SpILLO
TARGETS THAT MATTER

Member
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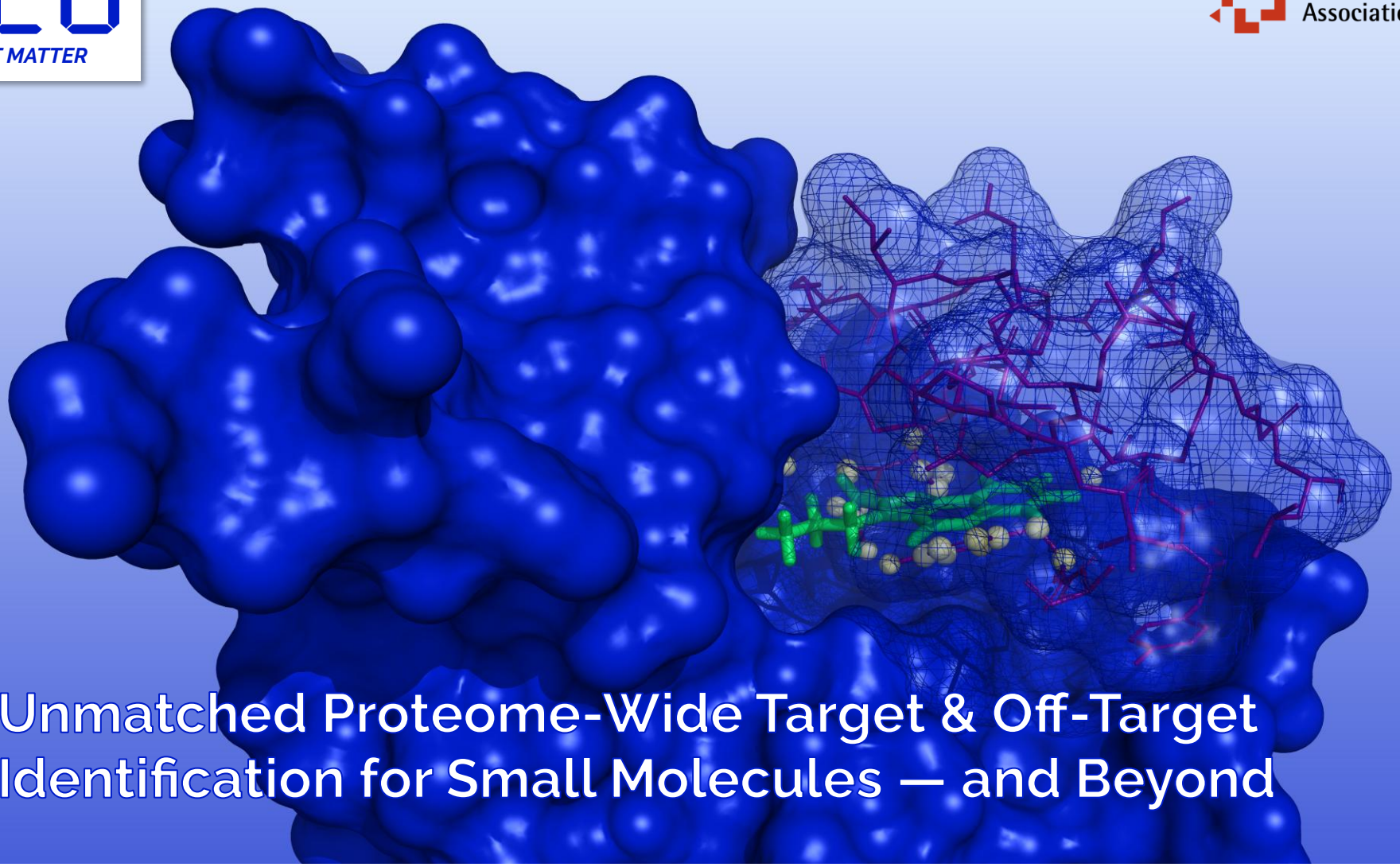

✅ Unmatched Proteome-Wide Target & Off-Target Identification for Small Molecules — and Beyond

High-Value, Experimentally Validated Solutions for Drug Development

— Physics-Based, Unbiased, and Trusted for Over a Decade —

Free from the Limitations of Current AI

Version: May 2025

SPILLO creates strategic value through unbiased, molecular-scale analysis — delivering critical advantages across the drug development pipeline.

Based in Switzerland, SPILLO is a science-driven company offering exclusive in silico services powered by proprietary, rigorously validated, **physics-based technology**. We support pharmaceutical, biotech, academic, and public institutions worldwide.

SPILLO analyzes the **entire 3D structural proteome** to identify:

- **"Target Proteins"**
- **"Off-Target Proteins"**

for small molecules and selected modalities, across advanced stages of drug development — from preclinical to post-market.

This is crucial to predict and understand:

- **"Therapeutic Effects"**
- **"Adverse Drug Reactions (ADRs)"**

and to unlock:

- **"Drug Rescue"**
- **"Drug Repurposing"**
- **"Drug Repositioning"**

— all at the **biomolecular mechanism level**.

SPILLO empowers critical decision-making across the drug development pipeline — reducing risk and revealing new opportunities.

- **Reduce development risk and late-stage attrition**
  through early, **proteome-wide** insight into off-target liabilities and ADRs.
- **Support better go/no-go decisions**
  with unbiased, quantitative selectivity profiles for each molecule.
- **Increase asset value and strengthen confidence**
  during investment and licensing — providing critical support for accurate asset assessment in due diligence.
- **Unlock rescue and repurposing opportunities — and strategic IP**
  by discovering novel targets and mechanisms, even for shelved or off-patent compounds — enabling faster approval and longer patent value.
- **Enable smarter trial design and patient stratification**
  by anticipating biomolecular causes of adverse effects.

These strategic benefits show how SPILLO supports better decisions — from early discovery to post-market optimization.

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The Problem — A Critical, Long-Standing Challenge Still Unsolved

Why it still matters

- Despite decades of research, determining which proteins a molecule truly interacts with — and how — remains an unsolved challenge, even for many approved drugs.
- In many cases, the molecular origins of adverse — and sometimes even therapeutic — effects are not fully understood.
- Among other consequences, this lack of molecular-level understanding contributes to high attrition rates and prevents full exploitation of the therapeutic and commercial potential of compounds — while also, and most critically, increasing risks to patients.

Experimental limits and in silico potential

- Experimental methods cannot scale to the full proteome.
- In silico methods are the only practical alternative, with structure-based approaches being the most direct and informative — despite important limitations.

The Problem — Why It Remains Unmanageable

Two key factors make this challenge truly unmanageable:

- **Flexibility** — Proteins are dynamic systems, yet experimental (or predicted) structures typically capture only one or a few conformations — static snapshots where relevant **binding sites** may appear **closed, distorted, or inaccessible**. Even when a ligand is present, the binding site conformation may not match the one relevant to the compound of interest. Modeling protein **flexibility is therefore essential to detect real interactions** with active compounds.

- **Scale** — The number of protein structures to analyze is large — tens of thousands for the human proteome alone, and even more when including model organisms such as mouse and rat, essential for preclinical research and cross-species translational insight.

The Problem — Why Existing Methods Fall Short

Efficient screening requires not only accuracy, but also speed and scalability — a combination that no current methods can achieve.

The main limitations of existing in silico methods are summarized below.

- **Molecular Dynamics** — The gold standard for modeling flexibility, but still far **too computationally intensive** for proteome-wide use — even in its enhanced sampling or accelerated forms.

- **Reverse Molecular Docking** and related approaches — Fast and scalable, but typically rely on static structures or model flexibility only to a limited extent — and therefore fail to capture large-scale conformational changes. They often **miss binding sites that are distorted, hidden, occupied, or ligand-induced** — unless already in an **ideal conformation** (**which is rare**). Even when a site is found, these methods struggle to assess if it represents a true binder (target or off-target), due to persistent limitations in binding affinity prediction — especially across structurally diverse proteins and ligands. Consequently, they remain unsuitable for proteome-wide use, with low accuracy and without **large-scale validation**.

- Current **AI-based methods** — While promising, and with some incorporating protein flexibility, they remain **limited by the quality and bias of their training data**. Performance often drops significantly when applied outside the training distribution — particularly for ligands or proteins that are underrepresented. Moreover, these models often function as **black boxes**, limiting interpretability and trust.

Accurately uncovering targets and off-targets across the proteome remains a major challenge — still unsolved by current computational approaches.

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A Unique Solution — Rooted in Science, Refined Through Time

SPILLO was specifically designed to overcome this long-standing challenge, with a unique, physics-based technology that captures system-level flexibility while remaining fast enough for proteome-wide scale.

