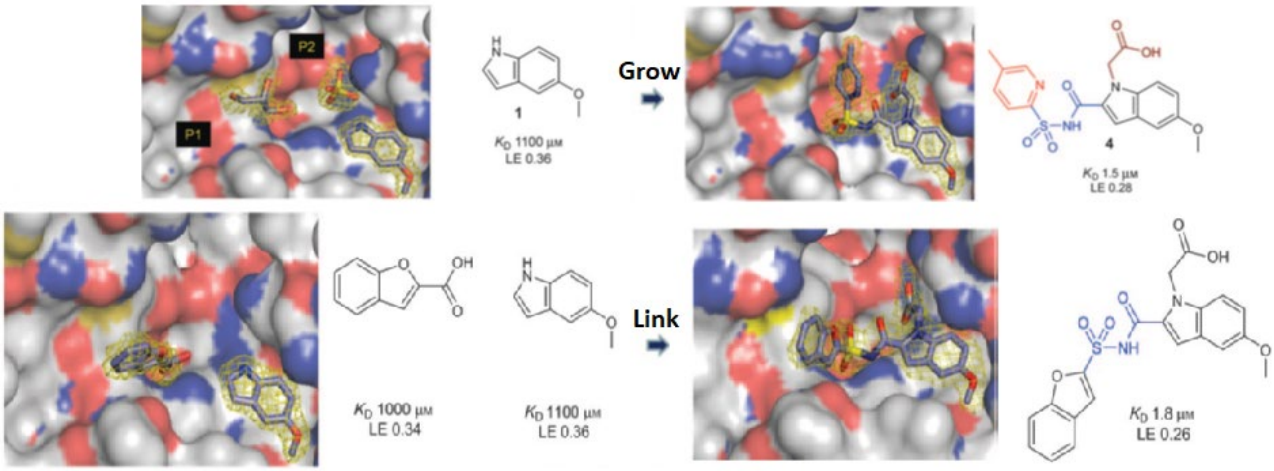

Approaches to Fragment-Based Drug Design

A comparison between fragment optimization methods

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1 SUMMARY

High Throughput Screening (HTS) has discovered a wide range of new chemical entities. However, with the exponentially increasing costs for drug development the industry is highly interested in alternative methods with lower costs. Over the past decades, Fragment-Based Drug Design (FBDD), which performs research on low molecular-weight molecules, has obtained this interest. Interestingly enough, this yielded a higher hit rate, higher binding efficiency & lower costs compared to the traditional HTS. Selected high-affinity fragments serve as great starting points for fragment optimization, a process that increases its size in the pursuit of higher potency. This study aimed to compare the widely used fragment growing and linking approaches and determine their potential for FBDD (1) (2).

After successfully comparing and assessing both optimization approaches for FBDD the following has been concluded.

- The fragment growing approach provides the most consistent results and is applicable for a wide range of targets. The precise alterations allow for a versatile exploration of the chemical space inside the target's binding pocket.
- The fragment linking approach contains a lot of potential and alterations to rapidly improve the binding affinity of fragments. Because of this potential, this optimization technique should always be taken into consideration.
- It is recommended to develop guidelines for the fragment optimization selection process to allow the suitability of each strategy to be estimated.

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1 INTRODUCTION

Hundreds of millions of dollars are invested into the development of new drugs. With the exponentially increasing costs of pharmaceutical design the industry has intensively explored alternative methods in drug design. High Throughput Screening (HTS) has discovered a fast array of new chemical entities. However, the downside of this technique is the amount of time, funding & risk required for performing experiments. After searching through huge collections of drug-sized molecules this technique might identify numerous leads & hits, however, few of these will pass clinical trials (1) (2).

Over the past decades, Fragment-Based Drug Design has become of increasing importance for the medicinal industry. An upcoming *in silico* and *In vitro* method utilizing low molecular-weight molecules which are referred to as “fragments” instead of intact compounds. It has gained interest because of its higher hit rate, higher binding efficiency & lower costs compared to the traditional HTS (1) (2).

Through the use of biochemical techniques, the potency and effectiveness of these fragments are determined. Selected through evaluation, high-affinity fragments serve as great starting points for fragment optimization methods. Using these methods the chemical space of the binding pocket can be explored and potent ligands can be developed. In this study, the fragment optimization methods growing and linking are assessed and compared on their effectiveness for FBDD.

