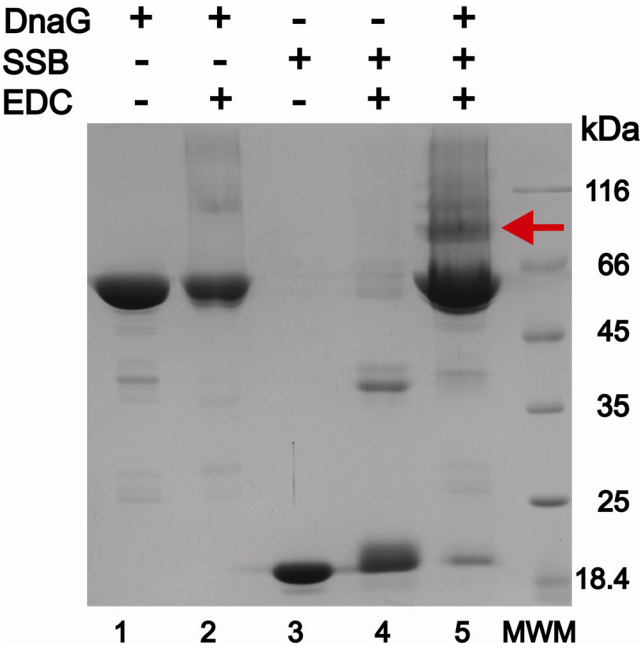
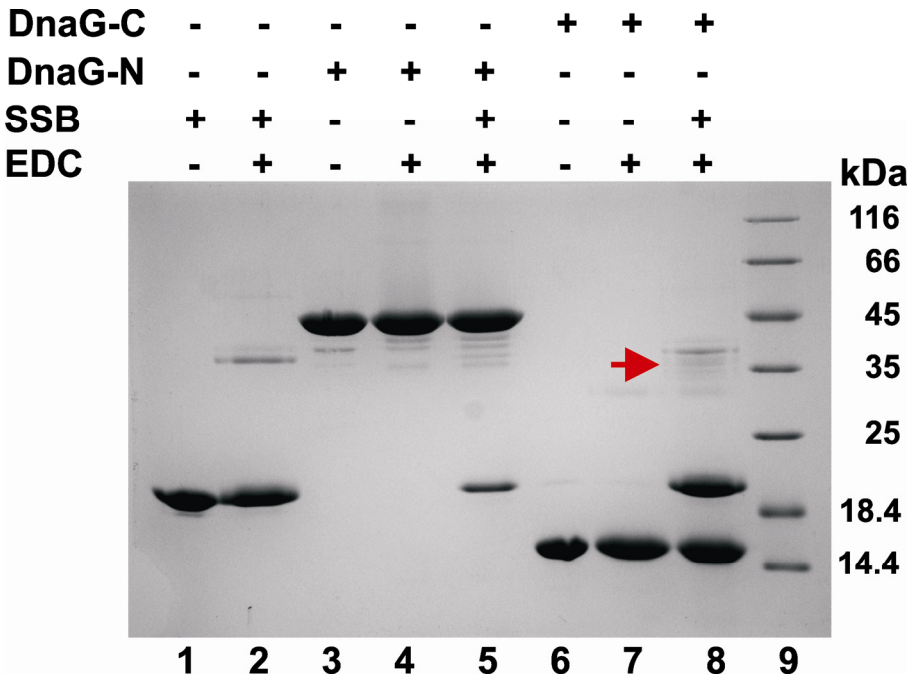
Supplementary Data



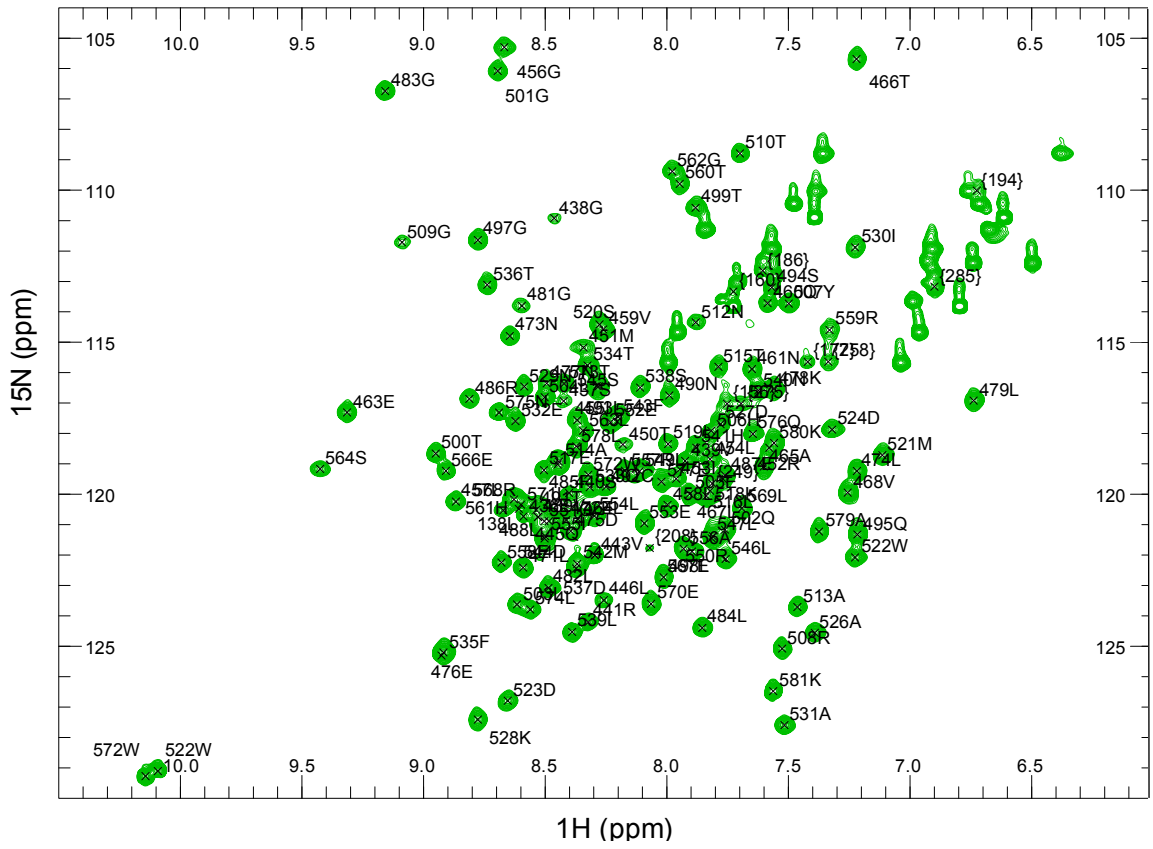
Supplementary Figure S1: **Cross-link of DnaG primase with EcoSSB.** Reactions were performed in 1 mM potassium phosphate buffer pH 7.4, 1 mM NaCl, 8% (w/v) glycerol and 1 mM DTT, using 25 μM DnaG, 5 μM EcoSSB and 50 mM EDC. After incubation of EcoSSB with DnaG and EDC (lane 5), cross-link bands were obtained (red arrow), which did not appear in reactions missing one of the respective protein partners (lane 2 and 4). 12% SDS-PAGE.



