

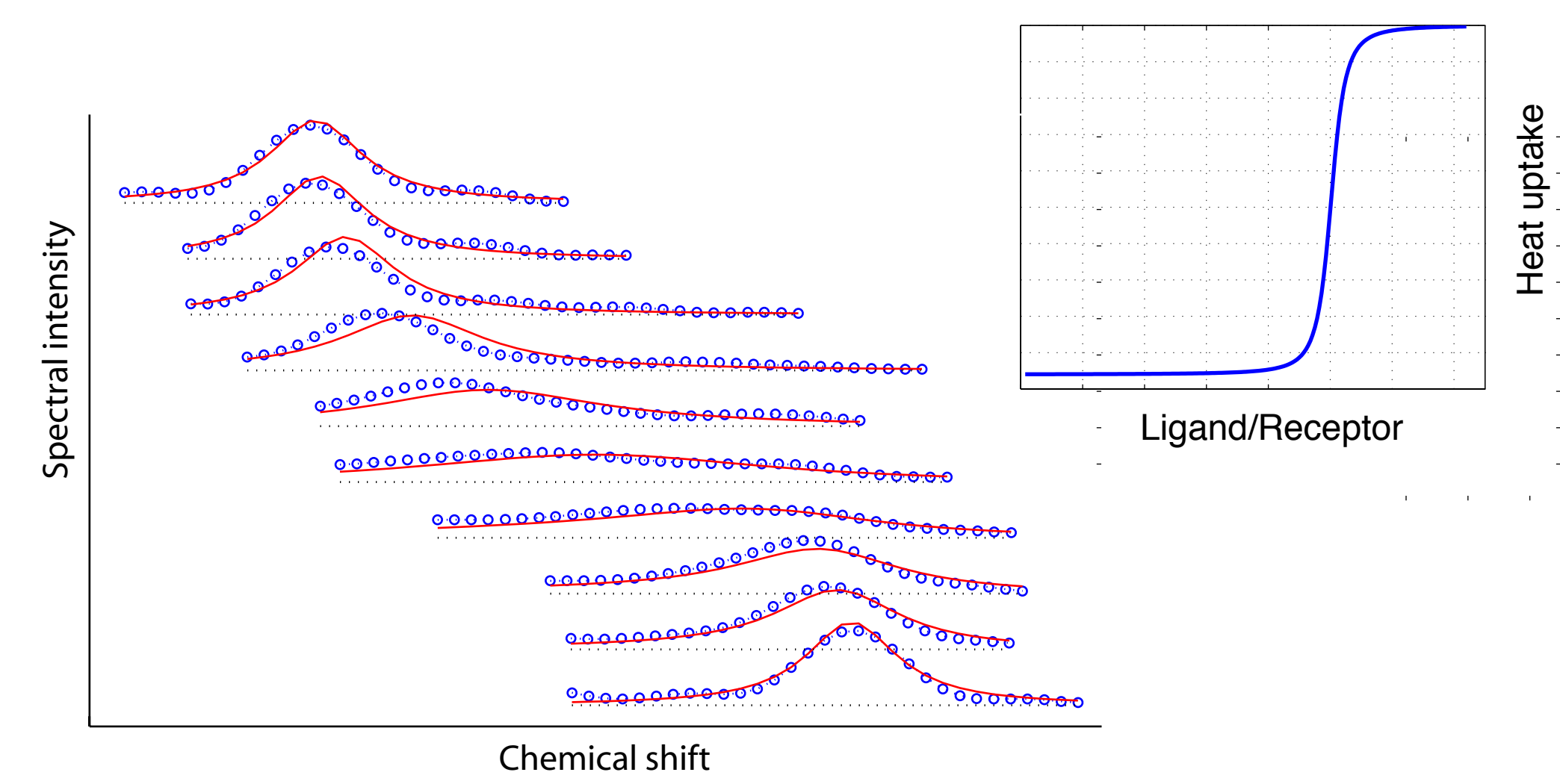
# Coupled binding and conformational equilibria in proteins: the NMR and ITC simulation study

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<http://LineShapeKin.net>

## SUMMARY

In this poster I computationally explore a variety of protein-ligand interaction mechanisms explicitly considering coupling of the conformational exchange or oligomerization in the receptor with the ligand binding. Systematic comparison of the NMR line shapes originating from different models reveals recognizable patterns usable in analysis of experimental systems. It is possible to discriminate between most of the alternative mechanisms on the basis of the HSQC titration experiments complemented with the relaxation dispersion measurements and Isothermal Titration Calorimetry (ITC). The utility of the analysis diminishes when the binding occurs in the slow exchange and the population balance is strongly shifted towards one of the conformers.

To perform the analysis I developed a software package **LineShapeKin Simulation**. It includes a variety of 3-site models discussed in the poster as well as a number of more complex multi-site mechanisms. It is easy to introduce any user-defined models and explore the expected NMR line shapes and the ITC profiles.

The software is freely available from <http://lineshapekin.net>.

The CD-ROMs (below) contain the **LineShapeKin Simulation 4.1** with the tutorials as well as the **LineShapeKin 3.2**, an NMR line shape analysis program to fit experimental spectral data with the 2-site exchange model. In the current analysis I used it as a diagnostic tool to observe how the line shapes due to the multi-site mechanisms deviate from the ones expected from the 2-site exchange.

**LineShapeKin Analysis** program capable of fitting experimental NMR line shapes, relaxation dispersion and ITC data with a variety of multi-site exchange mechanisms is currently being developed. If you are interested in receiving the update on its status, please, leave your email address below.

## Elementary 3-site mechanisms including intramolecular isomerization or dimerization processes coupled with the ligand binding 1

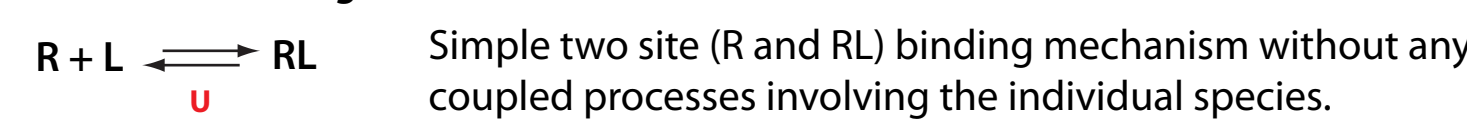
### Notation:

R - receptor, NMR observable species, L - ligand, NMR invisible species.

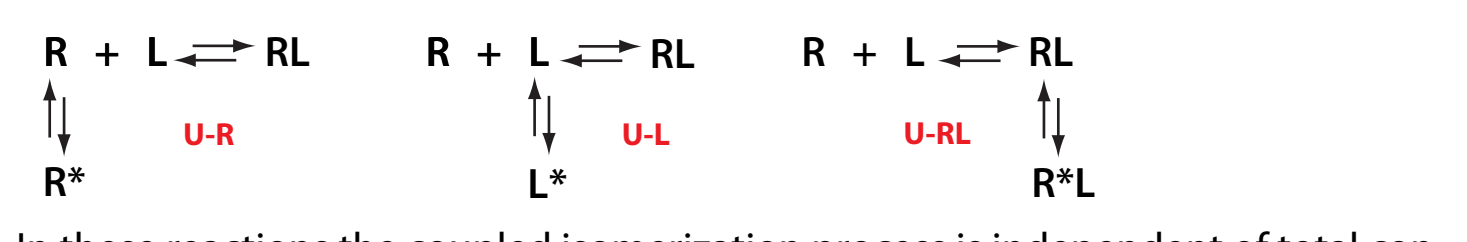
U-?? - my label for the mechanism.

The R and L labels are interchangeable. If it is possible to collect NMR data for both a ligand and receptor then the system will be most completely defined.

### Generic 1:1 binding

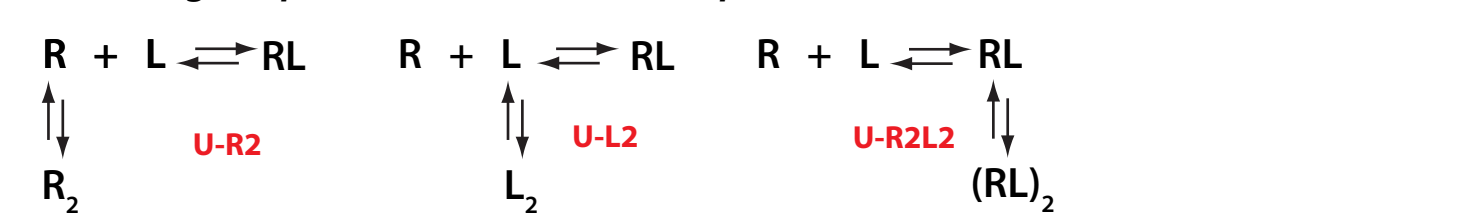


### 1:1 binding coupled with an intramolecular conformational change



In these reactions the coupled isomerization process is independent of total concentration of the species.