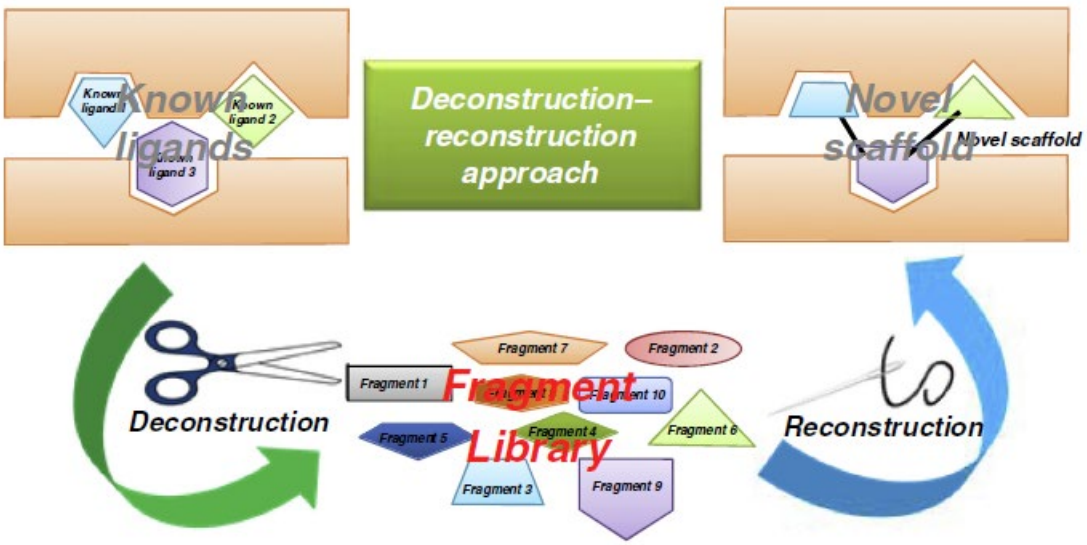
2 REVIEW SECTION

2.1 Fragment-Based Drug Design

Over the past decades, High Throughput Screening (HTS) has proven itself to be a useful approach in the discovery of new chemical entities. However, the downside of this technique is the amount of time, funding & risk required for performing experiments. After searching through huge collections of drug-sized molecules this technique might identify numerous leads & hits, however, few of these will pass clinical trials (3). Interestingly enough, screening a collection of small and simple molecules, hence the name drug "fragments", increased the amount of discovered hits compared to screening larger and more complex molecules (4). Compared to HTS, Fragment-Based Drug Discovery (FBDD) approaches have a higher hit rate, higher binding efficiency, and more effective optimization capacity. Because of this principle and its cost-effectiveness, FBDD has proven itself as an attractive alternative (3).

FBDD identifies several active fragments which can reach deep within the pocket of the active site. These identified fragments reveal useful information regarding the chemical space of the pocket, allowing the design of potent and efficient drug-like moieties. Generally, smaller fragments have more possibilities for further structural modifications, allowing them to search for more chemical space (3).

Many successful FBDD projects first selected fragments that contain high-quality interactions with the target and then further develop these fragments into complex molecules with improved potency & selectivity. In Figure 2-1 the FBDD deconstruction-reconstruction approach is shown, an alteration of the normal FBDD. This approach utilizes known ligands and a fragment library to construct potent drug-like moieties. The different segments & processes are further explained below (4).



2.1.1 ***Deconstruction***

Utilizing identified ligands containing high binding affinity to the target, knowledge regarding the chemical space and binding sites of the target can be obtained. Several fragments can be acquired through the deconstruction of these ligands. These fragments can be used to design key pharmacophores, which are of great importance for FBDD in combination with the fragment library, see Figure 2-1. The deconstruction approach is a valuable tool to probe multiple conserved and non-conserved binding pockets. Because of the reasons mentioned above, the deconstruction phase increases the likeliness of developing highly potent and efficient ligands and should be included in more standard approaches if possible (3).

2.1.2 ***Building & Selecting a fragment library***

The construction of a fragment library by screening low molecular weight compounds is usually the first step for FBDD. Fragments are detected and identified on their interaction with the macromolecule using biophysical techniques. Currently a few techniques are sensitive enough to screen weakly interactive fragments. This library is used to develop new drug-like moieties based on the pharmacophore models created after the deconstruction phase. This is done through fragment-based screening methods (FBS). Given the high sensitivity, FBS tends to deliver high hit rates, providing multiple starting points for further structural fragment optimizations. The screened compounds should be structurally diverse to cover a broad chemical diversity, allowing high-quality hits to be generated. The library composition should be carefully selected since it directly influences the quality of the outcomes (2) (3) (4) (5).

The selected fragments should ideally contain a balance between low molecular weight and large enough size to realize specific interactions with the target macromolecule. Fragments are smaller, and therefore generally more hydrophilic, compared to the drug-like moieties used in HTS. This increases the possibility of forming specific hydrogen bonds with the target. A downside to using fragments may be their ability to bind to multiple targets, making them less sensitive. However, their small size leaves many opportunities to increase selectivity through fragment optimization (2).

During drug discovery, the molecular weight and lipophilicity are often increased to improve the selectivity and affinity with the target. During this phase, the Lipinski's rule-of-five (RO5) is used as a tool to evaluate drug-likeness. However, fragment screening libraries should be more biased to lower molecular weight and lipophilicity. Hence the RO5 has been modified to Lipinski's rule-of-three (RO3), a set of rules for important physiochemical properties for FBDD library construction. In contrast to RO5, the RO3 was specifically designed as a tool for defining lead-like compounds. The RO3 can be used to gauge the expected effectiveness of fragments and improve the fragment library selection. This set of rules was stated as a useful tool for fragment selection by most medicinal chemists (3) (6). Overall, using this set of rules is recommended, however, it should not be overemphasized because of possible limitations. In addition to the RO3, the stability and synthetic routes should also be considered during the selection.

Molecules obeying Lipinski's rule-of-three contain:
Log P < 3
Molecular weight (Mw) < 300 Daltons
≤ 3 Hydrogen bond donors
≤ 3 Hydrogen bond acceptors
≤ 3 Rotatable bonds

Next to RO3, Verber's rules also reject compounds containing a polar surface area (PSA) higher than 140 angstroms. This guideline can be included next to the RO3 to improve fragment selection (3).

Fragments typically bind weakly to the target macromolecule, to increase the measurable interaction the concentration is often increased. To accomplish this a high solubility is needed, which is often correlated to a low Log P. This also shows the importance of utilizing the RO3 over the RO5 since lower lipophilicity is accepted (3).

2.1.3 ***Reconstruction***

After the deconstruction of known ligands (if possible) and the complete development of a fragment library the reconstruction phase is initiated. In this phase, the selected fragments from the library are reconstructed into new drug-like molecules and optimized using fragment optimization. During this phase, fragments are optimized to develop potent & efficient drug-like molecules with enhanced binding modes. The reconstruction phase often contains challenges, because by optimizing/further developing the fragments it is important to not disrupt the key binding modes with the target (3) and stay within the available chemical space.

