Title: Discovery of high-affinity (nanomolar) interactions between VEGFs and PDGFs

Authors

Shobhan Kuila[1], Yingye Fang[1], Princess I. Imoukhuede[1*]

Affiliations

1 Department of Bioengineering, University of Washington, Seattle, WA, USA

* Corresponding author: pii@uw.edu; +1 (206) 685-2002

Abstract

Angiogenic signaling research has conventionally focused on intrafamily molecular interactions, such as the binding of vascular endothelial growth factors (VEGFs) to VEGF receptors (VEGFRs), or of platelet-derived growth factors (PDGFs) to PDGF receptors (PDGFRs). However, the discovery of cross-family interactions, including VEGF:PDGFR and PDGF:VEGFR binding, challenges this convention and calls for investigation of other possible cross-family mechanisms. Notably, placental growth factor (PlGF), a member of the VEGF family, is known to form heterodimers with VEGF-A, suggesting a ligand–ligand hetero-oligomerization mechanism. We hypothesized that ligand–ligand hetero-oligomerization might also drive cross-family interactions. We thus measured the cross-family ligand hetero-oligomeric interactions between VEGF-A and five PDGF subtypes using surface plasmon resonance (SPR). Our findings reveal strong interactions between VEGF-A and PDGF-AA, -BB, -AB, and -DD, with high-nanomolar binding affinities of 0.92 nM, 2.2 nM, 4.8 nM, and 3.6 nM, respectively, whereas the VEGF-A:PDGF-CC interaction was weak or negligible (900 nM). We also characterized the established VEGF-A:PlGF binding and confirmed its strong affinity of 0.3 nM. Our discovery of these cross-family ligand hetero-oligomerizations suggests a new dimension in the control of vascular development in health and disease and may explain the discrepancies observed in growth factor therapies.

Keywords

Surface plasmon resonance, growth factor, vascular endothelial growth factor, platelet-derived growth factor, dissociation constant.

Introduction

VEGF-A (vascular endothelial growth factor A) and PDGFs (platelet-derived growth factors) are key signaling proteins involved in angiogenesis and vascular development[4]. VEGF-A primarily promotes the growth of blood vessels by stimulating endothelial cell proliferation, migration, and survival, making it a key molecule in tissue regeneration and wound healing. In diseases like cancer, VEGF-A facilitates tumor growth by enhancing blood supply. PDGFs, on the other hand, are essential for the recruitment and proliferation of pericytes and smooth muscle cells, supporting blood vessel maturation and stability. Together, VEGF-A and PDGFs coordinate the formation of robust and functional vascular networks, which establishes them as key targets in therapies aimed at modulating angiogenesis[1].

Notably, VEGF and PDGFs are structurally related. Phylogenetic analysis indicates that VEGF and PDGF have evolved from common precursors[2], with both families containing a highly conserved growth factor domain known as the PDGF/VEGF homology domain[3], characterized by a signature C-terminal cysteine knot formed by eight cysteine residues[4–6] (Figures A and C). Structural analyses show a root mean square deviation (RMSD) of 1.9 Å between the Cα atoms of VEGF and PDGF dimers when aligned, highlighting their structural similarity[7]. Moreover, their receptors, VEGFR and PDGFR, share their exon/intron structure[8]; both have a large extracellular region, with 7 (VEGFR) or 5 (PDGFR) immunoglobulin (Ig)-like domains; a single transmembrane spanning helix; and a cytosolic tail with a split Tyr kinase domain. An important feature is the transmembrane helix connecting D7 (in VEGFR) or D5 (in PDGFR) to the kinase domain[9] (Figure B). These structural similarities across families suggest the potential for cross-family signaling.

The structural and functional convergence between the VEGF and PDGF families underscores the need for deeper investigation into their cross-family interactions. This is particularly relevant in understanding resistance to VEGF-targeted anti-angiogenic therapies. PDGF upregulation has been implicated as compensating for VEGF inhibition and related anti-angiogenic therapy [10,11], suggesting PDGFs may replace VEGFs following VEGF inhibition[12]. Dual growth factor therapy with VEGF and PDGFs has yielded mixed results[13]; the combination increased both capillaries and arterioles but did not enhance angiogenesis more than either growth factor alone[14,15]. Some tissue-engineering studies coupling dual growth factors to biomaterials[16,17] showed improved wound healing, but in those studies the growth factors were released sequentially[13,14], limiting the potential for

cross-family ligand interaction. These mechanisms of cross-family compensation and discrepancies in dual-factor therapeutic advances underscore the importance of studying various cross-family interactions to better explore combined growth factor signaling pathways.

Surface plasmon resonance (SPR) is a powerful tool for understanding the dynamics and interactions of co-administered growth factors (Figure D). SPR has both advanced the study of ligand–receptor interactions and helped to optimize the targeting of ligands, receptors, and signaling[18–21]. SPR can detect interactions ranging from weak to strong (millimolar to nanomolar range)[22–24] and can be used as a primary tool to screen interacting partners or validate interactions identified by other methods. The affinity or kinetics of an interaction can be determined by SPR, so the approach is fundamental to understanding the nature of binding at the cellular level.

The established PDGF:VEGFR and VEGF:PDGFR cross-family interactions[25], along with the coordinating effects, structural similarities, and native dimeric forms of these growth factors, underscore the potential importance of cross-family interactions in angiogenesis[16]. Furthermore, the discovery of hetero-oligomeric interactions between VEGF-A and placental growth factor (PlGF) within the VEGF family inspired us to explore whether similar ligand–ligand interactions could occur across growth factor families. Such cross-family interactions may be critical regulators of angiogenesis and contribute to anti-VEGF therapy resistance. Toward this goal, we sought to examine whether cross-family interactions occur between VEGF-A and PDGF subtypes. We identified and characterized several high-affinity interactions via SPR, uncovering a novel mechanism in cross-family signaling and its implications for angiogenic therapies.

Results

Establishing VEGFA/PlGF binding kinetics to differentiate binders from nonbinders

The first step to understanding cross-family interactions and their possible clinical implications is examining the kinetics of known intra-family growth factor interactions, in this case, the known VEGF-A:PlGF interaction[26,27]. While VEGF-A monomers and PlGF monomers are known to assemble as VEGFA-PlGF, the kinetics of VEGFA dimer–PlGF dimer interaction (herein described as VEGF-A:PlGF) are unknown. We confirmed that the VEGF-A:PlGF interaction does exist and measured the dissociation constant (K_D) to be ~300 pM or 10^{-10} M, corresponding to a strong interaction, since antigen–antibody K_D values are usually in the range of 10^{-8} to 10^{-12} M [24] (Fig. 1). This strong VEGF-A:PlGF binding explains the formation of

their heterodimers when the two proteins are co-overexpressed in rat glioma cells and *E. coli* [28,29]. For the negative control, we used angiopoietin-4 (ANG4), an unrelated protein with no known interaction with VEGFA. While the VEGF-A:ANG4 affinity of 10^{-6} M is in the range of enzyme–substrate interactions, it is considered weak relative to antigen–antibody interactions (Fig. 2). We used the strong VEGF-A-PlGF interaction and the weak VEGF-A:ANG4 as reference points in comparing other interactions (Fig. 1). These data establish a baseline for the known intrafamily interaction (VEGF-A:PlGF) and provide a reference point for evaluating the strength of potential cross-family interactions.

