Development of FGF2 homologs for applications in human stem cell culture and the cultivated meat industry

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Abstract

Fibroblast growth factor 2 (FGF2 or basic fibroblast growth factor, bFGF), a multifunctional secreted signaling protein found in a variety of adult and embryonic cell types, has been instrumental in the maintenance and differentiation of stem cells, particularly in serum-free medium formulations. To explore evolution-driven changes in FGF2 across species relevant to the life science and cultivated meat fields, we produced homologs of FGF2 found in a range of mammalian, avian, and piscine species. These proteins, when validated in standard growth assays, highlight the versatility of growth factors produced from alternative species for use in human- and animal-derived stem cell applications. Subsequent efforts, complemented by a 3D crystal structure of hyper-stable human FGF2-G3 generated in-house, will explore inherent and mutation-driven hyper-stability of these factors for use in improving growth factor stability, shelf-life, and function.

## Workflow for FGF2 variant production and structure determination

Defined Bioscience leverages expertise in protein engineering and stem cell technologies to create fully-defined, highly-robust, and affordable solutions for stem cell culture, including purified growth factors, stem cell medium formulations, and protocols for downstream applications. With feedback from academic and industry professionals, we identified species and growth factors key to the life science and cultivated meat fields, and use our in-house pipeline to initiate production, purification, and validation trials.

Functional analysis
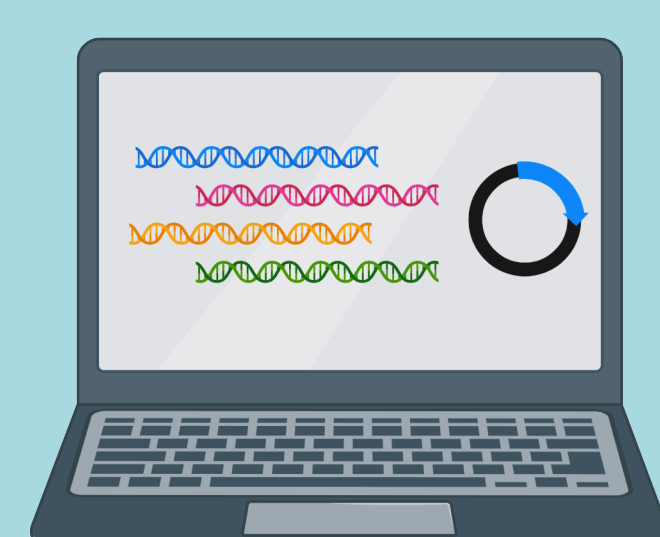
Identify growth factors of choice
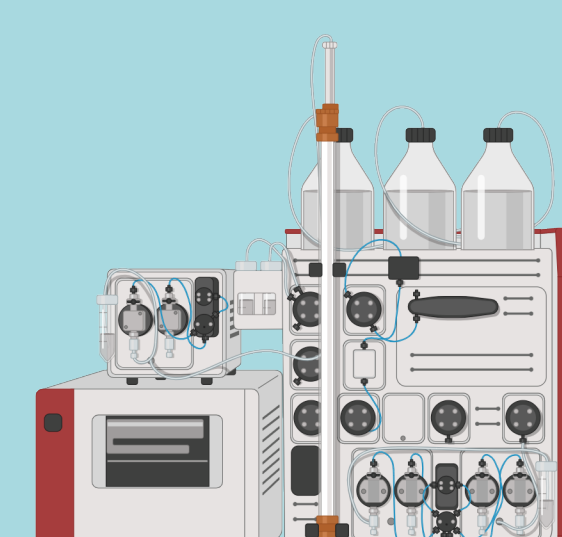
Produce and purify

