

1      **Global proteome response of *Escherichia coli* BL21 to production of**  
2      **human basic fibroblast growth factor in complex and defined medium<sup>#</sup>**

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17     <sup>#</sup>This manuscript is dedicated to Prof. Dr. Karl Schügerl on the occasion of his 90<sup>th</sup> birthday.

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19 **Abstract**

20 The global proteome response towards recombinant protein production in *Escherichia coli* BL21  
21 (DE3) grown in complex and defined medium was analyzed. Overproduction of human basic  
22 fibroblast growth factor (hFGF-2), a difficult-to-fold protein, led to a reconstruction of the  
23 bacterial proteome. For example, heat shock chaperones were highly up-regulated, especially  
24 when production occurred during fast growth in complex medium. Although heat shock  
25 chaperones increased to higher levels in complex medium more hFGF-2 accumulated within  
26 inclusion bodies indicating that the capacity to chaperone protein folding was not sufficient for  
27 high speed production. In both types of media, cellular proteins from substrate transport systems,  
28 central metabolic pathways, and by-product uptake (e.g. acetate) were down-regulated. This  
29 down-regulation was connected to growth inhibition and metabolic perturbations. For example,  
30 during production in complex and defined medium acetate re-assimilation and glucose uptake,  
31 respectively, were severely hampered. Cellular proteins for degradation of less favorable  
32 substrates, elimination of reactive oxygen species and DNA protection were also down-regulated  
33 in response to hFGF-2 production. The decrease of proteins involved in transport, central  
34 metabolic pathways and general cell protection was more pronounced in the fast producing  
35 culture in complex medium than in the slow producing culture in defined medium. In general,  
36 production of hFGF-2 seems to interfere with the adaptation process to changing growth  
37 conditions, in this case the adaptation from exponential growth to stationary phase.

38 **Keywords** Stress response / Metabolic burden / Two-dimensional gel electrophoresis / Defined  
39 medium / Complex medium

40

41    **1    Introduction**

42    Recombinant DNA techniques in combination with bioprocess engineering allow high level  
43    protein production using genetically modified microorganisms, such as *Escherichia coli*. Using  
44    strong inducible promoters, e.g. T7 promoter, heterologous proteins are typically produced at  
45    high levels resulting in physiological stress named metabolic burden often connected to the  
46    aggregation of the recombinant protein into inclusion bodies [1-6].

47    The metabolic burden caused by overexpression of heterologous genes has been reported many  
48    times [7-9]: it can lead to a decrease of the specific growth rate [1, 10-13] and biomass yield [11-  
49    13], and can result in an increase in maintenance energy [10] and enhanced acetate excretion  
50    [11]. The metabolic burden or the cellular response towards recombinant protein production in *E.*  
51    *coli* varies greatly for different target proteins and production conditions. Many reports state  
52    enhanced levels of heat shock proteins [14-15] or increased expression of heat shock genes in  
53    response to recombinant protein production [16-19]. However, there are also reports which did  
54    not notice an increase of heat shock proteins [20-21] or enhanced transcript levels of heat shock  
55    genes in response to protein overproduction [22]. Also, enhanced expression of ribosomal genes  
56    [17] but also lower levels of ribosomal proteins were reported as a result of recombinant protein  
57    overproduction [21-22]. Most studies describe a general down-regulation of central carbon  
58    metabolism, e.g. lower levels of enzymes involved in the pentose phosphate pathway [15], the  
59    glycolytic pathway [20], the TCA cycle [20] and of ATP synthase [14, 20] as well as decreased  
60    fluxes through the pentose phosphate pathway and the TCA cycle [23]. However, there are also  
61    reports describing enhanced levels of TCA cycle proteins [14-15] or a non-uniform regulation,  
62    e.g. simultaneous up- and down regulation of individual TCA cycle genes [22] or proteins [24]  
63    in response to recombinant protein overproduction. Contradicting findings also exist regarding  
64    transport systems. Baig *et al.*, observed down-regulation of genes related to transport of  
65    carbohydrates, amino acids and other substrates in response to recombinant protein production  
66    [17]. On the other hand, increased levels of proteins involved in carbohydrate [20] and peptide  
67    transport [15] have also been reported as a consequence of recombinant protein overproduction.  
68    A comparative examination of these seemingly contradicting findings appears impossible as  
69    above results were obtained with different proteins and host strains as well as different  
70    production conditions. Thus, a profound understanding of the cellular response towards  
71    recombinant protein production is still missing.

72    For recombinant protein production two different types of media are employed: complex  
73    medium (also referred to as rich medium) and defined mineral salt medium [25-30]. Complex

74 media are popular for the daily lab routine but lot-to-lot variations of raw materials (e.g., yeast  
75 extract, peptone or tryptone) can lead to poor production reproducibility in industrial scale  
76 cultures [31-32]. Moreover, the usage of defined medium is also a prerequisite in fed-batch  
77 cultures to reach cell densities above 100 g L<sup>-1</sup> dry cell mass [33]. Thus, chemically defined  
78 medium properly adjusted to the needs of the producing organism is gaining more and more  
79 popularity [34-36].

80 In the present study, human basic fibroblast growth factor (hFGF-2) was chosen as a “difficult-  
81 to-fold” model protein for production in shaken cultures using complex medium (Luria Bertani  
82 broth) and glucose-supplemented mineral salt medium with *E. coli* BL21 (DE3) as expression  
83 host. hFGF-2 is a cytokine which can be produced in *E. coli* in form of inclusion bodies but also  
84 as a correctly folded protein [3, 37]. During production of hFGF-2 in both types of media, a  
85 quantitative proteome analysis was performed for hFGF-2 producing cells and also for non-  
86 producing controls cells grown under identical conditions to get a deeper understanding of the  
87 production associated metabolic perturbations.

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89 **2 Materials and methods**

90 **2.1 Strain, media and cultivation conditions**

91 *E. coli* BL21 (DE3) with the plasmid pET-29c-hFGF-2 was used in this study [37]. The  
92 composition of Luria Bertani (LB) medium was as follows: 10 g L<sup>-1</sup> tryptone, 5 g L<sup>-1</sup> yeast  
93 extract and 5 g L<sup>-1</sup> NaCl. The composition of Defined Non-inducing Broth (DNB) was as  
94 follows: 10.91 g L<sup>-1</sup> glucose, 4 g L<sup>-1</sup> (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>, 13.3 g L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, 1.55 g L<sup>-1</sup> Citric acid,  
95 0.59 g L<sup>-1</sup> MgSO<sub>4</sub>, 100.8 mg L<sup>-1</sup> Fe(III) citrate, 2.1 mg L<sup>-1</sup> Na<sub>2</sub>MoO<sub>4</sub>.2H<sub>2</sub>O, 2.5 mg L<sup>-1</sup>  
96 CoCl<sub>2</sub>.6H<sub>2</sub>O, 15 mg L<sup>-1</sup> MnCl<sub>2</sub>.4H<sub>2</sub>O, 1.5 mg L<sup>-1</sup> CuCl<sub>2</sub>.2H<sub>2</sub>O, 3 mg L<sup>-1</sup> H<sub>3</sub>BO<sub>3</sub>, 33.8 mg L<sup>-1</sup>  
97 Zn(CH<sub>3</sub>COOH)<sub>2</sub>.2H<sub>2</sub>O, 14.10 mg L<sup>-1</sup> Titriplex III. The pH of all media was adjusted to pH 6.8  
98 using NaOH before sterilization. After sterilization, 50 mg L<sup>-1</sup> kanamycin was added. Details of  
99 medium preparation are described elsewhere [35-36].

100 Cultivations were carried out in duplicate using 1.8 L Fernbach flasks with three baffles  
101 containing 200 mL medium at 30°C and 180 rpm. When the culture reached the mid-exponential  
102 phase, IPTG was added to a final concentration of 0.25 mmol L<sup>-1</sup> for starting hFGF-2  
103 production. Non-producing control cultures were grown under identical conditions without the  
104 addition of IPTG. Culture samples were centrifuged at 17,000 g and 4°C for 3 min. After  
105 removal of the supernatant, cell pellets as well as supernatants were stored at -80°C until further  
106 analysis. Sampling points are indicated in the figure captions.

107 **2.2 Analysis of cell density, acetate and glucose concentrations, and SDS PAGE analysis**

108 Cell growth was monitored by measuring the absorbance at 600 nm (OD600). Acetate was  
109 analyzed using an acetic acid kit (Cat. No. K-ACETRM, Megazyme, Ireland). Glucose was  
110 determined by YSI 2300 STAT Plus (YSI Life Sciences, USA). For protein production SDS-  
111 PAGE analysis was performed in Mini-PROTEAN 3 Cell (Bio-Rad, USA) according to standard  
112 procedures and instructions from the manufacturer. BugBuster™ protein extraction reagent with  
113 rLysozyme and Benzonase (Novagen, USA) was used to generate cell extracts and to prepare  
114 soluble and insoluble fractions according to the instructions from the manufacturer. After  
115 electrophoresis, proteins were visualized by Coomassie blue staining [38]. Densitometry was  
116 carried out using ImageJ software (National Institutes of Health, USA).

117 **2.3 Two-dimensional gel electrophoresis**

118 Cell pellets were disrupted by BugBuster™ protein extraction reagent. The whole cell protein in  
119 the BugBuster suspension was precipitated as described previously [39]. The protein pellets

120 were re-solubilized in rehydration solution with IPG buffer (GE Healthcare, UK). About 280 µg  
121 protein for each sample were loaded onto Immobiline DryStrip gel (pH gradient 3-10NL, GE  
122 Healthcare, UK). The first-dimension using isoelectric focusing (IEF) and the second dimension  
123 using SDS-PAGE (10-15% gradient acrylamide gel) were run with the IPGphor™ Isoelectric  
124 Focusing System and Hoefer™ DALT System (GE Healthcare, UK), respectively. The detailed  
125 protocol has been described previously [40]. For each sample, two-dimensional (2D) gels were  
126 made in triplicate and the best two gels analyzed using Proteomweaver™ 4.0 software. Each  
127 spot's intensity was normalized by the whole spots intensity of the same 2D gel. The  
128 corresponding average intensity of the spot (or the sum of several spots representing the same  
129 protein in case of spot multiplicity) taken from the two duplicate gels was used to determine this  
130 protein's portion (%) of the relative protein mass (RPM).

131 **2.4 Protein identification and classification**

132 Proteins were identified by matrix-assisted laser desorption/ionization time-of-flight mass  
133 spectrometry (MALDI-TOF MS). Protein spots were excised from the 2D gels. After washing,  
134 reduction and alkylation, in-gel digestion was carried out by incubation with sequencing grade  
135 trypsin (Promega, USA). Obtained peptides were extracted and purified with reversed-phased  
136 ZipTips C18 (Millipore, USA). The resulting peptide solutions were mixed with a saturated  
137 matrix solution (acetonitrile 40%, α-Cyano-4-hydroxycinnamic acid 1% and trifluoroacetic acid  
138 0.06%) and spotted onto a 384 MTP target and dried at room temperature. A Bruker Ultraflex  
139 time-of-flight mass spectrometer (Bruker Daltonics GmbH, Germany) was used to obtain  
140 peptide mass fingerprints. Detailed experimental procedures have been described previously  
141 [40-41]. The MASCOT search program (Matrix Science, UK) was used for protein identification  
142 with the annotated *E. coli* genome Uniprot (<http://www.uniprot.org/>) serving as database. All  
143 proteins with a Mowse score greater than 54 were regarded as significant ( $p < 0.05$ ). For  
144 classification of identified proteins the EcoCyc (<http://ecocyc.org/>) [42] and KEGG  
145 (<http://www.genome.jp/kegg/>) databases [43] were used.

146

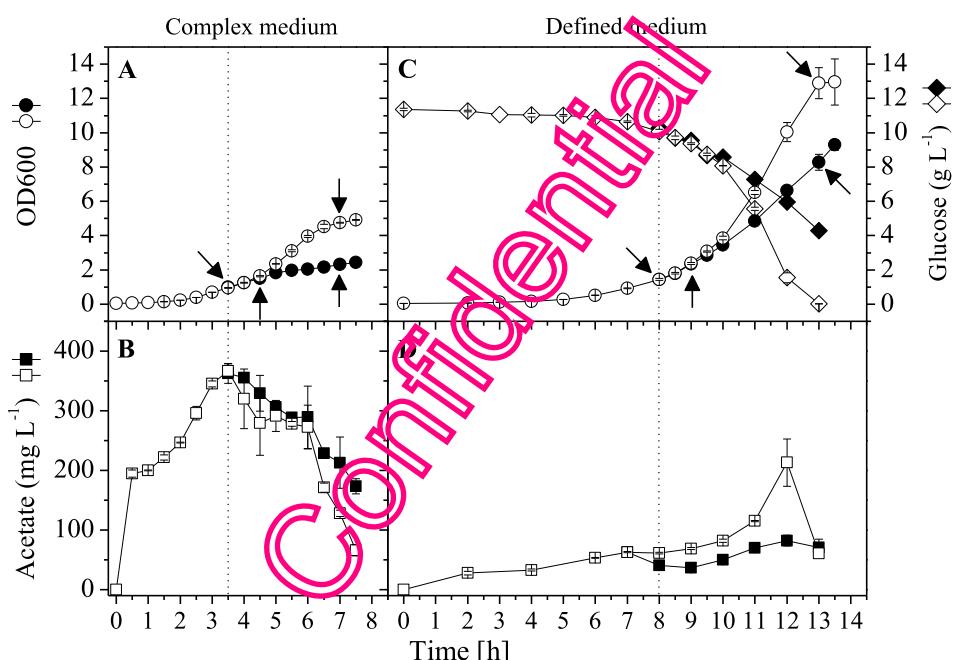
147 

### 3 Results

148 

#### 3.1 Overproduction of hFGF-2 in complex and defined medium

149 hFGF-2 production was carried out at 30°C in complex LB medium and defined glucose-  
150 supplemented mineral salt medium. Control cultures without production were grown under  
151 identical conditions without addition of IPTG. Cell growth prior to induced production of hFGF-  
152 2 was more rapid in complex medium than in defined medium (Fig. 1A,C, Table 1). After  
153 induction, growth inhibition was observed in both media, however, the production related  
154 growth inhibition was more severe in complex medium (Fig. 1A,C). SDS-PAGE analyses  
155 indicated more rapid hFGF-2 production in complex medium connected to a higher level of  
156 inclusion body formation (Fig. 2, Table 1).



157

158 **Fig. 1 Growth performance of *E. coli* BL21 (DE3) during hFGF-2 production in complex and**  
159 **defined medium.** hFGF-2 producing (closed symbols) and non producing control cells (open symbols)  
160 were grown in complex (LB, A and B) and defined glucose-supplemented mineral salt medium (DNB,  
161 C and D) at 30°C. The time-point of IPTG addition to the hFGF-2 producing culture is indicated by  
162 dotted lines. Sampling points for 2D proteome analysis are indicated by arrows (see also Supporting  
163 Information: Table S1).

164 Acetate formation was mainly observed during rapid growth in complex medium (Fig. 1B).  
165 During entry into stationary phase acetate re-assimilation occurred in producing and non-  
166 producing control cultures, however, acetate uptake was more delayed in the hFGF-2-producing  
167 culture (Fig. 1B). In defined medium, growth inhibition in the hFGF-2 producing culture

168 became apparent through inhibition of glucose uptake and not through enhanced acetate  
169 formation (Fig. 1B,D).

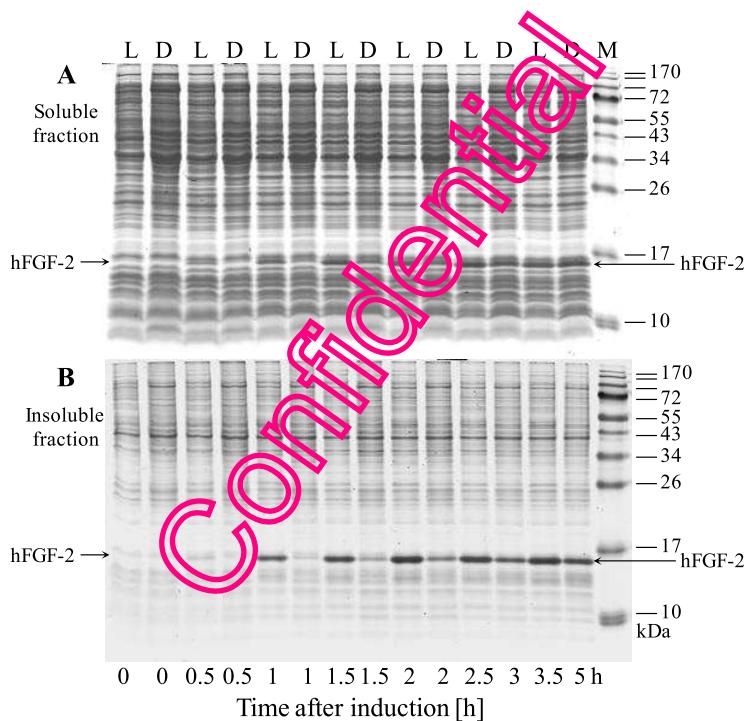
170 **Table 1 hFGF-2 production in defined and complex medium**

Medium	Specific growth rate before induction $\text{h}^{-1}$	Biomass before induction $\text{g DCM L}^{-1}$	Final biomass without induction $\text{g DCM L}^{-1}$	End of production <sup>a)</sup>		
				Biomass $\text{g DCM L}^{-1}$	Insoluble hFGF-2 %	Specific and volumetric hFGF-2 concentrations $\text{mg/g DCW}$ $\text{mg L}^{-1}$
Complex	0.92	0.37	1.78	0.85	55	45 38
Defined	0.52	0.53	4.77	3.07	46	38 117

171 <sup>a)</sup> End of production: 5 h and 3.5 h after IPTG addition in defined (DNB) and complex (LB) medium,  
172 respectively.

173 <sup>b)</sup> Biomass: One unit of OD600 corresponds to 0.37 g dry cell mass per liter for *E. coli* BL21(DE3) [35].

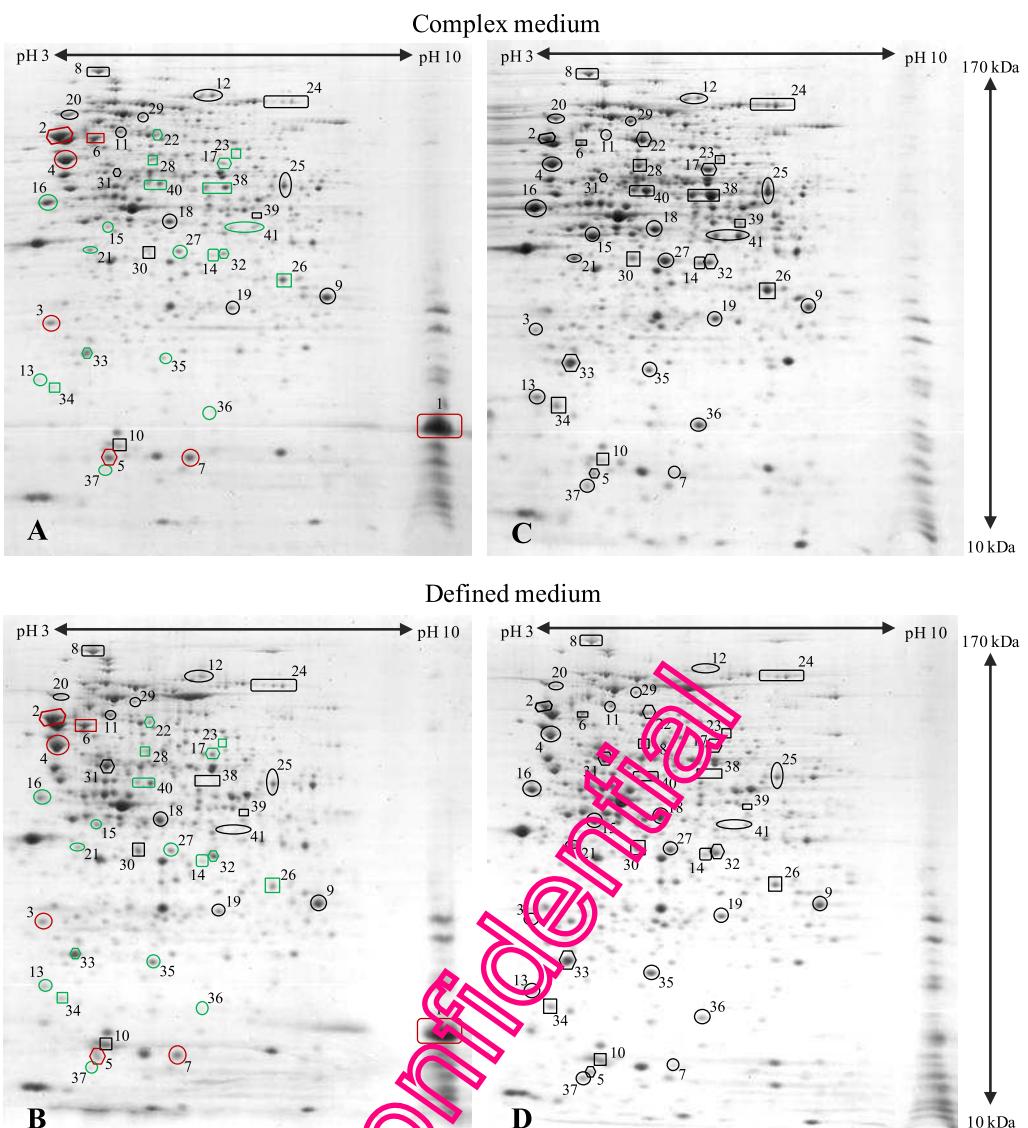
174 <sup>c)</sup> Specific and volumetric hFGF-2 concentrations were calculated assuming a constant cell protein content of  
175 550 mg protein per gram cell dry mass [53].



176 **Fig. 2 Production of hFGF-2 in complex and defined medium.** Soluble (A) and insoluble cell  
177 fractions (B) of hFGF-2 producing cells growing in complex (L = LB) and defined medium (D =  
178 DNB) were subjected to SDS-PAGE analysis. Time after induction is indicated.  
179

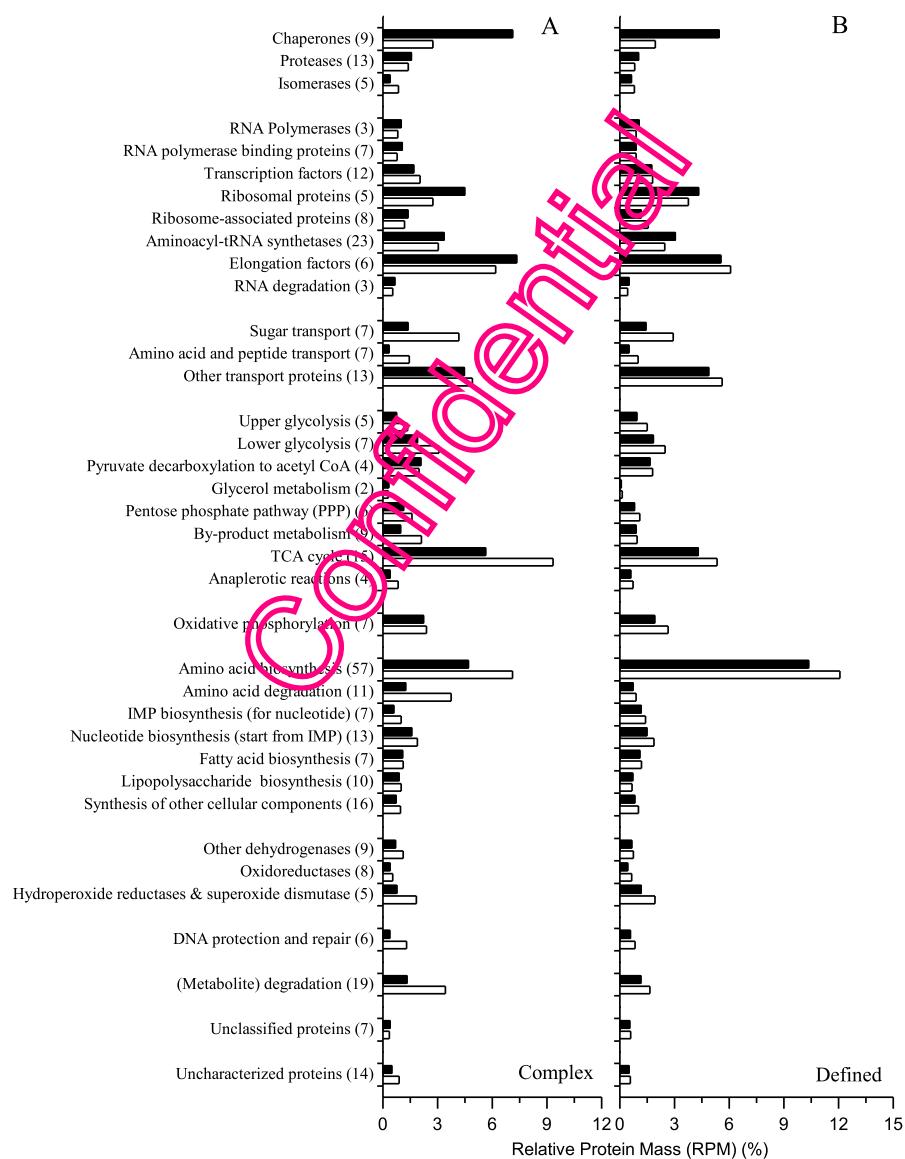
180 **3.2 Proteome analysis during recombinant protein production**

181 For a better understanding of the production associated metabolic perturbations in complex and  
182 defined medium a detailed comparative analysis of the bacterial proteome of producing and non-  
183 producing control cells was carried out using 2D gel electrophoresis.



**Fig. 3 Proteomic fingerprint of hFGF-2 producing cells.** The proteome of hFGF-2 producing cells at the end of production in complex (**A**, 3.5 h post-induction) and defined medium (**B**, 5 h post-induction) in comparison to non-producing control cells grown under identical conditions to the same sampling time point in complex (**C**) and defined medium (**D**), respectively. Gel images indicating representative proteins are shown: hFGF-2 1; chaperones, DnaK 2, GrpE 3, GroEL 4, GroS 5, HtpG 6, IbpA 7; transcription, RpoB 8; translation, RpsB 9, RpsF 10, GlyS 11, InfB 12; sugar uptake systems, Crr 13, ManX 14, MalE 15, LamB 16; peptide transport, OppA 17; upper glycolysis, FbaA 18; lower glycolysis, GpmA 19, PpsA 20; pentose phosphate pathway, TalB 21; by-product assimilation, Acs 22, PoxB 23, AdhE 24; TCA cycle: GltA 25, SucD 26, Mdh 27; anaplerotic reactions, PckA 28, MaeB 29; amino acid biosynthesis, IlvE 30, IlvC 31, CysK 32; removal of ROS, AhpC 33, Tpx 34, SodB 35; DNA protection, Dps 36, UspA 37; amino acid degradation, TnaA 38, AstA 39; metabolite degradation, GatZ 40, GatD, 41. Red and green colors indicate higher or lower levels, respectively, of the corresponding proteins in hFGF-2 producing than in non-producing control cells. Gel images indicating all identified proteins and representing other sampling times points (e.g. directly before and 1 h after induction of hFGF-2 synthesis) are shown in the Supporting Information: Figs. S1-S8.

200 A first brief visual examination of 2D gels revealed that heat shock proteins accumulated in both  
 201 media to higher levels in hFGF-2 producing cells compared to non-producing control cells (Fig.  
 202 3). In contrast, many metabolic enzymes were present in lower amounts in producing cells  
 203 compared to non-induced control cells (Fig. 3). A lumped quantitative analysis of the abundance  
 204 level of all identified proteins in hFGF-2 producing cells as well as in respective control cells  
 205 grown under identical conditions is given in Fig. 4. Following more detailed insights are given  
 206 to hFGF-2 production associated alterations of the cellular proteome with respect to (folding)  
 207 stress and protein synthesis associated processes as well as metabolic processes such as substrate  
 208 transport, central carbon metabolism, and metabolite degradation (for more detailed data refer  
 209 also to the Supporting information: Table S1).



210  
 211 **Fig. 4 Functional quantitative overview of the proteomic response to hFGF-2 production.**  
 212 Lumped quantitative proteome data sorted into functional categories comparing the proteome of *E.*  
 213 *coli* BL21 (DE3) producing hFGF-2 (black bars) in complex (A, 3.5 h post-induction) and defined

medium (**B**, 5 h post-induction) with non producing control cells grown under identical conditions to the same sampling time point (open bars). Numbers in brackets after each functional category are the number of proteins that were identified within this category. The entire data set is given in the Supporting Information: Table S1.