VEGFA exhibits strong interactions with PDGFs

We measured the binding rates and affinities for the different VEGF-A and PDGF pairs. We measured the VEGF-A:PDGF-AA binding affinity, K_D = 921 pM or 9.21×10^{-10} M, and found that it is very close to the PlGF:VEGF-A affinity (~300 pM); thus, VEGF-A:PDGF-AA can be considered a strong binding interaction (Fig. 3). We observed that VEGF-A:PDGF-BB affinity is slightly weaker than that of VEGF-A:PDGF-AA, with VEGF-A:PDGF-BB K_D = 2.2 nM or 2.2×10^{-10} M, followed by the VEGF-A:PDGF-DD interaction with K_D = 3.6 nM or 3.6×10^{-10} M. In contrast, the heterodimer PDGF-AB has lower binding affinity than homodimers of its component PDGFs, with K_D = 4.8 nM or 4.8×10^{-10} M (Fig. 3). The binding strength of PDGF-CC (with a K_D of 0.9 μM or 9×10^{-7} M) is similar to that of the negative control VEGF-A:ANG4 (Table 1). Therefore, we can consider VEGF-A:PDGF-CC to be a nonbinder. The association and dissociation constants and affinities for these interactions are compared in Table 1 and Fig. 4. The ability of VEGF-A to bind various PDGFs with high affinity suggests the formation of previously unrecognized hetero-oligomeric complexes that may modulate angiogenic signaling and therapeutic outcomes.

The association and dissociation rate constants provide insights into trends in affinity

The association rate constant (k_{on}) and dissociation rate constant (k_{off}) constants obtained from the SPR sensorgram analysis offer invaluable insights into the interaction dynamics between VEGF-A and PDGFs: k_{on} (M^{-1} s^{-1}) quantifies the rate at which two molecules interact to form a complex, while k_{off} (s^{-1}) measures the rate at which the complex dissociates into individual molecules. The affinity between two molecules or proteins is determined by the ratio of k_{off} to k_{on}. Notably, the association rates of VEGF-A to PDGF-AA, -BB, -CC, and -DD are all similar (Table 1), with the discrepancies in binding affinities predominantly attributable to their dissociation kinetics. With the highest affinity, PDGF-AA dissociates four times more slowly

than PDGF-BB, with a VEGF-A:PDGF-AA dissociation rate of 1.22×10^{-4} s^{-1}. Whereas, VEGF-A:PDGF-BB displays a dissociation rate of 4.11×10^{-4} s^{-1}. To put this in the perspective of exponential decay, the half-life of the VEGF-A:PDGF-AA complex is 1.57 hours, while that of the VEGFA:PDGF-BB is 0.46 hours. Just as the VEGF-A:PDGF-AB affinity was weaker, the dissociation rate is faster, with a dissociation rate of 7.86×10^{-4} s^{-1} and a half-life of 0.24 hours. VEGFA/PDGF-DD stands out as the fastest dissociator, with a rate of 1.01×10^{-3} s^{-1} and a half-life of 0.18 hours. It is important to note that the more slowly a complex dissociates, the longer its binding effects can be observed. These kinetic parameters offer a quantitative basis for understanding the stability and duration of VEGF-A:PDGF complexes.

When comparing these VEGF-A:PDGF data with the VEGF-A:PlGF interaction kinetics, we observed that the VEGF-A:PlGF association rate ($k_{on} = 1.12 \times 10^6$ M^{-1} s^{-1}) is faster than those of VEGF-A with all the PDGFs. One possible explanation for this could be the higher sequence identity between VEGF-A and PlGF (42–50%). Structural comparison supports this, with an RMSD of 1.9 Å between the Cα atoms of the VEGFA and PDGF-BB dimer[7], while the RMSD for VEGF-A and PlGF is 1.1 Å (analyzed using Chimera version 1.17.1) (Figure 5). The faster association rate of VEGF-A:PlGF compared with VEGF-A:PDGF correlates with the higher structural similarity between VEGF-A and PlGF, as evidenced by the lower RMSD value. This suggests that sequence and structural homology may play a role in determining the kinetics of these interactions.

Discussion

In this study, we used SPR to investigate ligand–ligand interactions within the VEGF family and across the VEGF and PDGF families. Our findings provide novel insights into the binding dynamics and affinity of these interactions, with three key outcomes: (1) We quantified, for the first time, the detailed kinetics and affinity of the known intrafamily ligand interaction between VEGF-A and PlGF. (2) We discovered high-affinity binding between VEGF-A and several PDGFs, revealing a new mechanism of cross-family VEGF–PDGF signaling with potential implications for disease prognosis and treatment. (3) We established the association and dissociation rates for each interaction, providing a quantitative basis for understanding the formation and stability of these ligand–ligand complexes. These findings expand our understanding of the complex interplay between angiogenic growth factors and also provide a new perspective on the inconsistencies observed in dual growth factor therapies, the potential

impact of cross-family interactions on disease prognosis, and the regulatory mechanisms governing vascular development and disease.

Strong interactions between VEGF-A and PDGFs shed new light on angiogenic cross-family signaling

Combinations of growth factors can promote or inhibit angiogenesis. For instance, the interaction between VEGF-A and PlGF leads to the formation of heterodimers (VEGF-A monomer forms a dimer with PlGF monomer), which exhibit 20–50-fold lower mitogenic activity compared with that of VEGF-A homodimers[29]. We measured the K_D of this known interaction and confirmed the strong binding affinity of 0.3 nM. Our findings on the high-affinity binding between VEGF-A and PDGFs, with K_D values ranging from 921 pM to 3.6 nM, provide a quantitative basis for understanding the potential formation of hetero-oligomeric complexes and their impact on dual growth factor targeting in angiogenic treatments. The affinities of these cross-family interactions are comparable with that of the VEGF-A:PlGF interaction, suggesting that VEGF-A:PDGF could play a significant role in modulating angiogenic signaling.

The clinical implications of these interactions are exemplified by a study on diabetic foot syndrome, where the modulation of angiogenic signaling by VEGF-A:PDGF complexes may contribute to impaired wound healing. The study reported that elevated levels of both VEGF-A and PDGF-BB correlate with the prognosis of diabetic foot syndrome. However, despite the increased presence of these growth factors, patients still exhibited impaired angiogenesis and compromised wound healing[15]. Our discovery of high-affinity interactions between VEGF-A (dimer) and PDGF-BB (dimer) offers a potential explanation for this observation. Similar to the reduced activity of VEGF-A:PlGF heterodimers[29], the individual signaling capacities of VEGF-A and PDGF-BB may decreased by their heterodimer formation, although further studies are needed to confirm the existence of such heterodimers in vivo.

Furthermore, the formation of hetero-oligomeric complexes between VEGF and PDGF in vivo may facilitate interactions between different receptor families. For example, VEGFR2 and PDGFRβ have been shown to associate with each other on tumor pericytes[30,31]. Our study demonstrates the possibility of VEGF-A:PDGF-BB hetero-oligomeric complex formation, which could alter their binding to respective receptors and subsequently modulate downstream angiogenic responses.

Many studies investigating the mechanisms, functions, and therapeutic potential of growth factors have overlooked the importance of cross-family ligand–receptor interactions [25] and ligand–ligand interactions. Consequently, various studies of dual growth factors have observed changes in receptor activation, signaling, and cellular responses without providing a clear mechanistic explanation for these effects. For instance, animal model experiments have shown that while VEGF mediates endothelial sprouting and the formation of new angiogenic vessels, it is not efficient in generating mature vascular networks[32]. In contrast, PDGF can induce mature vasculature in angiogenesis models. Interestingly, recent observations indicate that the treatment of tissues with subsaturating concentrations of both VEGF and PDGF fails to elicit a substantial angiogenic response compared with that induced by treatment with either growth factor individually. In fact, VEGF has been shown to suppress PDGF-mediated angiogenesis in vivo, an effect that can be reversed through genetic or pharmacological inhibition of VEGFR2[33].

