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ORIGINAL RESEARCH

Protein engineering in the computational age: An open source framework for exploring mutational landscapes *in silico*

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Abstract

The field of protein engineering has seen tremendous expansion in the last decade, with researchers developing novel proteins with specialised functionalities for a range of uses, from drug discovery to industrial biotechnology. The emergence of computational tools and high-throughput screening technology has substantially sped up the process of protein engineering. However, much of the expertise required to engage in such projects is still concentrated in the hands of a few specialised individuals, including computational biologists and structural biochemists. The international Genetically Engineered Machine (iGEM) competition represents a platform for undergraduate students to innovate in synthetic biology. Yet, due to their complexity, arduous protein engineering projects are hindered by the resources available and strict timelines of the competition. The authors highlight how the 2022 iGEM Team, 'Sporadicate', set out to develop InFinity 1.0, a computational framework for increased accessibility to effective protein engineering, hoping to increase awareness and accessibility to novel *in silico* tools.

KEYWORDS

biochemical engineering, synthetic biology

1 | PROTEIN ENGINEERING: AN OVERVIEW

Protein engineering is a rapidly evolving field in which efforts can be broadly categorised into two classes: the re-engineering of natural proteins to either fine-tune (substrate specificity and affinity, catalytic properties, stability) or introduce new functionality; or de novo protein design (referred to as design rather than engineering) where proteins are artificially built from first principles and engineered to perform a desired function [1] (Figure 1).

Within protein re-engineering, approaches range from the highly rational to solely combinatorial. Historically, researchers have relied on rational engineering approaches to introduce changes in natural protein structures [2]. Albeit requiring prior knowledge of the protein's folding and functional characteristics, these techniques rely on a small library size to screen for the desired behaviour, with many successful examples documented extensively in the literature [3].

When structural and functional information is not easily accessible, directed evolution strategies employ random mutagenesis such as error-prone PCR (epPCR) to generate a large protein library to screen for functionality. Existing directed evolution techniques, however, are limited in scalability due to the laborious process of *in vitro* high throughput screening and subsequent selection [4]. Additionally, directed

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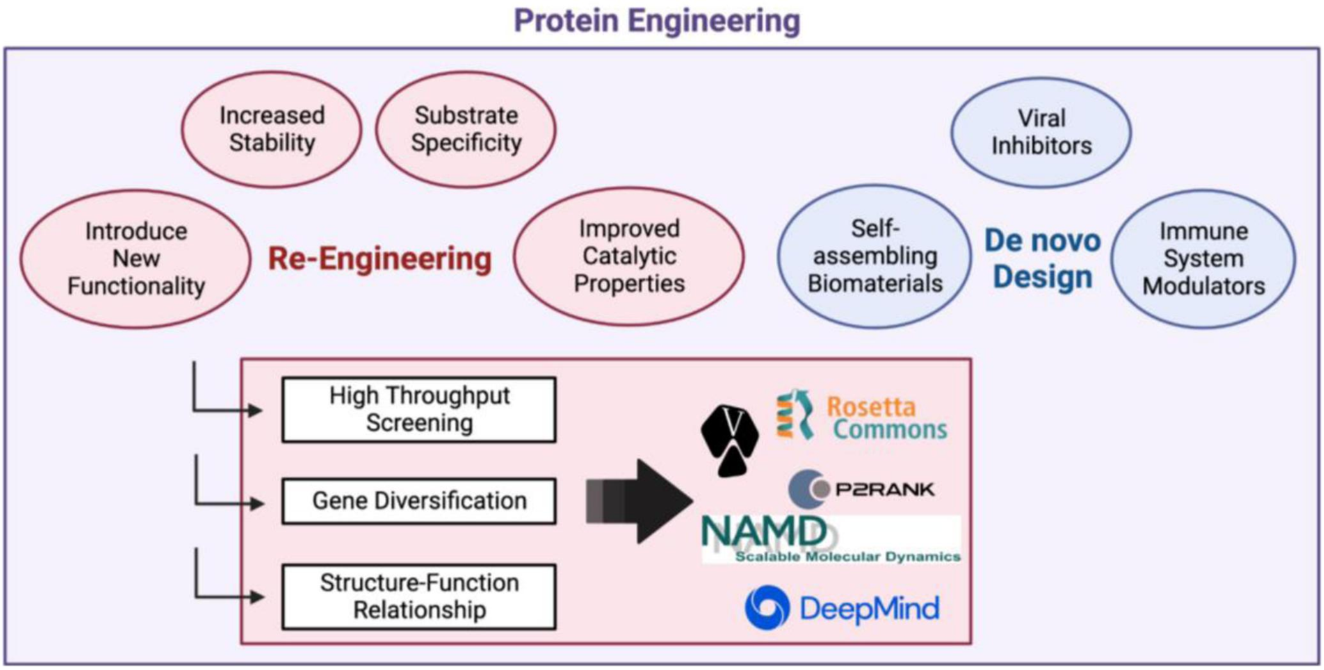


FIGURE 1 Areas of research in protein engineering.

evolution relies on manual staging of each cycle. This limits its ability to explore the functional sequence space deeply and extensively [5].

