Script for calculating different second order motifs

Brief description: This livescript imports the cell table (tcell) and the connection table (tconnection) and calculated the occcurence of different second order motifs. One can choose which cell types should be included at which position in the motif. Further the occurence of these motifs are compared to randomly generated networks which assume the overall subtype-specific connection probabilities.

Import excel tables and calculate degrees

```
n_celltypes = 2;    %choose PC/IN if IN are supposed to be pooled. Choose PC/FS/NFS if IN subty
layer = 0;          %choose layer. Publication mostly focused on superficial layer

central_celltype = 1;
partner_celltype = 0;

%these excel files need to be in the source folder
tcell = readtable('\tcell.xlsx');
tcell = sortrows(tcell,'IDcell','ascend');

tconnection = readtable('\tconnection.xlsx');
tconnection.IDpre = string(tconnection.IDpre);
tconnection.IDpost = string(tconnection.IDpost);
tconnection = tconnection(tconnection.synaptic_layer == layer,:); %remove cells without coordin

if n_celltypes == 2
    tconnection.stype = tconnection.Synaptic_Type_PCIN;
    tcell.ctype = tcell.cell_type_PC_IN;
elseif n_celltypes == 3
    tconnection.stype = tconnection.Synaptic_Type;
    tcell.ctype = tcell.cell_type_3;
end

for i = 1:size(tcell,1)
    pre = tconnection.IDpre == tcell.IDcell{i};
    post = tconnection.IDpost == tcell.IDcell{i};

    for s = 1:max(tconnection.stype)
        stype = tconnection.stype == s;
        tcell.in_found(i,s) = sum(tconnection.Connected(post & stype));
        tcell.in_tested(i,s) = numel(tconnection.Connected(post & stype));
        tcell.out_found(i,s) = sum(tconnection.Connected(pre & stype));
        tcell.out_tested(i,s) = numel(tconnection.Connected(pre & stype));
        tcell.in_recfound(i,s) = sum(tconnection.reciprocity(post & stype));
        tcell.out_recfound(i,s) = sum(tconnection.reciprocity(pre & stype));
    end
end
```

Connection type numbering correspond to either

PC-PC, PC-IN, IN-PC, IN-IN   -or-   PC-PC, PC-FS, PC-NFS, FS-PC, FS-FS, FS-NFS, NFS-PC, NFS-FS, NFS-NFS

```
if n_celltypes == 2
```

```
    synapse_matrix = [1 2; 3 4];
elseif n_celltypes == 3
    synapse_matrix = [1 2 3; 4 5 6; 7 8 9];
end
fil = tcell.ctype == central_celltype;
out_s = synapse_matrix(central_celltype+1,partner_celltype+1);
in_s = synapse_matrix(partner_celltype+1,central_celltype+1);
```

Calculate chain and doublerec motifs for each cell and calculate clusterwise aggregate of motifs

```
n_cell = numel(tcell.ctype(fil))
```

```
n_cell = 251
```

```
out_found = sum(tcell.out_found(fil,out_s))
```

```
out_found = 100
```

```
out_tested = sum(tcell.out_tested(fil,out_s))
```

```
out_tested = 506
```

```
out_prob = out_found/out_tested
```

```
out_prob = 0.1976
```

```

in_found = sum(tcell.in_found(fil,in_s))
```

```
in_found = 101
```

```
in_tested = sum(tcell.in_tested(fil,in_s))
```

```
in_tested = 506
```

```
in_prob = in_found/in_tested
```

```
in_prob = 0.1996
```

```

%calculate 2nd order motif for each cell
tcell.in_prob = tcell.in_found ./ tcell.in_tested;
tcell.out_prob = tcell.out_found ./ tcell.out_tested;

tcell.divergent_found = tcell.out_found .* (tcell.out_found-1) ./2;
tcell.divergent_tested = tcell.out_tested .* (tcell.out_tested-1) ./2;

tcell.convergent_found = tcell.in_found .* (tcell.in_found-1) ./2;
tcell.convergent_tested = tcell.in_tested .* (tcell.in_tested-1) ./2;

tcell.chain_found = tcell.in_found(:,in_s) .* tcell.out_found(:,out_s) - tcell.out_recfound(:,o
tcell.doublerec_found(tcell.out_recfound(:,out_s) >= 2) = tcell.out_recfound(tcell.out_recfounc

%subset tconnection
confil = tconnection.stype == out_s;
tconnection2 = tconnection(confil,:);
```

```
% create tcluster, only include cells with xy coordinates
tcluster = table();
tcluster.id = categorical(unique(tconnection2.Idslice));
for i = 1:size(tcluster,1)
    cell_central_filter = tcell.Idslice == tcluster.id(i) & tcell.ctype == central_celltype & t
    cell_partner_filter = tcell.Idslice == tcluster.id(i) & tcell.ctype == partner_celltype & t
    tcluster.n_central(i) = sum(cell_central_filter);
    tcluster.n_partner(i) = sum(cell_partner_filter);
    tcluster.rec_found(i) = sum(tcell.out_recfound(cell_central_filter,out_s));
    tcluster.doublerec_found(i) = sum(tcell.doublerec_found(cell_central_filter));
    tcluster.out_found(i) = sum(tcell.out_found(cell_central_filter,out_s));
    tcluster.in_found(i) = sum(tcell.in_found(cell_central_filter,in_s));
    tcluster.div_found(i) = sum(tcell.divergent_found(cell_central_filter,out_s));
    tcluster.con_found(i) = sum(tcell.convergent_found(cell_central_filter,in_s));
    tcluster.chain_found(i) = sum(tcell.chain_found(cell_central_filter));
end

rec_found = sum(tcluster.rec_found)
```

```
rec_found = 39
```

```
con_found = sum(tcluster.con_found)
```

```
con_found = 59
```

```
div_found = sum(tcluster.div_found)
```

```
div_found = 59
```

```
chain_found = sum(tcluster.chain_found)
```

```
chain_found = 87
```

```
doublerec_found = sum(tcluster.doublerec_found)
```

```
doublerec_found = 15
```

