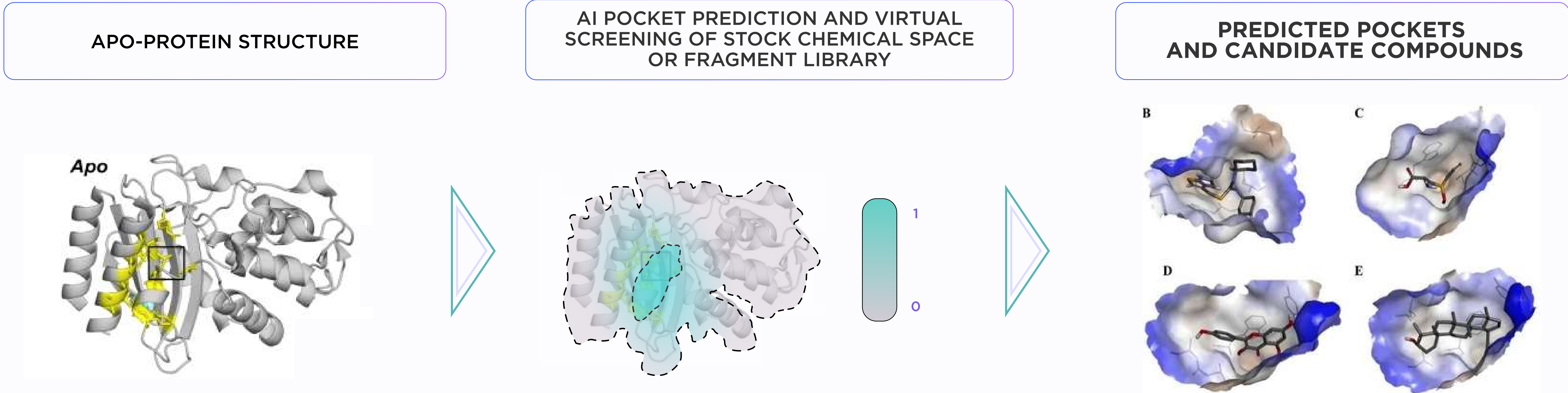# AI-POWERED WORKFLOW FOR CRYPTIC POCKET PREDICTION

The workflow of targeting the cryptic pockets consists of three stages:
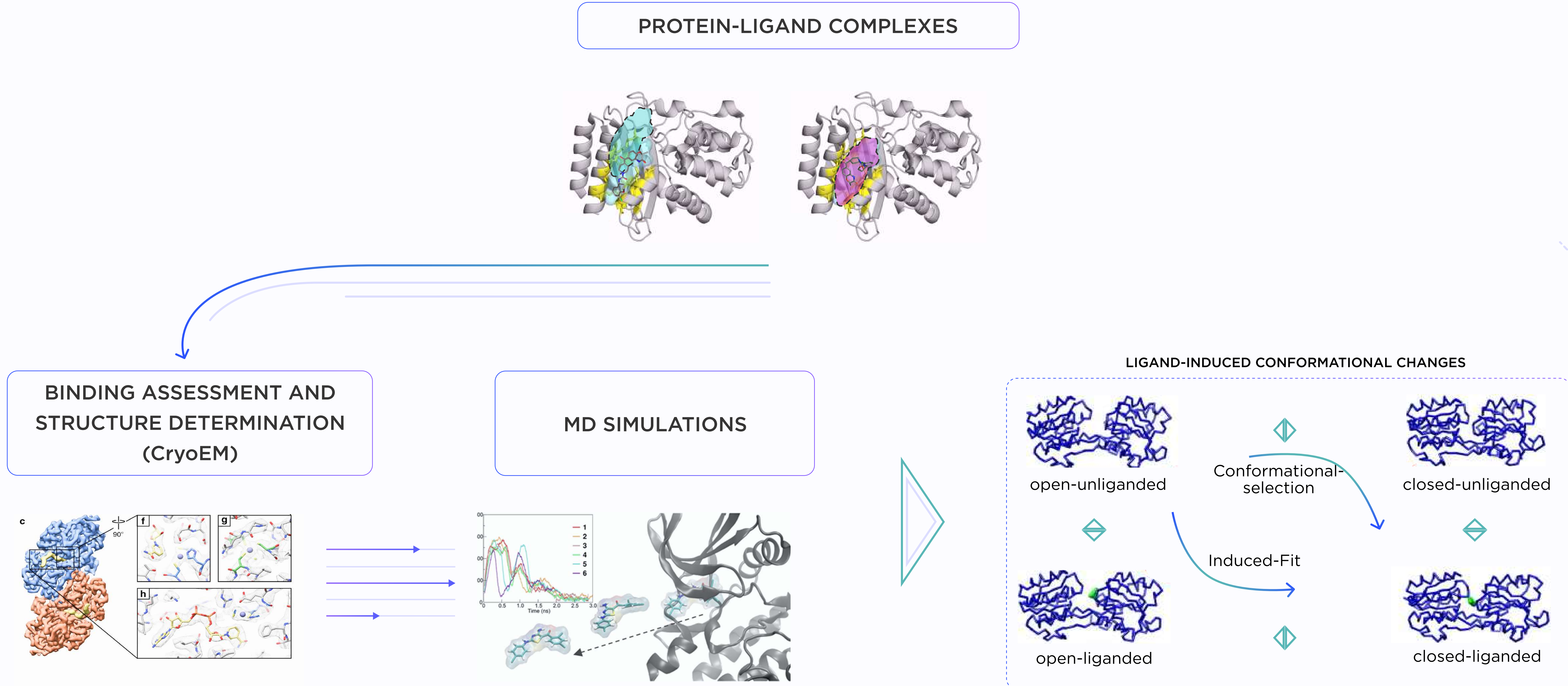
1. BOOTSTRAPPING