### 3.2.1 Protein folding and protein synthesis associated processes

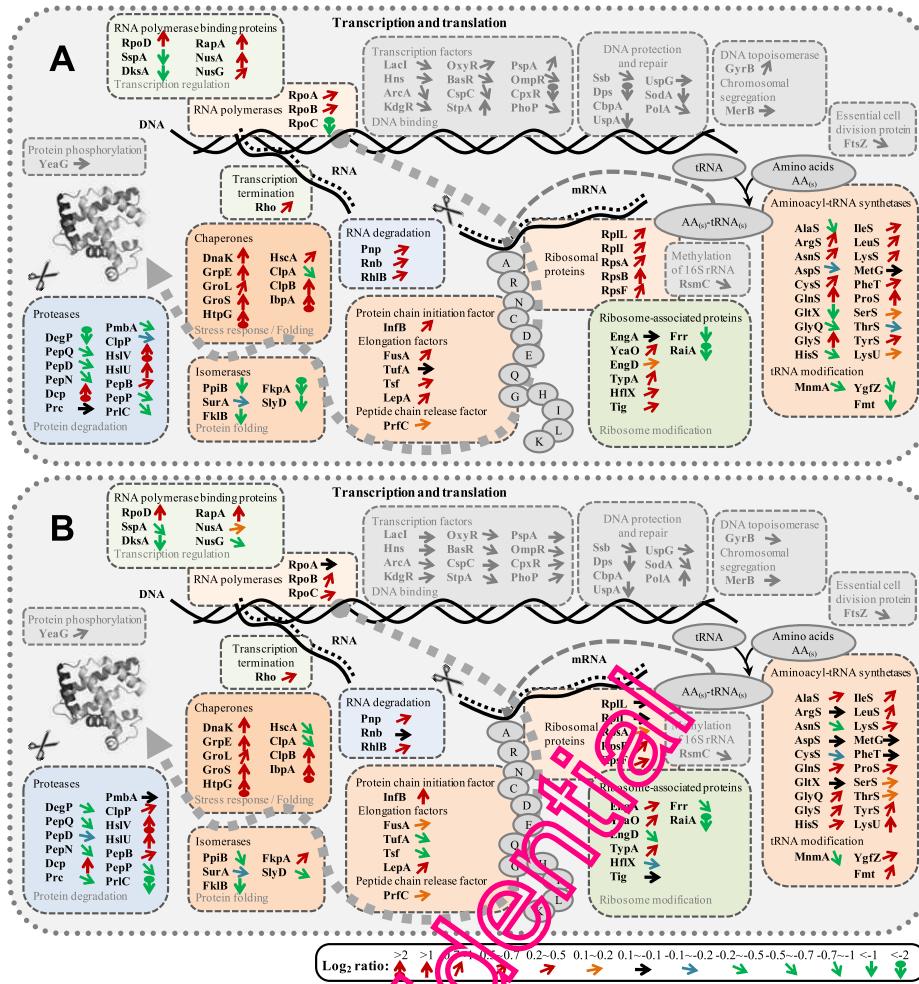
#### Protein folding and heat shock chaperones

Heat shock chaperones are crucially involved in helping protein folding by preventing protein misfolding and aggregation. The identified heat shock chaperones, DnaK, GrpE, GroEL/ES, HtpG, ClpB and IbpA increased in abundance in both media after induction of hFGF-2 synthesis, (Figs. 3-5). Lumped abundances of identified chaperones reached 7.1% and 5.5% of the relative protein mass (RPM) of hFGF-2 producing cells in complex and defined medium, respectively (Fig. 4). Nevertheless, higher amounts of chaperones in hFGF-2 producing cells growing in complex medium did not lead to higher amounts of soluble hFGF-2 (Fig. 2A). On the contrary, more hFGF-2 aggregated in form of inclusion bodies (IBs) during production in complex medium than during production in defined medium (Fig. 2B). In addition, the increase in the abundance of heat-shock chaperones was not only higher, it occurred also more rapidly during hFGF-2 production in complex medium (Supporting Information: Figs. S9 and S13). The heat shock proteases HslU/V also increased strongly in abundance in response to hFGF-2 production in both media (Fig. 5) suggesting that they may also play important roles in quality control of recombinant proteins.

Peptidyl prolyl *cis-trans* isomerases (PPIases) are important folding catalysts interconverting *cis/trans* peptide bonds adjacent to proline [44-45]. In both media, the identified PPIases (e.g. PpiB, SurA, FklB, and SlyD) decreased in abundance in response to hFGF-2 production (Fig. 5). The decrease was more pronounced in complex medium (with faster recombinant protein production) suggesting a potential bottleneck for correct folding.

#### Transcription and translation

Transcription and translation are the processes whereby cells generate new proteins. The amounts of identified proteins involved in these processes, e.g. RNA polymerase, ribosomal proteins, aminoacyl-tRNA synthetases and elongation factors, revealed in both media a slight increase in response to hFGF-2 production compared to the respective non-producing control cultures (Figs. 3-5). Again, this increase was slightly higher during production in complex medium (see Supporting Information: Table S1).



246

### 247 Fig. 5 Transcription, translation, and protein folding: proteomic response to hFGF-2 production.

248 Details of the subproteome related to transcription, translation, and protein folding of hFGF-2  
 249 producing cells in complex (A, 3.5 h post induction) and defined medium (B, 5 h post-induction) in  
 250 direct comparison to non-producing control cells grown under identical conditions to the same  
 251 sampling time point. Differences of the single protein abundances are expressed as Log<sub>2</sub>  
 252 (producing/non-producing) ratios using different color coded arrows for visualization [code on bottom,  
 253 e.g. Log<sub>2</sub> ratio > 2, indicated with a red circle with up-pointing arrow signifies that the abundance of  
 254 this protein is at least 4 times higher in the hFGF-2 producing culture than in the respective control  
 255 culture]. The entire comparative proteomic (pathway) data are visualized in Supporting Information:  
 256 Figs. S9-16 and the corresponding values are given in the Supporting Information: Table S1. The list  
 257 of abbreviations is found at the end of the Supporting Information: Figure S16.

### 258 3.2.2 Metabolic processes

#### 259 Substrate transport systems

260 Substrates need to be transported into cells prior to their catabolic breakdown or employment for  
 261 anabolic purposes. During growth in both types of media, proteins belonging to transport  
 262 systems were present in lower amounts in hFGF-2 producing cells than in the respective control

263 cells grown under identical conditions (Figs. 3, 4, and 6). This difference in abundance was more  
264 prominent for proteins from specific transport systems such as the sugar transporting  
265 phosphotransferase system (PTS) (Crr and ManX), and the maltose (MalE/K and LamB) and  
266 peptide transport systems (OppA/D) than for proteins belonging to unspecific transport channels  
267 of the outer membrane (OmpA/F/X, Supporting Information: Figs. S11 and S15). Thus, lower  
268 amounts of specific transport proteins point to a decreased capacity for substrate uptake after  
269 induction of hFGF-2 synthesis.

270 *Glycolysis, pentose phosphate pathway and pyruvate dehydrogenase*

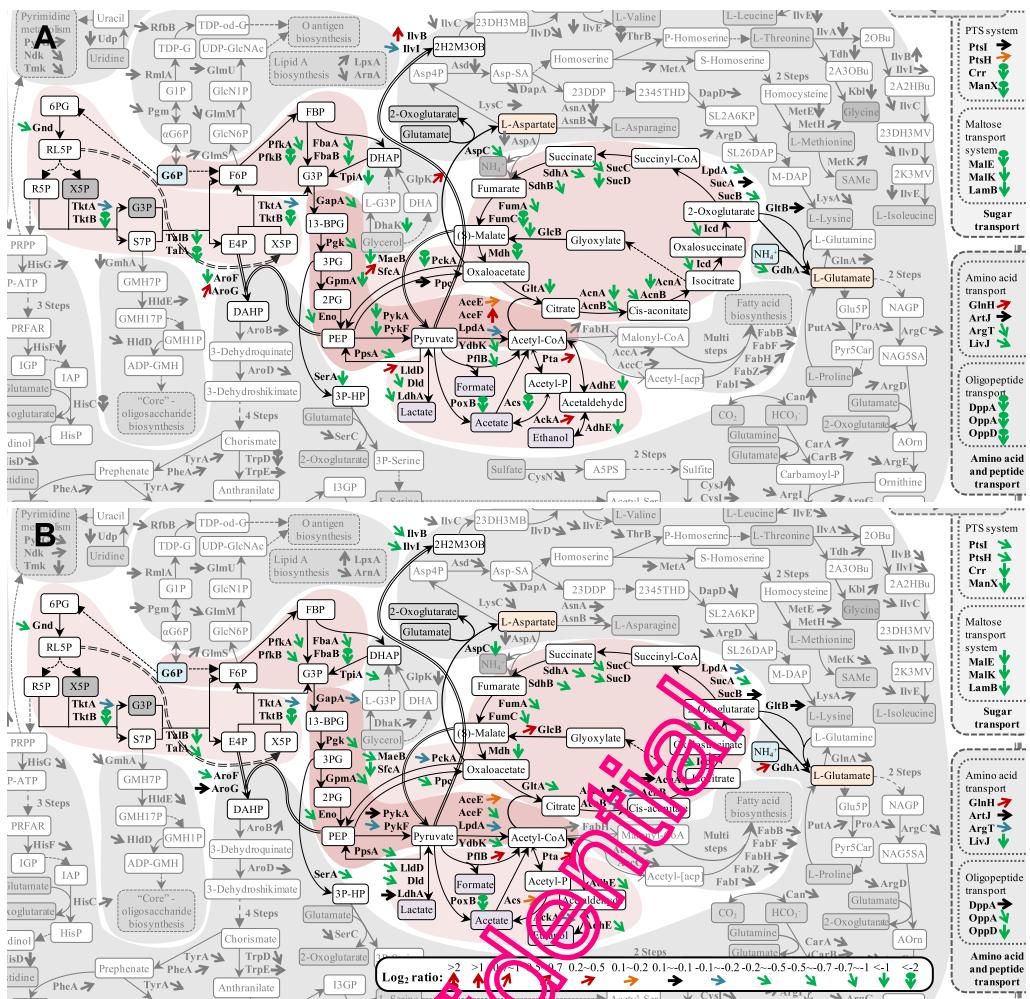
271 After entering into the cell, carbon substrates (e.g. glucose) are catabolized mainly through the  
272 glycolytic and the pentose phosphate pathways. In both types of media, enzymes belonging to  
273 these pathways decreased to lower levels in hFGF-2 producing cells compared to control cells  
274 grown under identical conditions (Figs. 4 and 6). Interestingly, pyruvate dehydrogenase, the  
275 enzyme connecting the glycolytic pathway to the TCA cycle did not reveal lower levels in  
276 hFGF-2 producing compared to the respective control cells (Figs. 4 and 6).

277 *Formation of acetate and other by-products*

278 Acetate formation occurs mainly from acetyl-CoA through the Pta-AckA pathway [46] and to  
279 minor amounts directly from pyruvate by oxidative decarboxylation through PoxB [47]. Acetate  
280 assimilation is exclusively catalyzed by Acs [46].

281 In complex medium, the enzymes of the Pta-AckA pathway were slightly higher in the hFGF-2  
282 producing culture than in the ~~control~~ culture not producing hFGF-2 (Fig. 6A). However, the  
283 acetate forming PoxB and, more importantly, Acs, the enzyme responsible for acetate utilization,  
284 reached considerably lower levels in the hFGF-2 producing cells than in the non-producing cells  
285 growing under identical conditions (Fig. 6A). Both enzymes accumulated to less than 5 times  
286 lower level in the hFGF-2 producing culture than in the non-producing control (Supporting  
287 Information: Table S1). In defined medium, the enzymes of the Pta-AckA pathway as well as  
288 Acs were at the same level in the producing as well as in the non-induced control culture (Fig.  
289 6B). Only PoxB was present in lower amounts in the hFGF-2 producing culture than in the non-  
290 producing control (Fig 6B).

291 Also, enzymes involved in the formation and degradation of lactate, formate and ethanol were  
292 found in both media at lower level in hFGF-2 producing cells than in the non-induced controls  
293 (Fig. 6).



295 **Fig. 6 Transport systems and central metabolic pathways: proteomic response to hFGF-2**  
296 **production.** Details of the subproteome related to transport and central metabolic pathways of hFGF-2  
297 producing cells in complex (A, 3.5 h post-induction) and defined medium (B, 5 h post-induction) in  
298 direct comparison to non-producing control cells grown under identical conditions to the same  
299 sampling time point. Differences of the single protein abundances are expressed as Log<sub>2</sub>  
300 (producing/non-producing) ratios using different color coded arrows for visualization [code on bottom,  
301 e.g. Log<sub>2</sub> ratio < -2, indicated with a green circle with down-pointing arrow signifies that the  
302 abundance of this protein is at least 4 times lower in the hFGF-2 producing culture than in the  
303 respective control culture]. The entire comparative proteomic (pathway) data are visualized in  
304 Supporting Information: Figs. S9-16 and the corresponding values are given in the Supporting Information:  
305 Table S1. The list of abbreviations is found at the end of the Supporting Information:  
306 Figure S16.

### 307 Tricarboxylic acid (TCA cycle) and oxidative phosphorylation

308 Major functions of the TCA cycle are the generation of biosynthetic precursors (e.g. for amino  
309 acid synthesis) and reducing equivalents (e.g. NADH) which are subsequently channeled into  
310 the oxidative phosphorylation pathway for the production of ATP. In both media, enzymes of the

311 TCA cycle were present at lower level in hFGF-2 producing cells compared to non-induced  
312 control cells (Figs. 3, 4 and 6). Again, this difference was more prominent in complex medium  
313 than in defined medium. Lumped abundances of identified TCA cycle enzymes were around 1.7  
314 times (or 40%) and 1.2 times (or 20%) lower in complex and defined medium, respectively, at  
315 end of production phase compared to the control culture grown under identical conditions (Fig. 4,  
316 Supporting Information: Table S1). Moreover, identified subunits of NADH dehydrogenase I  
317 and ATP synthase F1 complex decreased in abundance during production of hFGF-2 in both  
318 media compared to control cultures growing under identical condition, but the decrease was  
319 stronger in defined medium (Fig. 4).

### 320 **3.2.4 Amino acids biosynthesis**

321 Amino acids are the building blocks of proteins. In both media, enzymes of amino acid synthesis  
322 pathways (e.g. AroF: aromatic amino acids, Asd: lysine, AcnA: asparagine, CysK: cysteine,  
323 GlyA: glycine, IlvE: branched-chain amino acids, SerA: serine), were present at lower level in  
324 hFGF-2 producing cells compared to non-producing control cells (Supporting Information: Figs.  
325 S11 and S15). The observed difference was again more significant in complex medium than in  
326 defined medium. Lumped abundances of identified amino acids biosynthesis enzymes were  
327 present at around 1.5 times (or 34%) and 1.2 times (or 14%) lower level in complex and defined  
328 medium, respectively, at end of production phase compared to the control culture grown under  
329 identical conditions (Fig. 4, Supporting Information: Figs. S11 and S15 and Table S1). These  
330 findings show that the synthesis of protein building blocks is impaired by overproduction of the  
331 recombinant protein, especially when production occurs in complex medium.

### 332 **3.2.5 Utilization of less favorable substrates**

333 Enzymes for better nutrient scavenging and degradation of less favorable substrates (e.g.  
334 arginine, trehalose, galactitol) are required for starvation survival. Enzymes involved in these  
335 substrate utilization pathways (e.g. AstA/B/D, DadX, GatD/Z, MalP and TreA) also reached  
336 lower levels in hFGF-2 producing cells in both media compared to the respective non-producing  
337 control cultures (Fig. 4, Supporting Information: Figs. S11 and S15). The observed difference  
338 was again more pronounced in complex medium than in defined medium (Supporting  
339 Information: Table S1). Thus, the scavenging capacity for rare nutrients is impaired by the  
340 overproduction of the recombinant protein in particular during growth in complex medium.

342 **3.2.6 Other hFGF-2 production related stress responses**

343 Elimination of reactive oxygen species (ROS) and DNA protection are very important for *E. coli*  
344 especially during starvation survival. Many important enzymes for removal of ROS (e.g. AhpC,  
345 Bfr, CueO, Tpx and SodB) and DNA protection (e.g. Dps, SodA and UspA) were present in  
346 lower amounts in hFGF-2 producing cells in both media compared to the respective control  
347 cultures growing under identical conditions (Figs. 3 and 4, Supporting Information: Figs. S11  
348 and S15). Again, the difference was more prominent in complex medium than in defined  
349 medium (Supporting Information: Table S1). These findings indicate that recombinant protein  
350 overproduction may diminish the cellular capacity for general cell protection.

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352 **4 Discussion and conclusions**

353 The metabolic perturbations in *E. coli* BL21 (DE3) caused by hFGF-2 production in complex  
354 Luria Bertani and defined glucose-supplemented mineral salt medium were studied during batch  
355 growth in shaken cultures. Thus, protein production associated growth perturbations were  
356 investigated at conditions of initially non-limited nutrient supply. It was found that growth and  
357 production were more rapid and the growth inhibition more severe in complex medium (Table 1).  
358 The production associated stress response became also apparent in the reconstruction of the host  
359 cell proteome. In comparison to non-producing control cells grown under identical conditions  
360 strongly increased levels of heat shock proteins but also slightly higher levels of translation  
361 associated proteins (e.g. ribosomal proteins) were found in cells producing hFGF-2 with a more  
362 prominent increase during production in complex medium. An increase in heat shock protein  
363 synthesis in response to recombinant protein production is not unexpected and has been reported  
364 numerous times, e.g. [48-50]. However, larger amounts of heat shock chaperones in complex  
365 medium than in defined medium did not lead to higher amounts of soluble hFGF-2. On the  
366 contrary, more hFGF-2 was accumulating within inclusion bodies indicating that the capacity to  
367 chaperone protein folding was not sufficient for high speed production.

368 *E. coli* BL21 is known as a robust strain as it produces less acetate compared to *E. coli* K12  
369 strains [51-52]. Significant acetate formation was only observed during exponential growth in  
370 complex medium, followed by acetate re-assimilation during entry into stationary phase (Fig. 1).  
371 However, acetate re-assimilation was delayed in the hFGF-2 producing culture compared to the  
372 non-producing control. This observation was consistent with five times lower levels of Acs, the  
373 enzyme required for acetate uptake, in the culture producing hFGF-2. During production in  
374 defined medium uptake of glucose was severely hampered in hFGF-2 producing cells consistent  
375 with a more than two times lower level of the specific sugar transport porin LamB and sugar  
376 transporting phosphotransferase system than in the non-producing control cells. In addition to  
377 sugar transport proteins, other specific transport systems (e.g. peptide transport), but also  
378 enzymes of central carbon metabolism (e.g. TCA cycle) and amino acid biosynthesis were  
379 finally found in lower level in hFGF-producing cells than in the non-producing control cells.  
380 These findings point to a reduced capacity to generate building blocks required for protein  
381 synthesis and cell growth in producing cells. Also, enzymes involved in nutrient scavenging and  
382 stress protection (e.g. ROS, DNA damage) revealed lower levels in hFGF-producing cells than  
383 in non-producing control cells. Again, the difference to non-producing control cells was more  
384 prominent during production in complex medium. Interestingly, many of those proteins found at  
385 lower level in hFGF-2 producing cells usually increase during transition of *E. coli* BL21 (DE3)

386 from exponential to stationary phase [46]. This involves specific transport proteins, but also  
387 enzymes of central metabolic pathways and those involved in degradation of less favourable  
388 substrates, ROS elimination and DNA protection. Thus, the stress response towards production  
389 of hFGF-2 mimics – at least partly – an anti-stationary phase response. In this line, proteins  
390 involved in transcription and translation decrease during transition of *E. coli* BL21 (DE3) from  
391 exponential to stationary phase [46]. During production of hFGF-2 they do not show this decline  
392 and are finally present at higher level than in the non-producing control cells grown under  
393 identical conditions. In summary, production of hFGF-2 in *E. coli* BL21 (DE3) leads to the  
394 induction of the typical unfolded protein response, the enhanced synthesis of heat shock proteins.  
395 Under conditions employed during this study – induction of protein production during  
396 exponential growth in shaken cultures – it also leads to a kind of anti-stationary phase response  
397 in particular under conditions of rapid production in complex medium. The production of hFGF-  
398 2 seems to interfere with the adaptation process to changing growth conditions, in this case the  
399 adaptation from exponential growth to stationary phase in shaken cultures.

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404 **Conflict of interest**

405 The authors have declared no conflict of interest

406

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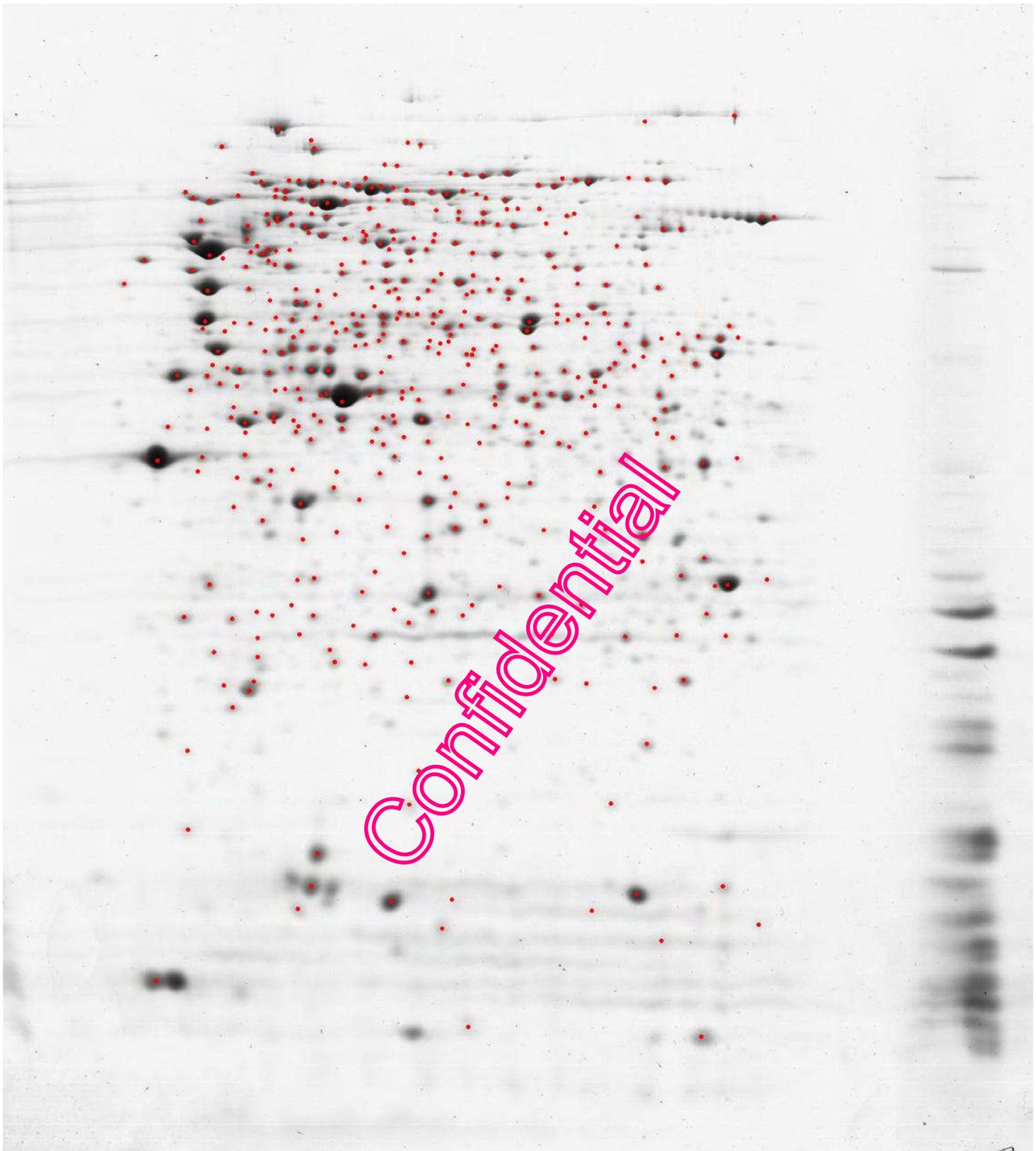
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546 **Supporting information**

547 **Supporting Information 1 (PDF file):** Figures S1-S8. Images of 2D gels of the proteome of *E.*  
548 *coli* BL21 (DE3) growing in complex and defined medium during production of hFGF-2 and of  
549 non-producing control cells grown under identical conditions. Identified proteins are marked and  
550 “clickable” to get access to further protein information (<http://www.uniprot.org>). Figures S8-S16:  
551 Detailed comparative scheme of the (proteomic) pathway regulation during hFGF-2 production  
552 in complex and defined medium (1 h after IPTG induction versus pre-induction, end of  
553 production versus pre-induction, end of production versus non-producing control at stationary  
554 phase, stationary phase versus exponential phase without induction). Table S1: Quantitative data  
555 of individual proteins of *E. coli* BL21 (DE3) growing in defined and complex medium during  
556 production of hFGF-2 and of non-producing control cells grown under identical conditions.

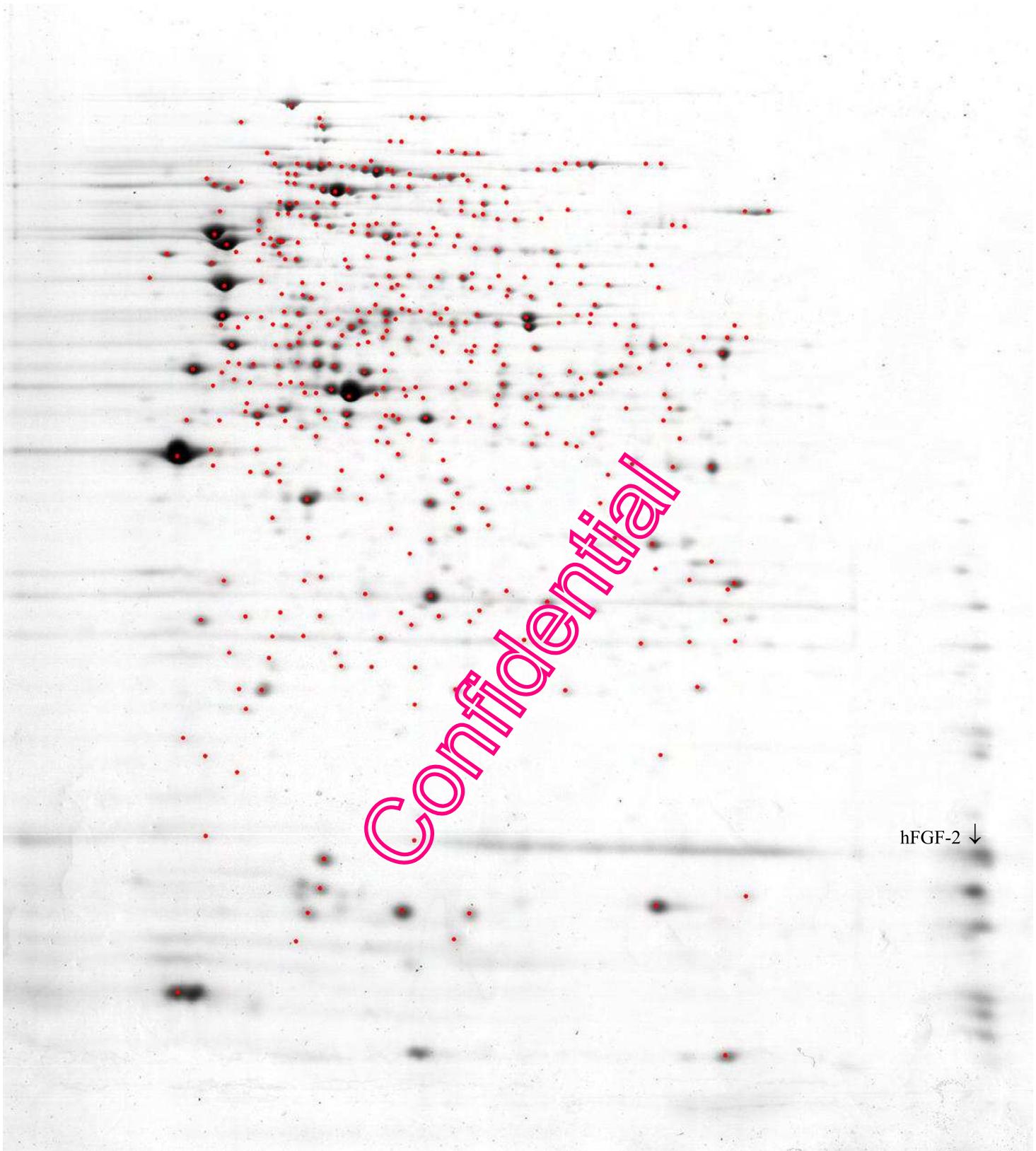
557 **Supporting Information 2 (Excel file):** Table S1: Quantitative data of individual proteins of *E.*  
558 *coli* BL21 (DE3) growing in defined and complex medium during production of hFGF-2 and of  
559 non-producing control cells grown under identical conditions.

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**Figure S1 2D gel of *E. coli* BL21 (DE3) harbouring plasmid pET-29c-hFGF-2  
in LB medium at exponential phase (before IPTG induction)**

Click on the red spots to get detailed information of identified spots (proteins). Cultivation was carried out in shaker flask with baffles at 30 °C and 180 rpm.



**Figure S2 2D gel of *E. coli* BL21 (DE3) harbouring plasmid pET-29c-hFGF-2  
in LB medium 1 h after induction with 0.25 mM IPTG**

Click on the red spots to get detailed information of identified spots (proteins). Cultivation was carried out in shaker flask with baffles at 30 °C and 180 rpm.



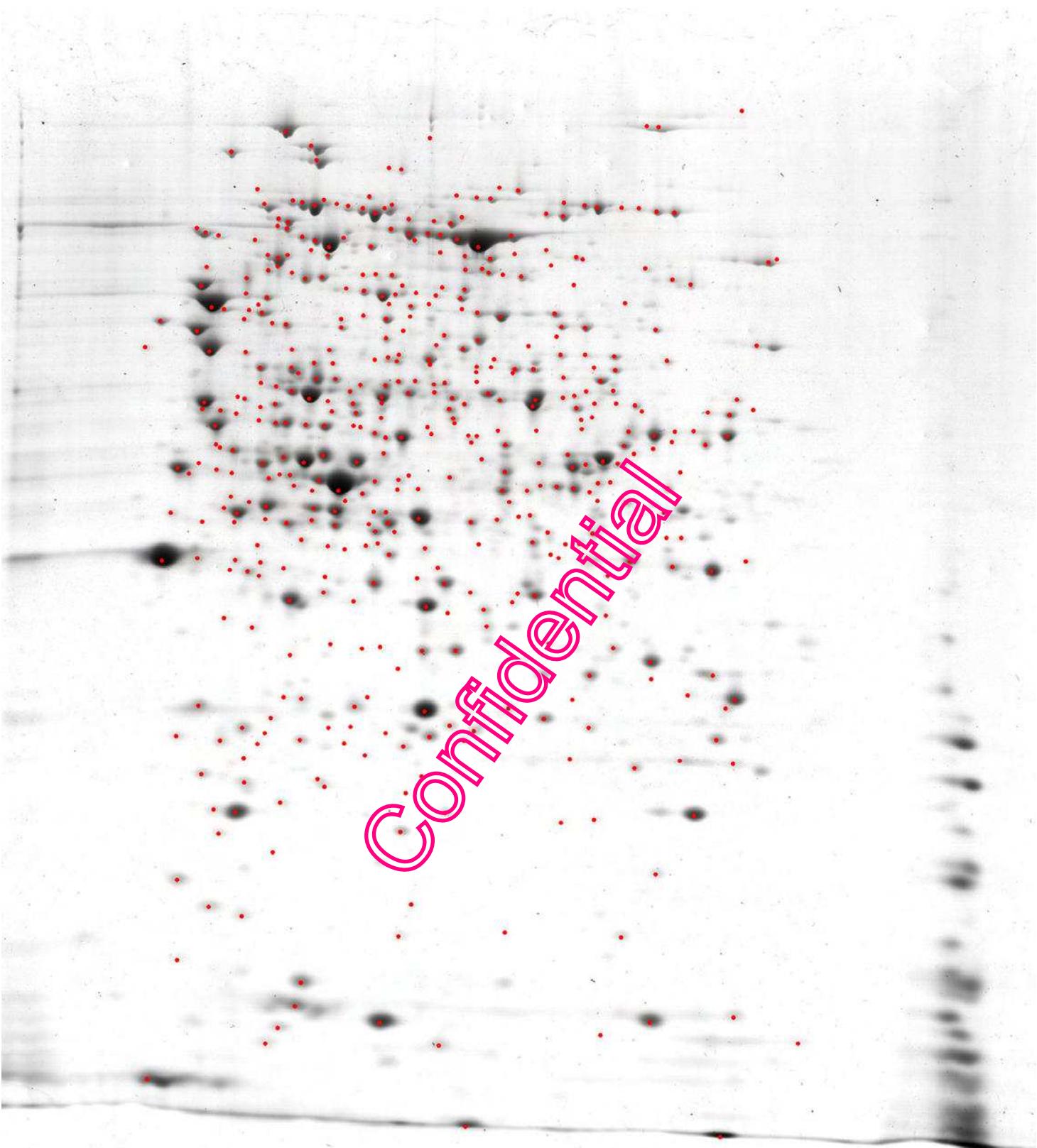
**Figure S3 2D gel of *E. coli* BL21 (DE3) harbouring plasmid pET-29c-hFGF-2  
in LB medium 3.5 h after induction with 0.25 mM IPTG**

Click on the red spots to get detailed information of identified spots (proteins). Cultivation was carried out in shaker flask with baffles at 30 °C and 180 rpm.



