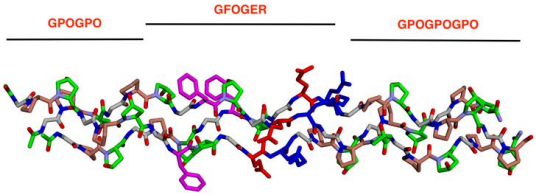
**Triple Helical Peptides Ltd**
www.triplehelical.com; sales@triplehelical.com

# GENERAL PEPTIDE PROPERTIES

Product information




Integrin-binding peptide. From Emsley, JMB 2004.

## General Organisation of Peptides:

Our synthetic collagen fragments generally contain real collagen primary sequence (above, GFOGER) within a Gly-Pro-Pro or Gly-Pro-Hyp (GPP or GPO) repeat sequence. The repeat sequences, known as hosts, confer triple-helical conformation upon the primary sequence, known as guests, which are too short to adopt triple-helical conformation spontaneously.

In the image above, from PDB **1Q7D,** GPOGPO and GPOGPOGPO hosts contain the integrin-binding GFOGER motif. The hosts adopt a tighter $7_2$ structure, whereas the guest sequence, usually relatively depleted in imino acids, tends towards the looser $10_3$ conformation.

The short integrin-binding peptide unfolds at ~ 22°C, and was designed for use in crystallography. All of our stock peptides are stable at 37°C.

In all of our peptides, the presence of Cys residues can be used for chemical crosslinking to deliberately polymerise the peptide, but intra-helix disulphide bonds also form which increase its thermal stability. Upon storage for short term use at 4°C, some inter-helix disulphide bonds may form, which can improve the avidity of the peptide for surfaces upon which it is to be coated. For longer-term storage, we recommend freezing in 0.01M acetic acid at 5mg/ml. Repeated freeze-thaw cycles should be avoided.

Custom synthesis allows us to introduce modification, such as **fluorophores** or **biotin** for specific needs.

## CRPs – Collagen-Related Peptides that are GPVI ligands:

Our **Collagen-Related Peptides** are based upon **GPO** polymers, where n = 10 or more. These are tight $7_2$ helices, with $T_m$ > 60°C.

## Target-specific Peptides:

An important example is our main integrin-binding peptide, **GFOGER**. Such peptides generally have 6 to 15 residue guest sequences within **GPC-[GPP]$_5$-** and **-[GPP]$_5$-GPCamide** hosts which have $7_2$ symmetry, with looser conformation in the central guest motifs. $T_m$ is generally about 45°C.

## The Collagen Ligands Collection:

These peptides were designed as **discovery tools**, to **map binding of receptors** and other molecules onto Collagen II or Collagen III. They also have **GPC-[GPP]$_5$-** and **-[GPP]$_5$-GPCamide** hosts, The guests are 27 residues of primary sequence, more likely to approximate to $10_3$ conformation. $T_m$ is generally about 45°C. The guest sequence advances by 18 residues along the Col domain of either Collagen II or Collagen III with each successive peptide, so that consecutive peptides share 9-residue overlaps.

This allows initial mapping of binding sites with 9-residue (3 nm) resolution. Next, truncation and substitution of the guest sequence will allow exact binding motifs to be identified.

## Control Peptide:

We supply a single control for all our peptides, **GPC-[GPP]$_{10}$-GPCGamide**, known as **GPP10**.

This combines the N- and C-terminal hosts into a continuous sequence.

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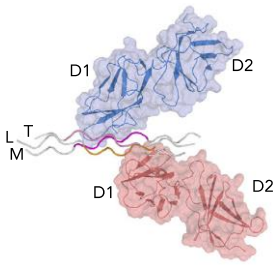
# GLYCOPROTEIN VI LIGANDS

Product information

**Glycoprotein VI:**

An immune receptor expressed on the platelet surface, GPVI is the key receptor that binds exposed collagen in the vessel wall and activates platelets. GPVI is a useful therapeutic target in thrombotic disease, and our GPVI ligands contribute to research in this area.




CRP–GPVI Co-crystal. From Feitsma, Blood 2022.

