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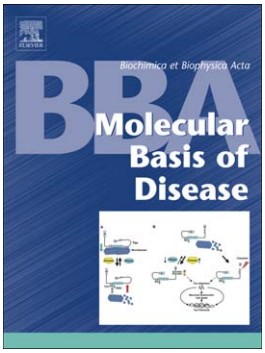

Differential activation of polymorphisms of the formyl peptide receptor by formyl peptides

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Differential activation of polymorphisms of the formyl peptide receptor by formyl peptides.

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Running title: Effects of polymorphisms on the Formyl Peptide Receptor

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Abstract. We have investigated the role of two polymorphic sites (R190W and N192K) on the binding and activation of the formyl peptide receptor (FPR) by viral and formyl peptides. WEDWVGWI, a peptide with antiviral activity derived from the membrane proximal region of feline immunodeficiency virus, binds with high affinity to FPR. The three tryptophans in the peptide are all essential for FPR binding, just as they were essential for antiviral activity (Giannecchini, S. *et al.*, J. Virol. 77 (2003) 3724). Formyl-NleWEDWVGWI behaved as a weak partial agonist with FPR W190/N192 but a stronger partial agonist with FPR R190/K192 and FPR R190/N192. Formyl-NleNleWEDWVGWI behaved as a full agonist toward all three FPRs but exhibited a much higher EC_{50} with W190/N192 FPR (300±45 nM) than for R190/K192 FPR (40±3 nM) or R190/N192 (60±8 nM). Formyl-MYKWPWYVWL preferentially activated R190/K192 and R190/N192 FPRs by >5 fold compared to W190/N192 FPR. Formyl-MFEDAVAWF, a peptide derived from a protein in *Mycobacterium avium* subsp. *paratuberculosis* and formyl-MFTFEPFPTN, a peptide derived from the N-terminus of chemotaxis inhibitory protein of *Staphylococcus aureus* with an added N-terminal methionine exhibited the greatest selectivity for R190/K192 and R190/N192 FPRs with ~10 fold lower EC_{50}s than that observed with FPR W190/N192. Thus, individuals with the W190 polymorphism may display a reduced ability to detect certain formyl peptides.

Keywords:

Formyl peptides, Signal Transduction, G protein-coupled receptor, polymorphism, feline immunodeficiency virus, chemotaxis inhibitory protein of *Staphylococcus aureus*.

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Introduction

Neutrophils play an essential role in innate immunity. In addition to activating phagocytosis and secreting superoxides and hydrolytic enzymes, the neutrophil must be able to chemotax, or migrate toward the source of a chemoattractant [1,2]. The formyl peptide receptor (FPR) is a chemoattractant G protein-coupled receptor found on the surface of phagocytes and it is thought to play an important role in allowing phagocytic cells to recognize the presence of bacteria [3] and damaged cells, since only eubacteria, mitochondria, and chloroplasts initiate their protein synthesis with formyl-methionine [4]. FPR expressing cells also exhibit chemotaxis toward peptides derived from the GP-41 envelope protein of HIV-1 [5,6]. A peptide derived from herpes simplex virus type 2 elicited chemotaxis and superoxide production in neutrophils in a process that appeared to involve FPR [7]. In addition, we recently observed that peptides from the proximal membrane region of the fusion proteins of human immunodeficiency viruses 1 and 2, severe acute respiratory syndrome coronavirus, coronaviruses 229E and HKU, and Ebola virus were potent inhibitors of FPR [8]. Thus FPR appears to respond to the presence of virally derived peptides in addition to bacterially derived ones (For a review of ligands affecting FPR see [9]). Two proteins secreted by *S. aureus* are inhibitors of FPR ([10-12]). Chemotaxis inhibitory factor of *S. aureus* (CHIPS) inhibits FPR and the C5a receptor and a homologue of CHIPS, FPRL1 inhibitory protein (FLIPr) inhibits FPRL1 and to a lesser extent FPR, but not C5a. The N-terminus of CHIPS is essential for binding to FPR since removal of the n-terminal phenylalanine reduces affinity for FPR ~1000 fold. Peptides derived from the N-terminus are also inhibitors of FPR but with ~10,000 fold lower affinity than CHIPS.

Polymorphisms in FPR are very common. Sahagun-Ruiz *et al.* [13] did an extensive haplotype investigation of FPR and reported finding at least 23 haplotypes for FPR. No polymorphisms were found in the closely related receptor FPRL1 [13]. At present there is no explanation for the wide sequence diversity of FPR. Numerous studies have indicated that patients with aggressive periodontitis exhibit a ~ 2 fold reduction in chemotaxis toward fMLF [14-19] indicating that a reduced ability to exhibit

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chemotaxis toward formyl peptides may be associated with this disease. Several studies have attempted to correlate FPR polymorphisms with aggressive periodontitis but have produced conflicting results [20-22].

Lentiviruses are associated with immunological impairment in their respective hosts, and both human immunodeficiency and feline immunodeficiency viral infections increase the likelihood of secondary bacterial infections [23,24]. Recently, Kubes *et al.* [25] demonstrated that feline neutrophils exhibited a marked (>90%) reduction of neutrophil chemotaxis toward fMLF following infection with FIV. Ueda *et al.* noted that the HIV-1 envelope GP-41 was able to down regulate the expression of FPR and several chemokine receptors at low nanomolar concentrations, and that the downregulation of FPR and the chemokine receptors was dependent upon expression of CD4 [26].

Peptides derived from the HR2 and proximal membrane regions of HIV-1, SIV, HRSV, human parainfluenza virus type 3, measles virus, and a coronavirus have all been shown to be able to block virus infection [27-30]. An eight mer peptide derived from the proximal membrane region of FIV GP-36, WEDWVGWI, was a potent (low nanomolar) inhibitor of FIV infection [31]. The three W residues were all essential for activity whereas the other residues were unimportant for activity.

We previously observed that FPR W190/N192 exhibited an enhanced affinity to bind some viral peptides but had a reduced affinity for the formyl peptide formyl-NleLeuPhe when compared with FPR R190/N192 or FPR R190/K192 [8]. Here we assessed whether the three W residues in WEDWVGWI essential to viral inhibition [31] are also are important in FPR binding. We also undertook a more extensive analysis of three FPR polymorphisms using a variety of formyl peptides of varying sequences in order to identify formyl peptides which might behave markedly different toward the different polymorphisms. We identified several formyl peptides that exhibit high selectivity for activation of R190/K192 and R190/N192 compared with W190/N192. We also identified possible secondary structure changes which might result from amino acid changes by secondary structure prediction [32] and correlated these structural changes with altered binding and activation of FPR.

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MATERIALS AND METHODS

FPR expressing cells. CHO S cells expressing W190/N192, R190/K192, and R190/N192 were prepared as described previously [8].

