BIOGRAPHICAL SKETCH

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NAME: Iris Lindberg

eRA COMMONS USER NAME (credential, e.g., agency login): ILINDB

POSITION TITLE: Professor, Anatomy and Neurobiology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing,* ***include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)***

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date	FIELD OF STUDY
Univ. of California, Berkeley, CA	A.B.	06/1976	Biochemistry
Univ. of Wisconsin, Madison, WI	Ph.D.	12/1980	Pharmacology
National Institute of Mental Health	Postdoc	09/1984	Neuropharmacology

A. Personal Statement

My research focuses on the molecules within the secretory pathway required for the successful production of bioactive peptide hormones and neuropeptides from precursor proteins. These include chaperones; abundant secretory proteins such as granins; and precursor processing enzymes – in particular, the proprotein convertases furin, PC1/3 and PC2, which I have studied for over 20 years. I am interested in the cellular regulation of convertase activity; establishing enzyme crystal structures; and identifying activators and inhibitors through various pharmacological collaborations. The widespread involvement of proprotein convertases in the physiology of nearly every tissue means that our work is directly linked to pathological processes in obesity and diabetes, as well as in Alzheimer's and other neurodegenerative diseases. Our work has resulted in the publication of 135 papers and 16 reviews/book chapters to date (see http://www.ncbi.nlm.nih.gov/sites/myncbi/collections/bibliography/40560230/), with over 5300 citations (Google Scholar).

As regards the attached proposal to crystallize the proPC2-7B2 complex, we have published methods to overexpress and purify many eukaryotic secretory proteins, including proenkephalin, furin, PC1/3 and proPC2. Our acquisition of an Akta Explorer through the ARRA program has speeded our protein purification efforts. The following reviews speak to my particular expertise in terms of successfully accomplishing the proposed project:

a. **Lindberg, I**. and Zhou, Y. (1995) "Overexpression of neuropeptide precursors and processing enzymes" in "Peptidases and neuropeptide processing. *In* Methods in Neuroscience, Vol 23, 94-108. (A. Ian Smith, Ed.) Academic Press, Orlando, FL

b. M. Vivoli, M. and **Lindberg, I.** (2012) "Prohormone convertase 2": *in* Handbook of Biologically Active Peptides. (A. Kastin, Ed). Academic Press.

c. Hoshino A., and **Lindberg, I.** (2012) The biochemistry and cell biology of prohormone convertases 1/3 and 2. *in* Neuropeptide Biosynthesis (E-Book) (L. Devi and L.D. Fricker, Eds). Morgan and Claypool Life Sciences Publishers.

B. Positions and Honors

9-75- 12-80 Graduate research assistant in Pharmacology, University of Wisconsin- Madison
1-81 - 6-81 Individual NIH F32 postdoctoral trainee, NIMH (Dr. Erminio Costa)
6-81 - 9-84 Pharmacology Research Associate Traineeship, NIMH
9-84 - 6-89 Assistant Professor of Biochemistry and Molecular Biology at Louisiana Health Sciences Ctr.

7-89 - 6-94 Associate Professor of Biochemistry and Molecular Biology; member, Neuroscience Center
7-94- 8/07 Professor of Biochemistry and Molecular Biology; Neuroscience Center member
8/07- present Professor, Department of Anatomy and Neurobiology, University of Maryland Medical School; Secondary appointment, Department of Biochemistry; Greenebaum Cancer Center Member

