# Estimation of stacking affinity of 7-methylguanosine in different protonation states

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**Dedicated to Professor Harri Lönnberg on the occasion of his 60th birthday**

**Abstract**
A new technique with which to estimate the affinity of any organic compound for participation in stacking interactions was applied to 7-methylguanosine. A microcalorimetric method was used. The compositions and apparent stability constants of the associations formed between caffeine and indole, 7-methylguanosine and indole, and caffeine and 7-methylguanosine were determined. It was concluded that, in respect of stacking affinity, 7-methylguanosine in protonated form behaves similarly to caffeine.

**Keywords:** 7-Methylguanosine, stacking interaction, caffeine, indole

## Introduction

Chemical bonds can be categorized into two main types: primary (including covalent, ionic, dative and metallic bonds) and secondary (including hydrogen bonds and van der Waals interactions (three types). This categorization of chemical bonds is sufficient for an interpretation of the chemical properties of many simple inorganic compounds at both a molecular and a macroscopic level. In contrast, in both inorganic and organic molecules and macroscopic matrices with more complicated compositions, different secondary interactions (chemical bonds) can be observed. This is especially true for organic systems, where many special types of interactions have been detected, both in relatively simple organic compounds and in macromolecules (e.g. proteins, DNA and RNA). These interactions include the formation of charge-transfer complexes, electrostatic-ionic interactions, hydrogen-bonding interactions, π–π interactions, cation–π interactions and stacking interactions.

Of these, my interest was attracted by the stacking interactions, observed in solid crystals, and in many biological and molecular recognition processes. [1-39] (My attention was called by

 

Professor Harri Lönnberg to the importance of stacking interactions in biological systems.) The presence and effects of such interactions have been presumed in the interpretation of many biological functions of the 7-methylguanosine, other alkylated nucleic acid bases and their nucleotide derivatives. One example is the eukaryotic mRNA with a special CAP structure (see [40-55] and the references therein) (the 5'cap is found on the end of mRNA molecule and consists of a guanine nucleotide connected to the mRNA via an unusual 5' to 5' triphosphate linkage). This guanosine is methylated on the 7 position directly after capping in vitro by a methyl transferase. It is referred to as 7-methylguanosine cap). This part of mRNA is responsible for many molecular recognition phenomena, e.g. binding to a CBP (Cap-binding protein: a protein (24 kD) with affinity for cap structure at 5' end of mRNA that probably assists, together with other initiation factors, in binding the mRNA to the 40S ribosomal subunit). Translation of mRNA in vitro is faster if it has a cap binding protein). This interaction is involved in the protein synthesis in eukaryotic cells. It has been postulated that the stacking interactions between the nucleic acid bases are the factors, responsible for the spatial geometrical arrangement of the CAP structure, which plays a special biological role in protein synthesis. To clarify the role of the 7-methylguanosine moiety of mRNA in this protein synthesis, the chemical properties of 7-methylguanosine and its ability to undergo stacking interactions, must be investigated.

7-Methylguanosine contains a quaternary N atom, which results in a protolytic equilibrium with relatively low pK (~ 7.2). (The pK of guanosine itself is ~ 8.5). In aqueous solution, therefore, 7-methylguanosine is present mostly in protonated and in unprotonated form at pH < 6.2 and pH > 8.2, respectively. The potentiometric titration curve of commercial 7-methylguanosine is presented in Figure 1.

