Supplementary Information

NF-κB Rel Subunit Exchange on a Physiological Timescale

Matthew Biancalana[1,2], Eviatar Natan[3], Michael J. Lenardo[2*], Alan R. Fersht[1*]

[1]Medical Research Council Laboratory of Molecular Biology, Cambridge Biomedical Campus, Cambridge CB2 0QH, UK
[2]Molecular Development of the Immune System Section, Laboratory of Immune System Biology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA
[3]Department of Chemistry, University of Oxford, Oxford OX1 3TA, UK

*co-last authors and co-supervisors

Keywords: DNA binding, native mass spectrometry, NF-kappaB, NF-κB, protein complex, protein dynamics, protein-protein interaction, Rel proteins, subunit exchange, transcription factor

Abbreviations: DSF; differential scanning fluorimetry, ESI; electrospray ionisation, GuHCl; guanidinium hydrochloride, Ig; immunoglobulin, IKK; Inhibitory κB Kinase, MALS; multi-angle light scattering, MS; mass spectrometry, NLS; nuclear localisation signal, RHD; Rel homology domain, SDS-PAGE; sodium dodecyl sulphate polyacrylamide gel electrophoresis, SEC; size-exclusion chromatography

Running title:
NF-κB Rel Subunit Exchange
NF-κB Rel Subunit Exchange on a Physiological Timescale

Statement of Significance:
Many of the fundamental biological processes across all organisms are directed by the expression of genes. Genes are, in turn, regulated by transcription factors that can either activate or silence regions of DNA. Here, we describe the interactions of a set of transcription factors that form a critical component in the genetic regulation of the immune system using a robust soluble expression system. The proteins p50 and p65 form both homodimers (p50/p50 and p65/p65) and heterodimers (p50/p65) that determine the DNA sequences these transcription factors bind to, as well as which genes they activate. We have defined the preferential association of p50 and p65 using a precision technique called “MS-MS.” These results shed further biophysical insight into how association of these proteins regulates gene transcription and protein expression.

Supplementary Figure 1. Comparison of human and mouse p50 RHD (residues 1-420).

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P50_HUMAN   MAEDDPYLGRPEQMFHLDPSLTHTIFNPEVFQPQMALPTDGPYLQILEQPKQRGFRFRYV 60
P50_MOUSE   MADDDPYG--TGQMFHLNTALTHSIFNAELYSPEIPLSTDGPYLQILEQPKQRGFRFRYV 58
            **:****     *****: :***:*** *::.*:: * **********************

P50_HUMAN   CEGPSHGGLPGASSEKNKKSYPQVKICNYVGPAKVIVQLVTNGKNIHLHAHSLVGKHCED 120
P50_MOUSE   CEGPSHGGLPGASSEKNKKSYPQVKICNYVGPAKVIVQLVTNGKNIHLHAHSLVGKHCED 118
            ************************************************************

P50_HUMAN   GICTVTAGPKDMVVGFANLGILHVTKKKVFETLEARMTEACIRGYNPGLLVHPDLAYLQA 180
P50_MOUSE   GVCTVTAGPKDMVVGFANLGILHVTKKKVFETLEARMTEACIRGYNPGLLVHSDLAYLQA 178
            *:************************************************** *******

P50_HUMAN   EGGGDRQLGDREKELIRQAALQQTKEMDLSVVRLMFTAFLPDSTGSFTRRLEPVVSDAIY 240
P50_MOUSE   EGGGDRQLTDREKEIIRQAAVQQTKEMDLSVVRLMFTAFLPDSTGSFTRRLEPVVSDAIY 238
            ******** *****:*****:***************************************

P50_HUMAN   DSKAPNASNLKIVRMDRTAGCVTGGEEIYLLCDKVQKDDIQIRFYEEEENGGVWEGFGDF 300
P50_MOUSE   DSKAPNASNLKIVRMDRTAGCVTGGEEIYLLCDKVQKDDIQIRFYEEEENGGVWEGFGDF 298
            ************************************************************

P50_HUMAN   SPTDVHRQFAIVFKTPKYKDINITKPASVFVQLRRKSDLETSEPKPFLYYPEIKDKEEVQ 360
P50_MOUSE   SPTDVHRQFAIVFKTPKYKDVNITKPASVFVQLRRKSDLETSEPKPFLYYPEIKDKEEVQ 358
            ********************:***************************************

P50_HUMAN   RKRQKLMPNFSDSFGGGSGAGAGGGGMFGSGGGGGGTGSTGPGYSFPHYGFPTYGGITFH 420   . . .
P50_MOUSE   RKRQKLMPNFSDSFGGGSGAGAGGGGMFGSGGGGGSTGSPGPGYGYSNYGFPPYGGITFH 418   . . .
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Supplementary Figure 2. Comparison of human and mouse p65 RHD (residues 1-360).

