

Supplemental tables and figures for:

Structural basis for parathyroid hormone-related protein binding to the parathyroid hormone receptor and design of conformation selective peptides

Augen A. Pioszak¹, Naomi R. Parker¹, Thomas J. Gardella², and H. Eric Xu¹

¹Laboratory of Structural Sciences, Van Andel Research Institute, 333 Bostwick Avenue, N.E., Grand Rapids, MI 49503

²Endocrine Unit, Massachusetts General Hospital, Boston, MA 02144.

Table S1. Summary of the R⁰/RG binding of hybrid PTH/PTHrP(1-34)NH₂ peptides.

peptide	IC ₅₀ Log M, n=3 (nM)			R ⁰ /RG
	R ⁰		RG	
hPTH(1-34)	-8.5 ± 0.12 (3.9±0.9)		-9.6 ± 0.16 (0.28±0.09)	13.9
W23F	-7.9 ± 0.06 (13.5±2.1)		-9.7 ± 0.03 (0.23±0.01)	59
K27L	-8.2 ± 0.09 (6.4±1.2)		-9.3 ± 0.13 (0.54±0.15)	11.9
L28I	-8.4 ± 0.03 (4.5±0.5)		-10.0 ± 0.07 (0.10±0.02)	45
V31I	-7.5 ± 0.04 (34±2.2)		-9.6 ± 0.15 (0.31±0.10)	110
W23F/K27L/L28I/V31I	-7.3 ± 0.04 (53±3.4)		-9.2 ± 0.14 (0.78±0.27)	68
W23F/K27L	-8.1 ± 0.02 (8.7±0.4)		-9.6 ± 0.18 (0.33±0.13)	26.4
W23F/L28I	-7.4 ± 0.04 (38±3.2)		-9.7 ± 0.10 (0.20±0.04)	190
K27L/L28I	-7.8 ± 0.02 (16±0.7)		-9.5 ± 0.20 (0.38±0.16)	42
W23F/V31I	-7.1 ± 0.01 (81±2.1)		-9.7 ± 0.12 (0.21±0.06)	386
L28I/V31I	-7.4 ± 0.10 (42±8.6)		-9.6 ± 0.13 (0.31±0.10)	135
K27L/V31I	-7.6 ± 0.01 (25±0.7)		-9.3 ± 0.23 (0.68±0.33)	36.8
PTHrP(1-34)	-7.6 ± 0.02 (27±1.4)		-10.0 ± 0.08 (0.11±0.02)	245
F23W	-7.8 ± 0.02 (16±0.9)		-9.8 ± 0.08 (0.15±0.03)	107
L27K	-5.9 ± 0.02 (1214±60)		-9.8 ± 0.15 (0.16±0.05)	7588
I28L	-7.7 ± 0.06 (21±2.5)		-10.0 ± 0.17 (0.11±0.03)	191
I31V	-7.9 ± 0.07 (14±2.2)		-9.8 ± 0.06 (0.15±0.02)	93
F23W/L27K/I28L/I31V	-7.4 ± 0.05 (41±4.2)		-10.1 ± 0.09 (0.07±0.01)	586
F23W/L27K	-7.1 ± 0.05 (77±9.3)		-10.0 ± 0.08 (0.10±0.02)	770
L27K/I28L	-6.2 ± 0.06 (598±84)		-9.8 ± 0.19 (0.17±0.06)	3518
L27K/I31V	-6.2 ± 0.06 (624±73)		-10.1 ± 0.24 (0.11±0.04)	5673

