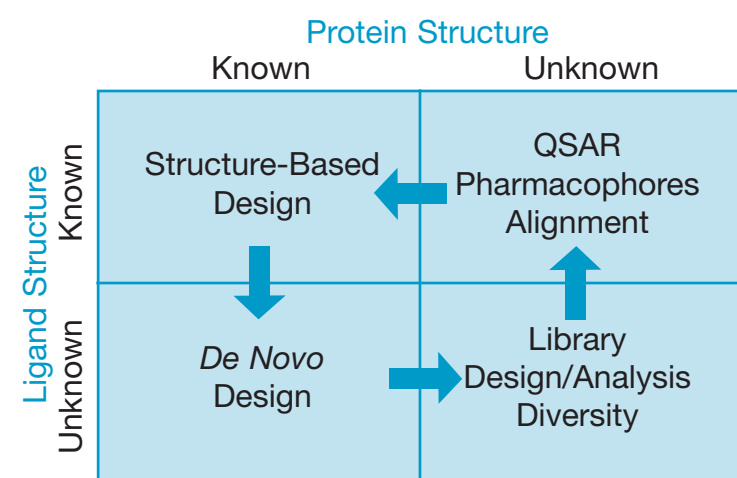


ABSTRACT

Affinity chromatography at an early stage in the processing of expressed proteins can increase yields and reduce the overall costs of goods significantly. As the therapeutic protein market becomes ever more valuable and diverse, the need for new affinity absorbents with the required selectivity and robustness increases. Prometic Biosciences Ltd is committed to the development of new stable synthetic affinity ligands derived from combinatorial ligand libraries. Synthetic affinity ligand discovery is facilitated by a ASW2000 ChemSpeed robot. This equipment allows combinatorial synthesis of libraries of potential affinity matrices to meet ever more stringent timelines for process development. This approach has now been used to identify affinity ligands for the capture of a variety of protein targets from differing sources including a tPA-Urokinase fusion protein from mammalian cell culture, and human plasma proteins including fibrinogen, plasminogen, α -1-Antitrypsin, and vWF/FactorVIII complex. Custom Research and Development Programmes have afforded affinity matrices capable of delivering considerable cost savings to end users of the absorbent.

DESIGN TOOL SELECTION

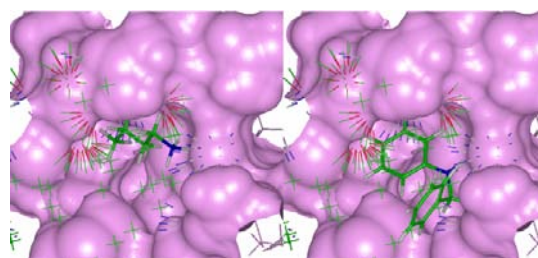


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DESIGN TOOLS

De Novo Design

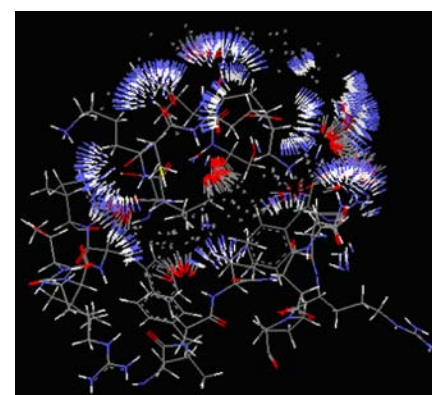
- Identify protein-ligand interactions necessary for strong binding
- Fit potential ligands into the binding site and score with tools including Ludi



Known Interaction Site

Structure-Based Design

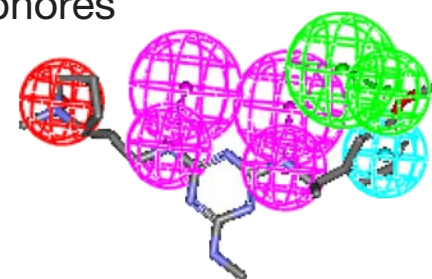
- Use the binding site to select compounds from the virtual library
- Interaction map defines site features



- Lipophilic
- Hydrogen donor
- Hydrogen Acceptor

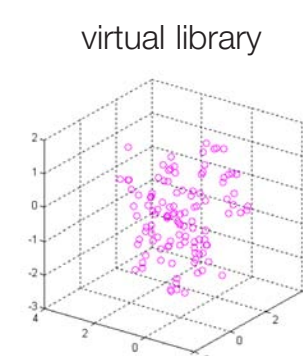
QSAR Pharmacophores Alignment

- Find chemical features shared by a series of active molecules
- Use the pharmacophore generated to search a database of compounds for predicted actives