**GPVI-binding products:**

We offer several **Collagen-Related Peptides**, triple-helical peptides that bind to GPVI, for use in research and diagnostic applications.

- **mCRP - Monomeric CRP.** GCO-$(GPO)_{10}$-GCOGamide.

  The active sequence of our CRPs is OGPOGP, which docks onto D1 of GPVI. The Cysteine residues can be used for crosslinking, or to derivatise the peptide. mCRP can be used in platelet binding assays, and for coating of surfaces for shear flow experimentation. It will coat onto glass surfaces for microscopic analysis or plastic surfaces for binding assays. No use for aggregometry or flow cytometry.

- **CRP-XL - Crosslinked CRP.** Sequence as for mCRP.

  A potent activator of platelets, as a consequence of chemical crosslinking using the free cysteine residues and N-terminal amino groups. The polymerisation so introduced allows CRP-XL to bind and cluster GPVI on the platelet surface, a process that is a prerequisite for platelet activation. CRP-XL is used worldwide in haemostasis research, finding application in aggregometry and flow cytometry.

- **CRP-QZ - A novel GPVI ligand.** A GPO polymer.

  CRP-QZ is also a potent activator of platelets through GPVI. No crosslinking step is involved in its production, and it is more stable on storage. Useful in aggregometry, flow cytometry and other platelet applications.

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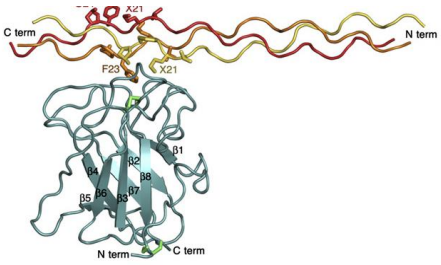
# DISCOIDIN DOMAIN RECEPTOR LIGANDS

Product information

In the image above, from **PDB 2WUH**, GPOGPOGPO and GPOGPO hosts contain the active **GPRGQOGVNleGFO** motif from Collagen III in complex with the DS domain of DDR2. Residues in red contribute to binding and signalling (Konitsiotis, JBC 2008). Note that in this version of the peptide, the native sequence methionine is replaced by norleucine, increasing the peptide's affinity and avoiding oxidation of methionine.

The main contacts between the collagen peptide and the DS domain surface are within the short GVMGFO motif.




DDR2 DS Domain–peptide co-crystal.

From Carafoli, Structure, 2009.

## Discoidin Domain Receptors:

Widely and differentially expressed receptor tyrosine kinases (RTKs), linked to several pathologies including inflammation, fibrosis, arthritis and cancer. The only RTKs known to bind collagen, recognising the conserved site (above) in collagens I, II and III, and receptor-specific sites in collagen IV (DDR1) and collagen X (DDR2).

## Selectivity of DDR-binding peptides:

Similar considerations apply to binding of DDR1 and DDR2: the same collagen residues are involved (Xu, Mat Biol 2011). Triple Helical Peptides will supply several DDR-binding peptides:

## Availability:

The above peptides are available in standard format, i.e., as

**GPC-[GPP]$_5$-[Gxx']$_n$-[GPP]$_5$-GPCGamide** from stock.

Derivatised peptides (biotinylated or with fluorophores) or other formats (shorter forms for structural studies) – **please enquire**

| Collagen source | Motif |
|---|---|
| **III** | GPRGQOGVNleGFO (recommended) |
| **III** | GPRGQOGVMGFO |
| **II** | GARGQOGVMGFO |
| **II and III** | GVMGFO |

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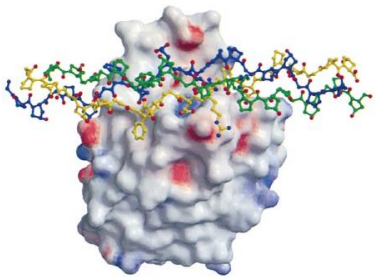
# INTEGRIN LIGANDS

Product information

In the image to the right, from **PDB 1DZI,** GPOGPO and GPOGPOGPO hosts contain the integrin-binding GFOGER motif in a peptide designed for crystallography and unfolds at ~ 22°C. Binding is divalent cation-dependent; in nature a $Mg^{2+}$ ion occupies the integrin I domain MIDAS, and is co-ordinated by the incoming ligand glutamate residue.



