HELIX BIOSTRUCTURES

Structural Biology Made Simple

Services Packet

Core Areas: Protein Production, Cryo-EM, X-Ray Crystallography

HELIX BIOSTRUCTURES
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Purpose

Enclosed you will find an explanation of Helix's services with information detailing our process and customer interaction. We are unique in the industry, and it shows. Being founded and operated by scientists, we are keenly aware of the issues among CROs, and we pride ourselves on doing things differently.

Engage with us to find out more about this difference.

Table of Contents

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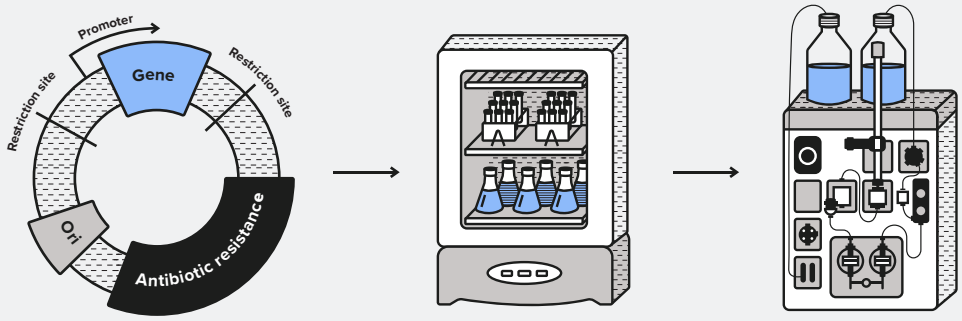
Protein Synthesis Services

CUSTOM PROTEIN SYNTHESIS

Early drug discovery projects require identification of key proteins that play pivotal roles in potential therapeutic pathways. In order determine the activity and function of a protein target it must be analyzed through biophysical and structural characterization in parallel with activity assays. Attaining a sufficient amount of purified protein is a prerequisite to completing this analysis. Helix's custom protein synthesis services produce crystallization grade recombinant protein having more than 95% purity suitable for any analytical technique.

CUSTOM GENE CONSTRUCT TO PURIFIED PROTEIN



1 Construct design

- Custom construct design enhances experimental success
- Fusion partner and affinity tag selection
- Codon optimization
- Post-translational modifications
- DNA isolation and subcloning expertise
- Construct optimization paired with a small-scale pilot expression

2 Protein expression

- Bacterial, mammalian, and insect expression platforms
- Small-scale preliminary runs validate constructs & conditions
- Scale-up expression tailored to customer requirements
- Western Blot analysis
- Quick turn-around time for literature/SOP reproduction
- Isotopically labeled (^{13}C/ ^{15}N; ^{15}N) protein for NMR.
- Pf (Pseudomonas fluorescens) expression system via Corteva AgriScience

3 Protein purification

- Custom-made purification schemes to provide high purity and homogeneity
- Significant experience with protein complex, monoclonal antibody expression & purification
- Quality control including SDS-Page, DLS, ITC, and Mass Spec
- Typical turn-around time for full-service protein production is ten weeks

PROTEIN SYNTHESIS TIMELINE

- **Construct design** 1 week
- **Vector construction/ optimization** 2 weeks
- **Cloning** 1 week
- **Small scale expression pilot** 2 weeks
- **Large scale expression** 2 weeks
- **Purification** 1 week
- **Validation/delivery** 1 week
- **Average completion time** 10 weeks

Crystallography Cryo-EM Biophysical characterization Cell assays NMR

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Protein Synthesis Budget Estimate

Date:

To: _____
_____
_____

From:

Helix BioStructures, LLC

RE: Budget Estimate Proposal for Target ExpiCHOTM Expression Strain Creation and Purification Services.

Dear _____,

From our discussion Date of conversation, this is our understanding of your objectives for this project:
1. Obtain cost and timeline estimates for Target baculovirus expression construct creation.
2. Establish expression and purification strategies for up to X constructs.
3. Establish relationship with expert protein production and structural biology CRO.