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P65_HUMAN   MDELFPLIFPAEPAQASGPYVEIIEQPKQRGMRFRYKCEGRSAGSIPGERSTDTTKTHPT 60
P65_MOUSE   MDDLFPLIFPSEPAQASGPYVEIIEQPKQRGMRFRYKCEGRSAGSIPGERSTDTTKTHPT 60
            **:*******:*************************************************

P65_HUMAN   IKINGYTGPGTVRISLVTKDPPHRPHPHELVGKDCRDGFYEAELCPDRCIHSFQNLGIQC 120
P65_MOUSE   IKINGYTGPGTVRISLVTKDPPHRPHPHELVGKDCRDGYYEADLCPDRSIHSFQNLGIQC 120
            **************************************:***:*****.***********

P65_HUMAN   VKKRDLEQAISQRIQTNNNPFQVPIEEQRGDYDLNAVRLCFQVTVRDPSGRPLRLPPVLS 180
P65_MOUSE   VKKRDLEQAISQRIQTNNNPFHVPIEEQRGDYDLNAVRLCFQVTVRDPAGRPLLLTPVLS 180
            *********************:**************************:**** * ****

P65_HUMAN   HPIFDNRAPNTAELKICRVNRNSGSCLGGDEIFLLCDKVQKEDIEVYFTGPGWEARGSFS 240
P65_MOUSE   HPIFDNRAPNTAELKICRVNRNSGSCLGGDEIFLLCDKVQKEDIEVYFTGPGWEARGSFS 240
            ************************************************************

P65_HUMAN   QADVHRQVAIVFRTPPYADPSLQAPVRVSMQLRRPSDRELSEPMEFQYLPDTDDRHRIEE 300
P65_MOUSE   QADVHRQVAIVFRTPPYADPSLQAPVRVSMQLRRPSDRELSEPMEFQYLPDTDDRHRIEE 300
            ************************************************************

P65_HUMAN   KRKRTYETFKSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPFTSSLSTINY 360   . . .
P65_MOUSE   KRKRTYETFKSIMKKSPFNGPTEPRPPTRRIAVPTRNSTSVPKPAPQPYTFPASLSTINF 360   . . .
            ******************.***:**** ******:*.*:********** * :******:
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Supplementary Figure 3. Comparison of human p65 RHD (residues 1-359) and p50 RHD (residues 1-410).

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P65_HUMAN      ----------------------MDELFPL-IFPAEPAQASGPYVEIIEQPKQRGMRFRYK 37
P50_HUMAN      MAEDDPYLGRPEQMFHLDPSLTHTIFNPEVFQPQMALPTDGPYLQILEQPKQRGFRFRYV 60
                                        : *  : *     :.***::*:*******:****

P65_HUMAN      CEGRSAGSIPGERSTDTTKTHPTIKINGYTGPGTVRISLVTKDPPHRPHPHELVGKDCRD 97
P50_HUMAN      CEGPSHGGLPGASSEKNKKSYPQVKICNYVGPAKVIVQLVTNGKNIHLHAHSLVGKHCED 120
               *** * *.:**  * ...*::* :** .*.**..* :.***:.   : * *.****.*.*

P65_HUMAN      GFYEAELCPDRCIHSFQNLGIQCVKKRDLEQAISQRIQTNN----N-------------- 139
P50_HUMAN      GICTVTAGPKDMVVGFANLGILHVTKKKVFETLEARMTEACIRGYNPGLLVHPDLAYLQA 180
               *:  .   *.  : .* ****  *.*:.: :::. *:        *

P65_HUMAN      --------------PFQVPIEEQRGDYDLNAVRLCFQVTVRDPSGR-PLRLPPVLSHPIF 184
P50_HUMAN      EGGGDRQLGDREKELIRQAALQQTKEMDLSVVRLMFTAFLPDSTGSFTRRLEPVVSDAIY 240
                              ::    :*  : **..*** * . : * :*    ** **:*. *:

P65_HUMAN      DNRAPNTAELKICRVNRNSGSCLGGDEIFLLCDKVQKEDIEVYF-----TGPGWEARGSF 239
P50_HUMAN      DSKAPNASNLKIVRMDRTAGCVTGGEEIYLLCDKVQKDDIQIRFYEEEENGGVWEGFGDF 300
               *.:***:::*** *::*.:*.  **:**:********:**:: *     .*  **. *.*

P65_HUMAN      SQADVHRQVAIVFRTPPYADPSLQAPVRVSMQLRRPSDRELSEPMEFQYLPDTDDRHRIE 299
P50_HUMAN      SPTDVHRQFAIVFKTPKYKDINITKPASVFVQLRRKSDLETSEPKPFLYYPEIKDKEEVQ 360
               * :*****.****:** * * .:  *. * :**** ** * ***  * * *: .*:..::

P65_HUMAN      EKRKRTYETFKSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPFTSSLSTIN 359   . . .
P50_HUMAN      RKRQKLMPNFSDSFGGGSGAGAGGG-----GMFGSGGGGGGT-GSTGPGYSF----PHYG 410   . . .
               .**::   .*.. :  .  :*  .       :   . .....   :   * *       .
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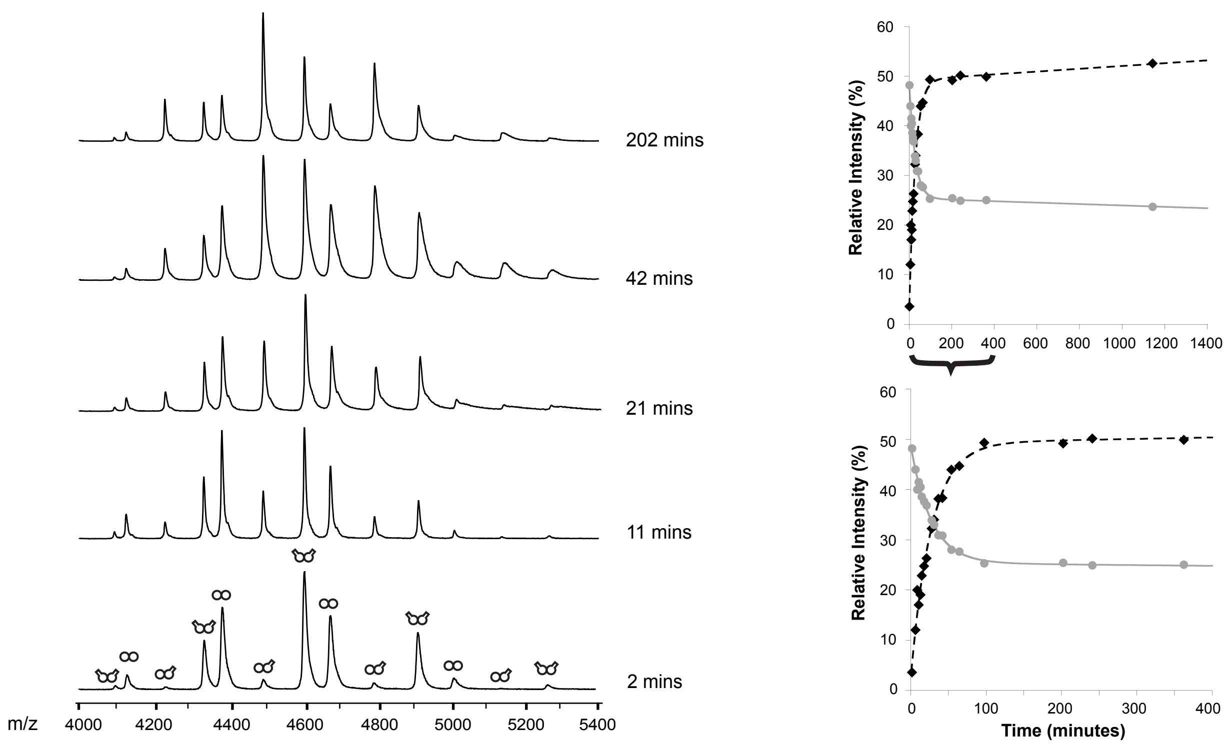
p50-1/p50-1 + p50-2/p50-2