Materials. Peptides were obtained from Genscript and were >75% pure. Mass spectral data was provided with each peptide and the major mass peak matched the expected mass of the peptide in all cases. WEDWVGWI, AEDWVGVWI, WEDAVGWI, and WEDWVGAI were N acetylated and amidated at the C-terminus. All formyl peptides and had free C-termini. FTFEPFPTN had a free N-terminus and a free C-terminus. Formyl-Nle-Leu-Phe was obtained from Sigma and used without further purification. CHIPS was purchased from Cell Sciences.

FACScan analysis of ligand binding to FPR W190/N192, R190/K192 or R190/N192 expressed in CHO-s cells. CHO S cells (100ul in Gibco CHO-S-SFMII) were added to 3 mM KCl, 100mM NaCl, 10 mM sodium phosphate, pH 7.4, 1 mM Mg^{++}, 1 mM Ca^{++} containing 5% fetal bovine serum and incubated at 4° C for 60 min with varying concentrations peptide and 0.38 nM formyl-Nle-Leu-Phe-Nle-Tyr-Lys-FITC. The mean fluorescence of the cells was determined, and the mean fluorescence observed in non expressing CHO S cells (non-specific binding) was subtracted. The K_i was then determined by nonlinear least squares analysis using the known K_d for formyl-Nle-Leu-Phe-Nle-Tyr-Lys-FITC for each FPR variant [8] and the observed EC_{50} of each peptide. The K_I was determined from the equation $K_I = EC_{50}/(1+(\text{ligand}/K_d)$ using the graphics/statistics program Graphpad Prizm.

Downregulation of surface FPR. CHO S cells expressing FPR were incubated in Gibco CHO-S-SFMII at 37 ^{0} C for 1 hr (or the indicated time for Figure 2A) with the indicted concentration of peptide. DMSO was 0.4% in all cases. The cells were washed 2x with 10 ml 10 mM phosphate, pH 7.4, 3 mM KCl, 100 mM NaCl at 4^{0} C. The cells were resuspended in 100 mM NaCl, 3 mM KCl, 10 mM sodium phosphate, pH 7.4, 1 mM Mg^{++}, 1 mM Ca^{++}, 5% fetal bovine serum and 50 nM formyl-Nle-Leu-Phe-Nle-Tyr-Lys-FITC and incubated for 1hr at 4^{0} C and analyzed by FAScan as describe above. For the receptor recovery experiment, the cells were washed 2x with 10 ml 10 mM

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phosphate, pH 7.4, 3 mM KCl, 100 mM NaCl at 4^0 C after incubation with peptide, resuspended in Gibco CHO-S-SFMII at 37 ^{0} C for the indicated time. Then the cells were washed with 10 ml 10 mM phosphate, pH 7.4, 3 mM KCl, 100 mM NaCl at 4^0 C and then analyzed by FACScan as above. The mean fluorescence of the cells was determined, and the mean fluorescence observed in non expressing CHO S cells (non-specific binding) was subtracted. The data were then analyzed by nonlinear least squares analysis and the EC_{50} and D_{max} determined. For partial agonist activity, D_{max} was the % downregulation of FPRs W190/N192, R190/K192, or R190/N192 compared with 20 μM formyl-Nle-Leu-Phe carried out on the same day.

Statistical analysis.

Experimental results are expressed as the mean± standard error of the mean. The significance was evaluated by unpaired t test (2 tailed) using the computer program Graphpad Prizm 2.0.

Results and Discussion

FIV peptides (N-acetylated and C-amidated) derived from the proximal membrane region of GP-36 have been assessed for antiviral activity [31]. Therefore N-acetylated and C-amidated, WEDWVGWI, AEDWVGVWI, WEDAVGWI, and WEDWVGAI were synthesized and evaluated for their ability to inhibit formyl-Nle-Leu-Phe-Nle-Tyr-Lys-FITC binding to FPR. Figure 1 shows the effect of these peptides on binding to W190/N192, R190/K192, and R190/N192. Only WEDWVGWI exhibited appreciable inhibition similar to what Giannecchini *et al.* [31] observed for inhibition of viral infection by FIV inhibition. The dose dependence of WEDWVGWI, formyl-NleWEDWVGWI, and formyl-NleNleWEDWVGWI inhibition of formyl-Nle-Leu-Phe-Nle-Tyr-Lys-FITC binding to W190/N192, R190/K192, and R190/N192 was determined. The results are shown in Table 1. Thus the addition of a formyl-Nle had no affect on affinity and the addition of formyl-NleNle increased the affinity to FPR ~ 2-fold. W190/N192 exhibited a ~ 2 fold lower affinity for all three peptides.

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Since downregulation is a hallmark of receptor activation [33] and the trafficking of FPR expressed in CHO cells to endocytotic vesicles following treatment with fMLF has been thoroughly investigated [34], we chose this assay as a highly precise way to evaluate ligand activation of receptor. Figure 2A shows a time course of loss of surface receptor for the three FPR variants following treatment with 3 μM formyl-Nle-Leu-Phe. All exhibited $t_{1/2}$ of ~ 5-6 min but W190/N192 downregulated to a lesser maximum extent. Figure 2B shows recovery of surface receptor after treatment with formyl-Nle-Leu-Phe for 1 hr. All three variants exhibited partial recovery of surface receptor after downregulation with slightly higher recovery observed with W190/N192. We then determined the peptides concentration dependent downregulation surface expression of the receptor to assess whether the peptides exhibited agonist, partial agonist, or antagonist activity. As we observed for other viral peptides [8], WEDWVGWI did not cause any down regulation at concentrations up to 25μM, but 25μM WEDWVGWI inhibited the downregulation with 300 nM formyl-Nle-Leu-Phe by ~ 50% in all three FPRs (data not shown). However, formyl-NleWEDWVGWI behaved as a partial agonist (figure 2B) when compared with formyl-Nle-Leu-Phe (figure 3A). Only weak partial agonist activity was observed with W190/N192 (34±3%, $P<0.0001$ vs either R190/K192 or R190/N192), and greater partial agonist activity was observed with R190/K192 (55±2%) and R190/N192 (58±4%). W190/N192 also exhibited a higher EC_{50} for downregulation than R190/K192 or R190/N192 but the difference was not significant. Formyl-NleNleWEDWVGWI behaved as a full agonist for all three FPRs (figure 4A), but the EC_{50} for W190/N192 (300±40 nM, $P<0.0001$ vs either R190/K192 or R190/N192) was 5-7 fold higher than either R190/K192 (40±3 nM) or R190/N192 (60±8 nM) despite the observation that the K_is differed by only two fold. The observed EC_{50}s were more than 10 fold below the respective K_is for R190/K192 and R190/N192 and 3 fold below the K_i of W190/R190, implying that formyl-NleNleWEDWVGWI was 3 times more effective in activating R190/K192 and R190/N192 than W190/N192. This large difference in EC_{50} vs K_i is in marked contrast to what we observe with formyl-Nle-Leu-Phe (figure 3A) where the EC_{50}s are similar to the K_i s we previously reported [8]; EC_{50}=175±20 vs K_i=160±25 for W190/N192; EC_{50}=75±5 vs K_i=62±8 for R190/K192; EC_{50}=50±7 vs K_i=67±16 for R190/N192. Thus, formyl-