A Solid Scientific Foundation

- **SPILLO's unique capabilities were first described in a peer-reviewed study** [A. Di Domizio et al., *J. Comp. Chem.*, 2014], and have since evolved well beyond their original formulation — not only through continuous refinement, but also through entirely newly conceived key functionalities that have profoundly transformed the platform.
- These are based on an innovative implementation of core principles of **statistical thermodynamics**, enabling SPILLO to operate as a powerful and flexible platform for **proteome-wide**, **unbiased**, **structure-based analysis**.
- The platform is under continuous development to further enhance **performance** and broaden applicability across diverse **molecular types and biological systems**.

SPILLO Sees What Others Miss

- SPILLO identifies targets and off-targets through a **systematic, unbiased analysis of both protein surface and internal regions**, aiming to detect binding sites for the compound of interest.
- Importantly, it incorporates a level of protein **flexibility far beyond that modeled by other tools** (e.g., flexible docking) — allowing it to explore a wider **conformational space** and capture sites not yet formed or visible in the available structures.
- **This unique ability** — illustrated in the following slides and supported by the reference publication — **enables accurate target and off-target detection across the proteome**.
- **Unlike other structure-based tools**, which are not suitable for proteome-wide blind searches, SPILLO combines **extensive conformational insight** with **the performance needed for large-scale application**.

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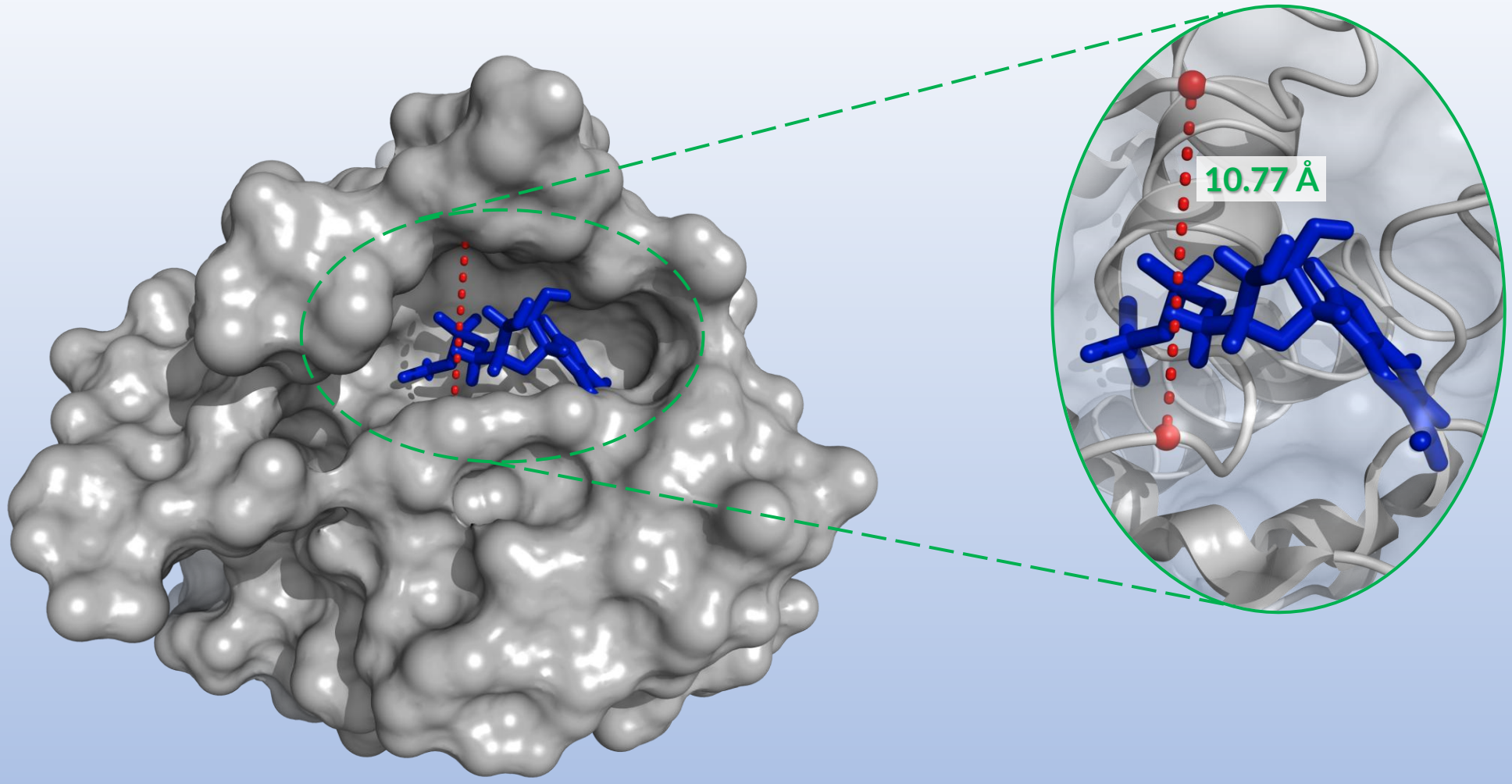
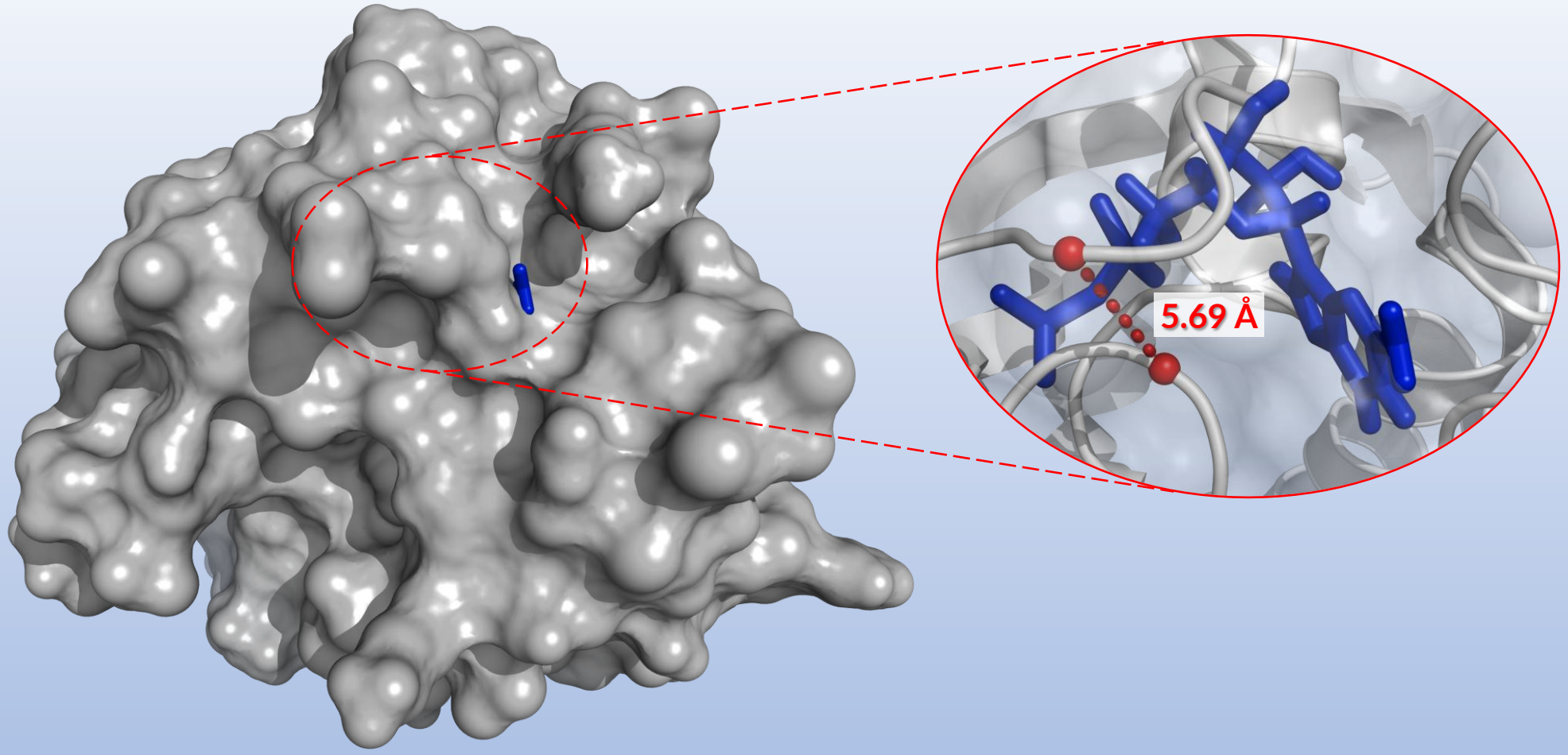
The Ideal Binding Site — Open, Clear, and Detectable by All Tools



