CAA ACC | Construction of $R^{2} R^{3} M A_{I I}$ and $R^{1} R^{2} R^{3} M A_{I I}$ from pPANK_N ${ }_{\mathrm{I}} \mathrm{M} 328 \mathrm{MC}_{\mathrm{II}}$ |
| 090_MRRRMAm_v10_FWD | GAA AAT TTA TAT TTT CAG GGT CGT GAT GGT AAC GAA CAA | Construction of $M R^{1} R^{2} R^{3} M A A ~ I I ~_{\text {II }}$ from pPANK_N ${ }_{\mathrm{I}} \mathrm{M} 328 \mathrm{MC}_{\mathrm{II}}$ |
| 088_RxMAm_v3_REV | AGA TGA GAG TAA GGC TAT CAT TAG TGG GAC TGC AG | Reverse primer for $077\left(\mathrm{R}^{2} \mathrm{R}^{3} \mathrm{MA}_{\text {II }}\right), 078\left(\mathrm{R}^{3} \mathrm{MA}_{\text {II }}\right)$ and $090\left(\mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{R}^{3} \mathrm{MA}_{\mathrm{II}}\right)$ |
| 053_B328_YMR ${ }_{\text {x_p }}$ pPANK_Fwd | TAA TGA GGT ACC CCG GGT CGA CCT GCA GCC | Construction of $Y_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{R}^{3}$ and $\mathrm{Y}_{\mathrm{I}} M \mathrm{R}^{1}$ from pPANK_NyM328MCa encoding VG_328 |
| 054_B328_YMRRR_pPANK_REV | ```CAG TTC AGC GAT GTT AGT CAG AGC GTC CAG``` | Reverse primer for $053\left(\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{R}^{3}\right)$ and 101 $\left(Y_{I} M R^{1} R^{2} R^{3} A_{I I}\right)$ |
| 056_B328_YMR_pPANK_REV | ```AGC GAA AGC GAT GTT GTT CAG AGC GAT AAG``` | Reverse primer for $101\left(\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{~A}_{\mathrm{II}}\right)$ and $053\left(\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1}\right)$ |
| 063_YMRR_REV | TTC CAT AGC GAT GTT AGT C | Reverse primer for $101\left(\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{~A}_{\mathrm{II}}\right)$ and 65 $\left(\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1}{ }^{1}{ }^{2}\right)$ |
| 065_YMRR_FWD | TAA TGA GGT ACC CCG Gg | Construction of $Y_{1} M R^{1} \mathrm{R}^{2}$ from pPANK_ $\mathrm{Y}_{\mathrm{I}} M \mathrm{R}^{1} \mathrm{R}^{2} \mathrm{R}^{3}$ |
| 101_AtypeC-cap_FW | GGT AAC GAA CAG AAA CAG GCT GTT AAA GAA G | Construction of $Y_{I} M R^{1} R^{2} R^{3} A_{I I}, Y_{I} M R^{1} R^{2} A_{I I}$, $\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{~A}_{\text {II }}$ from $\mathrm{pPANK} \mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{R}^{3} \mathrm{MA}_{\text {II }}$ |
| 118_delR42_FW | TGA TGG TAA CGA ACA AAT CC | Deletion of R42 from $\mathrm{Y}_{\mathrm{I}}$-cap |
| 119_delR42_RV | GAC AGG ATC TGA CGG AAT TTA AC | Reverse primer for 118 |
| 121_R42A_FW | AGA TCC TGT CTG CTG ATG GTA ACG A | Introduction of R42A into $\mathrm{Y}_{\mathrm{I}}$-cap |
| 122_R42A_RV | GAC GGA ATT TAA CGG TAG CAG | Reverse primer for 121 |
| 124_V34R_R37S_RV | AgC TGT TCC TGC ATG TCG TCG GAG TTC | Reverse primer for 139 and 140 |
| 139_Yf_V24R_FWD | GTC TGC TAC CCG TAA ATT CCG TCA GAT CCT G | Introduction of V34R into $\mathrm{Y}_{\mathrm{I}}$-cap and $\mathrm{Y}_{\mathrm{I}}$-cap_R42del |
| 140_Yf_R27S_FWD | GTC TGC TAC CGT TAA ATT CTC TCA GAT CCT G | Introduction of R37S into $\mathrm{Y}_{\mathrm{I}}$-cap and $\mathrm{Y}_{\mathrm{I}}$-cap_R42del |
| 141_M_E46A_FWD | GTC TCG TGA TGG TAA CGC ACA <br> AAT CC | Introduction of E46A into $\mathrm{Y}_{\mathrm{I}}$-cap |


| $142 \_M \_E 46 A \_R E V$ | AGG ATC TGA CGG AAT TTA ACG <br> GTA GC | Reverse primer for 141 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| $152 \_V 34 R \_F W D$ | GTC TGC TAC CCG TAA ATT <br> TCA G CTC | Introduction of V34R into Y Y-cap_R37S and <br> $Y_{\text {I }}$-cap_R37S_R42del |
| $153 \_V 34 R \_R E V$ | AGC TGT TCC TGC ATG TCG TCG | Reverse primer for 152 |

## 2. Details of Cloning procedures

Polymerase chain reactions (PCR) were usually performed with $200 \mu \mathrm{M}$ of total dNTPs, $1.5 \mu \mathrm{M}$ of forward and reverse primers and 50 ng of template. Taq polymerase was used for colony-PCRs, Pfu polymerase for site-directed mutagenesis and general high fidelity DNA amplification. Point mutations were introduced by whole plasmid amplification using non-overlapping primers, one of which introduced the mutation, and blunt end ligation. All plasmids were verified by DNA sequencing.