At the intersection between directed evolution and rational design lie combinatorial approaches, which combine stochastic elements and rational information to generate small yet effective protein pools, able to constrain the protein folding space and optimise the chances of success [6].

1.1 | Computational tools for addressing challenges in protein engineering

Two key challenges in protein engineering are: (1) deciphering the sequence-structure-function relationship in proteins and (2) effectively navigating the protein design space defined as 20^n, where n is the number of amino acids [7].

Computational tools addressing both these issues have been developed extensively in the last decade, with *in silico* strategies for predicting protein structure (AlphaFold, RoseTTAFold), their interactions with other ligands (AutoDock Vina, UCSF Dock, P2Rank), as well as dynamic system simulations (molecular dynamics software, such as GROMACS, NAMD, and AMBER) finding regular applications in the cutting edge.

In particular, AI-based methods have revolutionised the field of rational design through use of the ever increasing amount of data provided by -omics projects to investigate rules for engineering functional biomolecules [8]. Machine learning approaches have been applied to a range of processes that span all areas of protein design, including protein secondary structure prediction, fold recognition, contact matrix calculations, genomics, proteomics, and systems biology [8]. The development of deep learning approaches in particular, driven by the increasing availability of computer power and the development of GPU-based calculations has resulted in the release of several landmark rational design papers within the last decade [9, 10].

Adoption of these tools unlocks the possibility of semi-rational engineering approaches, characterised by smaller and enriched variant protein libraries. This not only mitigates the limitation of screening numerous variants, a time and resource-intensive step in wet lab settings, but increases the likelihood of engineering a functional protein.

As highlighted by Walker, Yallapragada and Tangney [11], one of the main limitations concerning protein engineering and its widespread adoption is the lack of intuitive workflows for *in silico* tools. In recent years, there have been efforts to tackle this issue (Table 1), an in depth discussion of tools considered approachable to non-specialists can be found in recent reviews [24, 25]. Yet, none of these address the specificity and affinity engineering problem in a manner that is one-stop and intuitive to non-specialists, whilst being open-source. Indeed, to exploit the capabilities of ever-evolving algorithms, expertise in both computational biology and structural biochemistry is required, resulting in a high barrier to entry. A great case study of the phenomenon is iGEM—(International Genetically Engineered Machine competition) an annual synthetic biology competition, wherein multidisciplinary student teams pursue a project of their own design.

In 2022, we set out to compete in the iGEM competition with Sporadicate, a novel, broadspectrum, and timely crop biofungicide system [26]. In order to target pathogens over both space and time, we designed a *Bacillus subtilis* spore-based system that would trigger the release of our biocontrol

TABLE 1 Recent computational tools considered approachable to non-specialist protein engineers.

Tool	Functionality	Features and limitations	Reference
AlphaFold 2.0	Computational tool designed for predicting three-dimensional protein structure using a deep learning system trained on structures in the PDB.	Features: Provides protein structure prediction with high accuracy. Limitations: Does not model the dynamics and mechanics of the protein. Low confidence in unstructured regions.	[12]
ROSIE	The gold-standard, a web platform hosting dozens of tools from the Rosetta suite of programs that enable modelling and protein design, including: Molecular docking, prediction and design for protein stability and solubility.	Features: Enables access to multiple tools a protein engineer would require from the Rosetta suite under one user-friendly environment. Limitations: Platform has been developed by different groups over time, thus it can be difficult to implement and utilise different modules seamlessly. Deep investigation into limitations provided by [13].	[14]
HotSpot Wizard 3.0	Web application that allows identification of hot spots for mutagenesis.	Features: User can input either the sequence or three-dimensional structure of the target protein. The tool enables development of smart libraries, integrating considerations on function, stability and evolutional variability. Limitations: The aim of the tool is to identify highly mutable functional residues that are unlikely to impair function, thus the design philosophy may not be in line with all protein engineering strategies. For example, the library generated may not be effective if screening for altered ligand specificity.	[15]
FuncLib	To redesign an active site and create multiple-point designs. Based on conservation analysis and energy calculations.	Features: Algorithm designed specifically to output a small ranked set of stable, multipoint active-site mutants that are functionally diverse, enabling efficient low-throughput wet-lab testing. Limitations: As input, it requires a molecular structure and diverse set of sequence homologues. It works better with a pre-stablised protein scaffold. In the absence of sufficient knowledge of the protein being engineered, poor results are likely.	[16]
CaverWeb 1.2	To calculate trajectory and interaction energy profiles of a ligand travelling through a protein tunnel.	Features: Integration with other CaverSuite tools, including Caver (software for identification and geometric analysis of tunnels) and CaverDock (software for docking-based analysis of ligand transport) allows deeper analysis of the ligand transport process by users with limited bioinformatics knowledge and experience using computational tools. Found to be more robust and provide higher resolution than other state-of-the-art tools such as SLITHER and MoMA-LigPath (doi: 10.1093/bioinformatics/btz386). Effective without extensive knowledge of studied system. Limitations: Lacks the possibility of calculating pores.	[17]
DockingApp (Autodock Vina)	Platform-independent application for setting up, performing and analysing results from AutoDock Vina.	Features: Provides a user-friendly graphical interface, enabling easier access to AutoDock Vina. Limitations: Slow docking procedure compared to EquiBind.	[18]
LoopGrafter	For transplanting loops between two structurally related proteins, with a focus on the analysis of dynamic properties of the selected loops to transplant.	Features: Enables optimised transplant of loops between structurally related but functionally different proteins. Limitations: Necessitates both the template and the scaffold proteins as inputs to function.	[19]
Protein Repair One-Stop-Shop (PROSS)	Automated web platform aimed at improving protein thermostability and functional yield.	Features: Was found to be successfully implemented by scientists without a background in protein designs. Method has been found reliable enough to only require screening of a limited number of output designs [20].	[21]

