Drug discovery aided by NMR- and enthalpy-based screening of combinatorial libraries

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We demonstrated that combinatorial libraries, arranged in positional scanning fashion (POS), can be screened by NMR (**HTS by NMR**)[1, 2] allowing the identification of potent antagonists of protein-protein interactions (PPIs).[3] The approach, schematically illustrated in **Figure 1**, merges the principles of positional scanning combinatorial chemistry and fragment-based drug design with protein-based NMR screening to iteratively identify and optimize antagonists from collections of >100,000 peptide mimetics and/or non-peptide POS libraries.[1-3] The approach seems also particularly effective in the fragment-hit-to-lead optimization process, when a positional scanning library is generated from an initial weak binder, perhaps common to a class of protein targets and/or previously identified from a FBDD campaign, and tested by biophysical methods including not only NMR[4, 5] but also ITC.[7] Our examples will illustrate that the proposed approach is effective and perhaps more amenable to academic research laboratories than the medicinal chemistry intensive SAR by NMR method, and it is also a powerful alternative to phage display approaches.

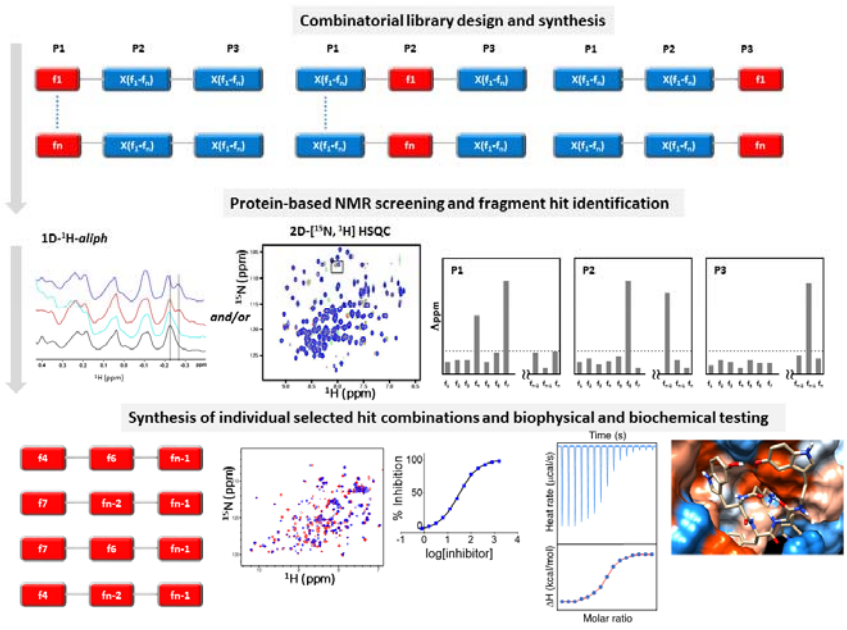

Figure 1. **Schematic representation of the HTS by NMR approach**. a) A general POS library can be comprised of three elements in positions P1, P2 and P3, and synthesized in a positional scanning (POS) fashion: each sample is a mixtures of compounds in which one position is fixed f1..fn, while other positions consist of all components X(f1..fn). In the example of 3-positions POS library, and fn-components per position, the library will contain 3 x n mixtures, and each mixture will contain a total n x n compounds. This reduces the number of compounds to be synthesized and tested from *n x n x n* to *n + n + n*. For example, for n = 50, we have 50x50x50 = 125,000 compounds contained in 50 + 50 + 50 = 150 mixtures, that are amenable to NMR screening. b) The designed library is next screened by collecting either 1D-1H-aliph, or 2D-[^{15}N, ^{1}H], or 2D [^{13}C,^{1}H] correlation spectra of target macromolecule (using uniformly or amino-acid selective labeling)[6] in presence and absence of each mixture. c) The library is deconvoluted by evaluating chemical shit perturbations (Δppm) induced by each mixture. Significant perturbations in the macromolecule spectra are attributable to the fixed component of the given hit mixture. Hence, components at each position are selected as hits based on their ability to induce significant chemical shift perturbations. d) Finally, individual compounds are synthesized with a proper combination of hit components and tested using biophysical and biochemical assays. The latter steps will identify positive hits for continues SAR and/or structure based optimizations. In the fPOS approach, the entire library can be derivatized with a common or know binding fragment, resulting in library that is highly enriched in more potent positive hit compounds.

References:

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