DNA restriction, phosphorylation and ligation were performed according to the manufacturer's instructions (New England Biolabs, USA, Fermentas, Lithuania or Promega, USA). DNA quality and size was assessed on ethidium bromide stained ( $0.1 \mu \mathrm{~g} / \mathrm{ml}$ ) $1 \%$ agarose gels. PCR products were purified using standard DNA kits (Macherey-Nagel, Germany, or Roche, Switzerland). DNA concentrations were determined by UV absorption spectroscopy at 260 nm (ND-1000 spectrophotometer, Nanodrop Technologies, USA).
pLIC_CR [31] and pPANK, a pQE30 derivative lacking the BpiI and BsaI endonuclease restriction recognition sites (GenBank accession number AY327140) were used as cloning and protein expression vectors. All pPANK-based vectors introduce an $\mathrm{MRGSH}_{6}$-tag at the N-terminus of the proteins. pLIC-based vectors carry a TEV protease cleavable N-terminal $\mathrm{H}_{6}$-tag (see Figure S 1 ).

## Cloning of $\mathbf{Y}_{\mathbf{I}} \mathbf{M R}^{1} \mathbf{R}^{\mathbf{2}} \mathbf{R}^{\mathbf{3}} \mathbf{M A}_{\text {II }}$ Fragments

pPANK_YIMR ${ }^{1} \mathrm{R}^{2} \mathrm{R}^{3} \mathrm{MA}_{\text {II }}$ has previously been established from pPANK_NyM328MCa [23] by introducing two point mutations (Q292L, F293Q) [25] in the $\mathrm{A}_{\mathrm{I}}$-type C-cap.

N-terminal fragments ( $\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1}, \mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{R}^{2}, \mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{R}^{3}$ ) were cloned by deleting the unrequired C-terminal repeats from previously established pPANK_NyM328MCa encoding $Y_{I} M R^{1} R^{2} R^{3} M A A_{I}$. C-cap primers 053 and 056 were used to create $\mathrm{YMR}^{1} \mathrm{R}^{2} \mathrm{R}^{3}$ (template: pPANK_NyM328MCa), 054 and 056 for YMR $^{1}$ (template: pPANK_NyM328MCa) and 63 and 65 for $Y_{M R}{ }^{1} R^{2}$ (template: pPANK_Y $Y_{I} M{ }^{1} R^{2} R^{3}$ ) (Table S2). Primers were designed to
allow amplification of the whole expression plasmid excluding the sequence to be deleted. The PCR product was then treated with DpnI (Fermentas) to remove template DNA, treated with PNK T4-Kinase (Promega) and blunt end ligated to re-create a circular pPANK based plasmid. C-terminal fragments ( $R^{3} M A_{I I}, R^{2} R^{3} M A_{I I}, R^{1} R^{2} R^{3} M A_{\text {II }}$ and $M R^{1} R^{2} R^{3} M A_{I I}$ ) were amplified from pPANK_N $\mathrm{N}_{\mathrm{I}} \mathrm{M} 328 \mathrm{MC}_{\text {II }}$ using primers 078 and 088 , ( $\mathrm{R}^{3} \mathrm{MA}_{\text {II }}$ ), 077 and 088 ( $R^{2} R^{3} M A_{\text {II }}$ and $R^{1} R^{2} R^{3} M A_{\text {II }}$ ) and primers 090 and $088\left(M R^{1} R^{2} R^{3} M A_{\text {II }}\right.$ ) (Table S2). Primers were designed to allow ligation independent cloning (LIC) [Aslanidis and Jong, 1990, Nucl. Acids. Res. 18(1990), 6069-74] into the pLIC_CR vector [31] to introduce a cleavable N-terminal His -tag for IMAC purification and undisturbed fragment interaction. pLIC_CR $^{\text {-ta }}$ contains an $\mathrm{N}^{-t e r m i n a l ~ M R G S H} 6$-tag followed by the TEV protease recognition site and the $\operatorname{sac} B$ gene as an additional negative selection marker. pLIC_CR_MA for the expression the C-terminal MA has been previously established by Watson et al. [31]. For LIC cloning 100 ng of pLIC_CR was BsaI digested, and treated with T4-DNA-polymerase in the presence of dCTP. The target PCR fragment was treated with T4-DNA-polymerase and dGTP. Prepared plasmid and insert DNA were column purified (High Pure PCR Product Purification kit, Roche), mixed at approximately 1:1 molar ratio and incubated for 10 min at room temperature and then transformed into E. coli XL1-blue cells. Plasmids obtained from colonies on LB agar plates containing $100 \mu \mathrm{~g} / \mathrm{ml}$ ampicillin and $7 \%$ sucrose were sequenced for correctness.