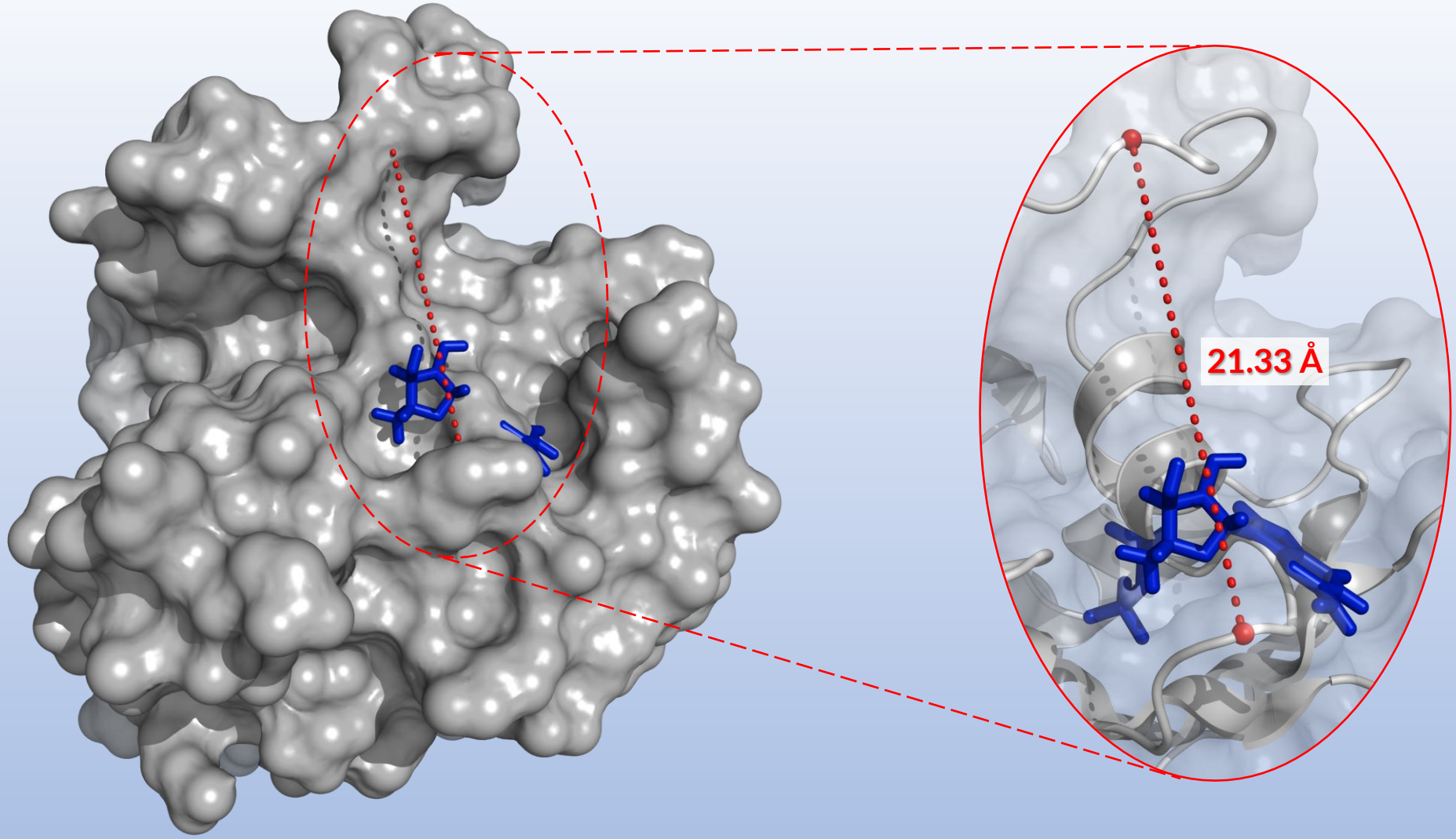
- Example of a PROPERLY OPEN binding site — the cavity is present and clearly accessible.
- Opening: 10.77 Å.
- Representative of an ideal case — easily DETECTABLE by any tool

SPILLO Detects What Others Miss — Fully Closed Binding Site



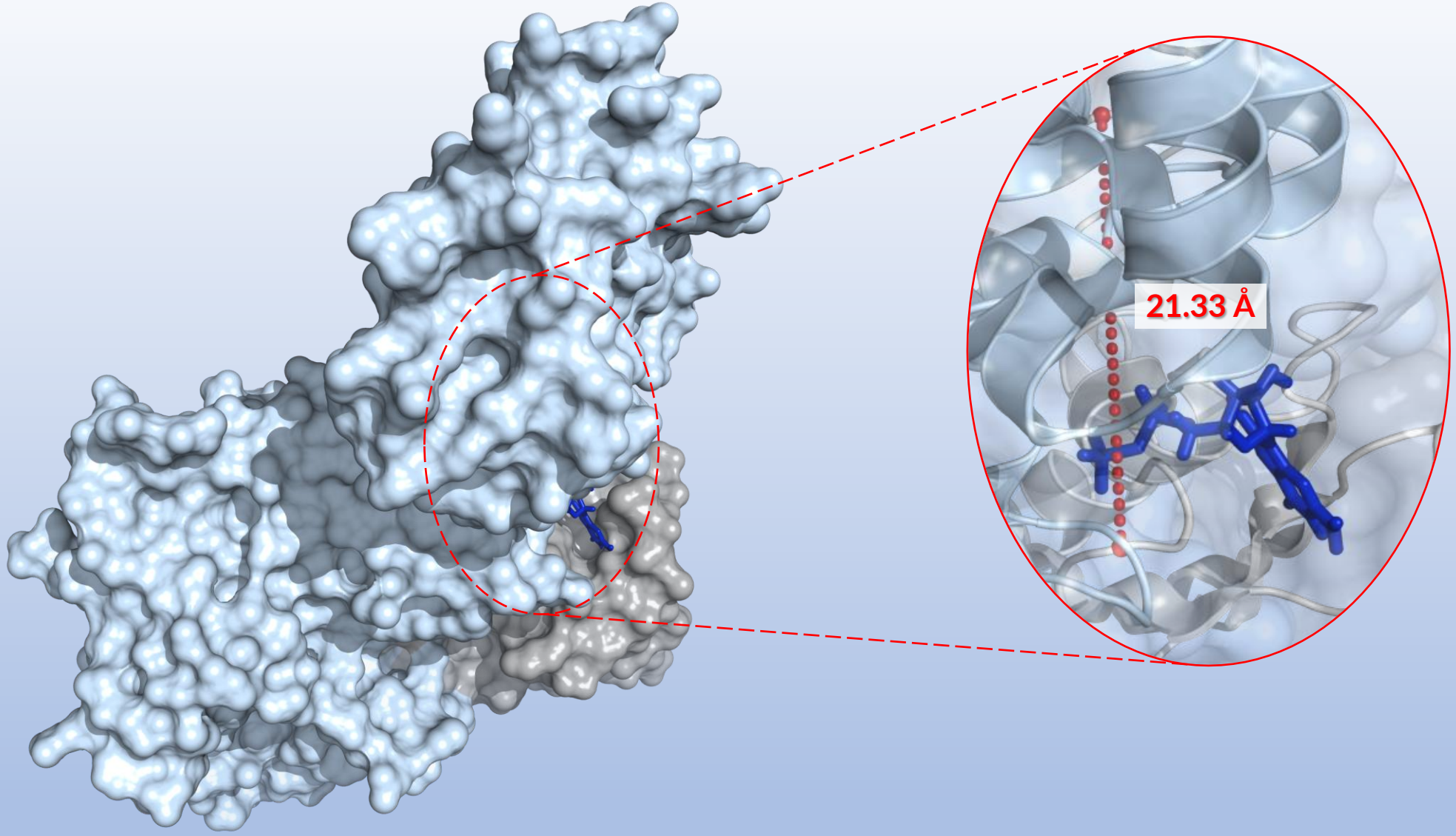
- Example of a FULLY CLOSED binding site — no actual cavity is present.
- Opening: 5.69 Å — nearly half the size of the properly open conformation (10.77 Å).
- NOT DETECTABLE by other tools.
- CORRECTLY DETECTED by SPILLO — despite multiple steric clashes.

SPILLO Detects What Others Miss — Too Open and Structurally Distorted Binding Site



- Example of a binding site that is EXCESSIVELY OPEN and DISTORTED — no actual cavity is present.
- Opening: 21.33 Å — nearly twice as wide as the properly open conformation (10.77 Å).
- NOT DETECTABLE by other tools.
- CORRECTLY DETECTED by SPILLO — despite multiple steric clashes.