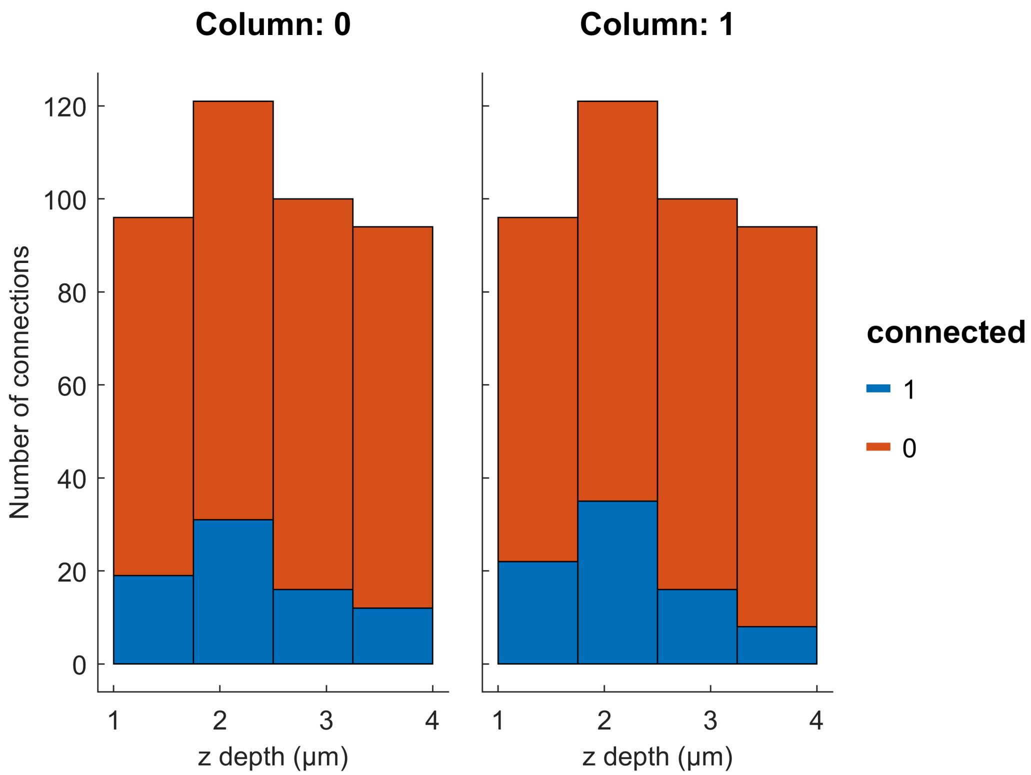
Simulate random networks with distance dependence