## Cloning of $\mathbf{Y}_{I} \mathbf{M R}{ }^{1} \mathbf{R}^{2} \mathbf{R}^{\mathbf{3}} \mathbf{A}_{\text {II }}, \mathbf{Y}_{\mathrm{I}} \mathbf{M R}{ }^{\mathbf{1}} \mathbf{R}^{\mathbf{2}} \mathbf{A}_{\text {II }}$ and $\mathbf{Y}_{\mathrm{I}} \mathbf{M R}{ }^{\mathbf{1}} \mathbf{A}_{\text {II }}$

Vectors for $Y_{I} M R{ }^{1} R^{2} R^{3} A_{I I}, Y_{I} M R{ }^{1} R^{2} A_{I I}$ and $Y_{I} M R{ }^{1} A_{\text {II }}$ were cloned by deleting the unrequired internal repeats from $Y_{I} M R^{1} R^{2} R^{3} M A_{I I}$ via a blunt end ligation strategy. Primers were designed to allow amplification of the whole expression plasmid excluding the sequence to be deleted. The PCR product was then treated with DpnI (Fermentas) to remove template DNA, treated with PNK T4-Kinase (Promega) and blunt end ligated to re-create a circular pPANK based plasmid. pPANK_YIMR ${ }^{1} R^{2} R^{3} A_{I I}$, was amplified from pPANK_ $Y_{I} M R^{1} R^{2} R^{3} A_{I I}$ using primers 101/054. pPANK_Y $\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{~A}_{\text {II }}$ and $\mathrm{pPANK} \mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{~A}_{\text {II }}$ were amplified from pPANK_Y $\mathrm{Y}_{\mathrm{I}} M \mathrm{R}^{1} \mathrm{R}^{2} \mathrm{R}^{3} \mathrm{~A}_{\text {II }}$ using primers 101/063 and 101/056 respectively (See Table S 2 ).

## Cloning of $\mathbf{Y}_{I} \mathbf{M R}^{1} \mathbf{R}^{2} \mathbf{A}_{\text {II }}$-Mutants

The following single, double and triple point mutations in $\mathrm{pPANK} \mathrm{Y}_{\mathrm{I}} M \mathrm{R}^{1} \mathrm{R}^{2} \mathrm{~A}_{\text {II }}$ were introduced by whole plasmid amplification and blunt end ligation as described above using primers indicated in Table S2: V34R, R37S, R42A, R42A, E46A, V34R/R37S, V34R/R42A, R37S/R42 $\Delta$ and V34R/R37S/R42 $\left(=Y_{\text {II }} M R^{1} \mathrm{R}^{2} \mathrm{~A}_{\mathrm{II}}\right)$.

## 3. Spectroscopic details



Figure S2: $\left[{ }^{15} \mathrm{~N},{ }^{1} \mathrm{H}\right]$-HSQC spectra showing chemical shift perturbations of $400 \mu \mathrm{M}{ }^{15} \mathrm{~N}$-labelled $Y_{I} M R^{1} R^{2} R^{3} \mathrm{MA}_{\text {II }}$ (VG_328) without (black) and with (red) two equivalents of NT. Details for the Gly (A) and Trp indole (B) regions are shown in Figure 3A and B.


Figure S3: $\left[{ }^{15} \mathrm{~N},{ }^{1} \mathrm{H}\right]$-HSQC spectra of ${ }^{15} \mathrm{~N}$-labelled N -terminally truncated $\mathrm{Y}_{\mathrm{I}} \mathrm{MR}{ }^{1} \mathrm{R}^{2} \mathrm{R}^{3} \mathrm{MA}_{\text {II }}$ fragments. All spectra were recorded at $32^{\circ} \mathrm{C}, 300 \mu \mathrm{M}$ concentration.


Figure $\mathbf{S 4}\left[{ }^{15} N,{ }^{1} \mathrm{H}\right]$-HSQC spectra of $\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{R}^{3} \mathrm{MA}^{\text {II }}$ (top left), $\mathrm{Y}_{\mathrm{I}} M \mathrm{R}^{1} \mathrm{R}^{2} \mathrm{R}^{3} \mathrm{~A}^{\text {II }}$ (top right), $\mathrm{Y}_{\mathrm{I}} M \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{~A}^{\text {II }}$ (bottom left) and $\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{~A}^{\mathrm{II}}$ (bottom right).


Figure S5: $\left[{ }^{15} \mathrm{~N},{ }^{1} \mathrm{H}\right]$-HSQC spectra showing chemical shift perturbations of $400 \mu \mathrm{M}{ }^{15} \mathrm{~N}$ labelled $\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{~A}_{\text {II }}$ without (black) and with (red) two equivalents of NT.

## 4. SPR Data:



Figure S6: SPR response curves (top) and fitted data (bottom) for $\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{~A}_{\mathrm{II}}(\mathrm{A}, \mathrm{B}, 14 \mu \mathrm{M})$ and $\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{R}^{3} \mathrm{MA}_{\text {II }}(\mathrm{C}, \mathrm{D}, 18 \mu \mathrm{M})$ at $8{ }^{\circ} \mathrm{C}$.

## 5. Chemical shift perturbation (CSP) data



Figure S7: Representative fits from CSP raw data of $Y_{I} M R{ }^{1} R^{2} A_{\text {II }}$ residues used for $k_{d}$ determination; G86 (empty circle), G128 (empty diamond), L76 (filled diamond), S66 (filled triangle), A59 (filled circle), A92 (empty triangle).