Sample analysis
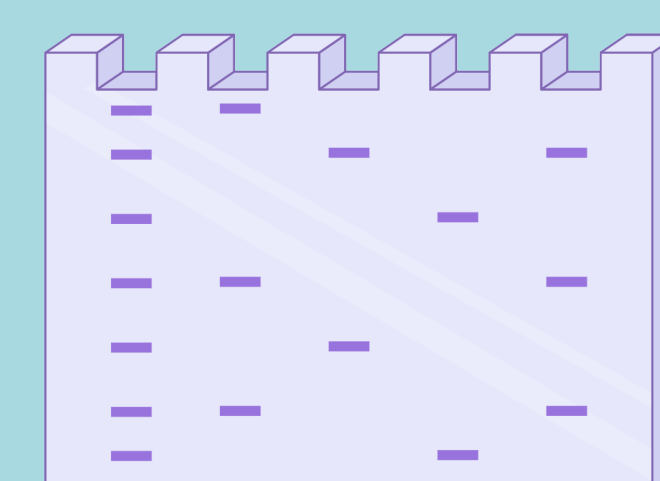
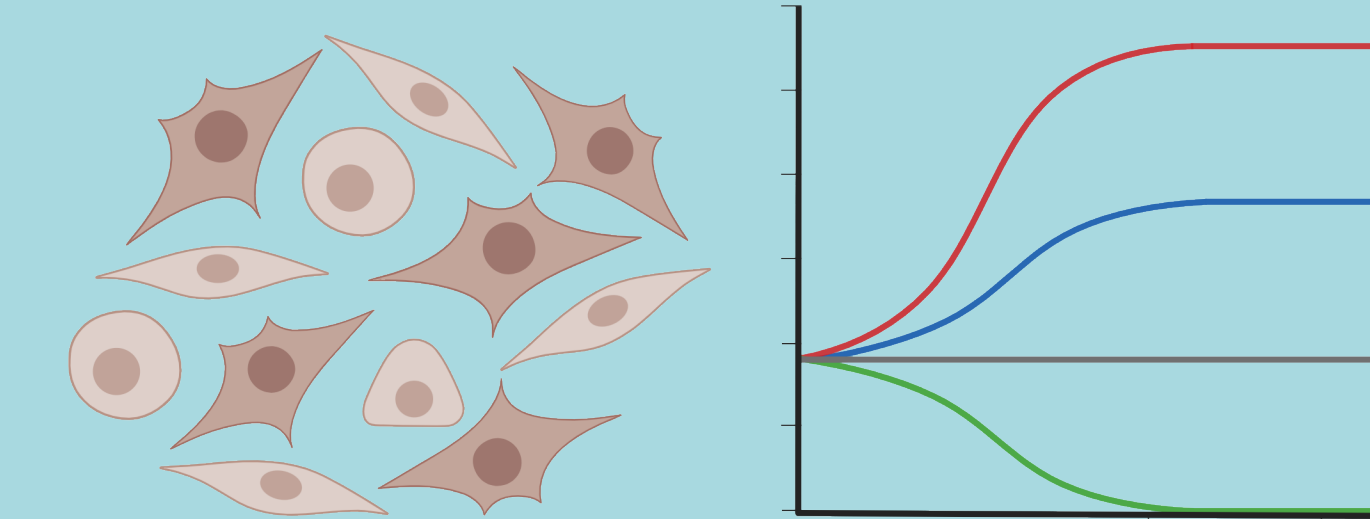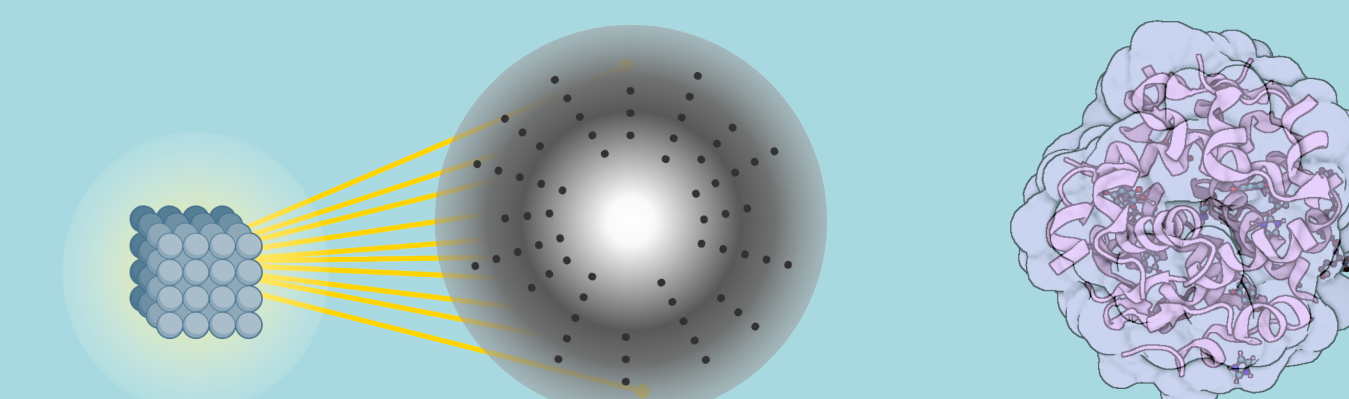Structural analysis