### 1:1 binding coupled with a dimerization process



Since the dimer formation depends on the monomer concentration it will introduce non-linearity in population profiles (ITC, chemical shift changes). Models with dimerization may also be viewed as representing the first step towards the higher oligomer formation.

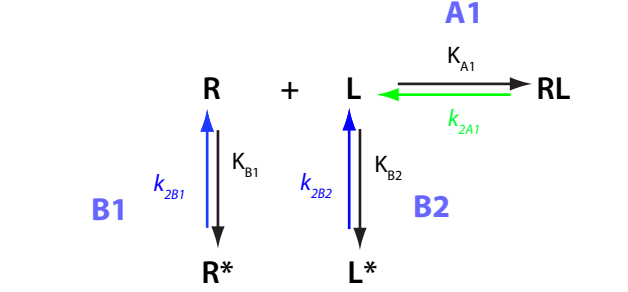
### Generic 1:2 binding



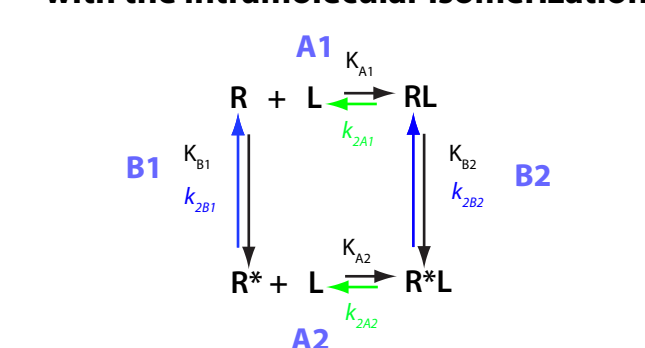
This binding mechanism will produce non-linear population changes because both transitions are directly dependent on the ligand concentration (unlike the coupled isomerization/dimerization equilibria considered above).

## Examples of complex mechanisms in LineShapeKin Simulation 2

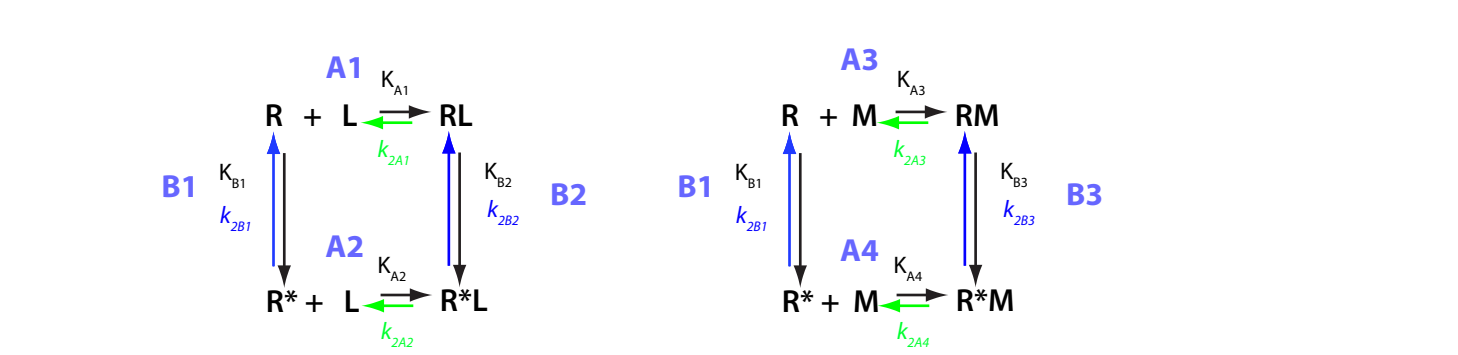
The binding process coupled with the intramolecular isomerization in both the receptor and ligand



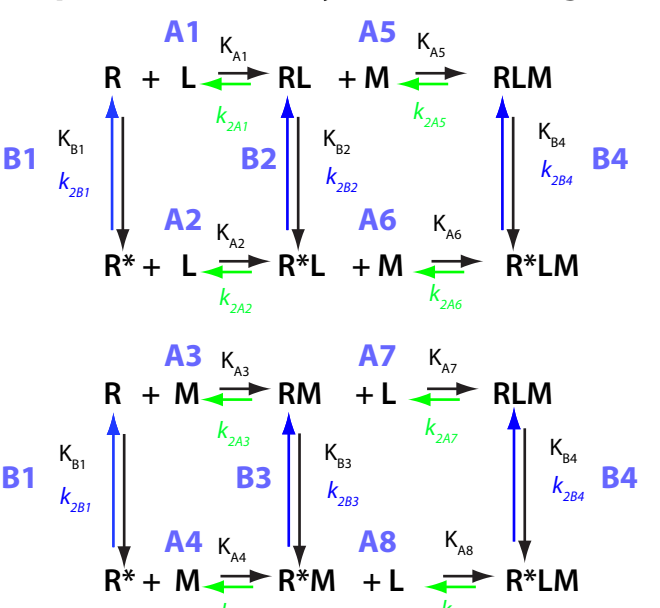
Binding of one ligand coupled with the intramolecular isomerization



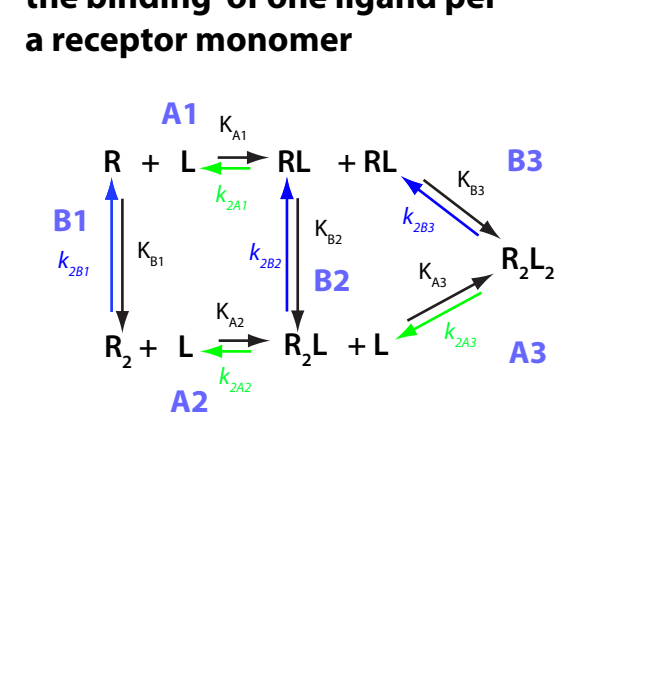
Binding of the two mutually exclusive ligands coupled with intramolecular isomerization of the receptor (competitive ligand binding)



Binding of two ligands at non-mutually exclusive binding sites coupled with intramolecular isomerization of the receptor (allosterically linked binding sites)

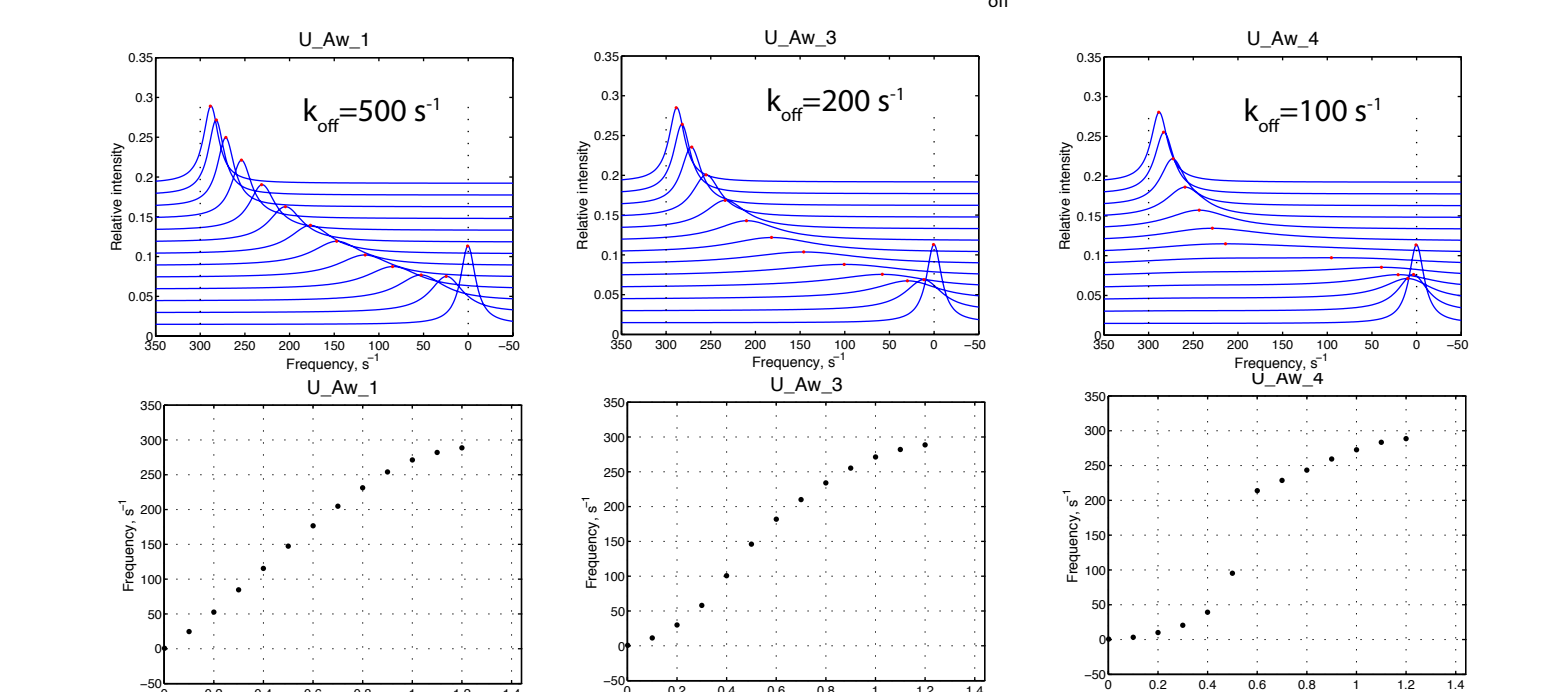


Receptor dimerization coupled with the binding of one ligand per a receptor monomer