SPILLO Detects What Others Miss — Too Open, Distorted, and Occupied



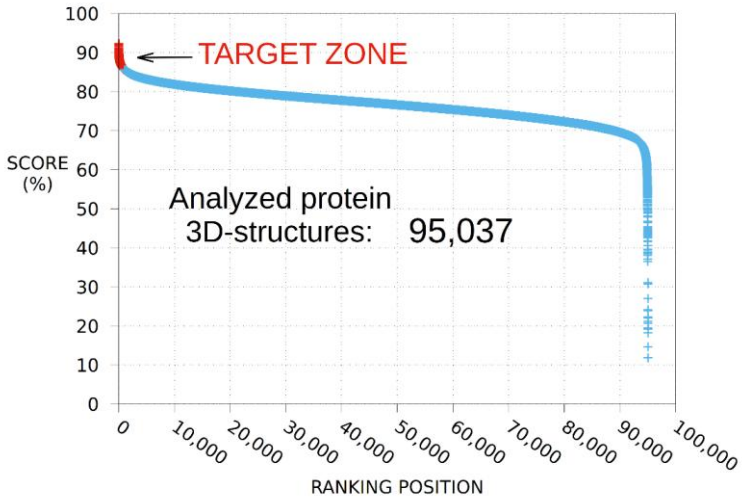
- Example of an EXCESSIVELY OPEN and DISTORTED binding site, OCCUPIED by another protein — no actual cavity is present.
- Opening: 21.33 Å — nearly twice as wide as the properly open conformation (10.77 Å).
- NOT DETECTABLE by other tools.
- CORRECTLY DETECTED by SPILLO — despite multiple steric clashes.

Targets and Off-Targets Emergence — The Typical SPILLO Output

How SPILLO Ranks the Proteome

SPILLO computes a quantitative score for each protein in the structural proteome, reflecting its propensity to act as a target or off-target for the compound of interest.

- SPILLO's output typically follows a **distinctive pattern**: a small number of proteins display **markedly higher scores**, standing out from the broad, low-score background of non-targets.
- This top-scoring region — the **Target Zone** (in red) — emerges naturally, allowing the prioritization of targets and off-targets **without arbitrary thresholds**, and relying entirely on the **intrinsic quality of their binding sites**.
- This behavior is key to enabling **reliable, large-scale, proteome-wide applications**.



From Prediction to Insight and Design

Beyond scoring, SPILLO provides **detailed structural information** for each identified interaction — including the **binding site position** and the **orientation of the compound** within it.

These insights provide a strong foundation for:

- Proposing **mechanistic hypotheses** based on the identified interactions.
- Supporting **structure-based drug design** and compound optimization.
- Enabling **more rational preclinical study design, including animal testing, through a Multilevel Cross-Organism Transferability Analysis (MCOTA)** — e.g., from *Homo sapiens* to various model organisms, or vice versa — where the comparison extends all the way down to binding-site-level resolution across organism-specific target proteins *(see Finasteride study in the next section)*.

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Key Differentiators

SPILLO combines physics-based modeling with structural proteomics and delivers capabilities unmatched by conventional and AI-based tools — grounded in scientific rigor.

- **Enables physics-based discovery**
  Unbiased, systematic search — no AI, no reliance on data quality, no black boxes

- **Handles protein FLEXIBILITY — sees what others miss**
  Detects even the most elusive targets and off-targets by uncovering binding sites in both accessible and challenging conformations — including distorted, hidden, or buried sites — **typically missed by conventional or AI methods**.

- **Scales to the entire proteome**
  Enables target and off-target discovery across the **entire structural proteome** of *Homo sapiens* and other organisms

- **Proven and published**
  Continuously improved since 2014 — with **multiple** peer-reviewed publications and **independent experimental validations**

- **Delivered by experts**
  Delivered through SPILLO's expert-led services — delivering clear, consistent, and scientifically grounded insights

These strengths set SPILLO apart — scientifically, strategically, and operationally.

SPILLO's unique approach to protein flexibility is what enables it to detect critical binding sites missed by other methods.

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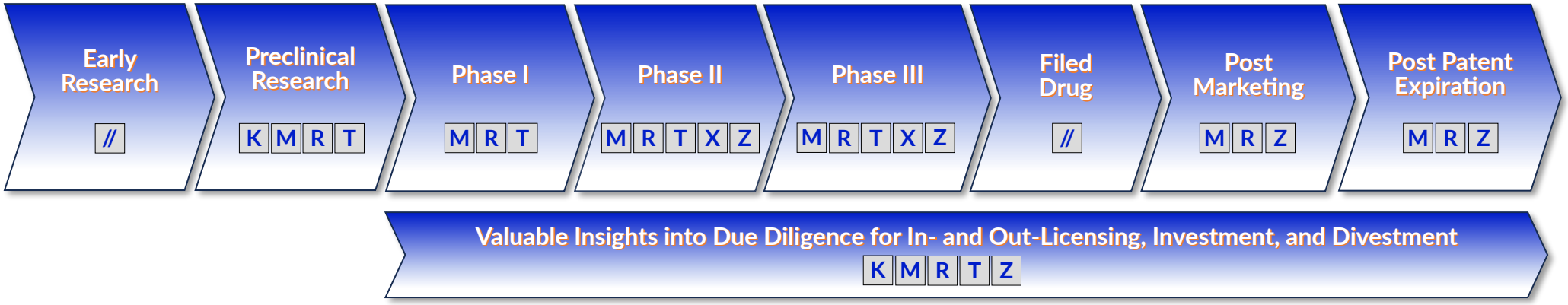
SPILLO R&D Services Across the Pipeline



K	Strategic Prioritization of Molecules by Selectivity Profile
M	Identifying Therapeutic Targets to Clarify Mechanisms and Unlock Asset Value
R	Finding Off-Targets Behind Known Adverse Drug Reactions (ADRs) to Enhance Drug Safety and Use
T	Cutting Attrition by Finding Off-Targets Behind Future Adverse Drug Reactions (ADRs)
X	DRUG RESCUE: Unlocking Unexpected ROI from Shelved Drug Assets
Z	DRUG REPURPOSING: Unlocking Strategic Value from On-, Late-, and Off-Patent Molecules

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K	Strategic Prioritization of Molecules by Selectivity Profile

Key Challenges

- It is often necessary to select, from a pool of active molecules, only a subset to advance to later stages of drug development.
- Making the right choice at this early stage is critical, as advancing suboptimal candidates may result in downstream failures with significant financial and time-related consequences.
- From a safety standpoint, selectivity is a key criterion — both in terms of the number of off-targets and their potential harmfulness.
- The challenge lies in being able to account for molecular selectivity — by reliably predicting it — in order to reduce the likelihood of downstream safety issues and avoid wasted time and resources.

Services

- For each active molecule in the pool, the number of off-target interactions is estimated through an unbiased, systematic analysis at the proteomic scale.
- Molecules are then ranked according to their selectivity, defined as negatively correlated with the number of predicted off-targets.
- When relevant, the identities of off-target proteins can be retrieved to assess potential safety concerns based on their biological roles.
- Structural insights can also be provided to support the understanding of mechanisms underlying adverse effects or to guide rational drug design strategies aimed at reducing undesired interactions.

Technical Advantages

- Provides an unbiased, physics-based criterion to assess molecular selectivity at the proteomic scale — supporting decisions on which compounds to advance or discard within a pool of active molecules.
- Enables early identification of potential off-targets, allowing for preliminary safety assessment based on their biological relevance and, when needed, structural analysis of the interaction.
- When appropriate, supports rational optimization of high-priority compounds by leveraging structural insights to reduce undesired off-target interactions through structure-based design.

Value Proposition

- Supports informed decision about which active compounds to advance or discard, based on a quantitative selectivity profile at the proteomic scale.
- Helps reduce safety-related failures and avoid costly downstream setbacks.
- SPILLO's technology detects off-targets often missed by conventional methods — enabling a more reliable selectivity profile for strategic decision-making.
- Also highly valuable during technical due diligence for **in-/out-licensing**, **investment**, or **divestment** — enabling more accurate assessment of asset quality and associated risk.

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M — Identifying Therapeutic Targets to Clarify Mechanisms and Unlock Asset Value

Key Challenges

- It is not unusual for a compound to show therapeutic efficacy while its biomolecular mechanism remains only partially understood — or even entirely unknown.
- This can occur when the effect is not due to the expected target or mechanism, or when the compound shows unexpected activity in another context, with insufficient data to explain it.
- As a result, development may stall, and the asset's value may remain underexploited — requiring costly and time-consuming efforts to uncover the molecular origin of the beneficial effect.
- The key challenge lies in efficiently discovering the responsible targets, binding sites, and mechanisms.

Services

- For the compound of interest, potential target proteins are systematically and unbiasedly searched across the available structural proteome of *Homo sapiens* and relevant model organisms.
- The most likely target proteins responsible for the observed therapeutic effect(s) are identified, together with their corresponding binding sites.
- Structural data can be provided to identify key interactions that support a mechanistic understanding of the observed effects.

Technical Advantages

- Provides a detailed target map for the compound, offering valuable insights into the biomolecular origins of its known therapeutic effects.
- Enables more effective planning of the compound's subsequent development steps.
- Supports rational drug design to improve affinity for the newly identified target(s).
- Facilitates optimized use of the compound within precision medicine strategies.