FGF2-G3, a thermostable variant of human FGF2[1], was concentrated and crystallized, and its atomic structure was determined using x-ray crystallography (2.2A). Meanwhile, **variants of native FGF2** from 11 other species were identified, produced, and analyzed in downstream functional assays.

Acknowledgements

Virginia Polytechnic Institute:
Reza Ovissipour PhD, Lexi Duscher PhD

2021 RFP Award No.
REES042021

References

[1]Dvorak P, Bednar D, Vanacek P, Balek L, Eiselleova L, Stepankova V, Sebestova E, Kunova Bosakova M, Konecna Z, Mazurenko S, Kunka A, Vanova T, Zoufalova K, Chaloupkova R, Brezovsky J, Krejci P, Prokop Z, Dvorak P, Damborsky J. Computer-assisted engineering of hyperstable fibroblast growth factor 2. Biotechnol Bioeng. 2018 Apr;115(4):850-862. doi: 10.1002/bit.26531. Epub 2018 Jan 24. PMID: 29278409.

FGF2-G3
Growth Factor

Sa/Su		
Weekend free Daily medium change not required	**Hyper stable** 20+ times the standard half-life in human cell culture incubated at 37°C	**Exceptional cell growth** Engineered with fully retained biological function

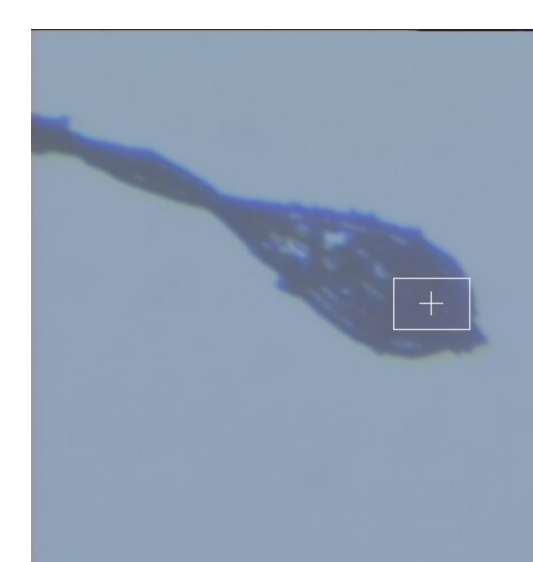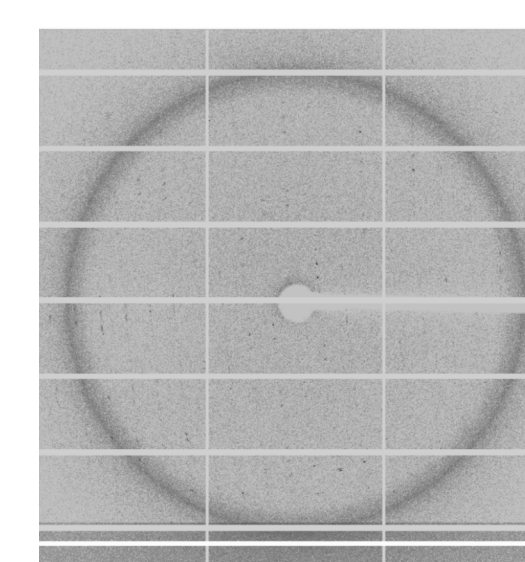Learn more about FGF2 and our other products at the Defined Bioscience website!

