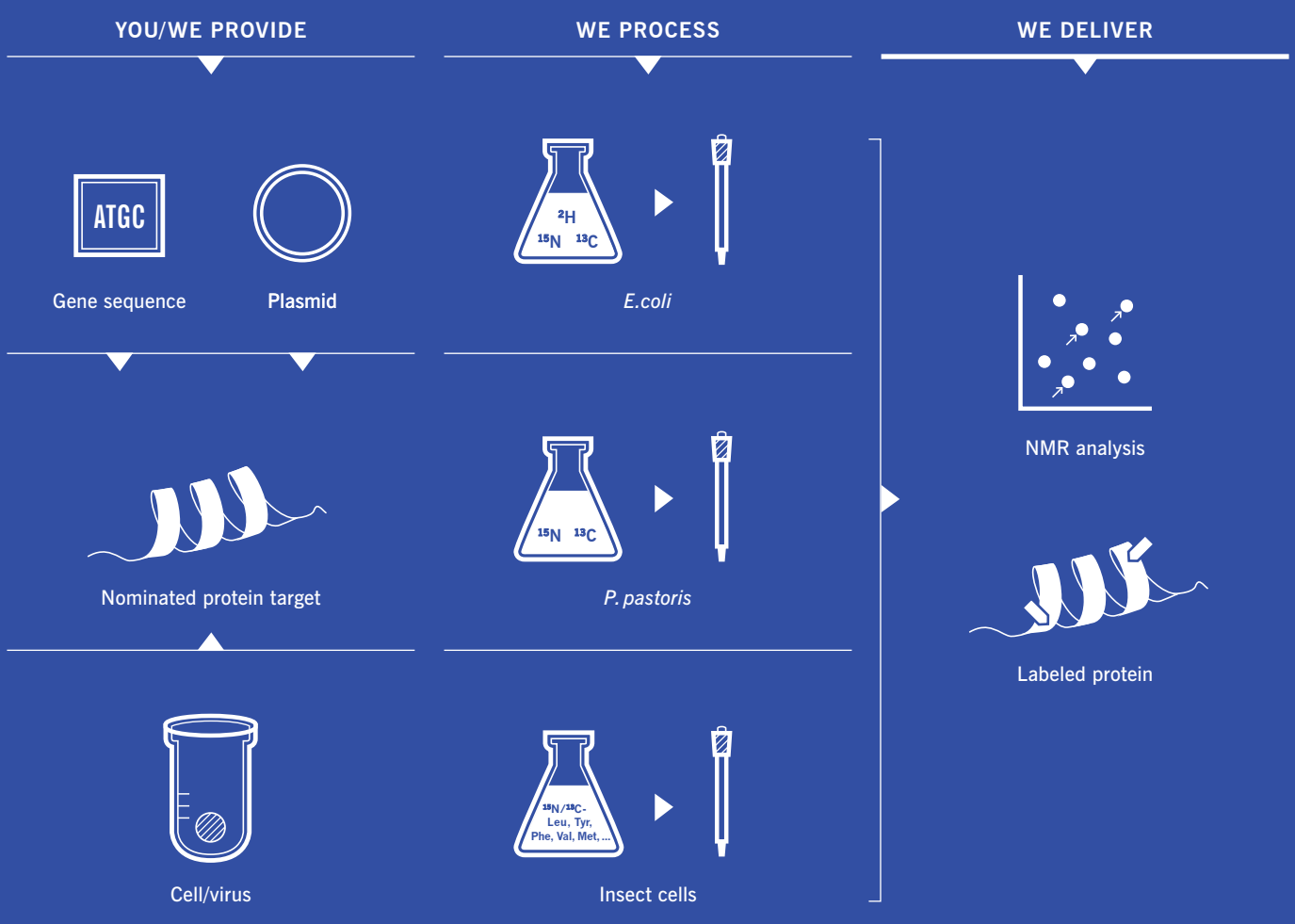
evotec

'RESEARCH NEVER STOPS'

info@evotec.com *www.evotec.com*

ISOTOPE-LABELED PROTEINS FOR NMR

- High-yield production of isotope-labeled protein for NMR
- Optimised expression protocols for prokaryotic and eukaryotic expression
- Bespoke labeling strategies to facilitate specific NMR assays
- State of the art purification and protein analysis
- Short turn-around times to obtain high-quality, labeled protein at low cost
- Over ten years experience in isotope-labeling & a track record of >100 labeled proteins



evotec

'RESEARCH NEVER STOPS'