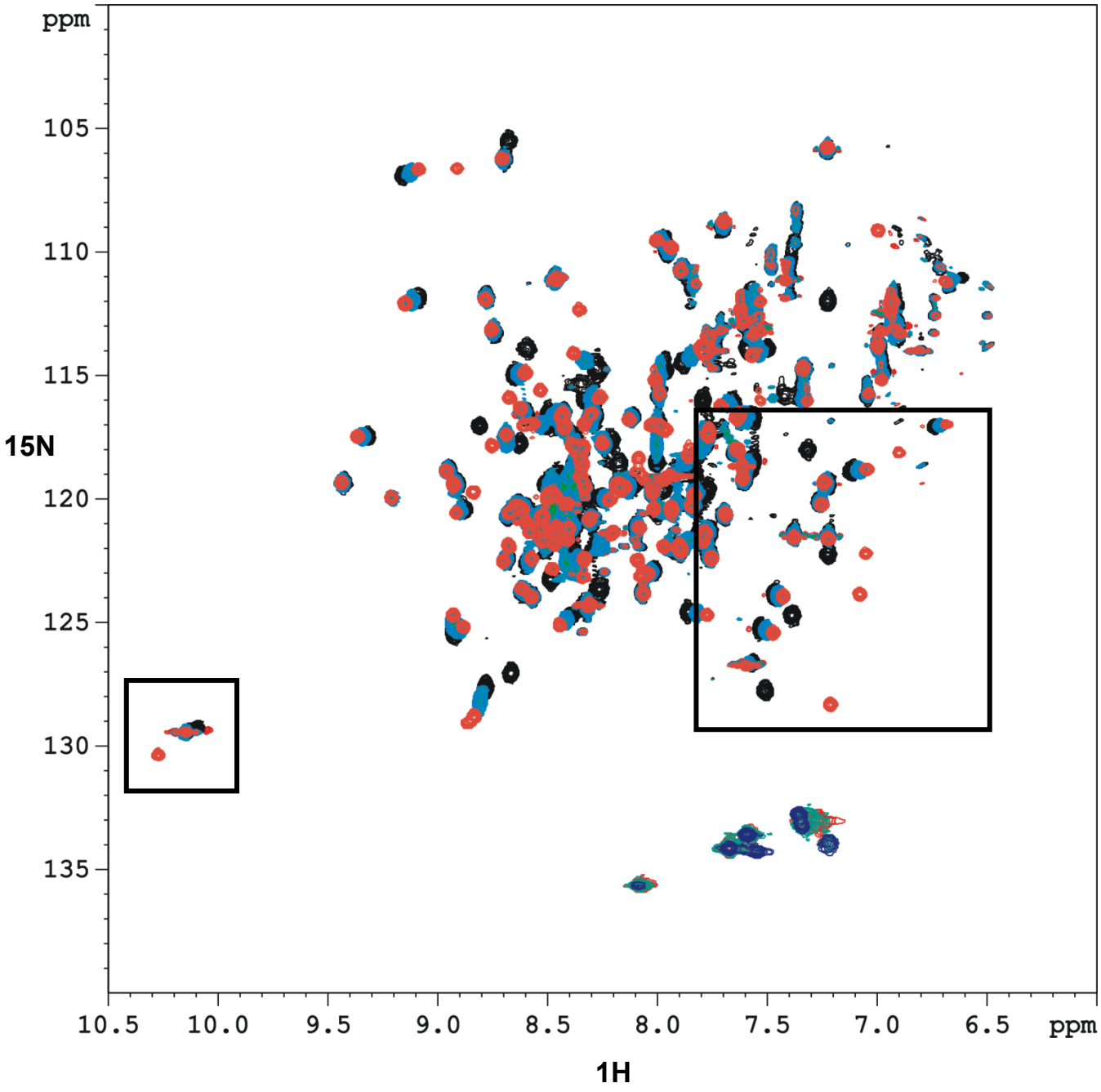
Supplementary Figure S2: **Cross-link experiments of DnaG-N and DnaG-C with EcoSSB.** Reactions were performed in 1 mM potassium phosphate buffer pH 7.4, 1 mM NaCl, 8% (w/v) glycerol and 1 mM DTT, using 25 µM DnaG variant, 5 µM EcoSSB and 50 mM EDC. Aliquots corresponding to 7.4 µg of the respective DnaG variant were applied to a 13.5% SDS PAGE. After incubation of EcoSSB with DnaG-C and EDC (lane 8), additional cross-link bands of higher apparent molecular weights were obtained (red arrow), which did not appear in reactions missing DnaG-C (lane 2). In the case of DnaG-N, no such cross-link bands could be detected.