**Figure S4** 2D gel of *E. coli* BL21 (DE3) harbouring plasmid pET-29c-hFGF-2  
in LB medium at stationary phase (without IPTG induction)

Click on the red spots to get detailed information of identified spots (proteins). Cultivation was carried out in shaker flask with baffles at 30 °C and 180 rpm.



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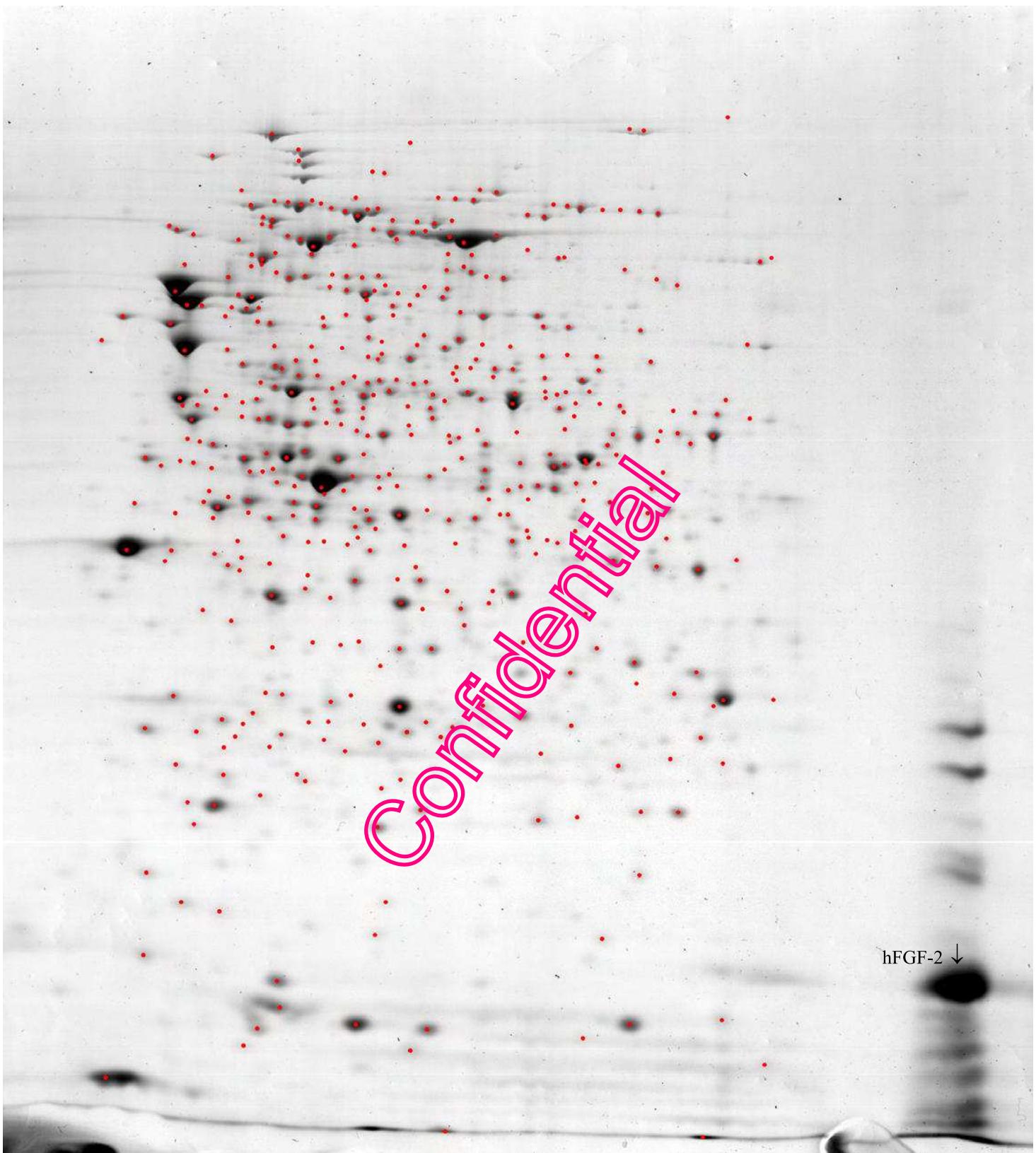
**Figure S5 2D gel of *E. coli* BL21 (DE3) harbouring plasmid pET-29c-hFGF-2 in DNB medium at exponential phase (before IPTG induction)**

Click on the red spots to get detailed information of identified spots (proteins). Cultivation was carried out in shaker flask with baffles at 30 °C and 180 rpm.



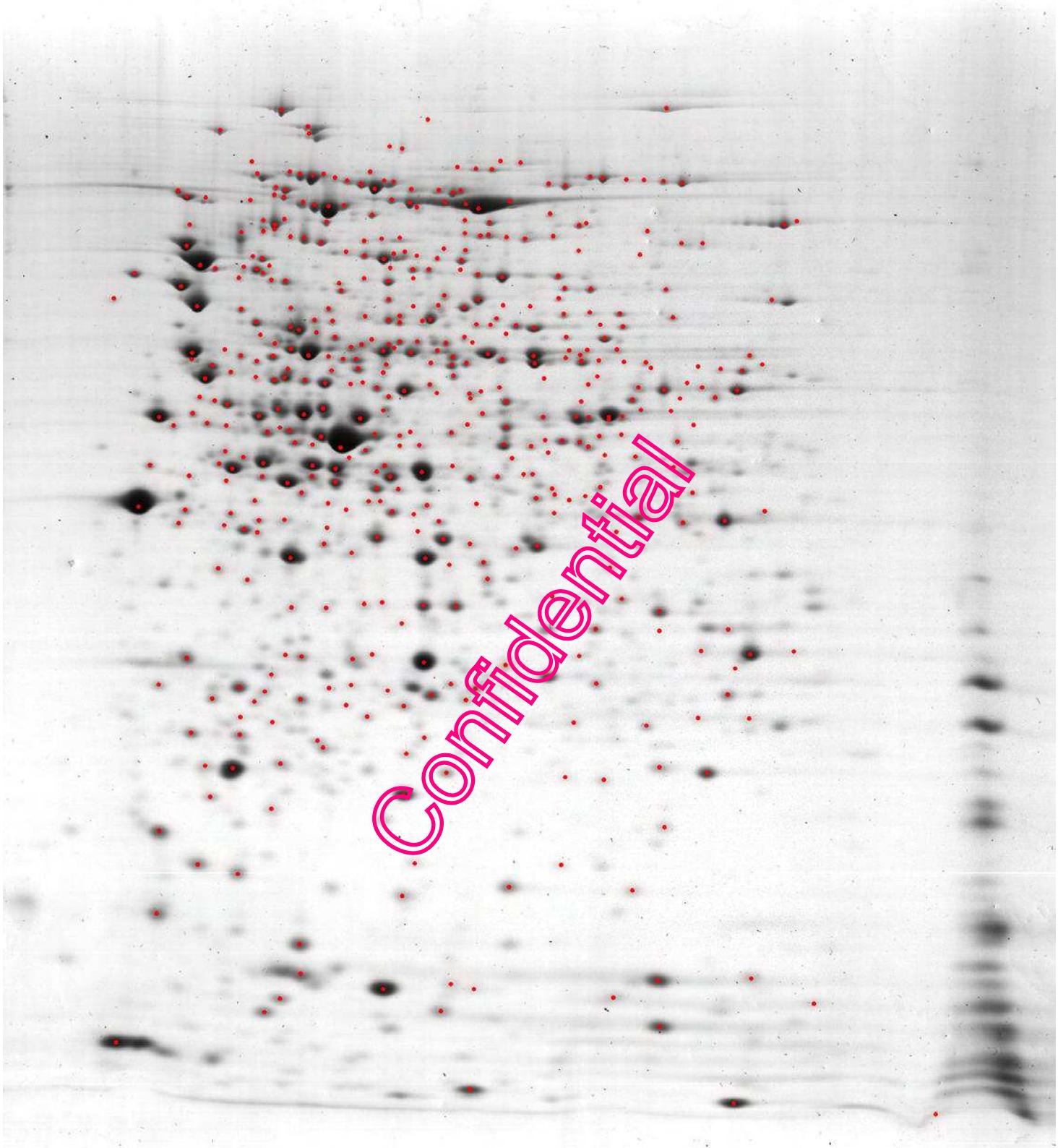
**Figure S6 2D gel of *E. coli* BL21 (DE3) harbouring plasmid pET-29c-hFGF-2  
in DNB medium 1 h after induction with 0.25 mM IPTG**

Click on the red spots to get detailed information of identified spots (proteins). Cultivation was carried out in shaker flask with baffles at 30 °C and 180 rpm.



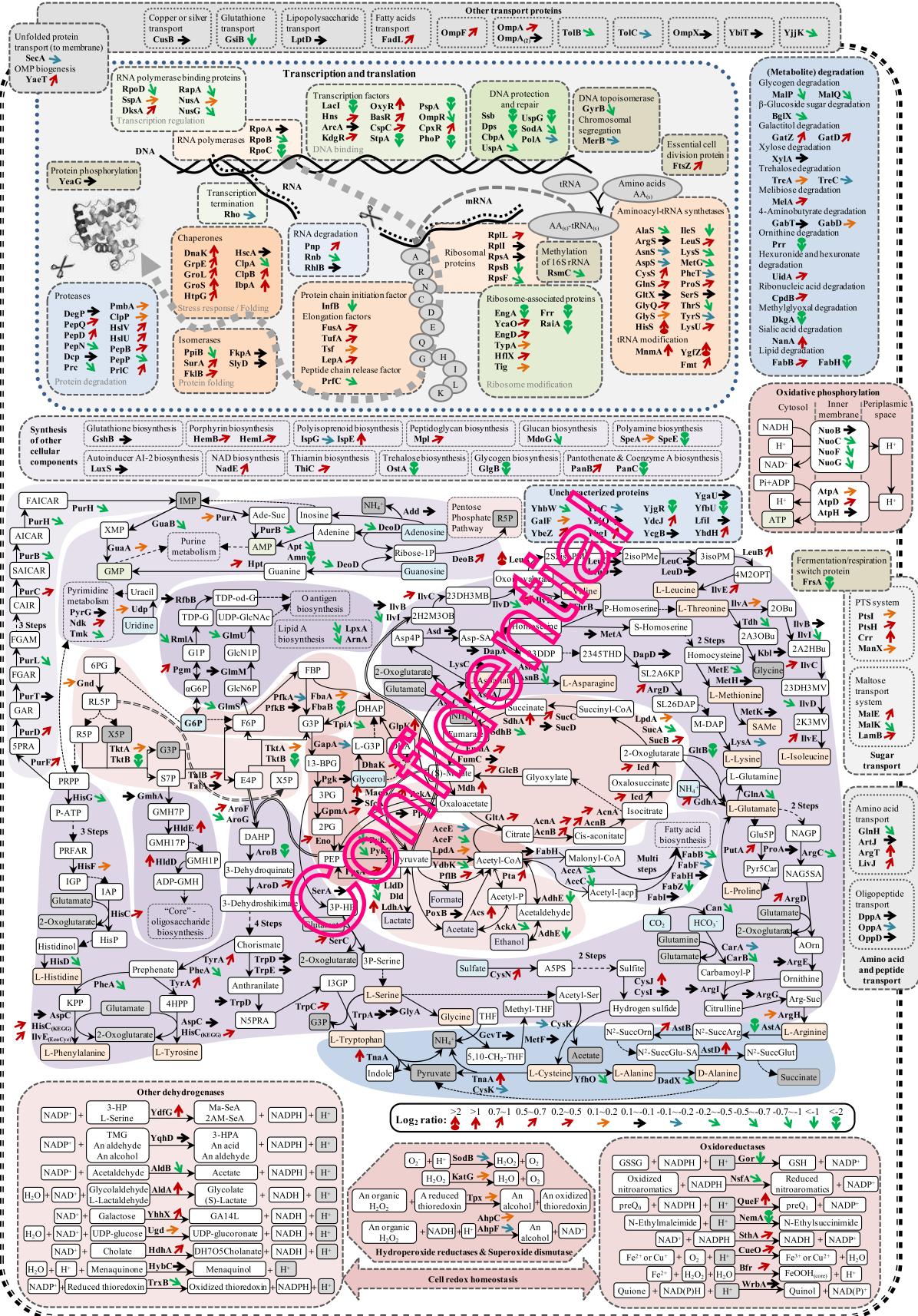
**Figure S7 2D gel of *E. coli* BL21 (DE3) harbouring plasmid pET-29c-hFGF-2  
in DNB medium 5 h after induction with 0.25 mM IPTG**

Click on the red spots to get detailed information of identified spots (proteins). Cultivation was carried out in shaker flask with baffles at 30 °C and 180 rpm.



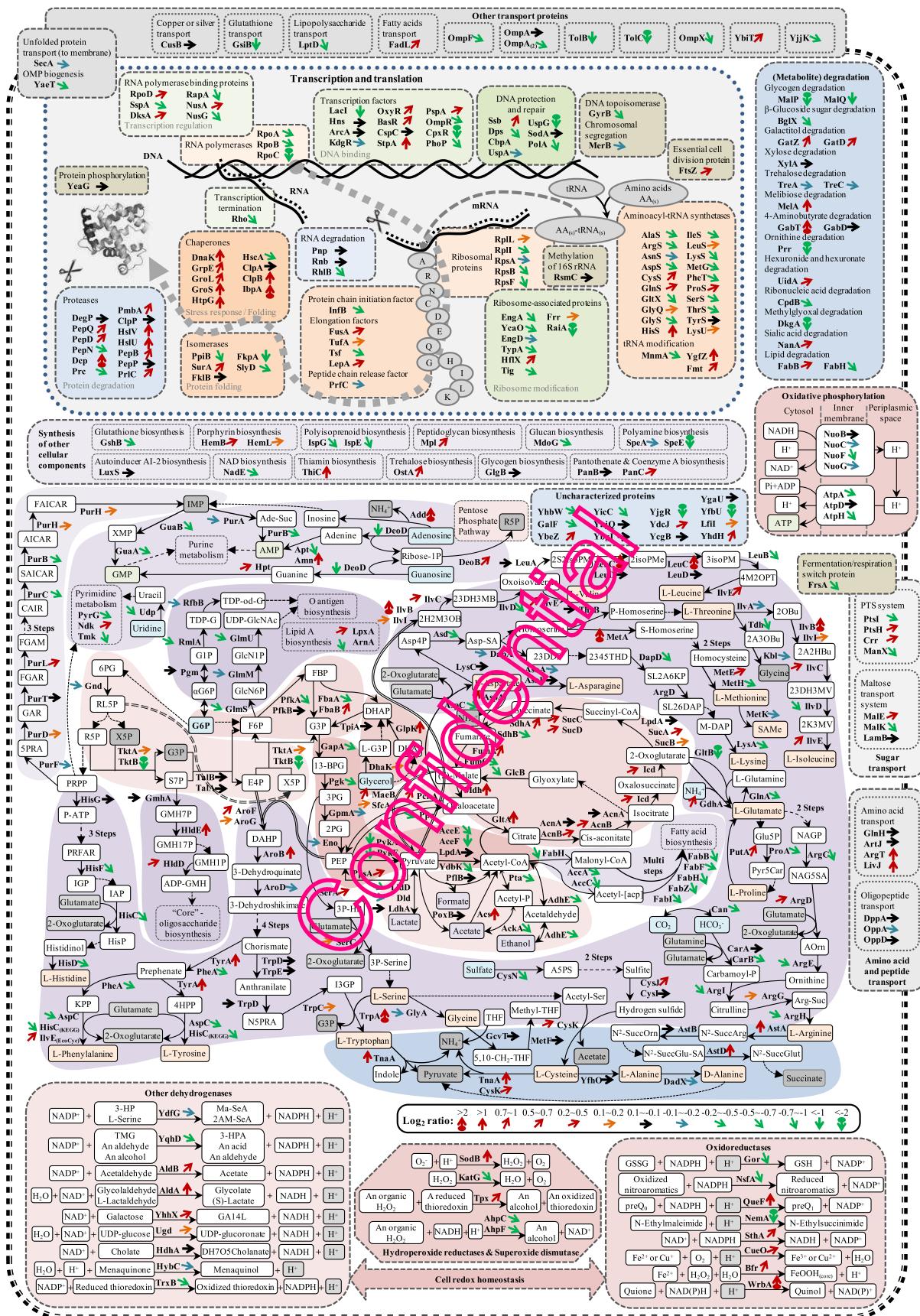
**Figure S8** 2D gel of *E. coli* BL21 (DE3) harbouring plasmid pET-29c-hFGF-2  
in DNB medium at stationary phase (without IPTG induction)

Click on the red spots to get detailed information of identified spots (proteins). Cultivation was carried out in shaker flask with baffles at 30 °C and 180 rpm.



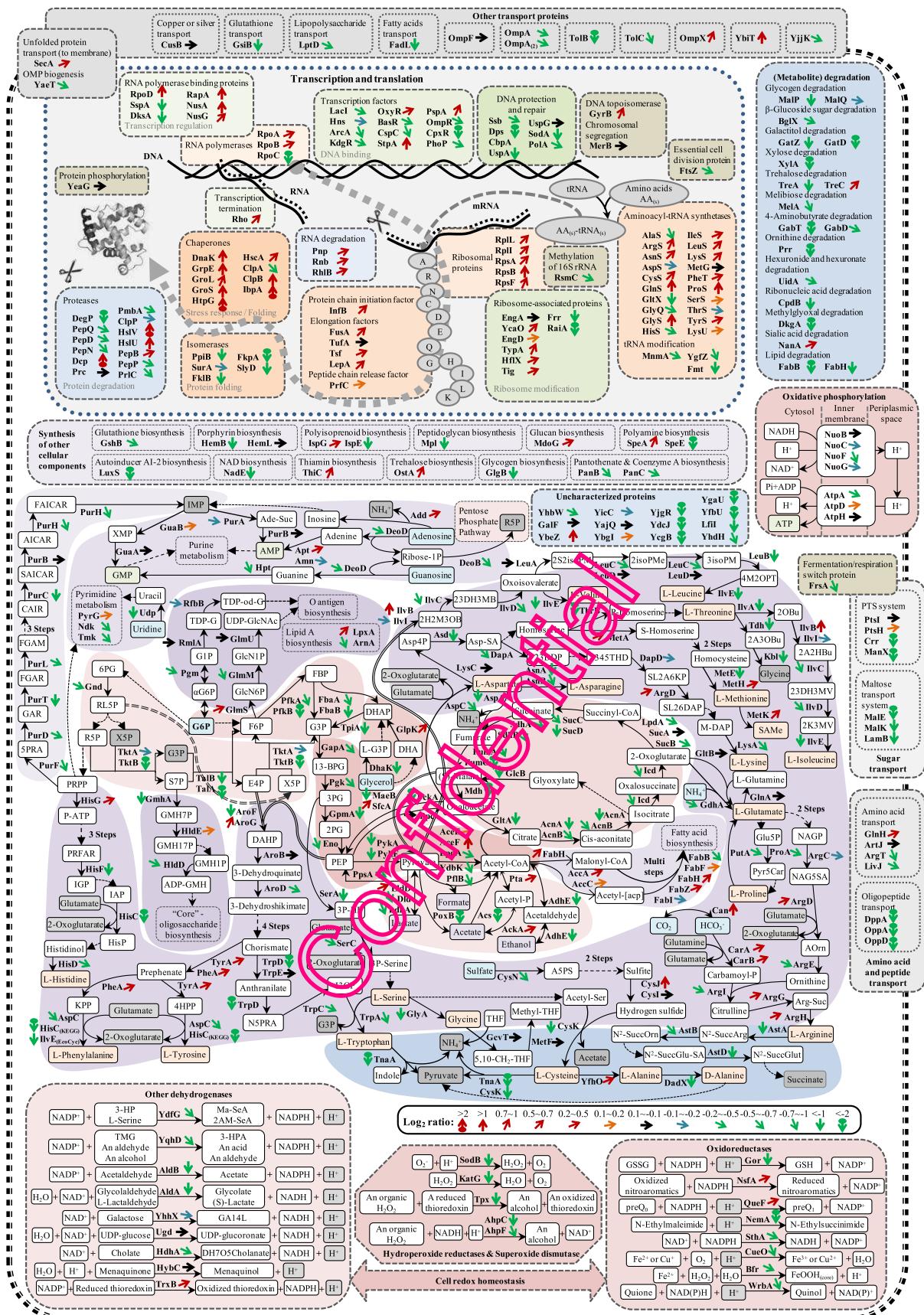
**Figure S9 Comparative proteome analysis of *E. coli* during hFGF-2 production (1h after IPTG induction versus exponential phase (before induction) in complex medium)**

Arrow indicates relative change of each protein ( $\log_2$  ratio). Color code is shown in the figure. Value is given in Table S1 (Supporting Information).



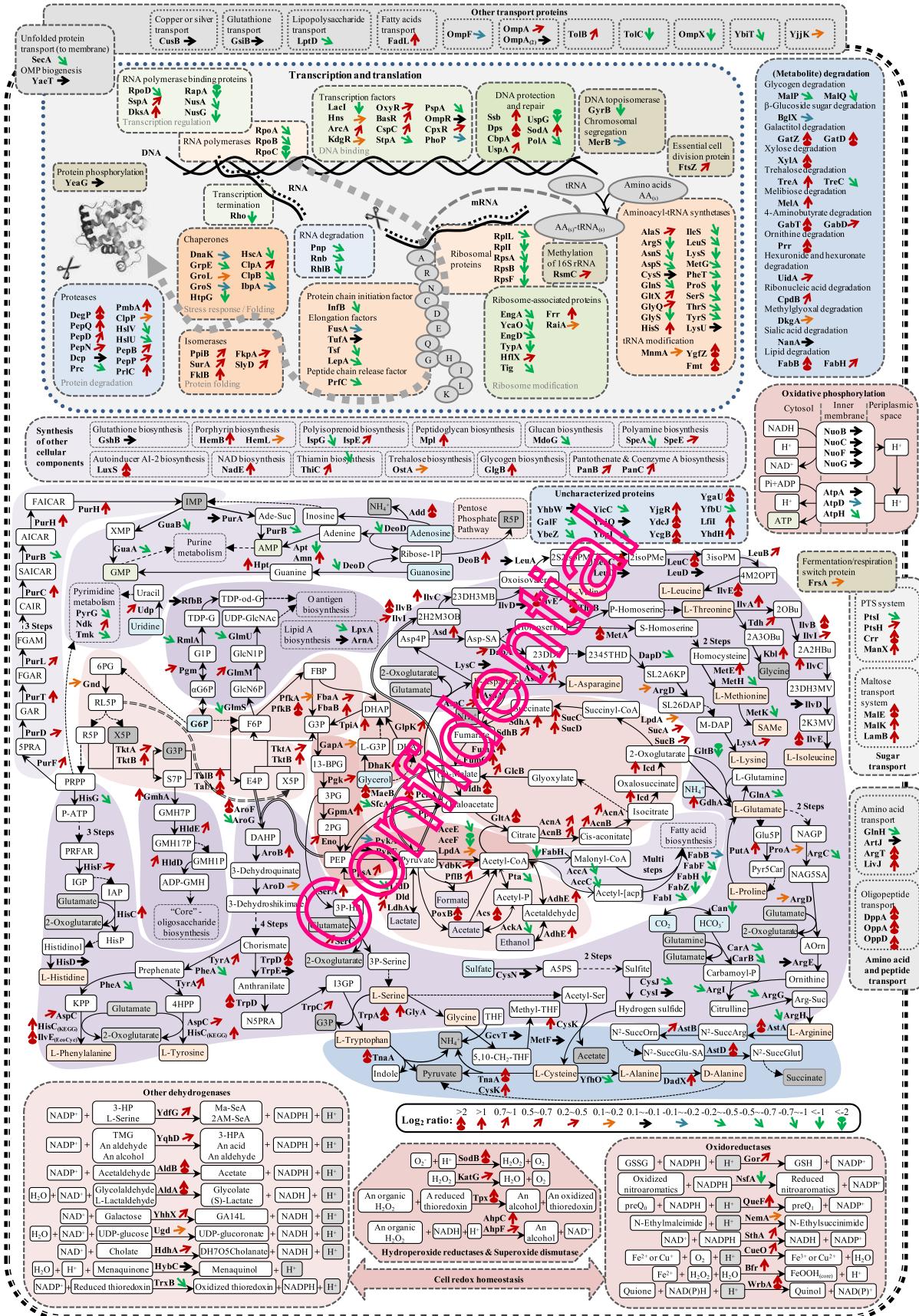
**Figure S10 Comparative proteome analysis of *E. coli* during hFGF-2 production  
(3.5h after IPTG induction versus exponential phase (before induction) in complex medium)**

Arrow indicates relative change of each protein ( $\text{Log}_2$  ratio). Color code is shown in the figure. Value is given in Table S1 (Supporting Information).



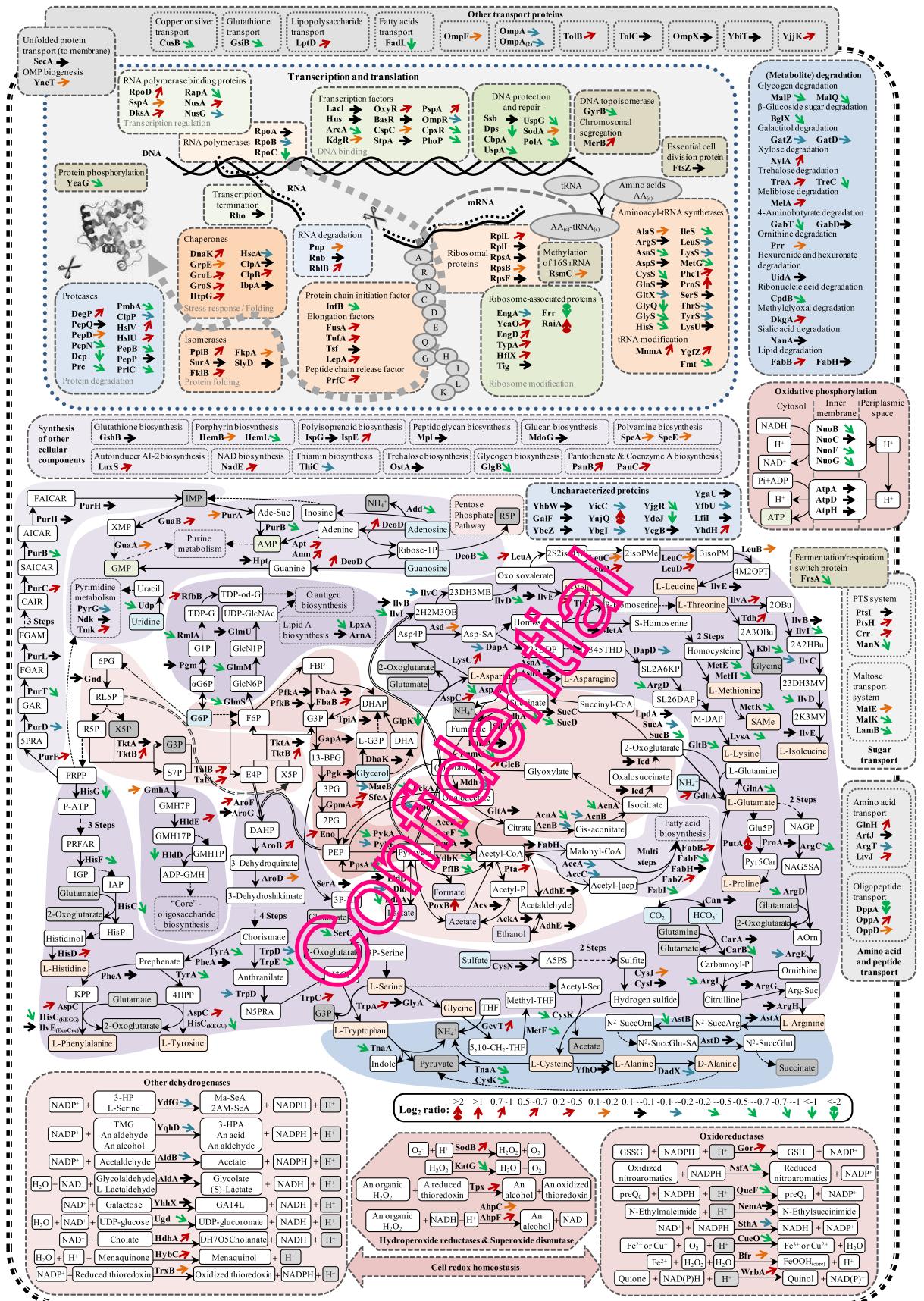
**Figure S11 Comparative proteome analysis of *E. coli* during hFGF-2 production  
(3.5h after IPTG induction versus stationary phase (without induction) in complex medium)**

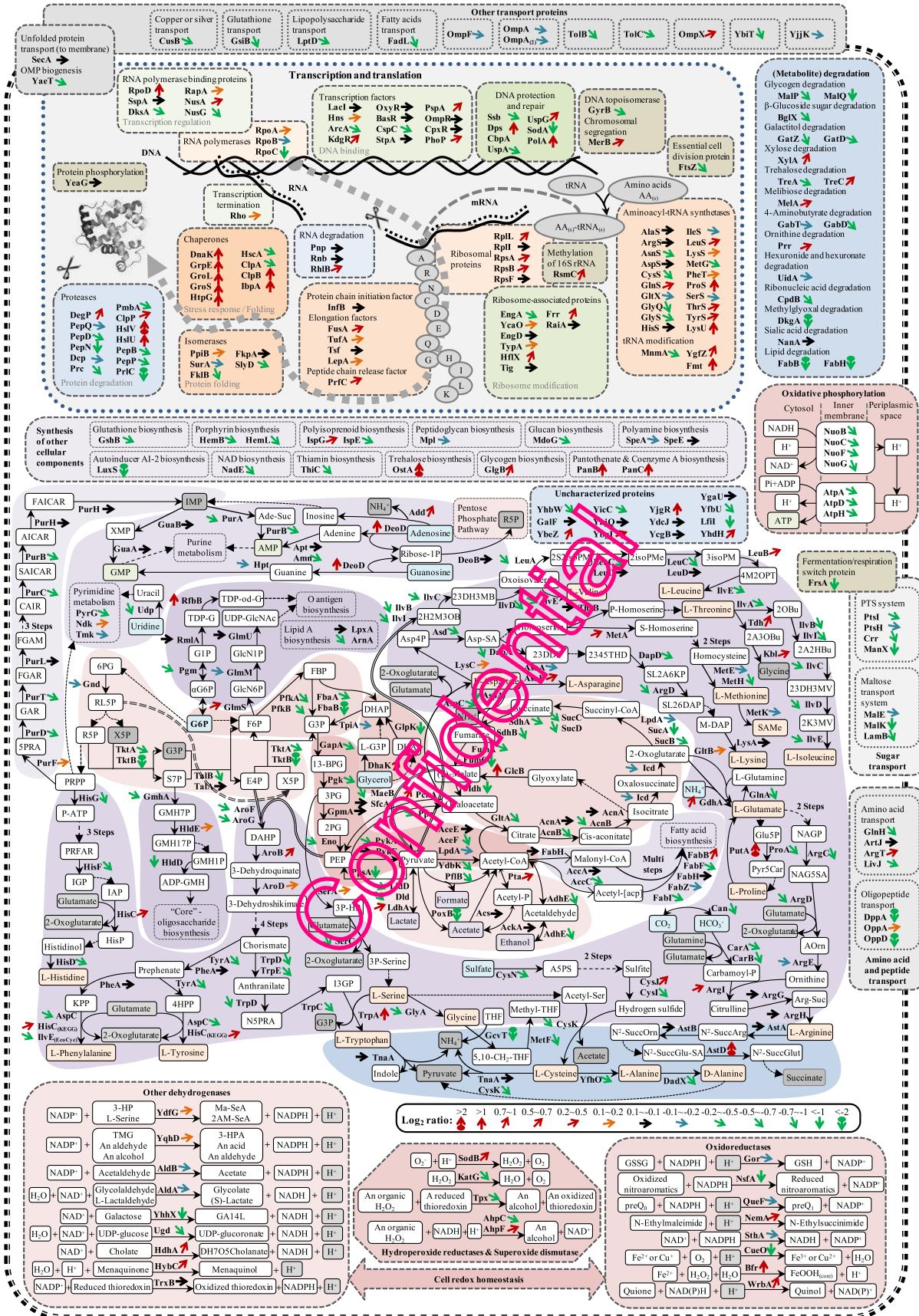
Arrow indicates relative change of each protein ( $\log_2$  ratio). Color code is shown in the figure. Value is given in Table S1 (Supporting Information).