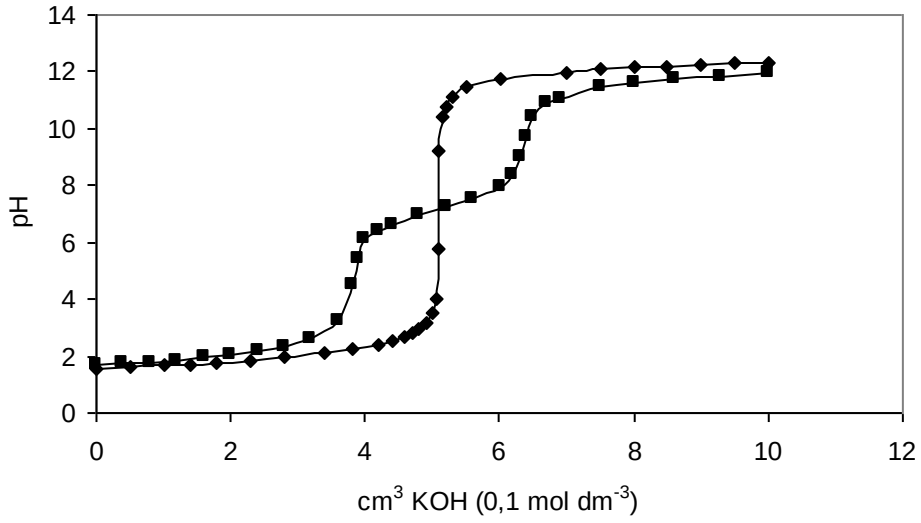


**Figure 1.** Potentiometric titration curves of HCl solution (◆) and of the 7-methylguanosine in HCl solution (■).

**The ability to undergo stacking interactions**

Lawaczek and Wagner[37] attempted to define the exact nature of stacking interactions, suggesting, that dipole-dipole interactions are the most important factors determining the effectiveness of the stacking of aromatic rings. Such interaction was defined synonymously as stacking, base-stacking, aromatic stacking or vertical stacking, the word stack being used to describe the resulting association. They postulated that the measure of the stacking interaction between two aromatic compounds depends on the polarizabilities and polarizing powers of the partners. The polarizability of a compound in turn depends on the mobile π-electron density, this being well characterized by the ring current effect (intermolecular shielding values), which can be determined by NMR spectroscopy. The polarizing power of a compound involved in a stacking interaction is determined by the dipole moments that exist in the molecule. If many C–O or C–N bonds are present in a molecule, a high stacking affinity (polarizing power) of the molecule is observed[37].

Lawaczek and Wagner suggested sequences for the nucleic acid bases and related compounds as concerns both the polarizing power and the polarizability:

Polarizing effect: caffeine > guanine > adenine > purine > indole

Polarizability: indole > adenine > purine > guanine > caffeine

**Estimation of the stacking affinity of organic compounds**

Setting out from the concept of Lawaczek and Wagner[37], we postulated that the stacking affinity of organic compounds, might be determined via their interactions with the extreme members of the above series, i.e. caffeine and indole, which would reflect their polarizing powers and polarizabilities.

**Estimation of the stacking affinity of 7-methylguanosine**

As an illustration, we report here an investigation of the interactions between caffeine, indole and 7-methylguanosine, with a view to determining the stacking affinity of 7-methylguanosine. Microcalorimetry was used as this method has sufficient sensitivity to detect such weak interactions. The question arises of whether there is a difference between the stacking affinities of the protonated and unprotonated forms of 7-methylguanosine. This may be important in respect of the biological roles of the 7-methylguanosine derivatives of mRNA, which are pH-dependent. In an attempt to answer this question and to determine the relative stacking affinity of 7-methylguanosine, the interactions (reaction heats) between 7-methylguanosine, indole and caffeine were measured in aqueous solution (pH=7), in HCl acid solution (pH=2) and in KOH solution (pH=12).

## Results and Discussion

### Interaction between caffeine and indole in aqueous solution

The heat of dilution of indole in the applied concentration range (<0.01 mol $dm^{-3}$) proved to be negligible, whereas that of caffeine (0.001-0.1 mol·$dm^{-3}$) depends on its concentration, and must be measured in each case. The interaction between caffeine and indole results in an endothermic effect (Figure 2). At low molar ratios (2, 4, 8) of indole:caffeine (lower curve in Figure 2), the heat of interaction ($Q_{react}$) clearly depends almost linearly on the concentration of indole. At high molar ratios (20, 40, 80) of indole:caffeine (upper curve), $Q_{react}$ tends to increase with increasing concentration of indole. This indicates that the mechanism of the interaction differs at low and high molar ratios.