Figure S8: A Appropriately weighted combined chemical shift changes $\Delta \delta(\mathrm{HN})$ in ${ }^{15} \mathrm{~N}$ and ${ }^{1} \mathrm{H}$ chemical shift (see Methods and Materials) upon addition of 2 equiv. of NT to $\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{~A}_{\mathrm{II}}$. Red bars indicate unquantifiable CSPs when assignments are only available in the presence of NT. Prolines are indicated by "P", unassigned residues by "*" B Difference between ${ }^{1} H$ chemical shift changes $\Delta \Delta \delta(H)$ of $Y_{I} M R{ }^{1} R^{2} A_{I I}$ in presence of 2 equiv. NT and in presence of 2 equiv. NTK6C-MTSL

## 6. Molecular Docking results



Figure S9: Parallel (A) and antiparallel (B) binding poses of NT7-13 (sticks) from automatic docking to $\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{~A}_{\text {II }}$ (green ribbon). The peptide was initially placed arbitrarily within the indicated box (see methods).

## 7. PRE results from additional spin-labeled NT forms



Figure S10: Interaction of $\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{~A}_{\text {II }}$ with NTQ1C-MTSL (A) and with NTL13C-MTSL (B) as probed by PREs. For further details see the caption of Figure 5 in the main paper.


Figure S11: Location of bound MTSL by differential chemical shift perturbation ( $\Delta \Delta \delta\left({ }^{1} \mathrm{H}\right)$, see Methods) mapped onto $\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{~A}_{\mathrm{II}}$ complexed with either unlabeled NT or with NT coupled to quenched MTSL. Positive and negative values of $\Delta \Delta \delta$ are depicted in blue and yellow, respectively for MTSL attached at position 6 (A) or at position 13 (B).

## 8. MD Simulations

Multiple MD runs were carried out in the absence and presence of the peptide. The MD simulations for determining the binding mode of $\mathrm{NT}(7-13)$ to $\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{~A}^{\mathrm{II}}$ were started from the parallel or the antiparallel orientation of $\mathrm{NT}(7-13)$. The same simulation protocol was used in both cases (see Molecular Dynamics Simulations section in Materials \& Methods). The two independent simulations of $2 \mu$ s each for the parallel orientation with the wild type $Y_{I} M R{ }^{1} R^{2} A^{\text {II }}$ used different random seeds for the initial assignment of the velocities.

Table S3: MD simulations performed to probe the intrinsic plasticity of armadillo proteins in the absence of peptide (apo), and to determine the binding mode of the NT7-13 peptide.

| system | Binding mode | Time <br> ( $\mu \mathrm{s}$ ) | Temperature <br> (K) | Force field | $\begin{aligned} & \text { vdW cutoff } \\ & (\mathrm{nm}) \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathrm{Y}_{\mathrm{I}} \mathrm{MR}{ }^{1} \mathrm{R}^{2} \mathrm{~A}_{\text {II }}$ | apo | 2 | 310 | Charmm36 | 1.0 |
|  |  | 1 | 330 | OPLS | 0.9 |
| $\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{~A}_{\text {II_ }}$ V34R | apo | 2 | 310 | Charmm36 | 1.0 |
| $\mathrm{Y}_{\mathrm{I}} \mathrm{MR}{ }^{1} \mathrm{R}^{2} \mathrm{~A}_{\text {II_ }} \mathrm{R} 42 \Delta$ | apo | 2 | 310 | Charmm36 | 1.0 |
| $\mathrm{Y}_{\text {III }} \mathrm{M}_{3} \mathrm{~A}_{\text {II }}$ | apo | 2 | 310 | Charmm36 | 1.0 |
|  |  | 1 | 330 | OPLS | 0.9 |
| $\mathrm{Y}_{\mathrm{I}} \mathrm{MR}{ }^{1} \mathrm{R}^{2} \mathrm{~A}_{\text {II_ }} \mathrm{R} 37 \mathrm{~S}$ | apo | 2 | 310 | Charmm36 | 1.0 |
| $\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{~A}_{\text {II }}-\mathrm{NT} 7-13$ | parallel | $2 \times 2$ | 310 | Charmm36 | 1.0 |
| $\mathrm{Y}_{\mathrm{I} M R}{ }^{1} \mathrm{R}^{2} \mathrm{~A}_{\text {II }}-\mathrm{NT} 7-13$ | antiparallel | 2 | 310 | Charmm36 | 1.0 |
| $\begin{gathered} \mathrm{Y}_{\mathrm{I}} \mathrm{MR}{ }^{1} \mathrm{R}^{2} \mathrm{~A}_{\mathrm{II}-} \mathrm{R} 37 \mathrm{~S}- \\ \mathrm{NT} 7-13 \end{gathered}$ | parallel | 2 | 310 | Charmm36 | 1.0 |

Table S4: Average interaction energy between residues of NT(7-13) and their main interacting partners of $\mathbf{Y}_{\mathbf{I}} \mathbf{M} \mathbf{R}^{\mathbf{1}} \mathbf{R}^{\mathbf{2}} \mathbf{A}_{\text {II }}$ (parallel orientation). The interaction energy is the sum of the Coulomb energy and the van der Waals energy. The energies are averaged over a $2-\mu \mathrm{s}$ trajectory and the values larger than $4 \mathrm{~kJ} / \mathrm{mol}$ are shown; the errors are the standard deviation. Values from two independent simulations are shown in plain and italics letters, respectively. Energies larger than $12 \mathrm{~kJ} / \mathrm{mol}(\sim 5 \mathrm{kT})$ are shown in bold letters.