**Figure S12 Comparative proteome analysis of *E. coli* during hFGF-2 production (stationary phase versus exponential phase in complex medium without protein production)**

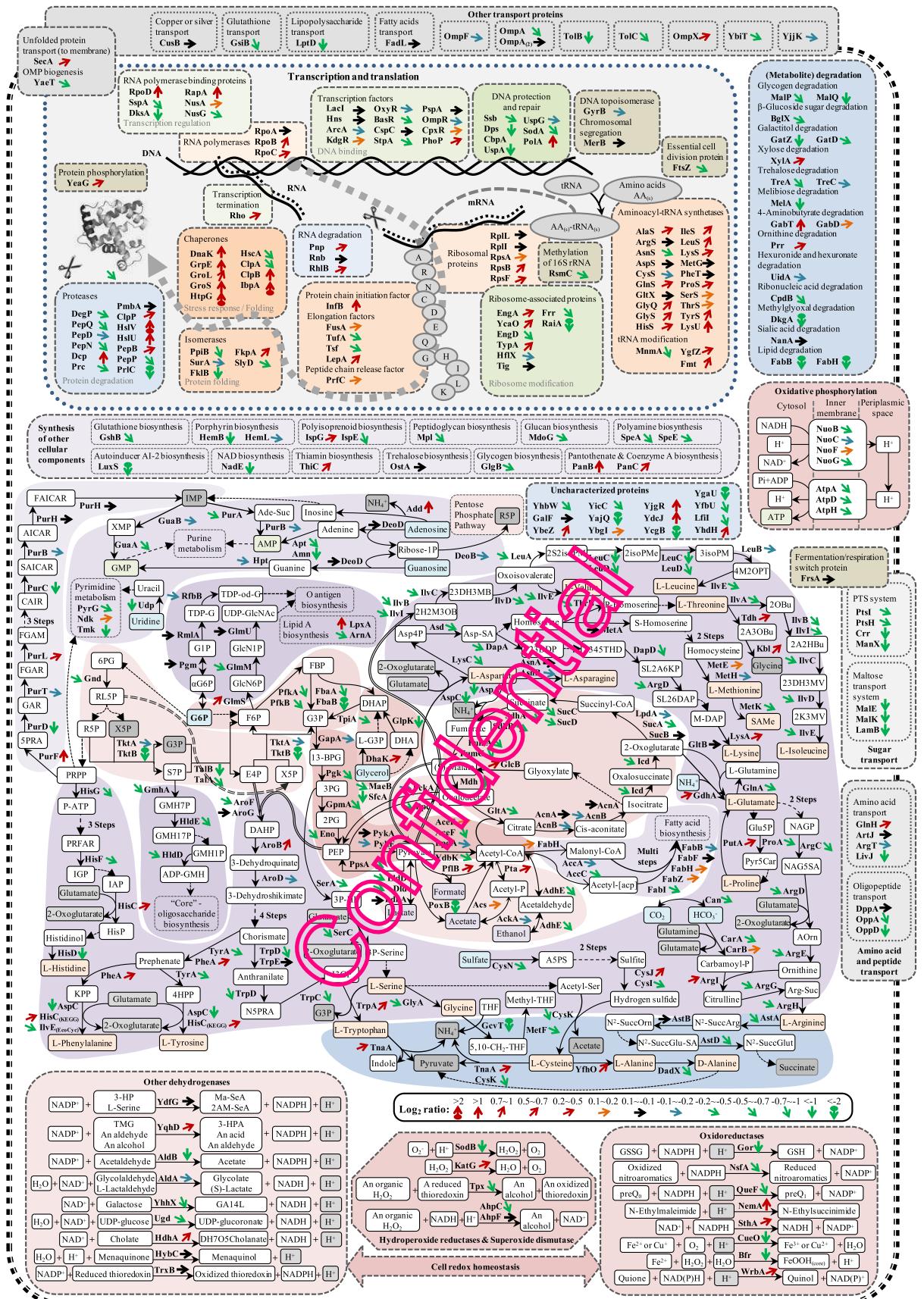
Arrow indicates relative change of each protein ( $\log_2$  ratio). Color code is shown in the figure. Value is given in Table S1 (Supporting Information).





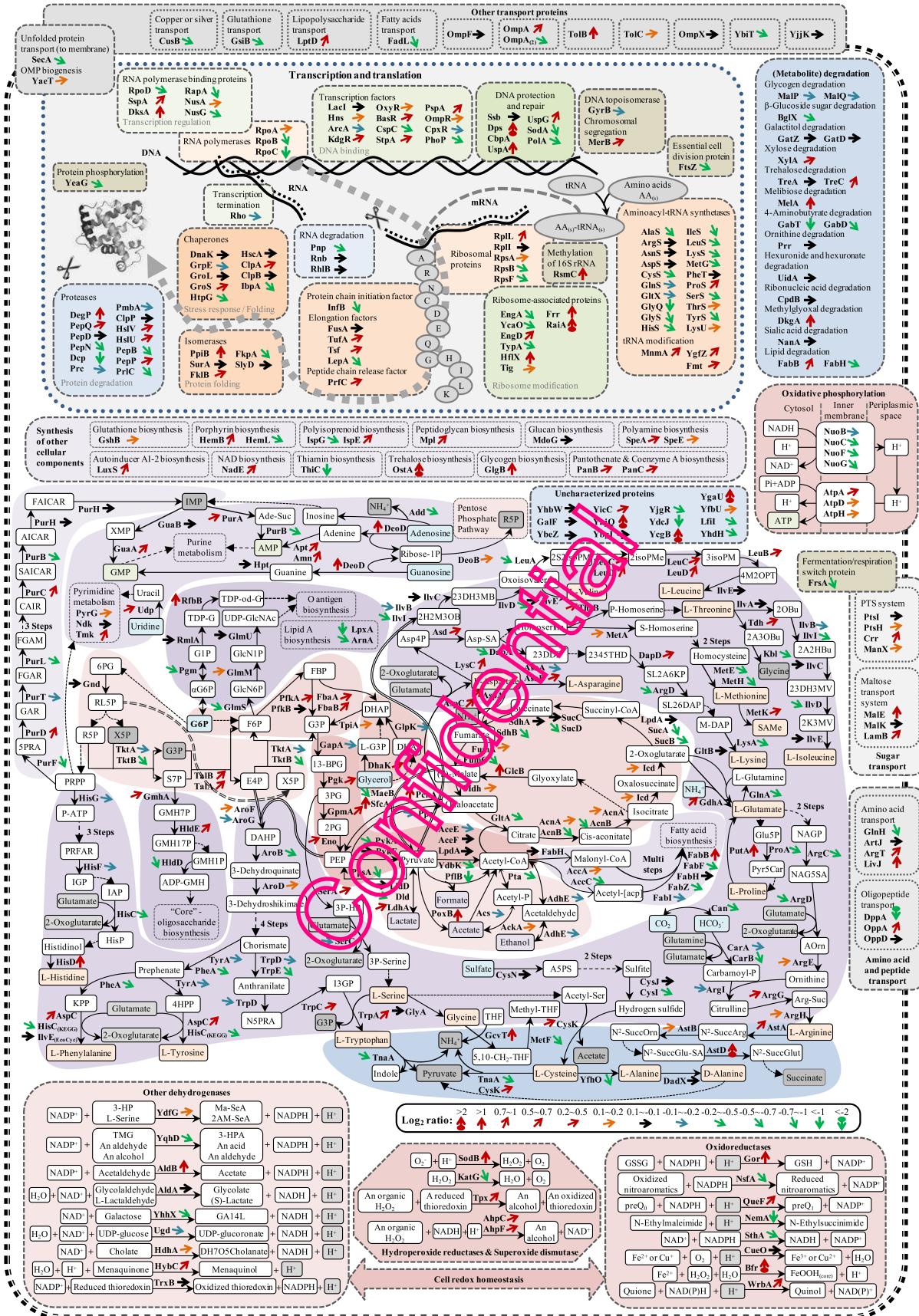
**Figure S14 Comparative proteome analysis of *E. coli* during hFGF-2 production (5h after IPTG induction versus exponential phase (before induction) in defined medium)**

Arrow indicates relative change of each protein (Log2 ratio). Color code is shown in the figure. Value is given in Table S1 (Supporting Information).



**Figure S15 Comparative proteome analysis of *E. coli* during hFGF-2 production  
(5h after IPTG induction versus stationary phase (without induction) in defined medium)**

Arrow indicates relative change of each protein ( $\log_2$  ratio). Color code is shown in the figure. Value is given in Table S1 (Supporting Information).



**Figure S16 Comparative proteome analysis of *E. coli* during hFGF-2 production (stationary phase versus exponential phase in defined medium without protein production)**

Arrow indicates relative change of each protein ( $\log_2$  ratio). Color code is shown in the figure. Value is given in Table S1 (Supporting Information).

## Figure captions for Figures S9-S16

A detailed direct comparison of pathway regulation at the proteomic level at different growth conditions is given in a pathway map using different color coded arrows for the representation of the comparative Log<sub>2</sub> ratios of the single protein abundances (code given in lower right of figure). Pathway map is drawn according to EcoCyc database (<http://ecocyc.org/>) [confirmed by KEGG database (<http://www.genome.jp/kegg/>)] based on current identified proteins. LB medium was employed to indicate the yeast extract- and tryptone-supplemented rich medium (complex medium). DNB medium was employed to indicate the glucose-supplemented defined mineral salt medium (defined medium). The corresponding values are given in the Table S1.

## Abbreviation used in Figures S9-S16

13-BPG	1,3-bisphospho-D-glycerate
2345THD	(S)-2,3,4,5-tetrahydroadipicoline
23DH3MB	2,3-dihydroxy-3-methylbutanoate
23DH3MV	2,3-dihydroxy-3-methylvalerate
2A2HBu	2-aceto-2-hydroxy-butanoate
2A3OBu	2-amino-3-oxobutanoate
2AM-SeA	2-aminomalonate-semialdehyde
2H2M3OB	(S)-2-hydroxy-2-methyl-3-oxobutanoate ( $\alpha$ -acetolactate)
2isoPMe	2-isopropylmaleate
2K3MV	2-keto-3-methyl-valerate
2OBu	2-oxobutanoate
2PG	2-phospho-D-glycerate
2S2isoPM	(2S)-2-isopropylmalate
3-HP	3-hydroxypropionate
3-HPA	3-hydroxypropionaldehyde
3isoPM	(2R,3S)-3-isopropylmalate
3PG	3-phospho-D-glycerate
3P-HP	3-phospho-hydroxypyruvate
3P-serine	3-phospho-L-serine
4H-2345THD	(2S,4S)-4-hydroxy-2,3,4,5-tetrahydroadipicoline
4HPP	4-hydroxyphenylpyruvate
4M2OPT	4-methyl-2-oxopentanoate
5,10-CH <sub>2</sub> -THF	5,10-methylenetetrahydrofolate
5PRA	5-phospho- $\beta$ -D-ribosyl-amine
6PG	6-phospho-D-gluconate
A5PS	adenosine 5'-phosphosulfate
Acetyl-P	acetylphosphate
Acetyl-Ser	O-acetyl-L-serine

Ade-Suc	adenylo-succinate
ADP-D-GMH	ADP-D-glycero- $\beta$ -D-manno-heptose
ADP-L-GMH	ADP-L-glycero- $\beta$ -D-manno-heptose
AICAR	aminoimidazole carboxamide ribonucleotide
AOrn	N-acetyl-L-ornithine
Arg-Suc	L-arginino-succinate
Asp4P	L-aspartyl-4-phosphate
Asp-SA	L-aspartate-semialdehyde
CAIR	5-amino-1-(5-phospho-D-ribosyl)imidazole-4-carboxylate
Carbamoyl-P	carbamoyl-phosphate
DAHP	3-deoxy-D-arabino-heptulosonate-7-phosphate
DH7O5Cholanate	3 $\alpha$ ,12 $\alpha$ -dihydroxy-7-oxo-5 $\beta$ -cholanate
DHA	dihydroxyacetone
DHAP	dihydroxyacetone phosphate
E4P	D-erythrose-4-phosphate
F6P	D-fructose-6-phosphate
FAICAR	phosphoribosyl-formamido-carboxamide
FBP	fructose-1,6-bisphosphate
FGAM	5-phosphoribosyl-N-formylglycineamidine
FGAR	5'-phosphoribosyl-N-formylglycineamidine
G1P	$\alpha$ -D-glucose 1-phosphate
G3P	D-glyceraldehyde-3-phosphate
G6P	$\beta$ -D-glucose-6-phosphate
GA14L	D-galactono-1,4-lactone
GAR	5-phospho-ribosyl-glycineamide
GlcN1P	D-glucosamine-1-phosphate
GlcN6P	D-glucosamine-6-phosphate
Glu5P	L-glutamate-5-phosphate
GMH17P	D-glycero- $\beta$ -D-manno-heptose 1,7-bisphosphate
GMH7P	D-glycero-D-manno-heptose-7-phosphate
GSH	glutathione
GSSG	glutathione disulfide
HisP	L-histidinol-phosphate
I3GP	(1S,2R)-1-C-(indol-3-yl)glycerol 3-phosphate
IAP	imidazole acetol-phosphate
IGP	D-erythro-imidazole-glycerol-phosphate
IMP	inosine-5'-phosphate
L-G3P	sn-glycerol-3-phosphate
L-G3P	sn-glycerol-3-phosphate
Ma-SeA	malonate semialdehyde
M-DAP	meso-diaminopimelate
Methyl-THF	5-methyl-tetrahydrofolate

N <sup>2</sup> -SuccArg	N <sup>2</sup> -succinylarginine
N <sup>2</sup> -SuccGlu-SA	N <sup>2</sup> -succinylglutamic-semialdehyde
N <sup>2</sup> -SuccGlut	N <sup>2</sup> -succinylglutamate
N <sup>2</sup> -SuccOrn	N <sup>2</sup> -succinylornithine
N5PRA	N-(5'-phosphoribosyl)-anthranilate
NAG5SA	N-acetyl-L-glutamate 5-semialdehyde
NAGP	N-acetylglutamyl-phosphate
Nitroaromatics	nitroaromatic compound
OC1D5P	1-(o-carboxyphenylamino)-1'-deoxyribulose-5'-phosphate
2O3PP	2-oxo-3-phenylpropanoate
P-ATP	phosphoribosyl-ATP
PEP	phosphoenolpyruvate
P-Homoserine	O-phospho-L-homoserine
preQ <sub>0</sub>	7-cyano-7-deazaguanine
preQ <sub>1</sub>	7-aminomethyl-7-deazaguanine
PRFAR	phosphoribulosylformimino-AICAR-P
PRPP	5-phospho- $\alpha$ -D-ribose 1-diphosphate
Pyr5Car	(S)-1-pyrroline-5-carboxylate
R5P	D-ribose-5-phosphate
Ribose-1P	$\alpha$ -D-ribose-1-phosphate
RL5P	D-ribulose-5-phosphate
S7P	D-sedoheptulose-7-phosphate
SAICAR	5'-phosphoribosyl-4-(N-succinocarboxamide)-5-aminoimidazole
SAMe	S-adenosyl-L-methionine
S-Homoserine	O-succinyl-L-homoserine
SL26DAP	Nsuccinyl-L,L-2,6-diaminopimelate
SL2A6KP	Nsuccinyl-2-amino-6-ketopimelate
TDP-G	dTDP- $\alpha$ -D-glucose
TDP-od-G	dTDP-4-dehydro-6-deoxy-D-glucose
THF	tetrahydrofolate
TMG	1,3-propanediol
UDP-GlcNAc	UDP- $\alpha$ -N-acetyl-D-glucosamine
X5P	D-xylulose-5-phosphate
$\alpha$ G6P	$\alpha$ -D-glucose 6-phosphate

**Table S1 - Quantitative data of individual proteins of *E. coli* BL21 (DE3) growing in defined and complex medium during production of hFGF-2**

Name <sup>1</sup>	Protein name <sup>1</sup>	Uniprot ID <sup>1</sup>	Molecular mass <sup>2</sup> (Da)	Calculated pI value <sup>3</sup>	Mascot Score <sup>2</sup>	Mascot Expect Value <sup>2</sup>	Mascot Seq. Cover. <sup>2</sup>	Defined (DNB) medium				Complex (LB) medium				Log <sub>2</sub> ratio in Defined (DNB) medium				Log <sub>2</sub> ratio in Complex (LB) medium				Log <sub>2</sub> (LB medium/ DNB medium) exponential before IPTG									
								exponential phase before IPTG induction	1h after IPTG induction	5h after IPTG induction	stationary phase without IPTG	exponential phase before IPTG induction	1h after IPTG induction	3.5h after IPTG induction	stationary phase without IPTG	(1h after IPTG/ exponential before IPTG)	(5h after IPTG/ stationary without IPTG)	(stationary phase before IPTG)	(1h after IPTG/ exponential before IPTG)	(3.5h after IPTG/ stationary without IPTG)	(stationary phase before IPTG)	(1h after IPTG/ exponential before IPTG)	(3.5h after IPTG/ stationary without IPTG)										
								Relative Protein Mass (RPM) <sup>3</sup> - %																									
<b>Central carbon metabolism (24 proteins): Upper and lower glycolysis, pyruvate decarboxylation to acetyl CoA, glycerol metabolism and pentose phosphate pathway</b>									Total RPM:	6.56	6.60	5.30	7.00	7.36	7.70	6.16	8.30	0.0	-0.3	-0.4	0.1	0.1	-0.3	-0.4	0.2	-0.2							
<b>Sub-group: Upper glycolysis (5 proteins)</b>																																	
PfkA	6-phosphofructokinase	P0A797	35162	5.47	105	2.70E-06	0.41	0.12	0.12	0.08	0.14	0.09	0.08	0.06	0.10	0.0	-0.6	-0.8	0.2	-0.2	-0.6	-0.7	0.2	-0.4	-0.4								
PfkB	6-phosphofructokinase isozyme 2	P06999	32664	5.25	145	7.20E-11	0.83	0.038	0.039	0.024	0.039	0.00	0.00	0.00	0.044	0.0	-0.7	-0.7	0.0	0.0	0.0	<5	>5	<5	<5								
FbaA	fructose-bisphosphate aldolase class 2	P0AB71	39351	5.52	80	2.30E-04	0.25	0.86	0.87	0.62	1.03	0.67	0.77	0.51	0.85	0.0	-0.5	-0.7	0.3	0.2	-0.4	-0.7	0.3	-0.4	-0.4								
FbaB	fructose-bisphosphate aldolase class 1	P0A991	38313	6.25	59	2.80E-02	0.29	0.019	0.024	0.00	0.030	0.018	0.00	0.033	0.07	0.3	<-5	<-5	0.7	<-5	0.9	-1.1	2.0	-0.1	-0.1								
TpiA	triosephosphate isomerase	P0A858	27126	5.64	77	4.40E-04	0.34	0.23	0.23	0.21	0.26	0.14	0.12	0.14	0.29	0.0	-0.1	-0.3	0.2	-0.2	0.0	-1.1	1.1	-0.7	-0.7								
<b>Sub-group: Lower glycolysis (7 proteins)</b>																																	
GapA	glyceraldehyde-3-phosphate dehydrogenase A	P0A9B2	35681	6.61	58	3.80E-02	0.22	0.64	0.63	0.53	0.58	0.64	0.56	0.48	0.72	0.0	-0.3	-0.1	-0.1	-0.2	-0.4	-0.6	0.2	0.0	0.0								
Pgk	phosphoglycerate kinase	P0A799	41264	5.08	231	6.60E-11	0.66	0.58	0.60	0.47	0.73	0.55	0.58	0.45	0.69	0.0	-0.3	-0.6	0.3	0.1	-0.3	-0.6	0.3	-0.1	-0.1								
GpmA	phosphoglyceromutase	P62709	28539	5.85	64	3.60E-02	0.27	0.18	0.21	0.17	0.26	0.18	0.25	0.16	0.37	0.2	-0.1	-0.6	0.5	0.5	-0.2	-1.2	1.0	0.0	0.0								
Eno	enolase	A7ZQM2	45683	5.32	106	5.80E-07	0.41	0.06	0.05	0.049	0.047	0.09	0.027	0.033	0.08	-0.3	-0.3	0.1	-0.4	-1.7	-1.4	-1.3	-0.2	0.6	-0.4								
PykA	pyruvate kinase II	P21599	51553	6.24	115	7.30E-08	0.36	0.06	0.05	0.049	0.047	0.09	0.027	0.033	0.08	-0.3	-0.3	0.1	-0.4	-1.7	-1.4	-1.3	-0.2	0.6	-0.4								
PykF	pyruvate kinase I	P0AD61	51039	5.77	70	2.10E-03	0.31	0.18	0.16	0.15	0.19	0.25	0.17	0.12	0.24	-0.2	-0.1	-0.2	0.1	-0.6	-1.1	-1.0	-0.1	0.5	-0.5								
PpsA	phosphoenolpyruvate synthase	P23538	87836	4.93	208	3.60E-17	0.32	0.12	0.06	0.07	0.24	0.33	0.30	0.41	-1.0	-1.3	-0.5	-0.8	0.5	0.3	-0.5	0.8	1.0	1.0	-0.5								
<b>Sub-group: Pyruvate decarboxylation to acetyl CoA (4 proteins)</b>																																	
AceE	pyruvate dehydrogenase E1 component	B3XKK6	99978	5.46	179	1.10E-13	0.28	0.87	0.96	0.82	0.74	1.51	1.45	1.02	0.92	0.1	-0.1	0.1	-0.2	-0.2	-0.6	0.1	-0.7	0.8	0.8								
AceF	pyruvate dehydrogenase, dihydrolipoyltransacetylase component E2	P06959	66112	5.09	156	2.10E-11	0.32	0.35	0.28	0.22	0.36	0.37	0.23	0.08	-0.3	-0.7	-0.7	0.0	-0.3	-1.0	1.5	-2.6	0.4	0.4	0.4								
LpdA <sup>4</sup>	dihydrolipoamide dehydrogenase	P0A9P2	50942	5.79	155	2.60E-11	0.43	0.64	0.63	0.57	0.62	0.83	0.89	0.90	0.93	0.0	-0.2	-0.1	0.0	0.1	-0.1	-0.2	0.2	0.4	0.4								
YdbK	pyruvate flavodoxin/ferredoxin oxidoreductase domain protein	A7ZZU0	129871	5.51	76	2.00E-03	0.14	0.07	0.06	0.043	0.050	0.05	0.04	0.033	0.06	-0.2	-0.7	-0.2	-0.5	-0.2	-0.6	-0.9	0.3	-0.5	-0.5								
<b>Sub-group: Glycerol metabolism (2 proteins)</b>																																	
GlpK	glycerol kinase	C5A093	56480	5.36	129	2.90E-09	0.28	0.10	0.032	0.026	0.09	0.12	0.43	0.29	0.20	-1.6	-1.9	-1.8	-0.2	1.8	1.3	0.5	0.7	0.3	0.3								
Dhak	dihydroxyacetone kinase subunit DhaK	A7ZZD5	38620	4.82	104	3.40E-06	0.44	0.030	0.030	0.05	0.042	0.027	0.036	0.031	0.09	0.0	0.7	0.3	0.5	0.4	0.2	-1.5	1.7	-0.2	-0.2								
<b>Sub-group: Pentose phosphate pathway (PPP) (6 proteins)</b>																																	
Gnd	6-phosphogluconate dehydrogenase, decarboxylating	Q59414	51563	5.06	133	1.10E-09	0.33	0.30	0.30	0.27	0.32	0.39	0.42	0.36	0.42	0.0	-0.2	-0.2	0.1	0.1	-0.1	-0.2	0.1	0.4	0.4								
TktA	transketolase 1	P27302	72451	5.43	75	7.20E-04	0.2	0.46	0.45	0.38	0.42	0.53	0.57	0.58	0.65	0.0	-0.3	-0.1	-0.1	0.1	0.1	-0.2	0.3	0.2	0.2								
TktB	transketolase 2	P33570	73225	5.86	190	2.30E-15	0.27	0.017	0.028	0.00	0.011	0.013	0.00	0.00	0.05	0.7	<-5	<5	-0.6	<-5	<5	<5	1.9	-0.4	-0.4								
TalB	transaldolase B	P0A870	35368	5.11	136	5.70E-10	0.56	0.13	0.15	0.07	0.21	0.15	0.18	0.16	0.37	0.2	-0.9	-1.6	0.7	0.3	0.1	-1.2	1.3	0.2	0.2								
TalA	transaldolase A	P0A867	35865	5.89	211	1.80E-17	0.62	0.048	0.06	0.045	0.07	0.00	0.00	0.06	0.041	0.3	-0.1	-0.6	0.5	0.0	0.0	<-5	>5	<5	<5								
Eda	KHG/KDPG aldolase	P0A955	22441	5.57	108	3.60E-07	0.49	0.032	0.039	0.031	0.06	0.029	0.035	0.029	0.041	0.3	0.0	-1.0	0.9	0.3	0.0	-0.5	0.5	-0.1	-0.1								

**Table S1 - Quantitative data of individual proteins of *E. coli* BL21 (DE3) growing in defined and complex medium during production of hFGF-2**