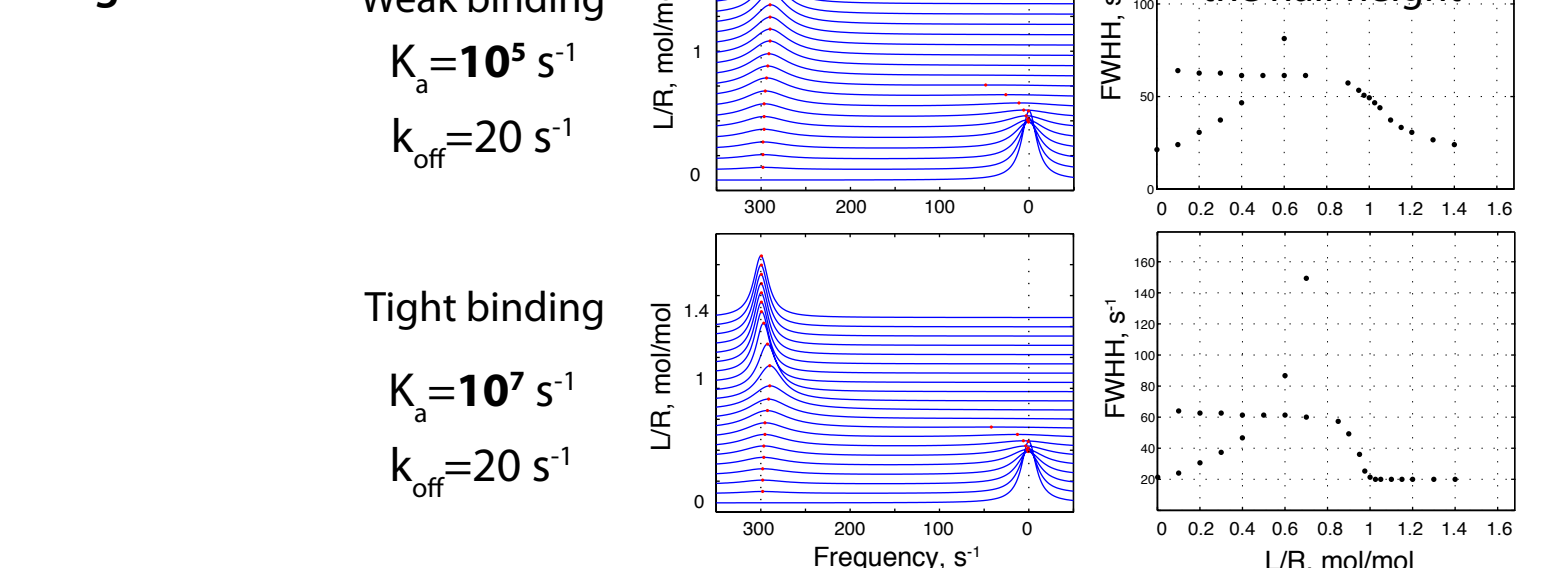
## Basic spectral patterns (the 2-site exchange, U model) 3

### Fast-intermediate exchange regime



Take home: The chemical shift changes in intermediate exchange become non-linear deceptively pointing at the 2:1 binding mechanism.

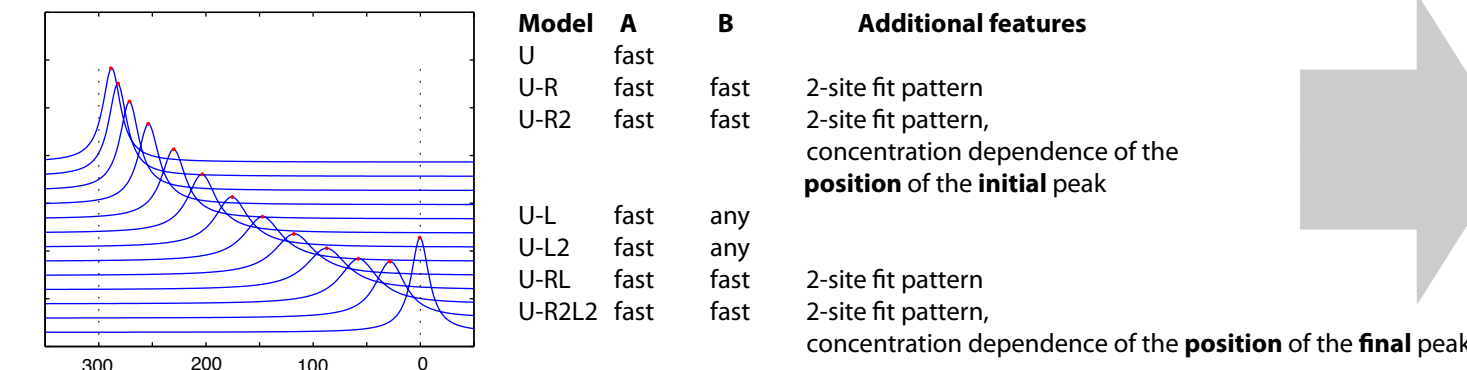
### Slow exchange regime



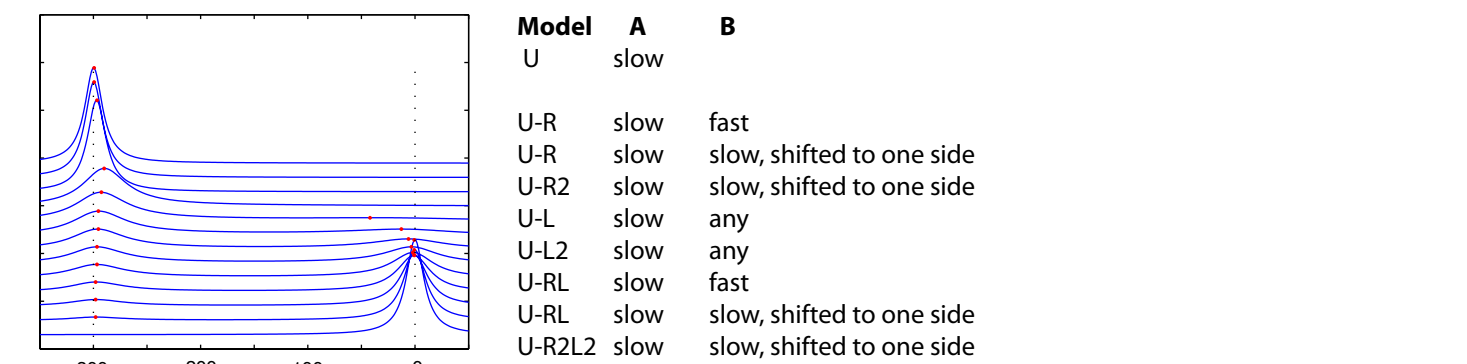
Take home: It is possible to fit slow exchange data to obtain accurate  $K_d$  if the titration series accurately covers the near-equivalence region.

