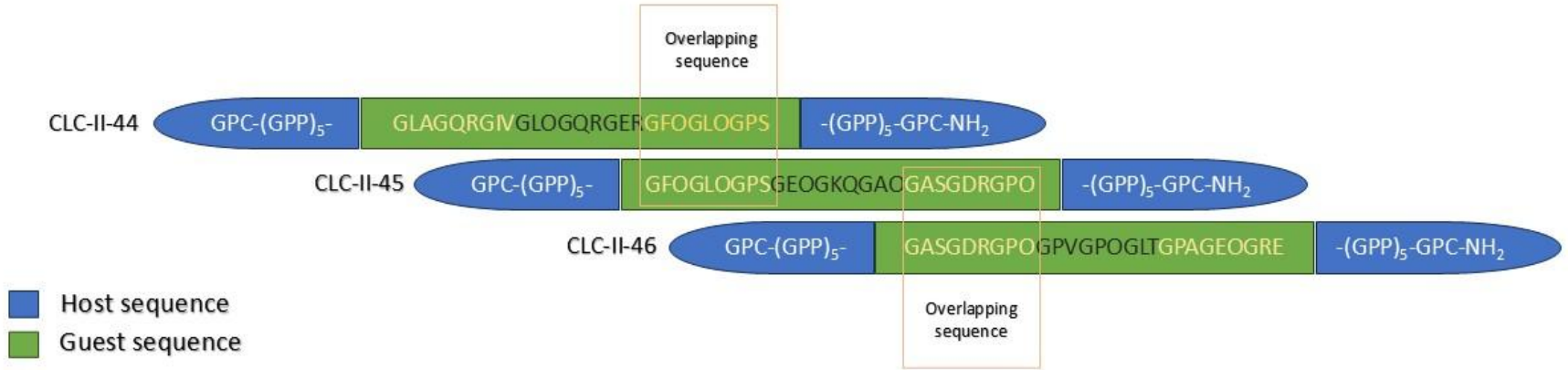
The Collagen Ligands Collection

A White Paper

Collagen Ligand Collection Design

The collagens are fundamental components of the extracellular matrix, and are highly interactive with many partners within the ECM and several sets of receptors on the cell surface. Collagen-like peptides open the way to rapid and independent verification of putative protein-collagen binding sites. The scientists at *Triple Helical Peptides Ltd* developed the Collagen Ligands Collection (previously described as collagen Toolkits) within the University of Cambridge and have synthesised these peptides for over 20 years. The Ligands encompass the entire triple-helical domains of collagens II and III can be used **to map the binding of receptors and other proteins onto the tropocollagen molecule** (the triple-helical collagen monomer).

The Guest sequences of the Ligands are each 27 residues long; initiating at the N-terminus of the collagen COL domain and advancing successively by 18 residues, allowing a 9-residue overlap between adjacent peptides. The collagen primary sequence is flanked on each side by five GPP triplets (the Hosts) to ensure triple-helical conformation. GPC extensions on each end of the Ligand allow crosslinking of peptides (if desired), making each 63 residues long. Collagen Ligand Collection III comprises 57 peptides, and II, 56 peptides. The triple-helical but inert ligand GPP_{10} is also included as a negative control. Others are available on request from our website https://www.triplehelical.com/products.html



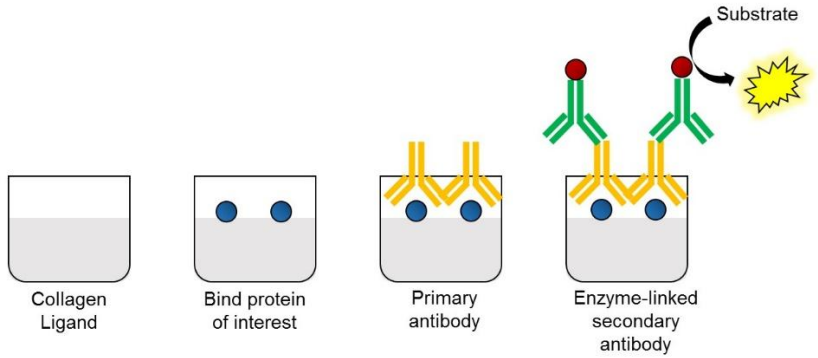
The successes of the Collagen Ligands Collection

To date 170 sites where over 30 proteins bind to collagen II have been mapped, providing firm conclusions about the amino acid distribution within such binding sites. Ligand II-44 is highly promiscuous; it alone binding over 20 different proteins. The Ligands have been used to determine atomic level resolution of interactions between collagen and other proteins, advancing our understanding of ECM assembly for applications such as tissue engineering.

