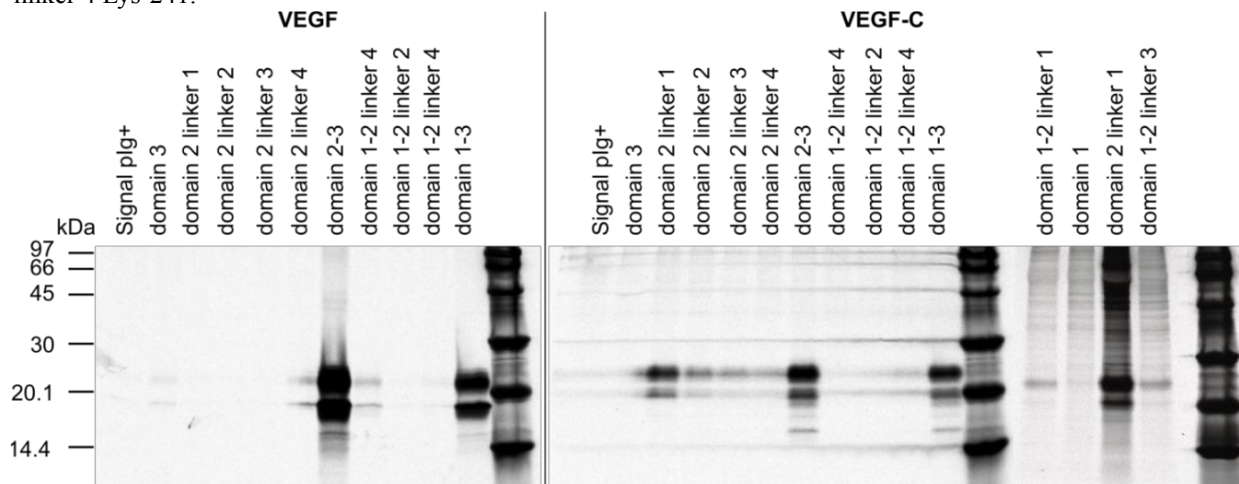


Binding of VEGF and VEGF-C to soluble VEGFR-2.

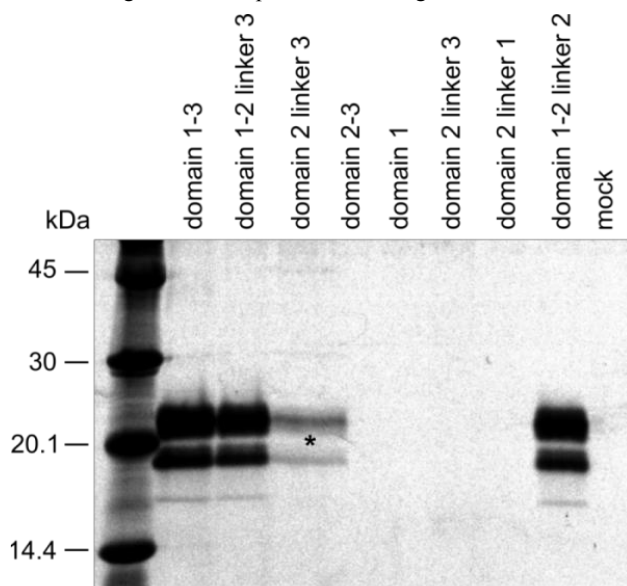
Conditioned medium from metabolically labeled 293T cells transiently expressing mature human VEGF or VEGF-C was incubated with unlabeled conditioned medium of 293T cells expressing human VEGFR-2/IgGfc fusion proteins. Lanes are labeled according to the extent of the extracellular domain of VEGFR-2 included in the fusion protein. For constructs in which the 2nd domain of VEGFR-2 was followed by the Fc domain of IgG the last C-terminal amino acid derived from VEGFR-2 differs as follows: for linker 1 Gly-220, for linker 2 Val-226, for linker 3 His-232 and for linker 4 Lys-241.



Binding of VEGF-C to soluble VEGFR-3

Conditioned medium from metabolically labeled 293T cells transiently expressing mature human VEGF-C was incubated with unlabeled conditioned medium of 293T cells expressing human VEGFR-3/IgGfc fusion proteins. Lanes are labeled according to the extent of the extracellular domain of VEGFR-3 included in the fusion protein. For constructs in which the 2nd domain of VEGFR-3 was followed by the Fc domain of IgG the last C-terminal amino acid derived from VEGFR-3 differs as follows: for linker 1 Lys-247, for linker 2 Leu-229, for linker 3 Gly-226, for linker 4 His-223 and for linker 5 Phe-220.

*A spillover from lane 3 to 4 and partially to lane 5 occurred after loading and the sample was loaded again on lane 7.



Covalent versus non-covalent dimerization of VEGF-, VEGF-C and selected VEGFR-3 binding VEGF/VEGF-C mosaic molecules

Conditioned media from metabolically labeled 293T cells transiently expressing human VEGF₁₀₉, VEGF-C₁₀₉ and four selected VEGF/VEGF-C mosaic molecules were immunoprecipitated with monoclonal anti-pentahistidine antibody (Qiagen). The immunoprecipitate was resolved using reducing and non-reducing 15% SDS PAGE. Bands were visualized by exposure on X-ray film.

