UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

MYLAN PHARMACEUTICALS INC.
Petitioner,

v.

BAUSCH HEALTH IRELAND LIMITED,
Patent Owner.

IPR2022-00722
Patent No. 7,041,786

DECLARATION OF BLAKE R. PETERSON, PH.D.

TABLE OF CONTENTS

Page

I. QUALIFICATIONS 1

II. SCOPE OF WORK 4

III. LEGAL STANDARDS 5

IV. OVERVIEW OF SHAILUBHAI 7

A. Claims of Shailubhai 12

B. Prosecution History of Shailubhai 13

V. LEVEL OF ORDINARY SKILL 14

VI. CLAIM CONSTRUCTION 15

VII. THE STATE OF THE ART 16

A. Peptide Hormones in the Body and in Medicine 16

B. Biochemistry Foundation 20

C. Biology of Human Uroguanylin 23

D. The Availability of Solid-Phase Peptide Synthesis for Preparation of Synthetic Analogs of Peptide Hormones 30

E. Strategies for Engineering Peptides 34

F. Assays for Measuring Therapeutic Activity for Clinical Constipation Were Well-Known in the Art 39

G. Formulating Peptides with Intestinal Targets for Oral Delivery 49

H. Treating Constipation and Inflammation 52

VIII. THE ASSERTED REFERENCES DISCLOSE OR SUGGEST EACH OF THE CLAIMED FEATURES OF SHAILUBHAI 53

A. Currie 53

B. Li 55

C. Narayani 56

D. Campieri 57

E. Ekwuribe 58

IX. GROUND 1. CLAIM 1 WAS OBVIOUS OVER CURRIE AND LI 58

A. Currie Suggests Uroguanylins For Treating Constipation 63

B. Reason to Look to Synthetic Analogs of Human Uroguanylin 66

C. Currie and Li Suggest a Glu^3 Substitution as in Rat Uroguanylin 69

D. The Known Chemical Properties of Uroguanylins and Amino Acids Would Have Further Provided a Skilled Artisan with a Reasonable Expectation of Improving Constipation-Related Activity through Glu^3 Substitution 77

E. Routine and Conventional Testing of Synthetic Analogs 85

F. The Known Issue of Aspartimide Formation in Solid-Phase Peptide Synthesis Would Have Further Supported a Glu^3 Analog of Human Uroguanylin 86

G. Minimal Difference Between Currie's Human Uroguanylin and the Claimed [Glu^3]-Human Uroguanylin. 87

X. GROUND 2. CLAIMS 2, 4, AND 5 WERE OBVIOUS OVER CURRIE, LI, AND NARAYANI 88

A. Formulating [Glu^3]-Human Uroguanylin in a Unit Dose Form as Recited in Claim 2 Was Obvious 93

B. Formulating [Glu^3]-Human Uroguanylin in a Capsule as Recited in Claim 4 Was Obvious 97

C. Formulating [Glu^3]-Human Uroguanylin in a Unit Dose Form along with One or More Excipients as Recited in Claim 5 Was Obvious 98

XI. GROUND 3. CLAIMS 3-5 WERE OBVIOUS OVER CURRIE, LI, NARAYANI, AND CAMPIERI 100

A. Formulating [Glu^3]-Human Uroguanylin in a Unit Dose Form as Recited in Claim 3 Was Obvious 103

B. Formulating [Glu^3]-Human Uroguanylin with an Anti-Inflammatory Agent in a Capsule with One or More Excipients as Recited in Claims 4 and 5 Would Have Been Obvious 107

XII. GROUND 4. CLAIM 6 WAS OBVIOUS OVER CURRIE, LI, AND EKWURIBE 108

XIII. SECONDARY CONSIDERATIONS 116

XIV. CONCLUDING STATEMENTS 119

XV. APPENDIX – LIST OF EXHIBITS 121

I, Blake R. Peterson, declare as follows:

I. QUALIFICATIONS

1. I am the John W. Wolfe Chair in Cancer Research with a focus on Medicinal Chemistry and Chemical Biology at The Ohio State University (OSU). I have held faculty appointments from 1998 to the present. My research for over two decades has been directed toward understanding and developing small molecule probes for biological systems. This research included the development of small molecules and peptides that promote cellular uptake of proteins, the synthesis and evaluation of antiviral agents and anticancer agents, the identification of biological targets of small molecules, and the construction of new types of fluorescent probes for immunology and cancer biology.

2. I also currently serve as a Professor of Medicinal Chemistry and Pharmacognosy at OSU, as well as Chair of the Division of Medicinal Chemistry and Pharmacognosy at OSU. I also serve as Co-Leader of the Translational Therapeutics Program of the OSU Comprehensive Cancer Center (CCC) and as Co-Director of the Medicinal Chemistry Shared Resource of the OSU CCC.

3. I previously served as a Regents Distinguished Professor in the Department of Medicinal Chemistry at the University of Kansas (KU) School of Pharmacy from 2008-2019. I also served for seven years as Co-Leader of the Synthetic Chemical Biology Core Facility at the KU.

4. Prior to joining the faculty at the KU, I served as an Assistant Professor (1998-2004) and Associate Professor with tenure (2004-2007) in the Department of Chemistry at The Pennsylvania State University (PSU). While at PSU, I was a member of the Life Sciences Consortium, of the Center for Biomolecular Structure and Function, of the Cancer Center, and of the Experimental Therapeutics Program of the PSU Hershey Medical School.

5. Thus, my faculty appointments encompass 23 years of experience with teaching and research in organic chemistry, medicinal chemistry, and chemical biology.

6. I earned my Ph.D. in Chemistry from the University of California, Los Angeles in 1994. My Ph.D. research was in bioorganic chemistry, where I used organic chemistry methods to synthesize small molecules termed "synthetic receptors," followed by evaluation of their affinities for steroids, such as cholesterol and steroid hormones in aqueous solution.

7. I was subsequently a postdoctoral fellow in Chemical Biology at Harvard University from 1995-1998. My postdoctoral research was in biochemistry, molecular biology, and chemical biology, and involved using genetic assays and biochemical systems to investigate the molecular basis of interactions of transcription factor proteins that control activation of T-cells. This research identified specific amino acids that mediate cooperative binding of the

transcription factors activator protein 1 (AP-1) and nuclear factor of activated T-cells (NFAT) to DNA. These proteins are involved in regulating expression of the cytokine IL-2, which plays a central role in the T-cell-mediated inflammatory response.

8. I have received many research grants and have been the principal investigator for multiple major research and training grants such as: "Synthetic Lethal Targeting of Growth Factor Receptors" NCI R01; "Tissue-Specific Delivery of Probes by Control of Membrane Trafficking of Endoprotease Substrates" NIH RC1; and "A New Approach for Systemic Delivery of siRNA: Cholesterylamine Conjugates that Target and Selectively Disrupt Early / Recycling Endosomes" Novartis Institutes for Biomedical Research.

9. Additionally, over the past ten years, I served as a co-investigator on five grants, including: "Molecular Analysis of Disease Pathways" NIH COBRE and "Development of Antiviral Therapeutics for Dengue" National Institutes of Health-U01. As part of the Molecular Analysis of Disease Pathways COBRE grant, I was funded to create and co-lead a new core facility at KU that offers the synthesis of molecular probes and associated fluorescent imaging services to faculty researchers at KU and beyond.

10. My work has been published in numerous prestigious journals, including *Journal of the American Chemical Society* and *Angewandte Chemie*. I

have over 80 peer reviewed publications, as well as over 10 non-peer reviewed publications and abstracts. I have also authored or co-authored two book chapters. I have additionally provided manuscript reviews for over 200 publications in upwards of 60 scientific journals, including *Proceedings of the National Academy of Sciences USA*, *Nature Methods*, and *Journal of the American Chemical Society*.

11. For a more detailed listing of my credentials and publications, please see my curriculum vitae, EX1003.

II. SCOPE OF WORK

12. I understand that Mylan Pharmaceuticals Inc. ("Mylan") is filing a petition with the United States Patent and Trademark Office for *Inter Partes* Review of U.S. Patent No. 7,041,786 to Shailubhai ("Shailubhai," EX1001). Mylan retained me as a technical expert in this matter to provide my opinions regarding Shailubhai related to my experience and expertise.

13. My opinions are based on my skills, knowledge, training, education, and experience in matters of this nature, and my examination of the materials used in preparing this testimony. In addition to Shailubhai, I have also reviewed and considered various other documents in arriving at my opinions and cite them in this declaration. For convenience, documents cited in this declaration are listed in the Appendix in Section XV. My opinions are based on the current record, so I reserve the ability to refine my opinions based on additional facts.

14. Mylan is compensating me at the rate of $475 per hour for services. No part of my compensation is dependent on my opinions or the outcome of this proceeding, and I have no other financial interest in the outcome of this matter.

III. LEGAL STANDARDS

15. I have been advised that the burden in this proceeding is on Mylan to demonstrate the unpatentability of the challenged claims.

16. I have been advised that a claimed invention is not patentable for obviousness if the differences between the claimed invention and the prior art are such that the subject matter as a whole would have been obvious at the time the claimed invention was made the person of ordinary skill in the art to which the subject matter of the invention pertains.

17. I understand that a determination of obviousness requires inquiries into: (*i*) the scope and content of the art when the claimed invention was made; (*ii*) the differences between the art and the claims at issue; (*iii*) the level of ordinary skill in the pertinent art when the claimed invention was made; and, to the extent any exist, (*iv*) secondary considerations indicating non-obviousness.

18. I understand that hindsight must not be used when comparing the prior art to the claimed invention for obviousness. Thus, a conclusion of obviousness must be firmly based on the knowledge and skill of the artisan at the time the claimed invention was made, without the use of post-filing knowledge.

19. I understand that in order for a claimed invention to be considered obvious, there must be some rational underpinning for combining cited references as proposed. I further understand that obviousness may also be shown by demonstrating that it would have been obvious to modify what is taught in a single piece of prior art to create the claimed invention. Obviousness may be shown by demonstrating that the skilled artisan would have found it obvious to combine the teachings of more than one element disclosed by prior art.

20. I understand that the following examples are approaches and rationales that may be considered in determining whether a piece of prior art could have been combined with other prior art or with other information within a skilled artisan's knowledge:

(i) combining prior-art elements according to known methods to yield predictable results;

(ii) substituting one known element for another to obtain predictable results;

(iii) using a known technique to improve similar devices (methods, or products) in the same way;

(iv) applying a known technique to a known device (method, or product) that was ready for improvement to yield predictable results;

(v) applying a technique or approach that would have been "obvious to try" (*i.e.*, choosing something from a finite number of identified, predictable solutions, with a reasonable expectation of success);

(vi) applying variations based on known work in one field of endeavor for

use in either the same field or a different one, based on design incentives or other market forces, if the variations would have been predictable to one of ordinary skill in the art; or

(vii) acting upon some teaching, suggestion, or motivation in the prior art to modify the prior-art reference or to combine prior-art reference teachings thereby arriving at the claimed invention.

21. I have been instructed that "secondary considerations" will be considered when present. Counsel have informed me that such secondary considerations, where evident, may include: (*i*) commercial success of a product due to the merits of the claimed invention; (*ii*) a long-felt but unsatisfied need for the claimed invention; (*iii*) failure of others to find the solution provided by the claimed invention; (*iv*) deliberate copying of the claimed invention by others; (*v*) unexpected results achieved by the claimed invention; (*vi*) praise of the claimed invention by others skilled in the art; (*vii*) lack of independent, simultaneous invention within a comparatively short span of time; and (*viii*) teaching away from the claimed invention in the prior art. I am informed that secondary considerations are relevant where there is a nexus between the evidence and the claimed invention.

22. I am informed that the patent owner, Bausch Health Ireland Ltd. ("Bausch"), bears the burden to establish any secondary considerations indicating non-obviousness.

IV. Overview of Shailubhai

23. Shailubhai is entitled "Guanylate Cyclase Receptor Agonists for the Treatment of Tissue Inflammation and Carcinogenesis." EX1001, [54]. Shailubhai is generally directed to a 16-residue peptide and compositions thereof for treating cancer. EX1001, [57], 2:1-61, 3:61-4:42. The peptide is defined in the patent as "consisting of the amino acid sequence of SEQ ID NO: 20," which is the sequence shown below.