| $\begin{aligned} & \hline \text { NT- } \\ & Y_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{~A}^{\mathrm{II}} \\ & \hline \end{aligned}$ | res-res ${ }^{1}(\mathrm{~kJ} / \mathrm{mol})$ | $\mathrm{bb}-\mathrm{bb}^{2}(\mathrm{~kJ} / \mathrm{mol})$ | $\mathrm{bb}-\mathrm{sc}^{3}(\mathrm{~kJ} / \mathrm{mol})$ | $\mathrm{sc}-\mathrm{bb}^{4}(\mathrm{~kJ} / \mathrm{mol})$ | $\mathrm{sc}-\mathrm{sc}^{5}(\mathrm{~kJ} / \mathrm{mol})$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| P7-W77 | -14 $\pm 6$ | $-1 \pm 1$ | -4 $\pm 4$ | -2 $\pm 2$ | -7 $\pm 4$ |
|  | $-13 \pm 4$ | $0 \pm 0$ | $-2 \pm 1$ | -4 $\pm 2$ | $-7 \pm 3$ |
| P7-S80 | $-13 \pm 11$ | -2 $\pm 2$ | -10 $\pm 10$ | -1 $\pm 1$ | $0 \pm 2$ |
|  | $-22 \pm 11$ | $-2 \pm 1$ | $-19 \pm 10$ | $-1 \pm 1$ | $0 \pm 1$ |
| P7-N81 | $-4 \pm 3$ | $0 \pm 0$ | $-1 \pm 2$ | $-1 \pm 1$ | $-2 \pm 2$ |
|  | $-7 \pm 2$ | $-1 \pm 0$ | $-1 \pm 1$ | $-2 \pm 1$ | $-4 \pm 2$ |
| P7-Y116 | -4 $\pm 3$ | $0 \pm 0$ | $-1 \pm 2$ | $0 \pm 0$ | $-2 \pm 2$ |
|  | -- | -- | -- | -- | -- |
| P7-I119 | $-5 \pm 2$ | $0 \pm 0$ | $-3 \pm 2$ | $0 \pm 0$ | $-1 \pm 1$ |
|  | $-4 \pm 2$ | $0 \pm 0$ | $-3 \pm 1$ | $0 \pm 0$ | $0 \pm 0$ |
| P7-N123 | $-7 \pm 6$ | $0 \pm 0$ | $-7 \pm 6$ | $0 \pm 0$ | $0 \pm 0$ |
|  | $-10 \pm 4$ | $0 \pm 0$ | $-9 \pm 4$ | $0 \pm 0$ | $0 \pm 0$ |
| R8-S84 | -4 $\pm 4$ | $0 \pm 0$ | $-3 \pm 3$ | $0 \pm 0$ | $-1 \pm 1$ |
|  | $-8 \pm 3$ | $0 \pm 0$ | $-7 \pm 3$ | $0 \pm 0$ | $0 \pm 0$ |
| R8-I119 | $-9 \pm 3$ | $0 \pm 0$ | $-1 \pm 1$ | $-2 \pm 1$ | $-6 \pm 2$ |
|  | -9 $\pm 3$ | $0 \pm 0$ | $-1 \pm 0$ | $-2 \pm 1$ | $-5 \pm 3$ |
| R8-N122 | $8 \pm 4$ | $0 \pm 0$ | $0 \pm 0$ | $0 \pm 0$ | $8 \pm 4$ |
|  | $6 \pm 4$ | $0 \pm 0$ | $0 \pm 0$ | $0 \pm 0$ | $6 \pm 4$ |
| R8-N123 | $-11 \pm 6$ | $0 \pm 0$ | $-4 \pm 5$ | $0 \pm 0$ | $-7 \pm 5$ |
|  | $-12 \pm 4$ | $0 \pm 0$ | $-5 \pm 3$ | $0 \pm 0$ | $-7 \pm 3$ |
| R8-E158 | $-147 \pm 7$ | $0 \pm 0$ | $0 \pm 0$ | $0 \pm 0$ | $-147 \pm 7$ |
|  | $-138 \pm 19$ | $0 \pm 0$ | $0 \pm 0$ | $0 \pm 0$ | $-138 \pm 19$ |
| R8-W161 | -- | -- | -- | -- | -- |
|  | -4 $\pm 4$ | $0 \pm 0$ | $0 \pm 0$ | $0 \pm 0$ | $-4 \pm 4$ |
| R9-S41 | $-6 \pm 9$ | $0 \pm 0$ | $0 \pm 0$ | $-6 \pm 9$ | $-1 \pm 2$ |
|  | -- | -- | -- | -- | -- |
| R9-R42 | $-4 \pm 8$ | $0 \pm 0$ | $0 \pm 0$ | $-3 \pm 8$ | $0 \pm 0$ |
|  | - | - | - | - | - |
| R9-D43 | -40 $\pm 52$ | $0 \pm 0$ | $0 \pm 0$ | $0 \pm 2$ | -41 $\pm 52$ |
|  | -- | -- | -- | -- | -- |
| R9-N81 | $-8 \pm 12$ | $0 \pm 0$ | $-1 \pm 2$ | $-2 \pm 2$ | $-6 \pm 10$ |
|  | -- | -- | -- | -- | -- |
| R9-A83 | -- | -- | -- | -- | -- |
|  | $-6 \pm 7$ | $0 \pm 0$ | $0 \pm 0$ | $-5 \pm 7$ | $0 \pm 0$ |