Many studies reporting positive outcomes of dual growth factor therapy either lack robust in vivo data or involve the use of modified growth factors to achieve desired outcomes, thus failing to provide a clear mechanistic understanding[34,35]. One exception is a study demonstrating that sequential infusion of VEGF-A followed by PDGF-BB using alginate hydrogels induced angiogenic and functional improvements after myocardial infarction[14]. Our results showing a direct interaction between VEGF-A and PDGF-BB could explain the suppressed angiogenic response observed in some studies as a consequence of ligand sequestration. Furthermore, the detailed binding kinetics provided in our data might help to address challenges encountered in anti- or pro-angiogenic treatments. However, further studies are needed to elucidate the positive or negative regulatory potential of VEGF:PDGF interactions and their implications for angiogenic therapies. The possible formation of hetero-oligomeric complexes between VEGF and PDGF in vivo may promote interactions across different receptor families. For example, VEGFR2 (vascular endothelial growth factor receptor 2) and PDGFRβ (platelet-derived growth factor receptor beta) can associate with each other on tumor pericytes, the mural cells that are essential for blood vessel stabilization[30,31]. Our study demonstrates that the formation of VEGF-A:PDGF-BB hetero-oligomeric complexes is possible and might alter these ligands' binding to their respective receptors and the downstream angiogenic response.

Future studies

The kinetics measurements in these studies focused on the growth factors among VEGF-A, PDGF, and PlGF that are most often used for treatment[5,11,17,36–38]. Several other growth factors still require such interaction studies, such as fibroblast growth factors (FGFs)[39], transforming growth factors-beta (TGF-beta), and tumor necrosis factor-alpha (TNF-α)[1], because many studies show their synergistic roles in therapeutic angiogenesis. Such studies would reveal which growth factors can engage in heterologous interactions that might interfere with or potentially enhance signaling. Indeed, in some studies the combination of growth factors, including VEGF-A, PlGF, and PDGF-BB, did not enhance angiogenesis[40–42].

The detailed kinetics values we have established provide an opportunity to model the complex binding network in silico. This will allow for better prediction of signaling outcomes in a multi-ligand environment and a better understanding of how these interactions influence angiogenic therapy outcomes. Furthermore, our findings highlight the importance of considering cross-family ligand–ligand interactions in the design and interpretation of studies involving multiple growth factors, as these interactions may have significant effects on signaling and therapeutic efficacy.

Material and methods

Surface plasmon resonance (SPR)

SPR experiments were conducted at 25 °C using the Reichert 4SPR (Reichert, Inc., USA). For the studies, planar polyethylene glycol (PEG)-coated gold sensor chips containing 10% COOH (Reichert, Inc., USA) were employed. Reichert 4SPR divides the chip into four flow cells: we immobilized different growth factors in channel 1 or channel 3 while leaving either channel 2 or channel 4 blank for reference (Supplementary figure). Running buffer: 1x HBS-EP pH 7.4 (10 mM HEPES, 3 mM EDTA, 150 mM NaCl, 0.005% Tween-20; cat. # BR100188, GE Life Sciences).

Protein immobilization

Growth factors, including VEGFA (R&D Systems, Cat. #293-VE-010) were immobilized using an amine-coupling method. We dissolved 40 mg of EDC (1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride, #22980 Thermo Scientific) and 10 mg of NHS (N-hydroxysuccinimide, #130672 Sigma Aldrich) in 1 mL of ddH2O. The solution was mixed and injected quickly at a 10 µL/min flow rate for 7 minutes to activate the sensor surface. Proteins were diluted to 30 µg/mL with 10 mM acetate buffer pH 4.0 and injected at 10 µL/min

until the desired immobilization level was reached (about ≥2000 RU). The desired immobilization level was calculated based on the expected Rmax, which is generally less than 200 RU to avoid a mass transfer effect[43]. After the protein was immobilized, the surface was deactivated by injecting ethanolamine for 7 min at 10 μL/min (1M ethanolamine hydrochloride-NaOH pH 8.5, #E9508, Sigma Aldrich).

Kinetic analysis

We measured the kinetic rate constants between PDGFs and VEGF growth factors using an SPR assay. We injected the ligands at 50, 25, 12.5, 6.25, and 3.12 nM and fit their association and dissociation curves using a 1:1 Langmuir binding model in TraceDrawer ver.1.8.1. Each fitting was analyzed by visual inspection and the ratio of χ^2-to-Rmax (<0.10). The χ^2-to-Rmax ratio can help validate the 1:1 binding interaction because this value describes how well the obtained sensorgram curves fit a 1:1 Langmuir interaction model [43,44]. All the analysis was performed in TraceDrawer ver.1.8.1.

The raw sensorgrams across the concentrations tested (3.12–50 nM) were aligned, and the background nonspecific binding was subtracted using the sensorgrams traced from the reference channel. Global analysis is considered to produce more accurate results than fitting a single response curve [45]. Global fitting was performed with TraceDrawer ver.1.8.1 (Ridgeview Instrument, Sweden). The software applies nonlinear least squares analysis to determine association (ka) and dissociation (kd) rates that best fit multiple response curves. Additionally, the software provides the goodness-of-fit parameter χ^2 and the peak magnitude of the signal response, Rmax.

Quality criteria for SPR data

The following are the standards used to judge the quality of SPR results:

Visual inspection: the lines of the resulting fit should pass through the experimental sensorgrams. If the fitting lines do not visually overlap the experimental data, either the interactions do not follow Langmuir 1:1 stoichiometry or there are more complex interactions (including nonbinding or nonspecific interactions) occurring (Luo, 2013).

The Rmax value is the maximum response expected from an analyte for a one-to-one interaction for a fixed amount of immobilized ligand on an SPR. The Rmax value should be within the range of a few hundred RU, ideally less than 200 RU, to minimize the impact of mass-transport effects (i.e., the analyte, while dissociating, will repeatedly attach to the next

ligand if we overcrowd the SPR chip surface during immobilization). Higher Rmax denotes promiscuous or nonspecific binding or interaction not following 1:1 Langmuir interaction.

χ^2 is the average of the squared residuals (the average of the squared differences between the measured data points and the corresponding fitted values). The lowest value that can be expected is the baseline noise. Empirically, these values should be less than 10% of Rmax regardless of units [43].

Acknowledgements

This material is based upon work supported by the National Science Foundation under Grant No. 1923151. Research reported in this publication was also supported by the National Institute of Heart, Lung, and Blood of the National Institutes of Health under Award Number 7R01HL159946-02. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation or the National Institutes of Health.

Conflict of interests

The authors declare no conflicts of interest.

Author contribution

Shobhan Kuila, Yingye Fang, and Princess I. Imoukhuede designed and conceptulized the research. Shobhan Kuila acquired, analysed, and interpreted the data. Shobhan Kuila, Yingye Fang, and Princess I Imoukhuede were involved in drafting and revising the manuscript.

References

[1] Liu ZL, Chen HH, Zheng LL, Sun LP, Shi L. Angiogenic signaling pathways and anti-angiogenic therapy for cancer. Signal Transduction and Targeted Therapy 2023 8:1 2023;8:1–39. https://doi.org/10.1038/s41392-023-01460-1.

[2] Rauniyar K, Bokharaie H, Jeltsch M. Expansion and collapse of VEGF diversity in major clades of the animal kingdom. Angiogenesis 2023 26:3 2023;26:437–61. https://doi.org/10.1007/S10456-023-09874-9.