Asn^{1} Asp^{2} Glu^{3} Cys^{4} Glu^{5} Leu^{6} Cys^{7} Val^{8} Asn^{9} Val^{10} Ala^{11} Cys^{12} Thr^{13} Gly^{14} Cys^{15} Leu^{16}
(asterisks: Cys^{4} *, Cys^{7} **, Cys^{12} *, Cys^{15} **)

EX1001, 5:5-16, claims 1-3, 6. As the asterisk notations indicate, a first disulfide linkage exists between the cysteines at positions 4 and 12 in the sequence, and a second disulfide linkage exists between the cysteines at positions 7 and 15. *Id.*

24. I note that Shailubhai acknowledges that the sequence of this claimed peptide (reproduced below, top) differs only from the naturally-occurring human uroguanylin sequence (reproduced below, bottom) by ***one*** amino acid, shown in bold in the sequences below:

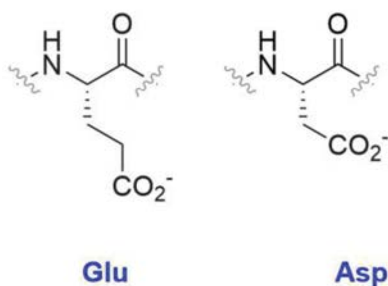
Asn^{1} Asp^{2} **Glu^{3}** Cys^{4} Glu^{5} Leu^{6} Cys^{7} Val^{8} Asn^{9} Val^{10} Ala^{11} Cys^{12} Thr^{13} Gly^{14} Cys^{15} Leu^{16}

Asn^{1} Asp^{2} **Asp^{3}** Cys^{4} Glu^{5} Leu^{6} Cys^{7} Val^{8} Asn^{9} Val^{10} Ala^{11} Cys^{12} Thr^{13} Gly^{14} Cys^{15} Leu^{16}

Id., 7:55-58.

25. Thus, the claimed peptide differs from the native human uroguanylin sequence only in that a glutamic acid (Glu) residue has been swapped in for the

aspartic acid (Asp) residue in the third position. As I discuss in more detail below, these are the only acidic amino acid residues with negatively-charged side chains at physiologically-relevant pH values. That is, both of these amino acids possess carboxylic acid groups that become deprotonated under physiological conditions. Upon deprotonation, glutamic and aspartic acid may also be referred to as glutamate and aspartate. I use both of these terms throughout this declaration. For convenience, and to assist in visualizing these amino acids, I provide the chemical structures of these residues below, shown in their deprotonated state.



26. As can be seen above, these amino acids differ structurally from one another in that the Glu residue, shown on the left, has an additional methylene unit (-CH_2-) in its side chain compared to Asp, shown on the right.

27. Conventional amino acid nomenclature provides one- and three-letter codes for each amino acid (*e.g.*, E or Glu for glutamic acid). I refer to the claimed peptide consisting of the amino acid sequence of SEQ ID NO:20 throughout this declaration as "[Glu^3]-human uroguanylin"—indicating the sequence is that of human uroguanylin where the aspartic acid (Asp) at the third position has been

swapped for a glutamic acid (Glu).

28. I note that Shailubhai acknowledges that these types of synthetic analogs could be synthesized and purified using known, published procedures. EX1001, 15:53-55, *see also id.*, 16:1-19, Table 4, 18:32. Shailubhai further acknowledges that the resulting peptides could be formulated and made into different dosage forms "using methods well known in the art." *Id.*, 13:27-30 (citing *Remington's Pharmaceutical Sciences*, 16*th* ed., A. Oslo ed., Easton PA. (1980)); *see also* EX1010[1], *generally*. I agree that a skilled artisan would have been able to formulate the peptides described in Shailubhai into different dosage forms using methods well known in the art, and discuss such formulations in Sections VII.G and X-XII, below.

29. As discussed in more detail below, a skilled artisan would have had good reason to make [Glu^3]-human uroguanylin by replacing the aspartic acid at human uroguanylin's third position with a glutamic acid, and further to formulate the resulting peptide using known, routine, and conventional methods to yield a composition for potential medical applications. In particular, a skilled artisan would have reasonably expected such a substitution to maintain or improve human

---

[1] King, R. E., *Chapter 89: Tablets, Capsules, and Pills*, REMINGTON'S PHARMACEUTICAL SCIENCES, 16*th* ed., (ed. A. Oslo, ed., Mack Publishing Co.) **1980** ("Remington's," EX1010).

uroguanylin's activity in treating inflammatory bowel disease and related constipation, as well as to improve the stability of uroguanylin during synthesis. *See* my discussion in Sections VII-XII. A skilled artisan would have seen the claimed invention as simply an obvious combination of familiar elements using known methods to achieve a predictable result.

30. I also note that Shailubhai describes the development of [Glu^3]-human uroguanylin as being the result of computational chemistry calculations. As is readily apparent from the Shailubhai disclosure, this computational work involved only known methods of performing computational analyses to identify energetically interesting analogs of human uroguanylin. *See*, *e.g.*, EX1001, 7:52-12:45; *see also id.*, 7:52-55 ("Molecular modeling was applied to the design of novel guanylate cyclase receptor agonists using methods detailed in (30)."), 8:9-12 ("Energy calculations were performed by use of build-up procedures (30). The ECEPP/2 potential field (31,32) was used…"), 8:22-28 ("At this step, all possible combinations…were considered…according to the notation in (33)."), 8:50-58 ("[M]issing side chains in the model fragments were restored, and energy calculations were performed again…employing an algorithm previously described (34)."), 9:2-5 ("The best fit in the superposition…was assessed…according to (35)."), 18:20-29 (providing citations for these known computational methods). Thus, rather than creating any novel analysis or method for analog exploration,

Shailubhai merely performed conventional calculations in a routine way to confirm the potential utility of an already obvious structural analog.

A. Claims of Shailubhai

31. I understand that Shailubhai includes 6 claims. Four of those claims—claims 1, 2, 3, and 6—are independent, while the remaining two claims are dependent claims. I understand a dependent claim includes all limitations of the claim from which it depends.

32. Each claim is directed to a 16-residue peptide or compositions thereof. The peptide, which I refer to as "[Glu^3]-human uroguanylin," is defined in the patent as "consisting of the amino acid sequence of SEQ ID NO: 20," which is the sequence shown below.

$$\mathrm{Asn^1\ Asp^2\ Glu^3\ \underset{*}{Cys^4}\ Glu^5\ Leu^6\ \underset{**}{Cys^7}\ Val^8\ Asn^9\ Val^{10}\ Ala^{11}\ \underset{*}{Cys^{12}}\ Thr^{13}\ Gly^{14}\ \underset{**}{Cys^{15}}\ Leu^{16}}$$

EX1001, 5:5-16, claims 1-3, 6. As the asterisk notations indicate, [Glu^3]-human uroguanylin has a first disulfide linkage between the cysteines at positions 4 and 12 in the sequence, and a second disulfide linkage between the cysteines at positions 7 and 15. *Id.*

33. Independent claim 1 recites:

1. A peptide consisting of the amino acid sequence of SEQ ID NO:20.

34. Independent claim 2 recites:

2. A composition in unit dose comprising a guanylate cyclase receptor

agonist peptide consisting of the amino acid sequence of SEQ ID NO:20.

35. Independent claim 3 recites:

3. A composition in unit dose form comprising:

> ***a) a guanylate cyclase receptor agonist peptide consisting of the amino acid sequence of SEQ ID NO: 20; and***
>
> ***b) at least one compound selected from the group consisting of: a cGMP-dependent phosphodiesterase inhibitor, an anti-inflammatory agent, an antiviral agent and an anticancer agent.***

36. Independent claim 6 recites:

6. A peptide conjugate comprising polyethylene glycol (PEG) attached to a peptide consisting of the amino acid sequence SEQ ID NO:20.

37. Claim 4 depends from claim 2 or 3 and further recites known unit dose forms. Claim 5 also depends from claim 2 or 3 and further recites that the composition further comprises "one or more excipients." I discuss these limitations in more detail in my detailed claim analysis below.

B. Prosecution History of Shailubhai

38. I have been advised that the examination process, or prosecution history of the application that led to the Shailubhai patent (EX1004) may be relevant to my analysis of the patentability of the claims. I understand that the

application that led to Shailubhai was filed on March 28, 2002. EX1001, [22]. I understand that Shailubhai purports to claim priority to U.S. Provisional Patent Application No. 60/348,646, filed January 17, 2002. *Id.*, [60]; EX1054 ("the '646 provisional"). For the purposes of my opinions, I have assumed that Shailubhai is entitled to claim priority to the '646 provisional.

39. However, I have reviewed the '646 provisional and note that it does not mention treating constipation or the use of [Glu^3]-human uroguanylin as a therapeutic for treating constipation. *See* EX1054.

V. LEVEL OF ORDINARY SKILL

40. I have been advised that the person of ordinary skill in the art is a hypothetical person who is presumed to have known the relevant art at the time of the claimed invention. The skilled artisan is also a person of ordinary creativity. I have been advised that the skilled artisan to whom one could assign a routine task with reasonable confidence that the task would be successfully carried out.

41. I have been advised that the relevant timeframe for my analysis is the time period prior to January 17, 2002—the earliest claimed priority date for Shailubhai. *See* Section IV.B, *above*. Unless otherwise specifically noted, all of my opinions expressed here regarding the skilled artisan apply to the skilled artisan as of January 17, 2002.

42. By virtue of my education, experience, and training, I am familiar

with the level of ordinary skill in the art of Shailubhai. A skilled artisan as of January 17, 2002 would typically have a Ph.D. in chemistry or protein engineering or a related field. Skilled artisans could also include individuals with a master's degree in one of these fields plus two-to-five years of experience in drug development. This individual would have worked in consultation with a team including, *e.g.*, a pharmaceutical chemist or a pharmacist familiar with formulating peptides for administration. This level of skill is consistent with that presumed by Shailubhai itself. *See* Section IV.

43. Furthermore, skilled artisans would be familiar with prior art pertaining to signaling peptides and their biochemistry, including the patents and publications discussed in this declaration. My education, experience, and training qualify me to opine as a skilled artisan regarding the understanding of a skilled artisan at the relevant time, as I am and was by 2002 a person of ordinary skill in the art.

VI. CLAIM CONSTRUCTION

44. I have been advised that the claim terms of Shailubhai are to be given their plain and ordinary meaning, *i.e.*, the meaning that the terms would have had to a skilled artisan at the time of the claimed invention.

45. I understand that this analysis focuses on intrinsic evidence, including how the patentee used the claim term in the claims, specification, and prosecution

history. I also understand that dictionaries or other extrinsic sources may assist in determining the plain and ordinary meaning but cannot override a meaning that is unambiguous from the intrinsic evidence. I have followed these principles in my analysis throughout this declaration.

VII. THE STATE OF THE ART

A. Peptide Hormones in the Body and in Medicine

46. Peptides are short chains of amino acids linked together covalently through peptide bonds. *See*, *e.g.*, EX1011, 1088 (describing, *e.g.*, "peptides of some 20-30 amino acid residues").[2] Peptide chains fold into three dimensional structures that are commonly called proteins. Peptide function involves the reversible and specific binding of peptide ligands to the binding site of a receptor. EX1012, 203.[3] A peptide ligand "discriminate[s] among the thousands of different molecules in its environment and selectively bind[s] only one or a few" based on the physical and chemical properties of the receptor and the ligand. *Id.*

47. Peptides that function as chemical messengers are called peptide

---

[2] Rehfeld, J. F., *The New Biology of Gastrointestinal Hormones*, PHYSIOL. REV., 78(4), **1998**, 1087-1108 ("Rehfeld", EX1011).