Name <sup>1</sup>	Protein name <sup>1</sup>	Uniprot ID <sup>1</sup>	Molecular mass <sup>2</sup> (Da)	Calculated pI value <sup>3</sup>	Mascot Score <sup>2</sup>	Mascot Expect Value <sup>2</sup>	Mascot Seq. Cover. <sup>2</sup>	Defined (DNB) medium				Complex (LB) medium				Log <sub>2</sub> ratio in Defined (DNB) medium				Log <sub>2</sub> ratio in Complex (LB) medium				Log <sub>2</sub> (LB medium/ DNB medium) exponential before IPTG	
								exponential phase before IPTG induction	1h after IPTG induction	5h after IPTG induction	stationary phase without IPTG	exponential phase before IPTG induction	1h after IPTG induction	3.5h after IPTG induction	stationary phase without IPTG	(1h after IPTG/ exponential before IPTG)	(5h after IPTG/ stationary without IPTG)	(stationary phase before IPTG)	(1h after IPTG/ exponential before IPTG)	(3.5h after IPTG/ stationary without IPTG)	(stationary phase before IPTG)	(1h after IPTG/ exponential before IPTG)	(3.5h after IPTG/ stationary without IPTG)		
								Relative Protein Mass (RPM) <sup>3</sup> - %																	
<b>By-product metabolism (9 proteins)</b>																									-0.2
Acs	acetyl-coenzyme A synthetase	P27550	72447	5.5	224	9.10E-19	0.42	0.10	0.10	0.09	0.05	0.10	0.13	0.71	0.0	0.0	0.2	-0.2	1.0	1.4	-2.4	3.8	-1.0		
PoxB	pyruvate dehydrogenase [ubiquinone]	Q47520	62542	5.86	160	2.30E-12	0.44	0.012	0.037	0.00	0.025	0.00	0.00	0.047	1.6	<-5	<-5	1.1	0.0	0.0	<-5	>5	<-5		
Pta	phosphate acetyltransferase	P0A9M8	77466	5.28	72	1.30E-03	0.07	0.13	0.16	0.15	0.11	0.17	0.30	0.14	0.12	0.3	0.2	0.4	-0.2	0.8	-0.3	0.2	-0.5	0.4	
AckA	acetate kinase	P0A6A3	43605	5.85	64	1.00E-02	0.18	0.08	0.08	0.08	0.09	0.21	0.17	0.14	0.11	0.0	0.0	-0.2	0.2	-0.3	0.3	-0.9	1.4		
AdhE	bifunctional acetaldehyde-CoA/alcohol dehydrogenase	P0A9Q8	96580	6.32	192	5.30E-15	0.26	0.33	0.31	0.18	0.29	0.21	0.09	0.15	0.46	-0.1	-0.9	-0.7	-0.2	-1.2	-0.5	-1.6	1.1	-0.7	
PflB	formate acetyltransferase I	P09373	85588	5.69	91	1.70E-05	0.2	0.41	0.32	0.26	0.20	0.32	0.38	0.32	0.55	-0.4	-0.7	0.4	-1.0	0.2	0.0	-0.8	0.8	-0.4	
LldD	L-lactate dehydrogenase	A7ZTF9	42902	6.33	107	1.10E-05	0.22	0.05	0.05	0.039	0.05	0.049	0.029	0.042	0.031	0.0	-0.4	-0.4	0.0	-0.8	-0.2	0.4	-0.7	0.0	
Dld	D-lactate dehydrogenase	Q8FFW1	66546	6.39	106	2.10E-06	0.15	0.047	0.044	0.031	0.039	0.031	0.016	0.029	0.05	-0.1	-0.6	-0.3	-0.3	-1.0	-0.1	-0.8	0.7	-0.6	
LdhA	D-lactate dehydrogenase	P52643	36854	5.29	115	7.20E-08	0.12	0.032	0.018	0.043	0.042	0.017	0.036	0.017	0.033	-0.8	0.4	0.0	0.4	1.1	0.0	-1.0	1.0	-0.9	
<b>TCA cycle (15 proteins)</b>																									-0.5
GltA	citrate synthase	P0ABH7	48383	6.21	118	3.60E-08	0.42	0.69	0.65	0.43	0.55	0.29	0.37	0.61	1.36	-0.1	-0.7	-0.4	-0.3	0.4	1.1	-1.2	2.2	-1.3	
AcnA	aconitate hydratase	P25516	98015	5.59	75	6.80E-04	0.17	0.12	0.10	0.16	0.17	0.21	0.26	0.20	0.41	-0.6	0.1	-0.1	0.2	0.3	-0.1	-1.0	1.0	0.5	
AcnB	aconitate hydratase 2	B1LGR9	91078	5.22	213	4.20E-17	0.46	0.86	0.76	0.55	0.59	0.61	0.93	0.85	1.21	-0.2	-0.6	-0.1	-0.5	0.6	0.5	-0.5	1.0	-0.5	
Icd	isocitrate dehydrogenase	P08200	46070	5.15	151	1.80E-11	0.4	0.94	0.85	0.82	1.02	0.59	0.79	0.79	1.35	0.0	-0.2	-0.3	0.1	0.4	0.4	-0.8	1.2	-0.7	
SucA	2-oxoglutarate dehydrogenase E1 component	P0AFG5	105566	6.04	99	1.00E-05	0.14	0.47	0.41	0.37	0.37	0.45	0.37	0.54	0.56	-0.2	-1.1	-0.3	-0.8	-0.3	0.3	-0.1	0.3	-0.1	
SucB	dihydrolipoamide succinyltransferase	A7ZXY7	44014	5.58	127	1.70E-08	0.38	0.48	0.37	0.37	0.37	0.43	0.57	0.46	0.54	-0.4	-0.4	0.0	-0.3	0.4	0.1	-0.2	0.3	-0.2	
SucC	succinyl-CoA synthetase subunit beta	P0A838	41652	5.37	256	2.10E-21	0.55	0.44	0.43	0.30	0.41	0.28	0.39	0.59	0.0	-0.6	-0.6	0.0	0.7	0.5	-0.6	1.1	-0.7		
SucD	succinyl-CoA ligase [ADP-forming] subunit alpha	P0AGE9	30044	6.32	72	1.50E-03	0.29	0.39	0.30	0.22	0.27	0.25	0.33	0.66	-0.4	-0.8	-0.3	-0.5	0.1	0.2	-1.0	1.2	-0.5		
SdhA	succinate dehydrogenase flavoprotein subunit	P0AC41	65008	5.85	59	2.70E-02	0.13	0.40	0.33	0.31	0.39	0.18	0.53	0.46	-0.3	-0.4	-0.3	0.0	1.0	0.9	-0.5	1.4	-1.2		
SdhB	succinate dehydrogenase and fumarate reductase iron-sulfur protein	Q8X9A8	27335	6.81	102	5.30E-06	0.34	0.16	0.12	0.09	0.11	0.07	0.05	0.11	-0.4	-0.8	-0.3	-0.5	-0.2	-0.2	-0.9	0.7	-1.2		
FumA	fumarate hydratase (fumarate A), aerobic Class I	P0AC33	60774	6.11	116	2.10E-07	0.26	0.029	0.030	0.021	0.032	0.025	0.035	0.044	0.08	0.0	-0.5	-0.6	0.1	0.5	0.8	-0.9	1.7	-0.2	
FumC	fumarate hydratase (fumarate C), aerobic Class II	P05042	50856	6.12	127	1.70E-08	0.29	0.16	0.11	0.06	0.10	0.08	0.08	0.06	0.27	-0.5	-1.4	-0.7	-0.7	0.0	-0.4	-2.2	1.8	-1.0	
Mdh	malate dehydrogenase	P61891	32488	5.61	79	1.00E-03	0.5	0.32	0.28	0.14	0.35	0.07	0.18	0.15	0.74	-0.2	-1.2	-1.3	0.1	1.4	1.1	-2.3	3.4	-2.2	
LpdA <sup>4</sup>	dihydrolipoamide dehydrogenase	P0A9P2	50942	5.79	155	2.60E-11	0.43	0.64	0.63	0.57	0.62	0.83	0.89	0.80	0.93	0.0	-0.2	-0.1	0.0	0.1	-0.1	-0.2	0.2	0.4	
GlcB	malate synthase G	P37330	80780	5.79	222	1.40E-18	0.37	0.015	0.022	0.041	0.034	0.039	0.05	0.024	0.07	0.6	1.5	0.3	1.2	0.4	-0.7	-1.5	0.8	1.4	
<b>Anaplerotic reactions (4 proteins)</b>																									-0.9
Ppc	phosphoenolpyruvate carboxylase	C5A0C2	99456	5.52	66	5.20E-03	0.07	0.46	0.42	0.36	0.42	0.24	0.23	0.20	0.20	-0.1	-0.4	-0.2	-0.1	-0.1	-0.3	0.0	-0.3	-0.9	
PckA	phosphoenolpyruvate carboxykinase [ATP]	C4ZUQ8	59891	5.46	219	2.90E-18	0.45	0.09	0.07	0.16	0.18	0.046	0.08	0.08	0.43	-0.4	0.8	-0.2	1.0	0.8	0.8	-2.4	3.2	-1.0	
MacB	malate dehydrogenase (oxaloacetate-decarboxylating) (NADP(+)), phosphate acetyltransferase	A8A2V3	82908	5.34	185	2.70E-14	0.46	0.09	0.08	0.048	0.06	0.040	0.08	0.07	0.17	-0.2	-0.9	-0.3	-0.6	1.0	0.8	-1.3	2.1	-1.2	
SfcA	NAD-dependent malic enzyme	A7ZLS1	63481	5.19	88	3.20E-05	0.24	0.034	0.041	0.033	0.07	0.033	0.035	0.037	0.026	0.3	0.0	-1.1	1.0	0.1	0.2	0.5	-0.3	0.0	
<b>Oxidative phosphorylation (7 proteins)</b>																									0.0
AtpA	ATP synthase subunit alpha	A7ZTU6	55416	5.8	60	2.60E-02	0.26	1.13	1.12	0.91	1.38	1.06	1.19	0.90	1.06	0.0	-0.3	-0.6	0.3	0.2	-0.2	-0.2	0.0	-0.1	

**Table S1 - Quantitative data of individual proteins of *E. coli* BL21 (DE3) growing in defined and complex medium during production of hFGF-2**

Name <sup>1</sup>	Protein name <sup>1</sup>	Uniprot ID <sup>1</sup>	Molecular mass <sup>2</sup> (Da)	Calculated pI value <sup>3</sup>	Mascot Score <sup>2</sup>	Mascot Expect Value <sup>2</sup>	Mascot Seq. Cover. <sup>2</sup>	Defined (DNB) medium				Complex (LB) medium				Log <sub>2</sub> ratio in Defined (DNB) medium				Log <sub>2</sub> ratio in Complex (LB) medium				Log <sub>2</sub> (LB medium/ DNB medium) exponential before IPTG	
								exponential phase before IPTG induction	1h after IPTG induction	5h after IPTG induction	stationary phase without IPTG	exponential phase before IPTG induction	1h after IPTG induction	3.5h after IPTG induction	stationary phase without IPTG	(1h after IPTG/ exponential before IPTG)	(5h after IPTG/ stationary without IPTG)	(stationary phase before IPTG)	(1h after IPTG/ exponential before IPTG)	(3.5h after IPTG/ stationary without IPTG)	(stationary phase before IPTG)	(1h after IPTG/ exponential before IPTG)	(3.5h after IPTG/ stationary without IPTG)	(stationary phase before IPTG)	
								Relative Protein Mass (RPM) <sup>3</sup> - %																	
AtpD	F0F1 ATP synthase subunit beta	P0ABB6	50351	4.9	113	4.20E-07	0.48	0.59	0.60	0.48	0.67	0.80	0.94	0.78	0.70	0.0	-0.3	-0.5	0.2	0.2	0.0	0.2	-0.2	0.4	
AtpH	F0F1 ATP synthase subunit delta	P0ABA5	19434	4.94	81	6.80E-04	0.41	0.07	0.07	0.06	0.08	0.09	0.09	0.06	0.06	0.0	-0.2	-0.4	0.2	0.0	-0.6	0.0	-0.6	0.4	
NuoF	NADH dehydrogenase I subunit F	Q8XCX1	49802	6.44	117	1.70E-07	0.33	0.14	0.11	0.12	0.11	0.14	0.08	0.08	0.14	-0.3	-0.2	0.1	-0.3	-0.8	-0.8	-0.8	0.0	0.0	
NuoG	NADH dehydrogenase subunit G	A8A2F2	101129	5.83	200	8.40E-16	0.35	0.24	0.16	0.13	0.17	0.21	0.12	0.19	0.21	-0.6	-0.9	-0.4	-0.5	-0.8	-0.1	-0.1	0.0	-0.2	
NuoC	bifunctional NADH:ubiquinone oxidoreductase subunit C/D	Q0TFG0	68451	5.98	64	3.10E-02	0.16	0.18	0.18	0.13	0.14	0.16	0.12	0.14	0.15	0.0	-0.5	-0.1	-0.4	-0.4	-0.2	-0.1	-0.1	-0.2	
NuoB	NADH-quinone oxidoreductase subunit B	C4ZUD0	25325	5.58	74	8.70E-04	0.36	0.10	0.07	0.07	0.09	0.07	0.07	0.07	0.07	-0.5	-0.5	-0.4	-0.2	0.0	0.0	0.0	0.0	-0.5	
<b>Amino acid biosynthesis and metabolism (68 proteins): Amino acid biosynthesis and amino acid degradation</b>								Total RPM:	13.39	12.33	11.08	12.95	5.51	5.81	5.92	10.84	-0.1	-0.3	-0.2	0.0	0.1	0.1	-0.9	1.0	-1.3
<b>Sub-group: Amino acid biosynthesis (57 proteins)</b>								Total RPM:	12.62	11.63	10.36	12.07	4.72	4.79	4.68	7.10	-0.1	-0.3	-0.2	-0.1	0.0	0.0	-0.6	0.6	-1.4
AroD	3-dehydroquinate dehydratase	A8A0N9	27459	5.3	68	3.80E-03	0.44	0.14	0.15	0.15	0.16	0.10	0.14	0.09	0.11	0.1	0.1	-0.1	0.2	0.5	-0.2	-0.3	0.1	-0.5	
AroB	3-dehydroquinate synthase	C4ZUP4	39141	5.72	91	1.70E-05	0.37	0.024	0.036	0.034	0.019	0.014	0.00	0.029	0.030	0.6	0.5	0.8	-0.3	<5	1.1	0.0	1.1	-0.8	
AroF	phospho-2-dehydro-3-deoxyheptonate aldolase	Q3YYP3	39079	5.42	131	6.70E-09	0.35	0.16	0.19	0.13	0.18	0.07	0.09	0.10	0.38	0.2	-0.3	-0.5	0.2	0.4	0.5	-1.9	2.4	-1.2	
AroG	phospho-2-dehydro-3-deoxyheptonate aldolase, Phe-sensitive	P0AB91	38385	6.14	131	1.80E-09	0.53	0.22	0.22	0.18	0.19	0.07	0.06	0.08	0.048	0.1	-0.2	-0.1	-0.1	-0.2	0.2	0.7	-0.5	-1.6	
TrpD	bifunctional glutamine amidotransferase/anthranilate phosphoribosyltransferase	B3HP12	57176	6.26	157	1.70E-11	0.37	0.09	0.08	0.047	0.08	0.00	0.00	0.00	0.022	-0.2	-0.9	-0.8	-0.2	0.0	0.0	<5	>5	<5	
TrpE	component I of anthranilate synthase	P00895	58142	5.32	138	1.30E-09	0.3	0.16	0.12	0.10	0.10	0.00	0.00	0.00	0.00	-0.4	-0.7	0.0	-0.7	0.0	0.0	0.0	0.0	<5	
TrpC	anthranilate isomerase	C5W377	49774	5.43	191	6.70E-15	0.56	0.10	0.04	0.07	0.12	0.09	0.12	0.10	0.13	0.4	-0.5	-0.8	0.3	0.4	0.2	-0.4	0.5	-0.2	
TrpA	tryptophan synthase alpha chain	B7L492	28905	5.31	107	4.60E-07	0.38	0.030	0.035	0.030	0.06	0.05	0.042	0.027	0.033	0.027	0.2	1.2	0.6	0.6	0.0	>5	-0.9	>5	<5
PheA	P-protein	P0A9J8	43312	6.21	154	9.10E-12	0.4	0.06	0.06	0.06	0.06	0.05	0.042	0.027	0.033	0.0	0.0	0.3	-0.3	-0.6	-0.3	0.3	-0.6	-0.5	
TyrA	T-protein	P07023	42187	5.68	165	7.20E-13	0.47	0.12	0.10	0.08	0.1	0.02	0.02	0.039	0.043	0.036	-0.3	-0.6	-0.5	-0.1	1.0	1.1	0.3	0.8	-2.6
AspC	aspartate aminotransferase, PLP-dependent	P00509	43831	5.54	151	6.70E-11	0.46	0.19	0.22	0.15	0.30	0.30	0.34	0.14	0.19	0.2	-0.3	-1.0	0.7	0.0	-0.2	-0.7	0.4	-0.4	
SerA	D-3-phosphoglycerate dehydrogenase	P0A9T2	44376	5.92	100	8.30E-06	0.32	0.58	0.59	0.54	0.69	0.23	0.22	0.27	0.64	0.0	-0.1	-0.4	0.3	-0.1	0.2	-1.2	1.5	-1.3	
SerC	phosphoserine aminotransferase	Q8FBJ7	39974	5.36	169	1.10E-10	0.41	0.31	0.23	0.18	0.27	0.10	0.14	0.11	0.15	-0.4	-0.8	-0.6	-0.2	0.5	0.1	-0.4	0.6	-1.6	
GdhA	glutamate dehydrogenase, NADP-specific	P00370	48778	5.98	179	1.10E-13	0.52	0.09	0.12	0.17	0.14	0.030	0.045	0.05	0.06	0.4	0.9	0.3	0.6	0.6	0.7	-0.3	1.0	-1.6	
GlnA	glutamine synthetase	P0A9C7	52099	5.26	70	2.10E-03	0.15	0.41	0.35	0.25	0.31	0.19	0.13	0.15	0.16	-0.2	-0.7	-0.3	-0.4	-0.5	-0.3	-0.1	-0.2	-1.1	
GltB	glutamate synthase [NADPH] large chain	P09831	164335	6.15	343	1.10E-30	0.38	0.15	0.12	0.17	0.16	0.037	0.00	0.00	0.00	-0.3	0.2	0.1	0.1	<5	<5	0.0	<5	-2.0	
HisG	ATP phosphoribosyltransferase	Q8X8T4	33615	5.59	104	9.10E-07	0.38	0.10	0.05	0.06	0.09	0.07	0.06	0.07	0.06	-1.0	-0.7	-0.6	-0.2	-0.2	0.0	0.2	0.2	-0.5	
HisF	imidazole-glycerol phosphate synthase subunit hisF	C4ZSB4	28722	5.03	80	2.50E-04	0.38	0.09	0.07	0.06	0.08	0.08	0.09	0.05	0.10	-0.4	-0.6	-0.4	-0.2	0.2	-0.7	-1.0	0.3	-0.2	
HisC	histidinol-phosphate aminotransferase	B7NQG9	39907	5	144	9.10E-11	0.51	0.06	0.035	0.07	0.05	0.038	0.048	0.025	0.11	-0.8	0.2	0.5	-0.3	0.3	-0.6	-2.1	1.5	-0.7	
HisD	histidinol dehydrogenase	A1ACN2	46939	5.13	125	2.70E-08	0.34	0.022	0.031	0.016	0.046	0.030	0.019	0.024	0.032	0.5	-0.5	-1.5	1.1	-0.7	-0.3	-0.4	0.1	0.4	
GlyA	serine hydroxymethyltransferase	C4ZXC6	45459	6.03	103	1.10E-06	0.31	0.71	0.68	0.59	0.75	0.32	0.31	0.28	0.67	-0.1	-0.3	-0.3	0.1	0.0	-0.2	-1.3	1.1	-1.1	
CysK	cysteine synthase A	P0ABK5	34525	5.83	176	5.70E-14	0.6	0.41	0.35	0.28	0.49	0.11	0.10	0.13	0.40	-0.2	-0.6	-0.8	0.3	-0.1	0.2	-1.6	1.9	-1.9	
CysN	sulfate adenylyltransferase, subunit 1	P23845	52640	4.98	56	5.60E-02	0.19	0.12	0.12	0.09	0.12	0.020	0.035	0.013	0.019	0.0	-0.4	-0.4	0.0	0.8	-0.6	-0.5	-0.1	-2.6	
CysJ	sulfite reductase subunit alpha	A8A3P5	66415	4.92	161	6.60E-12	0.33	0.07	0.08	0.10	0.07	0.024	0.05	0.041	0.020	0.2	0.5	0.5	0.0	1.1	0.8	1.0	-0.3	-1.5	
CysL	sulfite reductase subunit beta	Q8X7U2	64262	7.27	79	9.90E-04	0.21	0.05	0.049	0.031	0.037	0.00	0.00	0.00	0.00	0.0	-0.7	-0.3	-0.4	0.0	0.0	0.0	0.0	<5	
LysC	aspartokinase III	P08660	48787	5.03	237	1.70E-19	0.62	0.034	0.06	0.039	0.06	0.00	0.00	0.00	0.00	0.8	0.2	-0.6	0.8	0.0	0.0	0.0	0.0	<5	

**Table S1 - Quantitative data of individual proteins of *E. coli* BL21 (DE3) growing in defined and complex medium during production of hFGF-2**

Name <sup>1</sup>	Protein name <sup>1</sup>	Uniprot ID <sup>1</sup>	Molecular mass <sup>2</sup> (Da)	Calculated pI value <sup>3</sup>	Mascot Score <sup>2</sup>	Mascot Expect Value <sup>2</sup>	Mascot Seq. Cover. <sup>2</sup>	Defined (DNB) medium				Complex (LB) medium				Log <sub>2</sub> ratio in Defined (DNB) medium				Log <sub>2</sub> ratio in Complex (LB) medium				Log <sub>2</sub> (LB medium/ DNB medium) exponential before IPTG	
								exponential phase before IPTG induction	1h after IPTG induction	5h after IPTG induction	stationary phase without IPTG	exponential phase before IPTG induction	1h after IPTG induction	3.5h after IPTG induction	stationary phase without IPTG	(1h after IPTG/ exponential before IPTG)	(5h after IPTG/ stationary without IPTG)	(stationary phase without IPTG)	(1h after IPTG/ exponential before IPTG)	(3.5h after IPTG/ stationary without IPTG)	(5h after IPTG/ stationary without IPTG)	(stationary phase without IPTG)			
								Relative Protein Mass (RPM) <sup>3</sup> - %																	
Asd	aspartate-semialdehyde dehydrogenase	P0A9Q9	40221	5.37	128	3.60E-09	0.53	0.25	0.27	0.21	0.29	0.13	0.13	0.11	0.26	0.1	-0.3	-0.5	0.2	0.0	-0.2	-1.2	1.0	-0.9	
DapA	dihydronicolinate synthase	A7ZPS4	31549	5.98	119	2.90E-08	0.42	0.10	0.09	0.06	0.08	0.08	0.08	0.07	0.10	-0.2	-0.7	-0.4	-0.3	0.0	-0.2	-0.5	0.3	-0.3	
DapD	2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase	P0A9D8	30045	5.56	169	1.00E-12	0.63	0.18	0.16	0.13	0.25	0.13	0.13	0.09	0.10	-0.2	-0.5	-0.9	0.5	0.0	-0.5	-0.2	-0.4	-0.5	
LysA	diaminopimelate decarboxylase	P00861	46377	5.63	106	1.30E-05	0.45	0.06	0.05	0.06	0.05	0.10	0.09	0.08	0.13	-0.3	0.0	0.3	-0.3	-0.2	-0.3	-0.7	0.4	0.7	
ThrB	homoserine kinase	A7ZH92	34101	5.44	90	2.30E-05	0.34	0.039	0.045	0.039	0.05	0.00	0.00	0.00	0.037	0.2	0.0	-0.4	0.4	0.0	0.0	<5	>5	<5	
MetA	homoserine O-succinyltransferase	CSA0V0	35819	5.06	106	5.70E-07	0.35	0.036	0.034	0.042	0.040	0.00	0.00	0.035	0.025	-0.1	0.2	0.1	0.2	0.0	>5	0.5	>5	<5	
MetH	methionine synthase	B3XC96	136639	4.97	291	6.70E-25	0.38	0.12	0.09	0.07	0.08	0.06	0.06	0.05	0.038	-0.4	-0.8	-0.2	-0.6	0.0	-0.3	0.4	-0.7	-1.0	
MetE	5-methyltetrahydropteroylglutamate-homocysteine S-methyltransferase	B3XGD6	85074	5.61	107	1.70E-06	0.31	3.00	2.58	2.71	2.38	0.09	0.07	0.12	0.24	-0.2	-0.1	0.2	0.1	-0.4	0.4	-1.0	1.4	-5.1	
IlvA	threonine dehydratase biosynthetic	P04968	56559	5.57	213	2.70E-16	0.48	0.15	0.18	0.13	0.16	0.08	0.09	0.07	0.16	0.3	-0.2	-0.3	0.1	0.2	-0.2	-1.2	1.0	-0.9	
IlvB	acetolactate synthase I, large subunit	P08142	60915	5.3	85	2.40E-04	0.37	0.05	0.05	0.030	0.045	0.00	0.00	0.05	0.018	0.0	-0.7	-0.6	-0.2	0.0	>5	1.5	>5	<5	
IlvC	ketol-acid reductoisomerase	C4ZZ44	54376	5.2	93	1.10E-05	0.38	1.01	0.91	0.78	1.06	0.06	0.07	0.07	0.13	-0.2	-0.4	-0.4	0.1	0.2	0.2	-0.9	1.1	-4.1	
IlvD	dihydroxyacid dehydratase	P05791	66174	5.59	235	2.60E-19	0.41	0.31	0.26	0.20	0.25	0.07	0.06	0.048	0.07	-0.3	-0.6	-0.3	-0.3	-0.2	-0.5	-0.5	0.0	-2.1	
IlvE	branched-chain-amino-acid aminotransferase	P0AB80	34112	5.54	108	1.30E-06	0.44	0.33	0.33	0.24	0.33	0.048	0.07	0.07	0.23	0.0	-0.5	-0.5	0.0	0.5	0.5	-1.7	2.3	-2.8	
IlvI	acetolactate synthase isozyme 3 large subunit	P00893	63286	5.88	81	4.30E-03	0.25	0.16	0.16	0.12	0.15	0.00	0.07	0.00	0.00	0.2	-0.6	-0.3	-0.3	-0.7	0.2	-0.2	-0.2	0.4	-1.6
LeuA	2-isopropylmalate synthase	A7ZH6	57590	5.47	156	5.80E-12	0.46	0.18	0.21	0.12	0.15	0.00	0.07	0.00	0.00	0.1	0.3	-0.1	0.4	0.9	-0.5	-1.1	0.5	-0.5	
LeuB	3-isopropylmalate dehydrogenase	P30125	39834	5.14	72	5.60E-03	0.27	0.10	0.11	0.12	0.13	0.07	0.13	0.048	0.10	0.1	0.3	-0.1	0.4	0.9	-0.5	-1.1	0.5	-0.5	
LeuC	isopropylmalate isomerase large subunit	A7ZW23	50377	5.9	162	5.30E-12	0.42	0.12	0.13	0.10	0.13	0.00	0.00	0.015	0.018	0.1	-0.6	-1.0	0.4	0.0	>5	-0.3	>5	<5	
LeuD	3-isopropylmalate isomerase subunit	P30126	22587	5.16	128	1.30E-08	0.56	0.06	0.08	0.06	0.06	0.00	0.00	0.00	0.00	0.4	0.0	-0.7	0.7	0.0	0.0	0.0	0.0	<5	
AsnA	aspartate-ammonia ligase	B1LL71	36770	5.55	89	1.10E-04	0.33	0.09	0.08	0.08	0.08	0.03	0.03	0.02	0.11	-0.2	-0.2	0.0	-0.2	-0.8	-0.2	-1.8	1.6	-1.3	
AsnB	asparagine synthase	P22106	63075	5.55	70	2.00E-03	0.27	0.10	0.10	0.13	0.12	0.05	0.05	0.048	0.08	0.0	0.4	0.1	0.3	-1.0	0.1	-0.7	0.8	-1.2	
CarA	carbamoyl phosphate synthase small subunit	B7NHD6	41677	5.91	107	1.70E-06	0.39	0.15	0.15	0.11	0.14	0.15	0.15	0.13	0.13	0.0	-0.4	-0.3	-0.1	-0.1	0.0	0.2	-0.2	0.0	
CarB	carbamoyl phosphate synthase large subunit	B7MNN9	118633	5.22	103	4.20E-06	0.14	0.38	0.26	0.22	0.20	0.42	0.2	0.33	0.28	-0.5	-0.8	0.1	-0.9	-0.6	-0.3	0.2	-0.6	0.1	
ArgC	N-acetyl-gamma-glutamyl-phosphate reductase	B1LNR8	36328	5.58	105	2.70E-06	0.33	0.06	0.043	0.034	0.05	0.07	0.06	0.042	0.047	-0.5	-0.8	-0.6	-0.3	-0.2	-0.7	-0.2	-0.6	0.2	
ArgD	acetylornithine/succinylaminopimelate aminotransferase	P18335	44081	5.79	141	6.60E-10	0.6	0.25	0.18	0.16	0.19	0.09	0.14	0.12	0.10	-0.5	-0.6	-0.2	-0.4	0.6	0.4	0.3	0.2	-1.5	
ArgE	acetylornithine deacetylase	CSA0C3	42777	5.54	99	3.00E-06	0.37	0.043	0.039	0.040	0.047	0.045	0.046	0.035	0.048	-0.1	-0.1	-0.2	0.1	0.0	-0.4	-0.5	0.1	0.1	
ArgI	ornithine carbamoyltransferase chain I	P04391	37112	5.46	93	1.20E-05	0.35	0.041	0.031	0.05	0.037	0.06	0.06	0.038	0.049	-0.4	0.3	0.4	-0.1	0.0	-0.7	-0.4	-0.3	0.5	
ArgG	argininosuccinate synthase	B1XGY3	50038	5.23	143	2.60E-09	0.35	0.20	0.21	0.19	0.25	0.22	0.23	0.25	0.18	0.1	-0.1	-0.4	0.3	0.1	0.2	0.5	-0.3	0.1	
ArgH	argininosuccinate lyase	P11447	50686	5.11	128	1.30E-08	0.38	0.16	0.17	0.15	0.18	0.25	0.27	0.21	0.15	0.1	-0.1	-0.3	0.2	0.1	-0.3	0.5	-0.7	0.6	
AspA	aspartate ammonia-lyase	P0AC38	52950	5.19	135	7.20E-10	0.29	0.12	0.08	0.06	0.17	0.14	0.30	0.24	0.50	-0.6	-1.0	-1.5	0.5	1.1	0.8	-1.1	1.8	0.2	
ProA	gamma-glutamyl phosphate reductase	B3XF57	45031	5.42	205	2.70E-16	0.52	0.06	0.06	0.037	0.05	0.039	0.039	0.032	0.043	0.0	-0.7	-0.4	-0.3	0.0	-0.3	-0.4	0.1	-0.6	
IscS (Yfho)	cysteine desulfurase	C4ZXAS	45232	5.94	127	4.60E-09	0.57	0.32	0.32	0.26	0.18	0.39	0.27	0.38	0.28	0.0	-0.3	0.5	-0.8	-0.5	0.0	0.4	-0.5	0.3	
<b>Sub-group: Amino acid degradation (11 proteins)</b>								Total RPM:	0.78	0.70	0.72	0.88	0.79	1.02	1.24	3.74	-0.2	-0.1	-0.3	0.2	0.4	0.7	-1.6	2.2	0.0
DadX	alanine racemase, catabolic	P29012	39048	6.56	60	2.60E-02	0.31	0.038	0.035	0.026	0.037	0.039	0.028	0.036	0.08	-0.1	-0.5	-0.5	0.0	-0.5	-0.1	-1.2	1.0	0.0	
TnaA	tryptophanase	B1LL35	53107	5.88	97	4.80E-06	0.2	0.06	0.05	0.06	0.05	0.16	0.34	0.57	2.59	-0.3	0.0	0.3	-0.3	1.1	1.8	-2.2	4.0	1.4	
MetF	5,10-methylenetetrahydrofolate reductase	P0AEZ1	33253	6	165	7.30E-13	0.52	0.17	0.13	0.09	0.11	0.00	0.00	0.00	0.00	-0.4	-0.9	-0.3	-0.6	0.0	0.0	0.0	0.0	<5	

**Table S1 - Quantitative data of individual proteins of *E. coli* BL21 (DE3) growing in defined and complex medium during production of hFGF-2**

