Rethinking Library Strategy for Drug Discovery

LS: Mar 22, 2017

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Failure of Early-Stage Combinatorial Chemistry

"The perceived failure of combinatorial chemistry, for example, was primarily due to unrealistic expectations. Provided a large enough library was screened, it was assumed that useful leads would emerge, causing an unhealthy emphasis on compound numbers rather than quality or purity."[1)]

Several new methodologies arose from the failure[2)]

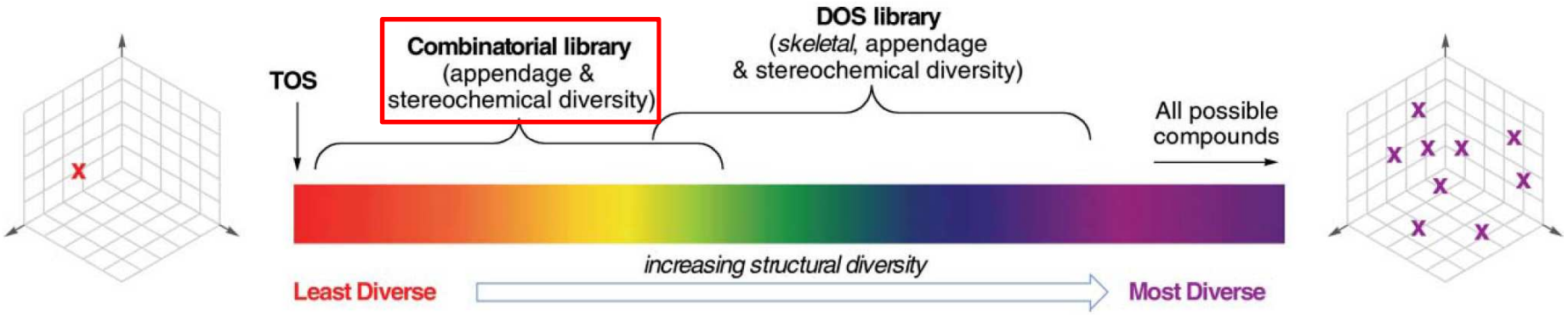
- **diversity-oriented synthesis (DOS)**
- **biology-oriented synthesis (BIOS)**

rethinking the future of focused (combinatorial) library in drug discovery field

1) Ortholand, J.-Y.; Ganesan, A. *Curr. Opin. Chem. Biol.* **2004**, *8*, 271. 2) see also Hoshikawa, T. LS 20080830.

Molecular Diversity Spectrum

1)



Focused library shares common skeleton

Focused library strategy is suitable for oligomeric compounds such as peptides, peptidomimetics, and nucleic acids, because these classes of compounds are able to be synthesized by reliable reactions

1) Spandl, R. J.; Bender, A.; Spring, D. R. *Org. Biomol. Chem.* **2008**, *6*, 1149.

Drug-Like Chemical Space: Lipinski's Rule of Five

Lipinski's rule of five for oral medication:[1)]

Mw <500

H-bond donors <5

H-bond acceptors <10

clogP <5

Many types of oligomeric compounds violate Lipinski's rules

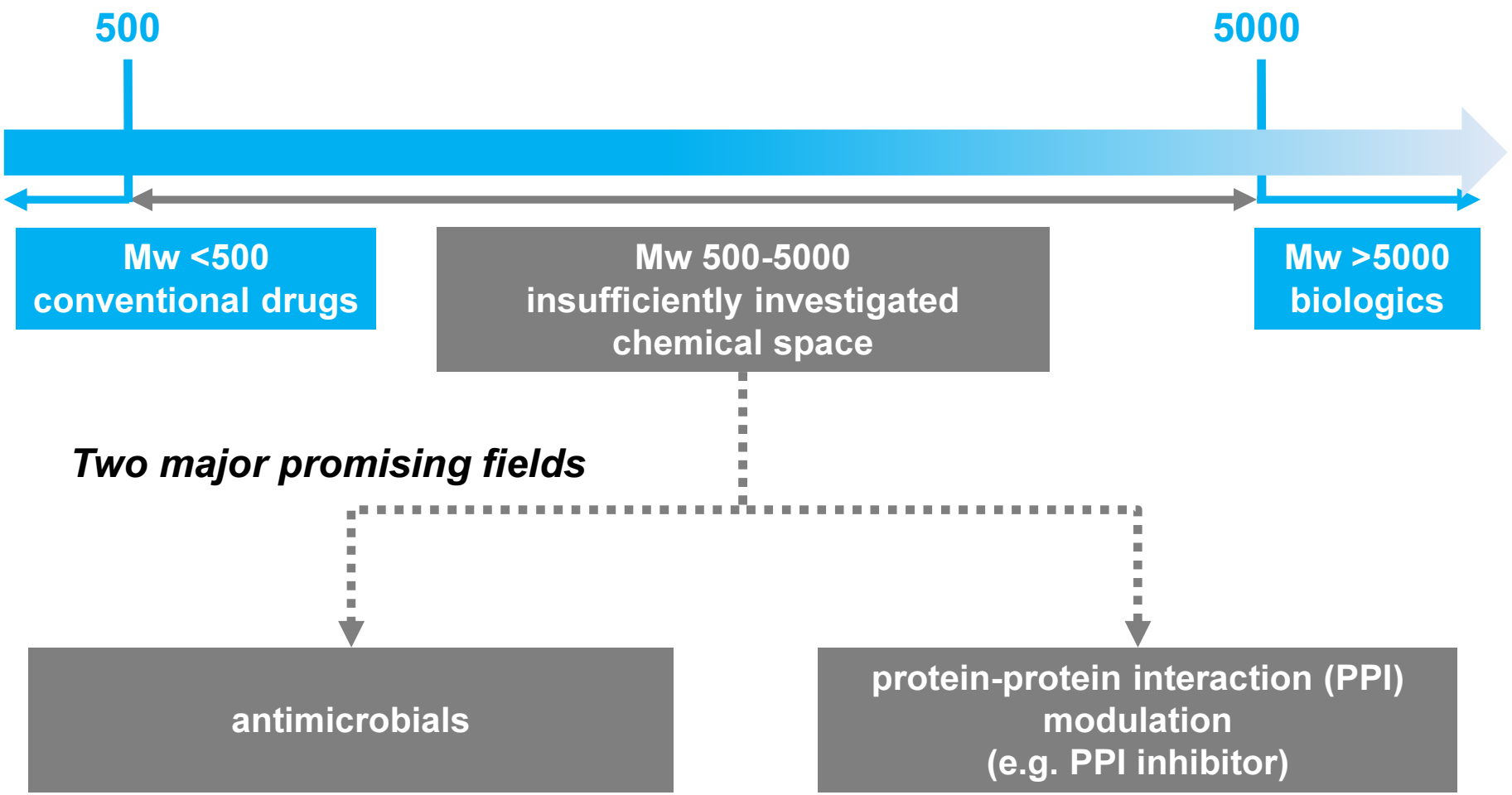
larger molecular mass (>500)

many H-bond donors/acceptors (amides, OH etc.)

Synthetic libraries had been constructed as small molecules for conventional druggable targets

1) a) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug. Delivery Rev.* **1997**, *23*, 3; b) Lipinski, C. A., *Drug Discovery Today: Technologies* **2004**, *1*, 337.

Redefinition of “Drug-Like” Chemical Space



Emergence of Antibiotic-Resistant Bacteria

Weekly July 5, 2002 / Vol. 51 / No. 26

Staphylococcus aureus Resistant to Vancomycin — United States, 2002

Staphylococcus aureus is a cause of hospital- and community-acquired infections (*1,2*). In 1996, the first clinical isolate of *S. aureus* with reduced susceptibility to vancomycin was reported from Japan (*3*). The vancomycin minimum inhibitory concentration (MIC) result reported for this isolate was in the intermediate range (vancomycin MIC=8 μg/mL) using interpretive criteria defined by the National Committee for Clinical Laboratory Standards (*4*). As of June 2002, eight patients with clinical infections caused by vancomycin-intermediate *S. aureus* (VISA) have been confirmed in the United States (*5,6*). This report describes the first documented case of infection caused by vancomycin-resistant *S. aureus* (VRSA) (vancomycin MIC ≥32 μg/mL) in a patient in the United States. The emergence of VRSA underscores the need for programs to prevent the spread of antimicrobial-resistant microorganisms and control the use of antimicrobial drugs in health-care settings.

appeared infected. VRSA, vancomycin-resistant *Enterococcus faecalis* (VRE), and *Klebsiella oxytoca* also were recovered from a culture of the ulcer. Swab cultures of the patient's healed catheter exit site and anterior nares did not grow VRSA. To date, the patient is clinically stable, and the infection is responding to outpatient treatment consisting of aggressive wound care and systemic antimicrobial therapy with trimethroprim/sulfamethoxazole.

- **Vancomycin-resistant *S. aureus* (VRSA) infection was firstly reported in 2002[1)]**
- **Development of new antimicrobials for emerged drug resistance is urgently required**

1) *Morbidity and Mortality Weekly Report* 2002, *51*, 565.

WHO Priority List of Antibiotic-Resistant Bacteria

Publication: Feb 27, 2017

Priority 1: Critical

***Acinetbacter baumanii*, carbapenem-resistant**
***Pseudomonas aeruginosa*, carbapenem-resistant**
***Enterobacteriaceae*, carbapenem-resistant, 3rd generation cephalosporin-resistant**

Priority 2: High

***Enterococcus faecium*, vancomycin-resistant**
***Staphylococcus aureus*, methicillin-resistant, vancomycin Intermediate and resistant**
***Hilicobacter pylori*, clarithromycin-resistant**
***Campylobacter*, fluoroquinolone-resistant**
***Salmonella* spp., fluoroquinolone-resistant**
***Neisseria gonorrhoeae*, 3rd generation cephalosporin-resistant, fluoroquinolone-resistant**

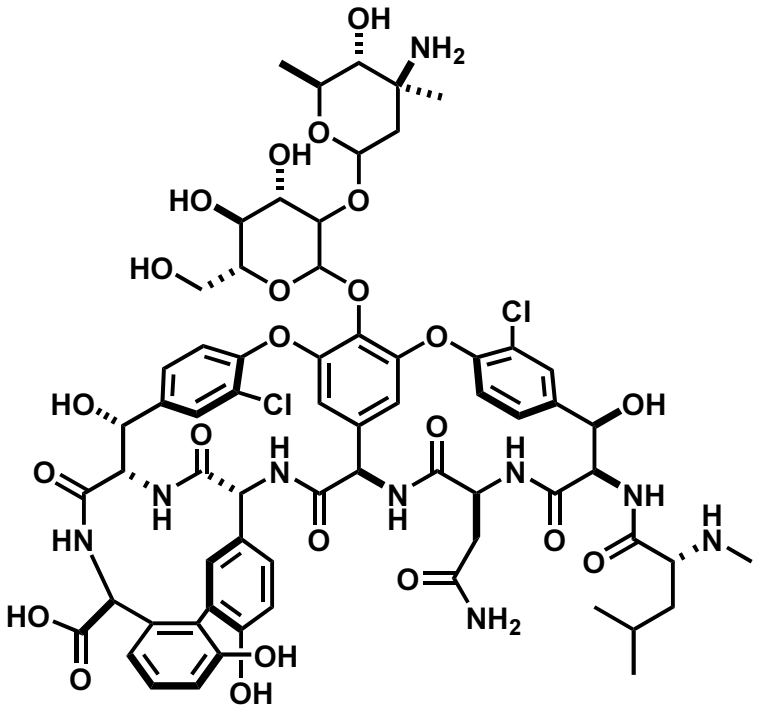
Priority 3: Medium

***Streptococcus pneumoniae*, penicillin-non-susceptible**
***Haemophilus influenzae*, ampicillin-resistant**
***Shigella* spp., fluoroquinolone-resistant**