The fragments are often optimized by growing and/or linking them to more complex molecules with enhanced binding affinity & selectivity to the target protein. These optimization methods are further elaborated in section 2.3 page 10. The techniques and methods to evaluate & rank the developed drug-like moieties are shown in section 2.2 page 8.

As earlier explained, the developed molecules need to be managed by the Lipinski's rule-of-three and Verber's rules guidelines to confirm suitable drug-like properties. This is crucial for the project since of the numerous hit and lead compounds few reach the market due to unfavorable absorption, distribution, metabolism, excretion and/or toxic (ADMET) properties (3).

2.2 Fragment-Hit Qualification techniques

During Fragment-Based Drug Design (FBDD) multiple fragments need to be evaluated on their ability to interact with the macromolecule. A few of the commonly used fragment evaluation techniques will be presented in this section. In sections 2.2.1 & 2.2.2, the X-ray and NMR techniques are shown respectively can determine the structural data of the protein and ligand-protein complexes. This provides insight into the mechanisms of binding. In section 0, the isothermal titration calorimetry technique is shown which thoroughly assesses the binding properties of the fragment.

*2.2.1 **X-ray crystallography***

Commonly used in structural biology and key in FBDD, X-ray crystallography can be applied to large proteins to obtain high-resolution structural data. This can be done by adding fragments to the protein solution or soaking fragments into the crystallized protein. After diffraction, the high-affinity fragments will be visible in the pocket as an electron density cloud. X-ray has become one of the preferred methods to obtain ligand-protein structures for FBDD. This technique is also used during the fragment optimization processes and the identification of hit fragments. During fragment optimization processes, the high-resolution images provide structural insight on how the fragment should be evolved. To speed up and reduce the cost of these X-ray experiments, a "cocktail" mix of, often approximately ten, different fragments are soaked into the crystal. After processing the X-ray data, the bound fragments inside the binding pocket can be identified (5).

The X-ray technique is widely applicable, but the methods however also contain their drawbacks. First of all, the protein crystals are required for X-ray crystallography. This however might prove challenging, because of the lumpy surface the proteins do not crystallize easily. In some cases, the addition of certain salts might reduce the protein's solubility and therefore aid the crystallization process. Protein targets that are unable to crystallize can not be measured using this technique. X-ray crystallography also cannot be used to determine the affinity of the bound fragments, therefore secondary methods are required like ITC, see 0 (5). The combination of two or more techniques could alleviate the cons of each technique, resulting in more promising outcomes (3).

2.2.2 **Nuclear Magnetic Resonance Spectroscopy (NMR)**

By exploiting the magnetic properties of specific atomic nuclei using NMR spectroscopy, the structural, dynamic & chemical environment of these atoms can be obtained. NMR spectroscopy was first described by S.W. Fesik *et al.* in their work "SAR by NMR", which demonstrated the first practical success of FBDD. By observing the changes in protein amide chemical shifts the presence and absence of fragments can be determined. This type of fragment screening is called protein-detected NMR, next to this type of NMR, ligand-detected NMR focuses on the changes in fragment NMR properties instead of the protein. Ligand-detected NMR cannot determine the binding site but can more precisely detect changes in fragment signals(5) (7).

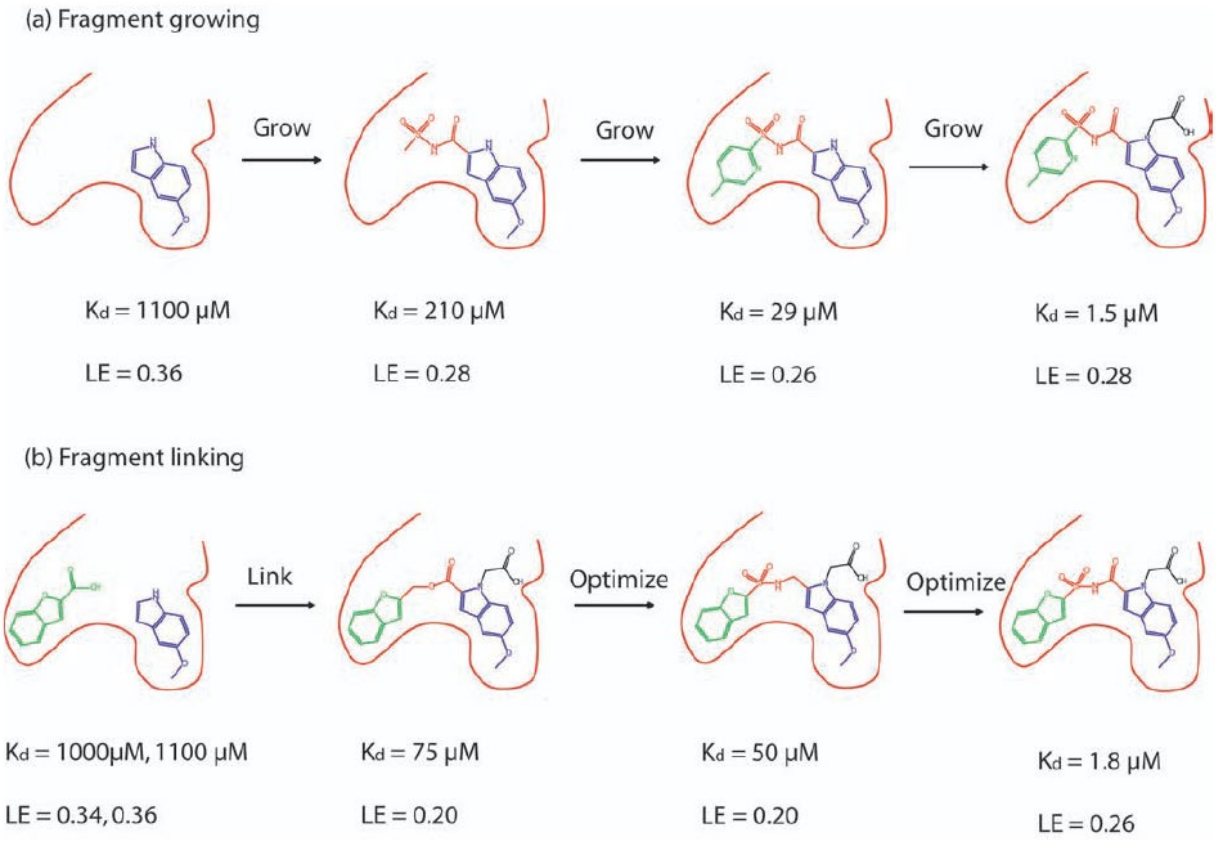
2.2.3 ***Isothermal Titration Calorimetry (ITC)***

