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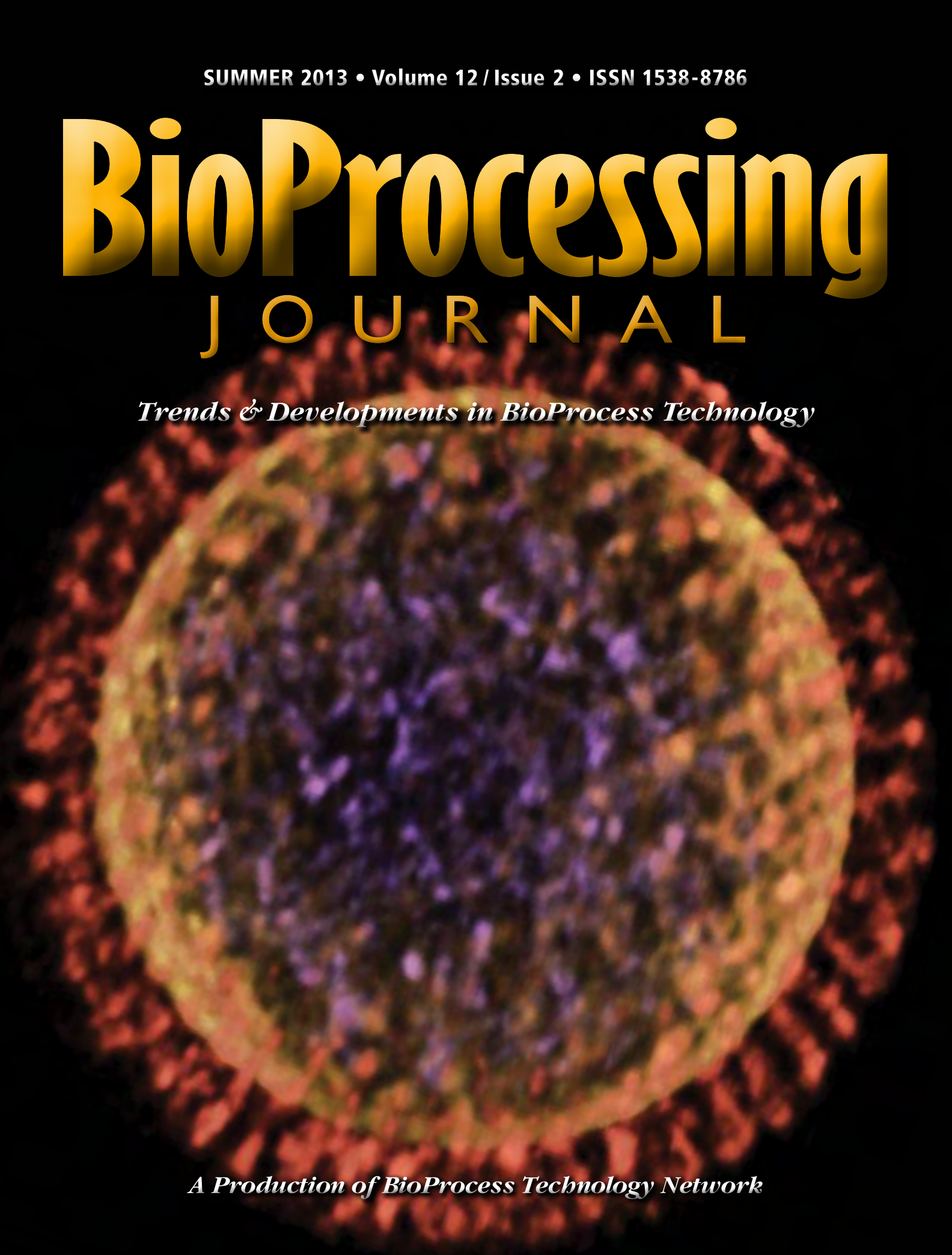
Trends & Developments in BioProcess Technology



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TRENDS & DEVELOPMENTS IN BIOPROCESS TECHNOLOGY

An Overview of Metal–Peptide Complexes Used in Biomedical Research

By ARCHANA GANGAKHEDKAR

Metal peptide complexes play an important role in understanding the mechanisms of complex diseases at a molecular level. Peptides are good chelators for metal ions and can form important tools in the drug and medical device industries. Transition metal ions are characterized by their ability to assume different oxidation states and serve as centers for metalloenzymes that catalyze electron transfers. Some transition metals act as Lewis acids and become an active site on enzymes and also catalyze reactions (*e.g.*, peptidases, phosphatases). Metal peptide complexes are involved in Alzheimer's disease (AD) and are used as cancer diagnostics.