Please find the Milestone based costs and timelines estimations to help you with your project (scope of work):

SERVICE	DESCRIPTION	COST	TIMELINE
Expression Plasmid Synthesis	Design single ExpiCHOTM expression plasmid with cleavable N-terminal 6His purification tag.	$TBD	3 weeks
Small Scale Transfection Expression/Purification Development	- 1 L expression. - Cell lysis, capture ion exchange and/or gel filtration.	$TBD	5 weeks
Large Scale Transfection, Expression and Purification	- 2 L, expression - Lysis, capture, tag removal, ion exchange and/or gel filtration.	$TBD	4 weeks

Estimated cost for one iteration of preliminary expression and purification method development:
Subsequent purifications utilizing the same protocol will be less.

The volume of culture required, and amount of method development will substantially impact the final price.

We're excited about working with _____ as this is our specialty and passion. If you have any questions or concerns, please let me know.

Sincerely,

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Protein Synthesis Budget Estimate

Date:

To: _____
_____
_____

From:

Helix BioStructures, LLC

RE: Budget Estimate for Protein target name E. coli Expression Strain Creation and Purification Services.

Dear _____,

From our discussion Date of conversation, this is our understanding of your objectives for this project:
1. Obtain cost and timeline estimates for Target, E. coli expression construct creation.
2. Establish expression and purification strategies for up to X constructs.
3. Add additional goals here, if needed
4. Establish relationship with expert protein production and structural biology CRO.

Please find the Milestone based costs and timelines estimations to help you with your project (scope of work):

SERVICE	DESCRIPTION	COST	TIMELINE
Expression Plasmid Synthesis	Design single E. coli expression plasmid with cleavable N-terminal purification tag.	$TBD	3 weeks
Small Scale expression/purification development	- 2L, LB expression test. - Cell lysis, capture, ion exchange and/or gel filtration.	$TBD	5 weeks
Large Scale Expression/Purification	- 4L, LB expression - Lysis, capture, tag removal, ion exchange and/or gel filtration.	$TBD	4 weeks

Estimated cost for one iteration of preliminary expression and purification method development:
Subsequent purifications utilizing the same protocol will be less. The expression screening will employ an overnight growth at low temperature with an IPTG induction.

We're excited about working with _____ as this is our specialty and passion. If you have any questions or concerns, please let me know.

Sincerely,

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Cryo-EM Services

CRYOGENIC ELECTRON MICROSCOPY

Cryo-EM is a powerful tool to structurally characterize soluble protein without the requirement to grow individual crystals such as with X-ray crystallography. Helix provides full-service cryo-EM services to resolve 3-D structures of protein complexes, viruses, antibodies, membrane proteins, ion channels, and more. Helix scientists provide weekly grid screening and collection services using state-of-the-art cryo-EM microscopes delivering high-resolution processed data sets with competitive turnaround times and rates.

SOLUBLE PROTEIN TO 3-D STRUCTURE

- **Protein production** Step 1
- **Grid preparation** Step 2
- **Grid screening** Step 3
- **Data collection** Step 4
- **Data processing** Step 5
- **Structure solution** Step 6

AFFORDABLE HIGH-THROUGHPUT CRYO-EM PACKAGES

A Protein-to-structure Cryo-EM

- Obtain high-resolution 3-D structure from soluble protein
- Competitive packages compared to other CROs
- In-house expression and purification of proteins

Timeline: 16 weeks

B High-throughput Grid Screening

- Preliminary screening to determine optimal grid conditions
- Precise grid preparation utilizing a Thermo Fisher Vitrobot with by-the-grid pricing

Timeline: 1 week

C High-resolution Data Collection

- Utilize Thermo Fisher Krios–Gatan K3 camera for data collection
- Collection speed of approximately 3,000 micrographs per day with by-the-day pricing

Timeline: 1 week

D Cryo-EM Data Processing

- Expertise with resolving flexible regions of protein and protein-complexes
- In-house cryo-EM data processing and analysis with by-the-dataset pricing

Timeline: 8 weeks

PREVIOUS WORK

Challenging 3-D structures

A protein target only provided partial 3-D structure resolution. Relying on in-house cryo-EM single particle processing expertise, Helix was able to elucidate 80% of the protein structure, providing novel high-resolution structural information on previously unresolved areas of the protein. The client was able to analyze important interactions in the processed protein structure that led to expanded cryo-EM data collection and processing.