(Continues)

TABLE 1 (Continued)

Tool	Functionality	Features and limitations	Reference
		Limitations: Structure is required for stability calculations, although this could be computationally generated if not available this may not always be reliable.	
SoluProt	Predicts the solubility of a protein specified by input sequence, in *Escherichia coli.*	Features: Only sequence required as input. Has a user-friendly web-server. Has the potential to reduce the cost of experimental studies via proritisation of highly soluble proteins. Limitations: Although higher than predecessors, accuracy is 58.5%.	[22]
DeepSoluE	Predicts the solubility of a protein specified by input sequence, in *Escherichia coli.*	Features: Similar advantages to SoluProt, with improved accuracy, 59.5%. Limitations: Focus on only *E.coli*	[23]

agent—vegetative *Bacillus subtilis* cells—in response to a common biomarker of pathogenic fungi (N-acetylglucosamine—i.e. chitin monomer). Our sensing system required transduction of our desired input signal into germination, the process by which dormant spores awaken into cells. Natively, protein receptors in the coat of spores known as germinant receptors perform this task, sensing for nutrients such as glucose as well as amino acids [27]. We planned to modify native germinant receptor GerA (Figure 2), a sensing protein detecting L-alanine [28], with enhanced specificity towards N-acetylglucosamine.

Given the limited available information on the protein structure and how this relates to function—we needed to adopt a combinatorial strategy. However, as GerA comprises 1220 residues, a protein design space of 20^{1220} would have been impossible to explore in practice. Hence, we devised a potential workflow integrating novel computational tools that would enable us to design a smaller, richer protein library. This not only would have maximised our chances of success, but could lay out the foundations of accessible protein engineering research, in iGEM and beyond.

2 | DESIGN

With the advent of computational tools that can accurately predict protein structures and significant progress in the field of molecular docking, we pose the question if said developments could be incorporated into a framework for streamlined protein engineering [12, 30, 31]. We propose In-Finity 1.0, an open-source computational framework, mimicking *in vivo* techniques (Figure 3). Specifically, this consists of mutant library generation, positive and negative selection finally followed by analysis of commonly occurring motifs. Here, we present how such a framework could be constructed (Figure 3).

First, taking either a predicted (e.g. Alphafold) or experimentally derived protein structure, mutations should combinatorically be introduced within the targeted ligand binding pocket. Sequence-wise this can be done quickly and efficiently with simple combinatorics of a supplied FASTA sequence. Accurately modelling the mutations structurally is perhaps the greatest challenge, and a compromise has to be met between computational efficiency and accuracy. For example, PyMol's mutagenesis tool can efficiently model changes to side-chains, avoiding steric clashes but does not account for changes to the scaffold and effect on local and global energetics as molecular dynamic simulations do. Nonetheless, at this step, the desired input is simply an initial protein structure and residue positions targeted for mutagenesis, while the output is a library of mutant structures.