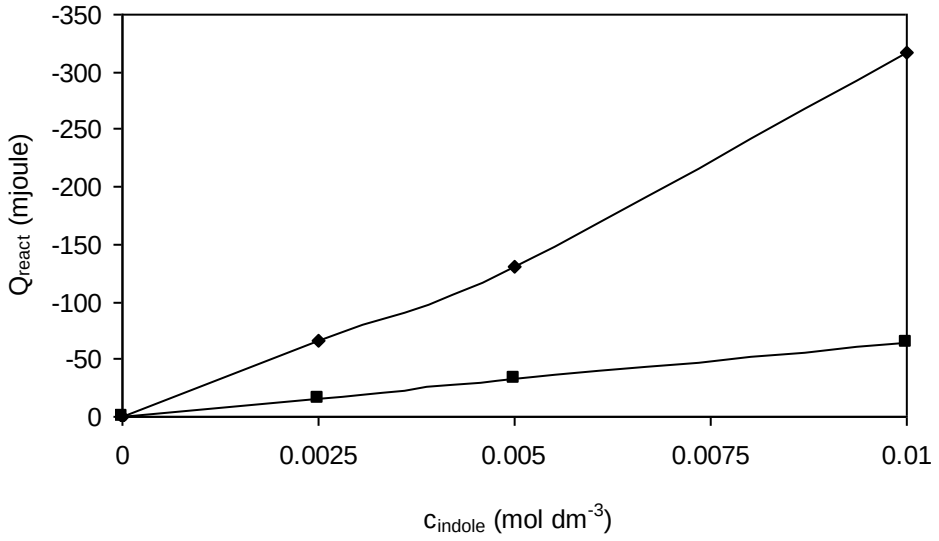


**Figure 2.** Heats of interaction of caffeine and indole at caffeine:indole molar ratio of 1 to 2, 4, 8 (■) and 1 to 20,.40 and 80 (◆) (1 caloria = 4.184 joule).

In the biological systems of interested, the molar ratios of the molecules, or moieties of the molecules, involved in the presumed stacking interactions are low.

The linear plateau observed in Figure 3 points to a definite composition of the association in this concentration range. To determine this composition, the method of Job was used. The plot to be seen in Figure 4 indicates a 1:1 composition. The above data suggest an interaction between caffeine and indole, which presumed to be a stacking interaction.