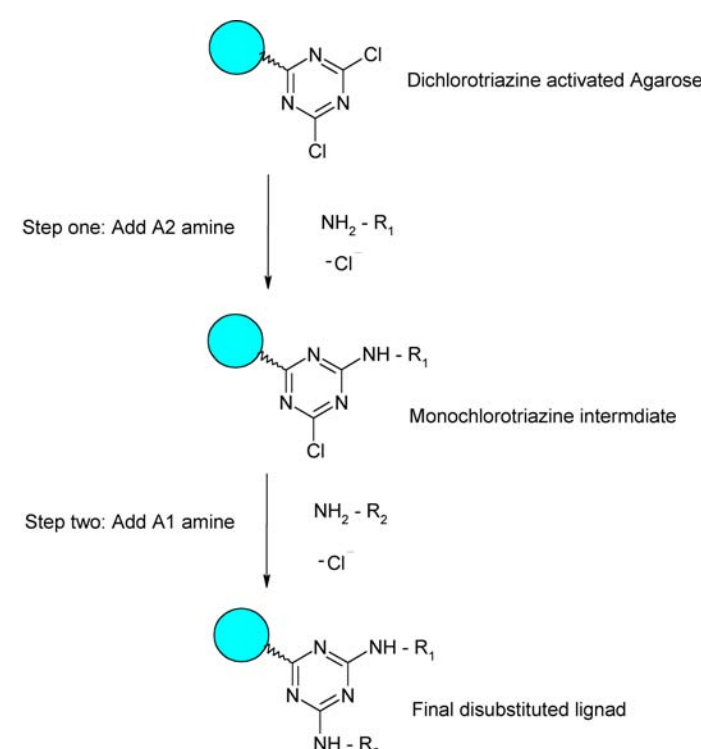
Diversity Selection

- Default spatial descriptors calculated for ProMetic Virtual Library of >50,000 compounds using Accelrys Cerius2 software
- Diverse combinatorial selection made using these descriptors



TRIAZINE LIGAND LIBRARIES

Combinatorial libraries can be constructed containing multidimensional triazine ligands derived from amines with chemically diverse 'R' groups. They are obtained by stepwise reaction of amine-containing compounds with immobilized di-chlorotriazine groups [1]. Such Mimetic™ ligands have proven utility for downstream process applications and are inherently inert due to the lack of scissile bonds. 96 well, fritted plate libraries are used in the synthesis process to produce an 8 by 8 array of 64 different compounds.



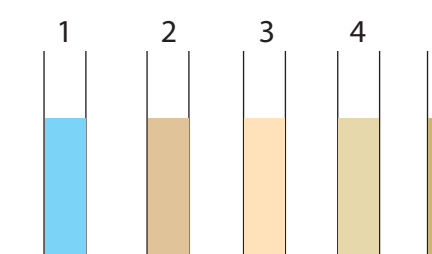
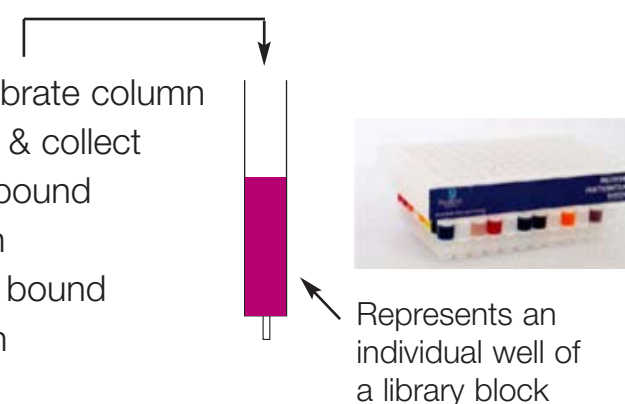
CHEMSPEED



An ASW2000 instrument is primarily used for time consuming liquid dispensing operations and is often left to run unattended. An array of 8 x 27 mL double jacket reactors, the vortex and a Huber Unistat are also required to carry out a set of intermediate reactions in the synthesis process. Applications typically range between 4 and 9 hours in length, but offer considerable time savings when compared to the manual method.

SCREENING

- Equilibrate column
- Load & collect non bound
- Wash
- Elute bound
- Clean



Each well of the library block contains 250 μ L of absorbent. The block is screened using a set of standard conditions across the whole plate and the whole screen. Screening is a simple process carried out under gravity. A Tecan Genesis liquid handling work station set up in a chromatography mode is used to automate the process. Equilibration and elution buffers are selected to maintain the target protein integrity during binding and elution.

