Supporting Information: Enhanced biosynthetically directed fractional carbon-13 enrichment of proteins for backbone NMR Assignments

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Supplemental Information Contains Three Figures with explanatory notes:

Figure S.1. Cell density monitoring for 4 g/L glucose levels as a function of induction time.

Figure S.2 : The suitability of biosynthetically fractionally enriched protein samples for typical 3D-NMR experimentation is tested by performing 3D-HNCA and 3D-HN(CO)CA.

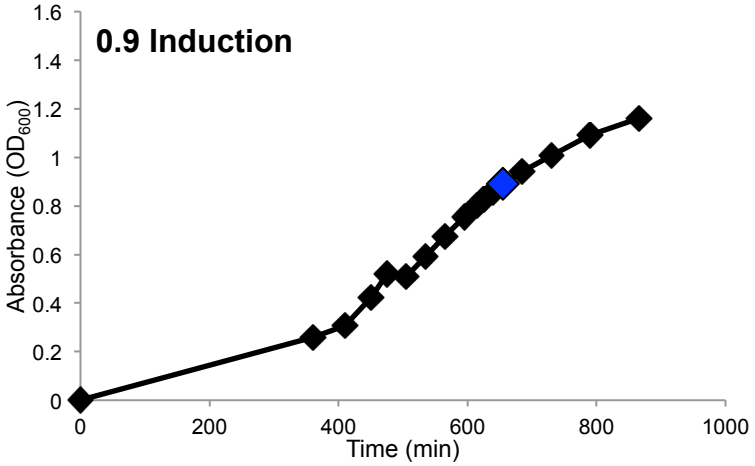
Figure S.3 Comparison of 10%-BDF and *opt*-10%-BDF when thiamine is supplemented at the induction point.

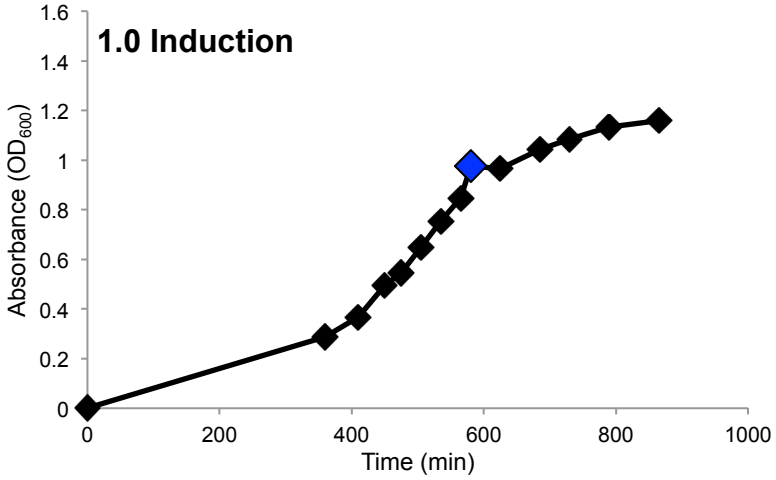
Figure S.1. Cell density monitoring for 4 g/L glucose levels as a function of induction time. A series of tests was conducted to probe bacterial growth as a function of the time of induction, as measured by the optical absorbance at 600 nm. A 25 mL overnight culture was inoculated into 1 L of minimal media containing 4 g glucose (M9, see experimental details in text), which was allowed to grow for approximately 1 hour before being decanted into smaller flasks containing 100 mL each, which grew at 37 ˚C in an incubating shaker (180 rpm). Absorbance values for six trials were then monitored (e.g. OD_{600}), with 10 μL IPTG added at distinct stages of growth as indicated on the individual panels below. In all figures below, the blue symbol indicates the time of induction.

