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Udenfriend S, Stein S, Böhlen P, Dairman W, Leimgruber W & Weigele M.
Fluorescamine: a reagent for assay of amino acids, peptides, proteins, and primary amines in the picomole range. *Science* 178:871-2, 1972.
[Roche Inst. Mol. Biol., and Chem. Res. Dept., Hoffmann-La Roche, Inc., Nutley, NJ]

Fluorescamine reacts rapidly and stoichiometrically with amino acids, peptides, and proteins to yield highly fluorescent derivatives. Since neither fluorescamine nor its hydrolysis products are fluorescent, it is an ideal reagent for online monitoring of eluates from rapidly flowing high-pressure liquid chromatography columns. [The SCI® indicates that this paper has been cited in over 1,180 publications since 1972.]

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Phenylalanine yields a weakly fluorescent product with ninhydrin. However, McCaman and Robins[1] noted that in deproteinized plasma more fluorescence was attained than could be accounted for by its phenylalanine content. They found the assay specific for phenylalanine, but some substance(s) in blood increased fluorescence manyfold. They later found that almost any peptide added to phenylalanine and ninhydrin increased fluorescence. McCaman and Robins's method for blood phenylalanine measurement uses a peptide to intensify the fluorescence. In my book on fluorescence[2] I made the following prediction, "The requirement of a peptide to produce phenylalanine fluorescence with ninhydrin suggests, too, that the procedure can be modified to detect peptides."

In 1968 K. Samejima found that the phenylacetaldehyde formed on reaction with ninhydrin could react in the presence of excess ninhydrin with any amino acid, peptide, or primary amine to yield highly fluorescent products.[3] We then showed that the fluorescent product formed on treating ethylamine with ninhydrin and phenylacetaldehyde arose from the condensation of one equivalent each of ethylamine, ninhydrin, and acetaldehyde with the concomitant elimination of water. From this I postulated a chemical structure for the fluorophor. Arnold Brossi, who was then head of the Chemistry Division at Hoffmann-La Roche, told me that he considered the proposed mechanism invalid and asked me to hold off publication. He then assigned the problem to an associate, Manfred Weigele. After several months, Weigele called to tell me that he had solved the mechanism and had synthesized the intermediate (later named fluorescamine) presumed to be formed in the reaction between ninhydrin and phenylacetaldehyde. Fluorescamine, which is itself nonfluorescent, reacts instantaneously and stoichiometrically with primary amines at neutral pH and room temperature to form highly fluorescent derivatives. Hydrolysis products of the excess fluorescamine are also nonfluorescent. Weigele and his colleagues had produced the ideal reagent for detection and assay of amino acids, peptides, and proteins in the picomole range.[4] Later Böhlen and Stein worked out optimal conditions for reacting fluorescamine with amino acids and polypeptides and developed assays for proteins and amino acids (reported in the *Citation Classic* paper). M. Rubinstein worked out conditions for the use of fluorescamine to monitor high-pressure liquid chromatography (HPLC) of proteins that we later applied to our studies that led to the discovery of proenkephalin.

Fluorescamine was developed jointly between scientists at the nonmission-oriented Roche Institute of Molecular Biology and the Chemistry Division of Hoffmann-La Roche. The question may be asked did Hoffmann-La Roche gain more than scientific prestige from fluorescamine? The annual sales of fluorescamine are probably equivalent to the hourly sales of Valium. However, if one looks more carefully, one finds applications of great value to Hoffmann-La Roche. In 1977 I assigned Rubinstein to work with Sidney Pestka and use fluorescamine along with HPLC in an attempt to purify human interferon α. At that time no one had succeeded in purifying any form of interferon, although many outstanding laboratories had been attempting to do so for years. In an amazingly short time the two succeeded in purifying and characterizing each of the several different forms of human interferon α.[5] This was the basis for the patent issued to Hoffmann-La Roche for interferon α. Human growth hormone releasing factor, which was purified in Roger Guillemin's laboratory at the Salk Institute by my former colleague Böhlen, also using fluorescamine and related methodologies, is now under development at Hoffmann-La Roche for human and animal use.

1 **McCaman M W & Robins E.** Fluorimetric method for the determination of phenylalanine in serum. *J Lab Clin Med* 59 885-90, 1962 (Cited 380 times)
2 **Udenfriend S.** *Fluorescence assay in biology and medicine* New York: Academic Press, 1969. Vol 2 p 205-7
3 **Samejima K, Dairman W & Udenfriend S.** Condensation of ninhydrin, aldehydes and primary amines to yield highly fluorescent ternary products. I. Mechanism of the reaction and partial characterization of the condensation product. *Anal Biochem* 42 222-36, 1971 (Cited 55 times)
4 **Weigele M, DeBernardo S L, Tengi J P & Leimgruber W.** The fluorogenic ninhydrin reaction. Structure of the fluorescent principle. *J Amer Chem Soc* 94 4052-4, 1972 (Cited 60 times)
5 **Rubinstein M, Rubinstein S, Familletti P C, Gross M S, Miller R S, Waldman A A & Pestka S.** Human leukocyte interferon purified to homogeneity. *Science* 202 1289-90, 1978