[3] Bergsten E, Uutela M, Li X, Pietras K, Östman A, Heldin CH, et al. PDGF-D is a specific, protease-activated ligand for the PDGF β-receptor. Nature Cell Biology 2001 3:5 2001;3:512–6. https://doi.org/10.1038/35074588.

[4] Iyer S, Acharya KR. Tying the knot: The cystine signature and molecular-recognition processes of the vascular endothelial growth factor family of angiogenic cytokines. FEBS Journal 2011;278:4304–22. https://doi.org/10.1111/J.1742-4658.2011.08350.X.

[5] Reigstad LJ, Sande HM, Fluge Ø, Bruland O, Muga A, Varhaug JE, et al. Platelet-derived Growth Factor (PDGF)-C, a PDGF Family Member with a Vascular Endothelial Growth Factor-like Structure. Journal of Biological Chemistry 2003;278:17114–20. https://doi.org/10.1074/JBC.M301728200.

[6] Li X, Eriksson U. Novel PDGF family members: PDGF-C and PDGF-D. Cytokine Growth Factor Rev 2003;14:91–8. https://doi.org/10.1016/S1359-6101(02)00090-4.

[7] Muller YA, Christinger HW, Keyt BA, De Vos AM. The crystal structure of vascular endothelial growth factor (VEGF) refined to 1.93 Å resolution: multiple copy flexibility and receptor binding n.d.

[8] Kondo K, Hiratsuka S, Subbalakshmi E, Matsushime H, Shibuya M. Genomic organization of the flt-1 gene encoding for Vascular Endothelial Growth Factor (VEGF) Receptor-1 suggests an intimate evolutionary relationship between the 7-Ig and the 5-Ig tyrosine kinase receptors. Gene 1998;208:297–305. https://doi.org/10.1016/S0378-1119(98)00006-7.

[9] Shaik F, Cuthbert GA, Homer-Vanniasinkam S, Muench SP, Ponnambalam S, Harrison MA. Structural Basis for Vascular Endothelial Growth Factor Receptor Activation and

Implications for Disease Therapy. Biomolecules 2020;10:1–25. https://doi.org/10.3390/BIOM10121673.

[10] Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. Nature Reviews Cancer 2003 3:6 2003;3:401–10. https://doi.org/10.1038/nrc1093.

[11] Erber R, Thurnher A, Katsen AD, Groth G, Kerger H, Hammes H-P, et al. Combined inhibition of VEGF-and PDGF-signaling en-forces tumor vessel regression by interfering with pericyte-mediated endothelial cell survival mechanisms. The FASEB Journal 2003. https://doi.org/10.1096/fj.03-0271fje.Published.

[12] Moench R, Gasser M, Nawalaniec K, Grimmig T, Ajay AK, Souza LCR de, et al. Platelet-derived growth factor (PDGF) cross-signaling via non-corresponding receptors indicates bypassed signaling in colorectal cancer. Oncotarget 2022;13:1140–52. https://doi.org/10.18632/ONCOTARGET.28281.

[13] Bai Y, Bai L, Zhou J, Chen H, Zhang L. Sequential delivery of VEGF, FGF-2 and PDGF from the polymeric system enhance HUVECs angiogenesis in vitro and CAM angiogenesis. Cell Immunol 2018;323:19–32. https://doi.org/10.1016/j.cellimm.2017.10.008.

[14] Hao X, Silva EA, Månsson-Broberg A, Grinnemo KH, Siddiqui AJ, Dellgren G, et al. Angiogenic effects of sequential release of VEGF-A165 and PDGF-BB with alginate hydrogels after myocardial infarction. Cardiovasc Res 2007;75:178–85. https://doi.org/10.1016/J.CARDIORES.2007.03.028/2/75-1-178-FIG8.GIF.

[15] Drela E, Kulwas A, Jundzill W, Góralczyk B, Boinska J, Drewniak W, et al. VEGF-A and PDGF-BB - Angiogenic factors and the stage of diabetic foot syndrome advancement. Endokrynol Pol 2014;65:306–12. https://doi.org/10.5603/EP.2014.0042.

[16] Shah NJ, Hyder MN, Quadir MA, Courchesne NMD, Seeherman HJ, Nevins M, et al. Adaptive growth factor delivery from a polyelectrolyte coating promotes synergistic bone tissue repair and reconstruction. Proc Natl Acad Sci U S A 2014;111:12847–52. https://doi.org/10.1073/PNAS.1408035111/SUPPL_FILE/PNAS.201408035SI.PDF.

[17] Hsu C-W, Poché RA, Saik JE, Ali S, Wang S, Yosef N, et al. Improved Angiogenesis in Response to Localized Delivery of Macrophage-Recruiting Molecules 2015. https://doi.org/10.1371/journal.pone.0131643.

[18] Zhang F, Zheng L, Cheng S, Peng Y, Fu L, Zhang X, et al. molecules Comparison of the Interactions of Different Growth Factors and Glycosaminoglycans 2019. https://doi.org/10.3390/molecules24183360.

[19] Decker S, Taschauer A, Geppl E, Pirhofer V, Schauer M, Pöschl S, et al. Structure-based peptide ligand design for improved epidermal growth factor receptor targeted gene delivery. European Journal of Pharmaceutics and Biopharmaceutics 2022;176:211–21. https://doi.org/10.1016/J.EJPB.2022.05.004.

[20] Mamer SB, Wittenkeller A, Imoukhuede PI. VEGF-A splice variants bind VEGFRs with differential affinities. Sci Rep 2020;10. https://doi.org/10.1038/s41598-020-71484-y.

[21] Mamer SB, Page P, Murphy M, Wang J, Gallerne P, Ansari A, et al. The Convergence of Cell-Based Surface Plasmon Resonance and Biomaterials: The Future of Quantifying Bio-molecular Interactions—A Review. Ann Biomed Eng 2020;48:2078–89. https://doi.org/10.1007/S10439-019-02429-4/FIGURES/1.

[22] Fägerstam LG, Frostell-Karlsson Å, Karlsson R, Persson B, Rönnberg I. Biospecific interaction analysis using surface plasmon resonance detection applied to kinetic, binding site and concentration analysis. J Chromatogr A 1992;597:397–410. https://doi.org/10.1016/0021-9673(92)80137-J.

[23] Malmqvist M. Surface plasmon resonance for detection and measurement of antibody-antigen affinity and kinetics. Curr Opin Immunol 1993;5:282–6. https://doi.org/10.1016/0952-7915(93)90019-O.

[24] Perera T, Gunasekara H, Hu YS. Single-molecule interaction microscopy reveals antibody binding kinetics. BioRxiv 2020:2020.09.21.306605. https://doi.org/10.1101/2020.09.21.306605.

[25] Mamer SB, Chen S, Weddell JC, Palasz A, Wittenkeller A, Kumar M, et al. Discovery of High-Affinity PDGF-VEGFR Interactions: Redefining RTK Dynamics. Scientific Reports 2017 7:1 2017;7:1–14. https://doi.org/10.1038/s41598-017-16610-z.

[26] Lennikov A, Mukwaya A, Fan L, Saddala MS, De Falco S, Huang H. Synergistic interactions of PlGF and VEGF contribute to blood-retinal barrier breakdown through canonical NFκB activation. Exp Cell Res 2020;397:112347. https://doi.org/10.1016/J.YEXCR.2020.112347.

[27] Bottomley MJ, Webb NJA, Watson CJ, Holt L, Bukhari M, Denton J, et al. Placenta growth factor (PlGF) induces vascular endothelial growth factor (VEGF) secretion from mononuclear cells and is co-expressed with VEGF in synovial fluid. Clin Exp Immunol 2000;119:182. https://doi.org/10.1046/J.1365-2249.2000.01097.X.