Poor resolution crystals

A client's protein complex yielded low-resolution crystals (~4.5Å). In order to analyze ligand density a higher resolution was required. Helix vitrified the samples optimized the grid conditions and obtained a 3000 micrograph data set. Helix's in-house cryo-EM expertise yielded a complete and refined 2.8Å protein complex revealing atomic resolution density for the ligand of interest.

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Cryo-EM Services

Cryo-EM Feasibility

1. Particle mass/size

A) Recommended minimum size of a particle is around 80kDa for Cryo-EM. Preferred particle size is > 150 kDa. Lower sizes may be possible but likelihood of achieving resolutions better than 4Å drops off significantly as mass drops below 80 kDa.
B) The metrics above refer to contiguous ordered mass, i.e. mass that behaves roughly as a single rigid body.

2. Symmetry

Internal symmetry within the target particle is highly beneficial.

3. Purity

Can tolerate somewhat lower purity than crystallization, especially with large particles. Small particles < 150 kDa should still be at similar purity as for crystallography (at least 90% purity).

4. Particle properties

Unfolded/disordered regions are particularly prone to destructive interactions with the air-water interface. Strongly recommend construct design and/or protein engineering to remove these if possible. If there are no related experimental structures available, AlphaFold is useful for this analysis.

5. Air-water interface

At least 75% of projects we see are not well behaved when frozen on normal holey grids, due to destructive interactions at the surface of the thin film, during the hundreds of milliseconds between blotting and vitrification. Amelioration requires sub-CMC detergent to act as surfactant (preferred, a normal part of our Milestone 1 screening below), or adsorption to a continuous surface prior to blotting (unpredictable, grids are more difficult to work with, only used as necessary as an extension to Milestone 1 grid screening).

Sample Requirements:

1. Preferred particle concentration and volume

for Cryo-EM work is 100 µl of 3 to 4mg/ml. Lower concentrations are not a problem in principle, however lower concentrations mean fewer particles per imaged area, which can (a) limit resolution or (b) drive up costs if we need to compensate by adding extra days of data collection to reach a target resolution.

2. Sample buffer

A) Excess sample buffer must be provided for making dilutions during screening.
B) Salts (e.g., NaCl) concentration > 0.5 M results in contrast reduction, avoid if at all possible.
C) Must keep any ingredients that increase solution viscosity at low concentration. Notable examples are glycerol or sucrose. Can tolerate up to ~ 4% glycerol if needed, however this carries the risk of extending timelines if this negatively impacts grid preparation reproducibility (i.e.., if we need to prepare many duplicate grids in order to catch one that freezes nicely). If shipped at very high sample concentration, dilution into a glycerol-free buffer has been a successful strategy; otherwise recommend gel-filtration polishing into a more compatible buffer.

Comparison Table: *This is a high-level comparison of two imaging methods detailing their attributes as well as differences. This is a VERY generalized means of comparing the two services, however, it is important to list these items as well as highlight the most critical aspects of each imaging option.*

X-Ray Crystallography	Cryo-EM
Shorter time to structure	Longer time to structure
Lower up front costs	Higher up front costs
Crystallization dependent	Shape, size dependent
Resolution to < 2.0 angstrom	Resolution to < 3.5 angstrom
High target concentration required	Low target concentration required
Low overall costs	High overall costs

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Cryo-EM Services

Milestone-based Project Workflow

Milestone 1: Grid screening

A) Sample is vitrified over 2-3 dilutions and a detergent screen
B) A small number (3-5 per grid square) of images are taken from 3 grid squares representing the range of apparent ice thickness available on the grid
C) Evaluation by eye for optimal particle monodispersity, density, and morphology
D) Deliverables:
  i. The images taken, in .mrc and .png formats
  ii. Written report on expert opinion and next steps
E) If prognosis is poor but there is high confidence in the input material: recommend extended milestone 1 to examine behavior on continuous surface grids (amorphous carbon, graphene oxide)