Value Proposition

- Unlocks new strategic and IP opportunities through clarified molecular interactions and mechanisms.
- Increases asset value for out-licensing and divestment, and supports informed evaluations during due diligence for in-licensing and investment.
- Improves trial design and strengthens regulatory submissions, increasing the likelihood of approval.
- Enables more precise therapeutic use, aligned with precision medicine.
- This, like other services powered by SPILLO, is based on a unique structure-based approach that works without prior data (unlike AI) and enables scalable proteome-wide analysis — detecting targets missed by conventional methods.
- SPILLO's results on challenges relevant to this service have been independently validated and published in peer-reviewed journals (see next section).

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R	Finding Off-Targets Behind Known Adverse Drug Reactions (ADRs) to Enhance Drug Safety and Use

Key Challenges

- Even well-characterized compounds may cause ADRs whose underlying biomolecular mechanisms are still unclear or unknown.
- Such effects may emerge during preclinical studies, clinical trials, or post-approval use — even for off-patent drugs — and can be common or rare, immediate or delayed, and variable across patient populations.
- While therapeutic effects typically rely on strong target engagement, ADRs may arise from weak off-target interactions, making them even harder to interpret mechanistically.
- The challenge is to discover the off-targets and binding sites responsible for the ADRs, to clarify mechanisms and guide redesign, mitigation, or safer therapeutic use.

Services

- For the compound of interest, potential off-target proteins are systematically and unbiasedly searched across the available structural proteome of *Homo sapiens* and relevant model organisms.
- The top-ranked off-targets potentially responsible for the observed ADRs are identified, together with their corresponding binding sites.
- Structural data can be provided to identify key interactions underlying the specific adverse effects under investigation.

Technical Advantages

- Enables a rational explanation for observed adverse effects.
- SPILLO's structural insights enable rational modifications to reduce off-target affinity while preserving binding affinity to therapeutic targets.
- The generated knowledge helps identify ADR risk in different patients and supports personalized treatment decisions for the drug being considered — supporting precision medicine.
- Provides a basis for developing strategies to mitigate adverse effects in affected patients — enabling access to a safer, more tolerable treatment.

Value Proposition

- Provides concrete, safety-related molecular insights to guide key decisions in drug development.
- Enables further development of the molecule through focused structural modifications that enhance safety.
- Enhances the value and responsible use of existing drugs — improving patient outcomes and satisfaction, and ultimately benefiting the company.
- SPILLO offers unique capabilities through unbiased, proteome-wide searches that include protein flexibility — as shown in peer-reviewed studies, where it was the first to uncover off-target ADR mechanisms in widely used, off-patent drugs, revealing insights that had remained elusive for decades.

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SPILLO R&D Services Across the Pipeline

T	Cutting Attrition by Finding Off-Targets Behind Future Adverse Drug Reactions (ADRs)

Key Challenges

- An active compound may cause Adverse Drug Reactions (ADRs) that only emerge during clinical trials or post-marketing surveillance, escaping standard preclinical toxicity studies.
- Rare but serious ADRs may appear only after widespread use in large patient populations.
- Insufficient early analysis of off-target interactions and potential ADRs increases the risk of clinical and commercial failure, with potentially serious consequences for both the company and patients.
- The key challenge is to identify, as early as possible, off-targets potentially responsible for yet unidentified — even rare — ADRs, in order to reduce the risk of future failure and financial impact.

Services

- For the compound of interest, potential off-target proteins are systematically and unbiasedly searched across the structural proteome of *Homo sapiens* and relevant model organisms.
- SPILLO identifies the top-ranked off-targets and their corresponding binding sites, providing structural data on the key amino acid interactions that stabilize the binding.
- This unbiased, bottom-up analysis, enables the prediction of potential ADRs resulting from off-target interactions — including those potentially responsible for both common and rare ADRs — specific to the compound under investigation.

Technical Advantages

- SPILLO identifies off-targets responsible for both common and rare ADRs, for small molecules and selected modalities — including first-in-class candidates — in a way not achievable at scale by other in silico or experimental methods.
- Returns ranked off-targets, their binding sites, and poses in a single analysis — enabling immediate interpretation and experimental validation.
- Provides structural insights that support rational modification of the compound to reduce undesired off-target interactions while preserving affinity for the intended therapeutic target.

Value Proposition

- Without dependence on prior knowledge, SPILLO uncovers if and why a drug may cause adverse effects — including rare ones — by identifying their biomolecular causes across the proteome. This enables earlier decisions and reduces safety-related risks and attrition.
- Physics-based and flexibility-aware, SPILLO discovers off-targets even when their binding sites are highly distorted — a unique capability unmatched by other technologies.
- By anticipating potential ADRs, it enables smarter trial design, refined patient monitoring, and improved risk mitigation.
- Supports more informed investment, divestment, and licensing decisions.

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X	DRUG RESCUE: Unlocking Unexpected ROI from Shelved Drug Assets		
Key Challenges	**Services**	**Technical Advantages**	**Value Proposition**
📌 Many molecules reaching Phase II or Phase III trials are discontinued due to limited efficacy in the intended indication — even when their safety profile is favorable — ultimately failing to deliver ROI, even after substantial investment. 📌 Even in successful cases, for every approved molecule, there are usually others discarded along the way — some in the final stages of clinical trials — despite a good safety profile, leaving potentially valuable therapeutic effects unexplored. 📌 The key challenge lies in uncovering new therapeutic opportunities — beyond the original indication — by identifying previously unknown targets, with the goal of unlocking the compound's hidden value, turning past investments into future ROI, and delivering new benefits to patients.	🖥 The structural proteome of *Homo sapiens* and relevant model organisms is systematically and unbiasedly analyzed by SPILLO to discover potential target proteins for the compound of interest. 🖥 This bottom-up approach enables the identification of previously unknown targets that may be responsible for additional therapeutic effects — either previously unknown or unrecognized. 🖥 For all identified targets, the corresponding binding sites are provided, along with structural data on the amino acids that stabilize the interaction.	🍃 Entirely new targets can be identified by SPILLO starting from the compound's structure alone — through a proteome-wide analysis that includes protein flexibility and does not depend on the quality or completeness of any training data. 🍃 Results include detailed structural information on binding sites — supporting mechanistic understanding and further validation. 🍃 Newly discovered targets may match known therapeutic targets or reveal novel ones not yet listed in disease databases, which may still offer valuable opportunities worth exploring.	📈 SPILLO revives shelved compounds previously abandoned due to lack of efficacy — despite confirmed safety — by uncovering new therapeutic indications. 📈 Reduces time, cost, and risk, as only late-stage development is typically required. 📈 Entering the pipeline closer to approval allows for a longer effective patent exploitation period than a typical new drug. 📈 Opens new therapeutic directions and unlocks unexpected ROI from previously discarded compounds. 📈 Identifies entirely new targets and their associated biomolecular mechanisms through a flexible and unbiased proteome-wide structural analysis, unaffordable with other methods.

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Z — DRUG REPURPOSING: Unlocking Strategic Value from On-, Late-, and Off-Patent Molecules

Key Challenges

- Many drug compounds still hold untapped therapeutic and strategic potential. In particular:
  - **On-patent compounds** can benefit from indication expansion — such as new brand uses — to enhance market potential and maximize ROI.
  - **Late-patent compounds** face the loss of exclusivity; repurposing offers a chance to secure new IP and extend strategic value.
  - **Off-patent compounds** may hold unexplored therapeutic value beyond their current indications.
- The key challenge lies in identifying new, biologically relevant targets — along with binding site and mechanism of action — that enable indication expansion at relatively low additional cost.

Services

- The structural proteome of *Homo sapiens* and relevant model organisms is systematically and unbiasedly analyzed by SPILLO to discover potential target proteins for the compound of interest.
- This bottom-up approach enables the identification of previously unknown targets that may be responsible for additional therapeutic effects — either previously unknown or unrecognized.
- For all identified targets, the corresponding binding sites are provided, along with structural data on the amino acids that stabilize the interaction.

Technical Advantages

- SPILLO identifies entirely new targets starting from the compound's structure alone — through a proteome-wide analysis that includes protein flexibility and does not rely on existing data.
- Results include detailed structural information on binding sites — supporting mechanistic understanding and further validation.
- Newly discovered targets may match known therapeutic targets or reveal novel ones not yet listed in disease databases, which may still offer valuable opportunities worth exploring.

Value Proposition

- SPILLO enables strategic repurposing of on-, late-, and off-patent compounds by uncovering new therapeutic indications.
- Reduces time, cost, and risk, as only late-stage development is typically required.
- Repurposed drugs benefit from faster approval and a longer effective patent exploitation period than a typical new drug.
- Opens new therapeutic directions and unlocks unexpected ROI from underused or declining assets.
- Identifies new targets and their biomolecular mechanisms through a flexible, unbiased, proteome-wide structural analysis — unaffordable with other methods.