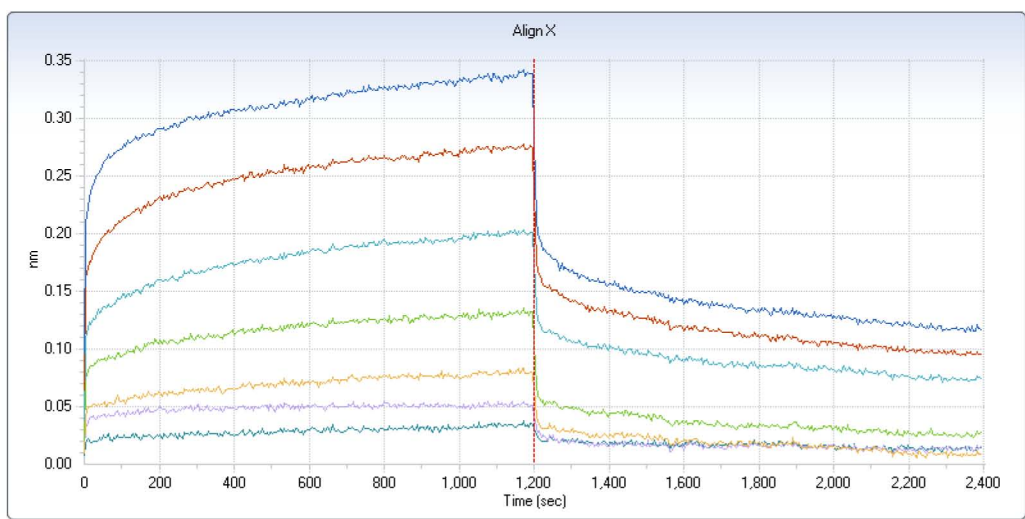
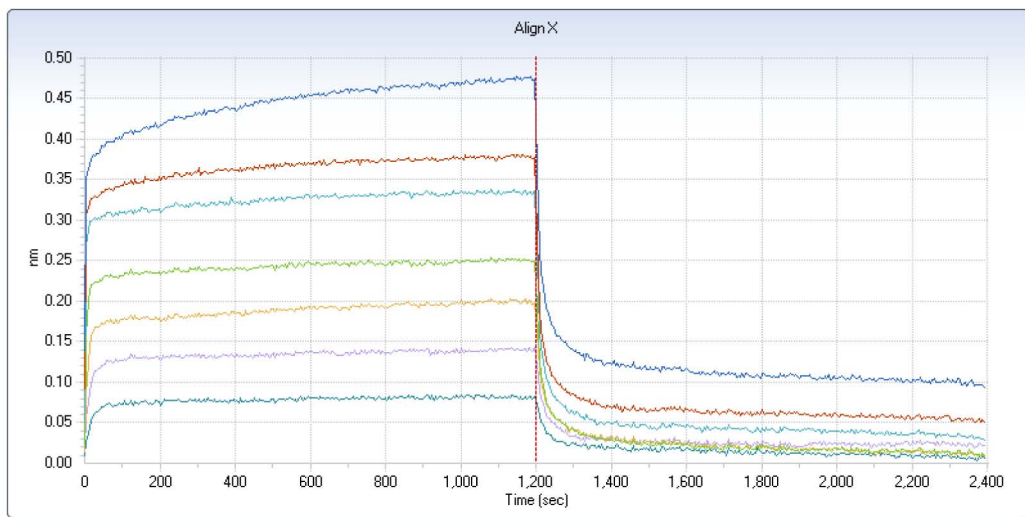
A**B**

Fig. S1. Octet Red real-time analysis of the binding of PTH and PTHrP to the PTH1R ECD. (A) Biotinylated PTH1R ECD was immobilized on streptavidin sensor tips and the binding of PTH(1-34)NH₂ was assessed at the following concentrations: 156.25 nM, 312.5 nM, 625 nM, 1.25 μ M, 2.5 μ M, 5 μ M, and 10 μ M. (B) Same as panel A, except the binding of PTHrP(1-34)NH₂ was assessed.