[28] DiSalvo J, Bayne ML, Conn G, Kwok PW, Trivedi PG, Soderman DD, et al. Purification and characterization of a naturally occurring vascular endothelial growth factor · placenta growth factor heterodimer. Journal of Biological Chemistry 1995;270:7717–23. https://doi.org/10.1074/jbc.270.13.7717.

[29] Cao Y, Chen H, Zhou L, Chiaug MK, Anand-Apte B, Weatherbee JA, et al. Heterodimers of placenta growth factor/vascular endothelial growth factor: Endothelial activity, tumor cell expression, and high affinity binding to Flk-1/KDR. Journal of Biological Chemistry 1996;271:3154–62. https://doi.org/10.1074/jbc.271.6.3154.

[30] Greenberg JI, Cheresh DA. VEGF as an inhibitor of tumor vessel maturation: implications for cancer therapy. Expert Opin Biol Ther 2009;9:1347–56. https://doi.org/10.1517/14712590903208883.

[31] Greenberg JI, Shields DJ, Barillas SG, Acevedo LM, Murphy E, Huang J, et al. A Role for VEGF as a Negative Regulator of Pericyte Function and Vessel Maturation. Nature 2008;456:809. https://doi.org/10.1038/NATURE07424.

[32] Chen RR, Silva EA, Yuen WW, Mooney DJ. SpatioYtemporal VEGF and PDGF Delivery Patterns Blood Vessel Formation and Maturation n.d. https://doi.org/10.1007/s11095-006-9173-4.

[33] Greenberg JI, Shields DJ, Barillas SG, Acevedo LM, Murphy E, Huang J, et al. A role for VEGF as a negative regulator of pericyte function and vessel maturation. Nature 2008 456:7223 2008;456:809–13. https://doi.org/10.1038/nature07424.

[34] Marushima A, Nieminen M, Kremenetskaia I, Gianni-Barrera R, Woitzik J, Von Degenfeld G, et al. Balanced single-vector co-delivery of VEGF/PDGF-BB improves functional collateralization in chronic cerebral ischemia n.d. https://doi.org/10.1177/0271678X18818298.

[35] White MJ V, Briquez PS, White DA V, Hubbell JA. VEGF-A, PDGF-BB and HB-EGF engineered for promiscuous super affinity to the extracellular matrix improve wound healing in a model of type 1 diabetes n.d. https://doi.org/10.1038/s41536-021-00189-1.

[36] Pennock S, Kazlauskas A. Vascular Endothelial Growth Factor A Competitively Inhibits Platelet-Derived Growth Factor (PDGF)-Dependent Activation of PDGF Receptor and Subsequent Signaling Events and Cellular Responses 2012. https://doi.org/10.1128/MCB.06668-11.

[37] Attanasio S, Snell J. Therapeutic angiogenesis in the management of critical limb ischemia: Current concepts and review. Cardiol Rev 2009;17:115–20. https://doi.org/10.1097/CRD.0B013E318199E9B7.

[38] Ball SG, Shuttleworth CA, Kielty CM. Vascular endothelial growth factor can signal through platelet-derived growth factor receptors. J Cell Biol 2007;177:489. https://doi.org/10.1083/JCB.200608093.

[39] Asahara T, Bauters C, Zheng LP, Takeshita S, Bunting S, Ferrara N, et al. Synergistic effect of vascular endothelial growth factor and basic fibroblast growth factor on angiogenesis in vivo. Circulation 1995;92. https://doi.org/10.1161/01.CIR.92.9.365/FORMAT/EPUB.

[40] Tarallo V, Vesci L, Capasso O, Esposito MT, Riccioni T, Pastore L, et al. A placental growth factor variant unable to recognize Vascular Endothelial Growth Factor (VEGF) receptor-1 inhibits VEGF-dependent tumor angiogenesis via heterodimerization. Cancer Res 2010;70:1804–13. https://doi.org/10.1158/0008-5472.CAN-09-2609.

[41] Mao Y, Liu X, Song Y, Zhai C, Zhang L. VEGF-A/VEGFR-2 and FGF-2/FGFR-1 but not PDGF-BB/PDGFR-β play important roles in promoting immature and inflammatory intraplaque angiogenesis. PLoS One 2018;13. https://doi.org/10.1371/journal.pone.0201395.

[42] Yang Y, Mao W, Wang L, Lu L, Pang Y. Circular RNA circLMF1 regulates PDGF-BB-induced proliferation and migration of human aortic smooth muscle cells by regulating the miR-125a-3p/VEGFA or FGF1 axis. Clin Hemorheol Microcirc 2022;80:167–83. https://doi.org/10.3233/CH-211166.

[43] Luo R. Guide to SPR Data Analysis on the Pr oteOn™ XPR36 System. Ruben Luo, Bio-Rad Laboratories, Inc., 2000 Alfred Nobel Drive, Hercules, CA 94547: 2013.

[44] Kinetics and affinity measurements with Biacore systems 2. n.d.

[45] Bli S/, Reiser J-B. Biacore T200 Getting Started Guide Biacore T200 Getting Started Guide SPR Platform. n.d.

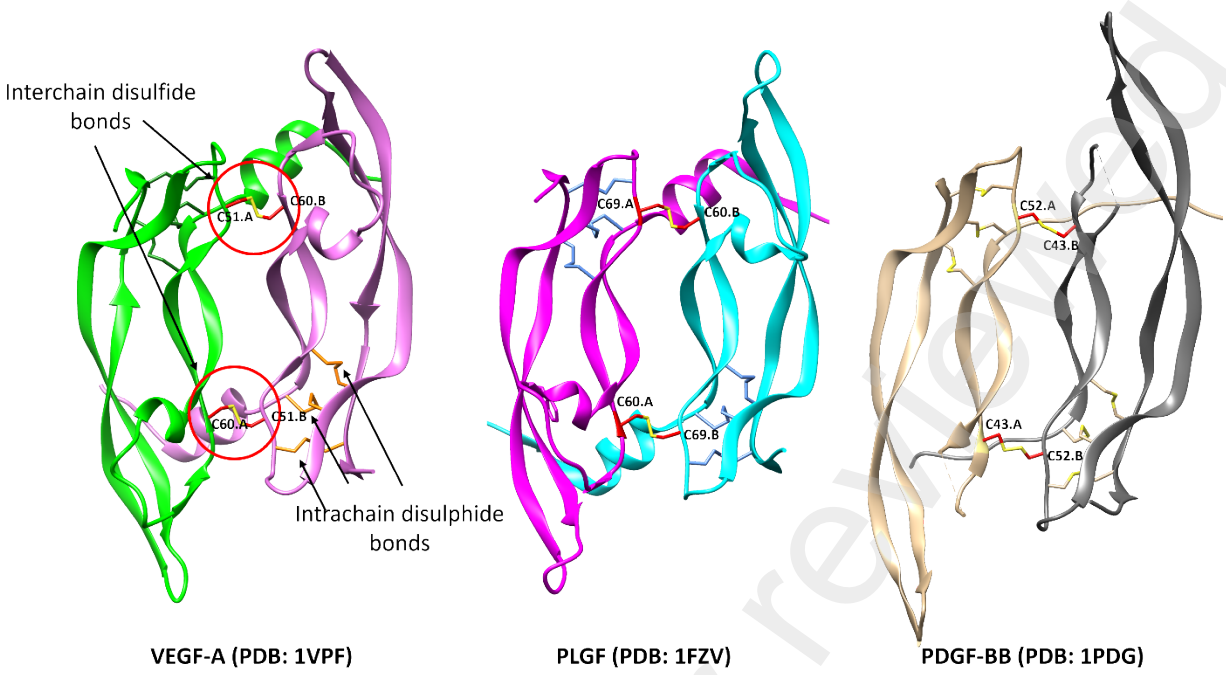

Figure A. Ribbon structures of different growth factors. The figures depict the similarities in the crystal structures of these growth factors, including their common intra- and interchain disulfide bonds, which contribute to the tertiary folding of the proteins and dimer formation. (**a**) VEGFA (PDB: 1VPF), is very similar to (**b**) PLGF (PDB: 1FZV), and (**c**) PDGF-BB (PDB: 1PDG).

