Discovery of High-Affinity PDGF-VEGFR Interactions: Redefining RTK Dynamics

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Supplementary Materials

Supplementary Tables

Supplementary Table S1. Classification of true and false interactions via χ^2-to-R_{max}. The ratio reflects how well a global kinetic analysis of a multi-concentration sensogram series can fit to a simple 1:1 Langmuir interaction model can fit. True interactions were distinguished from those due to non-specific binding patterns where χ^2-to-R_{max} < 1.0 (indicated by green shading and polka dot texturing). Fitted curves where χ^2-to-R_{max} > 1.0 (in red-shaded, grid-pattern textured cells) were considered to be dominated by non-specific binding. Ligand-receptor pairs that produced negative association curves were not analyzed with global fitting (indicated by "NI" for no interaction).

	VEGFR1	**VEGFR2**	**VEGFR3**	**PDGFRα**	**PDGFRβ**
VEGFA	0.17	0.52	6.03	34.4	0.95
PDGFAA	NI	0.082	NI	0.023	8.6
PDGFAB	NI	0.29	NI	0.0042	0.19
PDGFBB	NI	0.029	NI	0.0082	0.0064
PDGFCC	NI	0.33	NI	0.43	0.58
PDGFDD	NI	NI	NI	1130	2.2

Supplementary Table S2. VEGF and PDGF binding kinetics and affinities. Sensograms for ligand injections at 10 nM, 20 nM, and 40 nM, two replicates each, for each ligand-receptor pair. Kinetic constants were obtained by performing global kinetic analysis using the BIAevaluation software across several kinetic binding sensograms. Affinity constants were calculated as the ratio of the association and dissociation rates. Rate constants where the χ^2-to-R_{max} ratio exceeds 1.0 are shown *in italics*, indicating interactions that are dominated by non-specific interactions. Ligand-receptor pairs that produced negative association curves were not analyzed with global fitting (indicated by "NI" for no interaction). Mean values are reported for each kinetic fit for N=3. Uncertainty presented using the standard error of the mean.

	VEGFR1			VEGFR2			VEGFR3		
	k_a (M^{-1} s^{-1})	k_d (s^{-1})	K_D (M)	k_a (M^{-1} s^{-1})	k_d (s^{-1})	K_D (M)	k_a (M^{-1} s^{-1})	k_d (s^{-1})	K_D (M)
VEGFA	$4.0 \pm 0.04 * 10^{5}$	$4.0 \pm 0.1 * 10^{-7}$	$1.0 \pm 0.03 * 10^{-12}$	$9.7 \pm 0.3 * 10^{5}$	$9.5 \pm 0.2 * 10^{-6}$	$9.8 \pm 0.4 * 10^{-12}$	*2.2*	*$1.3 * 10^{-5}$*	*$5.2 * 10^{-6}$*
PDGFAA	NI	NI	NI	$1.6 \pm 0.1 * 10^{3}$	$8.6 \pm 0.1 * 10^{-4}$	$5.3 \pm 0.4 * 10^{-7}$	NI	NI	NI
PDGFAB	NI	NI	NI	$1.4 \pm 0.3 * 10^{3}$	$1.6 \pm 0.6 * 10^{-6}$	$1.1 \pm 0.5 * 10^{-10}$	NI	NI	NI
PDGFBB	NI	NI	NI	$3.5 \pm 0.7 * 10^{3}$	$1.3 \pm 0.1 * 10^{-4}$	$3.7 \pm 0.9 * 10^{-8}$	NI	NI	NI
PDGFCC	NI	NI	NI	$3.6 \pm 0.2 * 10^{4}$	$2.6 \pm 0.3 * 10^{-5}$	$7.0 \pm 0.9 * 10^{-11}$	NI	NI	NI
PDGFDD	NI	NI	NI	NI	NI	NI	NI	NI	NI