Protein	Binding constant (K_A)
DnaG-C wild-type	$8.6 \cdot 10^4$ M^{-1}
K447A	n/d
T450A	$2.8 \cdot 10^4$ M^{-1}
R452A	n/d
K478A	$6.2 \cdot 10^4$ M^{-1}
K518A	n/d
K528A	$7.4 \cdot 10^4$ M^{-1}

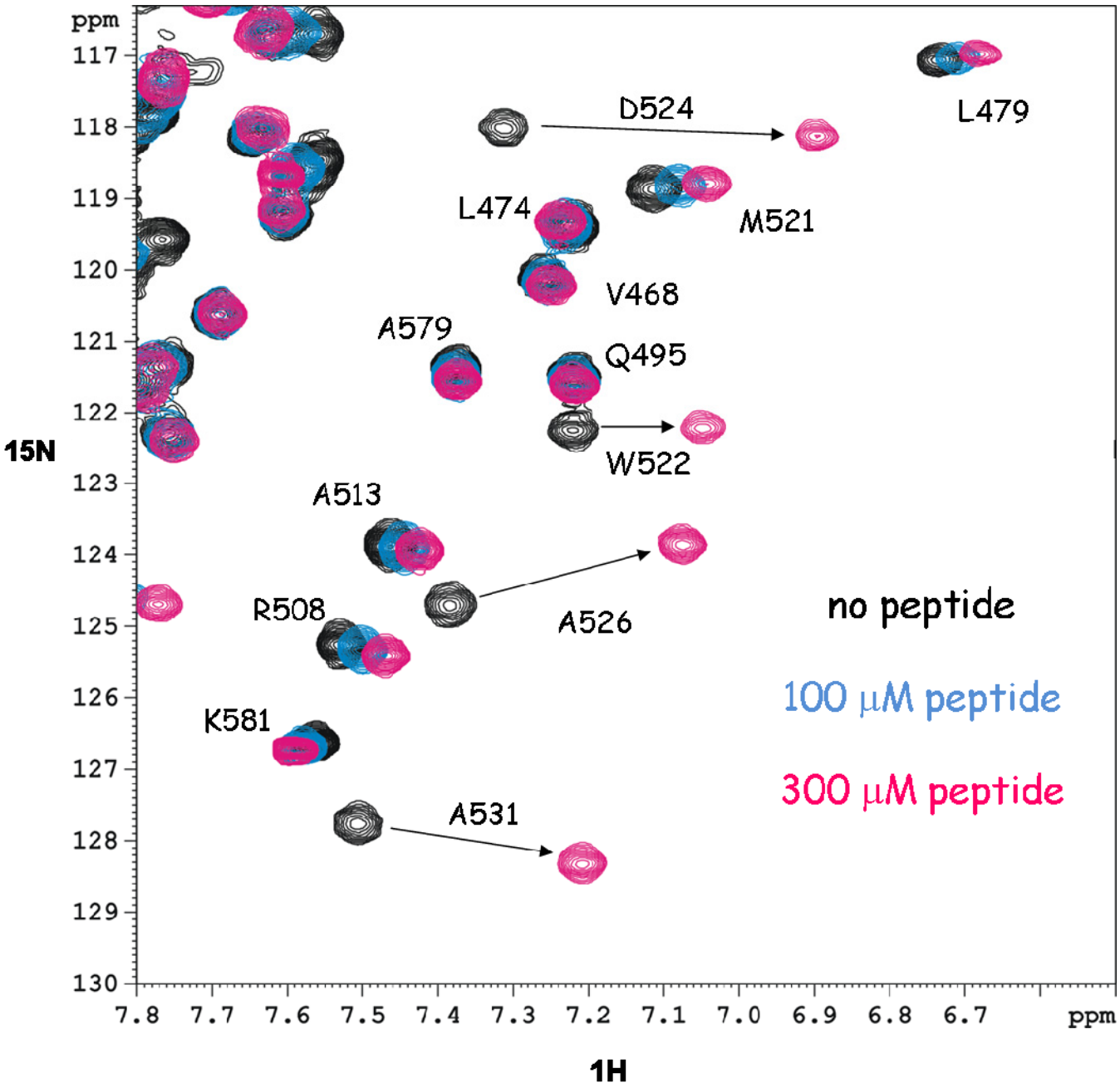
Supplementary Table S1: Overview of binding constants of DnaG-C variants and EcoSSB as determined by analytical ultracentrifugation. In the case of the K447A, R452A and K518A mutants of DnaG-C, the binding affinity was so weak that no binding constant could be determined (n/d). For details see Figure 3.