1) http://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/

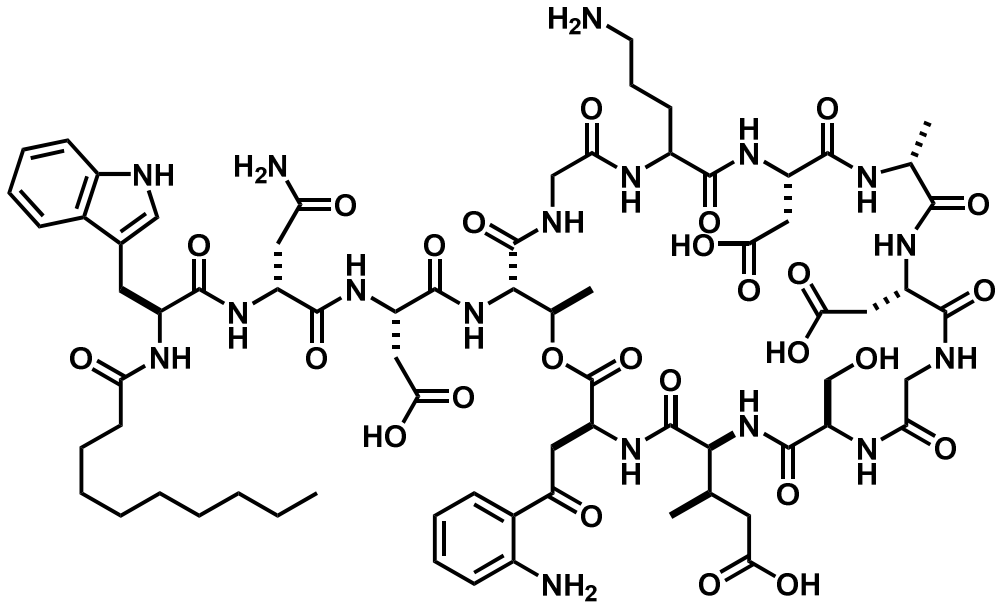
Chemical Diversity of Antimicrobials Is Different to Other Drugs

glycopeptides



vancomycin: Mw 1449.3

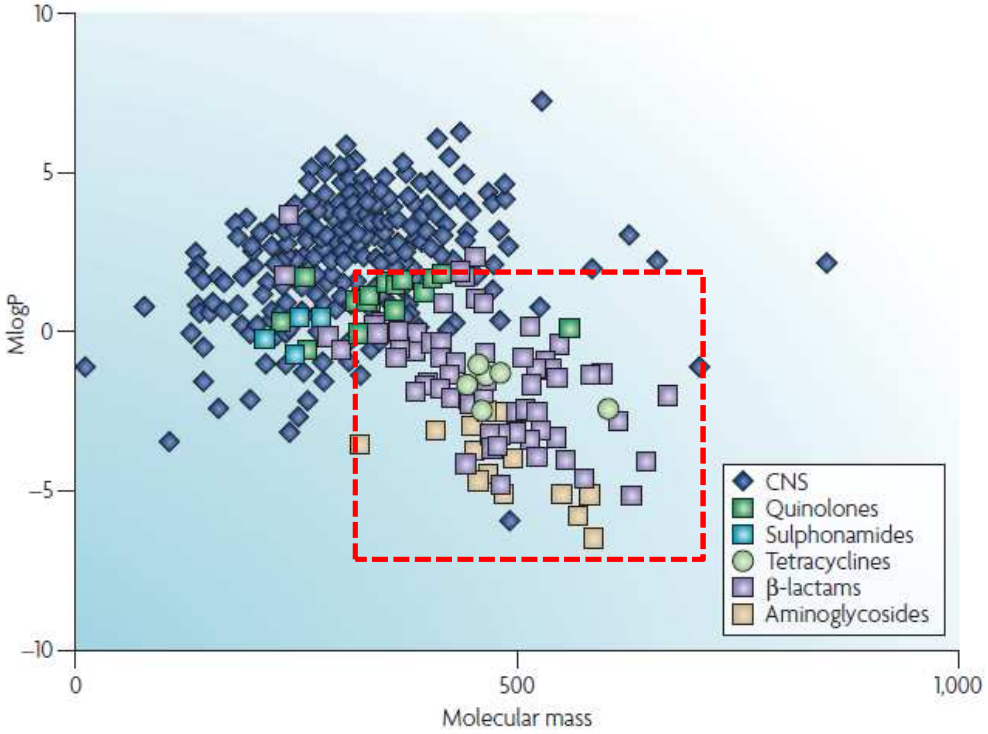
lipopeptides



daptomycin: Mw 1619.7

Chemical Diversity of Antimicrobials Is Different to Other Drugs

MlogP vs molecular mass of marketed drugs[1)]



Other classes of antibiotics:

macrolides (clarithromycin): Mw 748.0

glycopeptides (vancomycin): Mw 1449.3

lipopeptides (colistin): Mw: 1155.4

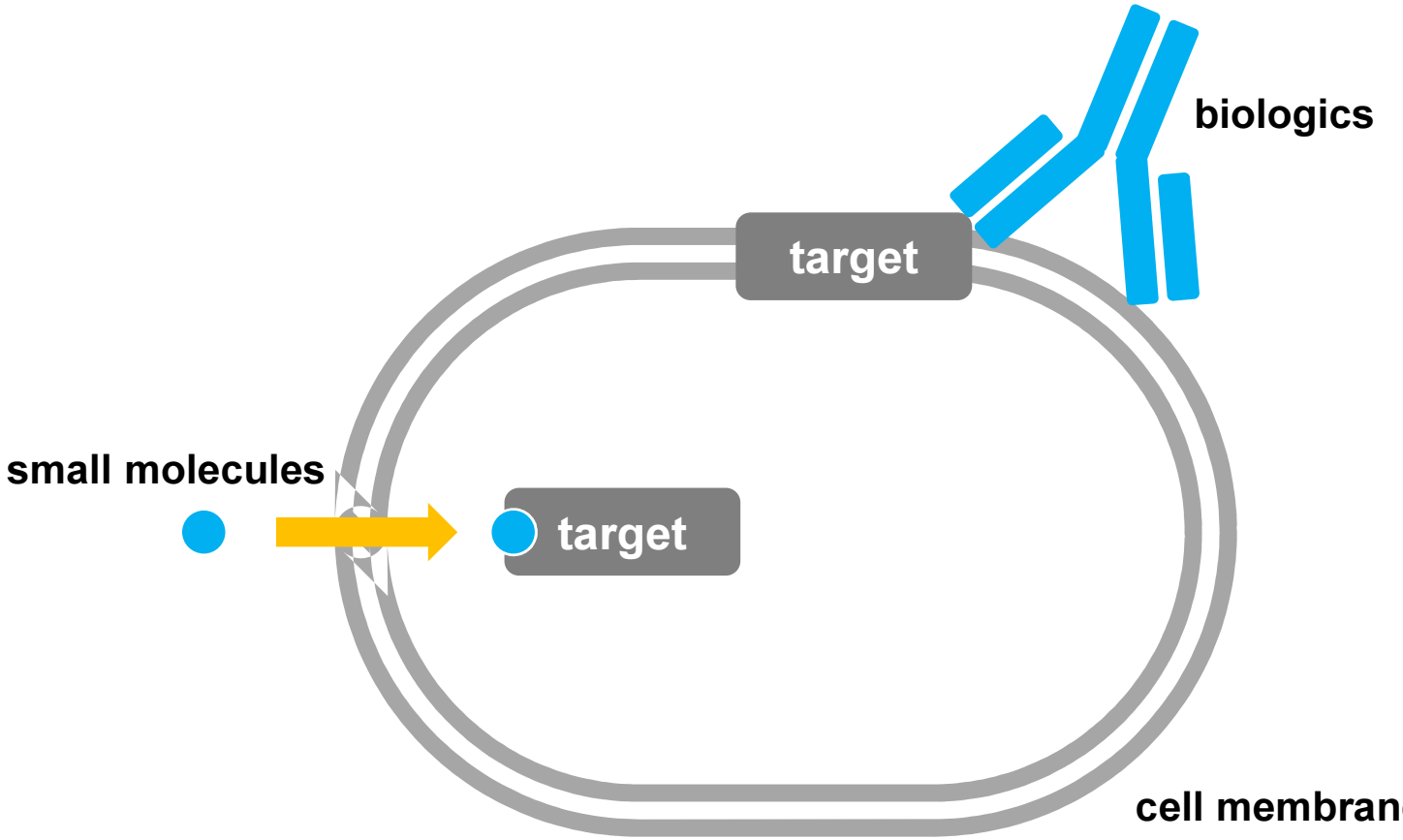
(daptomycin): Mw 1619.7

Known antibacterials do not generally follow Lipinski's rule of five[1)]

"Compound libraries are typically focused by filters such as Lipinski's rules on desirable physicochemical properties to improve the likelihood of oral bioavailability. However, these rules are not useful for identifying good antibiotic lead compounds, which need to satisfy a different requirement"[2)]

1) Payne, D. J.; Gwynn, M. N.; Holmes, D. J.; Pompliano, D. L. *Nat. Rev. Drug Discov.* **2007**, *6*, 29.
2) Lewis, K. *Nat. Rev. Drug Discov.* **2013**, *12*, 371.

“Druggable” Target



1. Extended/shallow protein surface is difficult to be bound by small molecules
2. Use of biologics is restricted for extracellular targets (no membrane permeability)

Challenging intracellular targets are not approachable for small molecules and biologics[1)]
They had been categorized into “undruggable” targets

1) Cromm, P. M.; Spiegel, J.; Grossmann, T. N. *ACS Chem. Biol.* **2015**, *10*, 1362-1375.

Larger Molecules for PPI Modulators

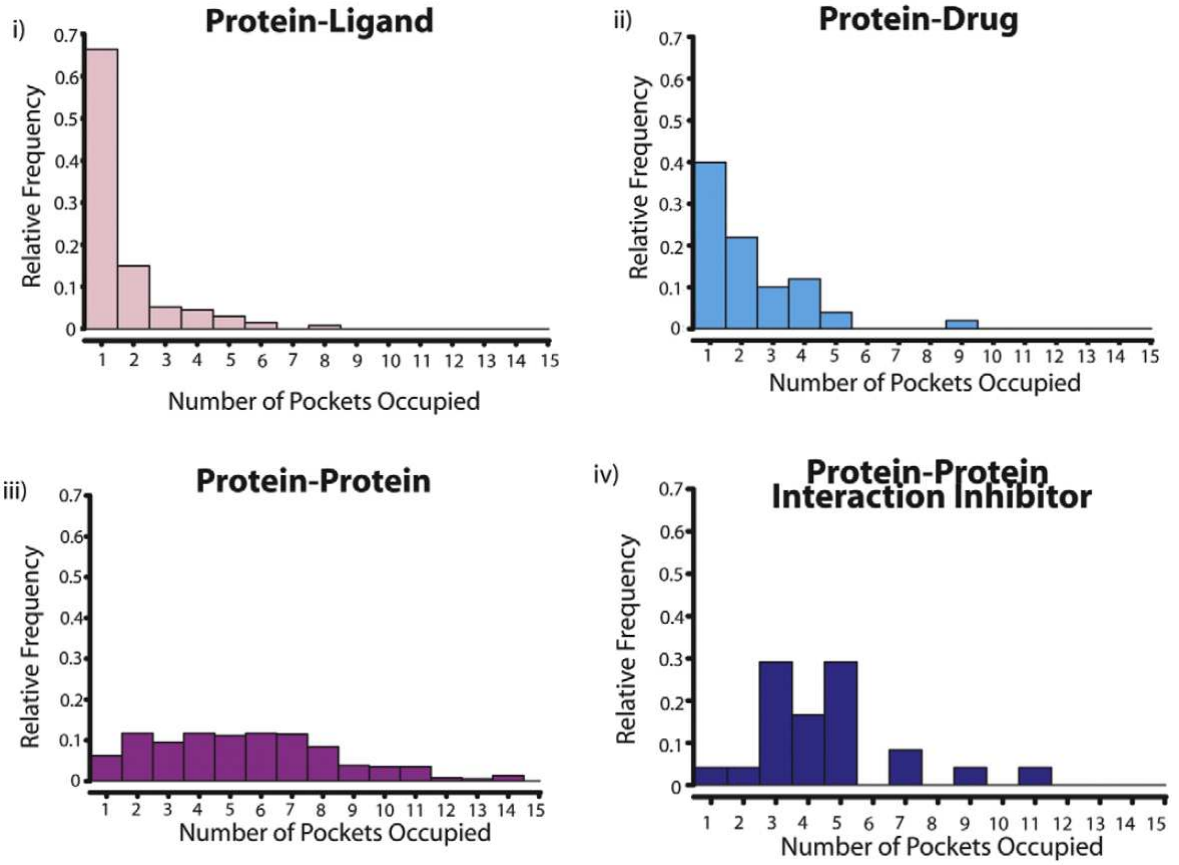
Protein-ligand interaction (134 complexes)

Average pocket volume: 77 angstrom^3
Average volume of occupied pockets: 260 angstrom^3

Protein-protein interaction (96 complexes)

Average pocket volume: 55 angstrom^3
Average volume of occupied pockets: 54 angstrom^3

number of occupied pockets



PPI modulators can achieve adequate affinity by being large enough to reach several smaller pockets of protein surface

1) Fuller, J. C.; Burgoyne, N. J.; Jackson, R. M. *Drug Discov. Today* **2009**, *14*, 155-161

Quality over Quantity...Is It Enough?