Many biomolecules (molecules produced by living cells) like metabolites, hormones, and vitamins are capable of forming strong, multidentate metal ion complexes. Species in the plant and animal kingdoms synthesize specific metal ion chelators that bind to essential metals with extremely high affinities. Plants and animals including humans possess a number of unique biomolecules that can coordinate metal ions. Peptide conjugation is a widely used and effective method for improving both cellular and nuclear entry of a variety of molecules.

Rhodium Metal Peptide Complexes

Researchers at Ruhr University and UC Berkeley have established a solid foundation for the development of better medicines. They used metal peptide complexes to modify biomolecules like peptide hormones.[1] In some areas of research, bioconjugation of the metal peptide complexes is proving to be a novel approach for drug discovery. A peptide hormone can generate signals for sensations like pain when it couples with receptors in the body and these signals are transported to different cells.

To improve the therapeutic value of these peptide hormones, chemical modifications are possible at specific sites. Researchers at Ruhr and UC Berkeley have successfully reacted the precious metal rhodium (Rh) with the amino acid tyrosine. The formation of the coordination complex of rhodium is highly selective—the rhodium compound is coordinated with the phenolic ring of tyrosine in spite of the presence of other reactive groups. They demonstrated this as a chemoselective reaction of tyrosine-containing G-protein coupled receptor (GPCR) peptides with (Cp*Rh [H_2O] 3) (OTf) 2 and water at room temperature. Researchers used three GPCR peptides with tyrosine positioned at different locations, and it was noted that all other functional groups present in peptides like amino, carboxyl, disulfide, phenyl, and indole were not prominent sites of reactivity for the Cp*Rh tris aqua complex. The structure of the resulting metal peptide complex was clarified by nuclear magnetic resonance (NMR).

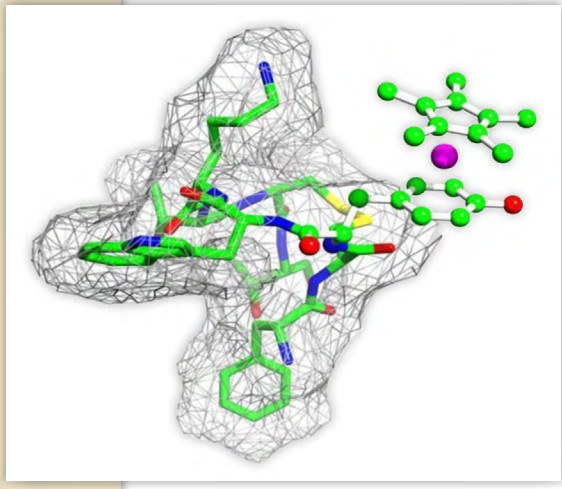

FIGURE 1. Metal-peptide complex–Cp*Rh-tyr-octreotide complex: With NMR spectroscopy, the Ruhr University research team determined the three-dimensional structure of the metal-peptide complexes. The metal atom, rhodium (magenta), binds to the peptides's amino acid tyrosine, specifically to the phenol moiety. The five-membered ring structure (green) above the phenol ring represents a pentamethylcyclopentadienyl (Cp*) group. Via metal coordination, rhodium is bound between the two aromatic rings. The gray net symbolizes the surface of the molecule. (*Credit: © Florian Wieberneit & Raphael Stoll.*)

Among the compounds analyzed, one was peptide hormone encephalin, important for pain sensation, and the other was octreotide, a synthetic derivative of the growth hormone somatostatin.