Integrin α2 I Domain–peptide co-crystal.

From Emsley, Cell, 2000.

**Collagen-binding Integrins:**

**α1β1, α2β1, α10β1 and α11β1** are widely-expressed heterodimeric adhesion receptors, where the α-subunit I-domain contains the ligand binding site. Expression varies with cell type and state of differentiation. We can provide peptides recognising each integrin which contain a 6-residue guest sequence (such as **GFOGER**) within **GPC-$[GPP]_5$-** and **-$[GPP]_5$-GPCGamide** hosts. The sequence **GFOGER** was the first such motif to be discovered, in a CB peptide of α1(I), and occurs in a conserved locus in Collagen II, and in Collagen IV. Our peptides are used for coating surfaces to support cell attachment, for example.

**Selectivity of integrin-binding peptides:**

Peptides containing the **GFOGER** motif bind with high affinity to **each of the collagen-binding integrins**.

Other motifs with the same generic sequence occur elsewhere in the collagens. **GLOGEN** (collagen III) has greater affinity for α1β1 and α10β1 than GFOGER, whilst **GROGER** (collagens I and III) does not bind α10β1 but is a good ligand for the other three integrins. **GVOGEA** (collagen II) is a moderate ligand for both α1β1 and α10β1 but not the other two. Several lower affinity motifs exist, such as **GMOGER** and **GAOGER**. When expressed on a cell, affinity of these integrins can be increased by cell activation.

**Availability:**

The above peptides are available in standard format, i.e., as

**GPC-$[GPP]_5$-Gxx'GEx''-$[GPP]_5$-GPCGamide** from stock.

Derivatised peptides (biotinylated or with fluorophores) or other formats (shorter forms for structural studies) – **please enquire**.

| Integrin | Preferred motif |
|---|---|
| **α1β1** | GLOGEN > GFOGER = GLOGER = GROGER > GVOGEA |
| **α2β1** | GFOGER > GLOGER ≥ GROGER ≥ GLOGEN |
| **α10β1** | GLOGEN > GFOGER = GLOGER > GVOGEA, but not GROGER |
| **α11β1** | GFOGER > GLOGER ≥ GROGER ≥ GLOGEN |

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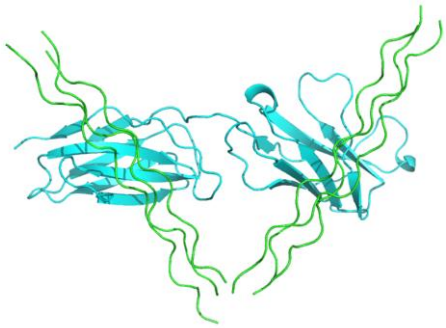
# OSCAR LIGANDS

Product information

In the image (right), from **PDB 5EIV,** GPOGPO hosts contain the active **GPOGPAGFO** motif from Collagen III in complex with both D1 and D2 domains of **OSCAR**.

The main contacts between the collagen peptide and the receptor surface are hydrophobic.



OSCAR–peptide co-crystal. From Zhou, Blood, 2016)

## OSCAR – Osteoclast-Associated Receptor:

Expressed on the monocyte lineage, OSCAR is a co-receptor for the differentiation of monocytes into osteoclasts. OSCAR is an immune receptor with two Ig-like domains, showing homology with GPVI. Each domain can bind collagen, and it seems likely that receptor clustering is important in monocyte osteoclast progression.

## Diversity of OSCAR-binding motifs:

Several conserved loci in Collagens I, II and III contain OSCAR binding motifs.

## Availability:

The above peptides are available in standard format, i.e., as

**GPC-[GPP]$_5$-[Gxx']$_n$-[GPP]$_5$-GPCGamide** from stock.

Derivatised peptides (biotinylated or with fluorophores) or other formats (shorter forms for structural studies) – **please enquire**.

| Collagen source | Motif (with alternatives) |
|---|---|
| **I and II** | GAOGPQGFQ, and GP[A/O]G[P/S][O/S]GFQ |
| **I, II & III** | GPOGPAGF[A/O] |
| **II** | GASGDR |