High throughput protein workflows for antimicrobial drug design

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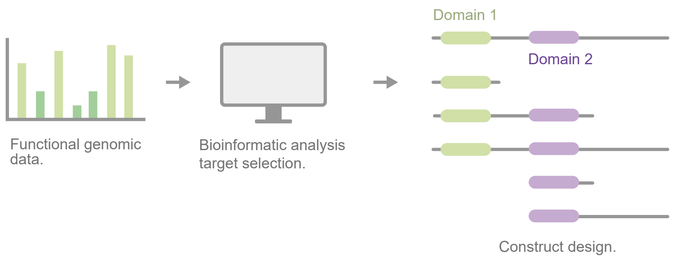
Introduction

At LifeArc we have developed a platform for the prosecution of multiple target proteins in parallel. We use a high throughput approach across the different processes to test different protein constructs, run fragment screens, and prioritise targets for further progression. We access large datasets of potential target proteins, such as gene essentiality data derived from TraDIS library screening. We carry out high-throughput cloning and expression testing to identify target protein constructs that deliver good levels of soluble protein expression with a range of domain boundaries and tag systems. Purified proteins are submitted to a range of biophysical experiments to check the quality of the purified material, including mass spectrometry, SEC-MALS and thermal melt assays. Proteins are tested for activity and positive controls identified and characterised by Biacore™. Usually we rely on biotinylated AviTags to immobilise target proteins on streptavidin sensor chips. We typically run high-throughput fragment screens on our Biacore™ 8K, but with options for alternative screening by thermal shift or microscale thermophoresis (MST). Fragment hits are confirmed in Biacore™ dose-response experiments where possible. Analysis of screening results allows us to prioritise target proteins by assessing their ligandability and identify fragment starting points for fragment-based drug design. Selected protein constructs are produced in larger scale, including by automated multi-step purification on the Akta™ Pure, and purified proteins used for biochemical assays and structural biology. Initial fragment hits are expanded on using an SAR-by-catalogue approach. We take an integrated approach to progress projects further, driving chemistry development with biochemical assays, biophysics data and structural biology. We have deployed this workflow for antimicrobial projects at LifeArc to combat the problem of antimicrobial resistance, a major growing public health crisis. Multiple target proteins have been expressed, purified, fragment screened and then preferred targets selected for further progression. However, our workflow is applicable to any drug design projects where a large number of targets are available and could be addressed in parallel.

Target selection & construct design → **Cloning & expression testing** → **Protein QC** → **Positive control testing** → **Fragment screening & confirmation** → **Large scale protein production** → **Fragment characterisation** → **Fragment hit expansion** → **Integrated project progression**

Target selection & construct design

Targets selected from large datasets, multiple constructs designed per target:



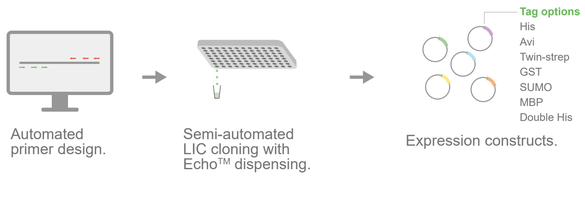
Functional genomic data.

Bioinformatic analysis target selection.

Construct design.

Cloning & expression testing

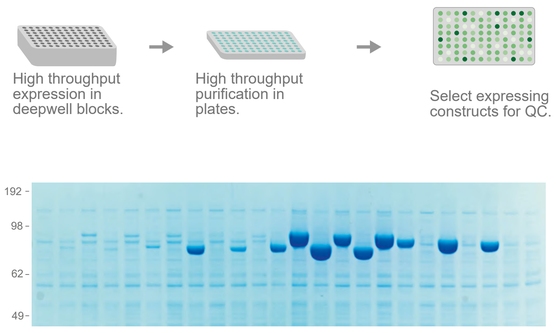
High throughput cloning and expression testing establishes useful constructs:



Automated primer design.

Semi-automated LIC cloning with Echo™ dispensing.

Expression constructs.



High throughput expression in deepwell blocks.

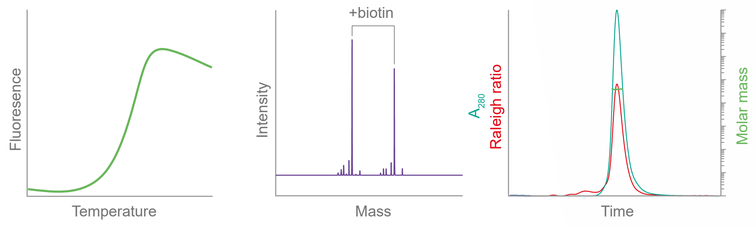
High throughput purification in plates.

Select expressing constructs for QC.

Expression test results.

Protein QC

A panel of biophysical experiments to test the quality of purified proteins:



Thermal melt curve:
Confirm folded protein.