Name <sup>1</sup>	Protein name <sup>1</sup>	Uniprot ID <sup>1</sup>	Molecular mass <sup>2</sup> (Da)	Calculated pI value <sup>3</sup>	Mascot Score <sup>2</sup>	Mascot Expect Value <sup>2</sup>	Mascot Seq. Cover. <sup>2</sup>	Defined (DNB) medium				Complex (LB) medium				Log <sub>2</sub> ratio in Defined (DNB) medium				Log <sub>2</sub> ratio in Complex (LB) medium				Log <sub>2</sub> (LB medium/ DNB medium) exponential before IPTG	
								exponential phase before IPTG induction	1h after IPTG induction	5h after IPTG induction	stationary phase without IPTG	exponential phase before IPTG induction	1h after IPTG induction	3.5h after IPTG induction	stationary phase without IPTG	(1h after IPTG/exponential before IPTG)	(5h after IPTG/stationary without IPTG)	(stationary exponential before IPTG)	(1h after IPTG/exponential before IPTG)	(3.5h after IPTG/stationary without IPTG)	(stationary exponential before IPTG)	(1h after IPTG/exponential before IPTG)	(3.5h after IPTG/stationary without IPTG)	(stationary exponential before IPTG)	
								Relative Protein Mass (RPM) <sup>3</sup> - %																	
MetK	chain A, S-adenosylmethionine synthetase	P0A817	42022	5.1	155	2.60E-11	0.61	0.27	0.21	0.24	0.32	0.19	0.18	0.17	0.11	-0.4	-0.2	-0.4	0.2	-0.1	-0.2	0.6	-0.8	-0.5	-0.5
GcvT	glycine cleavage system T protein	B2ND95	40251	5.36	88	1.30E-04	0.34	0.036	0.07	0.00	0.09	0.00	0.00	0.00	0.00	1.0	<-5	<-5	1.3	0.0	0.0	0.0	0.0	<-5	-0.5
AstA	arginine N-succinyltransferase	C4ZZA3	38831	6.02	172	1.40E-13	0.58	0.030	0.031	0.031	0.045	0.015	0.00	0.032	0.10	0.0	0.0	0.0	-0.5	<5	1.1	-1.6	2.7	-1.0	
AstB	N-succinylarginine dihydrolase	C4ZZA1	49439	5.74	116	5.70E-08	0.41	0.033	0.017	0.035	0.036	0.026	0.038	0.027	0.040	-1.0	0.1	0.0	0.1	0.5	0.1	-0.6	0.6	-0.3	
AstD	N-succinylglutamate 5-semialdehyde dehydrogenase	C4ZZA2	53278	5.69	85	6.80E-05	0.29	0.00	0.00	0.031	0.05	0.022	0.08	0.06	0.15	0.0	>5	-0.7	>5	1.9	1.4	-1.3	2.8	>5	
Tdh	L-threonine 3-dehydrogenase	C4ZXK8	37557	5.94	126	5.70E-09	0.56	0.021	0.031	0.037	0.029	0.08	0.05	0.048	0.12	0.6	0.8	0.4	0.5	-0.7	-0.7	-1.3	0.6	1.9	
Kbl	2-amino-3-ketobutyrate coenzyme A ligase	P0AB77	43432	5.64	127	4.60E-09	0.47	0.11	0.09	0.14	0.09	0.18	0.17	0.16	0.37	-0.3	0.3	0.6	-0.3	-0.1	-0.2	-1.2	1.0	0.7	
PutA	bifunctional protein PutA	P09546	144467	5.69	148	3.60E-11	0.13	0.007	0.035	0.033	0.026	0.08	0.13	0.14	0.18	2.3	2.2	0.3	1.9	0.7	0.8	-0.4	1.2	3.5	
<b>IMP biosynthesis (for nucleotide) (7 proteins)</b>								Total RPM:	1.27	1.22	1.16	1.40	0.59	0.59	0.60	0.99	-0.1	-0.1	-0.3	0.1	0.0	0.0	-0.7	0.7	-1.1
PurF	amidophosphoribosyltransferase	P0AG16	56852	5.33	82	4.70E-04	0.22	0.07	0.09	0.08	0.039	0.019	0.029	0.017	0.033	0.4	0.2	1.0	-0.8	0.6	-0.2	-1.0	0.8	-1.9	
PurD	phosphoribosylamine-glycine ligase	A7ZUM2	46326	4.89	80	8.90E-04	0.36	0.23	0.21	0.18	0.39	0.14	0.21	0.15	0.18	-0.1	-0.4	-1.1	0.8	0.6	0.1	-0.3	0.4	-0.7	
PurT	phosphoribosylglycinamide formyltransferase 2	C4ZZK6	42692	5.48	178	3.60E-14	0.56	0.09	0.06	0.07	0.08	0.05	0.047	0.05	0.19	-0.6	-0.4	-0.2	-0.2	-0.1	0.0	-1.9	1.9	-0.8	
PurL	phosphoribosylformyl-glycineamide synthetase	B3Y153	142036	5.23	149	1.00E-10	0.24	0.56	0.36	0.36	0.28	0.13	0.08	0.15	0.19	0.0	0.0	0.4	-0.4	-0.7	0.2	-0.3	0.5	-1.5	
PurC	phosphoribosylaminoimidazole-succinocarboxamide synthase	A7ZPS1	27149	5.07	136	5.70E-10	0.51	0.12	0.10	0.10	0.22	0.036	0.05	0.029	0.10	0.2	-0.3	-0.3	-1.1	0.9	0.5	-0.3	-1.8	1.5	-1.7
PurB	adenylosuccinate lyase	Q8X737	51652	5.68	173	4.20E-13	0.45	0.14	0.11	0.12	0.11	0.09	0.09	0.09	0.09	-0.3	-0.3	-0.1	-0.2	-0.3	-0.3	0.0	-0.3	-0.3	
PurH	bifunctional phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase	Q1R5X1	57747	5.53	81	6.40E-04	0.19	0.26	0.25	0.26	0.10	0.08	0.11	0.21	-0.1	0.0	-0.1	0.1	-0.3	0.1	-0.9	1.1	-1.4		
<b>Nucleotide biosynthesis (start from IMP) (13 proteins)</b>								Total RPM:	1.53	1.66	1.49	1.87	1.90	1.83	1.58	1.90	0.1	0.0	-0.3	0.3	-0.1	-0.3	-0.3	0.0	0.3
PurA	adenylosuccinate synthetase	A7ZV47	47543	5.31	172	1.40E-13	0.51	0.41	0.45	0.35	0.48	0.42	0.46	0.38	0.43	0.1	-0.2	-0.5	0.2	0.1	-0.1	-0.2	0.0	0.0	
GuaA	GMP synthetase (glutamine aminotransferase)	P04079	59041	5.24	178	1.30E-13	0.41	0.14	0.15	0.14	0.20	0.14	0.21	0.12	0.12	0.1	0.0	-0.5	0.5	0.1	-0.2	0.0	-0.2	0.0	
GuaB	inositol-5-monophosphate dehydrogenase	P0ADG8	52275	6.02	94	3.70E-05	0.35	0.16	0.19	0.15	0.17	0.22	0.15	0.14	0.13	0.2	-0.1	-0.2	0.1	-0.6	-0.7	0.1	-0.8	0.5	
Add	adenosine deaminase	C4ZY85	36603	5.36	72	1.60E-03	0.4	0.037	0.028	0.07	0.031	0.00	0.00	0.031	0.026	-0.4	0.9	1.2	-0.3	0.0	>5	0.3	>5	<5	
DeoB	phosphopentomutase	C4ZT65	44684	5.11	158	3.60E-12	0.44	0.042	0.030	0.041	0.045	0.046	0.07	0.07	0.10	-0.5	0.0	-0.1	-0.1	0.1	0.6	0.6	-0.5	1.1	0.1
DeoD	chain A, purine nucleoside phosphorylase	P0ABP8	26030	5.42	130	8.30E-09	0.62	0.048	0.09	0.10	0.10	0.21	0.15	0.10	0.12	0.9	1.1	0.0	1.1	-0.5	-1.1	-0.3	-0.8	2.1	
Hpt	hypoxanthine phosphoribosyltransferase	B1LGS6	20315	5.09	76	2.00E-03	0.56	0.15	0.16	0.14	0.16	0.07	0.09	0.09	0.16	0.1	-0.1	-0.2	0.1	0.4	0.4	-0.8	1.2	-1.1	
Apt	adenine phosphoribosyltransferase	P69503	19847	5.26	71	6.50E-03	0.52	0.06	0.07	0.06	0.09	0.10	0.07	0.06	0.049	0.2	0.0	-0.6	0.6	-0.5	-0.7	0.3	-1.0	0.7	
Udp	uridine phosphorylase	Q8X8L3	27304	5.71	81	6.80E-04	0.54	0.07	0.06	0.042	0.09	0.11	0.12	0.07	0.16	-0.2	-0.7	-1.1	0.4	0.1	-0.7	-1.2	0.5	0.7	
PyrG	CTP synthase	A7ZQM3	60792	5.63	74	1.00E-03	0.15	0.19	0.17	0.16	0.21	0.37	0.36	0.28	0.25	-0.2	-0.2	-0.4	0.1	0.0	-0.4	0.2	-0.6	1.0	
Tmk	thymidylate kinase	A7ZKK1	23768	5.33	65	7.60E-03	0.48	0.046	0.06	0.040	0.08	0.07	0.06	0.042	0.06	0.4	-0.2	-1.0	0.8	-0.2	-0.7	-0.5	-0.2	0.6	
Ndk	nucleoside diphosphate kinase	C4ZX93	15511	5.54	120	2.30E-08	0.62	0.15	0.15	0.17	0.15	0.11	0.15	0.13	0.21	0.0	0.2	0.2	0.0	0.4	0.2	-0.7	0.9	-0.4	
Amn	AMP nucleosidase	P0AEI3	54246	5.9	104	3.40E-06	0.35	0.031	0.05	0.024	0.06	0.034	0.00	0.07	0.08	0.7	-0.4	-1.3	1.0	<5	1.0	-0.2	1.2	0.1	
<b>Fatty acid biosynthesis (7 proteins)</b>								Total RPM:	1.14	1.09	1.10	1.18	1.63	1.30	1.08	1.12	-0.1	-0.1	-0.1	0.0	-0.3	-0.6	-0.1	-0.5	0.5
AccA	acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha	A7ZH55	35333	5.76	212	1.40E-17	0.57	0.09	0.08	0.09	0.10	0.14	0.11	0.11	0.08	-0.2	0.0	-0.2	0.2	-0.3	-0.3	0.5	-0.8	0.6	
AccC	acetyl-CoA carboxylase, biotin carboxylase subunit	P24182	49745	6.65	210	8.50E-17	0.43	0.14	0.13	0.10	0.12	0.25	0.13	0.15	0.14	-0.1	-0.5	-0.3	-0.2	-0.9	-0.7	0.1	-0.8	0.8	

**Table S1 - Quantitative data of individual proteins of *E. coli* BL21 (DE3) growing in defined and complex medium during production of hFGF-2**

Name <sup>1</sup>	Protein name <sup>1</sup>	Uniprot ID <sup>1</sup>	Molecular mass <sup>2</sup> (Da)	Calculated pI value <sup>3</sup>	Mascot Score <sup>2</sup>	Mascot Expect Value <sup>2</sup>	Mascot Seq. Cover. <sup>2</sup>	Defined (DNB) medium				Complex (LB) medium				Log <sub>2</sub> ratio in Defined (DNB) medium				Log <sub>2</sub> ratio in Complex (LB) medium				Log <sub>2</sub> (LB medium/ DNB medium) exponential before IPTG		
								exponential phase before IPTG induction	1h after IPTG induction	5h after IPTG induction	stationary phase without IPTG	exponential phase before IPTG induction	1h after IPTG induction	3.5h after IPTG induction	stationary phase without IPTG	(1h after IPTG/exponential before IPTG)	(5h after IPTG/stationary without IPTG)	(stationary exponential before IPTG)	(1h after IPTG/exponential before IPTG)	(3.5h after IPTG/stationary without IPTG)	(stationary exponential before IPTG)	(1h after IPTG/exponential before IPTG)	(3.5h after IPTG/stationary without IPTG)	(stationary exponential before IPTG)		
								Relative Protein Mass (RPM) <sup>3</sup> - %																		
FabH	3-oxoacyl-[acyl-carrier-protein] synthase 3	P0A6R0	33779	5.08	63	1.00E-02	0.19	0.045	0.045	0.047	0.043	0.07	0.07	0.041	0.029	0.0	0.1	0.1	-0.1	0.0	-0.8	0.5	-1.3	0.6		
FabB	3-oxoacyl-[acyl-carrier-protein] synthase I	P0A953	42928	5.35	259	1.10E-21	0.64	0.18	0.25	0.33	0.36	0.36	0.30	0.22	0.33	0.5	0.9	-0.1	1.0	-0.3	-0.7	-0.6	-0.1	1.0		
FabF	3-oxoacyl-[acyl-carrier-protein] synthase 2	P0AAJ5	43247	5.71	86	5.50E-05	0.41	0.28	0.22	0.21	0.20	0.35	0.32	0.26	0.23	-0.3	-0.4	0.1	-0.5	-0.1	-0.4	0.2	-0.6	0.3		
FabI	enoyl-[acyl-carrier-protein] reductase [NADH] FabI	P0AEK4	28074	5.58	117	4.60E-08	0.51	0.28	0.22	0.21	0.26	0.32	0.30	0.22	0.25	-0.3	-0.4	-0.3	-0.1	-0.1	-0.5	-0.2	-0.4	0.2		
FabZ	(3R)-hydroxymyristoyl-[acyl-carrier-protein] dehydratase	A7ZHS0	17136	6.84	71	4.50E-02	0.25	0.12	0.14	0.11	0.10	0.14	0.07	0.08	0.06	0.2	-0.1	0.1	-0.3	-1.0	-0.8	0.4	-1.2	0.2		
<b>Lipopoly saccharide biosynthesis (10 proteins)</b>																										
GmhA	phosphoheptose isomerase	C2DM95	21686	5.97	142	5.30E-10	0.63	0.037	0.040	0.029	0.046	0.023	0.024	0.024	0.06	0.1	-0.4	-0.7	0.3	0.1	0.1	-1.3	1.4	-0.7		
HldE	bifunctional heptose 7-phosphate kinase/heptose 1-phosphate adenyltransferase	Q8FDH5	51232	5.29	122	5.30E-08	0.31	0.039	0.045	0.042	0.06	0.025	0.05	0.05	0.046	0.2	0.1	-0.5	0.6	1.0	1.0	0.1	0.9	-0.6		
HldD	ADP-L-glycero-D-manno-heptose-6-epimerase	C4ZXL1	34985	4.8	83	1.20E-04	0.41	0.31	0.11	0.15	0.18	0.20	0.49	0.26	0.34	-1.5	-1.0	-0.3	-0.8	1.3	0.4	-0.4	0.8	-0.6		
LpxA	acyl-[acyl-carrier-protein]-UDP-N-acetylglucosamine O-acyltransferase	C4ZRS3	28348	6.63	58	3.80E-02	0.32	0.10	0.07	0.10	0.045	0.06	0.032	0.08	0.05	-0.5	0.0	1.2	-1.2	-0.9	0.4	0.7	-0.3	-0.7		
ArnA	fused UDP-L-AraN formyltransferase/UDP-GlcA C-4'-decarboxylase	P77398	74869	6.39	185	2.70E-14	0.39	0.06	0.06	0.039	0.048	0.08	0.043	0.043	0.08	0.0	-0.6	-0.3	-0.3	-0.9	-0.9	-0.9	0.0	0.4		
GlmS	glucosamine-fructose-6-phosphate aminotransferase [isomerizing]	P17169	67081	5.56	220	2.30E-18	0.38	0.11	0.11	0.19	0.12	0.27	0.22	0.22	0.16	-0.3	0.4	0.7	-0.2	-0.3	-0.3	0.2	0.0	-0.2	0.1	0.2
GlmM	phosphoglucomamine mutase	P31120	47799	5.71	140	8.50E-10	0.36	0.042	0.045	0.039	0.047	0.05	0.05	0.045	0.08	-0.3	-0.1	-0.3	0.2	0.0	-0.2	-0.8	0.7	0.3		
GlmU	bifunctional N-acetylglucosamine-1-phosphate uridylyltransferase/glucosamine-1-phosphate acetyltransferase	P0ACC8	49384	6.2	116	2.10E-07	0.3	0.00	0.00	0.00	0.00	0.06	0.05	0.049	0.047	0.0	0.0	0.0	0.0	-0.3	-0.3	0.1	-0.4	>5		
RmlA	glucose-1-phosphate thymidyltransferase	P55253	32703	5.27	114	9.10E-08	0.48	0.049	0.047	0.05	0.049	0.05	0.033	0.038	0.040	-0.2	0.0	0.0	0.0	-0.6	-0.4	-0.1	-0.3	0.0		
RfbB	dTDP-glucose 4,6-dehydratase	P55293	40787	5.09	122	1.40E-08	0.44	0.032	0.039	0.039	0.039	0.09	0.09	0.08	0.09	0.3	1.1	-0.2	1.3	0.0	-0.2	-0.2	0.0	1.5		
<b>Synthesis of other cellular components (16 proteins)</b>																										
HemB	delta-aminolevulinic acid dehydratase	B2NAC8	35962	5.25	95	2.40E-05	0.31	0.11	0.12	0.09	0.19	0.0	0.06	0.06	0.15	0.1	-0.3	-1.1	0.8	0.3	0.3	-1.3	1.6	-1.1		
HemL	glutamate-1-semialdehyde 2,1-aminomutase	C4ZRP7	45907	4.73	127	4.60E-09	0.36	0.12	0.09	0.08	0.09	0.08	0.11	0.08	0.09	-0.4	-0.6	-0.2	-0.4	0.5	0.2	0.0	0.2	-0.6		
IspG	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase	A7ZPV8	40943	5.87	146	5.70E-11	0.44	0.06	0.06	0.07	0.05	0.09	0.03	0.06	0.047	0.0	0.2	0.5	-0.3	-0.2	-0.6	0.4	-0.9	0.6		
IspE	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase	Q8FI04	31182	5.13	69	2.90E-03	0.33	0.042	0.06	0.035	0.06	0.031	0.07	0.019	0.05	0.5	-0.3	-0.8	0.5	1.2	-0.7	-1.4	0.7	-0.4		
Mpl	UDP-N-acetylmuramate:L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase	P37773	50298	5.53	64	8.30E-03	0.28	0.035	0.034	0.032	0.05	0.043	0.06	0.07	0.15	0.0	-0.1	-0.6	0.5	0.5	0.7	-1.1	1.8	0.3		
GshB	glutathione synthetase	P04425	35766	5.11	154	9.10E-12	0.47	0.046	0.045	0.034	0.05	0.05	0.048	0.037	0.05	0.0	-0.4	-0.6	0.1	-0.1	-0.4	-0.4	0.0	0.1		
MdoG	glucan biosynthesis protein, periplasmic	P33136	57876	6.7	89	1.00E-04	0.22	0.045	0.048	0.032	0.043	0.06	0.035	0.05	0.041	0.1	-0.5	-0.4	-0.1	-0.8	-0.3	0.3	-0.5	0.4		
SpeA	biosynthetic arginine decarboxylase	P21170	74308	4.83	76	5.50E-04	0.16	0.08	0.09	0.07	0.11	0.15	0.17	0.14	0.08	0.2	-0.2	-0.7	0.5	0.2	-0.1	0.8	-0.9	0.9		
SpeE	spermidine synthase	B1XC96	32643	5.33	87	4.30E-05	0.38	0.044	0.047	0.043	0.05	0.031	0.00	0.00	0.042	0.1	0.0	-0.2	0.2	<-5	<-5	<-5	0.4	-0.5		
LuxS	S-ribosylhomocysteine lyase	C4ZYT7	19575	5.18	77	4.40E-04	0.54	0.035	0.043	0.00	0.06	0.00	0.00	0.00	0.047	0.3	<-5	<-5	0.8	0.0	0.0	<-5	>5	<5		
NadE	NH(3)-dependent NAD(+) synthetase	B1XGK1	30789	5.41	63	1.00E-02	0.39	0.034	0.046	0.024	0.05	0.020	0.036	0.015	0.040	0.4	-0.5	-1.1	0.6	0.8	-0.4	-1.4	1.0	-0.8		
PanB	3-methyl-2-oxobutanate hydroxymethyltransferase	A7ZHM4	28389	5.16	63	1.10E-02	0.26	0.026	0.037	0.07	0.032	0.026	0.037	0.026	0.039	0.5	1.4	1.1	0.3	0.5	0.0	-0.6	0.6	0.0		
PanC	pantothenate synthetase	C4ZRMM6	31692	5.91	109	2.90E-07	0.42	0.028	0.038	0.06	0.039	0.026	0.00	0.034	0.046	0.4	1.1	0.6	0.5	<-5	0.4	-0.4	0.8	-0.1		
ThiC	phosphomethylpyrimidine synthase	B7NRS7	71320	5.66	66	6.30E-03	0.11	0.13	0.12	0.09	0.06	0.022	0.027	0.07	0.040	-0.1	-0.5	0.6	-1.1	0.3	1.7	0.8	0.9	-2.6		
GlgB	1,4-alpha-glucan branching enzyme	P07762	84398	5.91	71	7.00E-03	0.19	0.017	0.012	0.031	0.036	0.010	0.00	0.010	0.022	-0.5	0.9	-0.2	1.1	<-5	0.0	-1.1	1.1	-0.8		
OtsA	alpha,alpha-trihalose-phosphate synthase [UDP-forming]	B1X658	53749	6.37	91	1.70E-05	0.35	0.00	0.00	0.045	0.043	0.023	0.00	0.043	0.026	0.0	>5	0.1	>5	<-5	<-5	0.9	0.7	0.2	>5	

**Table S1 - Quantitative data of individual proteins of *E. coli* BL21 (DE3) growing in defined and complex medium during production of hFGF-2**

Name <sup>1</sup>	Protein name <sup>1</sup>	Uniprot ID <sup>1</sup>	Molecular mass <sup>2</sup> (Da)	Calculated pI value <sup>3</sup>	Mascot Score <sup>2</sup>	Mascot Expect Value <sup>2</sup>	Mascot Seq. Cover. <sup>2</sup>	Defined (DNB) medium				Complex (LB) medium				Log <sub>2</sub> ratio in Defined (DNB) medium				Log <sub>2</sub> ratio in Complex (LB) medium				Log <sub>2</sub> (LB medium/ DNB medium) exponential before IPTG		
								exponential phase before IPTG induction	1h after IPTG induction	5h after IPTG induction	stationary without IPTG	exponential phase before IPTG induction	1h after IPTG induction	3.5h after IPTG induction	stationary phase without IPTG	(1h after IPTG/exponential before IPTG)	(5h after IPTG/stationary without IPTG)	(stationary exponential before IPTG)	(1h after IPTG/exponential before IPTG)	(3.5h after IPTG/stationary without IPTG)	(stationary exponential before IPTG)	(1h after IPTG/exponential before IPTG)	(3.5h after IPTG/stationary without IPTG)	(stationary exponential before IPTG)		
								Relative Protein Mass (RPM) <sup>3</sup> - %																		
<b>(Metabolite) degradation (19 proteins)</b>																									-0.1	
MalP	maltodextrin phosphorylase	P00490	90865	6.94	118	3.60E-08	0.2	0.11	0.09	0.07	0.10	0.22	0.13	0.05	0.18	-0.3	-0.7	-0.5	-0.1	-0.8	-2.1	-1.8	-0.3	1.0		
MalQ	4-alpha-glucanotransferase	P15977	79080	6.14	123	1.10E-08	0.28	0.13	0.09	0.06	0.12	0.40	0.28	0.19	0.21	-0.5	-1.1	-1.0	-0.1	-0.5	-1.1	-0.1	-0.9	1.6		
Pgm	phosphoglucomutase	P36938	58610	5.43	75	7.80E-04	0.19	0.15	0.15	0.12	0.12	0.09	0.14	0.09	0.12	0.0	-0.3	0.0	-0.3	0.6	0.0	-0.4	0.4	-0.7		
BglX	periplasmic beta-glucosidase precursor	Q8CVX0	83562	5.85	113	4.20E-07	0.27	0.06	0.041	0.037	0.046	0.05	0.042	0.032	0.045	-0.5	-0.7	-0.3	-0.4	-0.3	-0.6	-0.5	-0.2	-0.3		
GatZ	putative tagatose-6-phosphate kinase	B3X944	47440	5.52	74	3.80E-03	0.33	0.54	0.48	0.28	0.57	0.18	0.31	0.34	1.16	-0.2	-0.9	-1.0	0.1	0.8	0.9	-1.8	2.7	-1.6		
GatD	galactitol-1-phosphate 5-dehydrogenase	P0A9S3	37822	5.94	61	2.00E-02	0.17	0.14	0.13	0.10	0.14	0.07	0.11	0.10	0.60	-0.1	-0.5	-0.5	0.0	0.7	0.5	-2.6	3.1	-1.0		
XylA	xylose isomerase	C4ZXF6	49939	5.75	63	1.20E-02	0.24	0.022	0.038	0.042	0.031	0.00	0.00	0.00	0.037	0.8	0.9	0.4	0.5	0.0	0.0	0.0	<5	>5	<5	
TreA	periplasmic trehalase	C4ZTN8	63825	5.6	213	1.10E-17	0.46	0.037	0.046	0.027	0.039	0.014	0.016	0.013	0.045	0.3	-0.5	-0.5	0.1	0.2	-0.1	-1.8	1.7	-1.4		
TreC	trehalose-6-phosphate hydrolase	P28904	64082	5.51	122	1.40E-08	0.25	0.05	0.026	0.08	0.09	0.11	0.10	0.10	0.07	-0.9	0.7	-0.2	0.8	-0.1	0.5	-0.7	1.1			
MelA	alpha-galactosidase	P06720	51309	5.52	109	2.90E-07	0.23	0.030	0.037	0.035	0.08	0.06	0.07	0.13	0.23	0.3	0.2	-1.2	1.4	0.2	1.1	-0.8	1.9	1.0		
GabT	4-aminobutyrate aminotransferase GabT	P22256	46202	5.78	227	4.60E-19	0.47	0.16	0.09	0.14	0.047	0.00	0.00	0.022	0.09	-0.8	-0.2	1.6	-1.8	0.0	>5	-2.0	>5	<5		
GabD	succinate-semialdehyde dehydrogenase [NADP+]-GabD	P25526	52030	5.44	125	7.20E-09	0.28	0.07	0.07	0.049	0.045	0.10	0.11	0.10	0.12	0.0	-0.5	0.1	-0.6	0.1	0.0	0.0	-0.3	0.3	0.5	
Prr	gamma-aminobutyraldehyde dehydrogenase	C4ZV13	51197	5.65	80	2.10E-04	0.41	0.34	0.38	0.43	0.04	0.020	0.00	0.00	0.20	0.2	0.3	0.3	0.0	<5	<5	<5	3.3	-0.8		
UidA	beta-glucuronidase	P05804	68917	5.24	80	2.30E-04	0.21	0.05	0.05	0.046	0.05	0.05	0.06	0.06	0.07	0.0	-0.1	-0.1	0.0	0.3	0.3	-0.2	0.5	0.0		
CpdB	2',3'-cyclic-nucleotide 2'-phosphodiesterase/3'-nucleotidase	P08331	70902	5.45	114	9.10E-08	0.34	0.026	0.02	0.01	0.025	0.021	0.025	0.015	0.040	-0.4	-0.7	-0.6	-0.1	0.3	-0.5	-1.4	0.9	-0.3		
DkgA	2,5-diketo-D-gluconic acid reductase A	Q46857	31147	6	151	1.80E-11	0.53	0.024	0.028	0.00	0.045	0.00	0.00	0.05	0.05	0.2	<5	<5	1.3	<5	<5	<5	<5	0.2	0.9	
NanA	N-acetylneuraminate lyase	C4ZSW3	32801	5.61	119	2.90E-08	0.36	0.00	0.00	0.00	0.00	0.42	0.09	0.05	0.043	0.0	0.0	0.0	0.0	1.1	0.3	0.2	0.0	>5		
FadB	fatty acid oxidation complex subunit alpha	C5A020	79829	5.84	131	1.80E-09	0.29	0.023	0.028	0.00	0.049	0.01	0.018	0.016	0.09	0.3	<5	<5	0.9	0.5	0.3	-2.5	2.8	-0.8		
FadH	2,4-dienoyl-CoA reductase [NADPH]	P42593	73203	6.11	89	3.20E-05	0.09	0.011	0.011	0.00	0.008	0.040	0.03	0.029	0.0	<5	<5	<5	-0.5	<5	-0.5	-1.1	0.5	0.9		
<b>Sugar transport (7 proteins)</b>																										-0.6
PtsI	phosphoenolpyruvate-protein phosphotransferase	Q8XB3	63722	4.78	109	1.00E-06	0.28	0.56	0.54	0.42	0.54	0.22	0.25	0.16	0.16	-0.1	-0.4	-0.4	-0.1	0.2	-0.5	0.0	-0.5	-1.3		
PtsH	phosphotidinoprotein-hexose phosphotransferase component of PTS system (Hpr)	P0AA06	9114	5.65	123	4.20E-08	0.9	0.33	0.45	0.30	0.37	0.10	0.13	0.13	0.12	0.4	-0.1	-0.3	0.2	0.4	0.4	0.1	0.3	-1.7		
Crr	glucose-specific enzyme IIA component of PTS	P69783	18240	4.73	104	3.60E-04	0.74	0.18	0.22	0.10	0.33	0.031	0.08	0.041	0.29	0.3	-0.8	-1.7	0.9	1.4	0.4	-2.8	3.2	-2.5		
ManX	PTS system mannose-specific EIIB component	P69797	35026	5.74	143	1.10E-10	0.42	0.12	0.07	0.06	0.13	0.045	0.05	0.031	0.17	-0.8	-1.0	-1.1	0.1	0.2	-0.5	-2.5	1.9	-1.4		
MalE	maltose-binding periplasmic protein	P0AEX9	43360	5.53	65	6.90E-03	0.16	0.28	0.31	0.25	0.76	0.13	0.23	0.17	1.37	0.1	-0.2	-1.6	1.4	0.8	0.4	-3.0	3.4	-1.1		
MalK	maltose/maltodextrin import ATP-binding protein MalK	P68188	41136	6.23	88	8.00E-04	0.33	0.07	0.05	0.029	0.07	0.13	0.09	0.09	0.25	-0.5	-1.3	-1.3	0.0	-0.5	-0.5	-1.5	0.9	0.9		
LamB	maloporin (lambda receptor protein)	P02943	47469	4.72	97	1.70E-05	0.35	0.50	0.36	0.26	0.73	0.71	0.94	0.75	1.80	-0.5	-0.9	-1.5	0.5	0.4	0.1	-1.3	1.3	0.5		
<b>Amino acid and peptide transport (7 proteins)</b>																										-1.6
GlnH	glutamine ABC transporter periplasmic protein	P0AEQ5	27173	8.44	75	2.80E-03	0.48	0.11	0.18	0.07	0.06	0.07	0.046	0.07	0.05	0.7	-0.7	0.2	-0.9	-0.6	0.0	0.5	-0.5	-0.7		
ArtJ	arginine transporter subunit	P30860	26927	6.84	90	8.10E-05	0.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
ArgT	lysine-arginine-ornithine-binding periplasmic protein	P09551	28088	5.62	94	8.50E-06	0.51	0.08	0.07	0.11	0.12	0.027	0.06	0.07	0.12	-0.2	0.5	-0.1	0.6	1.2	1.4	-0.8	2.2	-1.6		
LivJ	leucine/isoleucine/valine transporter subunit	P0AD96	39223	5.54	131	6.60E-09	0.55	0.26	0.31	0.19	0.55	0.05	0.09	0.10	0.12	0.3	-0.5	-1.5	1.1	0.8	1.0	-0.3	1.3	-2.4		
DppA	periplasmic dipeptide transport protein	P23847	60483	6.21	161	1.80E-12	0.44	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.43	<5	<5	0.0	<5	<5	0.0	0.0	<5	>5	<5	