Calculate distance-dependence of connectivity

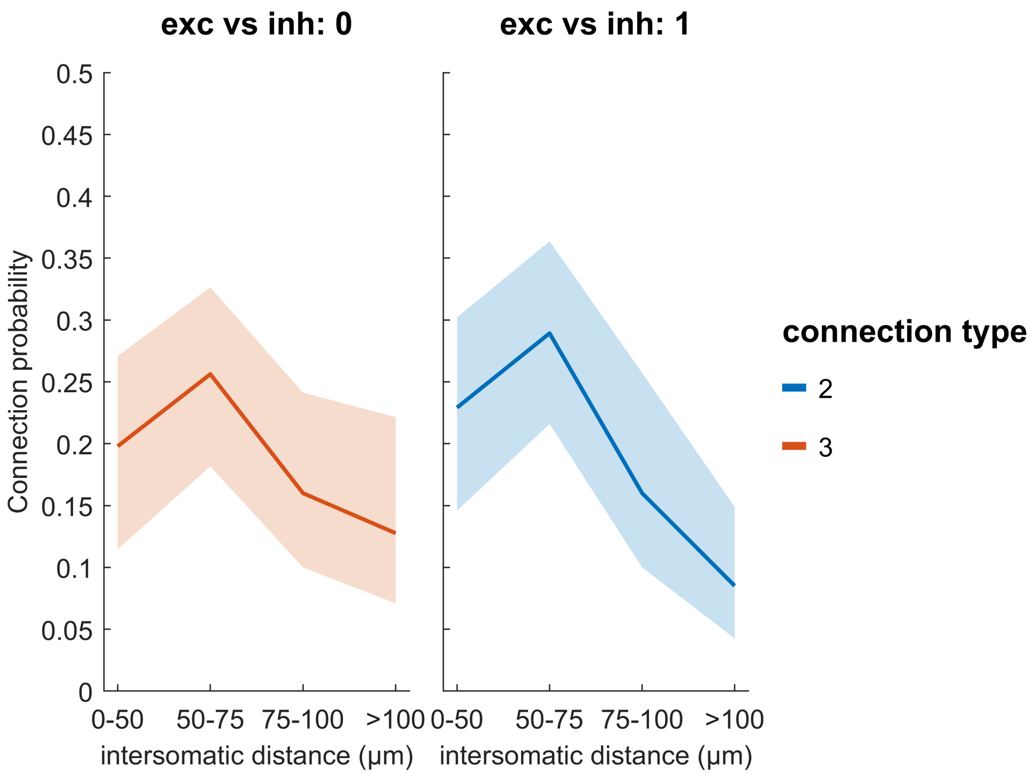
```
confil = tconnection.synaptic_layer == layer & ~isnan(tconnection.Distance);
tconnection_xy = tconnection(confil,:);
if n_celltypes == 2
    subset = tconnection_xy.stype == 2 |tconnection_xy.stype == 3;
    column = tconnection_xy.stype == 2;
elseif n_celltypes == 3
    subset = tconnection_xy.stype == 2 |tconnection_xy.stype == 3 |tconnection_xy.stype == 4 |t
    column = tconnection_xy.stype == 2 |tconnection_xy.stype == 3;
end

bins = [0 50 75 100 300];
dist_bin = discretize(tconnection_xy.Distance,bins);
```

```
%number of found/tested connection
figure
g = gramm('x',dist_bin,'color',tconnection_xy.Connected,'subset',subset,'column',column);
g.stat_bin('geom','stacked_bar','nbins',4);
g.set_color_options('map','matlab');
g.set_order_options('color',-1);
g.set_names('x','z depth (µm)','y','Number of connections','color','connected');
g.draw;
```




```
%connection probability
figure
g = gramm('x',dist_bin,'y',tconnection_xy.Connected','color',tconnection_xy.stype,'subset',subs
g.stat_summary('type','bootci');
g.axe_property('YLim',[0 0.5]);
g.set_names('x','intersomatic distance (µm)','y','Connection probability','color','connection t
g.set_color_options('map','matlab');
g.axe_property('XTickLabel',{'0-50','50-75','75-100','>100'},'XTick',[1:length(bins)-1]);
g.draw();
```



Get distance-dependent connectivity and bins. Only include connections with xy coordinates of cells

```
confil = (tconnection.stype == in_s | tconnection.stype == out_s) & tconnection.synaptic_layer
tconnection_xy = tconnection(confil,:);

bins = [0 50 75 100 300];
tconnection_xy.dist_group = discretize(tconnection_xy.Distance,bins);

tdist = groupsummary(tconnection_xy,{'dist_group','stype'},{'sum','mean'},'Connected');
dist_out_prob = tdist.mean_Connected(tdist.stype == out_s);
dist_in_prob = tdist.mean_Connected(tdist.stype == in_s);
```

Simulate random count of motifs from 10,000 random simulations

```
repeats = 10000;
[erdist_div_found,erdist_con_found,erdist_chain_found,erdist_rec_found,erdist_doublerec_found]
```

```
Starting parallel pool (parpool) using the 'local' profile ...
Connected to the parallel pool (number of workers: 4).
```

Calculates mean and std of random simulations.