For positive selection, this can be performed with relatively computationally efficient docking and scoring. In recent years, we have seen advances in the use of machine-learning based docking and scoring functions for computational drug discovery, and some have shown significant improvements in the predictive accuracy of ligand-binding affinities; for example,

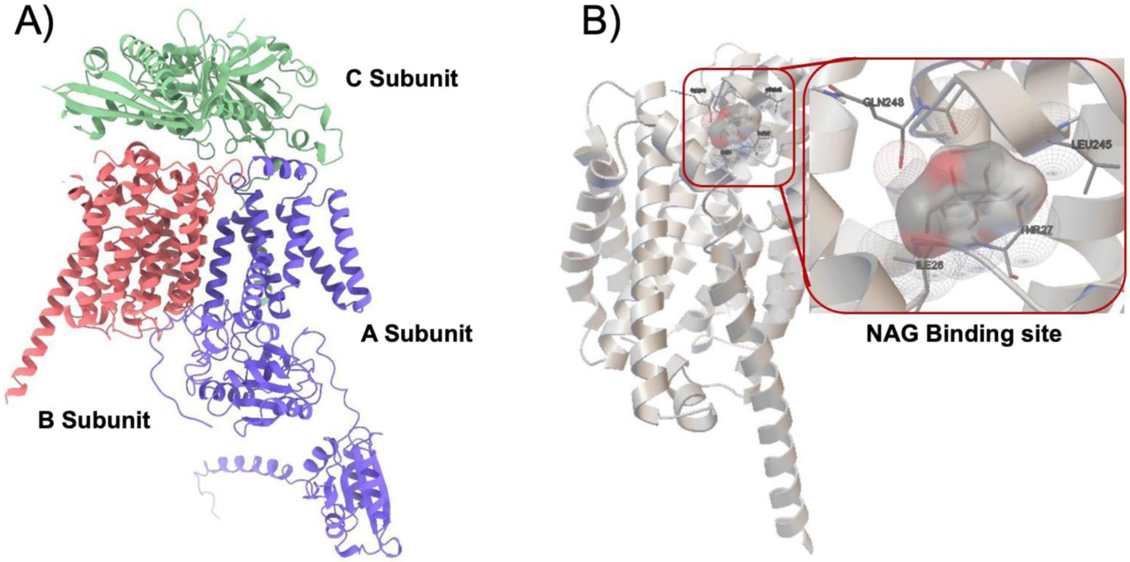


FIGURE 2 Binding site identification. (a) GerA complex obtained through AlphaFold. The protein consists of three smaller subunits, AA, AB and AC. The B subunit is thought to sense and bind with the germinant L-alanine, whereas the A subunit is responsible for transducing the signal from B [28] upon activation. The function of the C subunit is unclear, but it is not necessary for germinant receptor activation [29]. (b) NAG Binding site identified in GerAB through Autodock. NAG, n-acetylglucosamine.

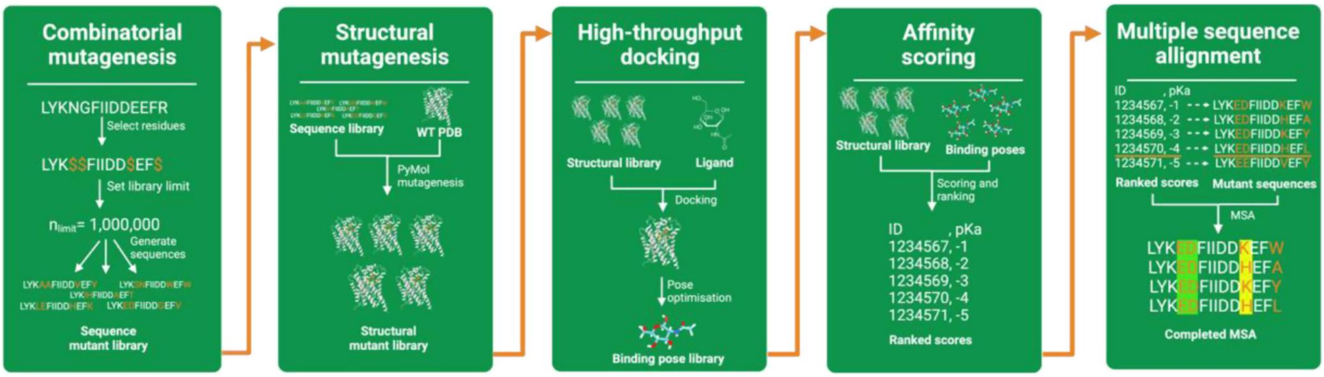


FIGURE 3 Overview of proposed framework for computational protein engineering. The framework will benefit from advances in structural modelling and molecular docking. Adapting these for use in computational protein engineering could allow for high-throughput screening of mutants, aiding in design and testing to be carried out in the lab.