In the first "bootstrapping" stage the conformational changes in the target protein are induced to reveal the cryptic pockets and make them accessible to the drugs.

APO-PROTEIN STRUCTURE	AI POCKET PREDICTION AND VIRTUAL SCREENING OF STOCK CHEMICAL SPACE OR FRAGMENT LIBRARY	PREDICTED POCKETS AND CANDIDATE COMPOUNDS

These molecules could either bind to the cryptic pockets themselves stabilizing them in open conformation or bind to the remote allosteric sites which induce corresponding changes in protein conformation or dynamics. Bootstrapping binders are chosen from the stock chemical spaces or readily available fragment libraries, which makes them cheap and fast to deliver.

2. CRYPTIC POCKET DISCOVERY

In the second stage the protein complexes with the best predicted bootstrapping binders are assessed by molecular simulations and the high throughput structure determination (CryoEM).

PROTEIN-LIGAND COMPLEXES

| BINDING ASSESSMENT AND STRUCTURE DETERMINATION (CryoEM)	MD SIMULATIONS	LIGAND-INDUCED CONFORMATIONAL CHANGES

This allows to elucidate the conformational changes, induced by the binders and to generate an ensemble of representative protein conformations, which contain open and accessible cryptic pockets. This ensemble is then passed through AI-based pocket prediction and tentative cryptic pockets are identified and annotated.

The production virtual screening using the full power of the Receptor.AI platform is performed against each of the tentative pockets and the candidate compounds are selected from the large and diverse combinatorial chemical space.

FREE ENERGY (G)

AI VIRTUAL SCREENING	TENTATIVE CRYPTIC POCKETS	AI POCKET PREDICTION

CANDIDATE COMPOUNDS
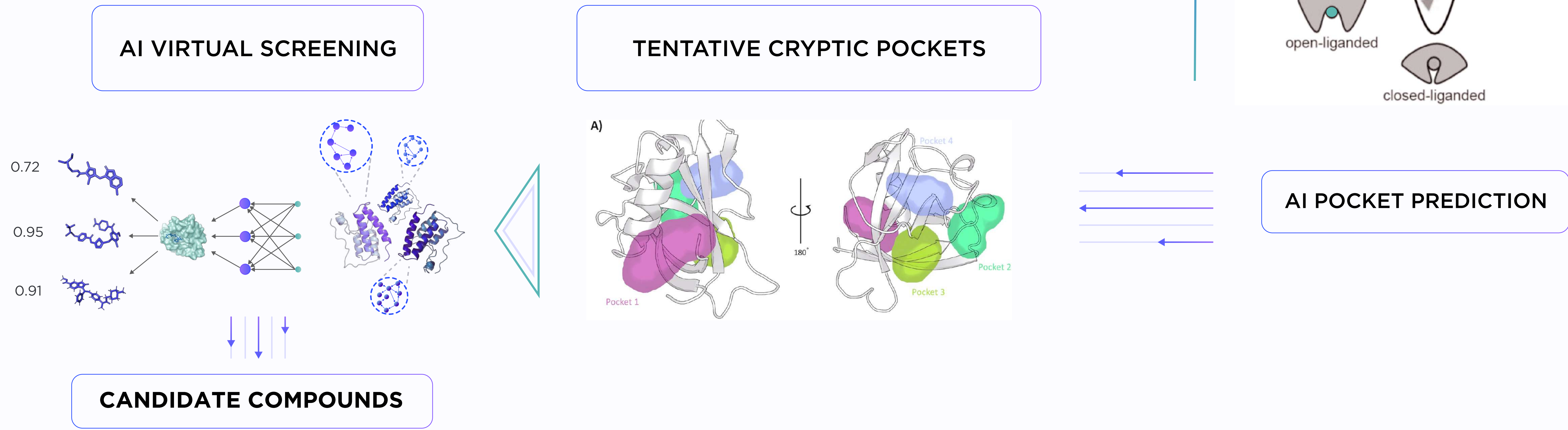
3. CRYPTIC POCKET CONFIRMATION