Calculates rank-based p-value for comparison (see methods).

```
figure
random_rec = nansum(erdist_rec_found,[2 3]);
```

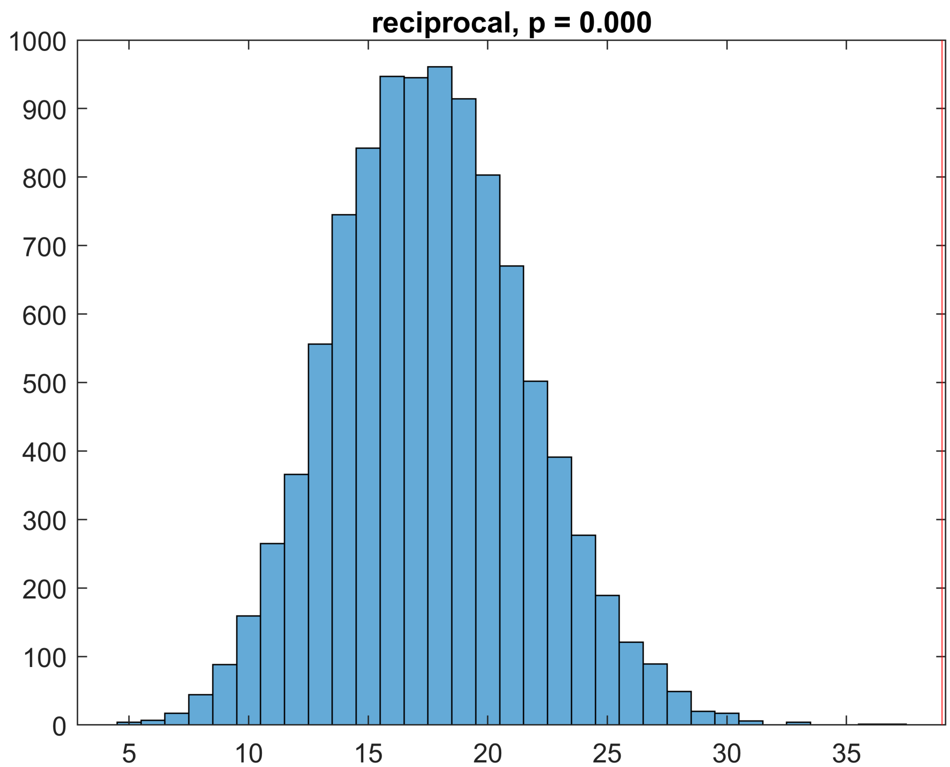
```
histogram(random_rec);
random_rec_mean = mean(random_rec)
```

```
random_rec_mean = 17.6333
```

```
random_rec_std = std(random_rec)
```

```
random_rec_std = 4.0364
```

```
xline(rec_found,'r',rec_found);
p_rank = max(sum(random_rec>rec_found),1/numel(random_rec))/numel(random_rec);
title(sprintf('reciprocal, p = %.3f',p_rank));
```