## Common spectral patterns originating from different mechanisms 4

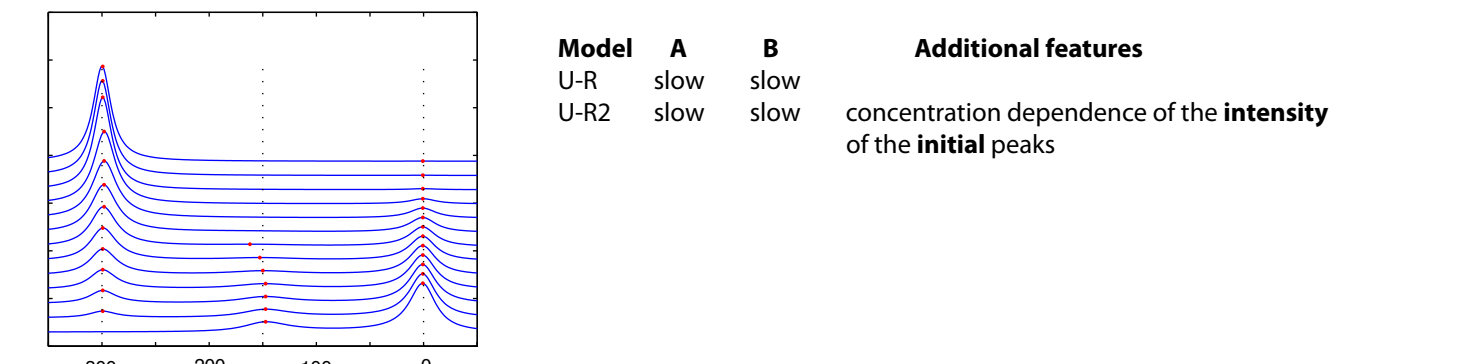
### Fast exchange between the two peaks



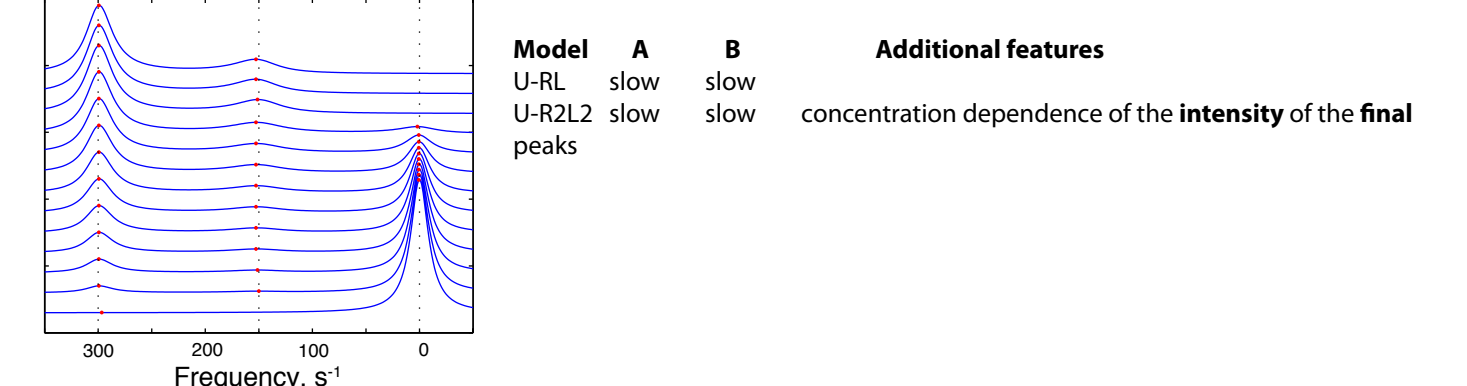
### Slow exchange between two peaks



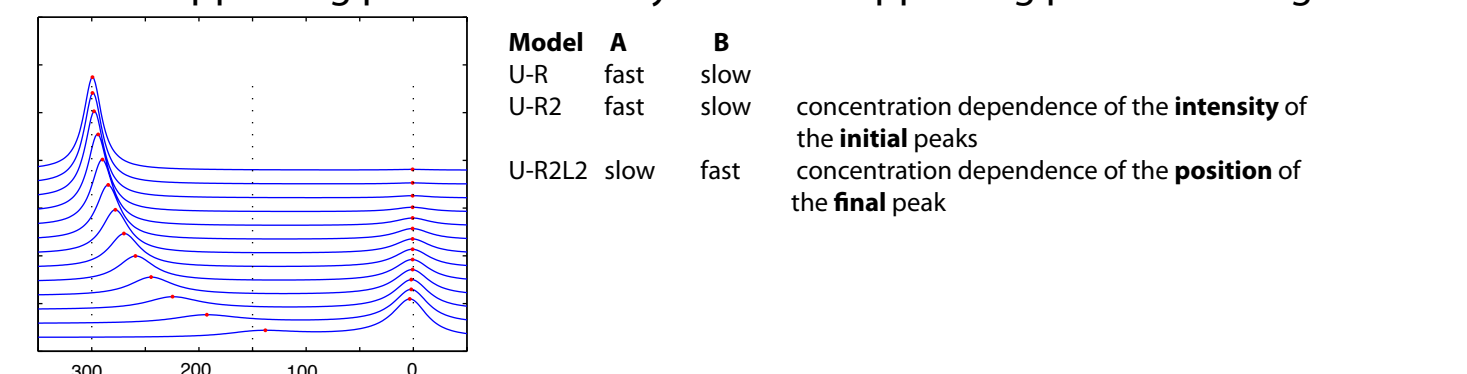
### More peaks disappear than appear in slow exchange



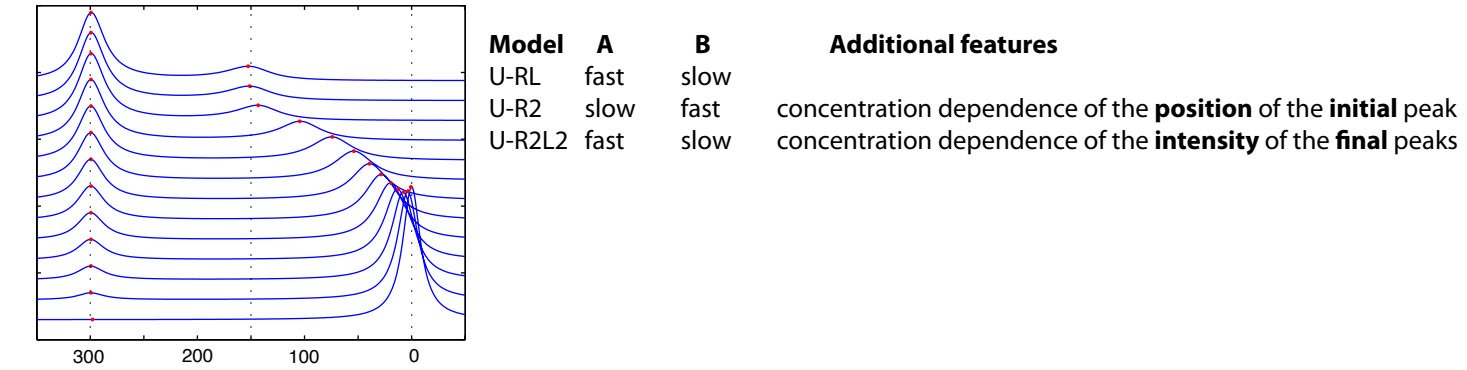
### More peaks appear than disappear in slow exchange



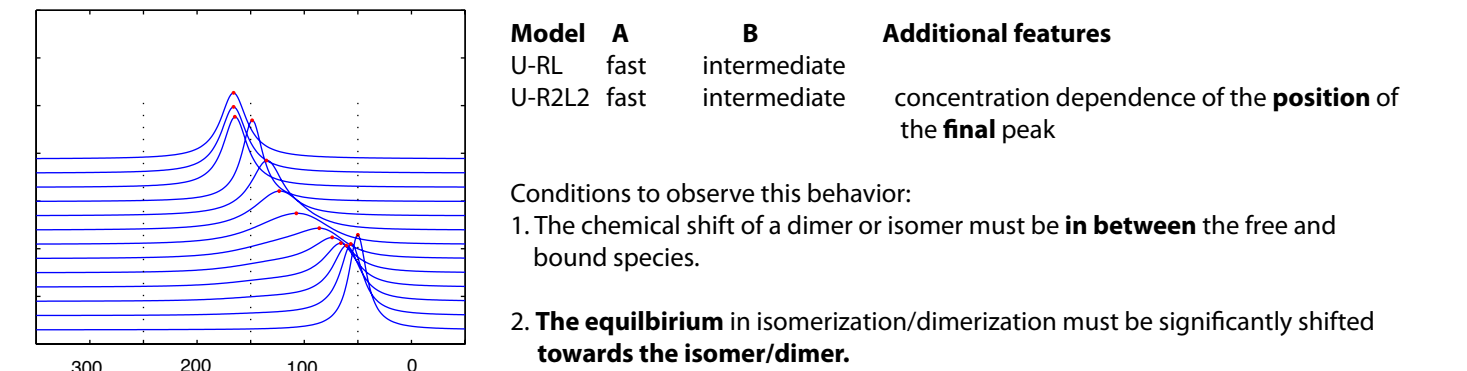
### The disappearing peak is stationary while the appearing peak is moving