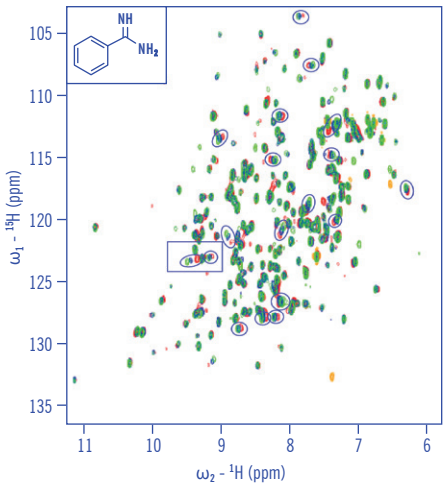
info@evotec.com *www.evotec.com*

IN-HOUSE OPTIMISED CULTURE MEDIA AND PROTOCOLS FOR COST-EFFICIENT EXPRESSION OF ISOTOPE LABELED PROTEINS:

	E. coli	*P. pastoris*	Insect cells	Mammalian cells
Ubiquitous labeling	^{15}N, ^{13}C, ^{2}H	^{15}N, ^{13}C	^{15}N, ^{13}C-labeling possible, but affiliated with high media costs	
Site-specific labeling*	^{13}C- Val, Leu/ Ile using α-keto -isovalerate/ -butyrate	–	^{15}N- and/or ^{13}C for: Val, Leu, Met, Tyr, Phe, Ile, His, Trp, Thr	–
Media properties	NH^4 as N-source, glucose *(E. coli)* and methanol *(P. pastoris)* as C-source, dedicated trace metal and vitamin mixes		taylor-made minimal media	–

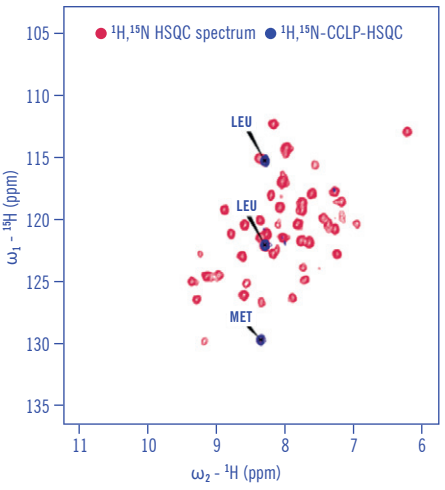
CASE STUDY 1

- Expression of a ^{15}N-labeled serine protease in Pichia pastoris was optimised, e.g. expression time / temperature
- The purified protease was submitted to an NMR fragment screen using ^{15}N-HSQC
- Active-site ^{1}H-^{15}N-resonances could be identified by analysing chemical shift perturbations induced by benzamidine binding:



CASE STUDY 2

- Expression of a ^{15}N/^{13}C-Met/Leu-labeled kinase in insect cells
- Due to the presence of a unique Leu-Met dipeptide, the ^{1}H-^{15}N-signal of the gatekeeper Met could be identified using CCLP-HSQC (see below). This facilitated compound binding studies where X-ray crystallography failed



Evotec Protein Production Services

- *Highly experienced team and state-of-the-art technology platform for membrane protein production, isotope-labeled protein preparation, high throughput protein purification and structural biology services*
- *Direct communication with project scientists and full access to raw data*
- *Reagent production services can be accessed as a standalone function or as integral part of wider collaborative programmes*
- *Links to key academic groups from Oxford University & Diamond light source, UK*