

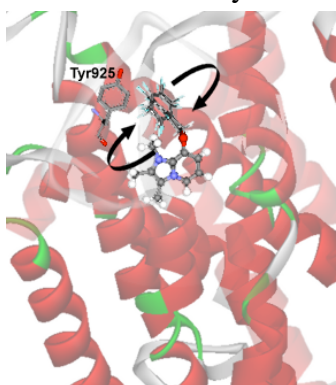
Pushing the limits: macromolecular biological solid state NMR

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Macromolecular interactions are at the heart of biological cell function, and many conventional structural biology approaches require either purified or single component (macro)molecules for study, and often in a specialized form. For study by nuclear magnetic resonance, size limitations and sensitivity can also be a challenge. Solid state NMR is an approach that can provide dynamic and structural information for both purified, as well as heterogeneous and biologically fully functioning systems, at sub-Å resolution and in a wide dynamic range (ms – ps), providing new insights into biomolecular assembly and function.

A brief review of some highlights of biological solid state NMR will be given, including novel fast (60kHz) magic angle spinning [1] and NMR crystallography [2], and then an extension into drug design for major pharmaceutical targets.

It is now possible to resolve ligand (or drug) conformation, binding site environment and local dynamics within a membrane bound target at near physiological and functionally relevant conditions in natural membranes, to inform design and mode of action, using solid state NMR approaches [3, 4]. This information is obtained by (non-perturbing) isotopically (^2H , ^{13}C , ^{19}F , ^{15}N , ^{17}O) labeling selective parts of either a ligand or the protein under study, and observing the nucleus in non-crystalline, macromolecular complexes [5, 6].



Conformational information and details come from precise (sub-Å, at $\leq 0.5\text{Å}$) distance measurements between defined sites within the bound ligand. A novel observation is that ligands with complex structure have differential mobility at their binding sites, which has implications for efficacy and action. Substituted imidazole pyridines, for example, which inhibit the H^+/K^+ -ATPase and have therapeutic use, are constrained in the imidazole moiety, but shows significant flexibility at the pyridine group [7] (see figure). It is this group which has a direct interaction with an aromatic (Phe198) residue, with concomitant π -electron sharing [8]. Similarly, the steroid moiety of ouabain undergoes motions that are similar to those of the target

protein, the Na^+/K^+ -ATPase, but the rhamnose undergoes a high degree of flexibility at fast rates of motion whilst interacting with Tyr198 [9]. For acetyl choline when bound in the nicotinic acetyl choline receptor (nAChR), the quaternary ammonium group undergoes fast rotation at an aromatic binding site, driven by thermal fluctuations that are functionally significant [10].

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