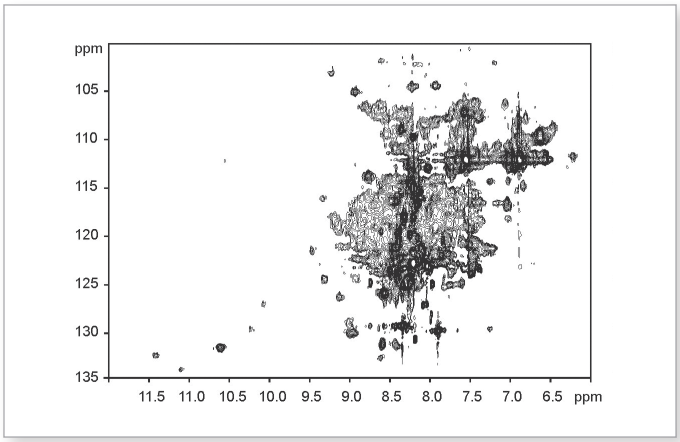
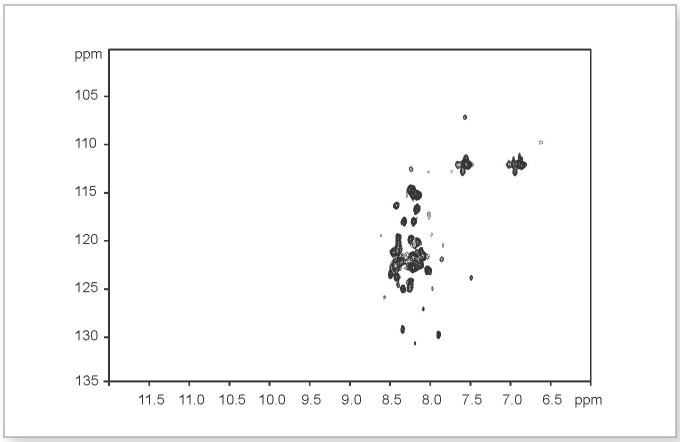
BioExpress® 6000 Mammalian Cell Growth Media

CIL is pleased to announce these recent NMR results of ^{15}N or ^{13}C,^{15}N-labeled rhodopsin obtained in collaboration with Prof. Harald Schwalbe, Karla Werner and Prof. Judith Klein-Seetharaman at the Goethe University in Frankfurt, Germany. The CIL media, labeled with either ^{15}N or ^{13}C; ^{15}N Gly, Lys, Leu, Gln, Ser, Thr, Val and Trp, was used to express rhodopsin from the HEK293 mammalian cell system.



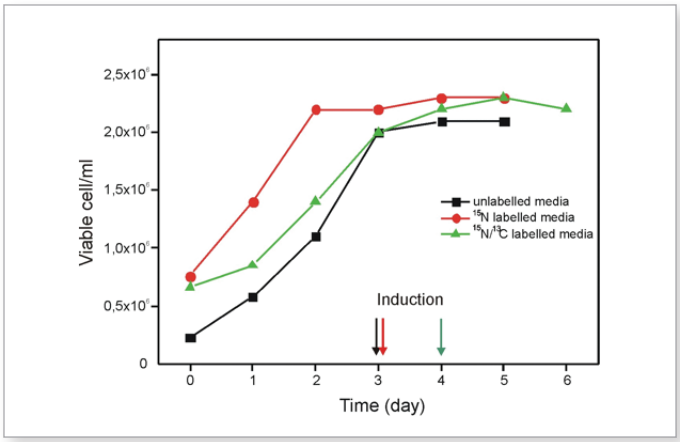


^{1}H; ^{15}N HSQC spectrum plotted at different threshhold levels of 200 µM sample of rhodospin expressed in CIL's ^{15}N-labeled media. The tryptophan sidechain signals are clearly visible, and the protein is folded in its native conformation. The broad peaks observed are expected for a large membrane protein. Also observed are some very sharp and intense peaks, which are believed to arise from the flexible C-terminus.

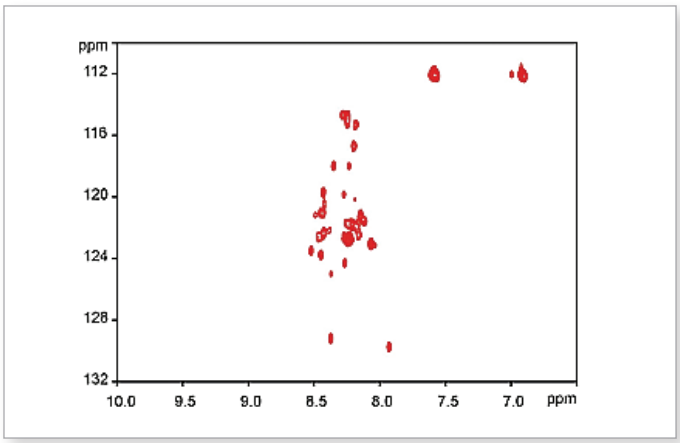
• Labeled amino acids: GKLQSTVW
(^{15}N and $^{15}N/^{13}C$)