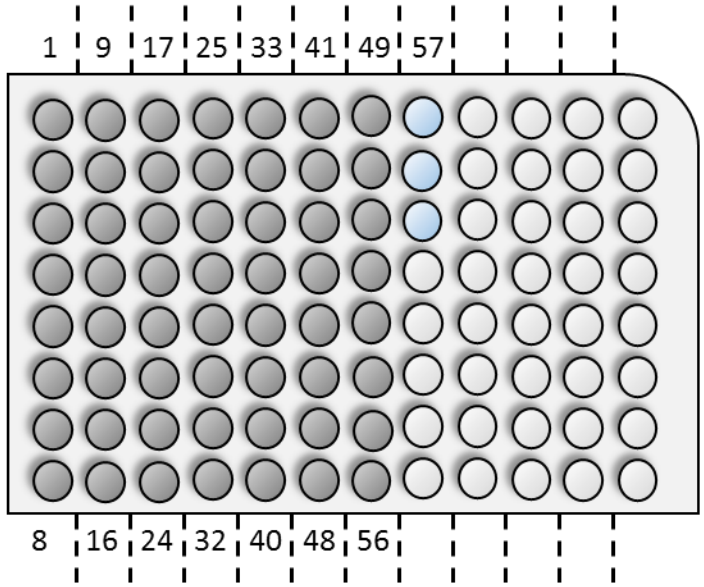
The Collagen Ligands Collection: A microplate-based assay

The Collagen Ligand Collection comes pre-coated on 96 well plates, in a ready-to-use adhesion assay format, and can be applied in solid-phase binding assays to map sites where collagen receptors and extracellular matrix components bind to collagens. Once a binding site is located, truncation and substitution allows exact residues involved to be determined, and corresponding minimal peptides to be synthesised for use in structural and functional studies.

We recommend blocking the peptide-coated with 5% BSA prior to binding and detecting your target protein according to the following protocol. BSA (only) coated wells, along with GPP_{10} may also serve as a negative control.



Collagen Ligands Collection II



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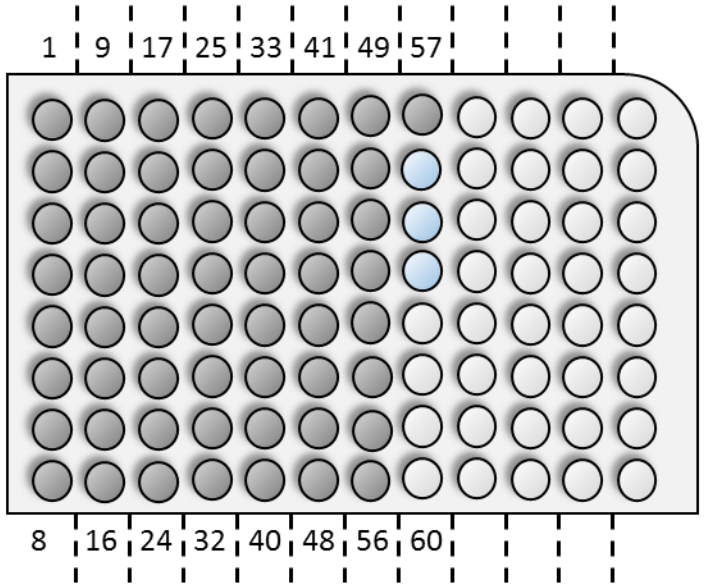

Collagen Ligands Control Peptides Empty wells for BSA block

(https://www.triplehelical.com/products.html)

Assay Protocol

Collagen Ligands are pre-coated on the plate at a saturating concentration of 10 µg/ml.