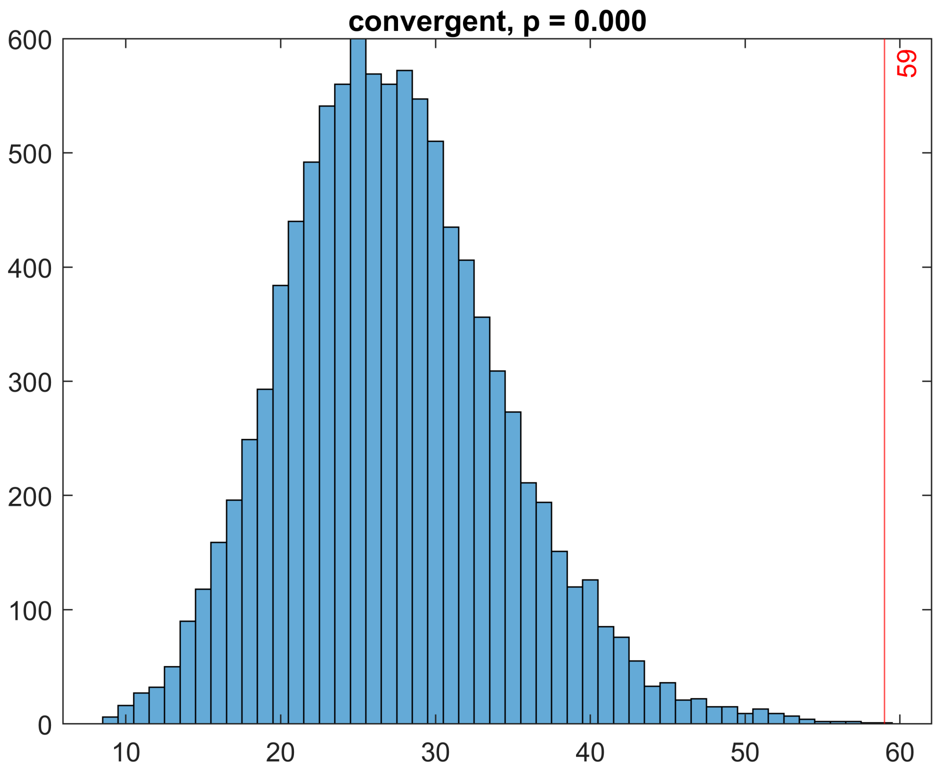

```
figure
random_con = nansum(erdist_con_found,[2 3]);
histogram(random_con);
random_con_mean = mean(random_con)
```

```
random_con_mean = 27.2928
```

```
random_con_std = std(random_con)
```

```
random_con_std = 7.0593
```

```
xline(con_found,'r',con_found)
p_rank = max(sum(random_con>con_found),1/numel(random_con))/numel(random_con);
title(sprintf('convergent, p = %.3f',p_rank));
```



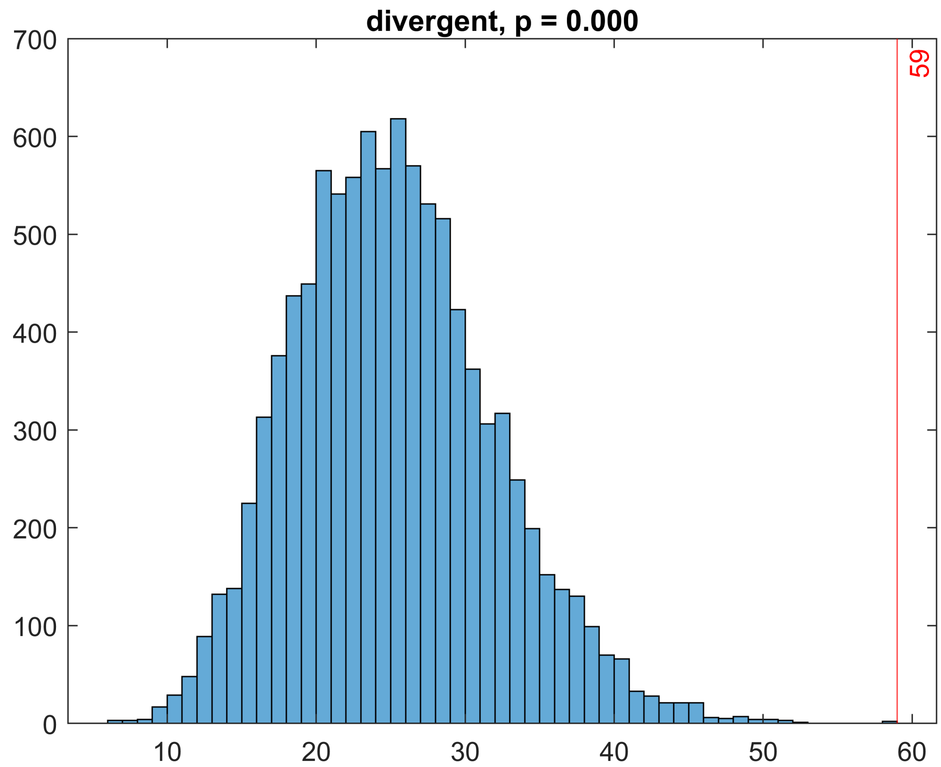

```
figure
random_div = nansum(erdist_div_found,[2 3]);
histogram(random_div);
random_div_mean = mean(random_div)
```

```
random_div_mean = 24.7246
```

```
random_div_std = std(random_div)
```

```
random_div_std = 6.7033
```

```
xline(div_found,'r',div_found)
p_rank = max(sum(random_div>div_found),1/numel(random_div))/numel(random_div);
title(sprintf('divergent, p = %.3f',p_rank));
```



