Theoretical background

Consider a ligand-receptor system with atomic coordinates $\mathbf{x}^T = [\mathbf{x}_R^T \ \mathbf{x}_L^T]$.

The potential energy $E^{(X)}$ of the system is expanded around each X metastable state: $E^{(X)}(\mathbf{x}) = E^{(X)}(\mathbf{x}_0) + \frac{1}{2}(\mathbf{x} - \mathbf{x}_0)^T H^{(X)}(\mathbf{x} - \mathbf{x}_0)$. We drop the state index X for now, just mentioned it for later use.

We have noted H the Hessian of E, which can be seen as a block matrix: $H = \begin{bmatrix} H_{RR} & H_{RL} \\ H_{LR} & H_{LL} \end{bmatrix}$.

Free energy and ensemble averages

The partition function Z of metastable state X is (noting $\beta^{-1} = k_B T$):

$$\begin{aligned} Z &= e^{-\beta E(\mathbf{x}_0)} \int d\mathbf{x} \, e^{-\frac{1}{2}\mathbf{x}^T H \mathbf{x}} \\ &= e^{-\beta E(\mathbf{x}_0)} \int d\mathbf{x}_R \, e^{-\frac{1}{2}\mathbf{x}_R^T H_{RR} \mathbf{x}_R} \int d\mathbf{x}_L \, e^{-\frac{1}{2}\mathbf{x}_L^T H_{LL} \mathbf{x}_L - \frac{1}{2}\mathbf{x}_L^T H_{LR} \mathbf{x}_R - \frac{1}{2}\mathbf{x}_R^T H_{RL} \mathbf{x}_L} \end{aligned}$$

couple technical points

> The hessian is hermitian so $\mathbf{x}_L^T H_{LR} \mathbf{x}_R = (\mathbf{x}_R^T H_{LR}^T \mathbf{x}_L)^T = \mathbf{x}_R^T H_{RL} \mathbf{x}_L$
>
> Gaussian integral: $\int d\mathbf{x} \, e^{-\frac{1}{2}\mathbf{x}^T A \mathbf{x} + B^T \mathbf{x}} = \sqrt{\frac{(2\pi)^n}{|A|}} e^{\frac{1}{2} B^T A^{-1} B}$.

We first integrate over the ligand degrees of freedom, before integrating over the receptor's:

$$\begin{aligned} Z &= \sqrt{\frac{(2\pi)^{N_L}}{|H_{LL}|}} e^{-\beta E(\mathbf{x}_0)} \int d\mathbf{x}_R \, e^{-\frac{1}{2}\mathbf{x}_R^T H_{RR} \mathbf{x}_R} \, e^{\frac{1}{2}\mathbf{x}_R^T H_{RL} H_{LL}^{-1} H_{LR} \mathbf{x}_R} \\ &= \sqrt{\frac{(2\pi)^{N_L + N_R}}{|H_{LL}||H_{RR,eff}|}} e^{-\beta E(\mathbf{x}_0)} \\ &\quad \text{where } H_{RR,eff} = H_{RR} - H_{RL} H_{LL}^{-1} H_{LR} \end{aligned}$$

We have thus derived an effective potential for the receptor where the interactions with the ligand have been integrated out.

The free energy of the system (assuming it has only one metastable state) is thus:

$$F = E(\mathbf{x}_0) + \frac{k_B T}{2}[\log \mathrm{Det}(H_{LL}) + \log \mathrm{Det}(H_{RR,eff}) - (N_L + N_R) \log 2\pi].$$

Going further, it is possible to compute the equilibrium value of any function of the receptor's degrees of freedom (noting $Z_R = \sqrt{\frac{(2\pi)^{N_R}}{|H_{RR,eff}|}}$):

$

<f(\mathbf{x}{R})>\ =\ Z{R}^{-1} \int d\mathbf{x}{R}\ e^{-\frac{1}{2}\mathbf{x}{R}^{T}H{RR,eff}\mathbf{x}{R}}\ f(\mathbf{x}_{R}) $

Coupling perturbation

Assuming that one perturbs the coupling between ligand and receptor while keeping their structure constant (constant number of atoms whose average position is maintained, up to a rotation or translation of the ligand), only the effective Hessian is affected, and the free energy change is:

$$\partial F = k_B T |H_{RR,eff}|^{-1} \partial |H_{RR,eff}|$$

This expression could be used to probe the stabilizing or destabilizing role of individual coupling.