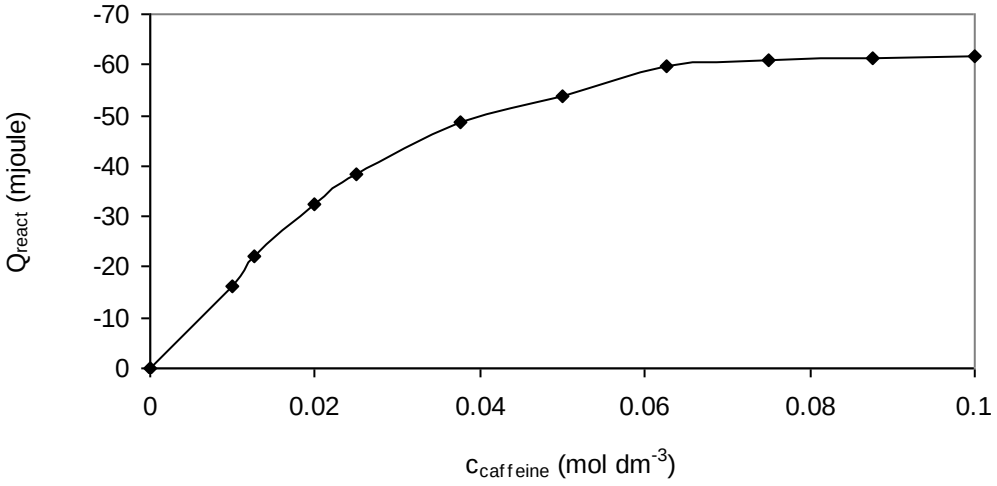


**Figure 3.** Heats of interaction of caffeine and indole at caffeine:indole molar ratios of 1:2 to 1:20. (■)

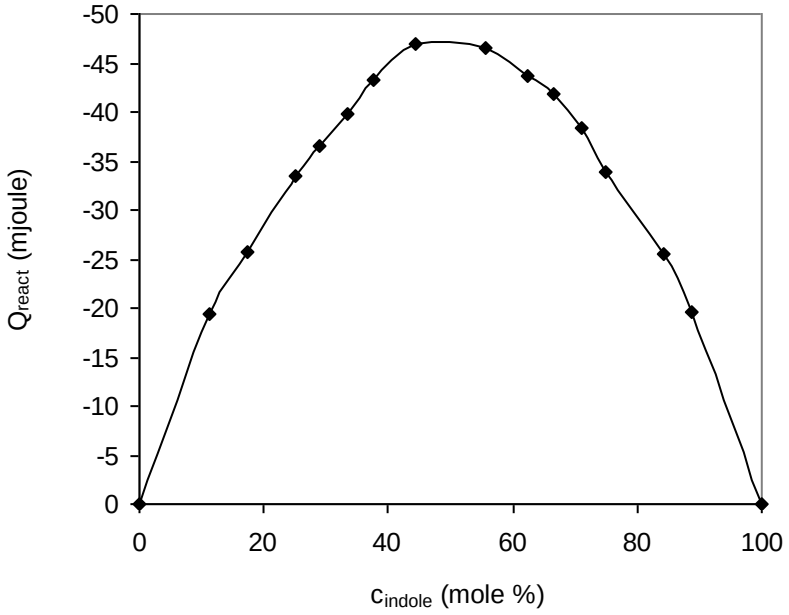


**Figure 4.** The Job-plot of the interaction between caffeine and indole at concentrations of [caffeine] + [indole] = 0.0075 mol $dm^{-3}$

**Interaction of 7-methylguanosine with indole in aqueous solution**

The plot for 7-methylguanosine and indole in Figure 5 resembles those for caffeine and indole in Figure 2, the mechanism of the interaction here too depending on the molar ratio of the reactants. The Job plot of the interaction between 7-methylguanosine and indole in Figure 6 indicates a 1:1

composition. The heat of interaction between caffeine and indole is higher than that between 7-methylguanosine and indole: at the maximum, –46 mjoule and –12 mjoule, respectively.

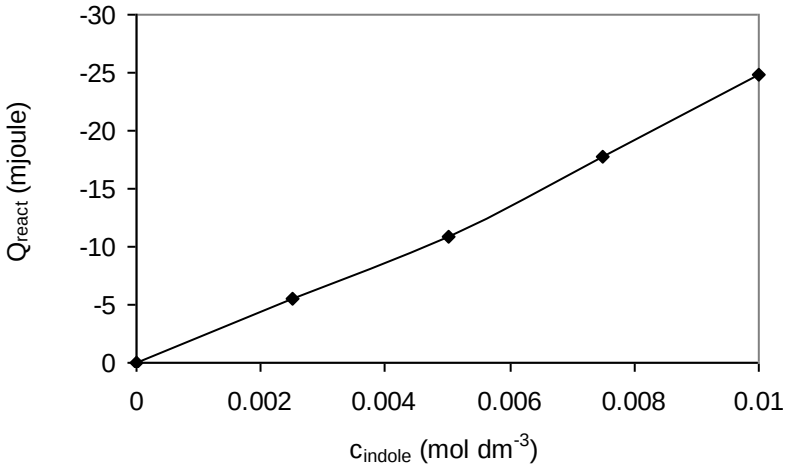


**Figure 5.** Heats of interaction of m7-methylguanosine and indole at indole:7-methylguanosine molar ratios of 1:10 to 1:40.