Milestone 2: Pilot dataset and processing

A) Collect ~ 500 exposure movies, using the same conditions/setup as would be used for a full dataset. From either the best milestone 1 grid or a new grid based on optimization from milestone 1 results.
B) Microscope used (Krios versus Glacios) will depend on project needs and/or microscope availability.
C) Data is also processed like a full dataset (see notes for milestone 3), as far as it will go. Goals are to determine:
  i. Are the visible particles/material capable of being aligned and averaged (2D classification)?
  ii. Do the 2D class averages meet the expectations for the target particle (e.g., is a subunit missing, is the conformation as desired)?
  iii. Is there a diversity of particle views, or is preferred orientation problem?
  iv. If all is well in 2D, attempt to jump to 3D for better insight into (i) and (ii) above.
D) Deliverables:
  i. Written report with expert analysis
  ii. Images of 2D classes if successful (.mrc and .png)
  iii. 3D refined volume or classes (.mrc)
  iv. Raw data available upon request

Milestone 3: Full dataset collection and processing

A) Collect ~ 4000 to 5000 exposure movies using a 24 hour time slot on a Titan Krios microscope. When possible, grid is prepared fresh, incorporating any optimizations indicated by milestones 1 and 2.
B) Data is processed to its full potential -- when refinement (including CTF refinement, per-particle motion correction, and multi-body refinement, where appropriate) and classification cease to yield resolution improvements.
C) Data processing pipeline is based primarily but not exclusively on the Relion software package. Exact pipeline is proprietary and varies with the needs of the target particle, but if Helix is included in journal publications, full assistance will be provided including figures, methods text, etc.
D) Deliverables:
  i. Written report with expert analysis, including key facts and figures (final particle count, resolution, FSC curves, particle angular distribution, sharpening and local resolution information)
  ii. Images of 2D classes from final particle set (.mrc and .png)
  iii. Final 3D refined volume(s) (.mrc):
    • Half maps
    • Full map
    • Sharpened map
    • Sharpened map, filtered to local resolution
  iv. Raw data available upon request

Milestone 4: Atomic model building

A) Requires starting model/fragment, or high enough resolution to 'read' sequence directly from the map.
B) Deliverables:
  i. "Table 1" summary of model geometry statistics and map-model fit
  ii. Atomic in .pdb and .cif format

Cryo-EM Software

- Data processing pipeline is based primarily but not exclusively on the Relion software package. Exact pipeline is proprietary and varies with the needs of the target particle.
- CryoSparc industrial license is prohibitively expensive. In the hands of experienced users, Relion provides all the utility and effectiveness of Cryosparc, albeit without the professionally designed user interface.

Microscopes

Helix relies on ThermoFisher Krios and Glacios microscopes. All 24-hour data acquisitions are completed on a Krios with K3 energy filter and camera, while most screening is performed on Glacios or the Krios K3 based on availability. Helix maintains primary scopes as well as contingency options to assure data screening and collection can occur without significant interruption.

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Cryo-EM Budget Estimate

Date:

To: _____
_____
_____

From: Joshua Carter
CEO
Helix BioStructures, LLC

RE: Budget Estimate Proposal for Cryo-EM Services – a la carte Based

Dear _____,

From our discussion, this is our understanding of your objectives for this project:
1. Establish Cryo-EM collection & structure solution services for key therapeutic targets.
2. Establish relationship with expert Structural Biology CRO.

Please find the **a la carte** costs and timelines estimations to help you with your project (scope of work):

SERVICE	DESCRIPTION	COST	TIMELINE
Grid Preparation	- Setup of dilution series and detergent additives - Grid preparation using Thermo Fisher Vitrobot	$ / Grid	> 1 week
Grid Screening	- Characterization of grid conditions and images - Determination of optimal target concentration - Determination of appropriate detergent/additive if required	$ / Hour 4-hour Min.	2 – 3 weeks
High-Res Data Collection	- High-Res Cryo-EM Data Collection on Thermo Scientific - Titan Krios G3i	$ / Hour	1 – 2 weeks
Raw Data Processing	- 2D Class averages of particle - Particle class distribution information - 3D class average map - Refined 3D maps of best class(es) - Globally Sharpened 3D Maps	$ / Data set	4 – 5 weeks
3D Model / Building	- Hand built / checked structure - Refined 3-D model and electron density maps	$ / Model	4 – 5 weeks

We're excited about working with _____ as this is our specialty and passion. If you have any questions or concerns let me know.