[3] Nelson, D. L., *et al.*, *Chapters 4-5, 7*, LEHNINGER PRINCIPLES OF BIOCHEMISTRY, 3rd ed. (eds. Ryan, M., *et al.*, Worth Publishers) **2000** ("Nelson," EX1012).

hormones. *See generally* EX1013.[4] In the paragraphs that follow, I will explain this process in greater detail. For summary purposes, however, it is helpful to understand that a particular peptide hormone may bind with the extracellular portion of a protein structure that extends through a membrane (membrane-bound protein). This binding interaction may change the shape (conformation) of the membrane-bound protein, thus changing the way the intracellular portion of the membrane-bound protein interacts with other proteins within the cell, for example. In this way, the body uses peptides to generate signaling cascades to activate (agonist hormones) or deactivate (antagonist hormones) functions within the body.

48. Well before 2002, skilled artisans knew many examples of naturally-occurring peptide hormones. Insulin is an example discovered more than 100 years ago. Rehfeld confirms that skilled artisans were aware of many peptide hormones, including peptide hormones that act on receptors located in the gut. EX1011, 1087. According to Rehfeld, "more than 30 peptide hormone genes are known to be expressed throughout the digestive tract, which makes the gut the largest endocrine organ in the body." EX1011, 1087. Rehfeld also reports that skilled artisans had identified "many more gut hormones, and each has its own or even more receptors,

---

[4] Segaloff, D. L., *et al.*, *Chapter 9: Internalization of Peptide Hormones and Hormone Receptors*, HORMONES AND THEIR ACTIONS, PART I, (eds. Cooke, B. A., *et al.*, Elsevier) **1988**, 133-149 ("Segaloff," EX1013).

although, vice versa, there are also examples showing that different gastrointestinal peptides may act on the same receptor." EX1011, 1088.

49. By 2002, skilled artisans understood that peptide hormones, including those regulating the gut, transmit signals by binding to their receptors expressed on the surface of certain cells. Segaloff confirms that the "ability of a particular peptide hormone to elicit an effect in the appropriate target cell is dictated by the presence of receptors on the surface of the target cell which specifically bind that hormone." EX1013, 133. It also was known that the sensitivity of any given cell to a particular hormone depends on the number of receptors that cell expressed for the particular hormone. Segaloff states, for example, that "the amount of hormone that is processed in this case is dictated by the number of hormone receptors." EX1013, 139.

50. It was well known before 2002 that hormone-receptor binding is driven both by the physical shape of the binding sites of the hormone and the receptor as well as by their chemical properties. For example, it was known that complementary electrostatic interactions between the hormone and receptor, across their interface, promote the binding relationship of ligand to receptor, thereby promoting their signaling activity. In addition to other physical and chemical forces, the binding interaction may be promoted when water is excluded from the binding region as the hormone locks together with the receptor. Chipens, for

example, describes hormone-receptor binding as both a mutual recognition between the molecules and a thermodynamically driven desolvation (water removal) at the hormone-receptor interface. EX1014, 100.[5] As Chipens published in 1978, skilled artisans long understood the power of properly designed counterion interactions between hormone and receptor.

51. Unson similarly explains that it was known that charged residues could "contribute strongly to the stabilization of the binding interaction with the [peptide] receptor that leads to maximum biological potency." EX1015, 10308.[6] As I will discuss below, certain amino acids, such as aspartate and glutamate, have charged side groups that can contribute to hormone-receptor binding.

52. Skilled artisans also knew that hormone-receptor binding and agonism (*e.g.*, initiation of biochemical signaling) depend on the physical structure and characteristics of the hormone and the receptor at the binding site in addition to the electrostatic interactions discussed above. For example, binding of the hormone with the receptor depends on the conformational fit of hydrophobic residues of the

---

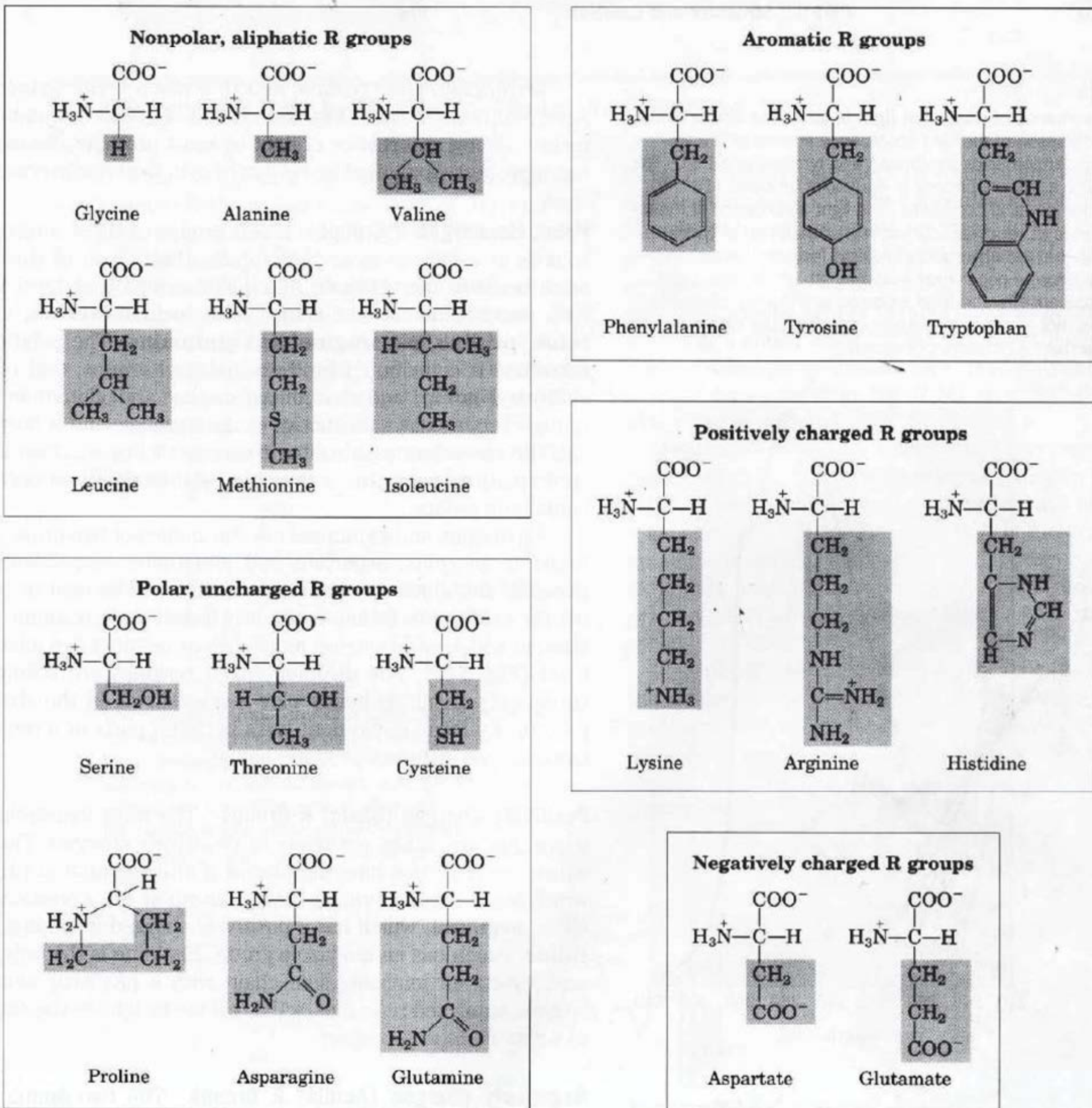
[5] Chipens, G., *et al.*, *Recognition of Peptide Hormones and Kinins: Molecular Aspects of the Problem*, FRONTIERS OF BIOORGANIC CHEMISTRY AND MOLECULAR BIOLOGY, (ed. Ananchenko, S. N., Pergamon Press) **1980**, 99-103 ("Chipens," EX1014).

[6] Unson, C. G., *et al.*, *Positively Charged Residues at Positions 12, 17, and 18 of Glucagon Ensure Maximum Biological Potency*, J. BIOL. CHEM., 273(17), **1998**, 10308-10312 ("Unson," EX1015).

hormone and the receptor, which contribute to the fit between a hormone and its receptor's binding pocket. EX1014, 101. Chipens, for example, links hormone-receptor selectivity and specificity to this process of mutual recognition. *Id.* Accordingly, the physical properties of the binding regions of the hormone and the receptor impact the functionality of the hormone for triggering the signaling pathway of the receptor.

B. Biochemistry Foundation

53. It was known well before 2002 that peptide chains were formed from 20 known proteogenic (protein-making) amino acids. Figure 5-5 from Nelson, reproduced below, shows the chemical structures of these amino acids. The "unshaded portions are those common to all the amino acids." EX1012, 119, FIG. 5-5. Species of amino acids are differentiated by the side chains (shaded in the figure below).



54. The chemical characteristics of the different amino acids depend on the side chains, which therefore may be referred to as the functional groups of the amino acid. For example, aspartic acid and glutamic acid, lower right in the figure above, are the only amino acids having a second carboxyl group within the side chain and therefore were known for their characteristic, negatively charged

functional groups. Indeed, among the 20 amino acids that make up natural peptides, only aspartic acid and glutamic acid are negatively charged at neutral pH. EX1012, 120. As can be seen in the figure above, the only structural difference between them is that the carboxylic acid at the end of glutamic acid's side chain is one additional methylene further from the backbone.

55. Chemical groups each have a value called a pKa. EX1012, 98-99. This value is the negative logarithm of the dissociation constant, the ratio at equilibrium of the product of concentrations of free protons and the ionized groups to the concentrations of protonated groups. Stronger acids have a high dissociation constant and thus a low pKa. In other words, the "stronger the tendency to dissociate a proton, the stronger is the acid and the lower its pK_a." *Id.*, 99. According to Nelson, aspartic acid has a pKa of 3.65 for the carboxylic acid functional group of its sidechain. EX1012, 118, Table 5-1. Nelson further notes that the pKa of glutamic acid's functional group is 4.25. *Id.*

56. For aqueous solutions, pH is a scale that designates the concentration of hydrogen cations, or free protons, in the solution. EX1012, 97. The pH scale is the negative logarithm of free protons molar in solution. Neutral solution has a free proton concentration of 10^{-7} molar and a pH of 7. Acidic solutions have free proton concentrations greater than 10^{-7} molar and a pH less than 7. For example, if an acidic solution has a pH of 5, the pH of black coffee, the free proton concentration

is 10^{-5} molar. *Id.*, 98, FIG. 4-13.

57. As I explained above, the lower the pKa value, the stronger the tendency to dissociate a proton. Similarly, a higher pKa value indicates a compound that has a weaker tendency to dissociate a proton. In other words, a molecule with a lower pKa, like aspartic acid, has a greater tendency to dissociate a proton than glutamic acid. EX1012, 98-99. Thus, between the two amino acids, because it has a higher pKa, glutamic acid is more prone to be protonated than aspartic acid at the same pH. As the pH of a given environment approaches neutral, glutamic acid thus remains protonated to a greater extent than does aspartic acid.

C. Biology of Human Uroguanylin

58. Uroguanylin is a small peptide that stimulates intestinal guanylate cyclase (GC-C), a receptor displayed in the mucosa of the intestinal endothelium. *See, e.g.*, EX1016, E957.[7] Thomson reports that skilled artisans knew by 2000 that "signal density for uroguanylin is greatest in the small intestine." EX1017, 807[8]; *see also* EX1018, G635-36, G639-41 (noting high activity in the proximal duodenal epithelium due to "a higher receptor density compared with other

---

[7] Fan, X., *et al.*, *Structure and Activity of Uroguanylin and Guanylin from the Intestine and Urine of Rats*, AM. J. PHYSIOL. ENDOCRINOL. METAB., 273(5), **1997**, E957-E964 ("Fan," EX1016).

[8] Thomson, A. B. R., *et al.*, *Small Bowel Review: Part I*, CAN. J. GASTROENTEROL., 14(9), **2000**, 791-816 ("Thomson," EX1017).

segments of the intestinal tract").[9] And as others noted by 1996, "All species of mammals and birds examined express GC-C-like receptor activity on the apical surface of enterocytes throughout the intestine." EX1019, G708.[10] Furthermore, according to Hamra 1996, intestinal guanylate cyclase is expressed on "apical membranes of cells throughout the entire length of the small and large intestine." *Id.*, G714. Accordingly, uroguanylin was known before 2002 to target signaling pathways in the intestines.