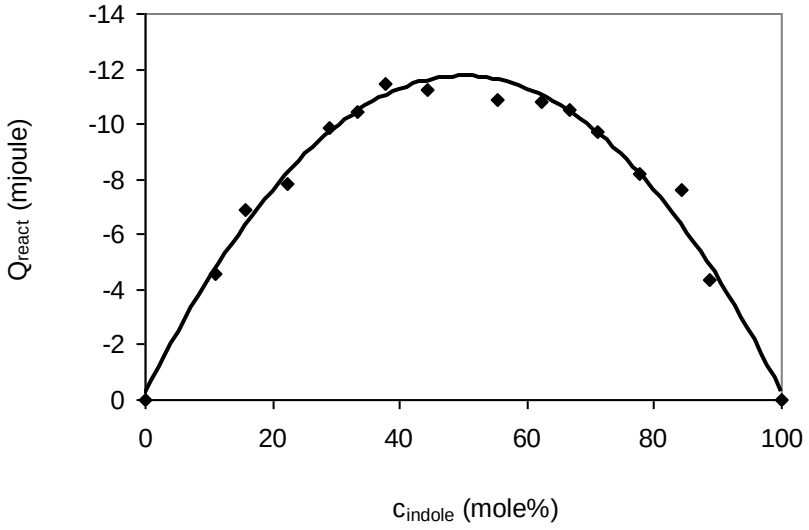


**Figure 6.** Job-plot of the interaction between 7-methylguanosine and indole in water at [7-methylguanosine] + [indole] = 0.0075 mol dm.$^{-3}$

**Interaction of 7-methylguanosine with indole in acidic solution (HCl, pH=2)**

At pH=2, 7-methylguanosine is present practically completely in protonated form, and thus the interaction with indole will relate to the stacking affinity of the protonated form. The Job plot of the interaction in Figure 7 (similarly as in aqueous solution) demonstrates a 1:1 composition of

the association, but with a higher heat of interaction at the maximum: -20 mjoule instead of -12 mjoule, pointing to the higher stacking affinity of the protonated form of 7-methylguanosine.

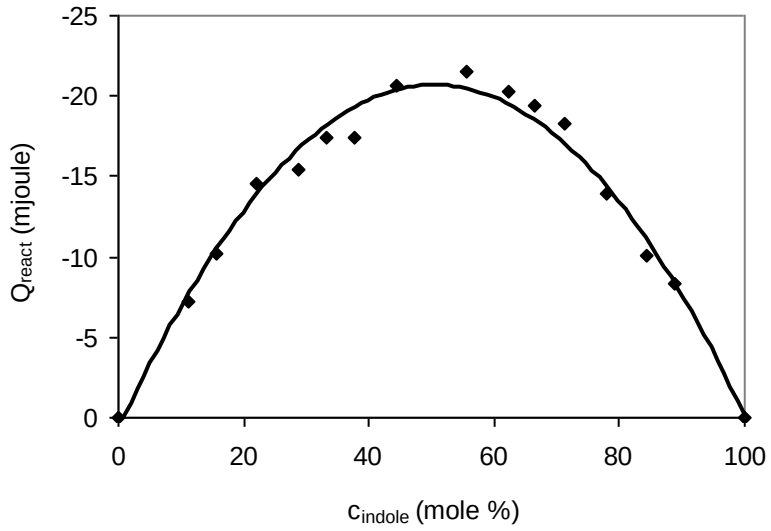


**Figure 7.** Job-plot of the interaction between 7-methylguanosine and indole in acidic solution (pH=2) at [7-methylguanosine] + [indole] = 0.0075 mol $dm^{-3}$

**Interaction of 7-methylguanosine and indole in alkaline solution (KOH, pH=12)**

In alkaline solution, the heat of interaction between 7-methylguanosine and indole is only slighthy larger than the sensitivity of the method (Figure 8). The suggested stoichiometry of the 7-methylguanosine:indole association is 2:1. It was concluded that the protonated form of the 7-methylguanosine has a higher stacking affinity than that of unprotonated 7-methylguanosine.