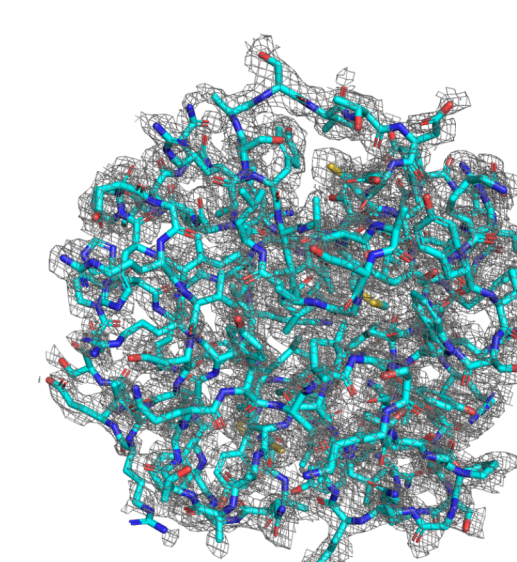## Structural elucidation of human FGF2-G3
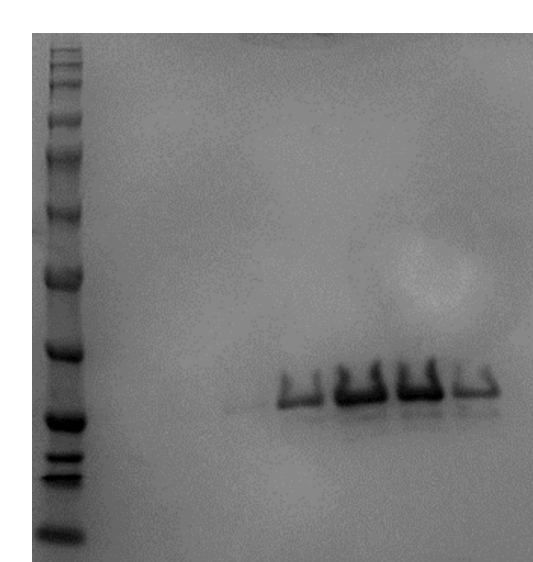
SDS-PAGE analysis, coupled with SEC and MS/MS, confirmed 98% purity and monodispersity.

FGF2-G3 crystals were grown from highly concentrated protein samples, and frozen with liquid nitrogen.

X-ray diffraction was measured for two samples, and the data merged for completeness to 2.2A resolution.