**Table S1 - Quantitative data of individual proteins of *E. coli* BL21 (DE3) growing in defined and complex medium during production of hFGF-2**

Name <sup>1</sup>	Protein name <sup>1</sup>	Uniprot ID <sup>1</sup>	Molecular mass <sup>2</sup> (Da)	Calculated pI value <sup>3</sup>	Mascot Score <sup>2</sup>	Mascot Expect Value <sup>2</sup>	Mascot Seq. Cover. <sup>2</sup>	Defined (DNB) medium				Complex (LB) medium				Log <sub>2</sub> ratio in Defined (DNB) medium				Log <sub>2</sub> ratio in Complex (LB) medium				Log <sub>2</sub> (LB medium/ DNB medium) exponential before IPTG		
								exponential phase before IPTG induction	1h after IPTG induction	5h after IPTG induction	stationary phase without IPTG	exponential phase before IPTG induction	1h after IPTG induction	3.5h after IPTG induction	stationary phase without IPTG	(1h after IPTG/ exponential before IPTG)	(5h after IPTG/ stationary without IPTG)	(stationary phase before IPTG)	(1h after IPTG/ exponential before IPTG)	(3.5h after IPTG/ stationary without IPTG)	(stationary phase before IPTG)	(1h after IPTG/ exponential before IPTG)	(3.5h after IPTG/ stationary without IPTG)			
								Relative Protein Mass (RPM) <sup>3</sup> - %																		
OppA	periplasmic oligopeptide-binding protein	P23843	60975	6.05	150	2.30E-11	0.38	0.12	0.17	0.13	0.23	0.11	0.10	0.10	0.70	0.5	0.1	-0.8	0.9	-0.1	-0.1	-2.8	2.7	-0.1		
OppD	oligopeptide transport ATP-binding protein OppD	P76027	37506	5.78	67	4.20E-03	0.41	0.040	0.045	0.00	0.038	0.00	0.00	0.00	0.031	0.2	<-5	<-5	-0.1	0.0	0.0	<5	>5	<5		
<b>Outer membrane proteins and other transport proteins (13 proteins)</b>								Total RPM:	5.40	5.46	4.88	5.61	5.46	7.43	4.47	4.90	0.0	-0.1	-0.2	0.1	0.4	-0.3	-0.1	-0.2	0.0	
<b>Sub-group: Outer membrane proteins (8 proteins)</b>								Total RPM:	5.10	5.17	4.65	5.31	5.13	7.16	4.19	4.61	0.0	-0.1	-0.2	0.1	0.5	-0.3	-0.1	-0.2	0.0	
LptD	exported protein required for envelope biosynthesis and integrity	P31554	89843	4.94	158	1.30E-11	0.27	0.06	0.08	0.047	0.10	0.08	0.08	0.044	0.06	0.4	-0.4	-1.1	0.7	0.0	-0.9	-0.4	-0.4	0.4		
FadL	long-chain fatty acid transport protein	Q8XCN6	48509	5.19	84	7.10E-05	0.34	0.10	0.05	0.06	0.06	0.06	0.09	0.09	0.20	-1.0	-0.7	0.0	-0.7	0.6	0.6	-1.2	1.7	-0.7		
OmpF	outer membrane porin 1a (la;bf;F)	P02931	39309	4.76	197	1.70E-15	0.69	2.63	2.89	2.33	2.65	2.93	4.70	2.51	2.55	0.1	-0.2	-0.2	0.0	0.7	-0.2	0.0	-0.2	0.2		
OmpA	outer membrane protein A	P0A911	37292	5.99	92	1.30E-05	0.32	0.69	0.61	0.62	0.99	0.47	0.65	0.49	0.59	-0.2	-0.2	-0.7	0.5	0.5	0.1	-0.3	0.3	-0.6		
OmpA (2) <sup>5</sup>	outer membrane protein A	ZP_03085789(NCBH11)	28830	5.98	114	9.20E-07	0.50	1.10	1.01	1.01	0.95	0.76	0.73	0.57	0.80	-0.1	-0.1	0.1	-0.2	-0.1	-0.4	-0.5	0.1	-0.5		
TolC	outer membrane protein TolC	P02930	53708	5.46	79	3.00E-04	0.23	0.08	0.08	0.06	0.09	0.10	0.09	0.021	0.038	0.0	-0.4	-0.6	0.2	-0.2	-2.3	-0.9	-1.4	0.3		
OmpX	outer membrane protein X	P0A919	18648	6.56	110	8.30E-07	0.68	0.30	0.30	0.40	0.31	0.54	0.51	0.31	0.18	0.0	0.4	0.4	0.0	-0.1	-0.8	0.8	-1.6	0.8		
YaeT	outer membrane protein assembly factor YaeT	A7ZHR7	90611	4.93	161	1.50E-12	0.26	0.11	0.15	0.12	0.16	0.19	0.31	0.15	0.19	0.1	-0.2	-0.4	0.2	0.7	-0.3	-0.3	0.0	0.4		
<b>Sub-group: Other transport proteins (5 proteins)</b>								Total RPM:	-0.30	-0.2	0.23	0.30	0.33	0.27	0.28	0.29	0.0	-0.4	-0.4	0.0	-0.3	-0.2	-0.1	-0.2	0.1	
CusB	copper/silver efflux system, membrane fusion protein	P77239	44277	5.93	253	4.20E-21	0.62	0.07	0.08	0.06	0.06	0.00	0.00	0.00	0.00	-0.2	-0.2	0.0	-0.2	0.0	0.0	0.0	0.0	<-5		
GsbB	glutathione ABC transporter, periplasmic glutathione-binding protein GsbB	B3IL13	56479	8.23	80	7.70E-04	0.22	0.042	0.034	0.024	0.024	0.040	0.016	0.019	0.038	-0.3	-0.9	-0.7	-0.2	-1.3	-1.1	-1.0	-0.1	-0.1		
TolB	translocation protein TolB	A7ZJC2	41502	6.22	100	8.50E-06	0.31	0.05	0.06	0.032	0.040	0.06	0.048	0.023	0.11	0.3	-0.6	-1.6	1.0	-0.3	-1.4	-2.3	0.9	0.3		
YbiT	putative ATP-binding component of a transport system	P0A9U5	59877	4.99	123	4.20E-08	0.31	0.05	0.047	0.027	0.027	0.06	0.06	0.09	0.036	-0.1	-0.9	-0.5	-0.4	0.0	0.6	1.3	-0.7	0.3		
SecA	preprotein translocase subunit, ATPase	P10408	102187	5.43	196	2.10E-15	0.36	0.09	0.09	0.09	0.07	0.11	0.15	0.11	0.0	0.0	0.4	-0.4	-0.4	-0.2	-0.2	0.4	-0.6	0.9		
<b>RNA Polymerases (3 proteins)</b>								Total RPM:	1.07	1.00	1.06	0.89	1.35	1.20	1.00	0.81	-0.1	0.0	0.3	-0.3	-0.2	-0.4	0.3	-0.7	0.3	
RpoA	DNA-directed RNA polymerase subunit alpha	P0A7Z6	36717	4.98	247	1.70E-20	0.69	0.54	0.54	0.60	0.61	0.71	0.74	0.57	0.44	0.0	0.2	0.0	0.2	0.1	-0.3	0.4	-0.7	0.4		
RpoB	DNA-directed RNA polymerase subunit beta	P0A8V4	150935	5.15	117	1.70E-07	0.18	0.49	0.44	0.44	0.26	0.57	0.46	0.43	0.36	-0.2	-0.2	0.8	-0.9	-0.3	-0.4	0.3	-0.7	0.2		
RpoC	DNA-directed RNA polymerase subunit beta'	B1XBZ0	155918	6.67	160	2.30E-12	0.18	0.043	0.020	0.021	0.017	0.07	0.00	0.00	0.008	-1.1	-1.0	0.3	-1.3	<-5	<-5	<-5	-3.1	0.7		
<b>RNA polymerase binding proteins (7 proteins)</b>								Total RPM:	0.82	0.92	0.88	0.88	1.23	1.07	1.05	0.78	0.2	0.1	0.0	0.1	-0.2	-0.2	0.4	-0.7	0.6	
RapA	RNA polymerase-associated protein RapA	A7ZHF0	110057	5.04	65	6.90E-03	0.12	0.029	0.018	0.031	0.015	0.08	0.043	0.048	0.020	-0.7	0.1	1.0	-1.0	-0.9	-0.7	1.3	-2.0	1.5		
NusA	transcription elongation protein NusA	P0AFF8	55008	4.53	77	4.80E-04	0.2	0.12	0.16	0.14	0.13	0.19	0.21	0.22	0.10	0.4	0.2	0.1	0.1	0.1	0.2	1.1	-0.9	0.7		
NusG	transcription termination/antitermination protein NusG	P0AFG0	20518	6.34	141	1.80E-10	0.69	0.09	0.08	0.06	0.07	0.09	0.06	0.06	0.042	-0.2	-0.6	-0.2	-0.4	-0.6	-0.6	0.5	-1.1	0.0		
Rho	transcription termination factor Rho	P0AG32	47032	6.75	166	5.70E-13	0.33	0.37	0.38	0.40	0.34	0.62	0.55	0.42	0.29	0.0	0.1	0.2	-0.1	-0.2	-0.6	0.5	-1.1	0.7		
RpoD	RNA polymerase sigma-subunit	Q59371	69157	4.87	97	1.60E-05	0.31	0.05	0.09	0.11	0.039	0.15	0.08	0.21	0.10	0.8	1.1	1.5	-0.4	-0.9	0.5	1.1	-0.6	1.6		
SspA	stringent starvation protein A	P0ACA3	24346	5.22	128	3.60E-09	0.58	0.08	0.09	0.08	0.12	0.07	0.08	0.05	0.11	0.2	0.0	0.6	-0.6	0.2	-0.5	-1.1	0.7	-0.2		
DksA	DnaK transcriptional regulator DksA	P0ABS3	17745	5.06	72	6.00E-03	0.64	0.08	0.10	0.06	0.17	0.031	0.044	0.039	0.12	0.3	-0.4	-1.5	1.1	0.5	0.3	-1.6	2.0	-1.4		

**Table S1 - Quantitative data of individual proteins of *E. coli* BL21 (DE3) growing in defined and complex medium during production of hFGF-2**

Name <sup>1</sup>	Protein name <sup>1</sup>	Uniprot ID <sup>1</sup>	Molecular mass <sup>2</sup> (Da)	Calculated pI value <sup>3</sup>	Mascot Score <sup>2</sup>	Mascot Expect Value <sup>2</sup>	Mascot Seq. Cover. <sup>2</sup>	Defined (DNB) medium				Complex (LB) medium				Log <sub>2</sub> ratio in Defined (DNB) medium				Log <sub>2</sub> ratio in Complex (LB) medium				Log <sub>2</sub> (LB medium/ DNB medium) exponential before IPTG	
								exponential phase before IPTG induction	1h after IPTG induction	5h after IPTG induction	stationary phase without IPTG	exponential phase before IPTG induction	1h after IPTG induction	3.5h after IPTG induction	stationary phase without IPTG	(1h after IPTG/exponential before IPTG)	(5h after IPTG/stationary without IPTG)	(stationary exponential before IPTG)	(1h after IPTG/exponential before IPTG)	(3.5h after IPTG/stationary without IPTG)	(5h after IPTG/exponential before IPTG)	(stationary exponential before IPTG)			
								Relative Protein Mass (RPM) <sup>3</sup> - %																	
<b>Transcription factors (12 proteins)</b>																									
LacI	lactose operon repressor	P03023	38737	6.39	58	4.10E-02	28%	0.00	0.00	0.00	0.11	0.022	0.033	0.042	0.0	0.0	0.0	0.0	0.1	0.0	-0.3	0.3	-0.1	>5	
Hns	global DNA-binding transcriptional dual regulator H-NS	P0ACG0	15587	5.43	92	5.90E-05	0.62	0.71	0.73	0.77	0.80	0.75	0.90	0.75	0.85	0.0	0.1	-0.1	0.2	0.3	0.0	-0.2	0.2	0.1	
ArcA	aerobic respiration control protein ArcA	P0A9Q1	27389	5.21	156	5.70E-12	0.46	0.09	0.07	0.07	0.08	0.08	0.08	0.08	0.14	-0.4	-0.4	-0.2	-0.2	0.0	0.0	-0.8	0.8	-0.2	
KdgR	transcriptional regulator KdgR	P76268	30067	5.43	164	9.10E-13	0.74	0.031	0.034	0.048	0.043	0.029	0.037	0.027	0.032	0.1	0.6	0.2	0.5	0.4	-0.1	-0.2	0.1	-0.1	
OxyR	hydrogen peroxide-inducible genes activator	P0ACQ4	34596	5.96	95	7.10E-06	0.34	0.038	0.05	0.039	0.043	0.029	0.06	0.046	0.037	0.4	0.0	-0.1	0.2	1.0	0.7	0.3	0.4	-0.4	
BasR	transcriptional regulatory protein BasR	P30843	25072	5.66	70	2.10E-03	0.31	0.06	0.06	0.06	0.07	0.042	0.06	0.06	0.08	0.0	0.0	-0.2	0.2	0.5	0.5	-0.4	0.9	-0.5	
CspC	CspC	Q1PG46	7497	8.09	56	2.20E-01	0.6	0.55	0.61	0.42	0.43	0.34	0.43	0.33	0.62	0.1	-0.4	0.0	-0.4	0.3	0.0	-0.9	0.9	-0.7	
StpA	DNA binding protein, nucleoid-associated	P0ACG2	15338	7.93	50	8.10E-01	0.33	0.06	0.06	0.06	0.07	0.07	0.00	0.22	0.06	0.0	0.0	-0.2	0.2	<-5	1.7	1.9	-0.2	0.2	
PspA	phage shock protein A	P0AFM6	25477	5.39	76	6.30E-04	0.55	0.028	0.040	0.043	0.041	0.027	0.00	0.034	0.018	0.5	0.6	0.1	0.6	<-5	0.3	0.9	-0.6	-0.1	
OmpR	transcriptional regulatory protein OmpR	P0AA16	27393	6.04	132	1.40E-05	0.52	0.11	0.10	0.11	0.12	0.10	0.06	0.08	0.10	-0.1	0.0	-0.1	0.1	-0.7	-0.3	-0.3	0.0	-0.1	
CpxR	transcriptional regulatory protein CpxR	P0AE88	26296	5.39	71	1.90E-03	0.36	0.044	0.038	0.046	0.041	0.026	0.05	0.00	0.035	-0.2	0.1	0.2	-0.1	0.9	<-5	<-5	0.4	-0.8	
PhoP	transcriptional regulatory protein PhoP	P23836	25519	5.1	123	1.10E-08	0.51	0.0	0.045	0.07	0.05	0.031	0.00	0.020	0.028	-0.4	0.2	0.5	-0.3	<-5	-0.6	-0.5	-0.1	-1.0	
<b>Ribosomal proteins (5 proteins)</b>																									
RplL	50S ribosomal protein L7/L12	P0A7K2	12069	4.6	70	7.80E-03	0.44	0.51	0.56	0.82	0.87	0.91	1.17	1.00	0.65	0.4	0.7	-0.1	0.8	0.4	0.1	0.6	-0.5	0.8	
RplI	50S ribosomal protein L9	P0A7R3	15759	6.17	197	1.70E-15	0.81	0.54	0.53	0.57	0.61	0.78	0.76	0.59	0.37	0.0	0.0	0.1	0.0	0.0	-0.4	0.7	-1.1	0.5	
RpsA	30S ribosomal protein S1	P0AG69	61235	4.89	189	1.00E-14	0.35	1.38	1.42	1.63	1.74	1.99	2.04	1.75	1.16	0.0	0.2	0.1	0.1	0.0	-0.2	0.6	-0.8	0.5	
RpsB	30S ribosomal protein S2	C4ZRR1	26784	6.61	68	3.20E-03	0.29	0.66	0.74	0.87	0.93	1.28	1.20	0.87	0.41	0.2	0.4	0.7	-0.3	-1.7	-0.6	1.1	-1.6	1.0	
RpsF	30S ribosomal protein S6	A7ZV71	15177	5.26	61	1.70E-02	0.39	0.45	0.43	0.45	0.33	1.36	1.26	0.27	0.15	-0.1	0.0	0.4	-0.4	-0.3	-0.8	0.8	-1.6	0.0	
<b>Ribosome-associated proteins (8 proteins)</b>																									
EngA	predicted GTP-binding protein	P0A6P5	55058	5.6	150	8.40E-11	0.32	0.039	0.036	0.032	0.027	0.035	0.00	0.023	0.023	-0.1	-0.3	0.2	-0.5	<-5	-0.6	0.0	-0.6	-0.2	
YeaO	UPF0142 protein YeaO	P75838	66009	4.38	75	7.80E-04	0.13	0.021	0.027	0.024	0.017	0.06	0.08	0.049	0.031	0.4	0.2	0.5	-0.3	0.4	-0.3	0.7	-1.0	1.5	
EngD (YchF)	GTP-dependent nucleic acid-binding protein EngD	P0ABU3	39984	4.87	211	6.70E-17	0.6	0.06	0.09	0.06	0.09	0.10	0.13	0.09	0.08	0.6	0.0	-0.6	0.6	0.4	-0.2	0.2	-0.3	0.7	
TypA	GTP-binding protein TypA/BipA	P32132	67542	5.16	71	4.10E-02	0.22	0.20	0.27	0.23	0.16	0.31	0.34	0.26	0.15	0.4	0.2	0.5	-0.3	0.1	-0.3	0.8	-1.0	0.6	
HflX	GTP-binding protein hflX	P25519	48468	5.68	88	8.60E-04	0.21	0.021	0.027	0.039	0.043	0.037	0.05	0.06	0.05	0.4	0.9	-0.1	1.0	0.4	0.7	0.3	0.4	0.8	
Tig	trigger factor	Q1RFA0	47836	4.83	205	2.60E-16	0.46	0.65	0.66	0.69	0.72	0.98	1.12	0.85	0.63	0.0	0.1	-0.1	0.1	0.2	-0.2	0.4	-0.6	0.6	
Frr	ribosome recycling factor	A1A7L6	19269	6.43	66	2.30E-02	0.4	0.05	0.00	0.09	0.14	0.030	0.00	0.034	0.11	<-5	0.8	-0.6	1.5	<-5	0.2	-1.7	1.9	-0.7	
RaiA	ribosome-associated inhibitor A	P0AD51	12777	6.2	62	1.40E-02	0.46	0.00	0.06	0.00	0.36	0.11	0.00	0.00	0.12	>5	0.0	<5	>5	<5	<5	<5	0.1	>5	
<b>Aminoacyl-tRNA synthetases (23 proteins)</b>																									
AlaS	alanyl-tRNA synthetase	B1XCM5	96315	5.53	99	2.80E-06	0.16	0.22	0.24	0.21	0.15	0.45	0.33	0.36	0.60	0.1	-0.1	0.5	-0.6	-0.4	-0.3	-0.7	0.4	1.0	
ArgS	arginyl-tRNA synthetase	Q8XCH2	64851	5.31	131	6.70E-09	0.26	0.049	0.05	0.05	0.049	0.08	0.08	0.06	0.033	0.0	0.0	0.0	0.0	0.0	-0.4	0.9	-1.3	0.7	
AsnS	asparagine-tRNA ligase	A7ZK21	52766	5.17	131	1.80E-09	0.32	0.22	0.18	0.19	0.22	0.28	0.25	0.25	0.17	-0.3	-0.2	-0.2	0.0	-0.2	-0.2	0.6	-0.7	0.3	
AspS	aspartyl-tRNA synthetase	Q8XC17	66115	5.47	59	3.00E-02	0.2	0.18	0.18	0.19	0.18	0.20	0.18	0.15	0.17	0.0	0.1	0.1	0.0	-0.2	-0.4	-0.2	-0.2	0.2	

**Table S1 - Quantitative data of individual proteins of *E. coli* BL21 (DE3) growing in defined and complex medium during production of hFGF-2**

Name <sup>1</sup>	Protein name <sup>1</sup>	Uniprot ID <sup>1</sup>	Molecular mass <sup>2</sup> (Da)	Calculated pI value <sup>3</sup>	Mascot Score <sup>2</sup>	Mascot Expect Value <sup>2</sup>	Mascot Seq. Cover. <sup>2</sup>	Defined (DNB) medium					Complex (LB) medium					Log <sub>2</sub> ratio in Defined (DNB) medium					Log <sub>2</sub> ratio in Complex (LB) medium					Log <sub>2</sub> (LB medium/ DNB medium) exponential before IPTG						
								exponential phase before IPTG induction	1h after IPTG induction	5h after IPTG induction	stationary without IPTG	exponential phase before IPTG induction	1h after IPTG induction	3.5h after IPTG induction	stationary phase without IPTG	(1h after IPTG/ exponential before IPTG)	(5h after IPTG/ stationary without IPTG)	(stationary without IPTG/ exponential before IPTG)	(1h after IPTG/ exponential before IPTG)	(3.5h after IPTG/ stationary without IPTG)	(3.5h after IPTG/ stationary without IPTG)	(stationary without IPTG/ exponential before IPTG)												
								Relative Protein Mass (RPM) <sup>3</sup> - %																										
CysS	cysteinyl-tRNA synthetase	Q8FK44	52436	5.33	76	2.00E-03	0.28	0.12	0.08	0.08	0.09	0.09	0.16	0.14	0.09	-0.6	-0.6	-0.2	-0.4	0.8	0.6	0.6	0.0	-0.4										
GlnS	glutaminyl-tRNA synthetase	Q8X9H8	64040	5.88	109	1.10E-06	0.31	0.09	0.09	0.11	0.08	0.13	0.18	0.16	0.08	0.0	0.3	0.5	-0.2	0.5	0.3	1.0	-0.7	0.5										
GltX	glutamyl-tRNA synthetase	B1X9R9	54181	5.59	58	3.20E-02	0.24	0.10	0.09	0.09	0.09	0.10	0.10	0.07	0.16	-0.2	-0.2	0.0	-0.2	0.0	-0.5	-1.2	0.7	0.0										
GlyQ	glycyl-tRNA synthetase alpha subunit	A7ZTA6	34979	4.94	106	5.70E-07	0.45	0.12	0.05	0.07	0.044	0.08	0.10	0.09	0.11	-1.3	-0.8	0.7	-1.4	0.3	0.2	-0.3	0.5	-0.6										
GlyS	glycyl-tRNA synthetase beta subunit	P00961	76936	5.29	181	1.80E-14	0.28	0.15	0.12	0.13	0.09	0.21	0.24	0.18	0.09	-0.3	-0.2	0.5	-0.7	0.2	-0.2	1.0	-1.2	0.5										
HisS	histidyl-tRNA synthetase	P60908	47285	5.65	148	1.30E-10	0.44	0.08	0.06	0.08	0.06	0.017	0.08	0.044	0.06	-0.4	0.0	0.4	-0.4	2.2	1.4	-0.4	1.8	-2.2										
IleS	isoleucyl-tRNA synthetase	P00956	105042	5.7	169	1.10E-12	0.33	0.16	0.13	0.14	0.09	0.35	0.15	0.25	0.18	-0.3	-0.2	0.6	-0.8	-1.2	-0.5	0.5	-1.0	1.1										
LeuS	leucine-tRNA ligase	A7ZJ31	97768	5.16	103	1.10E-06	0.21	0.26	0.24	0.36	0.21	0.30	0.40	0.34	0.21	-0.1	0.5	0.8	-0.3	0.4	0.2	0.7	-0.5	0.2										
LysS	lysyl-tRNA synthetase	Q8XD57	57652	5.11	141	1.80E-10	0.36	0.13	0.12	0.14	0.11	0.25	0.20	0.17	0.12	-0.1	0.1	0.3	-0.2	-0.3	-0.6	0.5	-1.1	0.9										
MetG	methionyl-tRNA synthetase	C4ZSJ8	76662	5.56	88	3.80E-05	0.31	0.12	0.10	0.10	0.10	0.14	0.10	0.11	0.11	-0.3	-0.3	0.0	-0.3	-0.5	-0.3	0.0	-0.3	0.2										
PheT	phenylalanyl-tRNA synthetase subunit beta	Q8XE32	88119	5.12	181	6.60E-14	0.31	0.17	0.20	0.19	0.18	0.15	0.14	0.13	0.11	0.2	0.2	0.1	0.1	-0.1	-0.2	0.2	-0.4	-0.2	-0.2									
ProS	proline-tRNA ligase	C4ZRT7	63710	5.12	214	9.10E-18	0.37	0.11	0.24	0.22	0.17	0.19	0.23	0.22	0.07	1.1	1.0	0.4	0.6	0.3	0.2	1.7	-1.4	0.8										
SerS	serine-tRNA ligase	A7ZJW2	48669	5.34	84	1.00E-04	0.35	0.11	0.11	0.10	0.09	0.14	0.14	0.11	0.10	0.0	-0.1	0.2	-0.3	0.0	-0.3	0.1	-0.5	0.3										
ThrS	threonyl-tRNA synthetase	Q8XE27	74722	5.8	52	5.80E-01	0.17	0.12	0.08	0.14	0.13	0.19	0.10	0.15	0.15	-0.1	0.2	0.1	0.1	-0.9	-0.4	-0.1	-0.3	0.7										
TyrS	tyrosyl-tRNA synthetase	A1ABI2	48368	5.49	83	1.10E-04	0.39	0.13	0.12	0.15	0.09	0.18	0.16	0.19	0.14	-0.1	0.2	0.7	-0.5	-0.2	0.1	0.4	-0.4	0.5										
LysU	lysyl-tRNA synthetase, heat inducible	P0A8NS	57847	5.1	166	5.70E-13	0.4	0.036	0.025	0.09	0.041	0.07	0.09	0.08	0.07	0.0	1.3	1.1	0.2	0.4	0.2	0.2	0.0	1.0										
MnmA	tRNA-specific 2-thiouridylase MnmA	A7ZKS3	41333	4.94	69	2.90E-03	0.33	0.05	0.09	0.02	0.03	0.043	0.12	0.036	0.05	0.8	-0.3	-0.9	0.7	1.5	-0.3	-0.5	0.2	-0.2										
YgfZ	tRNA-modifying protein YgfZ	C5A0H0	36185	5.17	211	1.80E-17	0.54	0.040	0.06	0.07	0.05	0.016	0.13	0.045	0.08	0.6	0.8	0.2	0.6	3.0	1.5	-0.8	2.3	-1.3										
Fmt	methionyl-tRNA formyltransferase	P23882	34318	5.56	65	7.80E-03	0.22	0.046	0.036	0.10	0.04	0.018	0.082	0.029	0.09	-0.4	1.1	0.7	0.4	0.8	0.7	-1.6	2.3	-1.4										
<b>Elongation factors (6 proteins)</b>								Total RPM:	4.93	6.00	5.54	6.08	6.27	8.12	7.39	6.18	0.3	0.2	-0.1	0.3	0.3	0.1	0.2	-0.1	0.5	0.1	0.2	-0.1	0.5					
InitB	initiation factor IF2-gamma	P0A705	78978	5.65	79	1.10E-03	0.26	0.15	0.11	0.16	0.08	0.28	0.38	0.24	0.15	-0.4	0.1	1.0	-0.9	-1.6	-0.2	0.7	-0.9	0.9										
FusA	elongation factor G	A7ZSL5	77704	5.24	102	1.40E-06	0.23	1.30	1.72	1.58	1.38	1.67	2.15	2.12	1.49	0.4	0.3	0.2	0.2	0.1	0.4	0.3	0.5	-0.2	0.4									
TufA	elongation factor Tu	A7ZSL4	43427	5.3	109	2.90E-07	0.28	2.77	3.48	3.08	3.76	3.72	4.70	4.12	3.89	0.3	0.2	-0.3	0.4	0.3	0.1	0.1	0.1	0.1	0.4									
Tsf	elongation factor Ts	P0A6P1	30387	5.22	245	2.60E-20	0.73	0.65	0.62	0.64	0.80	0.97	1.08	0.76	0.58	-0.1	0.0	-0.3	0.3	0.2	-0.4	0.4	-0.7	0.6										
LepA	GTP-binding protein LepA	P60787	67099	5.4	193	4.20E-15	0.4	0.041	0.048	0.045	0.029	0.05	0.06	0.06	0.037	0.2	0.1	0.6	-0.5	0.3	0.3	0.7	-0.4	0.3										
PrfC	peptide chain release factor 3	Q1R270	59632	5.66	75	2.90E-03	0.2	0.022	0.026	0.031	0.028	0.046	0.039	0.041	0.037	0.2	0.5	0.4	0.1	0.3	-0.2	-0.2	0.1	-0.3	1.1									
<b>RNA degradation (3 proteins)</b>								Total RPM:	0.51	0.56	0.51	0.43	0.68	0.98	0.64	0.53	0.1	0.0	0.2	-0.2	0.5	-0.1	0.3	-0.4	0.4									
Pnp	polyribonucleotide nucleotidyltransferase	B1IQV7	77093	5.09	100	2.60E-06	0.15	0.44	0.48	0.43	0.36	0.56	0.88	0.53	0.44	0.1	0.0	0.3	-0.3	0.7	-0.1	0.3	-0.3	0.3										
Rnb	exoribonuclease II	B3XC13	72829	5.44	136	2.10E-09	0.3	0.05	0.05	0.048	0.047	0.09	0.07	0.09	0.07	0.0	-0.1	0.0	-0.1	-0.4	0.0	0.4	-0.4	0.8										
RhlB	ATP-dependent RNA helicase	P0A8J8	47325	7.29	104	3.40E-06	0.29	0.020	0.030	0.028	0.021	0.032	0.032	0.021	0.018	0.6	0.5	0.4	0.1	0.0	-0.6	0.2	-0.8	0.7										
<b>Isomerases (5 proteins)</b>								Total RPM:	0.72	0.79	0.63	0.79	0.56	0.57	0.38	0.86	0.1	-0.2	-0.3	0.1	0.0	-0.6	-1.2	0.6	-0.4									
PpiB	peptidyl-prolyl cis-trans isomerase B (rotamase B)	Q8XCU0	18270	5.52	62	5.50E-02	0.38	0.07	0.10	0.08	0.15	0.08	0.047	0.048	0.12	0.5	0.2	-0.9	1.1	-0.8	-0.7	-1.3	0.6	0.2										
SurA	peptidyl-prolyl cis-trans isomerase SurA	P0ABZ8	47254	6.48	135	2.70E-09	0.28	0.09	0.09	0.08	0.09	0.07	0.10	0.10	0.11	0.0	-0.2	-0.2	0.0	0.5	0.5	-0.1	0.7	-0.4										

