

The most diverse libraries available for discovery of biologics

Phylogica is a biopharmaceutical company engaged in the discovery and development of novel peptide-based biopharmaceuticals. Phylogica uses its proprietary Phylomer® peptide libraries to target proteins and their interactions found within cells (intracellular interactions), on the cell surface or in the extracellular space (extracellular interactions).

Opportunity

Phylogica can screen Phylomer® libraries in partnership with companies which have disease protein targets; both to validate such targets and to generate lead therapeutics. Phylomer® libraries can be integrated with a number of screening platforms and are available for license from Phylogica.

Phylomer® Libraries

A core component of Phylogica's suite of technologies is the unique Phylomer® peptide libraries. These libraries consist of peptide sequences, which have been sourced from an evolutionary diverse range of bacterial genomes. [Watt *Nature Biotech* 24: 177-183(2006)]

From these bacterial genomes Phylogica has derived billions of distinct Phylomer® peptides covering thousands of distinct classes of protein structural folds.

These bacteria have been collected from diverse and often harsh environments, in which their genomes have been subject to intense

natural selection to evolve the optimal protein structures required for cell survival. Phylomer® libraries consist of fragments of these naturally occurring proteins, referred to as Phylomer peptides, which are highly enriched for stable secondary and tertiary structures found in abundance in biological genomes. Consequently, Phylomer peptides are a rich source of bioactive peptides capable of high affinity disruption of protein interaction. Screens of Phylomer® libraries yield very high hit rates of biologically active peptides even in the absence of affinity maturation. While Phylomer® peptides can be used as drug leads, these stable structures are also ideally suited as target validation tools, acting via "protein interference" to allow functional validation of protein interactions (the "interactome").

Phylomer® libraries available in different screening platforms

Phylogica has integrated Phylomer® technology with high throughput genetic screening platforms such as phage display and yeast two-hybrid. Phylomer® can also be used in phenotypic screens. Phylogica has successfully screened its Phylomer® libraries to isolate potent Phylomer® leads against challenging intracellular targets, (including signaling adaptor proteins and transcription factors) and conventional extracellular drug targets (e.g. receptor - ligand interactions). Phylomer® libraries have also yielded in high quality hits in cell-based screens.

