2. Objectives and Strategy

The goal of this study is the development of a method that enables the parallel purification of recombinant human proteins from bacteria. This task will be approached in the following four steps.

2.1. Development of a method for parallel protein expression and purification.

Denaturing protein purification conditions using the His_6-tag will be developed for first to address fundamental issues of parallel protein production.

2.2. Identification of a purification chemistry suited for parallel protein purification under non-denaturing conditions.

A test set of sequence verified human proteins will be used to evaluate different protein purification tags and their influence on yield, purity and functionality of the purified proteins.

Tag	Size	Affinity Matrix	Criteria for Selection
His_6-	<1kD	Ni^{2+} or Co^{2+}	• Small size may be beneficial for large proteins • minimal sterical interference
CBP	4kD	Calmodulin	• Second small tag with alternative binding chemistry
GST	26kD	Glutathione	• Frequently yields soluble and functional protein
MBP	42kD	Amylose	• Exceptional solubilizing properties

2.3. Evaluate the performance of the identified chemistry in the context of large sets of proteins.

CDNAs for 1000 human proteins selected without any deliberate bias, will be expressed and the proteins will be purified using the purification chemistry identified for question two.

2.4. Identification of protein properties that correlate with high or low chance of purification success from bacteria.

All experimental data from the data set in question 3 will be related to biochemical, biological and biophysical properties of the proteins.