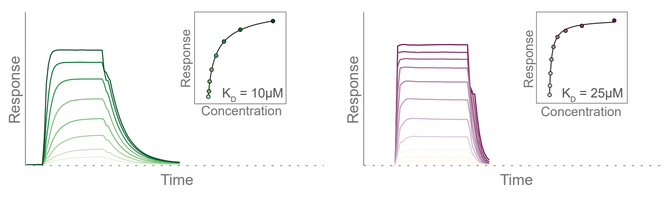
Mass spec:
Confirm intact mass / biotinylated AviTag.

SEC-MALS:
Check oligomeric state / monodispersity.

Positive control testing

Test known ligands by Biacore™ to confirm protein activity and identify suitable controls:

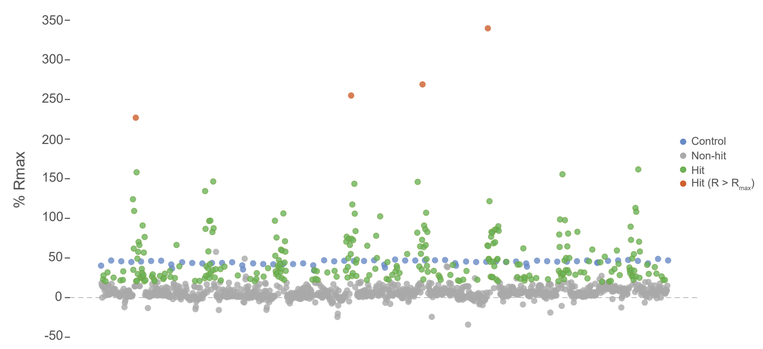
Immobilise target protein via AviTag + biotin.



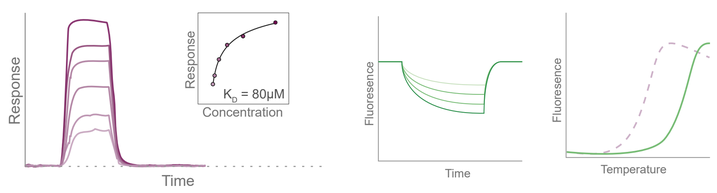
Confirm affinity & behaviour of prospective control ligands.

Fragment screening & confirmation

Biacore™ is first choice for fragment screening. Usually immobilisation is via AviTag:



High-throughput fragment screening on Biacore™ 8K using in-house fragment library.



Hits confirmed in dose-response experiments.

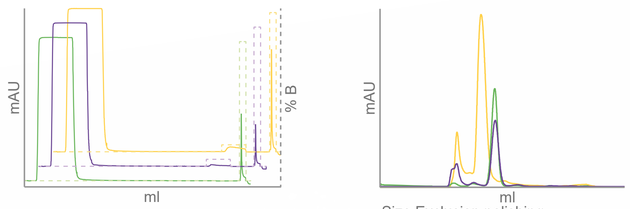
Options to screen by thermal shift and MST.

Large scale protein production

Selected constructs are scaled up to produce large quantites for follow up experiments:

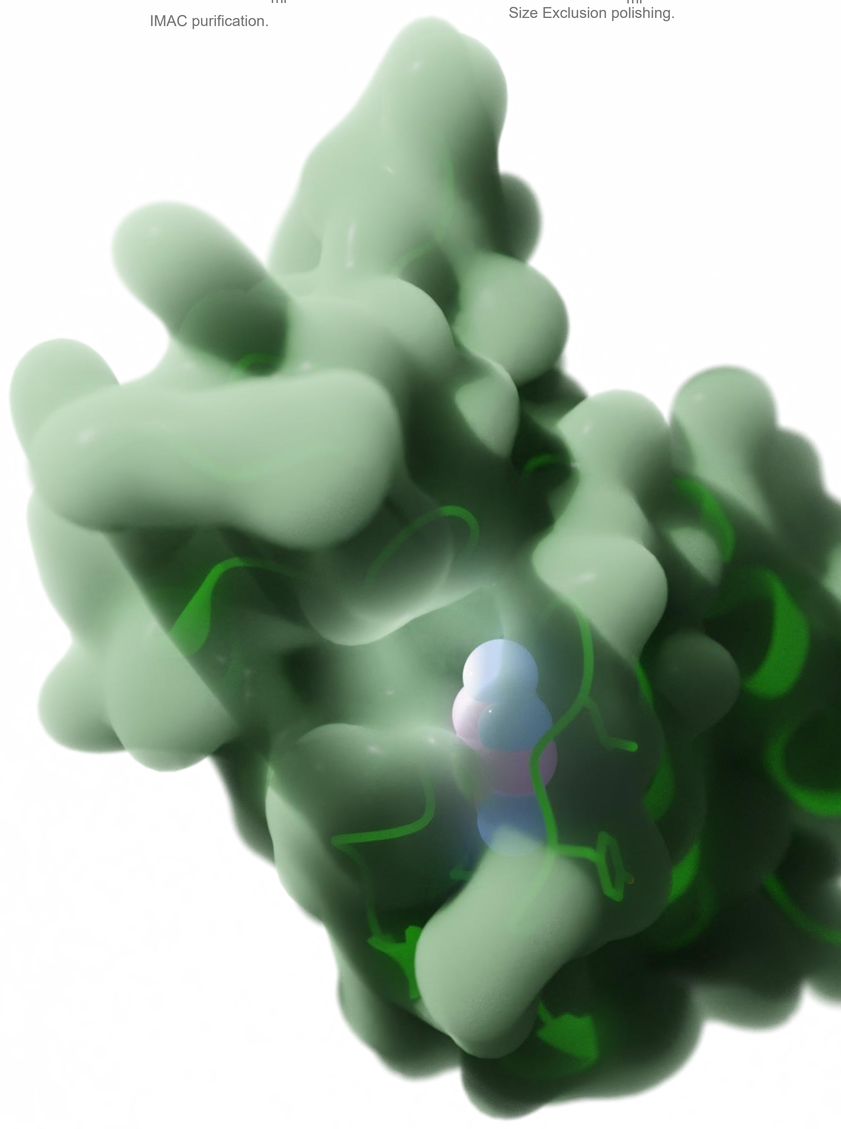
Scale up selected constructs, optional co-expression with BirA.