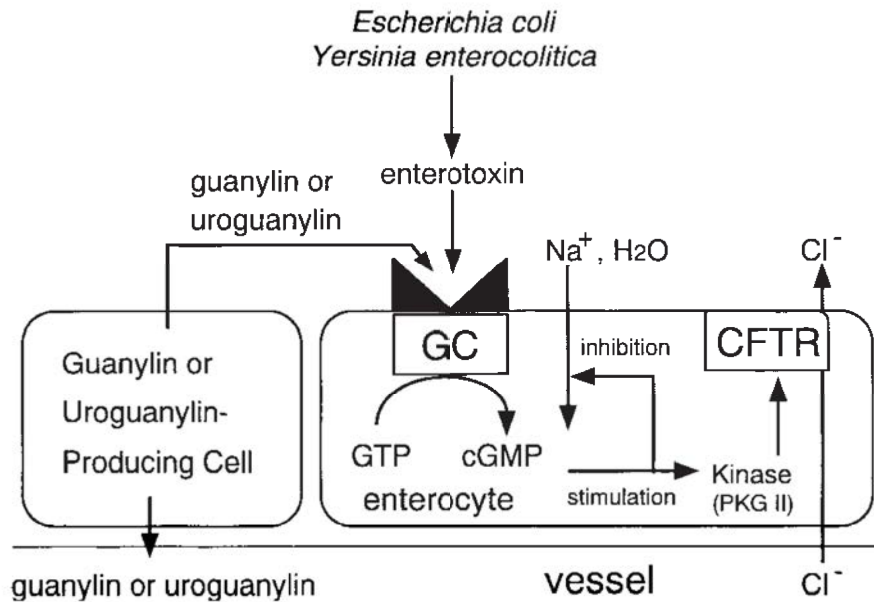
59. Known uroguanylin signaling suggested the peptide as a treatment for constipation. Once uroguanylin binds intestinal guanylate cyclase, cyclic guanosine monophosphate (cGMP) builds up in the cell. EX1016, E957, E962. This secondary messenger causes the cell, through a biochemical pathway, to secrete chloride and other anions. *Id.* The increasing solute concentration in the intestinal lumen draws water into the lumen from the surrounding tissue. *Id.*, E957. When too much water is drawn from the surrounding tissue into the intestinal lumen, the result is the disease commonly called diarrhea. *Id.*; *see also* EX1020,

---

[9] Joo, N. S., *et al.*, *Regulation of Intestinal Cl^- and HCO_3^- Secretion by Uroguanylin*, AM. J. PHYSIOL., 274(4), **1998**, G633-G644 ("Joo," EX1018).

[10] Hamra, F. K., *et al.*, *Opossum Colonic Mucosa Contains Uroguanylin and Guanylin Peptides*, AM. J. PHYSIOL. GASTROINTEST. LIVER PHYSIOL., 270, **1996**, G708-G716 ("Hamra 1996," EX1019).

222, FIG. 2 (reproduced below)[11]. Uroguanylin's known agonist activity thus suggested its usefulness for treating constipation by increasing water content in the intestine.



60. Skilled artisans discovered the diarrhea-producing heat-stable enterotoxins (STs) before they discovered uroguanylin. EX1019, G708. STs are produced by various pathogenic microorganisms. *Id.* STs evolved as molecular mimics of uroguanylin. *Id.* They produce a pathologically strong increase in cGMP via uroguanylin's binding to the receptor intestinal membrane guanylate cyclase and, hence, diarrhea. *Id.* At least as early as 1993, treating constipation using a more controlled version of this pathway had already been suggested. *See, e.g.*,

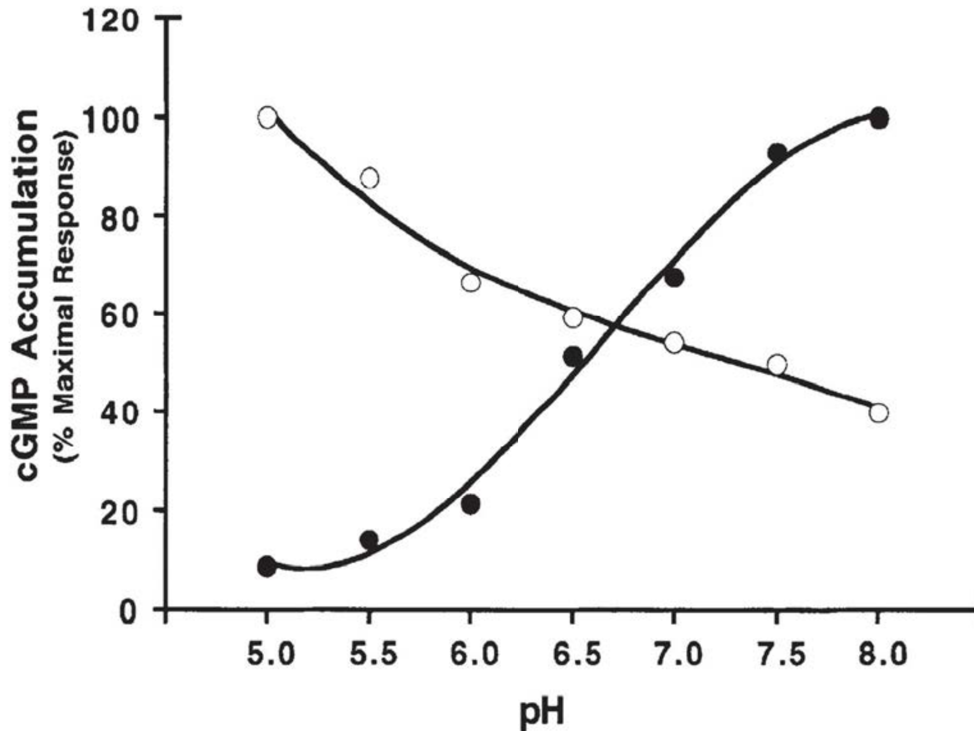
[11] Nakazato, M., *Guanylin Family: New Intestinal Peptides Regulating Electrolyte and Water Homeostasis*, J. GASTROENTEROL., 36, **2001**, 219-225 ("Nakazato," EX1020).

EX1005, 2:20-25 (teaching that "human uroguanylin may thus act as a laxative and be useful in patients suffering from constipation")[12].

61. The potency of uroguanylin for this known anti-constipation molecular signaling pathway was known to markedly enhance where the intestinal mucosal pH is more acidic (and to be less potent where the intestinal mucosal pH is less acidic). *See, e.g.*, EX1016, E962. Thomson confirms that "[u]roguanylin is most effective in acidic regions of the small intestine." EX1017, 807. Fan confirms that rat uroguanylin "stimulated cGMP accumulation greater at the medium [though acidic] pH of 5.0 than at pH 8.0." EX1016, E960.

62. Likewise, Hamra 1996 demonstrates that uroguanylin signaling depends on pH. EX1019, G710, FIG. 1 (reproduced below). In Hamra 1996 Figure 1, for example the horizontal axis depicts pH from an acidic 5.0 to a mildly alkaline 8.0. The vertical axis depicts cGMP response, normalized. Uroguanylin is charted as empty circles. (Another peptide, guanylin is indicated by filled circles.) As Figure 1 of Hamra 1996 shows, uroguanylin activity gradually decreases with increasing pH and thus increases as the pH decreases (*i.e.*, becomes more acidic). In other words, Hamra 1996 demonstrated that uroguanylin activity increases as the intestinal environment becomes more acidic.

[12] [12] U.S. Patent No. 5,489,670, *Human Uroguanylin*, issued **Feb. 6, 1996** to Currie, M. G., *et al.* ("Currie," EX1005).



EX1019, G710, FIG. 1.

63. Because uroguanylin activity is directly related to increased acidity of the intestinal environment, skilled artisans were aware that uroguanylin activity is strongest closest to the stomach where acidic conditions prevail and decreases further away from the stomach (closer to the colon) where the environment becomes more neutral or even alkaline. This relationship was known in the art. For example, Hamra 1996 teaches that the relevant microclimate is more acidic near the duodenum (the first part of the intestine proximate the stomach) because of highly acidic chyme from the flow of gastric contents. EX1019, G714. Fan similarly teaches that the "lumen of the intestine and the mucosal (microclimate)

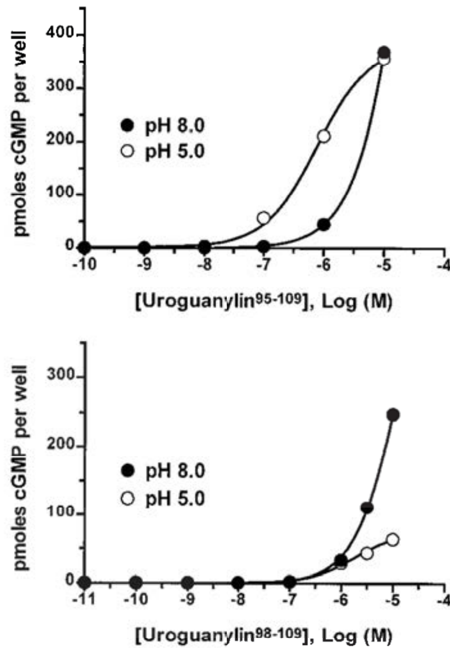
surface is acidified when chyme containing HCl enters the duodenum" from the stomach. EX1016, E963.

64. Hamra 1997 similarly confirms that uroguanylin signaling activity is enhanced under acidic conditions. Hamra 1997 tested opossum uroguanylin and a truncated analog in a model of the human intestinal endothelium. EX1021, 2705[13]. According to Hamra 1997, "High mucosal acidity, similar to the pH occurring within the fluid microclimate domain at the mucosal surface of the intestine, markedly enhances the cGMP accumulation responses of T84 human intestinal cells to uroguanylin." *Id.* Hamra 1997 further shows, in Figure 5 reproduced below, that "the N-terminal acidic residues of uroguanylin are required for the increased binding affinities, and therefore, the enhanced potency of uroguanylin for activation of receptors under acidic conditions." *Id.*, 2709. "All uroguanylin peptides have aspartate or glutamate residues at these positions," which suggests that acidic conditions enhance uroguanylin special activity because these residues are protonated at low pH. *See id.* Hamra 1997 thereby expressly suggests the importance of employing aspartate or glutamate residues at positions 2 and 3 of the amino acid chain of uroguanylin in order to conserve the physical and chemical

[13] Hamra, F. K., *et al.*, *Regulation of Intestinal Uroguanylin/Guanylin Receptor-Mediated Responses by Mucosal Acidity*, PROC. NATL. ACAD. SCI. USA, 94, **1997**, 2705-2710 ("Hamra 1997," EX1021).

properties of the chain to promote its signaling activity.

65. In further detail, Hamra 1997 Figure 5 compares full-length opossum uroguanylin ("95-109," top graph) with a truncated analog ("98-109," bottom graph) for pH dependence. EX1021, 2709, FIG. 5. Signaling activity is assessed by measuring cGMP, vertical axis, generated by treating model human intestinal endothelium. For both peptides, signaling activity is assessed as dose response over increasing peptide concentrations. Between 0.1 μM and 1 μM, full-length uroguanylin shows enhanced activity at pH 5 (empty circles) compared to pH 8 (full circles). At those concentrations, the truncated variant (lacking the N-terminal aspartate or glutamate residues) has the same activity at both acidities. Accordingly, Hamra 1997 demonstrates that the N-terminal acidic residues (aspartate or glutamate) are responsible for the enhanced signaling activity observed with uroguanylin.



EX1021, 2709, FIG. 5 (cropped).

D. The Availability of Solid-Phase Peptide Synthesis for Preparation of Synthetic Analogs of Peptide Hormones

66. Decades before 2002, skilled artisans routinely prepared peptide hormones by linking proteogenic amino acids together one at a time to form the peptide chain using a widely available process called solid-phase peptide synthesis. In solid-phase synthesis, an amino acid molecule is covalently bound to a solid support material and amino acids are added to the chain residue by residue. Solid-phase synthesis methods improved the efficiency, throughput, simplicity, and speed of the synthesis of earlier solution phase methods where the synthesized amino acid chains were not bound to a solid support. The chemistry and processes involved in solid-phase peptide synthesis were developed decades ago and were quite routine before 2002.