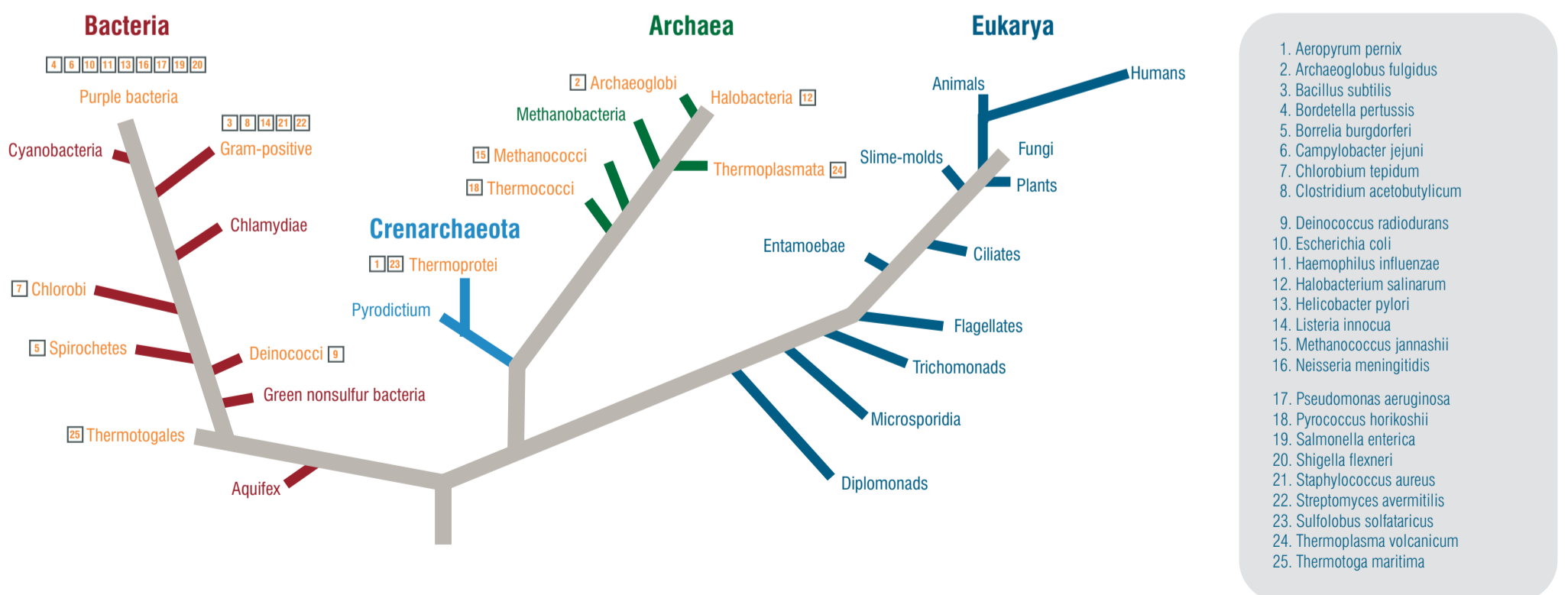
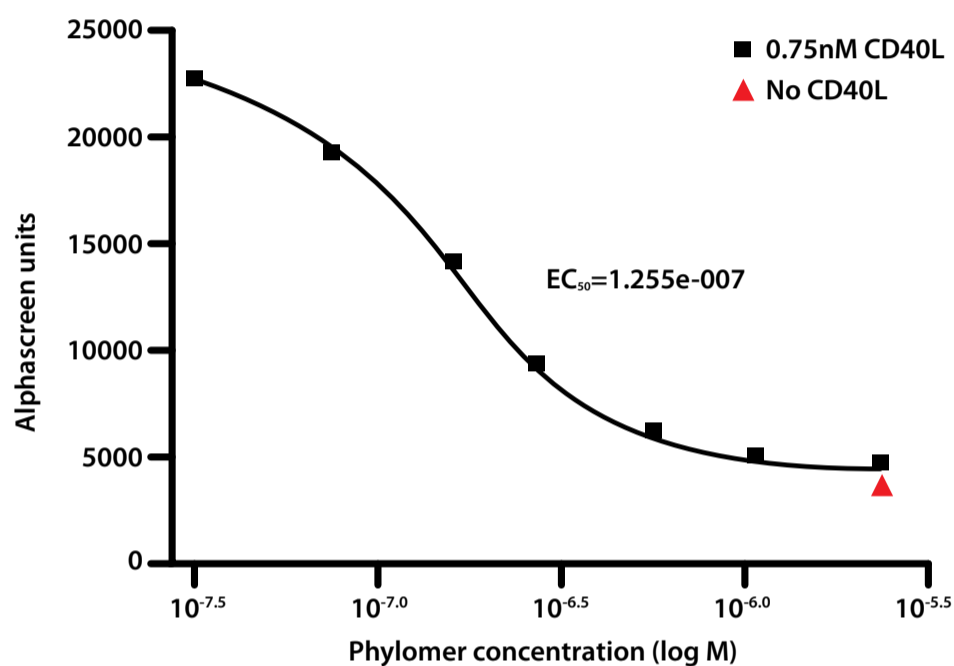


Figure 1: Phylogica Phylomer® libraries are composed of 25 characterised bacterial genomes from ancient bacteria that have evolved over millions to billions of years

Screening Extracellular Targets using Phage Display

Phylogica uses phage display to screen Phylomer[®] libraries against extracellular targets such as TNF- α , thrombin and CD40 Ligand (CD40L). For example, a phage display screen against CD40L identified 80 unique primary Phylomer[®] peptides that bound to CD40L, of which a subset of 56 peptides have been synthesized in full length as well as in high throughput Pepset[™] format (23 amino acid in length). The 56 peptides synthesized were screened for CD40L antagonism using AlphaScreen[®] and Bioassays screens to test for biological activity and specificity (Figure 2 & 3).



AlphaScreen[®] is a solution based proximity assay developed by Perkin Elmer.

*Pepset[™] is proprietary peptide synthesis platform developed by Mimotopes Pty Ltd.