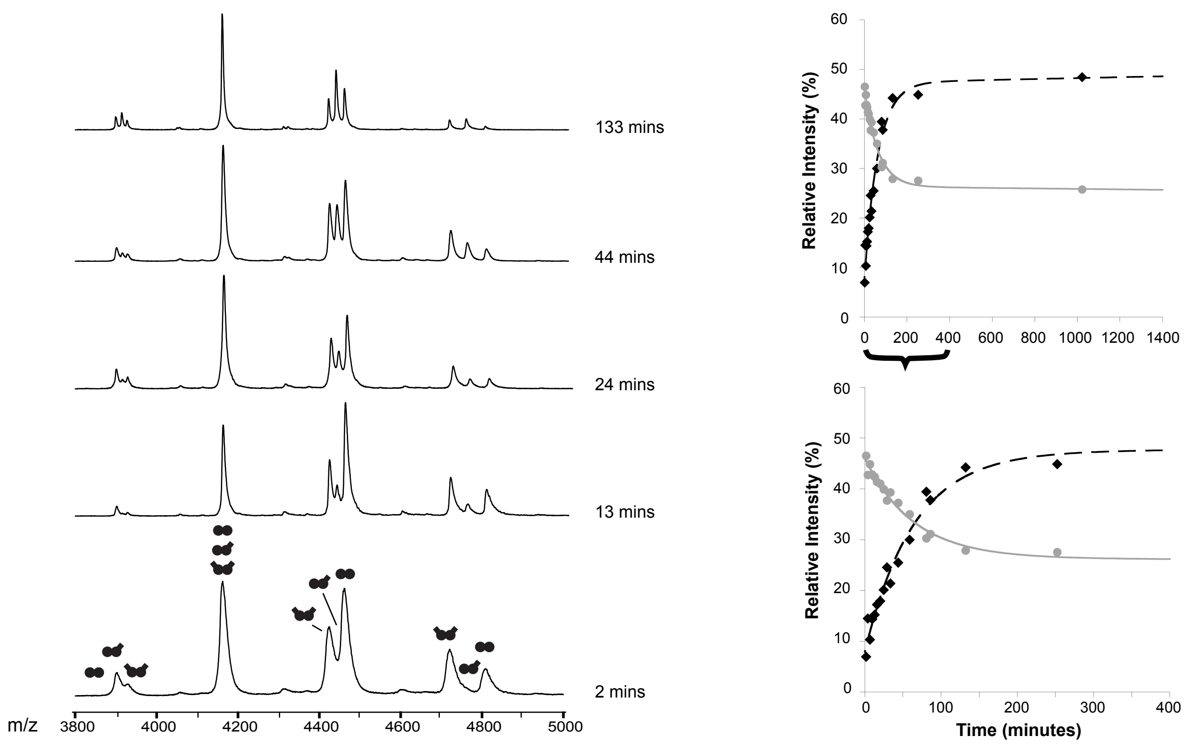
$t_{1/2}$ = 18 mins

Supplementary Figure 4

Subunit exchange between p50 RHD dimers at room temperature.

Native MS (ESI-MS) time-course of subunit exchange between p50-1/p50-1 (white circles) and p50-2/p50-2 (white circles with tails) RHD dimers. The upper right panel shows the relative populations of each species from 0-1400 minutes. Note that values obtained for the homodimeric species have been averaged to generate the decay curve. The lower right panel shows the data corresponding to the region bracketed above, showing the relative populations of each species from 0-400 minutes.