67. Karten[14] demonstrates how solid-phase peptide synthesis lowered the bar to synthesizing hormone peptide sequences. Karten observed, for example, that "rapid development of GnRH [Gonadotropin-Releasing Hormone] analogs was made possible through the extensive use of solid phase peptide synthesis," or, SPPS. EX1025, 44. Moreover, it was "quite clear" to Karten "that the use of SPPS and [chromatography] were essential for the rapid exploration of structure-activity relationships as well as providing investigators with relatively large amounts of these substances for pharmacological, toxicological, and clinical studies." *Id.* Skilled artisans thus did not wait for proof of efficacy to make a new synthetic peptide but instead were quite willing to synthesize even very large numbers of analogs simply to learn more about the functionality of the peptide and its interactions with the receptor.

68. On the other hand, solid-phase peptide synthesis did not completely eliminate the need that existed with solution phase peptide synthesis to decrease side reactions during synthesis. Karten warned, for example, that the "purity of the agonists synthesized either by SPPS or classical (solution) methods was always of concern in terms of the concomitant biological activity of potential racemization

[14] Karten, M. J., *et al.*, *Gonadotropin-Releasing Hormone Analog Design. Structure-Function Studies Toward the Development of Agonists and Antagonists: Rationale and Perspective*, ENDOCR. REV., 7(1), **1986**, 44-66 ("Karten," EX1025).

products." EX1025, 44. By 2002, skilled artisans understood that aspartic acid residues were prone to chemical reactions that generated impurities under the conditions involved in solid-phase peptide synthesis. EX1022, 63[15] ("Aspartimide formation is one of the best-documented side reactions in peptide synthesis. Even bulky side-chain protecting groups such as OtBu do not prevent this undesired reaction.").

69. During solid-phase peptide synthesis, "repetitive piperidine treatments needed for Fmoc removal leads to aspartimide formation and further by-products." *Id.* Others observed that "Aspartimide (cyclic imide) formation is a long-recognized side reaction that can occur both during solid phase peptide synthesis (SPPS) and storage of peptides, and may be either acid- or base-catalyzed." EX1023, 107.[16] Still others observed that "cyclization of aspartate to form aspartimide has long been recognized as a substantial side reaction occurring during both synthesis and storage of peptides." EX1024, 197.[17] These side

---

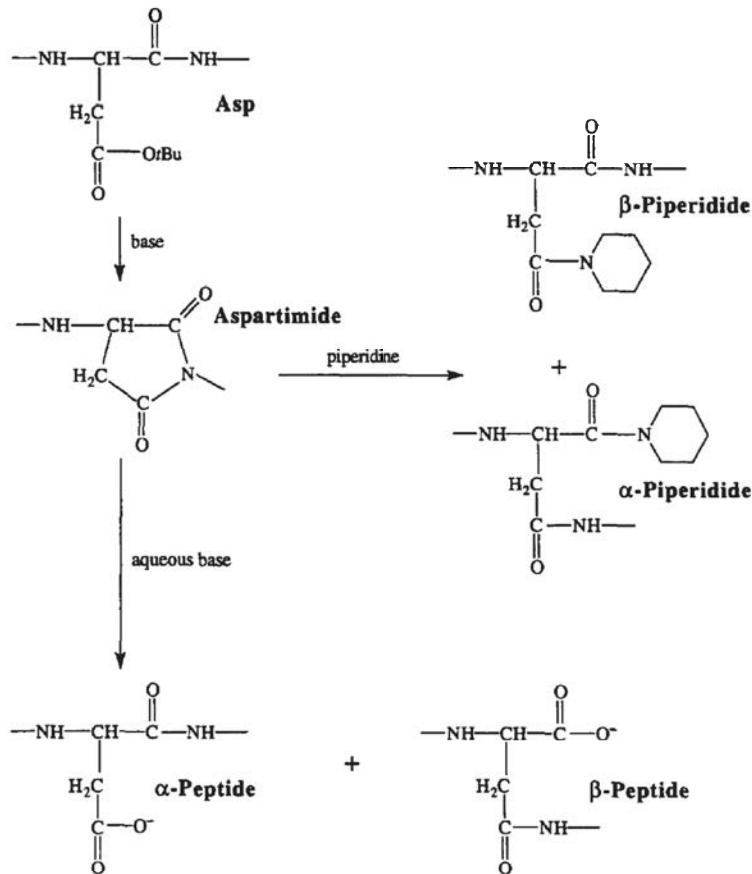
[15] Mergler, M., *et al.*, *Systematic Investigation of the Aspartimide Problem*, PEPTIDES: THE WAVE OF THE FUTURE, (ed. Lebl, M., *et al.*, American Peptide Society) **2001**, 63-64 ("Mergler," EX1022).

[16] Wade, J. D., *et al.*, *Base-Induced Side Reactions in Fmoc-Solid Phase Peptide Synthesis: Minimization by Use of Piperazine as N^{α}-Deprotection Reagent*, LETT. PEPT. SCI., 7, **2000**, 107-112 ("Wade," EX1023).

[17] Lauer, J. L., *et al.*, *Sequence Dependence of Aspartimide Formation during 9-Fluorenylmethoxycarbonyl Solid-Phase Peptide Synthesis*, LETT. PEPT. SCI., 1, **1994**, 197-205 ("Lauer," EX1024).

reactions were "difficult to both anticipate and control." EX1023, 107.

70. Below I have reproduced Scheme 1 from Lauer, which depicts the formation of aspartimide from aspartic acid.



Scheme 1. Formation and ring opening reactions of aspartimide.

EX1024, 198, Scheme 1. As explained by Lauer, aspartimide formation was observed in peptide residues which had an aspartic acid residue next to a protected arginine, aspartic acid, cysteine, or threonine residues (as well as glycine, serine, or unprotected threonine), and "[n]o single approach was found that could inhibit this side reaction for all sequences." EX1024, 197. However, no aspartimide formation

was observed when a protected glutamic acid residue was placed next to an aspartic acid residue. *Id.*, 199, 201.

E. Strategies for Engineering Peptides

71. Since the 1980s, skilled artisans made synthetic analogs of naturally occurring peptide hormones for better stability and activity, among other reasons. EX1025, 44. Karten reviews the historical progress skilled artisans made on synthetic analogs of GnRH, a 10-residue peptide hormone naturally occurring in humans. *Id.* According to Karten, artisans created more than 2000 synthetic analogs following the discovery of the natural GnRH. *Id.* For GnRH, Karten notes that "synthetic chemical efforts [were] devoted largely to increasing the affinity of the peptides to the GnRH receptor." *Id.* These efforts were directed to "more potent," that is, high-activity, "and longer acting," or, high-stability "analogs." *Id.* Enhanced activity or stability are desirable "for practical clinical utility" of peptide hormones. *Id.*, 45.

72. By 2002, skilled artisans had developed several strategies for designing peptide drugs from naturally occurring peptide hormones.

1) Conservative Substitutions for Synthetic Analogs

73. Skilled artisans long knew from evolution that "a substitution of one amino acid residue for another has a far greater chance of being accepted if the two

residues are similar in properties." EX1026, 171.[18] Accordingly, a primary strategy employed by protein engineers was making conservative substitutions.

74. Because the chemical and physical properties of the amino acids involved in a conservative substitution are similar, their impact on the structural and chemical properties of the protein may be similar enough to preserve functionality (*e.g.*, ligand binding activity) of the protein. Where conservative substitutions are found at a given position in natural homologs, especially across closely-related species, this provided a strong indication to skilled artisans that the particular substitution was biologically acceptable to retain or even improve functionality.

75. An example of a conservative substitution is replacing an aspartic acid with a glutamic acid. *See*, *e.g.*, Section VII.B (discussing the chemical similarities between these groups). Because of the structural similarity between these groups, replacing, *e.g.*, an aspartic acid with a glutamic acid at a given position was viewed as likely to preserve or even enhance functionality of a given peptide. In contrast, exchanging the acidic, ionizable R-group of an aspartic acid residue with, *e.g.*, the basic, ionizable R-group of a histidine residue would not be considered conservative, given the complete reversal of the electronic charge at physiological

[18] French, S., *et al.*, *What is a Conservative Substitution?*, J. MOL. EVOL., 19, **1983**, 171-175 ("French," EX1026).

pH (negative to positive) and the stark differences in the physical structures of these amino acids' side chains.

2) Preparing recombinants from naturally occurring homologs

76. In addition to looking to amino acids that would be conservative substitutions for the amino acids found at a given position of a peptide, scientists also studied similarities and differences in the sequences of particular proteins across different species to learn about how the particular amino acid at a particular position impacts or does not impact the function of the protein. Homologous proteins, or, *homologs*, are proteins that are evolutionarily related. EX1012, 139. They usually perform the same function in different species and are then called *orthologs*. Homologous proteins that perform similar or related functions in the same species are called *paralogs*. *See*, *e.g.*, EX1052 (describing paralogous loci). When closely related species have different amino acids at a particular position (variant residues), this often indicates that the particular observed differences preserve the functionality of the proteins. For example, homologs often will differ by *conservative substitutions*, that is, substitutions involving "similar amino acid residues (for example, positively charged Arg might replace positively charged Lys)." EX1012, 139.

77. In contrast, amino acid residues that do not vary at a particular position between closely related species often are indicative of criticality. Skilled

artisans assume that random mutations are likely to occur at each position of a protein over a sufficiently long period of time but that mutations that critically undermine the functionality of the protein will be disfavored by evolutionary pressures over time. Skilled artisans thus recognized that strong uniformity of peptide sequence at a given position among related species strongly indicated criticality, meaning that substitutions at those positions were more likely to detrimentally impact the functionality of the peptide. As Nelson, explains, invariant residues among species are thus understood to be "more critical to the structure and function of a protein than the variable ones." EX1012, 139.

78. As Karten explains, skilled artisans routinely investigated orthologous peptides, the same peptide hormone but in a different animal, for potential amino acid substitutions. When investigating GnRH, for example, Karten sequenced sheep and pig orthologs. EX1025, 44. Skilled artisans had good reason, as Karten documents, to believe that chicken GnRH would have mammalian activity because, it was "isolated on the basis of its ability to release LH in an *in vitro* dispersed cell culture assay" using mammalian cells. *Id.* And so, Karten suggested a "lead brought about by this observation, that [His^5, Trp^7, Tyr^8] GnRH (or chicken II GnRH) can still result in an analog with high *in vitro* relative potency" against the human receptor. *Id.* Accordingly, evaluating uniformity verses variance in the sequence of orthologs across various species was a routine practice by skilled

artisans at the time in order to develop synthetic analogs of natural peptide hormones for therapeutic purposes.

79. Moreover, as more animal genomes were sequenced, the library of homologous peptide hormones available as orthologs to a skilled artisan only increased. EX1027, 509.[19]

80. Skilled artisans also routinely investigated paralogous peptides for mechanistic insight into peptide binding. For example, skilled artisans prepared synthetic analogs for insulin because naturally occurring insulin assembles into hexamers and only slowly dissociates into the bioactive monomers. EX1028, 279.[20] Galloway notes that, to overcome this tendency in naturally occurring insulin, researchers reversed "the Pro, Lys sequence in the B28-29 region of insulin" by mimicking the natural sequence of insulin-like growth factor I (IGF-I), which "has many structural similarities to insulin … but does not self-associate." (reproduced below). EX1029, 19-20, FIG. 6.[21]

---

[19] Tager, H. S., *et al.*, *Peptide Hormones*, ANN. REV. BIOCHEM., 43, **1974**, 509-538 ("Tager," EX1027).

[20] Noble, S. L., *et al.*, *Insulin Lispro: A Fast-Acting Insulin Analog*, AM. FAM. PHYSICIAN, 57(2), **1998**, 279-286 ("Noble," EX1028).

[21] Galloway, J. A., *New Directions in Drug Development: Mixtures, Analogs, and Modeling*, DIABETES CARE, 16(Supp 3), **1993**, 16-23 ("Galloway," EX1029).