Figure 2: AlphaScreen[®] test for antagonistic activity

AlphaScreen[®] is a solution-based proximity assay, which is used to measure the ability of Phylomer[®] peptide to block CD40L-CD40Fc interaction. CD40L Phylomer[®] peptide 1 is one out of 26 peptides which blocked the CD40L-CD40Fc interaction when tested using AlphaScreen[®].

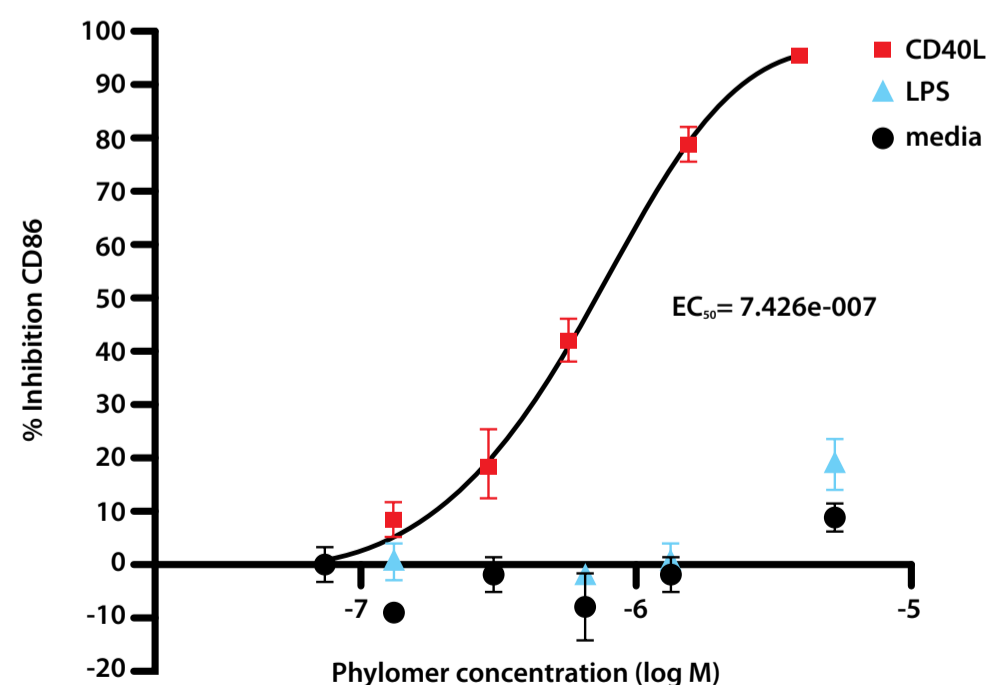
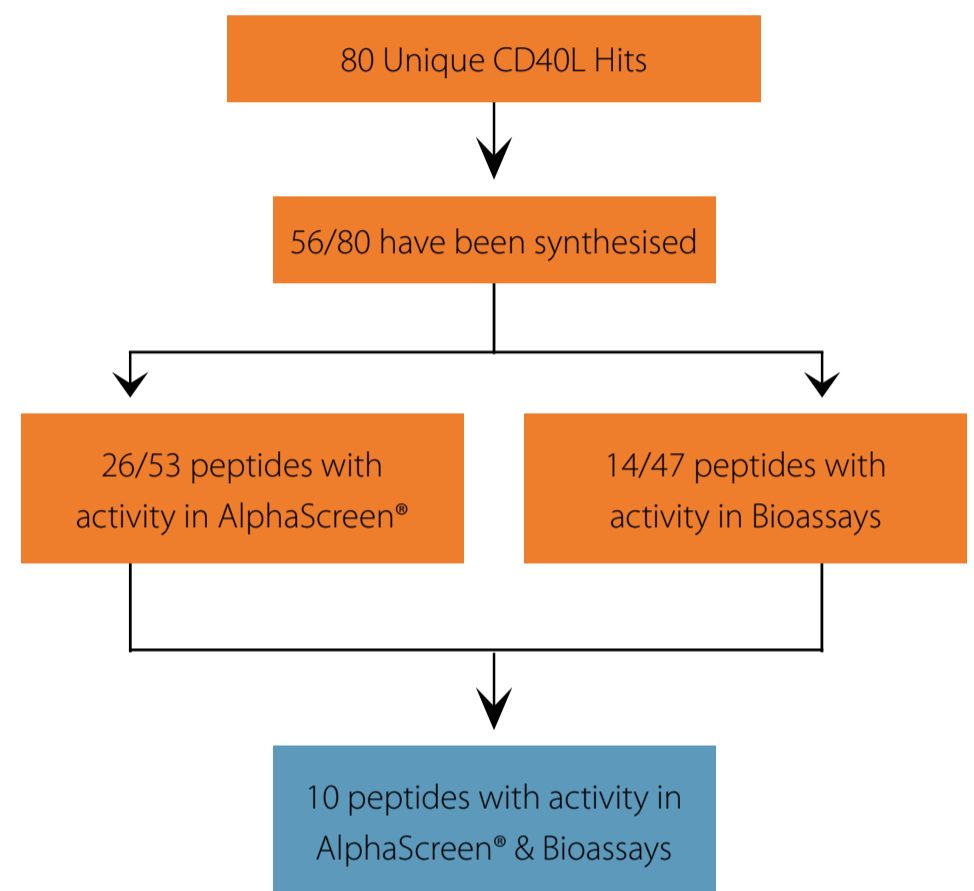