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NleNleWEDWVGWI appears to behave as a "super agonist" with R190/K192 and R190/N192, so that occupation of only 5% of the receptors is able to bring about the internalization of 50% of them. We also tested formyl-NleNleWED**A**VGWI (figure 3B) for its ability to cause down regulation of the receptor. Its behavior was essentially identical to that seen with formyl-NleNleWEDWVGWI . This is in contrast to WEDAVGWI which was >10 fold weaker than WEDWVGWI in binding to the three FPRs. We also tested formyl-NleNleWED. This peptide exhibited very little downregulation at concentrations up to 50 μM with all three FPRs. Thus the VGWI portion of the peptide, formyl-NleNleWEDWVGWI, appears to be essential for FPR activation.

We tested formyl-MYVKWPWYVWL, which we had previously shown to have essentially identical K_is for W190/N192, R190/K192 and R190/N190 [8] of 130 nM, for its ability to downregulate surface receptor (figure 4A). R190/K192 exhibited the lowest EC_{50} of 44±4 nM about 3 fold below the observed K_i and a maximum downregulation very similar (89%) to that seen with formyl-Nle-Leu-Phe (figure 2A). R190/N192 had a 2 fold higher EC_{50} (p=0.005) than R190/K192 and maximum down regulation similar to that observed with formyl-Nle-Leu-Phe. W190/N192 had a EC_{50} 14 fold greater than K192/N192 (p=0.0002) and 6 fold greater than R190/N192 (p=0.0006) and a maximum downregulation slightly reduced (74%) compared to that seen with formyl-Nle-Leu-Phe. This reduction is consistent with the reduced (65%) stimulation of GTPγS binding of W190/N192 FPR by 300 nM formyl-MYVKWPWYVWL compared to R190/K192 or R190/N192 FPRs reported previously [8].

We carried out a protein blastp search (http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi?PAGE=Proteins&PROGRAM=blastp&BLAST_PROGRAMS=blastp&PAGE_TYPE=BlastSearch&SHOW_DEFAULTS=on) with the sequence MMWEDAVGWI (Nle is a oxidation resistant analog of M) to identify sequences which were most similar in sequence to formyl-NleNleWEDAVGWI. Bacteria (taxid:2) was used as the search organism. Two sequences of reasonable similarity to MMWEDAVGWI at their N-terminus were identified (Only N-termini would be formylated). One was protein MAP 4176 from *Mycobacterium avium* subsp. *paratuberculosis* and the other was protein JNB 01080 from *Janibacter*. Only the MAP 4176 sequence

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was investigated and it had MFEDAVAWF as its N-terminal sequence. Formyl-MFEDAVAWF was synthesized and evaluated for binding and downregulation. Formyl-MFEDAVAWF's K_i was 4 fold higher for W190/N192 than for R190/K192 or R190/N192 (Table 1). We also determined the EC_{50} for downregulation. Formyl-MFEDAVAWF behaved as a full agonist with all three FPRs (figure 4B) and this peptide activates W190/N190 ~10 fold more weakly than either R190/K192 or R190/N192. EC_{50}s for W190/N192, R190/K192 and R190/N192 were 13,000±1000 nM, 1000±80 nM, and 1500±100 nM, respectively (P<0.0001, W190/N192 vs either R190/K192 or R190/N192).

Formyl-MFEDAVAWF exhibited some similarity to the N-terminus of CHIPS so we synthesized the peptide FTFEPFPTN, which corresponds to the N-terminus of CHIPS, and also formyl-MFTFEPFPTN. We tested commercially available CHIPS for its ability to inhibit formyl-Nle-Leu-Phe-Nle-Tyr-Lys-FITC binding but could not detect any inhibition at 50 nM with any of the FPR variants. FTFEPFPTN produced very little inhibition of formyl-Nle-Leu-Phe-Nle-Tyr-Lys-FITC binding also with at most 30% inhibition at 100 μM with the three FPR variants. Formyl-MFTFEPFPTN was very effective at inhibiting all three variants (Table 1) and exhibited significantly different K_is for W190/N192, R190/K192 and R190/N192 with K_is of 71±4 nM, 16±0.7 nM, and 9.5±0.2 nM, respectively (P<0.0001 for each variant vs each of the other two). We also tested formyl-MFTFEPFPTN for its ability to downregulate W190/N192, R190/K192 and R190/N192 (Figure 4C.) The EC_{50}s were 19±1 nM, 3.1±0.2 nM, and 6.8±100 nM, respectively (P<0.0001 for each variant vs each of the other two) and the maximum downregulation was very similar to that see with formyl-Nle-Leu-Phe. The addition of formyl-methionine to the N-terminus of FTFEPFPTN enhanced the affinity ~10,000 fold, and formyl-MFTFEPFPTN exhibited an affinity similar to that seen with CHIPS (K_d~35 nM)[11]. It should be noted that while CHIPS was originally isolated as a secreted protein from *S. aureus* [35], many of the experiments were done using CHIPS expressed in *E. coli*[10,11,36], and the properties of FLIPr were determined only on the *E. coli* expressed protein [12]. *E. coli* expressed proteins would be expected to have an N terminal formyl-methionine. However, the authors noted no difference with the *E. Coli* expressed CHIPS [11].