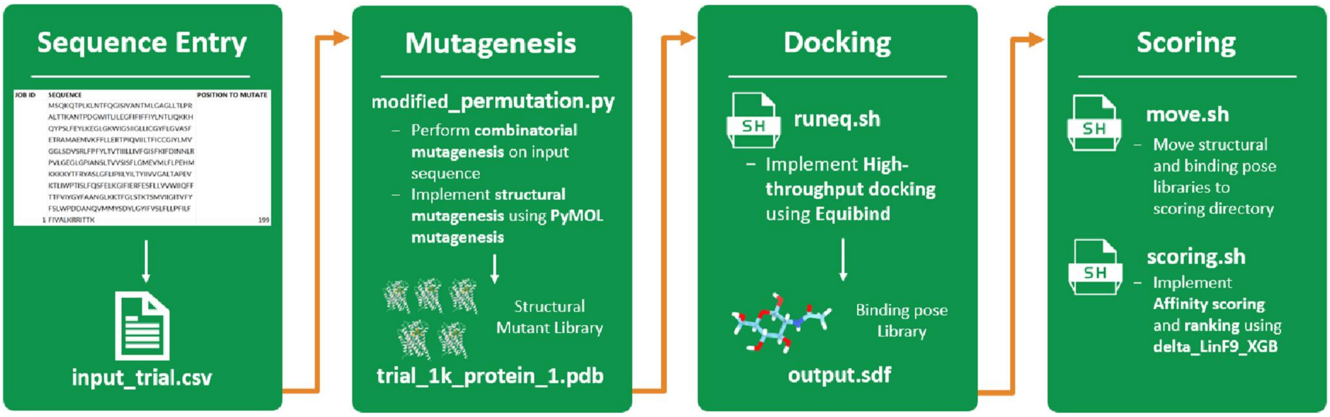
DiffDock, GNINA and scoring functions adapted with an XGB model [30, 32, 33]. Taking the mutant library previously derived, the desired ligand could be docked and affinities are calculated using a concoction of the leading docking tools and scoring functions. The final output would then be a ranked list of which mutants' structures showed the greatest binding affinities.

The use of negative selection will be desirable especially in cases where computational efficiency has been prioritised over accuracy in earlier steps. Here, we aim to investigate top scoring mutants from positive selection, specifically screening how mutants could have had detrimental effects on overall protein folding. Missense3D [34] is one example of a computationally efficient way of achieving this while Molecular Dynamics simulations could be used to gain more granularity.

The final step would then be ranking and consensus evaluation of common motifs. For use in iGEM and other protein engineering projects, a selection of the top motifs could then potentially be tested *in vivo*. The specific benefit of this framework over current implementations is its use of open source tools and modularity, opening the door for the integration of novel tools that could improve the performance of the overall pipeline.

3 | INFINITY 1.0 PIPELINE

Throughout the iGEM competition period, we produced an initial proof of concept prototype for the InFinity 1.0 pipeline (Figure 4). Our prototype links several established open-source tools, which allows inexperienced users to achieve high-


Sequence Entry
input_trial.csv
Mutagenesis
modified_permutation.py
– Perform combinatorial mutagenesis on input sequence
– Implement structural mutagenesis using PyMOL mutagenesis
Structural Mutant Library
trial_1k_protein_1.pdb
Docking
SH
runeq.sh
– Implement High-throughput docking using Equibind
Binding pose Library
output.sdf
Scoring
SH
move.sh
– Move structural and binding pose libraries to scoring directory
SH
scoring.sh
– Implement Affinity scoring and ranking using delta_LinF9_XGB

JOB ID	SEQUENCE	POSITION TO MUTATE
1	MSQKQTPLKLNTFQGISIVANTMLGAGLLTLPR ALTTKANTPDGWITLILEGFIFIFFIYLNTLIQKKH QYPSLFEYLKEGLGKWIGSIIGLLICGYFLGVASF ETRAMAEMVKFFLLERTPIQVIILTFICCGIYLMV GGLSDVSRLFPFYLTVTIIILLIVFGISFKIFDINNLR PVLGEGLGPIANSLTVVSISFLGMEVMLFLPEHM KKKKYTFRYASLGFLIPIILYILTYIIVVGALTAPEV KTLIWPTISLFQSFELKGIFIERFESFLLVVWIIQFF TTFVIYGYFAANGLKKTFGLSTKTSMVIIGITVFY FSLWPDDANQVMMYSDYLGYIFVSLFLLPFILF FIVALKRRITTK	199

FIGURE 4 Overview of implemented Infinity 1.0 pipeline. Process begins with a csv file in which users can input the sequence and mutations of their choice, and ends with the corresponding ranked affinity scores with the ligand of choice. For each stage, a script has been created to interface between the user and incorporated tool. Aside from the initial sequence information and computational resource specification, the pipeline requires minimal user input to run.

throughput and accurate mutant screening for protein design. Users can input sequence information which is then used to generate a mutant library, this is then sampled for high-throughput ligand docking which produces binding affinity information. Binding affinities are scored and ranked, and finally multiple sequence alignment is applied to the highest-ranked mutants to derive information about common motifs which is passed back to the user. Our pipeline along with a user guide can be found on our team's GitHub Page (See Code Availability below).