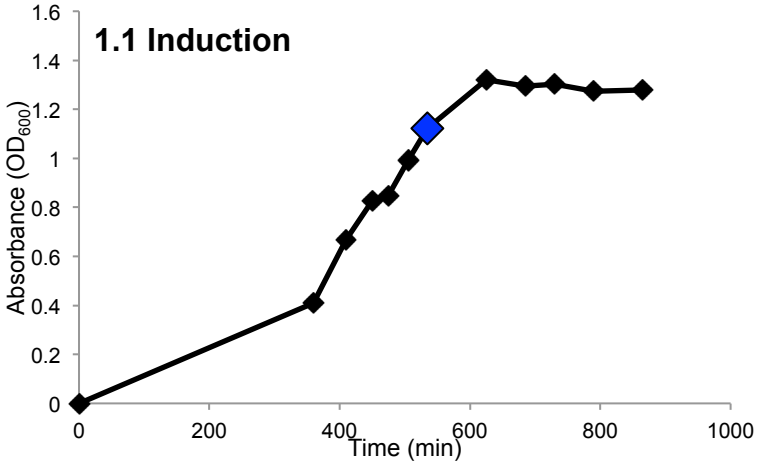
The goal of this test is to characterize the time of optimal induction for fractional isotope enrichment of cultures which employ 4 g glucose / L. Specifically, our results (**Figures 2** and **3**) support that the fractional quantity of ^{13}C-enriched glucose should be added before bacteria enter stationary phase so that cultures have consumed as much natural abundance glucose as possible but are still metabolizing glucose strongly. Early induction (OD_{600} = 0.9) shows cultures proliferating significantly after induction, consistent with expectations that large amounts of glucose are still available at this cell density for 4 g/L glucose conditions.

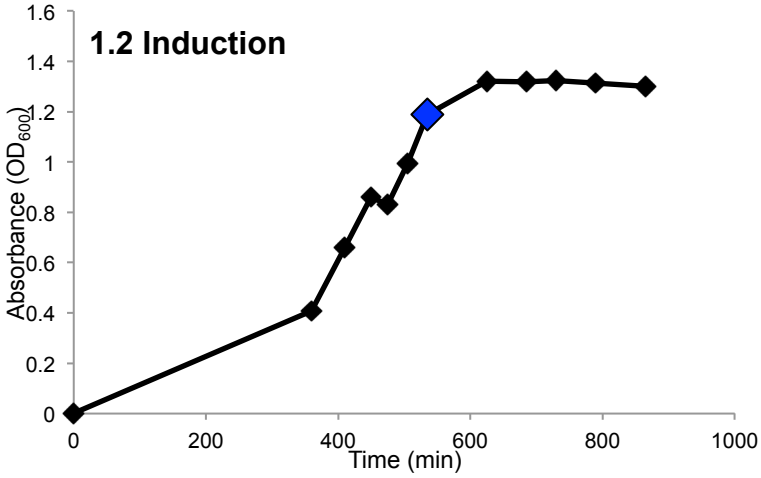
However, for induction times as early as OD_{600}= 1.0, we observe cultures rolling over in to stationary phase shortly after induction, supporting that these conditions should help to minimize the background glucose levels during growth. Indeed, in two separate trials reported in the main text, approximately 18% ^{13}C-isotope incorporation was measured for adding 10%$^{w/w}$ ^{13}C-glucose at either OD_{600} = 1.0 or at OD_{600} = 1.1 (see **table 1** in text).

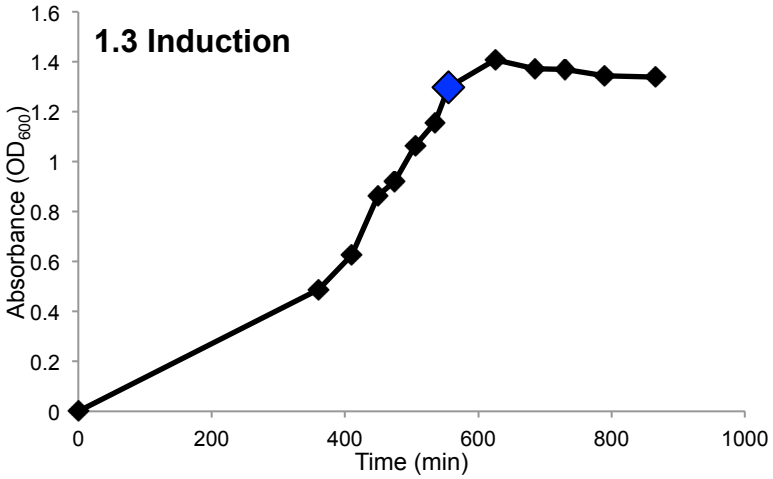
Notice that cultures show the onset of stationary phase prior to induction when allowed to grow to OD_{600}=1.2-1.4, suggesting these cell densities would not be favorable for enhanced isotope incorporation (e.g. **Figure 4**).


