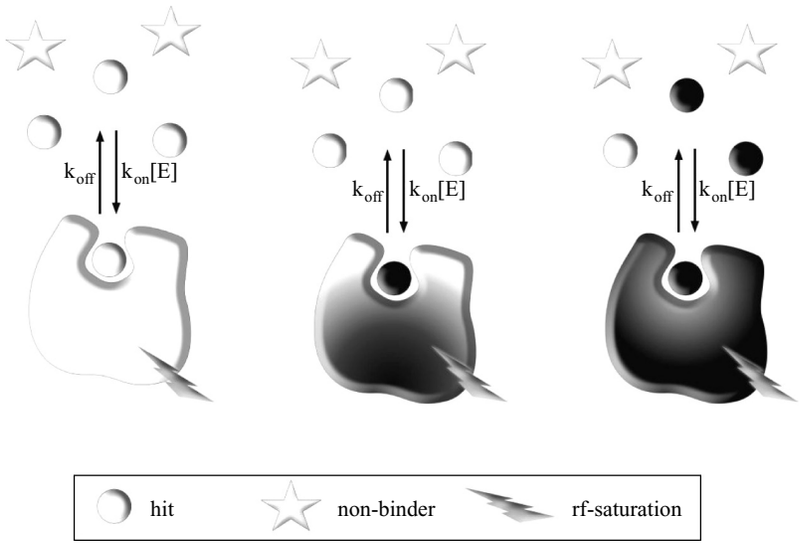

Figure 4.4. Detection of binding using the STD experiment. Circles and stars indicate binding and nonbinding compounds, respectively. Frequency selective irradiation (lightning bolt) causes selective ^{1}H saturation (shading) of the target receptor. Irradiation is applied for a sustained interval during which saturation spreads throughout the entire receptor via ^{1}H-^{1}H cross-relaxation (spin diffusion). Saturation is transferred to binding compounds (circles) during their residence time in the receptor binding site. The number of ligands having experienced saturation transfer increases as more ligand exchanges on and off the receptor during the sustained saturation period. Nonbinding compounds (stars) are unaffected. Reprinted with permission from *Chemical Reviews* (2004), 104 (8) 3641–3675. © 2004 American Chemical Society.

the bulk solvent is employed to create nonequilibrium magnetization on the target receptor, which is then transferred to a weakly binding ligand. At the beginning of the experiment, the magnetization of the bulk solvent (and protons of the target receptor whose resonance frequency is near that of bulk water) is either saturated or inverted by a selective 180° RF pulse. During the mixing time of the experiment, magnetization is transferred to the target protein by chemical exchange of the labile HN and OH protons of the target protein, as well as via cross-relaxation between the bulk solvent and protein protons. Magnetization is also transferred to ligands by either direct cross-relaxation with bulk solvent or relayed cross-relaxation via the target receptor. The magnetization transfer pathways are depicted in Figure 4.6. The resulting spectrum contains resonances from all the ligands in the fragment mixture. The binding ligands can be distinguished from the nonbinding ligands from the difference in the sign of the resonance peak as shown in Figure 4.7. Although a somewhat more technically challenging approach than STD methods, the WaterLOGSY experiment provides an excellent alternative when spin diffusion in the target receptor is inefficient. This situation typically arises where the target has a low proton density (i.e., nucleic acids).[68,69]

Relaxation and diffusion-based methods

There are also a number of ligand-directed methods that do not rely on intermolecular magnetization transfer between the fragment and target receptor but instead take advantage of a change in the translational and/or rotational mobility of the small molecule resulting from target binding. For example, the apparent diffusion coefficient of a small molecule is altered as a consequence of its interaction with a large molecule, while that of a noninteracting molecule is unaffected. Pulsed-field-gradient stimulated-echo experiments may be used to measure the difference in diffusion rates for ligands in the presence and absence of target protein receptor and thus detect binding ligands in a mixture of compounds.[70,71]

Interaction of a ligand with a large target molecule also alters the average rotational correlation time of the small molecule, and as a result, its relaxation properties. Shortened transverse relaxation times (due to longer average rotational correlation times and chemical exchange processes) are manifested in line broadening of the spectrum, which is often easily observable in a one-dimensional proton spectrum. Alternatively, the same phenomena can be observed by employing a CPMG (Carr-Purcell-Meiboom-Gill) pulse train (or, in the case of measuring $T_{1\rho}$, a spin lock) and determining the difference in peak intensity of ligand resonances in the presence and absence of the target of interest.[72] This effect can be enhanced by incorporation of one or more paramagnetic spin labels on the target protein by covalent modification of side chains such as lysine, tyrosine, cysteine, histidine, and methionine. This method (known as SLAPSTIC or Spin Labels Attached to Protein Side chains as a Tool to identify Interacting Compounds)[73,74] has a distinct advantage in sensitivity over detecting exchange line broadening in native proteins. Because the magnitude of the proton/electron dipole-dipole interaction is much larger than the proton-proton interaction, the effect extends over a much larger distance and allows lower bound ligand fractions, resulting in a lower protein concentration requirement. To apply this method, it must first be demonstrated that attachment of the spin label does not affect the binding properties or activity of the target receptor, which in turn requires detailed knowledge of the target's three-dimensional structure.

Longitudinal relaxation rates (R_1) can also be affected by binding to the target receptor and can be used to identify fragment binding in mixtures. As described by Peng et al.,[52] only selective R_1 measurements are useful in detecting ligand binding. However, it is impractical to have a different selective inversion RF pulse for every compound in a fragment library. An alternative method is to collect two-dimensional nuclear overhauser effect (NOE) spectra to identify those ligands that exhibit transferred