In a more operative fashion for the overall ligand binding event, we consider the change:

$$F(\text{liganded}) - F(\text{unliganded}) = \frac{k_B T}{2} [\log \mathrm{Det}(H_{RR,eff}) - \log \mathrm{Det}(H_{RR})]$$

Noting $\lambda_i = \omega_i^2$ the eigenvalues of H_{RR}, the free-energy change upon binding is :

$$\Delta F = k_B T \sum^{3N_R} \log \frac{\omega_i^*}{}$$

Implementation

[before running through the cells below, we load the necessary libraries, and run the Toolkit cells at the end of the notebook]

In [100]:
```
%matplotlib inline
import numpy as np                             # load numerical library
from matplotlib import pyplot as plt  # load plotting library
from prody import *                            # load prody library
```

Define parameters and variables...

In [101]:
```
pdbs_folder  = 'pdbs'           # local folder where PDB files can be fo
und
pdb_filename = '4FZ0.pdb'       # name of PDB considered for the analysi
s (must be found in pdbs_folder)
cutoff=15.0                     # distance cutoff to couple pairs of ato
ms
gamma=1.0                       # see ProDy
whole_selection     ='protein and name CA'              # set of all ato
ms considered
receptor_selection =whole_selection+' and not chain A'  # subset of whol
e_selection, for receptor
ligand_selection   =whole_selection+' and chain A'      # subset of whol
e_selection, for ligand
```

Load PDB object

```
In [102]: pdb1 = parsePDB(pdbs_folder+'/'+pdb_filename)
```

```
@> 10557 atoms and 1 coordinate set(s) were parsed in 0.08s.
```

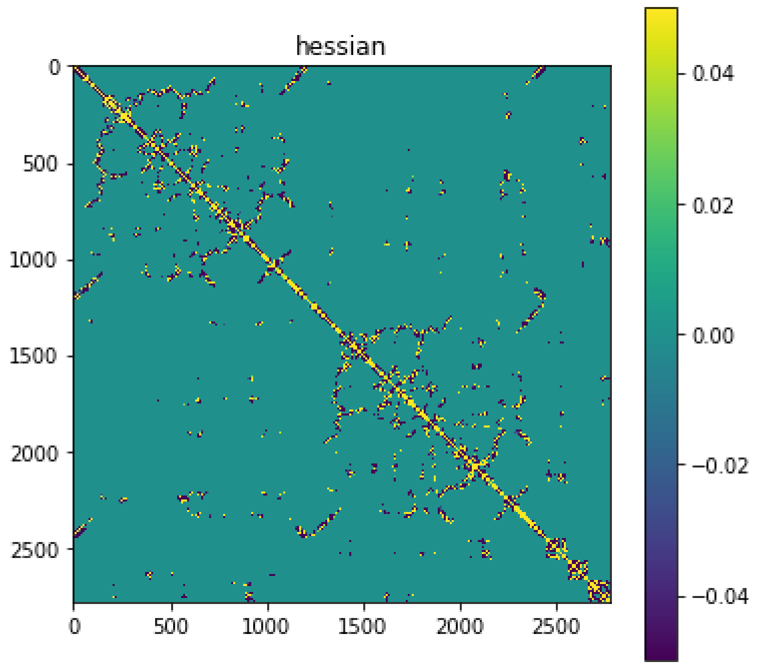
Compute uncoupled and effective receptor-receptor Hessians:

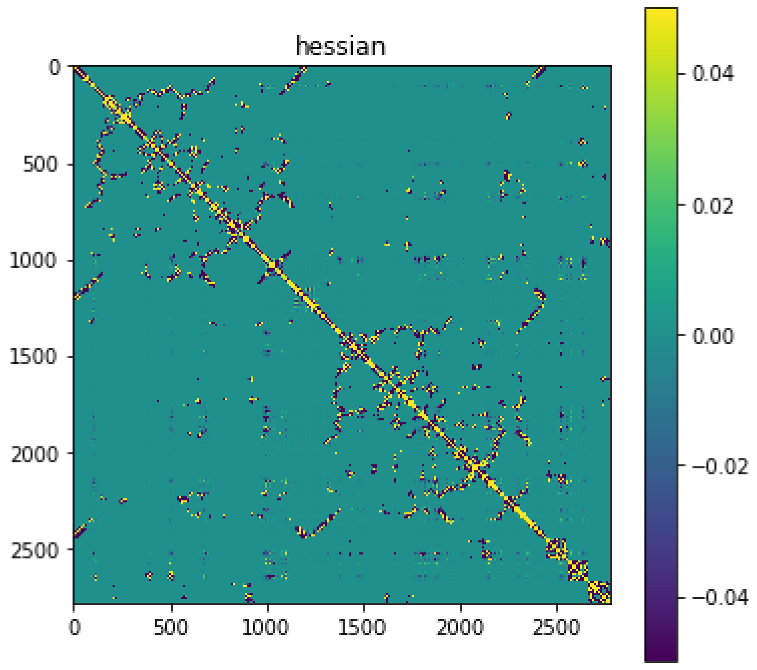
```
In [103]: HRR     = get_effective_hessian(pdb1,whole_selection=whole_selection,lig
and_selection=ligand_selection,coupling=0.0)
HRR_eff = get_effective_hessian(pdb1,whole_selection=whole_selection,lig
and_selection=ligand_selection,coupling=1.0)
```