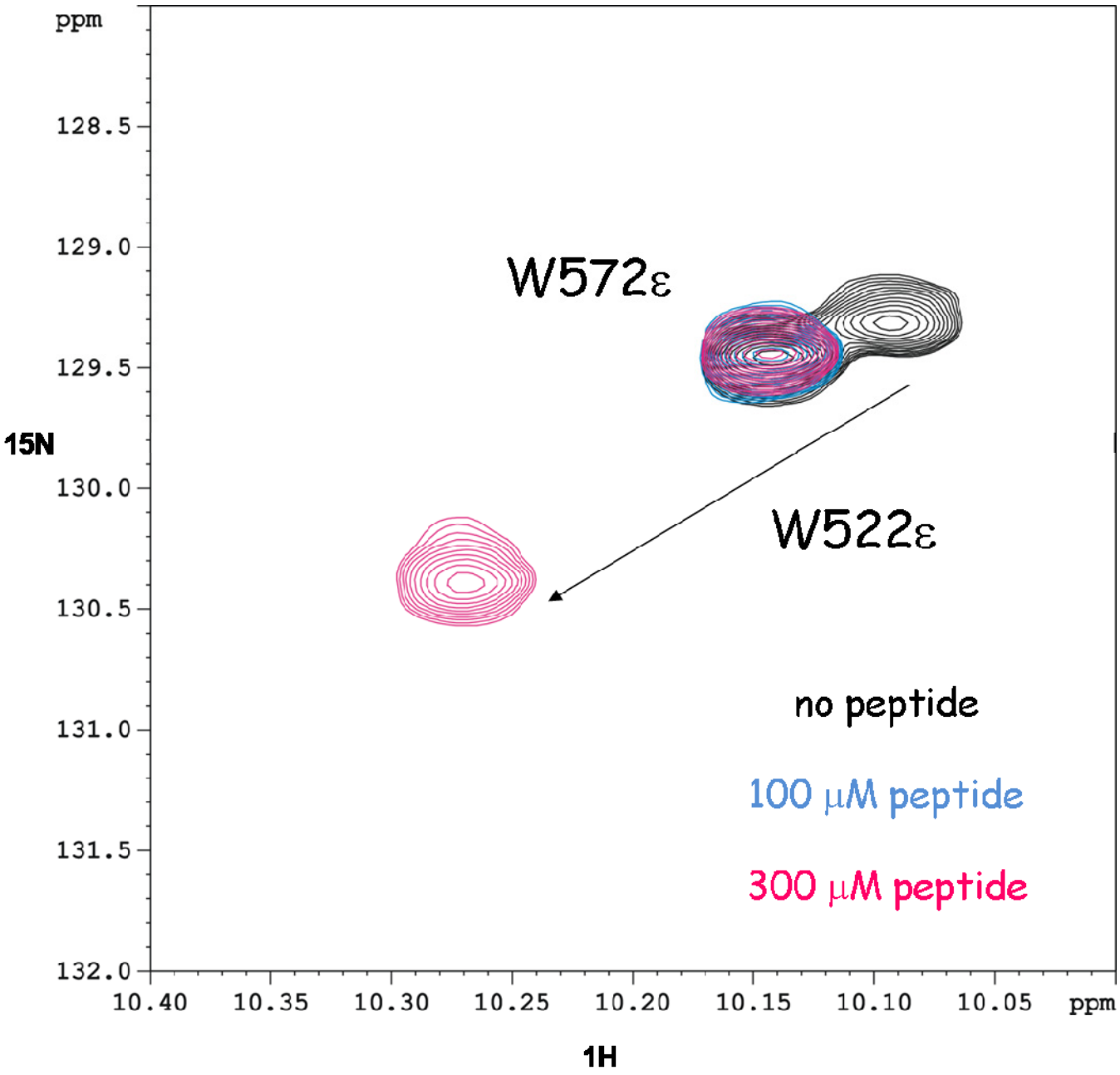
Supplementary Figure S3: ^{15}N-SOFAST-HMQC-spectra of ^{15}N-DnaG-C (300 µM) without SSB-Carb peptide. The spectrum was recorded at 600 MHz and 295 K. The assignment is indicated by labelling the respective peaks with the names of the amino acids and was repeated in presence of 1 mM SSB-Carb peptide. Differences in chemical shift are shown in Supplementary Table S2 and Supplementary Figure S7.