0.9 Induction
Absorbance (OD600)
Time (min)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
0
200
400
600
800
1000


1.0 Induction
Absorbance (OD600)
Time (min)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
0
200
400
600
800
1000


1.1 Induction
Absorbance (OD600)
Time (min)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
0
200
400
600
800
1000


1.2 Induction
Absorbance (OD600)
Time (min)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
0
200
400
600
800
1000


1.3 Induction
Absorbance (OD600)
Time (min)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
0
200
400
600
800
1000

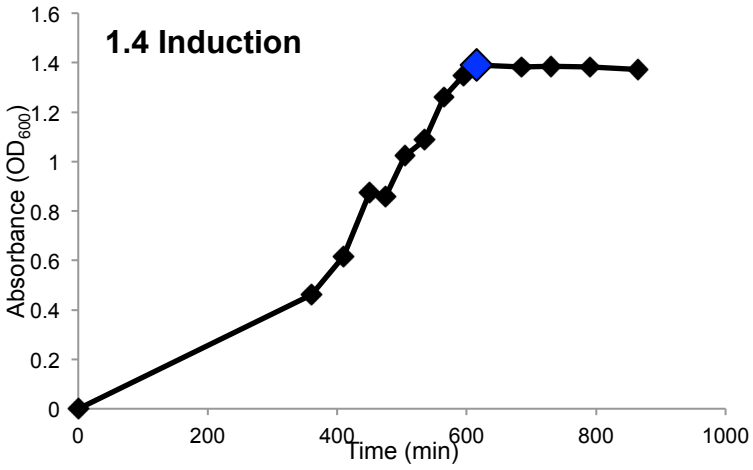
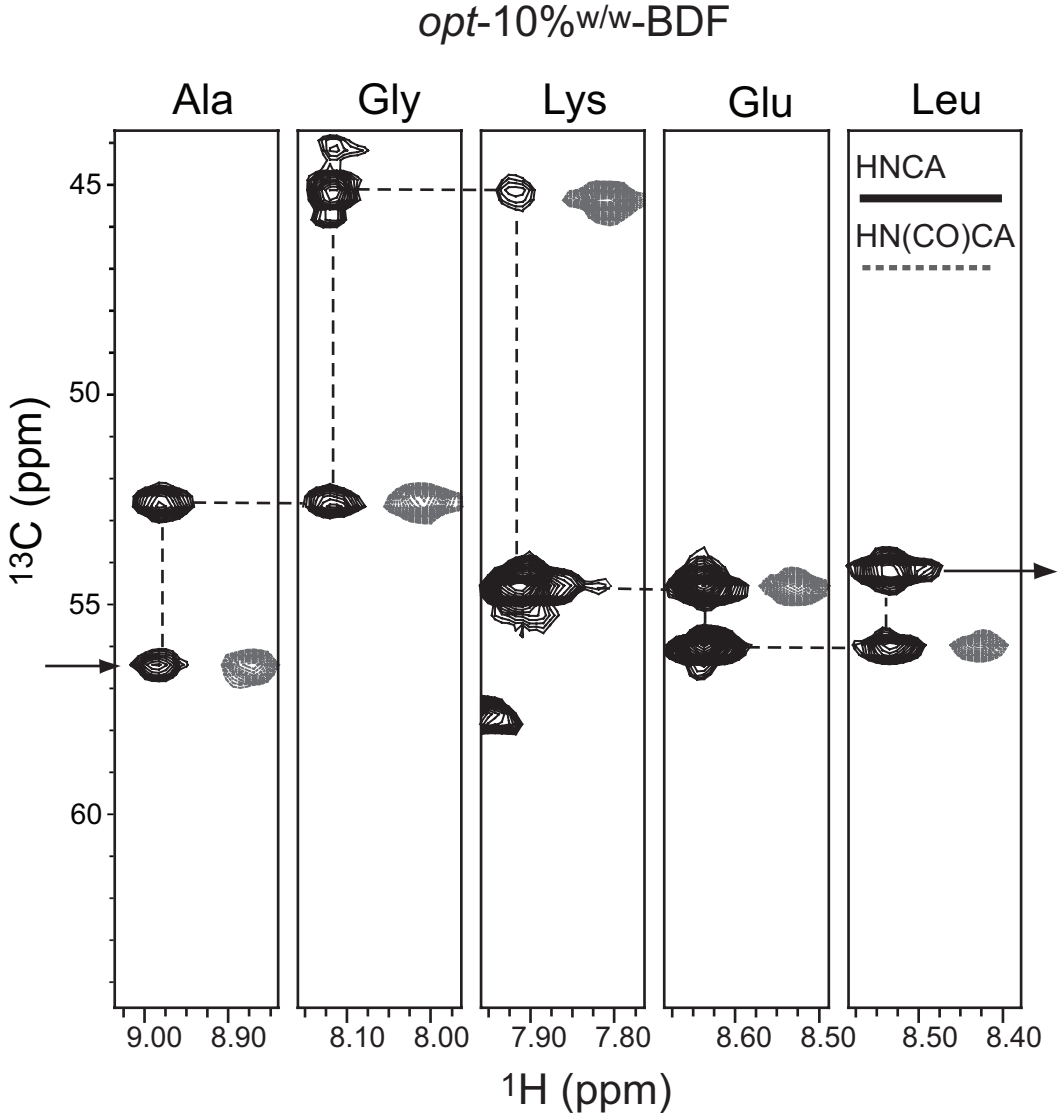