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Effects of variants W190 and K192 on predicted secondary structure. We analyzed the possible effects on secondary structure that might occur in response to changes at positions 190 or 192 using the program PSIPRED which gives a quantitative estimation of secondary structure (http://bioinf.cs.ucl.ac.uk/psipred/) [32]. The results are shown in Table II. The sequence prediction of rhodopsin is also given since its crystal structure is known [37]. TMH V in rhodopsin begins at E202 and is the first residue predicted to have a non zero value for helix propensity. Underlined residues in the rhodopsin sequence are identical in FPR. W190/N192 exhibits a reduced helix propensity of 113 vs 140 for R190/N192 or 137 for R190/K192 (summation of helix propensity for residues N-terminal to FPR P213 or rhodopsin P215; underlined in Table II). PRISPED analysis of all three FPR sequences suggest that TMH V is longer than that observed with rhodopsin as we previously suggested [8] based on Kyte and Doolittle [38] analysis, but the predicted beginning of helix V varies depending upon the FPR sequence. Most interesting is the predicted effect W190/N192 has on the helix propensity of the residues N-terminal to FPR P213. P213 was previously shown to be important in FPR structure, since P213A did not fold properly and was retained in the endoplasmic reticulum [39]. Since proline is secondary amine, it cannot hydrogen bond and this destabilizes the helix N-terminal to the proline. In rhodopsin, the carbonyl of H211 hydrogen bonds to the carboxylate of E122 (which can only occur if H211 is not hydrogen bonded to P215, a residue highly conserved in GPCR [40]). The H211 carbonyl is important in rhodopsin activation, since its hydrogen bonding with E122 is disrupted upon rhodopsin activation [41]. We have previously shown that F110, which is analogous to E122 of rhodopsin, is important in FPR activation, since the F110A mutant exhibits very poor coupling to G protein, and very poor chemotaxis toward fMLF [42]. While FPR F110 cannot hydrogen bond to the carbonyl of FPR G209 (analogous to rhodopsin H211), aromatic rings do exhibit energetically favorably interactions with carbonyl oxygens [43]. F110 interaction with the G209 carbonyl and disruption of the F110-G209 interaction by ligand binding could be important in FPR activation. The fact that W190/N190 is predicted to exhibit lower helicity near G209 may alter its ability to be activated in response to ligand binding.

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We had previously observed that the polymorphism W190/N192 exhibited slightly reduced (~2 fold) affinity for formyl-Nle-Leu-Phe but a 4 fold higher affinity for several virally derived peptides when compared with either R190/K192 or R190/N192 [8]. Here we observed that the three W in the peptide WEDWVGWI are all essential for FPR binding, just as they are essential for antiviral activity[31]. This could be due to changes in structure of the peptide since WEDWVGAI was shown by NMR in DMSO-water solution to adopt a different structure than WEDWVGWI [31]. Nonetheless, the correlation between antiviral activity and ability to interact with FPR is an intriguing one, especially since FPR is able to interact with the proximal membrane region of many viruses [8].

We also evaluated several formyl-Nle derivatives of WEDWVGWI and formyl-MYVKWPWYVWL in order to identify possible formyl peptide ligands which exhibit markedly different activities toward the W190/N192 polymorphism. We also identified a similar peptide from blastp bacterial sequence data bases. Most of the peptides evaluated interacted similarly with R190/K192 and R190/N192 FPRs, but both formyl-MYVKWPWYVWL and formyl-MFTFEPFPTN activated R190/K192 ~ 2fold more effectively than R190/N192. W190/N192 was activated much more poorly than either R190/K192 or R190/N192 by all the formyl peptides investigated here and exhibited much greater differences in EC_{50}s than observed for formyl-Nle-Leu-Phe. This was true even though W190/N192 bound formyl-NleNleWEDWVGWI and formyl-MYVKWPWYVWL with similar affinity to that observed with R190/K192 or R190/N192. This implies that a W at position 190 interferes with the activation process but does not alter the binding constant appreciably with these peptides.

We also identified a formyl peptide derived from a protein in *Mycobacterium avium* subsp. *paratuberculosis*, formyl-MFEDAVAWI, which activates R190/K192 and R190/N192 ~10 fold better than W190/R190. This could be important since *Mycobacterium avium* subsp. *paratuberculosis* is a facultative intracellular pathogen of macrophages which is associated with Johne's disease in cattle and may be associated with Crohn's disease in humans [44,45].

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Thus, the replacement of R190 by W190 produces profound effects on the activation of the receptor by six different formyl peptides composed of nine or more residues but a much lesser effect on the tri-peptide formyl-Nle-Leu-Phe. For formyl-MYVKWPWYVWL, formyl-NleNleWEDWVGWI, formyl-NleNleWED**A**VGWI, and formyl-NleWEDWVGWI, the major difference between W190/N192 and R190/N192 or R190/K192 is the ability to activate the receptor and the difference in K_i is small (~2 fold or less). For formyl-MFTFEPFPTN and formyl-MFEDAVAWF both K_i and EC_{50} were markedly increased in W190/N192 compared to either R190/N192 or R190/K192. It should be remembered that K_i does not reflect the binding energy used to promote conformational change in the receptor, since the latter is potential energy. The EC_{50} for downregulation, therefore, may be a better representation of the total binding energy.

The fact that the addition of formyl-Nle to WEDWVGWI had little effect (<20%) on its affinity but the addition of formyl-Met to FTFEPFPTN enhanced the affinity ~10,000 fold implies a marked difference in the mode of binding of the two peptides. One possibility is that WEDWVGWI occupies the formyl-Met (or formyl-Nle) binding pocket but FTFEPFPTN does not. The fact that formyl-MFTFEPFPTN binds with similar affinity to CHIPS may indicate that in the intact protein, some residue of the protein is able to interact with the formyl-Met binding site and substitute for it but not activate the receptor.

We had previously proposed that R205 in TMH V interacts with the C-terminus of fMLF [46] and is essential for binding this tri-peptide, but is much less essential in binding larger peptides like formyl-Nle-Leu-Phe-Nle-Tyr-Lys-FITC [39]. We have also shown that W190 quenches the fluorescence of formyl-Nle-Leu-Phe-Nle-Tyr-Lys-AlexaFluor, indicating W190 must reside close to the AlexaFluor moiety when this peptide is bound to FPR [8]. These findings are both consistent with the larger effects observed with W190/N192 with longer peptides since only peptides much longer than three residues are likely to interact near R or W190. In addition, provided that formyl-MFTFEPFPTN is a good model for CHIPS binding, this might indicate that the polymorphism W190/N192 would be more resistant to inhibition by CHIPS and thus those with this

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polymorphism might control infections with *S. aureus* better than those with the R190 polymorphism. Recent studies have indicated that a high percentage of normal individuals (24/24) express antibodies to CHIPS [47], so that a polymorphism that was more resistant to its effect might exhibit a selective advantage, since W190/N192 retains most of its ability to interact with formylated tri peptides.

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Abbreviations: FPR, formyl peptide receptor; CHIPS, chemotaxis inhibitory protein of *Staphylococcus aureus*; CHO S, Chinese hamster ovary cells designed for suspension culture; HRSV: human respiratory syncytial virus; FIV: feline immunodeficiency virus; fMLF: N-formyl-methionyl-leucyl-phenylalanine; FLIPr, FPRL1 inhibitory protein; AIDS, Acquired Immunodeficiency Syndrome; SIV: Simian Immunodeficiency Virus; HIV: human immunodeficiency virus; SARS: severe acute respiratory syndrome; GP-41, 41 kilodalton glycoprotein; GP-36, 36 kilodalton glycoprotein. HR: Heptade Repeat; FITC: Fluorescein isothiocyanate; formyl-Nle-Leu-Phe-Nle-Tyr-Lys-FITC, formyl-Nle-Leu-Phe-Nle-Tyr-Lys labeled at the Lys residue with Fluorescein isothiocyanate; formyl-Nle-Leu-Phe-Nle-Tyr-Lys labeled at the Lys residue with Alexa Fluor N-hydroxy-succinimide; fMLF, formyl-Met-Leu-Phe; TMH, transmembrane helix; FPRL1, formyl peptide receptor like 1; GTPγS, guanosine 5'-3-O-(thio)triphosphate.

