From screening to structure - Boosting NMR-based drug discovery with hyperpolarization and label-free structure determination

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Nuclear Magnetic Resonance (NMR) is the gold standard for fragment-based drug discovery (FBDD). However, due to its low sensitivity and tedious analysis, it needs high sample concentrations and has a low throughput. We present the use of photo-Chemically Induced Nuclear Polarization (photo-CIDNP) [1] and NMR Molecular Replacement (*N*MR²) [2] to establish a workflow from screening to structure-activity relationship for FBDD, overcoming the above-mentioned limitations. Photo-CIDNP drastically reduces the time and amount of material needed and *N*MR² makes protein-fragment structures accessible within a few days without the need for isotope labelling.

A photo-CIDNP fragment library is screened against the cancer target PIN1. Photo-CIDNP enables accelerated measurements of a few seconds. Furthermore, we demonstrate the possibility to screen at nanomolar concentrations and cryogen-free benchtop NMR spectrometers within a few minutes. Hyperpolarization also enables to determine the affinity of the hits within minutes. We present a new method analogous to STD-NMR to measure the affinity of protein-ligand interactions with photo-CIDNP. Several examples are presented. Finally, we show the implementation of a T_1, T_2-filtered 2D-NOESY pulse sequence to measure fragment-protein distance restraints. *N*MR² does not need a protein assignment and the distance restraints are directly converted to a protein-fragment complex structure. More than 10 complex structures of the oncogenic protein K-Ras have been elucidated this way.

This study shows how new NMR methods are implemented into a FBDD workflow. Lowering the target concentration for screening and K_D measurements into the nanomolar regime, moving away from cryogenic magnets, and elucidating structures without isotope labelling could help to tackle new targets that were up to now out of reach for NMR and medicinal chemists.

[1] Felix Torres, Matthias Bütikofer, Gabriela R. Stadler, Alois Renn, Harindranath Kadavath, Raitis Bobrovs, Kristaps Jaudzems, Roland Riek, *Journal of the American Chemical Society*, **2023**
[2] Felix Torres, Dhiman Ghosh, Dean Strotz, Celestine N. Chi, Ben Davis, Julien Orts, *RSC Medicinal Chemistry*, **2020**, 11, 591