Possible size of "focused library" of a larger molecule should be vast.

Reliable synthetic methods and efficient screening are necessary.

Mw 500-5000
insufficiently investigated chemical space

good (biologically relevant) scaffold

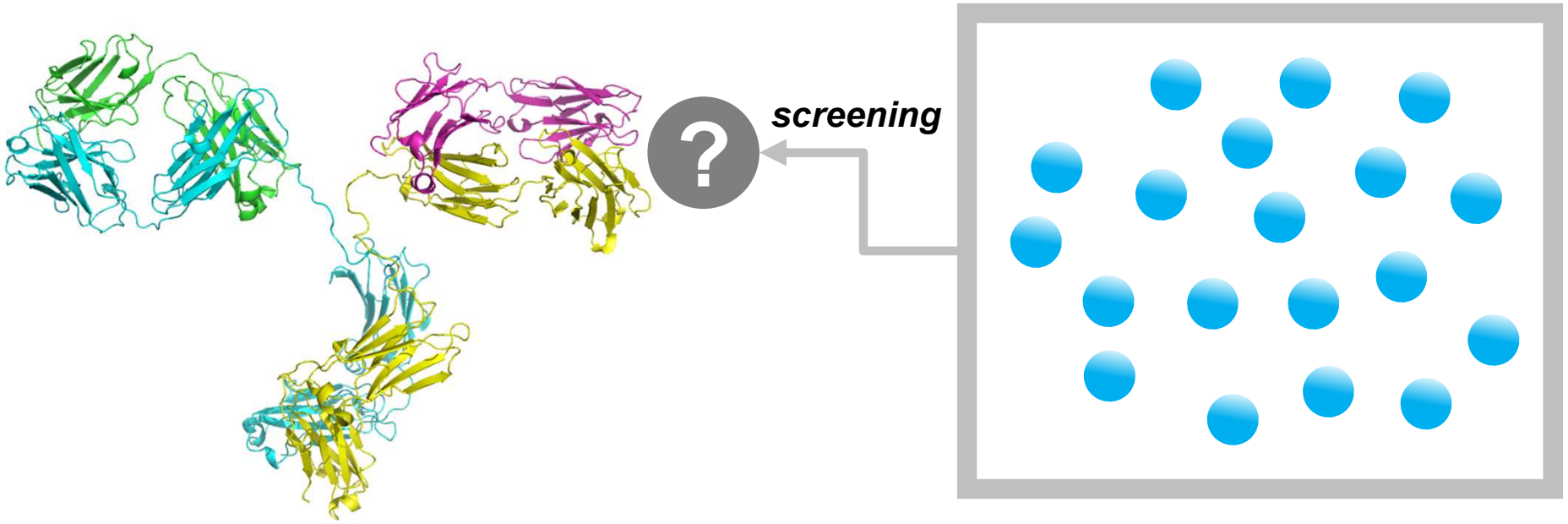
good biological target

practical synthetic methods for quality and quantity

practical and efficient screening methods (HTS, high sensitivity)

practical "decoding" (structural determination) method

Antigen Surrogates



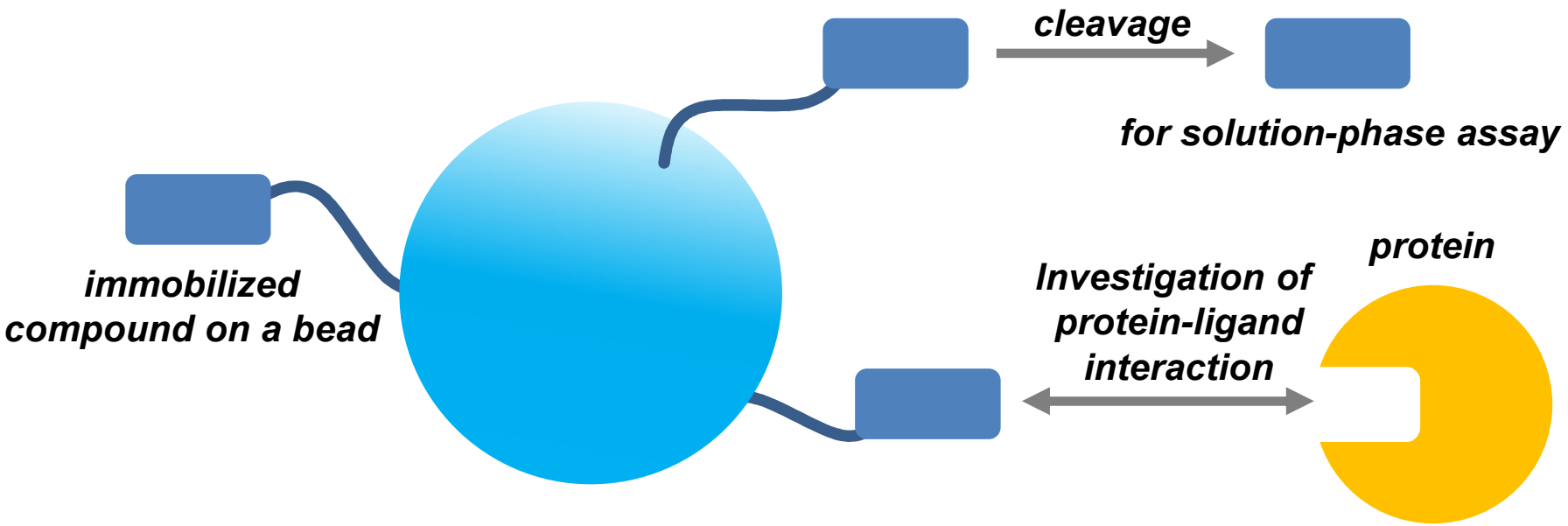
antibody (PDB ID: 1IGT)

bead-based library

1) Doran, T. M.; Sarkar, M.; Kodadek, T. *J. Am. Chem. Soc.* **2016**, *138*, 6076-6094.

Efficient Synthesis and Screening

Immobilized compounds are suitable to binding assay
(Washed beads after binding assay can be used for other assays)



OBOC library: bead-based library, which is constructed by split-pool synthesis[1)]

Easy separation of the beads before/after assay

(cf. SPR, BLI, ELISA etc.)

Beads are potentially compatible with fluorescent microscopy and flow cytometry

Successful application: protein-oligomeric ligand interaction

1) Lam, K. S.; Lebl, M.; Krchňák, V. *Chem. Rev.* **1997**, *97*, 411.

Chronic Lymphocytic Leukemia

Chronic lymphocytic leukemia (CLL)[1)]

- **CLL is the most common leukemia in the Western countries (4.2:100,000 per year)**
  ***CLL is rarely seen in Asia and Africa (0.3:100,000 per year in Japan)**
- **The median age at diagnosis is 72 years**
- **About 10% of the CLL patients are reported to be younger than 55 years**

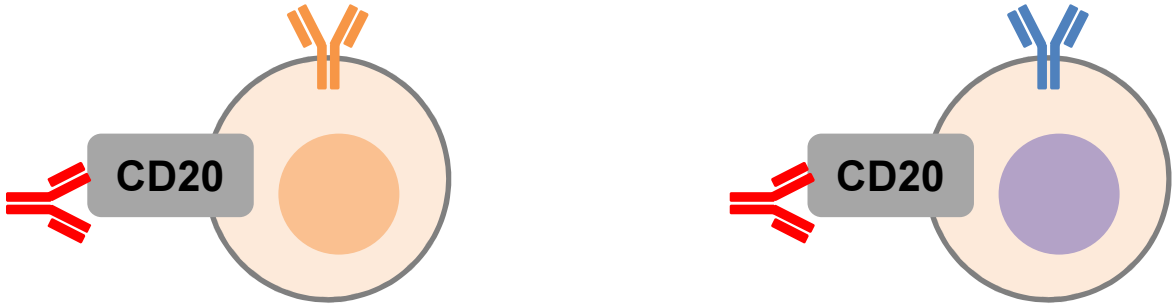
- **CLL B cells co-express the CD5 antigen and B-cell surface antigen CD19, CD20, and CD23**

1) Eichhorst, B.; Robak, T.; Montserrat, E.; Ghia, P.; Hillmen, P.; Hallek, M.; Buske, C.; on behalf of the ESMO Guidelines Committee *Ann.Oncol.* **2015**, *26 (supplement 5)*, v78.

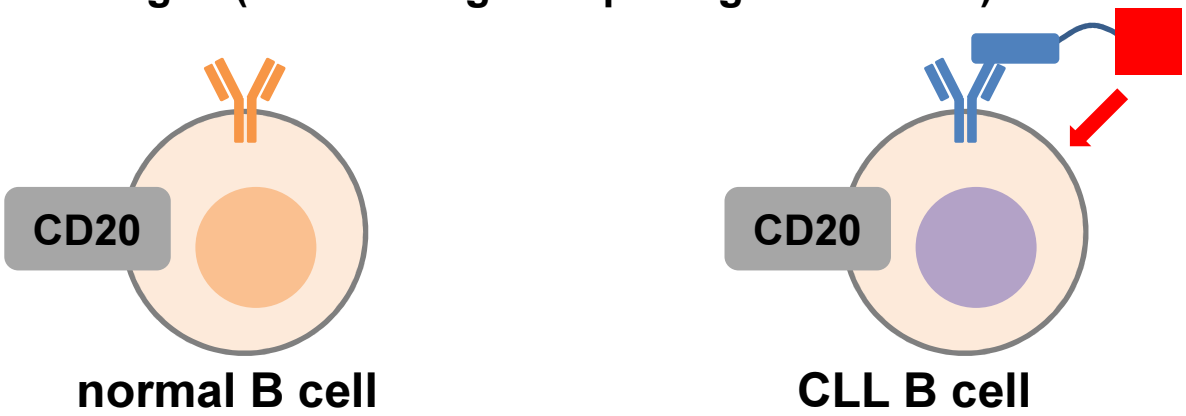
Aim of the Study

- CLL is often treated with immunosuppressive compounds such as monoclonal antibody (rituximub)
- Rituximub eliminate all B cells (CD20 is also expressed on normal B cells)
- Antigen-specific surface membrane immunoglobulin (smIg) component of the B cell receptor is intriguing target