	PDGFRα			PDGFRβ		
	k_a (M^{-1} s^{-1})	k_d (s^{-1})	K_D (M)	k_a (M^{-1} s^{-1})	k_d (s^{-1})	K_D (M)
VEGFA	*$1.1 * 10^{3}$*	*$2.7 * 10^{-5}$*	*$2.6 * 10^{-8}$*	$1.2 \pm 0.1 * 10^{4}$	$4.0 \pm 0.5 * 10^{-6}$	$3.4 \pm 0.5 * 10^{-10}$
PDGFAA	$6.8 \pm 1.3 * 10^{2}$	$4.5 \pm 1.3 * 10^{-4}$	$6.6 \pm 2.3 * 10^{-7}$	*$8.8 \pm 0.4 * 10^{3}$*	*$1.5 \pm 0.4 * 10^{-4}$*	*$1.7 \pm 0.4 * 10^{-8}$*
PDGFAB	$1.2 \pm 0.1 * 10^{3}$	$1.0 \pm 0.2 * 10^{-3}$	$8.4 \pm 1.7 * 10^{-7}$	$1.3 \pm 0.3 * 10^{3}$	$2.9 \pm 0.7 * 10^{-3}$	$2.2 \pm 0.7 * 10^{-6}$
PDGFBB	$2.0 \pm 0.1 * 10^{4}$	$8.6 \pm 0.1 * 10^{-3}$	$4.2 \pm 0.3 * 10^{-7}$	$1.9 \pm 0.1 * 10^{3}$	$1.6 \pm 0.2 * 10^{-3}$	$8.3 \pm 1.3 * 10^{-7}$
PDGFCC	$5.0 \pm 1.6 * 10^{3}$	$2.0 \pm 0.1 * 10^{-4}$	$3.4 \pm 1.8 * 10^{-9}$	$7.4 \pm 0.4 * 10^{5}$	$1.4 \pm 0.06 * 10^{-3}$	$1.9 \pm 0.1 * 10^{-9}$
PDGFDD	*18.8*	*$1.0 * 10^{-5}$*	*$5.4 * 10^{-7}$*	*$3.8 * 10^{4}$*	*$2.6 * 10^{-3}$*	*$6.7 * 10^{-8}$*

Supplementary Table S3. Literature mined VEGF and PDGF from SPR and cell-based assays. Values determined with cell assays indicated with † and SPR indicated with an asterisk***.** Currently, few interaction kinetics have been determined.

Interaction	Measured K_D (M)
VEGF-A:VEGFR1	$7.4*10^{-11}$ ***-** $7.5*10^{-12}$ M ([1])†
VEGF-A:VEGFR2	$5.2*10^{-11}$ M ([1]) †
PDGF-AA:PDGFRα	$1.34*10^{-8}$ M ([2])*****
PDGF-AA:PDGFRβ	$4.53*10^{-7}$ M ([2])*****
PDGF-BB:PDGFRα	$1.5*10^{-7}$ M ([2])*****
PDGF-BB:PDGFRβ	$1.6*10^{-9}$ M ([2])*****

Supplementary Table S4. Model kinetic parameters and initial values.

	Benchmark	Healthy	Cancer	Units
VEGFA:VEGFR1 coupling				
$k_{1,f}$ (coupling)	$3.8 * 10^6$ ([3])	$4.0 * 10^5$ (via SPR)		$M^{-1} s^{-1}$
$k_{1,r}$ (decoupling)	$95 * 10^{-6}$ ([3])	$4.0 * 10^{-7}$ (via SPR)		s^{-1}
VEGFA:VEGFR2 coupling				
$k_{2,f}$ (coupling)	$1.2 * 10^6$ ([3])	$9.7 * 10^5$ (via SPR)		$M^{-1} s^{-1}$
$K_{2,r}$ (decoupling)	$410 * 10^{-6}$ ([3])	$9.5 * 10^{-6}$ (via SPR)		s^{-1}
PDGF:VEGFR2 coupling	*N/A*	See **Supplementary Table S1**		
Receptor and ligand-receptor complex internalization				
k_{int}	10^{-5} ([3])			s^{-1}
Receptor insertion				
$k_{insert,R1}$	0.8 ([3])			s^{-1}
$k_{insert,R2}$	2.3 ([3])			s^{-1}
Extracellular volume				
V_{mem}	10^{-10} ([4])			L
VEGFA-bevacizumab coupling				
$k_{7,f}$	4.10×10^7 [(5)]	$5.3 * 10^5$ ([6])		$M^{-1} s^{-1}$
$k_{7,r}$	2.01×10^{-5} [(5)]	$3.1 * 10^{-5}$ ([6])		s^{-1}
VEGFR1 concentration/EC				
D_{11}	80,000 ([3])	990 ([7])	8200 ([8])	receptors
VEGFR2 concentration/EC				
D_{22}	230,000 ([3])	1890 ([7])	1100 ([8])	receptors
Healthy serum concentrations				
VA	2.2 ([3])	See **Supplementary Table S3**		nM
P_{AA}	N/A			nM
P_{AB}	N/A			nM
P_{BB}	N/A			nM
P_{CC}	N/A			nM

Supplementary Table S5. Serum concentrations for VEGF-A and PDGFs under physiological and pathological conditions. All values are means (± SD where provided) unless otherwise noted.