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Custom Solutions — Designed to Fit Your Goals

Our platform isn't just flexible — it's built to be **adapted and tailored**. Because SPILLO is fully developed and maintained in-house, we can offer customized services with unmatched agility.

- **Custom workflows** aligned with your project's specific goals
- **Fast feature adaptation** thanks to full control of the technology
- **No black-box limitations** — full interpretability and traceability
- **Innovative use cases** co-designed with our clients
- **Results easily integrated** into your existing decision-making or development pipelines

This makes SPILLO the ideal partner for teams looking for high-precision insights — without the need to change their infrastructure.

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Trusted Technology — Proven by Independent Validations

At SPILLO, we prioritize **quality and scientific rigor**. Our technology has been rigorously tested in **real-world scientific challenges** of interest to both **public and private research institutions**.

- **Tackling unanswered questions**
  We addressed biomedical challenges — including drugs under development and already on the market, **some even off-patent** — that were of interest to leading research centers, where key uncertainties had remained elusive.

- **Discovering brand new targets and off-targets**
  Our research revealed, for the first time, **critical targets and off-targets** — **not previously reported** in the scientific literature for the compounds studied — opening entirely new perspectives on their mechanisms of action.

- **Independent experimental validations**
  Our findings were **independently experimentally confirmed** by the research centers we worked with — often leading to co-authored publications. Importantly, these validations came from independent collaborations — with no SPILLO funding involved — further reinforcing the **objectivity and credibility of our results**.

- **Publication in peer-reviewed scientific journals**
  The following slides present **peer-reviewed publications** where SPILLO's **results** were **experimentally validated in diverse, real-world scenarios.** These studies showcase applications of SPILLO's analytical approach — the underlying framework applied across SPILLO's entire service portfolio.

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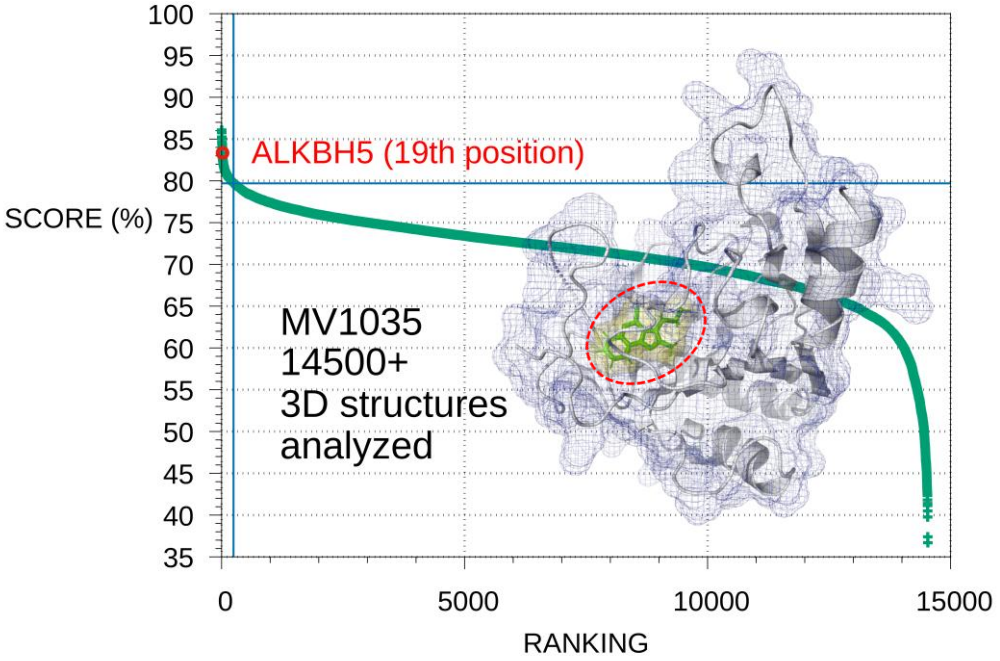
MV1035: SPILLO Identifies a Biomolecular Mechanism for Its Anti-Glioblastoma Effects - Experimentally Validated

3D proteome-wide scale screening and activity evaluation of a new ALKBH5 inhibitor in U87 glioblastoma cell line

BIOORGANIC AND MEDICINAL CHEMISTRY (2020)

DOI: 10.1016/j.bmc.2019.115300



- **Unsolved Scientific Question.** The small molecule MV1035 has been found to reduce migration and invasiveness in U87 glioblastoma cells; however, its mechanism of action remains unknown.

- **The Key Role of SPILLO.** Through an unbiased proteome-scale analysis, SPILLO identified the RNA demethylase ALKBH5 as a top-ranked target of MV1035. It also determined that the binding site partially overlaps with one of its substrates within the enzyme's catalytic site, where m6A mRNA demethylation occurs. Remarkably, the binding site was identified despite being inaccessible to the MV1035 molecule.

- **SPILLO-Proposed Biomolecular Mechanism.** This discovery led to the hypothesis that MV1035 inhibits ALKBH5 by competing with its substrate, 2-oxoglutarate, and consequently reducing the enzyme's catalytic activity.

- **Experimentally Confirmed Biomolecular Mechanism.** Experimental validations confirmed the mechanism predicted by SPILLO, demonstrating that MV1035 reduces glioblastoma cell migration and invasiveness by inhibiting ALKBH5's catalytic activity.

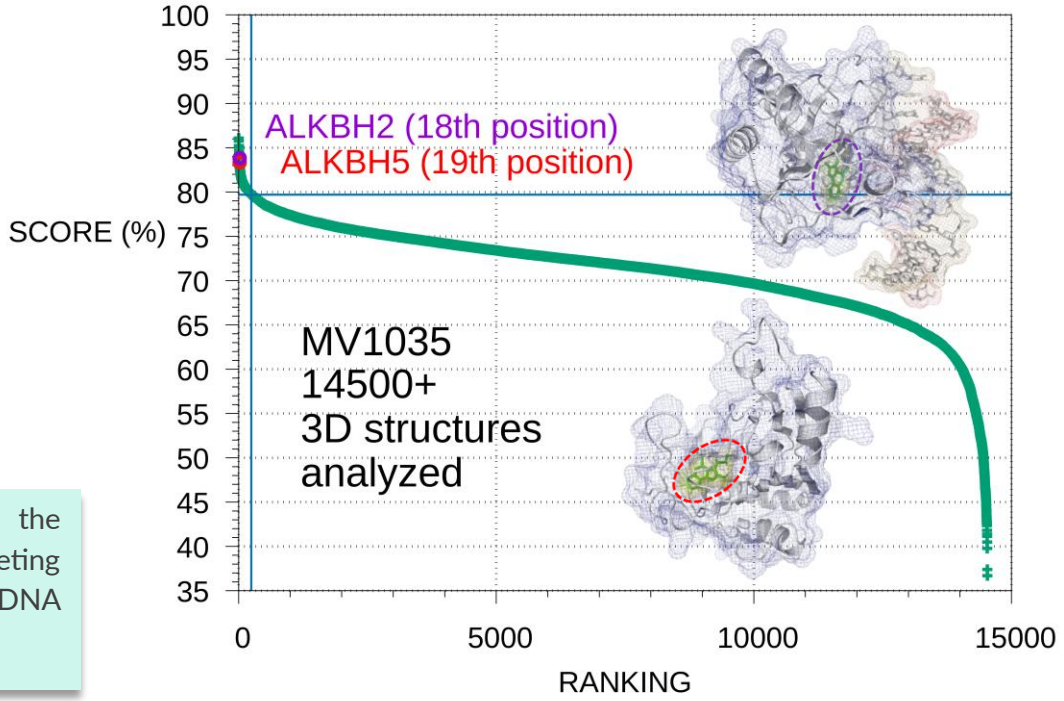
MV1035: SPILLO Identifies a Biomolecular Mechanism to Overcome Temozolomide Resistance in Glioblastoma - Experimentally Validated

- **Further Unsolved Scientific Problem**. Temozolomide (TMZ) is the standard first-line treatment for glioblastoma (GBM, grade IV glioma), but tumor recurrences and TMZ resistance are common and the prognosis is very poor.

MV1035 Overcomes Temozolomide Resistance in Patient-Derived Glioblastoma Stem Cell Lines
BIOLOGY (2022)
DOI: 10.3390/biology11010070

- **The Key Role of SPILLO.** Among the top-ranked targets identified by SPILLO for MV1035 through the same screening that led to the discovery of ALKBH5, the DNA repair protein AlkB homolog 2 (ALKBH2) was also found, with a binding site that partially overlaps with one of its substrates.

  ALKBH2 is known to contribute to resistance to temozolomide. Remarkably, in this case as well, the binding site was identified despite its conformation being inaccessible to the molecule.



- **SPILLO-Proposed Biomolecular Mechanism.** This discovery led to the hypothesis that MV1035 inhibits ALKBH2's enzymatic activity by competing with its substrate within the enzyme's catalytic site, where DNA demethylation occurs.