| R9-S84 | -13 $\pm 10$ | $-1 \pm 1$ | $-5 \pm 7$ | -4 $\pm 6$ | $-2 \pm 4$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  | $-14 \pm 6$ | $-1 \pm 1$ | $-3 \pm 3$ | $-7 \pm 4$ | $-3 \pm 3$ |
| R9-G85 | $-5 \pm 9$ | $0 \pm 0$ | $0 \pm 0$ | $-6 \pm 9$ | $0 \pm 1$ |
|  | $-11 \pm 8$ | $0 \pm 0$ | $0 \pm 0$ | $-11 \pm 8$ | $0 \pm 0$ |
| R9-N87 | $-10 \pm 20$ | $0 \pm 0$ | $0 \pm 0$ | $0 \pm 1$ | $-10 \pm 19$ |
|  | -- | -- | -- | -- | -- |
| R9-N123 | $-10 \pm 13$ | $0 \pm 0$ | $-5 \pm 7$ | $-3 \pm 5$ | $-2 \pm 5$ |
|  | $-20 \pm 8$ | $0 \pm 0$ | $-8 \pm 5$ | -7 $\pm 4$ | $-5 \pm 3$ |
| R9-F126 | $-4 \pm 6$ | $0 \pm 0$ | $-1 \pm 1$ | $-1 \pm 2$ | $-2 \pm 4$ |
|  | $-8 \pm 4$ | $0 \pm 0$ | $-1 \pm 1$ | $-3 \pm 4$ | $-4 \pm 3$ |
| Y11-S84 | $-6 \pm 8$ | $-2 \pm 4$ | $-1 \pm 1$ | $-2 \pm 4$ | $-2 \pm 3$ |
|  | -- | -- | -- | -- | -- |
| Y11-I119 | -- | -- | -- | -- | -- |
|  | $-4 \pm 2$ | $0 \pm 0$ | $0 \pm 0$ | $-3 \pm 1$ | $-1 \pm 1$ |
| Y11-N122 | -- | -- | -- | -- | -- |
|  | $-10 \pm 4$ | $0 \pm 0$ | $0 \pm 2$ | $-3 \pm 2$ | $-7 \pm 3$ |
| Y11-N123 | $-9 \pm 13$ | $0 \pm 0$ | $0 \pm 0$ | $-2 \pm 5$ | $-7 \pm 12$ |
|  | $-31 \pm 11$ | $0 \pm 0$ | $0 \pm 0$ | -2 $\pm 1$ | $-29 \pm 11$ |
| Y11-F126 | $-10 \pm 6$ | $0 \pm 0$ | $-2 \pm 3$ | $-1 \pm 1$ | $-7 \pm 5$ |
|  | $-15 \pm 4$ | $0 \pm 0$ | $-6 \pm 3$ | $-1 \pm 0$ | $-9 \pm 3$ |
| Y11-W161 | -- | -- | -- | -- | -- |
|  | $-9 \pm 4$ | $0 \pm 0$ | $-1 \pm 1$ | $-1 \pm 0$ | $-8 \pm 4$ |
| Y11-N165 | -- | -- | -- | -- | -- |
|  | -6 $\pm 4$ | $0 \pm 0$ | $0 \pm 1$ | $0 \pm 0$ | $-5 \pm 4$ |
| I12-S84 | $-5 \pm 9$ | $-4 \pm 7$ | $0 \pm 1$ | $-1 \pm 1$ | $0 \pm 0$ |
|  | -- | -- | -- | -- | -- |
| L13-E203 | -- | -- | -- | -- | -- |
|  | $5 \pm 14$ | $0 \pm 0$ | $5 \pm 14$ | $0 \pm 0$ | $0 \pm 1$ |

${ }^{1}$ interaction energy between an $N T$ residue and a $Y_{I} M R{ }^{1} R^{2} A^{I I}$ residue
${ }^{2}$ interaction energy between the backbone atoms of an NT residue and the backbone atoms of a $Y_{I} M R^{1} R^{2} A^{I I}$
residue
${ }^{3}$ interaction energy between the backbone atoms of an $N T$ residue and the side chain atoms of a $Y_{I} M R^{1} R^{2} A^{I I}$ residue
${ }^{4}$ interaction energy between the side chain atoms of an NT residue and the backbone atoms of a $Y_{\mathrm{I}} M R^{1} \mathrm{R}^{2} \mathrm{~A}^{\text {II }}$ residue
${ }^{5}$ interaction energy between the side chain atoms of an NT residue and the side chain atoms of a $\mathrm{Y}_{\mathrm{I}} M \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{~A}^{\mathrm{II}}$ residue

Table S5: Average interaction energy between residues of $\mathrm{NT}(7-13)$ and their main interacting partners of $\mathbf{Y}_{\mathbf{I}} \mathbf{M R}{ }^{1} \mathbf{R}^{\mathbf{2}} \mathbf{A}_{\text {II }}$ (antiparallel orientation). The interaction energy is the sum of the Coulomb energy and the van der Waals energy. The energies are averaged over a $2-\mu \mathrm{s}$ trajectory and the values larger than $4 \mathrm{~kJ} / \mathrm{mol}$ are shown; the errors are the standard deviation. Energies larger than $12 \mathrm{~kJ} / \mathrm{mol}(\sim 5 \mathrm{kT})$ are shown in bold letters.

