香 港 大 學
THE UNIVERSITY OF HONG KONG

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Synthetic combinatorial OPA-cyclic peptide library

Kin Nam Adrian Chu[1], Carina Hey Pui Cheung[1], Xuechen Li[1*]
[1]Department of Chemistry, State Key Laboratory of Synthetic Chemistry, The University of Hong Kong, Hong Kong, P. R. China, SAR

Abstract
Cyclic peptides have a rising popularity as novel drug due to its improved stability and lower entropic cost for binding. In order to look for potential cyclic peptide drug, a high-throughput screening on various drug target can be done using a cyclic peptide library. Among various types of peptide library, we have chosen to synthesize a cyclic peptide library using the One-Bead-One-Compound (OBOC) strategy. In the design of our cyclic peptide library, we utilized ortho-phthalaldehyde (OPA) to do an on-resin peptide cyclization[1]. After the cyclic peptide library has been synthesized, we conducted a bead-based biological screening on various drug targets, for example, BamA and Walk-EPAS. We have successfully screened a few potential binders through this technique.

OPA chemistry:
Ortho-phthalaldehyde (OPA) can react with an amine group to form a phthalimidines [Two-Component product]. (Figure 1b)[1]
When there is a thiol group, OPA will react with both amine and thiol to form an isoindole ring [Three-Component product]. (Figure 1a)[1]

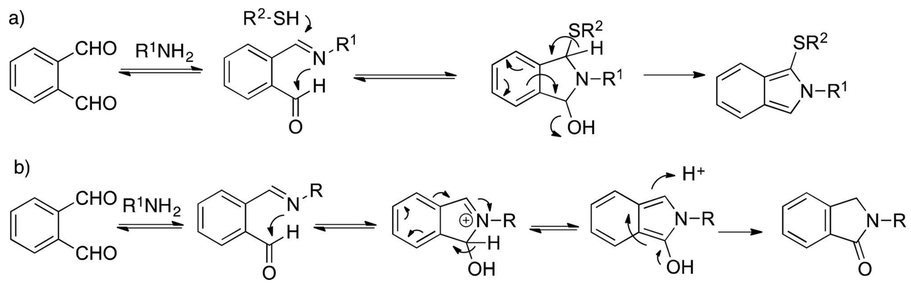

Figure 1: (a) Proposed Three-Component Reaction Mechanism; (b) Proposed Two-Component Reaction Mechanism

Library design:
- Synthesize a One-Bead-One-Compound (OBOC) OPA-cyclic peptide library. (Figure 2)
- Two layers in our library: outer layer is the cyclic peptide; inner layer is the linear peptide, which is the coding tag.

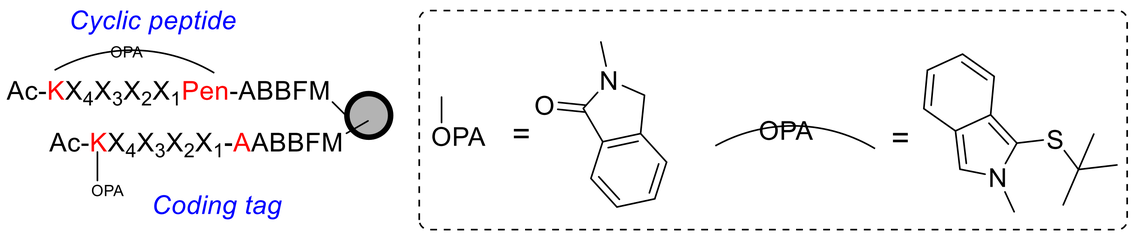

Figure 2: OBOC OPA-cyclic peptide library , B = β-Alanine, Pen = Penicillamine, X_n = canonical amino acids

- 16 canonical amino acids (Lys, Cys, Met, Ile not included) were used to form a random tetrapeptide region.
- Theoretical diversity of the library = 16^4 = 65536 peptides

Library construction:
- 3 key steps for library construction:
  - Spatial segregation: split the beads into 2 layers[2]
  - Split-and-pool synthesis: generate the random region[2]
  - On-resin OPA cyclization: cyclize the outer layer