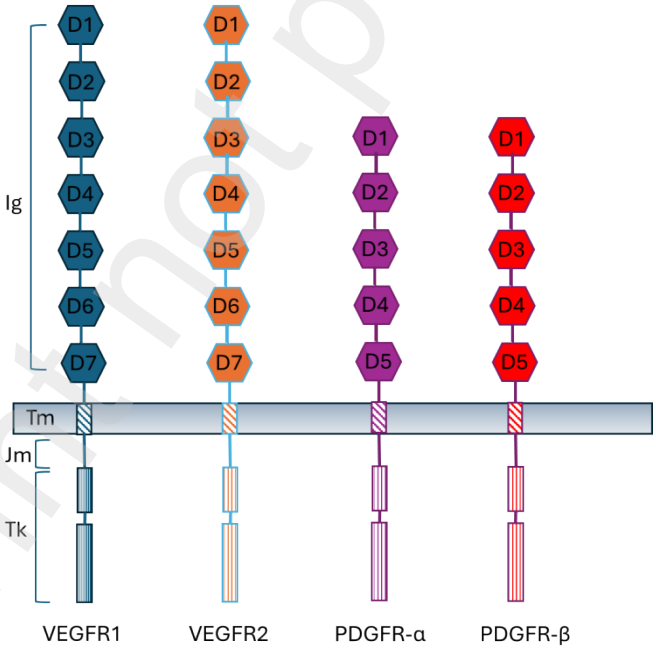

Figure B. Comparison of basic domains in VEGFR1, VEGFR2, PDGFR-α, and PDGFR-β; the various domains are Ig-like loops (Ig), juxtamembrane domain (Jm), and split tyrosine kinase domain (Tk). Tm denotes the transmembrane region.

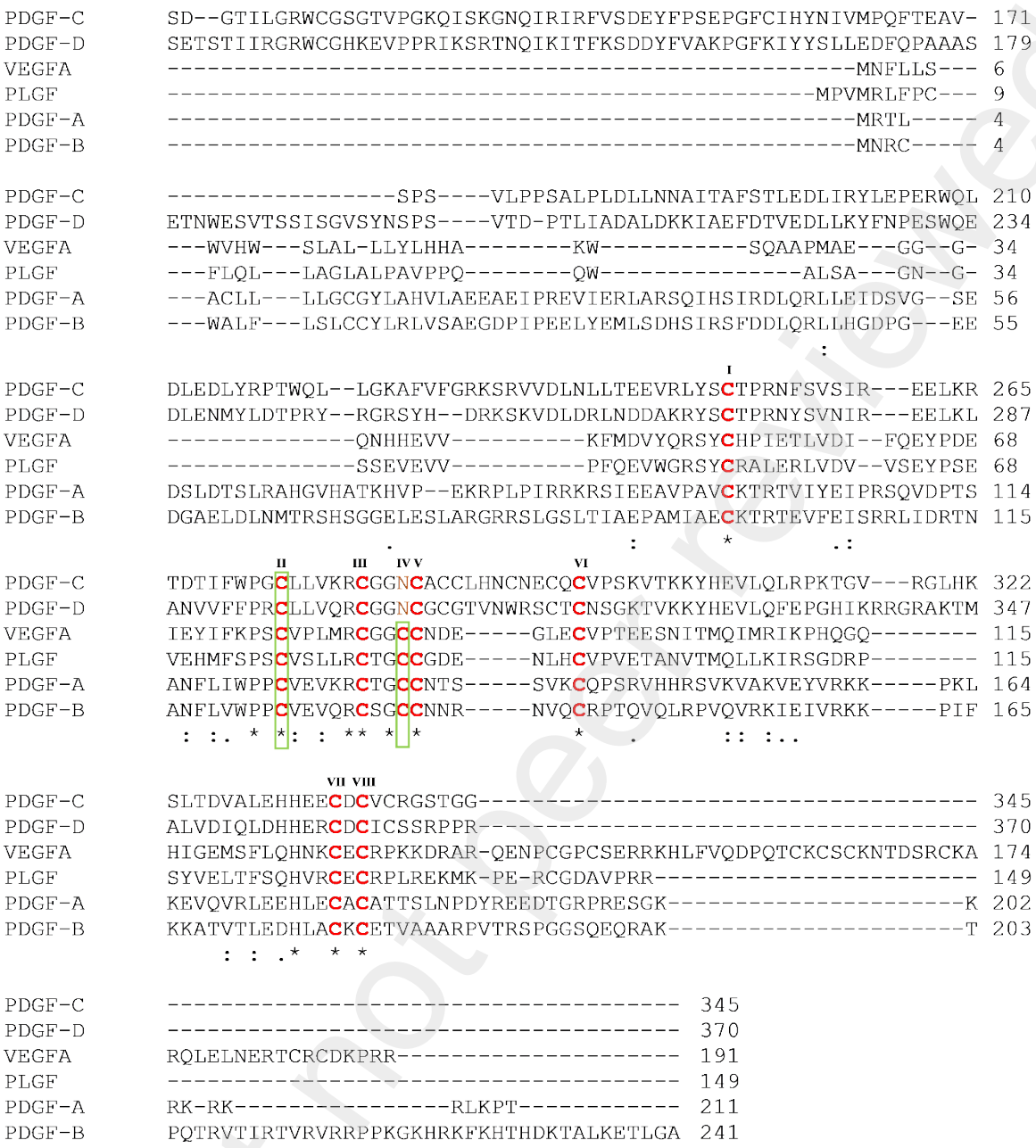

Figure C. Sequence alignment of human growth factors. The alignment was created using the program Clustal Omega with default parameters. The amino acid residues have been marked based on similarity. Identical residues are marked "*", residues of highly similar character are marked ":", and weakly similar residues are marked ".". The cysteine residues involved in the formation of the knot are in red. They are numbered in order from the N-terminus to the C-terminus. The three intrachain disulfide bridges are formed between Cys I and Cys VI, Cys III and Cys VII, and Cys V and Cys VIII. The interchain disulfide bonds are formed between Cys II of one monomer and Cys IV of another monomer (marked with green boxes) which are exactly 8 residues apart in VEGFA, PLGF, and PDGF-A and -B, while PDGF-C and -D lack a cysteine at the IV position.