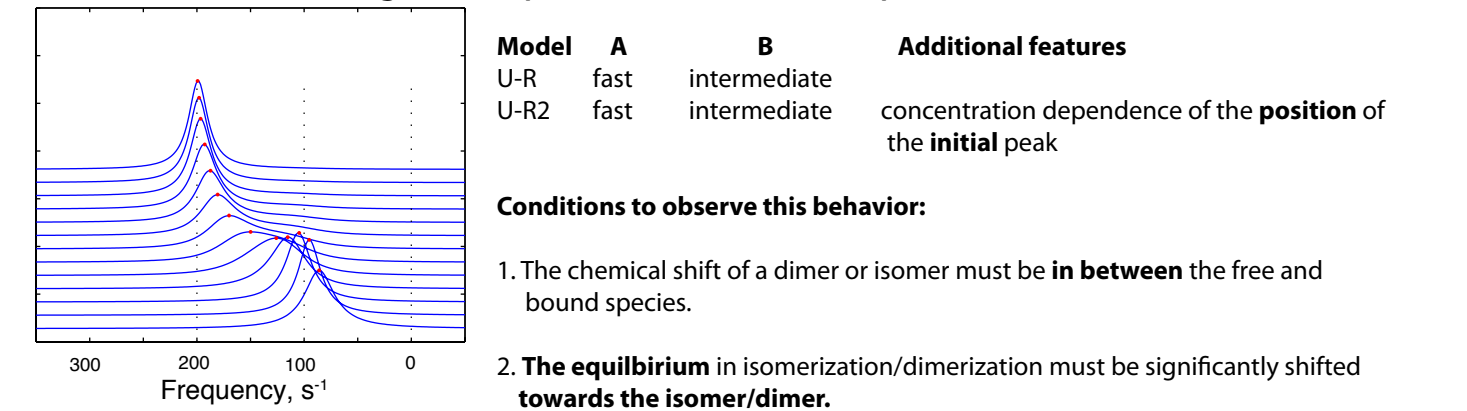
### The disappearing peak is moving while the appearing peak is stationary