Awards: Pharmacology Research Associate Traineeship, 1981-1984; Research Career Development Award, 1988-1993; Research Scientist Development Award, 1993-1998; 1998-2003.
Scientific Societies: ASBMB, 1985 – present; Society for Neuroscience, 1982- present; WCBR, 1985-present; Endocrine Society, 2001- present.
Study section service: *ad hoc* reviewer on NLS-1, 1994, 1995, 1996; NIDA-B, 10/97; DK Program Project Review 12/98; ACS, 6/99; MCDN5, 12/02; Endocrinology, 1989, 1990, 1995; 10/00; 2/02; 6/02; 2/03;10/03; 6/04; 10/04; 6/05; 2/06; 2/07; Standing Member, Endocrinology (renamed MCE) 1996- 2000 and 2006- 2010; Eureka SEP 3/2011; SBIR, 3/2013; MCE 6/2015.
Reviewer, international grants: Finnish National Academy of Sciences; Canada Research Chairs; Wellcome Trust; Belgian Foundation for Scientific Research; Canadian Institutes of Health Research; Danish Research Council
Editorial Board, J. Biol. Chem. 2000 – 2005; 2012- 2017
Chair, Gordon Conference on Proprotein Processing, Trafficking, and Secretion (formerly Hormonal and Neural Peptide Synthesis) 2004; GRC Proprotein Processing Advisory Committee 2002, 2006, 2008, 2010, 2014, 2016).

C. Contributions to Science

C1. Proprotein convertase biosynthesis and structure. In 1990 the first proteolytic steps in the biosynthesis of all peptide hormone signaling molecules were still completely unknown. The discovery by Steiner and Seidah of the family of enzymes known as the *proprotein convertases* in endocrine and neural tissues meant that this initial cleavage step could finally be studied in detail. Our contribution to this field was to biochemically characterize two prohormone convertases, especially with regard to their action on the neuropeptide precursor proenkephalin. We showed that only prohormone convertase 2 (PC2) is able to produce the cleavages which ultimately generate opioid-active enkephalins. We described the biosynthesis of PC1/3 in both endocrine and constitutive cell lines, demonstrating that the active enzyme is first produced as a stable 87 kDa precursor which gives rise to a C-terminally truncated form with very different biochemical properties. We used the dihydrofolate reductase-coupled method of overexpression to massively overexpress and purify this protein, as well as the related convertase furin, in CHO cells. Our soluble furin preparation yielded the first structure of a proprotein convertase, generated by our collaborators – including Dr. Manuel Than, a collaborator on the current proposal. The furin structure, still one of only two – the yeast protein was crystallized almost simultaneously – has guided structural analyses and inhibitor development of all convertases during the past dozen years. We further contributed to the structural biochemistry of proPC2, first by showing that the zymogen requires the presence of a specific chaperone, 7B2, for acquisition of enzymatic activity (see below); and then by establishing that proPC2 activation is a pH-dependent process that occurs spontaneously within secretory granules. The following four original research papers have been cited 632 times at this time, supporting their importance to the field.

a. Vindrola, O., and **Lindberg, I.** (1992) Biosynthesis of the prohormone convertase mPC1 in AtT-20 cells. Mol. Endocrinol. 6, 1088-1094.

b. Zhou, Y., and **Lindberg, I.** (1993) Purification and characterization of the prohormone convertase PC1 (PC3) J. Biol. Chem. 268, 5615- 5623.

c. Lamango, N., Zhu, X., and **Lindberg, I.** (1996) Purification and enzymatic characterization of recombinant PC2: stimulation of activity by 21 kDa 7B2. Arch. Biochem. Biophys. 330, 238-250.

d. Henrich, S., Cameron, A., Bourenkov, G.P., Kiefersauer, R., Huber, R., **Lindberg, I.,** Bode, W., and Than, M.E. (2003) The crystal structure of the proprotein processing proteinase furin explains its stringent specificity. Nature Structural Biology 10, 520-526.

C2. Identification of potent convertase inhibitors. The only convertase inhibitor available for over a decade was a highly cytotoxic chloromethyl ketone. We discovered that polyarginine-rich peptides represent potent furin inhibitors that can be used *in vitro*; in cell lines; and in animals to combat disease – for example anthrax and *Pseudomonas* toxicity. Our stable polyarginine inhibitor, hexa-D-arginine amide (D6R), is now