Isothermal Titration Calorimetry (ITC) is a thermodynamic method to determine thermodynamic properties by measuring the absorbance and release of heat during a biomolecular binding event. ITC allows the measurement of binding constant (K_B), Gibbs free energy (of binding), enthalpy, entropy in one experiment. Using this data, important metrics such as the IC50 and Ligand Efficiency (LE) can be calculated. Similar to the binding constant, the IC50 is a measurement of potency. FBDD intends to increase the potency as much as possible/required. The LE is used to determine if the increase of binding affinity is justified by the increase in heavy atoms (excluding hydrogen). FBDD aims to increase the binding affinity while maintaining or increasing the LE (4). Because of these important pieces of information, ITC is widely used during FBDD to determine the binding affinity of fragments and ligands. ITC equipment like AutoITC200 allows 50-100 samples to be measured in one single day (5).

2.3 Fragment optimization methods for (re)construction

Fragment optimization methods are crucial for Fragment-Based Drug Discovery (FBDD) since it enhances the potency and selectivity of selected fragments. In this segment, the fragment optimization methods growing (a) and linking (b) are further elaborated on their potential in FBDD, see Figure 2-2 for an example. In this thesis, the method "merging" is not further explained because of its large similarities with linking. With merging two separate molecules are bound to each other where the outcome structurally looks like an overlap of both molecules. (8)

Before the optimization procedures can be initiated the starting fragment(s) has to be selected. It is recommended to perform multiple binding affinity and molecular docking experiments to ensure that the most optimal initial fragment is selected. This is the most important step since this fragment will lay the fundament for the optimization approaches. Molecular docking is performed to predict the ligand-receptor interaction and yields important information regarding the chemical space of the binding pocket. Because of this, molecular docking is also frequently performed during the optimization process.



2.3.1 ***Fragment Growing***

The most common fragment optimization approach is “fragment growing”, see (a) in Figure 2-2. A. Kumar *et al.* states that a large number of reported studies were successfully utilizing this approach. This approach adds (functional) groups to the fragment’s core to improve its potency, multiple substituents can be added to the fragment to let it grow. It is important to stay within the available chemical space of the pocket during this process. One major benefit of fragment growing is that subtle stepwise changes are performed. This allows the corresponding effects on affinity to be monitored, yielding information regarding the binding pocket of the macromolecule (2) (5) (8).

To guide the growing process and conserve the binding mode, the structural information of the binding pocket can be obtained using X-ray and NMR. Utilizing this information certain growth vectors can be assigned to the fragment. This indicates locations on the molecule where it can grow inside the pocket and form new interactions with the target molecule, improving its affinity. The identification of these growth factors might be challenging during the project, especially if co-crystal structures cannot be obtained (2) (5) (8). However, P. Kirsch *et al.* states that even without structural information fragment growing can be attempted. This of course will require more effort and is less likely to deliver successful outcomes (8).

The medicinal chemistry guidelines should always be taken into consideration during the optimization process to increase the possibilities of favorable drug-like properties.

2.3.2 ***Fragment Linking***

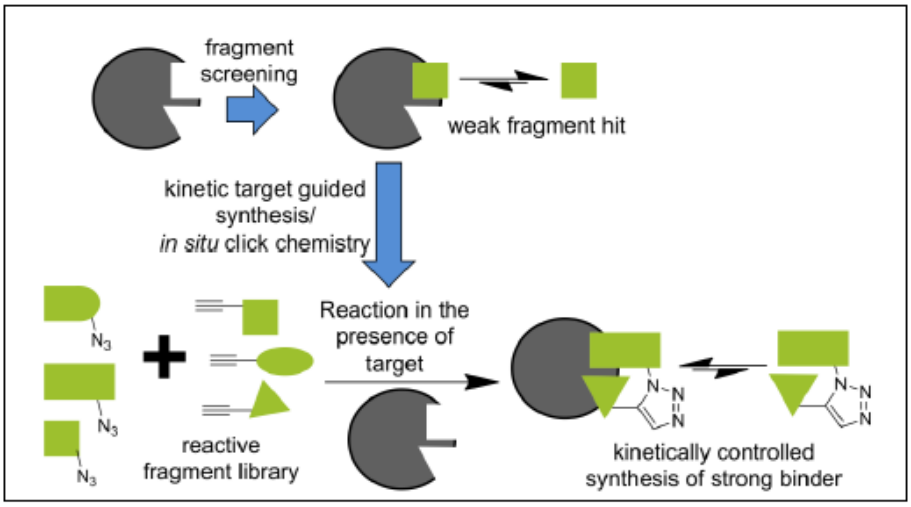
A less common fragment optimization approach is “fragment linking”, also known as “fragment joining”, see (b) in Figure 2-2. This approach was first successfully demonstrated by S.W. Fesik *et al.* utilizing “SAR by NMR” (7). Two or more fragments that occupy different adjacent sub-regions of the binding pocket must be identified, this can be realized using X-Ray crystallography or NMR. Fragment linkers are introduced to link the separate fragments (4) (5).