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Figure legends.

Figure 1. Inhibition of formyl-Nle-Leu-Phe-Nle-Tyr-Lys-FITC binding by 5μM WEDWVGWI, AEDWVGVWI, WEDAVGWI, and WEDWVGWI in FPRs R190/N192, R190/K192, or W190/N192.

Figure 2. (A) Downregulation of FPR surface expression in response to preincubation at 37^0 C for the indicated times with 3 μM formyl-Nle-Leu-Phe for FPR R190/N192 (■), R190/K192(○) or W190/N192 (♦). (B) Recovery from downregulation with 3 μM formyl-Nle-Leu-Phe for 60 min at 37^0 C after its removal for FPR R190/N192 (■), R190/K192(○) or W190/N192.

Figure 3. Downregulation of FPR surface expression in response to preincubation with varying concentrations of formyl-Nle-Leu-Phe (A) or formyl-NleWEDWVGWI (B) with FPR R190/N192 (■), R190/K192(○) or W190/N192 (♦). Maximum downregulation with formyl-Nle-Leu-Phe was 83±2%, 74±1%, and 60±2% for R190/N192, R190/K192, and W190/N192, respectively.

Figure 4. Down regulation of FPR surface expression in response to preincubation with varying concentrations of formyl-NleNleWEDWVGWI (A) or formyl-NleNleWED**A**VGWI (B) with FPR R190/N192 (■), R190/K192(○) or W190/N192 (♦).

Figure 5. Down regulation of FPR surface expression in response to preincubation with varying concentrations of formyl-MYKWPWYVWL (A) or formyl-MFEDAVAWF (B) or formyl-M MFTFEPFPTN (C) with FPR R190/N192 (■), R190/K192(○) or W190/N192 (♦).

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Table 1.
Ki (nM±SEM) for binding of peptides to R190/N192, R190/K192, or W190/N192 FPR

	R190/N192	R190/K192	W190/N192
WEDWVGWI	1300±200	1300±300	2600±400
Formyl-NleWEDWVGWI	1600±100	1700±100	2800±200*
Formyl-NleNleWEDWVGWI	480±30	480±20	1000±100*
Formyl-MFEDAVAWF	7700±500	7000±400	30,000±2000 **
FTFEPFPTN	>50,000	>50,000	>50,000
formyl-MFTFEPFPTN	16.0±0.7***	9.5±0.2***	71±4***

* P<0.001 W190/N192 vs either R190/N192 or R190/K192

** P<0.0001 W190/N192 vs either R190/N192 or R190/K192

***P<0.0001 vs both other variants

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Table II.
Structure prediction by PSIPRED

```
R190/N192 Conf: 754234000221000589999999998655778 8777 (140)
          Pred: CCCCCCCCCCCCEEHHHHHHHHHHHHHHHHHHHHHHH
            AA: SPWTNDPKERINVAVAMLTVRGIIRFIIGFSAPMSIV

W190/N192 Conf: 776433001100013557899999997741007 8998 (113)
          Pred: CCCCCCCHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH
            AA: SPWTNDPKEWINVAVAMLTVRGIIRFIIGFSAPMSIV

R190/K192 Conf: 754346410122588899999975434555447 8764 (137)
          Pred: CCCCCCCCCHHHHHHHHHHHHHHHHHHHHHHHHHHHH
            AA: SPWTNDPKERIKVAVAMLTVRGIIRFIIGFSAPMSIV
                        190       200       210

rhodopsin Conf: 542000245444443200247899999999999999999 (120)
          Pred: CCCEECCCCCCCCCCCHHHHHHHHHHHHHHHHHHHHH
           AA:  MQCSCGIDYYTPHEETNNESFVIYMFVVHFIIPLIVI
                     190       200       210       220
```

Conf,confidence; Pred,predicted secondary structure; AA,amino acid; C,coil; H,helix; E,extended. Numbers in parentheses are the summation of the underlined confidence prediction. Underlined bold; identical between rhodopsin and FPR; underlined; similar between rhodopsin and FPR. The highly conserved proline is indicated in italics [40].

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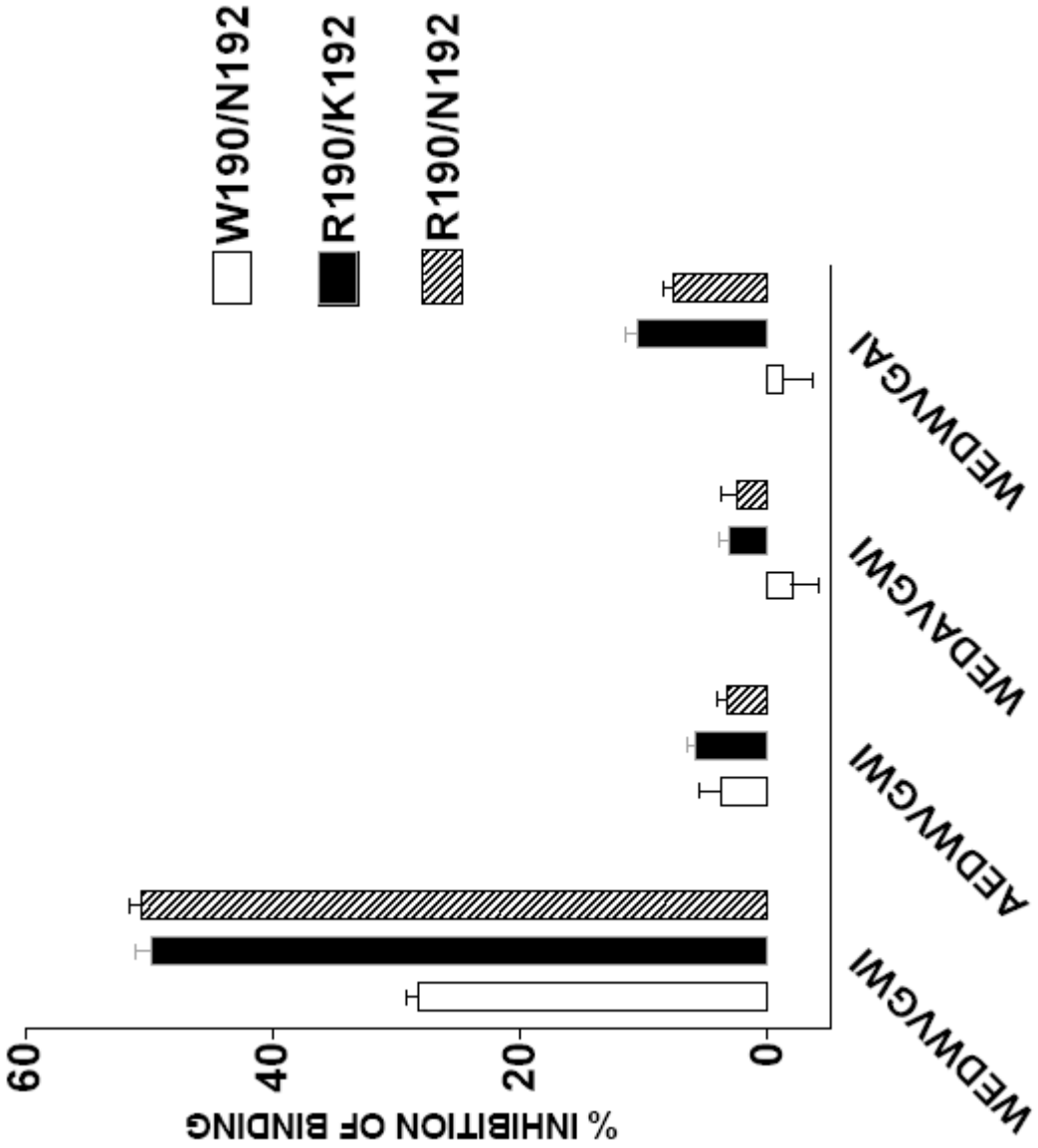