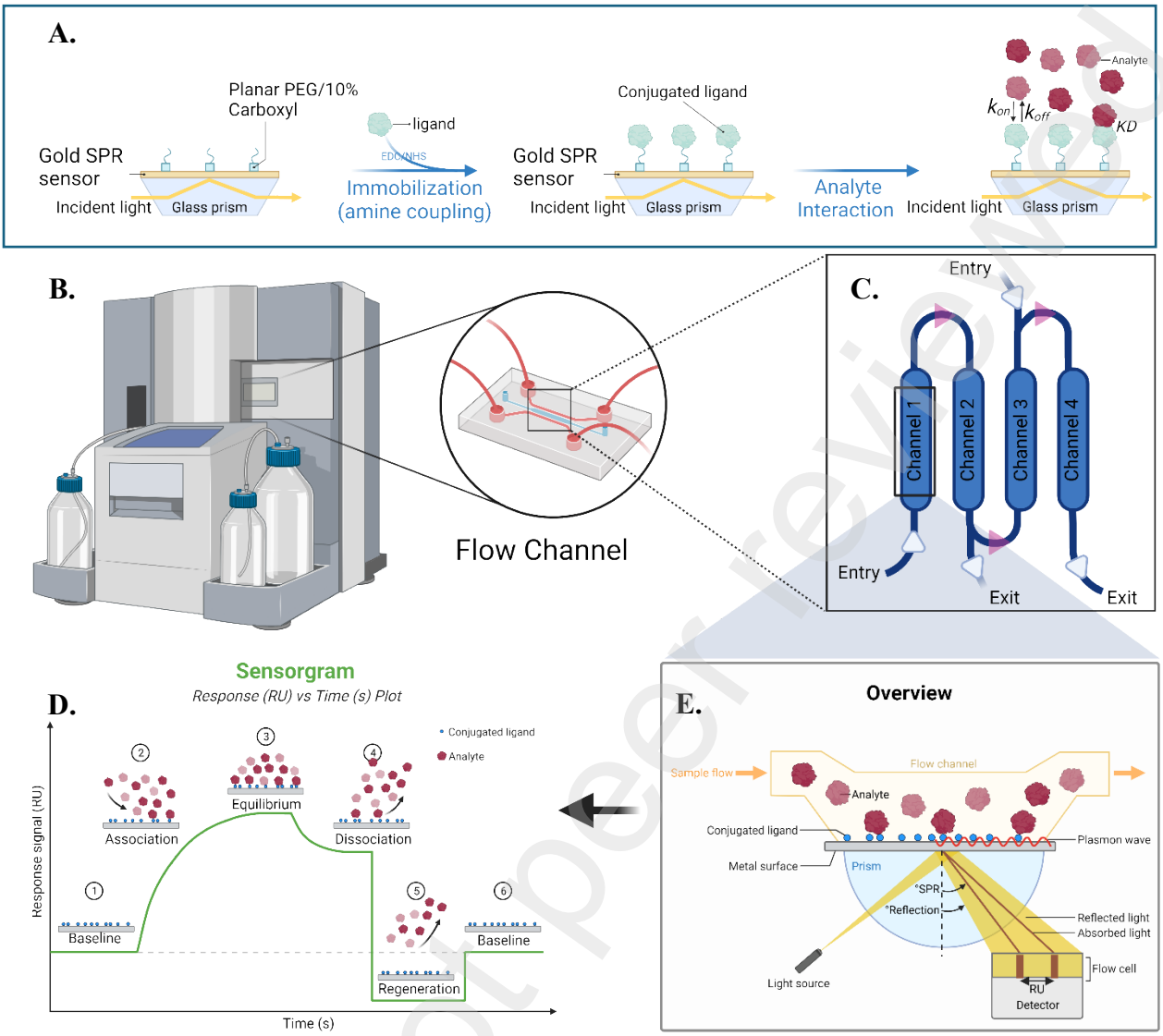

Figure D. Surface plasmon resonance (SPR) workflow. Using a planar polyethylene glycol/carboxyl-type sensor chip, SPR was performed as follows: (**a**) Carboxyl groups on the sensor surface were activated with EDC/NHS, enabling semipermanent amine coupling between the ligand and the PEG/carboxyl matrix. Such binding allows multiple kinetic cycles to be performed without requiring remobilization of the target. (**b**) SPR experiments were carried out at 25 °C using the Reichert 4SPR (Reichert, USA). (**c**) The Reichert 4SPR divides the chip into four flow cells; channels 1 and 3 are used for immobilization, and channels 2 and 4 are left blank for reference (matrix binding subtraction). **(d)** Kinetic study cycle: ligand is injected across the bound target in the *association phase*, in which binding events are sensed and detected in real time. Next, running buffer (HBS-EP) alone is injected. In this *dissociation phase*, the instrument detects only the dissociation of ligand from the target. (V) A *regeneration* solution is injected to clear any remaining bound ligands before another kinetic study is performed. (**e**) General principle of SPR, which includes a light source reflecting through a gold sensor surface and prism on a detector. The change in molecular interaction on the other side of the gold chip leads to a change in plasmon resonance, and light is absorbed. This change in absorption is recorded as a response unit (RU) and plotted against time in an interaction study.

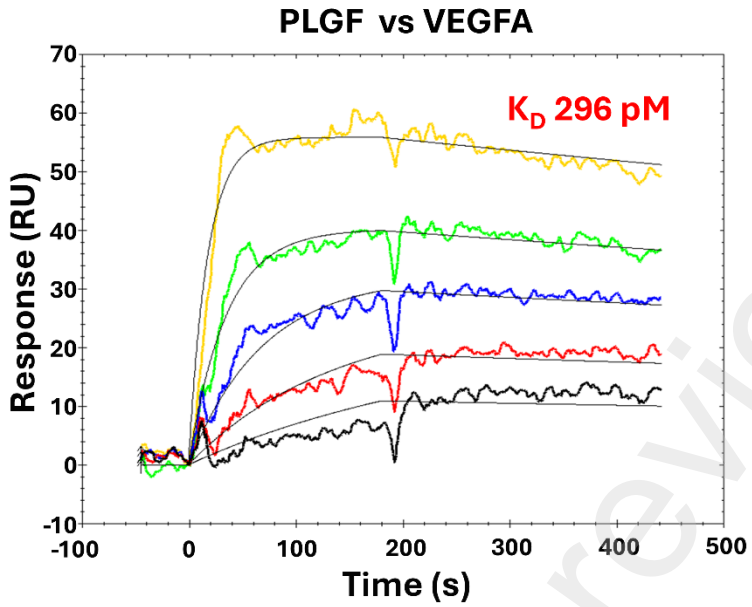

Figure 1. Interaction kinetics of known interacting growth factors VEGFA and PLGF, where VEGFA was immobilized and PLGF as analyte was passed over it at different concentrations: 50 nM (yellow), 25 nM (green), 12.5 nM (blue), 6.25 nM (red), and 3.125 nM (black). Note: the thin black overlapping lines are fitted curves of a 1:1 Langmuir model drawn with TraceDrawer ver. 1.8.1 software.

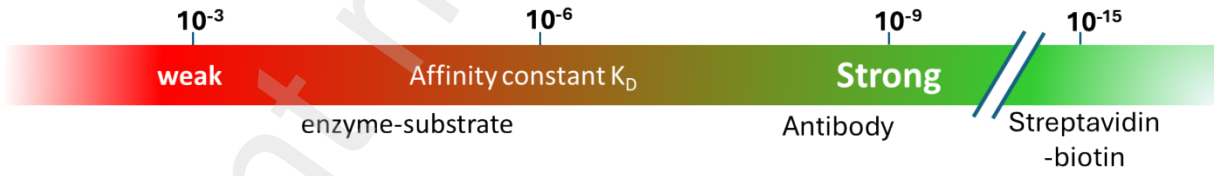

Figure 2. Typical ranges of affinities in biological interactions. Weak Enzyme–substrate interactions have affinities around 10^{-3} M to 10^{-7} M, and strong antibody–antigen interactions have affinities in the range of 10^{-8} to 10^{-12} M.

