Guidelines for Manual Annotation for eFIP evaluation

1. The Purpose of eFIP

The purpose of eFIP is to find abstracts refering to **protein-protein interactions (PPIs) involving phosphorylated proteins**, and also to identify specific sentences stating the interactions. This type of information is currently not captured in public databases; however, it is critical to the interpretation of PPIs and the prediction of functional outcomes. eFIP captures this information to support curation of the Protein Ontology (PRO) knowledge base.

There are two types of information that we would like to capture regarding a phospho-protein:
    (1) the proteins with which it interacts while in the phosphorylated state
    (2) the proteins with which it ceases (or fails) to interact as a consequence of phosphorylation

One example sentence mentioning a PPI involving a phospho-protein is:

    The interaction of phosphorylated Bad and 14-3-3 increased in the presence of estradiol.

The phospho-protein in this case is "Bad" and the interactant is "14-3-3". Another example is:

    BAD Ser-155 phosphorylation **regulates** BAD/Bcl-XL interaction and cell survival.

Here, we are additionally informed of the impact of phosphorylation on the interaction ("regulates").

We are also interested in the negative effect of the phosphorylation on PPI. Some examples here are:

    Phosphorylated Bad **cannot** bind to Bcl-XL.
    Survival-factor-induced phosphorylation of Bad **results in** its dissociation from Bcl-x(L).

2. The Annotation Task

The bio-curator will be given a set of PMIDs to be annotated for the task mentioned in the previous section. The annotator will read each PMID using PubMed, and will store in a spreadsheet the sentences that mention protein-protein interactions involving phosphorylated proteins. The following columns are required in the spreadsheet:

a) PMID
b) Phospho-protein: the protein that is mentioned to be phosphorylated
c) Phospho-site: the site of phosphorylation of the phospho-protein, if applicable
d) Interactant: the protein with which the phospho-protein is reported to interact (or not interact)
e) PPI Type: the verb or noun used to indicate the interaction (e.g., binding, interacts, forms complex)
f) Impact: the word(s) in the sentence that are used to indicate the impact of phosphorylation on the interaction, if applicable
g) Sentence: the sentence (or sentences) containing the evidence, as it appears in the abstract

3. Examples of relevant sentences (containing PPIs involving phosphorylated proteins)

3.1 PPI mentioning one interactant as being phosphorylated

    Phosphorylated BAD binds to the cytosolic 14-3-3 protein and is sequestered from the apoptotic machinery of the mitochondrial membrane.

Information to be stored in the spreadsheet:

- Phospho-protein: BAD
- Phospho-site: N/A
- Interactant: cytosolic 14-3-3 protein
- PPI Type: binds
- Impact: N/A

3.2 PPI mentioned as a consequence of phosphorylation

It is known that <u>Bad phosphorylation</u> induced by survival factors **leads to** <u>its preferential binding to 14-3-3</u> and supression of the death-inducing function of Bad.

Information to be stored in the spreadsheet:
- Phospho-protein: BAD
- Phospho-site: N/A
- Interactant: cytosolic 14-3-3
- PPI Type: binding
- Impact: leads to

Survival-factor-induced <u>phosphorylation of Bad</u> **results in** <u>its dissociation from Bcl-x(L)</u>.

Information to be stored in the spreadsheet:
- Phospho-protein: BAD
- Phospho-site: N/A
- Interactant: Bcl-x(L)
- PPI Type: dissociation
- Impact: results in

3.4 Multiple PPIs involving the phospho-protein

Mentions of multiple PPIs should be all captured.

Our data demonstrated that Akt1 mediated the <u>phosphorylation of Bad at serine 136</u>, which **increased** <u>the interaction of serine 136-phosphorylated Bad with 14-3-3 proteins</u> and **prevented** the <u>dimerization of Bad with Bcl-Xl</u>, inhibited the release of cytochrome c to the cytosol and the death effector caspase-3 activation, leading to the survival of neuron .

Information to be stored in the spreadsheet:
- Phospho-protein: BAD
- Phospho-site: serine 136
- Interactant: 14-3-3 protein
- PPI Type: interaction
- Impact: increased

Information to be stored in the spreadsheet:
- Phospho-protein: BAD
- Phospho-site: serine 136
- Interactant: Bcl-Xl
- PPI Type: dimerization
- Impact: prevented