- **Experimentally Confirmed Biomolecular Mechanism.** Experimental validation confirmed that MV1035 binds to ALKBH2, inhibits DNA demethylation, and exhibits a synergistic effect with temozolomide, enhancing its efficacy when both drugs are present simultaneously in experimental tests.

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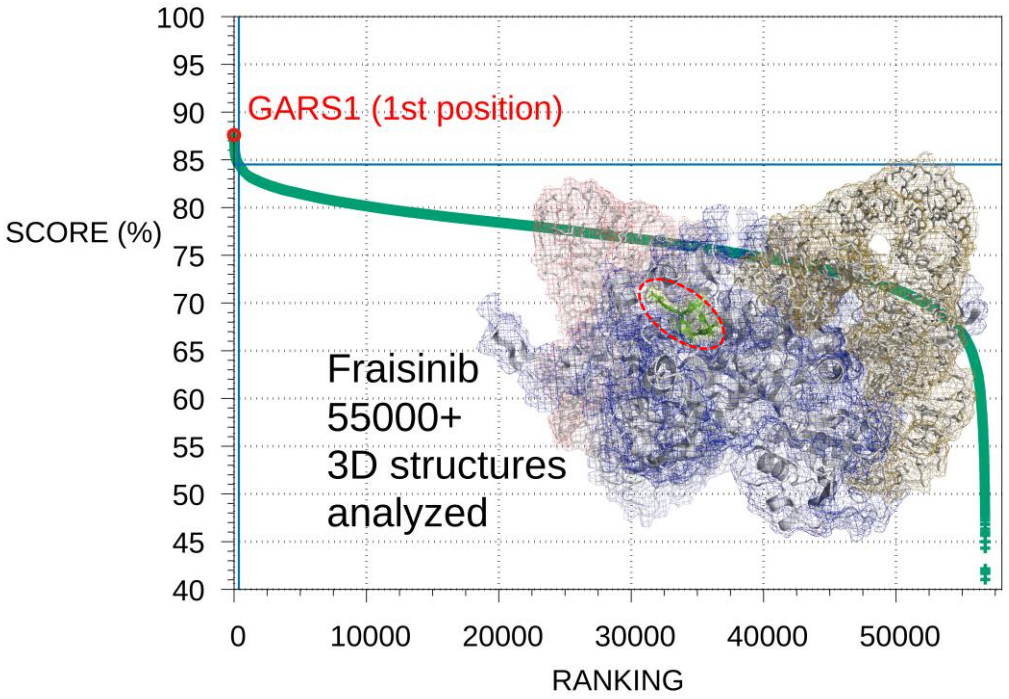
FRAISINIB: SPILLO Identifies a Biomolecular Mechanism for its Anti-NSCLC Effects - Experimentally Validated

Fraisinib: a calixpyrrole derivative reducing A549 cells-derived NSCLC tumor in-vivo, acts as ligand of the Glycine-tRNA Synthase, a new molecular target in oncology

FRONTIERS IN PHARMACOLOGY (2024)

DOI: 10.3389/fphar.2023.1258108



- **Unsolved Scientific Question.** The small molecule Fraisinib was in development following its demonstrated efficacy against several tumor cell lines, particularly non-small-cell lung cancer (NSCLC). However, its underlying molecular mechanism remained unclear.

- **The Key Role of SPILLO.** SPILLO performed an unbiased analysis of over 55,000 protein structures from *Homo sapiens*, *Mus musculus*, and *Rattus norvegicus*, identifying GARS1 as the top potential target for Fraisinib, despite significant steric clashes at the binding site.

- **SPILLO-Proposed Biomolecular Mechanism.** These findings enabled the formulation of the hypothesis that Fraisinib inhibits GARS1 catalytic activity. Furthermore, its binding may reduce GARS1 flexibility, potentially disrupting its interactions within the neddylation pathway.

- **Experimentally Confirmed Biomolecular Mechanism.** Experimental tests confirmed that Fraisinib suppresses GARS1-mediated Ap4A synthesis, validating GARS1 as the target protein of this lead compound, as predicted by SPILLO.

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FINASTERIDE: SPILLO Identifies a Biomolecular Mechanism for Its Adverse Effects - Experimentally Validated

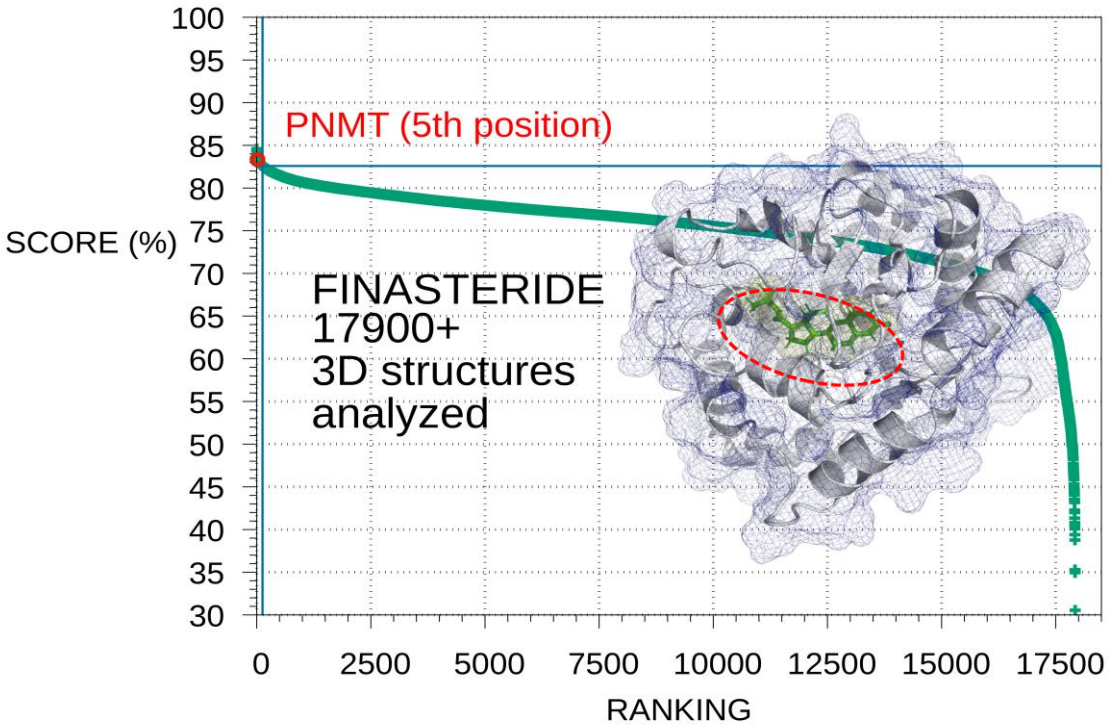
- **Unsolved Scientific Question.** Finasteride, a 5α-reductase inhibitor, has been on the market for many years and is widely used to treat benign prostatic hyperplasia (BPH) and androgenetic alopecia (AGA). However, its use is often associated with sexual, psychological, and physical side effects, while the molecular mechanisms underlying these effects remain unknown.

Three-dimensional proteome-wide scale screening for the 5-alpha reductase inhibitor Finasteride: identification of a novel off-target"
JOURNAL OF MEDICINAL CHEMISTRY (2021)
DOI: 10.1021/acs.jmedchem.0c02039

- **The Key Role of SPILLO.** An unbiased 3D proteome-wide analysis led to the first-ever identification of a novel off-target of Finasteride: the enzyme PNMT, which catalyzes the conversion of noradrenaline into adrenaline. Notably, PNMT was ranked 5th out of 17,925 and was identified despite its binding site conformation being unsuitable for binding.

- **SPILLO-Proposed Biomolecular Mechanism.** The discovery by SPILLO led to the hypothesis that Finasteride inhibits PNMT's catalytic activity by competing with its substrates, thereby reducing adrenaline levels while increasing noradrenaline.

- **Experimentally Confirmed Biomolecular Mechanism.** SPILLO's predictions have been experimentally validated through in vitro and rationally designed in vivo tests, which have confirmed PNMT as an off-target of Finasteride and its inhibitory mechanism of action.