Table 1 VEGFA binding kinetics and affinities with different PDGFs

Growth Factor	Rmax (RU)	ka (1/(M*s))	kd (1/s)	K_D (M)	χ^2 (RU)	U-value
PlGF	56.16	1.12E+06	3.33E-04	**2.96E-10**	5.24	17
PDGF-AA	316.15	1.32E+05	1.22E-04	**9.21E-10**	10.63	11.5
PDGF-BB	177.17	1.81E+05	4.11E-04	**2.27E-09**	7.63	2.2
PDGF-DD	167.21	2.75E+05	1.01E-03	**3.66E-09**	15.55	3.1
PDGF-AB	128.165	1.64E+05	7.86E-04	**4.80E-09**	3.41	2.8
PDGF-CC	**999.17***	2.00E+03	1.84E-03	**9.19E-07**	1.33	12.8
Ang-4	**3571.88***	1.12E+03	1.14E-03	**1.02E-06**	11.57	14.4

! *A very high Rmax (relative to expected Rmax) denotes promiscuous or low binding, which can also be judged by visual inspection (see the methods).

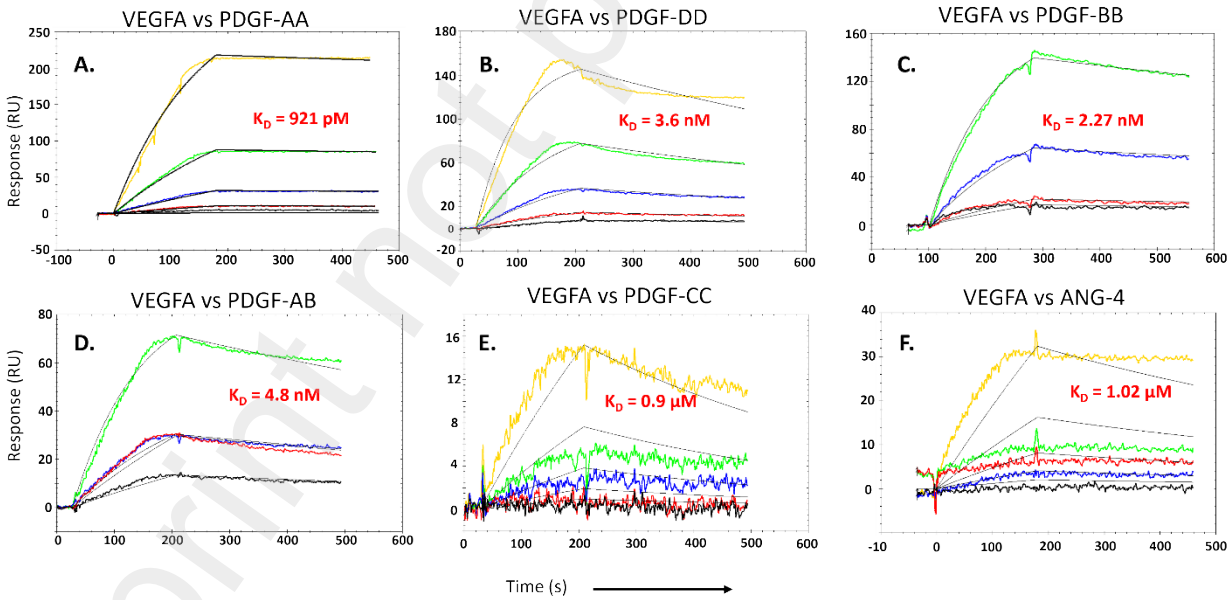

Figure 3. Kinetics of PDGFs' binding to immobilized VEGFA: Different concentrations of analyte were passed: 50 nM (yellow), 25 nM (green), 12.5 nM (blue), 6.25 nM (red), and 3.125 nM (black). (**a**) VEGFA vs PDGF-AA, (**b**) VEGFA vs PDGF-DD, (**c**) VEGFA vs PDGF-BB, (**d**) VEGFA vs PDGF-AB, (**e**) VEGFA vs PDGF-CC, and (**f**) VEGFA vs ANG4. Note: the thin black overlapping lines are fitted curves of a 1:1 Langmuir model on TraceDrawer ver. 1.8.1 software.

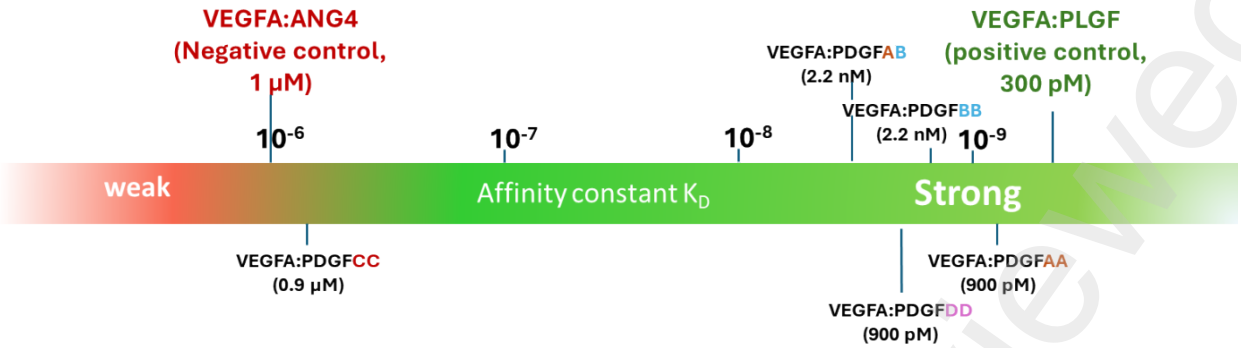

Figure 4. Affinity comparison of VEGFA interactions with PDGFs, with VEGFA:PLGF as a positive interacting partner and VEGFA:ANG4 as a negative interacting partner.

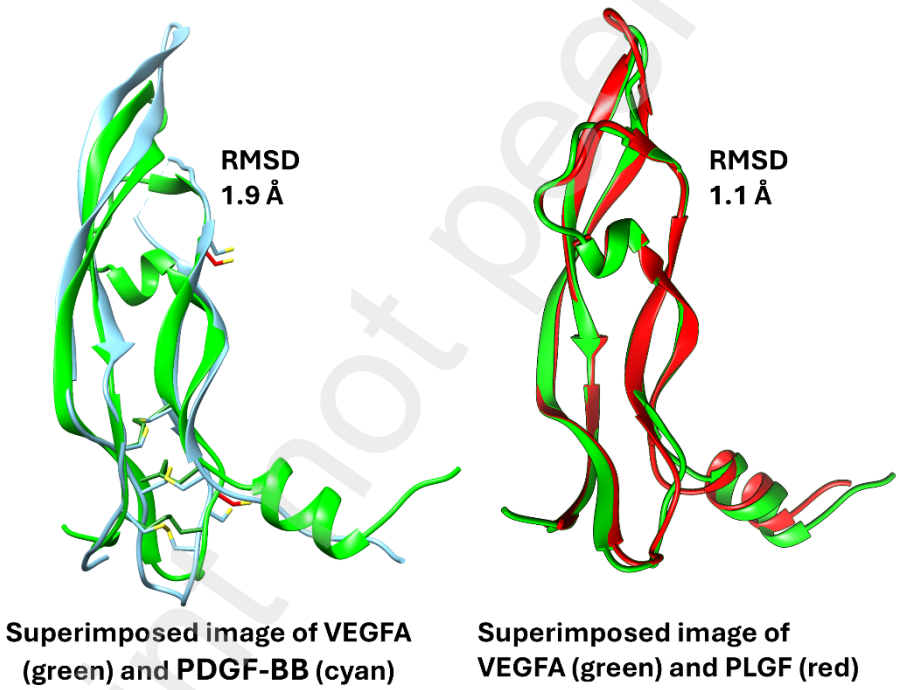

Figure 5 Structural comparison of monomers of VEGFA, PLGF, and PDGF-B