Figure 3: Bioassays to test biological activity and specificity

LPS or CD40L is used to upregulate expression of CD86 on murine B cells. Phylomer[®] peptide 2 is one out of 14 peptides which specifically inhibited CD40L-induced upregulation of CD86 on murine B cells. This effect was not seen for LPS-induced upregulation of CD86.

The AlphaScreen[®] and Bioassays were used to filter the primary hits to identify potential drug leads (Figure 4). To date, 10 peptides have been identified which have antagonistic activity in both AlphaScreen[®] and Bioassays.

Figure 4: Filtering the CD40L Phylomer[®] hits for drug leads



For more stringent functional specificity testing, peptides were further assessed in a TNF- α dependent cytotoxicity screen and also in LPS assay which measures CD86 upregulation in a CD40L-independent manner. Eight of the 10 Phylomer[®] peptides had no effect in either assay, highlighting the specificity of the primary hits for CD40-CD40L interaction. Several of these primary hits against CD40L have binding affinities in the low nanomolar to picomolar range (Figure 5).

The drug-like properties of the Phylomer[®] peptides can be tuned to adjust for desirable affinities and pharmacokinetics. The 8 CD40L-specific bioactive peptides are being affinity-matured using a number of approaches such as mutagenesis and multimerisation. Importantly, the peptides are also amenable to half-life extension technologies employing PEGylation and Human Serum Albumin binding. **This screen is an example of how Phylogica's drug discovery platform can be used identify quality primary hits with potent activity against extracellular targets.**