3.1 | Creating a library of mutants

Our approach to combinatorial mutagenesis was highly customisable, allowing the user to select which residues to mutate and wrote a script that generated a random subset of mutants from the associated combinatorial space. To make our software accessible to iGEM users and individuals without a background in structural biology or protein engineering, we added a simple .csv file that can be easily filled and is seamlessly integrated into the software. Users can select their desired sequence and specify which amino acids they want to mutate, as well as the positions of these amino acids.

We used PyMol's mutagenesis tool [35] in our proof of concept to introduce the generated mutant sequences into the wildtype protein structure. The tool substitutes residue side-chains and adjusts using the most optimal rotamer for each, and as such, is best suited for fewer and less structurally significant mutations, such as those required to alter protein specificity or affinity slightly which would be accounted for in a negative selection step.

As our implementation of the pipeline was more of an exploratory proof of concept within the limited iGEM time frame, we did not perform the negative selection step of the design, which would involve modelling the relaxed structure for each mutant and screen for large structural disruptions. We instead relied on the less computationally expensive positive selection and affinity score ranking processes to select our final mutants of interest.

3.2 | Positive selection

After obtaining the structural library, high-throughput rigid docking was performed using an adapted version of EquiBind [36], a recently released deep learning-based docking tool. EquiBind's use of a SE(3)-equivariant geometric deep learning model significantly improves the computational efficiency of docking and has higher accuracy of binding poses compared to comparable baselines. Using GPU acceleration, a single GPU can evaluate approximately 200,000 mutants per day. This step can also be parallelised using multiple GPUs by splitting the mutant dataset.

Affinity scoring was then applied to the high-throughput docking results using the ΔLin_F9XGB scoring function [33], an improved version of the linear empirical scoring function Lin_F9, utilising extreme gradient boosting and Δ-machine learning. ΔLin_F9XGB is open-source and has been shown to be on par or superior to some of the leading scoring functions when tested against the CASF-2016 benchmark [37]. The scoring function takes the .mol2 binding poses generated using equibind and can be massively parallelised according to the maximum number of CPU processing cores available to the user.

3.3 | Ranking

Finally, alignment and consensus screening were performed on the top-ranking mutants to identify trends in the specific types of residue substitutions. Through deriving consensus motifs, desirable properties of the binding pocket can be derived, and in turn aid in the selection of mutants to be tested experimentally.

3.4 | Validation

To test the protocol's ability to enrich for mutants with altered binding affinity/specificity, the protocol must be run to completion and a subset of top mutants would have to be synthesised and tested *in vitro*. Owing to the time and resource constraints of iGEM, we sought to instead investigate possible approaches to testing the protocol *in silico* using test sets from literature. The closest alternative to *in vitro* testing would in this case be evaluating the protocol's accuracy of affinity predictions following *in silico* mutagenesis. We attempted this on a test set of 26 ABL kinase mutants (PDB ID: 4WA9) with associated binding affinities from a study by Hauser et. al. [38]. First, taking the wild type ABL kinase structure, in-silico structural mutations were introduced to match those from the test-set. Affinity prediction was then carried out on these *in silico* mutants and changes in affinity between the wild type and mutants were compared to those same differences, determined experimentally. With this limited test-set, we were unable to show a significant correlation between predicted and literature affinities. However, this was an expected result as a much greater test-set would be required to detect meaningful relationships owing to the inherent low accuracy of current scoring functions. Yet, datasets of mutants and associated binding affinities are much sparser in literature as compared to wild-type datasets, and the largest mutant databases with experimental affinities have become outdated or are no longer publicly available [39, 40] To aid in the development of future protein engineering tools aimed at altering binding affinity we therefore call for the development and curation of new and comprehensive databases of mutants akin to PDBBind [41] in scope. However, even with such a database, evaluation would be restricted to affinity prediction and fail to address the specific aim of enriching for mutants with improved ligand binding affinity/specificity and as such *in vitro* evaluation is still necessary, and future work should attempt this.

In terms of computational efficiency we were able to screen 20,000 mutants/day with a quad-core intel-based system. With the structural mutagenesis step being rate limiting, we expect significant improvements could be made by better parallelising this step or utilising different mutagenesis tools.

4 | CASE STUDY: INFINITY 1.0 COMPARISON TO COMMERCIAL TOOLS

Tools similar to those integrated into the InFinity 1.0 pipeline, such as those for docking and scoring, are often commercially applied within the context of drug discovery services. One such company offering this service is MedChemExpress (MCE), which offers precise virtual screening (VS) as an efficient alternative to high-throughput screening of ligands in early-stage drug discovery. MCE's VS uses structure-based virtual screening (SBVS) to dock and rank molecules using 3D target structures.