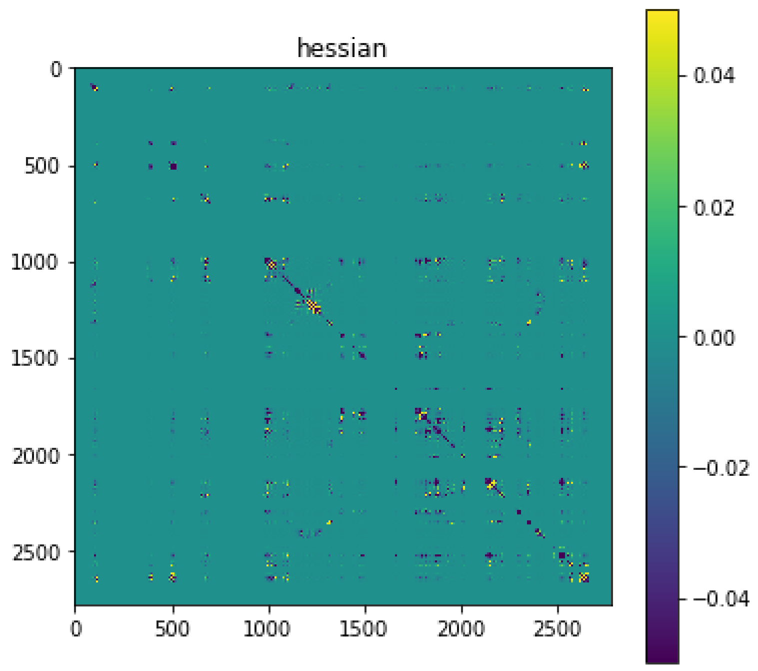
```
@> Hessian was built in 0.65s.
@> Hessian was built in 0.62s.
```

In [104]:
```
for Hessian in HRR,HRR_eff,HRR_eff-HRR:
    plot_hessian(Hessian,vrange=[-0.05,0.05],figsize=6)
```

```
Size of the hessian:  (2781, 2781)
Size of the hessian:  (2781, 2781)
Size of the hessian:  (2781, 2781)
```