### Transient narrowing of the peak before the full saturation is achieved

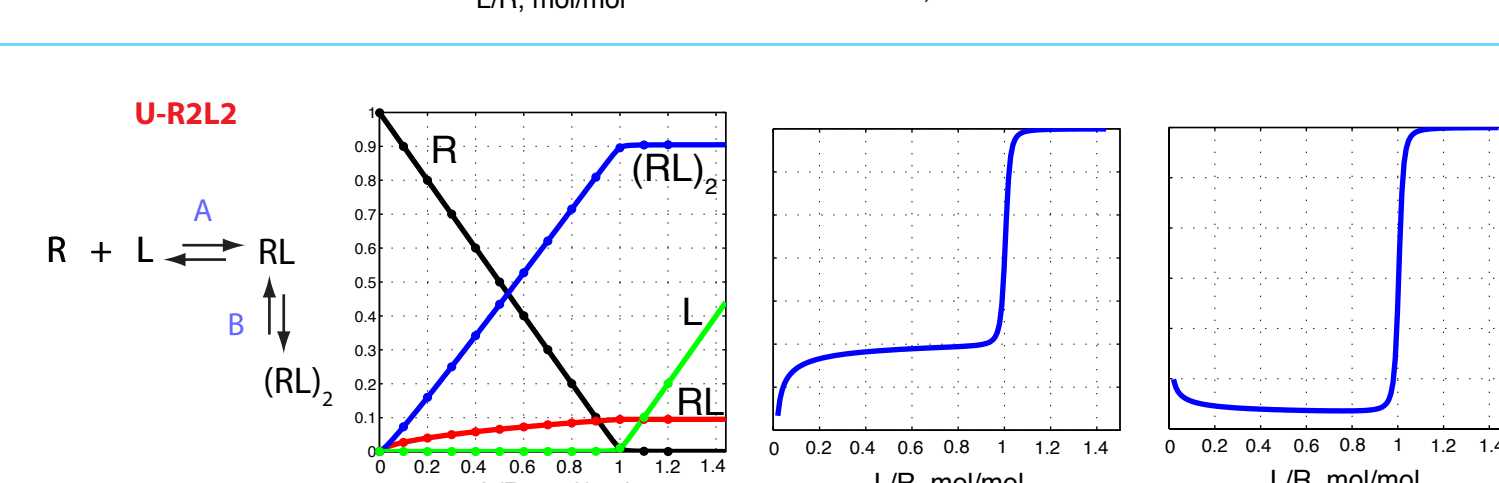
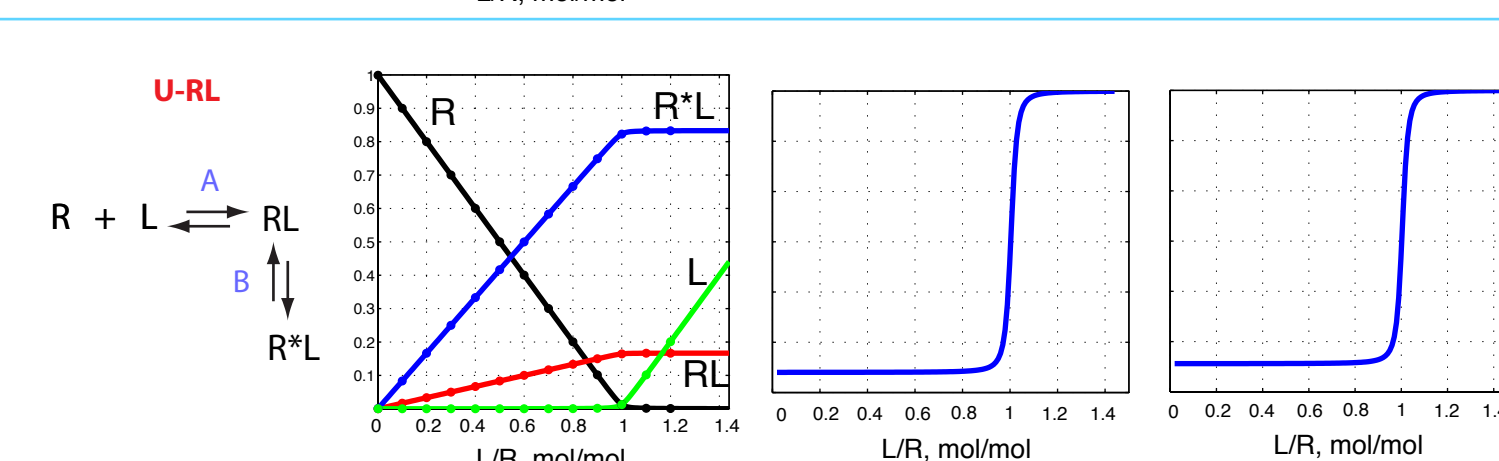
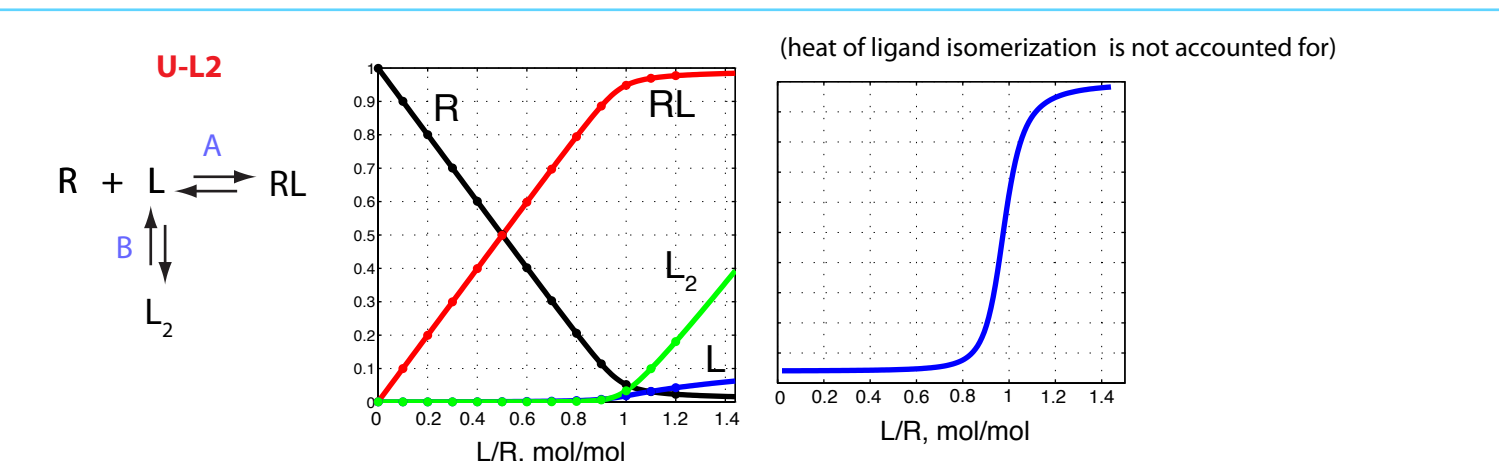
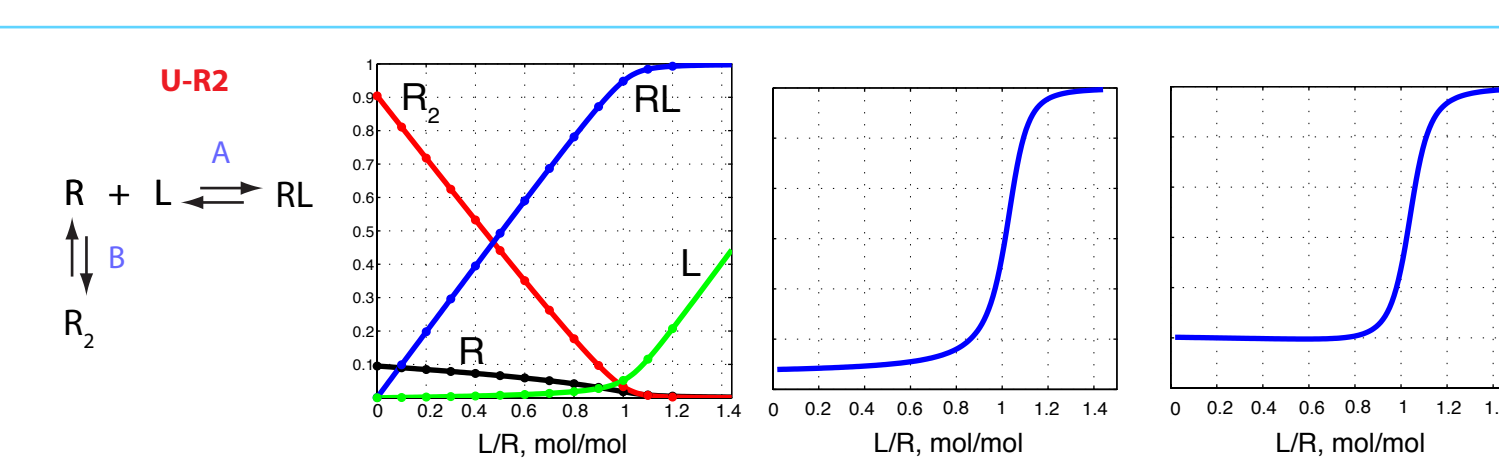
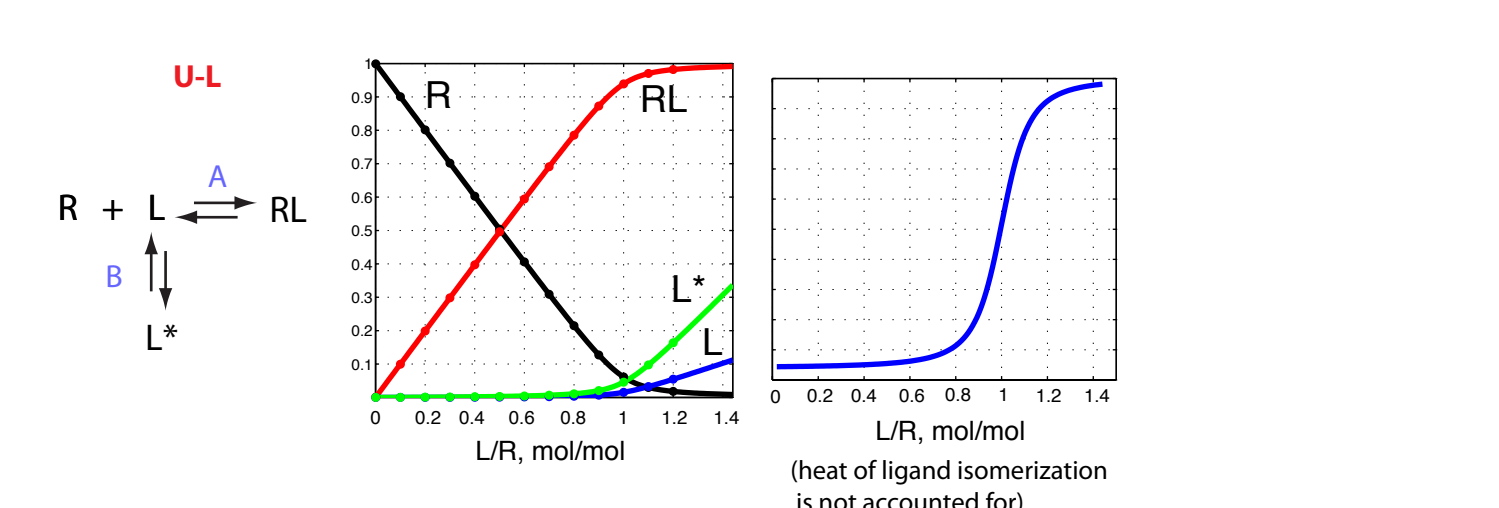
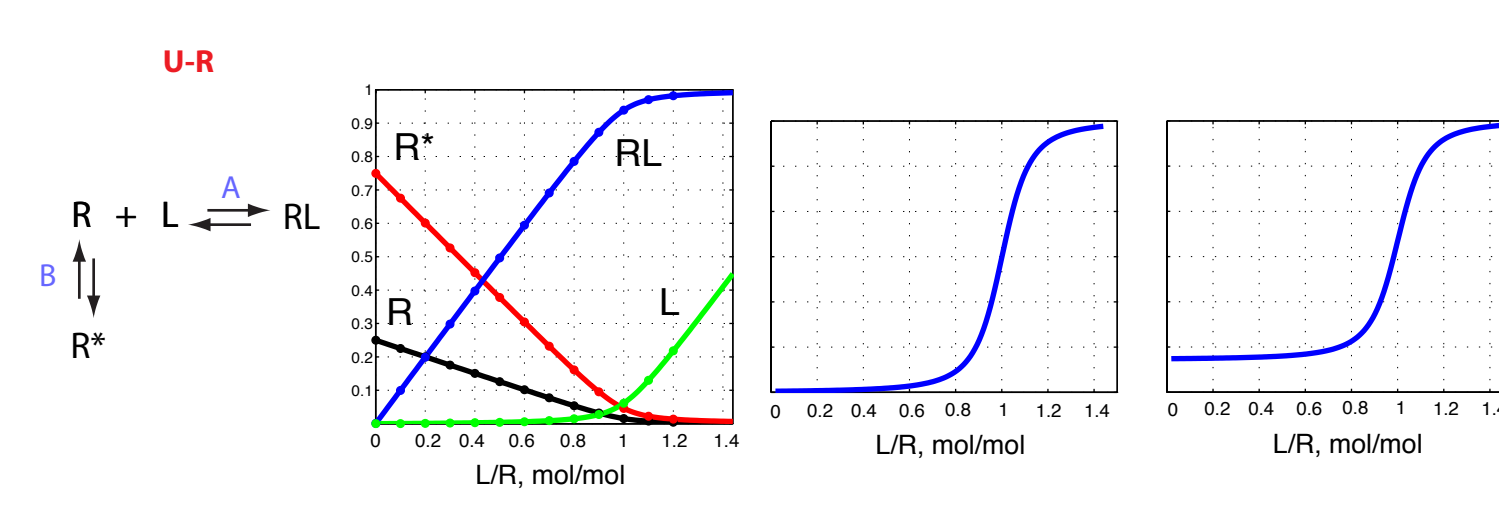
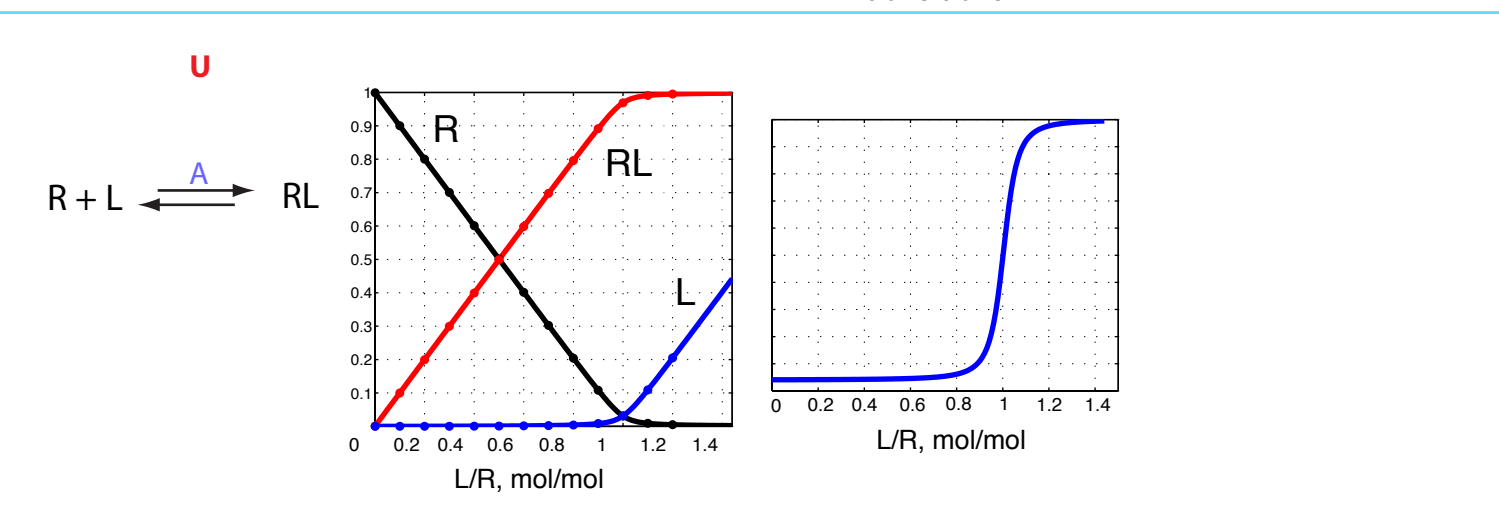