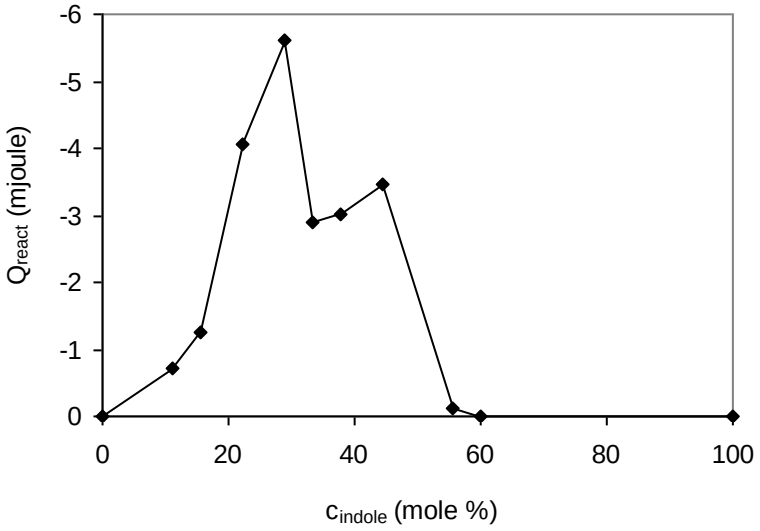


**Figure 8.** Job-plot of the interaction between 7-methylguanosine and indole in alkaline solution (pH=12) at [7-methylguanosine] + [indole] = 0.0075 mol $dm^{-3}$

**Interaction between 7-methylguanosine and caffeine in aqeous solution**

The plot of the heat of interaction of these two compounds is depicted in Figure 9, and a Job plot in Figure 10. The stoichiometry of the association formed between caffeine and 7-methylguanosine was calculated to be 2:1.

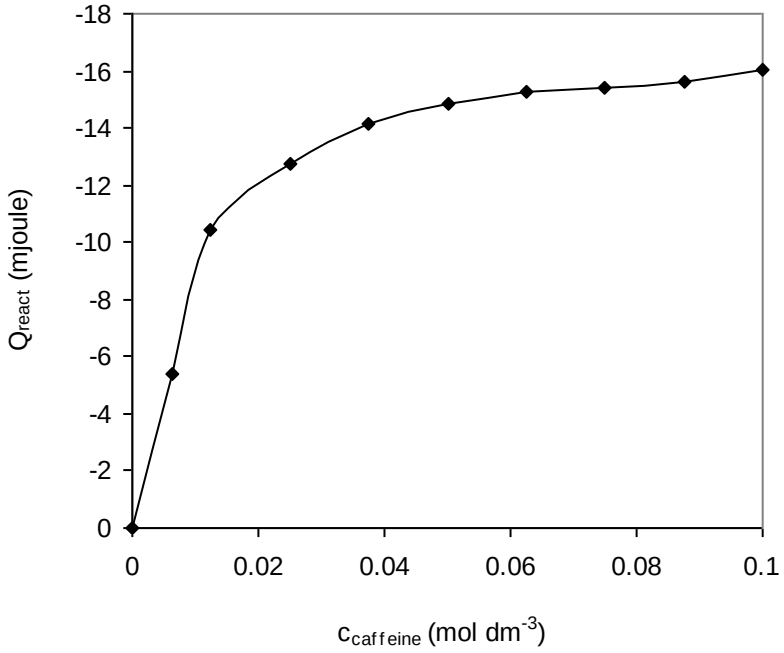


**Figure 9.** Heats of interaction between caffeine and 7-methylguanosine at 7-methylguanosine:caffeine molar ratios of 1:5 to 1:80.