The structure shown in Figure 1 is a metal peptide complex of

rhodium-octreotide. Metal peptide complexes of octreotide have a metal chelator that carries a radionuclide and octreotide (an octapeptide that is a potent somatostatin [SST]) analog. Studies have shown therapeutic benefits of this complex which include treatment and diagnosis of neuroendocrine tumors and cancers.[1]

Copper Metal Peptide Complexes in Alzheimer's Disease

Alzheimer's disease (AD) is the most common type of dementia and accounts for an estimated 60–80% of reported cases. Dementia is characterized by a loss or decline in memory and other cognitive abilities. AD is an irreversible, progressive neurodegenerative disorder.[2]

Researchers report that there is a plausible role for metals (particularly copper, zinc, and iron) in the pathogenesis of Alzheimer's disease. Research on metal ion complexes like copper oxide (Cu[II]) has shown that the role of metal ions is critical in synaptic function and their homeostasis is strictly governed. A synaptic dysfunction associated with AD is possible with disruption in Cu(II) homeostasis and generation of toxic amyloid beta (Aβ) oligomers. The presence of senile plaques, neurofibrillary tangles, neutrophil threads, Aβ peptide deposits, and selective loss of neurons are some of the distinctive characteristics of the disease. The most common feature of the disease is amyloid plaque deposits. Aβ peptides that are proteolytically cleaved from the membrane bound amyloid precursor protein (APP) are major constituents of these deposits. The familial AD genetic evidence shows a relation between metabolism of Aβ and AD disease. Although the role of APP is debated, some researchers hint that APP plays important role in maintaining metal homeostasis and maintains the copper concentration in cells. Amyloids (or fibrous aggregates) of abnormally folded proteins are a part of degenerative diseases like Alzheimer's and diabetes, but the mechanism of formation of these amyloids is still a mystery and is a hurdle in finding the right cure for these chronic diseases.

On one side, researchers say that Cu(II) is responsible for extracellular high concentrations of Aβ peptides which are implicated in the pathogenesis of Alzheimer's disease.[2] The interaction of Cu-Aβ is not clear but researchers have demonstrated that oligomeric complexes of Cu-Aβ may increase the formation of reactive oxygen species which are neurotoxic (Figure 2). Also, Cu(II) accelerates formation of insoluble Aβ oligomers, and if Cu(II) is removed, the oligomer dissolves. It is known that the presence of low order oligomers coordinated with or without Cu(II) are important factors in nerve degeneration of Alzheimer's patients. It is also known that Cu(II) binds to the N-terminal part of Aβ oligomers and the 16-residue fragment Aβ1–16 in the brain. Cu(II) reacts with the amino acid residues D1, H6, H13, and H14 and most likely with the N-terminal amino group of Aβ1–16. There is speculation that the high concentration of Cu(II) in amyloid plaques in the brains of AD patients emphasizes the Cu(II) binding with Aβ. Outside the cell, the Cu(II) concentrations can transiently reach micromolar concentrations. However, other recent publications have shown evidence of lower concentrations of Cu(II) in human brain tissue of Alzheimer's patients,[3] and also have shown an inverse correlation between Cu(II) content in brain tissue and amyloid pathologies in AD. Researchers have found that lower concentrations of copper in the brain leads to aggressive amyloid pathology and that there is no evidence to support the effect of copper on rapid formation of Aβ oligomers under physiological conditions.[3] Detailed information about the series of steps involved in binding of Cu(II) and Aβ leading to the formation of high-order oligomers is not known. This missing information is important to researchers for developing a therapeutic plan.

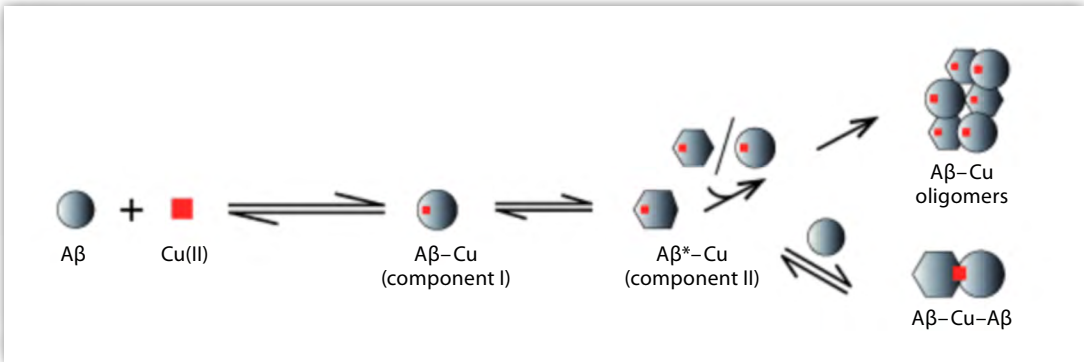

FIGURE 2. Cu(II) connects peptides: A proposed mechanism of binding of Cu(II) to Aβ peptides implicated in AD was elucidated by stopped-flow spectroscopy, NMR relaxation, and simulation of the kinetics on a millisecond timescale. Two monomeric Cu–Aβ species and a dimeric Aβ–Cu–Aβ species were identified. Aberrant aggregation apparently occurs from the monomeric species.