An atomic model of FGF2-G3 offers insight into this variant's high thermostability and potential means of further improvements in later constructs.
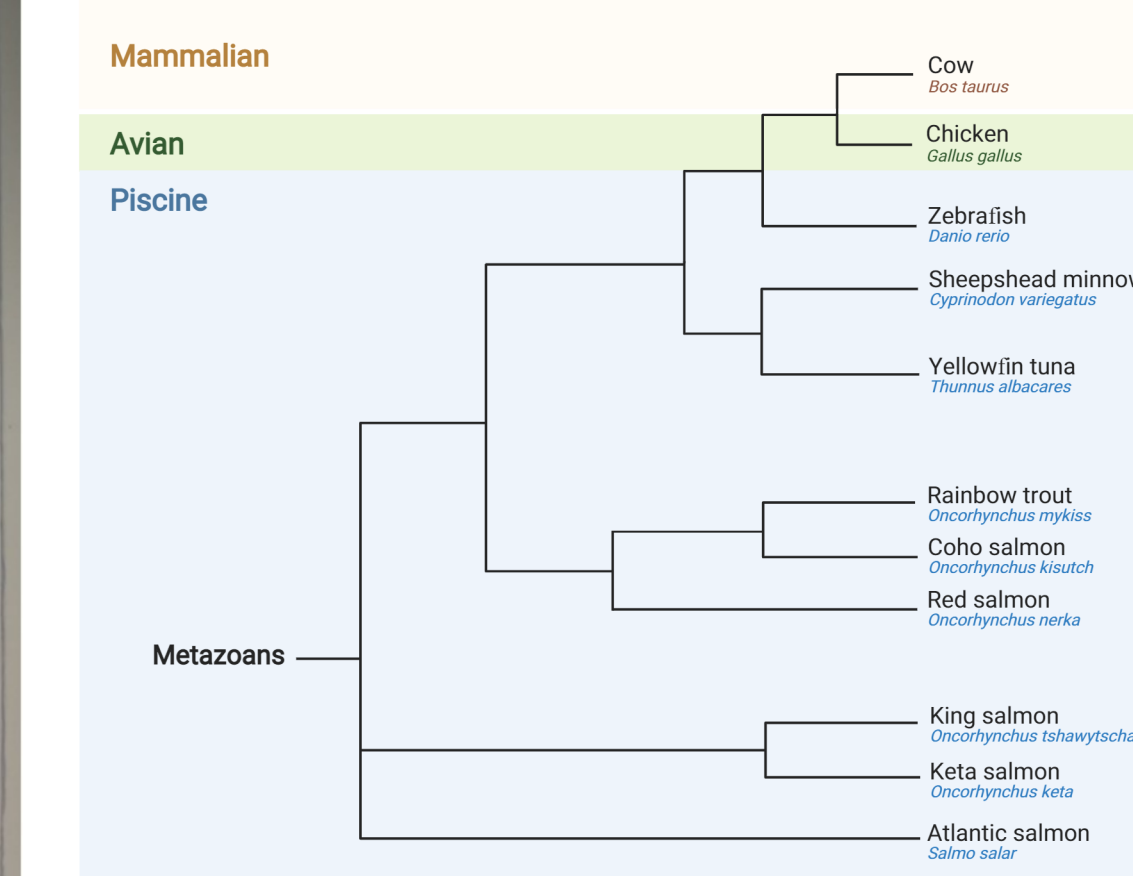
Stereo walleye images of FGF2-G3 crystal structure, shown as a ribbon model, generated using PyMOL. Coloring denotes N (blue) --> C (red) orientation.

Purification of FGF2-WT homologs

	cow	chicken	zebrafish	sheepshead	tuna	rainbow	red	coho	king	atlantic	keta
cow	100.00	87.74	73.38	74.84	78.06	74.19	74.19	74.19	73.55	75.48	74.19
chicken	87.74	100.00	74.68	72.26	76.77	72.90	72.90	72.90	74.19	76.13	74.84
zebrafish	73.38	74.68	100.00	78.57	79.87	77.27	77.27	77.27	77.92	79.22	77.92
sheepshead	74.84	72.26	78.57	100.00	89.03	84.52	85.16	85.16	84.52	87.10	85.81
tuna	78.06	76.77	79.87	89.03	100.00	87.10	87.10	87.10	89.03	89.68	88.39
rainbow	74.19	72.90	77.27	84.52	87.10	100.00	98.71	99.35	94.19	95.48	95.48
red	74.19	72.90	77.27	85.16	87.10	98.71	100.00	99.35	94.19	95.48	95.48
coho	74.19	72.90	77.27	85.16	87.10	99.35	99.35	100.00	94.19	95.48	95.48
king	73.55	74.19	77.92	84.52	89.03	94.19	94.19	94.19	100.00	97.42	98.71
atlantic	75.48	76.13	79.22	87.10	89.68	95.48	95.48	95.48	97.42	100.00	98.71
keta	74.19	74.84	77.92	85.81	88.39	95.48	95.48	95.48	98.71	98.71	100.00