The fragment linking method can be a powerful approach that turns low-affinity fragments into high-affinity leads. Each individual fragment loses rotational and translational entropy after binding. By linking these fragments in an ideal way the singular compound only loses rigid body entropy once. The linker must contain a sufficient amount of flexibility to not alter the binding pose of the fragments. However, adding (too much) flexibility will also result in an entropic penalty. The additional binding energy gained is also referred to as “linker energy”. However, despite its potential B.C. Doak *et al.* also mentioned that relatively few studies apply this approach with successful outcomes. P. Kirsch *et al*. & O. Ichihara *et al*. broadly explain that the addition of a linker moiety can cause major disturbances. The linker must not disturb the original binding modes with the target. This may prove challenging since, for instance, hydrogen bonds require a specific distance and angle for full potency. In contrast, hydrophilic interactions are more tolerant to changes in binding pose. This shows that the fragment linking approach is more suitable for binding pockets which require little to no hydrogen bonding.

O. Ichihara *et al*. explains that by analyzing the binding energy characteristics of the fragments the type of binding mode can be determined (H-bond, hydrophobic, etc). They recommend applying fragment linking on one fragment with strong H-bonds and a fragment that is more tolerant with binding mode modifications (Hydrophobic, vdW, etc) (4) (2) (5) (8) (9). Linking two fragments with H-bonds will require more extensive linker design.

A protein can also be utilized as a scaffold for the fragment linking approach, also known as "fragment *in situ* self-assembly" or "Target guided synthesis" (TGS). This approach depends on the simultaneous binding of two ligands with complementary reactive groups, to adjacent sites on the protein. The co-localization of the fragments is then likely to accelerate the reaction that connects them. TGS can be used to select the optimal fragments for a given linker and *vice versa*. The most common version of TGS is the *in situ* click chemistry method, see Figure 2-3. Here the formation of stable covalent bonds between fragments is kinetically stimulated by the presence of the protein. Components used for TGS must be hydro conductible, easily accessible for library creation & must not directly react with the protein (bio-orthogonal) (4) (5).

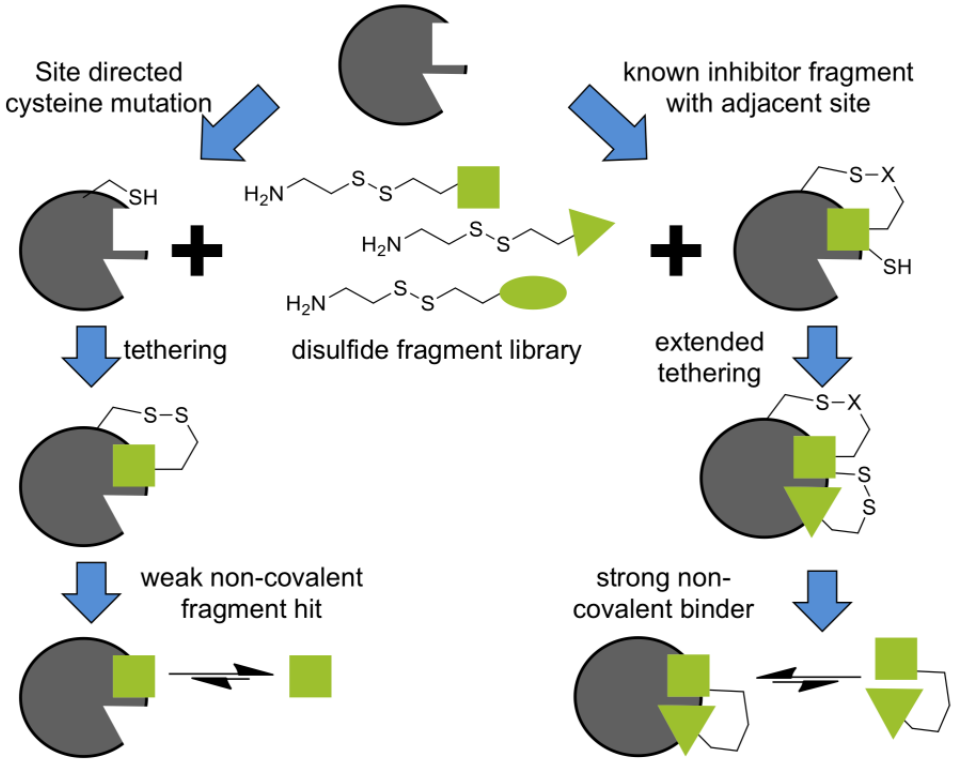
W.G. Lewis *et al*. successfully applied the *in situ* click chemistry approach where acetylcholinesterase combined azide and alkyne fragments into a very potent inhibitor (10). Utilizing the same approach, X. Hu *et al.* used BcI-X_L as a scaffold to perform an amidation reaction between sulfonyl azides and thio acids to successfully form an SMPPII, containing a higher binding affinity (11).