Automated multi-sample multi-step purification on Akta™, optional TEV cleavage.



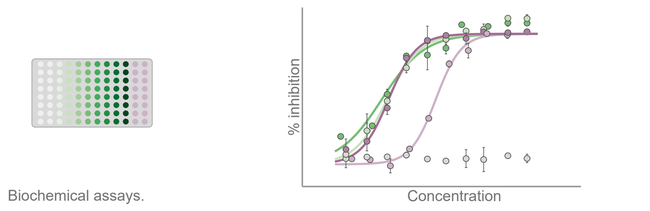
IMAC purification.

Size Exclusion polishing.

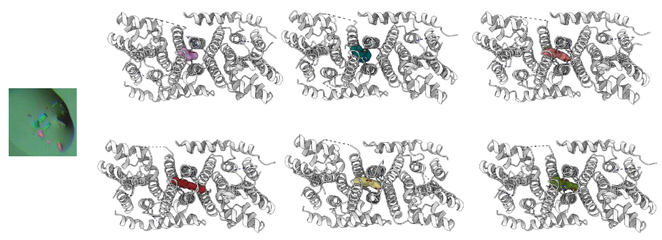


Fragment characterisation

High quality protein generated to support:



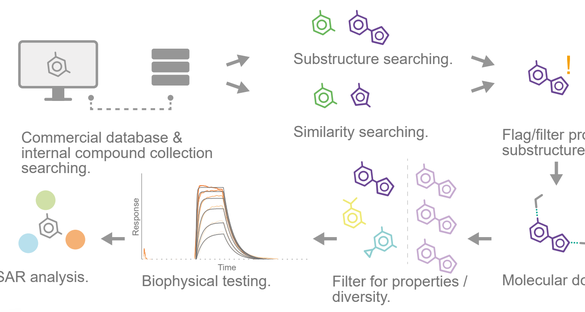
Biochemical assays.

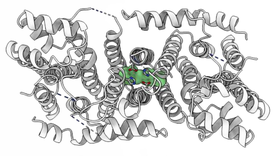


Structural biology.

Initial fragment expansion

SAR-by-catalogue:

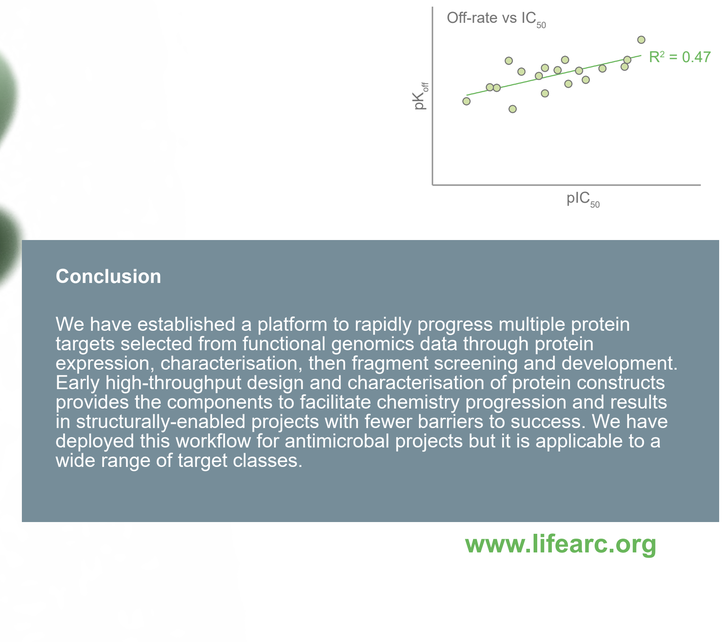




Structural biology.

Integrated project progression

Multiple techniques are used in parallel to advance projects further, providing data to support chemistry optimisation:



Conclusion

We have established a platform to rapidly progress multiple protein targets selected from functional genomics data through protein expression, characterisation, then fragment screening and development. Early high-throughput design and characterisation of protein constructs provides the components to facilitate chemistry progression and results in structurally-enabled projects with fewer barriers to success. We have deployed this workflow for antimicrobal projects but it is applicable to a wide range of target classes.

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