In a 2022 study, Kong et al. used MCE for molecular coupling, a tool to align substrates to protein binding sites with the aim of identifying potential binding pockets on amyrin synthase, amyrin synthase enzyme (CrMAS), for the substrate 2,3-oxidosqualene. The method revealed five binding pockets on CrMAS (Figure S1), with Site 1 showing the highest affinity to 2,3-oxidosqualene.

Our case study aimed to compare these findings with affinity predictions on the same sites using InFinity 1.0. The outcome of the scoring function delta_LinF9_XGB, used following docking performed by Equibind, was converted from its native units to Gibbs Free energy (ΔG) using a rearranged formula from the paper by Yang and Zhang [33].

Figure 5 and Table 2 show that the results from Autodock Vina, Kong et al. [42] and Equibind exhibit a similar trend in binding affinity, a property that is inversely proportional to ΔG value. Additionally, our tool effectively differentiated between sites of high (Sites 1, 2, and 5) and low binding affinity (Sites 3 and 4), mirroring the findings of Kong et al. [42].

To demonstrate the modularity of Infinity 1.0, we incorporated another scoring function, GNINA. GNINA, a fork of smina [43] and Autodock Vina [30], integrates convolutional neural networks (CNNs) for advanced scoring refinement. This integration of GNINA resulted in the identification of the top three highest (Sites 1, 2, and 5) and lowest (Sites 3 and 4) affinity sites, as shown in Table 2. These findings align with the results documented by Kong et al. [42]. The observed low binding affinity at Site 4, relative to the other sites, may be attributed to steric hindrance, as illustrated in Figure S1. Steric hindrance arises when atoms or groups within a molecule obstruct each other, thereby inhibiting optimal intermolecular interactions (e.g., hydrogen bonds, van der Waals forces, or ionic bonds) and preventing the establishment of efficient binding [44].

Our study demonstrates that InFinity 1.0 identifies sites exhibiting both high and low binding affinity to a given ligand, as shown when compared to the findings of Kong et al. [42]. InFinity 1.0 yielded more positive ΔG values for Site 1 and Site 2, indicating a heightened affinity of CrMAS for 2,3-oxidosqualene at these sites relative to Sites 3 and 4, which aligns with Kong et al. [42] finding. Furthermore, InFinity 1.0's rapid docking and scoring capabilities surpass those of traditional methods, such as Autodock Vina, allowing quick screening within proteins to pinpoint sites for further investigation. This accelerated screening process not only reduces the time required but also minimises the financial expenditure for iGEM teams. Furthermore, as our program is open-source, it provides iGEM competitors with free access for use in their projects, allowing them not only to use but also to expand upon the software without incurring costs.

5 | DISCUSSION

Our proposed implementation serves the intended purpose of demonstrating how a pipeline could conceivably be constructed, repurposing breakthrough tools utilised for structural prediction and computational drug discovery. As more effective tools are developed, they can replace those in our current implementation, with the aim to suggest a small subset of potential mutants