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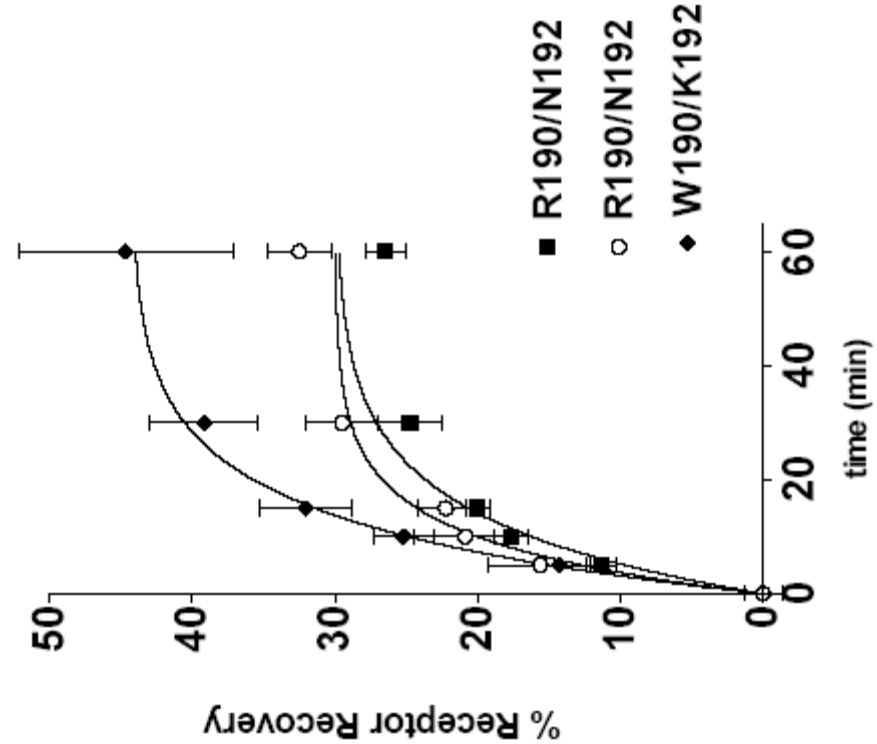