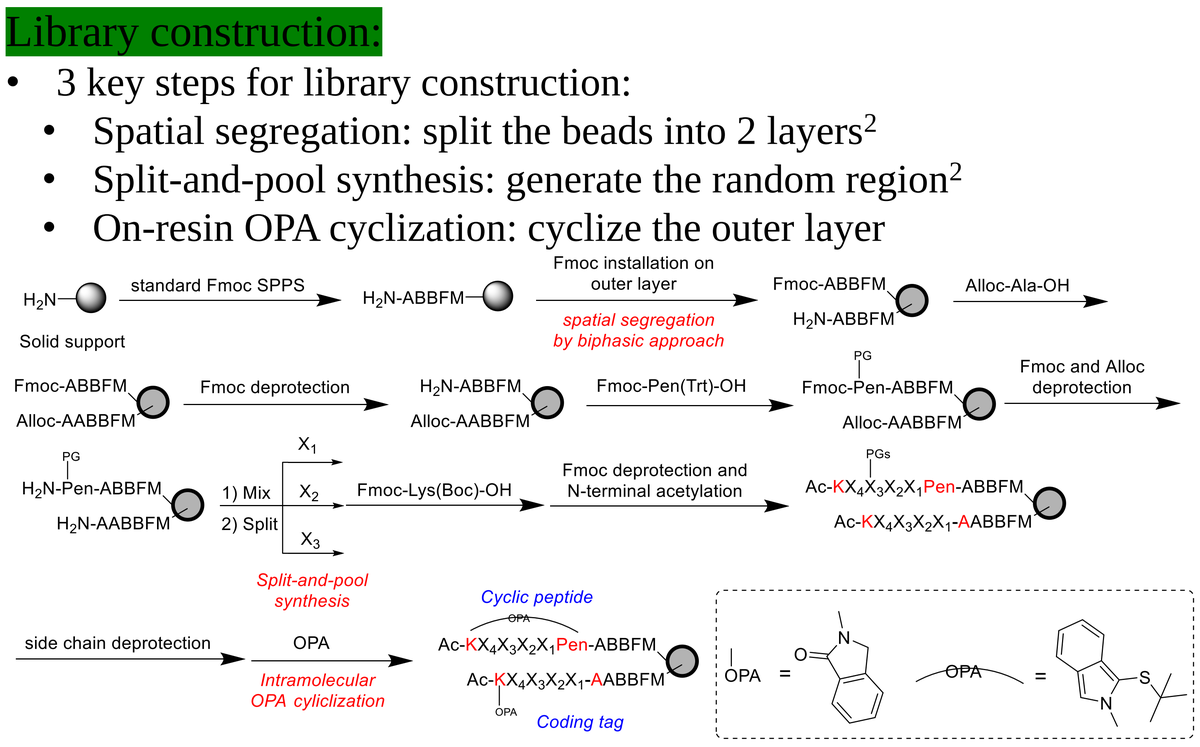

Figure 3: Synthetic scheme of the OBOC OPA-cyclic peptide library, B = β-Alanine, Pen = Penicillamine

Library screening & isolation of +ve hits:
- The OPA-cyclic peptide library will incubate into labelled target protein of interest (POI). (Figure 4)

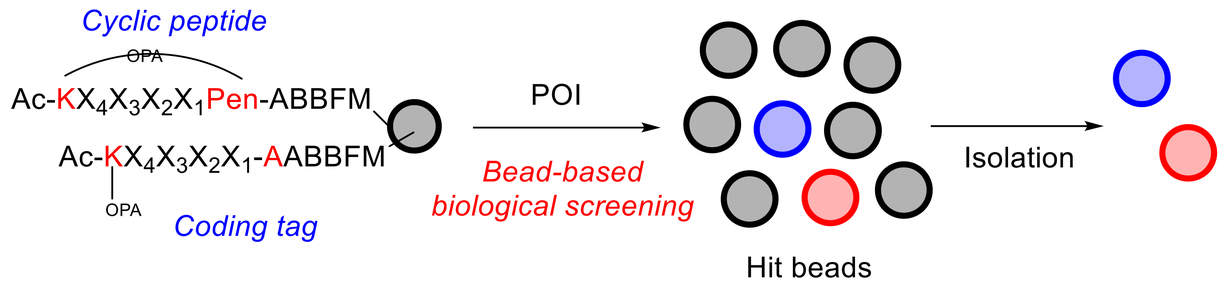

Figure 4: General scheme for library screening & isolation of positive hits

- Do magnetic screening & fluorescent screening to isolate positive hits
  - Magnetic screening: use biotin-labelled POI, isolated by streptavidin magnetic beads and a strong magnet.
  - Fluorescent screening: use fluorescent dye-labelled POI, isolated by picking the beads under confocal microscope (Figure 5)