commercially available and is widely used by others as a convertase inhibitor (*e.g*. Virol. J. 11:165, 2014). Our recent work shows that popular cationic protein transduction agents, such as Chariot and the TAT-derived peptide, also represent potent furin inhibitors (Ramos-Molina *et al*., *in press*.) We have also collaborated with various institutes, companies, and research groups to identify novel furin and prohormone convertase inhibitors; these collaborations have collectively resulted in 23 papers to date, and include small molecule inhibitors of PC1/3 and PC2 in addition to furin. Perhaps the most impressive collaboration in this regard was our 1998 collaboration with the Torrey Pines Institute for Molecular Studies, in which we screened a hexapeptide combinatorial library for inhibitors of our recombinant PC1/3. Two years later, the precise sequence of the best inhibitory peptide we identified, LLRVKR, was found within the natural PC1/3 inhibitor, proSAAS, by the Fricker group. The four inhibitor papers listed below have been cited 368 times to date, with the polyarginine papers gaining steady interest over the years with increasing use of polyarginines as protein transduction tags. We have patented polyarginine convertase inhibitors as potential antibacterial and anticancer therapeutics.

a. Apletalina, E., Appel, J., Lamango, N.S., Houghten, R., and **Lindberg, I.** (1998) Identification of potent inhibitors of prohormone convertases 1 and 2 using a peptide combinatorial library. J. Biol. Chem. 273, 26589-26595.

b. Cameron, A., Appel, J., Houghten, R.A. and **Lindberg, I.** (2000) Polyarginines are potent furin inhibitors. J. Biol. Chem. 75, 36741-36749.

c. Sarac, M.S., Peinado, J.R., Leppla, S.H., and **Lindberg, I**. (2004) Protection against anthrax toxemia by hexa-D-arginine *in vitro* and *in vivo*. Infection and Immunity 72, 602-605.

d. Kacprzak, M., Peinado, J.R., Than, M., Appel, J., Henrich, S., Bode, W., Houghten, R.A., and **Lindberg, I.** (2004) Inhibition of furin by polyarginine-containing peptides: nanomolar inhibition by nona-D-arginine. J. Biol. Chem. 279, 36788-9463

C3. Production of active PC2 requires the 7B2 protein. Surprisingly, overexpression of the convertase proPC2 in CHO cells resulted only in catalytically inert protein; our enzymatic studies to characterize this enzyme therefore first focused on immunopurified enzyme, precipitated from a beta cell line-conditioned medium with our new PC2 antiserum. Using this enzyme source, in collaboration with the Martens group, we found that the small neural and endocrine protein 7B2 represents a nanomolar inhibitor of active PC2, with all of the inhibitory activity contained within the C-terminal 31-residue peptide. In 1995 we discovered that co-expression of proPC2 with the remaining 21 kDa domain of 7B2 is both necessary and sufficient to produce enzymatically active PC2. Structure-function studies on 7B2 revealed that it contains a 36-residue internal peptide with three biochemical features (polyproline helix; alpha helix, and disulfide bond) required to maintain proPC2 in an activatable state. The mechanism by which this occurs took over ten years to solve, but in 2006 we published work showing that in the absence of 7B2, proPC2 becomes severely aggregated, which renders it incapable of maturation to an enzymatically competent species.

In 1998 we were contacted by Dr. Philip Leder (Harvard) who had created a 7B2 knockout mouse that inexplicably died between 5-8 weeks of age. Upon receipt of the mice, I noticed an unusual dorsal fat pad reminiscent of the "buffalo hump" present in patients with Cushing's disease. This observation led us to diagnose multiple endocrinological problems, which all originate from overproduction of intermediate lobe ACTH (due to the inability of PC2 to cleave - and thereby inactivate- ACTH in the absence of 7B2). The high circulating levels of ACTH in this null mouse result in adrenal cortex hypertrophy, generating corticosterone levels 50-fold above normal. Given the total inability to generate glucagon from proglucagon – again, due to a lack of active PC2 – this eventually culminates in a lethal hypoglycemic crisis. Our group later showed that adrenalectomy can rescue the 7B2 null; that intrapituitary adenoviral administration of 7B2 can extend its life span and partially reverse its endocrinological deficiencies; and that the lethal phenotype of the 7B2 knockout is extremely background-specific. The 7B2 knockout mouse was jointly patented by the Leder group and our group as a partial model for Cushing's disease, and the animals archived as a public resource at Jackson Laboratories. The following four papers have collectively been cited 548 times.