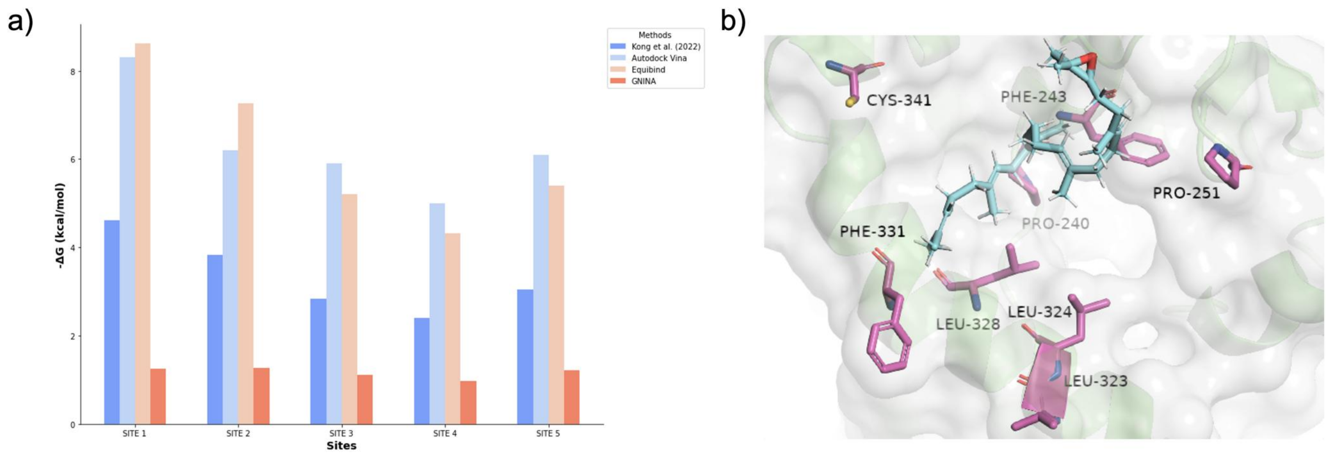


FIGURE 5 Comparison of CrMAS Binding Sites between MedChemExpress and InFinity 1.0 with 2,3-oxidosqualene as the Ligand. (a) Comparative analysis of various scoring functions—EquiBind, Autodock Vina, and GNINA—against the findings presented in Kong et al. [42] following the docking process. (b) PyMOL visualisation of the binding site at Site 1 with 2,3-oxidosqualene inside. CrMAS, amyrin synthase enzyme.

TABLE 2 Gibbs energy scores derived from various scoring functions for different sites during the docking of 2,3-oxidosqualene to CrMAS.

	$-\Delta G$ (kcal/mol)			
Sites	**Kong et al. [42]**	**Autodock Vina**	**Equibind (docking) and delta_LinF9_XGB (scoring)**	**GNINA**
Site 1	4.62	8.3	8.62	1.25
Site 2	3.83	6.2	7.27	1.26
Site 3	2.84	5.9	5.21	1.12
Site 4	2.41	5.0	4.32	0.97
Site 5	3.05	6.1	5.4	1.22

Abbrebiation: CrMAS, amyrin synthase enzyme.

to test *in vitro*. This future improved implementation would then open the door for researchers with time and funding-constrained projects such as those in iGEM to perform specificity and affinity engineering. The main aspect that calls for improvements is accuracy in modelling the structural impact of combinatorial mutants. In our study, we have employed PyMOL mutagenesis to introduce novel mutations into the 3D structure, due to its open-source nature and quick integration into our pipeline. However, this approach has its limitations as it does not alter the underlying scaffold and does not consider steric clashes. To overcome these limitations, an alternative software tool, Rosetta CoupledMoves could be used [45]. This tool couples protein side-chain, backbone flexibility, as well as ligand degrees of freedom for the improved redesign of viable mutants. The adoption of this alternative tool promises to enhance the accuracy of our structural modelling efforts, enabling us to more effectively investigate protein function and interaction. We have included command-line instructions for its use within the InFinity 1.0 pipeline context in our Github repository (see Code Availability).

Finally, seeing inherent flexibility and potential inaccuracies in structural prediction, we propose docking programs should utilise flexible docking. One interesting avenue would be utilising the open-source GNINA [46] platform which builds on AutoDock Vina [30], by using ML-based scoring functions.

As opposed to the currently available dominant *in silico*-based protein engineering tools that focus on enzyme engineering, InFinity 1.0 aims to alter binding affinity and specificity. This has applications namely for biosensor design, with use-cases readily seen in iGEM, and can include sensors for pollutant detection [47], medical diagnostic tests [48], agricultural use and microbiology research with the possibility of monitoring metabolites in real time [49]. Additionally it can be applied to alter cellular functions, by changing natural-sense and response systems. Its applicability could perhaps also translate to drug design, investigating mutants' effect on drug binding-specificity, which could prove useful in for example, antiviral drug development [50].

6 | CONCLUSION

In silico tools have developed exponentially in the last 5 years, with innovation in the sector set to revolutionise the current biotech landscape. In this study, we proposed a framework to increase accessibility and effectiveness of protein engineering techniques, stemming from our individual experience in the iGEM landscape. Focusing on altering ligand binding specificity, we enable users to generate a pool of combinatorial protein variants, which are then screened and ranked based on binding affinity predictions and common motifs, allowing for an output consisting of a library of 20,000 variants.

Seeing that our developed framework takes care of combinatorial mutagenesis, future work could adapt the software pipeline, simply changing the tools used for structural mutagenesis and docking/scoring without too extensive modification.

AUTHOR CONTRIBUTIONS

Shirin Bamezai: Conceptualisation; funding acquisition; investigation; methodology; writing – original draft. **Giovanni Maresca di Serracapriola**: Conceptualisation; funding acquisition; investigation; methodology; writing – original draft. **Freya Morris**: Conceptualisation; investigation; methodology; software; writing – original draft. **Rasmus Hildebrandt**: Conceptualisation; investigation; methodology; software; writing – original draft. **Marc Augustine Sojerido Amil**: Conceptualisation; investigation; methodology; software; writing – original draft. **Sporadicate iGEM Team**: Conceptualisation. **Rodrigo Ledesma-Amaro**: Funding acquisition; project administration; resources; supervision; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Access at: https://github.com/MarcAmil30/InFinity1.0.

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