Another alteration of the linking approach is called tethering, see Figure 2-4. Tethering is an approach based on covalent linking to a cysteine residue adjacent to the binding site. When the cysteine residue is not present it can be introduced using site-directed mutagenesis. This technique is can be used to identify fragments that bind to allosteric sites, secondary sites & PPI interfaces. It has often been observed that the tethered disulfide-linked covalent compounds have an identical binding pose as their non-covalent fragments. If so, tethering can be used to generate structural target data where co-structures of bound fragments cannot be obtained otherwise (4).

To investigate secondary binding sites which are separated by a greater distance from the cysteine residue, the extended tethering technique was introduced, see Figure 2-4. Extended tethering utilizes a modified cysteine residue called an "extender", which has an affinity for the protein and contains its own thiol functional group. As the name suggests, the thiol of the extender unit provides another reactive site for covalent linking, which is comparable to TGS. This development strategy, however, requires an extended library of disulfide fragments (100's-1000's) next to the design and expression of the cysteine mutants needed for screening (4).

During fragment screening and optimization, the medicinal chemistry guidelines should always be taken into consideration during the process to increase the possibilities of favorable drug-like properties.

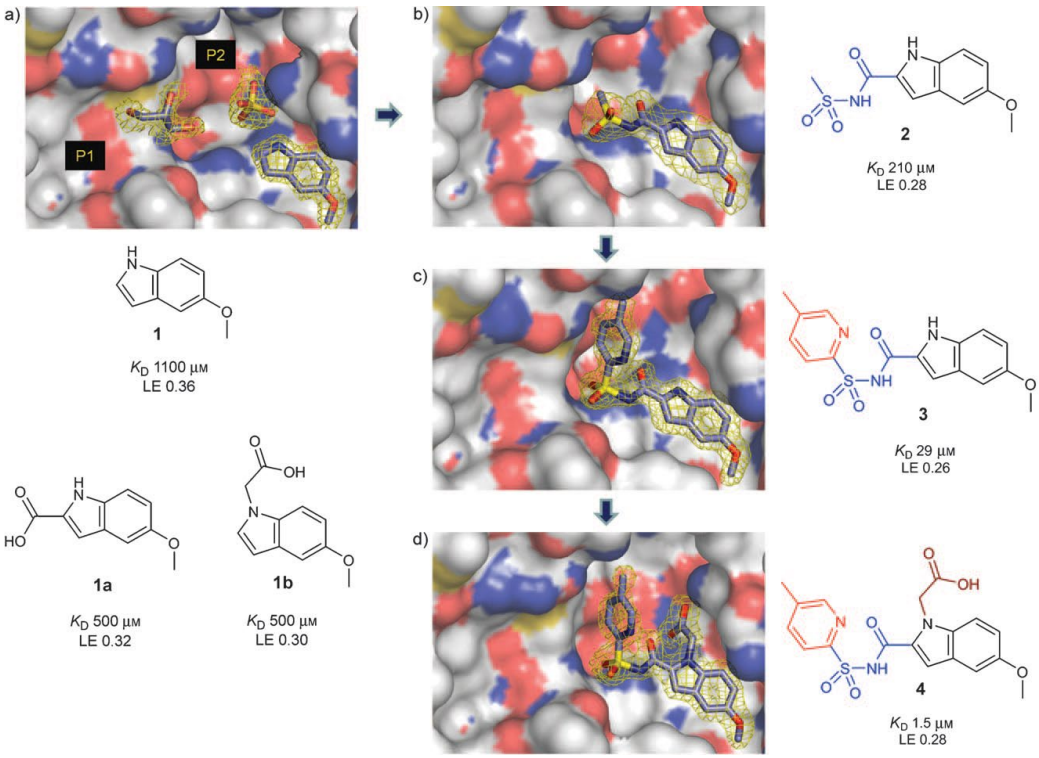


2.3.3 ***Fragment Growing vs Linking***

After individually elaborating the two fragment optimization approaches, see sections 2.3.1 and 2.3.2, both methods will be compared in this section. To develop a reliable comparison between these fragment optimization procedures, both individual procedures should be performed on the same target. A.W. Hung *et al*. selected the pantothenate synthase from Mycobacterium tuberculosis. The biophysical techniques X-ray crystallography, NMR, isothermal titration calorimetry & thermal shift assay were used for fragment screening and characterization. After determining the binding modes of the target protein, the indole fragment (K_D 1100 µM, LE 0.34) was selected for the growing approach while for the linking approach an indole and less potent benzofuran fragment (K_D 1000 µM, LE 0.36), see Figure 2-2. As seen in the figure, the indole and benzofuran fragments both interact with a different sub-region of the binding pocket. This enables the fragment linking approach to be applied (12).