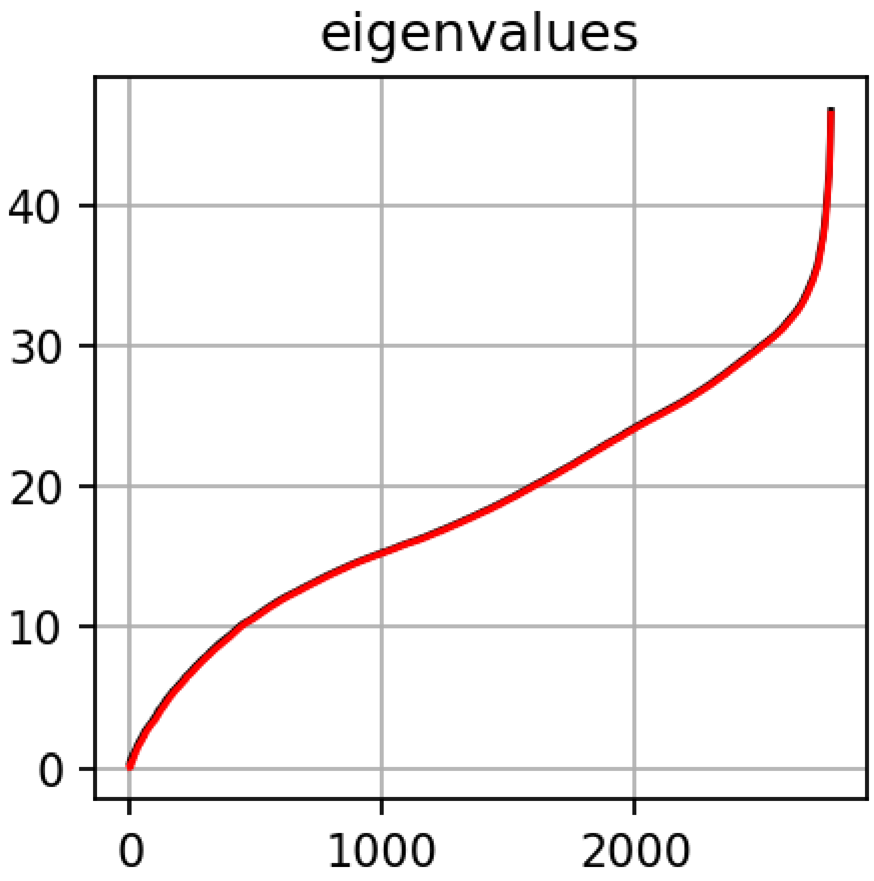


Compute eigenvalues and associated normal modes

```
In [105]: l,v         = get_eigen(HRR)
          l_eff,v_eff = get_eigen(HRR_eff)
```

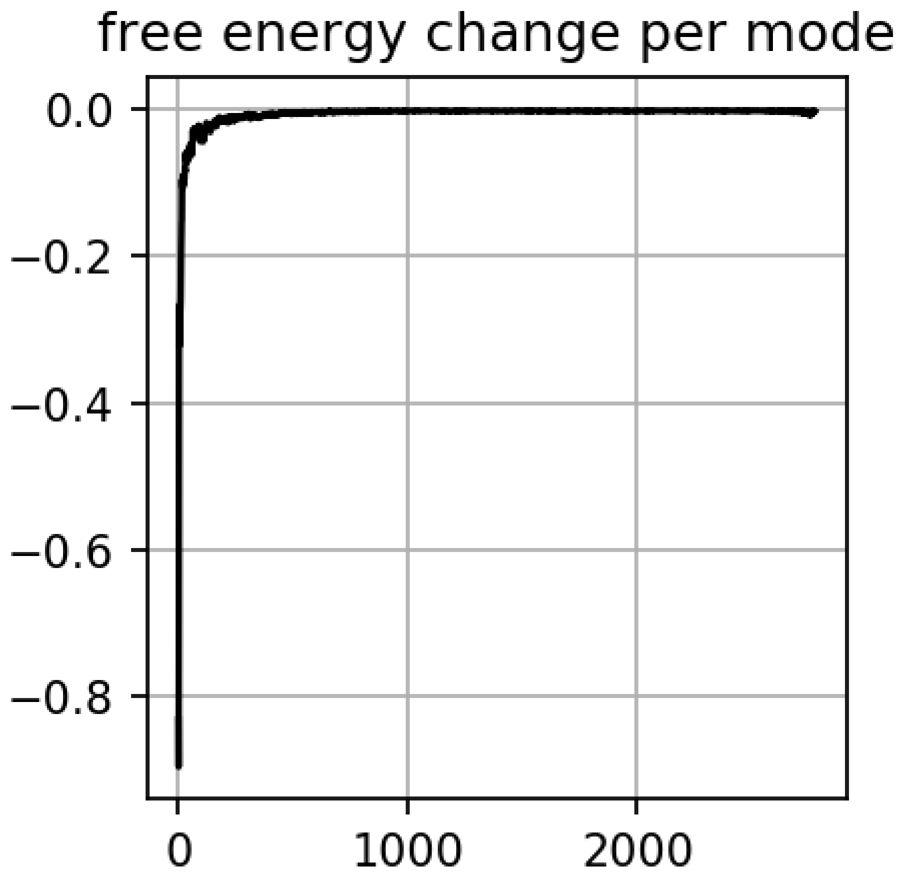
Plot uncoupled (black) and coupled (red) spectrum:

In [106]:
```
plot_spectrum(l,l2=l_eff,figsize=3)
```



Now plot $\log \frac{\omega_{eff}}{\omega}$

```
In [112]: plot_spectrum(np.log(l_eff/l)/2,figsize=3,title='free energy change per
           mode')
```



Finally, give the total free energy change upon binding:

```
In [114]: deltaF = get_binding_energy(spectrum_unbound=l,spectrum_bound=l_eff)

          Binding free energy (in units of kT):  -19.9180863239
```