1. Block all wells to be used with 200µL 50 mg/ml BSA in Tris-buffered saline (TBS) for 1h at room temperature.
2. Wash all wells three times with 200µL adhesion buffer (1 mg/ml BSA in TBS containing 0.1% (v/v) Tween-20).
3. To each well, add 50-100µL target protein. We recommend a starting concentration of 100nM, but this may require optimisation. Leave for 1h at room temperature.
4. Wash all wells three times with 200µL adhesion buffer.
5. To each well, add 100uL primary antibody against your target protein in TBS containing 0.1% (v/v) Tween-20. We recommend an antibody dilution factor of 1:2000, but this may require optimisation. Leave for 1h at room temperature.
6. Wash all wells three times with 200µL TBS containing 0.1% (v/v) Tween-20
7. To each well, add 100µL enzyme-conjugated secondary antibody (of your choice) in TBS containing 0.1% (v/v) Tween-20. We recommend an antibody dilution factor of 1:10,000, but this may require optimisation. Leave for 1h at room temperature.
8. Wash all wells three times with 200µL TBS containing 0.1% (v/v) Tween-20.
9. Add substrate as appropriate and according to the manufacturer's instructions.

Q&A

- *How should I store the plates?*
  The plates should be stored with plate sealers intact at 4˚C

- *How long can I store the plates?*
  The plates are shipped at ambient temperature and can be stored at 4°C for up to three months.

- Can I store the plates in blocking buffer?
  No. To avoid microbial growth, we recommend storing the plates 'dry' as received until used.

- *What blocking agent should I use?*
  We recommend 50mg/mL BSA in TBS

- *What is TBS?*
  We recommend 50 mM Tris-Cl, 150 mM NaCl, **pH 7.4**

- *What concentration of target protein should I use?*
  We recommend 100nM, **but optimisation may be required**.

- *Can I use different substrate conjugated antibodies?*
  Yes, the substrate can be tailored to your secondary antibody conjugate.

- *Can I re-use the plates?*
  No. The plates are single use only and cannot be stripped or re-used.

- *Can I get XCELLigence plates coated with peptides from Collagen Ligands Collections for studies with cells or bacteria?*
  YES. We coat AGILENT's XCELLigence plates (E-PLATE 96 PET).

Troubleshooting

Problem	Solution
Weak/no signal	Omission of key reagent Washes too long/stringent Increase incubation time of target protein or antibody
High background	Decrease target protein concentration Decrease antibody concentration(s) Optimise blocking buffer Increase percentage of Tween-20 or use alternative detergent Increase wash times between steps
Uneven colour development	Incomplete/uneven washing of wells Pipetting (user) error