a. Martens, G.M., Braks, A.M., Eib, D., Zhou, Y., and **Lindberg, I.** (1994) The neuroendocrine polypeptide 7B2 is a naturally occurring inhibitor of the prohormone convertase PC2. Proc. Nat. Acad. Sci. 91, 5784-5785.

b. Zhu, X., and **Lindberg, I.** (1995) 7B2 facilitates the maturation of proPC2 in neuroendocrine cells and is required for the expression of enzymatic activity. J. Cell Biol. 129, 1641-1650.

c. Muller, L., Zhu, X., and **Lindberg, I.** (1997) Mechanism of facilitation of PC2 maturation by 7B2: involvement in PC2 transport and activation, but not folding. J. Cell. Biol. 139, 625-638.

d. Westphal, C.H., Muller, L., Zhou, A., Zhu, X., , Bonner-Fraser, S., Schambelan, M., Steiner, D.F., **Lindberg, I.***, and Leder, P.* (1999) The neuroendocrine protein 7B2 is required for peptide hormone processing *in vivo* and provides a novel mechanism for pituitary Cushing's disease. Cell 96, 689-700. (*co-senior authors)

C4. Anti-aggregant chaperones in the secretory pathway. While the regulatory secretory pathway contains extremely high concentrations of proteins destined for packaging and export, no specialized anti-aggregant proteins had ever been identified within the lumen of this pathway. Based on our work demonstrating that 7B2 is a highly effective anti-aggregant for proPC2, we surmised that it might also represent a general secretory anti-aggregant, and this hypothesis was borne out by experiments using Abeta and other fibrillating secretory peptides and proteins such as hIAPP and synuclein. We extended this work to proSAAS, a protein with structural similarities to 7B2 whose expression is also widespread in (and restricted to) neural, neuroendocrine, and endocrine tissues. Our recent work has shown that 7B2 is an intrinsically disordered protein which itself forms oligomers; we believe that this oligomerization process may play a role in its anti-aggregant activities. These results are significant in their identification of an entirely new group of protein anti-aggregant chaperones that are specific to neural and endocrine secretory tissues.

a. Dasgupta, I.*, Sanglas, L.*, Enghild, J. and **Lindberg I.** (2012) The neuroendocrine protein 7B2 is an intrinsically disordered protein. (*co-first authors) Biochemistry 51(38):7456-64. PMID:22947085

b. Helwig, M., Hoshino, A., Berridge, C., Lee, S.N., Lorenzen, N., Otzen, D., Eriksen, J., and **Lindberg, I**. (2013) The neuroendocrine protein 7B2 suppresses neurodegenerative disease-related protein aggregation. J. Biol. Chem. 288:1114–1124. PMID: 23172224

c. Peinado, J.R., Sami, F., Rajpurohit, N., and **Lindberg, I.** (2013) Blockade of islet amyloid polypeptide fibrillation and cytotoxicity by the secretory chaperones 7B2 and proSAAS. FEBS Lett., Nov 1;587(21):3406-11 PMID: 24042052

d. Hoshino, A., Helwig, M., Rezaei, S., Berridge, C., Eriksen, J.L., and **Lindberg, I**. (2013) A novel function for proSAAS as an amyloid anti-aggregant in Alzheimer's disease. J. Neurochem. 128(3):419-30 PMCID: PMC3946950

C5. Mutations in PC1/3 result in deleterious dominant-negative interactions. Mutations in secretory proteins which result in poorly folded proteins are responsible for a variety of genetic diseases, for example the proinsulin mutations which cause neonatal diabetes. Because secretory proteins form oligomers, folding mutations can cause dominant-negative effects on wild-type proteins. We showed that PC1/3 also naturally oligomerizes, with oligomers being much less active than monomers. Biochemical and cell biological studies demonstrated that a portion of the N222D mouse mutant PC1/3 protein undergoes endoplasmic reticulum (ER) retention and increased ER-associated degradation. When mutant protein oligomerizes with wild-type PC1/3, it both become degraded. These dominant-negative results may explain the obesity which occurs in N222D PC1/3 heterozygote mice -- but not PC1/3 knockout heterozygotes.