Specificity of Phylomers

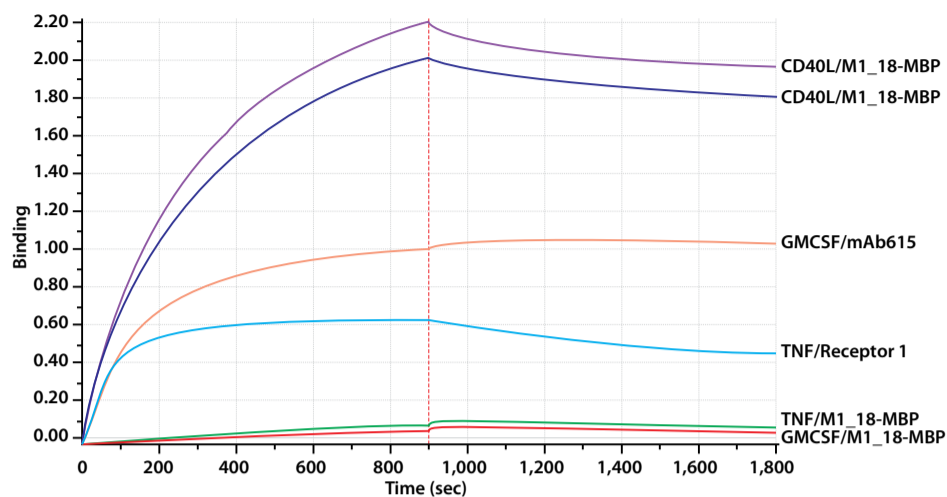
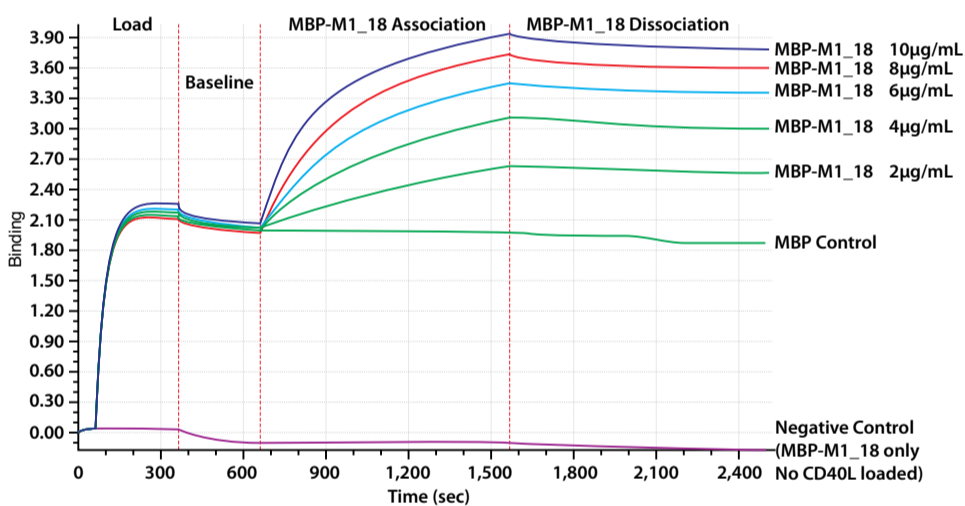


Figure 5. Phylomer peptide hits can have high target affinity before any sequence maturation. The upper panel shows Phylomer® specificity to the CD40L target. The lower panel shows affinity determination. Concentration titration of the maltose binding protein fusion MBP-M1_18 against CD40L using the ForteBio Octet®-RED to determine affinity for the target of 770pM. The table shows a range of affinities of other MBP-Phylomer fusions against this target, in addition to a CD40 positive control and a MBP negative control.

Specificity of Phylomers



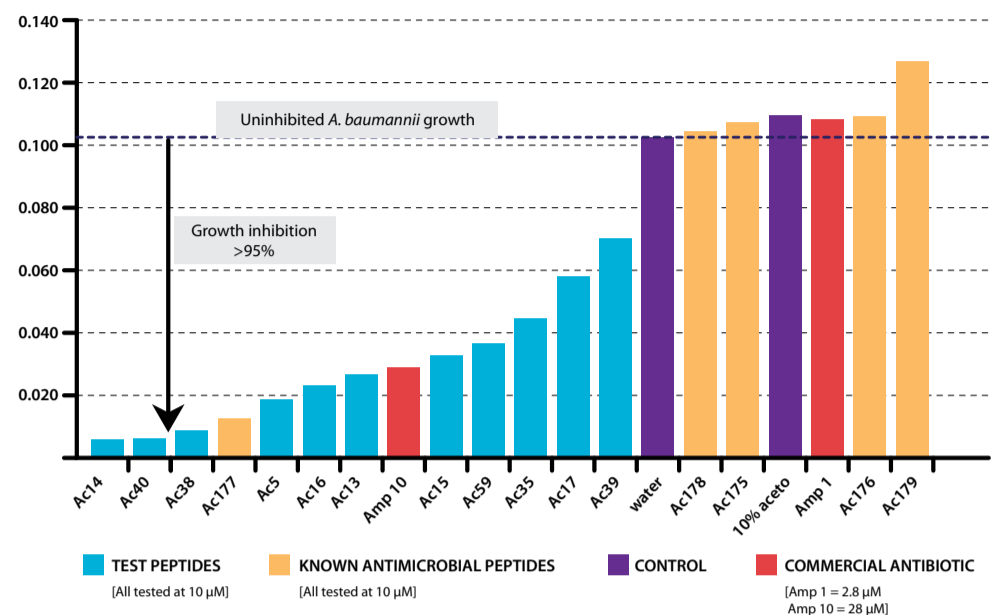
MBP-Peptide	M1_2	M1_4	M1_6	M1_7	M1_9	M1_18	Positive Control Fc-CD40 R	Negative Control MBP
K_D	8.5 nM	10 nM	11 nM	106 nM	8.3 nM	770 pM	5.6 nM	Undetectable

Cell-based Screening Using Phage Display

Phylogica's Antimicrobial Discovery subsidiary, Dynamic Microbials Ltd, has conducted a direct, cell-based screen to identify Phylomer® peptides which have activity against bacteria involved in multidrug resistant nosocomial infections.