	[VEGF-A]	[PDGF-AA]	[PDGF-AB]	[PDGF-BB]	[PDGF-CC]	[PDGF-DD]
Normal (serum)	81.7-91.83 pg/m[9,10]	250.0-1700 pg/mL[10–12]	250.0-2830 pg/mL[11]	8506 ± 550 pg/mL[13]	None	1170 ± 460 pg/mL[14]
Exercise (serum)	165.61 pg/mL[10]	4640 pg/mL[10]				
Wound fluid	1033 pg/mL[15]	8604 pg/mL[15]				
Stroke (0hr) (serum)	410 ± 71 pg/mL[16]	7300 ± 5100 pg/mL[17]	52600 ± 18900 pg/mL[17]	10100 ± 7700 pg/mL[17]	66900 ± 41200 pg/mL[17]	
Stroke (24hr) (serum)	416 ± 64 pg/mL[16]	5500 ± 3900 pg/mL[17]	48000 ± 16400 pg/mL[17]	7000 ± 4700 pg/mL[17]	123600 ± 64000 pg/mL[17]	
Breast Cancer (serum)	305.9 pg/mL[18]	11900 ± 5100 pg/mL[19]	11900 ± 5100 pg/mL[19]	7623 pg/mL[20]		7500 pg/mL[21]

Supplementary Table S6. Isoelectric points of immobilized proteins and optimal immobilization pHs.

Protein	pI	Optimal pH
VEGFR1	8.7[22]	3.7
VEGFR2	5.6[23]	4.0
VEGFR3	5.9[24]	5.0
PDGFRα	5.0[25]	4.5
PDGFRβ	4.9[26]	3.7
Angiopoietin-4	9.1[27]	4.0