| NT- <br> $\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{~A}^{\mathrm{II}}$ | res-res $(\mathrm{kJ} / \mathrm{mol})^{1}$ | $\mathrm{bb}-\mathrm{bb}(\mathrm{kJ} / \mathrm{mol})^{2}$ | $\mathrm{bb}-\mathrm{sc}(\mathrm{kJ} / \mathrm{mol})^{3}$ | $\mathrm{sc}-\mathrm{bb}(\mathrm{kJ} / \mathrm{mol})^{4}$ | $\mathrm{sc}-\mathrm{sc}(\mathrm{kJ} / \mathrm{mol})^{5}$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| P7 - S84 | $-6 \pm 7$ | $-3 \pm 3$ | $-3 \pm 3$ | $-1 \pm 1$ | $0 \pm 1$ |
| P7 - N122 | $-4 \pm 5$ | $0 \pm 0$ | $-1 \pm 2$ | $-1 \pm 1$ | $-2 \pm 2$ |
| P7 - N123 | $-7 \pm 7$ | $0 \pm 0$ | $-4 \pm 5$ | $-1 \pm 1$ | $-1 \pm 2$ |
| P7 - F126 | $-4 \pm 4$ | $0 \pm 0$ | $-1 \pm 1$ | $0 \pm 0$ | $-3 \pm 3$ |
| R8 - D43 | $\mathbf{- 1 5} \pm \mathbf{4 2}$ | $\mathbf{0} \pm \mathbf{0}$ | $\mathbf{0} \pm \mathbf{0}$ | $\mathbf{0} \pm \mathbf{0}$ | $\mathbf{- 1 5} \pm \mathbf{4 2}$ |
| R8 - A83 | $\mathbf{- 1 3} \pm \mathbf{1 5}$ | $\mathbf{0} \pm \mathbf{0}$ | $\mathbf{0} \pm \mathbf{0}$ | $\mathbf{- 1 3} \pm \mathbf{1 5}$ | $\mathbf{0} \pm \mathbf{0}$ |
| R8 - S84 | $\mathbf{- 2 6} \pm \mathbf{1 9}$ | $\mathbf{- 5} \pm \mathbf{7}$ | $\mathbf{- 5} \pm \mathbf{4}$ | $\mathbf{- 1 3} \pm \mathbf{1 1}$ | $\mathbf{- 3} \pm \mathbf{3}$ |
| R8 - G85 | $-11 \pm 11$ | $-1 \pm 1$ | $0 \pm 0$ | $-11 \pm 11$ | $1 \pm 1$ |
| R8 - G86 | $-4 \pm 4$ | $0 \pm 0$ | $0 \pm 0$ | $-5 \pm 4$ | $2 \pm 2$ |
| R8 - N87 | $-11 \pm 15$ | $0 \pm 0$ | $0 \pm 0$ | $-1 \pm 1$ | $-10 \pm 14$ |
| R8 - N123 | $\mathbf{- 1 6} \pm \mathbf{1 2}$ | $\mathbf{0} \pm \mathbf{0}$ | $\mathbf{- 7} \pm \mathbf{9}$ | $-\mathbf{- 7} \pm \mathbf{8}$ | $\mathbf{- 2} \pm \mathbf{3}$ |
| R8 - F126 | $-7 \pm 6$ | $0 \pm 0$ | $-2 \pm 2$ | $-1 \pm 3$ | $-5 \pm 4$ |
| R8 - E169 | $-5 \pm 24$ | $0 \pm 0$ | $0 \pm 0$ | $0 \pm 0$ | $-5 \pm 24$ |
| R9 - S84 | $-8 \pm 9$ | $-1 \pm 1$ | $-6 \pm 8$ | $0 \pm 0$ | $-1 \pm 1$ |
| R9 - I119 | $-6 \pm 3$ | $0 \pm 0$ | $0 \pm 0$ | $-2 \pm 2$ | $-4 \pm 2$ |
| R9 - N122 | $6 \pm 7$ | $0 \pm 0$ | $0 \pm 0$ | $0 \pm 0$ | $6 \pm 7$ |
| R9 - N123 | $-9 \pm 9$ | $0 \pm 0$ | $-1 \pm 1$ | $0 \pm 0$ | $-8 \pm 9$ |
| R9 - E158 | $\mathbf{- 1 4 3} \pm \mathbf{2 2}$ | $\mathbf{0} \pm \mathbf{0}$ | $\mathbf{0} \pm \mathbf{0}$ | $\mathbf{0} \pm \mathbf{1}$ | $\mathbf{- 1 4 3} \pm \mathbf{2 3}$ |
| Y11 - W77 | $-10 \pm 9$ | $0 \pm 0$ | $-2 \pm 3$ | $-1 \pm 2$ | $-7 \pm 6$ |
| Y11 - S80 | $-6 \pm 8$ | $0 \pm 0$ | $0 \pm 0$ | $-2 \pm 3$ | $-4 \pm 7$ |
| Y11 - N81 | $-10 \pm 10$ | $0 \pm 0$ | $0 \pm 1$ | $-8 \pm 9$ |  |
| Y11 - S84 | $-4 \pm 8$ | $0 \pm 0$ | $0 \pm 0$ | $-4 \pm 8$ |  |