To identify peptides which bind to bacteria, a phage display library of 51 million Phylomer® peptides was selected sequentially by biopanning on live *Acinetobacter baumannii*, a species of bacteria involved in nosocomial infections. A total of 101 Phylomer® peptides were obtained which bound to *A. baumannii*. These peptides were used to design a series of 280 overlapping synthetic peptides, which were then tested for antimicrobial activity (Figure 6).

Figure 6: Selected Phylomer® peptides inhibit growth of *Acinetobacter baumannii*



Several of these Phylomer® peptides (shown in blue) could inhibit the growth of bacteria as well as or better than known antimicrobial peptides (shown in brown) at an equivalent concentration or Ampicillin which was tested at two different concentrations.

Bactericidal activity was confirmed in live cell plating assays, in which 100% of bacteria were killed in the presence of Phylomer® peptides at concentrations as low as 5 µM. The active Phylomer® peptides were divided into the rapid bactericidal, slow bactericidal and bacteriostatic/delayed bactericidal peptides. Rapid bactericidal activity occurred within 5 minutes of co-incubation and was observed for 3 out of 14 active Phylomer® peptides.

Assessment of mammalian toxicity by red blood cell haemolysis showed no measurable toxicity for 7 of the active Phylomer® peptides (a selection of peptides shown in Figure 6) while 4 of the peptides exhibited the high toxicity commonly associated with this peptide class. Amino acid substitutions in two of the highly haemolytic peptides showed that cysteine residues seemed to be involved in the haemolytic activity; when replaced by serine residues the haemolytic activity dropped by a factor of 3–10 fold while the antimicrobial activity was unaffected (Ac13 and Ac 38 in Figure 7).

Figure 7: Selection of Phylomer® peptides with potent antimicrobial activity and varying degrees of haemolytic activity (MHC₁₀ = Minimal Haemolytic Concentration, i.e. high MHC₁₀ is considered to be favourable since a higher concentration is required to cause damage)

Synthetic Peptide	Minimal Inhibitory Concentrations (MIC) for complete inhibition of growth				MHC ₁₀ (concentration causing 10% haemolysis)
	<i>Acinetobacter baumannii</i>		<i>Pseudomonas aeruginosa</i>		
	<i>in NB</i>	<i>in MHB</i>	<i>in NB</i>	<i>in MHB</i>	
Ac5r	10 µM	15 µM	>60 µM	>60 µM	>100 µM
Ac14r	1.75 µM	1.75 µM	7.5 µM	60 µM	70 µM
Ac35	5 µM	15 µM	30 µM	20 µM	70 µM
Ac59	3.75 µM	10 µM	>60 µM	>60 µM	>100 µM
Ac195r	3.75 µM	7.5 µM	>60 µM	>60 µM	>100 µM
Ac259	2.5 µM	5 µM	25 µM	40 µM	30 µM
Ac476r	1.8 µM	15 µM	30 µM	30 µM	80 µM

The screen resulted in the isolation of 14 Phylomer® peptides with potent antimicrobial activity against the gram-negative bacterium *A. baumannii*. Further characterisation showed that biopanning the Phylomer® library had produced a diversity of antimicrobial peptide with varying degrees of cross-activity against the gram-positive bacterium *S. aureus* (Figure 7). **Pilot experiments with the most potent Phylomer® peptide Ac13 showed high antimicrobial activity at low micromolar concentration against clinical multi-resistant bacterial isolates. This peptide has activity *in vivo* in an animal model of pneumonia.**

Screening Intracellular Targets using yeast two-hybrid assays

Phylogica uses yeast two-hybrid screening platforms to identify high-quality Phylomer® hits against intracellular targets. These screens have yielded high functional hit rates, with a large percentage of primary hits showing activity in biological assays. For example, one such screen against an intracellular adaptor protein involved in Toll-Like Receptor (TLR signalling) yielded 58 primary Phylomer® hits that interacted with the target protein. Sixteen (16) of these hits (28%) showed functional activity in TLR-specific mammalian reporter gene assays where they inhibited activity of the reporter (i.e. TLR signalling).