Supplementary Figure S4: Overlay of ^{15}N-SOFAST-HMQC-spectra of ^{15}N-DnaG-C (300 µM) titrated with increasing concentrations of the SSB-Carb peptide. The spectra were recorded at 600 MHz and 295 K. Spectra with peptide concentrations of 0, 100 µM and 300 µM are shown in black, blue and orange, respectively. Hardly any changes were visible at higher concentrations of the peptide, the spectra are therefore omitted. Regions in black frames are shown in more detail in Supplementary Figures S5 and S6.



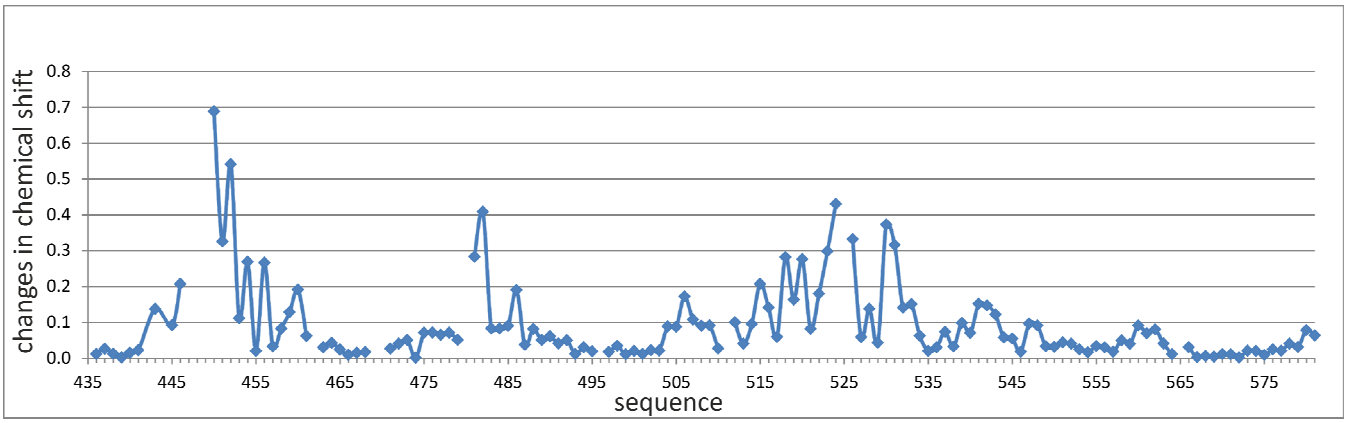
Supplementary Figure S5: Overlay of a region of the ^{15}N-SOFAST-HMQC-spectra of ^{15}N-DnaG-C (300 µM) titrated with increasing concentrations of SSB-Carb. Note that several peaks are missing in the blue spectrum (100 µM SSB-Carb peptide) which is typical for a ligand in fast exchange.



Supplementary Figure S6: Overlay of the region of the $HN^{\varepsilon 1}$ resonances in the ^{15}N-SOFAST-HMQC-spectra of ^{15}N-DnaG-C (300 µM) titrated with increasing concentrations of SSB-Carb. While the peak of the W572 side chain shows no changes, the peak of W522 exhibits a strong shift upon an increase of the peptide concentration to 300 µM. Note that this peak is missing in the blue spectrum (100 µM SSB-Carb peptide) which is typical for a ligand in fast exchange.