Sequence percentage identity matrix (PIM) of variants of native FGF2 (see below for scientific name designation).Generated using Clustal Omega 2.1.
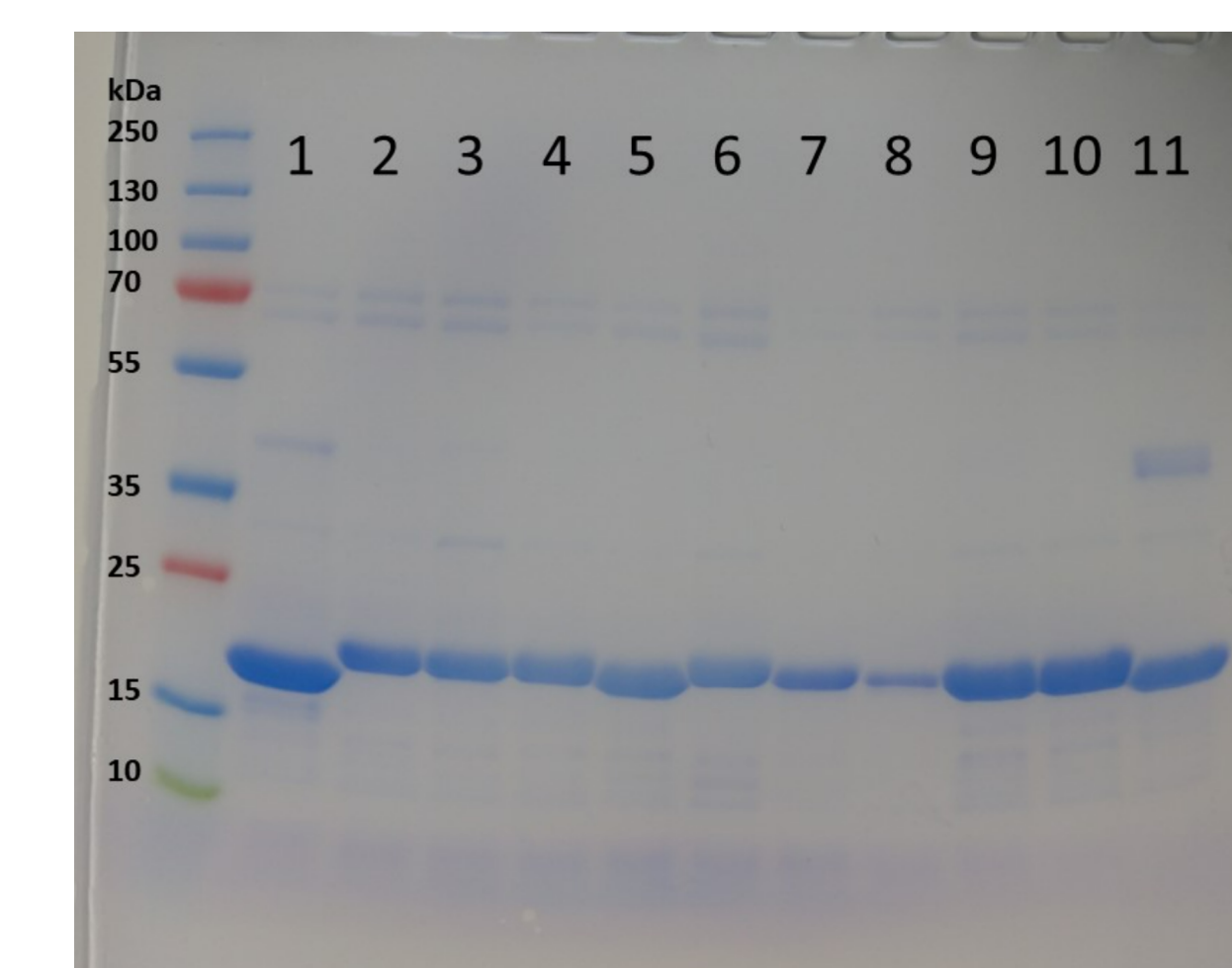
SDS-PAGE analysis of 11 variants of FGF2. Respectively: cow (1), atlantic salmon (2), sheepshead minnow (3), king salmon (4), red salmon (5), chicken (6), keta salmon (7), rainbow trout (8), yellowfin/bluefin tuna (9), coho salmon (10), and zebrafish (11).

Phylogeny of native FGF2 sequences, including common and scientific nomenclature. Generated using Clustal Omega v2.1 in conjunction with Simple Phylogeny, and designed using BioRender.