Matterworks™

APPLICATION NOTE

High-throughput metabolic screening for therapy candidate selection

Rapid experimental triage of novel AI-assisted engineered GLP-1 and incretin mimetics

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Challenge: experimental testing of AI-assisted therapy design

Generative AI empowers drug designers to reimagine protein therapeutics and engineer optimal pharmacological properties.

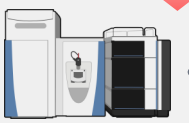
How do you efficiently and cost-effectively screen lead candidates to experimentally evaluate pharmacological properties, target activation, and toxicity?

Design candidates with improved drug properties using AI

AI-powered in vitro metabolic screening

Cellular metabolism provides an analytical roadmap for investigating drug-dependent perturbations and assessing efficacy signals.

Leveraging a proprietary Large Spectral Model (LSM), Pyxis rapidly and accurately converts raw mass spectrometry (MS) data into actionable metabolic insights regarding therapies, targets, and off-targets.



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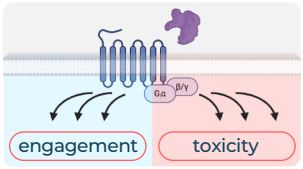
Rapid in vitro metabolic screening of synthesized candidates

Application: incretin receptor agonists

Peptides targeting pancreatic incretin pathways, including glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) receptor agonists are revolutionizing obesity and diabetes care, yet stability, multiple target activation, and side effect deficiencies limit their potential.

Scientists at 310.ai designed a suite of incretin mimetics engineered to reduce dosing frequency using their MP4 generative AI protein model. The performance of a half-life extension (HLE) incretin mimetic designed to agonize multiple receptors was evaluated against control peptides in a pancreatic cell model, with Pyxis-powered MS metabolite data providing the readout.

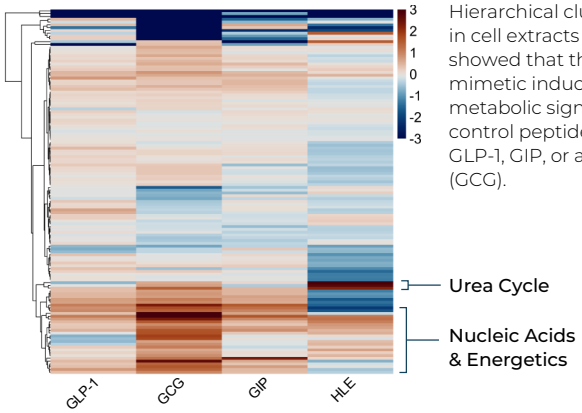


Multiple metabolic pathways inform on target engagement (efficacy) and cell health (safety)

One-day functional analysis reveals multiple signatures of drug action

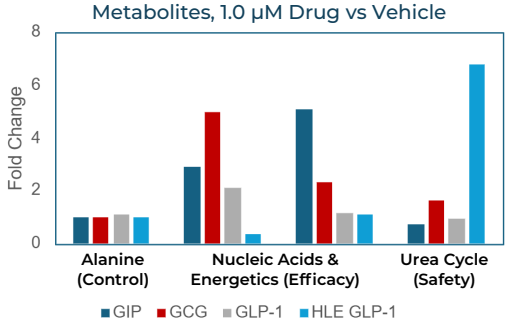
Pyxis reported confident metabolite identities and concentrations for 135 biochemicals in cell extracts and 65 biochemicals in supernatants within 15 minutes of uploading LC-MS data. Broad coverage of cellular metabolic pathways provided multiple downstream assessments of receptor activation and cell health. The HLE mimetic metabolic signature deviated from that of other incretin RAs, including in pathways indicative of cell toxicity. These signatures suggested that targeting multiple incretin receptors triggered unanticipated pathway activations. Discontinuation of the candidate as a lead was recommended, which was corroborated by standard assays conducted at a CRO.

Cell Extracts, 1µM Drug vs Vehicle



Hierarchical clustering analysis in cell extracts immediately showed that the HLE novel mimetic induced a distinct metabolic signature from control peptide mimetics GLP-1, GIP, or a dual GLP-1+GIP (GCG).

Metabolites, 1.0 µM Drug vs Vehicle



HLE failed to induce the expected changes indicative of robust target engagement (e.g., nucleic acid and energetic compounds), while simultaneously increasing the concentrations of cell toxicity markers (e.g., urea cycle).

Experimental details: AI design meets AI in vitro screening

The 310.ai team engineered their incretin mimetic de novo with structural properties to address drug stability and metabolism deficiencies, thereby extending its bioavailability and reducing dosing frequency.

Incretin mimetics were applied to a pancreatic adenocarcinoma cell line (CFPAC-1) at concentrations of 0.1 µM and 1.0 µM and incubated overnight. Cell extracts and supernatants were prepared and analyzed by LC-MS using the standard Pyxis method. Briefly, analytes were separated using a Waters Atlantis Premier BEH Z-HILIC column with a 6.5-minute gradient. MS1 spectra were acquired in positive-negative switching mode on a ThermoFisher Scientific Orbitrap Exploris™ 120 mass spectrometer. Pyxis returned confident metabolite identities and concentrations in minutes (detecting 135 compounds in cell extracts and 65 compounds in supernatants).

These data served as the basis for the functional characterization of various incretin mimetics, including those targeting multiple receptors.

Scale pass or fail with metabolic endpoints

Metabolic screening with Pyxis transforms routine LC-MS workflows into comprehensive, simultaneous assessments of cellular efficacy and safety endpoints. Overall, 96 conditions can be processed with data retrieval in 12 hours, providing a cost-effective, efficient, and high-throughput method of identifying top candidates for further in vitro validation. Notably, accurate metabolite concentrations enable the benchmarking of results across multiple studies, so you can leverage all your data to iteratively refine lead candidates.

Integrate metabolic screening into your preclinical pipeline to evaluate:

- Therapies incorporating multiple mechanisms of action
- Lead candidates & controls
- Cell lines & organoids
- Drug concentrations & combinations
- Functional genetics & genetic variants

Inquiries

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