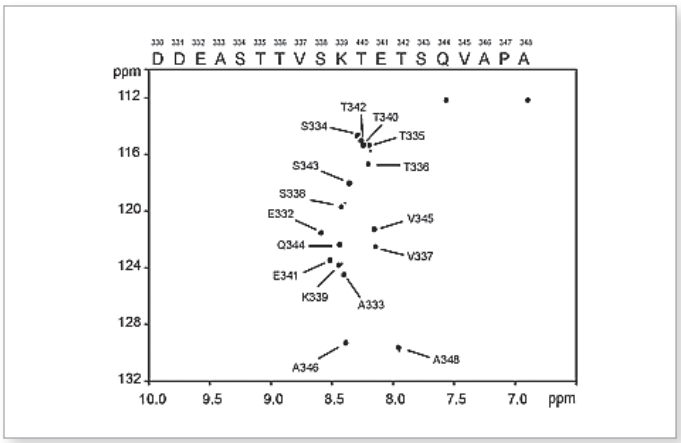
Amino acid composition:

Ala (A)	29	8.3%
Arg (R)	7	2.0%
Asn (N)	15	4.3%
Asp (D)	5	1.4%
Cys (C)	10	2.9%
Gln (Q)	12	3.4%
Glu (E)	17	4.9%
Gly (G)	23	6.6%
His (H)	6	1.7%
Ile (I)	22	6.3%
Leu (L)	28	8.0%
Lys (K)	11	3.2%
Met (M)	16	4.6%
Phe (F)	31	8.9%
Pro (P)	20	5.7%
Ser (S)	15	4.3%
Thr (T)	27	7.8%
Trp (W)	5	1.4%
Tyr (Y)	18	5.2%
Val (V)	31	8.9%
Labeled:	**170**	**48.8%**

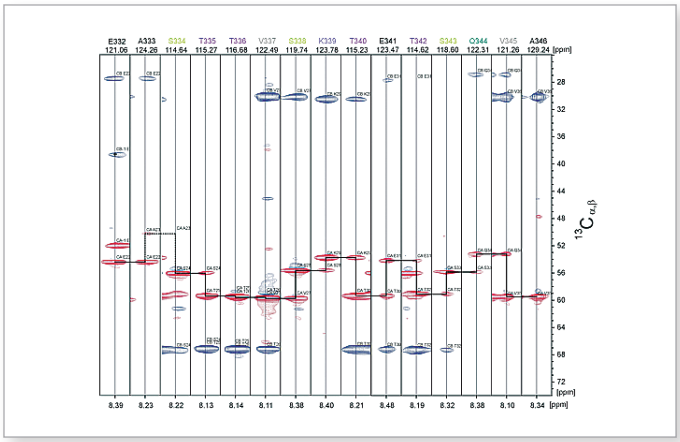


This graph shows the number of viable cells per mL of culture for differently labeled CIL media. Cells are induced on day 3 or 4 and harvested 2 days later. No differences in cell densities are seen so far. Protein yield is in all cases is approx. 2.2 mg/L cell culture.

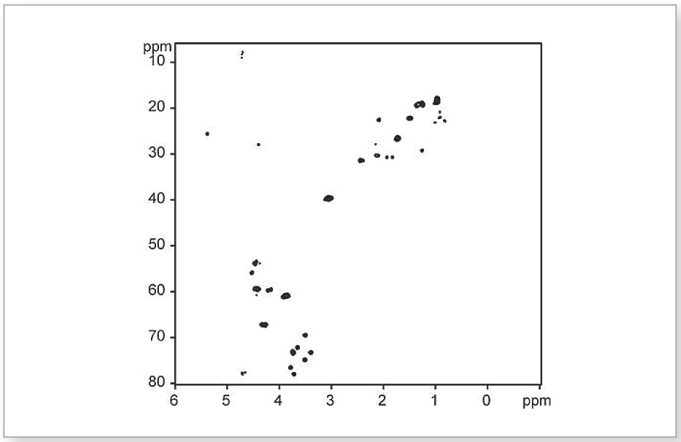




In order to prove if some of the sharp resonances in rhodopsin originate from the C-terminus, a peptide representing the last 19 amino acids of rhodopsin was synthesized, measured and assigned (right spectrum). The rhodopsin HSQC (left spectrum) shows very similar chemical shifts for many of the observed peaks. To further confirm this result the C-terminus of rhodopsin itself was assigned by running an HNCACB on $^{13}C/^{15}N$-labeled protein (below).



Strip plots of residue E332 to residue A346 extracted from an HNCACB spectrum run on $^{13}C/^{15}N$-labeled rhodopsin. Each strip shows resonances for the CA and the CB of the respecting residue and the preceding residue. Horizontal lines connect strips of a certain residue between the i and the i-1 strip, while vertical lines connect the i peak and the i-1 peak within one strip. Connected are only the CA resonances. Dashed lines show expected signals, but not seen because of not present isotope labeling.



^{13}C HSQC spectrum of ^{13}C, ^{15}N-labeled rhodopsin showing the sharper peaks due to the more flexible region of the spectrum.

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