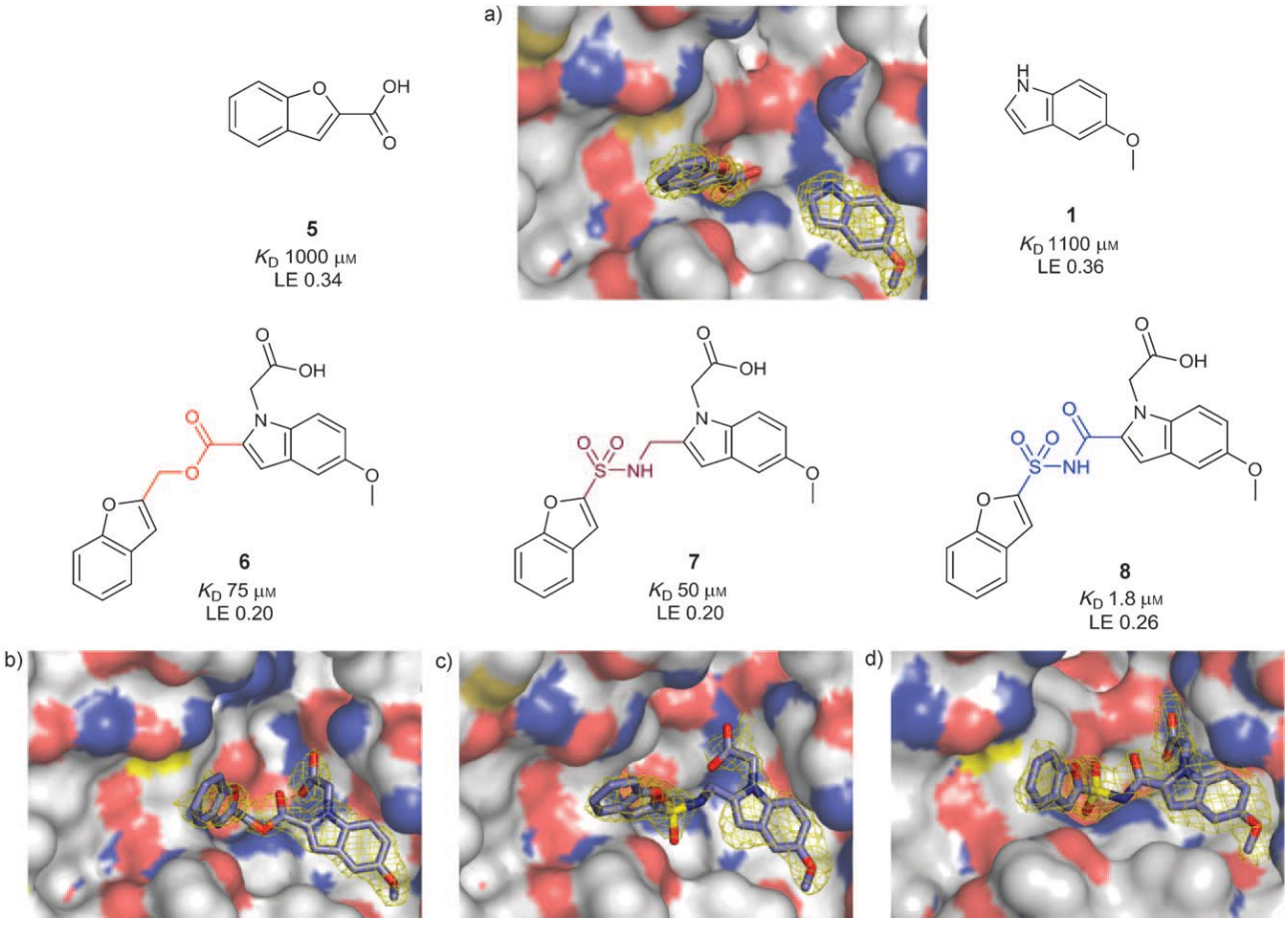
The indole fragment revealed a specific structure-activity relationship with the macromolecule. This was observed after modifying the structure of the indole fragment. The replacement of the methoxy group with a hydroxy or methyl group decreased the binding affinity 10-fold. After X-ray crystallography one hydrogen-bond interaction was observed between the methoxy group and the backbone, clarifying the structure-activity relationship. Because of this specificity in binding affinity the indole fragment was selected for fragment optimization approaches.

For the first growth step an acetate group was added, improving the binding affinity & Ligand Efficiency to K_D 500 µM, LE 0.3, see 1a & 1b in Figure 2-5. However, analysis of the structures suggests that these compounds may be too planar and rigid for the ligand to optimally interact with the P1 & P2 pockets, see Figure 2-5. To increase the flexibility of the ligand an acyl sulfonamide was introduced instead, improving the binding affinity & Ligand Efficiency to K_D 210 µM, LE 0.28, see 2 in Figure 2-5. After reviewing the crystal structure of this ligand bound to PS, this improvement can be credited to additional H-bonds between the sulfone oxygen with both the Met40 amine group and His47 nitrogen atom. The binding mode of this ligand was confluent with the indole binding mode. This confirms that the indole fragment was a good anchor for fragment optimization. After this a 4-methylpyridine group was introduced for hydrophobic interactions, see 3 in Figure 2-5, and an acetate group for more interactions with the His44 side chain, see 4 in Figure 2-5. These improvements yielded a ligand containing a binding affinity & Ligand Efficiency of K_D 1.5 µM & LE 0.28 (12).



For the linking approach, the indole and benzofuran fragments were first linked using a flexible alkyl ester into compound 6, see Figure 2-6. This ligand binds an order of magnitude better than the two fragments individually. After X-ray crystallography it was noticed that the ligand bends sharply to avoid the Met40 wall, causing the benzofuran to lose its H-bonds. To incorporate a bend and retain the H-bonds of benzofuran, the alkyl ester linker was exchanged for an alkyl sulfonamide, see 7. This improved the ligand properties to K_D 50 µM & LE 0.20. Because of its success during the growth strategy, for the final step the acyl sulfonamide was used as a linker to restrict the flexibility adjacent to the indole ring, see 8 in Figure 2-6. Removing unnecessary flexibility from the molecule also reduces the enthalpy penalty it induces. The final ligand 8 contains the following properties: K_D 50 µM & LE 0.20 (12).

A.W. *et al*. successfully linked both fragments into a ligand containing significantly improved binding properties with PS. Both the indole and benzofuran fragments interact with the target through H-bonds. O. Ichihara *et al*. would not recommend the linking approach because these types of binding modes are sensitive to structural change and can therefore prove difficult to maintain.



Generally, of both strategies the growing approach has proven itself to be more popular than the linking approach. The growing approach allows much freedom in choice for the medicinal chemist and is an attractive method when there's an obvious place to grow. Fragment linking is not as successful as growing because of challenges of introducing linkers without disturbing binding modes. O. Ichihara *et al*. emphasize that this especially becomes challenging when both fragments have specific binding modes which are sensitive to structural modifications.