Sequence	Difference
435 Ala	
436 Glu	0.01
437 Ser	0.03
438 Gly	0.01
439 Val	0.00
440 Ser	0.01
441 Arg	0.02
442 Pro	
443 Val	0.14
444 Pro	
445 Gln	0.09
446 Leu	0.21
447 Lys	n.a. - w.p.
448 Arg	n.a. - w.p.
449 Thr	n.a. - w.p.
450 Thr	0.69
451 Met	0.32
452 Arg	0.54
453 Ile	0.11
454 Leu	0.27
455 Ile	0.02
456 Gly	0.27
457 Leu	0.03
458 Leu	0.08
459 Val	0.13
460 Gln	0.19
461 Asn	0.06
462 Pro	
463 Glu	0.03
464 Leu	0.04
465 Ala	0.02
466 Thr	0.01
467 Leu	0.01
468 Val	0.02
469 Pro	
470 Pro	
471 Leu	0.03
472 Glu	0.04
473 Asn	0.05
474 Leu	0.00
475 Asp	0.07
476 Glu	0.07
477 Asn	0.07
478 Lys	0.07
479 Leu	0.05
480 Pro	
481 Gly	0.28
482 Leu	0.41
483 Gly	0.08
484 Leu	0.08
485 Phe	0.09
486 Arg	0.19
487 Glu	0.04
488 Leu	0.08
489 Val	0.05
490 Asn	0.06
491 Thr	0.04
492 Cys	0.05
493 Leu	0.01
494 Ser	0.03
495 Gln	0.02
496 Pro	
497 Gly	0.02
498 Leu	0.03
499 Thr	0.01
500 Thr	0.02
501 Gly	0.01
502 Gln	0.02
503 Leu	0.02
504 Leu	0.09
505 Glu	0.09
506 His	0.17
507 Tyr	0.11
508 Arg	0.09
509 Gly	0.09
510 Thr	0.03
511 Asn	n.p. - w.p.
512 Asn	0.10
513 Ala	0.04
514 Ala	0.10
515 Thr	0.21
516 Leu	0.14
517 Glu	0.06
518 Lys	0.28
519 Leu	0.16
520 Ser	0.28
521 Met	0.08
522 Trp	0.18
523 Asp	0.30
524 Asp	0.43
525 Ile	not assigned
526 Ala	0.33
527 Asp	0.06
528 Lys	0.14
529 Asn	0.04
530 Ile	0.37
531 Ala	0.32
532 Glu	0.14
533 Gln	0.15
534 Thr	0.06
535 Phe	0.02
536 Thr	0.03
537 Asp	0.07
538 Ser	0.03
539 Leu	0.10
540 Asn	0.07
541 His	0.15
542 Met	0.15
543 Phe	0.12
544 Asp	0.06
545 Ser	0.05
546 Leu	0.02
547 Leu	0.10
548 Glu	0.09
549 Leu	0.03
550 Arg	0.03
551 Gln	0.04
552 Glu	0.04
553 Glu	0.02
554 Leu	0.02
555 Ile	0.03
556 Ala	0.03
557 Arg	0.02
558 Glu	0.05
559 Arg	0.04
560 Thr	0.09
561 His	0.07
562 Gly	0.08
563 Leu	0.04
564 Ser	0.01
565 Asn	n.p. - w.p.
566 Glu	0.03
567 Glu	0.00
568 Arg	0.01
569 Leu	0.00
570 Glu	0.01
571 Leu	0.01
572 Trp	0.00
573 Thr	0.02
574 Leu	0.02
575 Asn	0.01
576 Gln	0.02
577 Glu	0.02
578 Leu	0.04
579 Ala	0.03
580 Lys	0.08
581 Lys	0.06

Supplementary Table S2: Changes in chemical shift for the amino proton and nitrogen resonances in the backbone of DnaG-C after addition of 1 mM SSB-Carb peptide. The shift was calculated using the formula: shift = sqrt[$(\Delta\delta(^{1}H))^2$ + $(\Delta\delta(^{15}N)/10)^2$] (where $\Delta\delta$ is the difference in chemical shift in the respective dimensions). I525 could not be assigned in either spectrum ("not assigned"), N511 and N565 did not show a peak in the ^{1}H,^{15}N correlation without peptide ("n.p. – w.p."). K447, R448 and T449 could not be assigned unambiguously in the spectra without peptide ("n.a. – w.p."). The nitrogen bound proton in the side chain of W522 showed a significant shift as well, larger than that of the backbone amide proton (see Supplementary Figure S6).



AAESGVS	RPVPQLKRTT	MRILIGLLVQ	NPELATLVPP	470
LENLDENKLP	GLGLFRELVN	TCLSQPGLTT	GQLLEHYRGT	510
NNAATLEKLS	MWDDIADKNI	AEQTFTDSLN	HMFDSLLELR	550
QEELIARERT	HGLSNEERLE	LWTLNQELAK	K	581

Supplementary Figure S7: Graphical representation of the data from Supplementary Table S2. A change in shift above 0.1 ppm indicates an interaction, those parts of the protein that show an interaction with SSB-Carb can be easily recognized. For comparison, the amino acid sequence of DnaG-C is given.