Sincerely,

Joshua Carter
CEO

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Cryo-EM Budget Estimate

Date:

To: _____
_____
_____

From: Joshua Carter
CEO
Helix BioStructures, LLC

RE: Budget Estimate for Cryo-EM Services – Milestone Based

Dear _____,

From our discussion, this is our understanding of your objectives for this project:
1. Establish Cryo-EM structure solution services for key therapeutic targets.
2. Establish relationship with expert Structural Biology CRO.

Please find the Milestone based costs and timelines estimations to help you with your project (SOW):

SERVICE	MILESTONE	DESCRIPTION	COST	TIMELINE
Grid Preparation	1	- Setup of dilution series and detergent additives - Grid preparation using Thermo Fisher Vitrobot	$ / Grid	2 - 3 weeks
Grid Screening	2	- Characterization of grid conditions and images - Determination of optimal target concentration - Determination of appropriate detergent/additive if required	$ / Hour 4-hour Min.	2 - 3 weeks
High-Res Data Collection	3	- High-Res Cryo-EM Data Collection on Thermo Scientific - Titan Krios G3i	$ / Hour	1 - 2 weeks
Raw Data Processing	4	- 2D Class averages of particle - Particle class distribution information - 3D class average map - Refined 3D maps of best class(es) - Globally Sharpened 3D Maps	$ / Data set	4 - 5 weeks
3D Model / Building	5	- Hand built / checked structure - Refined 3-D model and electron density maps	$ / Model	4 - 6 weeks

Estimated total cost for a well-working novel system: $ / Cryo-EM Structure
Estimated total cost for a ligand optimization structure: $ / Cryo-EM Structure

We're excited about working with _____ as this is our specialty and passion. If you have any questions or concerns let me know.

Sincerely,

Joshua Carter
CEO

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X-Ray Crystallography Services

X-RAY CRYSTALLOGRAPHY

3-D structures of proteins or other biological targets confirm the mechanism and engagement of the target, enabling the advancement and/or acceleration of R&D. Helix's crystallography expertise can provide 3-D structures for a variety of target types. These efforts have been acknowledged in recent publications in which high-resolution structures revealed the exact identity of a specific target.

EXPERTISE ENCOMPASSING:

- Kinases
- GPCRs
- DNA–RNA
- Nuclear receptors
- Docking
- Virtual libraries
- Fragment–based
- New active sites
- Peptidases
- Phosphatases
- Small molecules
- De-novo projects

GENE TO STRUCTURE DETERMINATION

1 Protein production

- Custom construct design
- Bacterial and Insect expression systems
- Purified to homogeneity by in-house protein scientists

2 High-throughput crystallization

- Screening of up to 3600 crystallization conditions
- Robotic automation allows for small protein volume
- Automated imaging of crystallization process
- Meticulously optimized crystallization conditions

3 3-D structure & analysis

- Weekly synchrotron access with no down time
- In-house automated software for accelerated data processing
- Structures are assembled and refined by our crystallographer specialists

PREVIOUS WORK

Structural antibodies

Helix co-crystallized, obtained high resolution X-ray diffraction, and computationally solved the 3-D structure of five antigen-binding fragments in complex with antagonists. Helix's work and expertise resulted in a 2019 publication in mAbs (journal) as well as the customer receiving "Two Fast Track Designations from the FDA" and $105M Series C Financing.

Challenging crystals

Helix obtained a high-resolution X-ray data set from small, non-single crystal samples. Helix X-ray data collection expertise and novel in-house data processing software produced two high resolution (1.7 and 2.3Å) co-crystal structures, resulting in a 2019 publication in *Structural Dynamics*.

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X-Ray Crystallography Services

Feasibility

1. Target mass/size

X-Ray Crystallography serves a wide range of protein sizes, even being amenable to protein complexes 150 kDa or above. While larger proteins certainly crystallize, the smaller, more globular proteins generally are more crystallizable due to their ability to pack in nice lattices (i.e., crystal packing).