1.4 Induction
Absorbance (OD600)
Time (min)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
0
200
400
600
800
1000

Figure S.2 : The suitability of biosynthetically fractionally enriched protein samples for typical 3D-NMR experimentation is also tested by performing 3D-HNCA and 3D-HN(CO)CA on a 0.4 mM *opt*-10%$^{w/w}$-BDF (comparable to ca. 18% carbon-13 incorporation) sample utilizing a 600 MHz spectrometer and a room-temperature probe in Shigemi 5 mm NMR tubes.



Specifically, a high concentration of $^{13}C'$-$^{13}C\alpha$ spin labels is demonstrated by the HN(CO)CA (light contour lines) experiment which is superimposed with the HNCA (dark contour lines) experiment. The 3D-HNCA (2 scans per increment nonuniformly spanning a $64_{[13C]}$ x $40_{[15N]}$ matrix with 500 samples, sw_{13C}=30 ppm, sw_{15N}=35 ppm, 2.0 second recycle time, 150 ms acquisition) was acquired in only 2.6 hrs with non-uniform sampling and processed via maximum entropy reconstruction. The HN(CO)CA (16 scans per increment non-uniformly sampling a $64_{[13C]}$ x $40_{[15N]}$ matrix with 500 samples, sw_{13C}=30 ppm, sw_{15N}=35 ppm, 2.0 second recycle time, 150 ms acquisition) was acquired in 19 hrs and processed via maximum entropy reconstruction using the Rowland NMR Toolkit (RNMRTK).

Figure S.3 Comparison of 10%-BDF and *opt*-10%-BDF when thiamine is supplemented at the induction point. Both cultures were induced identically at OD_{600}=0.8, however the *opt*-10%-BDF sample received 0.2 g *u*-^{13}C-glucose and 1 ml 0.1% thiamine at induction. The observed ^{13}C incorporation in the *opt*-BDF sample is 17%, consistent with prior *opt*-10%-BDF samples (see manuscript body) and indicates that this strategy did not appear to improve the utilization in protein synthesis of the 0.2 g ^{13}C glucose added at induction. This single test does not rule out that thiamine could still be a useful additive in *opt*-BDF strategies, but indicates that more work is needed, for example to test different levels of thiamine additions. Each sample was adjusted to a concentration of 0.8 mM. Each spectrum was acquired with 512 transients to facilitate accurate integration.