Lys (B28), Pro (B29) – Human Insulin

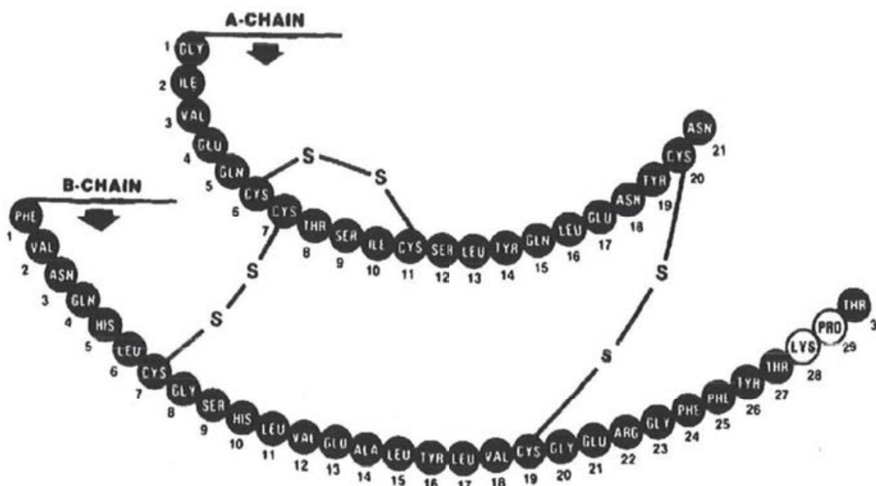


Figure 6—*Lys(B28), Pro(B29)-Human insulin. This analogue is identical to human insulin except at positions B28 and B29 where the sequence of the two residues has been reversed and are in the same order as in IGF-I, a polypeptide which does not self-associate. Courtesy of R.E. Chance.*

3) Engineering the biochemistry of the ligand-receptor interaction

81. Skilled artisans were also interested in exploring electrostatic complementarity of hormones and their receptors, thus, charge modulation was also used to control peptide binding. *See*, *e.g.*, EX1030, 7185.[22] Accordingly, optimizing the charge of the peptide within the binding site was a well-known strategy for engineering synthetic peptides by 2002.

F. Assays for Measuring Therapeutic Activity for Clinical

[22] Mishra, V. K., *et al.*, *Interactions of Synthetic Peptide Analogs of the Class A Amphipathic Helix with Lipids: Evidence for the Snorkel Hypothesis*, J. BIOL. CHEM., 269(10), **1994**, 7185-7191 (“Mishra,” EX1030).

Constipation Were Well-Known in the Art

82. Assays for measuring the therapeutic activity of peptides for clinical constipation were well known before 2002. For example, Currie discloses three methods for testing synthetic analogs to human uroguanylin for constipation-related activity. EX1005, 3:65-4:9; 4:62-5:16. By 2002, skilled artisans knew how to implement these conventional methods, which were also disclosed elsewhere. *See* EX1031, 947-48.[23] A skilled artisan would have recognized that these assays were useful for confirming the constipation-related activity of synthetic analogs to human uroguanylin because Currie used them to compare human uroguanylin to its natural analogs, as discussed in detail below. Because of these assays, and their known track record, a skilled artisan would have had a reasonable expectation of success in identifying [Glu^3]-human uroguanylin to treat clinical constipation.

1) Radioligand Binding Assay for Measuring Binding Affinity of Uroguanylin Analogs

83. Currie first discloses the "Radioligand Binding Assay." EX1005, 4:62-5:7. This assay is a competitive binding assay. EX1005, 6:16. It measures how strongly candidate analogs bind to, that is, their binding affinity for, human uroguanylin's receptor at that receptor's pocket of interest in treating clinical

[23] Currie, M. G., *et al.*, *Guanylin: An Endogenous Activator of Intestinal Guanylate Cyclase*, PROC. NATL. ACAD. SCI. USA, 89, **1992**, 947-951 ("Currie 1992," EX1031).

constipation. *See id.*, 4:62-5:7.

84. By 2002, it was known that this assay takes non-radioactive candidate analogs as a variant and outputs a dose-dependent "radioactivity by a multigamma counter." *See* EX1005, 5:5-7. The non-radioactive candidate analogs compete against a radioactive peptide hormone for binding the receptor. The signal from the radioactive peptide hormone that remains on cells depends on the concentration of the candidate analog and how strongly the non-radioactive candidate analog binds to the pocket of the receptor of interest. *See id.* 5:5-6 ("Results are expressed as the percentage specifically bound."). The produced radiosignal attenuates as the concentration and binding strength of the analog increase. If an analog is likely to be a human therapeutic for treating constipation, it eliminates the radiosignal at lower concentrations. EX1031, 949-950 ("Guanylin was also found to displace the specific binding of ^{125}I-STa from T84 cells. Therefore, these data support our proposal that guanylin is an endogenous activator of the intestinal guanylate cyclase and suggest that this peptide may influence intestinal fluid and electrolyte transport.").

85. A skilled artisan would have known how the Radioligand Binding Assay worked in detail. The Radioligand Binding Assay was conventional by 2002 and skilled artisans used it in a variety of contexts. It directly measures the competition between the uroguanylin analog and ^{125}Iodine-labeled heat-stable

enterotoxins to bind the intestinal guanylate cyclase as expressed by the T84 cell line—a cell line derived from a human colonic carcinoma. EX1005, 2:53-65; EX1032, 727; EX1031, 949. This concentration, at which competitive binding is halfway effective, is then used to evaluate uroguanylin analogs for how tightly they bind to their receptor intestinal guanylate cyclase. *See id.; see also id.*, FIG. 3B (reproduced below, showing displacement of the radio-labelled enterotoxin with increasing concentration of peptide hormone; human uroguanylin displacing the enterotoxin from its intestinal guanylate cyclase receptor establishes that human uroguanylin binds to the same receptor).

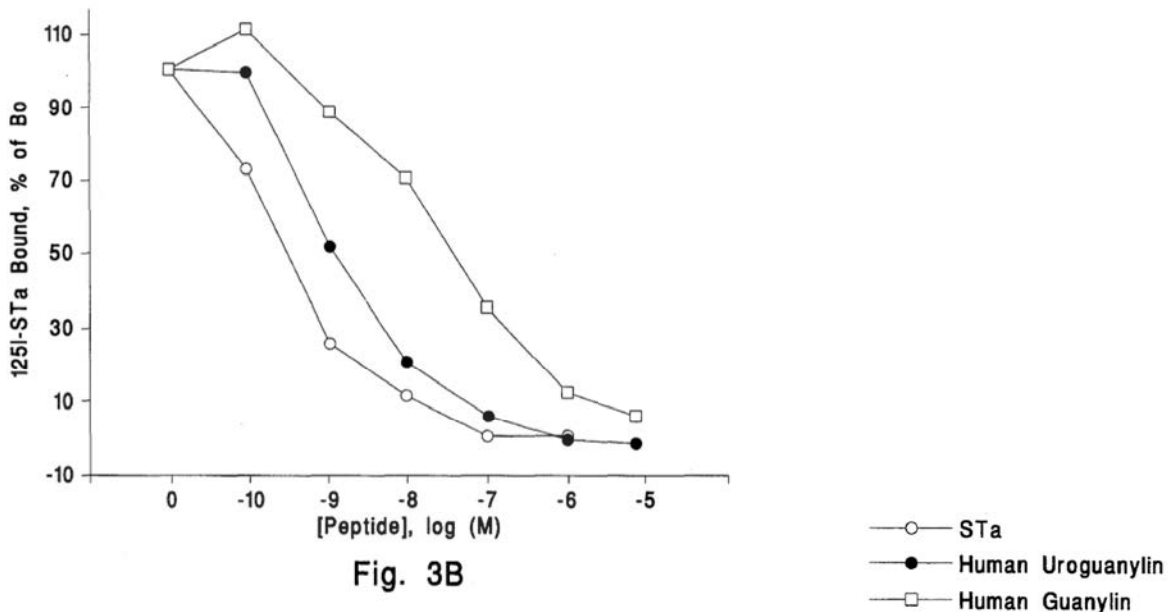


Fig. 3B

86. Using this assay, Currie was able to link signaling via intestinal guanylate cyclase, whether using STa or human uroguanylin, to increased fluid flow in the bowel, thus suggesting human uroguanylin as a human therapeutic for

treating constipation. EX1005, 2:20-24, 2:53-65, 5:2-7, 6:15-22; *see also*, *e.g.*, EX1032, 728-29[24] (using the same radio-ligand displacement method to measure binding affinity of variants of the *E. coli* enterotoxin, which binds to the same receptor as human uroguanylin); EX1033, 210-212[25] (employing this assay in evaluating ST, uroguanylin, and guanylin); EX1034, F874-F875[26] (employing this assay and evaluating dependency of binding affinities on temperature); EX1035, G623-G624[27] (employing this assay for an interaction involving a large number of high-affinity binding sites).

87. As measured in the Radioligand Binding Assay, displacement also increases with the selectivity of the uroguanylin analog for the enterotoxin-receptor interaction, in addition to the strength of that interaction. Non-specific interaction, that is, binding elsewhere on the cell, would soak up the uroguanylin analog,

---

[24] Visweswariah, S. S., *et al.*, *Characterization and Partial Purification of the Human Receptor for the Heat-Stable Enterotoxin*, EUR. J. BIOCHEM., 219, **1994**, 727-736 ("Visweswariah," EX1032).

[25] Krause, W. J., *et al.*, *Distribution of* Escherichia coli *Heat-Stable Enterotoxin/Guanylin/ Uroguanylin Receptors in the Avian Intestinal Tract*, ACTA ANAT., 153, **1995**, 210-219 ("Krause," EX1033).

[26] Forte, L. R., *et al.*, Escherichia coli *Enterotoxin Receptors: Localization in Opossum Kidney, Intestine, and Testis*, AM. J. PHYSIOL., 257(2), **1989**, F874-F881 ("Forte 1989," EX1034).

[27] Hyun, C. S., *et al.*, *Interaction of Cholera Toxin and* Escherichia coli *Enterotoxin with Isolated Intestinal Epithelial Cells*, AM. J. PHYSIOL., 247(6:1), **1984**, G623-G631 ("Hyun," EX1035).

attenuating it from displacing the radioligand, and requiring a higher concentration of the uroguanylin analog to displace a certain amount of the radioligand. The Radioligand Binding Assay thus provides a measure of both strength and selectivity of the ligand.

2) Assay for Measuring Activity of Uroguanylin Analogs from cGMP Induced in Human T84 Cell Monolayers

88. By 2002, skilled artisans routinely tested peptide hormones for activity by measuring how much cGMP the hormone induced from treated cell-culture models. Currie applied this widely available activity assay to uroguanylin and analogs on a cell-culture model of the human intestinal endothelium. EX1005, 3:65-4:9, 6:11-15, FIG. 3A. The T84 cells are exposed to the uroguanylin analogs of a series of escalating concentrations. *Id.*, 2:56-58. A good uroguanylin analog stimulates the production of cGMP in T84 cells. *See id.*, FIG. 3. Currie teaches that "Synthetic human uroguanylin caused a concentration dependent increase in T84 cell cyclic GMP (FIG. [3]a[28])." *Id.*, 6:11-13. It also teaches that "Human uroguanylin appeared to be more potent than human guanylin, but less potent than ST for activation of GC-C in T84 cells." *Id.*, 6:13-15.

89. From Currie, Figure 3A is reproduced below. The figure shows the production of cGMP on the vertical axis as a function of increasing peptide

[28] This is a typo in Currie.

hormone, left to right on a logarithmic scale from 0 to 10 µM. Human uroguanylin is shown to stimulate cGMP. This result shows that human uroguanylin stimulates the same receptor as human guanylin and STa. This activity leads Currie to suggest human uroguanylin as a human therapeutic for treating constipation. *See* EX1005, 2:20-24.