Rituximub (no selectivity)



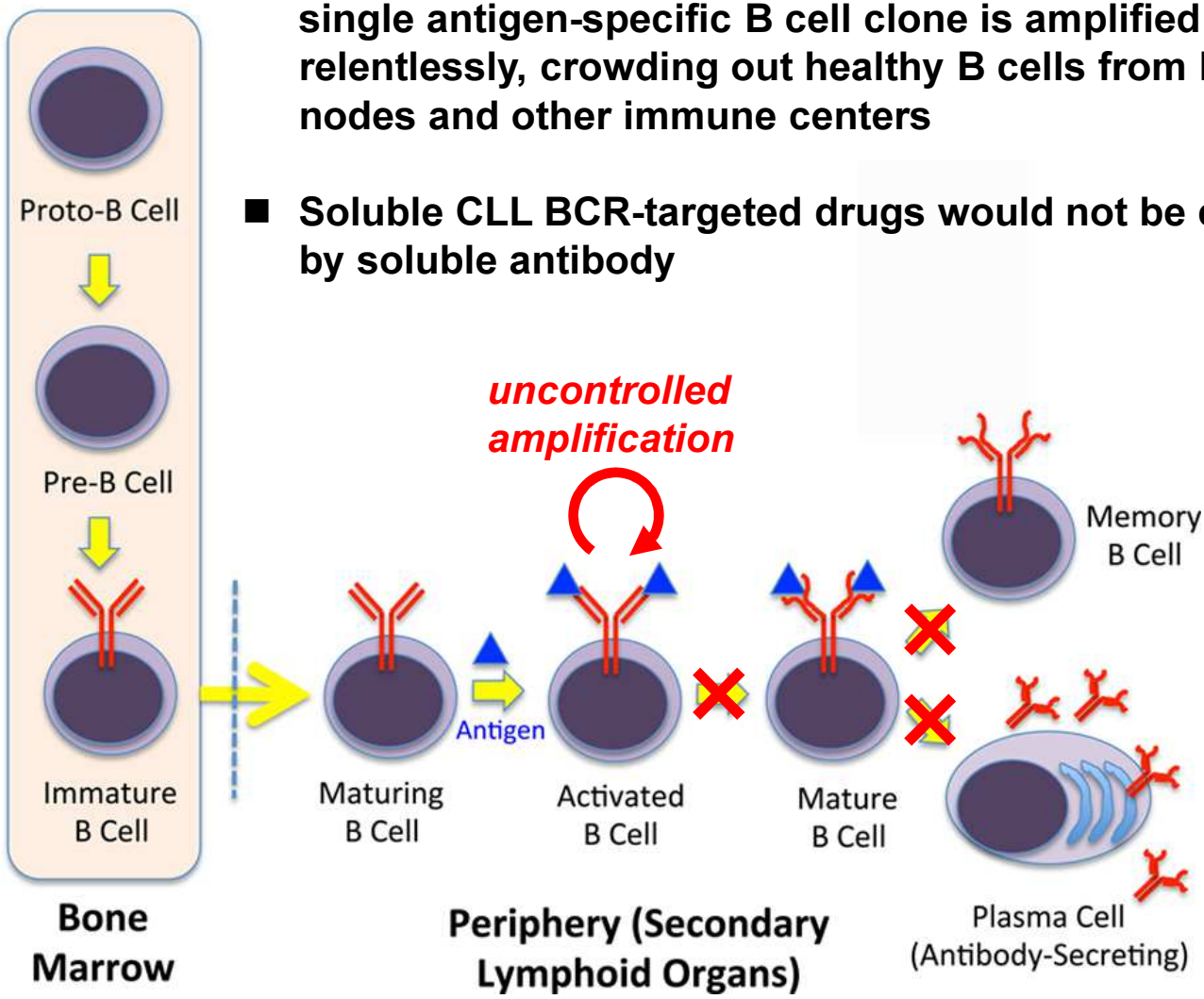
CLL B cell receptor antigen (selective against pathogenic B cells)



1) Sarker, M.; Liu, Y.; Morimoto, J.; Peng, H.; Aquino, C.; Rader, C.; Chiorazzi, N.; Kodadek, T. *Chem. Biol.* **2014**, *21*, 1670.

Development of B Cells and CLL

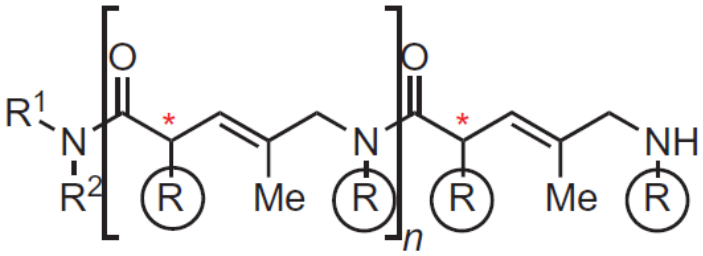
- **CLL is a hybrid of cancer and autoimmune disease, a single antigen-specific B cell clone is amplified relentlessly, crowding out healthy B cells from lymph nodes and other immune centers**
- **Soluble CLL BCR-targeted drugs would not be captured by soluble antibody**



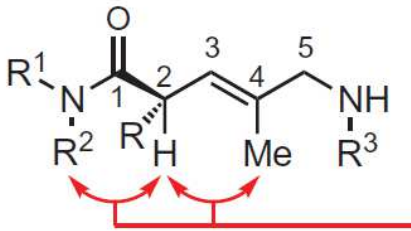
1) Doran, T. M.; Sarkar, M.; Kodadek, T. *J. Am. Chem. Soc.* **2016**, *138*, 6076.

COPAs

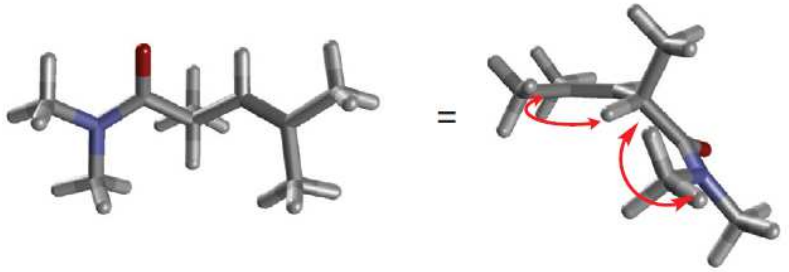
COPA: chiral oligomers of pentenoic amides[1)]
(polyketide-inspired oligomeric molecules)



Chiral oligomers of pentenoic amides
(COPAs)



Conformational control
by minimization
of A-1,3 strain

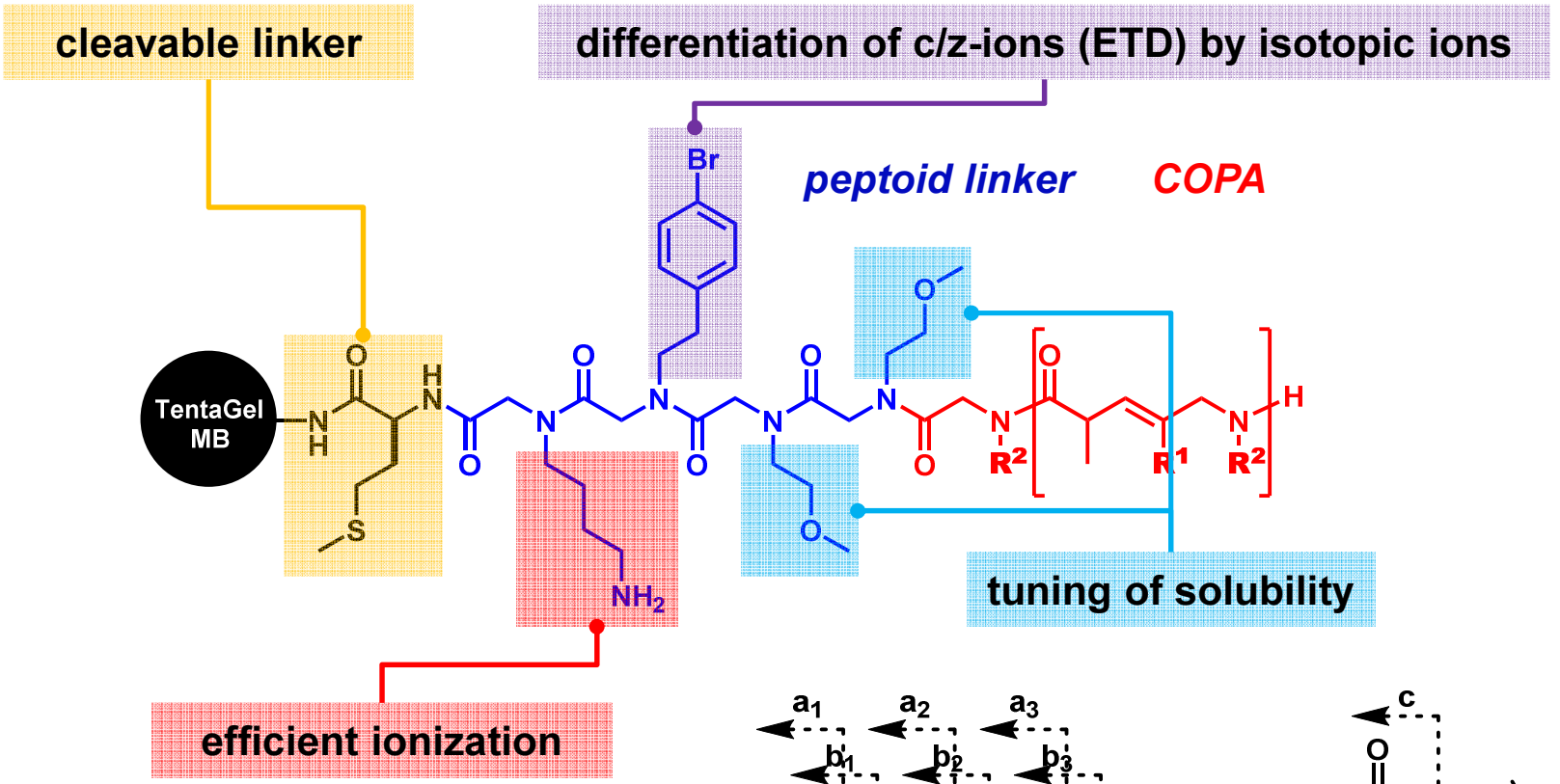


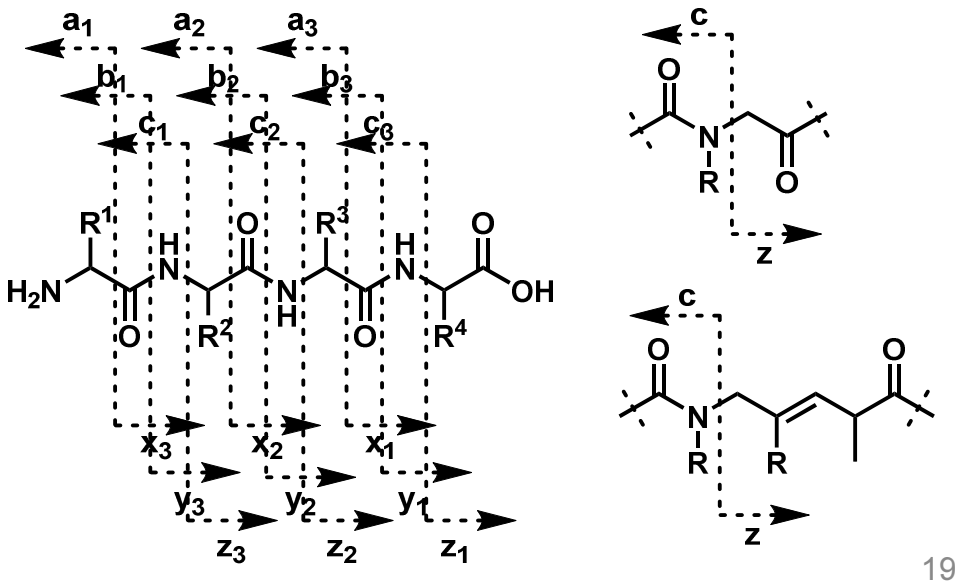
- **Relatively stable under proteolytic conditions**
- **Secondary structure of COPA is resistant to denaturing conditions**