Mutations in the *PCSK1* gene have been shown to be strongly associated with human obesity in over a dozen genome-wide association studies, indeed, PC1/3 mutations are found in up to 5% of a highly obese population. Our collaboration with the group of Dr. M. Martin (UCLA) has shown that *PCSK1* mutations are responsible for the loss of PC1/3 enzyme activity in a pediatric cohort who first exhibit severe malabsorptive diarrhea and then go on to develop obesity. We showed that the most severe human cases exhibit inactivating mutations in PC1/3 which result in ER retention. Our new work indicates that these human mutations also interact in a dominant-negative fashion with wild-type enzyme. These results extend an underlying cause of human obesity to PC1/3 trafficking problems, rather than to a simple lack of PC1/3 activity.

a. Hoshino, A., Kowalska, D., Jean, F., Lazure, C., and **Lindberg, I.** (2011) Modulation of PC1/3 activity by self-interaction and substrate binding. Endocrinology 152:1402-11

b. Pickett, L.A., Yourshaw, M., Chen, Z. Solorzano-Vargas, R.S., Nelson, S.F., Martín, M.G., and **Lindberg, I.** (2013) Functional consequences of a novel variant of *PCSK1*. PLoS One 8(1):e55065. PMCID: PMC3557230

c. Martín, M.G., **Lindberg, I**, Solorzano-Vargas, R.S., Wang, J., Avitzur, Y., Bandsma, R., Sokollik, C., Lawrence, S., Pickett, L.A., Chen, Z., Egritas, O., Dalgic, B., Albornoz, V., de Ridder, L., Hulst, J., Gok, F., Aydoğan, A., Al-Hussaini, A., Gok, D.E., Yourshaw, M., Wu, S.V., Cortina, G., Stanford, S., and Georgia, S.

(2013) Congenital proprotein convertase 1/3 deficiency causes malabsorptive diarrhea and other endocrinopathies in a pediatric cohort. Gastroenterology 45:138-48 PMCID:PMC3719133

d. Prabhu, Y., Blanco, E.H., Liu, M, Peinado, J.R., Wheeler, M., Gekakis, N., Arvan, P. and **Lindberg, I.** (2014) Defective transport of the obesity mutant PC1/3 N222D contributes to loss of function. Endocrinology 155:2391-401 PMID: 24828610

D. Research Support

Ongoing Research Support

Opioid Peptide Synthesizing Enzymes 4/88- 2/16 **(in unfunded extension)**
R01 DA05084-27 I. Lindberg (PI)
NIH/NIDA
This grant is to study the regulation of PC1/3 activity through oligomerization, and the role of proSAAS in mediating this process; to crystallize PC1/3; and to identify small molecule convertase inhibitors using combinatorial compound screening and molecular modeling. **NO OVERLAP**.

The Secretory Chaperone 7B2 as an Endogenous Regulator of Amyloid Pathology 9/01/14- 4/30/16
R21 AG045741-01 I. Lindberg (PI)
NIH/NIA
This 2-year grant is to explore the idea that brain 7B2 levels modulate the pathologic aggregation of beta amyloid-derived peptides by crossing 7B2 null and transgenic mice with AD-model mice. **NO OVERLAP.**

Completed Research Support in Last Five Years

Control of Peptide Hormone Biosynthesis by PC2 and 7B2
R01 DK49703-16 I. Lindberg (PI) 9/1996 - 3/2014
NIH/NIDDK
This grant investigated the role of the neuroendocrine protein 7B2 in PC2-mediated peptide hormone synthesis.

De-Orphanizing the Peptidome
R01 DA27170-05 I. Lindberg and B. Roth (co-PIs) 7/2009 - 6/2014
NIH/NIDA
This grant was to identify novel ligand-receptor pairs through systematic screening of novel and known peptide products against known and orphan GPCRs**.**