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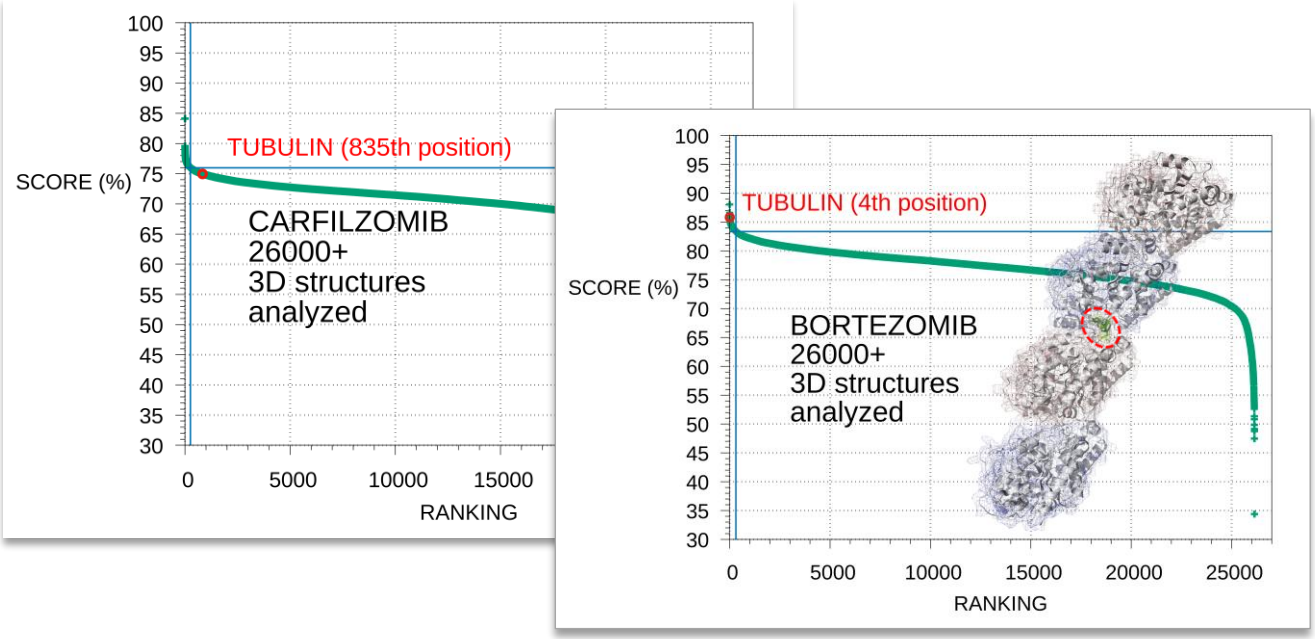
BORTEZOMIB: SPILLO Identifies a Biomolecular Mechanism for Its Adverse Effects - Experimentally Validated

Tubulin binding potentially clears up Bortezomib and Carfilzomib differential neurotoxic effect
SCIENTIFIC REPORTS (2021)
DOI: 10.1038/s41598-021-89856-3



- **Unsolved Scientific Question.** Bortezomib (BTZ) and Carfilzomib (CFZ) are used to treat multiple myeloma. Despite targeting the proteasome at the same binding site, they exhibit different toxicological profiles. The key challenge is understanding the molecular basis of why BTZ often induces severe peripheral neuropathy (PN), whereas CFZ induces a much milder form of PN.

- **The Key Role of SPILLO.** Two independent, unbiased 3D proteome-wide analyses — one for each drug — identified Tubulin as an off-target of BTZ, ranking 4th out of 26,000+ 3D structures, but not of CFZ, which ranked 835th. For the first time, BTZ neurotoxicity has been directly linked to its interaction with microtubules.

- **SPILLO-Proposed Biomolecular Mechanism.** The discovery by SPILLO led to the hypothesis that BTZ's interaction with tubulin — but not CFZ's — may induce a perturbation of microtubule dynamics, potentially explaining the different neurotoxic profiles of BTZ and CFZ.

- **Experimentally Confirmed Biomolecular Mechanism.** SPILLO's predicted off-target of BTZ has been experimentally validated: direct binding of BTZ to tubulin was demonstrated through STD-NMR ligand-receptor interaction studies. In contrast, CFZ did not exhibit the same behavior. Furthermore, unlike BTZ, CFZ did not increase the rate of tubulin polymerization in both DRG sensory neurons and a cell-free in vitro assay.

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PAROXETINE: SPILLO Identifies a Biomolecular Mechanism for Its Adverse Effects - Experimentally Validated

- **Unsolved Scientific Question.** Antidepressants, particularly paroxetine, are often associated with various side effects, with sexual dysfunction being one of the most prevalent. However, the biomolecular mechanisms underlying these effects are not fully understood.

- **The Key Role of SPILLO.** SPILLO conducted an unbiased analysis of 64,268 protein 3D structures (as of October 2021) from *Homo sapiens*, *Mus musculus*, and *Rattus norvegicus*. Among the top-10, phenylethanolamine N-methyltransferase (PNMT; PDB code: 3KQW) was identified as a potential off-target.

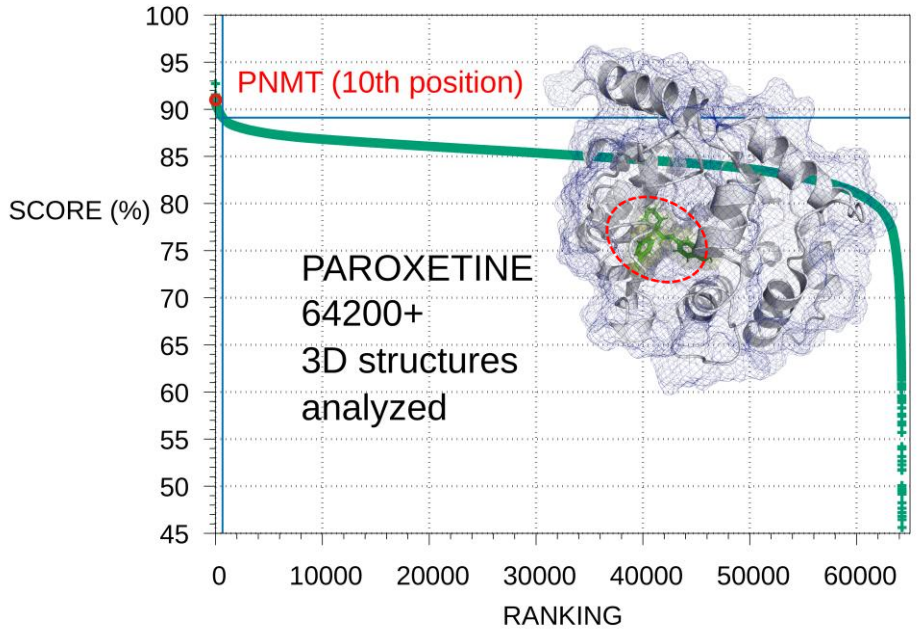
NOTE: Interestingly, PNMT was previously identified by SPILLO as an off-target of finasteride, another drug associated with sexual side effects. This suggests that PNMT inhibition may be a key factor in the mechanisms underlying sexual dysfunction caused by both drugs.

- **SPILLO-Proposed Biomolecular Mechanism.** A competitive inhibition hypothesis was formulated within PNMT's catalytic site, suggesting that the conversion of noradrenaline into adrenaline may be inhibited due to the overlap of Paroxetine with the noradrenaline binding site.

- **Experimentally Confirmed Biomolecular Mechanism.** SPILLO's predictions have been experimentally validated through in vitro and in vivo tests, which have confirmed PNMT as an off-target of Paroxetine and its inhibitory mechanism of action.

Identification of a novel off-target of paroxetine: Possible role in sexual dysfunction induced by this SSRI antidepressant drug"

JOURNAL OF MOLECULAR STRUCTURE (2022)
DOI: 10.1016/j.molstruc.2022.133690



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Protein Structural Database Coverage

SPILLO's structural database delivers near-complete coverage of the human proteome — enabling powerful, proteome-wide target and off-target discovery.

- SPILLO typically analyzes all **experimentally determined 3D protein structures** available in the RCSB Protein Data Bank for *Homo sapiens* and selected model organisms (e.g., *Mus musculus*, *Rattus norvegicus*) — which are among the most commonly studied in biomedical research.
- For *Homo sapiens*, these **experimental structures** currently cover approximately **40% of the proteome** (based on unique proteins).
- Since mid-2021, the integration of **AI-predicted structures** from the **AlphaFold Protein Structure Database** has extended the overall structural coverage of the human proteome to **over 99%**.

Organism	Structural Coverage — Protein 3D Structures from Experimental and AI-Based Sources	
	RCSB Protein Data Bank Solved by X-ray diffraction, solution NMR, cryo-EM **(With sequence redundancies)**	**AlphaFold Protein Structure Database** Predicted by AI **(Without sequence redundancies)**
Homo sapiens	73,400+ Structural coverage of the human proteome: ~ 40%	23,391 Structural coverage of the human proteome: > 95%
Mus musculus	9,700+	21,615
Rattus norvegicus	*4,300+*	21,270
Total:	**87,400+** (*April 2025*)	**66,276** (*July 2021*)

SPILLO maintains and regularly updates the database it analyzes — offering **near-complete proteome coverage** by integrating **experimental and AI-predicted data**, and supporting analysis of **custom datasets** when needed.

SPILLO

TARGETS THAT MATTER

Contact us to explore how SPILLO can support your scientific and business objectives

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