because of no H-bond within the main chain

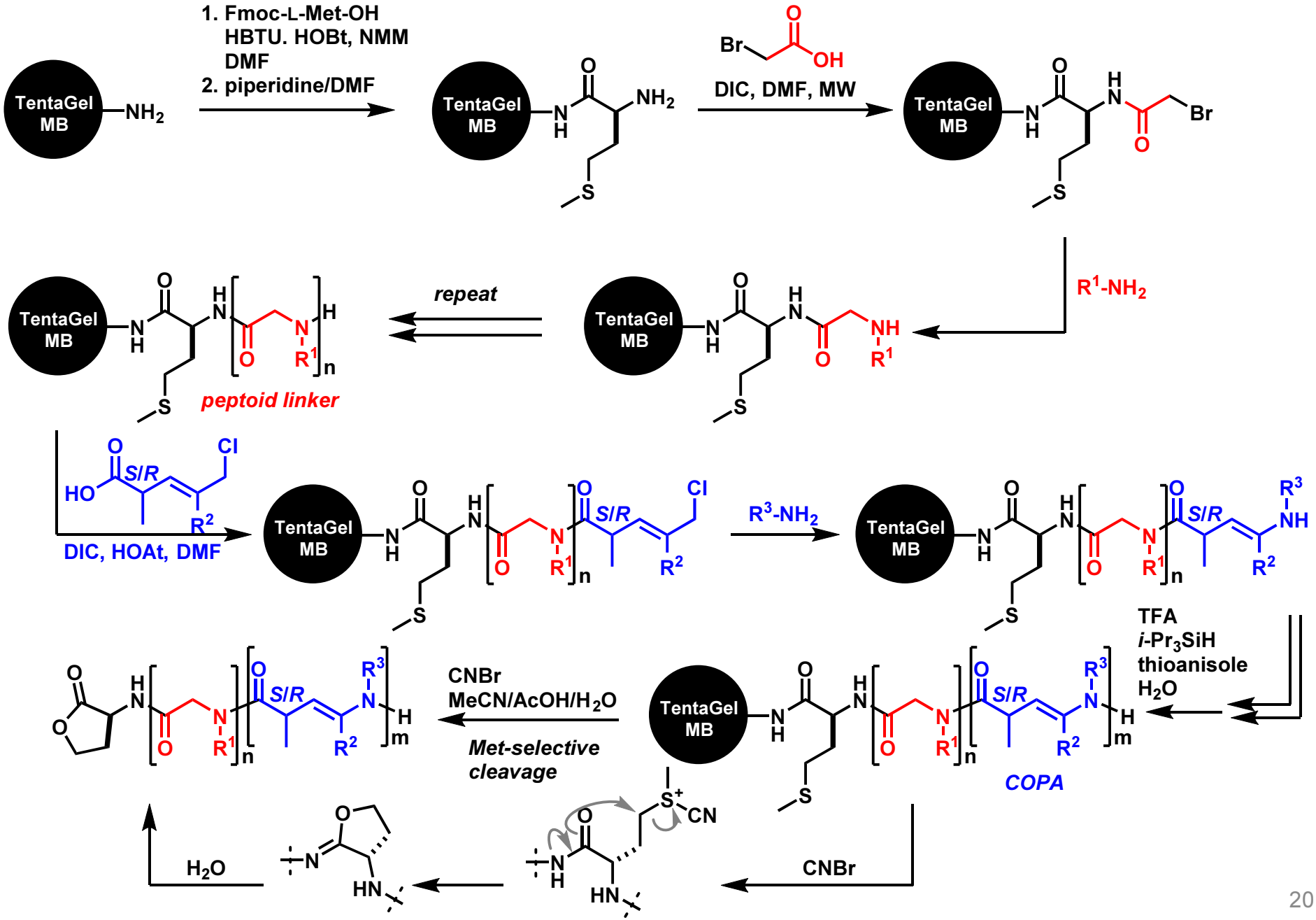
1) Aquino, C.; Sarkar, M.; Chalmers, M. J.; Mendes, K.; Kodadek, T.; Micalizio, G C. *Nat. Chem.* **2012**, *4*, 99.