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25. PDGFRA (human). *PhosphoSitePlus* **2014,**
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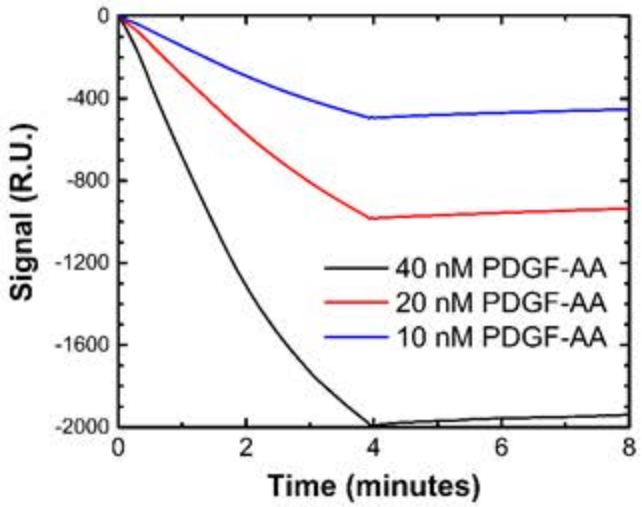
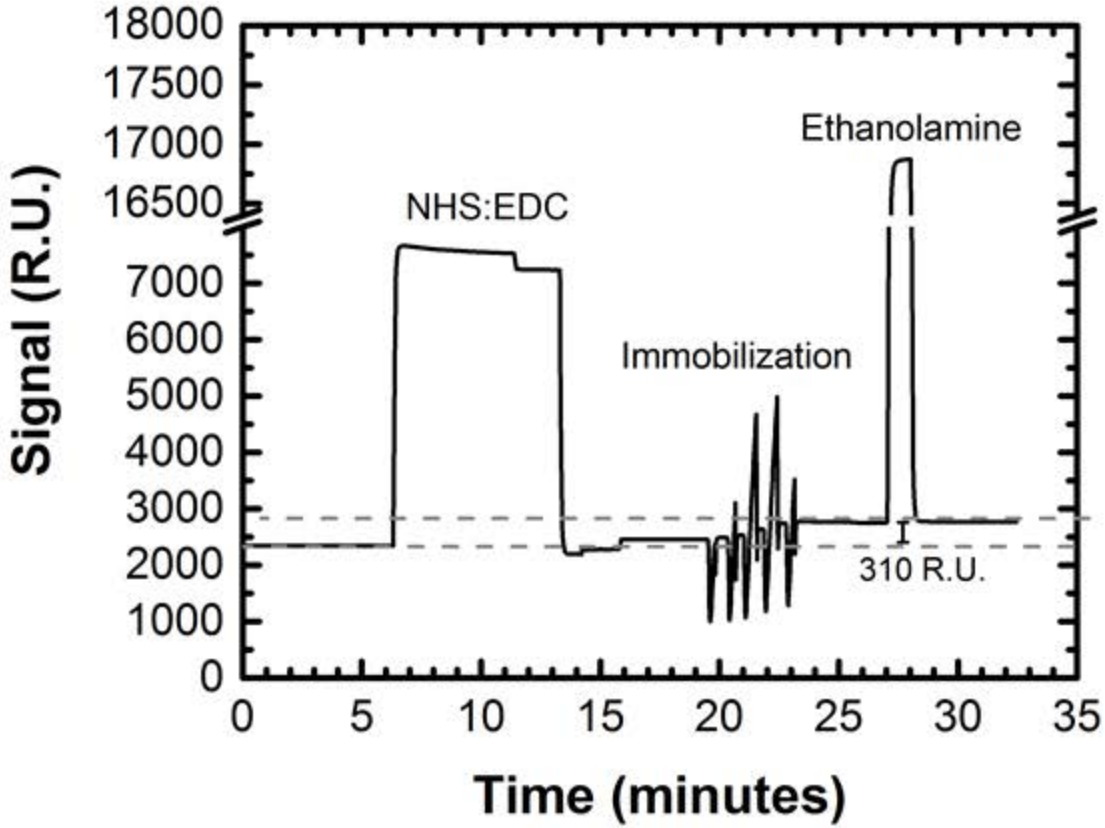
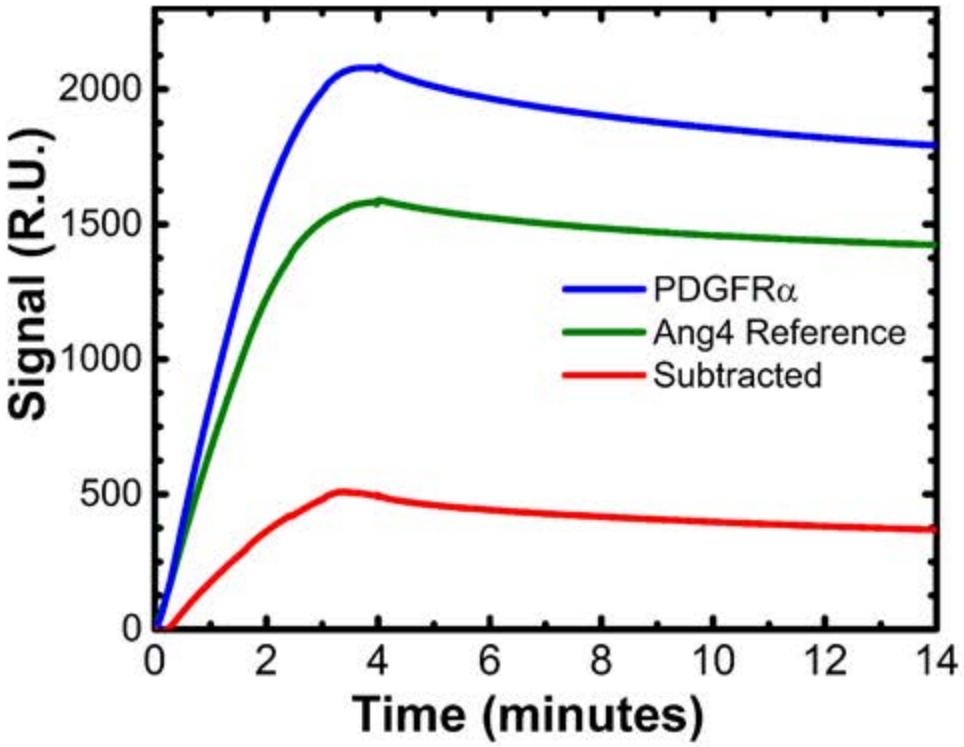
Supplementary Figures



Supplementary Figure S1. Non-specific signal example--PDGF-AA: VEGFR3. Sensogram depicts example negative reference-subtracted curve produced when subtracting Ang-4 reference signal from binding signal. The negative response signal suggests than non-specific binding effects are greater than specific, 'true' binding effects.



Supplementary Figure S2. SPR immobilization example—PDGFRα binding to the dextran matrix. Surface activated by injecting 35 µL 1:1 NHS:EDC solution at flow rate of 5 µL min^{-1}. Injected 20 µg mL^{-1} PDGFRα in 10mM pH 3.7 acetate buffer until 310 R.U. of receptor immobilized. Surface deactivated by injecting 5 µL ethanolamine-HCL at 5 µL min^{-1}.



Supplementary Figure S3. Non-specific binding reference signal subtraction. Sensogram depicts the injection of 40 nM PDGFAA across immobilized angiopoietin-4 and PDGFRα simultaneously. Ang-4, a protein with no known interaction with either PDGF or VEGF ligands, was immobilized at levels similar to the receptors as a background reference signal. The reference subtracted curve was obtained by subtracting this reference signal from the raw receptor-ligand interaction curve, removing signals associated with non-specific interactions.