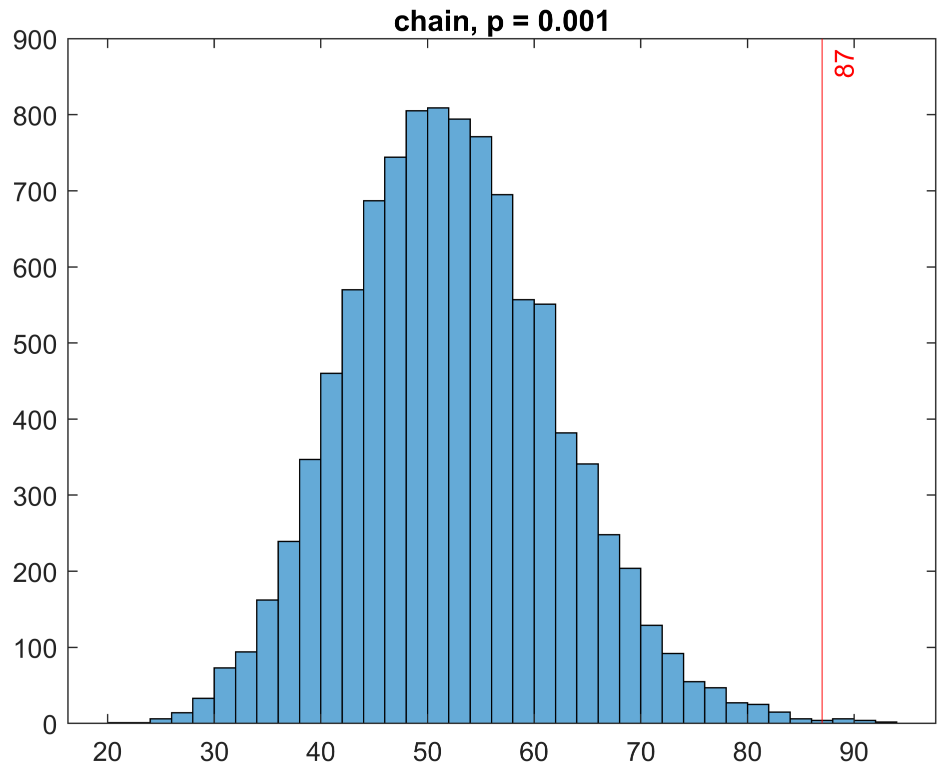

```
figure
random_chain = nansum(erdist_chain_found,[2 3]);
histogram(random_chain)
random_chain_mean = mean(random_chain)
```

```
random_chain_mean = 51.8352
```

```
random_chain_std = std(random_chain)
```

```
random_chain_std = 9.8006
```

```
xline(chain_found,'r',chain_found)
p_rank = max(sum(random_chain>chain_found),1/numel(random_chain))/numel(random_chain);
title(sprintf('chain, p = %.3f',p_rank));
```




```
figure
random_doublerec = nansum(erdist_doublerec_found,[2 3]);
random_doublerec_mean = mean(random_doublerec)
```

```
random_doublerec_mean = 2.8944
```

```
random_doublerec_std = std(random_doublerec)
```

```
random_doublerec_std = 3.2182
```

```
p_rank = max(sum(random_doublerec>doublerec_found),1/numel(random_doublerec))/numel(random_doub
```

Plot Fig 1E: random distribution as box plot and normalized overrepresentation of data count to mean random count

```
found_motif = [rec_found con_found div_found chain_found doublerec_found];
random_motif = [random_rec_mean random_con_mean random_div_mean random_chain_mean random_double
random_motif_std = [random_rec_std random_con_std random_div_std random_chain_std random_double

labels = {'rec','convergent','divergent','chain','doublerec'};

random_motif_count = [random_rec random_con random_div random_chain random_doublerec];
random_motif_norm = random_motif_count ./ mean(random_motif_count);
data_motif_count = [rec_found con_found div_found chain_found doublerec_found];
data_motif_norm = data_motif_count ./ random_motif;

figure
```

```
boxplot(random_motif_norm,'Widths',0.2)
hold on
plot(data_motif_norm,'+','MarkerSize',10,'MarkerEdgeColor','black')
hold off
ylim([0 8]);
xticklabels(labels)
ylabel('Random motif count normalized to mean random count')
title('Random distribution, data as +')
```