${ }^{1}$ interaction energy between an $N T$ residue and a $Y_{I} M R^{1} \mathrm{R}^{2} \mathrm{~A}^{11}$ residue
${ }^{2}$ interaction energy between the backbone atoms of an NT residue and the backbone atoms of a $\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{~A}^{\mathrm{II}}$ residue
${ }^{3}$ interaction energy between the backbone atoms of an $N T$ residue and the side chain atoms of a $\mathrm{Y}_{\mathrm{I}} M \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{~A}^{\mathrm{II}}$ residue
${ }^{4}$ interaction energy between the side chain atoms of an NT residue and the backbone atoms of a $\mathrm{Y}_{\mathrm{I}} M \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{~A}^{\mathrm{II}}$ residue
${ }^{5}$ interaction energy between the side chain atoms of an $N T$ residue and the side chain atoms of a $\mathrm{Y}_{\mathrm{I}} M \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{~A}^{\text {II }}$ residue


Figure S12: Time series of root-mean-square deviations (RMSD) of the C $\alpha$ atoms of NT(7-10) or NT(7-13) from two independent MD simulations. Data are shown with $\mathrm{NT}(7-13)$ in the parallel orientation, calculated upon fitting $M R^{1} R^{2}$ repeats of $Y_{I} M R^{1} R^{2} A_{I I}$ to the $M R^{1} R^{2}$ repeats of the structure obtained at the end of the second simulation ("sim2") (upper panel), or from a MD simulation with $\mathrm{NT}(7-13)$ in the antiparallel orientation, calculated upon fitting $M R^{1} \mathrm{R}^{2}$ to that of the structure at the end of the simulation (lower panel).


Figure S13: Time evolution of distances between $\operatorname{Pro}^{\mathrm{NT}}, \operatorname{Arg}^{\mathrm{NT}}, \operatorname{Arg}^{\mathrm{NT}}$ and $\mathbf{T y r 1 1}{ }^{\mathrm{NT}}$ and main interacting residues of $\mathbf{Y}_{\mathbf{I}} \mathbf{M R} \mathbf{R}^{\mathbf{1}} \mathbf{R}^{\mathbf{2}} \mathbf{A}_{\text {II }}$. Data are shown when $\mathrm{NT}(7-13)$ is in the antiparallel orientation. In (A) the distances are calculated between the center of mass of the two residues. In (B) and (C), explicit distances between the indicated atoms are given. In (D) the distance between $\operatorname{Tyr} 11^{\mathrm{NT}}$ and $\operatorname{Trp} 77$ is the distance between the center of masses of the aromatic rings.


Figure S14: Evolution of atomic distances during MD simulations: (A) Phe126 (center of mass of ring) to C $\gamma 1$ of Ile166 and (B) Phe126 (center of mass) to Trp161 (center of mass).


Figure S15: Inter-helical angle between helix 2 of the N -cap and helix 3 of the first internal repeat. The angle $\theta$ reflects the orientation of the N -cap relative to the first internal repeat (left). (Middle and right) Time series and probability density, respectively, of the inter-helical angle $\theta$ for $Y_{I} M R^{1} R^{2} A_{\text {II }}$ (blue lines) and $Y_{\text {III }} M_{3} A_{I I}$ (green lines) at 330 K . The simulations were run using OPLS force-field, with the same simulation set-up as described in Molecular Dynamics Simulations section in Materials \& Methods, except for the differences summarized in the Table S3.


Figure S16: Distance between residues R37 and Trp77 in the two simulations in presence of NT (black and red line) as well as in absence of NT (green line). The distance was measured between the guanidinium group of Arg37 and the center of mass of the aromatic ring of Trp77.

## 9. ELISA Data:



Figure S17: ELISA signal when adding $\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{~A}_{\text {II }}$ mutants and VG_328 based reference proteins (all at 200 nM protein) to NeutrAvidin-coated surface, to which biotinylated target peptide NT1-13 (200 nM) had been immobilized. Binding was detected by anti- $\mathrm{RGSH}_{6}$ mouse antibody followed by goat anti mouse IgG alkaline phosphatase conjugate, and signals were read out at 405 nm after 2 h , after signals at the ,reference wavelength
and the background have been subtracted. Since the protein concentrations are very high and the affinities are rather weak, the signals correspond to affinities. We found that $Y_{I} M R{ }^{1} R^{2} A_{I I}$ gave a significantly higher signal than the original $V G_{-} 328$ and $Y_{I} M R^{1} R^{2} R^{3} \mathrm{MA}_{\text {II }}$ indicating a size-dependent effect between the surface bound ELISA method and in-solution NMR method. For proteins of the same size a similar intensity of the ELISA signal translates reliably into a range of similar $K_{\mathrm{d}}$ values in NMR experiments. VG_306 is a previously published and characterized variant of VG_328 ( $\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{R}^{3} \mathrm{MA}_{\mathrm{I}}$ ) carrying the point mutation Y 116 H . The mutation is located in the first randomized repeat $\mathrm{R}^{1}$ in a randomized position on helix H 3 at position 30 of the repeat that was observed to have a negative effect on peptide binding [23].

## 10. MEXICO results:



Figure S18: Effect of NT on $\mathrm{Y}_{\mathrm{I}} \mathrm{MR}^{1} \mathrm{R}^{2} \mathrm{~A}_{\text {II }}$ solvent accessibility (MEXICO data). The thickness of the backbone denotes the amplitude of normalized ratio of the proton exchange rates in presence to those in absence of NT. The precision of the data is indicated by gradients from gray to red (decreased exchange rate in presence of NT) and gray to blue (increased exchange rate in presence of NT), respectively; maximum color saturation reflects minimal fitting error. Prolines and residues that could not be analyzed are shown in thin black trace.