The Collagen Ligands Collection Peptide Sequences

Collagen Ligands Collection II		Collagen Ligands Collection III	
1	GPC-(GPP)5-GPMGPMGPRGPOGPAGAOGPQGFQGNO-(GPP)5-GPC-NH2	1	GPC-(GPP)5-GLAGYOGPAGPOGPOGPOGTSGHOGSO-(GPP)5-GPC-NH2
2	GPC-(GPP)5-GPQGFQGNOGEOGEOGVSGPMGPRGPO-(GPP)5-GPC-NH2	2	GPC-(GPP)5-GTSGHOGSOGSOGYQGPOGEOGQAGPS-(GPP)5-GPC-NH2
3	GPC-(GPP)5-GPMGPRGPOGPOGKOGDDGEAGKOGKA-(GPP)5-GPC-NH2	3	GPC-(GPP)5-GEOGQAGPSGPOGPOGAIGPSGPAGKD-(GPP)5-GPC-NH2
4	GPC-(GPP)5-GEAGKOGKAGERGPOGPQGARGFOGTO-(GPP)5-GPC-NH2	4	GPC-(GPP)5-GPSGPAGKDGESGROGROGERGLOGPO-(GPP)5-GPC-NH2
5	GPC-(GPP)5-GARGFOGTOGLOGVKGHRGYOGLDGAK-(GPP)5-GPC-NH2	5	GPC-(GPP)5-GERGLOGPOGIKGPAGIOGFOGMKGHR-(GPP)5-GPC-NH2
6	GPC-(GPP)5-GYOGLDGAKGEAGAOGVKGESGSOGEN-(GPP)5-GPC-NH2	6	GPC-(GPP)5-GFOGMKGHRGFDGRNGEKGETGAOGLK-(GPP)5-GPC-NH2
7	GPC-(GPP)5-GESGSOGENGSOGPMGPRGLOGERGRT-(GPP)5-GPC-NH2	7	GPC-(GPP)5-GETGAOGLKGENGLOGENGAOGPMGPR-(GPP)5-GPC-NH2
8	GPC-(GPP)5-GLOGERGRTGPAGAAGARGNDGQOGPA-(GPP)5-GPC-NH2	8	GPC-(GPP)5-GAOGPMGPRGAOGERGROGLOGAAGAR-(GPP)5-GPC-NH2
9	GPC-(GPP)5-GNDGQOGPAGPOGPVGPAGGOGFOGAO-(GPP)5-GPC-NH2	9	GPC-(GPP)5-GLOGAAGARGNDGARGSDGQOGPOGPO-(GPP)5-GPC-NH2
10	GPC-(GPP)5-GGOGFOGAOGAKGEAGPTGARGPEGAQ-(GPP)5-GPC-NH2	10	GPC-(GPP)5-GQPGPOGPOGTAGFOGSOGAKGEVGPA-(GPP)5-GPC-NH2
11	GPC-(GPP)5-GARGPEGAQGPRGEOGTOGSOGPAGAS-(GPP)5-GPC-NH2	11	GPC-(GPP)5-GAKGEVGPAGSOGSNGAOGQRGEOGPQ-(GPP)5-GPC-NH2
12	GPC-(GPP)5-GSOGPAGASGNOGTDGIOGAKGSAGAO-(GPP)5-GPC-NH2	12	GPC-(GPP)5-GQRGEOGPQGHAGAQGPOGPOGINGSO-(GPP)5-GPC-NH2
13	GPC-(GPP)5-GAKGSAGAOGIAGAOGFOGPRGPOGPQ-(GPP)5-GPC-NH2	13	GPC-(GPP)5-GPOGINGSOGGKGEMGPAGIOGAOGLM-(GPP)5-GPC-NH2
14	GPC-(GPP)5-GPRGPOGPQGATGPLGPKGQTGEOGIA-(GPP)5-GPC-NH2	14	GPC-(GPP)5-GIOGAOGLMGARGPOGPAGANGAOGLR-(GPP)5-GPC-NH2
15	GPC-(GPP)5-GQTGEOGIAGFKGEQGPKGEOGPAGPQ-(GPP)5-GPC-NH2	15	GPC-(GPP)5-GANGAOGLRGGAGEOGKNGAKGEOGPR-(GPP)5-GPC-NH2
16	GPC-(GPP)5-GEOGPAGPQGAOGPAGEEGKRGARGEO-(GPP)5-GPC-NH2	16	GPC-(GPP)5-GAKGEOGPRGERGEAGIOGVOGAKGED-(GPP)5-GPC-NH2
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18	GPC-(GPP)5-GERGAOGNRGFOGQDGLAGPKGAOGER-(GPP)5-GPC-NH2	18	GPC-(GPP)5-GANGLOGAAGERGAOGFRGPAGPNGIO-(GPP)5-GPC-NH2
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23	GPC-(GPP)5-GQOGVMGFOGPKGANGEOGKAGEKGLO-(GPP)5-GPC-NH2	23	GPC-(GPP)5-GPOGPSGPRGQOGVMGFOGPKGNDGAO-(GPP)5-GPC-NH2
24	GPC-(GPP)5-GKAGEKGLOGAOGLRGLOGKDGETGAA-(GPP)5-GPC-NH2	24	GPC-(GPP)5-GPKGNDGAOGKNGERGGOGGOGPQGPO-(GPP)5-GPC-NH2
25	GPC-(GPP)5-GKDGETGAAGPOGPAGPAGERGEQGAO-(GPP)5-GPC-NH2	25	GPC-(GPP)5-GGOGPQGPOGKNGETGPQGPOGPTGPG-(GPP)5-GPC-NH2
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57	-	57	GPC-(GPP)5-GERGSEGSOGHOGQOGPOGPOGAO-(GPP)5-GPC-NH2