TOOLKIT

In [113]:
```
def get_binding_energy(spectrum_unbound=None,spectrum_bound=None):
    """get_binding_energy
    """
    deltaF=None
    if spectrum_unbound is not None and spectrum_bound is not None:
        deltaF = np.sum(np.log(spectrum_bound/spectrum_unbound))/2.0
        print('Binding free energy (in units of kT): ',deltaF)
    return deltaF

def get_effective_hessian(pdb,whole_selection='protein',ligand_selection
=None,coupling=1.0,cutoff=15.0,gamma=1.0):
    """ get_effective_hessian
    """
    whole_hessian = get_hessian(pdb1,whole_selection,cutoff=cutoff,gamma
=gamma)
    receptor_idx,ligand_idx = get_idxs(pdb,whole_selection=whole_selecti
on,subset_selection=ligand_selection)
    HRR = whole_hessian[receptor_idx,:][:,receptor_idx]
    HLL = whole_hessian[ligand_idx,:][:,ligand_idx]
    HLR = coupling*whole_hessian[ligand_idx,:][:,receptor_idx]
    HRL = coupling*whole_hessian[receptor_idx,:][:,ligand_idx]
    HRR_eff = HRR - np.dot(HRL,np.dot(np.linalg.inv(HLL),HLR))
    return HRR_eff

def get_idxs(pdb,whole_selection='protein',subset_selection=None):
    """
    """
    whole_selected  = pdb.select(whole_selection)
    subset_selected = pdb.select(subset_selection)
    whole_indices   = whole_selected.getIndices()
    subset_indices  = subset_selected.getIndices()
    mask = np.in1d(whole_indices,subset_indices,invert=True)
    complement_indices = whole_indices[mask]
    ligand_idx   = get_hessian_idx(whole_indices,subset_indices)
    receptor_idx = get_hessian_idx(whole_indices,complement_indices)
    return receptor_idx,ligand_idx

def get_hessian_idx(big_array, subset_array):
    """
    """
    index = np.argsort(big_array)
    sorted_big_array = big_array[index]
    sorted_index = np.searchsorted(sorted_big_array, subset_array)
    yindex = np.take(index, sorted_index, mode="clip")
    mask = big_array[yindex] != subset_array
    result = np.ma.array(yindex, mask=mask)
    idx = []
    #n=0
    for i in np.arange(result.shape[0]):
        for k in np.arange(0,3):
            #n+=1
            idx.append(3*(result[i])+k)
    return idx

def get_hessian(pdb,atom_selection,cutoff=15.0,gamma=1.0):
    """ get_hessian
    """
    atom_selected = pdb.select(atom_selection)
    anm = ANM(atom_selection)
    anm.buildHessian(atom_selected,cutoff=cutoff,gamma=gamma)
    return anm.getHessian()

def get_eigen(Hessian,keep_6first=False):
    eigenvalues, eigenvectors = eigenpb(Hessian)
    index = np.argsort(eigenvalues)
    l = eigenvalues[index]
    v = eigenvectors[:,index]
    if keep_6first:
        istart=0
    else:
        istart=6
    return l[istart:],v[:,istart:]

def eigenpb(matrix):
    """ eigenpb
    """
    eigenvalues, eigenvectors = np.linalg.eig(matrix)
    return eigenvalues, eigenvectors
```

In [109]:
```
def plot_hessian(hessian,xyrange=None,sampling=1,vrange=[0,1],figsize=12
):
    """ plot_hessian: show Hessian matrix
    Parameters
    ----------
    - xyrange  : the first and last index you want to plot. Namely hessi
an[range[0]:range[1],range[0]:range[1]]
    - sampling : only show every sampling-th index
    - vrange   : restrict the color value to that range
    """
    #hessian = anm.getHessian()
    print("Size of the hessian: ",hessian.shape)
    if xyrange is None:
        xrange=[0,hessian.shape[0]]
        yrange=[0,hessian.shape[1]]
    fig = plt.figure(figsize=(figsize,figsize))
    plt.imshow(hessian[xrange[0]:xrange[1]:sampling,yrange[0]:yrange[1]:
sampling],cmap='viridis',vmin=vrange[0],vmax=vrange[1])
    plt.colorbar()
    plt.title('hessian')

def plot_spectrum(eigenvalues,l2=None,figsize=12,title='eigenvalues'):
    """ plot_spectrum: ...
    """
    nrow=1
    ncol=1
    fig = plt.figure(figsize=(figsize, figsize), dpi= 160, facecolor='w'
, edgecolor='k')
    plt.subplot(nrow,ncol,1)
    plt.title(title)
    plt.grid()
    plt.plot(eigenvalues,'-',color='k')
    if l2 is not None:
        plt.plot(l2,'-',color='red')
    plt.tight_layout()
    plt.show()
```