p65-1/p65-1 + p65-2/p65-2

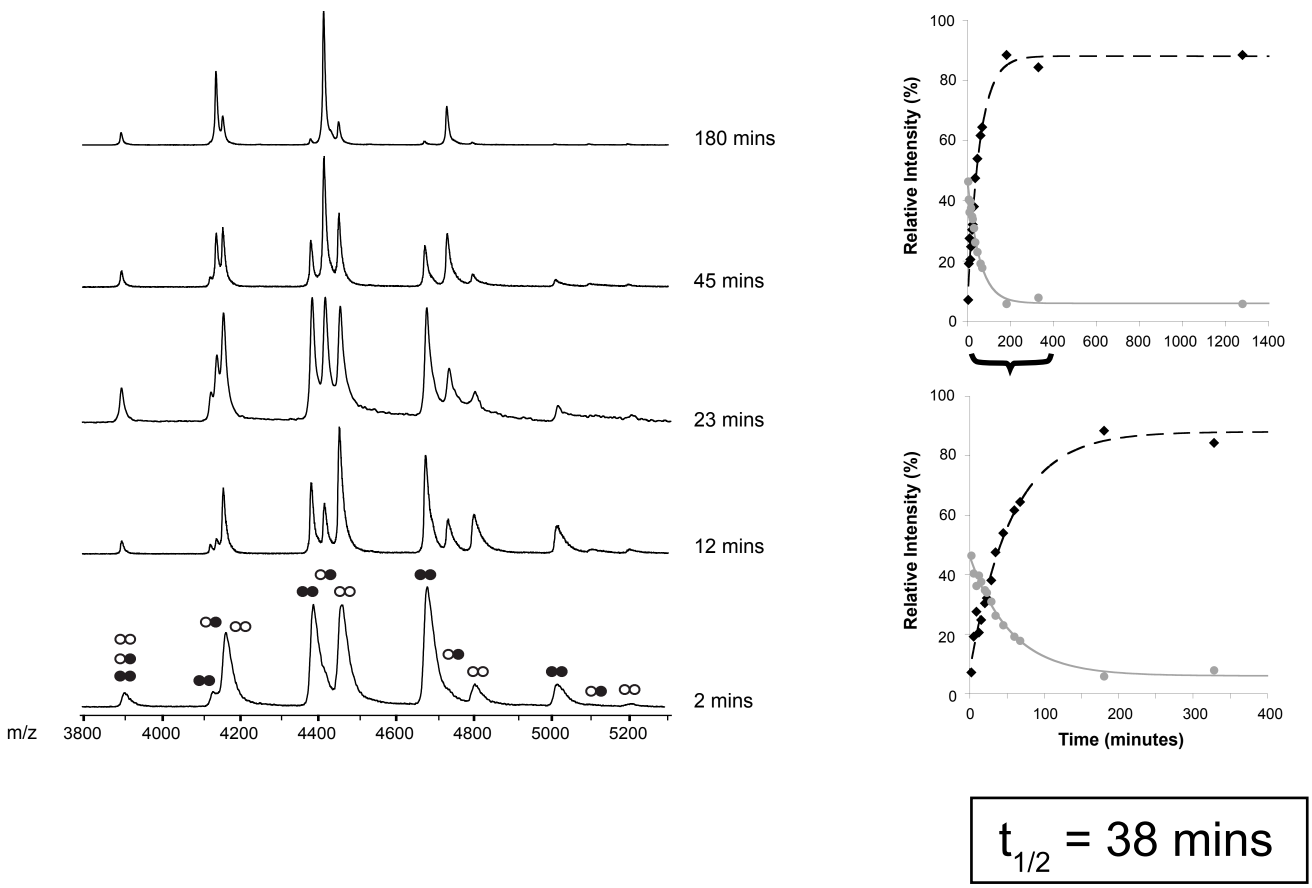


$t_{1/2}$ = 44 mins

Supplementary Figure 5
Subunit exchange between p65/p65 RHD dimers at room temperature.

Native MS (ESI-MS) time-course of subunit exchange between p65-1/p65-1 (black circles) and p65-2/p65-2 (black circles with tails) RHD dimers. The upper right panel shows the relative populations of each species from 0-1400 minutes. Note that values obtained for the homodimeric species have been averaged to generate the decay curve. The lower right panel shows the data corresponding to the region bracketed above, showing the relative populations of each species from 0-400 minutes.