2. Purity

Purity is the most critical variable for crystallization. Having aggregates or multiple species in solution (polydispersity) can lead to every crystallographer's nightmare... no crystals. Samples should be at 95% purity or above.

3. Solubility

Much like purity, the sample needs to be soluble upon crystallization setup. If the sample is aggregated before it touches the crystallization solution, the likelihood of it forming diffraction-quality crystals is close to zero.

4. Unstructured Regions

To increase a sample's ability to crystallize, it is many times necessary to truncate unstructured or flexible regions from the full-length molecule. These unstructured regions or flexible parts perturb the molecule's ability to pack in a crystal lattice.

For this reason, scrutinizing homology models in the PDB or predicted AlphaFold models is a primary tactic to increase success rates in crystallography.

Sample Requirements

1. Preferred protein concentration and volume

The standard "go-to" concentration for protein & nucleotide crystallography is 10 mg/mL; however, there are plenty of cases where the optimal concentration is less than or greater than 10 mg/ml. It is common for proteins to require 20, 50, or even 100 mg/mL concentrations before saturation levels are conducive for crystallization to occur.

Before setting up high throughput commercial screens, Helix uses a PCT (Pre-Crystallization Test) and a commercial PEG screen to analyze the concentration and determine whether it suffices for full commercial screening.

The volume of protein required depends on how many crystallization screens are being set up. For ten commercial screens + crystal optimization, 750 µl of the sample at crystallization concentration is required.

2. Sample buffer

The sample buffer should be as simple as possible to keep the sample happy and in solution (i.e., stable and soluble). pH, ionic strength, excipients, and additives should be refined to promote sample stability, solubility, and homogeneity.

It is important to include ionic strength, often in the form of NaCl, to keep the sample soluble and table. We recommend keeping the salt in the sample buffer 150mM or below to avoid salt crystal formation during commercial screening.

Additives or excipients, such as ligands, metals, detergents, sugars, etc., should be considered, especially if the inclusion of these in the sample buffer aids in sample stability, solubility, or homogeneity. These additives can also be added during the crystallization setup.

Reducing agents (i.e., BME, DTT, TCEP) may be included, but preferably at low concentrations that do not negatively impact the sample's ability to crystallize.

Glycerol or other cryo-preservatives should be avoided in the sample buffer if possible or dialyzed out before crystallization. These additives, especially 5% or greater, can inhibit the sample from crystallizing.

Protein Crystallography Milestone-based Project Workflow

Milestone 1: High-throughput Commercial Crystallization

Milestone 2: Crystal Optimization

Milestone 3: Synchrotron X-Ray Data Collection

Milestone 4: Atomic Structure Solution & Refinement

Crystallography Software

- AlphaFold – Construct design and homology model generation
- XDS/DIALS – Synchrotron X-Ray data processing
- AlphaHelix – Automated Structure Solution Pipeline
- CCP4 – Data Analysis, Molecular Replacement, Autobuilding, Refinement
- Phenix – Data Analysis, Molecular Replacement, Autobuilding, Ligand Fitting, Refinement, Structure Analysis

Instruments and Synchrotron Facilities

- ARI Gryphon LCP – Robotic setup of crystallization plates
- ARI CrysCam – Automated imaging of crystal plates
- Leica M205C – Crystallography Microscope
- Synchrotron Facilities – NSLS2, APS, CLS, PetraIII, ESRF, SOLEIL, ALBA

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APS Shutdown Concerns? *Helix has you Covered.*

- Helix maintains weekly beamtime, quite often, there are two collection days per week.
- Customers book beamtime through an online booking portal, reservation is simple and easy.
- Customers are free to pick from available slots, Helix pre-populates all the shipping labels required.
- Helix primarily collects in the U.S. (NSLS2 (AMX/FMX), APS); however, during NSLS2/APS maintenance, Helix does collect abroad (CLS, ESRF, Petra III, etc.)
- Sample information and diffraction characterization are communicated through secure online spreadsheets allowing customers to follow along “live” during data collection if desired.
- Our team has a combined data collection experience of 30 years, is rooted in the industry, and are experts with difficult samples that require special attention (helical, multi-point, mini beam, etc.)
- Helix processes results faster than other CROs using our in-house proprietary automation software.
- Helix serves many customers ranging from startups to Fortune 500s and everything in between.