Ruthenium Metal Peptide Complexes

Ruthenium (Ru) complexes containing polyamino carboxylate (PAC) ligands are suitable for various biological applications. One of the applications is binding of Ru-PAC complexes to biomolecules through a rapid and high-yielding aquo-substitution reaction. Ru-PAC has a range of accessible oxidation states and it has some catalytic properties that can mimic cytochrome P450. This property of Ru-PAC complexes makes them suitable as agents for oxidative cleavage of DNA and artificial nucleases in DNA footprinting experiments.

It has been discovered that the Ru-PAC complexes possess cysteine protease inhibition activity (Figure 3).[4,5] Understanding the cysteine protease inhibition through the interaction of Ru-PAC complexes with cysteine and other thioamino acids was studied by researchers and concluded that these reactions lead to the formation of S-coordinated species. The high affinity of ruthenium complexes make the binding facile with the –SH group of cysteine residue of enzymes. This affinity of Ru-PAC towards –SH binding is believed to be responsible for the inhibition of cysteine protease via a rapid aquo-substitution reaction.

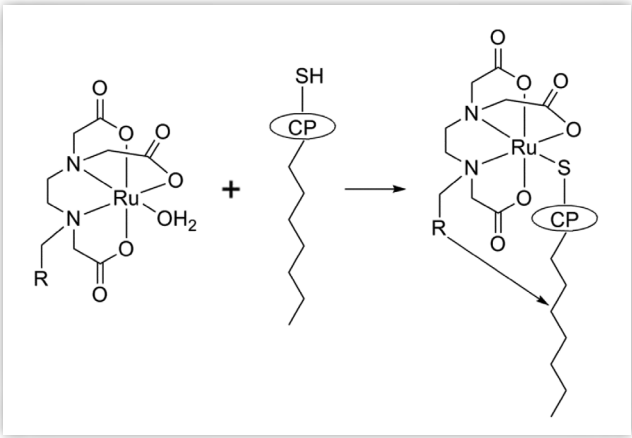

FIGURE 3. Protease activity of the enzyme, cysteine protease (CP) bearing an SH group, is inhibited by Ru-PAC to form a stable Ru (EDTA)-enzyme complex. R is a peptidal unit that can recognize enzymes selectively. If R were known, then a reactive drug could be developed.

Targeted Delivery and Controlled Release of Therapeutic Metals

The discovery of metal abstraction peptide (MAP), a novel tripeptide-metal complex at the School of Pharmacy at the University of Kansas has useful applications in targeted delivery for the drug and diagnostic industries. When MAP[6,7] extracts a metal ion from a chelator, it forms a stable complex. This metal ion complex has resistance towards high temperature and light waves. Though extraction of metal can take place only in acidic conditions, it can bind with a variety of metal ions like nickel (Ni) and platinum (Pt). Due to these unique properties, MAP complexes can be used as tags for achieving targeted and controlled release of metals in therapeutic and diagnostic applications. MAP tags can be beneficial in encoding site specific tripeptide sequences into proteins during their production to generate a homogeneous single product.

Metal Peptide Complexes in Diagnostics

The use of metal complexes in clinical diagnostic imaging such as gamma scintigraphy, positron emission tomography (PET), magnetic resonance imaging (MRI), and metal-based radiopharmaceuticals is rapidly increasing.

Neuroendocrine tumors (NETs) are composed of a heterogeneous group of tumors which frequently express cell membrane-specific peptide receptors, such as SST receptors (SSTRs).[8] Since there are high levels of SSTRs expressed on tumor cells, radiolabeled octreotide and lantreotide analogs are useful in detecting tumors. There is a special interest from researchers in biomedical applications of peptides as effective ligands for radiolabeled molecules and imaging agents. Peptide complexes are frequently attached to other biomolecules to be used as sensors or markers for analytical and biomedical purposes. Various octreotide analogs like ^{111}indium-octreotide (OctreoScan), ^{111}indium-diethylene triamine pentaacetic acid (DTPA)^0, ^{90}Y, a pure β-emitter, ^{90}Y–1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*–tetraacetic acid (DOTA)^0, Tyr^3, octreotide (^{90}Y–DOTATOC, OctreoTher) are being used as imaging or biomarkers for tumors.[9]