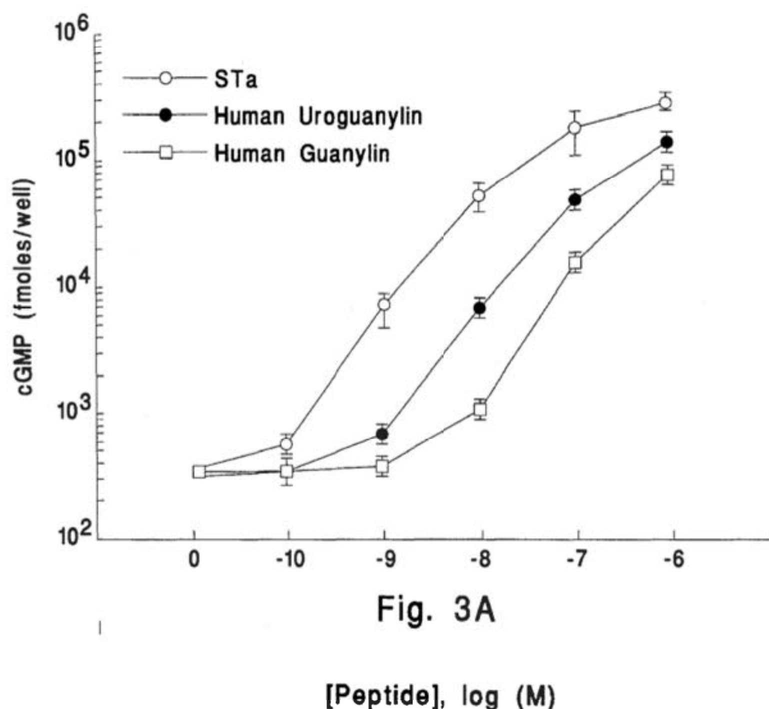


Fig. 3A

[Peptide], log (M)

90. Because the activity assay measures cGMP produced in response to uroguanylin and analogs, it is associated with the peptide hormone's potential as a human therapeutic for treating constipation. *See* EX1006.[29] Li provided good

---

[29] Li, Z., *et al.*, *Purification, cDNA Sequence, and Tissue Distribution of Rat Uroguanylin*, REGUL. PEPT., 68, **1997**, 45-56 ("Li," EX1006).

reason for concluding that data, such as that obtained in Currie's assays for cGMP, suggested the use of uroguanylin as a human therapeutic for treating constipation. Li instructs, for example, that "[e]levation of intracellular cyclic GMP levels in intestinal epithelial cells enhances secretion of chloride into the intestinal lumen …, and diminishes absorption of sodium and chloride …." *Id.*, 45. Li elaborates that "increased secretion and decreased absorption elevates the osmolarity of the lumen, and drives the luminal accumulation of water." *Id.* These phenotypes are associated with remediating constipation through intestinal control of water flux.

91. By 1991, T84 cells were already routinely being used to assess treatments for clinical constipation. *See, e.g.*, EX1036, 1885.[30] Well before 2002, skilled artisans had also found conventional activity assays that measure cGMP. *See*, *e.g.*, EX1037, G776[31] (measuring cGMP signaling from model intestinal tissue). Skilled artisans also routinely measured cGMP signaling to determine hormone activity on the target receptor. *See, e.g.*, EX1038, 345[32]; EX1021, 2705;

---

[30] Nguyen, T. D., *et al.*, *Stimulation of Secretion by the T_{84} Colonic Epithelial Cell Line with Dietary Flavonols*, BIOCHEM. PHARMACOL., 41(12), **1991**, 1879-1886 ("Nguyen," EX1036).

[31] Guarino, A., *et al.*, *T_{84} Cell Receptor Binding and Guanyl Cyclase Activation by* Escherichia coli *Heat-Stable Toxin*, AM. J. PHYSIOL., 253, **1987**, G775-G780 ("Guarino," EX1037).

[32] Bakre, M. M., *et al.*, *Dual Regulation of Heat-Stable Enterotoxin-Mediated cGMP Accumulation in T84 Cells by Receptor Desensitization and Increased Phosphodiesterase Activity*, FEBS LETT., 408, **1997**, 345-349 ("Bakre," EX1038).

EX1039, 23.[33] According to Bakre, "STh binding to its receptor, guanylyl cyclase C (GCC), leads to elevated intracellular levels of cGMP." EX1038, 345. According to Hamra 1997, "High mucosal acidity, similar to the pH occurring within the fluid microclimate domain at the mucosal surface of the intestine, markedly enhances the cGMP accumulation responses of T84 human intestinal cells to uroguanylin." EX1021, 2705. And according to Lin, "Heat-stable toxin (STa) increases cyclic GMP (cGMP) in isolated intestinal cells and in T84 cells, a colonic secretory cell line." EX1039, 23.

3) Assay for Measuring Chloride Secretion Induced by Uroguanylin Analogs in Rats

92. Currie also used a third assay to evaluate uroguanylin analogs' effects on chloride secretion. Currie measured the "Short-Circuit Current (ISc) in Rat Colon." EX1005, 5:8-16. By using this assay, Currie taught how to identify uroguanylin analogs, using a rat model, for their ability to treat clinical constipation. In this assay, "Rat proximal colon tissue" is mounted and cultured and used to "assess the effect of human uroguanylin on well characterized ST and guanylin-sensitive transport functions," *Id.*, 5:9-12, 6:23-26. This assay's measurements are an "indicator of transepithelial chloride secretion." *Id.*, 6:25-30.

---

[33] Lin, M., *et al.*, *Heat-Stable Toxin from* Escherichia coli *Activates Chloride Current via cGMP-Dependent Protein Kinase*, CELL PHYSIOL. BIOCHEM., 5, **1995**, 23-32 ("Lin," EX1039).

In fact, skilled artisans had already consulted "[p]revious studies in these preparations" which "indicated that the charge in Isc elicited by ST and guanylin is mostly accounted for by an increase in chloride secretion." *Id.*, 6:27-30. Currie illustrates the desired outcome from this assay in the output measured in microamperes per square centimeter after treatment of the tissue with a concentration of an uroguanylin analog. *Id.*, FIG. 4.

93. Human uroguanylin is "believed to be the physiologic driving force for eliciting chloride secretion and ultimately decreased water absorption," as evidenced by stimulation of the Short-Circuit Current in Rat Colon. EX1005, 2:18-21. Decreased water absorption indicates that a uroguanylin analog may "thus act as a laxative and be useful in patients suffering from constipation, e.g., cystic fibrosis patients who suffer with severe intestinal complications from constipation." *Id.*, 2:20-24.

94. By 2002, skilled artisans routinely practiced the assay for short-circuit current across rat intestine. *See*, *e.g.*, EX1040, 341[34]; EX1041, 101[35]; EX1042,

---

[34] Tien, X.-Y., *et al.*, *Neurokinin A Increases Short-Circuit Current Across Rat Colonic Mucosa: A Role for Vasoactive Intestinal Polypeptide*, J. PHYSIOL., 437, **1991**, 341-350 ("Tien," EX1040).

[35] Muflih, I. W., *et al.*, *Sugars and Sugar Derivatives which Inhibit the Short-Circuit Current of the Everted Small Intestine of the Rat*, J. PHYSIOL., 263, **1976**, 101-114 ("Muflih," EX1041).

81.[36]

G. Formulating Peptides with Intestinal Targets for Oral Delivery

95. Developing formulations in the form of, *e.g.*, tablets, capsules, and pills was routine well before 2002. *See*, *e.g.*, EX1010. Remington's notes, for example, that "[d]rug substances are most frequently administered orally by means of solid dosage forms such as tablets and capsules." EX1010, 1553. These dosage forms often included substances other than just the active pharmaceutical ingredient. As explained by Remington's, "[l]arge-scale production methods" used for the production of tablets, capsules, and pills "require the presence of other materials in addition to the active ingredients. Additives may also be included in the formulation to enhance the physical appearance, improve stability, and aid in disintegration after administration." EX1010, 1553. These materials are known in the art as excipients.

96. An excipient is a substance that is included in a pharmaceutical dosage form not for any direct therapeutic effect but to aid in manufacturing or delivery of the dosage form, such as by providing stability or protection for the active ingredient, to improve palatability of the dosage form for the patient, or to

---

[36] Helbock, H. J., *et al.*, *The Mechanism of Calcium Transport by Rat Intestine*, BIOCHIM. BIOPHYS. ACTA, 126, **1966**, 81-93 ("Helbock," EX1042).

increase bioavailability of the active ingredient. EX1043, 210[37]. Excipients may serve as the vehicle or medium for the active ingredient, and may include fillers, preservatives, stabilizers, coloring agents, and protective coatings. *Id.*; *see also, e.g.*, EX1044, 237[38] (stabilizing a vaccine with "an excipient blend of cellulose, starch, sucrose and gelatin"); EX1045, 1063[39] (noting that the "excipients used were … sodium alginate").

97. Oral delivery routes were also known to be useful in the administration of peptide-based drugs that targeted the intestinal endothelium. *E.g.*, EX1046, 28.[40] For example Mynott 1996 describes orally delivering a protein to reduce binding activity of the receptor from enterotoxin on the intestinal endothelium. *Id.* In more detail, Mynott 1996 describes delivering bromelain, a "glycoprotein" and "a cysteine protease obtained from pineapple stems." EX1046,

---

[37] Baldrick, P., *Pharmaceutical Excipient Development: The Need for Preclinical Guidance*, REGUL. TOXICOL. PHARMACOL., 32, **2000**, 210-218 ("Baldrick," EX1043).

[38] Duncan, J. D., *et al.*, *Comparative Analysis of Oral Delivery Systems for Live Rotavirus Vaccines*, J. CONTROL. RELEASE, 41, **1996**, 237-247 ("Duncan," EX1044).

[39] Gerogiannis, V. S., *et al.*, *Floating and Swelling Characteristics of Various Excipients Used in Controlled Release Technology*, DRUG DEV. IND. PHARM., 19(9), **1993**, 1061-1081 ("Gerogiannis," EX1045).

[40] Mynott, T. L., *et al.*, *Oral Administration of Protease Inhibits Enterotoxigenic* Escherichia coli *Receptor Activity in Piglet Small Intestine*, GUT, 38, **1996**, 28-32 ("Mynott 1996," EX1046).

29. The bromelain "preparation was suspended in water (l g per 5 ml) and administered by mouth." *Id.* Also, "enteric protected bromelain (Detach, Cortecs Ltd, Middlesex UK; 1 g of Detach contains 125 mg of bromelain) was administered." *Id.* A skilled artisan would have understood Mynott 1996 as showing that gelatin capsules allowed the administered bromelain to effectively target intestinal guanylate cyclase in that, via gelatin capsules, "administration of bromelain can inhibit ETEC receptor activity in vivo." *Id.*, 28.

98. Mynott 1991 explains that, to apply enteric protection to orally delivered peptides, skilled artisans used gelatin capsules. EX1047, 3708.[41] As above, an "enterically protected protease preparation" was administered orally. *Id.* This preparation involved "a single oral dose of 0.42 g of enteric-coated protease granules (Detach containing 25% protease; Enzacor Technology Pty. Ltd.)." *Id.*, 3709. "The protease granules were placed in gelatin capsules and administered by placing the capsules at the back of the throat." *Id.* For the peptide used in Mynott 1991, delivery to the intestine worked by simply encapsulating enough peptide in a gelatin capsule. In fact, via the gelatin capsule "efficacy of protease treatment was 99.5%, with very wide confidence limits (>0 to 99.9%)." *Id.*, 3708.

---

[41] Mynott, T. L., *et al.*, *Efficacy of Enteric-Coated Protease in Preventing Attachment of Enterotoxigenic* Escherichia coli *and Diarrheal Disease in the RITARD Model*, INFECT. IMMUN., 59(10), **1991**, 3708-3714 ("Mynott 1991," EX1047).

99. Skilled artisans also recognized that "oral administration of uroguanylin markedly stimulates intestinal fluid secretion." EX1018, G641. More specifically, Joo teaches that "orally administered uroguanylin resists luminal proteolysis and stimulates net intestinal fluid accumulation." *Id.*, G642.

H. Treating Constipation and Inflammation

100. Constipation and inflammation were long known to be symptomatic of some intestinal disorders by 2002. For example, in patients with an inflamed colon, "Twenty seven per cent of patients with active colitis (chronic inflamed colon) voided hard stools indicative of constipation," according to Rao.[42] EX1048, Abstract. Prior studies had found that "44% of colitics were constipated at the time of presentation, and in a larger retrospective enquiry, constipation was reported by 20% of patients." *Id.*, 342. In patients with ulcerative colitis, Rao "observed that transit through the small intestine and proximal colon was paradoxically slow." *Id.* This observation means that "at least a sizeable proportion of colitics are constipated." *Id.*, 344.

