Structural Dynamics of G Protein-Coupled Receptors and Mutated Amyloid β Fibrils

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G protein-coupled receptors (GPCRs) constitute a large group of membrane proteins, known to undergo a set of well-defined structural transitions upon activation and signaling. In our work, we address the molecular dynamics of peptide ligand GPCRs using solution and solid-state NMR. We work with human class A GPCRs that are activated by peptide hormones, such as neuropeptide Y (NPY) or ghrelin. The GPCRs are expressed in prokaryotic systems or by cell-free synthesis. In the talk, results on three research topics will be discussed. (*i*) Studies on the equilibrium dynamics of GPCRs using static ^{15}N CP NMR, ^{15}N NMR spectra acquired as a function of the CP contact time, and ^{13}C MAS NMR experiments confirm the high molecular dynamics of three peptide ligand GPCRs. Quantitative determination of ^{1}H-^{13}C order parameters through measurement of the ^{1}H-^{13}C dipolar couplings in separated local field NMR experiments revealed axially symmetric motions of the GPCRs and molecular fluctuations of large amplitude. (*ii*) Data will be reported that led to the development of structural models of NPY bound to the Y_1 and the Y_2 receptors. Isotope-labeled NPY was used to determine the secondary structure of the receptor bound ligand. Upon binding, the C-terminal α-helix of NPY, formed in membrane environment in the absence of receptor, is unwound starting at Thr^{32} to make optimal contact of the C-terminal residues within the binding pocket. The NMR signals of several hydrophobic residues in the α-helical region of NPY were broadened upon receptor binding. The ligands are tethered to the second extracellular loop by hydrophobic contacts, with the N-terminal part of its helix facing the solvent. The C-terminal pentapeptide of NPY inserts deeply into the transmembrane bundle, making optimal contacts to the Y_2 receptor including a contact NPY's amidated C-terminus with $Gln^{3.32}$ in a polar cluster within helices 2 and 3 of the receptor.

The second part of the talk is devoted to amyloid β fibrils. A series of peptide mutants was studied to understand the influence of local physical interactions on the fibril formation mechanism of amyloid β (Aβ) (1-40). In the peptide variants, the well-known hydrophobic contact between residues phenylalanine 19 and leucine 34 was rationally modified. In single site mutations, residue 19 was replaced by amino acids that introduce higher structural flexibility or restrict the backbone flexibility. Next, the aromatic phenylalanine was replaced by aromatic residues to probe the influence of hydrogen bond forming capacity in the fibril interior. Furthermore, charged residues were introduced to probe the influence of electrostatics. While the fibrillation kinetics and the local structure and dynamics of the peptide variants were influenced by the introduction of these local fields, the overall morphology and cross-β structure of the fibrils remained very robust against all the probed interactions. However, characteristic local structural and dynamical changes indicate that amyloid fibrils show an astonishing ability to respond to local perturbations but overall show a very homogenous mesoscopic organization. Interestingly, we find that even conservative mutations perturbing an early folding contact can drastically reduce the membrane-affinity and toxicity of Alzheimer's Amyloid-β oligomers, without substantially affecting the end-state structure. Given such a significant biological importance of putatively minor sequence alterations, we currently focus on finding the minimally tolerated variation of the F19-L34 hydrophobic contact in Aβ (1-40).