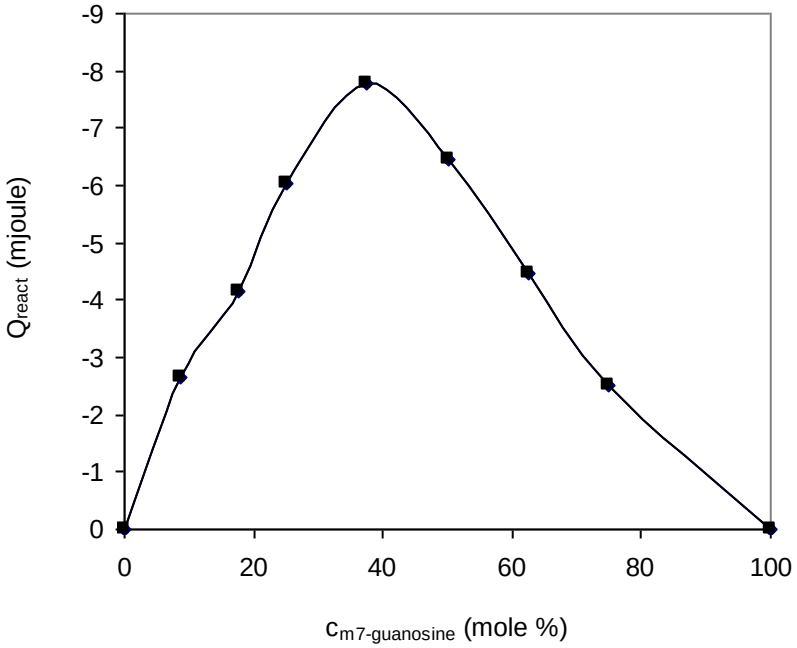


**Figure 10.** Job-plot of the interaction between caffeine and 7-methylguanosine in water solution, at concentration [caffeine] + [7-methylguanosine] = 0.00667 mol $dm^{-3}$

## Conclusions

The interaction observed between caffeine and indole by means of microcalorimetry was asssumed to be a stacking interaction. 7-Methylguanosine similarly forms an association with indole as does caffeine, but with a lower heat of interaction (see Figures 4 and 6). The protonated form of 7-methylguanosine exhibits a higher stacking affinity, than that of the unprotonated form. This could explain the pH-dependence of the effect of the 7-methylguanosine-containing CAP structure.

## Experimental Section

**Microcalorimetric measurements**

Measurements were made at 25 ºC with an LKB 10700-2 Batch Microcalorimeter, designed for study of the thermodynamic properties of biochemical reactions in aqueous solutions. In consequence of its high sensitivity, only small amounts of substance are needed for an investigation. Heats ranging from 200 $10^{-6}$ to 2000 $10^{-3}$ joules can be determined with high accuracy.

In studies of the heat evolved or absorbed when solutions of A and B are mixed, the heats of dilution of solutions A and B must be measured. The heat of interaction of solutions A and B was calculated via the following formula:

$$Q_{react} = Q_{measured} - Q_{dil}\,(\text{solution A}) - Q_{dil}\,(\text{solution B})$$

In the course of microcalorimetric measurements, two methods were used:

**Method 1**

Solutions of the reaction partners with different concentrations were prepared and mixed. In each series of experiments, the concentration of one of the partners was fixed (in view of the solubility limits, this partner was generally indole), and the concentration of the second partner was varied (changes in molecular ratio method).

**Method 2**

The well-known Job-method was used, where the sum of the concentrations of the partners is fixed, their molar ratio (0-100%) being varied, and the stoichiometry of the association is determined from the shape of the $Q_{react}$ vs. molar ratio (%) function.

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