Synchrotrons Available: *Helix has access to numerous beamlines providing constant access without interruption.*

SOURCE	LOCATION	# OF BEAMS	BEAMLINE
NSLS II	Brookhaven, New York	3	AMX, FMX, NYX
ESRF	Grenoble, France	6	ID30B, ID30A1, ID30A3, ID23-1, ID23-2, ID29
CLS	Saskatchewan, Canada	2	CMCF-08ID, CMCF-08BM
ALBA	Barcelona, Spain	1	BL13
SOLEIL	Saint-Aubin, France	2	PROXIMA1, PROXIMA2A
DESY	Hamburg, Germany	3	Petra III: P11, P13, P14
ANSTO	Sydney, Australia	2	MX1, MX2
APS*	Lemont, Illinois	15	14BM-C, 14ID-B, 17ID-B, 19DM-D, 19ID-D, 21ID-D, 21ID-F, 21ID-G, 22BM-D, 22ID-D, 23ID-B, 23ID-D, 24ID-C, 24ID-E, 31ID-D
ALS	Berkeley, California	8	BL 8.3.1, 5.0.1, 5.0.2, 5.0.3, 8.2.1, 8.2.2, 4.2.2, 2.0.1
DLS	Oxfordshire, England	3	TBD - Pending
SLS	Villigen, Switzerland	2	XO6SA, XO6DA
11	TOTAL	47	

Helix has partnered with an unparalleled number of synchrotrons.
*Expected shut down for 2023 - 2024

Note: If switching from ALS, it is important that you have both Unipucks and Spine caps since most modern robots here in the US and around the world only work with the Unipuck system and the pin caps must be Spine standard.

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X-Ray Crystallography Budget Estimate

Date:

To: _____
_____
_____

From: Joshua Carter
CEO
Helix BioStructures, LLC

RE: Budget Estimate Proposal for Crystallization Services – 'a la carte

Dear _____,

From our discussion, this is our understanding of your objectives for this project:
1. Establish crystallization conditions for key therapeutic targets.
2. Elucidate 3-D crystallographic structures of key therapeutic targets.
3. Establish relationship with expert protein crystallography CRO.

As discussed, these are costs and timelines estimations to help you with your project (scope of work):

SERVICE	DESCRIPTION	COST	TIMELINE
Crystallization Screening	- Provide crystallization screening/optimization of target using robotics - Observe crystallization screens to identify positive hits - Provide images of crystals and report on crystallization conditions / experiment setup	$ TBD / Screen	2 – 8 weeks (Crystal formation dependent)
Crystal Harvesting	- Harvest protein crystal for synchrotron data collection - Domestic Shipping = $750 International Shipping = $1500	$TBD / Crystal	1 day
Synchrotron X-Ray Data Collection	- Screen/collect harvested crystal samples - Diffraction characterization (.xls)	$TBD / Crystal	< 1 week
X-Ray Data Processing	- Fully processed data for full data collection(s) (.mtz) - Processing statistics (.xls)	$TBD / Data Set	1 day
Atomic Structure Solution & Refinement	- Molecular replacement of processed data sets - Hand built / checked structure - Fully refined final structure - Ligand docking / fitting (if applicable)	$TBD / Structure	2 – 3 weeks

We're excited about working with _____ as this is our specialty and passion. If you have any questions or concerns let me know.

Sincerely,

Joshua Carter
CEO

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helixbiostructures.com
info@helixbiostructures.com

X-Ray Crystallography Budget Estimate

Date:

To: _____
_____
_____

From: Matt Kelker, Ph.D.
V.P. of Research and Development
Helix BioStructures, LLC

RE: Budget Estimate Proposal for Protein Crystallization Services – Milestone Based

Dear _____,

From our discussion, this is our understanding of your objectives for this project:
1. Establish crystallization conditions for key therapeutic targets.
2. Elucidate 3-D crystallographic structures of key therapeutic targets.
3. Establish relationship with expert protein crystallography CRO.