CANDIDATE SELECTION

To identify candidates for selection the amount of target protein in the elution fractions from each well of a single library is measured. The example below shows a set of results measured by ELISA.

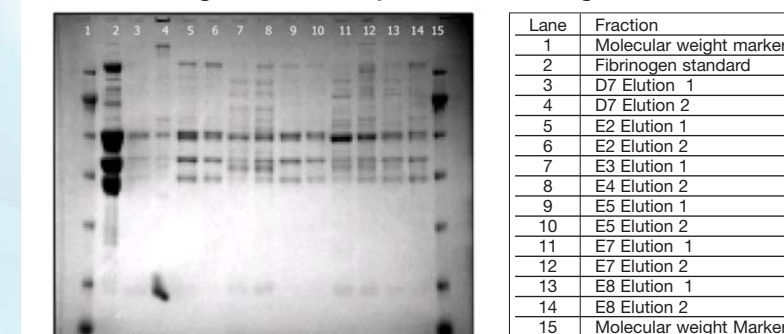
	1	2	3	4	5	6	7	8	scale lowest
A	41	19	99	21	>500	15	98	142	highest
B	250	38	134	26	>500	45	76	57	
C	39	21	157	27	>500	27	63	166	
D	>500	33	333	15	>500	34	138	52	
E	60	>500	137	>500	83	43	223	>500	
F	>500	9	57	11	>500	66	67	44	
G	386	26	51	10	417	73	92	29	
H	>500	14	35	14	388	65	120	34	

(μ g/mL) Target in elution

Sub-Library Screening for Fibrinogen recovery from human plasma

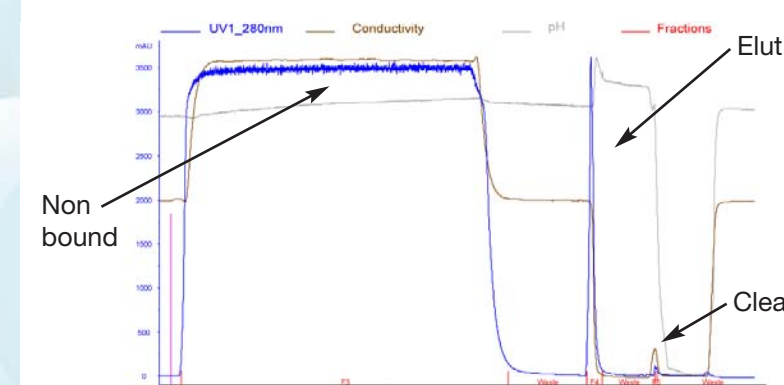
Candidate fractions showing high recovery of target protein are run on SDS PAGE to assess purity.

Reducing SDS-PAGE (10% Bis-Tris) for selected fractions from Fibrinogen Sub-library #172 screening



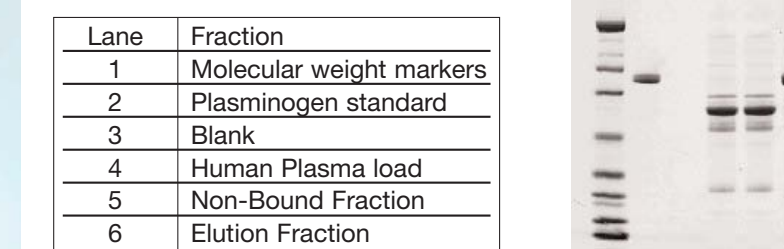
The most promising of these candidates were scaled up, and their performance verified by column chromatography.

PERFORMANCE VERIFICATION



Example: Chromatographic purification of plasminogen directly from human plasma using ligand #254/F4

- Capacity: 5 mg/mL
- Purity: \geq 95%
- Recovery: 90%
- Loading flow rate: 100 cm/hr
- Alkali Stable (0.5 M NaOH)



SUMMARY

By screening combinatorial libraries of triazine based affinity ligands, compounds can be identified which provide improved capture and purification of diverse proteins from relatively clean cell culture media or highly complex feedstock such as plasma. This work demonstrates the benefits of an automated combinatorial approach to ligand discovery and the utility of triazine ligands in downstream process applications for purification of therapeutic proteins.

REFERENCES

- Patent applications PCT/DK96/00399, WO2004/052870
- We thank Dev Baines and Steve Burton for their assistance in this work