### Transient narrowing of the peak at the low receptor saturation



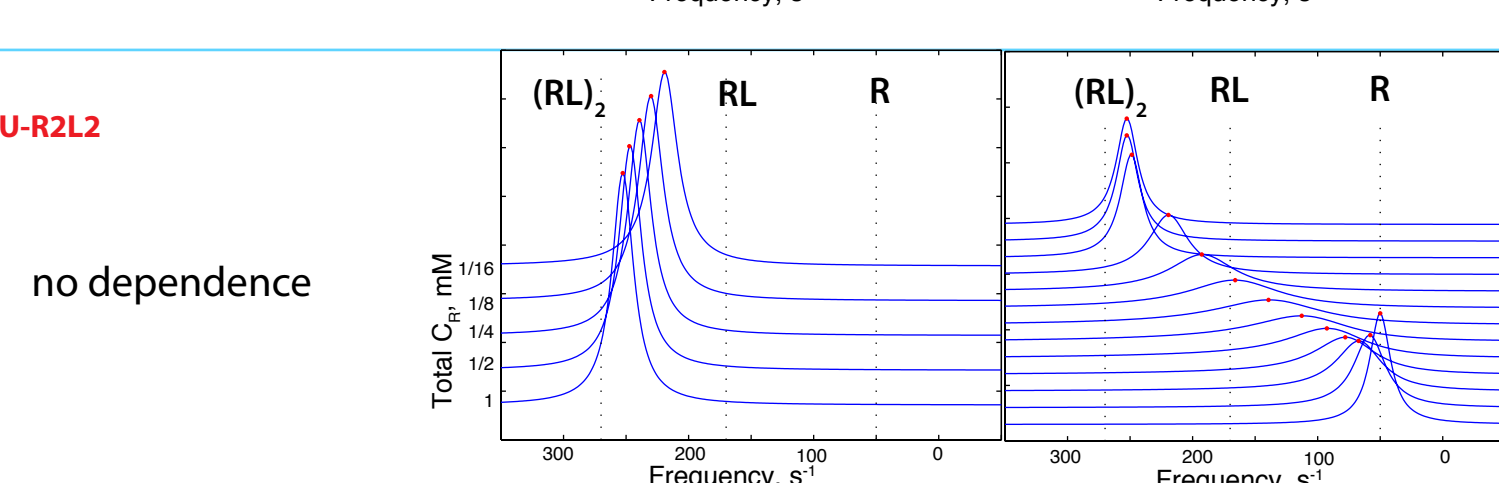
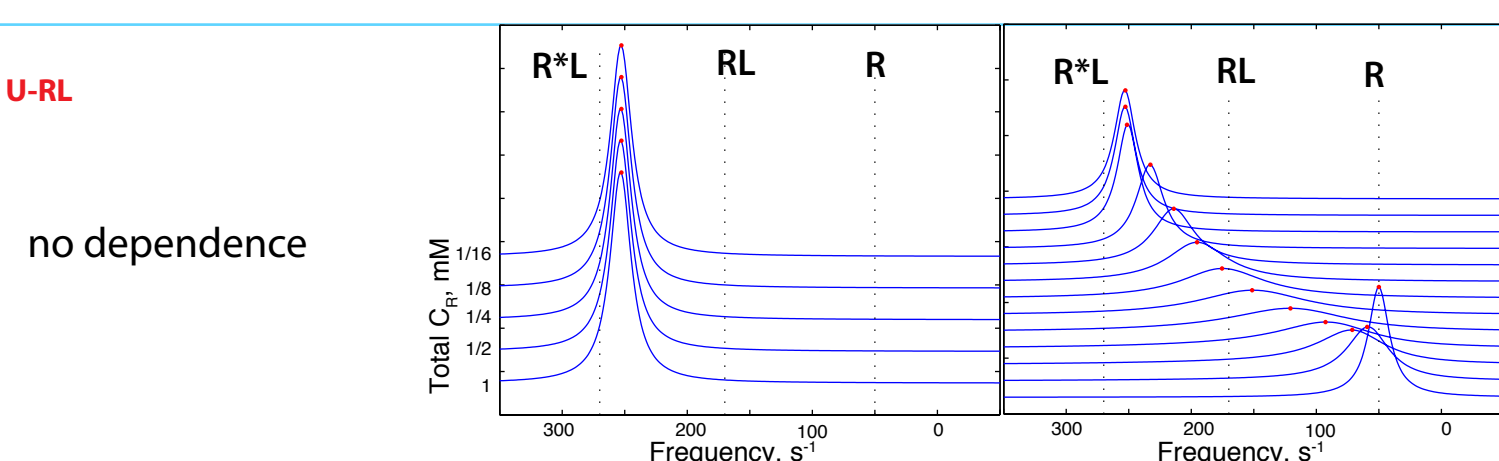
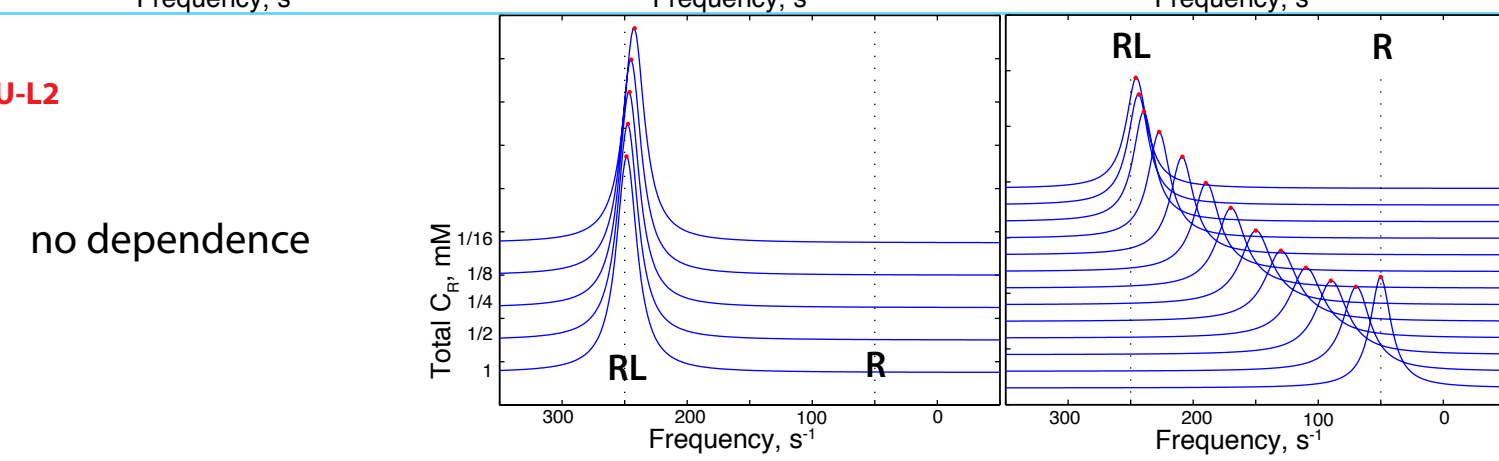
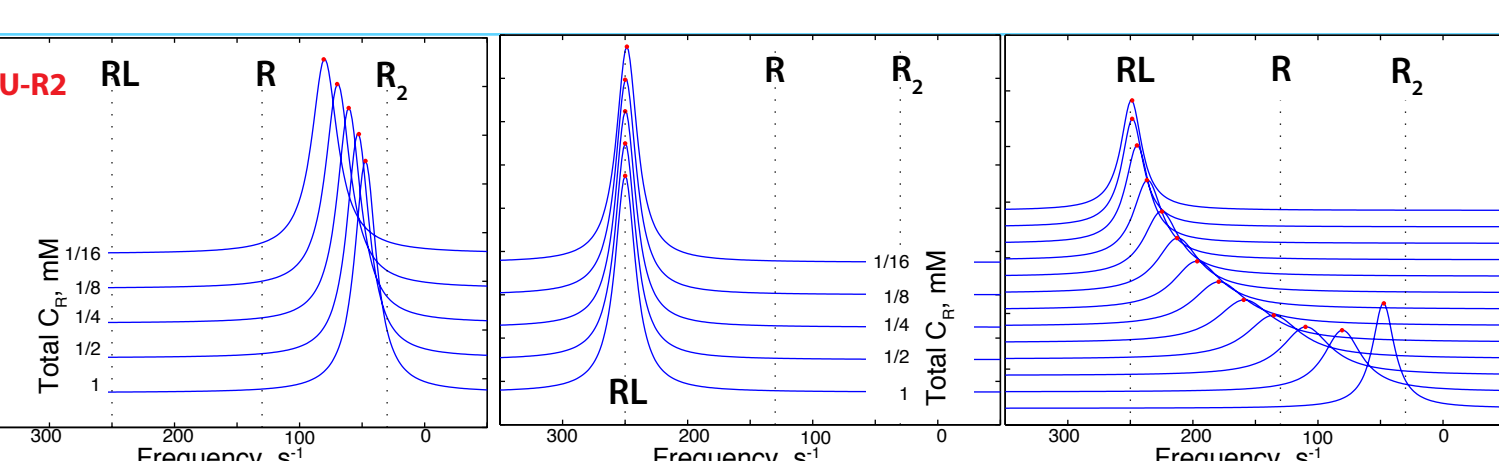
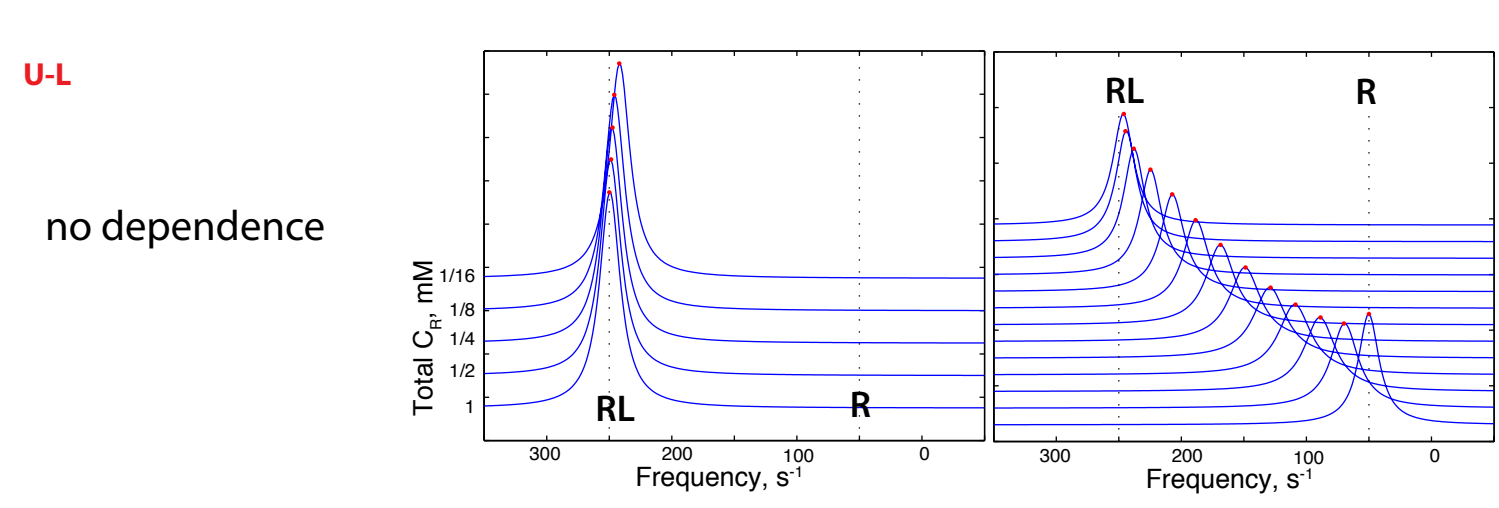
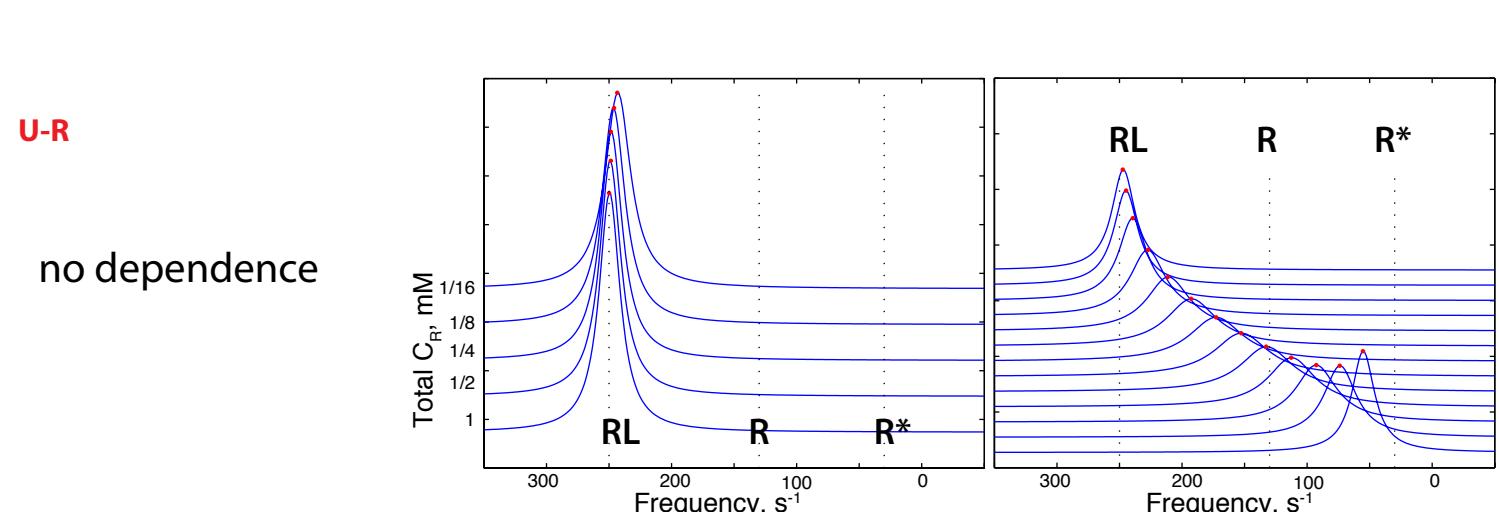
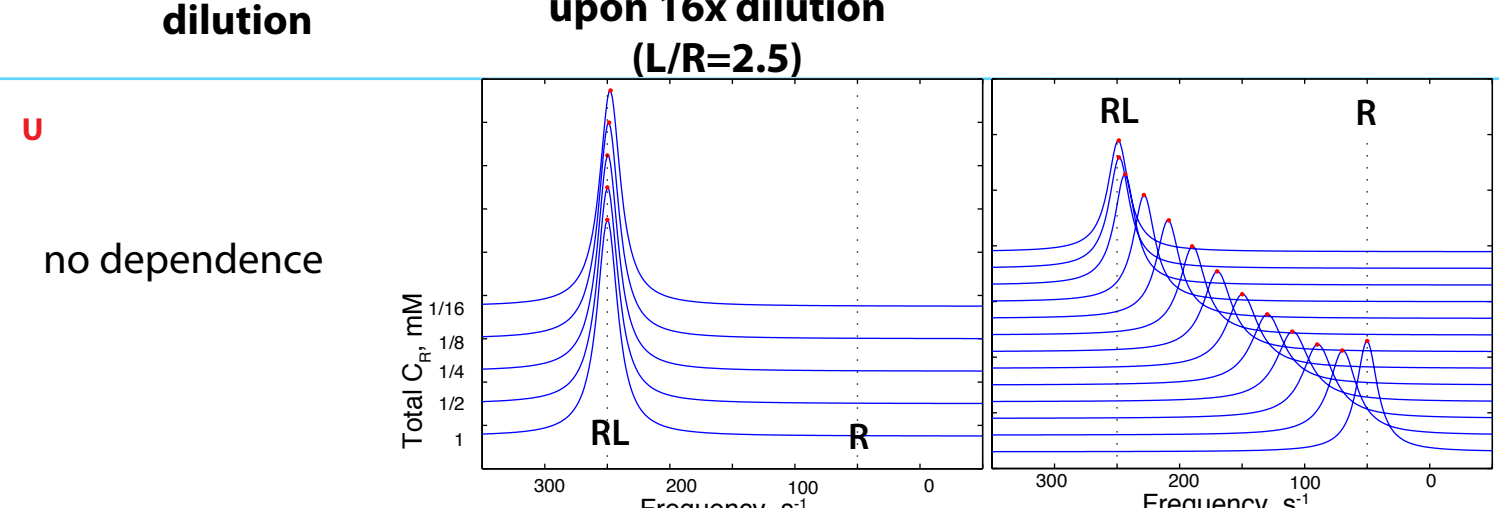
## Thermodynamic and spectral features of the fast-exchange systems I 5

**Model** **Population graph** **ITC profiles**



## Thermodynamic and spectral features of the fast-exchange systems II 6

**Dependence of the position of the unbound receptor peak upon 16x dilution** **Dependence of the position of the fully saturated receptor peak upon 16x dilution (L/R=2.5)** **Titration series**



## Thermodynamic and spectral features of the fast-exchange systems III 7

**Chemical shift change** **Line broadening** **Fit with the U model**