Single-photon emission computed tomography (SPECT) and PET imaging provide accurate data on radionuclide distribution at the desired target tissue by detection of the gamma photons that result from radionuclide decay. Radiopharmaceuticals are synthesized to be used as ligands for specific hormone, neurotransmitter, and cell surface or drug receptors as well as specific high-affinity transport systems or enzymes. Low molecular weight peptides and antibody fragments provide rapid tumor targeting and uniform distribution in tumor

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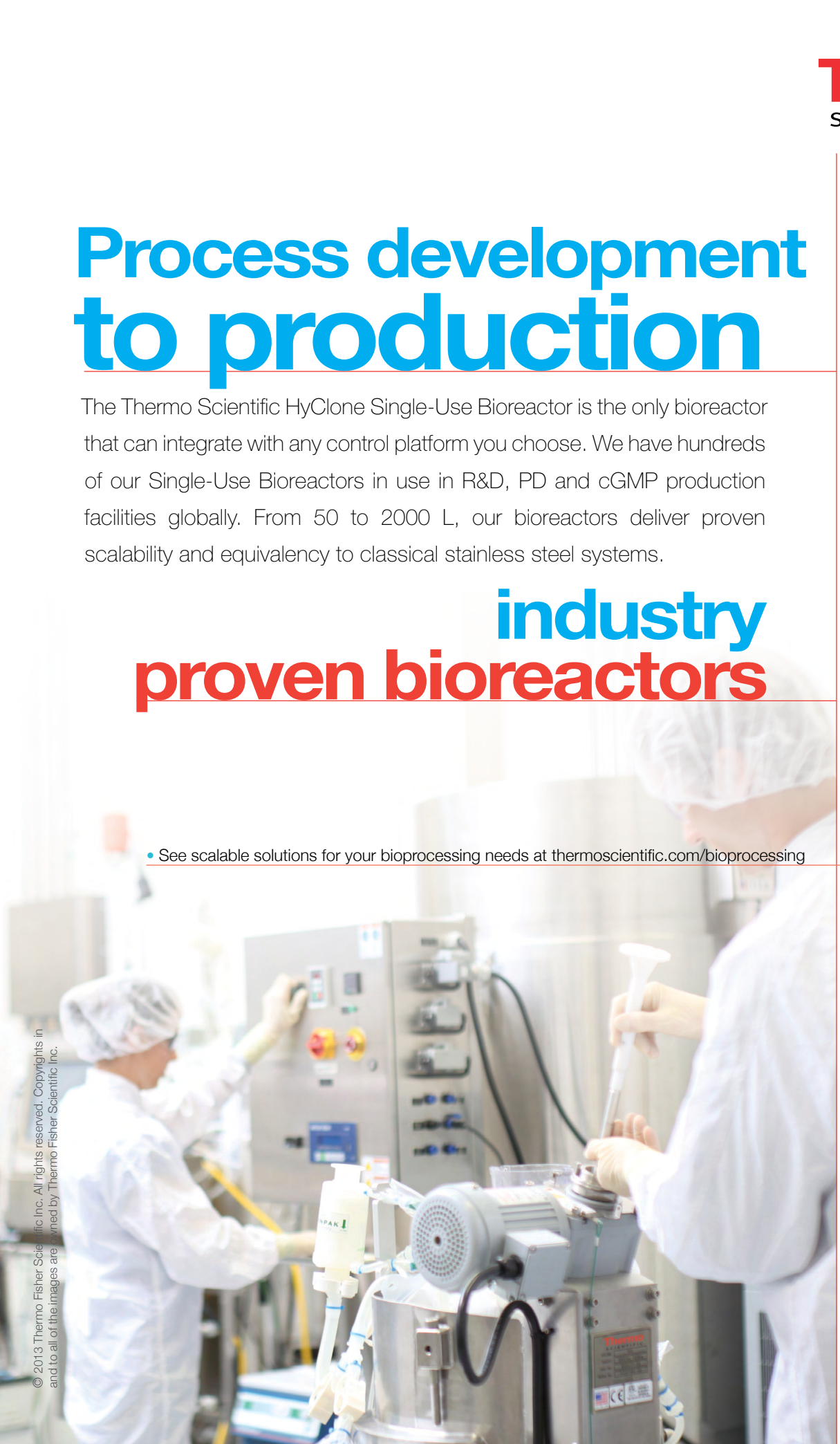