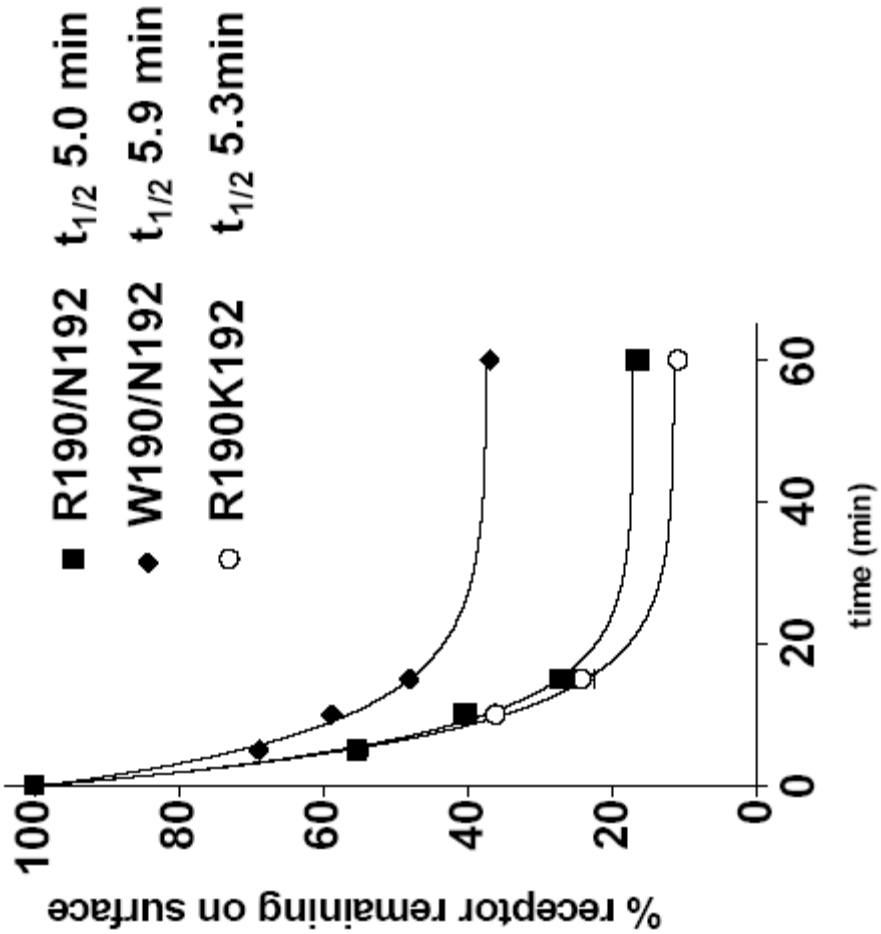

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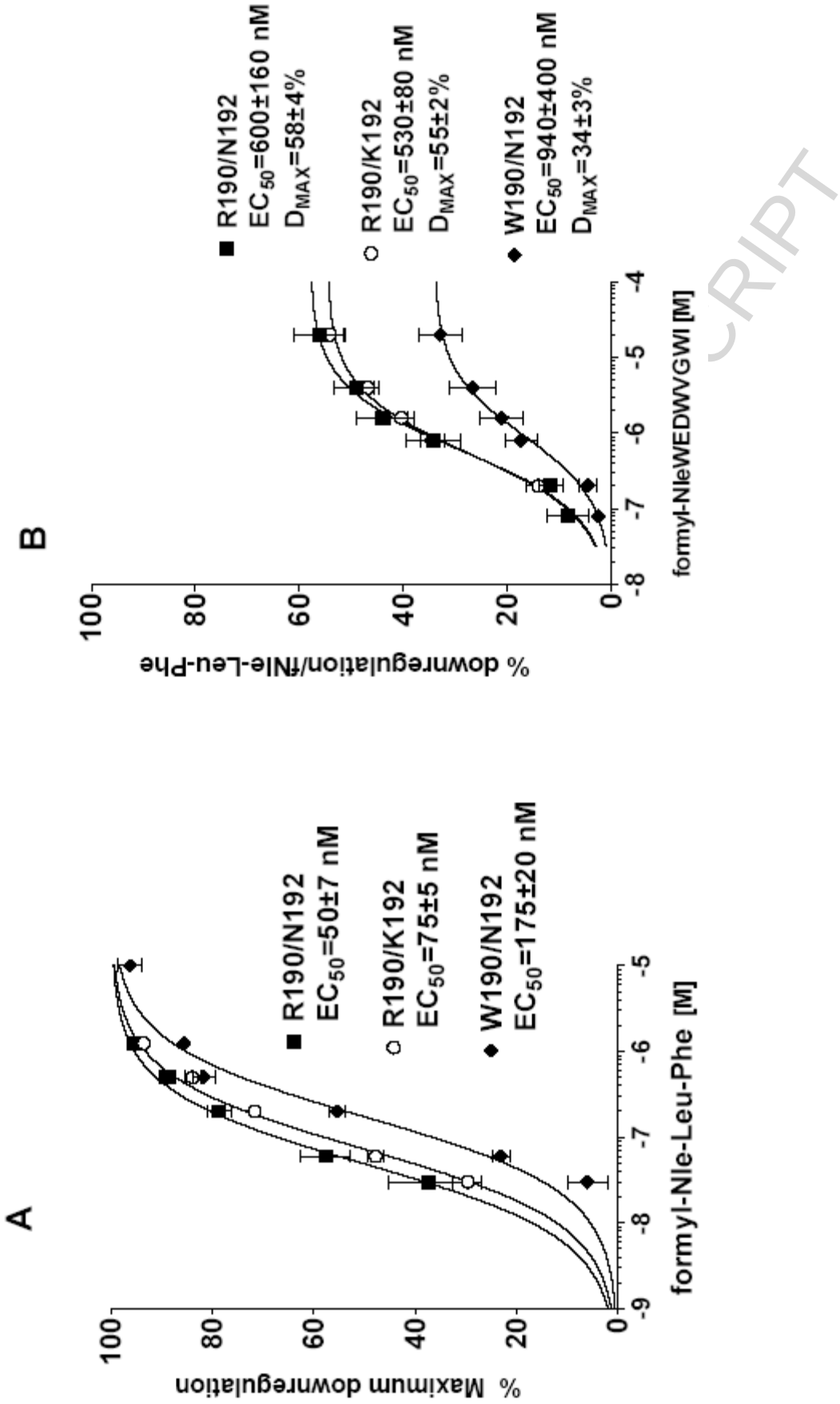