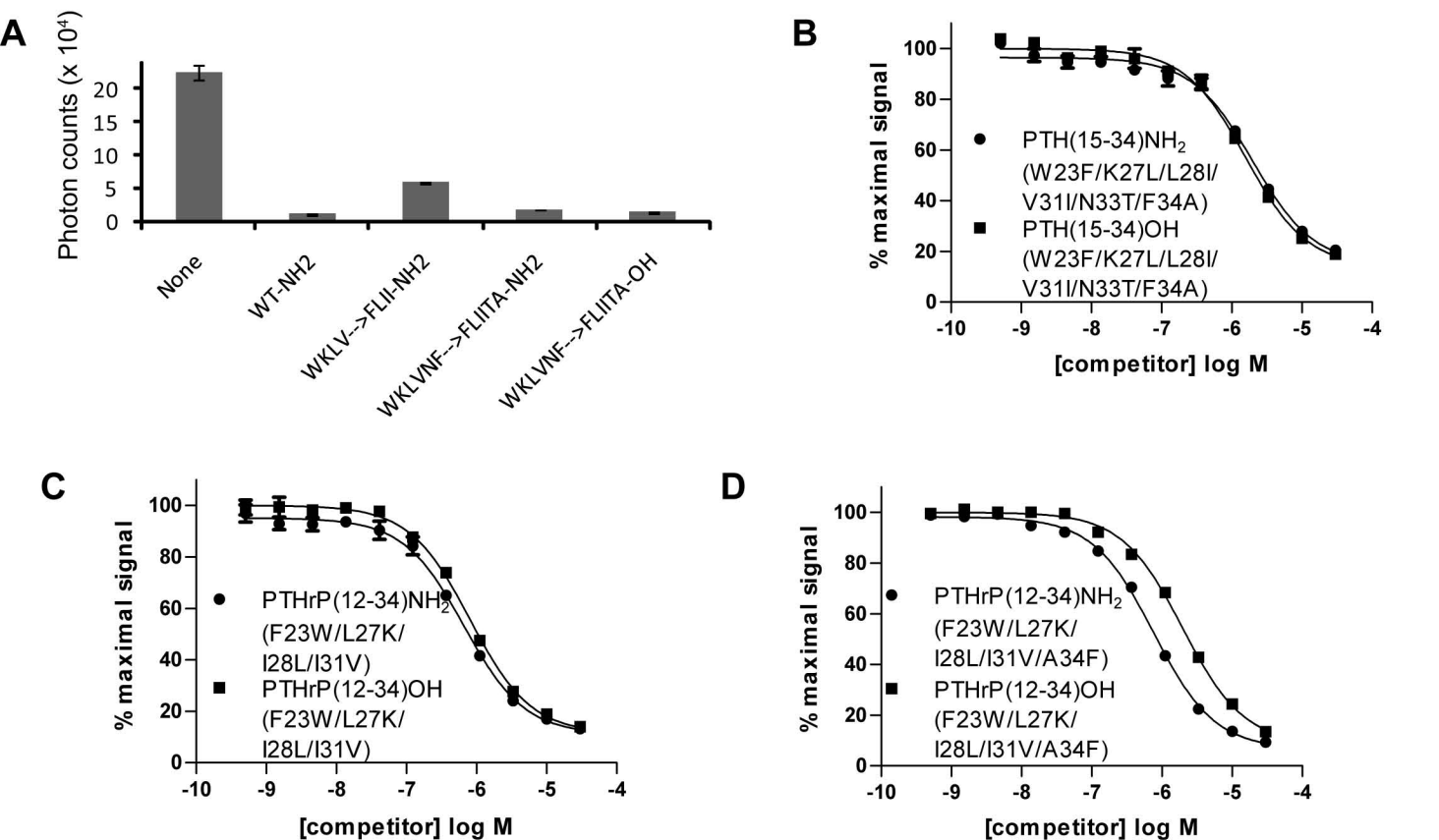


Fig. S2. AlphaScreen analysis of the binding of hybrid PTH/PTHrP peptides to the MBP-PTH1R ECD fusion protein. (A) Single point competition assay assessing the ability of the indicated peptides in the PTH(15-34) scaffold (20 μ M) to compete the interaction of biotin-PTH and the fusion protein. (B, C, D) Competition curves for the indicated peptides.