As discussed, these are costs and timelines estimations to help you with your project (scope of work):

SERVICE	DESCRIPTION	COST	TIMELINE
Crystallization Screening	- Provide crystallization screening of target using robotics (10X Commercial Screens) - Observe crystallization screens to identify positive hits - Optimize crystal hits to obtain high quality crystals - (2X Optimization Screens) - Provide images of crystals and report on crystallization conditions / experiment setup	$TBD	2 – 8 weeks (Crystal formation dependent)
Crystal Harvesting & X-Ray Data Collection	- Harvest/ship protein crystals for synchrotron data collection - Screen/collect harvested crystal samples - Fully processed data for full data collection(s)	$TBD	1 week
Atomic Structure Solution & Refinement	- Molecular replacement of processed data sets - Hand built / checked structure - Fully refined final structure - Ligand docking / fitting (if applicable)	$TBD	4 - 5 weeks

We're excited about working with _____, as this is our specialty and passion. If you have any questions or concerns let me know.

Sincerely,

Matt Kelker, Ph.D.
V.P. of Research and Development

HELIX BIOSTRUCTURES
helixbiostructures.com
info@helixbiostructures.com

X-Ray Crystallography Budget Estimate

Date:

To: _____
_____
_____

From: Joshua Carter
CEO
Helix BioStructures, LLC

RE: Budget Estimate Proposal for Structure Solution Services – 'a la carte

Dear _____,

From our discussion, this is our understanding of your objectives for this project:
1. Elucidate 3-D crystallographic structures of key therapeutic targets.

As discussed, these are costs and timelines estimations to help you with your project (scope of work):

SERVICE	DESCRIPTION	COST	TIMELINE
Raw Data Processing	- Fully processed synchrotron data (*.mtz, *.log, etc). - Data Statistics from processed data sets (*.xls).	$ / Data Set	1 day
Molecular Replacement	- Complete molecular replacement model and electron density map (*.pdb, *.mtz). - Refined model and electron density map (*pdb, *.mtz). - Refinement statistics for each sample (*.xls)	$ / Data Set	1 day
Autobuild	- Refined model and electron density map (*.pdb, *.mtz) - Refinement statistics for each structure (*.xls)	$ / Structure	< 1 week
Ligand Fitting	- Fully processed data for full data collection(s) (.mtz) - Processing statistics (.xls)	$ / Structure	1 day
Customer Structure Checklist	- 30-40 manual checks/actions customized to the Customer's needs (i.e. small stretches of residues to check, naming of chains/ligands, water placement, sequence check, etc). - Checklist table with Helix observations (*.xls).	$ / Structure	1 - 2 days
Manual Structure Check	- 2-3 manual step-throughs of the entire model - Refined model and electron density map (*.pdb, *.mtz) - Refinement statistics for each structure (*.xls)	$ / Structure	3 - 4 days
Atomic Structure Solution & PDB Refinement	- Hand built / checked structure - Fully refined final structure (PDB Quality) - Ligand docking / fitting (if applicable)	$ / Structure	2 - 3 weeks

We're excited about working with _____ as this is our specialty and passion. If you have any questions or concerns let me know.

Sincerely,

Joshua Carter
CEO

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X-Ray Crystallography Budget Estimate

Date:

To: _____
_____
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From: Joshua Carter
CEO
Helix BioStructures, LLC

RE: Budget Estimate Proposal for X-Ray Data Collection Services

Dear _____,

From our discussion, this is our understanding of your objectives for this project:
1. Establish on-demand X-Ray data collection services for internal protein crystal samples.
2. Establish relationship with expert protein crystallography CRO.

As discussed, these are costs and timelines estimations to help you with your project (scope of work):

SERVICE	DESCRIPTION	COST	TIMELINE
Synchrotron X-Ray Data Collection	- Screen/collect harvested crystal samples at premier synchrotron - If collectable resolution, ≥200-degree data set - Diffraction characterization (.xls) - AWS S3 secure data storage and transfer	$ / Crystal	< 1 week
Crystal Harvesting	- Fully processed data for full data collection(s) (.mtz) - Processing statistics (.xls)	$ / Data Set	1 day

We're excited about working with the _____ as this is our specialty and passion. If you have any questions or concerns let me know.

Sincerely,

Joshua Carter
CEO