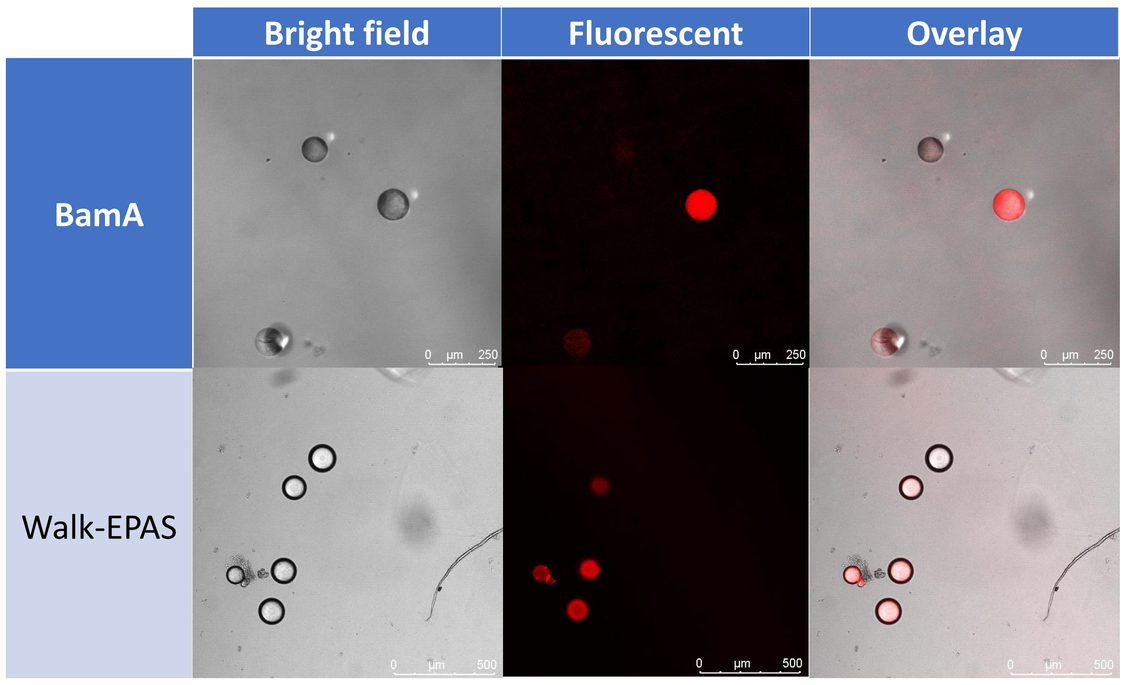

Figure 5: Visualization of positive beads under confocal microscope

Structural determination:
- Positive hits are cleaved from the beads by CNBr and send to Tandem MS/MS to obtain the sequence of positive hits. (Figure 6)
  - CNBr cleaves the C-terminal of Met to a homoserine lactone

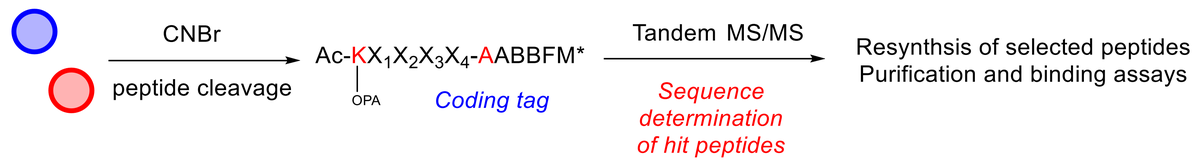

Figure 6: General scheme for structural determination, M* = homoserine lactone

- Results of BamA and Walk-EPAS screening (Table 1)

Proteins	Number of peptide sequences
Walk-EPAS	19
BamA	18

Table 1: Number of peptide sequences obtained from bead-based screening

References
[1] Zhang, Y., et al., Journal of the American Chemical Society, **2019**, 141(31), p. 12274-12279.
[2] Liu, Ruiwu et al., QSAR & combinatorial science, **2005,** 24(10), p. 1127–1140.