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tissues. Radiolabeled low molecular weight peptides and antibody fragments are useful for targeted imaging and therapy. There exists a continued need for peptide-based radiopharmaceuticals that are rapidly cleared and target intracellular receptors or enzymes.[8,9]

Copper Peptide Complexes in the Personal Health and Cosmetics Industry

Copper peptide (GHK-Cu) is widely used in anti-aging cosmetics. Several controlled facial studies confirmed anti-aging, firming, and anti-wrinkle activity of GHK-Cu. The numerous clinical studies that show GHK-Cu based cosmetics produce a significant reduction in wrinkles, skin roughness, skin slackening, mottled pigmentation, and other age-related skin problems. GHK-Cu greatly improved skin appearance, increased elasticity and skin thickness, and restored resilience and a healthy complexion.[10]

Copper peptide and its analogs were found to stimulate hair growth. In some circumstances, the efficiency of synthetic analog of GHK-Cu was similar to that of 5% minoxidil.[11]

There is ongoing research on copper peptide in DNA repair, nerve regeneration, anti-cancer effect, and genomic studies. GHK-Cu has a stimulatory action on wound healing.[12] A variety of copper binding analogs of GHK-Cu have potent activity in the inhibition of fibroblasts and fibrosarcoma cells at lower concentrations.[13] Hydrophobic analogs of the GHL-Cu structure are inhibitory to cell DNA synthesis and growth.

References

[1] Bauke Albada H *et al*. The chemoselective reactions of tyrosine-containing G-protein-coupled receptor peptides with [Cp*Rh(H_2O)3](OTf)2, including 2D NMR structures and the biological consequences. *J Am Chem Soc*, 2012; 134 (25): 10321-4.

[2] Hemmingsen L *et al*. Rapid formation of a preoligomeric peptide-metal-peptide complex following copper(II) binding to amyloid β peptides. *Angew Chem Int Ed Engl*, 2011; 50: (11) 2532-2535. http://dx.doi.org/10.1002/anie.201006335.

[3] Mold M *et al*. Copper prevents amyloid-β(1-42) from forming amyloid fibrils under near-physiological conditions *in vitro*. *Sci Rep*, 2013; 3:1256. http://dx.doi.org/10.1038/srep01256.

[4] Jain S *et al*. Metal complexes as magic bullet—an overview. *People's Journal of Scientific Research*, 2009; 2: (1) 37-40.

[5] Chatterjee D *et al*. Ruthenium polyaminocarboxylate complexes: prospects for their use as metallopharmaceuticals. *Platinum Metals Review*, 2006; 50: (1) 2-12. http://dx.doi.org/10.1595/147106705X82874.

[6] Laurence J *et al*. Univ. of Kansas, World Patent No. WO2009140408 (2009).

[7] Laurence J *et al*. Univ. of Kansas, US Patent No. US20120174712 (2012).

[8] Kaltsas GA *et al*. Treatment of advanced neuroendocrine tumours with radiolabelled somatostatin analogues. *Endocr Relat Cancer*, 2005; 12: 683-699. http://dx.doi.org/10.1677/erc.1.01116.

[9] Piwnica-Worms D, Washington University, US Patent No. US6589503 (2003).

[10] Pickart L *et al*. Biological activity of human plasma copper-binding growth factor glycyl-l-histidyl-l-lysine. *Methods Enzymol*, 1987: (147) 314-328. http://dx.doi.org/10.1016/0076-6879(87)47121-8.

[11] Uno H, Kurata S. Chemical agents and peptides affect hair growth. *J Invest Dermatol*, 1993 Jul;101 (1 Suppl): 143S-147S.

[12] Pickart L *et al*. Inhibition of the growth of cultured cells and an implanted fibrosarcoma by aroylhydrazone analogs of the gly-his-lys-Cu(II) complex. *Biochem Pharmacol*, 1983; 32: (24) 3868-3871. http://dx.doi.org/10.1016/0006-2952(83)90164-8.

[13] Johnson DK *et al*. Cytotoxic chelators and chelates inhibition of DNA synthesis in cultured rodent and human cells by aroylhydrazones and by a copper(II) complex of salicylaldehyde benzoyl hydrazone. *Inorg Chem Acta*, 1982: (67) 159-165.

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