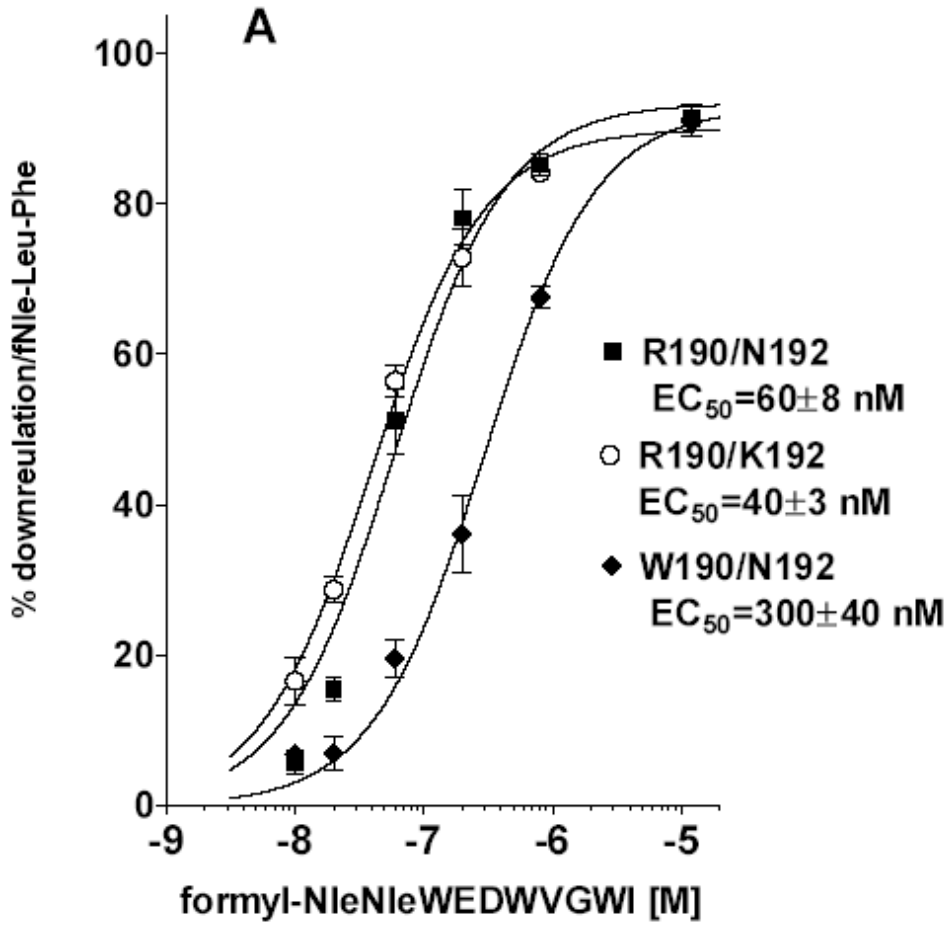

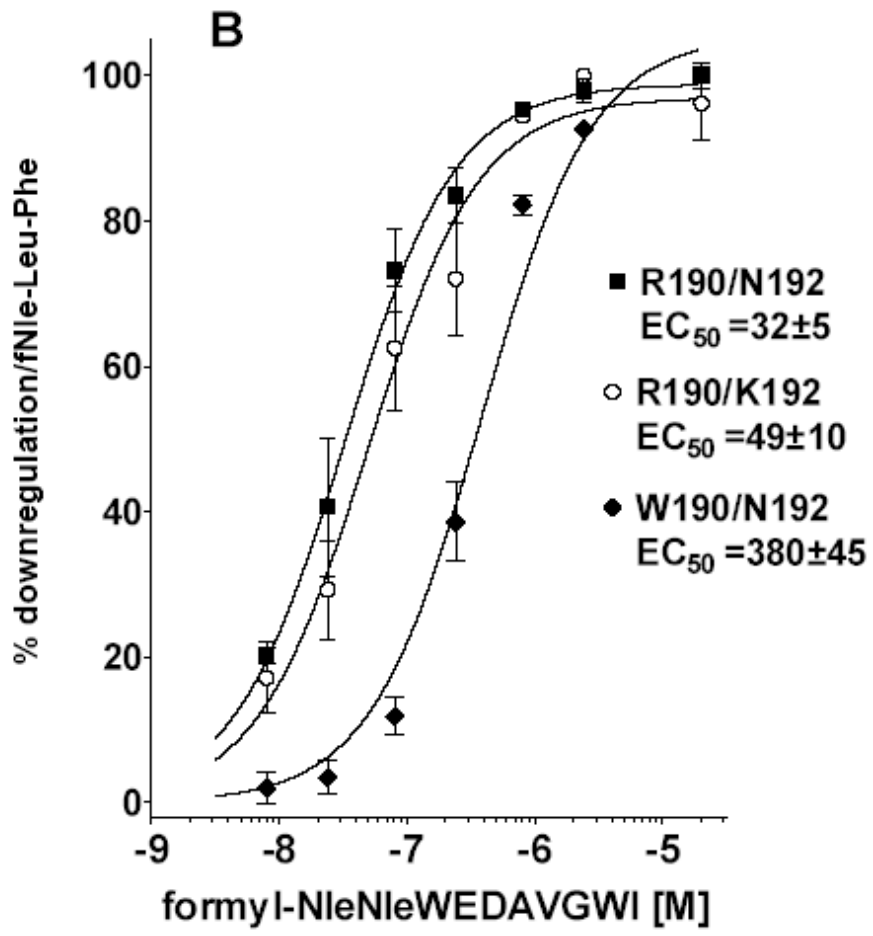

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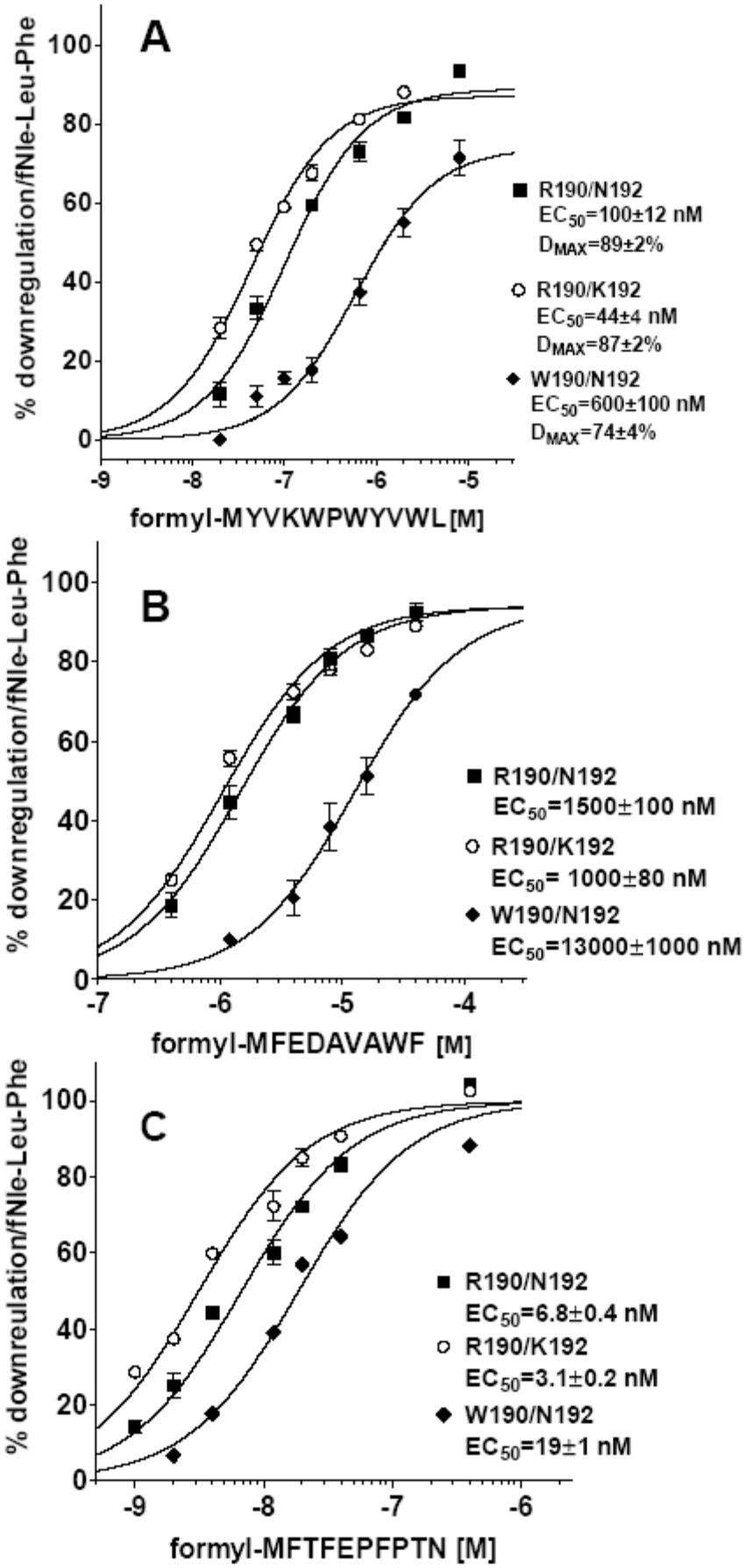
A
% downregulation/fNle-Leu-Phe
formyl-MYVKWPWYVWL [M]
R190/N192
EC50=100±12 nM
DMAX=89±2%
R190/K192
EC50=44±4 nM
DMAX=87±2%
W190/N192
EC50=600±100 nM
DMAX=74±4%
B
% downregulation/fNle-Leu-Phe
formyl-MFEDAVAWF [M]
R190/N192
EC50=1500±100 nM
R190/K192
EC50= 1000±80 nM
W190/N192
EC50=13000±1000 nM
C
% downreulation/fNle-Leu-Phe
formyl-MFTFEPFPTN [M]
R190/N192
EC50=6.8±0.4 nM
R190/K192
EC50=3.1±0.2 nM
W190/N192
EC50=19±1 nM