CANDIDATE COMPOUNDS	PROTEIN-LIGAND COMPLEXES
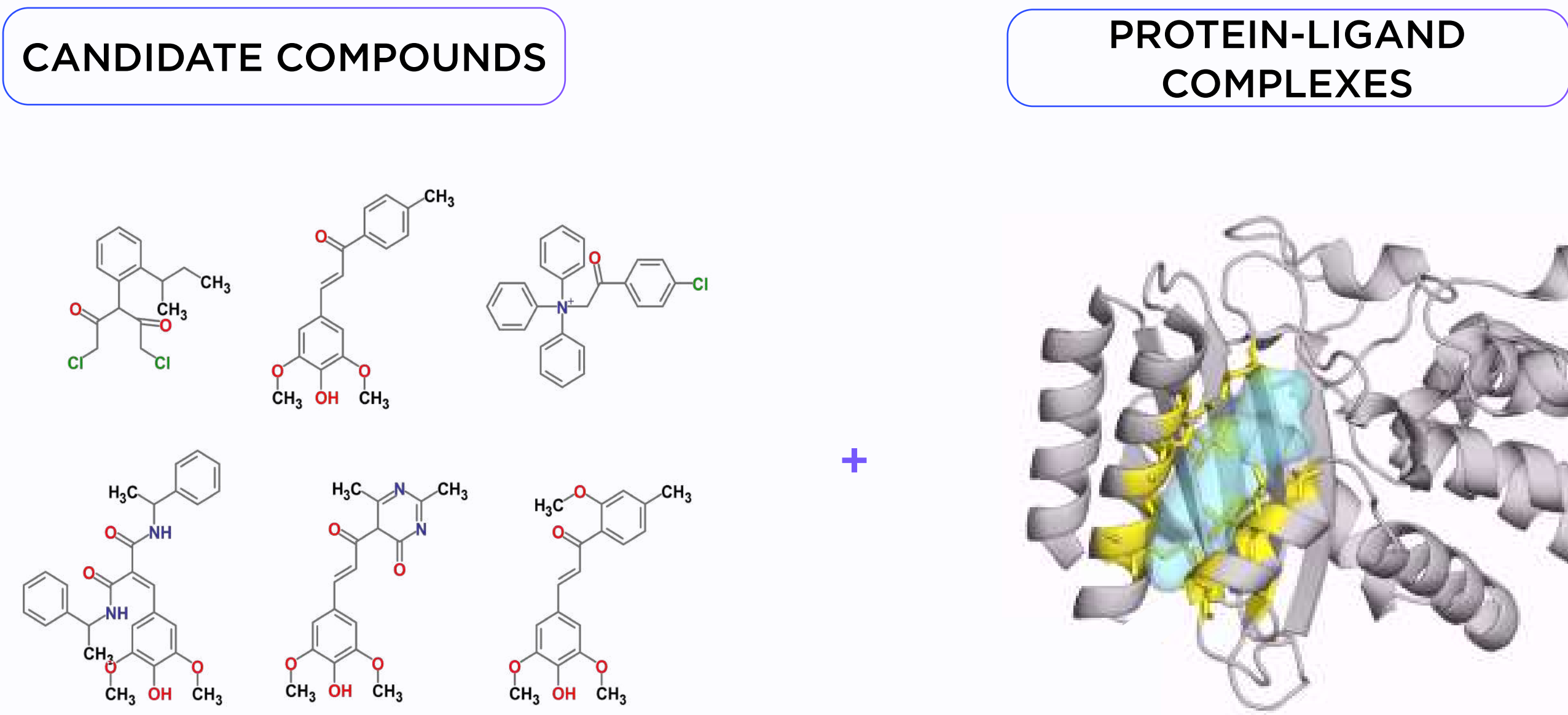
The third stage starts from validation of the candidate compounds' biological activity. The protein complexes with the active compounds are subject to another round of structure determination to confirm their binding to predicted cryptic pockets and to obtain additional data about binding mechanisms.

Confirmed compounds are designated as hits and are passed to the lead discovery and lead optimization stages using the standard Receptor.AI workflow.

BIOLOGICAL VALIDATION OF ACTIVITY	STRUCTURE DETERMINATION CryoEM	CONFIRMED CRYPTIC POCKETS	HIT-TO-LEAD DISCOVERY