**Table S1 - Quantitative data of individual proteins of *E. coli* BL21 (DE3) growing in defined and complex medium during production of hFGF-2**

Name <sup>1</sup>	Protein name <sup>1</sup>	Uniprot ID <sup>1</sup>	Molecular mass <sup>2</sup> (Da)	Calculated pI value <sup>3</sup>	Mascot Score <sup>2</sup>	Mascot Expect Value <sup>2</sup>	Mascot Seq. Cover. <sup>2</sup>	Defined (DNB) medium				Complex (LB) medium				Log <sub>2</sub> ratio in Defined (DNB) medium				Log <sub>2</sub> ratio in Complex (LB) medium				Log <sub>2</sub> (LB medium/ DNB medium) exponential before IPTG		
								exponential phase before IPTG induction	1h after IPTG induction	5h after IPTG induction	stationary phase without IPTG	exponential phase before IPTG induction	1h after IPTG induction	3.5h after IPTG induction	stationary phase without IPTG	(1h after IPTG/ exponential before IPTG)	(5h after IPTG/ stationary without IPTG)	(stationary phase before IPTG)	(1h after IPTG/ exponential before IPTG)	(3.5h after IPTG/ stationary without IPTG)	(5h after IPTG/ stationary without IPTG)	(stationary phase before IPTG)				
								Relative Protein Mass (RPM) <sup>3</sup> - %																		
FklB	FKBP-type 22 kDa peptidyl-prolyl cis-trans isomerase	P0A9L3	22203	4.85	146	4.70E-11	0.37	0.10	0.12	0.06	0.13	0.06	0.07	0.06	0.15	0.3	-0.7	-1.1	0.4	0.2	0.0	-1.3	1.3	-0.7		
FkpA	FKBP-type peptidyl-prolyl cis-trans isomerase	P65765	28894	8.39	94	3.30E-05	0.44	0.15	0.17	0.16	0.10	0.17	0.17	0.043	0.20	0.2	0.1	0.7	-0.6	0.0	-2.0	-2.2	0.2	0.2		
SlyD	FKBP-type peptidyl-prolyl cis-trans isomerase SlyD	P0A9L1	21182	4.86	100	2.60E-06	0.31	0.31	0.31	0.25	0.32	0.18	0.18	0.13	0.28	0.0	-0.3	-0.4	0.0	0.0	-0.5	-1.1	0.6	-0.8		
<b>Chaperones (9 proteins)</b>								Total RPM:	1.94	2.64	5.45	1.95	2.93	5.93	7.11	2.74	0.4	1.5	1.5	0.0	1.0	1.3	1.4	-0.1	0.6	
DnaK	molecular chaperone DnaK	P0A6Z0	69130	4.83	192	5.30E-15	0.39	0.56	0.87	2.19	0.56	0.89	2.10	2.53	0.81	0.6	2.0	2.0	0.0	1.2	1.5	1.6	-0.1	0.7		
GrpE	heat shock protein GrpE	A7ZQ54	21727	4.68	54	3.30E-01	0.32	0.08	0.09	0.19	0.07	0.14	0.27	0.27	0.10	0.2	1.2	1.4	-0.2	0.9	0.9	1.4	-0.5	0.8		
GroL	60 kDa chaperonin (GroEL protein)	P0A6F5	55224	4.84	219	1.00E-17	0.52	0.83	1.13	1.71	0.88	1.22	2.26	2.28	1.32	0.4	1.0	1.0	0.1	0.9	0.9	0.8	0.1	0.6		
GroS	co-chaperonin GroES	P0A6G1	10381	5.15	100	8.30E-06	0.64	0.11	0.14	0.26	0.13	0.15	0.36	0.54	0.14	0.3	1.2	1.0	0.2	1.3	1.8	1.9	-0.1	0.4		
HtpG	heat shock protein 90	P0A6Z5	71378	5.09	262	5.30E-22	0.5	0.16	0.21	0.62	0.13	0.28	0.48	0.66	0.14	0.4	2.0	2.3	-0.3	0.8	1.2	2.2	-1.0	0.8		
HscA	Fe-S protein assembly chaperone HscA	B3XDI5	65726	5.02	85	2.90E-04	0.21	0.040	0.036	0.029	0.042	0.05	0.05	0.040	0.027	-0.2	-0.5	-0.5	0.1	0.0	-0.3	0.6	-0.9	0.3		
ClpA	ATP-dependent Clp protease ATP-binding subunit	P0ABI1	84326	5.91	87	1.60E-04	0.21	0.05	0.049	0.039	0.06	0.037	0.025	0.038	0.06	0.0	-0.4	-0.6	0.3	-0.6	0.0	-0.7	0.7	-0.4		
ClpB	protein disaggregation chaperone	P63285	95697	5.37	160	8.30E-12	0.21	0.021	0.027	0.08	0.021	0.06	0.11	0.14	0.042	0.4	1.9	1.9	0.0	0.9	1.2	1.7	-0.5	1.5		
IbpA	small heat shock protein IbpA	A7ZTP1	15764	5.57	168	8.40E-12	0.7	0.09	0.09	0.33	0.06	0.10	0.27	0.61	0.10	0.0	1.9	2.5	-0.6	1.4	2.6	2.6	0.0	0.2		
<b>Proteases (13 proteins)</b>								Total RPM:	0.89	0.84	1.02	0.82	1.02	1.27	1.55	1.38	-0.1	0.2	0.3	-0.1	0.3	0.6	0.2	0.4	0.2	
DegP	serine endopeptidase	P0C0V1	49438	8.65	119	1.10E-07	0.31	0.017	0.055	0.033	0.037	0.00	0.00	0.00	0.027	0.6	0.8	-0.3	1.1	0.0	0.0	<-5	>5	<-5		
PepQ	Xaa-Pro dipeptidase	A7ZU52	50315	5.6	103	1.10E-06	0.28	0.08	0.08	0.07	0.07	0.08	0.10	0.13	0.16	0.0	-0.2	-0.5	0.3	0.3	0.7	-0.3	1.0	0.0		
PepD	aminoacyl-histidine dipeptidase	P15288	53110	5.2	78	8.00E-03	0.27	0.11	0.12	0.09	0.10	0.13	0.22	0.20	0.24	0.1	-0.3	-0.2	-0.1	0.8	0.6	-0.3	0.9	0.2		
PepN	aminopeptidase N	B7UN18	99343	5.09	131	6.70E-09	0.22	0.12	0.09	0.05	0.08	0.11	0.13	0.14	0.20	-0.4	-1.3	-0.7	-0.6	-0.4	-0.3	-0.5	0.2	0.5		
Dep	dipeptidyl carboxypeptidase II	Q8XB30	77516	5.35	189	1.10E-14	0.33	0.037	0.016	0.034	0.017	0.01	0.00	0.00	0.00	-1.2	-0.1	1.0	-1.1	0.0	>5	>5	0.0	<-5		
Pre	carboxy-terminal protease	Q321Y5	76514	6.38	105	2.70E-06	0.28	0.031	0.025	0.021	0.028	0.043	0.029	0.032	0.032	-0.3	-0.6	-0.4	-0.1	-0.6	-0.4	0.0	-0.4	0.5		
PmbA	protein PmbA	P0AFK0	48625	5.4	124	9.10E-09	0.35	0.039	0.030	0.033	0.034	0.041	0.045	0.08	0.10	-0.4	-0.2	0.0	-0.2	0.1	1.0	-0.3	1.3	0.1		
ClpP	ATP-dependent Clp protease proteolytic subunit	P0A6G9	23286	5.52	63	4.60E-02	0.3	0.08	0.07	0.11	0.08	0.09	0.10	0.09	0.10	-0.2	0.5	0.5	0.0	0.2	0.0	-0.2	0.2	0.2		
HslV	ATP-dependent protease peptidase subunit	P0A7C0	19138	5.96	79	1.00E-03	0.46	0.030	0.05	0.14	0.035	0.06	0.09	0.14	0.032	0.7	2.2	2.0	0.2	0.6	1.2	2.1	-0.9	1.0		
HslU	ATP-dependent protease ATP-binding subunit	P0A6H6	49677	5.24	166	2.30E-10	0.48	0.10	0.14	0.30	0.12	0.18	0.29	0.38	0.13	0.5	1.6	1.3	0.3	0.7	1.1	1.5	-0.5	0.8		
PepB	peptidase B	B7UGW9	46483	5.49	69	2.60E-03	0.29	0.12	0.09	0.10	0.08	0.12	0.14	0.20	0.17	-0.4	-0.3	0.3	-0.6	0.2	0.7	0.2	0.5	0.0		
PepP	Xaa-Pro aminopeptidase	P15034	50012	5.25	84	8.50E-05	0.27	0.047	0.048	0.037	0.06	0.06	0.05	0.06	0.08	0.0	-0.3	-0.7	0.4	-0.3	0.0	-0.4	0.4	0.4		
PrfC	oligopeptidase A	P27298	77461	5.15	209	2.90E-17	0.41	0.08	0.06	0.00	0.05	0.049	0.08	0.07	0.11	-0.4	<-5	<-5	-0.7	0.7	0.5	-0.7	1.2	-0.7		
<b>Other dehydrogenases (9 proteins)</b>								Total RPM:	0.69	0.66	0.65	0.74	0.61	0.75	0.68	1.12	-0.1	-0.1	-0.2	0.1	0.3	0.2	-0.7	0.9	-0.2	
YdfG	3-hydroxy acid dehydrogenase	Q8X505	27360	5.65	86	2.10E-04	0.4	0.10	0.09	0.11	0.11	0.049	0.10	0.044	0.07	-0.2	0.1	0.0	0.1	1.0	-0.2	-0.7	0.5	-1.0		
YqhD	alcohol dehydrogenase, NAD(P)-dependent	Q46856	42128	5.72	83	4.50E-04	0.32	0.11	0.10	0.12	0.09	0.14	0.14	0.12	0.18	-0.1	0.1	0.4	-0.3	0.0	-0.2	-0.6	0.4	0.3		
AldB	aldehyde dehydrogenase B	P37685	56670	5.44	62	1.30E-02	0.17	0.031	0.027	0.029	0.11	0.037	0.022	0.06	0.16	-0.2	-0.1	-1.9	1.8	-0.8	0.7	-1.4	2.1	0.3		
AldA	lactaldehyde dehydrogenase	P25553	52411	5.07	61	1.80E-02	0.2	0.10	0.10	0.09	0.10	0.047	0.10	0.12	0.36	0.0	-0.2	-0.2	0.0	1.1	1.4	-1.6	2.9	-1.1		
YhhX	predicted oxidoreductase with NAD(P)-binding Rossmann-fold domain	P46853	38912	6.07	131	6.70E-09	0.32	0.046	0.044	0.023	0.038	0.033	0.06	0.044	0.049	-0.1	-1.0	-0.7	-0.3	0.9	0.4	-0.2	0.6	-0.5		

**Table S1 - Quantitative data of individual proteins of *E. coli* BL21 (DE3) growing in defined and complex medium during production of hFGF-2**

Name <sup>1</sup>	Protein name <sup>1</sup>	Uniprot ID <sup>1</sup>	Molecular mass <sup>2</sup> (Da)	Calculated pI value <sup>3</sup>	Mascot Score <sup>2</sup>	Mascot Expect Value <sup>2</sup>	Mascot Seq. Cover. <sup>2</sup>	Defined (DNB) medium				Complex (LB) medium				Log <sub>2</sub> ratio in Defined (DNB) medium				Log <sub>2</sub> ratio in Complex (LB) medium				Log <sub>2</sub> (LB medium/ DNB medium) exponential before IPTG	
								exponential phase before IPTG induction	1h after IPTG induction	5h after IPTG induction	stationary phase without IPTG	exponential phase before IPTG induction	1h after IPTG induction	3.5h after IPTG induction	stationary phase without IPTG	(1h after IPTG/exponential before IPTG)	(5h after IPTG/stationary without IPTG)	(stationary phase before IPTG)	(1h after IPTG/exponential before IPTG)	(3.5h after IPTG/stationary without IPTG)	(stationary phase before IPTG)				
								Relative Protein Mass (RPM) <sup>3</sup> - %																	
Ugd	UDP-glucose 6-dehydrogenase	P76373	43744	6.06	76	1.50E-02	0.33	0.16	0.12	0.11	0.14	0.12	0.13	0.13	0.13	-0.4	-0.5	-0.3	-0.2	0.1	0.1	0.0	0.1	-0.4	
HdhA	7-alpha-hydroxysteroid dehydrogenase	P0AET8	26990	5.22	67	4.30E-03	0.36	0.030	0.048	0.05	0.033	0.05	0.08	0.05	0.07	0.7	0.7	0.6	0.1	0.7	0.0	-0.5	0.5	0.7	
HybC	hydrogenase-2 large chain	P0ACE0	62908	5.84	60	2.30E-02	0.25	0.028	0.038	0.042	0.041	0.041	0.041	0.038	0.040	0.4	0.6	0.0	0.6	0.0	-0.1	-0.1	0.0	0.6	
TrxB	thioredoxin reductase	P0A9P4	34829	5.3	128	3.60E-09	0.48	0.08	0.09	0.08	0.08	0.09	0.08	0.07	0.06	0.2	0.0	0.0	0.0	-0.2	-0.4	0.2	-0.6	0.2	
<b>Oxidoreductases (8 proteins)</b>								Total RPM:	0.39	0.36	0.43	0.65	0.33	0.34	0.39	0.54	-0.1	0.1	-0.6	0.7	0.0	0.2	-0.5	0.7	-0.2
Gor	glutathione reductase	P06715	49084	5.64	78	8.40E-03	0.29	0.028	0.034	0.026	0.07	0.047	0.022	0.028	0.07	0.3	-0.1	-1.4	1.3	-1.1	-0.7	-1.3	0.6	0.7	
NfsA	oxygen-insensitive NADPH nitroreductase	P17117	27069	6.45	117	4.60E-08	0.4	0.09	0.07	0.039	0.06	0.09	0.07	0.05	0.043	-0.4	-1.2	-0.6	-0.6	-0.4	-0.8	0.2	-1.1	0.0	
QueF	NADPH-dependent 7-cyano-7-deazaguanine reductase	C4ZZU9	32909	5.73	131	1.80E-09	0.39	0.05	0.041	0.046	0.08	0.019	0.046	0.05	0.041	-0.3	-0.1	-0.8	0.7	1.3	1.4	0.3	1.1	-1.4	
NemA	N-ethylmaleimide reductase	P77258	39492	5.8	90	2.20E-05	0.38	0.031	0.030	0.039	0.012	0.023	0.00	0.00	0.025	0.0	0.3	1.7	-1.4	<-5	<5	<5	0.1	-0.4	
SthA	soluble pyridine nucleotide transhydrogenase	A7ZUI2	51984	6.08	109	6.60E-06	0.22	0.08	0.07	0.07	0.06	0.07	0.09	0.10	0.13	-0.2	-0.2	0.2	-0.4	0.4	0.5	-0.4	0.9	-0.2	
CueO	blue copper oxidase CueO	P36649	53557	6.07	63	4.40E-04	0.18	0.033	0.028	0.016	0.034	0.025	0.030	0.029	0.049	-0.2	-1.0	-1.1	0.0	0.3	0.2	-0.8	1.0	-0.4	
Bfr	bacterioferritin, iron storage and detoxification protein	P0ABD3	18483	4.69	156	2.10E-11	0.03	0.044	0.05	0.13	0.28	0.06	0.08	0.10	0.13	0.2	1.6	-1.1	2.7	0.4	0.7	-0.4	1.1	0.4	
WrB	flavoprotein wrB	C4ZQD2	20832	5.59	85	7.60E-05	0.53	0.03	0.038	0.06	0.05	0.00	0.00	0.028	0.05	0.3	1.0	0.3	0.7	0.0	>5	-0.8	>5	<5	
<b>Hydroperoxide reductases and superoxide dismutase (5 proteins)</b>								Total RPM:	1.26	1.46	1.16	1.92	0.83	0.88	0.75	1.84	0.2	-0.1	-0.7	0.6	0.1	-0.1	-1.3	1.1	-0.6
AhpF	alkyl hydroperoxide reductase subunit F	B3XD66	56496	5.47	101	6.70E-06	0.28	0.08	0.11	0.10	0.10	0.11	0.10	0.08	0.15	0.5	0.3	0.0	0.3	-0.1	-0.5	-0.9	0.4	0.5	
AhpC	alkyl hydroperoxide reductase subunit C	P0AE08	20862	5.03	108	3.60E-07	0.7	0.79	0.89	0.85	0.85	0.53	0.57	0.46	1.10	0.2	-0.3	-0.8	0.5	0.1	-0.2	-1.3	1.1	-0.6	
Tpx	thiol peroxidase	P0A864	17995	4.75	103	1.10E-06	0.48	0.13	0.18	0.11	0.20	0.44	0.49	0.07	0.22	0.5	-0.2	-0.9	0.6	0.2	0.7	-1.7	2.3	-1.6	
SodB	superoxide dismutase [Fe]	P0AGD5	21310	5.58	125	2.60E-08	0.87	0.13	0.19	0.21	0.45	0.00	0.028	0.06	0.21	0.5	0.7	-1.1	1.8	-0.1	1.0	-1.8	2.8	-2.1	
KatG	catalase-peroxidase	B1XBA8	80031	5.14	125	7.20E-09	0.2	0.13	0.09	0.09	0.07	0.13	0.03	0.01	0.16	-0.5	-0.5	0.4	-0.9	0.1	-0.6	-1.0	0.4	-0.1	
<b>DNA protection and repair (6 proteins)</b>								Total RPM:	0.84	0.85	0.58	0.84	0.52	0.21	0.36	1.29	0.0	-0.5	-0.5	0.0	-0.9	-0.5	-1.8	1.3	-0.7
Ssb	single-stranded DNA-binding protein	P0AGE0	18963	5.44	132	1.40E-09	0.41	0.046	0.045	0.037	0.047	0.024	0.00	0.044	0.06	0.0	-0.3	-0.3	-0.3	0.0	<-5	0.9	-0.4	1.3	-0.9
Dps	DNA starvation/stationary phase protection protein Dps	P0ABT3	18684	5.72	142	5.30E-10	0.66	0.032	0.015	0.09	0.21	0.031	0.00	0.020	0.42	-1.1	1.5	-1.2	2.7	<-5	-0.6	-4.4	3.8	0.0	
UspA	universal stress protein A	P0AED2	16113	5.11	110	8.30E-07	0.79	0.07	0.06	0.05	0.15	0.09	0.07	0.08	0.16	-0.2	-0.5	-1.6	1.1	-0.4	-0.2	-1.0	0.8	0.4	
UspG	universal stress protein G	P39177	15925	6.03	101	1.80E-06	0.52	0.06	0.041	0.09	0.10	0.10	0.00	0.00	0.00	-0.5	0.6	-0.2	0.7	<-5	<5	0.0	<5	0.7	
SodA	superoxide dismutase [Mn]	P00448	22952	6.44	64	3.60E-02	0.44	0.57	0.64	0.19	0.29	0.18	0.12	0.17	0.59	0.2	-1.6	-0.6	-1.0	-0.6	-0.1	-1.8	1.7	-1.7	
PolA	DNA polymerase I	P00582	103168	5.4	119	2.40E-08	0.25	0.06	0.049	0.12	0.045	0.09	0.08	0.048	0.06	-0.3	1.0	1.4	-0.4	-0.2	-0.9	-0.3	-0.6	0.6	
<b>Unclassified proteins (7 proteins)</b>								Total RPM:	0.63	0.58	0.53	0.59	0.47	0.36	0.39	0.35	-0.1	-0.2	-0.2	-0.1	-0.4	-0.3	0.2	-0.4	-0.4
GyrB	DNA gyrase subunit B	P0AES6	90153	5.68	135	2.70E-09	0.24	0.29	0.23	0.24	0.27	0.12	0.07	0.08	0.046	-0.3	-0.3	-0.2	-0.1	-0.8	-0.6	0.8	-1.4	-1.3	
MreB	regulator of FtsI, penicillin binding protein 3, septation function	Q8X9C9	37140	5.19	101	6.70E-06	0.34	0.05	0.08	0.07	0.07	0.09	0.08	0.08	0.08	0.7	0.5	0.0	0.5	-0.2	-0.2	0.0	-0.2	0.8	
FtsZ	cell division protein FtsZ	P0A9A8	40299	4.65	138	1.30E-09	0.39	0.07	0.07	0.044	0.06	0.06	0.09	0.07	0.09	0.0	-0.7	-0.4	-0.2	0.6	0.2	-0.4	0.6	-0.2	
RsmC	ribosomal RNA small subunit methyltransferase C	B1XF10	37829	6	156	5.70E-12	0.51	0.017	0.019	0.029	0.034	0.040	0.034	0.042	0.05	0.2	0.8	-0.2	1.0	-0.2	0.1	-0.3	0.3	1.2	
Can	carbonic anhydrase	P61517	25366	6.16	89	9.80E-05	0.35	0.10	0.10	0.06	0.08	0.13	0.09	0.10	0.05	0.0	-0.7	-0.4	-0.3	-0.3	-0.5	-0.4	1.0	0.4	
FrsA	fermentation/respiration switch protein	Q8FKM5	47336	6.47	75	2.50E-03	0.24	0.030	0.021	0.015	0.016	0.029	0.00	0.018	0.032	-0.5	-1.0	-0.1	-0.9	<-5	-0.7	-0.8	0.1	0.0	

**Table S1 - Quantitative data of individual proteins of *E. coli* BL21 (DE3) growing in defined and complex medium during production of hFGF-2**

Name <sup>1</sup>	Protein name <sup>1</sup>	Uniprot ID <sup>1</sup>	Molecular mass <sup>2</sup> (Da)	Calculated pI value <sup>3</sup>	Mascot Score <sup>2</sup>	Mascot Expect Value <sup>2</sup>	Mascot Seq. Cover. <sup>2</sup>	Defined (DNB) medium				Complex (LB) medium				Log <sub>2</sub> ratio in Defined (DNB) medium				Log <sub>2</sub> ratio in Complex (LB) medium				Log <sub>2</sub> (LB medium/ DNB medium) exponential before IPTG	
								exponential phase before IPTG induction	1h after IPTG induction	5h after IPTG induction	stationary phase without IPTG	exponential phase before IPTG induction	1h after IPTG induction	3.5h after IPTG induction	stationary phase without IPTG	(1h after IPTG/ exponential before IPTG)	(5h after IPTG/ stationary without IPTG)	(stationary phase before IPTG)	(1h after IPTG/ exponential before IPTG)	(3.5h after IPTG/ stationary without IPTG)	(stationary phase before IPTG)	(1h after IPTG/ exponential before IPTG)	(3.5h after IPTG/ stationary without IPTG)	(stationary phase before IPTG)	
								Relative Protein Mass (RPM) <sup>3</sup> - %																	
YeaG	uncharacterized protein YeaG	P0ACY3	74776	5.63	66	5.20E-03	0.1	0.07	0.06	0.07	0.06	0.00	0.00	0.00	0.00	-0.2	0.0	0.2	-0.2	0.0	0.0	0.0	0.0	<-5	
<b>Uncharacterized proteins (14 proteins)</b>																									
YhbW	uncharacterized protein YhbW	P0ADV5	37163	5.99	89	2.90E-05	0.33	0.028	0.028	0.019	0.027	0.049	0.041	0.036	0.047	0.0	-0.6	-0.5	-0.1	-0.3	-0.4	-0.4	-0.1	0.8	>5
GalF	UTP-glucose-1-phosphate uridylyltransferase	P0AAB6	32979	5.73	61	1.90E-02	0.26	0.00	0.00	0.00	0.00	0.035	0.040	0.027	0.026	0.0	0.0	0.0	0.0	0.2	-0.4	0.1	-0.4	0.4	0.2
YbeZ	putative ATP-binding protein in Pho regulon	Q0T6R9	39129	5.71	84	3.80E-04	0.41	0.06	0.06	0.11	0.06	0.07	0.10	0.11	0.044	0.0	0.9	0.9	0.0	0.5	0.7	1.3	-0.7	0.2	0.3
YicC	UPF0701 protein YicC	P23839	33211	5.1	86	6.20E-05	0.43	0.036	0.033	0.029	0.042	0.045	0.040	0.028	0.032	-0.1	-0.3	-0.5	0.2	-0.2	-0.7	-0.2	-0.5	0.0	0.0
YajQ	UPF0234 protein YajQ	C4ZTI3	18333	5.96	178	3.60E-14	0.64	0.00	0.034	0.00	0.031	0.00	0.00	0.00	0.00	>5	0.0	<5	>5	0.0	0.0	0.0	0.0	0.0	0.0
YbgI	UPF0135 protein YbgI	P0AFP6	26990	5.07	89	2.70E-05	0.46	0.041	0.038	0.049	0.043	0.05	0.09	0.047	0.041	-0.1	0.3	0.2	0.1	0.8	-0.1	0.2	-0.3	0.3	0.3
YjgR	uncharacterized protein YjgR	P39342	54355	5.91	84	9.30E-05	0.28	0.016	0.011	0.037	0.012	0.012	0.00	0.00	0.024	-0.5	1.2	1.6	-0.4	<5	<5	<5	1.0	-0.4	0.4
YdcJ	uncharacterized protein ydcJ	P76097	51353	5.35	58	3.60E-02	0.34	0.08	0.031	0.08	0.039	0.018	0.031	0.022	0.10	-1.4	0.0	1.0	-1.0	0.8	0.3	-2.2	2.5	-2.2	0.2
YegB	uncharacterized protein YegB	P29013	61117	5.66	69	2.70E-04	0.15	0.00	0.00	0.00	0.028	0.00	0.00	0.00	0.046	0.0	0.0	<5	>5	0.0	0.0	<5	>5	0.0	0.0
YgaU	uncharacterized protein YgaU	P0ADE6	16053	5.71	70	2.10E-03	0.26	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.16	0.0	0.0	<5	>5	0.0	0.0	<5	>5	0.0	0.0
YfbU	hypothetical protein	P0A8W8	19638	6.06	111	6.60E-07	0.5	0.09	0.07	0.05	0.09	0.037	0.00	0.00	0.028	-0.2	-0.7	-0.8	0.2	<5	<5	<5	-0.4	-1.1	0.4
Lfil	lateral flagellar export/assembly protein Lfil	B1LHP6	48085	6.53	77	1.60E-03	0.32	0.07	0.06	0.032	0.06	0.07	0.07	0.08	0.16	0.0	-1.1	-0.9	-0.2	0.0	0.2	-1.0	1.2	0.0	0.0
YhdH	putative quinone oxidoreductase YhdH	P26646	34873	5.63	111	1.80E-07	0.33	0.031	0.04	0.03	0.026	0.019	0.034	0.035	0.06	0.5	0.7	0.9	-0.3	0.8	0.9	-0.8	1.7	-0.7	0.4
YjjK	uncharacterized ABC transporter ATP-binding protein YjjK	P0A9W3	62518	5.43	118	3.60E-08	0.34	0.047	0.06	0.02	0.07	0.11	0.09	0.09	0.12	0.4	-0.2	-0.2	0.0	-0.3	-0.3	-0.4	0.1	1.2	0.4

<sup>1</sup> Name, protein name and Uniprot ID are according to Uniprot database (<http://www.uniprot.org/>). Functional classifications are mostly according to EcoCyc database (<http://ecocyc.org/>). The classifications are confirmed by KEGG database (<http://www.genome.jp/kegg/>).

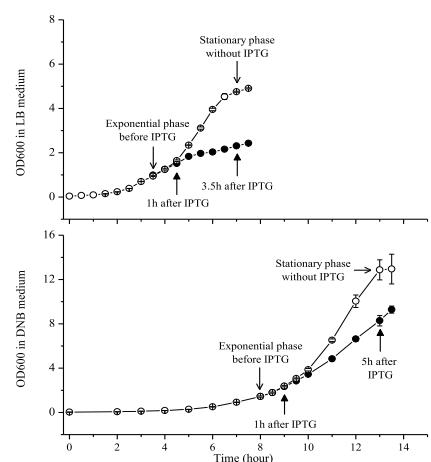
<sup>2</sup> Molecular mass, calculated pI, Mascot score, expect and sequence coverage are from MASCOT search program (Matrix Science, UK, <http://www.matrixscience.com/>), based on the annotated *E. coli* genome [Uniprot (<http://www.uniprot.org/>) serving as database].

<sup>3</sup> Relative Protein Mass (RPM)-%: Each spot's intensity was normalized by the whole spots intensity on each Coomassie blue stained 2D gel. The corresponding average from duplicate gels was used indicating each spots' protein fraction (% of Relative Protein Mass (RPM)). The RPM (%) of all spots representing the same protein was used for indicating the abundance of the corresponding protein.

<sup>4</sup> LpdA is assigned to both groups, pyruvate decarboxylation to acetyl CoA and TCA cycle.

<sup>5</sup> A truncated outer membrane protein A is identified in the NCBI database (<http://www.ncbi.nlm.nih.gov/>).

### Sampling times for proteome analyses



*E. coli* BL21 (DE3) with the plasmid pET-29c-hFGF-2 was cultivated in Luria-Bertani (LB) medium and Defined Non-inducing Broth (DNB). Cultivations were carried out in 1.8 L Fernbach flasks with three baffles containing 200 ml medium at 30°C and 180 rpm. When the cultures reached the mid-exponential phase, IPTG was added to a final concentration of 0.25 mmol L<sup>-1</sup> to start hFGF-2 production. Non-producing control cells were grown under identical conditions without addition of IPTG. Culture samples, as indicated by arrows in the Figure below, were centrifuged at 17,000 g and 4°C for 3 min. After removal of the supernatant, cell pellets were stored at -80°C until further analysis.