Design of Library

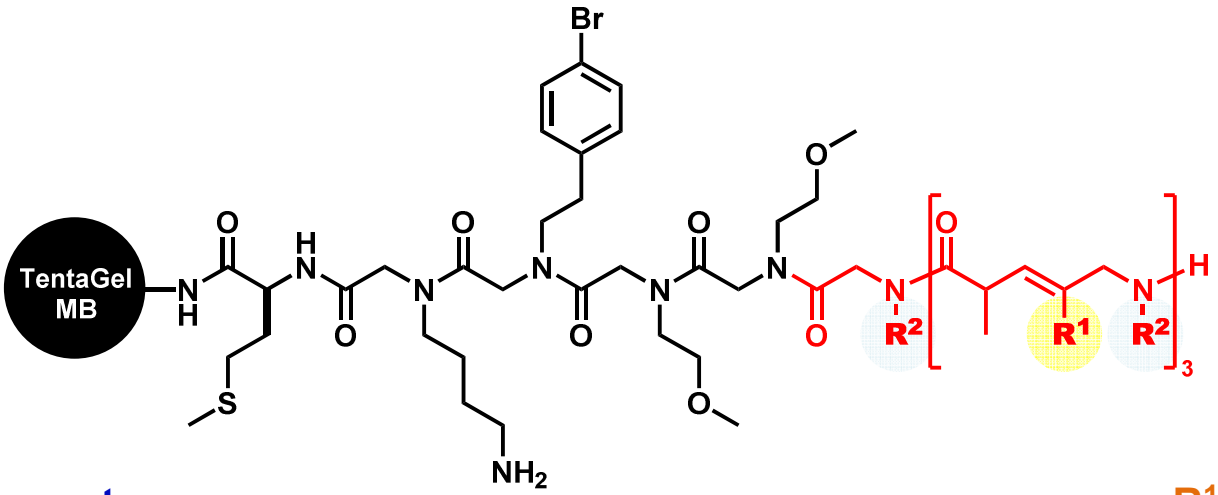




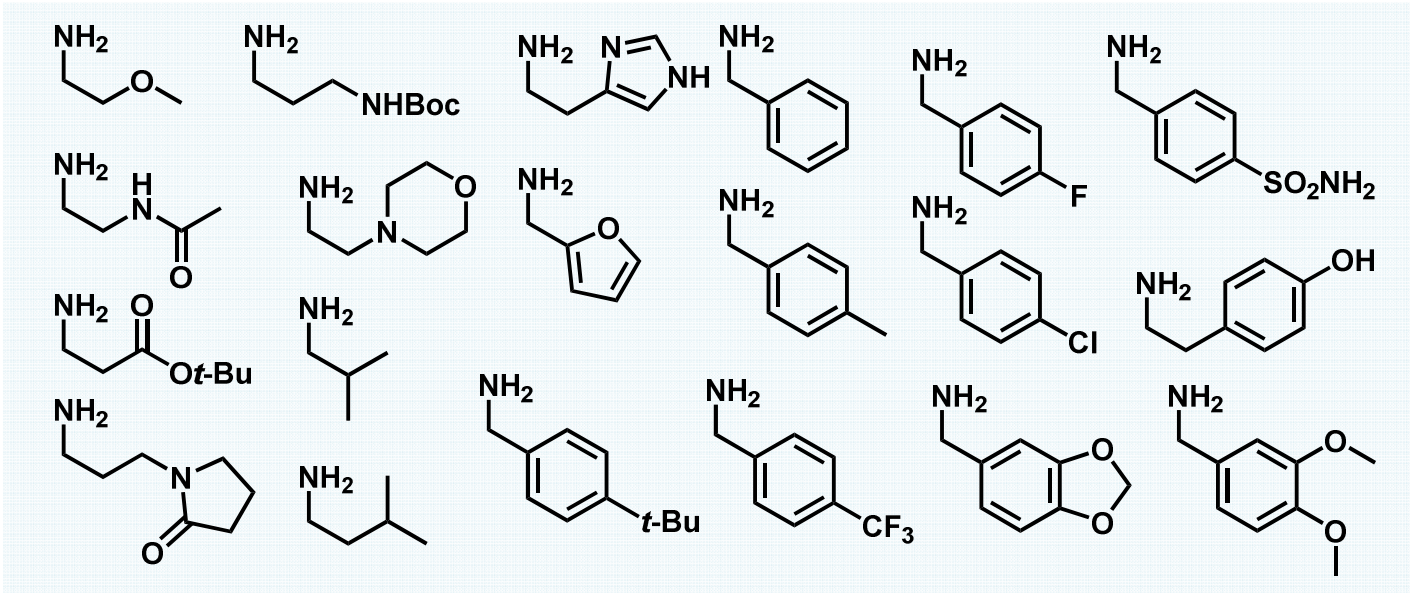
Synthesis of COPA Library



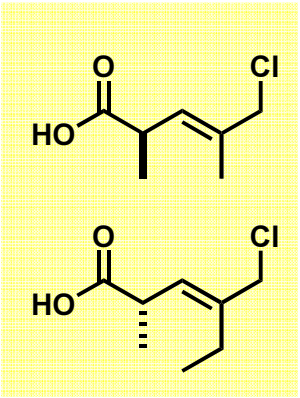
Library Diversity

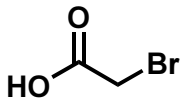


R^2: 20 components



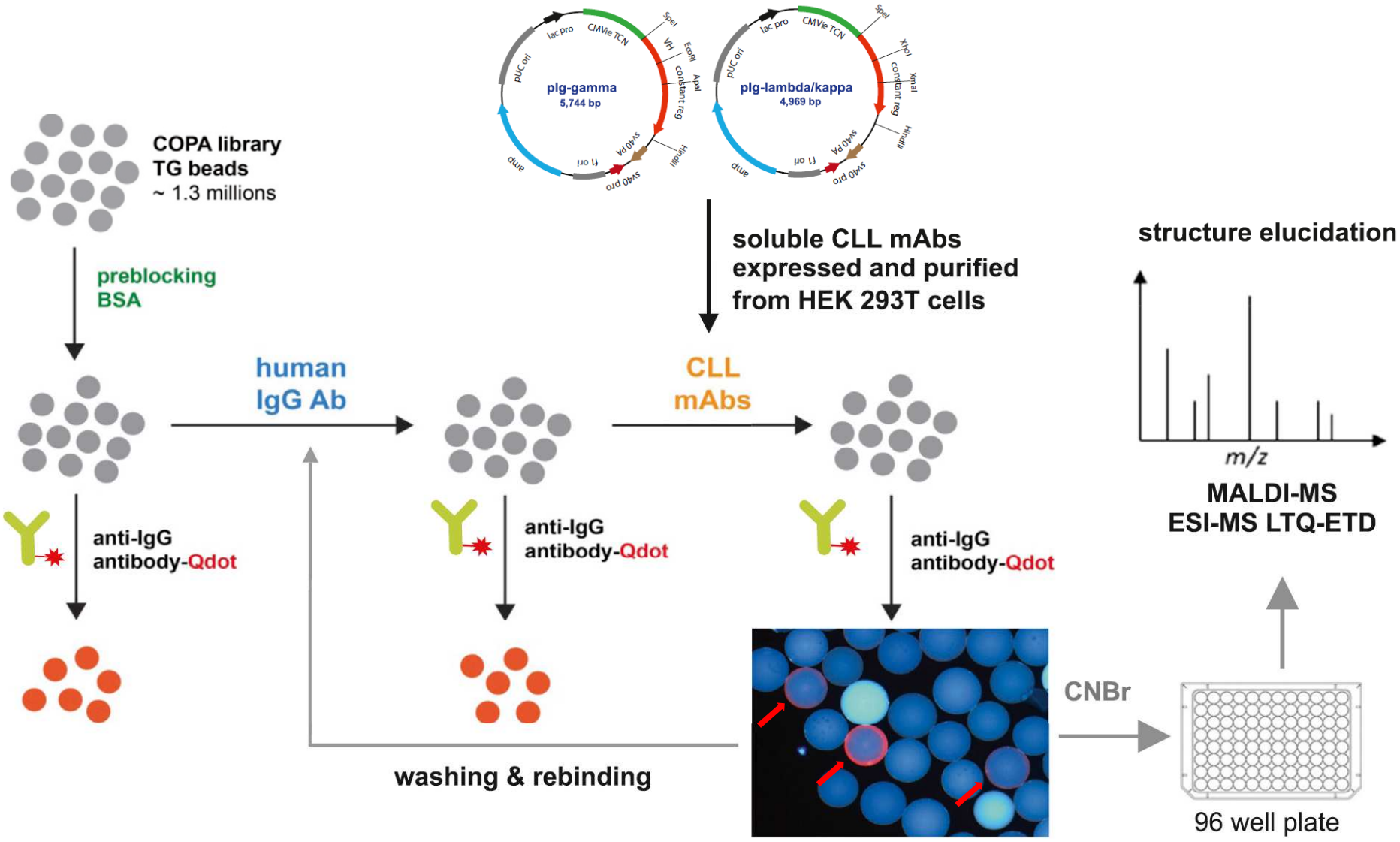
R^1: 2 components





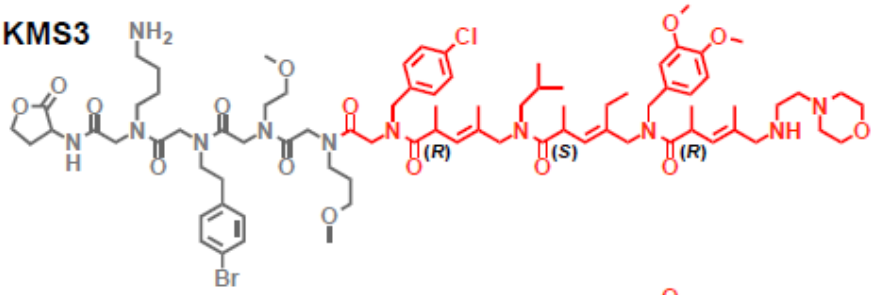
theoretical library diversity: $20^4 \times 2^3 =$ 1,280,000

Screening Protocol

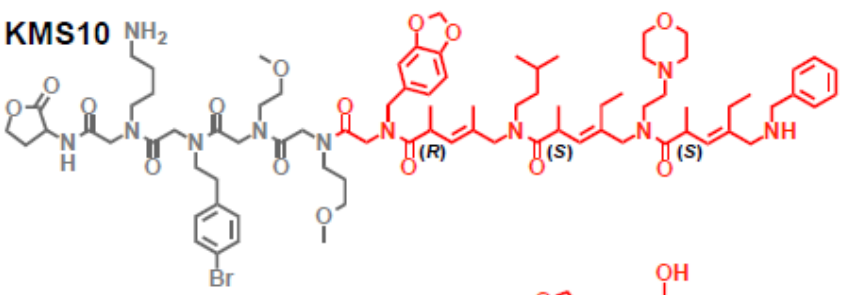


Hit Compounds against CLL 169 mAb

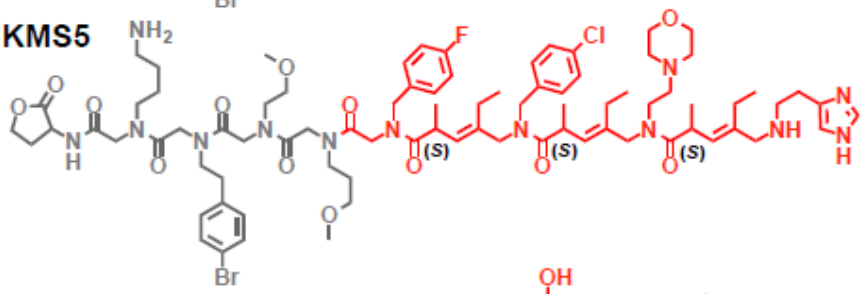
KMS3



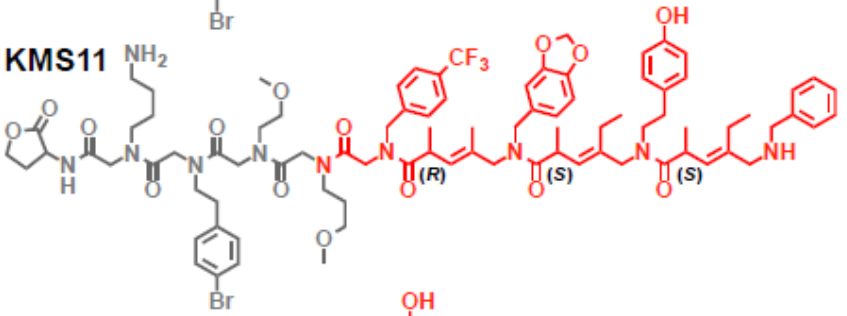
KMS10



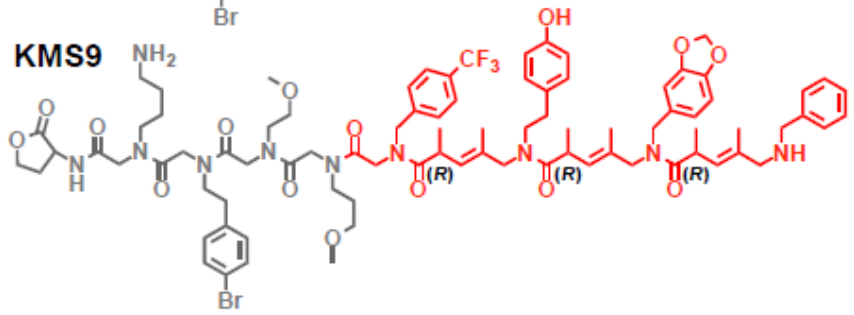
KMS5



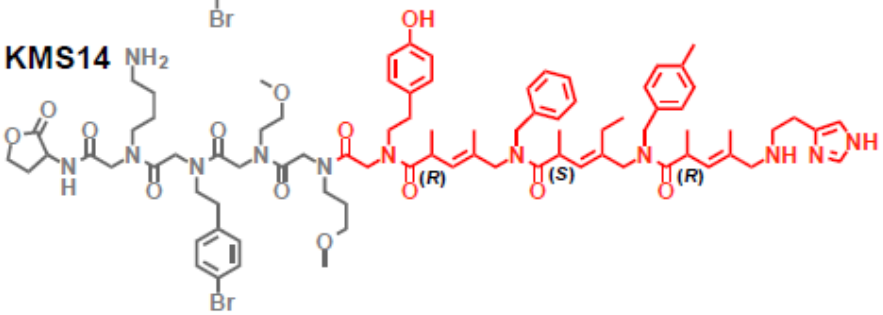
KMS11



KMS9



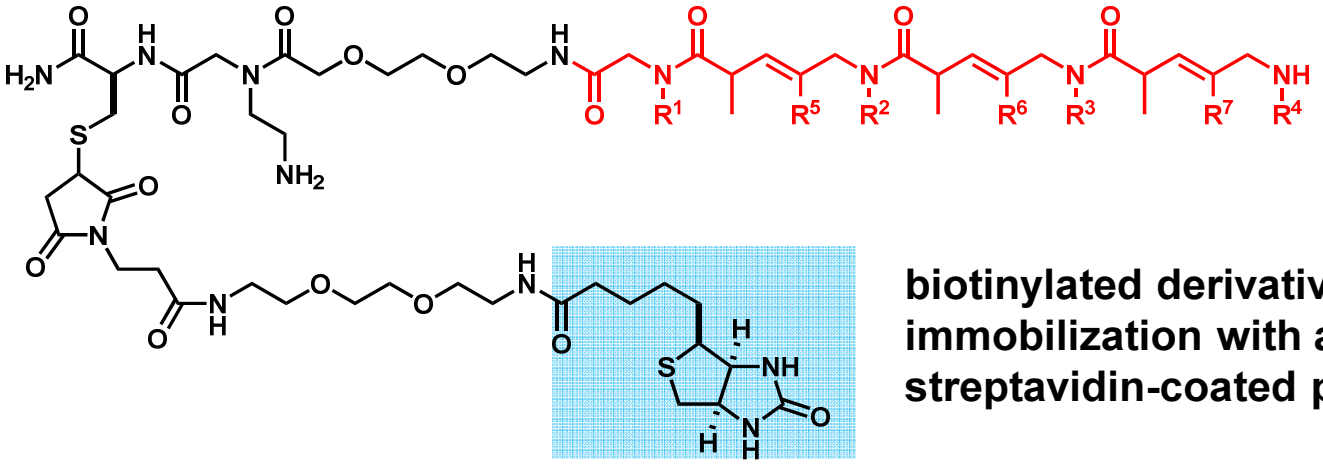
KMS14



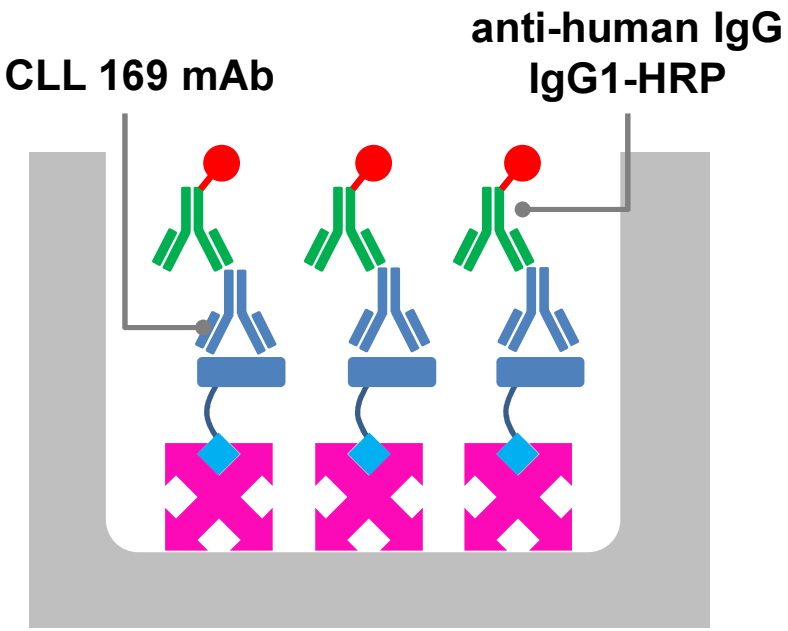
Hit structures were synthesized and purified for further evaluation