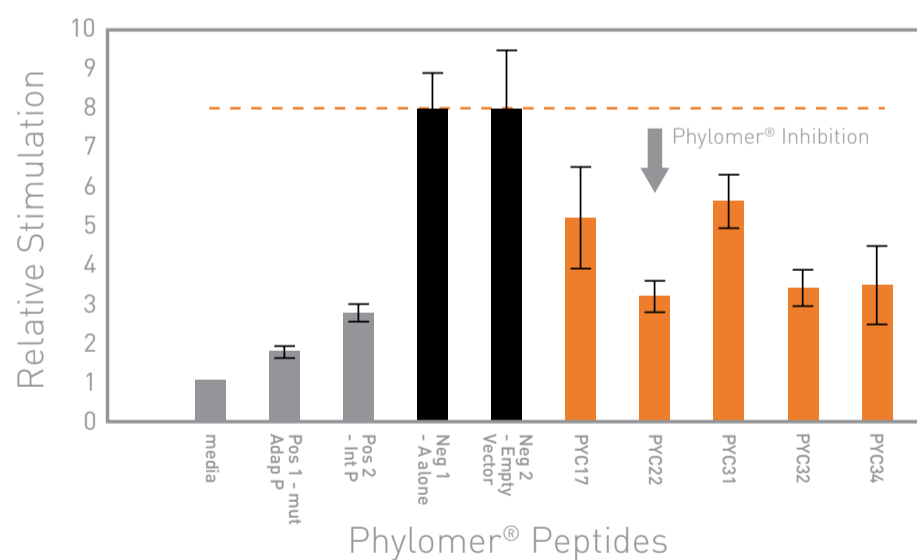


Figure 8: A subset of functional Phylomer® peptides that inhibited expression in the TLR-specific mammalian reporter gene assays. A reporter gene cell line was transfected with DNA constructs and stimulated with LPS in media. The negative controls contained either agonist (LPS) alone in media (Neg 1- A alone), or cells containing reporter genes and empty pcDNA3 (Neg 2 – Empty Vector).

The background control was media alone (media). Additional positive controls included cells transfected with dominant negative mutants of an adaptor (Pos 1- mutAdap P) or a known interacting protein (Pos 2– Int P).

Phylogica has a collaboration with Nexigen GmbH (Bonn, Germany) which provides it access to automated, scalable membrane-bound yeast two-hybrid screens. Phylogica and Nexigen have successfully integrated their technologies and identified specific Phylomer® peptides against c-Jun, one component of the AP-1 family of transcription factors. AP-1 pathway is validated target in models of inflammation including rheumatoid arthritis.

Why Choose to Partner with Phylogica?

- Phylogica offers a uniquely efficient system for identification and optimisation of drug-like peptide leads, based on the capture of primordial structures which have evolved within biodiverse proteins [see: Watt (2006) Nature Biotechnol. 24, 177-183, for review]
- Highly developed technology for Phylomer library construction and a versatile and robust screening process
- Phylomer libraries offer the biologics equivalent of diversity oriented synthesis – more structures with potential to bind challenging targets such as protein interactions
- Best primary hit-rates for peptides which are functional *in vitro* or *in vivo* (low nM)
- Effective against intracellular or extracellular targets and in direct phenotypic screens
- Good *in vitro* and *in vivo* evidence that the phylomer drug candidates work in multiple disease models, even prior to any affinity maturation
- Control of synthetic chemistry allows manipulation of peptides, just like small molecules, to enhance their drug-like characteristics (eg. stability, solubility, affinity and half-life)
- Established pathway through to GMP manufacturing via collaborations
- Data packs now available for several products for out licensing and/or for fee for service co-development proposals with associated milestones
- Directors with extensive big Pharma experience who understand the needs of partners.

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