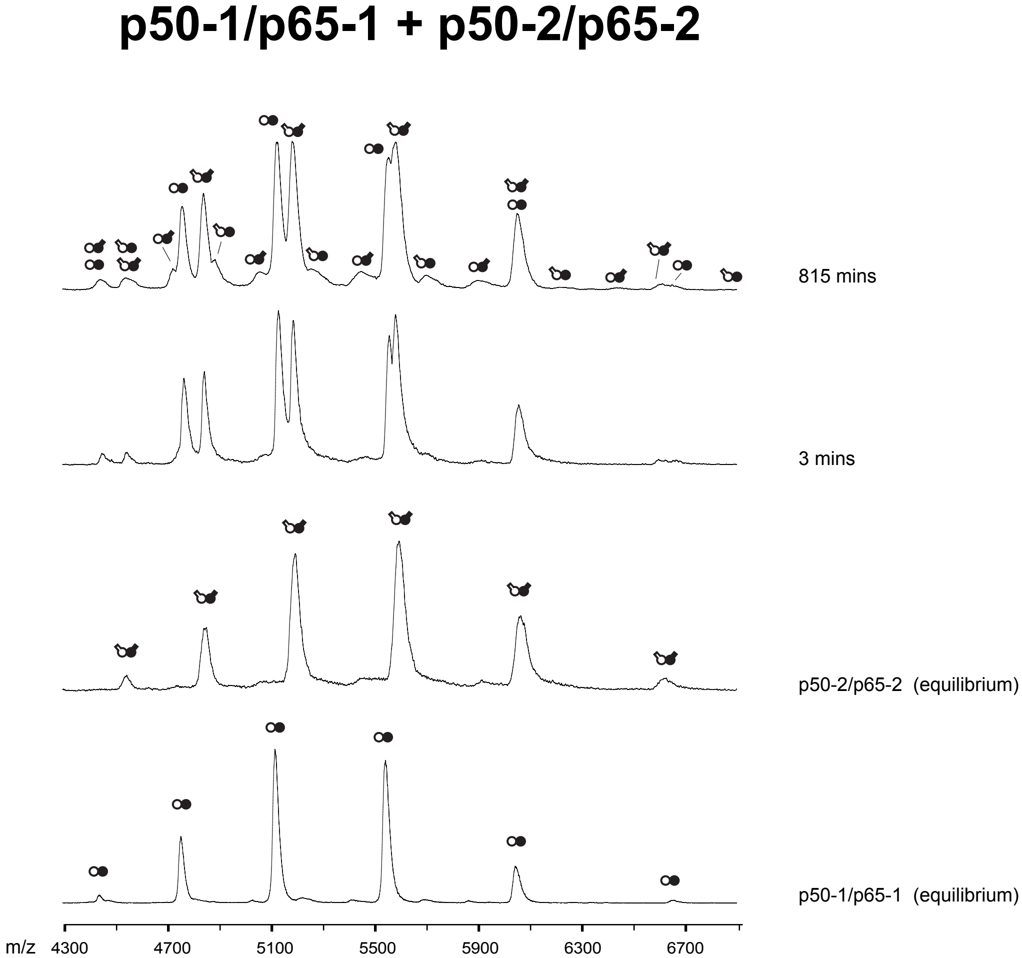
p50-1/p50-1 + p65-1/p65-1



Supplementary Figure 6

Subunit exchange between p50/p65 RHD dimers at room temperature.

Native MS (ESI-MS) time-course of subunit exchange between p50-1/p50-1 (white circles) and p65-1/p65-1 (black circles) RHD dimers. The upper right panel shows the relative populations of each species from 0-1400 minutes. Note that values obtained for the homodimeric species have been averaged to generate the decay curve. The lower right panel shows the data corresponding to the region bracketed above, showing the relative populations of each species from 0-400 minutes.



Supplementary Figure 7
Subunit exchange between p50-1/p65-1 and p50-2/p65-2 RHD dimers at room temperature.

Native MS (ESI-MS) time-course of subunit exchange between p50-1/p65-1 (white and black circles) and p50-2/p65-2 (white circles with tails and black circles with tails) RHD dimers. These data demonstrate the remarkable kinetic stability of the p50/p65 RHD heterodimers at RT. Only negligible subunit exchange is observed over the crouse of the experiment (exceeding 800 seconds).

Supplementary Table 1. DSF thermal melts conducted on p50 RHD.

	50 mM NaCl (°C)	150 mM NaCl (°C)
Protein alone	48.2 +/- 0.4	48.6 +/- 0.4
Heparin	53.0 +/- 0.3	52.9 +/- 0.4
κB_20mer ds oligo	58.6 +/- 0.3	52.4 +/- 0.2
κB_20mer ss oligo	48.7 +/- 0.4	49.2 +/- 0.4
non-specific 20mer ds oligo	50.6 +/- 0.4	49.4 +/- 0.3

Supplementary Table 2. DSF thermal melts conducted on p65 RHD.

	50 mM NaCl (°C)	150 mM NaCl (°C)
Protein alone	51.2 +/- 0.3	50.8 +/- 0.4
Heparin	55.9 +/- 0.2	56.5 +/- 0.4
κB_20mer ds oligo	61.5 +/- 0.4	55.5 +/- 0.3
κB_20mer ss oligo	55.8 +/- 0.4	50.8 +/- 0.3
non-specific 20mer ds oligo	52.6 +/- 0.4	52.4 +/- 0.3