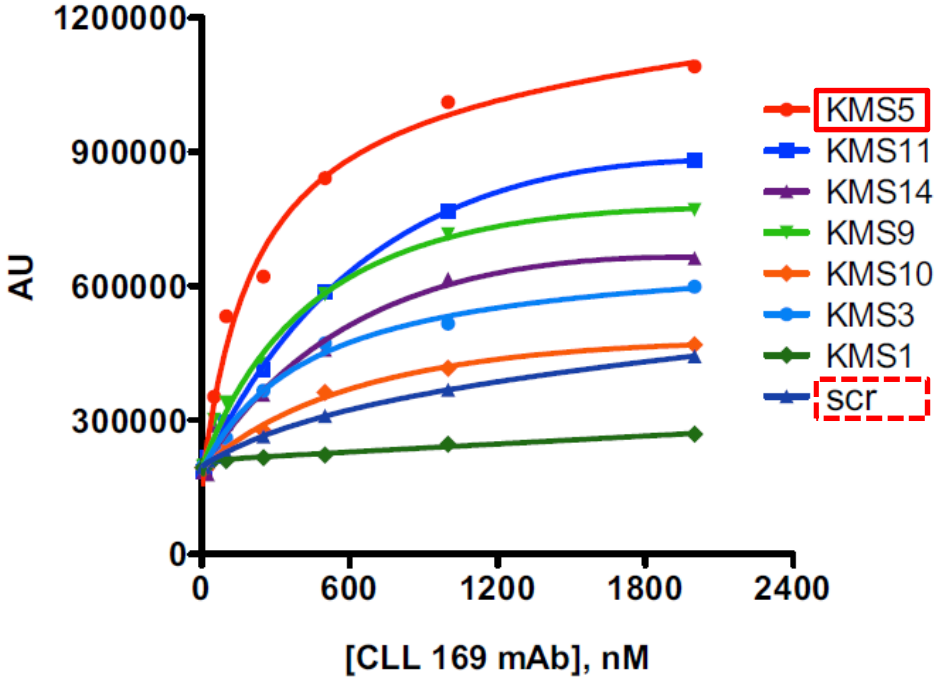
Affinity to CLL 169 mAb

Affinity was evaluated by ELISA:

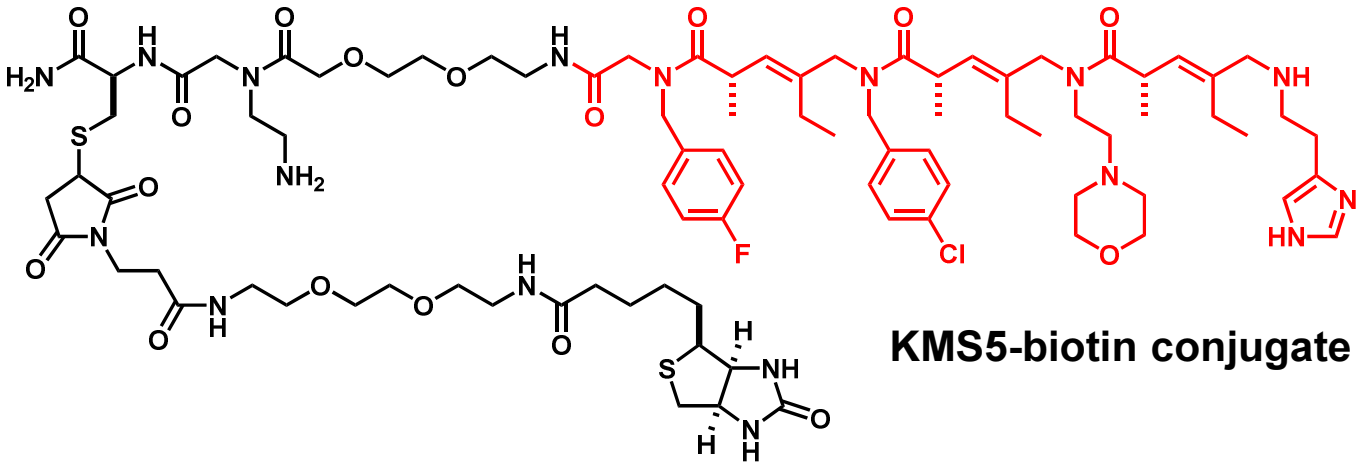


biotinylated derivatives for immobilization with a streptavidin-coated plate

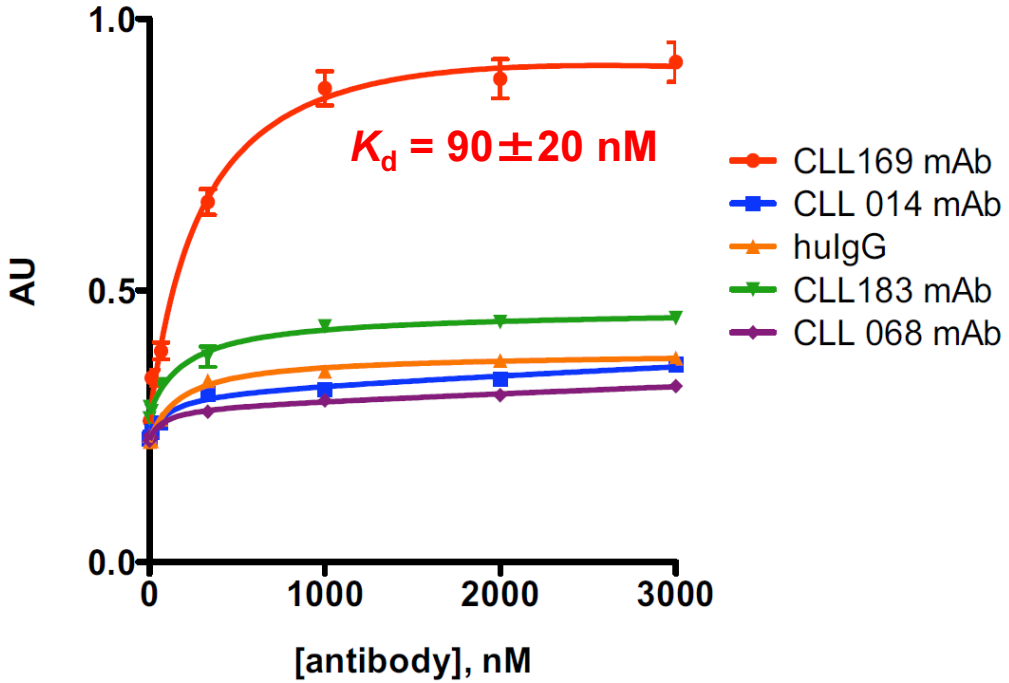




Affinity to CLL 169 mAb



KMS5-biotin conjugate

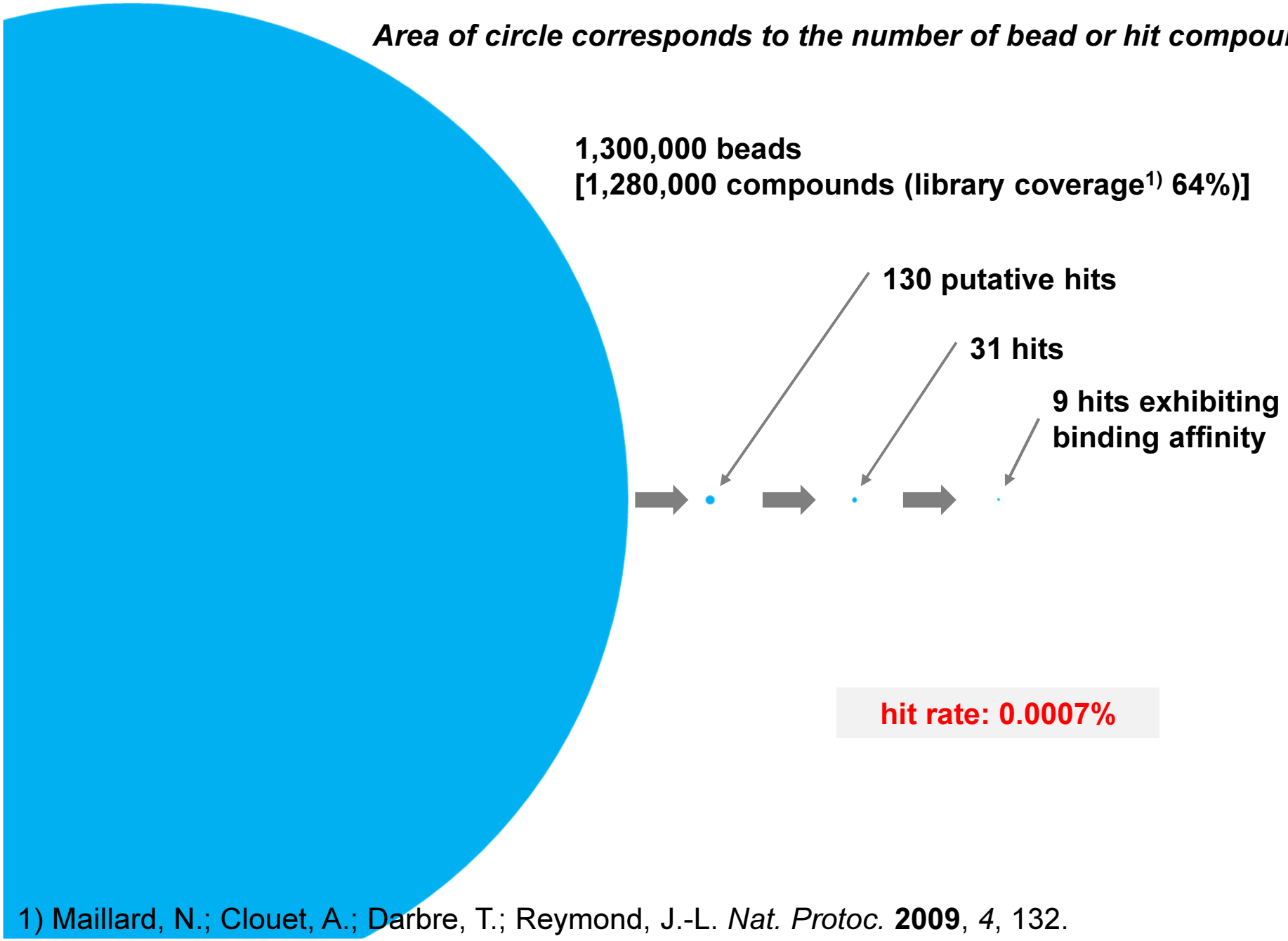


CLL 169 mAb bound to KMS5 with dissociation constant 90±20 nM

***general antigen-antibody reaction: K_d = 0.01-0.1 nM**

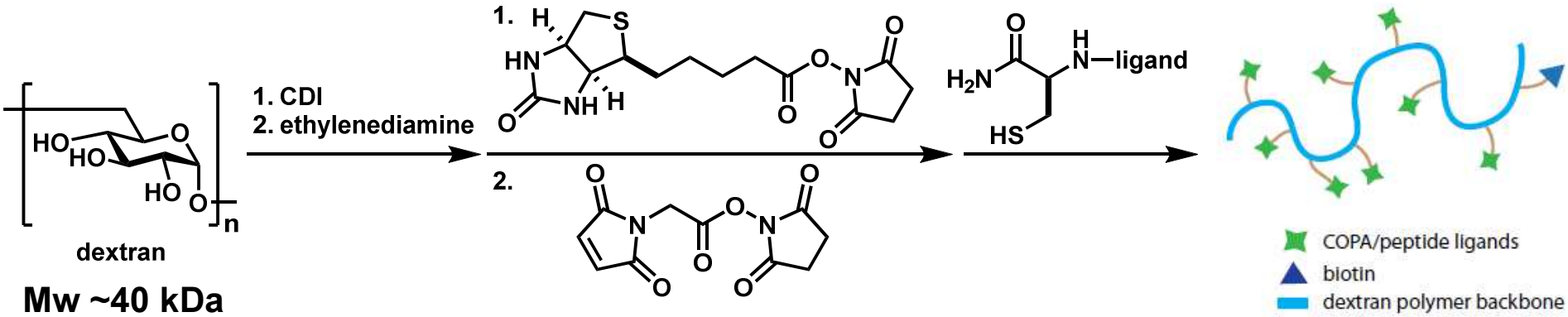
Hit Rate of the Library

Area of circle corresponds to the number of bead or hit compound



1) Maillard, N.; Clouet, A.; Darbre, T.; Reymond, J.-L. *Nat. Protoc.* **2009**, *4*, 132.