A.W. *et al*. successfully developed ligands with similar binding affinity utilizing both fragment optimization techniques individually. This group also emphasizes the importance of enzyme-ligand complex analysis to terminate incorrect assumptions quickly during the optimization process. During the growth strategy, this importance was noticed after discovering growth opportunities in the P2 pocket. For the linking strategy, the complex analysis was useful for determining the conformational constraints the linkers induce. After experiments A.W. *et al*. conclude that the linking approach appears more elegant, but that the limited repertoire of linkers is likely to compromise the binding of the original fragments. While the fragment growing approach provides more freedom for optimization during the complete process and enables more room for further optimization (12). Another benefit of fragment growing is that it can be performed without X-ray and/or NMR, however, this will make the process more challenging and therefore is not recommended.

Overall, the linking method contains the potential to provide improvements to the selected fragments but, because of the risk of altering binding modes, lacks the ability to do this consistently. This approach is less ideal to incorporate H-bonds into the ligand, this could decrease the specificity of the outcome molecule making growing. It was expected that fragment linking could decrease the experimental time and resources because of its potential fast progression, however, this was not confirmed nor denied by A.W. *et al*. or other sources. The introduction of linkers can still be a challenging process because of the limited repertoire of linkers, hopefully future research will improve the number of options available. The linking approach does have alterations available like *in situ* click chemistry and tethering. The applications and usability of these alterations are expected to improve over time. Because of this, the linking approach might become more interesting in the future, currently it's recommended to keep linking as an available option.

Because of its track record, low requirements & ability to allow precise alterations for a more flexible binding site exploration, the fragment growing approach has proven itself to be the most reliable choice of both optimizations. After the literature study, it is expected that all ligands developed through linking can also be developed through growing, and not always *vice versa*. This makes the fragment growing approach very attractive.

As said above, the fragment linking approach can be interesting for certain specific conditions. Similarly for the medicinal chemistry guidelines for fragments/molecules (RO3, RO5, Verber's rules), It is recommended to develop guidelines for selecting fragment optimization techniques. For example, O. Ichihara *et al*. states that fragments with specific binding modes sensitive to structural changes are more difficult to link. These guidelines will allow the expected success to be estimated and streamline choices during optimization.

3 CONCLUSION

The fragment growing and linking approaches have successfully been compared for their potential and reliability for Fragment-Based Drug Design. Multiple literature sources have been researched and the following can be concluded:

- The fragment growing approach provides the most consistent results and is applicable for a wide range of targets. The precise alterations allow for a versatile exploration of the chemical space inside the target's binding pocket.
- The fragment linking approach contains a lot of potential and alterations to rapidly improve the binding affinity of fragments. Because of this potential, this optimization technique should always be taken into consideration.
- It is recommended to develop guidelines for the fragment optimization selection process to allow the suitability of each strategy to be estimated.

4 BIBLIOGRAPHY

(1) Wasko MJ, Pellegrene KA, Madura JD, Surratt CK. A Role for Fragment-Based Drug Design in Developing Novel Lead Compounds for Central Nervous System Targets. Frontiers in neurology 2015;6:197.

(2) Kirsch P, Hartman AM, Hirsch AKH, Empting M. Concepts and Core Principles of Fragment-Based Drug Design. Molecules 2019 -11-26;24(23).

(3) Chen H, Zhou X, Wang A, Zheng Y, Gao Y, Zhou J. Evolutions in fragment-based drug design: the deconstruction–reconstruction approach. Drug discovery today 2015 Jan;20(1):105-113.

(4) Doak BC, Norton RS, Scanlon MJ. The ways and means of fragment-based drug design. Pharmacology & therapeutics (Oxford) 2016 Nov;167:28-37.

(5) KUMAR A, VOET A, ZHANG KYJ. Fragment Based Drug Design: From Experimental to Computational Approaches. Current medicinal chemistry 2012 Oct;19(30):5128-5147.

(6) Jhoti H, Williams G, Rees DC, Murray CW. The “rule of three” for fragment-based drug discovery: where are we now? Nature reviews. Drug discovery 2013 Aug;12(8):644.

(7) Fesik SW. Discovering high affinity ligands for proteins SAR by NMR.

(8) Bian Y, Xie X(. Computational Fragment-Based Drug Design: Current Trends, Strategies, and Applications. AAPS J 2018 Apr 09,;20(3):1-11.

(9) Ichihara O, Barker J, Law RJ, Whittaker M. Compound Design by Fragment-Linking. Molecular informatics 2011 Apr 18,;30(4):298-306.

(10) Lewis WG, Green LG, Grynszpan F, Radić Z, Carlier PR, Taylor P, et al. Click Chemistry In Situ: Acetylcholinesterase as a Reaction Vessel for the Selective Assembly of a Femtomolar Inhibitor from an Array of Building Blocks. Angew Chem Int Ed 2002;41(6):1053.

(11) Hu X, Sun J, Wang H, Manetsch R. Bcl-XL-Templated Assembly of Its Own Protein−Protein Interaction Modulator from Fragments Decorated with Thio Acids and Sulfonyl Azides. Journal of the American Chemical Society 2008 Oct 22,;130(42):13820-13821.

(12) Hung AW, Silvestre HL, Wen S, Ciulli A, Blundell TL, Abell C. Application of Fragment Growing and Fragment Linking to the Discovery of Inhibitors ofMycobacterium tuberculosisPantothenate Synthetase. Angewandte Chemie (International ed.) 2009 Oct 26,;48(45):8452-8456.