101. Skilled artisans had long treated constipation in patients suffering from colonic inflammation and constipation due to that inflammation. For example, Jalan "found both liquid paraffin and oral dioctylsodium sulphasuccinate

---

[42] Rao, S. S. C., *et al.*, *Symptoms and Stool Patterns in Patients with Ulcerative Colitis*, GUT, 29, **1988**, 342-345 ("Rao," EX1048).

to be helpful" in treating inflammation-driven constipation. EX1049, 693.[43]

102. Thus, by 2002, a skilled artisan would have understood the benefits of administering dosage forms comprising anti-inflammatory agents to treat constipation and related intestinal disorders. As Jalan noted, "constipation due to stasis in proctocolitis is usually a transient affair which remits when the inflammation is brought under control." EX1049, 693. This relationship would have made sense to a skilled artisan because the "cause is functional obstruction to passage of faeces associated with relatively normal proximal colon retaining the capacity to absorb water so that hard, scybalous masses are formed." *Id.*, 694.

VIII. THE ASSERTED REFERENCES DISCLOSE OR SUGGEST EACH OF THE CLAIMED FEATURES OF SHAILUBHAI

103. I have reviewed several references that I believe teach or suggest the compositions recited in claims 1-6 of Shailubhai. By virtue of their publication dates, I understand that these references, described in more detail below, may be considered in evaluating the validity of the claims of Shailubhai.

A. Currie

104. U.S. Patent No. 5,489,670, *Human Uroguanylin* to Currie, M. G., *et al.* ("Currie," EX1005) issued February 6, 1996. Accordingly, I have been advised that Currie is prior art to Shailubhai under pre-AIA 35 U.S.C. §102(b) because

[43] Jalan, K. N., *et al.*, *Faecal Stasis and Diverticular Disease in Ulcerative Colitis*, GUT, 11, **1970**, 688-696 ("Jalan," EX1049).

Currie published more than one year prior to January 17, 2002.

105. Currie discloses the peptide known as human uroguanylin and its use in controlling intestinal fluid absorption. EX1005, [54], [57]. As set forth by Currie, human uroguanylin is a 16-residue peptide with the following sequence:

$Asn^1\ Asp^2\ Asp^3\ Cys^4\ Glu^5\ Leu^6\ Cys^7\ Val^8\ Asn^9\ Val^{10}\ Ala^{11}\ Cys^{12}\ Thr^{13}\ Gly^{14}\ Cys^{15}\ Leu^{16}$

Id., [57], *see also id.*, 1:45-55 (noting the human uroguanylin sequence may also be denoted as NDDCELCVNVACTGCL). Currie notes that, "[i]n its oxidized active biologic form," human uroguanylin "has two disulfide bridges, one between cysteine residues at positions 4 and 12 and the other between cysteine residues at positions 7 and 15." *Id.*, 1:59-63.

106. Currie teaches that human uroguanylin is a naturally-occurring peptide that can be isolated from human urine or chemically synthesized by solid phase peptide synthesis. EX1005, 1:56-59. Currie also notes that preparing human uroguanylin synthetically, as opposed to isolating it from urine, yields a "homogenously purified form which did not exist in human urine from which it was initially obtained. That is, it has been prepared in a form which is essentially free of other low molecular weight peptides, and free from higher molecular weight material and other cellular components and tissue matter." *Id.*, 1:64-2:3.

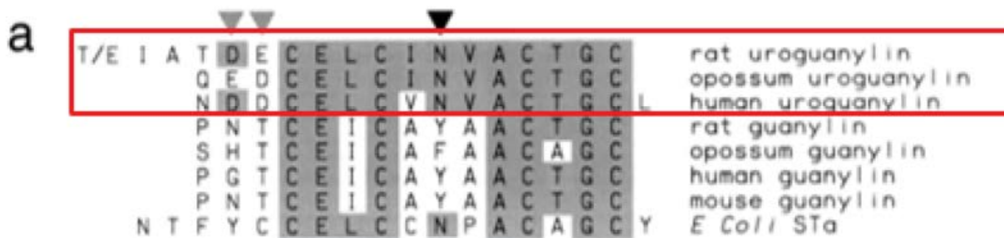
107. Currie teaches that human uroguanylin is useful for a variety of

therapeutic applications, including regulating intestinal fluid and electrolyte transport. EX1005, 1:34-44, 2:6-15. In particular, human uroguanylin can "act as a laxative and be useful in patients suffering from constipation, e.g. cystic fibrosis patients who suffer with severe intestinal complications from constipation." *Id.*, 2:16-24.

B. Li

108. Li, Z., *et al.*, *Purification, cDNA Sequence, and Tissue Distribution of Rat Uroguanylin*, REGUL. PEPT., 68, **1997**, 45-56 ("Li," EX1006) published in 1997. Accordingly, I have been advised that Li is prior art to Shailubhai under pre-AIA 35 U.S.C. §102(b) because Li published more than one year prior to January 17, 2002.

109. Li teaches human uroguanylin as a naturally occurring peptide that can be found in human urine. EX1006, 46. Li discloses the alignment and comparison of the peptide sequences for human, rat, and opossum uroguanylins. EX1006, 52, FIG. 6(a) (reproduced below, with the human, rat and opossum uroguanylin sequence alignment shown in the red box).



110. Li also teaches that "[t]he affinity of GCC for uroguanylin (opossum or human) is about 10-fold higher than its affinity for guanylin (rat or human). Thus, features that are found in uroguanylin, but not in guanylin, offer information about structural elements that specify the strength of the ligand/receptor interaction." *Id.*, 54. In particular, Li notes that "[o]f particular interest are two residues that are basic or uncharged in guanylin but acidic in uroguanylin (stippled [*i.e.*, grey] arrowheads)" in Figure 6(a), above. *Id.*

C. Narayani

111. Narayani, R., *et al.*, *Polymer-Coated Gelatin Capsules as Oral Delivery Devices and their Gastrointestinal Tract Behaviour in Humans*, J. BIOMATER. SCI. POLYM. ED., 7(1), **1995**, 39-48 ("Narayani," EX1007) published in 1995. Accordingly, I have been advised that Narayani is prior art to Shailubhai under pre-AIA 35 U.S.C. §102(b) because Narayani published more than one year prior to January 17, 2002.

112. Narayani discloses the formulation of peptides into capsules with excipients for oral administration for delivery to the intestines. EX1007, 39. More specifically, Narayani teaches an "enteric coated" delivery system for peptide-based therapeutics that may be "enclosed in a gelatin capsule." *Id.*, 40, 47. The capsule comprises excipients including a coating made of a "natural polymer such as alginate and cross-link[ed] with calcium chloride" to enhance delivery to the

intestine. *Id.*; *see also id.*, 39 (noting such capsule-based dosage forms "are becoming an increasingly popular method for providing controlled drug release in the gastrointestinal (GI) tract").

D. Campieri

113. Campieri, M., *et al.*, *Oral Budesonide Is as Effective as Oral Prednisolone in Active Crohn's Disease*, GUT, 41, **1997**, 209-214 ("Campieri," EX1008) published in 1997. Accordingly, I have been advised that Campieri is prior art to Shailubhai under pre-AIA 35 U.S.C. §102(b) because Campieri published more than one year prior to January 17, 2002.

114. Campieri teaches the administration of anti-inflammatory agents such as budesonide or prednisolone to patients suffering from constipation resulting from active Crohn's disease. EX1008, 209 (noting also that Crohn's disease is a chronic inflammatory disorder). Campieri teaches remission of inflammatory symptoms in "60% of patients receiving budesonide once daily or prednisolone and in 42% of those receiving budesonide twice daily." *Id.*, Abstract. According to Campieri: "As one of the first aims in treating patients with inflammatory bowel disease is the prompt disappearance of symptoms, this goal was most clearly achieved with budesonide once daily and prednisolone within the first two weeks." EX1008, 213.

E. Ekwuribe

115. U.S. Patent No. 5,359,030, *Conjugation-Stabilized Polypeptide Compositions, Therapeutic Delivery and Diagnostic Formulations Comprising Same, and Method of Making and Using the Same*, to Ekwuribe, N. N. ("Ekwuribe," EX1009) issued October 25, 1994. Accordingly, I have been advised that Ekwuribe is prior art to Shailubhai under pre-AIA 35 U.S.C. §102(b) because Ekwuribe published more than one year prior to January 17, 2002.

116. Ekwuribe discloses a "conjugated peptide complex comprising a peptide conjugatively coupled to a polymer," and specifically to polyethylene glycol. EX1009, [57], 7:65-8:8, claim 15. As explained by Ekwuribe, "[l]ipophilic and absorption enhancing properties are imparted by the oleic acid chain, while the polyethylene glycol (PEG) residues provide a hydrophilic (hydrogen bond accepting) environment." *Id.*, 13:47-50.

IX. GROUND 1. CLAIM 1 WAS OBVIOUS OVER CURRIE AND LI

117. As set forth below, claim 1 of Shailubhai claims a 16-residue peptide "consisting of the amino acid sequence of SEQ ID NO: 20," which is the sequence shown below.

$$\mathrm{Asn^{1}\ Asp^{2}\ Glu^{3}\ \underset{*}{Cys^{4}}\ Glu^{5}\ Leu^{6}\ \underset{**}{Cys^{7}}\ Val^{8}\ Asn^{9}\ Val^{10}\ Ala^{11}\ \underset{*}{Cys^{12}}\ Thr^{13}\ Gly^{14}\ \underset{**}{Cys^{15}}\ Leu^{16}}$$

EX1001, 5:5-16, claim 1.

118. As I noted in Section IV, above, Shailubhai acknowledges that the

sequence of this claimed peptide differs from the naturally-occurring human uroguanylin sequence by ***one*** amino acid. *Compare* EX1001, 5:5-16 sequence *with id.*, 7:55-58 sequence. That is—the claimed peptide differs from the native human uroguanylin sequence only in that a glutamic acid (Glu, D) residue has been swapped in for the aspartic acid (Asp, E) residue. These amino acids differ structurally from one another only in that a glutamic acid residue has an additional methylene unit ($-CH_2-$) in its side chain compared to aspartic acid. *See* Section IV (showing the chemical structures of these residues to assist in visualizing these amino acids). Thus, in view of the structural similarity of Glu and Asp residues, human uroguanylin differs from the claimed peptide by a single methylene unit ($-CH_2-$).

119. As I also noted in Section IV, above, I refer to the claimed peptide consisting of the amino acid sequence of SEQ ID NO:20 throughout this declaration as "[Glu^3]-human uroguanylin"—indicating the sequence is that of human uroguanylin where the aspartic acid at the third position has been swapped for a glutamic acid (Glu).

120. As set forth in my analysis below, Currie teaches human uroguanylin, and further suggests this peptide for use as a human therapeutic for treating constipation. Currie also teaches rat uroguanylin, a peptide with an amino acid sequence that is slightly different from that of human uroguanylin, as inspiration

for activity-enhancing amino acid substitutions that could be made to human uroguanylin. Currie also discloses three assays for investigating analogs of human uroguanylin, establishing that these assays were conventional and known to those of ordinary skill in the art for evaluating a given peptide's potential for treating clinical constipation.

121. Li provides further teachings on both human and rat uroguanylin. In particular, Li teaches that the replacement of an aspartic acid residue at the third amino acid position with a glutamic acid residue occurs naturally between human and rat uroguanylin aligned peptide sequences.

122. As I discuss throughout the course of my analysis, a skilled artisan would have had good reason to combine Currie and Li with a reasonable expectation of success. In particular, I discuss why a skilled artisan would begin with human uroguanylin, as taught by Currie, when developing a treatment for constipation. I also explain why, in view of Li's alignment of the human and rat uroguanylin sequences, a skilled artisan would have had good reason to substitute glutamic acid for aspartic acid at the third position.

123. In particular, I note that a skilled artisan would have had at least three good reasons to make [Glu^3]-human uroguanylin with a reasonable degree of success. First, directed evolution provided reason to make the conservative glutamic acid substitution based on the alignment of the naturally occurring human