Dextran-KMS5 Conjugate



Mw ~40 kDa
200-250 glucose/molecule
from *Leuconostoc mesenteroids*

1 ligand/7-10 glucose unit

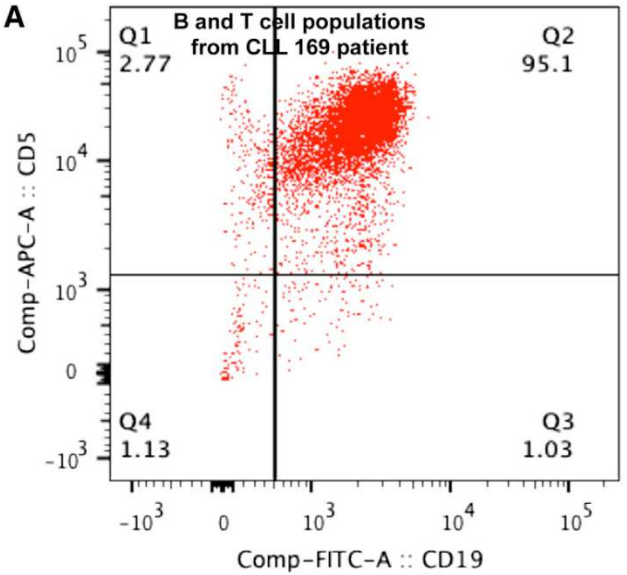
Ligand-dextran-biotin conjugate were incubated with CLL cells, streptavidin-phycoerythrin (PE)

flow cytometry (PE: binding of ligands)

1) Nakamura, J.; Nakajima, N.; Matsumura, K; Hyon, S. H. *Anticancer Res.* **2010**, *30*, 903.

Affinity to CLL B Cell from Patient

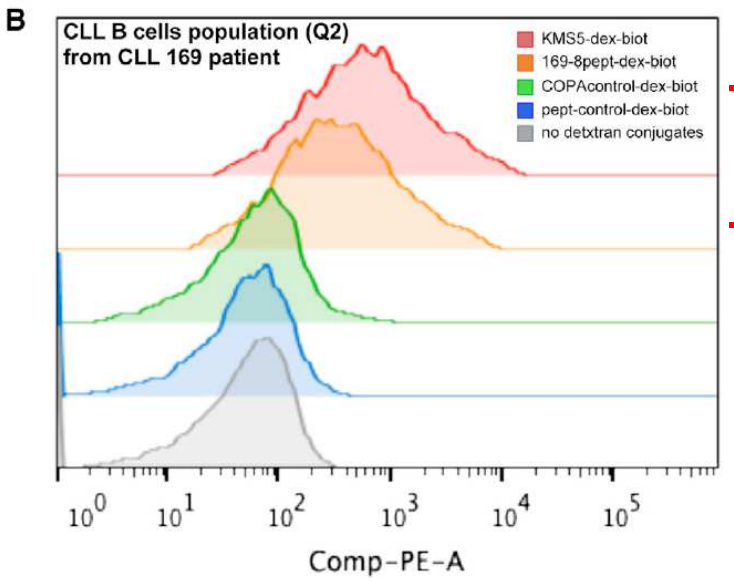
CD5+/CD19-
(T cell)



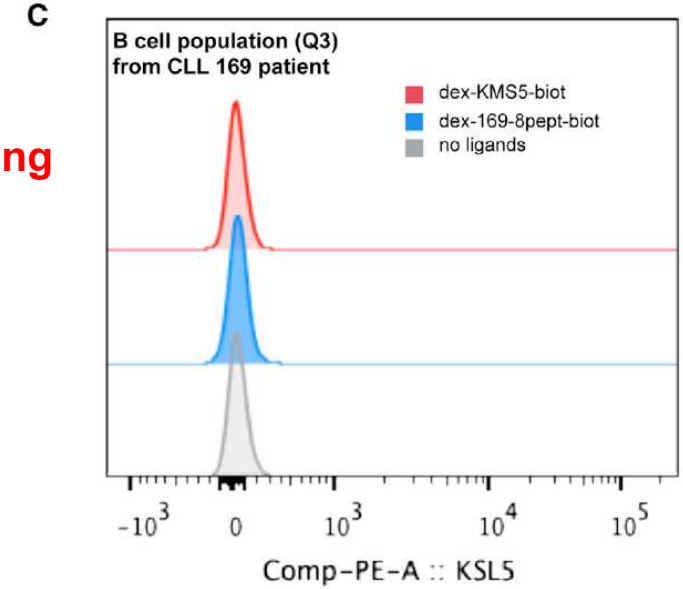
CD5+/CD19+
(CLL B cell)

CD5-/CD19+
(normal B cell)

169-8:
DNYAAALAQRARGSSGC[1)]
control peptide:
FLAQKLWSALEYGSSGC



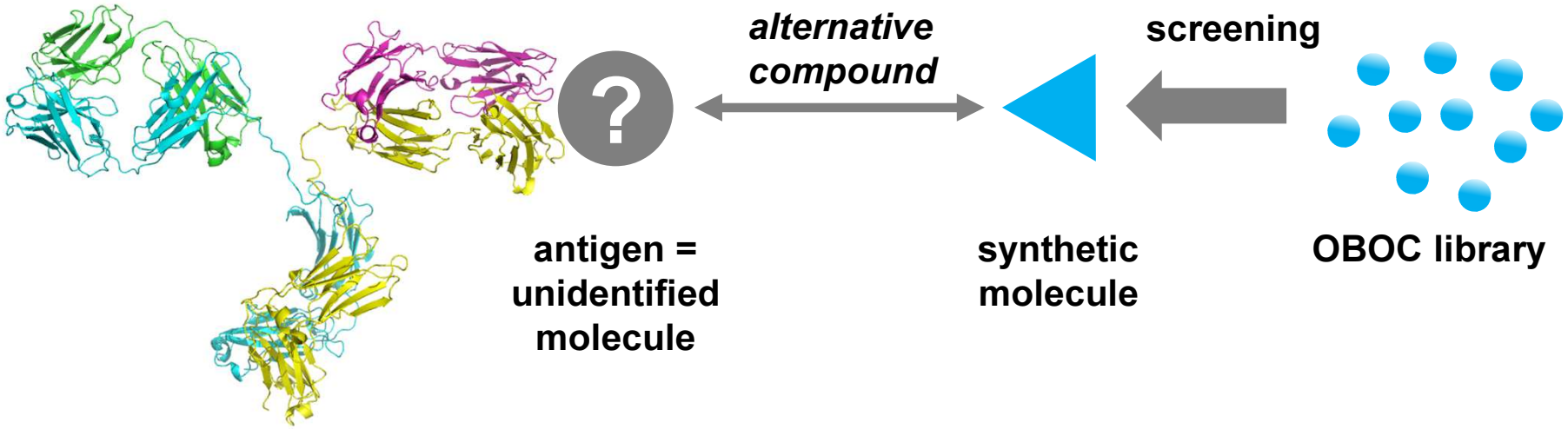
binding



1) Seiler, T.; Woelfle, M.; Yancopoulos, S.; Catera, R.; Li, W.; Hatzi, K.; Moreno, C.; Torres, M.; Paul, S.; Dohner, H.; Stilgenbauer, S.; Kaufman, M. S.; Kolitz, J. E.; Allen, S. L.; Rai, K. R.; Chu, C. C.; Chiorazzi, N. *Blood* **2009**, *114*, 3615.

Mimicking Antigen Structure by Focused Library

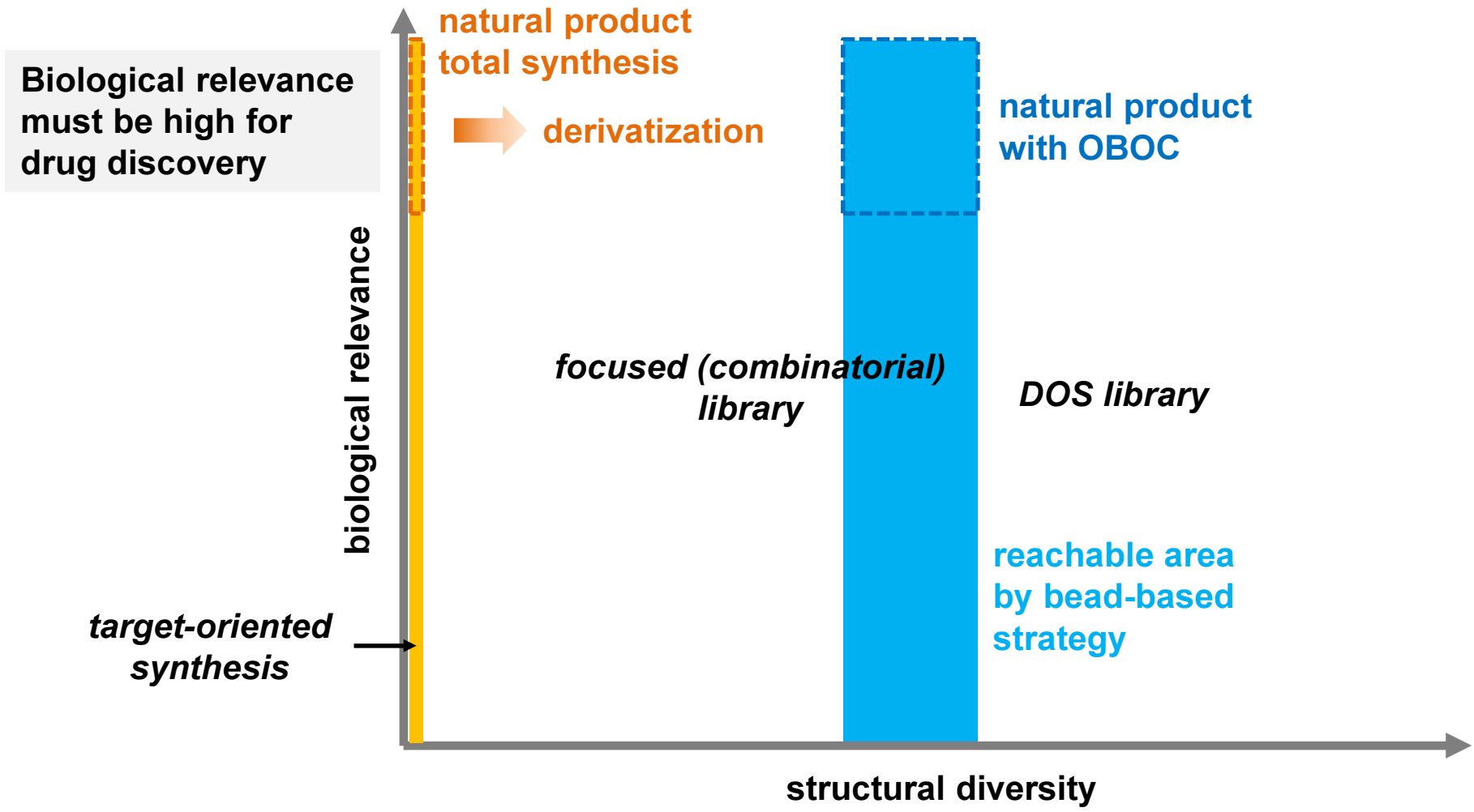
What is the significance of the study from viewpoint of library strategy?



Specific antigen-antibody interaction could be mimicked by artificial molecules derived from "focused library" of COPAs

other examples (discovery of autoantigen of type 1 diabetes):
Doran, T. M.; Morimoto, J.; Simanski, S.; Koesema, E. J.; Clark, L. F.; Pels, K.; Stoops, S. L.; Pugliese, A.; Skyler, J. S.; Kodadek, T. *Cell Chem. Biol.* **2016**, *23*, 618-628.

Structural Diversity and Biological Relevance



Towards the discovery of useful seeds from pool of natural product derivatives constructed by focused library strategy